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Frequency of Dividing Cells as an Estimator of Bacterial Productivity†

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It has recently been proposed that the frequency of dividing bacterial cells (FDC) can be used to predict growth rates of natural aquatic bacterial assemblages. We have examined the relationship between FDC and growth rate in bacteria from southern-temperate, coastal marine waters by using incubation under conditions of manipulated nutrient availability and exclusion of bacterivores. The regression of the natural logarithm of bacterial instantaneous growth rate (μ) on FDC resulted in a better fit than regression of untransformed μ on FDC. The regression equation was $\ln \mu = 0.299\text{FDC} - 4.961$. The coefficient of variation for predicted $\ln \mu$ at mean FDC was 7%. The range of FDC-estimated bacterial instantaneous generation times for coastal Georgia waters was 12 to 68 h, and range of calculated bacterial production rates was 0.6 to 17.6 mg of $C \cdot m^{-3} \cdot h^{-1}$. Unresolved problems of and suggested improvements on the FDC method of predicting growth rate are discussed.

The fluorescent-staining technique for acridine orange direct count (AODC) estimation of standing stocks of aquatic bacteria (8, 18, 20, 36) has been verified as accurate by comparison with electron micrographic, biochemical, and separate epifluorescence techniques and with plate counts from samples with known bacterial density (2, 17, 31, 35, 37, 48). The AODC technique has been applied to samples from a wide variety of geographical sites, and so sizes of bacterial standing stocks are rather well known for many types of aquatic environments (7, 17, 21, 39, 41). However, knowledge of rates of bacterial production is necessary for quantitation of the flow of material and energy through the bacterial compartment of the ecosystem (5, 33, 38, 47). A variety of techniques have been used to estimate or measure bacterial production, including cultural methods (4), radioisotopic methods (13, 22, 23, 42), and methods based on changes in AODC (6, 9, 13, 28) or adenosine triphosphate (39) values. A simplified (no incubation) AODC method of estimating bacterial production has been proposed by Hagström et al. (14). These authors made use of the relationship between frequency of dividing bacterial cells (FDC) and bacterial growth rate. Theoretical considerations and experimental evidence indicate that the proportion of cells in the dividing state at any one time in a bacterial population is dependent upon the growth rate, at least over a certain range of rates (50; B. Westling-Häggström, Ph.D. dissertation, University of Umeå, Umeå, Sweden, 1979). Hagström et al. (14) found a linear relationship between FDC and growth rate in the laboratory of bacteria from the coastal Baltic Sea and used this relationship to calculate environmental bacterial growth rates from measured FDC.

We report here an examination of the relationship between FDC and growth rate of bacteria from southern-temperate coastal Atlantic waters. We have tested this relationship over a range of growth rates induced by manipulation of growth conditions (elimination or reduction of predation and addition of nutrients). We compare our findings with those of Hagström et al. (14) and other investigators of bacterial productivity, and we discuss unsolved problems of the FDC method.

MATERIALS AND METHODS

Sites. Experiments were conducted with water column bacteria from two sites in the vicinity of the University of Georgia Marine Institute, Sapelo Island, Ga. (31°23'N, 81°17'W; for site description, see reference 49), and from the Skidaway River at the Skidaway Institute of Oceanography, Savannah, Ga. (31°59'N, 81°01'W; sampled July 1980). The Sapelo Island sites were: (i) the nearshore station, 300 m offshore of the barrier beach at the southern end of the island in the Atlantic Ocean, depth at mean low water, 3 m, sampled April and May 1980; and (ii) the

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headwaters of the Duplin River (station 174 of reference 15), sampled June 1980. The Skidaway and Duplin rivers are tidal marsh channels which are varyingly influenced by freshwater from the Ogeechee, Savannah, and Altamaha rivers. See Table 3 for hydrographic data taken at sampling times.

