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CORAL DISEASE DYNAMICS AND ENVIRONMENTAL DRIVERS IN THE NORTHERN FLORIDA KEYS AND LEE STOCKING ISLAND, BAHAMAS

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Joshua Daniel Voss

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To: Interim Dean Mark Szuchman
College of Arts and Sciences

This dissertation, written by Joshua Daniel Voss, and entitled Coral Disease Dynamics and Environmental Drivers in the Northern Florida Keys and Lee Stocking Island, Bahamas,having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

James Fourqurean

Peter Glynn

Walter Goldberg

DeEtta Mills

Laurie Richardson, Major Professor

Date of Defense: July 5, 2006

The dissertation of Joshua Daniel Voss is approved.

Interim Dean Mark Szuchman
College of Arts and Sciences

Interim Dean Stephan L. Mintz
University Graduate School

Florida International University, 2006

DEDICATION

I dedicate this dissertation to the late Joseph Carpineto, better known as "Mr. C." For twenty-five years Mr. C was a science teacher, soccer coach, and beloved friend to the community of St. Margaret Mary School in Winter Park, Florida. Of his many passions, science, teaching, and soccer became my passions. To this day I'm not sure if it was simply because of our common interest or because I admired him so greatly that I wanted to emulate him. His inspiration and enthusiasm, be it in the classroom, on the sideline, or knee deep in the muck on Big Pine Key, were key influences in my decision to pursue a career in academia. I last saw Mr. C at a wedding in Orlando where we shared a long chat about what each of us had been doing over the previous few years. As we left he turned to me and said plainly, "I always new you could do it... it was just my job to bring it out of you." The phrase was the essence of a true mentor who made it his life's work to improve the lives of all his students, young and old. I, along with the thousands of students and friends whose lives he touched, will miss Mr. C deeply.

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ABSTRACT OF THE DISSERTATION

CORAL DISEASE DYNAMICS AND ENVIRONMENTAL DRIVERS IN THE NORTHERN FLORIDA KEYS AND LEE STOCKING ISLAND, BAHAMAS

by

Joshua Daniel Voss

Florida International University, 2006

Miami, Florida

Professor Laurie Richardson, Major Professor

Coral reefs are experiencing declines worldwide and recently coral diseases have been identified as significant contributors to coral mortality. However, little is known regarding the factors that drive coral disease distributions and dynamics. Current knowledge of the organisms that cause coral diseases is also limited, with pathogens having been identified for only 5 of the 21 described coral diseases. The study presented here describes coral disease dynamics in terms of occurrence, prevalence, spatial distribution, and host species susceptibility from 2002-2004 on reefs of the Northern Florida Keys (NFK) and Lee Stocking Island (LSI) in the Bahamas' Exuma chain. In addition, this research investigated the influence of temperature, sediment, and nutrient availability on coral disease prevalence and severity. Finally, microbial communities associated with a polymicrobial disease, black band, were examined to address spatial and temporal variability.

Four scleractinian diseases were observed in repeated surveys conducted during June-August of each year: black band disease (BBD), white plague type 2 (WP), dark spots syndrome (DSS), and yellow band disease (YBD). Coral disease prevalence was

generally low in both the NFK and LSI as compared to epizootic levels reported previously in the NFK and other regions of the Caribbean. Disease prevalence and species susceptibility varied spatially and temporally. Massive framework species, including *Siderastrea siderea*, *Colpophyllia natans*, and *Montastraea annularis*, along with relatively smaller colonies of *Meandrina meandrites* and *Dichocoenia stokesi*, were most susceptible to disease. Temperature, sedimentation, and dissolved inorganic nitrogen were positively correlated with BBD infections. Furthermore, experimental nutrient enrichment exacerbated coral tissue loss to BBD both *in situ* and *in vivo*. Profiling of BBD microbial communities using length heterogeneity PCR revealed variation over space and time, with significantly distinct bacterial assemblages in the NFK, LSI, and US Virgin Islands.

This study contributes to knowledge of the relationship between coral diseases and the environment, and facilitates predictions regarding potential changes in coral reef communities under differing environmental conditions. Additionally, this research provides further understanding of coral disease dynamics at both the host and microbial pathogen levels.

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CHAPTER 1

INTRODUCTION

Coral reefs are experiencing a recent period of severe decline with losses of coral cover estimated to be 27% worldwide (Wilkinson 2002) and 80% in the wider Caribbean (Gardner et al. 2003). During this same time coral diseases and their contribution to the degradation of coral reef ecosystems have increased dramatically (Epstein 1998, Goreau et al. 1998, Hayes & Goreau 1998, Richardson 1998, Harvell et al. 1999, Porter et al. 2001). Published studies have demonstrated increases in the number of coral diseases, the number of reported cases of infection, and the number of coral species affected (Porter et al. 1999, Green & Bruckner 2000, Weil 2004). Similarly, the range of coral diseases has expanded from initial observations in the greater Caribbean (Antonius 1973, Dustan 1977, Gladfelter et al. 1977) to a worldwide distribution (Sutherland et al. 2004). Although the specific long-term effects of coral diseases on reef ecosystems are poorly understood, coral disease is known in at least one case to have caused an apparently permanent shift in the coral community structure of a Belizean reef (Aronson & Precht 2001). Knowledge of the causes and resulting distributions of coral diseases is fundamental for determining their potential impact on coral communities and necessary for any attempts to mitigate coral loss.

To date 21 coral diseases and syndromes have been observed (Weil et al. 2006), with four of these diseases found globally: black band disease (BBD), white plague (or plague-like infections), shut down reaction, and skeletal anomalies (such as neoplasia) (Sutherland et al. 2004). Globally at least 150 species of scleractinians, octocorallians,

and hydrozoans are susceptible to one or more coral diseases. The Caribbean has been identified as a coral disease "hot spot" due to the rapid emergence of diseases and relatively high frequency of epizootics (Epstein 1998, Weil 2004). By comparison, a relative paucity of epizootics have been reported on Indo-Pacific reefs, with low disease prevalence and limited geographic distribution (Willis et al. 2004). Four coral diseases, black band disease (BBD), white plague type II (WPII), dark spots syndrome (DSS), and yellow band (or blotch) disease (YBD) were observed in the studies that comprise this dissertation and are discussed in detail below.

BBD was the first coral disease to be described (Antonius 1973) and is the most widely distributed disease affecting reefs worldwide (Sutherland et al. 2004). At least 64 species of scleractinian corals are susceptible to BBD and of these 19 are found in the Caribbean (Sutherland et al. 2004). BBD primarily infects massive framework-building scleractians throughout the Caribbean including *Colpophyllia natans, Diploria labyrinthiformis, Montastraea annularis, M. cavernosa, M. faveolata,* and *Siderastrea siderea* (Antonius 1981, Rützler et al. 1983, Edmunds 1991), threatening not only the physical structure of the reef but also the principal contributors to coral gamete production. In contrast, *Acropora* spp. are most commonly affected by BBD in the Indo-Pacific (Page & Willis 2006). Six octocorallian species have also been observed with BBD on Caribbean reefs (Weil et al. 2006)

BBD is a polymicrobial disease that functions as a microbial consortium (Carlton & Richardson 1995, Richardson et al. 1997). The pathogenic assemblage of microbes migrates across an infected coral colony destroying coral tissue and leaving behind bare limestone skeleton (Carlton & Richardson 1995). Infections appear as a darkly pigmented

mat, or band, which can be several mm to several cm wide and approximately 1 mm thick. The visually dominant members of the BBD consortium are gliding, filamentous, non-heterocystous cyanobacteria that were originally identified as *Oscillatoria submembranaceae* (Antonius 1973) and later reclassified as *Phormidium corallyticum* based on morphological characteristics (Rützler & Santavy 1983). However, 16S rRNA gene sequencing has failed to identify cyanobacteria from the *Phormidium* genus in BBD samples (Cooney et al. 2002, Frias-Lopez et al. 2002, 2003, 2004, Ragoonath 2005). Likewise, the sulfide-oxidizing bacteria *Beggiatoa* spp. reported by a number of investigators (Garrett & Ducklow 1975, Ducklow & Mitchell 1979, Carlton & Richardson 1995, Richardson 1996) have not been observed in molecular studies based on DNA sequencing of the BBD microbial community (Cooney et al. 2002, Frias-Lopez et al. 2004). Sulfate-reducing bacteria including *Desulfovibrio* spp. (Garrett & Ducklow 1975, Schnell et al. 1996, Cooney et al. 2002, Viehman et al. 2006) as well as at least 50 other heterotrophic bacterial species (Cooney et al. 2002, Frias-Lopez et al. 2002) have also been identified using both standard microbiological and culture-independent techniques. The microbial communities associated with healthy coral tissue, dead coral skeletons, and overlying seawater are distinct from BBD bacterial assemblages (Frias-Lopez et al. 2002). Collectively within these studies, there are discrepancies regarding the composition and variation of the microbial consortia associated with BBD. Reservoirs of this consortium have been observed as non-pathogenic biofilms on healthy coral colonies (Richardson et al. 1997).

The distribution of BBD may be correlated with human influence. Outbreaks of BBD have been recorded near sewage outflows or other areas with increased pollution

(Antonius 1988, Bruckner & Bruckner 1997, Goreau et al. 1998). One study has quantitatively documented increased BBD prevalence at a sewage-impacted site as compared to a non-impacted site in St. Croix, USVI (Kaczmarsky et al. 2005). Additionally, water samples taken near colonies infected with BBD showed significantly higher nutrient levels than areas near healthy colonies (Kuta & Richardson 2002). Given this correlation, the anthropogenic eutrophication of specific areas through pollution may be influencing the virulence of BBD. A more detailed discussion concerning the role of environmental conditions in BBD activity is found below.

First described by Dustan (1977), the early outbreaks of white plague (now referred to as type I) were not studied on a regional basis and little is known about this disease's etiology. In 1995 a massive epizootic occurred on reefs of the middle and northern Florida Keys reef tract (Richardson et al. 1998b). Based on disease signs that were similar to those observed in the initial plague outbreak, this disease was named white plague type II (WPII) (Richardson et al. 1998a). The diseases appear as a sharply demarcated line between healthy coral tissue and bare coral skeleton, with no visible accumulation of microorganisms (Richardson et al. 1998a). Koch's postulates were fulfilled for an isolate collected during the 1995 outbreak (Richardson et al. 1998b), and the primary WPII pathogen, named *Aurantimonas corallicida*, comprises a new bacterial genus in the Alphaproteobacteria (Denner et al. 2003). The initial outbreaks of white plague (type I and II) were centered in the reefs of the Florida Keys, but more recent studies indicate that the range of WP II has greatly expanded to a Caribbean-wide distribution (Weil et al. 2002). Furthermore, the number of coral species infected by plague is also on the rise. The initial observations of white plague type I infected 6

species (Dustan 1977). Richardson et al. (1998a,b) observed WPII on 17 coral species during the 1995 epizootic. To date 41 scleractinian species are now known to be susceptible to WPII infections, making this the largest known host range for any coral disease (Weil et al. 2006). A third form of white plague, type III, was observed in the Florida Keys in 1999 causing rapid tissue loss on large colonies of *Montastraea annularis* and *Colpophyllia natans* (Richardson et al. 2001).

Dark spots syndrome (DSS), first observed in 1990 (Solano et al. 1993), is characterized by dark pigmented, roughly circular areas of depressed tissue on colonies of *S. siderea, Stephanocoenia intersepta, M. annularis, M. faveolata, M. franksi,* and *M. cavernosa* (Borger 2003, Gil-Agudelo et al. 2004, Borger 2005a). Previous studies have shown that *S. siderea* is the most susceptible species in the eastern Caribbean (Cervino et al. 2001, Borger 2003), the Bahamas (Gochfeld et al. 2006), and South Florida (Borger 2005b). In contrast, on Colombian Caribbean coastal reefs 62.9% of the DSS infections were found on *M. annularis* and 31.2% were on *S. siderea*, with the remainder on other *Montastraea* spp. and *S. intersepta* (Gil-Agudelo & Garzon-Ferreira 2001). Thus far no pathogen has been identified for dark spots but tissue loss has been documented (Cervino et al. 2001, Gochfeld et al. 2006). Because DSS affects three of the most important Caribbean reef-building species, *S. siderea, M. annularis,* and *M. cavernosa*, it has the potential to negatively impact the ecological processes and productivity of reefs throughout this region. Monitoring of DSS over time has revealed that these patches can be caused by a wide range of organisms including calcareous tube worms, boring sponges, algae, or BBD, and, therefore, are most likely a general stress response rather than a biological infection (Borger 2005a).

Yellow band disease (YBD) is characterized by circular or irregular lesions or bands of yellow or discolored coral tissue, commonly on the upper surface of colonies (Santavy et al. 1999). Initially observed on *M. faveolata*, YBD is now known to infect at least 9 scleractinian species (Garzon-Ferreira et al. 2001). No pathogen has been associated with YBD thus far. However, there is evidence that this disease affects the zooxanthellae within coral tissue directly due to both the loss of zooxanthellate pigments and cells and the lower mitotic indices of zooxanthellae associated with the disease (Cervino et al. 2001). In addition, while zooxanthellae of *Symbiodinium* clade C were common in healthy tissues from *Montastraea* spp., *Symbiodinium* of a different clade (A) were more abundant in YBD infected coral tissues and responsible for the yellow coloration (Toller et al. 2001)

Global climate change (including increased temperature), increased sedimentation, and anthropogenic nutrient inputs have all been suggested as potential causes of increased coral disease incidence (Harvell et al. 1999, Porter et al. 1999, Green & Bruckner 2000). The increased frequency and severity of coral bleaching events worldwide (Hoegh-Guldberg 1999), combined with data that indicate sea-surface temperatures are reaching levels not seen in the past 100 years (Lough 2000), provide evidence that global warming is negatively impacting coral reefs on a broad scale. In addition to environmentally induced coral bleaching (Brown 1997, Fitt et al. 2001) several coral diseases have been associated with relatively higher water temperature including BBD (Edmunds 1991, Kuta & Richardson 1996, Bruckner et al. 1997, Voss & Richardson 2006), WP II (Richardson et al. 1998b), aspergillosis (Alker et al. 2001), bacterial bleaching (Rosenberg & Ben-Haim 2002), and DSS (Gil-Agudelo & Garzon-

Ferreira 2001, Borger 2005a). Likewise, several studies have shown that the pathogens for gorgonian aspergillosis (Alker et al. 2001), bacterial bleaching (Banin et al. 2002), WP II (Remily & Richardson 2006), and a cyanobacterial member of the BBD consortium (Richardson & Kuta 2003) grow optimally at or above 30°C. In addition, Fitt et al. (2001) demonstrated that some corals become physiologically stressed above 30°C, potentially causing an increased susceptibility to infections. Synergistic interactions between increased pathogen growth or virulence and decreased coral immunity (Toren et al. 1998, Alker et al. 2001) may explain, at least in part, why coral diseases are most often observed during warmer periods of the year.

Sediments are capable of harming corals through a number of processes. Abrasion and/or smothering directly damage coral tissues. Indirect stresses to corals include decreased light availability, reducing photosynthetic rates of zooxanthellae, and increased mucus production, requiring additional energy expenditures by the coral (Riegl & Branch 1995). Damage and stress to corals may make them more susceptible to infections by microbial pathogens. Furthermore, it has been hypothesized that coral disease pathogens, *Aspergillus sydowii* spores in particular, may be transported to coral reefs by local runoff (Smith et al. 1996) or deposition of African dust in the Caribbean (Shinn et al. 2000). In addition to its potential role in disease, increased sedimentation has been shown to reduce reproductive success in corals (Gilmour 1999), exacerbating its effect on coral reef health.

Numerous studies have demonstrated that both increased nutrient availability and decreased herbivory can lead to phase shifts from coral-dominated to macroalgal-dominated communities (Littler & Littler 1984, Done 1992, Hughes 1994, Steneck &

Dethier 1994). However there is considerable debate regarding the relative roles of nutrients and herbivory in coral reef community dynamics (Lapointe 1997, Hughes et al. 1999, McCook 1999). The only long-term, large scale experiment designed to assess the effect of nutrient enrichment on coral community composition has provided only minimal evidence that increased nutrients lead to algal dominance (Koop et al. 2001).

Despite emphasis among researchers on the role of nutrification in coral reef declines, only a limited number of studies have assessed the potential interactions between increased nutrients as related to reef degradation other than phase shifts. One potential effect may be an enhancement of pathogen-associated coral diseases. Four previously published studies have included a quantitative assessment of the relationship between water quality and coral disease. Kim and Harvell (2002) in a field study of aspergillosis (a fungal infection of seafans), found that although the overall disease prevalence did not vary throughout the Florida Keys, higher levels of dissolved inorganic nitrogen and reduced water clarity (as indicated by chlorophyll *a* and turbidity) were correlated with increased disease severity (i.e. percent of colony area affected by aspergillosis). They emphasized that although an association was established, demonstration of a causative relationship would be premature due to limited sample size (5 locations) and the fact that water quality parameters were determined using samples that were not collected at the same time or location as the disease surveys.

Kuta and Richardson (2002) examined the relationship between BBD incidence and the environment in the Florida Keys by measuring eleven environmental factors within localized (2 m diameter) habitats surrounding colonies of both diseased and healthy (but BBD-susceptible) corals. That study (n = 190 colonies) revealed that BBD

infected colonies were on average found at shallower water depths with higher water temperature, higher levels of nitrite, and lower levels of ortho-phosphate than areas near healthy colonies. They concluded, however, that the increased nitrite concentration may have been a result of microbial metabolic processes within the black band consortium itself, leading to an accumulation of nitrite (Kuta & Richardson 2002).

Kaczmarsky et al. (2005) observed that both BBD and WPII were more prevalent at a sewage-impacted site as compared to an ecologically similar non-impacted site in St. Croix, USVI. In this study, a site near Frederiksted was exposed to episodic flushing of untreated waste water with concurrent increases in both fecal coliforms (>1400 CFUs 100 mL^{-1}) and *Enterrococci* (>1800 CFUs 100 mL^{-1}). In contrast Butler Bay, lying upcurrent of the sewage outflow, exhibited low densities (<5 CFUs 100 mL^{-1}) of both sewage indicator groups during bypass events. They found that BBD prevalence among susceptible coral species was 2.7 times greater in the Frederiksted site and that WPII prevalence was 3.7 times greater. Although this study did not include measurements of nutrient concentrations during or between sewage bypass events, it provides direct evidence that untreated sewage runoff is associated with increased coral disease prevalence.

Only one previously published study has examined the effects of nutrient enrichment on coral diseases using an experimental manipulation. Bruno et al. (2003) exposed both aspergillosis in seafans and YBD on two *Montastraea* species to artificial nutrient enrichment in Akumal, Mexico. In this study seafans experimentally exposed to both aspergillosis (infected tissue) and increased nutrients experienced significantly higher proportions of infected tissue and diagnostic purple galls. Likewise, nutrient

enrichment of *M. annularis* and *M. franksii* colonies with naturally occurring YBD infections resulted in increased rates of disease progression and host tissue loss.

Unrelated to local environmental conditions, various aspects of coral population and community structure may also influence the incidence, prevalence, and severity of coral diseases. Aspergillosis infections (number of lesions) were observed to be higher in number on individual sea fan colonies with relatively larger surface area (Kim & Harvell 2002). Quantitative observations in Dominica and St. Lucia revealed an increased incidence of WPII (Nugues 2002, Borger 2003), BBD, and DSS on relatively larger scleractinian colonies (Borger 2003). The probability of contact with water-borne pathogens, or other infectious vectors, is potentially increased for colonies of greater surface area. Additionally, because larger colonies are likely older than their smaller neighbors, they may have higher accumulated exposure to pathogens over time. However, it has also been hypothesized that under increased threat of mortality smaller colonies may allocate more resources toward defense mechanisms rather than reproduction (Bak & Meesters 1998, Kim & Harvell 2002).

Disease severity (percent of coral tissue lost), in contrast to disease incidence, may be negatively correlated to colony size as observed in white plague disease by Nugues (2002). The potential etiologically significance of this phenomenon is that while relatively large, ecologically important species (and colonies) are at an increased risk of infection, these colonies also may have a greater chance of recovering as compared to small colonies that succumb to the disease more quickly, given constant tissue degradation rates. This could be particularly important for diseases with seasonal

occurrence patterns related to water temperature. Large colonies may avoid complete tissue degradation but they may also act as reservoirs for coral pathogens and could lead to recrudescent infections in subsequent years.

The various external drivers, both environmental and biotic, discussed above are not inclusive of all factors that potentially influence coral diseases. Some aspects of coral diseases dynamics are determined directly by the organism (or organisms) that cause pathogenesis and their physiological capabilities. Therefore analyses of the microorganisms associated with diseases are fundamental to understanding mechanisms of infection. As discussed previously, discrepancies exist in published reports regarding the microorganisms that are associated with BBD. The same is true for other coral diseases including plague, white band, and red band. In addition to standard microbiological techniques, molecular approaches can provide significant insight into the identity of microbial pathogens that affect corals

One promising molecular method with applications in characterizing microbial communities is length heterogeneity polymerase chain reaction (LH-PCR). This technique differentiates organisms based on the natural variation in the sequence lengths of 16S rRNA genes (Suzuki et al. 1998). LH-PCR profiling provides relative abundance measures of 16S rDNA types and permits comparisons of whole community samples (Suzuki et al. 1998, Mills et al. 2003). Amplicons derived from LH-PCR often indicate phylogenetic differences. However, it is possible for multiple, distantly related organisms to give rise to amplicons of identical length (Mills et al. 2003). LH-PCR profiles are highly reproducible and economically feasible, making them ideal for

monitoring structural differences and dynamics in BBD (or any) microbial communities (Mills et al. 2003, Mills et al. 2006). Recently LH-PCR techniques have proven useful for profiling microbial communities in various investigations and monitoring efforts (Ritchie et al. 2000, Tiirola et al. 2003, Mills et al. 2006).

The studies presented here are the results of a multidisciplinary approach designed to investigate coral disease dynamics over space and time, the factors (described above) that may influence disease prevalence and severity, and biological variation within a specific coral disease (i.e. BBD). These findings are presented in four chapters:

Chapter Two is formatted for publication in the Journal *Diseases of Aquatic Organisms*. This monitoring study provides the first quantitative reports of coral disease occurrence, prevalence, recurrence, and species susceptibility for reefs near Lee Stocking Island (LSI) in the Bahamas' Exuma Chain over two summers (2002 and 2003). I assessed the potential roles of temperature and sedimentation on coral disease activity. Additionally, this study examined the relationship between coral colony size, disease occurrence, and disease severity (i.e. percent coral tissue lost).

Chapter Three has been formatted for submission to the Journal *Marine Ecology Progress Series*. The first objective of this study was to compare coral disease dynamics in the Northern Florida Keys (NFK) and LSI. Quantitative surveys were conducted in each region in June-August of 2002 and 2004 for evaluation of changes in coral disease prevalence and species susceptibility over space and time. Using dissolved inorganic nitrogen and total phosphorus as proxy variables, this study quantitatively tests the relationship between nutrient availability and coral disease prevalence in both regions.

Chapter Four is formatted for publication in the Journal *Coral Reefs*. This study was designed to determine the effects of nutrient enrichment on BBD progression across coral colonies and subsequent coral tissue loss. *S. siderea* colonies with naturally occurring BBD infections were exposed to elevated nutrient concentrations *in situ* using commercial time release fertilizer. Complementary laboratory experiments were conducted using artificially infected *S. siderea* colonies exposed to elevated concentrations of nitrate. In both the *in situ* and laboratory experiments, BBD migration in nutrient dosed colonies was compared to migration observed in control colonies exposed to ambient nutrient conditions.

Chapter Five is formatted for the Journal *Microbial Ecology*. The goal of this study was to discriminate overall structural patterns in the BBD microbial community, and to determine variation within and between microbial communities across space and time. LH-PCR was used to generate BBD community profiles from various coral hosts in three wider Caribbean regions: the NFK, LSI, and USVI. In addition to spatial variation, this report assesses potential variation in BBD communities over time (20 days) under both ambient and nutrient enriched conditions. Nonmetric multidimensional scaling based on similarity was used to compare samples between regions, host coral species, across time, and under each nutrient condition.

