Stimulation of Heterotrophic Microplankton Production by Resuspended Marine Sediments

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Resuspended material experimentally derived from natural marine sediments and added to dark microcosms containing natural seawater stimulated the suspended microheterotrophs (bacteria and protozoa) to attain 2.6 times the biovolume of controls after 32 hours. Free bacteria benefited most from the stimulus, both numerically and volumetrically. Attached suspended bacteria also increased in number during the first 64 hours of the experiment; particles remaining in suspension became more densely packed with bacteria. This increased microbial production may be an important source of high-quality biomass for consumers in the nearshore zone, depending on the frequency, duration, and intensity of resuspension events in a given region.

RESUSPENDED MARINE SEDIMENTS are an intermittent source of material to coastal waters. The nutritional quality of resuspended material varies widely, but it may be rich in algal biomass and other particulate organic matter (*1*). Resuspension may supply nutrients to phytoplankton (*2, 3*), and may enhance benthic suspension-feeder production (*4*). To determine if resuspended material provides a source of nutrition for the heterotrophic portion of the planktonic food web, the microbial response to this material was examined. Microbes are likely to react quickly to this material because of their rapid rates of growth and physiological diversity, thereby controlling the quality and quantity available to other tropic levels (*5*). Microbially mediated transformations of dissolved substances and particulate detritus into living biomass provide a potential link between these substances and macroconsumers (*6*). Although there is some evidence that detritus and dissolved organic matter may be directly used by macroconsumers (*7*), microbial "conditioning" probably makes these materials more available to macroconsumers (*8*). Since marine sediments typically contain 1000 times as many bacteria per unit volume as the water column (*9*), resuspension should immediately supplement bacterioplankton standing stocks. Furthermore, the release of benthic nutrients may stimulate microheterotrophic production.

To assess the microbial response to resuspended sediments, microcosms containing four experimental treatments were used: natural unmodified seawater, seawater with added resuspended sediments, and killed versions of both of these treatments (*10*). The experimental design attempted to control for both sinking and containment artifacts. An annular flume (*11*) was used to suspend surficial sediments. A box core of sediment (*12*) was inserted through a false bottom in the flume channel into a core-holding chamber. The flume channel was filled with unmodified seawater, and a bottom shear velocity (u_*) of about 1.8 cm per second (*13*) maintained for 30 minutes to resuspend sedimentary material. The resulting suspension was noticeably turbid (*14*), and sediment erosion was less than 1 mm. Storm-related sediment mixing to 25 cm has been reported in the study area (*3*).

Microcosms were incubated at 12°C in the dark, with no agitation, because settlement was an issue in this experiment. Subsamples were fixed and refrigerated for later microbial and particle analyses. Acridine orange direct counts were used to estimate bacterial abundance and biovolume, protozoan biovolume, and seston particle size and abundance (*15*). Water in some microcosms was filtered; retained material was then used for determination of total seston by weight, and particulate carbon and nitrogen (*16*).

The concentration of total microbial biovolume (bacteria plus protozoa, Fig. 1A) increased in live microcosms with and without added resuspended material, as compared with killed controls. These treatments were treated identically except for the addition of resuspended material; the statistically significant difference between them (*17*) can thus be attributed to a stimulus to the microbial community provided by resuspended material. Maximum stimulus occurred at 32 hours, where microbial volume was 2.6 times the volume in the live treatment without sediment (3.8 times the initial volume in the sediment suspension). These results agree with those of previous experiments (*18*). It is not known whether the stimulated microbes were benthic or pelagic in origin, but treatments with added resuspended material did not initially contain more bacteria or protozoans than those without added resuspended material (*19*). Bacteria benefited most from the resuspended nutrients (Fig. 1B), as indicated by the proportion of the total volume made up of bacteria. The net bacterial production rate, based on increase in bacterial volume, was maximum early in the experiment, followed by a net loss after 32 hours. In contrast, protozoa maintained their elevated biovolume at least until 128 hours, and equaled or exceeded bacterial biovolume after approximately 100 hours. Protozoa may have derived this prolonged benefit from grazing the larger (Fig. 1C) and more abundant bacteria, rather than from uptake of resuspended nutrients directly.

