***Hydrobiologia* 460:** 53-63.2001.
J. W. Porter (ed.), The Ecology and Etiology of Newly Emerging Marine Disease.*
<D 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Yellow Band and Dark Spot Syndromes in Caribbean Corals: Distribution, Rate of Spread, Cytology, and Effects on Abundance and Division Rate of Zooxanthellae

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Key words: mitotic index, zooxanthellae. yellow band, and dark spot

Abstract

Yellow band and dark spot syndromes have been frequently observed to affect coral species throughout the Caribbean within the last 10 years. These syndromes significantly impair at least three important reef-building species. Yellow band (also known as yellow blotch) appears as rings or blotches on *Montastrea an/uilaris* throughout the Caribbean. The coral tissue necrosis occurs at a rate of approximately 0.6 cm/month. Transect measurements at various locations indicated that as many as 90% of *M. aninilaris* were affected by yellow band during 1997-98. Tissue samples reveal a 41-96.9% decrease in zooxanthellae/sample compared to healthy specimens, depending on distance from healthy tissue. Mitotic indices (MI) of zooxanthellae (symbiotic algae appearing as doublets) for *M. aninilaris* are 2.5%. MI in yellow band samples directly bordering healthy tissue are less than 0.9%, and zooxanthellae directly within the band bordering exposed skeleton had a mitotic index of 0.0%. This indicates impairment of zooxanthellae cell division in yellow band specimens. Zooxanthellae are not expelled and appear vacuolated and devoid of organelles. Dark spot, characterized by tissue necrosis as well as a depression of the colony surface, affects *Stephanocoenia michelinii* and *Siderastrea siderea* throughout the Caribbean. Transects showed that as many as 56% of *S. michelinii* and *S. siderea* showed signs of dark spot during 1997-98. Affected tissues of *S. siderea* died at a rate of 4.0 cm/month. In dark spot samples from *S. siderea,* the total number of zooxanthellae was 56% of that in healthy tissue; dark spot-affected specimens of 5. *michelinii* showed a 14% decrease in the number of zooxanthellae compared to healthy tissue samples. Mitotic indices of zooxanthellae from healthy specimens of *S. siderea* were 1.20% compared to 0.40% in dark spot samples. Mitotic indices of healthy *S. michelinii* were 1.54% compared to 0.23% in dark spot samples, also indicating a decrease in algal cell division. Zooxanthellae from dark spot tissue are swollen and darker in pigment. Due to the changes that are evident in the symbiotic algae, we suggest that both syndromes act primarily on the zooxanthellae symbiont, and secondarily on the cnidarian host.

Introduction

Over the past 20 years, there has been an increase in possible new diseases (Antonius, 1981 a,b; Williams & Bunkley-Williams, 1990; Santavy & Peters, 1996), which are currently referred to as syndromes affecting hermatypic corals (Goreau et al., 1998; Richardson, 1998). Some of these epizootics are associated with unusual stresses from natural or man-made causes (e.g. sedimentation, temperature fluctuations and pollutanls (Mitchell & Chet, 1975; Antonius, 1977), suggesting that external stress may lower coral resistance or stimulate the growth of pathogenic organisms. However, most reef epizootics show little obvious linkage to local spatial and temporal stresses, and thus may be due to the dissemination of new or newly

adapted pathogenic agents in the marine environment (Goreau et al., 1998).

Some postulated anthropogenic stresses linked to coral reef disease include de-forestation and soil erosion. Also wind or ocean transport of dust could potentially result in the introduction of terrestrial microbes into the marine environment (Smith et al., 1996; Nagelkerken et al., 1997; Geiser et al, 1998). *Aspergillus sydowii* has been shown to be the pathogen associated with diseased sea fan tissue throughout the Caribbean, affecting *Gorgonia ventalina* and *G. flabellum* (Cnidaria:Gorgoniidae) (Smith et al., 1996; Nagelkerken et al., 1997; Smith et al., 1998). *A. sydowii* is not a common marine fungus, but it is a typically found in soil and other habitats.

Yellow band (YB) syndrome primarily affects the rounded, multilobate morphotypes of the major Caribbean reef building coral *Montastrea annularis,* but is also found less commonly on other *Montastrea* species and/or morphotypes. Yellow band was first reported as ring bleaching in the 1970s by Dr Phil Dustan, and is now thought to be a disease affecting an important keystone species throughout the Caribbean (Goreau et al., 1998; Cervino & Smith 1997; Santavy et al., 1999). Yellow band begins as a small pale round blotch that expands in diameter as a ring of pale tissue encircling an increasingly large area of dead coral, (Fig. la, b). In advanced stages, the ring becomes less defined, but continues to increase in diameter. Histological investigations revealed degenerative changes in the tissues and cells in affected areas, and the presence of pockets of unusual crystalline-like material in the gastric cavity (Santavy & Peters, 1997). Currently no pathogens have been identified in samples of YB.

