AN ABSTRACT OF THE THESIS OF Paula Wendy M. Lozada for the Master of Science in Biology presented November 28, 2011.

Title: Characterization of White Syndrome (WS) affecting *Porites* spp. in Guam and the Effect of Colony Morphology on Disease Dynamics

Approved: _____
Laurie J. Raymundo, Chairperson, Thesis Committee

Over the last two decades, there has been an increase in the reports of coral epizootics worldwide. These diseases have appeared with progressively greater frequency and with wider host and geographic range contributing to the observed global decline in reef-building corals. Thus, the emergence and increased incidence of coral diseases have placed coral reefs as one of the most vulnerable ecosystems on the planet.

Recently, the initial results from a long-term coral reef monitoring in Guam reported a highly prevalent, previously uncharacterized White Syndrome type disease affecting several dominant reef-forming species. The disease was also found to be prevalent in two Poritid growth forms (branching *Porites cylindrica* and massive *Porites* spp.). The aims of this study were then to characterize the disease in both Poritid growth forms by describing the gross and histological manifestations of the disease and investigate the role of coral morphology in tissue recovery. Infections experiments were also undertaken to attempt to identify etiologic agent associated with White Syndrome.

The results of a 7-month census revealed that the Poritid White Syndrome is a chronic disease appearing as irregular areas of tissue loss, small and diffuse in branching colonies, and multifocal to coalescing in massive colonies. Lesions were often

overgrown by filamentous algae within a few weeks, which may have contributed to secondary infections. The histopathology of branching *P. cylindrica* samples revealed tissue necrosis associated with endolithic algae and characterized by dense aggregations of eosinophilic granular ameobocytes, and a proliferation of pigment cells and bacterial aggregates near algal infiltrates indicative of an immune-related response. This study also found that White Syndrome is infectious within the species of *P.cylindrica* and is transmissible both through direct contract and through the water column. Partial sequencing of 16S rRNA reported that White Syndrome is associated with a member of the bacteria family Vibrionaceae that is 99% similar to the coral bleaching pathogen, *Vibrio coralliilyticus.*

The results of this study revealed that the rate of tissue recovery is directly proportional to lesion size and that recovery is linked to the amount of tissue bordering the injury from where regeneration, through a proportionate number of polyps, is initiated *(R^2=0.518, $F_{1,86}$ =92.33, p<0.001)*. This geometric relationship explains why lesions in branching *P. cylindrica* colonies, having fewer polyps at the lesion perimeter, were found to have slower recovery rates than in massive *Porites* spp. colonies. While tests showed no significant difference in the rate of tissue recovery and disease severity between the two Poritid growth forms, this study presented evidence that branching *P. cylindrica* colonies tend to develop small lesions that are able to fully recover while massive *Porites* spp. colonies develop larger lesions that tend to remain in stasis that would then require high resource allocation for regeneration. Hence, massive *Porites* spp. colonies are likely to be at a greater risk of total tissue mortality from possible secondary infections and from the constant reallocation of resources to healing, thus reducing overall fitness.

TO THE OFFICE OF GRADUATE STUDIES

The members of the committee approve the thesis of Paula Wendy Lozada presented November 28, 2011.

_____
Laurie J. Raymundo, Chairperson

_____
Alexander M. Kerr, Member

_____
David R. Burdick, Member

ACCEPTED:

_____ _____
John A. Peterson, Ph.D. Date
Assistant Vice President
Of Graduate Studies,
Sponsored Programs and Research

CHARACTERIZATION OF WHITE SYNDROME (WS) AFFECTING *PORITES* SPP. IN GUAM AND THE EFFECT OF COLONY MORPHOLOGY ON DISEASE DYNAMICS

BY

PAULA WENDY M. LOZADA

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM

DECEMBER 2011

For my father,

Pablito Silva Lozada

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Laurie Jeanne Raymundo, for giving me the opportunity to work with her on coral disease and for introducing me to this field. I thank her for her insight and unwavering support of my work and for her patience and willingness to invest her time in my research projects. Most importantly, I would like to thank Dr. Raymundo for her friendship, generosity, and her continuous encouragement and guidance that transcend beyond the school walls.

I would like to extend my gratitude to my graduate committee, Dr. Alexander Kerr and David Burdick of the Guam Coastal Management Program for their invaluable input and generous assistance on my thesis. I would also like to thank Dr. Drew Harvell of Cornell University who, through the Coral Disease Working Group (CDWG), generously provided the scholarship funding for the histopathology and a majority of the laboratory experiments conducted in this study. Histopathology was carried out at the USGS Field Station in Honolulu under the training and supervision of Dr. Thierry Work, to whom I am grateful for his expertise and guidance in coral pathology. I would also like to thank Courtney Couch of Cornell University for her advice and patience as I sought assistance on scholarship funding and general histological procedures. I also thank Nancy Douglas of Cornell University for her assistance and excellent work in processing the slide samples needed for histopathology.

I wish to express my special gratitude to my friend and colleague, Susanna Whitfield, for her expertise in microbiology and whom I am greatly indebted to for her collaboration on the laboratory infection experiments, for her assistance in the field, and

for her invaluable contribution to the microbiological aspects of my thesis. I would also like to thank Dr. Ernest Matson and Dr. Sloan Siegrist for lending their expertise in microbiology. This study would not have also been possible without the generous assistance and support of my friends and colleagues at the University of Guam Marine Laboratory, most especially to: Pablo Rojas, Jr. and Roxie Diaz for their willingness to spend long hours in the field to collect data; Maia Raymundo for her assistance in the laboratory experiments; and Ciemon Caballes, Roxanna Miller, and Brett Taylor for their professional advice. I would also like to thank the UOG Marine Laboratory Technicians, Jason Miller, Joe Cummings, and Chris Bassler, for their technical assistance at the wet laboratory facility.

Finally, I wish to thank my husband, William Misa, for his assistance in the field, for reviewing my drafts, and for his genuine interest in my work. I thank him, most especially, for his personal support, his unwavering encouragement and his confidence in my success. I would also like to thank my family for providing their wholehearted support and encouragement despite the distance, and a special thank you to my mother, Hidelisa, for her guidance and wisdom throughout this experience. This Master's thesis is dedicated to my late father, Pablito, for encouraging me to become a Marine Biologist.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. Introduction

1.1. DISEASES AND ENVIRONMENTAL STRESS

Disease is a natural aspect of populations and is one of the many population-regulating mechanisms that keep many living organisms in balance with one another (Raymundo et al., 2008). However, anthropogenic stressors and environmental contaminants have increased substantially and have amplified the role of disease in both wildlife and human populations. For example, the infectious oocytes of the protozoan parasite, *Toxoplasma gondii,* are shed in the fecal material of domesticated and wild felid and are transmitted to otters, spinner dolphins (Migaki et al., 1990), and Beluga whales (Mikaelian et al., 2000) via exposure to soil runoff and sewage effluent (Dubey et al., 2004). Furthermore, toxoplasmosis infection rates in sea otter populations were found to be three times higher in areas of maximum freshwater runoff along the California shoreline, a significant portion of which are urbanized and highly populated (Miller et al., 2002). Concentrated heavy metal and pesticide pollutants around developed areas were also found to be detrimental to vertebrate immune function (Bernier, et al., 1995; Krzystyniak, et al., 1995). The marine toad *Bufo marinus* and the whistling frog, *Eleutherodactylus johnstonei,* were reported to have decreased B cell-mediated immunity and increased helminth infections due to exposures to high levels of copper, cadmium, and byproducts of dichlorodiphenyltrichloroethane (DDT) in the soil (Linzey, et al., 2003). Global climate change and exposure to industrial, agricultural, and urban pollution have also been implicated in a number of infectious diseases such as

herpesvirus in pilchards (Whittington et al., 1997); canine distemper virus (CDV) and influenza B virus in seals (Harder and Osterhaus, 1997; Osterhaus et al., 2000); and morbilliviruses in dolphins, porpoises and seals (Dietz et al., 1989; Heidejorgensen et al., 1992). Similar to its effect on coral reef systems, these environmental stressors, not only influence the host's immune reactivity and susceptibility to infections, but also exacerbate the virulence of pathogens (Daszak et al., 2001; Bradley et al., 2007).

1.2. THE EMERGENCE OF CORAL DISEASE

The emergence of a number of coral diseases and syndromes has been correlated with increasing anthropogenic impacts and global climate change, however, the links are not always clear and the pool of quantitative data is at the moment too limited on a temporal scale to allow significant correlations. It is nonetheless safe to suggest that these diseases may be related to changing and deteriorating environmental conditions that could affect basic biological and physiological balances in pathogen-host populations (Harvell et al., 2002; Kuta and Richardson, 2002; Rosenberg and Ben-Haim, 2002).

The increasing prevalence of coral diseases and thermal stress have severely impacted reef-building corals over the last 20 years (Harvell et al., 1999; Cervino et al., 2004). This increase may be attributed to poor water quality, habitat degradation, overfishing, and global climate change that affect the basic biological and physiological balances in pathogen/parasite-host populations (Harvell et al., 2007; Kuta and Richardson, 2002; Edinger et al., 1998; Roberts, 1995; Hughes, 1994). In the last few decades, there has been a worldwide increase in the reports of diseases contributing to the

observed global decline in reef-building corals (Harvell et al., 1999; Ward and Lafferty, 2004). These diseases have appeared with progressively greater frequency and wider host and geographic range. In the Caribbean, diseases have wiped out large colonies of the main reef-building corals (i.e. *Acropora* spp., *Montastraea* spp., *Colpophyllia* sp., and *Diploria* spp.) and have persisted over the last two decades with local epizootic events in many localities influencing the composition of many coral reef communities (Weil, 2004). A recent study (Yakob and Mumby, 2011) suggests that due to the increasing incidence of coral epizootics, Caribbean coral assemblages that were once dominated by large long-lived species (*Acropora cervicornis* and *Montastrea annularis*) are now comprised of small-bodied, fast growing species that brood and recruit frequently (*Porites astreoides* and *Agaricia agaricites*) (Green et al., 2008). The study's epizoological model proposes that the high population turnover rate enhances coral resistance to secondary epizootics by reducing the ability of any disease to spread within a population that consists of diminutive colonies under high flux. In this case, disease has reshaped the Caribbean coral community resulting in the emergence of novel coral assemblages that are more equipped to cope with hostile changes in the environment. In the Indo-Pacific, recent surveys of coral diseases in well-sampled regions such as the Great Barrier Reef and the Philippines indicate that disease frequency has also increased dramatically over the last five years (Raymundo et al., 2009; Bruno and Selig, 2007). In East Africa, Black Band, White Band, and Yellow Band diseases were reported in isolated outbreaks, as well as a newly described white syndrome outbreak that occurred off the coast in Kenya. This outbreak, associated with an infection of fungal hyphae,

almost eliminated *Montipora* from affected Kenyan reefs (Korrubel, 2000; McClanahan, 2004).

Several factors are thought to be responsible for the emergence of new diseases and the increase in prevalence and virulence of existing diseases. Current research suggests that since tropical reef-building corals are extremely susceptible to temperature stress due to their narrow range of thermal tolerance (between 18° and 30°C), climate variability, particularly anomalous high temperature (1-2°C above daily average), is a key factor (Harvell et al., 2002; Selig et al., 2006; Brown, 1997). In 2005, a warm thermal anomaly in the Caribbean brought about a widespread bleaching event and was immediately followed by outbreaks of white plague and yellow blotch (Harvell et al, 2007). In this case, opportunistic infectious pathogens whose virulence was enhanced by increased temperatures caused an increase in coral mortality during the warming event. This correlation between increased seawater temperature and infectious disease could be the result of decreased host immuno-defense, increased pathogen virulence, higher frequency of transmission via a vector, or a combination of all three (Harvell et al., 2007; Bruno, 2007; Rosenberg and Ben-Haim, 2002).

Anthropogenic environmental stresses can also influence the severity and dynamics of infectious diseases by undermining host resistance and increasing or decreasing pathogen virulence (Harvell, et al., 1999; Bruno et al., 2007). While the link between anthropogenic stress and disease susceptibility is currently poorly documented for marine diseases, one hypothesis is that coral disease is facilitated by a decrease in water quality, particularly due to eutrophication, excessive sedimentation and nutrient enrichment (Raymundo et al., 2008; Kim and Harvell, 2002). Formation of Growth

Anomalies in *Acropora* (AGA) for example, is strongly linked to high levels of sedimentation, turbidity, and seasonal temperature extremes (Peters et al., 1986)) while Growth Anomalies in *Porites* (PGA) is strongly associated with human population size (Aeby et al., 2011). Nutrient enrichment (increases in the concentration of inorganic nitrogen and phosphorous) can occur on reefs due to anthropogenic input from rivers, effluents, local upwelling and internal tidal bores (Shinn et al., 1994; Nixon 1995; Szmant 2002; Leichter et al., 2003). Two recent studies suggest that nutrient enrichment is linked to the prevalence of infectious disease in corals: Black Band Disease (BBD) has been detected from sites with high nitrite concentrations in the Florida Keys (Kuta and Richardson, 2002), and seafan aspergillosis is correlated with poor water quality conditions including elevated nitrogen concentrations and turbidity (Kim and Harvell, 2002). Furthermore, an experimental study by Bruno et al. (2003) demonstrated that a modest increase in nutrient concentrations significantly increased the severity of sea fan aspergillosis and Yellow Band Disease by increasing pathogen fitness and virulence. This indicates that while corals are generally able to grow in high-nutrient waters (Atkinson et al., 1995), the combination of an existing infection and elevated nutrients could increase the disease progression rates and impact of some syndromes.

1.3. PATHOGENS, VECTORS AND DISEASE RESERVOIRS

Despite several years of ongoing research, causative pathogens have been documented for few coral diseases due to the complexity of establishing disease causation (Table 1). The knowledge of coral disease pathology (isolation and identification of the pathogen), etiology (disease signs and relationships between the host and pathogen), and epizootiology (e.g., geographic distributions, environmental factors, host ranges, prevalence, vectors, reservoirs, and spatial and temporal variability) is at present, very limited (Harvell, 2007).

Disease reservoirs are alternate hosts or passive carriers of disease-causing organisms (Dorland, 2000). Biofilms in reef sediments, which contain nonpathogenic versions of the BBD consortium (Richardson, 1997) and colonies that survived BBD infections (Sato et al., 2009), for example, have recently been proposed as disease reservoirs. Vectors on the other hand, are involved in the processes of both disease transmission and spread on both local and regional scales. To date, the only coral disease vectors that have been positively identified are the fireworm, *Hermodice carunculata* in the Mediterranean, which has been found to harbor *Vibrio shiloi* in its gut (Sussman et al., 2003) and the damselfish, *Stegastes planifrons*, which harbors one-life history stage of a digenean (trematode) that infects *Porites compressa* (Aeby and Santavy, 2006). Predators and resource competitors of Acroporids and other reef-forming corals such as snails (*Coralliophyila abbreviata* and *C. caribbea*), corallivores (*Acanthaster planci* and *Drupella* sp.), nudibranchs (*Phestilla* sp.), green calcareous algae (*Halimeda opuntia*), parrotfishes (*Sparisoma viride*) and several species of butterflyfishes have also been shown or suggested to play a role in disease transmission, either by acting as vectors

and/or stressors (Raymundo et al., 2009; Aeby and Santavy, 2006; Dalton and Godwin, 2006; Williams and Miller, 2005; Weil, 2004; Nugues et al., 2004; Sussman et al., 2003; Aeby, 1998).

1.4. HOST RANGE

Some diseases, such as White Band II, are very host-specific and only infecting single coral species (*Acropora* spp.) despite widespread and virulent epizootics. While highly host-specific diseases have the ability to only infect certain coral species, they can still play an important role in the reduction of coral cover and decimation of coral reefs especially when key, reef-building genera such as *Acropora* are affected. Most reported coral diseases, however, have remarkable wide host ranges when compared to terrestrial pathogen-host interactions. White Plague II, for example, is the most virulent in the Caribbean with the widest host range of 39 species, followed by Black Band affecting 21 species (Weil, 2004). In the Indo-Pacific, Skeletal Eroding Band (SEB) has been detected in 12 families and at least 82 scleractinian species with Pocilloporids and Acroporids being the most susceptible to the disease (Page and Willis, 2008). A comprehensive assessment study in 2007 also identified skeletal growth anomalies (SGA) to be the most geographically and taxonomically widespread disease in the U.S. Pacific, observed on nearly 40% of the surveyed sites and affecting at least six different anthozoan genera (Vargas-Angel, 2009). White syndromes and other types of white diseases both in the Caribbean and in the Indo-Pacific have been reported to affect at least 40 scleractinian, hydrozoan, and gorgonian species (Sutherland et al., 2004; Willis

et al., 2004). Some of these diseases with wide host ranges are responsible for intensive epizootic events that contribute to significant mortalities in coral populations on local and regional scales. In 1995, White Band disease spread over 200 kilometers in the Florida Key within 11 weeks and eliminated *Acropora palmata* and *Acropora cervicornis* from the reefs leading to the listing of White Band-susceptible corals species on the endangered species list (Precht et al., 2004). Between 1999 and 2003, a 20-fold increase in White Syndrome abundance was recorded on the Great Barrier Reef at almost 50 cases per reef following the 2001 to 2002 mass-bleaching event (Willis et al., 2004). Other White Syndrome outbreaks have also been reported elsewhere in the Indo-Pacific, including the Northern Hawaiian Islands, Marshall Islands, and Palau (Aeby, 2005; Sussman et al., 2008). Field observations on diseases with wide host ranges show that infection can occur across colonies of the same coral species, colonies of different coral species, and even across higher taxa (i.e., crustose algae and corals) (Weil, 2004).

1.5. MITIGATION

As a developing science, the current research trend in coral disease is mostly directed towards traditional surveillance (Morens et al., 2004), with comparatively less research directed towards developing strategies for active engagement in coral reef health management, disease prevention and cure (Efrony et al., 2007). However, innovative microbial approaches to coral defense coupled with improved molecular diagnostics of pathogenic microorganisms and attempts to approach coral resistance with genomics tools, are steadily emerging areas in the study of coral disease (Harvell et al., 2007). For

example, new advances in enhancing coral immunity are emerging through designing microbial defense systems such as phage therapy. Phage therapy inhibits the spread of a disease by isolating phage viruses, found in nature, that consume pathogenic bacteria (Efrony et al., 2007). Furthermore, phages have limited host ranges and are therefore unable to harm animal cells and mutualistic bacteria making them well-suited for the targeted control of pathogens (Matsuzaki et al., 2005; Jensen et al., 2006). An experimental study by Efrony et al. (2009) reported that a pathogen-specific phage (BA3) multiplies and lyses the γ-proteobacterium *Thalassomonas loyana*, the coral pathogen responsible for the white plague-like disease affecting *Favia favus*. However, it was also reported that the bacteriophage could no longer prevent infection two days after treatment, which suggests that phage therapy may be more valuable in preventing the spread of disease rather than cure an already infected coral. Another potential form of biological control is probiotics. Coral mutualistic bacteria (probiotics) have the potential to exclude pathogens from host surfaces via the coral mucus (surface mucopolysaccharide) layer (Reshef et al., 2006; Shnit-Orland and Kusmaro, 2009; Ritchie, 2006). Two studies (Shnit-Orland and Kushmaro, 2009; Ritchie, 2006) suggest that culturable microbes found on the mucus layer of stony and soft corals produce antimicrobial substances that can inhibit growth of potentially invasive microbes. This suggests that coral mucus plays a role in the structuring of beneficial coral-associated microbial communities and that this probiotic-related function could be used as indicators of a recovering reef ecosystem (Teplitski and Ritchie, 2009). Although these forms of biological tools are feasible and reasonably effective in mitigating coral diseases, much of the research is still in the early stages and transferring such a technology to a reef

system will have serious logistical and ethical issues and to date, this has not been attempted.

While some marine diseases may be caused by pathogens moving from terrestrial to marine systems, marine systems are qualitatively different from terrestrial environments in significant ways that can affect disease processes (McCallum, 2004). Marine host organisms have diverse phyla with equally diverse body plans and life histories that probably have different disease transmission modes than their terrestrial counterparts (de Meeus and Renaud, 2002; Bush et al., 2001). Furthermore, marine populations are typically more open than terrestrial ones with the potential for long-distance dispersal of larvae and pathogen, which in turn facilitates the rapid propagation of epidemics in marine systems (Kinlan and Gaines, 2003). Hence, traditional methods used in terrestrial disease management such as culling and vaccination are not practical in ocean systems and have currently limited applications in coral disease management (Weil, 2004; Harvell et al., 2007). More applicable management options for coral diseases are currently being developed. For example, there is evidence to suggest that corals may recover faster and prevent secondary opportunistic infections following a bleaching episode if anthropogenic stress is reduced (Carilli et al., 2009). In such cases, imposing quarantine on a reef acutely impacted by either bleaching or disease may be a viable option (Raymundo et al., 2008). In the case of White plague, Black band, Yellow band and White band diseases, the application of clay or underwater epoxy putty directly over the band can reduce bacterial progression (Bruckner and Bruckner, 1998d; Miller et al., 2003). New infections can also be reduced through the re-introduction of herbivorous urchins *(Diadema antillarum)* into habitats reducing the potential for algal competition

with corals and the likelihood of injuries that might facilitate invasion by pathogens (Bruckner, 1999; Nugues et al., 2004). The removal of corallivore species that undergo "boom and bust" population cycles such as *Acanthaster planci* and *Drupella* spp. could also potentially control the spread of disease and prevent the introduction of entry wounds (Antonius and Riegl, 1997). However, a full understanding of these species' role in the disease process is needed and caution must be exercised, as there may be other impacts to the ecosystem from such practices.

A potentially viable tool in coral diseases management is the establishment of Marine Protected Areas (MPA). The ability of reefs to recover from bleaching events, coral disease outbreaks and other acute disturbances is profoundly affected by the level of chronic anthropogenic disturbance. Through the establishment of well-managed MPAs, where there is increased fish stock and fish diversity, reduced biological stress and anthropogenic impacts, corals may recover quickly and may be more resilient to regional-scale waterborne pathogens (Page et al., 2009; Raymundo et al., 2009; Mumby and Steneck, 2008). The results of an extensive monitoring program in the Great Barrier Reef, for example, suggest that MPAs protect coral reefs from outbreaks of *Acanthaster planci,* and by extension, ease biological stress on the coral population (Sweatman, 2008). Another study by Page et al. (2009) shows that although disease prevalence between MPAs and non-protected reefs in Palau was not significantly different, their study did demonstrate the potential for increased fish diversity to reduce the occurrence of coral diseases in protected areas. This was further supported by the results of a study in the Philippines that show that disease prevalence was significantly negatively correlated with fish taxonomic diversity and positively correlated with corallivorous

butterflyfish abundance and that there was less coral disease observed in MPAs with high fish diversity (Raymundo et al., 2009).

At large scales, remote pristine areas may have greater capacity to absorb climate impacts and maintain a coral dominated and diverse ecosystem (Sandin et al., 2008). However most MPAs are small and embedded in heavily fished and degraded environments (Bellwood et al., 2004; McClanahan et al., 2007). Hence, there is a need to further determine whether MPAs do in fact have the ability to enhance resilience and recovery of coral reefs across regional spatial scales and following large-scale disease outbreaks. At this point, while the effect of MPAs in disease ecology have been demonstrated, at present, the mechanisms behind the observed patterns and the final link to enhanced recovery of an entire coral population has yet to be fully investigated.

1.6. THE EFFECT OF MORPHOLOGY ON HOST SUSCEPTIBILITY

Several studies suggest that corals have varying susceptibilities to damage or injury due to their different morphological attributes (Woodley et al., 1981; Hughes, 1989; Glynn, 1990; Chadwick-Furman, 1995; Diaz and Madin, 2011). A coral's ability to recover from tissue or skeletal damage depends on its priority to invest its resources either to growth or to maintenance and defense (Bak et al., 1977; Bak and Steward-Van Es, 1980; Meesters and Bak, 1993; Meesters et al., 1993). A poor regenerative ability could then potentially lead to reduced colony fitness since regeneration diverts resources away from growth and reproduction, survival, feeding, and photosynthetic capacity (Bak et al., 1977; Rinkevich and Loya, 1979). A ranking in regenerative ability proposed by

Jackson (1979) suggests that corals with increasing morphological complexity such as arborescent or branching forms have a more integrated response to resource allocation and invest more energy to growth, while simpler forms such as massive and encrusting corals that are more committed to its place of settlement invest its resources into somatic maintenance and repair to survive in their habitat. In agreement with Jackson's hypothesis, Diaz and Madin (2011) established that there is a general increase in coral disease potential in branching and corymbose forms than robust forms due to less allocated resource to defense mechanisms, and additionally because of the high proximity between branches or colonies that could potentially act as a mechanism for pathogen transmission (Jackson 1979; Soong and Lang, 1992). In contrast, Hall (1997) demonstrated that arborescent corals are able to regenerate a greater proportion of their injuries than massive corals, in part, because massive corals have a generally denser tissue and skeletal material that may incur a large drain on resources to regenerate. This suggests that although branching corals have a greater disease risk than massive corals, its high regenerative potential allows it to overcome total colony mortality. Massive corals, on the other hand, invest more energy in the implementation of a stronger defense and regeneration systems such as the production of melanin, phenoloxidase, and special proteins (Jackson 1979; Palmer 2008). Massive corals also tend to dominate in habitats with high wave action, which decreases the potential for disease through the constant flushing of accumulated materials that potentially carry pathogens (Diaz and Madin, 2011).

Previous studies also suggest that the characteristics of the injury or the wound can also influence the recovery/regeneration process of experimentally injured corals. Recovery of injuries was found to be size-specific with regeneration being higher for small (1 cm^2) injuries compared to large (5 cm^2) injuries since smaller injuries require fewer resources than larger injuries (Bak et al., 1977; Bak and Steward-Van Es, 1980). For example, Oren et al. (1998) demonstrated that significantly more carbon products were transported from healthy to damaged areas when corals (*Favia favus, Platygyra lamellina* and *Porites* spp.) developed large wounds. A positive relationship between wound perimeter and lesion regeneration capacity was also demonstrated in several regenerative studies in scleractinians (Meesters et al., 1996, Van Woesik, 1998; Lirman, 2000). A lesion with a relatively long perimeter was found to have a greater regeneration potential since it is associated with more healthy tissue bordering the wound that obtain a higher energetic allocation from the colony (Oren et al., 1997). Short-term regeneration was largely influenced by wound perimeter, after which time, regeneration was determined more by the surface area of the lesion and its surface area/perimeter ratio (Oren et al., 1997).

1.7. WHITE SYNDROMES

Prior to 2000, there were relatively few comprehensive detailed studies of coral disease in the Pacific. As efforts increase to document coral diseases from more locations within the Pacific, the lists of species affected by disease, locations where diseases are reported, and prevalence of those diseases, are also steadily increasing (Raymundo et al., 2008). Furthermore, the profusion of names describing these "new diseases" which may represent conditions caused by pathogens, predators, environmental perturbations, pose as a challenge in identifying and differentiating diseases (Goreau et al., 1998; Bruckner and Bruckner; 1998, Richardson, 1998). For instance, the term White Band disease affecting Caribbean Acroporids has been used to describe similar signs in massive and plating corals that are reported elsewhere as White Plague (Gladfelter et al., 1977; Dustan, 1977). In the case of White Syndromes (WS), prior to comprehensive disease characterization, the term is used transitorily to describe different diseases that share similar gross signs of coral abnormalities in the Caribbean and the Indo-Pacific (Willis et al., 2004; Blythell et al., 2004). The proliferation of names for different syndromes has also produced confusion when trying to evaluate host ranges and geographical distribution for diseases. In most instances the description of a disease/syndrome is based on limited etiological and ecological observation, often lacking pathological information and missing the temporal variability in signs and interaction between the host and pathogen (Work and Aeby, 2006).

Regardless of their limited etiological and pathological information, White Syndromes (WS) are among the most prevalent coral diseases responsible for the reef demise in the Caribbean and on the Great Barrier Reef (Weil, 2004; Ainsworth et al.,

2007). Recently, White Syndrome has been reported from numerous locations throughout the Indo-Pacific, constituting a growing threat to the coral reef ecosystem (Myers and Raymundo, 2009; Sussman et al., 2008; Ainsworth et al., 2007). Several years of rapid environmental assessments and coral disease monitoring (Myers and Raymundo, 2009) reveal that WS is also the most prevalent diseases in Guam found in 14 of the 15 surveyed sites, and affecting 8 genera from 5 families including several dominant, reef-forming coral species (*Porites* and *Acropora* spp.)

Generally, WS manifests as a clear differentiation between healthy tissue and recently denuded skeleton and is characterized by rapid rates of tissue loss and high levels of colony mortality (Roff et al., 2006; Ainsworth et al., 2007). This broad characteristic, however, varies between each species in the Indo-Pacific and the Caribbean. In the Caribbean, there are at least seven different types of white diseases that have been described, all of which are commonly characterized in the field by distinct and rapid sloughing of the coenosarc, exposing the underlying white skeleton (Blythell et al., 2004). These white diseases include White Band I (Gladfelter, 1982; Peters et al., 1983), White Band II (Ritchie and Smith, 1998, Gil-Agudelo et al., 2006), White Plague I (Dustan, 1977), White Plague II (Richardson et al., 1998b; Richardson et al., 1998c), White Plague III (Richardson and Aronson, 2002) and additional white plague "like" diseases from the Caribbean (Rodriguez-Martinez et al., 2001, Pantos et al., 2003).

In the Indo-Pacific, White Syndrome is a collective term to describe conditions resulting in white bands of recently-exposed skeleton on corals distinguished from feeding scars by the narrow width of the zone of recently exposed white skeleton and the relatively irregular appearance of the tissue front (Willis et al., 2004; Raymundo et al.,

2008; Myers and Raymundo, 2009). White Syndrome affecting tabular *Acropora* spp. in the Great Barrier Reef is relatively well-characterized (Roff et al., 2006; Work and Aeby, 2006; Ainsworth et al., 2007a). In these infected Acroporids, apparently healthy tissue borders a clear line of exposed skeleton, and tissue loss from the coral colony is acute, reaching rates of > 400 cm^2 wk^{-1} (Roff et al., 2006). Coral pathogens from WS epizootics in the Indo-Pacific were also investigated. Based on near complete 16S rRNA gene sequence comparisons, six coral pathogens were members of the γ-Proteobacteria family Vibrionaceae, and identified to share between 98-99% sequence identities with the previously characterized coral-bleaching pathogen *Vibrio coralliilyticus* (Sussman et al., 2008).

