

Monitoring the coral disease, plague type II, on coral reefs in St. John, U.S. Virgin Islands

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Abstract: In July 1997, conspicuous white patches of necrotic tissue and bare skeleton began to appear on scleractinian corals in several bays around St. John, US Virgin Islands. Analysis of diseased coral tissue from five different species confirmed the presence of a *Sphingomonas*-like bacterium, the pathogen for plague type II. To date, 14 species of hard corals have been affected by plague type II around St. John. This disease was monitored at Haulover and Tektite Reefs at depths of 7-12 meters. The study site at Tektite Reef has >50% cover by scleractinian corals with 90% of hard corals being composed of *Montastraea annularis*. Monthly surveys at Tektite Reef from December 1997 to May 2001 documented new incidence of disease (bare white patches of skeleton) every month with associated loss of living coral and 90.5% of all disease patches occurred on *M. annularis*. The frequency of disease within transects ranged from 3 to 58%, and the area of disease patches ranged from 0.25 to 9000 cm². The average percent cover by the disease within 1 m² ranged from 0.01% (\pm 0.04 SD) to 1.74% (\pm 9.08 SD). Photo-monitoring of 28 diseased corals of 9 species begun in September 1997 at Haulover Reef revealed no recovery of diseased portions with all necrotic tissue being overgrown rapidly by turf algae, usually within less than one month. Most coral colonies suffered partial mortality. Very limited recruitment (e.g., of *Agaricia* spp., *Favia* spp. and sponges) has been noted on the diseased areas. This coral disease has the potential to cause more loss of live coral on St. John reefs than any other stress to date because it targets the dominant reef building species, *M. annularis*.

Key words: Coral disease, plague type II, coral reef monitoring, St. John US Virgin Islands.

Diseases affecting stony corals have increased over the past 20 years (Peters 1996, Bruckner and Buckner 1997, Richardson 1998, Harvell *et al.* 1999), contributing to reef degradation (Richardson 1998) and causing changes in community structure (Aronson and Precht 1997). Within the last few years, diseases have increased in intensity, variety and frequency (Harvell *et al.* 1999, Jaap *et al.* 2000, Weil *et al.* 2000, Santavy *et al.* 2001). One of the most virulent was first reported in 1995 along the Florida reef tract (Richardson *et al.* 1998a, b). This new condition was simi-

lar to the disease ("plague type I") reported for Florida reefs in 1977 (Dustan 1977). In both cases, a sharp line differentiated apparently healthy tissue from diseased tissue. However, the new disease (referred to as plague type II) was affecting more species, progressing at a much faster rate (1-2 cm vs. 3.1 mm per day), and had a wider geographic distribution than the original plague ("type I"). The pathogen was initially identified as most closely related genetically (based on sequence data) to the bacterium *Sphingomonas* (Richardson *et al.* 1998a, b), but it appears to be a new genus of

bacteria (Richardson, unpublished data). Initial reports from Florida reefs indicated the species most affected was *Dichocoenia stokesi*, a relatively small species that is not a major reef-builder (Santavy and Peters 1997, Richardson 1998a, b). Currently this disease is reported throughout the Florida reef tract (Santavy *et al.* 2001, Bruckner and Green 2001) and the Caribbean (Weil *et al.* 2000).

This disease was first observed around St. John in the summer of 1997. It has now caused rapid and extensive mortality to major reef-building species, primarily *Montastraea annularis* (complex), but also *M. cavernosa*, *Colpophyllia natans*, *Siderastrea siderea*, and *Diploria* spp. It has been observed on most reefs around the island in shallow backreef habitats (depth < 3 m), sloping forereefs (depth 6 m -12 m), and on the mid-shelf reef 8 km off the south shore at depths 24 m to 32 m (J. Miller, pers. obs.). Recently, researchers believe there to be a new, even more virulent strain of plague, termed plague III (Richardson and Smith, pers. com.). It is unknown as to whether it has the same pathogen as plague II. It is differentiated from plague II in its faster rate of progression (decimeters per day vs. centimeters per day for plague II) and has been observed affecting large *C. natans* and *M. annularis* colonies. The majority of plague observed at our study sites has been type II, but since both have been observed they are grouped and termed "plague" hereafter. In St. John, plague appears to have resulted in greater coral tissue loss than any other stress in the last five years (USGS, NPS unpublished data).

