2007-12-11

Coral Disease Epizootiology in the Florida Keys (U.S.A.) and Cayman Islands (British West Indies), and the Development of the Simulation of Infected Corals Model

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By

Marilyn Elizabeth Brandt

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2007

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
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Coral Disease Epizootiology in the Florida Keys (U.S.A.) and Cayman Islands (British West Indies), and the Development of the Simulation of Infected Corals Model

(December 2007)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor John W. McManus.
No. of pages in text. (180)

Understanding coral disease dynamics within the heterogeneous populations in which they act is critical for predicting how the structure of reefs may change as a result of enzootic or epizootic levels of these important sources of mortality. This work focused on combining field studies and the development and testing of a spatially-explicit, individual-based epizootiological computer model with the aim of gaining a greater understanding of the dynamics and impact of white plague, a significant source of mortality on reef-building corals in the Caribbean region. Field studies focused on the incidence and distribution of all sources of coral mortality, including suspect white plague *in situ*, at two locations; the Florida Keys (United States of America) and Little Cayman Island (Cayman Islands, British West Indies). Results indicated that in both regions disease was the most significant source of mortality during the monitoring time periods, and that suspect white plague type II in Cayman is likely contributing to major structural changes. In Florida, observations made during a mass bleaching event indicated that a significant relationship exists between bleaching severity and disease incidence, and that mortality during the event was largely the result of disease and not bleaching. The simulation model was developed using a long-term data set from Little Cayman, and

results of calibration indicated that suspect white plague type II on these reefs is transmissible between colonies within a limited field and require a yearly input from an outside source, and that host susceptibility to infection is low and likely not variable among species. Parameters describing the distribution and composition of the coral population were varied, and results indicated a significant effect of colony density, aggregation, and mean size on the impact of disease. Scenario testing of various disease management strategies indicated that should local prevention measures be developed in the future, it is they, and not treatment, that will likely be the most effective in limiting the impact of disease.

To my family,
Mom, Dad, Will, and Ed,
who have supported me through everything

Acknowledgements

My committee: Dr. John McManus, Dr. Mike Schmale, Dr. Esther Peters, Dr. Peter Glynn, Dr. Bernhard Riegl, and Dr. Don DeAngelis

Aletta Yñiguez, for being an incredible friend

Dr. Carrie Manfrino and Dr. Robert Ginsburg, who inspired me and got me here

My support in and out of the field in Florida:
Tyler Smith
Erica Key
Wade Cooper
Cara Cooper
Felimon (Nonong) Gayanilo
Adrienne (Romanski) Simoes Correa
Johnathan Kool
Rick Gomez
Alison Moulding
Michelle Paddack
Kirsten Grorud-Colvert
Jill Borger
The Keys Marine Laboratory staff (Lisa, Lonny, Todd, Chris, Steve)
The FKNMS staff, especially Don Mooney and Bill Valley
CREM lab interns: Paige Smith, Lynsay Callies, Casey Gioia, Tom Murphy

My support in and out of the field in Cayman:
Jon Clamp
Peter Hillenbrand
Walter Rhian and family
Vania Coelho
John Bothwell
CCMI staff and interns, especially Alexander Soranno and Koby Elias
Staff of the Little Cayman Research Centre
DWAR program participants, especially Diana (Dina) Schmitt and Jim Kennedy

My funding sources:
The RSMAS fellowship
The National Center for Coral Reef Research (NCORE)
The Central Caribbean Marine Institute (CCMI)
The International Society for Reef Studies & Ocean Conservancy
The Project Aware Foundation
The Propeller Club of the Port of Miami
The Southern Cross Club, Little Cayman
Head O' Bay, Little Cayman

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

The research presented herein is an epizootiological examination of the disease white plague on scleractinian corals in two regions, the Cayman Islands (British West Indies) and the Florida Keys (U.S.A.) (Figure 1), and a description of the development and first application of the Simulation of Infected Corals (SICO) model, a spatially-explicit, individually-based epizootiological coral disease model. Additional information is presented on other coral diseases and on their relationships to coral bleaching. Worldwide, the emergence and spread of marine diseases has increased, and the need for understanding the dynamics and impact of these syndromes at the population level is essential to their containment and prevention, and ultimately to the conservation of the ecosystems in which they are present.

Coral reefs are among the most biologically diverse and economically significant ecosystems on the planet. However, the future of coral reefs is uncertain. Scleractinian zooxanthellate corals (i.e., reef-building corals), which both literally and figuratively form the base of these valuable ecosystems, have experienced considerable losses in the Caribbean in the last three decades (Gardner et al. 2003). Increased stress on reefs stemming from both natural (e.g., hurricanes, El Niño effects) and anthropogenic (e.g., sedimentation, eutrophication, overfishing, habitat destruction) sources has been correlated to an increase in coral disease (Harvell et al. 1999, Ward & Lafferty 2004), which is thought to be a significant contributor to these losses (Porter & Meier 1992, Porter et al. 2002, Sutherland et al. 2004, Weil 2004, Aronson & Precht 2006). The threat of coral disease is of increasing concern as pressure from human populations intensifies

and climate change alters the general physical characteristics of the marine environment (Harvell et al. 2002, Harvell et al. 2004).

Coral Disease

Corals, like any other organism, are susceptible to infectious diseases (Rosenberg & Loya 2004). However, several characteristics of corals make them especially sensitive to the influences of infectious disease. Corals are sessile benthic dwellers and do not have the ability to change their position if the environmental conditions surrounding them deteriorate, which could potentially lead to physiological stress and a decline in their overall health. Additionally, corals are invertebrates, possessing only innate immunity characteristics while lacking the ability to produce cells specific to particular pathogens, otherwise known as adaptive immunity (Mullen et al. 2004). Instead, corals rely mainly on such immune responses as the production of microbe-catching mucus, the mechanical activity of cilia and tentacles, and general phagocytosis of foreign objects by amoeboid cells to defend against disease (Bigger & Hildemann 1982).

As such, it is surprising that coral diseases were only first recorded in the early 1970s with the identification of black-band disease by Antonius (1973). However, since then, 29 syndromes have been reported. Of these, only a few have been intensively characterized and the extent of their impact remains in debate (Green & Bruckner 2000, Weil et al. 2002, Sutherland et al. 2004). For those diseases for which pathogens have been characterized, the causal agents have primarily been bacterial (Richardson et al. 1997, Richardson et al. 1998b, Patterson et al. 2002). One exception is the Caribbean sea-fan disease, aspergillosis, which is known to be caused by the terrestrial fungus *Aspergillus sydowii* (Smith et al. 1996, Petes et al. 2003). As yet, none of the identified

coral diseases have been attributed to a virus. Alternatively, others have suggested that what have been previously thought to be infectious coral diseases may instead be the result of predation (Bruckner & Bruckner 1998), or secondary infections and/or general reactions to increasing levels of stress (Lesser et al. 2007). This is supported by the recent finding that rapid tissue loss lesions on table acroporid corals (white syndrome) on Australian reefs were not associated with significant microbial activity but rather programmed cell death (Ainsworth et al. 2007b).

Disease is the result of the interaction between host, pathogen or pathogenic agent (i.e., an organism or agent "capable of causing disease"(Last 2001)) and the environment. The incidence (i.e., rate of occurrence within a population) and distribution of disease is directly based on this interaction, and the interaction of hosts at the population level. Therefore, understanding the factors influencing each component of these interactions is critical to predicting and ultimately containing the spread and impact of disease. Unfortunately, an understanding of the etiology and epizootiology of the various coral diseases has been elusive for several reasons, including the rare and patchy nature of the incidence of coral disease; a deficient understanding of coral defenses (humeral, cellular and mechanical); the difficulty involved in the *in situ* distinction between disease-related tissue loss and other externally-induced mortality, as well as between similar mortality manifestations caused by different pathogenic agents; the complexity involved in culturing potential pathogenic marine organisms; a lack of coral culture facilities able to provide genetically uniform experimental animals for infection and transmission studies; and the potential influence of other components of the coral holobiont including the

endosymbiotic microalgae and microorganisms colonizing the coral's surface (Kuta & Richardson 2002, Nugues 2002, Harvell et al. 2004).

Epizootiological Modeling

Despite the perceived sporadic incidence and random distribution of disease in the environment, information on the factors and interactions leading to disease can be inferred from an understanding of the patterns of its emergence and distribution in a population. John Snow, considered the founder of the field of epidemiology, was able to determine through the spatial mapping of infected individual's homes a significant correlation of the deadly disease cholera with contaminated drinking wells, thereby identifying cholera as a waterborne transmissible disease (Snow 1855). Since that time, inferring process from pattern has been an essential part of epidemiological and epizootiological investigations, and modeling has been the main tool with which to accomplish this task.

Modeling coral disease requires the incorporation of spatial heterogeneity of coral distribution, both within and among populations. Recent increases in computer processing power have allowed the more widespread application of simulation modeling in the fields of epidemiology and epizootiology (Bagni et al. 2002), thereby allowing populations and disease infection dynamics within them to be modeled in a spatially-explicit context that more closely approximates reality. This need to incorporate a spatial context when modeling population characteristics of corals has been recognized for several decades (Maguire & Porter 1977).

Epizootiological modeling provides a means for addressing what has not been possible before in coral disease research, namely the ability to test hypotheses of disease

spread and distribution at a reef scale. Spatially-explicit, individual-based simulation modeling specifically is uniquely capable of incorporating several key features of coral reef communities (i.e., multiple species and their characteristics, spatial distributions) that are not possible with other types of modeling. This project represents what appears to be the first attempt to apply a field-based individual-based epizootiological modeling to a coral disease, with the main objective of increasing an understanding of the dynamics of white plague disease, an important source of coral mortality in the Caribbean region.

Rapid tissue loss and the coral "plague" diseases

Rapid tissue loss in corals was first characterized as a disease by Dustan in (1977), who coined the term "white plague" to describe what he had observed on corals in the Florida Keys. Since then, terminology such as "white plague", "plague type X", and "plague-like" have been used to describe common signs of rapid tissue loss on scleractinian zooxanthellate corals in the Caribbean not of the genus *Acropora*. Similar signs recorded on species of the genus *Acropora* in the Caribbean have been given a separate designation, "white band" (Gladfelter 1982, Peters 1984, Ritchie & Smith 1998). More recently, "plague-like" signs have presented themselves on corals in other parts of the world (Barash et al. 2005, Ainsworth et al. 2007b). All of these conditions present as a sharp line of tissue loss delineating the intersection between live tissue and denuded white skeleton, that travels rapidly (on scales of mm to cm/day), and typically begins at the base or margin of the colony (Bythell et al. 2004). While the signs are similar, in some cases they have been attributed to multiple causes (Ainsworth et al. 2007a).

One of the most commonly noted plague diseases is "white plague type II", discovered in 1995 affecting colonies in the Florida Keys, whose defining signs are tissue

loss that initiates basally and progresses at a rate averaging 2 cm/day (Richardson et al. 1998a, 1998b). This rate is so rapid that small corals will often experience total mortality in a matter of days. Algal colonization of the denuded skeleton left behind after tissue loss is much slower than the advancing front of tissue mortality and therefore affected corals are generally easily identifiable by the presence of extensive recent mortality (Figure 2). When first identified, a Gram-negative bacterium related to the genus *Sphingomonas* was described as the etiologic agent of white plague type II through the fulfillment of Koch's postulates in a laboratory experiment (Richardson et al. 1998b). Subsequent work showed that this bacterium was of a new genus and species and was classified as *Aurantimonas coralicida* (Denner et al. 2003). Later, other work in the Caribbean region found no evidence of *A. coralicida* in the diseased tissue, despite that the authors identified the signs as consistent with that defined for white plague type II (Pantos et al. 2003). As is typical in pathology, similar disease signs may be the result of different pathogenic agents, and gross observations *in situ* is not enough to distinguish different syndromes. Today, few studies have combined field observations with laboratory microbiological and histopathological analyses to identify whether *A. coralicida* is present in corals having active signs of white plague type II. Work is therefore ongoing to distinguish whether similar disease signs are the result of different pathogens, and it is necessary to consider this in any study of these disease signs in the field.

In 1995, white plague type II appeared to be most severely affecting corals of the species *Dichocoenia stokesi* in the Florida Keys (Richardson et al. 1998a, Richardson et al. 1998b). Today, suspect white plague type II (based on gross signs only) and other

types of white plague (similar gross signs but slower or more rapid tissue loss rates) are known to affect coral species from multiple families over a large geographic extent and the most affected species seem to be the major reef-building genera, *Montastraea, Diploria,* and *Siderastrea* (Green & Bruckner 2000, Sutherland et al. 2004), in addition to *Dichocoenia*. The typical prevalence of white plague-like signs across the Caribbean region appears to be low (Weil et al. 2002); however, occasional epizootics have been shown to have a significant impact on isolated coral communities (Nugues 2002, Richardson & Voss 2005). These factors have caused great concern within the scientific and management communities regarding the overall impact of rapid tissue loss on the species composition of coral reefs in the Caribbean.

Techniques to identify the causal agent of "plague-like" signs in Little Cayman and the Florida Keys were not undertaken here, as this study was initiated only as a quantitative study that aimed to contribute to the understanding of the disease sign's epizootiology and provide a tool for predicting the impact of disease on Caribbean reefs. Therefore, field identifications were made based on published descriptions and training received by the author during two coral health and disease training workshops. During field studies, affected colonies were monitored weekly during defined periods to determine tissue loss rates. Methods involved in this monitoring are described in depth in the following chapters, but the results of the observations indicated that white plague-like signs observed in Little Cayman differed from those encountered in south Florida. Affected colonies in Little Cayman experienced tissue loss on the order of cm/day and were observed to have large areas of recently denuded skeleton with little algal colonization (Figure 2). These signs are consistent with what has been described for

white plague type II (Richardson et al. 1998a, b). Affected colonies in south Florida showed tissue loss rates on the order of mm/day and exhibited a smaller region of un-colonized recently dead skeleton near the tissue loss interface (Figure 3), consistent with what was originally described as plague by Dustan (1977) and later classified as white plague type I (Bythell et al. 2004).

Summary

It is critical to attempt an understanding of coral disease dynamics to predict potential changes in the future and potentially contain or prevent further outbreaks. This work combines field studies and the development and testing of a spatially-explicit, individual-based epizootiological computer model with the aim of investigating the dynamics and impact of white plague, a significant source of mortality on scleractinian corals in the Caribbean region. Field studies focused on the incidence and distribution of all sources of coral mortality, including suspect white plague *in situ*, at two locations; the Florida Keys (United States of America) and Little Cayman Island (Cayman Islands, British West Indies). Described herein is the epizootiology of suspect white plague type II for the forereefs of Little Cayman Island and suspect white plague type I for two coral habitats off the upper and middle Florida Keys. The simulation model was specifically developed to test scenarios of the incidence of suspect white plague type II and its consequent impact on coral communities of Little Cayman Island. Consequently, the components and operations of the model and its application to Little Cayman reefs are described in chapters 3 and 4 of this volume. The overall goal of developing this model is to provide an opportunity to examine several aspects of coral disease dynamics that have not been

possible with standard field observations, thereby bringing key concepts from the established discipline of epidemiology to the field of coral disease research.

The emergence and spread of multiple new syndromes, combined with the recent alteration of coral reef communities around the Caribbean, has led to concern within the scientific and management communities that disease may change the structure and function of coral reef ecosystems as presently known. Understanding coral disease dynamics has become increasingly urgent as environmental changes from human activities might increase the virulence, rate of spread, and geographic distribution of diseases within the next few decades. This study has resulted in the development of a tool to study suspect white plague dynamics under varying scenarios. Hopefully this contribution will advance our state of knowledge of this important source of mortality and its role in shaping the reefs of today and tomorrow.

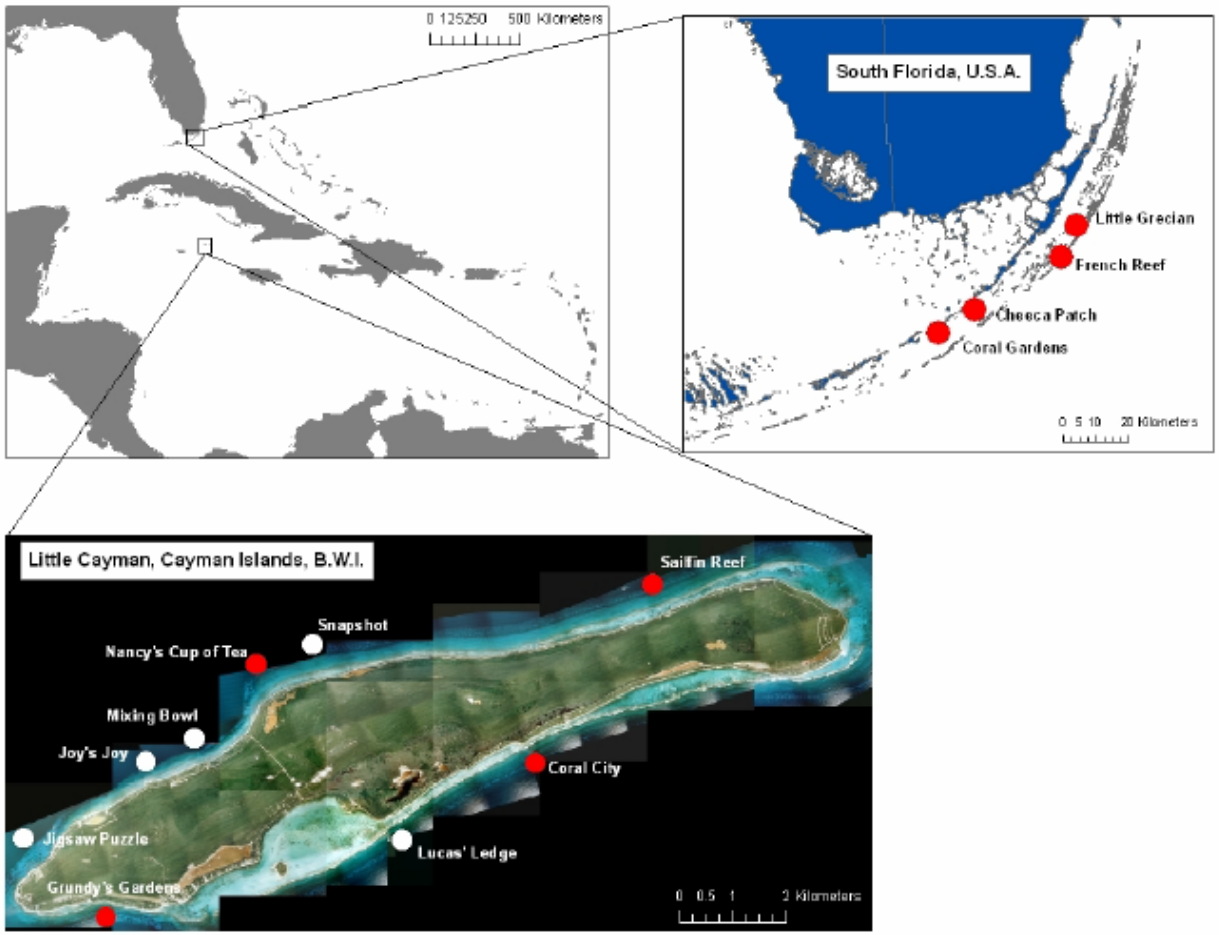

Figure 1: A map of study sites in Little Cayman (Cayman Islands, B.W.I.) and south Florida (U.S.A.). Red dots indicate sites at each of which 3 (Cayman) and 5 (Florida) 16m^2 permanent monitoring quadrats were installed in 2004. White dots on the Little Cayman map indicate sites where other sampling methodologies were used (Chapter 2, Table 1). In Cayman, Nancy's Cup of Tea, Mixing Bowl, Joy's Joy, and Grundy's Gardens all lay within the Cayman Islands Department of Environment's designated marine park areas. In Florida, French Reef and Cheeca Patch are both "Special Protected Areas" (SPAs) as designated by the Florida Keys National Marine Sanctuary; however quadrats were installed outside of the protected zone at Cheeca Patch.

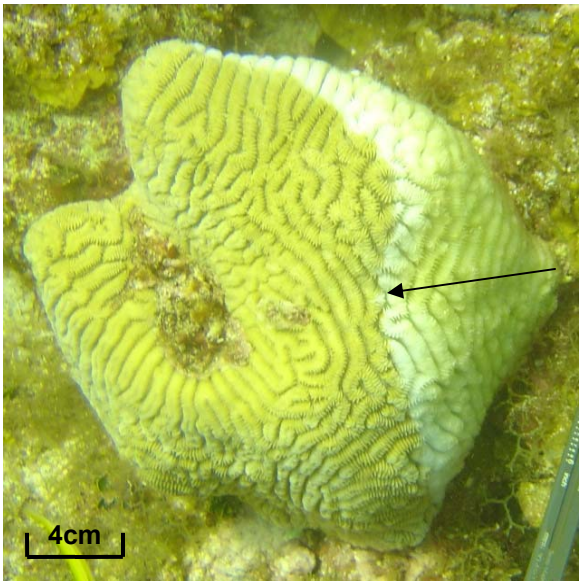

Figure 2: Advancing tissue loss (assumed direction of lesion progression indicated by arrow) on a colony of *Meandrina meandrites* attributed to suspect white plague type II (SWP2). Photograph taken July 6th 2006, at 7.5 m depth at Nancy's Cup of Tea dive site, Little Cayman, Cayman Islands, B.W.I.

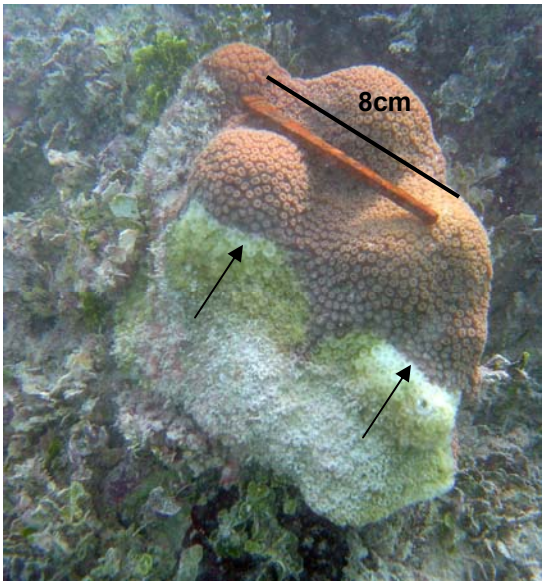

Figure 3: Advancing tissue loss (assumed direction of lesion progression indicated by arrows) on a colony of *Montastraea faveolata* attributed to suspect white plague type I (SWP1). Photograph taken November 9th 2005, at 3 m depth at Cheeca Patch (inshore patch reef), Florida Keys, U.S.A.

Chapter 2: Coral community change and the epizootiology of the coral disease white plague in Little Cayman, Cayman Islands, British West Indies

INTRODUCTION

Caribbean coral communities have recently experienced significant changes including a dramatic loss of live coral cover (Gardner et al. 2003). These changes have in some cases been associated with causes both natural (e.g. hurricanes, El Niño effects) and anthropogenic (e.g. sedimentation, eutrophication, habitat destruction, overfishing). However, the recent emergence of new syndromes causing reef-building coral tissue loss has also been implicated as a contributor to the alteration of Caribbean reef structures (Porter & Meier 1992, Aronson & Precht 2001, Porter et al. 2002, Weil et al. 2002, Sutherland et al. 2004).

The significance of emerging coral diseases in the Caribbean is a highly contested issue. The impact is thought to be great on the scale of the individual colony, as the rate of tissue loss on a colony due to disease greatly exceeds rates of tissue growth (Hayes & Goreau 1998). How this impact is translated to the level of the coral community and the reef overall is difficult to determine due to the high variability of coral disease in both time and space. In one instance, an "outbreak" of black band disease in the back-reef environment of Jamaica was documented to have affected a large number of the reef's dominant species of scleractinian zooxanthellate corals. However, this outbreak was limited both spatially and temporally and the back-reef community at large was not altered (Bruckner & Bruckner 1997). In contrast, white band disease has contributed significantly to widespread losses of *Acropora cervicornis* and *A. palmata* throughout their historic range in the tropical north Atlantic region (Aronson & Precht 2001). The impact of this disease has been a radical alteration of the shallow marine environments of

the Caribbean as these two species are major reef-builders on many shallow reefs. In both cases, it was difficult to determine how rates of disease mortality on individual colonies translated to an impact on the overall community until the "end" of the outbreak (Aronson & Precht 2006).

The coral disease identified as white plague is also thought to be severely impacting scleractinian corals throughout the Caribbean (Feingold & Richardson 1999, Nugues 2002) and possibly in other parts of the tropics (Dalton & Smith 2006, Ainsworth et al. 2007). This disease appears as a characteristic pattern of tissue loss that can progress rapidly and is easily identifiable by the appearance of large areas of recently dead coral skeleton (Figure 1). Rates of tissue loss attributed to suspect white plague "type II" (the most common type of plague recorded) range between 2 and 10 linear centimeters (cm) per day, making it one of the most acute causes of tissue loss recorded on scleractinian corals (Sutherland et al. 2004). In contrast to white band disease, which is known to affect only two species, white plague type II has been recorded on a wide range of species, although its overall impact appears to differ between species and regions (Weil et al. 2002). For example, in Florida Richardson *et al.* (1998a) reported white plague type II to be most commonly affecting *Dichocoenia stokesi* (Milne Edwards & Haime 1848) and Borger (2005) reported it primarily on *Diploria strigosa* (Dana 1848). In Puerto Rico, however, Bruckner and Bruckner (1997) found it predominantly on *Diploria labyrinthiformis* (Linnaeus 1758). In St. Lucia, *Montastraea faveolata* (Ellis & Solander 1786) and *Colpophyllia natans* (Houttuyn 1772) were most severely affected (Nugues 2002), while in Dominica, *Siderastrea siderea* (Ellis & Solander 1786) is the main host of the disease (Borger 2003). With the exception of *D. stokesi*, all of these

species are framework builders of Caribbean reefs, and this leads to concern that coral mortality due to white plague has the potential ability to alter the structure of reefs.

Monitoring data from Little Cayman Island, the smallest and least populated of the three-island nation of The Cayman Islands, British West Indies, are presented here, focusing on the decline of live coral tissue and the epizootiology of suspect white plague.

SPECIES AND METHODOLOGY

In June of 1999, Little Cayman Island was host to an Atlantic and Gulf Rapid Reef Assessment (AGRRA) survey (Manfrino et al. 2003, Pattengill-Semmens & Semmens 2003) for which the author was a participant. The methods of AGRRA include a benthic assessment protocol that uses 10-m transects to collect measures of coral cover (measured as the percent of the available hard substrate occupied by live coral), and coral densities, community characteristics, size distributions, partial mortality, and the prevalence of several described coral diseases (Kramer & Lang 2003). With the establishment of a monitoring program by the Central Caribbean Marine Institute (CCMI) in June, 2002, four of the sites surveyed in 1999 were re-surveyed with the AGRRA protocol and were again re-surveyed in February, 2004 by the author and employees of CCMI. The four sites are distributed around the island, with two each on the leeward and windward facing sides (Chapter 1, Figure 1). At three of these sites and one other site, permanent quadrats were installed by the author in July of 2004 and were repeatedly monitored for the incidence of disease and mortality for three consecutive weeks. Sites were revisited and repeatedly monitored again for three weeks in 2005 (June-July). Sites were revisited for the last time in July of 2006 although funding and time constraints prohibited weekly monitoring at that time. All sites and an additional four were also used to collect data on

the spatial distribution of suspect white plague in June of 2005. Site names and locations are shown in figure 1 of chapter 1, and are also listed in Table 1. Methods used are described in more detail below.

AGRRA survey methods (1999, 2002, 2004)

In 1999, survey sites were selected from known dive sites so that their distribution would encompass the entire shelf and be relatively evenly spaced around the island. Time and resources limited the number of sites that could be resurveyed in 2002 and 2004; therefore a sub-sample of sites was made so that as spatially wide a range of sites as possible was sampled. Each year, at least 6 10-m line transects were assessed at each site by a trained diver on SCUBA. These transects were haphazardly placed perpendicular to shore so that transects would lay parallel with spurs on the spur and groove formations and when possible avoid sandy substrate. For each transect, the diver first estimated live coral cover by recording the length of live coral on potential hard substrate directly underneath the transect line to the nearest 10 cm and substrate occupied by sand was subtracted from the total length of the transect On a second pass, any coral colony greater than 10 cm in diameter that lay directly under the transect line was independently assessed. These colonies were identified to species-level, the maximum height and diameter were measured to the nearest 5 cm, the percent of the colony that appeared long dead (old mortality) and the percent that appeared to have recently died (recent mortality defined where corallite structure was identifiable to genera and no or only recently colonized algae were present) was recorded, and any diseases affecting the colony were documented. All participants recording data were trained in the identification of coral species with aid of the *Reef Coral Identification* books (Humann & DeLoach 1992,

Humann & DeLoach 2001). At least one day was dedicated each year to consistency training so that the variability of estimates of mortality was kept to a minimum (5% difference or less between observers was considered acceptable). Identification of diseases was based on the NOAA coral disease identification cards. A more detailed description of the AGRRA methodology can be found in Lang (2003).

In the 2004 AGRRA survey, more detailed descriptions of coral condition were made during monitoring efforts. Certain factors (Table 2) were specifically recorded for all colonies, including algal associations or overgrowth, invertebrate associations or overgrowth, presence of coral predators, skeletal anomalies, tissue coloration, and presence and origin (if possible to determine) of damage to the tissue or skeleton.

Disease Monitoring Sites (2004, 2005, 2006)

At each of the sites, three permanent 16-m^2 quadrats were installed in July 2004 under the auspices of Cayman Islands Marine Conservation Board permit # MCB-300704. The first corner of each of the 16-m^2 quadrats was randomly located using a table of random compass directions and distances and setting out from the mooring pin at each location. The first corner was marked with a nail hammered into the substrate and a numbered tag was attached to the nail using a plastic cable tie. From this first corner, the second corner was located using another random compass direction and was placed in this random direction, four meters from the first corner. To locate the third corner, a nail was placed four meters away from the second corner at a right angle direction to the compass heading used to locate the second corner. Whether the right angle was directed to the right or left was alternated on the installation of each quadrat. The last corner of the quadrat was then located 4 m away from the third corner using the opposite compass

heading to that used to locate the second corner. The quadrat was then confirmed to be an approximate 4 by 4-m square by checking all right angles made by the nails. Nails were only hammered into bare substrate and precaution was taken to avoid installing nails into any living animal tissue. Positions of the corners were altered slightly if it was not possible to find bare substrate for a corner.

On site visits, each quadrat was outlined using a measuring tape wrapped around the corner nails. Permanent quadrats were further sub-divided into 16 smaller 1-m^2 quadrats using a measuring tape for ease of re-locating monitored corals. On the establishment of the quadrats, all corals greater than 5 cm in maximum diameter within these plots were recorded to species level, maximum diameter and maximum height were recorded, associated features were described and they were assigned to a 1-m^2 quadrat (numbers 1-16). Corals that exhibited signs of bleaching, disease or any other source of mortality were marked using numbered tags attached to galvanized masonry nails hammered into the adjacent substrate and were then photographed. These corals were then monitored through observation, measurement, and re-photographing on subsequent site visits. All other non-marked corals within the 16-m^2 quadrat were also monitored for the incidence of new mortality or stress signs and newly affected corals were then also tagged and monitored using photographs. Photographs were taken from the same angle on each visit (Figure 2) and then later, tissue loss estimates were approximated from the analysis of photos using ImageJ v1.37, a free open-source Java-based image analysis software program developed by the National Institutes of Health (http://rsb.info.nih.gov/ij/). All sites were accessed by boat and surveyed on SCUBA. Typically, two sites were visited one day and the other two sites were visited the next day

or at least within one or two days of the first visit. Sites were visited once a week for three consecutive weeks during one month in both the summer of 2004 and 2005 and then were visited once or twice in July of 2006.

Disease Spatial Distribution (2005)

To examine the spatial distribution of diseased colonies, randomly placed, 5-m radius arc transects were used. Again, the mooring pin at each site was used as a starting point for each survey, and a table of random directions and distances was used to bring an observer to points on the reef. Multiple test transects, in which a random affected colony was the center of transects, and control transects, in which a random unaffected colony was the center of transects, were both performed at each site surveyed (Table 1). For test transects, once at the random point designated by the table of directions and distances, the observer would use the closest colony exhibiting signs consistent with white plague within 5 m of that point. If a white plague-affected colony could not be located, the point was skipped and the diver continued to the next point. Once a colony was located, it would then become the center of a circular transect with a 5-m radius, within which all other white plague-affected colonies were recorded, identified to species, the maximum height and width were recorded, and any associated factors were noted. The linear distance between the center of the central colony and the center of the secondary diseased colony was then measured and recorded. Once a radial transect was completed and to ensure that no diseased colonies were counted more than once using this methodology, the table of random directions and distances was again used from that point to find a new point at least 20 m away. Control transects were performed in an identical manner as test

transects except with colonies apparently unaffected by white plague as the center of transects.

Data Analysis

Comparisons among species, years and sites of data on coral abundances, disease levels, sizes, percent mortality estimates and coral cover were performed using repeated-measures analysis of variance (RM-ANOVA) tests (Sokal & Rohlf 2001). Data were first tested to see if they conformed to assumptions of normality and transformations of data occurred when data did not. Arcsin-transformations were used for percentage data, square root-transformations for density data, and $\log_{10}$-transformations for all other data. When the assumption of equal variances was met, post-hoc pair-wise comparisons were made using the Bonferroni test, which is based on a Student's *t* statistic (Sokal & Rohlf 2001). When equal variances could not be assumed, Tamhane's conservative pair-wise comparison test, also based on a *t* test, was used instead (SPSS v15.0). When normality could not be achieved through transformation, non-parametric methods were used and are designated. This occurred when comparing percent recent mortality estimates among disease types or between diseased and unaffected colonies. In this case a Kruskal-Wallis test was employed for the test among disease types and a Kolmogorov-Smirnoff test was used for two sample tests. Univariate ANOVAs were used to investigate differences among tissue loss rates attributed to different sources of mortality including disease. Regression analyses were performed to determine the degree of relationship between SWP2 prevalence within transects and community factors, including coral species diversity, coral abundance, average size of colonies, and coral cover.

To analyze the spatial data on SWP2-affected colonies, the number of colonies was calculated for each distance category (0-1 m, 1-2 m, 2-3 m, 3-4 m, 4-5 m) for test and control transects, and the mean proportion of diseased colonies found within each category, normalized for the area of the arc represented by that distance category was determined. The distributions produced were then compared using a chi-square test (Sokal & Rohlf 2001). The distribution of diseased colonies in test transects was also compared to a hypothetical distribution of diseased colonies created using the mean density of colonies recorded in permanent quadrats and the mean prevalence of disease following the methods of Pielou (1977) and Diggle (1983). Spatial distributions of test and control arcs were tested using a chi-square goodness of fit test (Snedecor & Cochran 1989).

All statistical analysis was performed using the statistical software package SPSS v15.0 for Windows (SPSS Inc.), and graphs were produced using SigmaPlot 8.0 (SPSS Inc.). The particular observer for each observation was found to have no significant effects on the results of the analyses of AGRRA data and is therefore not indicated.

RESULTS

AGRRA Surveys (1999, 2002, 2004)

Suspect white plague distribution and abundance

AGRRA surveys identified 24 species of reef-building corals (Table 3). Of the 1167 corals surveyed during all three years at the four sites, 43 (approximately 4%) of them exhibited signs consistent with that described for white plague type II (Richardson et al. 1998a, b), although presence of the pathogen of white plague type II, *Aurantimonas coralicida*, was not confirmed. Prevalence of this suspect white plague (SWP2) was

significantly different through time (RM-ANOVA $F_{[2,4.657]} = 8.391$, $p < 0.001$), and was highest in 2002 (post-hoc pair-wise comparisons with Tamhane's test, $p<0.01$). Although no differences were detected among sites, SWP2 was prevalent all three sampling years only at lee sites (Figure 3). In 1999, SWP2 was found on five species of coral, and in 2002 and 2004 on six species (Table 4). Of the total number of SWP2-affected colonies documented, 58% were belonged to the *Montastraea annularis* species complex including the species *Montastraea annularis* (diseased=16, *n* =312), *M. faveolata* (diseased=6, *n* =164), and *M. franksi* (diseased=3, *n* =30). *Agaricia agaricites* composed 14% (diseased=6, *n* =313) of affected colonies, and the remaining 29% were *Porites porites* (diseased=6, *n* =129), *Montastraea cavernosa* (diseased=1, *n* =49), *Colpophyllia natans* (diseased=1, *n* =9), *Diploria labyrinthiformis* (diseased=1, *n* =28), *D. strigosa* (diseased=1, *n* =61), *P. astreoides* (diseased=1, *n* =93), and *Siderastrea radians* (diseased=1, *n* = 21).

Results of regression analyses showed no significant trends between SWP2 prevalence (% of population affected) and coral species diversity, coral abundance, average size of colonies, or coral cover. When SWP2 prevalence was further tested against the abundance of its two most preferred hosts (*Montastraea* and *Agaricia*), there was again no significant association.

Two other coral syndromes were recorded affecting colonies in AGRRA surveys: black band (Rutzler et al. 1983) and dark spot (Gil-Agudelo et al. 2004). On the 1167 corals surveyed, only 2 cases of black band were recorded and 23 cases of dark spot. All AGRRA data were pooled and mean percent recent partial mortality estimates on colonies was calculated based on disease category (black band-affected, dark spot-

affected, SWP2-affected, and no disease) for each of the 11 species affected by SWP2. Colonies affected by SWP2 were associated with higher percent recent partial mortality estimates than dark spot-affected colonies, black band-affected colonies, and colonies with no apparent disease (Table 5). Due to low sample sizes within disease categories for most species, significance tests for differences of mean percent recent mortality between no-disease and SWP2-affected colonies were performed only for four species (*Montastraea annularis*, *M. faveolata*, *M. franksi*, and *Porites porites*), and tests for differences among no disease, SWP2-affected, and dark spot-affected colonies were performed only for *Agaricia agaricites* colonies. Percent recent mortality was significantly different among no-disease, dark spot-affected and SWP2-affected colonies of *A. agaricites* (Kruskal-Wallis: $H = 57.6$, $p<0.001$, d.f. = 2), and was highest for SWP2-affected colonies. For the other four species, mean percent recent mortality was significantly greater for SWP2-affected colonies versus no-disease colonies (*M. annularis*: Kolmogorov-Smirnov test: $Z = 2.521$, $p<0.001$; *M. faveolata* $Z = 2.206$, $p<0.001$; *M. franksi* $Z = 1.521$, $p<0.05$; *P. porites* $Z = 1.400$, $p<0.05$. Mean percent old mortality was found to be similar among disease categories for each of the species.

The 2004 AGRRA data indicated that SWP2-affected colonies did not share any of the potentially associated features (Table 2), and only one condition, the presence of red/purple algal filaments and clusters (Figure 4), was noted *only* on colonies exhibiting active SWP2 signs. Of the SWP2-affected colonies recorded during this survey, this condition was noted in 20% of the cases. Algal colonization ranged in size from several millimeters (essentially tufts of algal filaments) up to 5 cm in diameter. These algal balls

always appeared on the newly dead skeletal area, typically at least 1 cm away from the disease line.