Microbial volume significantly declined with time in the killed sediment suspension, indicating settlement. The sinking effect was statistically undetectable until 256 hours (*20*), indicating the relative slowness of this process. The large difference between microbial production rates and sinking rates indicates that losses of microbial production due to sinking may be offset by even modest production.

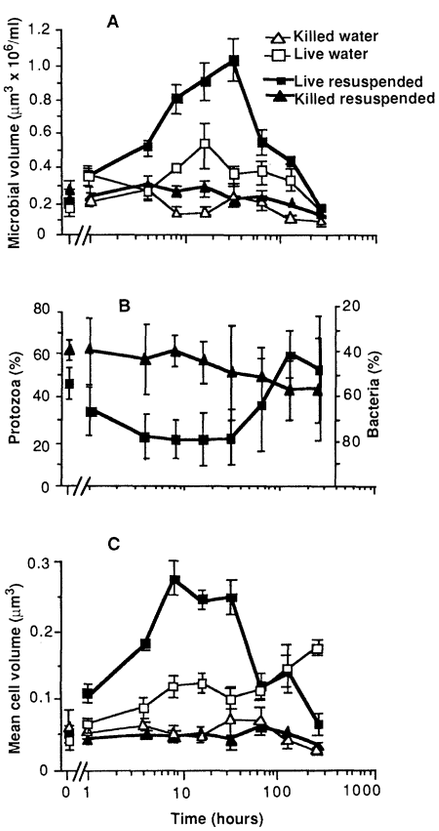

Fig. 1. (**A**) Total microbial cell volume (bacteria plus protozoa) per milliliter in the four experimental treatments. The time axis is disjunct to accommodate 0 hours on a logarithmic time scale. (**B**) The percentage of the total microbial volume made up of bacteria and protozoa in the live and poisoned sediment suspensions. (**C**) Average bacterial cell volume in the four experimental treatments. Plotted values in all cases are means (n = 6; 3 microcosms per treatment, 2 counts per microcosm) ±1 SE of the mean.

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Attached bacterial abundance increased (Fig. 2A) in the live sediment suspension, despite the sinking of particles (Fig. 2B) (*21*). Newly resuspended sediment particles were relatively devoid of bacteria, being colonized later, as evidenced by the aerial density of bacteria on particles (Fig. 2C). The bacteria:particle area ratio of live sediment suspensions increased to levels comparable with the live water treatments, and then declined, possibly due to protozoan grazing. Thus, resuspended sediment particles became more nutritious (in terms of bacteria per unit surface area or weight) with time, until 64 hours.

The maximum percentage of the bacteria which were attached was 13.6% at 8 hours in live suspensions. Although this is a conservative estimate, since only half the surface of a particle is visible during counting, even twice this estimate would not explain the increase in microbial volume in the live sediment suspension. Therefore, free bacteria and protozoans, rather than attached bacteria were the main beneficiaries of the resuspension stimulus, and particles themselves or particle-associated substances were probably not the principal stimulant (*22*).

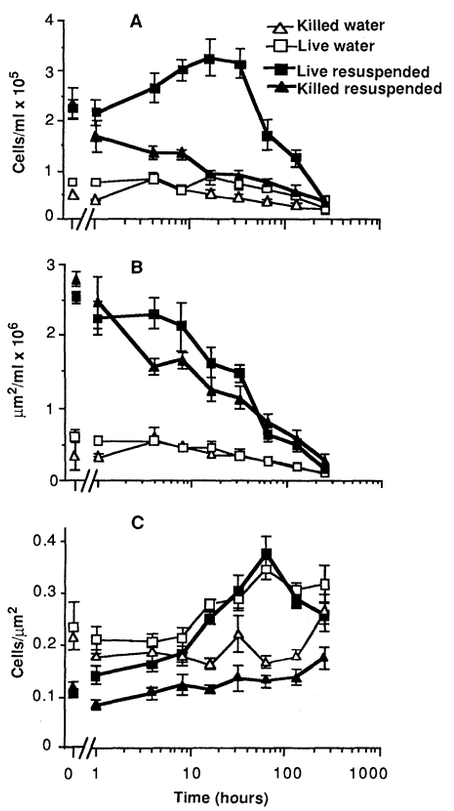

Fig. 2. (**A**) Numbers of attached bacteria per milliliter in the four experimental treatments. The time axis is disjunct to accommodate 0 hours on a logarithmic time scale. (**B**) Total cross-sectional area of particles per milliliter, as determined by microscopic counts and measurements. (**C**) Bacterial cell density on particles (areal density). Plotted values in all cases are means (n = 6; 3 microcosms per treatment, 2 counts per microcosm) ±1 SE of the mean.

