

BEHAVIORAL AND CHEMICAL ASPECTS OF BLACK BAND DISEASE
OF CORALS: AN *IN SITU* FIELD AND
LABORATORY STUDY

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*Black band disease of corals consists of a dense microbial community dominated by the cyanobacterium ("bluegreen alga") Phormidium corallyticum. The community, which is present as a line or band, migrates across the surface of infected scleractinian coral colonies, completely degrading coral tissue and leaving behind bare coral skeleton. Studies were performed on chemical microenvironments present within black band and their relation to behavioral patterns exhibited by microorganisms associated with the band. Vertical profiles of oxygen and sulfide within the top 500 micrometers of freshly collected intact band, measured using microelectrodes, demonstrated that the base of the band, near coral tissue, is both anoxic and contains sulfide. Oxygen microelectrodes were also used underwater to investigate vertical gradients of oxygen in black band in the natural environment. The *in situ* measurements corroborated the laboratory study, and showed that strong oxygen gradients were present throughout the band. The chemical dynamics and biological interactions within black band are similar to those reported from a wide variety of attached microbial communities found in diverse aquatic environments.*

INTRODUCTION

Black band disease of corals was first reported on reefs in Belize by Antonius (1973). Subsequently, black band has been observed throughout reefs of the Caribbean (Garrett and Ducklow, 1975; Rutzler et al., 1983; Edmunds, 1991), the Indo-Pacific (Antonius, 1985) and the Red Sea (Antonius, 1988). It is thought that the disease is contributing to an observed overall degradation of coral reefs (Williams and Bunckley-Williams, 1990; Porter and Meier, 1992; Peters, 1993). The disease is characterized by a dense band, 1 mm to 4 cm wide and <1 mm thick, which can be seen between apparently healthy coral tissue and freshly exposed, pure white coral skeleton. The band is composed of a microbial consortium dominated by a gliding, filamentous cyanobacterium, originally described as Oscillatoria submembranaceae by Antonius (1973), and

redescribed as Phormidium corallyticum by Rutzler and Santavy (1983). The band appears dark due to the cyanobacterial photosynthetic accessory pigment phycoerythrin. The band also contains numerous heterotrophic bacteria (Garrett and Ducklow, 1975), fungi (Ramos-Flores, 1983), and the sulfide-oxidizing filamentous bacterium Beggiatoa (Rutzler et al., 1983).

In the disease process, black band migrates across the surface of coral colonies, completely degrading coral tissue. Rates of movement have been observed up to 2 cm/day (Rutzler et al., 1983), which is highly destructive in that many scleractinian corals grow at rates of approximately 1 to 2 cm in circumference per year (Hudson, 1981). Typically, the disease is active only during the warmer months of the year when water temperature is at or above 25°C (Rutzler et al., 1983). The disease occurs on scleractinian corals, most commonly species of Montastraea, Diploria and Colpophyllia. Many, but not all, scleractinian coral are affected (see Antonius, 1981 and Rutzler et al., 1983 for details about susceptible colonies). Death of an entire colony can occur in one active season for small colonies (less than one meter), or after repeated infection in successive seasons (Richardson, personal observation).

It has been suggested (Rutzler and Santavy, 1983) that the cause of coral mortality is via cyanobacterial toxins, however this has never been tested experimentally. An alternate hypothesis is that the chemical environment of black band, i.e. anoxic, sulfidic conditions at the base of black band where the band contacts the coral, is responsible for coral death. Preliminary laboratory work by Richardson (1992) demonstrated anoxia at depths below 500 μ m into the band, measured using oxygen microelectrodes on intact black band sampled using an underwater coring device. Indirect evidence that sulfide is present in the band microcosm consists of observations of the sulfide-oxidizing bacterium Beggiatoa sp. within black band (Rutzler et al., 1983). At times, dense populations of Beggiatoa are present on the surface of well-developed black band (Richardson, 1992). Demonstration that the cause of coral death is by chemical microenvironments produced by the microbial consortium present in black band requires, as a first step, direct measurement of the chemicals on a microscale coupled with an investigation into the microbiology of the consortium.

