Assessment of Coral Stressors on St. Lucie Reef: Florida's Northernmost Coral Reef

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Summary

The main objective of the project was to assess and define the effects (stressors) of acute blackwater events from the St. Lucie River on coral health at the 4.5-mile long St. Lucie Reef during low (dry season) versus high (wet season) levels of precipitation with associated varying influences from river/estuarine water discharge. This project used bacterial community profiling, gene expression, and reproductive histological methods to quantify and compare coral health on St. Lucie Reef overtime. Light and temperature data collected on the reef were compared with data collected at the inlet to assess the relative contributions of multiple environmental parameters over time and space. Furthermore, data from St. Lucie Reef corals were compared to those from the Lower Florida Keys and Lee Stocking Island, Bahamas. By characterizing the current condition and ecology of this reef, this project provides baseline data for adaptive management of ongoing and proposed regional restoration efforts.

Introduction

Coral reefs are economically and ecologically important resources in Florida, providing valuable ecosystem services in terms of productivity, diversity, tourism, fishing, and coastal protection. Natural and anthropogenic stressors have contributed to a decline in coral reefs worldwide, with an estimated 80% loss of coral cover in Florida and the Caribbean since the 1970s (Gardner et al., 2003). Dramatic land-use changes in Florida have occurred over the past 100 years, resulting in increased run-off of pollution, nutrients, and freshwater, exposing coral to further anthropogenic stressors (Kautz et al., 2003). Coral reef communities found in close proximity to riverine outflows face a unique suite of stressors. Depending upon key hydrodynamic conditions such as flow, tide, and wave action, the gradient effects of land-based sources of pollution can be acute or chronic along the reef tract. Understanding the role of these effects and abating their impacts are of critical importance to the future of coral reef communities. Riverine outflow influences coral reef communities by reducing Photosynthetically active Radiation (PAR) through increased color and turbidity, inducing osmotic stress through variations in salinity, altering ecological balance through increased nutrient concentrations, and by introducing a host of xenobiotic pollutants from urban and agricultural sources such as heavy metals, hydrocarbons, pesticides, and herbicides (see reviews by Fabricius 2005; Jameson and Kelty 2004). The synergistic effects of these stressors greatly influence coral health and coral reef community productivity.

Quantifying reef community stress using various techniques has been a recent focus of coastal resources managers worldwide to quantify populational and organismal responses to stressors (Fabricius 2005; Hoegh-Guldberg & Jones 1999; Maxwell & Johnson 2000). Understanding the role and relative contribution of each stress factor is critical to the protection and restoration of these declining habitats (Anthony & Connolly 2004). Newly-developed independent, but complementary, technologies such as coral DNA microarray gene expression and length-heterogeneity PCR bacterial community profiling have proved to be useful tools for quantifying coral health (Edge et al. 2005; Morgan et al.

2001; Morgan et al. 2005; Snell et al. 2003; Mills et al. 2006; Voss et al. 2007). DNA microarrays provide a unique tool for investigating the expression of multiple coral genes related to environmental and physiological stresses. Because changes in gene expression can precede, underlie, and control changes in the physiological well-being and functional adaptation of an organism (Tsuji et al. 2000), changes in gene expression are logical foci for characterizing short-term responses to environmental variables. This research identifies stress at a sub-lethal level (i.e., in the absence of active tissue loss or bleaching) and can aid resource managers in decision making by prioritizing the stressors impacting particular coral reef ecosystems to formulate measures to abate coral health degradation. The microarray has been developed to study how corals respond to natural and anthropogenic stressors including various toxicant exposures, salinity, ultraviolet radiation and thermal stress in terms of the relative expression of genes with known metabolic roles (Edge et al., 2005; Morgan et al., 2005; Edge, 2007).

Stress operates at the organismal level, but is reflected at population and ecosystem levels; thus, measuring sub-lethal stress of individuals within a population can indicate the future of an ecosystem if mitigation efforts are not in practice. Molecular biomarkers are valuable early diagnostic tools to measure detrimental changes in an organism's physiological state due to chemical, physical or biological stress. Corals harbor bacterial symbionts, particularly in their mucus layer. These bacterial assemblages are generally consistent on individual coral species, and some coral associated microbes are known to harbor antimicrobial properties (Ritchie 2006) that potentially protect corals from infection. Shifts in bacterial community profiles, and comparison over time and space, can provide information on the underlying mechanisms driving coral health. Length heterogeneity polymerase chain reaction (LH-PCR) is a bacterial community profiling technique that differentiates organisms based on the natural variation in the sequence lengths of 16S rRNA genes. LH-PCR profiling provides relative abundance measures of 16S rDNA types to permit rapid, robust comparisons of whole microbial community samples (Mills et al. 2006) and has previously been optimized by for coral disease investigations (Voss et al. 2007). Corals are known to harbor predictable bacterial communities (Rohwer et al. 2002), and deviations from this normal or healthy condition indicate 1) significantly stressed coral colonies, and 2) the potential mechanisms of stress including temperature, salinity, sedimentation, nutrients, Dissolved Organic Carbon, and disease. Furthermore, LH-PCR permits rapid comparisons of similarities between disease- and mucus-associated bacteria communities among sites and regions, providing relative measures of coral health across time and space. In this manner, the collection of coral mucus samples over wide geographic areas is used to develop a unique coral health diagnostic.

Study Objectives

1). The main objective of the project is to assess and define the effects (stressors) of acute blackwater events from the St. Lucie River on coral health at the 4.5-mile long St. Lucie Reef during low (dry season) versus high (wet season) levels of precipitation with associated varying influences from river/estuarine water discharge. In addition this project will determine the relative health of corals on St. Lucie Reef versus those in the Lower Florida Keys and Lee Stocking Island, Bahamas. Finally, the reproductive condition of corals on St. Lucie Reef will be assessed during the spawning season. This will be accomplished via tissue coral sampling and various analyses of the samples.

2). By characterizing the current condition and ecology of this reef, this project will provide data for adaptive management of ongoing and proposed regional restoration efforts by producing a final report and publishing findings and associated management recommendations. The coral program at HBOI/FAU is committed to developing these novel diagnostic tools and providing information that resource managers need to conserve and protect coral reef ecosystems worldwide.

Methods

The Robertson Coral Reef Program (RCRP) at Harbor Branch Oceanographic Institution at Florida Atlantic Univ. (HBOI-FAU) has a state-of-the-art microarray and molecular analysis facility as well as expertise in designing and using DNA microarrays to assess coral stress gene expression. Previous and ongoing projects have investigated coral gene expression patterns in response to disease, temperature, UV, salinity, and land-based sources of pollution from dredge activities in Florida and a municipal dump in Bermuda (Edge et al. 2005, Morgan et al. 2005). The program has also developed LH-PCR profiling techniques specifically designed to understand coral mucus bacterial community ecology and stress response mechanisms (Mills et al. 2006; Voss et al. 2007).

Study Site

St. Lucie Reef in northern Martin County, east-central Florida, contains 21 scleractinian coral species, 100 other invertebrates, 23 algal species, 4 sea turtle species, and ~250 fish species within the 4.5-mile long reef tract. The northern third of the reef, proximal to St. Lucie Inlet, contains predominantly wormrock (*Phragmatopoma lapidosa*) and scleractinian corals with generally increasing diversity and abundance of corals (and other organisms) from north to south, likely the result of freshwater influence from the adjacent inlet. The reef is encompassed within Florida's State Park system, extending 1.23 miles offshore, and assessing the effects of freshwater runoff is a goal of Park managers (FDEP 2002). The reef "represents the absolute known northern limit of several species of hard and soft tropical reef corals" (Reed 1982) and was therefore proposed for protection as an Atlantic Hardbottom Sanctuary in 1982. These hardbottom communities also represent the northern limit of the Southeast Florida Coral Reef Initiative (SEFCRI), designated to assess threats and to promote protection/awareness of reefs through the US Coral Reef Task Force (Collier et al. 2008). Numerous partners have conducted resource management activities over the past decade including: buoy installation; marine debris removal (Herren et al. 2007); REEF's Great Annual Fish Count; annual assessment of bleaching through The Nature Conservancy's Florida Reef Resiliency Program (Wagner et al. 2010); and inclusion in the Southeast Coral Rees Evaluation and Monitoring Program (SECREMP) effort starting in 2006 (Gilliam 2011). In 2006, regional resource managers conducted the first St. Lucie Reef Symposium for 70 participants. During 2009, cores of the largest corals on the reef were taken to assess coral extension, density, and calcification history through a partnership with FWC and Nova Southeastern Univ. (Beal and Helmle, unpubl. data).

The St. Lucie Reef is strongly influenced by freshwater discharges through the St. Lucie Inlet (Fig. 1) from the St. Lucie River (with Lake Okeechobee providing a third of the river volume over the POR 1965-2005 via the C-44 canal connection) and its widely fluctuating water quality (Chamberlain &

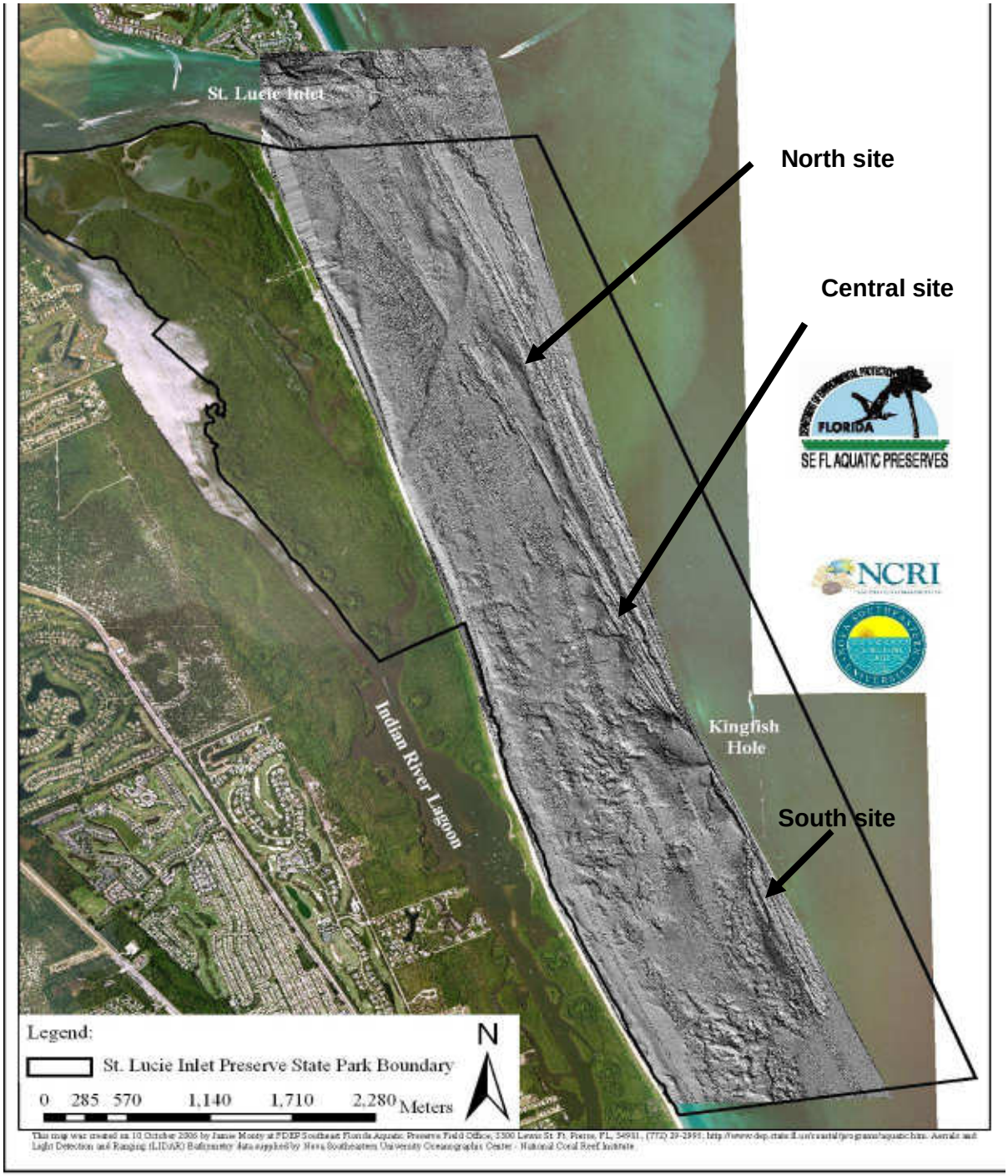
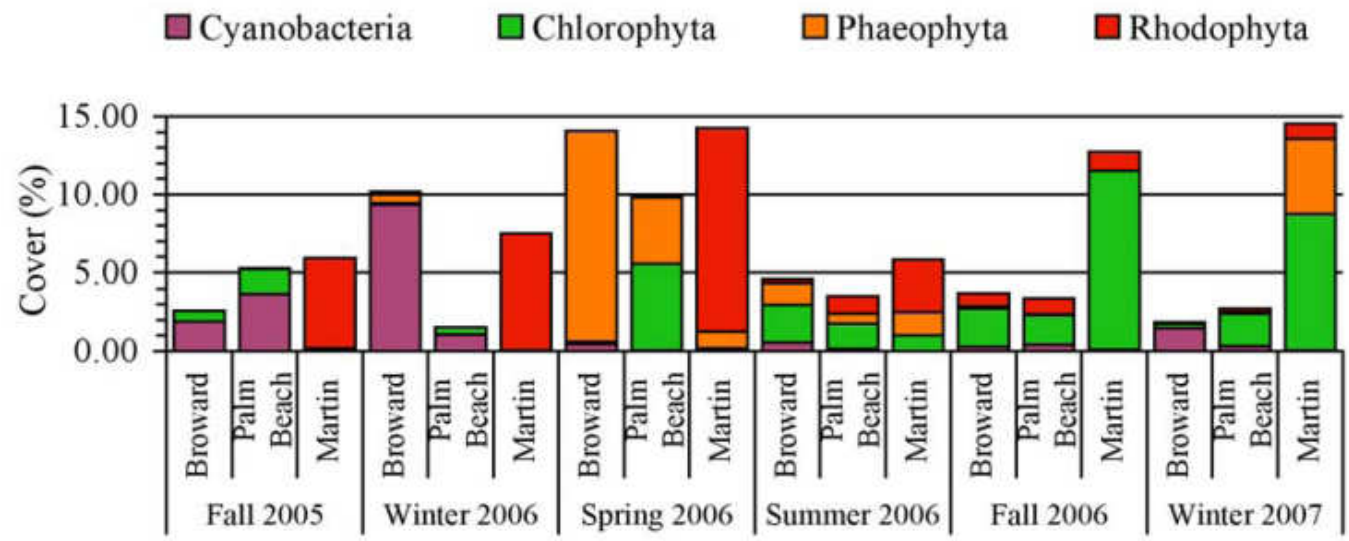


Fig. 1. LIDAR image of St. Lucie Inlet Preserve State Park boundary and three coral sampling stations.

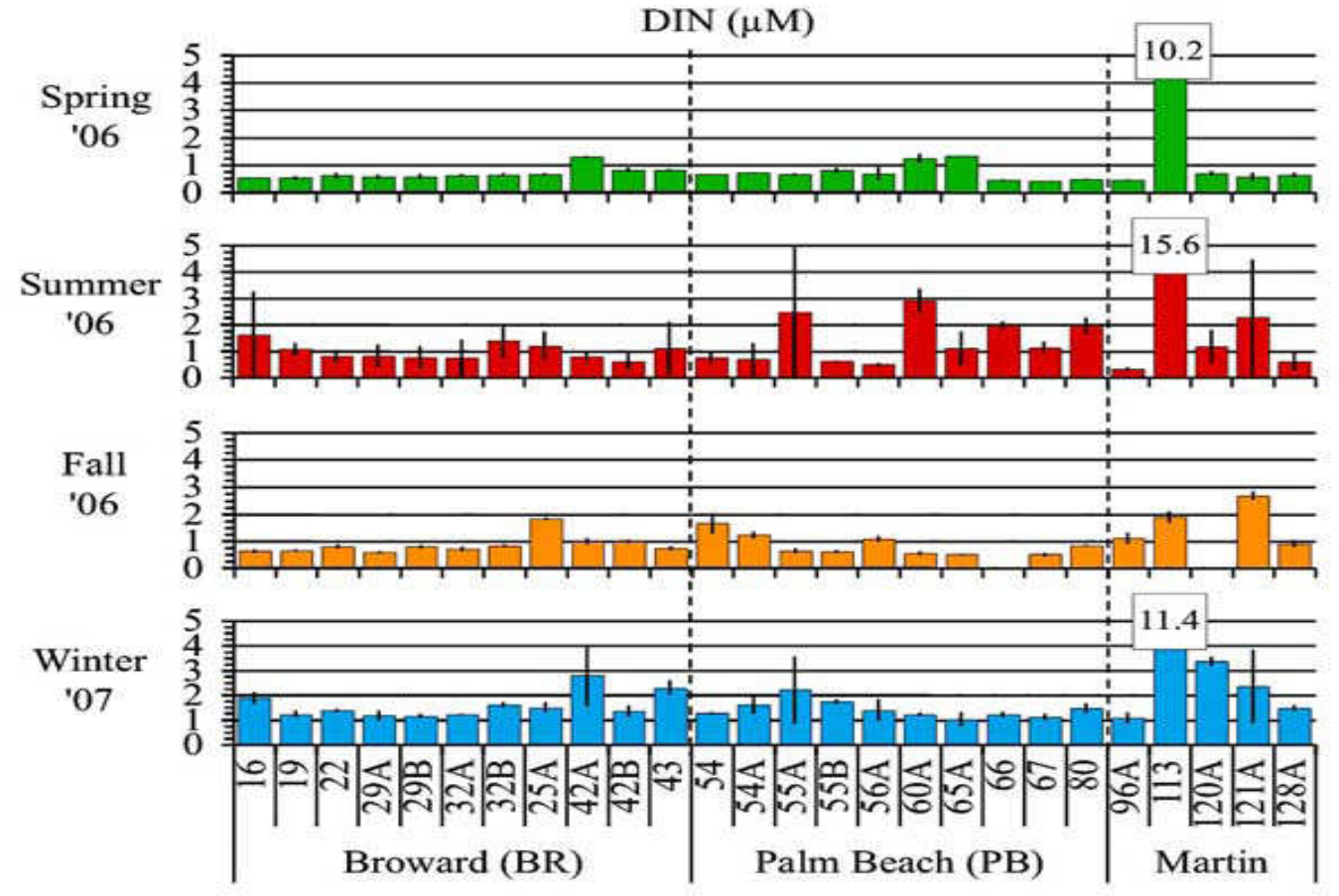
Hayward 1996). Blackwater events occur regularly (especially wet season) as the prevailing long-shore current moves inlet discharge onto the reef, persisting for days, weeks, or months. Latitudinal trends in numerous water quality parameters persist and secchi depth at the southern end of the reef was as little as 1.5m in fall 2008 following major discharges (FDEP, unpubl. data). The single known bleaching event on the reef occurred in February 2006, ~12 months after lake discharges following three 2004-05 regional hurricanes, and most of the corals recovered. During certain seasons, water samples at St. Lucie Reef had relatively high levels of Dissolved Inorganic Nitrogen, Soluble Reactive Phosphorous, Total Dissolved Phosphorous and the algal community showed high percent cover and ^{15}N values compared with counties further south (Figure3 adapted from LaPointe 2007).

