Size of Suspended Bacterial Cells and Association of Heterotrophic Activity with Size Fractions of Particles in Estuarine and Coastal Waters

By: Anthony V. Palumbo, Randolph L. Ferguson, and Parke A. Rublee

Palumbo, A.V., R.L. Ferguson, and P.A. Rublee. 1984. Size of suspended bacterial cells and association of heterotrophic activity with size fractions of particles in estuarine and coastal waters. *Appl. Environ. Microbiol.* 48(1):157-164.

Abstract:

The size of bacteria and the size distribution of heterotrophic activity were examined in estuarine, neritic, and coastal waters. The data indicated the small size of suspended marine bacteria and the predominance of free-living cells in numerical abundance and in the incorporation of dissolved amino acids. The average per-cell volume of suspended marine bacteria in all environments was less than 0.1 μm^3. Cell volume ranged from 0.072 to 0.096 μm^3 at salinities of 0 to 34.3960 in the Newport River estuary, N.C., and from 0.078 to 0.096 μm^3 in diverse areas of the Gulf of Mexico. Thus, the free-living bacteria were too small to be susceptible to predation by copepods. In the Newport River estuary, ca. 93 to 99% of the total number of cells and 75 to 97% of incorporated tritium (from ^3H-labeled mixed amino acids) retained by a 0.2-μm-pore-size filter passed through a 3.0-μm-pore-size filter. Although the amino acid turnover rate per cell was higher for the bacteria in the >3.0-μm size fraction than in the <3.0-μm size fraction, the small number of bacteria associated with the >3.0-μm size particles resulted in the low relative contribution of attached bacteria to total heterotrophic activity in the estuary. For coastal and neritic samples, collected off the coast of Georgia and northeast Florida and in the plume of the Mississippi River, 56 to 98% of incorporated label passed through a 3.0-μm-pore-size filter. The greatest activity in the >3.0-μm fraction in the Georgia Bight was at nearshore stations and in the bottom samples. Our data were consistent with the hypothesis that resuspension of bottom material is an important factor in influencing the proportion of heterotrophic activity attributable to particle-associated bacteria.

Article:

Bacteria are an abundant and productive food resource for fine-particle-feeding organisms in marine water. Bacteria have been found to comprise 10 to 83% of the total suspended biomass (13, 30, 33, 34, 42). These bacteria efficiently transform dissolved free amino acids into particulate organic nitrogen and, therefore, may be important in making this critical resource available to higher trophic levels (31). Paerl (29) demonstrated that bacteria confined in dialysis bags transform dissolved organic matter into particulate organic carbon. The particulate organic carbon consisted of free-living cells and diffuse particles composed of extracellular secretions and associated bacterial cells. Free-living bacteria are grazed by a variety of organisms including larvaceans (1, 14, 22), phagotrophic flagellates (16), marine suctorians (34), and the ribbed mussel Geukensia demissa (46) but appear to be too small to be cropped by most copepods (23). Studies in which both direct microscopic counts of bacteria and [^3H]thymidine-labeled bacterial cells were used have con-firmed the absence of bacterial cropping by copepods in samples of water from the Newport River estuary (J. Ustach and A. V. Palumbo, unpublished data). The availability of bacterial biomass as food for particle-feeding organisms depends on the size of bacterial cells and the extent of association of the bacteria with larger particles in the water.

The principal techniques used to determine bacterial size and to demonstrate the association of cells with particles are direct microscopic examination and differential filtration of samples. Both epifluorescent light microscopy and scanning electron microscopy allow direct measurement of bacterial cell dimensions (9, 13).

Scanning electron microscopy may be more precise and less subjective than epifluorescent microscopy, but it has been criticized because of cell shrink-age during sample preparation (15). Differential filtration combined with microscopic observation provides estimates of bacterial numbers associated with different size fractions (19) and also is used to determine the heterotrophic activity (incorporated ^{14}C- and ^{3}H-labeled organic compounds) retained by filters with different pore sizes (2, 8, 18, 41). Most of the heterotrophic activity in a wide variety of aquatic environments passes through filters with a 3.0-μm pore size (3, 4, 8, 10, 21; P. A. Rublee, S. M. Merkel, M. A. Faust, and J. Miklas, Microbial. Ecol., in press) except, perhaps, in estuarine and coastal waters off Georgia (17, 18).

To determine the size distribution of bacterial numbers, heterotrophic activity, and per-cell activity, we used epi-fluorescent microscopy, radiotracer techniques, and differential filtration. We report spatial and temporal differences both in the size of suspended bacterioplankton and in the association of heterotrophic activity and bacterial numbers with size fractions of suspended particulate matter for water samples from the Newport River estuary, N.C., and from the Gulf of Mexico. We also report differences in the size fractionation of heterotrophic activity associated with sample depth and distance from shore in coastal waters off Georgia and northeast Florida.

MATERIALS AND METHODS

Water was sampled from the Newport River estuary of North Carolina (September 1977 to September 1978), from the Georgia Bight off the coasts of Georgia and Florida (5 to 9 November 1977), and from the northeastern Gulf of Mexico (April to May 1980), including riverine, estuarine, neritic, and oceanic stations. Surface-water samples were collected by plastic bucket from a small boat or dock in the Newport River estuary. Samples were collected in 5-liter Niskin bottles on a rosette sampler from the R/V Columbus Islen (Georgia Bight) or in a 30-liter Teflon-lined Go-Flo bottle on a plastic hydrowire from the R/V Researcher (Gulf of Mexico).

