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Is There a Relationship between Proximity to Sewage Effluent and the Prevalence of Coral Disease?

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Abstract.—We studied the prevalence of black-band disease (BBD) and white plague type II (WP) in two sites (Frederiksted and Butler Bay) within a St. Croix coral community that varied in relative exposure to sewage outflow. During sewage discharge events, fecal coliform and *Enterococci* data indicated impact area was limited to Frederiksted. We gathered data from seven belt transects in each of the two sites during the summer of 2001. We sampled 1046 colonies in 343 m^2 in Frederiksted and 2399 colonies in 302 m^2 in Butler Bay. There was significantly (Chi-square: df = 1, p = 0.0001) more disease in the impacted site with 13.6% of colonies of locally susceptible species infected (n = 566) versus the up current site that had 3.7% (n = 1344). Prevalence was highest for *Diploria clivosa*, with 23.7% of total colonies (n = 76) infected in the sewage impact site, which was significantly (Chi-square: df = 1, p = 0.0001) more than in the Butler Bay site where only 2.5% of the total colonies (n = 320) were infected. Recorded mortality, due to WP type II, was most severe for *Dichocoenia stokesi* with 26% of the infected colonies (n = 38) dying in just two months or 9.4% of the total *D. stokesi* sample population (n = 107). The results of the study suggest a relationship between a high prevalence of BBD and WP type II and exposure to sewage.

Keywords.—St. Croix, coral-disease, sewage, white-plague, black-band-disease

INTRODUCTION

Reports of coral reef disease have risen in the last two decades (Hayes and Goreau 1998; Richardson 1998; Harvell et al. 1999; Williams and Bunkley-Williams 2000; Porter et al. 2001). Disease events are recognized as important factors affecting coral community composition, structure, and dynamics (Weil et al. 2003). While the exact causes of coral diseases are largely unknown (Goreau et al. 1998; Richardson 1998), they are often assumed to be linked to either direct or indirect anthropogenic stresses (Hallock et al. 1993; Santavy and Peters 1997; Geiser et al. 1998; Williams and Bunkley-Williams 2000). Despite the contention that human-caused environmental perturbations are associated with higher levels of coral disease, there is minimal quantitative support for the hypothesis (Harvell et al. 1999; Williams and Bunkley-Williams 2000; Kuta and Richardson 2002) and links to specific disturbances are unclear (Bruckner 2002).

Sewage is one of the most significant pollutants affecting the coastal environments of the Wider Caribbean Region, with only about 10% of the sewage generated in the Central American and Caribbean island countries being properly treated (UNEP 1994). In the U.S. Virgin Islands alone, waste loads from domestic sources contain 440 tons per year of suspended solids, 250 tons per year of nitrogen and 132 tons of phosphates (UNEP 1994). Pollution, sewage, and/or elevated nutrient levels (the usual result of sewage discharges) have been linked by a number of studies to coral mortality (Smith et al. 1981; Jokiel 1986; Mate 1997).

While causal connections had not been established, two studies did make rigorous quantitative correlations between elevated nutrients and disease (Kim and Harvell

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2002; Kuta and Richardson 2002). In a more recent study, however, nutrient enrichment was shown to significantly increase the severity of coral disease progression in two Caribbean coral diseases, aspergillosis and yellow band disease (Bruno et al. 2003). Hatcher et al. (1989) predicted because of variations in background nutrient levels coral community responses to nutrient loading from sewage input would have no simple pattern.

A number of researchers have made the link between nutrient inputs from such sources as sewage and alterations in marine bacterial communities that result in environmental degradation and coral disease (Mitchell and Chet 1975; Colwell and Sayler 1978; Brown and Howard 1985; Dustan and Halas 1987; Hayes and Goreau 1998).

Sewage effluent may be a possible source of coral pathogens (Patterson et al. 2002). Pathogen host shifts or expansions of their host ranges that might include corals are distinct possibilities (Smith et al. 1996; Harvell et al. 1999; Weir et al. 2000). Despite a few studies that have attempted to examine the relationships between coral disease and water pollution (Mitchell and Chet 1975; Antonius 1981), the role of anthropogenic influence remains unclear (Green and Bruckner 2000). These relationships however, are beginning to be recognized as some of the most important yet poorly understood aspects of coral disease.

The first author (LK) had lived on St. Croix from 1986 to 2000 and in that time observed numerous outbreaks and mortality events affecting the local marine life (gorgonians, urchins, sea grass, and coral species in the genera *Diploria, Porites*, and *Acropora*), particularly around the Frederiksted sewage outfall. During many of those events visual swim surveys of other nearby sites revealed substantially less or no impact. One of the most striking mortality events in the Frederiksted site occurred in the fall of 1999 when a large portion of the dominant gorgonian, the Rough sea plume, *Pseudopterogorgia acerosa*, was killed off by an *Aspergillosis* outbreak (laboratory confirmed; unpublished data). This was the first time this species was observed infected by this disease. These events were the motivation for a more scientific investigation of the site.

Thus, our primary goal was to examine the relationship between the prevalence of coral disease and the relative exposure level to sewage effluent in two ecologically similar coral reef sites on the west shore of St. Croix. Using a matched pair comparative approach, we tested the simple prediction that disease prevalence is higher in a site that is adjacent to a frequently used sewage bypass outfall (Frederiksted) than in a site within the same coral community that is remote and upstream from the same sewage outfall (Butler Bay).

Methods and Materials

Study Sites

This study examined coral disease frequency in two geomorphologically and ecologically similar sites within a continuous hard pavement fringing coral community, which extends for approximately 10 km along the west coast of St. Croix in the U.S. Virgin Islands (Fig. 1). Site selection minimized natural variability, while highlighting the difference in sewage exposure. Transects were permanently marked and locations recorded using GPS for future community and population studies.