Three sites (Fig. 1) representing the northern (nearest the inlet), central and southern range of the St. Lucie Reef tract were chosen for this study based upon historical reconnaissance, existing datasets, and similar depths (7-13' at MLW). The sites coincide with existing (SECREMP, fishes, water quality) and historical (isotopic) sampling sites. All three sites contain *Diploria clivosa* and the central and southern stations contain *Montastraea cavernosa*. The *D. clivosa* at the northern site reach a maximum diameter of ~2.5ft. This site also contains abundant wormrock, algal cover, few sponges/tunicates, and occasional *Siderastrea* spp. and *Oculina diffusa* corals. The central and southern sites contain a greater diversity and abundance of organisms than the northern site including certain corals (e.g., *Siderastrea* spp., *Oculina diffusa*, *Porites astreoides*, *Dichocoenia stokesii*, *Isophyllia sinuosa*). The central site contains *D. clivosa* reaching a maximum of ~13.5ft in diameter.

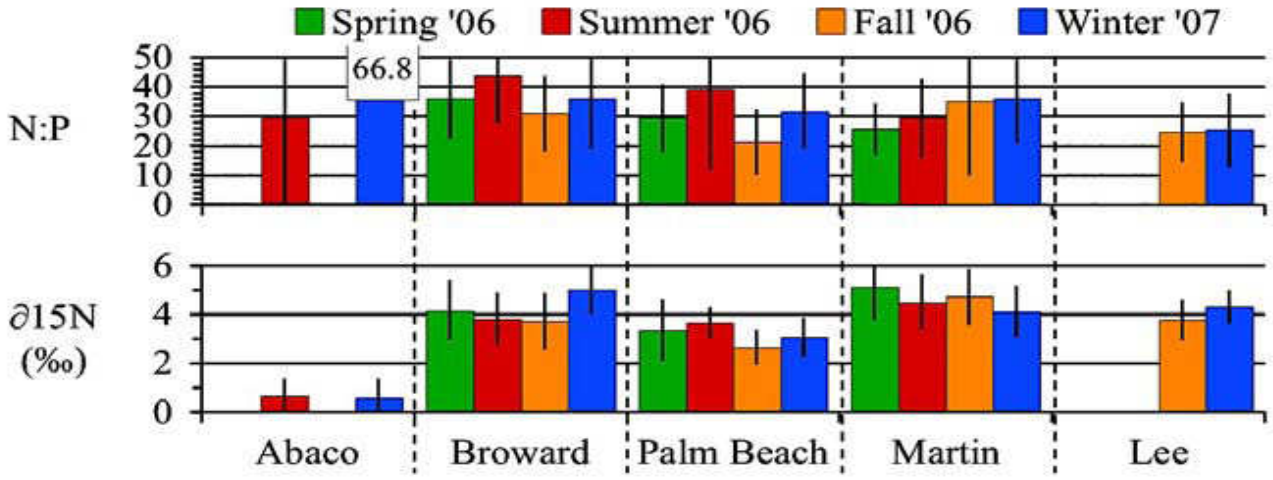
Fig. 2. Largest *D. clivosa* (~13.5ft maximum diameter) found at the reef (central site).


Cyanobacteria
Chlorophyta
Phaeophyta
Rhodophyta
Cover (%)
15.00
10.00
5.00
0.00
Broward
Palm Beach
Martin
Fall 2005
Winter 2006
Spring 2006
Summer 2006
Fall 2006
Winter 2007

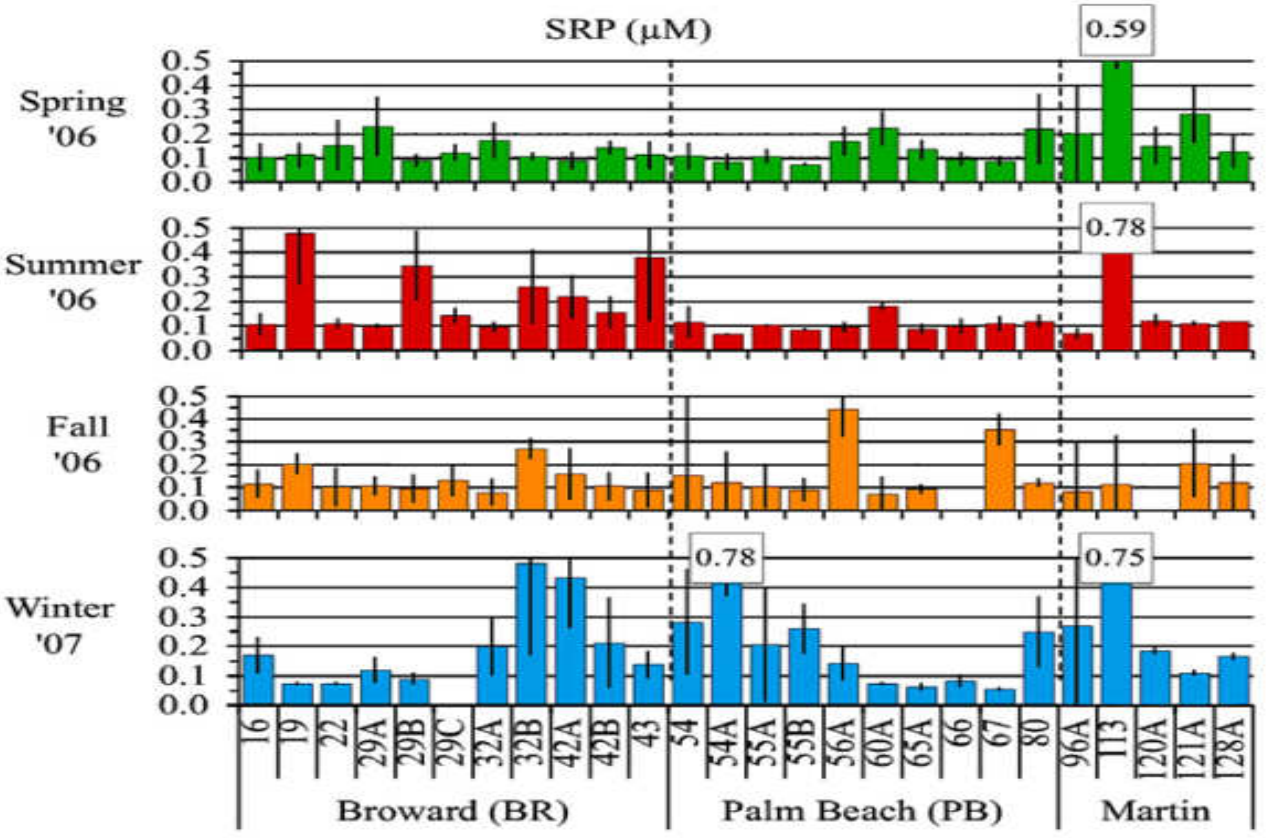
A


DIN (µM)
Spring '06
Summer '06
Fall '06
Winter '07
10.2
15.6
11.4
16
19
22
29A
29B
32A
32B
25A
42A
42B
43
54
54A
55A
55B
56A
60A
65A
66
67
80
96A
113
120A
121A
128A
Broward (BR)
Palm Beach (PB)
Martin

B



C



D

Fig. 3. Data from southeast Florida reefs including St. Lucie Reef in Martin County (LaPointe 2007). A). Algal percent cover by taxa 2005-07; B). DIN concentrations from water samples 2006-07; C). Algal N:P ratios and ^{15}N values 2006-07; D). SRP concentrations from water samples 2006-07.

Coral Sampling and Sample Processing

The first tissue sampling effort was conducted on August 13, 2010 with a subsequent sampling effort on October 12, 2010 (see Table 1). *Montastraea cavernosa* and *Diploria clivosa* coral fragments (3-5 cm^2) were collected at St. Lucie Reef with hammer and chisel and stored in sterile plastic bags during the dive. Immediately upon return to the boat, the *M. cavernosa* fragments were subsampled for DNA (stored in 10-20 ml Trizol and frozen upon return to the lab) and fragments of both coral species were preserved for histological analysis (stored in 10-30 ml Zenker's fixative). In addition, any remaining coral tissue was stored on ice and frozen upon return to the lab for potential zooxanthellae density analysis in future experiments. Duplicate coral mucus samples for bacterial community profiling analysis were also collected from each study colony by aspirating the surfaces of the corals with sterile 10 ml syringes. These samples were maintained at ambient *in situ* temperature for < 5 hours during transport and then allowed to settle before 2 ml was transferred to vials and stored at -80°C. Wounds created from tissue removal were monitored to track regrowth over the study period and beyond

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	Montastraea cavernosa	*Diploria clivosa*
North Site	none observed	4
Central Site	4	5
South Site	4	5

Table 1. The number of coral colonies sampled for each species at each site on Aug. 13, 2011. The same colonies were mapped and resampled Oct.12, 2010. Note that no *M. cavernosa* colonies were observed or sampled at the North Site.

Gene Expression Profiling using Microarray Technology

In a clean RNase free lab setting, phenol-chloroform extractions were performed on all 16 *M. cavernosa* samples. Trizol-preserved samples were allowed to slowly thaw at room temperature before aliquots of were removed and chloroform was added at a ratio of 1:5. Trizol volume was replaced and samples were stored at -80ºC for potential future studies. RNA was separated in the aqueous layer, precipitated with ethanol and purified with RNeasy MinElute spin columns (Qiagen, Venlo, Netherlands). A NanoDrop® 1000 spectrophotometer (Thermo Fisher Scientific, Inc., MA USA) was used to quantify total RNA at A_{260} and A_{280}. Samples with low RNA concentrations and purities were extracted 4-5 times then pooled and purified. Acceptable nucleic acid purity ratios ($A_{260/280}$) were between 1.88 and 2.17 yielding between 0.3-4 µg/µL total RNA with an average of 0.49-2.9 µg/µL per treatment group (Table 2). RNA quality for each sample was verified visually from a formaldehyde agarose electrophoresis gel imaged with Stratagene Eagle Eye Mini Darkroom Cabinet and Software (Agilent, USA).

Site	Date	mean µg/µL	± sem	n
South	August	2.87	0.48	4
South	October	0.64	0.20	4
Middle	August	1.52	0.31	4
Middle	October	0.49	0.07	4

Table 2. Mean concentrations of total RNA extracted from each group of *M. cavernosa* samples.

Label-IT© (Mirus Bio LLC, Wisconsin, USA) nucleic acid labeling kit (MIR 3700) was used to covalently attach Cy™ 5 (excitation 650 nm/ emission 670 nm) cyanine fluorescent dye at a frequency of one label about every 20-60 base pairs to 5µg of all 120 samples. Fluorescent dye labeling will allow detection if binding occurs between complimentary RNA fragments in the sample and the known oligonucleotides spotted on the microarray. Labeling was done according to the standard manufacturer's protocol (Lit# ML002, Rev 11/15/06) and quantified on a NanoDrop® 1000 spectrophotometer from wavelengths 225-750 nm. Concentrations of Cy 5 fluorescent dye ranged from 8.5 to 20 pmol/µL (Table 3) with frequency of incorporation (FOI) ratios between 8.47 and 13.3.

Site	*Date*	*mean RNA ug/ul*	*± sem*	*mean Cy™ 5 pmol/ul*	*± sem*	*N*
South	August	0.475	0.041	13.40	0.69	4
South	October	0.388	0.020	13.13	1.60	4
Middle	August	0.375	0.036	12.31	1.15	4
Middle	October	0.371	0.022	12.97	0.95	4

Table 3. Mean concentrations of Cy™ 5 labeling were uniform for each group of *M. cavernosa* samples.

Custom anthozoan holobiont microarrays consisting of 2240 features generated from multiple anthozoan and symbiont (*Symbiodinium*) species' gene sequences were used (Edge, 2007; Electronic Supplemental Material, ESM Table S1). More than 50 of the 148 genes represented on the array were isolated from scleractinian corals, Acropora cervicornis or Montastraea faveolata, exposed to natural or anthropogenic stressors (Morgan et al., 2001; Morgan and Snell, 2002; Edge et al., 2005). Additional genes, involved in various cellular functions, were identified using a bioinformatics approach and literature search resulting in an array consisting primarily (73%) of sequences from *Acropora* and

Montastraea species. One to five oligonucleotide sequences (35 base probes) from different regions of each gene were incorporated onto the array platform. In addition, two Arabidopsis spike mRNAs were included as positive controls and several phage sequences were included as negative controls. Array genes were categorized based on their primary cellular activity according to published research and the Gene Ontology database (Ashburner et al., 2000). All Cy™ 5-labeled total RNA samples were hybridized to the custom anti-sense 4x2K oligonucleotide CustomArrays™ using methods described in the hybridization and imaging protocol (PTL005, Combimatrix™, California, USA)(Ecogenomics, Inc., Japan). Total RNA samples were fragmented with 10X fragmentation reagent (buffered zinc solution) and stop solution (200 mM EDTA, pH 8.0) (Catalog #AM8740, Ambion®, Applied Biosystems, Texas, USA). Microarray chips were blocked for 30 min at 45ºC using pre-hybridization solution. Samples were then hybridized for 15 hours, washed and imaged at 635 nm using a high-resolution fluorescent GenePix® 4200A microarray scanner (Axon, Molecular Devices, California, USA).

Data were extracted from images using GenePixPro 6.0 software and analyzed using JMP® Genomics 4.1 (SAS Institute, Inc., North Carolina, USA). Background intensity was subtracted from each oligonucleotide feature and resulting feature intensities were averaged to produce a fluorescence measurement for each probe. Data were log base 2-transformed and normalized with an ANOVA model (JMP Genomics, SAS Institute). Replicate probes for each gene were averaged and multivariate repeated measures analysis of variance (corresponding to gene by gene comparison) was used to quantify significant differences in gene expression (JMP Genomics, SAS Institute). The model incorporated fixed effects and least squares effects as site (n=2), collection period (n=2) and an interaction between site and collection period (site x collection period; n=4). The cut-off value for significance was $p<0.001$. As an adjustment for multiplicity of testing, the false discovery rate was Q=0.05 (Benjamini and Hochberg 1995). Hierarchical cluster analysis of significant genes using Ward's method and geometric spacing was performed between sites, collection periods and samples (site x collection period) resulting in hierarchical clustering based on similarities in gene expression patterns (Ward 1963). Tukey-Kramer HSD post-hoc test was used to determine which genes demonstrated significant variability across samples ($p \leq 0.05$).

Bacterial Community Profiling using Length Heterogeneity PCR

The FastDNA® SPIN Kit for Soil (MPBio), with slightly modified protocols (Voss et al. 2007; Mills et al. 2003), was used to extract whole-community genomic DNA from the coral mucus samples. The genomic DNA extracts were quantified, verified, and assessed for 260/280 ratio using a Nanodrop 100 (ThermoScientific). The PCR primers used in this study were designed to amplify the domain of the 16S rRNA gene that included hypervariable domains V1 + V2. The fluorescently labeled forward primer 27F-6-FAM (5'-6-FAM-AGA GTT TGA TCM TGG CTC AG-3') was used with the non-fluorescent reverse primer, 355R (5'-GGT GCC TCC CGT AGG AGT-3'). The final concentrations of the PCR reactions were: 1x PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 µM forward and reverse primer, 0.25 U AmpliTaq® Gold LD DNA polymerase (Perkin Elmer, Wellesley, MA), 0.1% bovine serum albumin (BSA), fraction V (Sigma-Aldrich St. Louis, MO), 3 ng genomic DNA, and DEPC-treated water for a final volume of 22 µL. The PCR reactions were carried out in a Peltier Thermal Cycler under the following run conditions: 94°C for 11 min, 25 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C

for 10 min. Cycling parameters were optimized to address template reannealing (Suzuki & Giovannoni 1996) and kinetic biases (Suzuki et al. 1998). All PCR products were verified with 1% TBE agarose yield gels and Nanodrop analysis. Capillary electrophoresis was performed by the Forensic DNA Profiling Facility (FDPF) at Florida International University. The PCR products were denatured by adding 9.5 µl of 96:1 Hi-Di® deionized formamide: GeneScan® ROX 600 standard solution (Applied Biosystems, Foster City, CA) to 0.5 µl of each PCR product. The PCR products were then separated on a capillary electrophoresis Applied Biosystems (ABI) 3100 DNA genetic analyzer using filter set D and module DS-30. Injection time was set to 5 sec and run time to 28 min.

LH-PCR electropherograms (profiles) from each sample were analyzed using ABI's Prism GeneMapper® software. The analysis parameters were set to the Local Southern Method and peak amplitude threshold was set at 75 fluorescent units. No peak correction or smoothing was applied in order to increase peak resolution. Analysis was limited to fragments 300 to 400 base pairs (bp) in length. The size (to nearest bp) and relative abundance (i.e. relative fluorescence intensity) of each peak were determined for each profile. Amplicons that comprised less than 0.5% of total relative abundance were eliminated from the analysis. The relative abundance data were averaged among replicates and square root transformed before calculating Bray-Curtis similarity matrices (Bray & Curtis 1957). Non-metric multidimensional scaling (MDS) (Kruskal 1964) was used to visualize differences in the LH-PCR profiles of the samples. Each ordination was run with 25 random starting configurations to determine the best-fit model for non-parametric regression between the distance among samples in the plot and the Bray-Curtis similarity matrix. Dimensions were selected to minimize final stress, a goodness-of-fit measure related to the relationship between original p-dimensional space and the dissimilarity within the new ordination space. Variations among profiles from different geographic regions and different host species were tested using an analysis of similarity (ANOSIM), a robust analysis method requiring neither normally distributed abundance data nor balanced replicates between groups (Clarke & Warwick 2001). Similarity percentage (SIMPER) (Clarke & Warwick 2001) was used to determine the relative contribution of each amplicon to the average dissimilarity between the different groups. Only those amplicons that contributed >5% to the dissimilarity are discussed in the results section below. Non-metric MDS ordinations, ANOSIM, and SIMPER were conducted using Primer 6 (Primer-E Ltd., Plymouth, UK). This method was used to compare samples within this study, and to compare samples from this study to those previously collected in the Florida Keys and Lee Stocking Island, Bahamas.

