THE RELATIONSHIP BETWEEN CLIMATE-DRIVEN OCEAN WARMING AND CORAL DISEASES IN THE CARIBBEAN

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Dissertation Proposal

Submitted to the committee on _____
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B. PROJECT SUMMARY

Overview

Over the last half-century, new and emerging diseases have become a significant threat to the health of Caribbean corals, resulting in major declines in coral cover and in shifts in species composition. Since the earliest reported coral-disease outbreaks in the 1960s, researchers have been searching for key pathogens that cause coral diseases, yet many pathogens have remained elusive. Less attention has been given to the changing environment. Coral-disease outbreaks may be triggered by environmental stressors, such as high ocean temperatures, which weaken the corals' immune system or increase the virulence of pathogens. It is therefore possible that recent diseases outbreaks are a secondary effect of compromised corals that have been subjected to thermal stress as a result of rapidly warming oceans. Yet, ocean temperatures, and rates of change in ocean temperatures are spatially heterogeneous. In addition, thermal anomalies vary spatially and temporally, with the frequency of thermal-stress events ranging from every 4-6 years, to every 50-60 years in the Caribbean. These temperature patterns may, consequently, be reflected in contemporary populations of coral species.

My doctoral research will address the following four research questions:

(1) Do coral diseases cluster and follow a contagious-disease model that varies with thermal history?
(2) Are localities in the Caribbean with a history of frequent thermal anomalies (every 4-6 years) more likely to have higher disease prevalence than localities without a history of frequent thermal anomalies?
(3) What is the relationship between temperature and coral diseases over the past two decades?
(4) Are coral diseases, directly or indirectly, contagious?

Specifically, my doctoral research will use three complementary research methods to test two central hypotheses: (1) coral diseases are not infectious and transmissible, and (2) thermal stress is a significant driver of coral diseases in the Caribbean.

First, a hierarchical sampling design will be used to determine whether coral diseases cluster over two spatial scales. This sampling will be conducted in four locations in the Caribbean, two with and two without a history of frequent thermal anomalies. Second, the relationship between environmental predictors and outbreaks of coral disease in the Caribbean over the last two decades will be hindcasted using historical-disease data and satellite temperature records. Third, a series of laboratory experiments will be conducted to examine direct and indirect transmission of coral diseases.

Intellectual Merit

Coral diseases are devastating coral populations throughout the Caribbean region. For example, in the summer of 2014, diseases killed 70% of the acroporid corals (~28,000 colonies) in nurseries in the Florida Keys. There is a real urgency to identify coral-disease etiologies, predict their prevalence, and determine whether these diseases are infectious and contagious. This research will advance our understanding of the etiology of Caribbean-coral diseases, and will examine the role that ocean warming has played in driving outbreaks of disease. By understanding the etiology of coral diseases, and the degree to which the thermal environment drives these diseases, we will increase our capacity to predict and manage contemporary and future coral-disease outbreaks.

C. PROJECT DESCRIPTION

Background

Outbreaks of coral diseases have been a major cause of modern reef-coral decline in the Caribbean (Aronson and Precht 2001). These coral losses have also changed the community structure of many reefs (Aronson and Precht 2001, Cruz et al. 2014, Loh and Pawlik 2014). There are now approximately fourteen described stony-coral diseases in the Caribbean, many of which have become widespread (Table 1; Sutherland et al. 2004, Weil and Rogers 2011). Despite their wide geographic distributions, and their decades-long histories, little is known about the etiology of most coral diseases.

Table 1. Described diseases of Caribbean stony corals. If known, their etiologic agents are listed. Known abiotic stressors associated with the disease also are listed, if known, and references.

Disease	Known Etiology?	Abiotic Stressors	References
1. Bleaching	Yes	Osmotic shock, elevated temperature and light, cold-temperature stress	Goreau 1964; Glynn 1993
2. Dark-spot syndrome	No	Elevated temperature	Garzon-Ferreira and Gil 1998; Goreau et al. 1998
3. Black-band disease and red-band disease	Cyanobacterial spp., *Desulfovibrio* and *Beggiatoa* spp.	Elevated temperature, eutrophication, high light, sedimentation, pollution	Antonius 1973; Rutzler and Santavy 1983
4. Yellow-band and yellow-blotch disease	*Vibrio* spp. (?)	Elevated temperature	Santavy et al. 1999; Cerrano et al. 2000; Cervino et al. 2001, 2004
5. White-band disease type I	No	?	Gladfelter 1982; Peters et al. 1983
6. White-band disease type II	*Vibrio charcharii* (?)	?	Ritchie and Smith 1998
7. White-pox disease	*Serratia marcescens* (?)	Human sewage, elevated temperature, precipitation	Patterson et al. 2002; Sutherland and Ritchie 2004; Muller and van Woesik 2014
8. White-patch disease	No	?	Rodriguez-Matrinez et al. 2001
9. White-plague disease type I	No	?	Dustan 1977
10. White-plague disease type II	*Aurantimonas coralicida*	Elevated temperature (?)	Richardson et al. 1998; Denner et al. 2003
11. White-plague disease type III	No	?	Richardson et al. 2001
12. Caribbean white-syndromes	No	?	Weil and Rogers 2011
13. Caribbean-ciliate infection	*Halofolliculina* spp.	?	Croquer et al. 2006
14. Growth anomalies	No	UV Radiation	Squires 1965; Peters 1984; Peters et al. 1986

Etiological studies of coral diseases

Although outbreaks of coral diseases have been occurring since the 1960s, researchers are still trying to determine whether these diseased are indeed infectious and contagious. An infectious disease is caused by a micro-organism, such as a bacterium, protest, fungus, or virus; a contagious disease is an infectious disease, which is communicable by direct contact or through secretions from a diseased individual. Much research over the past several decades has focused on identifying putative pathogens of coral diseases and fulfilling Koch's postulates. Still, it is unclear whether most coral diseases are indeed transmissible (i.e., contagious). Of the 14 described Caribbean stony-coral diseases (Table 1), Koch's postulates have been fulfilled for very few coral-disease pathogens.