The west coast of St. Croix is in the lee of the island and is generally less exposed to oceanic swells and the high-energy wind-generated waves found on the east end. The pavement of the narrow shelf (<150 m) has a gentle to moderate slope with a sparsely to moderately developed fringing coral community. Geomorphologically, the western coast is fairly similar along most of its length. A typically weak to sometimes-moderate current running parallel with the shoreline regularly reverses but usually runs north to south. Daily tidal range is small, typically 0.2 m (Kjerfve 1981). Biological connectivity between the sites appears high thus ecological differences between the sites would not likely be due to spatially broad factors such as water temperature, hurricanes, overfishing, or major recruitment events but rather would be

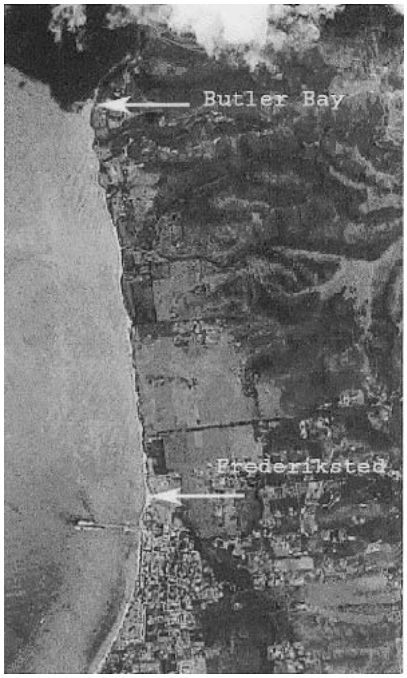

FIG. 1. Map and photo of study sites. The map of St. Croix (top) and the aerial photo (left) show the location of the two study sites (approx. 2.5 km apart). The town of Frederiksted can be seen at the bottom of the photograph.

more consistent with small-scale and short-term local factors.

The Frederiksted site, at 17°74.0′N, 64°87.5′W, is close to the town of Frederiksted (>10,000 people). The sewage system normally carries all city sewage to a central island treatment facility. An increasing trend in sewer system failures over the past 15 years has caused, at times, approximately half the islands untreated sewage to be diverted from the treatment facility and funneled to Frederiksted's sewage bypass outfall (A. Hutchins, V.I. Dept of Planning and Natural Resources; J. Bradford, St. Croix Dept of Public Utilities; pers. comm. 2001). Many cases of sewage bypasses in St. Croix are not reported (pers. obs., 1990-2002; J. Casey, US EPA Regional Director, pers. comm. 2001). EPA records show that between March 19, 1997 and June 27, 1999 (the only USEPA records available for the period closest to the time of the study), 90 sewage bypass events were reported for all of St. Croix, which totaled 15.3 million gallons of untreated sewage discharged at several different sites along the shorelines. Between 1997 and 2000, the only volumes reported by the Virgin Islands Department of Planning and Natural Resources (VIDPNR) at the Frederiksted study site were for two events: October 1, 1997 totaling 434 375 liters, and 3 270 586 liters on September 14, 2000. Although five other bypass events were recorded during the first half of 2000 in Frederiksted, no volumes were documented. These figures are presented here as an indication of the severity of some sewage system failures. The sewage-influenced study site (Frederiksted) begins about 7 m west of the sewage outfall where bypass events, as frequent as three times a month, are observed. Visible plumes associated with these events are only noticeable when it rains and are relatively small (<100 m wide). Additionally, an indeterminate volume of sewer line leakage from corroded pipelines flows continuously into the outfall drainage (pers. obs.; Hutchins, pers. comm.).

The Butler Bay study site is near Estate Butler Bay, which is a very lightly populated area, and is located 2.5 km north (up current) of the sewage outfall in Frederiksted at 17°75.3′N, 64°88.8′W. While it is ecologically similar it is shielded from regular exposure to sewage discharge, as indicated by the water quality tests that follow.

As an indication of the limited spatial impact of these sewage bypass events, which shows a pattern restricting it to the Frederiksted study site, we used recent laboratory analyses on water samples taken from sites along the west coast that were supplied by the VIDPNR (unpublished data, V. Vilanueva-Mayor). They reported on November 18, 2003, during a sewage bypass event, that seawater fecal coliform levels in Frederiksted were 1460/100 mL and *Enterococci* levels at 1880/100 mL while at the same time just 0.5 km north samples were 1/100 mL for fecal coliforms and 0/100 mL for *Enterococci* and at Butler Bay fecal coliforms and *Enterococci* were 0/100 mL and 5/100 mL, respectively. During an event on December 17, 2002 at Frederiksted, fecal coliforms and *Enterococci* were 3760/100 mL

and 7560/100 mL, respectively, while at the same time 1.5 km north fecal coliforms were 4/100 mL and 0/100 ml for *Enterococci*, and just 0.5 km to the south of Frederiksted, fecal coliforms were 2/100 mL and 3/100 mlL for *Enterococci*. The combination of the narrow shelf, wave action and the straight, open coastline appears to limit the spread of the effluent along the coast.

Survey and Analytical Techniques

Seven 2 meter wide belt transects approximately 10 meters apart were used in each site with depth ranging from 1 to 4 meters. The beginning of the first transect was determined haphazardly by swimming out to the beginning of the coral communities and blindly dropping the rolled up transect measuring tape from the surface and beginning where it landed. Following a north compass heading, roughly parallel with the shoreline, each subsequent transect began approximately 10 meters away. Transects lengths varied slightly because permanent transect end markers could not always be adequately secured at the 20 m mark (our first choice) because of the presence of sand or loose rubble but were approximately 20 m each. Generally, we extended the transect more than 20 m rather than shortening it. This is what accounted for the difference between sites in total reef area examined (see below). Each transect was divided into numbered 2 m^2 plots to help relocate individual colonies for long-term monitoring. Positioning of the survey areas deliberately avoided large areas of sand and seagrass.

