Limnol. Oceanogr., 50(1), 2005, 128–133

Wave-induced H_2S flux sustains a chemoautotrophic symbiosis

Kay Vopel[1]
National Institute of Water & Atmospheric Research, Hamilton, New Zealand

David Thistle
Department of Oceanography, Florida State University, Tallahassee, Florida 32306-4320

Jörg Ott and Monika Bright
Institute of Ecology and Conservation Biology, University of Vienna, 1090 Vienna, Austria

Hans Røy
Max Planck Institute for Marine Microbiology, 28359 Bremen, Germany

Abstract

Symbioses involving sulfur-oxidizing bacteria and invertebrate hosts require a source of reduced sulfur, a source of O_2, and transport mechanisms that ensure them a supply of both. We investigated these mechanisms using the symbiosis between the sessile ciliate *Zoothamnium niveum* (Hemprich and Ehrenberg 1831) and bacteria living on its surface. The stalked colonies of *Z. niveum* grow on peat walls around the openings of centimeter-scale conduits created when mangrove rootlets decompose. Using in situ, time-series measurements with fast-responding amperometric microelectrodes, we found that the conduits were charged with H_2S by diffusion from the decaying rootlets during periods of low boundary-layer flow speed. During these times, the feeding current of the zooids transported oxygenated seawater from outside the peat wall toward the ectobiotic bacteria. During periods of high flow speed, H_2S-rich seawater from the conduits was drawn along the colonies and over the bacteria. We conclude that this symbiosis exploits a combination of two transport mechanisms: (1) venting of H_2S-rich seawater due to pulsating boundary-layer current over ciliate groups and (2) the continuous and rapid feeding current generated by the host's cilia. This discovery raises the possibility that other systems in which pockets of decay are exposed to pulsating flow could support similar symbioses.

Symbioses involving sulfur-oxidizing bacteria and various invertebrates or protists are found in many habitats, ranging from the ''hot vents'' along the axes of midoceanic ridges to the ''cold seeps'' of deep-sea and continental slope sediments (Paull et al. 1984; Suess et al. 1985) and shallow-water habitats, such as sheltered sediments in inter- and subtidal zones (Ott 1995), organic-rich mud (Reid 1980), and mangrove peat (Ott et al. 1998). These symbiotic associations show great diversity. For example, Vestimentifera (Jones 1984), Pogonophora (Southward 1982), and the nematode *Astomonema* (Ott et al. 1982; Giere et al. 1995) have intracellular symbionts. In gastropods and the bivalves, the symbiotic bacteria are associated with gill tissue, either intracellularly in special bacteriocytes or enclosed in intercellular spaces (Windoffer and Giere 1997). In *Rimicaris* (Polz and Cavanaugh 1995), the Stilbonematinae (Ott and Novak 1989), and ciliates (Fenchel and Finlay 1989; Bauer-Nebelsick et al. 1996*a*,*b*), the bacteria are ectosymbionts; they cover parts of the external surface of the host. Such associations, in which the morphological modifications are often minor and the physiological dependences are presumably less strict, can serve as model systems to study the circumstances that set the stage for the development of the early evolutionary stages of endosymbiosis (Smith 1979).

Symbioses involving sulfur-oxidizing bacteria require a source of reduced sulfur, a source of O_2, and transport mechanisms that ensure a supply of both. Because such symbioses often exploit a spatially and temporally complex chemocline, where oxic and sulfidic conditions may change over a few millimeters and within seconds, mechanisms that let the sulfur-oxidizing bacteria overcome the diffusion limitation of nutrient supply are difficult to study and are therefore largely unknown. In this study, we used a sessile symbiosis from a shallow-water habitat, the *Zoothamnium niveum* (Hemprich and Ehrenberg 1831) ectosymbiosis, as an example to investigate these mechanisms.

The *Z. niveum* symbiosis was discovered in coves and channels of red mangrove (*Rhizophora mangle* Linnaeus) islands of the barrier reef off Dangriga, Belize (Ott et al.

[1] Corresponding author (k.vopel@niwa.co.nz).

Acknowledgments

G. Arlt assisted with previous laboratory studies and underwater measurements, G. Cocco helped with analyses of time series measurements, and A. Vopel contributed to the model. The comments of P. Jumars, L. Mullineaux, A. B. Thistle, and one anonymous reviewer improved the manuscript. D. Oliff built the underwater housing for the data logger. M. Carpenter and D. Miller provided technical service at the field station of the Caribbean Coral Reef Ecosystems program of the Smithsonian National Museum of Natural History (Washington, DC).