One of the best described infectious coral diseases is bacterial bleaching in the eastern Mediterranean, Indian Ocean, and Red Sea (Rosenberg et al. 2007). Koch's postulates were fulfilled for bacterial bleaching on two species when *Vibrio shiloi* was identified as the infectious agent for *Oculina patagonica* (Kushmaro et al. 2001), and *Vibrio coralliilyticus* was identified as the infectious agent for *Pocillopora damicornis* (Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003). Unexpectedly, a recent study showed that the putative pathogens that caused bacterial bleaching in the previous studies were no longer found in diseased samples, even though the corals appeared bleached (Ainsworth et al. 2007). Similarly, in 2002 Patterson et al. identified the fecal enterobacterium *Serratia marscescens* as the causal agent of white pox on acroporid corals in the Caribbean Sea, by fulfilling Koch's postulates. Yet, the same authors who first described the etiological agent of white pox only found the putative pathogen in only some diseased samples from the same location years later (Sutherland et al. 2010). Furthermore, in a study of white-pox disease on *Acropora palmata* in 2014, *Serratia marscescens* was not recoverable in any of the disease samples either through culture-dependent or culture-independent methods (Lesser and Jarett 2014). Therefore, even these "best-described" coral diseases are still not well understood. A final example of this is *Aurantimonas coralicida*, the pathogen that appeared to cause white-plague disease on the coral *Dichoceonia stokesi* (Denner et al. 2003). Although white-plague disease caused significant mortality of this species in the Florida Keys during the late 1990s (Richardson et al. 1998), subsequent laboratory testing suggested *A. coralicida* was not always pathogenic (Richardson and Aronson 2002). Although white-plague disease is now thought to affect at least 41 different coral species within the Caribbean (Sutherland et al. 2004), *A. coralicida* was not found on samples taken from *Orbicella faveolata* that showed signs of the disease (Sunagawa et al. 2009). These results suggest that *A. coralicida* may have been a pathogen that caused a disease outbreak on *D. stokesi* in the past, but the term "white-plague disease" may be a general description of tissue loss caused by several different pathogens.

Climate warming and coral diseases

While some researchers suggest that these emerging coral diseases are the result of an increase in human-introduced pathogens (Kaczmarsky et al. 2005, Sutherland et al. 2010, 2011), other researchers argue that these disease outbreaks are the result of immunocompromised corals, which have been subjected to increased environmental stressors (Lesser et al. 2007, Muller et al. 2008, Muller and van Woesik 2012, Miller and Richardson 2014).

Indeed, thermal stress has been implicated as a driver of several coral diseases using both correlative-field studies and experimental manipulations (Harvell et al. 2002, Bruno et al. 2007, Sokolow 2009; Muller et al. 2008, Muller and van Woesik 2012, Miller and Richardson 2014). For example, *in situ*, black-band disease has been shown to: (i) progress and transmit faster in the summer than in the winter (Boyett et al. 2007), (ii) increase in prevalence after a temperature threshold of 28 ˚C is surpassed (Kuta and Richardson 2002), and (iii) increase in incidence in direct relation to the rate of change in seawater temperature (Muller and van Woesik 2011). Similarly, experimental manipulations show that the rate of progression of Caribbean yellow-band disease increases with increasing temperature (Cervino et al. 2004). Although there is building evidence for thermal stress driving coral diseases, there is little conclusive evidence for any one defining mechanism that causes disease.

First, elevated temperature may influence coral diseases by increasing the growth rate and virulence of pathogens (Toren et al. 1998, Harvell et al. 2002). Some evidence suggests that the growth rate of *Vibrio* spp., and the expression of genes, which are thought to relate to their virulence, increase with increasing temperature (Kushmaro et al. 1998, Rosenberg et al. 2007). Second, elevated temperatures appear to compromise coral immunity (Toren et al. 1998, Lesser et

al. 2007, Muller et al. 2008, Mydlarz et al. 2010, Reed et al. 2010). For example, heat stress has been shown to down regulate lectin proteins that bind to microbes in the coral mucus, lowering coral-disease resistance (Ritchie 2006, Rodriguez-Lanetty et al. 2009). Third, thermal stress may affect vectors that transmit coral diseases (Harvell et al. 2002). Fourth, Harvell et al. (2002) suggested that warmer winters than in the past, have the potential to relax over-wintering dormancy, allowing infections to remain actively transmissible throughout the year. Finally, thermal stress frequently leads to coral bleaching, which is the loss of symbiotic dinoflagellates (*Symbiodinium* spp.) and their photosynthetic pigments from the coral host, which further compromises the health of the coral host, increasing disease susceptibility (Glynn 1984, Brown 1997).

Several studies have shown that disease outbreaks often coincide with or closely follow thermal stress events (Patterson et al. 2002, Weil 2004, Willis et al. 2004, Muller et al. 2008, Brandt and McManus 2009, Miller et al. 2009). The relationship between thermal stress (expressed as coral bleaching) and disease has been documented for yellow-band disease and white plague in the Caribbean (Cróquer and Weil 2009, Miller et al. 2009), 'atramentous necrosis' and white syndrome on the Great Barrier Reef (Jones et al. 2004, Bruno et al. 2007), dark-spot syndrome in the Caribbean (Brandt and McManus 2009) and white-pox disease in Florida and the Caribbean (Patterson et al. 2002, Muller et al. 2008, Rogers and Muller 2012). As the oceans continue to warm (Hansen et al. 2006, Hansen et al. 2010), thermal anomalies will most likely continue to cause coral bleaching (Hoegh-Guldberg 1999, Donner et al. 2005, Hoegh-Guldberg et al. 2007), and may consequently increase the prevalence of coral diseases (Harvell et al. 2002, Muller & van Woesik 2012).

While it is clear that elevated temperatures are associated with some coral diseases, the associations are not uniform across diseases or even uniform for single diseases across coral hosts. For example, Williams et al. (2010) studied the environmental drivers of four diseases on four coral species in Hawaii, and found that the diseases differed in their response to temperature. Furthermore, when disease prevalence was modeled by combining data for all diseases together, the predictive accuracy of the model decreased considerably. The results from Williams et al. (2010) indicate that each disease is likely to have a different set of environmental drivers. Such variability is also likely a result of distinct etiologies and of complex relationships among the different coral species, the pathogens, and the environment.

Furthermore, some studies have found no relationship between episodes of thermal stress and disease, including other studies on 'atramentous necrosis' and white syndrome on the Great Barrier Reef (Anthony et al. 2008, Ban et al. 2012). The lack of any relationship between thermal stress and disease is not, however, necessarily evidence of coral resistance. For example, no relationship between thermal stress and disease, in some studies, may be an artifact of sampling frequency (Muller et al. 2008). Indeed, continuous monitoring through thermal-stress events is rare. It is therefore conceivable that mismatches may occur between annual coral monitoring programs and the rapid rate at which some diseases spread (Dalton et al. 2010, Roff et al. 2011). Unless sampling is frequent, monitoring programs may miss the effect of thermal stress on the subsequent change in the prevalence of coral diseases. Alternatively, there may be temperature thresholds, below which diseases are rare and above which diseases increase (Lesser et al. 2007).

These examples highlight inconsistencies and a need for more comprehensive monitoring of both coral diseases and abiotic factors that potentially affect the expression of diseases.