The objectives of this continuing study are (1) to photographically monitor corals affected with plague and resulting areas of mortality over time (2) to evaluate the frequency and size of plague occurrences on a coral reef dominated by *M. annularis*, and (3) to see how this disease affects coral cover on this reef. Here we are reporting the results obtained from September 1997 to May 2001. We selected our study sites because they are located within the boundaries of Virgin Islands National Park, and represent two reefs with high coral cover composed of major reef-building corals that were apparently most vulnerable to plague in this region.

MATERIALS AND METHODS

Microbiology: In September 2000, samples from five coral species, *M. annularis*, *M. cavernosa*, *C. natans*, *S. siderea*, and *Dendrogyra cylindrus*, were taken for laboratory analysis to confirm the presence of plague. The authors and Dr. Laurie Richardson collected samples using sterile syringes from the active disease lines as well as adjacent, healthy coral tissue and bare coral skeleton from the same colonies. Upon return to shore (<1 hr) samples were diluted into sterile seawater in a 1:10 (dilution) series to a final dilution of 10^{-6} . Dilutions at 10^{-4} , 10^{-5} and 10^{-6} were plated (in triplicate) onto marine agar plates and incubated at 30° C. After two days, colonies that represented the most prevalent colony type from disease line samples (when compared to healthy tissue and bare skeleton samples) were picked and inoculated (streak plated) onto fresh marine agar plates to isolate pure colonies. Pure colonies were then selected and inoculated onto marine agar slants (Richardson pers. comm.). These were given to Dr. Garriet Smith for comparison with his microbial database, and the presence of the plague pathogen was confirmed (Table 1).

Field studies: Two different field methods are being used during this study: photo-monitoring of tagged corals at Haulover Reef, and surveys of quadrats within transects at Tektite Reef. Both sites are fringing reefs dominated by *M. annularis*, with depth ranges of 6 m -12 m and high coral cover (Haulover estimated as 20%, and Tektite quantified as 51% [\pm 19 SD]). Both reefs are within the boundaries of Virgin Islands National Park in relatively undeveloped watersheds with no obvious major sources of pollutants. However, they are "downstream" from the British Virgin Islands and other Eastern Caribbean islands, and, under some conditions, influenced by the plume of the Orinoco River.

Haulover Reef: In September 1997, red, numbered plastic tags were attached to the substrate to designate the locations of 28 coral colonies affected with plague. (Because of its multi-columnar growth form, it was not possi-

TABLE 1

List of scleractinian coral species affected by the coral disease plague type II on reefs around St. John, U.S. Virgin Islands

Coral Species	Plague II presence	Laboratory Confirmation	Tektite Reef	Photo-monitoring
<i>Montastraea annularis</i> (complex)	X	X	X	X
<i>Montastraea cavernosa</i>	X	X		X
<i>Colpophyllia natans</i>	X	X	X	X
<i>Siderastrea siderea</i>	X	X	X	X
<i>Dendrogyra cylindrus</i>	X	X	X	
<i>Mycetophyllia lamarckiana</i>	X			X
<i>Agaricia agaricites</i>	X		X	X
<i>Eusmilia fastigiata</i>	X			X
<i>Madracis mirabilis</i>	X		X	
<i>Madracis decactis</i>	X		X	
<i>Porites porites</i>	X		X	
<i>Porites astreoides</i>	X		X	X
<i>Leptoseris cucullata</i>	X		X	
<i>Stephanocoenia michelinii</i>	X			X

ble to ascertain if each *M. annularis* was a distinct colony.) Colonies ranged in size (diameter) from a 5 cm *Mycetophyllia lamarckiana* to >1 m multi-lobate *M. annularis*, and were chosen based solely upon the presence of plague. As this was a pilot photo-monitoring study, the species and colony sizes selected do not necessarily provide a quantitative representation of the distribution of plague on this reef. Photographs of each colony were taken approximately monthly for 6 months, and then quarterly, with a Nikonos-V camera/28 mm lens, and with ASA 200 Ektachrome film. For some colonies, a Nikonos close-up lens/framer was used for better resolution. Developed slides are labeled, cataloged and viewed on a light table. Slides were projected to facilitate comparison to slides from previous sample periods.