Stations within the Newport River estuary were located along the longitudinal axis of the estuary in a wide range of salinities (0 to 35‰). Samples for determination of the size distribution of heterotrophic activity and bacterial numbers were taken nearly simultaneously (within 2 h) at one to six stations. Variations in bacterial size were determined by using samples taken over a 24-h period at each of three stations (Fig. 1) during different seasons. The number of samples taken over the 24-h period was increased from 9 to 17 after the first sampling season, September and October 1977. The estuary (Fig. 1) is small (31 km^2), shallow (average depth at mean low water, 1 m), and unstratified (43). Circulation is predominantly tidal (tide range at Beaufort inlet, 0.9 m) but highly variable due to wind and irregular freshwater runoff. Its watershed is predominantly low-lying pine-cypress pocosin, pine forests, and farmland. The aver-age flushing time is 12 tidal cycles or about 6 days (R. A. Hyle, Ph.D. thesis, University of North Carolina, Chapel Hill, 1976). Water available for runoff from the Newport River watershed was calculated (35) from rainfall and temperature data (27, 28).

Stations in the Georgia Bight were sampled once in November in three transects of three stations, each aligned normal to the coastline. The transects originated at a water depth of 12 to 17 m and extended seaward to water depths of 125 m off Savannah, Ga., 41 m off Brunswick, Ga., and 45 m off Jacksonville, Fla. Duplicate samples were collected at one depth (surface of the Brunswick transect) or at three depths (surface, middepth, and within 2 to 4 m of the bottom of the Savannah and Jacksonville transects). In November 1977 the Georgia Bight was unstratified (isotherms were virtually vertical) due to seasonal storms. For surface waters, salinities ranged from 32.9 to 35.0‰ nearshore and from 36.2 to 36.4‰ offshore. Temperature ranged from 19.9 to 21.3°C nearshore and from 25.7 to 27.2°C offshore (L. Atkinson, unpublished data).

In the Gulf of Mexico, three water samples were collected (one per day) from the estimated depth of maximum productivity at each of three stations (13a). Stations varied widely in geographical location and primary productivity and included a low-productivity (<0.1 μg of C $liter^{-1}$ h^{-1}) oceanic station within the loop of the Gulf Loop current, an intermediate-productivity (0.5 to 1.8 μg of C $liter^{-1}$ h^{-1}), post-phytoplankton-bloom, neritic station near Cape San Blas, Fla., and a high-productivity (3 to 32 μg of C $liter^{-1}$ h^{-1}) neritic station in the plume

of the Mississippi River (Table 1).

Bacterial size measurements and counts were made by using the acridine orange direct count (AODC) technique as modified by Hobbie et al. (19). The measurements were made on 25-mm Nuclepore filters (pore size, 0.2 μm) except where noted below. The filters were stained with irgalan black (Ciba-Geigy) for 2 to 48 h and rinsed in distilled water immediately before use. Average cell volume was deter-mined based on the frequency of cells (100 cells from each water sample) in five size and shape categories and on the calculated mean cell volume of the categories (Table 2). Many of the bacteria observed were less than one-half the size of the smallest division (about 1.2 μm at × 1,250) on the Whipple disk, and precise measurement of individual bacteria was not attempted. We assumed a spherical diameter of 0.5 μm to estimate the average volume (0.065 μm^3) of cells in this smallest size category (<0.6 μm diameter). Thus, changes in average cell volume reflect changes in relative abundance of cells among size categories but would not reflect shifts in size of cells within the smallest size category.

Heterotrophic activity associated with particle size fractions was measured in neritic-zone and estuarine samples. Neritic water, sampled during October 1977 in the Georgia Bight, was filtered through 0.2- and 3.0-μm-pore-size Nucle-pore filters. Mississippi River plume water was filtered through 0.2-, 1.0-, and 3.0-μm-pore-size filters. Estuarine water, sampled during March, July, August, and November 1978, was filtered through 0.2-, 0.4-, 0.6-, 3.0-, and 8.0-μm-pore-size Nuclepore filters. In August, bacterial numbers were counted on the same pore-size filters that were used in the uptake experiments. From size fractionation data for bacterial amino acid uptake and cell numbers specific activity (44), the amino acid turnover rate per cell was calculated for the cells in the 0.2- to 0.6-, 0.6- to 3.0-, and >3.0-μm size classes. The amino acid turnover rate per cell was calculated as the volume of water from which a single bacterium theoretically could remove all of the amino acids in 1 h. In all cases water was incubated with tritiated amino acids (net 250; New England Nuclear Corp.). Triplicate subsamples (5 to 20 ml) and one formaldehyde-fixed control were incubated with radioactive label (4 × 10^5 to 1 × 10^6 dpm or ≃0.4 to 1 nmol $liter^{-1}$ [final concentration added]) and incubated for 30 to 60 min within 2°C of ambient temperature. After incubation, incorporation of label was terminated by adding formaldehyde to a final concentration of 0.6% or by flooding the sample with unlabeled substrate (31). Size fractions were obtained after incubation with label by filtering independently incubated subsamples through the Nuclepore filters. The filters then were placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear Corp.) and counted in a Beckman LS-200 liquid scintillation counter. Counts were corrected for quench, and control counts were subtracted.

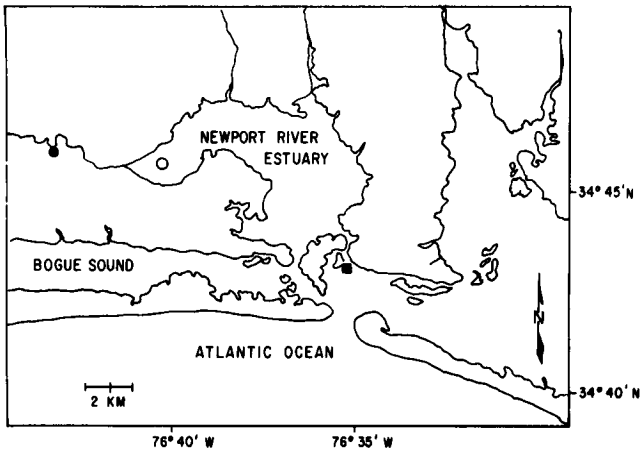

FIG. 1. Map of the Newport River estuary indicating the low (●)-, mid (○)-, and high (■)-salinity sites sampled for cell volume estimates.

