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

J. Miller[1], C. Rogers[2] & R. Waara[1]

[1]National Park Service, Virgin Islands National Park, 130 Cruz Bay Creek, St. John, VI 00830 USA
ph: 340-693-8950, ext 227; fax: 340-693-8950; e-mail: William_J_Miller@nps.gov
[2] US Geological Survey, 130 Cruz Bay Creek, St. John VI 00830 USA

Abstract: In July 1997, conspicuous white patches of necrotic tissue 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 *Sphingomonas*, 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 ranging from 7-12 meters. The study site at Tektite Reef is a nearly monospecific stand of *Montastraea annularis* with percent cover by hard coral of >50%. 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. The frequency of disease within transects ranged from 3 to 58%, and the area of disease patches ranged from 0.25 to 9000 cm^2. The average percent cover by the disease ranged from 0.01% (± 0.04 SD) to 1.74% (± 9.08 SD). Photo-monitoring of 28 diseased corals of 9 species (begun in September 1997) at Haulover Reef (estimated hard coral cover 20%) revealed no recovery of diseased portions with all necrotic tissue being overgrown rapidly, usually within less than one month, by turf algae. Most coral colonies suffered partial mortality yet some colonies greater than 1.5 m in diameter were completely consumed in less than six months. Some limited recruitment (e.g., *Agaricia* spp., *Favia* spp. and sponges) has been noted on the diseased areas.

Key Words: Coral Disease, plague type II, Coral 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), contributed to reef degradation (Richardson 1998) and caused changes in community structure (Aronson & 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 similar to the disease, plague, reported by Dustan in 1977 (Dustan 1977). In both cases, a sharp line differentiated apparently healthy tissue from diseased tissue. However, the new disease 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"). This new disease was termed "plague type II". The pathogen was identified as most-closely related genetically (based on sequence data) to the bacterium *Sphingomonas*, but may be a new species of bacteria (Richardson *et al.* 1998a, b). 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, where it was causing rapid and extensive mortality to tissue of major reef-building species, primarily *Montastraea annularis* (complex), but also *Montastraea cavernosa*,

Colpophyllia natans, *Siderastrea siderea*, and the *Diploria* species. 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.). In St. John, plague type II has resulted in greater coral tissue loss than any other stress in the last five years.

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

MATERIALS AND METHODS

Microbiology: In September 2000, microbiological 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 type II. The authors and Dr. Laurie Richardson collected samples using sterile syringes from the active disease lines as well as adjacent, healthy coral tissue and exposed 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 exposed 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 type II 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% [± 19 SD]). Both reefs are within the boundaries of Virgin Islands National Park in relatively undeveloped watersheds with no obvious major sources of pollutants.

Haulover Reef: On September 19, 1997, locations of 28 coral colonies affected with plague type II were tagged with red plastic tags. (Because of its multi-columnar growth form, it was not possible to ascertain if each *M. annularis* was a distinct colony.) Photographs of each colony were taken approximately monthly for 6 months, and then quarterly, with a Nikonos-V camera/28 mm lens, loaded 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 area (depth: 7 m to 10 m; est. size: 40 m x 20 m) of very dense coral cover (predominantly *M. annularis* and *C. natans*). A 1-meter 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 (due to area, data collection and time constraints) with eight transects sampled in December 1997, 11 transects in January 1998, nine in March, April and May 1998 and eight from June 1998 to May 2001. Within each quadrat, researchers recorded depth, percent live coral, macroalgae, and *new* disease cover (planar view). New disease was defined as being bright white bare coral skeleton. If algal turf was seen growing over the skeleton, it was not recorded as evidence of disease since the cause of mortality could not be determined. Observers were careful to discriminate between new disease, tissue loss from fish bites and discoloration from bleaching.

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

Table 1: List of scleractinian coral species affected by the coral disease plague type II on reefs around St. John, U.S. Virgin Islands.

Each patch of disease on any coral surface (planar or vertical) was measured along the longest length and perpendicular axis to produce a diseased area measurement. Frequency of occurrence, distribution, and area of disease patches within each quadrat was calculated for each sampling period by transect. Mean and standard deviation for percent live cover by coral, macroalgae and disease were calculated 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 every two hours daily (using Ryan Industries RTM 2000 Temp Mentors) at two sites around St. John. Temperature ranged from 25.1 °C to 29.6 °C. Data on monthly frequency of occurrence and percent cover by disease were compared to mean monthly temperature to look for seasonal patterns.

