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Bacterial activity and community structure in the Columbia River estuarine turbidity maxima

by

Byron C. Crump

A dissertation submitted in partial fulfillment of the
requirements for the degree of

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Doctoral Dissertation

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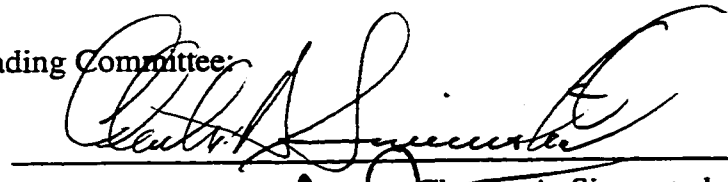

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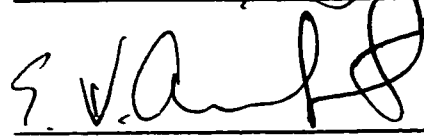

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Date: 9-9-99

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Abstract

BACTERIAL ACTIVITY AND COMMUNITY STRUCTURE IN THE COLUMBIA RIVER ESTUARINE TURBIDITY MAXIMA

By Byron C. Crump

Chairperson of the Supervisory Committee: Professor John A. Baross
School of Oceanography

The food web of the Columbia River estuary is centered in estuarine turbidity maxima (ETM) where allochthanous particulate organic material (POM), particle-attached bacteria, and consumers such as copepods and rotifers are trapped and concentrated by the hydrodynamics of the system. Bacteria play a central role in this food web as the principle consumers of dead and degraded organic matter and as a trophic link to protozoa and metazoa. Particle-attached bacteria dominated bacterial activity in all seasons of the year, subsisting primarily on river-borne POM. Most of this activity was associated with a subset of the particles in the water column characterized by small (< 10 μm) aggregates of mineral grains and broken diatom frustules bound together with an organic matrix. These 'bacterially-active' micro-aggregates had a slow settling rate (<0.07 mm s^{-1}), but often appeared to be associated with larger, faster-settling macro-aggregates in ETM. Phylogenetic analysis showed that ETM particles were substrata for many types of bacteria that were different than those in the river or the coastal ocean. These organisms are thought to compose the most active fraction of the bacterial community. Most archaea and free-living bacteria identified through phylogenetic analyses were similar to those in the river and the coastal ocean, suggesting that they

washed through the estuary quickly, and contributed little to estuarine bacterial and archaeal production. So bacterial production and consumption of POM in the Columbia River estuary were probably restricted to a specific estuarine community of particle-attached microorganisms. Analysis of seasonal and inter-annual data revealed that the production rate of these organisms was dependent on the quantity and quality of river-borne POM. Bacterial activity was greatest in the Spring and Summer when the principle source of fresh, high quality organic matter to the estuary, freshwater phytoplankton, was at its highest. The dominance of particle-attached bacteria, the concentration of bacterially-active particles, and the presence of a uniquely-adapted estuarine community of bacteria all indicate that Columbia River ETM have a major influence on the composition of the estuarine food web and the consumption and transformation of detrital organic matter.

Table of Contents

List of Tables

List of Figures

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The research presented in this document and the Columbia River ETM project as a whole would have been impossible without the efforts of its chief scientist, Charles Simenstad. I can only hope to emulate his extraordinary ability to keep track of the details of research and still remain focused on the big picture. Ginger Armbrust provided the encouragement and the expertise I needed to begin and complete the phylogenetic analysis research project. Her guidance and enthusiasm were invaluable as I learned how to use molecular methods and how to think about the results.

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Introduction

Research on microbial communities the Columbia River estuary began in 1990 with the first cruise of the Columbia River Estuarine Turbidity Maximum Land Margin Ecosystem Research project (CRETM-LMER), funded by the National Science Foundation. The research presented in this dissertation was conducted as part of this interdisciplinary study under the comprehensive hypothesis: *Physical, chemical and biological processes occurring in estuarine turbidity maxima (ETM) control food web dynamics of river-dominated estuaries and determine the nature of organic matter and biota exported to the nearshore ocean.*

Before 1990, very little was known about the microbial ecology of the Columbia River estuary (Wiebe & Liston 1972), and nothing was known about bacteria in the ETM. Most biological research had focused on the distribution and activity of phytoplankton and macrozooplankton. The first multidisciplinary, process-oriented research on the Columbia River estuary was the Columbia River Estuary Data Development Program (CREDDP). This project culminated in 1990 with a compilation of publications (Progress in Oceanography vol. 25) presenting an integrated description of the hydrology, sedimentary geology, biogeochemistry and biology of the estuary including models of carbon budget and food web structure. These studies identified the potential importance of ETM, and prepared the groundwork for the initial 4-year CRETM-LMER study (1990 to 1994) and subsequently to its 6-year renewal (1995-2000).

ETM Dynamics

ETM are common features in river-dominated estuaries created by the interaction

between river flow and tidal forcing. They trap and concentrate negatively buoyant particulate material near the head of the salt wedge, extending the residence time of particles over that of water and dissolved substances. ETM provide relatively persistent environments in which organisms may congregate and subsist on the high concentration of particulate organic material. By controlling the residence time and transport of particulate organic material, ETM are thought to influence the consumption and transformation of detrital organic carbon and the structure of estuarine food webs.

ETM in the North and South channels of the Columbia River estuary are maintained by daily tidal variations in bed stress and water column stratification which result in net upstream transport of suspended particulate material (SPM) near the bed (Jay & Musiak 1994). On flood tides, shear advection minimizes stratification near the bed and increases bed stress, which resuspends particles and carries them landward. In contrast, shear advection on early ebb tides increases stratification and minimizes bed stress, leaving ETM particles on or near the bed. Only late during strong ebb tides when salt water is forced downstream of the ETM regions are particles resuspended and mixed up in the water column. Thus, ETM particles are not carried too far ocean-ward on ebb tides before they are carried landward again by the incoming flood tide. So particles associated with ETM cycle between the surface sediments and the water column with each tide, and provided they are not mixed up too high in the water column, they will remain in the ETM.

Particle trapping in the estuary varies on a tidal monthly scale due to differences in tidal strength between spring and neap tides (Jay & Smith 1990). Research conducted as part of the CRETM-LMER project produced a model to explain this phenomonon. On neap tides, bed stress is minimized and water column stratification is maximized, and

therefore particles tend to accumulate in the ETM regions of the estuary where they are only rarely resuspended from the bed. Then as the spring tide develops, tide strength increases and stratification is reduced. At some point bed stress reaches a critical strength and ETM particles are readily resuspended. Reduced stratification allows particles to mix higher in the water column, and therefore become vulnerable to being washed out of the estuary. Massive fluxes of particles out of the system are thought to occur during the strongest spring tides when salt water is washed out of the estuary. This model predicts the residence time of ETM particles to be approximately 2 to 4 wks., which is much longer than the flushing time of the estuary (1 to 2 d)(Neal 1972).

ETM bacteria

Measurements made during the initial CRETM-LMER investigations in 1990 and 1991 established that bacterial activity was higher in the estuary than in the river or the coastal ocean, and was highest in the ETM (Baross et al. 1994). Also, some ETM samples were found to contain elevated concentrations of bacteria, ciliates and metazoa. We developed a hypothesis based on the microbial loop (Azam et al. 1983) in which dissolved organic matter (DOM) released from resuspended ETM particles stimulated free-living bacterial production, and enhanced the microbial food web, which in turn provided food for larger metazoan grazers (Baross et al. 1994).

In testing this hypothesis, we found that flagellates and ciliates, the primary consumers in our hypothesized microbial loop, were not significantly more abundant in the ETM than outside the ETM (Crump & Baross 1996). We also could not detect grazing on bacteria by protozoa with either dilution experiments (Landry & Hassett 1982) or with the fluorescently-labeled bacteria technique (Sherr et al. 1987). Moreover, there

was no evidence that DOC was released from ETM particles (Prahl & Coble 1994), and we found that a significant fraction of bacterial activity (measured as ^{3}H-thymidine incorporation rate) was associated with particles captured with a 20-μm Nytex screen (Crump & Baross 1996). We therefore abandoned our DOM-based 'enhanced microbial loop' hypothesis and turned our attention to particle-attached bacteria.

Chapter 1 of this dissertation presents the discovery that most of the bacterial production in the Columbia River estuary in May 1995 was due to particle-attached bacteria (> 3 μm) both within and outside the ETM. A gentle filtration method was used to remove particles from water samples, and the difference between filtered and unfiltered samples was defined as 'particle-attached'. Particle-attached bacteria were approximately as abundant as free-living bacteria, but accounted for almost 90% of ^{3}H-thymidine incorporation, indicating that average per-cell growth rate was much greater for particle-attached bacteria. We also found that the majority of extracellular enzyme activity was associated with particles, suggesting that particulate organic matter was the primary food source for bacteria in the estuary.

Elevated bacterial activity has been identified in the turbid regions of a number of estuaries including the Tamar (Plummer et al. 1987), the Humber (Goulder 1977), and the Severn (Joint & Pomroy 1982) rivers estuaries in Britain, and the Bay of Fundy (Cammen & Walker 1982), the Fraser (Bell & Albright 1981) and St. Lawrence (Painchaud & Therriault 1989) rivers estuaries in Canada. In most of these systems, particle-attached bacteria were important to total bacterial activity, but only in highly turbid systems did they dominate total bacterial activity (Bent & Goulder 1981, Plummer et al 1987). Turbidity in the ETM of the Columbia River estuary is comparable to many of these highly turbid estuaries, sometimes reaching 1000 mg l^{-1} (dry wt.), but outside the

ETM turbidity is much lower; generally less than 50 mg l^{-1}. Therefore, the dominance of particle-attached bacteria was not due to the overwhelming abundance of particles and particle-attached bacteria, as is the case in highly turbid estuaries, but rather to the combination of a highly active particle-attached community and a relatively inactive free-living community.

Particle-attached bacterial activity was correlated with the concentration of suspended particulate matter (SPM), but the relationship was highly variable due to a number of samples that contained relatively low SPM but supported very high bacterial activity (Crump & Baross 1998). These samples were collected in moderately turbid water just before or just after the most turbid part of the ETM. Chapter 2 describes the discovery that the majority of bacterial activity was associated with a small fraction of the particles in the water column. These 'bacterially-active' particles passed through a 10-μm Nytex screen, and appeared to be 'micro-aggregates' of smaller mineral grains and broken pieces of diatom frustules held together with an organic matrix. This demonstrates the heterogeneity that exists among particles in the estuary in terms of microbial colonization and perhaps organic matter quality. Moreover, it suggests that particulate organic matter associated with bacterially-active particles is consumed very rapidly, and therefore contributes disproportionately to bacterial production and the estuarine food web.

Variability in the relationship between bacterial activity and SPM also suggests that bacterially-active particles cycle in ETM differently than less bacterially-active material. The *in situ* settling velocity of bacterially-active particles was highly variable, ranging from < 0.07 mm s^{-1} in some samples to greater than 0.75 mm s^{-1}. This was thought to be caused by aggregation and disaggregation of rapidly-settling 'macro-aggregates' composed of bacterially-active particles and other less bacterially-active material. High

resolution sampling of ETM showed that bacterially-active particles were sometimes resuspended early during ETM events, prior to the resuspension of less bacterially-active material, and that bacterially-active particles remained suspended longer than less bacterially-active material. In chapter 2, a model of bacterially-active particle cycling in ETM is proposed.

Chapter 3 pursues the hypothesis that particle-attached bacteria in the ETM are different than free-living bacteria in their phylogeny. Particles and particle-attached bacteria trapped in the ETM are thought to remain there two to four weeks, but dissolved material and free-living bacteria are subject to the flushing time of the estuary, and therefore have a residence time of only one to two days (Neal 1972). The average doubling time of the free-living bacterial community in May 1995 was 223 h (Chapter 1), which is longer than the flushing time of the estuary. In contrast, the average doubling time of the particle-attached bacterial community was 9.4 h, which is less than the flushing time of the estuary and much less than the residence time of particles in the ETM. We hypothesized that particle-attached bacteria trapped in the ETM form a community that develops and is adapted to life in the estuary, and is different than source communities in the river and the coastal ocean. We also hypothesized that free-living bacteria grow too slowly to develop into an estuarine community and would therefore be composed of bacteria from the river and the coastal ocean. The research presented in Chapter 3 supports these hypotheses by identifying the dominant bacteria in the particle-attached and free-living fractions in the river, estuary, and adjacent coastal ocean. About half of the free-living bacterial types in the estuary were very similar to types found in the river and the coastal ocean. Some particle-attached bacterial types in the estuary were also similar to river and coastal ocean bacteria, but most were unique to the estuary

including an organism related to *Marinomonas* and *Oceanospirillum* spp., and diverse assemblages of organisms related to *Cytophaga* spp., γ-Proteobacteria and α-Proteobacteria. These organisms may represent the most active fraction of the bacteria, and may be responsible for the majority of bacterial production.

We further hypothesized that archaea in the estuary would match those in the river and coastal ocean, and we hoped to find uniquely estuarine archaea. Archaea in moderate temperature aerobic environments were discovered only recently (DeLong 1992, Fuhrman et al. 1992), and very little is known about their role in planktonic ecosystems. Chapter 4 describes the diversity of archaea in the Columbia River, estuary, and adjacent coastal ocean. The results showed that most of the archaea in the estuary appeared to be imported from the river and coastal ocean, and that there was no clear evidence of an estuarine community of archaea.

Four seasonal research cruises, embarked in 1997 and 1998 provided an opportunity to observe how changes in bacterial activity throughout the year related to changes in physical and chemical characteristics in the river and the estuary. Chapter 5 compares results from these four cruises and four earlier cruises (Spring 1992 & 1995, and Summer 1991 & 1996), and describes how bacterial activity in the estuary responds to seasonal changes in the quantity and quality of organic matter supplied by the river. Particle-attached bacteria accounted for 69 to 80% of bacterial activity throughout the year, and bacterial activity was positively correlated with POC in all seasons, indicating the importance of food supply to estuarine bacterial production. The concentrations of the two major pools of organic matter in the river, derived from soil and from freshwater phytoplankton, were found to be variable throughout the year. Bacterial activity was generally high in the Spring and Summer and low in the Fall and Winter, except in

Spring of 1997 when samples were collected prior to the onset of the Spring phytoplankton bloom in the river. Bacterial activity per mg POC was positively correlated with chlorophyll-a per mg POC in all seasons, demonstrating the importance of organic matter quality.

The goals of this project were to determine the influence of ETM on bacterial activity in the estuary, understand the role of bacteria in organic matter transformation and the transfer of that material to the estuarine food web, and identify the most active fraction of the bacterial community. In the process, we discovered an extraordinary diversity in particle composition, organic matter quantity and quality, bacterial activity, and species richness. We are only beginning to investigate the implications of this heterogeneity, but recognizing it allowed us to focus on the fractions most important to the functioning of the estuary. Particle-attached bacteria are the principal consumers of organic matter in the estuary, and ETM are the means by which these organisms maintain a community in the estuary. ETM also concentrate bacterially-active particles for grazing by particle-consuming copepods and rotifers. The dominance of particle-attached bacteria, the trapping of bacterially-active particles, and the presence of a uniquely-adapted estuarine community of bacteria all indicate that ETM are essential for the Columbia River estuary and its detrital food web to function as a 'filter' (Schubel & Kennedy 1984) for the comsumption and transformation of terrestrial organic matter.

Chapter 1. Dominance of Particle-Attached Bacteria

Abstract

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

bacteria by extending the residence time of particles in the ETM, and by quickly flushing free-living cells through the estuary, perhaps preventing the development of an estuarine population of free-living bacteria.

Introduction

Bacteria are the base of detrital food webs that recycle and consume dead and degraded organic matter. These food webs are particularly active in estuaries, thriving on the terrestrial and fluvial organic matter that passes through these systems to the ocean. In many estuaries a significant fraction of total bacterial carbon production is due to particle-attached bacteria (Bell & Albright 1981, Ducklow & Kirchman 1983, Iriberri et al. 1987, Kirchman & Ducklow 1987, Griffith et al. 1994), which are generally larger and more active per cell than free-living bacteria (Goulder 1977, Kirchman and Mitchell 1982, Unanue et al. 1992). Particle-attached and free-living bacteria may be considered separate populations in estuaries because very different environmental factors control their contribution to the degradation of detritus and their roles in the detrital food web. Particle concentration and composition and the physical factors that control particle dynamics in estuaries have a strong influence over the concentration, rate of production, and the availability to consumers of particle-attached bacteria. In some highly turbid river estuaries like the Humber and the Tamar in England particle-attached bacteria have been found to dominate microbial activity (Bent & Goulder 1981, Plummer et al. 1987). Work on the Columbia River estuary, USA, found elevated microbial activity associated with estuarine turbidity maxima (ETM)(Baross et al. 1994) and that a significant fraction of this activity was associated with particles retained by a 20-μm screen (Crump & Baross 1996). Our understanding of the hydrodynamics of this system suggests that free-

living bacteria and dissolved substances are rapidly flushed into and out of the estuary, but particles and particle-attached bacteria are retained in the estuary by ETM. For these reasons we hypothesized that most of the bacterial carbon production in the Columbia River estuary is due to particle-attached bacteria.

The Columbia River estuary (Fig. 1.1), in the northwestern United States, is a turbid, partially-mixed system with a flushing time of 1-3 d (Neal 1972). Its hydrodynamics support large ETM at the upstream limit of the salinity intrusion in both of the main (North and South) channels (Jay 1994, Jay & Musiak 1994). ETM result from net upstream flow in the lower layers of an estuary, and act as particle trapping mechanisms that extend the residence time of negatively buoyant particles in the estuary over that of water, dissolved material, and neutrally buoyant particles. In the Columbia River estuary, particles in the ETM average 3-10% organic material by weight (Reed & Donovan 1994) and appear to undergo some microbial transformation in a sub-oxic environment (Prahl & Coble 1994). Water-column turbidity in the Columbia River estuary averages about 20 mg dry weight l^{-1}, higher than in the river or the coastal ocean, and can exceed 1000 mg l^{-1} in the ETM. *In situ* primary production (31 x 10^6 kg C yr^{-1}) is dwarfed by inputs of allochthonous organic material and river phytoplankton (21 x 10^7 kg C yr^{-1})(Small et al. 1990). These conditions support a thriving detrital food web including detritivorous copepods and particle-attached bacteria that are most active when associated with the ETM (Baross et al. 1994, Simenstad et al. 1994a, Crump & Baross 1996).

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

carbon in the water column of the Columbia River estuary, and discusses the detrital food web and its connection to the estuarine hydrodynamics that control particle cycling.

Materials & Methods

Sample collection and particle fractionation

Sampling strategy. Eulerian sampling series were conducted in May 1995 in the North Channel of the estuary at a location where the ETM passed below the anchored ship with every tide (Fig. 1.1). Samples were collected from about 1 m off the bed every 2 h during a 30-h and a 148-h sampling series using a high-volume, low-pressure pump coupled to a conductivity-temperature-depth (CTD) meter and an optical backscatter sensor (OBS) for measuring turbidity in relative OBS units (Simenstad et al. 1994b).

Particle fractionation. All samples were filtered gently by floating a plastic 100-ml filter tower (Millipore) fitted with a 3-µm polycarbonate filter (Poretics) on 1 l of sample water in a 2-l beaker. Large particles settled to the bottom of the beaker and therefore did not clog the filter. Water flowed up through the filter into the filter tower as the tower sank, and was drawn out of the tower with a pipette.

Measurements

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

from the unfiltered water production.

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

thymidine incorporation per liter.

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

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

The concentration of suspended particulate matter (SPM) in unfiltered samples was measured by pressure filtering 2 to 4 l of sample onto pre-weighed 90-mm diameter polycarbonate filters and correcting the gravitational particle weight for the salt content using a neutron activation analysis of chloride (Prahl & Coble 1994) and the assumption of constant salt composition for seawater (OPEN University 1989). Particulate organic

carbon (POC) was measured in unfiltered samples filtered from a known volume onto a precombusted (450°C for 2 h) GF/F filter. Filters were fumed with concentrated HCl for 4 h to remove any inorganic carbonate, oven-dried at 60°C overnight, and cut in half. The POC and particulate nitrogen content of the packaged filter was determined by high temperature combustion using a Carlo Erba 1500 CNS analyzer setup and calibrated with the acetanilide standard as described by Verardo et al. (1990).

Results

Concentrations of free-living bacteria varied from 0.61×10^9 to 2.42×10^9 cells l^{-1}, with a mean of 1.25×10^9 (SD = 0.40×10^9, n = 54) cells l^{-1}, and were negatively correlated with salinity (Table 1.1, Fig. 1.2). Particle-attached bacteria concentrations varied from $<2.60 \times 10^6$ (detection limit) to 5.10×10^9, with a mean of 1.02×10^9 (SD = 1.00×10^9, n = 52) cells l^{-1}, and were positively correlated with turbidity (OBS and SPM) and POC (Table 1.1, Fig. 1.2).

Bacterial carbon production ($\mu g\ l^{-1}\ h^{-1}$) in unfiltered samples was always much higher than in 3-μm filtered samples, and tended to peak at or around the maximum ETM turbidities (Figs. 1.2 & 1.3). Particle-attached bacterial carbon production ranged from 0.13 to 4.50 $\mu g\ l^{-1}\ h^{-1}$, with a mean of 1.61 $\mu g\ l^{-1}\ h^{-1}$ (SD = 1.10, n = 90), and represented on average 90% (SD = 9%) of total bacterial production. Particle-attached bacterial carbon production was positively correlated with turbidity (OBS and SPM) and with POC, but free-living bacterial carbon production was not (Table 1.1, Fig. 1.4a).

There was a marginally significant positive correlation between salinity and particle-attached bacterial carbon production (Table 1.1). This relationship was driven by low bacterial carbon production in freshwater samples (Fig. 1.4a). When freshwater samples

were excluded, there was a significant negative correlation between salinity and both particle-attached bacterial carbon production and turbidity (Table 1.1). This was because the highest measured turbidities and attached bacterial carbon production rates occurred at intermediate salinities (Fig. 1.4b).

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

Thymidine incorporation per cell for free-living bacteria averaged 2.8×10^{-9} (SD = 3.2×10^{-9}, n = 54) pmol TdR $cell^{-1}\ h^{-1}$. Thymidine incorporation per cell for particle-attached cells was 10 to 100 times higher (Fig. 1.5), averaging 4.79×10^{-8} (SD = 4.17×10^{-8}, n = 47). In estuarine water (salinity > 1), specific growth rates averaged 0.15 (SD = 0.16, n = 46) d^{-1} for free-living bacteria and 2.6 (SD = 1.7, n = 37) d^{-1} for particle-attached bacteria.

The rate of extracellular enzymatic hydrolysis of MCA-L-leucine, MUF-D-glucoside and MUF-cellobioside increased with turbidity in unfiltered samples, and was greatly reduced in filtered samples (Fig. 1.6). Hydrolysis of MCA-L-leucine was less reduced by

3-μm filtration than hydrolysis of the carbohydrate compounds.

Discussion

Bacterial carbon production

An average of 90% of the bacterial carbon production in the water column of the Columbia River estuary was due to particle-attached bacteria. This was true not only in the ETM, where total bacterial carbon production was enhanced, but also in less turbid estuarine water and fresh water (Figs. 1.2 & 1.3). Other river estuaries where attached bacteria dominate total bacterial activity in the water column, such as the Humber and the Tamar in England, are generally more turbid than the Columbia River estuary (Goulder 1977, Bent & Goulder 1981, Plummer et al. 1987, Uncles & Stephens 1993). It appears that in the Columbia River estuary, the fast flushing time and the presence of ETM particle trapping combine to create conditions where particle-attached bacteria thrive on trapped particulate organic material while free-living bacteria are washed out of the system too quickly to establish an active estuarine community. Painchaud and Therriault (1989) describe such systems as accumulative for particle-attached bacteria and dispersive for free-living bacteria.

The relative contribution of particle-attached bacteria to bacterial carbon production in coastal and estuarine systems is variable, and depends on the concentration and growth rate of both particle-attached and free-living bacteria (Griffith et al. 1990, Unanue et al. 1992). For example, in the upper Chesapeake Bay, particle-attached bacterial production was highest during the summer months, but the relative contribution of particle-attached bacteria to total bacterial production was highest in winter, when free-living bacterial production was lowest (Griffith et al. 1994). In Spring and Summer, the Columbia River

estuary had high particle-attached bacterial carbon production and low free-living bacterial carbon production (Crump & Baross 1996, this study). A seasonal study has not yet been completed to determine if similar results also occur during the late fall and winter.

The assumption of a single average value of carbon per cell in the calculation of bacterial carbon production may have introduced some bias to the results. Since particle-attached bacterial cells are generally thought to be larger than free-living bacteria, estimates of bacterial carbon production may be relatively low for particle-attached bacteria and relatively high for free-living bacteria. Estimates of bacterial carbon production also assumed that the supply of tritiated thymidine to all bacterial cells was the same during the incubations. However, the supply of thymidine to bacteria on the inside of particles may have been limited, giving an underestimate of production by particle-attached bacteria. Another potential source of error was that some of the particle-attached cells could have detached from particles while passing through the plankton pump or while the particles were being filtered, giving an underestimate of particle-attached bacterial carbon production and concentration and an overestimate of free-living bacterial carbon production and concentration. No significant correlation was found between free-living and particle-attached bacterial concentrations or production (Table 1.1), suggesting that the pump and the 3-μm filtration method did not cause many particle-attached cells to release from their particles and enter the free-living fraction. Nevertheless, our estimates of particle-attached bacterial concentration and production should be considered conservatively low, and estimates of free-living bacterial concentration and production should be considered conservatively high.

There are many other reports where particle-attached and free-living bacteria were

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

Bacterial concentration

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

living bacterial concentration in the Columbia River estuary was constant throughout the system and, except in some highly turbid samples, was about the same or greater than particle-attached bacterial concentration.

The average free-living bacterial doubling time in samples with estuarine salinity was 223 h (SD = 190, n = 47, assuming 2 x 10^{18} cells produced mol TdR^{-1} incorporated (Fuhrman & Azam 1982), doubling time = ln2/specific growth rate), which is much longer than the flushing time of the estuary (about 1 to 3 d; Neal 1972). This suggests that free-living bacteria were native to river and coastal waters and simply washed in and out of the estuary. The average doubling time of the estuarine population of particle-attached bacteria, however, was 9.4 h (SD = 7.5, n = 37), which is less than the flushing time of the estuary, and potentially much less than the residence time of particles trapped in the ETM. Particles are thought to accumulate in the Columbia River estuary during neap tides, and to disperse on spring tides when shear forces are greater and salt water is washed out of the estuary. So, particles trapped in the ETM potentially remain in the estuary for two to four wk or longer if they are not washed out during the spring tide (D.J. Reed, pers. comm.). This is enough time for an estuarine population of particle-attached bacteria to develop before being washed out of the system. Therefore, it is likely that this population is composed of bacterial types native to the estuary and adapted for growth on particulate organic material under estuarine conditions.

Thymidine incorporation per cell

Specific thymidine incorporation rates were much higher for particle-attached bacteria than for free-living bacteria in both freshwater and estuarine samples (Fig. 1.5). This result has also been found in many other coastal and estuarine systems from measurements of the maximum incorporation rate of radioactive organic compounds

(Hodson et al. 1981, Cammen & Walker 1982, Kirchman & Mitchell 1982, Kirchman & Ducklow 1987). An important caveat regarding these calculations is that they produce an average thymidine incorporation rate per cell. Individual thymidine incorporation rates probably varied over a large range within both groups of bacteria. It is possible that a large percentage of free-living cells were inactive, and that the active free-living and particle-attached cells incorporated thymidine at the same rate. But if this were the case, active particle-attached cells would far outnumber active free-living cells.

Specific thymidine incorporation rate in estuarine water samples (salinity > 1) were significantly higher than in freshwater samples for both groups of bacteria (Mann-Whitney test, free-living $p < 0.00001$, attached $p < 0.00001$). The methodological error associated with these rates was often quite high after propagating methodological error from bacterial carbon production and bacterial concentration. However, the trend in the particle-attached bacteria data (Fig. 1.5) suggests that riverine particles undergo changes when mixed into the estuary that affect bacterial carbon production rates. These changes may involve some form of interaction between riverine particles and particles either generated in the estuary or particles that have resided in the estuary for sufficient time to acquire an estuarine bacterial fauna. These estuarine particles may host faster growing bacteria, giving a higher average rate of production when combined with riverine particles. Flocculation has been shown to make some organic material more available to bacteria (Tranvik and Sieburth 1989). It is possible that flocculation of dissolved organic material and aggregation with other particles in brackish water promotes bacterial colonization and growth. Another possibility is that organic material available to heterotrophic bacteria could be more labile in the estuary than in the river, accelerating bacterial growth rates. For example, freshwater phytoplankton are a major source of

organic matter to the estuary (Small et al. 1990) and have been observed microscopically to be a significant component of ETM particles (Crump, unpubl. results). Although these phytoplankton can survive at low salinities (Jackson et al. 1987), they probably perish at higher salinities and provide fresh, labile organic material to bacteria in the estuary.

Turbidity & salinity

Although there is a correlation between particle-attached bacterial carbon production and turbidity, variability in the relationship and the outlying values give indications of the heterogeneity of particles in this system (Fig. 1.4a). First, samples with relatively low bacterial carbon production per unit turbidity were collected in fresh water (salinity < 1) at the end of strong ebb tides, suggesting that a shift occurred between fresh and estuarine water that affects bacterial production rates. Second, samples that showed relatively high bacterial carbon production occurred just prior to peak turbidity of flood ETM, as though particles supporting unusually high bacterial carbon production were resuspended first by the incoming tide, followed by the resuspension of less bacterially-active particles. These very bacterially-active particles were probably organic rich and had a relatively low density and therefore were the last to settle out after resuspension events, putting them in position to be resuspended early during the next tide. Finally, the relationship between bacterial carbon production and turbidity appeared to diminish at high turbidity (Fig 1.4a). At times when ETM were most turbid, they often contained resuspended sand grains and bits of wood that added to the overall turbidity of the samples, but did not host an active bacterial population based on microscopic examination and direct measurements of ^{3}H-TdR incorporation by rapidly-settling particles (data not shown). These particles may have been responsible for the variation in bacterial carbon production estimates at the higher turbidities.

In May, 1992 another study documented that less than 50% of the bacterial carbon production in the ETM of the Columbia River estuary was associated with particles retained by a 20-µm screen (Crump & Baross 1996), implying that particles that host the active population of bacteria both in and outside the ETM were between 3 µm and 20 µm. *In situ* observation with a video camera (data not shown) and particle analyses in the lab (Reed and Donovan 1994) has shown that many particles in ETM are larger, rapidly sinking aggregates composed of smaller particles. These 'macro-aggregates' are common in estuaries (Kranck 1973, Eisma & Li 1993, Li et al. 1993, Fennessy et al. 1994, ten Brinke 1994), are formed by collision and adhesion of smaller particles (Eisma 1986), and are thought to be trapped in ETM due to increased sinking rate (Kranck 1981, Reed & Donovan 1994). Aggregates are also known to be sites of intense biological activity (Alldredge & Silver 1988, Muschenheim et al. 1989, Zimmermann & Kausch 1996). It is possible that small, bacterially-active particles in the Columbia River estuary are incorporated and retained in the ETM by aggregation with other ETM particles, and our sampling procedure causes disaggregation into individual particles and more cohesive 'micro-aggregates'.

The highest levels of particle-attached bacterial carbon production were found at intermediate salinities (Fig. 1.3), and appeared to be due in part to the coincidence of intermediate salinities and elevated turbidity (Fig. 1.4b). High-turbidity ETM, with associated high bacterial carbon production, were most often found at intermediate salinities. Lower-turbidity samples with unusually high bacterial carbon production were also found at these intermediate salinities. In fresh water and at higher salinities, turbidity and particle-attached bacterial carbon production were reduced. A similar result was found at higher salinities in the plume of the Hudson River estuary, USA, where

particle-attached bacterial activity was correlated with POC concentration but not with salinity (Ducklow & Kirchman 1983).

First difference analysis (Table 1.2) showed that changes in salinity over 2 h periods did not correlate with changes in carbon production by particle-attached bacteria in estuarine water. Changes in turbidity over those same 2 h periods, however, did correlate with changes in carbon production by particle-attached bacteria. So, other than the difference in particle-attached bacterial carbon production between fresh and estuarine water (discussed earlier), there did not appear to be a direct relationship between salinity and particle-attached bacterial carbon production. The relationship with salinity is instead a product of the cycling of particles by the hydrodynamics of the estuary because ETM tend to be located around the head of the salt wedge at intermediate salinities, and the strength of resuspension events increases with the velocity of water moving over the bed and the extent of mixing between fresh water and salt water.

The positive correlation between free-living bacterial carbon production and salinity was driven by low production estimates in freshwater samples (Table 1.1). Bacterial activity is often greater at intermediate salinity in estuaries than in fresh water or sea water (Griffith et al. 1990, Chin-Leo & Benner 1992). Ducklow & Kirchman (1983) found free-living bacterial activity to be negatively correlated with salinity in the Hudson River plume at high estuarine and coastal water salinities, and suggested that bacterial activity was enhanced by high levels of organic matter in the estuary. In the Columbia River estuary, inputs of allochthonous organic matter and freshwater phytoplankton are much greater than *in situ* primary production, so free-living bacterial activity at estuarine salinity may have been enhanced by the release of organic matter from particles or from dying freshwater phytoplankton.

Degradation of detritus

As a central component of the detrital food web, bacteria degrade insoluble organic polymers into compounds small enough for transport into the cell using degradative enzymes either attached to their membranes or released into the environment (Hollibaugh & Azam 1983, Hoppe 1983, Hoppe 1986). In the ETM region of the Elbe River estuary, Germany, proteolytic enzyme activity was found to correlate with the concentration of SPM and dissolved free amino acids (Bernat et al. 1994). In the Columbia River estuary, extracellular enzyme activity was detected principally on particles (Fig 1.6), suggesting that particulate organic material was the primary polymeric food source for bacteria in the estuary. The relatively high activity of particle-attached bacteria and their close association with particles suggests that they produced most of these enzymes, and therefore were probably responsible for most of the bacterial degradation of POC in the estuary.