Tektite Reef: Beginning in December 1997, 10-meter transects were haphazardly placed on a constant compass heading (i.e., parallel to each other) within a small, nearly rectangular patch reef (depth: 7 m to 10 m; est. size: 40 m x 20 m) of very dense coral cover (predominantly *M. annularis* and *C. natans*). Initially transects were not fixed, however, the transects generally encompassed the entire patch reef which is surrounded by sand. In March 2001, transect locations were permanently fixed using laminated photographs of

transect endpoints for relocation. For each survey, a 1 m square PVC quadrat was placed on both sides of each transect, at each meter, producing a 10 m x 2 m belt transect. Sampling occurred monthly from December 1997 to May 2001 (with the exceptions of Sept. and Nov. 1998, Jan., May and Oct. 1999, and April and June 2000). The number of transects varied initially from eight to eleven until June 1998 when eight transects consistently used. The same two researchers have collected all field data with the exception of April 2001, when a third investigator was used. Each observer is trained in disease identification, and has extensive experience with visual percent cover estimates. Each quadrat was subdivided by string into 100, 10 cm x 10 cm squares. For each quadrat, researchers recorded substrate depth, and estimates on percent cover of live coral, macroalgae, and *new* disease (not just planar). Percent cover was estimated by visually. New disease was defined by recent coral mortality and was characterized as being bright white bare coral skeleton (and meeting other criteria defined by Richardson 1998a). If algal turf was seen growing over freshly killed skeleton, it was not recorded as evidence of new disease. Observers were careful to discriminate between new disease, and tissue loss from fish bites, snail predation and discoloration from bleaching.

Each patch of disease on any coral surface (planar or vertical) was measured along the maximum length and perpendicular axis to produce a diseased area measurement. Frequency of occurrence, distribution, and area of disease patches within each quadrat were calculated for each sampling period by transect. Twenty quads from each transect were used to derive an overall mean per category for that transect. Then the grand mean and standard deviation for percent live cover by coral, macroalgae and disease for all transects were calculated (N=8-11) per sample period from the mean and standard deviation (of each category) for each transect using data from individual quadrats (N=20). Monthly mean percent live coral cover data were arcsine-square root transformed for regression analysis (Zar 1984). The Kolmogorov-Smirnov goodness of fit for two-sample test (Campbell 1994) was used to compare the distribution of percent live coral cover by quadrat between 1998 and 2001.

Mean monthly temperature data were calculated from data recorded daily every two hours (using Ryan Industries RTM 2000 Temp Mentors) at two sites around St. John. Temperature ranged from 25.1 to 29.6 °C. Data on monthly frequency of occurrence and percent cover by disease were compared to mean monthly temperature using a correlation analysis to look for seasonal patterns.

Additionally, a small-scale field test was conducted in which a two-part marine epoxy was applied to the disease line on colonies of two species (three *C. natans*, one *M. annularis*) in an attempt to smother the aerobic bacteria associated with this disease. The epoxy was 2.5-5 cm wide, extending into both the apparently healthy and diseased areas. Divers revisited the site to monitor and photograph the colonies.

RESULTS

Visual observations from reefs surrounding St. John, reveal that plague has affected 14 species of hard corals, including the primary reef building corals. *M. annularis* is the species most affected by plague (91% of plague at

Tektite was on *M. annularis* complex), followed by *Porites porites* (3%), and *C. natans* (2%). Laboratory analysis of tissue from five species (*M. annularis* complex, *M. cavernosa*, *C. natans*, *S. sidereal*, and *D. cylindrus*) confirmed the presence of the *Sphingomonas*-like bacterium, the pathogen associated with plague. *In situ* identification of plague was based upon external signs, species affected and rate of progression (Richardson 1998a). Rate of disease progression across a colony was not specifically measured, however, we observed large colonies of *M. annularis* (>1.5 m diameter) killed by plague in less than six weeks.

