Nutrient flux in the Rhode River: Tidal transport of microorganisms in brackish marshes

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Abstract:
Concentrations of bacteria, chlorophyll a, and several dissolved organic compounds were determined during 11 tidal cycles throughout the year in a high and a low elevation marsh of a brackish tidal estuary. Mean bacterial concentrations were slightly higher in flooding ($7.1 \times 10^6$ cells ml$^{-1}$) than in ebbing waters ($6.5 \times 10^6$ cells ml$^{-1}$), and there were no differences between marshes. Mean chlorophyll a concentrations were $36.7 \mu$g l$^{-1}$ in the low marsh and $20.4 \mu$g l$^{-1}$ in the high marsh. Flux calculations, based on tidal records and measured concentrations, suggested a small net import of bacterial and algal biomass into both marshes. Over the course of individual tidal cycles, concentrations of all parameters were variable and not related to tidal stage. Heterotrophic activity measured by the uptake of $^3$H-thymidine, was found predominantly in the smallest particle size fractions ( <10 μm). Thymidine uptake was correlated with temperature ($r=0.48$, $P<0.01$), and bacterial productivity was estimated to be 7 to 42 μg C l$^{-1}$ day$^{-1}$.

Keywords: bacteria, estuaries, microorganisms, salt marshes, nutrients, tidal cycles, wetlands, Chesapeake Bay

Article:

Introduction

Several decades of marsh research have revealed that it is difficult to draw simple generalizations about structural and functional attributes of marsh systems (Nixon, 1980; Odum, 1980). Specifically, the paradigm that marsh systems export carbon and nutrients has been reevaluated and recent work has demonstrated that detrital export, at least from some marsh systems, may not be large (e.g. Haines, 1979). In contrast, the importance of within-system detrital food webs has been emphasized since the magnitude of belowground production and decomposition appears to be large (e.g., Howarth & Teal, 1979). Thus, an understanding of nutrient dynamics in marshes has been the focus of much of the recent marsh and estuarine research.

Microbial production in marshes is significant. Decomposition of macrophyte production in marshes is an important process (Howarth & Teal, 1979) and heterotrophic microbial biomass production must be high as bacteria and fungi are the primary decomposer organisms. Additionally, microalgal production on marsh surfaces is important (Ribelin & Collier, 1979; Rublee, 1982). This represents a primary production source in addition to macrophytes that might be exported. Finally, Howarth & Hobbie (1982) speculate that chemosynthetic bacterial production is large: another source of microbial biomass.

One component of particulate export from marshes may be microbial cells. Stevenson et al. (1974) suggested that the sediments of marshes might be a source of suspended bacteria based on the characteristics of bacterial isolates from tidal waters. In later studies from the same system Erkenbrecher & Stevenson (1975) found that while ATP was exported from the marshes, there was an import of aerobic, heterotrophic bacteria. Thus, the net exchange may be inward rather than the marsh acting as a source. Rublee et al. (1983) have found that movement of bacteria through the sediments appears minimal, and therefore, sediment bacteria are probably not important agents of organic material transport.

This study examined the hypothesis that transport of suspended microbial biomass was a significant mechanism
of elemental movement from marshes in tidal waters of the Rhode River, a subestuary of Chesapeake Bay. We describe the dynamics of the suspended bacterial and algal components in tidal waters, bacterial activity, and their relationships to some nutrient and physical parameters.

**Materials and methods**

The Rhode River is (Fig. 1) a subestuary of Chesapeake Bay on the east coast of North America (38°51′ N, 75°32′ W). It is surrounded by a mixed use watershed of about 3200 ha and is typical of temperate coastal plain estuaries. Salinity in the river ranged from 0 to 17 ppt, slightly higher than usual because of drought conditions during the course of the study. Water temperatures ranged from near 0 to 30 °C. Tidal amplitude is approximately 35 cm in the river although meterological conditions may alter the water level by more than 1 m. Thus, tidal flooding of marshes in the system is frequently irregular and flooding and ebbing tidal volumes do not always balance on any given tidal cycle.