Dark spot syndrome (DS) was first observed in 1990 in *Siderastrea siderea* and *Stephanocoenia michelinii,* and is now confirmed throughout the Caribbean (Garzon-Ferreira & Gil, 1998; Goreau et al., 1998). DS begins as small dark pigmented circles whose surface becomes depressed below the height of unaffected surrounding tissue (Fig. 2a, b).

Analysis of changes in the frequency of zooxanthellae abundance and division and mitotic index have been used as tools to record stress in corals (Wilkerson et al., 1988; Suharsono & Brown, 1992; Jones, 1997; Jones & Yellowlees, 1997). Both increases and decreases in mitotic index (MI) have been observed during and/or after an external stress (Jones, 1997). The purpose of this study was to investigate the distribution of YB & DS on coral reefs of various Caribbean islands, document the rate of tissue distruction, and quantify the affect on zooxanthellae. Both the abundance and mitotic index of symbiotic algae in normal and affected tissues were determined.

Materials and methods

YB and DS surveys

Surveys were conducted using belt transects, each covering an area measuring 15 m x 1 m. Reefs were surveyed in Bonaire (Netherlands Antilles), Grenada, Providenciales (Turks and Caicos) and St. John (US Virgin Islands) to determine the prevalence of YB and DS. Approximately five horizontal transects in between 10-12 m were completed at 6-10 dive locations on each island. Study sites were chosen based upon reef visitation frequency, selecting sites that were both heavily visited and less frequently visited. Transects were run beginning at the shore-dive entry point, and were continued at increasing depth and distance from shore.

Photo documentation and tissue loss

To conduct the survey, a waterproof, tape-measured line was stretched vertically and horizontally at each coral reef location. All transects were between depths of 3 and 16 m. Counts of YB and DS corals were compared to those of healthy corals of the affected species. Stainless steel nails with numbered tags were placed at the affected edge to monitor the progression of DS in Bonaire and Curacao. Colonies in Bonaire were photographed from November 1997 to June 1998 using an Underwater Nikon RS (SLR System). The living affected tissue in YB-stressed corals and DS-stressed corals was measured for their rate of retreat from the lesion's edge, using a metric ruler (cm) during that 6-month period. Removal of tags and nails by SCUBA divers cut the surveys short. Adverse weather conditions were also a prohibitive factor.

Sample collection of YB and DS

Total numbers of zooxanthellae and mitotic indices were determined from 2.5 cm diameter tissue samples taken using a stainless steel hole-punch from control and affected colonies in Bonaire, Grenada, Providenciales and St. John. Coral tissue samples were collected while SCUBA diving from a small boat. Samples of YB and DS were taken within the moving lesion of the affected colonies and in normal tissue nearby

