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Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-series Study (BATS) site

Abstract—Accurate assessments of the true elemental cellular content of carbon (C), nitrogen (N), and phosphorus (P) in bacteria have been a major difficulty in microbial research for some time. This study is the first to present single-cell elemental C, N, and P measurements on natural populations of bacteria from the Sargasso Sea using a transmission electron microscope (TEM) equipped with an X-ray detector unit. Elemental cell content showed a best fit as a power function of the cell size, and smaller bacteria had significantly more elemental C, N, and P per cell volume than the larger ones (scaling factor $a < 1$). Contrary to coastal, brackish, and freshwater systems, the bacteria collected in this study appeared to have a molar elemental P:N close to the Redfield ratio of 0.063 (N:P = 16). The geometric mean of C per cell volume analyzed in this study (148 fg C μm^{-3}) was higher than any other estimates reported from coastal and brackish water systems. Total amount of bacterial C calculated from abundance estimates requires an average conversion factor, and the choice of a representative bacterial cell volume is therefore critical. We were able to demonstrate that the outcome of biomass assessments is highly dependant on the choice of cell-specific conversion factors. By applying cell volumes previously reported from the BATS site, the average 0–250 m depth integration of bacterial C between 1991 and 1996 ranged between 1.7 and 2.5 times less than other recent biomass estimates for these waters.

Bacteria are important in ocean biogeochemical cycles and considered key players in the transfer of dissolved organic matter into particulate organic matter. Ducklow and Carlson (1992) noted in their overview that a lot of attention has been focused on thymidine conversion factors in bacterial growth measurements during the last couple of decades, while choices of bacterial C per cell have been poorly investigated. Only recently, specific studies have focused on how to accurately measure individual elements in single-cell bacteria (Heldal 1993; Norland et al. 1995; Tuomi et al. 1995; Fagerbakke et al. 1996).

Early estimates of the elemental composition of individual bacteria were done by a combination of epifluorescence microscopy (EFM) for bacterial sizing and conventional carbon and nitrogen (CHN) elemental bulk analysis. This approach was most successful in cultures because debris and other plankton would interfere with the analysis of samples from natural seawater. Since Heldal et al. (1985) first introduced the X-ray microanalysis detection (XRMA) method, a limited number of studies (Tuomi et al. 1995; Fagerbakke et al. 1996) have been able to measure elemental C, N, and P in bacteria simultaneously. The elemental sensitivity was, in the beginning, limited to an atomic weight of 11 [Na] because of the absorption of low-energy X-ray photons by the protective window (Heldal et al. 1985). Since then, a more sensitive detector was installed, and lighter elements such as C and N could be measured accurately and with adequate precision (Heldal 1993; Norland et al. 1995). In this study, we applied the XRMA technique to measure the elemental C, N, and P cell content of individual bacteria collected at the BATS site. The critical choices of conversion factors and accurate cell volumes in comparative studies of bacterial and phytoplankton C biomass is also demonstrated.

Materials and methods—Seawater samples were collected at discrete depths from the surface down to 250 m at the BATS site (31°50′N, 64°10′W) using 12-liter Niskin water bottles. The water samplers were attached to a 12-position General Oceanics Model 1015 rosette. Samples for bacterial abundance and the chlorophyll *a* (Chl *a*) estimates were collected between January 1991 and December 1996. The bacteria samples were preserved in glutaraldehyde (2.5% final concentration) and stored at +4°C. Aliquots of 20 ml were filtered no longer than 2 d after collection, and the bacterial cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) as described by Porter and Feig (1980). Microplankton cells containing Chl *a* were identified using an EFM with a fluorescein isothiocyanate (FITC) filter combination (red fluorescence by ultraviolet excitation), and only the nonpigmented DAPI-stained bacterial cells were enumerated. The Chl *a* samples were filtered onto 47-mm Whatman GF/F filters (4 liters per depth) and stored in liquid nitrogen. The filters were extracted in 90% acetone for 24–48 h at −20°C and analyzed on a Turner Design 110 Fluorometer.

Samples for the XRMA were taken at five depths between 20 and 140 m in 1992 using Niskin water bottles. Aliquots of 90 ml were collected, and glutaraldehyde was immediately added (2.5% final concentration) to the samples. The preserved samples were kept at +4°C and processed 1 week later at the University of Bergen, Norway.