![Diagram of the intertidal zone of the Rhode River including marshes and mudflat. Study sites refer to marsh areas where tidal exchanges were monitored. Inset shows the location of the Rhode River.](image)

We sampled eleven tidal cycles from both a high and a low marsh station (Fig. 1). The high marsh was of mixed vegetation, including *Spartina cynosuroides, S. patens, Distichlis spicata, Scirpus spp.*, *Iva frutescens*, and *Hibiscus* spp. The low marsh, located further up the estuary, was dominated by *Typha angustifolia* and *Scirpus* spp. Each marsh was sampled from a flume built across the mouth of tidal creeks draining the marshes. Sampling generally began at slack low tide and continued until the next slack low. All water samples were taken just below the surface by immersion of 11 linear polyethylene bottles. Water depth and velocity were determined at 5 min intervals throughout the sampling period which allowed calculation of volume of flow for each interval. Further information on flow characteristics are given in Jordan et al. (1983).

Water samples for bacterial enumeration were taken at hourly intervals and preserved with formaldehyde (2% final concentration). Samples were stored on ice in the field and at 4 °C in the laboratory. Within two days the bacterial samples were composited into volume integrated flood and ebb samples and analysed. Preliminary testing demonstrated that compositing in this manner yielded bacterial concentrations statistically equivalent to those determined by counting the hourly samples followed by a mathematical integration of the hourly counts.

Water samples for chlorophyll determinations were taken on all tidal cycles at both marshes. Each half hour, 1 or 2 bottles were filled with water and stored on ice in an ice chest. At two hour intervals a composite sample was made from the half hour samples based on the volume of flow over the half hour interval they represented. Samples were then stored on ice and returned to the laboratory for analysis of chlorophyll *a*.
Microbial biomass, bacterial activity, and dissolved organic nutrient concentrations were determined from water samples taken at 1-2 hour intervals over some tidal cycles at the low marsh. Samples were either processed in the field and stored on ice or taken to the lab and processed within 45 min. In either case samples for dissolved organic nutrients were filtered through 0.45 pm pore cellulose acetate filters and frozen until analysis. Microbial activity was determined within 1 h of sample collection, and microbial biomass samples were stored at 4 °C until analysis.

An array of techniques was used for analysis. Bacteria were enumerated by the acridine orange direct count method (AODC) of Hobbie et al. (1977). Chlorophyll a was determined by sample filtration (Gelman type A/E glass fibre filter) followed by a DMSO-acetone extraction (Shoaf & Lium, 1976) and spectrophotometric analysis (American Public Health Association, 1975). Other measurements were dissolved primary amines (North, 1975), dissolved monosaccharides (Johnson & Sieburth, 1977), dissolved total carbohydrates (Burney & Sieburth, 1977), and particulate carbohydrates (Dubois et al., 1956) after collection of particulates on glass fibre filters.

Heterotrophic activity was estimated by the ³H-thymidine method of Fuhrman & Azam (1980) on seven tidal cycles from the low marsh station. For each sample, 5 ml aliquots (three live and one killed control) were incubated for 30 min with 1 μCi ³H-methylthymidine (specific activity 20 Ci per mmole) after which cells were collected on 0.2 pm pore Nuclepore filters and washed with cold trichloroacetic acid (Fuhrman & Azam, 1980). On some occasions we increased the number of replicates and collected the incubated samples on 5.0, 3.0, or 1.0 μm pore size filters in addition to the 0.2 μm pore size Nuclepore filters. Label incorporation from activity measurements was determined by liquid scintillation counting using channels ratio for quench correction.

