EFFECTS OF ST. LUICE ESTUARINE DISCHARGE WATER AND THERMAL STRESS ON THE CORAL *MONTASTRAEA CAVERNOSA*

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Joshua D. Voss, Department of Biological Sciences and Harbor Branch Oceanographic Institute, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

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Coral reef declines, particularly in coastal zones, have been linked to thermal stress and anthropogenic impacts on water quality. St. Lucie Reef near Stuart, Florida receives increased estuarine efflux as a result of watershed changes and management policies that have substantially altered historic, natural flows. This research used ambient and elevated temperatures (25°C and 30°C, respectively), and offshore versus St. Lucie Estuarine discharge water to investigate the individual and interactive effects of thermal and water quality stress on *Montastraea cavernosa*, a dominant scleractinian coral species at St. Lucie Reef. These goals were accomplished using *ex-situ*, factorial, experimental design that was supplemented with existing *in-situ* monitoring on St. Lucie Reef. Zooxanthellae density and chlorophyll content were evaluated to determine effects on the corals and their symbionts. Zooxanthellae populations were significantly affected by thermal stress. Significant interactions between temperature and water treatment were

observed, suggesting that the impacts of discharge water may be supplanted when corals are exposed to thermal stress. In a supplement to the experiment, *M. cavernosa* colonies transplanted from Palm Beach to St. Lucie Reef demonstrated resilience despite exposure to more variable environmental conditions. Collaborative partnerships with multiple state agencies and local government offices facilitated data sharing to inform decision making for South Florida's resource management strategies. Creating effective resource management is crucial for the conservation of coastal ecosystems impacted by land-based sources of pollution both locally and globally.

EFFECTS OF ST. LUICE ESTUARINE DISCHARGE WATER

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INTRODUCTION

Multiple stressors on coral reefs

Coral reefs are increasingly subject to anthropogenic stressors including exposure to land runoff, freshwater discharges, and elevated temperatures. Watershed manipulation, agriculture, and coastal land development can elevate amounts of nutrients, toxins, and sedimentation in freshwater runoff (Fabricius 2005; Shantz and Burkepile 2014). Similarly, anthropogenic changes to watersheds can also introduce large amounts of low-salinity water, creating osmotic stress in both estuaries and on reefs (Kimmerer 2002; Beal et al. 2012), especially in sessile organisms, such as oysters and corals, that cannot escape environmental fluctuations (Chamberlain and Hayward 1996). Persistent salinity variations can reduce species diversity and abundance of an ecosystem (Chamberlain and Hayward 1996). Globally, coral reefs are also being exposed to climate changes such as increased temperatures, sea level rise, and ocean acidification (Baker et al. 2004; Barshis et al. 2013; Palumbi et al. 2014). The threats of multiple stressors can lead to complex ecosystem dynamics, and effects on coral reef ecosystems are often not easily predictable (Breitburg et al. 1999; Crain et al. 2008). Stressors often interact with each other, and the combined effects are not always simply additive. Combined effects may be synergistic, greater than an individual stressor acting alone, or antagonistic, less than a single stressor acting alone (Ban et al. 2014). Combined disturbances could make a reef less resilient to future stress (Goreau et al. 2000), and stress tolerances can vary among species (Crain et al. 2008; Muthukrishnan and Fong 2014). A dominant effect

could also be seen where although an organism might be exposed to several stressors, effects from one could mask any observable effects from the other lesser stressors.

Some of the variance in stress tolerance among corals is due to symbiotic dinoflagellates from the genus *Symbiodinium* that live within the gastrodermal cells of many coral hosts. These dinoflagellates, known as zooxanthellae, comprise a portion of the coral holobiont, which is the host coral and its associated microorganisms. Zooxanthellae are often cited as being one of the major factors allowing corals to thrive in tropical waters, enhancing productivity and resulting in reefs that can maintain high levels of biodiversity (Brown 1997; LaJeunesse et al. 2010; Burriesci et al. 2012). These zooxanthellae are taxonomically divided into nine clades (A-I), each containing subdivisions of the overall known diversity (LaJeunesse 2002; Pochon and Gates 2010). Using chlorophyll *a* and c_2, and peridinin pigments, zooxanthellae carry out photosynthesis within host coral cells and generally produce more carbohydrates than is required to support their own metabolic needs. The excess, sometimes over 90%, is transferred to the host coral mostly in the form of sugars (Dubinsky and Jokiel 1994; Burriesci et al. 2012). In this symbiosis, the zooxanthellae are protected from predation and harmful UV radiation (Mieog et al. 2009), and can obtain nitrogen and phosphorus made available as metabolic waste products from the host coral (Rahav et al. 1989). Many corals supplement their energy budget via heterotrophic feeding, using tentacles on the coral polyps, which provides nitrogen for proteins and amino acids (Houlbreque and Ferrier-Pages 2009). The ability to form reefs, nutrient cycling, and having multiple forms of feeding contribute to the success of zooxanthellate corals in tropical waters, which can sometimes be nutrient-poor (Muscatine and Porter 1977). Corals form reefs by

precipitating calcium carbonate that forms a supportive skeleton beneath the visible, living animal tissue. The calcification process, which requires energy to actively transport calcium to the subcalicoblastic space, is fueled at least in part from energy created by photosynthesis within zooxanthellae cells (Al-Horani et al. 2003).

Within this symbiosis, the genetic diversity of zooxanthellae can affect holobiont thermal tolerance, photosynthetic capabilities of the symbiont, and growth of the host coral (Fitt and Warner 1995; Bhagooli and Hidaka 2004; Mieog et al. 2009; Baker 2014; LaJeunesse et al. 2014). These effects can influence the response of the holobiont to environmental disturbance.

Different coral species, and even different individual colonies within a species, can display differential tolerances to stress (Bhagooli and Hidaka 2004), and the elicited responses can be quite complex. For example, initially, increased nutrient loads can have a positive effect on the coral holobiont. Elevated nitrogen has been shown to increase the density of the endosymbiotic zooxanthellae, in turn increasing photosynthetic rates and available sugars (Muscatine et al. 1989; Fabricius 2005). However, over time the increase in zooxanthellae density can result in a depletion of phosphorus and leave the coral host more susceptible to light and temperature stress, leading to bleaching and mortality (Wiedenmann et al. 2012). Bleaching is the expulsion of the zooxanthellae from the host cells, a loss of photosynthetic pigment within the zooxanthellae, or a combination of both. Nutrient enrichment can also lead to increased macroalgal cover and denser phytoplankton populations. These in turn increase shading and reduce the amount of light, and thus photosynthetically active radiation (PAR), available to the corals

(D'Angelo and Wiedenmann 2014), while greater macroalgal cover increases competition for space (Smith et al. 1999; Liu et al. 2009a).

In addition to nutrient inputs, thermal anomalies and solar radiation are also known to disrupt coral-zooxanthellae symbioses (Douglas 2003; Berkelmans and van Oppen 2006), and large scale bleaching events are often attributed to elevated seawater temperatures, increased solar radiation, or the synergistic effects of both (Lesser 1997). Under high light conditions corals can experience hyperoxic conditions as a result of zooxanthellae photosynthesis (Kuhl et al. 1995), under which increased absorption of excitation energy from solar ultraviolet (UV) radiation can cause the formation of reactive oxygen species (ROS) that can damage photosystem II (Lesser et al. 1990). Concurrent temperature increases can inhibit a cell's ability to repair damage to photosystem II, lowering the threshold of light required to cause chronic photoinhibition (Bhagooli and Hidaka 2004; Takahashi and Murata 2008). This leads to a decrease in photosynthetic yield and possible expulsion of the zooxanthellae from the host (Bhagooli and Hidaka 2003; Fujise et al. 2014). Zooxanthellae cultured *ex vivo*, but in an environment similar to that found within a host coral, showed declines in photosynthetic yield and decreased cellular growth of zooxanthellae when exposed to elevated temperatures and UV radiation (Lesser 1996). Similar decreases in photosynthetic rates were seen in Caribbean corals exposed to simultaneous thermal stress and solar radiation, however, access to antioxidant enzymes that can inactivate harmful active oxygen species prevented such drops in photosynthetic rate (Lesser 1997).

When coral reefs are exposed to a combination of increased nutrients, thermal stress, and increased solar radiation, cumulative effects on coral can be different than the

effects of the same stressors acting alone (Crain et al. 2008). As explained above, eutrophication, which can result from land runoff, can cause increases in symbiont densities. Concomitantly, the total amount of ROS produced by zooxanthellae in response to thermal stress has been reported to be directly related to symbiont density (Cunning and Baker 2013). The combination of increased nutrients and thermal stress as coral tissue becomes overwhelmed with ROS can increase bleaching susceptibility in corals with high symbiont densities (Weis 2008). Zooxanthellae in corals exposed to both nitrate enrichment and elevated temperatures exhibit strongly reduced primary production rates compared to those in corals exposed to either increased nitrate or elevated temperatures alone (Nordemar et al. 2003). In time, lower primary production rates could lead to decreased host tissue growth and calcification. Similarly, *Porites lobata* and *Pocillopora damicornis*, two Pacific coral species, both exhibited decreases in zooxanthellae density when exposed to both elevated temperatures and nitrate enrichment. However, zooxanthellae density did not decrease significantly in *P. damicornis* when only exposed to elevated temperature, and did not decrease significantly in *P. lobata* when only exposed to nitrate enrichment (Schlöder and D'Croz 2004). Increases in zooxanthellae density were reported after low salinity estuarine discharge events in both *Montastraea cavernosa* and *Pseudodiploria clivosa* on a reef in South Florida (Klepac et al. 2015). Beyond single colony effects, coral cover and community susceptibility to bleaching has been predicted using both a multivariate stress model and three different thermal threshold indices (McClanahan et al. 2015). The threshold indices predicted global reef loss once sea surface temperature thresholds were

exceeded, however the multivariate model predicted more spatial and temporal variability in reef responses based on other heterogeneous environmental variables.

Multiple stressors not only cause complex responses on a reef, but can also impede coral recovery from stress events. A coral may bleach in response to an increase in temperature, but still have a good chance at recovery if no more environmental disturbances occur. However, concomitant rising temperatures can, for example, increase susceptibility to viral infection (Correa et al. 2016). The combination of environmental stressors might lead to coral death, where a coral similarly exposed to thermal stress, but with no other environmental disturbance, could recover (McClanahan et al. 2015). The implication that multiple stressors acting simultaneously on a reef can lower recovery thresholds should be considered when planning resource management strategies. In the interest of better understanding effects of multiple stressors on coral reefs, this study focused on the combination of thermal stress and exposure to estuarine discharges. This study did not attempt to identify causative agents of impacts of stress from individual elements of discharge water (i.e. increased nitrogen or pesticide pollution), rather it was designed to explore biological responses of corals to thermal stress and estuarine discharge water both individually and in combination.

Coral reefs along the Florida coastline are exposed to a variety of environmental stress, many caused by anthropogenic changes in land use, restructuring of the South Florida Watershed, and current water management practices. In areas like Florida Bay, such changes have led to warm, hypersaline water and large cyanobacterial blooms that can effect coral reefs in the Florida Keys National Marine Sanctuary (Porter et al. 1999; Zhao et al. 2013). Watershed restructuring has drastically changed the quality and volume

of water entering the St. Lucie Estuary and Indian River Lagoon along Florida's east coast (Sime 2005; Beal et al. 2012; Lapointe et al. 2012), as well as the Caloosahatchee River and Estuary on Florida's west coast (Liu et al. 2009b), and the coral reefs just offshore near both coasts. Globally, reefs are facing climate changes including increased water temperatures and sea level rise. The complex relationship of multiple stressors on coral reefs, especially along the Florida coastline, needs to be studied in depth, and with increasing urgency. Being able to predict and understand the relationships among multiple environmental stressors is key for effective management strategies and to determine where and which conservation efforts will have the greatest impact. It is important to learn how to mitigate the most severe interactions, and would prove beneficial if early signs of ecosystem decline can be recognized and preventative action taken.

Study site

This study focused on a northern section of the Florida Reef Tract located within the St. Lucie Inlet State Park in Stuart, FL. St. Lucie Reef (SLR) is a nearshore reef subject to seasonal estuarine discharges and thermal variability, making it an appropriate location to study the effects of multiple stressors on corals. SLR, as do most coral reefs along Florida's coastline, provides economical services such as tourism and fishing (Beal et al. 2012), as well as providing protection for the shore against wind and waves (Koop et al. 2001). St. Lucie Reef contains 24 species of scleractinian corals, and represents the northern habitat limit for many of these species, as most are not found on reefs further north along the Florida Reef Tract (Sime 2005; Beal et al. 2012). The region serves as a feeding ground for endangered sea turtle species, and supports large populations of

sponges, crustaceans, and other invertebrates, as well as several species of soft corals and commercially important fish such as mackerel (Beal et al. 2012). This project falls within the framework of NOAA's Florida Coral Reef Management Priorities, which aim to assess impacts of freshwater and pollutants on the Florida Reef Tract including nutrients, sedimentation, pesticides, and herbicides. SLR is also the northernmost geographic area included in the Southeast Florida Coral Reef Initiative (SEFCRI), whose goals include promoting conservation and management support of coral reefs in Southeast Florida. Managers at St. Lucie Inlet State Park, whose boundaries include SLR, have also identified assessing the effects of freshwater runoff on the reef as a primary goal (FDEP 2014).

Starting in the 1890's and continuing in the early 1900's, the St. Lucie Inlet was made permanent and canals were constructed to drain Lake Okeechobee and portions of the surrounding Everglades to provide land for agriculture. The C-23, C-24, and C-44 canals were built to regulate water levels of Lake Okeechobee in order to prevent the lake from overflowing and flooding residential and agricultural areas adjacent to the lake. Historically, this overflow water would flow south through the Everglades. Canal construction diverted the flow of water east and west instead of south through the Everglades. When Lake Okeechobee exceeds a depth of approximately 5.6 meters, risk of failure of the Herbert Hoover Dike increases as described in the Lake Okeechobee Regulation Schedule. Therefore water control gates are opened and the lake can drain through the canals, which empty straight into the St. Lucie Estuary (Chamberlain and Hayward 1996), without traveling through the Everglades first. Ultimately these water

masses mix with seawater and move out of the St. Lucie Inlet, at times extending south over SLR (Figure 1).

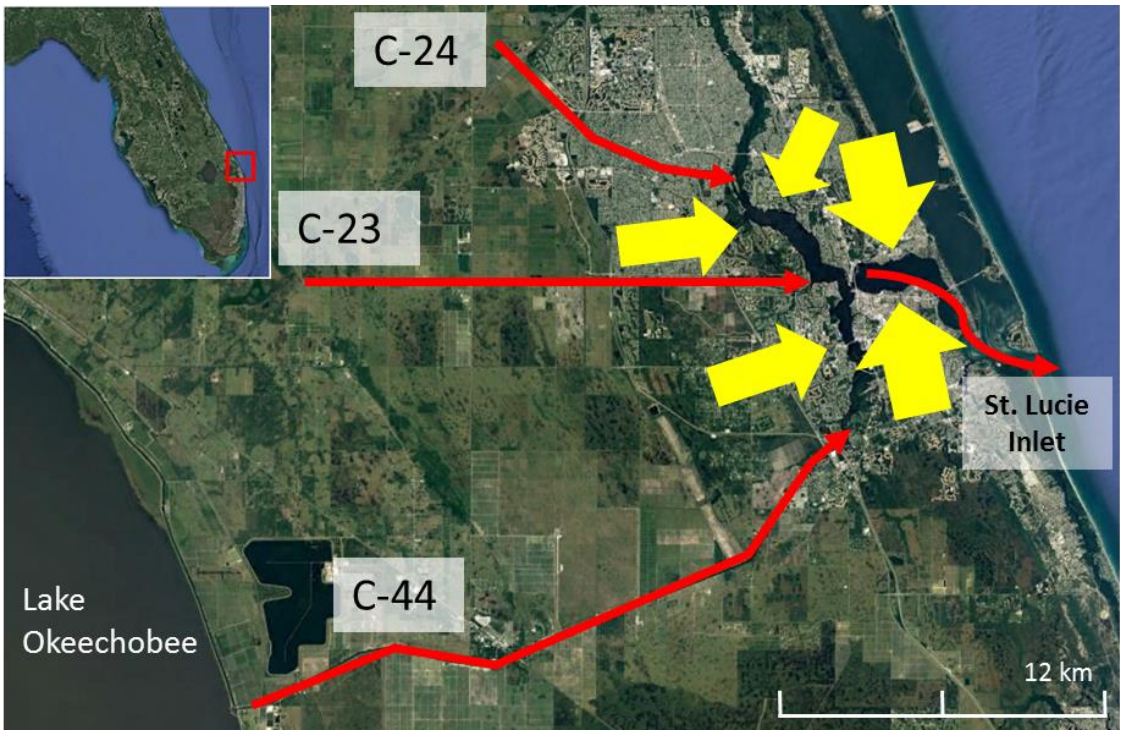

Figure 1: Primary canal drainage into the St. Lucie Estuary. Yellow arrows indicate runoff from land surrounding the St. Lucie Estuary. Red arrows show flow of water into St. Lucie Estuary through the C-23, C-24, and C-44 canals. Water from the canals combines with runoff from the surrounding watershed and flows into the St. Lucie Estuary. These water masses mix with seawater via tidal influence through the St. Lucie Inlet. After dispersing out of the inlet, depending on flux, tides, and wind conditions, these water masses can move south and over the St. Lucie Reef.