Each coral colony including gorgonians and milleporid were identified to species using Veron (2000) and the maximum height and width were recorded to the nearest cm on all colonies 3 cm wide or larger. Each colony (infected and uninfected) was assigned a logbook number with an exact location. In total, 302 m^2 were examined in Butler Bay and 343 m^2 in Frederiksted. Data were recorded using snorkel and SCUBA surveys and conducted over a two three week period from the last week of June to the second week of July and in August 2001. The diseases and syndromes were identified using Richardson (1998), NOAA (1999), and Williams and Bunkley-Williams (2000). For each infected colony numbered, aluminum tags were attached nearby to the substratum with masonry nails and photographs were taken. Two small nails were also inserted a few centimeters behind the area of active disease progression (or necrotic zone) in a line perpendicular to the area infected. Using a flexible ruler, with its edge flush with the two nails, the distance from the first nail to healthy tissue was recorded. After at least a month and as sea conditions permitted, tagged colonies were visited a second time, and again photographed and measured to estimate the rate of disease progression on a per day basis. All diseased corals were photographed with a Sea and Sea Motormarine 35 mm camera and lens; model MX-10 with 32 mm lens and close-up attachment. To verify and characterize the putative pathogens, samples of black-band disease and white-plague-infected tissues were collected and examined microscopically and cultured by the authors and at the laboratory of Dr. L. Richardson, an expert in pathogens of Caribbean corals.

Disease prevalence data were divided into the following categories : 1) All species observed with BBD, 2) All species observed with WP type II, 3) Elliptical star coral, *Dichocoenia stokesi* with WP type II, 4) Knobby brain coral, *Diploria clivosa* with BBD, 5) *D. clivosa*, with WP type II, 6) Symmetrical brain coral, *Diploria strigosa*, with BBD, 7) *D. strigosa*, with WP type II, 8) Massive starlet coral, *Siderastrea siderea*, with WP type II, 9) Great star coral, *Montastrea cavernosa*, with BBD, and 10) Blushing star coral, *Stephanocoenia michelinii*, with WP type II.

We reviewed the results of the only available water quality tests closest to the time of our study, which were conducted by the EPA (March 2000) and DPNR (Department of Planning and Natural Resources of the Virgin Islands; June 2000 to June 2001) on the Frederiksted site and from a sampling site located one km south of our Butler Bay site. Water temperature was recorded daily between 12 pm and 5pm during the study using a laboratory thermometer made and

calibrated by H-B Instruments Co. and tested against thermometer standards traceable N. I. S. T. with an accuracy of ±1 degree. Because of the openness of St. Croix's west coast, its lack of tidal flats, and small tidal range (<0.2 m) the temperature changes very slowly thus the time range here makes little difference. Water samples were collected from the two sites almost simultaneously and tested for total suspended solids and turbidity on June 21 2001 and July 3 2001. Benthic sediment samples were analyzed for grain size composition from nine locations in the Frederiksted site and four from the Butler Bay site during the June/July survey period. Rainfall records for the survey periods were supplied by a private field station on Hermon Hill, St. Croix operated by Dr. Ken Haines. Rainfall data for the survey period was compared to eight year averages in order to assess potential influence of any major run-off events occurring during the survey.

Statistical Methods

Chi-square contingency tables (Yates corrected) (Zar 1996; Fowler et al. 1998) were used to determine if the prevalence of diseased colonies significantly differed between sites and size-classes. Spearman's rank correlation analysis (Fowler et al. 1998) was used to determine if a significant relationship existed between colony density and prevalence of disease. Differences in densities of each species were analyzed using the Mann-Whitney U-test. Scleractinian coral diversity was determined by the Shannon-Weiner diversity index (H'n) and evenness was determined using Pileou's evenness component (Magurran 1988). Rarefaction analysis was used to compare the species richness of the two sites (Krebs 1999). Rarefaction standardizes samples from different sites to a common sample size and estimates the number of species expected in a random sample of individuals taken from a site.

Results

Three types of disease/syndrome were observed: 1) black-band disease (BBD), 2) a form of white plague type II (WP type II), and 3) dark spot syndrome (DS). There was a high prevalence of DS observed on colonies of *Siderastrea siderea*, and Lesser starlet coral, *S. radians*, in both sites. Other coral diseases/syndromes observed less frequently: aspergillosis on a few gorgonians, sponge overgrowth on a few corals, and a few cases of blotchy partial bleaching were recorded. These were excluded as too few for statistical analysis. No white-band or yellow-band disease were observed in the study sites but were observed elsewhere around St. Croix during the study period.

Between Site Differences

A higher prevalence of disease occurred in the Frederiksted site, with 7 of 10 species or categories examined had significantly more disease (Table 1, Fig. 2). Of the 21 species of scleractinian coral recorded and examined in the transects of both sites, 8 had BBD and/or WP type II (Table 2). Of 1046 colonies examined in Frederiksted, 566 colonies were of species susceptible to disease, 77 (13.6%) of which were infected. Of the 2399 colonies examined in Butler Bay, 1344 colonies were of species susceptible to disease of which 50 (3.7%) were infected.

Frederiksted had significantly more coral disease than Butler Bay for 1) BBD 2.7% (14 of 521) versus 1.0% (13 of 1255) (Chi-square: $df = 1$, $p < 0.013$); 2) WP type II 11.4% (63 of 552) versus 3.1% (38 of 1223) (Chi-square: $df = 1$, $p < 0.0001$); and 3) BBD and WP type II combined 13.6% (77 of 566) versus 3.7% (50 of 1344) (Chi-square: $df = 1$, $p < 0.0001$).