Like the previously described Indo-Pacific and Caribbean white diseases, White Syndrome observed in Guam could comprise a group of distinct diseases with similar signs that are either caused by one causative agent, or several. It is presumably infectious and its characteristics are similar to that of white syndromes observed in the Great Barrier Reef and to the Caribbean white diseases such as white band and white plague. The disease also manifests differently between species. In *Acropora* spp. for example, the lesion begins at the base and circumscribes the branch, while in branching *Porites* spp., the lesion is discrete and appears on any area of the colony, and multiple lesions may later on coalesce. At present, the disease is inadequately characterized and while WS is among the growing threats affecting several dominant, reef-forming species in Guam, the ecological impacts to its coral hosts are unknown.

1.8. OBJECTIVES AND HYPOTHESES

The etiology and mechanisms of coral death of Indo-Pacific White Syndrome observed in Guam is unknown. A series of long-term monitoring indicate that White Syndrome is the most prevalent disease in Guam observed in 9 out of 10 sites with high prevalence rates in several dominant, key reef-building corals such as Poritids, Pocilloporids, and Acroporids (Burdick et al., 2008; Myers and Raymundo, 2009). Hence, the purpose of this study is to investigate aspects of the histology, etiology, epizootiology, and pathology of this poorly-described, but highly prevalent disease. In addition, the results of this study will further elucidate the Indo-Pacific White Syndrome observed in Guam from previously described white diseases in the Great Barrier Reef and the Caribbean in order to facilitate the development of diagnostic tools that could be implemented in a coral reef health management plan.

The aims of the study, therefore, were to: i) describe the gross and histological characteristics of the disease in *Porites* spp.; ii) determine WS disease transmissibility and modes of transmission; iii) if transmissible, attempt to determine the causative agent; and iv) determine the effect of colony morphology on disease progression.

Table 1. Diseases for which pathogens have been established via Koch's Postulates

Disease	Host	Pathogen	References
White Band Type II	*Acropora cervicornis*	*Vibrio carchariae*	Ritchie and Smith,1998
Bacterial Bleaching (BBL)	*Oculina patagonica*	*Vibrio coralliilyticus* *Vibrio shiloi*	Kushmaro et al., 1997
Black Band Disease (BBD)	Scleractinian corals (42 species)	*Phormidium corallyticum* (microbial consortium)	Richardson et al., 1998 Richardson & Kuta 2003
Sea Fan Aspergillosis	*Gorgonia ventalina* *Gorgonia flabellum*	*Aspergillus sydowii*	Smith et al., 1998 Nagelkerken et al., 1997 Geiser et al., 1998
Tissue Lysis	*Pocillopora damicornis*	*Vibrio coralliilyticus*	Ben-Haim & Rosenberg, 2002
Serratiosis	*Acropora palmata*	*Serratia marcescens*	Patterson et al., 2002 Sutherland et al., 2011
White Plague Type II	Scleractinian corals (17 Caribbean species)	*Aurantimonas coralicida*	Richardson et al., 1998
White Syndrome (Palau, GBR, Marshall Is.)	*Pachyseris speciosa* *Montipora aequituberculata* *Acropora cytherea*	*Vibrio coralliilyticus*	Sussman et al., 2008

Chapter 2. Methods

2.1. INTRODUCTION AND TERMINOLOGY

Many existing coral disease and syndromes have been described on the basis of macroscopic features (Green and Bruckner, 2000; Sutherland et al., 2004; Weil et al., 2006). While these characteristics allow a broad characterization of the disease and one that facilitates monitoring and assessment in the field, most are ambiguous or open to subjective interpretation, making geographic comparisons problematic (Richardson, 1998; Work and Aeby, 2006). Therefore, in order to understand disease processes and causation in corals, it is necessary to understand disease terminologies, apply generally accepted nomenclature, and systematically identify and describe coral diseases from a standardized biomedical perspective (Peters, 1984; Work and Aeby, 2006). The following definitions and terms have been applied to animal disease research and provide an important framework for disease characterization. *Disease* is any impairment (interruption, cessation, proliferation, or other disorder) of vital body function, systems, or organs (Stedman, 2000). *Infectious biotic diseases* caused by microbial agents such as bacteria, fungi, viruses or protists spread between host organisms and negatively impact the host's health, while *non-infectious biotic disease* are not transmissible between organisms, though they may be caused by a microbial agent (Raymundo et al., 2008). *Abiotic diseases* on the other hand, are caused by environmental agents such as temperature stress, sedimentation, toxic chemicals, nutrient imbalance and UV radiation, and do not involve a microbial agent but impair a host's health nonetheless (Raymundo et

al., 2008). The term *syndrome* is a group of signs or symptoms that together comprise disease (Dorland, 2000; Stedman, 2000). A *sign* is any disease-related abnormality discoverable by objective examination of the organism. An example of a disease sign is the presence of a *lesion*, which is a functional and morphologic change in tissue when affected by a disease (Thompson, 1978). *Etiology* is the investigation of causes, development, and effects of a disease wherein causation may be attributed to pathogens, environmental stressors, or a combination of biotic and abiotic factors (Kinne, 1980). *Epizootiology* is the study of the occurrence, distribution, and control of a disease in an animal population and is synonymous with epidemiology in human populations (Stedman, 2000). *Incidence* measures the number of new cases of disease over a defined time period and is a useful indicator of whether or not disease is spreading (Raymundo et al., 2008)

A critical first step in understanding a disease is to provide a gross morphologic description of the disease sign in the field (Ainsworth et al., 2007). A typical sign of a diseased coral, which may not provide any clue regarding causation, is the presence of a lesion (Work and Aeby, 2006). Some lesions in corals may have known causes such as predation, abrasion, or competition and are not attributable to disease but may compromise a coral's health nonetheless. Given the potential for environmental stressors, between species differences, and diversity of coral morphologies to influence the progression of a disease, lesions may take on gross morphologies that differ between species or that vary temporally or spatially. In suspected disease cases such as White Syndromes, it is often impossible to determine the cause of the lesion (and disease) without additional laboratory or experimental efforts (Raymundo et al., 2008). Hence, a

description of the lesion at tissue and cellular levels (histopathology) is a necessary step following a gross morphological description. Histological analyses can characterize the morphology of the tissue and describe cellular changes associated with disease at the microscopic level (Galloway et al., 2006). Additionally, disease monitoring when targeted to address specific questions, can provide data on the status of a particular disease or coral species, seasonality, incidence and effects of diseases at a local scale, and the role of localized stressors on disease processes and impacts. Disease monitoring on the colony level can document patterns of spread, rates of tissue destruction, impact of diseases at a colony level or population level, and the fate of affected colonies (Raymundo et al., 2008). By providing these types of epizootiological observations following a systematic gross and microscopic morphologic description, an accurate and standardized comparison of the disease is possible within and among geographic regions

Determining a specific cause of a disease is considered a major goal in all disease characterization efforts. However, it is a challenging and lengthy process. To determine whether there is evidence of an infectious agent, a transmission experiment must first be conducted. If an infectious agent is suspected, an attempt to isolate and identify the pathogen can be done through a step-by-step process to prove what is known as Koch's Postulates (Raymundo et al, 2008). In proving Koch's Postulates, (1) diseased and healthy tissue samples are collected along with descriptions of the gross and microscopic morphology of the lesion, which in some cases, may reveal the presence of a potential causative agent, (2) various laboratory tools are then used to culture, isolate and identify suspected causative agents, (3) the cultured putative pathogens should cause disease signs (lesion) when introduced into a healthy host tissue under controlled experimental

conditions, (4) if the microorganism is successfully re-isolated from the diseased lesion, it can then be classified as the causal agent for the disease (Koch, 1891).

These procedures fundamental to the characterization of White Syndrome disease observed in Guam are described in detail in the following sections.

2.2. STUDY SITE

Guam, the southernmost island of the Mariana Archipelago, is the largest and most economically developed island in Micronesia. It is the regional hub for shipping and travel and has long been the center of regional activities (Kirch, 2000). Guam reefs are currently exposed to a multitude of anthropogenic effects while simultaneously serving as an economic resource via tourism (Myers and Raymundo, 2009).

Disease surveys were conducted on the reef flats of Luminao, southern Guam between October 2009 and June 2010 (Figure 1). Luminao Reef (N 13° 27' 52" E 144° 38' 38") is a submarine reef extending west from the Guam's seaward end behind Glass Breakwater, a one-mile breakwater built in 1941 using limestone blocks quarried on Cabras Is. (Rottman, 2002). The shallow (1-2 m) reef flat is characterized by scattered coral heads/patches interspersed with sand near the breakwater and with more consolidation seaward, towards the reef crest. Reef-building corals such as *Porites cylindrica, Porites rus, Porites convexa,* massive *Porites* spp., and *Acropora aspera* thickets dominate the reef (Raymundo, unpubl. data). Initial results from long-term monitoring have shown that disease prevalence at Luminao Reef is high (>30%) with a mean prevalence of 6% (Burdick et al., 2008; Myers and Raymundo, 2009). The data

also show that the highest disease prevalence occurred during the period of warmest temperature suggesting a correlation between temperature and disease (Burdick et al., 2008).

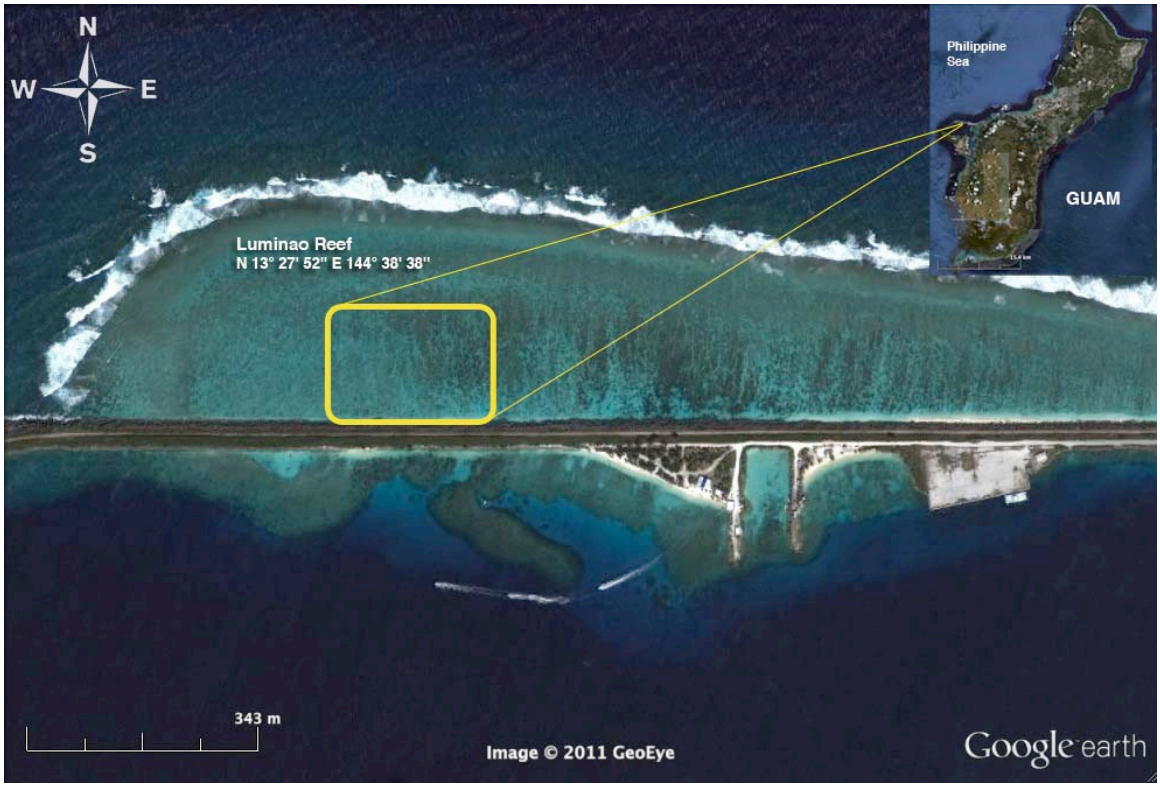


Figure 1. Approximate location of study site in Luminao Reef, Glass Breakwater, Piti, southern Guam

2.3. DISEASE CHARACTERIZATION

2.3.1. Gross Description of White Syndrome on *Porites* spp.

The first step to describing a coral disease is to formulate a good morphologic description of the lesion in order to provide the best tangible objective data regarding the disease (Raymundo et al., 2008). Ten colonies of *Porites cylindrica* and 10 colonies of massive *Porites* spp. were monitored in Luminao Reef from October 2009 to June 2010. A series of systematic observations and terminologies were then used based on the framework recommended by Work and Aeby (2006) (Table 2). Information on the distribution and location of lesions on each colony was noted along with descriptions of the lesion edge, margin, shape, relief, texture, color, and structures affected. A general size description as well as the average measurement and number of lesions in each colony per growth form were also provided. The descriptions of the lesions focused on their physical characteristics to avoid subjective interpretation.

Following a gross description of the lesions, a gross morphological diagnosis was formulated based on six components: extent, time, distribution, lesion, location, and structures affected. Extent of the lesion is an estimate of coral surface area occupied by lesion. Terms to describe time refer to the rapidity of the onset of the lesion. Lesions in coral can be categorized as tissue loss, growth anomaly, or discoloration (Work and Rameyer, 2005). Structures affected include polyps, coenosarc or skeleton (Work and Aeby, 2006).

Table 2. Terminologies used to describe lesions (Work and Aeby, 2006)

Category	***Term***
Distribution (Di)	Focal, multifocal, multifocal to coalescing, diffuse
Location (Lo)	Basal, medial, apical, peripheral, central, colony-wide
Edges (Ed)	Distinct, indistinct, annular
Margins (Ma)	Serrated, undulating, smooth, serpiginous
Shapes (Sh)	Circular, oblong, pyriform, cruciform, linear, lanceolate, irregular
Relief (Re)	Umbonate, bosselated, nodular, exophytic
Size (Si)	Small, medium, large, actual measurement
Number (Nu)	Small, medium, large, actual count
Color (Co)	White, black, tan, brown, red, green, orange, pink, purple, blue, yellow
Texture (Te)	Rugose, smooth
Extent (Ex)	Mild (1–20%), moderate (21–50%), severe (51–100%)
Time (Ti)	Acute, subacute, chronic
Lesion (Le)	Tissue loss, discoloration, growth anomaly
Structures affected (Su)	Polyp, coenosarc, skeleton

2.3.2. Histological Description of White Syndrome on *Porites cylindrica*

To investigate microbial populations associated with the lesions and the histological features of White Syndrome, and in addition, look for evidence suggesting causation, tissue samples of the branching *Porites cylindrica* were collected from Luminao Reef, Guam where White Syndrome is observed to be widespread. Prior to collection, corals were visually surveyed for signs of disease. Corals were assessed for typical macroscopic signs of White Syndrome including tissue loss and apparent rapid exposure of coral skeleton. All colonies exhibiting signs of disease were photographed prior to collection. Light microscopy usually requires 2 cm^2 of apparently healthy tissue taken several centimeters from the diseased tissue and another sample that includes the disease margin (i.e. bare skeleton intact, diseased tissue) (Raymundo et al., 2008). Replicates (n=5) of approximately 2.5 cm in length of (1) clinically healthy fragments from remote colonies (RH), (2) healthy (H) fragments from colonies displaying signs of WS, and (3) fragments with active lesions (D) from WS-infected colonies were collected at depths of 3-5 m using wire cutters. Each fragment was then placed in a pre-labeled 4-oz Whirl-Pak® filled with buffered zinc-formaldehyde fixative (Z-Fix, Anatech). Coral fragments were then soaked in the fixative for 24 h.

Prior to decalcification, fragments were repeatedly rinsed in slow-running tap water for a minimum of 30 min and then soaked in tap water for 24 h to remove fixative residues. The fragments were then placed in a glass culture dish and immersed in 5% HCl was changed periodically over time for 5 to 8 h until the entire tissue was released from the skeleton. The decalcified samples were washed several times with distilled water to remove salts and were dehydrated in 70% EtOH. Each sample was then placed

in macrocassettes packed with 70% EtOH for tissue and slide processing at the Animal Health Diagnostic Center at Cornell University. Instructions were given to cut tissue as 4-5 μm cross-sections showing both normal and abnormal borders in the diseased (D) tissues. Tissue samples were stained in Mayer's Hematoxylin & Eosin (MHE) and mounted onto slides for histological examination. (Szmant and Gassman, 1990; Work, pers. comm., 2010; Angel-Vargas, pers. comm., 2010; Couch, pers. comm., 2010).

Through the supervision of Dr. Thierry Work at the USGS NWHC Honolulu Field Station, samples were viewed with a light microscope for general tissue organization and cellular damage. Microscopic changes in the coenosarc, polyp, and in areas of denuded tissue associated with the disease were described. The presence of potential etiologic agents such as bacterium, fungus, and parasite virus were also noted. A glossary of commonly used histological terms, as compiled in the Coral Disease and Health Workshop: Coral Histopathology II report is presented in Appendix 9.

2.4. DISEASE ECOLOGY

2.4.1. Disease Infectiousness

2.4.1.1. Test for Transmissibility of Disease

The establishment of a disease in a host population would not only require the ability to invade a host but also a mechanism to transfer between hosts (Aeby and Santavy, 2006). Transmission of disease can occur either by direct contact, through the water column, or by vectors (Ewald 1987, 1994). Thus, this experiment tested the transmissibility of WS and whether WS is a waterborne disease, transmitted via direct contact or both. This experimental design is a modification of the transmission experiments of Raymundo *et al.* (2003), Kaczmarsky and Richardson (2007), and Williams and Miller (2005).

Fragments of *Porites cylindrica* (yellow morph) were collected from Luminao Reef, Guam. Sixteen fragments (7-10 cm in length) displaying White Syndrome disease signs and 80 clinically healthy fragments (7-10 cm length) were collected from depths between 2-4 m. Two diseased fragments were collected from eight donor colonies and 10 healthy fragments from eight donor colonies to ensure non-clonality of grouped fragments. Using wire cutters, fragments were collected by clipping a single or bifurcated branch at the base. Each fragment was placed in a pre-labeled 4-oz Whirl-Pak® filled with fresh seawater and then brought to the UOGML wet lab facility.

Two concrete tanks 2.5-meter long, 1-meter wide, and 1.25-meter deep with an open seawater system served both as water baths and holding tanks. Sixteen well-aerated plastic aquaria (9.46 l) filled with fresh seawater were randomly arranged between the

two holding tanks with eight aquaria per tank. Each fragment was fixed upright with non-toxic modeling clay into PVC cups. Eight aquaria served as the experimental set-up with each aquarium containing four clinically healthy, non-clonal fragments and two diseased, non-clonal fragments (n=16) (Figure 2). The experimental design is summarized in Table 2. To increase the probability of a result, two sets of each treatment were placed in each aquarium: (1) two healthy fragments in each aquarium were placed in direct contact with a single lesion on each of the two diseased fragments; (2) the two remaining healthy fragments were positioned at opposite ends of the aquarium (Table 3 and Figure 2). This allowed assessments of whether direct contact or waterborne contact were required for lesions to appear. Although pseudoreplication was not intended, the study design required two sets of each treatment (direct contact and freestanding) per aquarium as a standard procedure to increase the probability of seeing a result in transmission experiments. To offset pseudoreplication, only one set per treatment was statistically tested to compare microbial concentrations among samples of bacterial sources. As a procedural control, a total of six non-clonal fragments were positioned similar to the experimental set-up in the remaining eight aquaria and monitored for signs of tissue loss (n=16) (Table 3). Two temperature loggers (HOBO®) were placed in two randomly selected aquaria to monitor daily temperature changes. Seawater was replaced and fragments were photographed and checked every 3 d for appearance, location of lesions and overall health, and monitored for 39 d (6 wk). After each water change, healthy fragments were placed in contact in the same position as previous to maximize exposure to the lesion. Lesion size was digitally measured from macrophotographs using Image J™ (v.144) to estimate lesion progression rates.

At the onset of lesions, mucus samples were extracted from the margin between the progressing front and the healthy tissue from each infected fragment in the transmission experiment. To allow a comparison of the bacterial concentrations between diseased and healthy individuals both experimental and *in situ*, mucus samples were also collected *in situ* from *P. cylindrica* colonies at Luminao Reef. Using a sterile syringe, mucus samples were extracted from the margin of the exposed skeleton of lesions in three colonies showing signs of the disease (D). A control mucus sample was also extracted from a healthy region (H) on each of the diseased colonies. In addition, mucus was sampled from three remote colonies (RH) with no disease signs. Samples were mixed to a 1:1 ratio with filtered, sterile seawater and then vortexed. Samples were then serially diluted to 10^{-4} and 100 µl of each dilution was spread-plated onto marine agar (MA) and thiosulfate-citrate-bile-salt (TCBS), which is a *Vibrio*-selective media. Since most suspected putative pathogens in coral disease are caused by *Vibrios*, TCBS permitted the selective isolation of these pathogens by growing them in pure culture. Two replicates of each dilution were prepared and plates were incubated at 30˚C for 24 to 48 hours. Cultivable strains were then quantified as CFU ml^{-1} (colony-forming units) on both media types.

Microbial densities in infected fragments were hypothesized to be similar to densities in diseased colonies in the field. On the other hand, microbial densities in healthy colonies were expected to be significantly less than those in experimentally infected fragments and in diseased colonies in field. To test these hypotheses, microbial concentrations cultured on marine agar (MA) and TCBS of the following source types were compared: 1) experimentally infected fragments (E); 2) diseased colonies the field

(D); and 2) apparently healthy colonies in the field (RH, H). A 2-sample T-test (JMP 9.0) was performed on the microbial concentration grown on MA of each paired source type since the datasets were log-normally distributed (Shapiro-Wilk W Test) and the variances were equal (Levene's Test) after log transformations (ln Y) and after removing two outliers whose CFU counts were higher than the rest by 2-3 orders of magnitude. (Appendix 1). Microbial concentrations cultured on a *Vibrio*-selective media (TCBS) were also compared using a 2-sample T-test (JMP 9.0) since the datasets were homogenous and log-normal (Appendix 1).

Table 3. Experimental design of laboratory transmission experiment

Treatment	***Contact between fragments (fragment aquarium $^{-1}$)***	
Experimental aquaria Containing diseased fragments (n=16)	Direct physical (n=2)	Waterborne (n=2)
Control aquaria Containing healthy fragments (n=16)	Direct physical (n=2)	Waterborne (n=2)

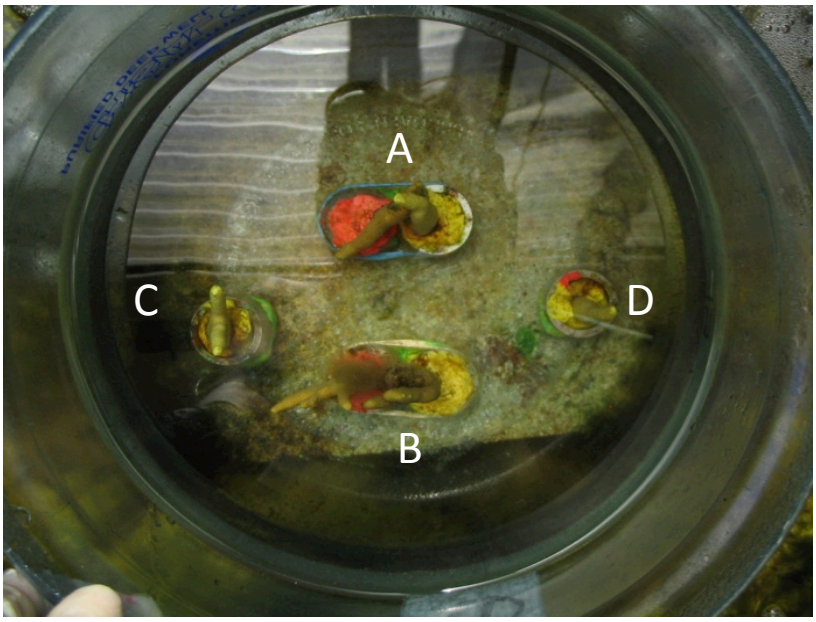


Figure 2. Placement of fragments to test modes of transmission. (A,B) healthy-diseased pairs to test direct contact transmission, (C,D) freestanding fragments to test waterborne transmission.

2.4.1.2. Isolation and Identification of Putative Pathogen

Two inoculation methods, each with their own advantages and disadvantages, were tested on *P. cylindrica*: a) Inoculation via liquid inoculum mixed with seawater in the aquarium allowed an exact computation of microbial cell density per inoculum (CFU ml^{-1}). This method however, assumes that White Syndrome is waterborne and may therefore, be unsuccessful in transmitting the disease or produce a false negative result; b) Inoculation by directly applying the inoculum via a solid media (sterile gauze strip) onto healthy tissue, on the other hand, only allows an approximation of the inoculum concentration and it is unknown how this concentration varies from that in nature.

a. Inoculation via Liquid Inoculum

Using aseptic techniques, 36 clinically healthy *Porites cylindrica* branches (7-10 cm in length) were collected from four non-clonal colonies (9 fragments per colony) in Luminao Reef. Using wire cutters, each branch/fragment was collected by clipping a single or bifurcated branch at the base. Each fragment was then placed in a pre-labeled Whirl-Pak® filled with ambient seawater and then brought to the UOGML wet lab facility.

A fiberglass tank 2.5-meter long, 1-meter wide, and 1-meter deep with an open seawater system served both as a water bath and a holding tank to 36 randomly-arranged, aerated 2000 ml plastic aquaria with a closed seawater system (Figure 3). Two temperature loggers (HOBO®) were placed in the holding tank to monitor daily temperature changes. Each fragment was then fixed upright with modeling clay into PVC cups and individually placed inside each aquarium. Fragments were allowed to

acclimate for 7 d and fed daily with live, 1-day old *Artemia* nauplii. During inoculation feeding was discontinued to discourage fouling of the aquaria. Seawater was replaced and the fragments were photographed and monitored every 3 d for size, appearance and location of lesions, and overall health for a period of 42 d (6 wk).

Mucus samples of the infected fragments from the previous transmission experiment were previously plated on a *Vibrio*-selective agar (TCBS), from which five experimental bacterial isolates were obtained. Aside from the five bacterial isolates, treatments included, a healthy control (untreated/no inoculum), a procedural control (1:10 marine broth/aseptic inoculum), a non-pathogenic strain (*Pseudomonas* sp.), and a pathogenic strain (*Vibrio coralliilyticus*) previously demonstrated to cause WS disease signs in *Montipora* spp. (Sussman, 2008) (Table 4).

To establish a growth curve for each strain, single isolates were grown in 10 ml Marine Broth (MB) diluted to 1:10 (7.5 g agar, 1.87 g MB 2216, 45 ml RO water, 450 ml sterile seawater), and incubated at 27°C for 24 h. All cultures were serially diluted by 1/3 out to 1/27. The absorbance of the original culture and each dilution were measured via spectrophotometer (550 nm). Ten-fold dilutions could not be used since the absorbance decreased too rapidly to obtain enough data points for the standard curve.

Each strain was streaked onto MB 1:10 agar plates. After 24 h, single colonies were inoculated into 100 ml of liquid MB 1:10 and incubated at 27°C for approximately 20 h to end the microbial log-phase growth. Concentrations of pure cultures were determined by comparing the isolates' absorbency measurements to its standard growth curve. After 20 h, all strains were approximately 10^8 CFU ml^{-1}. Cultures were then

centrifuged (2000 rpm for 10 min), the supernatant decanted, and the pellet re-suspended in 10 ml sterile seawater, with a concentration of approximately 10^9 CFU ml^{-1}. It was determined that approximately 2 ml of inoculum was needed, per strain, to achieve a final concentration of 10^6 CFU ml^{-1} in each 2000 ml aquaria.

Corals were injured by scraping the tissue surface with sterile forceps, introducing an approximately 1 cm x 1 cm point of entry for the bacteria. Each inoculum was aseptically added in the aquarium using a micropipette (Figure 4). Mucous from a healthy coral can inhibit the growth of bacteria (Geffen and Rosenberg, 2005; Ritchie, 2006), hence the pure cultures/inocula were only added into each aquaria after 45 min to allow anti-microbial compounds from the corals' surface mucus layer (SML) to dissipate. Of the 36 fragments, 16 were randomly selected as controls. The remaining pure cultures were serially diluted in sterile seawater to 10^{-9} and spread-plated in triplicate onto MA agar plates. The inoculated concentration for each strain was then determined based on the number of CFUs on the plates.

Using a sterile syringe, mucus was sampled from the progressing front of infected fragments. To allow a comparison between bacterial densities in the infected fragments and those in the field, mucus samples were also collected *in situ* from healthy *P.cylindrica* colonies and from colonies showing WS disease signs in Luminao Reef (n=3). Samples were then mixed to 1:1 ratio with filtered, sterile seawater and then vortexed. Samples were serially diluted to 10^{-4}. One hundred microliters of each dilution, with two replicates per dilution, were spread-plated onto marine agar (MA) and a *Vibrio*-selective media (TCBS). Plates were incubated at 27˚C for 48 hours. Culturable strains were quantified by counting CFUs on both media types. T-tests (JMP 9.0) were

then performed to determine if culturable bacterial densities from the infected fragments were significantly different from the healthy and diseased colonies in the field for both media types (MA and TCBS). Prior to analyses, MA datasets were log (ln)-transformed to improve normality (Shapiro-Wilk W Test) and homogeneity of variances (Levene's Test) (Appendix 2).