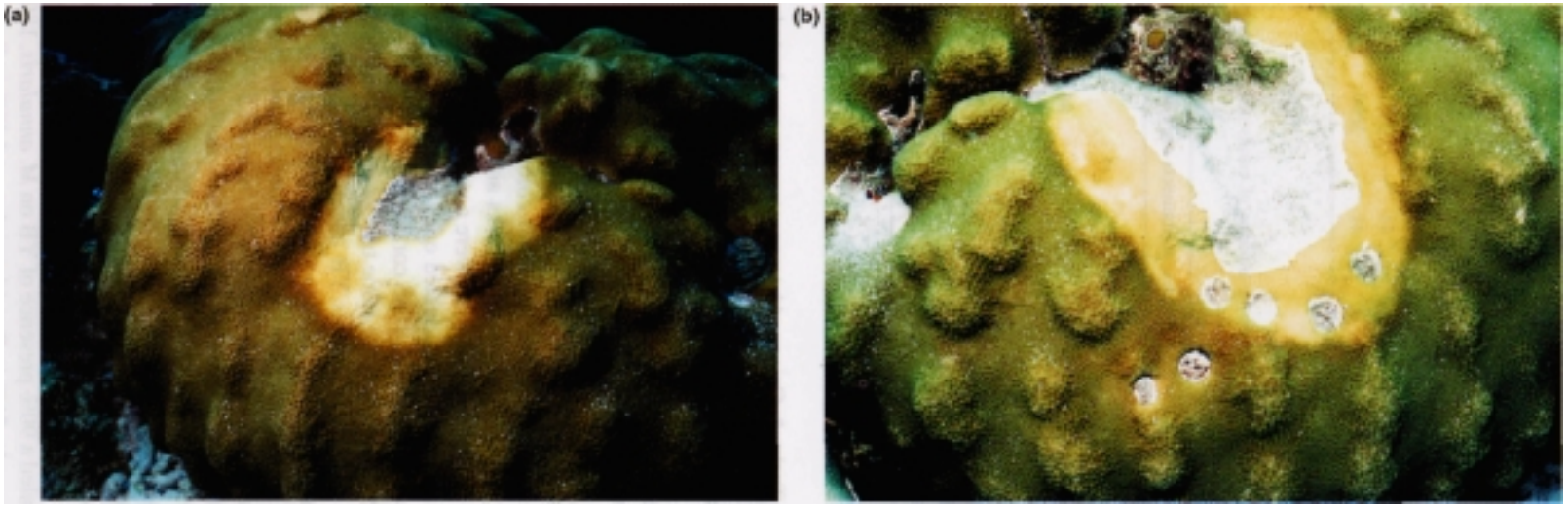


Figure 1. Appearance of yellow band in (he field (Fig. la). As yellow band expands outward the tissue generally dies in the center and expands as a yellow pigmented ring of necrotic tissue (Fig. Ib). The exposed skeleton can be quickly coloni/.ed by algae.

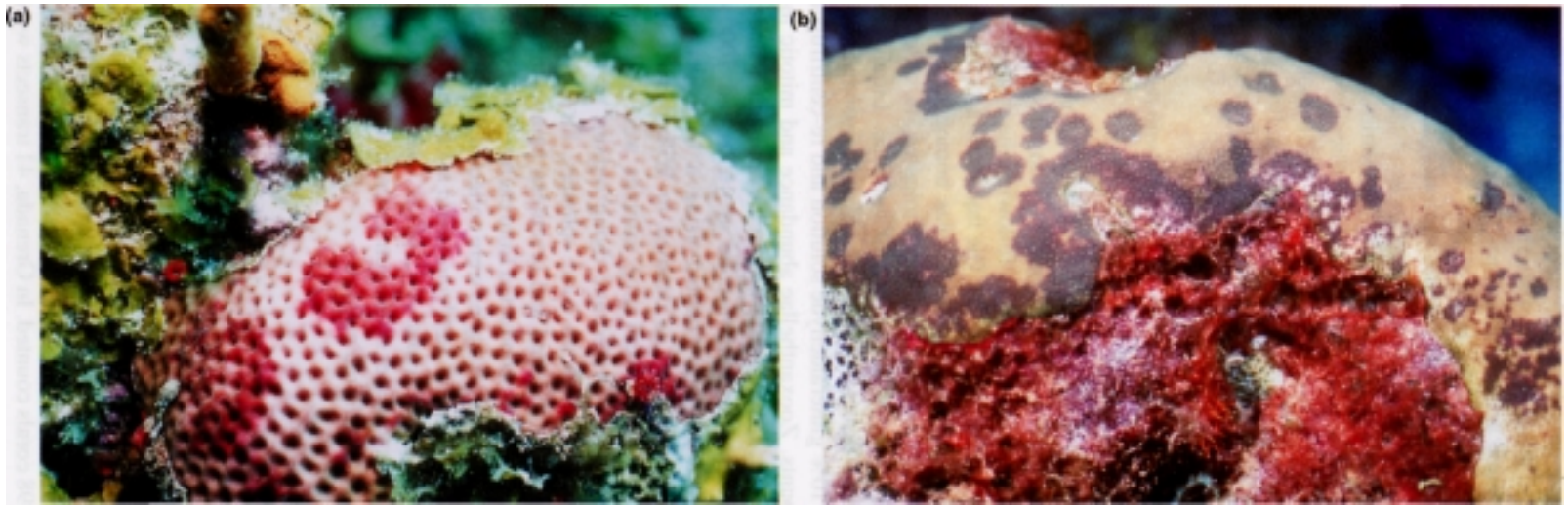


Figure 2. Appearance of dark spot during 1997-98 at various locations in the Caribbean on *Sidera.tlrea siderea* (Fig. 2a) and ***Stephanocoenia*** *Michelinii* in the field (Fig. 2b). As these circles expand outward they generally die in the center and expand as a dark pigmented ring of necrotic tissue. Sloughing of tissue can be seen in the early and later stages of DS.

on the same colony. YB samples were taken outside the band lesion (healthy tissue), from tissue bordering healthy and actively spreading YB, and from directly in the center of the band or blotch for analysis. At each survey site. 20 samples each of normal and affected tissue of each coral species were taken. These were placed in 100 ml polyethylene bottles. Samples were kept in ice coolers, in fresh filtered seawater (FSW). Samples for mitotic indices (Wilkerson et al., 1988; Jones. 1997) were preserved in 10% gluteraldehyde seawater solution, which had an original gluteraldehyde concentration of 90%.