Over the next century, the oceans will continue to warm (Hansen et al. 2010, Figure 1), and this warming may consequently increase the prevalence of coral diseases (Harvell et al. 2002, Muller and van Woesik 2012). Yet, thermal stresses vary spatially and temporally (Thompson and van Woesik 2009, Burrows et al. 2011; Figure 2). For example, some localities have historically experienced frequent thermal anomalies approximately every 4-6 years, whereas other localities have experienced infrequent thermal anomalies, every 50-60 years (Figure 1; Thompson and van Woesik, 2009). Two main regions in the Caribbean have experienced high-return frequencies of thermal anomalies. The first region is centered on Puerto Rico, and extends west to the Dominican Republic and east to the Virgin Islands. The second region of high-return frequencies is centered on eastern Costa Rica, and extends north to Nicaragua and south to Panama. Previous research has found that the same locations that experienced high-frequency return periods in the past few centuries have also most recently experienced the most severe thermal stress (Figure 1; Thompson & van Woesik 2009). If these patterns persist into the near future, then some localities will receive more intense and more frequent thermal stress than other localities.

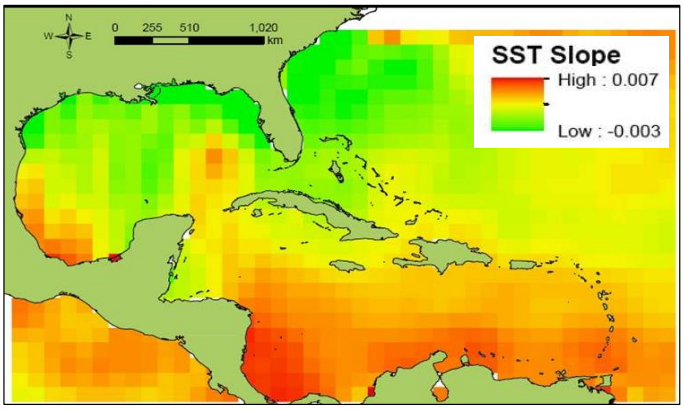

Figure 1. The rate of change in sea surface temperature per degree Celsius per year, using resolved 1° by 1° globally gridded HADISST sea-surface temperature data over the common time period (1870 to 2012).

Yet, populations have the potential to become locally adapted to their abiotic environment (Brown et al. 2002, Mitchell et al. 2005). Indeed, recent studies have shown evidence of coral acclimatization and, potentially, adaptation to thermal stress (Brown et al. 2002, Maynard et al. 2008, Thompson and van Woesik 2009, Kenkel et al. 2013, Palumbi et al. 2014). For example, Maynard et al. (2008) identified increased thermal tolerance in three major coral genera four years after mass bleaching on the Great Barrier Reef (after accounting for differential mortality), suggesting that the corals had acclimatized to their thermal environment. Similarly, Brown et al. (2014) identified evidence for environmental 'memory' in corals that had experienced high irradiance

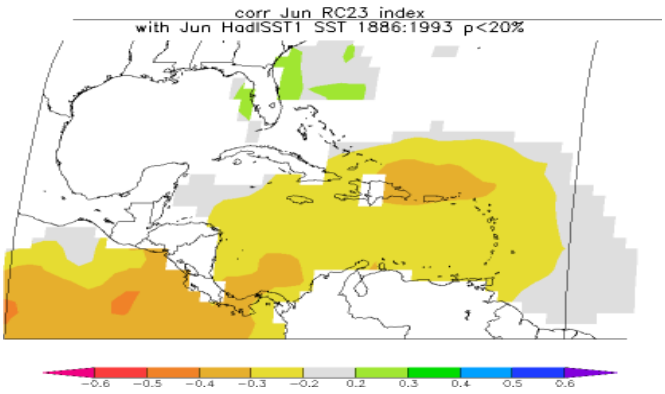

Figure 2. Correlation of the annual mean of the high-frequency (~6 year) component (based on detrended $\delta^{18}O$ and Sr/Ca records used in Multichannel Singular Spectrum Analysis to determine the significant modes of SST variability), with the detrended, annually resolved 1° by 1° gridded HADISST sea-surface temperature data over the common time period (1886-1993). Shading represents the strength of the correlation (from Thompson and van Woesik 2009).

10 years earlier, which were less susceptible to coral bleaching than corals which lacked the environmental 'memory' of high-irradiance stress. As the oceans continue to warm, considering historical trends in temperature (Figure 2) and the frequency of thermal-stress events (Figure 1) may both prove to be critical for accurately forecasting marine diseases.

Primary Research Questions

***Research Question 1*:** Do coral diseases cluster and follow a contagious-disease model that varies with thermal history?

There are limitations to the application of Koch's postulates, especially within the marine environment. Indeed, identifying coral pathogens has proven difficult and evasive (Ritchie et al 2011; Richardson 2004). A substantial limitation is the requirement to grow potential pathogens in pure culture, which eliminates many organisms, including viruses, protozoa, and many fungi and 99% of marine bacteria (Ritchie et al. 2001). Additionally, Koch's postulates cannot determine causative agents of diseases that require a consortium of bacteria, such as black-band disease (Richardson 2004). An alternative to testing Koch's postulates is through the study of spatial epidemiology, which examines the distribution of diseased individuals and attempts to highlight the factors that might control disease presence.

Spatial epidemiology, or disease mapping, can determine whether the distribution of diseased individuals is consistent with a contagious mode of transmission. Disease clusters represent abnormally high numbers of individuals within a defined area, which exhibit similar disease signs (Lawson 2009). Coral diseases that are found in clusters are likely to be either: (1) infectious diseases that are transmitted from colony to colony (e.g. direct contact or water-borne pathogens), or (2) a disease caused by some environmental factor associated with the disease cluster (e.g., sewage outflow). Therefore, mapping coral diseases quantifies the spatial variation of disease prevalence, which in turn may provide insight into the potential mechanisms that cause outbreaks of coral disease. Spatial epidemiology will be used to address the following hypotheses: (1) coral diseases cluster both within and among sites, and (2) the prevalence of coral diseases will increase with increasing coral-colony density and percent-coral cover.

Methods

The spatial distribution of four signs of coral disease will be examined at two different spatial scales in the Caribbean: (i) among sites within a location (1-10 km), and (ii) within sites (100 m). The benthic structure of the reef will also be recorded within each site and compared with disease data. These surveys will: (1) determine the extent to which coral diseases cluster, (2) determine the co-occurrence of multiple diseases, and (3) determine whether there are any relationships between spatial patterns and coral-species diversity, coral cover, and coral density. To assess the prevalence of coral diseases at each location, a survey area (~ 1–10 km^2 depending on the region's geographic features) of hard-bottom habitat will be visually defined using Google Earth (http://earth.google.com/). The survey area will be divided into 100 m^2 grid cells (using Google Earth Path 1.4.4). Within each location, twenty-five 1000 m^2 grid cells will be randomly selected as *sites* (Figure 3). These *sites* will be defined as the primary sampling unit (Cochran 1977, Smith et al. 2011). A single 100 m^2 quadrat will be haphazardly placed within each *site* for field-data collection. According to previously published studies (Jolles et al. 2002, Zvuloni et al.