This study was supported by the Austrian Science Foundation, the CCRE program of the Smithsonian National Museum of Natural History, and Unisense A/S (Aarhus, Denmark).

Contribution 657 of the CCRE program.

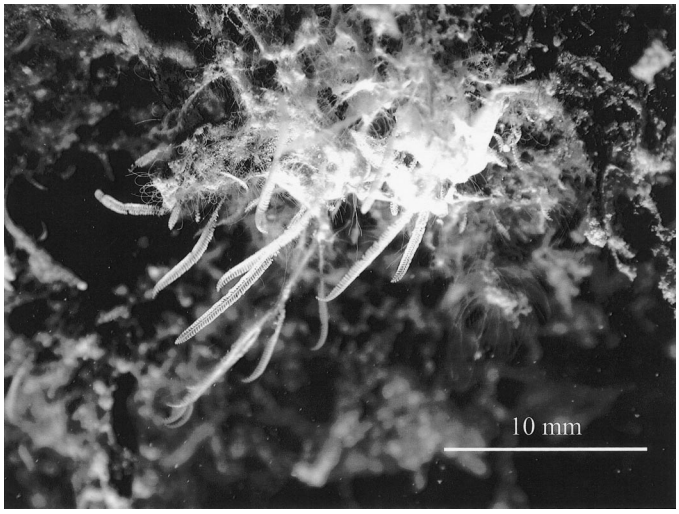

Fig. 1. A H_2S microvent at the surface of a vertically overhanging wall of red mangrove (*R. mangle* Linnaeus) peat at the mangrove island Twin Cays, Tobacco Reef section, Belize (16°48′N, 88°05′W). The tissue of a mangrove rootlet has rotted and partially fallen out, creating a conduit whose opening is surrounded by mats of unicellular and filamentous sulfur bacteria and the feather-shaped colonies of the peritrich ciliate species *Z. niveum*. The surrounding, intact surface of the peat is overgrown with diatoms and cyanobacteria.

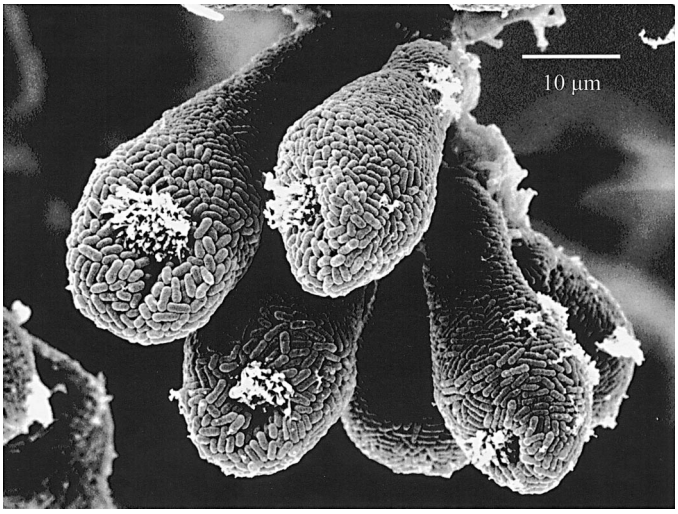

Fig. 2. *Z. niveum.* Scanning electron microscope picture of six contracted microzooids attached to a branch of a ciliate colony. The microzooids are overgrown by coccoid sulfur bacteria.

1998). The stalked colonies of *Z. niveum* grow on vertical walls of peat banks around the openings of centimeter-scale conduits (Fig. 1). The conduits form when mangrove rootlets die and decay, leaving only tubes of bark in the peat. The large (up to 15 mm) conspicuous colonies of *Z. niveum* occur in groups (~10 groups m^{-2}) of up to 100 colonies. The average life span of a group is ~3 weeks (Ott et al. 1998). The feather-shaped colonies are composed of polymorphic zooids (filter-feeding microzooids, dividing terminal zooids, and motile macrozooids) attached to a branched stalk. A single layer of coccoid and rod-shaped, sulfur-oxidizing bacteria covers all but the most basal parts of the colonies (Bauer-Nebelsick et al. 1996*a,b;* Fig. 2). Their white appearance stems from intracellular sulfur globules associated with the oxidation of sulfur compounds (Bauer-Nebelsick et al. 1996*b*).

