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# Quantitative assessment of coral diseases in the Florida Keys: strategy and methodology

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**Abstract**

Natural incidences of disease among scleractinian corals are unknown, since most studies have been initiated in response to specific disease outbreaks. Our ability to distinguish elevated disease incidences influenced by anthropogenic and climatic factors is limited since current estimates are probably inflated for extrapolation to larger areas. In our study, we used quantitative assessment methods to characterize the distribution and frequency of scleractinian and gorgonian coral diseases in the south Florida region. This paper is the first in a series that will detail different aspects of our studies. In this paper, we examined the strategy and methodology developed over 2 years to optimize the experimental design of our study. Pilot surveys were conducted in 1997 to develop and test methods, select and determine suitability of sites, and obtain preliminary data to assess the variance and efficiency of the sampling design. Survey periods targeted late spring, the time when coral diseases are believed to emerge, and late summer, the time when coral diseases are believed to be most prevalent. Two strata were chosen to evaluate patterns of coral disease: the first, geographic area, consisted of reefs in the vicinity of Key West, New Grounds and the Dry Tortugas; and the second, reef type, consisted of back, fore and transitional reefs. Random radial arc transects (10 m diameter) were used to quantify 10 diseases affecting 18 species of stony corals and gorgonian sea fans over a large geographical region. During the pilot survey, we demonstrated that the outer 8–10 m segment (113 $m^2$) was an adequate sampling area. The survey implemented important quality assurance measures for data quality control. Power analysis determined that future studies should adopt $\alpha$=0.10, $\beta$=0.0383, and $1 - \beta = 0.9617$ in our experimental design. The highest prevalence of disease in our study was during the 1997 summer survey, with a mean percent coral disease (MPCD) of 28% occurring at Key West area reefs, or 55% of all back reef stations. Our results do not show a clear pattern of seasonality in coral diseases within either stratum, although differences in disease distribution between reef types and geographic areas were apparent in some of the spring and summer surveys.

## Introduction

Most studies of coral disease are initiated to describe an observed disease outbreak. These studies often have focused on the incidence of a single disease within a limited geographic area, such as a single location or several proximal reefs (Gladfelter et al., 1977; Gladfelter, 1982; Feingold, 1988; Edmunds, 1991; Kuta & Richardson, 1996; Bruckner et al., 1997; Richardson et al., 1998a,b). The earliest coral disease surveys employed a qualitative approach to characterize black-band disease, first in South Florida, then in the Caribbean (Antonius, 1973, 1977), and later in regions of the Indo-Pacific (Antonius, 1985, 1988). The first quantitative coral disease study assessed the distribution of black-band disease on massive scler-

actinian corals by employing a 10 m radius circle (Edmunds, 1991). Subsequently, others have used a 10 m radius circle to study black-band disease (Kuta & Richardson, 1996; Bruckner et al., 1997) and white plague (Richardson et al., 1998a,b). Our study has developed a radial arc transect approach to assess multiple coral diseases on many species of scleractinian and gorgonian corals using a random statistical design.

The long-term goals of our study are to assess annual coral disease trends to understand the epizootiology of each coral disease, and to determine if the occurrences of specific coral diseases are related to water quality and climate change. Our study used quantitative assessment methods to characterize the distribution and frequency of scleractinian and gorgonian coral diseases in the south Florida region. This paper is the first in a series that will detail different aspects of our studies. We examine the strategy and methodology developed over 2 years and formulated to optimize our study's experimental design. Pilot surveys were conducted in 1997 to develop and test methods, select and determine suitability of sites, and obtain preliminary data to assess the variance and efficiency of the sampling design. In 1998, the study established permanent survey stations and incorporated multiple strata into the experimental design. The first stratum, geographic area, was used to determine whether the pattern and prevalence of coral diseases were related to different land-use characteristics. The second stratum, reef type, was used to determine whether the pattern and prevalence of coral diseases were associated with depth-related parameters.

## Materials and methods

### *General approach*

The SCUBA-based field study was developed and implemented over a 2-year period in the south Florida region. Survey areas were selected in the Lower Florida Keys in the vicinity of Key West, the New Grounds and the Dry Tortugas (Fig. 1). Zones that contained hard coral bottom were demarcated within each geographic area. These coral reef zones were located using a prototype of the Florida Marine Research Institute (FMRI) Benthic Habitats Map of the Florida Keys (FMRI, 1998). Potential stations for the pilot surveys were selected using a stratified random design, within the three regional areas. Individual stations were chosen by placing a random grid pattern that incorporated a hexagonal overlay over the individual coral reef zones contained within each geographic area (Summers et al., 1995). Surveyors went to randomly selected locations and assessed their suitability for sampling. If the location had sufficient coral coverage ($>5\%$), the site was surveyed; if it was not suitable, the next location on the list was assessed for sampling suitability. Twenty-one stations were surveyed in the 1997 spring pilot survey.

Survey periods targeted late spring, the time when coral diseases are believed to emerge, and late summer, the time when coral diseases are believed to be most prevalent. The 1997 spring and summer pilot surveys were conducted during 1–8 June 1997 and 6–14 September 1997. Stations assessed as suitable during the spring pilot could not be permanently established at that time, but permission was granted for permanent installation of stakes during the summer pilot. In September 1997, stations assessed during the spring pilot were relocated using GPS coordinates, and permanent sites were established by installing stakes to be used for future surveys in 20 of these stations (1 being omitted at New Grounds). At the same time, 6 new stations were added in the Key West and Dry Tortugas areas.

### *Survey strategy*

Based on results from the 1997 pilot survey, reef type was added as a stratum to the 1998 sampling design (see 'Results'). For the 1998 surveys, 6 additional stations were selected to balance the sample design across two strata: (1) three geographic areas established in the pilot surveys and (2) three reef types. The three reef types – fore reef, back reef and transitional reef, as defined in the Florida Keys National Marine Sanctuary (FKNMS) Management Document (Dobbin, 1983; Jaap, 1984) – were used for comparison among the areas. However, not all areas contained all reef types; for example, only deep transitional reefs were found in the New Grounds area. The 1998 spring survey was conducted from 25 May to 1 June 1998 and the 1998 summer survey from 2 to 11 September 1998. Thirty-two stations were assessed in the spring and summer of 1998. A power analysis was performed on the data acquired from both spring and summer 1998 surveys to determine the optimal number of sampling stations and the appropriate $\alpha$ level for data analysis (Sokal & Rohlf, 1981).

