The Transport of Bacteria in The Sediments of a Temperate Marsh

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Abstract:
The number of bacteria in sediments, interstitial water and overlying tidal water of an oligohaline marsh system are about 10^9, 10^6, and 10^6 cells cm^{-3}, respectively. Average cell size in the overlying water (about 0•06 μm^3), is much smaller than that in sediments and interstitial water (about 0•18 μm^3). Most bacterial cells in sediments are bound to sediment particles and less than 1% of the cells were displaced by percolating water through sediment columns. Concentration of bacteria in flooding tidal waters is generally higher than that in ebbing waters. Movement of bacterial biomass does not appear to be a significant mechanism of particulate organic transport in marsh sediments and marsh sediments do not appear to be a source of suspended bacteria for estuaries.
Keywords: bacteria; micro-organisms; salt marshes; estuaries; sediment

Article:

Introduction

Microbial biomass movement may be an important mechanism of organic nutrient transport in estuarine systems. Correll et al. (1975) followed movement of radioactively labelled orthophosphate applied to a marsh surface. They found that the label moved rapidly into the acid labile phosphorus pool, which is indicative of organically incorporated compounds. Significant vertical and horizontal movement of the pool was observed over a four month period. They concluded that orthophosphate underwent rapid uptake by micro-organisms with subsequent movement of the microbial cells. Such microbial transport might aid in resolving the controversy regarding export of macrophyte production from marshes. If particulate detrital transport from marshes is indeed less than originally thought (c.f. Haines, 1979) and the organic content of marsh sediments is not increasing dramatically (Valiela et al., 1976) then alternate pathways of organic losses must exist. Microbial breakdown of organic matter within the marsh followed by movement of microbial biomass out of the marsh may be one such pathway of export. Stevenson et al. (1974), using plate count methods, found higher bacterial densities in ebbing tidal waters and suggested that sediments were the source.

Previous studies of microbial transport have dealt with planktonic portions of estuarine systems or have traced movement of various innocula through soils. One study in South Carolina (Erkenbrecher & Stevenson, 1978) examined biomass fluxes of the total suspended microbial community (measured by adenosine triphosphate concentrations) and of aerobic heterotrophic bacteria (by plate counts). They found a net inward transport of aerobic heterotrophic bacteria, but some net outward movement of the total microbial biomass. Investigations of the movement of micro-organisms through soils appears restricted to amendments of organisms with unique characteristics or abiotic models such as latex spheres and monitoring their displacement from the source (e.g. Wollum & Cassel, 1978; Lahav & Tropp, 198o). To our knowledge movement of endemic microbial populations in sediment environments has not been examined.

The objective of this study was to examine the question of microbial transport in marsh sediments. Our hypothesis, based primarily on the results of Correll et al. (1975), was that microbial movement acts as a mechanism of nutrient transport in sediments. We chose to look specifically at one component of the microbial

community, the bacteria, since they are the dominant constituent of microbial biomass in marsh sediments, particularly below the surface centimetre (Rublee, 1982a, b). Bacterial movement was studied by three means: (i) comparative bacterial concentrations and cell sizes were measured in sediments and in interstitial, seepage and tidal creek waters; (ii) the number of bacteria flushed from sediment cores by percolation of filtered water through the core was determined; (iii) bacterial concentrations were measured in flooding and ebbing tidal waters as an index of mass flux.