Central experimental design. Three experiments were conducted, one at each of the above sites. The general design of the three experiments was the same. All glassware to be used in fixation of samples was acid washed and rinsed with a bacteria-free (0.2-μm-filtered) seawater solution of 2% formaldehyde. Pipettes for periodic sampling of cell suspensions were rinsed with bacteria-free distilled water (no formaldehyde). All glassware and filtration equipment for collection, processing, and incubation of samples of living bacteria were acid washed and autoclaved. Water was collected from just below the surface, and triplicate 18-ml samples were immediately preserved by bringing them to 2% formaldehyde. The remaining water (~ 4 liters) was immediately transported to the laboratory for incubation experiments. One-half of the unpreserved water collected was filtered through coarse glass fiber (P-100 Uni-Pore) and 3-μm pore-size polycarbonate membrane filters (Nuclepore Corp.), and 400 ml was added to each of three or four 1-liter screw-cap bottles. The remaining water was filtered through coarse and then fine (P-040 Uni-Pore) glass fiber filters and finally through 0.4-μm pore-size and then 0.2-μm pore-size Nuclepore membrane filters. Four hundred milliliters of this 0.2-μm-filtered water was added to each of the 1-liter bottles of 3.0-μm-filtered water. The rationale for these filtrations was that 3.0-μm filtration would remove predators and eukaryotic algae (24, 39), and 0.2-μm filtration of half of the water would dilute original inoculum and thereby encourage growth. Yeast extract (filter sterilized in 10 ml of distilled water) was added to the bottles in a range of final concentrations from 0 to 100 mg/liter. Zero-time samples (18 ml) were taken (after agitation) from each bottle and fixed (2% formaldehyde, final concentration), and the bottles were incubated on a rotary shaker (100 rpm) in the dark at the water temperature recorded at collection (26 to 29°C; see Table 3). Additional 18-ml samples were taken and fixed from each bottle at 2.5- to 10-h intervals until the turbidity of the water in the bottles indicated that a stationary growth phase had been reached.

Nearshore experiment. For the nearshore experiment (near the barrier beaches of Sapelo Island), concentrations of yeast extract used in the 800-ml incubations were 0, 50, and 100 mg/liter. A fourth incubation treatment of this experiment involved the inclusion of natural particulate matter and predators. For this treatment, no 3.0-μm filtration of original water was performed, but 1:1 dilution with 0.2-μm-filtered water was carried out. Bottles were sampled at 0, 10, 20, 25, 30, 35, and 40 h after initiation of incubation. At the time and site of collection of water for incubation and at 3 and 6 h before this time at the same site, triplicate field samples were collected from just beneath the surface and near the bottom.

Duplin experiment. For the Duplin experiment (tidal-drainage river west of Sapelo Island), yeast extract concentrations used were 25, 50, and 100 mg/liter. Bottles were sampled at 0, 8, 10.5, 13, 15.5, and 18 h after initiation of incubation. Field samples were taken from subsurface water only, in triplicate.

Skidaway experiment. For the Skidaway experiment (tidal-freshwater river west of Skidaway Island), yeast extract concentrations used were 0, 25, 50, and 100 mg/liter. Sampling of bottles was at 0, 6, 9, 12, 15, and 18 h after initiation of incubation. Field samples were collected from subsurface water only, in triplicate.

Other field samples. In addition to the field samples taken during collection of water for the incubation work, samples of subsurface water were taken during April 1980 at the nearshore site, and total AODC and FDC were determined.

Counts and calculations. The numbers of bacteria in the fixed samples were counted within 1 week of fixation, using an AODC method similar to that of Hobbie et al. (18). One milliliter of appropriately diluted sample was added to 1.5 ml of acridine orange solution (0.01% in bacteria-free seawater solution of 2% formaldehyde). The mixture of sample and stain was allowed to stand for 60 s and was then drawn through a 0.2-μm Nuclepore filter (25-mm diameter) which had been previously stained in irgalan black for at least 5 min. A 5-μm-mesh nylon support screen was used under the Nuclepore filter to foster random distribution of bacteria on the filter. A maximum vacuum of 18 cm of Hg was used in the filtrations. The vacuum was applied until no surface liquid remained on the filter; then the filter was placed on a slide with immersion oil (Cargille type A) above and below it. A cover slip was applied, and the filter was examined at ×1,000 under epifluorescent illumination (Zeiss; HBO 50 lamp, BG 12 excitation filter, FT 510 beam splitter, LP 520 barrier filter). Bacteria framed within an eyepiece grid were counted in each of 30 fields chosen by moving the microscope specimen holder through a standard path of progressive positions which included representative portions of most of the filter area. Objects which fluoresced green, yellow, or red were counted as bacteria if they had a definite cellular outline and a cell width less than 2 μm and were not arranged in such a way that they appeared to be organelles of protozoa. A cell was counted as dividing if a clear invagination of the cell wall could be seen, but not a clear separatory space between daughter cells (14). All bacteria were counted in the first 15 fields for each filter, and dividing bacterial cells were counted in all 30 fields. This resulted in total counts greater than 300 per filter and dividing cell counts greater than 30 per filter. Two operators (S.Y.N. and R.R.C.) separately counted bacteria in the same 120 microscope fields for both field and incubated samples, enumerating both total and dividing cells; their mean counts in both categories differed by less than 0.7% and were not significantly different from one another (analysis of variance, $P > 0.75$). Bacteria upon or within the faintly red-staining, amorphous particles of the field samples were counted separately from bacteria free of the particles. It was possible to see bacteria within these flimsy, transparent particles (the "floc" of reference 34). Numbers of bacteria per milliliter of original sample were calculated from the mean count per microscopic grid field, using values for grid area,