These alterations in the flow of water through the South Florida Watershed along with changes in land use since the 1890's, including coastal development and increased agriculture, have led to an increase in low salinity runoff that reaches SLR. These discharges and runoff have been shown to expose coastal environments to increases in nutrients, sediments, pollutants, and lower salinities (Chamberlain and Hayward 1996; Lapointe et al. 2012). SLR is exposed to a suite of other stressors as well, including

temperature variations, physical damage from fishing gear and storm-driven waves, increased sedimentation from local beach renourishment projects, and freshwater runoff that could introduce high turbidity and osmotic stress. Most of the scleractinian corals at SLR are living at their northern latitudinal limits along the Florida Reef Tract, yet they are persisting despite the apparently challenging conditions. A multifaceted relationship arises between protecting human health and economic interests inland, and managing environmental and economic impacts in the coastal zone. The connectivity of this system and costs and benefits that may impact areas differently can make decisions regarding management practices contentious and complex. However, access to detailed water quality data coupled with knowledge regarding the responses of critical species, like corals and their symbionts, are important for making informed resource management decisions. For example, if thermal stress exacerbates the negative effects of estuarine discharge, then freshwater releases should be scheduled so as not to happen during the hottest times of the year. Understanding how a reef's inhabitants respond to various combinations of both extrinsic and locally controlled environmental stresses will help shape local management and conservation.

There is a suite of water quality monitoring being done by several different agencies in the watershed near SLR. South Florida Water Management District's (SFWMD) DBHYDRO database reports flow rates of water through the control structures in the anthropogenically created canals in South Florida as well as rainfall in the region. Land/Ocean Biogeochemical Observatory (LOBO) units, which are maintained by Dr. Dennis Hannisak's lab at Harbor Branch Oceanographic Institute (HBOI) at Florida Atlantic University, record a suite of water quality data within the

Indian River Lagoon and St. Lucie Estuary. The nearest LOBO to SLR is located in the St. Lucie Inlet (Figure 2), and there is also one located in the C44 canal east of the S-80 control structure. Odyssey™ data loggers that record temperature and salinity were maintained at four sites (named North, Central, Ledge, and South; Table 1) along St. Lucie Reef and at one site inside the St. Lucie Inlet starting in October of 2013 (Figure 2). The northernmost of the four reef sites is just south of the St. Lucie Inlet.

	Latitude	Longitude
Inlet	27° 9' 54.72" N	80° 9' 47.21" W
North	27° 8' 46.608" N	80° 8' 20.976" W
Central	27° 7' 54.0114" N	80° 8' 2.5074" W
Ledge	27° 7' 16.104" N	80° 7' 38.748" W
South	27° 6' 42.7314" N	80° 7' 31.8714" W

Table 1: Locations of Odyssey temperature and conductivity data loggers on St. Lucie Reef and in St. Lucie Inlet.

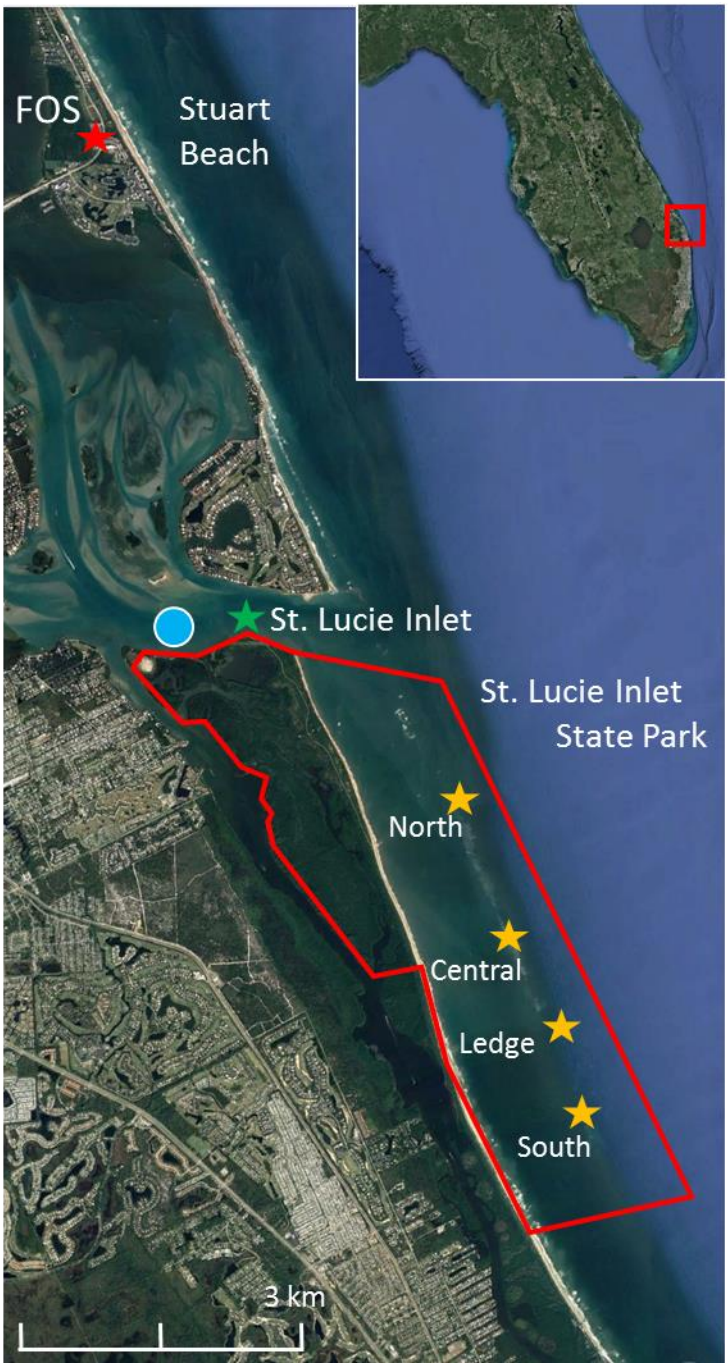

Figure 2: Map of monitored St. Lucie Reef sites and offshore water collection location. Duplicate sensors were deployed at four sites along St. Lucie Reef (yellow starts; site names from north to south: North, Central, Ledge, South) and at one site inside the St. Lucie Inlet (green star) from October 2013 to October 2014 to determine environmentally relevant ranges for the ex-situ experiment. Water used in the control offshore treatments was collected from Florida Oceanographic Society (FOS), who takes water from near Stuart Beach, north of the St. Lucie Inlet. The blue circle marks the location of the LOBO in the St. Lucie Inlet.

Several confounding trends in environmental parameters have been elucidated by comparing data recorded by these different agencies. Comparing data from SFWMD's DBHYDRO database with data from the LOBO located in the St. Lucie Inlet shows that levels of dissolved organic matter (CDOM), nitrate, chlorophyll, dissolved oxygen (DO), pH, and phosphate concentrations can all increase in the inlet following a discharge event, with concomitant salinity reductions and temperature increases (fau.loboviz.com; Figure 3). During long-term releases from the C-44, C-23, and C-24 canals in December 2015 through October 2016 (DBHYDRO database, SFWMD), increased nitrate and phosphate concentrations were recorded by LOBO's in both the St. Lucie Inlet and the C-44 canal (fau.loboviz.com; Figure 4), with levels in the C-44 canal higher than in the inlet.

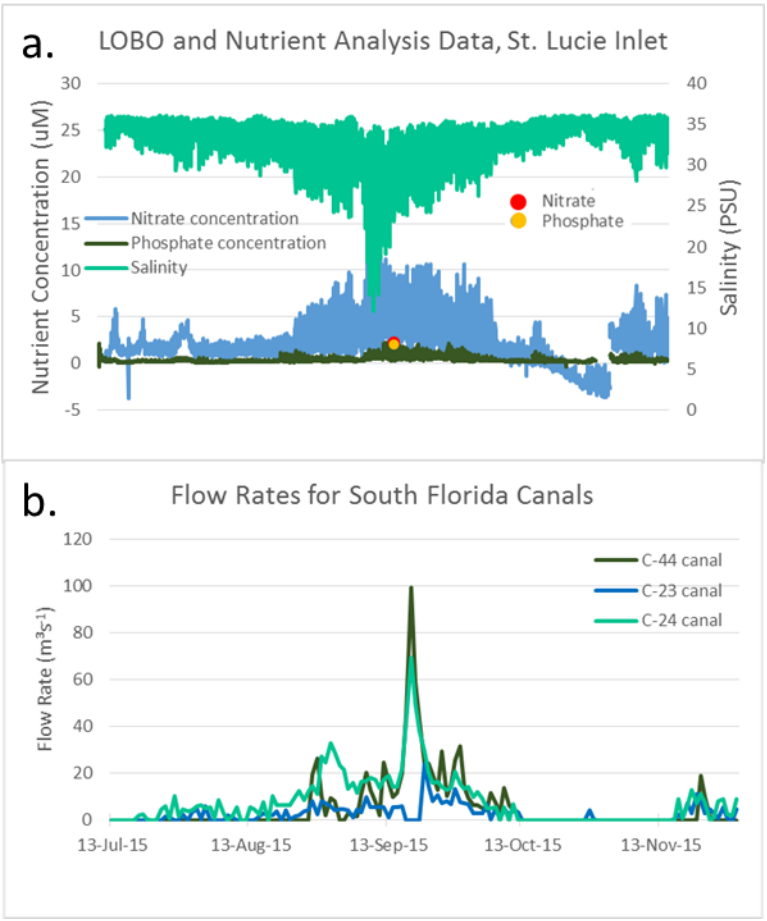

Figure 3: Nutrients in the St. Lucie Inlet during freshwater releases. (a.) Nitrate and phosphate concentrations and salinities recorded by the LOBO in the St. Lucie Inlet are reported (loboviz.fau.edu). Red and yellow dots represent nitrate and phosphate concentrations (respectively) of water samples taken on 9.24.15 and sent to CBL for LOBO validation. (b.) Flow of water released from the C-44, C-23, and C-24 canals (DBHYDRO, SFWMD).

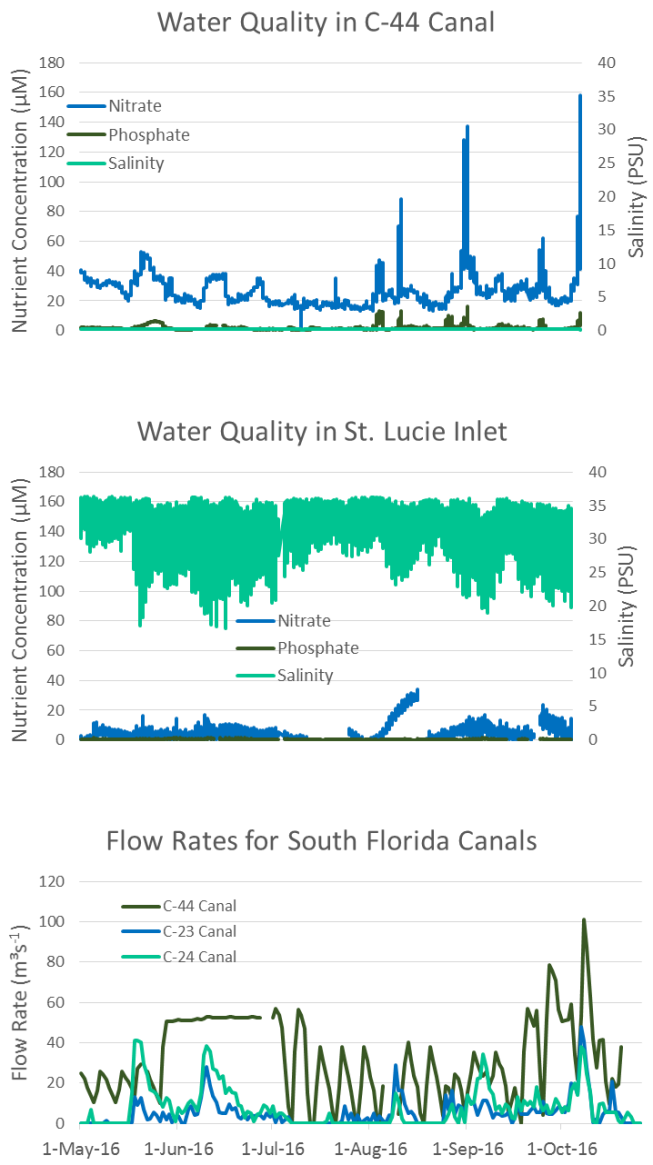

Figure 4: Nutrients in the C-44 canal and St. Lucie Inlet during freshwater releases. Nitrate concentration, phosphate concentrations, and salinities recorded by the LOBO's in the C-44 canal and St. Lucie Inlet are reported along with simultaneous released form the C-44 canal (DBHYDRO, SFWMD) for May through October of 2016.

Comparing temperature recorded by the Odyssey™ data loggers at SLR, with water flow from the canals reported by DBHYDRO shows that discharge events tend to occur during South Florida's rainy season (June-November), which is also the warmer

months of the year (Figure 5). The increase in rainfall and estuarine discharge are also associated with drops in salinity most notably in the Inlet, but also at SLR (Figure 5).

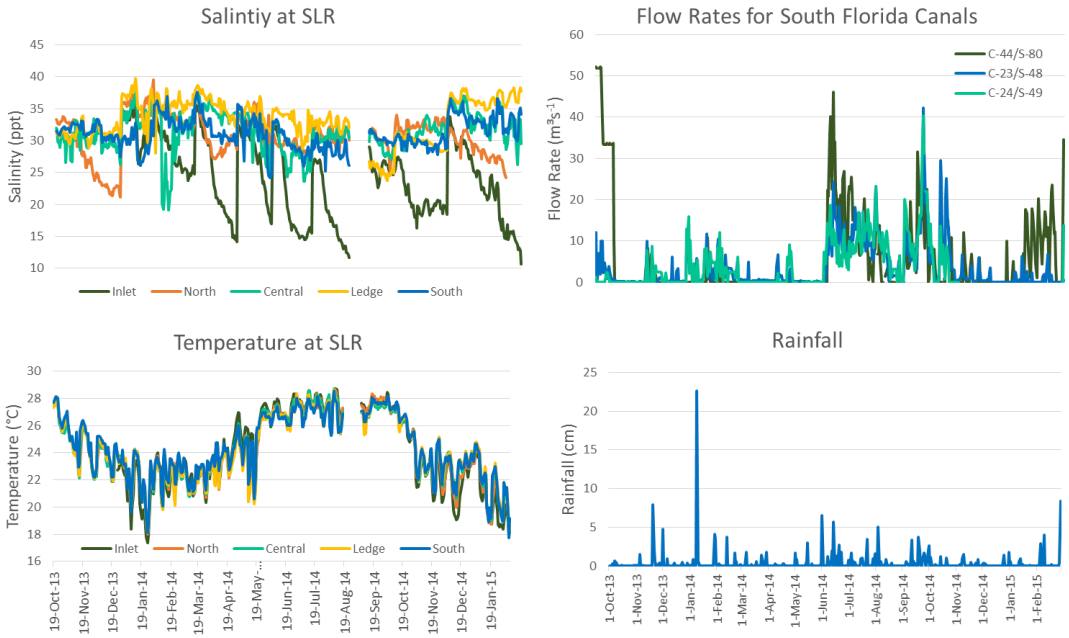

Figure 5: Temperature and salinity at SLR, discharge flow rate into St. Lucie Estuary, and rainfall from October 2013 to February 2015. Temperature and salinity were recorded at four reef sites and one inlet site with duplicate Odyssey™ data loggers; daily averages are reported. Discharge flow rates (DBHYDRO, SFWMD) are reported as average daily flow through S-80, S-48, and S-49 control structures in the C-44, C-23, and C-24 canals, respectively. Average daily rainfall is reported from the nearest weather station to SLR (DBHYDRO, SFWMD) located in Savanna Preserve State Park, Port St. Lucie, FL (27° 17' 25.18" N 80° 15' 13.17" W).

In addition to these confounding environmental trends, previous work at SLR has shown that the type of zooxanthellae in St. Lucie corals remains stable throughout both the wet and dry seasons, and slight changes in chlorophyll were observed after discharge events in 2013 (Klepac et al. 2015). Also, elevated expression of stress genes was seen in St. Lucie corals during discharge events (Beal 2012). However, because the rainy season has previously coincided with warmer months, which is also when water needs to be

released from the canals, it could not be determined if these coral responses were naturally occurring seasonal changes or effects from stress. Therefore, the primary focus of this project was to assess the effects of the observed environmental trends on corals at SLR through experimental design.

