ity. However, in all groups exposure to JP5 WSF began during the first day of zoeal development. The progeny from three females were used for each group. The larvae were reared in two incubators set at a nominal temperature of 25°C. (Differences in development rate suggest that the incubator containing the groups at 15 per mil salinity either had wider variation or maintained slightly lower temperatures than the incubator containing the groups at 5 per mil salinity.)

9. Water-soluble fractions of JP5 were made by gently stirring one part JP5 over nine parts seawater of the appropriate salinity at room temperature (*1*). We ensured a uniform stirring rate in each bottle by using a stirring table with multiple heads revolving at identical speeds. Because higher salinity causes a decrease in the time-dependent dissolution of hydrocarbons, the 15 per mil WSF were stirred for 3 to 4 hours longer each day to more closely equalize exposure concentrations. Ultraviolet spectrophotometry [J. M. Neff and J. W. Anderson, *Bull. Environ. Contam. Toxicol.* **14**, 122 (1975)] of the daily WSF preparations gave total aromatic hydrocarbon values of 2.62 ± 0.15 parts per million (ppm) (N = 17) in 5 per mil salinity and 2.70 ± 0.16 ppm (N = 18) in 15 per mil salinity, expressed as tetralin equivalents. Tetralin is a major ultraviolet-absorbing constituent of JP5 WSF. Gas chromatographic analysis indicated that alkyl-substituted monocyclic aromatics predominate. Fortuitously, concentrations of total aromatics determined here by ultraviolet spectrophotometry are similar to those determined by gas-liquid chromatography.
10. Megalops were rinsed briefly in tap water to remove adsorbed sea salts, dried for at least 2 days at 60°C, and weighed to the nearest 0.1 μg on a microbalance (Cahn 21). Generally, 15 megalops were weighed and three means determined as replicates for further statistical analysis. In several instances when fewer than three megalops per mean were available, the number of replicates was reduced from three, as generally used. Figure 3 shows the means across all hatches.
11. Percentage of survival was determined for each hatch to give three replicates per factor combination and transformed to the arc sin $x^{1/2}$ [R. G. D. Steele and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960)]. We performed statistical tests, using the "statistical package for the social sciences" computer programs [N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, D. H. Bent, *Statistical Package for the Social Sciences* (McGraw-Hill, New York, 1975)]. A regression analysis approach, as explained in the manual, was used. Data for the development rate and megalopal dry weights were treated similarly, except that they required no transformation.
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Methane Production from Acetate and Associated Methane Fluxes from Anoxic Coastal Sediments

Abstract. *The apparent microbial conversion of acetate to methane ranges seasonally from 0.7 to 88 micromoles per liter of whole wet sediment per hour in the top 5 centimeters of methane-producing sediments underlying sulfate-reducing sediments in Cape Lookout Bight, North Carolina. The associated methane flux across the sediment-water interface into overlying waters exhibits the same seasonal pattern. Significant methane production from acetate is observed only in sulfate-depleted sediments.*

Methane (CH_4) production is known to occur in organic-rich anoxic sediments with low concentrations of nitrate and sulfate (*1*, *2*). This process is the terminal step in the anaerobic decomposition of organic matter (*3*) and is consequently a major component of both global and local carbon budgets. Microbially mediated methanogenesis has been estimated to account for more than 80 percent of the total global flux to the troposphere (*4*). Sedimentary methanogenesis results in both the accumulation of CH_4 in the sediment and its transport into the overlying water (*5*). It is thought that the CH_4 produced in these environments arises from two distinct microbially mediated processes: the fermentation of acetate and the reduction of CO_2 by H_2 (*2*, *3*). The rates of CH_4 production from precursor molecules such as acetate in freshwater sediments have been measured (*6*, *7*), but this has not been done with marine sediments, nor have the values obtained been compared with changes in the in situ CH_4 concentrations or with fluxes of CH_4 out of the sediment being studied.

We report here the first seasonal measurements of microbial CH_4 production rates from acetate in a sediment conducted in association with in situ measurements of fluxes of CH_4 out of the sediment into the overlying water (*5*). We have found a strong seasonal correlation between these two processes in the sediments of a small coastal basin on the Outer Banks of North Carolina. We have also measured the depth dependency of CH_4 production from acetate and found that significant production occurs only when sulfate is totally depleted.