These studies were primarily carried out in two geographically distinct regions, the Northern Florida Keys and Lee Stocking Island in the Bahamas's Exuma Chain. Additional coral disease samples were collected from St. John in the US Virgin Islands

but no surveys were conducted. Coral reefs near Key Largo in the Northern Florida Keys are easily accessible to researchers, boating enthusiasts, divers, and sport fishers located in South Florida. This accessibility makes the reefs of the Florida Keys some of the most visited in the world. Coral diseases in the Florida Keys have been well documented and monitored over the past decade by the Coral Reef Evaluation and Monitoring Project (coordinated by the EPA, NOAA, and FWRI) and additional studies (Kuta & Richardson 1996, Porter et al. 2001, Richardson & Voss 2005). The Southeastern Environmental Research Center Water Quality Monitoring Network (SERC-WDMN) at Florida International University provides data that are useful for investigating the role of environmental conditions in coral disease activity on these reefs.

In contrast to coral reefs of the Florida Keys, those near LSI are relatively pristine. There is no environmental stress from sewage discharge, pesticide pollution, or river runoff (Dennis & Wicklund 1993). The area is geographically isolated from human population centers and receives little commercial or sport fishing since a no-take zone surrounds LSI. These factors make it an ideal region to study coral diseases, environmental conditions, and coral populations, with negligible human influence.

Study sites in the NFK and LSI were selected haphazardly based on observed coral cover and logistical accessibility (Figure 1). Study sites in the Florida Keys included many of which have been continually monitored by Richardson's lab over the past 10 years. Additional BBD samples were collected on three reefs near St. John in the US Virgin Islands.

In summary, this research is aimed at elucidating coral disease dynamics and the environmental factors that control infections in two tropical coral reef regions with variable levels of human impact. Furthermore, it describes biological variation in the microbial community structure associated with BBD and helps to explain discrepancies from previous reports concerning the identity of BBD microorganisms.

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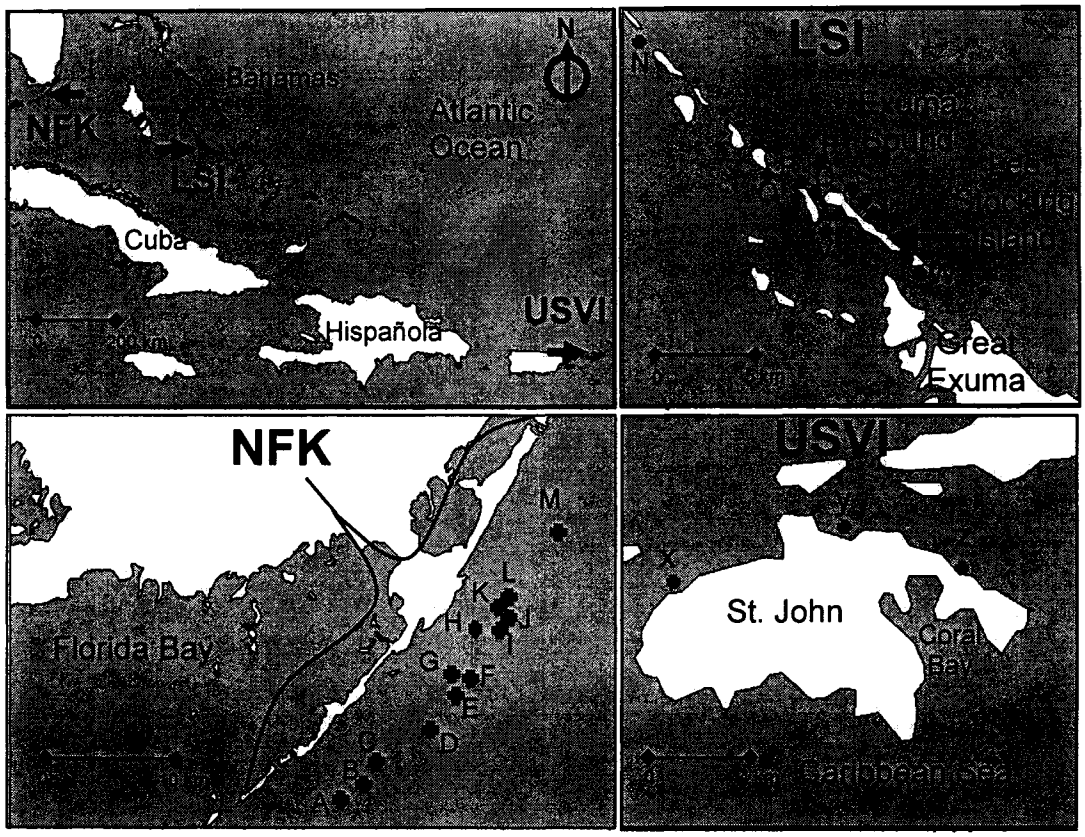

Figure 1. Studies sites in the northern Florida Keys (NFK), Lee Stocking Island (LSI), and St. John, US Virgin Islands (USVI). (A) Crocker, (B) Davis, (C) Conch, (D) Pickles, (E) Molasses, (F) French, (G) White Bank, (H) Cannon Patch, (I) Grecian, (J) Dry Rocks, (K) Horseshoe, (L) Algae, (M) Carysfort, (N) Goby Spot, (O) Rainbow Gardens, (P) North Norman's, (Q) Big Point, (R) White Horse, (S) North Perry, (T) South Perry, (U) Horseshoe, (V) Tug and Barge, (W) Square Rock, (X) Hawksnest Bay, (Y) Watermelon Cay, (Z) Haulover Bay.

CHAPTER 2

CORAL DISEASES NEAR LEE STOCKING ISLAND, BAHAMAS: PATTERNS AND POTENTIAL DRIVERS

ABSTRACT

The number of coral diseases, coral species they infect, number of reported cases, and range over which they are distributed have all increased dramatically in the past three decades, posing a serious threat to coral reef ecosystems worldwide. While some published studies provide data on the distribution of coral diseases at local and regional levels, few studies have addressed the factors that may drive these distributions. I recorded coral disease occurrence, prevalence, and severity along with temperature, sedimentation, and coral population data (species abundance and colony size) over two consecutive summers on reefs near Lee Stocking Island (LSI) in the Bahamas' Exuma Chain. In 2002 a total of 11,092 coral colonies (all species present) were examined within a survey area of 9,420 m^2, and 13,973 colonies within 10,362 m^2 in 2003. Similar to other reports, relatively large, framework species including *Siderastrea siderea*, *Colpophyllia natans*, and *Montastraea annularis*, along with the smaller *Dichocoenia stokesi*, were the species most susceptible to coral disease. Recurring infections were observed on individual colonies from 2002 to 2003, and were more likely for black band disease (BBD) than for either white plague (WP) or dark spots syndrome (DS). In 2002, WP and DS demonstrated clumped distributions, while BBD was randomly distributed. However,

in 2003 BBD and WP were clumped. This is the first study, to my knowledge, that quantitatively documents coral disease dynamics on reefs surrounding LSI.

INTRODUCTION

Over the past three decades both the number of coral diseases and their contribution to the degradation of coral reef ecosystems have increased dramatically (Goreau et al. 1998, Richardson 1998, Harvell et al. 1999, Porter et al. 2001, Rosenberg & Loya 2004). In addition to increases in the number of diseases and syndromes reported, the number of reported cases and species affected has also risen (Porter et al. 1999, Green & Bruckner 2000, Porter et al. 2001, Weil 2004). During this same period the geographical range of coral diseases has expanded from initial observations in the greater Caribbean (Antonius 1973, Dustan 1977, Gladfelter et al. 1977) to a worldwide distribution (Green & Bruckner 2000, Sutherland et al. 2004). Knowledge of the causes and resulting distributions of coral diseases is fundamental for the determination of their potential impact on coral communities. In this study I report the occurrence, prevalence, and distribution of coral diseases in the Bahamas' Exuma Cays near Lee Stocking Island (LSI). Furthermore, I discuss the roles of three potential factors influencing coral diseases in this region: (1) temperature, (2) sedimentation, and (3) coral colony size.

Lee Stocking Island is relatively pristine, with little local environmental stress from, for example, sewage discharge, pesticide pollution, or river runoff (Dennis and Wicklund 1993). The area is geographically isolated from human population centers, the nearest being Georgetown, Great Exuma (pop. ~1100) approximately 45 km to the southeast. This region receives little commercial or sport fishing, and a voluntary no-take

zone surrounds LSI. These factors make it an ideal region to study coral diseases with negligible human influence.

To date little is known about environmental factors or conditions that promote pathogenic infections of corals. While previous and ongoing studies, including this one, provide data on the distribution and abundance of diseases, few studies have addressed the factors that may drive these distributions. Global climate change (including increased sea surface temperature) and increased sedimentation have been suggested, among other factors, as potential causes of the increase in coral disease incidence (Harvell et al. 1999, Green & Bruckner 2000, Rosenberg & Ben-Haim 2002). In addition to environmentally induced coral bleaching (Brown 1997, Fitt et al. 2001), black band disease (BBD) (Edmunds 1991, Kuta & Richardson 1996, Bruckner et al. 1997), white plague (WP) (Richardson et al. 1998), aspergillosis (Alker et al. 2001), bacterial bleaching (Rosenberg & Ben-Haim 2002), and dark spots syndrome (DS) (Gil-Agudelo & Garzon-Ferreira 2001) have been associated with relatively higher water temperature. One quantitative study (Kaczmarsky et al. 2005) has shown a correlation between BBD and proximity to sewage outflow.

Coral colony size may also potentially influence the occurrence and severity of coral diseases. Water-borne pathogens, or other infectious vectors, have an increased probability of coming into contact with colonies of greater surface area. Larger colonies are also likely to be older than their smaller neighbors, and may have higher accumulated exposure to pathogens over time. This relationship may transcend a simple correlation with surface area. For example, it has been hypothesized that smaller coral colonies may allocate more resources toward defense mechanisms rather than reproduction due to an

increased threat of total mortality (Kim & Havell 2002). It is also possible that relatively larger colonies achieve their size through natural resistance to disease.

METHODS

Surveys

I quantitatively surveyed the same 10 reef sites at LSI in June-July of both 2002 and 2003 (Figure 1). Five of these reefs were located at 8-20 m depths on the northern side of the Exuma Chain, facing the Exuma Sound. The other five were located in shallower (2–8 m) more protected areas. Three haphazardly located surveys were conducted at each reef, totaling 9,420 m^2 in 30 surveys during 2002, and 10,362 m^2 in 33 surveys in 2003. In 2003, one additional survey was conducted at Horseshoe, North Perry, and South Perry for a total of four surveys on these three reefs. The surveys were conducted within circular sites (20 m diameter) using five 2 m wide concentric radial transects following the method of Edmunds (1991). Within each survey every scleractinian coral colony was identified to species and measured (maximum diameter) to the nearest 5 cm. Infected colonies were tagged and disease type and severity (estimated percent tissue loss by class: 1-10%, 11-20%, etc. to 91-100%) were recorded. The presence of disease was designated as disease occurrence, since it was unknown if a diseased colony represented a new case of disease in the population (defined as incidence). Disease prevalence was calculated as the proportion of infected colonies (percentage) both for all species and individually for susceptible species. Disease recurrence from 2002 to 2003 was assessed by examining previously diseased and tagged

colonies. It is not know whether recurrence was due to a new infection or reemergence of the same infection.

Sediment and temperature data

PVC pipe sediment traps (5 x 30 cm) secured to a steel stake at the center of each survey site were used to collect sediments over four consecutive one week intervals (with accumulated sediments collected at the end of each week) from June 22 to July 20, 2002. Sediment samples were dried at 60°C until constant weights were recorded. Temperature was recorded using Stowaway TidbiT® temperature loggers (resolution = 0.1°C, calibrated by the manufacturer) placed at Horseshoe (8 m) and North Perry (22 m) reefs (Figure 1).

Statistical analyses

Mean disease prevalence values for pooled species in each year were ArcSine transformed for normality and compared using a T-test. Distributions of BBD, WP, and DS were tested against the Poisson distribution using the goodness-of-fit G-test (with 5 levels: 0, 1, 2, 3, 4 or more infections per site) to determine if diseases were clumped or randomly distributed. Within each species the mean size of infected individuals was compared to the mean size of healthy, non-infected individuals using the nonparametric Mann-Whitney U-test. For BBD and WP, coral species were pooled and colony size was tested for correlation with disease severity class (categorical) using Spearman rank correlation analysis. Only two cases of DS with quantifiable tissue degradation were observed. Therefore this disease was omitted from the severity-size regression analysis. Sedimentation rates were compared using the Mann-Whitney U-test. All statistical procedures were conducted using SAS 9.0.

RESULTS

The overall occurrence, prevalence, and recurrence of coral diseases on reefs near LSI in 2002 and 2003 are given in Table 1. This table presents overall values within the total coral population, with n = 11,092 colonies in 2002 and 13,973 colonies in 2003 examined. From 2002 to 2003 I observed an increase in BBD prevalence, a decrease in DS prevalence, and no change in WP prevalence. There was no significant change over the 2 year period when considering all 3 diseases together. Disease recurrence (same colony) in 2003 was recorded in 13 of 17 (76%) colonies previously infected (2002) with BBD and in 11 of 38 (29%) colonies previously infected with DS. WP infections did not recur.

BBD was observed on 3 coral species in 2002 (*Colpophyllia natans, Siderastrea siderea,* and *Montastraea annularis*) and 4 species in 2003 (also *Diploria strigosa*). While the greatest number of infections (15 of 1335 colonies in 2002, 35 of 1924 colonies in 2003) was found on *S. siderea* (Table 2), these values were < 2% of the *S. siderea* population. The *C. natans* population showed the greatest percentage of infected colonies (1.4%, or 1 of 71 colonies in 2002, and 7.6%, or 6 of 79 colonies in 2003; Table 2, Figure 2A). For both species, disease prevalence increased from 2002 to 2003 (Figure 2A). BBD was present at extremely low values for *D. strigosa* and *M. annularis* (prevalence < 0.15 % for each species). WP was observed on 4 species in both 2002 and 2003. For 2002 and 2003 there were, respectively, 10 and 13 infected colonies of 374 and 477 total *Dichocoenia stokesi*; 2 and 1 infected colonies of 793 and 802 total *Montastraea cavernosa*; 3 and 6 infected colonies of 1685 and 1787 total *M. annularis*; and 3 and 4 infected colonies of 1335 and 1924 total *S. siderea* [Table 2]. WP was most

prevalent among the *D. stokesi* population (Figure 2B), exhibiting prevalence values of 2.7% for both years.

The species *S. siderea* was most susceptible to coral diseases in general, with colonies exhibiting signs of DS as well as both WP and BBD (Table 2). This is the only species that was observed to be infected with DS (38 of 1335 colonies in 2002, and 17 of 1924 colonies in 2003, for prevalence values of 2.8 and 0.88% respectively). When summing all three diseases, *S. siderea* exhibited an overall disease prevalence of 4.2 and 2.9% in 2002 and 2003.

Coral colony abundances (summed) for the 6 species observed to be infected with disease was 4811 in 2002 and 5799 in 2003 (Table 2). The total reef areas surveyed each year varied (30 sites, or 9,420 m^2, in 2002 and 33 sites, or 10,362 m^2, in 2003). The overall density of locally susceptible colonies was 160 per site in 2002 and 176 in 2003. While the overall abundance of the susceptible species (6 out of 31 species present) represented 43% percent of total colonies in 2002 and 42 % in 2003, observed disease prevalence was only 1.5 % of the susceptible populations in both 2002 and 2003. The observed disease prevalence for the entire coral population (susceptible and non-susceptible species) was 0.66 % in 2002 and 0.61 % in2003. (Table 1)

The pattern of disease distribution in terms of disease occurrence per reef varied per disease (Table 3). In 2002, G-tests of observed site frequencies against those predicted by the Poisson distribution indicated a clumped distribution for WP ($G = 10.51$ $df = 4$, $p < 0.05$) and DS ($G = 12.47$, $df = 4$, $p < 0.05$). The distribution of BBD was not significantly different from the Poisson distribution ($G = 1.69$, $df = 4$, $p > 0.05$). However in 2003 disease distributions shifted. Both BBD ($G = 20.52$, $df = 4$, $p<0.001$)

and WP ($G = 16.20$ df = 4, $p<0.01$) exhibited clumped distributions while DS was randomly distributed ($G = 1.20$ df = 4, $p>0.05$).

Spearman rank correlation analyses indicated that disease severity (% of tissue lost) was negatively correlated with colony size for both BBD ($\rho = 0.34$, $p < 0.01$) and WP ($\rho = 0.47$, $p < 0.01$; Figure 3). In all but two cases tissue loss was not observed with DS. Therefore severity measures were not recorded.

Within populations of individual coral species infected colonies did not differ in size from healthy individuals ($p > 0.05$ for all species; data not shown). However, when comparing different coral species there was a distinctive general trend in which the relatively larger species were observed to be susceptible to disease (Figure 4). Only one relatively smaller species, *Dichocoenia stokesi*, was observed with disease (WP).

During the survey period in 2003 water temperatures on the outer reefs dropped from 28°C to below 27.5°C from June 4 to June 14 as a result of a weak cold front (Figure 5). During this period BBD infections became visually undetectable and coral tissue loss ceased on all *C. natans* colonies that had been infected (n = 6). Similar cessation of disease signs occurred in 7 out of 35 BBD infected *S. siderea* colonies. As temperatures increased following June 14, BBD infections began to reappear on the previously infected colonies. By June 24, all colonies, aside from one *S. siderea* colony exhibited BBD in the same areas that had been previously infected on each colony.. The BBD-free colony did not exhibit signs of BBD for the remainder of the study.

Sedimentation rates recorded in 2002 were significantly higher (Mann-Whitney U = 109, $p < 0.05$) on sites in which BBD was observed (n = 21, median = 0.13 g cm^{-2} wk^{-1}) than on sites with no signs of disease (n = 9, median = 0.06 g cm^{-2} wk^{-1}).

DISCUSSION

Only one study (Weil et al. 2002) has been conducted in the Caribbean that reports disease prevalence for all scleractinian coral species. The overall (total) disease prevalence values I report for LSI are low compared to the prevalence values observed by Weil et al. (2002). While I recorded total disease prevalence values of 0.66% and 0.61% in 2002 and 2003, respectively, (Table 1) Weil et al. (2002) observed prevalence values ranging from 0.07% to 9.78% throughout the Caribbean, with a mean total disease prevalence of 3.02%.

Overall BBD prevalence values near LSI were similar to those reported in most previous BBD surveys, which are generally less than 1% (Edmunds 1991, Kuta & Richardson 1996, Weil et al. 2002). In contrast, Bruckner et al. (1997) observed BBD prevalence of over 7% in an outbreak on reefs of Jamaica. The approximate doubling in BBD prevalence near LSI from 2002 to 2003 warrants continued monitoring to document whether BBDoccurrence is progressing to an outbreak, i.e. a large number of cases of disease in a short period of time, in this region.

The recurrence of BBD infections on individual colonies suggests that bacteria associated with the infection may remain on or near the colony in a nonpathogenic state during the colder months of the year. BBD reservoirs have been observed as non-pathogenic biofilms in sediment pockets on apparently healthy coral colonies of BBD susceptible species (Richardson 1997). On reefs of the Florida Keys, individual corals with BBD often exhibit BBD signs at the same site on the colony during the warm summer months of each year (pers. obs).

The WP prevalence values I report did not differ from 2002 to 2003 and are comparable to Weil et al. (2002), who observed prevalence values ranging from 0% to 3.7% (mean = 0.77%). During outbreaks of WP, prevalence has been reported as high as 33% among susceptible species (Richardson et al. 1998). The lack of observed recurrence of WP may be related to its rapid progression, up to 2 cm d^{-1}, across colonies (Richardson et al. 1998), which often results in the complete mortality of the infected colony. In this study, six of the white plague infected colonies at LSI were completely denuded by the end of July, 2002.

While I measured DS prevalence values of 0.34% and 0.12% in 2002 and 2003 (Table 1), Gil-Agudelo & Garzon-Ferreira (2001) and Garzon-Ferreira and Gil (1998) reported prevalence values of 16.45% and 2.85% on reefs off the Columbian coast and four southwestern Caribbean atolls, respectively. Additionally, the DS infections I observed were found exclusively on *Siderastrea siderea.* Of the 1,545 DS-infected colonies observed on Colombian coastal reefs, 62.9% were *M. annularis* and 31.2% were *S. siderea*, with the remainder present on other *Montastraea* spp. and *Stephanocoenia intercepta* (Gil-Agudelo and Garzon-Ferreira 2001). Thus far no pathogen has been identified for DS. Tissue loss has been documented at rates of 3.99 cm/month (Cervino et al. 2001).

I found that disease distributions were variable in time. While both WP and DS demonstrated clumped distributions in 2002, BBD and WP were clumped in 2003. Clumped distributions are consistent with an infectious model of disease transmission. Previous studies have shown clumped distributions for both WP (Richardson et al. 1998, Borger 2003) and DS (Gil-Agudelo & Garzón-Ferreira 2001). One study of WP reported

that disease distribution was neither clumped nor density dependent (Nugues 2002). BBD has been observed in clumped distributions in numerous studies (Edmunds 1991, Kuta and Richardson 1996, Bruckner and Brucker 1997, Borger 2003). At this time, the mechanisms of disease transmission and infection are not known for any of these diseases.

It has been proposed that increased sea water temperature associated with global warming is a direct and important factor in the overall increase in coral disease incidence (Rosenberg and Ben-Haim 2002). Several studies have shown that the pathogens for gorgonian aspergillosis (Alker et al. 2001), bacterial bleaching (Banin et al. 2001), WP (Remily 2004), and a cyanobacterial member of the BBD consortium (Richardson and Kuta 2003) grow optimally at or above 30°C. In addition, Fitt et al. (2001) demonstrated that corals become physiologically stressed above 30°C, potentially causing an increased susceptibility to infections. Such data may explain, at least in part, why coral diseases are generally most active during warmer months of the year.

The observations of BBD cessation on corals when temperatures dropped below 27.5°C, and its subsequent return as temperatures warmed, suggest that temperature tightly controls disease activity. This observation is in agreement with those of Rützler et al. (1983) who documented BBD activity as limited to water temperature at and above 28°C.

Increased sedimentation rates on sites with disease as compared to healthy sites indicates that sediments may play a role in coral infections. Sediments are capable of harming corals in myriad processes. Abrasion and/or smothering directly damage coral tissues. Indirect stresses to corals include decreased light availability, reducing

photosynthetic rates of zooxanthellae, and increased mucus production, requiring additional energy expenditures by the coral (Riegl & Branch 1995). Damage and stress to corals may make them more susceptible to infections by microbial pathogens. Furthermore, sediments may act as vectors of coral disease. In addition to its potential role in disease, increased sedimentation has been shown to reduce reproductive success in corals (Gilmour 1999), exacerbating its effect on coral reef health. I stress, however, that these results consist of short-term data and that experimental studies are needed to definitively assess the role of sedimentation in coral diseases.

Although within species there was no statistical difference in the sizes of infected and healthy individuals, coral diseases primarily infected those species with larger mean colony sizes: *Colpophyllia natans, Diploria strigosa, Montastraea annularis, M. cavernosa,* and *Siderastrea siderea*. This pattern has been reported in other regions for a number of coral diseases including WP (Borger 2003, Nugues 2002), BBD (Borger 2003), DS (Borger 2003), and gorgonian aspergillosis (Kim & Harvell 2002). An exception appears to be *Dichocoenia stokesi*, a relatively small coral species which has been reported as most susceptible to WP in this study, and was also reported as such by Richardson et al. (1998).