In order to identify and compare the bacteria isolated from the infected fragments to the experimental inoculum, five bacterial colonies were randomly chosen from the TCBS media for PCR amplification. Universal bacterial primers 27F and 1492R (Lane, 1991) were used for amplification of 16S rRNA genes of bacterial isolates. PCR was performed on a thermal cycler (BioRad MyCycler v.1.065) as follows: 1.5 μl of template, 15 pmol of each primer, 10xPCR buffer and 0.5 U of *Taq* DNA polymerase, and was adjusted to a final volume of 50 μl sterile seawater. The cycle sequence were as follows: 1 cycle (hot start) at 95°C for 5 min; 30 cycles at 95°C, 55°C, and 72°C for 1 min each; and 1 final cycle at 72°C for 10 min. PCR products were purified using a Gel/PCR DNA Fragments Extraction Kit (IBI Scientific) and was checked using NanoDrop® 2000c Spectrophotometer. PCR products were sent to Macrogen Corp. for DNA sequencing. Sequences were corrected using FinchTV (v.1.4.0) and were then compared to sequences in the NIH BLAST server.

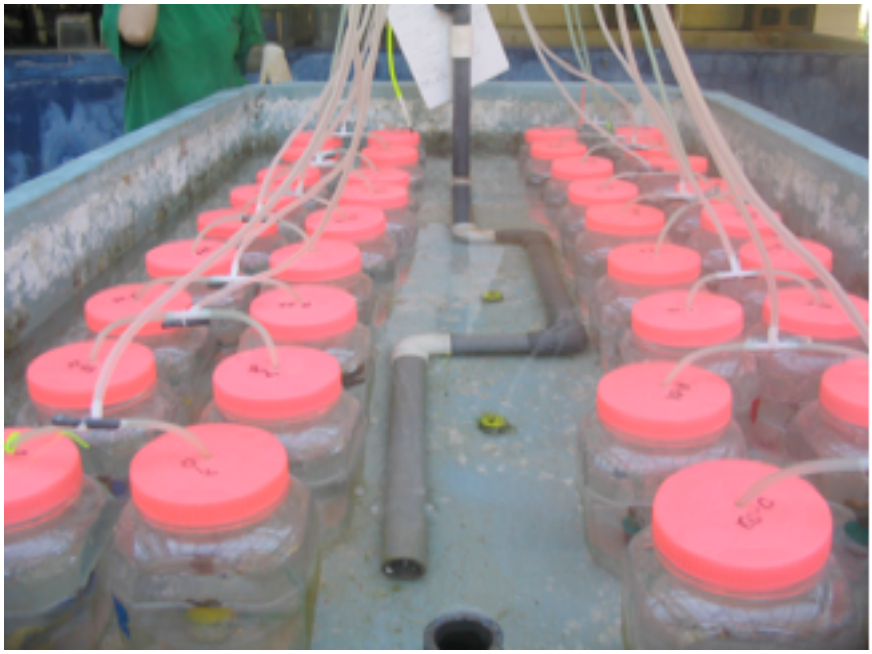


Figure 3. Laboratory setup of aquaria (n=36) in the holding tank

Figure 4. Inoculation of bacterial strain via liquid medium (marine broth)

Table 4. Experimental design of laboratory inoculation using liquid inoculum. Number of replicates per inoculum type is given.

Treatment	LIQUID INOCULUM			
Experimental aquaria (n=20)	Treated with 5 bacterial strains (n=4)			
Control aquaria (n=16)	**Control** untreated (n=4)	**Procedural Control** 1:10 marine broth (n=4)	**Positive Control** *Vibrio coralliilyticus* (n =4)	**Negative Control** *Pseudomonas* sp. (n=4)

b. Inoculation via Solid Inoculum at two temperatures

Using aseptic techniques, 40 apparently healthy *Porites cylindrica* branches were collected from four non-clonal colonies (10 fragments per colony) in Luminao Reef. Using wire cutters, each branch/fragment was collected by clipping a single or bifurcated branch at the base. Each fragment was then placed in a pre-labeled Whirl-Pak® filled with ambient seawater and then brought to the UOGML wet lab facility.

Two fiberglass tanks 2.5-meter long, 1-meter wide, and 1-meter deep with an open seawater system served both as a water bath and holding tank to 40 randomly-arranged, 2000 ml aerated plastic aquaria with closed seawater systems (Figure 5). To test the effect of temperature on the susceptibility of *P. cylindrica* to White Syndrome, one tank was heated to 32 °C using four aquarium heaters with two thermometers placed at opposite ends of the tank to monitor seawater temperature. Another tank was chilled to 26 °C using an aquarium water chiller (Aqualogic® Cyclone Chiller ¼ HP) with an LCD electronic temperature controller. In addition, two temperature loggers (HOBO®) were also placed in each holding tank to monitor minor daily temperature fluctuations. Each fragment was then fixed upright with modeling clay into PVC cups and individually placed inside each aquarium. From an ambient 28°C, fragments in the heated tank were gradually acclimated to 32°C seawater temperature at 1°C-increments per day for 3 d. Simultaneously, fragments in the chilled tank were gradually acclimated to 26°C at 1°C-increments per day for 3 d. Fragments were also fed daily with live, 1-day old *Artemia* nauplii during the acclimation period, with feeding discontinued during inoculation. Aeration was temporarily discontinued at inoculation and resumed 2 h post-inoculation. Fragments were monitored daily for size, appearance and location of lesions. To

optimize chances of transmission of inocula, seawater was replaced 3 d after inoculation with subsequent water changes done every 3 d. Appearance of lesions and overall coral health were monitored for 30 d (4 wk). At the onset of lesions, macrophotographs were taken in order to digitally measure lesion size using Image J™ (v.10.2) software.

Bacterial isolates needed for inoculation were grown on marine agar and TCBS plates. Treatments included an experimental bacterial isolate (D2AH8) from the previous inoculation experiment, a healthy control (no bacteria/inoculum), a procedural control (agar on filter paper), a non-pathogenic strain (*Pseudomonas* sp.), and a pathogenic strain (*Vibrio coralliilyticus*) previously demonstrated to cause WS disease signs in the Indo-Pacific (Sussman, 2008) (Table 5). Using marine agar to aid in adhesion, bacterial isolates were aseptically smeared onto a strip of sterilized gauze (15 x 15 mm). Sterilized stainless-steel forceps were then used to attach gauze strips to the basal, medial and apical regions of each of the four fragments in each treatment/inoculum type (Figure 6). After 48 h, gauze strips were removed to avoid further damage to the live tissue.

Using a sterile 5-ml syringe, mucus was sampled from the skeleton-lesion interface of fragments that developed disease signs. Mucus samples were then serially diluted and 100 μl was spread-plated in triplicate onto marine agar and TCBS media that were supplemented with 100 μg/ml cyclohexamide to inhibit fungal growth.

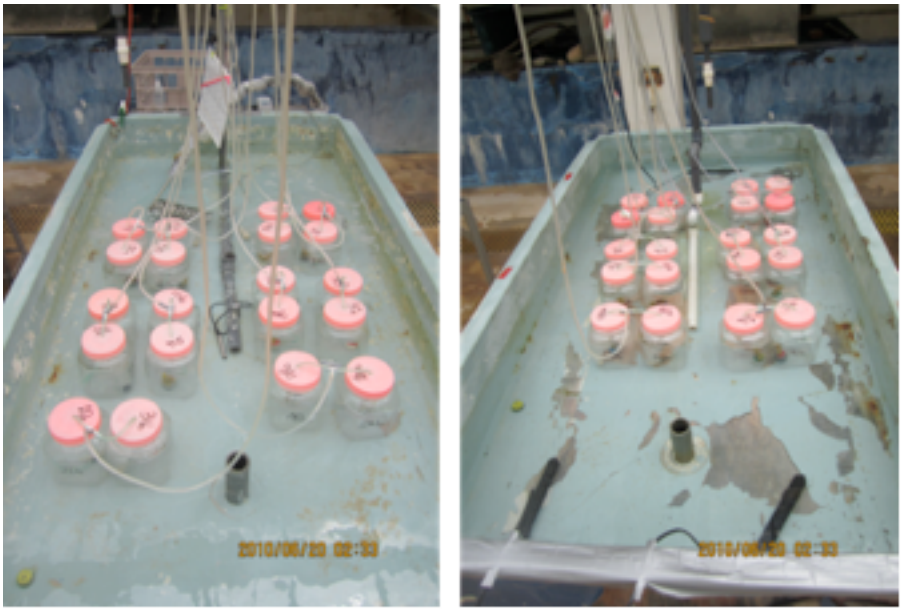


Figure 5. Laboratory setup of inoculation experiment with solid inoculum using chilled (left) and heated (right) tanks

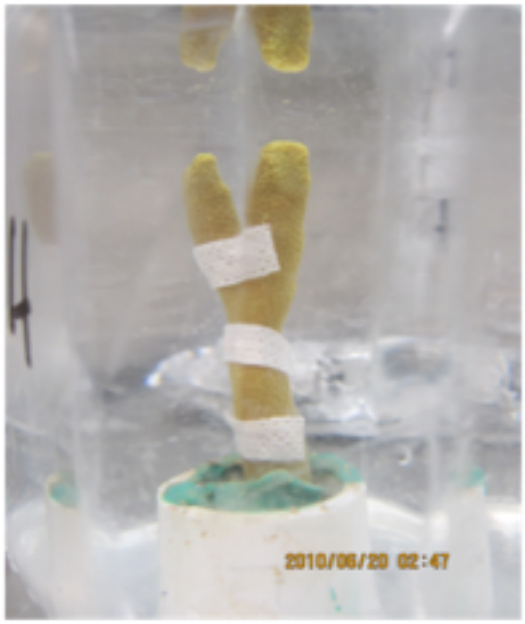


Figure 6. Gauze strips with inoculum attached to 3 regions on *P. cylindrica*

Table 5. Experimental design of laboratory inoculation using solid inoculum at two temperatures. The number of replicates per inoculum type is given.

Treatment	SOLID INOCULUM				
Heated (32°C) aquaria (n=20)	**Experimental** D2AH8 strain (n=4)	**Control** untreated (n=4)	**Procedural Control** 1:10 MB (n=4)	**Positive Control** *Vibrio coralliilyticus* (n =4)	**Negative Control** *Pseudomonas* (n=4)
Chilled (26°C) aquaria (n=20)	**Experimental** D2AH8 strain (n=4)	**Control** untreated (n=4)	**Procedural Control** 1:10 MB (n=4)	**Positive Control** *Vibrio coralliilyticus* (n =4)	**Negative Control** *Pseudomonas* (n=4)

2.4.2. Disease Severity Monitoring

To determine the frequency and severity of WS in each growth form and across census periods, 10 branching *P. cylindrica* colonies and ten massive *Porites* spp. colonies were censused monthly for the number of active lesions, and overall colony health for a period of 7 months. Lesions in massive colonies were photographed with a scale bar to record lesion size (cm^2) while the number of active lesions was counted on each branching colony. In addition, initial measurements to estimate colony size were undertaken during the first census period (described below).

WS disease severity was computed as the percentage of diseased surface area versus total surface area/colony size and is expressed as Percent Tissue Loss (%TL). Massive colony size (MCS) was estimated assuming an oval (i.e. elliptical) colony shape using the equation: MCS= π *(L/2) (W/2),* where *L* is the length of the axis of a colony (maximum diameter) and *W* is the width measured perpendicular to the length axis (cm). Alternatively, branching colony size was estimated as the product of the total branch count and the branch size proxy/constant (Mean Branch Size) wherein Mean Branch Size (MBS) was derived from the average measurement of 5 branches from each of the 10 *P.cylindrica* colonies (n=50). Colony-size measurement in branching colonies was done differently than in massive colonies for two reasons: 1) *P.cylindrica* colonies have complex geometric shapes (i.e. submassive or arborescent) that contribute to the logistical difficulty of measuring each branch; 2) *P.cylindrica* branches are fairly uniform in size and structure, which allows for a decent approximation of branch size using a mean of a subsample. Likewise, a proxy Mean Lesion Size (MLS) was used to represent all lesions in branching colonies. The constant (MLS) was derived from 5 lesion size

samples (cm^2) from 10 *P.cylindrica* colonies (n=50). For consistency, both branch and lesion sizes were digitally measured using Image J™ (v1.41) software from photographs (Canon G10) taken *in situ*.

Based on the data obtained above, %TL per colony was computed using the following equations:

$$\textbf{Branching \%TL} = \left[\frac{(MLS)(LesionCount)}{(MBS)(BranchCount}\right]100$$

Key:
MLS – Mean Lesion Size constant of 50 lesion samples (5.00 cm^2, SE= 0.46)
MBS – Mean Branch Size constant of 50 branch samples (19.65cm^2, SE=1.44)
Lesion Count – total # of lesions
Branch Count – total branch count per colony

$$\textbf{Massive \%TL} = \left[\frac{(TotalLesionSize)}{MCS}\right]100$$

Key:
Total Lesion Size – total lesion size (cm^2) per colony
MCS - colony size (cm^2) of massive colonies using area of an ellipse formula

To compare WS disease severity and incidence between branching and massive *Porites* spp. colonies, three analyses were done:

1) Repeated measures ANOVA (SPSS®) was performed on the %TL (Monthly Mean %TL) of each colony across 7 months to address: (1) whether there is an overall change in %TL over time for each growth form; (2) whether the change in %TL is the same between growth forms. Prior to the analysis, an ln Y power transformation was applied to both branching and massive colony datasets to meet the assumptions of normality (Shapiro-Wilk W Test and Levene's Test) (Appendix 3). Mauchly's Test of Sphericity

indicated that the assumption of sphericity was violated ($p<0.005$) and a subsequent correctional adjustment (Greenhouse-Geisser) was used.

2) The Total % Change in Tissue Loss (%TC) of 10 colonies per growth form was calculated for the entire study period (239 d) as the difference between the last and the first %TL. The mean %TC of each growth form and across colonies were then compared using a two-way nested ANOVA (JMP 9.0). Datasets in some colonies comprised of multiple negative values due to lesions that healed, hence, a constant (+10) was added to allow a subsequent ln Y data transformation. The power transformation did not improve normality in the branching colony dataset (Shapiro-Wilk W Test) but improved variances (Levene's Test) in both growth forms (Appendix 3).

3) Colony-level Disease (lesion) Incidence was assessed in both growth forms and was expressed as the number of acute or new lesions observed in each colony at each census period (n=10). Acute lesions were identified as areas of recent tissue loss displaying bare white skeleton or areas of tissue loss with an active disease progressing front or margin in the case of most lesions in the massive colonies. A two-way nested ANOVA (JMP 9.0) was performed to test differences between the mean incidence of acute lesions between the two growth forms and across colonies. Both branching and massive colony datasets were homogenous and normally distributed (Shapiro-Wilk W Test, Levene's Test) (Appendix 4).

2.4.3. Temperature Effects on Lesion Size

In order to assess whether lesion progression has a seasonal component arising from a temperature-related influence, a graphical comparison was done on the mean colony lesion size (cm^2) of each growth form (see Sec 2.4) and the mean monthly temperature (°C) taken during a 7-mo census period (Fig.1). Lesion size measurements were averaged across 5 lesions from 10 colonies of each growth form (n=10).

2.5. TISSUE LOSS AND RECOVERY DYNAMICS

To determine WS lesion dynamics on the two growth forms of *Porites* spp., colony-level monitoring and morphometrics were done on 10 randomly selected colonies each of *Porites cylindrica* of massive *Porites* spp. in Luminao Reef. Five lesions from each colony were monitored every month for size and appearance over a 7-month period. Concurrently, underwater temperature data loggers (HOBO®) were also deployed throughout the census period to allow a testing for a seasonal component in the disease progression, severity and incidence (see Sec. 2.4.3.)

2.5.1. Tissue Recovery Rate

In testing for differences in recovery rates (rate of lesion reduction via resheeting of new tissue) between growth forms, only lesions that, on average, did not increase in size were assessed. Hence, the mean (tissue) recovery rates over 7 months ($cm^2\ d^{-1}$) of 50 lesions in branching colonies and 38 lesions in massive colonies were compared to determine between-growth form/morphology variations. Mean Tissue Recovery Rate was computed as follows:

$$\textbf{Mean Tissue Recovery Rate (cm}^2\ \textbf{d}^{-1}\textbf{)} = \sum_{i=1}^{n}\left(\frac{L(i+1)-Li}{days}\right)$$

* **L*i*** – lesion size (cm^2) at time *i*
* ***Days*** - length of census period
* ***n*** =50 per growth form

A two-way nested ANOVA (JMP 9.0) was conducted on the mean recovery rate ($cm^2\ d^{-1}$) of lesions in both growth forms to determine variations that were either due to between-colony differences or differences due to morphology. Since this analysis is only testing for lesions that recovered and given that some of the lesions in 5 of the 10 massive colonies progressed, a balanced design was achieved by randomly selecting only 5 of the 10 colonies in the branching *P. cylindrica* (n=25 per growth form).

Based on the computed lesion recovery rates, a Pearson's Chi-square test (JMP 9.0) was used to determine the proportion of lesions that, on average, increased or decreased in size, or healed completely (i.e., lesion fate) over the 7-month census period, as well as determine whether the proportions differed between the two growth forms

To determine whether recovery rate is a function of lesion size, the initial lesion size (cm^2) (i.e., lesion size measured at day 0) of all lesions that decreased in size (branching n=50, massive n=38) was regressed against its corresponding mean recovery rate ($cm^2\ d^{-1}$) using a simple linear regression analysis. When the regression were significant for each growth form, an analysis of covariance (ANCOVA) was subsequently carried out on mean recovery rate, with lesion size as the covariate. This analysis factored out the effect of lesion size on recovery.

To satisfy the assumptions of normality, ln Y transformations were applied to the mean recovery rates of both growth forms, which improved the distributions (Shapiro-Wilk W Test) and equality of variances (Levene's Test) of both branching and massive colony datasets (Appendix 6). Initial lesions sizes of each growth form also exhibited

normal distributions and equal variances after subsequent ln Y transformations (Appendix 6).

2.5.2. Lesion Size Transition Probability

To examine the possible outcomes of a given lesion size (with lesion fate as an effect) in branching and massive colonies, a transition probability matrix was done based on the lesion size classes measured at each month over a 7-month census period. Size classes per growth form were formulated based on the range of the smallest and largest lesion size (cm^2) observed from a population of 50 pooled lesions (5 monitored lesions in each of 10 colonies per growth form). The probability of transitions, or fates, within each size class included growth to the next size class, stasis (remaining within a size class), shrinkage, or total shrinkage (healed), and were ordered according to the number of lesions experiencing each fate within a 7-mo period (Raymundo and Maypa 2004). In addition, lesion size subsets randomly selected from each growth form (n=10) were binned in three size classes (small, medium and large) based on their size range and compared using Fisher's Exact Probability test (2x3) to determine whether the proportion of lesions in each size class is significantly different between morphs.

2.5.3 Corallite Properties of branching and massive *Porites* spp.

In order to rule out an effect of corallite density and size on lesion size dynamics, corallite properties in *Porites* spp. were taken into account. Branching (*P.cylindrica*) and massive *Porites* spp. corallites were tested for significant differences in corallite density and size. Corallite properties were examined digitally from 10 colony macrophotographs (Canon G10) of each growth form using Image J™ (v. 144h) software. Corallite density of 10 colonies of each growth form was quantified within the area bounded by a fixed grid (0.50 cm^2). Subsequently, 20 corallites were randomly selected from each colony macrophotograph (n=200) and diameters (cm) were measured from the opposite ends of the corallite walls.

Corallite densities of both growth forms satisfied the assumptions of normality (Shapiro-Wilk W Test) and were analyzed using a 1-way ANOVA (JMP 9.0) (Appendix 7). Corallite sizes, on the other hand, while exhibiting homogenous variances, failed normality tests after a subsequent lnY power transformation (Appendix 8). A 1-way ANOVA was then used to detect differences between growth forms.

Chapter 3. Results

3.1. DISEASE CHARACTERIZATION

3.1.1. Gross Description of White Syndrome on *Porites* spp.

Fifty lesions in various states of progression chosen from 10 haphazardly selected colonies of *Porites cylindrica* and 10 colonies of massive *Porites* spp. were examined in Luminao Reef, southern Guam. Based on gross morphological observations, lesions in *P.cylindrica* were characterized as small (0.06 – 16.65 cm^2), irregular, diffuse areas of acute to subacute tissue loss revealing recently exposed white skeleton. In subacute infections, tissue loss revealing green or brown skeleton, indicative of algal colonization, was separated from intact tissue by a margin of bare white skeleton, displaying a progressing front (Figure 7). In massive *Porites* spp., lesions were characterized as medium to large (0.11 – 976.71 cm^2), multifocal to coalescing, irregular areas of tissue loss revealing (algae-covered) green or brown skeleton separated by a band of bare white skeleton. New lesions continuously appeared and then gradually increased in size within 1 to 4 weeks suggesting a chronic disease process (Figure 8).

Lesions in both growth forms varied in size and appearance during various states of disease progression (i.e., active or progressing, stasis, healing). Lesions that were in stasis were often overgrown with filamentous algae, crustose coralline algae (CCA), or mats of cyanobacteria while lesions that were healing showed newly re-sheeted pale or yellow or brown tissue surrounding the lesion perimeter.

Figure 7. WS lesions on branches of *P. cylindrica* colonies in various states of progression

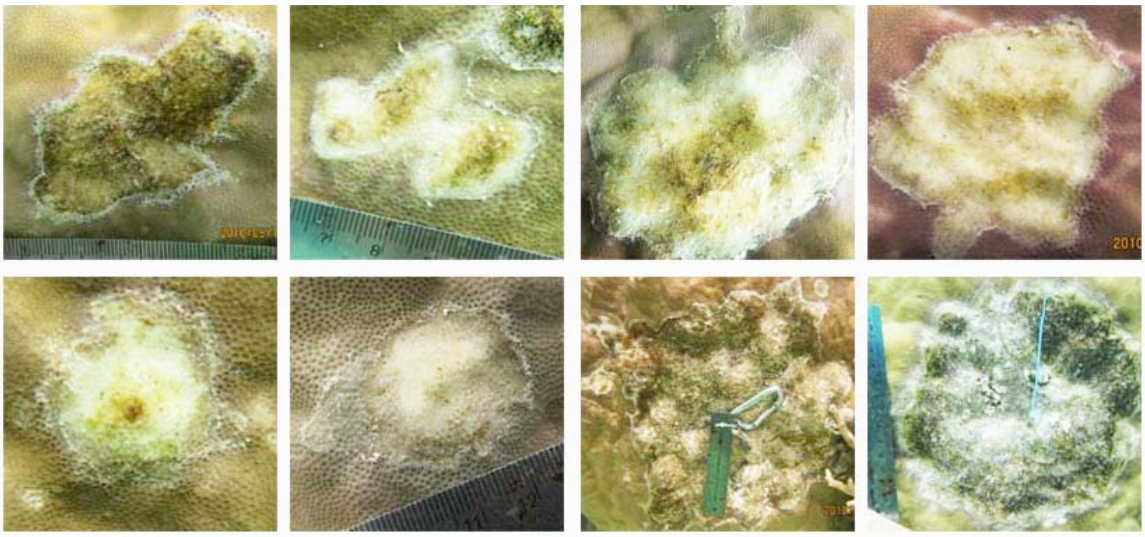


Figure 8. WS lesion on massive *Porites* spp. colonies in various states of progression

3.1.2. Histological Description of White Syndrome on *Porites cylindrica*

Five samples each of branching *P. cylindrica* were collected from: (1) fragments in remote healthy (RH) colonies, (2) healthy fragments (H) from WS-infected colonies, and (3) diseased fragments (D) (i.e., with active lesions) from WS-infected colonies. For each type, five histological tissue sections were observed under the microscope for general tissue organization, cellular damage, and presence of possible etiologic agents.

Histological examinations were recorded from anatomical structures within the coral tissue layers. The coenosarc is the overlying layer of living tissue connecting each polyp (Peters, 2001). The oral disc is the part of the polyp through the center of which the mouth opens (Peters, 1984). Connecting the mouth and the gastric cavity is an invagination of the epidermis called the actinopharynx (Fautin, 2005; Peters, 2001). Within the gastric cavity are longitudinal partitions called mesenteries that extend through its mesenterial filaments that aid in the capture and digestion of food (Peters, 1984). These six structures consist of three tissue layers: the epidermis; the mesoglea; and the gastrodermis, while the mesenteries and mesenterial filaments are each composed of two layers of gastrodermis separated by a mesoglea (Peters, 2001)

Histological sections of healthy (RH and H) tissues of *P. cylindrica* revealed intact epithelia with basally located golden-brown pigmented granules, or *pigment cells*, that surrounded the epidermis (Figure 9). Some supporting structures observed within the tissue layers were: columnar *supporting cells* with a central nucleus in the epidermis (Goldberg, 2002a); *nematocysts* (macrobasic mastigophores) and *spirocysts* (holotrichous isorhizas); and a few ovoid *bacterial aggregates* (Figure 9 and 10). *Zooxanthellae* found

in the gastrodermis were round and uniformly shaped with a visible *pyrenoid* (Figure 10), an important proteinaceous body found within the cytoplasm of zooxanthellae that is involved in the synthesis and deposition of polysaccharides (Dorland, 2000; Leggat et al., 1999).

Histopathology Description

Necrosis, the pathologic death of cells and tissues (Stedman, 1995) was evident on all diseased (D) tissue samples. Encompassing the polyp and coenosarcs were multicellular structures with cell walls (algal filaments) and sinuous eosinophilic structures with parallel striations (cyanobacteria) (Figure 11 and 13). Near the lesion, the zooxanthellae, surrounded by granular hypereosinophilic cytoplasm, were irregularly shaped with absent or obscured pyrenoids (Figure 12). Bacterial aggregates were ill-defined and fragmented while some were surrounded by necrotic debris and hyaline membranes (Figure 12). In some tissue samples, bacterial aggregates were found to proliferate in the outer tissue layers where algal infiltrates were numerous (Figure 11). Similarly, dense aggregations of eosinophilic granular ameobocytes and a proliferation of pigment cells were abundant near the algal filaments (Figure 11 and 12).

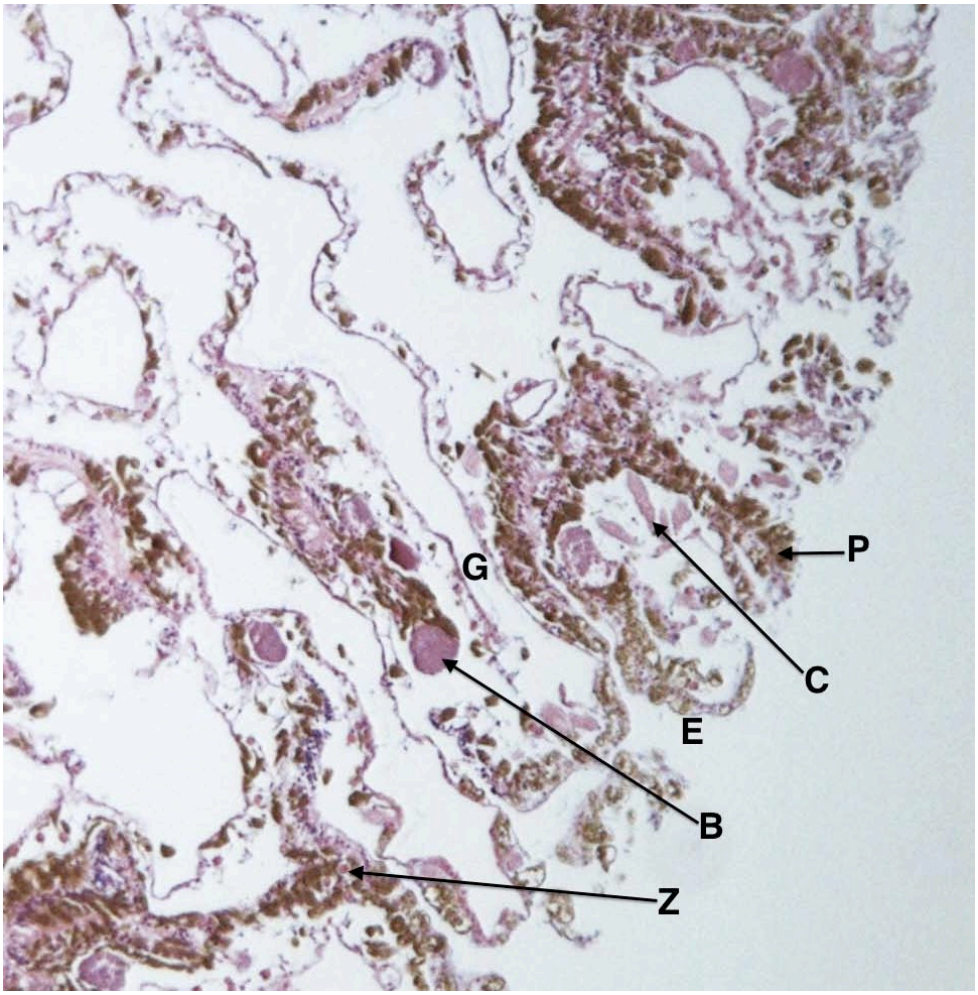


Figure 9. Photomicrograph of healthy (RH) *P. cylindrica* tissue section stained with MHE (10X). E - epidermis, G - gastrodermis, P - pigment cells, C - cnida (spirocyst), B - bacterial aggregate, Z - zooxanthela

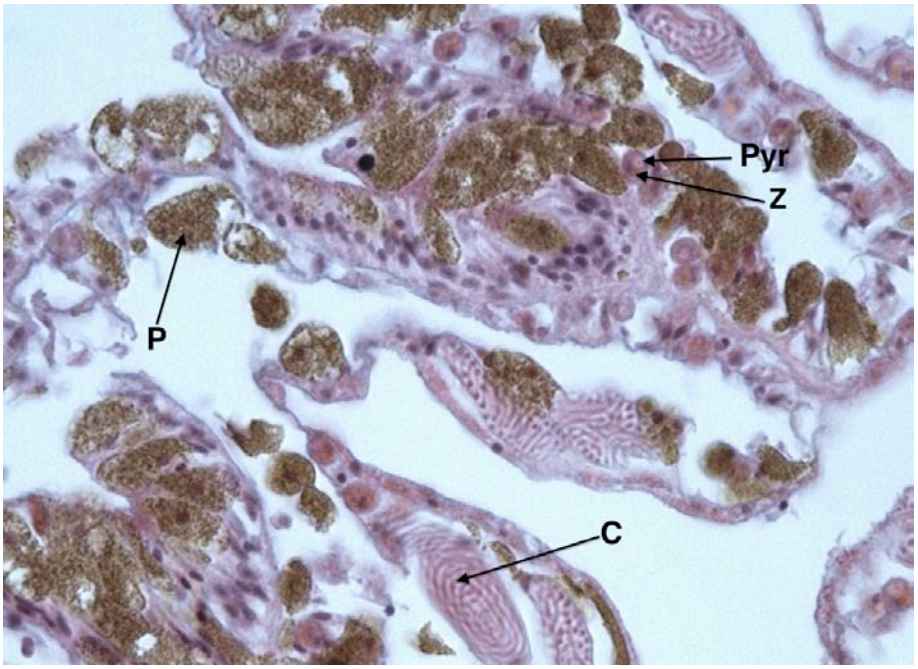


Figure 10. Photomicrograph (40X) of healthy *P. cylindrica* tissue section stained with MHE with associated supporting structures. P - pigment cells, C - cnida (spirocyst), Z -zooxanthella, Pyr - pyrenoid.