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

A rough estimate of POC turnover time by particle-attached bacterial decomposition can be made using the rate of bacterial carbon production and the concentration of POC in individual samples (Ducklow et al. 1985). Assuming a relatively high carbon conversion efficiency of 50% (g carbon produced g^{-1} carbon consumed), conservatively

long estimates of average POC turnover time due to bacterial decomposition were 71 d (SD = 38, n = 51) in estuarine water samples and 355 d (SD = 119, n = 15) in fresh water. Using a 10% conversion efficiency, turnover times decrease to 8 d (SD = 4, n = 51) in estuarine water and 39 d (SD = 13, n = 15) in fresh water. The extent to which particle-attached bacteria alter the flux of allochthonous detrital organic carbon through the estuary will depend on how long that carbon remains in the estuary, which cannot be estimated at this point. However, as previously discussed, the fraction of allochthonous organic material that is trapped in the ETM is thought to remain in the estuary on average 2 to 4 wk, or long enough for a significant fraction to be decomposed by particle-attached bacteria.

Enzymes associated with marine snow have been shown to hydrolyze much more POC than is required by particle-attached bacteria, possibly providing food for free-living bacteria (Smith et al. 1992). If this is true for particles in the ETM of the Columbia River estuary, then the turnover time of POC may be more rapid than calculated above. The fact that we did not detect enhanced free-living bacterial activity in turbid water samples suggests that freshly hydrolyzed dissolved organic matter (DOC) was not leaving the particles or that it was not being taken up by free-living bacteria. However, ETM are very dynamic in this system, and free-living bacteria probably do not remain in the ETM long enough to detectably change in their growth rate. It is more likely that DOC released from ETM particles is mixed into the estuary and contributes to the general enhancement of free-living bacterial carbon production in estuarine water over that in fresh water (Figs. 1.2 & 1.3).

Detrital food web

Particle-attached bacteria can have a very different role in a food web than free-living

bacteria because they may be directly consumed by larger metazoans, bypassing consumption by protozoan consumers and 'short-circuiting' the microbial loop (King et al. 1980, Baross et al. 1994). This appears to be the case in the Columbia River estuary where epibenthic copepods were found to feed directly on particle-attached bacteria (Simenstad et al. 1994a), and metazoans known to be capable of feeding directly on free-living bacteria such as larvaceans (King et al. 1980) are not present. The dominance of particle-attached bacteria in the Columbia River estuary suggests that the contribution of bacteria to the estuarine food web is mediated by particle dynamics. Bacteria are a high quality food source, and when they are attached to particles they increase the quality of detrital material as food (Heinle et al. 1977). Detritivorous metazoans may therefore be the principle consumers of bacterial biomass in this system (Simenstad et al. 1994a).

Up to 75% of the primary consumption by metazoans in the Columbia River estuary can be attributed to estuarine zooplankton (Jones et al. 1990, Simenstad et al. 1990). Due to low *in situ* primary production, allochthonous organic material is thought to be the dominant food source for these consumers (Simenstad et al. 1990). The system supports thriving populations of the copepods *Eurytemora affinis* and *Coulana canadensis* (Simenstad et al. 1994a). In Spring of 1992, a species of the rotifer Keratella was also found to be abundant at all salinities in the estuary with concentrations as high as 2200 l^{-1} (Crump & Baross 1996). These organisms are capable of consuming detritus (Heinle et al. 1977, Bogdan et al. 1980, Starkweather 1980), and were found to be concentrated in the ETM where they may take advantage of the high concentration of food particles (Morgan 1993, Simenstad et al. 1994a, Crump & Baross 1996). Preliminary experiments demonstrated that *E. affinis* and *C. canadensis* could consume directly on these particles (Simenstad et al. 1994a). In low salinity waters of the Elbe River estuary, 50% to 75% of

the rotifers were found to be associated with suspended aggregate particles (Zimmermann & Kausch 1996). This may also be the case in the Columbia River estuary, where the concentration of rotifers correlated with SPM and bacterial carbon production (Crump & Baross 1996). During our study, direct consumption of particles and particle-attached bacteria by metazoans was potentially the key step in the transfer of detrital organic matter into the food web of the Columbia River estuary.

Table 1.1. Spearman rank correlation coefficients (ρ), number of paired comparisons (n), and p-values for relationships between turbidity (OBS and SPM), POC, and salinity, and free-living and particle-attached bacterial concentration, production, and ^{3}H-thymidine (TdR) incorporation per cell. Calculations with bacterial production (l^{-1}) were made for all samples, freshwater samples (salinity < 1), and estuarine water samples (salinity > 1). Highly significant correlations ($p < 0.001$) are presented in bold type.

Spearman correlation matrix	OBS			SPM			POC			Salinity		
	ρ	n	p	ρ	n	p	ρ	n	p	ρ	n	p
Free-living bacteria												
Concentration	0.226	52	-	0.270	47	-	0.090	44	-	-0.419	52	<0.005
Production l^{-1}	-0.123	88	-	-0.002	71	-	-0.115	69	-	**0.507**	**88**	**<0.001**
fresh	0.217	19	-	0.052	17	-	-0.133	16	-			
estuarine	-0.151	68	-	-0.188	52	-	0.350	51	-	0.178	68	-
TdR incorporation $cell^{-1}$	-0.183	52	-	-0.231	47	-	-0.133	44	-	**0.612**	**52**	**<0.001**
Particle-Attached bacteria												
Concentration	0.443	51	<0.002	**0.505**	**45**	**<0.001**	0.575	42	<0.02	-0.094	51	-
Production l^{-1}	**0.425**	**88**	**<0.001**	**0.545**	**71**	**<0.001**	**0.593**	**69**	**<0.001**	0.293	88	<0.02
fresh	0.337	19	-	0.228	17	-	0.106	16	-			
estuarine	**0.652**	**68**	**<0.001**	**0.567**	**52**	**<0.001**	**0.694**	**51**	**<0.001**	**-0.506**	**68**	**<0.001**
TdR incorporation $cell^{-1}$	-0.161	47	-	-0.166	41	-	-0.096	38	-	0.385	47	<0.01
SPM	**0.761**	**69**	**<0.001**									
POC	**0.806**	**67**	**<0.001**	**0.862**	**67**	**<0.001**						
Salinity	-0.320	88	<0.005	0.051	69	-	-0.135	67	-			

Table 1.2. Spearman rank correlation coefficients (ρ), number of paired comparisons (n), and p values for relationships between the magnitude of change (first differences) in bacterial and physical data over two hour intervals. Highly significant correlations ($p < 0.001$) are presented in bold type

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

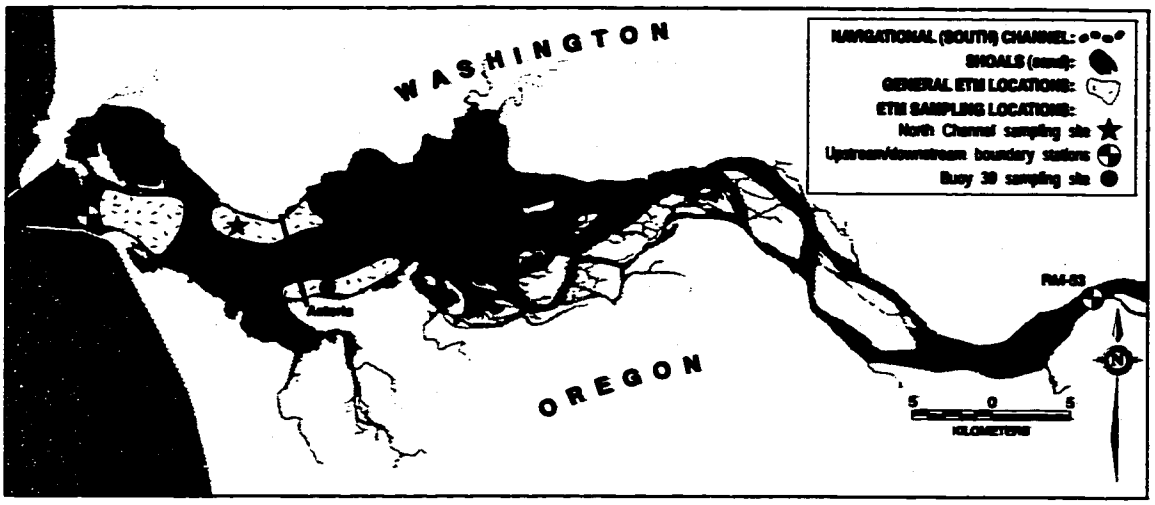


Fig. 1.1. Columbia River estuary (USA) with location of sampling site in the North Channel and extent of the estuarine turbidity maxima (ETM)

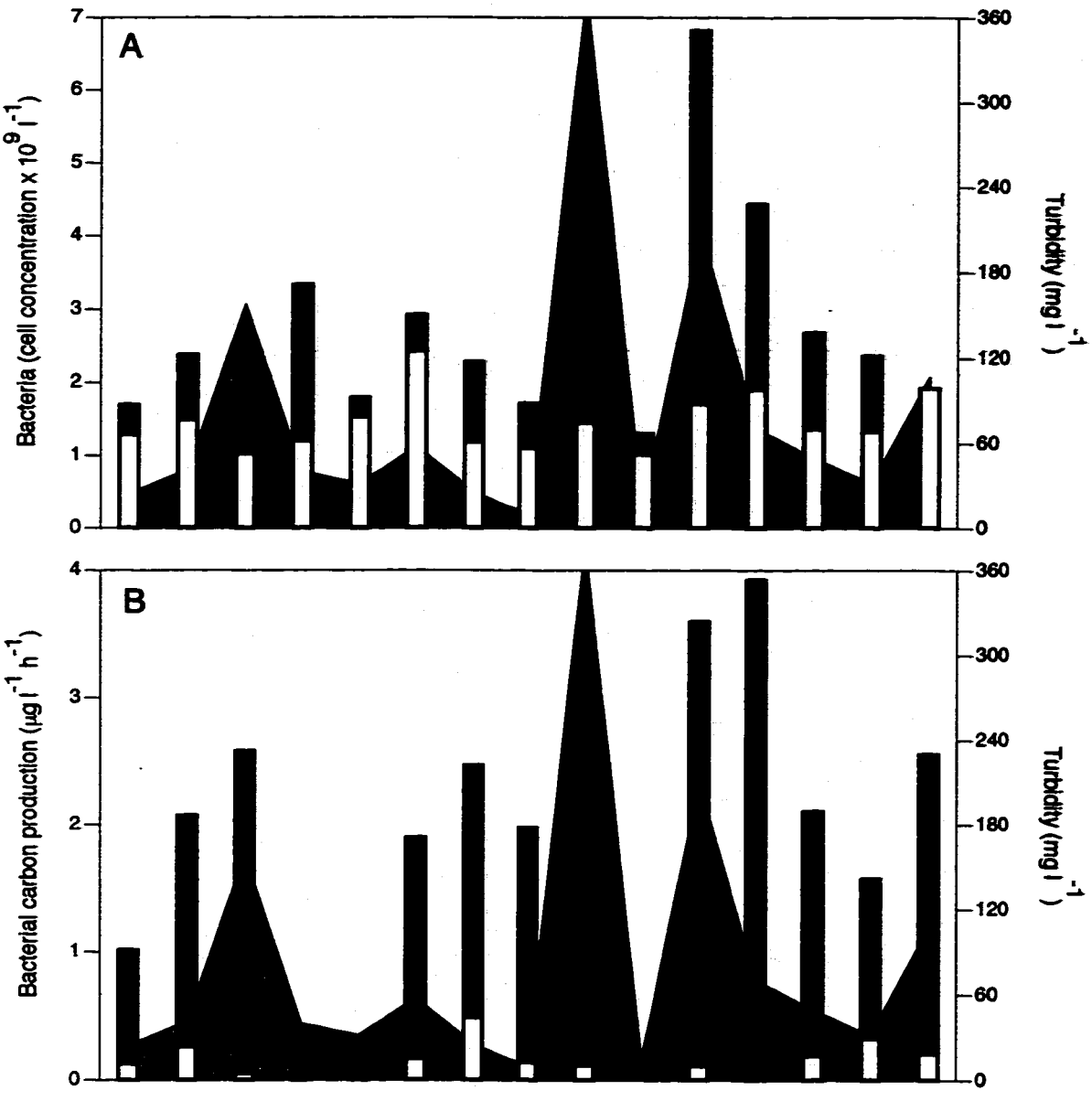


Fig. 1.2. (A) Bacterial concentration and (B) carbon production, and turbidity (solid line, shaded below for clarity) in near-bottom samples collected every 2 h during a 28-h sampling series in the North Channel of the Columbia River estuary. The open areas of the columns represent free-living bacteria and the shaded area represent particle-attached bacteria (> 3 µm)

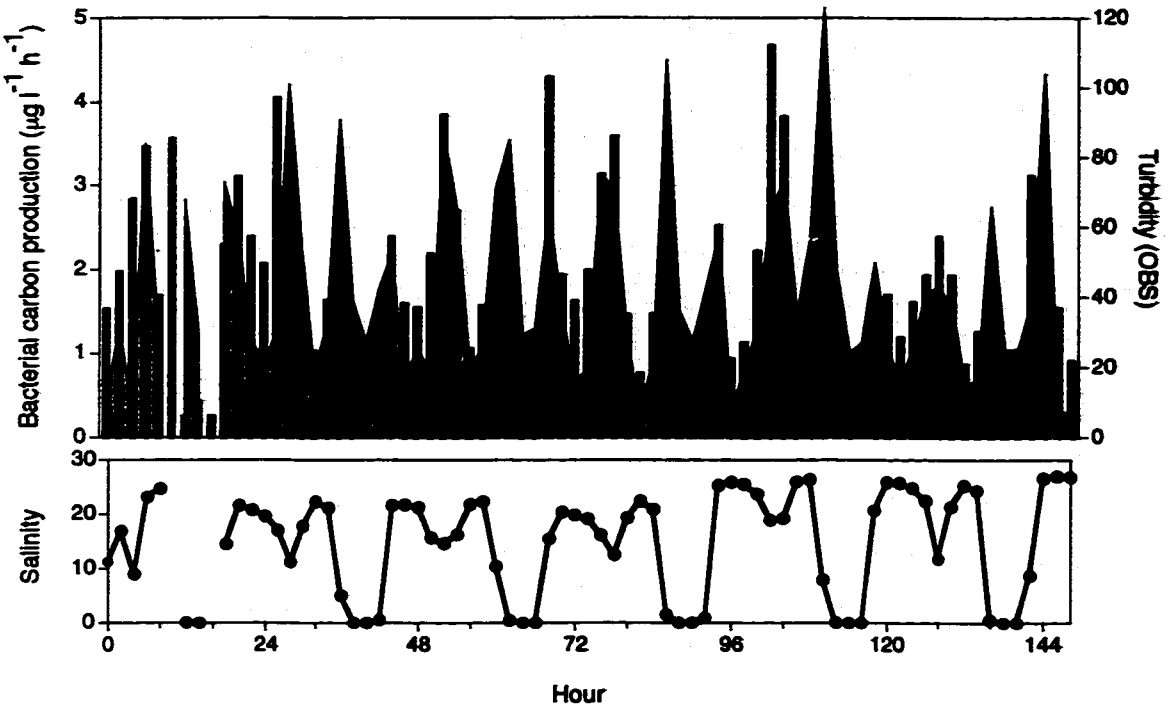


Fig. 1.3. (A) Bacterial carbon production and turbidity (solid line, shaded below for clarity), and (B) salinity in near-bottom samples collected every 2 h during a 148 h sampling series in the North Channel of the estuary

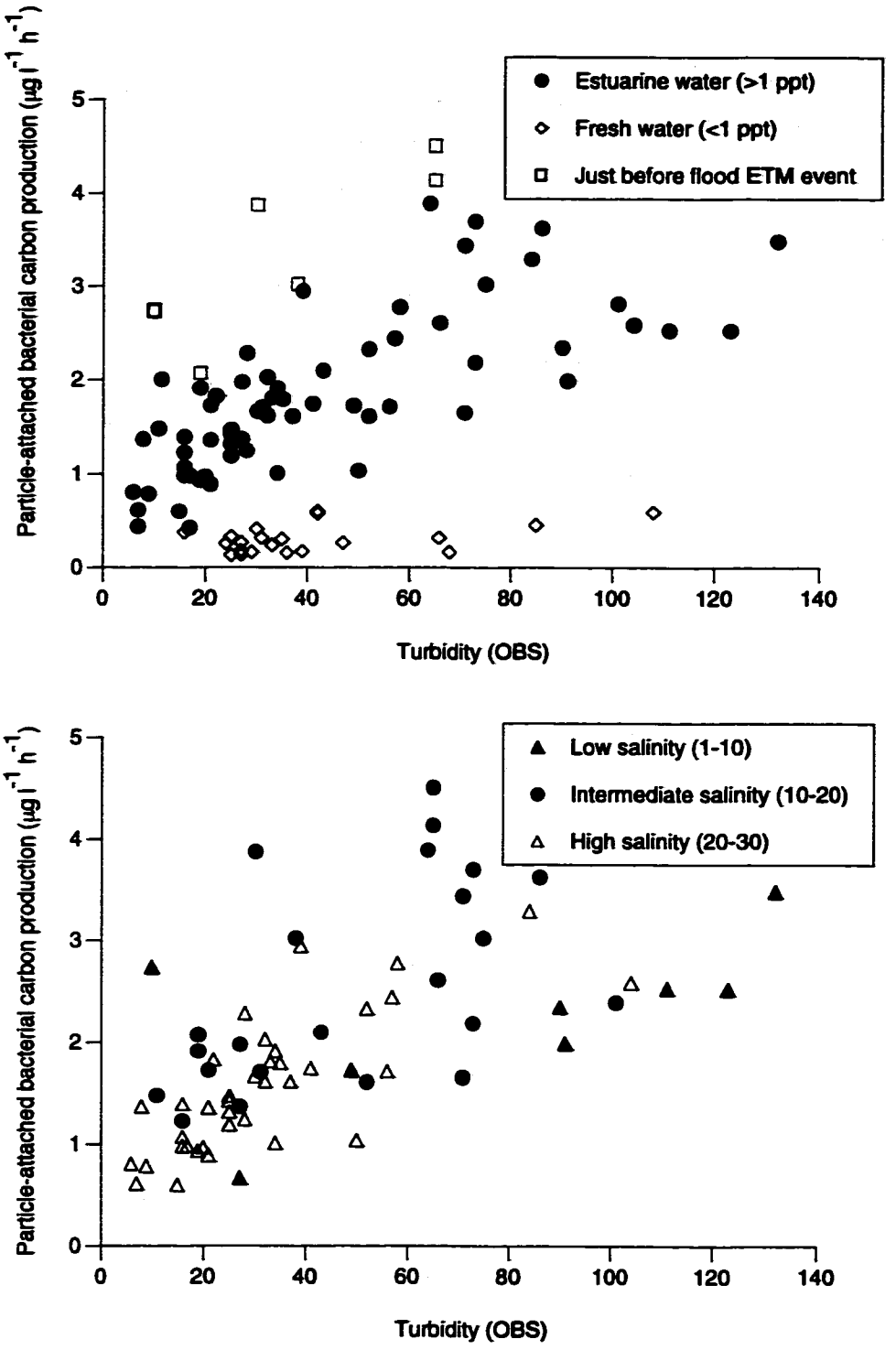


Fig. 1.4. Particle-attached bacterial production vs. turbidity (OBS) for (A) all data from Figs. 2 & 3, and (B) data for samples with estuarine salinity

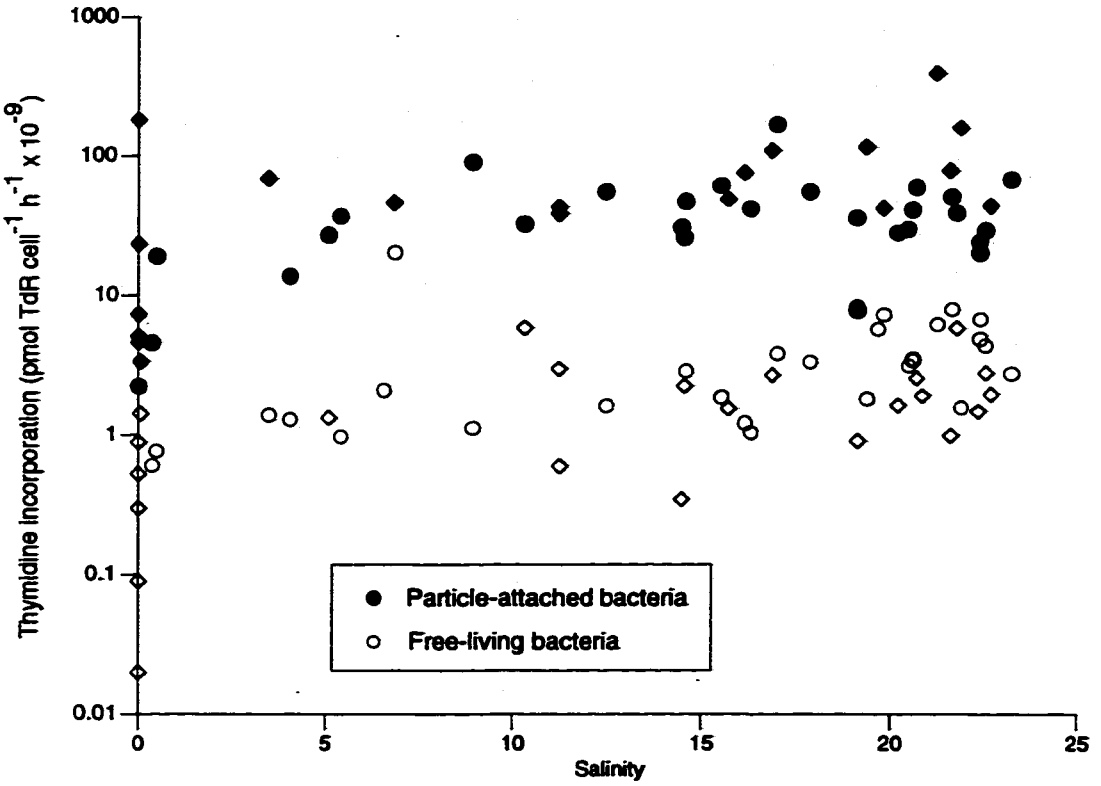


Fig. 1.5. Average thymidine incorporation rate per cell for all samples from the 28-h sampling series and the first 4 d of samples from the 148-h sampling series. Methodological error (95% confidence intervals) represented 61% (SD = 24%) of the values for circles and >100% for diamonds

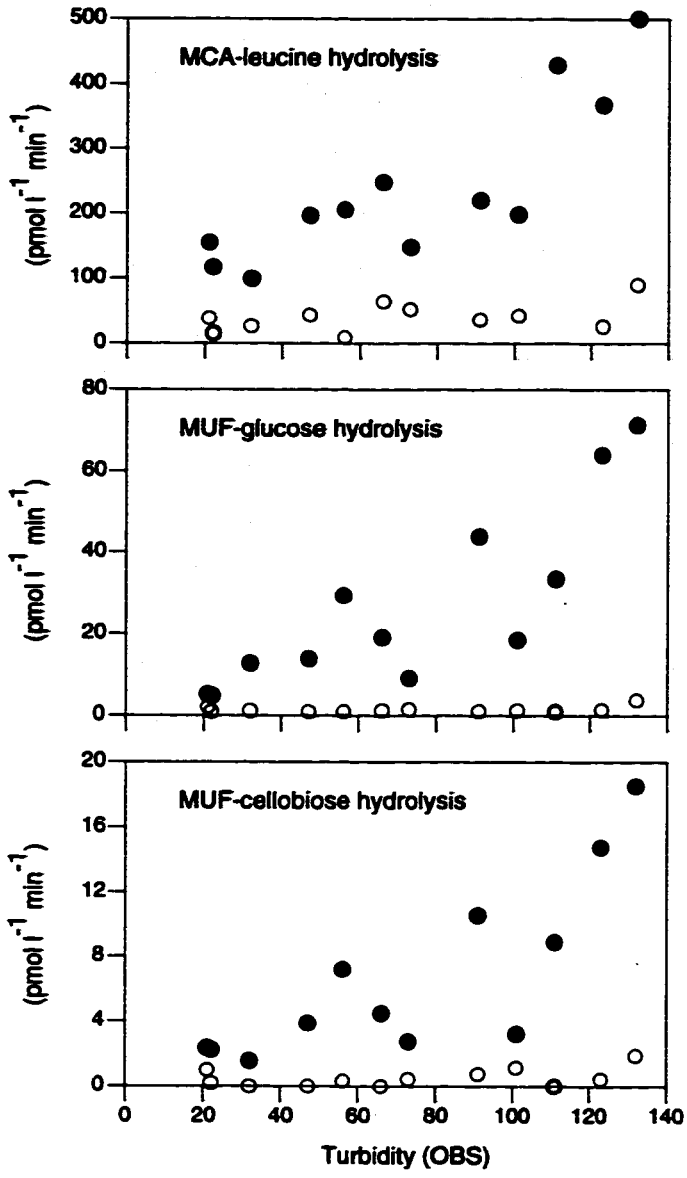


Fig. 1.6. Hydrolysis rates of fluorescently-labelled substrates in untreated water samples (solid circles) and 3 μm filtered water samples (open circle) plotted against turbidity of samples (OBS)

Chapter 2. Characterization of the Bacterially-Active Particle Fraction

Abstract

The Columbia River estuary is dominated by a detrital food web that is supported primarily by river-borne particulate organic material in the estuarine turbidity maxima (ETM). Approximately 90% of the bacterial activity and most of the extracellular enzyme activity in the water column of the estuary were associated with particles captured by a 3-μm filter. Earlier studies determined that the relationship between particle-attached bacterial activity and suspended particulate mass (SPM) was variable, suggesting that some particles supported a larger fraction of bacterial activity. In order to characterize these bacterially-active particles, suspended particulate material from the estuary was fractionated by size and *in situ* settling velocity, and analyzed for ^{3}H-thymidine incorporation rate, total particle mass and particulate organic carbon (POC) concentration. The location and movement of bacterially-active particles in the estuary was traced by measuring ^{3}H-thymidine incorporation rate and SPM in near-bottom depth profiles collected at 4 or 5 time points during ETM resuspension events. The smallest particle size fraction (3-10 μm) supported 87% (SE=13) of bacterial activity, but contained only 38% (SE=5) of total POC and 38% (SE=6) of total particle mass. However, when particles were separated by *in situ* settling velocity using a method that preserved the integrity of aggregated particles, the settling velocity of the majority of bacterially-active particles varied from < 0.07 mm s^{-1} in some samples to greater than 0.75 mm s^{-1}. Microscopic analysis of bacterially-active particles revealed that they were aggregates of smaller (<10 μm) particles held together by a transparent matrix. This study shows that small, slow-settling particles host most of the bacterial activity in the

Columbia River estuary, and suggests that they contain the most rapidly consumed pool of organic matter in the system. Furthermore, it suggests that these particles may exist in ETM as faster-settling macro-aggregates. In the estuary, these particles often appeared in the water column early during developing flood tides prior to the appearance of the most turbid part of ETM, and sometimes remained in the water column after most ETM particles had settled back to the bed. This cycle places bacterially-active particles in the water column longer than most ETM particles, making them more likely to be washed out of the estuary, but also making them more available to suspension-feeding detritivores. Formation of large, fast-settling macro-aggregates is probably the mechanism by which bacterially-active particles are trapped in the ETM, and may be essential to maintaining the estuarine community of particle-attached bacteria.

Introduction

Particle formation in estuaries and particle cycling in estuarine turbidity maxima have been extensively studied (Schubel 1971, Kranck 1981, Gibbs et al. 1989, Eisma & Li 1993, Li et al. 1993, Chen et al. 1994), but relatively little has been presented on the connection between these processes and the estuarine food web. A principle complicating issue in studying the ecology of estuarine turbidity maxima (ETM) is that ETM are not homogeneous, well-mixed masses of particles. Particles composed of labile organic material that host actively growing bacteria coexist with mineral grains, refractory organic material and other less active particles. Fluxes of particles into and out of ETM vary on many time scales, and the composition of the suspended particle field varies in space and time during individual ETM events (Li et al. 1993). Physical and chemical measurements of particles in estuaries, most often conducted on bulk particles,

are likely to be misleading when trying to interpret biological data. For example, the presence of large amounts of refractory organic matter could mask the composition and rate of consumption of relatively labile organic matter.

Past work on the Columbia River estuary indicated that the estuarine food web is dependent on the physical and geochemical environment of ETM (Simenstad et al. 1990, Simenstad et al. 1994b Morgan et al. 1997, Crump et al. 1998). The hydrodynamics that trap and concentrate particles in the ETM also trap and concentrate particle-attached bacteria, copepods, and other members of the food web. In this system, particle-attached bacteria dominate bacterial activity (Crump & Baross 1996, Crump et al. 1998), and detritivorous copepods are the most important metazoan consumers (Simenstad et al. 1994b). These bacteria and copepods feed primarily on detrital organic material concentrated in the ETM (Simenstad et al. 1994b Crump & Baross 1996, Crump et al 1998). The ETM environment is therefore the center of the planktonic food web in the Columbia River estuary and is probably important in other river-dominated estuaries.

The relationship between bacterial activity and suspended particle mass (SPM) in the Columbia River estuary is highly variable even within a single ETM. We have often found peaks in bacterial activity, measured as ^{3}H-thymidine incorporation rate, in samples collected just prior to peak water column turbidity (Crump et al. 1998). Moreover, in depth profiles of bacterial activity through an ETM, highest bacterial activity was often found in relatively less turbid water above the highest concentration of ETM particles (Crump and Baross 1996). We hypothesized that a fraction of the particles in the ETM hosts the majority of the bacterial activity, and that these particles do not cycle within ETM like most ETM particles. In this study, particles were fractionated by size and *in situ* settling velocity to characterize the most bacterially-active

particles. Also, ETM events were intensely sampled for bacterial activity, SPM and POC in order to track the cycling of bacterially-active particles. A model of bacterially-active particle cycling in the ETM is proposed.

Materials & Methods

Sample collection and particle fractionation

Size fractions. In July 1996, near-bottom water samples were collected from ETM with a high-volume, low-pressure pump coupled to a conductivity-temperature-depth (CTD) meter and an optical backscatter sensor (OBS) for measuring turbidity in relative OBS units (Simenstad et al. 1994a). They were then poured through a series of Nytex screens (63 μm, 20 μm, 10 μm) and were subsampled after each screen. Some of the filtrate was then filtered through a 3-μm polycarbonate filter as previously described (Crump et al, 1997). The rate of ^{3}H-thymidine incorporation, suspended particulate mass (SPM), and particulate organic carbon concentration (POC) were measured in untreated water and in each filtrate,

Particles collected from each Nytex screen and < 10-μm subsamples were fixed in formaldehyde (2%), collected onto 0.2-μm polycarbonate filters (Poretics), and viewed with an environmental scanning electron microscope (FIE Company).

Owen tube settling velocity fractionation. *In situ* settling velocity of ^{3}H-thymidine incorporation rate, SPM and POC was determined with a "Quad Owen tube" consisting of four individual Braystoke Sk 110 settling columns (Owen 1976, Pejrup 1988, Reed & Donovan 1994) attached side to side. Each clear plastic tube was 1 m in length with a 5.1-cm internal diameter. For each sample, the set of tubes was lowered over the side of the ship. Stabilizing fins aligned the tubes to the flow of water in a horizontal position

allowing water and particles at a discrete depth to pass through all four tubes. The ends of the tubes were simultaneously closed with a messenger, brought on deck, and set in a special rack that holds them in a vertical position. Sample fractions were drawn from the bottom of the tube at fixed time intervals (Table 2.1). The Quad Owen tube was used in order to have enough material for multiple analyses. Generally, one tube was used for SPM, a second tube was used for ^{3}H-thymidine incorporation rates, and a third tube was used for POC. ^{3}H-thymidine incorporation rate and SPM were measured in Owen tube samples collected in May 1995 and October 1997. POC was measured in Owen tube samples from 1997.

Settling velocity spectra were calculated using a mathematical version of Owen's (1976) original graphical method (Larry Sanford Pers. Comm.).

High resolution ETM sampling. Thymidine incorporation rate, SPM and POC were measured in water samples collected from 0.5, 1, 1.5, 2, and 2.5 m above the bed of the estuary in the North channel (Fig. 2.1) at 4 or 5 time points in rapid succession during flood and ebb ETM events in May, July and October 1997 and February 1998. Sampling times were selected to bracket ETM events by sampling before, during and after peak water column turbidity, and were therefore not always at fixed intervals. Water samples were collected with the pump system described above.

Measurements

Thymidine incorporation rates were determined by measuring the incorporation of methyl-tritiated thymidine (TdR., Fuhrman & Azam 1982) (20-nM final conc. at 64 Ci mmol^{-1}) into the cold trichloroacetic acid (TCA) insoluble fraction of macromolecules in four 10 ml (1995) or six 5-ml (1997-8) subsamples incubated with constant agitation at *in situ* temperature. Duplicate (or triplicate) incubations were terminated at 0 and 60

minutes with 0.25-M NaOH (final conc., Zweifel et. al 1995). TCA precipitated macromolecules were washed twice with ice cold 5% TCA and twice with ice cold 80% ethanol (Crump & Baross 1996, Crump et al. 1998).

SPM in size fraction experiments was determined by filtering the material in a known volume of sample onto pre-weighed 0.45-µm, 47-mm diameter polycarbonate filters. SPM in Owen tube experiments was determined by filtering each fraction onto pre-weighed 0.45-µm, 47-mm diameter cellulose acetate filters. Filters were washed with fresh water, dried and weighed (Reed & Donovan, 1994).

POC and PON were measured by filtering samples through precombusted GF/F filters, drying, and processing with a Leeman Labs CE440 CHN analyzer calibrated with an acetanilide standard.

