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Bacterial Productivity in the Water Column and Sediments of the Georgia (USA) Coastal Zone: Estimates via Direct Counting and Parallel Measurement of Thymidine Incorporation

Steven Y. Newell and Robert D. Fallon

University of Georgia Marine Institute, Sapelo Island, Georgia 31327, USA

Abstract. Three methods of estimating bacterial productivity were compared using parallel samples of Atlantic Ocean water (within 0.25–15 km of the Georgia coast). The frequency-of-dividing cells (FDC) method and the [^{3}H]thymidine incorporation method gave results which were strongly correlated ($r = 0.97$), but the FDC estimates were always higher (X2 to X7) than the [^{3}H]thymidine estimates. Estimates of bacterial productivity ranged from 2–4 $\times$ 10^8 cells·l^{-1}·h^{-1} at 0.25 km from shore to 1–9 $\times$ 10^7 cells·l^{-1}·h^{-1} at 15 km. A method involving incubation of 3-μm filtrates and direct counting gave results that could not be easily translated into estimates of bacterial productivity. Application of the FDC method to sediment samples gave high productivity estimates, which could be not reconciled with productivity estimates based on sediment oxygen uptake.

Introduction

Studies of secondary productivity by bacteria in the marine environment appear to show that, at least in some water masses, it is substantial relative to primary productivity taking place in the same water masses. For example, Williams [46] cited investigations that have led to the conclusion that 20–30% of marine phytoplankton production is utilized by bacteria, and in the same communication Williams demonstrated by size fractionation of total marine water-column respiratory activity that greater than 50% of the activity was often if not always associated with bacteria and small protozoa (microheterotrophs passing 5-μm filters). In some instances it has been estimated that bacterial productivity may equal or exceed phytoplankton primary productivity [14, 39, 41]. This type of finding has led to the hypothesis that marine bacteria provide a "mechanism for keeping dissolved organic carbon in the mainstream of trophodynamics" [40], i.e., that bacteria are not simply a sink for some fraction of primary production, but are intermediates in a food web [31, 34].

Several investigators have proposed methods for determination of aquatic bacterial productivity which could be used to test the validity of the hypothesis that bacteria are important producers and intermediates in marine food webs [see Table 5 of ref. 28: 3, 8, 20, 27], but at present it is probably fair to state that none of these is generally accepted as accurate. There is no standard method, without serious questions regarding its logical

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foundation, against which other methods can be compared. In this regard, the problem is analogous to that of determination of aquatic productivity [32].

Two methods that have been used for estimating marine bacterial productivity are the frequency-of-dividing-cells (FDC) method [12, 28] and the thymidine incorporation method [8, 9]. These authors discuss the realized and potential faults of the two methods. The FDC method is a no-incubation, direct-count method, and has its primary productivity analog in the dividing-cells method of Weiler and Eppley [45]. The thymidine incorporation technique involves incubation of samples with radioactive thymidine, and determination of rate of increase of radioactivity in the portion of the samples which is insoluble in cold trichloracetic acid. This insoluble portion is assumed to be largely deoxyribonucleic acid.

One means of helping to determine whether methods of estimating bacterial productivity are accurate is to compare their results in parallel samples. We report here a comparison of the FDC and thymidine incorporation methods in parallel samples taken in coastal marine waters of the southeastern United States. In addition, we examine the use of a second incubation technique (incubation of 3-μm filtrates with direct-count biomass determination [8]), and describe our attempt at applying the FDC method to estimation of sediment bacterial production.

Methods

Site and Sample Collection

Samples were collected on June 8, 1981, at 5 stations along a west-east transect running perpendicular to the shoreline of the southeastern portion of Sapelo Island, Georgia, USA (31°23′N, 81°17′W; site description: ref. 35). Distances of stations from shoreline ranged from 0.25 to 15 km. Basic data are presented in Table 1. Salinity and temperature were relatively uniform with depth and over the transect ($S^o/_{oo}$, 35–36, °C, 27.4–27.9). Water samples were collected with 1 liter Niskin bottles (rinsed with 70% ethanol prior to use) on 2 replicate casts at each station from 0.5 m above the bottom and 0.5 m below the surface. Sediment samples were collected from shipboard with a Reineck box corer (box size: 10 × 17 × 35 cm) [14]. Sediments along the transect are 90–98% sand and 5–10% silt and clay [17, 18]. Organic carbon content of these sediments ranges from 0.1% to 0.4% with pockets of high concentration (to 2.4%) between 0 and 2 km from shore, and is generally uniform with depth to 20 cm, indicating high levels of bioturbation [33].