RESULTS

Cell volume. Mean cell volume was variable within similar ranges in the Newport River estuary and the Gulf of Mexico. The range of cell volume for 15 sets of observations in the estuary (five sampling seasons at each of three stations) was 0.072 to 0.096 μm^3, compared with 0.078 to 0.096 μm^3 for the three stations in the Gulf. Coccoid rods which were <0.6 μm in major dimension comprised on the average about 85% of the total cell

number in both areas.

TABLE 1. Locations and mean cell volume estimates, with standard deviations, for three stations in the Gulf of Mexico[a]

Station	Latitude	Longitude	Salinity (‰)	Cell vol (μm^3)	
				$\bar{x}$[b]	SD
Mississippi plume	28°41′ N	89°16′ W	28.9–33.2	0.089	0.006
Cape San Blas	29°29′ N	86°08′ W	26.8–31.1	0.096	0.005
Gulf Loop	25°25′ N	86°59′ W	36.1	0.078	0.005

[a] Analysis of variance indicated that there were significant differences ($P < 0.05$) in mean cell volume among stations (F, 10.6; df, 2, 6) but not among samples at a station (F, 0.86; df, 6, 9).
[b] $\bar{x}$, Mean cell volume estimates.

Two-way analysis of variance of the cell volume data indicated a significant effect ($P \leq 0.01$) of both sampling period and location but not of their interaction on bacterial size in the Newport River estuary (Fig. 2). For the lower-estuary high-salinity site, there was relatively little variation in cell volume through the year for the five sampling periods (Fig. 2). There were larger differences in mean cell volume through the year at the upper-estuarine and riverine sites (Fig. 2). In June the average cell size was uniform through-out the estuary. The two highest values occurred at the river site in the falls of 1977 (0.096 μm^3) and 1978 (0.086 μm^3).

Temperature, salinity, and water available for runoff from the Newport River drainage basin also varied during the sampling of bacterial cell volume. During the fall, calculated runoff was low, and during the December, February, and June sampling periods, runoff was higher (Fig. 2). The average daily water temperature was lowest on 23 February at the river site (4.5°C) and highest on 5 September 1978 at the river site (26.4°C) and on 21 June at the upper-estuary site (28.4°C). Temperatures were between 25.5 and 28.4°C at all locations in June and September 1978. There was a sharp temperature increase from 7.0 to 17.3°C among the samplings from the three sites in February and March 1978. Water available for runoff varied inversely with temperature (Fig. 2). As a result, the average salinity changed seasonally. The mean salinity at the river site was greatest (4.2%) in October 1977.

In the Gulf of Mexico the mean size for cells at all sites was 0.088 μm^3; the lowest mean cell volume for a station was at the Gulf Loop, and the highest was at Cape San Bias (Table 1). Differences in the mean cell volumes among the stations were significant, but there were no significant differences among the samples at a station (Table 1). Both the Mississippi plume and the Cape San Bias stations had significantly greater mean cell volumes than the Gulf Loop station (95% confidence interval, ±0.0098 μm^3).

Association with particles. Bacterial-sized particles (i.e., <3 μm) accounted for 75 to 98% of the total amino acid incorporation in samples from the Newport River estuary, Georgia Bight, and Mississippi River plume. In the Newport River estuary, 75 to 96% of the label retained on the 0.2-μm filter (total incorporation) passed through a 3.0-p.m filter in samples collected only for size fractionation of heterotrophic activity (Table 3). The percentage of total incorporation that passed through filters with a pore size of 0.6 μm was highly variable, however, ranging from 0.2 to 70%. Similar results were observed when both amino acid incorporation and cell counts were measured in samples collected along the salinity gradient of the estuary in August (Table 4). More than 92% of the cell numbers and 84% of the heterotrophic activity were in the <3.0-μm fraction at all stations. The percentage of total cells in the 0.2- to 0.6-μm fraction and 0.6- to 3.0-μm size fraction was generally consistent throughout the estuary (Table 4), but the percentage of total amino acid incorporation was not (Table 4). Amino acid incorporation in the 1.4 and 8.0%c samples was relatively low for the 0.2- to 0.6-μm fraction and relatively high for the 0.6- to 3.0-μm fraction compared with that in the higher-salinity samples. Incorporation in the >3.0-μm fraction was greatest (≃15%) at salinities of ≤24.4‰ and least (≃5%) at salinities of >24.4‰ (Table 4).

Plots of the August data with distance upstream from Beaufort inlet suggest that intermediate salinities in the estuary contained the highest abundance of bacteria and chlorophyll, the most rapid amino acid turnover rates, and the highest per-cell turnover rates for the 3.0-μm size fraction (Fig. 3). The fastest turnover rate, the highest bacterial numbers, and the highest per-cell amino acid turnover rate in the >3.0-μm size fraction was just down-

stream from the peak in chlorophyll *a*. Minimum chlorophyll *a* was at the farthest upstream, lowest-salinity station, and minimum turnover rate was immediately downstream of the chlorophyll *a* minimum. The lowest bacterial numbers (Fig. 3) were at high-salinity stations nearest the inlet. There were significant negative correlations ($P < 0.05$) of percent heterotrophic activity and percent AODC in the >3.0-μm size fraction within salinity (Table 4). There were no significant correlations ($P > 0.05$) of percent activity and percent AODC in the >3.0-μm size class with either chlorophyll *a* or total AODC (Table 4).