*Table 1.* Species and diseases of scleractinian and gorgonian corals that were enumerated in the 1997 and 1998 surveys. The X designates the species enumerated in the study that are affected by the specific diseases. The references detail the specific signs used in assessing the health condition of the corals

| Species | Disease | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aspergillosis | Black-Band | Dark Spots | Hyperplasia | Patchy Necrosis/ White Pox | Red-Band | White Plague | | White-Band | | Yellow Blotch |
| | | | | | | | Type 1[b] | Type 2 | Type 1 | Type 2[b] | |
| *Acropora cervicornis* | | | | | | | | | X | X | |
| *Acropora palmata* | | | | | X | | | | X[c] | | |
| *Colpophyllia natans* | | X | X | | | X | X | | | | |
| *Dendrogyra cylindrus* | | | | | | | | X | | | |
| *Dichocoenia stokesii* | | | | X | | | | X | | | |
| *Diploria labyrinthiformis* | | X | | X | | | | X | | | |
| *Diploria strigosa* | | X | | X | | | | X | | | |
| *Gorgonia* spp. | X | X | | | | X | | | | | |
| *Montastraea annularis*[a] | | X | X | | | | | X | | | X |
| *Montastraea faveolata*[a] | | | X | | | | | X | | | X |
| *Montastraea franksii*[a] | | X | X | | | | | X | | | |
| *Montastraea cavernosa* | | X | | | | | | X | | | |
| *Mycetophyllia danaana*[d] | | | | | | | | | | | |
| *Mycetophyllia ferox* | | | | | | | X | | | | |
| *Mycetophyllia lamarckiana*[d] | | | | | | | | | | | |
| *Siderastrea siderea* | | X | X | | | | | X | | | |
| *Solenastrea bournoni* | | | | | | | | X | | | |
| *Stephanocoenia michelini* | | | X | | | | | X[e] | | | |
| References | Kim et al. (1997) Nagelkeren et al. (1997a,b) Smith et al. (1996) | Antonius (1981) Rützler et al. (1983a,b) | Garzón-Ferreira & Gil (1998) | Cheney (1975) Loya et al. (1984) Peters et al. (1986) | Bruckner & Bruckner (1997) Holden (1996) | Rützler et al. (1983a,b) Richardson (1993) | Dunstan (1977) | Richardson et al. (1998a,b) | Gladfelter (1982) Peters et al. (1983) | Ritchie & Smith (1998) | Santavy et al. (1999b) |

[a] All analyzed as *M. annularis* complex (Weil & Knowlton, 1994).
[b] Did not find in any of our surveys.
[c] Also reported to affect *Acropora prolifera*.
[d] Included in surveys because white plague reported to affect other species in genus, *M. ferox*.
[e] Also reported to affect *Agaricia agaricites, A. lamarcki*, *Eusmilia fastigiata, Madracis decactis, M. mirabilis, Manicina areolata, Meandrina meandrites* and hydrocoral *Millepora alcicornis*.