Additionally, a small-scale field test (n = 4) 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 cm - 5 cm wide, extending into both the apparently healthy and diseased areas. Divers re-visited the site to monitor and photograph the progression of the disease.

RESULTS

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

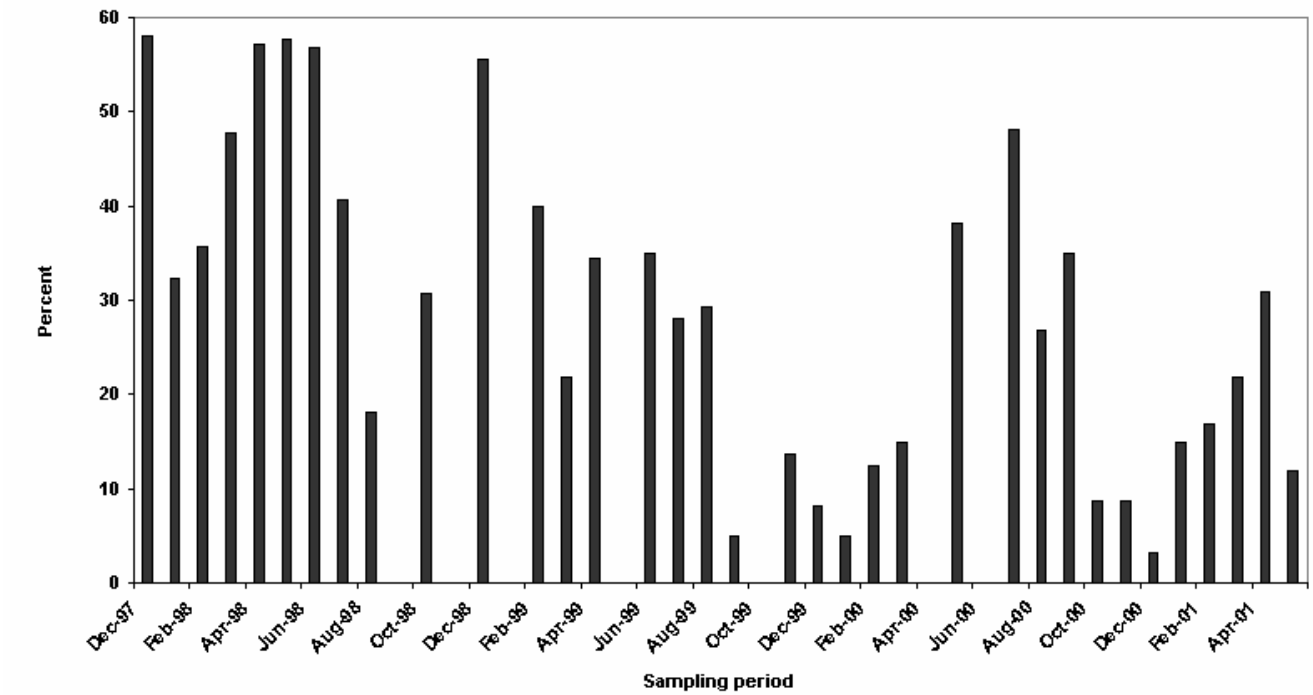

Figure 1. Mean monthly percent frequency of occurrence of disease incidences on Tektite Reef, St. John, over 42 months.

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 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 1.74% with > 1% new disease only observed in two months (Dec. 1998 = 1.23% and Sept. 2000 = 1.74)(Fig. 2).

Throughout this study, 3 555 patches of new disease were measured with a mean size of 32 cm^2 and a range from 0.25 cm^2 to 9 000 cm^2. The two largest patches occurred in August 2000 and were 9 000 cm^2 (slightly <1.0 m^2) and 6 000 cm^2 (slightly >0.5m^2). The size distribution of new disease patches reveals the majority (53%) of disease patches are <10 cm^2 (Fig. 3) and within that group 32% are 1 cm^2 (Fig. 4).