It is important to note that most of the coral species that were not diseased at LSI have been found to be susceptible to diseases in other areas of the Caribbean (Sutherland et al. 2004, Weil 2004). It is unknown whether disease occurrence per species for multi-host coral diseases is correlated with the overall prevalence of disease in the population, or whether the individual susceptibility of coral colonies controls coral disease patterns.

Disease severity (percent of coral tissue lost) was negatively correlated with colony size for both BBD and WP. A similar negative correlation between severity and large size has been observed previously in WP (Nugues 2002). This may be etiologically significant in that while relatively large, ecologically important species (and colonies) may be at an increased risk of infection, they may also have a greater chance of recovering and surviving. Small colonies,on the other hand, succumb to disease more quickly given constant tissue degradation rates. This could be particularly important for diseases with seasonal patterns of occurrence. While large colonies may not experience total mortality, they can potentially act as reservoirs for coral pathogens and thus promote disease recurrence. Furthermore, due to variation in reproductive capabilities and stress resistance among size classes within a population, partial mortality in large colonies and total mortality in small colonies may have effects beyond quantifiable tissue loss. As coral reef environmental quality is reduced, we are likely to see greater impacts on recruits and a resultant loss of the smallest size classes of coral colonies in a population (Bak & Meesters 1999). Such an event would exacerbate subsequent detrimental effects by limiting the recovery of the population in the future, particularly if recruitment is limited.

The roles of global environmental change and local anthropogenic effects in coral disease distributions remain unclear. However, this and other studies provide mounting evidence that environmental drivers, including temperature and sedimentation, and coral community characteristics (e.g. locally dominant species) may both impact coral diseases and reef health. Identification of factors influencing coral diseases and coral degradation

is important for understanding the decline of coral reefs worldwide and developing viable strategies for the management of this important resource.

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Table 1. Occurrence, prevalence, and recurrence (percentage of colonies previously infected in brackets) of coral diseases for all coral species observed near LSI in 2002 (area surveyed = 9,425 m^2 ; n = 11,092 coral colonies) and 2003 (area surveyed = 10,362 m^2; n = 13,973 colonies). Asterisks indicate significant differences (T-test with ArcSine transformed values) in mean prevalence values between years ($\alpha = 0.05$).

	2002		2003		
Disease	Occurrence	Prevalence (%)	Occurrence	Prevalence (%)	Recurrence
Black band	17	0.15*	44	0.31*	13 (76)
White plague	18	0.16	24	0.17	0(0)
Dark spots	38	0.34 *	17	0.12*	11(29)
All diseases	73	0.66	85	0.61	24(33)

Table 2. Disease occurrence, prevalence, and abundance of diseased corals near LSI in 2002 and 2003. Prevalence values (in percent) are under number of observed colonies with each disease.

	2002				2003			
Coral species	No. of colonies	BBD	WP	DS	No. of colonies	BBD	WP	DS
Colpophyllia Natans	71	1 (1.4)	0	0	79	6 (7.6)	0	0
Dichocoenia Stokesi	374	0	10 (2.7)	0	477	0	13 (2.7)	0
Diploria Strigosa	553	0	0	0	730	1 (0.14)	0	0
Montastraea Cavernosa	793	0	2 (0.25)	0	802	0	1 (0.12)	0
M. annularis	1685	1 (0.06)	3 (0.18)	0	1787	2 (0.11)	6 (0.33)	0
Siderastrea siderea	1335	15 (1.1)	3 (0.2)	38 (2.8)	1924	35 (1.8)	4 (0.20)	17 (0.88)
Totals	4811	17 (0.35)	18 (0.37)	38 (0.79)	5799	44 (0.76)	24 (0.41)	17 (0.29)
Total diseases (6 coral spp)			73 (1.5)				85 (1.5)	

Table 3. Number of diseased colonies (all species) observed at each reef (pooled survey sites) near LSI in 2002 and 2003. Clumped distributions were present for WP and DS in 2002 ($p < 0.05$) and BBD and WP in 2003 ($p < 0.01$). BBD was random in 2002 and DS was random in 2003. (See text for statistics)

		2002			2003		
Site	Map Icon	BBD	WP	DS	BBD	WP	DS
Goby Spot	A	2	4	1	1	8	1
Rainbow Gardens	B	1	0	6	3	0	4
North Norman's	C	1	0	6	2	3	3
White Horse	D	3	3	7	4	4	3
North Perry	E	2	4	5	6	0	2
South Perry	F	1	4	8	5	1	2
Horseshoe	G	4	2	3	14	6	2
Horseshoe Shallow	G	2	0	2	9	0	1
Tug and Barge	H	0	0	0	0	2	0
Square Rock	I	1	1	0	0	0	1

FIGURE LEGENDS

Figure 1. Locations of study reefs near Lee Stocking Island, Bahamas. At each reef site, represented by labeled crosses, 3-4 surveys were conducted. A = Goby Spot, B = Rainbow Gardens, C = North Norman's, D = White Horse, E = North Perry, F = South Perry, G = Horseshoe and Horseshoe Shallow, H = Tug and Barge, I = Square Rock. Note that two of the study reefs (Horseshoe and Horseshoe Shallow) are depicted as "G" on this diagram.

Figure 2. Percent of colonies infected with black band disease (A) and white plague (B) in 2002 and 2003. Note different coral species and scales for each graph.

Figure 3. Colony size (maximum diameter, cm) vs. severity (percent tissue loss) for black band disease (A) and white plague (B). Spearman Rank correlation analysis (ρ), asterisk indicates $p < 0.01$. Note different scales for each graph.

Figure 4. Mean colony size (maximum diameter, cm) for all coral species in all surveys. Occurrence of diseases within a species is noted by BB (black band), WP (white plague), and/or DS (dark spots). Asterisks indicate species observed with disease near LSI but outside survey areas.

Figure 5. Mean daily temperature (°C) for two outer reefs near Lee Stocking Island and associated black band disease cessation event in 2003. BBD monitoring began on June 1, 2003.

Figure 1

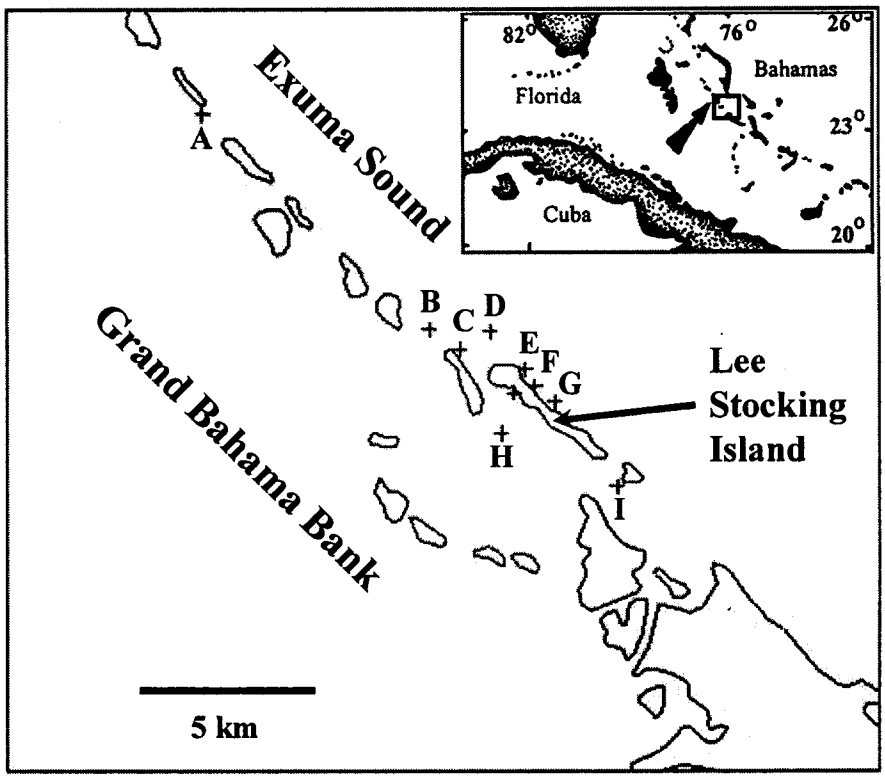
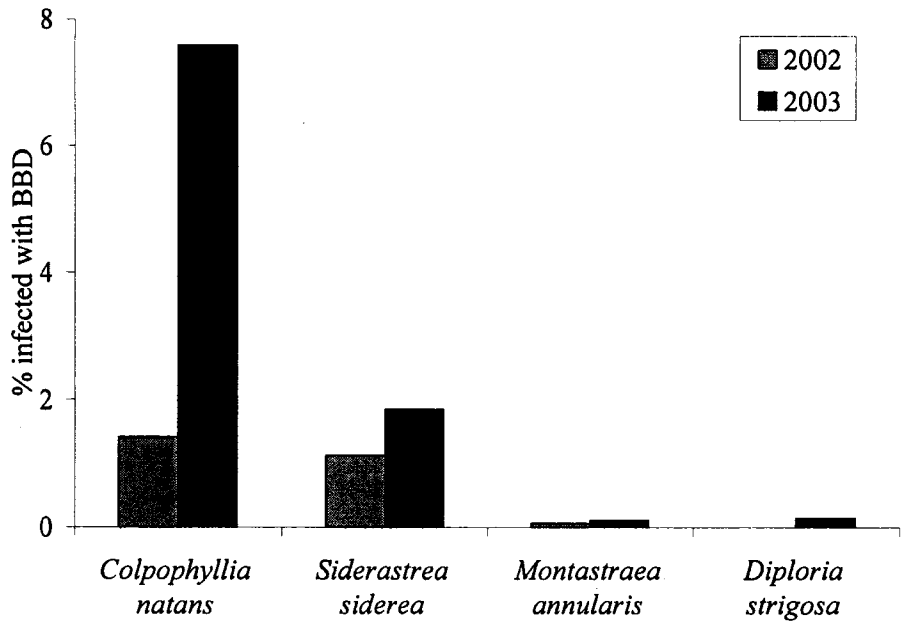


Figure 2

A



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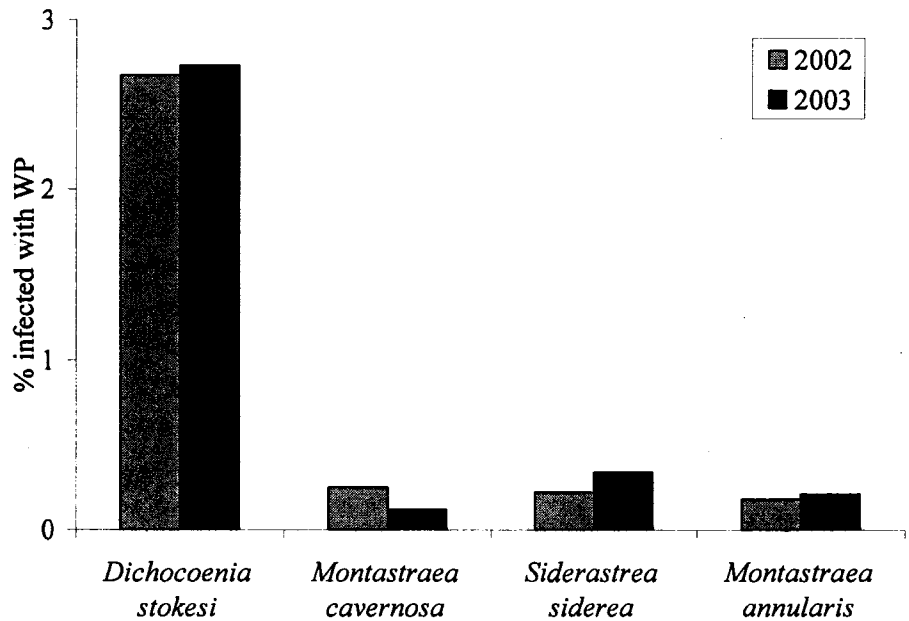
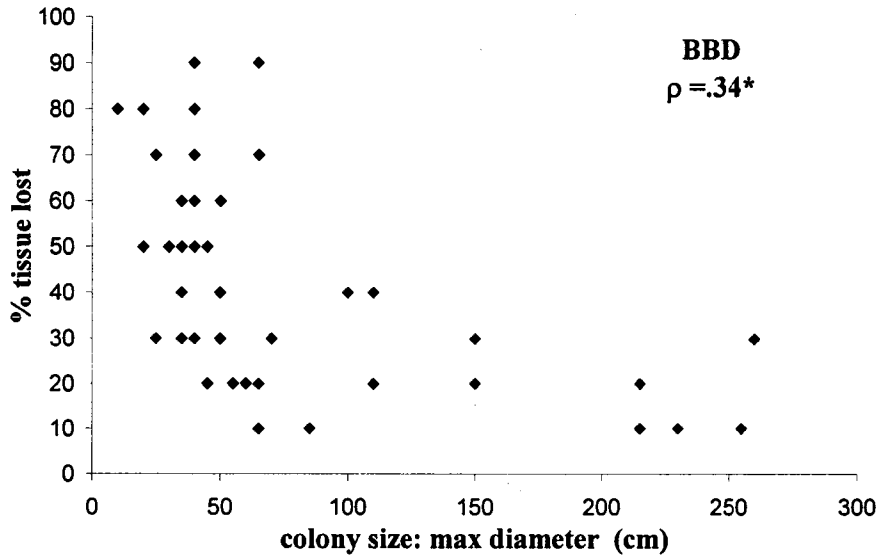


Figure 3

A



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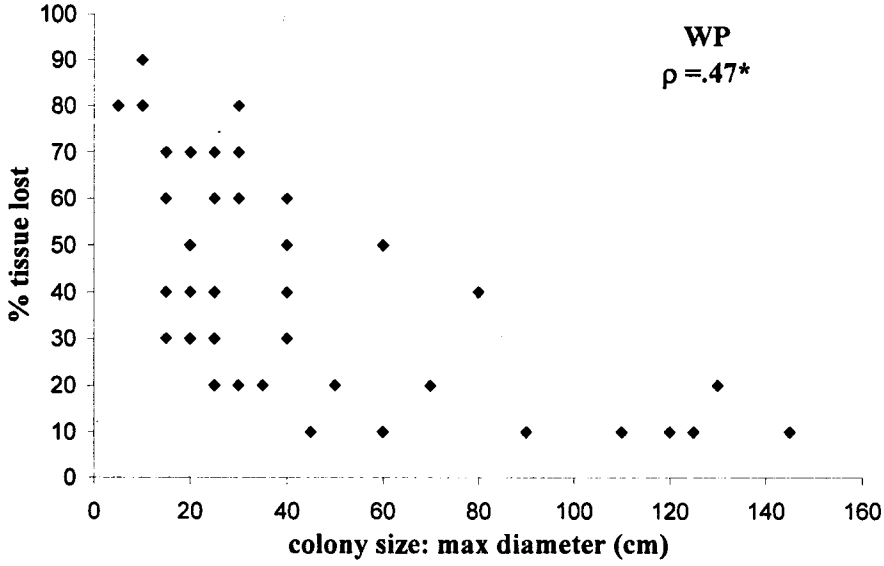


Figure 4

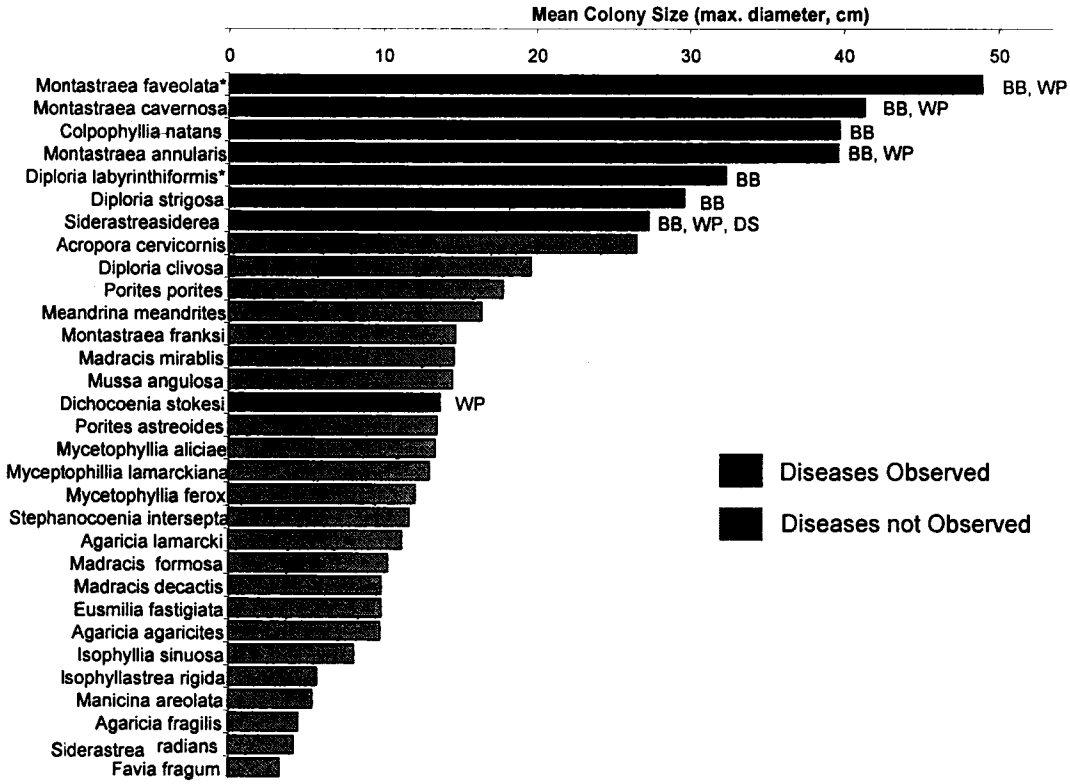
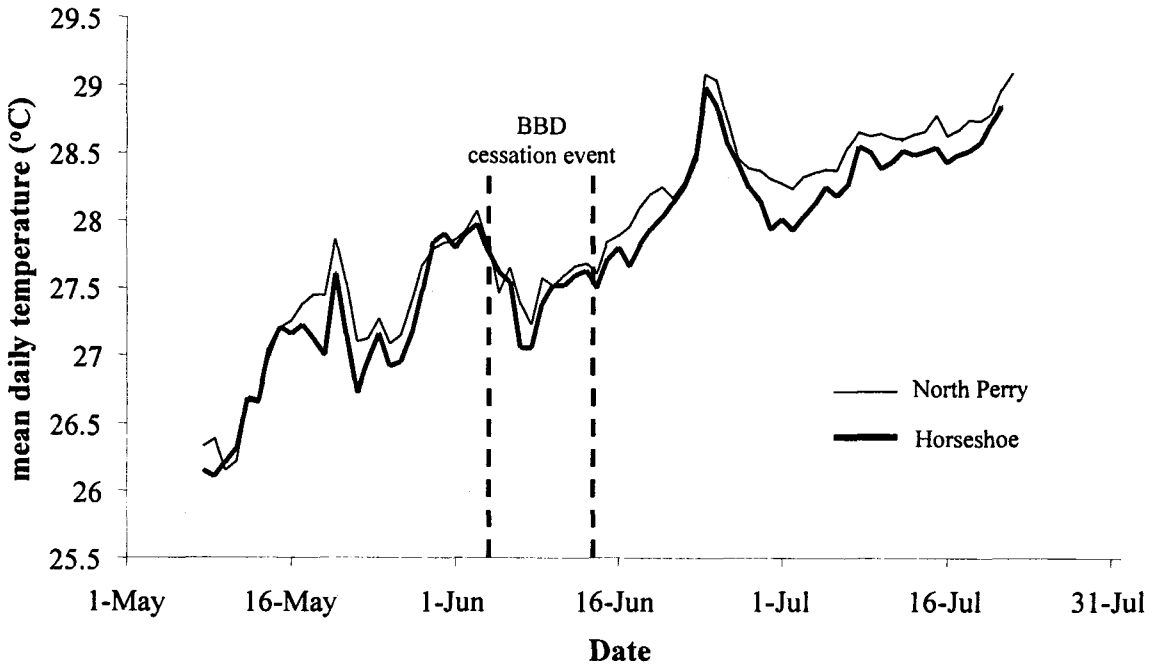


Figure 5



CHAPTER 3

CORAL DISEASE DYNAMICS AND NUTRIENT AVAILABILITY ON REEFS OF THE NORTHERN FLORIDA KEYS AND LEE STOCKING ISLAND, BAHAMAS

ABSTRACT

Coral reefs in the northern Florida Keys (NFK) and those near Lee Stocking Island (LSI) in the Bahamas' Exuma Chain, were quantitatively surveyed in June-August of both 2002 and 2004 to assess the relationship between coral disease and ambient nutrients. Coral disease dynamics, including occurrence, prevalence, and spatial distributions, were recorded for black band disease (BBD), dark spots syndrome (DSS), white plague type II (WP), and yellow band disease (YBD). Total disease prevalence was greater in the NFK than LSI in both 2002 and 2004, while overall nutrient concentrations were similar in both regions. Although we observed variability in host species susceptibility across space and time, massive framework scleractinians, including *Montastraea annularis* and *Siderastrea siderea*, along with relatively smaller *Meandrina meandrites* and *Dichocoenia stokesi*, were most susceptible to disease. DSS lesions were found exclusively on *S. siderea* in both regions. Based on correlations among measured nutrients (dissolved inorganic nitrogen, nitrate, nitrite, ammonium, and soluble reactive phosphate), dissolved inorganic nitrogen (DIN) and total phosphorous (TP) were selected as proxy variables for nutrient availability. Regression analyses indicated significant positive correlations between DIN and BBD prevalence in both the NFK and LSI, and

negative correlation with TP near LSI. This study is one of the few that has quantitatively addressed the potential difference in coral diseases between human-impacted (NFK) and relatively pristine (LSI) reef systems.

INTRODUCTION

Coral reefs are experiencing declines in coral cover and diversity on a global basis (Hoegh-Guldberg 1999, Hughes et al. 2003, Pandolfi et al. 2003), and recently there has been much interest concerning the role of human impacts in this decline (Harvell et al. 1999). One aspect of particular interest is the potential relationship between anthropogenic influence, especially nutrients, and coral disease. Recently both coral diseases (Rosenberg & Loya 2004) and nutrient input (Szmant 2002) have been recognized as contributors to coral mortality and phase shifts in coral communities. For example, white band disease was reported to have instigated a phase shift from acroporid to small encrusting corals (Aronson & Precht 2001, Rosenberg & Loya 2004), and a phase shift from a coral to macroalgal dominated community was attributed directly to human-based nutrient input in Kaneohe Bay, Hawaii (Smith et al. 1981). .

Several studies have suggested links between anthropogenic activities and coral disease. Green and Bruckner (2000) conducted a meta-analysis to examine the relationship between coral disease occurrence at locations throughout the Caribbean and perceived relative risk, which was assessed by four potential anthropogenic threats: coastal development, marine-based pollution, land-based pollution, and over fishing (Bryant et al. 1998). They found that at least 97% of locations with reports of coral disease were "high" or "medium" risk. Similarly, but on a smaller spatial scale, a

gradient of anthropogenic influence was correlated with the prevalence of two coral diseases (tumors and *Porites* ulcerative white spots syndrome) in the Philippines (Kaczmarsky 2006). Initial outbreaks of coral diseases have been reported near sewage outflows or other areas with increased pollution in several areas of the Caribbean (Antonius 1988, Bruckner & Bruckner 1997, Goreau et al. 1998). One study provides quantitative evidence that black band disease (BBD) and white plague type 2 (WP) were more prevalent at a sewage-impacted site as compared to an ecologically similar non-impacted site in St. Croix, USVI (Kaczmarsky et al. 2005).

Some researchers have suggested that sewage input may facilitate the transmission of disease-associated bacteria to the reef (Frias-Lopez et al. 2002, Patterson et al. 2002). Another possibility is that increased nutrient concentrations, associated with human influence, may affect coral disease dynamics. There is evidence that increased nutrient availability can exacerbate disease severity of aspergillosis (Kim & Harvell 2002, Bruno et al. 2003), BBD (Voss & Richardson in press), and yellow band disease, (Bruno et al. 2003). Additional evidence has shown that the presence of infection can be correlated with increased nutrients. Kuta and Richardson (2002) reported higher levels of nitrite, and lower levels of dissolved inorganic phosphate near black band-infected corals compared to healthy but BBD-susceptible colonies in the northern Florida Keys. While these studies demonstrate a relationship between elevated nutrients and disease severity and occurrence, there are no published quantititative studies regarding nutrient concentration and disease prevalence (i.e. percent of corals infected) within coral populations.