Another explanation for the increased microbial production is that dissolved substances were released from the sediments into the water column, where they would presumably benefit both free and attached microbes. Dissolved nutrient release from sediments is well documented (*2, 3*), and is usually implicated as a stimulant to phytoplankton production. Results reported here suggest that resuspended nutrients may take a heterotrophic route through the planktonic food web.

The nutritive value of the resuspension stimulus to higher order consumers may be related to the ratio of biomass to total seston (*23*). At 32 hours, biovolume concentration in the sediment suspensions was maximal, consisting mostly of protozoans and relatively large free bacteria, while total seston concentration was about 30% of initial, yielding 12 times the initial biovolume: seston ratio. A decline in the carbon:nitrogen ratio of the seston, often used as an indicator of nutritional quality, was significant just 8 hours into the experiment. At that point, microbial carbon and nitrogen made up 36 and 55% of the total particulate carbon and nitrogen, respectively (initial microbial contributions were 6.5 and 12.5%, respectively) (*24*).

Pathways of energy flow in the nearshore foodweb may vary considerably, depending on whether the dominant pathways are autotrophic or heterotrophic (*5*). The role of resuspension in a given region will depend on sedimentary characteristics as well as the frequency, duration, and intensity of resuspension events. However, present results indicate that the increased microheterotrophic production attributable to resuspended sediments may provide consumers with a high-quality food source (*25*) which did not previously exist.

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10. Two-liter polycarbonate bottles served as incubation vessels (microcosms). Killed controls contained 5% formalin (1.85% formaldehyde, final concentration). Seawater was the same as that used to fill the flume.
11. Patterned after one in the laboratory of A. Nowell and P. Jumars (personal communication); M. K. Fukuda and W. Lick [*J. Geophys. Res.* **85**(C5), 2813 (1980)] discuss operating principles.
12. Moderately sorted medium sand; 1% organic content; redox potential discontinuity at 2 to 5 cm; observed macrofauna: tube-building amphipods and polychaete worms, cumaceans; collected 14 February 1986 off Savannah, GA, 20-m water depth, 31°55.9′N, 80°48.5′W. The possibility that surface sediment scouring occurred during collection, with the accompanying loss of any flocculent layer cannot be excluded. However, typically surface-dwelling fauna were present, and the core surface appeared undisturbed. Considering the generally well-mixed nature of the water in the study area, the presence of a flocculent layer is considered unlikely. If a surface flocculent layer was lost during collection, then the initial contribution of particulate material (including microbes) to the overlying water may be conservative.
13. Average u_* calculated from velocity profiles at three points across the flume channel (heated thermistor anemometer probe).
14. The suspension contained 5.5 mg of seston per liter by filtration (SE, 0.5; n = 8); water without resuspended material contained 1.93 mg of seston per liter (SE, 0.07, n = 4). Water in the study area may contain up to 200 mg of seston per liter. Initial concentrations of particulate carbon and nitrogen in sediment suspensions [358.7 µg of carbon per liter (SE 17.6, n = 8); 45.5 µg of nitrogen per liter (SE 1.7, n = 8)] were comparable to values reported for Georgia coastal waters. E. B. Haines and W. M. Dunstan [*Estuar. Coastal Mar. Sci.* **3**, 431 (1975)] reported 35 to 800 µg of carbon per liter and 2 to 76 µg of nitrogen per liter for this study area).
15. S. F. Nishino, *Appl. Environ. Microbiolol.* **52**, 603 (1986). Microbial volumes were calculated assuming cocci and spherical flagellates to be spherical, rods and ellipsoid protozoans to be ellipsoid. Average bacterial cell volumes were computed as weighted averages, based on the fraction of the total in different size classes.
16. Water was filtered through preashed and preweighed Reeve-Angel 984H glass fiber filters and the filters were dessicated and reweighed, yielding total seston by difference. Filters were then leached with 0.58*N* HCl [M. D. Berner, R. A. Berner, *J. Sediment. Petrol.* **53**, 660 (1983)] for 5 minutes to dissolve carbonate material, rinsed three times with distilled water, dessicated, and subjected to CHN analysis (Perkin-Elmer 240C elemental analyser).
17. Two-way ANOVA; time and time by treatment interaction effects were also statistically significant.
18. Three previous experiments (S. Wainright, unpublished data) in which sediment types collected in summer rather than winter were used and were subjected to lower and higher shear stresses, yielded similar patterns—that is, rapid bacterial growth followed by a slower decline.
19. If the sediment contained 10^9 bacteria per gram [typical for the study area (S. Wainright, unpublished data)], then based on seston concentrations (*14*) only about 10^4 bacteria per milliliter were added initially. Bacterial and protozoan counts were 1×10^6 to 3.2×10^6 per milliliter and 0.8×10^3 to 3.2×10^3 per milliliter, respectively in the experimental systems. Typical field densities in the study area are 0.5×10^6 to 5×10^6 per milliliter and 0.01×10^3 to 2.5×10^3 per milliliter, respectively [L. R. Pomeroy, in *Oceanography of the Southeastern*