Photographs of 28 individual coral colonies (of nine species) at Haulover Reef affected with plague (Table 1) were analyzed. Sixty-one percent of the colonies had active disease for < 1 month, 36% between 1-3 months, and one *M. annularis* for 4 months, revealing the relatively short-lived nature of this disease. None of the tagged corals have shown a re-occurrence or re-activation of disease. Recovery (new growth) of coral tissue onto the diseased area has never been observed although limited recruitment by *Agaricia* spp., *Favia* spp., and sponges onto the new substrate has occurred.

At Tektite Reef new patches of disease were found during every month of the study and throughout the study area. Although substantial variability exists among months, the frequency of occurrence of disease patches (determined by number of quadrats with plague /total number of quadrats) has been decreasing over the study period (Fig. 1). The mean occurrence of new disease each month was 29%, ranging from 3% (December 2000) to 58% (December 1997). Mean percent cover by new disease was 0.3% and ranged from just over zero to 1.7% with > 1% new disease only observed in two months (Dec. 1998 = 1.2% and Sept. 2000 = 1.7) (Fig. 2).

Throughout this study, 3 555 patches of new disease were measured with a mean size of 32 cm² (range = 0.25 cm² to 9 000 cm²). The size distribution of new disease patches reveals the majority (53%) of disease patches are <10 cm² (Fig. 3) and with 32% of these 1 cm² (Fig. 4). Ten scleractinian coral species were found with plague at Tektite Reef (Table 1). Disease

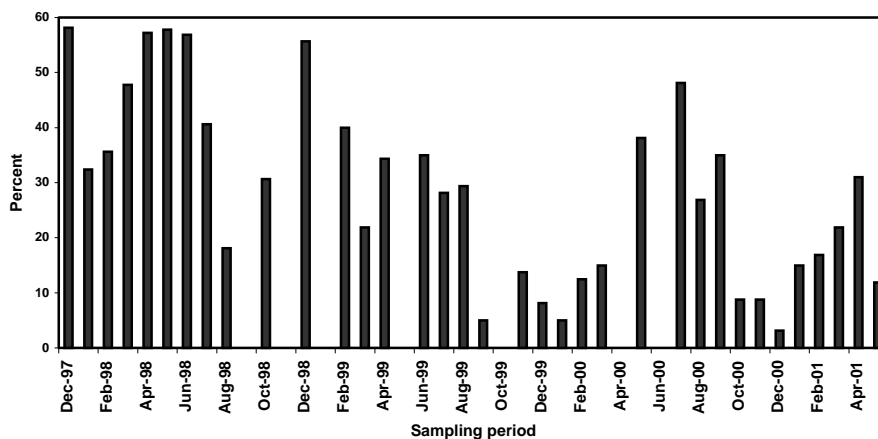


Fig. 1. Mean monthly percent frequency of occurrence of disease incidences on Tektite Reef, St. John, over 42 months (Dec. 1997 – May 2001). Frequency of occurrence is defined as the ratio of the number of quadrats with disease to total number of quadrats per transect.

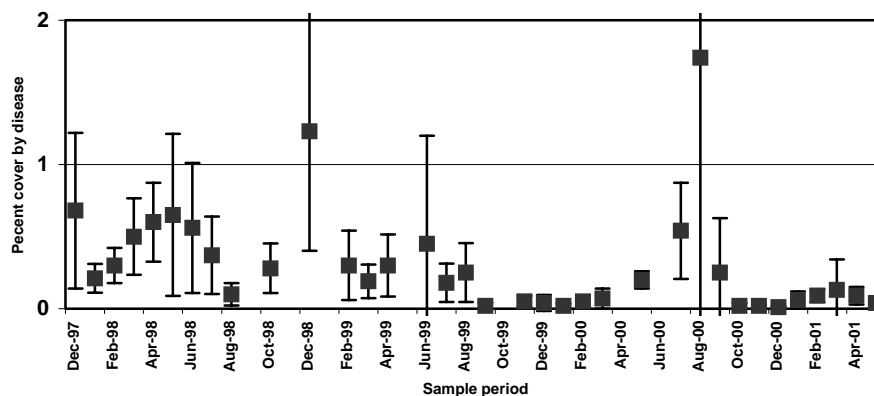


Fig. 2. Mean monthly percent cover by coral disease plague type II on Tektite Reef, over 42 months.