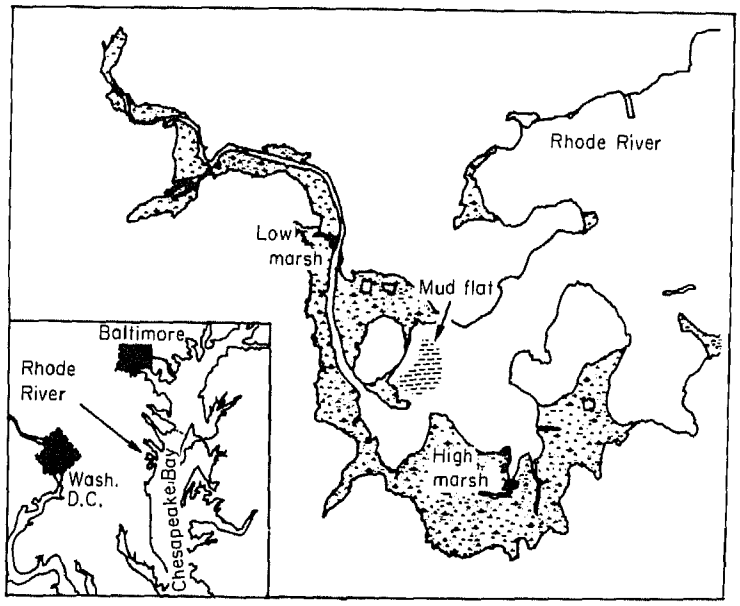

Figure 1. Map of Rhode River, illustrating location of the sampling stations. Inset shows relative location of Rhode River.

Materials and methods

The site of this study was the Rhode River Estuary, an oligohaline temperate subestuary of Chesapeake Bay in Maryland (Figure I). Tidal amplitude in the Rhode River is about 35 cm, although meteorological conditions may alter the water level by as much as 1•5 m. Three stations were studied. The first, a high marsh station, consisted of a tidal creek surrounded by mixed macrophyte vegetation including *Hibiscus, Scirpus olneyi, Spartina patens, Distichlis spicata, Spartina cynusoroides* and *Iva frutescens*. Second, a low marsh station included a tidal creek surrounded by a *Typha angustifolia* and *Scirpus olneyi* dominated marsh. The third station was a subtidal mud flat which occasionally exhibits submerged macrophyte vegetation. A series of groundwater wells were placed in a transect perpendicular to the tidal creek in each marsh. The wells were perforated PVC pipes of 3•8 cm inside diameter. The bottom of each tube was sealed and tubes were placed in the sediment so that the perforations extended from 5 to 60 cm below the surface. Interstitial water flow in the low marsh is predominantly between 20 to 40 cm of depth below the marsh surface (T. Jordan, personal communication).

Bacteria were enumerated and sized using the acridine orange direct count (AODC) method (Hobbie et al., 1977; Rublee & Dornseif, 1978). Each sample was stained for i min with acridine orange (0•01% in water) and filtered through a 0•2 μm pore Nuclepore filter, previously counterstained with irgalan black. A minimum of 10 microscope fields were examined or 200 cells enumerated on each filter. Cell sizes were determined by measuring the diameter (cocci) or length and width (rods) of at least too cells per sample. Cell volumes could then be estimated and an average cell volume determined as well as the distribution of cell sizes. Recently Fuhrman (1981) has determined that epifluorescent determinations of cell sizes are similar to those found by scanning electron microscopy. Interstitial water was sampled from the groundwater wells by first pumping the wells dry, and then sampling the recharged wells 15 to 45 min later. On two occasions we were also able to sample interstitial water from seeps located on the banks of marsh creeks. Interstitial and tidal water samples were preserved with formaldehyde (2% final concentration). All samples were stored at 4° C and counted within two weeks of collection.

Bacterial concentrations in marsh tidal waters were determined from volume-integrated composites of flooding

and ebbing water on 11 tidal cycles over one and a half years. Hourly water samples were taken at flumes built across the high and low marsh creek mouths. Each flume was calibrated and flow measurements were taken at 5 min intervals. Samples were composited from this information based on the total flow. Preliminary studies confirmed that volume-integrated composite water samples yielded mean bacterial concentrations statistically similar to that found by counting hourly samples and correcting for the contribution of each based on flow.