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; $p < .001$) (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, $p < .001$)(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

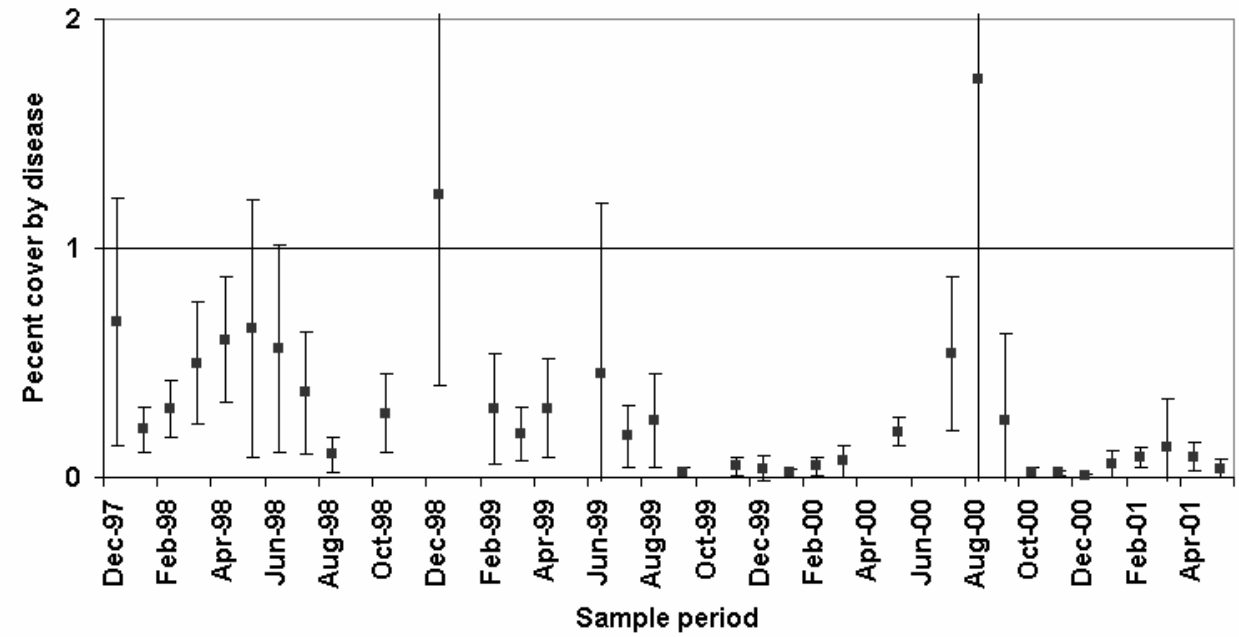

Figure 2: Mean monthly percent cover by coral disease plague type II on Tektite Reef, over 42 months.

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 mortality failed to advance beyond the epoxy line. However, given the 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 type II has caused extensive morality 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. Surviving tissue demonstrated viability through continued vertical growth, however we have not observed it growing onto adjacent formerly-diseased dead portions. This does not imply 'immunity' for surviving tissue as we observed plague type II affecting the surviving tissue at a later date. Whether this is a new infection or re-activation of the initial disease is undetermined. The presence of disease every month of the study period suggests a "reservoir" of disease might exist, where a population of the pathogen is always present. Laboratory experiments suggest that this disease is transmissible between adjacent colonies (Richardson *et al.* 1998b). Larger outbreaks might occur when adverse environmental conditions or stresses inhibit the coral's natural defense mechanisms (Peters 1996, Hayes and Goreau 1998).

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. Whether this is a "sign" of a different, more virulent pathogen, or just an atypical response

