Distribution and Activity of Bacteria in the Headwaters of the Rhode River Estuary, Maryland, USA

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Abstract:
A transect along the axis of the headwaters of a tidal estuary was sampled for microbial, nutrient, and physical parameters. Chlorophyll *a* averaged 42 μg 1^{-1} and phytoplankton comprised an estimated 80% of the total microbial biomass as determined by adenosine triphosphate (ATP). Bacterial concentrations ranged from 0.3-53.9 $\times 10^6$ cells ml^{-1} and comprised about 4% of the total living microbial biomass. Bacterial production, determined by 3H-methyl-thymidine incorporation was about 0.05-2.09 $\times 10^9$ cells 1^{-1} h^{-1}, with specific growth rates of 0.26-1.69 d^{-1}. Most bacterial production was retained on 0.2 μm pore size filters, but passed through 1.0 μm filters. Significant positive correlations were found between all biomass measures and most nutrient measures with the exception of dissolved inorganic nitrogen nutrients where correlations were negative. Seasonal variability was evident in all parameters and variability among the stations was evident in most. The results suggest that bacterial production requires a significant carbon input, likely derived from autotrophic production, and that microbial trophic interactions are important.

Article:
Introduction
Coastal and estuarine systems are sites where competition for nutrients by microorganisms and remineralization of photosynthetically fixed carbon are important processes. High rates of bacterial activity have been found in such systems for both inorganic nutrients and organic compounds [10, 29]. For example, Faust and Correll [10, 11] measured the uptake of orthophosphate by 2 size fractions of suspended plankton in the Rhode River over a period of 12 months. Except for summer months, the uptake of orthophosphate was predominantly by the smallest size fraction (< 5.0 μm), which they observed was primarily bacteria. Generally, only 1-6% of the uptake was by the larger size fraction (> 5.0 μm), composed primarily of phytoplankton. Palumbo [29] found rapid turnover times (1-2 days) but a high degree of variability in kinetic parameters of bacterial uptake of mixed amino acids in the Newport River estuary, North Carolina. Salinity, temperature, heterotrophic biomass, and supply of organic material all seemed to be important factors in regulating the bacterial activity.

Utilization of carbon fixed by primary production is a primary role of heterotrophic microorganisms, and recent studies suggest that the magnitude of this role has been underestimated [26, 36]. Williams [36] used a model to suggest that bacteria may utilize as much as 50% of the primary production of phytoplankton in coastal areas, and that this magnitude of uptake is compatible with current trophic models. Similarly, Nixon [26] has pointed out that earlier conceptualizations of remineralization pathways did not include bacteria as major contributors to that process, but that recent experimental work has pointed to its fundamental importance in some systems. The sources of dissolved organic carbon for bacterial uptake include excretion by primary producers, "sloppy eating" by zooplankters, and zooplankton fecal material. The fate of this carbon material is not only mineralization to inorganic carbon, but also production of bacterial biomass. Several authors [2, 36] have suggested the trophic importance of bacteria as a link between phytoplankton and higher consumers.

Estuarine systems may exhibit even greater dependence upon trophic links through microheterotrophs than

oceanic systems since detrital pathways in estuarine systems are frequently well developed, especially where surrounding marshes are extensive [15]. In these systems, important interactive mechanisms for microbial activity include not only the detrital pathway but also bacteria-particle relationships.

In this study we have utilized acridine oragne direct counts (AODC) and ^{3}H-thymidine incorporation to determine bacterial abundance and activity in the headwaters of a brackish tidal estuary, the Rhode River, in Maryland. We have also assessed some of the physical and chemical factors that may be important in determining bacterial distribution, activity, and the importance of these organisms to nutrient flux in the Rhode River.

Materials and Methods

Samples were collected at 7 stations in the Rhode River, a subestuary of Chesapeake Bay, on the east coast of North America (38°51'N, 76°32'W). Five of these stations were along a transect which ran up the axis of the river and headwater creeks (Fig. 1). The transect began in the river at station 4, continued upstream past a mudflat, station 5, and into a tidal creek, Muddy Creek, surrounded by an irregularly flooded *Typha angustifolia* marsh, station 6. The transect continued up 2 tributaries of Muddy Creek, Main Branch and North Branch. Stations 7 and 8 are located in these 2 branches, respectively, at the position where there is a transition from marsh to forested wetland. Salinity along the transect ranged from 18 ppt at station 4 during the summer to 0 ppt at stations 7 and 8 during spring runoff periods. Although there is a tidal influence in the Rhode River (approximately 35 cm), regional meteorological events such as wind and changes in barometric pressure can cause changes in water level of over 1 m.