In 1995, surface seawater samples were collected at 10 m depth on five cruises to the BATS site using a GoFlo bottle attached to a Kevlar wire. These samples were stored at +4°C and processed 6–12 h after sampling; details of the preparation method is given in Heldal (1993).

The bacterial cells were collected on an aluminum grid coated with a carbon-stabilized formvar polymer membrane using a Sorvall Combi Plus Ultracentrifuge (20,000 × g for 30 min). A careful examination of the supernatant in the microscope showed that all bacteria were collected from the water phase using this technique. The supernatant was removed and the aluminum grid with cells was rinsed with one drop of 0.2-μm filtered Milli-Q water and stored dry until analysis. The samples were analyzed in a JEOL 100CX transmission electron microscope. Cell volume (V) was calculated by assuming that all bacteria are essentially cylindrical shapes with two half-spherical ends (Heldal 1993); hence, only widths and lengths of the cells were measured.

Electron microscope specimens have a tendency to shrink during the process of air drying. Because of their reduced osmotic cell volume, the smaller sized bacteria that are abundant in the Sargasso Sea (Sieracki et al. 1995; Gin et al. 1999) are also known to shrink less than larger cells (Montesinos et al. 1983). Specific studies addressing the problem with shrinkage (Woldringh et al. 1977; Fagerbakke et al. 1996) have also concluded that the two dimensions used for cell volume calculations in scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies (width and length) are not significantly influenced by the process of drying.

The Tracor Z-MAX 30 energy dispersion detector was calibrated using a number of particles with known chemical composition (Coenzyme A, Ca-β-glycerophosphate, KNO_3, and $K_4[Fe(CN)_6]$) and Latex beads with a known carbon content (Norland et al. 1995). An earlier study noted that bacteria preserved in formaldehyde lost nearly all potassium and chlorine while other elements remained in the cells (Heldal et al. 1985). However, prolonged storage of bacterial cells in unbuffered glutaraldehyde can cause acid hydrolysis and significant loss of P (M. Heldal pers. obs.). Therefore, only elemental C and N was analyzed on the preserved cells collected from the depth profile samples in May 1992, whereas the air-dried surface samples from BATS in 1995 were analyzed for all three elements. Viewed by TEM, bacterial cells cannot be distinguished from pigmented cells such as the prochlorophytes. However, the abundance of prochlorophyte cells in surface waters at the BATS site is in the range of 0.001–0.5 $\times$ 10^8 cells L^{-1} (Sieracki et al. 1995; Gin et al. 1999), which is an order of magnitude less than bacterial abundance (Gundersen et al. 2001). Therefore, we did not expect any significant interference from the prochlorophytes in our single-cell elemental measurements.

Results and discussion—The bacterial cell volumes measured in this study varied by two orders of magnitude (0.01–2 μm^3), but the most frequent cell sizes were in the range of 0.03–0.1 μm^3 (Fig. 1). The geometric mean of all cell volumes measured (n = 164) was 0.048 μm^3. This estimate is within, but a little higher than, the average range of 0.033–0.057 μm^3 found by Carlson et al. (1996) from the Sargasso Sea. A recalculation of the Carlson et al. (1996) data set from the whole-depth profile shows a grand geometric mean of 0.042 μm^3. Carlson et al. (1996) measured the size of bacterial cells stained with acridine orange (AO) collected from seasonal (0–250 m) depth profiles at the BATS site. More comprehensive reports on the full size range of heterotrophic bacteria have been published by Sieracki et al. (1995) and Gin et al. (1999) using flow cytometry measurements of cells collected in the upper 250 m of the water column at BATS. The total size range of bacteria measured by Gin et al. (1999) (0.001–0.5 μm^3) also included the entire range of cells detected by Sieracki et al. (1995) and Carlson et al. (1996). Because flow cytometers are capable of measuring 10–100 times more cells per sample than EFM detection of AO-stained cells, we therefore find the Sieracki et al. (1995) and the Gin et al. (1999) studies more accurate and representative for the BATS site and conclude that the most frequently encountered bacterial cell volumes are in the range of 0.0025–0.062μm^3.

