Developing a Comprehensive Strategy for Coral Restoration for Florida

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Introduction

Coral reefs in the Florida Keys, like those elsewhere in the Caribbean, have become substantially degraded over recent decades due to numerous stressors that include hurricanes, overfishing, disease, thermal stress, and eutrophication (Dustan and Halas 1987, Jaap *et al*. 1988, Lessios 1988, Porter and Meier 1992, Hughes 1994, Porter *et al*. 2001). Florida's reefs now support a simpler coral assemblage that has lost the dominant reef-building coral species necessary to construct the reef framework that is fundamental to restoring the heath and resiliency of this ecosystem (Burman *et al.* 2012). Further, such is the present condition of Florida's coral reefs that there is now an ever-increasing acceptance by resource managers that threat abatement strategies developed to mitigate further degradation of the reef ecosystem are no longer solely sufficient to stem its progressive decline (*see*, US Coral Reef Task Force, Coral Reef Restoration and Mitigation Panel, 2011) at: http://www.coralreef.gov/meeting26/). In recognition of the condition of the state's coral reef ecosystem, the Florida Wildlife Legacy Initiative (FWLI), consistent with the guiding principles of Florida's State Wildlife Action Plan (Florida Fish and Wildlife Conservation Commission, 2012) that prioritizes within the coral reef ecosystem "the restoration of damaged areas and replacement of species lost", set as one of their goals to "improve coral reef restoration and conserve Species of Greatest Conservation Need (SGCN) through planning and research" at: http://www.myfwc.com/conservation/special-initiatives/fwli/taking-action/marine/ .

It is with that growing recognition that extensive effort in recent years has been directed at direct coral reef restoration efforts (Coral Recovery Program, American Recovery and Reinvestment Act, 2009 at: http://www.habitat.noaa.gov/pdf/tnc_noaa_arra_restoration_summary.pdf) and related ecosystem-based restoration research (*e.g.*, Yap, 2009; Burkepile and Hay, 2009; Baums *et al*, 2003; Miller *et al*., 2002). Although these efforts have undeniably enhanced our knowledge base required to ultimately develop and implement management actions designed to restore coral reef ecosystem health and resiliency, the lack of a unified vision toward that goal has resulted in a duplication of effort in some of these activities while vital gaps in information in other activities remain. This has ultimately hindered the development of a comprehensive coral reef restoration strategy. In recognition of this, the FWLI set as one of its priority objectives to "…develop a comprehensive coral reef restoration research plan for Florida that will outline the essential strategies necessary to affect a well coordinated, comprehensive coral reef restoration…" http://www.myfwc.com/conservation/special-initiatives/fwli/taking-action/marine/ .

This project directly addressed this priority objective. To achieve this objective, we first assembled a small group of key state and federal conservation managers and leading coral reef ecosystem researchers to form a steering committee (Table 1). This committee was tasked to work in close association with this project's Principal Investigators and identify needed topics of study related to coral reef ecosystem restoration research. The steering committee met on February 10, 2014 at Biscayne National Park Headquarters and prioritized four topics of study related to coral reef ecosystem restoration research. These four areas were: i) coral diseases; ii) coral reef ecosystem processes; iii) coral traits; and, iv) best restoration management practices.

The P.I.s then worked with a subset of the steering committee (Burkepile, Miller, and Whittle) to identify the most appropriate researchers to develop specific research and restoration activities associated with those topics. Once the researchers were identified, we invited them to participate in facilitated topical workshops to develop those research activities. We conducted workshops on three of the four topical research areas: coral diseases, coral reef ecosystem processes, and coral traits. The P.I.s consulted two Steering Committee members (Moore and Whittle) regarding a best management practices workshop. The Steering Committee members suggested that a best management practices workshop was not required and recommended that the essential first step regarding best management practices should focus on conducting a synthetic analysis of existing nursery and outplanting efforts. Accordingly, a best management practices research activity was developed. However, the research specified in that activity was also specified by the researchers that participated in the coral traits workshop. Consequently, the activities and approaches identified in the best management practices research activity have been incorporated into those developed during the coral traits workshop.

This document summarizes the results of the workshop process and outlines the essential research activities to guide the FWLI in setting funding priorities for future coral reef ecosystem activities during the remainder of its 2012-2017 funding cycle. The results of each topical workshop are summarized by chapter. Each of these chapters includes a summary of the state of knowledge of each topic found within the scientific literature and from presentations given by the workshop attendees. Each chapter concludes with a list of the research activities that were identified and drafted by the workshop attendees. These activities range from highly specific projects to broader approaches that describe the range of research needs required to understand these key areas of knowledge. Most of the highly specific research activities are found in the coral disease chapter. The remaining research activities do not focus on a specific research project. Rather, they were intended to be more general and drafted in a manner that, if selected by the FWLI standing team for inclusion in its announcements, could potentially elicit multiple different research project proposals from various researchers and have the potential to be used multiple times to elicit proposals. The year-to-year changes in the announcement could simply identify past FWLI funded research to ensure duplicative research does not occur. Finally, we have included as an appendix at the conclusion of the report the list of the identified research activities. We note that we have not ranked these activities in any particular order of priority.

Table 1. List of Steering Committee members and affiliations

Name	Organization
Erin McDevitt	Florida Fish & Wildlife Conservation Commission, Division of Habitat & Species Conservation
Amber Whittle	Florida Fish & Wildlife Conservation Commission, Fish & Wildlife Research Institute
Joanna Walczak	Florida Department of Environmental Protection, Coral Reef Conservation Program
Margaret Miller	National Oceanic and Atmospheric Administration, Southeast Fisheries Science Center
Deron Burkepile	Florida International University
Amanda Bourque	Biscayne National Park
Tom Moore	National Oceanographic and Atmospheric Administration

Literature Cited

Baums IB, MW Miller, and AM Szmant (2003) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. II. Feeding, respiration and growth. Marine Biology 142: 1093–1101.

Burman SG, RB Aronson, and R van Woesik (2012) Biotic homogenization of coral assemblages along the Florida reef tract. Marine Ecology Progress Series 457: 89-96.

Burkepile DE and ME Hay (2009) Nutrient versus herbivore control of macroalgal community development and coral growth on a Caribbean reef Marine Ecology Progress Series 389: 71-84.

Miller MW, IB Baums, DE Williams, and AM Szmant (2002) Status of candidate coral, *Acropora palmata*, and its snail predator in the upper Florida Keys National Marine Sanctuary:1998-2001. NOAA Technical Memorandum NMFS-SEFSC-479.

Dustan P, Halas JC (1987) Changes in the reef-coral community of Carysfort Reef, Key Largo, Florida: 1974 to 1982. Coral Reefs 6: 91-106.

Florida Fish and Wildlife Conservation Commission (2012) Florida's Wildlife Legacy Initiative: Florida's State Wildlife Action Plan. Tallahassee, Florida, USA. Available at: http://myfwc.com/media/2663010/StateWildlifeActionPlan.pdf

Hughes, TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reefs. Science 265: 1547-1551.

Jaap WC, Halas JC, Muller RG (1988) Community dynamics of stony corals (Scleractinia and Milleporina) at Key Largo National Marine Sanctuary, Key Largo, Florida during 1981-1986. Proc 6th Int Coral Reef Symp 2: 237-243.

Lessios HA (1988) Mass mortality of *Diadema antillarum* in the Caribbean: What have we learned? Annu Rev Ecol Syst 19: 371-393.

Porter JW, Meier OW (1992) Quantification of loss and change in Floridian reef coral populations. Am Zool 32: 625-640.

Yap HT (2009) Local changes in community diversity after coral transplantation. Marine Ecology Progress Series 374: 33-41.

Chapter 1. Coral Disease Workshop

Introduction

Extensive effort has been directed at growing and propagating predominantly *Acropora* spp. corals within *in situ* nurseries for outplanting onto the south Florida reef tract.
Presently, the Florida Fish and Wildlife Conservation Commission issues permits for such restoration efforts with only limited understanding of the risks of disease to the remnant wild population. However, there is a growing body of scientific literature, much of it still unpublished, that has evaluated disease transmission among *Acropora* spp. both in relation to nursery and outplanting work. In developing the concept for the coral disease workshop, the P.I.s recognized that an essential missing perspective in coral restoration, especially *Acropora* spp., is the degree and nature of the risk that nursery derived corals may cause to the wild populations of corals. Cognizant of that need to understand the health risks posed by outplanting of nursery-propagated corals, the coral disease workshop was designed bring together external independent experts in coral disease dynamics to provide their consensus recommendation regarding the degree of risk that nursery operations and outplanting pose to the wild *Acropora* spp. populations. Additionally, these researchers developed a suite of research activities designed both to better understand the risk factors and to better understand the factors that influence coral disease dynamics.

The coral disease workshop was held at the FWC's Fish and Wildlife Research Institute (FWRI) on April 25-26, 2014. This workshop gathered experts that study diseases affecting wild populations of Caribbean Staghorn and Elkhorn coral (*Acropora* spp.). Table 2 lists the workshop attendees and their affiliation. Erinn Muller, Staff Scientist with Mote Marine Laboratory, drafted a report summarizing the current state of knowledge regarding coral diseases from the both the scientific literature and from presentations and discussions by the workshop attendees of unpublished information. Additionally, her report provides a summary of qualitative risk assessment and the consensus opinion of the workshop participants regarding the risk of outplanting nursery corals on wild *Acropora* spp. Her report is below.

Table 2. List of Coral Disease Workshop attendees

Name	Title	Affiliation
John Hunt	Program Administrator	Florida Fish &Wildlife Conservation Commission
Margaret Miller	Ecologist	NOAA Southeast Fisheries Science Center
Erinn Muller	Staff Scientist	Mote Marine Laboratory
Cheryl Woodley	Research Microbiologist	NOAA Center for Coastal Environ. Health & Biodiversity
Esther Peters	Assistant Professor	George Mason University
Steven Vollmer	Assistant Professor	Northeastern University
Erin Lipp	Professor	University of Georgia
Jan Landsberg	Research Scientist	Florida Fish &Wildlife Conservation Commission
Cara Frankenfeld	Assistant Professor	George Mason University
Kristie Erickson	Biological Scientist	Florida Fish &Wildlife Conservation Commission

Report: *Acropora* disease risk workshop: estimating the risk of increased disease in wild Caribbean *Acropora* spp. because of restoration efforts

Glossary of terms

Disease terms

Disease – an abnormal condition, affecting any structure, part or system of an organism that impairs normal function

Direct transmission – the passage of the agents of disease from one organism to another through contact

Contagious – a disease transmissible by direct or indirect contact

Communicable – a disease capable of transmission from one organism to the other

Endemic disease – a disease that is prevalent continually in a particular locality

Epidemiology – the study of the frequency, distribution, and causation of disease, in a population, based upon the investigation of factors in the physical and social environment

Epizootiology – the science dealing with the character, ecology, and causes of diseases in animals, especially epizootic diseases

Epizootic – a disease that is temporarily prevalent and widespread in an animal population.

Etiology – the study of the cause or causes of disease

Fomes – any nonpathogenic substance or inanimate object other than food that is capable of harboring or transmitting pathogenic microorganisms. Example: doorknob for humans, or sediment in the marine environment

Horizontal disease transmission – transmission of disease or infection from direct contact with an infected individual, or by contact with infected excreta

Incidence – the number of cases of a disease arising in a defined population during a stated period of time, expressed as a proportion, such as *x* cases per 1000 population per year.

Indirect transmission – an infection transmitted by an intermediary such as food, water, air, fumes, or animate vectors, rather than transmitted directly from host to host

Infectious dose - the amount of pathogen required to cause an infection in the host

Infection – the invasion of the body by pathogenic microorgansims that reproduce, multiply, and cause disease

Infectious disease – a disease caused by the presence of a pathologic microorganism

Lesion – a morbid change in the structure of function of tissue due to injury or disease

Non-infectious disease - diseases that are not due to disease-causing organisms and are not liable to be spread through the environment

Pathogen – a microorganism capable of causing disease. It may be a primary pathogen, often associated with disease, or it may be an opportunist, becoming pathogenic in weakened hosts.

Pathogenesis – the mechanisms involved in the development of disease

Prevalence – the number of cases of disease which occur in a population at a single observation, expressed as a frequency with the total population as the denominator

Resistance – the ability to survive or flourish in spite of exposure to a disease

Susceptibility – liable to infection and lacking the capacity to respond effectively to a pathogen

Syndrome – a set of disease signs that appear together with reasonable consistency; often used when the cause of the condition is unknown

Transmission – the passage of the agents of disease from one organism to another

Vertical disease transmission – the direct passage of disease from a parent to an offspring

Vector – an organism capable of conveying an infection that produces the disease form one host to another.

Virulence – the pathogenicity or disease-producing capacity of any infectious agent

<u>Restoration terms</u>

Coral Gardening – a method where coral colonies or fragments are propagated and grown within a particular area (*i.e.*, nursery) for the purpose of transplanting the corals back onto degraded reefs for restoration purposes

In situ nursery – a coral nursery where colonies are collected and grown offshore, but near their natural habitat; often these nurseries are isolated over patches of sand away from the reef

In-land nursery – a coral nursery where colonies are collected offshore, but then are brought into land-based aquaria for propagation and grow out before being transplanted back into the reef environment.

Propagation – the process of cutting/pruning/fragmenting a large coral into smaller pieces called "fragments"

Fragment – a section of the coral colony branch used for nursery propagation and outplanting

Genet – a distinct genetic individual

Ramets – a population of colonies that share the same genotype, derived from asexual propagation

Clone – exact genetic copy of parent colony

Outplant – a coral fragment that has been grown within a nursery and then transplanted and reattached back onto a reef (*i.e.*, outplant site) to promote population enhancement and restoration

Partial mortality – the loss of living tissue from part, but not all, of the coral colony

Complete colony mortality – the loss of all living tissue within a coral colony

Risk Assessment Terms

Risk – the probability or expected frequency of occurrence of harmful effects as a result of exposure to a chemical, physical, or biological agent

Absolute Risk – the difference between the incidence of, or mortality from, a disease among exposed and unexposed subgroups of a population (*i.e.*, exposed – unexposed)

Acceptable risk – is a risk that is understood and tolerated because of the cost or difficulty of implementing an effective countermeasure for the associated vulnerability exceeds the expectation of loss

Relative risk – the ratio of the incidence of, or mortality from, a disease in a population exposed to the factor under consideration to the corresponding rate in a population not exposed. (*i.e.*, exposed/unexposed)

Risk assessment – the determination of quantitative or qualitative value of risk related to a situation and a recognized threat

Attributable risk – the fraction of the total incidence of, or mortality from, a disease which can be attributed to exposure to a given factor

Background risk – incidence of a disease found in the population which is not attributed to the exposure of a given factor

Risk Factor – an element thought to predispose an individual to the development of a disease

Risk Management – the identification, evaluation, and correction or mitigation of potential risks that could lead to the occurrence of harmful effects

Risk Communication – exchange of information and opinions, and establishment of an effective dialogue, among those responsible for assessing, minimizing, and regulating risks and those who may be affected by the outcomes of those risks.

Introduction

The populations of *Acropora cervicornis* and *A. palmata*, two major Caribbean reef-building corals, have declined so dramatically within the last several decades that active restoration efforts are taking place throughout Florida and the Caribbean. Disease was a major contributor to the decline of these two species and is still common today. Restoration efforts often use coral gardening and outplanting to increase the population of corals within reefs. However, it is unknown how outplanted colonies will affect the disease dynamics of the remnant wild population of *Acropora* corals. Outplanted corals may increase, decrease, or have no discernible effect on the disease prevalence and incidence of wild *Acropora* colonies. However, very little information on disease dynamics of nursery corals, outplanted corals, and even the wild populations has been published. Therefore, understanding how restoration efforts may affect disease dynamics of the wild population is difficult to discern. In April 2014, a panel of experts in the field of *Acropora* disease dynamics was gathered to provide a consensus on the potential for an increase in disease activity on wild corals based on the current state of knowledge. The objectives of the workshop were to: i) determine the current state of knowledge of *Acropora* disease dynamics based on published and unpublished data, ii) evaluate, using an ecological risk assessment, the level of increased risk of disease activity on wild populations because of restoration efforts, iii) report their findings and conclusions, and iv) identify research activities that would reduce uncertainty associated with the panel's disease risk assessment.

Status of Acropora populations in Florida and Caribbean

Acropora cervicornis and *A. palmata* were once two of the most common coral species found throughout the tropical Western Atlantic Ocean and Caribbean Sea. Indeed, for the last two million years the two species dominated most shallow-water reefs of the Caribbean (Pandolfi 2002). However, over the last several decades populations of these species dramatically declined, primarily from a disease outbreak that swept through the region in the late 1970s and early 1980s (Gladfelter 1982). Physical damage from storms, bleaching, predation, habitat degradation, eutrophication, sedimentation, overfishing, anchor damage and vessel groundings continue to contribute to the decline in these populations. Both *A. cervicornis* and *A. palmata* are now listed as threatened under the US Endangered Species Act of 1973 (Hogarth 2006), and both species have been listed as critically endangered on the International Union for the Conservation of Nature Red List.

Identifying diseases on *Acropora* spp. has been especially challenging because of the lack of diagnostic tools and consequently few pathogens have been identified. Most disease diagnostics include a set of signs that describe the pattern and rate of tissue loss. The diseases are often described as areas of 'tissue loss', usually without any macroscopic area of necrosis within the coral tissue. Therefore, the application of naming diseases on acroporid corals has been problematic. Several diseases, however, have been named and described within the literature, although characteristics described may apply to the different named diseases.

Tissue loss diseases were the primary cause of the region-wide decline of Atlantic *Acropora* spp. beginning in the late 1970s (*Acropora* Biological Review Team 2005) and continue to commonly affect colonies. Specifically, white band disease (WBD), which was first described in 1982 (Gladfelter 1982), was responsible for the majority of the disease-related coral loss on both *Acropora* spp. several decades ago (Aronson and Precht 2001). White band disease type II (WBDII) was described in the mid-1990s, but was only found on *A. cervicornis*. In the 1990s another disease was described affecting *A. palmata* and termed white pox disease (WPx, Holden 1996). Although WPx has only been described on *A. palmata*, recent evidence suggests that colonies showing WPx signs can transmit disease from *A. palmata* to *A. cervicornis* (Williams and Miller 2005). Often the signs of disease do not conform to the description of WBD, WBDII, or WPx, and may be characterized as 'rapid tissue loss'. Diseases not characterized as 'tissue loss' caused by ciliate infections have been recorded on Caribbean *Acropora* spp. (Croquer *et al.* 2006). However, whether ciliates are the primary cause of disease, or a secondary infection from an initial infectious disease, is unknown.

Tissue loss diseases on Acropora populations of Florida

White band disease

Description

WBD was first observed on *A. palmata* in Tague Bay, St. Croix, USVI (Gladfelter 1982), and was later observed on *A. cervicornis* (Peters 1983). WBD was also found throughout much of the Caribbean in the late 1970s and 1980s (Aronson and Precht 2001), and is observed within the Florida Keys as well (Williams and Miller 2005, Miller *et al.* 2014). WBD is characterized by "a shape line of advance where the distally located, brown zooxanthella-bearing coral tissue is cleanly and completely removed from the skeleton, leaving a sharp white zone about 1 cm wide that grades proximally into algal successional stages" (Gladfelter 1982). The classic description includes tissue loss that typically proceeds uniformly from the basal shaded portion of the colony to the branch tips, often causing complete colony mortality. However, recent descriptions include lesions along the base or in the middle of branches, with focal, multifocal, or diffuse lesions that completely encircle the branch (Miller *et al.* 2014).

'Classic' signs of WBD are not very common on contemporary reefs. On *A. palmata*, tissue loss that exhibits a banding pattern is often observed on the undersides of branches and moves up towards the surface of the colony. Many times progression ceases when the progression line reaches the upper, sun-facing, surface of the colony (Williams *et al.* 2006). For both species, but especially *A. cervicornis*, WBD can also begin in the middle of a branch and move toward the base or the branch tip (Peters *et al.* 1983, Santavy and Peters 1997). Tissue loss patterns that do

not follow the classic description of WBD are at times identified as 'rapid tissue loss' (see description below).

Pathogen

The primary pathogen of white band disease is unknown. Histological analyses from samples taken in the 1980s showed that bacterial aggregates were found within the mesoglea adjacent to the calicoblastic epidermis of corals affected with disease (Peters 1984). Alpha-proteobacteria similar to one that causes juvenile oyster disease and is closely associated with *Roseobacter* were also associated with diseased tissues and not found in healthy tissues using culture-independent techniques (Pantos and Bythell, 2006). More recent *in situ* transmission experiments indicate that WBD is caused by bacterial pathogen(s), which could be treated with ampicillin (Kline and Vollmer 2011, Sweet *et al.* 2014). Furthermore, Sweet *et al.* (2014) showed that both ampicillin and paromomycin arrested WBD progression completely and that one to three specific bacterial types were likely the causal agents of WBD. Sweet *et al.* (2014) also suggest that the ciliate *Philaster lucinda* plays a significant role in tissue loss associated with WBD. Interestingly, Casas *et al*. (2004) did not detect any specific pathogens associated with WBD tissue using culture-independent methods and suggested the disease may be non-bacterial. Vollmer *et al.* (unpublished) analyzed the 16S rRNA data from healthy and diseased corals sampled over two years and within four different sites. Results showed that 159 different operational taxonomic units (OTUs) were associated with diseased colonies, particularly Flavobacteriales, *Vibrio* spp., and Alteromonadales.

Prevalence and progression rates

WBD progresses at varying rates. Gladfelter (1982) reported that the average rate of tissue loss on *A. palmata* was 5.5 mm day^{-1}, ranging from 0.8 to 13.9 mm day^{-1}. A 2004 study from the Turks and Caicos Islands showed an average progression rate of 2.8 cm^2 day^{-1} (*i.e*., 1.7 cm day^{-1}) from WBD on *A. palmata.* On *A. cervicornis*, progression rates can reach up to several cm day^{-1} (Sweet *et al.* 2014).

The prevalence of WBD is also extremely variable. Monthly surveys of *A. palmata* colonies in Haulover Bay, St. John, US Virgin Islands showed <1% prevalence of WBD between 2003 and 2009 (Rogers and Muller 2012). However, during a similar time period, the prevalence of WBD averaged 3.2% on *A. palmata* around Buck Island Reef National Monument in St. Croix, USVI, located only 70 km from St. John (Mayor *et al.* 2006). One site surveyed for disease activity in a high density area of *A. palmata* called Limones Reef in Mexico showed 1% prevalence of WBD (Rodriquez-Martinez *et al.* 2014).

Transmission

Within the first report describing WBD, Gladfelter (1982) believed the disease to be contagious within small areas of reef; however, newly infected colonies were not necessarily adjacent to previously diseased ones, and healthy colonies were often immediately adjacent to diseased colonies and did not develop WBD through time. These observations suggest that WBD may indeed be transmissible, but other factors likely play a significant role that influences the health status of individual colonies. Additionally, the WBD outbreak along the northeast coast of St. Croix in the early 1980s appeared to move against the current, suggesting that the infectious agent was not a waterborne pathogen. Recent studies support this hypothesis.

Vollmer and Kline (2008) conducted an *in situ* transmission experiment where *A. cervicornis* fragments that were showing signs of WBD were artificially grafted to apparently healthy *A. cervicornis* fragments. Their experiment showed that direct transmission from diseased to healthy individuals was possible, and occurred approximately 45.5% of the time. Using a paired experiment, seven healthy fragments were held adjacent, but not touching diseased corals to determine whether indirect transmission was possible. None of the healthy fragments showed tissue loss after one month of exposure (Miller *et al*. unpublished). In fact, there has been no report of indirect transmission, in the absence of an animal vector, of WBD from a diseased individual to a healthy individual through controlled experiments, again suggesting that waterborne transmission is not likely.

Indirect transmission has occurred, however, through the animal vector, *Coralliophila abbreviata.* After consuming diseased tissue, *C. abbreviata* snails transmitted WBD to apparently healthy colonies when feeding (Gignoux-Wolfsohn *et al.* 2012). The coral-eating snails also acted as a reservoir for WBD by causing disease after being starved for two weeks (Gignoux-Wolfsohn *et al.* 2012). Interestingly, *C. caribaea*, another snail that at times consumes corals has not been identified as a vector (Gignoux-Wolfsohn *et al.* 2012). The coral-consuming fireworm, *Hermodice carunculata*, also may be a vector of disease transmission. Miller *et al.* (in press), showed that there was a ten times increase in frequency of disease-like tissue loss in the month following fireworm predation (21%) than on intact branches (2%). Therefore, fireworms may be a vector of disease transmission, or simply increase the risk of disease because predation causes lesions within the tissue, which may be an avenue for the infection of pathogenic agents.

Spatial clustering analysis of *A. palmata* showing signs of WBD within Buck Island Reef National Monument has also been conducted. When comparing the spatial pattern of diseased colonies with that of the naturally clustered distribution of the population, Lentz *et al.* (2011) showed that corals with WBD did not significantly cluster. However, when analyzing the data at the transect level (*i.e*., a more coarse spatial resolution), disease clustering did occur within 1 km (Lentz *et al.* 2011). Therefore, results of spatial clustering analysis will be dependent upon the spatial resolution of the data.