effective filter area, quantity filtered, and dilution factors. FDC was calculated as [(mean number of dividing cells per microscopic grid field)/(mean number of total cells per field)] × 100. Lengths and widths of bacterial cells were measured at ×2,000 (at least 50 per sample), and volumes were calculated considering each coccoid cell as a sphere or cylindrical cell as a cylinder plus two hemispheres (45).

Instantaneous growth rates (μ) were calculated from differences in mean densities of cells per milliliter in the incubated bottles according to the following formula (43): $\mu = [0.69 (\log_{10} N_{t2} - \log_{10} N_{t1})]/0.301\Delta \cdot t_{2-1}$, where N_{t2} is the mean cell density at time 2, N_{t1} is the mean cell density at time 1, and Δt_{2-1} = the time interval (hours) between t_1 and t_2. Linear regression analysis (40) was performed on the values for FDC at t_1, and μ or ln μ for Δ_{t2-1}. This was done only for time intervals which appeared to lie within the period of exponential growth for each treatment in each experiment. Zero-time values were not used in these calculations.

General. Coefficients of variation for bacterial counts averaged 29% for total AODC counts of incubated samples and 32% for total AODC counts of field samples. For dividing cells, the mean coefficient of variation was 90% for field samples and 125% for incubated samples. (The large coefficients of variation for counts of dividing cells are a consequence of the small number of dividing cells per field and their random distribution in any given sample. In a Poisson distribution, when the mean is near one, both the variance and standard deviation equal approximately 1. Thus, the expected coefficient of variation approximates 100%. In our analyses, however, the standard error of the mean [$s/\sqrt{n}$; reference 40] was no more than 0.18 of the standard deviation [$\sqrt{30}$ fields]. Increasing the quantity of sample filtered could decrease the counting variation, but it would also result in error due to crowding of cells and other particles in the microscope field. Counts of autofluorescent cells of bacterial size (19, 46) ranged from 1.4 to 2.2% of total AODC counts in field samples and were less than 0.6% of total AODC counts in incubated samples. Blank filtrations gave counts which were less than 1% of total AODC counts.

RESULTS

Bottle experiments. A summary of data from the 1-liter bottle incubations is presented in Table 1. Numbers of cells per milliliter increased by factors of 2.5 to 73 times, increasing with increase in yeast extract concentrations from 0 to 100 mg/liter (Fig. 1). This was true for all treatments except the one from the nearshore experiment which did not exclude particulate matter and predators (nearshore P of Table 1;