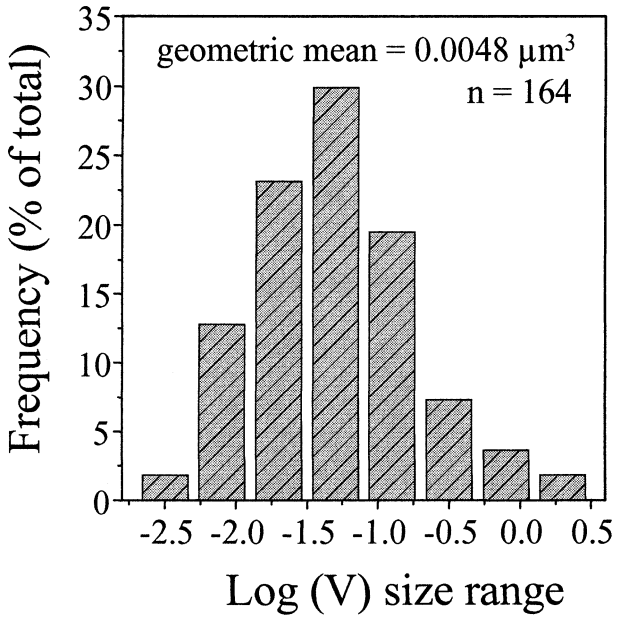

Fig. 1. Frequency of occurrence of cell volumes measured on bacteria collected from a depth profile at BATS in May 1992 and in surface waters from five BATS cruises between February and September 1995.

The elemental C, N, and P content of the bacteria in this study was plotted as a function of the cell volume (Fig. 2). The log-linear regression of the elemental cell content (fg $cell^{-1}$) showed a best fit as a power function of the cell volume. All the elements investigated in this study had volume scaling factors that were significantly less than unity (Fig. 2), suggesting that the larger bacteria had less elemental C, N, and P per cell volume than the smaller ones. The scaling factors found in this study (Fig. 2) are similar to the factor reported by Norland et al. (1987) for volume-specific dry weight but higher than the volume-specific protein estimates by Simon and Azam (1989). By doing a ''molecular inventory'' of the bacterial cell, Simon and Azam (1989) were also able to demonstrate that smaller bacteria are more compact than larger ones because of a certain minimum amount of space required for the DNA pool in each cell. The geometric mean of the volume-specific elemental C was 148 fg C μm^{-3} (Table 1), which is a factor of two to three times higher than similar direct measurements of bacteria collected in coastal marine waters. This finding concurs with Simon and Azam's (1989) suggestion that smaller cells have relatively higher elemental content per volume than larger ones.

By using the elemental power function from Fig. 2 and a cell volume of 0.025–0.062 μm^3 (Sieracki et al. 1995; Gin et al. 1999), an average elemental cell content of 4.0–8.9 fg C $cell^{-1}$ could be calculated. This range in elemental C per cell is lower but similar to the recent factors deduced by Caron et al. (1995; 10 fg $cell^{-1}$) and Zubkov et al. (2000; 7 fg $cell^{-1}$) and almost identical to the range inferred by Christian and Karl (1994) from the Hawaii Ocean Time-series (HOT) site (3.5–8.8 fg C $cell^{-1}$). In their inverse method approach to the microbial community structure, Christian and Karl (1994) reasoned that the elemental biomass for oce-