Coral diversity was lower in Frederiksted (H'n = 1.99) compared to Butler Bay (H'n = 2.28). Eveness was similar: 0.75 in Frederiksted and 0.73 in Butler Bay. Rarefaction analysis estimated species richness (including milleporids) was higher in Butler Bay, with 21 species, than Frederiksted, with 14 species. Using the Mann-Whitney U-test colony densities of large massive species were significantly lower in Frederiksted for *M. cavernosa* (93% fewer colonies;

TABLE 1. A comparison of White Plague type II (WP) and Black Band Disease (BBD) prevalence between Butler Bay and Frederiksted study sites. The "All species" groupings include only the species found to be susceptible to the disease(s) in this study. Asterisks denote significant differences. *D. labyrinthiformis* was only observed with disease outside the transects as was the case with BBD-infected *S. siderea* (only a few colonies), which is why there is limited data for these species.

Species or grouping	Butler Bay	Total colonies	Frederiksted	Total colonies	p value
All species, WP and BBD	3.7%	n = 1344	13.6%	n = 566	0.0001*
All species, BBD only	1.0%	n = 1255	2.7%	n = 521	0.013*
All species, WP only	3.1%	n = 1223	11.4%	n = 552	0.0001*
D. stokesi WP only	41.4%	n = 70	59.5%	n = 37	0.15
D. clivosa, WP and BBD	2.5%	n = 320	23.7%	n = 76	0.0001*
D. clivosa BBD	2.5%	n = 320	14.5%	n = 76	0.0001*
D. clivosa WP	0%	n = 320	9.2%	n = 76	0.0001*
D. strigosa WP and BBD	1.7%	n = 363	6.8%	n = 279	0.002*
D. strigosa BBD	1.4%	n = 363	0.7%	n = 279	0.93
D. strigosa WP	0.3%	n = 363	6.1%	n = 279	0.0001*
S. siderea WP	1.8%	n = 443	7.7%	n = 143	0.002*
M. cavernosa BBD	0%	n = 121	7.1%	n = 14	0.19
S. michelinii WP	0%	n = 18	57.1%	n = 7	0.004*

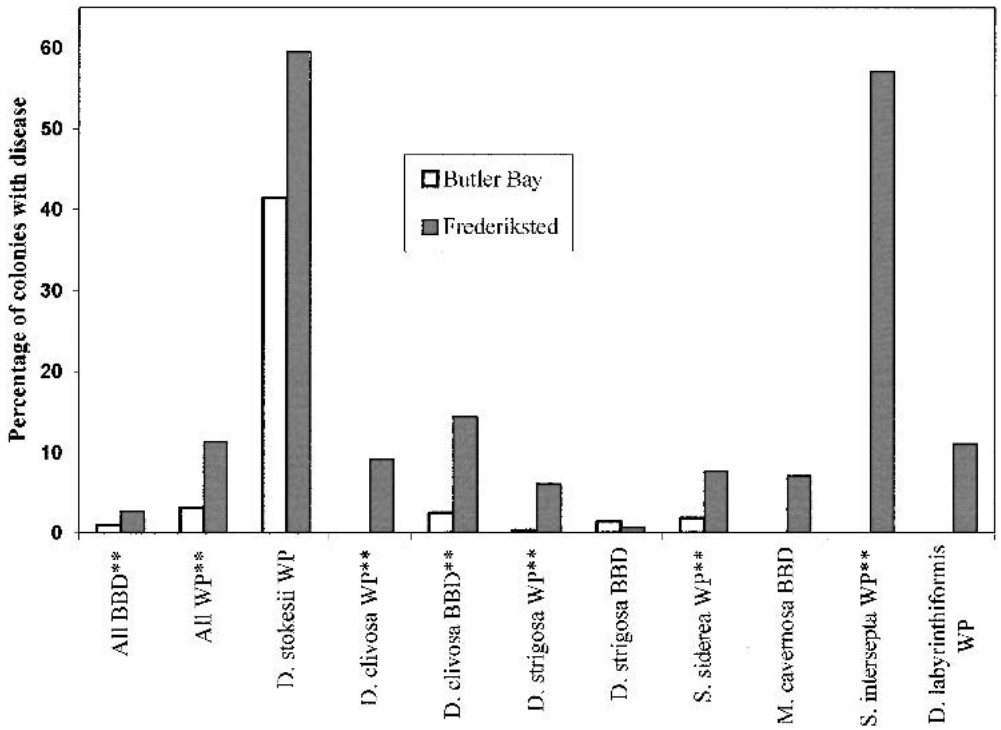

FIG. 2. Percentage of diseased coral colonies of common species. Only species that were susceptible to white plague type II (WP type II) and/or black band disease (BBD) in these study sites were included. ** indicates a statistically significant difference between sites using the chi-square test, $p < .05$.

p = 0.0209, n = 8), *S. siderea* (80% fewer colonies; p = 0.0209, n = 8), *S. michelinii* (77% fewer colonies; p = .0209, n = 8), and *D. stokesi* (67% fewer colonies; p = 0.0209, n = 8). The colony density of *Siderastrea radians*, a small massive brooder, was significantly higher in Frederiksted (394% more colonies; p = 0.0127, n = 14).

TABLE 2. Coral species found susceptible to disease in the St. Croix study sites, Frederiksted and Butler Bay. WP = white plague type II, BBD = black band disease, DS = dark spot syndrome.

Species	Diseases
Diploria clivosa	WP/BBD
Diploria strigosa	WP/BBD
Diploria labyrinthiformis	WP/BBD
Dichocoenia stokesi	WP
Siderastrea siderea	WP/BBD/DS
Siderastrea radians	DS
Stephanocoenia michelinii	WP
Montastrea cavernosa	BBD
Dendrogyra cylindrus	WP

WHITE PLAGUE TYPE II

Seven species of coral (*Dichocoenia stokesi*, *Diploria strigosa*, *D. clivosa*, *Diploria labyrinthiformis, Stephanocoenia michelinii, Siderastrea siderea,* and *Dendrogyra cylindrus*) had WP type II (Table 2, Fig. 3). The mean rate of disease progression for all tagged colonies infected with WP type II (n = 100) was 0.76 mm per day (S.D. = 1.26 mm: range 0.10-8.6 mm per day).