Risk factors

In situ grafting experiments in Panama showed that resistance to WBD within *A. cervicornis* followed a continuum, with some genotypes exhibiting high levels of susceptibility to disease and others showing high levels of disease resistance (Vollmer and Kline 2008). Approximately 6% of the population tested showed consistent resistance to WBD, suggesting that this natural disease resistance may allow for population persistence over time. In addition to an innate disease resistance of some corals, corals infected with WBD may differ in their gene expression. Next generation RNA-sequencing that produced transcriptome-wide profiles of the immune response of healthy and WBD-infected *A. cervicornis* corals showed significant differences in gene expression. Diseased corals exhibited a strong up-regulation of macrophage-mediated pathogen recognition and reactive oxygen species production, considered two hallmarks of phagocytosis. Key mediators of apoptosis and calcium homeostasis were also up-regulated (Libro *et al*. 2013). Libro *et al*. (in prep) also showed that there is a distinct pattern of the gene signatures of corals that are innately disease resistant compared with those that are disease susceptible. This pattern occurs whether the corals are exposed to disease homogenates or not,

indicating that this is a constitutive pattern in gene expression. If the pattern is consistent across *A. cervicornis* corals then there may be a tool to predict disease resistance and identify disease-resistant corals.

In addition to genotypic susceptibility, the physical state of the coral host also influences WBD dynamics. Open wounds within the coral tissue readily provide an entry site for infectious agents. This was observed when rates of WBD infection increased for corals with artificially induced lesions compared with those with intact tissue when aquaria were dosed with homogenates made from diseased coral tissue (Gignoux-Wolfsohn *et al.* 2012). Increasing disease activity is often associated with storm events when corals are frequently fragmented and abraded thus creating entry points for infectious agents (Miller *et al.* 2014).

Although many other coral diseases are often positively correlated with environmental variables, such as warm water temperatures, WBD does not consistently show a trend for increasing activity during the summer months (Miller *et al.* 2014).

White band disease type II

First described from the Bahamas in 1993, most of the information for white band disease type II (WBD type II) comes from Ritchie and Smith (1998). This disease appears to only affect *A. cervicornis,* and has not been reported for *A. palmata.* The disease is differentiated from WBD by the presence of a band of bleached tissue (2 – 20 cm wide) near the necrotic margin. Progression rates were approximately 0 to 10 cm/day reported by Ritchie and Smith (1998) and 0.2 to 1.2 cm/day reported by Gil-Agudelo *et al.* (2006). Often the necrotic margin "catches up" to the bleached margin, when this occurs there is no differentiation from WBD.

The causal agent for WBDII has been reported as *Vibrio carchariae*, which has been isolated from the surface mucopolysaccharide layer in diseased tissue (Ritchie *et al.* 1995, 1998, Gil-Agudelo *et al.* 2006). However, only 3 of the 4 Koch's postulates were fulfilled using *V. carchariae* on *A. cervicornis* and the reisolation and identification of the bacteria from experimentally infected corals has not occurred (Gil-Agudelo *et al.* 2006). WBDII has been rarely reported and there has been no report of prevalence, experiments to determine transmission, or identified risk factors from any location.

White pox disease

Description

In 1996 another disease, referred to as white pox disease (WPx), was first observed from Eastern Dry Rocks reef off of Key West, FL (Holden 1996). WPx has been reported on *A. palmata* only. Photographs from the 1970's, however, suggest that WPx may have been present on reefs several decades ago (Rogers *et al.* 2005). WPx is described as irregularly shaped white patches of recently exposed coral skeleton as a result of tissue loss with distinct smooth to serpiginous edges. Lesions, which can be focal or multifocal, are often completely surrounded by living tissue, but can also be along the base of a colony and coalesce into other lesions over time. The size of the lesions can vary from 3 to 80 cm^2 (Patterson *et al.* 2002).

Pathogen
As with many other types of tissue loss diseases on Caribbean *Acropora*, WPx may be a generalized disease description for several different pathogenic agents. *Serratia marcescens* has been identified as one possible causal agent for WPx, and Koch's postulates were fulfilled using this bacterium (Patterson *et al.* 2002). When there is a positive presence of *S. marcescens* within the WPx lesions, the disease is often referred to as acroporid serratiosis. Several studies, however, indicate that this bacteria is not often found on samples of WPx diseased tissues (Polson *et al.* 2009, Lesser and Jarett 2014). Polson *et al.* (2009) showed that *S. marcescens* was more often found within healthy tissue of *A. palmata* than in tissue adjacent to WPx lesions. Additionally, Lesser and Jarett (2014) could not detect *S. marcescens* in healthy or diseased samples of *A. palmata* from the Bahamas using both culturable and culture-independent techniques. Lipp *et al.* (unpublished) indicate that the signs and severity of WPx today has changed from the originally described disease and the authors suggest that the current episodes of WPx signs are different from those observed in the 1990s and early 2000s. The results of these studies suggest that there are likely other pathogens causing similar disease signs on *A. palmata.* What those other pathogens are, however, is currently unknown.

A significant roadblock to determine causative agents of disease within *Acropora* corals is that the natural microbiome of healthy corals is still not known. Recent work by Lipp *et al.* (unpublished) has shown that there is very little change in taxonomic groups of bacteria through time. However, there is an increase in Rhodobacterales in corals that are showing signs of disease compared with apparently healthy corals. Additionally, during one episodic monitoring event in July 2013, there was a substantial increase in Pseudomonadales and Vibrionales bacteria species within the microbiome of all samples. This monitoring event coincided with an African dust event. The effects of these changes, if any, are unknown.

Prevalence and progression rates
Progression rates of active WPx disease can reach 10.5 cm^2/day (Patterson *et al.* 2002). However, at times large lesions appear very rapidly (within days) and do not continue to progress (Muller unpublished). The lack of progression of disease lesions indicates that WPx may be less virulent than WBD because complete colony mortality does not often occur and WPx lesions that stop progressing can heal over time.

Monthly monitoring of individual colonies found in Haulover Bay, St. John, USVI showed that annual prevalence was approximately 12% within this location and annual disease incidence was around 8% (Rogers and Muller 2012). Monitoring efforts conducted from 2003 to 2009 revealed that overall annual disease prevalence and incidence did not change, but there was significant variation in prevalence and incidence within years. Disease prevalence ranged from 0 to 52% within this location and showed a positive correlation with average water temperature. An island-wide survey of *A. palmata* around St. John in 2004 and again in 2010 showed the average WPx prevalence was 19% and 3% respectively (Muller *et al.* 2014). The difference in these two averages was likely reflected by the time of year surveys took place -- June to September of 2004 and May of 2010. Prevalence values for the Turks and Caicos Islands showed that between 4.3% and 6.7% of colonies exhibited signs of WPx (Schelten *et al.* 2006). A survey of 107 sites along the Meso-American Reef System showed that the one site with high *A. palmata* cover, Limones

reef in Mexico, had approximately 14.7% WPx disease prevalence (Rodriquez-Martinez *et al.* 2014).

Transmission

Patterson *et al.* (2002) mentioned that WPx disease appeared to follow the nearest neighbor contagion model, however, the methods to assess disease clustering were not robust. In order to determine significant levels of disease clustering, which would infer contagion transmission, studies must first account for the natural clustering pattern of the species. A spatio-temporal model applied to monthly colony information over seven years from Haulover Bay, St. John, USVI showed that spatial location did not influence whether a colony showed signs of WPx disease (Muller and van Woesik 2014). The distance from a previously infected colony did not influence whether a healthy colony subsequently had WPx disease the next month, which suggests that WPx disease in St. John does not follow the nearest neighbor contagion model. Sutherland *et al.* 2011 showed that isolates from WPx lesions can infect other healthy fragments, suggesting that colony to colony transmission may be possible. Miller *et al.* (unpublished) showed that colonies of *A. palmata* with signs of WPx could directly transmit the disease to fragments of both healthy fragments of *A. palmata* and *A. cervicornis*. Disease transmitted to *A. cervicornis* 70% of the time, whereas *A. palmata* fragments became diseased only 30% of the time, and only 10% higher than controls. These data suggest that *A. cervicornis* may be more susceptible to disease than *A. palmata.*

WPx disease may be transmitted by *C. abbreviata.* Sutherland *et al.* 2011 showed that isolates of *S. marcescens* from the corallivorous snail, *C. abbreviata*, caused disease signs similar to WPx. These results again suggest that a coral predator has the potential to transmit disease. Controlled transmission experiments to test vector transmission have not been published.

Risk factors

The prevalence of WPx is positively correlated with water temperatures. Data from Patterson *et al.* (2002) indicated that WPx likely increased during warm summer months, but higher resolution sampling was needed to confirm these data. Monthly monitoring of colonies in the USVI often showed an increase of prevalence during periods of high water temperatures (Rogers and Muller 2012). However, other studies suggest it is not necessarily water temperature *per se* that drives an increase in disease activity, but that the change in temperature makes thermally intolerant individuals more susceptible to disease infection (Muller *et al.* 2008).

The prevalence of white pox disease also increases with colony size (Muller *et al.* 2013, Rodriquez-Martinez *et al.* 2014). Whether large sized colonies are more susceptible because they have a bigger ‘target’ area for pathogen transmission than small colonies, or if colony senescence plays a part in susceptibility has not been definitively determined. Since recent evidence suggests that WPx disease does not follow the contagion model *in situ* (Muller and van Woesik 2014), likely most large-sized individuals represent some of the oldest colonies within reef populations, which are also more susceptible to disease infection (Meesters and Bak 1995).

Fragmentation, or physical damage, can also increase susceptibility to WPx disease and lead to significant tissue loss (Williams and Miller 2012, Bright 2009). Storm-generated fragmentation of corals, which occurred because of large swells in March 2009 in St. John and St. Thomas

USVI, showed that corals suffering physical damage were more susceptible to disease (Bright 2009). Fragmented colonies were also more likely to suffer from snail predation (Bright 2009), identified as a potential vector of several tissue loss diseases on *Acropora* (Williams and Miller 2005, Sutherland *et al.* 2011, Gignoux-Wohlfson *et al.* 2013).

Rapid tissue loss

Description
Acute tissue loss on *A. cervicornis* and *A. palmata* often does not displays signs characteristic of WBD, WBDII, or WPx. Tissue loss disease on *Acropora* spp. was first documented on colonies after they were artificially fragmented (Bak and Creins 1981). Because tissue loss often displays an irregular pattern with indistinct edges, a more generic term has been applied describing disease as "rapid tissue loss" (Williams and Miller 2005). This term likely encompasses several different disease types, but is a more accurate categorization when the etiology of these tissue-loss diseases is unknown (*i.e.*, a syndrome). Outbreaks of rapid tissue loss have been documented in the northern Florida Keys and Broward County, FL (Williams and Miller 2005, Smith and Thomas 2008, Miller *et al.* 2014).

Prevalence and progression rates
Disease surveillance on 10 reefs within the Florida Keys showed highly variable prevalence over two survey periods in 2011 and 2012. Prevalence ranged from 0% to 70% over time. Progression rates reached up to 4 cm/day. Smith and Thomas (2008), however, showed that a tissue loss syndrome in Broward County FL showed a progression rate of ~1.5 mm/day. Therefore, like the other tissue loss diseases described for Caribbean *Acropora*, progression rates may vary, which may be from differing host conditions or virulence factors related directly to the pathogen or indirectly from the environment.

Transmission
Through manipulative field experiments, Williams and Miller (2005) showed that the rapid tissue loss disease was transmissible through direct contact between disease and healthy corals, as well as through the vector *C. abbreviata.* Interestingly, the disease could also be transmitted from a diseased colony of *A. cervicornis* to a healthy colony of *A. palmata*, indicating that the infectious agent was not necessarily host specific. Indirect transmission experiments were attempted, but did not show any sign of transmission without the use of an animal vector. Additionally, a tissue loss syndrome in *A. cervicornis* off Broward County, Florida showed 70% of active lesions caused severe tissue loss when a healthy fragment was grafted onto the lesion of a diseased colony (Smith and Thomas 2008).

Risk factors
Rapid tissue loss in the Florida Keys does not appear to have a seasonal cycle, and is often associated with physical damage associated with storm events (Miller *et al.* 2014). There was not a positive seasonal component to tissue loss in this study, but a rapid increase in disease prevalence was associated with fragmentation because of Tropical Storm Isaac in 2012 (Miller *et al.* 2014). Disease on *A. cervicornis* also appears to be site specific, with outbreaks occurring in some sites, but not in others (Miller *et al.* 2014). More frequent monthly monitoring of individual

sites year round in other places throughout the Caribbean would provide a more quantitative assessment of the potential for seasonal patterns for disease, particularly rapid tissue loss, on *A. cervicornis*.

Rickettsia-like organisms

In addition to gross disease signs, intracellular organisms may cause harm to *Acropora* corals without causing macroscopic disease signs. These include Rickettsia-like organisms (RLOs), which are obligate intracellular parasites that have been detected within the polyp mucocytes of histological samples (Miller *et al.* 2014, E. Peters, unpublished data). RLOs have been found in disease samples as well as healthy samples and within corals from different locations such as Panama, USVI, Florida, Belize, Cuba, and Puerto Rico. Often corals with RLOs have reduced zooxanthellae. The RLOs infect the coral polyp mucocytes, proliferate, causing cellular damage and then are shed into surrounding tissues. It is suggested that RLO infections could be causing cellular damage, reducing zooxanthellae concentrations, and increasing coral susceptibility to secondary infections under stressful environmental conditions (Miller *et al.* 2014, E. Peters unpublished).

Current state of *Acropora* nurseries in Florida

In an effort to mitigate the loss of acroporid corals throughout the Florida Reef Tract, *Acropora* corals are being propagated and used in restoration efforts to enhance the natural recovery of these threatened coral species. Several different restoration techniques have been applied and tested on Acroporid corals worldwide. The most common technique for *Acropora* spp. has been the use of *in situ* underwater nurseries where corals are propagated asexually through fragmentation. The fragments are then used as a sustainable source for restoration and population enhancement. *In situ* underwater nurseries use several designs for successful propagation, which are often based on each location's specific environmental conditions and project goals. These include floating or mid-water nurseries and frame nurseries. Floating nurseries include line or 'tree' nurseries where lines or frames are attached to the bottom with anchors and the corals are suspended off of the sea floor utilizing subsurface floats (Johnson *et al*. 2011). Anchor nurseries use blocks or frames that are directly attached to the substrate. The coral fragments are then attached to the blocks or frames using a coral attachment device such as a cement or PVC pedestal, puck, wire or plastic ties.

In 2009, The Nature Conservancy (TNC) received a $3.3 million dollar, 3-year grant from the National Oceanic and Atmospheric Administration (NOAA) through the American Recovery and Reinvestment Act (ARRA) to expand the existing *Acropora* restoration work. Currently, there are a total of 6 geographically differentiated *in situ* coral nurseries located throughout the state of Florida. These locations include Broward County, Dade County, the Upper Florida Keys, the Middle FL Keys, the Lower FL Keys, and the Dry Tortugas. Nurseries are also located offshore of St. Thomas and St. Croix, US Virgin Islands. In Broward County the nursery is managed by Nova Southeastern University Oceanographic Center, in Dade County the nursery is managed by the University of Miami Rosensteil School of Marine and Atmospheric Science and Biscayne National Park, the Upper Keys nurseries are managed by the Coral Reef Restoration Foundation

and the National Marine Fisheries Service-Southeast Fisheries Science Center, the Middle Keys are managed by the Florida Fish and Wildlife Conservation Commission, the Lower Keys nurseries are managed by Mote Marine Laboratory, and nurseries in the Dry Tortugas National Park are managed by The Nature Conservancy. Although there are some nurseries that are propagating *A. palmata*, such as those managed by the Coral Reef Restoration Foundation, a vast majority of the fragmentation and outplanting of nursery corals have been *A. cervicornis*. Each nursery contains individuals harvested within their specific region of Florida and contain anywhere between 18 and 100 different genotypes. Within the six nursery locations, there are approximately 50,000 coral colonies that have been propagated. NOAA and TNC continue to support several of these nurseries along with foundation funds from private donors.

Disease within nurseries

Corals grown in dense clusters, such as within an *in situ* nursery setting, may be more vulnerable to disease outbreaks and have high rates of disease transmission. Therefore, when disease occurs within the nurseries, preventative management methods are used to reduce the risk of exposure for other corals. Several methods can be used singly or in combination. Colony isolation occurs when managers remove the diseased corals from the main nursery area to a quarantine area at least 5 m away. A sandy buffer zone is typically used between the main nursery and the quarantine area. Pruning is another method that may reduce disease impacts by snipping off live tissue above the band of infection, which is then mounted in an area isolated from the parent colony. Banding is the application of underwater epoxy putty along the interface between apparently healthy tissue and recently exposed skeleton. The band should cover at least 1 cm of the adjacent live tissue. Unfortunately, success rates of both pruning and banding on outplanted colonies are not significantly more effective than controls (Miller *et al*. 2014). One final method that may reduce the probability of disease transmission is the management of vectors, such as various fish, snails, and worms. Removing these organisms, which may be carriers of disease pathogens, may increase the effectiveness of disease prevention and treatment.

For the most part, the use of these culling or preventative measures for disease transmission and outbreaks within nurseries has been considerably effective. Nursery managers have reported very little influence of disease (<1% prevalence), while the corals are being propagated and grown within the *in situ* nursery. Use of culling methods, however, provides a skewed perspective on the actual disease activity that may be occurring within the nursery populations, especially when attempting to compare disease activity within nurseries to that within wild populations. However, the vastly different environment in which corals are grown within a nursery setting (*e.g.*, often on vertical trees) makes this kind of comparison even more challenging. Corals grown within the water column are likely interacting with a very different microbial community compared with those attached and growing on reef substrate. Physiological differences in photosynthesis, growth, and respiration of corals grown under these different conditions will likely affect the overall health state of the coral, which may in turn influence disease susceptibility.

Disease on outplanted corals

Since 2012 thousands of individual corals have been outplanted from *in situ* nurseries back onto the reef environment throughout Florida's reef tract. The most common method of outplanting has been hammering a nail into the hard reef substrate and attaching the coral fragment to the nail using a plastic zip tie. Each outplanting site typically contains tens to hundreds of individual corals consisting of approximately 10 to 15 different genotypes. After the corals are outplanted, the corals are monitored one month later and then again at three months and at times six months after outplanting efforts were completed (C. Lustic personal communication). Survival of individuals as well as presence of tissue loss diseases was recorded.

Monitoring disease on outplanted corals is essential for understanding the risk that these individuals pose to the wild population of *Acropora* corals. Risks could increase immediately after outplantation, or there may be a lag of several years when population densities surpass a limiting threshold. The disease information gathered from monitoring efforts conducted by nursery manager's shows disease varies by site (C. Lustic personal communication). Disease prevalence has ranged from 0 to 25.6 % of the outplant population. Often disease appears during the spring and summer months, but the lack of long term monitoring precludes making conclusions on seasonal changes in disease prevalence of outplants. Distinction of disease types and progression rates has not been recorded. Furthermore, there is no monitoring of the wild populations that may be influenced by nearby outplanting efforts. Under the current monitoring protocol, it is difficult to quantify how disease risk to the wild population of *Acropora* corals may change because of outplanting efforts.

Assessing disease risk using our current state of knowledge

Since disease is a regular occurrence within *Acropora* spp. and has been frequently observed within outplant populations, concerns have been raised regarding the probability of increased disease risk for wild Caribbean *Acropora* populations because of restoration efforts. At present, permits to outplant *Acropora* spp. are being issued with limited understanding of these risks. To determine the level of risk associated with increasing disease activity on wild populations because of restoration efforts, a meeting of experts in the field of Caribbean *Acropora* diseases was held in St. Petersburg, Florida on April 25th and 26th, 2014. The goal of the workshop was to determine the probability of an increased disease risk in wild populations of acroporids because of outplanting efforts based on evidence within the published and unpublished literature. Workshop participants synthesized the existing research on *Acropora* disease dynamics, conducted a risk assessment exercise which provided the group's expert opinion on disease risk, and identified research activities for reducing uncertainties within the risk assessment.
A risk assessment is a systematic process of evaluating the potential risks that may be involved in a projected activity or undertaking. Risk assessments may be qualitative or quantitative, both of which require the consideration of two components of risk: the magnitude of the potential loss and the probability that the loss will occur. Part of the difficulties associated with risk management is that both magnitude of loss and probability of loss are difficult to measure.

Workshop participants discussed disease risk using an Ecological Risk Assessment framework. Ecological Risk Assessment (ERA) is a paradigm that deals with proposed action(s) and the uncertainty of the consequences (or outcomes) of the actions. The ERA framework provides a systematic approach for decision-making that is based on scientific knowledge. Within the workshop, participants used a qualitative ranking system (*e.g.*, high, high-medium, medium, medium-low, and low) to identify the qualitative risk of increased disease activity on wild populations of Caribbean *Acropora* corals because of restoration efforts. Workshop participants characterized their opinion of risk by discussing the current state of knowledge from published and unpublished literature. Participants anticipate using a more quantitative approach that incorporates more stringent analyses and assessments as a follow-up to the meeting. A future goal for workshop participants is to complete a quantitative risk assessment, where the results of the qualitative approach from the workshop can be applied to a more quantitative follow-up approach.

Qualitative risk assessment

A qualitative ranking system was used to identify the relative risk of increasing disease activity on wild populations of Caribbean *Acropora* spp. corals resulting from outplanting corals from *in situ* nurseries (Table 3). Four different risk scenarios, which identified potential adverse health effects for wild *Acropora* spp., were described and each participant received one vote to place within a risk category for each scenario. Five risk categories were used within the ranking system: *low*, when there was no reason to believe action would increase disease risk, *low-medium*, when there was likely no reason to be concerned, but increased disease risk may be possible, *medium*, when increased disease risk may be likely, *medium-high*, when an increased disease risk was highly likely, and *high*, when an increased disease risk was practically certain in the opinion of the participant. The participants then ranked the level of certainty behind their risk vote. Here the ranking system used only three categories: *low*, *medium*, and *high*. Low certainty suggested that there was little confidence within the risk vote, medium certainty indicated that the participant was fairly confident behind their risk vote, and high certainty was used when the participant was very confident with their risk vote. Within the scenarios, workshop participants addressed only one source location for outplants, *in-situ* coral nurseries. These locations are the most common form of practice currently occurring within the state of Florida. Within these nurseries, the corals collected remain offshore, and are only transported from the location of collection within the reef environment to the *in situ* nursery location. The corals are grown and propagated within the nursery, which is isolated but often near other reef patches. Two different outplant approaches were identified and considered. The first was when outplanting occurred *segregated* from natural populations of *Acropora.* In this situation, when corals are taken from the nursery and placed back into the reef environment the corals are isolated (*e.g.*, at least 500 m) from wild (naturally recruited) *Acropora* colonies. These often occur within patch reefs that historically had *Acropora*, but currently no living individuals are evident within the area. The second approach was identified as the *interspersed* outplanting method. This scenario describes practices when the outplanted corals are placed within reefs that also currently contain wild *Acropora.* In this situation there is less of a spatial buffer (*e.g.*, less than 50 m) between wild corals and outplanted corals.

Table 3. Ranking of risk associated with disease to wild populations of *Acropora* spp. because of restoration efforts.

Source of outplants	**Outplant Method (interspersed vs segregated)**	**Risk to Wild Population (concern about outplanting from nursery to reef)**	**Consensus ranking**	**Level of certainty**
In-situ nursery	segregated	Introduction of novel condition to wild populations of *Acropora* spp.	L	M
In-situ nursery	segregated	Increase from baseline *Acropora* spp. disease signs in wild populations	L	H
In-situ nursery	segregated	Increase from baseline *Acropora* spp. predation signs	L	H
In-situ nursery	segregated	Deleterious changes from wild *Acropora* spp. baseline health, additional (not disease, not predation)	L	H
In-situ nursery	interspersed	Introduction of novel condition to wild populations of *Acropora* spp.	L-M	M
In-situ nursery	interspersed	Increase from baseline *Acropora* spp. disease signs in wild populations	L-M	M
In-situ nursery	interspersed	Increase from baseline *Acropora* spp. predation signs	L-M	M
In-situ nursery	interspersed	Deleterious changes from wild *Acropora* spp. baseline health, additional (not disease, not predation)	L-M	M

The working group concluded there was a low risk to the wild population of *Acropora* corals when individuals from *in-situ* nurseries were outplanted into a segregated reef area. This conclusion was provided with high certainty from the current state of knowledge about disease within wild populations, nursery populations, and transmission probabilities. There was medium

certainty that there was a low risk of introduction of a novel condition to wild populations of *Acropora* spp., mainly because historical incidences of invasive and exotic organisms have been unpredictable.

