

Microbial Growth Rates and Biomass Production in a Marine Sediment: Evidence for a Very Active but Mostly Nongrowing Community

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Biomass, nucleic acid synthesis, and specific growth rates of the microbial communities were measured throughout a vertical profile of a coastal marine sediment. The microbial biomass, as determined by ATP concentrations, in the sediment-water interface was over twice that measured in the other horizons of a 10-cm profile. Likewise, biomass carbon production, as determined by DNA synthesis, and the specific growth rate, as determined from the kinetics of [³H]ATP pool labeling, were also elevated at the interface. These results indicate that, due to a large and active community in the interface, the greatest amount of microbial activity, growth, and biosynthesis occurs within the first few millimeters of sediment. These results notwithstanding, a combination of two independent techniques established that over 90% of the sediment-water interface community was not actively growing.

Individual microbial cells in a natural environment are either growing or nongrowing. It would seem to be an easy task to differentiate the two groups, but this has not been the case. Simple culturing techniques have not been able to duplicate the complex physical and chemical parameters of most natural environments. Since many determinations of microbial activity, productivity, and specific growth rate depend on measuring or estimating the magnitude of the living community, the accuracy of this estimation is crucial for assessing the role of microbes in natural environments. The problem is particularly complex in marine sediments, where the microbial community often exceeds 10^9 cells per g (2, 12). With such a large total population, even a small percentage of it would represent a substantial number of individuals. While several studies (9, 11, 17, 19) have measured the number of metabolically active individuals within the community, to my knowledge no studies have attempted to determine the proportion of nongrowing cells in any marine environment.

In an attempt to characterize the growth of microorganisms in a marine sediment, I used several techniques to measure specific microbial growth rates and biomass production in the surface sediments of Halifax Harbor. In addition, by combining a recently developed technique (5a) with direct microscopic enumeration and a sediment incubation method that I developed earlier (12), I report here the first calculation of the magnitude of the nongrowing portion of the microbial community in a natural environment.

MATERIALS AND METHODS

Sampling site and sample collection. The sampling site used in this study was the area of Halifax Harbor known as Eastern Passage, which was also the site of previous studies (7, 11, 12, 16). Samples of sediment were collected in plastic core tubes by hand by scuba divers, as described previously (11). Once collected, the sediment cores were transported on ice to the laboratory. Due to the proximity of the sampling site to the laboratory, experiments were begun within 1 h of sample collection. The sediment-water interface layer was removed by aspiration as described previously (11). Once the interface was removed, the overlying water was carefully

decanted and the sediment was extruded vertically by forcing a rubber plug through the bottom of the core tube. When 1 cm of sediment was extruded, it was removed with a spatula, and the process was repeated until 5 1-cm horizons had been collected. Unsieved sediment was then used directly (sediment plug) or suspended in filter-sterilized bottom water to yield a sediment slurry containing approximately 2.5 mg (dry weight) of sediment per ml of slurry.

ATP determinations. Sediment plugs (2 cm³) were placed directly into 10 ml of cold 1.47 M H₃PO₄ and vigorously mixed. After an extraction period of 10 min, the samples were centrifuged and 0.1 ml of the supernatant was diluted into 4.9 ml of phosphate buffer (60 mM, pH 7.4) and assayed for ATP by the methods of Karl and Craven (5). For interface and other slurry samples, 1 ml of the slurry was pipetted directly into 5 ml of boiling 60 mM phosphate buffer and extracted at 100°C for 5 min. For water samples, 100 to 250 ml of water was filtered through a Whatman GF/F glass fiber filter. Immediately after filtration, the filter was placed into boiling buffer and extracted as described for interface samples. Internal ATP standards were used to correct for real and apparent losses of extractable ATP.

Nucleic acid synthesis rates. DNA and RNA synthesis rates were determined by using 30-ml portions of the sediment slurries. [³H]adenine (specific activity, 17.1 Ci/mmol) was added to the slurries to a final concentration of 133 nCi/ml, and the samples were incubated with gentle shaking at the in situ temperature of 2°C in a Psychrotherm (New Brunswick Scientific Co.) incubator. During the incubation and sampling period, the headspace over the slurries was continuously gassed with a mixture of nitrogen, oxygen, and carbon dioxide to maintain the slurry at the in situ O₂ concentration (9). At various times duplicate 1-ml subsamples were injected into 10 ml of cold 1 N HCl containing nonradioactive adenine (2 mg/ml). The tubes were then capped and frozen. Radioactive RNA and DNA were separated and measured by the method of Karl (3) as adapted for sediment samples by Craven and Karl (1). The specific radioactivity of the ATP pool was measured at each time point by injecting a 0.5-ml portion of the sediment slurry into 5 ml of boiling phosphate buffer (60 mM, pH 7.4), followed by sample concentration

