

**Grantee:** Sara Edge, Harbor Branch Oceanographic Institute at Florida Atlantic University

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**Project Title:** Molecular diagnostics of coral exposed to oil & dispersants: a holobiont approach to investigate effects on the host corals, symbiotic algae, and mucus associated microbial communities

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Understanding the acute and chronic impacts to Florida's economically-important reef-building corals following an oil spill event, such as the Deepwater Horizon oil spill (April 2010), is of the utmost importance. This study has adapted and used existing advanced molecular biomarker tools to rapidly assess *ex situ* responses of one of Florida's cornerstone coral species, *Montastraea faveolata*, to crude oil, dispersant, and disease. The following objectives were proposed.

### ***Originally-proposed Objectives***

1. Modify current coral-stress microarray to include suites of toxicant specific biomarkers for acute and chronic oil/dispersant exposures.
2. Experimentally-determine acute susceptibility of coral colonies to crude oil and dispersants using an *ex situ* block design. Identify coral gene expression patterns associated with each exposure (oil, dispersant, oil and dispersant) versus controls. Evaluate the effects of oil/dispersant on the community composition of the mucus-associated microbial assemblages of using length heterogeneity PCR.
3. Determine if different coral genotypes have variable responses to disease, oil, and dispersant exposure in the experiment above.
4. Monitor coral exposure to crude oil and dispersants in the field through quarterly surveys and sampling at CREMP sites as well as sites established by the Harbor Branch's Robertson Coral Reef Program. This will include coral health surveys as well as tissue sample collections for genetic and microbial analysis in the event of oil/dispersants entering each site.
5. Compare gene expression data and microbial community profile data of coral colonies from the *ex situ* experiment to *in situ* data from oil/dispersant exposed and non-exposed coral sites. Additionally, the data will be compared to baseline *in situ* data from Harbor Branch's quarterly monitoring (2007 - 2009) of coral health and disease at monitoring sites in lower Florida and the Bahamas.
6. Provide rapid data analysis for managers and decision makers, as well as access to microarray technology by interested parties for further or auxiliary analyses.

### ***Project Collaborations***

Three months after the DWH oil spill, and before extensive oil impacts reached shelf-edge reefs in the eastern Gulf of Mexico, NOAA's Cooperative Institute for Ocean Exploration, Research & Technology (CIOERT) conducted a rapid response, multidisciplinary, multi-institutional expedition to assess the impacts of the DWH oil spill on mesophotic and deepwater ecosystems from the Florida Keys to the Mississippi coast. Habitat characterization, visual health

assessments, and reproductive status analyses of coral reefs were conducted during the expedition using the *Johnson Sea Link II* submersible and SCUBA. The coral toxicant-focused microarray developed with funds from the FIO project will be used to determine the relative levels of gene expression in representative scleractinian corals and octocorals collected from the CIOERT expedition. By comparing specimen collected during the CIOERT expedition with corals experimentally exposed to oil from the FIO funded project, we will be able to provide critical information on the underlying mechanisms of oil's impact on corals and other marine invertebrates.

Additionally, funds provided by FIO for this project were leveraged against funds provided by Mote Protect Our Reefs (POR) for a related project. (*Determining the Molecular Basis of Susceptibility to Disease in Floridian Corals*; Mote Protect Our Reefs funded project; \$34,603.00 awarded; PI Joshua Voss, Ph.D., Co-PI Sara Edge, Ph.D.). By combining the POR project with the FIO project we expect to gain significant insights into the roles of coral health, coral genotypes, and stress (oil/ dispersant related or otherwise) in the mechanisms that impact coral disease and mortality. Importantly, the combination of the POR and FIO objectives will allow us to answer additional critical research questions while requiring fewer corals to be sacrificed. For example, only one set of control coral fragments was required, as opposed to two if the project's objectives were addressed in separate experiments. In addition, combining the projects enables an investigation of the interactions between treatment factors that would not otherwise be possible. For example, *does dispersant exposure cause normally disease resistant corals to become susceptible?* We believe that leveraging the two projects has produced much more robust data that may help us to better understand coral resiliency and resistance (or susceptibility) to stress and disease.

#### ***Development of a Coral Toxicant-focused Microarray***

The initial steps in the development of a toxicant- and stress-focused coral microarray were performed using bioinformatics techniques. Available online databases were mined using search algorithms and browser tools for available genes from stony corals (Order Scleractinia) and their endosymbiotic zooxanthellae (Genus *Symbiodinium*). Nucleotide sequences for 57,153 Scleractinian coral (Taxonomy ID: 6125) and 4,810 *Symbiodinium* sp. (Taxonomy ID: 2949) matching previously characterized genes were obtained from the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). A high-throughput analysis tool for functional genomics research, Blast2GO (<http://blast2go.bioinfo.cipf.es/>), was used to characterize genes through the functional annotation of sequences and analysis of annotation data. Each sequence was characterized, assigned gene ontology annotations ([www.geneontology.org](http://www.geneontology.org)) and mapped to metabolic pathways based on function and gene interactions. Successful annotation was achieved for 12,086 Scleractinian coral sequences and 754 *Symbiodinium* sp. sequences. The remaining sequences did not receive positive gene alignments or ontology matches and were discarded.

The characterized sequences were further filtered and functions were verified using BioGPS gene annotation web portal (<http://biogps.gnf.org>, Novartis Institute for Functional Genomics, Inc.). Redundant, irrelevant, or inconsistent gene sequences were removed and genes were limited to Scleractinian species of interest. For example, only 12 of the 250 available green fluorescent protein sequences were selected based on coral species of interest, appropriate

sequence lengths and roles in multiple cellular processes. Sequences that were chosen represent approximately 50 stony coral species and over 100 biomolecular pathways. Selected sequences were divided into functional groups based on roles in normal cellular functioning or stress-related cellular processes. Scleractinian sequences were divided into 51 functional groups with 1,368 total sequences and 327 *Symbiodinium* sp. sequences from 13 functional groups. Multiple open reading frames (ORFs), or protein coding regions, were identified for each gene sequence using the NCBI Genome Workbench version 2.2.0. Probes, short gene sequences attached to the microarray platform, were designed from each ORF using eArray GE Probe Design program 7.3 (Agilent Technologies). Three different probes of 60 nucleotides each were generated using the “Base Composition” and “Best Probe” design settings for each ORF. The final microarray design includes 3 replicate spots of each probe representing 1,368 coral genes and 327 *Symbiodinium* sp. genes as well as positive and negative control sequences. The array design is stored on a secure server and coral microarray chips may be ordered through Dr. Sara Edge’s lab for coral health analysis (printed by Agilent Technologies).