This study examines coral disease dynamics in the Northern Florida Keys (NFK) reef tract and near Lee Stocking Island (LSI) in the Bahamas' Exuma Chain. Coral reefs near Key Largo in the NFK are easily accessible to boating enthusiasts, divers, sport and commercial fishermen, and researchers. Coral diseases in the NFK have been well documented and monitored over the past decade in the Coral Reef Evaluation and Monitoring Project coordinated by the EPA, NOAA, and FWRI (Porter et al. 1999, Porter et al. 2001), and by individual research groups (Dustan 1977, Dustan & Halas 1987, Kuta & Richardson 1996, Kim & Harvell 2002). In addition, the Southeast Environmental Research Center Water Quality Monitoring Network (SERC-WDMN) at Florida International University regularly monitors and disseminates nutrient data for many reefs in the Florida Keys (see methods). In contrast to the Florida Keys, coral reefs near LSI are relatively pristine and geographically isolated from human population centers. Little commercial or sport fishing takes place since a no-take zone surrounds LSI. There is no environmental stress from sewage discharge, pesticide pollution, or river runoff (Dennis & Wicklund 1993).

The purpose of this study was to determine scleractinian coral disease incidence, prevalence, and spatial distribution from surveys conducted in both the NFK and LSI. Furthermore, the research presented here assessed the potential influence of nutrient availability on coral disease prevalence in these two regions.

METHODS

Field surveys

In the NFK 7536 m^2 on 8 reefs were quantitatively surveyed during Aug 5-21, 2002, and 6594 m^2 on 14 reefs during June 14-27, 2004. Similarly, at LSI 9420 m^2 were surveyed on 10 reefs from June 29-July 26, 2002, and 9734 m^2 on 11 reefs from July 16-Aug 11. Reef locations are shown in Figure 1; the number of surveys conducted at each reef site are in Table 1.

Each survey was conducted within a haphazardly selected 20 m diameter circle using five 2 m wide concentric radial transects following the method of Edmunds (1991). Within each survey every scleractinian coral colony was identified to species. Infected colonies were tagged and monitored using digital photography. Disease prevalence was calculated as the proportion of infected colonies to total colonies (all species). Additional prevalence values were also calculated for individual locally susceptible species.

Nutrient analysis

Four 60 ml water samples were collected in the center of each site approximate 10 cm from the substrate using sterile plastic syringes, which were placed on ice upon return to the boat for transport. Immediately upon return to shore, two samples were filtered (0.45 μm GF/F filters) while the other two remained unfiltered. All samples were frozen (-40°C) for transport to Florida International University and storage (-20°C). The filtered samples were analyzed for nitrate, nitrite, ammonium, dissolved inorganic nitrogen ($NO_x^- + NH_4^+$), and soluble reactive phosphorus (SRP) using an ALPKEM Rapid Flow Analysis 300 (Alpkem Corp., Clackmas, OR). The unfiltered samples were analyzed for total phosphorus. All water samples were analyzed by the Southeast

Environmental Research Center (SERC, see Boyer 2005 for details of nutrient analysis). Additional nutrient data were provided from water samples collected during summers (July-September) in 2000-2004 as part of the SERC Water Quality Monitoring Network (WDMN). Six sites along the NFK reef tract,that were sampled by SERC correspond to sites in this study: Carysfort, Conch, Davis, Molasses, Grecian Rocks, and White Banks.

Statistically analyses

The distributions of BBD, WP, and DSS within each year and region were tested against the Poisson distribution using the goodness-of-fit G-test to determine if disease incidence was clumped or randomly distributed. In analysis of the 2004 data in the NFK, disease occurrence data were transformed to frequency of diseased corals per 314 m^2 in each site to control for variable sampling efforts at different reefs . T-tests with Bonferroni adjusted α-values were used to compare disease prevalence (arcsin transformed) between regions and years. Nutrient concentration data (and DIN:TP ratio) within each region were tested for correlations among one another. Based on the relationships observed in the correlation analysis (see results section) DIN and TP were chosen as proxy variables to represent nitrogenous and phosphorus compounds, respectively. These variables were then tested for potential relationships with disease prevalence in each region using multiple regression analysis. YBD was not included in statistical analyses since only two infections were observed. All statistical analysis was conducted using SPSS 13.0.

RESULTS

Coral disease occurrence and prevalence over time

In both the NFK and LSI, rates of infection were variable between 2002 and 2004 (Table 2). BBD was absent on reefs of the NFK in 2002. However, in the 2004 surveys 18 of 4088 colonies (0.44%) were infected with this disease. Conversely, 17 WP infections (0.46%) were recorded in 2002, but this disease was not observed on any NFK reefs in 2004. DSS prevalence in the NFK increased from 0.35% in 2002 to 1.05% in 2004 ($t = 3.77$, $p < 0.01$). YBD occurred at very low prevalence (0.05%) in the NFK in 2002 but was not observed in 2004. On LSI reefs BBD increased from 0.15% in 2002 to 0.35% in 2004 ($t = 4.20$, $p < 0.01$), while WP increased from 0.16% to 0.22% ($t = 2.64$, $p < 0.01$). The combined increases in these two diseases led to an overall increase in total disease prevalence ($t = 3.59$, $p < 0.01$). DSS prevalence did not differ between years near LSI.

Comparisons between regions

BBD prevalence was greater near LSI in 2002 ($t = 3.27$, $p < 0.02$) as this disease was absent in the NFK (Table 2). WP prevalence was higher in the NFK than LSI in 2002 ($t = 2.98$, $p < 0.01$). While DSS prevalence did not differ between the two regions in 2002, DSS was three times more prevalent in the NFK (1.05%) in 2004 compared to LSI (0.35%, $t = 4.56$, $p < 0.01$). In 2004 BBD prevalence did not differ between LSI and the NFK. WP was not observed in the NFK during the 2004 surveys, but DSS prevalence was significantly greater than observed in LSI ($t = 4.13$, $p < 0.01$). Total disease prevalence (sum of all diseases) was significantly greater in the NFK in 2002 ($t = 3.10$, p

< 0.02) and 2004 (2004: $t = 4.12$, $p < 0.02$). YBD was not observed in LSI in either 2002 or 2004.

In addition to differences in disease prevalence between the NFK and LSI, I observed variation in the species most commonly infected in each region (Table 3). In total, 12 of the 36 coral species observed in the surveys were susceptible to at least one coral disease. In the NFK, BBD affected primarily *Meandrina meandrites* and *Montastraea annularis*, while at LSI *Siderastrea siderea* was the species most often observed with BBD, followed by *Dichocoenia stokesi*. In the NFK *M. annularis*, *Porites astreoides*, and *S. siderea* were the species most commonly infected with WP while in LSI *D. stokesi* and *M. faveolata* were more often observed with this disease. Additionally, in both regions DSS was only observed on *S. siderea*.

Spatial distributions

Goodness of fit g-tests of observed site frequencies (Table 1) against those predicted by the Poisson distribution indicated clumped distributions for BBD in both the NFK ($G = 12.64$, $df = 4$, $p < 0.02$) and LSI ($G = 12.46$, $df = 4$, $p < 0.02$) in 2004. BBD distribution near LSI in 2002 demonstrated no significant pattern. WP infections were clumped near LSI in 2002 ($G = 10.51$, $df = 4$, $p < 0.05$) and 2004 ($G = 12.65$, $df = 4$, $p < 0.02$). Likewise DSS exhibited clumped distributions in both regions in 2004 (NFK: $G = 18.52$, $df = 4$, $p < 0.01$; LSI: $G = 18.87$, $df = 4$, $p < 0.01$), but only near LSI in 2002 ($G = 12.47$, $df = 4$, $p < 0.05$). No significant spatial distribution was observed in WP or DSS during the 2002 surveys in the NFK.

Nutrients and disease prevalence

Nutrient concentrations in 2002 and 2004 were generally similar in both the NFK and LSI (Table 4). .The nutrient concentrations observed in this study during 2002 and 2004 in the NFK did not differ significantly from those reported by the SERC- WQMN from summer sampling in 2000-2004. Significant correlations between individual nitrogenous species and DIN in both the NFK and LSI (Table 5) supported the use DIN as a proxy for nitrogen availability. Similarly, total phosphorous (TP) was correlated with soluble reactive phosphate in both regions (Table 5), and was selected as a proxy for phosphorous compounds.

In the NFK multiple regression analysis indicated a significant model ($n = 19$, $R^2 = 0.438$, $F = 6.633$, $p < 0.01$; 2002 data omitted as no BBD was observed) in which DIN ($B = 0.661$, $t = 3.633$, $p < 0.01$) was a significant predictor of BBD prevalence. However TP was not a significant predictor of BBD prevalence in the NFK._Near LSI (Figure 2B) both DIN ($B = 0.384$, $t = 2.761$, $p < 0.01$) and TP ($B = -0.125$, $t = -2.471$, $p < 0.02$) were significant predictor variables in this model ($n = 61$, $R^2 = 0.342$, $F = 5.756$, $p < 0.005$)._Regression analysis did not indicate significant relationships between the nutrient proxy variables and the other observed coral diseases (i.e. WP and DSS).

DISCUSSION

This study demonstrated significant variation in total coral disease prevalence across space and time in the NFK and LSI. Weil et al. (2002), the only other study to provide total disease prevalence for all coral species, observed values ranging from 0.07% to 9.78% throughout the Caribbean and with a mean of 3.02%. By comparison, the total disease prevalence values in the NFK and LSI in 2002 and 2004 were generally

low, ranging from 0.66% to 1.47% (Table 2). Of the 36 coral species surveyed in this study, 12 were observed with signs of at least one coral malady. In both regions *Siderastrea siderea* was susceptible to multiple diseases and accounted for > 60% of the observed infections in each region during both 2002 and 2004.

Black band disease

The BBD prevalence values <1% observed near LSI in both year and in the NFK in 2004 were similar to values reported in previous surveys (Edmunds 1991, Kuta & Richardson 1996, Weil et al. 2002). However, over 7% has been reported in Jamaica (Bruckner et al. 1997). While BBD prevalence did not vary between NFK and LSI when BBD was observed (i.e. 2004), species susceptibility to BBD differed between these regions. *Meandrina meandrites* and *Montastraea annularis* were the most commonly infected of 9 susceptible species in the NFK. While BBD affected 7 coral species in LSI, 79% of BBD infections occurred on *Siderastrea siderea* colonies. Similar high infection rates were observed on this species in Dominica (Borger & Steiner 2005) and Jamaica (Bruckner et al. 1997). Although host susceptibility may vary regionally, massive corals, like *S. siderea*, are generally the most commonly infected (Edmunds 1991, Kuta & Richardson 1996, Bruckner & Bruckner 1997). The occurrence of BBD on *Dichocoenia stokesi* in LSI and the NFK does not fit this pattern and has previously been reported on only one colony in a Dominican reef (Borger 2003). The clumped distributions of BBD reported in this and previous accounts (Edmunds 1991, Kuta & Richardson 1996, Bruckner & Bruckner 1997, Borger 2003) are in accordance with an infectious disease transmission model.

In this study BBD was completely absent during the 2002 NFK surveys. Additional evidence of low BBD activity in South Florida reefs was observed by Borger (2005) who reported only one case of BBD in surveys conducted north of my study area in July, 2002. The occurrence of black band disease generally corresponds to temperatures above 29°C (Kuta & Richardson 1996). Water temperatures during the 2002 surveys averaged 28.2 °C and a temperature >29 °C was recorded at only one site, Molasses Reef, which may explain the absence of BBD that year.

White plague

WP prevalence was higher in NFK as compared to LSI in 2002 (Table 2). However, in 2004 this disease was not observed in the NFK. The overall prevalence values in both of these regions were similar to Caribbean wide prevalence values ranging from 0% to 3.7% , with a mean of 0.77% (Weil et al. 2002). During outbreaks of white plague, prevalence has been reported as high as 11% among all susceptible species (Nugues 2002), and up to 33% in *Dichococenia stokesi*, the most susceptible species in the NFK (Richardson et al. 1998). During the surveys conducted in 2002, *Montastraea annularis*, *Porites astreoides*, and *Siderastrea siderea* were the species most commonly infected with WP in the NFK (Table 3). Rapid progression of WP resulted in the complete mortality of 4 *D. stokesi* and 1 *Eusmilia fastigiata* colonies within three weeks at LSI during 2004. Although WP was most common on *D. stokesi* near LSI, no WP infections were observed on this species in the 2002 or 2004 NFK surveys (Table 3).

Dark spots syndrome

DSS prevalence values were similar in the NFK and LSI, with the exception of 1.05% prevalence in the 2004 NFK surveys. These values were low in comparison to

previous studies by Gil-Agudelo and Garzon-Ferreira (2001) and Garzon-Ferreira and Gil (1998) who reported prevalence values of 16.45% and 2.85% on reefs of the Columbian coast and four southwestern Caribbean atolls, respectively. The DSS we observed were found exclusively on *Siderastrea siderea* (Table 3), as were those observed in Dominica (Borger & Steiner 2005). Likewise, other surveys in LSI (Gochfeld et al. 2006, Voss and Richardson 2006) and South Florida (Borger 2005) have indicated that *S. siderea* is most susceptible to DSS in these regions. In contrast, on Columbian coastal reefs 62.9% of the DSS infections were found on *Montastraea annularis* and 31.2% were on *S. siderea*, with the remainder on other *Montastraea* spp. and *Stephanocoenia intersepta*.(Gil-Agudelo & Garzon-Ferreira 2001). Although tissue loss has been documented in DSS (Cervino et al. 2001), no pathogen has been identified. Based on monitoring of lesions over three years, Borger (2005a) concluded that DSS is most likely a general stress response rather than a pathogenic infection.

Nutrient concentrations and influence on disease prevalence

Concentrations of inorganic nitrogenous compounds and phosphorous were variable across reef sites in both the NFK and LSI, but mean concentrations were consistent between 2002 and 2004 in both regions (Table 4). The concentrations reported in this study were similar to those observed during summer sampling events at six sites along the NFK reef tract from 2000 to 2004. Multiple regression analysis indicated a significant positive relationship between DIN concentration and BBD prevalence in both the NFK and LSI. These results in conjunction with previously reported data, provide evidence that increased nitrogen availability not only enhances BBD severity (Voss and Richardson in press) but also influences the prevalence of BBD infections on a reef.

However, the models for both the NFK and LSI failed to accurately predict BBD prevalence at or near zero. DIN is naturally in marine systems and detectable at all of the reef sites in this study. As a result, at least low levels of BBD were predicted for every site by the regression model. However, only a limited area of each reef was surveyed and in some cases no disease was observed. The models also moderately underpredicted BBD prevalence >1%. Variance not explained by the models may be related to additional environmental or coral community factors at each site such as temperature or abundance of susceptible species.

An inverse correlation between TP and BBD prevalence (and DIN) was observed near LSI. It is possible that the system is generally nitrogen limited and that increases in the concentrations of nitrogenous compounds facilitate increased phosphorus utilization by reef organisms. However, the study presented here is limited to nutrient samples collected at one point in time and provides no information on long-term trends or episodic nutrient delivery to these reefs. It is unlikely that the sources of elevated DIN near LSI are anthropogenically derived. LSI is relatively pristine with negligible effects from anthropogenic stressors such as sewage, pollution, or sedimentation (Dennis & Wicklund 1993). Increased DIN concentrations, and concomitant increases in BBD prevalence, were observed primarily on reefs exposed to upwelling from the Exuma Sound (i.e. Horseshoe, North Perry, South Perry, and White Horse; Figure 1).

In the NFK there has been considerable debate regarding sources of nutrients to the reef tract. Some scientists working in this area have speculated that sewage transport (LaPointe et al. 1990, Lipp et al. 2002) or water transfer from the Florida Bay (Lapointe & Clark 1992) might contribute to coral reef decline. Although phosphorus may be

retained in porous limestone that lies beneath the Florida Keys (Corbett et al. 2000), nitrogenous compounds from anthropogenic waste disposal can potentially seep into nearshore marine environments. Gradients of decreasing N:P ratios from onshore to offshore in the Florida Keys have been identified in sediments (Szmant and Forrester 1996), *Thalassia testudinum* leaves (Fourqurean and Zieman 2002), and macroalgae (Hannisak and Siemon 2000). Cook et al. (2002) observed that N:P ratios in zooxanthellae collected from experimental transplanted corals were higher for the colonies placed near shore than for those placed further offshore, near the reef tract. While Cook et al. (2002) suggest that the experimental corals placed near Long Key were phosphorus limited, all sites used in this study lie within areas defined as nitrogen-limited based on seagrass N:P ratios <30 (Fourqurean and Zieman 2002). In these areas even small increases in DIN concentration may have effects on BBD activity. Upwelling (Leichter et al. 2003), rather than outflows from Florida Bay, may also be an important source of nutrient along the NFK reef tract. Nutrient fluxes from Florida Bay into Hawk Channel and onto the reef are minimal (Boyer & Gibson 2005) compared to upwelling from offshore tidal bores (Leichter et al. 2003).

While overall levels of disease prevalence were low during 2002 and 2004, species susceptibility was variable between the NFK and LSI suggesting that diseases may affect coral community structure differently in these two regions. In both regions increased nitrogen availability was associated with elevated BBD prevalence. Although previous reports suggest that local anthropogenic influence may play an important role in coral disease, this report provides evidence that similar levels of disease prevalence can occur in regions with similar reef composition but widely differing levels of human

impact. While some localized human activities are known threats to coral reefs, additional factors that operate on broader spatial scales are likely influential in disease activity. The synergistic interactions between local nutrient availability and other environmental factors known to affect coral diseases, such as temperature and sedimentation, can be expected to impact disease dynamics over space and time.

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Table 1. Number of diseased colonies observed at each reef site (pooled surveys) in the Northern Florida Keys (NFK) in 2002 and 2004 and Lee Stocking Island (LSI) in 2004.

Site	Map Icon	2002					2004[a]			
NFK		Surveys	BBD	WP	DSS	YBD	Surveys	BBD	WP	DSS
Algae	L	3	0	5	7	0	1	0	0	3
Conch deep[b]	C	3	0	1	0	0	2	0	0	4
Conch shallow[b]	C	3	0	3	0	2	2	5	0	4
Cannon Patch	H	-	-	-	-	-	1	0	0	5
Carysfort	M	3	0	6	3	0	1	4	0	3
Crocker	A	-	-	-	-	-	1	0	0	0
Davis	B	-	-	-	-	-	1	3	0	0
Dry Rocks	J	-	-	-	-	-	2	1	0	6
French	F	3	0	2	0	0	2	0	0	5
Grecian	I	-	-	-	-	-	1	1	0	6
Horseshoe	K	3	0	0	2	0	1	0	0	2
Molasses	E	3	0	0	1	0	1	1	0	2
Pickles	D	3	0	0	0	0	2	2	0	2
White Bank	G	-	-	-	-	-	1	1	0	1
LSI[c]										
Big Point	Q	-	-	-	-	-	3	5	3	1
Goby Spot	N	3	2	4	1	0	3	0	6	0
Horseshoe deep[c]	U	3	4	2	3	0	3	7	0	6
Horseshoe shallow[c]	U	3	2	0	2	0	3	9	0	5
North Norman's	P	3	1	0	6	0	3	1	0	3
North Perry	S	3	2	4	5	0	3	3	0	9
Rainbow Gardens	O	3	1	0	6	0	3	3	3	4
South Perry	T	3	1	4	8	0	3	4	6	4
Square Rock	W	3	1	1	0	0	2	2	4	2
Tug and Barge	V	3	-	-	-	0	2	2	1	0
White Horse	R	3	3	3	7	0	3	3	2	5

[a] YBD was not observed in 2004
[b] Conch sites in NFK indicated by a single map icon
[c] Horseshoe sites in LSI indicated by a singe map icon

Table 2. Occurrence and prevalence of coral diseases for all coral species in the Northern Florida Keys (NFK) in 2002 (area surveyed = 7536 m^2, n = 3711 coral colonies) and 2004 (area surveyed = 6594 m^2, n = 4088 coral colonies) as well as Lee Stocking Island (LSI) in 2002 (area surveyed = 9420 m^2, n = 11092 coral colonies) and 2004 (area surveyed = 9734 m^2, n = 11133 coral colonies). Significant differences in mean (t-test of arcsine transformed values, $\alpha = 0.025$) prevalence are indicated by asterisks (between years within region) and crosses (between regions within years). (BBD) black band disease, (WP) white plague, (DSS) dark spots syndrome, (YBD) yellow band disease.

	NFK				LSI			
	2002		2004		2002[a]		2004	
Disease	Occur-rence	Preva-lence (%)	Occur-rence	Preva-lence (%)	Occur-rence	Preva-lence (%)	Occur-rence	Preva-lence (%)
BBD	0	0.00*†	18	0.44*	17	0.15*†	39	0.35*
WP	17	0.46*†	0	0.00*†	18	0.16*†	25	0.22*†
DSS	13	0.35*	43	1.05*†	38	0.34	39	0.35†
YBD	2	0.05	0	0.00	0	0.00	0	0.00
All diseases	32	0.86*†	61	1.49*†	73	0.66*†	103	0.93*†

Table 3. Disease occurrence, prevalence (in brackets, %), and number of corals observed in the Northern Florida Keys (NFK) in 2002 and 2004 and Lee Stocking Island (LSI) in 2004. Only species in which diseases were observed are shown. (BBD) black band disease, (WP) white plague, (DSS) dark spots syndrome, (YBD) yellow band disease. YBD was not observed in LSI.

	NFK									LSI[a]			
	2002					2004				2004			
Coral species	N	BBD	WP	DSS	YBD	N	BBD	WP	DSS	N	BBD	WP	DSS
Agaricia agaricites	572	-	-	-	-	491	1 (0.20)	-	-	1408	-	-	-
Colpophyllia natans	52	-	-	-	-	62	1 (1.61)	-	-	60	-	-	-
Dichocoenia stokesi	173	-	-	-	-	146	2 (1.37)	-	-	251	4 (1.59)	13 (5.18)	-
Diploria strigosa	32	-	-	-	-	67	-	-	-	616	2 (0.32)	-	-
Diploria labyrinthiformis	73	-	2 (2.74)	-	-	98	-	-	-	626	-	2 (0.32)	-
Eusmilia fastigiata	14	-	-	-	-	17	-	-	-	211	-	2 (0.95)	-
Meandrina meandrites	28	-	-	-	-	97	8 (8.25)	-	-	182	-	-	-
Montastraea annularis	293	-	4 (1.37)	-	1 (0.34)	328	5 (1.52)	-	-	1365	1 (0.07)	1 (0.07)	-
Montastraea cavernosa	338	-	-	-	1 (0.30)	288	-	-	-	716	-	-	-
Montastraea faveolata	na[a]	-	-	-	-	83	1 (1.20)	-	-	750	1 (0.13)	4 (0.53)	-
Porites astreoides	635	-	4 (0.63)	-	-	563	-	-	-	1385	-	-	-
Siderastrea siderea	1006	-	7 (0.70)	13 (1.29)	-	1124	-	-	43 (3.83)	1312	31 (2.36)	-	37 (2.82)

[a] For 2002 disease prevalence by species see Voss and Richardson 2006

[b] During the 2002 surveys in the NFK *Montastraea faveolata* was recorded as part of the *M. annularis* species complex.

Table 4. Mean nutrient concentrations (± SD) in the Northern Florida Keys (NFK) and Lee Stocking Island (LSI) in 2002 and 2004. Additional data for the NFK are from samples collected in 2000-2004 at six sites along the reef tract (see text).