Histological (Reproductive) Sample Processing

Histological samples taken from both coral species were processed using standardized techniques (described in Szmant 1986 and Szmant 1991). Following field collections, samples were placed in decalcification media (buffered HCL) for 24-48hrs until only soft tissue remained and then stored in ethanol. Each fixed and decalcified coral sample was trimmed to approximately 2 cm^2 and placed in an individual plastic cassette. The following washing protocol was used to impregnate the tissue samples with Paraffin; each step occurred under constant agitation using a shaker plate: 80% EtOH for 30 min (twice), 90% EtOH for 30 min (twice), 100% EtOH for 30 min (twice), Xylene for 30 min (three times), molten Paraffin (twice). After the second molten Paraffin incubation, each sample was embedded in additional molten Paraffin and manipulated to provide both transverse and horizontal

cross sections of the coral tissue. Once the embedded samples had been cooled and frozen, a microplane was used to create slide mounted 7 µm thick sections of embedded coral tissue. Each slide contained 2-4 sections and no less than four slides were created for each coral sample. The slides were dried over moderate heat (~40°C) to enhance bonding between the tissue sections and the slide. The tissue slides were stained using Heidenhain's Aniline Blue procedure with modifications recommended by Dr. Allison Moulding from the National Coral Reef Institute at Nova Southeastern University. The tissues were hydrated through a series of 2 minute washes as follows: xylene (3 times), 100% EtOH (3 times), 95% EtOH, 80% EtOH, and dH_2O (twice). During the initial staining run samples were incubated with azocarmine at ~60°C for 20 min, dipped twice in dH_2O, rinsed in aniline alcohol for 4 min, phosphotungstic acid for 15 min, dH_2O for 2 min, aniline blue for 20 min (dark), and dipped twice in dH_2O. Later, this protocol was revised to 30 min in azocarmine and only 15 min in aniline blue with better results. Each slide was dehydrated with 2 min washes: 95% EtOH, 100% EtOH (3 times), and xylene (3 times), before a permanent coverslip was affixed. Stained tissues were examined for the presence of gametes using light microscopy and archival photomicrographs of each sample were taken.

Environmental Parameters

General physical parameters (salinity, conductivity, temperature, DO, water column-profiled light intensity-PAR) were collected at times of coral tissue sampling and on other occasions during the project. PAR values, taken at high or low tide by quickly running a north to south transect, were collected using a LI-COR datalogger and 4pi sensor and used to calculate light attenuation coefficient (Kd). Dataloggers (PAR and temperature HOBOs) were installed at each site (two at each) and within the nearby beachside dunes (to assess cloud cover), collecting data every 30 min from early May to mid November, 2010. The submerged units were cleaned of fouling organisms every 13 days (average; range 4-22days). Data collected on the reef and in the dunes (PAR, temperature) during daylight were compared with data (temperature and conductivity at bottom and top, tidal stage) collected simultaneously (every 30 min) at a weather station located within St. Lucie Inlet through multi-variate analysis using Primer 6 (Primer-E Ltd., Plymouth, UK). Exceptions in the dataset include the first two weeks of August and the last two weeks of October when the weather station was down and did not collect data. The dataset was therefore divided into pre-upwelling period (early May to end of July) and post-upwelling (mid-August to mid Nov) which also mirrored the pre-blackwater and during-blackwater periods. The first major blackwater event witnessed occurred on August 17.

Results

Field observations

Despite the challenging environmental conditions corals are exposed to at St. Lucie Inlet, no active coral diseases or evidence of wide-scale bleaching was observed during sample collections. In general the corals were visually healthy with normal pigment concentration, no skeletal anomalies, and no active lesions. However, minor paling was observed on the margins of two *D. clivosa* at the north and central site, respectively, and cyanobacteria with associated partial mortality was observed on a single colony of *M. cavernosa* at the southern site. In addition, all of the corals sampled in October exhibited moderate-

heavy mucus production. Water temperatures experienced while diving during the first sampling event (August 13) revealed that a summer upwelling event had occurred as is typical for this region. Temperature data collected *in situ* by the HOBOs defined the extent of the ~10 day event. On August 17, we documented the first major blackwater event while diving the reef to clean the HOBOs. Subsequent blackwater episodes were seen until the HOBOs were pulled in mid-November. In general, the north site received the most colored water for the longest duration (sometimes throughout the entire water column, making dive operations difficult) though the central and southern sites also received colored surface water (upper 1-8ft) during low tide events stretching reef-long and occasionally as pockets of dark water even during higher tide stages. The concrete HOBO module units at the north site experienced the greatest abundance of fouling organisms settling, including 130 sq. cm of wormrock growth over the summer (Figure 4). Wound tracking of sampled corals revealed complete wound healing (tissue overgrowth) within 3-9 months. Clearly, the technique used for our sampling regime (removing minimal surface coral tissue without disturbing the underlying skeleton) is the preferred method.

Fig. 4. Concrete/PVC HOBO units from the north site with wormrock, barnacles, tunicates, algae, and sponges. Left unit shown cleaned and right unit fouled.

Gene Expression Analysis

Results of the ANOVA analysis reveal significant differences in expression of 31 of the 150 *M. cavernosa* genes on the microarray, with 29 genes exhibiting significant differences between samples after Tukey-Kramer post-hoc testing. Based on the number of genes expressed in each variable, there is a more significant difference in samples between sites than between dates (Figures 5 and 6).

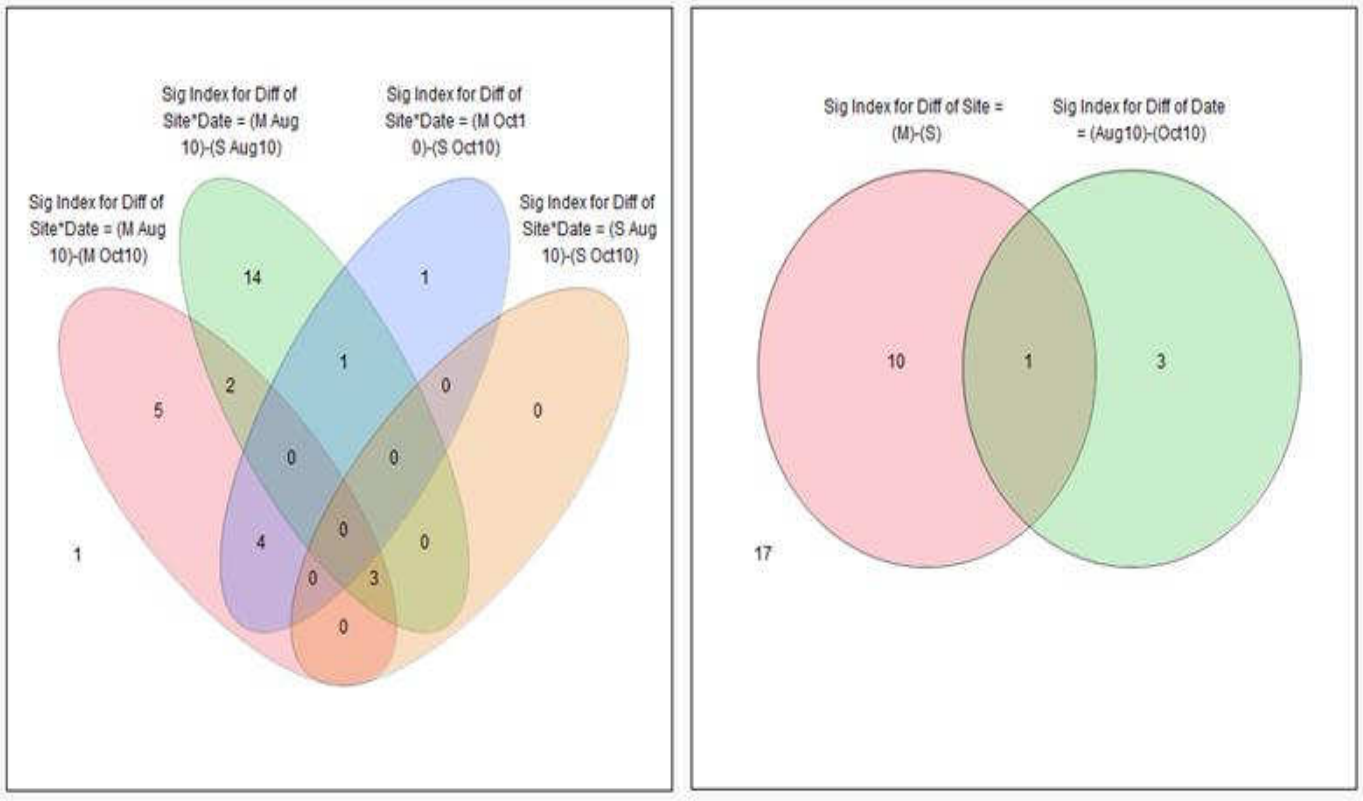


Fig. 5. Venn diagram showing the number and distribution of significant *M. cavernosa* genes between variables.

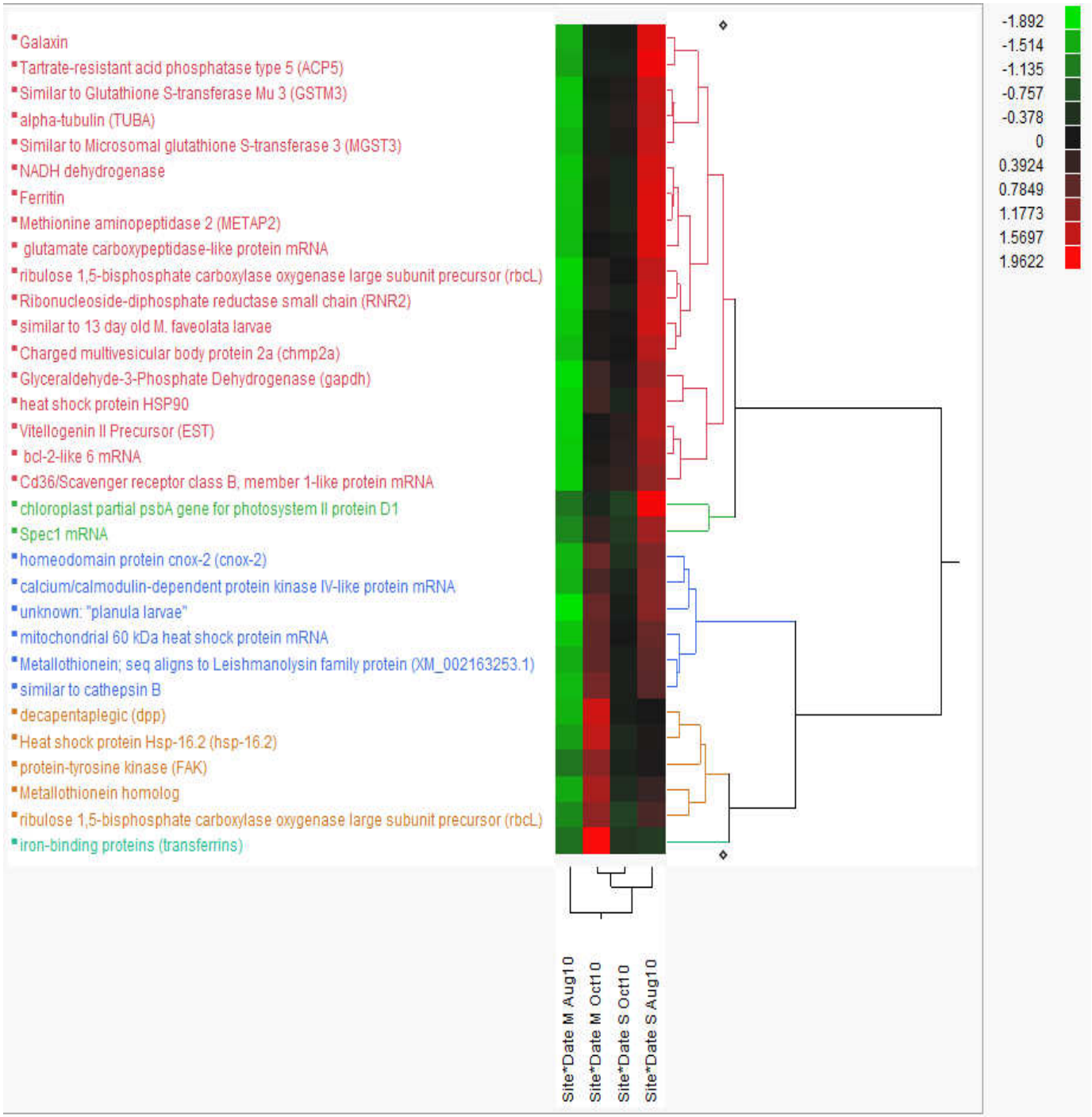
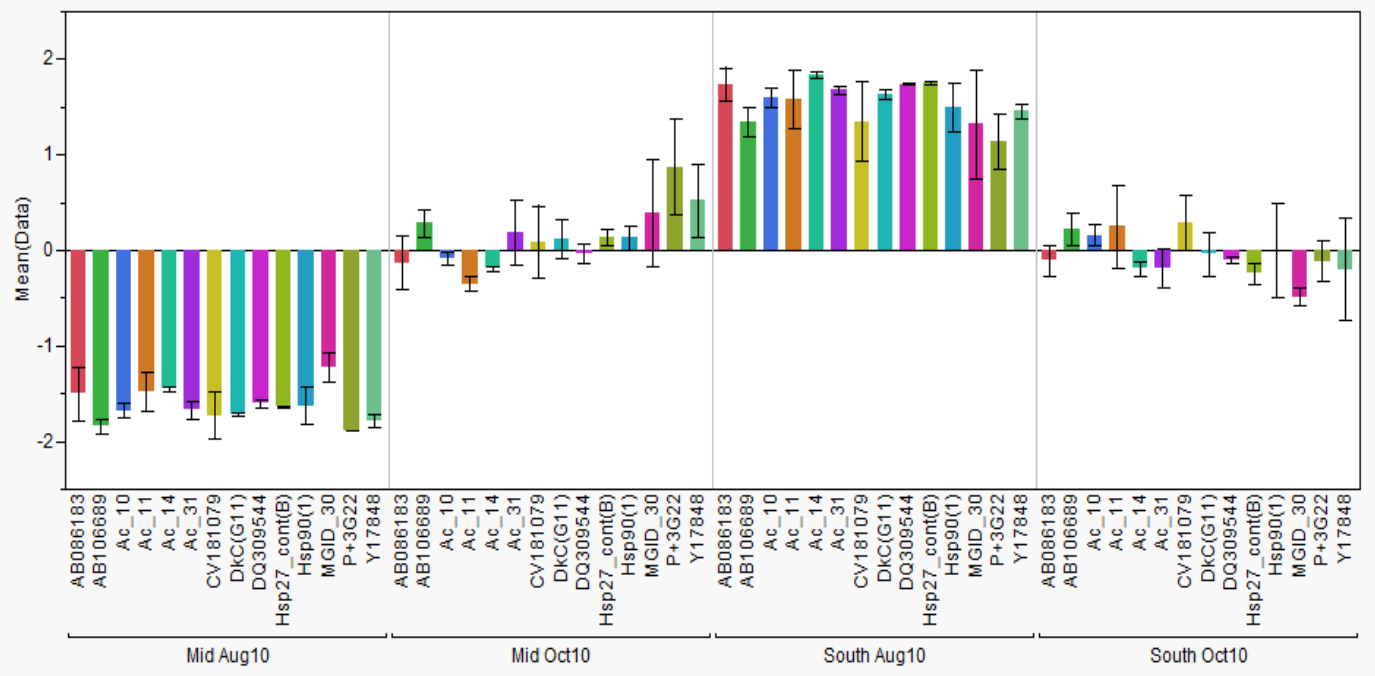


Fig. 6. Hierarchical clustering of significant *M. cavernosa* genes between variables. The standardized least squares mean for each gene was plotted against the variables of date crossed with site using the Fast Ward method. Genes cluster into 5 groups based on expression patterns. The variable clusters reveal distinct differences between the sites in August.

The majority of the genes (20) are significantly elevated at the south site in August and decreased at the middle site in August (Figure 7). Two of these genes have unknown functions, but are known coral genes (P+3G22, DkCG11) and both were isolated under stress conditions from coral tissues after exposure to pesticide (P+3G22) or extended periods of darkness (DkCG11). Several genes elevated at the middle site are induced by exposure to stressors. Two of these are specifically involved in xenobiotic metabolism (Ac_10, Ac_11). Others are responsible for the regulation of cell death (Ac_31, CV181079), response to inflammation (Ac_11, Ac_14), immune response (DkC(F1)) or chaperoning misfolded proteins (Y17848). Several of the genes have additional functions involved in cellular reorganization and tissue repair or regeneration (Hsp27cont(B), Hsp90(1), MGID_30, DkC(F1), DQ309525, MGID_344). Additionally, genes involved in cytokine signaling (Ac_14, AF285166), specifically one responsible for the regulation of symbiosis (DQ309525) are elevated at the south site in August. A recently identified coral gene, Galaxin (AB086183), is also significantly elevated in corals from the south site. Its function is not well characterized, but may be involved in skeletal deposition or biomineralization. The remaining genes showing elevated expression at the south site are involved in a variety of functions including metabolism (AB106689), signal transduction (DQ309544, MGID_30), DNA replication (MGIC_897), protein transport (Hsp90(1)) and proteolysis (Hsp27_cont(B), DQ309521).Table 4 shows the 31 genes found to have significant differences by ANOVA, their respective metabolic functions, and groupings found using Tukey-Kramer post-hoc analysis.