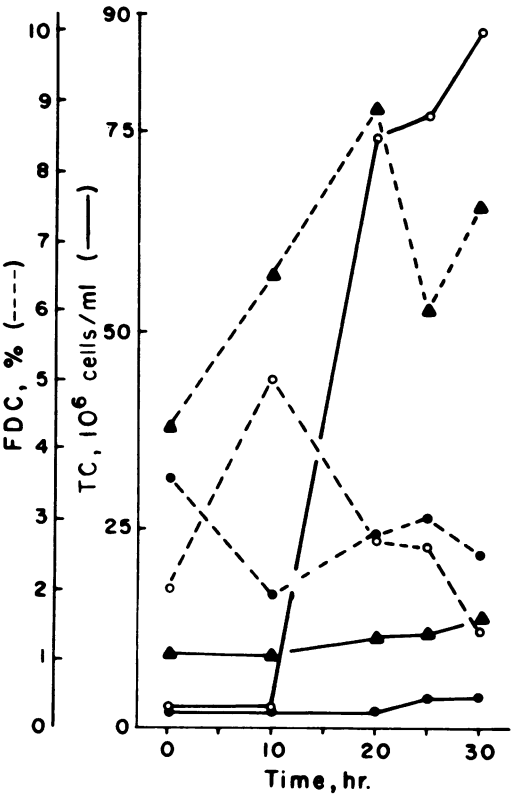

FIG. 1. *Changes with time in the FDC and in total acridine-orange bacterial counts (TC) for three treatments during the bottle incubations of the nearshore experiment. Treatments:* (○) *3-μm filtration, 1:1 dilution, 50 mg of yeast extract per liter;* (●) *3-μm filtration, 1:1 dilution, 0 mg of yeast extract per liter;* (▲) *no filtration, 1:1 dilution, 0 mg of yeast extract per liter.*

TABLE 1. *Data summary for the bottle incubation experiments*[a]

Experiment[b]	ICN	ICV	Range, FDC	Range, MCN	Range, MCV
Nearshore F	2.2	0.11	1.9–10.5	5.9–161.1	0.31–0.38
Nearshore P	9.5	0.10	6.0–8.9	15.5	0.19
Duplin	8.4	0.10	4.8–14.2	96.4–245.2	0.33–0.41
Skidaway	4.0	0.06	4.6–15.3	10.1–100.8	0.18–1.21

[a] Ranges presented are for mean values from each treatment (concentrations of yeast extract ranging from 0 to 100 mg/liter). Abbreviations: ICN, mean initial number of cells (× 10^6/ml) after filtration (3.0 μm) and 1:1 dilution in bacteria-free seawater; ICV, mean initial cell volume (cubic micrometers); MCN, maximum number of cells (× 10^6/ml); MCV, maximum cell volume (cubic micrometers).

[b] Nearshore F indicates data for treatments in which >3-μm particles were excluded; nearshore P indicates data for the treatment in which natural particulate matter >3 μm was included.

Fig. 1); for this treatment, increase from initial to maxium cell number was by a factor of 1.6 times. Also, cell volume did not increase as greatly (1.9 times) in the particulate-predator treatment as it did in the remaining treatments (3.0 to 20 times) (Table 1). Another clear difference between the data for the particulate and the nonparticulate treatments of the nearshore experiment could be seen in the relationship of net growth to FDC. The FDCs of the particulate treatment were consistently high, yet calculated growth rates were relatively small (Fig. 1).

Results of the regression of μ on FDC for the data from 3-μm-filtration treatments are presented in Table 2. When data from the three experiments were analyzed separately, a range of slopes was found, from 0.024 to 0.069, and these were each significantly different from zero slope ($P < 0.01$ to $P < 0.001$). However, when these three slopes were tested for equality (reference 40, box 14.8), the null hypothesis that $\beta_1 = \beta_2 = \beta_3$ could not be rejected ($0.1 < P < 0.25$). Therefore, the growth rate and FDC data from the experiments were pooled, and a regression of μ on FDC was performed (regression 4 of Table 2). With the formula derived from this regression, there appeared to be more departure of regression-estimated μ from measured μ as FDC increased (51), and the y-intercept (μ at 0 FDC) was substantially less than 0. For these reasons and theoretical considerations discussed below, a regression of the natural logarithm of μ on FDC was performed (regression 5 of Table 2). This regression yielded a slightly higher coefficient of determination (r^2), 0.60, as opposed to 0.54 for the pooled-data regression of μ on FDC. Also, the coefficient of variation of predicted $\hat{y}$ (ln μ) at $\bar{x}$ (FDC) for the natural-logarithm regression was less (0.07) than that for the untransformed data (0.21).

The regression of μ on FDC which we calculated for the data of Hagström et al. (14; continuous culture of mixed-enrichment strains, their Fig. 4 upper right) yielded the highest r^2 and lowest coefficient of variation for predicted growth rate ($CV_{\hat{y}}$) of any of the untransformed analyses (regression 6 of Table 2). However, the four data points of Hagström et al. (14) lay within the scatter at the low end of the range found for the pooled data of our incubation experiments, and a test of equality of slopes indicated that the slope of the data of Hagström et al. was not significantly different from that of our pooled data. When ln μ was regressed on FDC for the Hagström et al. data, the fit was nearly as good as had been found for the untransformed data (regression 7 of Table 2), and this regression was not significantly different from our ln μ-on-FDC regression ($0.25 < P < 0.5$).