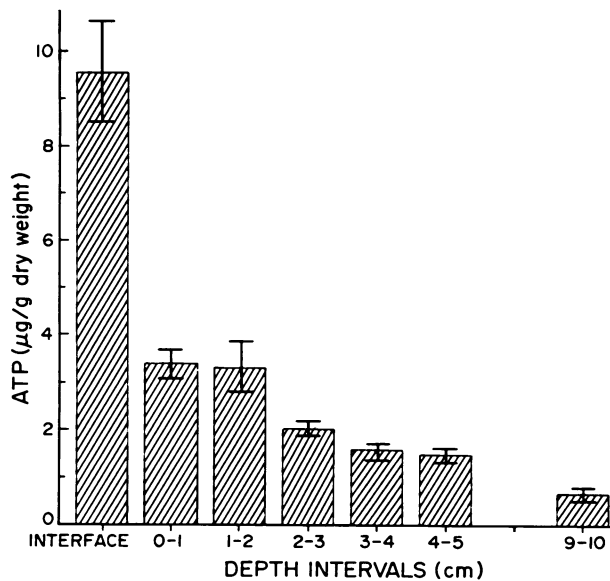


FIG. 1. ATP content of surface sediments of Halifax Harbor. The hatched bars represent the mean of five determinations; the vertical bars represent the standard error of the mean.

and purification as described previously (1). Rates of RNA and DNA synthesis were calculated from the rate of isotope incorporation during the incubation period (up to 12 h) divided by the integral of the ATP pool specific activity for that time period (18). The rate of biomass carbon production was extrapolated from the rate of DNA synthesis by assuming that 1 pmol of DNA is equivalent to 1,236 pg of DNA produced and that DNA constitutes 2% of total cell carbon. Residual [^3H]adenine and the production of $^3\text{H}_2\text{O}$ were also monitored in each time course experiment (3).

Specific-growth-rate determinations. The mean microbial community specific growth rate was estimated by three independent methods. The first estimate was derived from the measured rates of DNA synthesis and the ATP biomass data, i.e., (doublings/day) \times 0.693, assuming a DNA/ATP ratio of 5. The rationale for this extrapolation is presented elsewhere (6). The second estimate was derived from the methods of Karl et al. (5a) and Karl and Bossard (4) with direct measurement of the turnover time of the adenine nucleotide pool. Time-dependent changes in the specific activity of the ATP pool measured during the nucleic acid synthesis experiments were analyzed with a computer-assisted, nonlinear least-squares fit to the expected experimental relationship. From this analysis, T (the turnover time of the total adenine nucleotide pool) is derived. The specific growth rate is then calculated by assuming that the pool turns over 40 times per generation (5a). The third method, used only to determine the specific growth rate of the interface community, was the direct microscopic count method with epifluorescence microscopy or epifluorescence microscopy combined with autoradiography to determine total or active cell number increases over time. This method is used in conjunction with whole-core incubations and has been described in detail previously (12).

Direct microscopic cell counts and autoradiography. Epifluorescence microscopy after acridine orange staining was used for both cell enumeration and autoradiography as previously described (11). For interface autoradiography with whole-core incubations, [$2\text{-}^3\text{H}$]adenine (specific activ-

ity, 17.1 Ci/mmol) was added to the overlying water to a final concentration of 133 nCi/ml.

RESULTS

The ATP content of the sediment was determined by two different methods: acid extraction of sediment plugs and boiling-buffer extraction of sediment slurries. While both methods yielded comparable final results, the recovery efficiency of the boiling-buffer technique (average, 48.5%) was less than that recorded for the acid extraction technique (average, 67.5%). Accordingly, ATP was determined by the acid extraction technique for all sediment samples, while the boiling-buffer technique was used for ATP extraction from water and interface samples. All data were corrected for extraction efficiency according to the method used.

The ATP content of the various sediment horizons is shown in Fig. 1. The interface contained more than twice the ATP found in the deeper horizons. The ATP content generally decreased with depth, but detectable amounts were always present even in the deepest sediments examined (10 cm). The ATP content of the overlying seawater was 297.1 pg of ATP per ml, or over 4 orders of magnitude less than in the interface habitat.