We report here results of laboratory measurements of oxygen and sulfide microenvironments present in freshly collected, intact black band (measured using oxygen and sulfide microelectrodes), and field experiments on both oxygen microenvironments in in situ black band (measured using in situ microelectrodes) and in situ behavioral patterns of the black band community.

MATERIALS AND METHODS

The in situ study was performed on Grecian Rocks, ca. 3 miles offshore from Key Largo in the Key Largo National Marine Sanctuary (now part of the Florida Keys National Marine Sanctuary) during July of 1992. The site is dominated by large (many >2 m diameter) colonies of Montastraea annularis morphotype 3 (Knowlton et al., 1992), with more than 50% of the colonies infected with black band disease at this particular site. Water depth on the reef ranges from zero (when colonies of Acropora palmata are exposed at low tide) to 10 meters. Water depth at the study site was 2.7 m (high tide).

Water temperature was determined by a data logging Thermomentor (Ryan, Inc.) permanently installed by the Key Largo National Marine Sanctuary approximately 25 m from the study site, and at a similar depth. Underwater light levels were measured using a

diver operated Biospherical Instruments Inc. irradiance meter (Model QSI-140), which measures photosynthetically active radiation (PAR) from 400 to 700 nm. Laboratory light levels were determined using a Biospherical Instruments QSL-100 averaging quantum irradiance meter, which also measures PAR.

In situ oxygen dynamics of the black band community were investigated using oxygen-sensitive microelectrodes of the style designed by Revsbech and Ward (1983), modified by R.G. Carlton. Our probes were constructed (by R.G. Carlton) with thicker tip diameter (ca. 150 μm) to reduce chance of breakage if contact was made with the coral skeleton. In addition, a stronger adhesive (Torr Seal, Varian Associates) was used to seal probe components. Additional details of microelectrode use and construction are described in Revsbech and Jørgensen (1986) and Carlton and Wetzel (1987). During use the cathode was polarized at -0.75 Volts relative to the anode. The subnanoampere-level output signal, which is a linear function of oxygen concentration, was displayed on a Kiethley 480 picoammeter. The probe was electrically connected to the polarizing circuitry and picoammeter (both contained in a submersible acrylic pressure housing) by a 3 m long cable. A two point calibration of the probe was made before and after making the in situ measurements by alternately immersing the probe in air-equilibrated (21% O_2) or anoxic (N_2 sparged) sea water.

Accurate positioning of a microprobe can only be accomplished with a micromanipulator. For the in situ measurements a modified microscope stage micrometer was used, which costs much less than a laboratory micromanipulator and was easier to maintain after exposure to salt water. The micrometer/manipulator was mounted on a lattice of 13-mm diameter aluminum rods which were anchored ca. 1 m in the sand adjacent to the subject coral. In a laboratory situation, probes can be accurately positioned at the liquid-solid interface with the aid of fiber optics or magnifying devices, which cannot be used by a diver wearing a facemask. In this study we detected the interface (i.e. surface of black band) by watching for a characteristic output signal change which occurs upon contact. This was accomplished by moving the probe toward the band in increments of ca. 50 μm . Under quiescent conditions the diffusive boundary layer (DBL) can be several mm thick around solid immersed objects (Vogel, 1981). When approaching from outside the interface, this method first identifies the surface of the DBL (Revsbech, 1989). At our study site the water depth was ca. 2.7 m, the active black band was at a depth of 1.7 m, and sea state was running ca. 1 m. [These conditions dictated that we perform the experiment as rapidly as possible for diving safety. This was the last day of our field program, and we had been waiting unsuccessfully for conditions to improve for the in situ study.] The high turbulence and velocity of the surge (>1 m/sec) were probably sufficient to reduce thickness of the DBL to <50 μm (estimate based on unpublished data of R. Carlton). Therefore, our spatial resolution in terms of detecting the black band/water interface was, at best, ± 25 μm . After locating the black band/water interface, the probe was advanced in 100 μm increments. The visual contrast between the bright glass probe and the darkly pigmented microbial community made it possible to verify that the probe was inside the band. However, without being able to gauge the thickness of the band in situ, we opted to avoid breaking the probe by inserting it to a maximum depth of 400 μm .