Results

Bacteria associated with particles between 3 and 10 µm were responsible for the majority of bacterial thymidine incorporation in all samples, however a reduced fraction of total POC was associated with these particles (Fig. 2.2). Bacterial activity per unit POC was highest in the smallest particle size fraction. The smallest size fraction averaged 3.7% organic carbon by weight and had an average carbon to nitrogen ratio (C/N) of 7.6 (Table 2.2). The larger size fractions were more variable in percent organic carbon and C/N, and tended to be higher in percent organic carbon.

The settling velocities of bacterially-active particles were variable among ETM samples. In three ETM samples, over 70% of the bacterial activity was concentrated in the slowest-settling fractions (Figs. 2.3A to 2.3C), but SPM was more evenly distributed. In three other ETM samples, bacterial activity was concentrated in the faster-settling

fractions. In one sample, most of the bacterial activity was concentrated on particles with a settling velocity between 0.25 and 2.27 mm s^{-1} (Fig. 2.3D). In two other samples, bacterial activity was concentrated on particles with settling velocities greater than 0.75 mm s^{-1} (Figs. 2.3E & 2.3F). Organic carbon content was higher for particles with slower settling velocities (Table 2.3).

High resolution sampling of ETM show the highest measurements of water column bacterial activity occurred during ETM events, but were not always associated with peaks in turbidity. During a flood tide ETM event sampled in the Spring 1997 (Fig. 2.4A), maximum bacterial activity was measured in the last sample (panel 4) approximately 30 min after peak turbidity (panel 2). During a Summer flood tide, bacterial activity was highest in relatively low turbidity water 45 min prior to peak turbidity (Fig. 2.4B, panel 2). Moreover, when turbidity was at its maximum near the bed bacterial activity was often greatest in less turbid water higher in the water column (Fig. 2.4B, panel 3). Also, 30 min after peak turbidity bacterial activity remained relatively high (Fig. 2.4B, panel 4). During flood tide ETM sampled in the Fall and Winter (Fig 2.4C & 2.4D), bacterial activity appeared to follow turbidity, although there was a pulse of relatively high bacterial activity near the bed 15 min prior to the onset of the ETM in the Fall series (Fig 2.4C, panel 1). During a Spring ebb tide, bacterial activity was highest early in the ETM event and dropped off later when turbidity was slightly higher (Fig. 2.5A). During a Fall ebb tide, bacterial activity became very high 30 min prior to peak turbidity (Fig. 2.5B, panel 2), and remained high as turbidity began to drop off (panel 4). The Winter ebb tide ETM was not very strong, and bacterial activity in the water column was somewhat constant (Fig. 2.5C).

Salinity near the bed of the estuary at the North Channel sampling site (Fig. 2.1) can vary from 0 to nearly that of coastal seawater, and ETM generally appear in the water column at some intermediate salinity during the progression of flood and ebb. During flood tide, high resolution sampling series in the Spring, Summer and Winter (Fig. 2.4A, B, D), peak bacterial activity and peak water column turbidity appeared when salinity was between 10 and 15 psu. These series began at 5-10 psu, and ended at 21 to 22 psu. In the Fall series (Fig. 2.4C) salinity increased from 2 to 7 psu with peak turbidity appearing at 7 psu. Ebb tide ETM (Fig. 2.5) appeared at lower salinities between 1 and 5 psu, probably because near-bed shear stress is not very strong until later during an ebb tide. The Spring and Fall series (Fig. 2.5A, B) began at 8 to 10 psu and ended at 1 psu. The winter series (Fig. 2.5C) began at 2 psu and ended at 1 psu.

There was a strong correlation between SPM and POC in all high resolution samples from all four seasons (Fig. 2.6).

Discussion

Particle size spectra

It is clear that all particles in Columbia River estuary ETM do not equally support bacterial activity. Small particles (<10 μm) supported 45 to 100% of bacterial activity despite constituting only 14 to 65% of particulate organic carbon. These particles support 5 to 50 times more bacterial activity per mg POC than other particles, are probably composed of the most rapidly consumed fraction of organic matter in the estuary, and may be the most important food source to the detrital food web.

Observations using fluorescence microscopy and environmental scanning electron microscopy showed that most of the material in the <10-μm size fraction was composed

of small mineral grains and broken diatom frustules bound together by what appeared to be an organic matrix (Fig. 2.7). The 10- to 20-μm, 20- to 63-μm, and >63-μm size fractions contained some large aggregated particles, but were dominated by intact diatoms, individual mineral grains and large pieces of metazoans, which may explain why they tended to have a larger organic carbon content. The process of passing particles through Nytex screens was probably destructive to larger aggregated particles, breaking them up into individual mineral grains and cohesive micro-aggregates that could pass through the 10-μm screen. Previous work on the Columbia River estuary showed that these types of particles can be heavily colonized with bacteria (Crump and Baross, 1996). It is likely that bacterially-active particles in the estuary are composed of this aggregated material.

Aggregated particles are among the most abundant types of particles in ETM. The composition of estuarine aggregates has been extensively studied in laboratory experiments (Sakamoto 1972, Milligan 1995, Kranck 1973) and in the field (Gibbs et al. 1989, Eisma 1986, Li et al. 1993). Loosely-bound macro-aggregates, as large as several mm in diameter, are composed of smaller, more tightly-bound micro-aggregates (<100 μm) and larger mineral grains. These micro-aggregates are composed primarily of small mineral grains with organic coatings bound to phytodetritus, small bits of riverborne detritus, and perhaps flocculated DOC. Marine and lake "snow" particles differ from estuarine aggregates in that they are primarily composed of organic matter to which inorganic material subsequently attaches (Eisma 1986).

Bacteria are thought to play a key role in the formation of aggregates in estuaries. Particles encounter one another through Brownian motion, differential settling and the turbulent motion of water, but particle adhesion is facilitated in part by biologically

produced polymeric material (Eisma 1986). In laboratory incubations of DOC, granite-derived till and powdered salt marsh plant *Spartina* material, Muschenheim et al. (1989) found that larger aggregates formed after 24 to 48 hours coincidentally with the development of a microbial community, suggesting the importance of bacterially-produced exudates in "gluing" larger particles together. Bacterial production of exopolysaccharide probably helps bind together aggregates in the Columbia River ETM, especially while particles are concentrated on or near the bed during slack tide.

Besides their role in the formation and structural integrity of estuarine aggregates, bacteria on particles also contribute to the estuarine food web. Particle-attached bacteria increase the food value of particles to metazoan consumers as part of the detrital food web (Heinle et al. 1977). Flocculant particles in the Ems and the Elbe Rivers estuaries in Europe were shown to be colonized by bacteria and protozoa and in the Elbe River estuary to be colonized by rotifers (Zimmerman & Kausch 1996, Muschenheim et al. 1989). In the Columbia River estuary, copepods directly consume particles and particle-attached bacteria (Simenstad et al. 1994b). Bacterially-active particles probably contain some of the most labile organic matter in the estuary and therefore may be the best food source for metazoan detritovores.

Settling velocity spectra

The majority of bacterially-active particles in three ETM Owen tube samples had relatively slow-settling velocities of less than 0.007 cm s^{-1} (24.6 cm h^{-1}). Early work in the Tamar River estuary also found "non-sinking" particles to be much more bacterially-active than particles that sank (Goulder 1977). This seems to be in conflict with the idea that in order for particles to become trapped and concentrated in ETM and avoid being washed out of the estuary they must be able to sink into deeper water and settle to the bed

(Reed & Donovan, 1994). The most probable mechanism for concentrating slow and non-settling particles in ETM is aggregation with other particles to form rapidly-settling macro-aggregates. Cycles of aggregation and disaggregation as occur with every tide in the ETM of the Columbia River estuary (Reed & Donovan 1994) have been described for other systems. Research on the ETM of the Elbe River estuary found that aggregated particle size changed with the tidal cycle such that larger aggregates resuspended from the bed were disaggegated by shear stress during periods of peak flow, only to reform during slack tides and settle to the bed (Chen et al. 1994). In the Dollard River estuary, aggregation is thought to occur throughout the tidal cycle, and disaggregation to occur during or after particles settle to the bed (Eisma and Li, 1993). The presence of such cycles in the Columbia River estuary would account for the high concentration of slow-settling, bacterially-active particles found in Owen tube samples because the samples were collected during the intermediate stages of ETM events after particles would have disaggregated.

Most of the bacterial activity was associated with faster settling particles in three ETM Owen tube samples. Macro-aggregates exceeding 1 mm in diameter were often seen settling in Owen tube samples collected in some ETM and in images collected with an *in situ* video camera (data not shown). This shows that small, bacterially-active micro-aggregates combine to form larger, faster-settling macro-aggregates in the ETM, and that the degree of aggregation and disaggregation can vary during any particular ETM event. Two samples (Figs. 2.3e & 2.3f) were collected during a neap tide when the extent of disaggregation in the ETM was probably diminished because of reduced shear forces.

A large fraction of the suspended particulate material collected in Owen tube samples supported very little bacterial activity. Grain size analysis of particle settling fractions demonstrated that fast-settling particles were aggregates of smaller, slower-settling particles (Reed and Donovan 1994). So it is not clear why larger, faster-settling aggregates are not always bacterially-active. Faster-settling particles probably host less bacterial activity per mg SPM in part because they contain less organic matter (Table 2.3). It is also possible that faster settling particles are older aggregates that have been subjected to intense bacterial activity and no longer contain labile organic matter. Since less bacterially-active aggregates settled more rapidly than active ones it is tempting to speculate that older aggregates are larger, denser, and more tightly bound than aggregates that support a rapidly growing bacterial community. This would also explain why they are not broken up early in the ETM cycle, and why they settle out of the water column rapidly after a resuspension event.

Particle cycling in ETM

It is not unusual to find that different particles cycle through estuaries in different ways. In the Jiaojiang River estuary in China, small clay-dominated "dense" aggregates were preferentially mixed up from the ETM and carried oceanward of the ETM where they either sank back to deeper water and were re-entrained in the ETM or were washed out of the estuary (Li et al. 1993). However, large silt-dominated "loose" aggregates composed in part of dense aggregates, were not lifted far off the bottom because they had faster settling velocities, or because they broke up in the stronger currents. In the Gironde River estuary, large aggregated particles stayed in the ETM region by rapidly settling into deeper waters while smaller aggregates mixed into the upper layers of the

water column and traveled far down estuary before settling into deeper layers and being carried back up-estuary to the ETM (Gibbs et al. 1989).

In the Columbia River estuary, the appearance of high bacterial activity in the water column just before and just after peak water column turbidity during ETM events suggests that bacterially-active particles do not cycle between the water column and the surface sediments in the same way as the majority of ETM particles. We have learned that bacterially-active particles are either slow-settling or are part of faster-settling, loosely bound aggregates. Using this information we can construct a hypothetical model of how bacterially-active particles cycle in the ETM.

After a slack tide, most of the ETM material has settled to the bed or to a depth just above the bed. Sorting during the settling of these particles causes the slower-settling, bacterially-active particles to be on the surface of the sediments, and the faster-settling particles to be deeper. As bed shear increases during a flood or ebb tide the slowest-settling particles are the first to be resuspended. This would account for the appearance of high bacterial activity in the water column early during an ETM resuspension event. Particles that are aggregated after settling to the bed undergo some disaggregation as they are lifted off the bed and mixed up into the water column, releasing bacterially-active particles into suspension. As water velocity increases and shear over the bed reaches a maximum, the bulk of the particles that constitute the ETM are resuspended and mixed up in the water column. Then, as water column velocity begins to slow, particles with the fastest settling velocity are the first to settle to the bed. Slow-settling, bacterially-active particles remain in the water column until they settle to the bed either on their own or by forming loosely-bound macro-aggregates.

This model has implications for the estuarine food web which, in the Columbia River estuary, is centered in the ETM. Detritivorous copepods, the most important metazoan consumers in the system, feed directly on ETM particles, and have been shown to directly consume particle-attached bacteria (Simenstad et al. 1994b). The dominant copepod, *Eurytemora affinis*, migrates up and down in the water column with ETM particles in order to keep from being washed out of the estuary (Morgan et al. 1997, Simenstad et al. 1994). Various points of evidence suggest that these copepods rise into the near-bed water column prior to the appearance of peak ETM turbidity (Morgan et al. 1997, Simenstad & Cordell, unpub.). As suspension feeders, they may benefit from the sorting that occurs during resuspension events. They would encounter high concentrations of bacterially-active particles in the water column early during an ETM event when the particles are preferentially resuspended from the bed and again later when the particles stay in suspension longer than the less labile particles that make up the rest of the ETM.

Conclusions

Bacterially-active particles are concentrated in the ETM, but they do not cycle through the ETM like most particles because they tend to be small, slow-settling particles that are easily disaggregated from large, rapidly-settling macro-aggregates. The cycle followed by these particles places them in the water column longer than most ETM particles, making them more likely to be washed out of the estuary, but also making them more available to suspension-feeding detritivores. Formation of large, fast-settling macro-aggregates may be the mechanism by which bacterially-active particles are trapped in the ETM, and may be essential to maintaining the estuarine community of particle-attached bacteria.

Table 2.1. Time elapsed (s) before each fraction was collected from Owen tubes.

Owen tube fraction #	1995	1997-1998
1	1 m 14	1 m 2
2	12 m 1	5 m 45
3	28 m 29	12 m 3
4	final	19 m 18
5		27 m 48
6		40 m 36
7		41 m 48
8		final

Table 2.2. Organic carbon content and carbon to nitrogen ratios of particle size fractions.

Size fractions	Organic carbon (%)	SD	C/N ratio	SD
>63 µm	9.6%	6.5%	6.5	2.4
20-63 µm	5.2%	2.7%	9.5	3.9
10-20 µm	3.5%	2.5%	7.6	0.9
3-10 µm	3.7%	0.5%	7.6	1.4

Table 2.3. Percent organic carbon of particles in settling velocity fractions from 1997-1998 Owen tube samples

Settling Velocity mm s^{-1}	Organic Carbon (%) Fig. 3A	Fig. 3D
>14.71	1.6%	1.5%
2.31 - 14.71	1.0%	1.4%
0.93 - 2.31	0.4%	1.5%
0.47 - 0.93	0.8%	1.5%
0.25 - 0.47	1.9%	0.8%
0.12 - 0.25	2.6%	2.7%
0.07 - 0.12	4.6%	2.4%
<0.07	2.7%	3.8%

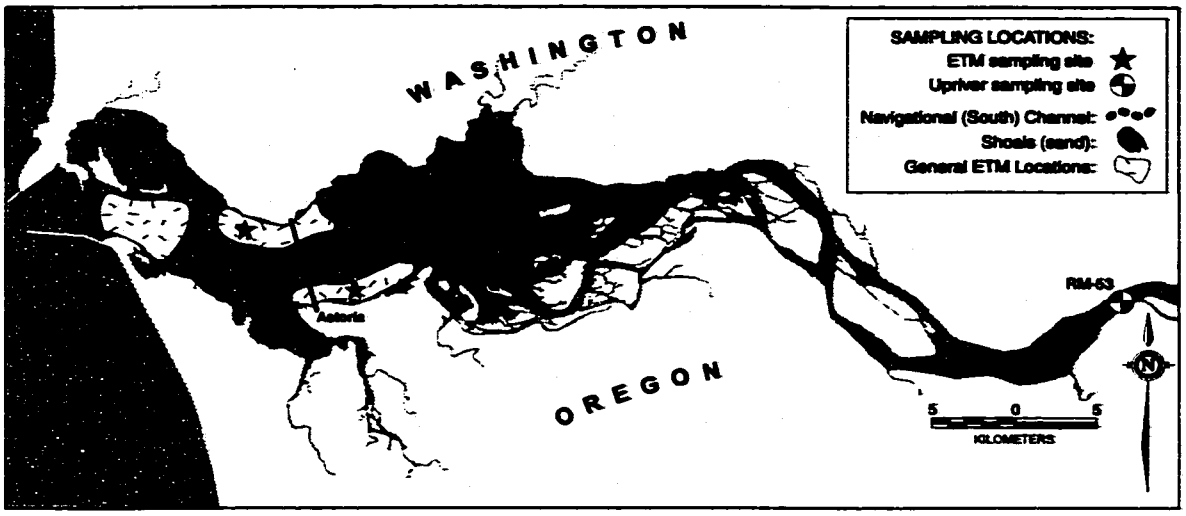


Fig. 2.1. The Columbia River estuary. Sampling sites in the North and South channels of the estuary and upriver are indicated.

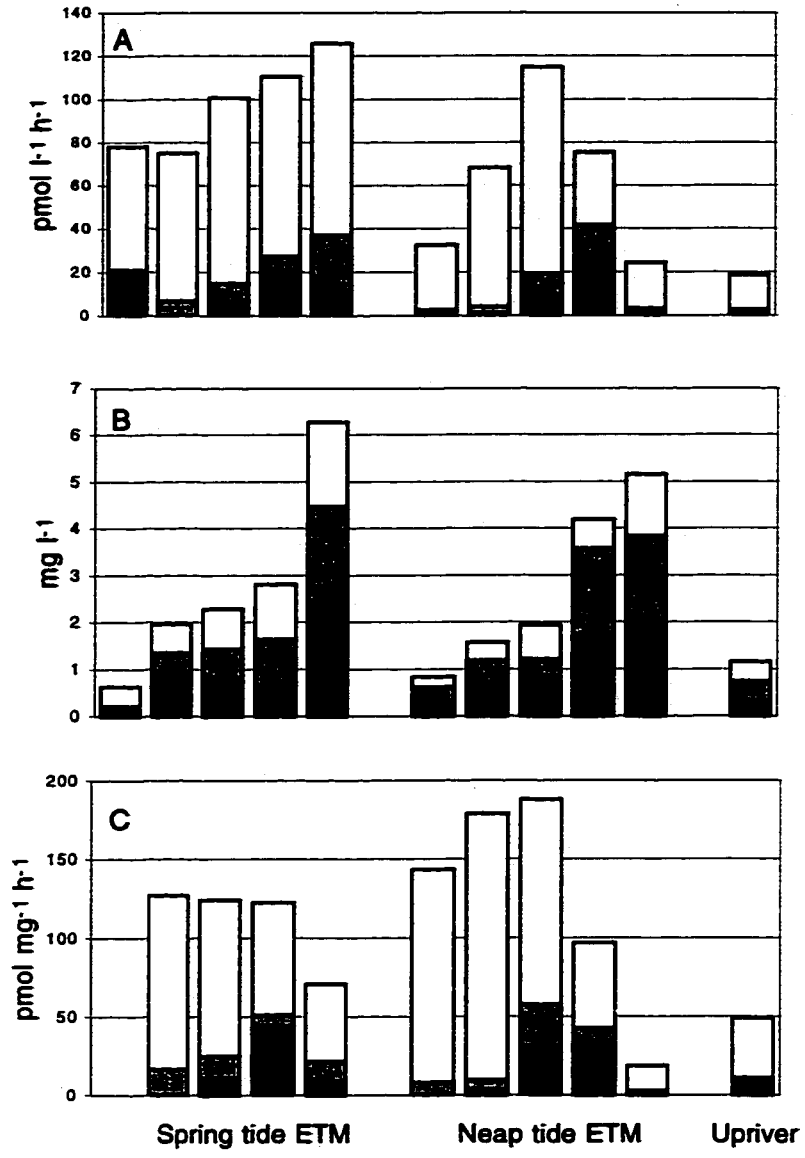


Fig. 2.2. Thymidine incorporation rate per liter (A), particulate organic carbon (POC) concentration (B), and thymidine incorporation rate per mg POC (C) for four size fractions (lightest to darkest shading: 3 to 10 μm, 10 to 20 μm, 20 to 63 μm, and >63 μm) in 5 spring tide ETM samples, 5 neap tide ETM samples, and 1 upriver sample.

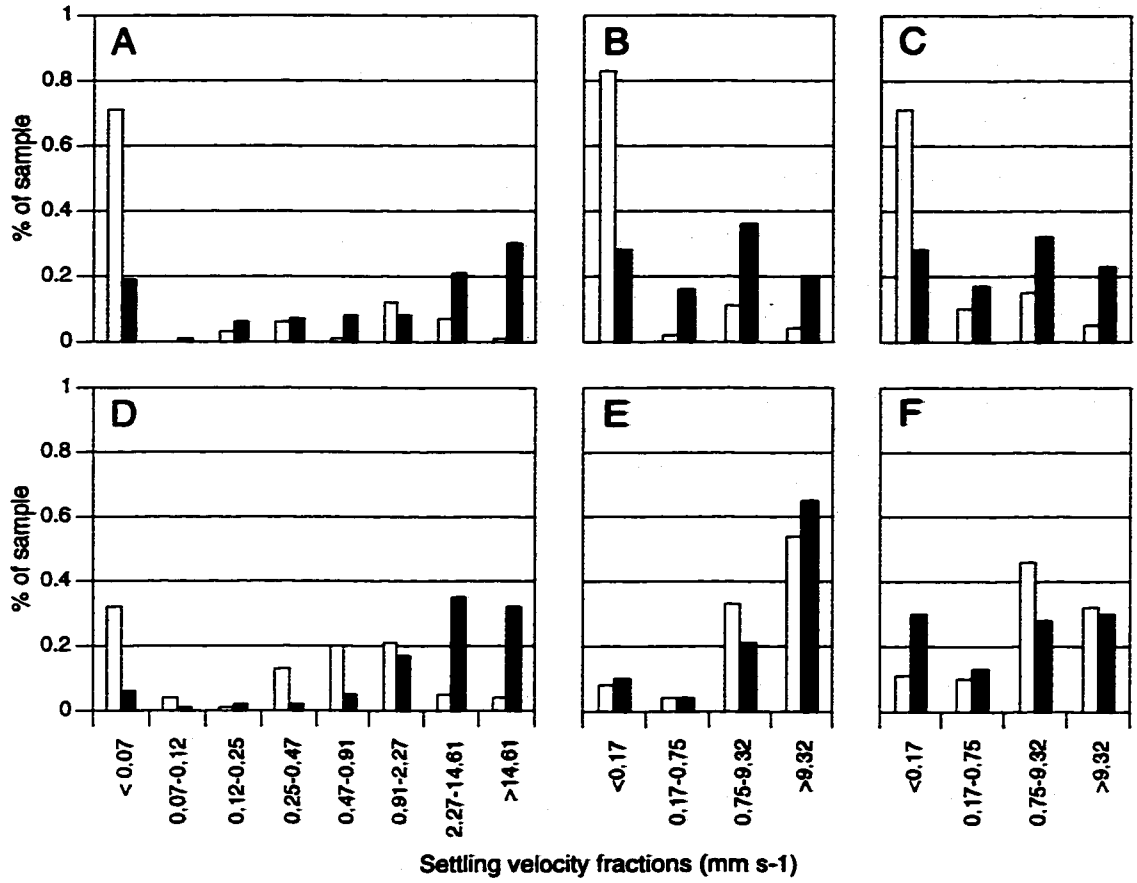


Fig. 2.3. Fraction of thymidine incorporation rate (white columns) and suspended particulate mass (dark columns) associated with settling velocity fractions in flood tide ETM samples collected in 1997-8 (A&D), and 1995 (B,C,E,F).

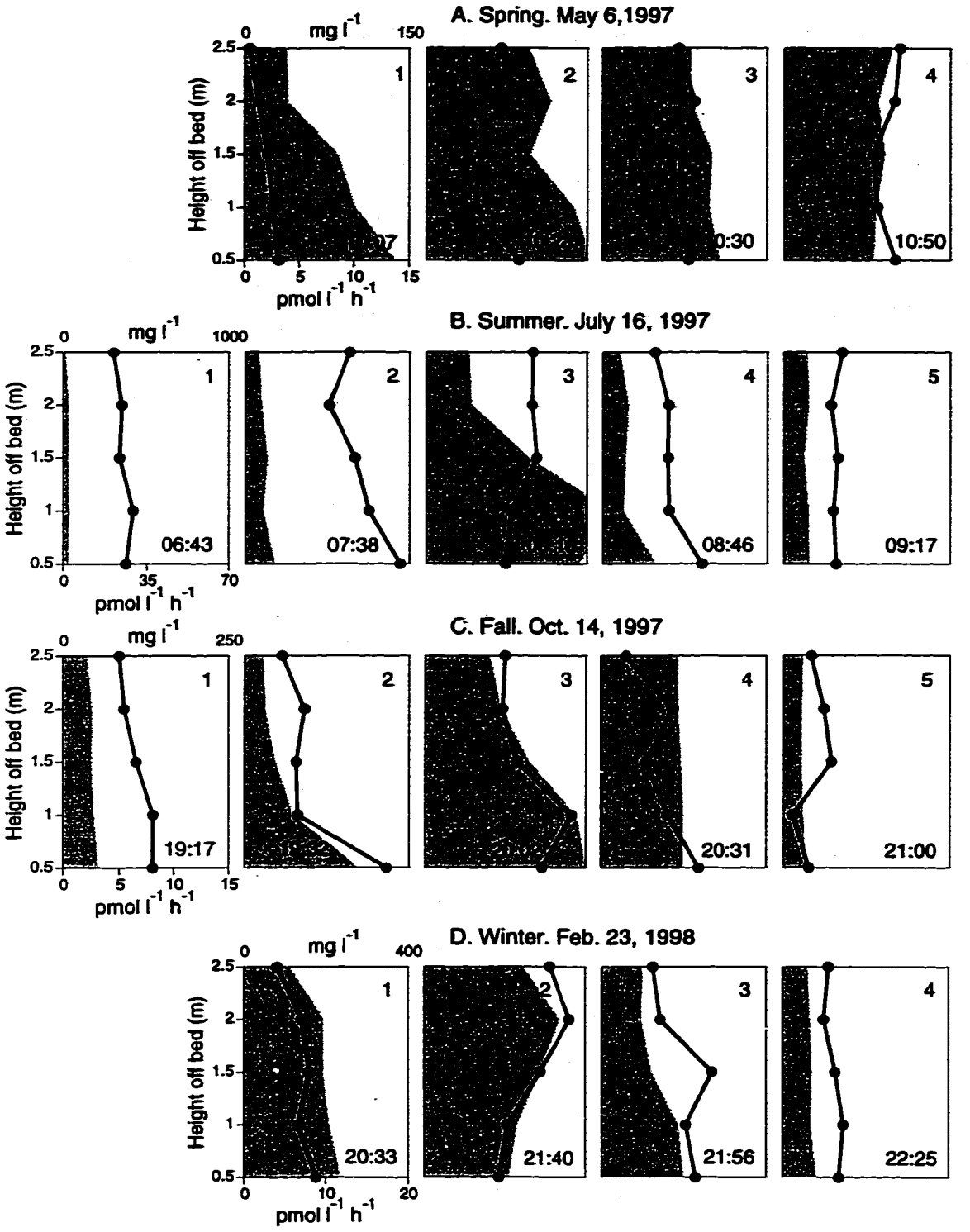


Fig. 2.4. Near-bottom depth profiles of thymidine incorporation rates (dots connected by lines) and suspended particulate mass (connected by line and shaded for clarity) during four flood tide ETM resuspension events sampled in Spring, Summer and Fall 1997, and in Winter 1998. The time on each panel indicates when the deepest sample was collected. Shallower samples were collected in sequence after collecting the deepest sample.

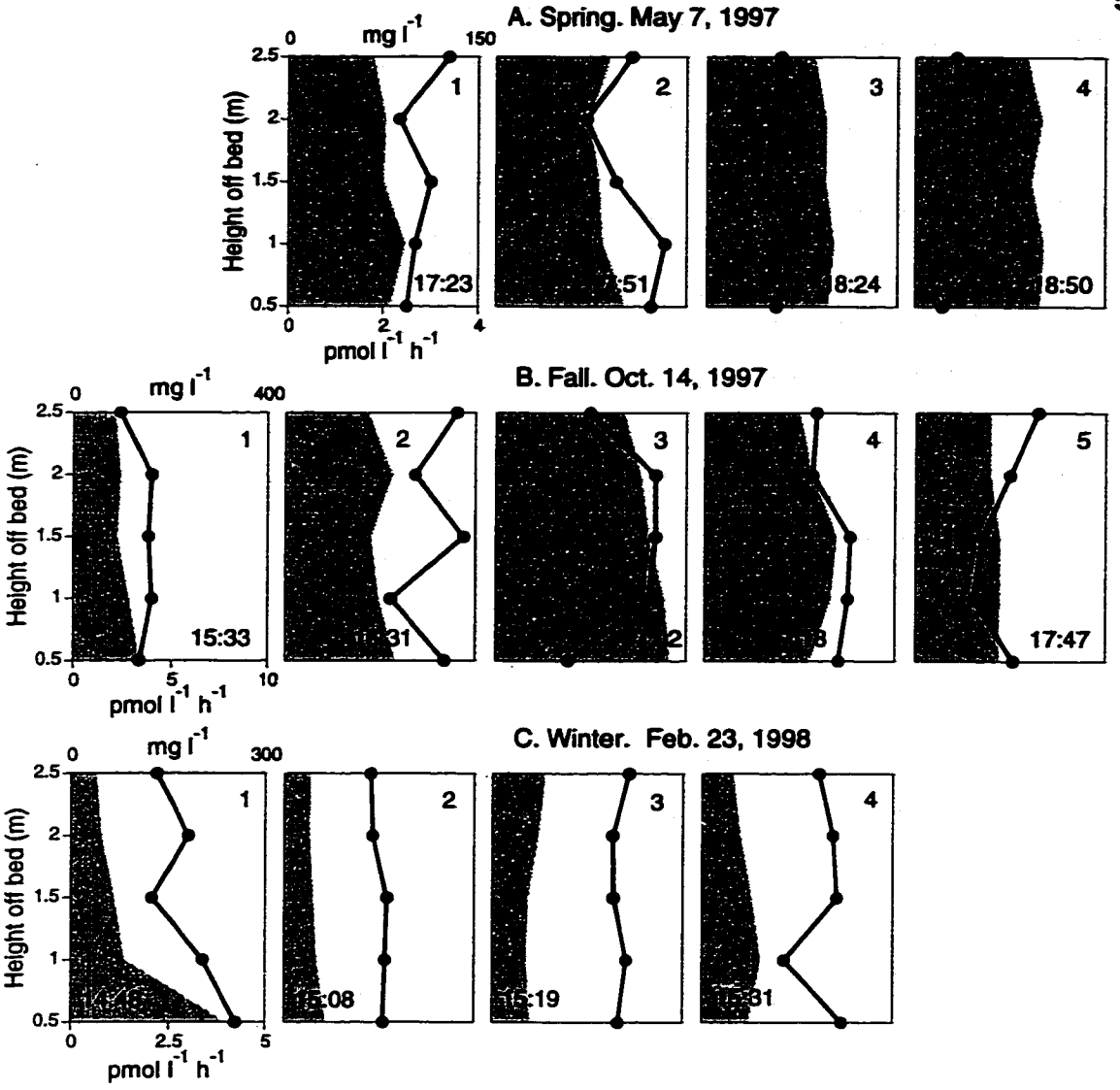


Fig. 2.4. Near-bottom depth profiles of thymidine incorporation rates (dots connected by lines) and suspended particulate mass (connected by line and shaded for clarity) during ebbtide ETM resuspension events.

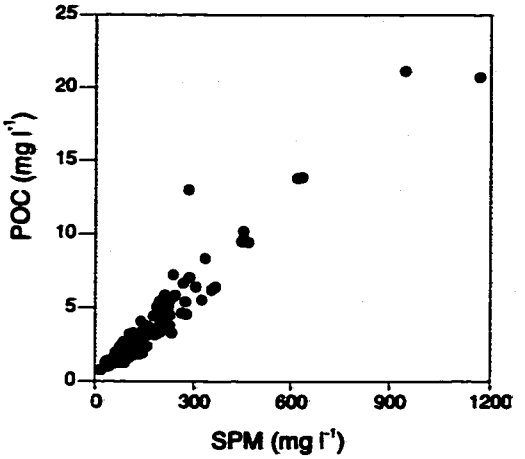


Fig. 2.6. Suspended particulate mass vs. particulate organic carbon concentration for samples on Figs. 2.4 & 2.5.

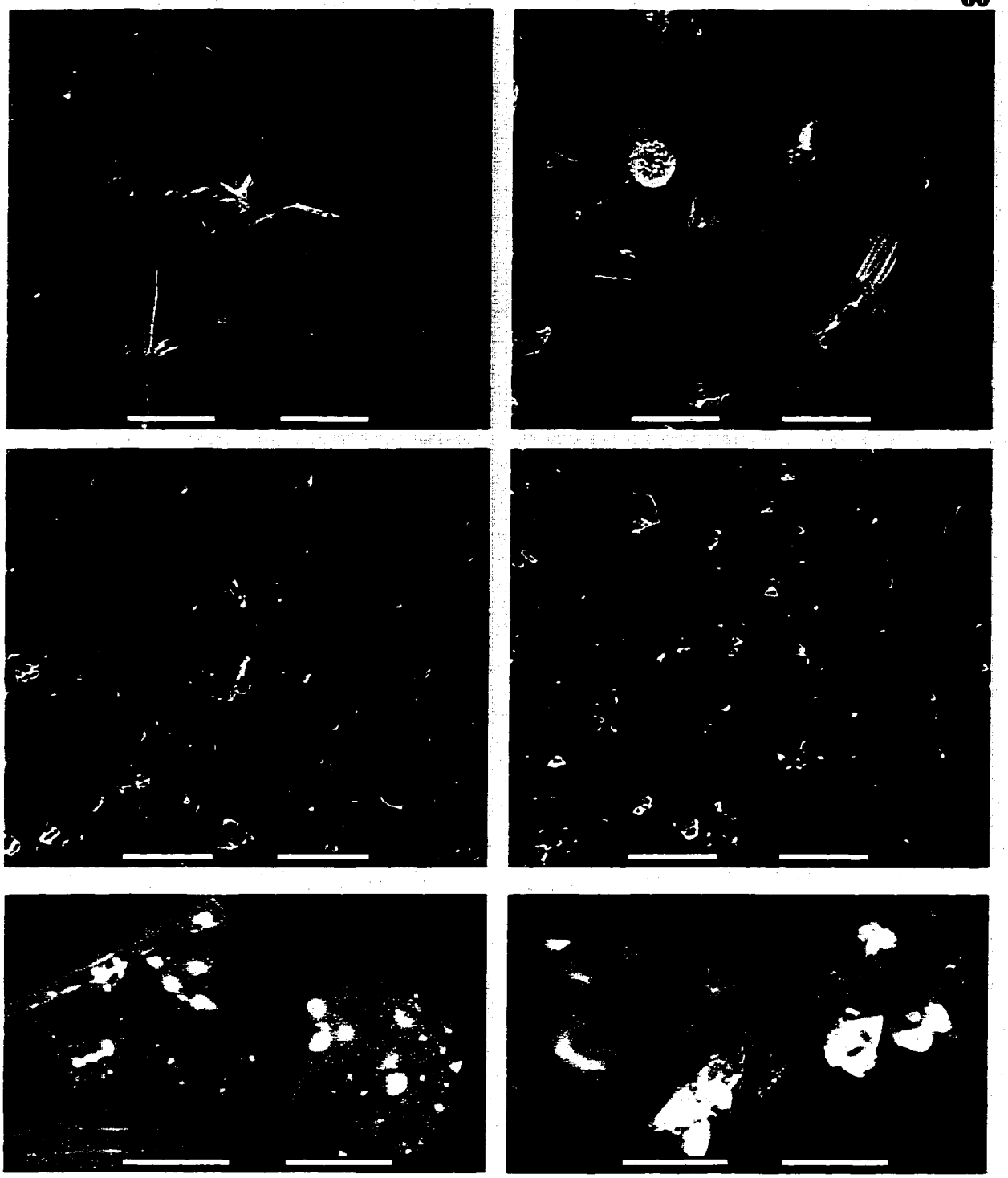


Fig. 2.7. Microscopic images of particles from the Columbia River estuary. Environmental scanning electron micrographs of size fractionated particles: > 63 um (A), 20 to 63 um (B), 10 to 20 um (C), < 10 um (D), and a close up of a particle from the < 10 um fraction (F). DAPI stained particle viewed with fluorescence microscopy (E).