Sediment cores for bacterial counts and those used in the sediment percolation experiments were taken to a depth of at least 20 cm with a 5•7 cm diameter plexiglass core tube. Cores were returned to the laboratory and extruded from the tube for analysis. For direct counts and sizing of bacteria, the cores were subsampled at the surface and at 20 cm by filling 0•1 cm^3 planchets with sediment and placing them in 20 ml of a prefiltered formaldehyde solution (2% final concentration v/v). For sediment percolation experiments no formaldehyde was used. Vertical and horizontal subcores from 2 to 3.6 cm in length were taken with 1.7 cm diameter tubes. Vertical subcores were taken downward from the sediment surface and horizontal subcores were taken parallel to and just below the surface. Ends of the subcore tubes were covered with 153 μm mesh Nitex netting. Tubes were oriented vertically and about 40 ml of prefiltered (0•2 μm pore filters) estuarine water was added to the tube taking care not to disturb the sediment surface. Column effluent was collected in a fraction collector and bacterial concentrations in fractions were determined immediately upon collection of each fraction by AODC. Columns were generally run for 3•5 to 4•5 h.

Results

The concentrations of bacteria were similar in tidal and interstitial water, but the concentration of bacteria in sediment samples was three to four orders of magnitude higher than the concentrations in the water (Figure a). The concentration of bacteria ($X \pm$ S.E.) found in tidal waters of the marsh sites was 6•81 ± 0•85 × 10^6 cells ml^{-1} (n=44). Interstitial water samples from the marshes had a mean concentration of 6•25 ± 0•85 × 10^6 cells ml^{-1} (n = 48). Concentrations in seeps from creek banks in the high marsh, measured on two occasions, were 2•49 and 3•17 × 10^6 cells ml^{-1}. In contrast, the concentration of bacteria in whole sediments (which includes those in the interstitial water of the sample) from both marshes ranged from an average of 1•38 ± 0•28 × 10^{10} cells cm^{-3} (n = 9) at the sediment surface to 0•55 ± 0•19 × 10^{10} cells cm^{-3} (n = 7) at a depth of 20 cm.

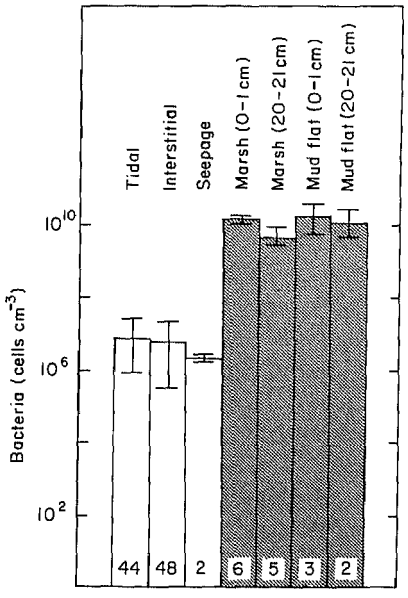

Figure 2. Distribution of bacteria in various samples from tidal water, interstitial water and sediments (▨) of the Rhode River. Mean and range of cell counts are indicated, number of samples is indicated by number at base of each bar.

Bacterial cell sizes were similar in whole sediments and interstitial water. The average volume of 900 cells from sediments was 0•17 μm^3 compared to 0•18 μm^3 for 600 cells from interstitial water samples. The mean cell volume of bacteria in overlying tidal waters, determined by examination of 400 cells was 0•06 μm^3, about one-third that of the sediment and interstitial cells. The range of individual cell volumes from all sources spanned

nearly three orders of magnitude (Figure 3). This range resulted in coefficients of variation for average cell size estimates of greater than 100%.

Sediment percolation studies to examine bacterial movement in soil columns indicated that only a small percentage of the bacteria were washed from the sediments. Bacteria concentrations in the effluent exhibited an initial peak concentration in the range of 10^7 cells ml^{-1}, followed by a sharp decrease to values in the range of 10^6 cells ml^{-1} (Figure 4). The total number of cells washed from each sediment column was less than 1×10^9 cells, which represented less than 1% of the total number of cells in the soil columns. This pattern was similar for all samples from the high marsh, low marsh, and the mud flat whether the subcore was taken vertically or horizontally from surface sediments. The rate of effluent flow and thus total volume of effluent collected from the soil columns differed between samples, a result of the different heights of sediment in the subcores and different proportions of organic matter, silt, and clay in various samples. In all cases, however, flow exceeded our best estimate of the natural rate of interstitial water flow (T. Jordan, personal communication).