2009), a 100 m^2 quadrat is large enough to capture clusters of coral disease, and small enough to completely videotape the area within a single dive.

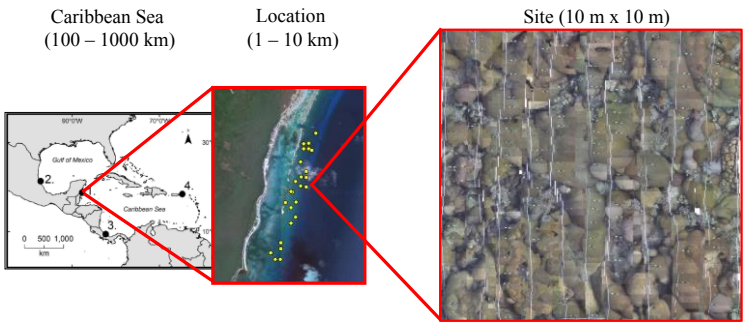

Figure 3. Twenty-five randomized sites (100 m^2) within one of four locations in the Caribbean Sea that will be surveyed.

Within each *site* divers will survey a 100 m^2 quadrat by systematically laying ten contiguous 1 x 10 m belt transects onto the reef substrate. Video transects of each *site* will be captured. Each coral colony with a disease sign will identified *in situ* and the species and disease signs will be recorded. Four signs of coral disease will be recorded: (1) **white sign** will be defined as a bright, white band or patch of recent mortality adjacent to healthy-appearing tissue (i.e., the tissue bordered a well-defined edge of exposed skeleton not yet colonized by algae or other biofouling organisms) (*sensu* Bythell et al. 2004) , (2) **dark spot** will be defined as tissue with purple, brown or black lesions, forming spots of irregular shapes (*sensu* Goreau et al. 1998), (3) **black band** will be defined as a black band over the coral tissue exposing white skeleton with different stages of biofouling (*sensu* Richardson 2004), and (4) **yellow sign** will be defined as a yellow discoloration of tissue forming a band or blotches (*sensu* Santavy et al. 1999). Bleached corals will also be noted, and any unknown signs will be recorded.

Still digital images will be captured from the video, stitched together using Adobe Photoshop®, and a mosaic of each quadrat will be created (Figure 3). Using the photo-mosaic, every coral colony within the quadrat will be outlined and measured, and the location of each colony will be mapped (Figure 4). A minimum of five 100 m^2 quadrats per location will be mapped in their entirety and used to test for disease clusters (approximately 14,000 colonies total). Metrics of coral-species diversity, coral cover, and coral density will be estimated for all quadrats, using Coral Point Count software.

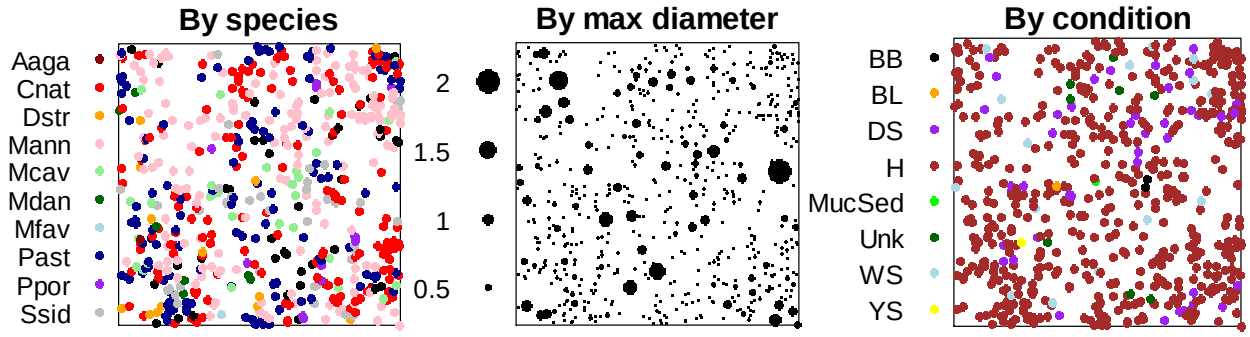

Figure 4. Mapped corals from one 10 m by 10 m site. Corals are plotted by species (left), by maximum diameter in meters (center) and by health condition (right). BB=black band, BL= bleached, DS = dark dpots, H=healthy, MucSed = mucous and sediment covered, Unk = unknown sign, WS = white signs, and YS = yellow signs.

Data analysis

The spatial distribution of coral disease will be analyzed at both scales (within sites and among sites) using the adjusted Ripley's K function (Figure 5). This function is defined as the expected number of diseased sites within a distance (r) from an arbitrary diseased site. Ripley's K analysis will identify areas of disease clusters by comparing the spatial distribution of diseased sites (or diseased corals) with the distribution of all sites (or all corals). Formally, Ripley's K will be calculated as:

$$K(r) = \frac{A}{n^2}\sum_{i=1}^{n}\sum_{j=1,j\neq 1}^{n}\frac{I_r(d_{ij})}{w_{ij}},$$

where *A* is the total area of the location, *n* is the number of diseased sites, and *dij* is the distance between any two diseased sites *i* and *j*. *Ir(dij)* indicates whether or not there is a diseased site within distance *r* from site *i*. Therefore, *Ir(dij)* has a value of 1 if *dij*<*r*, and 0 otherwise. Because the study area is finite, *wij* represents the portion of the circumference of each circle that falls outside of the previously defined location area. This statistic is standardized to account for the spatial aggregation of susceptible sites within the study area (see Zvuloni et al. 2009). Using a null model, a randomization technique will be applied to determine whether the *n* diseased sites found within the sample period are significantly spatially aggregated, when compared with the aggregation found in the population of all individual sites. A transformation, referred to as Besag's *L* function, will also be applied and is calculated as:

$$L(r) = \sqrt{\frac{K(r)}{\pi}} - r$$

With this scaling, sites that have a Poisson spatial distribution would result in the expected value of *L(r)*=0. The adjusted statistic compensates for the number of samples and stabilizes the variance (Diggle 1983). A null distribution for *L*(*r*) will be generated from a group of n sites and repeated 1,000 times so that *L*(*r*) is calculated for each group of n sites for any value of r. These results create a 95% confidence interval (CI) for *L*(*r*). *L*(*r*) is then calculated using only diseased sites to produce a new value, *LD*(*r*), which will then be compared with the *L*(*r*) null envelope. Any value that resides outside of the envelope indicates that either spatial clumping (above the *LD*(*r*)) or over-dispersion (below the *LD*(*r*)) of diseased sites is apparent. The spatial scale of disease clustering will be examined by increasing the distance from diseased sites (or individual corals within sites), within the sampling space over the two spatial scales.

