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The Ecology of a Novel Symbiosis Between a Marine Peritrich Ciliate and Chemoautotrophic Bacteria

JÖRG A. OTT, MONIKA BRIGHT & FRIEDRICH SCHIEMER

Institute of Zoology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria.
E-mail: joerg.ott@univie.ac.at

With 12 figures

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Abstract. A symbiosis between a sessile colonial ciliate, *Zoothamnium niveum* (HEMPRICH & EHRENBERG, 1831), and chemolithoautotrophic bacteria oxidizing reduced sulfur compounds is described. The ciliates and their ectosymbionts occur on mangrove peat in the Caribbean. The feather-shaped ciliate colonies expand and contract, thus exposing the microbes alternately to oxygenated water above and sulfidic water within the boundary layer developed on the surface of the highly sulfidic peat. The symbiotic ciliates colonize sites with high sulfide flux where the microbial surface mat has been disturbed and grow there until the microbe mat re-establishes itself.

Problem

Symbioses between chemolithoautotrophic sulfur-oxidizing bacteria and protists or invertebrates are common in marine habitats where reduced sulfur compounds are abundant. The discovery of animal communities around deep-sea hot vents and cold seeps (for reviews see TUNNICLIFFE, 1991, 1992; CHILDRESS & FISHER, 1992; CAVANAUGH, 1994; NELSON & FISHER, 1995; FISHER, 1996) which depend largely on the organic production of symbiotic bacteria that utilize sulfide as an energy source for the reduction of carbon dioxide, has spurred the search for such symbioses in shallow waters. Not surprisingly, similar microbe/animal associations have been found in all marine sulfidic habitats such as sheltered and organic-rich sediments, seagrass-rhizome mats and mangrove peat. The bacteria occur as ecto- or endosymbionts and the animal hosts are protists (FENCHEL & FINLAY, 1989; BAUER-NEBELSICK *et al.*, 1996a,b), plathelminthes (OTT *et al.*, 1982), nematodes (OTT *et al.*, 1982; OTT & NOVAK 1989), polychaetes (GIERE, 1996), oligochaetes (GIERE, 1981), echiurids (MENON & ARP, 1993), priapulids (OESCHGER & SCHMALJOHANN, 1988), bivalves (CAVANAUGH, 1985; FELBECK *et al.*, 1981), gastropods (STEIN *et al.*, 1988), echinoderms (TAMARA *et al.*, 1993) and pogonophorans (SOUTHWARD *et al.*, 1986).

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Among the protists, the genus *Kentrophoros* (Ciliophora, Karyorelictida) is known for a coat of bacteria on its dorsal side which act as a 'kitchen garden' for this free-living, mobile ciliate. Here, we report on the ecology of a symbiosis between a colonial, sedentary species of the genus *Zoothamnium* (Ciliophora, Peritrichida) – *Z. niveum* (HEMPRICH & EHRENBERG, 1831) – and sulfur-oxidizing chemolithoautotrophic bacteria. *Zoothamnium niveum* was described more than 150 years ago. Although the original authors paid tribute to the conspicuous white colour of their find by naming it '*niveum* = snowy', they failed to recognize that the colour was due to a complete coat of bacteria on the surface of the colony, covering all but the most basal part of the stalk. The redescription by BAUER-NEBELSICK *et al.* (1996a) showed that terminal zooids and microzooids are covered by coccoid bacteria, whereas the stalk, the branches and the macrozooids bear rod-shaped microbes. A complete series of intermediate shapes between those bacteria covering the branches and those attached to the microzooids suggest that they are morphotypes of the same bacterial species.

Colour, ultrastructure and the presence of the CO_2-fixing enzyme of the Calvin cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (H. FELBECK, pers. comm.), indicate that the bacteria are sulfur-oxidizing chemolithoautotrophs. The only bacteria detected so far in food vacuoles within the ciliates (TEM sections) show the typical ultrastructure of sulfur bacteria and appear to be identical to those growing on the pellicle of their host. This suggests that the symbionts are ingested by the microzooids and most likely play an important role in the nutrition of the ciliates (BAUER-NEBELSICK *et al.*, 1996b).

Zoothamnium niveum has so far been found in the Red Sea (HEMPRICH & EHRENBERG, 1831; BAUER-NEBELSICK *et al.*, 1996a) and the Caribbean (BAUER-NEBELSICK *et al.*, 1996a). In the Caribbean it is found growing in groups on mangrove peat along the banks of tidal channels and ponds. In this study we describe the ecology of a population living at Twin Cays, a mangrove island of the Belize Barrier Reef system, and report on the occurrence of this microbe/ciliate association at other locations in the area.