Ex-situ experimentation and coral holobiont analysis

Direct and interactive effects of discharge and thermal stress on corals and their symbionts have not been studied in depth. Many field-based approaches have included monitoring coastal sites exposed to freshwater runoff (Kimmerer 2002), precipitation or thermal stress (Dunne and Brown 2001; Edge et al. 2013). Such studies can provide an excellent picture of the current status of a reef and how major anthropogenic or natural events can change that status (Grigg 1995), however monitoring studies have limitations. Demonstrating causation, and therefore defensible recommendations for best management practices, becomes extremely difficult (Jameson et al. 2001). Confounding environmental variables often cannot be separated, and determining direct effects of a single variable, or how such effects could vary in the presence/absence of second variable, is often untenable. On the other hand, many laboratory experiments look at responses to changes in a single variable, but these changes rarely happen in isolation under natural conditions (Iglesias-Prieto et al. 1992; Dunn et al. 2004; Atkinson and Bilger 2006; Mayfield et al. 2013). *In-situ* observations can be most effective when they include diagnostic indices that aid in the determination of how coral reefs and their inhabitants respond to environmental changes (Jameson et al. 2001), and are supplemented by multivariate laboratory manipulations (Cadotte et al. 2005). The experimental objectives of this project are:

1. Determine direct effects of St. Lucie estuarine discharge water and thermal stress on *M. cavernosa* and its symbionts.

2. Determine interactive effects of St. Lucie estuarine discharge water and thermal stress on *M. cavernosa* and its symbionts.

3. Develop metrics to identify early signs of stress on nearshore reefs exposed to estuarine discharge water.

4. Assess feasibility of transplantation of *M. cavernosa* as a restoration strategy at St. Lucie Reef.

In addition to addressing the direct and combined impacts of thermal and estuarine discharge stress on corals, this study provides data that can be used to characterize the biological responses of corals *in-situ.* Biological indicators that allow early detection of declining coral health are of particular interest in nearshore reef systems exposed to highly managed estuarine efflux. St. Lucie Reef is not necessarily representative of reefs worldwide, but the ability to detect early signs of stress could be employed on reefs similarly exposed to large amounts of freshwater discharge and/or thermal stress. Such metrics would not only aid in identifying signs of distress, but would also be useful to distinguish reefs that are in danger of degradation and recognize where conservation efforts should be concentrated (Koop et al. 2001).

Confounding environmental variables can make ascertaining direct effects of stressors on corals difficult through *in-situ* experimentation. It is difficult to hold constant or effectively manipulate variables such as salinity, temperature, water quality, and turbidity in the field. At SLR, any *in-situ* experiment is faced with natural and anthropogenic influenced variability in several key environmental drivers. SLR is typically exposed to seasonal low-salinity discharges during Florida's rainy season, roughly June to November (Lapointe et al. 2012). Increased wind speeds in the winter

months can also affect sediment resuspension and water clarity (Beal et al. 2012). Water temperatures on the reef vary, and are influenced not only by seasons, but by freshwater discharges and mid-summer upwelling events (Beal et al. 2012). Another factor contributing to the difficulty of an *in-situ* experiment at SLR is the limitations on coral sampling due to the fact that SLR is within the St. Lucie Inlet State Park.

To address the impacts of thermal stress and estuarine discharge on *M. cavernosa*, this controlled study used an *ex-situ*, factorial, experimental design in ecologically relevant exposure ranges. In terms of abundance and size, the two most dominant coral species found at SLR are *Montastraea cavernosa* and *Pseudodiploria clivosa* (Beal et al. 2012), which have been found to associate with *Symbiodinium* clade C and B, respectively, at SLR (Klepac et al. 2015). In addition, previous work by our research team (Klepac et al. 2015) has shown that at SLR, *M. cavernosa* are principally associated with *Symbiodinium* clade C, and *P. clivosa* with clade B. Furthermore, these associations remained relatively stable across the reef and throughout seasonal temperature and precipitation fluctuations, however zooxanthellae densities increased in both species after discharge events in summer and fall of 2013. It was hypothesized that this stability, along with the detection of novel *Symbiodinium* associations in the two species at SLR, could indicate adaptations of SLR corals to local environmental conditions. An experimental aquarium system was constructed to control temperature, salinity, and light levels. Ambient and elevated temperatures, and offshore water versus estuary discharge water collected from the St. Lucie Inlet were used to investigate their individual and interactive effects on *M. cavernosa*. The *ex-situ* experiment was complemented by ongoing *in-situ* data collection on the St. Lucie Reef, including temperature and salinity monitoring, and

several different responses commonly associated with corals exposed to sublethal stress were measured. Samples were also collected to assess variations in gene expression for ongoing analysis at Florida Atlantic University (FAU), Harbor Branch.

METHODS

Experimental design

An *ex-situ* system was designed and constructed to achieve a two-by-two factorial design, with two levels of temperature and two levels of water type. Six 454 L, open-top raceways were set up as flow-through baths for temperature maintenance, using immersion heaters (Pentair Aquatic Eco-Systems® SmartOne® EasyPlug™ PTC, titanium) and external chillers to control temperature. Twelve 150 L, glass aquaria (90x50x40 cm) were placed in the water bath raceways, two per raceway. There were four treatments among the aquaria, and three replicates of each treatment. One preliminary and three experimental trials in total were initiated. The preliminary trial was conducted using the zooxanthellate sea anemone *Exaiptasia pallida* as a model organism (Grajales and Rodriguez 2014) to optimize the system parameters and operational logistics. Three subsequent experimental trials were initiated with *M. cavernosa* fragments. The first two trials, referred to as Trial 1 and Trial 2, were successful. However, the third experimental trial, Trial 3, was abandoned when more than one third of the incoming *M. cavernosa* fragments began to show signs of disease prior to experimental exposures.

The first treatment used offshore water as the control (explained below) maintained at a control temperature, the second treatment used control offshore water held at an elevated temperature, the third treatment used inlet discharge water at the control temperature, and the fourth treatment used inlet discharge water at the elevated

temperature. Control and elevated temperatures were chosen based on data recorded at SLR during times of no discharge and after a discharge event, respectively (Figure 5). Elevated temperature treatments were maintained at an average of 29.5°C for Trial 1 and 29.8°C for Trial 2, and temperatures remained stable around these averages over the entirety of both trials. Control treatment temperatures showed diel fluctuations due to the fact that the experiment was run in an un-air conditioned building during summer months. Control treatment temperatures ranged from 23.7-28.4 in Trial 1 and 23.6-27.1°C in Trial 2, but never overlapped with elevated treatment temperatures in either trial.

Salinities on the reef logged by the Odyssey™ recorders during and after large discharge events into the St. Lucie Inlet were used to determine salinities inside the *ex-situ* aquaria with inlet discharge water treatments. Odyssey™ data loggers at the northernmost site on SLR recorded a daily average salinity of 28.80 ppt on June 26, 2014, 5 days after the combined flow through C-44, C-23, and C-24 gates was 61.75 m^3s^{-1} (DBHYDRO, SFWMD). The same loggers recorded a daily average salinity of 28.49 ppt on October 1, 2014, 5 days after the combined flow through the gates was 118.87 m^3s^{-1} (DBHYDRO, SFWMD). Based on these observations, salinity in the aquaria with inlet discharge water was maintained at an average of 28.2ppt for Trial 1 and 27.8ppt for Trial 2. Salinity in the aquaria with control offshore water was maintained at an average of 31.6ppt for Trial 1 and 31.7ppt for Trial 2, which was the salinity of the water when collected.

Within the glass aquaria one AquaClear® Powerhead 30 with AquaClear® Quick Filter powerhead attachment, and one Marineland® Maxi Jet® Pro 400 powerhead were

placed on opposite ends in each aquarium to create a circular flow and provide mechanical filtration to remove debris from aquaria water (Figure 6). A single HydroGrow Sol™ 1 LED lighting unit was placed 15 cm above the water level in each aquarium. Temperature, pH, and ORP were constantly monitored by Neptune APEX aquarium controllers and salinity was taken daily with a refractometer. Light intensity was measured using waterproof data loggers (Onset UA-002-08 HOBO Pendant Temperature/Light Data Logger). Two layers of shade cloth were hung over the windows where the system was housed and all light bulbs immediately overhead of the aquaria were removed in order to minimize sunlight entering the building and reduce variability among aquaria.

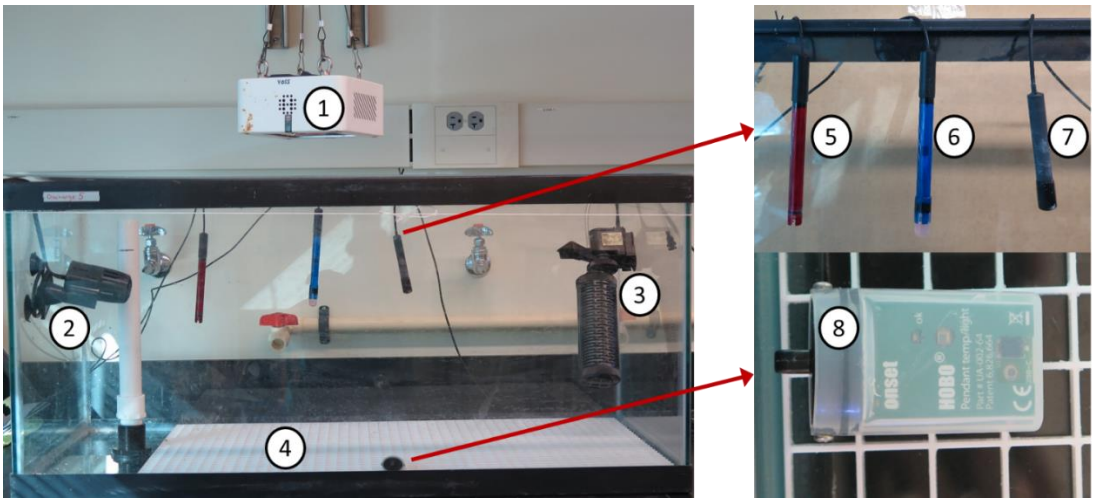

Figure 6: Glass aquaria used for the ex-situ experiments. The various components of the system are indicated in the figure. (1) a single HydroGrow Sol™ 1 LED lighting unit placed 15 cm above the water level (2) Marineland® Maxi Jet® Pro 400 powerhead (3) AquaClear® Powerhead 30 with AquaClear® Quick Filter powerhead attachment (4) plastic light diffusing panel (5) Neptune APEX ORP probe (6) Neptune APEX pH probe (7) Neptune APEX temperature probe and (8) Onset UA-002-08 HOBO Pendant Temperature/Light Data Logger.

LED lighting was chosen for this project because it provides a broad color spectrum and high enough PAR levels to maintain a live reef aquarium while being energy efficient. Before corals were collected, three different LED light fixtures were compared for spectral quality and quantity of light output: the Marineland reef capable 36-48", the Kessil® A360WE wide angle dimmable, and the HydroGrow Sol™ 1 grow light. An Ocean Optics Maya Pro 2000 was used to measure PAR levels (μmol/m²/s) and absolute irradiance (μW/cm²/nm) across the length, width, and height of one of the glass aquaria filled with seawater. The HydroGrow Sol™ had the most uniform PAR (Figure 7) and irradiance levels across the length of the aquaria. They also were the only ones with a peak in the red spectrum, not measure in the output of the other two fixtures, between 600 and 700 nm (Figure 8). This is critical as this peak corresponds to two of the major absorption peaks of chlorophylls *a* and c_2, and to the reaction center of photosystem II (Vinyard et al. 2013), and these light fixtures were used in this experiment.

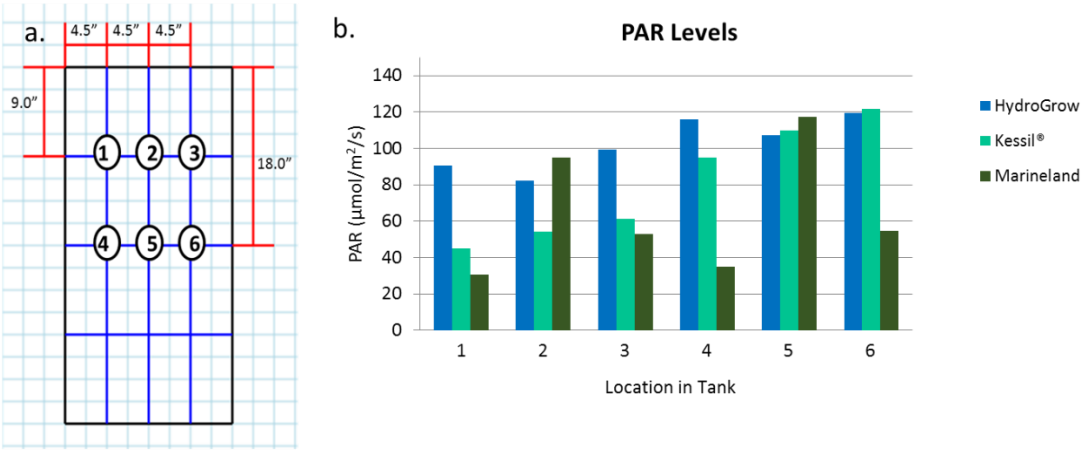

Figure 7: PAR levels of three LED lights. PAR was measured at six different locations inside of a glass aquaria filled with seawater. Measurements were taken 12 cm above the bottom. (a) The locations where each of the six measurements were taken. (b) PAR at those six location.

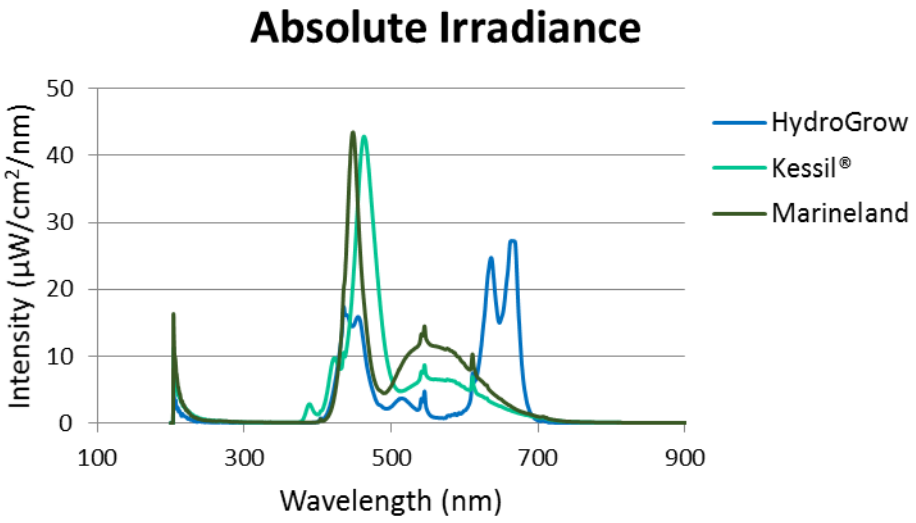

Figure 8: Absolute irradiance of three LED lights. Absolute irradiance was measured 12cm off the bottom at the same six locations where PAR was measured (Figure 7a) in a glass aquaria filled with seawater. Measurements taken at the center of the aquaria (location 5, Figure 7b) are shown in this graph; similar spectral patterns were recorded at the other locations in the aquaria.

Offshore seawater for control aquaria was collected from Florida Oceanographic Society (FOS), which uses a pump-driven system to pull nearshore seawater from

beneath the sand at Stuart Beach in Stuart, FL, just north of St. Lucie Reef (Figure 2). At the point in FOS's system where we collected water there was no further filtration other than the sand acting as a natural filter for large particulates. However, after transport to FAU Harbor Branch, the water was filtered for particulates and UV sterilized (see below). Stuart Beach was chosen as the location to collect control offshore water because it is near St. Lucie Reef, but north of the inlet, so it receives reduced influence from discharges through the inlet (Figure 2). Water was collected from FOS on June 24, 2015 (salinity 32 ppt) for Trial 1 and on August 20, 2015 (salinity 34 ppt, adjusted to 32 ppt) for the Trial 2. All offshore water was collected within 2 hours of high tide at Stuart Beach to avoid contamination from estuarine discharge.

Due to environmental conditions, inlet discharge water varied between the two trials. During the timeframe for Trial 1 preparations, despite being in the typical wet season, precipitation levels were unusually low and freshwater was neither currently being discharged from Lake Okeechobee, nor were there plans to discharge within the next month. Thus, the "inlet discharge water" used in the first trial was mixed from water taken on June 11, 2015 from the C-44 canal next to the S-80 control structure in Stuart, FL, and water taken from the St. Lucie Inlet near the FDEP weather station on June 17, 2015 (Figure 9). C-44 canal water (4 ppt) was added to the Inlet water (34 ppt) until the desired salinity of 28 ppt was achieved. The resulting mix, 85% Inlet water and 15% canal water, was used as the "inlet discharge water" substitute for the first trial. For the second trial, inlet discharge water (19 ppt) was taken from the St. Lucie Inlet at approximately low slack tide near the LOBO located in the St. Lucie Inlet (Figure 9) on September 23, 2015, five days after the flow through the S-80 gate of the C-44 canal

reached 99.02 m^3s^{-1}. This water was mixed with water taken from FOS until the desired salinity of 28 ppt was reached, resulting in a mix that was 16.67% discharge water and 83.33% FOS water.