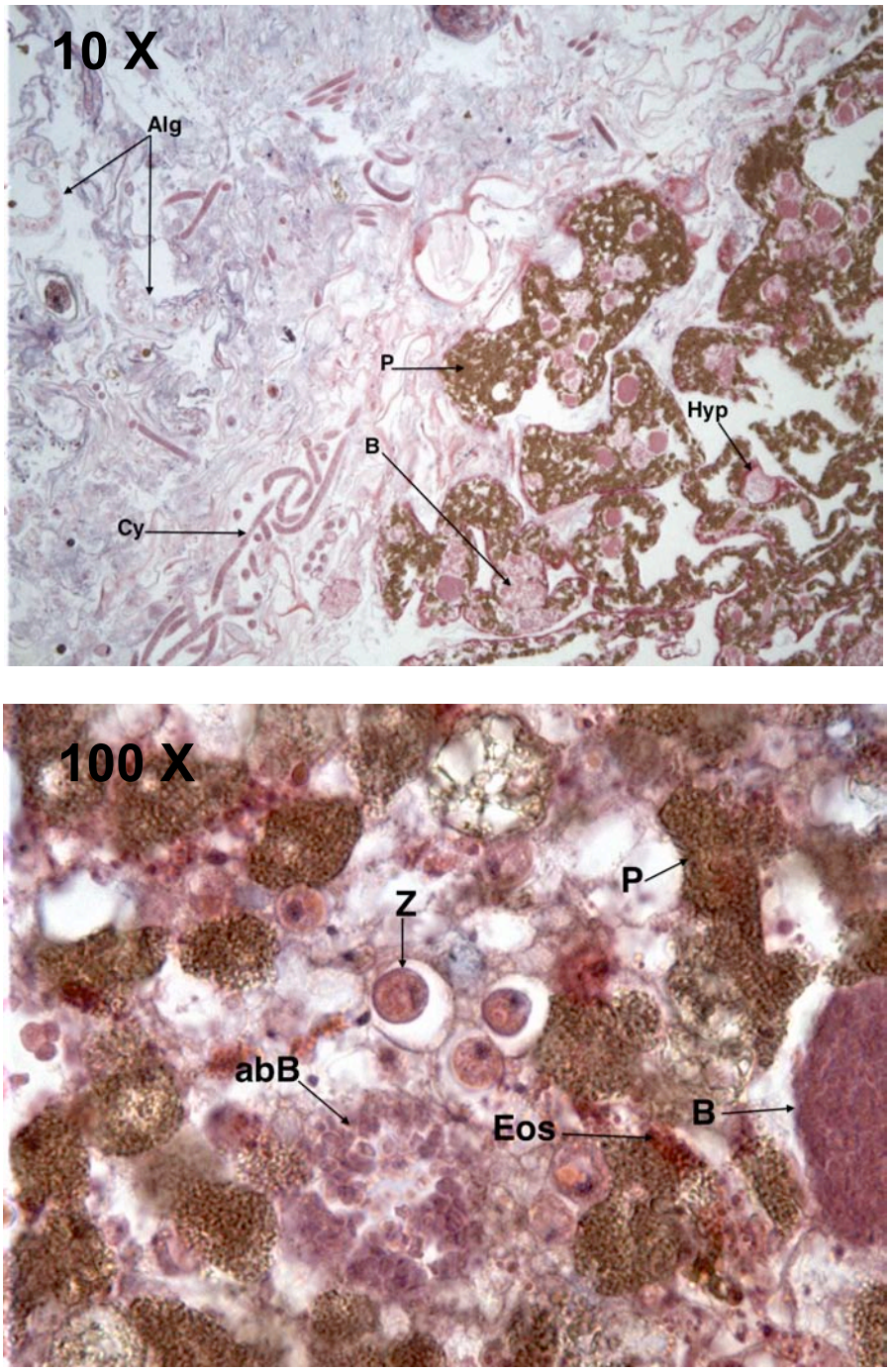


Figure 11. Photomicrographs of diseased *P. cylindrica* tissue section stained with MHE. Note increased number of swollen (B) and abnormal (abB) bacterial aggregates, hypereosinophilic cytoplasm (Hyp), marked infiltrates of granular pigmented cells (P), vacuolated zooxanthella (Z), numerous cyanobacteria (Cy) and algal filaments (Alg) at the disease front

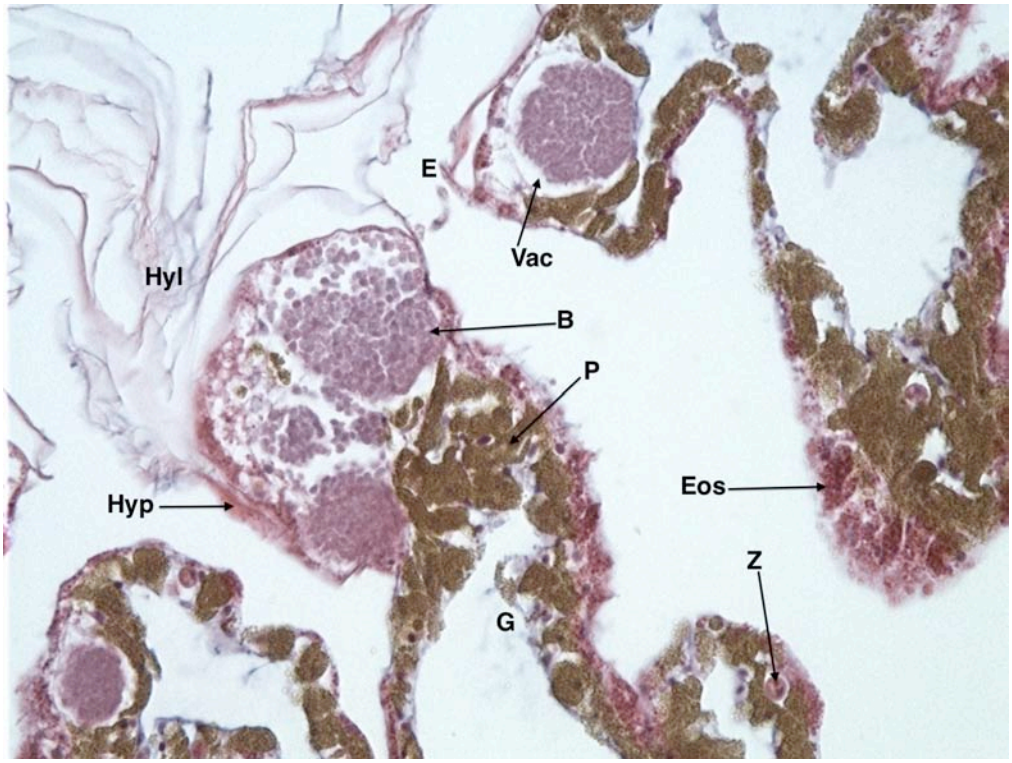


Figure 12. Photomicrograph (40X) of diseased *P. cylindrica* tissue section stained with MHE. Note increased hyalination (Hyl) and hypereosinophilic (Hyp) cytoplasm in vacuolated (Vac) bacterial aggregates (B), fragmented zooxanthella (Z), and abundant pigment cells (P) and eosinophilic granular amoebocytes (Eos) in the gastrodermis.

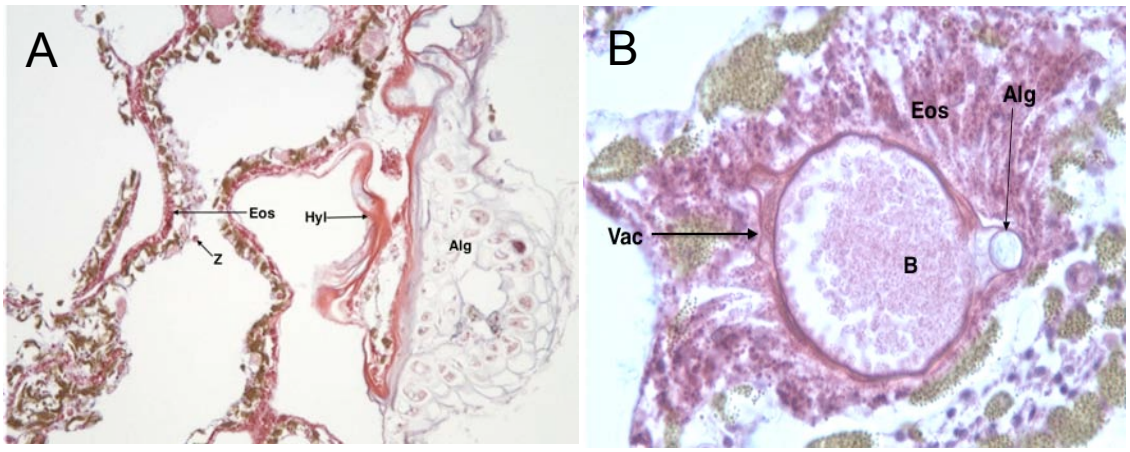


Figure 13. Photomicrograph of diseased *P. cylindrica* tissue section stained with MHE. A - 20X, note increased hyalination (Hyl) where algal filament (Alg) is present, free zooxanthela (Z) and merging eosinophilic granular ameobocytes (Eos); B - 100X, note algal cell (Alg) near degenerating and vacuolated (Vac) bacterial aggregate (B) with eosinophilic debris (Eos) and infiltrates of granular pigment cells

3.2. DISEASE ECOLOGY

3.2.1. Disease Infectiousness

3.2.1.1. Test for Transmissibility of Disease

In the direct contact experiment, 4 out of 16 healthy *P.cylindrica* fragments in direct contact with diseased fragments (D2, D4, D5, D7) developed lesions similar to those found in the field (Figures 14 -17). New lesions were grossly characterized as small, diffuse areas devoid of coral tissue leaving recently exposed white skeleton. On 3 out of the 4 infected fragments, the lesions appeared directly at the point of contact, while on one, the lesion appeared out at a break in the tissue and away from the point of contact suggesting transmission within the gastrovascular canals or waterborne transmission (Kaczmarsky, and Richardson, 2007). In addition, 1 out of the 16 healthy *P. cylindrica* freestanding fragments (D1) in the experimental setup developed a lesion within the census period (Figure 18). Of the five infected fragments, three (D4, D5, D1) developed a lesion within 3 d of the start of the experiment while two (D2 and D7) developed lesions later on (6 d). The lesions first appeared as small (1.04 cm^2, SE=0.37), diffused areas of tissue loss and increased in size (3.25 cm^2, SE=0.62) within 39 d at an average rate of 0.69 cm^2 d^{-1} (Figure 19). One of the infected fragments (D2) developed multifocal areas of tissue loss within 9 d, which later on coalesced and contributed to 90% tissue loss (Figure 14).

Within a 6-week period, the lesions on all infected fragments appeared necrotic and overgrown with filamentous algae and cyanobacteria. It is however important to note that on one fragment (D7), the lesion appeared to be in stasis on 13 d with no signs of further tissue loss and no new tissue growth, and appeared to heal by day 16 and 21. This

reaction is sometimes expected since most coral holobionts, are still able to restore tissue integrity and fully recover even under extreme stress (Visram et al., 2007; Palmer et al., 2011). However, on 24 d, the lesion on D7 progressed and gradually increased in size. Each lesion in the five infected fragments continued to progress throughout the remaining census period.

Mean seawater temperature (29 - 32°C) was fairly constant throughout the experiment and did not seem to influence the growth and spread of the lesions (Figure 19). None of the fragments in the control setup developed disease signs although some healthy-to-healthy paired fragments developed a small (0.2 cm^2), circular lesion at the point of contact. These lesions were likely caused by tissue damage due to physical abrasion or allorecognition interaction rather than disease since the lesions did not progress and eventually recovered by the end of the experiment. It was assumed however, that the healthy-to-diseased pairs in the experimental setup might have developed similar abrasions that aided in the transmission of the disease by introducing an entry wound.

Out of the 16 healthy fragments in direct contact with diseased fragments, 25% developed lesions while 6% of the 16 freestanding healthy fragments developed lesions from exposure to diseased fragments *($p=0.036$)*. Based on these results, waterborne transmission and direct contact to disease-affected areas were the sources of disease infection and suggests the involvement of a pathogenic microorganism. Furthermore, a comparison in microbial concentrations (CFU ml^{-1}) grown on marine agar using a Wilcoxon paired test show that concentrations were not significantly different in experimentally infected (E) fragments and in diseased colonies (D) in the field

(*t(23)=0.732, p=0.471)* (Figure 20). Consistent with Koch's postulate, microbial concentrations in diseased colonies (D) and in the experimentally infected fragments (E) were found to be significantly higher than in healthy colonies in the field *(*E>H*, t(23)=-8.338, p<0.001;* D>H*, t(23)=-6.369, p<0.001)*. Similarly, microbial concentrations grown on *Vibrio*-selective media (TCBS) were not significantly different between experimentally infected fragments and diseased colonies from the field $t(20)=0.099$, $p=0.922$, which indicates an association between *Vibrio* densities and WS disease lesions (Figure 20). Microbial concentrations in healthy colonies in the field were significantly lower than in the experimentally infected fragments (*t(20)=-2.022, p=0.056)* but not significantly different than from diseased colonies in the field (*t(20)=-1.442, p=0.165)*.

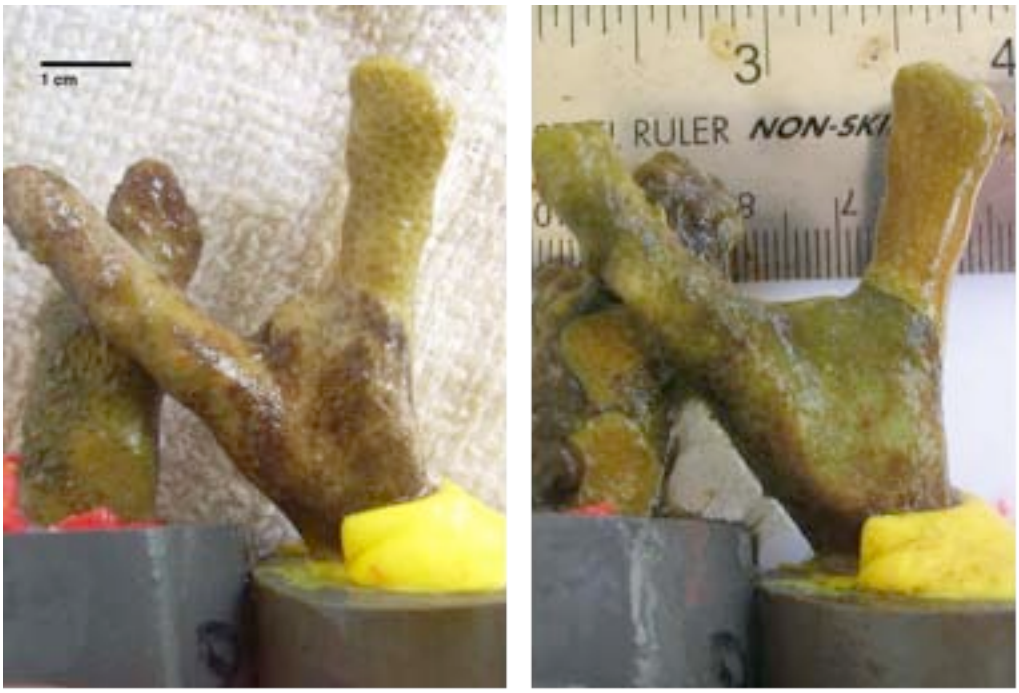


Figure 14. Experimentally infected fragment (**D2**) with multifocal lesion on 6 d (left) appearing necrotic and colonized by algae after 39 d (right)

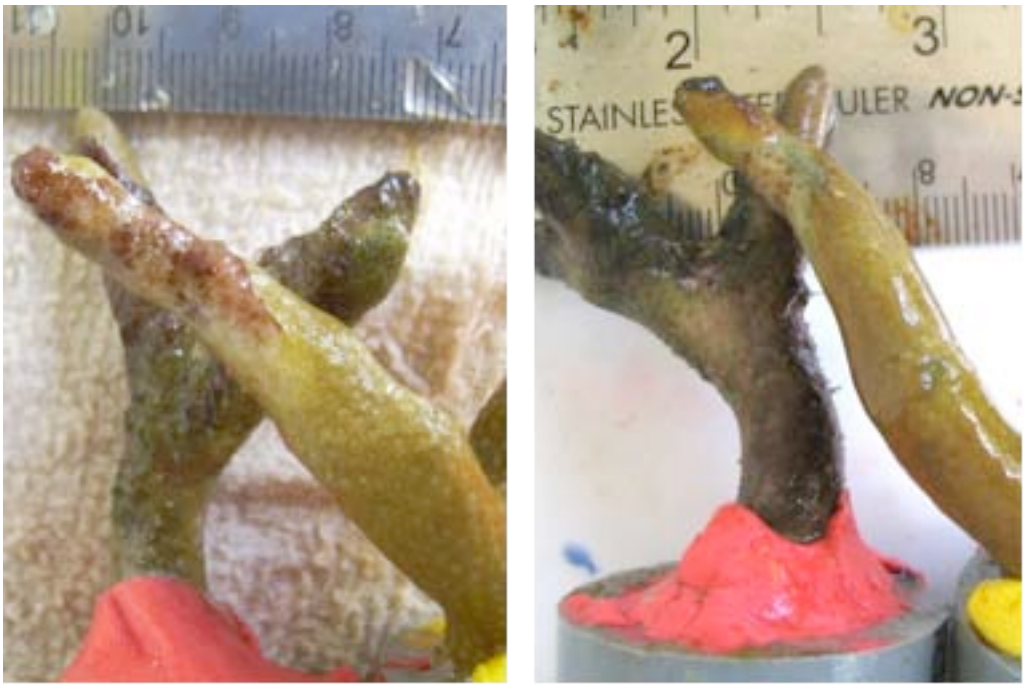


Figure 15. Experimentally infected fragment (**D4**) with irregular lesion on 3 d (left) with exposed skeleton colonized by algae after 39 d (right)

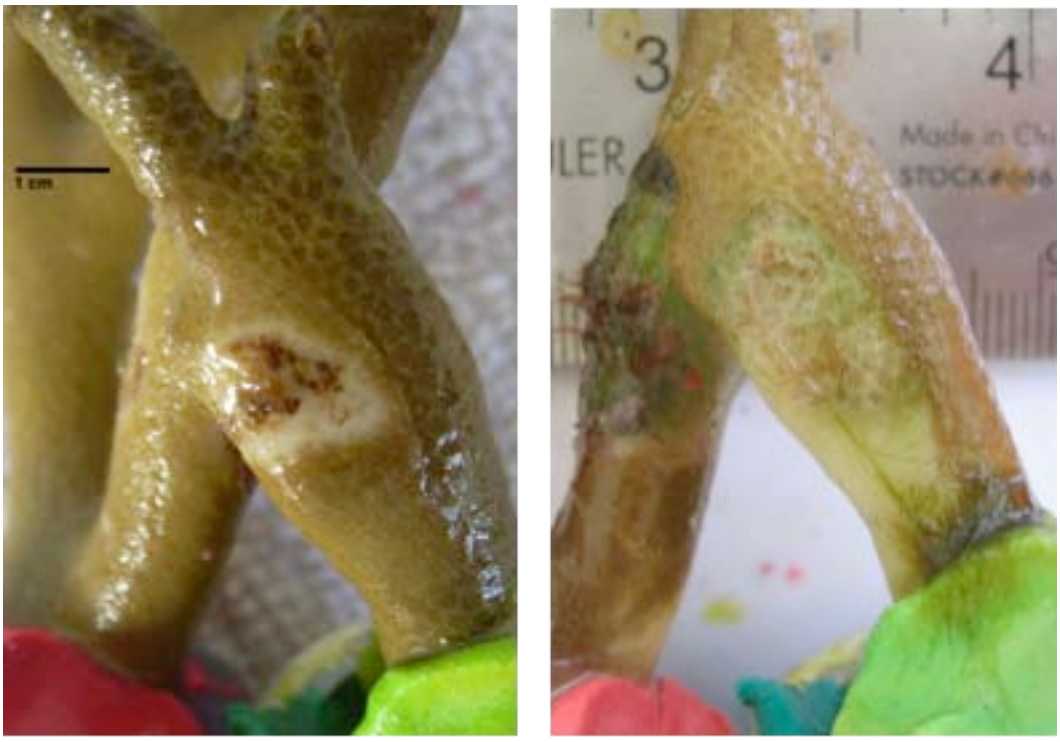


Figure 16. Experimentally infected fragment (**D5**) with irregular lesion circumscribed by progressing margin on 3 d (left) appearing necrotic and colonized by algae after 39 d (right)

Figure 17. Experimentally infected fragment (**D7**) with irregular lesion circumscribed by a disease progressing margin on 6 d (left) appearing necrotic and colonized by algae after 39 d (right)

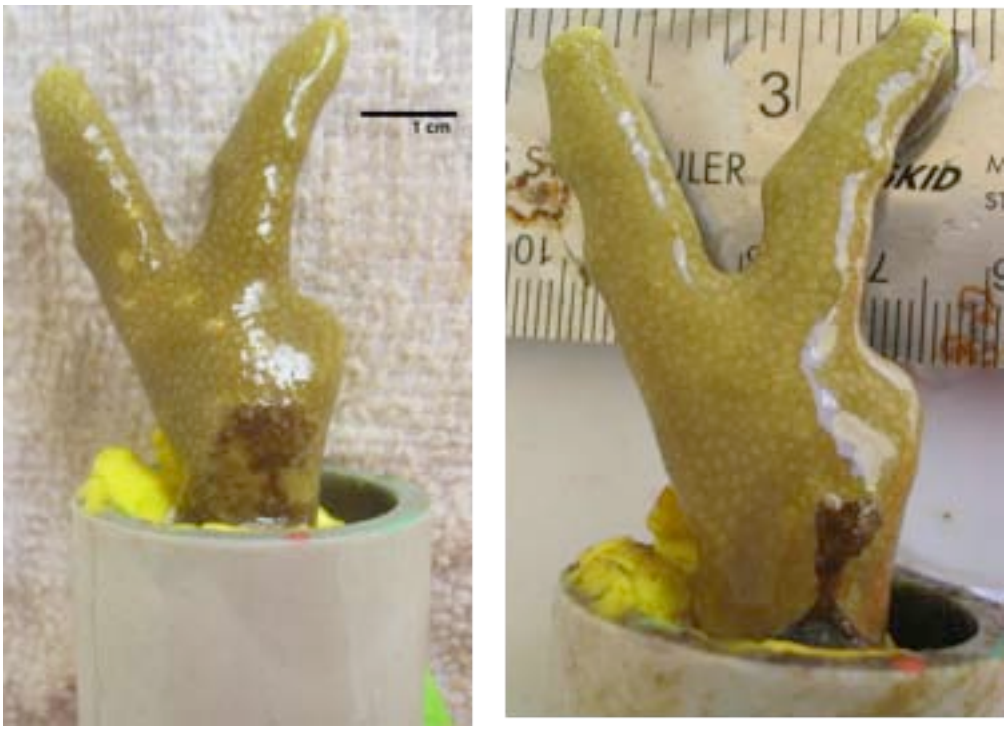


Figure 18. Experimentally infected freestanding fragment (**D1**) with irregular, basally lesion appearing on 3 d (left) that gradually recovered by 39 d (right)

3.2.1.2. Isolation and Identification of Putative Pathogen

a. Inoculation via Liquid Inoculum

By the end of the experiment (42 d), only one experimental strain (D2AH8) caused WS disease signs in 1 (C-05) out of 4 fragments: Twenty-seven days post-inoculation, the fragment developed 7 lesions, 3 of which developed medially, 3 at the basal region and 1 at the apical region (Figure 21). The lesions appeared as multifocal areas of light yellow tissue discoloration, which progressed to small multifocal areas of bare white skeleton. Within 29 d, the medial and basal lesions (0.29 cm^2) coalesced while one located apically (0.09 cm^2) was colonized by algae and appeared to be in stasis. During this time, new tissue growth was observed and the lesions appeared to be recovering. After 42 d, 5 lesions fully recovered and only 2 lesions remained discolored.

Within 15 d, all "entry" wounds completely healed on all fragments. None of the controls developed disease signs for the duration of the experiment. Furthermore, contrary to what was expected, fragments that were inoculated with *Vibrio coralliiltyticus*, previously demonstrated to cause WS in *Montipora* spp. (Sussman, 2008), did not develop any disease signs presumably because the strain was isolated from a marine sponge and may have been non-pathogenic. It is important to note, however, that bacterial concentrations (CFU ml^{-1}) were significantly higher in the infected fragment (C05) than in apparently healthy corals in the field $t(5)=-7.826$, $p<0.005$. Bacterial concentrations in the infected fragment were not significantly different from corals showing WS disease signs in the field $t(5)=-1.16$, $p=0.299$ (Figure 22). These results are congruent with the criteria that the microbial pathogen must be present in all

disease cases but absent from healthy organisms. However, *Vibrio* densities between the infected fragment and healthy corals in the field (Figure 22) were not significantly different, $t(5)=-0.160$, $p=0.880$.

Having successfully isolated the bacterium from the previous transmission experiment and reintroduced it this inoculation experiment, the following were accomplished contingent to fulfilling all of Koch's postulates:

1. Culturable bacteria were found in significantly higher numbers (CFUs) when compared to apparently healthy *P. cylindrica* samples obtained in the field (Figure 22).

2. Twelve strains of culturable *Vibrio* spp. were isolated from fragments showing WS disease signs (Sec. 3.2.1.1) and five were grown in pure culture and reinoculated.

3. After inoculation, White Syndrome disease signs appeared on a sample from an apparently healthy colony.

4. Using the NIH Blast Server, partial sequencing of the 16S rRNA on C05 reports a 99% similarity to *Vibrio coralliilyticus* (*Vibrio sp*. PMS20); the same pathogen identified for White Syndrome (WS) in the Great Barrier Reef (Sussman et al., 2008). A comparison of this isolate to the original inoculum, however, was not undertaken in this study due to logistical and time constraints.

Although *Vibrio* spp. was successfully re-isolated from a single experimentally infected fragment out of the 4 inoculated, this represents a very small sample size and repetition of the experiment is warranted. Various factors such as host susceptibility, environmental conditions, and weakening of the pathogenic agent may explain the

inability to replicate an infectious disease in controlled settings (Work et al., 2008). Hence, determining causality based on the fulfillment of Koch's postulates alone cannot be confidently assumed, and further testing, increased replication and a subsequent comparison of the bacterial isolate to the original inoculum are needed. However, these results are intriguing and consistent with out current understanding of causative agents of white syndromes elsewhere in the Pacific and on other species (Sussman et al., 2008)

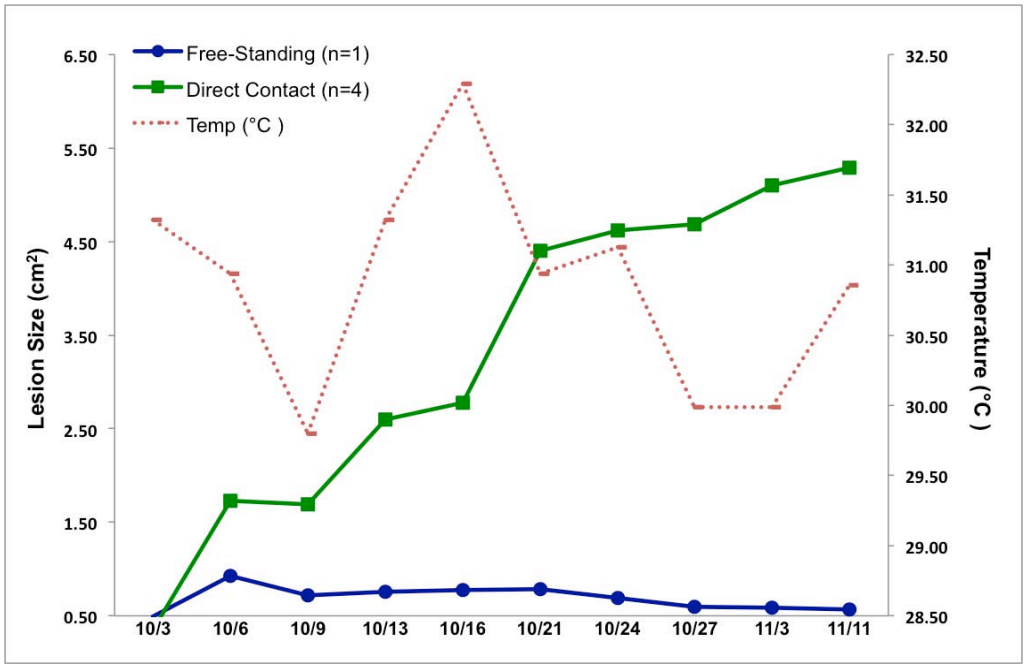


Figure 19. Mean size (cm^2) of 4 (direct contact) infected fragments and progression rate of one freestanding fragment over 39 d

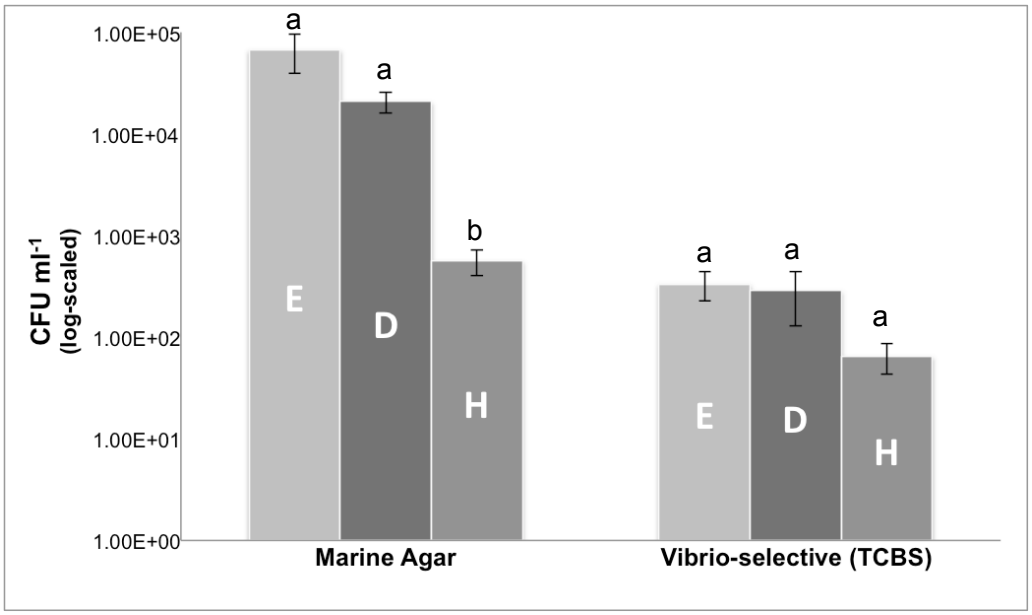


Figure 20. Mean microbial concentrations (CFU ml^{-1}) of experimentally infected fragments (E), diseased (D) and healthy (H) colonies in the field, cultured in marine agar and a *Vibrio*-selective media (TCBS). Dissimilar letters (a,b) are significantly different. Bars = SE.