Our sample site was Cape Lookout Bight, North Carolina, an organic-rich marine basin of approximately 2 km² located 115 km southwest of Cape Hatteras (*8*). The water column is well oxygenated (> 130 μM O_2) and low in CH_4 (< 5 μM) year-round (*5*, *9*) and is underlain by a fine-grained mud with an organic content ranging from approximately 3 percent (by dry weight) at the surface to 2 percent at a depth of several meters (*10*). The fine-grained sediment and presumably much of its organic content are derived from the tidal flushing of nearby barrier island lagoons (*5*). Because nutrient, dissolved gas, and sulfate vertical concentration profiles are reproducible in Cape Lookout sediments from year to year (*5*, *11*), we have combined data from 1976 to 1980 to demonstrate the seasonally dependent production and release of CH_4 from these sediments.

Methane has been observed (*5*, *8*) to be released from Cape Lookout sediments to the overlying water by two processes. The diffusive flux of dissolved CH_4, measured with the use of in situ benthic chambers, was found to have a large summer maximum (Fig. 1A). The quasi-advective flux of CH_4 arising from the release of gas bubbles was found to follow a similar seasonal pattern and to be approximately six times as large as the diffusive flux (Fig. 1A).

We measured the rates of CH_4 and CO_2 production from acetate by using methods described in (*12*). Briefly, both the whole sediment substrate concentrations (μmole $liter_s^{-1}$, where the subscript refers to the volume of whole wet sediment) and the corresponding first-order reaction rate constants ($hour^{-1}$) were measured periodically, and these parameters were multiplied to obtain apparent production rates (*13*).

Use of the term "apparent" is based on our measurements of whole sediment acetate concentrations (pool size). The methodology used in this study (see below) allows for determination of total "extractable" pool sizes of acetate; however, this should be distinguished from the "microbiologically available" pool size, which may be less than the total because of the partitioning of acetate between sediment pore water (both free and complexed), adsorption sites on sediment particle surfaces, and microbial cellular material. The actual microbial production rates may therefore be less than the apparent production rates.

In order to measure acetate concentrations, bulk wet sediment samples were basified, freeze-dried, extracted with methanol, derivatized to form methyl esters of the volatile fatty acids, and analyzed by gas chromatography with a hexanoate internal standard. Less than 1 percent of the acetate measured during the summer in Cape Lookout sediments was found to be dissolved in the pore water (*14*). The remainder was presumably

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bound to the solid phase and also present in cellular material.

We determined the rate constants for CO_2 and CH_4 production from acetate by anaerobically incubating samples at in situ temperatures with tracer concentrations of [1,2-^{14}C]sodium acetate, 53.5 mCi mmole^{-1} (New England Nuclear), using a modification of the methods of Christian and Wiebe (*15*). In situ acetate concentrations ranged from 34 μmole liter$_s^{-1}$ in March of 1979 to 660 μmole liter$_s^{-1}$ in August; 0.062 to 0.31 nmole of [^{14}C]acetate were added to each incubating tube containing 3 ml of wet sediment, thereby increasing the total acetate pool by less than 1 percent. During the 5-minute incubation, less than 5 percent of the added label was utilized. Reduction of $^{14}CO_2$ to $^{14}CH_4$ during the incubations was not considered important because of the very low ratio of $^{14}CH_4/^{12}CH_4$ ($< 10^{-6}$) at the end of the experiments and because the first-order rate constant for this reaction was less than 0.0001 hour^{-1} in October 1979 and less than 0.0003 hour^{-1} in February 1980 (*14*). Rate constants for acetate conversion to CH_4 were 0.063 and 0.010 hour^{-1}, respectively for these two dates. Based on the experimental conditions described above, we assumed that the rates measured were initial rates; that is, rates were not affected by changes in substrate or end product concentrations.