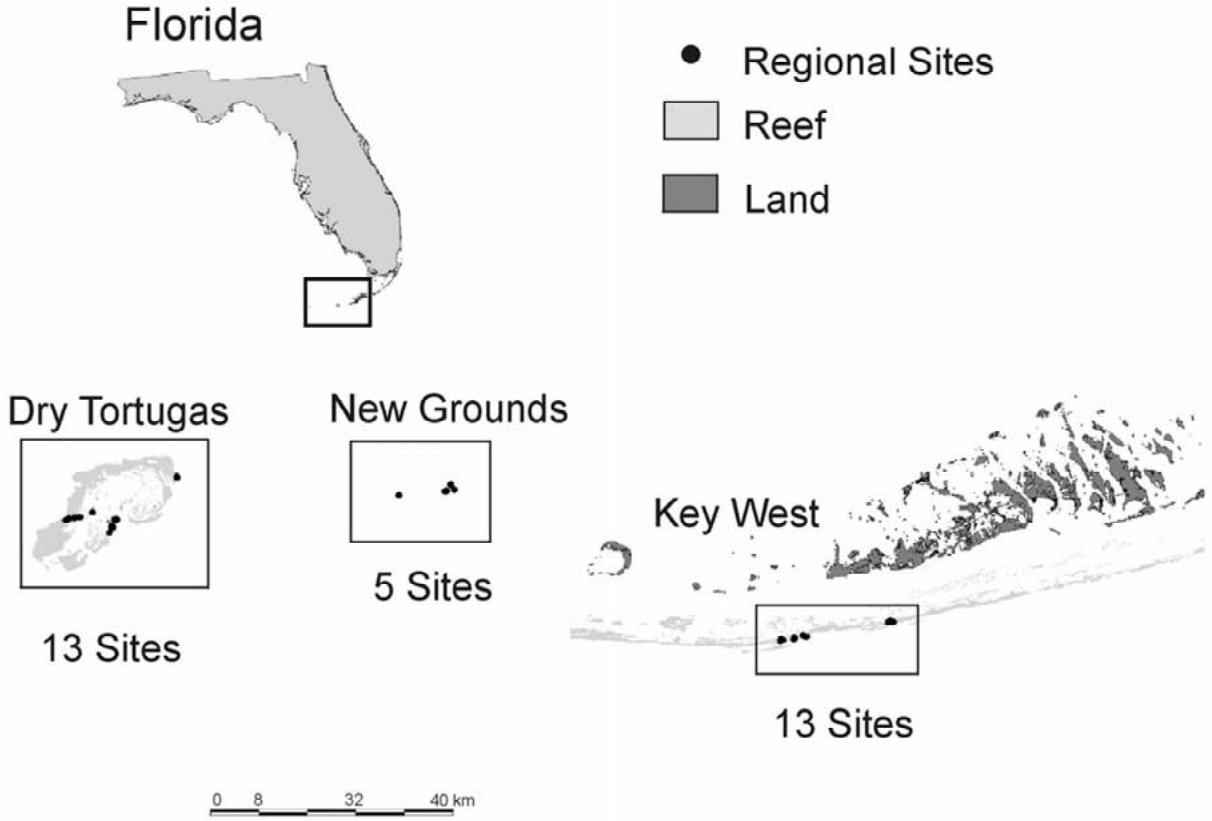


*Figure 1.* Map of all stations included in disease surveys of South Florida. The surveys contained 33 stations in a region from the Dry Tortugas to Western Sambo Reef in the Florida Keys National Marine Sanctuary (FKNMS). There were three areas surveyed, with 13 stations in the Dry Tortugas area, 5 stations in the New Grounds area, and 13 stations in the Key West area.

*Survey methodology*

All surveys were conducted using a radial arc transect method developed for this study. SCUBA was used on deeper reefs and snorkel was used on shallow back reefs. A stainless steel rod was positioned by driving it into the calcareous substratum for temporary sites (1997 spring pilot) or by permanently affixing a 12″ stainless steel pipe (all other surveys) at the designated site with underwater epoxy (Gunnebo® Liquid Roc 500, Gunnebo Fastening Corporation 800-336-1640). Site coordinates were determined by GPS technology during 1997, then by Differential-GPS (D-GPS) during 1998 surveys as it became available in our study region, improving our ability to easily locate stations. Surface maps with triangulation bearings and maps of underwater structures were generated, and a subsurface 3″ buoy was used to mark each station to enable us to return to each underwater stake.

The survey procedure required inserting a 6-foot pole into the stainless steel pipe. The pole had 2 adjustable collets with a carabineer and a snap shackle on one end of the pole. A 12 m Kevlar™ fishing line contained within a plastic housing reel (fly-fishing reel) was fastened to the snap shackle on the pole. The line was marked every meter and unreeled to desired lengths during the survey. Small fluorescent tags attached to the line were used to mark the 2 m wide transect area under the line. A line tender held the line taut above the reef structures and slowly moved the line in an arc around the fixed central stake, allowing time for the surveyors to record their data (Fig. 2). Two surveyors swam in concentric circles directly over the line, one recording the number of colonies of each coral species and the other recording the number of colonies of each species that displayed signs of a specific disease. The surveyors counted colonies larger than 10 cm that fell directly below each 2 m segment of the line, providing more than half of their area occurred within the segment. The originating point of the arc was marked with a weighted subsurface buoy

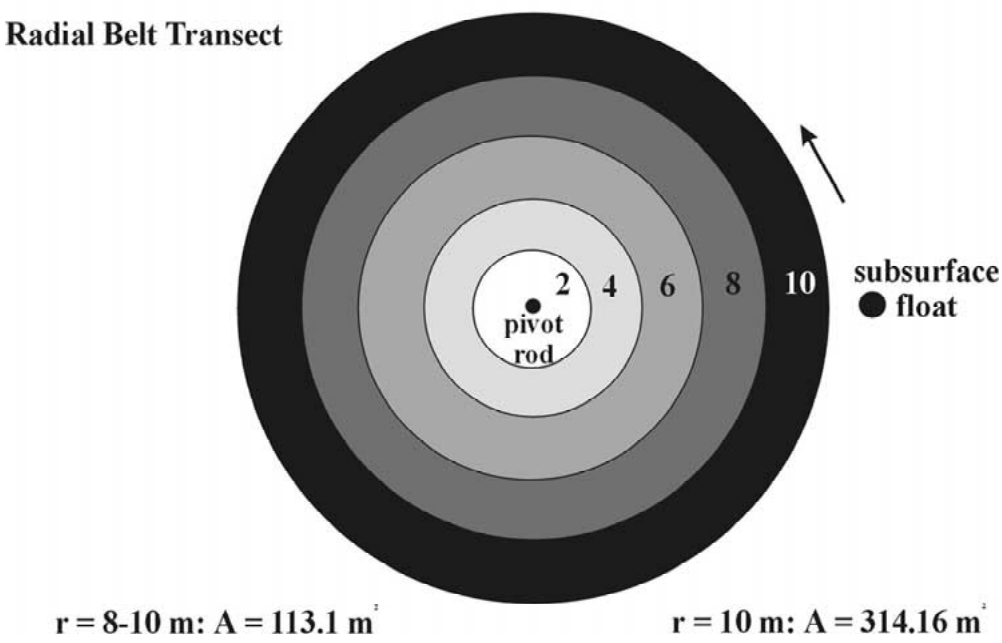


*Figure 2.* Diagram of the radial arc transect used for all surveys. The radius of the arc is 10 m, constituting an area of 314 $m^2$. The entire 10 m radius of the arc was surveyed during 1997, using 2 m increments on each sweep. Only the 8–10 m segment of the arc was used in 1998 surveys (113 $m^2$), an area determined to be sufficient to estimate the mean percent coral disease.

to alert the line tender when an entire arc segment had been completed.

During 1997, the surveys were conducted within the entire 10 m radius, in multiple radial increments that enlarged the arc by 2 m for each complete circle. For example, the first arc segment included the 0–2 m increment, the second arc segment included the 2–4 m segment, and so on until the entire 10 m radius of the arc had been completed, circumscribing a total area of 314 $m^2$. A species area curve was constructed to compare the June 1997 mean percent coral disease (MPCD) for increasing increments of the radial arc areas within the arc transect. Analysis of variance was used to determine whether the MPCD could be estimated using a portion of the arc, instead of the entire 10 m radius. Individual MPCD for each arc segment (0–2, then 2–4, and so on for each segment of 10 m radius) and cumulative MPCD for the cumulative areas of the arc segments (0–2, then 0–4, and so on for the entire 10 m) were used for the analysis, with arc area used as the class variable. This investigation determined that an area of 113 $m^2$, within the 8–10 m segment was sufficient for a reliable estimate of total MPCD (see 'Results'). Therefore, during 1998 surveys only the 8–10 m segment of the arc radius was used for assessment.