Direct Counts Without Incubation

All glass- and plasticware used in sample handling was acid-washed and rinsed with 0.2-μm filtered (bacteria-free) distilled water. Two 18-ml subsamples were taken from each near-surface and near-bottom Niskin bottle sample at each station (4 replicates per depth and station) and deposited in glass scintillation vials containing 1 ml of bacteria-free concentrated formaldehyde (final formaldehyde concentration = 2%). For collection of sediment samples, the box cores were subsampled immediately after collection by removal of 1 cm^3 samples (modified syringe method [25]) from the surface sediment and from 5- and 10-cm depths within the core. These were injected into scintillation vials, and 15 ml of a bacteria-free seawater solution of formaldehyde (2%) was added, with shaking. Vials of preserved water and sediment samples were stored at 4°C until processing for microscopy (within 1 month).

Samples were treated for bacterial direct counting by the acridine orange method of Hobbie et al. [13, 38] as described in detail by Newell and Christian [28] for water samples and by Robertson and Newell [37] for sediment samples. Briefly, these methods involve the following series of steps: (a) homogenization (5 min, about 15,000 r.p.m., sediment samples only); (b) dilution (to achieve approximately 20 cells/field in the final

Table 1. Basic data for stations along a west-east transect perpendicular to the shoreline of Sapelo Island.

Distance[a], km	Time[b]	Depth, m	Secchi depth, m
0.25	0915	2.4	0.4
3.0	1045	5.8	1.3
6.0	1200	6.7	1.7
9.0	1330	10.4	3.9
15.0	1530	15.8	5.0

[a] From shoreline

[b] Eastern Daylight; on this date (June 8, 1981) low tide was at 0656 and high tide at 1316 (range, 1.9 m).

preparation); (c) 60-second staining in acridine orange solution (0.01% in bacteria-free 2% formaldehyde solution in seawater); (d) filtration through 0.2 μm pore size Nuclepore polycarbonate membrane filters; and (e) counting of bacterial cells on the filter via epifluorescence microscopy (Zeiss; HBO 50 lamp, 2X BG12 excitation filter, FT 510 beam splitter, LP 520 barrier filter). Counts, cell volume measurements, and calculations were as described by Newell and Christian [28]; total cells were counted in 15 fields/sample and dividing cells in 30 fields/sample. Cell volume determinations were made via fluorescence microscopy; compare discussions of Fuhrman [6] and Krambeck et al. [21] on this method. Because of the uncertainty of applicability of the dividing-cells method to filamentous or chain-forming cells, cells in chains of >3 cells or longer than 13 μm (arbitrarily chosen) were not included in total cell counts for the purpose of calculating frequency of dividing cells. The proportion which these cells formed of the total cell counts in sediment samples ranged from 0.5% to 4.4% (They were virtually absent in water samples).

Because sample counts could decrease due to sample deterioration in storage ([36]; P. A. Rublee, personal communication), we counted one of our samples at 1, 2, and 7 weeks after collection. The individual mean counts (total cells/count $>$ 300) differed less than 0.3% from their grand mean and were not significantly different (ANOVA, $P > 0.75$).

Efficiency of Removal of Bacteria from Sand

Our use of homogenization to release bacteria attached to heavy particles, which sink out of suspension rapidly and are not included in the counted preparations, was based on the contention of Meyer-Reil et al. [25] that other techniques have not been shown to be more efficient. We needed estimates of total bacterial standing crop for calculation of productivity from FDC, so we tested the efficiency of bacterial removal from sand grains in one our sediment samples by collecting a parallel sample and omitting homogenization in its treatment. Sand grains from homogenized and untreated samples were rinsed in bacteria-free seawater and stained and prepared for microscopy as above. Preparations were read at X500. At higher magnifications, the irregular surfaces of the sand grains made it impossible to bring all cells with each field into focus. At X500, it was not possible to resolve individual cells within compact groups of cells well enough to count them. However, it was observed that few of the cells on the grains were free of the red-fluorescing patches of filmy debris on the grains, and that most cells came away with debris if it was removed. Therefore, the percent removal of the debris from the grains by homogenization was estimated as an indicator of degree of removal of bacterial cells from sand grains. Forty-five fields were read in each type of sample. In each field read, the eyepiece counting grid was entirely filled by a sand grain surface. The percent coverage of each grain by red debris was estimated visually in each field to the nearest 10%.