The risk to wild corals was slightly elevated to a low-medium rank when corals would be outplanted within interspersed situations. Here, the nursery corals would interact with wild corals with less spatial buffer, which may increase the potential for disease transmission. Within the interspersed outplanting situation the certainty identified with the low-medium risk was ranked with medium certainty. The amount of certainty was reduced because presently there have only been segregated outplanting efforts. Therefore, currently no data represent this situation within restoration efforts.

Future goal: Quantitative risk assessment using relative risk approximations

There are at least three steps involved for the quantitative estimation of risk. First, the hazard(s) must be identified, which aims to determine the potential adverse effects of a contaminant or action. Second, the dose-response is analyzed. The dose-response is the relationship between the dose of the contaminant or action and the probability of the effect. The dose-response relationship is often determined through controlled experiments and data mining from published literature. The third step is exposure quantification, which aims to determine the amount of the dose that individuals and populations will receive. The overall risk estimation will often vary within a population because of both different levels of susceptibility and exposure. Very little of the information needed to conduct a quantitative risk assessment for disease on *Acropora* corals from restoration efforts is known.

Relative risk assessments, as an example, are classically used in human epidemiology, where the incidence of a particular disease, also known as the attack rate, determines the absolute risk. The attack rate is the percentage of people that manifest a disease, some of which may have been exposed to an identified risk whereas others with the disease have not been exposed. The relative risk, or risk ratio, is the number of individuals with disease after exposure to the risk divided by the number of people with disease that have not been exposed to the risk:

Relative risk (RR) = $\frac{Risk\ in\ exposed}{Risk\ in\ non-exposed}$.

First, it is essential to identify the risk in each of the two cohorts, exposed and not exposed. A classic example would be the risk of developing coronary heart disease for a group of smokers and a group of non-smokers (Table 4).

Table 4. Data from Johns Hopkins Bloomberg School of Public Health (available at: http://ocw.jhsph.edu/courses/fundepiii/PDFs/Lecture16.pdf)

	Develop CHD	Do not develop CHD	Total	Incidence of disease	Risk
Smoke cigarettes	84	2916	3000	$\frac{84}{3000}$	0.0280
Do not smoke cigarettes	87	4913	5000	$\frac{87}{5000}$	0.0174

Relative risk (RR) = $\frac{0.0280}{0.0174}$ = 1.61

When RR=1 then there is no association between the risk and disease occurrence. However when RR>1 then there is a positive association and when RR<1 there is a negative association. From the data set above, it is clear that smoking increases the risk of developing coronary heart disease. If there is an association then the exposure is called a risk factor for the disease. The attributable risk is the incidence of the disease due to exposure to the risk factor (*i.e.*, the level of disease incidence above the background risk).

Risk assessment within a coral disease-and-restoration framework

A future goal for understanding the risk of increasing disease activity on wild populations because of restoration efforts would be to complete a relative risk assessment. However, the current data to properly conduct this undertaking is lacking. Current best practice scenarios advise outplanting efforts to take place on reefs that have very little to no wild *A. cervicornis* colonies present. This practice minimizes the potential risk of any negative impacts on an already threatened population. However, to conduct a risk assessment, data is needed to determine whether disease incidence changes on wild populations when outplants are placed within the same reef structure, or within a distance threshold. Within the context of disease risk related to restoration efforts, the exposed cohort would be considered wild corals that are located within a reef that also has outplanted fragments from an *in situ* coral nursery. The non-exposed cohort would be corals within a reef that does not have outplanted coral fragments. The prevalence of disease within both of the cohorts would need to be monitored at least monthly, and the two cohorts would be compared using the relative risk calculations illustrated above in Table 3. Replicates of each cohort would be essential to conduct the most powerful type of risk assessment analysis. Additionally, since at least some of the tissue loss diseases can be transmitted from *A. cervicornis* to *A. palmata* (Williams and Miller 2005), and *A. palmata* are also being cultured and outplanted in Florida, these controlled restoration activities should include reciprocal exposure among both wild colonies and outplants of both species.

Areas of uncertainty and research gaps

Although a significant amount of research has focused on *A. cervicornis* and *A. palmata*, there are still several areas of uncertainty and research gaps. Several areas of uncertainty were identified and discussed by the workshop participants. Once these gaps are filled, the level of certainty within the disease risk assessment will increase. Some areas in need of more research were generally discussed, while others were listed as key research priorities. All topics are described below.

Differences among species

A significant amount of restoration activity has focused on the propagation and outplanting of *A. cervicornis*. Much less work has involved *A. palmata* restoration, and virtually none on the genetic hybrid of *A. cervicornis* and *A. palmata*, *A. prolifera.* As the ESA precludes listing F1 hybrids as 'species' (in the ESA/legal sense), *A. prolifera* is not listed under the ESA, and primary efforts have centralized around the other two species. *Acropora cervicornis* is much easier to grow within a nursery setting and fragmentation and outplanting has been relatively inexpensive and successful, particularly because of this species growth morphology; thin and long branches. *Acropora palmata*, on the other hand, has larger, wider branches and a thicker growth form than *A. cervicornis*, which makes fragmentation, propagation, and outplanting more challenging. As much of the effort and research within Florida has focused on *A. cervicornis*, it is uncertain whether there may be differences in disease risk in *A. palmata* because of restoration efforts. We do know that Florida's wild *A. palmata* populations are more geographically concentrated (*i.e*., largely restricted to shallow fore-reef habitats (Miller *et al*. 2008) and likely less genotypically diverse than wild Florida *A. cervicornis* populations. How this may affect disease dynamics, and perhaps disease resistance within the population, is unknown.

Establish a coral disease data consortium

There are numerous researchers and nursery managers within the Caribbean and Western Atlantic that are presently collecting data on *Acropora* spp. within the region. The data may be informative to the particular site or region collected; however, it may be even more useful when combined with other researchers data for a regional perspective. There is a critical need to establish a coral disease database specific to Caribbean *Acropora* species, where information can be deposited, amalgamated, and used for meta-analyses within the region. Having a consortium of local researchers that commit to contributing to the database would help progress our current understanding of disease dynamics on Caribbean *Acropora* spp.

Development of strategic disease surveillance practices

One of the major limitations in understanding the risk associated with *Acropora* restoration efforts in Florida is the lack of baseline disease knowledge. Long-term, infrequent monitoring of reef areas provides very little insight into the shifting patterns of disease activity. Focused efforts on disease surveillance are essential for understanding how disease changes in the wild populations without the influence of restoration efforts. Documenting changes over time will help establish baseline health parameters in relation to key life-history traits (*i.e*., growth,

reproduction) of current populations without the influence of restoration activities. Surveillance efforts should also focus on wild populations both near outplanted corals and isolated from outplanted corals. Determining disease prevalence in populations that may be influenced by transplanted corals and those that are not likely affected will provide insight into whether restoration efforts have any impact on *Acropora* disease dynamics. Long-term surveillance information is critical to identify significant changes in disease activity.

Disease transmission

How diseases may be transmitted from one individual to another is presently not very well known. In fact, whether many tissue loss diseases are even transmissible is still under question. Some research is beginning to show the importance of suspect vector populations such as the predatory snail *Coralliophila abbreviata.* Further studies are necessary to i) determine whether direct or indirect transmission is a common characteristics of tissue loss diseases, ii) identify potential vectors of disease transmission, and iii) determine the overall impacts of previously identified vector densities.

Adverse changes in coral fitness and health

Disease within *Acropora* is only documented when tissue mortality occurs because currently researchers rely only on visual signs. Likely, disease that is non-lethal and negatively effects coral physiology is common, but not detected because of the lack of diagnostic tools. Understanding baseline physiology of healthy corals is essential to detect deleterious impacts from sub-lethal diseases and how that impacts population dynamics.

Risks to surrounding reef community

While the workshop participants focused on assessing the disease risk on wild *Acropora* populations because of restoration efforts, the team also noted that there is a need to understand the risks to the surrounding reef community. Increasing population density of *Acropora* spp. may affect other coral species as well as other reef organisms. If diseases can be transmitted across species, then other scleractinians could be at an increased disease risk. Higher densities of acroporids may increase vector populations, or even indirectly affect the health and fitness of non-*Acropora* corals and other reef organisms within restoration sites. Increasing the populations of *Acropora* corals within the Florida reef tract is assumed to create healthier reefs, but this assumption has not been tested.

Concerns regarding land-based restoration efforts

The risk assessment conducted by the workshop participants focused on *in situ Acropora* spp. nurseries, such as those described above. However, several organizations within Florida are implementing land-based coral nurseries to aid in restoration efforts. Corals within these nurseries are brought on land and propagated within tanks and raceways that often use salt water that is substantially different than the reef water these organisms typically experience (*i.e.*, instant ocean, marine well water). Land-based corals are also exposed to potential toxins that leach from the materials used to create the tanks and raceways. These corals often have higher

levels of human contact because they are more easily accessible than *in situ* coral nurseries. Finally, the risk of novel pathogens, and the introduction of invasive and exotic organisms that cause significant harm to wild populations (*e.g.*, corallivorous flatworms), is likely higher when corals are propagated within land-based systems rather than grown within *in situ* coral nurseries (Hume *et al.* 2014, Rawlinson and Stella 2012). Rigorous research, which identifies the risks associated with land-based coral propagation techniques, is essential before using these types of nurseries for outplanting corals onto Florida's reef tract.

Conclusions

Outplanting *Acropora* corals onto reefs of Florida may promote the restoration of two main reef-building species of the Caribbean. However, introducing corals that are artificially propagated within coral nurseries may have significant impacts on the wild populations present. One of those impacts may be an increase in disease risk of wild *Acropora* corals because of outplantation efforts. There is a substantial lack of information on the disease dynamics of *Acropora* necessary to conduct a quantitative reef analysis. The qualitative risk assessment completed by a panel of experts in the field of *Acropora* diseases determined that under the current state of knowledge there was a low risk for increased disease activity on wild populations when outplanted corals are segregated from the wild population, and a low-medium risk when outplanted corals are interspersed with the wild population. The workshop participants noted the significant lack of quantitative information and identified several general areas that need more attention as well as eight specific research activities. These activities were identified to address significant research gaps within our current state of knowledge. Filling these gaps would increase confidence in the qualitative risk assessment and provide information essential for completing a quantitative risk analysis. Understanding the baseline levels of disease within wild coral populations, within nurseries, and outplanted nursery-propagated corals, would provide researchers the data needed to determine whether disease risk increases within wild *Acropora* populations stemming from restoration efforts with more discernible confidence.

References

Acropora Biological Review Team (2005) Atlantic *Acropora* status review document. Report to National Marine Fisheries Service, Southeast Regional Office, March 3, 2005, p 152 + App

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25 – 38

Bak RPM, Criens SR (1981) Survival after fragmentation of colonies of *Madracis mirabilis*, *Acropora palmata* and *A. cervicornis* (Scleractinia) and the subsequent impact of a coral disease. Proc 4th Int Coral Reef Symp 2:221 – 227

Bright AJ (2009) The effect of swell-generated physical damage on disease prevalence and asexual reproduction in the coral, *Acropora palmata* (Lamarck). Master's Thesis, University of the Virgin Islands, St. Thomas, United States Virgin Islands.

Casas V, Kline DI, Wegley L, Yu Y, Breitbart M, Rohwer F (2004) Widespread association of a Rickettsiales-like bacterium with reef-building corals. Environ Microbiol 6:1137 – 1148

Croquer A, Bastida C, Lipscomb D (2006) Folliculinid ciliates: a new threat to Caribbean corals? Dis Aquat Org 69:75 – 78

Gignoux-Wolfsohn SA, Marks CJ, Vollmer SV (2012) White band disease transmission in the threatened coral, *Acropora cervicornis*. Scientific Reports 2: 804 doi:10.1038/srep00804

Gil-Agudelo DL, Smith GW, Weil E (2006) The white band disease type II pathogen in Puerto Rico. Rev Biol Trop 54:59 – 67.

Gladfelter WB (1982) White-band disease in *Acropora palmata*: implications for the structure and growth of shallow reefs. Bull Mar Sci 32:639 – 643

Holden C *(*1996*) Science* 274*:*2017

Hogarth WT (2006) Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. Fed Regist 71:26852–26861

Hume BC, D'Angelo C, Cunnington A, Smith EG, Wiedenmann J (2014) The corallivorous flatworm Amakusaplana *Acropora*e: an invasive species threat to coral reefs? Coral Reefs 33:267 – 272

Johnson ME, Lustic C, Bartels E, Baums I, Gilliam DS, Larson L, Lirman D, Miller MW, Nedimeyer K, Schopmeyer S (2011) Caribbean Acropora Restoration Guide. The Nature Conservancy. (online access at http://www.frrp.org/)

Kline DI, Vollmer SV (2011) White band disease (type I) of endangered Caribbean acroporid corals is caused by pathogenic bacteria. Scientific Reports 1:7 doi: 10.1038/srep00007.

Lentz JA, Blackburn JK, Curtix AJ (2011) Evaluating patterns of a white-band disease (WBD) outbreak in *Acropora palmata* using spatial analysis: a comparison of transect and colony clustering. PLoS ONE 6: e21830. doi:10.137/journal.pone.0021830.

Lesser MP, Jarett JK (2014) Culture-dependent and culture-independent analyses reveal no prokaryotic community shifts or recovery of *Serratia marcescens* in *Acropora palmata* with white pox disease. FEMS Microbial Ecology 88:457 – 467

Libro S, Kaluziak ST, Vollmer SV (2013) RNA-seq Profiles of Immune Related Genes in the Staghorn Coral *Acropora cervicornis* Infected with White Band Disease. PLoS ONE 8(11): e81821. doi:10.1371/journal.pone.0081821

Mayor PA, Rogers CS, Hillis-Starr ZM (2006) Distribution and abundance of elkhorn coral, Acropora palmata, and prevalence of white-band disease at Buck Island Reef National Monument, St. Croix, US Virgin Islands. Coral Reefs 25:239 – 242

Meesters EH, Bak RPM (1995) Age-related deterioration of a physiological function in the branching coral *Acropora palmata*. Mar Ecol Prog Ser 121: 203 – 209

Miller SL, Chiappone M, Rutten LM, Swanson DW (2008) Population status of *Acropora* corals in the Florida Keys. Proc 11th Int Coral Reef Symp: 775 – 779

Miller MW, Lohr KE, Cameron CM, Williams DE, Peters EC. (2014) Disease dynamics and potential mitigation among restored and wild staghorn coral, *Acropora cervicornis*. PeerJ 2:e541 http://dx.doi.org/10.7717/peerj.541

Miller MW, Marmet C, Cameron CM, Williams DE. (In Press) Prevalence, consequences, and mitigation of fireworm predation on endangered staghorn coral. Mar Ecol Prog Ser. doi: 10.3354/meps10996

Muller EM, Rogers CS, Spitzack A, van Woesik R (2008). Bleaching increases the likelihood of disease on *Acropora palmata* (Lamarck) at Hawksnest Bay, St. John, US Virgin Islands; Coral Reefs 27:191 – 195

Muller EM, Rogers CS, van Woesik R (2014) Early signs of recovery of *Acropora palmata* in St. John, US Virgin Islands. Marine Biology 161:359 – 365

Muller EM, van Woesik R (2014) Genetic Susceptibility, Colony Size, and Water Temperature Drive White-Pox Disease on the Coral *Acropora palmata*. PLoS ONE 9(11): e110759. doi:10.1371/journal.pone.0110759

Pandolfi J (2002) Coral community dynamics at multiple scales. Coral Reefs 21:13 – 23

Pantos O, Bythell JC. 2004. Bacterial community structure associated with white band disease in the elkhorn coral *Acropora palmata* determined using culture-independent 16S rRNA techniques. Dis Aquat Org. 69:79 – 88.

Patterson KL, Porter JW, Ritchie KB, Polson SW, Mueller E, Peters EC, Santavy DL, Smith GW (2002) The etiology of white pox a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. P Natl Acad Sci USA 99: 8725 – 8730.

Peters EC, Oprandy JJ, Yevich PP (1983) Possible causal agent of "white band disease" in Caribbean acroporid corals. J Invert Pathol 41:394 – 396.

Peters EC (1984) A survey of cellular reactions to environmental stress and disease in Caribbean scleractinian corals. Helgol Meeresunters 37:113 – 137.

Polson SW, Higgins JL, Woodley CM (2009) PCR-based assay for detection of four coral pathogens. Proc 11th Int Coral Reef Symp 1:251 – 255

Rawlinson KA, Stella JS (2012) Discovery of the Corallivorous Polyclad Flatworm, *Amakusaplana Acropora*e, on the Great Barrier Reef, Australia – the First Report from the Wild. PLoS ONE 7:e42240

Ritchie KB, Gerace DT, Smith GW (1995) Grouping of bacterial heterotrophs from scleractinian corals using metabolic potentials. In: Proc. 26th Meet. Associ. Mar. Lab. Caribbean. San Salvador, Bahamas: Bahamian Field Station, pp. 224 – 236

Ritchie KB, Smith GW (1998) Type II White-band disease. Rev Biol Trop 46:199 – 203

Rodriquez-Martinez RE, Bonaszak AT, McField M, Beltran-Torres A, Alvarez-Filip L (2014) Assessment of *Acropora palmata* in the Mesoamerican Reef System. PLoS ONE 9(4): e96140. doi:10.1371/journal.pone.0096140

Rogers CS, Sutherland KP, Porter JW (2005) Has white pox disease been affecting *Acropora palmata* for over 30 years? Coral Reefs 24:194 – 194

Rogers CS, Muller EM (2012) Bleaching, disease and recovery in the threatened scleractinian coral Acropora palmata in St. John, US Virgin Islands: 2003-2010. Coral Reefs 31:807 – 819

Santavy DL, Peters EC (1997) Microbial pests: coral disease research in the western Atlantic Proc 8th Int Coral Reef Symp Balboa 1:607 – 612

Shelten C, Brown S, Gurbisz CB, Kautz B, Lentz JA (2006) Status of *Acropora palmata* populations off the coast of South Caicos, Turks and Caicos Islands. *Proceedings of the Gulf and Caribbean Fisheries Institute.* 57:665 – 678

Smith AJ, Thomas JD (2008) White band syndromes in *Acropora cervicornis* off Broward County, Florida: transmissibility and rates of skeletal extension and tissue loss. Proc 11th International Coral Reef Symposium, Ft. Lauderdale, Florida

Sutherland KP, Shaban S, Joyner JL, Porter JW, Lipp EK (2011) Human pathogen shown to cause disease in the threatened elkhorn coral *Acropora palmata*. PLoS ONE 6:e23468 doi:10.1371/journal.pone.0023468

Sweet MJ, Croquer A, Bythell JC (2014) Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral *Acropora cervicornis*. Proc Royal Soc B 281: DOI: 10.1098/rspb.2014.0094

Vollmer SV, Kline DI (2008) Natural disease resistance in threatened staghorn corals. PLoS ONE 3:e3718. doi:10.1371/journal.pone.0003718.

Williams DE, Miller ME (2005) Coral disease outbreak: pattern, prevalence and transmission in *Acropora cervicornis*. Mar Ecol Prog Ser 301:119 – 128.

Williams DE, Miller ME (2012) Attributing mortality among drivers of population decline in *Acropora palmata* in the Florida Keys (USA). Coral Reefs 31:369 – 382

Coral Disease Workshop -- Identified Research Activities

<u>Research Activity</u>: Quantifying disease resistance and susceptibility of Florida *Acropora* spp. genets

Need
The degree of resilience to future disease outbreaks in remnant populations of *A. cervicornis* and *A. palmata* is a critical determinant of their recovery potential. Research from Panama, using simple transmission assays, indicates that ~6% of staghorn coral genotypes are disease resistant, but no similar information is available for any *Acropora* spp. genets in Florida. Transmission assays provide a cost-effective means of identifying disease resistant genets in both nursery stocks and in wild remnant populations. Identification of these resistant *Acropora* spp. coral genotypes could and should be used to inform on-going and future outplanting efforts and is a pre-requisite for further ecological and/or genomic studies to determine potential ecological tradeoffs of disease resistance needed to develop truly sustainable application of nursery restocking efforts, as well as molecular and physiological studies to elucidate functional mechanisms of disease resistance in *Acropora* spp.

Objective
Develop and apply standardized transmission assay criteria to assess the relative levels of disease resistance in nursery-stock genets of *Acropora* spp. to examine potential tradeoffs with other traits (*e.g.*, growth or other stress tolerance) and thereby improve outplanting success by focusing on resistant stocks.

Expected Benefits
Given the devastating effects of disease on *Acropora* spp. populations, the potential to increase disease resistance in restocked *Acropora* spp. populations may greatly increase the chances of these species' recovery. Data on disease resistance can be used to predict impacts of future resilience of these endangered coral populations in Florida as well as provide a starting point to more successfully investigate the functional mechanisms of disease and health in *Acropora* corals, which could lead to additional management tools such as effective disease prevention and/or treatment.

Approach
Grafting experiments (*i.e.*, an extant diseased 'inoculant' coral fragment placed in direct contact with the healthy 'target' fragment) should be applied in the field and/or laboratory (at the site of natural disease occurrence) to identify degrees of disease resistance of specific coral genets. Protocols to standardize and document the nature of the 'inoculant' disease, the time frame, and the specific parameters measured (*e.g.*, time to onset of disease signs, rate of tissue loss in infected target, etc.) will allow relative resistance to be compared among different nurseries and among nursery versus wild genets tested in different trials. Measures of disease performance should be correlated with coral performance (*e.g.*, growth rate, thermal tolerance, etc.). If tank-based transmission experiments are to be carried out, clear non-recirculating, biosecurity protocols need to be established to prevent potential spread of the disease.

<u>Research Activity</u>: Determine baseline disease dynamics in nurseries

Need

Tens of thousands of colonies of *A. cervicornis* and *A. palmata* are currently being propagated within *in situ* nurseries throughout the reefs of Florida. These corals are used as stock populations for the outplanting and restoration of the Florida Reef Tract. Since these corals are being grown within high density conditions, they may be more susceptible to disease outbreaks. To prevent outbreak conditions, nursery managers often employ a series of culling and mitigation methods to remove diseased individuals and limit transmission. These methods include colony isolation, pruning, banding and reduction of vector transmission and may reduce the probability of disease transmission to healthy individuals, but prevent an accurate assessment of the influence of disease within the nursery environment.

Objective

Compare disease prevalence within nursery populations that are actively culling diseased corals from nurseries to those that do not use culling practices. Compare disease prevalence in both nursery types to the wild population.

Expected Benefits

Corals grown within Florida's *Acropora* nurseries are the population stock being used for restoration of the species throughout the Florida Reef Tract. However, very little is known about the condition of the corals being used for outplanting as macroscopic characterizations of health provides minimal information on the physiology and microbial community that these corals harbor. Knowing to what extent disease affect the nursery stocks, among the different genotypes, and through time in the absence of intervention, will provide significant insight into the health status of the nursery population. Culling sick individuals may only mask the underlying influence of disease within the stock population. Understanding the effects of culling methods to reduce disease outbreaks will provide more informed health status information on the corals being used for propagation and restoration purposes and may enable improved health management within the nurseries as well.

Approach

Segregated nursery populations should be established and maintained under the current best practice scenarios used for *Acropora* spp. propagation and growth, except managers will refrain from culling or other active health management. Although this may yield higher disease toll in this nursery population, it will provide managers and scientists a better understanding of the influence of disease on corals being used for outplanting purposes and enhance understanding of genet-specific disease resistance traits. Additionally, managers will be able to compare disease activity within nurseries (by genet) with the populations of outplanted corals as well as in the wild population. Currently, because of culling practices that comparison does not provide an accurate representation of disease activity within a nursery setting.

<u>Research Activity</u>: Evaluate risk of disease to wild *Acropora* species when outplanting occurs interspersed with the wild populations vs. segregated from wild populations

Need

Currently, there is no direct evidence whether outplanting nursery corals will influence disease activity within the wild population of *Acropora* spp. in the Florida Reef Tract. If the tissue loss diseases that are commonly affecting *Acropora* populations are indeed transmissible, then adding individuals that may harbor potential pathogens, and also increasing population density, may inadvertently increase the probability of disease activity within the wild population. However, disease activity is currently commonplace within these two species and any potential increase in risk may be negligible and fall within a level of acceptable risk that is offset by the benefits associated with restoration efforts.

Objective

Determine whether the risk of disease activity on wild colonies of *Acropora* increases when nursery corals are outplanted within reefs that have *Acropora* colonies present (*i.e*., interspersed). Identify whether the risk is higher for wild populations on interspersed reefs compared with reefs isolated from outplanting activities (*i.e*., segregated).

Expected Benefits

Understanding whether outplanted *Acropora* colonies from nurseries impact the nearby wild populations either within the same or on adjacent reefs, is essential for establishing best practices for reef restoration and for improving the confidence in disease risk estimates related to different outplanting configurations. If outplanted corals do increase disease risk to wild populations then methods will need to be developed to reduce the identified risk to acceptable levels. If outplanted corals do not increase disease risk to wild populations then there is no need to develop or employ risk mitigation techniques.

Approach

Manipulative transplantation experiments should be conducted to compare disease dynamics and impacts to wild *Acropora spp.* that are ‘exposed’ (*i.e*., interspersed) versus ‘unexposed’ (*i.e*., segregated from) to outplanted *Acropora* colonies. Monitoring periodicity should be sufficient to capture disease onset and progression.

