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The Black Band Disease of Atlantic Reef Corals.

III. Distribution, Ecology, and Development

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With 15 figures and 3 tables

Key words: Coral disease, *Cyanophyta, Cyanobacteria, Scleractinia,* coral reef, ecology, toxicity, finestructure, disease community.

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Abstract. Some reef building corals in the western Atlantic are susceptible to an infection known as black band disease that is caused by the cyanophyte (cyanobacterium) *Phormidium corallyticum* RÜTZLER & SANTAVY. Field observations on the barrier reef of Belize and on reefs of the Bermuda platform indicate the disease is fairly common in susceptible species. Coral tissue destruction monitored *in situ* in Belize reveals rapid spreading rates and seasonality of the disease. Laboratory experiments confirm that *P. corallyticum* is the etiologic agent and we speculate that a toxic exudate is the cause of histolysis observed in coral penetrated by the organism. Coral tissue is further broken

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down by a rapidly developing disease microcommunity, including bacteria and ciliate protozoans, identified with the aid of light, SEM, and TEM photomicrography. Study of *P. corallyticum* in culture away from its host coral reveals several ecological requirements, including dependence for optimal growth on yet unidentified organic substances contained in coral tissue. Healthy corals inoculated with various control organisms – including the gliding bacterium *Beggiatoa,* a filamentous chlorophyte, and six cyanophytes other than *P. corallyticum* – did not develop the disease. Gorgonacean corals, too, can be artificially infected with *P. corallyticum*, but naturally occurring gorgonian bands are composed only of noninfectious cyanophyceans.

Problem

Diseases and other structural and functional anomalies are now recognized to be common occurrences among marine invertebrates. The latest survey (LAUCKNER, 1980) of the effects of various agents (ranging from viruses to fishes) on many types of invertebrate hosts concludes that scleractinian corals are remarkably unaffected by known diseases, particularly those caused by viruses and bacteria. Furthermore, it notes that many morphological abnormalities of these corals are traceable to nonpathological species interactions, for example, with facultative predators and temporary parasites, or merely with commensals and competitors for space.

A few recent reports of microbial attacks on stony corals, however, were not included in LAUCKNER's (1980) review. From field observations on the barrier reef of Belize (British Honduras) and on reefs off the Florida Keys, ANTONIUS (1973) identified the bluegreen alga [cyanobacterium] *Oscillatoria submembranacea* ARDISSONE & STRAFFORELLO as a coral killer. Unaware of ANTONIUS' surveys, GARRETT & DUCKLOW (1975) documented a dark brown to black disease line on coral species of the genera *Diploria* and *Montastrea* in Bermuda. Their microscope examination of these coral heads revealed the presence in considerable numbers of the gliding bacterium *Beggiatoa,* which they thought initiated the disease. The bacteria *Desulfovibrio* and *Beggiatoa,* both associated with the Bermuda disease, were also connected with coral death in the Red Sea (MITCHELL & CHET, 1975). There, corals of the genus *Platigyra* were observed to produce large quantities of mucus when they became exposed to petroleum hydrocarbons. Predatory bacteria attracted to the liberated mucus evidently invaded the stressed coral tissue and participated in the destruction of the cnidarian colonies.

Inoculation with a bluegreen alga identified as *Oscillatoria submembranacea* ("black band disease") in Florida and the Caribbean (summarized by ANTONIUS, 1976; 1981) indicated that infections take place where the coral tissue is injured. At the same time, other phenomena such as shutdown reaction and white band disease in scleractinians could not be related to microbial infection (ANTONIUS, 1977; 1981). Meanwhile, DUCKLOW & MITCHELL (1979) attempted to artificially trigger the "black line disease" in a zoanthid cnidarian in Bermuda. Scanning electron microscopy demonstrated that both cyanobacteria and sulfide oxidizing bacteria (*Beggiatoa* spp.) occur in the disease matrix, but neither of these microbes was identified as the etiologic agent.

Seeking further information about the cause and development of black band disease, we conducted comparative studies in Belize, where the disease was

discovered, and on the Bermuda platform, a geographically distant and climatically different site and location of past investigations. Our initial work (RÜTZLER & SANTAVY, 1983) on the morphology and finestructure of the suspected cyanophyte pathogen led to taxonomic reevaluation and comparison of Belize and Bermuda populations of this organisms, named *Phormidium corallyticum* RÜTZLER & SANTAVY. TAYLOR's (1983) culture work added information to the taxonomic characterization of the species. In the present study we examine factors such as distribution and spreading rates of the cyanophyte, along with experimental evidence for its coral-pathogenic properties, and describe disease community characteristics and development on the basis of light as well as scanning and transmission electron microscopy. In addition, we consider the ecological requirements of the black band cyanophyte and assess its actual and potential impact on the coral reef community.

Material and Methods

Field observations and collections were made in shallow reef areas (South Water and Carrie Bow cuts; back reef, near permanent transect; 0.5–3.0 m) near Carrie Bow Cay on the barrier reef of Belize (RÜTZLER & MACINTYRE, 1982) and on similar reefs (Three Hill and Sea Venture shoals; 2–4 m) on the Bermuda platform. Field work was conducted in January, February, April, May, June, and December of 1980–1983 in Belize, and in July of 1981, 1982 in Bermuda. Laboratory work took place near the field study locations, at the Smithsonian Institution's field laboratory at Carrie Bow Cay and at the Bermuda Biological Station. Live material transported to the National Museum of Natural History, Washington, was studied in further experiments and used in fixations for electron microscope observations.

Black band disease was sampled underwater by chiseling and scraping off afflicted parts of the corals and pipetting (with basting syringe) the loosened material into sealable plastic bags. These samples were kept in the open shade in covered glass dishes containing seawater (changed daily in the morning) at ambient temperature (28–30 °C) and served as stock cultures for experiments. Experiments involved inoculating primarily scleractinian corals, but some octocorals and one sponge were also tested. Inocula were the suspected pathogen *Phormidium corallyticum*, other members of the disease community, as well as some free living cyanophytes tentatively identified from HUMM & WICKS' (1980) manual. Inoculations were performed by puncturing the coral surface (including the surface of the limestone skeleton) with a stainless steel pick and inserting a plug of the foreign organisms (ANTONIUS, 1981). Clean, although not entirely axenic, cultures of *Phormidium* were obtained by repeatedly letting masses of filaments spread in filtered (0.45 μm) seawater and using a light gradient to attract the filaments away from associated nonautotrophic organisms (see Fig. 6 a). The remaining coral debris and associated microbes, which could be easily separated from the large residual *Phormidium* filaments under a microscope, were used as an infection control. The effect of antibiotics on *Phormidium* was tested using gentamicin sulfate (50 μg and 100 μg per ml seawater), but this broad spectrum bactericide was not employed in the purification of cultures.

The spreading of black band disease on coral heads in the field was monitored by marking its active edge (*Phormidium* mat bordering coral tissue) with nails and mapping and photographing the condition over time. Spreading rates were measured along unobstructed straight transects (2 cm apart) radiating from the original disease line. Area measurements were made planimetrically from true-area maps. In the laboratory the progress of the disease was documented by measurements from the point of inoculation and by close-up photography. Inoculated corals were kept either in running seawater at ambient temperature (30 °C) or in covered glass bowls, containing magnetically stirred seawater, at selected temperatures between 18 °C and 35 °C. Illumination was varied from full daylight to complete darkness and measured in lux by a Luna-Pro (Gossen) photographic exposure meter. The values were related to total radiation on a cloudless day in April (482 g cal $\cdot$ cm^{-2} $\cdot$ d^{-1}) recorded by a mechanical pyranograph (WeatherMeasure R401, Sacramento, California).

A number of attempts were made to culture *Phormidium* in seawater, enriched seawater, and on agar media but they were judged unsuccessful or only moderately successful. We have since learned that our expectations of marine cyanobacterial culturing may have been too high (C. LIPSCHULTZ, Smithsonian Radiation Biology Laboratory, and P. PIENTA, American Type Culture Collection; pers. comm.). Liquid media included plain filtered habitat seawater (changed daily), JONES', F/2, and ASN-III; each of these was also prepared with 1 % agar for plating. JONES' media were prepared according to JONES *et al.* (1963); the salts and trace element mix (courtesy C. LIPSCHULTZ) were dissolved in sterilized rain water. F/2 ingredients (MCLACHLAN, 1973; courtesy J. JOHNSON) were added in double concentration to autoclaved habitat seawater. We prepared ASN-III according to RIPPKA *et al.* (1979), but added filter-sterilized vitamin B_{12} to the autoclaved habitat seawater.

Electron microscope techniques included 3 % glutaraldehyde-1 % osmium tetroxide fixation in phosphate buffer-sucrose mixture, as outlined by RÜTZLER & SANTAVY (1983). Sections stained with uranyl acetate were photographed by a Zeiss EM9 S-2 transmission electron microscope (TEM). Critical point dried (in liquid CO_2) whole specimens were gold coated and photographed by a Cambridge Stereoscan Mark IIA scanning electron microscope (SEM). Minor experimental procedures and adaptations are included with the results.

Results

1. Field observations

a. Macroscopic appearance and distribution

Dead or partly dead corals are a common sight in reef areas, most of them still identifiable although their skeletons may be coated by algal turfs or crusts of sponges and other invertebrates. By that stage, the original cause of destruction is difficult to determine – it could have been disease, storm damage, low tide exposure, or one of many predators or competitors. Corals affected by active black band disease, on the other hand, are unmistakable. Progressing rapidly, the disease leaves behind a bright white zone of tissue-depleted coral skeleton that contrasts with the black band of intertwined *Phormidium corallyticum* filaments (Figs. 1, 2). Large coral heads of the species *Montastrea annularis* (ELLIS & SOLANDER) and *Diploria strigosa* (DANA) in depths of 0.5 m to 4.0 m appear to be particularly susceptible to the disease and are certainly the most conspicuous west Atlantic corals found with it. In Belize, *M. annularis* appears to be the species most commonly afflicted, whereas on Bermuda reefs *D. strigosa* is the regular victim. This observation, however, is probably a reflection of the relative abundance of the host species rather than geographical differences in coral susceptibility.

Typically, the top of a diseased coral head is more or less covered with turf, and a barren white zone one to several centimeters wide occurs just below it, followed by a black band and then the live coral. The algal turf is missing in most small (recent) and some larger (very active) areas of infection. The blackish brown *Phormidium* band is 1–40 mm wide or more (8 mm on the average), overlies decomposing coral tissue and is firmly anchored to the substrate along the edge bordering the live coral where strands of filaments extend into the host tissue. Dustings of white at the top of the band, usually at some distance from the active infection, are accumulations of sulfur-fixing bacteria (genus *Beggiatoa*). The cyanophyte mat readily flakes off with sudden water movement. Therefore, wide and continuous bands are most readily found during periods of

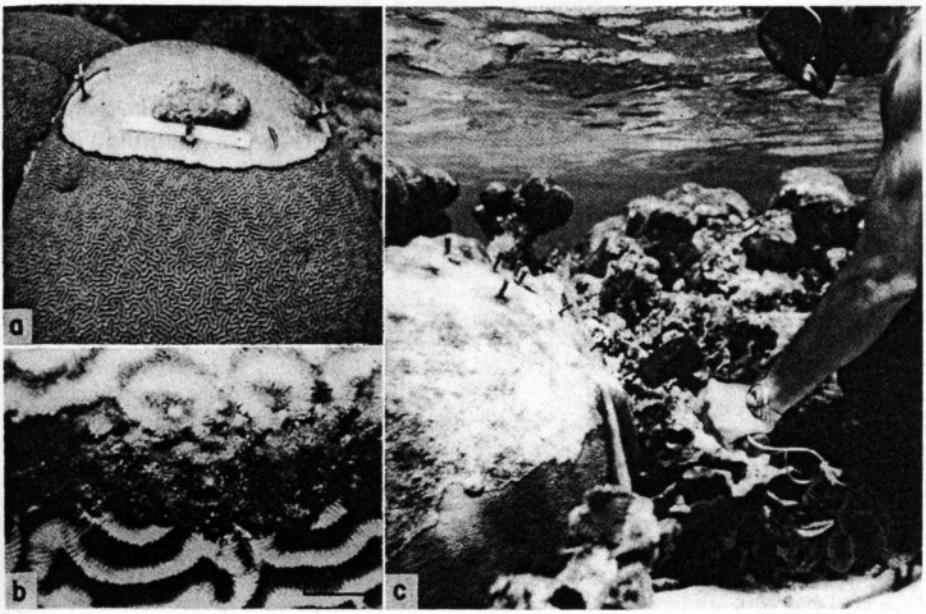


Fig. 1. Underwater views of the effect of black band disease on shallow (1 m) corals in the back reef near Carrie Bow Cay, Belize. a: *Diploria strigosa* photographed on 29 May, 1982; the three nails mark the disease line of 7 May, 1982; the scale weighted down by a piece of rubble is 15 cm long. b: Close-up of the disease line. (Scale = 10 mm). c: *Montastrea annularis* head marked at the disease line (nails on top) in May, 1972; rephotographed in May, 1974.

extended calm. At least eight other species of *Scleractinia* have been found infected with *Phormidium* (Table 1). Although reliable reports are available only for Bermuda, Florida, the Bahamas, Virgin Islands, Jamaica, and Belize (ANTONIUS, 1981; TAYLOR, 1983) it can be assumed that the problem occurs throughout the west Atlantic reef province. No group other than *Scleractinia* has yet been found naturally infected by *Phormidium,* but some gorgonians exhibit a similar condition that is briefly discussed below.