We used our figures for percent removal from sand grains to calculate the percent removal from wet sediments as follows. Previous work by Robertson and Newell [37] demonstrated that approximately 28% of the bacteria in samples of intertidal sandy sediment on Sapelo Island, homogenized in the same manner as our present samples, was initially present in the interstitial water as opposed to attached to sand grains. If 28% of the bacteria in the present samples were interstitial rather than attached to sand, and the grain size and grain density per cm^3 were similar to the samples of Robertson and Newell, then the fraction of the total cells per

sample left behind on sand (L/T) after homogenization is given by

$$L/T = [(R/H) - R] \div [(R/H) - R + C]$$

where L = number of cells remaining on sand after homogenization, T = total number of cells in the original sample, R = number of cells removed from sand by homogenization, H = fraction of cells originally on sand removed by homogenization (determined as described in the previous paragraph), and C = total cell count after homogenization. This equation is derived from the following equalities: $R = H(R + L)$ and $T = L + C$. If C is set = 100, then $R = C - 28 = 72$, since C is equal to the sum of R plus the interstitial cells (28% of C).

Direct Counting after Incubation

The 3-μm filtration/incubation method of Fuhrman and Azam [8] was used at the 0.25- and 6-km stations as an alternative means of estimating bacterial productivity by direct counting. Filtration through 3-μm pore size Nuclepore polycarbonate membrane filters theoretically removes bacterivores, and subsequent incubation allows consequent increase in bacterial cell numbers to be measured directly via acridine orange counting. The central assumption of the method is that the rate of increase in bacterial cells in the filtrate is equal to the rate of cell cropping in the whole sample.

About 150 ml of water from one of the near-surface bottles at each station was passed through the 3-μm filters. Application of slight (<3 cm Hg) vacuum was necessary to draw the samples through the filter. Each filtrate was dispensed into 6 glass scintillation vials at 18 ml/vial. Then 1 ml of bacteria-free concentrated formaldehyde was immediately added to 2 of the vials for each station. The remaining 4 vials for each station, which were taped to exclude light, were capped loosely and incubated at ambient seawater temperature (27°C). Formaldehyde was added to 2 replicate vials for each station after 5- and 10-hour incubations. Vials were stored and processed for estimation of bacterial cell number and biovolume as described above.

Use of 18-ml vials instead of 100-ml vessels was a departure from the method as originally described. However, Fuhrman and Azam [8] have demonstrated that for incubations of less than 15 h, surface growth (''bottle effect'') is negligible even in scintillation vials.

[^{3}H] thymidine Uptake

From each water sample 25 ml was removed to an acid-washed 40 ml glass vial. Duplicate experimental controls and 1 poisoned control (2% formaldehyde final concentration) were run for each depth at each station. At time zero, 50 μCi (0.86 nmol) of [^{3}H-methyl]thymidine (58 Ci/mmol)(ICN # 24042) was added to each vial with mixing. Vials were then incubated with agitation in the dark at ambient seawater temperature (27°C) for 1/2 to 4 hours. Incubation times were adjusted to compensate for expected changes in the rate of thymidine uptake with increasing distance from shore. Samples were harvested following the methods of Fuhrman and Azam [7]. Then 5 ml of sample was removed from the incubation vial and placed in an ice-water bath (2°C) for 5 minutes. Next, an equal amount of chilled (2°C) 10% w/v trichloracetic acid (TCA) was added to the chilled sample. After standing for 5 minutes in the ice-water bath, the combined contents were filtered through a 0.45-μm pore size, Gelman GN-6 membrane filter, and the filters were washed with 5 ml of chilled (2°C) 5% w/v TCA. The filters were then removed to scintillation vials.

To prepare for scintillation counting, 0.5 ml of 0.5N HCl was added, and the scintillation vials with filters were placed in a boiling water bath for 30 min in order to hydrolyse the DNA [8]. After cooling, 2 ml of ethyl acetate was added to dissolve the filter followed by 12 ml of scintillation cocktail. Radioactivity was counted on a Beckman 7500 scintillation spectrometer and quench correction was by the channels-ratio method. Counts in formaldehyde controls were subtracted from sample counts to correct for adsorption.

Statistical Analyses

Analysis of variance (ANOVA) was used in comparison of total cell counts and disintegrations per minute, after transformation ($\sqrt{X + 0.5}$) where necessary. The G-test (nonparametric frequency analysis) was used in

comparison of frequencies of dividing cells. Percentages of bacterial removal from sand were transformed ($\sin^{-1}\sqrt{\text{proportion}}$) before ANOVA. The authority for these analyses was Sokal and Rohlf [42].

Results

Direct Counts, Cell Volumes, and Thymidine Incorporation

Mean values for the data used in calculation of bacterial standing crop biomasses and productivities are presented in Table 2, with the exception of the data for 3-μm filtered, incubated samples (see below).