<u>Research Activity</u>: Influence of density dependence and corallivores on disease dynamics of *Acropora* spp.

Need

Predation, primarily by corallivorous snails and fireworms, is a significant chronic source of direct, though often partial mortality in Florida *Acropora* populations. Corallivory is also associated with increased subsequent disease risk in preyed colonies, either by vector transmission or simply by creating injury sites that enable disease infection. Recent Florida *A. cervicornis* studies suggest that high colony density may enhance colony condition and growth rate (likely due to positive feedbacks with resident fishes) as well as the attraction and impact of corallivores. Better mechanistic understanding of the complex interactions of colony density, corallivore attraction/impact, and disease risk/impact is needed to guide improved outplanting and potential predator control strategies.

Objective

Experimental field studies should be undertaken to evaluate the interactive effects of colony density and corallivore abundance on disease impacts and overall performance of outplanted *Acropora* spp. colonies.

Expected Benefits

The endeavor of 'creating' new *Acropora* spp. population patches via outplanting provides the opportunity to manipulate colony density to enhance performance. Relatedly, the substantial investment in said creation may warrant some additional effort in corallivore control if this may help limit the negative effects of predation, disease, or both. Teasing out these complex direct and indirect interactions of colony density, disease transmission risk, and corallivore attraction/impact are needed to prioritize and develop more effective strategies on outplant density and potential corallivore control in *Acropora* restoration efforts.

Approach

Experimental field studies should be undertaken to manipulate coral colony and corallivore density (and/or identity to include snails, *Coralliophila abbreviata*, and/or fireworms, *Hermodice carrunculata*) to understand the prevalence, timing, and mortality associated with disease and corallivory as well as the effects of these predators on coral colony performance (*e.g.*, growth, productivity, etc.). Ideally, experiments would examine these interactions in different habitat types (*e.g.*, shallow fore-reef versus patch reef/hardbottom) where corallivore dynamics may differ as well as elucidate the mechanism(s) of corallivory-associated disease risk (*e.g.*, vector transmission versus ancillary infection of injured tissue).

<u>Research Activity</u>: Evaluate the effect of genotypic diversity on disease risk of outplanted *Acropora* spp.

Need

The long-term success of restoration efforts may be influenced by the genetic and genotypic diversity of the restored coral populations. Inbreeding depression (the reduction of fitness from the mating of relatives) and outbreeding depression (the mating between individuals that are strongly adapted to divergent local conditions) are two substantial concerns. However, high genetic diversity will likely increase probability of success and species survival under our changing global environment. Evidence suggests that certain genotypes are more resilient to disease than others, although the proportion of known disease-resistant genotypes in *A. cervicornis* is less than 10%. The percentage and identity of coral genotypes currently being propagated within nurseries that may be resistant to disease is currently unknown. Outplanting a high diversity of genotypes may reduce disease activity if some genets are less likely to be infected with disease, or genotypic diversity may have little influence on disease risk.

Objective

Determine whether genotypic diversity of outplanted *Acropora* spp. corals affects disease incidence and prevalence of the transplanted corals through time.

Expected Benefits

Current best practice methods for *Acropora* spp. culture include collecting as much of the local genotypic diversity as possible for the nursery stock, while reducing the movement of individuals from distant populations. Also, genotypic diversity of colonies outplanted within a patch is generally maximized. Understanding how genotypic diversity may influence disease dynamics of corals used for restoration and ultimately influence wild populations of *Acropora* spp. is essential for optimizing best practices.

Approach

To determine whether genotypic diversity of outplanting sites influences disease activity, experimental plots representing a gradient of genotypic diversity should be established with corals from *in situ* nurseries and surveyed through time. The genotypic diversity of the coral plot should range from single clones within a plot to high genotypic diversity, incorporating documented susceptible and resistant genotypes into the design. These plots should be surveyed frequently enough to determine the relationship between genotypic diversity and disease prevalence, incidence, and impact (amount of mortality).

<u>Research Activity</u>: Verify the identity of 'Rickettsia-like organisms' seen in histology of *Acropora* spp. tissues

Need

The pathogens that are causing tissue loss diseases have not been identified, yet disease is one of the most significant threats that may lead to the extinction of *Acropora* spp. in the Caribbean. Histological samples from both diseased and healthy tissues indicate that there are Rickettsia-like organisms in mucocytes on polyp oral discs and tentacles and in cnidoglandular bands of mesenterial filaments. Examinations of Rickettsia-like organisms -infected tissues revealed that they infect the polyp mucocytes and alter the coral's mucus secretions without causing gross disease signs. The Rickettsia-like organism is infections, therefore, may increase the susceptibility of corals to other environmental stressors and tissue loss.

Objective

Undertake molecular identification of the suspected Rickettsia-like organisms to verify (or not) their identity as *Rickettsia*.

Expected Benefits

Unraveling the mystery of what causes tissue loss diseases on Caribbean *Acropora* species would provide significant insight into how to prevent or reduce future disease outbreaks. Much emphasis has been placed on identifying particular pathogens present in diseased corals, but absent in healthy corals. Recent evidence, however, suggests that Rickettsia-like organisms are present within both healthy and diseased tissues (*i.e.*, a chronic, virtually ubiquitous infection) and may be a significant contributor to reduced overall coral health under environmental stress scenarios. If true, this model of Acropora disease etiology may suggest alternative health management and mitigation strategies. Determining whether these Rickettsia-like organisms are a significant component of tissue loss diseases would be more difficult to ascertain, especially because even healthy samples contain Rickettsia-like organisms. An inventory of Rickettsia-like organisms in *Acropora* spp. from around the Caribbean may be necessary to first identify the distribution of these organisms and then potentially apply experimental manipulations to determine the relative contribution that Rickettsia-like organisms play in causing disease activity.

Approach

To determine whether Rickettsia-like organisms are in fact *Rickettsia* spp. within histological samples, suspect Rickettsia-like organisms must be isolated from the tissue sample and the DNA must be extracted. Primers specific for *Rickettsia* spp. could be used for positive identification using polymerase chain reaction. DNA from samples could also be sequenced for identification.

<u>Research Activity</u>: Identify the relationship between temperature tolerance and disease susceptibility

Need

Thousands of corals are currently being propagated and outplanted for restoration activities along the Florida Reef Tract. However, very little is known about the physiology of the genotypes that are being used for restoration. Although there are potentially thousands of *Acropora* genotypes within the reefs of FL, only a few hundred are being used for propagation and outplanting. Understanding which genotypes are resistant to disease is important (Research Activity1), but understanding the physiological tradeoffs associated with disease resistance is also essential. Some genotypes that are disease resistant may also be more susceptible to stress associated with high water temperature. Alternatively, corals that are more resilient to temperature stress may also be innately disease resistant. Climate change will continue to increase the oceanic water temperatures around the globe, and disease is one of the greatest threats to *Acropora* spp. Therefore, identifying coral genotypes that are resilient to thermal extremes and disease, or establishing the tradeoffs between these two physiological traits, will enable more informed restoration strategies.

Objective

Identify whether there is a tradeoff between disease resistance and resilience to high water temperatures for different coral genotypes currently being propagated within *in situ* nurseries.

Expected Benefits

Bleaching from high water temperatures and disease outbreaks are two of the greatest threats facing coral reefs around the world. These threats are particularly important for Caribbean *Acropora* species because *A. palmata* and *A. cervicornis* are highly susceptible to both. Understanding the phenotypic variability in temperature tolerance and disease resistance of the different genotypes currently being used for propagation and restoration will significantly increase the knowledge behind outplanting design. Knowing whether there is a tradeoff or independence between the two traits will provide considerable information for restoration science, but also on the natural level of phenotypic variability within remnant Florida *Acropora* populations.

Approach

Experimental manipulations within a laboratory would provide a robust platform to isolate these two traits. Individual genotypes from coral nurseries would first be tested for disease resistance. Likely, a continuum from disease susceptible to disease resistant would be detected. These same genotypes would then be exposed to high water temperature to determine relative resilience to thermal stress. If genotypes that are disease resistant are also more susceptible to temperature stress then there may be a tradeoff between these two variables. Experiments could further test whether disease resistant phenotypes change when these genotypes are exposed to warm thermal stress. Additionally, field observational data on disease incidence or prevalence among these genotypes and their relative level of thermal tolerance during a temperature anomaly would provide *in situ* information that could confirm the laboratory experimental results.

Chapter 2. Ecological Processes Workshop

Introduction

Coral reef restoration efforts have largely focused on the restoring of physical structure and individual coral colonies without directly addressing critical ecological processes such as herbivory, recruitment, predation, etc. that are vital to coral reef ecosystem function and consequently to coral reef restoration efforts. However, there is at present exceedingly limited knowledge regarding appropriate practices for enhancement of these ecosystem processes as part of proactive coral reef restoration efforts. The Steering Committee recommended a dedicated workshop to provide specific research activities to address how ecological processes can be actively managed to aid successful coral restoration on reefs.

The ecological processes workshop was held at the National Oceanographic and Atmospheric Administration's (NOAA) Southeast Fisheries Science Center in Miami, FL on September 25-26, 2014. Table 5 lists the workshop attendees and their affiliation. Mark Ladd of Florida International University drafted a report summarizing the current state of knowledge regarding coral reef ecological processes as they relate to coral reef ecosystem restoration. His report is below.

Table 5. List of Ecological Processes Workshop attendees

Name	Title	Affiliation
John Hunt	Program Administrator	Florida Fish &Wildlife Conservation Commission
William Sharp	Assoc. Research Scientist	Florida Fish &Wildlife Conservation Commission
Margaret Miller	Ecologist	NOAA Southeast Fisheries Science Center
Deron Burkepile	Assistant Professor	Florida International University
Mark Ladd	PhD Student	Florida International University
Stephanie Schopmeyer	Senior Research Associate	University of Miami
Lauren Toth	Post-Graduate Fellow	United States Geological Survey
Tom Adam	Post-Doctoral Fellow	University of California Santa Barbara
Valarie Paul	Head Scientist	Smithsonian Institute
Rob Ruzicka	Research Administrator	Florida Fish &Wildlife Conservation Commission
Kristie Erickson	Biological Scientist	Florida Fish &Wildlife Conservation Commission
Alejandro Acosta	Research Administrator	Florida Fish &Wildlife Conservation Commission
Mary Truglio	Wildlife Legacy Biologist	Florida Fish &Wildlife Conservation Commission

Report: Ecological processes and feedbacks in coral restoration

Background

Presently, coral reef restoration throughout Florida is focused on the outplanting of nursery-raised Acroporid coral colonies. This strategy is premised on the notion that augmenting coral populations can jumpstart coral reef community recovery. However, several years into coral

outplanting efforts it is becoming increasingly apparent that complimentary actions are required to restore coral reef community and ecological function. While there have been a host of studies investigating the growth and success of outplanted corals, there is much less research attention, especially in Florida, on the ecological processes and feedbacks that govern coral reef ecosystem structure, function and ultimately coral reef recovery. A better understanding of these processes and how they influence coral reef community structure and function is critical for developing effective coral reef restoration strategies. Specifically, identifying ecological processes that can be manipulated or utilized to facilitate coral reef community recovery is necessary to restore Florida's coral reefs.

Through a review of Caribbean coral reef ecology literature and a two-day workshop discussion among a group of Florida reef experts, a suite of ecological processes that might be enhanced or manipulated to benefit coral reef restoration efforts were identified and research activities developed. Herbivory, recruitment, competition, predation, disease, nutrient cycling, and bioerosion were identified as key processes that warrant further investigation in a reef restoration context, though other processes relevant to restoration likely exist. Many of these ecological processes affect each other, work synergistically, and can generate cascading impacts that alter coral reef community structure and function.

This document provides a review of relevant knowledge regarding the ecological processes of interest, specifically focused on Florida coral reefs. The goal is not to provide an exhaustive review, but rather to highlight manipulations and intervention points that could be used by those conducting coral reef restoration, as well as suggest important research topics to improve coral reef restoration approaches. Emphasis is placed on either breaking negative feedbacks curtailing the recovery of reef communities, or, promoting processes that support positive feedbacks that could facilitate the recovery of coral reef ecosystem function. The focus is on the entire coral reef community and ecological processes, not solely on restoring coral populations.

Herbivory

Herbivory is a key process on coral reefs that mediates competitive interactions between corals and macroalgae. Sufficient herbivory on reefs not only decreases macroalgal abundance, but has also been demonstrated to increase the presence of crustose coralline algae (CCA), a preferred settlement substrate for coral recruitment (Burkepile & Hay 2008), increase coral growth and recruitment and decrease coral mortality (Steneck 1988; Hughes *et al.* 2007). Herbivores can further enhance coral recruitment by decreasing the retention of sediments (Birrell, McCook & Willis 2005).

The critical role of herbivores in mediating coral-algal interactions has been demonstrated by a number of herbivore exclusion experiments, which have demonstrated increased coral mortality and decreased recruitment when herbivores are not present (Hughes *et al.* 2007; Burkepile & Hay 2008). Reduced macroalgal cover promotes the growth and recruitment of corals, which in turn increases topographic complexity on a reef, providing additional space for the recruitment of more fishes (herbivores and other trophic groups), while simultaneously intensifying herbivory on remaining macroalgal populations (Miller & Hay 1998; Hughes *et al.* 2007; Mumby &

Steneck 2008). Herbivory is the linchpin of this series of positive feedbacks that reinforce a topographically complex, coral-dominated system that supports diverse communities and ecosystem function.

Herbivory could be manipulated in a variety of ways to bolster coral restoration efforts and promote coral reef recovery. Developing methods to concentrate herbivory at selected restoration sites, for example, focusing existing grazing pressure by reducing grazeable substrate area or increasing grazer abundance could improve coral growth, recruitment and the aforementioned series of grazing-based positive feedbacks. Alternatively, restoration activities could focus on sites where herbivory is naturally elevated to take advantage of the benefits of high grazing intensity. Research is needed to identify herbivore community composition that provides sufficient herbivory to promote reef community recovery (*e.g.*, decrease competition with existing corals, enhance recruitment). Experiments that can test and refine methods to harness the benefits of herbivory would provide an extremely useful tool for restoration practitioners. Studies should consider the role of other processes and factors, such as nutrient cycling and structural complexity, in altering grazer behavior and influence.

Herbivores are commonly grouped into a single (or several) functional group(s). However, herbivores exhibit a diversity of jaw morphologies, feeding and habitat preferences that generate distinct, species-specific patterns in the influence herbivores have on benthic community structure (Bellwood *et al.* 2004; Burkepile & Hay 2010). Burkepile and Hay (2008) experimentally manipulated herbivore composition by caging different species combinations of herbivores and measuring changes in macroalgal community composition, coral growth and recruitment. They demonstrated that certain species (*e.g.*, the Redband Parrotfish, *Sparisoma aurofrenatum*) target specific algal groups such as upright macroalgae and are more effective at promoting beneficial benthic groups such as CCA. However, in the same experiment *S. aurofrenatum* was unsuccessful in removing turf algae, a poor settlement substrate for coral recruits, while the herbivorous Ocean Surgeonfish (*Acanthurus bahianus*) significantly decreased turf cover. These results demonstrate the importance of herbivore identity in affecting benthic community structure and mediating coral-algal interactions. Clearly, a diverse and ecologically relevant suite of herbivores is needed to maximize positive feedbacks that can promote and maintain functional coral reef communities. The ability to utilize diet specificity to remove specific impediments to recovery (*e.g.*, dominance by upright macroalgae) would be an invaluable reef restoration tool.

Beyond diet preferences, herbivores also differ in the spatial extent to which they graze and the intensity of grazing over that area. Grazing by herbivorous fishes is generally considered diffuse (*e.g.*, on the scale of an entire reef), while urchin grazing is spatially constrained to a relatively small area of the reef surface (~1m^2). The consistent source of herbivory provided by urchins has the potential to decrease competitive interactions and allow juvenile corals to reach a sufficient size to better compete with other benthic competitors (Sandin & McNamara 2012).

The benefits of grazing by the once Caribbean-wide abundant grazing urchin *Diadema antillarum*, which suffered a massive die-off in 1983, on coral growth and recruitment, are well documented (Lessios 1988; Edmunds & Carpenter 2001). Recently, *Diadema* has made several localized recoveries throughout the Caribbean. These recovery sites, compared to adjacent areas

with no *Diadema* present, are characterized by low (<15%) algae cover and significantly higher number of juvenile corals (Edmunds & Carpenter 2001; Carpenter & Edmunds 2006), although the vast majority of these recruits were brooding species and not reef-builders. These studies exemplify the massive influence that these grazers can have on coral reef community structure.

These small areas of high grazing intensity may represent ideal sites for coral restoration activities by taking advantage of the spatially constrained grazing of urchins to promote the survival and growth of outplanted corals. Such sites could serve as "recovery nodes" that spread out into adjacent degraded reef areas.

Recruitment

Successful recruitment is an essential characteristic of a healthy, self-sustaining coral reef community and a vital component of coral reef ecosystem recovery. Sexual recruits provide a supply of new genetic material to populations and introduce genotypes that may be better adapted to local conditions, especially in the face of global climate change. Recruitment involves three key phases: the larvae phase, settlement, and post settlement (Ritson-Williams *et al.* 2009). Each of these phases can be affected by a myriad of factors and therefore present multiple opportunities for restoration approaches to enhance this critical aspect of coral reef recovery.

The fecundity of a coral (*e.g.*, number of gametes produced) is largely size-dependent, with larger corals having a higher reproductive output than smaller corals (Szmant 1985; Soong 1993). Therefore, restoration actions could target efforts towards the maintenance and growth of large corals that will contribute most to reproductive output. Further, restoration efforts should aim to decrease competitive interactions between corals and other benthic components (*e.g.*, macroalgae, sponges, etc.), as competition has been demonstrated to decrease coral fecundity (Hughes *et al*. 2007).

In broadcast spawning species, once gametes have been released into the water column, fertilization is necessary for the development of larvae. Fertilization requires synchronization of gamete release as well as gamete compatibility, as not all gametes from the same species can successfully fertilize or have the same fitness depending on parent genotype (Palumbi 1994; Ritson-Williams *et al.* 2009; Baums *et al.* 2013). Additionally, gamete age can influence fertilization success, whereby gametes that spend more time in the water column are less likely to successfully fertilize, use more resources and have a lower chance of settlement and survival (Omori *et al.* 2001; Levitan *et al.* 2004). Thus, the density and identity of spawning adults can greatly influence the probability of successful fertilization. In combination, these results suggest that restoration activities that promote dense populations of reproductively compatible individuals may increase the chances of successful coral fertilization and reproduction (Baums *et al.* 2013).

Upon fertilization larvae must successfully settle to the benthos. The condition of larvae upon arrival to a settlement site can be influenced by a myriad of factors, including the time spent in the water column before settling (*e.g.*, depleted energy reserves), environmental stressors, microbial communities and proximity of conspecifics (Yakovleva *et al.* 2009; Marhaver *et al.* 2013). For example, Vermeij *et al.* (2006) demonstrated that salinity stress during the larval

phase decreased both pre- and post-settlement survival of *Orbicella faveolata* larvae. Consequently, pelagic conditions before settlement are an additional factor to take into account when considering restoration of coral recruitment processes.

Additionally, the distribution and availability of suitable settlement habitat can influence time spent in the larvae phase and ultimately influence recruitment success. Coral and fish larvae have evolved a suite of complex mechanisms to select suitable substrate for settlement. For benthic species such as coral, selection of a settlement site is especially crucial due to immobility after metamorphosis. Crustose coralline algae (CCA) as a single group are commonly referred as preferred settlement substrate for coral larvae. However, the ability of coral larvae to settle on CCA is species-specific, as many species of CCA posses chemical or physical (*e.g.*, sloughing) mechanisms to prevent successful recruitment by coral larvae, demonstrated by studies that have documented coral larvae exhibiting species-specific CCA preference for settlement (Raimondi & Morse 2000; Ritson-Williams *et al.* 2009). Beyond CCA-specific cues, coral larvae have shown preference for the protected undersides of substrates (Raimondi & Morse 2000) as well as preference for red and orange colored substrate (Mason, Beard & Miller 2011).

Coral reef restoration should take these factors that can influence recruitment success into consideration when developing restoration strategies. Processes that promote benthic cover of appropriate coral settlement substrate (*e.g.*, herbivory), physical structure that enhances fish recruitment, and reduce negative influences on the recruitment process (*e.g.*, preclusion of settlement space by macroalgae) should be a focus of restoration efforts. The identification of "good" CCA species that promote successful recruitment, either via attracting larvae or allowing for settlement and growth of corals, is an important first step, followed by research to measure the abundance and distribution of CCA species preferred for coral recruitment. An understanding of conditions and factors that promote preferred CCA species could allow the development of restoration approaches that target process promoting the growth of these beneficial substrates. Identification of characteristics at the micro-scale that enhance coral recruitment via settlement and survivorship is also needed. Incorporating factors such as the availability of cryptic/protected habitats at the micro-scale is an aspect of recruitment that warrants additional attention and could possibly be incorporated into the development of artificial recruitment structures.

Benthic species competing for space can reduce coral settlement via the direct preclusion of space (Mumby 2006). Kuffner *et al.* (2006) demonstrated decreased settlement of *Porites astreoides* larvae when the macroalgal species *Dictyota pulchella* and *Lobophora variegata* were present, and complete inhibition of settlement by the cyanobacteria *Lyngbia polychroa*. Facilitating or manipulating processes such as herbivory that reduce the presence of species that impede coral recruitment should be a component of a comprehensive coral reef restoration approach. Furthermore, selecting restoration sites that are less ideal for the growth of these negative species (*e.g.*, low-nutrient sites) may be a complementary strategy to reduce the negative effects benthic competitors can have on coral larvae settlement.

Newly settled corals must survive a host of acute and chronic stressors to successfully complete the recruitment process. Examples of acute stressors include bleaching events, which can result in wide spread mortality in a relatively short time period (*e.g.*, weeks) (Aronson *et al.* 2002; Hoegh-Guldberg 1999). Chronic stressors such as competition and sedimentation can also reduce

post-settlement survival rates. For example, competitive interactions can decrease recruit survival through direct overgrowth (Vermeij 2005), allelopathy (Pawlik, Steindler & Henkel 2007), shading, abrasion and contact (McCook, Jompa & Diaz-Pulido 2014), and vectoring pathogens (Nugues *et al.* 2004; Ritson-Williams *et al.* 2009).

The complexity of factors that contribute to settlement choices by both coral and fish larvae is exemplified by a recent study conducted by Dixson *et al.* (2014). The authors demonstrated that both fish and coral larvae possessed the ability to detect, and nearly exclusively prefer, water that came from areas with high coral cover and low algae cover, compared to adjacent sites with low coral cover and high algae cover. Further, Dixson *et al.* (2014) experimentally demonstrated that chemical cues from seaweeds that dominated low coral cover areas repelled coral and fish larvae, whereas chemical cues from corals found in the high coral cover sites attracted both types of larvae. This study, in combination with studies documenting microhabitat selection by larvae, suggests that recruitment decisions occur over a variety of spatial scales. Dixson *et al.* (2014) concluded that "for recovery, degraded reefs may need to be managed to produce cues that attract, rather than repel, recruiting corals and fishes. There is a need for restoration experiments to determine the appropriate scale at which restoration efforts will be effective in enhancing the natural recruitment of corals and fishes.

Benthic Invertebrate Competition

Coral cover on the Florida Reef Tract has dramatically declined over the last several decades, accompanied by a decrease in benthic diversity and an increase in "weedy" coral species, encrusting gorgonians and sponges (Burman, Aronson & van Woesik 2012; Ruzicka *et al.* 2013). Such declines in coral cover and diversity often lead to the loss of structural complexity, diminished fish populations, and decreased coral recruitment, ultimately reducing ecosystem function. In the context of coral reef restoration, understanding the outcomes of competitive interactions of different life history stages (larval, juvenile, adult) is needed to develop effective restoration approaches. For example, understanding competitive outcomes between outplanted corals and increasingly abundant benthic species could guide site selection to areas where outplanted corals have a higher chance of survival, growth and provide structural complexity. The outcomes of these competitive interactions can influence local conditions such as the development of topographic complexity and small-scale patterns in water flow, generating cascading effects that alter community structure and function.

Direct competition via space preclusion by competitors can reduce the amount of suitable recruitment substrate available for settling coral larvae. Alternatively, benthic components such as sponges can decrease post-settlement survival through allelopathy (Pawlik, Steindler & Henkel 2007). Competition between adult corals and other benthic species such as macroalgae and sponges can decrease growth and ultimately reduce reproductive output. Thus, competitive interactions can provide a series of negative feedbacks that can retard or inhibit coral reef community recovery. Research is needed to better understand competitive interactions affecting both outplanted and naturally occurring corals and what factors can mediate these interactions to produce a desired outcome. Such information could inform direct removal strategies or assist in selecting reef sites to avoid areas with benthic competing species that will impair outplanted

corals. Incorporating the process of succession, as is done by forest restoration practitioners, is a potentially fruitful research avenue with direct restoration application. Identifying competitive hierarchies and networks could provide a "road map" for a multi-step development of restored communities.