Nutrient Conc.	NFK			LSI	
(μmol/ L)	2000-2004[a]	2002	2004	2002	2004
	n = 30	n = 24	n = 19	n = 30	n = 31
NO_3^-	0.33 ± 0.24	0.43 ± 0.19	0.44 ± 0.20	0.34 ± 0.26	0.33 ± 0.17
NO_2^-	0.05 ± 0.02	0.12 ± 0.12	0.07 ± 0.03	0.06 ± 0.04	0.08 ± 0.02
NH_4^+	0.31 ± 0.12	0.40 ± 0.15	0.37 ± 0.18	0.35 ± 0.15	0.39 ± 0.26
DIN	0.68 ± 0.31	0.86 ± 0.25	0.88 ± 0.34	0.75 ± 0.31	0.81 ± 0.29
Total P	0.15 ± 0.09	0.16 ± 0.07	0.11 ± 0.06	0.25 ± 0.17	0.18 ± 0.14
SRP	0.04 ± 0.03	0.08 ± 0.04	0.04 ± 0.02	0.08 ± 0.05	0.04 ± 0.01
DIN:TP	5.97 ± 3.34	6.25 ± 4.6	7.31 ± 3.1	3.64 ± 2.79	3.83 ± 2.64

[a] Data provided by Florida International University's Southeast Environmental Research Center Water Quality Monitoring Network

Table 5. Correlation coefficients (Pearson) between nutrient concentrations and DIN:TP ratios in the NFK and LSI. Asterisks indicate significant correlations ($\alpha = 0.05$).

NFK	NO_3^-	NO_2^-	NH_4^+	DIN	Total P	SRP	DIN:TP
NO_3^-	1	0.194	0.319*	0.873*	0.210	0.221	0.459*
NO_2^-		1	0.347*	0.371*	0.124	-0.021	-0.108
NH_4^+			1	0.642*	0.074	0.068	0.413*
DIN				1	0.156	0.121	0.398*
Total P					1	0.324*	-0.141
SRP						1	-0.132
DIN:TP							1
LSI							
NO_3^-	1	-0.113	-0.039	0.245	-0.318*	-0.278	0.117
NO_2^-		1	0.459*	0.545*	0.207	0.187	-0.207
NH_4^+			1	0.972*	0.167	0.145	0.451*
DIN				1	-0.110	-0.091	0.409*
Total P					1	0.291*	-0.197
SRP						1	-0.136
DIN:TP							1

FIGURE LEGENDS

Figure 1. Studies sites in the northern Florida Keys (NFK) and Lee Stocking Island (LSI). (A) Crocker, (B) Davis, (C) Conch, (D) Pickles, (E) Molasses, (F) French, (G) White Bank, (H) Cannon Patch, (I) Grecian, (J) Dry Rocks, (K) Horseshoe, (L) Algae, (M) Carysfort, (N) Goby Spot, (O) Rainbow Gardens, (P) North Norman's, (Q) Big Point, (R) White Horse, (S) North Perry, (T) South Perry, (U) Horseshoe, (V) Tug and Barge, (W) Square Rock.

Figure 2. A comparison of observed and predicted BBD prevalence in the NFK (A) and LSI (B). Open symbols indicate 2002 data (no BBD observed in the NFK in 2002). Solid symbols indicate 2004 data. The dashed lines are 1:1 in each graph. Note different scales for NFK and LSI.

Figure 1

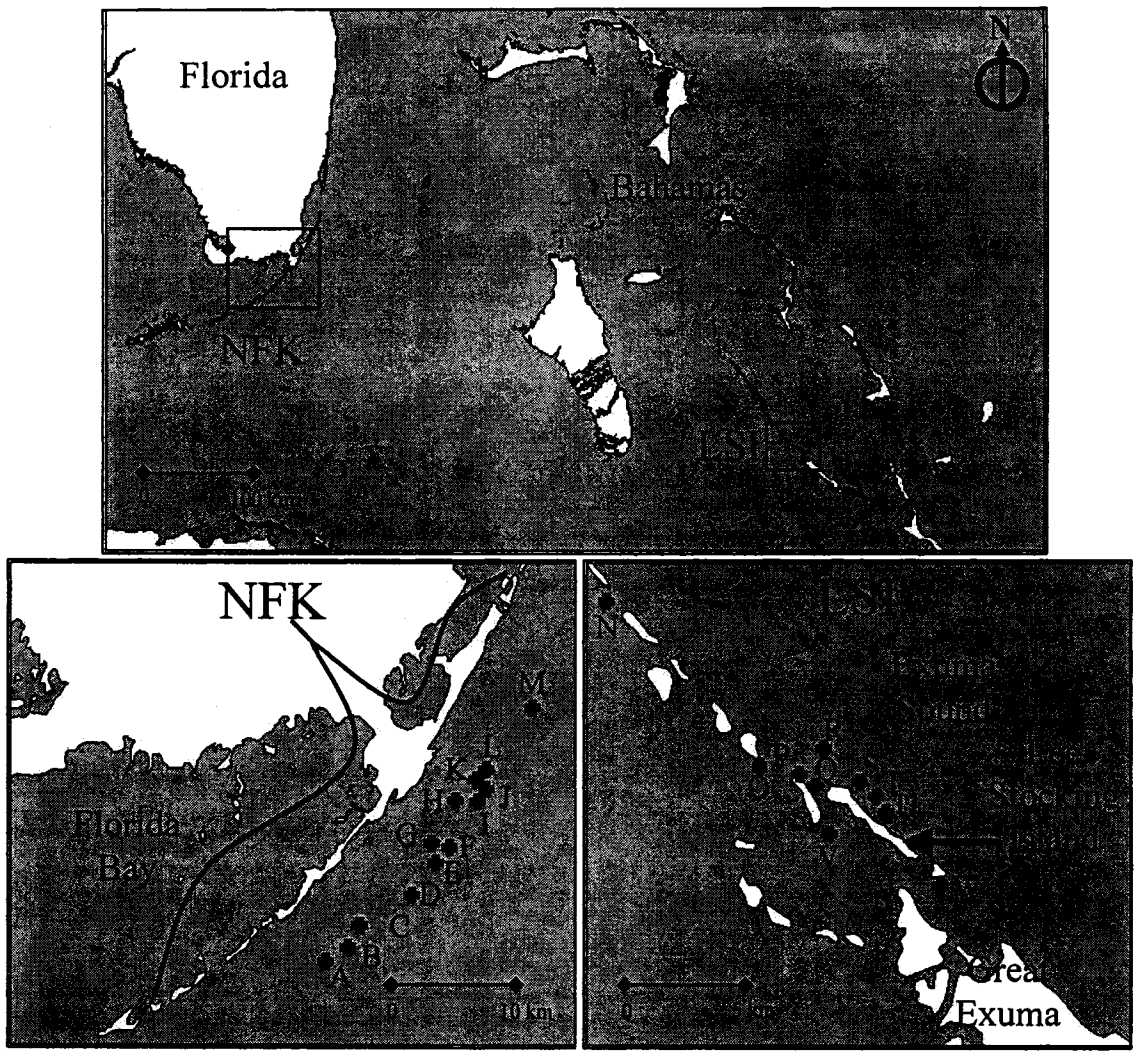
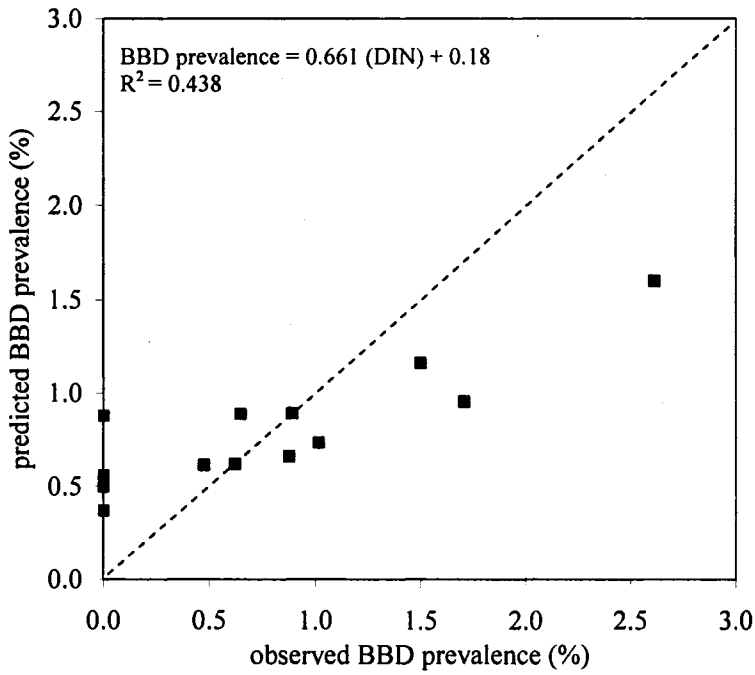
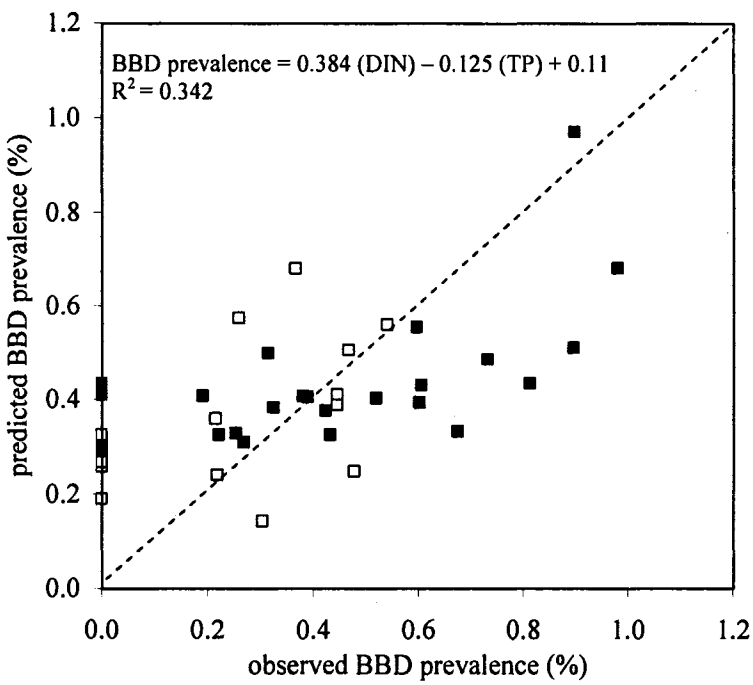


Figure 2
A. NFK



B. LSI



CHAPTER 4

NUTRIENT ENRICHMENT ENHANCES BLACK BAND DISEASE PROGRESSION IN CORALS

ABSTRACT

Infectious diseases are recognized as significant contributors to the dramatic losses of corals observed worldwide. However, the causes of increased coral disease prevalence and severity are not well understood. One potential factor is elevated nutrient concentration related to localized anthropogenic activities such as inadequate waste water treatment or terrestrial runoff. In this study the effect of nutrient enrichment on the progression of black band disease (BBD) was investigated using both *in situ* and laboratory experiments. Experimental increases in localized nutrient availability using commercial time released fertilizer *in situ* resulted in doubling of BBD progression and coral tissue loss in the common reef framework coral *Siderastrea siderea*. Laboratory experiments in which artificially infected *S. siderea* colonies were exposed to increased nitrate concentrations (up to 3 μM) demonstrated similar increases in BBD progression. These findings provide evidence that the impacts of this disease on coral populations are exacerbated by nutrient enrichment and that management to curtail excess nutrient loading may be important for reducing coral cover loss due to BBD.

INTRODUCTION

Globally, coral reefs are undergoing detrimental loss and degradation (Hughes et al. 2003). This trend has been particularly severe on reefs of the wider Caribbean, where an estimated 80% of coral cover has been lost over the past three decades (Gardner et al. 2003). Concurrent with losses in coral cover there have been dramatic increases in the number, frequency, geographic distribution, and host range of coral diseases (Richardson 1998; Harvell et al. 1999; Porter et al. 2001; Richardson and Aronson 2002; Sutherland et al. 2004; Weil 2004). Infectious diseases of corals are now recognized as significant contributors to the degradation observed in coral communities, particularly on Caribbean reefs (Rosenberg and Loya 2004). To date eighteen coral diseases have been described (Sutherland et al. 2004), but only six of these have been characterized in terms of pathogen identification and disease etiology.

Although the reasons for the increases in coral disease incidence and prevalence are largely unknown (Richardson et al. 1998), numerous factors including both natural and local anthropogenic impacts have been suggested as potential contributors to this phenomenon (Jackson et al. 2001; Rosenberg and Ben-Haim 2002). Of particular concern is anthropogenic nutrient enrichment. Potential human-related sources of elevated nutrients to the reef environment include inadequate sewage treatment (Lapointe et al. 1990; Paul et al. 1995; Szmant and Forrester 1996; Lipp et al. 2002) and increased terrestrial runoff related to development (Hallock et al. 1993; Costa et al. 2000). The most common natural, offshore contributions to nutrient enrichment originate from coastal upwelling, occasionally associated with internal tidal bores (Leichter et al. 1996; 2003) or volcanic events (Genin et al. 1995). Both increased nutrient availability and

decreased herbivory can lead to phase shifts from coral-dominated to macroalgal-dominated communities (Littler and Littler 1984; Done 1992; Hughes 1994; Steneck and Dethier 1994). Only a limited number of studies have assessed the potential interactions between increased nutrients and reef degradation other than phase shifts. One potential effect may be an enhancement of pathogen-associated coral diseases.

To date four published studies have included a quantitative assessment of the relationship between water quality and coral disease. Kim and Harvell (2002), in a field study of aspergillosis (a fungal infection of sea fans), found that although overall disease prevalence did not vary throughout the Florida Keys, higher levels of dissolved inorganic nitrogen and reduced water clarity (as indicated by chlorophyll *a* and turbidity) were correlated with increased disease severity (i.e. percent of colony area affected by aspergillosis). They emphasized that although a correlation was established, demonstration of a causative relationship would be premature due to limited sample size (5 locations) and the fact that water quality parameters were determined using samples that were not collected at the same time or location as the disease surveys. Kuta and Richardson (2002) reported that BBD infected corals occurred in shallower water depths with higher water temperature, higher levels of nitrite, and lower levels of ortho-phosphate than healthy (but BBD-susceptible) colonies in the northern Florida Keys. They concluded, however, that the increased nitrite concentration may have been a result of microbial metabolic processes within the BBD consortium itself, such as incomplete reduction of nitrate (Kuta and Richardson 2002). Kaczmarsky et al. (2005) observed that both BBD and white plague type II were more prevalent at a sewage-impacted site as compared to an ecologically similar non-impacted site in St. Croix, USVI. Although this

study did not include measurements of nutrient concentrations during or between sewage bypass events, the relative concentrations of indicator microorganisms provided evidence that untreated sewage runoff increased coral disease prevalence.

Only one published study has experimentally examined the effects of nutrient enrichment on coral diseases. Bruno et al. (2003) found that nutrient enrichment increased the severity of both aspergillosis in sea fans and yellow band disease (YBD) on two *Montastraea* species in Akumal, Mexico. In this study, sea fans experimentally exposed to both aspergillosis (infected tissue) and increased nutrients experienced significantly higher proportions of infected tissue and diagnostic purple galls. Likewise, nutrient enrichment of *Montastraea annularis* and *Montastraea franksii* colonies with naturally occurring yellow band infections resulted in increased rates of disease progression and host tissue loss.

The aim of this study was to experimentally determine the effect of nutrient enrichment on progression (and coral host tissue degradation) of BBD. This coral disease, caused by a pathogenic microbial consortium, is known to infect 64 scleractinian species, primarily those that form massive boulder-shaped colonies, and 6 octocoral species (Sutherland et al. 2004). BBD is found worldwide (Sutherland et al. 2004) and is responsible for persistent losses in coral cover (Edmunds 1991; Kuta and Richardson 1996; Borger and Steiner 2005). Furthermore, when coral tissue is lost to BBD, the exposed coral skeleton is commonly colonized by octocorallians and macroalgae and only rarely by scleractinian species (Kuta and Richardson 1997; Edmunds 2000). Outbreaks of BBD have been recorded near sewage outflows or other areas with increased pollution (Taylor 1983; Antonius 1988; Bruckner and Bruckner 1997; Goreau

et al. 1998), suggesting that the distribution of BBD may be correlated with human influence. However, with the exception of Kaczmarsky et al. (2005), no quantitative data regarding water quality were recorded in these studies. To specifically assess the effects of increased nutrient concentrations on the rate of BBD progression and host coral tissue loss manipulative experiments were conducted using both naturally occurring BBD infections *in situ* (modeled after Bruno et al. 2003) and artificially infected coral colonies in the laboratory.

MATERIALS AND METHODS

Study site

This study was conducted on Horseshoe Reef (23° 46'18" N, 76° 5'33" W) northeast of Lee Stocking Island (LSI) in the Bahamas' Exuma Chain. This reef is characterized by a complex fringing pattern with depths ranging from 4 to14 m. Reefs near LSI are relatively pristine with negligible environmental stress from sewage discharge, pesticide pollution, or river runoff (Dennis and Wicklund 1993). The area is geographically isolated from human population centers (45 km from Georgetown) and receives little commercial or sport fishing due to a voluntary no-take zone that surrounds LSI. These factors make it an ideal region to study coral disease dynamics with limited human influence.

In situ experiments

On July 16-17, 2004 and on July 12-14, 2005 *Siderastrea siderea* colonies with naturally occurring BBD infections were identified at Horseshoe Reef. Of these, 20 were selected in 2004 and 10 selected in 2005. The colonies were well isolated from one

another (at least 5 m apart), were within similar depth ranges (7-12 m), and had lost at least 5% of host coral tissue due to disease progression. The colonies ranged from 20 to 85 cm in diameter and had no other visual anomalies beyond BBD infection. Half of these colonies were randomly selected for experimental nutrient enrichment (10 in 2004, 5 in 2005) and the remaining half were used as controls. Colony size (approximated by elliptical area of maximum length and perpendicular width) was tested for differences in mean size between treatment groups (T-tests) and correlation with disease progression over the duration of the experiment (Spearman's rank).

To assess ambient (background) nutrient availability water samples were collected in 2004 approximately 10 cm above each of the BBD-infected colonies using sterile 60 ml plastic syringes before initiation of the nutrient dosing experiments. Additionally, water samples were collected at 5 other time points prior to and within the timeframe of the experiment (2004 only) to determine ambient nutrient variability over time. These samples were collected in locations >10 m from any experimental colonies. Water sample were immediately placed on ice upon return to the boat and, upon return to shore, filtered through 25 mm diameter GF/F filters before transfer to sterile 60 ml high density polyethylene bottles. The samples were then frozen (-40 °C) for transport to Florida International University (FIU).

Nutrient concentrations near experimental colonies were manipulated using diffusive nylon bags filled with 15 g Osmocote™ 9-6-12 time release fertilizer (Scotts, Maryville, OH). The bags were attached to bare coral skeleton 5-10 cm behind the advancing BBD line using a latex coated masonry nail and zip tie (Figure 1a). Empty nylon bags were used for the control colonies. The fertilizer filled bags were replaced

every 5 days to maintain elevated nutrient concentrations over the duration of the experiments (20 days). Empty nylon bags (controls) were physically touched at 5 day intervals to control for diver contact. At each 5 day interval BBD disease migration was quantified to the nearest mm using calipers and two latex coated reference nails (inserted at the beginning of the experiment). For these measurements, the caliper was placed against both the nail holding the fertilizer bag and one of the two nails marking the edge of the BBD infection at t = 0 to ensure that tissue loss at the exact same point was measured. The average migration distance was thus calculated at two points for each BBD infection (Figure 1a).

To assess the increase in nutrient availability near the nutrient dosed experimental corals, triplicate 60 ml water samples were collected in 2004 from directly above the colony. The samples were collected at three time points: immediately after fertilizer addition (t = 0); 1 day later; and 5 days later. These samples were processed in the same manner as the ambient nutrient samples described above. Nutrient concentrations were analyzed only in the first 5 day period of the experiment and with the assumption that reef dynamics were similar following each subsequent nutrient loader replacement.

Nutrient analysis

Samples for nutrient analysis were stored at -20 °C upon arrival at FIU for 61 days until analysis. The filtered samples were analyzed for total nitrogen, nitrate, nitrite, ammonium, and soluble reactive phosphate (henceforth referred to simply as phosphate) using an ALPKEM Rapid Flow Analysis 300 (Alpkem Corp., Clackamas, OR) at FIU's Southeastern Environmental Research Center (see Boyer and Jones 2002 for methods).

Laboratory nutrient enrichment

To determine the effects of nutrient addition on BBD progression under known, constant nutrient concentrations, a controlled laboratory experiment was conducted. Six colonies of healthy *S. siderea* (approximately 5 cm^2 each) were collected on July 14, 2005 from White Horse reef (23° 48'14" N, 76° 7'53" W, located 5.2 km NW of Horseshoe Reef), a spur and grove formation 4-13 m in depth with high wave action. A no-take zone surrounding LSI prevented coral collection from Horseshoe Reef (the site of the *in situ* experiment). Each fragment was placed in a 1 liter glass beaker inside a flow through seawater raceway in the wet laboratory (wet lab) facility on LSI. Air was bubbled at the surface to circulate water in each beaker. The wet lab was shaded by two layers of neutral density screen, reducing direct light exposure to the experimental colonies. After allowing the corals to acclimate for 1 day, four colonies were artificially infected with freshly collected BBD from a single naturally infected *S. siderea* colony on Horseshoe Reef. For inoculation, approximately 0.25 ml of clumped BBD was placed directly on the center of each colony and monitored visually to ensure the attachment of the BBD to the surface of each colony. The four artificially infected colonies were exposed to ambient, +1, +2, and +3 μM nitrate ($NaNO_3$) in filtered seawater. Two colonies were left as uninfected controls. Of these, one was exposed to ambient nutrient concentrations while the other was exposed to 2 μM nitrate. Time series photographs (e.g., Figure 1b) were taken twice daily (morning and evening) and used with a grid overlay to estimate band migration rates (to the nearest 0.5 mm) over 10 days. A water change was performed for all treatments at day 5.

Statistical analysis

Differences in nutrient concentrations between ambient and experimentally enriched conditions were tested using the nonparametric Mann-Whitney U-test and Bonferroni-adjusted significance levels. To test for differences in the BBD migration distance between nutrient enriched and control BBD infections *in situ,* and in the laboratory experiment, repeated measures analysis of variance (ANOVA) was used. Statistical procedures were conducted with SPSS 13.0.

RESULTS

Quantification of *in situ* nutrient enrichment

Ambient nutrient concentrations were generally low with minimal variability across Horseshoe Reef (Table 1). Furthermore, ambient nutrient concentrations appeared to remain relatively constant over the duration of the experiment. Nutrient levels in samples from five different time points on Horseshoe Reef, collected >10 m from the experimental colonies, did not differ significantly from the ambient conditions recorded at the onset of the study (one sample T-tests, $\alpha = 0.05$).

Immediately after the initiation of the nutrient dosing experiment on July 17, 2004 (Table 1) nitrate, nitrite, ammonium, and phosphate concentrations increased significantly compared to ambient conditions directly above the colony (0.1 m). After 1 day nitrite , ammonium, and phosphate concentrations were significantly greater than ambient. After 5 days concentrations of all three nitrogenous compounds were significantly greater than ambient conditions, however, there was no quantifiable phosphate enrichment.

***In situ* experiment**

Mean colony size did not differ between nutrient-dosed and control groups in 2004 or 2005 (2004: control 8364 ± 8319 cm^2, nutrient-dosed 8875 ± 9130 cm^2; 2005: control 4273 ± 1460 cm^2, nutrient-dosed 5576 ± 1972 cm^2 means ± SD). There was no significant correlation between colony size and total BBD migration distance in either year.