The rate of DNA synthesis, and hence of biomass carbon production, was also greatest in the interface (Fig. 2). Below the interface, biomass carbon production was low and uniform throughout the profile. The profile of RNA synthesis rates was similar to that for DNA synthesis, although the rate of RNA synthesis was always approximately 100 times that of DNA synthesis (data not shown). The biomass-specific rates of carbon production are shown in Fig. 3. The carbon production per unit biomass was twice as high in the interface as it was in the deeper horizons.

The specific growth rates of the various sediment communities are shown in Fig. 4. Like the other parameters measured, the specific growth rate was also higher for the interface community. Below the interface horizon, the spe-

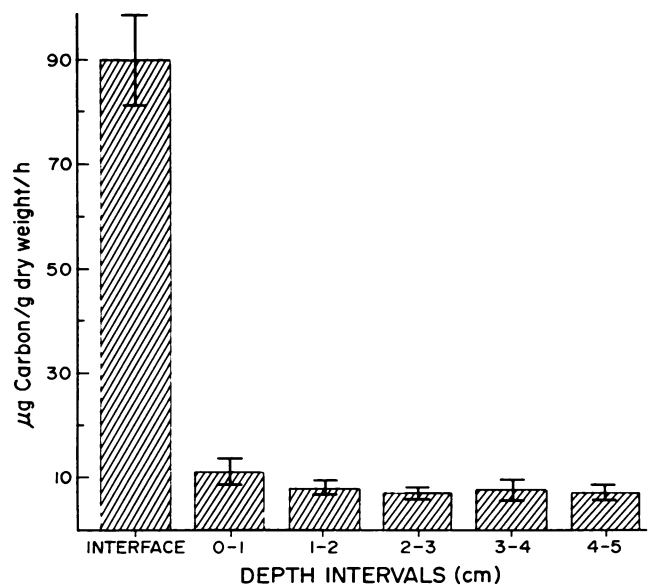


FIG. 2. Biomass carbon production in surface sediments as extrapolated from the incorporation of [^3H]adenine into DNA. The hatched bars represent the mean of three determinations; the vertical bars represent the standard error of the mean.

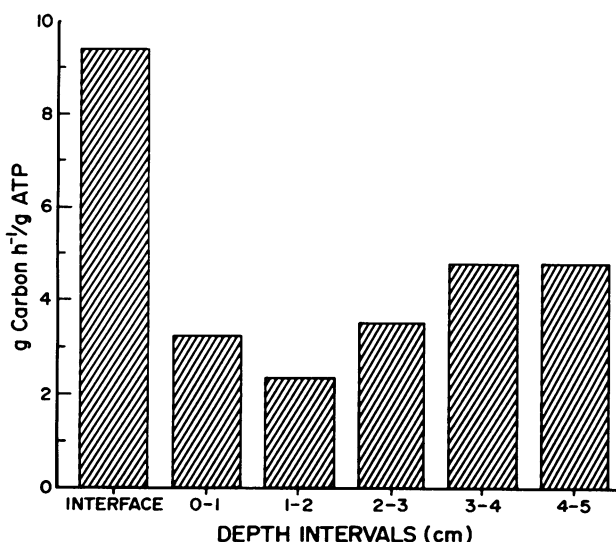


FIG. 3. Biomass (ATP)-specific carbon production in Halifax Harbor sediments as measured by the incorporation of [³H]adenine. For each horizon the bar represents the mean biomass carbon production (Fig. 2) divided by the mean ATP content (Fig. 1).

cific growth rate varied little, and with no apparent pattern, with depth.

DISCUSSION

A previous study (12) identified the sediment-water interface in Halifax Harbor as an environment containing a large number of bacterial cells with high heterotrophic activity. Both the direct counts and the heterotrophic activity were twice those measured in the sediment immediately below and orders of magnitude above those measured in the overlying water. The results of the present study agree with these results and confirm the increased standing-stock biomass and biomass production in the interface. Examining the deeper layers of sediment, it now appears that the top 5 cm (excluding the interface) of sediment are rather homogeneous with respect to biomass (Fig. 1) and biomass production (Fig. 2), indicating the sharp boundary between the interface and the sediment below. In addition to a high rate of biomass production in the interface due to the large resident microbial community, on a biomass-specific (and presumably a per cell) basis, the results indicate a more active community as well (Fig. 3). These data are in agreement with those reported by Novitsky and Karl (15) for a coastal subtropical sediment. Due to a large and active community in the interfaces of both these muddy sediments, the largest amount of cellular biosynthesis, heterotrophic activity, and probably organic matter mineralization occurs within the first millimeter of sediment.