Results
Bacterial concentrations in flooding and ebbing tidal waters ranged from 0.8 to 23.9 x 10⁶ cells ml⁻¹ (Fig. 2). Overall mean concentration in tidal waters was 6.8 x 10⁶ cells ml⁻¹ (n=44), and the concentrations of bacteria were similar in both marshes. Seasonally, there were three peaks in bacterial abundance, a moderate spring peak, and larger peaks in summer and late fall (Fig. 2). A paired t-test did not demonstrate consistent significant differences in concentration of bacteria in flooding and ebbing waters throughout the year (low marsh P=0.55; high marsh P=0.65), although the mean values for bacterial concentrations on flood tides were 7 to 10% higher than the mean concentrations on ebb tide for both marshes.
Chlorophyll $a$ concentrations ranged from about 2 $\mu$g l$^{-1}$ to 95 $\mu$g l$^{-1}$ (Fig. 2). Again, a paired $t$-test did not show a consistent significant difference between flood and ebb concentrations throughout the year (low marsh $P=0.95$; high marsh $P=0.78$). There was, however, a significant difference (ANOVA, $F=11.2$, $P<0.01$) in chlorophyll $a$ concentrations between the high marsh, 20.4 ± 17.9 $\mu$g l$^{-1}$ and the low marsh, 36.7 ± 32.8 $\mu$g l$^{-1}$.

Figure 3. Microbial and dissolved organic components of composited water samples during 8 September 1981 tidal cycle in low and high marshes of the Rhode River. The flood is upper figures: bacterial and chlorophyll $a$ concentrations. Lower figures: concentrations of dissolved monosaccharides (MCHO), dissolved total carbohydrates (TCHO), particulate carbohydrates (PCHO), and dissolved primary amines (DPA).

Variability in all parameters was evident over the course of tidal cycles, and is illustrated by results from the 8 September 1981 cycle (Fig. 3). During this particular tide more bacterial biomass was found in the flooding water than in ebbing water, and slightly lower concentrations were found in the high marsh than in the low marsh. Similarly, the chlorophyll $a$ values determined from two-hour composites were both lower and more variable in the high marsh than in the low marsh. On this date, in the high marsh there appeared to be a chlorophyll $a$ peak at high slack water. However, this was unusual and we did not notice any pattern of peaks of either chlorophyll or bacteria associated with specific tidal stage.

We also found that concentrations of organic nutrients varied two to four-fold over single tidal events (Fig. 3). There did appear to be a relationship between organic nutrients and other variables, but the only significant correlation was a slight positive correlation between chlorophyll $a$ and particulate carbohydrate concentrations ($r=0.37$, $n=80$, $p<0.001$). No significant correlations were found between bacterial counts and any of the organic nutrient concentrations. Variability of organic constituents over the year generally spanned one order of magnitude (Table 1).

Bacterial activity, measured by thymidine uptake, was determined on seven tidal cycles. Six of these were in the low marsh during daylight hours on 4 November 1980, 2 March 1981, 28 April 1981, 27 May 1981, 8 September 1981, and 21 October 1981. One was from the high marsh on 12 August 1980, and began at midnight and ran until 1200. The uptake of $^3$H-thymidine varied by almost two orders of magnitude with a mean value of 5.8 × 10$^4$ DPM 5 ml$^{-1}$ h$^{-1}$ (range 0.2 × 10$^4$-12.8 × 10$^4$ DPM 5 ml$^{-1}$ h$^{-1}$). There was no consistent pattern of uptake with time or stage of the tide, but a significant positive correlation was found with temperature ($r=0.48$, $n=72$, $P<0.01$). Uptake of thymidine was not significantly correlated with AODC ($r=0.11$, $n=69$, $P=0.18$), but $^3$H-thymidine uptake was found predominantly in the smallest size fractions. Generally, less than 10% of the total uptake retained on 0.2 $\mu$m filters, was found in size fractions > 3.0 $\mu$m (Fig. 4).
Discussion
Comparison of flood and ebb concentrations of bacteria and chlorophyll a did not demonstrate a consistent significant difference by either a paired t-test or comparison of annual means. There are, however, significant differences in concentrations on some dates (e.g. low marsh, AODC, 29 July 1980, high marsh, AODC, 4 November 1980). Similarly, Chrzanowski et al. (1982) found significant net exchanges of microbial biomass on only 4 of 22 tidal cycles studied in the North inlet system of South Carolina. Earlier, Erkenbrecher & Stevenson (1975) had found a net import of aerobic, heterotrophic bacteria into the North Inlet system, but they noted large variability among individual tidal cycles. Wright & Coffin (1983) also found variable fluxes on four spring tides in a Massachusetts marsh, although the average value indicated a net import of only 1.5% of the bacterial standing crop over a tidal cycle.