patches occurred most on *M. annularis* (90.5%), followed by *Porites porites* (3.2%), *C. natans* (2.1%), *Agaricia agaricites* (1.6%), *Madracis mirabilis* (1.4%) and *P. astreoides* (0.8%).

The highest monthly mean percent live coral cover occurred in the first month of the study (Dec. 1997 = 65.3%) with a significant negative regression from that point ($r^2 = 0.57$; $y = -0.178x + 51.4$; $df = 1$; $p < 0.001$) (Fig. 5). Grouping percent live coral cover into percent class 'bins' (e.g.: number of quadrats with 0 – 10% live coral cover, number of quadrats with 11 – 20%, etc.) and evaluating this distribution, showed a statistically significant difference

between 1998 and 2001 (Kolmogorov-Smirnov test, $p < 0.001$) (Fig. 6). Seasonal patterns of disease outbreaks were not evident as the highest percent cover by disease occurred in August 2000 (warmer water temperatures), while the second and third highest percent cover by disease occurred in December 1998 and 1997, respectively (colder water temperatures). Neither the frequency of disease occurrence or percent cover by disease was correlated to water temperature ($p > 0.05$).

In three of the four cases in which epoxy had been applied, the disease failed to advance beyond the epoxy line. However, given the

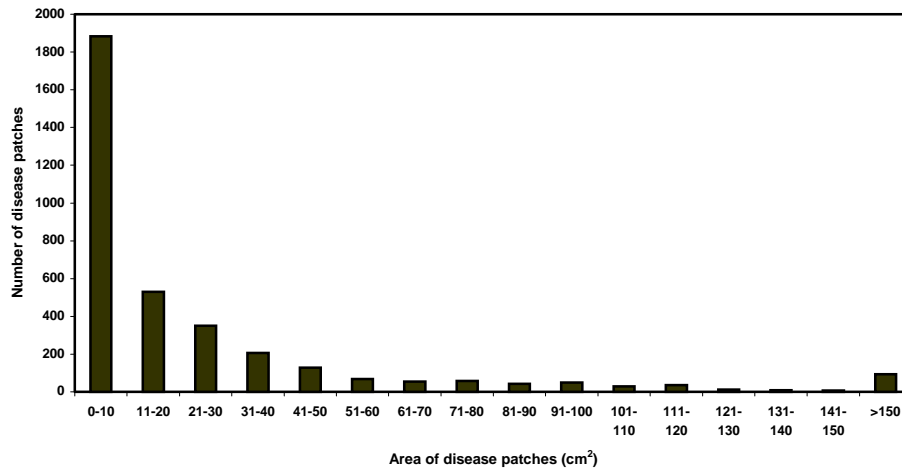


Fig. 3. Size distribution of disease patches measuring from <1 cm² to >150 cm² on Tektite Reef.