Like the biomass and biomass production data, the specific growth rate of the interface community was also greater than that measured for the deeper-sediment horizons. Thus, the increased biomass production is due not only to the large microbial community and its increased per cell production rate, but to an increased growth rate as well. Since the carbon and energy input to this sediment system is detritus that sediments from the water column, the interface community will receive more nutrient input than deeper-sediment communities. This input is probably responsible for maintaining the increased biomass, production, and growth rate

of this community. This is in contrast with the results reported by Novitsky and Karl (14), who found increased biomass and production but no increase in specific growth rates (above control values) for sediment communities receiving large amounts of organic matter. These results may indicate that microbial growth rates in natural environments are not controlled simply by a single parameter but may be controlled by a number of factors, some of which may not yet have been identified.

In a previous study (11) I reported the observation that only a small portion (<5%) of the interface community was active in glutamate uptake, as determined by autoradiography. This observation raised the question of the metabolic status of the large majority of the observed community. Assuming that the autoradiography technique is valid (for a critical discussion, see reference 11), these uptake-inactive cells may be nongrowing but capable of future growth (dormant) or dead. Using a whole-core incubation technique and a direct counting procedure (DC), I was able to measure the specific growth rate of the interface community ($\mu = 0.0067/h$ [12]). This figure, however, represents the average growth rate of the entire community, enumerated by direct microscopic counts which included dead and dormant cells. In the present study I again measured the specific growth rate of the interface community, this time using both the DC method and an adenine turnover (AT) method recently developed by Karl et al. (5a) and Karl and Bossard (4), based on the observation that microbial cells turn over their adenine nucleotide pools 40 times per generation independent of the specific growth rate. The specific growth rates determined by these two independent techniques for the interface communities on two sampling dates are presented in Table 1. On prima facie inspection, the values from both techniques appear unrelated. However, the DC-derived rates include the dead and dormant cells which are counted

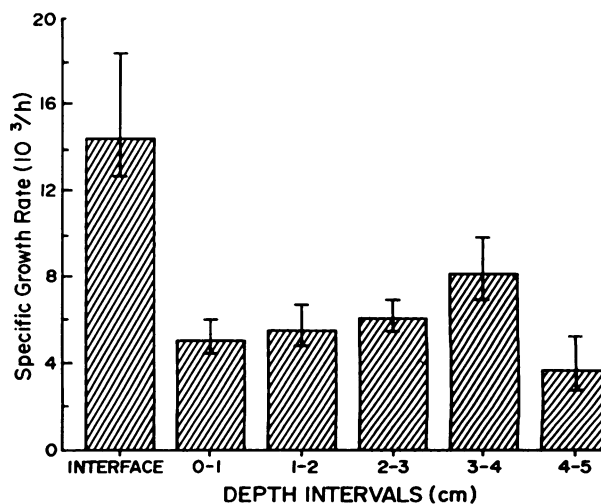


FIG. 4. Specific growth rates of the microbial communities in surface sediments as determined by the turnover rate of the adenine nucleotide pool. Results from time-dependent changes in the specific activity of the ATP pool after the addition of [³H]adenine were analyzed with a computer-assisted, nonlinear least-squares fit to the expected experimental relationship described by Karl et al. (5a). From this analysis the mean nucleotide turnover time and its standard deviation are derived. The hatched bars represent the specific growth rate extrapolated from the nucleotide turnover time; the vertical bars represent the 95% confidence intervals.

TABLE 1. Specific growth rates and generation (doubling) times for the microbial community in the sediment-water interface of Halifax Harbor

Date (mo-day-yr)	DC			AT			% Nongrowing ^a
	μ (h ⁻¹)	SE	Doubling time (h)	μ (h ⁻¹)	SE	Doubling time (h)	
10-10-85	0.0069	9.3×10^{-5}	100	0.030	2.8×10^{-2}	23.4	97.8
12-17-85	0.0037	3.2×10^{-5}	187	0.015	2.2×10^{-2}	46.1	90.0
12-17-85 (autoradiography)	0.0140	2.0×10^{-3}	49.5				

^a Determined by comparing the two growth rates with the relationship described by Fig. 5.