Concentrations of cells in the water column declined with distance from shore to 9 km from about 10×10^6 to about 3×10^6 cells $\cdot$ ml^{-1}). There was no large or consistent difference between near-surface and near-bottom cell concentration. Concentrations of cells in the surface sediments declined more sharply with distance from shore, from about $60–100 \times 10^8$ cells$\cdot$cm^{-3} of wet sediment at zero to 0.25 km, to about 10×10^8 at 6 km and 5×10^8 at 15 km. Decline in cell concentration with depth to 10 cm in the sediment was sharpest on the beach (by a factor of 30 in the first 5 cm), less marked at 0.25 km (by 1.2X in the first 5 cm, and by 6X over 10 cm), and not detectable at 6 and 15 km.

Frequencies of dividing cells in the water column were rather uniform, showing no clear trend with distance from shore or depth (range 5.0–7.3%). FDC was not significantly correlated with size of bacterial standing crop ($r = -0.22$; $0.2 > P > 0.1$) Sediment FDCs in general decreased with depth to 10 cm, and were somewhat higher than water column FDCs (range 5.6–12.1%), but showed no pattern of change with distance from shore.

Mean cell volume was greater in sediments (range $0.23 - 0.35\ \mu m^3$) than in the water column (range $0.12–0.17\ \mu m^3$), but showed no clear differences with distance from shore or depth in sediment.

Mean rates of thymidine uptake (Table 2) declined rather steadily with distance from shore (from about 150 pmol$\cdot$l$^{-1}\cdot$h^{-1} to about 10 pmol$\cdot$l$^{-1}\cdot$h^{-1}), but were highly variable between replicates, so that values for the 3–15-km stations could not be statistically differentiated ($P > 0.05$). Mean thymidine incorporation (10^{-21} moles$\cdot$h^{-1}) per bacterial cell was 7.3 at 0.25 km, 9.0–9.5 at 3–9 km, and 3.1 at 15 km.

Efficiency of Bacterial Removal from Sand

The percentage of attached bacteria which were removed from sand grains, as indicated by percent coverage ($\pm$95% confidence interval) of grains by red-fluorescing film before ($25.3 \pm 8.6\%$) and after ($10.4 \pm 5.9\%$) homogenization, was 59.3% (ANOVA, $P < 0.01$). Thus, based on the equation given above (Methods), 33% of the total sample cells (sand-bound plus interstitial) were left behind on sand grains after homogenization.

Calculated Bacterial Productivity, Water Column

We used the value at the high end of the range of factors given by Fuhrman and Azam [8; but see ref. 9] for conversion of quantity of thymidine incorporated into number of cells produced (1.3×10^{18} cells$\cdot$mole^{-1} thymidine). We used the equation ($\ln = 0.299$ FDC - 4.961) given by Newell and Christian [28] for calculation of growth rate from

Table 2. Acridine orange direct bacterial counts (AODC), frequency of dividing cells (FDC), mean cell volumes, and uptake of [^{3}H]thymidine (pmol) per hour of incubation for water column (W) and sediment (S) samples taken along a transect perpendicular to shore in the Georgia nearshore zone.[a]

		AODC			
		10^6 cells·ml^{-1} (W)	FDC	Volume	Thymidine[c]
Station[b]	Depth	10^8 cells·cm^{-3} (S)	%	μm^3·cell^{-1}	pmol·l^{-1}·h^{-1}
0.25 W	0.5 m	9.2± 1.0^{B}	5.4± 0.4^{AB}	0.15± 0.05^{BCe}	117± 57^{B}
0.25 W	1.9 m	11.4± 2.7^{A}	5.5± 0.9^{AB}		180± 134^{A}
3 W	0.5 m	6.0± 0.6^{D}	5.4± 0.4^{AB}	-[d]	71± 67^{C}
3 W	5.3 m	7.5± 1.4^{C}	5.0± 1.8$_{B}$	-	57± 38^{C}
6 W	0.5 m	4.9± 0.5^{E}	6.1± 0.7^{AB}	0.17± 0.4^{ABe}	50± 20^{C}
6 W	6.2 m	3.9± 0.5^{F}	5.7± 0.8^{AB}		29± 11^{C}
9 W	0.5 m	2.6± 0.5^{G}	5.7 1.0^{AB}	-	19± 9^{C}
9 W	9.9 m	3.1± 0.5^{G}	7.3± 1.8^{A}	-	34± 12^{C}
15 W	0.5 m	3.0± 1.1^{G}	6.5± 1.7^{AB}	0.12± 0.03^{Ce}	-
15 W	15.3 m	3.0± 0.2^{G}	4.7± 1.5^{B}		9± 2^{C}
0 S	1 cm	62.5^{U}	8.4^{XY}	-	-
0 S	5 cm	2.4^{YZ}	9.4^{XY}	-	-
0 S	10 cm	2.1^{Z}	6.3^{YZ}	-	-
0.25 S	1 cm	99.7^{S}	8.4^{XY}	0.23± 0.06^{ABC}	-
0.25 S	5 cm	85.7^{T}	5.6^{Z}	-	-
0.25 S	10 cm	16.6^{V}	12.1^{W}	0.32± 0.11^{AB}	-
6 S	1 cm	10.5^{W}	8.3^{XY}	-	-
6 S	5 cm	13.9^{VW}	5.7^{Z}	-	-
6 S	10 cm	15.0^{VW}	6.4^{YZ}	-	-
15 S	1 cm	5.6^{XY}	11.3^{WX}	0.35± 0.11^{A}	-
15 S	5 cm	3.8^{YZ}	9.3^{WX}	-	-
15 S	10 cm	6.1^{X}	6.8^{XYZ}	0.27± 0.10^{ABC}	-