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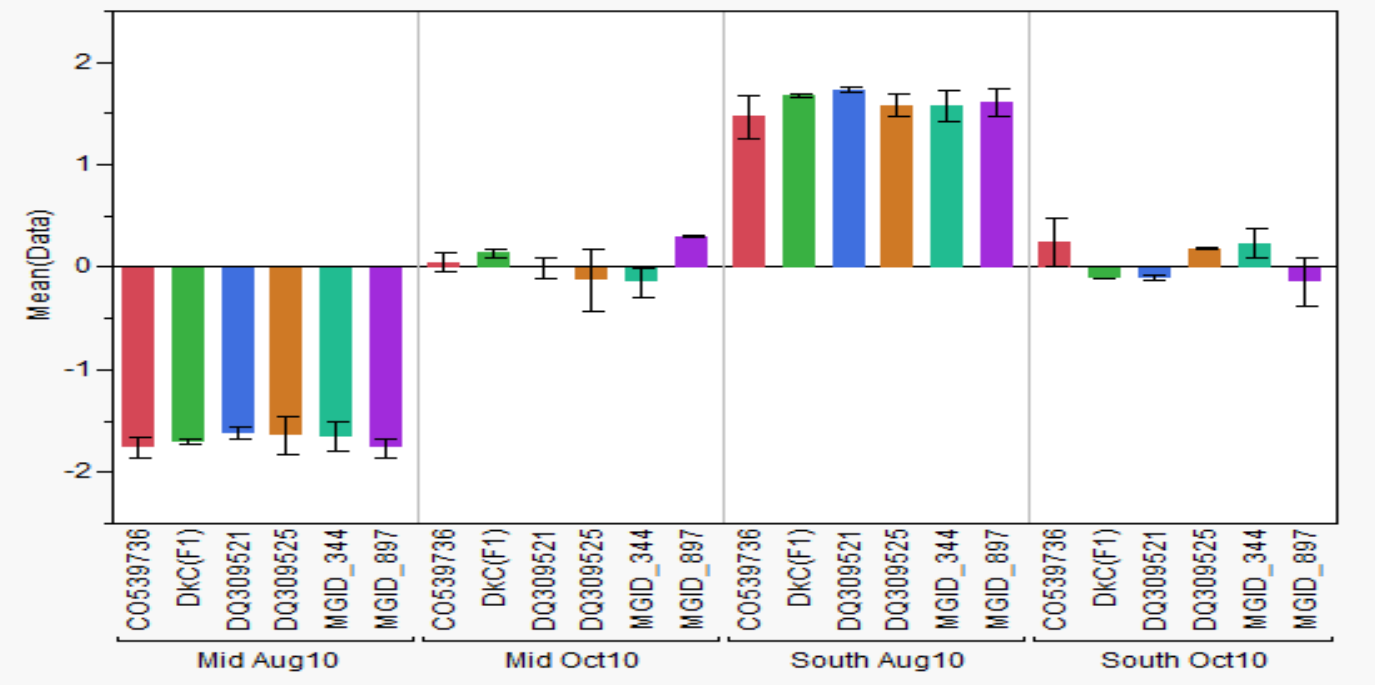


B

Fig. 7. Significant *M. cavernosa* genes between sites in August, 2010. The X axis shows gene names grouped by date and site. The Y axis indicates the level of the standardized least squares mean (Std Lsmean = 0). Greater deviation away from the standard least squares mean indicates increased significance. Error bars indicate the standard error.

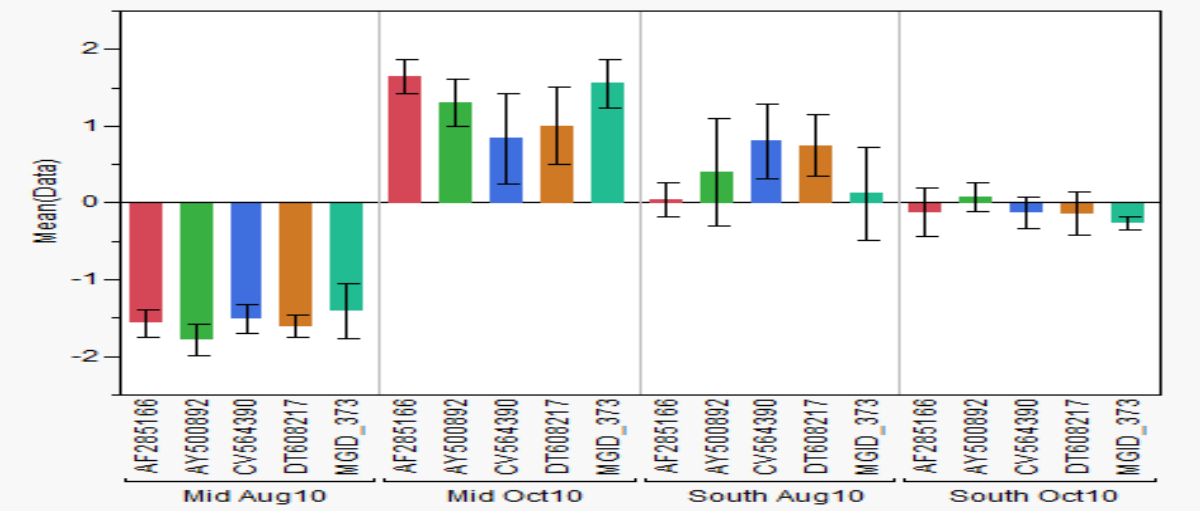
Table 4. List of significant genes by ANOVA with probe name, gene name, gene symbol, gene function and significance determination. Gene symbol is based on searches using NCBI (www.ncbi.nlm.nih.gov) and GeneCards (www.genecards.org). Gene function is based on searching the Gene Ontology database (www.geneontology.org). Letters indicate significant differences among samples based on Tukey-Kramer post-hoc test ($p \leq 0.05$).

Probe	Gene Name	Gene Symbol	Function	Mid, Aug10	Mid, Oct10	South, Aug10	South, Oct10
AB086183	Galaxin	GXN	not well characterized, but thought to be involved in skeletal deposition (biomineralization)	**B**	**AB**	**A**	**AB**
AB106689	Glyceraldehyde-3-Phosphate Dehydrogenase	GAPDH	glycolysis	**B**	**AB**	**A**	**AB**
Ac_10	Glutathione S-transferase Mu 3	GSTM3	detoxification of electrophilic compounds including carcinogens, therapeutic drugs, environmental toxins and oxidative stress products	**B**	**AB**	**A**	**AB**
Ac_11	Microsomal glutathione S-transferase 3	MGST3	xenobiotic metabolism; mediates inflammation	**B**	**AB**	**A**	**AB**
Ac_14	Tartrate-resistant acid phosphatase type 5; aka TRAP (glycosylated monomeric metalloenzyme)	ACP5	iron binding; response to cytokine stimulus; negative regulation of tumor necrosis factor, superoxide anion generation, and inflammatory response	**B**	**AB**	**A**	**AB**
Ac_31	NADH dehydrogenase 1	NDUFA9	electron transport chain component; *complex 1* is pro-apoptotic & produces reactive oxygen species (superoxide)	**B**	**AB**	**A**	**AB**
CV181079	B-cell CLL/lymphoma 2	BCL2	promotes cell survival (anti-apoptotic)	**B**	**AB**	**A**	**AB**
Hsp27cont(B)	Methionine aminopeptidase 2 (METAP2)	METAP2	proteolysis (processes N-terminal methionine from nascent proteins); cell proliferation; increased in cancer cells; substrate includes GAPDH	**B**	**AB**	**A**	**AB**

DQ309544	Calcium/calmodulin dependent protein kinase IV	CAMK4	transcriptional regulation; signal transduction	**B**	**AB**	**A**	**AB**
P+3G22	unknown: coral "planula larvae"	EST	unknown function	**B**	**AB**	**A**	**AB**
Y17848	Heat shock protein HSP90	HSP90AA1	inducible form (AA) expressed under stress conditions; protein folding; protein degradation	**B**	**AB**	**A**	**AB**
DkC(G11)	similar to 13 day old *M. faveolata* larvae	EST	unknown function	**B**	**AB**	**A**	**AB**
Hsp90(1)	Charged multivesicular body protein 2a	CHMP2A	protein transport; cellular membrane organization	**B**	**AB**	**A**	**AB**
MGID_30	Spec1	CDC42SE1	signal transduction; regulation of cell shape changes; phagocytosis	**B**	**AB**	**A**	**AB**
DkC(F1)	Ferritin (similar to soma ferritin & vertebrate H-ferritin or heavy chain ferritin)	FTH1	ferroxidase activity; oxidoreductase activity; immune response; negative regulation of cell proliferation; cellular membrane organization	**C**	**B**	**A**	**B**
DQ309521	Glutamate carboxypeptidase	PGCP	metallocarboxypeptidase activity; proteolysis; metal ion binding; tissue regeneration	**C**	**B**	**A**	**B**
DQ309525	Cd36/Scavenger receptor class B, member 1	SCARB1	symbiosis regulation; receptor activity; transporter activity; endothelial cell proliferation; lipopolysaccharide-mediated signaling	**C**	**B**	**A**	**B**
MGID_344	Alpha-tubulin (TUBA)	TUBA1A	cytoskeletal organization; microtubule movement	**C**	**BC**	**A**	**AB**
MGID_897	Ribonucleoside-diphosphate reductase subunit M2	RRM2	catalyzes formation of deoxyribonucleotides from ribonucleotides; DNA replication	**B**	**A**	**A**	**AB**
CO539736	Vitellogenin-2 Precursor (EST)	VTG	reproduction; gametogenesis	**B**	**A**	**A**	**A**

AF285166	Decapentaplegic (dpp) / Bone morphogenetic protein (BMP2 / BMP4)	BMP4	c ytokine activity (signaling); protein binding; transcription regulation; activation of MAPKK signaling	**B**	**A**	**AB**	**AB**
AY500892	Mitochondrial 60 kDa heat shock protein	HSPD1	prevent misfolding and promote the refolding of unfolded polypeptides generated under stress conditions in the mitochondrial matrix	**B**	**A**	**AB**	**AB**
CV564390	Leishmanolysin family protein (metallopeptidase M8 family)	LMLN	metalloendopeptidase activity; mitosis; cell migration and invasion	**B**	**A**	**AB**	**AB**
MGID_373	Heat shock protein Hsp-16.2	HSPB11	response to stress; protein chaperone; cell adhesion; intraflagellar transport	**B**	**A**	**AB**	**AB**
DT608217	Cathepsin B	CTSB	protein binding; response to wounding; response to organic cyclic compound; negative regulation of apoptosis	**B**	**A**	**AB**	**AB**
MGID_307	Ribulose 1,5-bisphosphate carboxylase oxygenase large subunit precursor	rbcl	RuBisCO; Calvin cycle; zooxanthellae; induced by multiple stressors (UV, temp, salinity, etc)	**B**	**A**	**B**	**B**
AY841903	Protein-tyrosine kinase	PTK2	signal transduction	**B**	**A**	**B**	**B**
DR681654	Metallothionein	MT1A	homeostasis of essential metals, cellular free radical scavenging, and metal detoxification	**B**	**A**	**B**	**B**
Dk(C)A2	Melano- or serotranferrin	TF	metal binding; ferric iron binding; ubiquitin protein ligase binding; cellular iron ion homeostasis	**B**	**A**	**B**	**B**
AF245689	Homeodomain protein cnox-2 (cnox-2)	HOXB1	DNA binding; transcription regulator activity; multicellular organismal development;	-	-	-	-
AJ884906	Chloroplast partial psbA gene for photosystem II, protein D1	psbA	important in photosynthesis	-	-	-	-

A secondary response was observed at the middle site between the two collection dates, August and October, 2010. Nine of the differentially expressed genes are significantly elevated at the middle site in October (Figure 8). Five of these are only significant compared to corals from the middle site in August (Figure 8A). The remaining 4 genes are significantly elevated at the middle site in October compared to all other samples (Figure 8B). Several of these genes are inducible upon stress conditions, including misfolded protein chaperone activity (AY500892, MGID_373), metal detoxification and homeostasis (DR681654, Dk(C)A2), negative regulation of cell death (DT608217) and general induction upon stress exposure (MGID_307). The remaining genes are involved in signal transduction (AF285166, AY841903) and cell migration (CV564390). Finally, vitellogenin (CO539736), a reproduction-associated gene, showed significantly lower expression in coral samples from the middle site in August compared to all other samples (Figure 8).



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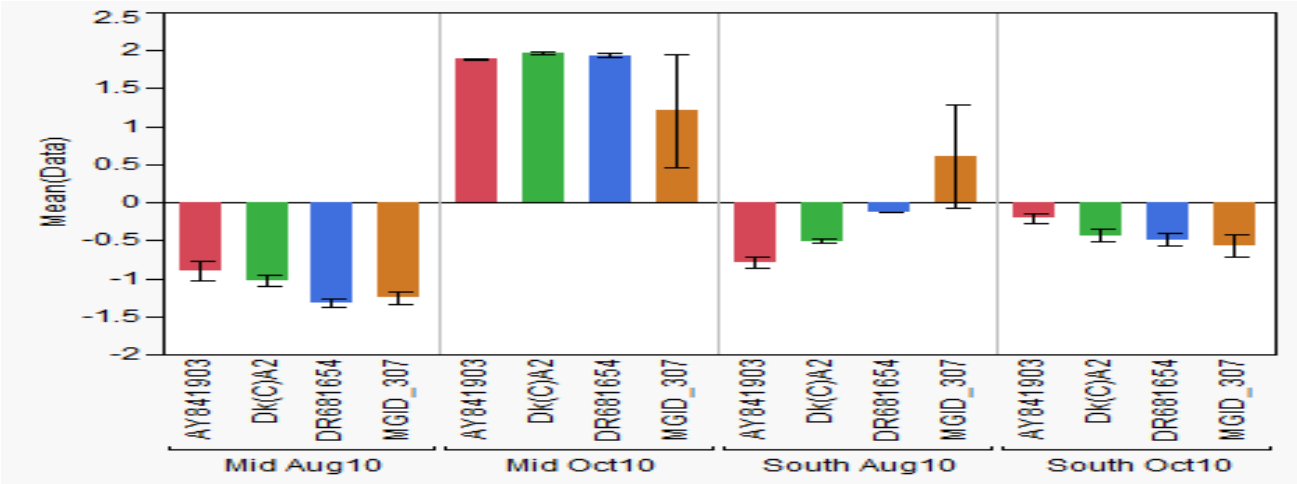


B

Fig. 8. Significant *M. cavernosa* genes at the middle site in October compared to A). August and B). all other sites/dates. The X axis shows gene names grouped by date and site. The Y axis indicates the level of the standardized least squares mean (Std Lsmean = 0). Greater deviation away from the standard least squares mean indicates increased significance. Error bars indicate the standard error.

Bacterial Community Profiling Analysis

The initial DNA extractions and LH-PCR amplifications did not produce via PCR products for capillary electrophoresis analysis. Two potential factors may have contributed to this issue. First, the DNA could have been degraded following extraction due to oscillations in the storage freezer. Alternatively, the primer set may have been degraded or improperly constructed by the supplier. The duplicate mucus samples were then processed and provided much better results, with all but one of the PCR products demonstrating > 500ng/µl in concentration. In addition, the majority of the PCR products were clearly visible on qualitative 1% agarose gels. While the *D. clivosa* samples yielded good sequence runs, FDPF reported that the *M. cavernosa* samples frequently misinjected into the sequencer, or yielded improper

sizes during the runs. Therefore only *D. clivosa* samples are included in the bacterial community profile analysis. LH-PCR profiles were reproducible both in terms of duplicate PCRs and triplicate sequencer runs. This reproducibility can be seen when viewing raw data as shown in the example in Figure 9, i.e. electropherograms, which reveal both the abundance of amplicons (relative peak height) and number of base pairs (amplicon length).

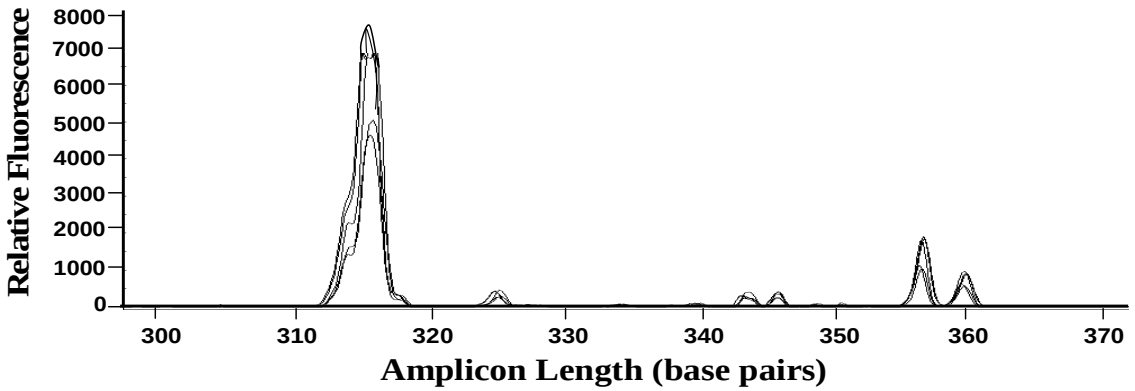


Fig. 9**.** Six superimposed LH-PCR electropherograms from replicate PCRs and sequence runs. Low variability in peak presence and amplitude demonstrate the reproducibility of this technique.

Comparison of LH-PCR profiles revealed variability in mucus microbial communities associated with *D. clivosa* at St. Lucie Reef. Representative profiles of two corals from each sampling event are shown in Figure 10. In this figure, unlike Figure 9 which shows raw data, individual peaks were binned with 1 bp resolution, and any overlapping peaks were resolved (see methods section). In total, 18 different amplicon lengths were observed within the samples, ranging from 302-357 base pairs (bp). These represent 18 different phyolotypes or ribotypes. Amplicons of 318 bp, 320 bp, 323 bp, 347 bp, and 357 bp were observed exclusively in samples collected in October. Amplicons of length 318bp, 324 bp, and 330 bp were unique singletons, each observed in only one of the samples collected. The 324 bp and 330 bp singletons were the only amplicons observed exclusively in the August sampling event. The remaining products (310, 312, 315, 322, 327, 335, 338, 343, 345, and 354 bp) were observed during both August and October sampling. Two amplicon lengths, 310 bp and 343 bp, were observed in all but one of the samples.