The per-cell turnover rate of the amino acid pool for the >3.0-μm size class (98.7×10^{-9} ml h^{-1} $cell^{-1}$) was significantly higher ($P > 0.05$) than that for the 0.2- to 0.6-μm size class (30.05×10^{-9} ml h^{-1} $cell^{-1}$) or that for the 0.6- to 3.0-μm size class (27.25×10^{-9} ml h^{-1} $cell^{-1}$) (Fig. 3). There was no significant difference between the uptake per cell in the 0.2- to 0.6-μm and the 0.6- to 3.0-μm size classes.

TABLE 2. Cell size and shape categories for determinations of average cell volume[a]

Cell shape	Diam or length (μm)	Width (μm)[a]	Vol for category (μm^3)
Coccoid rods	<0.6	<0.6	0.065
Cocci	0.6–1.2	0.6–1.2	0.320
Rod	0.6–1.2	0.38–0.44	0.111
Rod	1.2–1.8	0.44–0.5	0.254
Rod	1.8–3.0	0.54–0.63	0.574

[a] The volume of the smallest size category was calculated, assuming a cell diameter of 0.5 μm. Volumes of the other categories are the geometric mean of the calculated volumes of the largest and smallest cell in each class. Cell width for rods (needed for volume calculation) was determined by linear regression of the data of Zimmerman (47). The resulting equation was width = 0.101 (length) + 0.323 ($r = 0.923$; $n = 5$).

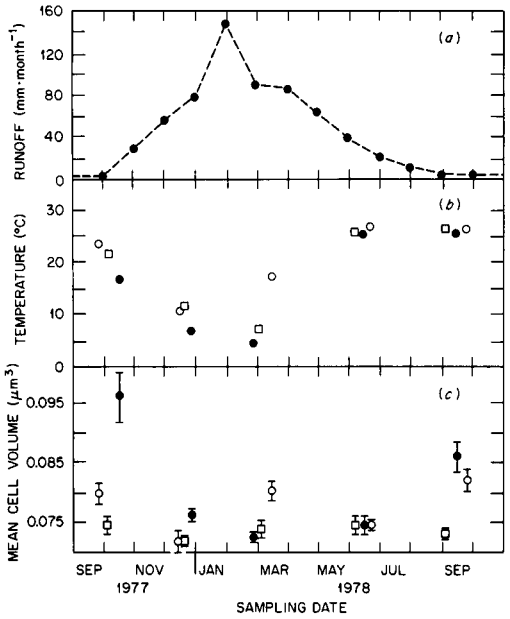

FIG. 2. Sampling data versus (*a*) estimated water available for runoff, (*b*) average temperature on the sampling day, and (*c*) mean cell volume (± standard error). Data are for low (●)-, mid (○)-, and high (□)-salinity sites in the Newport River estuary. Cell volume was significantly affected ($P \leq 0.05$) by the sampling period (F, 20.7; degrees of freedom [df], 4, 195) and location (F, 29.6; df 2, 195) but not by their interaction (F, 1.033; df, 8, 195).

In the neritic samples from Georgia Bight and from the plume of the Mississippi River the <3.0-μm size fraction also accounted for the greatest portion of the amino acid incorporation. In the Georgia Bight samples the association of heterotrophic activity with size fractions varied with relative sample depth (surface, middepth, or bottom) in the water column and relative distance from shore (coastal, midshelf, or shelf break) (Fig. 4). Two-way analysis of variance of the six stations where surface-, middepth-, and bottom-water samples were collected (Savannah and Jacksonville transects) indicated a significant effect of both relative distance from shore and relative depth of sample and no significant interaction between relative distance from shore and depth (Table 5). At these six stations, the incorporated label retained on the 3.0-μm-pore-size filter relative to total incorporation on 0.2-μm filters was about the same in the surface (9.3%) and middepth samples (11%) but was significantly higher (25.4%) in the bottom-water samples (Table 5). The incorporation in the >3.0-μm fraction for the two transects and all three depths also decreased significantly (Table 5) from nearshore (22.3%) to midshelf (13.4%) and shelf break (9.25%) stations. Two-way analysis of variance of the surface-water samples for all nine stations indicated a significant difference among stations associated with relative distance from shore but not with transect or the interaction of distance and transect (Table 5). In these surface samples the mean percentage of the total incorporation in the >3.0- μm size fraction was highest for the nearshore stations (15.2%), intermediate for the midshelf stations (11.7%), and lowest for the shelf break stations (5.5%). At the Mississippi station in April 1980 the <1.0-μm fraction accounted for 90.5% and the <3.0-μm fraction accounted for 98.4% of the amino acid incorporation.

DISCUSSION

Our data are consistent with data in the literature showing that in samples of natural water the mean bacterial cell volume varies within a relatively narrow range, but changes within that range may reflect differences in growth rate (25) or physiological state (36) of the bacterial community. There are changes in bacterial cell size in the Newport River estuary associated with a phytoplankton bloom and with seasonal changes, which could influence substrate availability and thus the growth rate or physiological state of the bacteria. In the Gulf of Mexico differences in bacterial size among stations may also be related to substrate availability.

The mean cell volumes calculated from AODC measurements of cells from the Newport River estuary (0.08 μm^3) and the Gulf of Mexico (0.088 μm^3) were similar and in the range (0.045 to 0.090 μm^3) reported by other investigators, who used epifluorescence techniques (13, 15, 47) in marine and estuarine waters. The variability in bacterial size and in the maximum cell size of suspended bacteria appears to be larger in freshwater environments (e.g., see reference 24). Bowden (9) measured bacterial cell size on scanning electron micrographs and reported an average volume of 0.047 μm^3 for samples from the Newport River estuary. Although we found differences in cell volume associated with time and location of sampling in the Newport River estuary (Fig. 2), it is unlikely that the differences between the scanning electron microscopy estimates of Bowden and our AODC estimates are due entirely to these factors. The difficulty in sizing the smallest cells with light microscopy and the problem of cell shrinkage during sample preparation for scanning electron microscopy (15) probably contribute to the discrepancy between the cell volume estimates.