### ***Coral Collections and Experimental Exposure***

#### *Coral Collections and Permitting*

A permit was obtained to collect fragments, tissue and mucus from the coral species *Montastraea faveolata* (#FKNMS-2011-060) in the Florida Keys for experimental exposures and field monitoring of coral health. For experimental exposures, 5 fragments (500 cm<sup>2</sup>) of *Montastraea faveolata* colonies were collected from 3 different sites in the lower Florida Keys (West Washerwoman, Wonderland and East of Looe; Table 1) and cut into 2.5 cm x 4.0 cm rectangular fragments. The fragments were tagged, distributed in a flow-through raceway and allowed to acclimate for five days at Mote Tropical Research Laboratory (Summerland Key, Florida). Corals were tagged and allowed to recover/acclimatize for 5 days before exposures began. For field monitoring purposes, tissue was collected from 5 colonies of *M. faveolata* at 9 sites in the Florida Keys (Table 1). Specific requirements of the permit required the use of a syringe biopsy method to sample coral tissue in the field for monitoring. This method of tissue collection uses a needleless syringe to extract individual, single polyps from a coral colony and is less invasive than traditional sampling methods.

**Table 1.** Collection sites in Florida Keys

<i>Upper Keys</i>	Depth (ft)	<i>Middle Keys</i>	Depth (ft)	<i>Lower Keys</i>	Depth (ft)
Molasses Deep	50	Sombrero Deep	50	Looe Key Deep	50
Molasses Shallow	25	Sombrero Shallow	20	Looe Key Shallow	25
Porter Patch	20	Dustan Patch	15	Wonderland	25

#### *Field Monitoring Collections*

Coral tissue was collected from colonies in the field to monitor for potential oil exposure (or other contaminants) during September 12 – 16, 2011. A needleless 60 ml syringe was pressed securely over a single corallite of an *M. faveolata* colony. Once a sufficient seal was obtained, the plunger was pulled out, sucking the polyp tissue into the syringe (Figure 1a). The syringe tip was inserted into a 25 mm plastic Swin-Lok filter holder (Whatman Ltd.) containing a glass fiber GF/F filter (0.7 μm pore size) and the tissue was expelled onto the filter. This was repeated on 3 – 10 polyps per colony from 5 colonies at each site. Filters were removed and placed into 2.0 ml tubes with a buffered guanidine-based preservative for future RNA extraction and analysis. The process went smoothly with sufficient tissue collected with minimal damage to coral colonies

(Figure 1b). Fish and Wildlife Research Institute (FWRI) South Florida Regional Laboratory, Marathon Key, provided dive support, boats and laboratory space for sample processing during the collection trip.

Extraction of RNA from coral for gene expression profiling has not been attempted before with tissue collected using the syringe biopsy method. Therefore, the ability to obtain sufficient quantities of pure, intact total RNA was tested. Filters containing coral polyps were removed from preservative and total RNA was extracted from each using a phenol-chloroform based method with column purification (Qiagen<sup>®</sup>, RNeasy Minelute). Electropherogram analysis of the RNA was performed using an Agilent<sup>®</sup> Bioanalyzer instrument which measures the yield and purity of samples. Results indicate that 3 – 5 polyps per filter extraction is optimal and that high quality, intact total RNA can be successfully extracted from small amounts of coral tissue using the syringe biopsy method.



**Figure 1. A) Syringe sampling of *M. faveolata* tissue from colony in Florida Keys; B) Red circles show bare coral skeleton after tissue extraction of 3 polyps.**

### *Experimental Exposures*

Experimental exposures of coral colonies to oil, dispersant and disease were performed at Mote Marine Research Station, Summerland Key, FL June 6 – 22, 2011 by Drs. Edge and Voss, our technician, Lisa Cohen, and three undergraduate summer interns, Maureen Williams, Elizabeth Fahsbender and Natalie Harrison. After the 5 day acclimatization period, one fragment from each sampled colony was randomly transferred into 24, 5.5 L aquaria (n =15 fragments per tank) in a factorial block design. For experimental exposures, Corexit 9500A dispersant obtained from the manufacturer (Nalco, Co.), weathered oil collected on June 10, 2010 off the coast of Pensacola (by Florida Fish and Wildlife) and naturally-occurring black band disease were used. For each treatment tank, oil was added to a final concentration of 25 ppm and dispersant to 1 ppm. For disease treatments, black band disease (BBD) was collected from 3 naturally-infected *M. faveolata* at East of Looe Key reef. The BBD samples were homogenized and applied to the 2.5 cm edge of coral fragments in treatment tanks. Experimental treatments were replicated three times and environmental parameters, including pH, temperature, salinity and sunlight, were monitored and controlled to resemble the conditions from local reef sites. Table 2 shows a list of the treatment groups in this experiment (N=45 per group):