Naturally occurring BBD infections on *S. siderea* migrated more rapidly when exposed to elevated nutrient concentrations (Figure 2). In 2004 (Figure 2a) repeated measures ANOVA indicate that time interval ($F = 20.90$, $p < 0.001$), treatment (between groups, $F = 80.42$, $p < 0.001$), and the interaction between the two ($F = 3.77$, $p < 0.02$) were all significant factors in BBD migration on *S. siderea* (sphericity assumed, Mauchly's $W = 0.445$, $p > 0.1$). At the conclusion of the experiment nutrient-dosed infections had migrated on average 25.4 mm compared to 10.8 mm in the controls. Likewise in 2005 (Figure 2b) differential migration was observed between control and nutrient-dosed BBD infections. Again time ($F = 53.77$, $p < 0.001$), treatment (between groups $F = 16.77$, $p < 0.001$), and the interaction between both factors ($F = 8.02$, $p < 0.01$) were significant (sphericity assumed, Mauchly's $W = .398$, $p > 0.2$). After 20 d nutrient-dosed BBD infections had migrated on average 24.8 mm while control infections had migrated only 11.2 mm.

Laboratory experiment

When artificially BBD-infected *S. siderea* colonies were exposed to elevated levels of nitrate in a laboratory setting, increasing rates of tissue loss were observed with increasing nitrate concentration (Figure 3). Repeated measures ANOVA indicated

differences in the slopes of BBD progression for the four nitrate treatments, evidenced by the significance of the interaction between treatment and time ($F = 6.01$, $p < 0.05$). BBD migration rates in the 2 μM and 3 μM treatments were approximately two and three times, respectively, the rate observed for BBD exposed to ambient nutrient conditions. Neither of the two non-infected control colonies experienced tissue loss over the duration of the experiment.

DISCUSSION

The BBD migration rates measured in this study under ambient nutrient concentrations both *in situ* at Horseshoe Reef (~0.6 mm day^{-1}) and in the laboratory (~0.5 mm day^{-1}) were generally low and independent of host colony size. These rates were similar to an average BBD progression of 0.81 mm day^{-1} observed by Borger and Steiner (2005) in Dominica. Only the nutrient-dosed BBD infections during the first 5 day interval in the 2004 *in situ* experiment (mean = 2.6 mm day^{-1}) approached the average migration rates of 3 mm day^{-1} reported elsewhere (Rützler et al. 1983; Richardson 1996). None of the infections observed in this study exhibited rates near the maximum of 6.2 mm day^{-1} reported by Rützler et al. (1983). With these generally slow migration rates over the 20 day duration of the *in situ* experiment, complete coral host mortality was not observed in control or nutrient-dosed colonies. The lack of total colony mortality within 20 days is not uncommon as medium to large colonies are more often affected by BBD (Borger 2005). It may take months for BBD to completely kill such coral colonies, or even years with recrudescent infections on large colonies.

The results of both the *in situ* and laboratory experiments in this study suggest that increases in nutrient availability can exacerbate the effect of BBD on coral hosts. *In situ* nutrient-dosed BBD infections migrated on average twice as quickly as control infections (1.3 mm day^{-1} vs. 0.6 mm day^{-1}). Nitrate concentrations also directly affected BBD migration rates in the laboratory experiment. Compared to an average rate of 0.5 mm day^{-1} under ambient nutrient conditions, rates in the nitrate treatments were 0.7 mm day^{-1} (1 μM), 1.0 mm day^{-1} (2 μM) and 1.7 mm day^{-1} (3 μM). The observed rates from the 3 μM nitrate and ambient nutrient treatments in the laboratory were similar to those observed *in situ* under nutrient enriched (N and P compounds) and control treatments, respectively. While this suggests that nitrogenous compounds may be limiting, additional investigations in which both nitrate and phosphate are manipulated independently are needed to elucidate the relative roles and potential synergistic effects of these nutrients on BBD migration.

Similar increases in disease migration rates have been observed during *in situ* nutrient dosing experiments for YBD in studies by Bruno et al. (2003). These investigators found that nutrient enrichment increased average rates of coral host tissue loss by 1.8x relative to controls over the 90 day experiment. Bruno et al. (2003) hypothesized that increased nutrient concentrations potentially enhance pathogen fitness and virulence. However, as there is no known pathogen for YBD, the mechanism(s) by which nutrient enrichment facilitates increased YBD progression rates remain unknown. This study differed from that of Bruno et al. (2003) in that the initial mass of nutrient addition was less (15 g compared to 30 g). This factor is likely responsible for the more moderate nutrient increases observed at deployment. Furthermore, in both studies

increases in nutrient availability diminished over time. However, while Bruno et al. (2003) reported nutrient concentrations after 4 days that were still greater than ambient, this study found that nitrate and phosphate concentrations returned to ambient concentrations more quickly over time.

The mechanisms that enhance BBD migration rates under increased nutrient exposure are not yet understood but may be related to the fact that BBD is a complex microbial infection. The BBD consortium includes two populations of gliding, filamentous microorganisms, cyanobacteria and sulfide-oxidizing *Beggiatoa* spp. (Garrett and Ducklow 1975; Rützler et al. 1983; Taylor 1983; Richardson 1996), along with sulfate-reducing bacteria (*Desulfovibrio* spp., Garrett and Ducklow 1975; Schnell et al. 1996; Cooney et al. 2002; Viehman et al. 2006) and at least 50 other heterotrophic bacterial species (Cooney et al. 2002; Frias-Lopez et al. 2002). An additional source of nutrients, i.e. other than those released by coral tissue lysis (from nutrient enrichment manipulations or other sources as discussed above), may enhance growth rates and/or motility (Grossart et al. 2001) for one or many of the various BBD microbes.

To date there has been only one published study concerning the ecological physiology of a BBD microorganism (Richardson and Kuta 2003). In this study it was found that a culture of one BBD cyanobacterial isolate, as well as freshly collected BBD samples (both from the Florida Keys), were unable to fix nitrogen as determined by the acetylene reduction technique (Richardson and Kuta 2003). Therefore an increase in available nitrogenous compounds, from any source, may release this organism (and other non-nitrogen fixing BBD cyanobacteria) from nitrogen limitation on the reef. The

relative contributions of water column nutrients and nutrients derived from lysed coral tissues to BBD virulence and migration rates remain unknown.

The increases in BBD severity observed in this study due to nutrient enrichment may also be related to coral host stress. Multiple studies have shown decreased coral growth rates when nutrient (inorganic nitrogen and phosphorus) concentrations are increased (Stambler et al. 1991; Ferrier-Pages et al. 2000; Renegar and Riegl 2005). These short-term reductions in coral growth are likely linked to increased competition for CO_2 between calcification and photosynthesis as zooxanthellae densities increase (Szmant 2002). Nutrient enrichment has also been shown to reduce coral fecundity, fertilization, and recruitment (Hunte and Wittenburg 1992) and can lead to increased coral mortality (Kuntz et al. 2005). Elevated nutrients may reduce the coral host's ability to counteract infection by pathogenic microorganisms. There are, however, some cases in which nutrients have had no effect (Taylor 1978) or even positive effects (Atkinson et al. 1995) on coral growth.

While nutrient inputs can increase disease prevalence (Kaczmarsky et al. 2005) and exacerbate infections (Bruno et al. 2003; this study), they are not necessary for coral diseases to occur. Multiple coral diseases have been observed near LSI (Voss and Richardson 2006) and in Bonaire (Weil et al. 2002), both relatively pristine regions of the Caribbean. Likewise, both BBD and aspergillosis have been reported in pristine areas by Edmunds (1991) and Nagelkerken et al. (1997), respectively. Therefore other environmental drivers with more widespread regional effects, such as rising sea surface temperatures, may be more important contributors to the observed increases in coral disease incidence and prevalence throughout the Caribbean. Nonetheless the results of

this study provide evidence that localized nutrient levels play a role in BBD dynamics and support the proposal of Bruno et al. (2003) that management to curtail excess localized nutrient loading may be important for reducing coral cover loss to disease.

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Table 1 *In situ* nutrient concentrations during the 2004 black band disease (BBD) nutrient dosing experiment. Ambient nutrient concentrations were measured before adding nutrient loaders at a distance of 0.1 m from the BBD-infected colonies (n = 20). Additional ambient nutrient concentrations are reported as averages of duplicate analyses on split single samples collected from locations > 10 m from experimental colonies Experimental nutrient concentrations were measured immediately after deployment of the nutrient loaders on July 17, and again 24 hours and 5d later. Concentrations are shown as mean ± SD (when available). Experimentally dosed nutrient concentrations that differ significantly from ambient are indicated by an asterisk (Mann-Whitney U-test, $p < 0.017$).

	Date	N	Nutrient Concentration (μM)			
			nitrate	nitrite	ammonium	sr phosphate
Ambient	July 12	1	0.31	0.03	0.18	0.02
	July 17	20	0.28 ± 0.09	0.05 ± 0.01	0.19 ± 0.08	0.03 ± 0.02
	July 18	1	0.36	0.04	0.19	0.04
	July 22	1	0.41	0.05	0.12	0.03
	July 27	1	0.30	0.05	0.11	0.01
	Aug 2	1	0.40	0.06	0.21	0.02
Experimental	July 17	3	1.36 ± 0.49*	0.14 ± 0.03*	2.49 ± 1.40*	0.25 ± 0.11*
	July 18	3	0.55 ± 0.11	0.15 ± 0.02*	2.11 ± 0.54*	0.10 ± 0.02*
	July 22	3	0.61 ± 0.15*	0.11 ± 0.01*	1.03 ± 0.25*	0.03 ± 0.00

FIGURE LEGENDS

Figure 1. *Siderastrea siderea* infected with black band disease (BBD). Advancing BBD separates dead coral skeleton from apparently healthy tissue. (a) *In situ* naturally infected colony with Osmocote™ filled nutrient loading bag. Migration measures were recorded at two points on each colony using reference nails (indicated by stars) as shown by the bracketed areas on the red lines. For each colony these two distances (D1 and D2) were averaged to determine migration at each time interval. (b) Artificial infection under 2 μM nitrate treatment at day 6. Note that BBD infections in the laboratory were thinner and less dense than those tested *in situ*.

Figure 2. Migration of *in situ* black band disease on nutrient dosed (○) and control (●) *Siderastrea siderea* colonies at 5 day intervals over 20 days (mean ± SD). Standard deviations shown represent the variation observed among colonies within each experimental group (controls and nutrient dosed) at each 5 day interval. (a) July 17 - August 7, 2004: experimental n = 10, control n = 10. (b) July 15 - August 5, 2005: experimental n = 5, control n = 5.

Figure 3. Tissue loss associated with migration of black band disease on artificially infected *Siderastrea siderea* colonies and non-infected control colonies exposed to nitrate enrichment ranging from ambient to + 3 μM in the laboratory.

Figure 1

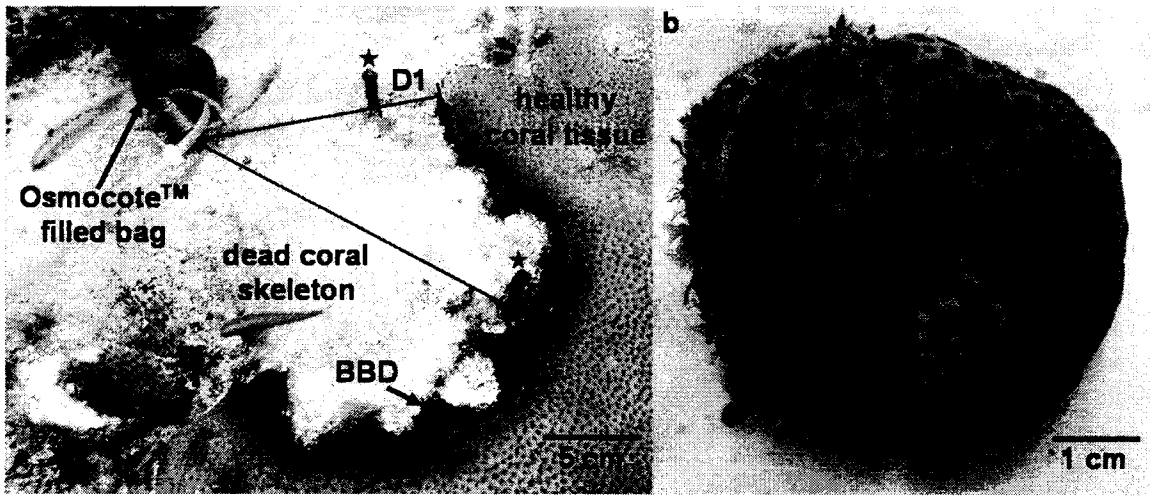
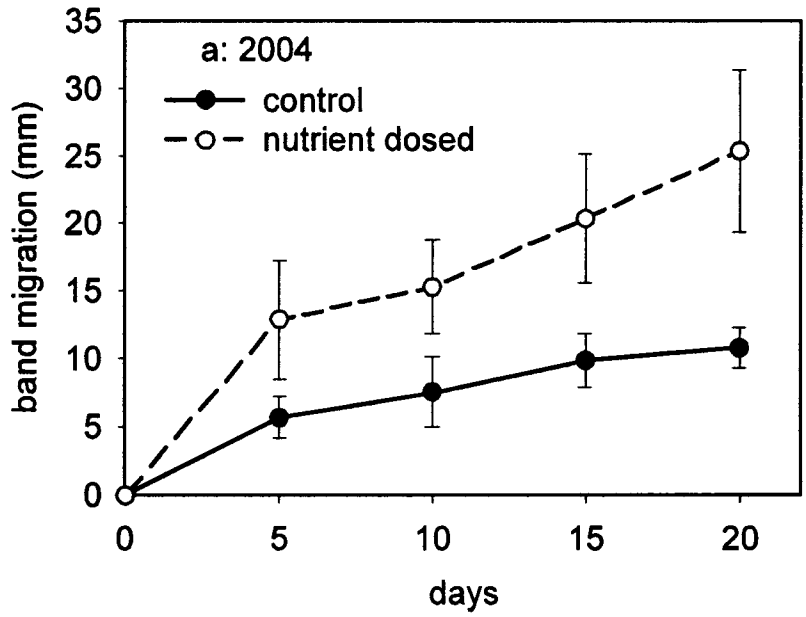


Figure 2



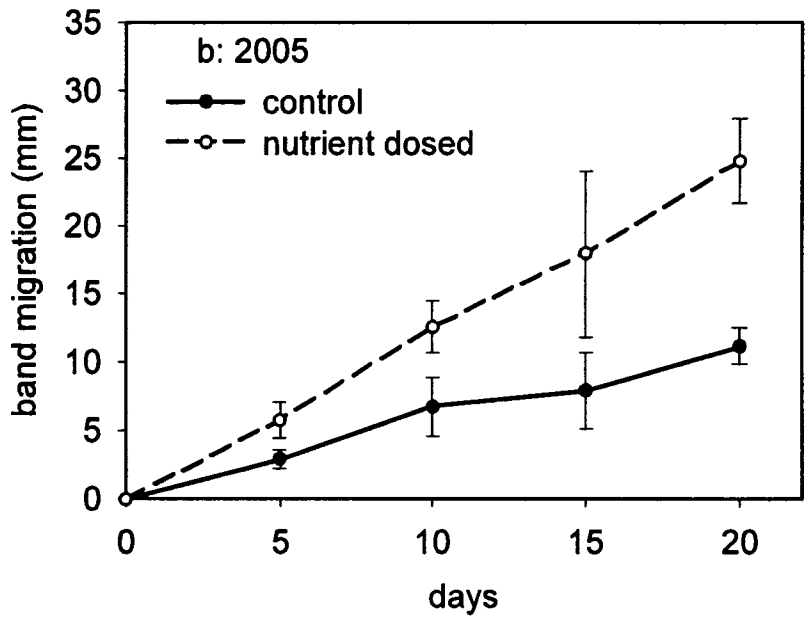
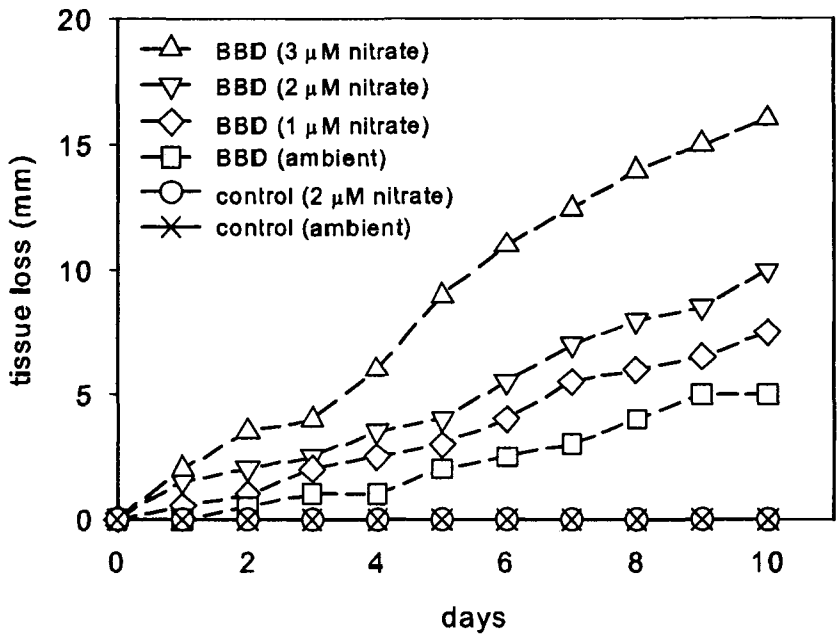


Figure 3



CHAPTER 5

BLACK BAND DISEASE MICROBIAL COMMUNITY VARIATION ON CORALS IN THREE REGIONS OF THE WIDER CARIBBEAN

ABSTRACT

Black band disease (BBD) is a pathogenic consortium of microorganisms that primarily affects massive framework-building scleractinian corals on reefs worldwide. There has been considerable debate concerning both the composition of the bacterial assemblage associated with BBD and the role of different organisms in the pathogenicity. The aim of this study was to utilize microbial profiling to assess overall variation in the BBD bacterial community with respect to geographic location, host coral species, time, and nutrient regime. Length heterogeneity polymerase chain reaction (LH-PCR) was employed to differentiate organisms based on the natural variation in the sequence lengths within hypervariable domains of the 16S rRNA gene. Comparison of LH-PCR profiles revealed much variability in BBD microbial communities among samples. Analysis of the LH-PCR profiles using multivariate ordination methods and analysis of similarity revealed significant differences in the BBD-associated bacterial communities sampled from reefs near Lee Stocking Island (LSI) in the Bahamas' Exuma Chain, the Northern Florida Keys (NFK), and St. John in the US Virgin Islands (USVI). Furthermore, there was significant reef-to-reef variation among BBD bacterial assemblages in the NFK. The observed differences among BBD microbial community profiles from LSI, NFK, and USVI, as well as site-to-site variation in the NFK, were

driven primarily by variation in relative abundance of 313-316 bp amplicons. The results obtained in this study, in concert with the findings of previous molecular analyses of the BBD bacterial community, attest to the intrinsic variability and complexity of the BBD microbial community.

INTRODUCTION

The dramatic decline of coral reefs over the past three decades (Hughes et al. 2003, Pandolfi et al. 2003) has focused much attention and research on the processes that potentially drive this degradation. Numerous factors of both natural and anthropogenic origin have been implicated in coral loss (Jackson et al. 2001, Rosenberg & Ben-Haim 2002), often noted to be accompanied by phase shifts toward algal-dominated communities (Done 1992). More recently, infectious diseases of scleractinian coral have been recognized as significant contributors to the decline of coral communities, particularly in the Caribbean (Rosenberg & Loya 2004). Concurrent with the decline of coral reefs, the number of coral diseases, number of coral species infected, number of reported cases, and the geographic distribution of diseases have all dramatically increased (Goreau et al. 1998, Richardson 1998, Harvell et al. 1999, Porter et al. 2001, Richardson & Aronson 2002, Sutherland et al. 2004, Weil 2004). Despite the documented and potential impacts on coral reef health and increased attention in this field, relatively little is known regarding the etiology and pathogenesis of many coral diseases (Richardson 1998).

Black band disease (BBD), first identified by Antonius (Antonius 1973), is the most widely distributed coral disease affecting reefs worldwide (Sutherland et al. 2004). BBD primarily infects massive framework-building sceleractians including *Colpophyllia natans, Diploria labyrinthiformis, Montastraea annularis, M. cavernosa, M. faveolata,* and *Siderastrea siderea* (Antonius 1981, Rützler et al. 1983, Edmunds 1991, Kuta & Richardson 1996, Voss & Richardson 2006) threatening both the physical structure of the reef and the principal contributors in coral gamete production. At least 64 species of scleractinian corals are susceptible to BBD and of these, 19 are found in the Caribbean (Sutherland et al. 2004).

BBD is a polymicrobial disease that functions as a microbial consortium (Carlton & Richardson 1995, Richardson et al. 1997). The pathogenic assemblage of microbes migrates across an infected coral colony destroying coral tissue and leaving behind bare limestone skeleton (Carlton & Richardson 1995). Infections appear as a darkly pigmented mat, or band, which can be several mm to several cm wide and approximately 1 mm thick. The visually dominant members of the BBD consortium are gliding, filamentous, non-heterocystous cyanobacteria that were originally identified as *Oscillatoria submembranaceae* (Antonius 1973) and later reclassified as *Phormidium corallyticum* based on morphological characteristics (Rützler & Santavy 1983). However, 16S rRNA gene sequencing has failed to identify cyanobacteria from the *Phormidium* genus in BBD samples (Cooney et al. 2002, Frias-Lopez et al. 2002, Frias-Lopez et al. 2003, Frias-Lopez et al. 2004, Ragoonath 2005). Likewise, the sulfide-oxidizing bacteria *Beggiatoa* spp. reported by a number of investigators (Garrett & Ducklow 1975, Ducklow &

Mitchell 1979, Carlton & Richardson 1995, Richardson 1996) have not been observed in molecular studies based on DNA sequencing of the BBD microbial community. Sulfate-reducing bacteria, including *Desulfovibrio* spp. (Garrett & Ducklow 1975, Schnell et al. 1996, Cooney et al. 2002, Viehman et al. 2006) as well as at least 50 other heterotrophic bacterial species (Cooney et al. 2002, Frias-Lopez et al. 2002), have been identified using both standard microbiological and culture-independent techniques. The microbial communities associated with healthy coral tissue, dead coral skeletons, and overlying seawater are distinct from BBD bacterial assemblages (Frias-Lopez et al. 2002). Collectively within these studies, there are discrepancies regarding the composition and variation of the microbial consortia associated with BBD.

Because BBD is comprised of multiple microbial species functioning as a consortium, geographic location, host coral species, nutrient availability, or other environmental drivers may potentially influence the relative abundance of various members of this pathogenic community. This study utilized a polymerase chain reaction-based technique based on amplicon length heterogeneity (LH-PCR) to assess variation in BBD microbial communities. This technique differentiates organisms as a result of the natural variation in the sequence lengths of hypervariable regions within the 16S rRNA genes (Suzuki et al. 1998). Analysis of microbial communities using LH-PCR profiles is based on the relative abundance of amplicons (the hypervariable regions) of different lengths within the 16S rRNA gene pool, extracted from the whole bacterial community (Suzuki et al. 1998, Mills et al. 2003). Amplicons derived from LH-PCR can, additionally, indicate phylogenetic differences. However, it is possible for multiple,

distantly related organisms to give rise to amplicons of identical base pair length but composed of different base sequences (Mills et al. 2003). Recently LH-PCR has proven useful in profiling microbial communities and in various monitoring efforts (Suzuki et al. 1998, Ritchie et al. 2000, Mills et al. 2003, Tiirola et al. 2003, Bernhard et al. 2005, Mills et al. 2006). The goal of this study was to discriminate overall patterns in the BBD microbial community, and to determine variation within and between microbial communities across space and time.