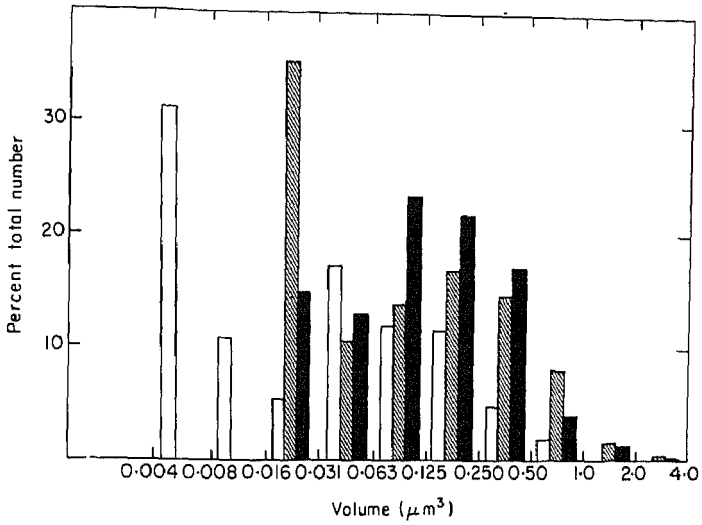

Figure 3. Cell volume distribution in tidal water (□), interstitial water (▨) and intertidal sediments (■) of the Rhode River.

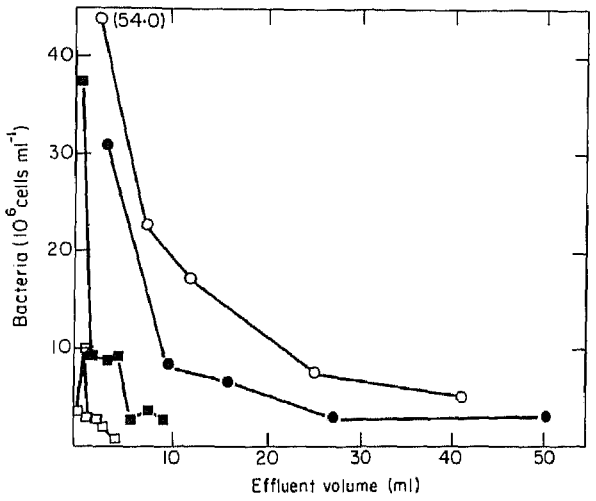

Figure 4. Bacterial cells recovered in effluent of soil percolation columns from high Marsh samples. Source of sample and the orientation of the original core: ○, surface →; ●, surface ↑; □, creek bed →; ■, creek bed ↑.

Tidal flux studies suggested an import of bacteria into the marshes, Concentrations of bacteria were generally higher on incoming than ebbing tides in both marshes (Table 1). Water flow on any tidal cycle may not balance in our marshes due to meteorological changes which can prevent flooding of the marshes for a number of tidal cycles. During these periods a constant slow drainage is maintained. On the next tide which does flood the marsh, a significant volume of water may be `absorbed', in the manner of a sponge, and retained through the

succeeding ebb period. Since such tidal asymmetry is common in these marshes, we have not calculated net flux of bacterial biomass. We do feel, however, that the bacterial concentration data are a reasonable indicator of flux.

TABLE 1. Bacterial concentration in flooding and ebbing waters of low and high marsh sites on the Rhode River. Values are mean (±S.E.) and are derived from flow-composited samples collected hourly over each tidal cycle. Values are 10^6 cells ml^{-1}. Positive net values indicate flux into the marsh