**Table 2.** List of treatment groups used to expose corals in a randomly distributed, factorial block design.

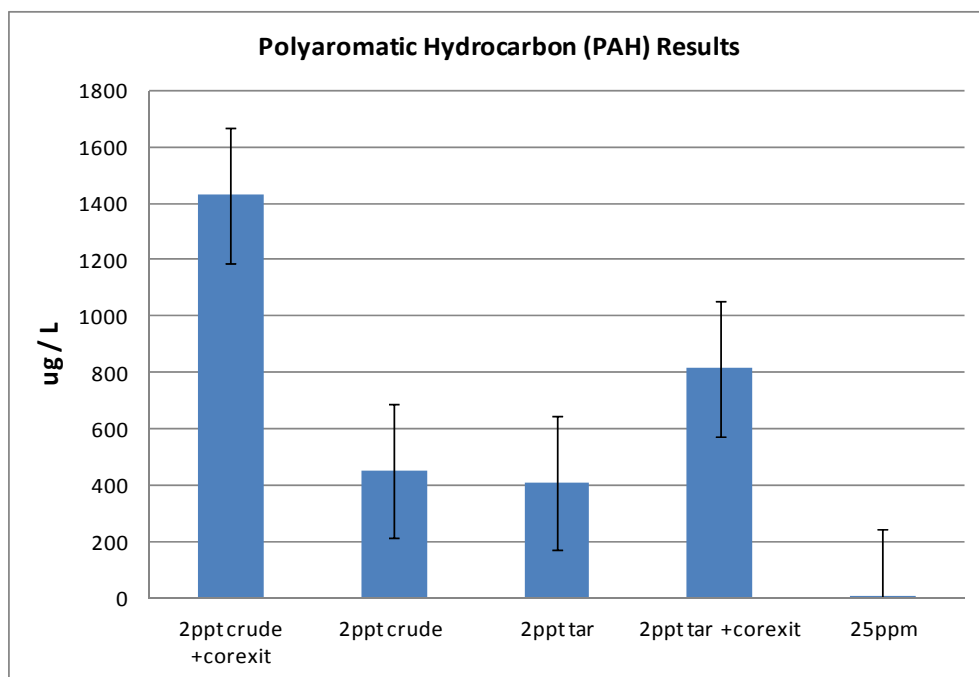
<b>HUC:</b> no disease, no oil, no dispersant	<b>BUC:</b> black band disease (BBD), no oil, no dispersant
<b>HUD:</b> no disease, no oil, dispersant	<b>BUD:</b> BBD, no oil, dispersant
<b>HOD:</b> no disease, oil, dispersant	<b>BOD:</b> BBD, oil, dispersant
<b>HOC:</b> no disease, oil, no dispersant	<b>BOC:</b> BBD, oil, no dispersant

After a seven-day treatment period, coral mucus and black band samples were collected from each fragment using sterile, plastic syringes, flash frozen in liquid nitrogen and stored at -20°C for bacterial community profiling analysis. Each coral fragment was then subdivided for gene expression analysis, zooxanthellae density and chlorophyll concentration measurements. Additionally, a subset of fragments for gene expression analysis was sampled using the polyp extraction method. This was done to confirm that gene expression by extracted polyps equates to that of the parent fragments.

**Results**

*Analysis of PAH in Oil Samples*

Oil and dispersant water samples were sent to Mote Marine Laboratory (care of Dana Wetzel) for total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon analyses (PAH). Mixtures of crude oil (2 ppt & 25 ppm), crude oil with Corexit 9500A (2 ppt + 1ppm), tar ball oil (2 ppt) and tar ball oil with Corexit 9500A (2 ppt + 1 ppm) were analyzed. A 2ppt stock solution was made by adding 2.0 g tar mat oil to 2 L of filtered full-strength seawater and stirred on low heat overnight. The following day, a 25 ppm solution was made in full strength seawater by pipetting below the upper layer of the stock solution. Dichloromethane was added 1:10, per the instructions of the analytical lab. Liquid samples were extracted at Mote using modified EPA

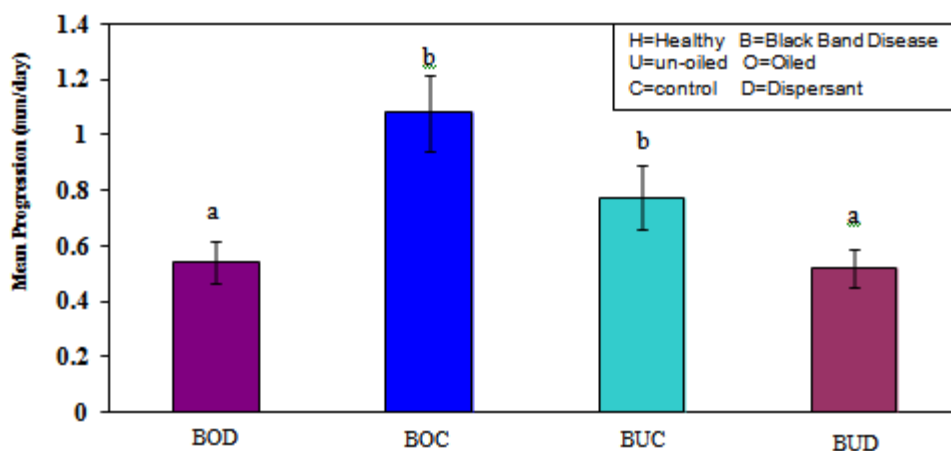


**Figure 2.** Polyaromatic hydrocarbon (PAH) content analysis in ug/L. Performed using gas chromatography / mass spec by Mote Research Labs.

Method 3510C and analyzed using gas chromatography/mass spectrometry. Results indicate significantly higher concentrations of both TPH's and PAH's in samples with dispersant added (Figure 2). More specifically, compounds significantly elevated in crude oil with Corexit (2 ppt + 1 ppm) include Naphthalene, Acenaphthylene, Fluorene, Fluoranthene, Benzo[b]fluorine and Pyrene. In tar ball with Corexit (2 ppt + 1 ppm) Dibenzothiophene, Benzo[a]anthracene, Phenanthrene and Anthracene exhibit the highest concentrations. Naphthobenzothiophene is significantly elevated in tar ball only, followed by crude oil (2 ppt) with Corexit (1 ppm). These results indicate that, in these samples, the addition of Corexit had an impact on the detectible availability of certain polyaromatic hydrocarbons.

### *Observed Physiological Responses*

No mortality, visible bleaching or excessive mucus production was observed during the study. After the experiment was complete, linear and spatial progression of black band disease, a sign of its pathogenesis, was measured using digital photographs and spatial analysis software (Coral Point Count with excel extensions). The ratio of healthy coral tissue to final black band area was calculated along with the total linear progression and a MANOVA was performed to determine significance. Results indicate that exposure to oil had no significant effect on progression of the disease. Conversely, treatments dosed with dispersant were shown to have lower linear and spatial progression (Figure 3). However, the interaction of oil and dispersant did not have an impact on disease progression. These results suggest that dispersant may have a greater impact on the BBD microbial community than on the coral or its associated bacterial symbionts.