Predation

Predation is a chronic source of mortality for many coral species found throughout South Florida, directly contributing to tissue removal and partial mortality. The five major predator groups affecting corals along the Florida Reef Tract are corallivorous snails, fireworms, butterflyfishes, damselfishes and corallivorous parrotfishes, each of which can negatively impact individual corals and coral populations in different ways.

Beyond reducing or halting growth rates (Rotjan and Lewis 2008), the corallivorous snail *Coralliophila abbreviata,* arguably the most common South Florida coral predator, can vector disease among both *Acropora cervicornis* and *A. palmata* colonies (Williams and Miller 2005). Similarly, the fireworm *Hermodice caranculata* has been identified as a reservoir for coral disease in the Mediterranean (Sussman *et al.* 2003) and *A. cervicornis* preyed upon by fireworms in the Florida Keys show greatly increased risk of subsequent disease-like tissue loss (Miller *et al. In Press*). Both of these predators can remove tissue at rates that far exceed coral growth rates, especially in the two most common coral species used for outplanting, *A. cervicornis* and *A. palmata* (Baums *et al.* 2003). Yet, little is known regarding what species prey on these corallivores. Outplanting efforts could greatly benefit if they could remove or reduce this chronic source of mortality and impediment to recovery.

Certain species of territorial damselfishes will kill large portions of live coral tissue to create algal gardens that are fiercely protected. This not only directly removes tissue, but the aggressive behavior of damselfish can reduce grazing rates, alter the spatial distribution of grazing, and shift grazer diets, magnifying the negative impacts of these fishes on coral reef recovery (Hixon and Brostoff 1983; Catano *et al.* 2014). These algal gardens are especially prevalent in the two most common coral species outplanted in coral reef restoration, *A. cervicornis* and *A. palmata*. Butterflyfishes, many of which primarily feed on coral tissue, have been observed to preferentially feed on coral polyps with the highest reproductive potential, amplifying the damage done to a coral colony by removing tissue in which corals have invested a large amount of energy (Rotjan 2007). While it little is known on fish vectoring coral disease, the injury sites created by these corallivores generates a potential opening for disease infection (Gignoux-Wolfsohn *et al.* 2012).

The extensive direct and indirect consequences of predation underscore the need to address this negative process in order to reduce damage to existing coral populations. Manipulating predator-prey dynamics could play a large role in the abundance, and subsequent influence of corallivorous snails, fireworms, damselfishes and butterflyfishes on coral populations. Research addressing this knowledge gap could aid reef restoration efforts to better identify sites where the negative impacts of these coral predators will be minimized. Outplanting corals in areas with naturally low abundances of coral predators may be one option to promote restoration success. However, there is an urgent need for research that investigates factors that can be manipulated to

decrease corallivore populations. For example, identification of organisms that prey on *C. abbreviata* and *H. caranculata* could allow restoration practitioners to outplant corals in locations with naturally high abundances of these predators to improve the probability of reef recovery.

Parrotfishes are critical herbivores on Caribbean reefs that regulate macroalgal populations, promote coral cover and growth and can prevent seaweeds from dominating coral reefs, especially after a large disturbance (Mumby, Hastings & Edwards 2007; Mumby 2009). However, parrotfish populations have been severely reduced throughout the majority of the Caribbean region due to overfishing (Hawkins & Roberts 2004). Conversely, on Florida reefs parrotfishes are extremely abundant relative to most Caribbean reefs, due to nearly non-existent fishing pressure for this group of fishes. The abundance of parrotfishes in Florida, in conjunction with some of the lowest coral cover found throughout the Caribbean, has generated a unique situation where parrotfish grazing intensity on corals has been documented to be up to 34 times higher than reported anywhere else in the Caribbean (Burkepile 2011). Corallivorous parrotfishes on Florida reefs have also shifted their diets towards species more commonly found on local reefs (*e.g.*, *Porites porites*, *P. astreoides*, and *Siderastrea siderea*), leading to an alarming pattern in which predation intensity on corals increases as coral cover further decreases (Burkepile 2011).

It is important to emphasize that parrotfish are *not* the problem on Florida coral reefs. The extremely low coral cover characteristic of Florida reefs is the major driver of this pattern. These findings suggest that the lowest coral cover reefs, commonly targeted by coral restoration efforts, may actually be poor choices for restoration sites as they exhibited the highest rates of corallivory by parrotfishes. Outplanting operations should incorporate information such as this into decision-making when selecting restoration sites. Research on the effects of existing coral cover on outplant predation and survival, or the effect of increased coral cover via outplanting on the survival of existing corals (*e.g.*, dilution of corallivory pressure) could help improve outplanting strategies.

Disease

Coral disease is another chronic source of partial and total colony mortality. There are at least 14 diseases recognized in South Florida, though the etiology of most remains unknown (Randall and Jordán-Garza 2014). However, studies have documented a number of mechanisms by which disease can be vectored, as well as factors that can exacerbate disease virulence. For example, *C. abbreviata* can vector disease between *A. cervicornis* colonies as well as transfer disease signs from *A. cervicornis* to *A. palmata* (Williams and Miller 2005). Similarly, butterflyfishes can increase black-band disease transmission among *Orbicella faveolata* colonies (Aeby and Santavy 2006). Macroalgae in competition with corals can also harbor and directly vector disease onto corals they are in contact with (Nugues *et al.* 2004), providing another pathway by which disease can spread through coral populations. Even if competing macroalgae do not directly vector disease to corals, they can drastically alter microbial community structure, likely leading to reduced ability to combat disease infection (Vega Thurber *et al.* 2012).

A myriad of factors can exacerbate disease virulence. Increased water temperatures and nutrient levels have both been demonstrated to increase the severity and prevalence of coral diseases (Bruno *et al.* 2003; Vega Thurber *et al.* 2014). High rates of corallivory can increase disease transmission (Sussman *et al.* 2003; Gignoux-Wolfsohn *et al*. 2012). All stressors impacting corals can reduce growth, competitive ability, and have the potential to exacerbate diseases by reducing the amount of energy a coral can devote towards fighting infection.

Given the potentially catastrophic effect of disease on coral populations, it is essential to develop restoration strategies that minimize disease transmission and prevalence. Spatial arrangement and the density of outplanted corals are two possible factors that could be manipulated to minimize the deleterious effects of coral disease. Methods to reduce the abundance and presence of organisms known to vector coral disease could further improve restoration efforts.

Bioerosion

Vertical accretion of reef framework is a critical process for the long-term survival of coral reefs and the crucial ecological, economic and social services they provide. Especially in the face of sea level rise projections, reefs must be able to accrete framework or risk "drowning", accompanied by decreased diversity and function (Hoegh-Guldberg *et al.* 2007). Beyond keeping up with sea level rise, coral reef framework is constantly being eroded by a suite of bioeroding organisms. Boring organisms, such as sponges, can directly dissolve reef substrate and weaken reef framework such that it is more susceptible to breakage by large disturbances such as hurricanes (Wisshak *et al.* 2014). Mobile animals, such as grazing urchins and parrotfishes, can also substantially contribute to bioerosion by removing massive amounts of carbonate (Bruggemann *et al.* 1996; Bellwood *et al.* 2003; Brown-Saracino *et al.* 2006).

It is imperative that restoration approaches address the issue of bioerosion to reverse trajectories of eroding reefs. While a number of studies have been conducted investigating historical and present day bioerosion rates, no studies have been done to examine methods to slow or stop bioerosion in a restoration context. Potential methods include "capping" reefs with unpalatable benthic organisms that prevent the removal of carbonate by parrotfish and urchins. However, these methods clearly must be developed with caution, as covering a reef with a benthic species that outcompete corals and inhibits coral recruitment would run counter to reef recovery. Therefore, coupling bioerosion reduction studies with competitive interaction studies to identify benthic components that can "hold" space to prevent bioerosion, and could eventually be replaced by hard corals would be an ideal restoration approach. Methods to promote the recruitment and growth of reef-building corals and maintaining are another important step to mitigate the erosion of reef framework.

Nutrient Cycling and Fish-based feedbacks

Nutrient cycling is a critical process in the nutrient poor waters in which coral reef ecosystems are found. Mobile organisms, such as fishes that exhibit consistent sheltering and foraging behaviors, can important significant amounts of nutrients from adjacent ecosystems. For example, grunts school during the daytime in structurally complex reef areas and leave the reef

during the nighttime to forage in nearby habitats. The excretory products of diurnally foraging grunts can vector significant amounts of nitrogen and phosphorus to the reef environment in a form that can accelerate growth of corals and macroalgae. This consistent behavior pattern can concentrate nutrient inputs to a relatively small area of the reef, potentially influencing the composition of benthic communities and the processes that regulate them. Meyer and Schultz (1985) found that growth rates, skeletal accretion and surface area expansion of *Porites furcata* were greater in colonies with resident schools of grunts compared to colonies without resident schools, likely a result of the nutrient subsidy received from the schooling grunts.

Shantz *et al.* (i*n review*) demonstrated that areas with resident schooling grunts had grazing rates approximately three times higher than structurally similar areas without resident schooling grunts. Furthermore, they found that these areas exhibited distinct benthic communities, with areas where schooling fishes were present containing roughly double the amount of CCA cover and half the turf-algal-sediment matrix cover compared to sites without fishes present. Lastly, the authors found that growth rates of transplanted *A. cervicornis* colonies were 150% higher at fish-schooling sites compared to sites without fishes. This study demonstrates the potentially multi-faceted benefits of fish-derived nutrients for coral reef restoration, as several processes and characteristics that promote recovery were present at these localized sites. These results also suggest that developing methods to attract schooling fishes could create areas where fish will school and jumpstart the positive feedbacks that promote coral reef recovery. This is only one example of a fish-based process that can be utilized to advance coral reef restoration efforts. However, a number of additional processes and feedbacks likely exist that could be taken advantage of by coral reef restoration approaches to mediate the negative effects of competition, predation and disease and/or promote recruitment and the development of topographically complex reefs.

References

Aronson, R.B., Precht, W., Toscano, M. and Koltes, K. (2002) The 1998 bleaching event and its aftermath on a coral reef in Belize. *Marine Biology*, **141**, 435–447.

Baums, I.B., Devlin-Durante, M.K., Polato, N.R., Xu, D., Giri, S., Altman, N.S., Ruiz, D., Parkinson, J.E. and Boulay, J.N. (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral, Acropora palmata. *Coral Reefs*, **32**, 703–717.

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

Bellwood, D.R., Hoey, A.S. and Choat, J.H. (2003) Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. *Ecology Letters*, **6**, 281–285.

Bellwood, D.R., Hughes, T.P., Folke, C. and Nyström, M. (2004) Confronting the coral reef crisis. *Nature*, **429**, 827–33.

Birrell, C.L., McCook, L.J. and Willis, B.L. (2005) Effects of algal turfs and sediment on coral settlement. *Marine pollution bulletin*, **51**, 408–14.

Brown-Saracino, J., Peckol, P., Allen Curran, H. and Robbart, M.L. (2006) Spatial variation in sea urchins, fish predators, and bioerosion rates on coral reefs of Belize. *Coral Reefs*, **26**, 71–78.

Bruggemann, J., van Kessel, A., van Rooij, J. and Breeman, A. (1996) Bioerosion and sediment ingestion by the Caribbean parrotfish Scarus vetula and Sparisoma viride:implications of fish size, feeding mode and habitat use. *Marine Ecology Progress Series*, **134**, 59–71.

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

Burkepile, D.E. (2011) Context-dependent corallivory by parrotfishes in a Caribbean reef ecosystem. *Coral Reefs*, **31**, 111–120.

Burkepile, D.E. and Hay, M.E. (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 16201–6.

Burkepile, D.E. and Hay, M.E. (2010) Impact of herbivore identity on algal succession and coral growth on a Caribbean reef. *PloS one*, **5**, e8963.

Burman, S., Aronson, R. and van Woesik, R. (2012) Biotic homogenization of coral assemblages along the Florida reef tract. *Marine Ecology Progress Series*, **467**, 89–96.

Carpenter, R.C. and Edmunds, P.J. (2006) Local and regional scale recovery of Diadema promotes recruitment of scleractinian corals. *Ecology letters*, **9**, 271–80.

Catano, L., Shantz, A. and Burkepile, D. (2014) Predation risk, competition, and territorial damselfishes as drivers of herbivore foraging on Caribbean coral reefs. *Marine Ecology Progress Series*, **511**, 193–207.

Dixson, D.L., Arbrego, D., Hay, M.E. (2014) Chemically mediated behavior of recruiting corals and fishes: A tipping point that may limit reef recoveryScience, **345**, 892–897

Edmunds, P.J. and Carpenter, R.C. (2001) Recovery of *Diadema antillarum* reduces macroalgal cover and increases abundance of juvenile corals on a Caribbean reef. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5067–71.

Gignoux-Wolfsohn, S., Marks, C.J. and Vollmer, S. V. (2012) White Band Disease transmission in the threatened coral, Acropora cervicornis. *Scientific reports*, **2**, 804.

Hawkins, J.P. and Roberts, C.M. (2004) Effects of Artisanal Fishing on Caribbean Coral Reefs. *Conservation Biology*, **18**, 215–226.

Hixon, M. and Brostoff, W. (1983) Damselfish as keystone species in reverse - intermediate disturbance and diversity of reef algae. *Science*, **220**, 511–513.

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**, 839–866.

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A. and Hatziolos, M.E. (2007) Coral reefs under rapid climate change and ocean acidification. *Science*, **318**, 1737–42.

Hughes, T.P., Rodrigues, M.J., Bellwood, D.R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., Moltschaniwskyj, N., Pratchett, M.S., Steneck, R.S. and Willis, B. (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current biology*, **17**, 360–5.

Kuffner, I., Walters, L., Becerro, M., Paul, V., Ritson-Williams, R. and Beach, K. (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. *Marine Ecology Progress Series*, **323**, 107–117.

Lessios, H. (1988) Mass mortality of Diadema Antillarum in the Caribbean: What Have We Learned ? *Annual Review of Ecology and Systematics*, **19**, 371–393.

Levitan, D.R., Fukami, H., Jara, J., Kline, D., McGovern, T.M., McGhee, K.E., Swanson, C.A. and Knowlton, N. (2004) Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the Montastraea annularis species complex. *Evolution*, **58**, 308–23.

Marhaver, K., Vermeij, M., Rohwer, F. and Sandin, S. (2013) Janzen-Connell effects in a broadcast-spawning Caribbean coral : distance-dependent survival of larvae and settlers. *Ecology*, **94**, 146–160.

Mason, B., Beard, M. and Miller, M.W. (2011) Coral larvae settle at a higher frequency on red surfaces. *Coral Reefs*, **30**, 667–676.

McCook, L., Jompa, J. and Diaz-Pulido, G. (2014) Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs*, **19**, 400–417.

Meyer, J.L. and Schultz, E.T. (1985) Tissue condition and growth rate of corals associated with schooling fish. *Limnology and Oceanography*, **30**, 157–166.

Miller, M. and Hay, M. (1998) Effects of fish predation and seaweed competition on the survival and growth of corals. *Oecologia*, 231–238.

Miller MW, Marmet C, Cameron CM, and Williams DE. (In Press) Prevalence, consequences, and mitigation of fireworm predation on endangered staghorn coral. *Marine Ecology Progress Series*. DOI: 10.3354/meps10996

Mumby, P. (2006) The Impact of Exploiting Grazers (Scaridae) on the Dynamics of Caribbean Coral Reefs. *Ecological Applications*, **16**, 747–769.

Mumby, P.J. (2009) Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. *Coral Reefs*, **28**, 761–773.

Mumby, P.J., Hastings, A. and Edwards, H.J. (2007) Thresholds and the resilience of Caribbean coral reefs. *Nature*, **450**, 98–101.

Mumby, P.J. and Steneck, R.S. (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. *Trends in ecology & evolution*, **23**, 555–63.

Nugues, M.M., Smith, G.W., 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.

Omori, M., Fukami, H., Kobinata, H. and Hatta, M. (2001) Significant drop of fertilization of Acropora corals in 1999 : An after-effect of heavy coral bleaching ? *Limnology and Oceanography*, **46**, 704–706.

Palumbi, S. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, **25**, 547–572.

Pawlik, J., Steindler, L. and Henkel, T. (2007) Chemical warfare on coral reefs: Sponge metabolites differentially affect coral symbiosis in situ. *Limnology and Oceanography*, **52**, 907–911.

Raimondi, P. and Morse, A. (2000) The Consequences of Complex Larval Behavior in a Coral. *Ecology*, **81**, 3193–3211.

Randall, C. and Jordán-Garza, A. (2014) Relationships between the history of thermal stress and the relative risk of diseases of Caribbean corals. *Ecology*, **95**, 1981–1994.

Ritson-Williams, R., Paul, V.J., Arnold, S.N. and Steneck, R.S. (2009) Larval settlement preferences and post-settlement survival of the threatened Caribbean corals *Acropora palmata* and *A. cervicornis*. *Coral Reefs*, **29**, 71–81.

Rotjan, R.D. (2007) *The Patterns, Causes, and Consequences of Parrotfish Corallivory in Belize*.

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

Ruzicka, R., Colella, M., Porter, J., Morrison, J., Kidney, J., Brinkhuis, V., Lunz, K., Macaulay, K., Bartlett, L., Meyers, M. and Colee, J. (2013) Temporal changes in benthic assemblages on Florida Keys reefs 11 years after the 1997/1998 El Niño. *Marine Ecology Progress Series*, **489**, 125–141.

Sandin, S. a and McNamara, D.E. (2012) Spatial dynamics of benthic competition on coral reefs. *Oecologia*, **168**, 1079–90.

Soong, K. (1993) Colony size as a species character in massive reef corals. *Coral reefs*, **12**, 77–83.

Steneck, R. (1988) Herbivory on coral reefs: a synthesis. *Proc 6th Int Coral Reef Symp*, **1**, 37–49.

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**, 250–5.

Vega Thurber, R., Burkepile, D.E., Correa, A.M.S., Thurber, A.R., Shantz, A. a, Welsh, R., Pritchard, C. and Rosales, S. (2012) Macroalgae decrease growth and alter microbial community structure of the reef-building coral, Porites astreoides. *PloS one*, **7**, e44246.

Vega Thurber, R.L., Burkepile, D.E., Fuchs, C., Shantz, A. a, McMinds, R. and Zaneveld, J.R. (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global change biology*, **20**, 544–54.

Vermeij, M. (2005) Substrate composition and adult distribution determine recruitment patterns in a Caribbean brooding coral. *Marine Ecology Progress Series*, **295**, 123–133.

Vermeij, M., Fogarty, N. and Miller, M. (2006) Pelagic conditions affect larval behavior, survival, and settlement patterns in the Caribbean coral *Montastraea faveolata*. *Marine Ecology Progress Series*, **310**, 119–128.

Williams, D.E. & Miller, M.W. (2005) Coral disease outbreak : pattern , prevalence and transmission in *Acropora cervicornis*. , **301**, 119–128.

Wisshak, M., Schönberg, C.H.L., Form, a. & Freiwald, a. (2014) Sponge bioerosion accelerated by ocean acidification across species and latitudes? *Helgoland Marine Research*, **68**, 253–262.

Yakovleva, I., Baird, A., Yamamoto, H., Bhagooli, R., Nonaka, M. and Hidaka, M. (2009) Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. *Marine Ecology Progress Series*, **378**, 105–112.

Ecological Processes Workshop -- Identified Research Activities

Research Activity: Identification of site characteristics driving coral reef restoration success and failure along the Florida Reef Tract

Need
Coral reef restoration efforts via the outplanting of nursery-raised Acroporid corals is rapidly increasing along the Florida Reef Tract, resulting in thousands of corals outplanted in an attempt to restore coral reef communities. Observations suggest that outplanted corals perform better at some sites compared to others, influencing the magnitude and speed of coral reef community recovery. However, to date studies of restoration efforts have been on small spatial- and time scales, and typically focus on the survivorship and growth of transplanted corals. Consequently, there is an urgent need to understand the role of underlying ecological processes and site characteristics that contribute to optimal restoration sites to better inform coral reef restoration efforts.

Objective
Utilize existing coral outplant sites to quantify relevant ecological processes and site characteristics influencing coral reef community recovery and increase our understanding of the ecology of restored areas.

Expected Benefits
Current efforts to restore Florida reefs have relied largely on outplanting corals and have placed little emphasis on process-based strategies. Understanding the drivers behind the success and failure of the most common coral restoration approach in Florida is critical for the development of effective restoration practices. Results from this project can be directly applied by coral reef restoration practitioners to focus restoration efforts in areas with the highest chances of success. Furthermore, the identification of key processes and factors influencing coral reef restoration will allow the for the development of complimentary process-based restoration actions that can be undertaken to advance coral reef restoration beyond only outplanting of coral colonies.

Approach
Field studies should be undertaken that quantify important ecological processes (*e.g.*, herbivory levels, recruitment, benthic and fish community composition) and site characteristics (*e.g.*, sedimentation rates, temperature, etc.) at sites where coral colonies have been outplanted for restoration, with the goal of identifying key drivers of outplant success. Ideally, experiments will compare ecological processes across different scales, *e.g.*, within-reef (unrestored vs. restored areas), within-region (*e.g.*, Upper Keys) and between regions (*e.g.*, Upper Keys and Broward County) spanning the entire Florida reef tract. Where possible, survey design should also include a range of reef habitats and restored coral densities likely requiring collaborations among universities and research groups working throughout South Florida.

<u>Research Activity</u>: Characterizing recruitment habitat of corals to facilitate settlement of coral larvae and promote growth and survivorship of juvenile corals

Need

Recovery of coral reef ecosystems requires the replenishment of coral populations via coral recruitment. Coral recruitment can be limited by the settlement of coral larvae as well as the growth and survivorship of newly settled corals. Coral larvae frequently exhibit strong settlement preferences for specific microhabitats where they can experience increased growth and survivorship. Although several studies have demonstrated the ability of CCA to induce the settlement of coral larvae, the effects are not ubiquitous among CCA species. Important reef-building Acroporid species, in both the Indo-pacific and Caribbean, exhibit settlement preferences for certain CCA species. Based on these preferences, it is clear that coral larvae are capable of recognizing and discriminating among CCA species, yet little is known about which species of CCA facilitate larval settlement or their abundance and distribution across reef habitats, Further, there is little information regarding the settlement preferences of most Caribbean corals or the suitability of different microhabitats for their growth and survivorship immediately post-settlement. Thus, there is a need for research that identifies microhabitat characteristics that can enhance coral recruitment by increasing coral settlement and facilitating the early growth and survivorship of juvenile corals.

Objective

Studies should be undertaken to evaluate how microhabitat characteristics of natural or artificial substrate can be manipulated to facilitate coral settlement and early post-settlement growth and survivorship. Additionally, studies should evaluate how specific species of CCA facilitate/impede coral larval settlement and whether CCA species assemblages can be manipulated to enhance the abundance of facilitative species.

Expected Benefit

Reduced populations of adult corals are likely to result in diminished availability of coral larvae and concomitant reductions in coral settlement. In addition, newly settled corals are especially vulnerable to predation, incidental mortality by grazers, sedimentation, and overgrowth by other benthic organisms during their early juvenile stage. Thus, reduced settlement and early survivorship of corals could be an important bottleneck limiting the recovery of corals on Florida's reefs. Developing methods that can enhance coral recruitment by facilitating the settlement and/or early post-settlement survivorship of reef-building corals could aid restoration efforts by helping to eliminate a key bottleneck that can limit coral recovery.

Approach

Experimental studies should be conducted to manipulate characteristics of natural or artificial substrate to increase coral settlement and/or facilitate early growth and survivorship of juvenile corals. Experiments could alter the biological, chemical, or physical environment of potential settlement habitat and quantify the impact on coral settlement and early growth and survivorship. Manipulations may include changes in the rugosity, texture, orientation, or location of potential settlement habitat, removal of potential competitors of corals such as macroalgae or invertebrates, or addition of benthic organisms or chemical cues known to facilitate corals. Field-based studies will be necessary to determine the distribution and abundance of CCA species in

different habitats along the Florida reef tract. Laboratory settlement assays with coral larvae may be necessary to discern their preferences for different CCA species and for biofilms and bacteria present on different species of CCA. An important aspect of these manipulations is the potential for them to be used in a restoration context. Thus, while settlement preferences could be studied in a laboratory setting, the ultimate consequences of settlement choices for the growth and survivorship of juvenile corals needs to be evaluated in the field.

<u>Research Activity</u>: Herbivory manipulations to facilitate algal removal and create coral-friendly habitat

Need

Grazing by herbivorous fishes and invertebrates is a key ecosystem process on coral reefs. When grazing rates are reduced due to overfishing or disease, or spatially diluted by reductions in coral cover, benthic algal communities transform from those dominated by crustose coralline algae and closely-cropped filamentous algae that are beneficial or benign for corals to communities dominated by upright macroalgae that can negatively affect corals at all life stages (*e.g.*, larvae, juveniles, adults). Yet, even on many reefs with robust herbivore populations (mostly fishes) grazing pressure is often too diffuse, especially when coral cover is low, to remove abundant macroalgae and promote coral settlement and growth. Thus, there is a need for research examining how local interventions may be able to facilitate local increases in the rates or quality of herbivory thereby facilitating macroalgal removal and creating positive feedbacks on coral recruitment and growth.