Prevalence of WP type II was also significantly higher in Frederiksted for the following infected species: *D. clivosa* 9.2% versus 0% ($p < 0.0001$); *D. strigosa* 6.1% versus 0.3 % ($p < 0.0001$); *S. siderea* 7.7% versus 1.8% ($p < 0.0015$); and *S. michelinii* 57.1% versus 0% ($p < 0.0038$). However, there was no significant difference in disease prevalence between sites for the most severely WP type II-infected species, *D. stokesi*, with 59.5% in Frederiksted and 41.4% in Butler Bay (Table 1, Fig. 2).

The prevalence of WP type II was significantly higher in small (<10 cm) colonies of D. *strigosa* than in larger colonies (chi-square: df = 1, $p < 0.0261$). All other species with disease were examined for size-related effects and results were inconclusive.

BLACK-BAND DISEASE

In the study sites, black band disease infected five species: *D. strigosa*, *D. clivosa, Montastrea cavernosa*, *S. siderea*, and *D. labyrinthiformis.* These were infected at least once (Table 2). The mean rate of disease progression for all tagged colonies infected with BBD (n = 27) was 1.45 mm per day (S.D. = 1.10; range 0.32-5.8 mm per day). Positive identification of the pathogens responsible for BBD was microscopically confirmed from the collected samples (L. Richardson, pers. comm.).

The prevalence of BBD was significantly higher in Frederiksted for the most severely BBD-infected species, D. *clivosa* 14.5% (11 of 76 colonies) versus 2.5% (8 of 320 colonies) (Chi square: df = 1, $p < 0.0001$). In Frederiksted the impact of combined colonies of D. clivosa with both type of infections (BBD and WP type II 23.7% of colonies diseased), in ecological terms, is severe and significantly higher than Butler Bay (2.5%) (Chi-square: df = 1, $p < 0.0001$). No WP type II was observed on *D. clivosa* in Butler Bay. There was no significant difference between sites in the prevalence of BBD for either *D. strigosa* or *M. cavernosa* (Table 1, Fig. 2).

MORTALITY RATE

During the survey periods high mortality occurred among the populations of *D. stokesi* infected with WP type II. During the second survey period in August, 10 of the 38 (26%) WP type II-infected colonies of *D. stokesi* tagged in the first survey had died. This translates to 9.4% of the entire sample population of *D. stokesi*, (n = 107) died in approximately 2 months.

DISEASE CLUMPING AND COLONY DENSITY

Spearman rank correlation analyses revealed no significant correlations between the prevalence of WP type II or BBD and colony density in either site. For example, for all species susceptible to WP type II in Frederiksted, Rs = 0.18 and p = 0.70, n = 7. Colony density for all species susceptible to WP type II in Frederiksted versus percent affected by WP disease was as follows: transect 1, 0.10 colonies per m^2 vs. 0.0%; transect 2, 0.31 colonies per m^2 vs. 16.6%; transect 3, 1.34 colonies per m^2 vs. 12.1%; transect 4, 2.22 colonies per m^2 vs. 21.0%;