Seasonality of the disease has been studied only to a small extent. In Belize we noted peak developments in May and June, lesser abundance in February, March and April, and only a few exceptional and insignificant infections during December and January (no surveys made in other months). In Bermuda the condition is prevalent between July and September, rare in November (ANTONIUS, 1981), and almost impossible to find in January (W. STERRER, pers. comm.).

b. Growth rates

No naturally occurring mechanisms of new black band infections have been directly observed. We have, however, monitored a number of existing conditions on a reef just south of Carrie Bow Cay (Figs. 1 a; 2) and measured the spreading over a period of several weeks to obtain average and maximum

values. Multiyear monitoring turned out to be pointless because in each new "season" the disease does not resume its activity in the same tissue area or coral colony where it stopped (Fig. 1 c).

In April and May 1982, three coral heads (two *Montastrea annularis* and one *Diploria strigosa*) with infections were mapped, photographed, and the disease

Table 1. Field observations on natural occurrence of black band disease and laboratory experiments testing susceptibility of various lower invertebrates to artificial infection by *Phormidium corallyticum;* some data from ANTONIUS (1981) and TAYLOR (1983). (++ = common [field], or fast growing [experimental]; + = rare, or slow growing; +? = disease nature uncertain; – = never found, or no disease development; 0 = no data).

Organism	Field observations	Laboratory experiments
Dictyoceratida		
Aplysina fistularis (PALLAS)	–	+[a]
Milleporina		
Millepora sp.	+?	–
Scleractinia		
Acropora cervicornis (LAMARCK)	–	–
Acropora palmata (LAMARCK)	–	–
Acropora prolifera (LAMARCK)	–	–
Agaricia agaricites (L.)	–	0
Agaricia tenuifolia DANA	–	0
Siderastrea radians (PALLAS)	–	–
Siderastrea siderea (ELLIS & SOLANDER)	+	0
Porites astreoides LAMARCK	–	–[b]
Favia fragum ESPER	+	0
Diploria clivosa (ELLIS & SOLANDER)	+	+
Diploria labyrinthiformis L.	+	+
Diploria strigosa (DANA)	++	++
Colpophyllia natans (HOUTTUYN)	–	0
Solenastrea hyades (DANA)	+	0
Montastrea annularis (ELLIS & SOLANDER)	++	++
Montastrea cavernosa (L.)	+	+
Meandrina meandrites (L.)	+	0
Dichocoenia stokesi MILNE EDWARDS & HAIME	+	+
Dendrogyra cylindrus EHRENBERG	–	+
Mycetophyllia ferox WELLS	–	0
Mycetophyllia lamarckiana MILNE EDWARDS & HAIME	–	0
Gorgonacea		
Plexaura homomalla (ESPER)	–	+
Plexaurid, unidentified	+?	0
Gorgonia flabellum L.	+?	+
Gorgonia ventalina L.	+?	+

[a] spreading without tissue destruction
[b] except in the presence of pollutants (ANTONIUS, 1981)

line marked with nails. One to four visits were made at intervals of 3–20 days in order to measure and photograph the progress of the disease (plotted in Table 2; coral specimen *Montastrea* I is depicted in Fig. 2, *Diploria* in Fig. 1 a, b). In December of the same year, we found the disease line had entirely disappeared, but the area of dead coral indicated that the disease had been active for several more months beyond May. When first marked, the semicircular disease line on all three coral heads was facing west; subsequent spreading moved in other directions, but to a lesser extent. Table 2 a shows a maximum linear spread of 6.2 mm per day, with a mean of 3.8 mm per day for the entire 24 cm band of the most active infection. Mean spreading rate for all three corals combined is 3.1 mm per day. The maximum distance covered by the disease on one coral was 160 mm during 41 days of observation in April–May, 400 mm for the duration of the infection (recorded in December 1982). Area measurements (Table 2 b) show maximum spreading of 28.5 cm^2 per day, with a mean of 20.3 cm^2 per day for the most seriously affected specimen. Combined mean spread is 14.1 cm^2 per day. Maximum surface area destroyed on one coral during the period of direct observation was 745.5 cm^2; during the entire disease period it reached 4820.8 cm^2. The shortest average "doubling time" of the diseased area is calculated as 2.1 days. Doubling time is the time it takes for the area of depleted tissue to equal the area of the originally recorded black band and does not imply the doubling of the *Phormidium* band. Despite certain fluctuations, the band had a mean width of only 8 mm and did not exceed 20 mm on the measured corals.

Table 2a. Linear field measurements of coral destruction caused by black band disease at Carrie Bow reef, April–May, 1982; maximum seasonal destruction recorded during re-survey in December, 1982.

Coral	Number of measurements	Time period recorded (d)	Spreading rate (mm · d^{-1})			Max. radius destr. (mm)	
			min.	max.	mean	May	Dec
Montastrea I	36	41	2.3	6.2	3.8	160	295
Montastrea II	28	20	0.3	6.0	2.3	48	400
Diploria	4	5	2.4	4.0	3.2	20	370

Table 2b. Area measurements (planimetric) from maps prepared of infected corals presented in Ta
(> = more than recorded; 0 = no data).

Coral	Number of measurements	Time period recorded (d)	Coral live at start (cm^2; approx.)	Black band at start (cm^2)	Spreading rate ($cm^2 \cdot d^{-1}$)			Coral killed (cm^2)		A d ti
					min.	max.	mean	May	Dec	
Montastrea I	4	41	1600	19.2	13.8	28.5	20.3	745.5	1509.6	
Montastrea II	2	20	> 5700	22.4	5.4	12.8	9.1	130.2	4820.8	
Diploria	1	5	> 7200	32.0	0	0	12.8	64.0	3961.6	

When the corals were examined again in February 1983, a few cases of black band disease had appeared elsewhere on the reef. None of the previously afflicted corals showed infections, however, and microscopic examination of tissue taken where the disease had stopped failed to reveal any residual *Phormidium* filaments.

According to the data from different observation periods, the rate of growth, or spread, of black band disease varies considerably. Its linear growth in *Montastrea* I increased from a mean of 3.5 mm per day in April to 4.7 mm in mid-May, then dropped to 2.9 mm during the second half of May. The drop can partly be explained by the fact that the disease encountered some dead areas of coral, deep furrows, and vertical areas having less light exposure. By December this coral head was almost entirely consumed, with only three small tissue blotches remaining on the eastern and northern base of the head. Although slowed growth was also evident in *Montastrea* II – from 3.1 mm per day in early May to 1.4 mm in the second half of the month – later the rate must have increased again because by December this coral had suffered more extensive damage than had *Montastrea* I. *Diploria* cannot be used for comparison because only one growth period was recorded in May.

2. Laboratory observations

a. *Phormidium corallyticum*, behavior

Subsequent to our detailed morphological study of this organism (see RÜTZLER & SANTAVY, 1983), we made the following observations from fresh scrapings of black band disease containing the entire microcosm as well as from coral tissue and skeleton fragments. After about an hour's time in a glass bowl, the dead and dying coral tissue, including mucus and free zooxanthellae, settles out on the bottom and serves as substrate for the microorganisms. Gliding bacteria (*Beggiatoa*) and other bacteria, together with small ciliates and some unicellular eukaryotic algae, remain closely associated with the organic debris. *Phormidium corallyticum* tends to separate and form a mat of interwoven filaments over the entire residue, or a thin veil that remains suspended in the surface film of the water (Fig. 6). When a flat object such as a microscope slide is brought into contact with a *Phormidium* mat, it, too, is soon covered by a film of entangled filaments. When a tuft of cyanophyte material is teased out with fine forceps and dropped into a dish with seawater, it first curls up into a tight ball. After about ten minutes, some probing filaments protrude from the coil, which then gradually flattens and becomes star shaped, with about 20 rays composed of 3–20 filaments each extending in all directions of the substrate plane. Most filaments that make up the rays are staggered and have pointed tips. Some are twisted around others but all are in constant swaying motion. Individual filaments glide as well as oscillate, their free ends tracing the surface of a cone; little or no rotation is evident. Gliding movement takes place predominately in one direction, but reversal is possible and spontaneous. Eventually, the intertwined filaments spread out and again form the familiar thin film.