Material and Methods

1. Sampling locality

Twin Cays (Fig. 1) is a mangrove island located 3.5 km landwards of Carrie Bow Cay, a small island on the reef edge of the Belize Barrier Reef off Dangriga, Belize, where a laboratory of the Caribbean Coral Reef Ecosystem program of the National Museum of Natural History (Washington, DC) is located (for a description of the area see RÜTZLER & MACINTYRE, 1982). Twin Cays is bisected by a tidal channel cut into Red Mangrove (*Rhizophora mangle*) peat with vertical to overhanging walls (Fig. 2) extending in some places down to 2 m depth. Stilt roots of Red Mangroves hang down into the channel and are covered by a remarkable diversity of algae and sedentary animals (RÜTZLER & FELLER, 1996). The peat walls in back of the curtain of hanging stilt roots are covered by a dense microbial mat and in places by sponges and ascidians. The peat surface is highly structured with depressions and holes, and in some places is deeply undercut. All quantitative data come from the walls of the main tidal channel close to its north end, where most of the experimental animals were also collected. Other collecting sites are similar mangrove islands in the vicinity (Fig. 1).

Observations and experiments reported in this paper were made during repeated visits of 3 weeks each during February to June in the years 1992–1997.

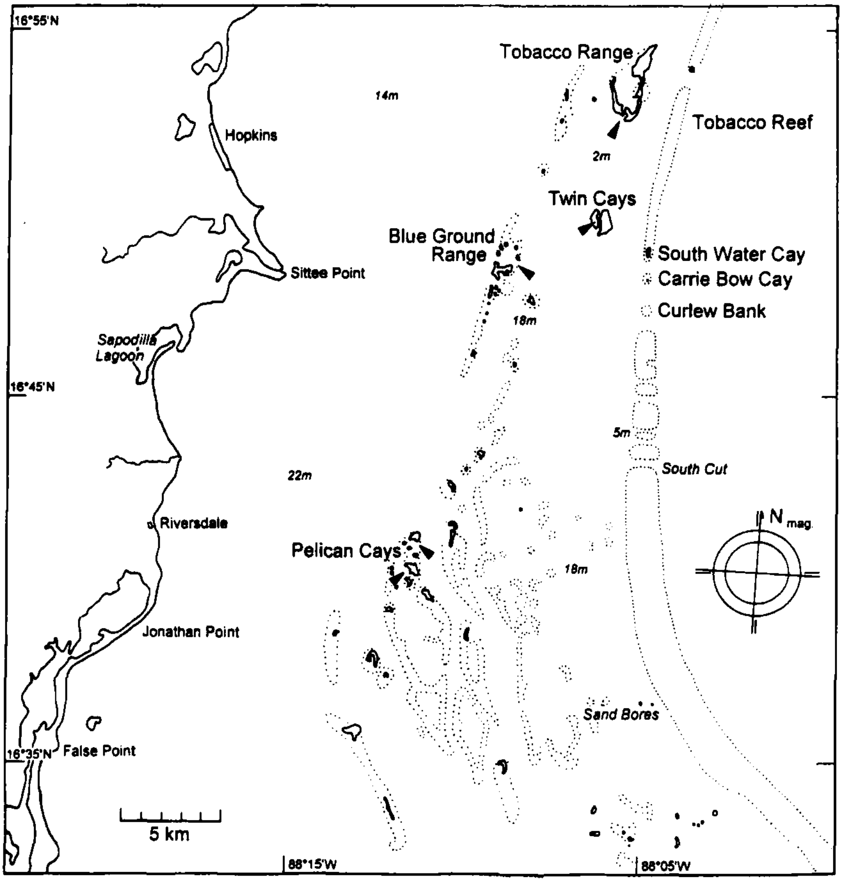

Fig. 1. Map of the vicinity of Twin Cays in the Belize barrier reef system. Sites where *Zoothamnium niveum* has been found are indicated by arrow heads.

2. Methods

Density was determined using a 50 × 50 cm frame divided in 25 squares of 100 cm^2 each and an underwater magnifying glass. Roughness of the peat surface was measured as the distance from the grid points of the frame to the surface of the peat wall. Likewise, the distance between those surfaces where ciliate groups were found and the plane of the frame was determined.

In order to estimate group life-span, groups were marked with small flags and visited daily over a period of 3 weeks.

For the micro-electrode profiles, peat blocks that were at least 30 cm deep and fitted tightly into a 10 litre bucket were cut from the peat wall with a machete, inserted into the bucket and sealed with a lid under water. Thus, blocks could be transported into the laboratory at Carrie Bow Cay without disturbing the microbial mat on the surface. Blocks were kept in flowing seawater between measurements. Sulfide and oxygen profiles next to and away from colony groups were measured with a combined needle electrode (VISSCHER *et al.*, 1991) under stagnant and flowing water conditions ($0.5–5\ cm \cdot s^{-1}$) within 24 h after collection. Sulfide concentrations in the pore water deeper than 5 cm in the peat were determined from water samples collected *in situ* by syringes and analyzed photometrically with a modification of the CLINE method (CLINE, 1969). Pore water sulfide concentrations measured with the

Fig. 2. Peat wall along the main tidal channel at Twin Cays, Belize. Colonies of *Zoothamnium niveum* can be seen on and around the white patch of sulfur bacteria in the centre (× 0.3).

Fig. 3. *Zoothamnium niveum*. Large group on mangrove peat (× 1.7).

electrode in the layer 10 mm below the peat surface were within 10% of those determined photometrically in samples from the same block.