Laboratory measurements of both oxygen and sulfide microprofiles were made from cores taken from the same *M. annularis* colony on which we conducted the in situ study. One inch cores were taken, which contained black band, exposed coral skeleton, and intact coral tissue, by using a pneumatic drill (attached to the first stage of a SCUBA cylinder with a high pressure hose) equipped with a hole saw. The remaining hole in the

coral colony was plugged with modeling clay to prevent infection. Experimental cores were immediately taken to Key Largo and placed in a 3 gallon aquarium with flow-through sea water. Light (natural sunlight) intensity was modified to that measured *in situ* by neutral density screens. The oxygen microelectrodes were calibrated in the same manner detailed above for the *in situ* study. The sulfide microelectrodes were calibrated using a set of different concentrations of sodium sulfide (NA2-9H2O) made up in anoxic (N2 sparged) seawater, which were in turn analyzed for sulfide concentration using a modified Pachmayr assay (see Richardson and Castenholz, 1987) to generate a standard curve. Calibration standards ranged from 0 to 2 mM sulfide. Both oxygen and sulfide microelectrodes were inserted by micromanipulators. Simultaneous, continual oxygen, sulfide, and light measurements were made and recorded on Kipp and Zonen chart recorders. Light was measured using the Biospherical Instruments QSL-100 sensor.

Underwater photography was carried out with a Nikonos 5 equipped with a 1:1 macrolens, and a Sea & Sea Motormarine II using both a 35 mm and a 1:2 macrolens.

RESULTS AND DISCUSSION

In situ microprofiles of oxygen concentration were measured in an intact section of black band growing on a large, otherwise healthy, colony of *Montastraea annularis*, morphotype 3 (Knowlton et al., 1992) as shown in Figure 1. The band ranged from <1 mm to 1 cm from front to back, and was <1.0 mm thick. Measurements were made near the active front (by intact coral tissue), middle, and back (by freshly exposed coral skeleton) of the band. Oxygen profiles at the three band sites are shown in Figure 2A. The front and back measurements were made ca. 1 mm from the actual edges of the band. A second O2 microprofile, measured 4 mm from the initial one, also 1 mm from the front of the band, was measured as a duplicate and agreed closely with the initial profile (data not shown). During the experiment, *in situ* light was 7.2×10^{-16} quanta/cm²/sec.

At each of the *in situ* microsites oxygen concentration at the black band/water interface exceeded that of the bulk water phase, which was 194 μ M. The highest observed oxygen concentration in the band, 315 μ M, occurred at the surface of the back edge (Figure 2A). At all band sites, oxygen concentration decreased with depth. The deepest (middle of band) measured profile showed that anoxic conditions existed at the base of the band. Although the oxygen concentration gradients at the front and back of the band had similar slopes between 0 and 200 microns, our data are not sufficient to conclude whether the front or back of the band are as strongly depleted in oxygen at their bases. [All laboratory experiments, however, showed anoxia at depth; see below.] Due to the sea conditions, we opted for limited depth in most of the measurements to avoid electrode breakage.

The laboratory experiments on chemical microenvironments present in black band on freshly collected, intact cores were conducted in much more detail due to the fact that we didn't have to contend with strong surge and minimal time. Figure 2B shows the front edge oxygen and sulfide profiles of a core collected from the same colony shown in Figure 1, but at a different site on the active band. Light intensity was 8×10^{-17} quanta/cm²/sec during the experiment (adjusted to approximate the *in situ* level, shielding the experiment with two layers of neutral density screen). The oxygen profile revealed a surface maximum of 500 μ M. Oxygen penetrated the band to a depth of 1 mm, below which conditions were anoxic. The laboratory cores did not show variation in oxygen levels as were measured *in situ*. While the middle profile showed similar (i.e. relatively lowest) oxygen penetration, the front edge was oxygenated as the back (Carlton and Richardson, unpublished data).