Objective

Experimental field studies should be undertaken to evaluate how levels of herbivory can be manipulated in order to reduce algal abundance and create habitat that is known to facilitate coral settlement, growth, and health.

Expected Benefit

Given that herbivory is one of the most important processes for creating reef environments where corals can thrive, it is critical for coral reef restoration efforts to manipulate levels of herbivory in order to help facilitate coral-friendly environments as part of a restoration strategy. Developing methods to manipulate the distribution and concentration of herbivory in conjunction with coral transplantation will increase the chances of success of reef restoration efforts.

Approach

Experimental field studies should be done to affect the spatial concentration and/or quality of herbivory and track the impact to the benthic community. Manipulations may include herbivory by fishes, urchins, or mesograzers (*e.g.*, crabs, snails). Experiments could target concentrating grazing pressure by increasing herbivore density, manipulating available space for grazing, removing competitors of corals such as macroalgae or invertebrates, introducing beneficial shelter for herbivores, etc. Key reef responses to herbivory enhancement such as (but not limited to) benthic community changes, coral recruitment, or quality of coral recruitment habitat must be examined.

<u>Research Activity</u>: Importance of structure and live coral cover in coral reef fish recruitment and community development

Need

Reef fish are heavily dependent upon coral reefs for food, shelter and the successful completion of life cycles. Similarly, fish-dependent processes can have profound impacts on coral reef community structure and function. Fish are fundamental components of many ecological processes on coral reefs, such as providing connectivity between adjacent systems via diurnal feeding habits and influencing benthic community structure via grazing. Most reef fish species have specific microhabitat requirements and many are highly dependent on a narrow suite of coral species or coral morphologies for shelter and reproduction sites. Since one of the major goals of outplanting corals is to restore structural complexity to reef sites to promote the recruitment of fishes, understanding the relative importance and interaction between topographic complexity, coral outplants, and coral species identity on the recruitment of ecologically important fishes is important.

Objective

Field experiments should be undertaken to elucidate the relative importance and interaction of structural complexity, coral outplant density (or arrangement) and coral identity in the recruitment and development of fish communities, and how these communities affect key ecological processes on coral reefs.

Expected Benefits

Topographic complexity and coral cover influence the recruitment, abundance and composition of fishes on coral reefs, which ultimately determines the ability of fishes to contribute to key ecological processes such as herbivory. Outplanting coral colonies provides the opportunity to manipulate colony density, arrangement, and identity and incorporate supplementary restoration actions such as the addition of artificial structures. Developing methods to enhance fish recruitment and the ecological processes fishes influence via outplanting corals in conjunction with natural or artificial structures could aid restoration efforts by promoting key ecosystems components that support reef recovery.

Approach

Field experiments should be undertaken manipulating structural complexity, coral colony density, arrangement and/or identity to examine response variables related to fish recruitment and community composition. Experiments could utilize natural variations in topography or incorporate artificial structures with coral outplanting to test for enhancement of the recruitment of important functional groups of fishes (*e.g.*, herbivores). Ideally, projects will examine one or several fish-dependent positive feedbacks that could enhance coral reef recovery (*e.g.*, nutrient cycling, grazing, predation, etc.). Ultimately, these projects will be able to determine outplanting or restoration approaches that are most effective in recruiting fishes that support ecological processes key to coral reef recovery.

<u>Research Activity</u>: Determinants of Bioerosion on Florida Reefs

Need

Positive reef accretion can only be maintained when the growth of calcifying organisms exceeds the rates of reef erosion. With the decline in scleractinian coral populations in recent decades, Florida's reefs are now eroding faster than they are growing, which is causing the net loss of reef structure. There is a critical need to understand the biotic and abiotic drivers on reef erosion both now and under increasingly acidic conditions expected in the future and to identify which taxa are most important bioeroders on Florida's reefs. Perhaps most critically, understanding the role that reef herbivores play in bioerosion is vital. These herbivores (especially parrotfishes and *Diadema* urchins) are important to recovery of corals, and consequently have been focus of both coral reef restoration theory and in manipulative reef restoration studies. However, these species are also potential bioeroders. A better understanding of the tradeoff between enhanced herbivory and bioerosion is needed before large-scale restoration efforts that manipulate these species are implemented. By better understanding the ecological interactions that modulate reef erosion we may find ways to slow, or even reverse reef erosion in the future.

Objectives

Identify the biotic and abiotic drivers of bioerosion across the Florida reef tract. Examine potential restoration actions that can impede or reverse bioerosion (*e.g.*, promoting CCA growth).

Expected Benefits

The maintenance and restoration of reef structure should be a central goal of coral-reef restoration. Coral restoration efforts may increase community calcification, but this is only one half of the accretion equation. By better understanding the natural variability in bioerosion along the Florida reef tract and the biotic and abiotic processes driving these rates could allow the identification of methods to slow or reverse bioerosion.

Approach

A study should be undertaken to examine the existing information of long-term bioerosion along the Florida reef tract. Small-scale experimental assays (*e.g.*, measuring erosion using coral tiles) should be developed to relate patterns of bioerosion to ecological characteristics of the reefs (*e.g.*, percent cover of important benthic organisms, herbivore abundance and identity, etc.) Studies should be conducted to measure bioerosion rates with regard to different abundant benthic species (*e.g.*, CCA. macroalgae, sponges, and zonanthids). Additionally focused, small-scale manipulative experiments should be conducted to investigate the ecological tradeoffs between herbivory and bioerosion.

Research Activity: Enhancing coral propagule supply and viability

Need

The multifaceted process of coral replenishment includes several bottlenecks, one of which is the supply of viable coral propagules (*i.e.*, gametes, planktonic larvae, and/or settlers) to a reef. As adult coral population density (both at the colony and the genet levels) has declined, the processes orchestrating spawning synchrony and successful fertilization also likely decline in effectiveness. The logistic difficulties of research on spawning species (*i.e.*, spawning/larval availability limited to a few nights per year) further challenges advancing knowledge of these processes. There is a need for research to address both the logistic and ecological aspects of propagule supply in order for restoration actions to more effectively foster coral population replenishment.

Objective

Develop and test ecological and/or technological approaches to improve the availability and viability of coral sexual propagules.

Expected Benefits

From a research perspective, improvements in propagule supply, including potential improved technologies such as spawning induction or cryopreservation, would provide vastly expanded scope and efficiency to advance and test ecological-scale interventions. Meanwhile, viable coral propagules are the bottom-line determinants of a self-sustaining, coral reef ecosystem.

Approach

A combination of field and laboratory studies should be undertaken to investigate methods to increase spawning effectiveness, which may include manipulations via staging adult populations at certain locations or induced spawning. Additionally, studies should test the success of concentrating viable propagules in habitats or conditions where they can successfully settle and survive, (*e.g.*, tenting).

Chapter 3. Coral Traits Workshop

Introduction

Extensive effort has been directed at growing and propagation of predominantly *Acropora* spp. corals within *in situ* nurseries for eventual outplanting onto the south Florida reef tract. There is a growing body of information evaluating the performance (*i.e.*, survival and growth) of coral colonies under a range of environmental conditions. Although this work has demonstrated measurable differences in genet-specific performance, information remains limited regarding the specific traits associated with that variation. The third workshop recommended by the Steering Committee focused on coral traits. The goal of this workshop focused on providing guidance and research activities to better understand the range of coral functional traits (*e.g.*, coral host/zooxanthellae dynamics, physiology, disease resistance, growth rates, etc.) and their potential interactions that could aid the performance of coral culture and restocking efforts.

The coral traits workshop was held at NOAA's Atlantic Oceanographic and Meteorological Laboratory on October 25-26, 2014. Table 6 lists the workshop attendees and their affiliation. Mark Ladd of Florida International University drafted a report summarizing the current state of knowledge regarding coral traits as they relate to coral reef ecosystem restoration. His report is below.

Table 6. List of Coral Traits Workshop attendees

Name	Title	Affiliation
John Hunt	Program Administrator	Florida Fish &Wildlife Conservation Commission
William Sharp	Assoc. Research Scientist	Florida Fish &Wildlife Conservation Commission
Margaret Miller	Ecologist	NOAA Southeast Fisheries Science Center
Deron Burkepile	Assistant Professor	Florida International University
David Gilliam	Associate Professor	Nova Southeastern University
Diego Lirman	Senior Research Associate	University of Miami
William Fitt	Professor	University of Georgia
Andrew Baker	Associate Professor	University of Miami
John Parkinson	Post-Doctoral Scholar	Penn State University
Mikhail Matz	Associate Professor	University of Texas
Ian Enochs	Assistant Scientist	NOAA
Elizabeth Larson	PhD Student	Nova Southeastern University
Kerry Maxwell	Research Associate	Florida Fish &Wildlife Conservation Commission
Alison Johnson	Biological Scientist	Florida Fish &Wildlife Conservation Commission
Brian Reckenbeil	Biological Scientist	Florida Fish &Wildlife Conservation Commission
Mary Truglio	Wildlife Legacy Biologist	Florida Fish &Wildlife Conservation Commission

Report: The importance of genetic diversity and coral traits for the restoration of coral reef ecosystems

Introduction

Significant effort has been directed at restoring depleted coral populations, in particular *Acropora cervicornis*, on reefs throughout the Florida reef tract using colonies propagated in underwater coral nurseries. Results from monitoring corals within these nurseries and outplanted colonies suggest that genet-specific differences in survival and colony performance exist for key functional traits (*e.g.*, growth rate, disease- and thermal-resistance). Yet, there has not been a large-scale, coordinated effort to systematically identify these genet-specific coral traits so that they can be incorporated into coral reef restoration strategies.

While the role of genetic identity, phenotypic expression and how they manifest in coral traits has received some attention by coral reef researchers, this information is rarely included in coral restoration outplant design and strategy (Baums 2008). There is an urgent need for studies that track and specifically tie coral outplant success to coral traits or differential performance by genotypes in order to incorporate this information into coral outplanting strategies. Research that uses common garden and reciprocal transplant experiments should be pursued to identify genotype-specific traits, differences in performance, as well as the heritability of traits (Baums 2008). For example, identifying genotypes that are resistant to disease or thermal stress can improve coral colony selection for transplant strategies and increase the probability of restoration success.

Sets of coral traits likely integral to successful reef restoration by outplanting coral colonies were identified trough a review of relevant coral reef studies focused on the role of genotype and phenotype in coral performance and a two-day workshop discussion among a group of Caribbean coral reef experts. Along with sets of coral traits, the attendees developed a suite of research activities that should be undertaken to address important information gaps. This document provides a brief introduction to the importance of genotype- and phenotype-based traits in the maintenance and survival of populations, with a specific focus on the role of coral traits on coral success in the context of coral reef restoration. First, key sets of coral traits are discussed with examples from published literature, followed by those research activities identified by coral reef experts as priorities for advancing our knowledge of coral traits and the role they can play in coral reef restoration efforts.

Background

Differences in individual performance due to genetic variation are a critical attribute for populations to resist, recover from or adapt to disturbances and changes, especially in the face of climate change. Genetic diversity has been demonstrated to enhance a multitude of processes and functions in a range of populations and entire communities. For example, populations of the green algae *Chlamydomonas reinhardtii* with high genetic diversity demonstrated higher productivity compared to populations composed of single genotype (Bell 1991). Schmitt and Antonovics (1986) found that stands of the grass species *Anthoxanthum odoratum* with high

genetic diversity had less damage from predatory aphids compared to low genetic diversity stands. Similarly, genetically diverse seagrass stands have demonstrated increased resistance to disturbance by grazing geese (Hughes & Stachowicz 2004). The effects of increased plant population genotypic diversity can transcend trophic levels. By influencing primary productivity, genetically diverse plant populations can support an increase in both herbivore and predator arthropod species richness (Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006). Clearly, genetically-based differences in individual performance play an important role in the provisioning and maintenance of community structure and ecological processes.

In the Caribbean, a number of studies have demonstrated the existence of a range of coral traits and variable performance by genotypes within the same species. For example, in Puerto Rico Griffin *et al.* (2012) found that mean linear extension rates of six different genotypes of *A. cervicornis* ranged two-fold, from a low linear extension rate of 40.3 cm/year to a more than double high of 90.2 cm/yr, exemplifying how genotypic differences can manifest in key coral traits such as growth. Baums *et al.* (2013) identified differences in sperm morphology, egg size and gamete compatibility between different genotypes of *Acropora palmata.* Furthermore, *A. palmata* larvae have demonstrated variable gene expression and subsequent success in response the thermal stress (Polato, Altman & Baums 2013). Sets of coral traits such as these (growth, stressor tolerance and reproductive ability) could be instrumental in advancing coral reef restoration approaches. By identifying coral traits and genotypes that perform best under different conditions, restoration efforts can inform colony genotype selection based on site characteristics to maximize the probability of restoration success.

Coral Traits: Growth

Traits related to coral growth have obvious implications for restoration potential. There is abundant evidence demonstrating that different genotypes of *A. cervicornis*, the primary coral for reef restoration in Florida, grow at different rates (*e.g.*, Johnson *et al.* 2011; Griffin *et al.* 2012; Lirman *et al.* 2014). Genotypes that can to grow quickly under variable conditions may present ideal restoration genotypes, as they can rapidly increase coral cover and spread asexually through fragmentation. Branching rates can also differ between genotypes. For structurally complex branching species such as *A. cervicornis* and *A. palmata*, genotypes with higher branching rates may afford a higher level of structural complexity and enhance shelter and refuge availability for organisms with important ecological roles (*e.g.*, herbivores) (Mumby & Steneck 2008; Lirman *et al.* 2014).

However, there is a paucity of research investigating the tradeoffs between high growth rates and other important coral traits. Skeletal density and strength are two additional growth traits that could ultimately influence coral outplant success. For example, fast growing corals with low skeletal density and strength may fragment more easily. This could potentially benefit restoration efforts if frequent fragmentation increases asexual reproduction, or may result in increased mortality through breakage and subsequent death. Conversely, genotypes with high skeletal density and strength may be better candidates for outplanting in high wave energy sites to decrease tissue loss and mortality from breakage. Recent research conducted at sites throughout the Caribbean has identified location-specific differences in tissue biomass of *A. palmata* and *Montastraea* spp., also finding that corals with higher tissue biomass also had higher

zooxanthellae densities. These higher biomass and zooxanthellae density species demonstrated lower mortality during thermal extremes compared to species like *A. cervicornis*, which had lower tissue biomass and zooxanthellae densities (Fitt, personal communication).

Coral Traits: Stress Resistance

Populations with intraspecific genetic variability contain some individuals that are better adapted to resisting certain stressors (*e.g.*, thermal stress or disease), while other individuals may excel in other areas such as growth or reproductive output. Anecdotal reports throughout the Florida reef tract suggest that certain individuals within restored *A. cervicornis* populations are less affected by thermal stress events than significantly impacted or killed conspecific neighbors (*e.g.*, the summer 2014 bleaching event). Identification of genets that can withstand stressors impacting restored reefs could help inform coral propagation priorities.

Polato *et al.* (2013) found that different genotypes of *A. palmata* larvae demonstrated variable expression of key stress response genes when exposed to thermal stress, suggesting that some genotypes may be better suited to dealing with temperature extremes than others. If these differences represent heritable traits, these results could help to identify genets that could be utilized by coral restoration practitioners for areas more likely to be subjected to thermal stress events.

A number of studies have documented differential gene expression by individual corals when exposed to stressors (*e.g.*, Bellantuono *et al.* 2012; Libro, Kaluziak & Vollmer 2013). Therefore, it is reasonable to expect that some genotypes may be better adapted to respond to, and deal with, specific stressors better than other genotypes. However, the overwhelming majority of these studies utilized few or only one genotype in experimental designs, and have focused nearly exclusively on thermal stress, likely because temperature is a relatively simple factor to control in experiments. For example, DeSalvo *et al.* (2008) investigated gene expression profiles of *Orbicella faveolata* colonies when subjected to thermal stress. However, this study was conducted with a single genotype of *O. faveolata*. Studies such as this should be expanded to include a range of known genotypes to elucidate genetic-based differences in response and resistance to thermal stress.

Vollmer and Kline (2008) provide a prime example of one such study. They investigated white band disease (WBD) transmission in a number of *A. cervicornis* genotypes to determine if differences in disease resistance existed, finding that 3 of 49 genotypes surveyed were resistant to WBD. Further, Libro *et al*. (2013) determined that the coral host, not algal symbionts, drove *A. cervicornis* response to WBD. Studies such as this provide invaluable information to coral reef restoration practitioners, as disease-resistant genotypes have clear advantages for restoration purposes in disease-prone areas over genotypes more likely to die from potential disease outbreaks.

Characterizing genotypic differences in response and resistance to pervasive stressors impacting Florida reefs could greatly benefit coral outplant strategies. There is an urgent need for research that systematically explores genotypic differences in response to common stressors such as: thermal extremes, disease, predation, sedimentation, bioerosion, ocean acidification and

competition. All of these stressors have been demonstrated to significantly negatively impact corals throughout the Florida reef tract. Since totally eliminating these stressors is unrealistic, finding genotypes that can survive, and even thrive, under these less than ideal conditions would allow restoration efforts to greatly enhance their effectiveness and expand the areas that can be successfully targeted for coral restoration.

Reproduction

The ultimate goal of coral reef restoration is to restore viable, self-sustaining coral populations that can provide the foundation upon which the rest of the coral reef community depends. Therefore, it is important to understand genetic differences in reproductive success and the heritability of coral traits. Identification of genotypes that demonstrate high fecundity and/or can contribute to reproductive events quickly (likely based on both colony size and age) would further assist in identifying genotypes that should be focused on for cultivation and outplanting.

Research has documented genetic differences in the reproductive potential within Caribbean coral species, finding that some offspring are more likely to succeed than others (Baums 2008; Baums *et al.* 2013). Baums (2008) documented differential performance of *Montastraea faveolata* larvae depending on parental identity. This corroborates with results demonstrating among colony differences in sperm morphology, swimming speeds and energy depletion rate of coral larvae, which can have significant consequences for population connectivity and ultimately the probability of recruitment success (Baums *et al.* 2013). Further, Baums *et al.* (2013) experimentally demonstrated increased fertilization rates with a higher diversity of parental gametes (density held constant), highlighting the importance of maintaining genetic diversity within coral populations. Spawning synchrony, *i.e.*, the timing of gamete release by reproductive conspecifics, can largely affect fertilization rates (Ritson-Williams *et al.* 2009). Outplanting different genotypes in close proximity that are known to spawn at the same time could increase the probability of successful fertilization and the production of novel genotypes to seed Florida reefs.

Asexual fragmentation and rapid reattachment to the substrate is one of the most common mechanisms for *A. cervicornis* to naturally propagate and spread (Tunnicliffe 1981). Research is needed to understand if, and why, some genotypes are more apt to fragment and spread (*e.g.*, low skeletal density). As part of this research, quantifying tradeoffs in growth and survival across a range of environments associated with frequent fragmentation will be important.

There is also a need for research in spawning timing between genotypes and if there are genotype-specific traits regarding time and/or age when colonies become reproductive. Due to the longer time-scale that such experiments require to produce results (several years), tracking a subset of colonies with known genotypes that have already been outplanted to see at what age and size they become reproductive would provide useful data. This information could be used to inform the size at which colonies are outplanted to decrease the time need to reach a reproductive status. Following colonies of the same genotypes at a range of sites and environmental conditions could allow for the identification of “spawning sites”, or areas that have ideal conditions for specific genotypes and allow colonies to reach reproductive status more rapidly.

Symbiodinium identity, performance and importance

Although differential performance and expression traits that are determined solely by the coral host represent critical knowledge gaps, the influence of the entire the coral holobiont on restoration potential; *i.e*., the combined community of the coral host, prokaryotic members, viruses and photosynthetic dinoflagellates, must be taken into account. *Symbiodinium*, the genus of photosynthetic endosymbiotic dinoflagellates that provide reef building corals with a significant portion of their energy, is comprised of a number of distinct groups know as clades. The *Symbiodinium* community of a single coral colony can be comprised of a mixture of clades (Baker 2001; Little, Oppen & Willis 2004), with specific clades commonly more prevalent in specific environments or in the presence of certain abiotic characteristics (*e.g.*, depth and light). For example, in *Montastraea* sp. it was found that *Symbiodinium* from clades A, B and D dominated corals in shallow waters, while clade C was found in corals at deeper depths (Rowan & Knowlton 1995; Baker 2003). Research has identified *Symbiodinium* clades that can contribute to, or detract from, resistance to stressors such as thermal extremes (Baker *et al.* 2004; Jones & Berkelmans 2010).

It has been suggested that corals can acquire stress tolerance by changes in the relative abundance of the *Symbiodinium* clades they host. One pathway by which *Symbiodinium* community composition can change is symbiont "switching", which involves expelling symbionts followed by the acquisition of new symbionts from the water column (Baker 2003). Alternatively, under the "shuffling hypothesis", *Symbiodinium* composition within in a coral shifts towards dominance by a more beneficial (under the new conditions) clade after exposure to a stressful event (Buddemeier & Fautin 1993; Baker 2003). Baker (2001) conducted reciprocal transplants between shallow and deep habitats and found that corals transplanted to shallow environments bleached and changed *Symbiodinium* composition, suggesting that bleaching can act as a mechanism to promote symbiont community composition shifts. Such shifts have also been observed after thermal stress events, representing a possible pathway by which individuals can increase the probability of surviving such events by acclimating to stressful conditions (Baker 2001; Baker *et al.* 2004; Cantin *et al.* 2009). However, Cantin *et al.* (2009) found that thermo-tolerant *Symbiodinium* provided less metabolic utility, therefore presenting a tradeoff of hosting thermally tolerant photosynthetic symbionts for lower metabolic inputs. These results are corroborated by Little at al. (2004), who found that juvenile *Acropora* spp. colonies hosting thermally-intolerant clade C *Symbiodinium* had 2-3x faster polyp budding than juveniles hosting thermally-tolerant clade D symbionts. Furthermore, Jones and Berkelmans (2010) observed that adults with clade D symbionts grew 29% and 28% (in the lab and field, respectively) more slowly than conspecifics hosting clade C symbionts.

A growing body of work suggests that clade D *Symbiodinium* are a more stress tolerant clade (Baker *et al.* 2004). They are one of the most commonly found clades in corals living under stressful conditions (reviewed in Baker 2003), and are more common in coral colonies recovering from thermal-induce bleaching (Baker 2001) and bleaching from disease (Toller, Rowan & Knowlton 2001).

More research is needed to understand several fundamental aspects of the role of *Symbiodinium* on coral performance and survival. Specifically, research that addresses the following questions would be of particular use in a restoration context:
(1) Are certain coral genotypes more likely to host or switch/shuffle to acquire symbionts that afford increased stress resistance? (2) Can nursery-raised corals be pre-conditioned or inoculated to possess thermal-, disease- or other stressor-tolerant *Symbiodinium* communities? (3) How do environmental conditions influence these interactions? Under and experimental framework, studies should track *Symbiodinium* composition and relate it to overall colony performance and stress resistance. Quantifying multiple traits (*e.g.*, growth, branching, etc.) along with stressor resistance will allow the quantification of tradeoffs associated with hosting distinct symbionts communities and could be done in conjunction with the approaches previously suggested in this document. Approaches should be developed to better understand how *A. cervicornis* genotypes are able to acclimatize via *Symbiodinium* shifts after a stressful event to be able to utilize this information in a restoration context.

Important considerations

The importance of each set of traits and individual traits discussed in this document will be context- and goal-dependent. It is apparent that a variety of tradeoffs between coral traits likely exist. Therefore, it is important to gather information on as many traits as possible to understand what tradeoffs exist and where certain genotypes are most likely to succeed, or under what conditions specific traits may be most beneficial.

There is a need to clearly define restoration "success" at the beginning of experiments. Success in coral restoration has been traditionally defined as colony survival, but this does not necessarily translate into the successful restoration of a reef community. Other possible measures of restoration success include reproductive output, recruitment or change in community and ecosystem structure or processes (*e.g.*, fish populations or herbivory rates). Defining success in these or other well thought out terms will allow better assessment of coral reef restoration efficacy.

Restoration approaches need to be planned with climate change in mind. Thermally induced bleaching is predicted to increase in severity in coming decades (Hoegh-Guldberg 1999). Therefore, there it is essential that we identify thermally resistant genets and/or mechanisms (*e.g.*, symbiont shuffling or switching) that allow restored corals to survive under stressful conditions. The high variability of sites within the Florida reef tract presents an ideal setting to test these questions. However, it is important to not only focus on fast growing and bleaching resistant genotypes. It is imperative to promote and maintain genetic diversity as much as possible for future, unknown conditions. Many genets that currently are not "top performers" may have hidden traits that aren't apparent under current conditions, but will be important in the future.