Animals for oxygen uptake measurements were collected by cutting out fist-sized peat blocks with a knife and transferred in sealed containers to the laboratory, where the respiration of whole colonies was measured using stoppered Cartesian divers (KLEKOWSKI, 1971, SCHIEMER *et al.*, 1990). Colonies were repeatedly rinsed in filtered seawater prior to introducing them into the diver in air-saturated water. This prevents sulfidic water from being transferred with the experimental animals. All measurements were taken at 28 °C, which was close to the temperature in the field.

Free-flow water movement along the channel walls was measured by timing dye (Fluorescin) markers along a measured distance. Additional current measurements were obtained from J. HAVENHAND (pers. comm.). Video recordings of the colonies in the laboratory were made with a Philips LDH 0640 CCD color camera; photos were taken with a Nikon EM, equipped with a Micro-NIKKOR 55 mm lens, bellows and ring flash using Kodachrome 64 film.

Results

1. Distribution

Zoothamnium niveum was found along both banks of the main tidal channel ('Main Channel') at Twin Cays. The primary collecting and experimental sites were north of the dock on the channel's west side and between the entrance to 'Turtle Pond' and 'Cassiopeia Cove' on the east side. In addition, *Z. niveum* colonies occurred practically everywhere where vertical or overhanging peat walls exist in nearby mangrove cays, such as Tobacco Range to the North and Blue Ground Range and Pelican Cays to the South of Twin Cays (Fig. 1). Especially conspicuous populations were found in two locations at the Pelican Cays, the undercuts in the double lagoon at Manatee Cay and a small cove at Northwest Cay.

Zoothamnium niveum occurs in groups (Fig. 3) which may contain more than 100 colonies (max. = 127, mean = 26 ± 17 SD, n = 34) from approximately 30 cm below mean low water level down to where the peat walls disappear in the soft sediment of the channel bottom in less than 2 m depth. The distribution of groups is random on a 100 cm^2 scale (mean = 0.48 ± 0.62, n = 150), but strongly aggregated on a m^2 scale (mean = 46 ± 20.8, n = 6). Groups of colonies do not prefer depressions in the peat, as we originally assumed. They are, however, predominantly found at sites where the microbial surface mat consisting of microscopic algae, cyanobacteria (*Oscillatoria*, *Spirulina*) and filamentous sulfur bacteria (*Beggiatoa*) has been disturbed. Most conspicuous are groups around holes in the peat where mangrove rootlets had rotten and fallen out (Fig. 4). Purple and white sulfur bacteria in many cases form conspicuous mats around these holes (Fig. 5). On the average there were about 1200 colonies per m^2.

Small groups with only a few colonies contained either only small colonies or predominantly large colonies often showing signs of deterioration (loss of microzooids on lower branches, dense overgrowth by microbes on the lower stalk) (Fig. 6); larger groups consisted of colonies of all sizes.

Based on the disappearance rate of marked groups (Fig. 7) a group life-time of *ca.* 20 days was estimated, assuming that groups were randomly marked and were at least 4 days old on the marking date which is the time required for a full grown colony to develop (M. BRIGHT, J. OTT, unpubl. obs.).

Fig. 4. *Zoothamnium niveum*. Colonies surrounding a hole in the peat. The small white dots are *Vorticella*-like solitary peritrichs, which are also covered by white bacteria (× 2).

Fig. 5. Site where a mangrove root has rotted and fallen out. The remainder of the root epidermis is covered by purple bacteria and filamentous white sulfur bacteria. *Zoothamnium niveum* colonies are visible on the left (× 2).

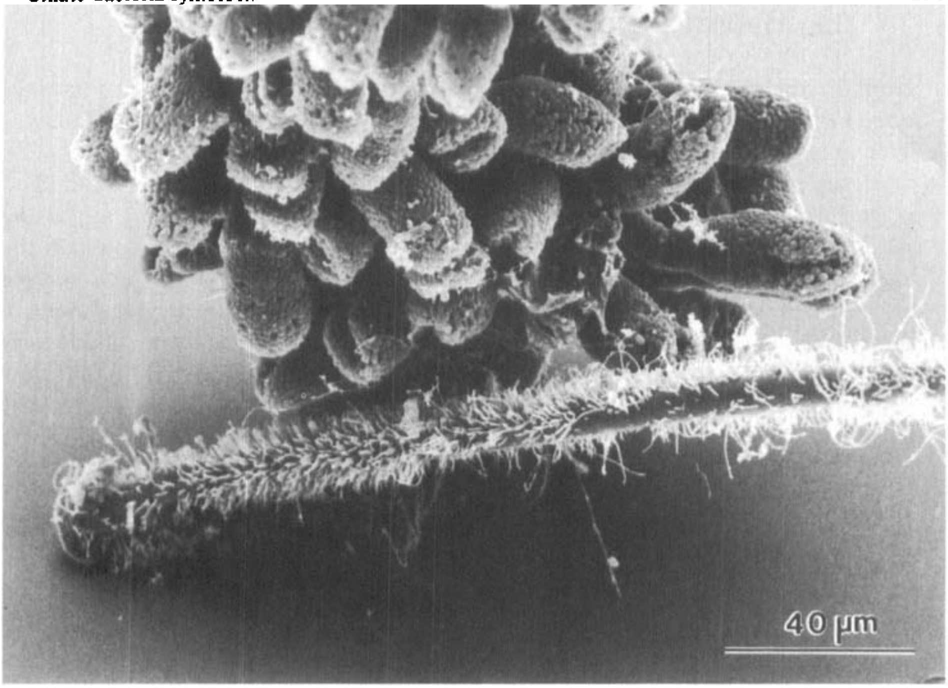

Fig. 6. SEM of the stalk and several microzooids of a contracted colony of *Zoothamnium niveum*. The stalk is overgrown by white filamentous bacteria, while coccoid symbionts cover the microzooids.