MATERIALS AND METHODS

Sample collection

BBD infected coral colonies were identified during surveys as part of multiyear monitoring programs near Lee Stocking Island (LSI) in the Bahamas Exuma Chain, on the Northern Florida Keys (NFK) reef tract, and near St. John in the US Virgin Islands (USVI, Figure 1). In this study 19 reef sites were sampled in the three geographic regions. Samples of BBD near LSI were collected on July 13 - 25, 2004 from seven reef sites: Big Point, Horseshoe, North Perry, Rainbow Gardens, South Perry, Tug and Barge, and Whitehorse (Figure 1). Big Point, Rainbow Gardens, and Tug and Barge are shallow (1 - 5 m), protected patch reefs dominated by large boulder corals and subject to strong tidal influences. Whitehorse is a well-demarcated spur and groove reef formation with depth ranging from 3 m to 13 m. Horseshoe, North Perry, and South Perry are complex fringing reefs with moderate wave action and 4 m to 20 m depths. These three sites, along with Whitehorse, are exposed to the deepwater Exuma Sound and potential cool

water and nutrient upwelling. BBD samples were also collected in the NFK during June 14 -19, 2004 and May 16 – 20, 2005 from nine reef sites: Carysfort South, Conch Shallow, Davis Ledge, French, Grecian Rocks, Key Largo Dry Rocks, Molasses, Watson, and White Banks (Figure 1). All of these sites are in patch reefs that lie along the reef tract in approximately 2 – 6 m of water. BBD samples were collected on September 6, 2005 from fringing reefs (2-5 m) at Haulover Bay, Hawknest, and Watermelon Cay off St. John in the USVI. A total of nine host coral species were sampled: *C. natans, Dichocoenia stokesi, D. labyrithiformis, D. strigosa, M. annularis, M. cavernosa, M. faveolata, Meandrina meandrites,* and *S. siderea.*

Duplicate BBD samples were collected from each infected coral colony on the LSI and NFK reefs using sterile 10 ml syringes while SCUBA diving. Only individual samples were collected on the USVI reefs. After the BBD sample was drawn into the syringe, it was allowed to clump before decanting approximately 8 ml of seawater from the syringe. This was done in order to limit the amount of seawater bacteria in the sample. BBD samples were stored in the dark on ice (no longer than 4 h) until processing at the field laboratory where the BBD samples were aseptically transferred into sterile 2 ml cryovials and frozen. All samples were stored at -20°C until DNA extraction.

Nutrient dosing and sampling over time

To assess variation in the BBD microbial community over time both with and without (controls) increased nutrient availability, 10 naturally occurring infections on *S. siderea* were selected at Horseshoe reef near LSI. *S. siderea* is the most BBD-susceptible species in this region, and in recent years BBD infections have been concentrated at this

reef (Voss & Richardson 2006). Five of these colonies were randomly chosen as control colonies while the remaining five were subjected to artificial increases in nutrient availability using time-release fertilizer (see Voss and Richardson, in press, for methods). BBD infections on all colonies were sampled at 5-day intervals from July 6-25, 2005 using the methods described above. These time series data were not included in overall comparison among regions, or among sites within LSI, as they were not independent sampling events.

DNA extraction and PCR conditions

The FastDNA® SPIN Kit for Soil (QBiogene, Vista, CA) with slightly modified protocols (Mills et al. 2003) was used to extract whole-community genomic DNA from the BBD samples. The genomic DNA extracts were verified using 1% TBE agarose yield gels and subsequently quantified using Picogreen dye on a Bio-Rad VersaFluor™ fluorometer (Richmond, CA) per the manufacturer's protocol.

The PCR primers used in this study were designed to amplify the domain of the 16S rRNA gene that included hypervariable domains V1 + V2. The fluorescently labeled forward primer 27F-6-FAM (5'-6-FAM-AGA GTT TGA TCM TGG CTC AG-3') was used with the non-fluorescent reverse primer, 355R (5'-GGT GCC TCC CGT AGG AGT-3'). The final concentrations of the PCR reactions were: 1 x PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 μM forward and reverse primer, 0.25 U AmpliTaq® Gold LD DNA polymerase (Perkin Elmer, Wellesley, MA), 0.1% bovine serum albumin (BSA), fraction V (Sigma-Aldrich St. Louis, MO), 1 ng genomic DNA, and DEPC-treated water for a final volume of 20 μL. The PCR reactions were carried out in a Peltier

Thermal Cycler (PTC-200, MJ Research, Waltham, MA) under the following run conditions: 94°C for 11 min, 25 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. Cycling parameters were tested using 15, 20, 25, 28, and 30 cycles to address template reannealing and kinetic biases (Suzuki & Giovannoni 1996, Suzuki et al. 1998). Likewise, various concentrations (0.5 ng, 1 ng, 1.5 ng, and 2 ng) of high molecular weight (HMW) community DNA were tested to reduce the likelihood of non-detection of relatively rare phylotypes. Twenty-five cycles and 1 ng were found to optimally produce consistent and representative profiles (data not shown). All PCRs were run in duplicate and PCR products were verified with 1% TBE agarose yield gels.

Electrophoresis and analysis

All samples were denatured by adding 9.5 μL of 96:1 Hi-Di® deionized formamide: GeneScan® ROX 500 standard solution (Applied Biosystems, Foster City, CA) to 0.5 μL of each PCR product. The PCR products were then separated on a capillary electrophoresis Applied Biosystems (ABI) 310 DNA genetic analyzer using filter set D and module DS-30. Injection time was set to 5 sec and run time to 28 min.

LH-PCR electropherograms (profiles) from each sample were analyzed using ABI's Prism GeneMapper® research version 3.7 software. The analysis parameters were set to the Local Southern Method and peak amplitude threshold was set at 50 fluorescent units. No peak correction or smoothing was applied in order to increase peak resolution. Analysis was limited to fragments 300 to 400 base pairs (BP) in length. The size (to nearest bp) and relative abundance (i.e. relative fluorescence intensity) of each peak was

determined for each profile. Amplicons that comprised less than 0.5% of total relative abundance were eliminated from the analysis. The relative abundance data were averaged among replicates and square root transformed before calculating Bray-Curtis similarity matrices (Bray & Curtis 1957).

Non-metric multidimensional scaling (MDS) (Kruskal 1964) was used to visualize differences in the LH-PCR profiles of the samples. Each ordination was run with 20 random starting configurations to determine the best-fit model for non-parametric regression between the distance among samples in the plot and the Bray-Curtis similarity matrix. Dimensions were selected to minimize final stress, a goodness-of-fit measure related to the relationship between original p-dimensional space and the dissimilarity within the new ordination space. Variation in profiles were tested using an analysis of similarity (ANOSIM), a robust analysis method requiring neither normally distributed abundance data nor balanced replicates between groups (Clarke & Warwick 2001). Variation with respect to geographic location and host coral species was tested using two-way crossed ANOSIM. The Global R-values reported in this study have potential ranges of -1 to 1, where values >0 indicate replicates within each group are more similar to one another than between groups, values <0 indicate the unlikely occurrence that samples between groups are more similar than samples within groups, and values near zero indicate similarities both within and between groups are approximately the same. Similarity percentage (SIMPER) (Clarke & Warwick 2001) was used to determine the relative contribution of each amplicon to the average dissimilarity between the different groups. Although all values were included in the analysis, only those amplicons that

contributed >5% to the dissimilarity are discussed. Non-metric MDS ordinations, ANOSIM, and SIMPER were conducted using Primer 5 (Primer-E Ltd., Plymouth, UK).

Although relatively precise amplicon size calling was observed in the data set (maximum SE of 0.3 bp), LH-PCR profiles were also analyzed using the strategy suggested by Hewson and Fuhrman (Hewson & Fuhrman 2006) to test for any potential effects from imprecision in amplicon size calling. Using this method the starting size of 2 bp wide bin window frames was shifted by 1 bp over 2 frames and similarities were calculated for each frame. The maximum similarity between each pair of samples was used for comparisons among community profiles. This technique is a theoretically more conservative approach that reduces the probability of type I error when testing for statistical differences among samples. Two-way crossed ANOSIM (region, host species) analysis and pairwise comparisons were repeated using the maximum similarity matrix for comparison to the standard 1 bp resolution analysis.

RESULTS

Reproducibility

LH-PCR profiles produced from subsamples of the same BBD sample were reproducible both in terms of triplicate samples analyzed and duplicate PCRs (Figure 2). This reproducibility can be seen when viewing the raw data as shown in Figure 2, i.e. electropherograms, which reveal both the abundance of amplicons (relative peak height) and number of base pairs (amplicon length).

Variability in BBD profiles

Comparison of LH-PCR profiles revealed much variability in BBD microbial communities among the different samples from the NFK, LSI, and the USVI and among different coral host species. Representative profiles from each geographic region, sampled from two host coral species, are shown in Figure 3 (amplicons that constitute less than 0.5% of the cumulative peak height are not shown, see methods). In this figure, unlike Figure 2 which shows raw data, individual peaks are binned with 1 bp resolution, and any overlapping peaks are resolved (see methods section). In the BBD profile from the NFK (on *M. annularis*), 13 amplicons were present with lengths ranging from 304 to 360 bp, while BBD profiles from *S. siderea* at both LSI and the USVI revealed 14 amplicons from 304 to 359 BP and 12 amplicons from 306 to 358 bp, respectively. In addition to the differences in total numbers of amplicons, the relative abundances (peak height) of amplicons in these three samples from each region were highly variable.

In this study 97 BBD samples were analyzed (not including time series/nutrient dosing experiment samples) to assess regional and coral host species variability. Within these samples, a total of 48 amplicons of different lengths were resolved ranging from 301 to 370 bp. Of these, 45 amplicons were present in the NFK samples, while 44 and 24 amplicons were observed in the LSI and USVI samples, respectively. There were 21 amplicons common to all three geographic regions, 42 common to the NFK and LSI, 23 common to NFK and USVI, and 21 common to LSI and USVI (the same 21 common to all regions). Each geographic region had unique amplicons: 301 and 318 bp in LSI, 325 bp in NFK, and 306 bp in USVI. While the unique amplicons in the NFK and LSI were

relatively rare (and not observed in the representative samples shown in Figure 3), the 306 bp amplicon was present in all profiles from the USVI (Figure 3).

Multidimensional comparison by geographic regions

Non-metric multidimensional scaling and ANOSIM analysis of the LH-PCR profiles indicated significant clustering with respect to geographic region (Figure 4A, two-way crossed ANOSIM [region, host species] Global $R = 0.501$, $p < 0.01$). While some overlap is apparent among the three regions, in pairwise comparisons, all regions were statistically discriminant from one another (NFK:LSI, $r = 0.473$, $p < 0.01$; NFK:USVI, $r = 0.931$, $p < 0.01$; LSI:USVI, $r = 0.574$, $p < 0.05$), suggesting that unique bacterial assemblages are associated with BBD in each of these three geographical groups.

SIMPER analyses identified the relative contributions of each amplicon to average dissimilarity of samples between regions (Table 1). LH-PCR profiles from BBD collected in the NFK were primarily differentiated from those at LSI by the greater relative abundance of amplicons 315 and 338 bp and lesser abundance of the 313 bp and 316 bp amplicons (Table 1A). Of these amplicons, those with 315, 316 and 313 bp were the most important, accounting for 21.4%, 16.0%, and 12.5% respectively, of the average dissimilarity. Differences in NFK profiles from samples in USVI were driven by greater abundance of the 315 bp amplicon and relatively lesser abundance of 355, 356, 316, and 306 bp amplicons (Table 1B). When comparing LSI to USVI, 313-316 bp amplicons were again more abundant at LSI while 306, 355, and 356 bp amplicons were more abundant in USVI (Table 1C). The 355 and 356 bp amplicons were common dominant

amplicons in the USVI samples and 306 bp amplicons were found exclusively in this region (e.g. Figure 3). Notably, the 315 bp amplicon was absent from the USVI profiles.

Multidimensional comparison by host coral species

MDS and ANOSIM analyses also indicated that LH-PCR profiles of BBD microorganisms differed with respect to host coral species (Figure 4B, two-way crossed ANOSIM [region, host species] Global $R = 0.184$, $p < 0.05$). Of the nine coral host species investigated, pairwise comparisons indicated that five pairs of host species could be discriminated from one another (Table 2). These were *M. annularis*: *M. cavernosa, M. annularis*: *M faveolata*, *M. annularis*: *S. siderea*, *M. faveolata*: *M. meandrites, M. faveolata*: *S. siderea.*

Multidimensional comparisons within geographic regions

In order to assess variation in BBD microbial communities at smaller spatial scales, MDS and ANOSIM analyses were conducted within each geographic region. Within the NFK region, LH-PCR profiles differed significantly with respect to site (Figure 4C, two-way crossed ANOSIM [site, host species] Global $R = 0.147$, $p < 0.05$) but not by host coral species (MDS not shown). Pairwise comparisons indicated six pairs of sites that differed significantly from one another (Table 2). Five samples, three from Davis and two from Molasses, are notably separate from the remaining NFK samples in the MDS plot (Figure 4C). These five samples were similar to samples from LSI in that they were dominated by the 316 bp amplicon. No significant differences were observed in two-way crossed ANOSIM test with respect to site and host coral species in either LSI or USVI.

Variation over time in control and nutrient dosed BBD infections

The LH-PCR profiles of samples from BBD infections on *S. siderea* at Horseshoe Reef in LSI collected at 5 day intervals demonstrated variability but did not reveal any significant patterns over the 20 day duration of the experiment (Figure 4D). Furthermore, there were no detectable trends or changes in the BBD microbial community following artificial increases in nutrient availability. Despite temporal variation and nutrient dosing, all of these samples were similar to the other samples from LSI as demonstrated by MDS ordination (Figure 4E).

Comparison to frame shift analysis method

Use of the analysis strategy described by Hewson and Fuhrman (2006) resulted in similar MDS patterns (Figure 4F) and increased discrimination among samples with respect to both region and host coral species. As in the standard analysis, profiles differed significantly by both region and coral host species (two-way crossed ANOSIM, region: Global $R = 0.607$, $p < 0.001$, species: Global $R = 0.381$, $p < 0.005$). With respect to both region and host species, the frame shift analysis method resulted in Global R-values greater than those obtained by 1 bp resolution analysis. Increased R-values were also observed in pairwise comparisons by region (NFK:LSI, $R = 0.578$, $p < 0.01$; NFK:USVI, $R = 0.839$, $p < 0.05$; LSI:USVI, $R = 0.716$, $p < 0.05$). Finally, while standard analysis methods identified five pairs of host species that were significantly different from one another (Table 2), frame shift analysis discriminated two additional pairs (*M. annularis*: *Meandrina meandrites* and *M. cavernosa*: *M. meandrites*).

DISCUSSION

LH-PCR profiling revealed that distinct microbial assemblages are associated with BBD infections in three different regions of the Tropical Western Atlantic: the NFK, LSI, and USVI. Geographically distinct BBD microbial communities have also been reported previously based on terminal restriction fragment length polymorphism (T-RFLP) community profiling (Frias-Lopez et al. 2004). The T-RFLP profiles for BBD samples collected from one *M. annularis* and nine *D. strigosa* colonies in Curaçao were similar to each other but differed significantly from BBD samples collected on *Porites lutea* in Papua New Guinea (Frias-Lopez et al. 2004). While similarly detecting significant variation in BBD microbial communities from different geographic regions, variability among conspecific coral hosts was also observed at the same reef as well as significant spatial patterns at a much smaller scale (i.e. when comparing BBD profiles from various reef sites in the NFK; Figure 4C).

LH-PCR profiles of the BBD microbial assemblage also differed with respect to host coral species. The interpretation of observed patterns among BBD profiles from various host species is complex, however, because this variable can be confounded with geographic location. For example, differential BBD prevalence among individual coral species in various locations (Voss & Richardson 2006) can prevent optimal sampling designs. As a result it is difficult to determine the relative contribution of the coral host to the structure of the BBD microbial community. However, geographic location (and most likely the associated environmental drivers in each locale) appears more important than host species in structuring BBD communities. For example, samples from five

different coral host species in the USVI were more similar to one another than to samples from conspecific coral hosts in either of the other two regions. This is in agreement with Frias-Lopez et al. (2004) who observed similar T-RFLP profiles on two host species in one geographic region. In apparently healthy corals distinct microbial communities have been identified on different coral species (Rohwer et al. 2002).

While the results of this study demonstrate that BBD communities are dynamic over relatively short time scales (Figure 4D), there were no clear trends or successional patterns in the relative abundance of various amplicons. Nonetheless, the temporal variability we observed has important implications regarding the community structure of BBD in that the sampling time point may influence both community profiling and clone library results. Despite this temporal variation, time series samples in this study remained clustered among other samples from LSI (Figure 4E). Furthermore, although experimental increases in nutrient availability caused accelerated rates of BBD disease progression and host coral tissue loss (Voss and Richardson, in press), there was no significant observable effect on the structure of the BBD microbial communities.

When comparing standard analysis methods with 1 bp resolution to the strategy suggested by Hewson and Fuhrman (Hewson & Fuhrman 2006), increased discriminatory power was observed among *a priori* sample groups. The frame shift strategy, using maximum similarity between samples, is designed to more conservatively address the null hypothesis that microbial communities do not differ among samples. In the BBD community profile data set using frame shift analysis increased similarity within each sample group. As a result greater ANOSIM Global R-values implied higher levels of

dissimilarity between groups. While the frame shift strategy may be highly effective in data sets where imprecise size calling is apparent, in certain cases, such as that described here, the method may understate community variation within *a priori* groups. Furthermore, use of the frame shift strategy precludes SIMPER analysis and its elucidation of the relative contributions each amplicon makes to dissimilarity between groups.

The SIMPER analyses revealed that the differences observed in BBD community profiles between regions were driven primarily by variation in the amplicons 306, 313, 315, 316, 355, and 356 bp in length. Amplicons of 313 and 316 bp were abundant at LSI, while 315 bp amplicons were more common in NFK samples and USVI profiles were dominated by 306, 316, 355, and 356 bp amplicons. There is evidence that the amplicons we observed in the 313-316 bp range are associated with cyanobacteria and alphaproteobacteria. For example previous studies using *in silico* analyses of sequences from both bacterial isolates and clones from seawater extractions associated V1+V2 amplicons with both cyanobacteria and alphaproteobacteria (Suzuki et al. 1998, Bernhard et al. 2005). Likewise, LH-PCR and sequence analyses of clones generated from BBD infections on two *S. siderea* colonies in LSI revealed both cyanobacteria and alphaproteobacteria with amplicons ranging from 313-316 (Sekar et al. in press). Furthermore, three BBD cyanobacteria from both the NFK (1) and LSI (2), as well as three non-BBD reef-associated cyanobacteria cultured in the laboratory and analyzed separately using LH-PCR, produced amplicons 313-316 bp in length (Ragoonath 2005,

Myers and Richardson, unpublished data). Further examination is needed to identify the species and genera of BBD microbes that are associated with these specific amplicons.

Previous molecular studies have identified multiple cyanobacterial genera associated with BBD infections (Cooney et al. 2002, Frias-Lopez et al. 2002, 2003, 2004). Since no single amplicon indicative of cyanobacteria was present in all samples, this study supports the hypothesis that the presence of any one single cyanobacterial species is not required for BBD to occur. Rather, different cyanobacterial species may possess the physiological capabilities required to function in the dynamic chemical environment of the BBD mat (Carlton & Richardson 1995, Richardson 2004). The presence or absence of these cyanobacteria, and their relative abundance in a single infected coral, may be influenced by such factors as environmental conditions or coral host response. The latter remains a poorly understood aspect of disease dynamics for BBD and other coral diseases.

This study demonstrates that LH-PCR is a robust technique for molecular profiling of BBD microbial communities. Because LH-PCR is a profiling technique that requires no further processing beyond PCR, it has the benefit of eliminating additional time, effort, and biases introduced by post-PCR processing commonly required with other profiling techniques. LH-PCR profiles indicated the BBD microbial communities were variable with a total of 48 amplicons observed in 97 samples from the NFK, LSI, and USVI. The conservative estimates of variability in this study did not include amplicons that contributed < 0.5% to the relative abundance of the entire sample. These amplicons represent additional organisms in the BBD community that were not only

relatively scarce in each individual sample, but also rarely observed among different samples.

In addition to identifying biogeographic trends in BBD microbial communities, the data presented here help explain the discrepancies between results of different molecular microbial community analyses of BBD (Cooney et al. 2002, Frias-Lopez et al. 2002, 2003, 2004, Viehman et al. 2006) Variability in the structure of the BBD microbial community over relatively small spatial and temporal scales may be responsible for the variation observed in BBD clone libraries and profiling efforts from different studies. Rather than contradictory, together these reports may attest to the intrinsic variability and complexity of the BBD microbial community. Further studies that examine not only the identity of BBD microbes, but also the mechanisms of pathogenicity and the potential drivers of variation in the BBD community are important for understanding the dynamics of this detrimental disease.

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Table 1. Results of SIMPER analyses for significant ANOSIM contrasts among regions.

A. Northern Florida Keys (NFK) v. Lee Stocking Island (LSI) **B.** NFK v. US Virgin Islands (USVI) **C.** LSI v. USVI.

Amplicon length (bp)	Mean relative abundance[1]	Mean relative abundance[1]	%[2]	% cummulative[3]
A	**NFK**	**LSI**		
315	41	18	21.4	21.4
316	3	25	16.0	37.4
313	4	21	12.5	49.9
338	8	0	5.2	55.1
B	**NFK**	**USVI**		
315	41	0	23.5	23.5
355	2	26	13.6	37.1
356	4	23	11.5	48.6
316	3	19	10.6	59.2
306	0	11	6.1	65.3
C	**LSI**	**USVI**		
355	1	26	14.9	14.9
356	0	23	13.4	28.3
316	25	19	13.4	41.7
313	21	1	12.1	53.9
315	18	0	10.6	64.5
306	0	11	6.4	70.9

[1] Mean relative abundance of each amplicon as a percentage of total amplicon abundance.

[2] Amplicon contribution as a percentage dissimilarity between the two groups. Lists are truncated to include only those amplicons that contribute >5% to the differences between groups.

[3] Cummulative contributions of amplicons to dissimilarity between groups.

Table 2. Distribution of BBD samples among reef and host coral species in the Northern Florida Keys (NFK), Lee Stocking Island (LSI), and US Virgin Islands (USVI). Significant differences in pairwise ANOSIM comparison between host coral species (among all regions) and reefs sites in the NFK are indicated by pairs with the same superscript letter. CN- *Colpophyllia natans,* DSI- *Dichocoenia stokesi,* DL- *Diploria labyrinthiformis,* DST- *D. strigosa,* MA- *Montastraea annularis,* MC- *M. cavernosa,* MF- *M. faveolata,* MM- *Meandrina meandrites,* and SS- *Siderastrea siderea.*

	Host Coral Species								
Reef Site	CN	DSI	DL	DST	MA[a,b,c]	MC[b]	MF[c,d,e]	MM[d]	SS[a,e]
NFK	-	-	-	-	-	-	-	-	-
Conch[u,v,w]	-	-	-	-	-	-	-	9	-
Carysfort	-	-	-	-	1	-	-	-	-
Davis	-	-	-	-	-	-	-	12	-
Dry Rocks[x]	-	-	-	-	2	-	-	-	-
French	1	-	-	-	-	-	-	-	-
Grecian Rocks[u,x,y,z]	-	-	-	-	5	-	-	-	-
Molasses[v,y]	-	2	-	-	-	-	2	-	-
Watson[w,z]	-	-	-	-	-	-	-	-	4
White Bank	-	-	-	-	-	3	-	-	-
LSI	-	-	-	-	-	-	-	-	-
Big Point	-	-	-	1	-	-	-	-	2
Horseshoe	-	-	-	-	1	-	-	-	31
North Perry	-	-	-	-	-	-	-	-	2
Rainbow	-	-	-	-	-	-	1	-	3
South Perry	2	-	-	-	-	-	-	-	2
Tug & Barge	-	-	-	-	-	-	-	-	3
White Horse	-	-	-	-	-	-	-	-	3
USVI	-	-	-	-	-	-	-	-	-
Hawksnest Bay	-	-	-	-	-	-	-	-	1
Haulover Bay	-	-	1	-	1	1	-	-	-
Watermelon Cay	-	-	-	1	-	-	-	-	-

FIGURE LEGENDS

Figure 1. Black band disease samples were collected from study reefs in the northern Florida Keys (A), near Lee Stocking Island in the Bahamas's Exuma Chain (B), and on the north side of St. John in the US Virgin Islands (C). Reef locations are represented by labeled crosses. NFK: CS = Carysfort South, CO = Conch, DL = Davis Ledge, DR = Key Largo Dry Rocks, F= French, GR = Grecian Rocks, M= Molasses, W= Watson, WB = White Bank. LSI: BP = Big Point, HS = Horseshoe, NP= North Perry, RB= Rainbow, SP= South Perry, TB= Tug and Barge, WH = White Horse. USVI: HB= Haulover Bay, HN= Hawksnest Bay, WC= Watermelon Cay. Note different scales on panels.