Temporal dynamics of coral communities

Coral cover varied among sites producing a significant effect of site (RM-ANOVA, $F_{[3,1.071]} = 6.739$, $p<0.001$). In general, coral cover significantly declined through time (Figure 5, RM-ANOVA, $F_{[2,1.825]} = 11.492$, $p<0.001$), but there were differences in the magnitude and timing of coral cover decline at each site (RM-ANOVA *time X site* $F_{[3,0.780]} = 4.909$, $p<0.001$). Coral City-W, located to the west on the windward side of the island, experienced no change in coral cover during the three years while all three other sites demonstrated major declines.

Coral abundances recorded in transects were significantly different among species through time (RM-ANOVA *species*, $F_{[5,10.643]} = 6.108$, $p<0.001$; *time X species* $F_{[4,3.059]} = 1.756$, $p<0.05$). Therefore, the six most abundant species (*Agaricia agaricites*, *Montastraea annularis, M. faveolata, Porites astreoides, P. porites,* and *Siderastrea siderea*), altogether representing > 90% of the coral community during any survey, were analyzed independently to test for changes through time and/or differences among sites. All selected species but *Montastraea faveolata* showed significant changes through time and all but *Porites porites* showed significant differences among sites (Table 6). The abundance of colonies of *Agaricia agaricites*, *Porites astreoides*, *P. porites*, and *Siderastrea siderea* increased through time, while abundances of *Montastraea annularis* and *M. faveolata* varied but generally stayed the same (Table 6)).

For the six most abundant species, maximum diameters for all six species were found to co-vary significantly with percent old mortality but not with percent recent

mortality which remained approximately the same throughout monitoring. Sizes of *Agaricia agaricites*, *Porites astreoides*, and *Siderastrea siderea* significantly declined, while sizes of *Montastraea annularis*, *M. faveolata*, and *P. porites* did not change significantly (Table 7). Following the observed decline in size, percent old mortality on *Agaricia agaricites* did not differ among sites but significantly declined between 1999 and 2004 (Figure 6). Mean percent old mortality of *P. astreoides* and *Siderastrea siderea* did not differ through time, and was less than 15% at all sites and for all years (Figure 6). Despite no change in size, percent old mortality on *Montastraea annularis* significantly increased through time although not at Grundy's Gardens (Figure 6). Similarly, percent old mortality on *M. faveolata* colonies was significantly greater in 2004 than in 2002. This change was greatest at Sailfin but was not apparent at Coral City (Figure 6).

Disease had significant effects on mean maximum colony size of *Agaricia agaricites* and *Siderastrea siderea* (Figure 7). Mean maximum diameter of SWP2-affected colonies of *A. agaricites* was significantly larger than unaffected colonies (Bonferroni adjustment on post-hoc pair-wise comparison: $p<0.05$). Similarly, dark spot-affected colonies of *S. siderea* were significantly larger than unaffected colonies (T-test, equal variances not assumed: $t = 3.205$, d.f. = 78, $p<0.01$).

Disease Monitoring (2004, 2005, 2006) Results

Disease incidence within permanent quadrats (4 sites targeted)

In the three years (2004-2006) of monitoring permanent quadrats 39 of the 835 coral colonies monitored were recorded as affected by SWP2. More than half of affected colonies were *Agaricia agaricites* (diseased=21, *n* =238). Other species affected included *Montastraea faveolata* (diseased=4, *n* =44), *Dichocoenia stokesi* (diseased=3, *n* =5), *M.*

annularis (diseased=3, *n* =30), *M. franksi* (diseased=2, *n* =8), *Siderastrea siderea* (diseased=3, *n* =84), *Porites astreoides* (diseased=2, *n* =161), and *P. porites* (diseased=2, *n* =54). The majority of SWP2 cases (87%) were recorded in quadrats at sites on the lee side of the island.

Dark spot (Garzon-Ferreira & Gil 1998) was also recorded in permanent quadrats, and 96 colonies exhibited signs of this syndrome over the course of monitoring. Of these, 83 were colonies of *Agaricia agaricites*, 10 were *Siderastrea siderea*, 2 were *S. radians*, and 1 was *Montastraea faveolata*. Of these cases 85 (89%) were recorded at sites on the lee side of the island.

Twenty-one active SWP2 cases were found in three weeks of monitoring in 2004, 2 cases in three weeks of monitoring in 2005, and 16 cases in single visits made to sites in 2006. Five cases developed during the monitoring period (were not active when sites were first visited) in 2004, and one developed during monitoring in 2005. All other SWP2 cases in these two years were active when sites were first visited at the beginning of monitoring periods. Additionally, eighty-five active dark spot cases were recorded in 2004, 89 in 2005 and 88 in 2006. Ten dark spot cases developed during monitoring in 2004, and four developed in 2005.

Dark spot prevalence was higher than SWP2 prevalence in all three years, and also when all cases are combined for the 3-year period (Table 9). However, dark spot incidence proportion (the mean proportion of the population becoming infected per day) was only 0.02% greater than SWP2 in 2004, and 0.007% greater in 2005 (Table 9). Both dark spot and SWP2 were more prevalent on colonies than other sources of mortality including predation, algal overgrowth or total colony loss which are combined as

"other/unknown" (Table 9). The mean incidence proportion of other/unknown conditions was much lower than for dark spot and SWP2 in 2004 and no new incidence of other sources of mortality was recorded during monitoring in 2005 (Table 9).

Tissue loss rate estimates attributed to SWP2, dark spot, and other (predation, algal overgrowth, etc.) or unknown causes of tissue loss were approximated on a per day basis by dividing the total tissue loss between site visits by the number of days passed between visits. For all three mortality types, lesions identified on one visit would often show no expansion between site visits. However, on one occasion a SWP2 lesion moved so quickly that an entire 20 cm maximum diameter colony of *Dichocoenia stokesi* experienced complete mortality in less than a week. When all actively affected colonies were considered for all three years the number of lesions per colony did not differ among mortality types. However, tissue loss rates per lesion significantly differed among mortality types (ANOVA, $F_{[2,151.986]} = 9.447$, $p<0.001$), and SWP2 tissue loss rates were significantly greater than tissue loss rates attributed to dark spot or other types of mortality (post-hoc comparisons, Tamhane's test: $p<0.05$ for both comparisons, Table 10). When lesions were combined by colony and the analysis was repeated, a significant difference among mortality types was also found (ANOVA, $F_{[2,274.628]} = 3.479$, $p<0.05$), and SWP2 tissue loss rates were again the fastest.

Spatial distribution of disease

The distribution of SWP2-affected colonies surrounding randomly-located SWP2-affected colonies (test) and randomly-located unaffected colonies (control) were significantly different (chi-square goodness of fit test: $\chi^2 = 27.04$, $p<0.001$). SWP2-affected colonies surrounding "test" colonies were found most often in the 0-1 m distance

category, while SWP2-affected colonies were more evenly distributed among distance categories when they surrounded "control" colonies (Figure 8). Also, total disease was much greater in test transects compared with control transects (Figure 8).

When the distribution of SWP2-affected colonies surrounding randomly-located SWP2-affected colonies (test) was tested against a distribution of SWP2-affected colonies predicted by the underlying density of coral colonies, these two distributions were also significantly different (chi-square goodness of fit test: $\chi^2 = 13.20$, $p<0.05$). Again, the shorter distance categories contained more SWP2-affected colonies surrounding test colonies than what would be predicted by a Poisson distribution of the underlying density of coral colonies (Figure 9).

DISCUSSION

These results have quantitatively described the significant changes to the coral communities of Little Cayman fore-reefs between 1999 and 2004, and the associated epizootiology of suspect white plague type II (SWP2).

For the most prominent coral species, colony abundances generally increased between 1999 and 2004. However, at three of the four sites, percent live coral cover declined dramatically. The greatest increases in abundances of colonies occurred for the species that typically reach a smaller adult size (*Agaricia agaricites*, *Porites astreoides, P. porites,* and *Siderastrea siderea*) and are not considered to be the dominant framework-builders of the reefs. Most of these species similarly experienced a decrease in mean maximum diameter. Additionally, species that typically reach a greater maximum diameter (*Montastraea faveolata* and *M. annularis*) did not experience declines in size, but showed an increase in the percent old mortality recorded on colonies.

Therefore, the seemingly contradictory finding of declining coral cover with increasing coral abundances could possibly be explained by the increased abundance of smaller colonies and colonies of species that do not reach a large adult size combined with an increase in the percent old mortality on species that have historically represented a large proportion of live tissue on the reef.

A syndrome with signs consistent with those described for white plague was the most abundant of the three identified syndromes on Little Cayman (SWP2, dark spot, and black band) between 1999 and 2004. However, between 2004 and 2006, monitoring of permanent quadrats revealed that SWP2 was less prevalent than dark spot and similar to other types of mortality combined, and black band was not recorded in quadrats during this time period. Correspondingly, the incidence of SWP2 from 2004 to 2006 was very low and was similar to the incidence of dark spot and to other types of partial or total coral mortality. Despite its low prevalence and incidence, SWP2 was significantly associated with large amounts of mortality and exhibited rates of tissue loss that were capable of causing complete mortality of small colonies in less than a week. These rates were rapid and similar to previously recorded rates associated with white plague type II (Richardson et al. 1998a, Sutherland et al. 2004), and which have been documented to cause changes in the community structures of some reefs (Nugues 2002, Richardson & Voss 2005).

Additionally, the spatial distribution of SWP2-affected colonies recorded in this study was clumped, which is typically indicative of an infectiously transmitted disease. This type of distribution has been documented in most previous studies (Borger 2003, Richardson et al. 1998a, Voss & Richardson 2006). However, another study did not show

a significant pattern that would suggest contagiousness (Nugues 2002), although tissue loss rates documented in this study were slower and similar to those documented for white plague type I (Dustan 1977). Based on the rates of tissue loss and the spatial distribution of affected colonies, it is likely that the SWP2 recorded during this study is more closely related to white plague type II (Richardson et al. 1998a, 1998b).

SWP2 was not significantly associated with any community factors, despite the fact that some of these, such as algal contact, have been linked with the white plague type II pathogen, *A. corallicida* (Nugues et al. 2004). Other factors, such as predation, have also been shown to be vectors for other coral diseases (Aeby 1991, Sussman et al. 2003), but the presence of coral predators was not significantly related to the presence of disease signs in this case. It is noteworthy that the majority of SWP2-affected and dark spot-affected colonies were documented at lee sites. These sites presumably experience different oceanographic dynamics than windward sites although this has not been well documented. Lee sites also support greater diving activity because of the typically calmer waters and because Bloody Bay, located on the lee of the island, enjoys widespread notoriety and popularity in the worldwide recreational diving community. Further studies of the variability in the physical dynamics impacting the reefs of Little Cayman (including currents, potential upwelling, temperature, water quality, etc.) in relation to disease occurrence would be necessary to determine the influence of these processes on the occurrence of disease-related mortality.

For the first two years of the AGRRA surveys, SWP2 was found principally affecting the framework-building species of the *Montastraea annularis* species complex: *M. annularis*, *M. faveolata*, and *M. franksi*. One or a combination of these three species

has previously been reported as the dominant host for SWP2 in many parts of the Caribbean region (Nugues 2002, Croquer & Bone 2003, Miller et al. 2003, Croquer et al. 2005, Jordan-Dahlgren et al. 2005, Miller & Williams 2007). Other studies have documented the dominant host of SWP2 to be *Siderastrea siderea* (Borger 2003), and *Dichocoenia stokesi* and *Diploria clivosa* (Borger 2005b, Richardson & Voss 2005). In Borger (2003) the coral community was naturally characterized by less dense populations of corals, while in Borger (2005b) and Richardson & Voss (2005) the reef communities had experienced major losses in coral cover in the last several decades.

In 2004, although SWP2 was still documented affecting *Montastraea annularis* and *M. faveolata*, the majority of affected colonies were *Porites porites* and *Agaricia agaricites*, species documented to have increased in abundance from 1999 to 2004. During monitoring from 2004 to 2006, the majority of SWP2 cases were also *A. agaricites*. In infectious multi-species host-pathogen systems, the pathogen population and, therefore, prevalence within each host population is often dependent on the relative density of each species. Similar to classical predator-prey dynamics, a higher density of the preferred host species is capable of supporting a higher prevalence of disease until that population declines. The pathogen will then target its less preferred host species population (May & Anderson 1979). While the abundance of *Montastraea* spp. colonies did not decline in Little Cayman, the amount of mortality on these species was found to significantly increase, thereby decreasing the amount of live tissue that was potentially available to become affected. Whether this variability in species susceptibility is (a) a product of species abundance, or (b) a reflection of other potential stressors variably impacting different species in this region, is difficult to determine with these data alone.

Overall, Little Cayman has experienced little development of the shoreline in the last 20 years, with the number of permanent residents increasing to only 129 from 12 in 1958 (pers. comm. Little Cayman resident P. Hillenbrand). Anthropogenic effects from eutrophication and/or sedimentation on the reef, though not officially documented, are therefore assumed to be low. Other than disease, a natural source of mortality that could potentially cause large-scale changes to the island's coral community is damage from hurricanes. In 2004, when Hurricane Ivan passed west of Grand Cayman, it was a category 5 storm. Before Ivan, the last hurricane to have impacted Little Cayman was Hurricane Gilbert in 1988 (NOAA hurricane records website). This hurricane represented the only other major extrinsic source of coral mortality to have occurred on the reefs between 1999 and 2006. However, these results suggest that significant changes to the coral community occurred prior to its passing. When sites were first re-visited in 2005, only 7 small (<35 cm) colonies were recorded as lost, likely due to hurricane impacts. No other partial or total mortality was observed that could be attributed to hurricane damage. Therefore, the overall influence of the passage of this hurricane on the fore-reefs of Little Cayman was thought to be relatively minor.

Coral reef ecosystems depend on scleractinian corals as the physical and biological foundation of the reef, providing homes and food for the great diversity of organisms that they host. Based on these results, signs consistent with those reported for white plague type II represent the greatest source of coral mortality on these reefs in the last several years. Its ability to cause rapid tissue mortality and propensity to affect the most dominant species means it can significantly alter the coral communities of Little Cayman. Diving tourism and recreational fishing, both dependent on a healthy

ecosystem, are dominant industries in the Cayman Islands. Therefore, further deterioration of these reefs due to disease-related mortality may have disastrous effects for the ecology and ultimately the economy of these islands.

Figure 1: *Montastraea faveolata* exhibiting signs of suspect white plague type II (SWP2). Tissue loss appeared to have initiated basally, and was progressing in the direction of the arrows. Photo taken on July 6th, 2006, at 10 m depth, Sailfin Reef, Little Cayman.

Table 1: Little Cayman study sites

Site	Latitude	Longitude	Orientation	Marine Park Designation	Diving Activity
Coral City[a,b,c]	19.68061	-80.02311	Wind	Non park	Low
Grundy's Gardens[a,b,c]	19.65725	-80.08966	Wind	Park	High
Jigsaw Puzzle[a,c]	19.66614	-80.10683	Lee	Park	High
Joy's Joy[c]	19.67863	-80.09312	Lee	Park	High
Lucas' Ledge[c]	19.66925	-80.04325	Wind	Park	High
Mixing Bowl (Bus Stop)[c]	19.68493	-80.07833	Lee	Park	High
Nancy's Cup of Tea[b,c]	19.69349	-80.06902	Lee	Park	Medium
Sailfin[a,b,c]	19.70688	-80.01219	Lee	Non park	Low
Snapshot[c]	19.70095	-80.05706	Lee	Non park	Medium

a. Sampled in 1999, 2002, and 2004 using AGRAA methods
b. Permanent quadrats installed and repeatedly sampled in consecutive three week periods in each of the summers of 2004 and 2005, and sampled once in the summer of 2006
c. Used for spatial sampling

Table 2: Recorded associated factors for corals surveyed in the AGRRA survey of 2004.

Coloration	Damage/Overgrowth/Smothering	Other Invertebrates	Vertebrates
A. Normal	A. Predation	A. Anemone	A. Damselfish
B. Pale bleaching	i. Fish bites w/skeletal damage	B. Zooanthid	Algae Associated
C. Partial bleaching	ii. Fish bites w/ damage to tissue	i. Palythoa	A. Green
D. Complete bleaching	iii. Snail scars	C. Sponge	i. Calcareous
E. Excess mucus	iv. Worm scars	i. *Cliona langae*	ii. Non-calcareous
F. Dark spot or areas	B. Other damage	ii. *Cliona delitrix*	B. Red
G. Splotchy/discolored	i. Fresh damage to tissue only	D. Worm	i. Calcareous
Behavior	ii. Fresh damage to skeleton	i. Calcareous	ii. Non-calcareous
A. Normal	iii. Damaged but healed	ii. Mobile	C. Brown
B. Polyps open in day	C. Algal overgrowth	E. Echinoid	i. *Dictyota* spp.
C. Mesenteries out	i. Algal tufts on surface	F. Snail	ii. *Lobophora* spp.
D. Some in, some out	ii. Obvious algal mat smothering	G. Crustacean	iii. *Sargassum* spp.
Morphology	iii. Damselfish garden	H. Tunicate	D. CCA
A. Normal	D. Sediment	i. *T. solidum*	
B. Skeletal anomaly	i. Excess sediment on live tissue	I. Gorgonian	
C. Damselfish chimneys	ii. Sediment on recently dead	J. Other hard corals	

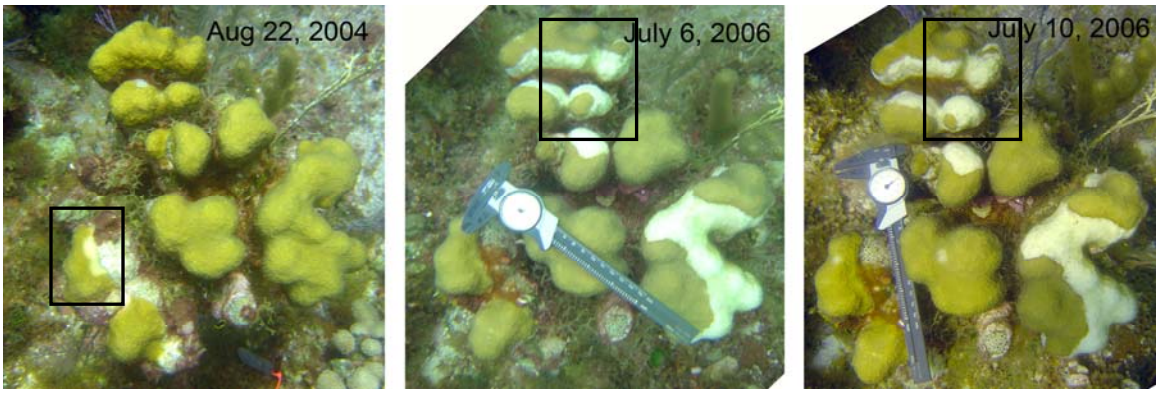

Figure 2: Time series of SWP2 tissue loss on a colony of *M. annularis*. The colony was originally photographed in 2004 due to partial bleaching (left photo, within box), but bleached tissue later recovered its pigment. In 2006, tissue loss was first noted (middle photo, within box), and the colony was subsequently photographed four days later (right photo). Colony located at 7.5 m depth, Sailfin Reef, Little Cayman. Calipers are 22 cm in length.

Table 3: Species occurring in transects at Little Cayman sites with taxonomic sources.

Suborder	Family	Species	Source
Astrocoeniina	Astrocoeniidae	*Stephanocoenia intersepta*	Milne Edwards & Haime (1848)
	Pocilloporidae	*Madracis mirabilis*	Duchassaing & Michelotti (1860)
	Acroporidae	*Acropora palmata*	Lamarck (1816)
		A. cervicornis	Lamarck (1816)
Fungiina	Agariciidae	*Agaricia agaricites*	Linnaeus (1758)
	Siderastreidae	*Siderastrea siderea*	Ellis & Solander (1786)
		S. radians	Pallas (1766)
	Poritidae	*Porites astreoides*	Lamarck (1816)
		P. porites forma *porites*	Pallas (1766)
Faviina	Faviidae	*Diploria clivosa*	Ellis & Solander (1786)
		D. strigosa	Dana (1848)
		D. labyrinthiformis	Linnaeus (1758)
		Manicina areolata	Linnaeus (1758)
		Colpophyllia natans	Houttuyn (1772)
		Montastraea annularis	Ellis & Solander (1786)
		M. faveolata	Ellis & Solander (1786)
		M. franksi	Gregory (1895)
		M. cavernosa	Linnaeus (1767)
	Meandrinidae	*Meandrina meandrites*	Linnaeus (1758)
		Dichocoenia stokesi	Milne Edwards & Haime (1848)
		Dendrogyra cylindrus	Ehrenberg (1834)
	Mussidae	*Mussa angulosa*	Pallas (1766)
		Mycetophyllia lamarckiana	Milne Edwards & Haime (1848)
Caryophylliina	Caryophylliidae	*Eusmilia fastigiata*	Pallas (1766)

Table 4: Species affected by SWP2 in each year of AGRRA sampling. The total number of SWP2-affected colonies was 10 in 1999, 25 in 2002, and 8 in 2004. Percentage is percent of total number of affected colonies recorded in each year represented by a given species. N is total number of colonies of each specie surveyed in that year.

1999	2002	2004
Montastraea annularis 50% (N=128)	*M. annularis* 38% (N=125)	*P. porites* 38% (N=52)
M. franksi 30% (N=25)	*M faveloata* 19% (N=46)	*A. agaricites* 13% (N=129)
M. cavernosa 10% (N=24)	*A. agaricites* 15% (N=100)	*C. natans* 13% (N=2)
Agaricia agaricites 10% (N=84)	*Porites porites* 12% (N=44)	*D. strigosa* 13% (N=18)
Colpophyllia natans 2% (N=6)	*Diploria labyrinthiformis* 4% (N=8)	*M. annularis* 13% (N=59)
	P. astreoides 4% (N=29)	*M. faveloata* 13% (N=60)

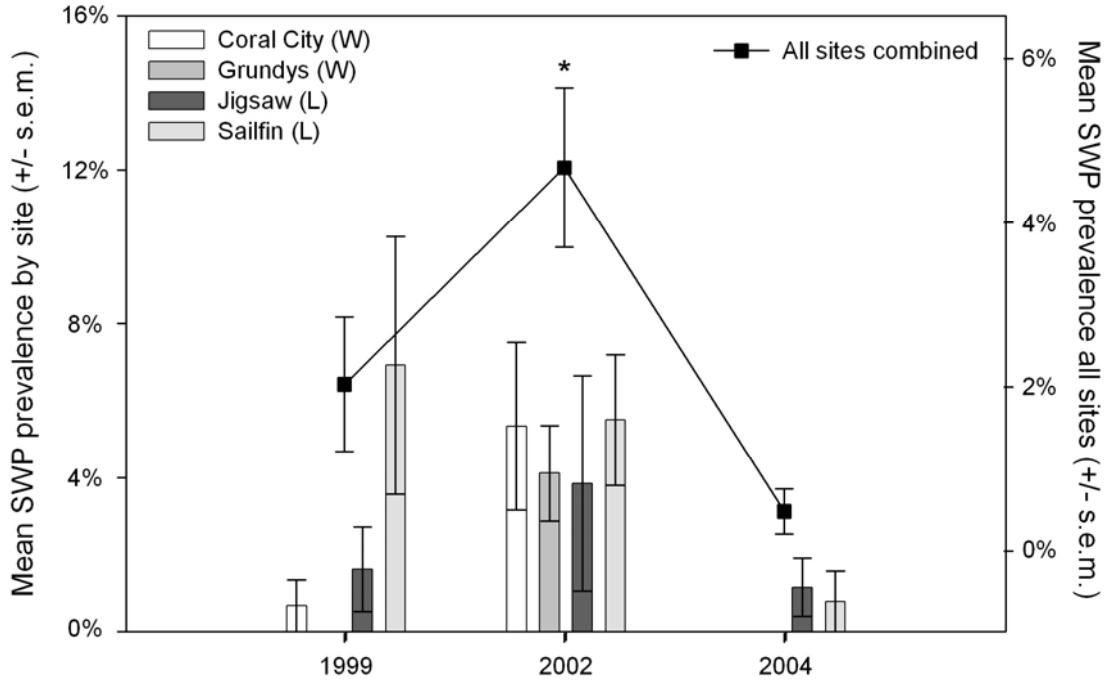

Figure 3: Mean SWP2 prevalence (± s.e.m.) in transects from repeated sampling of four sites in 1999, 2002, and 2004. Bars represent mean prevalence per site, per year. Boxes represent mean prevalence per year from combined transect data for all four sites (1999 N=50; 2002 N=40; 2004 N=32). Mean prevalence for all sites combined was significantly greater in 2002 than in 1999 or 2004 (Tamhane's test: p<0.01), indicated by the *.

Table 5: Mean % recent and old partial mortality of coral colony surfaces for all species affected by black-band (BB), dark spot (DS), or suspect white plague (SWP2), ± s.e.m. unless *n* =1. "No disease" indicates those colonies that were not exhibiting one of the three characterized conditions. Bold indicates a significantly greater value than other categories.

	Species	BB	DS	SWP2	No Disease
Mean % recent mortality (± s.e.m.)	*A. agaricites*		3.1%±1.7 (n=14)	**12.0%±8.7** (n=6)	0.6%±0.4 (n=293)
	C. natans			10.0%	1.3%±1.3 (n=8)
	D. labyrinthiformis			25.0%	0.2%±0.2 (n=27)
	D. strigosa			2.0%	0.1%±0.1 (n=60)
	M. annularis	10%		**8.1%±4.2** (n=16)	1.0%±0.2 (n=295)
	M. cavernosa			10.0%	0.6%±0.6 (n=48)
	M. faveolata	10%	0.0%±0.0 (n=2)	**5.3%±2.4** (n=6)	0.9%±0.5 (n=155)
	M. franksi			**10.0% ±0.0** (n=3)	0.7%±0.5 (n=27)
	P. astreoides			10.0%	0.3%±0.1 (n=92)
	P. porites			**3.8%±1.5** (n=6)	1.3%±0.5 (n=123)
	S. radians			0.0%	0.0%±0.0 (n=20)
	S. siderea		0.0%±0.0 (n=7)		0.5%±0.3 (n=73)
Mean % old mortality (± s.e.m.)	*A. agaricites*		27.9%±7.6(n=14)	15.8%±7.8 (n=6)	15.1%±1.3 (n=293)
	C. natans			40.0%	22.5%±9.4 (n=8)
	D. labyrinthiformis			25.0%	22.1%±6.0 (n=27)
	D. strigosa			0.0%	14.7%±2.9 (n=60)
	M. annularis	25%		28.9%±7.0 (n=16)	38.2%±1.7 (n=295)
	M. cavernosa			10.0%	15.0%±3.7 (n=48)
	M. faveolata	60%	45.0%±5.0 (n=2)	7.5%±4.0 (n=6)	26.6%±2.1 (n=155)
	M. franksi			38.3%±18.3 (n=3)	26.3%±5.7 (n=27)
	P. astreoides			0.0%	5.1%±1.4 (n=92)
	P. porites			20.8%±12.3 (n=6)	23.3%±2.6 (n=123)
	S. radians			100.0%	0.0%±0.0 (n=20)
	S. siderea		18.6%±5.2 (n=7)		9.7%±1.7 (n=73)

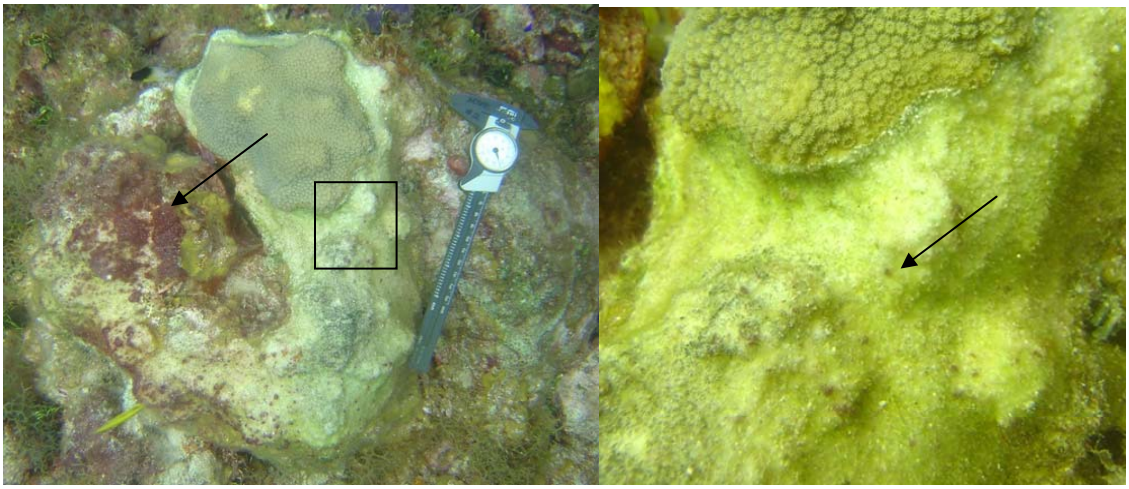

Figure 4: *Montastraea faveolata* colony with active SWP2. Left photo: arrow pointing to severe colonization by purple algal balls on recently dead surfaces (calipers are 22 mm in length). Right photo: close up of box in left picture showing small algal balls forming on recent tissue loss areas. Photo taken July 6, 2006, at 7.5 m depth, Sailfin Reef, Little Cayman. Calipers are 22 cm in length.

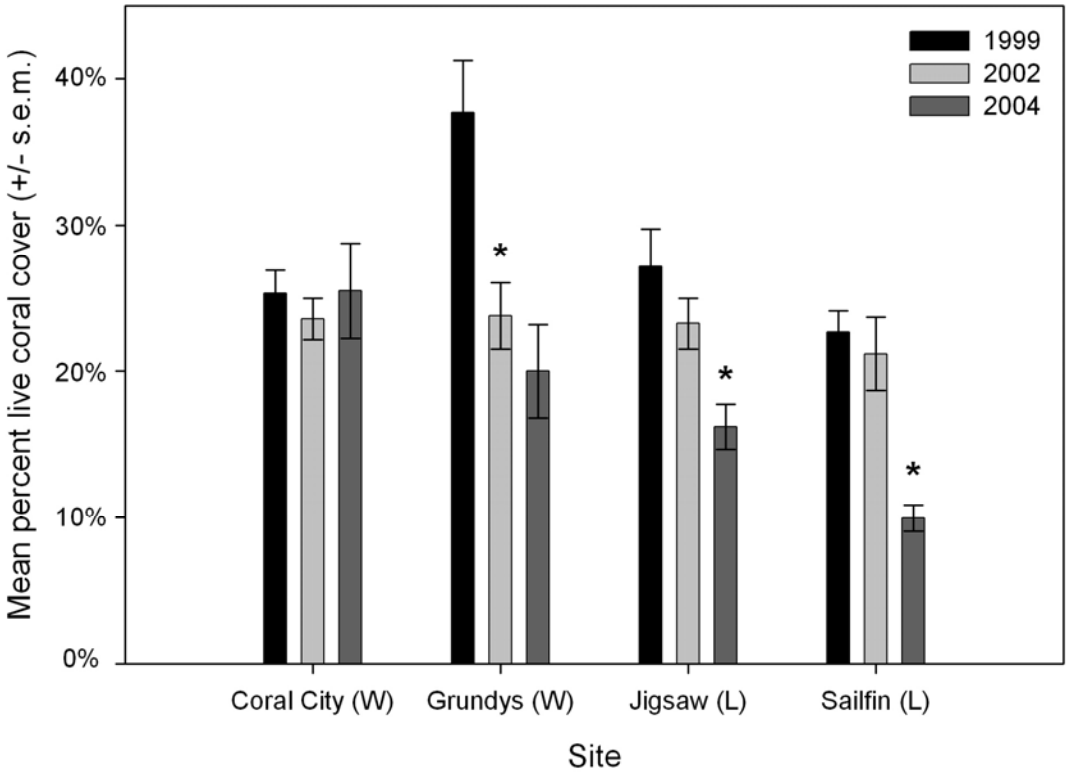

Figure 5: Mean percent live coral cover (± s.e.m.) in 1999, 2002, and 2004. * indicates significant difference between coral cover in that time period versus the previous time period (RM-ANOVA, *time* post-hoc pair-wise comparison $p < 0.05$). At Coral City, no significant coral cover change occurred while at the other three sites, significant declines were observed. Number of transects surveyed at sites – 1999: Coral City N = 14, Grundys N = 12, Jigsaw N = 14, Sailfin N = 10; 2002: Coral City N = 8, Grundys N = 11, Jigsaw N = 10, Sailfin N = 11; 2004: all sites N = 8.

Table 6: Mean colony densities (number/10 m) by survey year and results of RM-ANOVAs of the effects of site and year on colony abundances (number/10 m). Abundance data were square-root transformed. Bold indicates a significant effect. * indicates a significant post-hoc pair-wise comparison (Tamhane's test) between densities in that year compared with the previous year where p<0.05. *M. faveolata* and *P. astreoides* additionally showed significant interactive effects of *year X site* ($F_{[6,1.883]}$=4.013], p = 0.001 and $F_{[6,1.708]}$=6.161, p = 0.000, respectively).

			Mean number/10 m (± s.e.m.)		
Species	**Year**	**Site**	**1999 (N=50)**	**2002 (N=40)**	**2004 (N=32)**
Agaricia agaricites	**$F_{[2,4.523]}$=7.914 p = 0.001**	**$F_{[3,3.351]}$=5.862 p = 0.001**	2.1 ± 0.2	2.5 ± 0.4	4.0 ± 0.5*
Montastraea annularis	**$F_{[2.3.006]}$=6.753 p = 0.002**	**$F_{[3,2.183]}$=4.904 p = 0.003**	2.0 ± 0.2	3.2 ± 0.3*	1.8 ± 0.3*
M. faveolata	$F_{[2,1.190]}$=2.536 p = 0.083	**$F_{[3,2.550]}$=5.434 p = 0.002**	1.4 ± 0.2	1.1 ± 0.2	1.9 ± 0.3
Porites astreoides	**$F_{[2,4.206]}$=15.169 p = 0.000**	**$F_{[3,3.562]}$=12.846 p = 0.000**	0.3 ± 0.1	0.6 ± 0.1	1.5 ± 0.3
P. porites	**$F_{[2,4.519]}$=13.306 p = 0.000**	$F_{[3,0.869]}$=2.560 p = 0.058	0.5 ± 0.1	1.1 ± 0.2*	1.6 ± 0.2*
Siderastrea siderea	**$F_{[2,1.660]}$=4.317 p = 0.016**	**$F_{[3,1.168]}$=3.040 p = 0.032**	0.5 ± 0.1	0.5 ± 0.1	1.2 ± 0.2*

Table 7: Results of RM-ANOVAs of the effects of site and year on colony maximum diameter (cm) and % old mortality. Bold indicates a significant effect. Letters (a,b,c) indicate significant differences in post-hoc pair-wise comparisons (Tamhane's test) between years.

Species		Year	Site	1999	2002	2004
Agaricia agaricites	N			84	100	129
	Size (cm)	**$F_{[2,1.131]}$=5.034 p = 0.007**	$F_{[3,0.430]}$=1.913 p = 0.127	32.8 ±1.3^{a}	28.3 ±1.7^{b}	19.5 ±1.0^{c}
	% Old Mortality	**$F_{[2,14.935]}$=5.612 p = 0.004**	**$F_{[3,4.855]}$=1.824 p = 0.000**	19.7 ±2.2	18.5 ±2.8	10.8 ±1.8
Montastraea annularis	N			128	125	59
	Size (cm)	$F_{[2,0.149]}$=0.419 p = 0.658	$F_{[3,0.413]}$=1.157 p = 0.327	58.3 ±3.2	64.1 ±4.3	69.2 ±5.6
	% Old Mortality	**$F_{[2,16.588]}$=5.963 p = 0.003**	$F_{[3,0.683]}$=0.245 p = 0.865	29.4 ±2.3^{a}	44.9 ±2.9^{b}	40.4 ±3.5^{b}
M. faveolata	N			58	46	60
	Size (cm)	$F_{[2,0.039]}$=0.107 p = 0.889	**$F_{[3,4.978]}$=13.72 p = 0.000**	95.4 ±9.0	73.3 ±7.5	88.8 ±9.8
	% Old Mortality	**$F_{[2,20.862]}$=7.580 p = 0.001**	**$F_{[3,7.567]}$=2.749 p = 0.045**	26.6 ±3.0^{a}	20.7 ±4.1^{a}	30.3 ±3.7^{b}
Porites astreoides	N			16	29	48
	Size (cm)	**$F_{[2,3.140]}$=7.083 p = 0.001**	$F_{[3,0.259]}$=0.584 p = 0.627	30.3 ±2.7^{a}	24.0 ±2.9^{a}	16.1 ±1.7^{b}
	% Old Mortality	$F_{[2,0.447]}$=0.331 p = 0.719	$F_{[3,0.379]}$=0.280 p = 0.839	7.3 ±4.3	2.6 ±1.9	5.7 ±2.1
P. porites	N			33	44	52
	Size (cm)	$F_{[2,0.632]}$=2.027 p = 0.137	$F_{[3,0.725]}$=2.327 p = 0.079	40.0 ±2.6	35.6 ±4.2	29.0 ±3.1
	% Old Mortality	$F_{[2,8.907]}$=2.613 p = 0.078	**$F_{[3,9.669]}$=2.837 p = 0.041**	27.6 ±4.7	26.4 ±5.0	17.7 ±3.6
Siderastrea siderea	N			25	18	37
	Size (cm)	**$F_{[2,1.134]}$=3.292 p = 0.044**	$F_{[3,0.388]}$=1.127 p = 0.345	36.2 ±2.8	41.2 ±5.2	30.9 ±2.6
	% Old Mortality	$F_{[2,0.883]}$=0.470 p = 0.627	$F_{[3,1.738]}$=0.925 p = 0.433	10.1 ±2.6	8.6 ±4.0	11.6 ±1.3

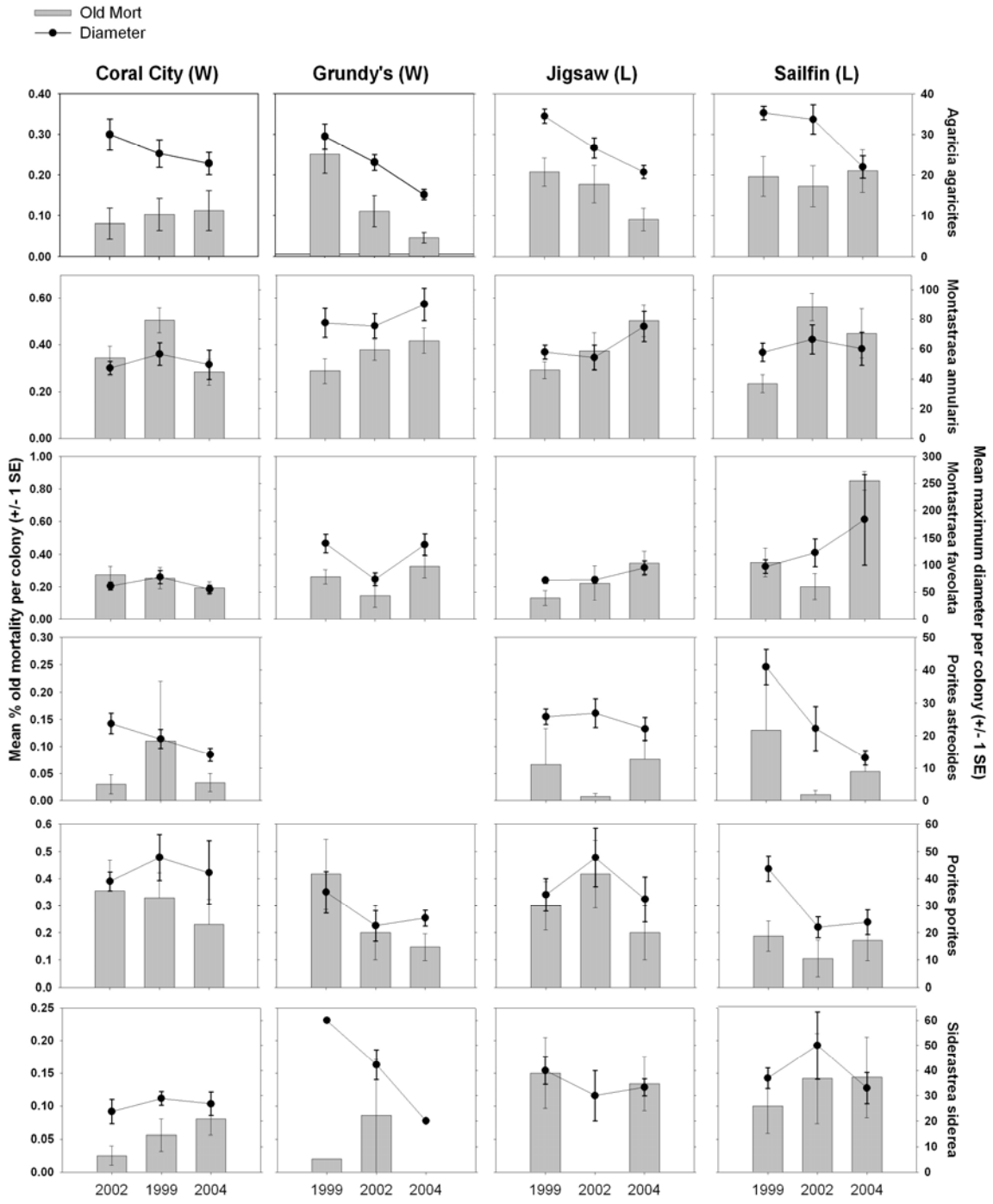

Figure 6: Mean percent old mortality and mean maximum diameter (± s.e.m.) of the six most abundant species by site and year (only three *P. astreoides* colonies were recorded at W2 so data are not displayed).