along with the actively dividing ones. The AT-derived rates, on the other hand, represent only those cells actively taking up and turning over their adenine nucleotide pools, i.e., the active population. In the DC calculations, the specific growth rate obtained assumes that the entire community is dividing at the same rate. If a portion of the community is not dividing, the growing portion of the community must be dividing at a rate higher than the calculated rate to offset that portion with a growth rate of zero. The theoretical relationship between an assumed nongrowing percentage of the community and the specific growth rate of the growing population for the total interface community measured on two sampling dates is presented in Fig. 5. Initially, direct counts of the interface community were conducted over a 7-day period as described previously (12). The graphs in Fig. 5 were then generated by assuming that an arbitrary portion of the initial community was not dividing and subtracting this

number of cells from all subsequent direct counts. The number of cells remaining (now assumed to be the actively growing portion of the community) was plotted against time to yield the specific growth rate and generation time of the actively growing portion of the community. It can be seen from Fig. 5 that as the nongrowing portion of the community increased, the growing portion had to divide proportionately faster to maintain the growth rate observed for the community as a whole. With this relationship, the specific growth rate calculations from the two techniques (DC and AT) can only be reconciled if 90 to 97% of the cells (6.26×10^9 to 6.74×10^9 cells/g [dry weight]) counted microscopically are nongrowing. Since the microbial communities in the sediment would be expected to vary slightly both spatially and temporally, DC data were collected for eight cores taken throughout the year. The average specific growth rate so calculated (0.0066/h; standard error, 1.3×10^{-3}) indicates that the growth rate reported in Table 1 for samples taken on 10 October 1985 should be considered as most representative of the average community. The relationship presented in Fig. 5 also brings into agreement the autoradiography data indicating that less than 10% of the community was active in uptake of glutamate, glucose, or thymidine (11). For the 17 December 1985 samples, only 3.9% of the community was active in taking up [³H]adenine, and the [³H]adenine autoradiography-derived growth rate of 0.014/h was almost identical to the AT-derived rate of 0.015/h (Table 1). Unfortunately, the whole-core incubation technique provides DC-derived growth rates only for the interface community. It is therefore impossible, at present, to estimate the nongrowing portions of the deeper-sediment communities. Since the interface community is metabolically more active than those immediately below it, it is reasonable to assume that an even greater portion of the deeper communities are nongrowing. Autoradiography of deeper-sediment horizons (to 100 cm) has consistently shown that <3% of the community is active in glucose, glutamate, or lactate uptake (data not shown).

Another method for determining the growth rate of microbial communities is through the measurement of DNA production as measured by the incorporation of [³H]adenine (6). This method depends on the measurement of biomass (ATP), so that a large amount of ATP in nongrowing cells will give an artificially low growth rate. However, the growth rate of the community (17 December 1985) determined in this manner was 0.018/h. This close agreement indicates that either all the living biomass is growing or that the dormant portion of the community contains only a small amount of ATP. While little is known about dormant cells, it is likely that their metabolism would be minimal. Dormancy is generally considered a likely survival strategy for marine microbes, especially in view of their longevity under low-nutrient and starvation conditions (10). The exact proportion of dormant and dead components in the community remains unknown. However, the observation that killed microbial cells in marine sediments are very labile (13) lends support to

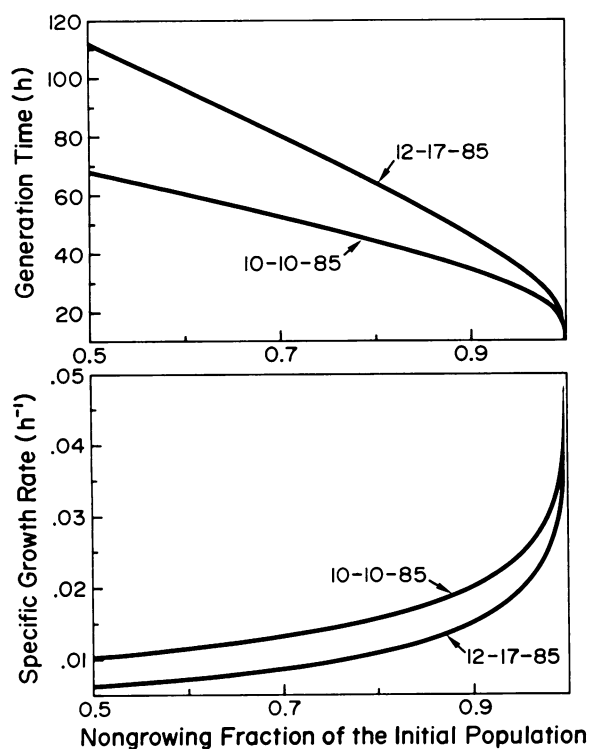


FIG. 5. Specific growth rate and generation (doubling) time of surface sediment microbial communities corrected for an assumed nongrowing fraction of the community. Both parameters were calculated for the entire community (from direct microscopic counts during whole-core incubations) and then recalculated after subtracting a fraction of the initial community, assumed to be nongrowing, from each direct microscopic count. Dates are presented as month-day-year.

the hypothesis that the nongrowing portion of the community is mainly dormant.