Lab preparation for zooxanthellae and mitotic index counts

Samples were stored in a freezer (—4.0 °C) until tissue processing. Tissue was removed from all specimens collected using a Water Pik following the methods of Johannes & Wiebe (1970). The liquid extract containing tissue and zooxanthellae was homogenized and centrifuged at 5000 rpm for 5 minutes, in order to separate host tissue from zooxanthellae. Liquid extract was discarded and pellet was re-suspended in FSW/10% gluteraldehyde solution and re-centrifuged for counts. Zooxanthellae abundance and mitotic index were determined by direct examination under a phase contrast microscope at 400 x and lOOOx magnification, and counted using a Neubauer ruling hemocytometer as detailed in Wilkerson et al., (1988). Three 2.5 cm plug samples were collected from healthy, stressed and dying regions of *M. annularis* from different coral colonies, for a total of 9 plugs. One hundred and seventeen hemocytometer slide counts were conducted from each region. For DS. three 2.5 cm plug samples were collected from healthy and DS lesions on *Stephanocoenia michelinii* & *Siderasirea sidereu,* a total of 12 plugs. One hundred and five hemocytometer slide counts were conducted from healthy and affected regions.

Results

Field data and tissue destruction for yellow band & dark spot

The following transects were conducted to determine the ratio of healthy *Montastrea* sp. vs. those affected with YB. In Bonaire, 102 transects were conducted. /;= 6298 corals counted. In Grenada, 41 transects were conducted, and a total of 2607 corals were counted.

Table I. Percentage of *Montastrea* sp.colonies affected by yellow hand during 1997-98

Locations	»=sites	/j=transects	//=colonics	ᶜ/t YB
Bonaire	10	102	6298	91%
Turks & Caicos	6	41	1318	56%
Grenada	6	41	2607	18%
St. John	6	41	2899	40%

Table 2. Percentage of the number of corals affected by dark spots during 1997-98

Locations	»=sites	»=transects	;i=colonies	% DS
Stephanocoenia michelenii				
Turks & Caicos	6	86	861	46%
Grenada	6	86	1046	43%
Siileraxlrea sitlerea				
Bonaire	10	101	1297	53%
Turks & Caicos	6	86	897	56%
Grenada	6	86	882	42%

In Turks & Caicos, 41 transects were conducted, and 1318 corals counted. In St. John, 51 transects were conducted, and 2899 corals counted (Table 1).

The following transects were conducted to determine the ratio of healthy *Siderastrea siderea,* and in some cases. *S. Michelinii,* vs. those affected with DS. In Bonaire, 101 transects were run and 1297 *S. siderea* corals counted. In Turks & Caicos, 86 transects were run and 897 *S. siderea* coral colonies were counted. At the same locations in Turks and Caicos, a total of 861 *S. michelinii* corals were counted. In Grenada, 86 transects were conducted on *S. siderea,* and 882 corals were counted; 1046 corals of *S. michelinii* species were counted at the same transect locations (Table 2).

Our study reveals YB and DS at sites across the Caribbean (Fig. 3). However, DS is fairly rare at sites in Mexico, and has not been reported from Bermuda. Transects in Bonaire during 1998 reveal 53% of the *S. siderea* colonies affected by DS. *S. michelinii* was too rare in Bonaire to estimate DS frequency. In Turks & Caicos, 56% of *S. siderea* and 46% of *S. michelinii* had signs of DS. In Grenada during 1997, DS lesions were seen on 43% of the *S. michelinii* colonies and 42% of *S. siderea* colonies. Transect data from Bonaire revealed a 90% presence of YB on *M. annularis,* 18% in Grenada, 56% in Providenciales. and 40% in St. John.