Because of their white appearance, the colonies stand out from the surrounding brownish peat, which may also be covered by thin films of diatoms and cyanobacteria (Fig. 1). Ciliary movement of their zooids transports seawater, which is filtered for suspended particles (clearance rate ≈ 0.45 mm^3 s^{-1}). The joint action of all zooids of a colony results in unidirectional flow through the colony, perpendicular to the longitudinal axis of the stalk, from the convex to the concave side of the feather-like colony (Vopel et al. 2002). The inflowing current comes from a broad area; the exiting jet leaves the colony as a narrow band along its center line at 0.3–0.6 mm s^{-1} (H.R. and K.V. unpubl. data). Filter feeding of the zooids is frequently interrupted (1.7 min^{-1}) by rapid stalk contractions, during each of which the mass of zooids bunches together and whips downward at 520 mm s^{-1} (Reynolds number [Re] ≈ 10^2). After a contraction, the colony extends slowly (Re ≈ 10^{-1}). High shear stress during stalk contraction, cell shrinkage, and bunching of the zooids presumably detach ectobiotic bacteria that, once suspended, can be transported with the feeding current toward the cytostome (Vopel et al. 2002).

Our previous laboratory studies failed to discover how reduced sulfur released by the decaying rootlets is transported to the ectosymbiotic bacteria of *Z. niveum*. In the present study, we used fast-responding, amperometric microelectrodes in situ to investigate the mechanisms that generate the physicochemical microenvironment for rapid symbiotic growth.

Study site and methods

Microelectrode measurements of $[O_2]$ and $[H_2S]$ were carried out by SCUBA divers at overhanging vertical walls of red mangrove peat at the north entrance (Batfish Point) of the main channel of the mangrove island Twin Cays, Belize, in April 2002. Twin Cays is situated inside the Tobacco Reef section of the Belize barrier reef (16°48′N, 88°05′W). Detailed information about the site and a description of the area are available in Rützler and Macintyre (1982) and Ott et al. (1998). The average temperature of the seawater recorded from February 2001 through January 2002 was 28.1 ± 1.7°C (maximum = 32.1°C, minimum = 22.3°C). The water depth was <2 m, the average salinity was 35.4 ± 1.4 (n = 25), and the pH was 8.2 ± 0.1 (n = 18). The maximum flow speed measured by tracking the movement of particles in the water 5 cm away from the peat wall over a horizontal distance of 10 cm was 2.4 cm s^{-1}. Flow along the peat wall oscillated as a result of infragravity waves in the sea surrounding the mangrove islands. That is, seawater alternately moved into and out of coves or channels at a period of 50–200 s. These oscillations were superimposed on higher-frequency oscillations resulting from a variety of mechanisms related to specific hydrodynamic conditions and geometry of the cove or channel.

A H_2S microelectrode (Jeroschewski et al. 1996) and a Clark-type O_2 microelectrode with internal reference and guard cathode (Revsbech 1989) were mounted on a manually operated micromanipulator (Märzhäuser Wetzlar) such that their movement (250- or 500-μm increments) was normal to the vertical peat surface. The amperometric microsensor for dissolved H_2S is based on the same principle as the Clarke-type O_2 microsensor. The sensor is equipped with a glass-coated platinum working electrode and a platinum guard electrode. Both the working electrode and the guard electrode were polarized at +85 mV with respect to a counter electrode. The sensor was sealed with a thin silicone membrane and filled with a buffered electrolyte solution that contained ferricyanide ($K_3[Fe(CN)_6]$) as redox mediator. Driven by the external partial pressure, H_2S from the environment penetrates the silicone membrane and is oxidized by ferricyanide, which results in the formation of elemental sulfur and ferrocyanide ($K_4[Fe(CN)_6]$). The latter is electrochemically reoxidized at the platinum working electrode, creating an electrical current that is directly proportional to the $[H_2S]$ at the sensor tip. Note that this sensor detects the partial pressure of H_2S gas, which is only one component of the total equilibrium system. Both microelectrodes had outside tip diameters of 40–60 μm; the 90% response time was <1 s, the stirring sensitivity was $<2\%$, and the detection limit was 0.3×10^{-6} mol L^{-1}. The micromanipulator was attached to an aluminum post that had been driven into the mangrove peat. Sensor signals, measured and digitized by an underwater picoammeter (PA 3000U; Unisense), were logged at a frequency of 3.3 Hz (data logger OM-3000 and measurement analysis software OM-3000 MAS version 4.01; Omega Engineering).