A summary of the data and results from analysis of our field samples is presented in Table 3. Instantaneous growth rate and generation time ($1/\mu$) were calculated from the formula ln μ = 0.299FDC − 4.961, derived from the regression of ln μ on FDC for the pooled data of our incubation experiments (regression 5 of Table 2). This regression was used because the individual experimental regression slopes could not be statistically differentiated from one another, and the regression of ln μ on FDC gave lower coefficients of variation for predicted values than did the regression of μ on FDC ($CV_{\hat{y}}$ of Table 2). The range of FDC in field samples was from 2.5 to 9.8%, and predicted instantaneous generation times ranged from 8 to 68 h. Total counts ranged from 1.9×10^6 to 21.4×10^6 cells per ml, and in general, high total counts were associated with

TABLE 2. *Linear regression statistics[a] for regressions of instantaneous growth rate (μ) or ln μ on the FDC from the bottle incubation experiments and from the continuous culture experiment of Hagström et al. (14)*

Experiment	Regression	n	b[b]	a	F_b[c]	r^2	$CV_{\hat{y}}$
1. Nearshore[d]	μ on FDC	10	0.054[A]	−0.091	29.96***	0.79	0.02
2. Duplin	μ on FDC	10	0.024[A]	−0.098	44.46***	0.85	0.01
3. Skidaway	μ on FDC	11	0.069[A]	−0.336	12.65**	0.58	0.08
4. 1-3 pooled	μ on FDC	31	0.047[A]	−0.161	27.53***	0.54	0.21
5. 1-3 pooled	ln μ on FDC	31	0.299[B]	−4.961	40.67***	0.60	0.07
6. Hagström et al.	μ on FDC	4	0.009[A]	+0.015	171.22***	0.99	0.00
7. Hagström et al.	ln μ on FDC	4	0.163[B]	−3.749	85.79**	0.97	0.02

[a] Abbreviations: n, number of samples; b, slope; a, y-intercept; F_b, calculated F value for test of the null hypothesis, $\beta = 0$; r^2 = coefficient of determination; $CV_{\hat{y}}$, coefficient of variation for estimated y at $\bar{x}$ ($s\hat{y}/Yi$) (40).

[b] Slopes with the same superscript letter are not significantly different from one another ($P > 0.05$).

[c] Asterisks indicate level of significance: **, $P < 0.01$; ***, $P < 0.001$.

[d] Excluding data for the treatment in which no 3-μm filtration was applied, i.e., in which natural particles and predators were present. For the particulate treatment, the null hypothesis $\beta = 0$ could not be rejected ($P > 0.05$).

TABLE 3. *Data summary for field samples based on the regression of ln μ on FDC (see Table 2)*[a]

Site	Date (1980)	Time	Tide	S(‰)	Temp (°C)	TC	FDC (%)	μ	1/μ
Nearshore	4 April	1427	ME	16	17[b]	4.1	5.2	0.03	30
Nearshore	24 April	1005	LE	16	19[b]	5.2	3.8	0.02	46
Nearshore	24 April	1306	MF	21	21[b]	4.5	2.5	0.01	68
Nearshore	24 April	1622	HS	21	20[b]	3.7	2.7	0.02	64
Nearshore, surface	27 May	0845	HE	29	24	9.3	5.7	0.04	26
Nearshore, bottom	27 May	0845	HE	—[c]	—	12.9	6.1	0.04	23
Nearshore, surface	27 May	1200	ME	29	24	8.0	4.5	0.03	37
Nearshore, bottom	27 May	1200	ME	—	—	9.1	5.5	0.04	28
Nearshore, surface	27 May	1525	LF	29	26	17.4	7.6	0.07	15
Nearshore, bottom	27 May	1525	LF	—	—	21.4	9.8	0.13	8
Nearshore, part.[d]	27 May	—	—	—	—	7.5	8.4	0.09	12
Nearshore, free[d]	27 May	—	—	—	—	6.0	5.7	0.04	26
Duplin	12 June	1335	LS	—	29	16.1	7.7	0.07	14
Duplin, part.[d]	12 June	1335	LS	—	29	4.5	8.8	0.10	10
Duplin, free[d]	12 June	1335	LS	—	29	11.6	7.3	0.06	16
Skidaway	1 July	1045	HS	22.5	29	16.7	4.9	0.03	33
Skidaway, part.[d]	1 July	—	—	—	—	1.9	7.1	0.06	17
Skidaway, free[d]	1 July	—	—	—	—	14.8	4.6	0.03	36