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LITERATURE CITED

- Craven, D. B., and D. M. Karl. 1984. Microbial RNA and DNA synthesis in marine sediments. *Mar. Biol.* **83**:129-139.
- Dale, N. G. 1974. Bacteria in intertidal sediments: factors related to their distribution. *Limnol. Oceanogr.* **19**:509-518.
- Karl, D. M. 1981. Simultaneous rates of ribonucleic acid and deoxyribonucleic acid synthesis for estimating growth and cell division of aquatic microbial communities. *Appl. Environ. Microbiol.* **42**:802-810.
- Karl, D. M., and P. Bossard. 1985. Measurement and significance of ATP and adenine nucleotide pool turnover in microbial cells and environmental samples. *J. Microbiol. Methods* **3**: 125-139.
- Karl, D. M., and D. B. Craven. 1980. Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities. *Appl. Environ. Microbiol.* **40**:549-561.
- Karl, D. M., D. R. Jones, J. A. Novitsky, C. D. Winn, and P. Bossard. 1987. Specific growth rates of natural microbial communities measured by adenine nucleotide pool turnover. *J. Microbiol. Methods* **6**:221-235.
- Karl, D. M., and C. D. Winn. 1984. Adenine metabolism and nucleic acid synthesis: applications to microbial oceanography, p. 197-215. *In* J. E. Hobbie and P. J. L. Williams (ed.), *Heterotrophic activity in the sea*. Plenum Publishing Corp., New York.
- Kepkay, P. E., R. C. Cooke, and J. A. Novitsky. 1979. Microbial autotrophy: a primary source of organic carbon in marine sediments. *Science* **204**:68-69.
- Kepkay, P. E., and J. A. Novitsky. 1980. Microbial control of organic carbon in marine sediments: coupled chemoautotrophy and heterotrophy. *Mar. Biol.* **55**:261-266.
- Meyer-Reil, L.-A. 1978. Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolizing bacteria in natural waters. *Appl. Environ. Microbiol.* **36**:506-512.
- Morita, R. Y. 1982. Starvation-survival of heterotrophs in the marine environment, p. 171-198. *In* K. C. Marshall (ed.), *Advances in microbial ecology*, vol. 6. Plenum Publishing Corp., New York.
- Novitsky, J. A. 1983. Heterotrophic activity throughout a vertical profile of seawater and sediment in Halifax Harbor, Canada. *Appl. Environ. Microbiol.* **45**:1753-1760.
- Novitsky, J. A. 1983. Microbial activity at the sediment-water interface in Halifax Harbor, Canada. *Appl. Environ. Microbiol.* **45**:1761-1766.
- Novitsky, J. A. 1986. Degradation of dead microbial biomass in a marine sediment. *Appl. Environ. Microbiol.* **52**:504-509.
- Novitsky, J. A., and D. M. Karl. 1985. Influence of deep ocean sewage outfalls on the microbial activity of the surrounding sediment. *Appl. Environ. Microbiol.* **50**:1464-1473.
- Novitsky, J. A., and D. M. Karl. 1986. Characterization of microbial activity in the surface layers of a coastal subtropical sediment. *Mar. Ecol. Prog. Ser.* **28**:49-55.
- Novitsky, J. A., and P. E. Kepkay. 1981. Patterns of microbial heterotrophy through changing environments in a marine sediment. *Mar. Ecol. Prog. Ser.* **4**:1-7.
- Tabor, P. S., and R. A. Neihof. 1982. Improved microautoradiographic method to determine individual microorganisms active in substrate uptake in natural waters. *Appl. Environ. Microbiol.* **44**:945-953.
- Winn, C. D., and D. M. Karl. 1984. Laboratory calibrations of the [³H]adenine technique for measuring rates of RNA and DNA synthesis in marine microorganisms. *Appl. Environ. Microbiol.* **47**:835-842.
- Zimmermann, R., R. Iturriaga, and J. Becker-Birck. 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Environ. Microbiol.* **36**:926-935.