Figure 1. In situ oxygen microelectrode study. A. Study coral with microelectrode apparatus. B. Photomacrograph (2:1) showing oxygen electrode with tip penetrating band.

The higher oxygen concentration at the surface of the core as opposed to the surface of *in situ* black band is probably due to the relatively lower turbulence in the aquarium, which allows accumulation of oxygen. The aquarium water was kept in motion by the flow-through system, but was obviously much less dynamic than that present in the turbulent conditions *in situ*. The high turbulence and surge present when we carried out the *in situ* study were not rare for this reef, however, they are hydrodynamically difficult to duplicate in the laboratory. Conversely, there are many days when the reef is calm, and one would expect surface black band oxygen levels to increase. Therefore, the surface oxygen levels in black band both *in situ* and in the cores must be considered as examples of a range of possible oxygen distributions controlled by an interaction between photosynthesizing *Phormidium corallyticum* and turbulent flow of oxygen away from the surface of the band.

The variation in oxygen profiles across the width of the band (Figure 2A) may be explained by a number of scenarios. There may be a higher biological demand for oxygen at the front and middle of the band, where, presumably, coral tissue degradation is actively occurring and heterotrophic oxygen demand, fueled by higher nutrient supply, is much greater. Or, there may be a true higher photosynthetic rate at the back of the band. Both the *in situ* and laboratory (data not shown) black band profiles revealed relatively higher oxygen levels associated with the back edge. This pattern may be related to higher light near the exposed coral skeleton, reflected from and through the calcium carbonate, which could potentially support a faster rate of oxygenic photosynthesis. There has been much work on the photic responses of cyanobacteria (Castenholz, 1982), and it has been demonstrated that natural populations of cyanobacteria position themselves at a light intensity which is optimal for support of maximal photosynthesis (Garcia-Pichel and Castenholz, 1990). Cyanobacterial strategies to maintain a preferred light regime include phototaxis and avoidance of very high light (which suppresses photosynthesis) by photophobic responses (Castenholz, 1982).

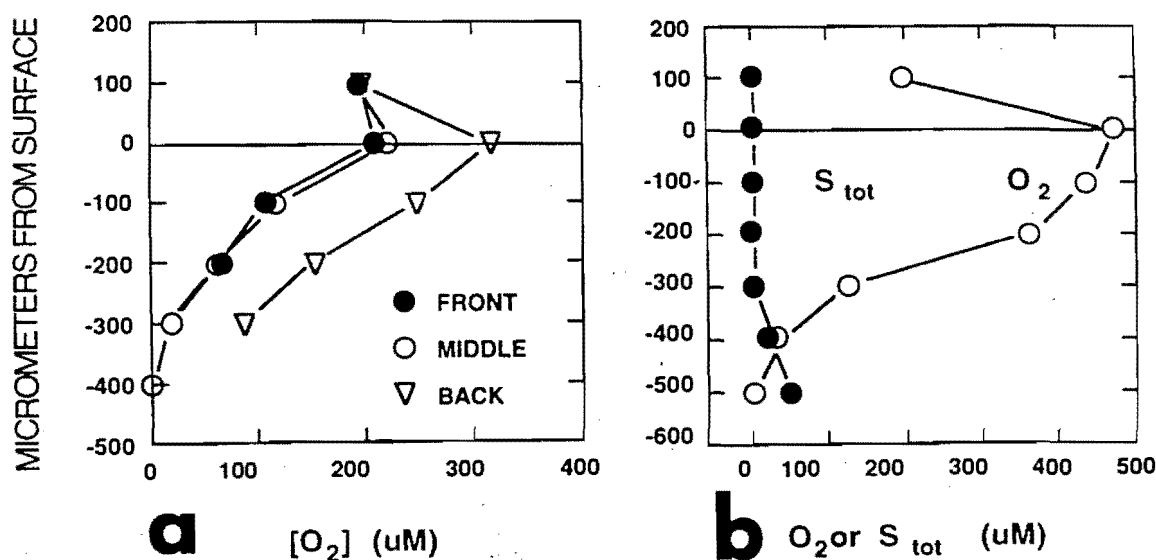


Figure 2. Oxygen and sulfide distributions in black band on *M. annularis*. A. Oxygen microprofiles, measured in situ. B. Oxygen and sulfide microprofiles, measured in a core.