At four randomly chosen locations, we measured one $[O_2]$ and one $[H_2S]$ profile across the intact surface of the peat near the opening of a rootlet conduit and repeated the measurements through the interface between the seawater and the opening of that conduit. We made four simultaneous time-series measurements of $[O_2]$ and $[H_2S]$ at one conduit opening on each of two consecutive days in April 2002. For these measurements, the two microelectrodes were positioned at an angle of 80° to the surface of the peat wall, so that their tips were both at the same distance from the opening of the rootlet conduit. The distance between the electrode tips was <1 mm. We measured at 1 mm inside the conduit, at the opening of the conduit, and 1 and 2 mm outside the opening. The measurements at each position required 10 min. During the time series on April 7, we observed that changes in the signals of the two microelectrodes correlated with changes in the displacement of the colonies from the vertical by the flow. During the time series measurements the following day, we estimated relative flow speed as follows. Using the method of La Fond (1967), we imagined a colony as hanging at the center of a clock face. The colony pointed to 6 o'clock when the water was still. As flow speed increased, the colony was deflected toward 3 or 9 o'clock. At regular intervals ($\sim$4 times min^{-1}), the diver wrote on a slate the clock position of a colony and the record number from the data logger. We used the recording number to match the $[O_2]$ and $[H_2S]$ values to estimated flow speed. Because our visual observations of deflection angles may misrepresent both the upper and lower flow speeds because of the inertia of the colony (low speed) and asymptotic relationship between flow and deflection (high speed), we used the data qualitatively.

The O_2 microelectrode was calibrated in fully oxygenated seawater and in seawater that had been deoxygenated with sodium sulfite. Microelectrodes were calibrated before each time series measurement at in situ temperature. The H_2S microelectrode was calibrated in a flow-through system that consisted of a peristaltic pump (Ismatec), a coulometric sulfide generator (AMT), and a flow-through chamber. The coulometric sulfide generator was equipped with a mixing cell for preparations of standard solutions in the micromolar range. H_2S was generated by cathodic reduction at an electrode made of HgS, S, and C in an oxygen-free, acid solution (0.01 mol L^{-1} H_2SO_4). This solution was pumped continuously through the coulometric cell and the flow-through chamber that enclosed the tip of the H_2S microelectrode. The $[H_2S]$ in the carrier solution was precisely adjustable through the flow rate (0.25–5 ml min^{-1}) and/or the current at the coulometric cell (0–4 mA). This technique allowed fast and accurate calibrations in the field and limited the loss of H_2S due to wall adsorption or exchange between the liquid and gas phases.

Spectral analyses of the time series measurements were done with the data analysis software system STATISTICA (StatSoft). The time series were transformed by simple exponential smoothing (no trend, no season, $\alpha = 0.1$), and the periodogram values were smoothed using a weighted moving average (Tukey window). Underwater photographs of groups of colonies of *Z. niveum* were made with a Nikon Coolpix 4500 digital camera. For scanning electron microscopy, specimens were fixed in 2.0% osmium tetroxide in natural seawater for 15 min at $\sim$30°C, then dehydrated in a graded series of ethanol-distilled water solutions, critical-point dried in CO_2 in a Polaron E3000 Series II critical-point drying apparatus, sputter-coated with gold/palladium with a Balzer SCD 030 Sputter-Coater, and viewed with a Cambridge Stereoscan 250 Mk2.

Results and discussion

Our in situ measurements showed that, under incident sunlight, the peat surface surrounding the openings of the conduits was supersaturated with O_2 (up to 724×10^{-6} mol L^{-1}) because of photosynthesis by mat-forming diatoms and cyanobacteria (Fig. 3A). Dissolved O_2 diffused through an $\sim$0.75-mm-thick diffusive boundary into the surrounding seawater. No H_2S was detected in the peat to a depth of 4 mm (Fig. 3A). In contrast, the seawater in the rootlet conduits was deoxygenated and contained up to 739×10^{-6} mol H_2S L^{-1} (Fig. 3B). The conduits were charged with H_2S by diffusion from the decomposing tissue and bark of the rootlets, where $[H_2S]$ was $>10^{-3}$ mol L^{-1}. At the peat-seawater boundary at the openings of the conduits, a maximum $[H_2S]$ of 639×10^{-6} mol L^{-1} was detected. $[H_2S]$ and $[O_2]$ outside the conduits and at the opening of the conduits varied rapidly. Simultaneous time series measurements of $[O_2]$ and $[H_2S]$ 1 mm outside of the conduit opening (Fig. 4A) and at