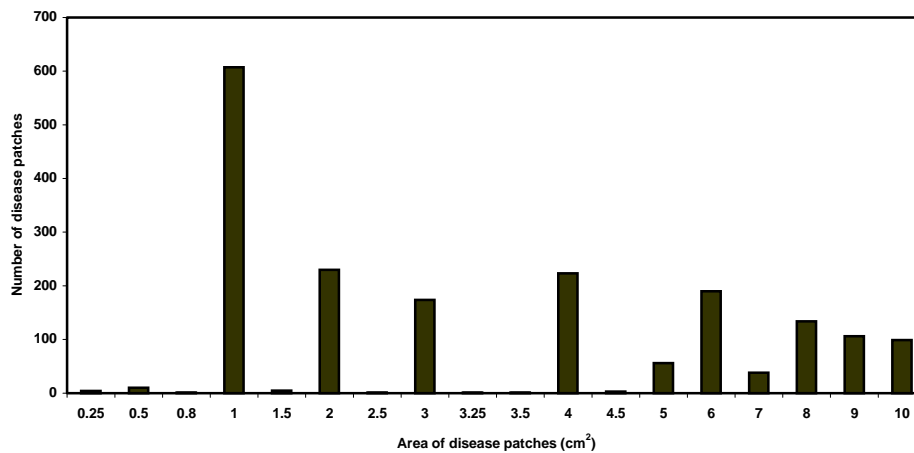


Fig. 4. Size distribution of disease patches measuring from 0.25 cm² to 10 cm² on Tektite Reef.

sporadic activity (rapid progression, sudden stop) of this disease no direct results can be drawn from these trials, and further field-testing is planned.

DISCUSSION

Plague has caused extensive mortality at Tektite and Haulover Reefs, affecting 14 different coral species (Table 1). Although patches of disease were typically very small, cumulative effects resulted in a significant decline in total live coral cover at Tektite Reef

from December 1997 through May 2001. The decrease in frequency of occurrence throughout the study period may result in a slower decline in coral cover than in previous years. However, with no evidence of recovery (new growth of coral tissue adjacent to areas killed by disease) and new patches of disease occurring every month, continued loss of live coral cover appears likely. We observed plague affecting the surviving tissue on previously diseased (but untagged) corals ($N < 5$; J. Miller pers. obs.). Whether this is a new infection or re-activation of the initial disease is undetermined. The presence of disease every month

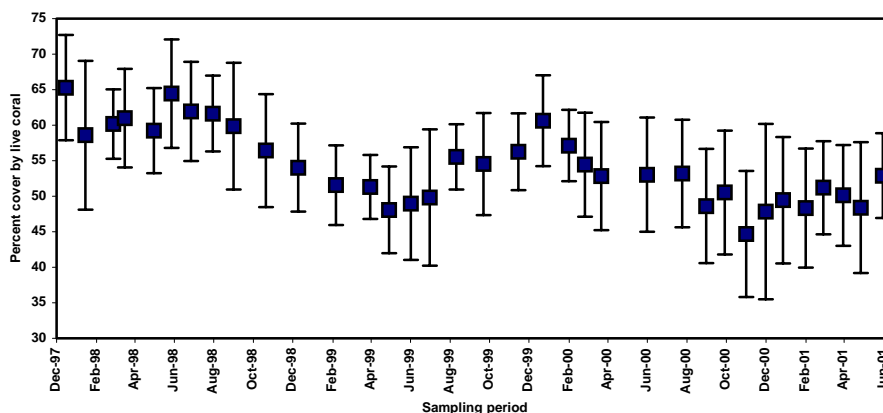
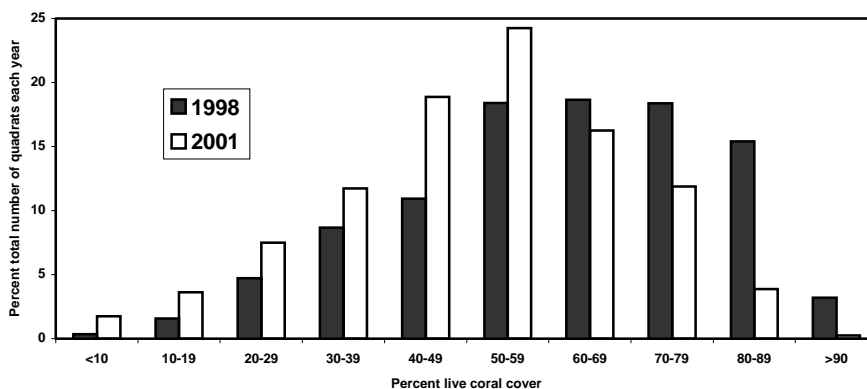


Fig. 5. Mean monthly live coral cover on Tektite Reef.



of the study period suggests a “reservoir” of disease might exist, where a population of the pathogen is always present.