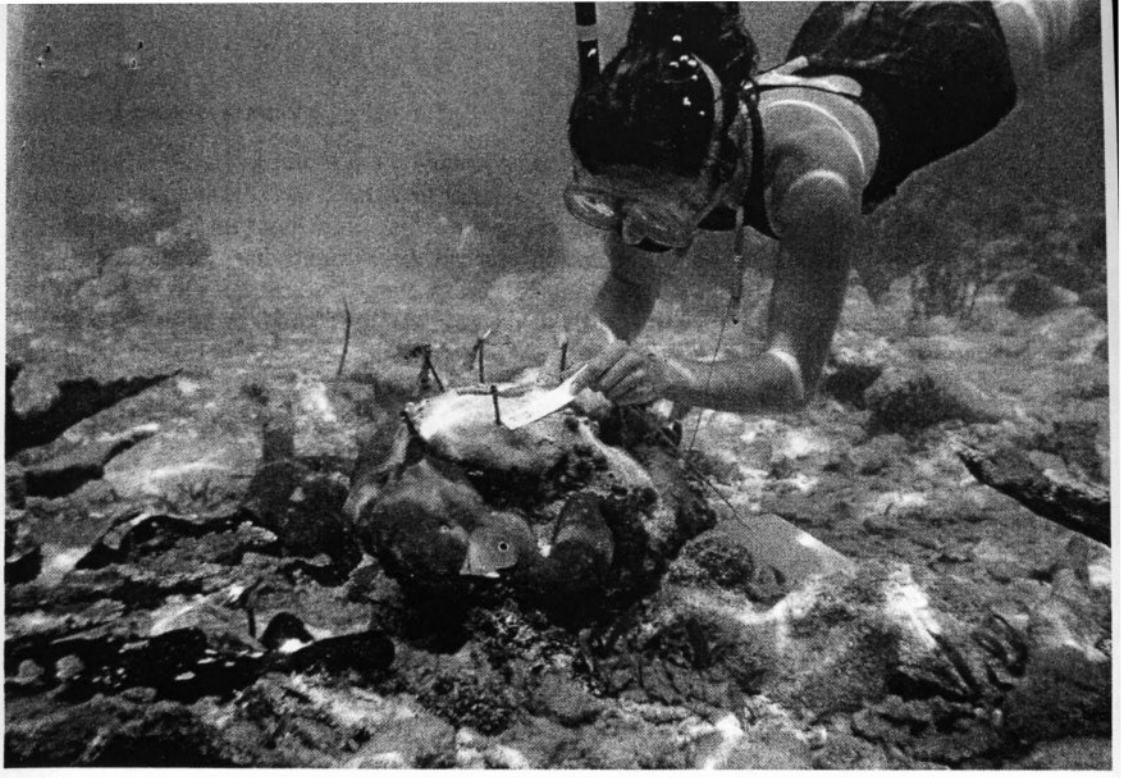
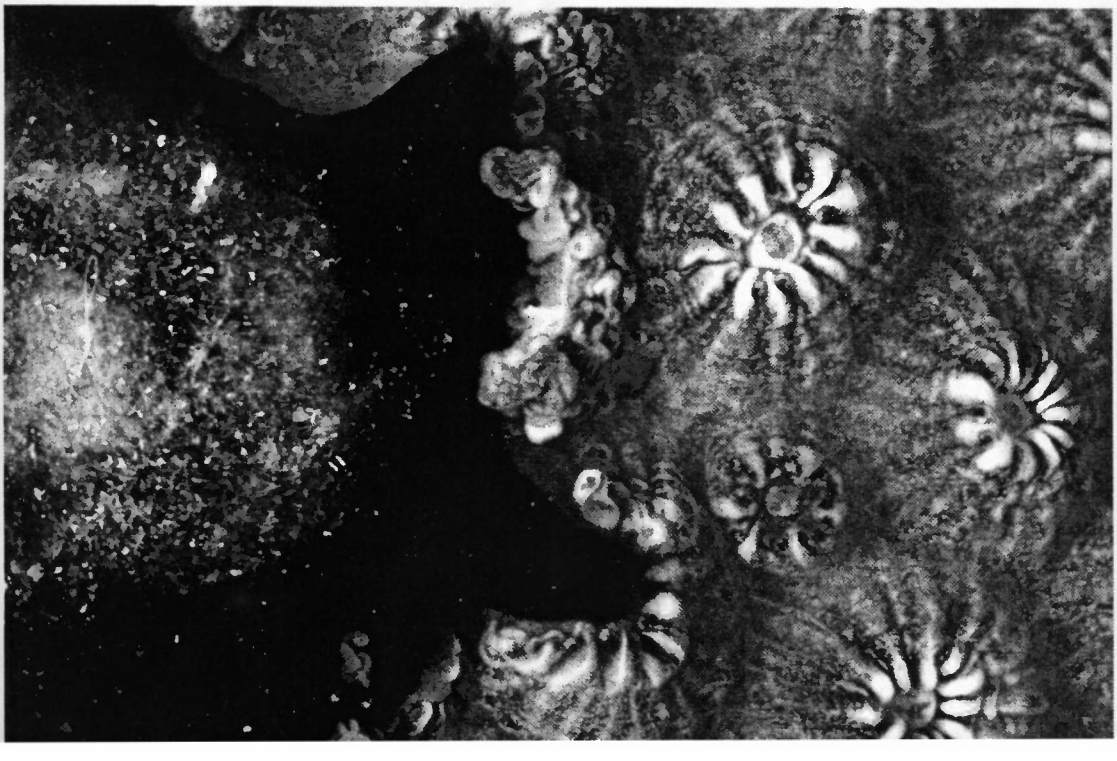


Fig. 2. Characteristic back reef environment with coral rubble, *Acropora palmata,* and diseased *Montastrea annularis* near the southern reef crest of Carrie Bow Cay; tagged nails mark progress of black band disease. (Scale in diver's hand is 15 cm).

Fig. 3. Photomacrograph of *Montastrea annularis* infected by *Phormidium corallyticum* black band disease. (Picture width = 35 mm).



b. Primary infection of *Scleractinia*

No direct evidence is available to explain where, how, and under what circumstances reef corals first become infected by black band disease. Plankton tows over reef areas affected by black band disease have so far been void of drifting bluegreen algae other than tufts of the planktonic *Trichodesmium* species. Field observations indicate, however, that bands of interwoven *Phormidium corallyticum* filamants are attached only along the boundary of living tissue in the host coral and that even moderate wave action can dislodge them in flakes or sheets.

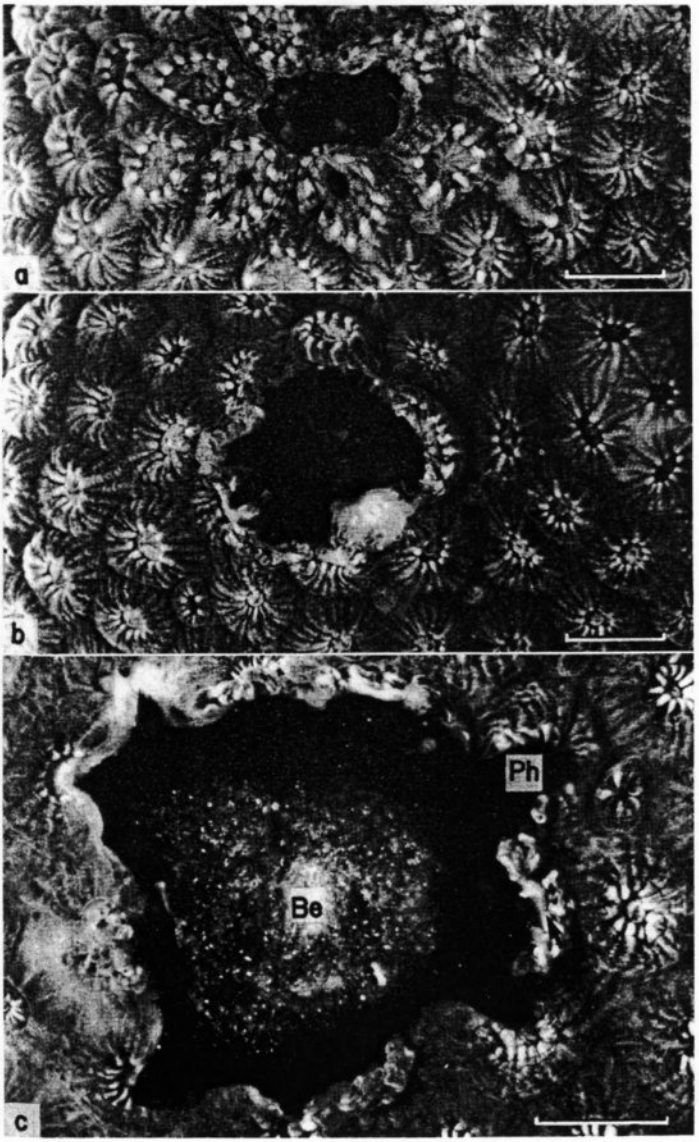


Fig. 4. Black band disease inoculation experiment using *Montastrea annularis* kept in trays with running seawater in the open shade (Carrie Bow Cay, 27 April–14 May, 1981). a: One day after infection; note *Phormidium* material appearing inside mouths of two polyps. b: After four days the initial infection area has increased about three times. c: After 16 days; tunneling *Phormidium* (Ph) strands at the periphery are causing coral mesenteries (arrow) to be expelled; whitish material in the center of the infection is *Beggiatoa* (Be). (Scales = 5 mm).

This light material can float great distances, particularly when aided by trapped gas bubbles from its own photosynthesis.

In the laboratory, all new primary infections were induced artificially. A number of tests were employed and their success or failure taken as an indication of what processes in nature might lead to similar results. Inoculations were used to identify the disease causing organism, to determine the susceptibility of different host species to infection, and to quickly survey the disease process under various conditions.

The most successful method for starting an infection on a suitable coral species has been to insert a wad of algae into a deep, narrow puncture that extends through the soft tissue into the underlying skeleton (ANTONIUS, 1981). A dense ball of cyanophyte filaments is obtained by teasing material out of the mat in the culture vessel and letting it aggregate in a separate dish for about an hour. Only minutes after the ball is wedged into the puncture, it flattens and bundles of filaments – spirally gliding along each other – start to penetrate the tissue lesion. In most cases this marks the beginning of the disease process, which becomes established within four to six hours (Figs. 3, 4). Only few healthy coral specimens infected in this way are able to shed the foreign organisms by themselves and only if tentacle and ciliary movements (which usually occur at night) begin shortly after infection. Even then, single filaments may remain in the wound and spread. On some stressed animals flakes of cyanophyte material, even though transported away from the lesion by ciliary action at night, have been observed to enter through the mouths of contracted polyps during the inactive period of the following day.

Infection can also take place by direct contact with the diseased portion of another coral. *Phormidium* trichomes enter at the point of contact where coral tissue is stressed or injured. When healthy and diseased corals are kept close together in the same aquarium but are not in direct contact (separated by a gap of no more than 2 mm), infection does not occur. Coils of the cyanophyte placed loosely on top of active or inactive healthy corals are enveloped in mucus and swept away by ciliary movement. *Phormidium* material placed on a 150 μm mesh net (bolting cloth) supported 5 mm above a healthy coral surface *(Diploria)* moves to the lower surface of the net and forms a mat there, but is unable to enter coral tissue. In similar experiments with injured corals, however, the cnidarian is readily infected even if the lesions are as much as 15 mm away from the cyanophyte web. Infection also takes place if the net supporting algal material touches and stresses the coral at any point.

c. Tissue penetration and development of disease community

Freshly harvested material from the black band is generally contaminated by decaying coral tissue and mucus and accompanied by a community of microscavengers (mainly bacteria and ciliates), and some unicellular algae. Although mechanical separation of the large *Phormidium corallyticum* is fairly easy (see Material and Methods), we obtained no axenic cultures for observation. Minutes after a tissue lesion is inoculated with *Phormidium,* bundles of 3 to 20 or more filaments separate from the cyanophyte mass and penetrate below the

coral ectoderm (Fig. 5 a, b). Individual filaments glide smoothly along each other with their free and mostly pointed ends oscillating somewhat jerkily (Fig. 5 c). Subectodermal tunneling takes place in all directions and can progress at a speed of as much as 20 mm during a 12 h period. Under the microscope it is