Two additional stations were sampled, a dock station and a low marsh tidal flume station. The dock station is located near station 4 (Fig. 1) and was sampled on only 1 day, primarily to examine the size distribution of heterotrophic activity. The low marsh station, located close to station 6, was sampled on 11 tidal cycles. Results of the tidal cycle studies are given elsewhere [30], but we have utilized some of the data on bacterial distribution and activity derived from that study to complement our observations from the other stations.

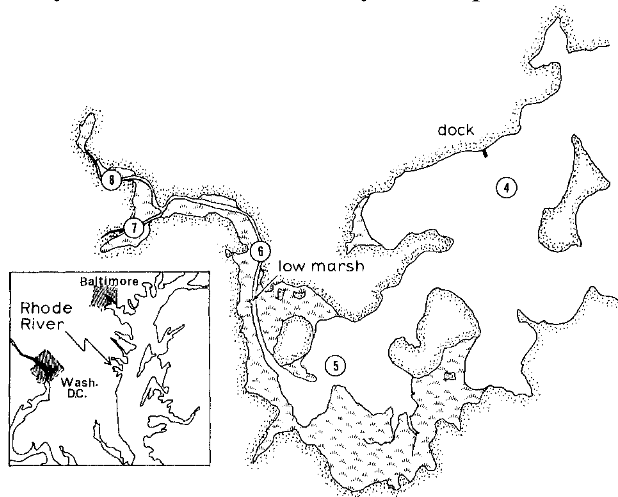

Fig. 1. Rhode River, MD (Latitude 38°51′N, Longitude 76°32′W), showing sampling locations.

The transect was sampled 18 times at 2-6 week intervals at high tide. Water for nutrient analyses was collected continuously as the boat moved from one station to the next. Water was pumped through a peristaltic pump into 5 gallon acid-washed polyethylene jugs, one for each segment of the transect. Microbial parameters and dissolved organic compounds were determined from surface samples taken by immersing 1 gallon polyethylene bottles just below the water surface. Subsamples for bacterial enumeration were fixed in the field with formaldehyde to a final concentration of 2% (v/v). Salinity and temperature were measured at each station with a Beckman RS5-3 salinometer, and dissolved oxygen was measured with a YSI Model 54 oxygen meter. All samples were returned to the laboratory within several hours, and microbial samples were kept on ice.

Concentrations of organic matter, nitrogen, and phosphorus were determined from the integrated samples. Total and dissolved organic matter were measured as chemical oxygen demand (COD) before and after filtration

through 0.4 µm membrane filters [23]. $HgSO_4$ was added to these samples prior to analysis to eliminate chloride interference [8]. Nitrate and ammonia were determined by a reduction and oxidation, respectively, to nitrite, followed by nitrite analysis [1]. Total phosphorus was determined by digestion with perchloric acid and measured as orthophosphate by the stannous chloride method [1, 20]. Total particulate phosphorus was calculated as the difference between total phosphorus and total dissolved phosphorus. Dissolved organic phosphorus was calculated as the difference between dissolved orthophosphate and dissolved total phosphorus. Total particulates were determined by weight after filtration onto Nuclepore filters (0.4 µm pore).

Various microbial parameters were determined from the surface samples. Bacterial concentrations were determined using the acridine orange direct count method (AODC) of Hobbie et al. [17]. Chlorophyll *a* was determined by sample filtration through a glass fiber filter followed by maceration and an acetone/DMSO extraction [33]. Absorption of the extract was determined at 663 nm before and after acidification with 1.0 N HCl, and pigment content determined by the equations given in Standard Methods [1]. Heterotrophic activity was measured as ^{3}H-methyl thymidine incorporation following the method of Fuhrman and Azam [13, 14]. Briefly, 5 ml samples were incubated with 1 µCi of labeled thymidine (20 Ci/mmol^{-1}, 1 nM final concentration) for 30 min at room temperature and then filtered onto a 0.2 µm pore size polycarbonate membrane filter. Incorporation of label was determined by liquid scintillation counting after extraction with trichloroacetic acid as recommended by Fuhrman and Azam [13, 14] and correction for quenching by the channels ratio method. All values were corrected to in situ temperature by assuming a Q_{10} factor of 2. Occasionally additional replicates were incubated for determination of the size partitioning of thymidine incorporation. Following incubation, replicates were filtered through 1.0, 3.0, or 5.0 µm pore Nuclepore membrane filters. Adenosine triphosphate (ATP) concentration was determined following extraction in a boiling Tris buffer by the method of Holm-Hansen and Booth 1181. Electron transport system (ETS) activity was measured by a tetrazolium reduction method 1281. Dissolved primary amines [27], dissolved monosaccharides 1191, and dissolved total carbohydrates [6] were also determined from surface samples.