U.S. Continental Shelf, L. P. Atkinson, D. W. Menzel, K. A. Bush, Eds. (American Geophysical Union, Washington, DC, 1985), pp. 118–129; L. R. Pomeroy and S. C. Wainright, unpublished data].
20. Linear regression of microbial volume on log(time).
21. While sediment suspensions contained more seston than treatments without resuspended material until the end of the experiment, average particle diameter (data not shown) was identical in these treatments after 4 hours (pooled mean, 4.95 μm). Therefore, sediment suspensions contained more particles, not larger particles.
22. Intense grazing of attached bacteria by protozoans could also have produced the relatively low net production rate of attached bacteria.
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24. Conversion factors for bacterial C and N (106 fg of carbon per cubic micrometer and 25 fg of nitrogen per cubic micrometer), taken from T. Nagata [*Appl. Environ. Microbiol.* 52, 28 (1986)], were applied to protozoans as well. Concentrations of particulate carbon and nitrogen declined in a pattern similar to particle area (Fig. 2B).
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26. L. R. Pomeroy, C. A. Butman, W. J. Wiebe and two anonymous reviewers greatly improved the manuscript; B. A. Biddanda assisted in the laboratory, and D. J. Douglas reviewed the experimental design. Supported by DOE grant DE-FG09-86ER60451 to L. R. Pomeroy.

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Quantal Release of Transmitter Is Not Associated with Channel Opening on the Neuronal Membrane

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The traditional view that quantal release of neurotransmitter results from the fusion of transmitter-containing vesicles with the neuronal membrane has been recently challenged. Although various alternative mechanisms have been proposed, a common element among them is the release of cytoplasmic transmitter, which, in one view, could occur through large conductance channels on the presynaptic membrane. Six nerve-muscle cell pairs were examined with a whole-cell patch clamp for the presence of such channels that are associated with the production of miniature end-plate potentials. Examination of the neuronal membrane current during the occurrence of 822 miniature end-plate potentials produced no evidence of large channels. Thus it is unlikely that quantal release is mediated by such channels in the neuromuscular junction.

FOR A NUMBER OF YEARS, QUANTAL release of neurotransmitter has been understood to occur through fusion of small (50 nm in diameter) transmitter-containing vesicles with the presynaptic membrane. Recent challenges to this explanation of quantal release consider the direct translocation of cytoplasmic transmitter to the extracellular region (*1*). One possible mechanism for such a translocation is via channels within the presynaptic membrane that open to "gate" the cytoplasmic transmitter. We have looked for the presence of such channels in the nerve membrane of nerve-muscle cell pairs produced from *Xenopus* nerve and muscle cells in culture.