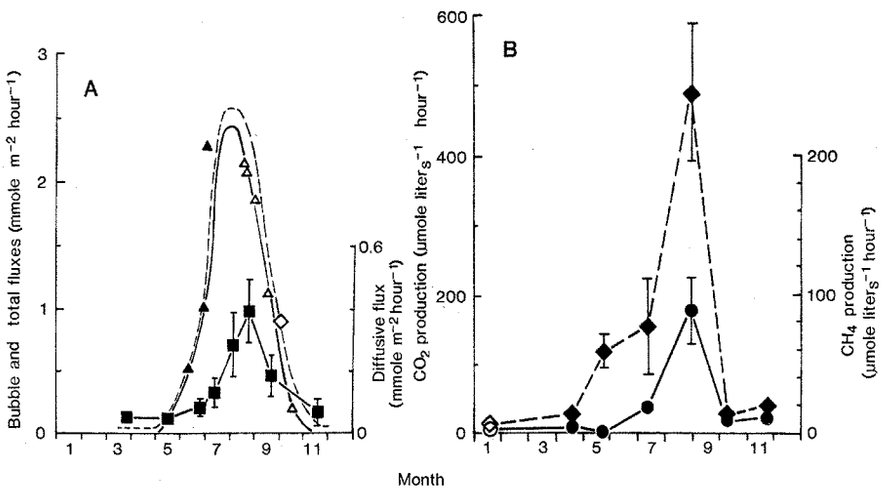

Fig. 1. (A) Seasonal variation in CH_4 fluxes: diffusive flux data (solid line with squares) are from 1977; bubble flux data (solid line with triangles and diamond) are from 1976 (◇), 1977 (▲), and 1978 (△); dashed line indicates combined diffusive and bubble fluxes. (B) Seasonal variation of apparent acetate turnover: CO_2 production data are from 1979 (◆) and 1980 (◇); CH_4 production data are from 1979 (●) and 1980 (○). The vertical bars in (A) are the 34 percent error (1 standard deviation) observed in multiple-chamber diffusive experiments; the vertical bars in (B) represent the standard deviation (1 standard deviation) of triplicate acetate utilization experiments. Error bars are not shown in cases where they are smaller than the symbols.

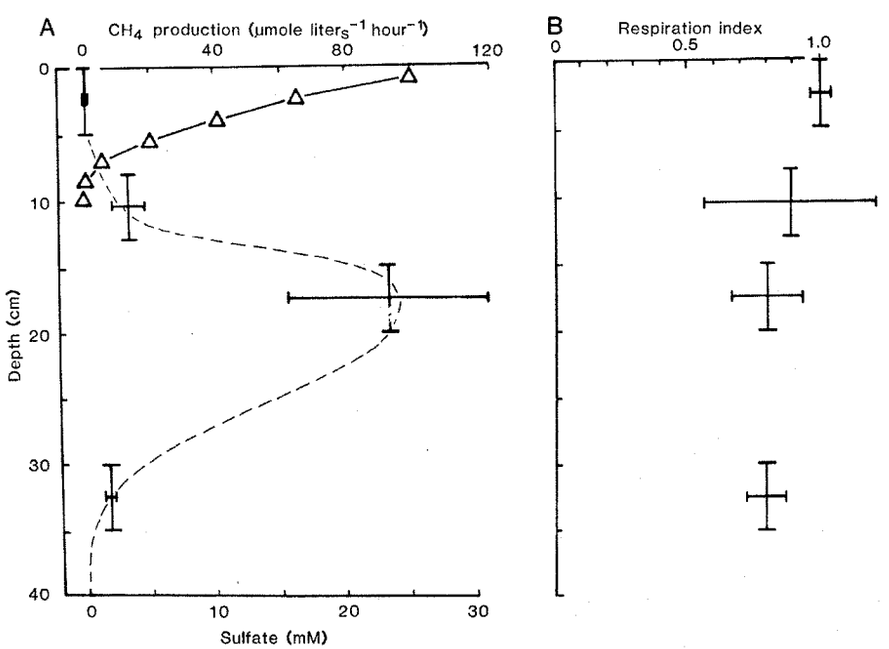

Fig. 2. (A) Depth variation of apparent $^{14}CH_4$ production from [1,2-^{14}C]acetate (dashed line); dissolved sulfate concentration (△). (B) [1,2-^{14}C]acetate respiration index. Data are from 13 to 15 July 1980 (26.5°C). Vertical bars indicate the depth interval from which samples were taken. Horizontal bars indicate the standard deviation (1 standard deviation) of triplicate acetate utilization experiments