The cyanobacterial light responses operating in microbial mat communities might control both black band migration patterns, and the differences in in situ oxygen across the band. If the cyanobacteria present in black band are migrating to maintain a light regime to support optimal photosynthesis, and optimal photosynthesis is revealed as highest concentrations of oxygen found at the back edge of the band, it would be expected that they remain at the high light (back of band) environment during daylight hours and not migrate across coral colonies. Such a pattern is suggested by the oxygen profiles. Conversely, if the population is avoiding high light and migrating away from the reflective coral skeleton, one would expect photosynthetic rate (thus oxygen levels) to be suppressed near the back of the band, which is not seen in the oxygen data. In the latter case, oxygen profiles would be more likely to be due to microbial interactions.

In a previous study (August, 1991), *in situ* patterns of the horizontal migration of black band in terms of light intensity and known cyanobacterial photic responses were measured on 6 coral heads with active black band (Richardson, unpublished data). A total of 536 measurements were made over a 100 hour period. The results of this study showed that none of the cyanobacterial photic responses are controlling the migration of black band across coral surfaces, thus the oxygen profiles are not the result of movement of *P. coralliticum* to optimize photosynthesis. These results suggest that control of oxygen dynamics within black band is apparently more complicated than in microbial mats, where virtually all known cyanobacterial motility patterns are ultimately controlled by light.

Sulfide was also measured in the laboratory cores. Figure 2B shows that sulfide was absent from the surface of the band to a depth of 300 μm . While some oxygen was present at this depth, it decreased rapidly to 0 by 400 μm . Sulfide increased with depth to a maximum measured value of 80 μM at a depth of 500 μm in the band (the deepest measurement taken due to potential electrode breakage). The direct measurement of sulfide by the sulfide-sensitive microelectrodes confirmed the presence of sulfide and anoxia within the black band microcosm, suggested by the previous observations of dense populations of sulfide-oxidizing bacteria. Figure 3 shows two macrophotographs (taken in August, 1991) of the vertical migration of a population of *Beggiatoa* sp., a gliding, sulfide-oxidizing bacterium which positions itself via negative aerotaxis (Jørgensen and Revsbech, 1983) at an oxygen/sulfide interface to optimize oxidation of sulfide as an energy yielding metabolic mode. Figure 3A shows the surface of black band at 8:00 am. The white surface is due to highly refractile granules of elemental sulfur present in cells of *Beggiatoa* as a result of intracellular sulfide oxidation. Figure 3B shows the same part of the band at 3:15 pm the same day. As light increased (and oxygen accumulated), *P. coralliticum* replaced *Beggiatoa* as the surface microbe. Note that black band has moved across the coral polyps.

The oxygen and sulfide microprofiles measured in this study are consistent with observations from other types of microbial mat communities such as epipelagic periphyton on lake sediments (Carlton and Wetzel, 1987), intertidal mud flats (Bebout et al., 1987; Pinckney and Zingmark, 1991), and mats found in sulfide-rich hot spring outflows (Richardson and Castenholz, 1987). Characteristics seen almost universally in photosynthesizing mat communities include oxygen supersaturation near the surface (where the rate of photosynthesis is maximal), a nearly linear oxygen concentration gradient just below the maximum, and low or zero oxygen concentration at the base of the community. The oxygen and sulfide profiles together are indicative of the respiratory oxygen demand extant in the lower strata of the black band community, as well as chemical reduction of oxygen by sulfide present near the base of the band. The

observation of movement of Beggiatoa to the surface of the band during darkness is also quite similar to vertical migrations in sulfide-rich microbial mats, which includes both migration of the microorganisms and vertical migrations of the oxygen/sulfide interface. Such profiles are controlled by diel changes in surface oxygenic photosynthesis coupled with bacterial sulfidogenesis at depth, (Jørgensen et al., 1979), and have been shown to control vertical migrations of cyanobacteria (Richardson and Castenholz, 1987) as well as Beggiatoa (Jørgensen and Revsbech, 1983).