Table 1. Mean values ± SE for physical and chemical characterization of transect stations

Station	Salinity (ppt)	Temperature (°C)	pH	Total suspended particulates (mg l^{-1})
4	12.3 ± 0.7	17.2 ± 2.2	8.13 ± 0.09	16.9 ± 1.4
5	10.9 ± 0.9	16.8 ± 2.4	7.84 ± 0.10	28.1 ± 3.2
6	7.5 ± 1.5	14.9 ± 2.1	7.39 ± 0.08	32.5 ± 3.6
7	5.3 ± 1.3	14.8 ± 2.1	7.15 ± 0.08	34.5 ± 5.2
8	5.6 ± 1.4	14.6 ± 2.0	7.11 ± 0.12	38.3 ± 6.7

Table 2. Mean values ± SE for dissolved nutrients by station along Rhode River transect

Station	NH_4 (µg l^{-1})	NO_3 (µg l^{-1})	PO_4 (µg l^{-1})	COD (as mg C l^{-1})
4	31 ± 5	49 ± 33	27 ± 7	13.8 ± 1.1
5	58 ± 13	50 ± 31	53 ± 10	15.6 ± 1.4
6	58 ± 12	97 ± 43	62 ± 10	16.5 ± 2.8
7	48 ± 10	101 ± 43	24 ± 6	14.5 ± 2.4
8	44 ± 11	144 ± 58	25 ± 5	14.5 ± 1.9

Results

Physical and chemical characteristics of the study site varied both seasonally and by station. Overall, salinity ranged from 0-17.6 ppt (mean = 8.4 ppt, SE = 0.6, n = 86). Both station and sampling date contributed significantly to the variability in salinity (ANOVA, $F = 33.5$, $P < 0.01$), and Duncan's multiple range test separated the stations into 2 groups. Stations 6, 7, and 8 comprised one group with a lower average salinity, and the downstream stations 4 and 5 comprised the second group. Salinity was occasionally high, however, even at the upstream stations during 1980 and 1981 which were drought years. pH ranged from 6.1-9.0 (mean = 7.5, SE = 0.06, n = 80) during the course of the study, and lowest values were found at the upstream stations. Mean total suspended particle concentration was 24.7 mg l^{-1} (SE = 2.0, n = 85), with a range from 4.7-108.5 mg l^{-1}. Temperature was generally higher at the downstream stations. Analysis of variance indicated that significant differences occurred among stations and sampling dates for total suspended particulates ($F = 3.8$, $P < 0.01$), pH ($F = 34.9$, $P < 0.01$), and temperature ($F = 54.7$, $P < 0.01$). Mean values for these parameters by station are given in Table 1.

Nutrient concentrations were also variable. All nutrient parameters had significant seasonal variation at the $P < 0.05$ level or better based on analyses of variance. Analyses of variance also indicated significant differences among stations for dissolved nitrogen nutrients and orthophosphate. Mean values for nutrient concentrations at

stations are given in Table 2.

The concentration of bacteria ranged from 0.3-53.9 × 10^6 cells ml^{-1}, with a mean value of 7.3 × 10^6 cells ml^{-1} (SE = 0.98 × 10^6 cells, n = 86). An analysis of variance indicated that there was no significant difference in bacteria concentration among stations 4 through 8 of the transect ($F = 1.11$, $P = 0.37$). Seasonal patterns were evident in bacterial concentrations, with fall peaks in bacteria in both 1980 and 1981 (Fig. 2).