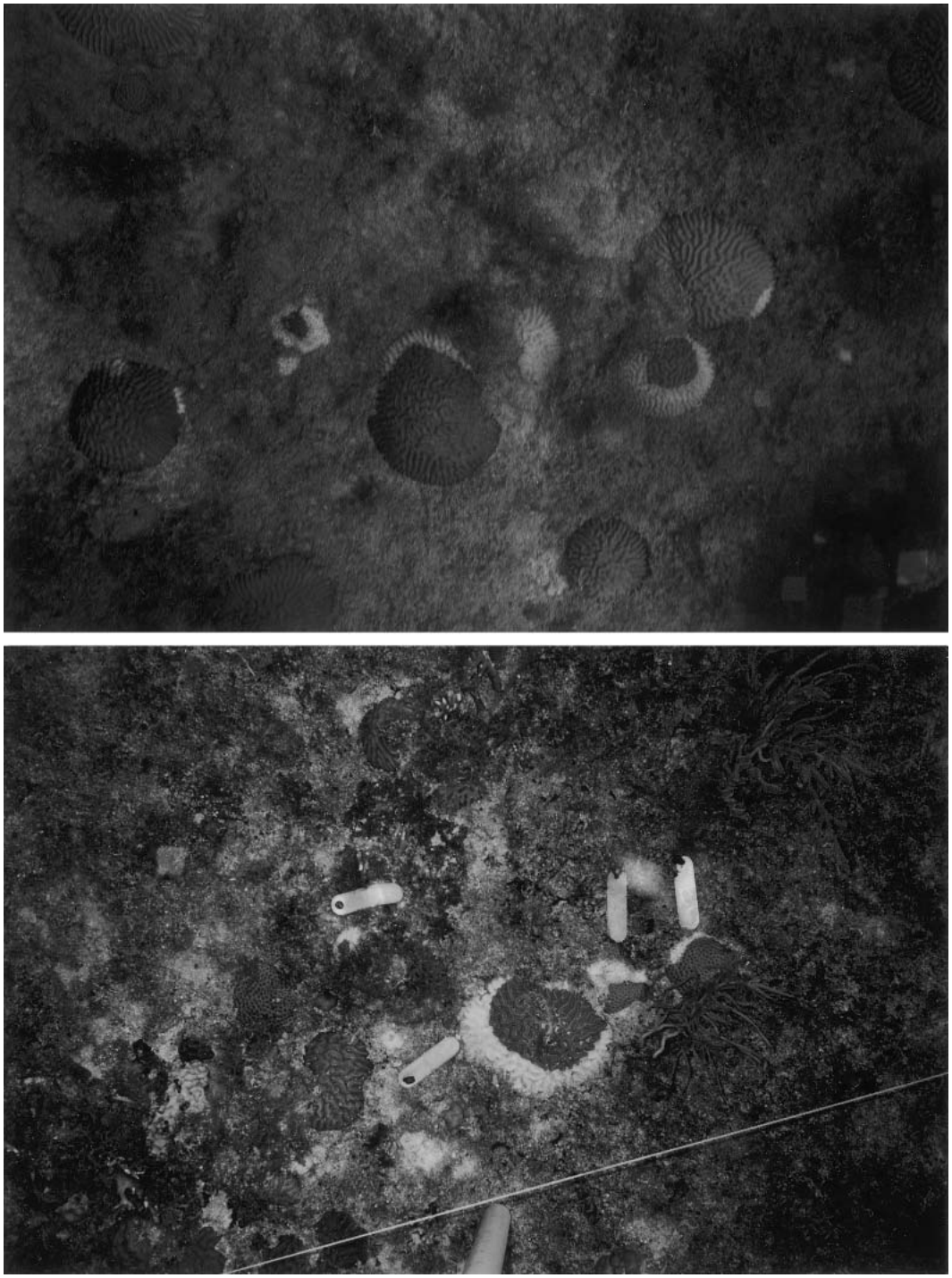

FIG. 3. Photographs showing patterns of contagiousness as observed in the Frederiksted study site. Viewed from above. Top) A cluster of seven colonies all with white plague type II, five *D. strigosa* and two smaller *S. siderae.* Bottom) Four WP type II-infected colonies in close proximity; *D. stokesi, D. strigosa,* and two *S. siderea* colonies (from left to right).

transect 5, 2.69 colonies per m^2 vs. 8.8%; site 6, 2.19 colonies per m^2 vs. 8.1%; site 7, 1.22 colonies per m^2 vs. 12.8%.

Environmental Data

Tested environmental parameters were as follows: turbidity averaged 0.31 NTU's (n = 2) in Frederiksted vs. 0.31 NTU's (n = 2) in Butler Bay, total suspended solids averaged 9.3 mg/L (n = 2) in Frederiksted vs. 15.8 mg/l in Butler Bay (n = 2) and the clay/silt fraction of the sediment (<0.0625 mm) averaged 5% (n = 9) in Frederiksted vs. 6% (n = 4). VIDPNR turbidity data averaged 0.78 NTU's (n = 7) in Frederiksted and 0.89 NTU's (n = 4) in Butler Bay (June 2000 to June 2001). The rainfall, June through August 2001,was normal (8 previous years average 5 to 9 cm per month). However, for May 2001 just prior to the survey, rainfall was more than three times higher than normal (27 cm vs. 8 cm). Water temperature averaged 29ºC during the June/July period and 30ºC in August.

Discussion

Our results indicate significantly higher prevalence of BBD and WP type II in a site exposed to persistent sewage discharge (Frederiksted) than in an ecologically similar up current site (Butler Bay). Environmental data during episodes of sewage bypasses show, by fecal coliform and *Enterococci* levels, that the impact appears to diminish after only a few hundred meters. Thus the Butler Bay site is clearly less exposed to effluent. The disease data was stronger for BBD than WP type II. Considering all the species that were susceptible to both diseases in this study there was a significant difference in overall disease prevalence, with nearly 14% of 566 susceptible colonies diseased in the Frederiksted site as compared with less than 4% of 1344 in Butler Bay. In fact all but one of the 8 species (i.e. *D. stokesi*) of coral susceptible to BBD and/or WP type II had statistically significant higher disease prevalence in the Frederiksted site. As non-parametric analysis was used and therefore Type II error is more likely, there's the possibility that WP II-infected *D. stokesi* colonies were in fact significantly more prevalent in Frederiksted as well. Acquiring normally distributed data from percentages (even after transformation) is not typical and applying parametric tests to data such as this one might not be appropriate. Using nonparametrics in our study was in fact more conservative than using parametrics in terms of finding the significant differences.

Diploria clivosa forms massive colonies in the shallow waters along the west end of St. Croix and is the most dominant coral species contributing to shallow-water community structure, possibly having replaced the once dominant *A. palmata*. Our results are particularly striking when we draw a comparison between disease prevalence of *D. clivosa* in the two sites. In Frederiksted, *D. clivosa* was more likely to be diseased than in the Butler Bay site. The prevalence of BBD on this species in the Frederiksted site (14.5%) is one of the highest levels reported for any single species to date. Other large massive coral species, *Siderastrea siderea*, *Stephanocoenia michelinii*, and *Diploria strigosa*, were also significantly more affected by one or both of the diseases in Frederiksted. We speculate that sustained levels of disease similar to those reported in this paper may lead to (and perhaps already have caused) considerable restructuring of the shallow coral community in the Frederiksted site. An increase in nutrients may play a role in increased virulence and fitness of coral pathogens (Bruno et al. 2003). We suspect that past sewage impacts have resulted in reductions in coral species richness, diversity and densities.

Because we are concerned with the potentially negative impact of sewage and dark spot syndrome is generally thought not to cause mortality (Bruckner 2002), the data collected for DS were not analyzed here. The results of this study would have been essentially the same as we obtained using just BBD and WP type II data alone even if all syndromes and diseases had been pooled because there were so few incidents of diseases/syndromes other than WP type II and BBD.