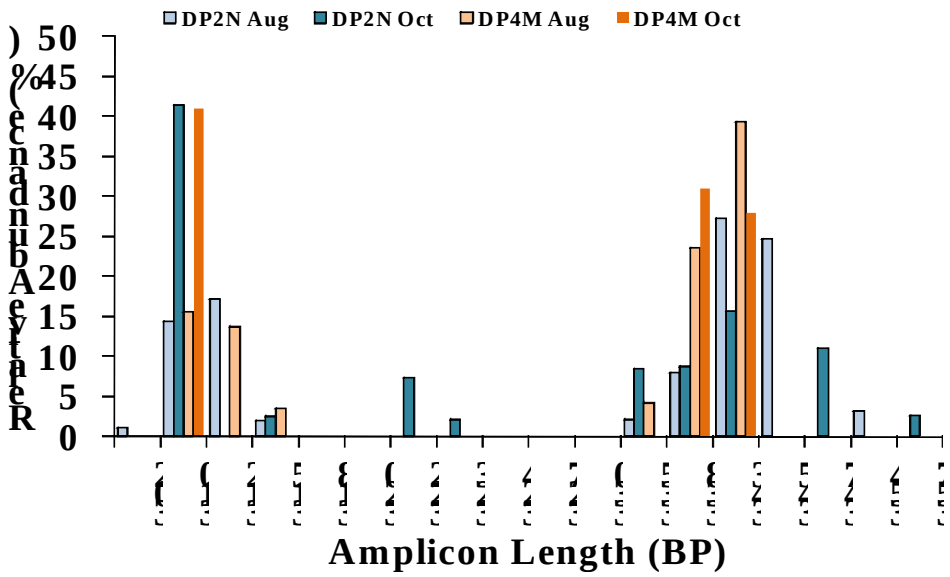
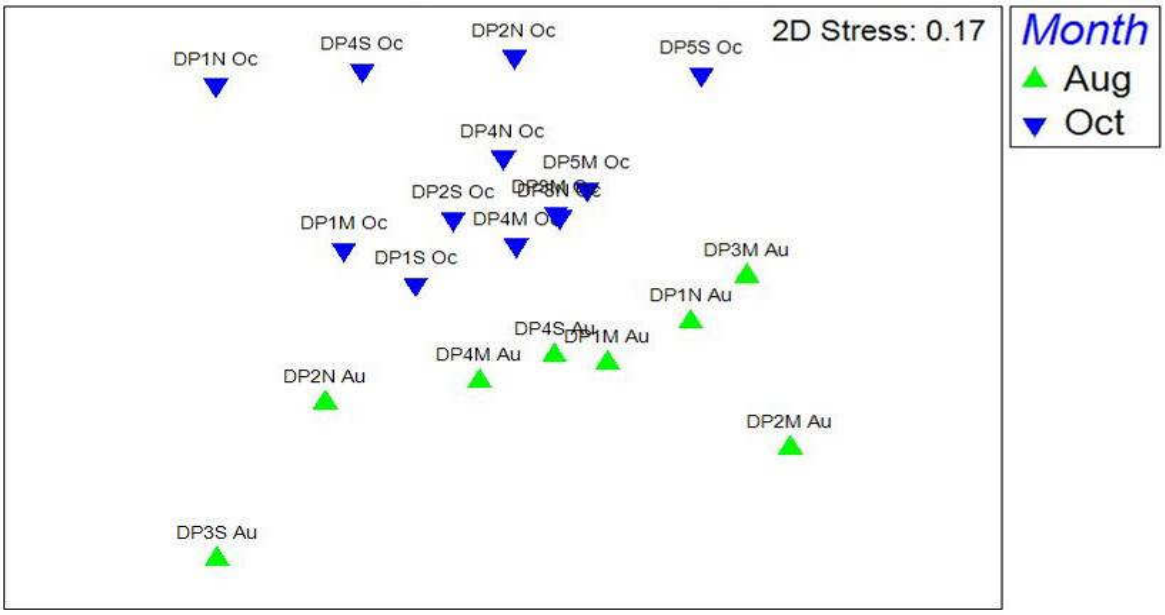
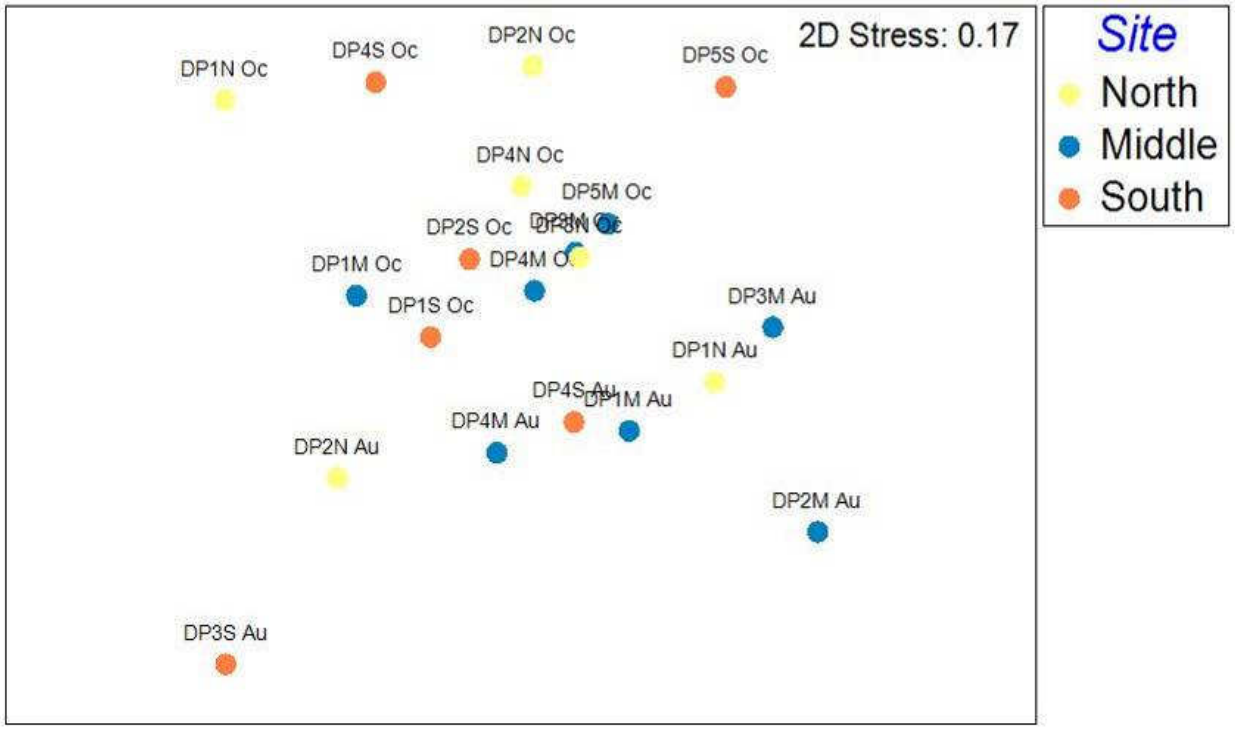


Fig. 10. Representative LH-PCR profiles from two *D. clivosa* at the northern and middle St. Lucie Reef sites during both August and October, 2010.

Non-metric multidimensional scaling and ANOSIM analysis of the LH-PCR profiles indicated significant clustering with respect to time of sample collection (Figure 11A, two-way crossed ANOSIM [site, time] Global R = 0.432, $p < 0.005$). However, there was no significant difference among sites (Figure 11B, two-way crossed ANOSIM [site, time] Global R = 0.022, $p = 0.366$).



A



B

Fig. 11. Non-metric MDS ordinations of LH-PCR profiles from *D. clivosa* mucus samples. Distance between samples represents similarity among LH-PCR profiles. Stress in each plot is a goodness-of-fit measure between the plot and the original p-dimensional matrix. (A) Samples labeled by time of collection. (B) Samples labeled by site.

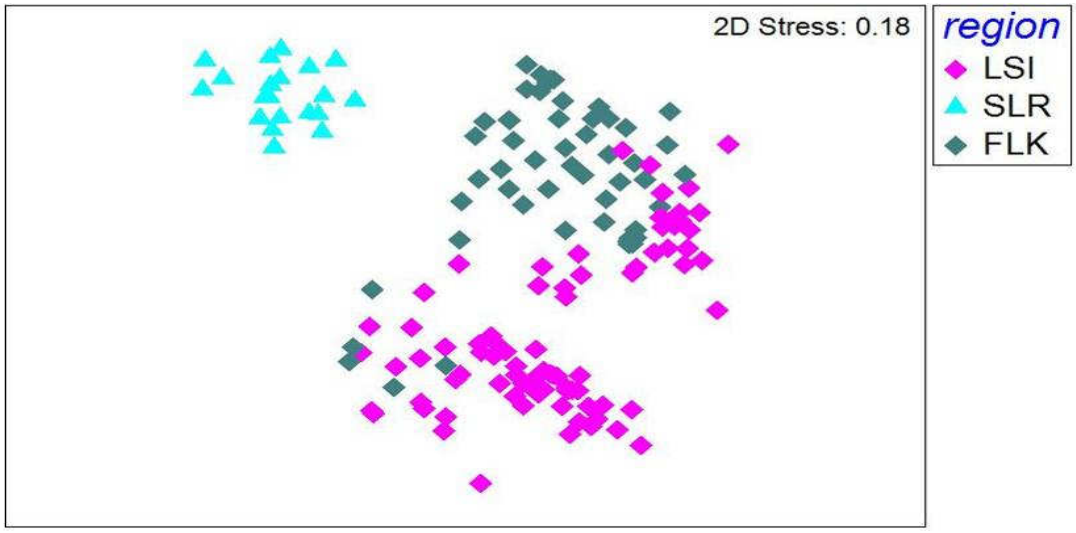
SIMPER analyses (Table 5) identified the relative contributions of each amplicon to average dissimilarity of samples between sampling times. Amplicons of 338, 310, and 322 bp were more abundant during October, while 343 and 312 bp amplicons were more abundant during August. The relative contribution to dissimilarity over time was not attributable to any single amplicon. However, variability in the 338 and 343 bp amplicons were the two most important drivers for the difference observed in August and October.

Amplicon Length (bp)	Mean relative abundance **August**	Mean relative abundance **October**	Percent Contribution	Cumulative Contribution
338	2.35	4.44	14.74%	14.74%
343	6.77	4.74	10.52%	25.25%
310	3.72	5.4	9.86%	35.11%
327	1.78	0.28	9.69%	44.8%
322	0	1.67	9.1%	53.9%
345	1.19	1.17	8.45%	62.35%
312	1.55	0	7.27%	69.62%

Table 5. Mean relative abundance of each amplicon as a percentage of total amplicon abundance. Amplicon contribution as a percentage dissimilarity between the two groups is shown in percent contribution. Lists are truncated to include only those amplicons that contribute >5% to the differences between groups.

The bacterial community profiles observed on *D. clivosa* at St. Lucie Reef were significantly different from those observed on multiple species in the Florida Keys and Lee Stocking Island, Bahamas. NMDS and ANOSIM indicated that mucus bacterial communities differed highly significantly by region (Figure 12A, two-way crossed ANOSIM [region, species] Global $R = 0.535$, $p < 0.001$), as well as by host coral species (Figure 12B, two-way crossed ANOSIM [region, species] Global $R = 0.227$, $p < 0.03$). However, these two factors confound since *D. clivosa* was not sampled elsewhere in the Florida Keys or Lee Stocking Island.

A



B

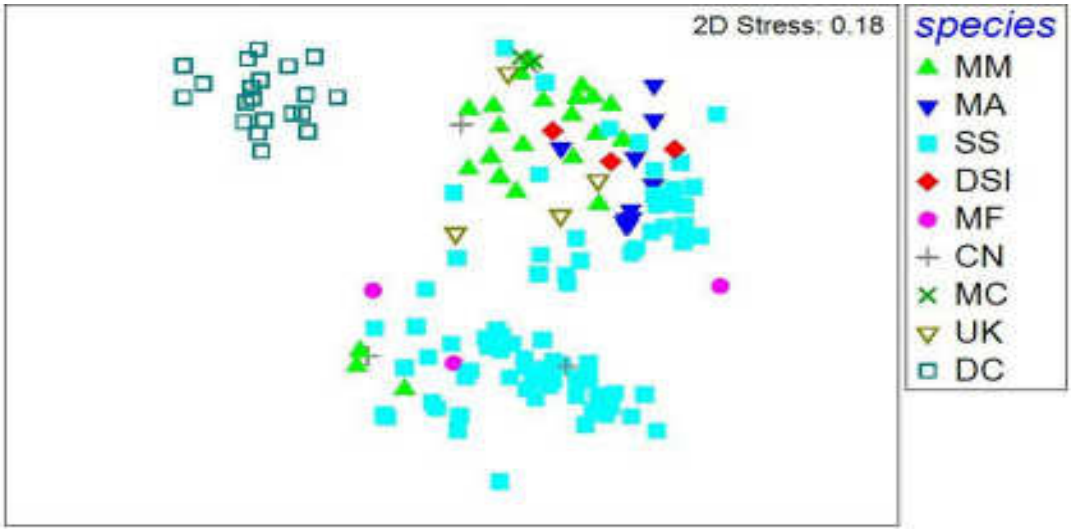


Fig. 12. Non-metric MDS ordinations of LH-PCR profiles from coral mucus samples. Distance between samples represents similarity among LH-PCR profiles. Stress in each plot is a goodness-of-fit measure between the plot and the original p-dimensional matrix. (A) Samples labeled by region of collection (FLK= Florida Keys, LSI = Lee Stocking Island, SLR = St. Lucie Reef). (B) Samples labeled by species (MM = *Meandrina meandrites*, MA = *Montastraea annularis*, SS = *Siderastrea siderea,* DSI = *Dichocoenia stokesii*, MF = *Montastraea faveolata*, CN = *Colpophyllia natans*, MC = *Montastraea cavernosa*, UK = unknown, DC = *Diploria clivosa*).

Histological (Reproductive) Sample Analysis

The coral histological samples revealed that only one colony (*M. cavernosa* colony #1 at the south site) contained identifiable gonads (stage 2 spermaries) at one sampling period (August). Figure 13 shows typical stage 2 spermaries. Due to inclement weather, no diving operations were conducted during the typical spawn window (8 days following the full moon was September 2 in 2010).

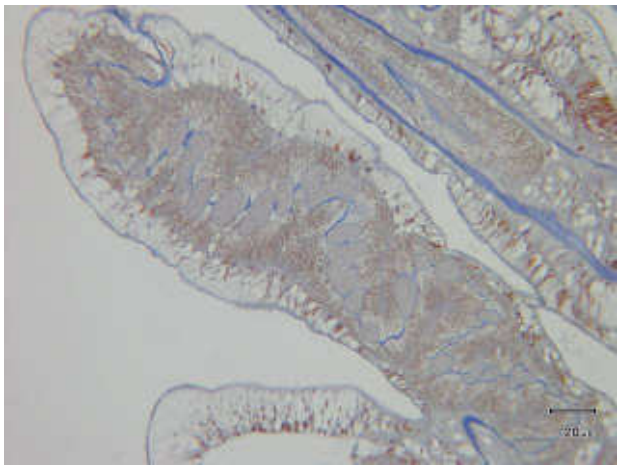


Fig. 13. Maturing gonads (stage 2 spermaries) from *M. cavernosa* colony (stock photo).

Environmental Parameters Analysis

Water quality data collected at the reef using a hand-held YSI on certain dates are shown in Table 6. Bottom temperatures ranged from 23 C (south site) to 30.6 C (north site) whereas the temperature data collected by the in situ HOBOs installed at the coral sites showed a range of 16.62 C (south site) during the upwelling period to 31.37 (north site) during the heat of late July. The upwelling dropped temperatures rapidly at all sites (e.g., 8.34 degrees C in 15 hours at the south site). The deeper the site (south site 13 feet at MLW; central site 11 feet; north site 7 feet), the greater the drop and more persistent the upwelling event. On August 17, immediately following the upwelling event, the lowest bottom dissolved oxygen readings were taken (Table 6). Salinity showed little variation over time and space and the lowest values occurred at the north including a bottom salinity low of 29.1 psu in early September.

Table 6. Water quality data collected at St. Lucie Reef during 2010 at coral sites (north, central, south), a historical State Park sampling station (SLPWQ1) 1.13 miles north of north coral site, and at the inlet weather station (inlet WS). Tide/time is published time for Sewall's Point just inside the inlet; Samp Time is sampling time; STemp C is surface temp in C; SSal is surface salinity in psu; Scon is surface conductivity in micro-Siemens; SDO is surface dissolved oxygen in mg/litre; columns beginning with B denote bottom readings.

Date	Tide/time	Samp Time	Site	STemp C	Ssal psu	SCon microS	SDO mg/L	BTemp C	BSal psu	BCon microS	BDO mg/L
5.14.10	HT at 10:38	10:45	north	27.6	34.2	54	6	27.5	34.8	53.7	5.8
5.14.10	HT at 10:38	11:00	central	27.8	34.3	53.3	6.1	27.4	34.8	54.3	5.8
5.14.10	HT at 10:38	11:20	south	27.3	34.8	54.8	6.5	27	34.3	53.1	6.4
5.19.10	LT at 9:05	9:45	SLPWQ1	24	27.8	45.5		23.6	31	47.5	
5.19.10	LT at 9:05	10:00	north	25.2	29.2	45		25.3	30.8	47.7	
5.19.10	LT at 9:05	10:28	central	26.2	32.2	50.4		26	32.1	49.5	
5.19.10	LT at 9:05	11:40	south	26.5	34.7	54.2		24.9	34.1	51.7	
6.15.10	HT at 12:44	11:40	north	31.3	33.8	53.2	5.01	30.6	34.2	52.4	4.92
6.15.10	HT at 12:44	12:05	central	30.5	34.1	52.8	5.1	30.5	35	51.8	5.22
6.15.10	HT at 12:44	12:30	south	29.8	34.3	53.7	5.13	29.1	34.8	52.5	4.99
7.27.10	HT at 10:02	10:15	north	29.4	33.8	51.8	4.94	29.5	34.3	52.3	5.05
7.27.10	HT at 10:02	11:30	central	29.9	34.6	52.6	5.08	29.8	34.7	52.9	4.65
7.27.10	HT at 10:02	12:45	south	30	34.7	52.9	5.3	29.5	34.9	53.2	4.9
8.17.10	HT at 14:30	11:05	south	26.7	39.7	59.3	4.43	23	41.4	61.5	3.81
8.17.10	HT at 14:30	14:00	central	27.8	33.2	55.2	4.37	24.2	35.1	58.8	4.16
8.17.10	HT at 14:30	14:50	north	28.2	29.2	50.4	4.04	27.1	31.4	51.1	4.12
8.27.10	HT at 11:35	12:00	south	27.5	32.2	49.3	5.54	25	34.7	52.7	4.17

8.27.10	HT at 11:35	12:20	central	27.7	33.2	49.4	5.5	25.2	34.5	52.3	5.53
8.27.10	HT at 11:35	12:31	north	25.2	34.6	52.6	5.64	25	34.6	52.5	5.37
9.4.10	LT at 13:19	13:00	north	25.5	28	46.2	5.24	25.1	29.1	50.3	4.51
9.4.10	LT at 13:19	13:20	central	27.9	31.7	5.71	5.71	26	33.4	52	5.48
9.4.10	LT at 13:19	13:55	south	26.4	32.5	50.4	5.82	25	34.2	51.8	4.03
Date	Tide/time	Samp Time	Site	STemp C	Ssal psu	SCon microS	SDO mg/L	BTemp C	BSal psu	BCon microS	BDO mg/L
10.1.10	LT at 10:30	11:15	south	27.3	34.3	52.2	5.15	27.6	34.8	52.9	4.99
10.1.10	LT at 10:30	11:30	central	27.4	31	48	5.16	27.6	34.6	52.5	4.98
10.1.10	LT at 10:30	12:07	north	27.4	28.1	44.7	5.07	27.6	34.3	52.3	4.8
10.1.10	LT at 10:30	12:20	SLPWQ1	27.6	31.7	47.4	4.91	27.8	34.6	52.7	4.18
10.1.10	LT at 10:30	12:35	inlet WS	27.4	26.5	40.12	4.58	27.4	29.3	45.9	4.89
10.12.10	HT at 14:06	10:15	south	27	35	53.1	4.68	27	35.1	55.4	4.3
10.12.10	HT at 14:06	13:15	central	27.3	34.1	52.5	5.12	27.1	34.1	52.8	4.43
10.12.10	HT at 14:06	15:45	north	27.2	34.4	53.4	4.88	27.1	34.1	52.5	4.64

Light data (PAR) collected by water-column profile technique on four occasions reveal a consistent north-south trend in light attenuation at the reef (Figure 14). Prior to the upwelling or major riverine releases, all sites showed relatively low Kd values. The highest Kd values occurred at the north site during blackwater events in mid-August and mid-September. All of the sites showed their highest Kd values during a low tide event in mid-September except for the north site which had a higher Kd at high tide during a major blackwater event in mid-August. Light data collected in situ using the HOBO meters were compared with concomitant tidal stage data collected at the inlet weather station. Before the upwelling and blackwater period, the north and central sites showed a weak positive correlation between tidal stage and light intensity whereas the south site showed a weak negative one (Figure 15A). During the balckwater period, all sites showed a weak negative relationship (Figure 15B). The relationship between stage and light was strongest at the south site after the upwelling (Pearson's r = -0.1311) followed by the north site before (Pearson's r= +0.1022).