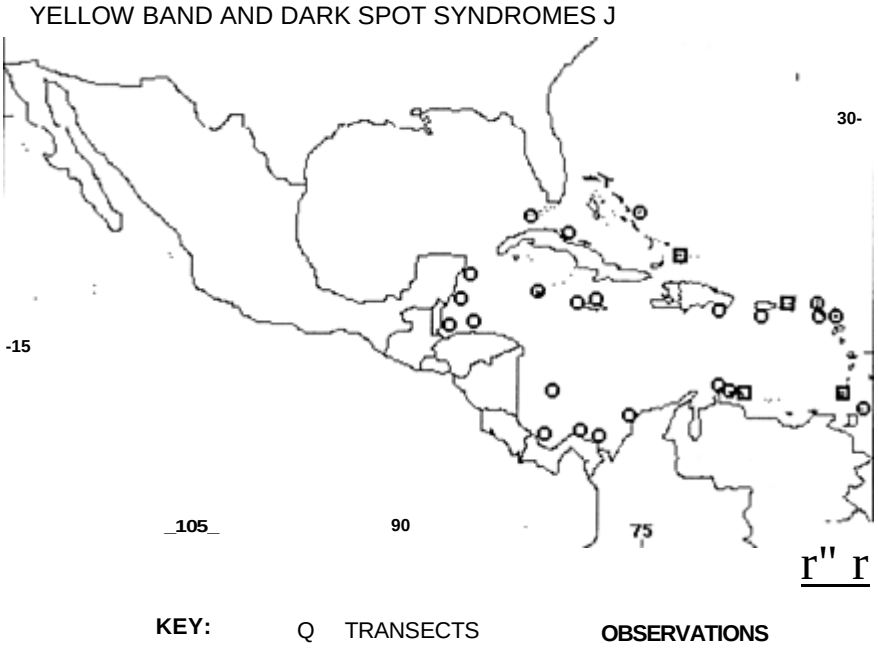


Figure 3. Map of Caribbean showing areas where yellow band and dark spot syndromes were reported in 1996—1998 (circles). Squares indicate locations where transects were run to determine frequencies of affected corals.

The rate at which tissue dies back from affected areas in corals with YB in Curacao is around 0.63±0.17 cm per month (Fig. 4).

DS is reported at sites across the Caribbean. The rate of tissue die back at the edges of DS affected specimens of *Siderastrea siderea* in Bonaire was around 3.99 cm/month, for 2 coral colonies counted. DS in 5. *michelinii* appears to be much slower, and in many cases the tissue does not immediately die back, but is seen to be uniformly depressed below the surface of unaffected surrounding tissue. DS tissue and skeleton depression ranges from I to 4 cm. The color of DS tissue is characteristically a uniform dark chocolate brown in *S. michelinii,* but can range from purple to

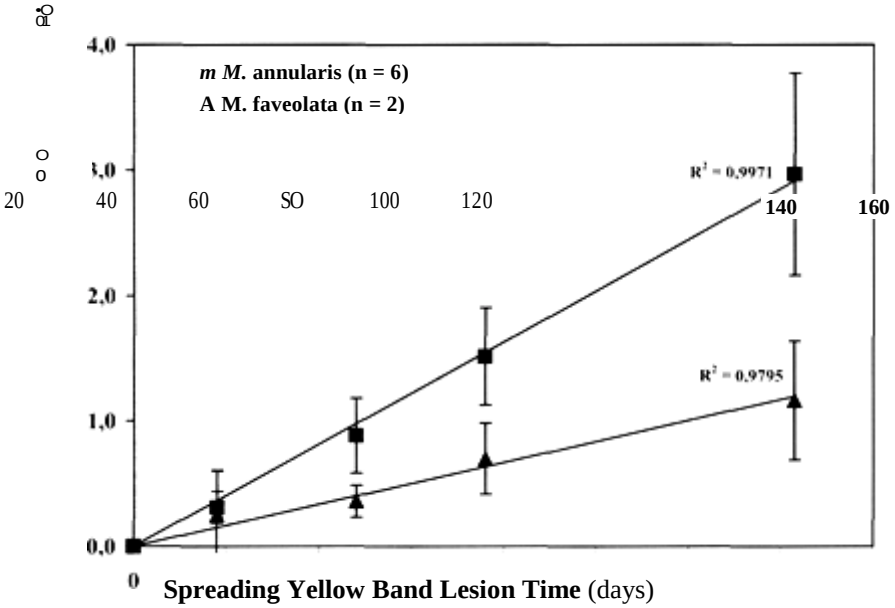
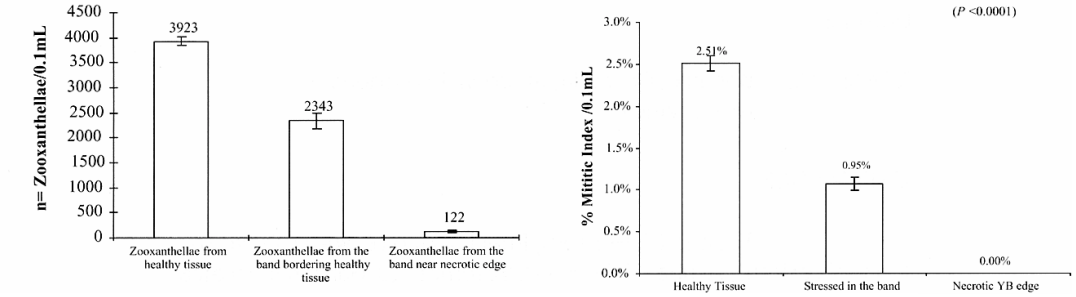


Figure 4. Rate of Spread of YB affected tissue. The rate at which YB spreads is 0.63±0.17cm per month.