**Figure 3.** Black band disease progression differed among treatment groups ( $F=13.77$ ,  $p<0.001$ ). Exposure to oil alone had no significant effect on coral BBD pathogenesis ( $F=2.396$ ,  $p=0.135$ ). Dispersant alone and dispersant with oil had a significant effect on the spatial progression of black band disease ( $F=13.766$ ,  $p=0.0003$ ).

Zooxanthellae density was measured for each coral replicate using a hemocytometer to count individual algal cells in a filtered and concentrated suspension. Five counts were averaged for each replicate. Chlorophyll 'a' and chlorophyll 'c' concentrations were determined by measuring light absorbance with a spectrophotometer at 750nm, 663nm, and 630nm following acetone extraction. Zooxanthellae density and chlorophyll density were scaled per  $\text{cm}^2$  of the surface area for each fragment. Correlations between the dependent variables were analyzed using Pearson's R and a four way MANOVA identified significant differences in zooxanthellae density,

chlorophyll 'a' density, chlorophyll 'c' density, chlorophyll 'a' per cell, and chlorophyll 'c' per cell. Oil treatment alone produced no significant effect on zooxanthellae density or chlorophyll concentrations. The presence of BBD increased zooxanthellae and chlorophyll 'a' densities; however chlorophyll 'a' decreased in concentration per cell (Table 3). Chlorophyll 'c' density and quantity per cell was not affected by BBD. Dispersant treatments resulted in a significant increase in chlorophyll 'a' density and quantity per cell, but did not affect chlorophyll 'c' or zooxanthellae density (Table 3). The only treatment interaction to show significance was chlorophyll 'a' concentration per cell in the BBD and dispersant exposure (Table 3). These results indicate that BBD increases zooxanthellae density while decreasing chlorophyll 'a' per cell. This is consistent with photoinhibition and rapid division of the algal symbionts during disease exposure. The increased density of algal cells may be due to spatial release or nutrient availability. Dispersant also significantly impacted the density of chlorophyll 'a' and amount per cell which may indicate an adaptive response or a symbiont clade shift, as different clades have unique physiological properties, including differences in photosynthetic abilities. These results need to be tested further and genetic analysis may help clarify some of the responses.

**Table 3.** ANOVA of zooxanthellae, chlorophyll 'a' & 'c' densities and chlorophyll 'a' & 'c' per cell. Treatments include BBD (black band disease), Oil, Disp (dispersant), BBD+Oil, BBD+Disp, Oil+Disp and BBD+Oil+Disp.

	Zooxanthellae Density		Chlorophyll 'a' Density		Chlorophyll 'a' per cell		Chlorophyll 'c' Density		Chlorophyll 'c' per cell	
	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F	F Ratio	Prob > F
<b>BBD</b>	58.8759	<.0001*	51.7687	<.0001*	8.3373	0.0041*	2.6462	0.1047	<i>unk</i>	<i>unk</i>
<b>Oil</b>	0.6994	0.4036	0.3204	0.5717	0.1900	0.6623	2.8253	0.0937	1.2444	0.2654
<b>BBD*Oil</b>	1.2738	0.2598	1.9197	0.1668	0.0267	0.8703	0.3796	0.5382	1.3353	0.2487
<b>Disp</b>	2.4516	0.1183	5.0249	0.0256*	3.4103	0.0656	4.4973	0.0347*	3.4769	0.0631
<b>BBD*Disp</b>	1.7823	0.1827	0.2233	0.6368	5.3391	0.0214*	0.9357	0.3341	2.7133	0.1004
<b>Oil*Disp</b>	0.2302	0.6317	0.4099	0.5224	0.8310	0.3626	3.4021	0.0660	3.6932	0.0554
<b>BBD*Oil*Disp</b>	0.8699	0.3516	0.0572	0.8111	0.7902	0.3746	2.5105	0.1140	3.2829	0.0709

### *Bacterial Community Profiling*

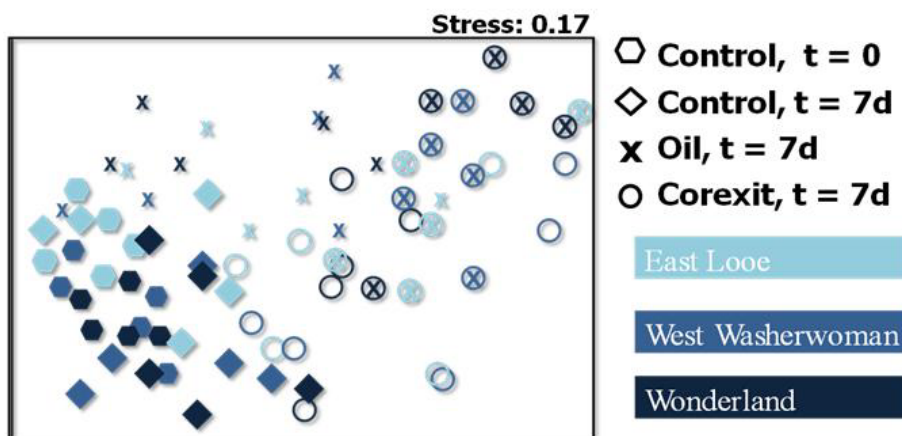
Coral mucus was collected via syringe from the surface of each *M. faveolata* coral fragment in all treatment groups to test bacterial community composition. Length heterogeneity PCR using universal 16S ribosomal DNA primers was performed to identify significant changes in the mucus-associated microbial community structure between treatments. Results indicate that oil and dispersant exposures significantly affect microbial community structure more than reef sampling site (Global R=0.52, p<0.01; Figure 4). Furthermore, dispersant plus oil resulted in greater changes in the microbial community than oil or dispersant alone.

### *Gene Expression Profiling*

#### *-Methods*

For each coral sample (N=360), total RNA was isolated from TRIzol aliquots following the manufacturer's protocol (based on Chomezynski and Sacchi 1987) and purified using lithium chloride (LiCl) precipitation. The concentration and purity of extracted RNA was determined using UV spectrophotometry (NanoDrop<sup>®</sup> 1000, Thermo Fisher Scientific<sup>™</sup>) and microfluidics-based electropherogram (Bioanalyzer 2100, Agilent Technologies). The RNA was then labeled with cyanine-3 (Cy<sup>™</sup>3) fluorescent dye using a LowInput QuickAmp Labeling Kit<sup>®</sup> according to the manufacturer's protocol (Agilent Technologies). Labeled RNA was quantified and dye

incorporation was measured using UV spectrophotometry. Preceding analysis, RNA was stored at  $-80^{\circ}\text{C}$  to prevent degradation.