Figure 2. Six superimposed LH-PCR electropherograms from triplicate BBD samples and independent PCRs. The low variability in peak presence and amplitude demonstrate the reproducibility of this technique.

Figure 3. Representative LH-PCR profiles: NFK-Grecian Rocks (BBD on *Meandrina meandrites*): LSI - Horseshoe (BBD on *Siderastrea siderea*); USVI- Hawksnest Bay (BBD on *S. siderea*). Means and standard error are shown for the NFK and LSI sample based on four profiles from duplicate extractions and duplicate PCRs. For the USVI duplicates were not available; mean and SE based on triplicate PCR.

Figure 4. Non-metric MDS ordinations of LH-PCR profiles from BBD samples. Distance between samples represents similarity in LH-PCR profiles. Stress in each plot is a goodness-of-fit measure between the plot and the original p-dimensional matrix.

(A) Samples labeled by region. (B) Samples labeled by host coral species. (C) Northern Florida Keys samples labeled by reef site. (D) Samples from the time series/nutrient experiment. In panel D, shaded symbols represent control colonies while open symbols indicate those colonies exposed to elevated nutrients. The numbers within the symbols indicated each 5 day interval (i.e. 1 = 5 days, 2 = 10 days, etc). The shapes represent individual *Siderastrea siderea* colonies in each treatment. (E) Samples from all regions, including those from time series/nutrient loading experiment. (F) Samples analyzed using frame shift method and labeled by region.

Figure 1

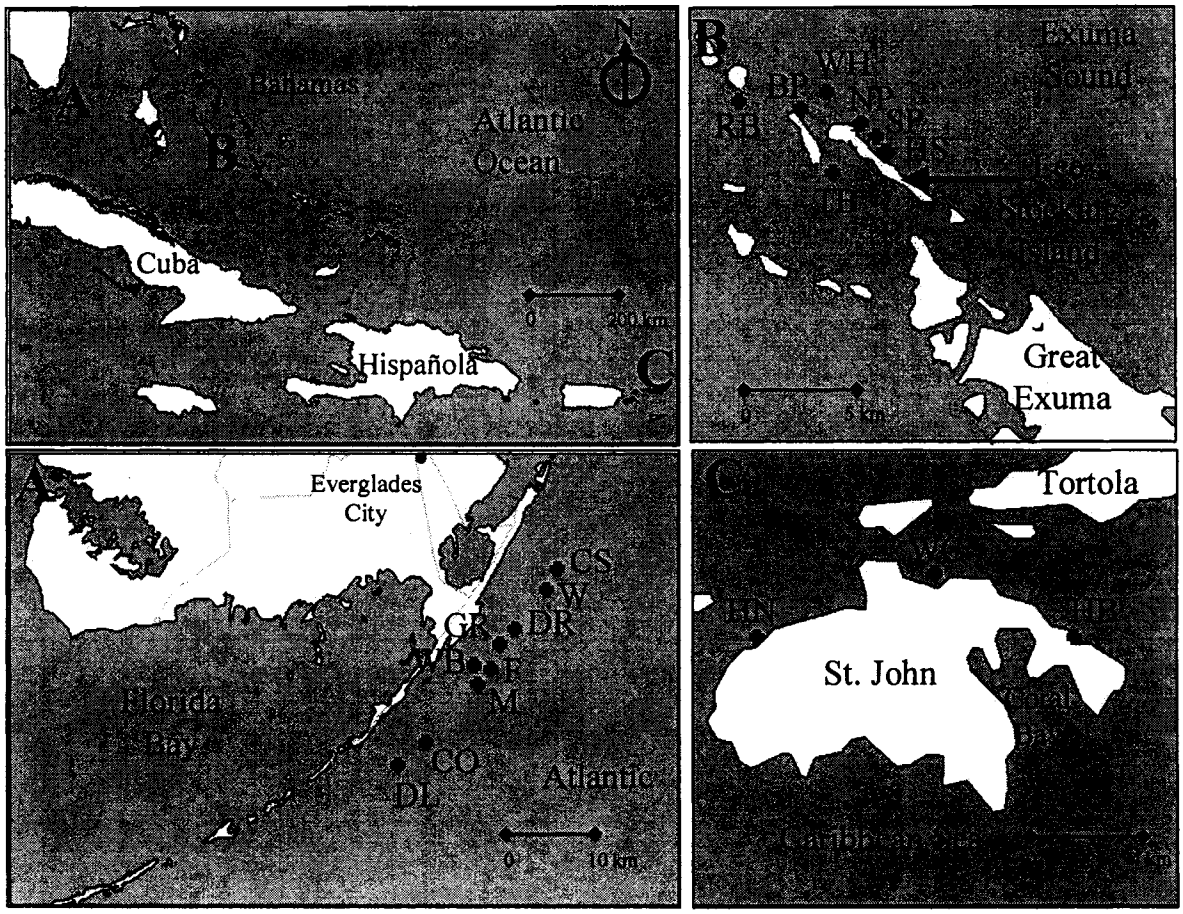


Figure 2

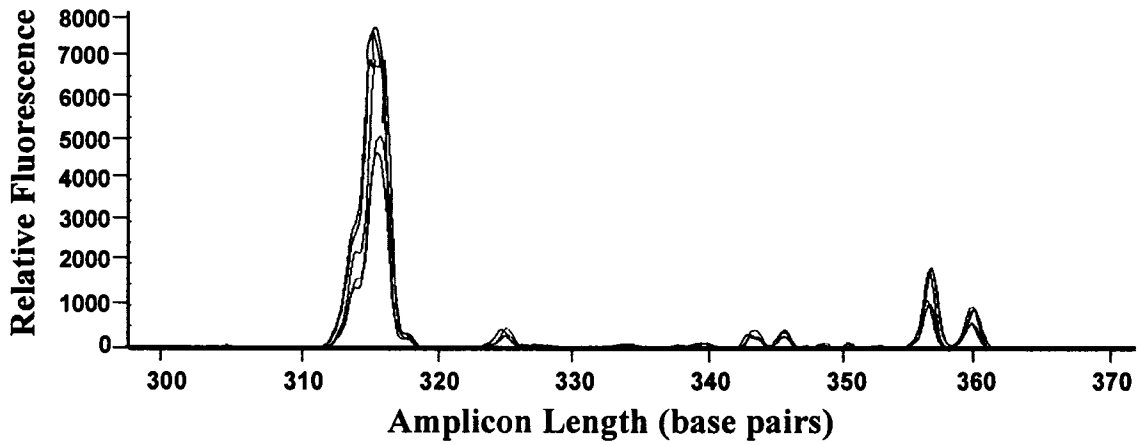


Figure 3

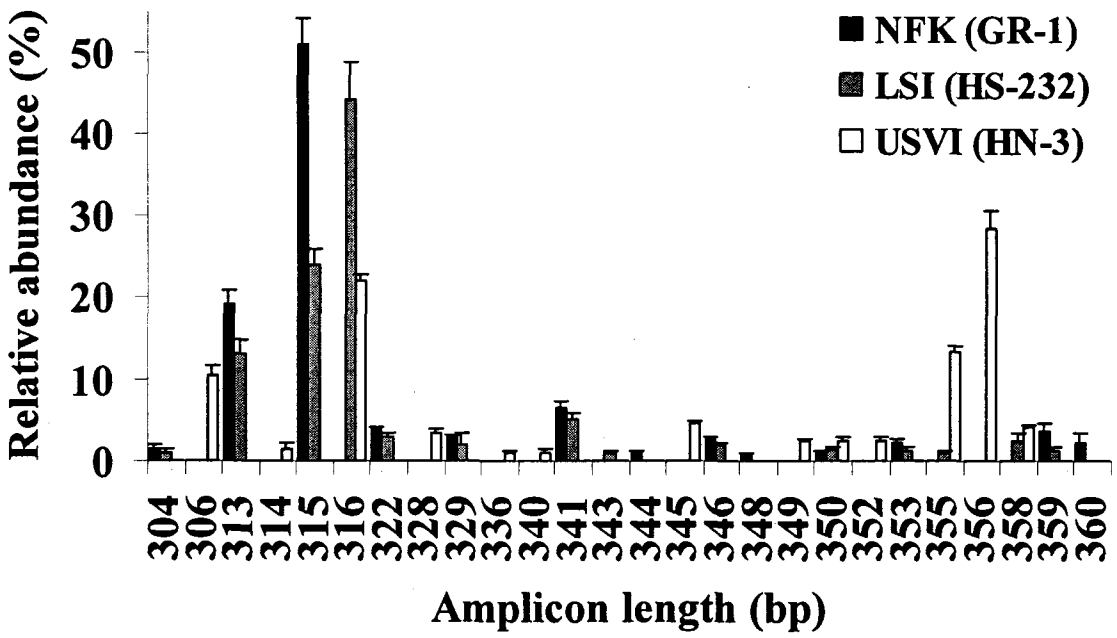
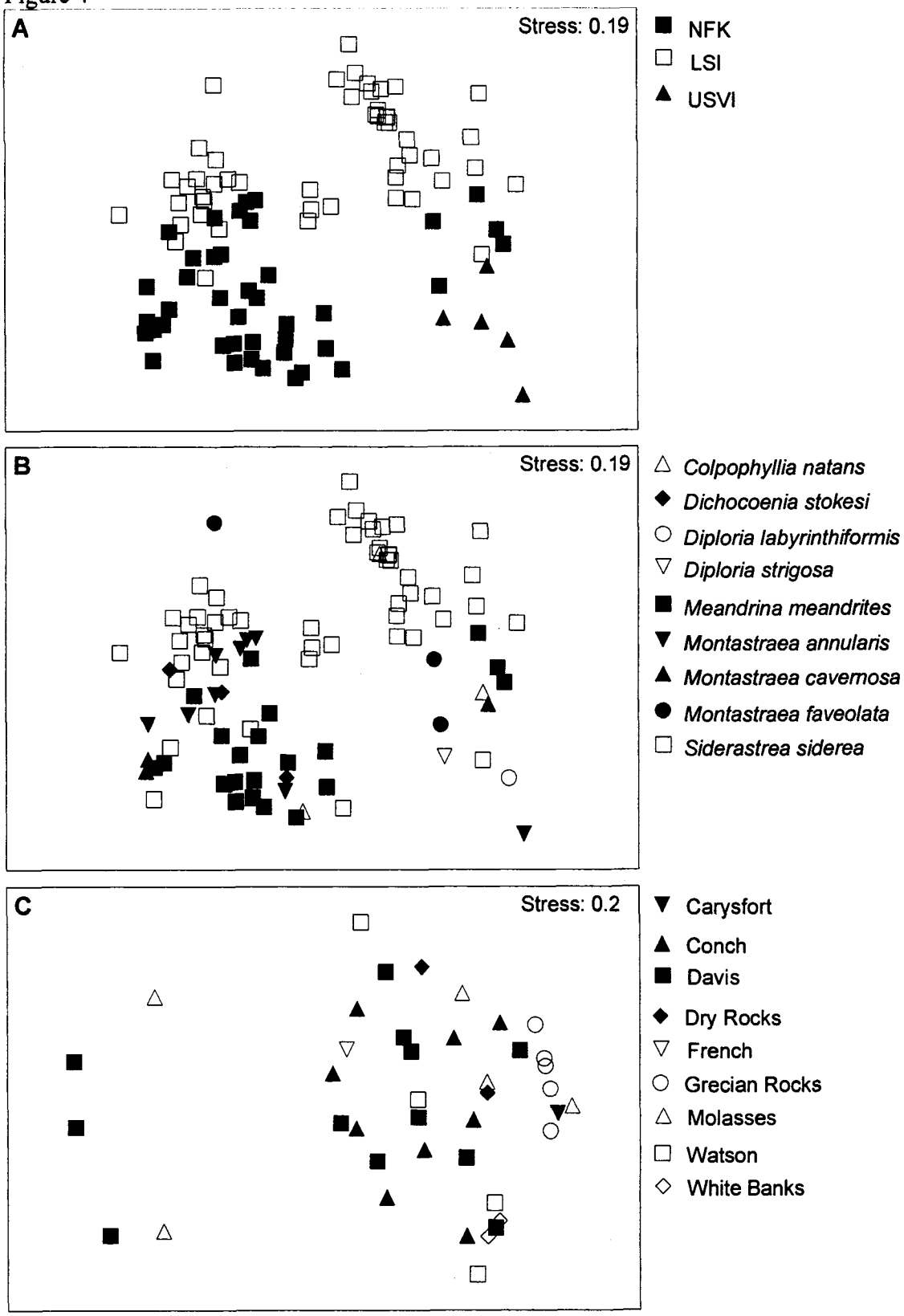
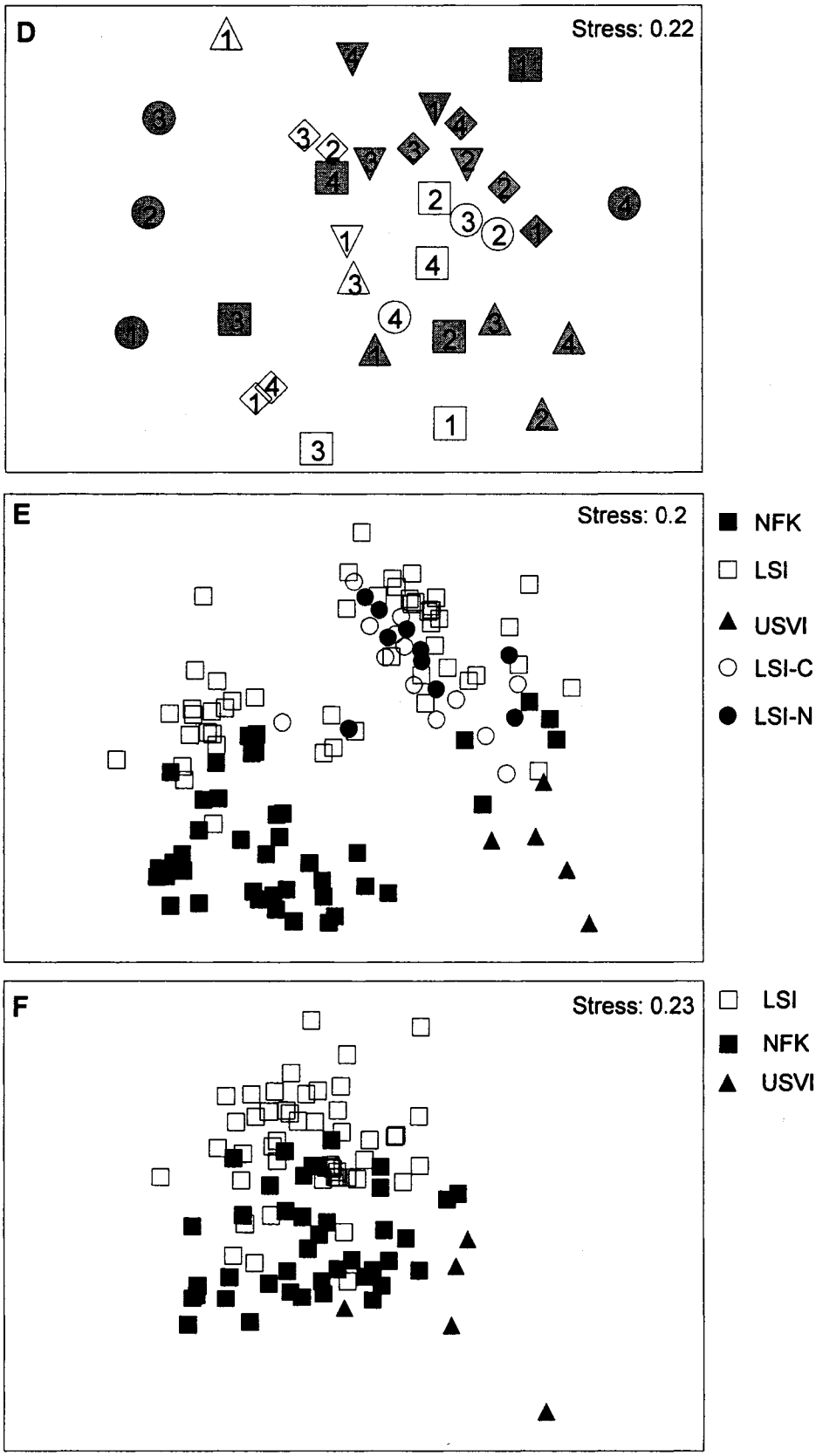


Figure 4





CHAPTER 6

SUMMARY

Coral reefs worldwide are under a real and perilous threat from pathogenic diseases. Studies have demonstrated that the number of coral diseases, the coral species they infect, the number of reported cases, and the range over which they are distributed have all increased rapidly over the past three decades. These diseases have resulted in losses of coral cover, losses of reproductively viable individuals, and in a least one case, lasting community phase shift. While there are clearly numerous threats to the health of coral reef ecosystems from global to local scales, numerous reports, including those presented here, suggest that diseases should be considered as significant agents of coral decline.

The studies presented here combine data regarding coral diseases from extensive field surveys, experimental manipulations both *in situ* and in the laboratory, and molecular investigation of microbial community structure. These reports demonstrate that coral diseases impact reefs in locations with both high and low levels of anthropogenic influence, i.e. the Northern Florida Keys (NFK) and Lee Stocking Island (LSI) in the Bahamas' Exuma Chain, respectively. Comparative studies across space and time, such as those presented here, can help elucidate the dynamics of coral diseases as well as their past and potential effects on reef communities.

In Chapter two, I examined patterns of coral disease near LSI over two consecutive summers in 2002 and 2003. Similar to previously published reports,

relatively large, framework species including *Siderastrea siderea*, *Colpophyllia natans*, and *Montastraea annularis*, along with the relatively smaller *Dichocoenia stokesi*, were the species most susceptible to coral disease near LSI. Recurring infections observed on individual colonies from 2002 to 2003 were more likely for black band disease (BBD) than for either white plague (WP) or dark spots syndrome (DSS). In 2002, WP and DS demonstrated clumped distributions, while BBD was randomly distributed. However, in 2003 BBD and WP were clumped. These clumped distributions are indicative of infectious models of disease transmission. This is the first study, to my knowledge, that quantitatively documents the prevalence and severity of coral diseases on reefs surrounding LSI.

My observation of BBD cessation on corals when temperatures dropped below 27.5°C, and its subsequent return as temperatures warmed, support previous hypotheses that temperature tightly controls disease activity. I found that the effect of temperature on BBD occurrence is differential depending on the host coral species. BBD infections found on *C. natans* were more sensitive to drops in temperature than infections found on *S. siderea*. The observation of increased sedimentation rates on sites with disease as compared to healthy sites supports the hypothesis that sediments may play a role in coral infections, although a causal relationship has not been established.

The results presented in Chapter Three indicate that total disease prevalence was greater in the NFK than LSI during my sampling periods in both 2002 and 2004. I observed variability in host species susceptibility across space and time. *M. annularis* and *Meandrina meandrites* were more commonly infected in the NFK,

while *S. siderea*, along with *D. stokesi*, were most susceptible to disease in LSI. DSS lesions were found exclusively on *S. siderea* in both regions. To assess the impact of nutrient availability on coral disease prevalence dissolved inorganic nitrogen (DIN) and total phosphorous (TP) were selected as proxy variables, based on correlations with all recorded nutrients (also nitrate, nitrite, ammonium, and soluble reactive phosphate). Regression analyses indicated significant positive correlations between DIN and BBD prevalence in both the NFK and LSI. The results of this study suggest that efforts to protect locally susceptible species by reduction of nutrient inputs may help to mitigate the effects of disease on coral communities.

In Chapter Four I report that experimental increases in localized nutrient availability using commercial time release fertilizer *in situ* resulted in doubling of BBD progression and coral tissue loss in the common reef framework coral *S. siderea*. Laboratory experiments in which artificially infected *S. siderea* colonies were exposed to increased nitrate concentrations (up to 3 μM) demonstrated similar increases in BBD progression as compared to controls exposed to ambient nutrient levels. The mechanisms that enhance BBD migration rates under increased nutrient exposure are not yet understood but may be related to the fact that BBD is a complex microbial infection. These findings provide direct evidence that the impacts of this disease on coral populations are exacerbated by nutrient enrichment and that management to curtail excess nutrient loading may be important for reducing coral cover loss due to BBD.

In Chapter Five analyses of the LH-PCR profiles using multivariate ordination methods and analysis of similarity indicated much variation in the BBD-associated bacterial communities across space and time. While some overlapping similarity occurs, distinct microbial assemblages were associated with BBD infections in three different Caribbean regions: the NFK, LSI, and USVI. At smaller spatial scales, I observed significant reef-to-reef variation among BBD bacterial assemblages in the NFK. Because coral species susceptibility was variable among regions, interpretation of observed patterns among BBD profiles from various host species was complex as this variable was confounded with geographic location. Differences among BBD microbial community profiles from LSI, NFK, and USVI, as well as site-to-site variation in the NFK, were driven primarily by variation in relative abundance of 313-316 bp amplicons.

In addition to identifying biogeographic trends in BBD microbial communities, the data presented in Chapter Five may help explain the discrepancies between results of different molecular microbial community analyses of BBD. Variability in the structure of the BBD microbial community over relatively small spatial and temporal scales may be responsible for the variation observed in BBD clone libraries and profiling efforts from different studies. Rather than contradictory, together these reports may attest to the intrinsic variability and complexity of the BBD microbial community. Further studies that examine not only the identity of BBD microbes, but also the mechanisms of pathogenicity and the potential drivers of variation in the BBD community, are important for understanding the dynamics of this detrimental disease.

Substantial efforts to identify coral pathogens, elucidate etiology, and understand and reduce the impacts of disease are necessary to give hope to conservation initiatives and potentially slow coral decline. The studies presented here contribute to our knowledge of the species and areas most susceptible to coral disease in the NFK and LSI, and facilitate predictions regarding potential changes in coral reef communities. Additionally they provide information concerning the environmental drivers and describe structural variation in the BBD microbial community. Continued efforts to integrate these various aspects of coral disease biology, both within and between research groups, is recommended to most effectively expand knowledge in the field of coral epizootiology.

VITA

JOSHUA DANIEL VOSS

December 19, 1978	Born, Louisville, Kentucky
1999	Bookhout Fellowship, Research Assistant Duke University Marine Laboratory Beaufort, North Carolina
2000	Perry Institute for Marine Science Intern Caribbean Marine Research Center Lee Stocking Island, Bahamas
2001	B.S. Biology, Magna cum Laude Outstanding Senior Biology Major Elon University Elon, North Carolina
2001-2006	Doctoral Student in Biological Sciences Florida International University Miami, Florida
2001-2005	Graduate Teaching Assistant Florida International University Miami, Florida
2002-2004	Science to Achieve Results MAI Fellowship United States Environmental Protection Agency
2002-2005	National Oceanographic and Atmospheric Administration/ Perry Institute for Marine Science Program Development Grants Caribbean Marine Research Center Lee Stocking Island, Bahamas
2004	Graduate Research Grant Smithsonian Tropical Research Institute Bocas del Toro, Panama
2004-2005	Budweiser Conservation Fellowship National Fish and Wildlife Foundation
2005-2006	Dissertation Year Fellowship Florida International University Miami, Florida

2006	Biomedical and Comparative Immunology Symposium Student Presentation Award Florida International University Miami, Florida
2006	L.L. McGee Outstanding Scientific Diver Award Florida International University Miami, Florida

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