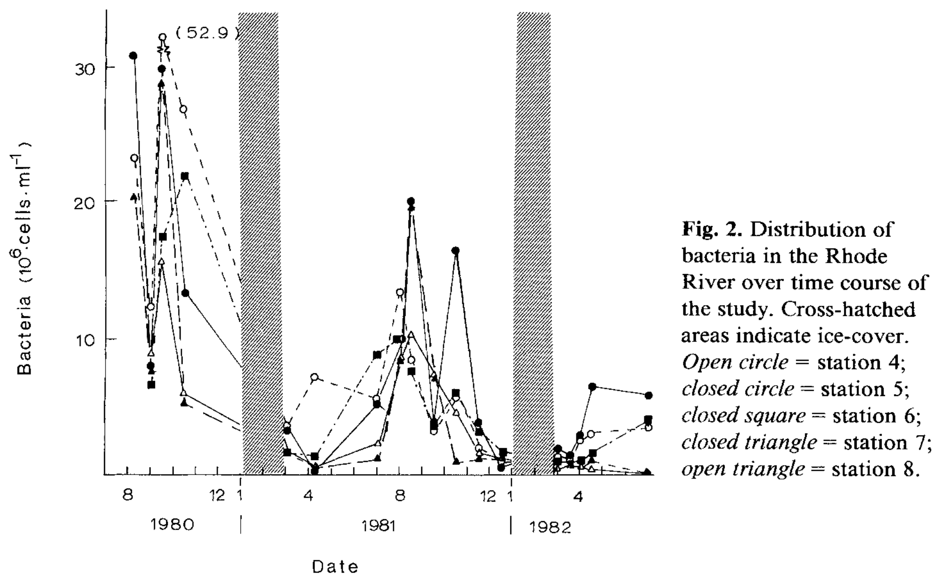

Fig. 2. Distribution of bacteria in the Rhode River over time course of the study. Cross-hatched areas indicate ice-cover. *Open circle* = station 4; *closed circle* = station 5; *closed square* = station 6; *closed triangle* = station 7; *open triangle* = station 8.

Incorporation of ^{3}H-thymidine followed a pattern similar to that of bacterial concentrations (Fig. 3). Mean incorporation of thymidine over the 30 min incubation period was 2.89 x 10^4 dpm 5 ml^{-1} (SE = 0.45 × 10^4 dpm, n = 60), with a range from 0.01-17.89 × 10^4 dpm 5 ml^{-1}. There was no difference among stations (ANOVA, $F = 2.00$, $P = 0.16$) but a strong seasonality was evident (ANOVA, $F = 8.92$, $P < 0.01$). Thymidine incorporation was found primarily in the smallest size fractions on 4 dates when replicate samples were filtered through different pore size filters (Table 3). Generally, 60-70% of the incorporated thymidine was found in the 0.2 µm-1.0 µm size fraction, and smaller percentages were found in larger size fractions up to >3.0 µm (Table 3).

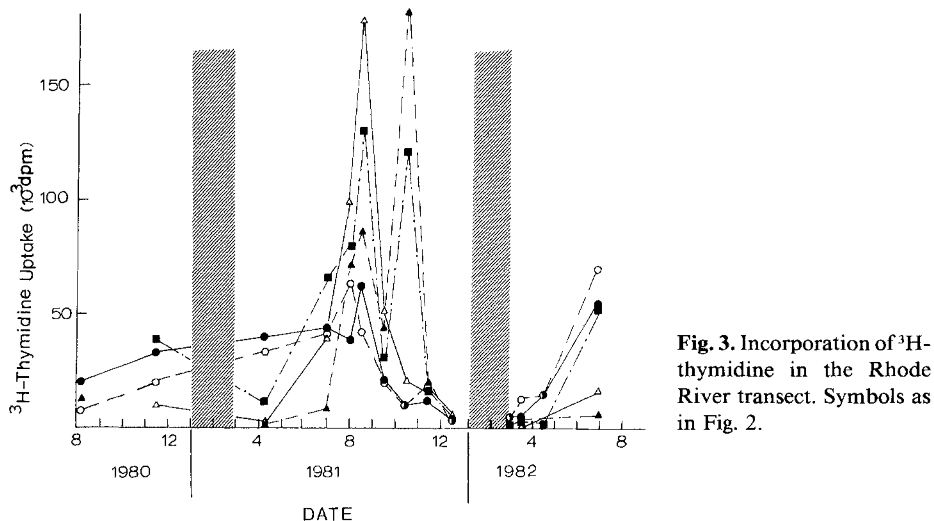

Fig. 3. Incorporation of ^{3}H-thymidine in the Rhode River transect. Symbols as in Fig. 2.

Chlorophyll *a* distribution was similar to that of bacteria, except that maximum values were found in the fall of 1981 rather than 1980, although peaks in concentration were evident in both years (Fig. 4). Mean chlorophyll *a* concentration for the data set was 42.1 µg 1^{-1} (SE = 5.9 µg, n = 84), with a range from 0.5-244.7 µg 1^{-1}. Variability in chlorophyll *a* concentration was attributable to both station and sampling date (ANOVA, $F = 10.6$, $P < 0.01$). Duncan's multiple range test found 2 groups, with stations 4, 5, 6, and 7 in the first group, and stations 5, 6, 7, and 8 in the second group. In general, the highest concentrations of chlorophyll *a* were found at station 8.