[a] Headings: site, experimental site, surface water unless otherwise noted; for tide, H = high, M = mid, L = low, S = slack, E = ebbing, and F = flooding; S ‰, salinity in parts per thousand of surface water; Temp, temperature of surface water; TC, total number of bacterial cells (× 10^6/ml); μ, instantaneous growth rate (h^{-1}); 1/μ, instantaneous generation time (h).

[b] Note that these temperatures are several degrees lower than those used (26 to 29°C) in the generation of our regression equation. This may result in error in prediction of μ (Å. Hagström, personal communication).

[c] —, No data, or not applicable.

[d] part., Cells on or in particulate matter; free, cells not associated with particulate matter.

high FDC ($r = 0.88$), low tidal levels, and near-bottom samples. The ratio of numbers of bacteria on particles to those away from particles in individual samples varied with sampling site and time, ranging from 0.1:1 (Skidaway samples) to 3.0:1 (nearshore, 27 May, surface, mid-ebbing tide). FDC was either higher (up to three times; data not shown) for bacteria associated with particles or was essentially equivalent between free and attached or associated bacteria.

DISCUSSION

Relationship between FDC and growth rate. Hagström et al. (14) introduced the determination of FDC as a novel means of expanding the scope of the AODC method of estimating bacterial standing stocks by adding capability of estimation of bacterial productivity. The FDC method is based on theoretical and experimental work which has shown that FDC is directly related to the growth rates of bacteria in culture (50). This direct relationship is a consequence of the constancy, over a range of growth rates, of the period of time between initiation of cell constriction and cell separation. However, as Woldringh (50) has shown, the time from constriction to division (T) is constant only for growth rates greater than $k = 1$ doubling per h (or $\mu = 0.69\ h^{-1}$). Below this growth-rate level, T increases as growth rate decreases (Fig. 4 of reference 50). Only two of the growth rates which we used in our regressions were greater than $\mu = 0.69\ h^{-1}$. Since increasing T would logically cause increasing FDC, this may explain, at least in part, our finding that a simple linear relationship between growth rate and FDC does not appear to be as good a fit for our data as the fit between ln μ and FDC. All of the growth rates (μ) for the continuous-culture experiments of Hagström et al. (14) were 0.1 or less. Therefore, the data of Hagström et al. (14) are nearly as well fit by the logarithmic regression as by the regression of untransformed growth rates on FDC, since all four data points are at the bottom of the μ/FDC curve. In other words, we suggest that the straight-line fit of FDC on μ found by Hagström et al. (14) was fortuitous and would not have been found if higher growth rates had been examined. This hypothesis is made more plausible by attempts to match our natural-logarithm formula and the untransformed formula which we calculated for the data of Hagström et al. to data of Woldringh (50) and Y. Feig and M. Pace (unpublished) (Table 4). With our formula, the range of deviation of predicted μ from measured or calculated μ is 10 to 14%; using the Hagström et al. formula, the range is from 20 to 76%.

In situ productivity. The considerable scatter around our regression of ln μ on FDC (indi-

TABLE 4. *Prediction of growth rates from frequency of dividing cells for data ($\mu \leq 0.69\ h^{-1}$) of Woldringh (50) and Y. Feig and M. Pace (unpublished data)*

Source	FDC	μ	ln μ on FDC[a]		μ on FDC[b]	
			$\hat{\mu}$	$(\hat{\mu} - \mu)/\mu$	$\hat{\mu}$	$(\hat{\mu} - \mu)/\mu$
Woldringh[c]	15%	0.69	0.621	−0.10	0.150	−0.76
Feig and Pace[d]	9%	0.12	0.103	−0.14	0.096	−0.20