The changes in cell volume with sampling time in the Newport River estuary may be related to seasonal changes in freshwater flow, temperature, and salinity. These factors may directly or indirectly influence the growth rate of the bacteria or their physiological state. The large temperature increase between the February and March samples (Fig. 2) cooccurred with an observed increase in mean cell volume from $\simeq$0.075 μm^3 in the river and lower-estuary sites to 0.080 pin3 at the upper-estuarine site (Fig. 2). During this temperature increase, a large phytoplankton bloom occurred in the estuary, and bacterial numbers increased rapidly (A. V. Palumbo, Ph.D. thesis, North Carolina State University, Raleigh, 1980). The increased size of the bacteria at the upper-estuarine site may have been in response to the phytoplankton bloom and faster bacterial growth due to increased organic nutrient availability during the bloom. This interpretation is consistent with our observation for the Gulf of Mexico where cells in highly productive (Mississippi plume) or postbloom (Cape San Blas) waters were larger than those in low-productivity water (Gulf Loop). Other data also indicate that water temperature alone does not control cell volume. In August and September of both 1977 and 1978, there were significantly higher mean cell volumes in the river and upper-estuarine sites than during a period of high temperature throughout the estuary in June (Fig. 2).

TABLE 3. Salinity, total number of bacteria (AODC), and percentage of amino acid incorporation by different size fractions in the Newport River estuary in 1978

Date	Salinity (‰)	AODC (10^6 ml^{-1})	% Amino acid incorporation by following size fractions:			
			0.2–0.6 μm	0.6–3.0 μm	<3.0 μm	>3.0 μm
27 March	11		18[a]	78[b]	96	3.8
31 July	0	2.54	0.2	75	75.2	24
31 July	21	4.01	50	38	88	12
31 July	34	0.87	70	18	88	12
12 November	34				83	17[c]

[a] Size fraction for this datum: 0.2 to 0.4 μm.
[b] Size fraction for this datum: 0.4 to 3.0 μm.
[c] Of the 17% in this fraction, 8% occurred in the 3.0- to 8.0-μm size fraction.

In August and September, seasonal Spartina alterniflora die-off combined with increased flushing time of the estuary during this period of reduced runoff (Fig. 2) probably in-creased substrate availability, which could explain the observed increase in bacterial size in the upper estuary. This results in an inverse relationship between the salinity of a site and the mean cell volume. Valdes and Albright (37) also found a general correspondence between increasing mean cell volume and decreasing salinity in March in the Fraser River estuary.

TABLE 4. Percentage of amino acid incorporation and cell number (AODC) in different size fractions in the salinity gradient of the estuary on 23 August 1978

Salinity (‰)	% Total in following size fractions[a]: 0.2–0.6 μm		0.6–3.0 μm		>3.0 μm		<3.0 μm[b]	
	Incorp	AODC	Incorp	AODC	Incorp[c]	AODC[d]	Incorp	AODC
1.4	27.4	62.7	57.6	29.6	15.0	7.7	85	92.3
8.0	27.5	52.7	57.4	43.5	15.1	3.8	84.9	96.8
24.4	54.1	53.3	31.3	43.5	14.6	3.2	85.4	98.9
29.2	64.3	62.1	29.2	36.8	6.5	1.1	93.5	98.7
34.2	77.8	55.3	19.2	43.4	3.0	1.3	97.0	97.5
34.3	62.3	63.8	32.9	33.7	4.8	2.5	95.2	96.7
Mean	52.2	58.3	37.9	38.4	9.8	3.3	90.2	96.8
SD	20.6	5.1	15.9	6.0	5.7	2.4	5.6	2.4

[a] Station locations identified on Fig. 1 were: low-salinity site, 8.0‰ sample; midsalinity site, 29.2‰ sample; high-salinity site, 34.3‰ sample. Incorp, Incorporation.
[b] Sum of incorporation of AODC in the 0.2- to 0.6-μm fraction and the 0.6- to 3.0-μm fraction.
[c] The percentage of the total incorporation in the >3.0-μm fraction was significantly correlated with salinity ($r = -0.853$) but not with AODC ($r = 0.283$) or chlorophyll *a* ($r = 0.336$).
[d] The percentage of the total AODC in the >3.0-μm fraction was significantly correlated with salinity ($r = -0.877$) but not with AODC ($r = -0.191$) or chlorophyll *a* ($r = -0.208$).

Association of bacteria cells and heterotrophic activity with large particulate matter probably has a greater effect on potential availability of bacteria to predators than do the changes we report in average cell volume. Individual bacterial cells are below the size that can be effectively utilized, for example, by adult copepods (22). Bacterial association with larger particles, however, might make them vulnerable to copepod predation (32). In the Newport River the association of bacterial activity with particles is dependent on location. Very small particles (<0.6 μm) accounted for ≥50% of the number of cells and heterotrophic incorporation of dissolved amino acids in all samples taken at salinities of >20% (Tables 3 and 4). At salinities of <20%, however, particles of >0.6 μm accounted for the majority of the activity and for more than 30% of the cell numbers (Table 4). Also, there were significant negative correlations of salinity and the percentage of the AODC and activity in the >3.0-μm fraction, which reflects the abrupt change from 29 to 25‰ (Table 4). Our observations of a correspondence between increasing salinity and decreasing importance of attached bacteria relative to free-living bacteria in an estuarine environment agree with observations by Bell and Albright (5, 6).