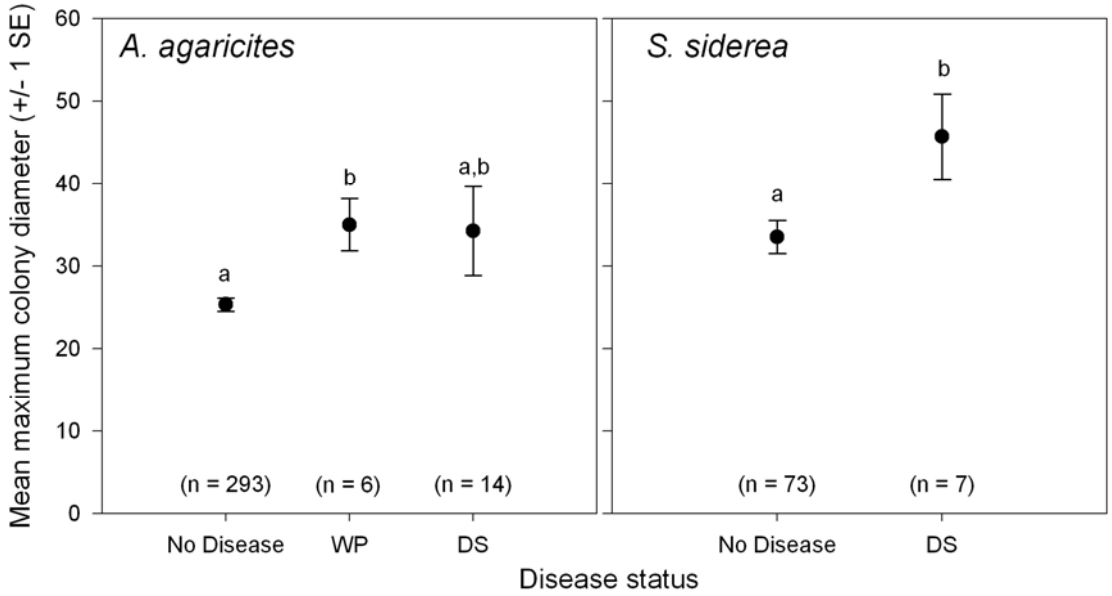

Figure 7: Mean maximum colony diameter by disease for *A. agaricites* and *S. siderea.* WP = suspect white plague type II, DS = dark spot.

Table 8: Results of RM-ANOVAs of the effects of site and year on % old mortality recorded on colonies. Bold indicates significant effects (p<0.05). Samples sizes as in Table 5.

Dependent variable	Source	Type III Sum of Squares	d.f.	Mean Square	F	Sig.
	Year	**29.870**	**2**	**14.935**	**5.612**	**0.004**
A. agaricites	Site	14.564	3	4.855	1.824	0.143
	Year * Site	22.502	6	3.750	1.409	0.211
	Year	**33.176**	**2**	**16.588**	**5.963**	**0.003**
M. annularis	Site	2.048	3	0.683	0.245	0.865
	Year * Site	**44.457**	**6**	**7.409**	**2.664**	**0.016**
	Year	**41.725**	**2**	**20.862**	**7.580**	**0.001**
M. faveolata	**Site**	**22.700**	**3**	**7.567**	**2.749**	**0.045**
	Year * Site	30.724	6	5.121	1.861	0.091
	Year	0.894	2	0.447	0.331	0.719
P. astreoides	Site	1.136	3	0.379	0.280	0.839
	Year * Site	4.666	5	0.933	0.691	0.632
	Year	17.814	2	8.907	2.613	0.078
P. porites	**Site**	**29.007**	**3**	**9.669**	**2.837**	**0.041**
	Year * Site	14.433	6	2.406	0.706	0.646
	Year	1.765	2	0.883	0.470	0.627
S. siderea	Site	5.214	3	1.738	0.725	0.433
	Year * Site	6.343	6	1.057	0.563	0.758

Table 9: Prevalence and incidence (± s.e.m.) of dark spot (DS), suspect white plague (SWP2), and other or unknown conditions (including predation, algal overgrowth, colony loss) in 16-m^2 permanent quadrats (N=12). Mean period prevalence is the average proportion of the initial population of sampled colonies that exhibited the indicated conditions at least once throughout the entire monitoring period. Mean point prevalence is the average proportion of the quadrat populations during that year that exhibited the conditions at least once. Mean incidence proportion rate is the average proportion of the quadrat populations becoming infected per day, and is calculated as the number of new cases of the condition divided by the total population and then by the number of days that the population was monitored in that time period.

	Mean period	Mean point prevalence			Mean incidence proportion rate	
Syndrome	prevalence	2004	2005	2006	2004	2005
DS	5.7% ± 1.6	5.0% ±1.5	5.3% ±1.5	5.2% ±1.5	0.04% ±0.02	0.01% ±0.01
SWP2	2.8% ± 1.2	1.6% ±0.7	0.1% ±0.1	1.1% ±0.5	0.02% ±0.01	0.003% ±0.003
Other/unkwn	1.3% ± 0.3	0.5% ±0.2	1.0% ±0.2	0.3% ±0.1	0.005% ±0.003	0

Table 10: Lesion numbers and tissue loss rates associated with dark spot (DS), suspect white plague (SWP2), and that attributed to other or unknown causal factors including total colony loss (± s.e.m.). Values for all years and species are combined. For mean number of lesions and number of dark spots, maximum values for repeatedly sampled colonies were used.

Syndrome	Mean # lesions/colony	Mean # DS/colony	Mean tissue loss (cm^2)/lesion/day*	Mean tissue loss (cm^2)/colony/day*
DS (N=45)	0.3 ± 0.1	4.2 ± 1.1	0.02 ± 0.01	0.05 ± 0.02
SWP2 (N=35)	1.7 ± 0.2	N/A	**4.01 ± 1.40**	7.79 ± 4.61
Other (N=13)	2.8 ± 1.2	N/A	0.08 ± 0.04	0.15 ± 0.06

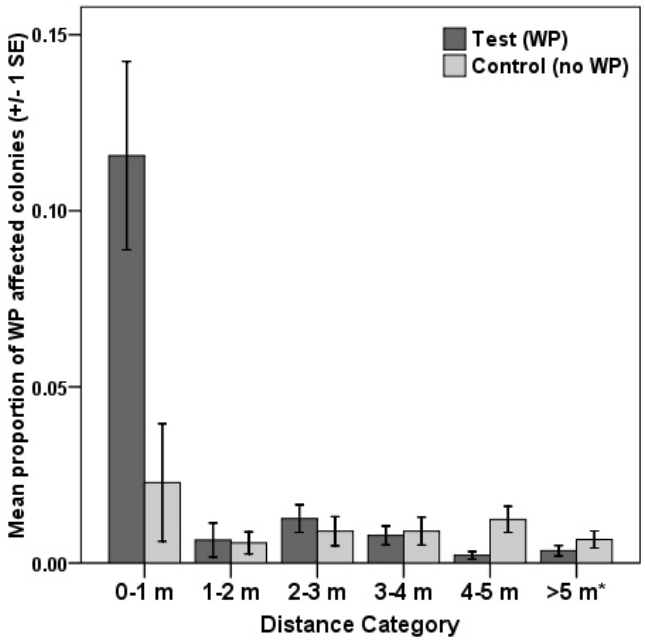

Figure 8: Mean proportion of SWP2-affected colonies surrounding SWP2-affected (test) and apparently healthy colonies (control). Proportion data were normalized for the total area within each distance arc. Test: N = 32, Control: N = 32.

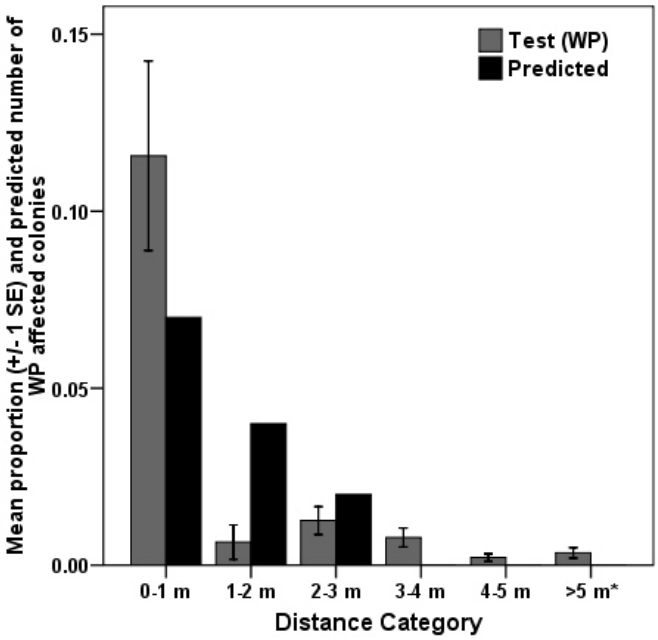

Figure 9: Mean proportion of SWP2-affected colonies surrounding SWP2-affected colonies (Test) and a predicted distribution based on the mean abundance of colonies found at sites. Test: N = 32.

Chapter 3: A Model for the Simulation of Infected Corals (SICO) – An overview

INTRODUCTION

A coral reef represents a highly complex community of organisms for which the scleractinian corals form the foundation. In the Caribbean region, over 60 species of scleractinian corals have been identified and many of these are known to exhibit signs of one or more of recently identified diseases (Sutherland et al. 2004). For instance, white plague type II is known to affect up to 30 species of scleractinian corals (Weil et al. 2002, Sutherland et al. 2004). This disease was first identified in an outbreak on Florida Keys corals and it is identified by its characteristic pattern of tissue loss (Richardson et al. 1998a), which is more rapid than the similar earlier-identified syndrome white plague (Dustan 1977). The pathogen of white plague type II was identified as a novel genus and species of bacteria, *Aurantimonas coralicida* (Richardson et al. 1998b, Denner et al. 2003); however, studies in different regions of the Caribbean have shown that this pattern of tissue loss may be the result of another pathogen (Pantos et al. 2003). On various Caribbean reefs white plague type II has been recorded at "epizootic" levels (Feingold & Richardson 1999, Nugues 2002, Croquer et al. 2003, Richardson & Voss 2005) and also "endemic" levels (Weil et al. 2002, Borger 2003, 2005), however there have been no epizootiological models validated with quantitative data to support these claims (Harvell et al. 2004).

Within the fields of epidemiology (the study of the spread and distribution of disease in human populations) and epizootiology (the analogous field for animal populations), modeling has played a significant role by providing a powerful means to investigate the dynamics, containment and prevention of disease. Epidemiological modeling has historically been mathematical in nature, relying primarily on deterministic equations to examine the parameters

that determine the dynamics of disease (Kermack & McKendrick 1927, Anderson & May 1979, Murray 1989). However, these models can be limited in their scope and application, as they become complicated and impractical when attempting to represent characteristics of disease in heterogeneous populations (Smith et al. 2002, Shirley et al. 2003). Recent strides in computer processing power have allowed simulation modeling to become more accessible and reasonable, and it is now possible to examine parameters of disease spread in heterogeneous populations which would not be achievable with deterministic modeling alone (Jeltsch et al. 1997, Bagni et al. 2002, Koopman 2002, Leung & Grenfell 2003).

It is possible to conceive of a model that would address the dynamics of disease in a coral community with a classical approach. For instance, the basic equations of Kermack and McKendrick (1927) could be applied to a coral population, where it is split into compartments of susceptible, infected and recovered/immune, and then average rates are applied to describe the change in distribution of the population into these compartments. However, the application of these models would be limited by factors such as the characteristics of individual species, such as size and density within the community, as well as characteristics yet to be defined that might describe differential susceptibility between species. An appropriate simulation model, however, could incorporate these characteristics and is therefore more likely to accurately portray actual coral disease dynamics so that real world applications of the model would be possible.

Here, I describe the framework and details of the Simulation of Infected Corals (SICO) model which was developed in response to the need to investigate coral disease dynamics within a framework that would allow the incorporation of these key factors of coral reefs, namely their spatial heterogeneity and species diversity. This model will be used to simulate the dynamics of the coral disease white plague type II; a deadly disease

that affects a wide variety of species and which has the capability of altering the structure of coral reef communities (Nugues 2002, Richardson & Voss 2005). Although the development of SICO has primarily focused on this important coral disease, it can be used as a basis for investigating transmission factors, infectivity, and impact dynamics of other coral diseases for which similar information is known. The model is programmed in Java 4.0 using the Repast component toolkit with a Mercenne Twister random number generator (Collier et al. 2003). The details of the model structure are presented below in similar fashion to the standards suggested by Grimm et al. (2006).

MODEL DETAILS AND FUNCTIONING

Scales, State Variables and Model Parameters

Spatial and temporal scales of the simulations

The objective of this project was to study the incidence of disease within a "local" population of coral, and so the spatial scale of the simulation landscape was set to the scale of a typical reef study site, approximately 100 x 100 m, within a larger area of similar community composition. The simulation landscape was represented by a square grid of 10,000 cells, on which individual coral colonies with unique characteristics were distributed. In order to avoid potential scale factors or edge effects, the landscape was represented as a torus, having continuous borders left to right and top to bottom (and the reverse).

Tissue loss on colonies affected by white plague type II has been shown to occur at rates of up to 2 cm/day (Sutherland et al. 2004). An implication of this level of tissue loss is that small colonies can potentially experience total mortality due to disease in a matter of days. Therefore, SICO's temporal resolution was set such that each time step

within the simulation represented one day. A resolution greater or less than a day would result in unnecessary time steps under the former condition or an inaccurate portrayal of the loss of small colonies under the latter.

Coral colony agent variables and model parameters

There exists for every simulation run, an array of coral colonies (agents) that consists of all living corals within the simulation and which are the basic units of the model. Each agent contains a common list of variables (Table 1A), and they are assigned values to their own specific variables based on certain initial population-level variables (Table 1B). The details of this process are covered in the section "Initiation". Variables can be fixed so that they do not change after the colony's creation or flexible which indicates that their values can vary from their initial value. This allows each colony to be distinct and acquire a unique history which will ultimately impact its fate within the simulation environment.

The x, y position variable of an agent determines its placement in a cell on the grid and is fixed throughout the simulation run. Each grid cell is capable of containing only one agent at a time. When a colony agent experiences complete mortality during a simulation, it is removed from the living coral array and from the grid, thereby freeing the grid cell to be occupied by a newly recruited "juvenile" coral. Based on the initial designation of the population-level variable that describes ***taxonomic compositions***, corals are given a taxonomic designation which can be defined to different levels of detail (e.g. family, genus, or species-levels) and which are also fixed throughout the course of the simulation run.

Flexible variables are capable of changing from their initially stated value to some other value over the course of the simulation. Of these variables, a colony's ***state*** variable

and its ***probability of infection*** variable are the most critical. In traditional "SIR" epidemiological models, populations consist of proportions of susceptible, infected, recovered and dead individuals, hence S.I.R. (susceptible, infected, and removed) (Kermack & McKendrick 1927). This fundamental aspect of traditional models is incorporated into SICO by the corals' ***state*** variable which defines each coral's status at any point in the simulation. A coral can have a ***state*** of: 1) not susceptible, 2) susceptible, 3) infected, 4) recovered and 5) dead. The transition of a colony's ***state*** variable from susceptible/recovered to infected during each time step is dependent on the value of its ***probability of infection*** variable. This value is reset at the start of each time step and then adjusted through interactions with other corals in the simulation during that time step. How the value of this variable is calculated is discussed in depth in the "disease parameters" section below and a more detailed discussion of the timing and details of the transition are covered in "scheduling".

Other variables within corals that are flexible over the course of a simulation include the colony's ***size***, which is the maximum size of the colony in centimeters, and a record of the amount of ***mortality*** that a colony experiences, which is the number of centimeters of live tissue lost due to natural or disease-related mortality. ***Size*** can be initially defined based on a coral's ***taxonomic designation*** while ***mortality*** is set to zero for all colonies at the initiation of a simulation. These variables can increase or decrease over time based on growth and/or on the colony's history within the simulation (e.g., infection by disease causes ***mortality*** to increase and ***size*** to decrease). The scheduling and rules by which these changes occur is discussed in more detail in the "process overview and scheduling" section below.

A colony can experience an increase in its ***mortality*** and a decrease in its ***size*** during a time step if it experiences natural mortality. Natural mortality is a daily probabilistic occurrence and dependent on two parameters: the probability of experiencing mortality designated *natural mortality percentage*, and the amount actually experienced by an agent designated *natural mortality amount* (Table 2A). A colony has an x% chance of experiencing mortality in a time step as defined by *natural mortality percentage*, and the amount of mortality experienced by the colony is based on random selection from a uniform range of numbers, the upper boundary of which is determined by *natural mortality amount*. Both parameters can be based field data. For instance, during two years of monitoring on Little Cayman reefs, it was documented that 1% of colonies experienced mortality attributable to causes other than disease (assumed to be "natural") in the monitoring time period and that this mortality ranged between 0 and 0.15 cm^2 per day. This information was used to parameterize the occurrence and amount of natural mortality within simulations presented in the following chapter.

A colony can also experience an increase in its ***mortality*** and a decrease in its ***size*** if its ***state*** is set to infected. The coral selects a value from a uniform distribution of random numbers defined by 0 and the disease parameter *mortality rate cap*, which describes the maximum rate of linear tissue loss in centimeters associated with disease (Table 2B). However, if this value is less than the parameter *recovery rate threshold*, the coral is directed to recover and change its ***state*** back to susceptible. If it is not less than the *recovery rate threshold*, the coral subtracts and decreases the random amount from its ***size*** and ***mortality*** variables, respectively. If, when this amount is subtracted, the coral's ***size*** becomes a value equal to or less than 0, the coral agent changes its ***state*** to dead.

The simulation rule by which a coral recovers, specifically that if a coral selects a mortality rate that is less than the *recovery rate threshold*, is meant to represent the observation that infected colonies in reality will often recover after tissue loss due to disease slows (Richardson et al. 1998a, Nugues 2002, Borger 2003, 2005). However, incidence and virulence of many marine diseases including coral diseases are known to increase with warmer water temperatures (Harvell et al. 1999, Ben-Haim & Rosenberg 2004). Therefore, the *recovery rate threshold* or *mortality rate cap* parameters can be set so that they are dependent on factors such as seasonality in order to reflect the increasing virulence of some pathogens during warmer months. For instance, in scenarios where *mortality rate cap* is affected by seasonality, this parameter can be larger during warmer simulation months (June – October) thereby increasing the probability that when a colony agent's ***state*** is infected, it will choose a value larger than that defined by *recovery rate threshold*. In this scenario, *mortality rate cap* in cooler simulation months (November to May) would be smaller thereby decreasing the probability that a coral will choose a value larger than the *recovery rate threshold*.

A colony's ***size*** can increase through growth as defined by the parameter *coral growth rate*, which can be species-dependent and derived from field observations or published growth rates. This parameter designates the average rate of linear increase in a coral's diameter in centimeters on a daily basis. Every time step (day) of the model, the model selects for each colony agent a random value to increase its ***size*** from a normal distribution defined by *coral growth rate*.

The reef area that the simulation grid represents is defined by the parameter *area*. In order to distribute colony agents within this area and to assign each colony initial

values for its variables, the model is initialized using input variables describing coral distribution that can be based on data collected in the field (Table 1B). These input variables include: 1) ***coral density*** or ***coral cover*** (a measure of the abundance of living coral colonies) 1) ***taxonomic identifications*** (a list of names of taxonomic groups), 2) ***taxonomic compositions*** (a list of proportions that each taxonomic group should occupy at initiation within the entire coral population), 3) ***size distribution of corals*** (can refer to all corals or can be based on ***taxonomic identifications,*** and includes average, minimum and maximum sizes), and 4) ***spatial aggregation of corals, alpha*** (***α***) (determines how aggregated coral colony agents are across the simulation landscape based on the methods of Lundquist and Botsford (2004)). The parameter *area* and the variables described above allow simulations to represent diverse types of coral communities.

After initiation of the simulation, new juvenile corals can be added to the simulation landscape at different time steps throughout the simulation and in different abundances. These are defined by the model parameters ***recruitment percentage*** which determines the number of juvenile corals to add to the simulation based on the abundance of living corals, and ***recruitment time-step*** which determines how often recruitment will occur (Table 2).

Disease incidence

Disease is not explicitly represented within the model as individual agents, and instead colonies become infected based on the value of their ***probability of infection*** variable which can be changed either by the model or through interactions with other corals. At the population level, there are two types of disease incidence that occur through either **disease input** or **infectious spread**. Disease input represents the number of cases of

disease incidence that occur through random selection by the model. This is a process by which to "seed" the model with diseased colonies, and can be considered the input of disease into the system from an outside source. The two model parameters that determine disease input include *disease seeding proportion* which is the amount of random disease input into the system based on the number of living corals, and *disease seeding time step* which defines the frequency of input (Table 2B). When disease is input into the system, *seeding proportion* is used to calculate the number of random cells to distribute disease to based on the abundance of living corals (e.g., if disease seeding proportion = 5%, and the number of living corals is 100, 5 random grid cells will be selected). If a living coral is found in the random grid cell selected, its ***state*** is changed to infected. The lesser and more spread out the living corals in the system are, the less likely disease input will occur. Therefore disease input is density-dependent.

Infectious spread is determined at the end of each time step and is dependent on the interactions of the corals during the time step; therefore it is not defined by any model-level parameters. Unlike disease input which is directly indicated through the designation of *seeding proportion* and *seeding time step*, infectious spread incidence is a population-level parameter that emerges from interactions between colonies within each simulation. Therefore, disease incidence caused through infectious spread cannot be designated at the start of a simulation. Infectious spread incidence is instead calculated as the proportion of living colonies that change from susceptible to infected through transmission of infection between colonies at any time, and it represents the probability of a colony becoming infected through infectious spread throughout the duration of a simulation.

Infectious spread between colonies is determined through interactions between susceptible and infected colonies within the simulation, the strength of which is defined by **m**, or the force of infection. During a time step, all infected colony agents interact with all other colony agents within a set distance defined by the disease parameter *range* (Table 2B). An infected colony will then calculate for every other colony with its *range* the force of infection, **m**, between itself (j) and the other colony (k) using the following equation:

$$m_{jk} = \frac{s_k}{d_{jk}^{\rho}} \qquad \text{(Equation 1)}$$

In equation 1, s_k is the susceptibility of the colony as defined by the disease parameter *susceptibility*, d_{jk} is the distance (grid cells) between the colonies, and ρ is a decay factor describing the influence of distance on the spread of disease as defined by the disease parameter *rho*.

The effect ρ on the value of m between two colonies is displayed graphically in Figure 1. When ρ is small, distance between colonies has less of an effect on interaction than when ρ is large. For example, when ρ is equal to 1 the force of infection decreases with distance. When ρ is smaller than 1, distance begins to have little effect on the incidence of disease, and instead a colony's inherent susceptibility becomes the more significant factor in determining whether it becomes infected or not. Therefore, when ρ is close to zero the force of infection within the population is dispersed widely, and represents a well-mixed system where all colonies are strongly associated and transport of the disease agents is far. Conversely, when ρ is much larger than 1 it has a large affect on the force of infection and causes it to decline significantly with increasing distance between colonies. The force of infection in this scenario is concentrated with small

distances, representing a system in which infectious agents are limited in their dispersion and colonies are assumed to be increasingly isolated with greater distances.

While ρ impacts the force of infection between two corals when at least one of them is diseased *range* sets the limit for the area in which the force of infection acts. This parameter was introduced to in order to cut processing power by the computer. However, by setting ρ equal to 1 and varying *range*, the effect of distance can still be investigated and various levels of infectious spread between colonies can be simulated. These scenarios include where infectious spread accounts for nothing (when *range* = 0) and disease incidence is entirely input, or where disease is infectious but there is no effect of distance (*range* = area, $\rho = 0$), and where infectious spread occurs only between immediately adjacent colonies (when $\rho >> 1$ and/or when *range* is small).

Initialization - building the simulation environment

The ***build model*** method of the program creates and distributes the initial population of coral colonies across the simulation grid. The model creates and distributes corals in sets, and in between the creation of each set the simulation calculates the simulation ***coral density*** or ***percent coral cover***. This value is compared to the input target value, and the model continues to create and distribute sets of corals until the simulation and target values are approximately equal. Each simulation may undershoot or overshoot the input density or coral cover by a small amount, therefore multiple simulation runs of the same scenario will have initial values for these variables that vary around the targets.

When a colony agent is "created" within the simulation, the model is actually creating a unique instance of a coral java class which contains a list of variables. Values are assigned to these variables when an instance of a coral is created but these values do

not have to be equal between instances. The model assigns values based on several input variables already discussed.

When ***taxonomic compositions*** of corals vary (i.e., when all colonies are not one species), each set of colony agents is created with different proportions of colonies initialized with different taxonomic designations as defined by ***taxonomic compositions***. Because colony agents are distributed on the grid individually and because the model will eliminate a coral if it cannot find an open cell on the grid after several tries, taxonomic groups can inadvertently be given precedence if they are the first corals to be distributed. They can then become more abundant than would otherwise be expected. For example, if taxonomic groups were to be distributed alphabetically, those beginning with the letter "a" would have a greater chance of occupying grid spaces than those beginning with the letter "b". Therefore, before creating each set of corals, the program shuffles the order by which the taxonomic groups will be created so as to ensure that no group is given precedence when distributed, which would possibly alter the initial ***taxonomic composition***. Once this is done, a taxonomic group is selected and the number of corals to create per set for that taxonomic group is determined by multiplying the number of corals to create per set by the probability that a coral is of that taxonomic group. This probability is entered into the model based on field data and is defined by the variable ***taxonomic composition***.

To test the ability of SICO to accurately create coral communities from data entered from field surveys, an analysis of similarities (ANOSIM) test using the statistics package PRIMER 5 (PRIMER E-Ltd, 2001) was used to compare simulated coral communities to data collected from a 1999 assessment of the benthic communities of

Little Cayman, Cayman Islands, B.W.I. (Manfrino et al. 2003). The ANOSIM test tests the null hypothesis that no differences exist between groups of community samples using a Bray-Curtis similarity index matrix created from species abundances lists. A statistic, Global R is calculated with a significance level of 0.1%. Therefore an ANOSIM between communities that returns a significance level > 0.1% indicates that they are not significantly different from each other.

The field data were collected following the benthic methods of the Atlantic and Gulf Rapid Reef Assessment (AGRRA) which includes recording species abundances with a line intercept method (Kramer & Lang 2003). Average community composition values by species from data collected at the site "Sailfin" during this survey were used to distribute simulated coral colonies within SICO (Table 3 – "field data"). Ten simulations using Sailfin parameter values were initiated with SICO. Average density and community composition values from these simulations are also displayed in Table 3 ("simulations"). Species abundances within simulations were compared to field data using the ANOSIM test and were not found to be significantly different (Global R = 1, significance of sample statistic = 9.1%).

In nature, coral colonies settle and recruit to the reef community based on the chemical and physical properties of the substrate. This typically results in an aggregate, as opposed to random, distribution. The degree of aggregation of colonies is also dependent on the character of the substrate and the oceanographic conditions of the environment. Coral patches or "patch reefs," which are typically found in protected, shallow reef zones such as lagoons, are generally highly aggregated collections of colonies separated by unsuitable substrate (e.g. loose sand/seagrass). Spur and groove, or

buttress, reef systems, which are found on wave exposed shelves, develop on large expanses of suitable substrate and though aggregation does occur, it is less significant than amid patch reefs. In order to represent the degree of aggregation of colonies at a site, the input variable ***alpha***, ***α***, is used. ***Alpha*** is the percentage of the total colonies that are distributed into randomly selected grid cells at first. To distribute the remaining colonies, the model randomly selects colonies already distributed on the grid and places colonies in any empty cell that is as close as possible to the selected colony's cell. Therefore, coral colonies in a simulation run where ***α*** = 50% (i.e., 50% of colonies are distributed randomly and 50% are distributed adjacently to random colonies) will be less aggregated then in a simulation where ***α*** = 2% (i.e., 2% of colonies are distributed randomly and 98% are distributed adjacently to random colonies). If ***α*** is 1, the distribution of colonies is completely random (Figure 2). When simulating reef systems that are fore-reef spur and groove reefs, ***α*** is set to a moderate level of 20%.

Process overview and scheduling

Processes built into the model include, at the population-level recruitment, mortality and disease incidence, and at the colony-level growth, tissue loss, and disease spread. Scheduling of these events is determined through the order in which various methods are accessed in each time step. The main program has methods that it follows during each time step which determine population-level processes. Each instance of a coral colony has its own set of methods that it carries out when directed to by the main program (Figure 3). All of these methods act to change variable values of the population (e.g., number of colonies infected, number of colonies alive) and of each of the colonies (e.g., amount of tissue lost due to disease, probability of infection for a time step).

The first method that the main program carries out is to reset the ***probability of infection*** variable for all living colonies to zero for that time step. This variable within each colony was altered in the previous time step depending on the force of infection between the colony and all infected colonies that it was within a set distance of defined by *range*.

Once all colony agents' ***probability of infection*** variable is reset to zero, the main program then directs each coral to follow its main method called "step" which contains many sub-methods described below. Each coral follows this method one at a time, such that it executes its step method before the next coral is directed to execute it and so on. The process of executing the step method within all coral agents is randomized so that the order of corals directed to step is not the same during each time step, eliminating any potential bias of the ordering of coral agents.

Coral colony step methods

The first sub-method that the coral is directed to follow under its main step method is growth. A coral experiences growth by increasing its ***size*** by an increment that can be generic or designated by its ***taxonomic identification*** and is defined by the parameter *coral growth rate*. The scheduling of this method before all other colony sub-methods has implications when a coral is infected and is undergoing mortality from the infection. If a coral's ***size*** is increased enough to offset the effect of its ***mortality*** during that time step, the coral remains living.

The second coral sub-method is accessed only if its ***state*** is set to infected. This method determines how much mortality the coral will experience due to disease in that time step. As previously described, the coral selects a value from a uniform distribution

of random numbers defined by 0 and the parameter *mortality range cap*. If this value is less than the *recovery rate threshold*, the coral recovers. If, when this amount is subtracted, the coral's ***size*** becomes a value equal to or less than 0, the coral is directed to execute its death method, which puts the coral into a queue to be removed from the live coral list by the model later in the time step.

The last sub-method executed by a coral when it is stepped is again only accessed if the coral's ***state*** is set to infected. This method directs the coral to access every other coral in the coral population within a distance defined by the model parameter *range* in order to add to their ***probability of infection*** variable based on the force of infection, m, between the two colonies as previously described (Equation 1). The execution of this method after the mortality method means that the coral will no longer contribute to the incidence of disease if it has been determined already to have recovered or died during that time step.

Population-level methods

Once all corals have stepped, the program begins to carry out population-level processes based on the results of the colonies changing their variable levels.

Disease Incidence

First, the model initiates the incidence of disease during that time step, which is dependent on the ***probability of infection*** of each coral. As covered previously, a coral's ***probability of infection*** is changed to 100% automatically if during a time step disease is input into the model and the coral is randomly selected to become disease. This process occurs before all corals execute their step method so that the randomly infected colonies' infection levels will influence other colony agents during that time step. However, if it is

not set to 100% at the beginning of a time step because of disease input, then each colony's *probability of infection* it is altered through the duration of the time step by infected corals accessing and adding to it based on the force of infection, m, between the two colonies. Therefore, at the end of every simulation step, all corals have a ***probability of infection*** (pI) based on the number and value of the force of infections between it (k) and any infected coral (j) that it is within the distance set by *range* such that:

$$pI_{k,t} = \sum_{j \neq k, d_{jk} < range} m_{jk,t} \qquad \text{(Equation 2)}$$

Note that the forces of infections are added because each is independent of the other. Specifically, the force of infection of an infected coral on a susceptible coral is independent of the force of infection of another infected coral on the same susceptible coral.

All colony agents' ***probability of infection*** values are translated into incidence by a stochastic process of random number selection. The model selects a random number from a uniform distribution of numbers between 0 and 1. If the number selected is less than the coral's ***probability of infection***, the colony becomes infected. Therefore, in the event that a coral has a high level of ***probability of infection***, for example 99%, it also has a 1% chance of not becoming infected depending on the random number selected by the model.

Recruitment

Another method carried out by the program is the population-level process of recruitment. However, this method is only activated as designated by the parameter *recruitment time step*. During the time step in which the recruitment process occurs, the recruitment method occurs before all other methods. This means that corals that are

removed from the grid due to total mortality experienced in that time step are not removed before the recruitment event and their grid spaces are not available for occupation by new corals. The scheduling of this event, though it has implications for the dynamics of the population, has not resulted in major changes to the outcome of simulations when placed before or after the process of removing corals from the grid.

Observation

Within SICO, each colony agent contains variables whose values can change over the course of a simulation and this includes some that keep records of its infection history. Each time a colony's ***state*** changes, the colony records the change and the time step at which it occurs. Therefore, the number of time a colony has become infected and the corresponding number of times it has recovered is recorded for every colony agent.

The records of infection for each colony within the simulation can be used to calculate population-level variables such as disease prevalence and the disease incidence proportion rate (Table 1B). These measures are often recorded in field studies aimed at quantifying disease in coral populations and can be important indicators of accurate model performance when compared with field data.

The spatial distribution of diseased individuals in relation to other diseased individuals or potential vectors and reservoirs is an often used parameter to determine infectiousness in populations, and dates back to the founding of the field of epidemiology and John Snow's mapping of cholera cases in relation to a London drinking well (Snow 1855). It is a powerful means of determining how interactions on the organism-level can lead to patterns of disease distribution at the population-level (Mausner & Kramer 1985, Murray 1989), though it must be used cautiously (Jolles et al. 2002). SICO incorporates

the ability to randomly select diseased colonies and record the distribution (a frequency histogram of relative distances) of diseased colonies surrounding these colonies in relation to the underlying distribution of susceptible colonies. This distribution (Table 1B) can then be compared to distributions in the field based on a similar method of data collection for validation of model functioning.

Model details and functioning summary

The goal of the development of SICO is to design a tool with which to investigate properties of coral disease which would not be possible with today's field sampling techniques. The design and functioning of SICO allows it to be flexible so that it can incorporate characteristics of coral populations that are important in considering the spread and impact of disease, namely the spatial heterogeneity and species diversity of coral reefs. Validation of SICO is possible by comparing the population-level phenomena between simulations and field data. Overall, the difficulty and limitations associated with the study of coral disease in the environment has limited the field of coral epizootiology. SICO provides the opportunity to move beyond those limitations and test hypotheses in ways that have not been possible with traditional field sampling.

DESIGN CONCEPTS

Emergence

Emergence is to "become manifest" or to "rise from an obscure or inferior position or condition" (Merriam-Webster online dictionary 2007). In the context of individual-based modeling, phenomena that emerge are the products of the interaction of the individuals with each other and with their environment and are not imposed in any way on the structure of the model. Examples of emergent phenomena in SICO include the

population-level patterns of disease incidence and prevalence, its rates and patterns of geographical dispersion and the dynamics of changes in coral density or cover and community and size structure of the entire population. These phenomena are the result of the interactions among individuals and the amount of stochasticity included in model functioning, and they are not imposed on the model. These phenomena are also the basis for comparison for matching field data with simulation outputs in order to examine the accuracy of model results.

Conversely, examples of imposed conditions on the model are the initial conditions of the population such as coral density or cover, the spatial distribution of colonies, the community structure of the corals and also their size structure. These conditions are imposed such that the simulation landscape reflects what is found in reality.

Stochasticity

Simulation modeling, unlike deterministic modeling, allows the incorporation of stochasticity into modeling functioning such that multiple simulation runs with the same parameter values will produce a variety of outcomes. Therefore, simulation scenarios can produce a set of probabilities for certain outcomes.

Stochasticity in SICO is incorporated in several ways. At initiation, when colonies are distributed across the simulation landscape, grid cells are selected randomly to distribute within them a designated portion of the population of corals. Because the exact location of colonies in the field is not known for the sites surveyed, this allows a range of distributions to be examined and parameterized. Multiple simulations are run in order to accommodate the variability in outcomes due to changes in the simulation landscape of

corals from the incorporation of this stochastic element. A major improvement to the model would be the coupling of SICO to a Geographic Information System (GIS) in which the geographic position of each colony is known. A further improvement would be the incorporation of coral size into the probability of infecting other corals – a factor that would require additional field parameterization and validation.

Stochasticity is incorporated in the initial seeding of disease within colonies. A designated portion of the population is randomly selected to become infected at the start of simulations and in some cases, throughout simulation runs. Due to the deficient state of knowledge of the factors responsible for the incidence of disease in the field, the incorporation of this element of stochasticity allows the model to investigate the effects of seeding different portions of the population with disease at different times without basing it on a specific responsible factor. This seeding can be based on an outside input of disease or the initiation of disease from within the population, both of which are possible as discussed below.