More extensive disease outbreaks might occur when adverse environmental conditions or stresses inhibit the coral’s natural defense mechanisms (Peters 1996, Hayes and Goreau 1998). We observed a peak in percent cover by disease at Tektite Reef in December 1998, three months after the major bleaching event of August-September 1998. However, we have no data or qualitative observations linking specific corals that bleached with their subsequent vulnerability to disease. During this study, no significant damage was observed on the reef (e.g. in 1998 and 1999 from Hurricanes Georges,

Jose, and Lenny, anchoring, or pollution spills) that might have obviously affected the corals natural defenses. Disease occurrence and percent cover were not correlated with temperature within temperature ranges existing around St. John. However, outbreaks in Florida from 1995-1997 were seasonal, occurring in warmer months from June to October (Richardson *et al.* 1998b).

Richardson *et al.* (1998a) described the progression of the disease line as typically starting at the base of the colony and progressing upward. We found corals that demonstrated this response, however, during extremely virulent outbreaks (e.g., August 2000) we observed tissue loss originating and progressing without

a consistent pattern. This is thought to be a new disease type/syndrome called "plague type III". However, whether this is a different pathogen than plague II or a different response by the same pathogen is not known. There are no apparent external cues or conditions to predict the rate of advance.

Monthly monitoring was necessary to track effects of plague at our study sites. Given that the primary distinction between plague II and III is the rate of advance, frequent monitoring is necessary to differentiate between the two types. Protocols for monitoring the number of diseased colonies over a reef area (Weil *et al.* 2000, Boger 2001, Croquer *et al.* 2001, Santavy *et al.* 2001) to determine incidence of disease are applicable in areas where discrete colonies can be determined. However, in reefs dominated by a high cover and density of *M. annularis*, where differentiating between individual colonies is not possible (without genetic testing), monitoring diseased colonies is not practical. For example, 178 ramets or lobes of *M. annularis* were counted in a typical meter square from Tektite reef.

If monitoring isn't frequent enough, the relationship between the cause of mortality and effect (loss of live coral cover) may be missed. The disease has the ability to both suddenly advance at a rapid rate and suddenly stop. Once mortality occurs from disease, the area is rapidly overgrown with turf algae, and in some cases eventually macroalgae. As with other sources of chronic tissue loss (e.g., sedimentation), this type of change (small areas of living tissue becoming colonized by turf or macroalgae) is difficult to document, especially with infrequent monitoring.

Boundaries of MPAs are not effective barriers to coral disease pathogens. Coral diseases can cause dramatic change in reef structure and relative abundance of species (Gladfelter 1982, Aronson and Precht 1997). Plague is currently causing mortality of this area's most abundant reef building coral, *M. annularis*. The slow growth rate (Gladfelter *et al.* 1978) and low number of recruits of this species (Rogers *et al.* 1984) hinder recovery. Additionally, the loss of tissue may reduce its reproductive capability (Szmant-Froelich 1985). Plague has affected 14 coral species and

caused a significant reduction in live coral cover on two of the most pristine reefs located in remote areas within Virgin Islands National Park. Although reefs in this Marine Protected Area are also stressed by hurricanes, overfishing and anchoring (Rogers and Beets in press) if plague continues to affect reefs around St. John, it has the potential to cause more coral mortality than any other factor to date.