Figure 9: Location of inlet discharge treatment water collection for Trials 1 and 2. Trial 1 used a combination of water collected from the C-44 canal near the S-80 control structure (star #1) at Phipps Park 27° 7'2.22"N, 80°16'26.69"W (star #2), and water collected near the FDEP weather station in the St. Lucie Inlet (star #3). Trial 2 used water collected near the LOBO unit located in the St. Lucie Inlet (star #4), and water collected from FOS (Figure 2).

All water was stored in 5,000 L polyethylene holding tanks after being filtered through a series of bag filters (50, 10, and 1 µm), and UV sterilized at a single pass dose of 50,000 µWs/cm^2. The holding tanks were painted dark blue first to block sunlight, then painted over with white to reduce solar warming. Prior to and during the experiments, water pumps circulated the water through the bag filters and UV sterilizer. This storage method was designed to reduce bacterial growth and contamination; as a result these

experiments do not assess potential impacts of bacteria from discharge events on coral or symbiotic algal responses. Between each trial, all holding tanks, bag filters, glass aquaria, light grading, PVC fittings, powerheads, and raceways were emptied, sterilized with bleach, rinsed in triplicate, and allowed to dry before being re-assembled for the next trial.

Sample collection

All coral samples were taken from Breakers Reef in West Palm Beach (26° 41' 27" N 80° 01' 05" W; Figure 10). While SLR is the focus of this project, it is within the borders of St. Lucie Inlet State Park so due to restrictions on collecting, we could not sample corals from within the park. Sampling was planned to take place just south of the State Park borders. However, after a few dives to locate potential collection sites there were very few *M. cavernosa* colonies found. Damage to the reef in that area had occurred as a result of washed out sediment from beach renourishment efforts that had previously taken place along the shoreline north of SLR. Further scouting dives identified Breakers Reef as a suitable alternative. Breakers Reef runs parallel to shore, about 1.5 km off the coast and ranges from about 12-25 meters in depth. It is roughly the same distance from shore as SLR, however *M. cavernosa* colonies were collected between 13-17 meters depth, where SLR ranges from 1.5-6 m (Beal et al. 2012). Breakers Reef is far enough south that effects from the discharge water exiting Palm Beach Inlet are limited. Before coral collection, two Odyssey™ data loggers and two HOBO data loggers were deployed at the site in June 2015, placed at a depth of 15 m to record temperature and light conditions on Breakers Reef. Temperatures at SLR and Breakers for June 2015 were similar for most of the month, with daily averages roughly 27-28°C, although there was

about a week where SLR temperatures dropped to averages around 20-21°C (Figure 11). The range of light intensity measured at Breakers Reef during this same month averaged around 3,300 lumens/m² and peaked around 16,000 lumens/m². Previously recorded light intensities at SLR from early May to late July from sunrise to sunset averaged around 3,700 lumens/m² and peaked around 50,000 lumens/m² (Beal et al. 2012).

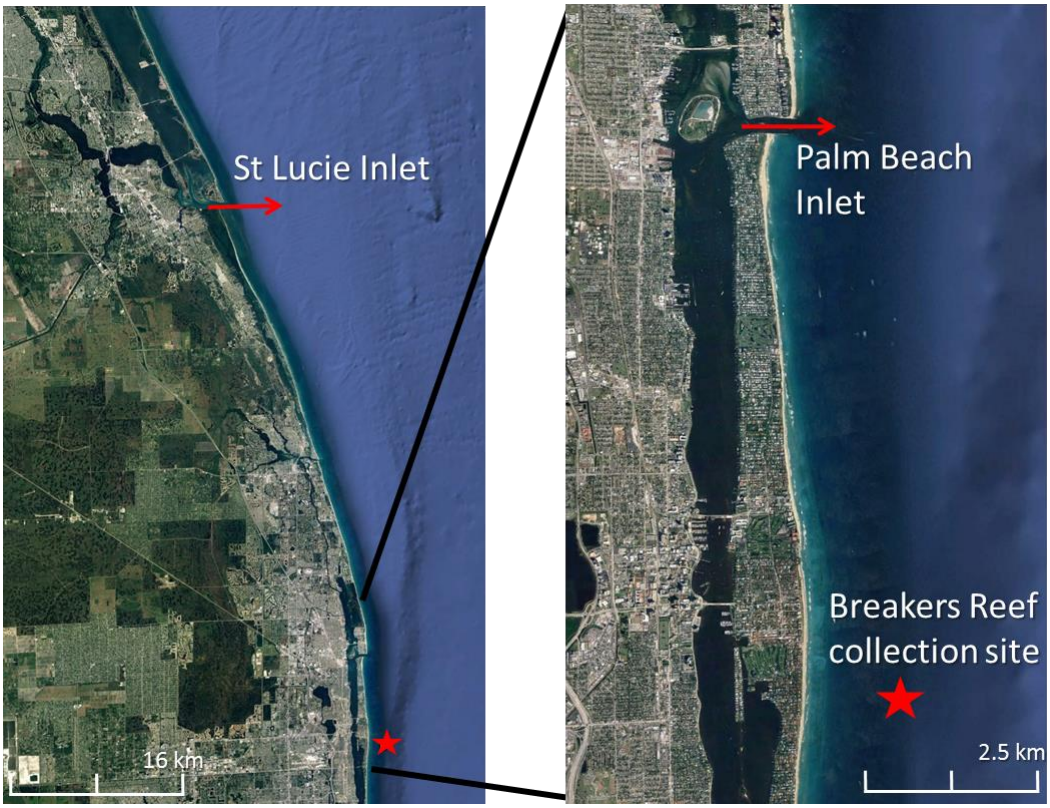

Figure 10: Location of Breakers Reef. Breakers Reef, where coral collection took place, is located south of St. Lucie Reef along the Florida Reef Tract offshore from West Palm Beach.

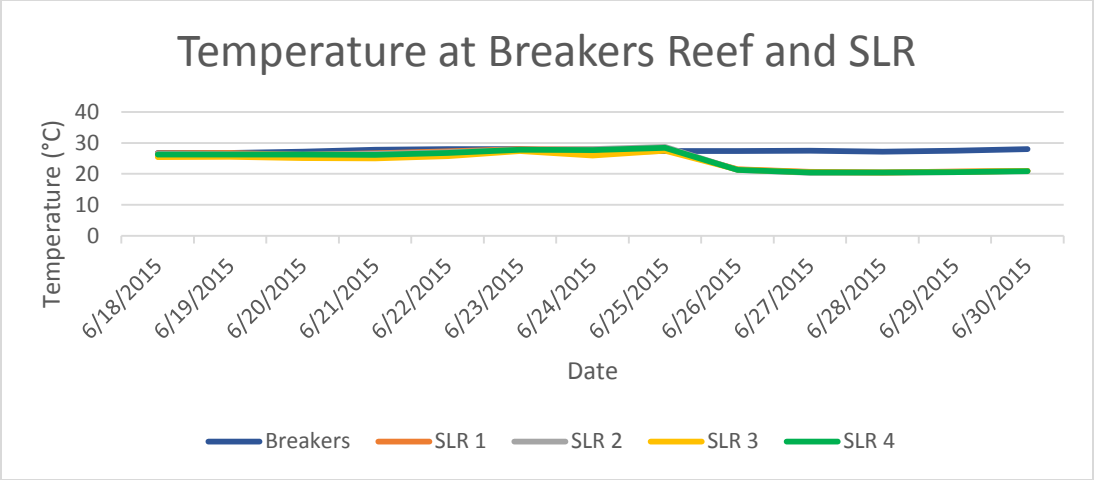

Figure 11: Temperatures at Breakers and St. Lucie Reef. Temperatures at Breakers were logged using duplicate HOBO recorders, and at SLR using duplicate Odyssey™ recorders.

Coral collection for Trial 1 was conducted on July 1, 2015, and for Trial 2 on October 1, 2015. Eleven *M. cavernosa* colonies, each approximately 150-175 cm^2, were sampled for each trial. For each colony, an initial subsample including a roughly 12cm^2 fragment was taken *in situ* to characterize initial conditions of the coral colonies in the field for comparison to the fragments used in the later experimental trials. The initial coral fragments were collected using a hammer and chisel, and stored individually in clean plastic bags filled with seawater until they were brought onto the boat. Then each fragment was split again yielding a smaller piece, 2-3 polyps, immediately preserved in TRIzol for ongoing genetic analyses at FAU Harbor Branch. The remainder of the fragment stored in a dry Whirl-Pak™. All samples were then immediately put on ice until being frozen upon reaching the lab, TRIzol samples at -80°C and Whirl-Pak™ bags at -20°C. After the subsamples were taken, each colony was removed, whole, from the reef and kept in coolers on the boat which were flushed with surface seawater every 5 to 10

minutes. The colonies were transferred to dry coolers and wrapped loosely in bubble wrap for transport to the lab (approximately 2 hours).

Once at the lab, coral colonies were placed in glass aquaria filled with water collected from FOS at 32 ppt and 28°C overnight. Subsequently, each colony was cut into 12 3x4 cm fragments using a rotary tile saw with a diamond encrusted blade lubricated by sterile 35 ppt Instant Ocean seawater (IOSW). All fragments were individually labeled with small plastic tags on the bottom of the skeleton to track each fragment's source colony. One fragment from each colony was placed in each of the 12 glass aquaria (Figure 12) on white, plastic light diffuser panels, elevated 1 inch off the bottom of the aquaria with PVC fittings.

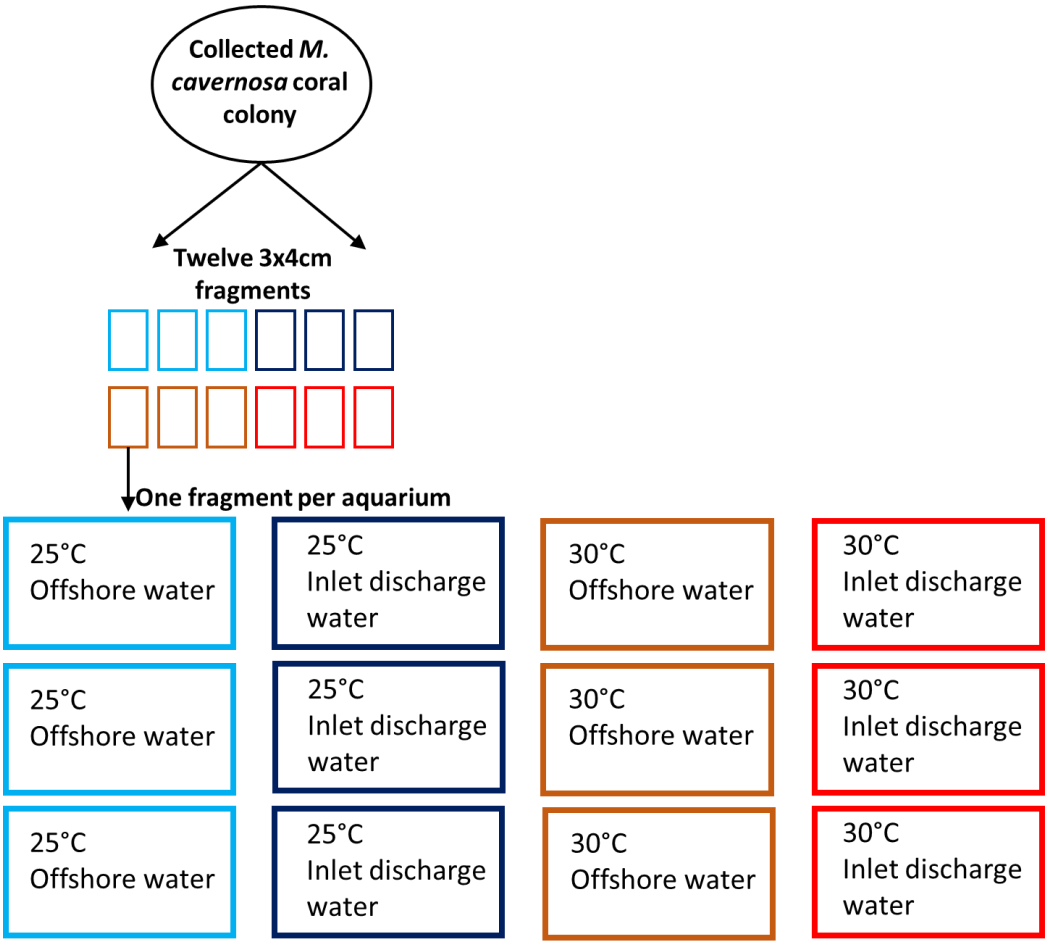

Figure 12: Coral fragmentation and placement. This diagram details how each collected M. cavernosa colony was fragmented into twelve 3 x 4cm fragments, and one of the twelve fragments was placed into each experimental aquarium. In total, there were three replicates of each of four treatments, and one fragment from each colony in each aquaria.

Experimental trials

For trials 1 and 2, all glass aquaria were initially filled with 130 L of control offshore water. One aquarium from each raceway was randomly assigned to receive control offshore water and the other to receive inlet discharge water (Figure 13). For controlling temperature, the raceways were split into two groups, half hooked up to an external chiller, and both had an immersion heater placed in a sump tank below each group of raceways. Each trial lasted approximately 32 days, during which time the coral

were fed brine shrimp every 3-4 days. For the first week, the coral fragments were allowed to recover and acclimate in control offshore water at 32 ppt and 28˚C, similar to ambient parameters when the corals were collected. The next five days in each trial were used to slowly change salinity and temperature within the aquaria until they reached desired experimental parameters. The change in salinity was driven by daily water changes, where during each of the 5 days, 15% of the water in the aquaria was removed and replaced with either discharge or offshore water, depending on the treatment designation. This lowered salinity in the aquaria designated to receive discharge water to 28 ppt, while salinity in the aquaria receiving more offshore water stayed constant at 32 ppt (Figure 14). Over the same 5 day period, temperature was changed gradually, less than one degree a day. Temperature, monitored using the Neptune APEX system, was lowered in half of the aquaria from 28 to 25°C using an external chiller to cool the waterbaths, and was increased in the other half of the aquaria from 28 to 30°C with an immersion heater to warm the waterbaths (Figure 14). Once the desired parameters were reached, the treatment period lasted for 20 days, during which 10% daily water changes with either 32 ppt offshore or 28 ppt discharge treatment water were performed to maintain biological integrity. During those 20 days, elevated temperature treatments were maintained at a relatively constant mean of 29.5°C for Trial 1 and 29.8°C for Trial 2. Control treatment temperatures ranged from 23.7-28.4 in Trial 1 and 23.6-27.1°C in Trial 2 due to diel fluctuations, but never overlapped with elevated treatment temperatures in either trial.

At the conclusion of each trial, samples were taken from all *M. cavernosa* fragments. Similar to the *in-situ* subsamples taken, each experimental fragment was split

with a hammer and chisel. A smaller piece, 2-3 polyps in size, was preserved in TRIzol and frozen at -80°C, and the rest of the fragment was frozen dry at -20°C for zooxanthellae counts and chlorophyll analyses. All fragments were photographed before and after the experimental trials. Photographs taken of the top and sides of coral fragments were used later in surface area analyses. TRIzol-preserved fragments from both *in-situ* and post-experimental sampling were set aside for ongoing gene expression analysis at FAU Harbor Branch.

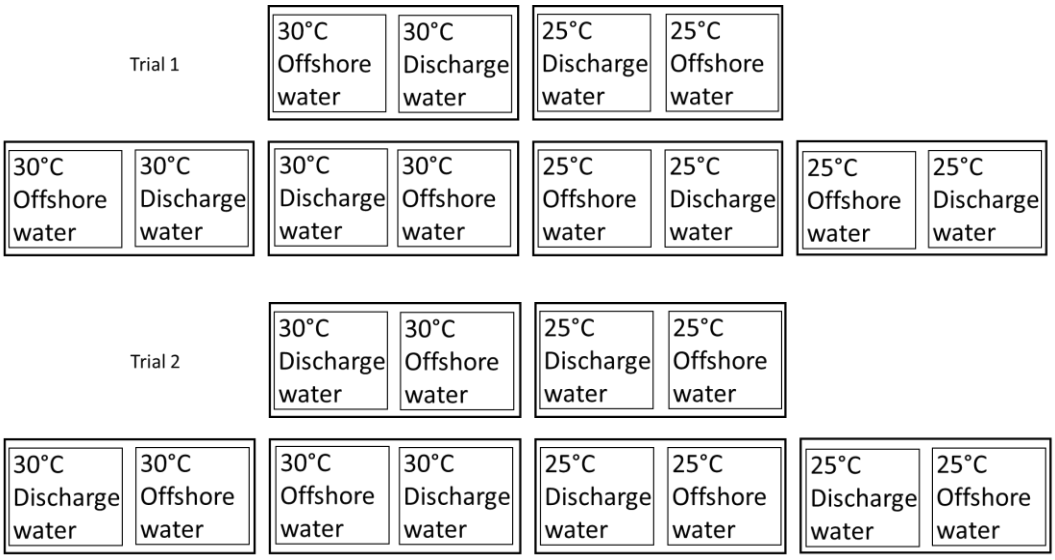

Figure 13: Designation of treatment factors for trials 1 and 2. Larger, darker rectangles represent raceways and smaller squares represent individual aquaria placed two within each raceway.