Many populations require an outside input of disease into the system in order to initiate epidemic or epizootic disease spread, the most common example being measles (Bartlett 1957, Bolker & Grenfell 1995). In this case, the properties of the pathogenic agent's transmission and incidence interacting with properties of the general population are such that the disease will "burn out" after a certain period of time (Black 1966). This may also occur in a metapopulation situation, which is often dependent on stochastic events (Grenfell & Harwood 1997), and it is not hard to imagine it occurring in a coral reef metapopulation. For instance, if the reefs surrounding individual islands within an island chain are considered a metapopulation, disease may go extinct on the reefs of one

island only to be repopulated through connectivity with another population where disease persists.

Disease can also be stochastically initiated from within a population. This type of scenario may occur when a stress event causes a population to become susceptible to infection by opportunistic pathogens. Corals have a diverse microbial community flora within their surface mucus layer, and this microbial community can vary among species (Meikle et al. 1988), with age (Ducklow & Mitchell 1979), with depth (Crossland 1987). Most recently it has been discovered that the microbial communities of the mucus layer can change under stress from bleaching (Ritchie 2006). It is highly possible that a normally present and benign microbe within the coral mucus could become pathogenic under stressful conditions (Harvell et al. 2004, Sutherland et al. 2004). In this case, disease input would result from initiation within the population.

Stochasticity is also incorporated in a colony's selection of its growth during each time step of the model. Coral growth as measured in the field can be highly variable. Acquiring specific growth rates of coral species was beyond the scope of this study and, therefore, rates of growth specific to species were derived from literature reviews which provided these rates in ranges. When a colony experiences growth, it randomly selects an increment to increase its size from a range of growth designated for its species. Designation of the range of this parameter can have implications of the survival of colonies within the model; for example, if the growth of a coral is capable of offsetting the amount of mortality that it experiences, it will continue to survive. Therefore, ranges of growth for species are, when feasible, as specific as possible.

Mortality due to disease also incorporates an element of stochasticity, which can have severe implications for the population-level processes of the mortality of colonies, the average duration of infections and the average amount of tissue lost per colony. Mortality due to disease can be highly variable between colonies and within colonies through time. Mortality rates can also vary between regions and between seasons. Because mortality rates in the field are measured for specific sites, the simulations are more specifically parameterized to the regions under study, thereby limiting the total amount of stochasticity that must be incorporated.

Stochasticity is also incorporated to a degree in the incidence of disease itself. All corals become infected during a time step dependent on their ***probability of infection*** variable value at that time step. Although the value that the ***probability of infection*** variable has at any time step is dependent on the interaction of that coral with other corals and with the environment, the actual determination of the incidence of disease within a coral is probabilistic. Therefore, in a time step a colony may have a 99% ***probability of infection*** variable value but it may not become infected.

SICO uses the selection of randomly generated numbers from mathematical distributions for stochastic processes or "decisions" within the model. For random numbers selected within a range of numbers (e.g., the selection of a growth increment for a species within a range designated for that species) the model will select the number from a uniform distribution of numbers between the lower and upper range values. For numbers that deal with population parameters the model selects a random number from a distribution corresponding to the population-level distribution of that parameter. For example, in assigning initial size variables to colonies of a certain species the model will

select a size value from a Poisson distribution of sizes created using the average and standard deviation of sizes for that species. However, all newly recruited colonies are designated a size of 2 mm as this is the typical size of colonies when they become apparent to the human eye. In determining actions based on probabilities, the model selects a random number from a uniform distribution of between 0 and 100; if the number is less than the probability of that action happening, the action occurs, and if not, the action does not occur.

Collectives

As in the field, coral colonies in the model can be analyzed or directed in terms of their species composition and/or size distribution. Colonies within the model have variables that are assigned at their initiation which identify them as a particular taxonomic affiliate. Also, although mutable, colonies contain a variable indicating their maximum diameter of tissue which allows them to be placed into a size category. For this reason, collectives of colonies can experience similar conditions based on their species or size despite the fact that they may be separated spatially within the model landscape. For example, the susceptibility of colonies to disease can be set to correspond to their species or size thereby allowing one species or size category to be more susceptible versus another. Also, the incidence of disease within a certain species or size group may be influenced by its dominance in the community of the simulation if disease spread is based on density (i.e., the more abundant a coral group, the more it is affected because the sheer numbers that can become affected).

SUMMARY

In order to determine the impact of coral disease on reefs today and their potential to alter the structure and function of reefs in the future, the development of models of coral disease has recently been described as a research priority (McManus 2001, Harvell et al. 2003, Weil et al. 2006). Although information on the epizootiology of coral diseases is fairly scarce at this point, it is possible to take what is known and place it into a framework such as SICO that allows the incorporation of new information as it becomes available. Within SICO, hypotheses about the spread and distribution of these diseases in the environment can be tested without the need for extensive field sampling. Overall, SICO will provide a tool for managers and scientists to understand some fundamental attributes of the diseases that may be playing a dominant role in shaping the reefs of tomorrow.

SICO is a spatially-explicit, individual-based model that is capable of representing several features of coral reefs that are not able to be represented using classical analytical modeling approaches. Coral variables are incorporated that determine the species composition, size distribution and spatial distribution of coral colony agents within the model, therefore allowing it to represent the variability of coral communities found in reality. Several parameters describe the interactions between colony agents within the model from which population-level phenomena emerge. However due to a lack of knowledge of many of the epizootiological characteristics of coral diseases several of the disease parameters can not be parameterized with today's knowledge of coral disease alone. The following chapter details the parameterization of these unknown parameters using a pattern oriented modeling (POM) approach.

Table 1: Coral population variables	
A) Colony agent-level variables	**B) Population-level variables**
Position on the grid (x, y)	Coral cover (% of simulated area composed of live coral) or coral colony density (#/simulated area)
Taxonomic designation	Taxonomic compositions (% of colony population of each taxa)
State	Average/Maximum/Minimum sizes (by taxa)
Probability of infection	Spatial aggregation (α) of colonies
Size	Disease prevalence (% of susceptible simulation population in a diseased state)
Mortality	Disease incidence proportion rate (% of the population becoming infected per day)
	Disease spatial distribution

Table 2: Model parameters and their sources

Model Parameter	Description	Source
A) Coral parameters		
Coral growth grate (by species)	Average daily growth rate of colonies based on defined rates for each species when available	Literature review
Natural mortality percentage	Percent of the population experiencing natural mortality on any simulation day	Field observations
Natural mortality amount	Average amount of mortality occurring on a colony that experiences natural mortality in a simulation day	Field observations
Area	Area meant to be represented by the grid	Input
Recruitment percentage	Percentage that determines the number of new colonies entering the simulation as juvenile corals dependent on the existing population	Assumed 1%
Recruitment time step	Frequency of recruitment events	Assumed once a year
B) Disease parameters		
Mortality rate cap	Maximum of the rate of mortality caused by disease during a simulation day. When a colony is infected the simulation picks a value between this number and 0	Field observations
Recovery threshold	The threshold below which if the simulation selects a value for mortality during a simulation day that the colony is disease the colony will recover.	Unknown
Seeding proportion	Number of random colonies selected to become infected by disease input based on the number of colonies that are alive on that day	Unknown
Seeding time step	How many simulation days in a year that disease is input into the system	Unknown
Range	Maximum distance (# of cells) of interaction between infected and susceptible colonies	Unknown
Susceptibility Probability (s)	Inherent susceptibility of colonies	Unknown
Rho	Distance effect on the force of infection between colonies	Unknown

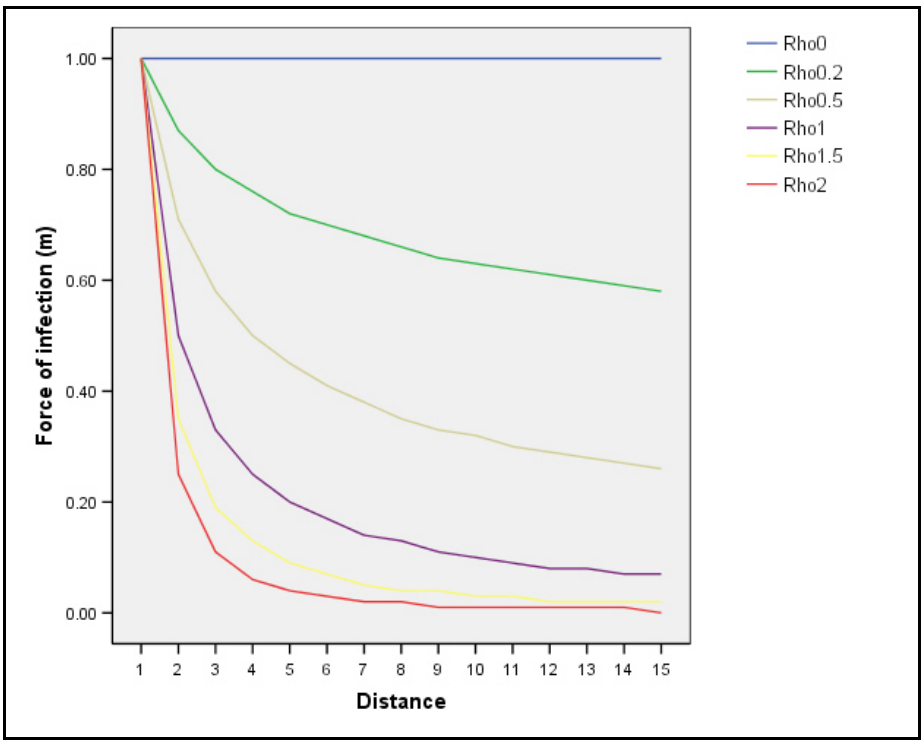

Figure 1 – Effect of distance and values of ρ on the force of infection between an infected colony and a colony with a susceptibility of 100%.

Table 3: Comparison of simulation and field coral communities

Coral Species	Avg. proportion in community from field data (± std error) (No. of transects = 16)	Avg. proportion in community from simulations (± std error) (No. simulations =10)
Agaricia agaricites….....	……..29% (±4.22%)……..	…..……24% (±0.02%)
Colpophyllia natans	1% (±0.78%)	2% (±0.02%)
Dichocoenia stokesii	1% (±0.69%)	2% (±0.01%)
Diporia labrynthiformis	1% (±0.70%)	2% (±0.01%)
D. strigosa	1% (±0.70%)	2% (±0.01%)
Eusmilia fastigiata	1% (±0.63%)	2% (±0.02%)
Meandrina meandrites	1% (±0.57%)	2% (±0.02%)
Montastraea annularis	7% (±2.16%)	7% (±0.02%)
M. cavernosa	7% (±2.26%)	7% (±0.01%)
M. faveolata	17% (±4.39%)	14% (±0.02%)
M. franksi	17% (±3.79%)	14% (±0.02%)
Mycetophyllia spp.	1% (±0.69%)	2% (±0.01%)
Porites astreoides	5% (±3.23%)	5% (±0.01%)
Porites porites	7% (±1.80%)	7% (±0.01%)
Siderastrea siderea	6% (±2.21%)	6% (±0.01%)

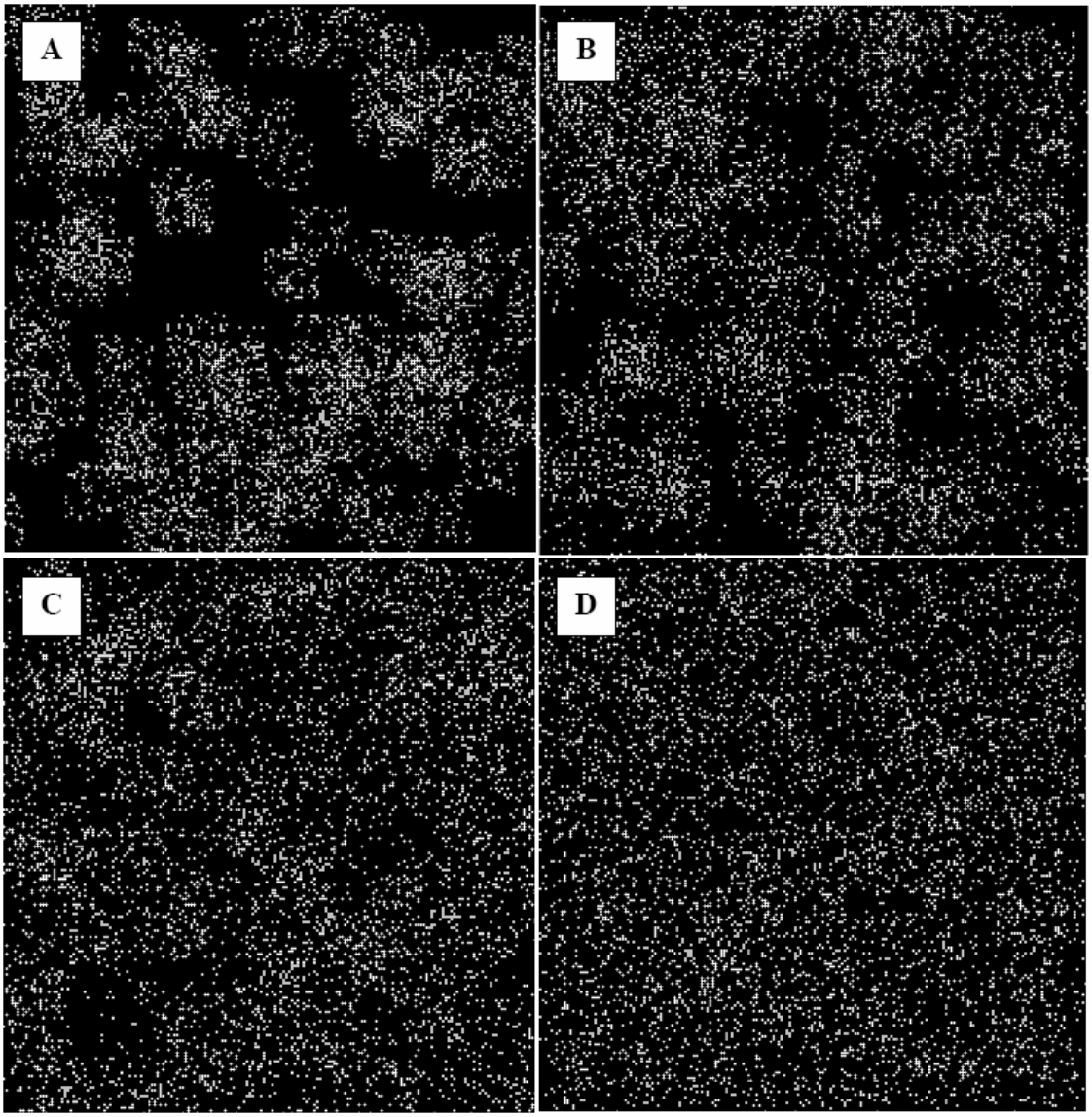

Figure 2: Output displays of four simulations parameterized with identical coral densities, community compositions and size distributions. Each box represents different degrees of aggregation with α set to different levels. A represents a highly aggregated coral population where α is set to 2%. In B α = 10%, and in C α = 50%. D represents a completely randomly distributed coral population with α set to 100%.

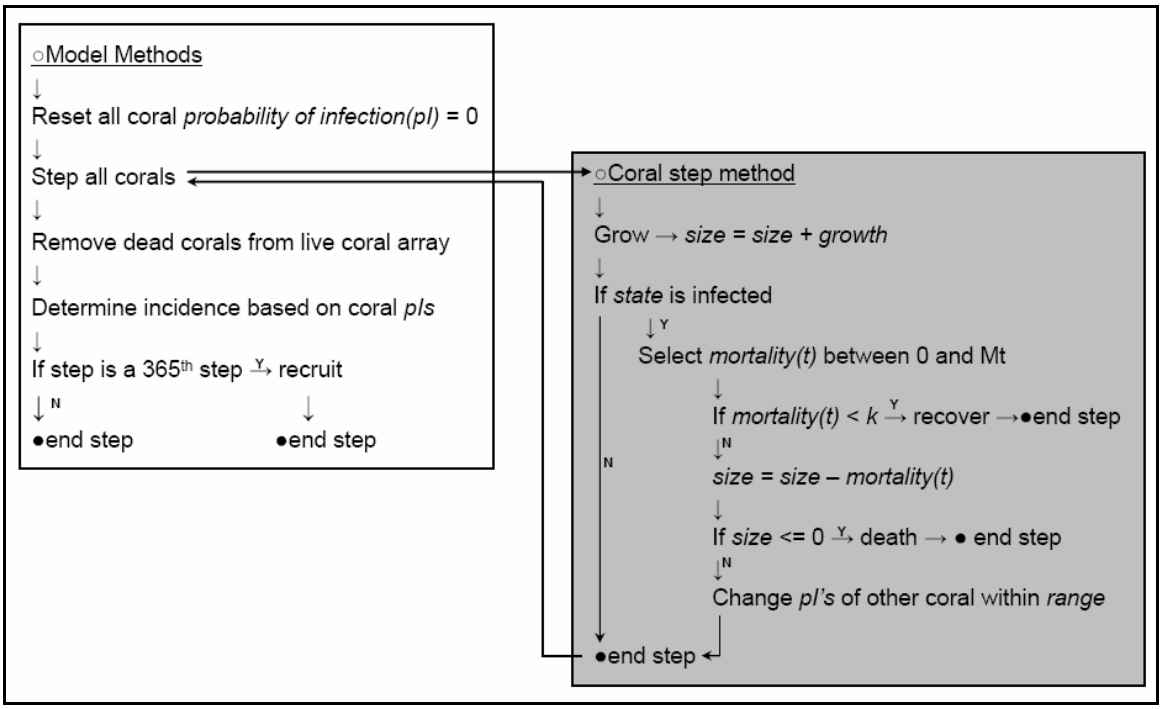

Figure 3: Flowchart depicting scheduling of model and coral methods during one simulation time step. Mt = mortality rate range cap and k = recovery threshold rate

Chapter 4: Investigating white plague disease dynamics through the development of the 'Simulation of Infected Corals (SICO)' model

INTRODUCTION

The first reporting of a coral disease occurred in the early 1970s when Antonius described black band as causing rapid tissue loss on colonies of scleractinian corals (Antonius 1973). Since then, more than 30 syndromes have been reported from coral reefs in all parts of the world and the number continues to grow (Ward & Lafferty 2004). Few of these syndromes have been intensively characterized and the extent of their impact on the reef communities that they affect remains debatable (Green & Bruckner 2000, Weil et al. 2002). Overall, the mechanisms behind the spread of these diseases and the factors controlling their distribution have been hard to determine due to their rarity and patchiness and the limited number of quantitative *in situ* studies (Kuta & Richardson 2002, McCallum et al. 2003). Therefore, studies are often limited in both space and time, and results of these studies are applicable only to specific reef areas and not capable of explaining disease dynamics in general.

In the field of human disease research, epidemiologists perform the essential task of studying the incidence and distribution of disease as it affects populations (Mausner & Kramer 1985). Modeling is a standard epidemiological tool used to understand disease dynamics in a population (Anderson & May 1979). Models have also historically been useful in identifying the underlying mechanisms behind the spread and distribution of a disease if they are unknown (Smith 2001). As yet there have been limited applications of the tools and methods from the discipline of epidemiology, including modeling, to the study of coral diseases (Green & Bruckner 2000, McCallum et al. 2003). By developing a

model capable of simulating disease dynamics in variable coral habitats, hypotheses concerning coral disease spread and impact can be tested.

Here, the development of an individual-based epizootiological coral disease model is described as it is applied to the dynamics of suspect white plague type II (SWP2) on the once *Montastraea* spp.-dominated forereefs of Little Cayman Island, the smallest and least populated of the three Cayman Islands, British West Indies (B.W.I.). Throughout a period of monitoring from 1999-2004, persistent levels of SWP2 coincided with significant changes in the structure of the coral communities (described in Chapter 2, this volume). The Simulation of Infected Corals (SICO) model is used here to investigate the dynamics and potential impact of SWP2 on a simulated reef environment corresponding to the reef environment of Little Cayman. Following the Pattern Oriented Modeling (POM) approach (Grimm 1994, Grimm et al. 1996), the effects of varying types of disease spread and the incorporation of different levels of host susceptibility were explored, and the outcomes of simulations were assessed in terms of their accuracy to field observations. Projections were then made based on today's incidence rates, and under various scenarios.

METHODOLOGY

SICO has previously been described in detail in this volume (chapter 3). Briefly, it is an individual-based model, implemented in Java and using class libraries from the RePast component toolkit (Collier et al. 2003). Within SICO, coral colonies are the fundamental units of the model and are distributed in cells on a grid whose dimensions were 200 by 200 grid cells (40,000 cells). The area of the grid represented a specific reef area of 100 m^2. Unnatural boundary conditions were reduced through the use of a toroidal grid.

Model functioning in SICO is determined by several coral variables and model parameters previously described (chapter 3, Tables 1 & 2). Values for several of the model parameters including *seeding proportion*, *seeding time step*, *range*, *susceptibility probability*, and *rho* are unknown due to the lack of knowledge of fundamental epizootiological characteristics of white plague. To narrow down the range of possible values for these parameters, a Pattern Oriented Modeling (POM) approach was applied (Grimm 1994, Grimm et al. 1996, Grimm & Railsback 2005). POM uses multiple patterns at the population-level to validate model functioning. This approach has been used successfully in several ecological studies where parameter values were unknown (Wiegand et al. 2003). In this study, multiple patterns including disease prevalence, incidence, spatial distribution, and changes in the community structure of various coral communities were used to parameterize and test the functioning of SICO.

First, the composition and distribution of simulated coral populations were initialized using 1999 field data from one site on Little Cayman, Sailfin Reef. This site was sampled in 1999, 2002, and 2004 using methods of the Atlantic and Gulf Rapid Reef Assessment program (described in methods of chapter 2), and a significant loss of coral cover (chapter 2, Figure 5) and consistent levels of SWP2 were recorded (chapter 2, Figure 2). Through further monitoring at Sailfin, rates of incidence (chapter 2, Table 9) and associated tissue loss (chapter 2, Table 10) for SWP2 and other types of mortality were quantified *in situ*. The spatial distribution of disease at this site was also quantified in 2005 (chapter 2, Figure 8). These population-levels patterns (i.e., coral cover loss, disease prevalences in 2002 and 2004, disease incidence and spatial distribution)in addition to changes in the relative abundance of the six most abundant species were used

for statistical comparison to simulation data. Equivalent data collected from three other sites in Little Cayman (Coral City, Grundy's Gardens, and Jigsaw Puzzle) were used for validation of the unknown parameter adjustment results. For further validation, data from monitoring in the Los Roques National Park of Venezuela, published in Croquer *et al.* (2005) were used, as this data set also documented coral cover change in relation to signs consistent with SWP2. Using disease parameter settings from the results of the unknown parameter adjustment and validation, simulations were projected 100 years to develop a range of potential impacts to the coral communities at current and possible disease incidence rates.

Sensitivity analysis

A sensitivity analysis was performed first to determine which disease parameters influenced model outcomes significantly. For this analysis, 200 parameter sets were created by randomly selecting values within uniform ranges for all disease parameters (Table 1). SICO distributes and assigns variable values to simulated coral colonies stochastically, and the development of infections within the population is probabilistic. Ten simulations were performed for each parameter set in order to develop a mean and standard deviation associated with each parameter set. The composition and distribution of the initial coral population were parameterized with field data from Sailfin Reef in 1999, and simulations were run for the length of time corresponding to the monitoring period (1999 – 2004). Those simulations that predicted a mean complete loss of coral cover were eliminated from further analysis as this outcome did not occur in reality. Multiple linear regressions were then run with the predicted mean coral cover loss as the

dependent variable and the parameter values as independent variables to determine which parameters significantly impacted the outcome of simulations.

Parameter adjustment (calibration)

Ranges were then established for the significant parameters to calibrate the model using data recorded between 1999 and 2004 at Sailfin Reef. Again, multiple simulations were performed for every parameter set. Simulations were run for a time period equivalent to the monitoring period in the field, 5 years, or 1825 simulation "steps", with each step equivalent to 1 day. Results of these simulations were statistically compared to field data that included coral cover change between 1999 and 2004 (loss between day 1 and day 1820 of the simulation time period), mean point prevalence of SWP2 in the summer of 2002 (compared to point prevalence on day 1090 of simulation time period), mean point prevalence of SWP2 in the winter of 2004 (compared to point prevalence on day 1650 of the simulation time period) and mean percent composition of the six most abundant species (*Agaricia agaricites*, *Montastraea annularis*, *M. faveolata*, *Porites astreoides*, *P. porites*, and *Siderastrea siderea*) in 2004 (on day 1820 of the simulation).

Statistical comparisons between results of field work and simulation runs were made using a special case of the Welch confidence-interval approach, which constructs a confidence interval for the difference in two expectations with unequal sample sizes (Law & Kelton 1982). In this approach, a confidence interval is developed for the difference between field and simulation means, $\zeta = \mu_x - \mu_y$, where x independent samples of a parameter are collected from the simulation system and y independent samples from the field (e.g., coral cover change estimates from x simulation repetitions and y field

samples). Letting $n_1 = x$ and $n_2 = y$, where 1 indicates simulation data and 2 indicates field data, the confidence interval is developed by first letting:

$$\bar{X}_i(n_i) = \frac{\sum_{j=1}^{n_i} X_{ij}}{n_i} \qquad \text{(Equation 1)}$$

and,

$$s_i^2(n_i) = \frac{\sum_{j=1}^{n_i} [X_{ij} - \bar{X}_i(n_i)]^2}{n_i - 1} \qquad \text{(Equation 2)}$$

for i = 1, 2.

The degrees of freedom are then estimated by

$$\hat{f} = \frac{[s_1^2(n_1)/n_1 + s_2^2(n_2)/n_2]^2}{[s_1^2(n_1)/n_1]^2/(n_1 - 1) + [s_2^2(n_2)/n_2]^2/(n_2 - 1)} \qquad \text{(Equation 3)}$$

and the confidence interval is formed as:

$$[\bar{X}_1(n_1) - \bar{X}_2(n_2)] \pm t_{\hat{f},1-\alpha/2} \left[\frac{s_1^2(n_1)}{n_1} + \frac{s_2^2(n_2)}{n_2} \right]^{1/2} \qquad \text{(Equation 4)}$$

Letting $l(\alpha)$ and $u(\alpha)$ be the respective lower and upper confidence interval endpoints, then the observed difference between μ_x and μ_y, $\bar{X}(x) - \bar{Y}(y)$, is considered statistically significant if $0 \notin [l(\alpha), u(\alpha)]$. Therefore, if 0 is contained between the lower and upper confidence interval endpoints, the simulation data are not significantly different from field data. Calculations were performed using Microsoft Office Excel 2003.

A clumped spatial distribution of disease in a population can be indicative of transmissibility between hosts, and the details of the distribution in relation to other factors can potentially reveal the corresponding mode of transmission. Reports of the

spatial distribution of SWP2 cases in the field have in some cases been conflicting (Borger 2005b, Nugues 2002, Richardson & Voss 2005), although this may be due to differing sampling regimes or the scale on which this sampling was performed. The spatial distribution of SWP2-affected colonies was quantified at Sailfin Reef on Little Cayman in the summer of 2005 using 5-m radius radial transects (methods described in Chapter II methodology). The number of diseased colonies was calculated for each distance category (0-1 m, 1-2 m, 2-3 m, 3-4 m, 4-5 m), and the mean proportion of diseased colonies found within each category, normalized for the area of the arc represented by that distance category, was determined. For further calibration, distributions of diseased colonies within simulations were sampled in a similar manner as in the field. Specifically, 20 diseased colonies were randomly selected at time step 10 in each simulation run. The number of other diseased colonies within similar relative distance categories to those sampled in the field was sampled in the simulation based on the average density of colonies. Then, the mean proportion of diseased colonies found within each category, also normalized for the area represented by that distance category, was calculated. Simulation and field distributions were then statistically compared using a chi-square goodness of fit test (Sokal & Rohlf 1995).

Further, incidence rates of disease were determined for SWP2 by monitoring permanent plots at Little Cayman sites in 2004 and 2005 (Chapter 2). A population of corals, sampled with three permanent 16-m^2 quadrats at each of four sites, was monitored through time for the rate of occurrence of new SWP2 cases. In both 2004 and 2005, these populations were monitored once weekly for three consecutive weeks during June and July. The incidence proportion rate was calculated as the number of new cases of SWP2

appearing in the quadrat divided by the total number of corals in the quadrat and then by the number of days of the monitoring period (7 days * 3 weeks = 21 days). These data are used here for comparison to incidence rates recorded in simulations during time steps equivalent to years 2004 and 2005. In simulations, the incidence proportion used for comparison to field data was calculated using the cumulative incidence of disease over 21 time step sets during summer months in simulation years 2004 and 2005 divided by the total number of live corals and then by 21.

Validation

Simulations were run using disease parameter settings based on the results of calibration, but the initial composition and distribution of coral colonies were initialized with 1999 field data from three other sites surveyed in 1999, 2002, and 2004: Jigsaw Puzzle, Coral City and Grundy's Gardens. 50 replicate simulations were performed to establish a range of outcomes. Results of these simulations and field data were also statistically compared for their accuracy using the methods and equations described above.

For further validation, an independent data set was used based on work published in Croquer *et al.* (2005). In this study similar data to that collected in the monitoring of Little Cayman sites and used in calibration here (community composition, coral cover change and disease prevalence) were recorded on the fore-reef of a fringing reef system located along Madrizquí Key, Los Roques National Park, Venezuela. Reef sites were surveyed in 2000, and at that time a high level of SWP2 was recorded. When reefs were re-surveyed one year later, coral cover had declined from 44% to 33%, and the average live tissue cover of the three most dominant species or species groups (*Montastraea annularis* spp. complex, *Colpophyllia natans*, and *Madracis mirabilis*) had significantly

declined. The composition and distribution of the simulated coral population was initialized with field data from 2000 presented in Croquer *et al.* (2005), and unknown disease parameter settings were based on the results of calibration. Simulations were run for one year time frames, and coral cover and community composition change during the simulation year were then compared with field data. In this case, only coral cover estimates between simulations and field data could be compared statistically. Standard deviations of the average live tissue cover of the three species groups were not presented within the publication; therefore confidence intervals could not be calculated, and instead only data trends were compared.

Projections

The disease parameter settings resulting from calibration were used to project forward in time the impact of SWP2 on the percent live coral cover at each site. Simulations were run for time periods equivalent to reaching the year 2100, and 50 replicate simulations were performed for each site to establish a range of projected changes.

Hypothetical scenarios were also implemented within the model that might represent natural environmental changes, anthropogenic change, or management strategies, and simulations were run again for each site for 100 years with 500 replicates. Each scenario was represented by altering one disease parameter. Scenarios included: 1) limiting disease spread by decreasing the transmission *range* of the disease, 2) increasing the resistance of corals to disease by decreasing their *susceptibility probability*, 3) decreasing the amount of mortality experienced by corals by decreasing the *mortality rate cap*, 4) lessening disease input into the system by decreasing the disease *seeding proportion*.

RESULTS

Sensitivity Analysis

The sensitivity analysis revealed that of the seven disease parameters tested, *range*, *susceptibility probability*, and *mortality rate cap* were the most sensitive (linear regressions produced significant results), disease *seeding time step* was intermediately sensitive ($p = 0.05$), and *seeding proportion*, *recovery threshold*, and *rho* had very little effect on the model output (Table 2). *Range* was by far the most influential of all of the parameters. The outcome of simulations with *range* >2 produced waves of infections that spread continuously and resulted in between 65-100% loss of coral cover by the end of each simulation (Figure 1).

Disease parameter adjustment (calibration)

Scenarios tested were based on the results of the sensitivity analysis and involved varying the disease parameters *range*, *susceptibility probability*, *mortality rate cap* and *seeding time step* within designated ranges, while all other disease parameters were held constant at assumed levels (Table 3). Including all combinations of these settings amounted to 1080 total sets. In order to determine whether species-dependent susceptibility affected the model, further parameter sets were developed with increased susceptibility of one of each of the six main species groups, while *range*, *seeding time step* and *mortality rate cap* were held at constant values based on the results of the initial parameter sets.

When *susceptibility probability* was equal among all species, outcomes from a subset (17%) of these parameter sets exhibited coral cover estimates at the end of year 5 (equivalent to 2004 sampling time) that were accurate to field data, (i.e., not statistically different from field data, Table 4A). All of these parameter sets had transmission *range*

settings of <2, a *seeding time step* equivalent to disease input events occurring yearly, and *susceptibility probability* that was <10%. Parameter sets with *range* >= 2, *seeding time step* that was monthly or daily, or *susceptibility probability* >= 1% typically resulted in near total coral cover loss by the end of year 5 which did not occur in reality.

Those parameter sets that produced accurate coral cover change also produced SWP2 prevalence levels that were accurate to that recorded in the field in the summer of 2002 or the winter of 2004 (Table 4B).

A subset (25%) of the parameter sets that produced accurate coral cover changes and SWP2 prevalence exhibited a spatial distribution of disease within the simulation landscape that was also accurate to field data. These parameter sets were those where *range* was greater than 0, indicating that the disease was contagious between adjacent colonies (Figure 2). These parameter sets in which coral cover, disease prevalence in 2002 and 2004, and disease spatial distribution were all accurate, represented only 4% of all parameter sets tested.

Using an equivalent *susceptibility probability* among all species, the end proportions of three of the six most abundant species within simulations were not significantly different from that recorded in the field in 2004 (Table 4C). However, no simulation outputs recreated accurate end proportions for all six species. When species-dependent *susceptibility probability* was instituted, there were again no parameter sets that were capable of reproducing all six species compositions at the end of year 5 accurately. Parameter sets where *susceptibility probability* for *Montastraea annularis* and *M. faveolata* were doubled versus all other species did produce a similar trend to what was observed in the field. Specifically, proportions of *M. annularis* and *M. faveolata*

declined while proportions of *P. astreoides*, *P. porites*, and *S. siderea* increased. However, the magnitude of this change was small, and it did not improve the fit of the model to field data above what was observed under the scenario of a species-independent *susceptibility probability*. Therefore, the incorporation of a species-dependent *susceptibility probability* did not increase the accuracy of simulations when compared with a species-independent *susceptibility probability*.

Incidence rates of simulations in year 5 and 6 were compared with field data to determine if the magnitude of the incidence of disease was similar. The mean incidence proportion rate of SWP2 recorded in the field was 0.02% ± 0.01 (mean ± SE) in 2004 and 0.003% ± 0.003 (mean ± SE) in 2005. Incidence rates recorded in simulations using parameter sets that had resulted in accurate coral cover change, disease prevalence and disease distribution were of similar magnitude to that recorded in the field in 2004, 0.01% ± 0.0003 (mean ± SE) in both simulation years 2004 and 2005.

Validation

Simulations were run with coral composition and distribution initialized using field data from the three other Cayman sites (Jigsaw Puzzle, Coral City, and Grundy's Gardens). Disease parameter settings were equal to those that reproduced most accurately coral cover change, disease prevalence, and disease distribution in the calibration exercises. Specifically: *range* = 1, *susceptibility probability* species-independent and = 5%, and a yearly *seeding time step*.

Simulations were capable of producing coral cover changes accurate to field data for all three sites (Table 5A). The magnitude of change at Jigsaw Puzzle was twice as much in simulations compared with the field, although this did not produce a significant

difference. It is important to note however that for Jigsaw Puzzle the confidence interval endpoints were much closer to producing a significant difference versus the other two sites due to the variability of simulation outputs (Table 5A). Simulations for all three sites also had disease prevalence levels in 2002 and 2004 that were not significantly different from field data (Table 6).

Similar to calibration results using Sailfin data, these simulations could not replicate the community changes recorded in the field, although for Coral City simulations, four of the six species proportions were not significantly different from field data at the end of the simulation time period (Table 7A). Grundy's Gardens and Jigsaw Puzzle simulations only replicated two and three end species compositions, respectively (Table 7A).

Simulations were also run with these settings and initialized with coral composition and distribution data from fore-reef sites in Los Roques National Park in northern Venezuela, based on Croquer *et al.* (2005). Simulations run with the unknown disease parameter settings equal to those used for Cayman simulations also produced coral cover changes that were accurate to what was recorded in the field (Table 5B).

Percent cover by species data were recorded in the Venezuela study, and it was possible to derive this information from simulations. The three most dominant species recorded in Venezuela were *Colpophyllia natans*, *Madracis* spp., and *Montastraea annularis* species complex (including *M. faveolata* and *M. franksi*), and each of these species experienced significant declines in percent coverage (Croquer et al. 2005). No standard deviation data were presented in the publication for this specific data set, therefore it was not possible to derive confidence intervals. However, declines in

coverage recorded in simulations were similar (within 1-2%) for two species, *C. natans* and *M. annularis* spp. complex, but not for *Madracis* spp. for which the simulation predicted a much larger decline (Table 7B).

Projection forward

For all sites, when simulations were projected to the year 2100 using parameterizations from the unknown parameter adjustment exercises, live coral cover was predicted to decline dramatically but then stabilize to an approximately constant level at each site (Figure 3). Corresponding with this decline and stabilization, disease prevalence levels dropped from 4-6% in the early years to close to zero (Figure 4). The decline in coral cover was much less at Coral City than at any other site despite a similar level of disease to other sites. This site maintained mean percent live coral cover levels >20% for the duration of the 100 year simulations. Grundy's Gardens experienced coral cover decline to near 12%, and all other sites experienced coral cover decline to 10% or less. The main reason behind the stabilization of coral cover at a higher level at Coral City was the persistence of the unsusceptible species *Acropora palmata* at this site. In addition to being unsusceptible to SWP2, this species is typically large and fast-growing, and by the end of the projected time period had doubled its representation in the community to 16% ± 8 (mean ± st. dev.) of the population. In AGRRA transects performed in the field at Coral City in 1999, *A. palmata* composed 7% of the population and this value was used to parameterize the initial population in Coral City simulations. In field transects by 2004, *A. palmata* represented 11% of the population, while at the same point in time in simulations the relative proportion of this species had also

increased, to an average of 9% of the population. Therefore, trends of increasing dominance by *A. palmata* were similar between simulations and field data.

The introduction of different scenarios for which disease parameter values were altered changed the outcome of the projections so that disease had less impact on coral cover in all cases (Table 8). Of the scenarios tested, reducing disease input (*seeding proportion*) into the system resulted in disease having the least impact on coral cover by the end of the simulation time frame at all sites but Coral City (Figure 5). Limiting disease transmission between colonies (decreasing *range*) and decreasing the susceptibility of colonies to infection (decreasing *susceptibility probability*) were next in ability to reduce the impact of disease in all but Coral City simulations (Figure 5). At Coral City, the scenario that resulted in the least impact by disease was when disease transmission was limited (*range* decreased). Next in reducing impact was decreasing *susceptibility probability*. For all sites, limiting the amount of mortality occurring on colonies once they became infected (decreasing *mortality rate cap*) had the least impact on reducing loss from disease (Figure 5).