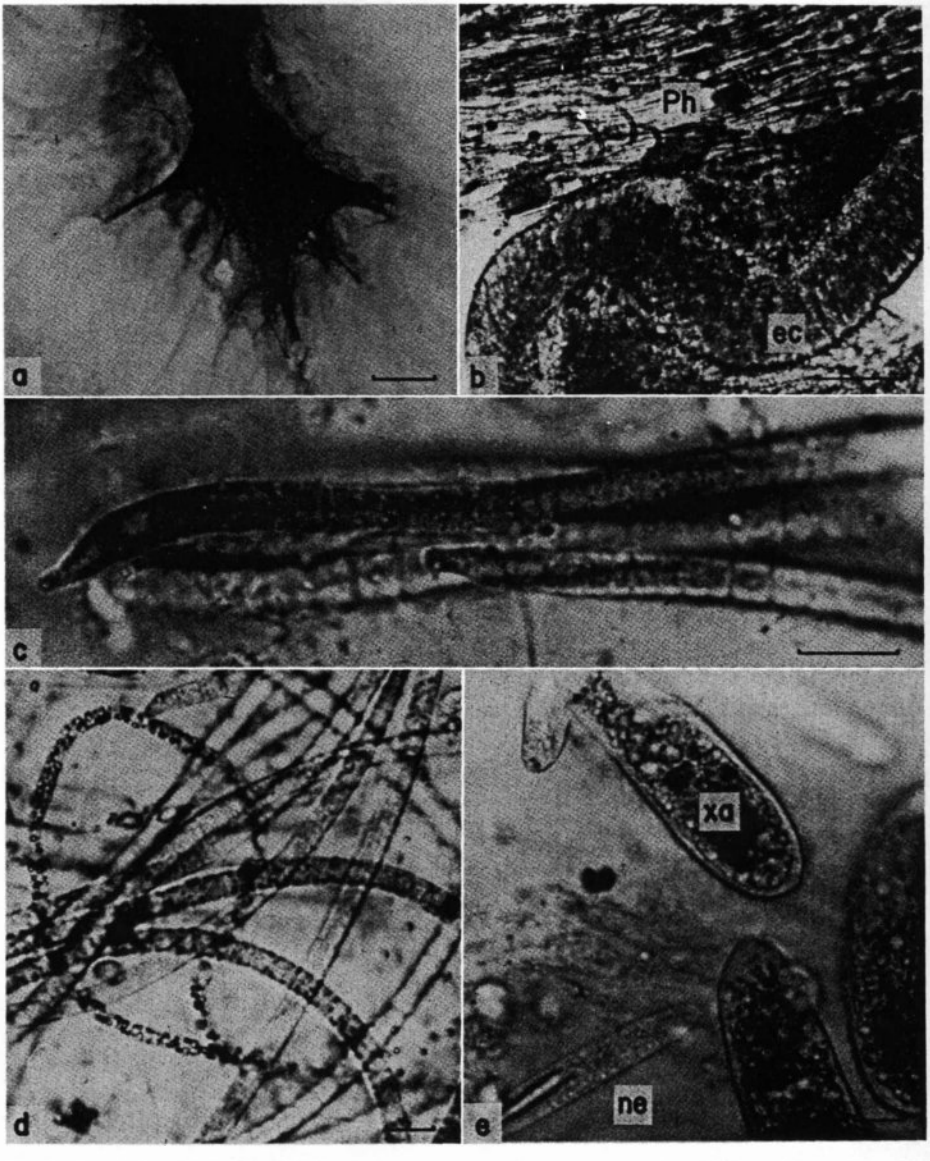


Fig. 5. Black band infection of *Diploria strigosa*. a: Photomacrograph of *Phormidium* filaments penetrating coral ectoderm. b: View similar to (a) in cross section, cyanophycean trichomes (Ph) are on top, coral ectoderm (ec) below. c: Photomicrograph of pioneer *Phormidium* filaments. d: *Phormidium* and *Beggiatoa* (granular) inside decomposing coral tissue. e: Ciliates (*Philaster* sp.) filled with consumed coral zooxanthellae (xa); note isolated unexploded nematocyst (ne). (Scales = 1 mm for a; 100 μm for b; 10 μm for c, d, e).

evident that tissue penetration is accomplished not so much by mechanical puncturing as it is by histolysis, presumably after cell membranes are broken down by chemical action. No intact coral cells can be seen in contact with or in the immediate vicinity of the *Phormidium* filaments. A mucus pulp containing numerous zooxanthellae and a number of free coral cells, including exploded and unexploded nematocysts, is characteristic of this stage (Fig. 8 a, c). Wherever *Phormidium* tunneling reaches the base of a polyp, entire mesenterial filaments become dislocated and protrude or are expelled from the polyp's mouth together with pioneering algal strands. Once histolysis is under way, algal filaments can break through the ectoderm anywhere from below. Reentry through this tough ciliated tissue layer, however, seems difficult or impossible, whereas penetration into the endoderm is readily accomplished through a polyp's stomodaeum.

At the inoculation site, the remaining *Phormidium* filaments form a thin blackish sheet over the puncture and bundles of the organism penetrate tissue along the edge of the wound, thus anchoring the delicate algal mat. The mat grows in area as coral tissue is broken down and the trichomes divide rapidly. The dense web of several layers of filaments stays on top of the mash of

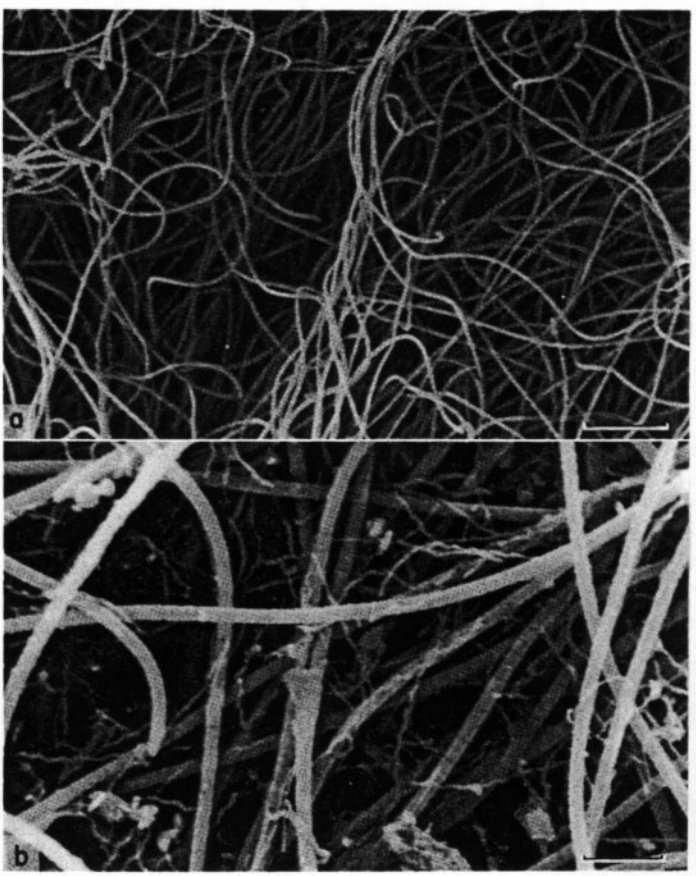


Fig. 6. Scanning electron micrographs of *Phormidium* mat. a: Pure spread on filtered seawater. b: Similar entangled growth over *Diploria*, with associated filamentous bacteria. (Scales = 50 μm for a; 20 μm for b).

decaying coral tissue and forms a tight seal over an oxygen-reduced microenvironment (Fig. 6 b). The mat becomes increasingly thinner near its center where nutrients are first depleted, and as it is reduced, the clean white coral skeleton is gradually exposed. On coral heads first infected at their tops, as is usually observed, the algal material forms a tonsure-like black band along the edge of live coral tissue.

Under the microscope, pioneering *Phormidium* strands teased out from coral tissue (Fig. 5 c) are found to have only a few associated bacteria. A few micrometers behind the tips of these filament bundles, however, histolysis is in full progress and supports a diverse population of scavaging microorganisms (Figs. 5 d, e; 6–10). Certain bacteria and ciliates are the first to appear in great numbers. The most conspicuous bacteria at this stage are unicellular gliding bacteria of the *Flexibacter* and *Saprospira* types and much smaller oval forms with characteristic single divisions along the longer axis, possibly *Nitrosomonas*. The most common ciliates belong to the genera *Philaster* and *Porpostoma* (E. SMALL, pers. comm.). They can be observed hovering over occasional openings in the *Phormidium* mat as well as diving down deep into the mucus cell mash of decaying coral tissue and devouring numerous whole zooxanthellae (Fig. 10 c). Some ciliates become so stuffed that they are incapable of directional swimming and merely rotate in place.

As the disease progresses, the number of pluricellular gliding bacteria filled with characteristic double refracting sulfur granules (*Beggiatoa* spp.) increases

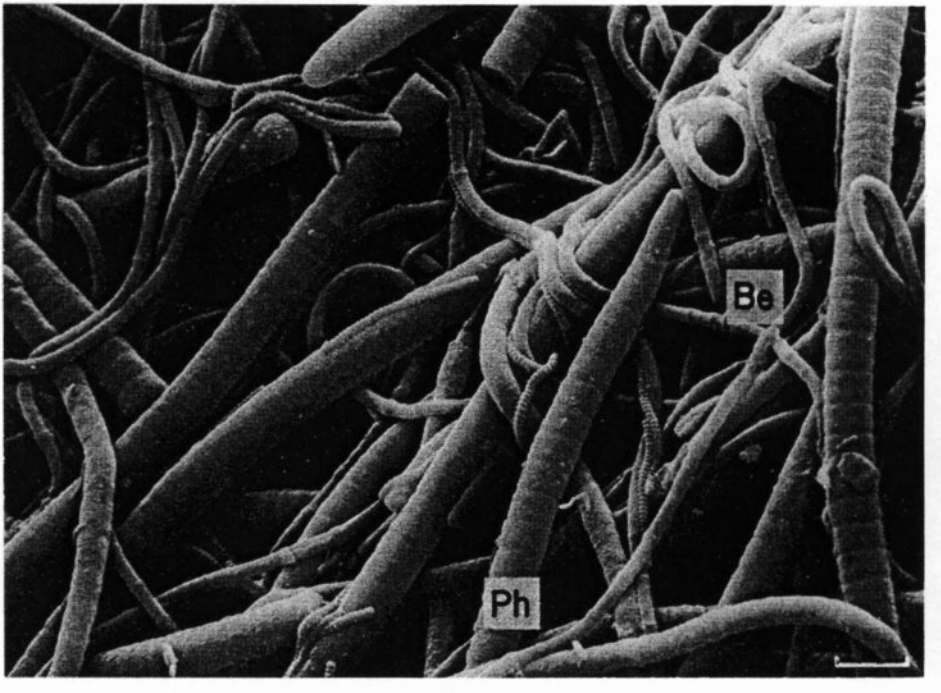


Fig. 7. Black band disease community from *Montastrea*, including *Phormidium corallyticum* (Ph) with round and tapered end cells, *Beggiatoa* sp. (Be), and spiral bacteria. (Scale = 5 μm).

sharply (Fig. 10a, b). The *Beggiatoa* trichomes move about but can also form a mat similar to *Phormidium.* In fact, the lower layers of a well-established black band consist of *Beggiatoa* (Fig. 8b). In the older portions of the mat (away from the live coral), these sulfur bacteria grow over the *Phormidium* carpet, where they form a whitish zone (Fig. 4c).

Various saprobic organisms now appear within the decomposing tissue layer under the *Phormidium-Beggiatoa* carpet: colorless flagellates, turbellarians, nematodes, purple bacteria, euglenoids, cyanophytes *(Oscillatoriaceae, Chroococcaceae),* diatoms, palmelloid *Volvocales,* and dinoflagellates. Where the decomposition process is almost completed, purple bacteria and palmelloid algae may appear as a pink and green layer. Several specimens of a macroscopic plant, *Enteromorpha* sp., were also found flourishing under the *Phormidium* mat of an experimentally infected *Diploria.*

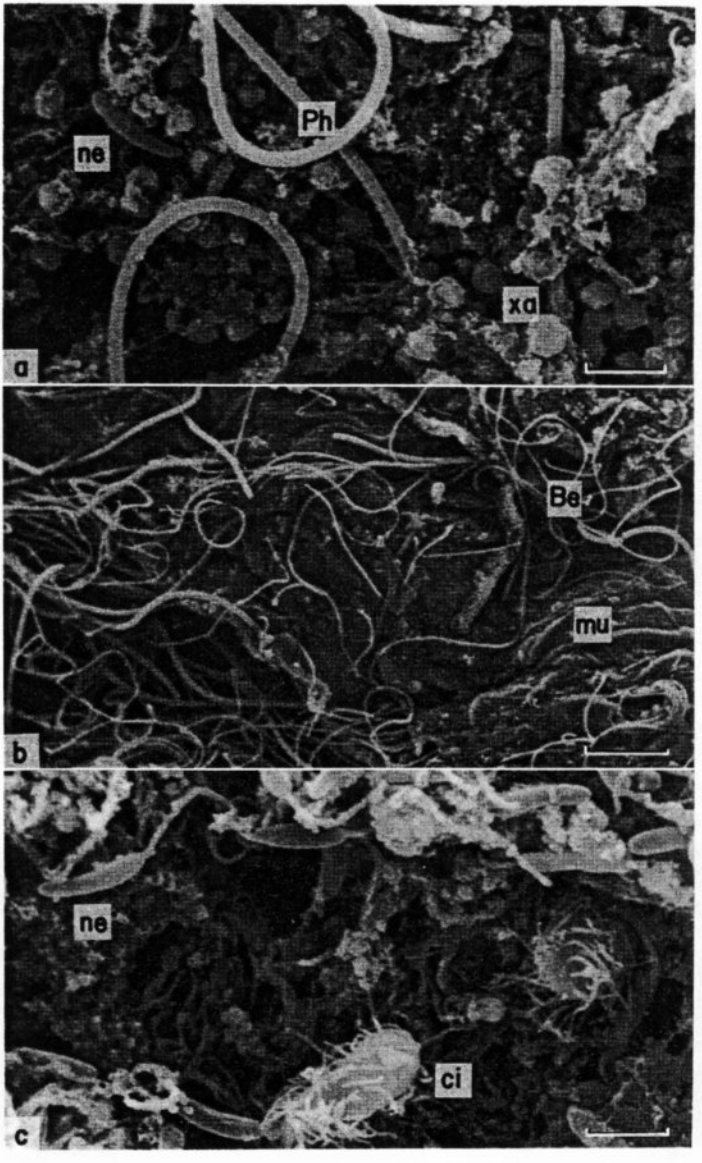


Fig. 8. Peels exposing three layers of diseased *Diploria* tissue under *Phormidium* mat shown in Fig. 6b. a: Liberated coral zooxanthellae (xa), unexploded nematocyst (ne), and *Phormidium* trichomes. b: *Beggiatoa* (Be) zone; filaments of these gliding bacteria are embedded in coral mucus (mu). c: Decomposing coral tissue with free, exploded nematocysts (ne) and saprophagous ciliates (ci). (Scales = 20 μm for a; 50 μm for b; 10 μm for c).