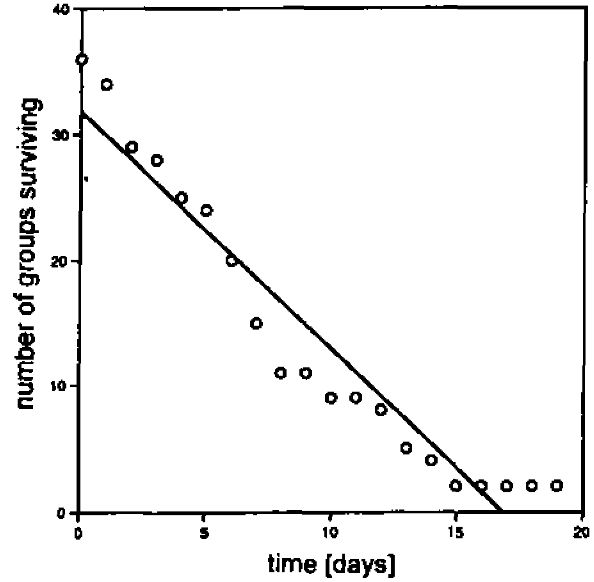

Fig. 7. Disappearance of marked groups of colonies. The regression line intersects the x-axis at 16.9 days. Adding 4 days to allow for the groups to contain fully grown colonies yields an average life expectancy of a group of 21 days.

2. Habitat characteristics

Sulphide concentrations in pore water from depths > 5 cm below the peat surface ranged up to 1.4 mM, whereas in water samples from the channel no sulfide was detectable even when taken with a syringe close to the peat surface.

Microelectrode profiles revealed extremely sharp gradients of both sulfide and oxygen at the peat surface (Fig. 8), with oxygen disappearing and sulfide appearing in a 3 mm thick boundary layer. In areas without ciliate colonies the shape of the gradient curves was rather insensitive to water movement over the peat surface and little sulfide was detectable 2 mm above the peat surface. Close to *Zoothamnium* groups, however, sulfide concentrations in the 2–4 mm layer were significantly higher in profiles under stagnant conditions compared to those at a free flow velocity of 0.5–5 cm·s^{-1}, which is in the range of the velocities measured in the field.

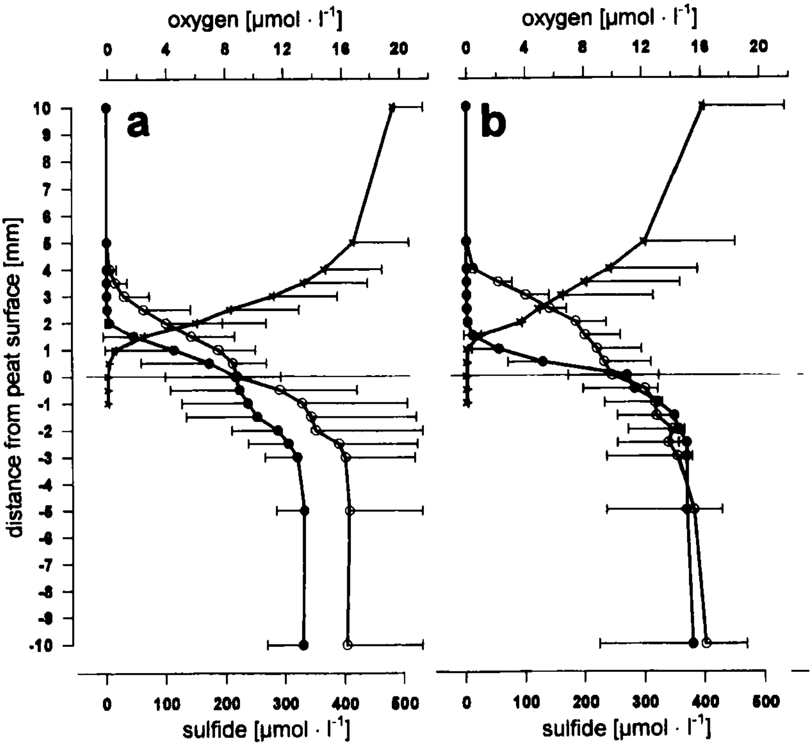

Fig. 8. Profiles of oxygen and HS$^-$ across the surface of mangrove peat measured with a combined needle electrode: (a) profiles through the surface without ciliates; (b) profiles at sites with groups of *Zoothamnium niveum* colonies. Error bars are standard deviations from the mean of several measurements (shown only to one side of the profile for clarity). Sulfide profiles were measured under stagnant conditions (empty circles) or flow over the surface (filled circles). All oxygen profiles (asterisks) were taken under stagnant conditions.