DISCUSSION

Calibration

Simulations which accurately replicated the largest number of patterns recorded in the field were those in which disease was transmissible, but only to within a short range. Contagiousness is often inferred by spatial patterns that reveal a clumped distribution (Diggle 1983), and this type of distribution, based on the underlying density of colonies, was recorded for SWP2 in field surveys on Little Cayman (chapter 2, this volume). However, the underlying spatial distribution of coral colonies at Little Cayman sites is

slightly aggregated, recreated in the simulation landscape by setting the coral parameter α = 50%, which describes the proportion of the initial total population of corals to distribute randomly on the grid and the remaining proportion is distributed aggregately around the initial set of colonies. Therefore, a simulated population with α = 5% is more aggregated than a simulated population with α = 75% (a more detailed description of α can be found in chapter III, this volume). It is possible that by random chance, incidence of disease in a population of colonies with an underlying spatial distribution that is aggregated may also produce spatial distributions of disease that are aggregated. However, aggregation of colonies was included in the simulations and a similar distribution of aggregated diseased colonies to that recorded in field surveys was only reproducible in simulations where transmission between colonies was allowed. Therefore, these results suggest that the spatial distribution of SWP2 on Little Cayman reefs is the result of transmission of disease among colonies, though whether this transmission is the result of direct transmission or transmission through a vector is not possible to discern.

The range of transmission was limited to only the immediate area of the colony, or else persistent disease fronts formed which would endure until the majority of susceptible colonies were killed within the five year time frame, which did not occur in reality. Based on these results, this syndrome is likely not transmissible by water movement between colonies, as the range of spread between colonies would be expected to be much greater in that case. Instead, other potential transmission scenarios are more likely, including where transmission is only possible through direct contact between susceptible individuals, or through a limited vector. The majority of coral colonies in Cayman do not come into direct tissue contact with each other (pers. obs.); therefore the

former scenario is unlikely. Several potential vectors exist however which could allow transmission within such a limited range. Nugues *et al.* (2004) demonstrated that algal contact with coral tissue could initiate white plague-like signs in susceptible colonies and that algae are potentially acting as a reservoir for pathogen populations. A scenario could therefore be imagined in which pathogenic transport between two colonies is facilitated by algal cover that spans the distance between colonies. The algal species targeted by Nugues *et al.* (2004) was *Halimeda opuntia*, which occurs in Little Cayman but was not found to be associated with SWP2 signs (results of chapter 2, this volume). Another potential scenario could exist in which an animal with a limited territorial range that extends over a few colonies but that comes into direct contact with diseased tissue (via predation) acts as the vector. This type of transmission has been demonstrated in other coral pathogen systems (Aeby 1991, Aeby 2003, Sussman et al. 2003, Aeby & Santavy 2006). Coral predators observed to be feeding on corals during monitoring on Little Cayman included individuals of *Coralliophila* spp., *Hermodice carunculata*, and *Chaetodontidae* spp. Any of these could potentially act as vectors for the disease.

Other potential scenarios which would produce aggregated patterns of disease that are limited locally include those where disease is not transmissible between colonies but acts through a common stress and/or susceptibility that is distributed aggregately along the reef tract. Such a case could occur through reproduction by asexual (fragmentation) or sexual (settlement of brooded larvae) that results in a clumped distribution of equally susceptible colonies based on common genes. Studies of genetic spatial distribution in relation to disease incidence would aid in elucidating whether this hypothesis is valid.

Comparison of multiple patterns also showed that simulations that were not significantly different from field data were those that incorporated a *susceptibility probability* setting that was extremely low, <5%. Although corals do not possess the strong pathogen defense capabilities stemming from adaptive immunity, they do possess several lines of defense from innate immunity including nonspecific cellular responses (phagocytic cells), physical barriers, and mechanical and chemical defenses (Mullen et al. 2004). These defenses have allowed corals to persist in coral reef environments for millennia, and it is safe to assume that they have been sufficient to allow corals to resist infection from epidemic disease. It is surprising, however, that species-dependent susceptibility did not improve the fit of the model, which suggests that coral resistance to this disease may be similar among species. Bacterial communities of the mucus layer of corals, which may play a role in resisting disease, are known to differ among species (Ritchie & Smith 1996, Klaus et al. 2005). However, if SWP2 is the result of mechanical injury, then no difference among species may be expected if resistance to such injury is similar among species. A better understanding of species-specific defenses to disease is expected in the near future, as work is ongoing to better describe the means by which corals defend against pathogen invasion (Israely et al. 2001, Geffen & Rosenberg 2005) and to determine what are "normal" versus "diseased" microbial communities of corals (Frias-Lopez et al. 2002, Ritchie 2006, Johnston & Rohwer 2007).

Accurate simulations also incorporated yearly input of disease into the system. Disease in scenarios in which this input was not incorporated tended to burn out after approximately one simulation year. It is not surprising that disease input would be required, as this has been demonstrated in other coral-pathogen systems. For example,

evidence suggests that *Vibrio shiloi*, the bacterial bleaching pathogen of *Oculina patagonica* in the Mediterranean Sea, does not appear to infect its host in the cool winter months but resides in its winter reservoir, the fireworm *Hermodice carunculata* (Sussman et al. 2003). The worm may then act as a vector for the bacteria, as it feeds on the corals in the spring when temperatures are warm enough to activate the bacteria's virulence factors (Toren et al. 1998, Banin et al. 2001, Sussman et al. 2003). Also, other disease systems have been demonstrated to be governed by metapopulation dynamics with some populations acting as sources for new disease in other distant but connected populations (Grenfell & Harwood 1997). These dynamics govern other ecological processes on reefs including the maintenance of diversity in coral communities (Van Woesik 2000).

Simulations were not able to reproduce community composition changes of the six most abundant species for any site, even with the incorporation of species-dependent susceptibility. This may have been a result of the representation of recruitment within the simulation environment. Recruitment is incorporated in the simulations as a yearly event, but it is not based on quantitative data collected in the field, as no such data exist for Little Cayman to my knowledge. The magnitude of recruitment that occurs at yearly time points is instead assumed to be a reflection of the existing population size. For these simulations, a quantity of colonies representing 1% of the existing simulation population is recruited as juvenile corals each year. Therefore, if the simulation population is zero, no new corals will be recruited. The composition of the juvenile population recruited is also based on the composition of the existing population. In reality, sexual reproduction of corals occurs through spawning and distribution of larvae by ocean currents, and recruitment may be density-independent (Hubbell 1997). The connectivity of coral

populations in the Caribbean is not well established, but populations on reefs of different areas that are connected by high speed currents have a higher probability of influencing each other than those which do not. Therefore, recruitment in this model may be over or underestimated depending on species and time period. Parameterization of the model would therefore benefit by further studies quantifying recruitment rates on Little Cayman.

Projections – today's incidence rates

Based on the assumptions of the model, the impact of SWP2 on the coral communities of Little Cayman and Venezuela, as predicted by today's incidence levels, is significant. A large percentage of the simulated coral cover was lost in all cases, except at one site, Coral City. At this site, the presence of a species, *Acropora palmata*, which is not described to be susceptible to the plague diseases, according to recent mantra (Sutherland et al. 2004) tended to compensate for the loss of corals of the other species. However, this species has historically been sensitive to the effects of disease and bleaching and a similar set of signs to that described for white plague which affects only *Acropora* spp. in the Caribbean has been distinguished as its own syndrome, white band (Aronson & Precht 2001). Both of these syndromes may in fact represent the same disease, or each set of disease signs on each affected species could be the product of separate causes (Bythell et al. 2004). In fact, the original description of "plague" by Dustan (1977) as describes it as affecting *A. palmata*. By allowing *A. palmata* to be susceptible to the disease within simulations, this would have changed the outcome of projected simulations of Coral City.

Additionally, these simulations were based on the incidence of disease as recorded today with recruitment rates that were relative to the existing population. As

mentioned previously, by incorporating recruitment rates that are based on quantitative data from the field, a better parameterization of the model is possible. However, recruitment rates in the Caribbean as compared to the Indo-pacific are quite low, and even when better parameterized are likely not high enough to compensate for great losses in adult coral populations (Hughes & Tanner 2000).

Projections – possible scenarios

Limiting disease input into the system each year caused the minimal amount of coral cover loss for all sites but Coral City. The initiation of disease on a yearly basis could be for several reasons as previously mentioned. Among them, a situation where rising temperatures each spring initiate virulence factors of the pathogen, such as in the case of bacterial bleaching by *Vibrio shiloi* (Sussman et al. 2003). In that particular case, a reduction in new infections each spring may occur if average annual spring and summer temperatures decrease. Also, adaptation or acclimatization on the side of the host may allow them to better resist infection. Recently, infection with *V. shiloi* has not been capable of inducing bacterial bleaching in *Oculina patagonica* colonies, which may be due to newly acquired coral resistance, possibly because of adaptation (Reshef et al. 2006, Rosenberg et al. 2007). Also, several years after the major incidence of white plague type II disease was recorded in the Florida Keys *Aurantimonas coralicida*, the proposed pathogenic agent responsible, was not capable of infecting corals (Richardson & Aronson 2002). This may have been due to coral adaptation, or it could have been the product of a remaining population composed of only naturally immune individuals. Disease input may also be from an outside source, such as from terrestrial runoff, which is potentially responsible for the initiation of aspergillosis in gorgonians (Jolles et al.

2002). If increased rainfall is seasonal, such as in a case where wet and dry seasons exist, runoff may increase dramatically in the wet season and could account for a yearly input of disease. In this case, drought years or a management implementation that reduces the amount of runoff may decrease disease input into the system.

Two scenarios that also decreased the impact of disease in simulations, but were less effective, included decreasing the susceptibility of colonies and reducing the transmission of disease between infected colonies. Decreasing the susceptibility of colonies may arise in a situation where hosts acquire better resistance to disease through adaptation or acclimatization, as mentioned above. The susceptibility of organisms to infection can also be dependent on their exposure to stress, either acute or chronic (Borger 2005a). Coral bleaching, the disruption of the symbiosis between the coral and their endosymbiotic algae, is considered a sign of stress in corals (Jokiel 2004), and can be induced by extreme changes in a coral's physical environment (Glynn 1993). Bleached corals may also lose the antibiotic activity of their mucus (Ritchie 2006), resulting in susceptibility to infection. Therefore a reduction in physical stressors and/or stresses that induce coral bleaching may reduce coral susceptibility to disease.

A reduction in the transmission of disease depends on the mode of transmission. If transmission occurs through a vector, such as a plant or animal vector, the reduction in the abundance of the vector would likely diminish transmission. For instance, if algae are indeed a reservoir and vector for the white plague type II pathogen (Nugues et al. 2004), reducing their abundance in the system would reduce infection. This reduction could be achieved through an increase in the abundance of herbivores or a decrease in the amount of limiting nutrients input into the system. If the vector is an animal, such as a coral

predator, an increase in the predators of such vectors would decrease the vector population or a reduction in the abundance of hosts to feed on may act to decrease the abundance of the vector following predator-prey dynamics.

The scenario of reduced mortality on colonies once they became infected had the least impact on limiting loss from disease, yet this type of scenario is often suggested as a means to control coral diseases. Specifically, by removing the infected part of coral tissue (e.g., the black band on black band infections through aspiration), some workers have demonstrated that the progression of tissue loss may be halted and that this may be an effective management tool (http://www.coral.noaa.gov/coral_disease/black_band_management.shtml). On an ecological time scale however, the results of projected simulations suggest that it has significantly less impact then other potential management applications.

Unlike at other sites, limiting the disease input at Coral City had less effect then the scenarios of reducing susceptibility and limiting transmission. This was due to the normally lower probability of disease input inducing infection at Coral City because of the large presence of an unsusceptible species. Instead, a greater reduction of disease impact was achieved by reducing disease spread within the population once it was introduced and decreasing the susceptibility of colonies disease.

Ultimately, all strategies were able to limit the impact of disease in the short-term. Considering the entire projected 100-year time frame however, which is more ecologically relevant, the prevention strategies were much more successful than the treatment.

Summary

The term epizootic is subjective, and is typically used to describe a level of disease significantly greater than what is normal, or enzootic, within a population (Last 2001). The field of coral disease is young, and it is not known what level of disease can be considered enzootic in a coral population. However, one can assume that because these reefs have historically been dominated by coral, a disease that is enzootic (i.e., not epizootic) would be one that does not drastically reduce the dominance of coral and ultimately alter the structure of reef. Here, however, simulation modeling has described how SWP2 is possibly acting to change the structure of the reef by drastically reducing coral cover. Based on these results, this disease is ecologically relevant and should be considered epizootic.

Potential scenarios were tested in which changes in the environment, adaptation to disease or various implemented management strategies were assumed to impact disease parameters within the model. The most "effective" strategy (i.e., where disease impact was curbed the most) resulted when yearly disease input into the system was limited. Other strategies also limited disease impact in the short term, but ultimately were less successful in preventing a considerable loss of coral.

Modeling provides a perspective not possible with laboratory or field measurements along. However, more quantitative studies on general coral population characteristics and disease parameters will increase the accuracy of the parameterization of models such as this thereby increasing their power. Ultimately, with better models, we will be able to better understand disease dynamics within and among coral populations today and ultimately in the future.

Table 1: Ranges for disease parameters used in the sensitivity analysis.

Parameter	Description	Range of variability
Range	Maximum distance (# of cells) of interaction between infected and susceptible colonies	0 – 12
Susceptibility Probability	Inherent susceptibility of colonies	0.01 – 1.0
Mortality rate cap	Maximum of the rate of mortality caused by disease. When a colony is infected the simulation picks a value between this number and 0	0 – 10
Seeding time step	How many simulation days in a year that disease is input into the system	0 – 365
Seeding proportion	Number of random colonies selected to become infected by disease input based on the number of colonies that are alive at that time step	0 – 0.5
Recovery threshold	Limit of the rate of mortality caused by disease. If the simulation selects a value for mortality in a time step that is below this threshold, the colony will recover.	0.0001 – 0.1
Rho	Distance effect on the force of infection between colonies	0 – 4

Table 2: Results of linear regressions of each parameter against change in coral cover documented during simulation runs. * indicates a significant relationship ($p<0.05$) between the parameter and changes in simulation coral cover over a 5 year simulated time period.

Parameter	Sensitivity coefficient β_i	MS	F	p	R^2
High sensitivity					
Range	0.37	0.777	33.91	0.000*	0.2737
Susceptibility Probability	0.15	0.159	5.33	0.023*	0.0559
Mortality rate cap	0.15	0.185	6.27	0.014*	0.0651
Intermediate sensitivity					
Seeding time step	0.14	0.119	3.92	0.051	0.0418
Low sensitivity					
Seeding proportion	0.04	0.013	0.40	0.527	0.0044
Recovery threshold	-0.05	0.016	0.51	0.479	0.0056
Rho	0.02	0.002	0.07	0.790	0.0008

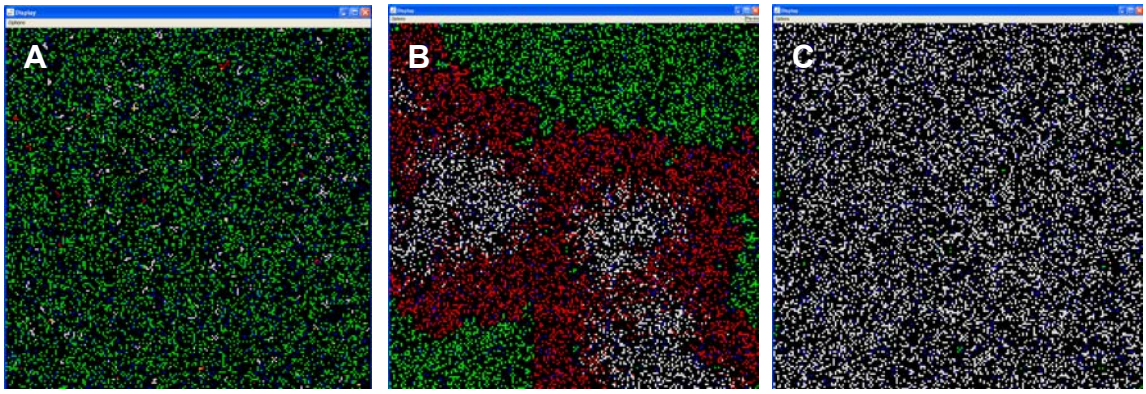

Figure 1– Model visualizations during a simulation with range = 3. A) time step = 2 (day 2); B) time step = 45; C) time step = 100. Each dot represents a coral colony with a list of variables with unique values, against a black background. Green indicates a susceptible, healthy colony; Red indicates an infected colony; Yellow indicates a recovered, susceptible colony; White indicates a dead colony.

Table 3: Values or ranges of values for disease parameters used in calibration simulations. 10 simulations were run for each parameter set in order to develop a range of outcomes under that set of values.

	Range of variation		
Parameter	**Lower boundary**	**Upper boundary**	**Increments**
Varied			
Transmission range	0	3	1
Susceptibility probability	0.01	1.0	<0.1 increments=0.01; >0.1 increments=0.2
Seeding time step	Daily (1)	Yearly (365)	Daily (1), monthly (30), yearly (365)

Constant	**Value**
Seeding proportion	0.10
Recovery threshold	0.001
Mortality rate maximum	5.0
Rho	0.0

Table 4: Field results from Sailfin Reef and simulation results from scenarios in which % live coral cover change, SWP2 prevalence in 2002 and 2004, and disease distribution did not significantly differ from field data. Bold indicates confidence interval endpoints which do not encompass 0, thereby signifying a significant difference between field and simulation samples.

	Field Results				Upper conf intvl endpt	Lower conf intvl endpt
	1999 ± St dev (N)	2004 ± St dev (N)	1999-2004 change Field	Sims		
A)						
% Live Coral Cover	22.7% ± 4.7 (10)	9.9% ± 2.5 (8)	-12.8%	-13.7%	0.1205	-0.1025

	Field	Simulation	Upper conf intvl endpt	Lower conf intvl endpt
B)				
SWP2 prevalence 2002, summer	5.5% ± 5.6 (11)	3.8% ± 0.3	0.0512	-0.0171
SWP2 prevalence 2004, winter	0.8% ± 2.2 (8)	0.003% ± 0.007	0.0234	-0.0078

	Field Results					
	1999 % species composition ± St dev	2004 % species composition ± St dev	1999-2004 change Field	Sims	Upper conf intvl endpt	Lower conf intvl endpt
C)						
A. agaricites	29.4% ± 16.9	25.9% ± 8.3	-3.5%	-5.7	0.0848	-0.0407
M. annularis	7.0% ± 8.7	9.9% ± 8.0	+2.9	-0.5	0.0948	-0.0266
M. faveolata	17.5% ± 17.6	3.0% ± 5.8	-14.5	-3.1	**-0.0701**	**-0.1580**
P. astreoides	5.4% ± 12.9	24.5% ± 9.3	+19.1	-0.3	**0.2648**	**0.1235**
P. porites	6.8% ± 7.2	14.8% ± 7.5	+8.0	-0.2	**0.1392**	**0.0257**
S. siderea	5.7% ± 8.9	12.4% ± 16.0	+6.7	+0.1	0.1867	-0.0555

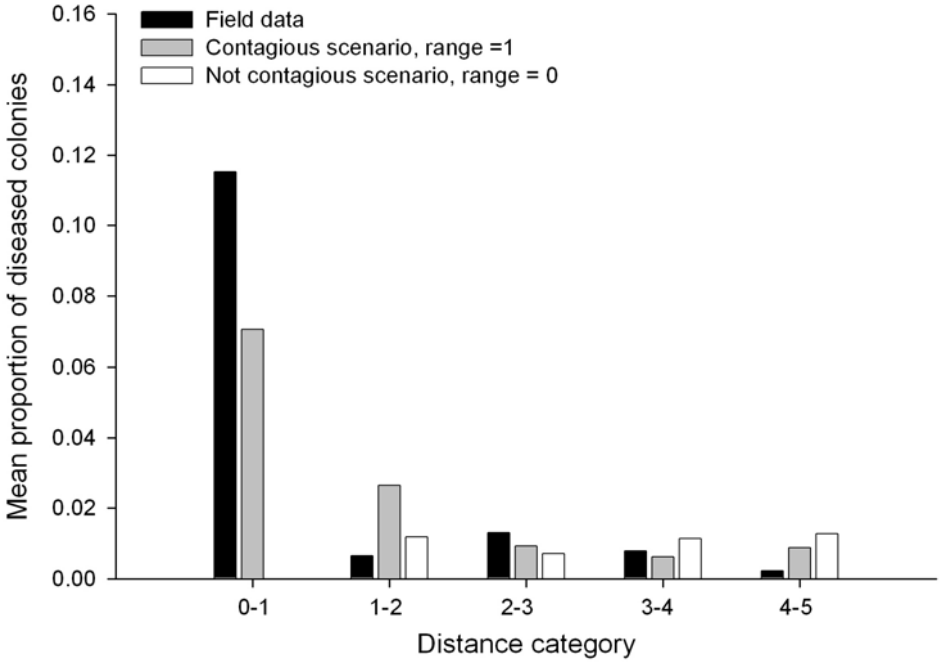

Figure 2 – "Field data" refers to the mean proportion of SWP2-affected colonies within 1 m distances out to 5 m from randomly selected SWP2-affected colonies at sites on Little Cayman, normalized for the total area of each 1 m arc (N=31). "Contagious scenario" and "Not contagious scenario" refer to the mean proportion of diseased colonies within similar relative distance categories to the field based on the average density of colonies, normalized for the area represented by each distance arc, and under different scenarios of disease spread within simulations (N=20 for each scenario). Comparisons between field distributions and range 0 were statistically significant ($\chi = 62.17$, $p<0.01$). Comparisons between field distributions and range = 1 were not statistically different ($\chi = 7.04$, $p > 0.05$).

Table 5: Percent live coral cover at sites in Cayman in 1999 and 2004 and for Venezuela in 2000 and 2001, along with absolute % change between years. Average absolute % change between years in simulations is given (Sims), along with upper and lower confidence interval endpoints calculated based on the deviation in means between field samples (Cayman: n = 15 in 1999, n = 9 in 2004, Venezuela: n = 9) and simulation samples (n = 10 both years). No significant differences were detected between field and simulation results based on the confidence interval endpoints.

	Field Results					
Site	1999 % live coral cover ± St dev (N)	2004 % live coral cover ± St dev (N)	1999-2004 change Field	Sims	Upper conf intvl endpt	Lower conf intvl endpt
A)						
Coral City	25.3% ± 6.0 (15)	25.5% ± 9.2 (8)	+0.2%	-0.4%	0.2087	-0.1967
Grundy's Gardens	37.7% ± 11.7 (11)	20.0% ± 9.1 (8)	-17.7%	-14.6%	0.1698	-0.2318
Jigsaw Puzzle	27.1% ± 9.7 (14)	16.2% ± 4.4 (8)	-10.9%	-20.1%	0.2402	-0.0562
	2000 % live coral cover ± St dev (N)	**2001 % live coral cover ± St dev (N)**	**2000-2001 change Field**	**Sims**		
B)						
Venezuela	34.5% ± 21.1 (16)	28.5% ±21.8 (16)	-6%	-10.3%	0.2706	-0.1826

Table 6: SWP2 prevalence recorded in field transects and in simulations in year 2002 and step 1090 (summer of sim year 2002) and in year 2004 and step 1280 (winter of sim year 2004), respectively with upper and lower confidence interval endpoints calculated based on the deviation in means between field samples. No significant differences were detected between field and these simulation results based on the confidence interval endpoints.

Site	Field SWP2 prevalence ± St dev (N)	Simulation SWP2 prevalence ± St dev (N = 25)	Upper conf intvl endpt	Lower conf intvl endpt
	2002 summer	**2002 summer**		
Coral City	5.3% ± 6.2 (15)	3.7% ± 0.2	0.0598	-0.0277
Grundy's Gardens	4.1% ± 4.1 (11)	2.3% ± 0.2	0.0430	-0.0068
Jigsaw Puzzle	3.8% ± 8.8 (14)	3.8% ± 0.3	0.0558	-0.0559
	2004 winter	**2004 winter**		
Coral City	0.0% ± 0.0 (8)	0.004% ± 0.008	0.000	-0.0001
Grundy's Gardens	0.0% ± 0.0 (8)	0.002% ± 0.007	0.000	-0.0001
Jigsaw Puzzle	1.1% ± 2.1 (8)	0.006% ± 0.011	0.0265	-0.0037

Table 7: Percent species composition (Cayman sites) and percent species cover (Venezuela sites) of the most abundant species. For Cayman data, 1999 and 2004 species compositions (± standard deviation) are given from field data along with the absolute % change between years. Average absolute % change between 1999 and 2004 species compositions recorded in simulation runs (Sims) is also given along with the upper and lower confidence interval endpoints calculated based on the deviation in means between field samples (n = 15 in 1999, n = 8 in 2004 and simulation samples (n = 10). Bold confidence interval endpoints indicate significant differences between field and simulation samples. For Venezuela, 2000 and 2001 % species cover is given from Croquer *et al.* 2003 along with the absolute % change. Average absolute % change in species cover is given from simulations. No confidence interval boundaries are given because necessary data (sample sizes, standard deviations) were not presented in Croquer *et al.* 2003.

Site	Species	1999 % species composition ± St dev	2004 % species composition ± St dev	1999-2004 change Field	1999-2004 change Sims	Upper conf intvl endpt	Lower conf intvl endpt
A)							
Coral City	*A. agaricites*	9.6% ± 9.5	17.2% ± 12.6	+7.6	-0.8	0.1796	-0.0115
	M. annularis	27.8% ± 19.2	14.4% ± 11.4	-13.4	-3.5	**-0.0127**	**-0.1854**
	M. faveolata	13.1% ± 10.0	22.5% ± 11.6	+9.4	-0.3	**0.1839**	**0.0090**
	P. astreoides	3.1% ± 5.9	4.7% ± 4.4	+1.6	+0.9	0.0406	-0.0258
	P. porites	7.7% ± 8.7	11.1% ± 9.1	+3.4	-0.5	0.1083	-0.0295
	S. siderea	4.8% ± 7.3	6.9% ± 11.1	+2.1	+1.3	0.0912	-0.0766
Grundy's Gardens	*A. agaricites*	19.4% ± 18.4	34.8% ± 18.4	+15.4	-1.5	**0.3077**	**0.0297**
	M. annularis	32.6% ± 21.0	15.7% ± 12.9	-16.9	-3.9	**-0.0324**	**-0.2274**
	M. faveolata	30.4% ± 22.3	21.2% ± 19.9	-9.2	-4.7	0.1058	-0.1950
	P. astreoides	0.6% ± 2.2	0.0% ± 0.0	-0.6	+1.8	**-0.0242**	**-0.0256**
	P. porites	2.8% ± 5.2	19.0% ± 12.3	+16.2	+1.2	**0.2433**	**0.0573**
	S. siderea	0.9% ± 3.2	2.0% ± 3.7	+1.1	+1.6	0.0229	-0.0331
Jigsaw Puzzle	*A. agaricites*	29.7% ± 16.2	36.2% ± 19.0	+6.5	-6.0	0.2692	-0.0182
	M. annularis	27.7% ± 18.5	11.1% ± 5.4	-16.6	-2.8	**-0.0964**	**-0.1784**
	M. faveolata	4.0% ± 6.9	13.6% ± 10.7	+9.6	+0.4	**0.1721**	**0.0108**
	P. astreoides	4.7% ± 6.7	13.8% ± 10.9	+9.1	+0.7	**0.1661**	**0.0006**
	P. porites	3.9% ± 5.6	4.6% ± 5.0	+0.7	+0.8	0.0360	-0.0401
	S. siderea	7.9% ± 9.0	12.9% ± 6.8	+5.0	+0.0	0.1027	-0.0005

		2000 % species composition	2001 % species composition	2000 – 2001 change Field	2000 – 2001 change Sims
B)					
Venezuela	*C. natans*	12.3%	8.1%	-4.2	-5.5
	Madracis spp.	12.6%	9.8%	-2.8	-11.3
	M. annularis spp. complex	38.3%	34.8%	-3.5	-1.2

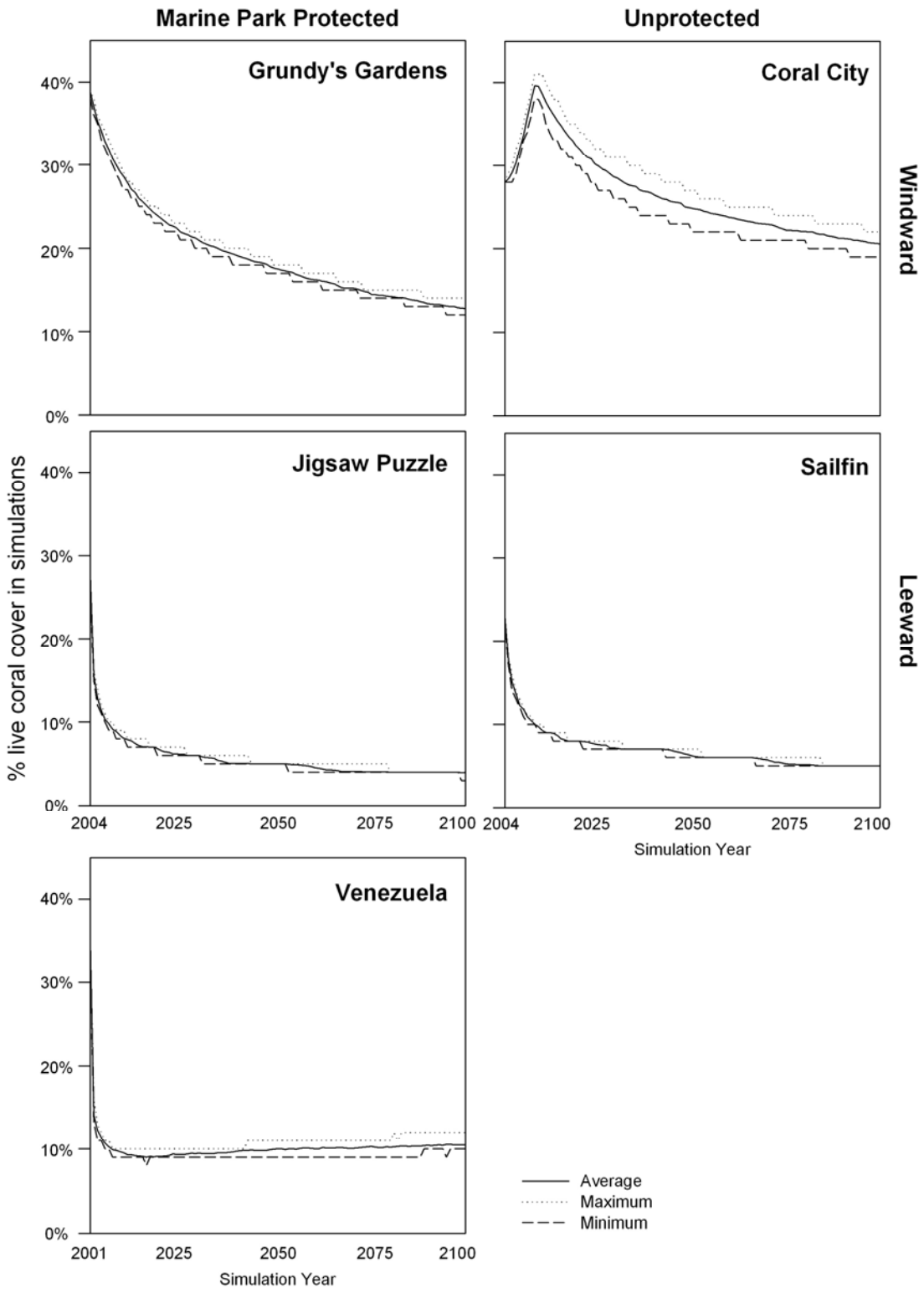

Figure 3 – 100 simulated year projections of percent live coral cover for each site using disease parameter values from calibration (Sailfin) and validation (all others) runs. Average, maximum and minimum values are given for 500 stochastic simulations, and percent coral cover of the simulation landscape was measured once every simulation year.

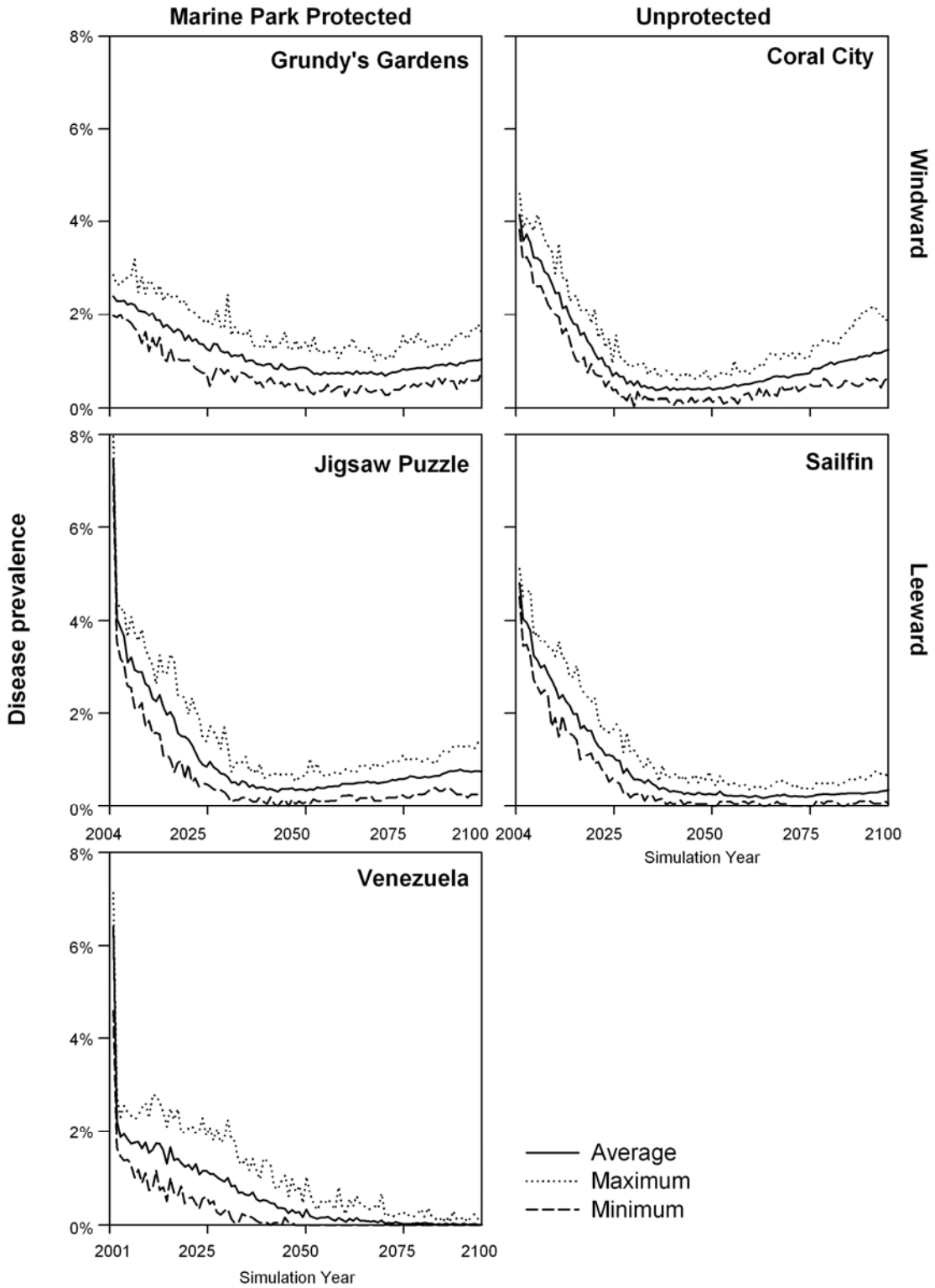

Figure 4 – 100 simulated year projections of disease prevalence each site using disease parameter values from calibration (Sailfin) and validation (all others) runs. Average, maximum and minimum values are given for 500 stochastic simulations. Disease prevalence was recorded once a year during the "summer" of simulations.

Table 8: Mean coral cover (N=500) at end of simulation time frame (year 2100) under scenarios of today's incidence and with various imposed "management schemes". (1) Transmission limited, (2) Disease input limited, (3) Colony susceptibility decreased, (4) Mortality reduced.

		Parameter altered, new value (old value)			
	Today's incidence	**(1) *Range*, 0 (1)**	**(2) *Seeding proportion*, 0.1% (15%)**	**(4) *Mortality rate cap*, 5.0cm (1.0cm)**	**(3) *Susceptibility probability*, 0.1% (5%)**
Sailfin	5.0%	9.0%	18.4%	6.1%	9.0%
Coral City	20.3%	53.3%	44.5%	28.3%	51.0%
Grundy's Gardens	12.6%	19.0%	43.0%	14.0%	18.4%
Jigsaw Puzzle	3.9%	8.0%	13.1%	5.0%	7.8%
Venezuela	10.4%	21.0%	35.4%	14.5%	20.4%

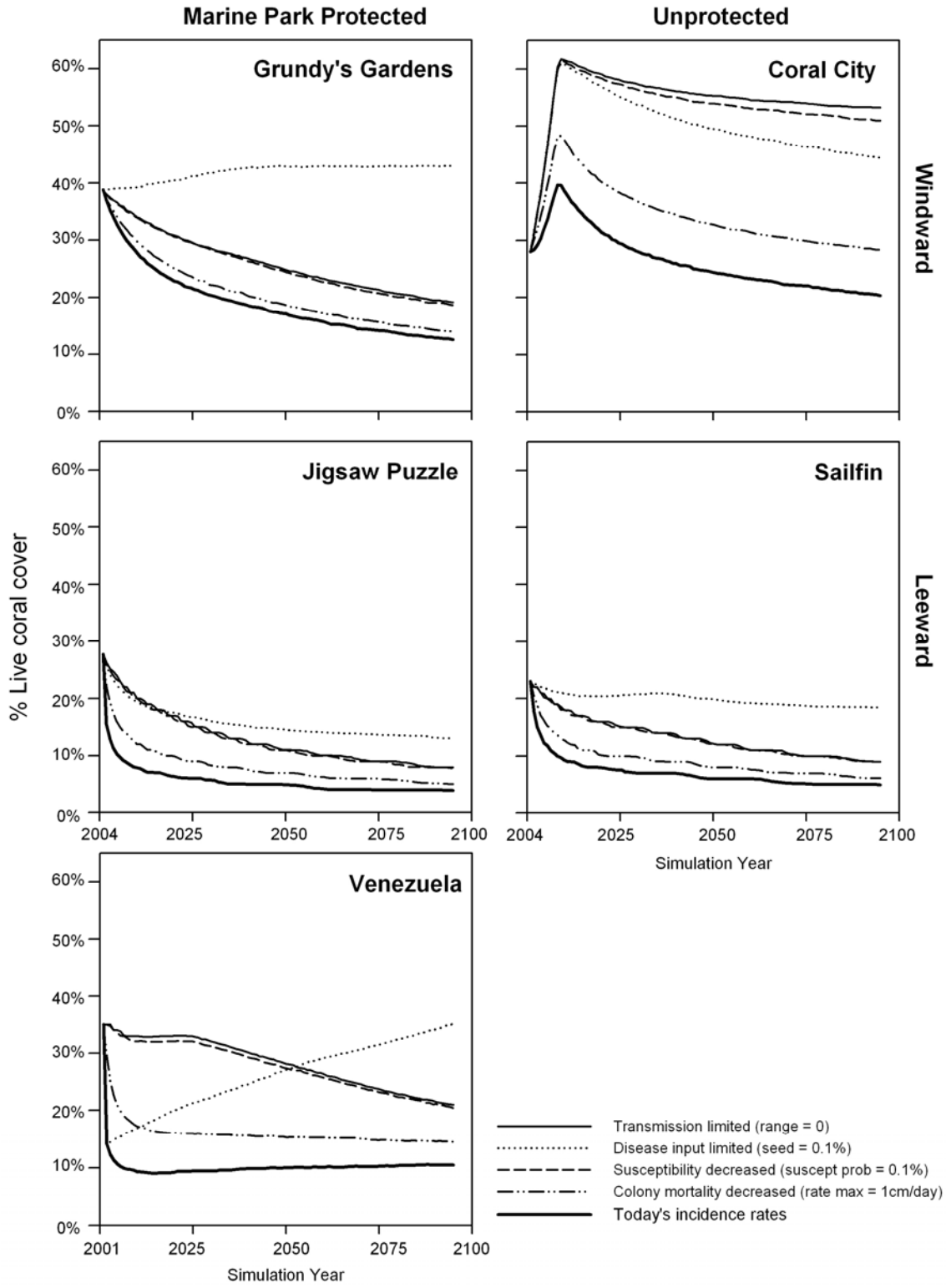

Figure 5: 100 simulated year projections of percent live coral cover for each site under scenarios of today's incidence (from calibration and validation results) and with various imposed "management schemes". Averages are given for 500 stochastic simulations, and percent coral cover of the simulation landscape was measured once every simulation year.