[a] Values are given with ± 95% confidence intervals, except for sediment samples, for which only 1 sample was taken per station. Average coefficient of variation for sediment counts was 32.9%. Four replicates were taken for direct count water column values, a minimum of 50 cells per sample were measured for cell volumes, and there were 2 replicates for ^{3}H analysis. Mean values bearing the same superscript letter could not be statistically differentiated ($P > 0.05$). Sediment values were statistically analyzed separately from water column values, except in the case of cell volumes.

[b] Numbers indicate distance from shoreline in km. 0 = level of mean neap low tide. S = sediment; W = water column.

[c] [^{3}H]thymidine uptake during 0.5–3.7-h incubations, corrected for formaldehyde-killed control values. Five of 25 ml filtered.

[d] No data.

[e] Near-surface and near-bottom samples pooled for analysis.

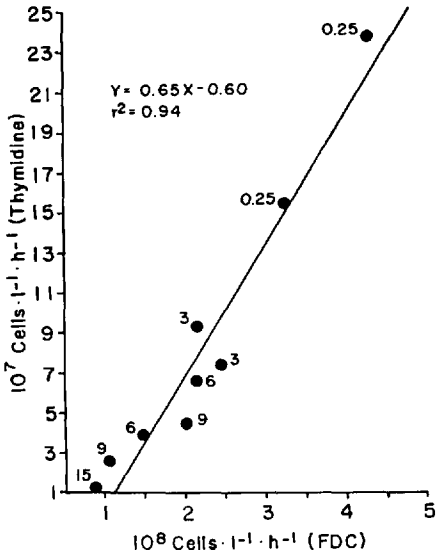

Fig. 1. The relationship between bacterial productivities as estimated in parallel samples by 2 independent methods: y-axis, productivity as estimated from [^{3}H]thymidine incorporation; x-axis, productivity as estimated by the frequency-of-dividing-cells method. Numbers adjacent to points indicate distance (km) of sampling from shoreline. Number of replicate samples per point: FDC, 4; [^{3}H]thymidine, 2.

frequency of dividing bacterial cells. In conjunction with our thymidine uptake rates and standing stocks of bacteria, we then calculated the water column bacterial productivity values (cells·l^{-1}·h^{-1}) displayed in Fig. 1. As measured by either method, bacterial productivity declined by roughly 50% per 3 km distance from shoreline, from about 2–4 $\times$ 10^8 cells·l^{-1}·h^{-1} at 0.25 km, to about 1–9 $\times$ 10^7 cells·l^{-1}·h^{-1} at 15 km (Fig. 1). The two measures of productivity were strongly correlated ($r = 0.97$, $P < 0.001$). However, as estimated from FDC, productivity ranged from 1.8 to 7.4 times greater than productivity estimated from thymidine incorporation rates. This ratio increased steadily with distance from shore. It may be that the relationship between thymidine-estimated productivity and FDC-estimated productivity is nonlinear. When the logarithm of thymidine-estimated productivity was regressed on FDC-estimated productivity, the correlation was found to be nearly as strong as the linear-linear one ($r^2 = 0.90$). In addition, there was a strong positive relationship between simple standing crop of bacteria and thymidine-estimated productivity ($r^2 = 0.91$).

Mean FDC-calculated instantaneous generation times for water column bacteria at each station ranged from 21.7 to 30.8 hours, without obvious relationship to distance from shore. Turnover times for bacterial standing crops based on thymidine-estimated productivities and assumption of steady state ranged from 53 to 252 hours, increasing with distance from shore.