3. Behaviour and metabolism

The colonies expand and contract, measuring up to 15 mm in height when expanded and less than 1 mm when contracted. Analysis of video recordings showed that colonies spend more time expanded with practically all zooids in contact with the oxic, non- or low-sulfidic water of the channel. Intervals between contractions typically range from 5 to 30 s. Contraction takes less than the time between video frames (1/24th of a second) and brings the colony into the anoxic and sulfidic boundary layer. Colonies usually remain contracted for less than 1 s and then expand again, which takes 1 to 3 s.

Freshly collected colonies which were introduced in the Cartesian diver within 15 min after removal from the habitat showed elevated rates of oxygen consumption. These rates decreased rapidly within the first four hours to 140–180 nl $O_2 \cdot mm^{-2}$ colony surface$\cdot h^{-1}$, a level sustained over at least 24 h. When colonies which had been maintained in normoxic seawater for 24 h were subsequently incubated in 100 μM sulfide for up to 3 h, they showed a restoration of higher oxygen uptake rates followed by a subsequent decrease (Fig. 9).

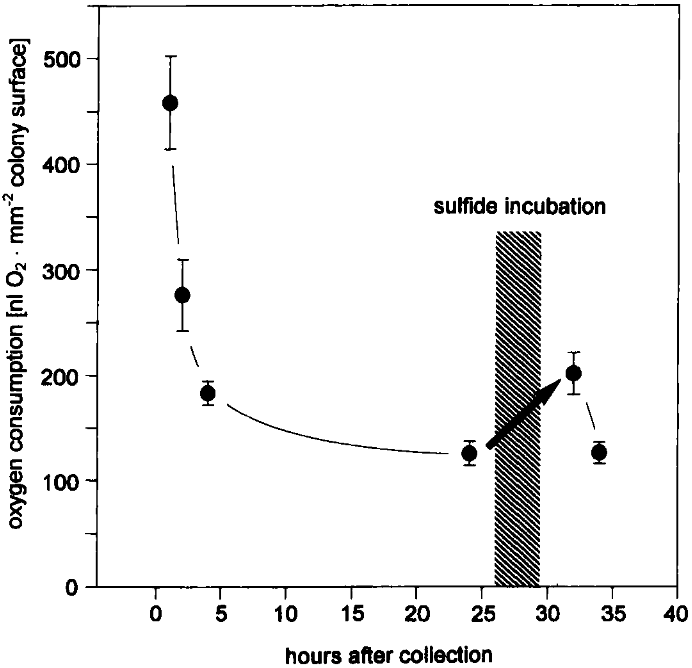

Fig. 9. *Zoothamnium niveum*. Hourly rates of oxygen uptake of colonies with symbionts measured in stoppered Cartesian divers. Rates are given per unit surface area, which is proportional to symbiont mass. The x-axis shows time after removal of the colonies from the peat. Colonies were introduced into the divers within 15 minutes after collection. Colonies used for the sulfide incubation (n = 5) were not the same as those used for the oxygen uptake determination of freshly collected material (n = 6), but had been removed from the peat and maintained in normoxic seawater for at least 24 h. Error bars indicate standard deviations.

Discussion

Zoothamnium niveum inhabits the spatially narrowest sulfide/oxygen gradient of all symbioses based on chemoautotrophic sulfur-oxidizing bacteria. Depending on the state of contraction or expansion the colonies are bathed in seawater containing no oxygen and sulfide up to 250 μM, or in normoxic sulfide-free seawater. The transition between the two media is rapid especially during contraction, which proceeds at a speed of 25 $\text{cm} \cdot \text{s}^{-1}$. Although colony expansion takes only about 1–3 s it is 25 to 80 times slower (0.3–1 $\text{cm} \cdot \text{s}^{-1}$). Calculating REYNOLDS numbers ($R = u \cdot l \cdot \nu^{-1}$ with $l = 0.01$ m, $\nu = 10^{-6}\ \text{m}^2 \cdot \text{s}^{-1}$) for both contraction and expansion suggests that contraction at $R = 2500$ effectively exchanges the water around the colony, whereas during expansion ($R = 30-100$) sulfidic water probably adheres to the surface of the ciliate due to viscous forces and is carried through the boundary layer, thus prolonging the time for uptake of sulfide by the bacteria. In sulfur symbioses the major problem of the animal host is to supply the sulfur-oxidizing symbionts with both sulfide and oxygen, which are mutually exclusive under most circumstances (Fig. 10). Rapid turbulent mixing at hot vents delivers sulfide and oxygen to the plume of vent vestimentifera; cold seep vestimentifera probably 'mine' sulfide in deeper sediment layers with root-like posterior processes (SCOTT & FISHER, 1995); bivalves such as *Calyptogena* (ARP *et al.*, 1984; see also review in CHILDRESS & FISHER, 1992) and *Thyasira* (DANDO & SOUTHWARD, 1986) use their foot to obtain sulfide from lava cracks or deep sediment, respectively, while pumping oxygenated water across their gills. Nematodes and oligochaetes migrate (OTT *et al.*, 1991; GIERE *et al.*, 1991) over a distance of several cm to dm between sulfidic layers deep in the sediment and the oxic surface. The extreme gradient at the peat surface allows *Zoothamnium niveum* to uncouple sulfide and oxygen uptake in space and time over a few mm and within seconds. The steep drop of oxygen uptake under oxic conditions indicates that there is a store of reduced sulfur compounds which is available to the bacteria and which is rapidly exhausted but may be replenished, as shown by the rise in respiration rate following sulfide incubation.