Since there are significant transports of microbes on some dates, the question arises does this constitute a significant portion of the flux of organic components in these marshes? Determination of net flux of bacterial biomass is difficult due to the asymmetry of tidal cycles. Our estimates are based on the procedure described by Jordan et al. (1983) which utilizes tidal heights and water flow measurements to calculate tidal volumes, and assumes that the measured values of bacterial and algal concentrations were representative of the months from which they were taken. Under these assumptions the net flux of bacterial and algal biomass can be determined from a conversion of cell numbers and chlorophyll to biomass and comparison to the net fluxes of nutrients from these marshes determined by Jordan et al. (1984). For bacteria we used an average cell volume of 0.06 [μm3 cell-1 (Rublee et al., 1983), a conversion of 8.6 × 10^{-14} g C μm^{-3} (Ferguson & Rublee, 1976) and a C: N : P
ratio of 100 : 28 : 3 (Ferguson, R. L., National Marine Fisheries Service, Beaufort, NC, personal communication). Similarly, we used a C: N : P ratio for algal biomass of 100: 16 : 2 (Strickland, 1960) and a chlorophyll a to carbon ratio of 1 : 30 (see Ferguson & Murdoch, 1975; Banse, 1977; de Jonge, 1980) to estimate algal contribution to nutrient pools. These estimations suggest that the bacterial contribution to particulate organic nutrients is small, on the order of a few percent of the total (Table 2). In contrast, algal biomass is a significant component of the particulate organic nutrients in both marsh sites. These estimations must be considered cautiously since 11 tidal cycles certainly do not constitute a very large sample. These marsh stations are now being automated and continuous data will provide significant improvement in the determination of net flux.

There are a number of potential transport mechanisms that we have not assessed which might lead to different conclusions regarding microbial transport. First, Odum et al. (1979) have argued that geomorphology of estuarine systems is an important consideration and that bed-load transport must be considered as a flux component. Since the tidal amplitude in the Rhode River is not large, bed load transport is probably not important. Second, episodic forcing events may be significant mechanisms of transport. Hackney & Bishop (1981) found significant transport of macroorganic material during storm events. Such detrital material contains large numbers of bacteria (Newell & Hicks, 1982) and several of these forcing events may alter considerably the net flux of various materials. Finally, surface films and aggregates floating near the surface of the water column may be mechanisms of microbial transport. Harvey & Young (1980) and Sewell et al. (1982) found elevated microbial biomass in surface films. The origin and composition of aggregates have been discussed by Ribelin & Collier (1979) although they did not quantify the biomass of the microbial component of these aggregates. We frequently find such material, in the form of floating diatom mats, in the tidal creeks of Rhode River marshes in spring. The net flux of these mats and hence microbial biomass remains a question.

The bacteria and chlorophyll a concentrations found in this study are typical for estuarine areas. The range of bacteria concentrations found in this study, 1-23X 106 cells m-1, is similar to the numbers found in other marshes and estuaries (Palumbo & Ferguson, 1978; Harvey & Young, 1980; Wilson & Stevenson, 1980; Kirchman & Mitchell, 1982; Ducklow, 1982). Similarly, chlorophyll a values 20-74 μg l-1, are in the range commonly reported in other tidal areas of Chesapeake Bay (e.g. Heinle & Flemer, 1976; Mountford, 1980).