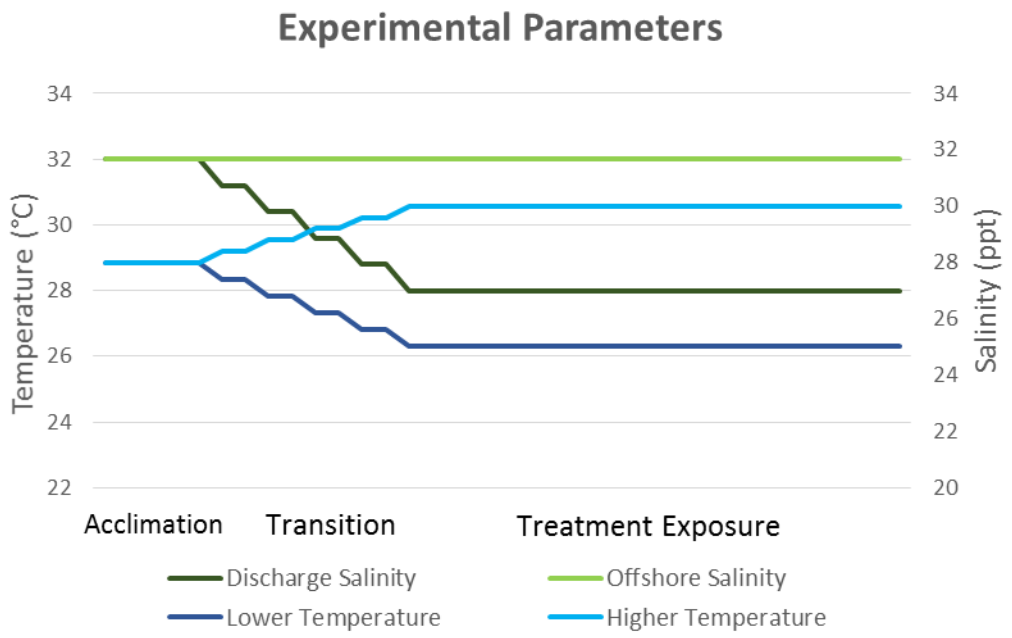

Figure 14: Phases of experimental trials. This diagram shows the four variables, two temperatures and two salinities/types of water, that were used to create a 2 x 2 factorial experimental design, and a mockup of desired values for each parameter during the experimental trials.

Response variables

Buoyant weight of the live *M. cavernosa* fragments was measured before and after the first trial by hanging each fragment from a scale in seawater of a known density. After Trial 1 it was determined that the precision of the method wasn't sufficient to measure potential differences in mass over the time of the experiment, so this time intensive method was abandoned for other trials. However scaled pictures were taken of all frags before and after the experimental trials.

Treatment effects on zooxanthellae populations

In order to determine zooxanthellae density and chlorophyll concentrations, within 30 days of collection coral tissue was removed from subsampled fragments that had been preserved at -20°C. A dental Waterpik® (waterflosser® Ultra) was used with

sterile 35 ppt IOSW to dissociate the coral tissue and zooxanthellae from the coral skeletons. The resultant slurry was centrifuged with an Eppendorf 5810R centrifuge at 3220 g for 30 minutes to concentrate the sample, and then filtered through 100µm Nitex mesh to remove any large skeletal particles. Samples were centrifuged again at 3220 g for 10 minutes, and excess water and coral tissue/mucus were removed. Each centrifuged zooxanthellae slurry was resuspended in IOSW for a total volume of 10 ml. A 2 ml aliquot of the resuspended slurry was removed and fixed in 10% formalin and preserved at 4°C for cell counts. For each *M. cavernosa* fragment, five photographs were taken of the fixed zooxanthellae cells on a hemacytometer at 400x magnification. Coral Point Count with Excel extensions (CPCe) software (Kohler and Gill 2006) was used to count the number of zooxanthellae cells within 0.1 mm^3. The five counts per fragments were averaged together and that average was multiplied by 10,000 to determine cells per milliliter, then multiplied again by the volume of the original sample (10 ml). For approximately 34% of Trial 1 fragments and 76% of Trial 2 fragments, samples still had debris so aliquots were thoroughly vortexed and diluted 50% with IOSW. The dilution factor was accounted for when calculating cell densities.

Surface areas for all of the denuded skeletal fragments were determined using a DAVID SLS-2 structured light 3D scanner (Polinski & Voss, in prep). The fragment was placed on an automated turntable (DAVID TT-1) and approximately 12-30 scans were taken over a 90° to 170° area, depending on the size of the skeletal fragment. The scans were then fused to create a 3D image of the coral skeleton. The 3D image was compared to photographs taken of the same coral fragment before the tissue was removed, and skeletal areas in the 3D image not covered by coral tissue in the photographs were

cropped out, leaving only the surface area previously covered by living coral tissue. The surface area of the cropped 3D image was calculated using Netfabb® Basic 3D printing software. The surface area of the fragment obtained with the 3D scanner was used with the zooxanthellae counts to estimate the number of zooxanthellae cells per cm^2 of coral tissue.

The remainder of zooxanthellae cells not fixed in formalin were used to determine chlorophyll content per zooxanthellae cell and chlorophyll concentration per cm^2 of coral tissue. A sterile 10 ml syringe was loaded with a 200-300 µL aliquot of the zooxanthellae cells and 3 ml IOSW (32 ppt), and used to push the sample through a 25mm diameter Whatman® GF/F glass filter. The filters were frozen overnight at -80°C then ground in the dark in 90% acetone using an Omni International General Laboratory Homogenizer on speed setting 5. Ground filters were stored in the dark at 4°C for 20-24 hours before the absorbance was measured on a Thermo Scientific™ Evolution™ 60S UV-Visible spectrophotometer at 750, 663, and 630 nm. The content of chlorophyll *a* and c_2 per zooxanthellae cell and per cm^2 of coral tissue (areal concentration) for each fragment was calculated using the equations of Jeffrey & Humphrey (1975). All chlorophyll assays were run in triplicate for each coral sample and averaged. There were three samples in Trial 1 that had failed absorbance readings, even after repeated measuring, possibly due to low readings below the detection limit of the machine. Those readings could not be used during statistical analysis.

Water quality and nutrient analysis

In order to better characterize the quality of the inlet discharge water collected, nutrient data was collected for Trial 2 only by Dr. Dennis Hanisak's research group (FAU

Harbor Branch Oceanographic Institute) on September 24 at low tide, one day after the inlet discharge water for Trial 2 was collected. The tested water was collected in triplicate from the St. Lucie Inlet, also next to the LOBO station, and sent to Chesapeake Biological Laboratory (CBL) Nutrient Analytical Services for nutrient analysis.

Coral transplants

For Trial 1, the portions of the coral colonies that were not used in the experimental trials were transplanted to SLR with permission from St. Lucie Inlet State Park. Eight of the eleven colonies had leftover pieces large enough to be transplanted. Any skeleton exposed from fragmentation with the tile saw was covered with All Fix waterproof epoxy to prevent erosion or biofouling, a suitable place was found on SLR near our Central reef site, and the coral piece was secured to a rock with epoxy where it would not harm nearby corals. Transplant locations were mapped and re-visited to monitor and photograph growth progression. For Trial 2 and 3, the leftover colony pieces had massive tissue sloughing off the skeleton by the third day after fragmentation, and were therefore not healthy enough to be transplanted onto SLR. In Trial 3, all of the experimental coral fragments showing signs of tissue loss and/or disease were removed from the aquaria, photographed, and preserved for possible characterization in the future. The rest of the coral fragments were kept alive in an aquaria together, but will not be transplanted to St. Lucie Reef to eliminate any risk of pathogen transmission.

Statistical analysis

Statistical analysis was performed with the R software package v3.2.5 to test the main and interaction effects in the experimental treatments on the dependent variables, including zooxanthellae density per cm² of host coral tissue, chlorophyll *a* and c_2 cellular

content per zooxanthellae cell and areal concentrations per cm^2 of host coral tissue of the three replicate fragments per sample. When testing assumptions of normality, cellular chlorophyll content and areal chlorophyll concentrations were kept separate because they were too closely correlated. Multivariate normality assumptions (*mardiaTest* function in the *MVN* package) were not met for either trial (Table 2), and several transformations did not improve data distributions. Therefore, a nonparametric, 3-factor permutational multivariate analysis of variance (PERMANOVA; using the *adonis* function from the *vegan* package; Euclidian distances; 9999 permutations) was run for each trial. Temperature, water type, and *M. cavernosa* colony were used as the three independent factors to assess variation in zooxanthellae density and chlorophyll *a* and c_2 cellular content and areal concentrations among the different experimental treatments. The PERMANOVA's were run using the change in response variable from *in-situ* conditions (final – initial). Two of the five response variables in Trial 1 and four of the five in Trial 2 demonstrated univariate normality (*shapiro.test* function in the *stats* package and *leveneTest* function in the *car* package; Table 3) even after attempting several transformations, however it is not uncommon for the assumptions of normality to be violated when analyzing multiple response variables with the potential to be correlated. So to better understand the differences driving significant factors in the PERMANOVA's, a 3-factor analysis of variance (ANOVA; using the *aov* function from the *stats* package) was run for each of the response variables for both trials. ANOVA was chosen to assess the interaction between temperature and water type that is a crucial focus of this project, and which is unique to a multi-factor ANOVA. Again, temperature, water

type, and *M. cavernosa* colony were the three independent factors, and the change in response variables from *in-situ* conditions (final – initial) was also used in the ANOVA's.

Multivariate normality tests (Mardia Test)					
	Trial 1			Trial 2	
Variables	skew	kurtosis		skew	kurtosis
Zoox cm^{-2}+Chl *a* (pg $cell^{-1}$)+Chl c_2 (pg $cell^{-1}$)	<0.0001*	0		0.023*	0.316
Zoox cm^{-2}+Chl *a* (pg cm^{-2})+Chl c_2 (pg cm^{-2})	<0.0001*	0		0.004*	0.684

Table 2: Multivariate normality tests. Multivariate normality was tested using the Mardia Test, where p-values for both skewness and kurtosis statistics should be greater than or equal to 0.05 to denote normality. An asterisk (*) denotes a significant result (α=0.05).

Univariate normality tests (Shapiro-Wilk, Levene Test)					
	Trial 1			Trial 2	
Variable	Normality	HOV		Normality	HOV
Zoox cm^{-2}	0.0001*	0.72		0.026*	0.917
Chl *a* (pg $cell^{-1}$)	<0.0001*	0.71		0.106	0.988
Chl c_2 (pg $cell^{-1}$)	<0.0001*	0.758		0.649	1.000
Chl *a* (pg cm^{-2})	0.379	0.772		0.434	0.992
Chl c_2 (pg cm^{-2})	0.142	0.893		0.474	1.000

Table 3: Univariate normality tests. Univariate normality was tested using the Shapiro-Wilk Test for normally distributed data and the Levene Test for homogeneity of variance (HOV). An asterisk (*) denotes a significant result (α=0.05).

RESULTS

Experimental Design

Preliminary trial

The preliminary trial performed using *E. pallida* provided a chance to make the following improvements in operational logistics that were incorporated in the subsequent two experimental trials. Response variable analysis was not performed for this preliminary trial; the focus was to test the new experimental system's capabilities. Timing of daily water changes was performed in the morning before the sun warmed the water in the storage tanks, when the temperatures of the water in the experimental aquaria was most similar to that of the water being added. The fluorescent lights directly above the aquaria were removed and two layers of shade cloth were hung over nearby windows in order to maintain more even light regimes among aquaria. There was a large, rolling door in one side of the facility where the aquaria were housed and this was kept shut in Trials 1 and 2 to improve the maintenance of stable, desired temperatures within the aquaria (Figure 15). Several small changes to the Neptune APEX monitoring system were made including the addition of an extra power source (enercell™ 12 V, 300mA, AC adapter), necessary due to the high number of probes that were being used. Also, the salinity probes were never able to be properly calibrated, so the decision was made to record salinity daily for all aquaria using a calibrated refractometer. The way the HOBO light and temperature loggers were secured in the aquaria was also altered because in the original design, powerheads caused the HOBO's to move, and a portion of the sensor

became covered by a zip tie. Therefore, light data from the preliminary trial are not reported. It was originally thought that additions of DI water would be necessary to replace water loss from evaporation in the aquaria, however because of the daily water changes and well-fitted transparent acrylic lids (90x50x0.3 cm), there was little to no change in salinity due to evaporation and additions were not necessary. Acrylic lids also prevented any debris from falling into the water. No analysis was done on the *E. pallida*, they were included in the preliminary trial to make sure that parameters could be maintained even in aquaria containing a bioload, not just in empty aquaria.

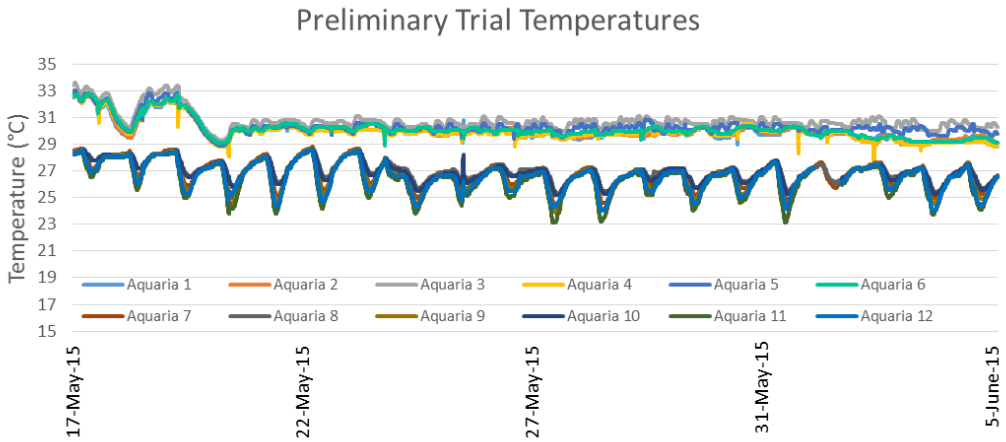

Figure 15: Preliminary trial experimental temperatures. Temperatures for the preliminary trial were reported during the 20-day treatment exposure. There were four treatments in total: elevated temperature/control offshore water (aquaria 1,3,6), elevated temperature/discharge water (aquaria 2,4,5), control temperature/control offshore water (aquaria 8,9,12), and control temperature/discharge water (aquaria 7,10,11).

Sample collection: baseline, in-situ conditions for collected coral colonies

Coral colonies collected from Breakers Reef were all chosen based on being roughly 144 cm^2 and their having no visual signs of declining health (e.g. disease and loss and/or paling of coral tissue). The subsamples taken from all *in-situ* colonies were analyzed to create a baseline condition that was later compared to the analyses from the experimental fragments. The baseline conditions of *in-situ* samples from Trial 1 and 2 indicated that, in terms of symbiont density and photosynthetic pigment content (Table 4), there was not a large difference between Trial 1 and 2, despite collection for Trial 1 occurring on July 1 and on October 1 for Trial 2.

	Trial 1 Baseline Conditions				
	Zoox cm^{-2}	Chl *a* (pg $cell^{-1}$)	Chl c_2 (pg $cell^{-1}$)	Chl *a* (pg cm^{-2})	Chl c_2 (pg cm^{-2})
Mean	1.46E+05	4.43	1.90	6.41E+06	2.76E+06
Std. Deviation	2.36E+05	1.02	0.44	1.31E+06	5.97E+05
Maximum	1.80E+06	7.20	3.09	8.56E+06	3.73E+06
Minimum	1.06E+06	3.33	1.45	4.63E+06	1.85E+06

	Trial 2 Baseline Conditions				
	Zoox cm^{-2}	Chl *a* (pg $cell^{-1}$)	Chl c_2 (pg $cell^{-1}$)	Chl *a* (pg cm^{-2})	Chl c_2 (pg cm^{-2})
Mean	1.36E+06	5.78	2.27	7.68E+06	3.00E+06
Std. Deviation	4.53E+05	1.29	0.48	2.59E+06	1.11E+06
Maximum	2.13E+06	8.90	3.10	1.22E+07	5.12E+06
Minimum	5.84E+05	4.40	1.64	3.04E+06	1.56E+06

Table 4: Baseline conditions for trials 1 and 2. Average, maximum, and minimum values are reported for zooxanthellae density (Zoox cm^{-2}), cellular chlorophyll a and c_2 content (Chl a (pg $cell^{-1}$) and Chl c_2 (pg $cell^{-1}$)), and areal chlorophyll a and c_2 concentrations (Chl a (pg cm^{-2}) and Chl c_2 (pg cm^{-2})).