The occurrence at disturbed sites on the peat surface together with the short life time of such aggregations suggest that *Z. niveum* is an opportunist which is able to colonize and exploit sites of high sulfide flux to the water. The sensitivity of the sulfide profile to water movement indicates that the flux there is under physical control with rapid leakage of sulfide from the peat. In contrast, sulfide flux to the water appears low and insensitive to flow along the undisturbed surface. Sulfide diffusing from deeper layers of the peat is probably either oxidized by the oxygen produced by cyanobacteria or taken up and eventually oxidized by sulfur bacteria in the undisturbed microbial mat and thus is under biological control (Fig. 11).

Groups consisting of a few small colonies are probably early stages of colonization of a newly formed hydrogen sulfide 'minivent'. Colonization occurs through a dispersal stage (swarmer, macrozooid) (BAUER-NEBELSICK *et al.*, 1996a). Larger groups consisting of colonies of all sizes represent a later, 'mature' stage of the sulfide point source. In time the microbial mat which covers the peat surface as the 'climax' stage re-establishes itself, reducing the sulfide flux and thus rendering the site unsuitable for the ciliates. Those small groups mostly consisting of large and degenerating colonies are a late stage where new recruitment of swarmers has ceased (Fig. 12). The high growth rates, short life span and the extraordinary