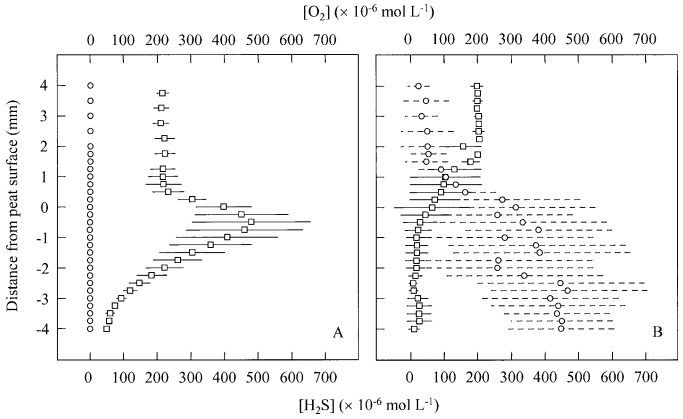

Fig. 3. Average $[O_2]$ (rectangles) and $[H_2S]$ (circles) profiles measured in situ normal to (A) the intact surface of the mangrove peat or (B) the opening of a rootlet conduit. The peat-water interface is located at 0 mm. Symbols indicate the mean ($n = 4$), and horizontal lines indicate standard deviations.

the conduit opening (Fig. 4B) showed alternating periods when $[H_2S]$ was high and $[O_2]$ was low and when $[H_2S]$ was low and $[O_2]$ was high. Measurements 1 mm inside the conduits revealed high $[H_2S]$ and low $[O_2]$; measurements 2 mm outside revealed high $[O_2]$ and low $[H_2S]$. Spectral analyses of the relatively short time series of $[H_2S]$ and $[O_2]$ revealed

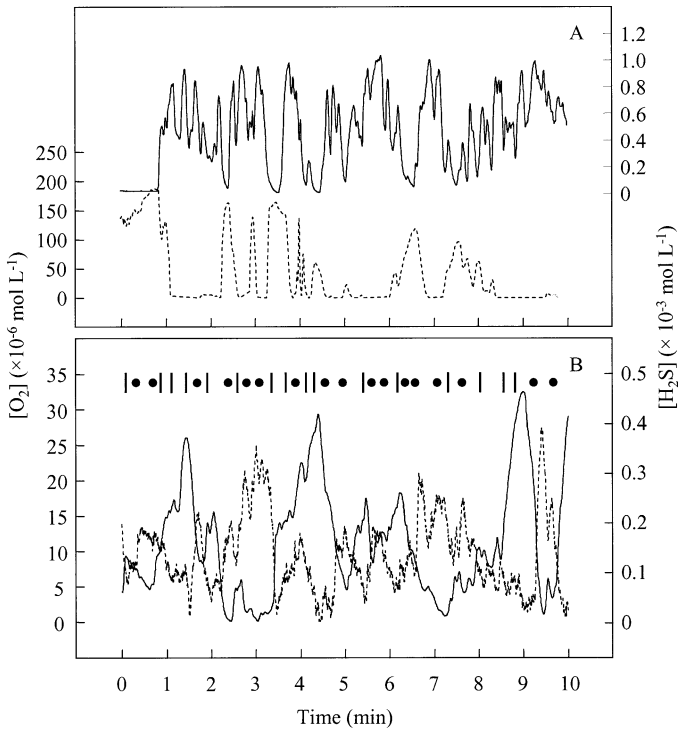

Fig. 4. Simultaneous time series of $[O_2]$ and $[H_2S]$ measured at the mouth of two mangrove-rootlet conduits on 2 consecutive d in April 2002. The two microelectrode tips were positioned at an angle of 80° to the surface of the peat wall at (A) 1 mm distance from the opening of the conduit and (B) the conduit opening. Dashed lines represent $[O_2]$. Filled circles indicate periods of relatively low flow speed, and vertical lines indicate periods of high flow speed.