[a] Regression 5 of Table 2, using data from the present study.
[b] Regression 6 of Table 2, using data from Hagström et al. (14).
[c] *Escherichia coli* was the bacterium used (culture temperature, 37°C).
[d] Calculated for the interval 8 to 44 h of a growth curve for *Pseudomonas aeruginosa* (culture temperature, 20°C).

cated by the r^2 of 0.6) hinders precise prediction of μ from FDC of field samples. Confidence intervals (95%) for predicted μ ($\hat{\mu}$ back transformed) at the low end of our observed FDC scale (1.9%) were 0.007 to 0.023; for the mean of observed FDC (6.9%), $\hat{\mu}$ was 0.037 to 0.081; for the upper end of the FDC scale (15.3%), $\hat{\mu}$ was 0.440 to 1.059. However, the range of measured or estimated marine bacterial growth rates listed by Sorokin (41) is 0.3 to 1.0 day^{-1}; the range which we predict from our field data (average for each sampling date) is 0.4 to 1.5 day^{-1}. We have calculated production rates of bacterial carbon from our regression-estimated instantaneous generation times, using the measured (AODC) standing stocks and mean cell volume (0.12 μm^3 for both nearshore and Duplin experiments, and 0.08 μm^3 for the Skidaway experiment) and a value for bacterial carbon per unit of cell volume (0.087 g of $C \cdot cm^{-3}$; reference 11). (Hagström et al. [14] used a value of 0.165 g of $C \cdot cm^{-3}$ in their similar calculations. Fuhrman and Azam [13] used 0.12 g of $C \cdot cm^{-3}$, and Jordan and Likens [22] used 0.10 g of $C \cdot cm^{-3}$.) This requires the assumption that the bacteria are in steady-state, output by predation equalling input by growth. The work of Meyer-Reil et al. (30) and Sieburth (39) has demonstrated that this is probably not always the case. However, it may be that bacterial population sizes are stable enough that they approximate steady-state conditions, and the steady-state assumption may be useful for approximating production rates (1, 3, 27). That substantial predation on bacteria does take place in waters sampled is indicated by our findings with the incubated samples when predators were not excluded; although high bacterial growth rates were apparently in process (FDC, 6 to 9%), increases in cell numbers per milliliter were much lower than predicted from the ln μ/FDC relationship observed in the incubations without predators.

Our calculated range for production of bacterial carbon, using data from Table 3, is 0.6 mg of $C \cdot m^{-3} \cdot h^{-1}$ (24 April, high slack tide) to 17.6 mg of $C \cdot m^{-3} \cdot h^{-1}$ (27 May, low flooding tide). For comparison, bacterial production values which we calculated from the data of other investigators are given in Table 5. The range for a wide variety of oligotrophic waters and north-temperate coastal marine waters is 0.00 to 5.5 mg of $C \cdot m^{-3} \cdot h^{-1}$ (24-h average). Our average value for one tidal cycle for 27 May, nearshore site, was 7.4 mg of $C \cdot m^{-3} \cdot h^{-1}$. If this was representative of the 24-h average rate, then our estimated bacterial production rates range higher than the others cited.

Reservations. Our own data for change in mean cell volume, and recent work by R. L. Ferguson, E. N. Buckley, and A. V. Palumbo (manuscript in preparation) raise an important question regarding validity of predictions of marine environmental bacterial growth rates using FDC/growth rate relationships determined in bottle incubation experiments. We found considerable increases in mean cell volume as incubation proceeded, and Ferguson et al. (in preparation) found similar increases in cell size corresponding to large increases in numbers of agar-culturable bacterial genera. Does this mean that bottle incubation induces development of bacterial populations which have FDC/growth rate relationships unlike those of naturally occurring marine bacterial assemblages? A related question (P. A. Rublee, personal communication) is, is it possible to detect dividing cells, if they are present, among the group of very small cells which are almost invariably present in marine environmental samples? (In the case of the present work, smallest cells were ~0.02 μm^3 in volume.) Other questions which require answering before the FDC method of predicting growth rates in the field can be accepted as valid are the following. The bottle incubations encourage development of populations of cells most of which are actively growing; if substantial fractions of naturally occurring cells were dormant (this is a controversial question; cf. references 10, 16, 17, 25, 26, 29, 44, 47, 52), would this cause underestimation of FDC for the active group and con-