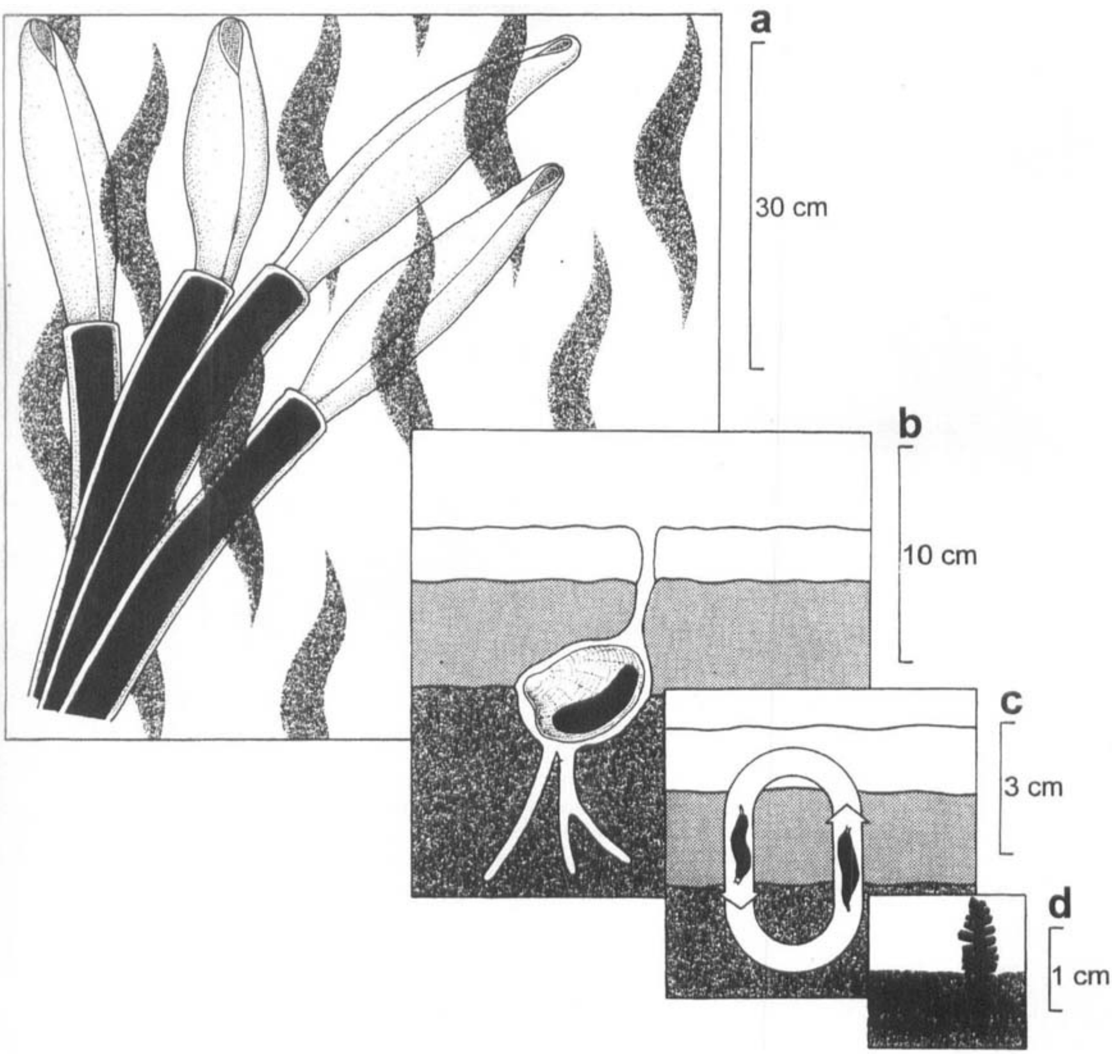

Fig. 10. The different strategies of hosts to supply their symbiotic sulfur-oxidizing chemoautotrophic bacteria with sulfide and oxygen. From top to bottom the gradient becomes more spatially defined and the role of the host in creating the proper environment decreases. (a) Vestimentiferan tube-worms at deep-sea hot vents are exposed to a turbulent mixture of sulfidic and oxic water. The supply rate of sulfide and oxygen to the internal symbionts is under tight bio-molecular control by the host haemoglobin. (b) Bivalves procure the necessary chemical compounds using special organs such as siphons, gills or foot to pump or mine sulfide and/or oxygen (in part the transport process is also dependent on blood compounds). (c) Interstitial meiofauna (gutless oligochaetes, stilbonematid nematodes) migrate through the chemocline of marine sediments and stay for some time alternately in sulfidic or oxic sediment layers. (d) The narrow gradient along its colony allows *Zoothamnium niveum* to rapidly change between high sulfide concentration and oxygen saturation without even having to resort to locomotion (symbionts – darkest shading, sulfidic water – medium shading, no oxygen/no-sulfide zone – light shading, oxygenated water – white).

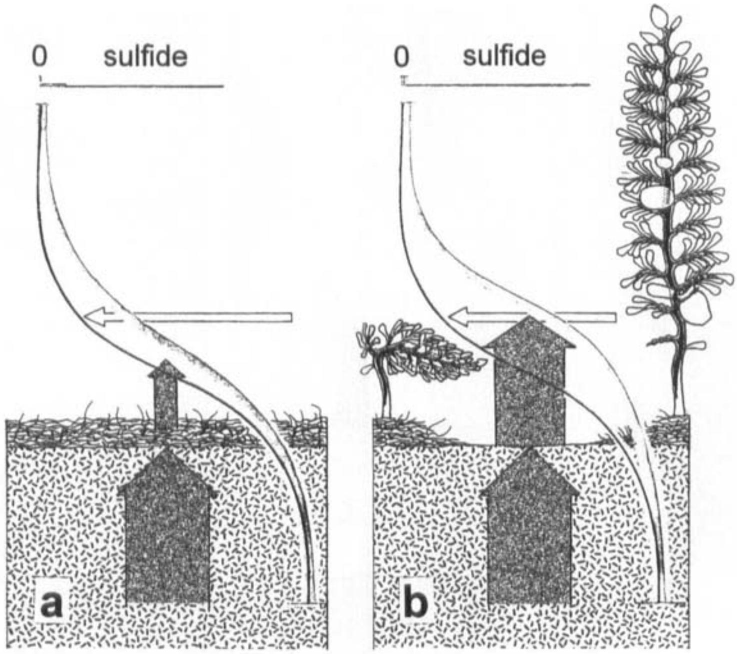

Fig. 11. Model of the flux of sulfide at sites with (a) intact and (b) disturbed microbial mat on the peat surface. In (a) the profiles of sulfide concentrations during stagnant (thick line) and flow (thin line) conditions do not differ much where the microbial mat keeps sulfide flux under biological control. In (b) sulfide flows freely creating a several mm thick sulfidic boundary layer under stagnant conditions which is markedly reduced by water flow. Expanded *Zoothamnium* colonies (right) extend into oxygen containing water with little or no sulfide. Contracted colonies (left) are completely immersed in sulfidic water. Dark arrows indicate magnitude of sulfide flux, white arrow symbolises current flow over the peat surface.

habitat locating abilities of the swarmers (M. BRIGHT, J. OTT, unpubl. obs.) are consistent with the life style of a pioneer in gap dynamics. The promotion of an opportunistic, fugitive life style through a symbiosis is contrary to the paradigm that symbioses are characteristic of climax stages (ODUM, 1969).

The genus *Zoothamnium* contains approximately 60 described species from many aquatic habitats, both freshwater and marine, benthic and pelagic. In a number of cases, associations with microorganisms have been reported, among others with cyanobacteria or bacteria – some in connection with occurrence in sulfidic habitats (see review in BAUER-NEBELSICK *et al.*, 1996a). A possible symbiosis has been suggested by LAVAL-PEUTO & RASSOULZADEGAN (1988) for the association of *Z. pelagicum* with cyanobacteria.