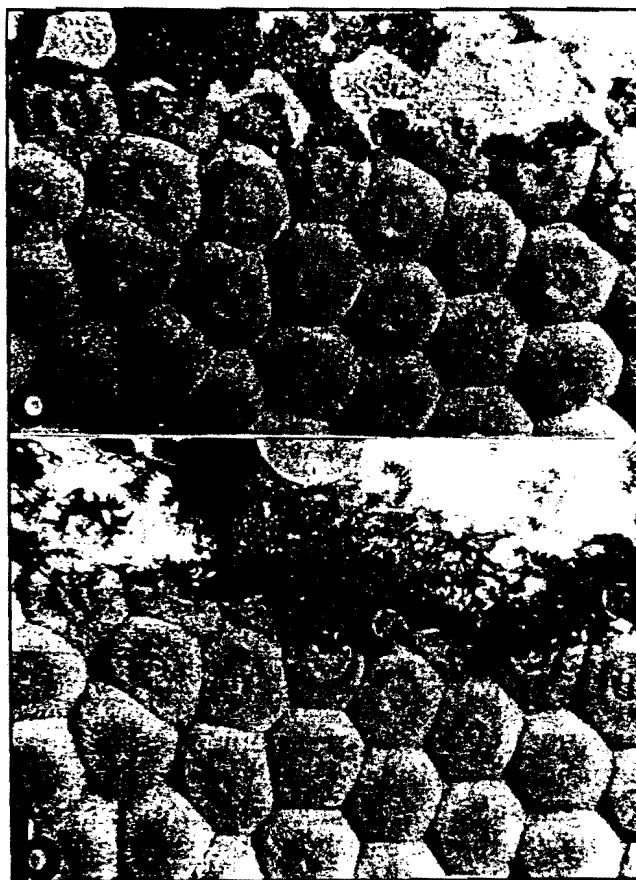


Figure 3. In situ vertical migration of Beggiatoa in black band on M. carvernosa. A. Beggiatoa on surface at 8:00 am. B. Phormidium on surface at 3:15 pm. Nail diameter is 9 mm.

Most microbial mat communities are present on sediment or accumulated mat material undergoing diagenesis, both of which serve as carbon sources to the surface, photosynthetically active layer. The black band community is the first case we know of a microbial mat community present on an animal, and actively killing the animal. The high concentrations of sulfide, plus anoxia, present in black band support the hypothesis that coral mortality associated with black band disease is related to chemical microenvironments. Sulfide is an inhibitor of aerobic respiratory electron transport, acting similar to cyanide as a respiratory poison. It is highly unlikely that coral tissue can survive exposure to anoxic conditions with 80 μ M sulfide. Thus, as black band moves over coral, it exposes coral tissue to a toxic chemical microenvironment which may very

well be the specific mechanism of death of the coral. Subsequent to coral death, the degradation of coral tissue may serve as a source of both organic carbon and nutrients to the black band community, thus ensuring a nutrient source in the nutrient poor environment of coral reefs.