	Low marsh			High marsh		
Date	Flood	Ebb	Difference	Flood	Ebb	Difference
6/23/80	2·1 (0·1)	1·6 (0·1)	+0·5	2·3 (0·2)	1·7 (0·1)	+0·6
7/7/80	5·1 (0·1)	5·2 (0·3)	+0·1	6·7 (1·5)	5·8 (0·5)	+0·5
7/29/80	23·9 (1·6)	17·0 (1·3)	+6·9	10·5 (1·0)	15·1 (2·9)	−4·6
8/12/80	13·4 (0·7)	11·7 (2·0)	+1·7	12·8 (1·0)	15·1 (2·7)	−2·3
11/4/80	13·0 (1·0)	10·0 (1·3)	+3·0	20·5 (2·3)	8·1 (1·5)	+12·4
12/17/80	6·2 (0·7)	11·2 (3·7)	−5·0	9·6 (1·1)	4·9 (1·0)	+4·7
3/2/81	3·3 (0·2)	3·8 (0·2)	−0·5	2·6 (0·2)	4·2 (0·6)	−1·6
4/28/81	5·1 (0·4)	3·8 (0·4)	+1·3	2·8 (0·3)	8·6 (1·3)	−5·8
5/27/81	3·9 (0·2)	4·5 (0·9)	−0·6	3·1 (0·6)	3·2 (0·6)	−0·1
9/8/81	2·4 (0·9)	2·2 (0·2)	+0·2	2·1 (0·2)	1·4 (0·2)	+0·7
10/21/81	0·8 (0·1)	2·8 (0·2)	−2·0	3·9 (0·3)	1·2 (0·1)	+2·7
Means for all samples	7·2 (2·2)	6·7 (1·6)	+0·5	7·0 (1·9)	6·3 (1·6)	+0·7

Discussion

Bacterial cell counts from tidal water, interstitial water, and sediments demonstrate marked differences in concentration between the sediments and the surrounding waters. The concentrations of bacteria found in this study are similar to those found in other estuarine systems (e.g. Palumbo, 198o; Rublee, 198za,b). The concentrations of bacteria in seepage water from the marsh sediments and in interstitial water are in the same range as the overlying water, and about three orders of magnitude less than in the sediments as a whole. This suggests a strong binding of bacterial cells to sediment particles.

Although there is a wide statistical confidence limit about the average bacterial cell sizes it seems apparent that the assemblage of cells found in sediments is different than the assemblage in overlying waters. Ferguson & Rublee (1976) found the average cell volume of bacteria in near shore coastal waters to be 0•09 μm^3. Bowden (1977) confirmed the small size (<0.05 μm^3) of planktonic estuarine bacterial populations with the use of scanning electron microscopy. These values compare favorably with determinations of a mean cell volume of 0•06 μm^3 from two water samples taken adjacent to the low marsh. In contrast, the average cell sizes found in interstitial waters and sediments were three times larger, 0•18 and 0•17 μm^3, respectively, values similar to those found by Rublee (1982b) in a North Carolina salt marsh and by J. E. Hobbie and J. Helfrich, (personal communication) in a Massachusetts marsh. Note, however, that the interstitial samples contain a similar proportion, 46%, of small cells (less than 0•06 μm^3) as the tidal water samples. Thus, the interstitial water cells may represent a gradient between total water and sediment assemblages. The high proportion of small cells in the overlying water may be an indication that sediments are not the primary source of suspended bacteria. An alternate explanation may be starvation and shrinkage of sediment bacteria, but this is not likely since the Rhode River is a eutrophic system (Correll, 1978).

The sediment percolation experiments support the concept of a sediment bound bacterial assemblage. The pattern of exponential decrease in bacterial numbers seen in all of the percolation experiments may stem from several processes. The initial peak may be the result of a flush of unattached or loosely bound cells and the displacement of small sediment particles (and associated bacterial cells) from the soil column. We noticed that initial fractions appeared turbid on some occasions. On one occasion we counted cells which were 'attached', that is, on or adjacent to sediment particles. The number of attached cells in the effluent was initially a large proportion of the total number of cells (about 80%), but after 2-3 ml of filtered water had passed through the column the proportion decreased to zero. An additional process is one of growth and sloughing off of actively metabolizing bacteria from particles. This may be the process which is achieved over the longer term, and may be likened to a continuous culture. We restricted our percolation runs to a maximum of six hours with the expectation that longer time periods would result in production and sloughing of bacterial cells. This process

undoubtedly occurs in the natural system, but the relatively low level of transport that we found after several hours in the percolation columns suggests that such production and transport is of the same order of magnitude as overlying water concentrations ($\approx 10^6$ cells ml^{-1}) and thus represents an insignificant loss from the sediments (< 0•1% per day).