Chapter 3. Phylogenetic Analysis of Particle-attached and Free-living Bacterial Communities

Abstract

The Columbia River estuary is a dynamic system in which estuarine turbidity maxima trap and extend the residence time of particles and particle-attached bacteria over that of the water and the free-living bacteria. Particle-attached bacteria dominate bacterial activity in the estuary and are an important part of the estuarine food web. PCR amplified 16S rRNA genes from particle-attached and free-living bacteria in the Columbia River, its estuary and the adjacent coastal ocean were cloned, and 239 partial sequences were determined. Wide diversity was observed at the species level within at least six different bacterial phyla, including most subphyla of the *Proteobacteria*. In the estuary, most particle-attached bacterial clones (75%) were related to *Cytophaga*, α-*Proteobacteria* , γ-*Proteobacteria* and δ-*Proteobacteria*. These same clones, however, were rare or absent from either the particle-attached or the free-living bacterial communities of the river and the coastal ocean. In contrast, about half (48%) of the free-living estuarine bacterial clones were similar to clones from the river and the coastal ocean. These free-living bacteria were related to groups of cosmopolitan freshwater bacteria from the β-*Proteobacteria*, Gram Positive bacteria, and *Verrucomicrobium*, and groups of marine organisms from the Gram Positive bacteria, and the α-*Proteobacteria* (SAR11 and Rhodobacter). These results suggest that rapidly growing particle-attached bacteria develop into a uniquely adapted estuarine community, and that free-living estuarine bacteria are similar to the river and the coastal ocean communities. The high

diversity in the estuary is the result of mixing of bacterial communities from the river, estuary and coastal ocean.

Introduction

Bacterial diversity in estuarine environments is expected to be high due to a combination of the mixing of sea water and fresh water, and the resuspension of sediments and particles from many sources including benthic zones, tidal mudflats, and seagrass beds. However, only a fraction of these bacteria may be active as consumers of detrital organic matter. Previous work in the Columbia River estuary showed that the fraction of bacteria attached to particles accounted for approximately 90% of heterotrophic bacterial activity in the water column, and were 10 to 100 times more active than free-living bacteria (Crump & Baross 1996, Crump et al. 1998). These bacteria are responsible for most of the degradation of detrital organic matter in the estuary (Crump et al. 1998), and are also part of a thriving estuarine food web in which they are consumed by detritivorous copepods, the dominant metazoan consumers in the system (Simenstad et al. 1994a). This food web is supported by allochthanous organic material and river phytoplankton, the supply of which far surpasses *in situ* primary production (Small et al. 1990). In the estuary, this material forms organic-rich particles (Prahl et al. 1997) that can be heavily colonized by bacteria and are the site of the majority of water column extracellular enzymatic activity (Crump et al. 1998).

The physical, chemical and biological environment of the Columbia River estuary is centered in the estuarine turbidity maxima (ETM), which are common, well-studied features of river-dominated estuaries, created by the interaction between river flow and tidal forcing (Berner & Berner 1996). In the Columbia River estuary, ETM trap and

extend the residence time of particles near the head of the salt wedge in the deeper regions of the two main channels. Organic and inorganic material from the river and the coastal ocean enter into these ETM regions and become part of tidal cycles of sedimentation and resuspension as they are advected up and down the estuary. The residence time of particles in the ETM is thought to be approximately 2 to 4 weeks (D.J. Reed, pers. comm.), which is much longer than the 1 to 2 day residence time of water (Neal 1972). The organic matter associated with these particles is thought to be the primary food source for the food web of the Columbia River estuary (Baross et al. 1994, Crump et al. 1998).

One goal of our research was to investigate whether estuarine hydrodynamics involved in ETM formation influence the composition of bacterial communities. We hypothesized that actively-growing, particle-attached bacteria trapped in the ETM form a community that develops and is adapted to life in the estuary, and is different than source communities in the river and the coastal ocean. We also hypothesized that the free-living bacterial community in the estuary grows too slowly to develop into an estuarine community and would therefore be composed of bacteria from the river and the coastal ocean.

Microbial community analyses using 16S rRNA sequencing have provided a picture of bacterial diversity in oceans, lakes, soils, sediments, aquifers, animal guts, terrestrial hot springs and sewage (Giovannoni et al. 1990, Liesack & Stackebrandt 1992, DeLong et al. 1993, Fuhrman et al. 1993, Stackebrandt et al. 1993, Pederson et al. 1996, Wilson & Blitchington 1996, Hiorns et al. 1997, Rappé et al. 1997, Suzuki et al. 1997, Felske et al. 1998, Hugenholtz et al. 1998, Nold & Zwart 1998). No such studies, however, have been conducted on planktonic bacteria in rivers or estuaries. Here we present the results of

community analyses of particle-attached and free-living bacteria in the Columbia River of the United States Northwest, its estuary and the adjacent coastal ocean.

Materials & Methods

Sample collection

The Columbia River is the second largest river in the United States, with a drainage basin of 660,480 km^2 (Simenstad et al. 1990). Impoundments occur along almost the entire length of the river, creating relatively still reservoirs where riverborne detritus sediments, and phytoplankton thrive. The river drains into a shallow, partially-mixed estuary (Fig. 3.1) with two main channels that are generally 20-25 m deep. The South Channel is dredged for navigation. Sediments in the main stem of the estuary are sand. The estuary is flanked by tidal mudflats in a few shallow peripheral bays. Salinity intrudes to the ETM regions in the North and South Channels with every tide, and can extend up to approximately 20 km from the mouth of the estuary depending on river flow and tide-stage.

Water samples were collected at three stations (Fig. 3.1) in May, 1997, with an high-volume low pressure pump system coupled to a conductivity-temperature-depth (CTD) sensor and an optical backscatter sensor (OBS) for detecting turbidity (Simenstad et al. 1994b). The coastal ocean sample was collected about 1 m above the bed at the end of a flood tide in order to collect high salinity (salinity (S) = 30 psu) low turbidity (Suspended Particulate Mass (SPM) =18.5 mg l^{-1}) marine water as it entered the mouth of the estuary. The freshwater river sample was collected at mid-depth (10 m) at a location above the influence of salinity (S = 0 psu, SPM = 29.8 mg l^{-1}). The estuarine sample was collected at an intermediate salinity (S = 9 psu) in the North Channel of the estuary about 1 m

above the bed (17.6 m) during a flood tide turbidity maximum resuspension event in order to collect ETM particles (SPM = 167.5 mg l^{-1}).

Samples were stored at 4°C for up to an hour before processing, and then prescreened with a 10-µm nytex mesh to exclude diatom chains and mesozooplankton. Free-living bacteria were gently separated by floating three plastic filter towers (47-mm dia.; Millipore) equipped with 3-µm polycarbonate filters (Poretics) on the surface of a sample contained in a 2-L beaker (Crump et al. 1998). Filtered water flowed up into the towers and was collected. This method allowed larger particles to settle to the bottom of the beaker and not interfere with the filtration of the sample by clogging the filter. Particle-attached bacteria were collected separately by vacuum filtration again using 3-µm polycarbonate filters. Samples were poured into a plastic filter tower and drawn down onto the filters. At intervals when flow through the filters slowed, particles were rinsed by drawing approximately 2 ml of sterile double-distilled water through the filter. Particles were then dislodged from the filter with a stream of sterile water from a squirt bottle, poured out of the filter tower and collected. Particle-attached and free-living bacteria were then concentrated onto separate 0.2-µm Sterivex filters (Millipore). The coastal ocean particle-attached sample was lost during processing, and so a whole water sample was used instead.

DNA extraction and purification

Sterivex filters were immediately flooded with approximately 2 ml of sarcosyl lysis buffer ("SLB": 0.14 M NaCl, 50 µM NaOAc (pH5.2), 0.3% sodium-N-lauroyl-sarcosinate (sarcosyl), autoclaved and filter sterilized), and 100 µl of proteinase-K (2%), agitated briefly and incubated at 38°C for 2 h. The filters were then frozen at –20°C until further processing.

The filters were defrosted and agitated, and each sample was drawn off along with resuspended particulate material. Particles carried off the filter with the extraction buffer were pelleted by centrifugation (6000 x g, 5 min). This was done for all samples. The resulting supernatant was combined with 0.5 ml of CTAB (5%) and 20 µl Proteinase-K (20%), incubated at 37°C on a rotating carousel for 30 min, and stored on ice.

DNA extraction buffer (DEB: 0.1M Tris-HCL (pH 8), 0.1M NaEDTA (pH 8), 0.1M $NA_2H_2PO_4$ (pH 8), 1.5M NaCl, 5% CTAB)(Zhou et al. 1996) and proteinase-K (2%) was added to both parts of each sample: the Sterivex filter (1.85 ml & 10 µl respectively) and pelleted particles (0.925 ml & 5 µl respectively). Samples were frozen at –70°C and thawed at 65°C three times, and then incubated on a rotating carousel at 37°C for 30 min. SDS (20% sodium dodecyl sulfate) was added (150 µl to filter, 30 µl to particles), and samples were incubated on a rotating carousel at 65°C for 2 h. The particles were centrifuged and the buffer was added to the supernatent from the first extraction. The extraction buffer from the Sterivex filter was then centrifuged over the particles and the supernatent was added to the supernatent from the first extraction. The extraction procedure was repeated once on the Sterivex filter and the particles.

An equal volume of chloroform:isoamyl alcohol (24:1) was added to the combined supernatents, vortexed, and centrifuged (3000 rpm in a Jouan microcentrifuge; 10 min). The aqueous layer was transferred to a sterile 50-ml glass Corex tube (Corning), combined with an equal volume of isopropanol and incubated at room temperature for 1 h. Precipitated DNA was centrifuged (16,000 x g, 20 min, room temp.), washed with 5 ml 70% ethanol, dried down, dissolved in 500 µl TE buffer (10 mM Tris-HCl, 1mM NaEDTA, pH8), and frozen.

Aliquots of each DNA extract was purified with Qiaquick PCR purification columns (Qiagen) following manufacturer's instructions except DNA was washed twice with PE buffer and eluted twice with EB buffer heated to 65°C (buffers provided by manufacturer).

Clone library construction

PCR was run in 8-10 separate 100-µl reactions for each DNA sample using "universal bacterial" primer 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and "universal" primer 1492r (5'-GGT TAC CTT GTT ACG ACT T-3') (Weisberg et al. 1991) (2.5 mM MgCl, 0.8 mM dNTPs, 1ng μl^{-1} of each primer, 2.5 U *Taq* DNA polymerase [Promega], 1X PCR buffer [Promega]). PCR amplification began with a 1 min denaturation at 94°C followed by 20 to 25 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min. The final cycle was extended at 72°C for 5 min. PCR cycles were stopped while the product concentration was still increasing exponentially. The resulting low concentrations of PCR product required us to run multiple PCR reactions to have enough product for cloning. PCR products were combined, concentrated, and purified with Qiaquick PCR purification columns (Qiagen) following manufacturer's instructions.

PCR products were ligated into pGEM-T cloning vectors (Promega) and used to transform JM109 competent cells (Promega) following manufacturers instructions. Positive colonies were picked and stored on agar plates and frozen in liquid media at –70°C.

The use of environmental clone libraries as a quantitative measure of diversity has fallen into question due to variable primer specificity and over-amplification of rare sequences (Suzuki & Giovannoni 1996, Wintzingerode et al. 1997, Hansen et al 1998). However, 16S rRNA clone libraries have provided valuable qualitative pictures of

microbial diversity that allow us to compare and contrast the communities in different environments (Fuhrman et al. 1993, Munson et al. 1997, Methe et al. 1998, Zwart et al. 1998). We attempted to minimize the amplification of contaminant genes and the overamplification of rare genes by using a reduced number of PCR cycles, stopping the amplification while the concentration of PCR product was still increasing exponenially.

RFLP analysis

Seventy-five clones from each of the estuarine clone libraries were randomly chosen to inoculate 100 µl LB medium (1% Tryptone, 0.5% yeast extract, 1% NaCl, pH7), and incubated at 37°C for 1 h. The plasmid inserts were PCR-amplified with the vector specific primers SP6 (5'-ATT TAG GTG ACA CTA TAG-3') and T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') (20-µl reactions with 1 µl of clone culture, 3 mM MgCl, 0.8 mM dNTPs, 1ng μl^{-1} of each primer, 2.5 U *Taq* DNA polymerase [Promega], 1X PCR buffer [Promega]). PCR amplification began with a 1 min denaturation at 94°C followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min folowed by a 5 min extension at 72°C. PCR products were restriction digested with Msp1 and Rsa1 (Boehringer-Mannheim) following manufacturer's instructions, electrophoresed on 2.5% agarose gels (Agarose 3:1, Amresco) prepared with TAE (0.04 M Tris-acetate, 1 mM EDTA), and stained with SYBR green (Molecular Probes). Gel images were digitized with a Fluorimager 575 fluorescent gel scanner (Molecular Dynamics) and band sizes were determined using FragmeNT Analysis 1.1 (Molecular Dynamics) based on 1 kb ladder size standard (GibcoBRL).

Sequencing and phylogenetic analysis

Estuarine clone inserts with unique RFLP patterns and 25 clones chosen randomly from each of the river and coastal ocean clone libraries were sequenced. Inserts were

PCR amplified as described above. PCR products were purified using Qiaquick PCR purification columns, sequenced bidirectionally using primers 8f (as above) and 519r (5'-GWA TTA CCG CGG CKG CTG-3') with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems Inc.), and resolved on a Model 373A automated DNA sequencer (Applied Biosystems Inc.).

We tested for chimeric sequences in two ways. First we ran sequences through the Chimera Check program of the Ribosomal Database Project website (RDP, http://www.cme.msu.edu/RDP; Maidak et al. 1997). We also analyzed the secondary structures by aligning bases 52 to 58 with 354 to 359, and bases 122 to 142 with 221 to 239 (E. coli numbering system) for each sequence (Kopczynski et al. 1994).

Unaligned sequences were submitted to the Sequence Match program of the RDP, and to the Advanced BLAST search program of the National Center for Biotechnology Information website (National Center for Biotechnology Information, 1999) to find closely related sequences. Related sequences were acquired using the Batch Entrez program (National Center for Biotechnology Information, 1999). Preliminary alignments were made using the Sequence Align program (RDP) and requesting that common gaps be preserved. Sequences were organized by phylum and alignments were completed manually using SeqApp (Gilbert, 1994). Some sections of the sequences in each phyla could not be aligned and were therefore removed from further analysis (Table 3.1).

Percent similarity between sequences was determined using the Distances program of the Wisconsin Package version 9.1-UNIX of the Genetics Computer Group, Inc. (GCG) set to calculate uncorrected distances.

Phylogenetic analysis was accomplished with the PAUP program (Smithsonian Institution, 1997) accessed through the Wisconsin Package. Consensus (50% majority

rule) trees were constructed using uncorrected neighbor-joining distances with 1000 bootstrap replicates. These trees exclude groupings that occurred in less than 50% of the replicates. Negative branch lengths were prohibited.

Phylum-specific trees were originally prepared with three different outgroup sequences from among the following organisms: *Roseobacter denitrificans, Rhodoferax fermentans, Escherichia coli, Pirellula staleyi, Cytophaga lytica,* and *Agrococcus jenensis*. Clones were placed on trees with their closest relatives identified with the database searches described above. Clones with no clear affiliation to a single phylum were put on a separate tree with a broad diversity of bacteria using an archaebacteria as an outgroup.

Nucleotide sequence accession numbers. The genBank nucleotide sequence accession numbers for sequences determined in these studies are as follows: for CR-FL1 to -6, -8 to -13, -15, -16, -18, -20 to -23, and –25 to –30, AF141387-AF141411; for CR-PA2, -6, -11, -13, -15, -16, -19 to -22, -24, -26, -27, -30, -36, -38, -40, -43, -44, -50, -53, -52, and -55, AF141412-AF141434; for CRE-FL1, -3, -4, -7, -8, -10, -11, -13, -14, -16, -18 to -26, -28, -31, -33, -35, -37 to -41, -43 to -47, -49, -50, -52 to -54, -56, -57, -59 to -64, -67 to -70, and -72 to –80, AF141435-AF141493; for CRE-PA2, -4, -6, -7, -9 to -11, -14 to -18, -21 to -27, -29, -30, -32, -34, -35, -37 to -42, -44, -45, -47, -49, -50 to -53, -58 to -60, -63, -64, -66, -69, -70, -72 to -80, and –82 to –89, AF41494-AF141556; for CRO-1, -2, -4, -6, -11, -13 to -19, -21, -22, -24, -27 to -29, and –31 to –35, AF141557-AF141579; for CRO-FL1 to -5, -7 to -18, and –22 to –26, AF141580-AF141601.

Results

Bacterial activity and RFLP analysis

Heterotrophic bacterial activity (^{3}H-thymidine incorporation rate) determined within one week of DNA sampling, one year, and two years prior to this study (Crump et al. 1998) are summarized in Table 3.2. Bacterial cell concentrations in the estuary were similar between particle-attached and free-living fractions, but the activity associated with the particle-attached bacteria was always greater than that associated with the free-living bacteria. Also, the overall bacterial activity in the estuary was greater than in the coastal ocean or the river.

RFLP patterns were used as an initial measure of diversity in the estuarine clone libraries. The goal was to sequence representatives from groups of clones with identical RFLP patterns. However, only 43 of 146 clones examined were part of groups with matching RFLP patterns, 25 of which were in one individual group. Representatives from three groups of clones with matching RFLP patterns were sequenced, and were found to be identical (CRE-FL4&7, CRE-PA2&16, and CRE-FL16&19). Based on this evidence 24 estuarine clones were catagorized by their RFLP patterns and were not sequenced (Table 3.3, Fig. 3.2).

All clone sequences from this study are presented on phylum- or sub-phylum-specific trees (Fig. 3.2), and listed by clone library (Table 3.3). Clones with sequences that could not be grouped with known phyla or subphyla were put on a tree with a diverse group of bacteria (Fig. 3.2I).

Riverine diversity

This study is the first to describe the planktonic bacterial community in a river using 16S rRNA clone libraries. Twenty-two of forty-eight clones from the two river clone

libraries were remarkably similar to sequences found in lakes in the Adirondack mountains, the Netherlands and Alaska, further confirming the existence of clades of "cosmopolitan" freshwater bacteria within the α–*Proteobacteria* , β–*Proteobacteria* , *Verrucomicrobiales* , and Gram Positive bacteria (Bahr et al. 1996, Hiorns et al. 1997, Methe et al. 1998, Zwart et al. 1998a, Zwart et ai. 1998b) (Figs. 3.2A,B,E&G). Twelve clones were related to soil isolates and clones, including TRS20 (γ–*Proteobacteria*), MC55 (*Planctomycete*), MC19 (Gram Positive), and clones related to the *Rhizobium-Agrobacterium* group (α-*Proteobacteria*) (Figs. 3.2A,C,E&G). Previously described "freshwater" bacteria clades *Rhizobium-Agrobacterium* and *Verrucomicrobiales* also include many isolates and clone sequences from soils. There is probably a close relationship and significant overlap in communities between soil and freshwater bacteria due in part to the interaction between the two environments.

The free-living and particle-attached clone libraries from the river contained clones from the same phyla and subphyla (Fig. 3.3B), but within these major groupings free-living and particle-attached clones rarely clustered together. For example, within the β-*Proteobacteria* clones related to *Polynucleobacter necessarius* were all free-living, but clones related to BAL47 were all particle-attached (Fig. 3.2B). Chloroplast clones were only found in the particle-attached fraction, probably because their phytoplankton hosts could not pass the 3-μm filter (Fig. 3.2H). Also, clones related to *Verrucomicrobiales spp.* were only found in the free-living fraction (Fig. 3.2E).

Coastal ocean diversity

Thirty-one of forty-five clones from the two coastal ocean libraries were closely related to coastal and marine clone sequences SAR11 and SAR7 (Sargasso Sea, Figs. 3.2A&H), OM42&81 (North Carolina coast, Fig 3.2A&H), OCS124&155 (Oregon coast,

Figs. 3.2A&G), BAL47 (Baltic Sea, Fig. 3.2B), and MED25 (Mediterranean Sea, Fig. 3.2F), and to marine isolates of *Prochlorococcus sp.* (Fig. 3.2H) and to NKB4 (deep sea sediment, Fig. 3.2C). Six clones, potentially of terrestrial origins, were related to the soil isolate *Planctomyces limnophilus*, to the plant pathogen *Pseudomonas syringae*, and to *Legionella sp.* (Figs. 3.2E&C).

Clones related to the marine organisms *Prochlorococcus*- and *Synechococcus* were very common in the coastal ocean clone library (Fig 3.2H). One cluster was very closely related to SAR7 (98.9-99.7%), an open ocean clone related to *Synechococcus sp.* The other cluster was most closely related to low light-adapted strains of *Prochlorococcus* (97.3-98.6%, MIT9303 & MIT9313), and less so to high light-adapted strains (95.1-96.4%, MIT9302 & MIT9312) (Moore et al. 1998).

The two coastal ocean clone libraries were very different (Table 3.3). The unfiltered coastal ocean clone library was dominated by Cyanobacteria (35%), Chloroplasts (22%) and γ–*Proteobacteria* (22%). The clone library made with 3-μm filtered water was dominated by α–*Proteobacteria* (52%) and contained only three clones that were related to clones from the unfiltered clone library.

Estuarine diversity

The free-living estuarine clone library was dominated by β–*Proteobacteria*, Gram Positive bacteria, α–*Proteobacteria*, *Cytophaga*, and one type of γ–*Proteobacteria* (Table 3.3). Twenty-one β–*Proteobacteria*, one *Verrucomicrobium* clone, and seven Gram Positive clones were related to clones from the river and belonged to clades of cosmopolitan freshwater bacteria or common soil bacteria (Fig. 3.2B,E,&G). Also, three α–*Proteobacteria* clones and two Gram Positive clones were related to clones found in

the coastal ocean clone library. A total of 48% of free-living estuarine clones were related to clones isolated from the river or the coastal ocean (Fig. 3.3B).

Of the remaining 52% of free-living estuarine clones, all γ–*Proteobacteria* clones, five *Cytophaga* clones, two *Verrucomicrobium* clones, and one δ–*Proteobacteria* clone (30%) had no relatives in the river or the ocean clone libraries, but were related to sequences in the particle-attached estuarine clone library (Fig. 3.2C,D,E&F). The remaining clones were unique to the free-living estuarine clone library.

The particle-attached estuarine clone library was dominated (75%) by clones that were rare or absent from the river or the coastal ocean including many clones related to *Cytophaga*, α–*Proteobacteria*, and a diverse assemblage of γ-*Proteobacteria*. Other particle-attached estuarine clones were related to clones in the particle-attached river library and the unfiltered coastal ocean library (Fig. 3.3B).

Discussion

Bacterial diversity in the Columbia River estuary appears to be influenced by the rapid movement of water through the system and the trapping of particles in ETM. Water masses entering the estuary from the river and the coastal ocean are mixed by tidal action and are then washed out of the estuary at the surface above the incoming layer of coastal marine water in an average of 1-2 d (Neal 1972) (Fig. 3.3A). The free-living bacterial communities associated with these water masses are also mixed by tidal action and presumably wash out of the estuary just as rapidly. Clones isolated from the river and the coastal ocean generally fell into distinct freshwater or marine phylogenetic clusters (Fig. 3.2), and were similar to organisms and environmental clones isolated from other freshwater and marine systems. Nearly half of the free-living clones from the estuary

were related to these freshwater and marine clones (Fig. 3.3B), demonstrating how this system acts as a mixing zone for bacterial communities, and suggesting that free-living bacteria wash into and out of the estuary too rapidly to develop into an estuarine community.

The movement of particles and particle-attached bacteria in the estuary is very different than the movement of water. Allochthanous particles can be trapped in ETM by attaching to other particles forming large, rapidly-settling "macro-aggregates" (D.J. Reed, pers. comm.). In the ETM, these particles settle to the bed at slack tide and are resuspended during flood and ebb tides. The formation of these particles and their cycling in the ETM brings together both allochthanous and estuarine particle-attached bacteria. The particle-attached estuarine clone library showed evidence of this mixing in that it contained river and coastal ocean clones as well as uniquely estuarine clones (Fig. 3.3B).

We hypothesized that ETM promote the development of an estuarine bacterial community by trapping particles in the estuary. Particles trapped in ETM are thought to remain there for 2 to 4 weeks (D.J. Reed pers. comm.), creating a relatively "stable" estuarine environment within this fast moving system on which estuarine organisms may have time to develop into a robust community. Estuarine clones unrelated to clones found in the river or the coastal ocean composed 75% of the particle-attached clone library (Fig. 3.3B), suggesting that the particle-attached fraction of bacteria in the ETM was composed of organisms that developed in the estuary.

Particle-attached bacteria play a critical role in the ecosystem of the Columbia River estuary due to their relatively high activity and their high concentration in ETM. They are the most important decomposers of organic matter in the system, turning over

particulate organic matter in an average of 8 to 71 days depending on conversion efficiency (Crump et al. 1998). They are also important in the estuarine food web as they are directly consumed by detritivorous copepods (Simenstad et al. 1994a), rotifers and protozoa (Crump & Baross 1996). We cannot say if the allochthanous particle-attached organisms remain active in the estuary. However, if organisms unique to the particle-attached estuarine clone library did not wash in from other sources, then they must have actively developed in the estuary.

Clones unique to the estuary were found in both the particle-attached and free-living fractions, making it unclear whether these organisms were originally free-living or particle-attached. However, the particle-attached fraction of bacteria had a much higher thymidine incorporation rate (Table 3.1) and extracellular enzyme activity (Crump et al. 1998) suggesting that uniquely estuarine organisms grew primarily on particles and were released into the free-living fraction *in situ* or perhaps during sample manipulation.

The largest groups of uniquely estuarine clones in the particle-attached fraction were related to *Cytophaga*, *α-Proteobacteria*, and *γ-Proteobacteria*. Environmental clones similar to *Cytophaga* and *γ-Proteobacteria* were also among the most abundant phylogenetic types found on marine snow particles in the Santa Barbara Channel (DeLong et al. 1993). All but four of the *Cytophaga-Flexibacter* clones were found in the estuary, and most were in the particle-attached fraction (Fig. 3.2F). *Cytophaga sp.* exhibit gliding motility and are therefore thought to live primarily on surfaces. They are also known for their abilities to produce exopolysaccharide slime and extracellular enzymes capable of degrading many different refractory biomacromolecules including cellulose and chitin (Reichenbach 1989). *Cytophaga* seem to be the ideal organisms to

thrive as particle-attached bacteria in the estuary, and may be one of the hallmark bacterial types for the Columbia River estuary.

The largest cluster of clones in both the particle-attached and the free-living estuarine clone libraries (17%) was most closely related to *Marinomonas vaga* (89.2-89.8%) and other members of the *Oceanospirillum* assemblage (Fig 3.2C). There are many oceanic environmental clone sequences from other studies that appear related to this assemblage, however most of these are only partial sequences that do not overlap with our sequences (NH16-1&18, NH29-6 & 17, NH49-13, BDA1-8 & -10). Most genera in the *Oceanospirillum* and *Alteromonas* assemblages require NaCl for growth (Krieg et al. 1984) but a subset can grow at the reduced salt concentrations typical of estuaries (Gauthier et al. 1992, Gonzalez et al. 1997). The sheer abundance of these clones in both estuarine libraries and the complete absence of them from river and coastal ocean libraries suggest that they are estuarine organisms.

The study of environmental clone libraries is starting to reveal the existance of environment-specific clades of microorganisms, such as the recently described clades of cosmopolitan freshwater bacteria. This suggests that 16S rRNA diversity may reflect metabolic diversity. Two clusters of α-*Proteobacteria* clones from this study provide examples of these environment-specific clades. Eleven clones were related to the *Rhodobacter* (Fig. 3.2A), a group that includes two clusters of phylogenetically distinct organisms from marine and freshwater environments (Hiraishi & Ueda 1994). Members of the marine *Rhodobacter* group were recently shown to dominate coastal bacterioplankton communities, accounting for 28% of the rDNA genes in coastal ocean water collected off Sapelo Island, Georgia, USA (Gonzalez & Moran 1997, Rappé et al.

1997). Members of the freshwater group have not been previously identified in environmental clone libraries, but are known from cultured organisms.

Three groups of clones belonging to the SAR11 cluster (Giovannoni et al. 1990, Mullins et al. 1995, Field et al. 1997) were identified, two marine and one freshwater. One clone collected in the Columbia River (CR-FL10), had a 100% sequence identity to LD12, a clone sequence collected from Lake Loosdrecht, The Netherlands. This clone is also closely related to lake clones ACK-M20, ARC22 and others that compose the freshwater SAR11 cluster. Marine clones from this cluster were very abundant in the coastal ocean clone library, and were also found in the Columbia River estuary.

Phenotypic capabilities cannot be determined directly from 16S sequences, but information about the environment and about related organisms in cultivation provide clues to the potential phenotypes of environmental clones. Some clusters of particle-attached clones from the Columbia River estuary were closely related to cultivated organisms with characteristics conducive to life on particles in ETM. Cultivated *Rhodobacter sp.* can grow aerobically and anaerobically, and many display some degree of halotolerence or osmotolerence (Hansen & Imhoff 1985, Imhoff 1988, Abee et al. 1990, Igeno et al. 1995). δ-*Proteobacteria sp.* include obligately anaerobic sulfate reducers, and may grow in low oxygen regions of ETM particles. *Cytophaga sp.*, as described earlier, are surface-associated bacteria known to produce exopolysaccharides as part of biofilm formation and to release extracellular enzymes for the degradation of particulate organic matter. It is reasonable to hypothesize that clones related to these cultivated organisms share some of the same phenotypic capabilities. Bacteria with these known phenotypes probably compose the most active fraction of bacteria on particles, and may be an important component of the estuarine food web.

Table 3.1. Bases used for phylogenetic analysis using the E. coli numbering system

Major grouping	Range of bases used for analyses	Bases omitted
α-Proteobacteria	32-479	71-98
β-Proteobacteria	33-514	76-93
γ-Proteobacteria	50-494	76-93, 199-220
δ-Proteobacteria	28-519	76-93, 184-193
Gram Positive bacteria	37-508	71-97, 450-479
Cytophaga-Flavobacteria	73-513	none
Verrucomicrobiales & *Planctomyces*	37-519	71-97, 184-193, 453-477
Cyanobacteria & Chloroplasts	94-451	none
Unknown	50-519	69-99, 184-220, 452-480

Table 3.2. Bacteria abundance and thymidine incorporation rate in samples collected in the Spring from the Columbia River, its estuary, and the adjacent coastal ocean. "n" indicates number of samples.

Sample location and site	Bacteria abundance ($x10^9$ l^{-1})			Thymidine incorporation rate (pmol l^{-1} h^{-1})		
	mean	range	*n*	mean	range	*n*
Columbia River						
1997 free-living				0.4	0.2-1.0	4
particle-attached				1.3	0.9-2.1	4
1996 free-living				0.9	0.8-1.0	2
particle-attached				13.7	11.5-15.8	2
1995 free-living				0.3	0.3-0.3	2
particle-attached				2.2	2.0-2.5	2
Columbia River Estuary						
1997 free-living	1.8	0.9-3.8	10	1.0	0.1-2.6	35
particle-attached	2.4	0.7-5.1	10	7.7	0.3-31.7	35
1996 free-living				8.9	0.2-37.3	20
particle-attached				57.4	12.8-104.4	20
1995 free-living	1.4	0.7-2.6	56	3.1	0.2-7.7	54
particle-attached	3.3	0.1-12.6	56	38.4	12.0-90.1	54
Coastal Ocean						
1997 free-living				0.5	0.2-1.0	2
particle-attached				1.3	1.2-1.7	2

Table 3.3. Clone sequences from each clone library listed with phylum affiliation, nearest neighbor from the global database, percent similarity based on alignable base pairs, and grouping within each phylum.