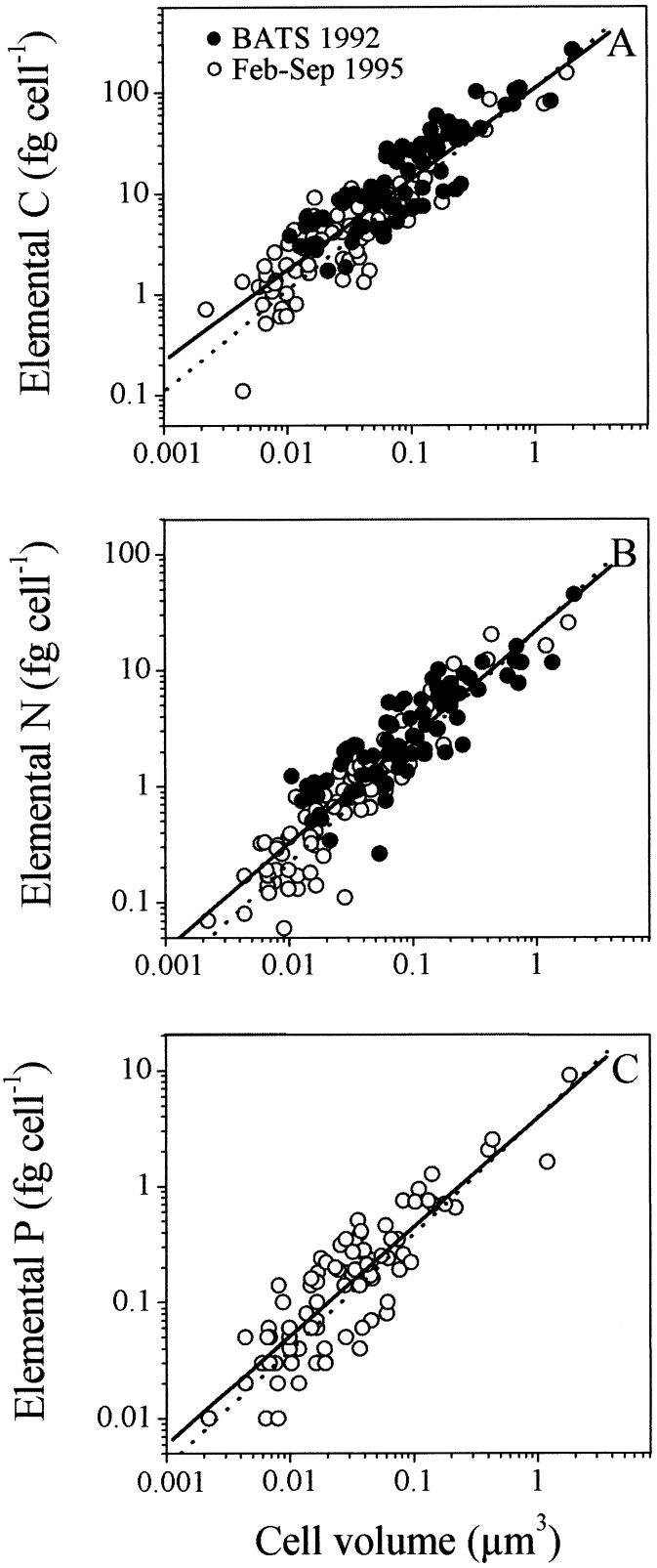

Fig. 2. Elemental cell content of (A) C, (B) N, and (C) P in bacteria, expressed as a log-linear function of the cell volume. The elemental cell content was expressed as a power function of the cell volume (V): fg C cell^{-1} = 108.8 (± 5.8) × V$^{0.898(\pm 0.035)}$ (r^2 = 0.894, $P < 0.0001$, n = 164); fg N cell^{-1} = 21.7 (± 0.6) × V$^{0.913(\pm 0.033)}$ (r^2 = 0.905, $P < 0.0001$, n = 164); fg P cell^{-1} = 3.89 (± 0.15) × V$^{0.937(\pm 0.058)}$ (r^2 = 0.873, $P < 0.0001$, n = 84). The dotted line was plotted using a scaling factor of one. All the scaling factors were significantly less than unity (Student's t-test of equality, $P \ll 0.001$).

anic bacteria at the HOT site could not be higher than 10 fg C cell^{-1} and that the widely used factor of 20 fg C cell^{-1} (Lee and Fuhrman 1987) was not plausible. A similar view has been argued for a number of open ocean studies of the microbial biomass (Joint and Pomeroy 1987; Caron et al. 1995; Fukuda et al. 1998) and Norland (1993) demonstrated that it was physiologically unreasonable to apply a constant conversion factor of 20 fg C cell^{-1} for a number of size classes. It should also be noted that Lee and Fuhrman (1987) warned against extrapolating their data to larger and smaller bacteria in microbial biomass assessments. Fukuda et al. (1998) showed recently that bacterial elemental C per cell was more than twice as high in coastal waters than in open ocean environments. A lot of emphasis has been put on the overestimation of bacterial biomass in open ocean waters. Fukuda et al. (1998), however, were also able to demonstrate that bacterial biomass in coastal environments might have been underestimated (by up to 40%) by applying the most commonly used conversion factor (20 fg C cell^{-1}). Cell volume estimates of bacteria and the use of element-per-volume conversion factors are therefore essential in order to obtain accurate estimates of bacterial biomass.