White Plague type II

The WP type II seen here formed a distinct white line or bleached band between

healthy tissue and exposed skeleton. The typical infection appears to start from the base. The combined disease prevalence of WP type II (5.7%; n = 1775) was higher in this study than in all other Caribbean studies except Nugues (2002), who found 11% of the susceptible colonies infected during an outbreak in St. Lucia in March 1998. Based on WP progression rate (a distinguishing characteristic of the WP disease types) in our two sites, which ranged between 0.1 mm to 8.6 mm per day, it is not clear whether the WP seen here is more similar to WP type I (3.0 mm/day, Dustan 1977) or type II (up to 2 cm/day, Richardson 1998). Alternatively, specific colonies, species, and/or microhabitats may vary in the expression of disease virulence (i.e. disease progression rates).

Ten tagged WP type II-diseased colonies were dead during the second survey period. The exact disease progression rates for these particular colonies could not be determined and could have been much faster for these colonies. Recorded per day rates for these colonies that died sometime between the two survey periods were thus, minimum values, which ranged from 0.18-1.23 mm per day. However, these minimum values were consistent with the other observed rates (0.10-8.6 mm per day) and thus were included in the calculated average rate.

It should be noted that in calculating the prevalence for the category "all species infected with WP", colonies of *D. labyrinthiformis* found in the transects were included for the following reason. While only healthy colonies of *D. labyrinthiformis* were found within the transects, infected colonies were found outside the transects, nearby. Since this makes it a locally susceptible species and it was found uninfected within the transects, the number of healthy colonies in the transects was added to the total number of susceptible colonies found in the transects (healthy and diseased) used in calculating the prevalence in the category "all species infected with WP". No colonies from outside the transect were included in the counts.

A sample examined microscopically had revealed numerous gram-negative rods and flexi-rods. The gross morphology of the rods appeared to be the same as the pathogen identified in the white plague type II outbreak in Florida coral reefs (Richardson 2001, pers. comm.). Plate culturing produced many uniform round whitish translucent colonies 1.5 to 3 mm in diameter after 4-day incubation at room temperature on nutrient agar. The colonies darkened slightly to yellow and became more opaque after one more week of incubation. Recently, Denner et al. (2003) identified and described the causative agent of white plague type II with similar gross morphology and characteristics and, based on detailed polyphasic taxonomic characterization, proposed a new genus and species name, *Aurantimonas coralicida.* In addition, *D. stokesi*, which was severely diseased in this study, appears to be susceptible to type II, and not to type I, disease (Richardson 1998).

The significantly higher prevalence of WP type II in small (<10 cm) colonies of *D. strigosa* might be explained by their greater contact with sediment-associated pathogens. However this was not tested; other factors that differ between small and large colonies could be involved. Since these smaller colonies would perish more quickly than larger colonies and be lost from the count, the apparent preference for small colonies was probably greater than our data suggested. All other species with disease were examined for size-related effects and results were inconclusive. While large colonies (>10 cm in height) for most species and disease type had significantly higher disease prevalence, this is not necessarily indicative of higher susceptibility, as they may simply take longer to die or have more time to recover than smaller colonies. However if small colonies have significantly higher disease rates then this variable is not relevant. This was the case only for *D. strigosa*.

BLACK-BAND DISEASE

Seven species of Caribbean coral were reported as the most susceptible to BBD: *Colpophyllia natans, D. strigosa, D. labyrinthifor-*

mis, Montastrea cavernosa, M. annularis, M. faveolata, and *M. franksii* (Rutzler et al. 1983; Edmunds 1991). Bruckner et al. (1997) and Kuta and Richardson (2002) recently included *D. clivosa* in the list of most susceptible. Our study supports the addition of *D. clivosa* to that list.

For *S. siderea*, and *D. labyrinthiformis*, infected colonies were only found outside transects, nearby, thus this makes these species locally susceptible. These were also species found uninfected within transects, so the number of healthy colonies found in the transects were included in the total number of colonies (healthy and diseased) used to calculate disease prevalence for the category "All species infected with BBD."

The mean rate of disease progression for all colonies infected with BBD in St. Croix was 1.45 mm per day (S.D. = 1.10), which is about half the reported average in other studies (Rutzler et al. 1983; Kuta and Richardson 1997). However the rates are typically highly variable within and between studies and can occasionally reach 2 cm/day.

Contagiousness

Antonius (1985) found BBD to be infectious *ex situ*. However, contagiousness of BBD and WP type II in the field is still in question (Edmunds 1991; Kuta and Richardson 1996; Bruckner and Bruckner 1997; Richardson et al. 1998a; Nugues 2002). Our correlation analysis found no statistical support of either disease being contagious. However, our qualitative observations lead us to think they might be. For example, several adjacent *D. clivosa* colonies were observed sharing circular patches of BBD infection indicating possible contagiousness. However this may also be explained by the two colonies sharing a common initial injury that led to disease. Although our data doesn't substantiate it, colonies infected with WP type II in St. Croix often visually appeared clumped (Fig. 3). Nugues (2002) also found no statistical evidence of clumping in the WP type II outbreak in St. Lucia. While clumped distributions would suggest contagiousness via the spreading of pathogen among adjacent colonies, alternately clumped distributions could result from adjacent colonies sharing an environment highly favorable to the pathogen.

Populations, Community Structure and Disease

We found that coral species richness, diversity and densities were lower in the Frederiksted site, which had significantly higher BBD and WP type II prevalence. We speculate that chronic exposure to sewage and the resulting high incidence of disease has contributed to this reduction. Kuta and Richardson (2002) also found coral diversity to be lower in sites with BBD-infected corals compared to sites without BBD. In St. Lucia, Nugues (2002) suggested WP type II could progressively deplete two of the most important reef frame-building coral species. Higher colony densities in our sites were not associated with higher disease prevalence as one might expect with density-dependent factors.