The amount of variability in the dissolved organic nutrient concentrations did not allow us to attach any statistical significance to these fluxes. Examination of the patterns of these fluxes, however, in conjunction with other reports yields some insight into the relationship of these dissolved organic constituents with the microbial components. First, Jordan et al. (1983) have pointed out that the Rhode River marshes generally import particulate material and export dissolved forms of nitrogen and phosphorus. Wright & Coffin (1983) have discussed the process of export of dissolved organic matter from marshes in Massachusetts and they concluded that not only was it characteristic of many marsh systems, but that it may fuel heterotrophic production within the adjacent estuarine basin. Palumbo (1980) has also suggested that this source of organic matter may be important. Our data are consistent with this interpretation, but do not demonstrate this coupling, since they are
Correlation coefficients between microbial biomass indicators and nutrient measures were calculated from nutrient data collected from the tidal cycles and provided by Jordan et al. (1983). Data were combined for both marshes since correlations were similar. More relationships were evident between chlorophyll a and nutrients than between bacterial concentrations and nutrients (Table 3). In the case of particulate nutrients this is expected since the phytoplankton biomass is greater than the bacterial biomass. Chlorophyll a also correlated positively with most phosphorus measures, but negatively with most dissolved nitrogen measures, which may result from rapid nitrogen uptake by phytoplankton. Nitrogen appears to be the limiting nutrient during most of the summer for Chesapeake Bay (Taft & Taylor, 1976). Bacteria numbers demonstrated weak relationships to nutrients and only two significant correlations were evident, positive with dissolved orthophosphate phosphorus and negative with particulate organic phosphate (Table 3).

Thymidine uptake was generally restricted to size fractions less than 3.0 pm (Fig. 4), suggesting not only that bacteria were indeed the primary agents of thymidine uptake, but also that bacteria attached to particulate matter were either inactive or represented only a small proportion of the active heterotrophic microbes. We did not make quantitative determinations of the number of bacteria associated with particles in our samples, but there never appeared to be significant numbers of cells attached to particles (i.e. greater than 5-10% of the total). The lack of obvious bacteria-particle associations combined with the uptake data suggests that most of our cells are not attached to particles. This result is rather surprising in light of the general expectation that a significant proportion of bacterial activity is found in particle associated cells in coastal and especially marsh/estuarine systems (Hanson & Wiebe, 1977; Goulder, 1977; Harvey & Young, 1980; Wilson & Stevenson, 1980; Kirchman & Mitchell, 1982). The characteristics of the Rhode River may be important in understanding this result. Tidal amplitudes are small, salinity is low, suspended particulates are small (primarily silts and clays of low organic content) and the system is eutrophic. Thus, there may be little advantage to attachment. Palumbo (1980) also found most bacterial activity in the Newport River to be in free-living cells and suggested that bacteria particle interactions may be most pronounced only during periods of sediment resuspension. Certainly this is an area for further study.

The size of the bacterial production estimated from $^3$H-thymidine uptake suggests an active heterotrophic assemblage. Conversion of the $^3$H uptake values to biomass as described by Fuhrman & Azam (1980) yields mean production values ranging from $1 \cdot 3-8 \cdot 2 \times 10^9$ cells $1^{-1} \text{day}^{-1}$. On a carbon basis these production estimates are about 7-42 µg bacterial carbon $1^{-1} \text{day}^{-1}$, which is about 5-25% of algal production values for the Rhode River (Correll, 1978). These values are equivalent to specific growth rates of bacteria ranging from $0 \cdot 2-1 \cdot 2$, day$^{-1}$ or turnover times from less than 1 to about 5 days based on the average bacterial cell concentrations in the tidal waters. Similar estimates have been reported by Fuhrman & Azam (1980) in coastal California.