**Figure 4.** Oil and dispersant exposure significantly altered the mucus microbial community structure after 7 days. Symbols with an “X” surrounded by a circle indicate exposure to both oil and dispersant. In this nonmetric scaling plot, distances between samples are indicative of dissimilarities in community structure (Global  $R=0.52$ ,  $p<0.01$ ).

Labeled RNA samples were hybridized to coral microarrays for 17 hours at  $65^{\circ}\text{C}$  with rotation and then washed according to the manufacturer’s protocol (Agilent Technologies). The arrays were then imaged with a GenePix 4200A<sup>®</sup> laser scanner (Axon Instruments). The laser scanner excites fluorescence, detects the signals and quantifies their intensities on each array. Fluorescent spots on the image correspond to specific genes on the microarray. The image software GenePix Pro (Axon Instruments) was used to measure the fluorescence intensity of each signal. Before analysis, each array was visually inspected to remove artifacts, such as scratches or smears. The software corrects for background intensities by subtracting local background from each spot. The resulting intensities are an average of the foreground pixels (the spot) minus the averaged local background pixels. Each array was visually inspected for hybridization anomalies using the imaging software. This data was discarded before the data was analyzed. After imaging, labeled RNA previously hybridized to the arrays was removed and arrays were reused up to two times (Zhang et al., 2009).

#### *-Statistical analysis*

Resulting data of spot intensities was transformed using log base 2 and loess normalized. The resulting normalized data distribution was assessed using box plots and overlaid density estimate curves. Principle component analysis and correlation scatter plots were run to verify the multivariate structure of the normalized data and associated patterns. Replicate spots for each gene were averaged and a multivariate, repeated analysis of variance was used to quantify significant differences between probes (ANOVA F-test). The model was set up with fixed effects and least squares effects by treatment. The cut-off value for significance was set at  $\log_{10}(p)=2.5$ , which corresponds to a significance value of  $p < 0.003$ . Residual diagnostics showed linearity and normality of the data validating the fit of the model.



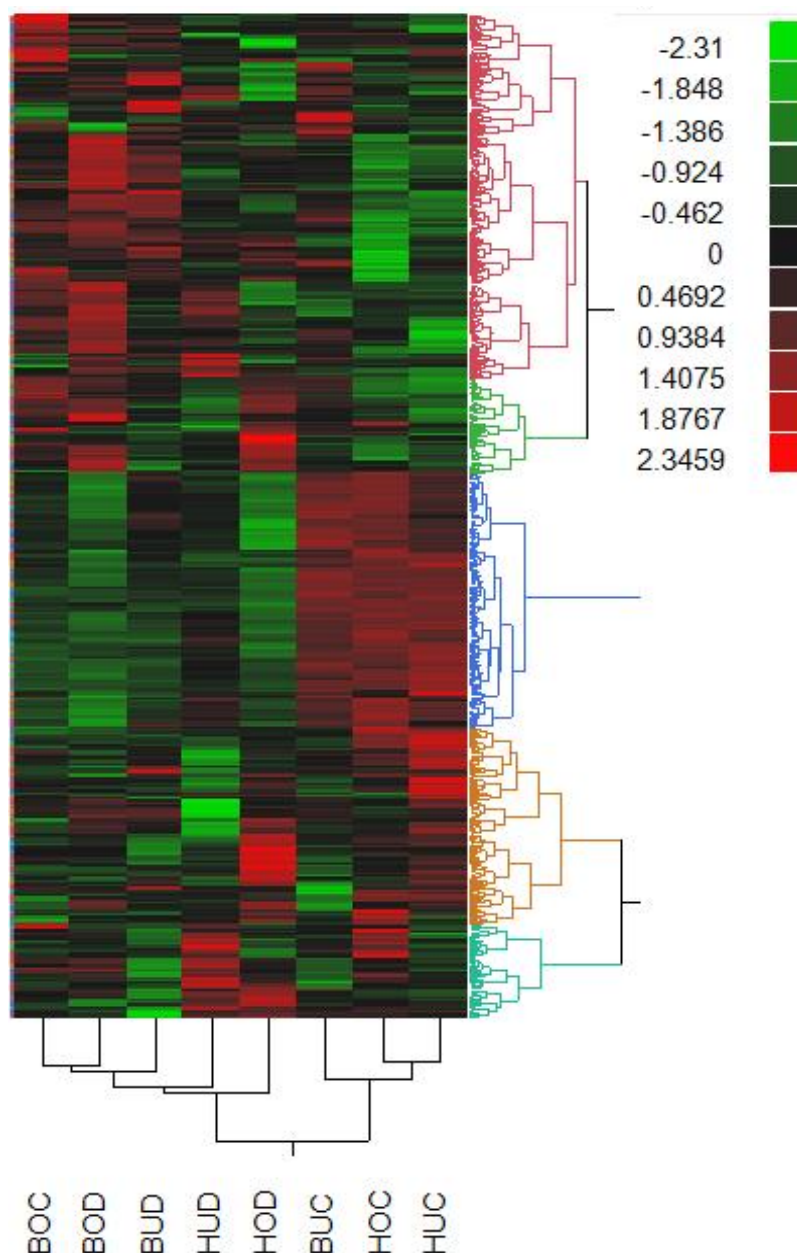
*-Preliminary results*

Because analysis and interpretation of the gene expression results are still ongoing, only the functional responses to oil and dispersant exposed samples are presented here. Samples treated with black band disease are still in progress. Additionally, general patterns of oil and dispersant treatments have been observed but require further analysis to determine specific responses and overall interpretation. Preliminary results indicate interesting responses to oil and dispersant exposures.