Tissue Samples From 6 Specimens (*P* <O.OOOI)

Figure 5. Mean and standard deviations of zooxanthellae numbers in normal and yellow band affected *Montastrea species.* This figure compares healthy zooxanthellae, with: zooxanthellae that borders healthy tissue and the advancing yellow blotch; and. with zooxanthellae in nccrotic areas.

Figure 6. Mean and standard deviations showing the mitotic indices of zooxanthellae in normal, YB stressed (advancing yellow blotch), and nccrotic surface areas on *Montastrea annularis.*

pink to brown in *S. siderea* with a strong increase in pigmentation towards the edge, and with surface depression greatest at the dying edge or center of the lesion.

Zooxanthellae abundance and mitotic index

Total cell counts of zooxanthellae in six 2.5 cm samples of YB-affected tissue near the healthy tissue had 40% less symbiotic algae (n=2343) within host tissue than did healthy specimens («=3923), (J9=<0.0001) (Fig. 5). There was a decrease in zooxanthellae density near the dead edge of yellow band-affected tissue, which had only 97%(n=122) fewer symbiotic algae than healthy tissue *(p=<0.*0001).The zooxanthellae in the necrotic edge of YB tissues showed no dividing cells and almost complete cessation in zooxanthellae cell division. The mitotic index of *Montastrea sp.* decreased from 2.51 % in healthy samples, to 0.95% in areas of YB near living tissue, to 0% in samples near the necrotic edge (p<0.0001) (Fig. 6).

DS-affected *Siderastrea siderea* show a significant decrease in zooxanthellae in host tissue (56% decrease; n=5029) compared to healthy specimens («=11343)(p=0.237935).

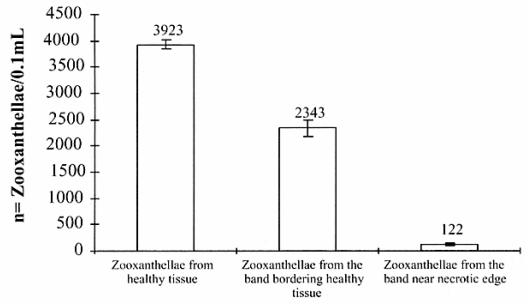
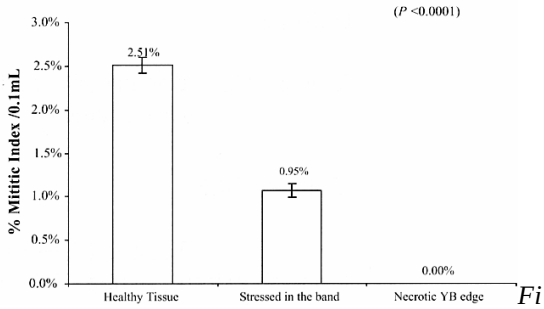


Figure 7. Mean and standard deviations of /.ooxanthcllae numbers in normal and dark spot affected *Siderastrea siderea* and *Stephanocoenia michelinii.*



Fi gure 8. Mean and standard deviations showing the mitotic indices of normal and dark spot affected *Siderasl mi siderea* and *Stephanocoenia michelinii.*

However, cell counts in *Stephanocoenia michelinii* tissue affected by DS reveal only a slight decrease in total cells (13% decrease; «=6453) compared to healthy specimens («=7415), (/?=<0.0001). (Fig. 7).

An average of 1.2% of the zooxanthellae in control samples of *S. siderea* were undergoing cell division, compared to 0.5% in DS-affected samples. The average in *S. michelinii* was 1.5% compared to 0.4% in DS-affected samples (P<0.0001) (Fig. 8).