*Coral species and coral disease identification*

Ten disease conditions affecting 18 species of scleractinian corals and gorgonian sea fans were enumerated (Table 1). Three species of coral contained within the *Montastraea annularis* complex (Weil & Knowlton, 1994) were combined as a single category for data analysis, because discrepancies in identification were noted among some of the surveyors. Two gorgonian species, *Gorgonia flabellum* and *Gorgonia ventalina*, were combined as *Gorgonia* spp. All diseases were scored only for colonies containing active lesions; diseases were not scored if mortality had occurred recently and the cause of death was not apparent. Signs used to distinguish most coral diseases have been detailed elsewhere (Table 1) (Santavy & Peters, 1997; McCarty & Peters, 1998). Similar conditions described in the literature as patchy necrosis disease (Bruckner & Bruckner, 1997) and white pox (Holden, 1996), might be the same disease. We could not distinguish between the two conditions based on Bruckner & Bruckner's (1997) mention of patchy necrosis or Holden's (1996) mention of white pox; therefore, we used the term patchy necrosis disease/white pox to describe the lesions found on *Acropora palmata* colonies that were not white-band disease or predation. We did not distinguish the differences between white plague

type 1 and 2, since the primary difference in distinguishing them in the literature is dependent on the rate of progression (Dustan, 1977; Richardson et al., 1998a,b). This could not be determined in our surveys; therefore, we identified these conditions only as white plague in our surveys. Finally, there is some uncertainty in identifying aspergillosis (Smith et al., 1996; Kim et al., 1997; Nagelkerken et al., 1997a,b). For our surveys, this disease was scored if one of the following conditions were met: white fungal-like filaments with active lesions (tissue loss) and major skeletal damage, or white fungal-like filaments with active lesions showing coenenchyme purpling, or white fungal-like filaments with active lesions and purple galls in the vicinity of the diseased area. There are some inconsistencies in the literature concerning the signs of this disease, therefore, one might prefer to refer to the condition simply as sea fan disease.

*Quality assurance*

A rigorous quality assurance (QA) plan was adopted to quantify surveyor error and minimize data processing errors. The data collection protocols required training to improve identification skills and familiarize participants with the survey procedures, including the completion of standardized data forms. Scientific experts assessed the coral species and diseases in the 1997 surveys. Coral surveyors in 1998 were evaluated by expert coral taxonomists and expert coral pathologists for their ability to identify coral species and to classify coral conditions as either healthy, affected by a specific disease, bleached or physically damaged. Only those individuals who had successfully passed a test (scoring 90% similarity or greater using the experts as truth) were employed to collect data for the surveys. To evaluate inter-surveyor error, we had multiple surveyors take repeated counts of coral colonies by species and of coral disease types by species. To evaluate intra-surveyor error, we had each surveyor count species and disease types multiple times at a single station. The QA plan included procedures for several levels of data verification, including checks made in the field, duplicate surveys, and independent validation of all electronically entered data.

*Data analysis*

Data were recorded on standardized data sheets printed on Dura/Copy™ (J.L. Darling Corp., Tacoma, WA.) underwater paper. All data were entered into a computerized database using a PerForm Pro™-generated template, exported into MS Excel® worksheets, and used to create SAS data sets. Electronic data quality was confirmed twice by someone other than the original data recorder. The parameter of interest was mean percent coral disease (MPCD) (i.e. number of affected colonies per total number of colonies) per unit area. Data were analyzed using one-way ANOVA for unbalanced design in the 1997 pilot surveys and for balanced design in the 1998 surveys. The assumptions for ANOVA were tested and met, including independence, homogeneity of variances, and normality. The class variables or strata used included geographical areas (Key West, New Grounds or Dry Tortugas) and reef types (back, fore or transitional reef). Statistical significance for type I error was designated as $\alpha$=0.05 level. Tukey's Studentized Range Test (HSD) was used for means separation at the $\alpha$=0.05 level. A power analysis was performed to determine appropriate type 1 and 2 errors, and statistical power to be employed for future analyses. All analyses for the study were performed using SAS Version 6.12 (Statistical Analysis System Institute Inc., Cary, N.C., 1989–1996).

## Results

The pilot surveys demonstrated that the radial arc method was suitable for our coral disease assessment. Surveyors were able to make appropriate measurements by circumscribing the arcs and working together with the line tender. They were able to consult with each other underwater to reconcile colonies contained in overlapping concentric arcs, as confirmed by QA/QC (quality control) procedures (Santavy et al., 1999b). The primary drawback of the method was the amount of time it took to complete an entire 10 m radial arc, often 1–2 h to finish the 5 concentric laps.

In the spring 1997 pilot, the mean percent coral disease (MPCD) was calculated for each arc segment (0–2 m, 2–4 m, etc.) (Table 2a), as well as for increasing cumulative area of the arc (Table 2b). Analysis of variance showed that there was no statistical difference in the MPCD along the cumulative areas within the arc segments ($p \leq 0.99$) or between individual areas within each arc segment ($p \leq 0.89$). Therefore, modification of the radial arc transect method was implemented in 1998 when only the outer 8–10 m segment (113 $m^2$) was used to assess each station, rather than 0–10 m segments (314 $m^2$) for the entire arc. This change de-

*Table 2a.* Arc segment, arc area and mean percentage (±standard error) of all coral diseases associated with each segment of the 10 m radial arc. *N*=21 for all means

| Arc segment | Arc area ($m^2$) | Mean%±SE |
|---|---|---|
| 0–2 m | 12.6 | 12.8±3.0 |
| 2–4 m | 37.7 | 12.3±3.2 |
| 4–6 m | 62.8 | 10.1±1.8 |
| 6–8 m | 88.0 | 11.7±2.4 |
| 8–10 m | 113 | 13.6±1.8 |

*Table 2b.* Arc segment, arc area and mean percentage (±standard error) of all coral diseases associated with increasing cumulative area of the 10 m radial arc. *N*=21 for all means

| Arc segment | Arc area ($m^2$) | Mean%±SE |
|---|---|---|
| 0–2 m | 12.6 | 12.8±3.0 |
| 0–4 m | 50.3 | 15.3±5.2 |
| 0–6 m | 113 | 13.9±3.9 |
| 0–8 m | 201 | 14.4±3.9 |
| 0-10 m | 314 | 15.6±3.6 |

creased the amount of time required to complete one station from 60 to 120 min to 30 to 60 min, allowing more stations to be assessed over a greater area for the same amount of time and resources by decreasing the length of time for individual dives. Moreover, the use of the outer 8–10 m diameter segment also helped to decrease or eliminate bias from any localized outbreaks of a single disease that might occur at the inner 0–2 m or 2–4 m segments.