For seasonal studies of acetate cycling in Cape Lookout Bight sediments, samples were taken over a 5-cm depth interval immediately below the 1 m*M* sulfate isopleth, that is, at the top of the zone of CH_4 production (*16*). The apparent production of both CH_4 and CO_2 from acetate showed the same seasonal pattern (Fig. 1B) as did the CH_4 flux out of the sediment (Fig. 1A). The production of CO_2 from acetate was approximately five times the production of CH_4. The earlier maxima for the CH_4 fluxes as compared to the maxima for the apparent production rates is most likely due to the earlier occurrence of peak summer air temperature in 1977 and 1978 (~ 15 July) as compared with 1979 (~ 15 August).

The depth dependency of CH_4 production from acetate measured on 13 July 1980 is illustrated in Fig. 2A. Maximum production occurred between 10 and 35 cm, where dissolved sulfate was undetectable (< 0.2 m*M*) by gravimetric analysis as $BaSO_4$. Production of CH_4 in the overlying sulfate-reducing zone between 0 and 5 cm (13 to 25 m*M* sulfate; Fig. 2) was undetectable (< 0.3 μmole liter$_s^{-1}$ hour^{-1}).

The respiration index (RI = $^{14}CO_2$ production/$^{14}CO_2$ + $^{14}CH_4$ production) from [1,2-^{14}C]sodium acetate is shown in Fig. 2B. The RI values ranged from 1.00 ± 0.04 between 0 and 5 cm in the sulfate reduction zone to 0.79 ± 0.07 at 30 to 35 cm in the CH_4 production zone. The RI values were invariant within experimental error from 8 to 35 cm, implying that the proportion of the methyl group of acetate oxidized to CO_2 did not vary significantly with depth within the CH_4 production zone at our study site.

Preliminary calculations (*17*) indicate that more than 50 percent of the observed summertime CH_4 flux (Fig. 1A) can be accounted for by CH_4 production from acetate in the upper 5 cm of CH_4-producing sediments underlying the sulfate reduction zone (Fig. 1B). Further evidence for high rates of methanogenesis down to at least 30 cm at our site is provided by recent studies of the stripping of the dissolved radioactive gas

^{222}Rn from interstitial waters by CH_4 bubbles (*18*). Our data suggest that a significant portion of the CH_4 released from Cape Lookout Bight sediments during the summer is derived from the fermentation of acetate to CH_4 in the sulfate-depleted sediments in the depth range from 8 to 35 cm.

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16. Preliminary anaerobic sediment incubation experiments by one of us (F.J.S.), using Cape Lookout sediments, indicated immediate net CH_4 accumulation only in samples with initial in situ sulfate concentrations less than 1 m*M*.
17. By numerically integrating the area under the total CH_4 flux curve (Fig. 1A) and the apparent acetate turnover to CH_4 curve (Fig. 1B) over equal time intervals, one can compare the measured rate of CH_4 production in the top 5 cm of the CH_4 production zone with the measured flux out of the sediment during the given interval.
18. G. W. Kipphut and C. S. Martens, in preparation. A deficit in the concentration of the dissolved radioactive gas ^{222}Rn between 10 and 30 cm during the summer months results from in situ stripping of this gas from the sediment interstitial waters by CH_4 bubbles. This provides direct evidence for CH_4 production in, and transport from, this depth interval.
19. We thank G. Kipphut and P. Crill for help in the collection of samples. We also thank the staffs of the University of North Carolina Institute of Marine Sciences and the National Oceanic and Atmospheric Administration National Marine Fisheries Service Laboratory, Beaufort, N.C. We thank C. D. Taylor of Woods Hole Oceanographic Institution and M. B. Goldhaber of the U.S. Geological Survey, Denver, for critical remarks on an early version of this report. Research supported by NSF grants OCE78-09485 and OCE80-09245 (C.S.M.) and OCE78-09507 (F.J.S.) from the Marine Chemistry Program, Oceanography Section.
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Membrane Conductance and Action Potential of a Regenerating Axonal Tip