Chapter 5: The dynamics and impact of white plague-like signs in varying habitats; insights from a simulation model

INTRODUCTION

Accounting for the spatial complexity of coral reefs can be critical when modeling important reef processes (Langmead & Sheppard 2004). A coral reef represents a highly complex community of organisms for which the reef-building scleractinian corals form the foundation. In the Caribbean region over 60 species of scleractinian corals have been identified and these species vary in size, distribution and abundance on a reef. These population characteristics can also vary within a species among reefs and reef habitats, which can depend on various physical features of the environment (Andréfouët & Guzman 2005). For these reasons, coral reefs exhibit wide-scale heterogeneity, which must be considered when modeling processes potentially related to the abundance, distribution, and composition of coral colonies on a reef, including the dynamics of disease.

It is possible to conceive of a model that would address the dynamics of disease in a coral community with a classical approach. For instance, it would be possible to apply the equations of Kermack and McKendrick (1927) to a coral population where the population is split into compartments of susceptible, infected and recovered (immune) and average rates describe the change in distribution of the population into these compartments. Some modifications would necessarily be made to take into account the particular characteristics of corals, such as the lack of an adaptive immune system, which would likely require the removal of any immune compartment. However, many species of coral are known to exhibit signs of one or more characterized coral diseases. The focal syndrome in this project, white plague type II (Richardson et al. 1998a, b), is known to affect up to 30 species of scleractinian corals (Sutherland et al. 2004). Therefore, characteristics of the individual susceptible species, such as

size and density within the community, and the spatial complexity of the reef itself would be difficult to incorporate into a classical analytical model. A simplified approach would be required that would limit the application of the model to real world situations. Simulation modeling on the other hand is capable of representing these characteristics, thereby providing the opportunity to test hypotheses that are both theoretical and applied.

Disturbance has long been recognized as an important structuring influence on marine communities (Dayton 1971). Sources of disturbance on coral reefs can be physical (Williams & Bunkley-Williams 1990, Glynn 1993, Connell 1997, Wesseling et al. 1999, Ostrander et al. 2000), biological (Riegl 2001, Knowlton 2004), and anthropogenically-induced (Jackson et al. 2001). Disease is possibly both an acute and chronic biological source of disturbance on a coral reef. It can act quickly to significantly alter a reef's composition by removing a key species (Lessios 1988), or it can act slowly but persistently to change its overall structure (Gladfelter 1982, Aronson & Precht 2001, Nugues 2002, Miller & Williams 2007). The extent to which disease acts as a disturbance, however, may be a function of the characteristics of the coral communities themselves, and the ability to incorporate these characteristics, such as density and distribution, has previously been important when modeling other sources of disturbance on reefs (Langmead & Sheppard 2004, Sleeman et al. 2005, Mumby et al. 2006).

Few field studies have evaluated the regional distribution of coral disease among variable coral reef habitats using standardized methods to determine whether the characteristics of the reef itself influence the distribution of disease (Green & Bruckner 2000). One such study focused on aspergillosis in Caribbean gorgonians and demonstrated a relationship between disease prevalence and the abundance of large

individuals (Kim & Harvell 2004). Another study was broadly based but included an analysis of white plague disease; however the results could only demonstrate that white plague levels were typically low but variable within the Caribbean region (Weil 2004).

Environmental quality parameters are most likely influencing the distribution of disease among coral reefs as they can affect pathogen load and infectivity as well as host susceptibility (Banin et al. 2000, Kuta & Richardson 2002, Nugues 2002). However, the assumption that the distribution and composition of potential hosts is a major determinant of the distribution and impact of disease is consistent with commonly held tenets of infectious disease epidemiology (Anderson, May 1986, Anderson & May 1991). To validate this assumption for white plague type II in variable coral reef habitats would take greater resources than are currently available, and may also result in highly variable data. With the development of the simulation model described in this volume which can simulate variable aspects of coral distribution and composition various scenarios of the impact of underlying coral distribution on disease can be tested.

METHODOLOGY

Disease parameter settings that described the dynamics and impact of suspect white plague on Little Cayman reefs (chapter 4, this volume) were used to run the Simulation of Infected Corals (SICO) model (described in chapter 3, this volume) while the values used to initialize the structure and distribution of the simulated coral population were varied. These variable initial values described coral colony features, including density, size distribution, and degree of colony aggregation. The dimensions of the grid on which coral colony agents were distributed were 200 by 200 cells (40,000 total cells). Each cell could only be occupied by one colony agent. The grid was a torus and its area was set so that it

represented a reef area of 100 m^2. Ranges of variation for each parameter (Table 1) were used to create parameter sets, and 10 multiple simulations were run for each set. Simulations ran for a simulated time period of 10 years with every time step of the model corresponding to one day.

Simulations were then run under hypothetical scenarios which altered the settings of specific disease parameters. These scenarios could represent natural environmental change, anthropogenically-induced change, or the implementation of various management schemes (e.g., the removal of diseased individuals). Simulations were run for all landscape parameter sets for a simulated time frame of 50 years. Each scenario was represented by altering one disease parameter (parameters indicated by italics). Scenarios included: 1) limiting disease spread by decreasing *range* from 1 to 0, 2) increasing the resistance of corals to disease by decreasing *susceptibility probability* from 0.5% to 0.01%, 3) decreasing the amount of mortality experienced by corals by decreasing *mortality rate cap* from 5 cm/day to 2.5 cm/day, 4) lessening disease input into the system by decreasing *seeding probability* from 5% to 1%.

Several factors were used to analyze the effect of variable landscape values on the outcomes of simulations. The impact of disease on the simulated populations was evaluated by calculating the percent of the initial number of colonies at the start of each simulation that experienced complete mortality due to disease over the course of the 10 year model time duration. Maximum disease prevalence (# colonies infected/# total living colonies) and the duration (# of simulation days) that disease prevalence >0% were also evaluated for each landscape parameter setting. The relative abundances of the two types of infection, primary and secondary, were compared. Primary infections were those

infections in corals arising from disease seeding in the model, and represented infections originating by input from an outside source. Secondary infections were those infections that occurred because of the value of a colony's ***probability of infection*** variable. This variable increases from zero during every time step in susceptible colonies through interactions with infected colonies (described in detail in chapter 2). In comparing simulation results with variable initial mean colony sizes, partial mortality on colonies within simulations at the end of the simulated time period was also evaluated. Percent loss of colonies in simulations was the only outcome evaluated under the four hypothetical scenarios.

RESULTS

Variable Colony Density

Increasing coral density had a profound impact on the percent of the total population lost by the end of the 10^{th} simulation year. The relationship between increasing coral density and the % loss of colonies was approximately one to one (Figure 1A). Percent loss of colonies was related the mean maximum prevalence of disease experienced by the population during the simulation time period which was also linearly related to coral density (Figure 1B). The period over which prevalence of disease in the population was >0% during the entire 10 year simulation period increased with increasing densities up to $1.5/m^2$ (Figure 1C). With colony densities $1.5/m^2$ or greater the mean number of simulation days that the disease was prevalent in the population remained approximately the same (Figure 1C). Infections that were initiated in colonies randomly were more common in low density populations than in high density populations, where instead

secondary infections, initiated through interactions among infected and susceptible colonies, were more common (Figure 1D).

Variable Mean Colony Size

As initial mean sizes of coral colonies in simulations were set at higher values, the percent of the initial population lost to complete mortality also increased, although this relationship was non-linear (Figure 2A). When average size at initiation was set at 10 cm the % loss of colonies after 10 years was less than 50%. By increasing average initial size to 20 cm, the percent loss of colonies increased 23%, from a mean of 48% to 59%. The percent loss also increased when initial colony size was increased to a mean of 30 cm, but only by 8% (from a mean of 59% to 63%). Only a 1% absolute difference between percent loss in simulations with initial mean size of 30 cm and 40 cm was found, and larger than 40 cm, percent loss of the initial coral population remained approximately the same (Figure 2A).

Mean maximum disease prevalence in these simulations followed trends of percent loss (Figure 2B). The highest disease prevalence levels were recorded in simulations with initial mean colony sizes of 30-40 cm or larger, and the magnitude of prevalence was approximately the same. Below initial size settings of 30 cm, mean maximum prevalence levels reached in simulations were considerably smaller.

The mean duration that colonies experienced infection in year 10 was also non-linearly related to coral size. Specifically, in simulations initialized with colonies that were on average < 30 cm, the duration of infection experienced by these colonies was shorter, < 7 days (Figure 2C). When the initial population mean colony size was 30 cm or

greater, the mean duration of infection was similar among size settings, and was approximately 8 days.

Partial mortality recorded on colonies in year 10 of the simulation showed effects of initial mean size also (Figure 2D). By the tenth year, mean partial mortality on colonies when populations were initialized with the smallest mean size was extremely low (<2%). When mean initial size was increased to 20 cm, mean partial mortality was nearly ten times greater, and was twice that when mean initial size was 30 cm. When mean colony size was 40 cm, however, partial mortality was similar to that in 30 cm simulations, and as mean initial size was set larger and larger, mean partial mortality declined.

The relative percent of primary and secondary infections was different among different initial mean size settings (Figure 2E). As initial mean colony size increased, the relative percent of secondary infections increased. This increase was greatest when size was increased between 10 and 40 cm.

Variable Degree of Colony Aggregation

As the distribution of colonies became less aggregated, the % of the initial population that died due to disease declined (Figure 3A). Mean maximum prevalence in these simulations was approximately the same, varying by <1% (Figure 3B). The mean duration of disease, or the number of days in which disease prevalence was >0%, differed between aggregation settings (Figure 3C). Simulations in which colonies were more aggregated experienced disease prevalence >0% for a longer duration. This duration declined as colony aggregation was set lower. The percent of infections due to primary infection (from seeding) versus secondary infection (due to contagious spread) differed

markedly between levels of aggregation, with the ratio of primary to secondary increasing as the degree of aggregation declined. Therefore, in highly aggregated simulated populations the majority of infections were due to infectious spread, while in less aggregated populations the majority were due to primary infection from seeding (Figure 3D).

Scenarios

All hypothetical scenarios decreased the mean % of colonies lost due to disease-induced mortality (Figure 4A-C). Decreasing the amount of mortality experienced by colonies after they had already become infected had the least effect of all scenarios. When compared with the landscape parameter settings, decreasing disease mortality had no effect in simulations with initial colony densities of 1 (Figure 4A). When initial coral colony density was increased to $2/m^2$, decreasing disease mortality exhibited some effect which was similar in magnitude to that recorded when colony density was increased to $3/m^2$. For each setting of spatial aggregation, decreased disease mortality lowered the overall loss of colonies due to disease by a slight amount (3-4% less than original), and this amount was similar in magnitude in all spatial aggregation categories (Figure 4B). Decreasing disease mortality had variable effects on simulated coral populations with different initial mean colony sizes (Figure 4C). The greatest effect of decreasing disease mortality was recorded when mean coral size was 20 cm. When mean size was 10cm or greater than 20cm, the decrease in overall colony loss was less. As mean initial colony size increased incrementally greater than 20 cm the magnitude of decrease in overall colony mortality decreased so that simulations with mean colony size = 50 cm experienced the least effect of decreased disease mortality (Figure 4C).

Decreasing colony susceptibility had the next lowest magnitude of effect in reducing overall colony mortality, although it was generally 10 times greater than decreasing disease mortality (Figure 4A-C). The magnitude of decrease in mortality due to disease increased as the initial density of colonies increased (Figure 4A), but the magnitude of decrease did not change with increasing aggregation of colonies (Figure 4B). When tested under scenarios of differing initial mean sizes, the effect of decreased colony susceptibility was least in simulations with the smallest mean size, 10 cm. This effect increased dramatically between 10 and 20 cm mean initial size settings, and then remained approximately the same for larger initial mean size settings (Figure 4C).

Limiting the transmission of disease such that no contagious spread could occur had the next greatest impact in reducing the % loss of colonies in simulations (Figures 4A-C). As density increased in simulations, this effect also increased, so that limiting secondary spread in more dense coral populations had a greater impact then limiting secondary spread in less dense coral populations (Figure 4A). There was little difference in the effect of limiting transmission among simulations with varying degrees of colony aggregation (Figure 4B). Similarly to trends described for the scenario of decreasing colony susceptibility, limiting transmission had the least effect when mean colony size was 10 cm, but increased greatly when size settings were incremented to 20 cm and 30 cm. Simulations with mean initial size set as 40 cm or greater showed similar decreases in the % of colonies lost versus “normal” incidence levels (Figure 4C).

Limiting the input of disease had the greatest impact on decreasing the number of colonies lost during simulations. In all simulations, by reducing the input of disease into the system, the percent of colonies lost to disease by year 10 decreased to 5% or less

(Figure 4A-C). This amounted to a difference in loss of 90% or greater between these simulations and those under the scenario of "normal" incidence. The magnitude of difference among landscape settings including variable densities, degrees of aggregation, and mean initial size, was very little (Figure 4A-C).

DISCUSSION

Coral reefs are notoriously diverse in their composition and distribution across and among regions, which makes characterizing and understanding them challenging (Knowlton & Jackson 2001). Here, some types of diversity were represented in a simulated landscape and disease dynamics among variable habitats were examined. All parameters including initial colony density, aggregation and mean colony size, were found to have large impacts on the outcome of disease.

Colony density was found to be linearly related to the number of corals lost to disease within simulations. As density increased, the relative proportion of the initial population that was lost also increased. These results are in line with a general tenet of infectious disease, that increasing population density will facilitate the outbreak and impact of epidemic disease (Anderson & May 1979). As colony density increased the maximum mean prevalence of disease and the proportion of infections due to transmission and not seeding also increased, indicating that disease within the population was capable of reaching higher levels in more dense populations than in more sparse populations due to the increased frequency of transmission.

Mean initial size of colonies also had an impact on the relative number of colonies lost due to disease, although a size effect was noted. When colonies were less than 40cm, the relative loss was lower. Greater than 40 cm, loss was approximately the same. Similar

to what has historically been observed in the field (Richardson et al. 1998), the rate of tissue loss associated with white plague type II was fast enough that small colonies were lost in a matter of days within simulations. In coral populations dominated by small colonies, this loss meant that fewer infected colonies in these simulations persisted long enough to cause the incidence of disease in other colonies. Therefore, the numbers of secondary infections were less and the duration of disease prevalence within the population was shorter.

Spatial aggregation impacted the relative loss of colonies as well. As colonies became more randomly spaced, the impact of disease decreased. This was primarily because greater aggregation facilitated increased numbers of secondary infections. In simulations with more randomly spaced populations, infected colonies were less capable of inducing incidence in other colonies because contact between them was limited.

The introduction of hypothetical scenarios produced results similar to that found in chapter 4. All scenarios reduced the impact of disease, but the scenario under which disease input into the system was reduced had the greatest impact, while the scenario of limiting mortality on colonies once they were infected had the least impact. The difference between these two scenarios was approximately 90% in all cases.

Lowering mortality due to disease had the greatest impact in scenarios where initial populations were dominated by small colonies. In these simulations, colonies were lost quickly due to their small size; therefore halting infections early reduced the overall loss of colonies by a large amount. In simulations with large colonies, overall loss of whole colonies was low but partial mortality on colonies was common. Therefore, although reducing mortality on colonies once they were infected decreased the amount of

partial mortality experienced by large colonies, it did not decrease the relative loss of whole colonies in these simulations.

Decreasing colony susceptibility had a greater impact in scenarios where the percent of infections that were spread from already infected colonies was dominant. These scenarios included those where the mean size of initial colonies was large, when the density of colonies was high, and when the degree of aggregation of colonies was high. Secondary infections declined when colony susceptibility was lowered, because this directly impacted the calculation of the force of infection between infected and susceptible colonies (chapter 3, Equation 1). By lowering the force of infection between colonies, this lowered the probability that a colony would become infected through contagious spread during the simulation. In simulations where disease within the population was dominated by primary infections, the difference in the force of infection between already infected and susceptible colonies had little impact, therefore reducing the susceptibility of colonies had little effect.

Limiting transmission predictably had a greater impact in scenarios where infections that were due to infectious spread were dominant. By not allowing disease to spread within simulations, secondary infections were reduced to zero and infection was only due to disease input. This scenario also represents the condition under which white plague disease is not contagious between colonies. If white plague is not an infectiously transmittable disease, the impact of disease under different landscape parameter settings varies little. The only differential impact on percent loss of colonies under this scenario is observed with different mean initial sizes of colonies. As mean initial size increases, the relative loss of whole colonies to disease decreases. Otherwise, there is no effect of

density or aggregation on the relative loss of colonies when disease was not transmissible.

Decreasing disease input, and therefore the number of primary infections in the system, had the greatest impact on disease in all scenarios and this impact was dramatic. Under this scenario the force of infection between colonies was not reduced, but the number of colonies interacting was. Therefore, secondary infections were reduced, which impacted simulations under all landscape parameter settings.

Potential causes for changes in disease parameter settings that would result in the hypothetical scenarios in reality are discussed in detail in the discussion section of chapter 4, and are therefore not repeated here.

Summary

Modeling allows the capability to hold certain parameters constant while investigating the sensitivity of outcomes to variation in other parameters. Here, parameters defining the distribution and composition of coral colonies were all found to cause significant changes in outcomes when varied. Although environmental parameters possibly influence the incidence of disease in a population of corals, the underlying distributions of the populations themselves can greatly affect the overall impact of disease. More work is needed on disease transmission both *in situ* and in laboratory studies to validate the results of this modeling. However, in general these results suggest that those reefs characterized by highly aggregated coral populations such as the inshore patch reefs in the Florida Keys (chapter 6), may be at greater risk from disease than those reefs characterized by more spatially disperse and populations, such as those in the forereef zone of Little Cayman (chapter 2).

Taking into consideration the risk of epidemic disease in populations is important when considering protective and conservation measures for any ecosystem (Scott 1988). To conserve the biodiversity of corals, the foundation organisms of coral reefs, including populations that may inherently be less susceptible to impact from disease may be crucial. As climate change acts to facilitate the spread and emergence of marine diseases (Harvell et al. 2002, McCallum et al. 2003), and susceptible populations are more heavily impacted, these reefs may become the dominant source populations for new corals.

Table 1: Values or ranges of values for coral colony parameters used simulations. 10 simulations were run for each parameter set in order to develop a range of outcomes under that set of values.

	Range of variation		
Parameter	**Lower boundary**	**Upper boundary**	**Increments**
Coral colony density ()	0.5/100m^2	4.0/100m^2	0.5/100m^2
Colony aggregation	α = 5%	α = 50%	α = 5, 10, 25, 50%
Avg. colony size	10cm	100cm	10cm

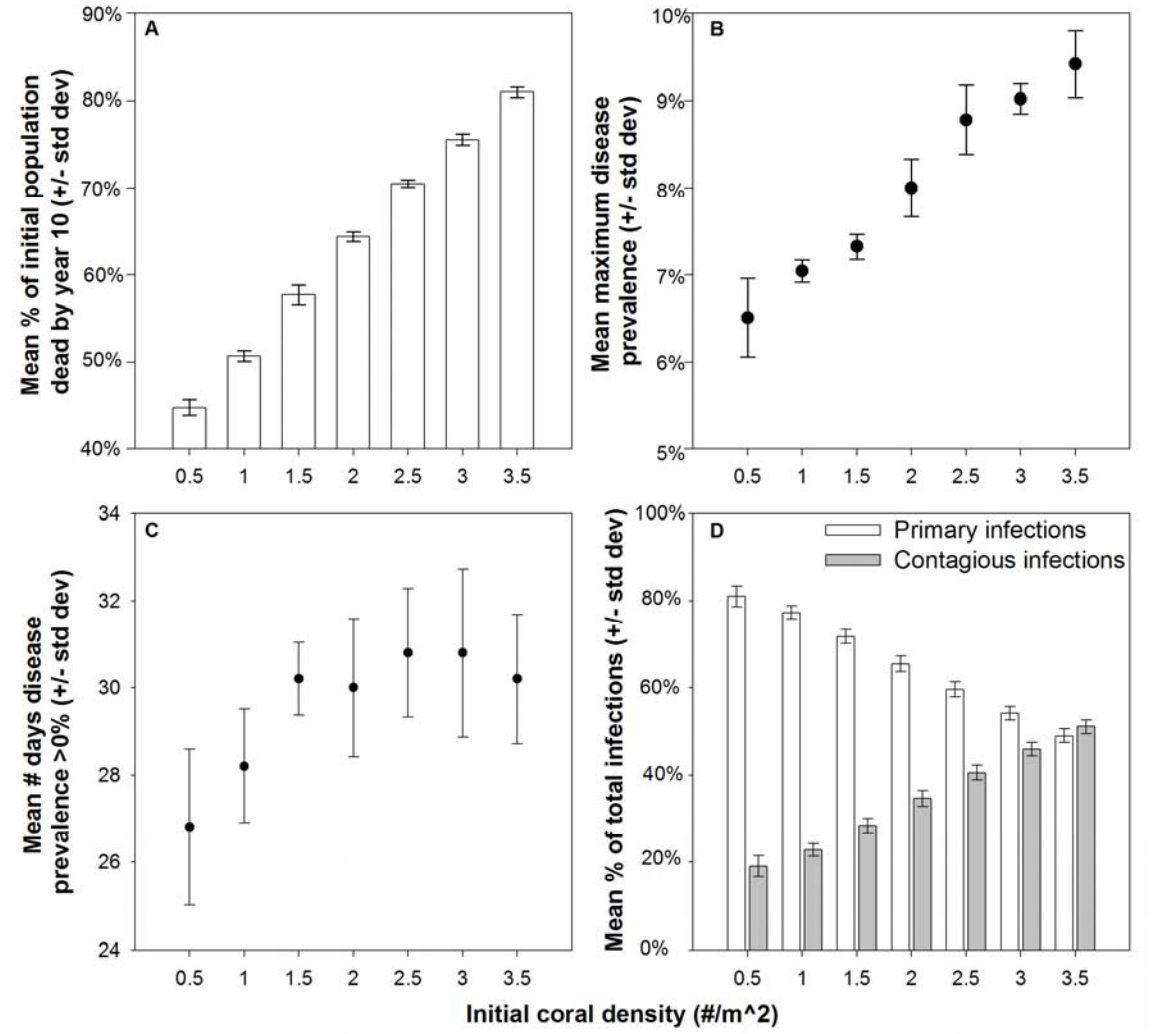

Figure 1: A) Mean % loss of initial populations of coral colonies in simulations with different initial coral densities, B) Mean maximum disease prevalence in simulations, C) Mean duration (# of days) within simulation where disease prevalence was >0%, D) Mean percent of infections attributed to primary infection (i.e., infection through random input into the system) and contagious infection (i.e., infection derived from an increased corals' probability of infection parameter due to the cumulative force of infection between colonies. All simulations used for this figure had initial mean sizes of corals = 50 cm, and spatial α's = 25% (N = 10).

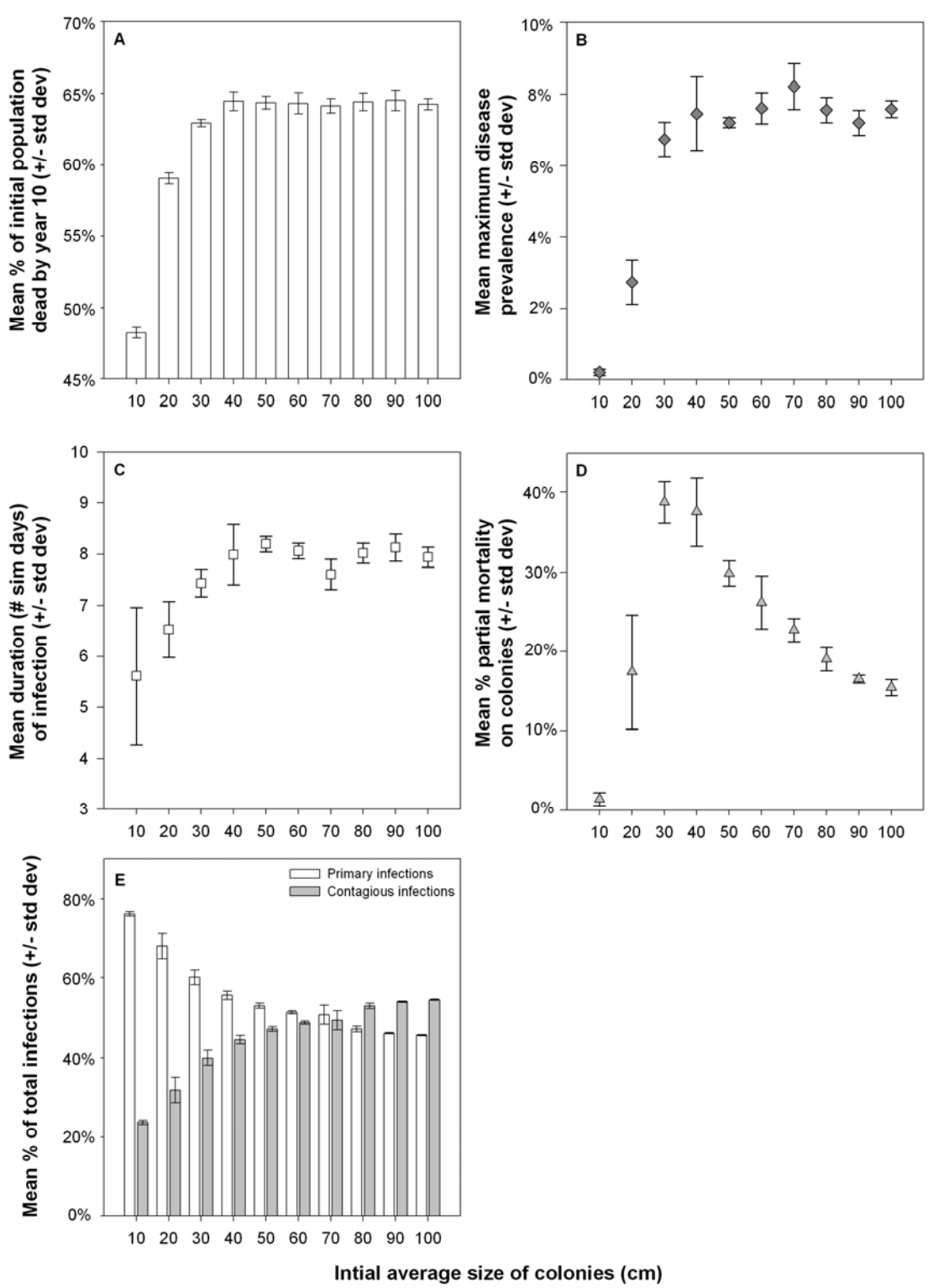

Figure 2: A) Mean % loss of the initial population in simulations with coral populations with different initial mean sizes, B) Mean maximum disease prevalence in simulations, C) Mean duration (# consecutive sim days) of infection for colonies infected in simulation year 10, D) Mean % of partial mortality recorded on colonies in year 10 (N = # corals alive in year 10). All simulations used in this figure had initial mean densities of colonies = 2.0/m^2, and initial spatial α's = 25% (N=10).

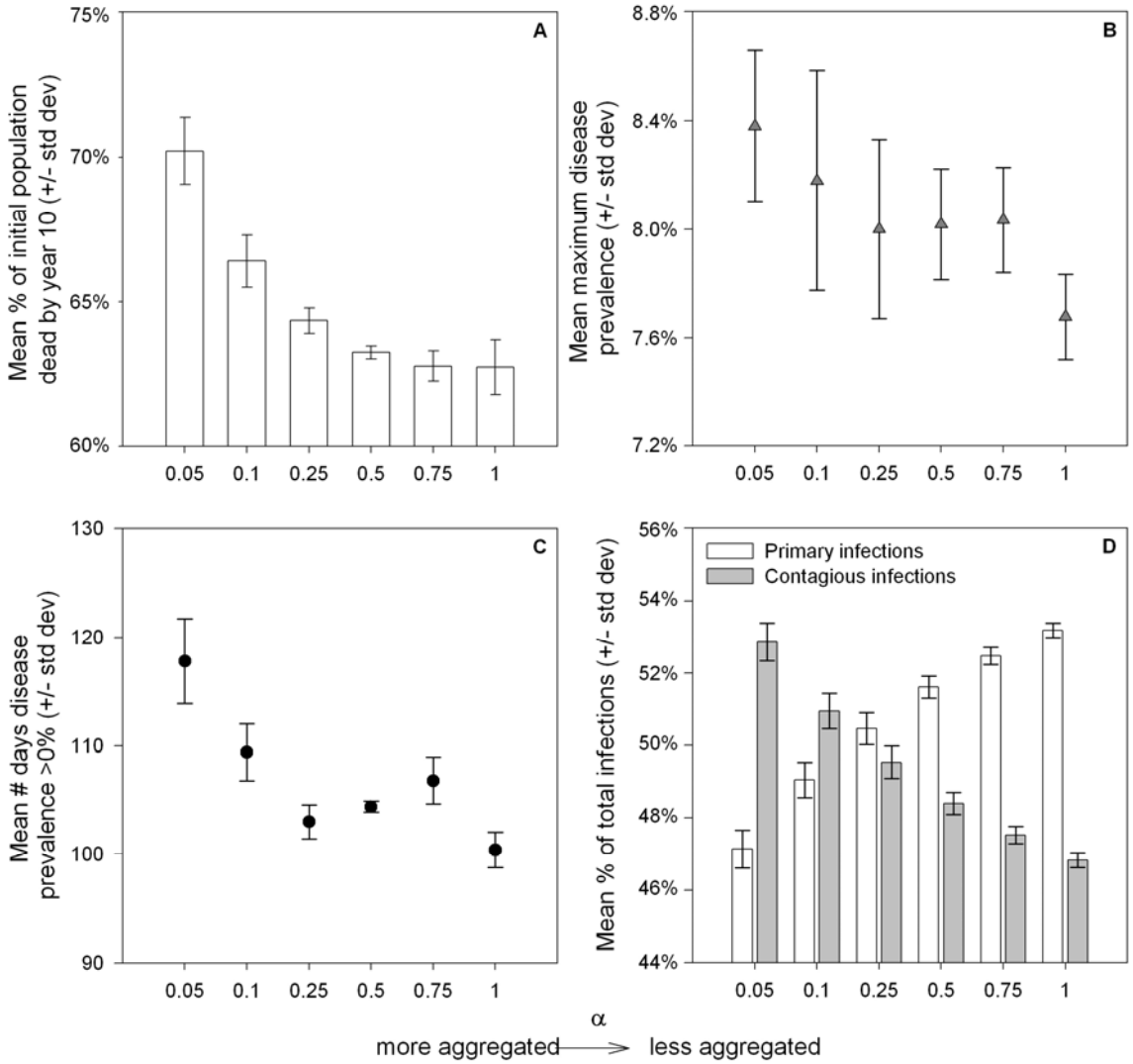

Figure 3: A) Mean % loss of the initial population in simulations with coral populations distributed with different degrees of aggregation (N = 10), B) Mean maximum disease prevalence in simulations, C) Mean duration (# consecutive sim days) of infection for colonies infected in simulation year 10, D) Mean % of infections in simulations attributed to primary infection (i.e., infection through random input into the system) and contagious infection (i.e., infection derived from an increased corals' probability of infection parameter due to the cumulative force of infection between colonies (N = 10 simulations). All simulations used in this figure had initial mean densities of colonies = 2.0/m^2, and mean sizes of corals = 50 cm.

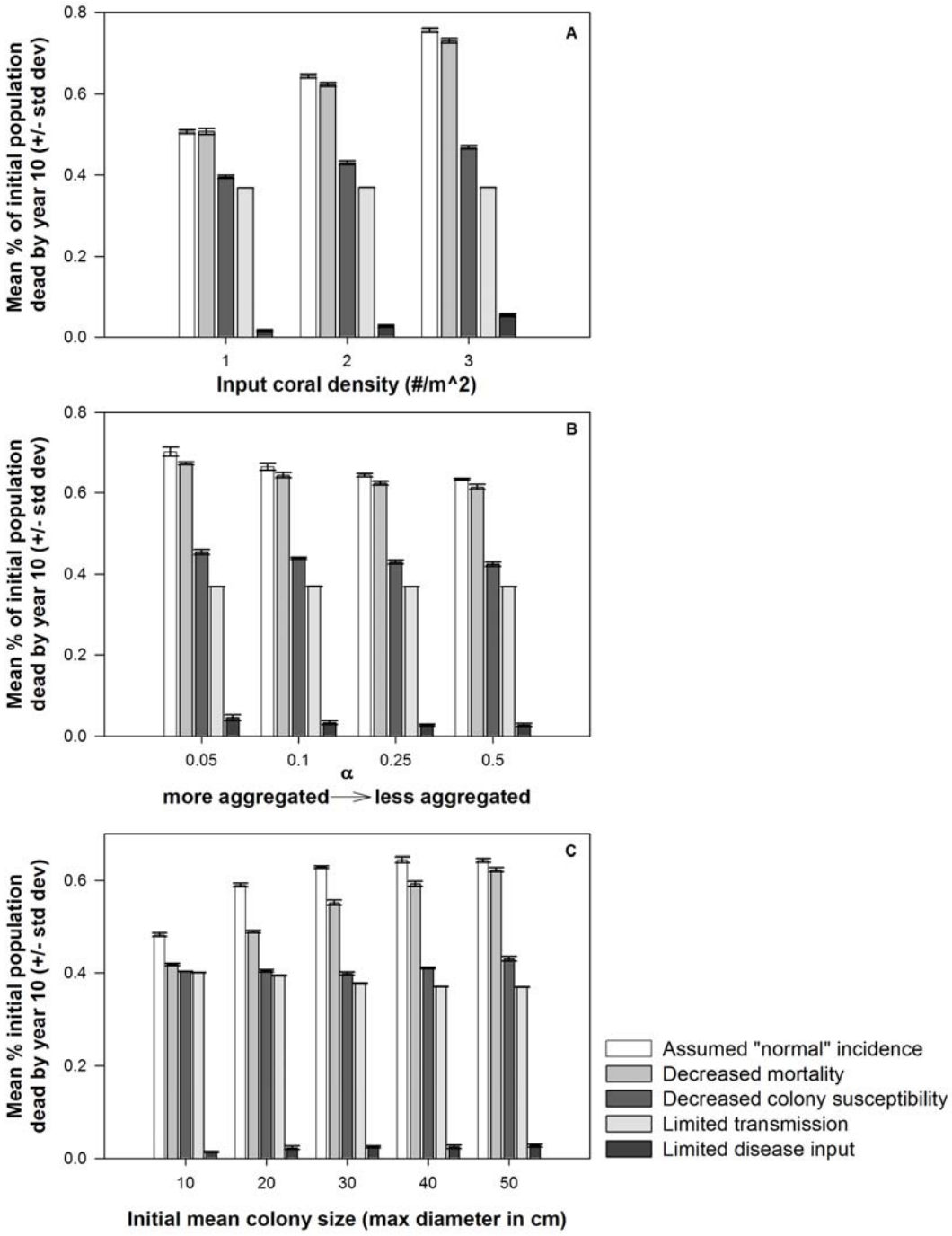

Figure 4: Mean % loss of the initial population in simulations vs. A) initial colony density, B) initial degree of aggregation of colonies, and C) initial mean colony size of the population, under scenarios including assumed "normal" incidence with settings equal to that parameterized in chapter IV, and with decreased mortality due to disease, decreased colony susceptibility to infection, limited transmission of disease, and limited disease input.

Chapter 6: Coral disease and bleaching in the Florida Keys, U.S.A. during the 2005-2006 mass bleaching event

INTRODUCTION

Reef-building corals rely on the microscopic symbiotic algae belonging to the group *Symbiodinium* that reside in their gastrodermal tissue layer to provide them with the additional energy necessary to survive and calcify in nutrient poor environments. Coral "bleaching" is the disruption of this symbiotic relationship, and is generally considered a reaction of the coral to an external stress. Bleaching can be induced by extreme changes in the coral's physical environment, including elevated water temperatures (Jokiel 2004), increased irradiance (Lesser et al. 1990), or excessive sedimentation (Philipp & Fabricius 2003). Bleaching can also be biologically-induced by bacteria, which has been demonstrated in two species of coral, *Oculina patagonica* (Kushmaro et al. 1996) and *Pocillopora damicornis* (Ben-Haim et al. 2002). Recently, with the identification of multiple algal clades within the *Symbiodinium* group, much work has been done investigating coral bleaching as a mechanism for acclimatization, where bleaching-tolerant species of *Symbiodinium* replace bleaching-susceptible species under times of stress (Buddemeier & Fautin 1993, Baker 2003). Regardless of the mechanism, the relative increase in the frequency and severity of mass bleaching events around the world in recent decades may mean that reef corals are experiencing levels of stress that are unprecedented in recent history (Hoegh-Guldberg 1999).

Coincident with increasing bleaching episodes, multiple new coral syndromes have emerged and subsequently expanded (Goreau et al. 1998, Hayes & Goreau 1998, Harvell et al. 1999, Porter et al. 2001, Harvell et al. 2003, Ward & Lafferty 2004). Many of these diseases have been linked to high temperatures (Jones et al. 2004), and recently it

has been suggested that these infections are secondary effects brought on by an increase in stressful conditions due to declining environmental conditions, including those that influence bleaching (Lesser et al. 2007).

Links between bleaching and disease have only been suggested as it is difficult to investigate any potential relationships experimentally or in the field. However, as part of an effort to document the enzootic incidence and mortality of corals due to disease for use in the calibration and validation of an epizootiological computer model, five permanent quadrats at each of four sites in the Florida Keys were monitored beginning in September 2004 and continuing into the spring of 2006. Therefore, this monitoring documented the *in situ* incidence of bleaching and disease and their associated mortality on corals during the height of the 2005 bleaching event that affected many parts of the northeastern Caribbean region (Miller et al. 2006). During this event, warmer than average temperatures were recorded throughout the Florida reef tract, which prompted the National Oceanic and Atmospheric Administration's Coral Reef Watch to issue coral bleaching alerts due to the accumulation of thermal stress by late August (coralreefwatch.noaa.gov/satellite/current/sst_series_24reefs.html). Although the passing of two major hurricanes (Rita and Wilma) likely resulted in early recovery from bleaching on Florida reefs compared to other parts of the Caribbean (Manzello et al. 2007), the bleaching documented was still severe. The temporal and spatial dynamics of bleaching and disease during this event at the four sites are presented here. This time series reveals species differences in timing and severity of bleaching and also suggests a relationship between the severity of bleaching and the incidence of disease.