Redfield et al. (1963) defined the ''normal'' molar elemental ratio of aquatic marine organisms (C : N : P = 106 : 16 : 1). For balanced growth, the fluxes of N and P into new cell growth should be in equal ratios to phytoplankton C incorporation (Dubinsky and Berman-Frank 2001). Because elemental N and P incorporation is equal to the rates of photosynthetic C incorporation, we have inverted the elemental molar ratios in this study (Fig. 3); hence, the ''Redfield ratio'' of elemental molar P : N is 0.063. The geometric means of the molar N : C and P : C ratios ranged between 0.11 and 0.19 (C : N = 5.3–9.1) and 0.007 and 0.017 (C : P = 58.8–142.9), and the bacterial cells measured between February and September 1995 appeared to follow the slope (molar P : N ratio) of 0.063 (Fig. 3, N : P = 16). Only a few studies have reported elemental C, N, and P in naturally occurring bacteria (Tuomi et al. 1995; Fagerbakke et al. 1996), and they are either from coastal marine, brackish, or freshwater environments. The site comparison made by Fukuda et al. (1998) showed that the elemental N : C ratios in coastal and open ocean bacteria were not significantly different from each other. This overlap in molar N : C ratios between coastal and open ocean environments is also evident for the bacteria collected in February and March 1995 in this study (Fig. 3) and in the cells measured by Tuomi et al. (1995) and Fagerbakke et al. (1996) from a fjord at the west coast of Norway. However, the further decline in both the N : C and the P : C elemental ratios in May–September (Fig. 3) suggest that the collected cells in this study might have been equally limited by N and P. This is in contrast to the cells collected in coastal marine and brackish–freshwater en-

Table 1. Direct measurements of elemental C and cell volumes in aquatic bacteria. SW, seawater; EFM, epifluorescence microscope; TEM, transmission electron microscope; CHN, carbon, hydrogen, and nitrogen analysis; FA, formaldehyde; GA, glutaraldehyde; AO, EFM detection of bacteria using acridine orange (AO); XRMA, X-ray microanalysis.

Reference	Source	C content, mean (range) (fg C μm^{-3})	Cell volume range (μm^3)	Fixation	Detection
Nagata 1986	Lake water	106(39–188)	0.10–0.25	2% FA	EFM, AO, CHN
Nagata and Watanabe 1990	Lake water	120(59–252)	0.17–1.80	1% GA	EFM, AO, CHN
Tuomi et al. 1995	Coastal SW*	81(63–127)	0.15–0.60	Air dry	TEM, XRMA
Fagerbakke et al. 1996	Coastal SW*	59(30–82)	0.10–0.36	Air dry	TEM, XRMA
Fagerbakke et al. 1996	Brackish*	103(94–112)	0.22–0.40	Air dry	TEM, XRMA
Fagerbakke et al. 1996	Lake water*	53(51–55)	0.38–0.44	Air dry	TEM, XRMA
This study	Sargasso Sea	148(24–537)	0.002–2.0	Air dry, 2.5% GA	TEM, XRMA

* Average calculated from estimates made at the growth and stationary phase. Ranges were calculated from the standard error of the two means of the stationary and growth phase.

vironments (Tuomi et al. 1995; Fagerbakke et al. 1996), where the bacteria appeared to have excess P relative to N (Fig. 3). Coastal and brackish shallow water environments are closer to and more frequently influenced by nutrient inputs from rivers and in closer proximity to sediments rich in phosphorus. This is in contrast to open oceanic environments like the Sargasso Sea and the BATS site, which are characterized by random seasonal intrusions of nutrient-rich deep water to the euphotic zone (Michaels et al. 1994). Our finding that the bacteria we collected between February and September 1995 had a molar N : P ratio close to the Redfield ratio (Fig. 3) might also indicate that cell growth was limited by C or other essential elements and that N or P was not the primary limiting element.