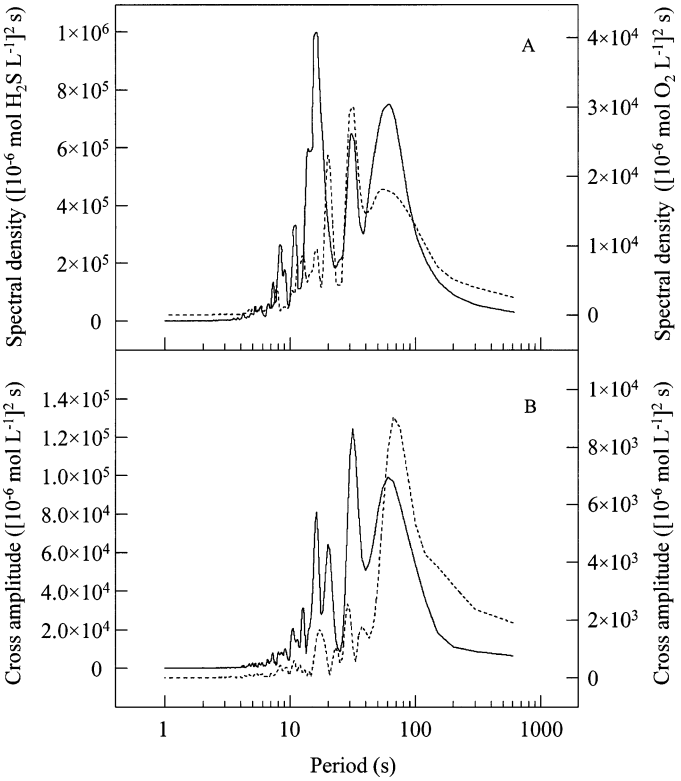

Fig. 5. (A) Spectral analyses of time series of $[O_2]$ (dashed line) and $[H_2S]$ presented in Fig. 4A. (B) Cross-spectrum of the two series shown in Fig. 4A (solid line, left *y*-axis) and 4B (dashed line, right *y*-axis).

peaks at periods of 10–100 s (*see Fig. 5 for examples*). The period with the greatest spectral density of the time series of $[H_2S]$ shown in Fig. 4A was 16 s (Fig. 5A). Smaller peaks occurred at periods of 31.7 and 61.4 s. The analysis of the simultaneously measured time series of $[O_2]$ (Fig. 4A) revealed a high spectral density at a period of 31.6 s (Fig. 5A). Cross-spectrum analysis confirmed the covariance between the respective frequency components in the two series (Fig. 5B). The measurement shown in Fig. 4B revealed temporal variation in seawater $[O_2]$ and $[H_2S]$ at periods of 18, 55, and 67 s that is superimposed over variation of shorter period. The cross-amplitude of these time series peaked at a period of 67 s (Fig. 5B, dashed line).

Our visual underwater observations revealed that the signals of the H_2S electrode were highest when colonies were horizontal (i.e., during periods of maximal flow speed) and lowest when colonies were hanging vertically (i.e., during periods of minimal flow speed). Matching the recorded $[O_2]$ and $[H_2S]$ values and the displacement of the ciliate colonies from the vertical confirmed this observation: $[H_2S]$ 1–2 mm outside the conduit opening was high during periods of high flow speed and low during periods of low flow speed. In contrast, measurements 1 mm inside the conduit revealed that $[H_2S]$ decreased and $[O_2]$ increased during periods of high flow speed. We conclude that fluctuations in $[H_2S]$ and $[O_2]$ at the openings of the conduits are caused by pulsed exchange of the deoxygenated, H_2S-containing seawater in

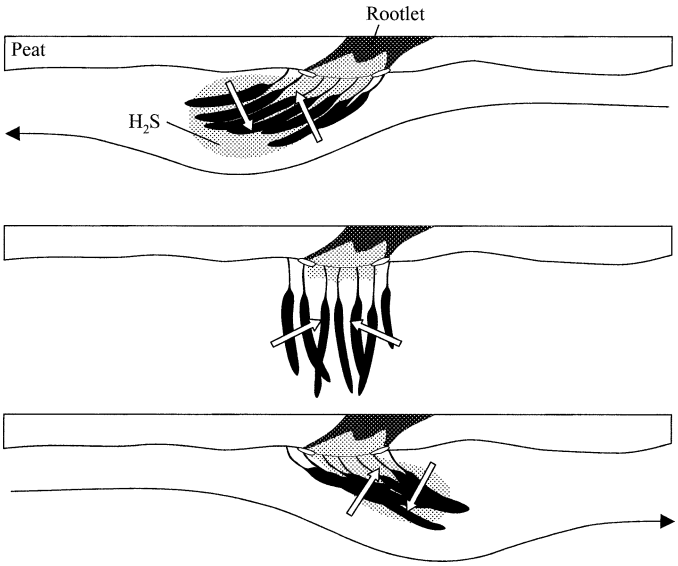

Fig. 6. Schematic drawing of cross section of peat matrix, a rootlet that had partially fallen out, and the attached group of *Z. niveum* colonies. Black arrows indicate boundary-layer flow. Arrows within the group of ciliate colonies indicate the feeding currents of the zooids.