Experimental trials

During the acclimation period in Trials 1 and 2, the only obvious signs of stress in the corals were mesenterial filaments that appeared on most of the fragments after fragmentation with the tile saw, but disappeared completely after a few days. Other than the one coral removed before the end of the experiment in Trial 2, there were no other observed instances of tissue necrosis or disease in Trials 1 and 2. This study examined effects from sublethal stress where live corals were maintained throughout both experimental trials, this was not a study looking at a controlled death in coral fragments. Overall, there was low variability in temperature (Figure 16), salinity (Figure 17), and light (Figure 18) among aquaria for both trials. The system was housed in a building without air conditioning, and thus received some day/night temperature variations. Temperature fluctuations were greater for Trial 1 (July 3 - August 3 2015) than for Trial 2 (October 16 - November 4 2015), and greater for the control temperature treatments compared to the elevated temperature treatments due to limitations of the external chiller. However, the mean elevated temperatures were 29.5°C for Trial 1 and 29.8°C for Trial 2, and the mean control temperatures were 26.8°C for Trial 1 and 25.2°C for Trial 2, and temperatures between treatments never overlapped. Light intensity across all aquaria that received control offshore water averaged 4101.21 lumens/m² for Trial 1 and 4026.89 lumens/m² for Trial 2 during the day, and lights were shut off overnight. Across aquaria that received discharge water, light intensity averaged 4322.37 lumens/m² for Trial 1 and 4246.94 lumens/m² for Trial 2. Water in aquaria receiving inlet discharge water was visibly, slightly cloudier than in aquaria receiving control offshore water, however according to the data recorded by the HOBO instruments, there was not a large difference

in light intensity between water treatment types. While we could not simulate the ramp up and down in light intensity of a natural sunrise and sunset, these averages are similar to the previously mentioned averages recorded at SLR during summer months. In Trial 2, there was one fragment from the elevated temperature/control offshore water treatment on October 29, 2015 (6 days before the end of Trial 2) that was pulled out of the aquarium, photographed, and samples taken and preserved for later analysis. This fragment had very pale coloring, visible tissue loss, and had not opened its polyps in several days. All samples had to be collected from living coral tissue, and there were only three replicates of an individual coral colony in each experimental treatment. The paling fragment was processed before the end of the trial in order to preserve the ability to obtain samples from living coral tissue. Fragments from that same colony in all elevated temperature treatments, regardless of water type, showed similar signs of stress, but not to the same extent and were still alive at the end of Trial 2.

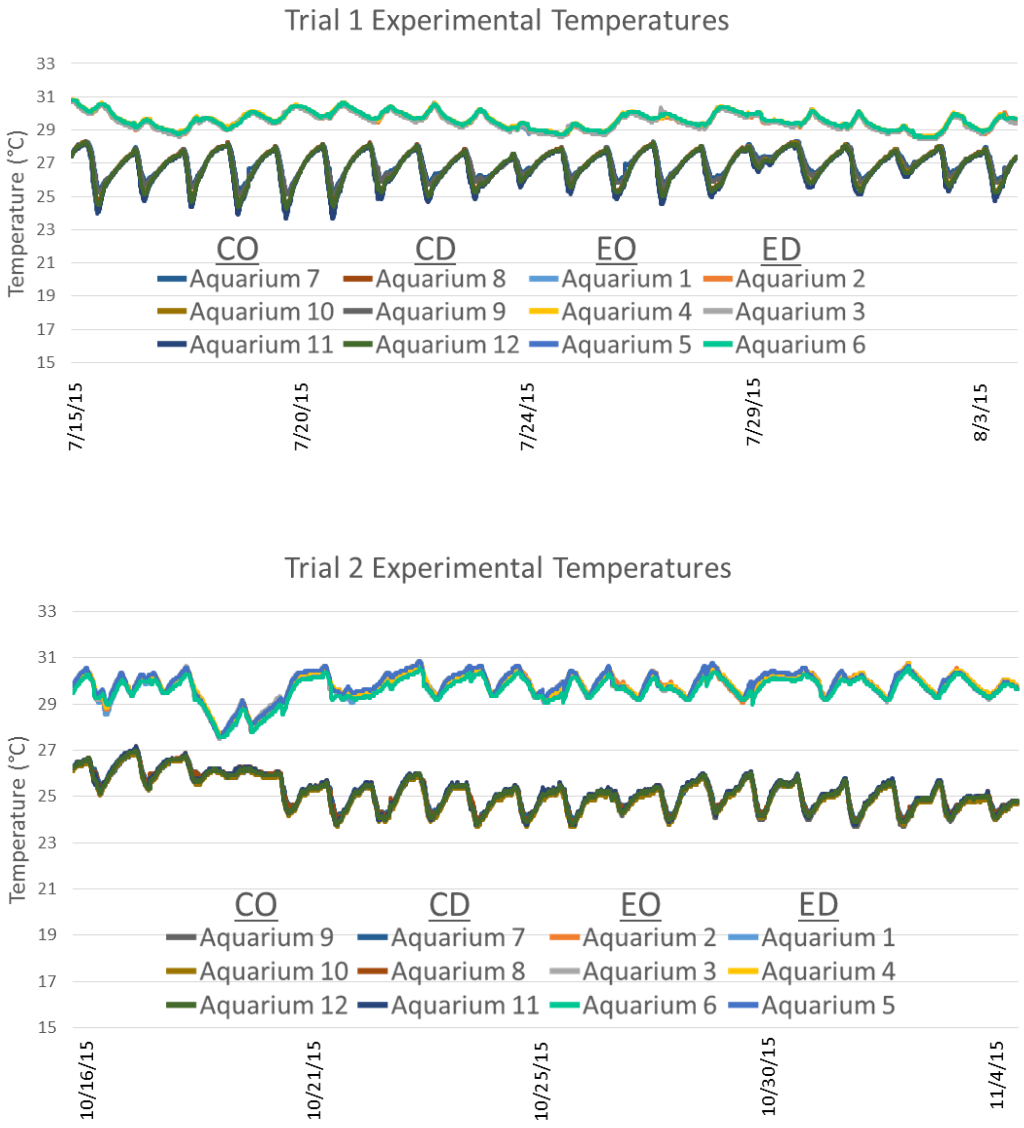

Figure 16: Trials 1 and 2 experimental temperatures. Temperatures for Trials 1 and 2 are reported during the 20-day treatment exposure. There were four treatments in total: control temperature/offshore water (aquarium 7,10,11 Trial 1 and 9,10,12 Trial 2), control temperature/discharge water (aquarium 8,9,12 Trial 1 and 7,8,11 Trial 2), elevated temperature/offshore water (aquarium 1,4,5 Trial 1 and 2,3,6 Trial 2), elevated temperature/discharge water (aquarium 2,3,6 Trial 1 and 1,4,5 Trial 2).

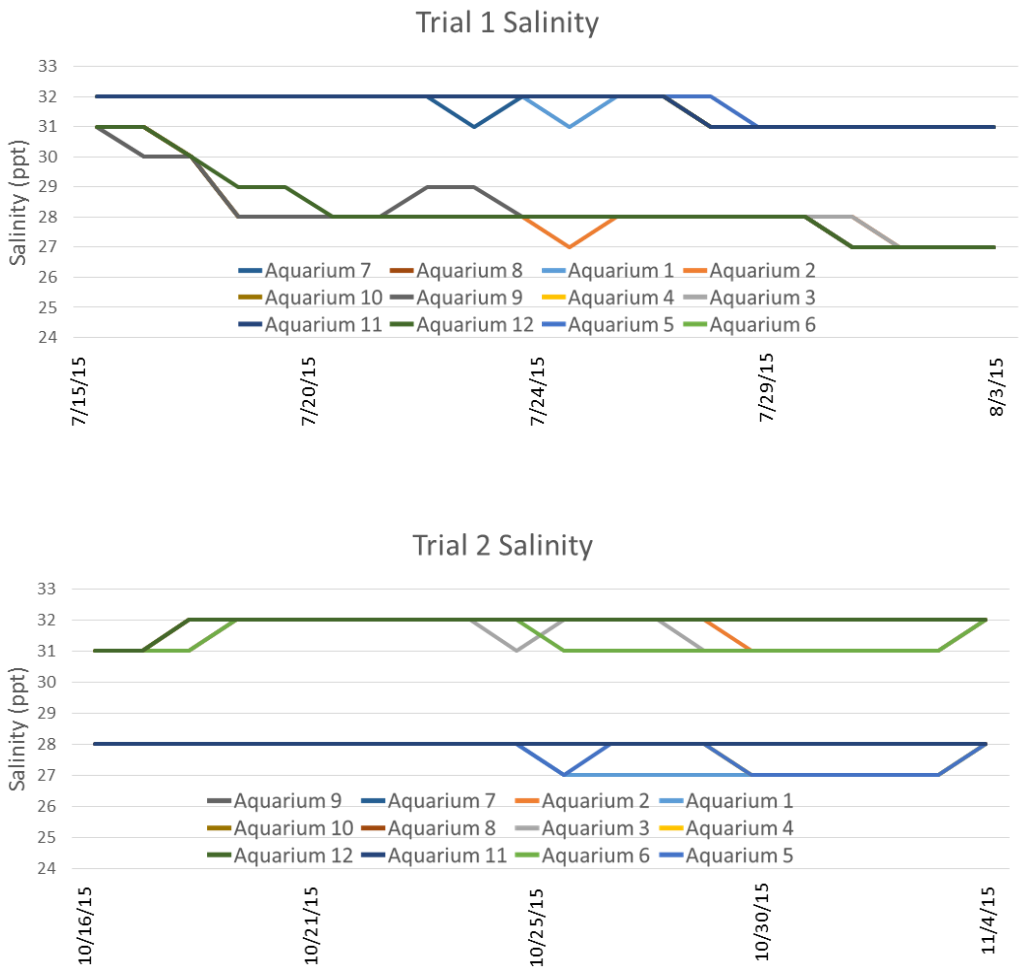

Figure 17: Trial 1 and 2 salinity. Salinities, collected by refractometer, for Trial 1 and 2 are reported during the 20-day treatment exposure. Treatment assignments are reported in Figure 16 caption.

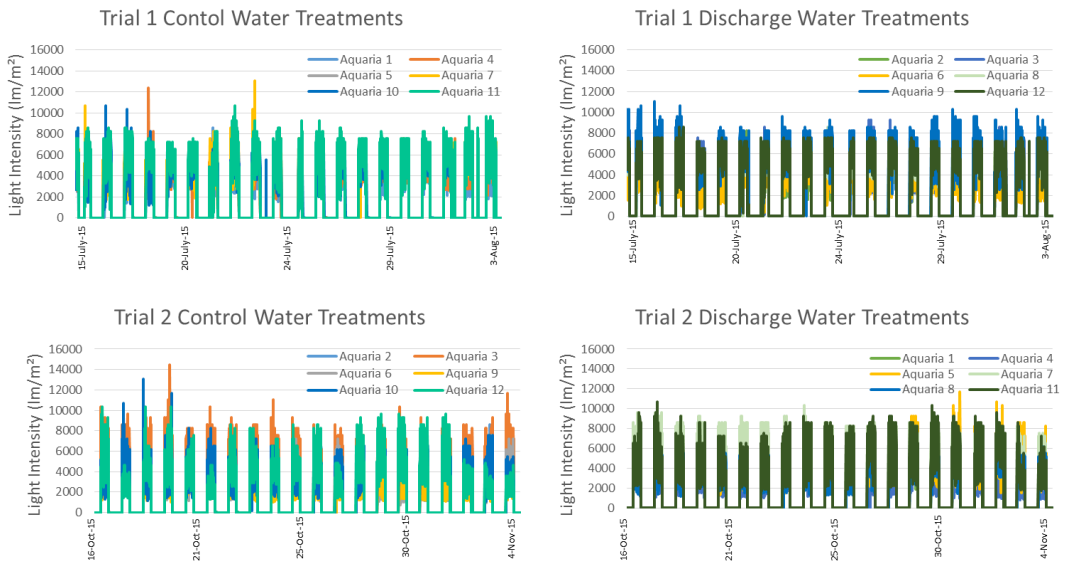

Figure 18: Trial 1 and 2 light intensity. Light intensity, logged by HOBO recorders, for Trial 1 and 2 are reported during the 20-day treatment exposure. Treatment assignments are reported in Figure 16 caption.

Response variables

Treatment effects on zooxanthellae populations and colony effects

The mean and range in response variables for Trial 1 and 2 are reported in Table 5 below for comparison to the range in baseline condition (Table 4). When assessing the overall treatment effects on the suite of zooxanthellae and chlorophyll response variables, we observed some similarities among the two *M. cavernosa* trials (e.g. a consistent effect of temperature) and some variability (e.g. significant interaction effects of temperature and water type in Trial 1). Assessing the observed changes in symbiont density along with cellular chlorophyll content (pg/zooxanthellae cell) and areal chlorophyll concentrations (pg/cm^2 coral tissue) from *in-situ* conditions to after experimental exposure, this study demonstrated significant effects (Table 6) of temperature for both trials (PERMANOVA; trial1: $F=40.02$, $p\leq0.0001$; Trial 2: $F=150.19$, $p\leq0.0001$), and coral colony for Trial 2 ($F=20.34$, $p\leq0.0001$). There were observed significant interaction effects for Trial 1 only: between temperature and water type ($F=7.16$, $p\leq0.008$), between temperature and colony ($F=3.33$, $p\leq0.001$), and between all three factors ($F=5.66$, $p\leq0.0001$).

	Trial 1 Mean and Range of Response Variables				
	Zoox cm^{-2}	Chl *a* ($pg\ cell^{-1}$)	Chl c_2 ($pg\ cell^{-1}$)	Chl *a* ($pg\ cm^{-2}$)	Chl c_2 ($pg\ cm^{-2}$)
Mean	1.43E+06	4.31	1.67	6.11E+06	2.36E+06
Maximum	3.71E+06	7.29	4.97	1.36E+07	7.24E+06
Minimum	7.55E+05	0.71	0.07	7.58E+05	6.54E+04

	Trial 2 Mean and Range of Response Variables				
	Zoox cm^{-2}	Chl *a* ($pg\ cell^{-1}$)	Chl c_2 ($pg\ cell^{-1}$)	Chl *a* ($pg\ cm^{-2}$)	Chl c_2 ($pg\ cm^{-2}$)
Mean	1.43E+06	4.51	1.59	6.30E+06	2.23E+06
Maximum	4.04E+06	8.11	3.19	1.31E+07	4.27E+06
Minimum	4.77E+05	2.28	0.36	1.66E+06	2.65E+05

Table 5: Mean and range of response variables in fragments after experimental trials 1 and 2. Average, maximum, and minimum values are reported for zooxanthellae density (Zoox cm^{-2}), cellular chlorophyll a and c_2 content (Chl a ($pg\ cell^{-1}$) and Chl c_2 ($pg\ cell^{-1}$)), and areal chlorophyll a and c_2 concentrations (Chl a ($pg\ cm^{-2}$) and Chl c_2 ($pg\ cm^{-2}$)).

3-factor PERMANOVA on Zooxanthellae and Chlorophyll												
		Trial1						Trial 2				
Response Variable	*df*	SS	MS	F	R^2	*p*		SS	MS	F	R^2	*p*
Temperature	1	1.37E+14	1.37E+14	40.02	0.153	<0.0001*		3.28E+14	3.28E+14	150.19	0.320	<0.0001*
Water Type	1	1.88E+12	1.88E+12	0.55	0.002	0.475		6.64E+12	6.64E+12	3.04	0.006	0.072
Colony	10	6.33E+13	6.33E+12	1.85	0.071	0.058		4.44E+14	4.44E+13	20.34	0.434	<0.0001*
Temperature x Water Type	1	2.45E+13	2.45E+13	7.16	0.027	0.008*		1.41E+12	1.41E+12	0.64	0.001	0.434
Temperature x Colony	10	1.14E+14	1.14E+13	3.33	0.127	0.001*		1.13E+13	1.31E+12	0.52	0.011	0.902
Water Type x Colony	10	5.95E+13	5.95E+12	1.74	0.066	0.076		1.65E+13	1.65E+12	0.76	0.016	0.693
Temperature x Water Type x Co	10	1.94E+14	1.94E+13	5.66	0.216	<0.0001*		2.43E+13	2.43E+12	1.11	0.024	0.358

Table 6: Results of 2 factor PERMANOVA. Analysis was performed using change (final – initial) in response variables: zooxanthellae density and chlorophyll a and c_2 cellular content and areal concentration. Results are based on Euclidean distances and 9999 permutations. An asterisk (*) denotes a significant result (α=0.05).