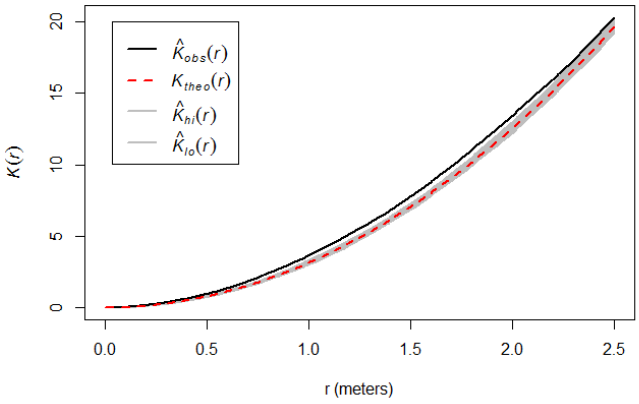

Figure 5. Ripley's K analysis of diseased colonies in Tuxpan, Mexico. The black line represents the observed spatial pattern of colonies with disease. The red line indicates the theoretical (Poisson) distribution. The gray area represents the 95% confidence intervals of the null distribution. The observed pattern is outside the theoterical distribution indicating clustering around a radius of 0.5-2 meters.

Research Question 2: Are localities in the Caribbean with a history of frequent thermal anomalies (every 4-6 years) more likely to have higher disease prevalence than localities without a history of frequent thermal anomalies?

The oceans are not homogenous, and thermal-stress events vary considerably both spatially and temporally (Figure 1; Thompson and van Woesik 2009, Burrows et al. 2011). Some localities in the Caribbean have historically experienced frequent thermal anomalies (~4–6 years), whereas other localities in the Caribbean have not. If these patterns persist into the near future, then some localities will receive more intense and more frequent thermal stress, and in turn, will experience higher selective pressure. Relative risk analyses will be used to address the following hypothesis: coral populations in localities with a history of frequent thermal anomalies will have an increased risk of developing a disease compared with coral populations in reference locations.

Methods

Based on the regions that were identified as either experiencing frequent or infrequent thermal anomalies in Thompson and van Woesik (2009), locations will be selected in the Caribbean region (Figure 3) and their thermal histories will be examined using data from the MetOffice HadISST records from 1870-2012 (Rayner et al. 2003). Wavelet analyses will be used to examine the frequency of thermal anomalies in those locations (Figure 6). Ultimately, four locations will be selected for survey, two with and two without a history of frequent thermal anomalies. (These four locations will be those same locations surveyed for *Research Question 1*). Two of these locations without a history of frequent thermal stress will be considered 'reference locations' and two locations with a history of frequent thermal stress will be considered 'frequent-anomaly locations'. To minimize the potential effect of spatial covariates, the locations will be separated by a minimum of 1,000 kilometers. Field surveys (as described above in Research Question 1) will be conducted at the four field locations in the Caribbean that have different thermal-anomaly frequencies.

Data analysis

The odds of corals developing signs of disease at reference locations will be compared with the odds of corals developing signs of disease at high-frequency locations. Formally, the odds will be examined using the relative risk (RR) assessment:

$$RR = \frac{\frac{a}{a+b}}{\frac{c}{c+d}}, \quad (3)$$

where a is the number of sites with a specific disease at a frequent-anomaly location, b is the number of sites without a specific disease at a frequent-anomaly location, c is the number of sites with a specific disease at a reference location, and d is the number of sites without a specific disease at a reference location (Sistrom and Garvan 2004). Relative risk will be calculated using a Bayesian approach (Gelman et al. 2004, Lawson 2009) and will be estimated using a binomial likelihood distribution and a uniform-Beta prior distribution. To obtain an estimate of relative risk, Markov Chain Monte Carlo simulations (100,000 iterations with a burn-in of 10,000) will be used with Gibbs sampling in OpenBUGS (MRC Biostatistics Unit, Cambridge, UK). This method will also determine the 95% credible intervals of the predicted relative risk for disease prevalence in the different coral taxa.

***Research Question 3*:** What is the relationship between temperatures and Caribbean-coral diseases over the past two decades?

Historical survey data from Caribbean coral reefs are available from the Atlantic and Gulf Rapid Reef Assessment Program (www.agrra.org), at least for the past two decades. These survey records provide data on a suite of coral diseases, and together with freely-available environmental data,

are an untapped resource for studying relationships between coral diseases and the environment. Historical data will be used to hindcast the underlying relationships between ocean warming and coral diseases to test the hypothesis that thermal stress is a primary driver of coral disease in the Caribbean.

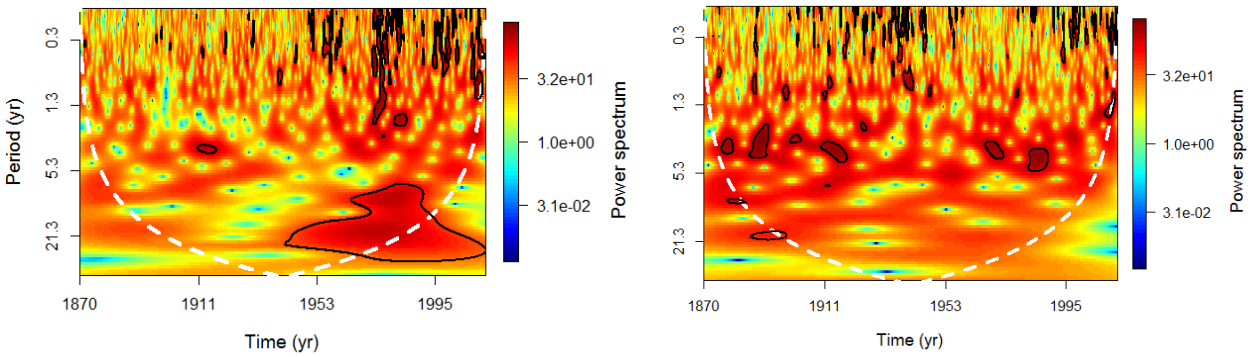

Figure 6. Morlet wavelet-transform analyses of records of detrended mean monthly sea surface temperature anomalies from Met Office HadISST data records from Jan. 1870 to Sept. 2012 for Mahahual, Mexico (left) and Bocas del Toro, Panama (right). The power spectra indicate the strength of the signals in time-frequency space. Black contour lines represent 95% confidence limits of significant periodicities (5% significance level against he red noise). White dashed lines indicate the one of influence.