the conduits with fully oxygenated seawater adjacent to the peat surface.

Given our results, we envision these stages in colony development. Flow across a sulfide-rich conduit that is not surrounded by ciliate colonies will draw some sulfide out of the conduit by viscous entrainment (Vogel 1996), attracting the motile swarmer stages of *Z. niveum*, which settle preferentially in the chemocline around such sources of reduced sulfur (M. Pöhn, K.V., and J.O. unpubl. data). As colonies grow larger, they interact more strongly with boundary-layer flow. By continuity, the flow will accelerate as it passes over and around the colonies. Resulting pressure differences should cause upwelling on the downstream face of the groups. As a result, the deoxygenated, H_2S-containing seawater in the conduit is transported along the ciliate colonies (Fig. 6). Flux of H_2S from a conduit should increase with the size and number of colonies around the conduit opening, because pressure differences would increase. The periods of reduced speed during flow reversals facilitate the transport of oxygenated seawater toward the outer surface of the zooids by the feeding current of the ciliates and allow recharge of the conduit void with H_2S by molecular diffusion from the tissue and the bark of the decomposing rootlet. In this model, the ectobiotic bacteria of *Z. niveum* are exposed to a rapid, cilium-generated current (the feeding current of the zooids) that alternately transports H_2S-rich and O_2-rich seawater. We suggest that these bacteria overcome the diffusion limitations of their substrate supply by a combination of two transport mechanisms: (1) a pulsed advection of H_2S-rich seawater caused by interaction of the boundary-layer flow with the ciliate groups and (2) the continuous and rapid feeding current of the host. We hypothesize that the recharge of the conduits with H_2S during periods of reduced flow speed is crucial for the maintenance of this symbiosis, so continuous flow artificially applied to ciliate groups would remove the colonies' white appearance. The fact that the flow in this particular mangrove habitat is oscillating is not necessary to our model. All that is required is quiescent periods between distinct flow periods—that is, this type of symbiosis can occur in habitats that do not have reversing flow.

Small-scale, pulsed microcirculations of oxygenated near-bottom water and sulfidic pore fluid have also been reported from the hydrothermal vent fields of Guaymas Basin, Gulf of California (Gundersen et al. 1992). In that case, the advective transport is driven by magmatic intrusion below 400-m-thick deposits. It increases the supply of substrate to communities of the sulfur-oxidizing bacterium *Beggiatoa* spp. Increased nutrient supply can also result from mass flow caused by the behavior of bacteria. For example, the sulfur-oxidizing bacterium *Thiovulum majus* forms veils on the sediment that generate the transport of oxygenated seawater through the 0.5-mm-thick water layer above the veil at rates ~40 times higher than that of molecular diffusion (Fenchel and Glud 1998). In the case we report, a combination of behaviorally and physically mediated transport mechanisms—the feeding current generated by the ciliate host and the pulsed release of sulfide due to distinct periods of flow over ciliate groups interrupted by periods quiescence—can sustain rapid growth of both the ciliate host and its ectosymbiotic sulfur-oxidizing bacteria. This discovery raises the possibility that other shallow-water systems where pockets of decay are bathed in pulsating flow could support similar symbioses.

References

BAUER-NEBELSICK, M., C. F. BARDELE, AND J. A. OTT. 1996*a*. Electron microscopic studies of *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. Eur. J. Protistol. **32:** 202–215.

———, ———, AND ———. 1996*b*. Redescription of *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg, 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. Eur. J. Protistol. **32:** 18–30.

FENCHEL, T., AND B. J. FINLAY. 1989. *Kentrophoros:* A mouthless ciliate with a symbiotic kitchen garden. Ophelia **30:** 75–93.

———, AND R. N. GLUD. 1998. Veil architecture in a sulphide-oxidizing bacterium enhances countercurrent flux. Nature **394:** 367–369.