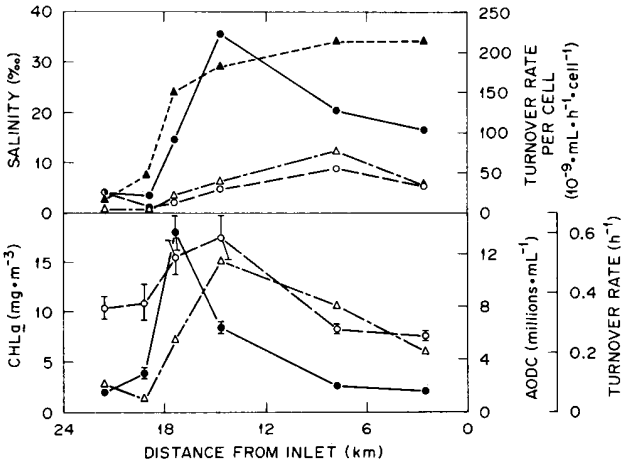

FIG. 3. Plots of salinity (▲); amino acid turnover rate per cell in the >3.0 (●)-, 0.6- to 3.0 (△)-, and 0.2- to 0.6 (○)-μm size fractions; and AODC (○), chlorophyll *a* (●), and total amino acid turnover rate (△) versus distance from Beaufort inlet for samples collected on 23 August 1978. AODC and chlorophyll *a* are given ± 1 standard error. There are significant differences ($P \leq 0.05$) in amino acid turnover rate per cell (F, 4.11; df, 2, 15) among the different size classes (95% confidence interval, 57.5×10^{-9} ml h^{-1} $cell^{-1}$.

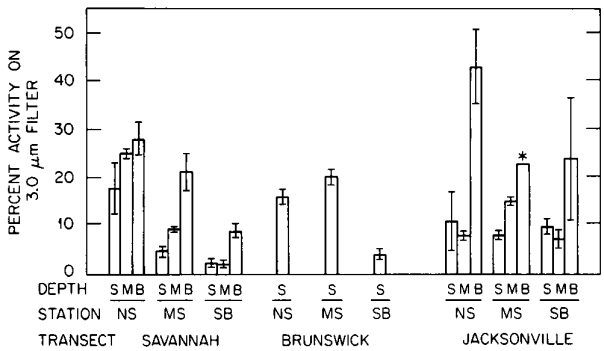

FIG. 4. Percentage of heterotrophic activity on the filters with a pore size of 3.0 μm in samples from Georgia Bight. Data are for surface (S)-, middepth (M)-, and bottom (B)-water samples collected at nearshore (NS), midshelf (MS), and shelf break (SB) stations. All values except the sample marked with an asterisk are means of two replicate samples (± 1 standard error) taken from different Nisken bottles.

Although cells associated with particles have a higher activity on a per-cell basis (Fig. 3) and may be consumed by a wider variety of zooplankton than are free-living cells, their contribution to estuarine food webs may be limited and difficult to assess. In the Newport River estuary, amino acid turnover rate per cell was greatest in the >3.0-μm fraction (Fig. 3), in agreement with the suggestion of Ferguson and Rublee (13) and the observations of later investigators (20, 24, 45) that the larger cells associated with particles may be more active on a per-cell basis than are the free-living bacteria. This observation may not hold when attached bacteria

predominate in bacterial activity and numbers and when there are very high concentrations of suspended solids (7). Despite the higher per-cell activity for bacteria associated with particles retained on a filter with a pore size of 3.0 μm, their incorporation of amino acids in the Newport River estuary was still only 3 to 24% (Tables 3 and 4) of the total.

Problems remain in assessing the relative contribution of attached and free-living bacteria to total heterotrophic activity (23). For example, direct counts underestimate the number of cells associated with particles, and incorporation of dissolved amino acids by particle-associated bacteria will be underestimated to the extent that these bacteria utilize amino acids directly from the particles upon which they reside. This pathway cannot be measured by the technique we employed but may not be significant in all cases or for all compounds.

In the Newport River estuary and in coastal waters of the Gulf of Mexico and Georgia Bight, we found that 85 to 98% of the heterotrophic activity was associated with the <3.0- μm size fraction. Other investigators (2, 3, 8, 10), utilizing techniques similar to ours have also reported that the smaller size fractions (e.g., <3 μm) are responsible for the majority of heterotrophic activity in coastal and oceanic water. Filtration of samples before incubation with label yields data (17, 18, 40) that apparently are not consistent with those derived from filtration of samples after incubation (e.g., see references 2, 3, and 8). There is evidence that filtration before the addition of the label can increase substrate levels and thus reduce the uptake of labeled substrate and that sample manipulation can also result in inadvertent increases in toxic trace metals such as copper (13a). However, filtration artifacts could affect filtration after incubation procedures (39), making the choice of technique an important factor in comparing results from different investigators.

The relatively high activity of large size fractions reported for the Georgia estuaries (17, 18) may nevertheless reflect real differences from other areas. When used in offshore water (2, 18), filtration before incubation has yielded estimates of activity in the >3.0-μm fraction of 14 to 50%, only slightly higher than the 10 to 30% obtained without prefiltration (2, 3, 8, 10). Although for coastal Georgia waters we found a general trend of decreasing activity in the larger size fractions with distance from shore, our measurements in near-surface samples (5.5 to 15.2% in the >3.0-μm fraction) were much lower than values obtained with prefiltration in the same area (18). Our measurements of heterotrophic activity in Georgia coastal waters in the >3.0-μm fraction are similar to the 3 to 17% we observed in the high-salinity area of the Newport River estuary.

Differences in the amount of resuspension of bacteria and detritus in the Georgia estuaries (tidal amplitude, >2.0 m [17, 18]) and the Newport River estuary (tidal amplitude, <1.0 m) may influence the size distribution of heterotrophic activity. Heavy particulate loads result in an increased percentage of heterotrophic activity or bacteria or both in larger size fractions (6, 7, 24). A significant portion of the bacteria in sediments is attached to particles (38), and bacterial density on sedimented particles is greater than the density on suspended particles in the same size range (26). Thus, resuspension of sediments would result in a greater proportion of the heterotrophic activity being associated with larger size fractions and would put particles with greater numbers of bacteria into the water column. Resuspension of bottom sediments and bacteria appears to be more important in Georgia (18) than in the Newport River estuary (Palumbo, Ph.D. thesis). The potential for resuspension in the Newport River estuary is greatest in the shallow upper estuary where the underlying sediment has a high silt and detritus content in contrast to that of the lower estuary, where the sediment is predominantly sand (11). The low-salinity upper estuary also has a higher proportion of activity and cells in the larger size fractions than does the high-salinity region of the estuary.