Sample location and type	Category	Clone no.a	Nearest neighbor	% similarity	Assemblage
Coastal Ocean, Free-living					
	α-Proteobacteria	CRO-FL4, -10, -15, -23	OCS12	97.6-99.7%	SAR11
		CRO-FL1, -3, -7, -12, -26	OCS154	96.3-98.4%	SAR11
		CRO-FL11	OCS124	99.7%	OCS124
		CRO-FL5, -16	?[d]	?	?
	β-Proteobacteria	CRO-FL2	BAL47	98.1%	*Rubrivivax* spp.
		CRO-FL17	*Hydrogenophaga flava*	93.7%	*Rubrivivax* spp.
		CRO-FL25	?	?	?
	γ-Proteobacteria	CRO-FL8	NKB4	90.5%	
	Gram Positive	CRO-FL9, -14	ACK-M1	94.2, 94.9%	ACK-4
		CRO-FL22	OCS155	99.8%	OM1
	Cyanobacteria	CRO-FL18, -24	*Prochlorococcus* sp. strain MIT9303	98.6, 97.8%	*Prochlorococcus* spp.
		CRO-FL13	SAR7	99.5%	SAR7
Coastal Ocean, Unfiltered					
	α-Proteobacteria	CRO-1	OM42	98.9%	Marine *Rhodobacter* spp.
	γ-Proteobacteria	CRO-2, -21	*Legionella lytica*	95.1, 94.7%	*Legionella* spp.
		CRO-33	*Legionella feeleii*	94.7%	*Legionella* spp.
		CRO-14, -19	*Pseudomonas syringae*	99.4%	*Pseudomonas* spp.
	CFB[b]	CRO-4	MED25	92.2%	*Cytophaga* spp.
	Planctomyces spp.	CRO-13	*Planctomyces limnophilus*	98.5%	*Planctomyces limnophilus*
	Cyanobacteria	CRO-15, -34	*Prochlorococcus* sp. strain MIT9303	98.1, 97.3%	*Prochlorococcus* spp.
		CRO-16, -24, -27, -29, -31, -35	SAR7	98.9-99.7%	SAR7
	Chloroplasts	CRO-11, -22, -28, -32	OM81	91.0%	*Chrysophyceae* spp.
		CRO-17	OM81	87.5-88.0%	*Chrysophyceae* spp.
	Unknown	CRO-6	?	?	?
		CRO-18	?	?	?
Columbia River, Free-living					
	α-Proteobacteria	CR-FL10	LD12	100.0%	Freshwater SAR11
		CR-FL11	soil clone (AF010012)	96.6%	*Rhizobium-Agrobacterium*
	β-Proteobacteria	CR-FL2, -6, -9	BAL47	96.3-96.5%	*Rubrivivax* spp.
		CR-FL8[c]	MT11	94.6%	*Rubrivivax* spp.
		CR-FL13, -22	LD17	97.0,96.7%	*Polynucleobacter necessarius*
		CR-FL23	ACK-L6	96.1%	*Polynucleobacter necessarius*
		CR-FL21	ACK-C30	99.8%	*Methylophilus* spp.
	γ-Proteobacteria	CR-FL28	*Vibrio marinus*	92.9%	?
		CR-FL29	*Pseudomonas* sp. clone (U63942)	95.8%	*Pseudomonas* spp.
	Gram positive	CR-FL16, -18	MC19	84.2, 86.3%	CR-FL16
		CR-FL3, -20	ACK-M1	88.6, 91.1%	ACK-4
		CR-FL30	*Agrococcus jenensis*	86.8%	?
	CFB	CR-FL26	*Capnocytophaga gingivalis*	85.4%	*Cytophaga* spp.
		CR-FL12	?	?	?
	Planctomyces spp.	CR-FL15	MC55	88.7%	*Isophaera* spp.
	Verrucomicrobiales	CR-FL1, -25, -27	VeSm13	86.8-87.6%	*Verrucomicrobiales*
		CR-FL5	MC18	93.3%	*Verrucomicrobiales*
	Unknown	CR-FL4	?	?	?
Columbia River, Particle-attached					
	α-Proteobacteria	CR-PA55	*Rhodobacter sphaeroides*	94.2%	Freshwater *Rhodobacter* spp.
		CR-PA22	*Beijerinckia indica*	95.3%	*Rhizobium-Agrobacterium*
		CR-PA53	MHP17	93.9%	*Rhizobium-Agrobacterium*
	β-Proteobacteria	CR-PA6, -11	*Rhodoferax fermentans*	98.3, 96.3%	*Rubrivivax* spp.

Continued on following page

Table 3.3. Continued

Sample location and type	Category	Clone no.a	Nearest neighbor	% similarity	Assemblage
		CR-PA24	*Alcaligenes denitrificans*	92.5%	*Bordetella* spp.
		CR-PA50	*Ralstonia pickettii*	99.3%	*Ralstonia* spp.
	γ-Proteobacteria	CR-PA40	*Methylobacter* sp. strain BB5.1	96.2%	*Methylomonas* spp.
		CR-PA44	*Legionella feeleii*	96.0%	*Legionella* spp.
		CR-PA27	TRS20	88.2%	?
	Gram positive	CR-PA52	MC19	86.5%	CR-FL16
		CR-PA21, -26, -38	MC19	86.1-87.3%	CR-FL16
		CR-PA36	ACK-M1	94.7%	ACK-4
		CR-PA13	OPB90	83.0%	?
	CFB	CR-PA19	soil clone C125 (AF013539)	93.4%	*Saprospira* spp.
	Planctomyces spp.	CR-PA16	MC55	88.7%	*Isophaera* spp.
	Chloroplast	CR-PA2, -20	Chloroplast (*Skeletonema pseudocostatum*)	98.6, 98.4%	*Bacillariophyta*
		CR-PA30, -43	Hstpl4	98.1, 97.8%	*Bacillariophyta*
	Unknown	CR-PA15	?	?	?
Columbia River estuary, Free-living					
	α-Proteobacteria	CRE-FL64	OM42	99.5%	Marine *Rhodobacter* spp.
		CRE-FL23	MED26	97.4%	Marine *Rhodobacter* spp.
		CRE-FL63	OCS12	98.7%	SAR11
		CRE-FL21	*Sphingomonas adhaesiva*	95.3%	*Sphingomonas*
		CRE-FL20	?		?
		CRE-FL1	?		?
	β-Proteobacteria	CRE-FL38, -49	BAL47	98.1%	*Rubrivivax* spp.
		CRE-FL16, -19, (-2, -65)	BAL47	95.5%	*Rubrivivax* spp.
		CRE-FL37c	MT11	95.0%	*Rubrivivax* spp.
		CRE-FL62	*Aquaspirillum delicatum*	92.0%	*Rubrivivax* spp.
		CRE-FL35, -41, -50	*Rhodoferax fermentans*	96.1-97.2%	*Rubrivivax* spp.
		CRE-FL14, -26, -79	ACK-L5	98.9-99.6%	*Polynucleobacter necessarius*
		CRE-FL11	LD17	97.0%	*Polynucleobacter necessarius*
		CRE-FL45, -78	ACK-L6	96.5, 96.7%	*Polynucleobacter necessarius*
		CRE-FL73	ACK-C30	100.0%	*Methylophilus* spp.
		(CRE-FL15)	ACK-C30	(98.1%)	*Methylophilus* spp.
		CRE-FL40	ACK-C30	95.0%	*Methylophilus* spp.
		CRE-FL44, -56, (-58)	*Alcaligenes denitrificans*	93.1, 92.2%	*Bordetella* spp.
		CRE-FL33	*Ralstonia pickettii*	98.9%	*Ralstonia* spp.
		CRE-FL22	*Ultramicrobacterium* sp. strain ND5	95.7%	?
		CRE-FL68	*Gallionella ferruginea*	95.5%	*Gallionella* spp.
	γ-Proteobacteria	CRE-FL4, -7, -61, -76, -77, -80, (-6, -9, -29, -30, -32, -34, -36, -42)	*Marinomonas vaga*	89.2-89.8%	*Oceanospirillum* spp.
		CRE-FL8	*Marinomonas aquaeolei*	88.6%	*Oceanospirillum* spp.
	δ-Proteobacteria	CRE-FL54	*Desulfosarcina variabilis*	93.1%	*Desulfobacter* spp.
	Gram positive	CRE-FL67	MC19	84.9%	CR-FL16
		CRE-FL47, -53	MC19	86.8, 85.8%	CR-FL16
		CRE-FL18, -70, (-66)	ACK-M1	90.8, 90.6%	ACK-4
		CRE-FL13	ACK-M1	88.6%	ACK-4
		CRE-FL43, -60	*Agrococcus jenensis*	93.3,94.2%	?
		CRE-FL10, -72	OCS155	99.5,99.8%	OM1
	CFB	CRE-FL46	sea ice psychrophile (U85888)	93.4%	*Cytophaga* spp.
		CRE-FL24, -25	TBS22	96.1,93.1%	*Cytophaga* spp.
		CRE-FL57	OM271	94.5%	*Cytophaga* spp.
		CRE-FL75	*Psychroserpens burtonensis*	88.1%	*Cytophaga* spp.
		(CRE-FL17)	SCB37	(93.1%)	*Cytophaga* spp.
		CRE-FL3, -39	*Flectobacillus major*	86.2, 86.4%	*Flexibacter flexilis*
	Verrucomicrobiales	CRE-FL31	TM18	88.5%	*Verrucomicrobiales*
		CRE-FL59	LD29	87.3%	*Verrucomicrobiales*

Continued on following page

Table 3.3. Continued

Sample location and type	Category	Clone no.a	Nearest neighbor	% similarity	Assemblage
		CRE-FL74	*Verrucomicrobium spinosum*	85.0%	*Verrucomicrobiales*
	Chloroplast	CRE-FL52	OM20	90.5%	*Bacillariophyta*
	Unknown	CRE-FL28	?	?	?
		CRE-FL69	?	?	?
Columbia River estuary, Particle-attached					
	α-Proteobacteria	CRE-PA76, -77	KAT10	95.0%	Marine *Rhodobacter* spp.
		CRE-PA4, -47, -80, -89	*Rhodobacter capsulatus* strain ATH	97.4-98.4%	Freshwater *Rhodobacter* spp.
		CRE-PA51	BAL27	95.0%	Freshwater *Rhodobacter* spp.
		CRE-PA70	OM188	100.0%	SAR11
		CRE-PA52, -53	*Blastobacter natatorius*	97.1%	*Sphingomonas* spp.
	β-Proteobacteria	CRE-PA69	*Rubrivivax gelatinosus*	93.3%	*Rubrivivax* spp.
		(CRE-PA65)	*Rhodoferax fermentans*	(96.1%)	*Rubrivivax* spp.
		CRE-PA22	?	?	?
		CRE-PA84	?	?	?
		CRE-PA45	ACK-C30	98.1%	*Methylophilus* spp.
	γ-Proteobacteria	CRE-PA2, -16, -49, -86, -87, -88 (-5, -8, -20, -33, -48)	*Marinomonas vaga*	89.2-89.8%	*Oceanospirillum* spp.
		CRE-PA40	OM23	97.9%	*Oceanospirillum* spp.
		CRE-PA14, -50	Symbiont (hydrothermal vent mussel)	92.3, 93.4%	*Thiothrix nivea*
		CRE-PA25	NKB4	88.2%	?
		CRE-PA78	OM60	92.8%	?
		CRE-PA17	TBS23	89.0%	?
		CRE-PA9	*Methylococcus capsulatus*	87.8%	*Methylomonas* spp.
		CRE-PA58, -74	TRS20	88.2, 89.4%	?
		CRE-PA35	*Xanthomonas vesicatoria*	92.2%	*Xanthomonas* spp.
	δ-Proteobacteria	CRE-PA6, -66	*Desulfurhopalus vacuolatus*	91.8, 93.1%	*Desulfobacter* spp.
		CRE-PA18	*Desulfovibrio sp.* str. STL6	94.5%	*Desulfovibrio* spp.
	Gram positive	CRE-PA41	MC19	87.3%	CR-FL16
		CRE-PA39	OCS155	99.5%	OM1
		CRE-PA63, (-67, -81)	OPB90	83.0%	?
		CRE-PA72	*Spiroplasma* sp. strain Y32	83.2%	Low G+C
		CRE-PA64	MB2424	88.5%	Low G+C
	CFB	CRE-PA32	BAL13	93.6%	*Cytophaga* spp.
		CRE-PA38	sea ice psychrophile (U85888)	93.4%	*Cytophaga* spp.
		CRE-PA10, -79, (-43)	MED25	92.0%	*Cytophaga* spp.
		CRE-PA44	MED18	92.5%	*Cytophaga* spp.
		CRE-PA37	SCB37	93.1%	*Cytophaga* spp.
		CRE-PA11, -15, -85	*Psychroserpens burtonensis*	93.0%	*Cytophaga* spp.
		CRE-PA7	*Flectobacillus major*	86.7%	*Flexibacter flexilis*
		CRE-PA83	soil clone C125	89.8%	*Saprospira* spp.
		CRE-PA30	?	?	?
		CRE-PA75	?	?	?
		CRE-PA82	?	?	?
	Planctomyces spp.	CRE-PA34	MC100	94.6%	*Planctomyces limnophilus*
	Verrucomicrobiales	CRE-PA23	LD29	87.9%	*Verrucomicrobiales*
		CRE-PA73	MC18	92.2%	*Verrucomicrobiales*
		CRE-PA29	?	?	*Verrucomicrobiales*
	Chloroplast	CRE-PA60	Chloroplast *(Skeletonema pseudocostatum)*	98.4%	*Bacillariophyta*
		CRE-PA42	Hstpl4	96.5%	*Bacillariophyta*
		CRE-PA59, (-19)	AGG56	97.3%	*Bacillariophyta*
		CRE-PA21	OM81	87.7%	*Chrysophyceae*
		CRE-PA27	*Chlorella sacharophila*	91.5%	Green Plant Chloroplasts
	Unknown	CRE-PA24, -26	?	?	?

[a] Clone numbers in parentheses were catagorized by RFLP pattern

[b] CFB, Cytophaga-Flexibacter-Bacteroides

[c] clone that is most closely related to known contaminants from a negative control library (43).

[d] ?, nearest neighbor could not be determined

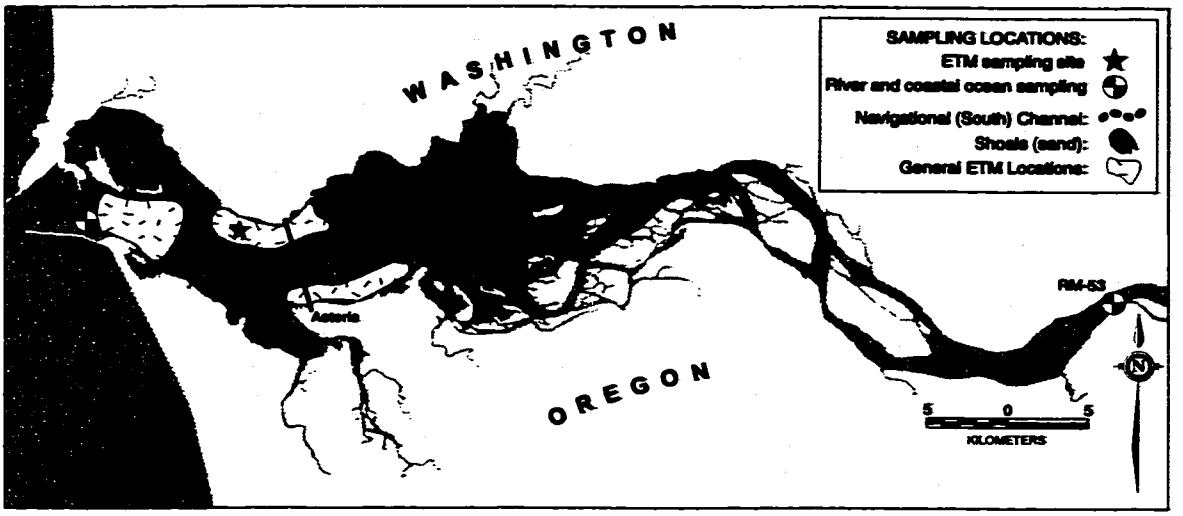


Fig. 3.1. The Columbia river estuary with sampling sites

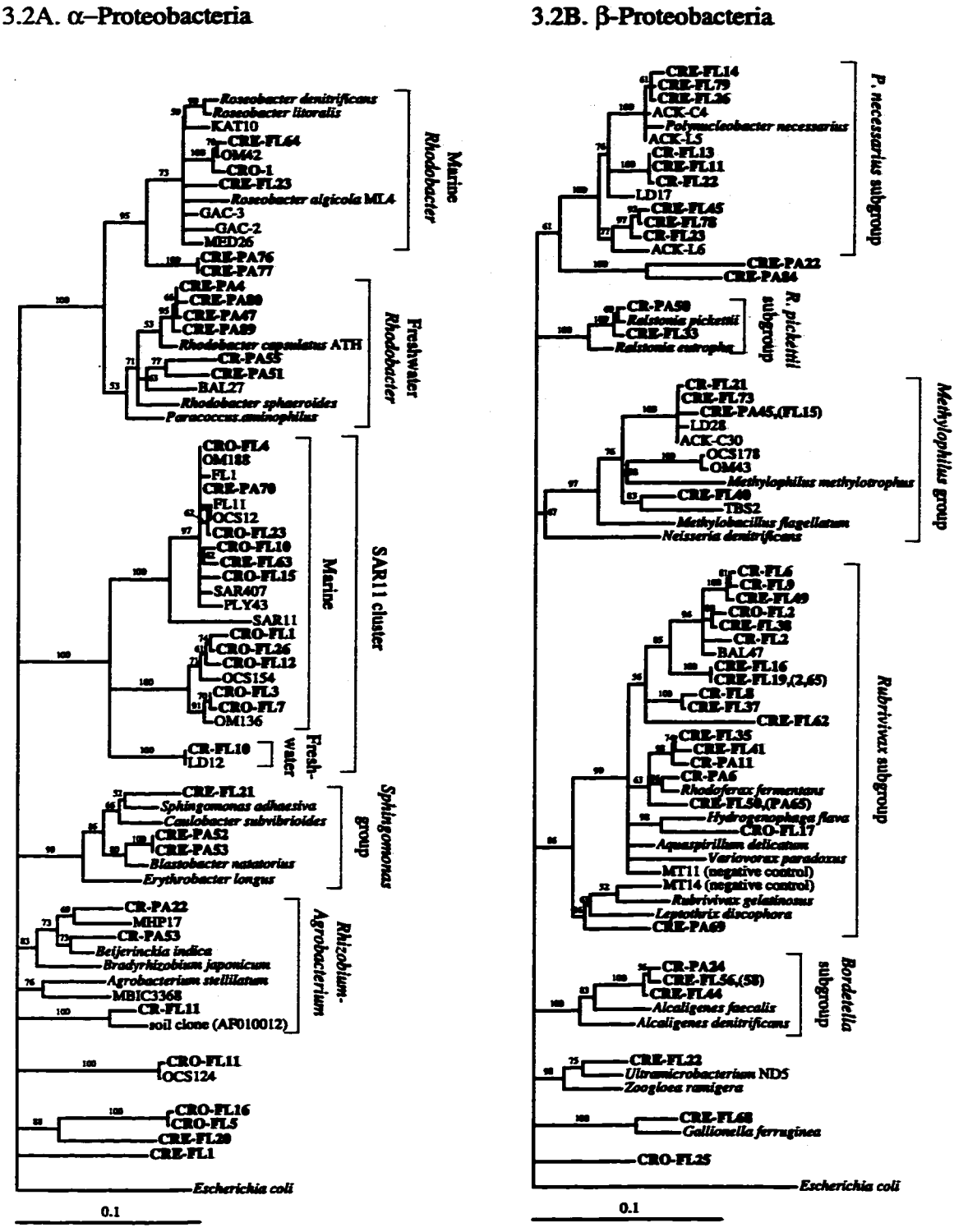


FIG. 3.2. Phylogenetic relationships among 16S rRNA sequences from Columbia River, estuary and adjacent coastal ocean clones and from other environmental clones and cultured organisms. (A) α-proteobacteria; (B) β-proteobacteria; (C) γ-proteobacteria; (D) δ-proteobacteria; (E) *Verrucomicrobiales* and *Planctomyces* clade; (F) *Cytophaga-Flexibacter* assemblage; (G) gram positive bacteria; (H) chloroplasts and cyanobacteria; (I) all other clones. (continued next page)

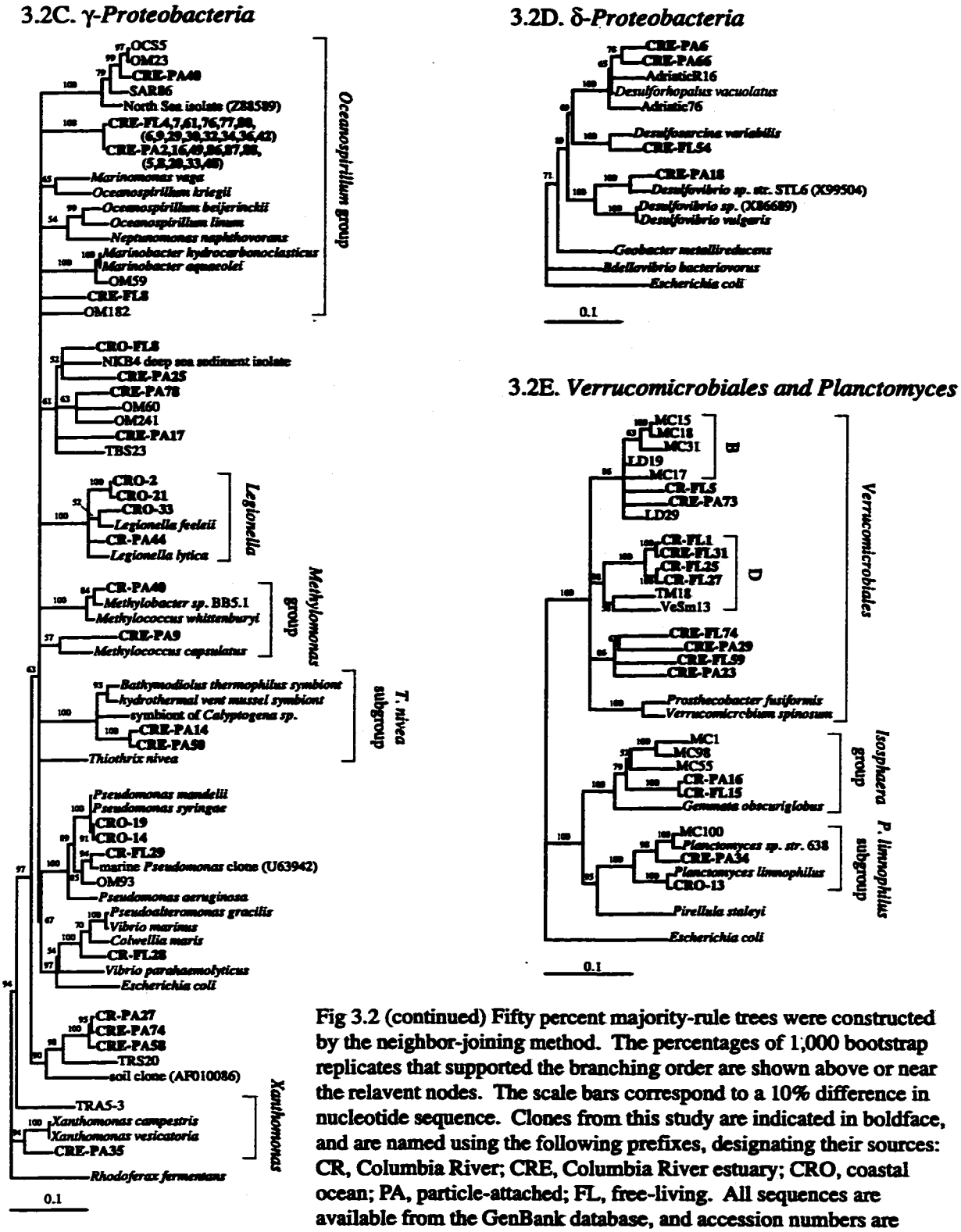
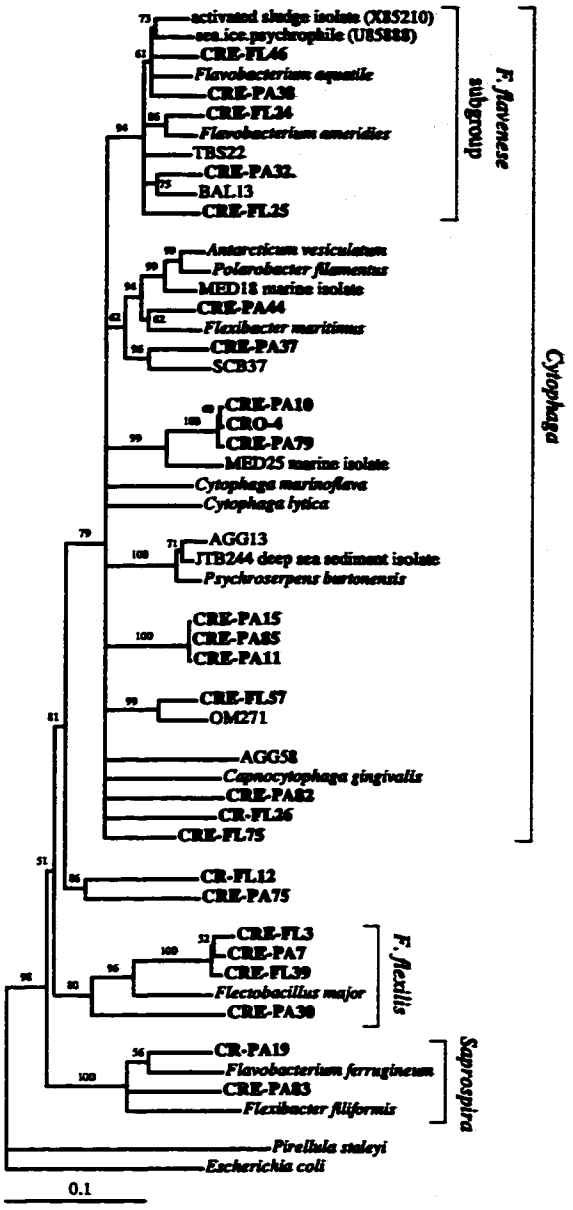


Fig 3.2 (continued) Fifty percent majority-rule trees were constructed by the neighbor-joining method. The percentages of 1,000 bootstrap replicates that supported the branching order are shown above or near the relavent nodes. The scale bars correspond to a 10% difference in nucleotide sequence. Clones from this study are indicated in boldface, and are named using the following prefixes, designating their sources: CR, Columbia River; CRE, Columbia River estuary; CRO, coastal ocean; PA, particle-attached; FL, free-living. All sequences are available from the GenBank database, and accession numbers are provided if the organism or clone name is not unique. CFB, *Cytophaga-Flexibacter-Bacteroides* Phylum.

3.2F. *Cytophaga-Flexibacter*



3.2G. Gram Positive

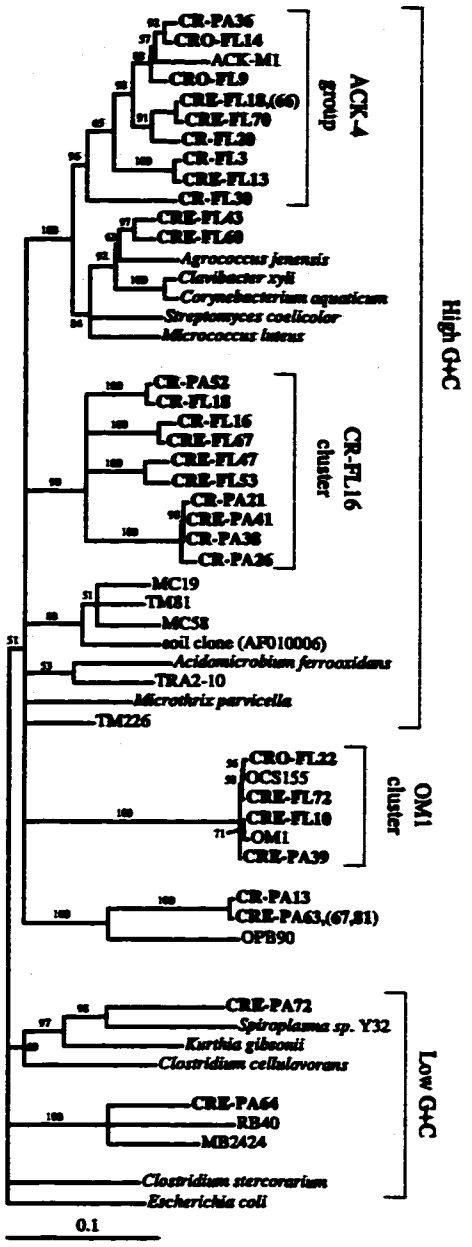
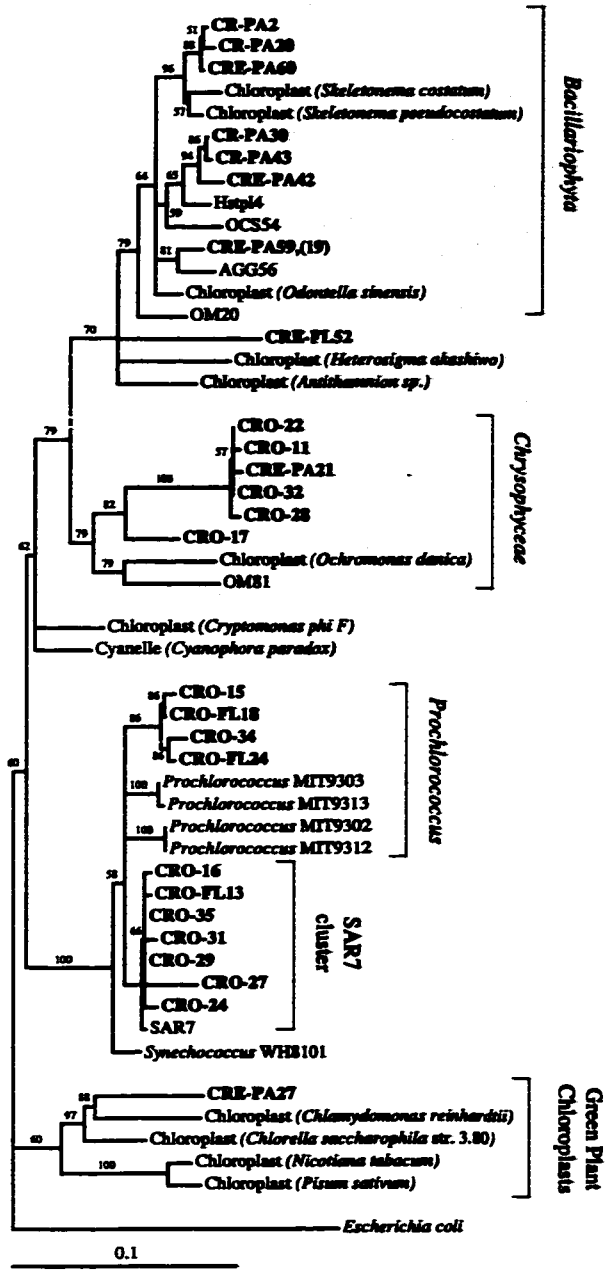


Fig. 3.2. Continued

3.2H. Chloroplasts and Cyanobacteria



3.2I. Unknown

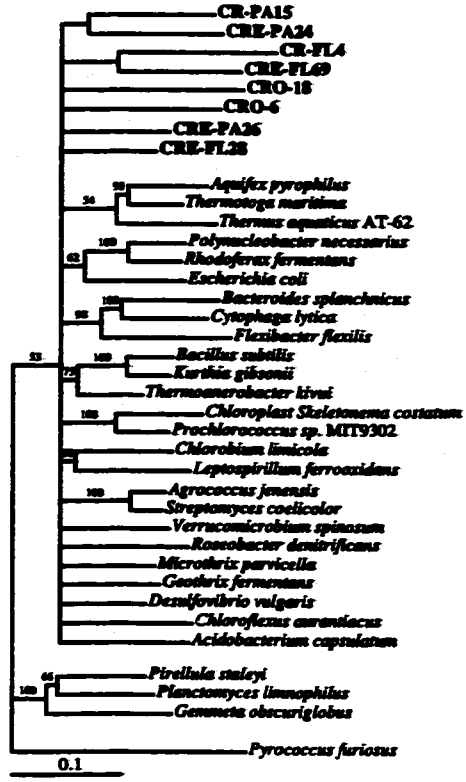


Fig. 3.2. Continued

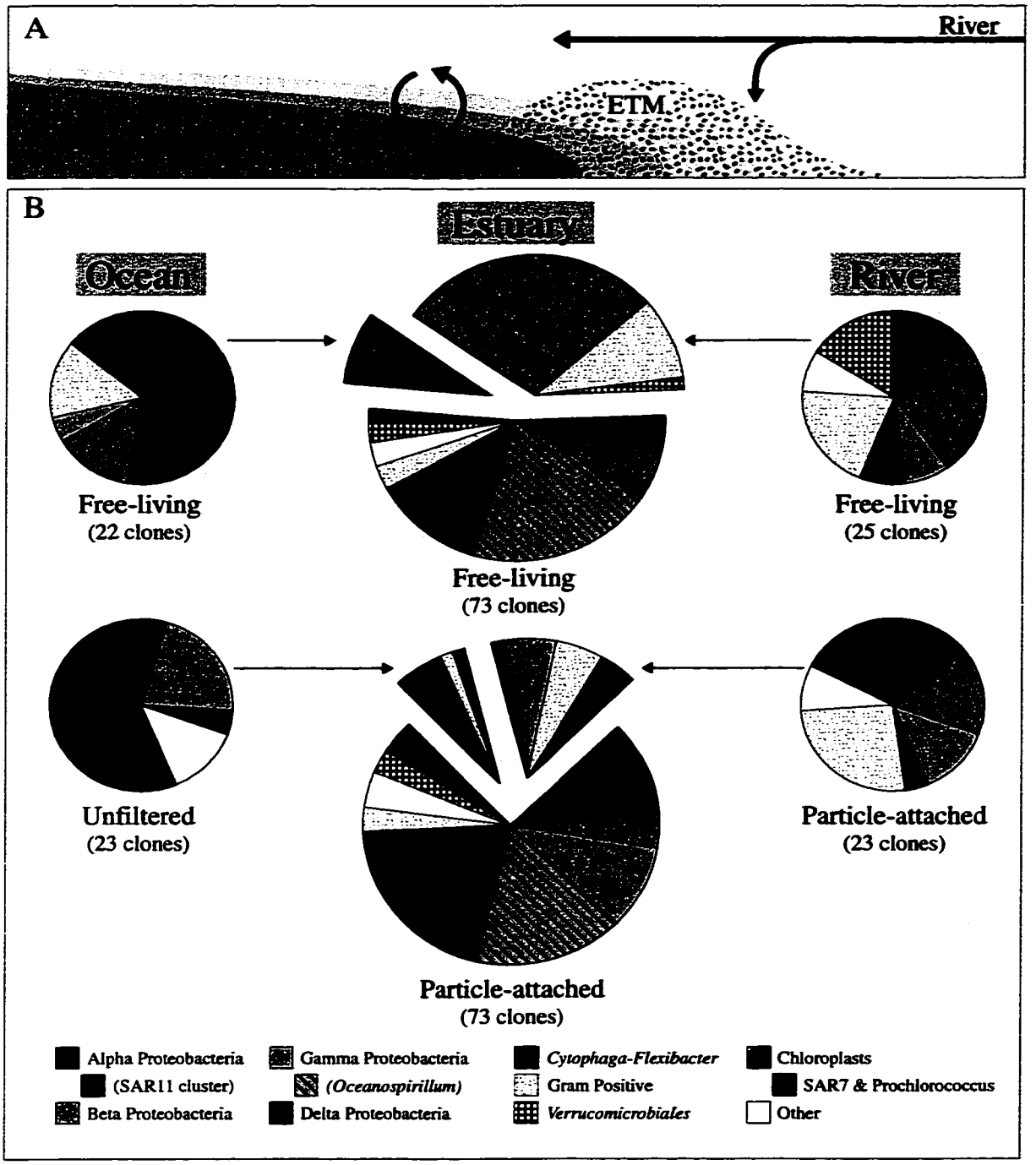


FIG. 3.3. (A) Longitudinal cross section of an ETM region of the Columbia River estuary showing inputs of river and coastal ocean water and particles, and the location of the ETM at the head of the salt wedge. Curved arrows indicate mixing of fresh water (white) and marine water (dark gray). (B) Composition of clone libraries at the phylum and sub-phylum level. Arrows show movement of bacterial types from source populations into the estuary. Estuarine clone libraries are separated into clones unique to the estuary (bottom section of free-living and particle-attached charts), clones similar to those found in the river (upper right sections) and clones similar to those found in the coastal ocean (upper left sections). Estuarine clones were designated river or coastal ocean when they clustered with clones from these source communities. Most had at least 96% sequence similarity to river and coastal ocean clones.