During the surveys, only 9 of the 10 disease conditions known to affect Caribbean scleractinian corals and gorgonian sea fans were detected. White-band disease type 2 was never observed during any of the surveys. Since it was unclear whether it would be encountered in the future, the surveyors continued to look for it in all surveys.

*Power analysis*

After review of 1998 survey data were complete, a power $(1-\beta)$ analysis was done in order to adopt acceptable $\alpha$ and $\beta$ levels (Table 3). Future studies would adopt $\alpha$=0.10 level with 5 replicates, $\beta$=0.0383, and a power $(1-\beta)$ of 0.9617. The 5 replicates or $(n)$ are the number of stations examined within each area and reef type.

*1997 surveys*

The overall prevalence of coral disease among the geographical areas during the spring pilot, expressed as MPCD, was 9.0%±1.4 (all values reported as $\bar{X}$±SE) (Table 4). There was no statistical difference in MPCD among the geographical areas, although Key West was considerably higher than the other areas. Dark spots disease, affecting *Siderastrea siderea*, was the most prevalent disease, being evenly distributed across the areas (9 Key West, 2 New Grounds and 5 Dry Tortugas stations), although it was not necessarily the most

*Table 3.* Results from power analysis to determine optimal number of replicates required for each stratum and to strengthen statistical inference ($\alpha$ and $\beta$ levels). Used $\bar{\times}$=10.3 and SD=9.0 for power analysis

| No. replicates ($n$) | Type I error ($\alpha$) | Type II error ($\beta$) | Power $(1-\beta)$ |
|---|---|---|---|
| 3 | 0.05 | 0.750 | 0.25 |
| 4 | 0.05 | 0.234 | 0.766 |
| 5 | 0.05 | 0.0820 | 0.918 |
| 3 | 0.10 | 0.3090 | 0.691 |
| 4 | 0.10 | 0.0952 | 0.9048 |
| 5 | 0.10 | 0.0383 | 0.9617 |

abundant disease at any given station. Aspergillosis-like signs affected sea fans at 8 Key West stations, 3 New Grounds stations and 4 Dry Tortugas stations. White-band disease type 1, affecting *Acropora cervicornis*, was found at 6 stations in the Key West area.

The overall prevalence of disease among the geographical areas during the summer pilot was 19.2%±4.2, due to a substantial but not a statistically significant increase in MPCD in the Key West area (Table 4). Aspergillosis, the most widely distributed disease observed at this time, affects gorgonian sea fans, and was observed at 9 Key West stations, 2 New Grounds stations and 7 Dry Tortugas stations. Acroporid species were affected by diseases occurring in all three areas, primarily with patchy necrosis/white-pox and white-band type 1 disease. Patchy necrosis/white-pox affected *A. palmata* at 8 Key West stations, where it was a newly emergent disease. White-band disease type 1 affecting *A. cervicornis* also occurred at 8 Key West stations. Dark spots disease affecting *S. siderea* occurred at 2 New Grounds stations.

An *a posteriori* analysis was conducted when it became evident that different reef types were being

*Table 4.* One-way ANOVA results for the 1997 Spring and Summer Pilot surveys, using area and reef type as the class variable for the strata examined. The mean percentage of coral disease for each survey period was analyzed separately. The mean percent diseased corals, standard error, (*n*), and (*N*) are presented. (*n*) is the number of stations examined within each area, at each survey period. (*N*) is the number of stations surveyed across all areas, at each survey period indicated. Tukey's Studentized Range Test was used to separate classes ($\alpha$=0.05). Unique integers represent significant differences among the classes

| Survey time (*N*) | | Stratum | Mean% Coral Diseased (*n*) ±SE | *p* value | Tukey's Studentized Range Test ($\alpha$=0.5) | |
|---|---|---|---|---|---|---|
| 1997 Pilot Spring | Area | Dry Tortugas | 6.5 (7)±1.7 | | 1 | |
| (21) | | Key West | 11.4 (11)±2.3 | $p \leq 0.21$ | 1 | |
| | | New Grounds | 6.0 (3)±1.7 | | 1 | |
| 1997 Pilot Summer | | Dry Tortugas | 8.9 (10)±3.2 | | 1 | |
| (26) | | Key West | 28.2 (14)±6.6 | $p \leq 0.06$ | 1 | |
| | | New Grounds | 7.8 (2)±0.2 | | 1 | |
| 1997 Spring Pilot | Reef Type | Back Reef | 10 (3)±2.5 | | 1 | |
| (21) | | Fore Reef | 8.4 (9)±1.1 | $p \leq 0.93$ | 1 | |
| | | Transitional Reef | 9.2 (9)±3.11 | | 1 | |
| 1997 Summer Pilot | | Back Reef | 54.6 (4)±7.6 | | 1 | |
| (26) | | Fore Reef | 19.2 (11)±5.8 | $p \leq 0.0001$ | | 2 |
| | | Transitional Reef | 6.3 (11)±1.2 | | | 2 |

compared among the geographical areas. During the 1997 spring pilot survey, 3 back reef, 9 fore reef and 9 transitional reef stations were surveyed. The coral disease distribution among the different reef types was not statistically significant (Table 4). Dark-spots disease was found at the greatest number of stations at both the fore and transitional reef stations; it occurred at 8 and 7 stations, respectively. On the fore reef, aspergillosis was observed at 4 stations, while white-band disease type 1 and yellow-blotch were found at only 1 station each. At back reef stations, white-band disease type 1 and patchy necrosis/white-pox were found at 2 stations each, while the only other disease observed was dark spots disease at 1 station. The transitional reef had 5 stations where white plague occurred and 3 stations where white-band disease type 1 occurred.