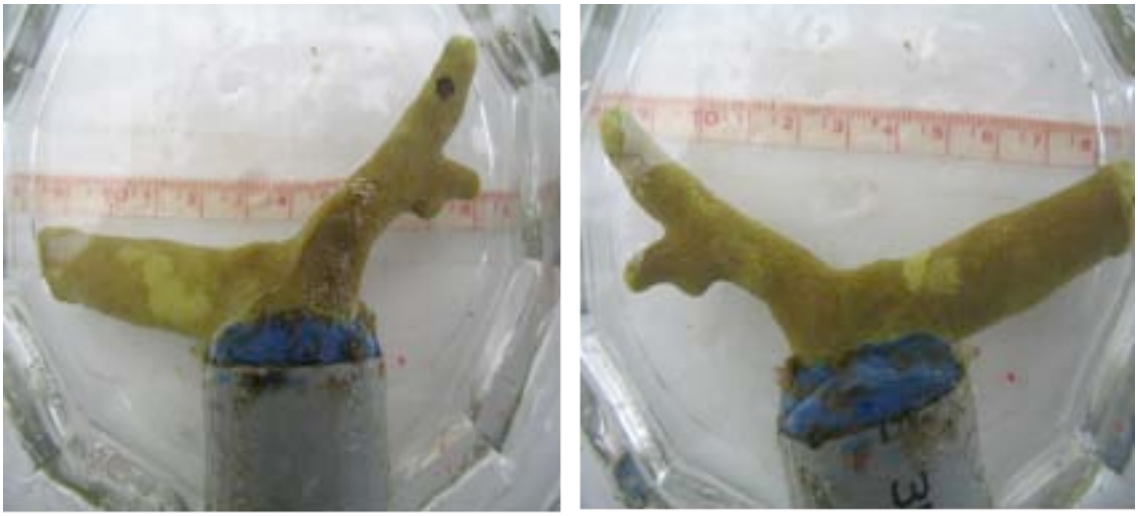


Figure 21. *P.cylindrica* fragment (C05) displaying WS disease signs after inoculation with bacterial strain (D2AH8).

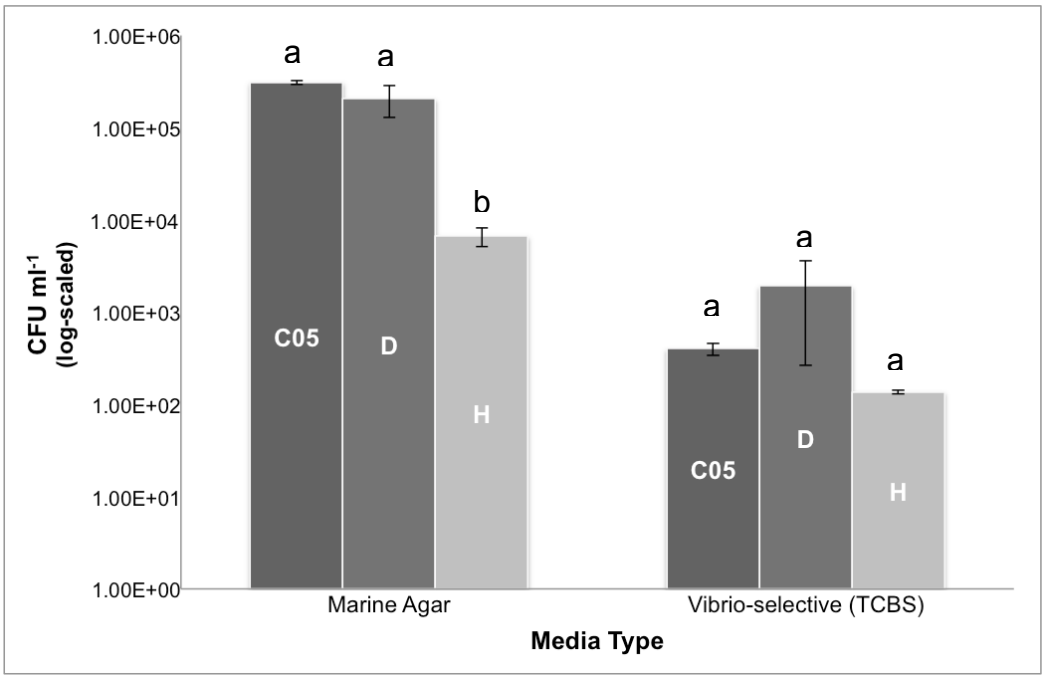


Figure 22. Mean microbial concentrations (CFU ml^{-1}) of the infected fragment (C05), diseased (D) and healthy (H) colonies in the field cultured in marine agar and a *Vibrio*-selective media (TCBS). Dissimilar letters (a,b) are significantly different. Bars = SE.

b. Inoculation via Solid Inoculum at two temperatures

At the end of the experiment (30 d), 2 of the 4 fragments that were inoculated with the bacterial strain (D2AH8) from the heated tank developed lesions at the regions where the solid inocula (sterile gauze) were attached. The lesions appeared as small (M=0.46 cm^2, SE=0.13), diffuse areas of tissue loss revealing brown or green skeleton where algae have colonized. Three days post-inoculation, one fragment (4DH) developed a small apical lesion (0.12 cm^2) (Figure 23). The lesion advanced at a rate of 0.002 $cm^2 d^{-1}$ to 0.14 cm^2 within 9 d, and then fully recovered/healed by 12 d (Figure 25). Another fragment (3DH) developed 2 medial lesions and 1 basal lesion 13 d post-inoculation (Figure 24). The 3 lesions advanced at an average rate of 0.04 cm^2 d^{-1} from a mean lesion size of 0.21 cm^2 (SE=0.02) to 0.68 cm^2 (SE=0.42) after 10 d (Figure 25). Other than these two infected fragments, none of the control fragments in the heated tank developed disease signs. Similarly, none of the fragments in the chilled tank developed disease signs including those inoculated with the experimental bacterial strain.

Mucus was sampled at the skeleton-lesion interface in both diseased fragments. Mucus samples were then serially diluted and 100 µl spread-plated in triplicate onto marine agar and TCBS media. After 24 to 48 h incubation period, no microbial growth was observed on all plates from both media types. It was suspected that the dose of cyclohexamide (100 µg/ml) added to each medium to inhibit fungal growth might have also inhibited the growth of non-target microorganisms. Hence, bacterial density could not be quantified and no colonies could be isolated for DNA sequencing and identification in this experiment.

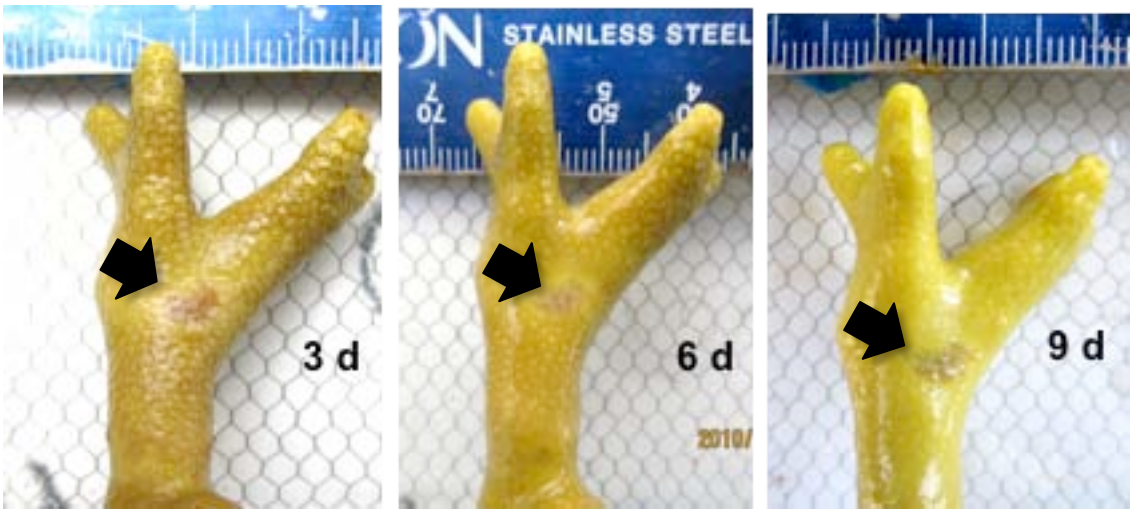


Figure 23. *P.cylindrica* fragment (4DH) from the heated tank displaying WS disease signs 3 d after inoculation with bacterial strain (D2AH8).

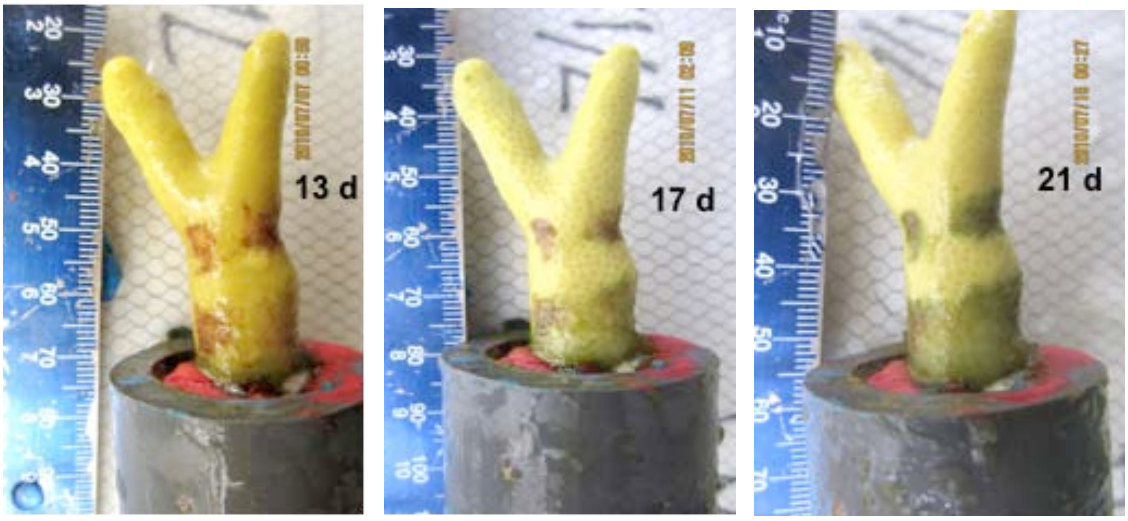


Figure 24. *P.cylindrica* fragment (3DH) from the heated tank displaying WS disease signs 13 d after inoculation with bacterial strain (D2AH8).

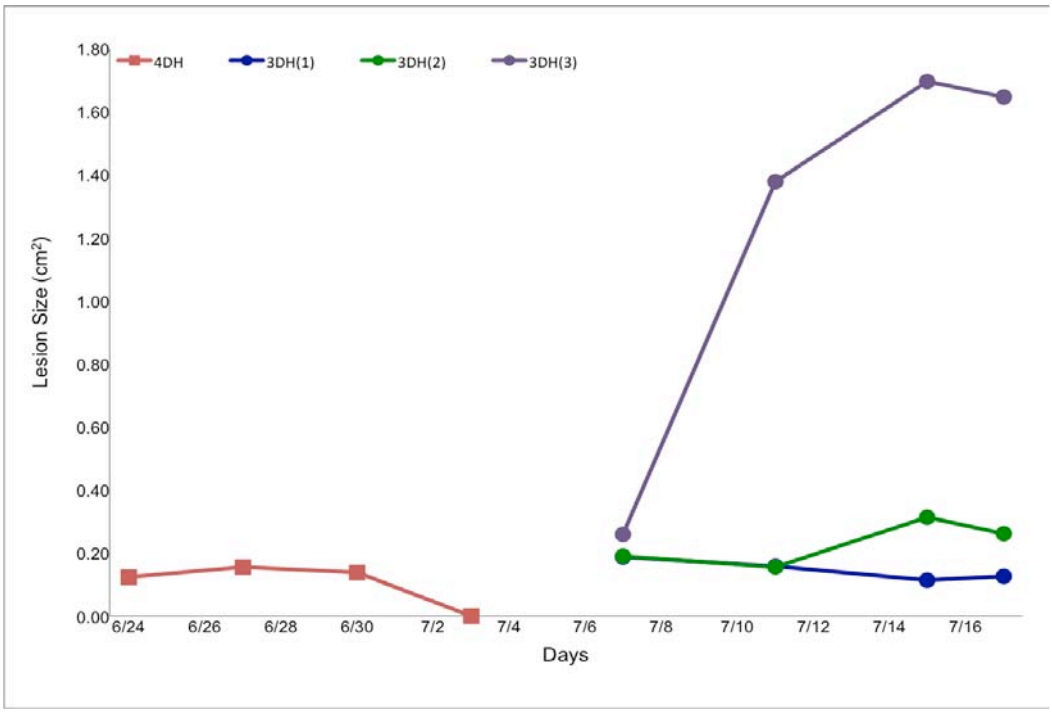


Figure 25. Lesion size (cm^2) progression in infected fragments 4DH (n=1) and 3DH (n=3) from a heated tank (32 °C) inoculated with bacterial strain (D2AH8)

3.2.2. Disease Severity Monitoring

The percent tissue loss experienced by each growth form during each census period and throughout the entire census period (relative to tissue loss at 0 d) is presented in Table 6. The results of a repeated-measures ANOVA with a Greenhouse-Geisser correction show that the mean % TL in both growth forms did not differ statistically between census periods *($F_{2.02,36.43} = 1.17, p = 0.321$)* (Figure 26). In addition, while tissue loss in both branching and massive colonies slightly relative to the first census period, there was no significant change over time and no seasonal or temporal trend was detected *($F_{2.02,36.43} = 1.23, p = 0.306$)* (Figure 27). Consequently, the Total % Change in Tissue Loss (% TC) showed no significant difference between growth forms *($F_{1,16} = 3.15, p = 0.090$)* or across colonies *($F_{2.,16} = 0.85, p = 0.450$)* (Figure 28). On the other hand, there was a significant difference *($F_{1,18} = 13.33, p = 0.002$)* in the mean incidence of new lesions between the two Poritid growth forms with branching colonies *($M = 0.374, SE = 0.322$)* experiencing a higher incidence of new (acute) lesions than massive colonies *($M = 0.208, SE = 0.322$)* over 7 months (Figure 29). These results suggest that although new lesions appeared more frequently in branching colonies, they were presumed to heal immediately and did not contribute to total tissue loss. In this case, White Syndrome equally affected both growth forms in terms of the proportion of healthy and diseased tissues over time.

Table 6. Mean tissue loss (%) (±SE) of 10 colonies per census period in branching and massive colonies

Mean Monthly Tissue Loss (%)		
Days	**BRANCHING**	**MASSIVE**
0 d	1.45±0.40	1.65±0.36
49 d	0.95±0.20	1.55±0.31
100 d	0.65±0.46	2.06±0.52
135 d	2.13±0.59	1.62±0.36
158 d	1.55±0.44	1.91±0.63
203 d	1.40±0.32	2.63±0.63
239 d	1.18±0.34	2.16±0.50
% TC (0-239 d)	-0.28±0.29	0.50±0.24

1) *One-way ANOVA with Repeated Measures:*
Monthly Tissue Loss (%)

a) Test of Within-subjects Effects

Source	SS	DF	MS	F	P
Time	1.72	2.02	0.85	1.17	0.321
Morph*Time	1.79	2.02	0.89	1.23	0.306
Error	26.36	36.43	0.72		

b) Test of Between-subjects Effects

Source	SS	DF	MS	F	P
Intercept	0.96	1	0.96	0.11	0.743
Morph	1.22	1	1.22	0.14	0.712
Error	156.29	18	8.68		

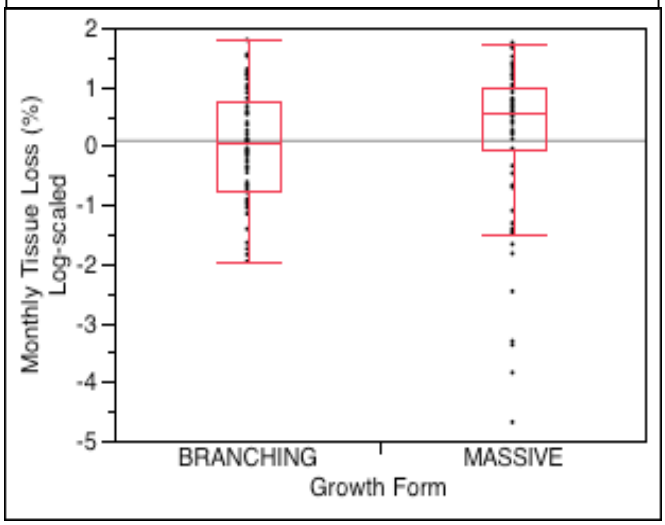


Figure 26. Analysis results and box plot of (ln transformed) Monthly Tissue Loss (%). Plot indicates no significant difference between growth forms but high variability in massive colonies.

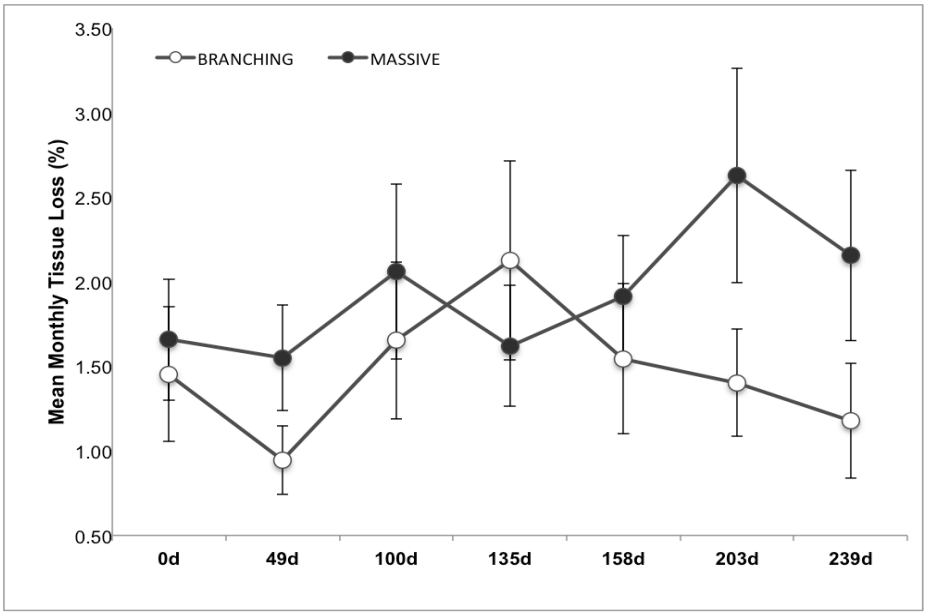


Figure 27. A comparison of the mean Monthly Tissue Loss (%) shows no significant difference between branching and massive colonies over time. Bars = SE. Data is ln(Y) transformed.

2) ***Two-way nested ANOVA Test:***
Total % Change in Tissue Loss

Source	DF	SS	MS	F	P
Growth Form	1	0.025	0.025	3.155	0.095
Colony	2	0.013	0.006	0.845	0.448
Error	16	0.141	0.008		
Total	19	0.172			

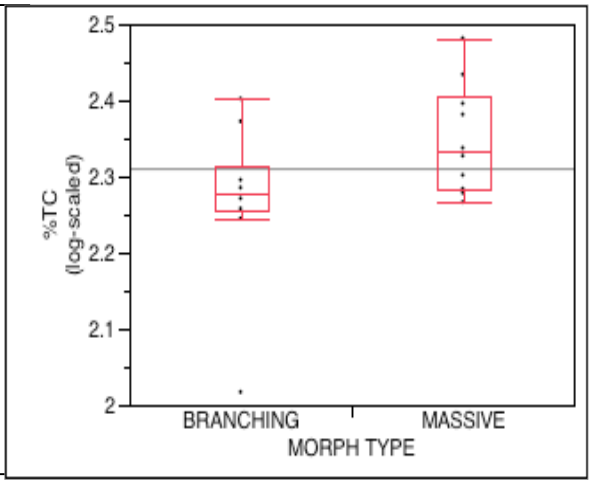


Figure 28. Analysis results and box plot of (ln transformed) Total % Change in Tissue Loss. Plot shows slight overlap and indicate no significant difference between branching and massive colonies.

3) *Two-way nested ANOVA Test:*
Disease Incidence per Colony

Source	DF	SS	MS	F	P
Growth Form	1	6.137	6.137	7.494	0.014
Colony	2	1.656	0.828	1.025	0.381
Error	16	12.929	0.808		
Total	19	20.723			

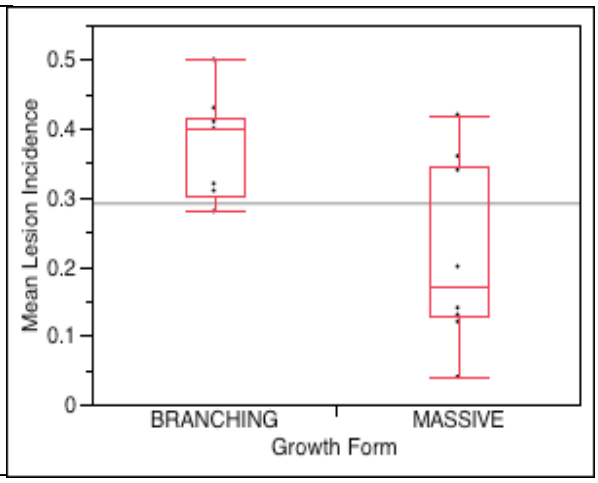


Figure 29. Analysis results and box plot of Disease Incidence per colony (n=10) between the two growth forms. Plot indicates a significant difference between branching and massive colonies. Data is Ln(Y) transformed

3.2.3. Temperature Effect on Lesion Size

Based on the run sequence plot, mean lesion sizes in both branching and massive colonies were relatively constant in size each month and no apparent seasonal pattern was observed relative to the monthly mean temperature during the entire census period. Hence, a correlation/regression analysis between lesion size and temperature was deemed not necessary. In conclusion, while temperature did not affect disease progression, temperature may play a role in other aspects of the disease not tested in this study.

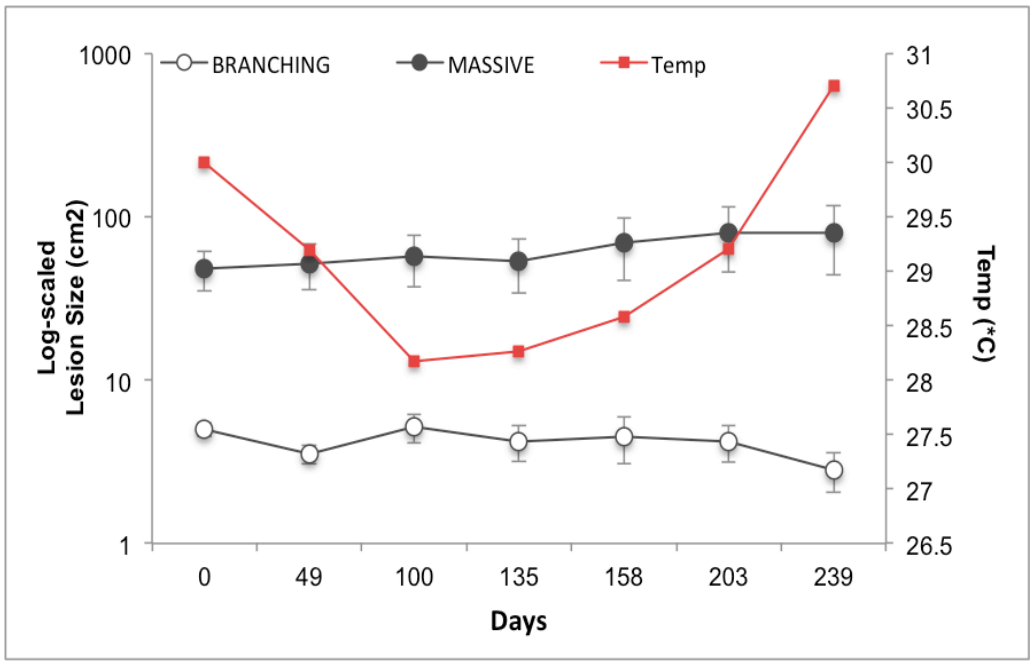


Figure 30. Semi-log (ln) plot of the colony mean lesion size (cm^2) measurements of both growth forms and temperature (°C) over a 7-mo census period (239 d). Bars = SE.

3.3. TISSUE LOSS AND RECOVERY DYNAMICS

3.3.1. Tissue Recovery Rate

The mean recovery rate ($cm^2\ d^{-1}$) achieved by each branching and massive colony (n=10) over the 7-month census period is presented in Table 7. Tissues affected by WS in branching colonies recovered at a rate of 0.06 cm^2d^{-1} (SD = 0.16) while massive colonies recovered at a rate of 0.11 cm^2d^{-1} (SD = 0.01) (Table 7). Lesion recovery rates ($cm^2\ d^{-1}$) differed significantly across colonies in both growth forms *($F_{8,40}$ =2.477, p=0.028)* but not between growth forms *($F_{1,40}$ = 4.210, p=0.070)* (Figure 31, Appendix 9). Furthermore, the results of a post-hoc test (Tukey-Kramer HSD) indicate that the source of colony variation were due to two massive colonies, MA1 *(M=0.561, SD=1.334*; colony A in Table 7) and MA2 *(M=0.087, SD=0.464;* colony B in Table 7), whose mean recovery rates varied significantly from the observed mean in massive colonies (Figure 32, Appendix 10).

The results of the linear regression show that initial lesion size (cm^2) explained a significant proportion of variance in the mean tissue recovery rate in the branching colonies *(b=0.69, t(48)=6.60, p<0.001)* and in the massive colonies *(b=0.76, t(36)= 7.09, p<0.001)* (Figure 33 and Appendix 11). The test demonstrates that the rate of recovery of the tissue surrounding an exposed coral skeleton is directly proportional to the size of the area that is available for re-sheeting. This correlation, however, explained a geometric rather than a disease-related issue. In addition, slopes are significantly different between growth forms indicating that the geometry of a lesion may not be the

only factor affecting tissue recovery once the effect of the amount of tissue available for recovery has been adjusted for by ANCOVA *($F_{1,85} = 42.45, p<0.001$)*.

A Pearson's Chi-square test was conducted to test the difference in the proportion of lesions that increased in size, decreased in size, or that healed completely between the two growth forms. The test revealed that the proportion of lesions in each state of progression significantly differed between branching and massive colonies $X^2(2, N=100)=29.08$, $p<0.05$. A significant proportion (86%) of lesions affected by WS in the branching colonies healed, while 14% decreased in size. On average, none of the lesions in the branching colonies increased in size within the 7-month census period. Conversely, 26% of the lesions in the massive *Porites* spp. increased in size while a substantial proportion either healed (42%) or decreased in size (32%). The results suggest that lesions affected by White Syndrome in both branching and massive *Porites* spp. colonies have a good chance of healing, however, lesions in massive colonies may also take longer to heal completely, allowing the disease a chance to progress given the right conditions.

Table 7. Mean (±SE) tissue recovery rate (cm^2 d^{-1}) of lesions in branching and massive colonies (n=10) that decreased in size after a 7-mo census period.