Correlation coefficients between microbial biomass indicators and nutrient concentrations for all samples from both marshes (values in parenthesis indicate number of samples)

<table>
<thead>
<tr>
<th>Dissolved Nutrients</th>
<th>Organic Nitrogen</th>
<th>Nitrate</th>
<th>Ammonia</th>
<th>Organic Phosphorus</th>
<th>Orthophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>0·196</td>
<td>-0·353**</td>
<td>-0·502**</td>
<td>0·089</td>
<td>0·529**</td>
</tr>
<tr>
<td>(44)</td>
<td>(44)</td>
<td>(41)</td>
<td>(44)</td>
<td>(44)</td>
<td>(44)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>-0·038</td>
<td>-0·185</td>
<td>-0·144</td>
<td>-0·232</td>
<td>0·371**</td>
</tr>
<tr>
<td>(44)</td>
<td>(44)</td>
<td>(41)</td>
<td>(44)</td>
<td>(44)</td>
<td>(44)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particulate Nutrients</th>
<th>Organic Nitrogen</th>
<th>Ammonia</th>
<th>Organic Phosphorus</th>
<th>Orthophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>0·812**</td>
<td>0·452**</td>
<td>-0·070</td>
<td>0·660**</td>
</tr>
<tr>
<td>(44)</td>
<td>(43)</td>
<td>(44)</td>
<td>(43)</td>
<td>(43)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>0·151</td>
<td>-0·178</td>
<td>-0·335*</td>
<td>0·190</td>
</tr>
<tr>
<td>(44)</td>
<td>(43)</td>
<td>(44)</td>
<td>(43)</td>
<td>(43)</td>
</tr>
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</table>

* P<0·05.
** P<0·01.
waters and Ducklow (1982) for suspended bacteria in the York River estuary in Virginia.

Since the suspended bacteria are actively growing, why are the concentrations of bacteria in tidal waters draining the marsh no larger than those in flooding waters? Three possibilities are that: (1) the marshes are sinks for bacteria; (2) predators consume suspended bacteria; and (3) some other natural death process occurs. Although there may be some deposition of bacteria, it is not large since both direct observations and activity measures indicate predominantly free living populations. Such small free living cells have low sinking rates and could not be expected to sediment out of the water column while over the marsh. The second possibility seems quite likely in light of the activity measures and concentration of organic substances. Production of the bacteria, allowing for 50% of even greater respiration is still small compared with the concentrations of dissolved monosaccharides and primary amines (Table 1, Fig. 3) which presumably constitute labile substrates. Thus, a nutrient limitation is not apparent and a likely alternative is predator limitation by micro-flagellates and other protozoans. We cannot evaluate the third possibility, but we think it is unlikely within this generally productive system.

In sum, we have found that most planktonic bacterial activity is by free living cells in the Rhode River and we have not found significant transport of microbial biomass between marshes and open estuary. These results seem consistent with other studies of marsh systems with small tidal amplitudes. We suspect that our results cannot be generalized to coastal marshes in areas with larger tidal amplitudes and different nutrient regimes. Our results tend to support the concept of detrital decomposition and nutrient cycling within the boundaries of the marsh itself (Jordan et al., 1983). Further emphasis on the microbial role in cycling of specific organic and inorganic compounds would appear an obvious next step in addressing the role of microorganisms in these brackish, estuarine marshes.

References
Chrzanowski, T. H., Stevenson, L. H. & Spurrier, J. D. 1982 Transport of microbial biomass through the North Inlet ecosystem. Microbial Ecology 8, 139-156.
de Jonge, V. N. 1980 Fluctuations in the organic carbon to chlorophyll a ratios for estuarine benthic diatom populations. Marine Ecology Progress Series 2, 345-353.


Shoaf, W. T. & Lium, B. W. 1976 Improved extraction of chlorophyll a and b from algae using dimethyl sulfoteide. Limnology and Oceanography 21, 926-928.