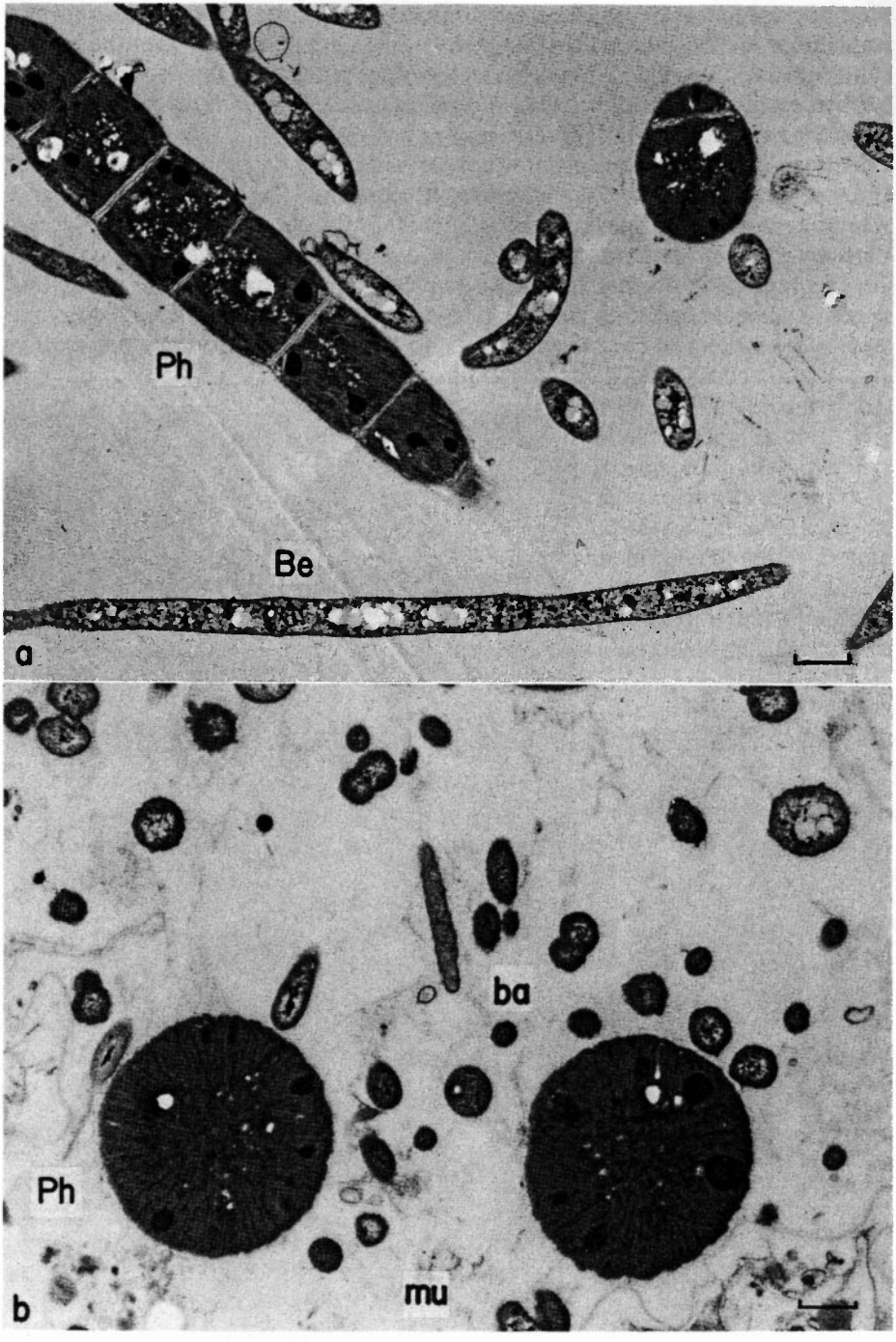


Fig. 9. Transmission electron micrographs of black band disease microcommunity. a: Predominately longitudinal sections of *Phormidium* (Ph) and *Beggiatoa* (Be). b: Cross sections of *Phormidium* (Ph) showing radial thylakoid, sections of bacterial associates (ba), and coral mucus (mu). (Scales = 2 μm for a; 1 μm for b).

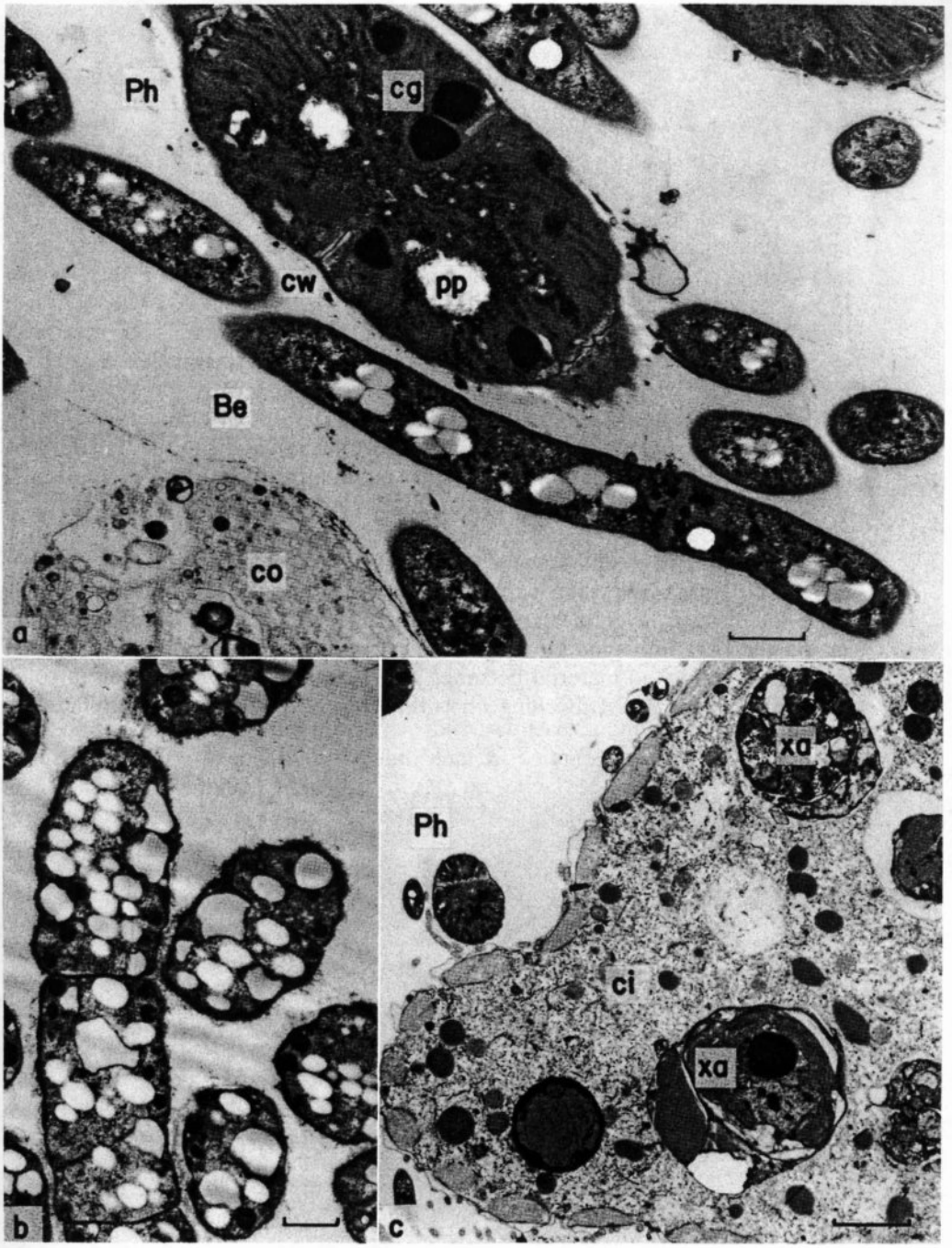


Fig. 10. Black band disease community. a: *Phormidium* (Ph) showing developing cross wall (cw), cyanophycin granules (cg), and polyphosphate bodies (pp); *Beggiatoa* (Be); and lysing coral cell (co). b: *Beggiatoa* with large electron transparent sulfur granules and smaller dense lipid globules. c: Ciliate (ci) next to *Phormidium* (Ph); note consumed coral zooxanthellae (xa) in various stages of digestion. (Scales = 1 μm for a; 0.5 μm for b; 5 μm for c).

3. Laboratory experiments

a. *Phormidium corallyticum*, growth in culture

Black band disease can be easily maintained on suitable live corals in laboratory aquaria. Open shade was found to provide sufficient light without excessive heating under low water flow. Average spreading rate of infections on five corals kept in the aquarium at Carrie Bow Cay was 2 mm per day, thus approaching conditions in the field. *Phormidium* mats scraped from live coral and kept in glass bowls thrive for a number of days over decaying coral tissue. Frequent changes of water keep associated bacterial populations low and assure sufficient oxygen supply. The isolated cyanophyte kept in plain seawater soon stops growing and within a week changes color from dark brownish black to pale brown.

Tested nutrient-enriched media did not noticeably enhance growth over plain seawater. Agar plates were more successful than liquid media and generated different spreading patterns, according to the additives used. Thick strands in a wispy growth pattern are produced on JONES' agar (Fig. 11 a); a thin and even spread develops on plain or F/2 enriched seawater agar (Fig. 11 b). The latter growth pattern is comparable to that of the active black band or to freshly collected *Phormidium* when it spreads over coral debris or another substrate, or even the surface layer of stagnant seawater (Fig. 11 c). In stirred or aerated media the organisms commonly cluster in radial bundles (Fig. 11 d). At least part of all cultivated material becomes pale brown or brownish green in color after a few days if not dwelling on coral tissue, a phenomenon unrelated to

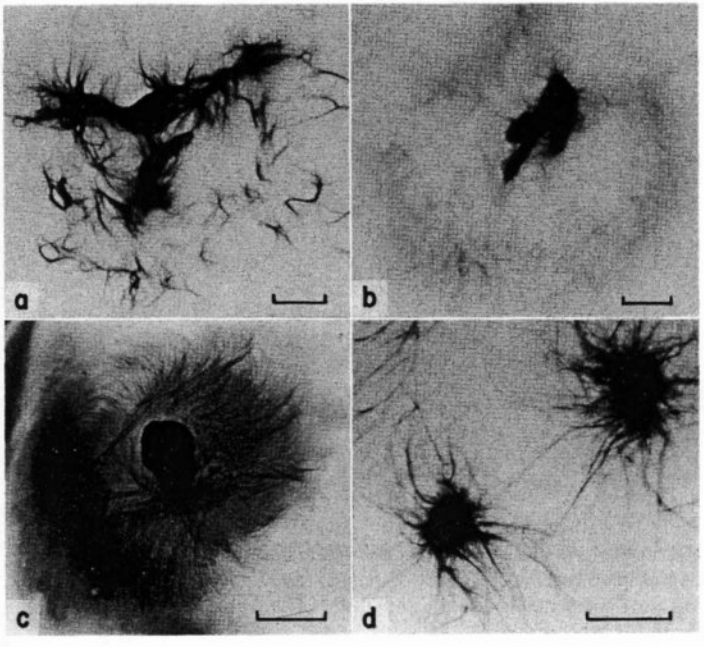


Fig. 11. Morphology of *Phormidium corallyticum* colony under different culture conditions. a: Spreading pattern characteristic for JONES' seawater agar. b: F/2 seawater agar. c: Spreading on surface of plain seawater. d: Stellate aggregates in agitated culture. (Scales = 5 mm).