Mean Tissue Recovery Rate (cm^2 d^{-1}) per Colony		
Colony	**BRANCHING** *(n=50)*	**MASSIVE** *(n=38)*
A	0.02 ± 0.01	0.32±0.16
B	0.04 ± 0.01	0.04±0.01
C	0.03 ± 0.01	0.11±0.04
D	0.10 ± 0.05	0.11±0.05
E	0.08 ± 0.02	0.02±0.01
F	0.10 ± 0.03	0.07±0.02
G	0.05 ± 0.01	0.06±0.01
H	0.06±0.01	0.12±0.03
I	0.02±0.01	0.04±0.00
J	0.05±0.02	0.19±0.10
Total Mean	**0.06±0.01**	**0.11±0.03**

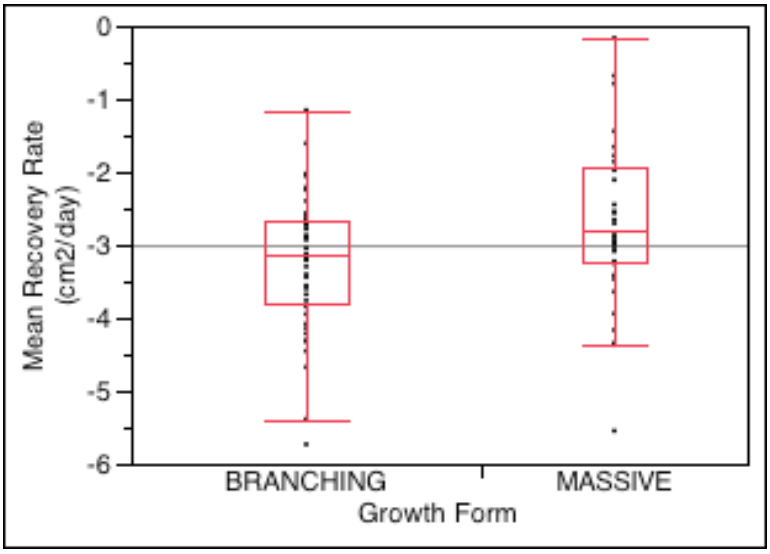


Figure 31. Box-plot of (ln transformed) mean recovery rates (cm^2 d^{-1}) of branching and massive colony lesions

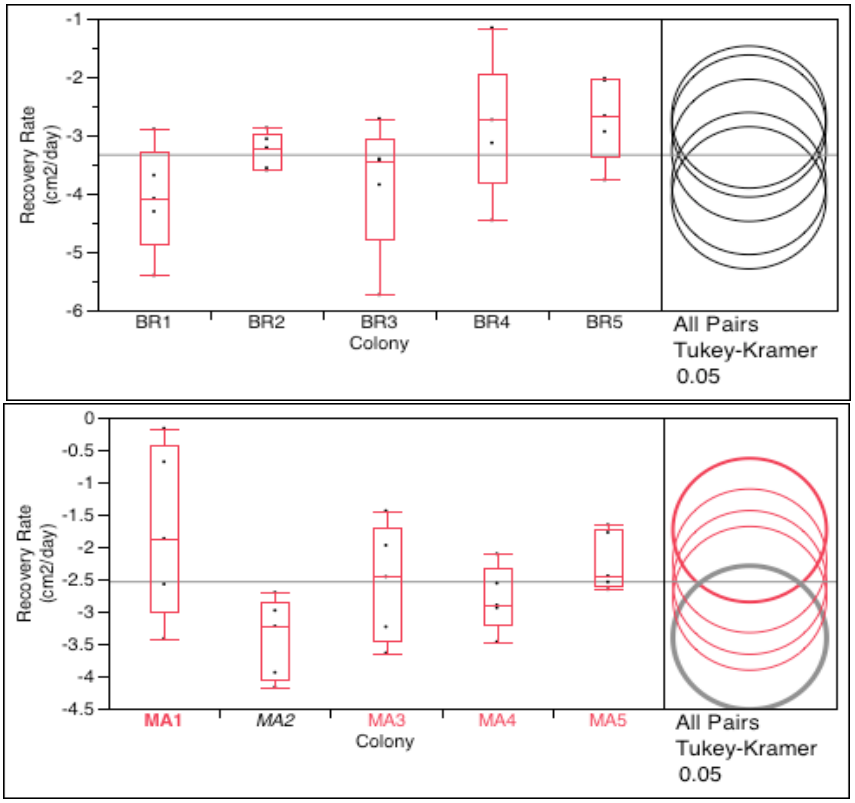


Figure 32. Comparison of mean lesion recovery rates in branching (top) and massive (bottom) colonies. Circles represent colony means wherein angle of intersection ≤ 90° indicate significant difference. Note colonies MA1 (bold red) and MA2 (grey) are significantly different from the observed mean.

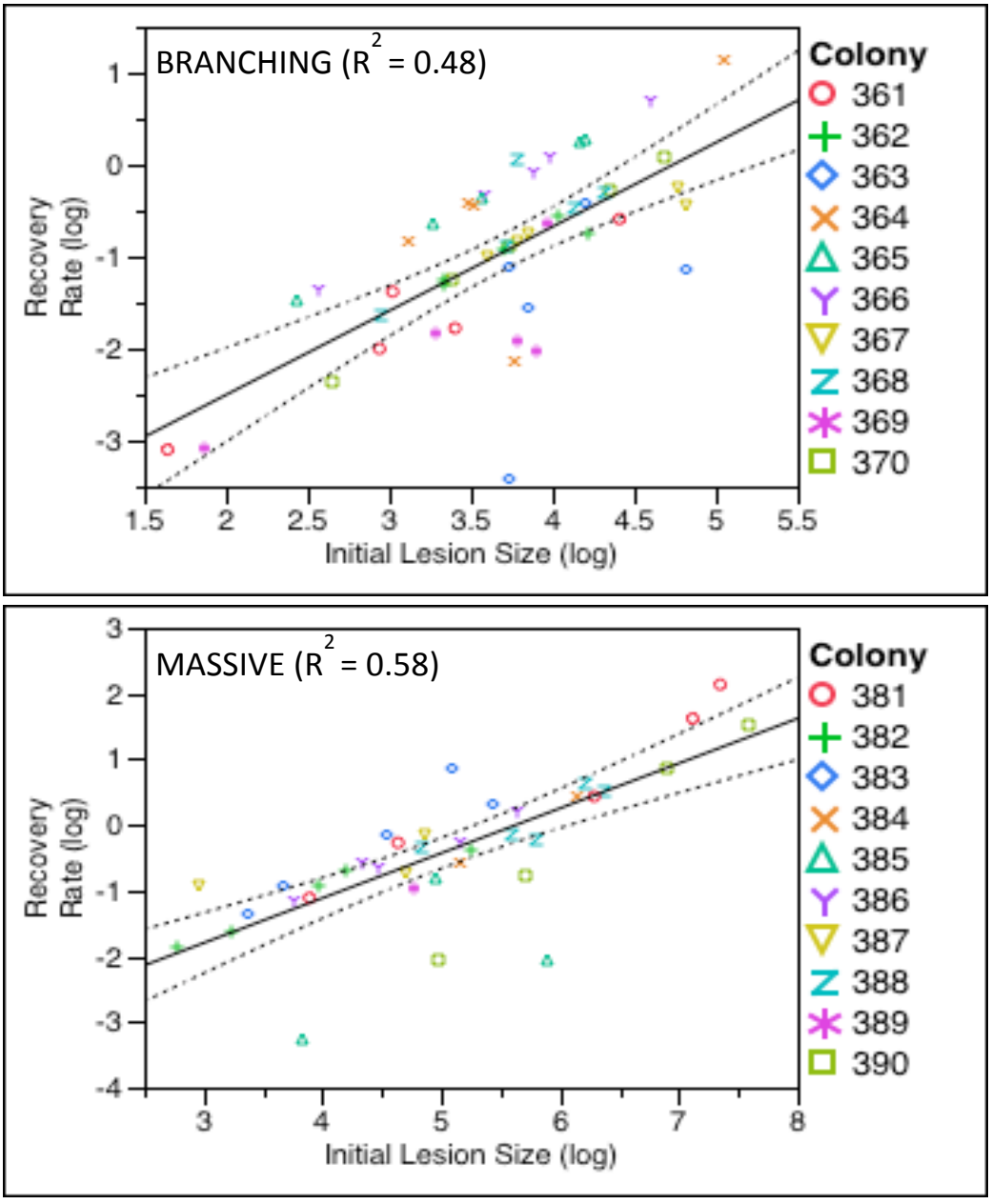


Figure 33. Linear relationship between mean recovery rate (cm^2/day) and initial lesion size (cm^2) of 50 lesions in branching colonies (top) and 38 lesions in massive colonies (bottom). Values are Ln (X,Y) scaled.

3.3.2. Lesion Size Transition Probability

BRANCHING COLONIES: Among the small lesions (0.1 to 5.9 cm^2), 36% of the lesions healed while 56% remained in stasis and only 8% increased in size. A majority (77%) of the medium-sized lesions decreased to the next size class while 17% remained in stasis and a few (7%) increased in size. Among the lesions that had already progressed or circumscribed to more than 50% the average branch size (19.65 cm^2, SE=1.43), a majority decreased size (71%) and only 29% remained in stasis.

MASSIVE COLONIES: Among the small lesions, a majority (80%) remained in stasis while 17% healed and only a few increased in size (3%). A significant proportion (91%) of the medium-sized lesions decreased in size while few progressed (8%). Notably, only a few (8%) of the larger lesions in massive colonies decreased in size. Unlike lesions in branching colonies, massive colony lesions that have attained their maximum size (71 - 1000 cm^2) remained in stasis (92%).

In addition, the result of a Fisher's Exact (2-tailed) test based on 10 lesion size subsets per growth form revealed that proportion of lesions in each size class significantly differed between the two growth forms *($p=0.019$)*

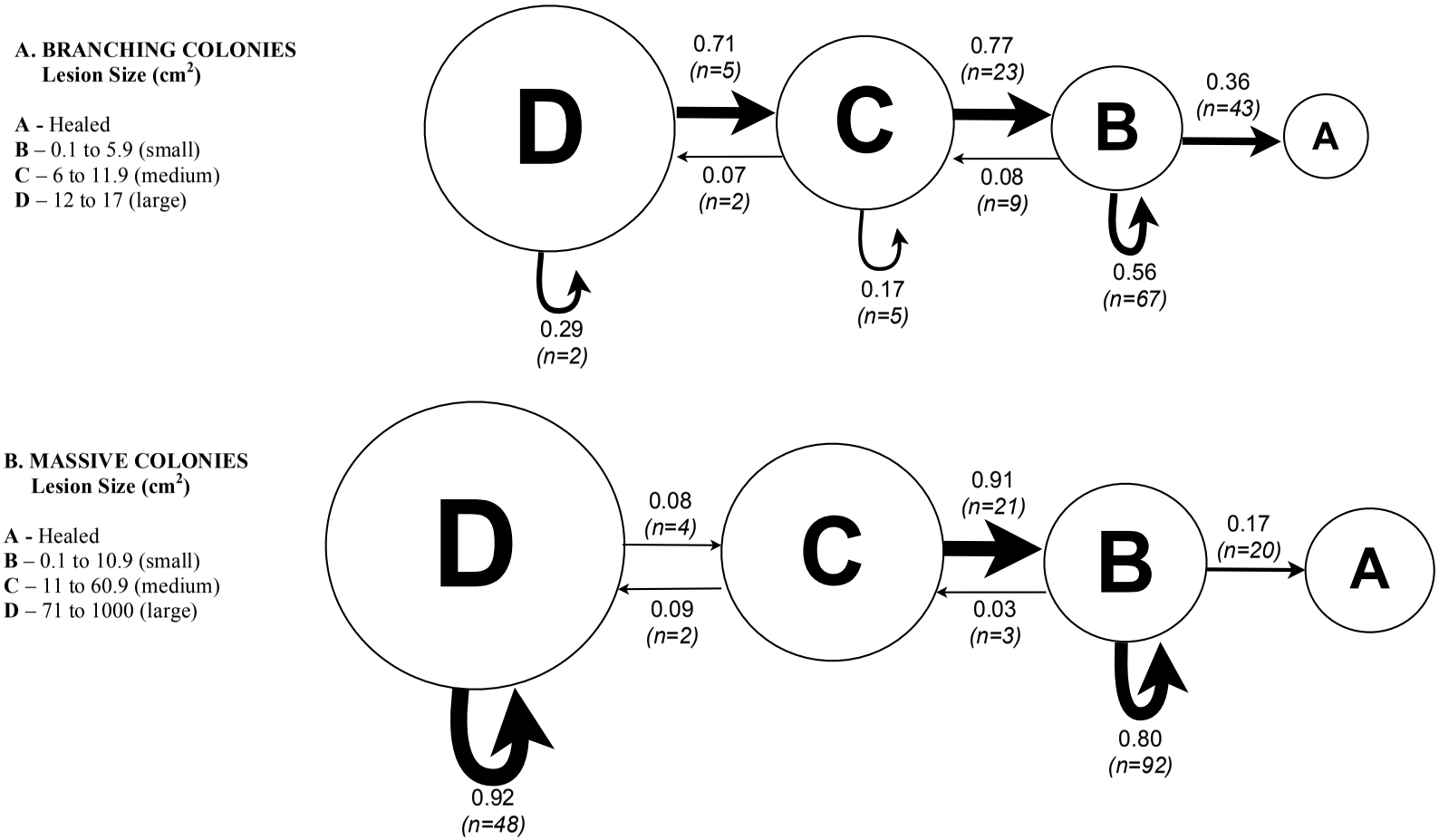


Figure 34. Probabilities of WS lesions in branching and massive *Porites* spp. transitioning between size classes in a 7-mo period. Size class D represents the largest lesion size attained after a 7-mo census. Lesion sizes ranged between **0.10 – 16.65 cm^2 in branching colonies** and **0.42 – 976.71 cm^2 massive colonies**. Note that scales vary between branching and massive colonies (see legend).

3.3.3 Corallite Properties of branching and massive *Porites* spp.

In order to rule out an effect of corallite density and size on lesion size dynamics, differences in corallite properties between the species tested were taken into account.

Branching colonies had a mean corallite density of 34 (SE= 0.94) and a mean corallite diameter of 0.11cm. Massive colonies had a mean corallite density of 34 (SE=0.62) and a mean corallite diameter of 0.12 cm. There were no significant differences in either corallite density *($F_{1,18}$ =0.125, p=0.728)* and in corallite size *($F_{1,398}$ =0.00, p=0.957)* between branching and massive colonies (Figure 35 and 36) indicating that the corallite properties, in terms of size and density, have no effect in lesion size dynamics and that differences in the tissue recovery, disease severity and lesion fate between growth forms were not attributed to variations in corallite characteristics.

One-way ANOVA

Source	DF	SS	MS	F	P
Growth Form	1	0.80	0.80	0.13	0.728
Error	18	115.00	6.39		
Total	19	115.80			

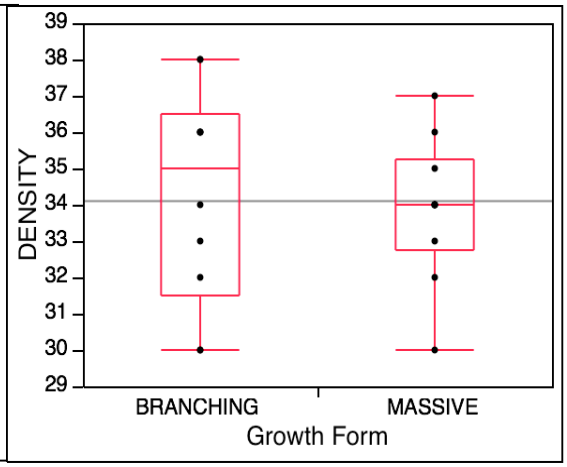


Figure 35. Box-plot of corallite densities showing a clear overlap indicating no significant difference between growth forms

One-way ANOVA

Source	DF	SS	MS	F	P
Growth Form	1	0.00	0.00	0.00	0.957
Error	398	0.04	0.00		
Total	399	0.04			

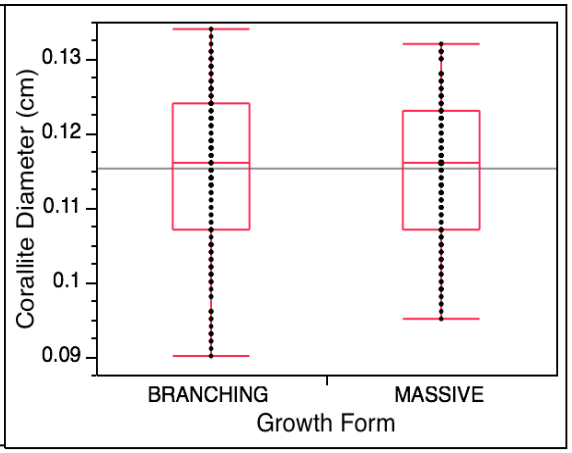


Figure 36. Box-plot of corallite size showing a clear overlap indicating no significant difference between growth forms

Chapter 4. Discussion

4.1. DISEASE CHARACTERIZATION

The Indo-Pacific White Syndrome affecting *Porites* spp. in Guam is characterized as acute and progressive tissue loss revealing small diffuse areas of bare white skeleton usually at the tips of *P. cylindrica* branches. Tissue loss is oftentimes followed by colonization and overgrowth of endolithic algae or cyanobacterial mats that give a green or brown coloration in old lesions. In massive *Porites* spp. colonies, the disease manifests as diffuse, multifocal, medium to large areas of tissue loss colonized by endolithic algae and bordered by a margin of recently exposed skeleton at the disease progressing front. Numerous lesions were observed to coalesce with adjacent lesions forming irregular patches of exposed skeleton that were invariably colonized by filamentous algae. In both growth forms, most lesions that were colonized by algae either remained in stasis or progressed to create a larger patch of tissue mortality.

Further histological examination of the tissue-lesion interface in the branching *P. cylindrica* revealed tissue necrosis associated with endolithic algae, characterized by dense aggregations of eosinophilic granular ameobocytes and a proliferation of pigment cells and bacterial aggregates where algal infiltrates were numerous.

Corals, like other invertebrates, have an immune system based on self or non-self recognition and cellular or humoral processes (Mydlarz et al., 2010). As clonal invertebrates, corals rely on physiochemical barriers and cellular processes as a first line

of defense against potential etiologic agents such as bacterium, fungus, and parasite virus. Amoebocytes, which are coral immune cells, wander throughout the mesoglea and have been demonstrated to aggregate near skeletal anomalies and hyperpigmented areas in *Porites* spp. (Domart-Coulon et al., 2006; Palmer et al., 2008). Prophenoloxidase (PPO) is also an integral part of the innate immune effector processes in invertebrates and is involved in wound healing, encapsulation, parasite and disease resistance, and the general coordination of immune responses (Mydlarz et al., 2008; Nappi and Christensen, 2005). Melanin, the end-point of the PPO cascade in corals is a potent physiochemical barrier (Mydlarz et al., 2008; Palmer et al., 2008). Consistent with the findings of this study, a recent study documenting the immune processes involved in the wound-healing of *P.cylindrica* suggests that the pronounced hyalination and aggregation of eosinophilic granular amoebocytes and pigment cells in injured tissue sections of the coral are indicative of increased immune-related activities in the coral host (Palmer, 2011). Another experimental study posits that that the colonization and overgrowth of bacterial aggregates in the tissues of thermally-stressed corals is a response to environmental stressors that severely alter the natural bacterial community dynamics in corals (Ainsworth and Hoegh-Guldberg, 2009). Based on these findings, it is suspected that endolithic algae, readily visible both in gross and microscopic coral tissues, play a role in the disease process either as a source of secondary infection or an opportunistic secondary colonizer of available substrate.

While full tissue necrosis could also be caused by viruses detected in light microscopy through the formation of intracytoplasmic or intranuclear inclusions (Sparks, 1985), there was no evidence of these structures in all of the diseased tissue samples

examined. The association of viruses with WS should, however, not be discounted at this time since *Vibrio coralliilyticus*, the causative agent of white syndrome affecting *Montipora aequituburculata* (Sussman et al., 2008), was isolated from mucus samples of *P. cylindrica* displaying disease signs in an inoculation experiment discussed in the following section.

4.2. DISEASE INFECTIOUSNESS

The results of the transmission experiment indicate that WS affecting *Porites cylindrica* is transmissible both by direct contact and through the water column. These findings are similar to the transmission experiments done by Raymundo et al. (2003) on *Porites* ulcerative white spot disease (PUWS) wherein infection of the disease was also observed to be possible both through direct contact and through the water column. Similarly, *Montipora* white syndrome (Aeby et al., 2010) and the Australian subtropical white syndrome (Dalton et al., 2010) were both found to be transmissible through direct contact, although waterborne transmission was not observed in both studies.

Corals are frequently subjected to tissue or skeletal damage or injuries from a variety of disturbances in the reef. Injuries may be caused by fish bites, boring and corallivorous organisms, algal abrasion and overgrowth, storm damage, and anthropogenic activities (Rotjan and Lewis, 2008; Jompa and McCook, 2003; Edmunds and Witman, 1991; Bythell et al., 1993; Bythell et al., 2000; Fang and Shen, 1988). Over the course of the census period, the coralliophilid gastropod, *Coralliophila violacea* was present in some of the tagged colonies with some colonies displaying numerous feeding

scars. In this case, *Coralliphila violacea* could have potentially facilitated the progression and prevalence of the disease by providing a point of entry for a waterborne pathogen via feeding scars. Furthermore, a study by Williams and Miller (2005) demonstrated that coralliophilid gastropods are capable of transmitting coral disease and have the tendency to move to adjacent healthy colonies after their host dies (Knowlton et al., 1990; Baums et al., 2003). This suggests that *Coralliophila violacea,* if exposed to the pathogenic agent, is capable of vectoring the disease across nearby *P. cylindrica* colonies as well. A laboratory-based experiment demonstrated that 26% of the feeding scars left by *Coralliophila violacea* showed progressing tissue loss after the snails were removed suggesting an association between corallivorous snails and disease (Raymundo, unpublished data). The implications of these findings suggest that the disease has the potential to spread across colonies and to geographically distant habitats through both modes of transmission and additionally through the aid of a disease vector.

4.3. CAUSATION

Following the transmission experiment, bacterial isolates from diseased fragments were reinoculated into healthy fragments both as a liquid and a solid inoculum. While mucus samples from infected colonies inoculated via solid media could not be cultured, the ones inoculated via liquid inoculum were successfully cultured, reisolated and analyzed for DNA sequencing. Based on the near complete 16S rRNA gene sequence comparison, the strain was found to share 99% sequence identities with *Vibrio coralliilyticus*, a pathogen previously demonstrated to cause bleaching and lysis of

Pocillopora damicornis in Zanzibar (Ben-Haim and Rosenberg, 2002) and was also confirmed to be the causative agent of another type of white syndrome affecting *Montipora aequituburculata* in the Great Barrier Reef (Sussman et al., 2008).

The fulfillment of Koch's postulates has classically been the 'gold standard' for determining causation in a number of suspected terrestrial wildlife and human diseases. However, fulfilling Koch's postulates is a challenging and lengthy process particularly for marine disease, in part due to the complex nature of the host-pathogen relationship, the possibility that most infectious diseases are polymicrobial and the inability of Koch's postulates to incorporate changes in host susceptibility or pathogen virulence with changes in the marine environment (Richardson 1998; Sutherland et al., 2004). Furthermore, pathogens that cause diseases and syndromes span most phyla. For instance, Yellow Band disease and several white diseases are caused by bacterial pathogens (Cervino et al., 2008; Denner et al., 2003; Rosenberg and Ben-Haim, 2002; Sussman et al., 2008), while sea fan Aspergillosis is caused by fungal pathogens (Kim and Harvell, 2004). Red and Black Band Disease are triggered by cyanobacteria (Page and Willis, 2006; Richardson, 1992; Richardson and Kuta, 2003) and Skeletal Eroding Band and Brown Band are caused by ciliates (Croquer, 2006; Page and Willis, 2008; Bourne et al., 2008). More confounding is the fact that many marine bacteria are viable but not culturable in the laboratory by conventional means (Rappe and Giovannoni, 2003) and only through culture-independent methods will the identification and quantification of bacteria associated with a disease be made possible (Fredricks and Relman, 1996; Ritchie et al., 2001).

Similar challenges were encountered in this study in the attempt to fulfill Koch's postulates. Inducing the disease signs in the laboratory was poorly replicated indicated by the few successfully infected fragments and necessitates a repetition of the experiments. Regardless, the application of Koch's postulates in this study aided in elucidating the etiology of WS by demonstrating that (i) WS disease signs, although not the etiologic agent, were present in each diseased individual; (ii) the pathogenic strain was isolated in pure culture; (iii) infection of healthy corals with the putative pathogen in controlled aquarium experiments resulted in WS disease signs; and that (iv) the pathogen was reisolated from infected corals. However, the challenges encountered during the laboratory experiments suggest that determining causality based on Koch's postulates alone is not ideal and that along with the results of these experiments, other underlying factors involved in the disease process must be carefully considered before causation is assumed. The fact that endolithic algae were found in the diseased tissues while *Vibrio* spp. was isolated from infected fragments but was not detected in histopathology suggest that WS may be a multifactorial disease. *Vibrio* spp. and endolithic algae could both be etiologic agents of WS but operate at different stages in the disease process, possibly initiated by an environmental stressor that was not detected in this study. It is also possible that *Vibrio* spp. directly induces the disease symptoms by some common pathogenic mechanism and that endolithic algae and other metazoans represent several environmental cofactors acting as sources of secondary infection in the disease process.

4.4. DISEASE SEVERITY, TISSUE RECOVERY, AND THE EFFECT OF MORPHOLOGY ON LESIONS SIZE DYNAMICS

Several studies suggest that regeneration in colonial corals is fueled by a definite and limited energetic resource and that lesions that are < 1 cm^2, such as those observed in the branching colonies, are well within the regeneration capabilities of corals (Bak et al., 1977; Bak and Steward-van Es 1980; Bak, 1983; Meesters et al., 1992; Meesters and Bak 1993). Lesions that are too large and are energetically costly to heal may result in a long-lasting dead spot prone to colonization by spatial competitors such as filamentous algae (Bak and Steward-van Es, 1980). Over time, algae can then impede the process of regeneration and interfere the re-sheeting of new tissue since it is predisposed to increase in biomass acting as a trap for sediment. The presence of a solid mat of algae and sediment may then present an obstruction difficult for the coral to overgrow, presumably as a consequence of the reallocation of available resources from tissue regeneration to competition (Hall, 1997, 2001). Therefore, lesions such as those observed in the massive colonies that are too large to be recovered within the regeneration time may never heal and can accumulate over time (Bak and Steward-van Es 1980). Based on field observations, algal overgrowth was observed in most of the massive colony lesions; however, the presence of these colonizers did not deter lesion recovery but may have impeded lesions from healing completely. This is consistent with the sizeable proportion of large lesions in the massive colonies that remained in stasis with only a few that recovered or healed, wheareas most lesions in the branching colonies, even after progressing to more than 50% the mean branch size, recovered or healed completely. Recovery is also influenced by the amount of tissue bordering the injury with

regeneration initiated by the polyps along the lesion perimeter (Meesters et al., 1997). This supports the concept that resources required for regeneration can be drawn from the tissue surrounding the lesion, and that if the ratio of damaged to undamaged polyps within a certain area of the colony is low enough to support regeneration, lesions may recover independently of colony size (Meesters et al., 1994). In the current study, given that recovery rate was computed as the change in lesion area over time, recovery is then influenced by the number of polyps producing new tissue, proportionate to the area of denuded skeleton, depositing tissue at a fairly constant rate along the lesion perimeter. It is then assumed that the geometry of a lesion would then strongly influence the rate of recovery and that large lesions, having more polyps around the lesion perimeter, would recover faster than small lesions (*sensu* Pain and Levin, 1981; Fonseca et al., 2004). This correlation was found to be consistent with the findings of this study wherein recovery rate was positively correlated to the lesion area when testing the relationship between initial lesion size and recovery rate in both growth forms. This geometric relationship also explains why lesions in branching colonies, having fewer polyps along the lesion perimeter, were found to have slower recovery rates than massive colonies. Based on the results gathered, it appears that recovery is not only linked to the geometry of a lesion but presumably on other physiological immune-defense mechanisms, as well its ability to outcompete organisms that have settled onto the injured area (Hall, 2001).

Given that massive colonies have larger surface areas than branching colonies, massive colonies typically developed larger lesions as shown on the lesion size transition probability matrices, indicating that lesion size is influenced by morphology. While significance tests did not find differences in the recovery rates and tissue mortality

between the two growth forms, presumably because massive *Porites* spp. lesions were highly variable in size, most of the large lesions were also shown to remain in stasis and may take longer to heal. Large wounds such as those in the massive *Porites* spp. colonies are then more prone to secondary infections from opportunistic pathogens and substrate colonizers, contributing to colony mortality. Corallivorous snails such as *Drupella* spp. have been demonstrated to occupy and graze over dead coral substrate rather than live coral tissue (Cumming, 2000). A colony with a large persistent lesion such as those in massive colonies would then be more susceptible to chronic predation by *Drupella* spp. Furthermore, corallivores such as chaetodontids that have been found to specifically target diseased tissue (Cole et al., 2009), can also vector the disease to nearby colonies or to geographically distant reefs increasing the prevalence and incidence of the disease.

Finally, it is also important to note that regeneration requires a trade-off in the energy allocation for colony growth, reproduction, resistance to disease and competition (Bak, 1983; Rinkevich and Loya, 1989; Harrison and Wallace, 1990, Van Veghel et al., 1994; Bak and Criens, 1981). A coral that is continuously regenerating large injuries will likely have reduced defenses and therefore susceptible to attack by predators, competitors and disease, and with less optimal microhabitat characteristics, leading to more abiotic impacts (Cumming, 2002). And while branching *P. cylindrica* colonies were found to have a higher WS disease incidence (expressed as the number of new acute lesions per colony), massive *Porites* spp. colonies are likely to be at a greater risk of total tissue mortality, especially during large-scale disturbances, due to the wide variability in the size of its injuries and its tendency to sustain large injuries that remain in stasis that are energetically costly to heal, thus reducing overall fitness.