The use of cultured cells of *Xenopus* has many advantages for this study. (i) Isolated nerve and muscle cells can be manipulated into contact with each other, and quantal release of transmitter can be detected within minutes of contact, from both the neuronal soma and the neurites (*2*). (ii) The approximately spherical soma may be placed under a whole-cell patch clamp, and the resting membrane impedance is high (>200 megohms) so that background current noise is low (<2.5 pA at 1 kHz). (iii) The transmitter is acetylcholine (ACh), which has a high enough molecular weight (AChCl, 181.7) that it will not pass through the large conductance (60 pS) ACh-receptor channel (*3*). Therefore, any channel that conducts ACh must be at least this large, which would make it detectable by our technique.

Xenopus cell cultures were prepared as described (*2*). Before the start of the recording period, the culture medium was replaced by external solution (125 m*M* NaCl, 2 m*M* KCl, 10 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 20 m*M* Hepes, at *p*H 7.8). An isolated spherical muscle cell was impaled with a microelectrode (resting potential, −60 to −100 mV) and moved into contact with the soma of a neuron (Fig. 1). If the neuron was cholinergic, miniature end-plate potentials (MEPPs) were recorded from the muscle within 1 to 5 minutes. After this, the soma was placed under a voltage clamp with a patch pipette and a patch-clamp amplifier (List EPC-7). The internal solution was 92 m*M* KCl, 40 m*M* KOH, 1 m*M* $CaCl_2$, 11 m*M* EGTA, 1 m*M* $MgCl_2$, 20 m*M* Hepes, at *p*H 7.8. The soma membrane voltage was held at various potentials between −50 and +34 mV. Simultaneous recordings of the neuronal somata membrane current and muscle membrane potential were stored on magnetic tape (RACAL Store 4 FM) for later playback and analysis.

Recordings of 822 muscle MEPPs were made simultaneously with associated neuronal membrane currents resulting from six nerve-muscle contacts. Large neuronal membrane channels (currents >2.5 pA) were not seen immediately before, during, or after the peak of the MEPPs. This lack of neuronal channel opening was a consistent finding despite the presence of very large depolarizations of the muscle membrane (30 mV, which indicates a large amount of ACh was released). Records from three different nerve-muscle contacts are shown in Fig. 2A. The bottom set exhibits the highest resolution of neuronal currents (<2.5 pA), and no neuronal single channels were detected. Records of neuronal single-channel currents that occurred at random, not correlated with the MEPPs, illustrate the high current resolution of the preparation (Fig. 2B). It is unlikely that we have failed to detect channel openings as a result of the transmitter having been released far from the soma along newly sprouted neurites for two reasons. (i) At the end of an experiment, when the muscle was pulled away from the neuron, no such neurites were observed. (ii) Neurite growth is slow [0.25 μm/min (*4*)] compared to the time course of our experiments (less than 5 minutes of nerve-muscle contact) so that even if new sprouting occurred instantly after contact, the length would be less than 1.25 μm at the end of the experiment. Because the reversal potential of such putative transmitter channels is unknown, the holding potential of the neuron was

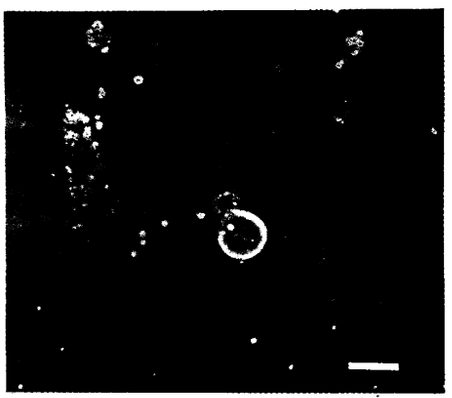

Fig. 1. Photomicrograph of manipulated nerve-muscle contacts in *Xenopus* cell culture. A spherical muscle cell (m) is impaled with a microelectrode (e) and manipulated into contact with a neuronal soma (n). After the appearance of MEPPs, the soma was placed under a whole-cell clamp by a patch electrode (p). Scale bar, 30 μm.

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