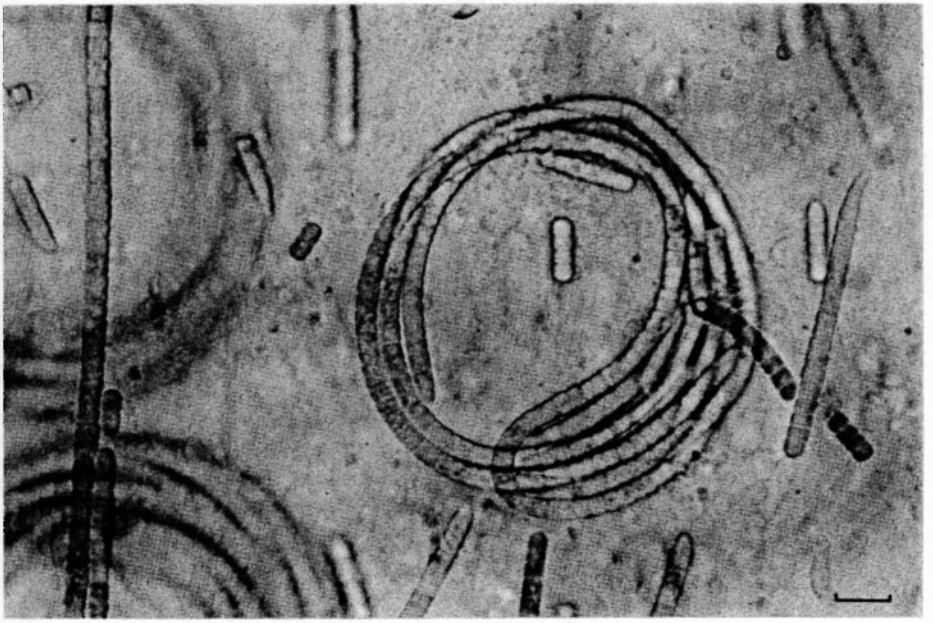


Fig. 12. Characteristic coiling of *Phormidium* filament under stress (this example, low nutrient level); note also fragmented trichomes. (Scale = 10 μm).

illumination level. Although *Phormidium* can be kept alive for many weeks without the proper organic nutrients, microscope examination shows sharply reduced mobility, fragmentation, and filament coiling, signs of stress that are also exhibited by material about to dry up on a microscope slide (Fig. 12). The process is reversible, at least to a certain degree, when *Phormidium* is reinoculated into live coral. We used pale green material from ten day old starved cultures to infect six heads of *Montastrea annularis*. Although it took 2–7 days for the implants to take effect all six infections succeeded and produced viable new blackish filaments and the symptoms of black band disease.

To confirm the nutrient value of coral for *Phormidium* growth we added autoclaved and untreated *Montastrea* tissue (10 each) to small clusters of the organism on agar plates. Growth was determined by measuring the longest perpendicular diameters of the *Phormidium* colonies; plain agar served as control. A one way analysis of variance (Sokal & Rohlf, 1969) demonstrates significantly greater growth ($p = 0.01$) on agar with tissue supplement than on plain agar. Autoclaving had no effect on the nutrient value of coral.

We also tested the response of fresh viable *Phormidium* material to antibiotics and to a number of physical factors. The broad spectrum bactericidal antibiotic gentamicin sulfate (100 μg/ml seawater) killed many small associated bacteria after 2 hours of exposure. After 12 hours, all bacteria were motionless, but *Phormidium* trichomes were also affected. The filaments were no longer entangled but separated and straight, so that it was convenient to measure their length (up to 2 mm). They still moved but did not form a coil or web when transferred to pure seawater.

Oxygen requirements of this organism are apparently high, as one might expect from its association with shallow reef corals. Despite their own photosynthetic production of oxygen – demonstrated by bubbles under illumination – the filaments always cluster near the water surface or around air bubbles when they are kept in dishes or on a microscope slide under a cover slip.

Phormidium's resistance to salinity changes was found to be high. During six days of exposure to 70‰, 44‰, 35‰ (normal *in situ* condition), 25‰ and 18‰ salinity, only the two extremes induced signs of stress such as coiling, fragmentation, and cell wall constrictions.

Temperature strongly influenced *Phormidium* growth in culture as well as its potency as a disease agent. Cultures maintained at 20 °C did not survive more than a week. Although no negative effects were noted at 25 °C, spreading on the substrate and growth occurred only at 28 °–30 °C, apparently the optimum range. Cultures at 35 °C were unsuccessful because of rapid bacterial takeover. *Phormidium*-infected corals showed similar results. At 20 °C and 25 °C, inocula did not spread but stayed in place and were not rejected, as was some other foreign material (see below). Maximum spread took place at 30 °C, with a fluctuation range of 28 °–32 °C. At 35 °C the corals *(Diploria strigosa)* started to die off from temperatures stress (they had been maintained in stirred, not in running seawater) and *Beggiatoa* rapidly took over.

Field observations have already shown that light is an important parameter for black band disease as its growth is vigorous on the top surfaces of shallow coral heads, whereas the process is slow along the sides and under overhangs where light intensity is reduced. Approximate *in situ* light levels at the study locations were 25–35 % of surface radiation, 120–170 $g \cdot cal \cdot cm^{-2} \cdot day^{-1}$. In the laboratory, fastest growth occurred on inoculated corals in aquaria fully exposed to sunlight, although the growth of bacteria (including *Beggiatoa*) was also promoted and at times overpowered the infected coral and destroyed it together with the cyanobacterial population. As a result, we obtained the best yield of *Phormidium* from infected corals maintained in the open shade, at about 40 % of full sunlight intensity (approximately 190 $g \cdot cal \cdot cm^{-2} \cdot day^{-1}$ at Carrie Bow Cay), but good growth was also obtained in deep shade at about 1 % sunlight (5 $g \cdot cal \cdot cm^{-2} \cdot day^{-1}$). Cultures in plain seawater, in or on any of the enriched media tested, or over rotting coral debris could only be maintained at reduced light levels; the best growth of cultured *Phormidium* away from live coral was recorded on coral debris at a light level of 6 % of full sunlight (30 $g \cdot cal \cdot cm^{-2} \cdot day^{-1}$). In the presence of a light gradient, observed in cultures as well as on inoculated corals, *Phormidium* filaments move and grow towards the light source.

b. Confirmation of primary disease agent

Field observations and initial laboratory trials (ANTONIUS, 1981) strongly suggested that *Phormidium corallyticum* is the cause as well as the most conspicuous component of black band disease. Nevertheless, we sought experimental evidence to clarify the role of accompanying microorganisms. For the purpose of inoculations, active *Phormidium corallyticum* was collected from black bands

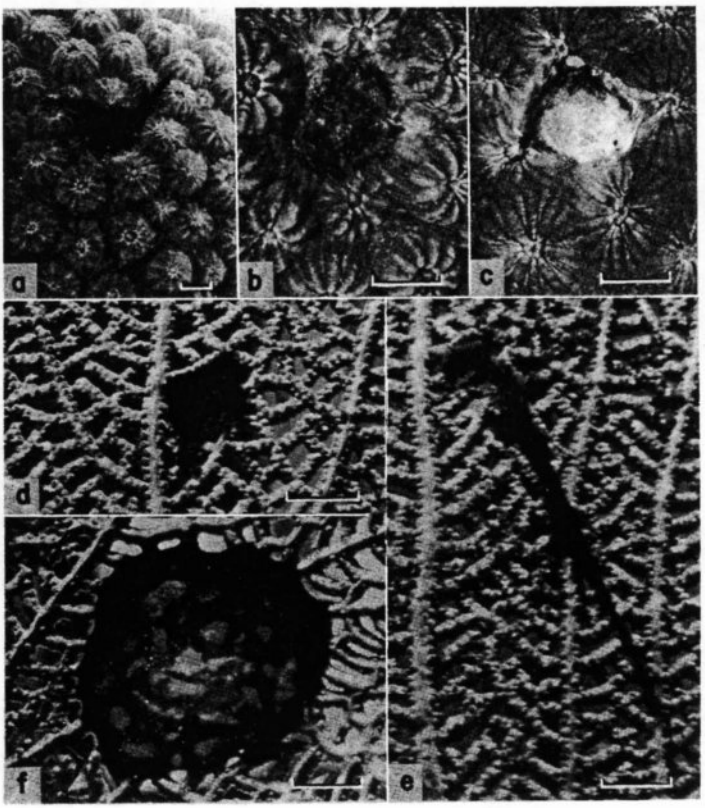


Fig. 13. Inoculation control experiments testing nonpathogenic *Oscillatoria lutea* from a Belizean mangrove against *Montastrea annularis* (a–c) and *Gorgonia ventalina* (d, e), and *Phormidium corallyticum* against *G. ventalina* (f). a: *M. annularis* one hour after inoculation; note probing *Oscillatoria* trichomes. b: After 1 day algal plug is faded in color and enveloped in coral mucus. c: After 3.5 days most algal material is rejected by coral, leaving inoculation lesion exposed. d: *G. ventalina* one hour after inoculation with *Oscillatoria*. e: After 2 days rejected algal plug is swept off wound tissue by ciliary movement. f: Successful infection by *Phormidium* 5 days after inoculation (this condition not yet confirmed as occurring without manipulation). (Scales = 2 mm for a–c; 5 mm for d–f).

on shallow (1 m) *Diploria strigosa* and moderately deep (3.5 m) *Siderastrea siderea* near Carrie Bow Cay, and from *D. labyrinthiformis* and *D. strigosa* (1 m and 3.5 m) off Bermuda (Table 3; tests no. 1–4). Fifty freshly collected specimens of three scleractinian species (4 *D. labyrinthiformis*, 24 *D. strigosa*, 22 *Montastrea annularis*) and 12 gorgonians (to be discussed below) served as hosts. Since we had not attempted to grow *Phormidium corallyticum* in pure culture and found it impossible to separate the filaments from adhering or accompanying bacteria, we separated the full spectrum of mucus eneveloped bacterial and eukaryotic associates from cyanophyte filaments and used this mixture as the inoculum. The assortment of unpigmented organisms was then divided into fractions that were either dominated by or free of the sulfur-fixing bacterium *Beggiatoa* (Table 3; tests no. 4, 5). As Table 3 shows, only inoculations involving *Phormidium corallyticum* were positive and triggered the previously described stages of the disease. Here and there, single filaments of the cyanophyte overlooked in preparations of "unpigmented mix" could be observed moving from the inoculation plug into the tissue of the wound but were unable to trigger tissue destruction. In fresh corals, plugs were rejected overnight (comparable to Fig. 13 a-c) – also a second time when corals were reinoculated at the same puncture – and the wound was found healed and uninfected after 3–9 days. In corals increasingly stressed because of insufficient

water circulation, the wounds eventually became infected by bacteria (remaining from the rejected plug or recruited from seawater) and overgrown by tufts of *Beggiatoa,* but characteristic black band symptoms never developed.

c. Suspected gorgonian black band disease

Near Carrie Bow Cay (South Water and Carrie Bow cuts, leeward of Curlew bank) a number of partly dead *Gorgonia ventalina* in depths of 2–4 m appeared to have the black band disease. On them, the brownish to blackish brown band is about 1 cm wide and borders the living part of the mixed algal turfs covering the dead portion of the sea fan (Fig. 14 a). Microscope observation of bands showed that they were made up of oscillatoriacean filaments – but not of *Phormidium corallyticum* – and contained isolated *Gorgonia* spicules indicating a possible disease process. Some bands contained numerous specimens of the nematode *Araeolaimus* sp., and a few bacteria, including *Beggiatoa* (Fig. 15), but no ciliates.