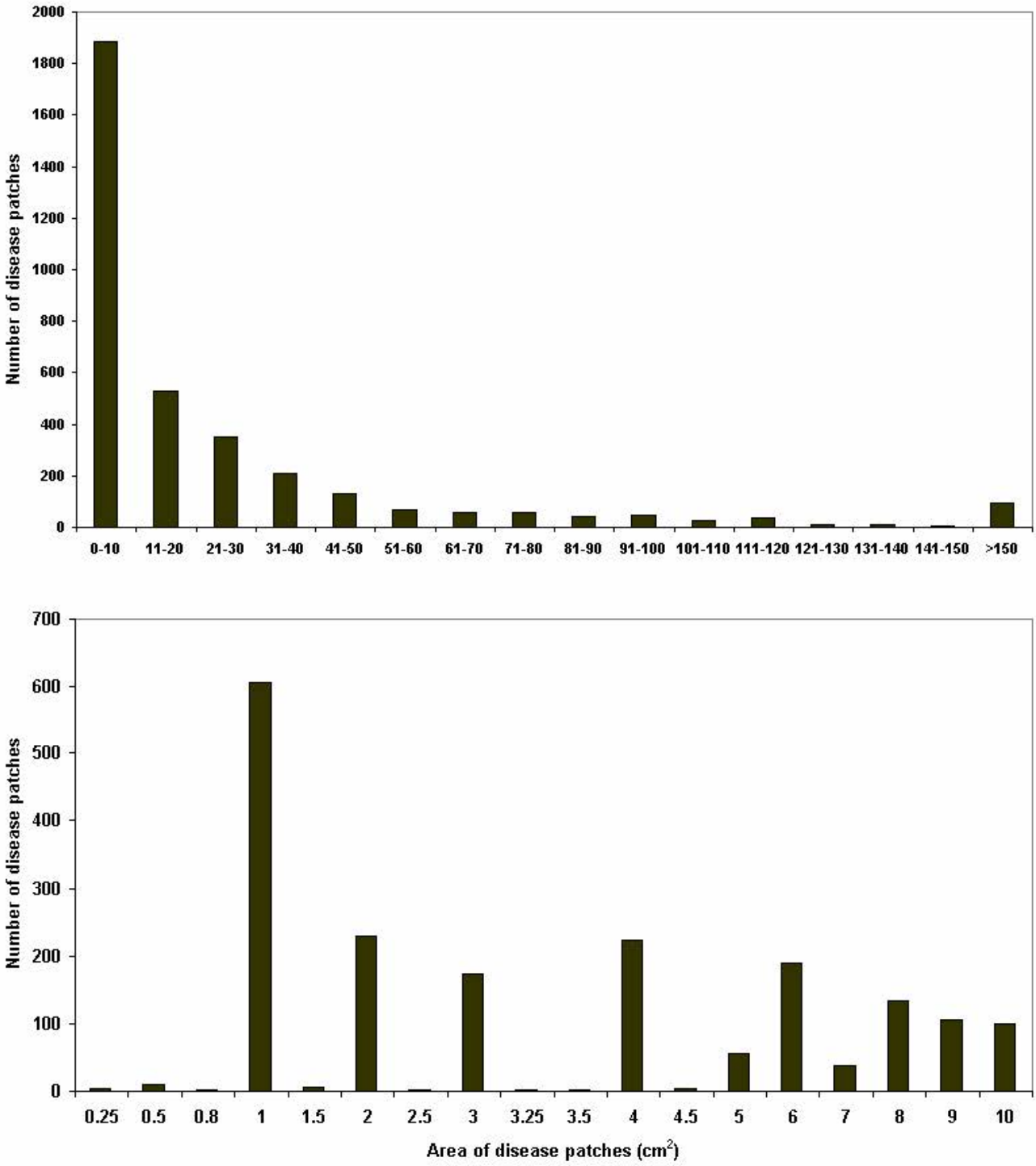

Figure 3 and 4: Size distribution of disease patches measuring from on Tektite Reef.