Table 3. Percent of total uptake in the size fraction for size partitioning of ^{3}H-thymidine incorporation

Site	Date	Size fraction (μm) 0.2–1.0	1.0–3.0	>3.0
Station				
Transect	10/16/80			
4		66	24	10
5		—	—	12
6		64	27	8
8		42	36	22
Transect	6/24/82			
4		58	28	13
5		73	19	8
6		71	23	6
7		60	35	4
8		16	70	14
Time				
Low marsh	9/8/81			
1050		63	28	9
1245		64	29	7
1426		61	33	7
1602		78	14	8
1730		66	27	7
Dock	7/1/82			
0930		48	33	20
1050		65	23	12
1302		60	28	12
1403		57	37	7
Mean for all data		60 ± 14	30 ± 12	10 ± 5

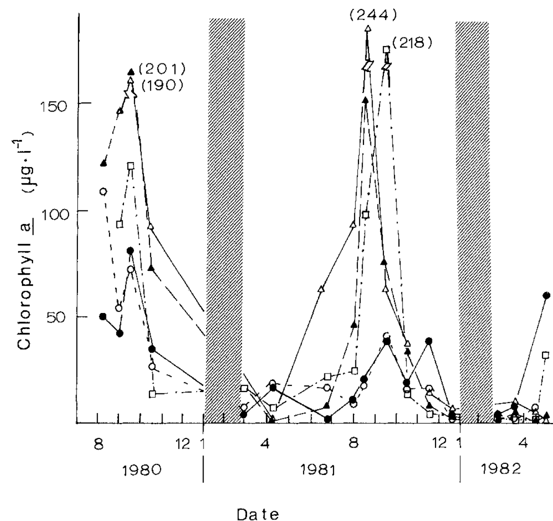

Fig. 4. Distribution of chlorophyll *a* in the Rhode River over the course of the study. Symbols as in Fig. 2.

ATP concentrations ranged from 0.3-43.1 μg ATP 1^{-1}, with a mean value of 5.4 μg ATP 1^{-1} (SE = 1.2 μg, n = 50). The highest ATP values were generally found at the upstream stations. Similarly, values for ETS, the community respiration measure, were highest at the upstream stations and had a highly significant positive correlation with ATP (r = 0.94, P < 0.01, n = 39). Mean ETS value was 26.02 μg O 1^{-1} (SE = 7.1 μg, n = 42) with a range from 0-251.2 μg O_2 1^{-1} h^{-1}. Strong positive correlations were found between chlorophyll *a* and both ATP (r = 0.929, P < 0.01, n = 48) and ETS (r = 0.885, P < 0.01, n = 40); correlations with bacteria were not as large for ATP (r = 0.453, P < 0.01, n = 50) or ETS (r = 0.308, P < 0.05, n = 42).

Table 4. Correlation coefficients between bacteria, chlorophyll *a*, and nutrient parameters

	Dissolved nutrients: Organic nitrogen	Nitrate	Ammonia	Organic phosphorus	Ortho-phosphate
Chlorophyll *a*	0.703[b] (84)	−0.363[b] (83)	−0.228[a] (66)	0.428[b] (84)	0.151 (84)
Bacteria	0.502[b] (86)	−0.352[b] (85)	−0.294[b] (68)	0.482[b] (86)	0.521[b] (86)

	Particulate nutrients: Organic nitrogen	Ammonia	Organic phosphorus	Ortho-phosphate
Chlorophyll *a*	0.853[b] (80)	0.563[b] (66)	0.751[b] (84)	0.570[b] (84)
Bacteria	0.439[b] (82)	0.464[b] (68)	0.481[b] (86)	0.234[a] (86)

[a] $P < 0.05$
[b] $P < 0.01$
Values in parentheses indicate number of observations

Significant correlation coefficients were found between chlorophyll *a* and many of the nutrient parameters (Table 4). Dissolved nitrogen components exhibited negative correlations with chlorophyll *a*, while dissolved phosphorus components exhibited positive relationships with chlorophyll *a*. Positive correlations were found between chlorophyll *a* and most particulate nutrient parameters. Similar significant correlations were found between bacteria and the nutrient parameters (Table 4).

Heterotrophic activity correlated significantly with chlorophyll *a* and with bacterial concentration in the overall data set (Table 5). However, if the correlations were determined by station, a different pattern emerged. Significant correlations between ^{3}H-thymidine incorporation and bacterial concentration were found only in the upstream stations 6, 7, and 8 (Table 5). Similarly, the only significant correlation between chlorophyll *a* and thymidine incorporation occurred at station 8.