Methods

White-band disease is one of the most severe and wide-spread diseases of Caribbean acroporids, yet there is a lack of empirical evidence identifying a relationship between elevated temperatures and white-band disease. To address research question 3, I will focus my efforts specifically on hindcasting the response of white-band disease to several metrics of sea-surface temperature.

Temperature data

AVHRR. Advanced Very High Resolution Radiometer (AVHRR) Pathfinder 5.2 (PFV5.2) nightly sea-surface temperature records will be obtained from the National Oceanographic Data Center and GHRSST (http://pathfinder.nodc.noaa.gov)[25], and monthly averages will be calculated for 1982-2012, at a 4 km by 4 km spatial resolution. Pathfinder records will be used to calculate all temperature predictors except for the 30-year climatology, which will require a long-term data set (Table 2).

HadISST. Mean monthly sea-surface temperatures at a 1˚ by 1˚ spatial resolution will be obtained from the MetOffice HadISST records to calculate the 30-year climatology (Rayner et al. 2003). All temperature predictors will be also calculated at the course-grained 1˚ by 1˚ spatial resolution, and the models will be run with both coarse-grained and fine-grained temperature data, and compared.

Eight metrics of temperature will be tested as predictors of disease. These metrics of sea surface temperatures are based on previous research and on proposed mechanistic effects of temperature on coral diseases. For example, the minimum temperature will be evaluated because warm winters have the potential to relax over-wintering restrictions (dormancy) on pathogens (Harvell et al. 2002), and winter cold snaps have been shown to reduce the risk of some coral diseases (Heron et

al. 2010). The maximum temperature will be evaluated because of the documented association between elevated temperature and some coral diseases (Harvell et al. 2002, Bruno et al. 2007, Muller et al. 2008, Miller and Richardson 2014). Also, the temperature during the month prior to survey will be evaluated because a temporal lag has been observed between thermal stress and the development of signs of bleaching and disease (Berkelmans and Willis 1999, Miller et al. 2009). Thermal anomalies are measures of above-average heat stress, and several anomaly calculations have been used successfully to predict coral bleaching and disease (Strong et al. 1997, Heron et al. 2010). Therefore, measures of thermal anomalies will be included as predictors of the disease. Rates of change in temperature also will be tested to determine whether disease is more likely to occur during periods of rapid temperature change. Lastly, the historical rate of change in temperature will be included to determine whether localities that have experienced a rapid increase in temperature during the 30 years prior to the surveys are more or less likely to have corals with white-band disease than other localities that have experienced less rapid increases in temperature.

Table 2. Definition of 10 predictor variables that will be used to hindcast white-band disease in the Caribbean using boosted-regression tree models.

	Predictor variables	Description
1.	Minimum temperature	Minimum monthly mean SST for the year prior to survey (˚C)
2.	Maximum temperature	Maximum monthly mean SST for the year prior to survey (˚C)
3.	Survey temperature	Mean SST for the month and year of survey (˚C)
4.	Prior-month temperature	Mean SST for the month prior to survey (˚C)
5.	Survey-temperature anomaly	Mean SST anomaly for the month of survey, calculated from the 10-year monthly means (˚C)
6.	Rate of change in temperature	Mean SST for the month prior to survey subtracted from the mean SST during the month of survey (˚C)
7.	6-month cumulative anomaly	Sum of the monthly SST anomalies for the 6 months prior to survey, calculated from the 10-year monthly SST means (˚C)
8.	30-year rate of change in temperature	Rate of change in mean monthly SST for the 30 years preceding survey (˚C)
9.	Depth	Maximum depth of survey site (m)
10.	Reef habitat	Categorical; Bank reef, reef crest, forereef, leeward reef, patch reef, or rhomboid reef (as per the AGRRA protocol)

Coral-disease data

Coral-disease data will be obtained from the Atlantic and Gulf Rapid Reef Assessment (AGRRA) survey program database (http://www.agrra.org/). Presence and absence of white-band disease on each colony will be used in the hindcasting model.

Data Analysis: The BRT Model

Identifying and predicting spatial patterns of coral disease in general, and white-band disease in particular, are challenging due to: (i) our lack of knowledge of disease etiology, and (ii) the complexity of the tripartite relationship among the hosts, the pathogens, and the environment. This complexity is rooted in non-linear relationships and interactions among predictor variables. Boosted regression tree (BRT) modeling is a statistical approach that is capable of incorporating

complex, non-linear relationships into a single, predictive model (De'ath et al. 2007, Elith et al. 2008). BRT modeling will be used to hindcast white-band disease on Caribbean acroporids.

The boosted-regression-tree technique combines regression trees and boosting, by iteratively fitting new trees to a model that best reduces the model's deviance. More formally, a boosted-regression-tree model is an additive regression model that takes the form:

$$f(x) = \sum_{m=1}^{n} \beta_m \, b(x; \gamma_m),$$

where β_m is a vector of weighted constants for each node of the tree, *x* is the predictive variable, and γ_m is a matrix that defines the splitting variables, their values at each node, and the predicted values; the function $b(x; \gamma_m)$, therefore, represents the 'tree' (De'ath et al. 2007). Trees are constructed recursively and added to the model sequentially from *m* to *n*, and each subsequent tree is added to minimize the loss function of the model. The loss function is defined as:

$$L\left(y, f(x)\right) = [r - \beta b(x;\, \gamma)]^2 \,,$$

where *r* is the least-squares residuals (De'ath et al. 2007). Stochasticity is incorporated into the model by bagging, which uses a bootstrapped subset of data (75%, with replacement) to fit each new tree. This probabilistic component, combined with a model-simplification procedure, reduces overfitting and improves the model's accuracy (De'ath et al. 2007, Elith et al. 2008). BRT models will be constructed using the package *'gbm'* version 2.1 (Ridgeway 2006), and using code written by Elith et al. 2008, in the R statistical program (R Core Team 2014). A k-fold cross-validation procedure will be used to train (90%) and test (10%) each model. The relative contribution of each predictor variable will be estimated (Elith et al. 2008), and any interactions between predictor variables will be examined (Ridgeway 2006; Figure 7).