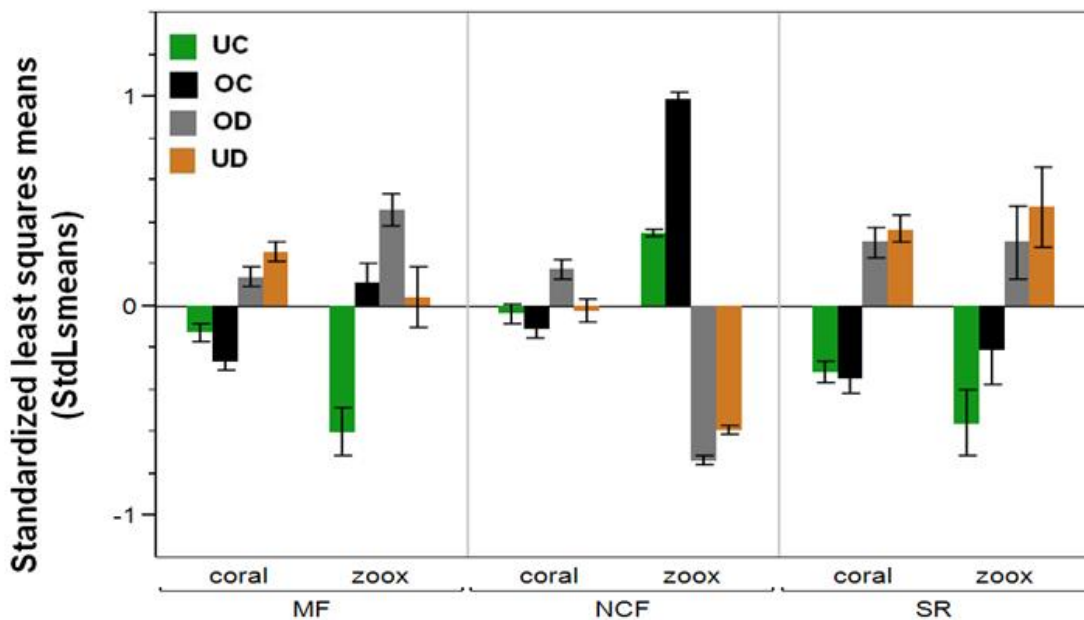
The ANOVA F-test reveals significant differences in expression of 727 of the 4,118 gene probes on the microarray. Hierarchical cluster analysis of these significant genes using geometric spacing was performed between treatments resulting in clusters of genes with similar expression patterns across treatments (y-axis) and clusters of treatments with similar expression patterns of genes (x-axis; Figure 5). Results indicate that the oil only treatment (HOC) clusters with control (HUC) and disease only (BUC) treatments, suggesting similar patterns of gene expression between the three treatment groups. In addition, treatment groups with dispersant added (BOD, BUD, HUD and HOD) cluster together indicating similar gene expression profiles among these samples. These results suggest that the presence of dispersant has a more significant impact on gene expression than oil exposure since the oil only treatment is most similar to the control. Additionally, the presence of oil and/or dispersant appear to impact gene expression of disease treated samples. Samples with black band disease combined with oil and/or dispersant cluster together (BOC, BOD, BUD), while the black band only treatment (BUC) is more similar to the oil only treatment and the control (Figure 5).

Genes were categorized based on primary cellular activity according to published research and the Gene Ontology database (Ashburner et al., 2000). Categories were further grouped based on primary function to provide a general overview of the coral holobiont response. These functional groups included normal cellular function (NCF), multifunctional response (MF) and stress response (SR). NCF genes are involved in such functions as transcription and translation, cellular respiration, metabolism, and signal transduction. MF genes include those with both normal cellular and stress response functions, such as molecular chaperones, heat shock elements and genes involved in the regulation of apoptosis, proteolysis and metal ion regulation. SR genes include those involved in DNA repair, wound healing, oxidative stress, and xenobiotic exposure. The least squares means of significant genes in each category were standardized to the overall mean expression resulting in standardized least squares means (StdLsmean). The StdLsmean data was graphed by organism and functional group to reveal deviations above (elevated expression) and below (decreased expression) the mean (Figure 6). Results indicate that exposure to dispersants has a more significant impact on coral and zooxanthellae genetic responses than oil only. This holds true for the multifunctional genes and the stress responsive genes. However, genes in the normal cellular function category do not deviate greatly from the StdLsmean for the coral. Zooxanthellae NCF genes are significantly decreased below the mean in corals that have been exposed to dispersant and increased in the oil only treatment (Figure 6). This response is likely due to highly significant differential expression of genes involved in cellular respiration by zooxanthellae. Figure 7 shows the variation in expression of two genes involved in cellular respiration, cytochrome oxidase and cytochrome b, in coral and zooxanthellae, between treatments. In coral, these genes are elevated in the dispersant only treatment, while in zooxanthellae expression is decreased when dispersant is present and

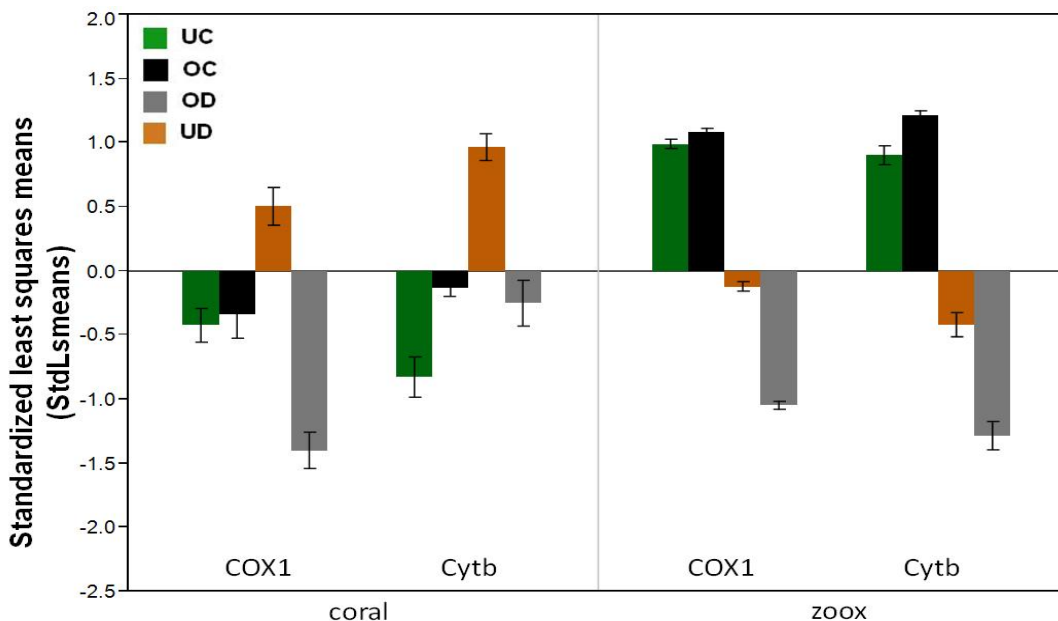
increased in the presence of oil only. Other NCF genes involved in cellular signaling and photosynthesis are also decreased in zooxanthellae when dispersant is present and increased in the oil only treatment (Figure 8). These results suggest suppression of genes involved in normal cellular functions in zooxanthellae exposed to dispersant. Further analysis will identify impacts to molecular pathways and highlight additional changes in gene expression associated with treatment interactions. Results will be published upon completion.