Experiments assessing the role of genetic diversity in affecting coral fitness and the ability to adapt to climatic changes highlight the importance of genetic diversity in the survival of coral populations in the face of global climate change. Consequently, it remains important to maintain genetic diversity as part of a comprehensive restoration strategy. Nevertheless, determining

whether coral genets can be environmental specialists (those that perform well only in certain environments) or generalists (those that perform well across a broad range of environments) is critical for development of more efficient coral reef restoration practices. An enhanced understanding of a full range of coral functional traits will improve both coral culture within nurseries and restocking efforts undertaken as part of a coral reef restoration effort.

References

Baker, A.C. (2001) Reef corals bleach to survive change. *Nature*, **411**, 765–6.

Baker, A.C. (2003) Flexibility and specificifity in coral-algal symbiosis: Diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 661–689.

Baker, A.C., Starger, C., McClanahan, T. and Glynn, P. (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741.

Baums, I.B. (2008) A restoration genetics guide for coral reef conservation. *Molecular ecology*, **17**, 2796–811.

Baums, I.B., Devlin-Durante, M.K., Polato, N.R., Xu, D., Giri, S., Altman, N.S., Ruiz, D., Parkinson, J.E. and Boulay, J.N. (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral, *Acropora palmata*. *Coral Reefs*, **32**, 703–717.

Bell, G. (1991) The Ecology and genetics of fitness in *Chlamydomonas* .IV. The properties of mixtures of genotypes of the same species. *Evolution*, **45**, 1036–1046.

Bellantuono, A.J., Granados-Cifuentes, C., Miller, D.J., Hoegh-Guldberg, O. and Rodriguez-Lanetty, M. (2012) Coral thermal tolerance: Tuning gene expression to resist thermal stress. *PloS ONE*, **7**, e50685.

Buddemeier, R. & Fautin, D. (1993) Coral bleaching as an adaptive mechanism. *Bioscience*, **43**, 320–326.

Cantin, N.E., van Oppen, M.J.H., Willis, B.L., Mieog, J.C. and Negri, a. P. (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs*, **28**, 405–414.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. and Sanders, N.J. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, **313**, 966–8.

DeSalvo, M.K., Voolstra, C.R., Sunagawa, S., Schwarz, J.A., Stillman, J.H., Coffroth, M.A., Szmant, A.M. and Medina, M. (2008) Differential gene expression during thermal stress

and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, **17**, 3952–71.

Griffin, S., Spathias, H. and Moore, T. (2012) Scaling up Acropora nurseries in the Caribbean and improving techniques. *Proceedings of the 12th International Coral Reef Symposium*, 9–13.

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**, 839–866.

Hughes, A.R. and Stachowicz, J.J. (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *PNAS*, **101**, 8998–9002.

Johnson, M.E., Key, S., Bartels, E., Beach, D. and Larson, L. (2011) *Caribbean Acropora Restoration Guide*.

Johnson, M.T.J., Lajeunesse, M.J. and Agrawal, A.A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters*, **9**, 24–34.

Jones, A. & Berkelmans, R. (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PloS ONE*, **5**, e10437.

Libro, S., Kaluziak, S. and Vollmer, S. (2013) RNA-seq profiles of immune related genes in the Staghorn coral *Acropora cervicornis* infected with White Band Disease. *PloS ONE*, **8**, e81821.

Lirman, D., Schopmeyer, S., Galvan, V., Drury, C., Baker, A.C. and Baums, I.B. (2014) Growth dynamics of the threatened Caribbean staghorn coral *Acropora cervicornis:* Influence of host genotype, symbiont identity, colony size, and environmental setting. *PloS ONE*, **9**, e107253.

Little, A., Oppen, M. Van and Willis, B. (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science*, **304**, 1492–1494.

Mumby, P.J. & Steneck, R.S. (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. *Trends in Ecology & Evolution*, **23**, 555–63.

Polato, N.R., Altman, N.S. and Baums, I.B. (2013) Variation in the transcriptional response of threatened coral larvae to elevated temperatures. *Molecular Ecology*, **22**, 1366–82.

Ritson-Williams, R., Paul, V.J., Arnold, S.N. and Steneck, R.S. (2009) Larval settlement preferences and post-settlement survival of the threatened Caribbean corals *Acropora palmata* and *A. cervicornis*. *Coral Reefs*, **29**, 71–81.

Rowan, R. and Knowlton, N. (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *PNAS*, **92**, 2850–2853.

Schmitt, J. and Antonovics, J. (1986) Experimental studies of the evolutionary significance of sexual reproduction .IV. Effect of neighbor relatedness and aphid infestation on seedling performance. *Evolution*, **40**, 830–836.

Toller, W., Rowan, R. and Knowlton, N. (2001) Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *The Biological Bulletin*, 360–373.

Tunnicliffe, V. (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. *PNAS*, **78**, 2427–2431.

Vollmer, S. V and Kline, D.I. (2008) Natural disease resistance in threatened staghorn corals. *PloS ONE*, **3**, e3718.

Coral Traits Workshop -- Identified Research Activities

<u>Research Activity</u>: Enhanced data collection on corals propagated and grown within *in situ* coral nurseries in south Florida to evaluate heritable traits

Need

Extensive effort has been directed at restoring depleted coral populations, primarily staghorn coral (*Acropora* spp.), on degraded coral reefs using colonies propagated within *in situ* nurseries located along the South Florida reef tract. A substantial body of information (much of it anecdotal) on the performance of individual coral genets has accumulated, suggesting that there are differences in the functional traits (*e.g.*, growth rate, disease resistance) among *Acropora* spp. genotypes. Still, many important traits related to coral fitness have not yet been systematically measured across nurseries. A rigorous evaluation of among-genet variation in fitness-related traits and between-trait interactions (reinforcements or tradeoffs) is needed to better predict coral performance in a reef restoration context under current and expected future South Florida reef environments.

Objective

Initiate enhanced and coordinated data collection across *in situ* coral nurseries to collect genet-specific fitness-related information to better evaluate the heritability of fitness traits and their potential interactions that could affect coral performance in a coral reef restoration context.

Expected Benefits

Current efforts to restore Florida reefs have relied largely on outplanting genotypically diverse coral colonies propagated and grown within *in situ* nurseries, but with little understanding of the genet-specific traits that potentially affect coral performance. *A posteriori* measurements of outplant success, largely limited to genet-specific survival and growth rates, have to date added relatively little to our understanding of the heritable traits responsible for coral outplanting success. An enhanced understanding of a full range of coral functional traits across several 'common gardens' represented by individual nursery sites will improve both coral culture within nurseries and restocking efforts undertaken as part of a coral reef restoration effort.

Approach

An expanded evaluation of the heritable traits of the *Acropora* spp. colonies maintained in the *in situ* coral nurseries located in south Florida (representing a range of 'distributed common gardens') should be conducted. Such an evaluation should entail a systematic monitoring of a suite of genet-specific traits across each nursery that could include host and *Symbiodinium* genotyping (down to individual clone level), growth rate (such as total linear extension or buoyant weight), skeletal density, tissue biomass, stress tolerance (particularly resistance to thermal extremes), disease resistance, photosynthetic performance, and fecundity. Concomitant environmental data (especially temperature and light) must also be collected. Data from these coordinated measurements should be synthesized across all the nurseries and evaluated for correlation among heritable traits.

<u>Research Activity</u>: A synthesis of the available data on *Acropora cervicornis* propagated and grown within *in situ* coral nurseries to evaluate heritable traits

Need

Extensive effort has been directed at restoring depleted coral populations, primarily staghorn coral (*Acropora cervicornis*), on degraded coral reefs using colonies propagated within *in situ* nurseries along the South Florida reef tract. Through monitoring efforts and observation within these nurseries a substantial body of information on the performance of individual coral genets has accumulated, suggesting that there are differences in the functional traits (*e.g.*, growth rate, growth pattern, disease resistance) among *A. cervicornis* genotypes. This genetically determined variation in traits related to coral fitness has clear implications for successful artificial propagation of this species and re-establishment of an ecologically functional population of *A. cervicornis* along the South Florida's reef tract. Although these findings are intriguing, a comprehensive quantitative analysis of genetically determined variation in coral functional traits across different nurseries has yet to be undertaken and is vital if we are to maximize coral restoration success.

Objective

Compile, analyze, and summarize the available information on *Acropora cervicornis* genets propagated in coral nurseries in south Florida to evaluate the extent of heritable variation in coral functional traits.

Expected Benefits

A comprehensive evaluation of the available data collected across the network of *in situ* coral nurseries will yield vital baseline information on the variation in *A. cervicornis* functional traits from which to develop an optimal design plan for propagating and outplanting this species for restoration activities. Such information could improve coral culture and restocking efforts undertaken as part of a coral reef restoration effort and provides an initial basis for exploring the potential correlation or anti-correlation (*i.e.*, tradeoffs) between traits.

Approach

An evaluation of the functional traits of the *Acropora cervicornis* colonies maintained in the *in situ* coral nurseries located in south Florida should be conducted. This evaluation would entail a coordinated effort with the individual nursery practitioners to compile the available monitoring data that have been collected to date from the nurseries maintained by Nova Southeastern University, the University of Miami, the Coral Restoration Foundation, the Florida Fish and Wildlife Conservation Commission, Mote Marine Laboratory, and The Nature Conservancy. Once compiled, these data should be analyzed in a quantitative genetics statistical framework to characterize among-genet variation in functional traits. Depending on nursery-specific monitoring protocols, these functional traits could include survival, growth (*e.g.*, total linear extension or area), growth patterning (*e.g.*, branching rate), and resistance to thermal and/or disease-related stress. Rigorous meta-analysis strategies should be employed to synthesize this information across nurseries despite differences in the precise methods of raw data collection. If adequate data are available, it is also desirable to explore possible correlations or tradeoffs among genet-specific traits.

<u>Research Activity</u>: Determine differences in performance of outplanted corals based on nursery growth platforms

Need

Acropora spp. within *in situ* coral nurseries are being grown using several different platforms that include blocks, lines, and trees. Corals on different platforms are exposed to different environmental conditions (*i.e.*, different selection regimes). Block corals are attached to pedestals that are anchored onto a concrete block, which is in turn attached to the (usually sand) substrate. The tissue of these corals interact with sediments and benthic organisms found along the substrate, including benthic-associated microbes. However, corals grown on lines or trees do not interact with the substrate. Line and tree corals are attached to filamentous line and are suspending within the water column. Corals attached to the substrate must withstand different current, temperature, and light regimes compared with those suspended within the water column. Evidence suggests that the corals grown on blocks have slower extension rates than those grown suspended in the water column. Microbial communities of corals grown under the two methods also likely differ. Whether the corals grown on the lines and trees differ in performance after outplanting from those grown on blocks is currently unknown.

Objective

Determine whether performance (measured as growth, survival and/or other traits) of outplanted corals differs based on initial nursery platform.

Expected Benefits

Methods being used for the propagation of nursery corals are currently optimized for branch production within the nursery environment. However, these methods create corals that may be conditioned for different ecological and environmental scenarios than those they will be exposed to after outplanting onto a reef. Testing the traits and performance of the corals that are being propagated using different techniques will determine whether those that are grown using line or tree-based methods are comparative to the outplants that are grown on blocks.

Approach

Several field and laboratory manipulations can be applied to determine genet-specific differences in physiological state of corals grown on different nursery platforms. Photochemical efficiency, photosynthetic rate, respiration rate, calcification rate, skeletal density, and surface microbial communities can all be measured on a subset of the corals grown under block, line, and/or tree conditions. Representatives from each grow-out method should then be outplanted within replicate reefs and monitored through time to elucidate the impact of nursery growth platform on colony performance and survival.

<u>Research Activity</u>: Evaluation of heritable traits of nursery-cultured corals after outplanting to coral reef environments

Need

Current efforts to restore Florida reefs have relied largely on outplanting coral (primarily *Acropora cervicornis*) maintained in the network of *in situ* coral nurseries located along the south Florida reef tract. An emphasis has been placed on ensuring that the colonies within these stocks are genotypically diverse, and consequently there is great opportunity to collect information characterizing among-genet variation in functional traits across the environmental gradient where these nurseries are located. However, to maximize the success in establishing of an ecologically functional coral population it is necessary to understand how this genetically determined variation in functional traits translates into variation in fitness once the corals are transplanted out of the nurseries and into a variety of reef habitats. Although restoration efforts to date have placed an emphasis on outplanting genotypically diverse coral colonies at restoration sites, properly matching the genotypic composition of outplanted sub-populations to the types of reef environment that are most conducive to their survival could substantially improve the long-term success of these projects.

Objective

Experimental field studies should use outplanted nursery-propagated corals to evaluate how various reef environments modulate genet-specific functional traits. The spatial resolution of the environmental influence should be determined, as well as the plasticity of functional traits (which might vary among genets and hence be one of the criteria for selecting genotypes for outplanting).

Expected Benefits

Determining whether coral genets can be environmental specialists (best performers only in certain environments) or generalists (performing well across a broad range of environments) is critical for development of more efficient coral reef restoration practices. Results from this project can be directly applied by coral reef restoration practitioners to focus restoration efforts using coral genotypes in environments where they have the highest chance of success.

Approach

Field studies should be undertaken using *Acropora* spp. genets propagated within *in situ* nurseries to assess performance variation of potential heritable traits when the corals are outplanted across a range of reef sites. Ideally, these studies would involve outplanting and evaluating a representative sample of the genotypes maintained in all of the nurseries (*i.e.*, representing both high and low growth rates, thermal tolerance, etc) pooled at sites across a gradient of environmental conditions (*e.g.*, nearshore vs. offshore, degraded vs. healthy, high vs. low turbidity, high vs. low herbivory, etc.). Important metrics to be monitored could include growth, host/zooxanthellae/microbe dynamics, skeletal density, tissue biomass, photosynthetic performance, fecundity, and prevalence and impact of predation, bleaching, and disease. In addition, detailed habitat characterization of the outplant sites should be conducted and key environmental parameters collected. The resulting information, in concert with FWRI's *Acropora* resilience data, can then be used to create an Optimal Design and Site Selection Plan.

Appendix 1. The list of research activities identified by the Principal Investigators and the attendees of the Coral Diseases, Ecological Processes, and Coral Traits workshops

This list is provided as an appendix for the ease of FWLI staff to extract these research activities for their use in developing announcements. These research activities are presented in no particular order of priority.

<u>Research Activity</u>: Quantifying disease resistance and susceptibility of Florida *Acropora* spp. genets

Need

The degree of resilience to future disease outbreaks in remnant populations of *A. cervicornis* and *A. palmata* is a critical determinant of their recovery potential. Research from Panama, using simple transmission assays, indicates that ~6% of staghorn coral genotypes are disease resistant, but no similar information is available for any *Acropora* spp. genets in Florida. Transmission assays provide a cost-effective means of identifying disease resistant genets in both nursery stocks and in wild remnant populations. Identification of these resistant *Acropora* spp. coral genotypes could and should be used to inform on-going and future outplanting efforts and is a pre-requisite for further ecological and/or genomic studies to determine potential ecological tradeoffs of disease resistance needed to develop truly sustainable application of nursery restocking efforts, as well as molecular and physiological studies to elucidate functional mechanisms of disease resistance in *Acropora* spp.

Objective

Develop and apply standardized transmission assay criteria to assess the relative levels of disease resistance in nursery-stock genets of *Acropora* spp. to examine potential tradeoffs with other traits (*e.g.*, growth or other stress tolerance) and thereby improve outplanting success by focusing on resistant stocks.

Expected Benefits

Given the devastating effects of disease on *Acropora* spp. populations, the potential to increase disease resistance in restocked *Acropora* spp. populations may greatly increase the chances of these species' recovery. Data on disease resistance can be used to predict impacts of future resilience of these endangered coral populations in Florida as well as provide a starting point to more successfully investigate the functional mechanisms of disease and health in *Acropora* corals, which could lead to additional management tools such as effective disease prevention and/or treatment.

Approach

Grafting experiments (*i.e.*, an extant diseased 'inoculant' coral fragment placed in direct contact with the healthy 'target' fragment) should be applied in the field and/or laboratory (at the site of natural disease occurrence) to identify degrees of disease resistance of specific coral genets. Protocols to standardize and document the nature of the 'inoculant' disease, the time frame, and the specific parameters measured (*e.g.*, time to onset of disease signs, rate of tissue loss in infected target, etc.) will allow relative resistance to be compared among different nurseries and among nursery versus wild genets tested in different trials. Measures of disease performance should be correlated with coral performance (*e.g.*, growth rate, thermal tolerance, etc.). If tank-based transmission experiments are to be carried out, clear non-recirculating, biosecurity protocols need to be established to prevent potential spread of the disease.

<u>Research Activity</u>: Determine baseline disease dynamics in nurseries

Need

Tens of thousands of colonies of *A. cervicornis* and *A. palmata* are currently being propagated within *in situ* nurseries throughout the reefs of Florida. These corals are used as stock populations for the outplanting and restoration of the Florida Reef Tract. Since these corals are being grown within high density conditions, they may be more susceptible to disease outbreaks. To prevent outbreak conditions, nursery managers often employ a series of culling and mitigation methods to remove diseased individuals and limit transmission. These methods include colony isolation, pruning, banding and reduction of vector transmission and may reduce the probability of disease transmission to healthy individuals, but prevent an accurate assessment of the influence of disease within the nursery environment.

Objective

Compare disease prevalence within nursery populations that are actively culling diseased corals from nurseries to those that do not use culling practices. Compare disease prevalence in both nursery types to the wild population.

Expected Benefits

Corals grown within Florida's *Acropora* nurseries are the population stock being used for restoration of the species throughout the Florida Reef Tract. However, very little is known about the condition of the corals being used for outplanting as macroscopic characterizations of health provides minimal information on the physiology and microbial community that these corals harbor. Knowing to what extent disease affect the nursery stocks, among the different genotypes, and through time in the absence of intervention, will provide significant insight into the health status of the nursery population. Culling sick individuals may only mask the underlying influence of disease within the stock population. Understanding the effects of culling methods to reduce disease outbreaks will provide more informed health status information on the corals being used for propagation and restoration purposes and may enable improved health management within the nurseries as well.

Approach

Segregated nursery populations should be established and maintained under the current best practice scenarios used for *Acropora* spp. propagation and growth, except managers will refrain from culling or other active health management. Although this may yield higher disease toll in this nursery population, it will provide managers and scientists a better understanding of the influence of disease on corals being used for outplanting purposes and enhance understanding of genet-specific disease resistance traits. Additionally, managers will be able to compare disease activity within nurseries (by genet) with the populations of outplanted corals as well as in the wild population. Currently, because of culling practices that comparison does not provide an accurate representation of disease activity within a nursery setting.

<u>Research Activity</u>: Evaluate risk of disease to wild *Acropora* species when outplanting occurs interspersed with the wild populations vs. segregated from wild populations

Need

Currently, there is no direct evidence whether outplanting nursery corals will influence disease activity within the wild population of *Acropora* spp. in the Florida Reef Tract. If the tissue loss diseases that are commonly affecting *Acropora* populations are indeed transmissible, then adding individuals that may harbor potential pathogens, and also increasing population density, may inadvertently increase the probability of disease activity within the wild population. However, disease activity is currently commonplace within these two species and any potential increase in risk may be negligible and fall within a level of acceptable risk that is offset by the benefits associated with restoration efforts.

Objective

Determine whether the risk of disease activity on wild colonies of *Acropora* increases when nursery corals are outplanted within reefs that have *Acropora* colonies present (*i.e*., interspersed). Identify whether the risk is higher for wild populations on interspersed reefs compared with reefs isolated from outplanting activities (*i.e*., segregated).

Expected Benefits

Understanding whether outplanted *Acropora* colonies from nurseries impact the nearby wild populations either within the same or on adjacent reefs, is essential for establishing best practices for reef restoration and for improving the confidence in disease risk estimates related to different outplanting configurations. If outplanted corals do increase disease risk to wild populations then methods will need to be developed to reduce the identified risk to acceptable levels. If outplanted corals do not increase disease risk to wild populations then there is no need to develop or employ risk mitigation techniques.

Approach

Manipulative transplantation experiments should be conducted to compare disease dynamics and impacts to wild *Acropora spp.* that are ‘exposed’ (*i.e*., interspersed) versus ‘unexposed’ (*i.e*., segregated from) to outplanted *Acropora* colonies. Monitoring periodicity should be sufficient to capture disease onset and progression.

<u>Research Activity</u>: Influence of density dependence and corallivores on disease dynamics of *Acropora* spp.

Need

Predation, primarily by corallivorous snails and fireworms, is a significant chronic source of direct, though often partial mortality in Florida *Acropora* populations. Corallivory is also associated with increased subsequent disease risk in preyed colonies, either by vector transmission or simply by creating injury sites that enable disease infection. Recent Florida *A. cervicornis* studies suggest that high colony density may enhance colony condition and growth rate (likely due to positive feedbacks with resident fishes) as well as the attraction and impact of corallivores. Better mechanistic understanding of the complex interactions of colony density, corallivore attraction/impact, and disease risk/impact is needed to guide improved outplanting and potential predator control strategies.

Objective

Experimental field studies should be undertaken to evaluate the interactive effects of colony density and corallivore abundance on disease impacts and overall performance of outplanted *Acropora* spp. colonies.

Expected Benefits

The endeavor of 'creating' new *Acropora* spp. population patches via outplanting provides the opportunity to manipulate colony density to enhance performance. Relatedly, the substantial investment in such an activity may warrant some additional effort in corallivore control if this may help limit the negative effects of predation, disease, or both. Teasing out these complex direct and indirect interactions of colony density, disease transmission risk, and corallivore attraction/impact are needed to prioritize and develop more effective strategies on outplant density and potential corallivore control in *Acropora* restoration efforts.

Approach

Experimental field studies should be undertaken to manipulate coral colony and corallivore density (and/or identity to include snails, *Coralliophila abbreviata*, and/or fireworms, *Hermodice carrunculata*) to understand the prevalence, timing, and mortality associated with disease and corallivory as well as the effects of these predators on coral colony performance (*e.g.*, growth, productivity, etc.). Ideally, experiments would examine these interactions in different habitat types (*e.g.*, shallow fore-reef versus patch reef/hardbottom) where corallivore dynamics may differ as well as elucidate the mechanism(s) of corallivory-associated disease risk (*e.g.*, vector transmission versus ancillary infection of injured tissue).

<u>Research Activity</u>: Evaluate the effect of genotypic diversity on disease risk of outplanted *Acropora* spp.

Need

The long-term success of restoration efforts may be influenced by the genetic and genotypic diversity of the restored coral populations. Inbreeding depression (the reduction of fitness from the mating of relatives) and outbreeding depression (the mating between individuals that are strongly adapted to divergent local conditions) are two substantial concerns. However, high genetic diversity will likely increase probability of success and species survival under our changing global environment. Evidence suggests that certain genotypes are more resilient to disease than others, although the proportion of known disease-resistant genotypes in *A. cervicornis* is less than 10%. The percentage and identity of coral genotypes currently being propagated within nurseries that may be resistant to disease is currently unknown. Outplanting a high diversity of genotypes may reduce disease activity if some genets are less likely to be infected with disease, or genotypic diversity may have little influence on disease risk.

Objective

Determine whether genotypic diversity of outplanted *Acropora* spp. corals affects disease incidence and prevalence of the transplanted corals through time.

Expected Benefits

Current best practice methods for *Acropora* spp. culture include collecting as much of the local genotypic diversity as possible for the nursery stock, while reducing the movement of individuals from distant populations. Also, genotypic diversity of colonies outplanted within a patch is generally maximized. Understanding how genotypic diversity may influence disease dynamics of corals used for restoration and ultimately influence wild populations of *Acropora* spp. is essential for optimizing best practices.

Approach

To determine whether genotypic diversity of outplanting sites influences disease activity, experimental plots representing a gradient of genotypic diversity should be established with corals from *in situ* nurseries and surveyed through time. The genotypic diversity of the coral plot should range from single clones within a plot to high genotypic diversity, incorporating documented susceptible and resistant genotypes into the design. These plots should be surveyed frequently enough to determine the relationship between genotypic diversity and disease prevalence, incidence, and impact (amount of mortality).

<u>Research Activity</u>: Verify the identity of 'Rickettsia-like organisms' seen in histology of *Acropora* spp. tissues

Need
The pathogens that are causing tissue loss diseases have not been identified, yet disease is one of the most significant threats that may lead to the extinction of *Acropora* spp. in the Caribbean. Histological samples from both diseased and healthy tissues indicate that there are Rickettsia-like organisms in mucocytes on polyp oral discs and tentacles and in cnidoglandular bands of mesenterial filaments. Examinations of Rickettsia-like organisms -infected tissues revealed that they infect the polyp mucocytes and alter the coral's mucus secretions without causing gross disease signs. The Rickettsia-like organism is infections, therefore, may increase the susceptibility of corals to other environmental stressors and tissue loss.

Objective
Undertake molecular identification of the suspected Rickettsia-like organisms to verify (or not) their identity as *Rickettsia*.