GIERE, O., R. WINDOFFER, AND E. C. SOUTHWARD. 1995. The bacterial endosymbiosis of the gutless nematode *Astomonema southwardorum:* Ultrastructural aspects. J. Mar. Biol. Assoc. U.K. **75:** 153–164.

GUNDERSEN, J. K., B. B. JØRGENSEN, E. LARSEN, AND W. JANNASCH. 1992. Mats of giant sulphur bacteria on deep-sea sediments due to fluctuating hydrothermal flow. Nature **360:** 454–456.

JEROSCHEWSKI, P., C. STEUCKART, AND M. KÜHL. 1996. An amperometric microsensor for the determination of H_2S in aquatic environments. Anal. Chem. **68:** 4351–4357.

JONES, M. L. 1984. The giant tube worms. Oceanus **27:** 47–52.

LA FOND, E. C. 1967. Movements of benthonic organisms and bottom currents as measured from the bathyscaph Trieste, p. 295–302. *In* J. B. Hersey [ed.], Deep-sea photography. Johns Hopkins Univ. Press.

OTT, J. A. 1995. Sulfide symbioses in shallow sands, p. 143–147.

In A. Eleftheriou, A. D. Ansell, and C. H. J. Smith [eds.], Biology and ecology of shallow coastal waters. Olsen & Olsen.

———, M. BRIGHT, AND F. SCHIEMER. 1998. The ecology of a novel symbiosis between a marine peritrich ciliate and chemoautotrophic bacteria. PSZN Marine Ecology **19:** 229–243.

———, AND R. NOVAK. 1989. Living at an interface: Meiofauna at the oxygen/sulfide boundary of marine sediments, p. 415–422. *In* J. S. Ryland and P. A. Tyler [eds.], Reproduction, genetics and distribution of marine organisms. Olsen & Olsen.

———, G. RIEGER, R. RIEGER, AND F. ENDERES. 1982. New mouthless interstitial worms from the sulfide system: symbiosis with prokaryotes. PSZN Marine Ecology **3:** 313–333.

PAULL, C. K., AND OTHERS. 1984. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. Science **226:** 965–967.

POLZ, M. F., AND C. M. CAVANAUGH. 1995. Dominance of one bacterial phylotype at a midatlantic ridge hydrothermal vent site. Proc. Natl. Acad. Sci. USA **92:** 7232–7236.

REID, R. G. B. 1980. Aspects of the biology of a gutless species of *Solemya* (Bivalvia; Protobranchia). Can. J. Zool. **58:** 386–393.

REVSBECH, N. P. 1989. An oxygen microsensor with a guard cathode. Limnol. Oceanogr. **34:** 474–478.

RÜTZLER, K., AND I. G. MACINTYRE. 1982. The habitat distribution and community structure of the barrier reef complex at Carrie Bow Cay, Belize, p. 9–45. *In* K. Rützler and I. G. Macintyre [eds.], The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize, I: Structure and communities. Smithsonian Institution Press.

SMITH, D. C. 1979. From extracellular to intracellular: The establishment of a symbiosis. Proc. R. Soc. Lond. B **204:** 115–130.

Southward, E. C. 1982. Bacterial symbionts in Pogonophora. J. Mar. Biol. Assoc. U.K. **62:** 889–906.

SUESS, E., B. CARSON, S. D. RITGER, J. C. MOORE, M. L. JONES, L. D. KULM, AND G. R. COCHRANE. 1985. Biological communities at vent sites along the subduction zone off Oregon. Bull. Biol. Soc. Wash. **6:** 475–484.

VOGEL, S. 1996. Life in moving fluids. The physical biology of flow, 2nd ed. Princeton Univ. Press.

VOPEL, K., C. H. REICK, G. ARLT, M. PÖHN, AND J. A. OTT. 2002. Flow microenvironment of two marine peritrich ciliates with ectobiotic chemoautotrophic bacteria. Aquat. Microb. Ecol. **29:** 19–28.

WINDOFFER, R., AND O. GIERE. 1997. Symbiosis of the hydrothermal vent gastropod *Ifremeria nautilei* (Provannidae) with endobacteria—structural analyses and ecological considerations. Biol. Bull. Mar. Biol. Lab. Woods Hole **193:** 381–392.

Received: 4 December 2003
Amended: 6 July 2004
Accepted: 28 August 2004