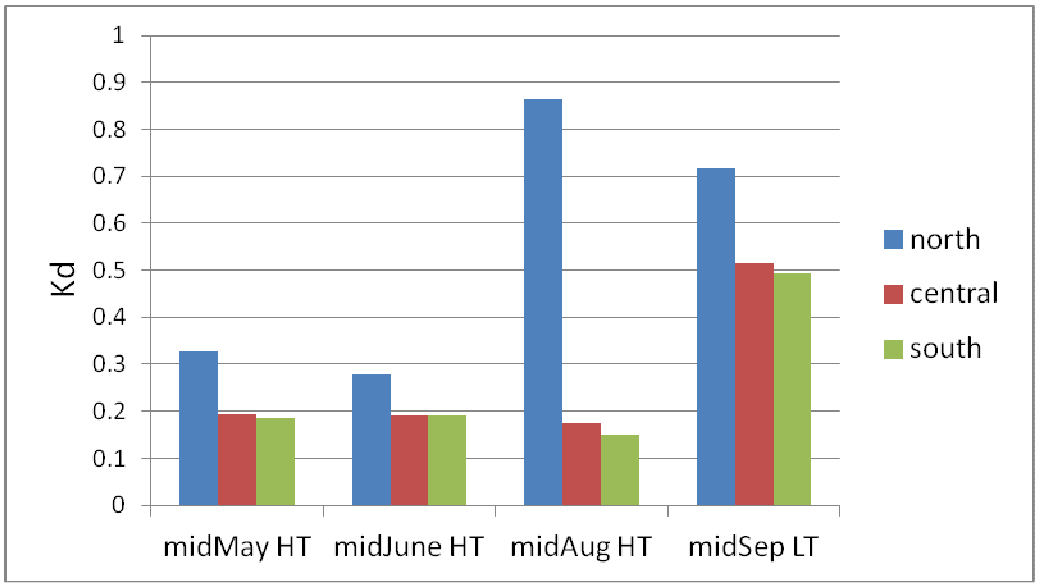


Fig. 14. Kd values calculated using water-column profile sampling at the coral sites in 2010. HT is high tide and LT is low tide.

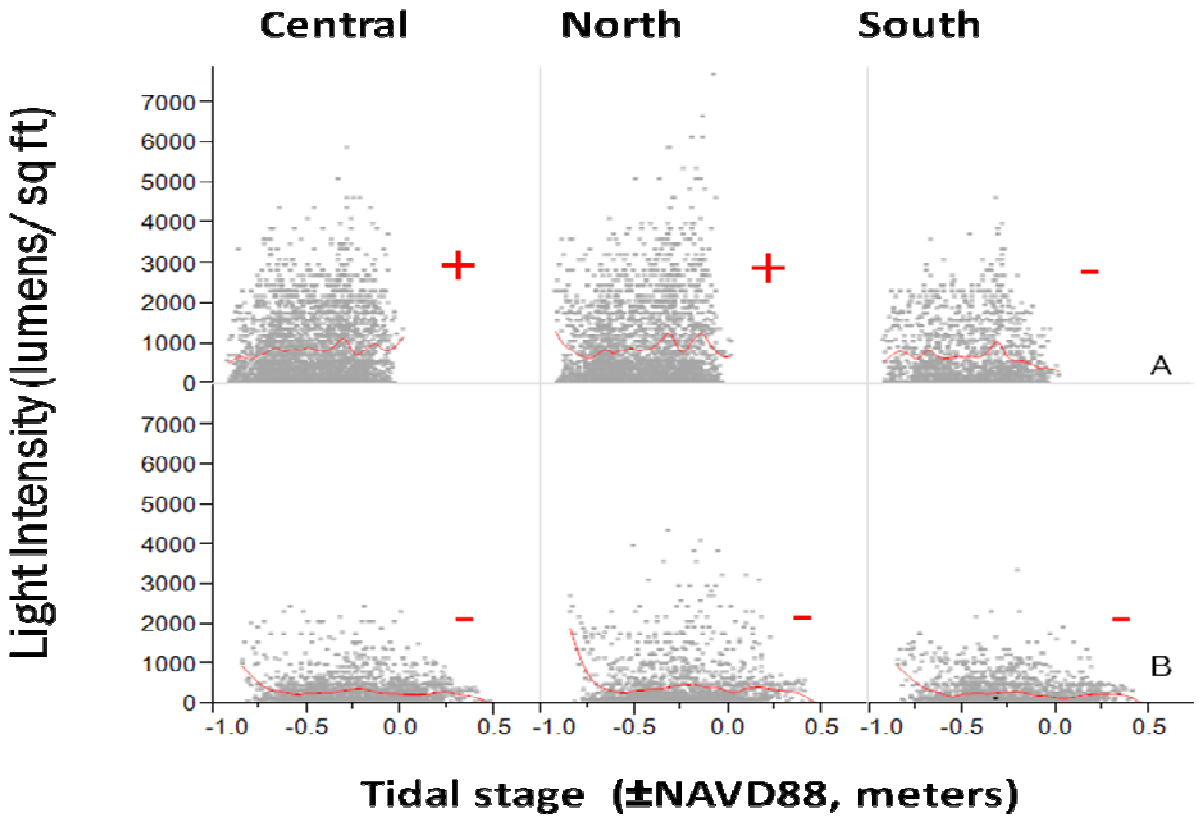


Fig. 15. Correlations between tidal stage at St. Lucie Inlet weather station and light intensity at each site A). from early May to late July and B). from mid August to mid November.

Analysis of in situ light versus dune light data (Appendix) revealed that before and during the upwelling period, coral site and dune site light readings showed very strong agreement (Pearson's r mean=0.6657; range 0.4243-0.7574). After the upwelling and during the blackwater events, the strength of the agreement became less pronounced (Pearson's r mean=0.5546; range 0.4840-0.6175). Throughout the entire study, the north site usually showed the weakest site/dune light relationship (before upwelling Pearson's r mean=0.6610; range 0.6606-0.6683; during upwelling Pearson's r mean=0.5791; range 0.4243-0.6943; after upwelling Pearson's r mean=0.5191; range 0.4840-0.5562). Temperature data showed very strong agreement between inlet (especially the bottom sensor) and the central site during the entire study whereas the north and south sites showed a weaker relationship generally which weakened for the north site during the blackwater period (Appendix). Temperature data at the central and south sites were weakly related to inlet bottom conductivity before the upwelling and moderately related after whereas the north site was weak-moderate throughout the study, never reaching the significance level of the other two sites during the blackwater period (Appendix).

Discussion

Watershed Effects

St. Lucie Reef in Martin County has been called Florida's northernmost coral reef. The reef contains 21 of the ~70 scleractinian coral species commonly found along the state's reef tract and hardbottom "reefs" just north of the area contain only 5 of those species, none of which are plate- or mound-forming. The reef is therefore an example of tropical coral species living at the extremes of their range (latitude 27deg 08min), exposed to a limited light regime, relatively cold winter water temperatures, and associated reduced growing season. In addition to these factors, the reef lies in close proximity to an inlet which conveys water from the Indian River Lagoon, St. Lucie River, and, under certain conditions, Lake Okeechobee via the C-44 canal. The net result of the major (C-44, C-23, C-24) and minor (secondary and tertiary) canals, outfalls, and creeks is a highly variable micro-climate on the reef sometimes making conditions unfavorable for tropical/subtropical organisms such as scleractinian corals (Figure 16).

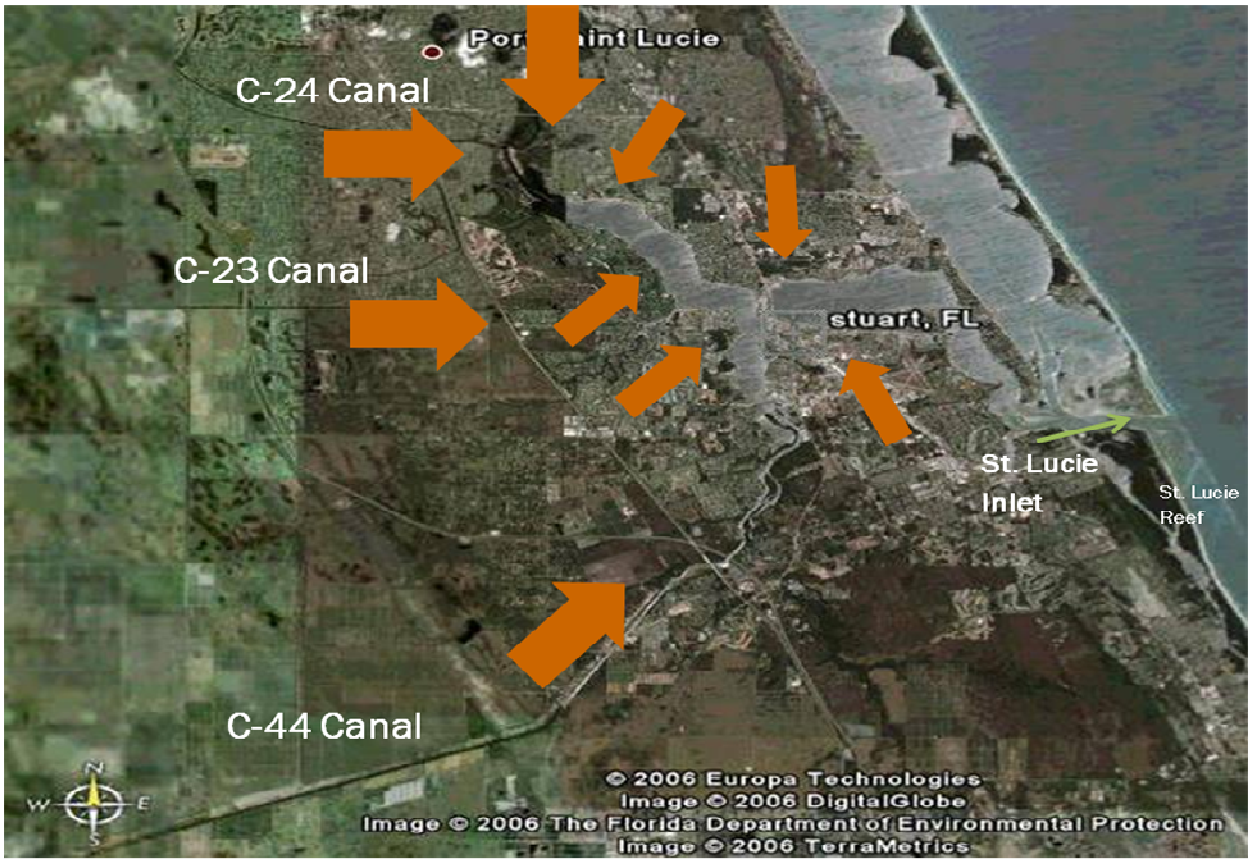


Fig. 16. Watershed draining to St. Lucie Reef. Arrow size depicts relative contribution of flow.

Evidence for water quantity (volume) and quality effects of discharge onto the reef is seen in a core extracted from a massive *D. clivosa* at the central coral site used in this study (Beal and Helmle, unpubl. data). Analysis of the core using x-ray revealed changes in coral density, calcification, and

extension rate after 1970 when major water management strategies involving rainfall retention commenced (Figure 17). Mean density decreased sharply while calcification decreased slightly, suggesting major changes in reef water chemistry (e.g., pH, salinity, micronutrients); whereas mean extension rate increased, suggesting improved conditions for coral growth.

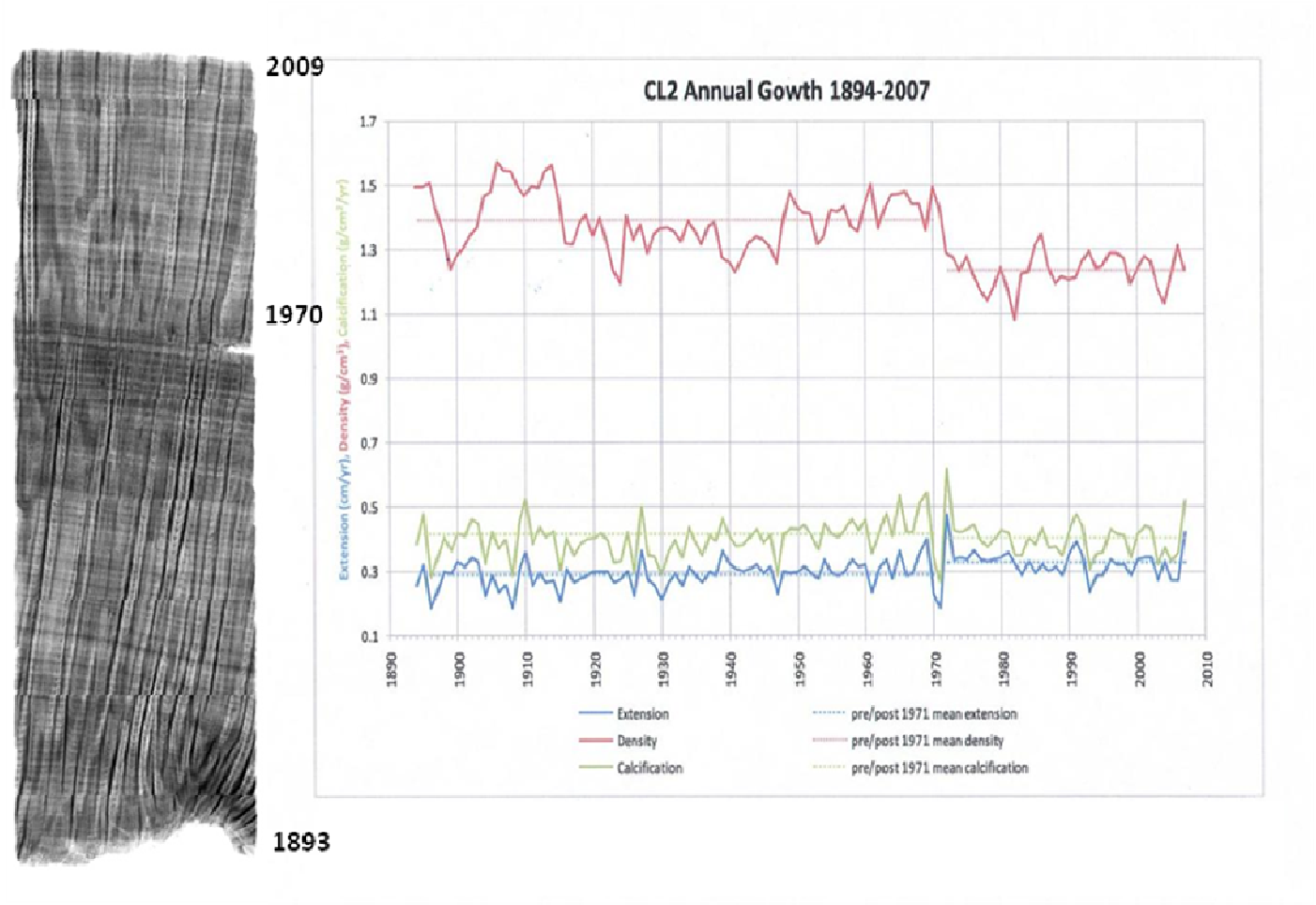


Fig. 17. X-ray analysis of a *D. clivosa* core taken from St. Lucie Reef central site, 2009.

The complete flow dataset for the period of record 1990-2007 (DBHYDRO, SFWMD) was used to analyze flow versus coral skeleton parameters, capturing significant wet (e.g., 1998-99, 2001, 2005, 2008) and dry (e.g., 2000, 2006, 2007) years. Flow negatively reduced calcification and extension rate to a significant degree but had little positive effect on density during this period (Figure 18). During extreme flow periods (i.e., consecutive days of tens of thousands of cfs net flow from the three main basins), salinity readings at St. Lucie Inlet have dropped to 1 psu during wet season and 8 psu during dry season releases (DBHYDRO, SFWMD). Secchi depth at the inlet usually drops below 1.0 meter and has reached 0.4 meters (DBHYDRO, SFWMD). During one moderately major release event in late summer, 2008, State Park staff recorded secchi depths of 0.75, 1.5, and 1.5 meters during high tide at the north, central, and south coral sites, respectively (Charley Jabaly, FDEP Parks, pers. comm.).