Univariate analysis indicated that *M. cavernosa* fragments exposed to elevated temperatures had significantly reduced symbiont density as well as reduced chlorophyll *a* and c_2 cellular content and areal concentrations as compared to fragments exposed to control temperatures in Trial 1 (Table 7; Figure 19). In Trial 2, symbiont density and chlorophyll *a* and c_2 areal concentrations were significantly different between

temperature treatments (Table 7; Figure 19). Coral colony was a significant factor for all response variables in Trial 2. In order to show some of this variability, a graph of the overall mean of areal chlorophyll for all 12 coral fragments in all treatments for each of the 11 colonies in Trials 1 and 2 (Figure 20). The variability is greater between the 11 colonies used in Trial 2 than those used in Trial 1. While coral colony did not meet the significance threshold in the less statistically powerful nonparametric multivariate analysis for Trial 1, parametric univariate analyses indicated that colony was a significant factor for all zooxanthellae and chlorophyll response variables in both trials except on areal chlorophyll c_2 concentration in Trial 1 (Table 7). A significant interaction between colony and water type was also observed among chlorophyll *a* cellular content and areal concentrations in Trial 1, and chlorophyll *a* and c_2 cellular content in Trial 2 (Table 7; Figure 19). There was also a significant interaction effect of temperature and water type in Trial 1 on zooxanthellae density ($F=5.57$, $p \leq 0.021$) and chlorophyll *a* areal concentration ($F=8.98$, $p \leq .004$), but not for Trial 2.

Trial 1: 3-way ANOVA on each response variable											
Response Variable	*df*	Zoox cm^{-2}		Chl *a* (pg $cell^{-1}$)		Chl c_2 (pg $cell^{-1}$)		Chl *a* (pg cm^{-2})		Chl c_2 (pg cm^{-2})	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Temperature	1	16.52	<0.0001*	15.79	0.0001*	19.37	<0.0001*	40.10	<0.0001*	44.50	<0.0001*
Water Type	1	0.47	0.496	0.11	0.745	0.25	0.616	0.49	0.484	0.78	0.380
Colony	10	2.53	0.001*	4.03	0.0001*	2.49	0.011*	2.13	0.030*	0.66	0.760
Temperature x Water Type	1	5.57	0.021*	0.17	0.680	1.20	0.277	8.98	0.004*	0.75	0.389
Temperature x Colony	10	1.40	0.193	4.13	0.0001*	2.31	0.019*	3.33	0.001*	3.75	0.0003*
Water Type x Colony	10	1.04	0.420	4.06	0.0001*	1.31	0.241	2.12	0.031*	0.48	0.898
Temperature x Water Type x Colony	10	3.03	0.002*	5.70	<0.0001*	2.50	0.011*	6.12	<0.0001*	4.50	<0.0001*

Trial 2: 3-way ANOVA on each response variable											
Response Variable	*df*	Zoox cm^{-2}		Chl *a* (pg $cell^{-1}$)		Chl c_2 (pg $cell^{-1}$)		Chl *a* (pg cm^{-2})		Chl c_2 (pg cm^{-2})	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Temperature	1	152.23	<0.0001*	0.04	0.846	0.33	0.569	148.97	<0.0001*	158.77	<0.0001*
Water Type	1	1.07	0.304	0.42	0.517	0.15	0.704	3.42	0.068	0.97	0.327
Colony	10	9.99	<0.0001*	12.11	<0.0001*	20.76	<0.0001*	19.93	<0.0001*	27.88	<0.0001*
Temperature x Water Type	1	0.16	0.690	0.32	0.575	0.04	0.846	0.65	0.422	0.79	0.376
Temperature x Colony	10	0.98	0.465	1.04	0.420	0.95	0.494	0.49	0.893	0.54	0.857
Water Type x Colony	10	1.61	0.117	2.60	0.008*	3.39	0.0009*	0.68	0.744	1.03	0.428
Temperature x Water Type x Colony	10	0.35	0.965	1.52	0.146	1.44	0.176	1.12	0.358	1.37	0.207

Table 7: Results of 2-way ANOVAs for trials 1 and 2. Analyses were performed using change (final – initial) in response variables: zooxanthellae density (Zoox cm^{-2}), chlorophyll *a* and c_2 per zooxanthellae cell (Chl *a* $zoox^{-1}$ and Chl c_2 $zoox^{-1}$), and chlorophyll *a* and c_2 per cm^2 coral tissue (Chl *a* cm^{-2} and Chl c_2 cm^{-2}). An asterisk (*) denotes a significant result (α=0.05).

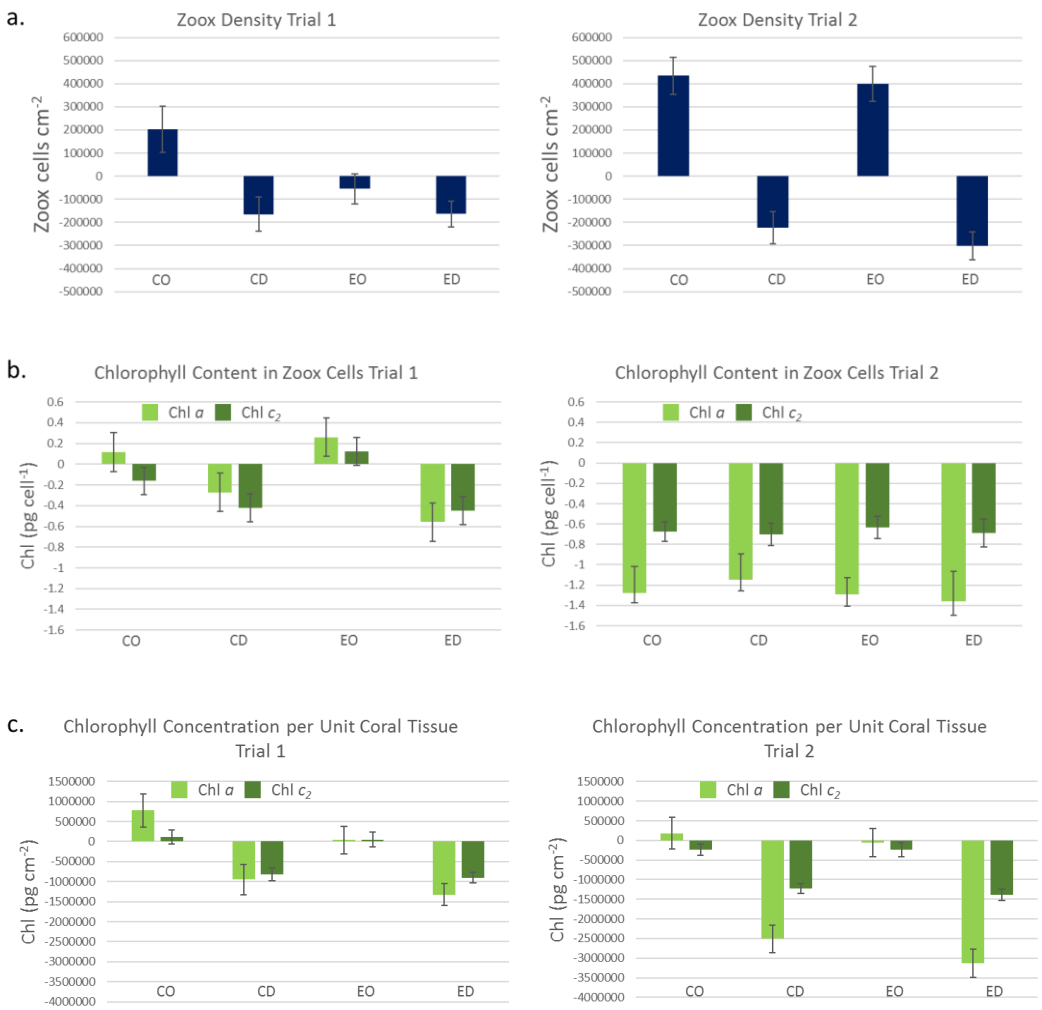

Figure 19: Mean of response variables for the four treatment types in Trials 1 and 2. Each bar represents the mean of the change from in-situ conditions (final-initial) for a certain treatment (CO: control temp/offshore water, CD: control temp/discharge water, EO: elevated temp/offshore water, ED: elevated temp/discharge water). Graphs are separated by response variable: (a.) zooxanthellae density (b.) chlorophyll a and c_2 content per zooxanthellae cell (c.) chlorophyll a and c_2 concentration per cm^2 of coral tissue. Error bars indicate standard error.

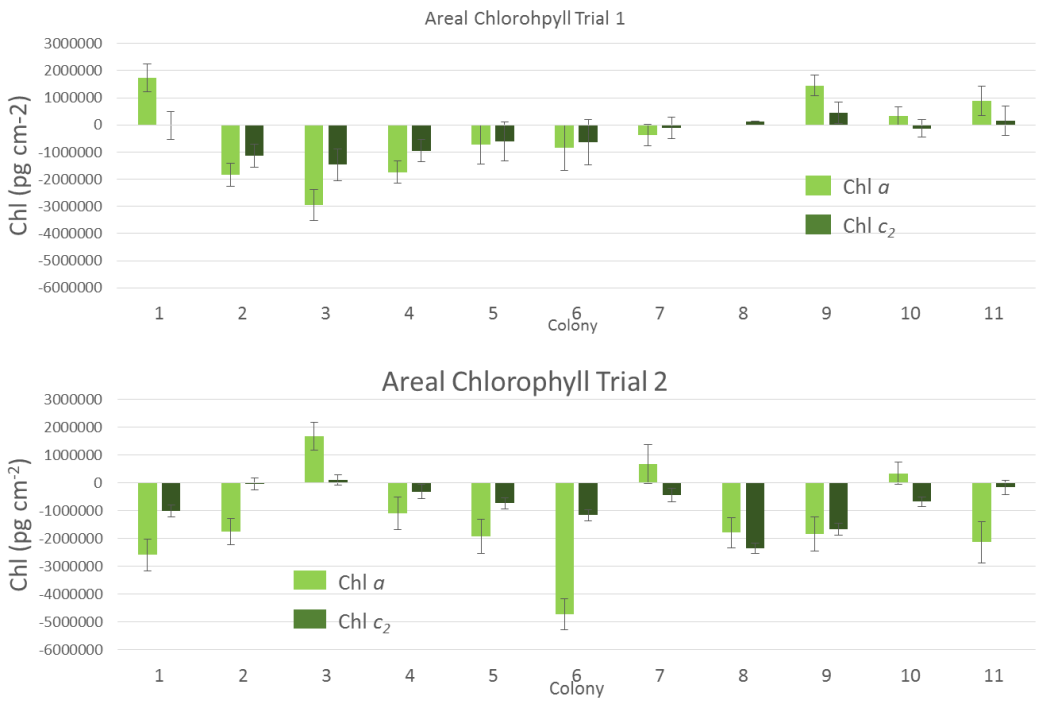

Figure 20: Overall mean change of areal chlorophyll concentration by colony. The overall mean change (final – initial) of areal chlorophyll a and c_2 concentrations for all 12 coral fragments in all treatment types for each of the 11 colonies use in (a.) Trial 1 and (b.) Trail 2.

A series of multi-dimensional scaling (MDS) plots were constructed that show the same general trends in effects from temperature, water type, and colony. MDS plots are a way to look at the amount of similarity between two data points, where the closer two points fall to each other on the plot, the more similar they are. When organized by treatment type, MDS plots show that for both Trial 1 and 2 there is a slight grouping of the data points by temperature, but not by water type (Figure 21). When the same MDS plots are labelled by colony instead, there are no obvious trends in colony effects in Trial 1 (Figure 22a), however there are definite patterns of grouping in Trial 2 that suggest colony would have a significant effect (Figure 22b).

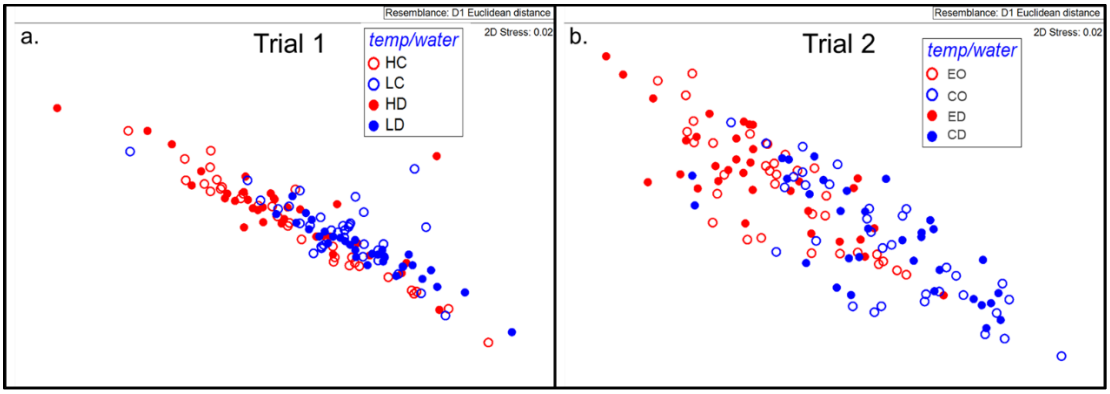

Figure 21: MDS plots of experimental coral fragments labelled by treatment type. MDS plots show a stronger correlation among samples exposed to different temperatures than samples exposed to different water types for both (a.) Trial 1 and (b.) Trial 2. The four treatments are EO: elevated temp/offshore water, CO: control temperature/offshore water, ED: elevated temp/discharge water, and CD: control temp/discharge water.

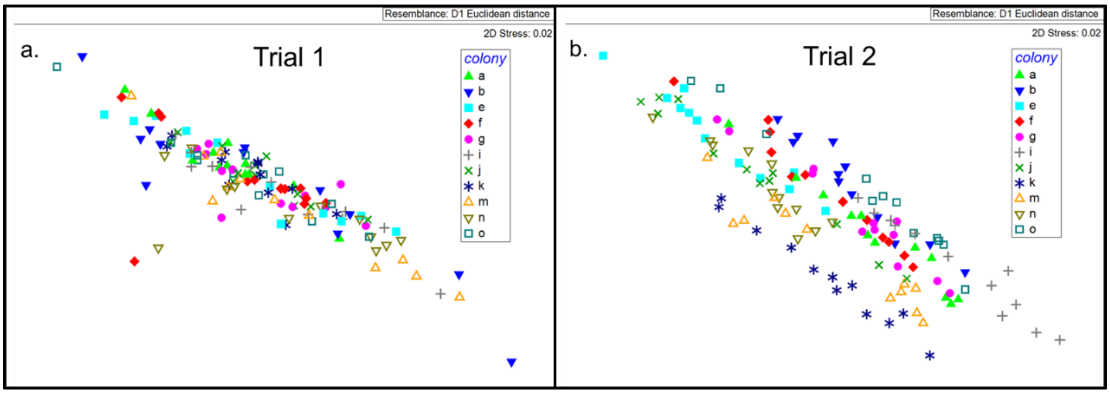

Figure 22: MDS plots of experimental coral fragments labelled by colony. MDS plots show effects from colony are not as strong in (a.) Trial 1 as they are in (b.) Trial 2.

Water quality and nutrient analysis

Water samples for nutrient analysis were taken from next to the LOBO located in the St. Lucie Inlet one day after inlet discharge water for Trial 2 was collected from the same location. The water collected contained the following concentrations: nitrate+nitrite nitrogen (NOx-N) at 2.87µM, nitrate (NO_3) at 2.31 µM, and phosphate (PO_4) at 1.99 µM (Figure 3). The LOBO recorded increased nitrate and phosphate concentrations as well as decreases in salinity in the St. Lucie Inlet in September and October of 2015, coinciding

with low-salinity releases from the C-44, C-23, and C-24 canals (DBHYDRO database, SFWMD; fau.loboviz.com; Figure 3). The LOBO also recorded fluctuations throughout the day in both nitrate and phosphate concentrations, with higher concentrations occurring at low tide. This could be due to the fact that at low tide there is the least offshore oceanic water coming into and mixing with the inlet water. Phosphate concentration in the sampled water was very close to that recorded by the LOBO at the same date and time. Nitrate concentrations were lower in the sampled water than were recorded by the LOBO, however according to the LOBO, the concentration dropped to the same level after a few hours. The discrepancy in nitrate concentration could be due to different levels in the water column depending on how the discharge water was mixing with the oceanic water in the inlet.

Coral transplants

Corals from Trial 1 collected on July 1, 2015 (Figure 23a) were transplanted to SLR on July 7, 2015 near the site where Odyssey data loggers were being maintained at the Central reef site (Figure 2). The transplants were visited and photographed on October 15, 2015 and July 5, 2016 (Figure 23b) in order to monitor their status for visual signs of stress. Transplantation at SLR seems to be feasible, as there were no visible signs of disease, tissue necrosis, or bleaching after one year.