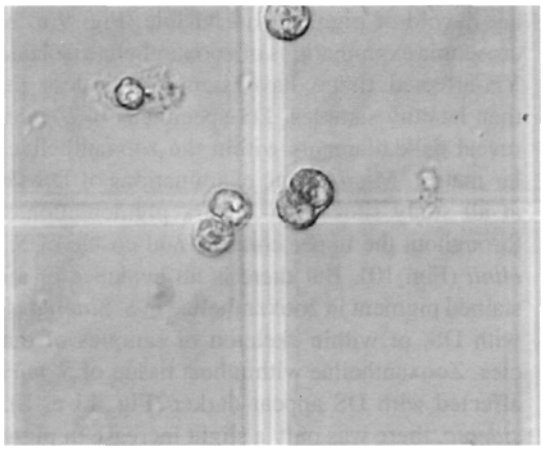
Light microscopy of affected tissue

Microscopic examinations were conducted on zooxanthellae isolated from healthy tissue, from healthy tissue bordering the yellow band, and from directly in the band bordering the necrotic edge (exposed skeleton). The healthy tissue reveals dark pigmented zooxanthellae, as well as the presence of distinct or-ganelles. However, zooxanthellae from the YB appear to be vacuolated and depleted of organelle structures. Remnants of vague 'ghost' zooxanthellae cells (which are devoid of pigment) are visible (Fig. 9 a, b). Microscopic examinations of zooxanthellae isolated from YB-affected tissue have significantly less pigment than healthy samples. DS specimens of 5. *michelinii* reveal dark filaments within the zooxanthellae cellular matrix. Microscopic examinations of DS skeleton at all study sites show a dark pigmentation stained throughout the upper corallite and costae of *S. michelinii* (Fig. 10). But there is no evidence of a darkly stained pigment in zooxanthellae in *S. Siderea* affected with DS, or within skeleton of samples of that species. Zooxanthellae within host tissue of 5. *michelinii* affected with DS appear darker (Fig. 11 a, b). In *S. siderea,* there was only a slight increase in pigment of the zooxanthellae compared to that of the healthy specimens. DS in *S. siderea,* and *S. michelinii* is slightly different in morphology. YB- and DS-affected specimens in the field exhibit polyp retraction and a visible increase in mucus production. Expulsion of zooxanthellae in mucus during *in situ* observations was not evident in YB and DS samples. Zooxanthellae seem to be dying inside host tissue. Zooxanthellae in DS tissue of *S. siderea* were swollen and internally disrupted. Future experiments will include electron microscopy and CHL *a* analysis.

Discussion

Cellular changes

Coral tissue has not been observed to recover from DS or from YB, and algae often colonize dead areas. Carbon fixed autotrophically by the coral's symbiotic alga is translocated to the animal host (Goreau & Goreau, 1960; Trench, 1971). The contribution of translocated carbon to the animal host may be 90% or more of coral growth requirements (Muscatine et al., 1983). Zooxanthellae loss can leave the coral with an insufficient energy budget that hinders growth. The host regulates algal population density by expelling excess algae, digesting degenerate algae (Steele & Goreau, 1977), or by controlling algal cell division to keep those cells from overgrowing the host (Muscatine & McNeil, 1989). Zooxanthellae normally resist digestion by the host lysosomes when alive. Further work is needed using electron microscopy and specific stains to determine if the degenerative fragmented algae seen in host tissue with YB are being digested by host lysosomes or if the pathogen is lysing the symbiotic algae directly.



(a)

(b)

Figure y. Appearance of x.ooxanthellae heallhy dividing (see photo a) and /ooxamhellae atTectcd by yellow band (see photo b) showing the membrane and vaeuolated structures, depleted organelles. and the early signs of 'ghosts'.

Figure It). Appearance of dark spot affected ***Stephanocoenia*** *miclielinii* skeleton, showing dark pigmentation stained below the septal margin.

Coral tissues affected by YB and DS show characteristic abnormalities that differ from those seen in bleaching. During bleaching, zooxanthellae are released into the coelenteron from the intracellular location in the endoderm as temperature increases (Yonge & Nicholls. 1931). The expelled algae are visible with the naked eye during temperature increase. Mucus collected from YB- & DS-affected tissues contains only isolated traces of zooxanthellae, in sharp contrast to bleaching stress responses (Hayes & King, 1995). It appears that in YB-affected tissue, necrotic zooxanthellae are being cytolysed *in-sitit.* leading to the formation of vaeuolated zooxanthellae, which are reduced in concentration and depleted of structural