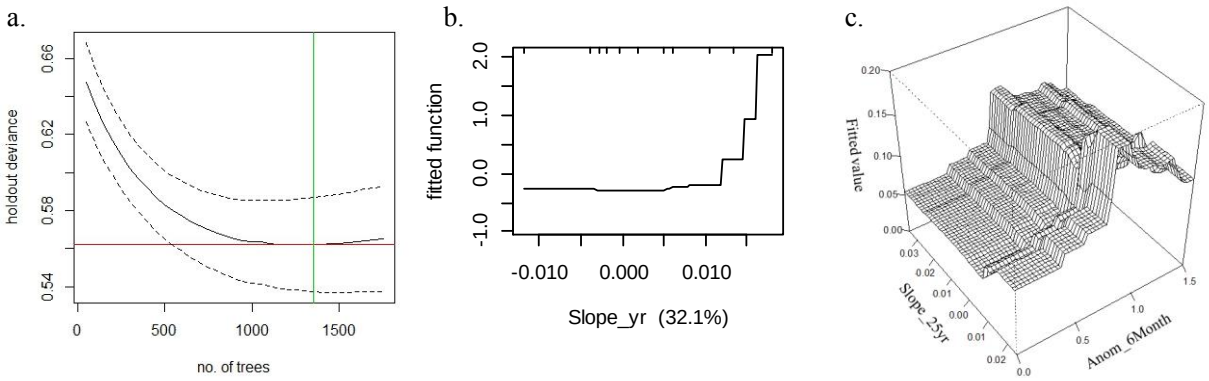

Figure 7. Various steps in the boosted regression tree modeling procedure. (a) Identification of the optimal number of trees (n) using the minimum cross-validation (holdout) deviance. The dark line is the mean deviance of the 10-fold cross-validation and dotted lines indicate standard error. The green line indicates the optimal number of trees (n) and the red line indicates the minimum mean deviance. (b) Partial dependency plot for a predictor (25-year rate of change) of white-band disease on *Acropora palmata*. (c) Partial dependency plot of the interaction between two predictor variables (25-year rate of change and 6-month heat accumulation) against the fitted values of the preliminary model.

***Research Question 4*:** Are coral diseases directly and indirectly contagious?

Laboratory transmission experiments can be used to determine whether diseases are transmissible, yet few studies have used this method to test coral-disease transmission. A series of experiments will be conducted to test the hypothesis that coral diseases are contagious and transmissible by

direct contact and through waterborne transmission. These experiments will provide insight into the potential mechanisms of transference, and determine whether these diseases are contagious.

Methods

Dark-spot syndrome on *Siderastrea siderea* and yellow-band disease on *Orbicella faveolata* will be the focus of these experiments, as they are prevalent and wide-spread diseases, which affect critical reef-building coral species. Dark spots will be defined as tissue with purple, brown or black lesions, forming spots of irregular shapes (*sensu* Gil-Agudelo and Garzon-Ferreira 2001), and yellow signs will be defined as yellow discoloration of the coral tissue forming bands or irregular blotches, congruent with Caribbean yellow-band disease or yellow-blotch disease (*sensu* Santavy et al. 1999). *S. siderea* and *O. faveolata* were selected for use in the transmission experiments because they are the most common hosts of dark-spot syndrome and Caribbean yellow-band disease, respectively (Garzon-Ferreira and Gil 1998, Santavy and Peters 1997, Cervino et al. 2001, Gil-Agudelo and Garzon-Ferreira 2001, Borger 2003, Weil 2004, Borger 2005, Bruckner and Bruckner 2006).

All transmission experiments will be conducted in an outdoor flow-through seawater facility at Mote Marine Laboratory in Summerland, Key, Florida. Appropriate permits will be obtained from the Florida Keys National Marine Fisheries Service for the collection of coral colonies. All healthy and diseased corals will be collected from Wonderland Reef in the lower Florida Keys using a sterilized hammer and chisel, and surgical gloves. Healthy fragments will be collected prior to diseased fragments, to prevent the potential exposure of healthy corals to disease during collection.

Experiment 1 – Direct-contact transmission

Two treatments will be included in the direct-contact transmission experiments: (1) direct-contact from a diseased fragment to a healthy fragment (experimental), and (2) direct-contact from a healthy fragment to a healthy fragment (control). Contact between the corals will be achieved through direct placement of one coral with disease on top of the other, without disease for a 24 hour period. Then, the direct contact colonies will be separated and the corals will be monitored twice daily for signs of disease, and will be photographed daily. A minimum of three replicate per treatments will be run in each experiment.

Experiment 2 – Indirect (waterborne) transmission

A cascading system has been designed to test for waterborne transmission of coral diseases. This design allows for uni-directional flow of UV-sterilized and filtered seawater to test for waterborne transmission by exposing corals in ‘lower chambers’ to water from diseased corals in ‘upper chambers’ (Figure 8). The experiments will consist of four treatments: (1) transmission from a diseased-coral fragment to a healthy-coral fragment (experimental), (2) transmission from a healthy-coral fragment to a healthy-coral fragment (control 1), (3) transmission from a rubble fragment to a healthy-coral fragment (control 2), and (4) transmission from an empty chamber to a healthy fragment (control 3). A minimum of seven replicates of each treatment pair will be tested in the experiment. Corals will be monitored twice daily for signs of disease, and will be photographed daily for up-to three weeks.

Experiment 3 – Antibiotic treatment

The effect of a broad-spectrum antibiotic on the progression and transmission of yellow-band disease will be tested using a fully-crossed experimental design with eight treatments: (1) healthy coral with no ampicillin (control 1), (2) healthy coral with ampicillin (control 2), (3) diseased coral with no ampicillin (control 3), (4) diseased coral with ampicillin (experimental 1), (5) healthy fragment in direct contact with a healthy coral with no ampicillin (control 4), (6) healthy fragment in direct contact with a healthy coral with ampicillin (control 5), (7) diseased fragment in direct contact with a healthy coral with no ampicillin (control 6), and (8) diseased fragment in direct contact with a healthy coral with ampicillin (experimental 2).

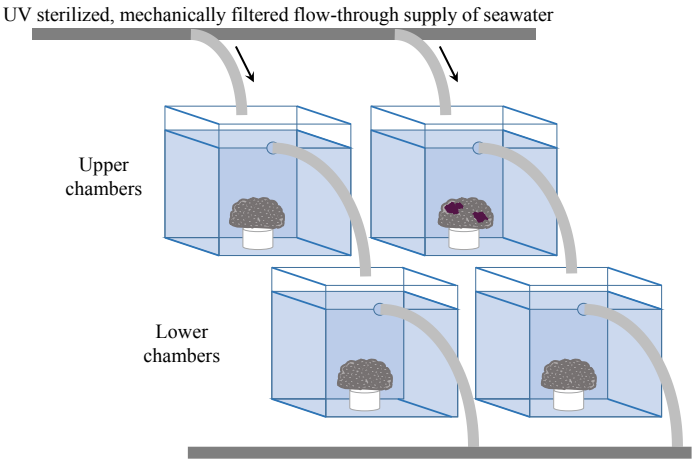

Figure 8. Cascading experimental design with uni-directional water flow from upper chambers to lower chambers (2.7 L each). This design will allow the healthy corals in lower chambers to be exposed to water from diseased corals in upper chambers. All upper chambers will be supplied with UV sterilized and mechanically filtered seawater.