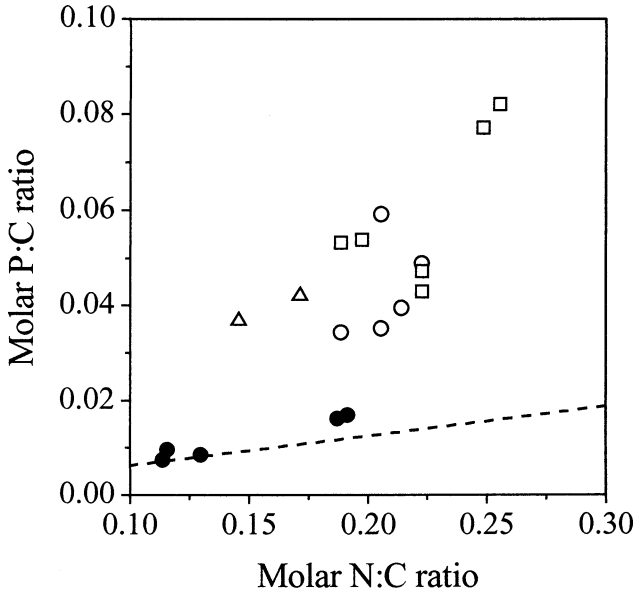

Fig. 3. The molar P : C ratio in bacterial cells as a linear function of the N : C ratio. The data in this study (closed circles) were collected from surface waters at BATS between February and September 1995. Data from the literature are from temperate coastal waters (open circles, Tuomi et al. 1995; Fagerbakke et al. 1996), fresh and brackish waters (open triangles, Fagerbakke et al. 1996) and in vitro bacterial cultures (open squares, Norland et al. 1995; Fagerbakke et al. 1996). Dashed line is the molar P : N ratio of 0.063 (the inverse relationship of the molar Redfield N : P ratio of 16).

Figure 4 shows the range of bacterial C : phytoplankton C (BC : PC) ratios that can be calculated from the same set of data and by using different sets of single-cell elemental conversion factors for the bacterial population. For this comparison, phytoplankton C was calculated from Chl *a* using the C : Chl *a* conversion factor developed for the BATS site

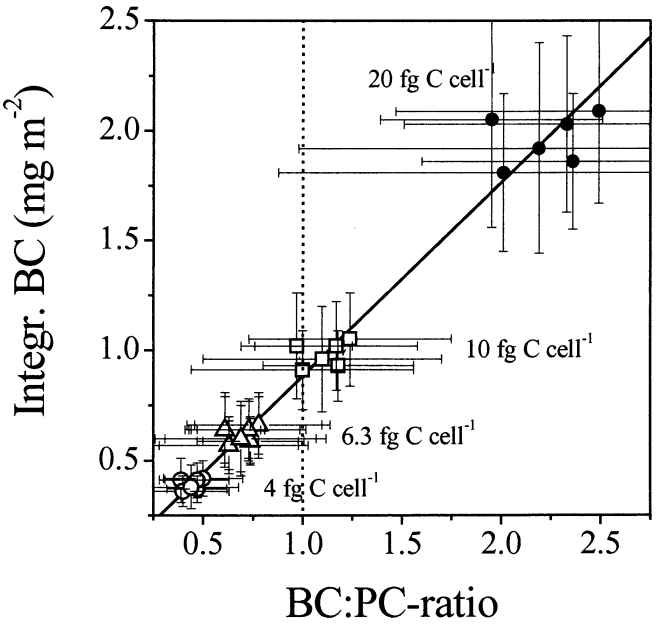

Fig. 4. Depth-integrated (0–250 m) bacterial C (BC) plotted as a function of the elemental BC : phytoplankton C (PC) mass ratio at the BATS site between 1991 and 1996. The depth integrations of BC were calculated from bacterial abundance and by assuming average C conversion factors of 4 fg C cell^{-1} (Gin et al. 1999; 0.025 μm^3; Fig. 2A), 6.3 fg C cell^{-1} (Carlson et al. 1996; 0.042 μm^3; Fig. 2A), and two general conversion factors of 10 fg C cell^{-1} and 20 fg C cell^{-1} that are commonly used in the literature. The linear regression (whole line) is the 6-yr average of BC as a function of the BC : PC ratio using the selection of elemental C conversion factors (BC = 0.88[BC : PC ratio]). Vertical and horizontal lines are standard errors of the seasonal variability within each annual estimate, and the dashed line is integrated BC = PC biomass.