During the 1997 summer pilot survey, the highest prevalence of MPCD was found at back reef stations (54.6%±7.6). There was a statistical difference in the MPCD between the back reef stations and the fore and transitional reef stations (Table 4). The second greatest prevalence of disease occurred at fore reef stations (MPCD=19.2%±5.8). Since there was a statistically significant difference in the MPCD among reef types during the 1997 summer survey, it was decided that reef type would be formally added as an additional stratum in the experimental design for the 1998 surveys. White-band disease type 1 and patchy necrosis/white-pox dominated the back reef, occurring at all 4 stations, while aspergillosis and black-band disease occurred at 1 station each. In contrast to the spring survey, aspergillosis dominated the other 2 reef types, occurring at 9 fore reef and 8 transitional reef stations. White plague occurred at 7 stations each on both the fore and transitional reefs. White-band disease type 1 occurred at 7 and patchy necrosis/white-pox occurred at 5 fore reef stations. White-band disease type 1 occurred at 4 transitional reef stations, affecting *A. cervicornis*.

*1998 surveys*

The overall prevalence of coral disease among the geographical areas was 4.5%±0.87 during the spring survey. Key West had the greatest MPCD, followed by the Dry Tortugas, and the New Grounds (Table 5). The MPCDs among the areas were not statistically significant. Patchy necrosis/white-pox disease affected *A. palmata* at 2 Dry Tortugas stations and 6 Key West stations. In the Key West area, disease was observed to

*Table 5.* One-way ANOVA results for the 1998 Spring and Summer surveys, using geographic area and reef type as the class variable for the strata examined. The mean percentage of coral disease for each survey period was analyzed separately. The mean percent diseased corals, standard error, (*n*), and (*N*) are presented. (*n*) is the number of stations examined within each area, at each survey period. (*N*) is the number of stations surveyed across all areas, at each survey period indicated. Tukey's Studentized Range Test was used to separate classes ($\alpha$=0.05). Unique integers represent significant differences among the classes

| Survey time (*N*) | | Stratum | Mean% Coral Diseased (*n*)±SE | *p* value | Tukey's Studentized Range Test ($\alpha$=0.5) | |
|---|---|---|---|---|---|---|
| 1998 Spring | Area | Dry Tortugas | 4.4 (13)±1.6 | | 1 | |
| (30) | | Key West | 6.0 (12)±1.1 | $p \leq 0.14$ | 1 | |
| | | New Grounds | 1.0 (5)±0.27 | | 1 | |
| 1998 Summer | | Dry Tortugas | 2.6 (13)±0.6 | | 1 | |
| (31) | | Key West | 10.1 (13)±3.2 | $p \pm 0.03$ | | 2 |
| | | New Grounds | 1.2 (5)±0.3 | | 1 | |
| 1998 Spring | Reef Type | Back Reef | 8.1 (6)±2.7 | | 1 | |
| (30) | | Fore Reef | 4.2 (11)±1.4 | $p \leq 0.084$ | 1 | |
| | | Transitional Reef | 3.0 (13)±0.88 | | 1 | |
| 1998 Summer | | Back Reef | 11.7 (6)±5.0 | | 1 | 2 |
| (31) | | Fore Reef | 6.5 (12)±2.7 | $p \leq 0.042$ | 1 | |
| | | Transitional Reef | 1.7 (13)±0.31 | | | 2 |

affect the large colonies of the *Montastraea annularis* complex, with white plague at 5 stations and yellow-blotch disease at 3 stations in Key West. Hyperplasms were observed at several newly installed stations in the New Grounds, affecting *Diploria strigosa* and *Dichocoenia stokesii* (Table 6).

The overall prevalence of coral diseases among the geographical areas was 5.5%±1.5 during the summer survey and was statistically significant, with a higher prevalence in Key West (Table 5). The most pervasive disease condition was white-band disease type 1, affecting *A. palmata*; it was observed at 8 Key West stations and 1 Dry Tortugas station. More white plague was observed on *S. siderea* at 3 Dry Tortugas stations and on *M. annularis* complex at 4 Key West stations. Yellow-blotch disease on *M. annularis* complex was observed at 3 Dry Tortugas stations, whereas it had not been previously observed in our survey stations. Gorgonian sea fans were observed to be affected with aspergillosis at 5 stations each, the Dry Tortugas, and 3 stations from Key West and at only 1 New Grounds station. Red-band disease on sea fans was observed during this time, recorded at 2 stations in New Grounds and at 1 station in Key West (Table 6).

During the 1998 spring survey, there was not a statistically significant difference in MPCD among the reef types. The back reef stations had the greatest prevalence of disease (Table 5), with white-band disease type 1 and patchy necrosis/white-pox occurring at 4 and 5 back reef stations respectively. No other diseases occurred at back reef stations during this survey. White plague was the most common disease, occurring on 8 fore reef stations and 5 transitional reef stations. Patchy necrosis/white-pox occurred at 3 fore reef stations and hyperplasms were observed at both fore and transitional reefs, at 1 and 4 stations respectively. On fore reefs, aspergillosis, red-band disease, white-band disease type 1, and yellow-blotch disease were found at only 1 station each. White-band disease type 1 was found at 4, red-band disease was found at 2, and yellow-blotch disease was found at 3 transitional reef stations (Table 7).

During the 1998 summer survey, the MPCD on the back reef stations was statistically significant and greater than the MPCD on the transitional reef stations, although neither was statistically different from MPCD on the fore reef stations (Table 5). White-band disease type 1 was the dominant disease at most back reef stations. Aspergillosis and patchy necrosis/white-

pox were present at 2 stations each in the back reef. For the first time in the study, black-band disease was found at 5 fore reef stations, although not at any other reef types. White plague was encountered at 7 stations on fore reefs and 8 stations on transitional reefs. Aspergillosis was very common, occurring at 4 fore reef and 3 transitional reef stations. Hyperplasms, red-band disease, and white-band disease were found at 2 transitional reef stations each (Table 7).