The broad-spectrum antibiotic ampicillin was chosen for this experiment for three reasons: (1) gram-negative marine *Vibrio* spp. bacteria are thought to be the pathogens of yellow-band disease (Cervino et al. 2004; Cervino et al. 2008) and ampicillin has a high action rate against gram-negative bacteria, (2) ampicillin is seawater soluble, and (3) ampicillin was the most effective drug at preventing the infection of another Caribbean-coral disease (white-band disease on *Acropora cervicornis*, Kline and Vollmer 2011). The target dose of ampicillin will be 100 µg L^{-1} hr^{-1}, for one hour per day, for an 8-day course of drugs, and was determined based on dosages used in previous studies that were shown to inhibit the infection of other coral diseases (Smith et al. 2006, Kline and Vollmer 2011). A minimum of three replicates for each treatment will be run. Corals will be monitored for signs of disease, and will be photographed daily for up-too two weeks.

Data Analysis

A chi-square goodness-of-fit test will be used to compare the observed number of transmissions (from diseased to healthy) with the expected number of transmissions (from healthy to healthy) to test the hypothesis that the disease is not transmissible.

The freeware ImageJ (National Institutes of Health, http://imagej.nih.gov/ij/), will be used to measure the change in the disease front (i.e. the edge of the lesion), from the daily images. A progression or regression rate will be calculated based on whether the disease progresses or regresses, and the rates will be averaged across colonies within each treatment.

D. PROPOSED DISSERTATION OUTLINE

Chapter 1. Introduction
Chapter 2. Spatial epidemiology (*Research Question 1*)
Chapter 3. Relative risk of disease based on thermal history (*Research Question 2*)
Chapter 4. Coral-disease hindcasting models (*Research Question 3*)
Chapter 5. Coral-disease transmission experiments (*Research Question 4*)
Chapter 6. Description of ciliated protist discovery
Chapter 7. Synthesis and conclusions

D. PROPOSED PROJECT TIMELINE

2012	**Summer**	Complete field surveys of four Caribbean locations
	Fall & Winter	Begin relative risk analysis and image processing
2013	**Spring**	Continue analyses and image processing
	Summer	Complete transmission experiments at Mote Marine Laboratory
	Fall & Winter	Continue image processing and begin transmission experiment analyses
2014	**Spring**	Continue image processing and transmission experiment analyses. Conduct hindcasting analyses. *Note: Publication of relative risk analyses in Ecology*
	Summer	Continue image processing and transmission experiment analyses.
	Fall & Winter	Continue image processing and transmission experiment analyses. *Note: Publication of ciliate discovery in Coral Reefs*
2015	**Spring**	Continue image processing and transmission experiment analyses. *Note: Publication of hindcasting in Nature Climate Change*
	Summer	Complete transmission experiment analyses. Submit for publication. Continue image processing.
	Fall	Complete image processing. Begin spatial analyses and dissertation writing.
	Winter	Complete spatial analyses. Submit spatial analysis manuscript for publication.
2016	**Spring**	Finish dissertation and defend.

E. PROPOSED BUDGET

YEAR 1			
Field Supplies	**COST PER UNIT**	**UNITS**	**TOTAL COST**
HOBO data logger Pendant temp/light	$59	15	$885
HOBO sofware	$45	1	$45
HOBO communication kit	$115	1	$115
Underwater transect tapes	$35	6	$210
Steel stakes	$6	30	$180
Dive thermometers	$50	10	$500
Underwater paper	$36	8	$288
Underwater slates	$15	16	$240
Miscellaneous field supplies	$250	1	$250
		subtotal	**$2,713**
Field Travel for Caribbean Surveys	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Accommodation Tuxpan + food ($65)	$145	10	$1,450
Accommodation Mahahual + food	$230	10	$2,300
Accommodation Bocas del Toro + food	$220	10	$2,200
Accomodation St. John USVI + food	$250	10	$2,500
USVI - (diving from shore, no boat costs), land travel	$45	10	$450
Gasoline per field trip Puerto Morelos	$45	10	$450
Gasoline per field trip Tuxpan	$58	10	$580
Air travel Orlando Cancun	$230	2	$460
Air travel Cancun Mexico City	$120	2	$240
Air travel Mexico City Orlando	$250	2	$500
Land travel Mexico City Tuxpan Mexico city	$85	2	$170
Air travel Orlando Panama City Orlando	$510	2	$1,020
Air travel Panama City San Blas Panama city	$220	2	$440
Air travel Orlando USVI Orlando	$425	2	$850
Travel to Orlando (return)	$125	2	$250
		subtotal	**$13,860**
Office Supplies	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Paper	$50	1	$50
Publication Costs	$700	2	$1,400
		subtotal	**$1,450**
Other Costs	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Conference registration fee	$300	1	$300
Conference travel costs	$1,400	1	$1,400
		subtotal	**$1,700**
	YEAR 1 TOTAL		**$19,723**

YEAR 2			
Field Supplies	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Acrylic tanks for transmission experiments	$100	20	$2,000
Wet tile saw for coral fragmentation	$245	1	$245
Mote boat use for coral collection (4 half days)	$280	4	$1,120
Tanks and dive gear rental	$166	1	$166
UV Sterilizers	$133	2	$266
Ampicillin	$75	2	$150
Contract for 12 hr system set-up @ $25/hr	$25	12	$300
Wet-lab space rental for 38 days @25/day	$25	38	$950
50 mL Centrifuge tubes, pack of 50	$36	1	$36
Whirl-Pak Write-On Bags 2-ox, pack of 500	$47	1	$47
Buckets, acrylic, and misc. field supplies	$250	1	$250
		subtotal	**$5,530**
Field Travel for Experimental Research	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Mote housing for 2 researchers 6 weeks @ $210/week	$210	6	$1,260
Per diem for 2 researchers for 6 weeks @ $24/day	$24	84	$2,016
Travel to Mote from FIT and locally	$250	1	$250
		subtotal	**$3,526**
Office Supplies	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Paper	$50	1	$50
Publication Costs	$700	2	$1,400
		subtotal	**$1,450**
	YEAR 2 TOTAL		**$10,506**

YEAR 3			
Office Supplies	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Computer for modeling	$1,200	1	$1,200
Paper	$50	1	$50
Publication Costs	$700	2	$1,400
		subtotal	**$1,450**
Other Costs	**COST PER UNIT**	**UNITS**	**TOTAL COST**
Conference registration fee	$300	1	$300
Conference travel costs	$1,800	1	$1,800
		subtotal	**$2,100**
	YEAR 2 TOTAL		**$3,550**

Total Budget: 33,997

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