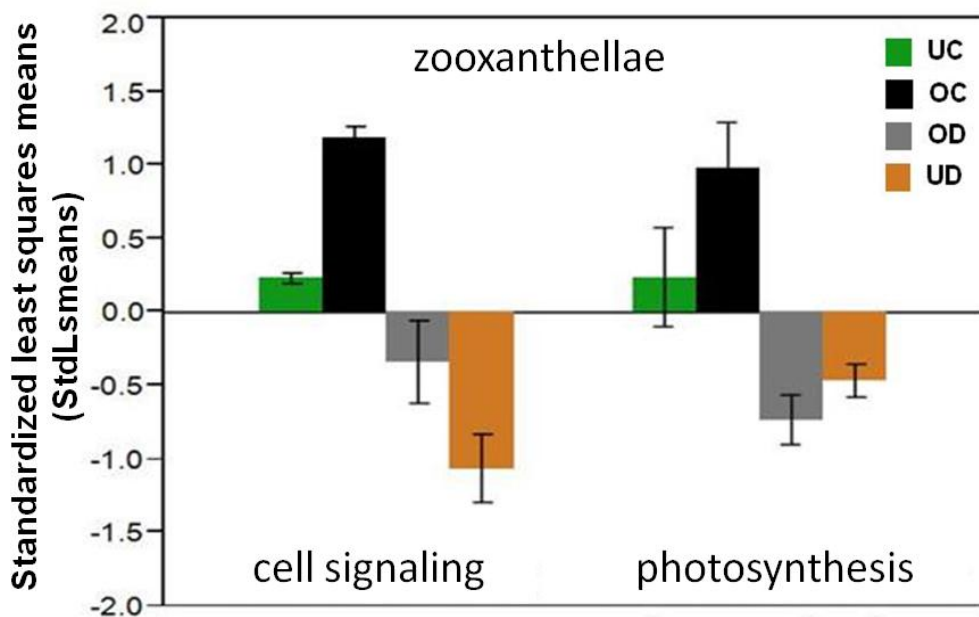
**Figure 5.** Hierarchical clustering of significant genes between treatments. The standardized least squares mean (StdLsmean) for significant genes from the ANOVA was plotted against treatments groups using the Fast Ward method. Treatment clusters reveal distinct groupings of oil, dispersant and black band exposures based on expression profiles. Shades of green to red indicate distance from the StdLsmean (-2.31 green to 2.3459 red). Black indicates no difference from the StdLsmean. Sample names indicate the presence or absence of treatment exposures: B = black band, H = healthy, O = oil, U = no oil, D = dispersant, C = no dispersant.



**Figure 6.** Deviations from the standardized least squares mean (Std Lsmean) of significant genes by ANOVA (F-test) within functional categories (MF = multifunctional, NCF = normal cellular functioning, SR = stress response). Least squares profiles (corresponding to normalized signal intensities) were standardized to mean 0 for all genes. The mean Std Lsmean value of genes within each functional category is graphed by organism (coral or zooxanthellae). Greater deviation away from the Std Lsmean indicates increased significance. Sample names indicate the presence or absence of treatment exposures: O = oil, U = no oil, D = dispersant, C = no dispersant.



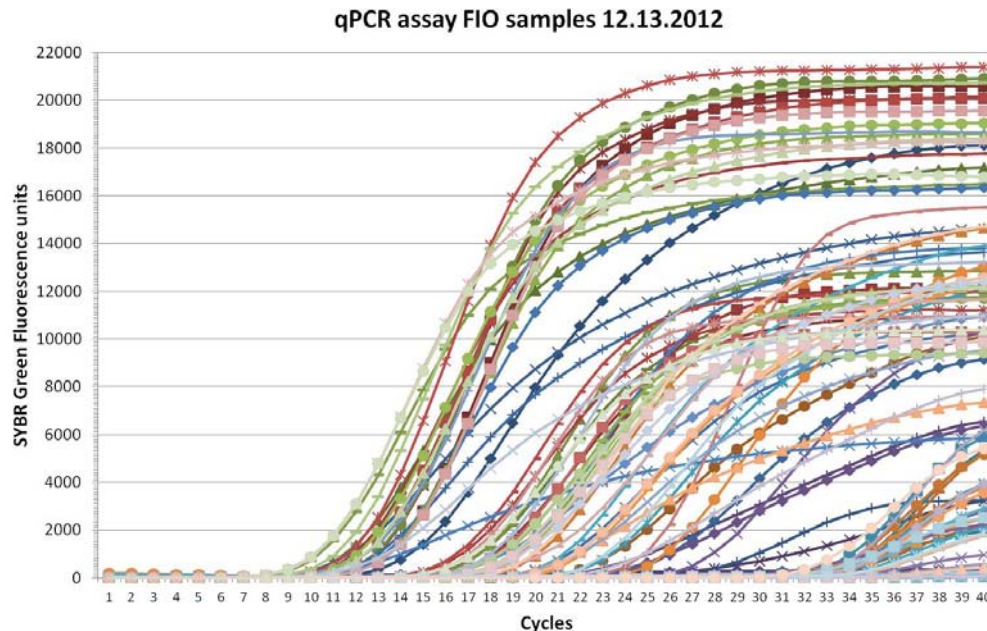
**Figure 7.** Deviations from the standardized least squares mean (Std Lsmean) of cellular respiration genes, cytochrome oxidase (COX1) and cytochrome b (Cytb) by organism (coral or zooxanthellae). Least squares profiles (corresponding to normalized signal intensities) were standardized to mean 0 for all genes. Greater deviation from the Std Lsmean indicates increased significance. Sample names indicate the presence or absence of treatment exposures: O = oil, U = no oil, D = dispersant, C = no dispersant.