4.5. CONCLUSIONS AND FUTURE DIRECTIONS

Overall, the findings of this study contribute substantially to our understanding of a previously uncharacterized coral disease that predominantly affects *Porites* spp., a key reef-building genus dominant not only in Guam, but most of the Indo-Pacific. The study described the gross and histological manifestations of the disease and demonstrated that disease dynamics, to some extent, is influenced by the host's morphology. While significance tests showed no difference in the rate of tissue recovery and mortality between branching and massive *Porites* spp., this study presented evidence, nonetheless, that branching colonies tend to develop small lesions that are able to fully recover while massive colonies develop larger lesions that tend to remain in stasis and require high resource allocation for regeneration. In addition, this study found that WS is infectious within species of *Porites cylindrica* and is transmissible both by direct contact and through the water column. An attempt to isolate the microbial pathogen suggests that WS may be associated with a member of the bacteria family Vibrionaceae that is 99% similar to the coral bleaching pathogen, *Vibrio coralliilyticus*. Histopathology also revealed an association of cellular structures involved in the activation of immune-related mechanisms in areas where algal infiltrates were numerous. Furthermore, the absence of viruses in diseased tissues and its presence in mucus samples in infected individuals suggest that WS may be a multifactorial disease that involves *Vibrio* spp. and endolithic algae operating at different stages in the disease process, coupled with other environmental factors that were not detected in this study. Future research will attempt to confirm these associations through additional testing and increased replication. Furthermore, due to logistical and time constraints, infection experiments and

histopathology examinations on massive *Porites* spp. were not undertaken in this study. However, it is important to know whether the same etiologic agents are present in both growth forms to allow for a better characterization of the White Syndrome.

Finally, the relationship between colony morphology and disease dynamics has implications for the management of coral reefs. If colony structure is a reliable predictor of resistance, it may be possible to use the information on the morphological composition of coral assemblages to predict the susceptibility of corals to possible epizootics or large-scale disturbances. An ability to predict the susceptibility of corals to disease, bleaching, or other forms of damage based merely on its morphologies is a novel and less invasive way to assess and manage reefs especially in small and locally-managed marine protected areas, and can be critical to informed and effective management

References

Aeby, G.S. and D.L. Santavy. (2006). Factors affecting susceptibility of the coral *Montastraea faveolata* to black-band disease. *Marine Ecology Progress Series* 318: 103-110.

Aeby, G, Ross, M., Williams, G., Lewis, T., and Work, T. (2010). Disease dynamics of Montipora white syndrome within Kaneohe Bay, Oahu, Hawaii: distribution, seasonality, virulence, and transmissibility. *Disease of Aquatic Organisms* 91: 1–8.

Ainsworth, T.D., Kramasky-Winter, E., Loya,, Y., and Hoegh-Guldberg, O. (2007). Coral Disease Diagnostics: What's between a Plague and a Band? *Applied and Environmental Microbiology* 73 (3): 981-992.

Ainsworth, T.D., Kvennefors, E.C., Blackall, L.L., Fine, M. and Hoegh-Guldberg, O. (2007). Disease and cell death in white syndrome of Acroporid corals on the Great Barrier Reef. *Marine Biology,* 151: 19-29.

Ainsworth, T., and Hoegh-Guldberg, O. (2009). Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. *Aquatic Biology* 4: 289–296.

Antonius, A. and B. Riegl (1997). A possible link between coral diseases and a corallivorous snail (*Drupella cornus*) outbreak in the Red Sea. *Atoll Research Bulletin* 447: 1-9.

Bak, R.P.M. (1977). Coral reefs and their zonation in Netherland Antilles. In *Reefa sn Related Carbonates–Ecology and Sedimentology,* eds. S. Frost, M. Weiss and J. Saunders, community patterns. Tulsa: American Association of Petroleum Geologists.

Bak, R.P.M. and Y. Steward-van Es (1980). Regeneration of superficial damage in the scleractinian corals *Agaricia agaricites f. purpurea* and *Porites astreoides*. *Bulletin of Marine Science* 30: 883-887.

Bak, R.P.M. and Criens, S.R. (1981). Survival after fragmentation of colonies of *Madracis mirabilis, Acropora palmata,* and *Acropora cervicornis* (Scleractinia) and the subsequent impact of a coral disease. In *4th Int'l. Coral Reef Symposium, Manila.* 221-227.

Bak R.P.M. (1983). Neoplasia regeneration and growth in the reef-building coral *Acropora palmata*. *Marine Biology* 77: 221-227

Banin, E., Israely, T., Kushmaro, A., Loya, Y., Orr, E., and Rosenberg, E. (2000). Penetration of the coral-bleaching bacterium *Vibrio shiloi* into *Oculina patagonica*. *Applied and Environmental Microbiology* 66(7): 3031-3036.

Baums I.B., Miller M.W., and Szmant A.M. (2003). Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on 2 scleractinian hosts. I. population structure of snails and corals. *Marine Biology* 142: 1083–1091.

Bayer, F.M., Grasshoff, M. and Verseveldt, J. (1983). Illustrated Trilingual Glossary of Morphological and Anatomical Terms Applied to Octocorallia. Leiden: E.J. Brill, Dr. W. Backhuys. The Netherlands.

Bellwood D.R., Hughes T.P., Folke C., Nystrom, M. (2004). Confronting the coral reef crisis. *Nature* 429: 827–833.

Ben-Haim, Y. and Rosenberg, E. (2002). A novel Vibrio sp. pathogen of the coral Pocillopora damicornis. *Marine Biology* 141: 47-55.

Bernier, J., Brousseau, P., Krzystyniak, K., Tryphonas, H., and Fournier, M. (1995). Immunotoxicity of heavy metals in relation to Great Lakes. *Environmental Health Perspectives* 103: 23–34.

Bourne, D., Boyett, H., Henderson, M., Muirhead, A. and Willis, B. (2008a). Identification of a ciliate (Oligohymenophorea: Scuticociliatia) associated with brown band disease on corals of the Great Barrier Reef. *Applications in Environmental Microbiology* 74: 883.

Bruckner, A.W. and R.J. Bruckner (1998d). Treating coral disease. *Coastlines*. 8.3: 10-11.

Bruckner, A.W. (1999). Black band disease (BBD) of scleractinian corals: occurrence, impacts and mitigation. Doctor of Philosophy Dissertation. University of Puerto Rico.

Bruno, J.F., Petes, L.E., Harvell, C.D., and Hettinger, A. (2003). Nutrient enrichment can increase the severity of coral diseases. *Ecology Letters* 6(12): 1056–1061.

Bruno, J.F., Selig, E.R., and Freckleton, R. (2007). Regional Decline of Coral Cover in the Indo-Pacific: Timing, Extent, and Subregional Comparisons. *PLoS One* 2(8): e711.

Burdick, D., Brown, V., Asher, J., Gawel, M., Goldman, L., Hall, A., Kenyon, J., Leberer, T., Lundblad, E., Mcilwain, J., Miller, J., Minton, D., Nadon, M., Pioppi, N., Raymundo, L., Richards, B., Schroeder, R., Schupp, P., Smith, E. and Zglickzynski, B. (2008). The State of Coral Reef Ecosystems of Guam. 1-45.

Bush, A.O. (2001). Parasitism: the Diversity and Ecology of Animal Parasites, Cambridge University Press.

Bythell, J.C., Bythell, M., and Gladfelter, E.H. (1993) Initial results of a long-term monitoring program-impact of hurricane Hugo at Buck Island reef national monument, St Croix, United States Virgin Islands. *Journal of Experimental Marine Biology and Ecology* 172: 171–183

Bythell, J.C., Hillis-Starr, Z.M., Rogers, C.S. (2000). Local variability but landscape stability in coral reef communities following repeated hurricane impacts. *Marine Ecology-Progress Series* 204: 93–100.

Bythell J.C., Pantos, O., Richardson, L. (2004). White plague, White Band and other "White" diseases. In: E. Rosenberg and Y. Loya (eds). Coral Health and Disease. Springer-Verlag, Berlin.

Carilli, J.E., Norris, R.D., Black, B.A., Walsh, S.M. and McField, M. (2009). Local Stressors Reduce Coral Resilience to Bleaching. *PLoS One* 4: 5.

Cervino, J., Hayes, R., Goreau, T.J. and Smith, G.W. (2004) Zooxanthellae regulation in Yellow Blotch/Band and other coral diseases contrasted with temperature related bleaching: In situ destruction vs. expulsion. *Symbiosis,* 37: 63-85.

Cervino, J.M., Thompson, F.L., Gomez-Gil, B., Lorence, E.A., Goreau, T.J., Hayes, R.L., Winiarski-Cervino, K.B., Smith, G.W., Hughen, K. and Bartels, E. (2008). The Vibrio core group induces yellow band disease on Caribbean and Indo- Pacific reef-building corals. *Journal of Applied Microbiology.* 105: 1658-1671.

Chadwick, N. and Loya, Y. (1990) Regeneration after experimental breakage in the solitary reef coral *Fungia granulosa* Kluzinger, 1879. *Journal of Experimental Marine Biology and Ecology,* 142: 221-234.

Cole A.J., Chong Seng K.M., Pratchett M.S., Jones G.P. (2009) Coral-feeding fishes slow progression of black band disease. *Coral Reefs* 28:965

Couch, C., (2010). Personal communication. Department of Ecology and Evolutionary Biology. Cornell University.

Croquer, A., Bastidas, C., Lipscomb, D., Rodriguez-Martinez. R.E., Jordan-Dahlgreen, E. and Guzman, H.M**.** (2006). First report of folliculinid ciliates affecting Caribbean scleractinian corals. *Coral Reefs* 25: 187-191.

Cumming, R.L. (2000) Distinguishing predation injuries inflicted by Drupella and Acanthaster. *Reef Encounter* 27: 12–13

Cumming, R.L. (2002). Tissue injury predicts colony decline in reef-building corals. *Marine Ecology Progress Series.* 242: 131-141.

Dalton, S. J., Godwin, S., Smith, S.D.A., and Pereg, L. (2010). Australian subtropical white syndrome: a transmissible, temperature-dependent coral disease. *Marine and Freshwater Research* 61: 342.

de Meeus, T., and Renaud, F. (2002). Parasites within the new phylogeny of eukaryotes. *Trends in Parasitology* 18: 247–251.

Denner, E.B.M., Smith, G.W., Busse, H.J., Schumman, P., Narzt, T., Polson, S.W., Lubitz, W., and Richardson, L.. (2003). *Aurantimonas coralicida* gen. nov. sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. *Internationl Journal of Systematic and Evolutionary Microbiology* 53: 1115-1122.

Diaz, M. and J. Madin (2011). Macroecological relatioships between coral species' traits and disease potential. *Coral Reefs* 30: 73-84.

Dietz, R., Ansen, C.T., Have, P., Heidejorgensen, M.P., (1989). Clue to seal epizootic. *Nature* 338: 627.

Domart-Coulon, I.J., Traylor-Knowles, N., Peters, E., Elbert, D., Downs, C. A., Price, K., Stubbs, J., McLaughlin, S., Cox, E., Aeby, G., Brown, P.R., Ostrander, G.K. (2006). Comprehensive characterization of skeletal tissue growth anomalies of the finger coral *Porites compressa*. *Coral Reefs* 25: 531-543.

Dorland, W.A.N. (2000). Dorland's Illustrated Medical Dictionary, 29th ed. W.B. Saunders and Company.

Dubey, J., Graham, D., De Young, R., Dahl, E., Eberhard, M., Nace, E., Won, K., Bishop, H., Punkosdy, G., and Sreekumar, C. (2004). Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. *Journal of Parasitology* 90: 67–71.

Duerden, J. (1902). West Indian madreporarian polyps. *Memoirs of the National Academy of Sciences* 8: 402-597.

Dustan, P. (1977). Vitality of reef coral populations off Key Largo, Florida: recruitment and mortality. *Environmental Geology,* 2: 51-58.

Edmunds P.J. and Witman J.D. (1991). Effect of Hurricane Hugo on the primary framework of a reef along the south shore of St. John, US Virgin Islands. *Marine Ecology Progress Series* 78: 201–204.

Efrony, R., Atad, I., and Rosenberg, E. (2009). Phage therapy of coral White Plague Disease: properties of Phage BA3. *Current Microbiology* 58: 139-145.

Efrony, R., Loya, Y., Bacharach, E. and Rosenberg, E. (2007). Phage therapy of coral disease. *Coral Reefs* 26: 7-16.

Ewald P. (1987). Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* 503: 295 – 306

Ewald P. (1994). Evolution of infectious disease. Oxford University Press, Oxford

Fautin, D. G. 2005. Interactive Glossary of Actinarian (Sea Anemone) Anatomical Terms.

Fang, L.S., Shen, P. (1988). A living mechanical file - the burrowing mechanism of the coral-boring bivalve *Lithophaga nigra*. *Marine Biology* 97: 349–354.

Fonseca, M. S., Whitfield, P.E., Judson Kenworthy, W., Colby, D.R., and Julius, B.E. (2004). Use of two spatially explicit models to determine the effect of injury geometry on natural resource recovery. *Aquatic Conservation: Marine and Freshwater Ecosystems* 14: 281–298.

Fredricks, D.N., and Relman, D.A., 1996. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews* 9: 18–33.

Galloway S.B., Work T.M., Bochsler V.S., Harley R.A., Kramarsky-Winters E., McLaughlin S.M., Meteyer C.U., Morado J.F., Nicholson J.H., Parnell P.G., Peters E.C., Reynolds T.L., Rotstein D.S., Sileo L., and Woodley C.M. (2007). Coral Disease and Health Workshop: Coral Histopathology II. NOAA Technical Memorandum NOS NCCOS 56 and NOAA Technical Memorandum CRCP 4. National Oceanic and Atmospheric Administration, Silver Spring, MD. 1-86.

Geffen, Y. and E. Rosenberg (2005). Stress-induced and rapid release of antibacterials by scleractinian corals. *Marine Biology* 146(5): 931-935.

Geiser, D.M., Taylor, J.W., Ritchie, K.B., and Smith, G.W. (1998). Cause of sea fan death in the West Indies. *Nature* 394: 137–138.

Gladfelter, W.B., Gladfelter, E.H., Monahan, R.K., Ogden, J.C., and Dill, R.F. (1977). Coral destruction, environmental studies of Buck Island Reef National Monument. Prepared for the US Nat Park Serv. U.S. Department of Interior. Biosphere Reserve Research Report No. 6.

Goldberg, W. M., and Taylor, G.T. (1996). Ultrastructure of the spirocyst tubule in black corals (Coelenterata: Antipatharia) and its taxonomic implications. *Marine Biology* 125: 655-662.

Green D.H., Edmunds, P.J., Carpenter, R.C. (2008). Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series* 359: 1–10.

Green, E. and A.W. Bruckner. (2000). The significance of coral disease epizootiology for coral reef conservation. *Biological Conservation* 96(3): 347-361.

Hall, V. (1997). Interspecific differences in the regeneration of artificial injuries on scleractinian corals. *Journal of Experimental Marine Biology and Ecology*. 212: 9-23.

Hall, V. (2001). The response of *Acropora hyacinthus* and *Montipora tuberculosa* to three different types of colony damage: scraping injury, tissue mortality and breakage. *Journal of Experimental Marine Biology and Ecology*. 264:209-223.

Harder, T.C. and A.D.M.E. Osterhaus (1997). Canine distemper virus — a morbillivirus in search of a new host? *Trends in Microbiology* 5: 120–124.

Harrison, P.L. and C.C. Wallace (1990). Reproduction, dispersal, and recruitment of scleractinian corals. In *Ecosystems of the World,* ed. Z. Dubinsky, 133-207. Amsterdam: Elsevier.

Harvell, C. D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hofmann, E.E., Lipp, E.K., Osterhaus, ADME, Overstreet, R.M., Porter, J.W., Smith, G.W., Vasta, G.R. (1999). Emerging Marine Diseases: Climate Links and Anthropogenic Factors. *Science* 285(5433): 1505-1510.

Harvell, C.D., Mitchell, C., Ward, J., Altizer, S., Dobson, A., Ostfeld, R., and Samuel, M. (2002). Climate Warming and Disease Risk for Terrestrial and Marine Biota. *Science* 296: 2158-2162.

Harvell, C.D., Jordan-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G.W., Weil, E. and Willis, B. (2007). Coral disease, environmental drivers, and the balance between coral and microbial associates. *Oceanography* 20: 172-195.

Heidejorgensen, M.P., Harkonen, T., Dietz, R., Thompson, P.M., (1992). Retrospective of the 1988 european seal epizootic. *Diseases of Aquatic Organisms* 13: 37–62.

Hudson, H. (2000). First aid for massive corals infected with blackband disease: an underwater aspirator and post-treatment sealant to curtail reinfection. In Diving for Science in the 21st Century (Hallock, P. and French, L., eds), American Academy of Underwater Sciences. 10–11

Hyman, L.H. (1940). The Invertebrates: Protozoa through Ctenophora. McGraw-Hill Book Company.

Jackson, J.B.C., 1979. Morphological strategies of sessile animals. In, Biology and Systematics of Colonial Organisms, edited by G. Larwood and B.R. Roser, Academic Press, London. 499–555.

Jensen, M.A., Faruque, S.M., Mekalanos, J.J., and Levin, B.R. (2006). Modeling the role of bacteriophage in the control of cholera outbreaks. *Proceedings of the National Academy of Sciences of the United States of America* 103: 4652.

Jones, R.J., Bowyer, J., Hoegh-Guldberg, O., and Blackall, L.L. (2004). Dynamics of a temperature-related coral disease outbreak. *Marine Ecology Progress Series* 281: 63-77.

Jompa J. and McCook L.J. (2003) Coral-algal competition: macroalgae with different properties have different effects on corals. *Marine Ecology-Progress Series* 258: 87–95.

Kaczmarsky, L. and L.L. Richardson (2007). Transmission of growth anomalies between Indo-Pacific Porites corals. *Journal of Invertebrate Pathology* 94: 218-221.

Kim, K., Harvell, C.D., Kim, P., Smith, G., and Merkel, S. (2000). Fungal disease resistance of Caribbean sea fan corals (*Gorgonia* spp.). *Marine Biology* 136: 259-267.

Kim, K. and C.D. Harvell (2002). Chapter 30. Aspergillosis of Sea Fan Corals: Disease Dynamics in the Florida Keys. The Everglades, Florida Bay, and Coral Reefs of the Florida Keys. An Ecosystem Sourcebook. J. W. Porter and K. G. Porter. Boca Raton, CRC Press. 813-824.

Kim, K. and Harvell, C.D. (2004). The rise and fall of a six-year coral-fungal epizootic. *The American Naturalist* 164: S52-S63.

Kinlan, B.P. and S.D. Gaines (2003). Propagule dispersal in marine and terrestrial environments: a community perspective. *Ecology* 84: 2007–2020.

Kinne, O. (1980). Diseases of marine animals: general aspects. In: Kinne O (ed) Diseases of marine animals, Vol 1. General aspects, Protozoa to Gastropoda. John Wiley & Sons, New York.

Kirch, P. (2000). On the roads of the winds: An archaeological history of the Pacific Islands. University of California Press, Berkeley.

Knowlton N., Lang, J.C., Keller, B.D. (1990). Case study of natural population collapse: post-hurricane predation on Jamaican staghorn corals. *Smithsonian Contributions to the Marine Sciences* 31:25

Koch, R. (1891). Über bakteriologische Forschung. Verhandlung des X Internationalen Medichinischen Congresses, Berlin, 1890. August Hirschwald, Berlin. *Xth International Congress of Medicine, Berlin, Germany*. 1-35.

Korrubel, J.L. (2000). Coral diseases in the Western Indian Ocean. In: McClanahan T.R., Sheppard, C.S., Obura D.O. (eds) Coral reefs of the Indian Ocean: their ecology and conservation. Oxford Univ Press, New York. 279-283.

Krzystyniak, K., Tryphonas, H., and Fournier, M. (1995). Approaches to the evaluation of chemical-induced immunotoxicity. *Environmental Health Perspectives* 103: 17–22.

Kushmaro, A., Rosenberg, E., Fine, M. and Loya. Y. (1997). Bleaching of the coral *Oculina patagonica* by Vibrio AK-1. *Marine Ecology Progress Series* 147: 159–165.

Kushmaro, A., Rosenberg, E., Fine, M., Ben-Haim, Y., and Loya, Y. (1998). Effect of temperature on bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. *Marine Ecology Progress Series* 171: 131-137.

Kuta, K. and L.L. Richardson (2002). Ecological aspects of black band disease of corals: relationships between disease incidence and environmental factors. *Coral Reefs* 21(4): 393-398.

Lane, D.J. (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester. 177–203.

Leggat, W., Badger, M. R., and Yellowlees, D. (1999). Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinium* sp. *Plant Physiology* 121: 1247-1255.

Leichter, J.J., Stewart, H.L. and Miller, S.L. (2003). Episodic nutrient transport to Florida coral reefs. *Limnology and Oceanography* 48: 1394–1407.

Linzey, D., Burroughs, J., Hudson, L., Marini, M., Robertson, J., Bacon, J., Nagarkatti, M., and Nagarkatti, P. (2003). Role of environmental pollutants on immune functions, parasitic infections and limb malformations in marine toads and whistling frogs from Bermuda. *International Journal of Environmental Health Research* 13: 125–148.

Lirman, D. (2000). Lesion regeneration in the branching coral *Acropora palmata:* effects of colonization, colony size, lesion size, and lesion shape. *Marine Ecology Progress Series* 197: 209-215.

Loya, Y., (1976). Skeletal regeneration in a Red Sea Scleractinian coral population. *Nature* 261: 490 – 491.

Mariscal, R.N. (1984). Cnidaria: Cnidae. In: J. Bereiter-Hahn, A. Matoltsy and K.S. Richards (eds.), Biology of the Integument. Springer-Verlag. 57-68.

Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., and Wakiguchi, H. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *Journal of Infection and Chemotherapy* 11: 211–219.

McCallum, H.I., Kuris, A., Harvell, C.D., and Lafferty, K. (2004). Does terrestrial epidemiology apply to marine systems? *Trends in Ecology and Evolution* 19: 585–591.

McClanahan, T.R (2004). Coral bleaching, diseases and mortality in the western Indian Ocean. In: Rosenberg, E. and Y. Loya (eds) Coral Health and Disease. Springer-Verlag. 157-176.

McClanahan, T.R., Graham, N.A.J., Calnan, J.M., and MacNeil, M.A. (2007). Toward pristine biomass: reef fish recovery in coral reef marine protected areas in Kenya. *Ecological Applications* 17: 1055–1067.

Meesters, E.H., Bos, A. and Gast, G.J. (1993). Effects of sedimentation and lesion position on coral tissue regeneration. *Proceedings of the 7th International Coral Reef Symposium, Guam* 681–688.

Meesters E.H., Noordeloos M., Bak R.P.M. (1994). Damage and regeneration: links to growth in the reef-building coral *Montastrea annularis*. *Marine Ecology Progress Series* 112: 119-128.

Meesters, E.H., Wesseling, I., and Bak, R.P.M. (1996). Partial mortality in three species of reef-building corals and the relation with colony morphology. *Bulletin of Marine Science* 58: 838-852.

Meesters E.H., Pauchli W., Bak R.P.M. (1997). Predicting regeneration of physical damage on a reef-building coral by regeneration capacity and lesion shape. *Marine Ecology Progress Series* 146: 91-99.

Migaki, G., Sawa, T.R., Dubey, J.P., (1990). Fatal disseminated toxoplasmosis in a spinner dolphin (*Stenella longirostris*). *Veterinary Pathology* 27: 463–464.

Miller, M., Gardner, I., Kreuder, C., Paradies, D., Worcester, K., Jessup, D., Dodd, E., Harris, M., Ames, J., and Packham, A. (2002). Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *International Journal for Parasitology* 32: 997–1006.

Miller, J., Rogers, C., and Waara, R. (2003). Monitoring the coral disease, plague type II, on coral reefs in St. John, US Virgin Islands. *Revisita de Biologia Tropical* 51: 47–55.

Morens, D.M., Folkers, G.K., and Fauci, A.S. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242–249.

Mumby, P.J. and R.S. Steneck (2008). Coral reef management and conservation in light of rapidly evolving ecological paradigms. *TREE* 23(10): 555-562.

Mydlarz, L., Holthouse, S., Peters, E. and Harvell, C.D. (2008). Cellular responses in sea fan corals: granular amoebocytes react to pathogen and climate stressors. *PLoS ONE* 3: e1811.

Myers, R., and L.J. Raymundo (2009). Coral disease in Micronesian reefs: a link between disease prevalence and host abundance. *Disease of Aquatic Organisms* 87: 97–104.

Nagelkerken, I., Buchan, K., Smith, G.W., Bonair, K., Bush, P., and Garzon-Ferreira, J. (1997). Widespread disease in the Caribbean Sea fans. II. Patterns of infection and tissue loss. *Marine Ecology Progress Series* 160: 255–263.

Nappi, A.J. and Christensen, B.M. (2005). Melanogenesis and associated cytotoxic reactions: Applications to insect innate immunity. Insect Biochem. *Molecular Biology* 35: 443- 459

Nixon, S.W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* 41: 199–219.

Nugues, M.M., Smith, G.W., van Hooidonk, R.J., Seabra, M.I. and Bak, R.P.M. (2004) Algal contact as a trigger for coral disease. *Ecology Letters* 7: 919-923.

Oren, U., Benayahu, Y., and Loya, Y. (1997). Effect of lesion size and shape on regeneration of the Red Sea coral *Favia favus*. *Marine Ecology Progress Series* 146: 101-107.

Osterhaus, A.D.M.E., Rimmelzwaan, G.F., Martina, B.E.E., Bestebroer, T.M., Fouchier, R.A.M., 2000. Influenza B virus in seals. Science 288, 1051–1053.

Page, C. and Willis, B. (2006). Distribution, host range and large-scale spatial variability in black band disease prevalence on the Great Barrier Reef, Australia. *Diseases of Aquatic Organisms*. 69: 41-51.

Page, C.A., and B.L. Willis (2007). Epidemiology of skeletal eroding band on the Great Barrier Reef and the role of injury in the initiation of this widespread coral disease. *Coral Reefs* 27: 257–272.

Paine R.T. and S.A. Levin (1981). Intertidal landscapes: disturbance and the dynamics of pattern. *Ecological Monographs* 51: 145–178.

Palmer, C.V., Mydlarz, L.D. and Willis, B.L. (2008). Evidence of an inflammatory- like response in non-normally pigmented tissues of two scleractinian corals. *Proceedings of the Royal Society B: Biological Sciences* 275: 2687-2693.

Palmer, C.V., Traylor-Knowles, N.G., Willis, B.L., and Bythell, J.C. (2011). Corals Use Similar Immune Cells and Wound-Healing Processes as Those of Higher Organisms. *PLoS ONE* 6: e23992.

Patterson, K.L., Porter, J.W., Ritchie, K.B., Polson, S.W., Mueller, E., Peters, E.C., Santavy, D.L., and Smith, G.W. (2002). The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proceedings of the National Academy of Sciences of the United States of America* 99(13): 8725-8730.

Peters, E.C. (1984). A survey of the normal and pathological histology of scleractinian corals with emphasis on the effects of sedimentation stress (*Astrangia danae,* Caribbean, Histopathology). ETD Collection for University of Rhode Island. Paper AA18501295.

Peters E.C., Halas J.C., McCarty H.B. (1986). Calicoblastic neoplasms in Acropora palmata, with a review of reports on anomalies of growth and form in corals. *Journal of the National Cancer Institute* 76: 895–912.

Peters E.C. (2001). Coral Histopathology. Coral Tissue Slide Reading Workshop Modules, p1-122. Distributed by EC Peters.

Pharma (2006). Medi-Lexicon, Medical Terms Dictionary.

Rappe, M.S. and S.J. Giovannoni, S.J. (2003). The uncultured microbial majority. *Annual Reviews in Microbiology* 57: 369–394.

Raymundo, L.J., Harvell, C.D., and Reynolds, T. L. (2003). *Porites* ulcerative white spot disease: description, prevalence, and host range of a new coral disease affecting Indo-Pacific reefs. *Diseases of Aquatic Organisms* 56 (2): 95–104.

Raymundo, L.J, Saltonstall, C, and Harvell C.D. eds. (2008). Coral Diseases: Guidelines for Assessment, Monitoring and Management. Currie Communications. Melbourne, Australia.

Raymundo L.J., Halford A.R., Maypa A.P., Kerr A.M. (2009) Functionally diverse reef fish communities ameliorate coral disease. *Proceeding of the National Academy of Sciences* 106:17067–17070

Raymundo, L.J. (2010). Personal communication. University of Guam, Marine Laboratory.

Raymundo, L.J. Unpublished data. University of Guam, Marine Laboratory.

Reshef, L., Koren, O., Loya, Y., Zilber Rosenberg, I., and Rosenberg, E. (2006). The coral probiotic hypothesis. *Environmental Microbiology* 8: 2068–2073.

Richardson, L. (1992). Red Band Disease: a new cyanobacterial infestation of corals. In Proceedings of the American Academy of Underwater Sciences Twelfth Annual Scientific Diving Symposium (ed. L. B. Cahoon), pp. 153-160. Wilmington, NC: American Academy of Underwater Sciences.

Richardson, L., Kuta, K., Schnell, S., and Carlton, R. (1997). Ecology of the black band disease microbial consortium. *Proceedings of the 8th International Coral Reef Symposium, Panama* 1, 597–600.

Richardson, L.L., W.M. Goldberg, K.G. Kuta, R.B. Aronson, G.W. Smith, and K.B. Ritchie. (1998). Florida's mystery coral-killer identified. *Nature* 392:557–558.

Richardson, L.S. (1998). Coral diseases: what is really known? *Trends in Ecology and Evolution* 13: 438–443.

Richardson, L.L. and K.G. Kuta (2003). Ecological physiology of the black band disease cyanobacterium *Phormidium corallyticum*. *FEMS Microbiology Ecology* 43: 287-298.

Rinkevich B., Loya Y. (1989). Reproduction in regenerating colonies of the coral *Stylophora pistillata*. In: Spanier, E., Stinberger, Y., Luria, M (eds) Environmental quality and ecosystem stability, 4B, Environmental quality. Hebrew University, Jerusalem. 257-265.