Chapter 4. Phylogeny of Archaeal Communities

Abstract

PCR-amplified 16S rRNA genes from particle-attached and free-living archaea in the Columbia River estuary, particle-attached archaea in the river, and archaea in the adjacent coastal ocean were cloned, and 43 partial sequences were determined. There was a high diversity of archaea in the estuary, especially among the particle-attached archaea, with representatives from 4 major phylogenetic clusters. Eighteen of 21 estuarine clones were closely related to clones from the river and the coastal ocean or to clusters of marine and soil clones identified in other studies. This result contrasts with a similar study of the estuarine bacterial community presented in Chapter 3 of this dissertation that found 62% of bacterial 16S rRNA clones to be unique to the estuary. Archaea in the estuary were primarily allochthonous, and therefore, unlike the bacteria, probably do not form a native estuarine community.

Introduction

Archaeal diversity in non-extreme habitats (moderate temperature, salinity, pH) measured by sequencing 16S rRNA genes is generally restricted to one or two phylogenetic clusters per environment. These uncultivated archaea tend to form phylogenetic clusters that are specific to individual environment types (Buckley et al. 1998) including soils (Jurgens 1996, Bintrim et al. 1997), freshwater and marine sediments (MacGregor et al. 1997, Munson et al. 1997, Vetriani et al. 1998), and marine plankton (DeLong 1992, Fuhrman et al. 1992). The majority of archaeal 16S rRNA sequences isolated from these environments are only distantly related to those of

cultivated archaea, and are often grouped with the thermophilic Crenarchaea, making it difficult to infer their metabolic capabilities.

Ecosystems that lie at the interface between different environments should contain bacteria and archaea from each source environment resulting in an enhanced local diversity. This has been suggested for a Yellowstone hot spring pool, which contained archaeal 16S rRNA genes related to the high temperature crenarchaeota and to archaea recovered from moderate temperature freshwater sediments. (Barnes et al. 1994, Buckley et al. 1998). Likewise, estuaries are located at the interface between freshwater and marine water systems and may, in some cases, receive inputs of archaea from soils, resuspended sediments and mudflats. Phylogenetic analysis of the bacteria in the Columbia River estuary revealed a remarkable diversity that resulted from the combination of native estuarine bacteria with bacteria from the river and the coastal ocean (Crump et al. 1999). This study also identified a group of bacteria that were only found associated with estuarine particles. We hypothesize that the archaeal community in the Columbia River estuary is composed of an equally diverse group of organisms, and that these archaea can be traced to their origins by analyzing communities in the river and the coastal ocean. Furthermore, analysis of these communities may reveal archaeal phylotypes specific to estuarine particles.

Materials & Methods

Clone library construction. DNA samples used for the preparation of archaeal clone library construction were the same as those used in Chapter 3. PCR was run in 2-7 separate 100 µl reactions for each DNA sample using "universal archaeal" primer 21f (5'-TTC CGG TTG ATC CYG CCG GA-3') and "universal archaeal" primer 958r (5'-YCC

GGC GTT GAM TCC AAT T-3') (2.5 mM MgCl, 0.8 mM dNTPs, 1 ng μl^{-1} of each primer, 2.5 U *Taq* DNA polymerase (Promega), 1X PCR buffer (Promega)). PCR amplification began with a 5 min denaturation at 94°C followed by 32-35 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 2 min. The final cycle was extended at 72°C for 5 min. PCR products were combined, concentrated and purified with Qiaquick PCR purification columns (Qiagen) following manufacturer's instructions.

PCR products were either ligated into pGEM-T cloning vectors (Promega) and used to transform JM109 competent cells (Promega) or were topoisomerized into TOPO-TA cloning vectors (Invitrogen) and used to transform TOP10 cells (Invitrogen). All cloning followed manufacturers' instructions. Positive colonies were picked and stored on plates and frozen in liquid media at -70°C.

The free-living fraction of the river sample did not amplify with archaeal primers, and so no clone library was constructed.

Sequencing. The inserts in 10 or 11 randomly selected clones from each library were sequenced. Inserts were PCR amplified with vector-specific primers, purified using Qiaquick PCR purification columns, sequenced bidirectionally using primers 21f (as above) and 519r (5'-GWA TTA CCG CGG CKG CTG-3') with the PRISM bigdye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.), and resolved on a Model 377 automated DNA sequencer (Applied Biosystems Inc.).

Phylogenetic analysis. Methods used for phylogenetic analyses were identical methods to those used in Chapter 3. Some sections of the sequences could not be aligned (*E. coli* base positions 68-100, 198-119, and 408-433) and were therefore removed from further analysis.

Results & Discussion

Estuaries can host a great amount of biological diversity because of their position at the interface between terrestrial and marine environments. Estuarine microbial communities are composed of organisms originating from other ecosystems such as rivers, coastal ocean waters, salt marshes, and mudflats, not all of which are active in the estuaries (Crump et al. 1998). Recent work on the Columbia River estuary revealed a very high diversity of planktonic bacteria resulting from the combination of at least three different bacterial communities: riverine, oceanic, and native estuarine (Crump et al. 1999). Archaeal diversity in the estuary followed a similar pattern, and included clone sequences closely related to microbes found in marine plankton, freshwater plankton, soils, and sediments (Fig. 4.1).

All 11 free-living estuarine archaeal clones were closely related to clones isolated from the river or the coastal ocean, and fell into the two major clusters of archaea originally identified as the two principle clusters of marine archaeaplankton (Group 1 and 2). Those related to Group 1 archaea (Fig 4.1C) separated into a marine cluster which included coastal ocean clones from this study and marine clones from other studies, and a freshwater cluster which included river clones from this study and clones isolated from freshwater Lake Michigan sediments (MacGregor et al. 1997). Those related to the Group 2 archaea (Fig. 4.1A) were very similar to a coastal ocean clone from this study or to a marine clone from the East Coast of USA (WHARN). It is likely that these archaea washed into the estuary from the river and the coastal ocean.

The particle-attached estuarine clones were much more diverse, falling into four major clusters of archaea. Seven of the ten clones were related to Group 1, Group 2 and the so-called "terrestrial" clusters, and were very closely related to marine or freshwater

clones (Figs. 4.1A & C). Three clones (CRE-PA2a, -7a and -10a), however, were not closely related to any specific clone sequence (<94% sequence similarity), but clustered with a diverse group of freshwater sediment and plankton clones that appear related to the thermophilic crenarchaea (Fig. 4.1B). These clones cannot be directly traced to the river or the coastal ocean, and may represent archaeal populations developed in the estuary. However, the three estuarine archaeal clones were not closely related to each other (maximum sequence similarity = 87.8%) and therefore did not form a conclusive "estuarine cluster" as was found among the bacteria (Crump et al. 1999).

Thus, most archaeal clones from the estuary appear to have arrived there from the river and the coastal ocean (Fig. 4.2). This is in contrast with bacteria from the estuary, many of which were different than those in the river or the coastal ocean. These uniquely estuarine bacteria were thought to constitute the actively growing fraction of the bacterial community. The absence of a similar community of estuarine archaea suggests that archaea cannot grow in the estuary, or grow too slowly to establish an estuarine community.

It was surprising to find that four of the coastal ocean clones in the "terrestrial" cluster (CRO-4a, -5a, -6a, -11a) were very similar to two river clones (CR-PA1a, -13a)(Fig 4.1C). In a similar study of bacterial clones from the Columbia River, its estuary and the adjacent coastal ocean, only once were there identical clones found in the river and the coastal ocean among 48 river and 44 coastal ocean clones (Crump et al. 1999). The coastal ocean sample was collected at the mouth of the estuary near the end of a flood tide in order to minimize the influence of river water on the sample. So it is unlikely that these clones originated from the river and were mixed into coastal ocean water off-shore.

A recent survey tentatively separated a large number of archaeal 16S rRNA sequences into broad environment-specific clusters including a diverse "freshwater" cluster of sediment clones, a "terrestrial" cluster of soil and freshwater sediment clones, and a "marine" cluster (Group 1)(Buckley et al. 1998)(Fig. 4.1). There is also a second "marine" cluster (Group 2) of planktonic clones related to the Euryarchaeota (DeLong 1992, Fuhrman et al. 1992)(Fig 4.1A). All 22 clones from the Columbia River and the coastal ocean fell into one of these four clusters, and 13 were closely related to groups of clones isolated from similar environments. However, 7 coastal ocean clones were related to the "terrestrial" cluster of environmental clones (Fig. 4.1C) perhaps indicating that this cluster extends beyond the terrestrial environment. The Group 1 "marine" archaeal cluster (Fig 4.1C), known to include a number of freshwater sediment clones from Lake Michigan, also separated into distinct freshwater and marine sub-clusters (Fig. 4.1C). Moreover, the "freshwater" cluster of sediment clones was recently shown to include marine clones from deep-sea sediments (Vetriani et al. 1998). Interestingly, the same is true for the SAR11 cluster of uncultivated bacteria which includes marine organisms from the Atlantic and Pacific oceans (Giovannoni et al. 1990, Fuhrman et al. 1993, Field et al. 1997) as well as freshwater organisms from lakes (Bahr et al. 1996, Zwart et al. 1998) and the Columbia River (Crump et al. 1999). The expansion of archaeal clusters to include sub-clusters of organisms from different systems suggests that each of these clusters may be widely distributed in nature, existing in many different natural environments.

It is impossible to determine the phenotypic characteristics of the archaea presented in this study because no closely related archaea have been cultivated from temperate environments, except that they are likely to be "non-thermophilic" (Buckley et al. 1998).

The clustering of many clones from the Columbia River and from the coastal ocean with clones from similar freshwater and marine environments suggests that these organisms develop in these environments and therefore can grow at the temperature and salt concentration typical of these environments. One study in the Antarctic Ocean determined that planktonic archaea form a dynamic community, implying that they are autochthanous organisms that can reproduce at oceanic temperature and salinity (Massana et al. 1998). However, results from the present study indicate that organisms found in some systems may be allochthanous. Archaea found in the Columbia River estuary probably did not develop in the estuary, but rather developed in the coastal ocean, the river or in some other terrestrial ecosystems farther upstream. Therefore it cannot be assumed that these archaea can grow under estuarine environmental conditions.

One metabolic characteristic we expected to exist among particle-attached estuarine archaea was methanogenesis. Clones related to cultivated methanogenic archaea are common in clone libraries prepared with DNA from salt marsh sediment (Munson et al. 1997), and were also found in Lake Michigan sediment (MacGregor et al. 1997). Analysis of ETM particles from the Columbia River estuary showed evidence of chemical processing that can only occur in reducing anaerobic environments (Prahl & Coble 1994). Moreover, clones related to anaerobic sulfur-reducers (δ-Proteobacteria) were recovered from ETM particles (Crump et al. 1999). It is thought that a fraction of these particles wash into the estuary from anoxic regions of surrounding tidal mudflats and salt marshes or that anaerobic micro-zones exist within the particles themselves. Since a phylogenetically diverse group of archaea produce methane, it is possible that some organisms within the clusters of uncultivated archaea may be methanogens.

New clusters of archaeal 16S rRNA sequences are usually placed tentatively into one

of the two archaeal divisions, Crenarchaea and Euryarchaea. However, the placement of Group 1 archaea and related clusters, which include most of the sequences identified in this study, has been uncertain (DeLong 1992, McInerney et al. 1997). Analyses of 12 relevant signature base pairs designed to distinguish between Crenarchaea and Euryarchaea (Winkler & Woese 1991) places these clusters somewhere between the two groups, with seven crenarchaeal signatures and five euryarchaeal signatures (DeLong 1992). In our analyses, these clusters did not group with the high temperature Crenarchaea (Fig. 4.3). The recent designation of a third archaeal division, Korarchaeota (Barnes et al. 1996) forecasts the tremendous diversity that exists among the Archaea, and supports the assertion that the separation of sequences into Crenarchaea and Euryarchaea is restrictive (McInerney et al. 1997). Moreover, during the preparation of Fig. 4.3, the inclusion of some intermediate clone sequences prevented the confident clustering of sequences into Crenarchaea and Euryarchaea. It is likely that the expansion of the uncultivated archaeal clusters with the addition of newly discovered organisms will blur the distinctions between these clusters.

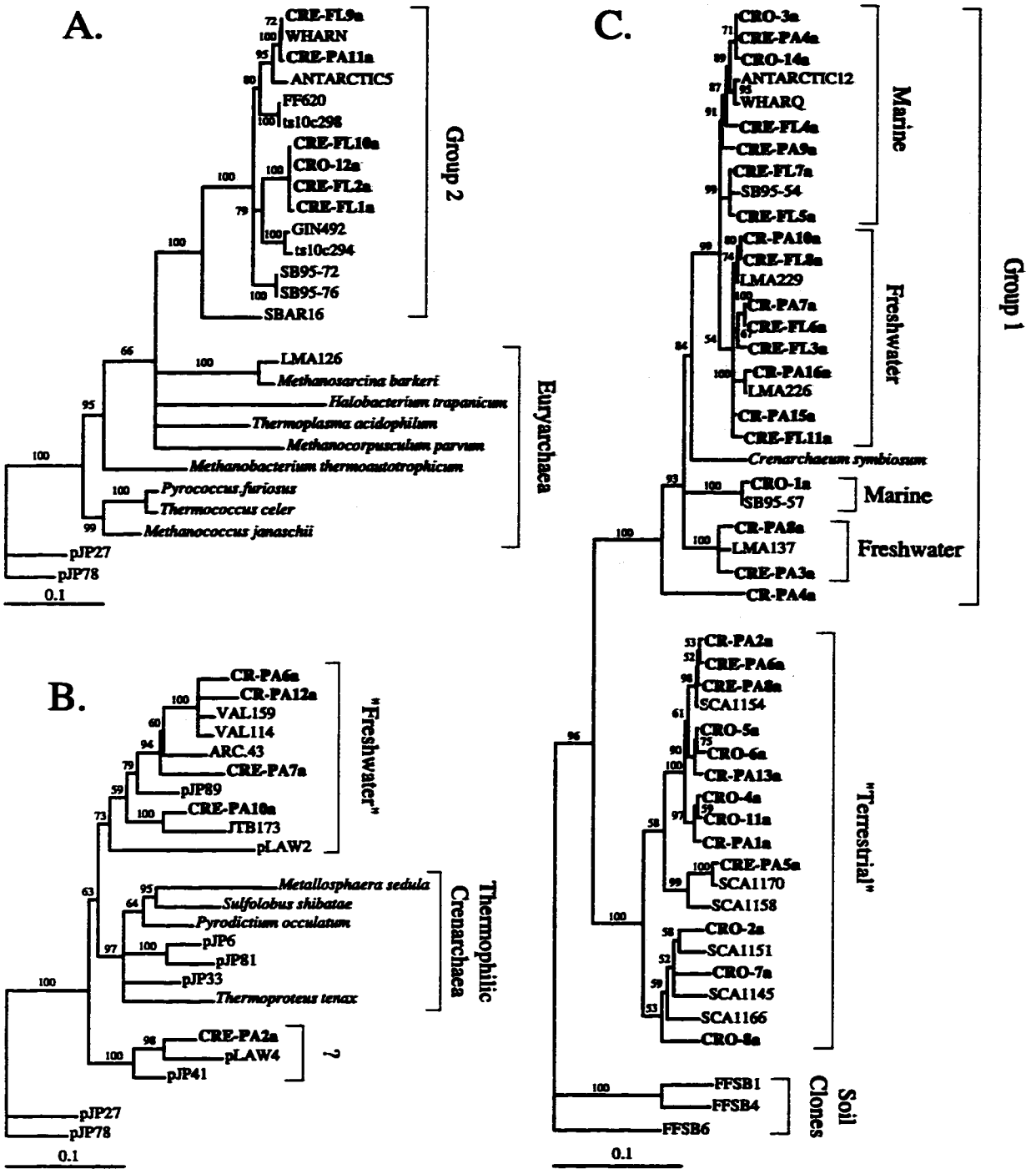


FIG. 4.1. Phylogenetic relationships among 16S rRNA environmental clones of Columbia River particle-attached archaea (CR-PA), Columbia River estuary particle-attached and free-living archaea (CRE-PA and CRE-FL), and coastal ocean archaea (CRO-), as well as environmental clones from other studies and cultivated organisms. (A) Euryarchaea and Group 2 archaea; (B) Crenarchaea and related environmental clones; (C) Group 1 archaea and related environmental clones. Fifty percent majority rule trees were constructed using the neighbor-joining method. The percent of 100 bootstrap replicates that supported the branching order are shown above or near the relevant nodes. The scale bars correspond to a 10% difference in nucleotide sequence. Clones from this study are indicated in bold type.

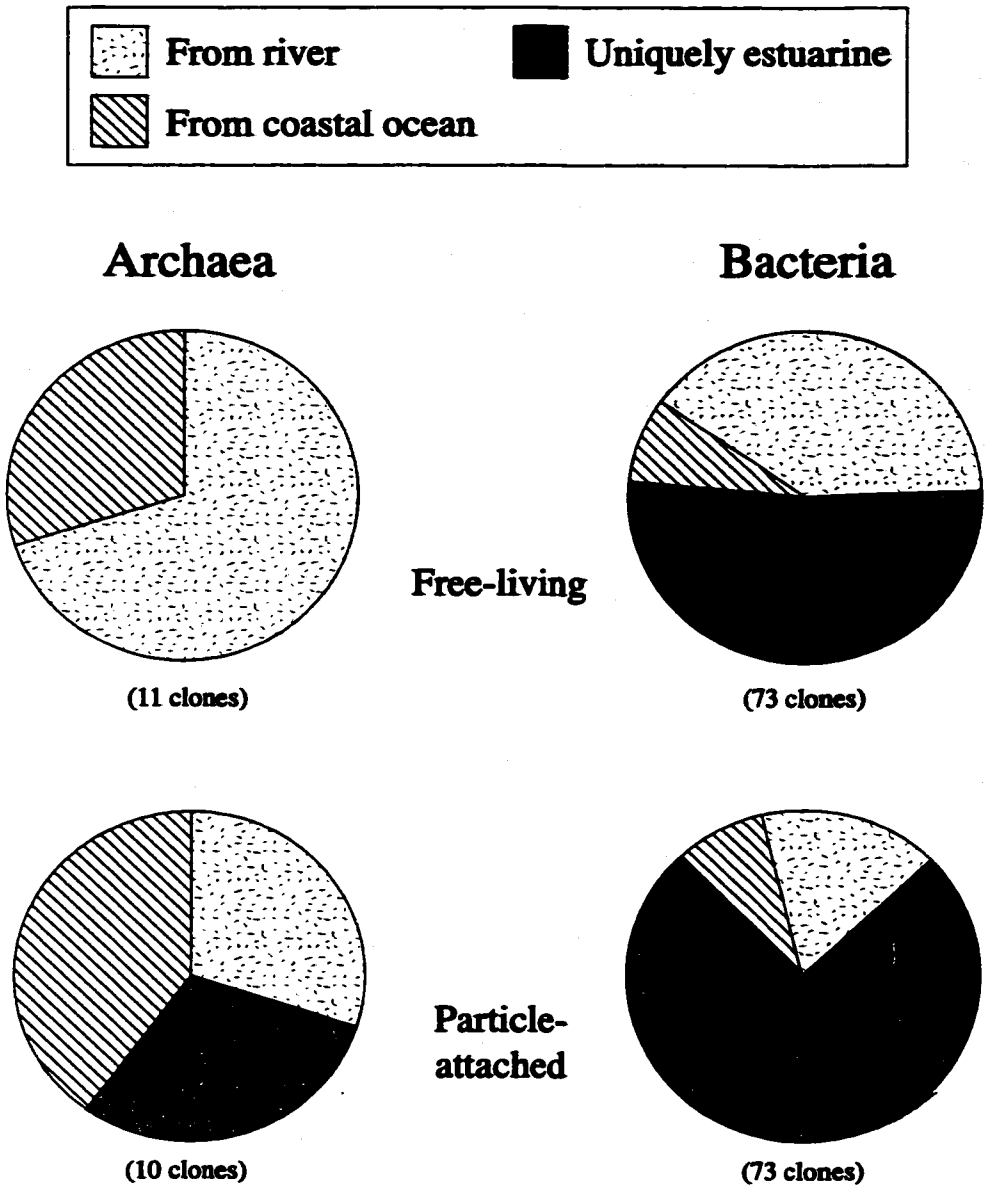


Fig. 4.2. Composition of particle-attached and free-living bacterial and archaeal clone libraries constructed from an estuarine DNA sample. Libraries are divided into clones related to river organisms, clones related to coastal ocean organisms, and clones unique to the estuary.

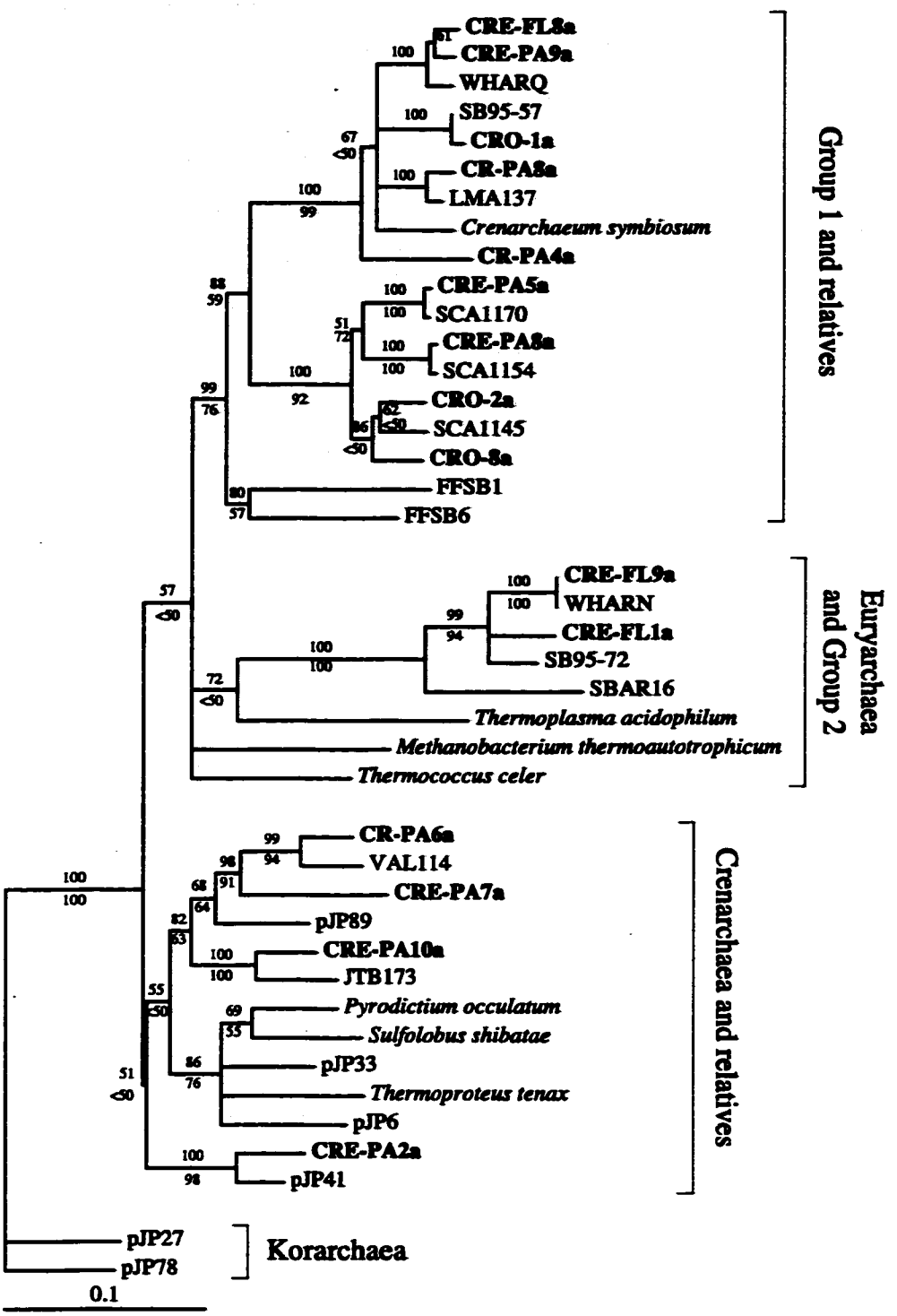


FIG. 4.3. Phylogenetic relationships among a sampling of 16S rRNA sequences from this study, other environmental clones, and cultivated organisms for the three groups of sequences displayed in Fig. 1. 50% majority rule tree was constructed using the neighbor-joining method. The percent of 100 bootstrap replicates that supported the branching order are shown above the relevant nodes. Bootstrap values (>50%) for a heuristic parsimony analysis consensus tree are shown below the neighbor-joining tree values. The scale bar corresponds to a 10% difference in nucleotide sequence. Clones from this study are indicated in bold type.

Chapter 5. Seasonal and Inter-annual Variability in Bacterial Activity

Abstract

Bacterial production in the water column of estuaries is variable throughout the year in response to changes in environmental conditions including food supply, grazing pressure, and temperature. In the Columbia River estuary, the principal sources of organic matter throughout the year are river-borne particulate material derived from soils and phytoplankton. Earlier research found that most of the bacterial production in the estuary in Spring and Summer was associated with particles > 3 μm. We hypothesized that particle-attached bacteria would dominate bacterial activity in all seasons of the year, and that bacterial production in the estuary would be dependent on the concentration and composition of river-borne particulate organic matter. Thymidine incorporation rate (TdR) of particle-attached and free-living bacteria, potential carbon fixation rate (at saturating light levels), concentrations of particulate organic carbon (POC), chlorophyll-a (CHL-a), and NH_4, salinity, and temperature were determined in river and estuarine samples during four seasonal cruises on the Columbia River estuary from May 1997 to March 1998. Results were also compared to those from four previous cruises embarked between 1991 and 1996. TdR was generally high in the Spring and Summer (averaged 26 to 69 pmol l^{-1} h^{-1}) and low in the Fall and Winter (4.5 pmol l^{-1} h^{-1}), except in Spring of 1997 when samples were collected prior to the onset of the Spring phytoplankton bloom in the river (6.4 pmol l^{-1} h^{-1}). Particle-attached bacteria accounted for 69 to 80% of TdR throughout the year, TdR was positively correlated with POC, and TdR per mg POC was positively correlated with CHL-a per mg POC in all seasons indicating the importance of both organic matter quantity and quality to estuarine bacterial production. Temperature

appeared to have little effect on bacterial production in the estuary, but the potential importance of grazing on bacterial production in Spring and Summer was suggested by a correlation between TdR and NH_4.

Introduction

Seasonal and inter-annual comparisons of biological and chemical characteristics can provide insight into how ecosystems change and clues to the controls on organic matter production and consumption. Bacterial abundance and heterotrophic activity in estuaries are variable throughout the year and between years in response to changes in temperature, particulate organic matter concentration and phytoplankton production (Bell & Albright 1981, Bent & Goulder 1981, Joint & Pomroy 1982, Hoch & Kirchman 1993, Sampou & Kemp 1994). Bacteria are important in estuarine ecosystems as the primary consumers of dead and degraded organic matter, as recyclers of nutrients, and as prey for protozoa and metazoa in estuarine food webs. Determining the controls on bacterial activity is essential to understanding how estuarine ecosystems function and how they change throughout the year. Towards this goal, as part of the National Science Foundation Land Margin Ecosystem Research (LMER) program, research in the Columbia River estuary and its turbidity maxima regions was conducted during nine research cruises between 1990 and 1998: three Spring seasons, three Summers, two Falls and one Winter,.

While we standardized chemical and biological measurements on each cruise in order to compare seasons and years, we also conducted experiments that added caveats to these "bulk" measurements and enhanced the interpretation of the results. First, we discovered that particle-attached bacteria accounted for the majority of bacterial production in the water column, and that free-living bacteria, though abundant, were relatively inactive

(Crump et al. 1998). We also found that only a fraction of the particles in the water column supported most of the bacterial production, and that these particles could be separated by size and, in some samples, by settling velocity from less bacterially-active material (See Chapter 2). We determined that one of the most abundant copepods in the estuary, *Eurytemora affinis*, directly consumes particle-attached bacteria (Simenstad et al. 1994a). We found that the phylogenetic composition of the free-living bacterial community in the estuary was very similar to those in the river and the coastal ocean, while the particle-attached community was different, and was composed of organisms unique to the estuary (Crump et al. 1999). These samplings and experiments demonstrated that the microbial contribution to the degradation of detritus and to the estuarine food web is due entirely to a small, specialized sub-population of particle-attached bacteria (and perhaps archaea).

The active fraction of the bacteria in the Columbia River estuary are concentrated in the estuarine turbidity maxima (ETM) regions near the head of the salt intrusion where particulate matter is trapped and concentrated by the interaction between river flow and tidal action. These particles and their associated bacteria are resuspended from the bed during flood and ebb tides, move up or down the estuary, and settle back to the bed during slack tide. The rapid flushing time of water and soluble constitutents in the estuary (1 to 2 d) causes organisms and organic material to wash through the system very rapidly, but organisms and organic material trapped in ETM are thought to remain there up to 2 to 4 wks. So ETM function as comparatively stable environments where the estuarine food web develops, feeding on trapped allochthanous detrital material.

The Columbia River supplies most of the organic matter found in the estuary. Phytoplankton production in the estuary is low due to water column turbidity, and is

dwarfed by inputs of freshwater phytoplankton that develop in the relatively still, clear waters behind the many impoundments behind hydroelectric power and flood control dams upriver. We hypothesized that particle-attached bacteria would dominate bacterial production in the estuary year round, but that the rate of production would vary in response to the quality and quantity of detrital organic matter supplied by the river. The goal of this project was to understand how changes in the river influence bacterial activity in the estuary.

Materials & Methods

Sample collection

The Columbia River is the second largest river in the United States, with a watershed of 660,480 km^2 (Simenstad et al. 1990). The river drains into a shallow, partially-mixed estuary (Fig. 5.1) cut with two main channels that are generally 20-25 m deep. The estuary is flanked by tidal mudflats in a few shallow peripheral bays, but sediments in the of the estuary are predominately sand except in peripheral habitats. Salinity intrudes to the ETM regions in the North and South Channels with every tide, and can extend up to approximately 20 km from the mouth of the estuary depending on river flow and stage of the tidal month.

Standard sets of water samples were collected during four two-week "seasonal" research cruises in May, July and October, 1997, and in February, 1998. On each cruise, a total of four 30-hr Eulerian sampling series were conducted on the spring and neap tides in both the North and South channels of the estuary. During these series, water samples were collected from approximately 0.5 m off the bed every 2 h using a high-volume, low-pressure pump coupled to a conductivity-temperature-depth (CTD) meter and an optical

backscatter sensor (OBS) for measuring turbidity (Simenstad et al. 1994b). Also, during each cruise water samples were collected rapidly from 0.5, 1, 1.5, 2, and 2.5 m above the bed of the estuary in the North channel at four or five time points during a flood and an ebb tide in order to more carefully sample ETM resuspension events (Simenstad et al. 1994b). Additionally, two or three freshwater samples were collected from middle depth at a station upstream of the influence of ocean water.

Inter-annual comparisons of biological and chemical measurements were made for the Spring season using samples collected in May to June 1992 (Crump and Baross, 1996) and May, 1995 (Crump et al. 1998), and for Summer using samples collected in July, 1991 (Baross et al, 1994) and July, 1996. These samples were collected in the same regions of the estuary and at the same depths as those collected in 1997 and 1998.

Measurements

Prokaryotic cells were enumerated by direct counts (Hobbie et al. 1977) on formaldehyde-fixed samples (2% final conc.) using DAPI stain (Sigma)(Porter & Feig 1980) and a Zeiss UEM epifluorescent microscope (Crump & Baross, 1996).