by Malone et al. (1993). In the equation given by Malone et al. (1993) the C : Chl *a* factor decreased as a negative power function between the surface (C : Chl $a = 100$) and 250 m (C : Chl $a = 3$) depth. Bacterial C was calculated from nonpigmented microbial cells enumerated at the BATS site between 1991 and 1996 and by using a range of single-cell conversion factors. The elemental conversion factors were chosen as the most frequently used values in open ocean studies (20 fg C cell^{-1} and 10 fg C cell^{-1}) and by assuming that the most frequent cell size at the BATS site was 0.042 μm^3 (compiled from Carlson et al. 1996) or 0.025 μm^3 (compiled from Gin et al. 1999) and using the equation in Fig. 2A. Very few open ocean studies in recent years have applied the constant conversion factor of 20 fg C cell^{-1} for bacterial biomass estimates, but the factor of 10 fg C cell^{-1} has been more frequently implemented (Caron et al. 1995; Gundersen et al. 2001; Steinberg et al. 2001). If we assume that the most frequently occurring bacterial cell volume in the Sargasso Sea is within the size range of 0.025–0.042 μm^3 (Carlson et al. 1996; Gin et al. 1999), these most recent assessments might have overestimated bacterial C by a factor of 1.7–2.5 times. This study clearly demonstrates the need for accurate elemental conversion factors and bacterial cell volume estimates in aquatic biomass assessments.

Summary: This study is the first report of elemental C, N, and P measurements of individual bacterial cells from the Sargasso Sea. The elemental C, N, and P content per bacteria showed a best fit as a power function of the cell volume. The elemental scaling factors of the cell volumes found in this study (Fig. 2) were all significantly less than unity for all three elements investigated, and the volume-specific elemental C content in this study (Table 1) is the highest reported from direct measurements. Because Fukuda et al. (1998) found that cell-specific elemental C varied by a factor of more than two in bacteria collected from coastal and open ocean waters, a general pattern can be observed. Because of their frequently smaller size, open ocean bacteria appear to have a cell-specific elemental C content in the range of 4–9 fg cell^{-1} (Christian and Karl 1994; this study). Bacteria in coastal waters are more frequently exposed to nutrients essential for cell growth; hence, they are larger and have a cell-specific C content in the range of 20–30 fg C cell^{-1} (Lee and Fuhrman 1987; Fukuda et al. 1998). The bacteria analyzed in this study appeared to have less P per biomass than coastal cells, suggesting the cells were constrained equally by P and N or that a third element might have been more limiting for cell growth. This study clearly demonstrates the need of using accurate and locally derived cell volume estimates and elemental conversion factors in biomass assessments of bacteria in aquatic biogeochemical studies.

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Dissolution of particle-reactive radionuclides in deposit-feeder digestive fluids

Abstract—Naturally occurring radionuclides such as ^{234}Th, ^{7}Be, and ^{210}Pb are important tracers for quantifying sediment-mixing and sediment-accumulation rates. Profiles of these radionuclides in marine sediments are strongly influenced by particle displacement due to deposit feeding. Observations of rapid dissolution and high concentrations of dissolved metals in deposit-feeder digestive fluids suggest that particle-bound radionuclides could also undergo dissolution during deposit-feeder gut passage. We investigated this possibility in laboratory experiments examining radionuclide dissolution into the digestive fluids of the lugworm, *Arenicola marina.* Experiments with artificially labeled particles indicated that significant fractions of ^{234}Th, ^{7}Be, and ^{210}Pb dissolved from labeled algal detritus and clay particles at low particle concentrations. ^{137}Cs was also dissolved from clays. However, if unlabeled sediment particles were added to reach sediment : fluid ratios similar to those in *A. marina* midguts, little net dissolution occurred, which implies resorption of dissolved radionuclides by the added solid phases. Partition coefficients of these radionuclides in mixtures of digestive fluid and the various solid phases imply that relatively more ^{234}Th resorbs to the residual organic phase following digestion, compared to ^{210}Pb and ^{7}Be, which partition more strongly to the inorganic sediment phases. Despite little net dissolution, the phase change from algal detritus to either mineral surfaces (for ^{210}Pb) or undigested organic matter (for ^{234}Th) implies that ^{234}Th would serve as a better tracer for organic-matter mixing in sediments compared to ^{210}Pb, which would better trace bulk sediment mixing.