Implications

From our study we speculate that BBD and WP type II may be killing a high proportion of ecologically important scleractinian corals (i.e. *D. clivosa* and *D. stokesi*) in some St. Croix near-shore coral communities and may be responsible for restructuring these communities. For example, the prevalence of WP type II among the *D. stokesi* population here is among the highest thus far reported in the Caribbean for any species. Because our sample area is small, however, this localized high level of infection would not necessarily parallel the larger scale trends showing lower prevalence seen in other studies (e.g. Weil et al. 2003). More than 25% of the infected *D. stokesi* colonies died in less than three months in our study. We speculate that this translates into an estimated mortality rate of almost 10% (both sites combined) for this species within at least the area between sites, which extends across approximately 25% of the fringing west coast coral community. *Dichocoenia stokesi* is the fourth

most abundant large massive coral in this community. This level of mortality due to WP type II could cause considerable restructuring. Despite a severe outbreak of white plague type II in the Florida Keys in 1995 that highly affected *D. stokesi,* Richardson and Aronson (2003) initially concluded that it had minimal long-term impact as this species began recolonizing these reefs within a year. However, since then the population of these new recruits has plummeted (Richardson, pers. comm.). Ongoing monitoring of the size-class frequencies in our study sites in St. Croix will reveal if any long-term impact occurs as a result of these disease events.

According to Kuta and Richardson (1997), BBD acts selectively on important reef framework species and stated that this may result in reef degradation and changes to the reef community structure. On a local scale, BBD appears to cause considerable restructuring in St.Croix by selectively removing *D. clivosa* from the sewage-impacted site. *Diploria clivosa* nears 100% cover in some areas of the up current Butler Bay site and although it is at a lower density in the shallow range of the Frederiksted site (1-1.5 m) it is still dominant there. There are several very large *M. cavernosa, S. michelinii* and *D. cylindrus* colonies in the deeper range (2-4 m) of both study sites. All three of these were susceptible to either WP type II or BBD. We speculate that the increasing trend in sewage discharges over the past 15 years (J. Casey, J. Bradford, A. Hutchins; pers. comm.) has contributed to a decline in these large massive species in Frederiksted. *M. cavernosa* and *S. michelinii* were much more abundant 5 to 15 years ago in Frederiksted (pers. obs.), to which some large dead colonies of these species now attest. While the exact cause of death is unknown, the death of these large colonies coincided with the period of increasing frequency of sewage bypasses. Also more abundant 10 to 15 years ago, in both Frederiksted and Butler Bay, were *Acropora palmata* (now completely absent in the Frederiksted site) and *D. stokesi* (large tracts of dead colonies provide evidence). We suspect the significantly higher density of *S. radians* in the sewage-impacted site reveals it is opportunistically making use of space recently made available as a result of coral disease, at least in part.

Sewage and Other Potentially Synergistic Influences

Our study documents significantly higher disease prevalence at the Frederiksted site, which is directly and chronically exposed to raw sewage discharge, than the similar nearby Butler Bay site that is not directly exposed to sewage. The March 2000 U. S. EPA water quality report on St. Croix documented the presence of high nutrient levels and toxic contaminants in the water and sediments in this sewage-impacted site, an indication of chronic sewage exposure. We propose our study links higher disease prevalence with exposure to sewage and hypothesize that chronic sewage exposure can lead to higher rates of coral disease, especially considering the consistently statistically lower prevalence of disease in the ecologically similar upstream study site that is not exposed to sewage discharge. However, cause and effect has not been conclusively shown. We do realize that the two study sites are not identical in every way except sewage discharge and thus recognize several other potential sources of stress, which may contribute to increased coral disease in Frederiksted. For example, foot traffic from recreational (i.e. snorkelers and bathers) and fishing activities (i.e. seine netters and spear fishers) is higher in Frederiksted and likely results in more physical damage to corals there. It has been shown that the BBD infection rate is higher among damaged corals (Antonius 1985). In addition, recent landscape modifications adjacent to the Frederiksted study site of both marine (the recent installation of a vertical sea wall without any energy-absorbing toe protection, i.e. boulders) and terrestrial features (the recent addition of a large paved lot and partial filling of an adjacent drainage canal) have likely increased total suspended solids (TSS) and terrestrial run-off in the waters around Frederiksted. While a link between TSS and disease may exist, we do not

believe it accounts for the significant differences in disease measured in this study since our water samples indicated that TSS might possibly be higher in Butler Bay (15.8 mg/l, n = 2) than in Frederiksted (9.3 mg/l, n = 2). There is severe shoreline erosion at the Butler Bay site that might account for the higher TSS levels there, as opposed to anthropogenic pollution. Our measures of the silt/clay fraction of the surrounding sediments and turbidity (as well as turbidity data from VIDPNR) were similar in both sites. However, environmental sampling was limited and a more thorough sampling would be required to draw more robust conclusions about the role of these parameters. On St. Croix's north shore near the town of Christiansted a similar chronic sewage system failure problem exists resulting in large volumes of raw sewage that are bypassed into coastal waters. It is quite possible that coral disease there is also found in higher prevalence than adjacent reefs.

Not all coral diseases are exacerbated by environmental impacts (Goreau et al. 2000). Mona Island in the Mona Passage off Puerto Rico represents a more pristine environment than the waters near the main island of Puerto Rico. However, the corals around Mona Island have in the last few years suffered more from the yellow-band syndrome and *Cliona* (a coral-killing sponge) damage than the main island (Williams and Bunkley-Williams 2000; Williams, unpubl. data).

Our study presents some evidence that supports the idea that some coral diseases with wide ranges can have more intense impacts at local scales in association with anthropogenic influence. Furthermore, while BBD and WP type II have been observed to occur in remote areas not influenced by exposure to sewage and/or high nutrient levels, the results presented here suggest that their prevalence may significantly increase in localized areas as a result of exposure to such insults.

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