Ritchie, K. B. and G. W. Smith (1998). Type II White Band Disease. *Revisita de Biologia Tropical*. 46(Suppl. 5): 199-203.

Ritchie, K.B., Polson, S.W., Smith, G.W. (2001). Microbial disease causation in marine invertebrates: problems, practices, and future prospects. *Hydrobiologia* 460: 131–139.

Ritchie, K. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series* 322: 1–14.

Roff, G., Hoegh-Guldberg, O., and Fine, M. (2006). Intra-colonial response to Acroporid "white syndrome" lesions in tabular *Acropora* spp. (Scleractinia). *Coral Reefs* 25: 255-264.

Rosenberg, E. and Y. Ben-Haim (2002). Microbial diseases of corals and global warming. *Environmental Microbiology* 4(6): 318-326.

Rotjan R.D., and Lewis S.M. (2008) Impact of coral predators on tropical reefs. *Marine Ecology-Progress Series* 367: 73–91.

Rottman, G.L. (2002). World War II Pacific Island Guide: A Geo-Military Study. Greenwood Press.

Sandin, S.A., Smith, J.E., De Martini, E.E., Dinsdale, E.A., Donner, S.D., Friedlander, A. M., Konotchick, T., Malay, M., Maragos, J.E., and Obura, D. (2008). Baselines and degradation of coral reefs in the northern Line Islands. *PLoS ONE* 3: e1548.

Selig, E.R., Harvell, C.D., Bruno, J.F., Willis, B.L., Page, C.A., Casey, K.S., and Sweatman, H. (2006). Analyzing the relationship between ocean temperature anomalies and coral disease outbreaks at broad spatial scales. Coral reefs and climate change: Science and Management. Washington (DC): American Geophysical Union. 111–128.

Shields, J.D., Scanlon, C., and Volety, A. (2003). Aspects of the pathophysiology of blue crabs, *Callinectes sapidus*, infected with the parasitic dinoflagellate *Hematodinium perezi*. *Bulletin of Marine Science* 72: 519–535.

Shinn, E.A., Reese, R.S., and Reich, C.D. (1994). Fate and pathways of injection-well effluent in the Florida Keys. Department of the Interior US Geological Survey, Open-File Report 94-276.

Shinn, E.A., Smith, G.W., Prospero, J.M., Betzer, P., Hayes, M.L., Garrsion, V., and Barber, R.T. (2000). African dust and the demise of Caribbean coral reefs. *Geophysical Research Letters* 27(9): 3029-3032.

Shnit-Orland, M. and Kushmaro, A. (2009). Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiology Ecology* 67: 371–380.

Smith, G.W., Harvell, C.D. and Kim, K. (1998). Response of sea fans to infection with *Aspergillus* sp. (fungi). *Revista Biologia Tropical* 46:205–208.

Soong, K. and J.C. Lang (1992). Reproductive integration in reef corals. *The Biological Bulletin* 183: 418-431.

Stachowitsch, M. (1992). The Invertebrates: An Illustrated Glossary. Wiley-Liss.

Stedman, T.L. (1995). Stedman's Medical Dictionary, 26th Edition. Lippincott Williams & Wilkins, Baltimore.

Stedman T.L. (2000). Stedman's Medical Dictionary, 27th Edition. Lippincott Williams & Wilkins, Baltimore.

Stenner, V.J., MacKay, B., King, T., Barrs, V.R.D., Irwin, P., Abraham, L., Swift, N., Langer, N., Bernays, M., Hampson, E., Martin, P., Krockenberger, M.B., Bosward, K., Latter, M., Malik, R. (2007). Protothecosis in 17 Australian dogs and a review of the canine literature. *Medical Mycology* 45: 249–266.

Sussman, M., Loya, Y., Fine, M., and Rosenberg, E. (2003). The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. *Environmental Microbiology* 5: 250–255.

Sussman, M., Willis, B.L., Victor, S., and Bourne, D.G. (2008). Coral pathogens identified for White Syndrome (WS) epizootics in the Indo-Pacific. *PLoS ONE* 3(6): e2393.

Sutherland, K.P., Porter, J.W. and Torres, C. (2004). Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Marine Ecology Progress Series* 266: 273–302.

Sutherland, K.P., Shaban, S., Joyner, J.L., Porter, J.W., and Lipp, E.K. (2011). Human pathogen shown to cause disease in the threatened Elkhorn coral *Acropora palmata. PLoS ONE* 6(8): e23468.

Sweatman, H. (2008). No-take reserves protect coral reefs from predatory starfish. *Current Biology* 18: R598–R599.

Szmant, A.M. (2002). Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries* 25: 743–766.

Thompson R.G. (1978) General veterinary pathology. WB Saunders, Philadelphia, PA

Van Veghel M.L.J. and R.P.M. Bak (1994). Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis*. III. Reproduction in damaged and regenerating colonies. *Marine Ecology Progress Series* 109: 229-233.

Van Woesik, R. (1998). Lesion healing on massive *Porites* spp. corals. *Marine Ecology Progress Series* 164: 213-220.

Vargas-Angel, B. and B. Wheeler (2009). Coral health and disease assessment in the US Pacific territories and affiliated states. *Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida* 175–179.

Vargas-Angel, B., 2010. Personal communication. NOAA Fisheries, Pacific Islands Fisheries Science Center.

Visram, S. and A.E. Douglas (2007). Resilience and acclimation to bleaching stressors in the scleractinian coral *Porites cylindrica*. *Journal of experimental Marine Biology and Ecology* 349(1): 35-44.

Ward, J. and K.D. Lafferty (2004). The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? *PLoS Biology* 2(4): 0542-0547.

Weil, E. (2004). Coral reef diseases in the wider Caribbean. In: E. Rosenberg and Y. Loya (eds). Coral Health and Disease. Springer-Verlag, Berlin, 35-68.

Weil, E., Smith, G.W., and Gil-Agudelo, D.L. (2006). Status and progress in coral reef disease research. *Diseases of Aquatic Organisms* 69:1–7.

Weir-Brush, J., Garrison, V., Smith, G.W., and Shinn, E. (2004). The relationship between gorgonian coral (Cnidaria: Gorgonacea) diseases and African dust storms. *Aerobiologia* 20: 119–126.

Whittington, R.J., Jones, J.B., Hine, P.M., Hyatt, A.D. (1997). Epizootic mortality in the pilchard *Sardinops sagax neopilchardus* in Australia and New Zealand in 1995. I. Pathology and epizootiology. *Diseases of Aquatic Organisms* 28: 1–16.

Williams, D.E. and M.W. Miller (2005). Coral disease outbreak: Pattern, prevalence and transmission in *Acropora cervicornis*. *Marine Ecology Progress Series* 301: 119–128.

Work, T.M., and G.S. Aeby (2006). Systematically describing gross lesions in corals. *Diseases of Aquatic Organisms* 70: 155–160.

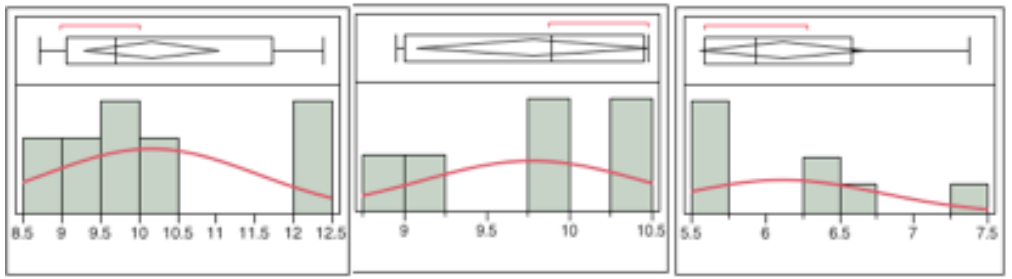
Work, T., 2010. Personal communication. USGS National Wildlife Health Center, Honolulu Field Station.

Yakob, L. and P.J. Mumby (2011). Climate change induces demographic resistance to disease in novel coral assemblages. *Proceedings of the National Academy of Sciences* 108(5), 1967–1969.

Appendix 1. Descriptive statistics of bacterial concentrations (CFU ml^{-1}) in experimentally infected samples and in diseased and healthy samples in the field, grown on both media types (MA and TCBS), using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

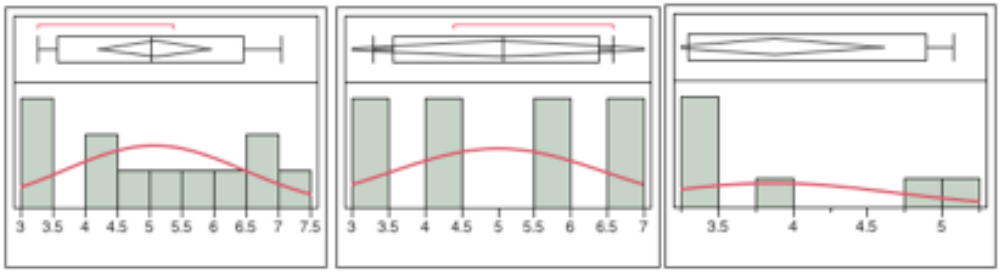
Tests and P-values	
Marine Agar	
EXPERIMENTAL	Non-normal; p=0.009
DISEASED	Normal; p=0.203
HEALTHY	Normal; p=0.047
Levene's Test	Homogenous; p=0.077
TCBS	
EXPERIMENTAL	Normal; p=0.201
DISEASED	Normal; p=0.848
HEALTHY	Non-normal; p=0.011
Levene's Test	Homogenous; p=0.115

MARINE AGAR



Experimentally infected **Diseased Colonies** **Healthy Colonies**

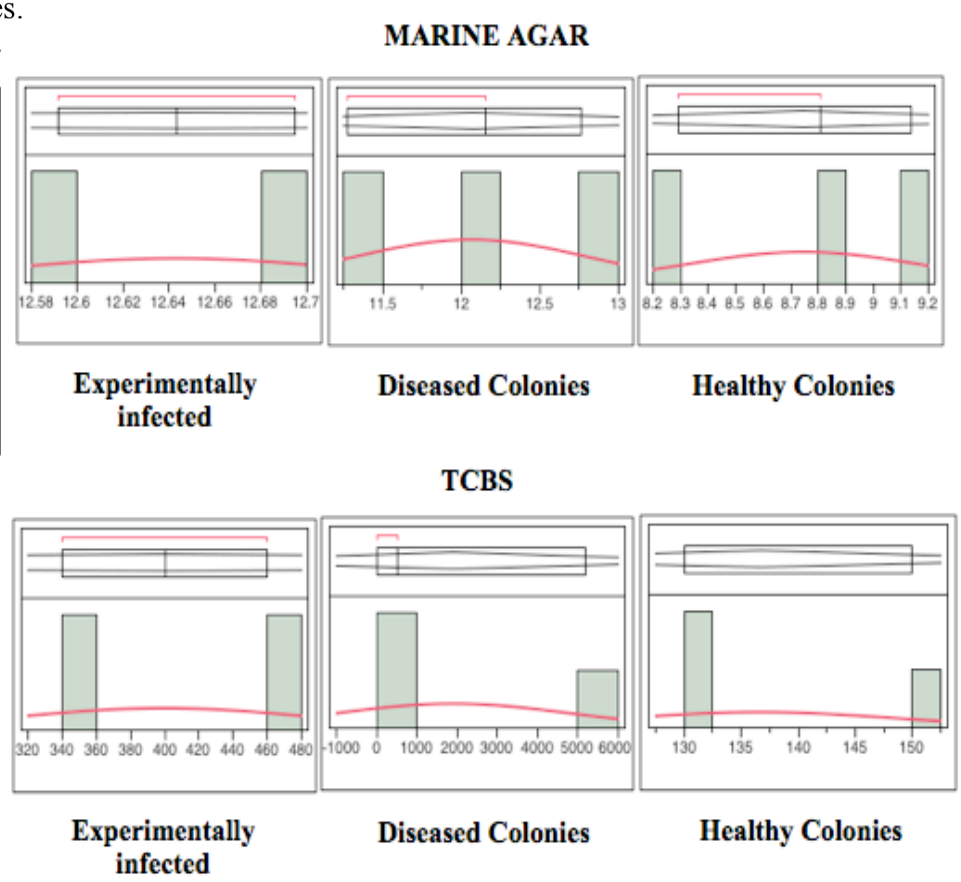
TCBS



Experimentally infected **Diseased Colonies** **Healthy Colonies**

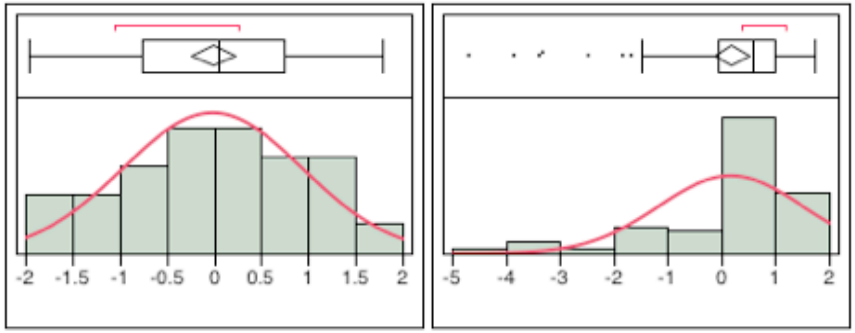
Appendix 2. Descriptive statistics of bacterial concentrations (CFU ml^{-1}) from the inoculated (diseased) sample (C05) and in diseased and healthy samples in the field, grown on both media types (MA and TCBS), using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

Tests and P-values	
Marine Agar	
EXPERIMENTAL	Normal; p=1.000
DISEASED	Normal; p=0.803
HEALTHY	Non-normal; p=0.756
Levene's Test	Heterogenous; p<0.025
TCBS	
EXPERIMENTAL	Normal; p=1.000
DISEASED	Normal; p=0.174
HEALTHY	Non-normal; p<0.005
Levene's Test	Homogenous; p=0.135



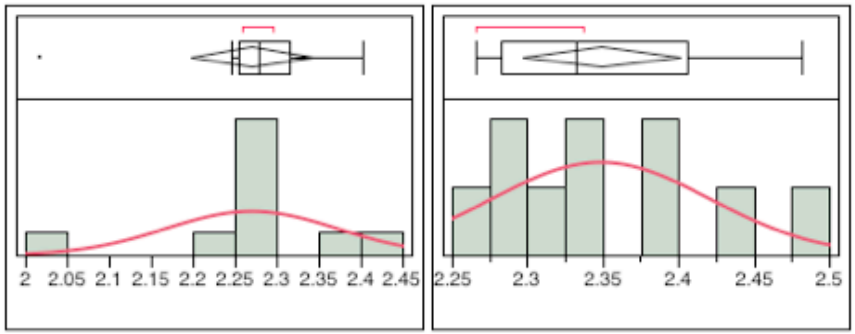
Appendix 3. Descriptive statistics of Monthly % Tissue Loss (%TL) and Total % Change in Tissue Cover (%TC) in branching and massive *Porites* spp. colonies using Shapiro-Wilk W Test for normality and Levene's Test for

Tests and P-values	
Monthly %TL: Repeated Measures ANOVA	
BRANCHING	Normal; p=0.149 Homogenous; p=0.691
MASSIVE	Non-normal; p<0.005 Homogenous; p=0.979
%TC: 1-way ANOVA	
BRANCHING	Non-normal; p=0.013 Homogenous; p= 0.961
MASSIVE	Normal; p=0.443 Homogenous; p=0.961



Monthly % TL
BRANCHING

Monthly % TL
MASSIVE

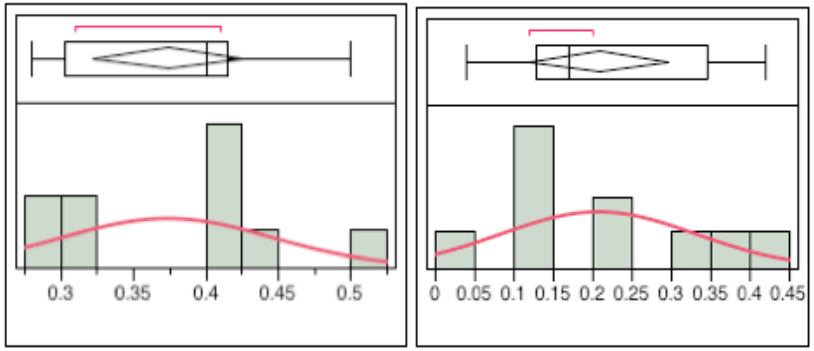


% TC
BRANCHING

% TC
MASSIVE

Appendix 4. Descriptive statistics of Disease Lesion Incidence in branching and massive *Porites* spp. colonies using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

Tests and P-values	
Disease Lesion Incidence	
BRANCHING	Normal; p=0.271
MASSIVE	Normal; p=0.222
Levene's Test	Homogenous; p=0.125



Incidence
BRANCHING

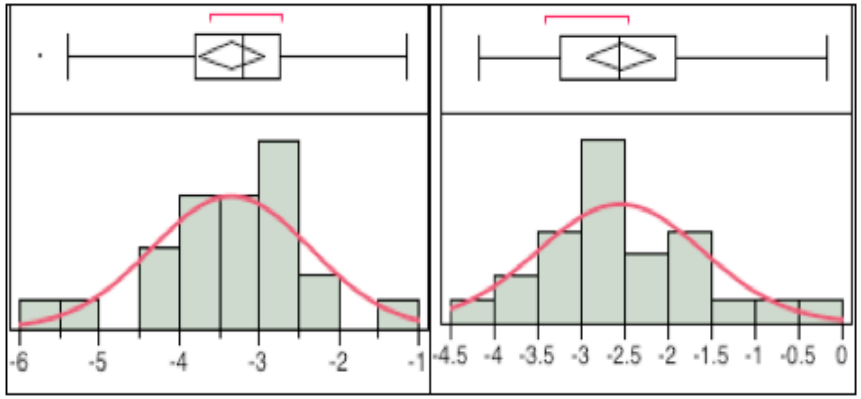
Incidence
MASSIVE

Appendix 5. Mean weekly temperature (°C) at Luminao Reef, Piti, southern Guam from October 19, 2009 to June 15, 2010.

Mean Weekly Temperature			
Week	Temp (°C)	Week	Temp (°C)
10/19/09 - 10/25/09	29.98	02/15/10 - 02/21/10	28.49
10/26/09 - 11/01/09	29.83	02/22/10 - 02/28/10	28.74
11/02/09 - 11/08/09	29.47	03/01/10 - 03/07/10	28.49
11/09/09 - 11/15/09	29.32	03/08/10 - 03/14/10	28.72
11/16/09 - 11/22/09	28.79	03/15/10 - 03/21/10	28.64
11/23/09 - 11/29/09	28.59	03/22/10 - 03/28/10	28.59
11/30/09 - 12/06/09	28.59	03/29/10 - 04/04/10	28.98
12/07/09 - 12/13/09	28.65	04/05/10 - 04/11/10	29.18
12/14/09 - 12/20/09	28.49	04/12/10 - 04/18/10	29.03
12/21/09 - 12/27/09	28.31	04/19/10 - 04/25/10	29.10
12/28/09 - 01/03/10	28.28	04/26/10 - 05/02/10	29.55
01/04/10 - 01/10/10	28.23	05/03/10 - 05/09/10	29.43
01/11/10 - 01/17/10	28.16	05/10/10 - 05/16/10	29.95
01/18/10 - 01/24/10	27.53	05/17/10 - 05/23/10	30.08
01/25/10 - 01/31/10	27.25	05/24/10 - 05/30/10	30.67
02/01/10 - 02/07/10	28.08	05/31/10 - 06/06/10	31.06
02/08/10 - 02/14/10	28.21	06/07/10 - 06/15/10	31.40

Appendix 6. Descriptive statistics of Mean Recovery Rate ($cm^2\ d^{-1}$) and Initial Lesion Size (cm) in branching and massive *Porites* spp. colonies using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

Tests and P-values	
Mean Recovery Rate	
BRANCHING	Normal; p=0.475 Homogenous; p=0.642
MASSIVE	Normal; p=0.593 Homogenous; p=0.056
Initial Lesion Size	
BRANCHING	Normal; p=0.119 Homogenous; p=0.924
MASSIVE	Normal; p=0.897 Homogenous; p=0.196

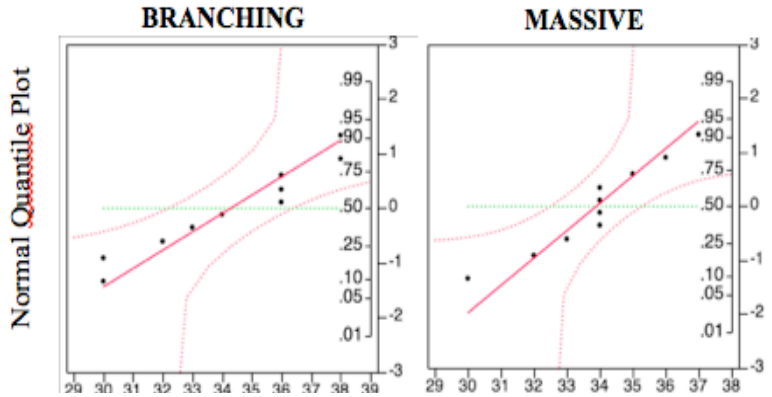


Mean Recovery Rate ($cm^2\ day^{-1}$)
BRANCHING

Mean Recovery Rate ($cm^2\ day^{-1}$)
MASSIVE

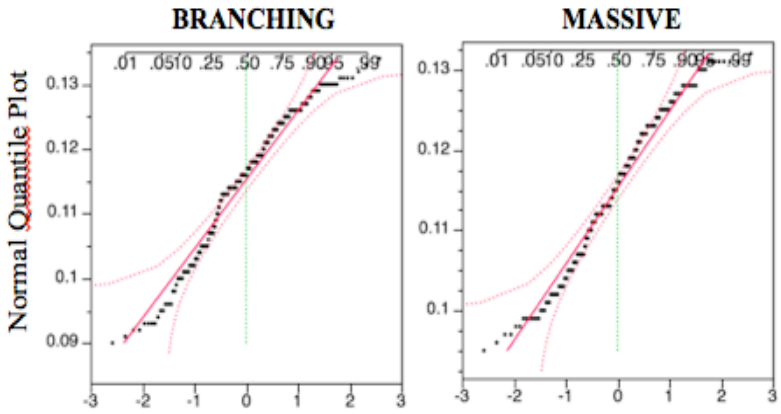
Appendix 7. Descriptive statistics of *Porites* spp. corallite density in branching (n=50) and massive colonies (n=50) using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

Tests and P-values	
Corallite Density	
BRANCHING	Normal; p=0.266 small sample size
MASSIVE	Normal; p=0.678 small sample size



Appendix 8. Descriptive statistics of *Porites* spp. corallite diameter in branching (n=10) and massive colonies (n=10) using Shapiro-Wilk W Test for normality and Levene's Test for equal variances.

Tests and P-values	
Corallite Diameter	
BRANCHING	Non-normal; p<0.005 Homogenous; p=0.094
MASSIVE	Non-normal; p<0.005 Homogenous; p=0.796



Appendix 9. A glossary of histological terms used in this study (Galloway et al., 2007). Sources of the definitions are cited.

Eosinophilic – cell or tissue elements staining readily with eosin dyes, appear pink to red when using a hematoxylin and eosin staining procedure; sometimes referred to as "acidophilic." (Pharma, 2006)

Filamentous, sinuous, eosinophilic structures with parallel striations - cyanobacteria

Hyalin – a translucent, homogenous, structureless, eosinophilic, albuminoid substance occurring in tissue degeneration. (Pharma, 2006; Stedman, 1995)

Hyalination – process of deposition of a cellular amorphous homogeneous substance, which stains bright red with hematoxylin and eosin. (Stedman, 1995)

Hyaline – having the properties of hyalin. (Stedman, 1995)

Multicellular structures with cell walls – algae; algal filaments

Necrosis – cell death characterized by irreversible damage, the earliest of which is mitochondrial. Changes visible with light microscopy are nuclear (pyknosis, karyolysis, or karyorrhexis) and generally accompanied by cytoplasmic hypereosinophilia, shrinkage, or fragmentation. After such changes, the outlines of individual cells are indistinct and affected cells may become merged, sometimes forming a focus of coarsely granular, amorphous, or hyaline material. (Stedman, 1995)

Necrotic – pertaining to or affected by necrosis. (Stedman, 1995)

Pigment cell – basally located epithelial cell that produces pigmented granules (e.g., green fluorescent protein-like pigments, animal coloration pigment). The genus Porites contains specialized pigment granule-producing amoeboid cells called chromophore cells. Note that phagocytes can contain lipofucsin pigment granules obtained from necrotic cells. (Duerden, 1902; Peters, 1984)

Polyp – the basic structural unit of an anthozoan, consisting of a sac-like cylindrical body, a basal (aboral) disk that may be modified to produce a calcium carbonate or gorgonin exoskeleton or attach the polyp to the substrate, and an oral disk bearing mouth and tentacles. (Peters, 1984)

Pyrenoid – a small proteinaceous body found within the cytoplasm of zooxanthellae (and other phytoflagellates) and closely associated with the chloroplasts. It contains the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), which adds carbon dioxide to the sugar ribulose-1,5-bisphosphate as it synthesizes and deposits polysaccharides. The pyrenoid is visible in fixed, stained sections of zooxanthellae as a small round refringent body surrounded by a pale staining starch sheath. (Dorland 2000; Leggat et al. 1999)

Section – a thin slice of tissue, cells, macroorganisms, or any material for examination under the microscope. (Stedman, 1995)

> **Cross** – sliced at right angles (or transverse) to the longitudinal axis of the organism. A cross-section of a polyp is one sliced at right angles to the longitudinal axis running in the oral to aboral direction. A cross-section of a

coral colony branch is one sliced at right angles to the longitudinal axis extending from the axial polyp to the base of the branch.

Sagittal – sliced along or parallel to the longitudinal axis of the polyp or branch (see Cross for explanation of axes).

Oblique – a diagonal cross section that is neither parallel to the longitudinal axis nor at right angles to this axis (see Cross for explanation of axes).

Skeleton – the structurally supporting matrix of aragonite crystals formed by a scleractinian on the outside of the polyp, technically an exoskeleton, or the structural support for an octocoral. (Bayer et al., 1983; Stachowitsch, 1992)

Spirocyst – single-walled capsule which contains a tightly coiled tubule bearing microtubules that form a web of fine, adhesive microfibrillae when discharged for prey capture or attachment, produced by a spirocyte. (Goldberg and Taylor, 1996; Mariscal, 1984; Peters, 1984)

Tissue – a collection of similar cells and the intercellular substances surrounding them united in the performance of a particular function. Cnidaria possess all four of the basic tissues: (1) epithelium, (2) connective, (3) muscle, and (4) nerve. (Dorland, 2000; Hyman, 1940; Stedman, 1995)

Vacuole – a tiny fluid-filled cavity or a membrane-bound vesicle formed in the protoplasm of a cell. (Dorland, 2000; Stedman, 1995)

Vacuolated – having vacuoles. (Stedman, 1995)

Vacuolization (or **Vacuolation)** – formation or multiplication of vacuoles. (Stedman, 1995)

Zooxanthellae – dinoflagellates (unicellular photosynthetic organisms) that live within the gastrodermal cells of some scleractinians, octocorals, sea anemones and other animals (not cnidarians), which give corals a characteristic brown coloration. Zooxanthellae provide energy in the form of photosynthate, use animal wastes (nitrogenous ones and carbon dioxide) and, in calcifying organisms, enhance calcification. (Peters, 1984)

Appendix 10. Testing between-colony and between-morph differences on the mean recovery rates (cm^2 d^{-1}) of healed lesions. **Tukey HSD**: positive values show pairs of means that are significantly different

Nested ANOVA:
between Growth Forms on HEALED lesions

Source	DF	SS	MS	F	P
Growth Form	1	7.921	7.921	4.214	**0.074**
Colony	8	15.037	1.880	2.477	**0.028**
Error	40	30.356	0.759		
Total	49	53.313			

a) ***Tukey-Kramer HSD Post-hoc:***
Branching Colonies (HEALED)

	BR5	**BR4**	**BR3**	**BR2**	**BR1**
BR5	-1.7226	-1.5695	-1.1523	-0.5845	-0.3381
BR4	-1.5695	-1.7226	-1.3054	-0.7376	-0.4912
BR2	-1.1523	-1.3054	-1.7226	-1.1548	-0.9084
BR3	-0.5845	-0.7376	-1.1548	-1.7226	-1.4762
BR1	-0.3381	-0.4912	-0.9084	-1.4762	-1.7226

b) ***Tukey-Kramer HSD Post-hoc:***
Massive Colonies (HEALED)

	MA1	**MA5**	**MA3**	**MA4**	**MA2**
MA1	-1.5713	-1.0971	-0.7625	-0.5176	0.0909
MA5	-1.0971	-1.5713	-1.2367	-0.9918	-0.3833
MA3	-0.7625	-1.2367	-1.5713	-1.3264	-0.7179
MA4	-0.5176	-0.9918	-1.3264	-1.5713	-0.9628
MA2	0.0909	-0.3833	-0.7179	-0.9628	-1.5713

Appendix 11. Testing the relationship between lesion size and recovery rate using simple linear regression for each growth form (R^2 values highlighted)

Linear Regression: BRANCHING COLONIES
Initial Lesion Size vs Mean Recovery Rate

Source	DF	SS	MS	F	P
Model	1	20.558502	20.5585	43.5907	<.0001
Error	48	22.638047	0.4716		
Total	49	43.196549			

RSquare - 0.475929
RSquare Adj - 0.465011
Root Mean Square Error - 0.68675
Mean of Response - -0.95131
Observations (or Sums Wgts) - 50

Linear Regression: MASSIVE COLONIES
Initial Lesion Size vs Mean Recovery Rate

Source	DF	SS	MS	F	P
Model	1	24.942548	24.9425	50.2431	<.0001
Error	36	17.871759	0.4964		
Total	37	42.814307			

RSquare - 0.582575
RSquare Adj - 0.57098
Root Mean Square Error - 0.704583
Mean of Response - -0.40638
Observations (or Sums Wgts) - 38