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REFERENCES

- Aronson, R. B. & W. Precht. 1997. Stasis, biological disturbance, and community structure of a Holocene coral reef. *Paleobiol.* 23: 326-346.
- Boger, J.L. 2001. Patterns of scleractinian coral disease infections in Dominica, West Indies. Abstract, 30th Scientific Meeting of the Association of Marine Laboratories of the Caribbean. La Parguera, Puerto Rico. 16 p.
- Bruckner, A.W. & R.J. Buckner. 1997. Outbreak of coral disease in Puerto Rico. *Coral Reefs* 16: 260.
- Bruckner, A.W. & E.P. Green. 2001. The Global Coral Disease Database. Abstract, 30th Scientific Meeting of the Association of Marine Laboratories of the Caribbean. La Parguera, Puerto Rico. 16 p.
- Campbell, R.C. 1994. *Statistics for Biologist*. Cambridge University, New York. 446 p.
- Croquer, A., S.M. Pauls & A.L. Zubillaga. 2001. Coral disease outbreak in a coral reef at Los Roques national Park, Venezuela. Abstract, 30th Scientific Meeting of the Association of Marine Laboratories of the Caribbean. La Parguera, Puerto Rico. 17 p.
- Dustan, P. 1977. Vitality of reef coral populations off Key Largo, Florida: Recruitment and mortality. *Environm. Geol.* 2: 21-58.
- Gladfelter, E. H., R.K. Monahan & W.B. Gladfelter. 1978. Growth rates of five reef-building corals in the north-eastern Caribbean. *Bul. Mar. Sci.* 28: 728-734.
- Gladfelter, W.B. 1982. White-band disease in *Acropora palamata*: Implications for the structure and growth of shallow reefs. *Bul. Mar. Sci.* 32: 639-643.

- Harvell, C.D., K. Kim, J.M. Burkholder, R.R. Colwell, P.R. Epstein, D. J. Grimes, E.E. Hofmann, E.K. Lipp, A.D.M.E. Osterhaus, R.M. Overstreet, J.W. Porter, G.W. Smith & G.R. Vasta. 1999. Emerging marine diseases-climate links and anthropogenic factors. *Science* 285: 1505-1510.
- Hayes, R.L. and N.I. Goreau. 1998. The significance of emerging disease in the tropical coral reef ecosystem. *Rev. Biol. Trop.* 46 (Supl. 5): 173-185.
- Jaap, W.C., J.W. Porter, J. Wheaton, K.Hacket, M. Lybolt, M. Callahan, C. Tsokos, G. Yanev & P. Dustan. 2000. Coral reef monitoring project executive summary. EPA Science Advisory Panel. 16 p.
- Peters, E.C. 1996. Diseases of coral-reef organisms, p. 114-139. *In* C. Birkeland (ed.). *Life and Death of Coral Reefs*, Chapman Hall, New York.
- Richardson, L.L. 1998. Coral diseases: what is really known? *Trends Ecol. and Evol.* 13: 438-443.
- Richardson, L.L., W.M. Goldberg, R.G. Carlton & J.C. Halas. 1998a. Coral disease outbreak in the Florida Keys: Plague Type II. *Rev. Biol. Trop.* 46 (Supl. 5): 187-198.
- Richardson, L.L., W.M. Goldberg, K.G. Kuta, R.B. Aronson, G.W. Smith, K.B. Ritchie, J.C. Halas, J.S. Feingold & S. Miller. 1998b. Florida's mystery coral killer identified. *Nature* 392: 557-558.
- Rogers, C. & J. Beets. (in press). Degradation of marine ecosystems and decline of fishery resources in Marine Protected Areas in the US Virgin Islands. *Environm. Conserv.*
- Santavy, D.L. & E.C. Peters. 1997. Microbial pests: coral disease in the western Atlantic. *Proc. 8th Int. Coral Reef Symp. Panamá* 1: 607-612.
- Santavy, D.L., E. Mueller, J.M. Hawkrige, L. MacLaughlin, J.W. Porter & E.C. Peters. 2001. The prevalence of ten coral diseases in the Florida Keys and Dry Tortugas. Abstract, 30th Scientific Meeting of the Association of Marine Laboratories of the Caribbean. La Parguera, Puerto Rico. 17 p.
- Szmant-Froelich, A. 1985. The effect of colony size on the reproductive ability of the Caribbean coral *Montastrea annularis* (Ellis and Solander). *Proc. 5th Int. Coral. Reef Symp.* 4: 295-300.
- Weil, E., I. Urreizieta, J. Garzon-Ferreira, P. Gayle, D.L. Gil-Aguedelo & S. Zea. 2000. Local and geographic variability in the incidence of diseases in western Atlantic coral reefs. Abstract, 9th Int. Coral Reef Symp. Bali. 286 p.
- Zar, J.H. 1984. *Biostatistical Analysis*. Prentice Hall, New Jersey. 718 p.