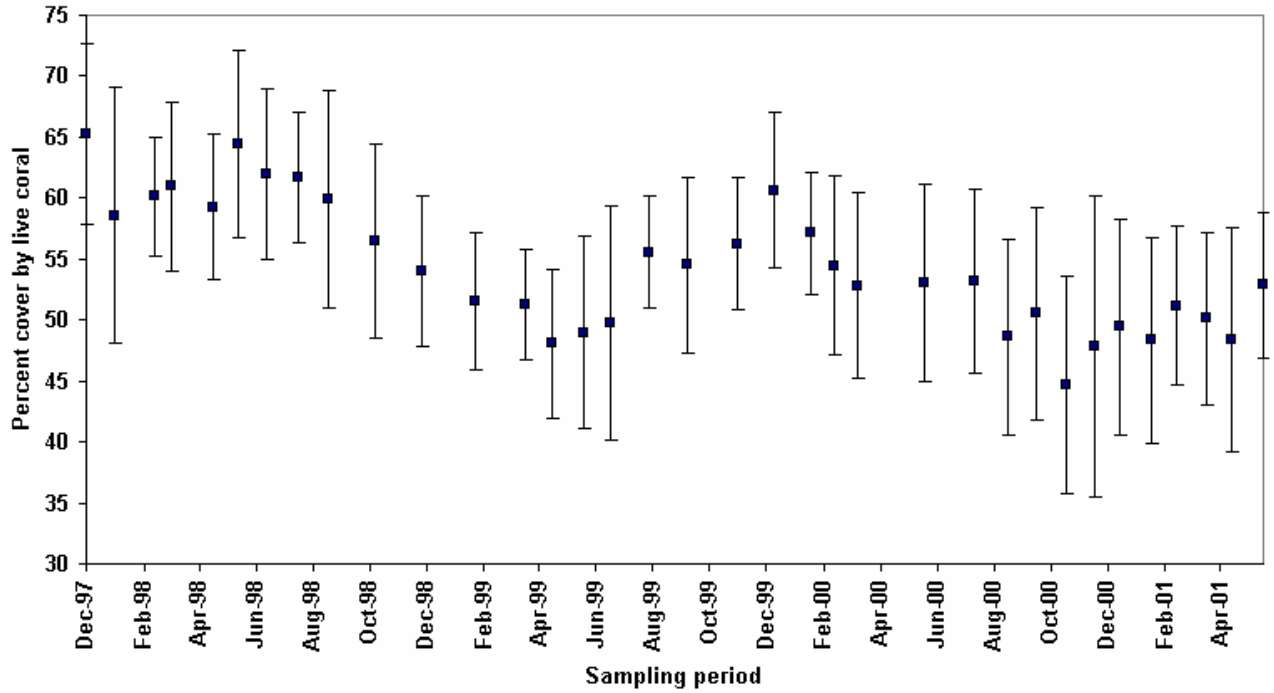

Figure 5. Mean monthly live coral cover on Tektite Reef.

is not known. There are no *apparent* external cues or conditions to predict the level of disease activity. 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).

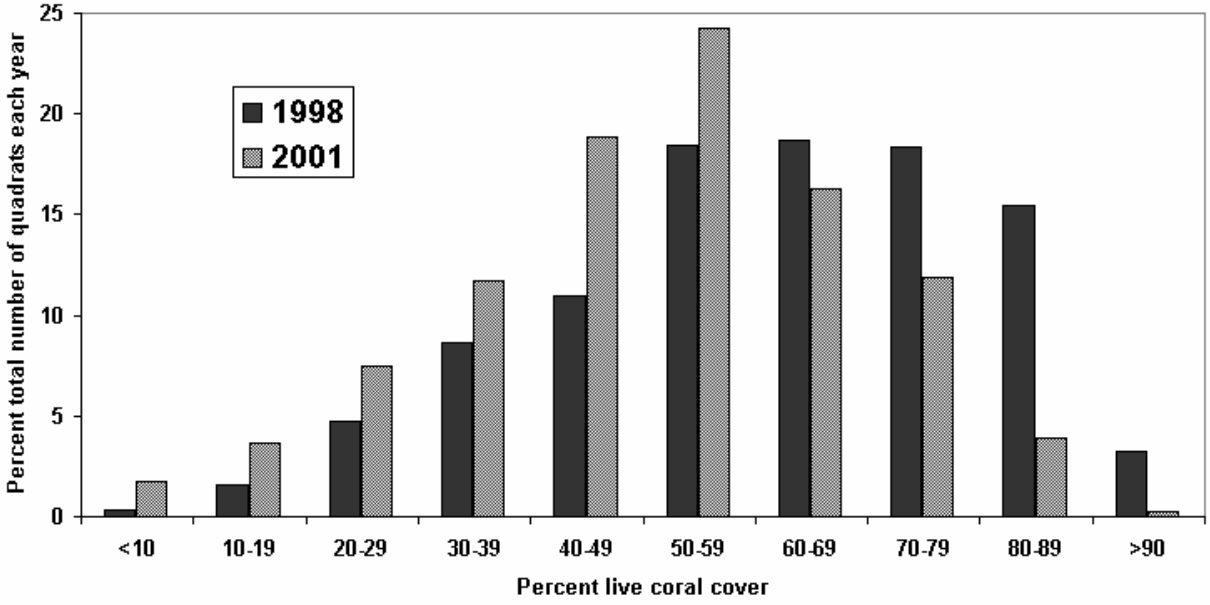

Figure 6: Comparison of the distribution of the percent of the total number of quadrats from 1998 and 2001 containing grouped percent live coral cover values from Tektite Reef.

Monthly monitoring was necessary to track effects of plague type II at our study sites. 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 feasible. The disease has the ability to both suddenly expand 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 turf or macroalgae) is difficult to document, especially with infrequent monitoring. Consequently, if monitoring isn't frequent enough, the cause (mortality from plague type II) – effect (loss of live coral cover) relationship may be missed.

Plague type II has caused a significant reduction in live coral cover on two of the most pristine reefs located in remote areas within Virgin Islands National Park. Reefs in this Marine Protected Area are also stressed by hurricanes, overfishing and anchoring (Rogers and Beets, in press). If plague type II 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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