METHODOLOGY

Four permanent 16-m^2 quadrats were installed at each of four sites, two offshore of the upper Florida Keys and two offshore of the middle Florida Keys (Chapter 1, Figure 1), in September, 2004 under the auspices of Florida Keys National Marine Sanctuary permit number 2004-071. An additional fifth permanent 16-m^2 quadrat was established at each of 4 sites in February of 2005. The first corner of each of the 16-m^2 quadrats was randomly located using a table of random compass directions and distances and setting out from a permanently located position (a mooring pin at French, and a randomly located coral head at each of the other three sites). The installation of permanent quadrats is identical to that described in the methods section of chapter 2, this volume.

All sites were accessed by boat and surveyed on SCUBA. Typically, two sites were visited one day and the other two sites were visited the next day or at least within one or two days of the first visit. Sites were visited once a week for at least four consecutive weeks during periods in late summer and winter. However, the ability to survey a site was highly dependent upon boat availability and weather and visibility conditions; therefore, in some cases visits are separated by several weeks. In all graphs and tables, survey dates are presented as inshore survey dates first and offshore survey dates second (e.g. Oct 12, Sep 29 = inshore, offshore).

Water temperatures were taken at the approximate top of the reef structure (5 m at Cheeca, Coral Gardens, and Little Grecian, and 10 m at French Reef) on each site visit using a YSI 30 Multiparameter probe.

Data Analysis

Coral communities at the four sites were compared using a one-way Analysis of Similarity (ANOSIM) test, calculated using the statistical software package PRIMER v5.2.2 (PRIMER-E Ltd. 2001). ANOSIM tests the hypothesis that there are no differences among groups of samples of assemblages, specified *a priori*. The test uses permutation/randomization methods on a Bray-Curtis similarity matrix to determine differences within and among sites. The calculated global test statistic *R* is a measure of the similarity of replicates within sites compared to the measure of similarity of replicates between sites. *R* is then compared to a permutation distribution of *R* and the hypothesis that there are no differences among groups can be accepted or rejected at a designated significance level, suggested to be at 0.1% (Clarke & Gorley 2001). Here, the Bray-Curtis similarity matrix was calculated using abundances of all species within quadrats (N = 5) at all sites.

Bleaching prevalence was recorded as a population-level index as the mean proportion of colonies within quadrat coral populations experiencing at least 10% partial bleaching (pale colonies were not included in the analysis), as estimated by visual inspection. All observations were made by the author, therefore there were no observer differences, and baseline "normal" colony color was established through observations throughout the previous year leading up to the bleaching event. The number of corals used as the denominator in calculating quadrat proportions included corals >10 cm, as variability in locating colonies <10 cm on each site visit was high. If colonies experienced complete mortality, they were removed from calculations, therefore the

denominator of proportions changed slightly through time for some quadrats, though complete mortality was rare.

Bleaching severity was calculated as a colony-level index, and was the mean percent partial bleaching exhibited by all coral colonies within quadrats combined. Percent partial bleaching was recorded on a colony by colony basis, and was the observed percent of the colony experiencing total loss of pigment to the nearest 10%. The denominator in the calculation of bleaching severity was the total population of coral colonies >10 cm, which also changed slightly as some colonies experienced complete mortality.

Disease prevalence was calculated similarly to bleaching prevalence, as the mean proportion of affected colonies within quadrats. Identification of diseases followed that described by the National Oceanic and Atmospheric Administration's Coral Health and Monitoring Program's Coral Disease Identification and Information website (http://www.coral.noaa.gov/coral_disease/). The author received additional training in the identification of disease in two international coral disease workshops, and through participation as a disease counter on two Florida Keys National Marine Sanctuary coral condition research cruises in the Florida Keys and Dry Tortugas (2005 and 2006). Specifically, black band was identified as a black mat that delineates live tissue from recently dead, dark spot was recorded as darkly discolored areas of tissue, which occasionally exhibited mortality, and white plague were those where a sharp line separated live tissue from recently dead tissue and the loss appeared to have originated from the base or margin of the colony.

Comparisons among dates and sites of data on bleaching prevalence, severity, and disease prevalence were performed using repeated measures analysis of variance (ANOVA) tests. A one-way ANOVA was also used to test differences among tissue loss rates associated with different mortality types. Arcsin transformations of proportion data (prevalence and severity) and natural log transformations of tissue loss estimates were performed in order to conform to assumptions of normality for these tests. When the assumption of equal variances for data was met, post-hoc comparisons were made using the Bonferroni test, which is based on a Student's *t* statistic (Sokal & Rohlf 2001). When equal variances could not be assumed, Tamhane's conservative pair-wise comparison test, also based on a *t* test, was used instead (SPSS v15.0).

For each of the main hosts of the three prevalent diseases (black band, dark spot, and white plague), the bleaching severity of colonies that became diseased during the bleaching event and those that did not were compared to determine whether disease status affected bleaching severity or vice versa. This data could not be normalized with transformations; therefore comparisons were made using the Mann-Whitney *U* test for pair-wise comparisons.

All statistical analyses other than the ANOSIM test were performed using the statistical software package SPSS v15.0 for Windows (SPSS Inc.)

Bleaching incidence and recovery proportions for each survey date were also calculated. Incidence proportion was the mean proportion of colonies within quadrats that exhibited bleaching on a date which were not exhibiting bleaching on the previous survey date. Similarly, recovery proportion was the mean proportion of colonies within quadrats that were not exhibiting bleaching on a date which had been on the previous survey date.

Disease incidence and recovery for diseases were not given as proportions, but instead as absolute numbers, due to the low numbers of cases of disease within the total population.

RESULTS

Site descriptions

The four study sites were located near the middle and upper Florida Keys (chapter 1, Figure 1) and differ with respect to species composition and community structure (Table 1). The middle Keys sites consisted of two shallow (average depth <5 m) inshore patch reef sites (Cheeca Rocks and Coral Gardens). The upper Keys sites included Little Grecian, a shallow (average depth <5 m) spur and groove reef, and French, a deeper (average depth 5-10 m) bank reef.

The inshore sites consisted of inshore coral patches (mean coral colony density 3.2 colonies/m^2 ± 0.4 and 4.8/m^2 ± 0.4 at Cheeca and Coral Gardens, respectively) surrounded by large expanses of sand. Coral colonies at these sites tended to be larger (mean maximum diameter of all colonies 36.9 cm ± 2.0 and 42.4 cm ± 1.7, respectively). The geomorphological structure of Little Grecian and French Reef were considerably different from each other. Little Grecian consisted of medium relief coral spurs separated by grooves of rippled sand with scattered gorgonians while French reef was composed of solid platforms at depths between 5 and 10 meters separated by scattered sandy patches. However, mean coral densities and sizes at these sites were similar, and much lower in comparison to the inshore patch reefs. Mean densities were 2.5/m^2 ± 0.7 and 2.2/m^2 ± 0.3, and mean maximum colony diameters were 14.7 cm ± 1.0 and 19.1 cm ± 1.0 at French and Little Grecian, respectively. Here, inshore sites are referred to as “inshore” sites and the two other sites as “offshore”.

The coral communities of the four sites were significantly different (ANOSIM, Global R = 0.302, $p<0.01$), but did not differ between sites within inshore and offshore categories (pair-wise tests Cheeca vs. Coral Gardens: R = 0.34, $p>0.05$; French vs. Little Grecian: R = 0.264, $p>0.05$). Dominant coral community members at inshore sites included species of *Montastraea* and *Siderastrea siderea*, while dominant species at offshore sites included *Agaricia agaricites*, *Siderastrea siderea*, and species of *Porites* (Table 1). Only two species represented >5% of the population at all sites, *S. siderea* and *Porites astreoides*.

Bleaching and Disease Time-series

The repeated measures ANOVAs of bleaching prevalence by quadrat showed differences between sites, survey dates and interactions between site and survey date. The analysis was therefore repeated for each site separately. This analysis revealed that for all sites, bleaching prevalence was significantly affected by survey date (Table 2). Post-hoc multiple comparisons were performed between consecutive survey dates using Tamhane's comparison, and revealed significant increases in bleaching prevalence at three of four sites in August and early September of 2005, and then significant decreases at the same sites in late fall and/or early spring (Figure 1).

Bleaching severity is a colony-level index and was analyzed for the effects of species in addition to that of site and survey date. The analysis revealed that all factors significantly affected bleaching severity, including species (mean square = 116.590, F = 98.718, d.f. = 18, $p<0.001$). Bleaching severity is therefore presented separately for the eight most abundant species (bold in Table 2), either at each of the four sites or by

density category if no differences in abundance and bleaching severity existed between sites within the category (Table 3a, b).

Bleaching prevalence remained low at all sites (<2%) in the fall and winter sampling periods (Figure 1). *Agaricia agaricites* was the most affected species although bleaching severity of colonies was low and this species was only affected at offshore sites (Table 3a). *Colpophyllia natans*, *Diploria strigosa*, *Montastraea cavernosa*, and *Porites porites* colonies also showed bleaching, although only at one inshore site, Coral Gardens, and the severity of bleaching on colonies was low. No bleaching was recorded at Cheeca. New incidence of bleaching was recorded only at French reef (Figure 2) during the fall and winter; therefore the constant level of bleaching prevalence during this time (Figure 1) was primarily due to colonies continuing to exhibit bleaching and not due to new incidences.

Bleaching was present at every site in several species when sampling began again at the beginning of August. Although average prevalence of bleaching remained low (Figure 1), between 3 and 11% of corals showed new incidence of bleaching at the four sites (Figure 2). Severity of bleaching experienced by colonies was also slightly higher than in previous sampling periods and the difference was greatest at inshore sites (Table 3b). Incidences of bleaching the following week were approximately the same so that average prevalence of bleaching corals doubled at inshore sites and increased slightly at offshore sites. At this time, the severity of the bleaching on colonies dramatically increased at inshore sites and at French Reef, but increased only slightly at Little Grecian (Table 3b).

The highest incidence of bleaching was recorded in the first week of September. At inshore sites, a mean of 60% of corals that were not previously bleached had begun bleaching (Figure 2), causing bleaching prevalence within the population to exceed 80% (Figure 1). Bleaching severity recorded on colonies was also high for some species at these sites, notably *Agaricia agaricites*, *Colpophyllia natans*, *Montastraea annularis*, *M. faveolata*, *Porites astreoides*, *P. porites*, and *Siderastrea siderea* (Table 3b). French Reef also experienced a heightened incidence of bleaching (Figure 2), causing bleaching prevalence to increase, though it remained lower than at inshore sites (Figure 1). Severity of bleaching on colonies at French Reef was similar to inshore sites for *A. agricites*, but not for other species, which exhibited less severe bleaching. Little Grecian experienced no new incidence of bleaching, and bleaching prevalence remained less than 5%.

Between the first and second week of September, some new incidences of bleaching were recorded at inshore sites (Figure 2), and average prevalence increased slightly (Figure 1). No new incidence was recorded at either offshore site, and recovery was noted for the first time at French Reef (Figure 2), This recovery occurred in three small colonies, two *Agaricia agaricites* and one *Siderastrea siderea* (all less than 15 cm in maximum diameter). Bleaching severity of all other colonies remained approximately the same at all sites (Table 3).

On 20 September 2005, Tropical Storm Rita (later Hurricane Rita) passed south of Key Largo and all dive operations were suspended prior to, during and for two weeks after its passing. The next dates that sites could be sampled were at the end of September and early October for offshore and inshore sites, respectively. At this time, bleaching prevalence had dropped significantly at both inshore sites and had declined slightly at

French Reef (Figure 1). No new bleaching was recorded on or after this date at any site for the rest of sampling in 2005 (Figure 2). At inshore sites, corals began to recover their pigment. A mean of 8% of corals bleaching on the previous sample date were noted as fully pigmented at this time (Figure 2). At French Reef, very little recovery occurred (Figure 2). Overall, the severity of bleaching remained high; therefore, there was full recovery in some corals (primarily *Agaricia*), but those corals that remained bleached remained intensely so (Table 3a).

On 24 October 2005, Hurricane Wilma passed over South Florida and diving operations were once again suspended. It was possible to visit sites at the end of the first week of November at which point bleaching prevalence had significantly declined to early-August levels at Cheeca and French and had declined to approximately 20% at Coral Gardens (Figure 1). Full recovery of corals was at its highest in this week at both inshore sites (Figure 2). The severity of bleaching had also declined significantly, although partial bleaching was still apparent at all three sites (Table 3b).

Sites were visited again in early spring of 2006. At this time, average bleaching prevalence at French Reef was higher than it had been when sites were visited in March of the previous year (Figure 1). Recovery to pre-bleaching event levels was not noted at this site until late spring when bleaching dropped below 10%. Overall, the only two species affected at this site were *Agaricia agaricites* and *Porites astreoides*.

When inshore sites were sampled in the spring, partial bleaching continued at Cheeca (Table 3b) and affected a mean of 10% of the population (Figure 1). Incidence indicates that this was due to continued partial bleaching of colonies that were bleaching in the fall, and not new cases of bleaching (Figure 2). Most corals had recovered

completely at this time and the species primarily affected by continued bleaching at Cheeca was *Colpophyllia natans* (Table 3b). No bleaching was recorded at Coral Gardens in the spring.

By April, bleaching prevalence had dropped to less than 5% at all sites (Figure 1), however, the severity of bleaching had increased at both inshore sites (Table 3b). There was very little new incidence of bleaching: on average 1% at Coral Gardens, less than 1% at Little Grecian, and none detected at Cheeca or French Reef (Figure 2). Therefore, the increase in average severity at inshore sites was due almost entirely to an increase in partial bleaching observed on *Colpophyllia natans* that had never fully recovered from the fall (Table 3b). Bleaching continued to affect only *Agaricia agaricites* and *Siderastrea siderea* at the offshore sites.

Species considered to be the most severely affected during the bleaching event were *Colpophyllia natans*, *Montastraea annularis*, and *Agaricia agaricites. C. natans* and *M. annularis* experienced the highest prevalence of bleaching, both reaching >90% at inshore sites in the first week of September (data not shown). These two species also exhibited the greatest severity of bleaching on colonies, with each species exhibiting >90% bleaching severity at some time during the bleaching event (Table 3b). *A. agaricites* and *C. natans* experienced the greatest duration of bleaching, each exhibiting bleaching prevalences of >30% bleaching for >3 weeks (data not shown).

The repeated measures ANOVA of the severity of colony bleaching within *S. siderea* and *P. astreoides*, the only two species to represent >5% of the population at all sites, revealed significant effects of date (*P. astreoides* mean square = 28.128, $F = 18.406$, d.f. = 14, $p<0.001$, *S. siderea* mean square = 32.198, 22.159, d.f. = 14, $p<0.001$)

and site (*P. astreoides* mean square = 32.438, F = 21.226, d.f. = 3, $p<0.001$, *S. siderea* mean square = 11.663, F = 8.027, d.f. = 3, $p<0.001$). Post-hoc pair-wise comparisons of bleaching severity between site pairs revealed that inshore sites experienced significantly higher mean bleaching severity versus offshore sites for both *S. siderea* ($p<0.01$ for comparisons: Cheeca vs French, Cheeca vs. Little Grecian, Coral Gardens vs. French, Coral Gardens vs. Little Grecian) and *P. astreoides* ($p<0.01$ for comparisons: Cheeca vs French, Cheeca vs. Little Grecian, Coral Gardens vs. French, Coral Gardens vs. Little Grecian). Mean bleaching severity of these species did not differ between inshore sites or between offshore sites.

Disease

Dark spot

Dark spot affected corals at three of the four sites (Coral Gardens, French and Little Grecian) when sites were established in late summer of 2004 (Figure 3). New cases of dark spot appeared during the fall at Coral Gardens but not at French or Little Grecian (Figure 4). One coral recovered from dark spot at Coral Gardens during the fall, however most cases noted when sites were established continued to show signs at the end of the monitoring period in November of 2004 (Figure 4). Therefore, average prevalence of dark spot remained constant at offshore sites but fluctuated slightly at Coral Gardens (Figure 3). When sites were visited in early February, recovery had occurred on most dark spot-affected colonies at the two offshore sites but not at Coral Gardens. Through the winter sampling, incidence and recovery occurred at these sites such that dark spot prevalence remained fairly constant (Figure 3).

During the bleaching "event" time period (the time period from early August to early November) no new incidence of dark spot was recorded at Little Grecian and very little was recorded at French or Cheeca, causing dark spot prevalence to remain approximately the same at these three sites (Figure 3). Incidence of dark spot at Coral Gardens was high in the first few weeks of the event, and caused prevalence levels to increase significantly (Figure 3, 4), though they remained approximately the same through to November when recovery was documented (Figure 4).

In the spring of 2006, new darks spots cases were identified at French Little Grecian, recovery was documented at French, and previously dark spot-affected corals at Coral Gardens recovered (Figure 4). Therefore, by spring, dark spot prevalence at all sites was similar or less than it had been prior to the bleaching event (Figure 3).

White plague

Signs consistent with that described for white plague type I (Dustan 1977) were only identified on corals at Cheeca until the spring of 2006 (Figure 5). The majority of cases identified when sites were established in 2004 continued to be active through the winter and into the spring of 2005, causing prevalence of this suspect white plague type I (SWP1) within the population to remain approximately constant at this site (Figure 3). Some recovery occurred and new incidence was recorded in mid-February when six new cases developed in the second week of sampling and two new cases developed in the third week (Figure 5).

On the first visit in August 2005, recovery of most colonies affected by SWP1 in the spring at Cheeca had occurred (Figure 5), and this caused SWP1 prevalence at this site to decline (Figure 3). During the height of the bleaching new incidence of disease

was low but continuous, as was recovery. Therefore, average prevalence of SWP1 remained lower during the bleaching event compared to its previous levels in the spring and winter. During dates (early October and early November) on which extensive recovery from bleaching was recorded (Figure 2), the highest number of new cases of SWP1 was recorded at Cheeca, causing the average prevalence of this disease to increase and reach its highest level since monitoring began (Figure 3, 6). Of the 24 new cases of SWP1 documented on these two dates, 11 were new cases and 13 were re-infections of previously infected colonies. However, by November 2005, SWP1 had still not been documented at any other site but Cheeca.

Many corals with active SWP1 in November 2005 had recovered when Cheeca was visited in March (Figure 5), and average prevalence levels dropped to what they had been prior to the bleaching event (Figure 3). In April, SWP1 then appeared on monitored corals for the first time at Coral Gardens and at Little Grecian.

Black band

Black band was recorded on a colony within quadrats for the first time at Coral Gardens at the beginning of August 2005 (Figure 6), in conjunction with the onset of increased levels of bleaching at this site (Figure 1). New cases of black band were found on each subsequent visit to Coral Gardens (Figure 6), so that black band prevalence increased continuously up to when sites were visited at beginning of October (Figure 3). On this date, black band was also recorded for the first time at Cheeca (Figure 6). On the next sampling date in November after the passing of Hurricane Wilma recovery from black band was documented on most previously affected corals at Coral Gardens and on the one coral at Cheeca (Figure 6).

In the spring, black band was again prevalent at Cheeca, Coral Gardens, and a new infection was noted at French (Figure 6). At Coral Gardens, two of the five infections were re-infections. No cases of black band were ever noted within quadrats at Little Grecian, although two were noted anecdotally outside of quadrats.

Bleaching and Disease

All three major syndromes showed species preferences (Table 4). Black band was primarily found on *Colpophyllia natans*, dark spot on *Siderastrea siderea*, and SWP1 on *Montastraea faveolata*. *C. natans* was the only species to exhibit signs of all three diseases, but no coral monitored exhibited signs of more than one syndrome at any time or over the duration of monitoring.

The majority of black band infections were recorded at Coral Gardens on *Colpophyllia natans* colonies. At this site, colonies that developed black band infections at some time in the course of the bleaching event were significantly more bleached than colonies that never developed infections when sites were visited in the second sampling period of early August (Figure 7A). The incidence of most black band infections occurred after this date, although no other effects of disease status were detected.

For *Siderastrea siderea*, bleaching severity was examined for inshore sites as no differences in bleaching severity in this species were found between Cheeca and Coral Gardens, and sample sizes were too low at offshore sites for analysis. On the second week of summer sampling (16, 17 August) and the subsequent two sampling periods, bleaching severity of dark spot affected colonies was significantly higher than bleaching severity of non-affected colonies (Figure 7B). The majority of those corals that exhibited

dark spot signs were identified on colonies in the first three weeks of sampling in the summer, and these signs persisted through the bleaching event.

Montastraea faveolata colonies that experienced SWP1 were primarily found at Cheeca, and bleaching severity on SWP1-affected and un-affected colonies also showed significant differences. Colonies that were unaffected by SWP1 during the monitoring time period did not show severe signs of bleaching until sites were visited in the first week of September (Figure 7C). However, colonies that developed SWP1 during the episode were significantly more bleached than unaffected colonies on each survey date beginning the second week of monitoring (16, 17 August) until the end of monitoring in November (Figure 7C).

Tissue loss estimates

Mean number of lesions and tissue loss rates per colony and per lesion were calculated for each major mortality type encountered, including black band, bleaching (Figure 8), dark spot, and SWP1. All other types of mortality were combined into the category "other/unknown", and included sponge overgrowth, total colony loss (possibly due to hurricane effects), predation, and any mortality not able to be attributed to a specific cause.

SWP1 was associated with the highest number of lesions per colony, followed by other/unknown and black band (Table 5). Mortality rates per lesion and per colony were significantly different among condition types (per lesion: mean square = 69.054, $F = 32.731$, d.f. = 4, $p<0.001$; per colony: mean square = 21.525, $F = 11.157$, d.f. = 4, $p<0.001$), and SWP1, other/unknown, and black band were also associated with the highest rates of tissue loss, both per lesion and per colony (Table 5). Black band had

significantly greater tissue loss per lesion than all other conditions, and significantly greater tissue loss per colony than all conditions but other/unknown (Table 5). Mortality was found rarely on bleaching or dark spot-affected colonies (mean number of lesions associated with both of these was <1), and tissue loss rates recorded for these lesions were low (Table 5).

DISCUSSION

These results highlight the importance of longitudinal studies in investigations of disease and bleaching *in situ*. Although the number of sites were limited and the resultant data were due to an unforeseen bleaching episode, it was possible to document previously unrecorded relationships between disease and bleaching by having monitoring in place before the onset of the event.

Bleaching

Bleaching prevalence and severity was much greater at the two inshore sites than at the two offshore sites. There are several factors which may have contributed to these observed differences, both ecological and physical.

Ecologically, the relative dominance of susceptible species within a community may determine the extent of bleaching recorded at the population level (McClanahan et al. 2005). Species compositions differed between inshore and offshore zones, so the abundance of more bleaching susceptible species inshore may have contributed to the greater amount of bleaching found at these sites. However, bleaching severity of *Porites astreoides* and *Siderastrea siderea* was significantly greater on colonies at inshore sites than at offshore sites. Additionally, one of the most bleaching susceptible species, *Agaricia agaricites*, was a dominant species at both offshore sites, yet these sites showed

lower bleaching prevalence. Therefore, it is likely that species composition is not the main reason for differences observed between inshore and offshore bleaching prevalence and severity.

Mass bleaching on the order of what was observed at the two inshore sites is typically thought to be caused by high water temperatures, and possibly the synergistic effects of temperature and high light conditions (Brown 1997, Berkelmans 2002). In this study, temperatures measured at the reef tended to be approximately 1.5ºC higher at inshore sites than at offshore sites (Table 6). Irradiance levels calculated at the level of the reef structure for all four sites indicated that inshore sites experienced lower irradiance during the study period than offshore sites (Yniguez in prep). Therefore, temperature was greater at inshore sites and might possibly explain the higher bleaching, but it is unlikely that light interacting with temperature played a large role.

Water flow is another potential physical factor contributing to the extent to which colonies bleach and/or recover during mass bleaching events (Nakamura & van Woesik 2001, Nakamura et al. 2003). Water flow was not measured directly during this study, but qualitatively inshore sites tended to be relatively calmer compared with offshore sites.

Overall, temperature and water flow were likely dominant factors influencing differences observed between inshore and offshore sites, but species composition potentially contributed to the higher population-level prevalence observed inshore.

Bleaching and disease

Bleaching and disease were both limited in their abundance and impact on colonies when sites were established in 2004. An increase in SWP1 and the appearance

and increase of black band occurred coincident with the bleaching event that more severely impacted inshore sites.

The coral species most impacted by both bleaching and disease was *Colpophyllia natans*. This species is a ubiquitous member of coral reef communities in the Caribbean region, but has recently been documented as a commonly affected species by diseases in several parts of the Caribbean (Nugues 2002, Croquer et al. 2005, Voss & Richardson 2006). *C. natans* here was the most affected by bleaching in terms of severity and duration and was also the only species documented to be impacted by all three diseases. After the bleaching event, the majority of colonies of *C. natans* at both Cheeca and Coral Gardens were observed with partial mortality that was in many cases extensive. Repeated episodes of the extent and severity of a bleaching episode such as this could have disastrous consequences for this species in the communities of the inshore patch reefs of this region.

Dark spot was present at all four sites but in low abundance (<2%) and remained low during and after the bleaching event, despite that recent work has suggested this syndrome to be a general response to stress (Borger 2005a). However, the main species host of this syndrome, *Siderastrea siderea*, experienced less severe bleaching at inshore sites during the height of the event versus all but one species, *Montastraea cavernosa*. This species also experienced severe bleaching (>50% mean severity) later and recovered from severe bleaching earlier than most other species. At the site most severely impacted by bleaching, Coral Gardens, dark spot was recorded to increase. Data at this site would tend to support the suggestion that dark spot can be a response to increasing stress. However, this was not documented at other sites.

The slow rate of tissue loss associated with SWP1 in this study correspond more closely with the slow rate of tissue loss (mm/day) associated with white plague "type I", originally described by Dustan (1977), on which little work has been done (Sutherland et al. 2004). White plague type II is the most well-studied of the Caribbean plague diseases (Sutherland et al. 2004), and has been associated with a microbial pathogen (Richardson et al. 1998), potentially initiated by contact with calcareous algae (Nugues et al. 2004), however this disease is associated with rapid tissue loss on the order of cm/day. It is unlikely then that the disease signs identified in this study are attributable to the white plague type II pathogen.

SWP1 was only present at one inshore site prior to the bleaching event. During the event, the prevalence of SWP1 was depressed at this site, and although new cases were identified, most cases recovered or were not active while mass bleaching was recorded. When significant recovery from bleaching was documented, a significant increase in SWP1 was noted and it appeared for the first time at two other sites.

There are several reasons that could be suggested for the depression of SWP1 during the height of the bleaching event in this case. For instance, mass bleaching is known to be a product of the combination of temperature extremes and high light conditions (Fitt et al. 2001). Most microbial activity, while promoted by warmer temperatures (Harvell et al. 2002) can be inhibited by exposure to ultraviolet radiation (UV). If the SWP1 documented during this study has a similar microbial-related etiology as white plague type II and the majority of characterized coral diseases (Rosenberg & Loya 2004), the high light conditions acting to promote mass bleaching could have acted to restrain the growth and activity of the hypothetical pathogen. The onset of infections

after the passing of Hurricane Wilma when temperatures cooled and light was reduced by increased turbidity would tend to support this. Alternatively, other coral diseases have been shown to affect primarily the symbiotic zooxanthellae within affected coral tissue (Banin et al. 2001, Cervino et al. 2001). If the pathogenic agent of these SWP1 signs is somehow linked to the abundance and/or activity of the zooxanthellae, a reduction in their abundance in the coral tissue during bleaching would act to slow or inhibit infections. With the onset of coral recovery and the re-acquisition of zooxanthellae, the pathogen would regain a population to infect.

Black band emerged at sites and then increased during the height of the bleaching event, but declined significantly coincident with significant recovery from bleaching. This emergence and increase could be related to the recent finding that the cyanobacterial member of the black band microbial consortium performs optimally at high temperatures, specifically at or above 30ºC (Richardson & Kuta 2003). The temperature recorded at Coral Gardens, the primary site of black band infections, at approximately 10:00 am on August 11th, 2005 when black band was first recorded was 31.8ºC (Table 6). Prior to this date, temperatures recorded at all sites on all dates remained <30ºC. Subsequent to this emergence of black band, temperatures remained at or above 30ºC until after the passing of Hurricane Wilma when significant black band recovery was noted, and the temperature recorded at the reef was <26ºC. However, a nearly identical temperature record was documented at Cheeca and only a few black band infections were recorded at this site, and not until the survey date right before Hurricane Wilma passed. The abundance of the primary host of black band, *Colpophyllia natans*, was also nearly identical at these sites (Table 1) and the severity of bleaching on colonies during the

event was not significantly different. Therefore, other factors were potentially involved in the high abundance of black band disease at Coral Gardens, potentially related to nutrient abundance, which has previously be been linked to black band infections (Kuta & Richardson 2002). Unfortunately, nutrient levels were not recorded in this study and are not available for comparison.

Although it is impossible to disentangle the potential influences of temperature, turbidity, nutrient loading, and other parameters that could have factored into the emergence and increase coral disease, data on the severity of bleaching on diseased colonies through time suggest a relationship between bleaching and disease. Specifically, higher severity of bleaching was recorded on diseased colonies versus non-diseased colonies. This relationship could be the result of three possible scenarios based on the susceptibility of the host; (1) the susceptibility of the host to disease could have been increased due to the high severity of bleaching that was experienced by the colonies (i.e. bleaching facilitate disease), (2) the susceptibility of the host to bleaching could have been increased due to the onset of disease in these colonies (i.e. disease facilitates bleaching), and (3) the mechanism(s) or factor(s) that influence increased susceptibility to bleaching also increase susceptibility to disease (i.e. some factor facilitates coincident severe bleaching and disease). For SWP1 and black band, the majority of infections were recorded *after* the bleaching event was already at its height, so therefore scenario 2 is unlikely. Conversely, the majority of dark spot infections were recorded *before* the onset of major bleaching, and scenario 1 would be unlikely. Scenario three could've occurred in all disease-bleaching relationships. Despite the number of possibilities that have yet to be explored, these results do indicate that severe bleaching and disease incidence are

somehow related, and that overall the physiological response of the corals to the factor or factors responsible for causing mass bleaching (potentially temperature and water flow) also facilitate the incidence of disease.

These results would not be counterintuitive in light of recent findings, which alternately support all three scenarios described above. In support of scenario 1 (bleaching facilitates disease), the stress of bleaching has been shown to alter the potentially beneficial microbial communities of the coral mucopolysaccharide (MPS) layer resulting in a loss of antibiotic activity (Ritchie 2006). If this shift in microbial community leaves the coral more vulnerable to infiltration by pathogenic agents, and assuming a more severely bleached coral will experience a greater loss of antibiotic activity, it is likely that those corals more severely bleached will experience a greater probability of developing an infection. In support of scenario 2 (disease facilitates bleaching), the MPS microbial community of diseased corals has also been shown to change during infection (Frias-Lopez et al. 2002), which could result in a similar but opposite situation where this alteration increases the corals vulnerability to bleaching. Finally, at the environmental level, physical factors such as temperature and anthropogenic impacts are known to influence the virulence and abundance of pathogens in the environment (Harvell et al. 1999, Harvell et al. 2002), as well as determine the severity of bleaching (Hoegh-Guldberg 1999), and so these factors may be acting at the same time but independently of each other, which would support scenario 3. All of these scenarios should be more thoroughly investigated to further understand the potential relationships between disease and bleaching.

Bleaching and disease are currently acting to change the structure and composition of coral reefs worldwide. Episodes of mass bleaching and the further emergence of epizootic diseases can be expected to increase as the planet faces increasing temperatures due to climate change (Harvell et al. 2002). This small scale study emphasizes how bleaching and disease can act in tandem on local coral reefs. Though hurricanes aided the recovery of these corals from bleaching versus other parts of the Caribbean (Manzello et al. 2007), mortality due to disease and bleaching was still recorded. It is likely that these reefs will not escape from further cycles of mass bleaching and disease and further work is necessary to address the potential links between bleaching and disease both at ecological and physiological scales.

Table 1: Mean proportion of colonies of each species in 5 16-m^2 quadrats installed at each site. Only species representing on average >1% of the population are shown. Species >5% are in bold

Cheeca	Coral Gardens	French	Little Grecian
***M. faveolata* 42.9%**	***S. siderea* 28.8%**	***A. agaricites* 46.2%**	***P. astreoides* 59.9%**
***S. siderea* 26.9%**	***M. cavernosa* 19.4%**	***P. porites* 18.8%**	***S. siderea* 11.1%**
***C. natans* 7.3%**	***M. faveolata* 18.9%**	***S. siderea* 13.0%**	***A. agaricites* 10.4%**
***P. astreoides* 6.2%**	***P. astreoides* 10.1%**	***P. astreoides* 7.6%**	***P. porites* 9.3%**
P. porites 4.5%	***C. natans* 7.1%**	*M. faveolata* 3.0%	*M. faveolata* 4.6%
S. radians 2.9%	***M. annularis* 5.2%**	*A. cervicornis* 2.7%	*D. labrynthiformis* 1.3%
D. strigosa 2.8%	*S. intersepta* 2.9%	*M. mirabilis* 2.0%	*M. cavernosa* 1.3%
M. cavernosa 2.8%	*D. strigosa* 2.8%	*S. intersepta* 1.4%	
S. intersepta 1.6%	*D. stokesii* 1.1%	*D. labrynthiformis* 1.3%	
A. agaricites 1.2%	*P. porites* 1.0%	*M. cavernosa* 1.3%	
		D. clivosa 1.1%	
		D. strigosa 1.1%	

Table 2: Results of the effect of survey date on RM-ANOVAs of bleaching prevalence (arcsin transformed data of proportion of bleached colonies in quadrats, N = 5/site).

Source	Site	Type III Sum of Squares	df	Mean Square	F	Sig.
Bleaching Prevalence	Cheeca	302.156	14	21.583	139.371	**.000**
	Coral Gardens	258.270	14	18.448	104.584	**.000**
	French	91.500	14	6.536	14.015	**.000**
	Little Grecian	5.366	14	.383	.646	.815

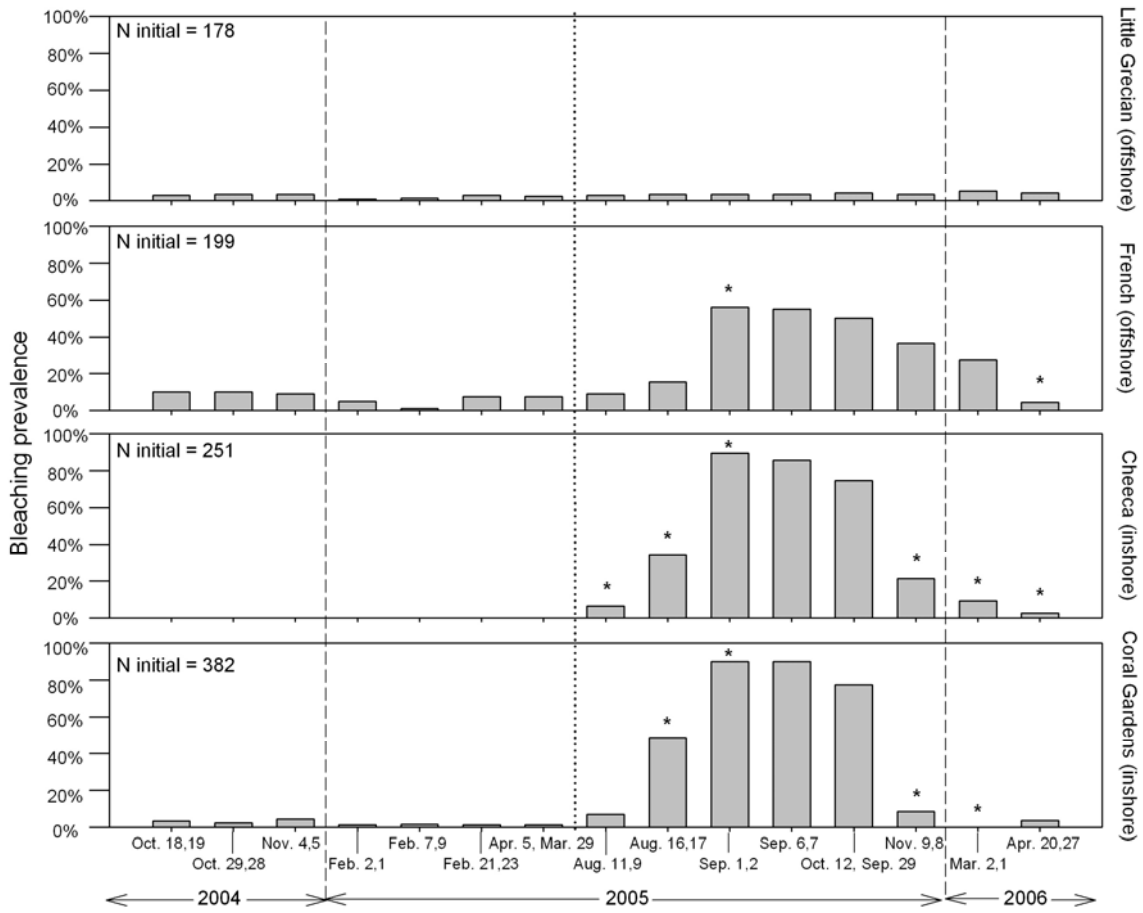

Figure 1 – Prevalence of bleaching in all quadrats combined at each site on sampling dates (inshore site sample dates first). Prevalence of bleaching is the total proportion of corals within quadrats exhibiting ≥10% partial bleaching. * indicates a significant difference between that time period compared with the previous time period based on the results of post-hoc Tamhane's comparison after repeated measures ANOVAs where $p<0.05$. Dashed line indicates different years, and dotted line indicates transition from spring to summer.

Table 3a: Mean bleaching severity (± 1 SE) recorded on colonies of the eight most abundant species by site or habitat for survey dates that occurred before the mass bleaching event. Mean bleaching severity is presented by site or combined by habitat depending on the results pair-wise comparisons. Bold indicates a survey date where bleaching was >0%, or where statistical tests produced a significant effect. * indicates a significant repetitive comparison (Tamhane's test) between mean bleaching severity of that date compared with the previous date.