It was not readily possible to calculate bacterial productivity based on the results of 10-hour incubation of 3-μm filtered water samples, as described by Fuhrman and Azam [8] (Table 3). For samples taken at 0.25 km from shore, no increase was detected in concentration of cells with time, FDC decreased with time, and the filtration reduced mean concentration of cells by about X4 and mean cell volume by about X2. At the 6-km station, the filtration reduced cell concentration and volume less sharply (X1.3 and X1.7), and although FDC decreased with time, there may have been (see superscript letters in Table 3) an increase in cell concentration over the first 5 hours of incubation,

Table 3. Results of incubation of 3-μm filtrates of water samples at 2 stations. See heading and footnotes to Table 2.[a]

Station	Hours[b]	AODC	FDC	Volume
0.25	0	2.4^{A}	3.8^{A}	0.07^{A}
	5	1.7^{A}	2.6^{B}	—
	10	2.3^{A}	2.3^{B}	0.06^{A}
6	0	3.9^{B}	5.1^{A}	0.10^{A}
	5	4.5^{AB}	3.8^{B}	—
	10	4.5^{A}	3.9^{B}	0.14^{A}

[a] Each value is the mean for 2 replicate incubation bottles; least-significant-range testing performed separately for each station. Mean values bearing the same superscript letter could not be statistically differentiated ($P > 0.05$).
[b] Hours of incubation.

but not over the final 5 hours. Small (2–3 μm diameter) flagellate-like cells were seen in acridine orange preparations for incubated samples from the 6-km station. If it was assumed for the 6-km samples that the increase over the first 5 hours of incubation reflected the rate of environmental cell growth, then the calculated rate of bacterial productivity at the 6-km station (near-surface) was 1.2×10^8 cells$\cdot l^{-1} \cdot h^{-1}$. Corresponding mean values for FDC-estimated productivity and thymidine-estimated productivity were 2.1×10^8 and 6.5×10^7 cells$\cdot l^{-1} \cdot h^{-1}$.

Bacterial Productivity, Sediment

Mean instantaneous generation times calculated from sediment FDC values (pooled over sediment depth) at the 0.25-, 6-, and 15-km stations were shorter by roughly X2 than corresponding water column generation times (range 9.3–18.7 h). When these times were used with mean sediment bacterial standing stocks and mean cell volumes to calculate sediment bacterial productivity, the resulting values seemed impossibly high. They are compared with corresponding values for the water column in Table 4, along with calculated values for bacterial productivity in terms of carbon.

Discussion

As Pamatmat et al. [30] recently stated, in introducing a multimethod analysis of total marine benthic metabolic activity, "it is necessary to find some agreement, or reasons for disagreement, among the different methods [because] the actual metabolic rates in natural sediments may be expected to lie somewhere in the area of convergence of various measures." We have adopted a similar tack in our attempts to determine bacterial productivity in coastal Georgia marine environments.

If the thesis of Pamatmat et al. [30] is true in our case, then the actual bacterial productivity in Georgia coastal waters at the time of our sampling lay between about $1–20 \times 10^7$ cells$\cdot l^{-1} \cdot h^{-1}$ ([^{3}H] thymidine estimates) and about 2–7 times greater values (FDC estimates), with a widening interval as distance from shore increased. At present

Table 4. Mean calculated bacterial productivities for water column and sediment (to 10-cm depth) at 3 stations, based on FDC-calculated instantaneous generation times. The values for sediments are bracketed because of their seemingly impossibly large size (see Discussion).

Station[a]	Cells·cm^{-3}·h^{-1}	mgC·cm^{-3}·h^{-1}[b]	mgC·m^{-2}·h^{-1}[c]
0.25 W	3.7×10^5	5.6×10^{-6}	13
0.25 S	[6.3×10^8]	[1.8×10^{-2}]	[1800]
6 W	1.8×10^5	2.7×10^{-6}	18
6 S	[7.0×10^7]	[2.0×10^{-3}]	[200]
15 W	1.1×10^5	1.7×10^{-6}	27
15 S	[5.6×10^7]	[1.6×10^{-5}]	[160]

[a] Numbers indicate distance from shoreline in km. W = water column; S = sediment.
[b] mgC·cm^{-3} cell taken as 100 [28].
[c] The productivity under 1 m^2 throughout the water column (W) or within the sediment to 10-cm depth (S).

we have no compelling reason to designate either type of estimate as the more accurate one (but see below). The lone estimate from the third method used (incubation of 3-μm filtrates) lay almost exactly equidistant from the FDC and [^{3}H]thymidine estimates made in parallel. However, 25% may be a fair estimate of the fraction of net phytoplankton production (particulate + exudate) that might be utilized by bacteria [see 15, 22, 23, 46; Chrost and Faust, 1981, Abstr. Ann. Meeting ASM, p. 186]. If so, then our FDC productivity estimates for the 0.25-km water-column samples (which are quite similar to those published by Newell and Christian [28] for the same site and season 1 year earlier] are not unrealistically high relative to estimates of net primary productivity at the same site and season. Net primary productivity at the 0.25-km station was 4 g C·m^{-2}·d^{-1} (D. W. Kinsey, unpublished data); FDC estimates of bacterial productivity are about 9% of this value (see Table 4, and Table 5 of ref. 28). FDC estimates of bacterial productivity at the 6- and 15-km stations (Table 4) are about 25% of the June rate of net primary productivity (particulate only) given for 5–10-m deep water of coastal Georgia by Turner et al. [44].