## Discussion

Natural incidences of disease among scleractinian corals are not known, since most studies of coral diseases have been initiated in response to increased observations of a specific disease outbreak in a specific location. Selecting such an area to study coral disease might lead to a biased estimate of disease(s) among pristine or adjacent areas, since the site was chosen for its significant presence of disease and hence estimates probably would reflect inflated values for larger areas. The use of incorrect or biased information might interfere with best management or land use decisions intended to mitigate small localized outbreaks. Most often the real factors leading to localized disease incidences are not determined and the importance of small localized activity might be overestimated.

In our study, we were interested in looking at the patterns of disease prevalence over a large geographic region, so we employed a stratified random design within the areas to reduce potential bias. Previous studies of coral diseases, especially those describing disease outbreaks, have used other methods. Investigators have chosen their area of interest, traveled to the site, then chosen a direction to begin swimming a given number of kicks or throwing a dive weight in a 'random' direction (Gladfelter et al., 1977; Gladfelter, 1982; Feingold, 1988; Edmunds, 1991; Kuta & Richardson, 1996; Bruckner et al., 1997; Richardson et al., 1998a,b). Unlike the other studies, we had the advantage of access to detailed benthic maps for much of our study area prior to implementation (FMRI, 1998). The ability to select stations prior to physically traveling to the site allowed us to preclude *a priori* observations of coral diseases and obtain more accurate estimates when assessing the coral disease prevalence. This strategy should allow us to distinguish native disease incidences from elevated disease incidences induced by anthropogenic and climatic factors.

*Pilot surveys*

The pilot study allowed us to achieve a more efficient design, approach, and implementation for our study than would have been possible if we had initiated our work without this information. We were confident that most of our goals could be attained, and we found that the overall approach was well suited for our study objectives. This time allowed us to test the approach and methods we had developed and to solve problems that required design or equipment modification. A 10 m radial area or some segment of it was preferred because all previous quantitative coral disease studies (Edmunds, 1991;, Kuta & Richardson, 1996; Bruckner et al., 1997; Richardson et al., 1998a) had used this method, with the exception of the sea fan disease studies (Nagelkerken et al., 1997a,b). It will be important for us to compare our results with those of published studies, when we identify the diseases by species and type.

Execution of site selection in the spring 1997 pilot survey was sometimes tedious and time consuming, since some of the locations selected by the random hexagon process did not contain any coral communities or suitable coral coverage. Time and effort were consumed by travel to find bare sand and sea grass bottoms, especially since the information available on coral coverage for the Dry Tortugas and New Grounds area was minimal. During implementation of the pilot study, we discovered that the amount of time required to examine one arc was so long that the number of locations which could be assessed was greatly restricted. Although we demonstrated that surveying only the outer 8–10 m segment was appropriate for our study, it might not be acceptable in all studies. For example, completing only one segment of the arc might not provide adequate areal coverage to predict the MPCD incidence for a study examining a single disease in a limited locale or describing a disease outbreak. A pilot survey assessing the conditions using the entire arc could be required to insure adequate sampling area.

Several investigators had concerns that reef types were important to consider in this survey. Their concerns were that many of the diseases are taxon-specific and that they might be confined to specific depths or be more prevalent in certain reef types. Although the survey was not designed or balanced to test reef types in 1997, the data were used to determine whether differences in coral disease among reef type might exist. Thus, our results led us to add stations to incorporate reef type as a stratum. The pilot results also suggested

that real biological differences might occur, but not at the type 1 error established for the study.

Concerns were expressed about the inclusion of aspergillosis in the study. Since signs to discern aspergillosis in the field used by other groups were inconsistent, we incorporated the most common signs for us to score the disease. Since previous studies by several groups (Smith et al., 1996; Kim et al., 1997; Nagelkerken et al., 1997a,b), had inferred their sea fans were diseased with aspergillosis using fewer signs than this study, we confer that the incidence of aspergillosis is most likely under estimated rather than over estimated in our study. If one questions the prevalence of aspergillosis on sea fans, they cannot dispute the prevalence of sea fans diseased.

Finally, the pilot survey allowed us to test and modify procedures for efficiency. Standardized data sheets were developed to maximize their use underwater, and they convinced us of the utility of electronic data entry shortly after data collection. The species and disease lists were modified as we encountered diseased coral species that we had not anticipated. In 1998, we implemented a more rigorous bleaching assessment in the arc survey when it became obvious that coral bleaching was a significant event in the study areas. We devised, tested and implemented a strict quality assurance and quality control (QA/QC) protocol for disease identification. QA/QC measures were first employed by taxonomy and disease experts to establish truth and train the other surveyors. The pilot survey provided the opportunity for potential surveyors to gain experience and demonstrate their abilities before they were allowed to collect data for the survey (Santavy et al., 1999a).

*1998 study*

Coral disease workers have inferred that seasonal differences occur in the incidence and prevalence of coral diseases (Rützler et al., 1983b; Kuta & Richardson, 1996; Santavy & Peters, 1997); our preliminary results do not show a clear seasonal pattern in coral diseases within either stratum. If there is any difference, it appears that the Key West reefs have an increased prevalence of coral diseases in the summer. It is interesting to note that the distribution of diseases differed within area in the spring and summer surveys. In spring, the prominent disease was white plague, occurring at 91.7% of the stations in the Key West area (Table 6). Patchy necrosis/white-pox occurred at 50%, white-band disease type 1 at 25%, and yellow blotch disease at 25% of the stations in the Key West area. In the summer, the prominent diseases were white plague and white-band disease type 1, each occurring at 69.2% of the stations in the Key West area. Aspergillosis appeared to be more prevalent at all the geographic areas in the summer, whereas black-band disease was more prevalent in the summer, with the exception of New Grounds.