Species	Effect of site	Habitat	Site	N	Effect of date	Pre-bleaching						
						2004			2005			
						Oct 18, 19	Oct 29, 28	Nov 4, 5	Feb 2, 1	Feb 7, 9	Feb 21, 23	Apr 5, Mar 29
A.agaricites	**F=42.2, p<0.05**	Inshore	Cheeca	3	**F=37.7, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
		Offshore	French	106	**F=167.7, p<0.05**	**10.9±2.6%**	**8.1±1.9%**	**6.4±1.7%**	**1.9±0.5%**	**0.6±0.2%**	**0.1±0.1%**	**1.0±0.4%**
			L. Grecian	14	F=1.0, p = 0.44	**13.4±9.1%**	**13.4±9.1%**	**10.7±7.4%**	**3.0±2.8%**	0.0±0.0%	0.0±0.0%	0.0±0.0%
C. natans	F=0.0, p=0.948	Inshore	combined	705	**F=176.8, p<0.05**	**0.4±0.3%**	**0.2±0.2%**	**0.3±0.2%**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
M. annularis		Inshore	C. Gardens	17	**F=317.7, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
M. cavernosa	**F=32.7, p<0.05**	Inshore	Cheeca	90	F=1.5, p = 0.118	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
			C. Gardens	1050	**F=1069, p<0.05**	**2.2±0.9%**	**1.1±0.6%**	**1.3±0.5%**	**0.2±0.2%**	**0.2±0.1%**	**0.2±0.1%**	**0.1±0.0%**
		Offshore	French	30	No change	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
			L. Grecian	30	No change	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
M. faveolata	**F=39.6, p<0.05**	Inshore	combined	2805	**F=719.6, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	**0.1±0.1%**	**0.1±0.1%**	**0.1±0.1%**	0.0±0.0%
		Offshore	combined	150	F=1.3, p=0.215	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
P. astreoides	**F=161.4, p<0.05**	Inshore	Cheeca	195	**F=22.5, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
			C. Gardens	585	**F=41.7, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
		Offshore	French	165	**F=3.1, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
			L. Grecian	1800	F=0.9, p = 0.606	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	**0.2±0.2%**	**0.2±0.2%**	**0.2±0.2%**
P. porites	**F=34.8, p<0.05**	Inshore	combined	240	**F=32.9, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
		Offshore	French	525	**F=4.8, p<0.05**	**2.3±2.3%**	**1.7±1.7%**	**0.9±0.9%**	**0.3±0.3%**	0.0±0.0%	0.0±0.0%	0.0±0.0%
			L. Grecian	225	No change	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
S. siderea	**F=55.9, p<0.05**	Inshore	combined	2670	**F=386.6, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	**0.2±0.2%**
		Offshore	combined	615	**F=4.4, p<0.05**	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	**0.5±0.3%**

Table 3b: Mean bleaching severity (± 1 SE) recorded on colonies of the eight most abundant species by site and habitat for survey dates that occurred during and after the mass bleaching event. N remains the same as Table 3a.

			Bleaching						Post-bleaching	
			2005 con't						2006	
Species	Habitat	Site	Aug 11, 9	Aug 16, 17	Sep 1, 2	Sep 6, 7	Oct 12, 29	Nov 8, 9	Mar 1, 2	Apr 20, 27
A.agaricites	Inshore	Cheeca	30.0±30.0%	32.7±32.7%	66.7±33.3%	100.0±0.0%	99.3±0.7%	48.7±27.7%	0.7±0.7%	0.0±0.0%
	Offshore	French	3.6±1.3%	18.4±3.4%*	80.3±3.2%*	82.5±3.4%	64.9±3.7%	13.1±1.5%*	1.3±0.3%*	0.3±0.1%
		L. Grecian	0.0±0.0%	13.4±9.1%	14.2±9.7%	14.3±9.7%	4.3±3.6%	4.6±3.3%	0.0±0.0%	0.0±0.0%
C. natans	Inshore	combined	8.3±2.9%	27.8±5.4%	90.1±3.9%*	95.4±2.5%	86.3±3.7%	16.9±3.9%*	1.8±0.8%*	3.5±1.2%
M. annularis	Inshore	C. Gardens	0.0±0.0%	0.0±0.0%	93.8±1.5%*	95.4±1.3%	56.5±7.8%	0.0±0.0%*	0.0±0.0%	3.5±2.4%
M. cavernosa	Inshore	Cheeca	0.0±0.0%	0.0±0.0%	16.3±16.3%	24.7±16.8%	18.0±16.1%	0.0±0.0%	0.0±0.0%	0.0±0.0%
		C. Gardens	4.9±2.4%	8.1±2.8%	64.4±4.2%*	78.1±3.0%	43.1±3.6%*	1.4±0.8%*	0.0±0.0%	0.0±0.0%
	Offshore	French	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
		L. Grecian	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
M. faveolata	Inshore	combined	0.1±0.1%	2.6±1.0%	75.4±2.6%*	80.3±2.3%	60.4±2.6%*	3.5±0.7%*	0.1±0.1%*	0.0±0.0%
	Offshore	combined	0.0±0.0%	1.0±1.0%	6.0±6.0%	9.0±9.0%	8.0±8.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
P. astreoides	Inshore	Cheeca	0.0±0.0%	7.7±5.2%	71.2±11.6%*	73.3±11.8%	32.3±11.1%	0.2±0.2%	1.5±1.5%	0.0±0.0%
		C. Gardens	0.0±0.0%	2.6±2.6%	42.1±7.4%*	48.9±6.6%	22.7±5.0%	0.0±0.0%*	0.0±0.0%	0.0±0.0%
	Offshore	French	0.0±0.0%	0.0±0.0%	19.1±10.1%	27.1±14.0%	24.5±12.7%	1.8±1.2%	0.0±0.0%	0.0±0.0%
		L. Grecian	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
P. porites	Inshore	combined	0.0±0.0%	3.1±3.1%	76.9±9.6%*	79.6±9.9%	41.3±10.9%	0.0±0.0%	0.0±0.0%	0.0±0.0%
	Offshore	French	0.0±0.0%	1.7±1.7%	17.2±6.0%	17.9±6.2%	10.0±4.8%	0.6±0.4%	0.6±0.6%	0.0±0.0%
		L. Grecian	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%	0.0±0.0%
S. siderea	Inshore	combined	1.1±0.6%	3.3±1.1%	65.6±2.9%*	69.0±2.7%	32.8±2.5%*	0.4±0.2%*	0.1±0.0%	0.2±0.1%
	Offshore	combined	2.2±1.0%	3.0±1.5%	7.4±2.7%	7.3±2.8%	4.0±1.7%	0.0±0.0%	1.7±0.7%	0.5±0.3%

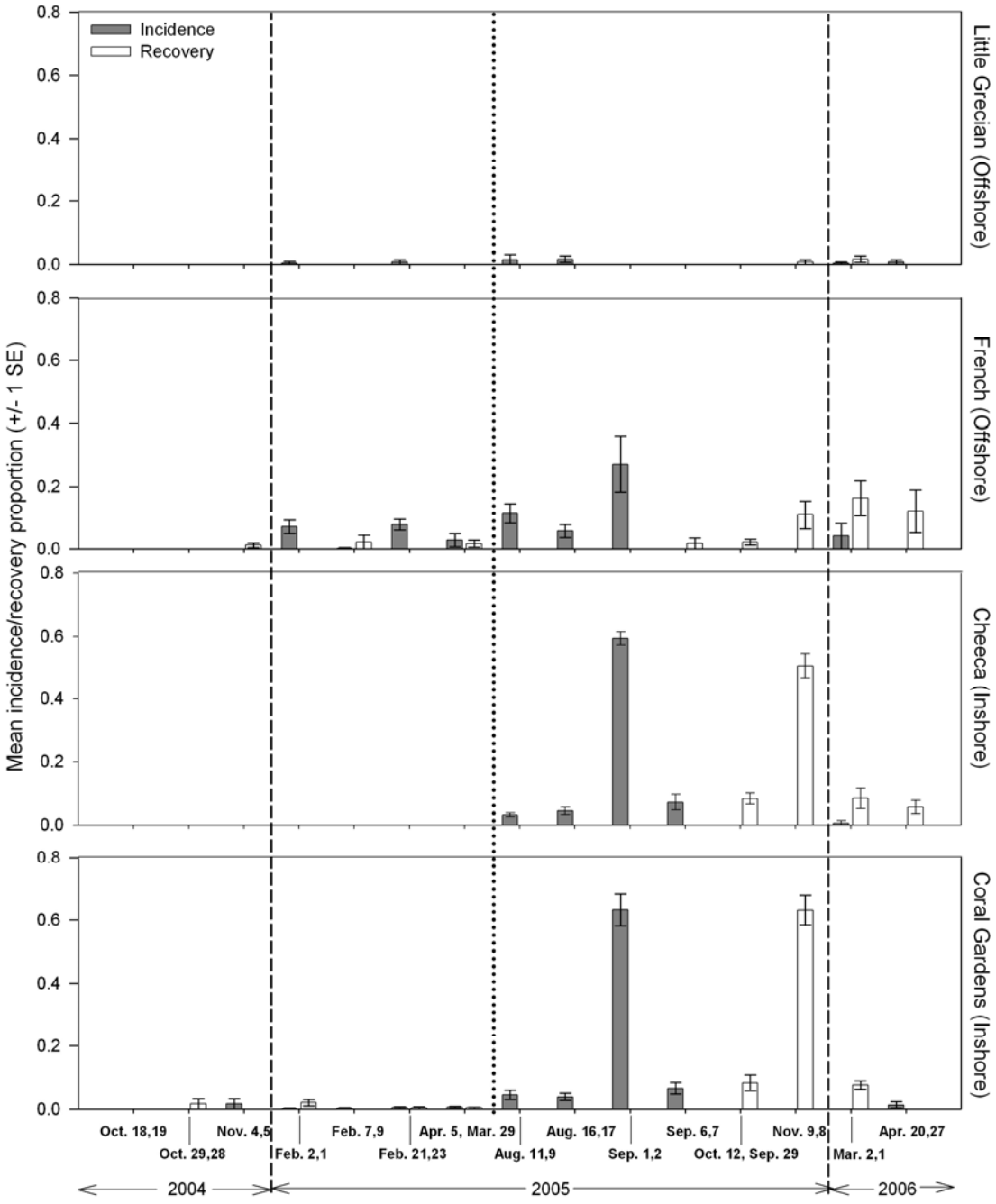

Figure 2 – Mean bleaching incidence and recovery proportions (+/- 95% confidence limits) within quadrats at sites (n = 4 quadrats/site). Incidence proportion is the proportion of colonies within a quadrat that showed bleaching that were not bleached in the previous sampling period. Recovery proportion is the proportion of colonies within a quadrat that were not bleached on the date sampled that were bleached on the previous sampling date.

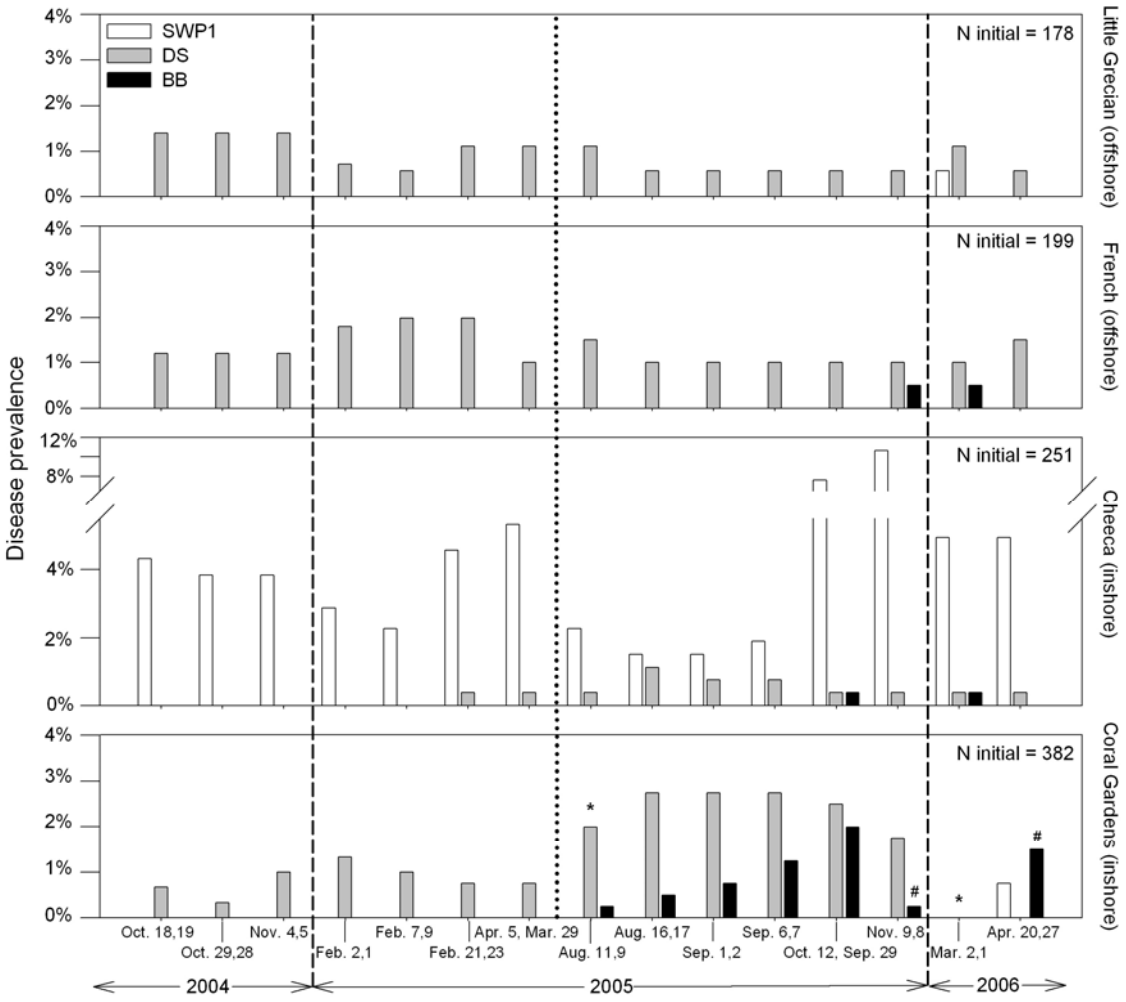

Figure 3 – Prevalence of the three characterized diseases on sample dates. SWP1 **indicates suspect white plague type I; DS indicates dark spot; and BB indicates black band. Prevalence is the proportion of all corals within quadrats exhibiting signs consistent with that described for each syndrome. '*' and '#' indicate a significant difference between dark spot prevalence and black band prevalence respectively in that time period compared with the previous time period based on a repeated measures ANOVA post-hoc Tamhane's pair-wise comparison where $p<0.05$.**

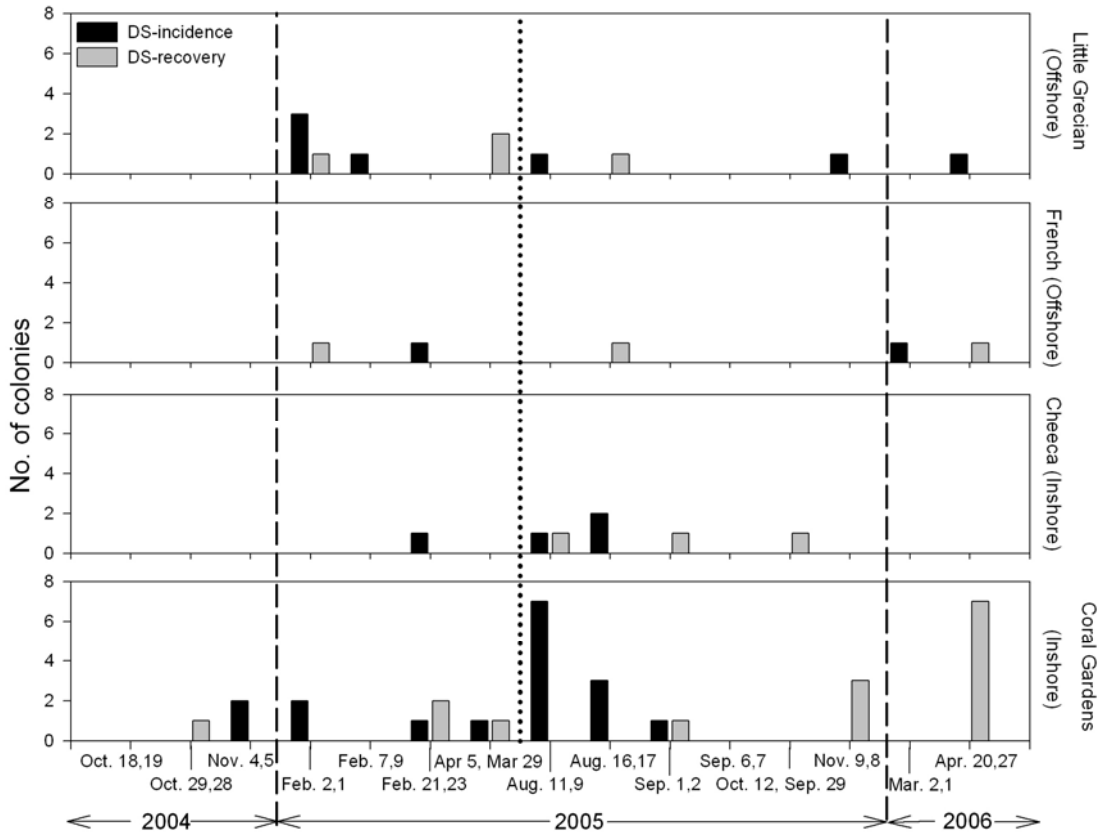

Figure 4 – Incidence and recovery of colonies from dark spot (DS) at all four sites. Incidence is the absolute number of colonies that exhibited signs of the disease on that site visit which were free of signs on the previous visit. Recovery is the absolute number of colonies free of signs that on the previous visit were showing signs of the disease.

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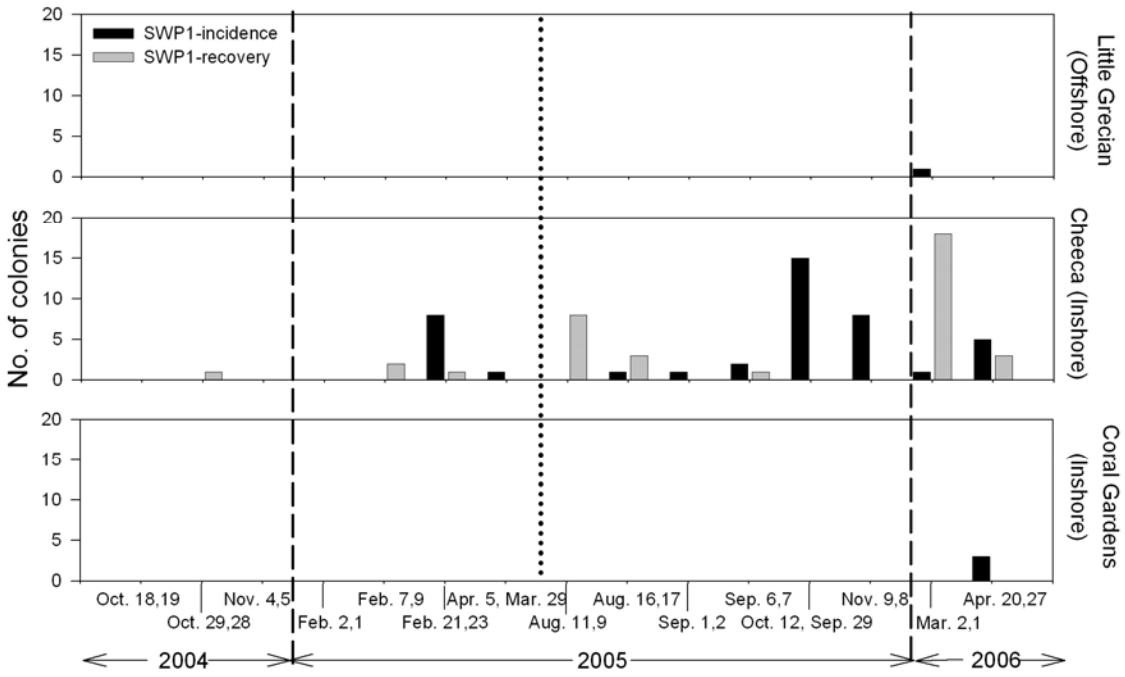

Figure 5 – Incidence and recovery (as defined in Figure 4) of coral colonies from suspect white plague type I (SWP1).

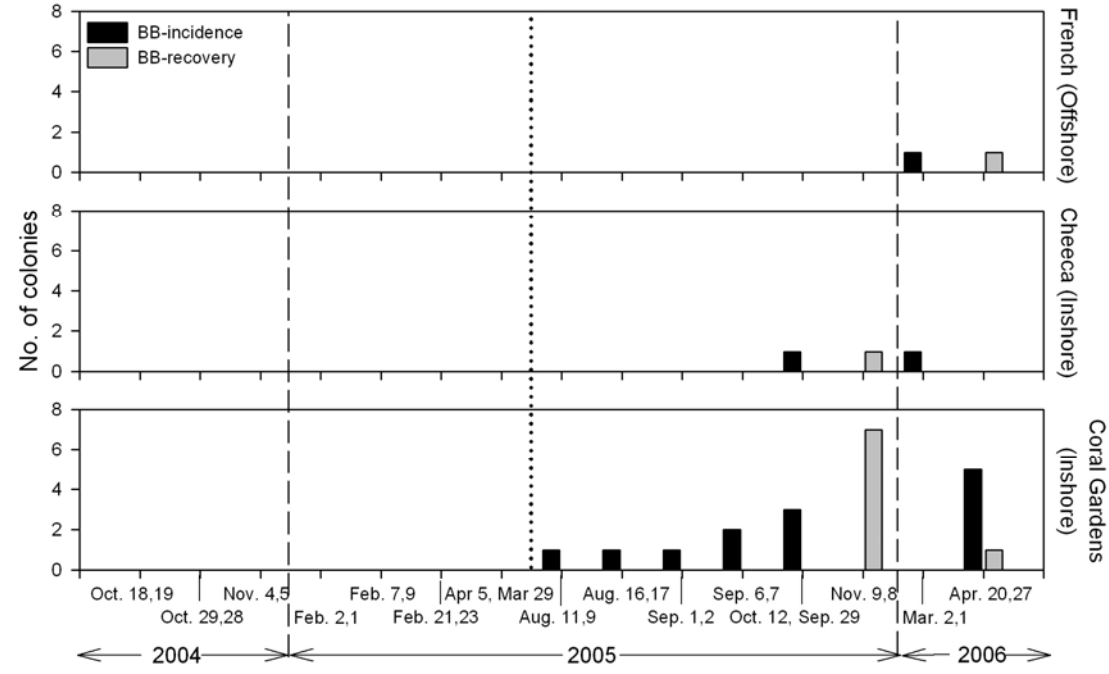

Figure 6 – Incidence and recovery of colonies exhibiting signs consistent with the description of black band disease (BB).

Table 4: Proportion of colonies of eight species affected by the three dominant recorded syndromes. Bold indicates highest proportion affected for that syndrome.

Species	N	BB	DS	SWP1
Agaricia agaricites	123		0.1%	
Colpophyllia natans	47	**14.5%**	6.7%	5.0%
Diploria strigosa	21	7.4%		1.6%
Montastraea cavernosa	80		0.5%	
Montastraea faveolata	197			**5.6%**
Siderastrea radians	10		6.3%	
Siderastrea siderea	219		**10.8%**	
Stephanocoenia intersepta	19		2.9%	

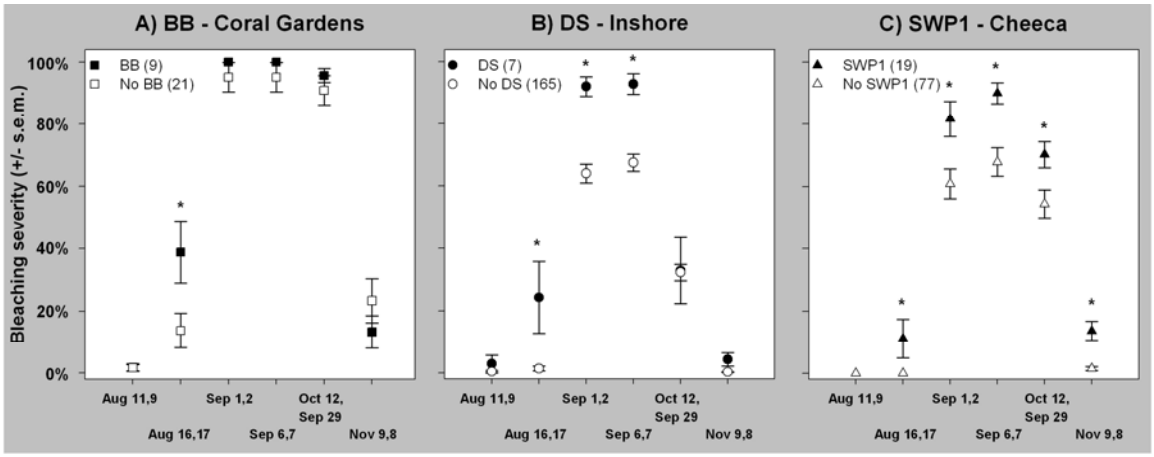

Figure 7 – Comparison of mean bleaching severity (± s.e.m.) recorded on 2005 bleaching event dates on colonies of A) *Colpophyllia natans* affected by black band during the bleaching event (BB) versus unaffected colonies (no BB) at Coral Gardens, B) *Siderastrea siderea* affected by dark spot during the bleaching event (DS) and unaffected colonies (no DS) at inshore sites combined, and C) *Montastraea faveolata* affected by suspect white plague type I during the event (SWP1) and unaffected colonies (no SWP1). N of colonies indicated in parentheses, * indicates a significant difference from results of Mann-Whitney pair-wise tests: $p<0.05$.

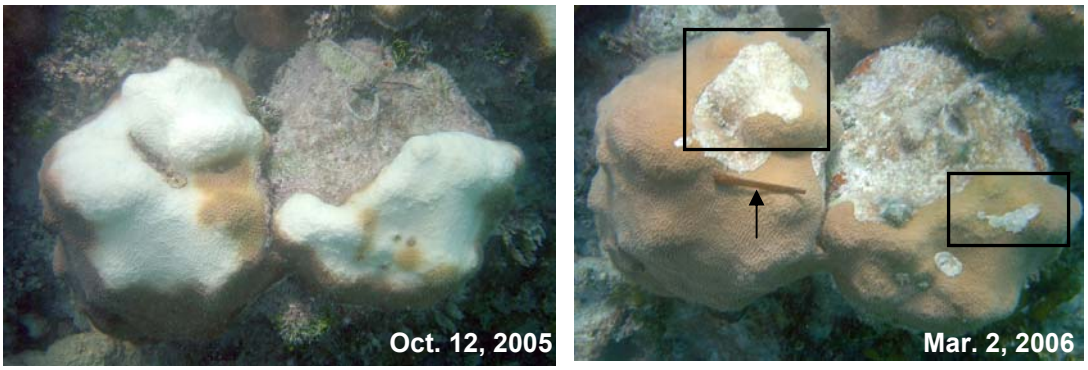

Figure 8 – *Montastraea faveolata* colony that experienced severe bleaching in the fall of 2005. The most severely bleached portions of the colony appear to have exhibited mortality due to bleaching (right photo, boxes) as this mortality could not be attributed to any characterized coral disease, predation or other type of mortality such as hurricane damage. Colony located at 4 m depth, Cheeca Rocks, middle Florida Keys. Scale bar in right photograph (indicated by arrow) is 8 cm long.

Table 5: Lesion numbers and tissue loss rates associated with identified syndromes and attributed to other or unknown causal factors including total colony loss. Values for all years and species are combined. For tissue loss rates only consecutive sampling periods separated by <1 month where was colony infected during both visits was used. Letters indicate significant groups as determined by post-hoc comparisons with Tamhane's pair-wise tests ($p<0.05$).

Syndrome	Mean # lesions with mortality / colony ± s.e.m.	Mean tissue loss (cm^2) / lesion / day* ± s.e.m.	Mean tissue loss (cm^2) / colony / day* ± s.e.m.
BB	3.0 ± 0.9	4.11 ± 0.8^{a}	8.93 ± 2.33^{x}
Bleaching	0.07 ± 0.01	0.98 ± 0.26^{bc}	1.88 ± 0.53^{y}
DS	0.9 ± 0.3	0.40 ± 0.19^{cd}	2.29 ± 1.09^{y}
SWP1	7.6 ± 1.4	0.72 ± 0.05^{b}	1.82 ± 0.20^{y}
Other/unk	3.5 ± 0.7	1.00 ± 0.42^{d}	3.58 ± 1.35^{xy}

Table 6: Water temperature (°C) measured at the tops of the reef structure. (I) and (O) indicate inshore and offshore sites, respectively.

	Pre-bleaching						
	2004			2005			
Site	Oct 18, 19	Oct 29, 28	Nov 4, 5	Feb 2, 1	Feb 7, 9	Feb 21, 23	Apr 5, Mar 29
Cheeca (I)	27.0	26.0	26.7	21.4	20.2	22.3	23.4
C. Gardens (I)	26.9	26.3	27.0	21.1	20.6	22.3	23.0
French (O)	27.8	27.1	27.6	22.7	23.6	24.0	24.0
L. Grecian (O)	27.9	26.4	27.8	23.2	23.1	23.8	25.8

	Bleaching						Post-bleaching
	2005 con't						2006
	Aug 11, 9	Aug 16, 17	Sep 1, 2	Sep 6, 7	Oct 12, 29	Nov 8, 9	Mar 1, 2
Cheeca (I)	31.8	31.7	31.1	30.6	29.3	25.5	22.7
C. Gardens (I)	31.8	23.0	31.4	30.3	29.5	25.4	22.7
French (O)	30.6	30.8	29.5	29.3	29.1	26.2	21.8
L. Grecian (O)	30.9	31.3	29.4	29.1	29.1	26.4	22.8

Chapter 7: Summary and synthesis

Background

The epizootiology of wildlife diseases is highly dependent on the ecology of the landscape and the variability found within populations of organisms. While all populations of living organisms experience disease, those living under stressful conditions or in degraded habitats are more susceptible to invasion by pathogens. Similarly, communities that are less diverse are more likely to be at risk from disease than are communities and populations that are genetically and/or phenotypically diverse. As the degradation of ecosystems and loss of biodiversity continues, all populations are increasingly at risk from epidemic disease.

In order to contain or prevent epidemic disease in wildlife populations, aspects of the host's susceptibility and the pathogen's transmission and virulence mechanisms must be understood. However, marine populations including populations of reef-building corals offer unique challenges in the study of the etiology and epizootiology of disease. First, seawater provides a potential breeding ground for pathogenic organisms, and all organisms living in the marine environment are constantly bathed in what amounts to a microbial soup, making the sterile sampling of diseased tissue and isolation of potential pathogens difficult and often confounded (Harvell et al. 2004). Also, ocean currents are major determinants of the distribution of organisms in the marine environment but they can accelerate the rate of spread of pathogens between hosts compared to similar host-pathogen systems on land (McCallum et al. 2003). Therefore, response times for studying events such as the emergence of a new disease or an epidemic outbreak must be rapid in order to fully document such occurrences. The loss of nearly 97% of the regional

population of *Diadema antillarum*, a major herbivore on Caribbean reefs, was likely due to a rapidly spreading, possibly viral, disease that followed the major current pathways in the Caribbean basin (Lessios 1988). The disease moved so rapidly that very little information on its characteristics was able to be collected. Reef-building corals specifically, like many other organisms in the marine environment, offer the added challenge of relying on a tight symbiosis with the unicellular algae of the genus *Symbiodinium* that live in the gastrodermal layer of their tissues. Therefore, any coral-pathogen system must take into consideration the potential role of the symbionts, and in some cases it has been demonstrated that the symbionts themselves are the target of pathogen invasion (Ben-Haim et al. 1999, Cervino et al. 2004). Also, the majority of coral diseases have been identified affecting multiple species, with varying characteristics that may make them more or less susceptible to pathogen invasion (Sutherland et al. 2004).

An understanding of coral diseases can therefore only result from a better understanding of coral biology and ecology, including the coral animal's interactions with its endosymbiotic zooxanthellae, endolithic organisms, and mucosal microbial communities. However, integrating all of this information at the population-level requires epizootiological models, like that developed in this study. These models provide a framework for combining what information is already known about the disease and using it to provide guidance for further targeted data gathering. Additionally, although much information on coral-pathogen interactions remains to be understood, by beginning to build these frameworks today they can provide a basis for applying current knowledge and testing hypothesis concerning the prevention and/or containment of diseases. They

are also flexible enough to incorporate data as it becomes available, further increasing the refinement of predictions and the ability to test hypotheses.

White plague-like signs on Little Cayman, trends and modeling

On Little Cayman, the smallest and least populated of the three Cayman Islands, a long-term data set revealed that suspect white plague type II (SWP2) was potentially having a significant impact on living coral cover and may have been contributing to a shift in the dominance of coral species away from those that contribute extensively to the framework of the reef to those considered to be more weedy species. Shorter term incidence data revealed that the occurrence of SWP2 and other types of mortality is rare; however, SWP2 caused excessive tissue loss in short periods of time on colonies that it affected.

The model in this project, the Simulation of Infected Corals, or SICO, model was developed using this long-term data set. This model incorporates features that allow it to be applied to specific reefs, with the capability to model reef sites of varying coral cover, community composition, size distribution, and spatial distribution, which may impact the dynamics of disease within a reef system. SICO was capable of recreating multiple patterns of disease and community dynamics at the population-level using simple disease interactions at the colony-level. The results of the calibration and validation of the model suggest that the incidence of SWP2 documented on Little Cayman corals occurred through primary infections originating either from an outside source (e.g., metapopulation connections) or from within the population (e.g., opportunistic infections) and also secondary infections, which occurred through contagious transmission within a limited distance of infected corals. Changes in the relative abundances of the six most abundant species that occurred at Little Cayman sites were not all accurately reproduced

in simulations, however this may have been due to inaccurate parameterization of recruitment of juvenile corals on the reef, for which no data were available. In applying various potential mediation scenarios that could be either anthropogenically or naturally induced, it was found that prevention strategies and not treatment strategies were most effective in reducing the impact of disease on ecological time scales.

The influence of population characteristics on the dynamics of coral disease

In applying the disease parameter settings that resulted from calibration and validation, the variability in the distribution and composition of coral populations was found to have significant impacts on the dynamics and impact of SWP2. The transmission ability of disease and the rapid rate of tissue loss associated with SWP2were strong determinants in the outcomes of simulations with varying coral population parameter settings. Although transmission was limited spatially, there was a strong effect of disease in coral populations that were more densely populated or aggregated due to the transmissible quality of the disease in simulations. The rapid rate of tissue loss allowed populations dominated by small corals to experience less impact from disease because infections persisted for shorter time periods, thereby limiting the interaction between infected and susceptible colonies and lowering the risk of infection through contagious spread. Overall, the characteristics of the populations themselves can play a large role in determining the overall influence of disease.

Disease and bleaching on Florida reefs

In Florida, the bleaching event of 2005 provided the opportunity to examine disease incidence in relation to bleaching severity and prevalence. Before the onset of bleaching, two syndromes, darks spot and white plague type I (SWP1), were identified occurring at

monitored sites but at very low incidences. With the onset of bleaching and during the height of bleaching the incidence of these two syndromes was also low. However, after bleached colonies recovered their color, SWP1 increased in both abundance and distribution. A third syndrome, black band, occurred for the first time coincident with the onset of bleaching and increased dramatically as the severity of bleaching increased. Those colonies that developed disease tended to be more significantly affected by bleaching than those that did not, suggesting a relationship between disease and bleaching.

The impact of disease and bleaching on the future of coral reefs

Reporting of coral diseases first began in earnest in the 1970s and 1980s. During this time, the majority of the reports were made by ecologists, and many of these reports included descriptions of the diseases' distribution in the populations. Yet despite this, much of the effort involved in studying coral diseases since then has been within the disciplines of microbiology, molecular biology, and histology. Some epizootiological information is collected however it is typically not based on common epidemiological sampling techniques, and tools such as modeling have not been applied. Only recently has the importance of studying the epizootiology of coral disease as a means to gaining an understanding the etiology, functioning and impact of disease been stressed in the literature (Green & Bruckner 2000, Jolles et al. 2002, Kim & Harvell 2004, Borger 2005a, 2005b).

Projections of the impact of disease on Little Cayman reefs by the model were dramatic, although they are in holding with suggestions by other authors that the collapse of coral dominated reefs in the near future is imminent due to several factors including

disease. For example, mortality due to mass bleaching has been predicted to cause the complete decline of corals within 50 years as temperatures rise above their postulated thermal tolerance limits (Hoegh-Guldberg 1999). Near total loss of coral cover has been documented (Glynn 1994) in the past. Also, regional populations of individual species have come close to extinction due to disease. For *Acropora palmata* and *A. cervicornis*, white band caused wide-scale mortality in less than a decade (Gladfelter 1982, Aronson & Precht 2001), while *Diadema antillarum* lost most of its regional population in close to one year (Lessios 1988). Yet the prediction of the complete decline of corals in the next several decades does not consider the ability of corals to change their species of algal symbiont for those that are more bleaching-tolerant (Baker 2003). This mechanism could potentially enable corals to acclimatize as ocean temperatures rise (Buddemeier & Fautin 1993).

In studies reported here, coral cover at Little Cayman sites and in Venezuela declined dramatically but then stabilized once the population hit a critical density, in which the likelihood of primary and secondary incidence became very low. Other factors not currently apparent may also act to prevent coral communities from even reaching this critical density. Corals, being invertebrates, do not possess adaptive immunity. Therefore, it is unlikely that a coral invoking an immune reaction in response to a pathogen will acquire long-lasting defensive capabilities when challenged with that pathogen again. However, innate immune reactions can be stimulated to high levels that leave residual influences if challenged again. Also, in the long term, the potential exists that a naturally (genetically) immune population which survives could continue and repopulate. This may be what is driving the recovery of *Acropora* spp. and *Diadema antillarum* populations in

the Caribbean (Hunte & Younglao 1988, Edmunds & Carpenter 2001, Miller et al. 2003, Lessios 2005). However, the combined influence of regional (e.g., temperature-induced mass bleaching) and local (e.g., point source pollution, coastal development) stressors on coral populations has already been enough to cause significant declines in the last few decades (Gardner et al. 2003).

In Florida, the passage of hurricanes relieved the temperature stress and resulted in recovery from bleaching, although in other systems no relief was observed and bleaching continued into the winter of 2005 (Manzello et al. 2007). In these regions, mortality from bleaching and disease was severe (Miller et al. 2006). The relationship between bleaching and disease documented in this study suggests that as climate change acts to increase the global average temperature of tropical oceans, disease and bleaching may act synergistically to impact coral populations. Although acclimatization to conditions causing bleaching can occur (Baker 2003), and populations may naturally become less disease-susceptible (Kim & Harvell 2004), the threshold for these two process may lie beyond the point at which reefs have transitioned from coral-dominated systems to alternate states (Knowlton 1992). Recovery from these alternate states back to coral-dominated systems is slow, and the impact of existing in this alternate state on other parts of the ecosystem and on reef-dependent societies of people is likely to be severe (Knowlton 2004).

The field data and modeling presented in this dissertation have furthered the knowledge of the epizootiological characteristics of the potentially devastating set of disease signs, popularly referred to as white plague disease, found on scleractinian reef-building corals of the Caribbean. This work would be advanced by more detailed studies

focused on validating local transmission dynamics among colonies. Also, linking the biological model developed in this study with physical models including those describing temperature dynamics and current flow would allow the model to be applied at larger temporal and spatial scales, where these physical factors begin to play major roles. With the increasing recognition of the important role of disease in influencing population dynamics and the threat of increased risk from disease in the future, tools such at the model developed in this study are important for helping understanding key features of disease dynamics in the marine environment. This understanding is critical to the management and conservation of reefs.

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