**Figure 8.** Deviations from the standardized least squares mean (Std Lsmean) of genes involved in cell signaling and photosynthesis in zooxanthellae. Least squares profiles (corresponding to normalized signal intensities) were standardized to mean 0 for all genes. Greater deviation from the Std Lsmean indicates increased significance. Sample names indicate the presence or absence of treatment exposures: O = oil, U = no oil, D = dispersant, C = no dispersant.

#### *-qPCR validation of microarray data*

Differential expression data obtained from microarray analyses will be validated using real-time, quantitative polymerase chain reaction (qPCR), which isolates and amplifies a sequence of interest and provides information on the amount of the sequence present in the sample. Primers were designed and validated for six gene sequences present on the microarray. These genes include a large ribosomal subunit, cytochrome c oxidase, ribulose-1,5-bisphosphate carboxylase oxygenase, multidrug-resistant protein, and a caspase-like gene. An initial assay was run on a subset of samples to test primer binding and gene amplification. Following the manufacturer's protocol, cDNA was converted from total RNA and amplified for 40 cycles (Express One-step SYBR Green kit, Invitrogen) (Figure 9). Aspects of this assay need troubleshooting and primers will be designed for additional sequences on the coral microarray. A subset of samples from each of the eight treatment groups will be selected for this assay to validate data collected from the microarray. Results are anticipated to be completed by March 2013.



**Figure 9.** Data following 40 cycles of a qPCR assay with 96 reactions. Computer software is used to calculate the mean fold change in gene expression of each sample following amplification of each sequence.

### *Transcriptome Sequencing*

Sequencing the transcriptome (total set of actively expressed genes) identifies novel, previously unknown gene sequences that are differentially expressed during different conditions. This experiment provides a unique opportunity to contribute important genetic information associated with oil, dispersant and BBD exposures of coral to Scleractinian and *Symbiodinium sp.* gene databases. Total RNA from each treatment group was pooled (10 µg) and sent for transcriptome sequencing (RNAseq) using an Illumina HiSeq instrument (BaseClear, The Netherlands). To date, 3 of the 8 samples have been sequenced. Library preparation will be repeated on the other 5 samples in the early part of January 2013 for sequencing. Sequenced samples are in the process of de novo transcriptome assembly and annotation by bioinformaticists at BaseClear. Results are anticipated in March 2013.

### *Coral Genotyping*

Genotyping is on-going and has been outsourced to Dr. Tonya Shearer at Georgia Institute of Technology. Genomic DNA will be isolated using standard phenol-chloroform extraction methods. Polymerase chain reaction (PCR) amplifications will be done for 5 mitochondrial loci using GeneAmpXL PCR kit (Applied Biosystems) under normal PCR conditions. The double-stranded PCR products will be visualized using 1% agarose gel electrophoresis and purified using a Qiagen PCR purification kit, following the manufacturer's protocols. Purified PCR products will be sequenced directly using the ABI BigDye Terminator version 3.0 (Applied Biosystems) and analyzed using an ABI Prism Genetic Analyzer at Florida International University's DNA CORE facility. Sequences will be edited and aligned in SEQUENCHER 4.7 (Gene Codes Corporation 1991-2007). Unique multilocus genotypes will be identified and analyzed for differences in their responses to treatment types.

**Presentations**

- Edge, SE. 2013. Molecular diagnostics of coral exposed to oil and dispersants. Gulf of Mexico Oil Spill & Ecosystem Science Conference, New Orleans, Louisiana, January 21-23, 2013.
- Edge, SE. 2012. Marine Ecological Genomics: Using Molecular Biomarkers for Health Assessment. Research Seminar in Marine Science, Harbor Branch Oceanographic Institute at Florida Atlantic University. Harbor Branch Oceanographic Institute at Florida Atlantic University, *invited presentation*, November 30, 2012.
- Edge, SE, Voss J., Semon K., Ruzicka R., Williams M., Fahsbender E., Cohen L. 2012. Molecular diagnostics of coral exposed to oil and dispersants: a holobiont approach to investigate potential effects on the host corals, symbiotic algae, and mucus associated microbial communities. 12<sup>th</sup> Annual International Coral Reef Symposium, Cairns, Queensland, Australia. July 9-13, 2012.
- Edge, SE. 2012. Oil, Dispersants, and Ecosystem Health. Ocean Science Lecture Series, Harbor Branch Oceanographic Institute at Florida Atlantic University. March 21, 2012.
- Voss J., S. Edge, L. Cohen, E. Fahsbender, M. Williams, N. Harrison. 2012. Oil, Dispersant, and Disease: Impacts and Interactions on Corals in the Florida Keys. 41<sup>st</sup> Benthic Ecology Meeting, Norfolk, VA. Mar 23, 2012.
- Voss J., S. Edge, L. Cohen, E. Fahsbender, M. Williams, N. Harrison. 2011. Oil, Dispersant, and Disease: Impacts and Interactions on Corals in the Florida Keys. Coastal And Estuarine Research Federation Meeting, Daytona Beach, FL. Nov 7, 2011.
- Edge, SE. 2010. Managing stress through transcriptional regulation: analyzing gene expression profiles of corals exposed to different stressors. USGS St. Petersburg Coastal and Marine Science Center and University of South Florida Joint Seminar Series. St. Petersburg, FL. October 29, 2010.
- Edge, SE. 2010. Detecting coral responses to stress using microarray technology. Smithsonian Marine Station Fall Seminar. Fort Pierce, FL. October 22, 2010.
- Edge, SE. 2010. Gene Expression Microarrays: Assessing Impacts from the Gulf of Mexico Oil Spill on Marine Benthic Invertebrates. Florida Atlantic University Fall Colloquium. Fort Pierce, FL. October 1, 2010.

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