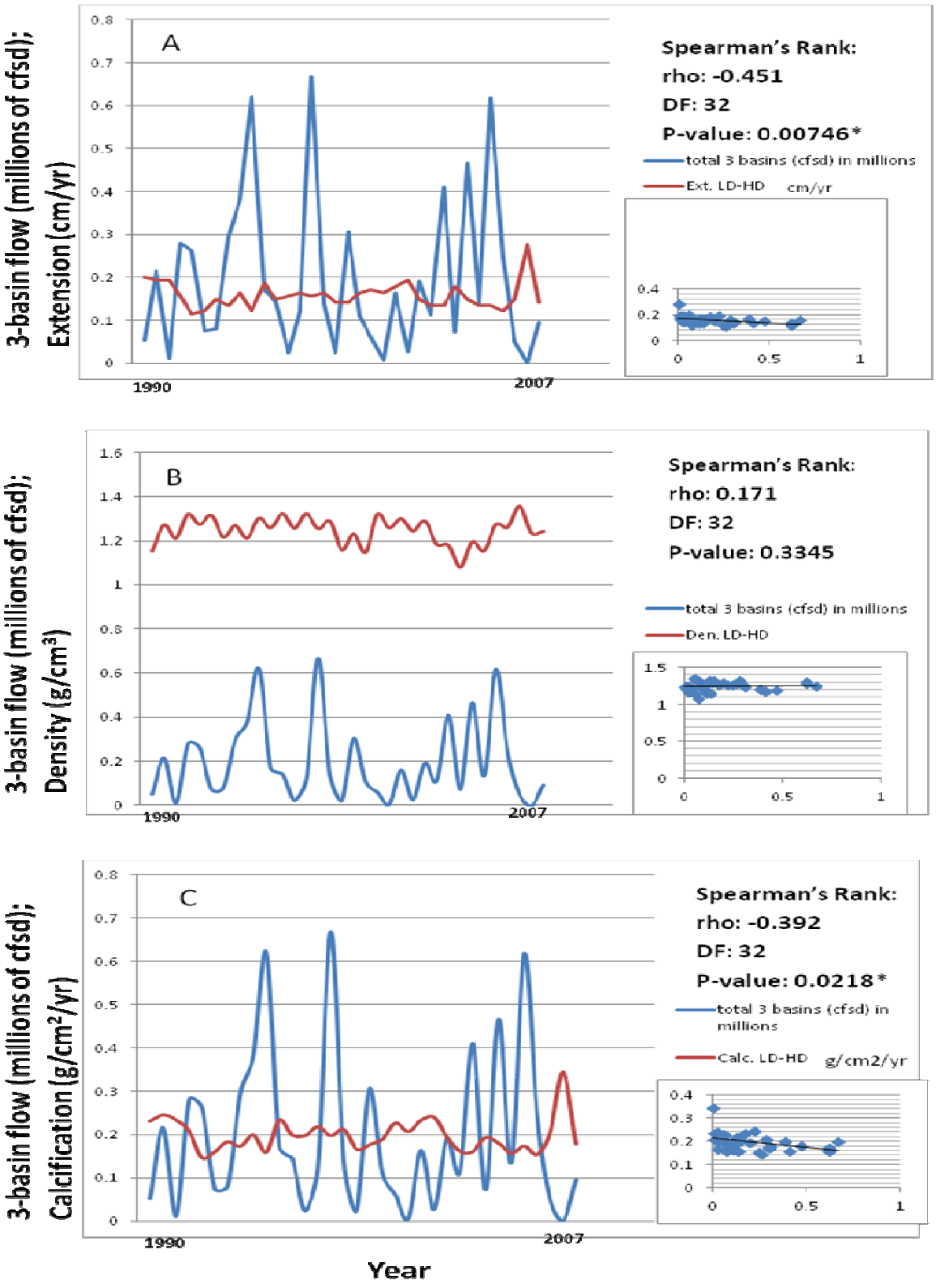


Fig. 18. Flow from the St. Lucie River's three main basins (C-44, C-23, C-24) versus *D. clivosa* skeleton core A). extension rate B). density C). calcification for 1990-2007.

Gene Expression and Bacterial Community Profiling: Evidence of Coral Stress

LH-PCR bacterial community profiling revealed that distinct microbial assemblages are present on *D. clivosa* colonies at St. Lucie Reef during different times of year (August and October). These shifts are linked to changes in the relative abundance of multiple bacterial types. Furthermore, despite the temporal variability observed at St. Lucie Reef, these samples were consistently different from those that have previously been sampled in the Florida Keys (FLK) and Lee Stocking Island, Bahamas (LSI). However, the interpretation of these regional differences is confounded by difference in coral species sampled. *D. clivosa* samples for bacterial community profile analysis have not been collected from either FLK or LSI, since these previous studies target coral species with relatively higher disease prevalence.

Nonetheless, the highly significant differences observed between St. Lucie Reef *D. clivosa* and corasl from other regions, suggest two possibilities. First, that *D. clivosa* Caribbean-wide harbor unique and distinct microbial assemblages in their mucus. Indeed, distinct microbial communities have previously been identified on different coral species, albeit only at one site (Rohwer et al. 2002). The alternative hypothesis is that *D. clivosa* at St. Lucie Reef harbor distinct microbial assemblages due to the variable and unique environmental conditions present on this reef, as compared to most coral reefs in the Tropical Western Atlantic. Overcoming the challenges encountered during sample analysis for *M. cavernosa* may allow us to further resolve this hypothesis. We plan to continue work with these colonies at St. Lucie Reef to address this question, as well as to further characterize changes that occur over time due to seasonal freshwater influence.

The SIMPER analyses revealed that the differences observed in *D. clivosa* mucus bacterial community profiles between sampling time points were driven primarily by variation in amplicons 338, 343, 310, 327, 322, 345 and 312 bp in length. Amplicons of 338, 310, and 322 bp were more abundant during October, while 343 and 312 bp amplicons were more abundant during August. Because the relative contribution to dissimilarity over time was not attributable to any single amplicon, it is not likely that these corals are being dominated by a single bacterial species or taxa during August or October. Rather, a more cosmopolitan and balanced bacterial assemblage is present. There is evidence that the amplicons we observed in the 310-312 bp range are associated with cyanobacteria and alphaproteobacteria. Previous studies using in silico analyses of sequences from both bacterial isolates and clones from seawater extractions associated these amplicons with both cyanobacteria and alphaproteobacteria (Suzuki et al. 1998, Bernhard et al. 2005).

In contrast to the bacterial community profiling data for *D. clivosa*, the gene expression data for *M. cavernosa* indicated that site was a relatively more important criterion, with time of sampling playing a secondary role at only one site. The gene expression results indicate that corals at the south site in August, 2010 are experiencing exposure to unknown xenobiotics. This is supported by the significant upregulation of two glutathione s-transferase (GST) genes (GSTM3, MGST3). GST functions in the detoxification and metabolism of xenobiotics or toxicants (Ji et al., 1994; Haynes et al. 2000). Additionally, a gene isolated from corals experimentally exposed to permethrin (P+3G22), a known pesticide, was upregulated in corals from the south site (Morgan and Snell, 2002). The upregulation of a

stress induced molecular chaperone (HSP90) and an immune response gene (FTH1) are also indicative of a cellular mediated stress response. Tissue necrosis and/or damage is also likely occurring based on the elevated expression of cell death mediator genes (NDUFA9, BCL2), inflammation responsive genes (MGST3, ACP5) and cellular reorganization or tissue repair genes (METAP2, CHMP2A, CDC42SE1, FTH1, SCARB1, TUBA1A). Additionally, increased signaling between cells, specifically by a gene involved in the maintenance and regulation of symbiosis (SCARB1), could indicate a breakdown in the symbiotic association between coral and zooxanthellae. This needs to be tested further, but could be an important indicator of the onset of paling or bleaching. Finally, elevated expression of genes involved in normal cellular functions, such as metabolism, DNA replication, protein transport and proteolysis indicate that although stressed, corals at the south site are still able to maintain functional cellular processes. However, due to the increased expression of stress genes, the homeostatic balance is likely shifted such that additional stress, even minor, could cause significant repercussions, such as paling, bleaching or mortality.

The results of this study are similar to another study investigating molecular responses of corals at reefs near point source inputs of pollution in Florida. In 2007, the same anthozoan microarray was used to identify molecular responses of *M. cavernosa* to point source inputs from the Port of Miami inlet and surrounding area (Biscayne Bay and Virginia Key sewage outfall) in south Florida (Edge, 2007). Gene expression patterns of corals at the site closest to the Port of Miami inlet revealed an acute localized stress response during February 2005. Gene profiling revealed highly differential expression in molecular chaperones, DNA repair, wound healing, metabolism, proteolysis, metal ion regulation, and xenobiotic metabolizing genes indicative of stress responses consistent with sediment stress and xenobiotic exposure (Edge, 2007). Specific similarities between the studies include elevated expression of cell death regulatory genes (NDUFA9, BCL2), an immune responsive gene (FTH1), and inflammation and xenobiotic responsive genes (GSTM3, MGST3, P+3G22) at sites nearest the point source of pollution. In addition, corals at the impacted site in the South Florida study appeared healthy (i.e. no visual signs of partial mortality or bleaching), although most exhibited full or partial covering by sediments (Dr. Sara Edge, pers. obser.). This is similar to observations of corals at all sites in the St. Lucie reef study. Little bleaching was observed, but sedimentation was evident. Additionally, both sites (St. Lucie reef and Miami) exhibited low species diversity and were dominated by sediment tolerant scleractinian coral species, including *M. cavernosa* and *Diploria sp.* (Bak & Elgershuizen, 1976; Lasker, 1980).

Another response observed in this study reveals the differential expression of genes at the central site between dates. These genes show an elevated level of expression in the month of October compared to August, 2005. At least three of these genes are stressed-induced, including two coral host molecular chaperones (HSPD1, HSPB11) and a zooxanthellae gene (RBCL). These genes are known to be induced by multiple stressors such as elevated temperatures, decreased salinity, ultraviolet radiation and pollution (Edge, 2007). Additionally, the elevated expression of two genes involved in metal detoxification and homeostasis (MT1A, TF) indicate exposure to a stimulus that effects metal ion homeostasis in the cell, such as osmotic or oxidative stress (Jamieson, 2002). In addition, vitellogenin (VTG), a gene involved in reproduction, which is also inducible upon exposure to endocrine disruptors and/or other xenobiotics, shows significantly lower expression in coral samples from the central site in

August compared to all other samples (Flouriot et al., 1995; Kloasa et al., 1999; Islinger et al., 2002) (Figure 4). These results could indicate xenobiotic exposure at the central site in October, but since no other xenobiotic responsive gene is elevated (GSTM3, MGST3 or P+3G22), it is hypothesized to be a more generalized stress response such as exposure to a low toxicity compound, a low-dose of a compound, or exposure for a short duration (Hall, 2002; Mandala, 2006; Edge, 2007). Finally, a cell death regulator (CTSB) is elevated in October at the central site, which is involved in a cellular response to wounding and acts as a negative regulator of apoptosis in cellular responses. The remaining gene responses are involved in normal cellular or multifunctional activities, such as signal transduction (BMP4, PTK2) and cell migration (LMLN). These results indicate a stress response by corals at the central site in October, but it is unclear if these patterns are related to the same or similar stressor as the south site in August. Based on the number and types of genes expressed in *M. cavernosa* samples, it appears that between sites, the south site is more stressed than the central site. But between collections dates, the central site shows elevated stress in October compared to August, indicating a temporal response. It is hypothesized that the south site is continuously stressed such that there is no change over time in stress response, whereas the central site is stressed due to a temporal effect such as freshwater influence, temperature, upwelling, salinity, etc. However, this hypothesis needs to be tested further. Regardless, coral colonies at both sites reveal some level of stress response, and those at the south site in August appear to be responding to an undetermined xenobiotic response.

The lack of significant differences in *D. clivosa*-associated bacterial communities among the north, central, and south sampling sites was unexpected. Likewise, the elevated levels of stress-related gene expression in *M. cavernosa* at the southern site contradict our initial hypothesis. We hypothesized that estuarine water influence would be variable along a north to south gradient, resulting in variable environmental conditions and corresponding differences in bacterial communities and gene expression. A more detailed study is needed to resolve the spatial and temporal scales of any environmental gradients that occur as a result of St. Lucie Estuary water influx. We must also determine with future sampling and analyses if compounds within the riverine waters are responsible for rapid decay of genetic material extracted from the mucus of *M. cavernosa* at St. Lucie Reef.

Conclusions and Recommendations

Based upon our findings in this study and the existing datasets regarding the St. Lucie River impairment (e.g., well-documented nutrient and dissolved oxygen problems associated with the state's Total Maximum Daily Load program) and its potential influence on St. Lucie Reef, the corals located on this reef are subject to a unique suite of stress-related factors. Clearly, the tropical/subtropical benthic organisms here exist in a balance of latitude (e.g., extremes of light and temperature requirements) and the pronounced influence of a proximal inlet (e.g., turbidity, sedimentation, color, nutrients, salinity, pollutants). For photosynthesizing organisms such as corals, these factors strongly determine ecological compensation depth (Kirk 1994). The corals of St. Lucie Reef have adapted a unique life history strategy, exhibiting low-relief plate-forming growth forms (esp. *D. clivosa*) which maximize exposure to sunlight while reducing drag. Coral survival in low light conditions via increased heterotrophy is well-documented (Porter 1976) and perhaps the St. Lucie River influence provides the right mix for a ready planktonic food source when conditions are unsuitable for appreciable photosynthesis. Our data shows

that light levels can drop to extremely low values for days/weeks at the reef during the blackwater period, even at shallow depths (7 feet MLW at north site) and proximity to the inlet is paramount.

The skeletal features of St. Lucie Reef corals also reveal the effects of landscape position. Coral adherence to the substrate is an ongoing concern for reef managers at St. Lucie Reef. Corals are regularly found dislodged and wedged into crevices. Of the 22 corals studied for this project, four have become dislodged and were reattached or lost following our sampling, including a *D. clivosa* that is ~1.5 meters in diameter and requires three divers to move. A clear link has been established at this reef between flow and calcification and extension rate. Mean extension rate at St. Lucie Reef for the *D. clivosa* core analyzed was 3.0mm/yr (Beal & Helmle, unpub. data) and this is similar to *D. strigosa* in Bermuda but less than values measured for *Diploria* in other Caribbean locations (Logan et al. 1994). Because corals excrete a calcium carbonate latticework "scaffolding" during the daytime photosynthesis period and fill in remaining gaps at night, corals living under stressful conditions adopt a strategy in which fragile, low-density skeletons are made. Yentsch et al. (2002) describe the relationship between maximum coral depth and skeletal density as the "jaws of a vice" and St. Lucie Reef corals are likely exhibiting this dilemma. At the north site, very small specimens of few species occur (4 total colonies of *D. clivosa*, numerous *Siderastrea* spp.) at very low cover/density in shallow water (7 feet MLW) and deeper (8-15 feet) hardbottom areas directly adjacent contain no corals. The central site (11 feet MLW) contains the greatest coral cover, density, size and diversity (*D. clivosa* up to 13.5 feet in diameter) on the reef while the south site (13 feet MLW) contains similar species that are more widespread and smaller (*D. clivosa* up to 6 feet in diameter) than the central site specimens.

Small decreases in Kd can have large effects on ecological compensation depth, coral percent cover, and skeletal features (Cook et al. 1997; Yentsch et al. 2002) and the St. Lucie Reef sites show evidence of these relationships with an obvious north-south increase in Kd values. Kd values at St. Lucie Reef (Figure 14) were comparable to values calculated in various sites in summer around Key West (mean 0.2722, range 0.0197-1.1537) during early summer but all sites showed levels during the blackwater period that likely exceed known coral needs (Yentsch et al. 2002). The north site showed Kd values slightly above the Key West mean early on and greatly above it late. Two key factors dictating maximum coral depth are solar irradiance at the surface influenced by seasonal sun angle and day length (latitude) and water transparency (Yentsch et al. 2002) and St. Lucie Reef corals are strongly influenced by both. Another key factor is that the deeper the site, the greater the temperature drop and more persistent the upwelling event. The south site therefore experienced the greatest effect of the mid-summer upwelling in terms of low temperatures and dissolved oxygen levels and perhaps responded to this influence in terms of stress-gene expression. St. Lucie Reef exhibits a unique hydrodynamic in that increased tidal stage can bring oceanic water (outside of upwelling events), the clarity of which is more important than the increases in overall volume. Evidence for this occurs at the north and central sites before the upwelling but not at the south site where the volume of persistently clear water is more important (Figure 15). During the blackwater period, all of the sites showed decreased light levels with increased tidal stage as colored water permeated the reef. Dune light levels were a good barometer for coral site light levels before the blackwater period with cloud cover having an obvious and predictable effect. The relationship was weakened during the blackwater event and the

north site showed the weakest link with dune light as inlet influence was more pronounced. The temperature connection between the inlet and central site was strong but weaker for the north and south sites, indicating other overriding spatial and/or temporal influences proximal and distal to the inlet.

When blackwater events occur, exacerbated in the fall by high wind events, the corals experience prolonged nighttime-like conditions which affects growth and survivorship. This major influence, coupled with other stressors such as upwelling events, likely explain the coral stress responses seen in this study. The few surviving corals at St. Lucie, however, remain resistant and resilient to perturbations, exhibiting relatively few incidences of disease and only occasional visible signs of stress (Figure 19). The only coral bleaching event documented at St. Lucie Reef occurred in February, 2006 following 12 months of releases from Lake Okeechobee into the St. Lucie River (Jeff Beal, pers. obser.). Low salinity bleaching events have been seen elsewhere (Kerswell & Jones 2003) and this was the likely cause in this case. The reef had minimal bleaching on one third of Siderastrea spp. specimens surveyed in fall 2006-07 (Wagner et al. 2010). No bleaching was documented during the cold winters of 2010-11, however, which caused major die-offs to corals in the Florida Keys (Jeff Beal, pers. obser.). Sebastian et al. (2009) suggest that corals at high latitude (such as those found at St. Lucie Reef) are protected from bleaching.

Fig. 19. Visible signs of stress on a *M. cavernosa* at the central site on 4/29/10. The coral recovered fully.

The effects of land-based sources of pollution on coral reefs is well-documented worldwide (see reviews by Fabricius 2005, Jameson and Kelty 2004, PBSJ 2008), with turbidity (Anthony & Connolly 2004, Anthony and Larcombe 2000, Rogers 1990, Telesnicki & Goldberg 1995) and salinity (Coles & Jokiel 1992) playing key roles in determining coral stress, growth, and survivorship. Up to certain thresholds which are largely undescribed, high turbidity can reduce high light, thereby protecting corals

at certain times/locations, and corals can use the suspended particles as a food source to increase lipid storage. This has been shown in mesocosm experiments (Anthony et al 2007) and field studies (Anthony 2006). When thresholds are reached, corals often respond to turbidity by producing excessive mucus, a metabolically-expensive loss of carbon (Reigl & Branch 1995). Corals at all sites in our study showed heavy mucus layers on the October sampling date during the blackwater period and this is an obvious stress response indicator, possibly due to turbidity and other water quality factors associated with riverine influence on the reef. Land-based sources of pollution from point sources such as inlets can establish recognizable gradients of reef community structure and ecology (Hennige et al. 2010, Van Woesik et al. 1999). Some aspects of St. Lucie Reef exhibit signs of a gradient (light data, coral cover, species, and size, certain genes expressed by site), while others are variable, likely caused by unique hydrodynamic forces. The metabolic demand of corals living in this stressful environment also affects reproductive capacity. Reproduction of corals on St. Lucie Reef remains a mystery as our study found only one coral with visible gonads, unlike a study conducted by Nova Southeastern Univ. in 2009 which found visible gonads in specimens of *D. clivosa, M. cavernosa*, and *Siderastrea siderea* (Adam St. Gelais, unpubl. data). St. Gelais (2010) recorded lower fecundity and oocyte volume numbers from St. Lucie Reef *S. siderea* colonies than those from Key Largo and Ft. Lauderdale, describing a latitude gradient. The riverine influence at St. Lucie Reef could disrupt well-known cues for spawning and conditions necessary for preparation to spawn such as temperature, solar insolation/gravity, tidal signature, and lunar cycle. The mid-summer upwelling likely plays a role during the critical pre-spawn window as well. Despite numerous night dives by researchers and local divers on the reef for several decades (mainly associated with recreational lobstering but also with coral research), no spawn has been witnessed on the reef. It remains unclear whether or not the coral population at St. Lucie Reef is a sink or a source of coral recruits. Certainly, upwelling and/or blackwater events which can occur during the spawning period (likely 8 days following the full moon in August and/or September) would have severely detrimental effects on coral reproduction metabolism, cues, and gamete survivorship. An example from our study is the down-regulation of vitellogenin at the central site in August when corals might usually be preparing for spawning. The reproductive strategies adopted by the species (Szmant 1986) inhabiting St. Lucie Reef (i.e., broadcasting gametes), developed by parent colonies to the south, might preclude these specimens from spawning successfully here due to the unique water quality conditions.