Expected Benefits
Unraveling the mystery of what causes tissue loss diseases on Caribbean *Acropora* species would provide significant insight into how to prevent or reduce future disease outbreaks. Much emphasis has been placed on identifying particular pathogens present in diseased corals, but absent in healthy corals. Recent evidence, however, suggests that Rickettsia-like organisms are present within both healthy and diseased tissues (*i.e.*, a chronic, virtually ubiquitous infection) and may be a significant contributor to reduced overall coral health under environmental stress scenarios. If true, this model of Acropora disease etiology may suggest alternative health management and mitigation strategies. Determining whether these Rickettsia-like organisms are a significant component of tissue loss diseases would be more difficult to ascertain, especially because even healthy samples contain Rickettsia-like organisms. An inventory of Rickettsia-like organisms in *Acropora* spp. from around the Caribbean may be necessary to first identify the distribution of these organisms and then potentially apply experimental manipulations to determine the relative contribution that Rickettsia-like organisms play in causing disease activity.

Approach
To determine whether Rickettsia-like organisms are in fact *Rickettsia* spp. within histological samples, suspect Rickettsia-like organisms must be isolated from the tissue sample and the DNA must be extracted. Primers specific for *Rickettsia* spp. could be used for positive identification using polymerase chain reaction. DNA from samples could also be sequenced for identification.

<u>Research Activity</u>: Identify the relationship between temperature tolerance and disease susceptibility

Need

Thousands of corals are currently being propagated and outplanted for restoration activities along the Florida Reef Tract. However, very little is known about the physiology of the genotypes that are being used for restoration. Although there are potentially thousands of *Acropora* genotypes within the reefs of FL, only a few hundred are being used for propagation and outplanting. Understanding which genotypes are resistant to disease is important (Research Activity1), but understanding the physiological tradeoffs associated with disease resistance is also essential. Some genotypes that are disease resistant may also be more susceptible to stress associated with high water temperature. Alternatively, corals that are more resilient to temperature stress may also be innately disease resistant. Climate change will continue to increase the oceanic water temperatures around the globe, and disease is one of the greatest threats to *Acropora* spp. Therefore, identifying coral genotypes that are resilient to thermal extremes and disease, or establishing the tradeoffs between these two physiological traits, will enable more informed restoration strategies.

Objective

Identify whether there is a tradeoff between disease resistance and resilience to high water temperatures for different coral genotypes currently being propagated within *in situ* nurseries.

Expected Benefits

Bleaching from high water temperatures and disease outbreaks are two of the greatest threats facing coral reefs around the world. These threats are particularly important for Caribbean *Acropora* species because *A. palmata* and *A. cervicornis* are highly susceptible to both. Understanding the phenotypic variability in temperature tolerance and disease resistance of the different genotypes currently being used for propagation and restoration will significantly increase the knowledge behind outplanting design. Knowing whether there is a tradeoff or independence between the two traits will provide considerable information for restoration science, but also on the natural level of phenotypic variability within remnant Florida *Acropora* populations.

Approach

Experimental manipulations within a laboratory would provide a robust platform to isolate these two traits. Individual genotypes from coral nurseries would first be tested for disease resistance. Likely, a continuum from disease susceptible to disease resistant would be detected. These same genotypes would then be exposed to high water temperature to determine relative resilience to thermal stress. If genotypes that are disease resistant are also more susceptible to temperature stress then there may be a tradeoff between these two variables. Experiments could further test whether disease resistant phenotypes change when these genotypes are exposed to warm thermal stress. Additionally, field observational data on disease incidence or prevalence among these genotypes and their relative level of thermal tolerance during a temperature anomaly would provide *in situ* information that could confirm the laboratory experimental results.

<u>Research Activity</u>: Identification of site characteristics driving coral reef restoration success and failure along the Florida Reef Tract

Need

Coral reef restoration efforts via the outplanting of nursery-raised Acroporid corals is rapidly increasing along the Florida Reef Tract, resulting in thousands of corals outplanted in an attempt to restore coral reef communities. Observations suggest that outplanted corals perform better at some sites compared to others, influencing the magnitude and speed of coral reef community recovery. However, to date studies of restoration efforts have been on small spatial- and time scales, and typically focus on the survivorship and growth of transplanted corals. Consequently, there is an urgent need to understand the role of underlying ecological processes and site characteristics that contribute to optimal restoration sites to better inform coral reef restoration efforts.

Objective

Utilize existing coral outplant sites to quantify relevant ecological processes and site characteristics influencing coral reef community recovery and increase our understanding of the ecology of restored areas.

Expected Benefits

Current efforts to restore Florida reefs have relied largely on outplanting corals and have placed little emphasis on process-based strategies. Understanding the drivers behind the success and failure of the most common coral restoration approach in Florida is critical for the development of effective restoration practices. Results from this project can be directly applied by coral reef restoration practitioners to focus restoration efforts in areas with the highest chances of success. Furthermore, the identification of key processes and factors influencing coral reef restoration will allow the for the development of complimentary process-based restoration actions that can be undertaken to advance coral reef restoration beyond only outplanting of coral colonies.

Approach

Field studies should be undertaken that quantify important ecological processes (*e.g.*, herbivory levels, recruitment, benthic and fish community composition) and site characteristics (*e.g.*, sedimentation rates, temperature, etc.) at sites where coral colonies have been outplanted for restoration, with the goal of identifying key drivers of outplant success. Ideally, experiments will compare ecological processes across different scales, *e.g.*, within-reef (unrestored vs. restored areas), within-region (*e.g.*, Upper Keys) and between regions (*e.g.*, Upper Keys and Broward County) spanning the entire FRT. Where possible, survey design should also include a range of reef habitats and restored coral densities likely requiring collaborations among universities and research groups working throughout South Florida.

<u>Research Activity</u>: Characterizing recruitment habitat of corals to facilitate settlement of coral larvae and promote growth and survivorship of juvenile corals

Need

Recovery of coral reef ecosystems requires the replenishment of coral populations via coral recruitment. Coral recruitment can be limited by the settlement of coral larvae as well as the growth and survivorship of newly settled corals. Coral larvae frequently exhibit strong settlement preferences for specific microhabitats where they can experience increased growth and survivorship. Although several studies have demonstrated the ability of CCA to induce the settlement of coral larvae, the effects are not ubiquitous among CCA species. Important reef-building Acroporid species, in both the Indo-pacific and Caribbean, exhibit settlement preferences for certain CCA species. Based on these preferences, it is clear that coral larvae are capable of recognizing and discriminating among CCA species, yet little is known about which species of CCA facilitate larval settlement or their abundance and distribution across reef habitats, Further, there is little information regarding the settlement preferences of most Caribbean corals or the suitability of different microhabitats for their growth and survivorship immediately post-settlement. Thus, there is a need for research that identifies microhabitat characteristics that can enhance coral recruitment by increasing coral settlement and facilitating the early growth and survivorship of juvenile corals.

Objective

Studies should be undertaken to evaluate how microhabitat characteristics of natural or artificial substrate can be manipulated to facilitate coral settlement and early post-settlement growth and survivorship. Additionally, studies should evaluate how specific species of CCA facilitate/impede coral larval settlement and whether CCA species assemblages can be manipulated to enhance the abundance of facilitative species.

Expected Benefit

Reduced populations of adult corals are likely to result in diminished availability of coral larvae and concomitant reductions in coral settlement. In addition, newly settled corals are especially vulnerable to predation, incidental mortality by grazers, sedimentation, and overgrowth by other benthic organisms during their early juvenile stage. Thus, reduced settlement and early survivorship of corals could be an important bottleneck limiting the recovery of corals on Florida's reefs. Developing methods that can enhance coral recruitment by facilitating the settlement and/or early post-settlement survivorship of reef-building corals could aid restoration efforts by helping to eliminate a key bottleneck that can limit coral recovery.

Approach

Experimental studies should be conducted to manipulate characteristics of natural or artificial substrate to increase coral settlement and/or facilitate early growth and survivorship of juvenile corals. Experiments could alter the biological, chemical, or physical environment of potential settlement habitat and quantify the impact on coral settlement and early growth and survivorship. Manipulations may include changes in the rugosity, texture, orientation, or location of potential settlement habitat, removal of potential competitors of corals such as macroalgae or invertebrates, or addition of benthic organisms or chemical cues known to facilitate corals. Field-

based studies will be necessary to determine the distribution and abundance of CCA species in different habitats along the Florida reef tract. Laboratory settlement assays with coral larvae may be necessary to discern their preferences for different CCA species and for biofilms and bacteria present on different species of CCA. An important aspect of these manipulations is the potential for them to be used in a restoration context. Thus, while settlement preferences could be studied in a laboratory setting, the ultimate consequences of settlement choices for the growth and survivorship of juvenile corals needs to be evaluated in the field.

<u>Research Activity</u>: Herbivory manipulations to facilitate algal removal and create coral-friendly habitat

Need

Grazing by herbivorous fishes and invertebrates is a key ecosystem process on coral reefs. When grazing rates are reduced due to overfishing or disease, or spatially diluted by reductions in coral cover, benthic algal communities transform from those dominated by crustose coralline algae and closely-cropped filamentous algae that are beneficial or benign for corals to communities dominated by upright macroalgae that can negatively affect corals at all life stages (*e.g.*, larvae, juveniles, adults). Yet, even on many reefs with robust herbivore populations (mostly fishes) grazing pressure is often too diffuse, especially when coral cover is low, to remove abundant macroalgae and promote coral settlement and growth. Thus, there is a need for research examining how local interventions may be able to facilitate local increases in the rates or quality of herbivory thereby facilitating macroalgal removal and creating positive feedbacks on coral recruitment and growth.

Objective

Experimental field studies should be undertaken to evaluate how levels of herbivory can be manipulated in order to reduce algal abundance and create habitat that is known to facilitate coral settlement, growth, and health.

Expected Benefit

Given that herbivory is one of the most important processes for creating reef environments where corals can thrive, it is critical for coral reef restoration efforts to manipulate levels of herbivory in order to help facilitate coral-friendly environments as part of a restoration strategy. Developing methods to manipulate the distribution and concentration of herbivory in conjunction with coral transplantation will increase the chances of success of reef restoration efforts.

Approach

Experimental field studies should be done to affect the spatial concentration and/or quality of herbivory and track the impact to the benthic community. Manipulations may include herbivory by fishes, urchins, or mesograzers (*e.g.*, crabs, snails). Experiments could target concentrating grazing pressure by increasing herbivore density, manipulating available space for grazing, removing competitors of corals such as macroalgae or invertebrates, introducing beneficial shelter for herbivores, etc. Key reef responses to herbivory enhancement such as (but not limited to) benthic community changes, coral recruitment, or quality of coral recruitment habitat must be examined.

<u>Research Activity</u>: Importance of structure and live coral cover in coral reef fish recruitment and community development

Need

Reef fish are heavily dependent upon coral reefs for food, shelter and the successful completion of life cycles. Similarly, fish-dependent processes can have profound impacts on coral reef community structure and function. Fish are fundamental components of many ecological processes on coral reefs, such as providing connectivity between adjacent systems via diurnal feeding habits and influencing benthic community structure via grazing. Most reef fish species have specific microhabitat requirements and many are highly dependent on a narrow suite of coral species or coral morphologies for shelter and reproduction sites. Since one of the major goals of outplanting corals is to restore structural complexity to reef sites to promote the recruitment of fishes, understanding the relative importance and interaction between topographic complexity, coral outplants, and coral species identity on the recruitment of ecologically important fishes is important.

Objective

Field experiments should be undertaken to elucidate the relative importance and interaction of structural complexity, coral outplant density (or arrangement) and coral identity in the recruitment and development of fish communities, and how these communities affect key ecological processes on coral reefs.

Expected Benefits

Topographic complexity and coral cover influence the recruitment, abundance and composition of fishes on coral reefs, which ultimately determines the ability of fishes to contribute to key ecological processes such as herbivory. Outplanting coral colonies provides the opportunity to manipulate colony density, arrangement, and identity and incorporate supplementary restoration actions such as the addition of artificial structures. Developing methods to enhance fish recruitment and the ecological processes fishes influence via outplanting corals in conjunction with natural or artificial structures could aid restoration efforts by promoting key ecosystems components that support reef recovery.

Approach

Field experiments should be undertaken manipulating structural complexity, coral colony density, arrangement and/or identity to examine response variables related to fish recruitment and community composition. Experiments could utilize natural variations in topography or incorporate artificial structures with coral outplanting to test for enhancement of the recruitment of important functional groups of fishes (*e.g.*, herbivores). Ideally, projects will examine one or several fish-dependent positive feedbacks that could enhance coral reef recovery (*e.g.*, nutrient cycling, grazing, predation, etc.). Ultimately, these projects will be able to determine outplanting or restoration approaches that are most effective in recruiting fishes that support ecological processes key to coral reef recovery.

<u>Research Activity</u>: Determinants of Bioerosion on Florida Reefs

Need

Positive reef accretion can only be maintained when the growth of calcifying organisms exceeds the rates of reef erosion. With the decline in scleractinian coral populations in recent decades, Florida's reefs are now eroding faster than they are growing, which is causing the net loss of reef structure. There is a critical need to understand the biotic and abiotic drivers on reef erosion both now and under increasingly acidic conditions expected in the future and to identify which taxa are most important bioeroders on Florida's reefs. Perhaps most critically, understanding the role that reef herbivores play in bioerosion is vital. These herbivores (especially parrotfishes and *Diadema* urchins) are important to recovery of corals, and consequently have been focus of both coral reef restoration theory and in manipulative reef restoration studies. However, these species are also potential bioeroders. A better understanding of the tradeoff between enhanced herbivory and bioerosion is needed before large-scale restoration efforts that manipulate these species are implemented. By better understanding the ecological interactions that modulate reef erosion we may find ways to slow, or even reverse reef erosion in the future.

Objectives

Identify the biotic and abiotic drivers of bioerosion across the Florida reef tract. Examine potential restoration actions that can impede or reverse bioerosion (*e.g.*, promoting CCA growth).

Expected Benefits

The maintenance and restoration of reef structure should be a central goal of coral-reef restoration. Coral restoration efforts may increase community calcification, but this is only one half of the accretion equation. By better understanding the natural variability in bioerosion along the Florida reef tract and the biotic and abiotic processes driving these rates could allow the identification of methods to slow or reverse bioerosion.

Approach

A study should be undertaken to examine the existing information of long-term bioerosion along the Florida reef tract. Small-scale experimental assays (*e.g.*, measuring erosion using coral tiles) should be developed to relate patterns of bioerosion to ecological characteristics of the reefs (*e.g.*, percent cover of important benthic organisms, herbivore abundance and identity, etc.) Studies should be conducted to measure bioerosion rates with regard to different abundant benthic species (*e.g.*, CCA. macroalgae, sponges, zonanthids). Additionally focused, small-scale manipulative experiments should be conducted to investigate the ecological tradeoffs between herbivory and bioerosion.

Research Activity: Enhancing coral propagule supply and viability

Need

The multifaceted process of coral replenishment includes several bottlenecks, one of which is the supply of viable coral propagules (*i.e.*, gametes, planktonic larvae, and/or settlers) to a reef. As adult coral population density (both at the colony and the genet levels) has declined, the processes orchestrating spawning synchrony and successful fertilization also likely decline in effectiveness. The logistic difficulties of research on spawning species (*i.e.*, spawning/larval availability limited to a few nights per year) further challenges advancing knowledge of these processes. There is a need for research to address both the logistic and ecological aspects of propagule supply in order for restoration actions to more effectively foster coral population replenishment.

Objective

Develop and test ecological and/or technological approaches to improve the availability and viability of coral sexual propagules.

Expected Benefits

From a research perspective, improvements in propagule supply, including potential improved technologies such as spawning induction or cryopreservation, would provide vastly expanded scope and efficiency to advance and test ecological-scale interventions. Meanwhile, viable coral propagules are the bottom-line determinants of a self-sustaining, coral reef ecosystem.

Approach

A combination of field and laboratory studies should be undertaken to investigate methods to increase spawning effectiveness, which may include manipulations via staging adult populations at certain locations or induced spawning. Additionally, studies should test the success of concentrating viable propagules in habitats or conditions where they can successfully settle and survive, (*e.g.*, tenting).

<u>Research Activity</u>: Enhanced data collection on corals propagated and grown within *in situ* coral nurseries in south Florida to evaluate heritable traits

Need

Extensive effort has been directed at restoring depleted coral populations, primarily staghorn coral (*Acropora* spp.), on degraded coral reefs using colonies propagated within *in situ* nurseries located along the South Florida reef tract. A substantial body of information (much of it anecdotal) on the performance of individual coral genets has accumulated, suggesting that there are differences in the functional traits (*e.g.*, growth rate, disease resistance) among *Acropora* spp. genotypes. Still, many important traits related to coral fitness have not yet been systematically measured across nurseries. A rigorous evaluation of among-genet variation in fitness-related traits and between-trait interactions (reinforcements or tradeoffs) is needed to better predict coral performance in a reef restoration context under current and expected future South Florida reef environments.

Objective

Initiate enhanced and coordinated data collection across *in situ* coral nurseries to collect genet-specific fitness-related information to better evaluate the heritability of fitness traits and their potential interactions that could affect coral performance in a coral reef restoration context.

Expected Benefits

Current efforts to restore Florida reefs have relied largely on outplanting genotypically diverse coral colonies propagated and grown within *in situ* nurseries, but with little understanding of the genet-specific traits that potentially affect coral performance. *A posteriori* measurements of outplant success, largely limited to genet-specific survival and growth rates, have to date added relatively little to our understanding of the heritable traits responsible for coral outplanting success. An enhanced understanding of a full range of coral functional traits across several 'common gardens' represented by individual nursery sites will improve both coral culture within nurseries and restocking efforts undertaken as part of a coral reef restoration effort.

Approach

An expanded evaluation of the heritable traits of the *Acropora* spp. colonies maintained in the *in situ* coral nurseries located in south Florida (representing a range of 'distributed common gardens') should be conducted. Such an evaluation should entail a systematic monitoring of a suite of genet-specific traits across each nursery that could include host and *Symbiodinium* genotyping (down to individual clone level), growth rate (such as total linear extension or buoyant weight), skeletal density, tissue biomass, stress tolerance (particularly resistance to thermal extremes), disease resistance, photosynthetic performance, and fecundity. Concomitant environmental data (especially temperature and light) must also be collected. Data from these coordinated measurements should be synthesized across all the nurseries and evaluated for correlation among heritable traits.

<u>Research Activity</u>: A synthesis of the available data on *Acropora cervicornis* propagated and grown within *in situ* coral nurseries to evaluate heritable traits

Need

Extensive effort has been directed at restoring depleted coral populations, primarily staghorn coral (*Acropora cervicornis*), on degraded coral reefs using colonies propagated within *in situ* nurseries along the South Florida reef tract. Through monitoring efforts and observation within these nurseries a substantial body of information on the performance of individual coral genets has accumulated, suggesting that there are differences in the functional traits (*e.g.,* growth rate, growth pattern, disease resistance) among *A. cervicornis* genotypes. This genetically determined variation in traits related to coral fitness has clear implications for successful artificial propagation of this species and re-establishment of an ecologically functional population of *A. cervicornis* along the South Florida's reef tract. Although these findings are intriguing, a comprehensive quantitative analysis of genetically determined variation in coral functional traits across different nurseries has yet to be undertaken and is vital if we are to maximize coral restoration success.

Objective

Compile, analyze, and summarize the available information on *Acropora cervicornis* genets propagated in coral nurseries in south Florida to evaluate the extent of heritable variation in coral functional traits.

Expected Benefits

A comprehensive evaluation of the available data collected across the network of *in situ* coral nurseries will yield vital baseline information on the variation in *A. cervicornis* functional traits from which to develop an optimal design plan for propagating and outplanting this species for restoration activities. Such information could improve coral culture and restocking efforts undertaken as part of a coral reef restoration effort and provides an initial basis for exploring the potential correlation or anti-correlation (*i.e*., tradeoffs) between traits.

Approach

An evaluation of the functional traits of the *Acropora cervicornis* colonies maintained in the *in situ* coral nurseries located in south Florida should be conducted. This evaluation would entail a coordinated effort with the individual nursery practitioners to compile the available monitoring data that have been collected to date from the nurseries maintained by Nova Southeastern University, the University of Miami, the Coral Restoration Foundation, the Florida Fish and Wildlife Conservation Commission, Mote Marine Laboratory, and The Nature Conservancy. Once compiled, these data should be analyzed in a quantitative genetics statistical framework to characterize among-genet variation in functional traits. Depending on nursery-specific monitoring protocols, these functional traits could include survival, growth (*e.g.,* total linear extension or area), growth patterning (*e.g.,* branching rate), and resistance to thermal and/or disease-related stress. Rigorous meta-analysis strategies should be employed to synthesize this information across nurseries despite differences in the precise methods of raw data collection. If adequate data are available, it is also desirable to explore possible correlations or tradeoffs among genet-specific traits.

<u>Research Activity</u>: Determine differences in performance of outplanted corals based on nursery growth platforms

Need

Acropora spp. within *in situ* coral nurseries are being grown using several different platforms that include blocks, lines, and trees. Corals on different platforms are exposed to different environmental conditions (*i.e.*, different selection regimes). Block corals are attached to pedestals that are anchored onto a concrete block, which is in turn attached to the (usually sand) substrate. The tissue of these corals interact with sediments and benthic organisms found along the substrate, including benthic-associated microbes. However, corals grown on lines or trees do not interact with the substrate. Line and tree corals are attached to filamentous line and are suspending within the water column. Corals attached to the substrate must withstand different current, temperature, and light regimes compared with those suspended within the water column. Evidence suggests that the corals grown on blocks have slower extension rates than those grown suspended in the water column. Microbial communities of corals grown under the two methods also likely differ. Whether the corals grown on the lines and trees differ in performance after outplanting from those grown on blocks is currently unknown.

Objective

Determine whether performance (measured as growth, survival and/or other traits) of outplanted corals differs based on initial nursery platform.

Expected Benefits

Methods being used for the propagation of nursery corals are currently optimized for branch production within the nursery environment. However, these methods create corals that may be conditioned for different ecological and environmental scenarios than those they will be exposed to after outplanting onto a reef. Testing the traits and performance of the corals that are being propagated using different techniques will determine whether those that are grown using line or tree-based methods are comparative to the outplants that are grown on blocks.

Approach

Several field and laboratory manipulations can be applied to determine genet-specific differences in physiological state of corals grown on different nursery platforms. Photochemical efficiency, photosynthetic rate, respiration rate, calcification rate, skeletal density, and surface microbial communities can all be measured on a subset of the corals grown under block, line, and/or tree conditions. Representatives from each grow-out method should then be outplanted within replicate reefs and monitored through time to elucidate the impact of growth platform on colony performance and survival.

<u>Research Activity</u>: Evaluation of heritable traits of nursery-cultured corals after outplanting to coral reef environments

Need

Current efforts to restore Florida reefs have relied largely on outplanting coral (primarily *Acropora cervicornis*) maintained in the network of *in situ* coral nurseries located along the south Florida reef tract. An emphasis has been placed on ensuring that the colonies within these stocks are genotypically diverse, and consequently there is great opportunity to collect information characterizing among-genet variation in functional traits across the environmental gradient where these nurseries are located. However, to maximize the success in establishing of an ecologically functional coral population it is necessary to understand how this genetically determined variation in functional traits translates into variation in fitness once the corals are transplanted out of the nurseries and into a variety of reef habitats. Although restoration efforts to date have placed an emphasis on outplanting genotypically diverse coral colonies at restoration sites, properly matching the genotypic composition of outplanted sub-populations to the types of reef environment that are most conducive to their survival could substantially improve the long-term success of these projects.

Objective

Experimental field studies should use outplanted nursery-propagated corals to evaluate how various reef environments modulate genet-specific functional traits. The spatial resolution of the environmental influence should be determined, as well as the plasticity of functional traits that might vary among genets and hence be one of the criteria for selecting genotypes for outplanting.

Expected Benefits

Determining whether coral genets can be environmental specialists (best performers only in certain environments) or generalists (performing well across a broad range of environments) is critical for development of more efficient coral reef restoration practices. Results from this project can be directly applied by coral reef restoration practitioners to focus restoration efforts using coral genotypes in environments where they have the highest chance of success.

Approach

Field studies should be undertaken using *Acropora* spp. genets propagated within *in situ* nurseries to assess performance variation of potential heritable traits when the corals are outplanted across a range of reef sites. Ideally, these studies would involve outplanting and evaluating a representative sample of the genotypes maintained in all of the nurseries (*i.e.*, representing both high and low growth rates, thermal tolerance, etc) pooled at sites across a gradient of environmental conditions (*e.g.*, nearshore vs. offshore, degraded vs. healthy, high vs. low turbidity, high vs. low herbivory, etc.). Important metrics to be monitored could include growth, host/zooxanthellae/microbe dynamics, skeletal density, tissue biomass, photosynthetic performance, fecundity, and prevalence and impact of predation, bleaching, and disease. In addition, detailed habitat characterization of the outplant sites should be conducted and key environmental parameters collected. The resulting information, in concert with FWRI's *Acropora* resilience data, can then be used to create an Optimal Design and Site Selection Plan.