Abstract. *The electrical membrane properties of axotomized and regenerating giant axons from the nerve cord of the cockroach* Periplaneta americana *were studied. Immediately after axotomy there was a decrease in resting potential, input resistance, and action potential amplitude near the cut end. This decrease was followed by the disappearance of the sodium-dependent action potential; an increase in the resting membrane conductance to K^+, Na^+, and Ca^{2+}; and the appearance of a calcium-dependent action potential.*

In many neurons, axonal transection leads to changes in morphology, metabolism, and membrane properties (*1*). Changes in input resistance, resting and action potentials, afterhyperpolarization, and excitability have been demonstrated in the somata and in dendrites remote from the site of transection (*2*). At the site of transection, a large injury potential and loss of excitability have been demonstrated (*3*). Recovery takes place gradually within a few days (*3*). Several investigators have suggested that ionic fluxes through the injured region and the ensuing change in intracellular ion composition may trigger the degenerative and regenerative responses. Membrane properties at the site of transection must

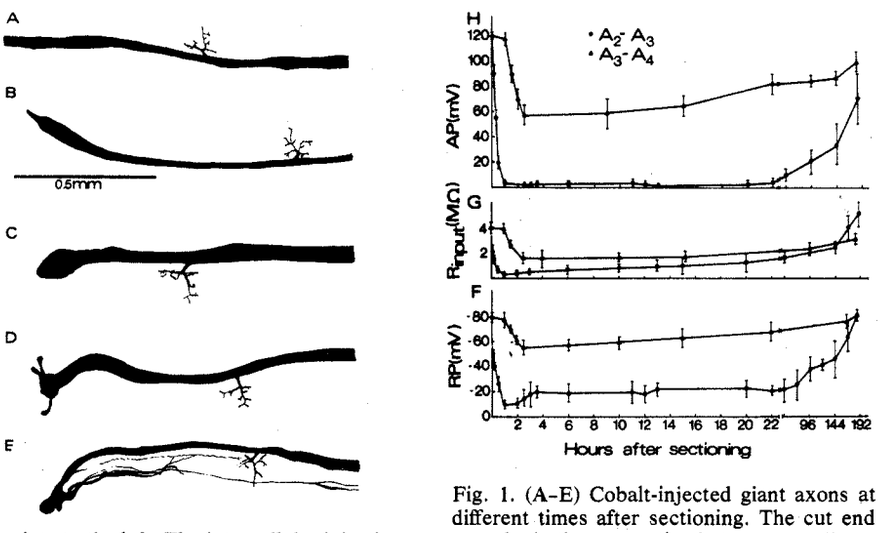

Fig. 1. (A–E) Cobalt-injected giant axons at different times after sectioning. The cut end points to the left. The intracellular injections were made, in the connective between ganglia A_3 and A_4. (A) A normal giant axon [number II in (*17*)] emits a neurite in ganglion A_3 (in the middle of the figure). (B) Twenty-four hours after sectioning, the cut end was sealed. (C) Forty-eight hours after sectioning, the cut end showed a swelling. (D) Seven days after sectioning, sprouts emerged from the bulging end. (E) Twenty-six days after sectioning, the sprouts elongated in a retrograde direction. Some variability in the length, shape, and number of sprouts has been observed in different preparations. Despite this, the figure is representative of the sequence of growth. (F–H) Membrane properties of the giant axons at different times after axotomy and at different distances from the cut end of the axon. Values of (F) resting potential, (G) input resistance, and (H) action potential amplitude rapidly fell during the first hour and then gradually recovered to normal by 8 to 10 days after sectioning. Intracellular recordings were made 0.2 to 0.3 mm caudal to the cut end, anterior to ganglion A_3 (closed circles), and more distant from it, close to ganglion A_4 (triangles), 5 ± 0.5 mm from the cut end. Each value is a mean ± standard deviation of results taken from 5 to 12 preparations. Input resistance was measured at membrane potentials more negative than −90 mV (*7*).

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