Table 5. Correlation of ^{3}H-thymidine incorporation with bacteria concentration and chlorophyll *a*, overall and by station

	Overall				
Bacteria	0.359[b] (60)				
Chlorophyll *a*	0.335[b] (58)				
	By station				
	4	5	6	7	8
Bacteria	0.239 (13)	0.214 (13)	0.796[b] (11)	0.649[a] (12)	0.876[b] (11)
Chlorophyll *a*	−0.042 (12)	−0.033 (12)	0.329 (11)	0.388 (12)	0.983[b] (11)

[a] $P < 0.05$
[b] $P < 0.01$
Values in parentheses indicate number of observations

Discussion

Microbial distribution and dynamics in the Rhode River estuary exhibit some similarities to other estuarine systems. For example, bacterial concentrations are similar to those found for many estuarine and nearshore systems [3, 4, 9, 21, 24, 25, 29]. The seasonal distribution of bacteria was also typical. Warmer summer temperatures foster larger bacterial populations [e.g., 29]. The spatial distribution of bacteria was not typical, however, as there was a significant positive correlation of bacterial numbers with salinity ($r = 0.446$, $P < 0.01$, $n = 86$). In most studies of estuarine systems, the numbers decrease with increasing salinity [e.g., 3, 29]. Ducklow [9] however, has documented a destratification event in the York River, Virginia that resulted in a doubling of bacterial concentrations although salinity changed less than 2 ppt. It may be that the complexity of our estuarine system, that is, high variability in salinity, nutrient concentrations, and freshwater inputs as well as the variability resulting from meteorological conditions either obscures, or more likely eliminates the generality.

The distribution of chlorophyll *a* is also not surprising. Phytoplankton populations are large in the Rhode River during the spring and summer seasons [7]. Phytoplankton blooms dominated by dinoflagellates occur frequently in the Rhode River and are often found following nitrogen inputs during spring runoff or rainfall. We did not see peaks in chlorophyll *a* during the spring of 1981, however, probably because it was a drought year and the usual input of nutrients during spring runoff did not occur. The highest chlorophyll *a* values (greater than 200 µg chlorophyll *a* 1^{-1} attest to the occurrence of such blooms.

The variability of both bacteria and chlorophyll *a* is related to nutrient concentrations as well as physical processes. Significant negative correlation coefficients were found for bacteria and chlorophyll with dissolved inorganic nitrogen components. Nitrogen is likely limiting during most of the summer in the Rhode River [7] as it is generally in Chesapeake Bay [35]. The negative correlation probably reflects the rapid uptake of nitrogen by the plankton community. Positive correlations of phosphorus nutrients with bacteria and chlorophyll reflect summer peaks of these parameters.

Phytoplankton comprise a significant portion of the microbial community, while bacteria contribute little. If we use a conversion factor of 250 to estimate total living microbial carbon from the ATP determinations [18], then the mean value for total living microbial carbon found in this study is 1.58 mg C 1^{-1} (range: 0.16-10.78 mg C 1^{-1}). Similarly, a conversion of 30 for chlorophyll a to carbon [34] yields a mean phytoplankton biomass of 1.26 mg C 1^{-1} (range: 0.02-7.34 mg C 1^{-1}). Thus, algal biomass constitutes approximately 80% of the total microbial biomass, and this explains the high positive correlations of chlorophyll a with particulate organic phosphorus

and nitrogen (Table 4), as well as the high positive correlations found with ATP and ETS. A conversion factor for bacteria, 5.16×10^{-15} g C cell^{-1}, derived from values given in Ferguson and Rublee [12] and a mean cell volume estimate of 0.06 μm^3 [31], yields a mean value of 0.06 mg C 1^{-1} (range: < 0.01-0.29 mg C 1^{-1}). Bacteria then, contribute only about 4% of the total living microbial carbon. The value for bacteria is similar to that found by Bell and Albright [3] in the Strait of Georgia, Canada, but is only about half that reported by Ferguson and Rublee [12] for North Carolina coastal waters. Note, however, that the conversion factor used in this study is conservative; most authors use factors about twice as large [e.g., 3, 9].