The rate of bacterial production was determined by measuring the incorporation of methyl-tritiated thymidine (Fuhrman & Azam 1982) (20 nM final concentration) into the cold TCA insoluble fraction in six 5-ml subsamples incubated with constant agitation at *in situ* temperature (Bell 1993, Crump et al. 1998). Triplicate incubations were terminated at 0 and 60 minutes with 0.25 M NaOH (final concentration, Zweifel et. al 1995).

Cell concentration and thymidine incorporation rate of free-living bacteria were measured in water samples gently filtered through 3-μm pore size polycarbonate membranes (Poretics) (Crump et al. 1998). These values were subtracted from those in

unfiltered water to calculate cell concentration and thymidine incorporation rate of particle-attached cells.

The concentration of suspended particulate matter (SPM) in unfiltered samples was measured gravimetrically (Prahl & Coble 1994). Particulate organic carbon (POC) was measured using a CHN analyzer (Prahl et al. 1997). Chlorophyll-a concentration was measured in acetone-extracted samples, and potential primary production at light saturation was measured by standard ^{14}C techniques (Small & Morgan 1994).

Ammonium was measured by autoanalyzer using standard methods (Strickland & Parsons 1972).

Results

Historically, Columbia River flow was extremely variable throughout the year and between years, but regulation of the river has reduced flooding during high flow years, and moderated flow throughout the year (Sherwood et al. 1990). River flow rates between 1991 and 1998 were generally higher and more variable from December to June and lower and more constant from July to November (Fig. 5.1). River flow in Spring 1997 was the highest encountered during our research on the estuary (Table 5.1). It was 1.5 times the flow in Spring 1995 and more than 2 times the rate in 1992. Summer flow rates were more constant, varying from 6000 to 8300 $m^3 s^{-1}$. Average flow rates during the cruises in Fall 1997 and Winter 1998 were nearly the same as Summer 1997 (Table 5.1).

Particle-attached and free-living cell concentrations were fairly constant throughout the year (Table 5.2). Particle-attached cell concentration was more variable than free-living cell concentration (Table 5.2) and appeared to be related to the concentration of

POC (Fig. 5.2). The number of cells per mg POC was similar during all four seasons (Fig. 5.2).

Bacterial activity (thymidine incorporation rate) was higher in the Spring and Summer 1997 than in the Fall and Winter in the North (Fig. 5.3) and South channels (Fig. 5.4) of the estuary. During each season, thymidine incorporation rates were highest in samples collected during ETM resuspension events, which generally occurred at intermediate salinity, and were lowest in freshwater samples collected at the end of stronger ebb tides. Thymidine incorporation rates in freshwater samples from the estuary were similar to those measured at the upstream station. Particle-attached bacteria dominated thymidine incorporation in most samples, being responsible for 69 % to 80 % of thymidine incorporation in the estuary, and 40 % to 66 % in the river (Table 5.3). These patterns are typical of all time series data in all years.

There was a significant positive correlation between particulate organic carbon concentration and thymidine incorporation rate in all four seasons (Table 5.4), but in the Spring and Summer this relationship was much more variable (Fig 5.5). Previous work, summarized in Table 5.5, explained this variability by showing that estuarine particles are heterogeneous and that a small fraction of these particles supports most of the thymidine incorporation (Crump & Baross, submitted). This is further demonstrated by the decrease in thymidine incorporation rate per mg POC at higher concentrations of POC (Fig. 5.6), which suggests that bacterially-active particles are diluted by less active particles in more turbid water.

Thymidine incorporation rate per mg POC was positively correlated ($p < 0.001$) with the concentration of chlorophyll-a per mg POC in all four seasons (Table 5.4, Fig. 5.7).

Mixing of river and coastal ocean water controlled the temperature and salinity in the

estuary. In Summer 1997 and Winter 1998, these measurements described mixing lines in the estuary (Fig. 5.8). In Spring and Fall 1997, river and coastal ocean water were similar in temperature. The temperature of the river in Spring 1997 changed during the sampling period from 11 to 14 °C.

The highest rates of thymidine incorporation per liter were found in samples collected within or near estuarine turbidity maxima, which generally occurred at intermediate temperature and salinity (Fig. 5.8). In Spring and Summer, thymidine incorporation rate per mg POC was also highest at intermediate temperature and salinity. However, during Fall and Winter there was a significant positive relationship between thymidine incorporation rate per mg POC and temperature (Table 5.4), suggesting that temperature and salinity have a greater influence over bacterial production in these seasons.

The difference in estuarine thymidine incorporation rate between the Springs of 1992, 1995 and 1997 appears to be related to the rate of primary production in the river and the concentration of chlorophyll per mg POC in the estuary. Thymidine incorporation rate in Spring 1997 was 7 to 8 times lower than in 1992 and 1995 (Table 5.1), and potential phytoplankton production in the Columbia River in 1997 was 4.2 times lower than in 1995 (Table 5.1). The concentration of chlorophyll-a per mg POC and rate of thymidine incorporation per mg POC in the estuary were greatest in 1992, less in 1995, and lowest in 1997 (Fig. 5.9). Thymidine incorporation rate per liter was greater in the summers of 1991 and 1996 than in the summer of 1997 (Table 5.1), but there was no difference in the concentration of Chlorophyll-a per mg POC and little difference in the rate of thymidine incorporation per mg POC (Fig. 5.10).

Discussion

Particle-attached bacteria dominated bacterial activity in both the river and the estuary in all seasons of the year (Table 5.3) despite the seasonal variability in total thymidine incorporation rate. This contrasts with the upper Chesapeake Bay, where particle-attached bacteria were responsible for more than 20% of bacterial production only in winter (Griffith et al. 1994). Earlier work in the Columbia River estuary revealed that most of the extracellular enzyme activity in the estuary was associated with particles (Crump et al. 1998), indicating that particulate organic matter (POM) supported most of the bacterial production. Therefore POM, supplied principally from the Columbia River, is an important control on bacterial production in the estuary.

The concentration of particulate organic carbon (POC) correlated well with thymidine incorporation rate in all seasons (Table 5.4). However, when we compared different seasons, the rate of thymidine incorporation was highly variable while the concentrations of POC remained approximately the same (Figs. 5.3 & 5.4). Therefore, it is important to consider both the quantity and the quality of organic matter in the estuary in order to understand the controls on bacterial production, the contribution of bacteria to the consumption organic matter, and the role of bacteria in the estuarine food web.

The contribution of particle-attached bacteria to bacterial production in aquatic environments depends on the concentration and growth rate of both particle-attached and free-living bacteria. The concentration of particle-attached and free-living cells was fairly constant throughout the year (Table 5.2), suggesting that per-cell production rates were higher in Spring and Summer. However, production per cell is known to be highly variable within individual samples (Crump et al. 1998). Thus it is equally as likely that

actively growing cells have the same per-cell production rate, and that active cells are simply more abundant in the Spring and Summer.

Particle variability

In Spring and Summer, the relationship between thymidine incorporation rate and particulate organic carbon was probably variable because of the heterogeneity of particles in the estuary (Crump & Baross, submitted). Bacterially-active particles combine with less active material in the estuarine turbidity maxima (ETM) regions of the estuary (Table 5.5). This was demonstrated by the negative relationship between thymidine incorporation rate per mg POC and POC concentration (Fig. 5.6). In turbid regions of the estuary, bacterially-active particles were diluted with less active particles associated with refractory organic matter. Samples exhibiting high rates of thymidine incorporation per mg POC were collected when high concentrations of bacterially-active particles are lifted off the bed early during ETM resuspension events, and again later when these particles remain suspended in the water column longer than the more rapidly-settling, less bacterially-active refractory material (See Chapter 2).

The relationship between thymidine incorporation rate and POC was much stronger in the Fall and Winter than in the Spring and Summer (Fig. 5.5, Table 5.4). This suggests that particle heterogeneity was reduced during these seasons, and that bacterial production was more evenly distributed among the different types of particles. Furthermore, total thymidine incorporation rate was much lower during Fall and Winter. Bacterially-active particles were probably less abundant during these seasons, and supported a slower growing community of organisms.

Organic matter quantity & quality

The quality, or lability, of organic matter is known to influence bacterial production in aquatic ecosystems (Hopkinson et al. 1998). The principle source of fresh detrital organic matter to the Columbia River estuary is river-borne phytoplankton, composing approximately 30% of annual organic carbon inputs from the river (Small et al, 1990). When these freshwater organisms are exposed to elevated estuarine salinity, they die and become a part of the detrital pool (Jackson et al. 1987). The other large pool of allochthanous organic matter is derived from soils (B. Sullivan, pers. comm.), and is probably much less labile. The relative concentration of these two organic matter pools varies with season and river flow rate (B. Sullivan, pers. comm.). The concentration of phytoplankton-derived carbon is generally highest in the Spring and Summer when primary production in the river is at its highest. The concentration of soil-derived POC is augmented by increased river flow and upstream flooding in Winter and Spring. When we compare seasons and years, there was a positive relationship between average primary production in the river and average thymidine incorporation rate in the estuary (Table 5.1), suggesting that the supply of fresh, labile POM from river phytoplankton influences bacterial production in the estuary.

The concentration of chlorophyll per mg POC is an indicator of the relative amounts of phytoplankton-derived organic matter and organic matter derived from soils and estuarine marshs. The transition from low phytoplankton production to high phytoplankton production in the Columbia River occurs in the Spring season. When we compare the three spring cruises, the concentration of chlorophyll-a per mg POC in the estuary is lowest in 1997 and highest in 1992 (Fig. 5.9) corresponding to the average rates of potential primary production in the river (Table 5.1). Thymidine incorporation

rate per unit POC increased with chlorophyll enrichment of estuarine POC among the Spring seasons (Fig. 5.9). Moreover, within each season in 1997 to 1998, thymidine incorporation rates per mg POC were strongly correlated with the concentrations of chlorophyll-a per mg POC (Table 5.4). A similar result was found in surface sediments on the shelf of the Bay of Biscay near the Gironde estuary, France, where electron-transport-system activity (an index of overall metabolism) was strongly correlated with chlorophyll-a per g dry sediment (Relexans et al. 1992). When we used chlorophyll-a per mg POC as an indicator of the freshness and lability of particulate organic matter in the Columbia River estuary, we found a relationship between thymidine incorporation rate and organic matter quality within each season and between different seasons.

After the response of the estuarine bacterial community to the Spring season phytoplankton bloom in the river, conditions appear to stabilize through the Summer. In the Summers of 1991, 1996 and 1997, river phytoplankton production, the concentration of chlorophyll per mg POC, and thymidine incorporation rate per mg POC in the estuary were nearly identical (Fig. 5.10). Differences in thymidine incorporation rate per liter among the three years (Table 5.1) was probably caused by differences in the concentration of POC in the estuary. Therefore, the supply of river phytoplankton to the estuary and the concentration of relatively fresh organic matter derived from phytoplankton are both important factors controlling the level of bacterial production in the estuary.

Temperature

Temperature is known to directly effect the growth rate of bacteria in culture and has been shown to control the growth rate of free-living bacteria in the Chesapeake Bay below 20° C (Shiah & Ducklow, 1994). Within each season in the Columbia River

estuary, the highest rates of thymidine incorporation per liter occurred at intermediate temperatures and salinities (Fig. 5.8) corresponding to higher concentrations of POC. However, there was a significant positive correlation between thymidine incorporation rate per mg POC and temperature in Winter and Fall (Table 5.4), suggesting that temperature influences estuarine bacterial production when total bacterial production is low. But, it is important to note that temperature and salinity correlate well in the Columbia River estuary due to the rapid flushing time of the system (Neal 1972). Therefore it is impossible to distinguish between the effects of temperature and salinity.

Grazing

One important control on bacterial production not directly addressed in this study is consumption by protozoans and metazoans. Mesocosym and modeling studies have demonstrated that food supply (bottom-up) and grazing (top-down) are both important controls on bacterial abundance and production (Wright 1988, Shiah & Ducklow 1995). Consumer control is thought to be more important in eutrophic environments while food supply is more important in oligotrophic environments (Sanders et al. 1992). Thymidine incorporation rates in the Columbia River estuary indicate that there is a shift from more oligotrophic to more eutrophic with the onset of the Spring bloom in the river (Table 5.1). In the Spring and Summer, thymidine incorporation rates were strongly correlated with ammonium concentration (Table 5.4); a value which is thought to be controlled by micro- and macrozooplankton grazing (Johannes 1965, Fenchel & Harrison 1976, Bidigare 1983). Also, thymidine incorporation rates in Spring and Summer were approximately an order of magnitude higher than in the Fall and Winter (Table 5.1), but bacterial abundance was about the same in all seasons. Thus, consumer control on bacterial abundance and production is probably more important in the Spring and Summer when

conditions in the estuary are more eutrophic and less important in the Fall and Winter when conditions are more oligotrophic.

River flow rate

The food web of the Columbia River estuary originates to a large degree from the estuarine turbidity maxima regions where allochthanous POM, particle-attached bacteria (Crump et al. 1998), detritivorous copepods (Morgan et al. 1997) and rotifers (Crump & Baross 1996) are trapped and concentrated by the hydrodynamics of the system (Simenstad et al. 1990). The concentration and residence time of organisms and organic material in the ETM is dependent on the strength of river flow, which is variable throughout the year. Flooding caused by high river flow increases the supply of riparian and terrestrial material to the estuary and therefore to the ETM (Sullivan et al. submitted). River flow also influences the trapping efficiency of ETM. Increased river flow results in greater stratification in the estuary and thereby increases the trapping efficiency of the ETM. However, at very high river flow, salt water can be washed very far down estuary, particularly in combination with large spring-tide tidal ranges, potentially carrying ETM pareticles out of the system and therefore decreasing the trapping efficiency of ETM. Therefore, during Winter and Spring, higher river flow may influence the estuarine food web by increasing the trapping efficiency of ETM. However, periodic spikes in river flow rates during these seasons may wash ETM particles and organisms out to sea. In May 1997, for example, the remarkably high flow rate may have reduced bacterial production in the estuary by shortening the residence time of the particles in the ETM. Furthermore, the high water column turbidity in the river may have delayed the Spring bloom of phytoplankton. It is not clear to what extent river flow rate influences bacterial production in the river, but its influence on the supply of organic matter to the estuary

and its role in estuarine hydrodynamics suggest that it has a major impact on the estuarine food web.

Conclusions

Bacterial activity in the Columbia River estuary is dominated by particle-attached bacteria in all seasons of the year, and is influenced by seasonal changes in the quantity and quality of particulate organic matter supplied by the river. The principal sources of particulate organic matter are river phytoplankton and organic matter derived from soils and other terrestrial materials. The supply of freshwater phytoplankton is highest in the Spring and Summer. The supply of terrestrial material varies with river flow, and is lowest in the Summer. The quality of organic matter in the estuary varies with the relative concentrations of these two organic matter pools. Seasonal variability in temperature was not an important influence on bacterial activity in the estuary, but consumption by grazers in the Spring and Summer could be an important control on bacterial production.

Table 5.1. Mean and standard deviation of daily river flow rates ($m^3 s^{-1}$), ^3H-thymidine incorporation rates (pmol $l^{-1} h^{-1}$), and potential carbon fixation rates (μg C $l^{-1} h^{-1}$, measured at saturating light conditions) for freshwater (salinity < 1 psu) and estuarine water (salinity > 1 psu) samples. Number of samples is in parentheses.

Season	Year	River flow		^3H-thymidine incorporation				Potential carbon fixation			
				freshwater		estuarine		freshwater		estuarine	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Summer	1991	6.0	1.0	29.9 (11)	10.1	69.1 (32)	46.4	n.d.		n.d.	
Spring	1992	5.8	0.7	10.6 (2)	1.1	49.2 (62)	27.5	n.d.		n.d.	
Spring	1995	8.9	0.5	6.4 (26)	5.7	43.0 (69)	21.5	75.0 (14)	13.6	33.7 (39)	29.0
Summer	1996	7.4	0.7	16.1 (10)	6.0	54.7 (45)	32.0	73.5 (6)	4.9	40.5 (22)	25.3
Spring	1997	13.4	1.3	1.0 (14)	0.7	6.4 (77)	6.0	18.2 (6)	1.3	14.1 (40)	7.7
Summer	1997	8.3	0.5	4.5 (4)	3.3	26.3 (92)	12.8	60.5 (7)	6.6	35.3 (65)	25.5
Fall	1997	7.2	0.7	1.7 (11)	0.7	4.5 (89)	3.0	27.7 (7)	4.1	8.2 (53)	2.7
Winter	1998	8.6	0.6	1.4 (27)	0.6	4.5 (81)	3.5	16.4 (7)	3.1	10.7 (43)	4.7

Table 5.2. Free-living and particle-attached cell concentrations (x 10^9 l^{-1}) for samples collected in the North Channel of the Columbia River estuary during spring tide sampling series.

Season	Free-living mean	SD	n	Particle-attached mean	SD	n
Spring 1997	2.20	0.87	(15)	2.65	1.42	(15)
Summer 1997	1.19	0.34	(16)	1.70	1.62	(15)
Fall 1997	1.98	0.75	(16)	3.45	2.97	(16)
Winter 1998	1.31	0.83	(14)	1.89	1.02	(13)

Table 5.3. Mean percent of thymidine incorporation due to particle-attached bacteria in freshwater and estuarine water samples.

Season	freshwater			estuarine		
	mean	SD	n	mean	SD	n
Spring 1997	66%	15%	(9)	80%	17%	(35)
Summer 1997	59%	37%	(4)	69%	22%	(50)
Fall 1997	40%	22%	(10)	78%	20%	(54)
Winter 1998	53%	22%	(25)	69%	21%	(42)

Table 5.4. Spearman rank correlation coefficients and number of paired comparisons (in parentheses), for relationships between ^{3}H-thymidine incorporation rate in estuarine samples (l^{-1} and mg POC^{-1}), POC, chlorophyll-a (l^{-1} and mg POC^{-1}), NH_4 concentration, salinity and temperature for data collected in May 1997 (Spring), July 1997 (Summer), October 1997 (Fall), and February 1998 (Winter). Only significant correlations ($p < 0.05$) are shown. Highly significant correlations ($p < 0.001$) are presented in bold type. (n.s. = not significant)

Spearman rank correlation	pmol l^{-1} h^{-1}	pmol mg POC^{-1} h^{-1}	POC	Chl-a	Chl-a mg POC^{-1} h^{-1}	NH_4	Salinity
Spring							
pmol mg POC^{-1} h^{-1}	**0.88 (79)**						
POC	0.24 (78)	n.s.					
Chlorophyll-a	**0.61 (47)**	**0.49 (45)**	n.s.				
Chl-a mg POC^{-1} h^{-1}	n.s.	**0.53 (45)**	**-0.78 (45)**	0.33 (45)			
NH_4	**0.64 (39)**	**0.57 (38)**	n.s.	0.30 (53)	n.s.		
Salinity	**0.57 (79)**	**0.61 (77)**	n.s.	n.s.	n.s.	0.34 (53)	
Temperature	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s. (94)
Summer							
pmol mg POC^{-1} h^{-1}	n.s.						
POC	**0.45 (96)**	**-0.71 (94)**					
Chlorophyll-a	**0.43 (77)**	**-0.42 (75)**	**0.85 (81)**				
Chl-a mg POC^{-1} h^{-1}	n.s. (75)	**0.57 (75)**	**-0.55 (75)**	n.s.			
NH_4	**0.71 (51)**	**0.48 (49)**	n.s.	n.s.	n.s.		
Salinity	n.s.	0.24 (94)	**-0.41 (100)**	-0.35 (81)	0.32 (75)	n.s.	
Temperature	n.s.	n.s.	0.24 (100)	0.24 (81)	n.s.	**-0.49 (55)**	**-0.90 (101)**
Fall							
pmol mg POC^{-1} h^{-1}	-0.24 (97)						
POC	**0.69 (99)**	**-0.81 (97)**					
Chlorophyll-a	**0.57 (74)**	**-0.50 (71)**	**0.69 (86)**				
Chl-a mg POC^{-1} h^{-1}	**-0.49 (70)**	**0.76 (70)**	**-0.86 (74)**	-0.35 (74)			
NH_4	n.s.	**0.45 (53)**	n.s.	n.s.	0.44 (54)		
Salinity	**-0.40 (101)**	**0.41 (97)**	-0.27 (114)	-0.31 (88)	**0.48 (74)**	0.38 (68)	
Temperature	**-0.42 (101)**	**0.44 (97)**	**-0.43 (114)**	-0.31 (88)	**0.63 (74)**	**0.59 (68)**	**0.59 (117)**
Winter							
pmol mg POC^{-1} h^{-1}	n.s.						
POC	**0.71 (81)**	**-0.45 (81)**					
Chlorophyll-a	**0.64 (59)**	-0.32 (58)	**0.68 (85)**				
Chl-a mg POC^{-1} h^{-1}	n.s.	**0.53 (58)**	**-0.66 (61)**	n.s.			
NH_4	n.s.	n.s.	n.s.	n.s.	n.s.		
Salinity	n.s.	**0.52 (81)**	n.s.	n.s.	n.s.	n.s.	
Temperature	n.s.	**0.51 (81)**	n.s.	-0.28 (85)	n.s.	n.s.	**0.94 (124)**

Table 5.5. Mean thymidine incorporation rate per mg particulate organic carbon (pmol thymidine mg POC^{-1} h^{-1}) in particle size fractions from estuarine turbidity maxima samples collected in July, 1996.

Size fractions	pmol mg^{-1} h^{-1}	SD	n
>63 µm	3.2	3.9	(8)
20-63 µm	11.9	17.1	(9)
10-20 µm	10.9	7.7	(9)
3-10 µm	92.9	49.0	(9)

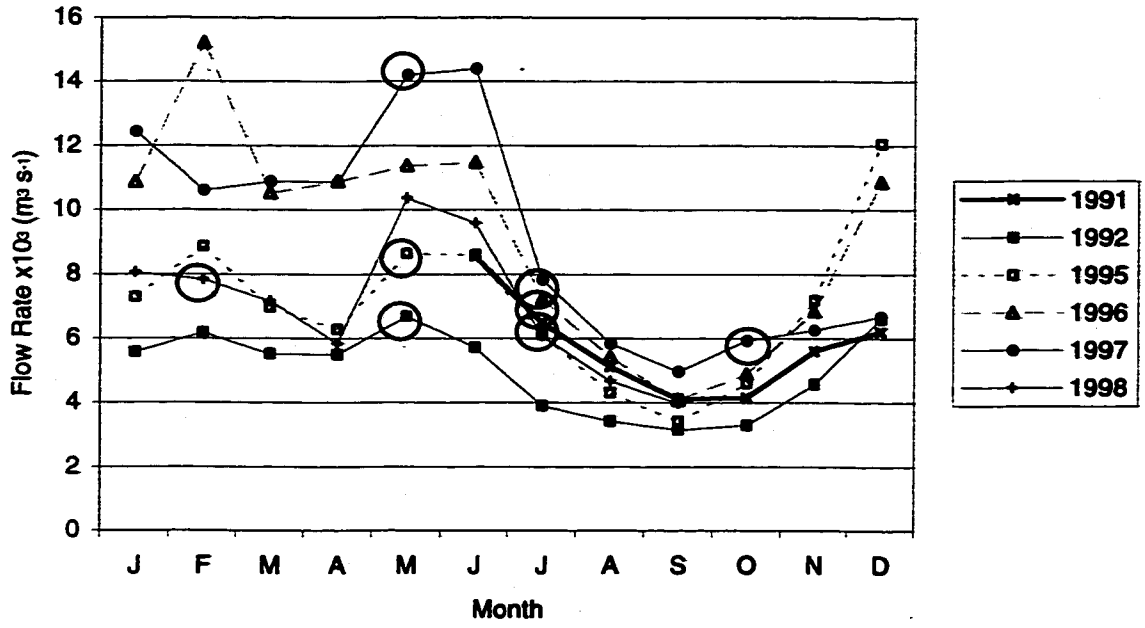


Fig. 5.1. Mean monthly river flow rates at near Quincy, Oregon (River Mile 53, from mouth). Months sampled for the present study are circled. Data for 1991 begins in June. Data for 1998 ends in September.

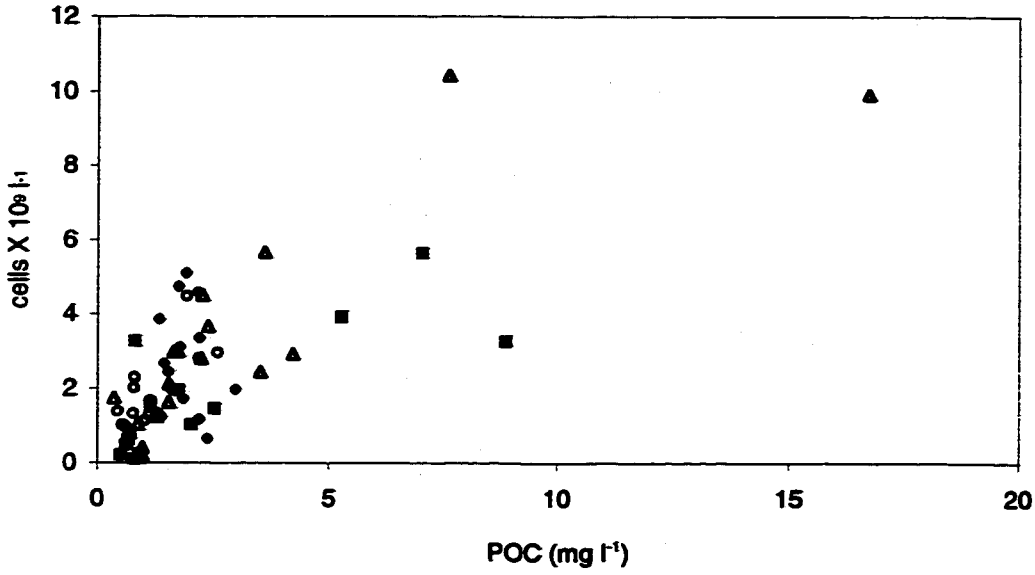


Fig. 5.2. Particle-attached cell concentration vs. POC concentration for Spring 1997 (diamonds), Summer 1997 (boxes), Fall 1997 (triangles), and Winter 1998 (circles).

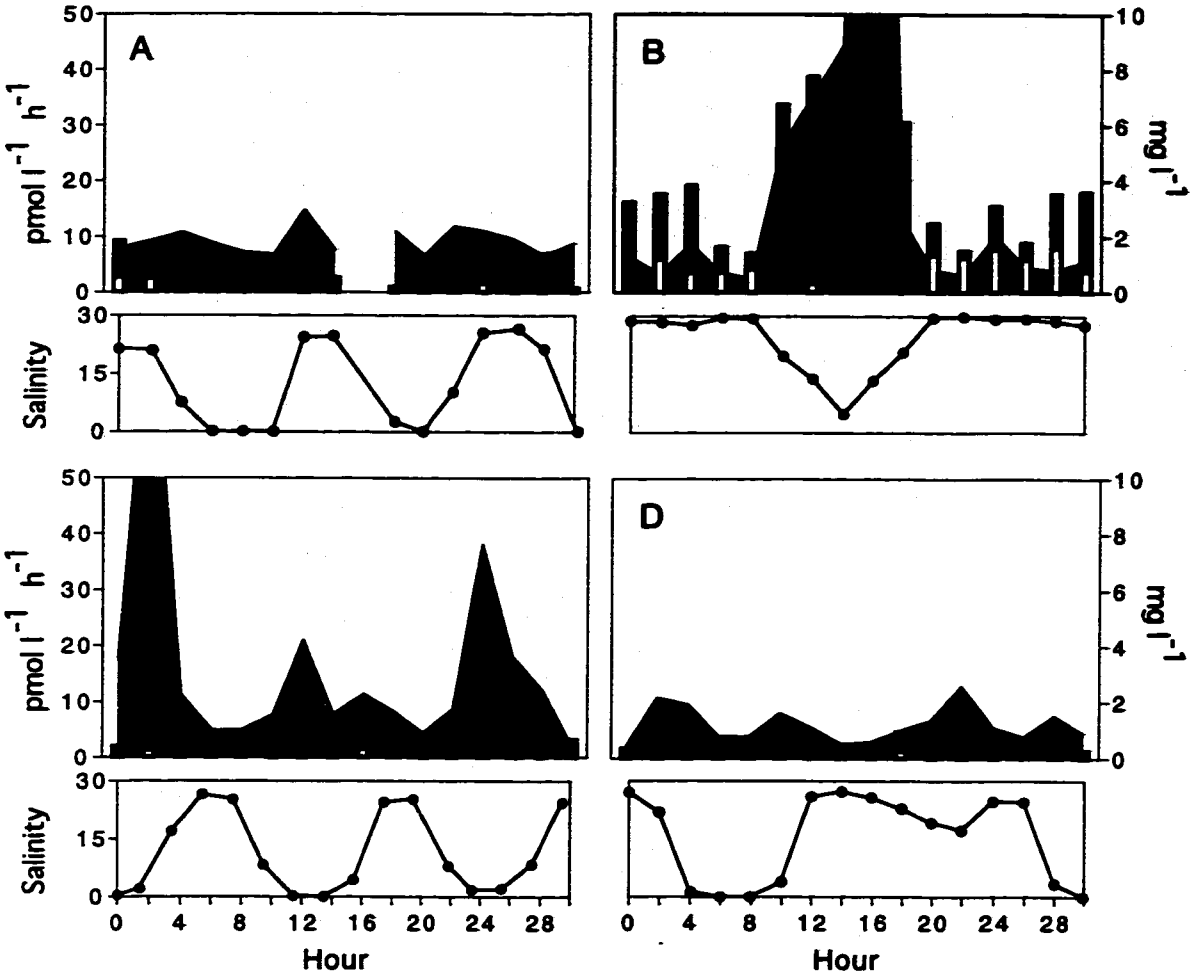


Fig 5.3. North Channel spring tide 30-hr series of bacterial thymidine incorporation rate (units on left axes) in the particle-attached (black columns) and free-living (white columns) fractions collected every two hours in Spring (A), Summer (B), and Fall (C) 1997, and Winter (D) 1998. Shaded regions indicate turbidity (units on right axes) in each sample (connected by a line and shaded below for clarity).

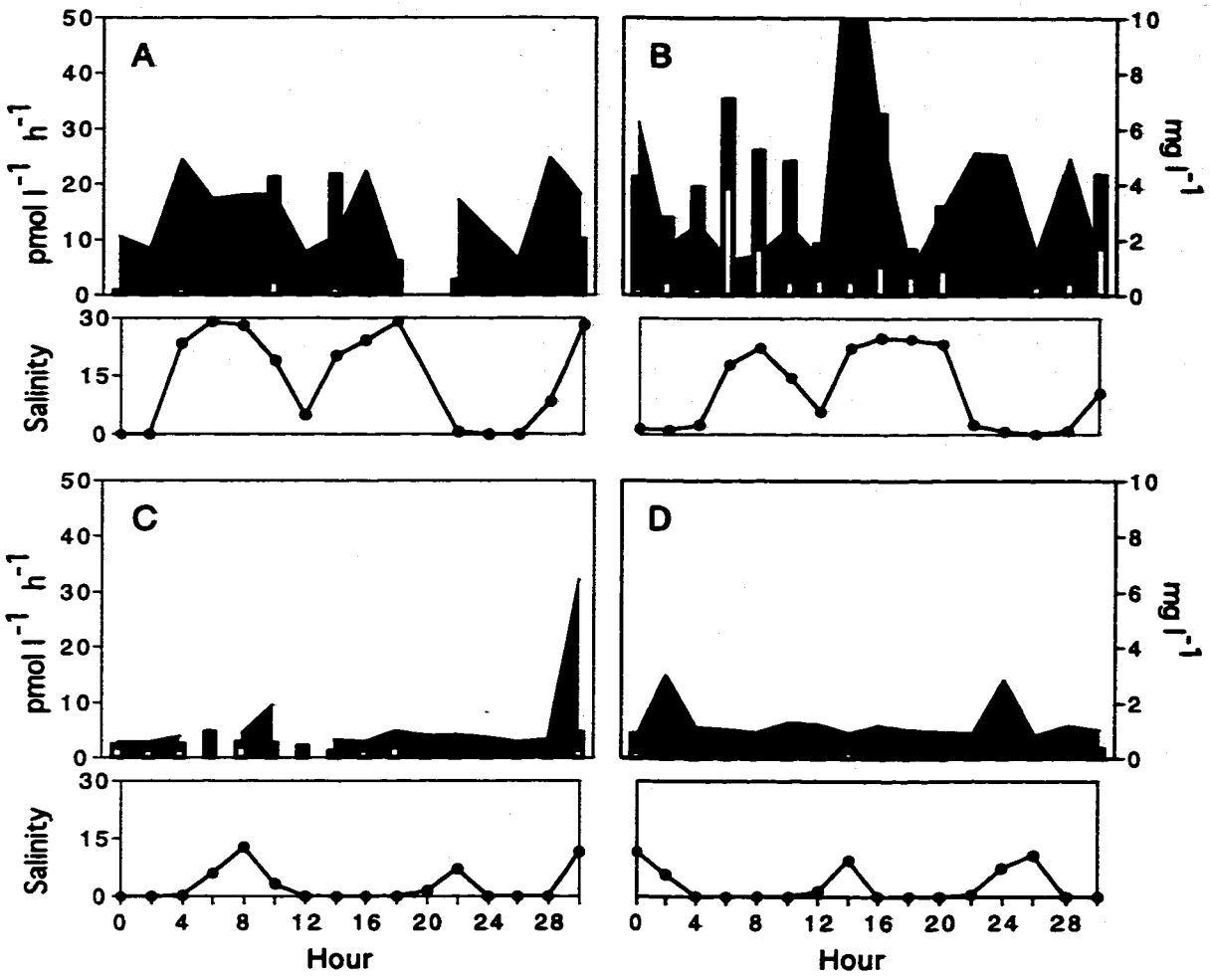


Fig 5.4. South Channel spring tide 30-hr series of bacterial thymidine incorporation rate (units on left axes) in the particle-attached (black columns) and free-living (white columns) fractions collected every two hours in Spring (A), Summer (B), and Fall (C) 1997, and Winter (D) 1998. Shaded regions indicate turbidity (units on right axes) in each sample (connected by a line and shaded below for clarity).