With catastrophic losses of corals worldwide during the past few decades, reef managers are encouraged to alleviate stressors to reef communities such as land-based sources of pollution in an effort to save these fragile habitats (Marshall & Schuttenberg 2006). The effects of inlets on reefs in south Florida has been identified as a key component of the Southeast Florida Coral Reef Initiative (SEFCRI) as well as a need for greater understanding of the existing state water quality rules and their relatedness to coral communities (Trnka et al. 2007). Improvements to the St. Lucie Estuary are proposed (Figure 20) through the Indian River Lagoon-South portion of the Comprehensive Everglades Restoration Plan (CERP) and certain aspects of the plan are ongoing through Acceler8 projects in the region (*viz*., reservoirs and Stormwater Treatment Areas). Associated RECOVER (CERP monitoring program) performance measures, including water quality targets (Sime 2005), will likely improve conditions on the reef but the RECOVER program is not providing any funds toward reef assessment or monitoring on St. Lucie Reef. Therefore, historical conditions and future with-project improvements on

the reef have been largely unknown and unpredictable, respectively. This study addresses the need for baseline information on the pre-restoration conditions along the reef tract and establishes clear linkages between releases from the St. Lucie River and key parameters of reef ecology. St. Lucie Reef corals show signs of stress at the genetic level and thresholds for coral health, though still undetermined to any large degree for the species in question, are being exceeded. Improvements in water delivery (timing, volume) and quality from the St. Lucie system onto the reef are needed to promote the continued survival of keystone species such as hard corals and to promote improved coral health and (ideally) the expansion of coral abundance, cover, density, and diversity. Evidence for improvements would include a reduction in sub-lethal indicators (gene expression) and the expansion of corals toward the inlet and into deeper hardbottom areas. Reductions in key indicators such as color, turbidity, and nutrients while maintaining bottom salinity above 30 psu and Kd values suitable for coral survival/growth on the reef tract are tantamount to restoration. This reef project represents a case-study for assessing the effects of land-based sources of pollution on Florida's coral reefs, an emphasis of the Florida Wildlife Legacy Initiative to define and abate coral reef threats/sources (incompatible release of water/stormwater management, lack in keystone species abundance, altered water quality, sedimentation), thereby supporting efforts of the SEFCRI Land-Based Sources of Pollution Subteam and the NOAA Coral Reef Conservation Program (CRCP). Methods such as the ones used in this study to assess sub-lethal effects are critically important and useful for identifying coral stress when no visible indicators are apparent. Integration of these independent methodologies is used to validate each process and determine the relative strengths and weaknesses of each method. Secondarily, these molecular methodologies are integrated with field observations of coral colonies and abiotic parameters in order to provide a robust assessment of coral health (Risk et al. 2001). St. Lucie Reef and its corals exhibit certain conditions unique to the wider Caribbean while sharing some key indicator species. Studying the extent of coral resistance and resilience to stressors at this reef will lend insight into reef management in Florida. We will expand this study with grant support from the Save Our Seas Specialty License Plate Program to include additional sites, species, and time points for sampling. Furthermore, we will install HOBO salinity and temperature loggers to more accurately determine the dynamics of freshwater influx and upwelling events.

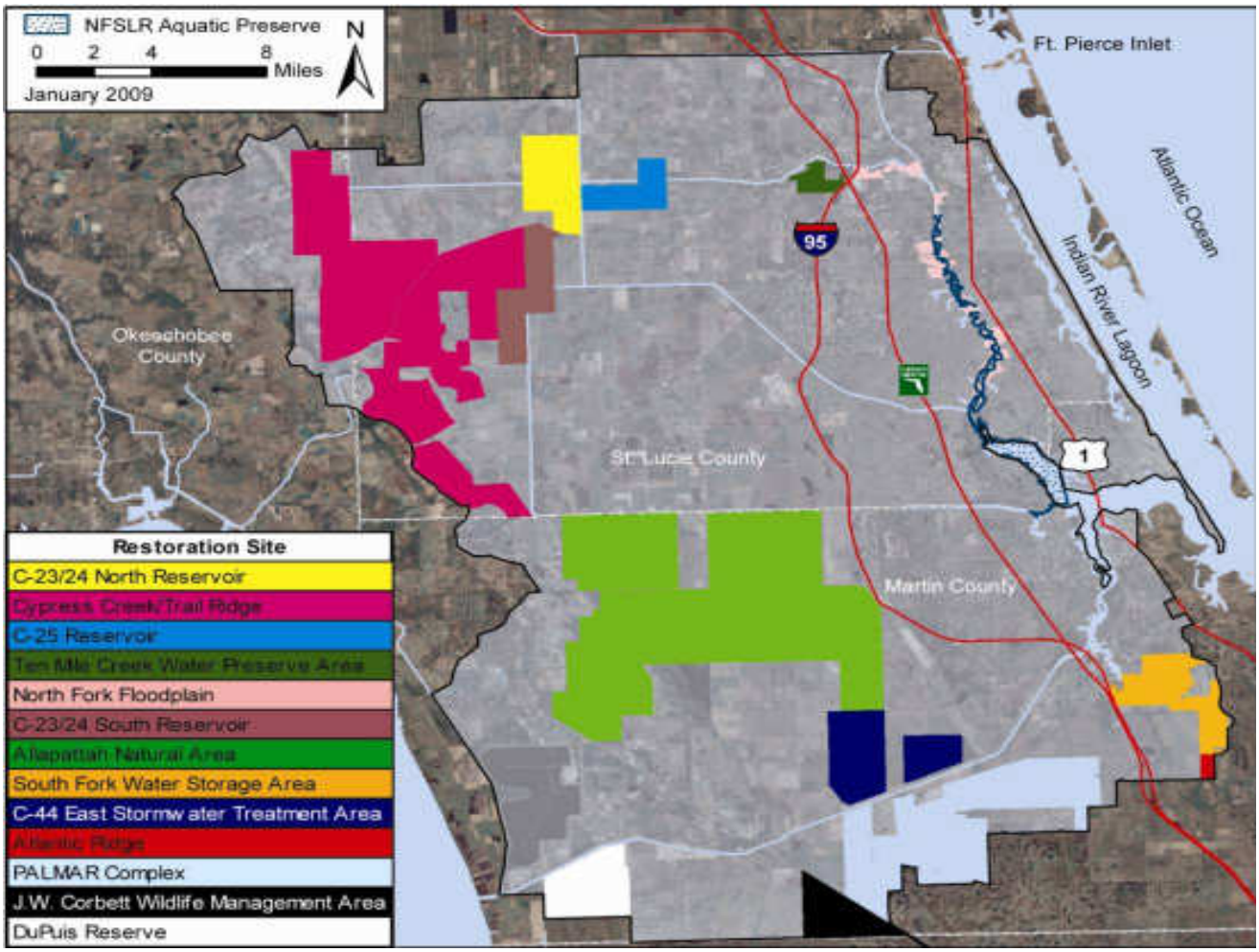


Fig. 20. Features associated with the St. Lucie River watershed to be developed as part of the Indian River Lagoon South Feasibility Plan (CERP) to improve water distribution and quality.

Acknowledgments

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Appendix

Multi-variate analysis of data collected on St. Lucie Reef (PAR, temperature; redundant HOBO units) versus St. Lucie Inlet weather station (various parameters) and at adjacent dunes (PAR); numbers are Pearson's r values

Pre-upwelling period at north site

	Temp1 C north site	Temp2 C north site	Intensity 1, lum/ft^2 north site	Intensity 2, lum/ft^2 north site
Time, GMT-04:00	0.5337	0.3761	0.1480	0.0007
Temp1 C north site	1.0000	0.9877	0.2459	0.2752
Intensity 1, lum/ft^2 north site	0.2459	0.2692	1.0000	0.7372
Temp2 C north site	0.9877	1.0000	0.2692	0.2827
Intensity 2, lum/ft^2 north site	0.2752	0.2827	0.7372	1.0000
inlet upper temp C	0.7382	0.6140	0.0499	0.0046
inlet lower temp C	0.7321	0.6012	0.0481	0.0163
inlet conduct upper	0.0960	0.1985	0.0487	-0.0206
inlet conduct lower	0.3087	0.2855	0.1102	0.0503
Dune Intensity 1, lum/ft^2	0.1174	0.1509	0.6606	0.6525
Dune Intensity 2, lum/ft^2	0.1150	0.1472	0.6624	0.6683
inlet level NAVD88 in meters	-0.0896	-0.1683	0.1022	0.0520

During upwelling period at north site (inlet weather station inoperable)

	Temp1 C north site	Temp2 C north site	Intensity 1, lum/ft^2 north site	Intensity 2, lum/ft^2 north site
Time, GMT-04:00	0.2980	0.2893	-0.1560	-0.2054
Temp1 C north site	1.0000	0.9992	0.1837	0.3245
Intensity 1, lum/ft^2 north site	0.1837	0.2058	1.0000	0.7930
Temp2 C north site	0.9992	1.0000	0.2058	0.3435
Intensity 2, lum/ft^2 north site	0.3245	0.3435	0.7930	1.0000
inlet upper temp C	0.0000	0.0000	0.0000	0.0000
inlet lower temp C	0.0000	0.0000	0.0000	0.0000
inlet conduct upper	0.0000	0.0000	0.0000	0.0000
inlet conduct lower	0.0000	0.0000	0.0000	0.0000
Dune Intensity 1, lum/ft^2	0.1630	0.1804	0.6943	0.5121
Dune Intensity 2, lum/ft^2	0.1039	0.1201	0.6855	0.4243
inlet level NAVD88 in meters	0.0000	0.0000	0.0000	0.0000

Post-upwelling period at north site

	Temp1 C north site	Temp2 C north site	Intensity 1, lum/ft^2 north site	Intensity 2, lum/ft^2 north site
Time, GMT-04:00	-0.2164	-0.1418	-0.3230	-0.1382
Temp1 C north site	1.0000	0.9987	-0.0152	-0.1439
Intensity 1, lum/ft^2 north site	-0.0152	-0.0510	1.0000	0.8588
Temp2 C north site	0.9987	1.0000	-0.0510	-0.1445
Intensity 2, lum/ft^2 north site	-0.1439	-0.1445	0.8588	1.0000
inlet upper temp C	0.5110	0.4571	0.0771	-0.1144
inlet lower temp C	0.6149	0.5888	-0.0503	-0.2355
inlet conduct upper	-0.1242	-0.1070	0.0874	0.1046
inlet conduct lower	0.3320	0.3558	0.0490	0.0255
Dune Intensity 1, lum/ft^2	0.0641	0.0491	0.5562	0.4840
Dune Intensity 2, lum/ft^2	0.0660	0.0522	0.5522	0.4841
inlet level NAVD88 in meters	0.2076	0.1756	-0.0534	-0.0538

Pre-upwelling period at central site

	Temp1 C central site	Temp2 C central site	Intensity 1, lum/ft^2 central site	Intensity 2, lum/ft^2 central site
Time, GMT-04:00	0.5676	0.5735	-0.0484	-0.0907
Temp1 C central site	1.0000	0.9990	0.1291	0.0031
Intensity 1, lum/ft^2 central site	0.1291	0.1051	1.0000	0.7702
Temp2 C central site	0.9990	1.0000	0.1051	-0.0085
Intensity 2, lum/ft^2 central site	0.0031	-0.0085	0.7702	1.0000
inlet upper temp C	0.8029	0.8105	-0.0990	-0.1552
inlet lower temp C	0.8007	0.8081	-0.0836	-0.1447
inlet conduct upper	-0.0916	-0.0970	0.0969	0.0134
inlet conduct lower	0.2076	0.2064	0.0644	-0.0394
Dune Intensity 1, lum/ft^2	0.1080	0.0856	0.7511	0.6529
Dune Intensity 2, lum/ft^2	0.1073	0.0851	0.7425	0.6419
inlet level NAVD88 in meters	-0.1045	-0.1053	0.0661	0.0205

During upwelling period at central site (inlet weather station inoperable)

	Temp1 C central site	Temp2 C central site	Intensity 1, lum/ft^2 central site	Intensity 2, lum/ft^2 central site
Time, GMT-04:00	0.3516	0.3483	-0.0124	-0.0555
Temp1 C central site	1.0000	0.9995	0.1260	0.0592
Intensity 1, lum/ft^2 central site	0.1260	0.1144	1.0000	0.8829
Temp2 C central site	0.9995	1.0000	0.1144	0.0477
Intensity 2, lum/ft^2 central site	0.0592	0.0477	0.8829	1.0000
inlet upper temp C	0.0000	0.0000	0.0000	0.0000
inlet lower temp C	0.0000	0.0000	0.0000	0.0000
inlet conduct upper	0.0000	0.0000	0.0000	0.0000
inlet conduct lower	0.0000	0.0000	0.0000	0.0000
Dune Intensity 1, lum/ft^2	0.0080	0.0002	0.7374	0.7120
Dune Intensity 2, lum/ft^2	-0.0260	-0.0339	0.7281	0.6297
inlet level NAVD88 in meters	0.0000	0.0000	0.0000	0.0000

Post-upwelling period at central site

	Temp1 C central site	Temp2 C central site	Intensity 1, lum/ft^2 central site	Intensity 2, lum/ft^2 central site
Time, GMT-04:00	-0.4506	-0.4480	-0.2967	-0.2356
Temp1 C central site	1.0000	0.9996	0.0340	0.0161
Intensity 1, lum/ft^2 central site	0.0340	0.0219	1.0000	0.8660
Temp2 C central site	0.9996	1.0000	0.0219	0.0073
Intensity 2, lum/ft^2 central site	0.0161	0.0073	0.8660	1.0000
inlet upper temp C	0.7569	0.7559	0.0621	0.0669
inlet lower temp C	0.8130	0.8124	0.0146	0.0151
inlet conduct upper	-0.1784	-0.1810	0.0356	0.0024
inlet conduct lower	0.5186	0.5160	0.0891	0.0410
Dune Intensity 1, lum/ft^2	0.0653	0.0584	0.5887	0.5605
Dune Intensity 2, lum/ft^2	0.0896	0.0822	0.6175	0.5713
inlet level NAVD88 in meters	0.0735	0.0739	-0.0722	-0.0663

Pre-upwelling period at south site

	Temp1 C south site	Temp2 C south site	Intensity 1, lum/ft^2 south site	Intensity 2, lum/ft^2 south site
Time, GMT-04:00	0.3331	0.1397	0.0357	0.0067
Temp1 C south site	1.0000	0.9931	0.1271	0.2226
Intensity 1, lum/ft^2 south site	0.1271	0.2150	1.0000	0.7924
Temp2 C south site	0.9931	1.0000	0.2150	0.1961
Intensity 2, lum/ft^2 south site	0.2226	0.1961	0.7924	1.0000
inlet upper temp C	0.7604	0.4440	-0.0701	-0.0235
inlet lower temp C	0.7733	0.4752	-0.0757	-0.0005
inlet conduct upper	-0.0793	-0.0206	0.0535	-0.0905
inlet conduct lower	0.1923	0.0338	0.0570	-0.0182
Dune Intensity 1, lum/ft^2	0.0708	0.0787	0.6987	0.6991
Dune Intensity 2, lum/ft^2	0.0685	0.0846	0.7033	0.6990
inlet level NAVD88 in meters	-0.1347	-0.1594	-0.0461	-0.0507

During upwelling period at south site (inlet weather station inoperable)

	Temp1 C south site	Temp2 C south site	Intensity 1, lum/ft^2 south site	Intensity 2, lum/ft^2 south site
Time, GMT-04:00	0.3170	0.3102	0.1377	0.0481
Temp1 C south site	1.0000	0.9941	0.2369	0.1661
Intensity 1, lum/ft^2 south site	0.2369	0.2241	1.0000	0.8432
Temp2 C south site	0.9941	1.0000	0.2241	0.1592
Intensity 2, lum/ft^2 south site	0.1661	0.1592	0.8432	1.0000
inlet upper temp C	0.0000	0.0000	0.0000	0.0000
inlet lower temp C	0.0000	0.0000	0.0000	0.0000
inlet conduct upper	0.0000	0.0000	0.0000	0.0000
inlet conduct lower	0.0000	0.0000	0.0000	0.0000
Dune Intensity 1, lum/ft^2	0.0705	0.0640	0.7115	0.6082
Dune Intensity 2, lum/ft^2	0.0434	0.0384	0.7208	0.5614
inlet level NAVD88 in meters	0.0000	0.0000	0.0000	0.0000

Post-upwelling period at south site

	Temp1 C south site	Temp2 C south site	Intensity 1, lum/ft^2 south site	Intensity 2, lum/ft^2 south site
Time, GMT-04:00	-0.2053	-0.2291	-0.2765	-0.3286
Temp1 C south site	1.0000	0.9981	-0.1111	-0.0746
Intensity 1, lum/ft^2 south site	-0.1111	-0.1104	1.0000	0.9200
Temp2 C south site	0.9981	1.0000	-0.1104	-0.0708
Intensity 2, lum/ft^2 south site	-0.0746	-0.0708	0.9200	1.0000
inlet upper temp C	0.5619	0.5745	0.0930	0.1213
inlet lower temp C	0.6124	0.6218	0.0480	0.0825
inlet conduct upper	-0.1328	-0.1335	0.0541	0.0220
inlet conduct lower	0.4646	0.4677	0.0739	0.0848
Dune Intensity 1, lum/ft^2	-0.0178	-0.0162	0.5377	0.5531
Dune Intensity 2, lum/ft^2	0.0094	0.0117	0.5661	0.5837
inlet level NAVD88 in meters	0.1127	0.1061	-0.1311	-0.0661