Although there are not many data available for comparison, the values presented in Table 5 indicate that our measured rates of thymidine incorporation per cell are within the range of rates found for marine water samples by other investigators. They are low, however, relative to some of the values found for areas of high primary productivity by Fuhrman and Azam [8], Fuhrman et al. [7], and Ducklow [4] (Table 5).

Neither the FDC nor [^{3}H]thymidine methodology is yet well enough understood for definite conclusions to be reached regarding their relative accuracies. Newell and Christian [28] list several uncertainties involved in applying the FDC method to bacterial ecological work, and suggest investigative approaches to their clarification. Fuhrman and Azam [8] present a detailed listing of the uncertainties associated with the chemical analysis and calculations of the thymidine-incorporation technique. Since Fuhrman and Azam [8, 9] took a conservative approach at each step in the series of calculations required to convert thymidine-incorporation values to cell-production values, it might be expected that the [^{3}H]thymidine method would yield low results relative to the FDC method, which is based on an experimental laboratory calibration. Any any rate, our comparison of FDC and [^{3}H]thymidine estimates of bacterial productivity appears to

Table 5. Rates of thymidine incorporation per cell (moles·h^{-1}) for some marine water and sediment samples.

Source	Type of sample	Moles·cell^{-1}·h^{-1}
Present study	Temperate zone coastal water, eastern USA	3 to 10 × 10^{-21}
[7]	Temperate zone coastal water, western USA	0.0 to 6.4 × 10^{-20}
[8]	Antarctic Ocean water	1.4 to 15 × 10^{-21}[a]
R. B. Hanson, in manuscript	Antartic Ocean water, upper 100 m	2 × 10^{-21}[a]
[8]	CEPEX[b] water	4.1 to 9.8 × 10^{-20}[a]
[4]	York River estuary	0.8 to 5.8 × 10^{-20}[a]
[25]	Subtropical seagrass sediments, summer	1.4 × 10^{-19}[ac]
D. L. Kirchman, pers. comm.	Surfaces of living seagrass leaves	5 × 10^{-19}[a]

[a] Calculated from data given in the reference or by the source cited.
[b] Controlled Ecosystem Pollution Experiment [9].
[c] Isotope dilution estimated and taken into account in calculations.

show that the two methods are measures of the same activity, viz., bacterial growth rates. We must point out, however, that the thymidine-estimated productivities were nearly as strongly correlated with bacterial standing crop values as with FDC productivities (Results), and that the frequencies of dividing cells were less variable over the transect examined than were total counts. Fuhrman et al. [7] also found a significant correlation between bacterial standing crop and rates of thymidine incorporation. In order to determine whether the close relationship between FDC and [^{3}H] thymidine estimates of productivity can be generally found, and to provide evidence for the relative accuracies of the two methods, the methods will have to be compared in a larger range of types of water samples, and contrasted with alternative methods in addition to or other than incubation of 3-μm filtrates.

With our water samples, the method of incubation of 3-μm filtrates [8] was not a useful means of estimating bacterial productivity. In the samples used by Fuhrman and Azam [8] in developing the method, most (91–97%) of the bacteria that could incorporate thymidine could be passed through a 1-μm poresize filter. At our 0.25-km station, there were 4 times fewer cells in the 3-μm filtrate than in the original sample, as a consequence of association of bacteria with the abundant floc present [28], and probably decreasing effective pore size with time of filtration due to clogging of pores (bacteria in the filtrate were twice as small as bacteria in the original sample). Although filtration effects were less marked at our 6-km station, we did observe what we believe were small heterotrophic flagellates in samples of the filtrates, suggesting that bacterial cell cropping might still have been taking place during incubation. The fact that FDC decreased significantly with time in samples from both stations suggests that the bacteria in the filtrates entered a lag phase during incubation, as Newell and Christian (28; see their Fig. 1) had found in their incubation of diluted, unenriched 3-μm filtrates of Georgia coastal water samples. We must note one difference between our incubations and those of Fuhrman and Azam [8]: we excluded light and they did not. However, since

3-μm filtration removes most algal cells, absence of light should not have influenced availability of algal excretory products.