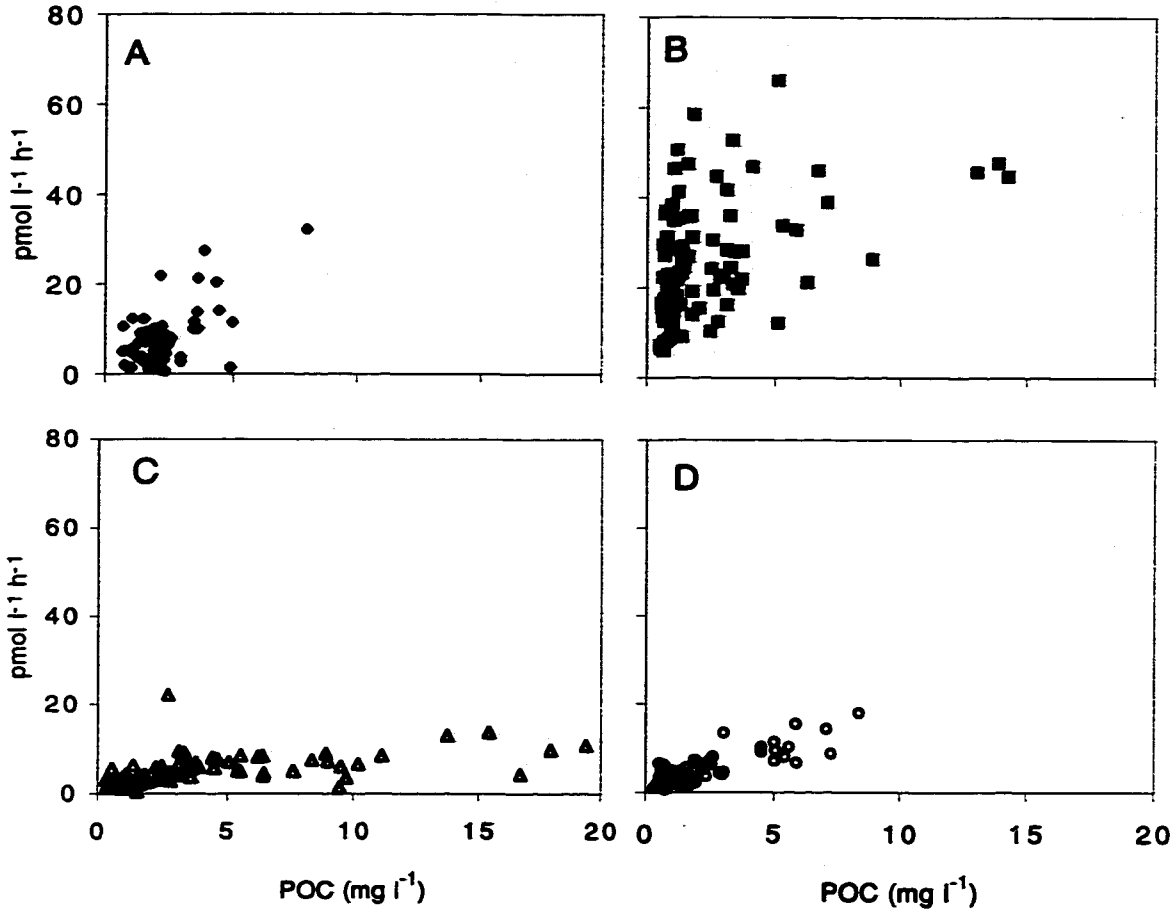


Fig. 5.5. Thymidine incorporation rate per liter vs. particulate organic carbon concentration per liter for estuarine samples collected in (A) Spring 1997, (B) Summer 1997, (C) Fall 1997, and (D) Winter 1998.

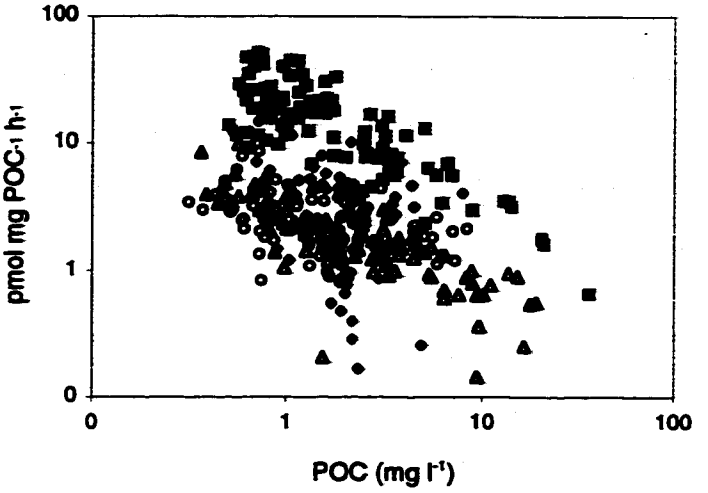


Fig. 5.6. Thymidine incorporation rate per mg particulate organic carbon vs particulate organic carbon concentration for Spring 1997 (diamonds), Summer 1997 (boxes), Fall 1997 (triangles), and Winter 1998 (circles).

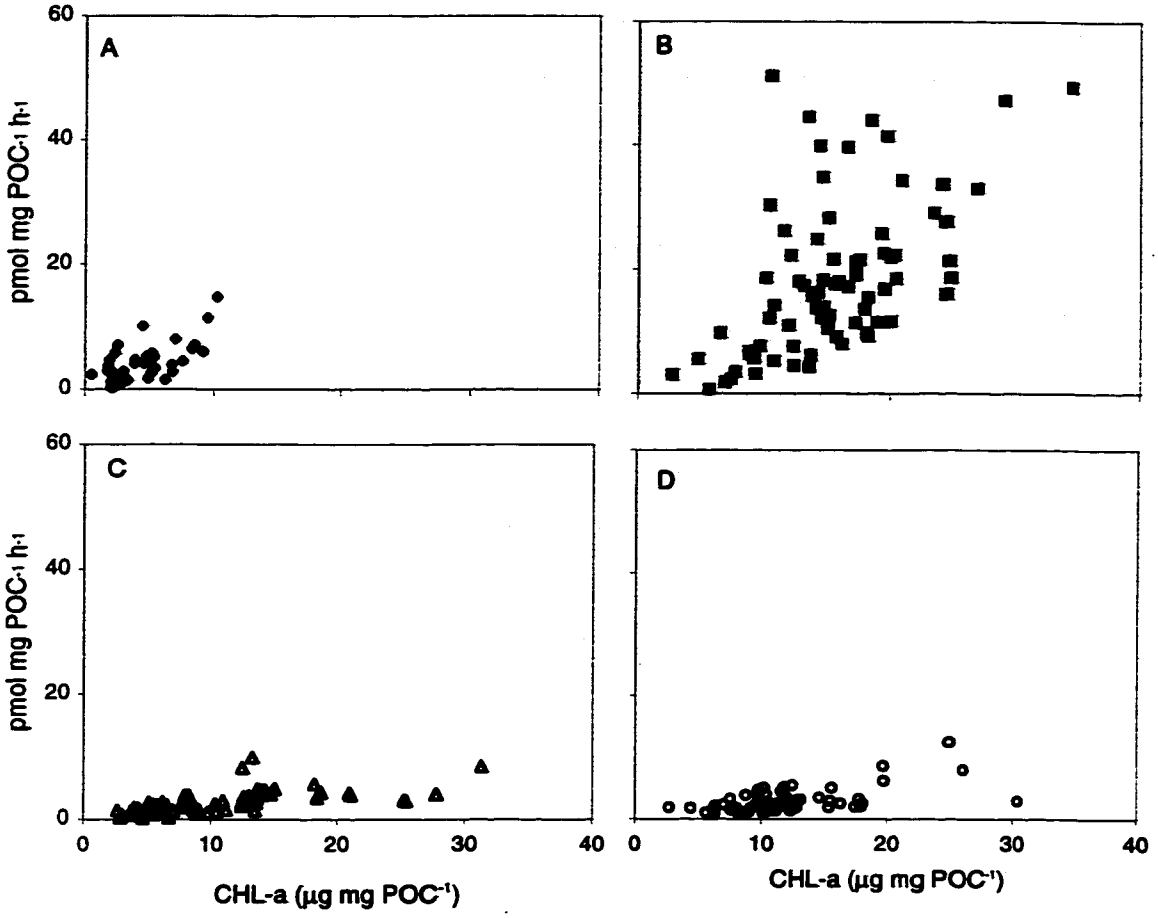


Fig. 5.7. Thymidine incorporation rate per mg POC vs. chlorophyll-a per mg POC for estuarine samples collected in (A) Spring 1997, (B) Summer 1997, (C) Fall 1997, and (D) Winter 1998.

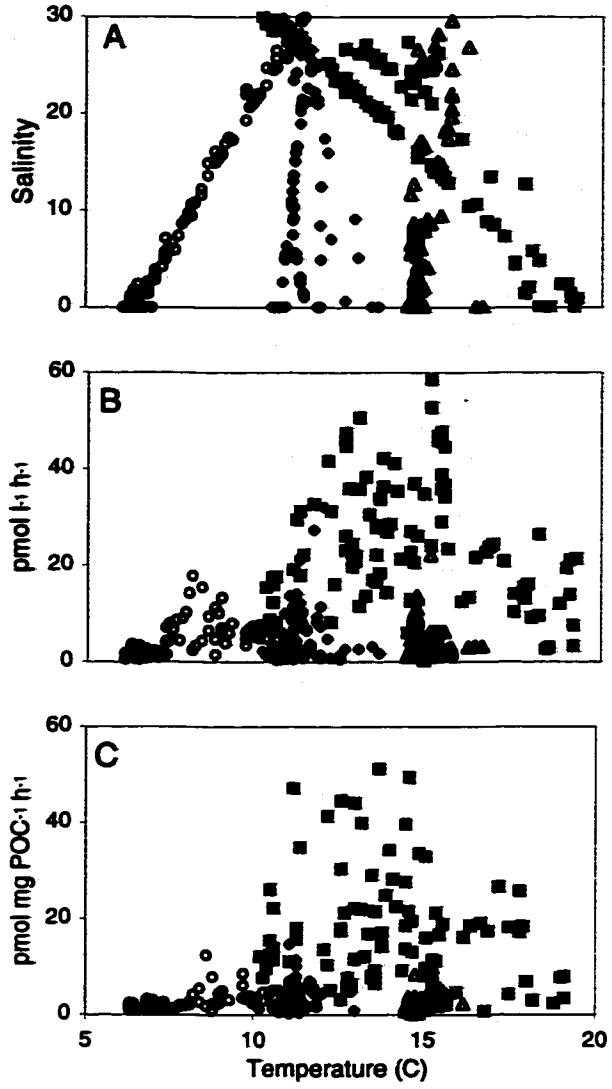


Fig. 5.8. Salinity (A), thymidine incorporation rate per liter (B), and thymidine incorporation rate per mg particulate organic matter (C) as a functions temperature for Spring (diamonds), Summer (boxes), and Fall 1997 (triangles), and Winter 1998 (circles).

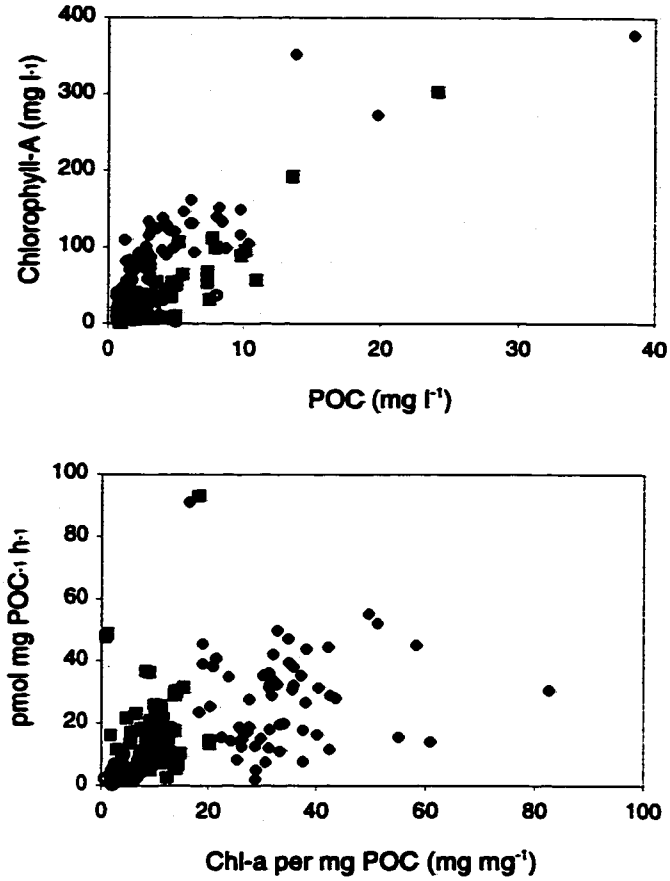


Fig. 5.9. Chlorophyll-a vs particulate organic carbon concentration and thymidine incorporation rate per mg POC vs Chlorophyll-a per mg POC for non-freshwater estuary samples from May, 1992 (diamonds), May, 1995 (boxes), and May, 1997 (circles).

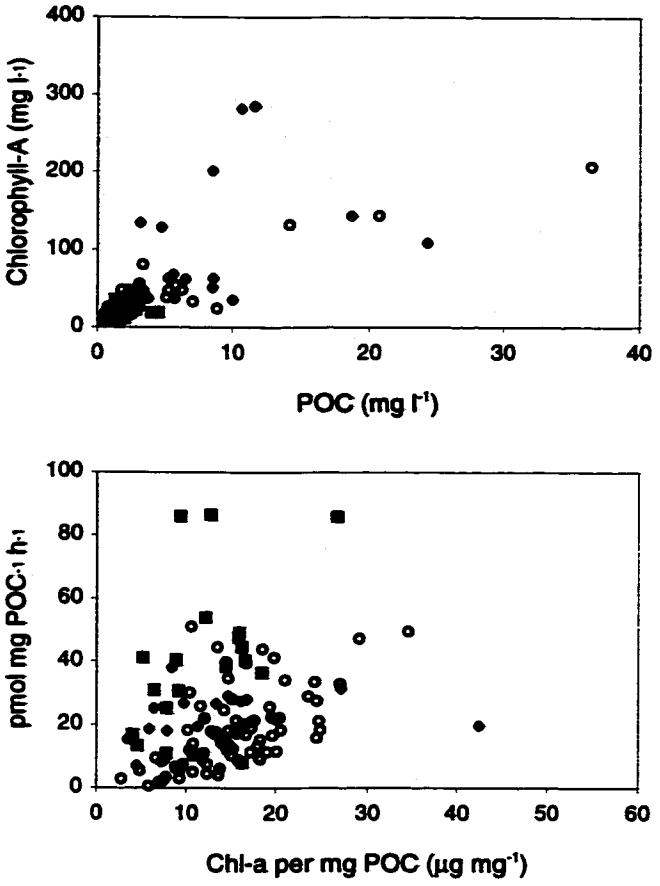


Fig. 5.10. Chlorophyll-a vs particulate organic carbon concentration and thymidine incorporation rate per mg POC vs Chlorophyll-a per mg POC for non-freshwater estuary samples from July, 1991 (diamonds), July, 1996 (boxes), and July, 1997 (circles).

Conclusions

The research presented in this dissertation takes a somewhat unusual approach to microbial ecology. Rather than relying entirely on "bulk" measurements of bacterial abundance and activity, we treated the microbial community in the Columbia River estuary as a complex mixture of different microbial assemblages and, using a number of techniques, identified the most active of these assemblages. We found that the majority of bacterial activity in the estuary is due to a sub-population of particle-attached bacteria associated with a sub-set of the particles. This assemblage is concentrated in ETM, and is composed, in part, of a native estuarine community of bacteria that may be uniquely adapted to the conditions of life on particles in the ETM. The particles that host these bacteria, called "bacterially-active" particles in this dissertation, are small (< 10 μm), slow-settling micro-aggregates that appear to aggregate into larger, faster-settling macro-aggregates in ETM.

The dominance of particle-attached bacteria in the Columbia River estuary in all seasons of the year links bacterial activity to the hydrodynamics of particle cycling in ETM. ETM particle trapping extends the residence time of particles in the estuary and therefore creates a relatively persistent habitat in an otherwise rapidly flushed system. Bacteria attached to these particles have more time relative to free-living bacteria to degrade and consume detrital organic matter, and to grow and develop in the estuary. Particle trapping in ETM also makes particles and particle-attached bacteria available to consumers in the estuary by concentrating them in the ETM regions.

The estuarine biological community augments the trapping efficiency of ETM by contributing to the formation of rapidly-settling aggregated particles. Particle-attached bacteria, particularly those related to *Cytophaga spp.* identified in Chapter 3

(Reichenbach 1989), produce exopolysaccharide material as part of the process of attachment and biofilm formation. This material can act as a glue that enhances particle aggregation (Eisma 1993). Polysaccharide material may also be released from freshwater phytoplankton as they expire in the elevated salinity of the estuary. Freshwater diatoms contribute dense silica frustules that increase the average density of aggregated ETM particles. Copepods directly create faster-settling aggregated particles by consuming ETM particles and releasing them as fecal pellets. ETM trapping is therefore more than just a physical-chemical phenomonon, and, moreover, the contribution of copepods and bacteria to particle formation feeds back to benefit the entire ETM community by maintaining the stability of the habitat.

The model presented in Chapter 2 brings together many of these discoveries and describes a hypothesis of how bacterially-active particles cycle in the estuary relative to the ETM and to the other members of the estuarine food web. A cartoon of a flood tide ETM event based on this model is displayed in Fig. 6.1. Bacterially-active particles are resuspended early during the tide and carried up or down estuary (Fig. 6.1A) along with particle-associated protozoa and rotifers, and aggregates are broken up by shear forces near the bed. Copepods swim up into the water column with these particles and graze on them. Then as the strength of the tide increases (Fig. 6.1B), bed stress is maximized and the rest of the ETM particles, including less-bacterially-active material, are resuspended, and aggregates are further broken up by shear forces in the water column. Later, as the tide slows down (Fig. 6.1C), less-bacterially-active particles settle out, leaving the slower-settling bacterially-active particles in the water column for further consumption by copepods. By slack tide (Fig. 6.1D), bacterially-active particles form more rapidly settling macro-aggregates and sink to the surface of the sediments where they will be the

first particles resuspended with the next tide. So, bacterially-active particles cycle differently in the estuary than the bulk of ETM particles, but they are still trapped and concentrated in the ETM regions. This model provides a conceptual framework connecting the research presented in this dissertation to research being conducted by other members of the Columbia River estuary ETM group on sediment dynamics, food web dynamics and biogeochemistry.

This model also provides a basis for hypotheses concerning the development and maintenance of the estuarine community of bacteria. The estuarine bacterial community in ETM identified in Chapter 3 could be maintained in the estuary by a number of different mechanisms. It could simply be passed from old ETM particles to new ETM particles as these particles aggregate in the water column or on the bed. It could also be seeded with bacteria from mudflats and marshes located in the estuary's peripheral embayments. Copepods may play an important role in maintaining the estuarine bacterial population by seeding particles with bacteria from their own gut flora as they consume particles and release fecal pellets. Multiple refugia exist for estuarine bacteria in this system. Enumerating these bacteria and tracing their movement in the estuary with the use of phylogenetic probes could reveal the importance of each potential refugia, and would help determine how anthropogenic changes to the ecosystem may influence the ETM community.

The relationship between bacteria, the principal consumer of detrital organic matter, and copepods, the most important metazoan consumer, is potentially very complex. Particle-attached bacteria probably provide some nutrient to particle-consuming copepods, but they may also provide active extracellular enzymes such as cellulase and other carbohydrate-ases that the copepods cannot produce but aid in the decomposition of

detritus in the copepod's guts.

The relationship between ETM and bacterial activity in the Columbia River estuary demonstrates the importance of multi-disciplinary research. Our understanding of microbial ecology in the estuary would have been severely limited without an understanding of the hydrodynamics involved in ETM particle trapping, the sources and composition of ETM particles, and the behavior of copepods. Ecosystems such as the Columbia River estuary are remarkably interconnected, and it seems likely that members of a biological community, including bacteria, evolve together to benefit from their physical and chemical environment.

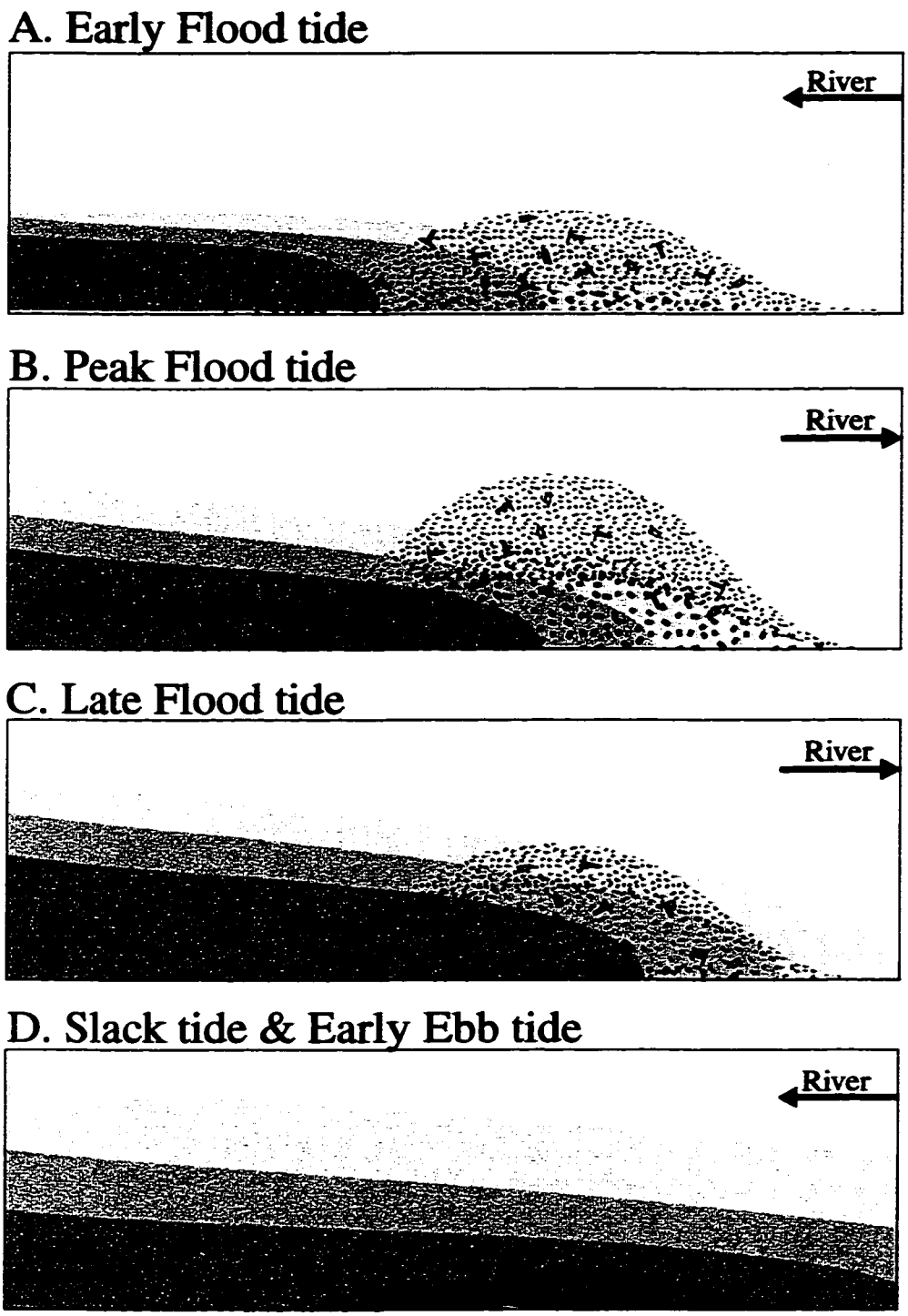


Figure 6.1. Conceptual model of bacterially-active particle cycling in the Columbia River ETM during a flood tide. Freshwater is in white and salt water is in gray. Bacterially active particles are in blue. Less bacterially-active particles are in black. Rapidly-settling aggregates of bacterially-active particles are in red. The drawing includes copepods and rotifers.

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Vita

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Education

1999 Doctor of Philosophy. University of Washington, School of Oceanography, Seattle, Washington.

1996 Master of Science. University of Washington, School of Oceanography, Seattle, Washington.

1990 Bachelor of Arts. Department of Biology, Oberlin College, Oberlin, Ohio

Professional experience

1995-1996 & 1996-1997 Teaching Assistant for Senior Research Project course for Oceanography majors. Mentored students as they designed research projects, wrote research proposals, conducted field and laboratory research, composed final papers and gave oral presentations. University of Washington, Seattle, Washington

1995 & 1996 Organizer for Annual University of Washington – University of British Columbia Biological Oceanography Student Exchange meeting. Hosted meeting in 1995.

1992-1999 Research assistant (Graduate Student), University of Washington, Seattle, Washington

1990-1992 Research scientist (Oceanographer I), University of Washington, Seattle, Washington

1989 Summer Intern, National Science Foundation REU program, laboratory of Evelyn J. Lessard, Horn Point Environmental Labs, Cambridge, Maryland.

Research interests

Microbial ecology. Organic matter cycling. Microbial food web structure. Influence of hydrodynamics and particle cycling on microbial activity. Composition and development of microbial communities using molecular methods.

Refereed publications

Crump BC, Armbrust EV, Baross JA (1999) Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol 65:3192-3204

Crump BC, Baross JA, Simenstad CA (1998) Dominance of particle-attached bacteria in the Columbia River estuary, USA. Aquatic Microbial Ecology 14:7-18

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Manuscripts submitted or in preparation

Crump BC, Baross JA. Characterization of the bacterially-active particle fraction in the Columbia River estuary. Submitted to Mar Ecol Prog Ser

Crump BC, Baross JA. Archaeaplankton in the Columbia River, its estuary, and the adjacent coastal ocean. In Prep

Crump BC, Simenstad CA, Bolton SA, Baross JA. Seasonal and inter-annual variability of bacterial activity in the Columbia River estuary. In Prep

Abstracts

Crump BC, Baross JA (1999) Characterization of the bacterially-active particle fraction in the Columbia River estuary. General Meeting of the American Society for Microbiology, Chicago, IL, May 30 to June 3, 1999 (Poster presentation)

Crump BC, Baross JA (1999) Archaea in the Columbia River, estuary, and adjacent coastal ocean. Sixth annual NSF Hyperthermophile Symposium, Athens, GA, May 19-21, 1999 (Poster presentation)

Crump BC, Armbrust EV, Baross JA (1998) Ecology and phylogeny of bacterial communities in the Columbia River estuary, USA. Eigth International Symposium on Microbial Ecology, Halifax, Nova Scotia, Canada. August 9-14, 1998 (Poster presentation)

Crump BC, Armbrust V, JA Baross JA (1998) The Influence of Hydrodynamics on Particle-Attached and Free-Living Bacteria in the Columbia River Estuary. American Society of Microbiology General Meeting, Atlanta, Georgia. May 17-21, 1998 (Poster presentation)

Crump BC, Armbrust EV, Baross JA (1998) The influence of hydrodynamics on particle-attached and free-living bacteria in the Columbia River estuary. Fifth Annual NSF Hyperthermophile Symposium, Seattle, Washington. May 27-29, 1998 (Poster presentation)

Crump BC, Baross JA, Simenstad CA (1997) Dominance of particle-attached bacteria in the Columbia River estuary, USA. Land Margin Ecosystems Research all scientists' meeting, Solomons, Maryland. November 13-15, 1997 (Poster presentation)

Crump BC, Baross JA (1996) Tidal variability in bacterial activity associated with particles and aggregates in the Columbia River estuarine turbidity maxima. ECSA 26 and ERF 96 Symposium, Middelberg, The Netherlands. September 16-20, 1996 (Oral presentation)

Crump BC, Baross JA (1996) Particle-attached bacteria in the Columbia River estuary. Pacific Estuarine Research Society annual meeting, Olympia, Washington. (Received 3rd place in student poster division)

Crump BC, Baross JA (1996) Particle-attached bacteria in the Columbia River estuary. Land Margin Ecosystems Research all scientists' meeting, Savannah, Georgia. November 7-9, 1996 (Poster presentation)

Holden JF, Crump BC, Summit M, Baross JA (1995) Microbial blooms at the CoAxial Segment, Juan de Fuca Ridge deep-sea hydrothermal vent site following a magma intrusion. Second Annual NSF Hyperthermophile Symposium, University of Georgia, Athens, Georgia. April 19-21, 1995 (Poster presentation)

Holden JF, Crump BC, Summit M, Baross JA (1995) Microbial blooms at the CoAxial Segment, Juan de Fuca Ridge deep-sea hydrothermal vent site following a magma intrusion. Fall meeting, Geology and Geophysics of the Juan de Fuca Ridge, University of Washington, Seattle, Washington (Poster presentation)

Crump BC, Baross JA (1994) Bacteria and their grazers in the turbidity maxima of the Columbia River estuary. Land Margin Ecosystems Research all scientists' meeting, Gloucester, Massachusetts. October 27-30, 1994 (Poster presentation)

Crump B, Baross JA (1994) Investigations of thermophilic and hyperthermophilic viruses. NSF Symposium on Hyperthermophiles - 1994, University of Washington, Seattle, Washington. March 18, 1994 (Poster presentation)

Crump BC, Baross JA, Morgan CA, Simenstad CA. (1993) Suspended particles and moderate salinity combine in the Columbia River estuarine turbidity maximum (ETM) to stimulate bacterial production: Microbial loop implications. Land Margin Ecosystems Research all scientists' meeting, Seaside, Oregon, October 16-20, 1993 (Poster presentation)

Baross JA, Holden JF, Pledger RJ, Crump BC, Summit M. (1993) Submarine vent *Archaea*: Ecology and strategies for growth above 90° C. Abstracts of papers American Chemical Society vol. 205, no. 1-2, p BTEC 7. 205th ACS national meeting, Denver, CO. March 28-Apr. 2, 1993

Baross JA, Holden JF, Pledger RJ, Crump BC, Summit M (1993) Submarine vent *Archaea*: Ecology and strategies for growth above 90° C. Proceedings of the International Summer Seminar on Deep-Sea Microorganisms of the Japan Marine Science and Technology Center. Deep Star Group, JAMSTEC. p 69-73

Baross JA, Holden JF, Crump BC, Summit M (1993) An examination of the evidence that Archaen microbial ecosystems were associated with deep-sea hot springs and implications for the existance of novel thermophiles and ancient genotypes.

Abstract, New Zealand workshop on extreme thermophiles, Hamilton, New Zealand. December 1993

Invited Presentations

Crump BC, Baross JA. Bacterial activity and community composition in the Columbia River estuarine turbidity maxima. Seminar given at the Ecosystems Center, Marine Biological Laboratories, Woods Hole, MA. June 7, 1999 (Oral presentation)

Crump BC. Analysis of Columbia River estuary bacteria using 16S rRNA clone libraries. University of Washington Oceanography graduate course Principles and Applications of Molecular Methods. November 18, 1998 (Oral presentation)

Crump BC, Armbrust EV, Baross JA. Using molecular techniques to study the influence of hydrodynamics on particle-attached and free-living bacteria in the Columbia River estuary. Seminar given at University of Georgia, May 22, 1998 (Oral presentation)

Crump BC, Armbrust EV, Baross JA. Using molecular techniques to study the influence of hydrodynamics on particle-attached and free-living bacteria in the Columbia River estuary. University of Washington Biological Oceanography Departmental Seminar. May 12, 1998 (Oral presentation)

Crump BC, Baross JA. Columbia River LMER Site Highlights presentation: Recent discoveries and future directions. LMER all scientists' meeting, Solomons, Maryland. Nov. 13-15, 1997 (Oral presentation)

Crump BC, Baross JA. Fractionating particles in search of attached bacteria in the Columbia River estuary. University of Washington Biological Oceanography Departmental Seminar. June 3, 1997 (Oral presentation)

Crump BC, Baross JA. Past, present and future of research into the microbial ecology of the Columbia River estuary. CRETM-LMER II Spring 1996 annual review meeting (Oral presentation)

Crump BC, Baross JA. Particle-attached bacteria in the Columbia River estuary. CRETM-LMER II Spring 1996 annual review meeting (Poster presentation)

Crump BC. Bacterial activity and community structure in the Columbia River estuarine turbidity maxima. General exam presentation. January 17, 1996 (Oral presentation)

Crump BC, Baross JA. Particle-attached bacteria and heterotrophic plankton associated with the Columbia River estuarine turbidity maxima. Masters defense. University

of Washington Biological Oceanography Departmental Seminar. September, 1995 (Oral presentation)

Reports

Crump BC, Baross JA (1998) Dominance of particle-attached bacteria in the Columbia River estuary, USA. LMER News - Newsletter of the Land Margin Ecosystem Research Program. Fall issue, p 2-5

Field work experience

Location	Year	Vessel
Columbia River estuary	1999	RV Robert Gordon Sproul
Fraser River estuary	1999	RV Robert Gordon Sproul
Columbia River estuary	1998	RV Wecoma
Columbia River estuary, 3 cruises	1997	RV Robert Gordon Sproul
Columbia River estuary	1996	RV Robert Gordon Sproul
Elliot Bay of Puget Sound	1996	RV Clifford Barnes
Budd Inlet of Puget Sound	1996	
Columbia River estuary	1995	RV Robert Gordon Sproul
Duwamish River estuary	1995	RV Weelander
Endeavour segment of the Juan de Fuca Ridge, NE Pacific Ocean	1995	RV Atlantis & DSV Alvin
Mt. St. Helens blast zone hot springs	1994	
Columbia River estuary	1992	RV Robert Gordon Sproul
Columbia River estuary	1991	RV Robert Gordon Sproul
Endeavour segment of the Juan de Fuca Ridge, NE Pacific Ocean	1991	RV Atlantis & DSV Alvin
Columbia River estuary	1990	RV Robert Gordon Sproul