TABLE 5. *Aquatic bacterial production rates*[a]

Reference	Site	mg of C·m^{-3}·h^{-1}
14	Photic zone, coastal Baltic Sea[b]	0.09
22	Oligotrophic freshwater lake[c]	0.13
23	450-m water, Caribbean Sea[d]	0.17
39	Coastal marine, northeastern United States[e]	3.5
9	Coastal marine, northern France[f]	0.03–1.83
41	Variety of oceanic sites[g]	0.00–2.92
13	Coastal marine, western North America[h]	0.03–3.0
28	Kiel Fjord and Kiel Bight[i]	0.00–3.25
H.W.D.[k]	York River estuary, Virginia[j]	0.04–5.5
—[l]	Nearshore, Georgia Bight[b]	0.8–7.4

[a] Values tabulated were calculated as 24-h averages from values published in the cited references.

[b–j] Methods used: *b*, FDC; *c*, $^{35}SO_4$ uptake; *d*, [^{3}H]adenine incorporation into ribonucleic acid; *e*, Δadenosine triphosphate in diffusion culture of 3-μm-filtered water; *f*, Δtotal AODC in 3-μm-filtered water in diffusion chambers; *g*, dark $^{14}CO_2$ uptake; *h*, [^{3}H]thymidine incorporation into deoxyribonucleic acid and Δtotal AODC in incubated 3-μm-filtered water; *i*, Δtotal AODC on membranes floating over natural seawater; *j*, [^{3}H]thymidine incorporation into deoxyribonucleic acid.

[k] H. W. Ducklow, unpublished data.

[l] Two averages from the present study, for sampling dates at which more than one point in the tidal cycle was sampled.

sequent error in calculation of production? How steady are marine bacterial steady-states, and how much inaccuracy could this factor cause in estimation of production from FDC? Does division with cell volume decrease of starving cells (32) ever introduce a confounding factor in coastal waters? Can a better fit be found for the FDC/growth rate relationship than the logarithmic one which we used? Can the scatter in the relationship be reduced methodologically so that small differences in growth rate might be resolvable with the FDC prediction method? In connection with these last two questions, we must note that there may be significant interactive effects of nutrient concentration and temperature upon the relationship between FDC and growth rate (Å. Hagström, personal communication). It may be that multiple regression including these two factors could reduce predictive error.

We suggest that the following approaches could provide answers to some or all of these questions. (i) The first approach is collection of more information than is currently available on dynamics of natural bacterial assemblages, including diel and seasonal changes in total cell numbers, the proportion of the total which are actively growing, and rates of bacterivorous predation. (ii) The second approach is comparison of FDC as detected by electron microscopy of samples in parallel with light-microscopic analysis. Hagström et al. (14) reported that very similar FDC values were found when this comparison was made, but more extensive work is needed here. (iii) The third approach is examination of the FDC/growth rate relationship in diffusion chambers (12, 29) in the field, so that natural proportions of inactive cells and natural nutrient changes would be involved, inhibiting development of non-representative bacterial populations. Alternatively, the brief (<15-h) bottle incubation of 3-μm-filtered water without nutrient addition or dilution, as used by Fuhrman and Azam (13), might serve this purpose; change in total counts was found to be reflected by deoxyribonucleic acid synthesis rates under these conditions. In this type of situation, the relationship between FDC and change in total AODC per unit time might be stronger than the relationship between FDC and the calculated exponential growth rate (μ or k), or the FDC/μ relationship might be more reliable if corrections to total counts were made for the inactive fraction. (iv) The fourth approach is comparison of production values resulting from FDC predictions and radioisotopic or other methods of measuring bacterial production. Although more testing is required, Hagström et al. (14) have already accomplished a comparison of this sort. They compared bacterial production estimated by bacterial algal exudate uptake with values resulting from their FDC predictions of growth rate. The two estimates were in agreement, and the FDC/growth rate plot of Hagström et al. is fit well by our ln μ-on-FDC regression, so that both their regression and ours predict virtually the same production value.

Summary. Our work has confirmed the contention of Hagström et al. (14) that there is a statistically significant mathematical relationship between FDC and growth rates of bacteria from marine environments. However, the constancy of the relationship which we have described, the extent of its applicability, and the validity of its use as a predictor of environmental bacterial growth rates need further examination.

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