The increased activity in the large size fractions in the near-bottom samples from coastal water off Georgia and northern Florida (Fig. 4) is also consistent with the hypothesis that sediment resuspension results in increased heterotrophic activity in large size fractions. In this unstratified water column, wind mixing and other water movements resuspend bottom sediments. Additional evidence for resuspension of sediments includes elevated bacterial counts in near-bottom samples in coastal waters (12) and shifts in heterotrophic activity to large size fractions in estuaries at low tide (A. V. Palumbo and D. Cooper, unpublished data).

In samples from the open ocean, coastal waters, and Newport River estuary, the majority of bacteria are associated with the <0.6-μm particulate fraction, and thus most of the potential flow of dissolved organic matter into the planktonic food web might be via the bacteria-microzooplankton or bacteria-larvacean link. However, because the activity per cell of particle-associated bacteria can be significantly higher than that of free-living bacteria, the particle-associated bacteria could contribute significantly to the flow of dissolved organic carbon. The proportions of free-living and particle-associated bacteria in estuaries and coastal marine waters may depend on the amount of resuspension of bottom sediments. Thus, in areas where resuspension is substantial, as in some estuaries and in near-bottom waters of the neritic zone, a high proportion of bacterial production may be available to benthic and planktonic organisms feeding on particles which are >0.6 μm in diameter.

TABLE 5. Analysis of the percent activity in the >3.0-μm size fraction from samples collected in Georgia Bight

Analysis[a]	Source	F	df	$P > F$[b]
A	Relative distance[c]	6.59	2, 26	0.01
A	Relative depth[d]	10.6	2, 26	0.001
A	Interaction	0.13	4, 26	NS
B	Relative distance[e]	5.11	2, 9	0.05
B	Transect	1.43	2, 9	NS
B	Interaction	2.41	4, 9	NS

[a] A. Analysis of six stations and three depths from the Savannah and Jacksonville transects; the weighted squares of means method was used due to a missing replicate from the midshelf bottom sample from the Jacksonville transect. B. Analysis of surface samples from all nine stations.
[b] NS. Not significant: $P > F$ is greater than 0.05.
[c] 95% confidence interval ± 5.3%.
[d] 95% confidence interval ± 5.1%.
[e] 95% confidence interval ± 4.9%.

LITERATURE CITED

1. Alldridge, A. L. 1981. The impact of appendicularian grazing on natural food concentrations in situ. Limnol. Oceanogr. 26:247- 257.
2. Azam, F., and O. Holm-Hansen. 1973. Use of tritiated sub-stances in the study of heterotrophy in seawater. Mar. Biol. 23:191-196.
3. Azam, F., and R. E. Hodson. 1977. Dissolved ATP in the sea and its utilization by marine bacteria. Nature (London) 267:696-698.
4. Azam, F., and R. E. Hodson. 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. 2:492-5(11.
5. Bell, C. R., and L. J. Albright. 1981. Attached and free-floating bacteria in the Frazer River Estuary. British Columbia. Canada. Mar. Ecol. Prog. Ser. 6:317-327.
6. Bell, C. R., and L. J. Albright. 1982. Attached and free-floating bacteria in a diverse selection of water bodies. Appl. Environ. Microbiol. 43:1227-1237.
7. Bent, E. and R. Goulder. 1981. Planktonic bacteria in the Humber estuary: seasonal variations in population density and heterotrophic activity. Mar. Biol. 62:35-45.
8. Berman, T. 1975. Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. Mar. Biol. 33:215-22(1.
9. Bowden, W. B. 1977. Comparison of two direct-count techniques for enumerating aquatic bacteria. Appl. Environ. Micro-biol. 33:1229-1232.
10. Derenbach, J. B., and P. J. LeB. Williams. 1974. Autotrophic and bacterial production: fractionation of plankton populations by differential filtration of samples from the English Channel. Mar. Biol. 25:263-269.
11. Ferguson, R. L., and M. B. Murdoch. 1975. Microbial ATP and organic carbon in sediments of the Newport River estuary. North Carolina, p. 229-250. in E. Cronic (ed.). Estuarine research. I. Chemistry biology and the estuarine system. Academic Press, Inc.. New York.
12. Ferguson, R. L., and A. V. Palumbo. 1979. Distribution of suspended bacteria in neritic waters south of Long Island during stratified conditions. Limnol. Oceanogr. 24:697-7(15.
13. Ferguson, R. L., and P. A. Rublee. 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21:141-145.
13a.Ferguson, R. L., and W. G. Sunda. 1984. Utilization of amino acids by planktonic marine bacteria: importance of clean technique and low substrate additions. Limnol. Oceanogr. 29:258- 275.
14. Flood, P. R. 1978. Filter characteristics of appendicularian food catching nets. Sep. Exp. 34:173-175.