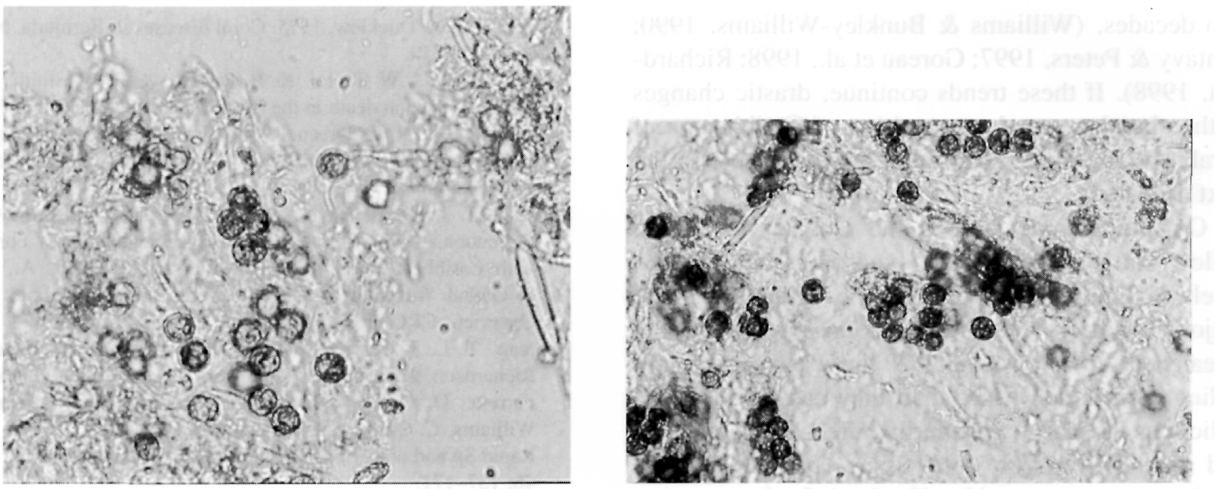


Figure *II.* Zzooxanthellae from healthy *S.* ***michelinii*** (see photo a) and zooxanthellae from 5. *michelinii* with dark spot (see photo h). Photo b clearly shows altered pigmentation of zooxanthellae as well as fragmentation.

content. Zooxanthellae viewed under light microscopy in DS samples reveal a slight increase in pigments. This dark pigment is evident on the surface of the skeletal matrix deep into the septal margin.

It may be significant that dark spots in *S. siderea* and *S. michellinii* exibit differences in morphology. This may represent different conditions or diseases, and should be further studied.

Ecological concerns

Yellow band lesions may often be confused with bleaching in *Montaxtrea* sp. During bleaching events, it is almost impossible to determine which corals are affected by YB because the entire colony exhibits a substantial decrease in pigment compared to healthy specimens. When the bleaching ends and the coral regains pigmentation, the signs of YB re-appear and continue to spread. YB was uncommon during the first few major Caribbean bleaching events, and was initially confused with a delayed recovery from bleaching. Had it been more prevalent, the connection between bleaching and temperature (Goreau et al., 1993) would have been much harder to recognize. DS-affected colonies also bleach, but the dark areas remain dark and continue to advance during and after bleaching. These data reveal that in coral tissue associated with of YB and DS syndromes, the number and division rate of intracellular Zooxanthellae are reduced. We are currently investigating the possibility that the etiology of the syndrome may be initiated in the Zooxanthellae. We are currently trying to isolate pathogens associated with YB and DS tissues. Corals with YB and DS reveal elevated mucus release, which is generally the first physiological response of corals to stress (Hayes & Goreau, 1998). Increased mucus production can alter the abundance and/or diversity of naturally occurring microbial epibiota that use mucus as component energy sources. It has been hypothesized that microbial proliferation can lead to coral necrosis by the release of toxins or the establishment of anoxic conditions (Di Salvo, 1971; Mitchell & Chet, 1975; Garrett & Ducklow, 1975; Ducklow & Mitchell, 1979; Rublee et al., 1980; Richardson, 1998). Further work is warranted to determine any pathogenic mechanisms involved with these two syndromes. The marked differences seen in DS syndrome between *S. siderea* and *S. michelinii* may indicate that different agents are responsible, that different zooxanthellae are affected, or that the coral host might be responding differently to DS.

YB and DS appear to be directly affecting the abundance of at least three major reef building coral species in the Caribbean. The growth rate and number of symbiotic algae in their tissues are reduced, decreasing skeletal growth rate and causing progressive coral tissue mortality at a rate much faster than growth. DS lesions appear to expand 6.65 times faster than YB.These effects could cause the progressive reduction of these species if these syndromes persist. Several other major framework-building reef coral species are also being severely affected by different syndromes and coral bleaching. Coral bleaching and diseases have only become widespread in the last

two decades, (Williams & Bunkley-Williams, 1990; Santavy & Peters, 1997; Goreau et al., 1998; Richardson, 1998). If these trends continue, drastic changes in the abundance and composition of Caribbean reef corals and reef framework integrity may occur in the next decade.

Our study indicates that the prevalence of both yellow band and dark spot syndromes has reached levels of significance throughout the Caribbean. Three major Caribbean reef-building coral species may be threatened. It is possible that these syndromes are killing them much faster than they can grow. These afflictions appear to primarily affect zooxanthellae and secondarily affect coral tissue. To remedy these syndromes, we must identify the infectious agents, understand their modes of action and their sources, and determine how they are transmitted. Such challenges will require extensive field analyses, controlled aquarium experiments, and detailed molecular and biochemical analyses.

Acknowledgements

We thank Derek Kelly (New York City Fire Dept.) for field assistance and, Ginger Garrison (USGS), Esther Peters (Tetra Tech), Robert Trench, Len Muscatine, Ross Jones and Ove Hoegh-Guldberg for discussions. We thank all field observers (listed in map) who contributed data on the distribution of YB and DS syndromes. We also thank Anthony Barricelli (St. Francis Prep), Kathryn Winiarski (Long Island University) and Stamatia Alexiou (Boston University) for assistance with laboratory work.

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