The most common cyanophyte identified from gorgonian bands is *Schizothrix mexicana* GOMONT (Fig. 14 b). Its trichomes measure 10–14 μm in width, and the cells 3–4 μm long. This organism was also responsible for the band on the only specimens of fire coral (*Millepora* sp.) observed with this condition. Another species, *S. calcicola* (C. AGARDH) GOMONT, was a less abundant associate in at least one of the gorgonian bands; its cells measure 2 μm in width, 5 μm in length. Almost the entire band of one gorgonian collected at Curlew bank

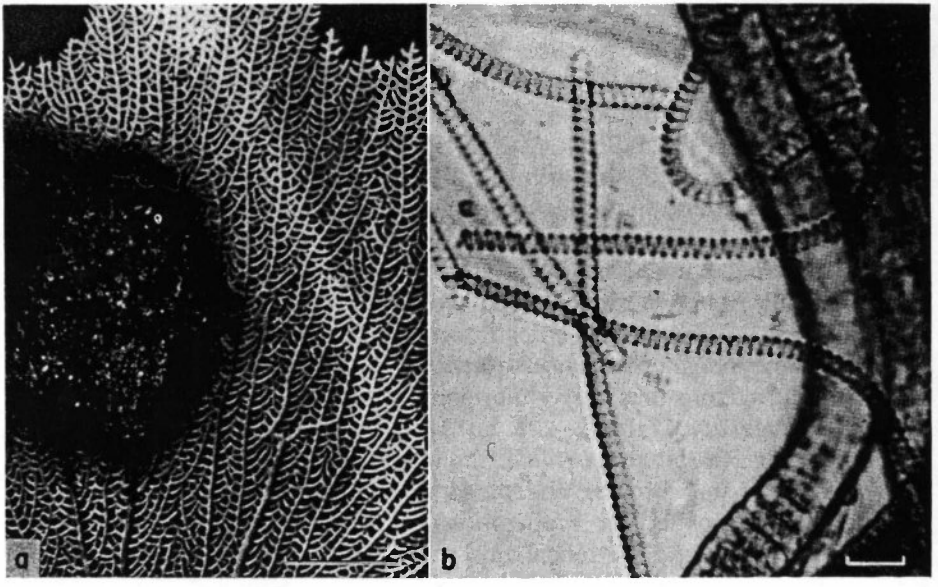


Fig. 14. Suspected black band disease of *Gorgonia.* a: Appearance on reef-dwelling *G. ventalina* (barrier reef channel North of Carrie Bow Cay, 4 m). b: Photomicrograph showing main components of dark brown zone adjacent to live gorgonian tissue, *Spirulina* sp. (center) and *Schizothrix mexicana* (right). (Scales = 50 mm for a; 10 μm for b).

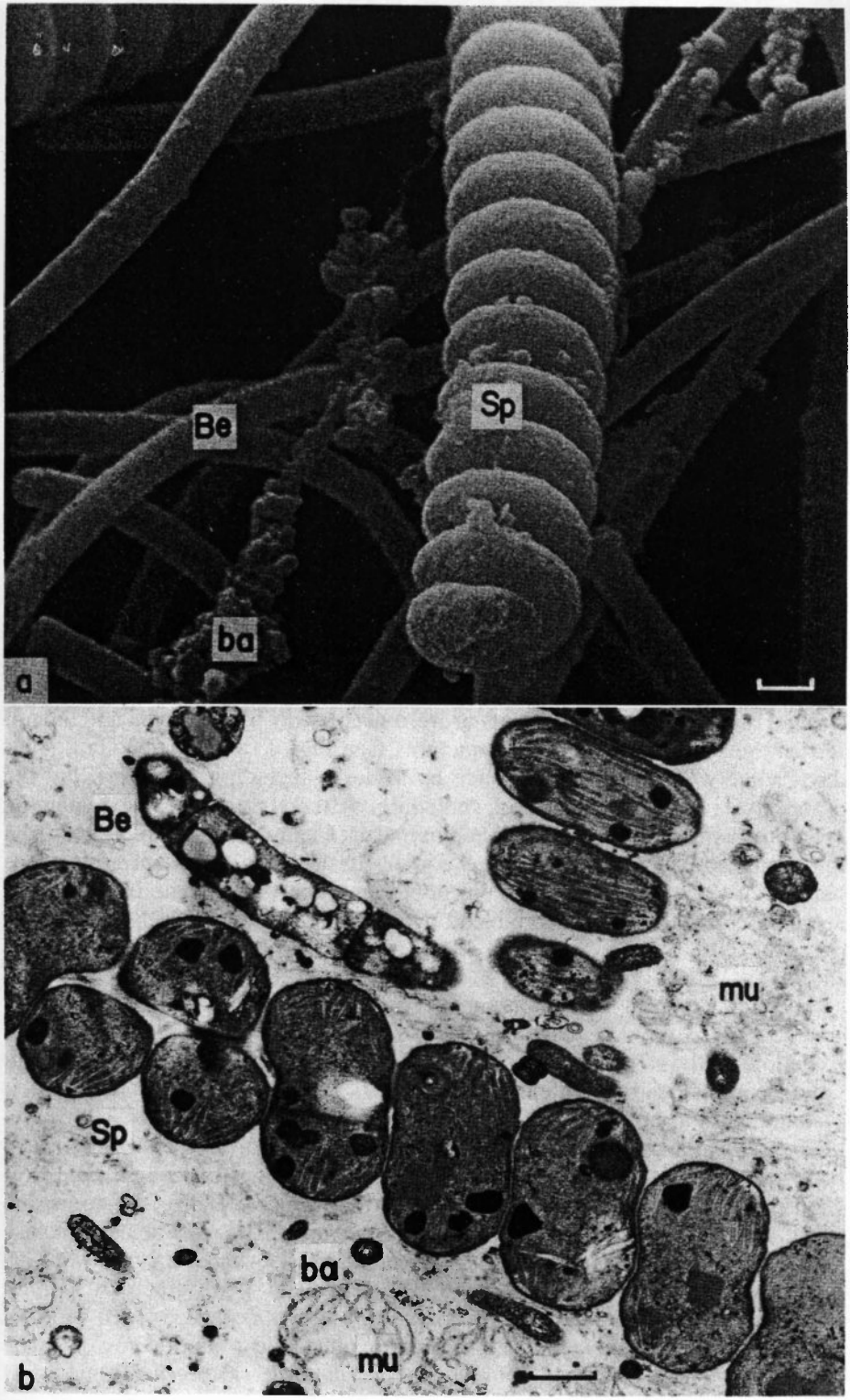


Fig. 15. Electron micrographs of *Spirulina* community from *Gorgonia ventalina*. a: SEM end on view of *Spirulina* (Sp), *Beggiatoa* (Be), and bacterial aggregates (ba). b: TEM, longitudinal sections of *Spirulina* (Sp) and *Beggiatoa* (Be), sections of bacteria (ba) and gorgonian mucus (mu). (Scales = 1 μm).

contained the spiral form, *Spirulina subsalsa* OERSTED. The cells of this species are 1 μm wide and form a tight spiral, 4 μm wide (Figs. 14 b; 15).

We attempted to infect fresh *Gorgonia ventalina* with coils of *Schizothrix mexicana* and *Spirulina subsalsa* obtained from bands appearing to be active (Table 3; tests no. 7, 9, 10). In all twelve inoculations the microorganisms stayed confined to the wound area or became enveloped in mucus and were sloughed off (see Fig. 13 d, e). After four days under low water flow conditions in the aquaria, *Beggiatoa* growth appeared along some margins of the wounds, but the cyanophytes neither spread nor grew. Eight controls with *Phormidium corallyticum* from scleractinian black bands (Table 3; test no. 1) showed instant settlement of the organism in the wound and subsequent growth of the infected area from 3–4 mm diameter initially to about 8 mm in two days, and 18 mm in four days (Fig. 13 f).

Similar inoculations were performed on five scleractinians, all but one species *(Porites astreoides)* of which are known for their susceptibility to black band disease (Table 3; tests 7–10). Not one of the twenty-seven gorgonian band inoculations took, whereas 100 % of the *Phormidium corallyticum* control infections were successful. Coils dominated by *Schizothrix mexicana* initially spread within the wound area and filaments concentrated peripherally, but the organism did not become established in any of the coral species. Plugs were washed out even by slight currents or were swept away by coral cilia and did not settle when reinserted into the punctures (see Figs. 13 a–c). After about four days, some wounds became infected by bacteria, including *Beggiatoa,* but the cyanophytes showed no sign of spreading. After eight days, the remaining *Schizothrix* filaments were enveloped in mucus, fragmented, and bleached from the pigment. *Spirulina* plugs were equally unsuccessful, the only difference being that they did not become enveloped in mucus; thus some of the mucus around *Schizothrix* may be produced by the cyanophytes themselves.

d. Control inoculations

Reactions of the scleractinian and gorgonian hosts to inoculations by foreign but not disease-associated organisms was tested. Inocula were selected for their systematic, morphological, or distributional similarities to *Phormidium corallyticum. Schizothrix mexicana* (cell width × length: 15–20 μm × 3–4 μm), a brownish filamentous mass, was collected from intertidal rocks on Carrie Bow Cay and from subtidal mangrove roots on Twin Cays, Belize. Diatoms and spiral and rod shaped bacteria were associated with these filaments. Blackish *Oscillatoria lutea* C. AGARDH (5 × 5 μm cells) also came from Twin Cay mangrove roots, along with some (10 %) *Spirulina* sp. Dark green *Oscillatoria (= Phormidium) submembranacea* ARDISSONE & STRAFFORELLO (cell width × length: 5 × 8 μm) was scraped from coral rock just below the tide line of Carrie Bow Cay. Radially aligned bundles of *Oscillatoria erythraea* (EHRENBERG) KÜTZING (4 × 5 μm cells) were collected from blue water southeast of Bermuda; they had a mucus center containing coccoid bacteria and cyanobacteria. Reddish brown streamers of *Porphyrosiphon miniatus* (HAUCK) DROUET (3 × 5 μm cells) originated at depths of 3 m on a patch reef east of Bermuda. The only

noncyanophyte test organisms were greenish filaments of *Cladophoropsis* sp. (*Chlorophyta;* cells not measured) from Carrie Bow Cay intertidal rocks.

In all, 61 inoculations were attempted and observed for 7–9 days (Table 3; tests no. 11–16). Active, unstressed corals and gorgonians were able to rid themselves of the foreign objects with the help of mucus and ciliary action and polyp movements (Fig. 13). With decreasing coral activity (aquarium stress) algal plugs remained but did not spread or invade tissue although filaments were in good condition, as judged from pigmentation and movement. *Schizothrix mexicana* plugs became enveloped in a polysaccharid substance when stressed

Table 3. Results of inoculation tests using microorganisms from different locations, substrates or hosts, and depths on scleractinian and gorgonian corals. (Numbers of inoculations attempted are accompanied by one of two symbols: + = positive; − = negative; 0 = not tested).