Production of the bacteria can be estimated from the thymide incorporation data. To do so we have followed the protocol of Fuhrman and Azam [13, 14], and used the complete range of conversion factors they suggested, although recent studies suggest that the high end of the range may be correct [5, 22]. Even under this conservative approach the productivity of bacteria is often high, with mean values for bacterial production of $0.05\text{-}2.09 \times 10^{9}$ cells 1^{-1} h^{-1}. These values compare favorably with those reported by Fuhrman and Azam [14] and Newell and Fallon [25] for coastal areas. They also span nearly the same range reported by Meyer-Reil [24] for the Kiel Bight, although he used a technique of microscopic observation of biomass production in incubated samples. Finally, the values for production are similar to those reported by Ducklow [9] for the York River estuary, Virginia, which is a subestuary of Chesapeake Bay as is the Rhode River. Ducklow also calculated specific growth rates for bacteria by division of the production estimate by the corresponding standing crop. In the Rhode River, mean values for such specific growth rates range from 0.26-1.69 d^{-1} over the range of conversion factors provided by Fuhrman and Azam [13] and these are similar to the range of 0.2-1.1 d^{-1} found by Ducklow.

With some estimate of the specific growth rate, speculative determinations of fluxes through the bacterial assemblage can be attempted. For example, the assimilation efficiency for bacteria is generally taken to be about 50% [36]. Under this assumption, the net bacterial production is in the range of 0.020.10 mg C 1^{-1} day^{-1} and gross production would be twice that. This result suggests several important interactions within the Rhode River system. First, the source of carbon for bacterial metabolism may be largely derived from algal production. Williams [36] and Newell and Fallon [25] have drawn this conclusion in their studies. The similar overall correlation between thymidine incorporation and chlorophyll *a* as compared to bacterial biomass supports this contention (Table 5). Further, higher correlations of thymidine incorporation with chlorophyll a at the upstream stations (Table 5) are also consistent with this idea. These stations had the highest chlorophyll values and therefore primary production which would in turn influence bacterial activity. Second, since production is not manifested in consequent large bacterial biomass downstream, then losses must be occurring due to settling, autolysis, or predation. Rublee et al. [30] have estimated minor losses from the tidal creek into adjacent marsh areas, but they are not of sufficient magnitude to account for the large production estimate. Settling directly to the sediment surface is not likely to account for the production either, as tidal and meterological forces would serve to maintain cells in suspension, and many free-living bacteria would have low sinking rates. Autolysis and predation have not been evaluated, but we favor predation as a major pathway for recycling. Protozoan populations are evident in the Rhode River [32] and many studies have suggested that these organisms are important trophic links within estuarine systems [e.g., 25, 36].

An additional important feature of the heterotrophic production is that the majority of activity appears to be associated with free-living bacteria, and not those attached to the particles. We found most cells to be free of particles during our study, and the highest percentage of activity was located in the 0.2-1.0 µm size fraction. These results provide an interesting point of comparison with a number of other studies that have assayed for bacterial numbers and biomass. Generally, in marine systems, less than 5-10% of the heterotrophic activity appears to be associated with particles [cf. 4, 21]. Hanson and Wiebe [16] found that most of the heterotrophic activity in nearshore waters of Georgia was associated with particles. Bell and Albright [4] found decreasing proportions of attached bacteria as they moved down the Fraser River estuary. Kirchman and Mitchell [21] did not always find the majority of bacteria associated with particles in New England estuarine systems, but they did find correlations of numbers of attached bacteria with tidal range and the amount of suspended particulate matter.

Our finding of relatively low thymidine incorporation associated with particles in an estuarine system does not entirely agree with these earlier results, but it is not totally at odds with them either. In fact, on a few occasions we did find large numbers of bacteria associated with particles and on those occasions the heterotrophic activity was also predominantly in the larger size range. For example, during the transect of 6/24/81 at station 8, we found high concentrations of suspended particulates and observed that about 50% of the bacteria were attached to particles, in contrast to the general pattern of predominantly free-living forms. The incorporation of thymidine in this sample was also predominantly in larger size fractions than generally expected (Table 3). Clearly, bacteria-particle interactions are complex and the factors that govern them remain fruitful areas for further study.