15. Fuhrman, J. A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescene microscopy compared to scanning electron microscopy. Mar. Ecol. Prog. Ser. 5:103-106.
16. Haas, L. W., and K. L. Webb. 1979. Nutritional mode of several non-pigmented microflagellates from the York River Estuary. Virginia. J. Exp. Mar. Biol. Ecol. 39:125-134.
17. Hanson, R. B., and J. Snyder. 1979. Microheterotrophic activity in a salt-marsh estuary. Sapelo Island. Georgia. Ecology 60:99- 107.
18. Hanson, R. B., and W. J. Wiebe. 1977. Heterotrophic activity associated with particulate size fractions in a Spartina alterni-flora salt-marsh estuary, Sapelo Island. Georgia, USA. and the continental shelf waters. Mar. Biol. 42:321-330.
19. Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence micros-copy. Appl. Environ. Microbiol. 33:1225-1228.
20. Hodson, R. E., A. E. Maccubbin, and L. R. Pomeroy. 1981. Dissolved adenosine triphosphate utilization of free-living and attached bacterioplankton. Mar. Biol. 64:43-61.
21. Kimmel, B. L. 1983. Size distribution of planktonic autotrophy and microheterotrophy: implications for organic carbon flow in reservoir food webs. Arch. Hydrobiol. 97:303-319.
22. King, K. R., J. T. Hollingbaugh, and F. Azam. 1980. Predator prey interactions between the larvacean Oikopieura choir(' and bacterioplankton in enclosed water columns. Mar. Biol. 56:49- 57.
23. Kirchman, D. 1983. The production of bacteria attached to particles suspended in a freshwater pond. Limnol. Oceanogr. 28:858-872.
24. Kirchman, D., and R. Mitchell. 1982. Contribution of particle-bound bacteria to total microheterotrophic activity in five coastal ponds and two marshes. Appl. Environ. Microbiol. 43:200-209.
25. Larsson, U., and A. Hagstrom. 1982. Fractionated phytoplankton primary production. exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67:57-70.
26. Marsh, D. H., and W. E. Odum. 1979. Effect of suspension and sedimentation on the amount of microbial colonization of salt marsh microdetritus. Estuaries 2:184-188.
27. National Climatic Center, National Oceanographic and Atmospheric Administration. 1977. Climatological data, North Carolina, vol. 82, no. 13. National Climatic Center. National Oceanographic and Atmospheric Administration, U.S. Department of Commerce, Washington. D.C.
28. National Climatic Center, National Oceanographic and Atmospheric Administration. 1978. Climatological data. North Carolina, vol. 83, no. 13. National Climatic Center, National Oceanographic and Atmospheric Administration, U.S. Department of Commerce, Washington, D.C.
29. Paerl, H. W. 1978. Microbial organic carbon recovery in aquatic ecosystems. Limnol. Oceanogr. 23:927-935.
30. Palumbo, A. V., and R. L. Ferguson. 1978. Distribution of suspended bacteria in the Newport River estuary. North Carolina. Estuarine Coastal Mar. Sci. 7:521-529.
31. Palumbo, A. V., R. L. Ferguson, and P. A. Rublee. 1983. Efficient utilization of dissolved free amino acids by suspended marine bacteria. J. Exp. Mar. Biol. Ecol. 69:257-266.
32. Roman, M. R., and P. A. Rublee. 1981. A method to determine in situ zooplankton grazing rates on natural particle assemblages. Mar. Biol. 65:303-309.
33. Sieburth, J. McN., V. Smetacek, and J. Lenz. 1978. Pelagic ecosystem structure: heterotrophic compartments of the plank-ton and their relationship to plankton size fractions. Limnol. Oceanogr. 23:1256-1263.
34. Sieburth, J. McN., P. J. Willis, K. M. Johnson, C. M. Burney, D. M. Lavoie, K. R. Hinga, D. C. Caron, F. W. French, P. W. Johnson, and P. G. Davis. 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayers of the North Atlantic. Science 194:1415-1418.
35. Thornthwaite, C. W., and J. R. Mather. 1955. The water balance. Publications in Climatology, vol. 8, no. 1. Drexel Institute of Technology Laboratory of Climatology, Philadelphia.
36. Torrella, F., and R. Y. Morita. 1981. Microcultural study of bacterial size changes and microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. Appl. Environ. Microbiol. 41:518-527.
37. Valdes, M., and L. J. Albright. 1981. Survival and heterotrophic activities of Fraser River water and strait of Georgia bacterio-plankton within the Fraser River plume. Mar. Biol. 64:231-241.
38. Weise, W., and G. Reinheimer. 1979. Fluoreszensmikrosko-piche untersuchungen uber die

Bakterienbesiedlung mariner Sandsediments. Bot. Mar. 22:99-106.
39. Wheeler, P., B. North, M. Littler, and G. Stephens. 1977. Uptake of glycine by natural phytoplankton communities. Lim-nol. Oceanogr. 22:900-910.
40. Wiebe, W. J., and D. F. Smith. 1977. Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. Mar. Biol. 42:213-223.
41. Williams, P. J. LeB. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. 1. Size distribution of population and relationship between respiration and incorporation of growth substrates. J. Mar. Biol. Assoc. U.K. 50:859-870.
42. Wilson, C. A., L. H. Stevenson, and T. H. Chrzanowski. 1981. The contribution of bacteria to the total adenosine triphosphate extracted from the microbiota in the water of a salt marsh creek. J. Exp. Mar. Biol. Ecol. 50:183-195.
43. Wolfe, D. A., F. A. Cross, and C. D. Jennings. 1973. The flux of Mn, Fe, and Zn in an estuarine ecosystem, p. 159-175. in Radioactive contamination of the marine environment. International Atomic Energy Agency, Vienna.
44. Wright, R. T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36:297-305.
45. Wright, R. T., and R. B. Coffin. 1983. Planktonic bacteria in estuaries and coastal waters of northern Massachusetts: spatial and temporal distribution. Mar. Ecol. Prog. Ser. 11:205-216.
46. Wright, R. T., R. B. Coffin, C. P. Ersing, and D. Pearson. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27:91-98.
47. Zimmerman, R. 1977. Estimation of bacterial numbers and biomass by epifluoresence microscopy and scanning electron microscopy, p. 103-120. In G. Rheinheimer (ed.), Microbial ecology of a brackish water environment. Springer-Verlag, Berlin.