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REFERENCES

- Antonius, A. 1973. New observations on coral destruction in reefs. Assoc. Isl. Mar. Lab. Carib., Univ. Puerto Rico, Mayaguez, Abst. 10:3.
- Antonius, A. 1981. The "Band" diseases in coral reefs. Proc. 4th. Intl. Coral Reef Symp. 2:7-14.
- Antonius, A. 1985. Coral diseases in the Indo-Pacific: A first record. PSZNI Mar. Ecol. 6:197-218.
- Antonius, A. 1988. Distribution and dynamics of coral diseases in the eastern Red Sea. Proc. 6th Intl. Coral Reef Symp. 3:145-150.
- Bebout, B.M., H.W. Paerl, K.M. Crocker and L.E. Prufert. 1987. Diel interactions of oxygenic photosynthesis and N₂ fixation (acetylene reduction) in a marine microbial mat community. Appl. Env. Microbiol. 53:2353-2362.
- Carlton, R.G. and R.G. Wetzel. 1987. Distributions and fates of oxygen in periphyton communities. Can. J. Bot. 65:1031-1037.
- Castenholz, R.W.. 1982. Motility and taxes, p. 413-440. In: N.G. Carr and B.A. Whitton [Eds.], The Biology of Cyanobacteria. University of California Press, Berkeley.
- Edmunds, P.J. 1991. Extent and effect of black band disease on a Caribbean reef. Coral Reefs. 10:161-165.
- Garcia-Pichel, F. and R.W. Castenholz. 1990. Comparative anoxygenic photosynthetic capacity in 7 strains of a thermophilic cyanobacterium. Arch. Microbiol. 153:344-351.
- Garrett, P. and H. Ducklow. 1975. Coral diseases in Bermuda. Nature. 253:349-350.
- Hudson, J.H. 1981. Growth rates in *Montastraea annularis*: A record of environmental change in the Key Largo Coral Reef Marine Sanctuary, Florida. Bull. Mar. Sci. 31:444-459.
- Jørgensen, B.B. and N.P. Revsbech. 1983. Colorless sulfur bacteria, *Beggiatoa* sp. and *Thiovulum* sp. in O₂ and H₂S microgradients. Appl. Env. Microbiol. 45:1261-1270.

- Jørgensen, B.B., N.P. Revsbech, T.H. Blackburn and Y. Cohen. 1979. Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. *Appl. Env. Microbiol.* 38:46-58.
- Knowlton, N., E. Weil, L.A. Weigt and H.M. Guzman. 1992. Sibling species in Montastraea annularis, coral bleaching, and the coral climate record. *Science* 255:330-333
- Peters, E.C. 1993. Diseases of other invertebrate phyla: Porifera, cnidaria, ctenophora, annelida, echinodermata. In: Couch and Fournie tEds.] *Adv. Fish. Science, Pathology of Marine and Estuarine Organisms*, p. 393-449. CRC Press.
- Pinckney, J. and R.G. Zingmark. 1991. Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. *Mar. Ecol. Prog. Ser.* 76:81-89.
- Porter, J.W. and O.W. Meier. 1992. Quantification of loss and change in Floridian reef coral populations. *Amer. Zool.* 23:625-640.
- Ramos-Flores, T. 1983. Lower marine fungus associated with black line disease in star corals (Montastraea annularis E.&S.) *Biol. Bull.* 165:429-435.
- Revsbech, N.P. and D.M. Ward. 1983. Oxygenic microelectrode insensitive to medium chemical composition: Use in an acid microbial mat dominated by Cyanidium caldarium. *Appl. Env. Microbiol.* 45:755-759.
- Revsbech, N.P. and B.B. Jørgensen. 1986. Microelectrodes: Their use in microbial ecology. *Adv. Microb. Ecol.* 9:292-352.
- Revsbech, N.P. 1989. Diffusion characteristics of microbial communities determined by use of oxygen microsensors. *J. Microbiol. Methods.* 9 ~ 122.
- Richardson, L.L. 1992. Horizontal and vertical migration patterns of microbial consortia associated with black band disease. *ASLO Abst.*
- Richardson, L.L. and R.W. Castenholz. 1987. Diel vertical movements of the cyanobacterium Oscillatoria terebriformis in a sulfide-rich hot spring microbial mat. *Appl. Env. Microbiol.* 53:2142-2150.
- Rutzler, K. and D.L. Santavy. 1983. The black band disease of Atlantic reef corals. I. Description of the cyanophyte pathogen. *P.S.Z.N.I. Mar. Ecol.* 4:301-319.
- Rutzler, K., D.L. Santavy and A. Antonius. 1983. The black band disease of Atlantic reef corals. III. Distribution, ecology, and development. *P.S.Z.N.I. Mar. Ecol.* 4:329-358.
- Vogel, S. 1981. *Life in Moving Fluids*. Willard Grant, Boston.
- Williams, E.H. and L. Bunkley-Williams. 1990. The world-wide coral reef bleaching cycle and related sources of coral mortality. *Atoll. Res. Bull.* 335:1-71.