References

1. American Public Health Association (1976) Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C.
2. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Progr Ser 10:257-263
3. Bell CR, Albright LJ (1981) Attached and free-floating bacteria in the Fraser River Estuary, British Columbia, Canada. Mar Ecol Progr Ser 6:317-327
4. Bell CR, Albright LJ (1982) Attached and free-floating bacteria in a diverse selection of water bodies. Appl Environ Microbiol 43:1227-1237
5. Bell RT, Ahlgren GM, Ahlgren I (1983) Estimating bacterioplankton production by measuring [3H] thymidine incorporation in a eutrophic Swedish lake. Appl Environ Microbiol 45:17091721
6. Burney CM, Sieburth J McN (1977) Dissolved carbohydrates in seawater. II. A spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. Mar Chem 5:15-28
7. Correll D (1975) The Rhode River program. In: Estuarine Pollution Control and Assessment. Vol I. Environmental Protection Agency Symposium. Pensacola, Florida, pp 19-27
8. Dobbs RA, Williams RT (1963) Elimination of chloride interference in the chemical oxygen demand test. Anal Chem 35:1064-1067
9. Ducklow HW (1982) Chesapeake Bay nutrient and plankton dynamics. I. Bacterial biomass and production during spring tidal destratification in the York River, Virginia estuary. Limnol Oceanogr 27:651-659
10. Faust MA, Correll DL (1976) Comparison of bacterial and algal utilization of orthophosphate in an estuarine environment. Mar Biol 34:151-162
11. Faust MA, Correll DL (1977) Autoradiographic study to determine metabolically active phytoplankton and bacteria in the Rhode River estuary. Mar Biol 41:293-305
12. Ferguson RL, Rublee P (1976) Contribution of bacteria to standing crop of coastal plankton. Limnol Oceanogr 21:141-145
13. Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl Environ Microbiol 39:10851095
14. Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66: 109-120
15. Haines EB (1979) Interactions between Georgia salt marshes and coastal waters: a changing paradigm. In: Livingston RJ (ed) Ecological processes in coastal and marine systems. Plenum Press, New York, pp 35-46
16. Hanson RB, Wiebe WJ (1977) Heterotrophic activity associated with particulate size fractions in a Spartina alterniflora salt marsh estuary, Sapelo Island, Georgia, USA, and continental shelf waters. Mar Biol 42:321-330
17. Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225-1228
18. Holm-Hansen 0, Booth CR (1966) The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol Oceanogr 11:510-519
19. Johnson KM, Sieburth J McN (1977) Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. Mar Chem 5:1-13
20. King EJ (1932) The colorimetric determination of phosphorus. Biochem J 26:292-297
21. Kirchman D, Mitchell R (1982) Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl Environ Microbiol 43:200-209

22. Kirchman D, Ducklow H, Mitchell R (1982) Estimates of bacterial growth from changes in uptake rates and biomass. Appl Environ Microbiol 44:1296-1307
23. Maciolek JA (1962) Limnological organic analyses by quantitative dichromate oxidation. Fish Wild Sery Publ
24. Meyer-Reil L (1977) Bacterial growth rates and biomass production. In: Rheinheimer G (ed) Microbial ecology of a brackish water environment. Springer-Verlag, Berlin, pp 223-236
25. Newell SY, Fallon RD (1982) Bacterial productivity in the water column and sediments of the Georgia (USA) coastal zone: estimates via direct counting and parallel measurement of thymidine incorporation. Microb Ecol 8:33-46
26. Nixon SW (1981) Remineralization and nutrient cycling in coastal marine ecosystems. In: Neilson BJ, Bronin LE (eds) Estuaries and nutrients. Humana Press, Inc, pp 111-138
27. North BB (1975) Primary amines in California coastal waters: utilization by phytoplankton. Limnol Oceanogr 20:20-27
28. Owens TG, King FD (1975) The measurement of respiratory electron transport system activity in marine zooplankton. Mar Biol 30:27-36
29. Palumbo AV (1980) Dynamics of bacterioplankton in the Newport River Estuary. PhD Thesis, North Carolina State University, Raleigh
30. Rublee PA, Merkel SM, Faust MA (in press) Nutrient flux in the Rhode River: tidal transport of microorganisms in brackish marshes. Est Coastal Shelf Sci
31. Rublee PA, Merkel SM, Faust MA (1983) Transport of bacteria in the sediments of a temperate marsh. Est Coastal Shelf Sci 16:501-509
32. Sawyer TK, MacLean SA, Coats W, Hilfiker M, Riordan P, Small EB (1976) Species diversity among sarcodine protozoa from Rhode River, Maryland following tropical storm Agnes. In: CRC Publ No 54, The effects of tropical storm Agnes on the Chesapeake Bay estuarine system, pp 531-543
33. Shoaf WT, Lium BW (1976) Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. Limnol Oceanogr 21:926-928
34. Strickland JDH (1960) Measuring the production of marine phytoplankton. Bull Fish Res Bd Canada 122:1-172
35. Taft JL, Taylor WR (1976) Phosphorus dynamics in some coastal plain estuaries. In: Wiley M (ed) Estuarine processes. Academic Press, New York, pp 79-90
36. Williams PJ leB (1981) Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kiel Meeresfor Sonderh 5:1-28