When the potentials for seasonal differences were examined among reef types, the pattern was more confusing. Although a significant difference did not exist among the reef types in spring ($\alpha$=0.05), there was a significant difference in summer. Once again, the distribution of certain diseases during the two sampling periods varied. During the spring survey, white-band disease type 1 and patchy necrosis/white pox were the most prevalent diseases, occurring at 67% and 83% of the back reef stations, respectively. White plague occurred at 73% of the fore reef stations. During the summer survey, white-band type 1 and white plague were the most prevalent diseases observed, with 83% on the back reefs and 62% on the transitional reefs, respectively. White plague also occurred at 58% of the fore reef stations. For the first time, significant black-band disease occurred at 42% of the fore reef stations. Aspergillosis did not appear to be confined to a specific reef type; it occurred on all reef types, but only during the summer survey. The results are suggestive of seasonality in some coral diseases. Additional studies are needed to determine if coral diseases are correlated to season, a more rigorous sampling regime would need to span different seasons (Table 7).

*Power analysis*

In the 1997 pilot and 1998 surveys, 0.05 was used as the type I error ($\alpha$), and no estimation was made for the type II error ($\beta$). After examining data from the 1998 survey, a power ($1 - \beta$) analysis (Table 3) was used to determine that $\alpha$=0.10, $\beta$=0.0383, and $1 - \beta = 0.9617$ levels should be adopted for future studies, with 5 replicates. This choice allowed for a 'safety factor' in the case that all 5 replicates within a given stratum could not be surveyed. This is a real possibility given the likelihood of complications due to weather, safety, time, or other logistical constraints that can occur during field work on research vessels. Closer examination of the relationships between MPCD and the strata (area and reef type) revealed that at most of the sampling times, except June 1997, there were probably real biological differences

*Table 6.* The percentage of stations within a single region that contained specific coral diseases in the 1998 surveys

| Disease | % Stations in Dry Tortugas | | % Stations in New Grounds | | % Stations in Key West | |
|---|---|---|---|---|---|---|
| | May 98 (13)[a] | Sept. 98 (13) | May 98 (5) | Sept. 98 (5) | May 98 (12) | Sept. 98 (13) |
| Aspergillosis | 8.7 | 38.5 | 0.0 | 20.0 | 0.0 | 23.1 |
| Black-band disease | 0.0 | 15.4 | 0.0 | 0.0 | 0.0 | 23.1 |
| Dark spots disease | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hyperplasia | 0.0 | 0.0 | 80.0 | 40.0 | 8.3 | 0.0 |
| Patchy Necrosis/White Pox | 15.4 | 0.0 | 0.0 | 0.0 | 50.0 | 30.8 |
| Red-band disease | 15.4 | 0.0 | 0.0 | 40.0 | 8.3 | 7.7 |
| White-band disease type 1 | 46.2 | 15.4 | 0.0 | 0.0 | 25.0 | 69.2 |
| White plague | 23.1 | 30.8 | 0.0 | 40.0 | 91.7 | 69.2 |
| Yellow blotch disease | 8.7 | 23.1 | 0.0 | 0.0 | 25.0 | 7.7 |

[a] ($n$) Number of stations sampled within each category.

*Table 7.* The percentage of stations within a single reef type that contained specific coral diseases for the 1998 surveys, presented by each survey period

| Disease | Back Reef | | Fore Reef | | Transitional Reef | |
|---|---|---|---|---|---|---|
| | May 98 (6)[a] | Sept. 98 (6) | May 98 (11) | Sept. 98 (12) | May 98 (13) | Sept. 98 (13) |
| Aspergillosis | 0.0 | 33.3 | 9.1 | 33.3 | 0.0 | 23.1 |
| Black-band disease | 0.0 | 0.0 | 0.0 | 41.7 | 0.0 | 0.0 |
| Dark spots disease | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hyperplasia | 0.0 | 0.0 | 9.1 | 0.0 | 30.1 | 15.4 |
| Patchy Necrosis/White Pox | 83.3 | 33.3 | 27.3 | 16.7 | 0.0 | 0.0 |
| Red-band disease | 0.0 | 0.0 | 9.1 | 8.3 | 15.4 | 15.4 |
| White-band disease type 1 | 66.7 | 83.3 | 9.1 | 33.3 | 30.1 | 15.4 |
| White plague | 0.0 | 0.0 | 72.7 | 58.3 | 38.5 | 61.5 |
| Yellow blotch disease | 0.0 | 0.0 | 9.1 | 25.0 | 23.1 | 7.7 |

[a] ($n$) Number of stations sampled within each category.

that were not always supported by statistical testing. If 5 replicates were completed using $\alpha$=0.10, $\beta$=0.0383 the power would be 0.9617; if only 4 replicates could be sampled at the same $\alpha$ level, the power would drop to only 0.9048. By comparison at $\alpha$=0.05, the power for $n$=5 would be 0.918, the power drops precipitously to 0.766 when $n$=4.

## Conclusions

The experience and information gained from the pilot surveys allowed us to improve our methodology in the 1998 surveys. Important changes allowed us to increase the number of stations by decreasing the area of the arcs examined. This allowed us to collect data from the necessary number of stations within our limited cruise schedule. Additional benefits were gained from improved protocols, modifications to data sheets, and implementation of an electronic data entry system. Altering QA/QC procedures allowed us to achieve higher data quality standards. Results obtained from the pilot allowed us to assess whether the interstation variability would be low enough to detect differences among the areas. Those data were also used to decide that reef type should be examined as another source of variability. The pilot surveys greatly improved our study but did not contribute to determining whether there were seasonal differences between spring and summer, thus whether two surveys per year were justified.

The results from the 1998 surveys show that the strata chosen to examine the epizootiology of coral diseases are valid and should be included in the ex-

perimental design. The data also suggest that there are differences in the distribution and frequency of specific coral diseases between the two seasons sampled. It is important to continue to sample at different seasons to fully understand the epizootiologies and to eventually link environmental and climatic factors to the frequency and distribution of coral diseases in the Florida Keys.

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