Zoothamnium niveum is another case of ectosymbiosis which provides us with a glimpse of how symbioses may have evolved. The special properties of the surface of Peritrichida and especially members of the genus *Zoothamnium* permit epigrowth by microorganisms – or rather the ciliates cannot prevent fouling, which appears to limit age and size of the colonies. Attaining selective control over the fouling organisms is certainly an advantage. In *Z. niveum* such control appears to exist at

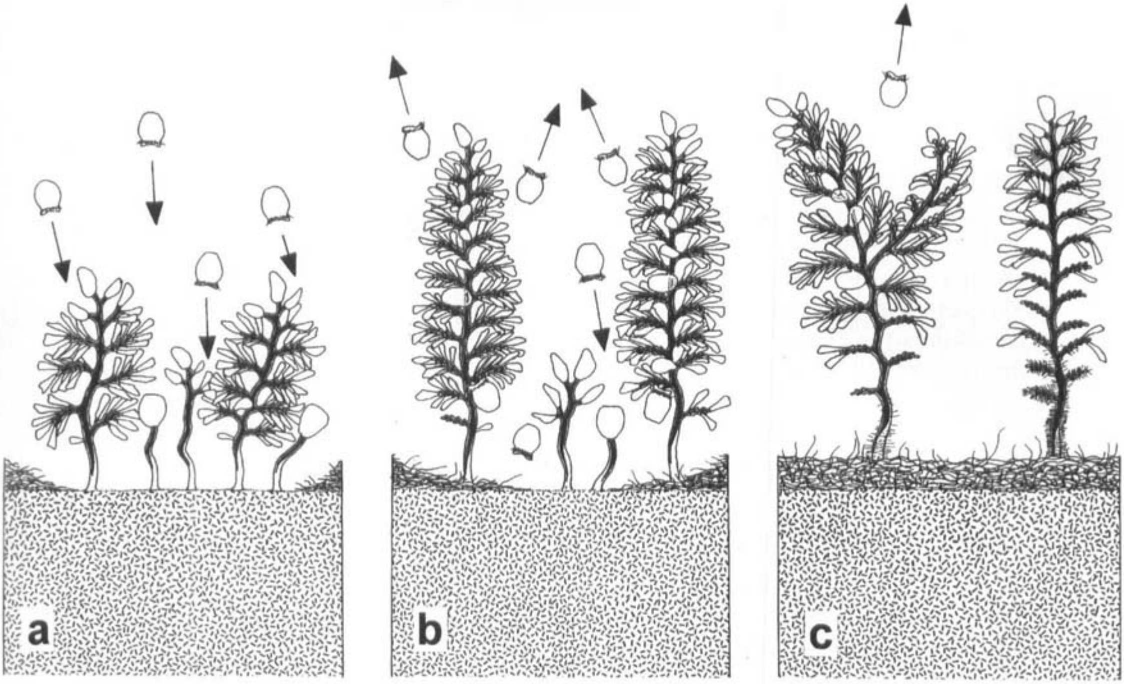

Fig. 12. Sequence of (a) colonization of a disturbed site, (b) maturation of a group and (c) senescence of the sulfide point source. During colonization the group comprises only young colonies and many freshly settled swarmers; a mature group shows a mixture of fully grown colonies already releasing swarmers and some young colonies; senescent groups are made up by mature colonies, many of which have already lost a large number of microzooids and show overgrowth by a variety of bacteria.

least in young parts of the colony and may be one of the reasons why such a large size can be attained. Whereas the main axis, the branches and the zooids are covered by a presumably monospecific coat of rod-shaped to coccoid bacteria (Bauer-Nebelsick *et al.*, 1996a), the basal portions of the stalk in fully grown colonies are overgrown by a variety of filamentous microorganisms. With onset of senescence, larger parts of the colony base lose the zooids and are subsequently fouled.

The occurrence of the ciliate close to sulfide gradients allowed sulfur-oxidizing bacteria to become members of the fouling community. The special trait to exchange surrounding water by rapid contraction of stalks – which is common in Peritrichida – may have led to selection for those bacteria which alternately need sulfide and oxygen by enhancing their growth. Ample nutrition from the 'gardened' bacteria provides a positive feedback allowing rapid growth of the ciliate. Schiemer *et al.* (1990) proposed a similar sequence for the evolution of an ectosymbiosis in the marine nematode subfamily Stilbonematinae. This taxon consists of several genera possessing a surface coat of ectosymbiotic bacteria within the family Desmodoridae, which is characterized by the frequent occurrence of fouling organisms (bacteria, diatoms, suctorians) on their cuticle (Ott, 1996).

Summary

The colonial ciliate *Zoothamnium niveum* lives on mangrove peat in densities of more than 1000 colonies·m^{-2}. Groups of a few to more than 100 colonies cluster around disturbed sites on the peat surface, which act as point sources for sulfide. Colonies retract and expand through a sharp gradient of water chemistry, where oxygen disappears and sulfide is present in concentrations of 250–300 μM within a 3–4 mm thick boundary layer on the peat surface. In this way the ciliate host supplies its ectosymbiotic chemoautotrophic bacteria alternately with sulfide and oxygen. A single point source for sulfide supports growth of the ciliate and its symbionts for about 3 weeks. At this point the microbial mat of the adjoining, undisturbed peat surface regrows over the disturbed site and reduces sulfide flux.

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