Transport of bacteria is a function of both physical and biological processes. Physical processes such as porosity and rate of water flow are intimately related and would be a major controlling factor on whether cells are filtered from the medium or pass freely. Biological factors include microbial attachment to particles, a process that is well documented in the literature (e.g. Costerton et al., 1978). Our use of undisturbed columns to monitor transport of the natural microflora represents a realistic approach to the estimation of the ecological importance of microbial transport. However, our method is still likely to over-estimate the true rate of microbial transport since the flushing rates of water we generated in most columns exceeded the interstitial water movement in our marshes. Our suggestion of a sediment bound bacterial assemblage is also consistent with previous results reported by Rublee (1982a). He found that homogenization was necessary for removal of bacteria from particles to effectively count cells using the AODC method. After gently shaking sediment samples, only 16% of the cells appeared free of particles. In contrast, a 1 min homogenization in a commercial blender yielded greater than 50% of the observable cells free of particles. Together with our data these results yield strong evidence for a tighly bound assemblage of natural sediment bacteria.

The tidal study data provides a further, indirect, but consistent piece of evidence for a lack of bacterial transport from marsh sediments. Our data indicate an import of bacteria into the tidal marshes (Table 1), a finding similar to those of Erkenbrecher & Stevenson (1978), who found an import of aerobic heterotrophic bacteria into a South Carolina tidal marsh, and R. B. Coffin & R. T. Wright (personal communication), who found an import of total bacteria into a New Hampshire marsh. Further, bacteria are not conservative particles. Determinations of heterotrophic activity by ^{3}H-thymidine uptake in tidal waters of our system indicate an active bacterial assemblage with estimated daily production to biomass ratios of 0•2-1•2 (Rublee et al., in prep.). Thus, if no net exchange occurred one would expect higher concentrations on ebb tides. Since we do not see this, some combination of sedimentation, cell lysis, or predatory loss must be occurring over the marsh. Our data also suggest that any exchange between suspended bacteria and sediment bacteria in the Rhode River is limited, since even a small loss from the surface sediments, say 1%, would represent a 10-fold increase in suspended bacterial concentrations. Three caveats must be stated, however. First, we have not disproved exchange between sediments and overlying water. It likely does occur but it must be on a relatively small scale. Second, we have not sampled tidal cycles during strong forcing events (e.g. storms) in which resuspension may be a dominant process. Preliminary evidence from shallow water riverine samples does suggest, however, that the fate of bacteria attached to resuspended sediment particles is resedimentation with the particle. A third caution is that since tidal amplitude in the Rhode River system is small, our system may not be typical of coastal tidal marshes with stronger tidal forces.

The lack of bacterial movement in sediments contrasts with the interpretation of phosphorus flux data presented by Correll et al. (1975). Their findings of a large and apparently mobile acid labile phosphorus pool (characteristic of prokaryotic organic phosphorus compounds) would seem at odds with our data. Although our data indicate that transport of bacterial biomass is probably not an important mechanism of elemental flux from sediments, we have no doubt of the high levels of bacterial activity. We tentatively suggest, therefore, rapid nutrient cycling mechanisms. For example, although the radioactively labelled phosphorus molecules are predominantly located in microbial cells, they may be rapidly cycled through a soluble organic phosphorus pool and then returned to the bacteria. In this manner the bacteria could play an extremely significant role in nutrient transformations and hence in elemental nutrient mobilization and immobilization. Certainly this simple hypothesis is speculative and more research will be necessary to confirm it or to find alternatives. A combination of biological and chemical approaches may lead us to a more factual understanding of the role of sediment bacteria in such nutrient cycling and transport.

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