No. Inoculum	Location	Host/Substrate	Depth (m)	*Porites astreoides*	*Favia fragrum*	*Diploria labyrinthi-formis*	*Diploria strigosa*	*Montastrea annularis*	*Plexaura homomalla*	*Gorgonia ventalina*
1. *Phormidium corallyticum*	Belize	*Diploria strigosa*	1.0	0	0	0	0	12+	0	8+
2. *Phormidium corallyticum*	Belize	*Siderastrea siderea*	3.5	−[a]	+[a]	2+	0	6+	0	0
3. *Phormidium corallyticum*	Bermuda	*Diploria labyrinthiformis*	1.0	0	0	0	4+	0	0	0
4. *Phormidium corallyticum*	Bermuda	*Diploria strigosa*	3.5	0	0	2+	11+	2+	4+	0
5. *Beggiatoa* sp.	Bermuda	*Diploria strigosa*	3.5	0	0	0	4−	0	0	0
6. Bacteria mix	Bermuda	*Diploria strigosa*	3.5	0	0	0	5−	2−	0	0
7. *Schizothrix mexicana*	Belize	*Gorgonia ventalina*	1.5	0	0	0	0	4−	0	4−
8. *Schizothrix mexicana*	Belize	*Gorgonia ventalina*	3.5	3−	2−	3−	0	6−	0	0
9. *Schizothrix mexicana*	Belize	*Gorgonia ventalina*	6.0	0	0	0	0	5−	0	4−
10. *Spirulina subsalsa*	Belize	*Gorgonia ventalina*	1.5	0	0	0	0	4−	0	4−
11. *Schizothrix mexicana*	Belize	coral rock, mangrove root	0.0–0.5	3−	2−	3−	2−	12−	0	4−
12. *Oscillatoria lutea*	Belize	mangrove root	0.5	0	0	0	0	6−	0	0
13. *Oscillatoria submembranacea*	Belize	coral rock	0.5	0	0	0	2−	4−	0	0
14. *Oscillatoria erythraea*	Bermuda	plankton	0.5	0	0	0	6−	0	0	0
15. *Porphyrosiphon miniatus*	Bermuda	coral rock	3.0	0	0	0	2−	0	0	0
16. *Cladophoropsis* sp.	Belize	coral rock	0.0	3−	2−	1−	2−	7−	0	0

[a] Not part of this experimental series but results known from previous inoculation tests (1980).

and were readily sloughed off the scleractinian or gorgonian substrate, occasionally with the help of trapped gas bubbles. *Oscillatoria erythraea,* too, was difficult to keep inside the coral puncture because of its ability to float; bundles remaining in wound tissue, however, still did not participate in infection. Again, 100 % of controls with *Phormidium corallyticum* were positive and developed black band disease.

Discussion

Our observations and the unpublished reports of divers indicate that the black band disease is a common affliction of Atlantic shallow reef corals. The characteristic black *Phormidium* band contrasted against newly exposed coral skeleton is highly noticeable *in situ,* but the inexperienced collector may find the cyanobacterial filaments elusive, particularly in agitated water. That may be why at least one earlier report did not mention cyanobacteria as part of the black line disease (GARRETT & DUCKLOW, 1975). A coral disease involving cyanobacteria has not yet been observed in the Indo-Pacific region. Most of the easily infected Atlantic corals belong to genera that do not occur in the Indo-Pacific, and Atlantic species of common Indo-Pacific genera, such as *Acropora* and *Porites,* are resistant to the disease (Table 1).

Spreading rate of the infection, once it is established on a coral, is remarkably fast compared with the growth of competitors for space, such as encrusting sponges. Under optimum conditions a black band may advance as much as 10 mm per day (ANTONIUS, 1981). Over the long term, we calculate an average of 3.1 mm per day (Table 2). At this rate, the disease covered the longest radius of live coral surface remaining on *Montastrea* I after our observation in May (135 mm) within 44 days, or by mid July. Since the tissue area was then depleted, the disease was halted by lack of substrate. On *Montastrea* II the disease took about 114 days to destroy the remaining 352 mm maximum radius, on *Diploria* about 112 days for 350 mm. Both these estimates project the seasonal decline of black band disease into late September, a reasonable prediction since its termination was confirmed by direct observation in early December.

It is noteworthy that the disease spreads primarily in the direction of due west in the three monitored corals (Table 2), and in four other coral heads (3 *Montastrea annularis,* 1 *Diploria strigosa*) for which this information was recorded. We assume that the predominantly east-west current flow on the reef together with favourable light exposure may have been responsible for this pattern.

Only three conditions seem to impede the progress of black band disease in nature: seasonal decline, lack of coral tissue, and decreased light level. It has already been noted that spreading speed is highest on horizontal coral surfaces in shallow water (ANTONIUS, 1981). Progress slows on vertical surfaces, and coral tissue survives most commonly under overhangs of coral heads (ANTONIUS, 1973).

Our observations and experiments have demonstrated beyond doubt that the cyanobacterium *Phormidium corallyticum* is the etiologic agent of black band

disease in scleractinian corals and not merely the organism that forms and maintains the black line, as has been suggested (DUCKLOW & MITCHELL, 1979). This organism can be grown autotrophically away from the host coral but certain organic substances available in coral tissues (TAYLOR, 1983) seem to be essential for its optimum development, as judged by growth rate, pigmentation, cell shape, and mobility. The fact that polyphosphate bodies (Fig. 10a), too, are larger and more common near the active edge of the disease than away from it – as in the *Beggiatoa* zone – indicates a build-up of energy storage (SHIVELY, 1974).

Many types of organisms associated with the disease by themselves cannot harm healthy, unstressed corals. Several species or size classes of *Beggiatoa* are among the most conspicuous microbes because they are large and bright white (owing to birefringent sulfur granules), and their movements and the mats they form are very similar to those of the cyanobacteria. *Beggiatoa* mats occur over anaerobic zones of decaying coral tissue where these organisms take advantage of the hydrogen sulfide-air interface. Concentrations of 30 nm lipid globules along with the sulfur granules (Fig. 10b) in *Beggiatoa* are indicative of increased sulfur metabolism (LANG, 1968). Other bacteria, such as *Desulfovibrio,* characterize this anaerobic zone (GARRETT & DUCKLOW, 1975). Distinctive spiral bacteria (*Saprospira;* Fig. 7) seen by us in all layers of the disease were also noted by DUCKLOW & MITCHELL (1979). Some of our tests indicate that antibiotics, if carefully applied, could eliminate most if not all bacteria without killing the *Phormidium* filaments, an observation consistent with that of VANCE (1966) in experiments with chroococcalean cyanophytes. Overexposure, on the other hand, leads to cell damage in *Cyanophycea* (ROSSNER, 1963) thereby demonstrating that antibiotics can control black band disease (ANTONIUS, 1981).

Bacterial associates have been shown to play an important role in the dissolved organic carbon secretion and utilization by some cyanophyte species (genera *Oscillatoria, Synechococcus)* (CHANG, 1981). Similar relationships conceivably exist within the black band disease community but experimental evidence to that effect is still lacking. Another unexplored question is whether *Phormidium corallyticum* is capable of fixing nitrogen. Aerobic nitrogen fixation is known for a planktonic oscillatoriacean in which the absence of heterocysts is compensated for by bundle formation and trichome differentiation into granulated and nongranulated regions. The mats (Figs. 4, 6, 11c) and radially aligned bundles (Fig. 11d) formed by *Phormidium* may be functionally comparable to the *Oscillatoria* bundles with parallel or radial trichome alignment shown in BRYCESON & FAY (1981: Fig. 1), but we noted no regional granulation in *Phormidium* trichomes.

We do not yet know why some coral species are so readily infected by black band disease whereas others are rarely or never infected. In initial experiments (briefly described by ANTONIUS, 1981), we placed homogenized coral tissue from *Montastrea annularis, Siderastrea siderea,* and *Acropora palmata* – representing easily, rarely, and never infected species – near (5 mm distance) freshly coiled balls of *Phormidium.* Whereas *Montastrea* tissue was immediately (within 15–30 min) invaded and enveloped by *Phormidium,* the cyanobacterial filaments clearly backed away from the other two species during the first 1–2 h of exposure. This inhibition eventually disappeared, however, and tissue invasion

was accomplished in all three species. The nature of the deterrent is not yet known. It is interesting to note, however, that species of *Acropora, Porites, Montastrea,* and *Millepora* tested for antimicrobial activity were inactive against all microorganisms employed, including members of *Clostridium, Micrococcus, Bacillus, Escherichia,* and various marine bacteria (BURKHOLDER & BURKHOLDER, 1958). Of the tested host species, all but *Montastrea* resist the cyanobacterial infection.

Two other questions concerning *Scleractinia* black band have yet to be answered: How is the disease transmitted in nature? How does *Phormidium* initiate the infection? We know from our experiments and from the work of others (*e.g.*, MITCHELL & CHET, 1975; DUCKLOW & MITCHELL, 1979) that bacterial infections can be triggered in corals that are under severe stress (owing to reduced oxygen, high temperature, the presence of pollutants). However, none of these conditions apply to the reefs of Carrie Bow Cay, which account for the highest incidence of black band disease of all areas examined (ANTONIUS, unpublished). Many infested parts of the reefs off Bermuda, are also distant from possible stress factors produced by these islands. One can assume that on the reef flakes of dislocated, floating *Phormidium* mat settle on fresh tissue lesions, as they are caused by parrot fish bites, storm damage, or dropped anchors. Only one of our observations and field experiments – invasion of a fresh wound by *Phormidium* filaments suspended in a net above the coral – supports this assumption. Of course, the probability of finding a fresh wound colonized by a flake of *Phormidium* filaments is obviously low, and lesions healed over by new ectodermal tissue are no longer accessible to the organism. Another stress situation in scleractinians that is still unexplained is the noninfectious white band disease and interestingly, monitored corals with this conditon were indeed observed to be infected by black band (ANTONIUS, 1981). More data are clearly needed on the entire subject of primary infection in the field.

Inoculation experiments have shown that access to subectodermal coral tissue is all that is needed for *Phormidium* filaments to cause histolysis, which is the beginning of the disease process. A toxic exudate is probably responsible for this effect, but bio-assays are needed to confirm this hypothesis. In the hundreds of thin sections of pioneering filament bundles that were viewed it was not possible to detect an intact coral cell within a radius of about 10 μm from *Phormidium* cells. Indeed, the presence of cell wall lytic enzymes generally known from bacteria have been demonstrated for certain bluegreen bacteria responsible for "water blooms" (INGRAM, 1973). Moreover, toxic substances extracted from some cyanophytes cause general cellular damage and severe injury to parenchymal cells of the liver of injected rats (ASHWORTH & MASON, 1946). Even human swimmers are affected by cyanobacterial *(Lyngbya)* toxins, which cause dermatitis on contact; in the laboratory the toxin has been shown to lyse a protozoan *(Tetrahymena)* and rabbit erythrocytes, and to possess antibacterial activity (MOIKEHA & CHU, 1971).

Some gorgonians on shallow reefs near Carrie Bow Cay exhibit a seemingly typical black band disease, but microscope examination of about a dozen cases revealed no *Phormidium corallyticum* filaments in their algal bands. At the same time, *Phormidium* does readily infect gorgonians *in vitro,* whereas organisms isolated from the natural cyanophyte band *(Schizothrix, Spirulina)* are

ineffective if implanted into healthy gorgonians or scleractinians. More band conditions will have to be studied in octocorals at different seasons and localities to confirm the obvious suspicion that *Phormidium corallyticum* is the primary agent, and *Schizothrix* and *Spirulina* are secondary colonizers.

Summary

The black band disease of several western Atlantic shallow reef corals, notably *Montastrea annularis* and *Diploria strigosa*, is caused primarily by the cyanophyte *Phormidium corallyticum*. In Belize this affliction is common and spreads at an average rate of 3.1 mm per day. About 14.1 cm^2 of tissue per day is destroyed on a single coral head. *Phormidium* filaments readily invade susceptible corals wherever lesions or stress have weakened the ectoderm. Histolysis occurs immediately around pioneering filaments, presumably via toxic cyanobacterial exudate. *Phormidium* eventually forms a mat over invaded areas, where oxygen-reduced conditions allow a saprobic microcommunity to flourish and further break down coral tissue. As the disease advances, sulfur-fixing *Beggiatoa* bacteria multiply and form mats along or under the *Phormidium* band. In both Belize and Bermuda the disease and cyanophyte almost disappear during periods of low water temperature. Some gorgonian octocorals in Belize have a similar black band disease, but *P. corallyticum* is not part of the band. The cause of gorgonian band disease is still unresolved.

Growth rates and survival of autotrophically cultured *Phormidium corallyticum* are considerably below values recorded in decomposing coral. Homogenized tissue on some corals *(Montastrea* and *Diploria)* is actively sought by separated filaments, whereas in other corals *(Acropora* and *Porites)* it is avoided until the unidentified inhibiting mechanism switches off. *Phormidium* filaments are resistant to salinity changes but display aerophilic behavior and require temperatures around 30 °C as well as high light levels (about 40 % of full sunlight) for optimum activity. In culture, however, best growth took place at only 6 % of full sunlight. Filaments exposed to antibiotics weaken and eventually die.

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