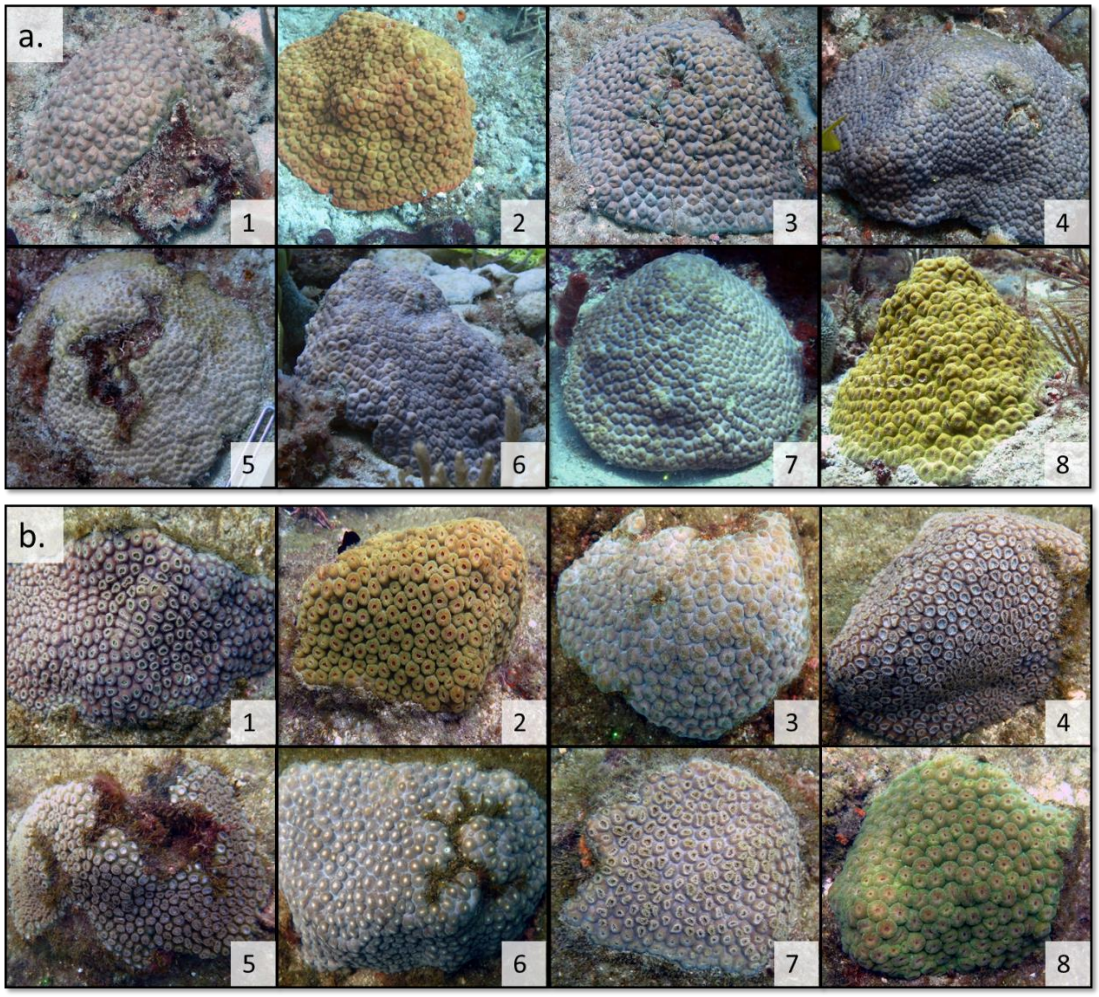

Figure 23: Transplanted M. cavernosa colonies. (a.) Images of eight M. cavernosa colonies at Breakers Reef on July 1, 2015 before being samples, collected, and fragmented for Trial 1. (b.) Images of those same 8 coral colonies on July 5, 2016 one year after being transplanted to SLR. Matching numbers correspond to the same colony.

DISCUSSION

Using *ex-situ* experimentation combined with *in-situ* monitoring can help reveal organismal responses to concomitant environmental stressors. Understanding such responses in habitats like the Florida Reef Tract is important in effectively managing resources and minimizing stress in coastal ecosystems. In this study, *M. cavernosa* exhibited significant differences among symbiont densities and chlorophyll concentrations between temperature treatments. While exposure to elevated temperatures (30°C) for three weeks resulted in reductions in symbiont densities and chlorophyll *a* and c_2 concentrations, corals incubated in near ambient temperatures (25°C) demonstrated no changes or increases in zooxanthellae density.

Treatment effects on zooxanthellae populations

Elevated temperature treatments

There was a general trend across elevated temperature treatments where reductions in zooxanthellae density and cellular chlorophyll content lead to an overall decrease in chlorophyll per unit coral tissue. These results reinforce previous studies that have reported corals being affected by thermal stress (Douglass 2003, Bhagooli and Hidaka 2004, Berkelmans and van Oppen 2006, Fujise et al. 2014), however the data does not indicate that discharge water, at the exposure level used in this project, causes stress at an elevated temperature of 30°C. Reduced zooxanthellae density and/or decreased chlorophyll pigmentation in corals as a result of thermal stress has been demonstrated in previous experiments and field studies. In some studies, corals exposed

to thermal stress (30°C) have been reported to release more photosynthetically degraded zooxanthellae cells compared to corals that were not thermally stressed (27°C), suggesting that the inability of the coral host to keep up with an increasing accumulation of damaged zooxanthellae after thermal stress exposure may contribute to coral bleaching mechanisms (Fujise et al. 2014). A significant, temperature dependent increase in the rate of symbionts being expelled from the host coral has also been reported when corals are exposed to ≥30°C, as compared to corals at 27°C (Hoegh-Guldberg and Smith 1989). The corals in the present study, whether exposed to control offshore water or inlet discharge water, showed significant effects in both the number of zooxanthellae cells and the total concentrations of chlorophyll *a* and c_2 pigments within the coral tissue under elevated temperatures. Elevated temperature has been linked to an inability of photosystem II in the zooxanthellae cells to repair damage from light irradiance (Takahashi and Murata 2008). Zooxanthellae cells, both damaged and undamaged, can then be expelled from the host coral (Brown et al. 1995) leading to decreased symbiont density. We did not quantify symbiont densities in the aquaria to verify expulsion as the mechanism of zooxanthellae reduction. An alternative mechanism could simply be degradation of zooxanthellae cells within host coral tissue. For the experimental factor ranges used in this study, thermal stress was a stronger driver of changes in zooxanthellae populations within corals than exposure to estuarine discharge water. On the reef, estuarine discharge at SLR can increase turbidity, thus reducing the amount of light reaching corals (Beal et al. 2012) that already have reduced resiliency due to thermal stress. If the corals at SLR have already reduced symbiont populations during the warmer months of the year,

exposure to estuarine discharge and subsequent shading of the colonies could reduce photosynthetic production and affect coral growth.

Control temperature treatments

In the control temperature treatments, there was a difference between Trial 1 and Trial 2. In Trial 1 control temperature treatments, significant interactions between temperature and water type were reported by both the PERMANOVA and ANOVA analyses. There were increases in both symbiont density and areal chlorophyll *a* concentration in the corals exposed to offshore water and no thermal stress which were not mirrored in the corals exposed to discharge water and no thermal stress. While elevated temperatures caused reduced zooxanthellae density and chlorophyll *a* and c_2 concentrations, in the control temperature treatments, the zooxanthellae density and areal chlorophyll *a* concentration responses varied based on water treatment.

In Trial 2, there was an average increase in symbiont density and decrease in cellular pigment content regardless of the type of water. One possible explanation could be a photoacclimatory response to increased light. Corals experienced average light levels in the aquaria that were characteristic of SLR, but slightly higher than average levels at Breakers Reef where the corals were collected. Decreases in pigment concentration unaccompanied by loss of zooxanthellae cell has been seen in corals in response to increased irradiance and UV levels (Lesser et al. 1990). If light regimes within the aquaria were higher than at Breakers Reef, but not beyond a photoinhibitory threshold, then it could explain the increased zooxanthellae densities. The amount of light reaching individual symbionts can also be reduced in coral tissues with symbiont densities high enough to cause self-shading (Kaniewska et al. 2011; Cunning and Baker 2014), which

could explain the decrease in cellular pigment seen in Trial 2 control temperature treatments. Lower zooxanthellae densities and increased chlorophyll *a* and c_2 concentrations were observed in corals transplanted into deeper habitats, believed to be a photoadaptation to lower light levels with depth (Kaiser et al. 1993). The opposite response, increased symbiont density and decreased cellular chlorophyll content, were seen in Trial 2 in the corals from Breakers Reef after they were put in the aquaria with increased light levels. However, this same pattern was not seen in Trial 1, suggesting there could be a difference in the type of discharge water used between the two trials. Trial one demonstrates that discharge water may have a direct effect in reducing zooxanthellae density, but this effect is masked or insignificant once thermal stress thresholds are exceeded. The differential effect in the control temperature treatments was not observed in Trial 2, which could suggest that this estuarine water had less negative impacts on zooxanthellae abundance than trail 1 water.

The type of inlet discharge water used in the trials seemed to elicit different responses in the corals, and could possibly explain the significant interaction factor between water type and thermal stress seen in Trial 1 only. In Trial 1, water was taken straight from the locks in the C-44 canal and mixed with water from the St. Lucie Inlet after a period of little to no freshwater discharges and very low precipitation. Water quality analysis was not done on this water, however it is likely that the long retention time in the canal without discharges from Lake Okeechobee allowed for higher nutrient levels in the stagnant canal water, which has been shown to have higher nitrogen and phosphorous concentrations than the lake water (FDEP 2001). Also, being retained in the canals could allow the water to percolate through the canal sediment, which in the C-23

canal has been shown to contain concentrations of copper and lead high enough to possibly be toxic to benthic organisms (FDEP 2000). The increasing nutrients in the canal have reflected the change in the surrounding land from wetlands to agricultural and residential areas, and these nutrients ultimately end up in the St. Lucie Estuary when water is released from the canal (FDEP 2003). In Trial 2, water was taken from the St. Lucie Inlet 5 days after a large release of freshwater through the C-44 canal, and mixed with water obtained from FOS. Nitrate and phosphorus levels have been recorded at significantly higher levels in the St. Lucie Estuary during large discharges from the C-44 canal compared to times with little freshwater discharged from the canal (Lapointe et al. 2012). There have also been higher levels of dissolved inorganic nitrogen, total dissolved phosphorus, and higher percent cover of algal communities on SLR than coastal areas in counties further south (Beal et al. 2012). The LOBO stationed in the St. Lucie Inlet recorded increases in nitrate and phosphate concentrations and decreased salinity in the Inlet coinciding with large freshwater releases from the C-44, C-23, and C-24 canals (fau.LOBOviz.com; Figure 3), during which time inlet discharge water was collected for Trial 2. Nutrient analysis done on water taken from near the same LOBO on September 24, 2015 reported levels within the same ranges (Figure 3).

Water taken from the C-44 canal was diluted with water from the St. Lucie Inlet before being used in Trial 1, however there was still a significant interaction between temperature and water type in that trial only. Compared to a lack of significant interaction in Trial 2, this could indicate a possible threshold was exceeded in Trial 1 and not Trial 2, where increases in nutrient concentrations begin to affect corals also exposed to increased temperatures. Negative effects of elevated temperatures and increased nutrients combined

has been recorded on primary production rates (Nordemar et al. 2003) and zooxanthellae densities (Schlöder and D'Croz 2004) in corals previously. Knowing this threshold would be imperative to local adaptive management strategies, keeping the freshwater discharge volume and timing, and concomitant nutrient exposure, below levels that could potentially negatively affect coral reef habitats during warmer, summer months.

Colony effects

Coral colony was a significant factor in Trial 2, but not in Trial 1. At elevated temperatures (30 and 32°C), genetically unique individuals of the species *Porites porites* have shown significantly different rates of zooxanthellae loss (Edmunds 1994). Subcladal genetic differences can vary in their tolerance to thermal stress and also cause intraspecific differences in responses to sub-lethal stress (Sampayo et al. 2008). If differential responses among coral colonies is expected, impact experiments and field studies should include high replication and colony-specific information such as coral genotyping and zooxanthellae typing in order to fully represent how a species responds to stress. Because including such information would require sampling from many colonies, steering toward non-consumptive sampling whenever possible would be ideal for coral preservation. For example, PAM flourometry could be used to measure photosynthetic efficiency instead of zooxanthellae counts that require removing a fragment of coral from the colony. Due to the potential for intraspecific variation in resilience to thermal stress, genotyping these colonies could tell us more about the underlying mechanisms of significant effects of individual colony on the response variables, and whether or not some of the corals within the *M. cavernosa* population at SLR are more likely to survive environmental fluctuations.

Coral transplants

This project also had success in transplanting corals between differing environments. Corals were taken from Breakers Reef, where they were existing at roughly 15 m, in relatively clear waters, without apparent exposure to estuarine discharge. These corals were transplanted to SLR, where they would have to adapt to living at 5 m depth, in waters with less clarity than at Breakers Reef, and being exposed to estuarine discharge and summer upwelling events. The period from May to October of 2016, after the corals were transplanted to SLR, was a time of almost constant discharge from the C-44, C-23, and C-24 canals into the St. Lucie Estuary (SFWMD, DBHYDRO database). Despite the change in environment and reduction in water quality, repeated monitoring of these colonies has shown that they are surviving quite well at SLR, with no visible tissue loss or bleaching since July of 2015. Corals grown in labs or coastal nurseries are often transplanted out onto reefs to help bolster coral reefs damaged from storms, anthropogenic influences, disease, and bleaching and mortality events (Shafir et al. 2006). Such active restoration activities are becoming more prevalently used where more passive conservation and management methods have not sufficiently enhanced reef quality after long-term degradation (Rinkevich 2014). While there are still many factors to work out in nursery design to improve survivorship of transplants once they are back on a reef, there has been some promising short-term successes along the Florida Reef Tract (Herlan and Lirman 2008; O'Donnell et al. 2017), in the greater Caribbean (Bowden-Kerby and Carne 2012), and even across the globe (Shaish et al. 2008; Nakamura et al. 2011; Mbije et al. 2013). *M. cavernosa* is even being used in some nurseries, and has shown higher survival rates among colonies that had become dislodged

and were transplanted to nurseries compared to becoming dislodged and left on the reef (Monty et al. 2006). However, transplanting corals often means taking organisms that were nurtured in a relatively sheltered environment and provided optimal growth conditions, and moving them to a reef with potentially poorer water quality and less optimal conditions. While the corals transplanted in this project are a single example in a unique environment, the fact that after one year at SLR there are no visible signs of bleaching, disease, or tissue necrosis is worth noting when considering local restoration efforts.

Conclusions

Corals at St. Lucie Reef are living at a latitudinal limit for the Florida Reef Tract, and are exposed to seasonal freshwater discharges that occur during the warmer months of the rainy season. Even moderate stress (i.e. 1-2°C above natural, ambient temperatures), when endured for prolonged periods of time, can cause enough stress to corals to cause bleaching (Fujise et al. 2014), and the presence of other possible environmental stressors, such as estuarine discharges, can decrease recovery potentials of corals. This would most drastically affect coral reefs such as SLR, existing at extremes and heavily influenced by anthropogenic and environmental changes. In order to properly manage a coral reef like St. Lucie Reef, a deep understanding of the ecological complexity at SLR is necessary. This project builds understanding, by providing information regarding the potential impacts of anthropogenic changes to and management of the South Florida watershed on corals in adjacent coastal zones. While this study did not identify causative agents of stress from estuarine discharge, it demonstrated the efficacy of diagnostic techniques to assess coral stress in combination with *in-situ*

environmental monitoring to determine coral responses to exposure to estuarine discharge and thermal stress. It has shown that elevated temperatures can be detrimental to holobiont functioning, which should be taken into account when scheduling South Florida watershed maintenance. Possible management actions could include proactive releases from the South Florida canals if a wet summer is predicted, avoiding the compounded stress of large amounts of low-salinity water in the estuary and on the reef during the warmer months of the summer when elevated temperatures are affecting the corals at St. Lucie Reef.

Globally, coral reef survival may depend on their ability to adapt to long-term changes in their environment. Corals along the Florida Reef Tract are faced with similar stressors as coral reefs globally, and a better understanding of how these stressors interact with one another and the effects they elicit in corals is necessary to reduce compounding anthropogenic influences on top of coral reefs already struggling to persist. Elevated temperatures are one of the most influential threats to coral reefs today (Hoey et al. 2016), and this study showed that elevated temperatures reduced zooxanthellae densities and chlorophyll concentrations in host corals. Corals may have a limited capacity to adapt to globally elevated temperatures based on their zooxanthellae assemblages (Chen et al. 2003), genotype (Sampayo et al. 2008), or differential expression of thermal tolerance genes (Barshis et al. 2013), and have rebounded after periods of environmental change in the past (Pandolfi and Kiessling 2014). Even if corals can adapt to an increasingly changing environment, concurrent anthropogenic stress can reduce survival and recovery (Doney et al. 2012; Hoey et al. 2016), displayed by this study in the significant effect low-salinity, estuarine discharge water in conjunction with elevated temperatures had on

zooxanthellae populations in Trial 1. This project also demonstrated short-term successes in site-to-site coral transplantation and the resilience of colonies even when moved onto a reef with increased environmental variability and stress. Pilot transplant projects to assess resilience may be an effective mechanism to gauge the potential success of restoration efforts whether they be site-to-site transplants, or from coral nurseries.

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