Based on our water-column results, then, one might tentatively conclude that the FDC method yielded the most acceptable estimates of bacterial productivity of the three methods tested. However, the FDC method was the only one which we applied to estimation of sediment bacterial productivity, and the resultant values raise serious questions about this usage of the FDC method. Our FDC values for sediment bacterial assemblages were higher than the corresponding water-column values, to the extent that calculated growth rates in the sediment to 10-cm depth were about twice as great as growth rates in the water column. Further, we found that standing crop was about $X10^3$ greater in sediments than in the water column, not decreasing sharply with depth to 10 cm, and that mean cell volume was about twice as great in the sediments.

These factors result in calculated bacterial productivities (mg $C \cdot h^{-1}$; table 4) which are much higher than calculable secondary productivity (little or no light reaches these sediments) based on sediment oxygen uptake measured for the same sites and seasons (C.S. Hopkinson and R. L. Wetzel, unpublished). For example, at the 0.25-km station, Hopkinson (personal communication) estimates that about 1 g of C is released as CO_2 per m^2 per day from the sediments. If this were entirely bacterial C, and if bacterial C conversion efficiency were 50% [17], then bacterial production as indicated by oxygen input would be about 1 g $C \cdot m^{-2} \cdot day^{-1}$. Our FDC estimate was 43 g $C \cdot m^{-2} \cdot day^{-1}$ (11-hour instantaneous generation time). Our estimate was based on sediment standing stocks to 10 cm only, and our percent-removal experiment indicated that our standing stock estimates were low by about X1.5 (Results). For comparison, Meyer-Reil et al. [24] found a 100-hour turnover time and 43 mg $C \cdot m^{-2} \cdot day^{-1}$ for summer sandy sediment heterotrophic microbial productivity (0–1 cm depth). Sulfate respiration without subsequent oxygen input in oxidation of reduced S might account for some bacterial production; however, it is unlikely that this production would be substantially greater than production ultimately resulting in oxygen input, especially considering the relative efficiencies of production associated with oxygen and sulfate respiration (5, 43). Furthermore, the FDC-calculated quantity of production appears absurdly high when attempts are made to reconcile it with calculated potential inputs of fixed carbon compounds from water-column productivity and landward salt marshes (D. W. Kinsey, unpublished data; 35).

One possible reason for the apparent inapplicability of the FDC method to sediments is the distinct possibility that the several physiological types of bacteria to be found in sediments (see Fig. 5 of ref. 29) have different FDC:growth rate relationships than that which Newell and Christian [28] found for water-column bacteria. It is also possible, however, that sediment environmental factors are the primary reasons for the high sediment FDC-productivity estimates, as discussed in the following paragraph.

Current limited knowledge of bacterial growth rates in sediments indicates that they are lower on a per-cell basis than those which occur in the water-column [10, 24, 25; but see ref. 1]. This is probably a consequence of limited access to oxygen, lower concentrations of available dissolved carbon per cell, and inhibitory factors due to cell crowding [2, 25, 47]. Pamatmat et al. [30] suggest that the heat production: ATP ratios which they found for sandy marine sediments are indicative of a largely stationary or senescent bacterial assemblage. Yingst and Rhoads [47] discuss the likelihood that bioturbation greatly enhances sediment bacterial growth rates. We speculatively suggest, as an explanation for our excessively high FDC productivity estimates for

sediments, that sediment bacterial assemblages (at least in aerobic sandy sediments like those we sampled) are inhibited by crowding and substrate limitation, but are poised to move quickly from a stationary phase into rapid growth rates, the potential magnitude of which is indicated by the high FDCs to be found in sediments. In other words, sediment FDCs, except in bioturbated patches, might be relections of past and potential future growth rates, so that the FDC method cannot be used in sediments. Results of a recent application of a [^{3}H]thymidine method to marine sediments by Moriarty and Pollard [26] may be in line with our speculative suggestion. Moriarty and Pollard slurried their samples in order to achieve even distribution of thymidine; this "bioturbation" may have been a major factor in their finding a summer rate of bacterial productivity of 3.7×10^8 cells·h^{-1}·g^{-1}. At 1.5 g·cm^{-3} sediment density [25] this would equal 2.5×10^8 cells·cm^{-3}·h^{-1}, 40% of our excessively (probably more than X10) high FDC estimate for our 0.25-km station (Table 4). On a per-cell basis, the thymidine incorporation rates of Moriarty and Pollard [26] are high relative to most of the water column values reported elsewhere (Table 5), and are similar to values found by D. L. Kirchman (personal communication) for bacteria on seagrass leaf surfaces, where oxygen availability and three-dimensional crowding are probably not limiting factors.

Summary

We have found that the FDC and [^{3}H]thymidine methods of estimating bacterial productivity in the water column can yield strongly correlated results. Direct counting of incubated 3-μm filtrates was not useful for estimating bacterial productivity when used with turbid water samples. The FDC method did not appear to be applicable to the types of sediment samples with which we worked.

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