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CHARACTERIZATION AND OIL RESPONSE OF THE DEEP SEA CORAL-ASSOCIATED MICROBIOME

A Thesis in

Biology

by

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ABSTRACT

Corals form habitats with high biodiversity, including a diverse microbiome, but we know relatively little about the microbes associated with deep-sea corals. Deep-sea corals are increasingly being exposed to anthropogenic impacts, including oil spills. Although acute effects of oil have been studied in the octocoral *Paramuricea biscaya*, we know little about the sub-lethal effects, or the effects on hexacorals. The microbiome of these corals is unexplored and may play important roles based on studies of shallow-water corals. Bacterial RNA was collected from 4 colonies (2 red and 2 white) of the black coral *Leiopathes glaberrima,* and these colonies were exposed to oil over the course of 3 experiments. Recovered genes were classified by primary function and those included nitrogen metabolism and oxidation/reduction. However, only 1,342 bacterial genes were recovered. None of these genes showed any significant differences in expression levels between either red and white corals or oil and control samples. Bacterial community composition was also measured in *L. glaberrima* collected from 3 sites separated by 375 km in the Gulf of Mexico and compared with 3 other genera of corals: *Sibopathes, Lophelia,* and *Paramuricea. L. glaberrima* oil and control samples were also compared from each of the 3 experiments. None of the 3 oil experiments showed any significant differences between oil and control fragments. Proportions of known oil-degrading genera were also examined for each experiment, but showed no significant differences between oil and control samples in any experiment. *L. glaberrima* harbored a specific microbial consortium that differed from other coral species, water samples, and sediment samples, and also varied within *L. glaberrima* by site. One operational taxonomic unit (OTU) in particular from the genus *Endozoicomonas* was found in every *L. glaberrima* sample. This genus has also been reported as a symbiont in shallow water corals, as well as being found in other marine organisms including sponges. This study lends support to the idea that corals have species-specific assemblages of bacteria that differ from water and sediment reservoirs, and hints that at least a few of them may be important to the coral host.

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Chapter 1. Characterization of the *Leiopathes glaberrima* Microbiome

Abstract: Corals form habitats with high biodiversity, including a diverse microbiome, but we know relatively little about the microbes associated with deep-sea corals. Their microbiomes may play important roles based on studies of shallow-water corals. The black coral, *Leiopathes glaberrima* is one of the most abundant black corals, found throughout the Atlantic, Mediterranean, and Gulf of Mexico. It has a variety of color morphs, the most visually distinct of which are red and white. Bacterial RNA was collected from 4 colonies (2 red and 2 white) of *L. glaberrima.* Recovered genes were classified by primary function and those included nitrogen metabolism and oxidation/reduction. However, only 1,342 bacterial genes were recovered. None of these genes showed any significant differences in expression levels between host color morphs. Bacterial community composition was also measured in *L. glaberrima* collected from three sites separated by 375 km in the Gulf of Mexico and compared with 3 other genera of corals: *Sibopathes*, *Lophelia*, and *Paramuricea*. *L. glaberrima* harbored a specific microbial consortium that differed from other coral species, water samples, and sediment samples, and also varied within *L. glaberrima* by site. One operational taxonomic unit (OTU) in particular from the genus *Endozoicomonas* was found in every *L. glaberrima* sample. This genus has also been reported as a symbiont in shallow water corals, as well as being found in other marine organisms including sponges. This study lends support to the idea that coral microbiomes include species-specific assemblages of bacteria that differ from water and sediment reservoirs, and suggests that at least a few of them may be important to the coral host.

Introduction:

Corals are well-known as foundation species because of the 3-dimensional structures they form. Coral reefs provide spatially complex habitats for many organisms; shallow water reefs support hundreds of thousands of animal and plant species (Reaka-Kudla 1997). In addition to this diversity of multicellular organisms, corals also harbor a diverse microbiome. In shallow-water corals, these microbes include *Symbiodinium* spp*.,* a dinoflagellate endosymbiont that provides up to 95% of its fixed carbon to the coral host – although one recent study suggests 78% is a more accurate number (Tremblay et al. 2012) – more than enough to meet all the coral's energy needs for respiration (Muscatine et al.

1984). Additionally, some coral-associated bacteria fix nitrogen (Lesser et al. 2007, Olson and Lesser 2013), as well as both contributing to and ameliorating coral disease (Roder et al. 2014, Rodriguez-Lanetty et al. 2013). Bacteria produce toxins as a means of limiting resource competition, and many of the putative disease-causing agents are present in healthy corals, although at much lower abundance (Rodriguez-Lanetty et al. 2013). Finally, both bacterial stress response genes and virulence genes are upregulated in response to various stressors (Thurber et al. 2009). Thus, host-prokaryote interactions are likely ecologically and perhaps evolutionarily Important in corals.

The coral microbiome is compartmentalized throughout the host, and these compartments include the mucus layer covering the coral's surface, intracellular and interstitial spaces, the gastrovascular cavity, and the skeleton. According to a recent review by Thompson and colleagues (2014), microbial community composition may differ between compartments, with mechanisms mediated by both the host and the microbial consortia that include contact-mediated signaling and bacteriocin production. For example, in shallow water scleractinians, the mucus layer helps trap *Roseobacter* spp. and *Alteromonas* spp. during spawning, while the interstitial spaces are dominated by *Symbiodinium* spp. and the skeleton harbors endolithic algae, as well as a distinct bacterial population (Thompson et al. 2014).

Deep-water corals lack the photoautotrophic *Symbiodinium* spp. found in many shallower corals, but harbor a bacterial community. This community, which is important in shallow water corals (Lesser et al. 2007, Olson and Lesser 2013, Sweet et al. 2014, Roder et al. 2014, Rodriguez-Lanetty et al. 2013), may also play a role in deep-water corals, and many of the functions seen in shallow-water corals, such as pathogen resistance and stress response, may apply to the deep-water coral microbiome as well. The analysis of deep-water coral microbiota has been for the most part limited to the scleractinian *Lophelia pertusa*, although the microbiomes of a few other species have been analyzed using clone libraries (Kellogg et al. 2009, Neulinger et al. 2008, Gray et al. 2011). *L. pertusa* has a characteristic microbiome that differs from both water and sediment, both in the Gulf of Mexico and Norwegian Sea (Neulinger et al. 2008, Kellogg et al. 2009). The bacterial community of *L. pertusa* in the Gulf is dominated by a combination of Tenericutes and Gammaproteobacteria, with small numbers of Alphaproteobacteria and Bacteroidetes present (Kellogg et al 2009). Norwegian Sea *L. pertusa* have two color morphotypes: white corals are dominated by Alphaproteobacteria, with Actinobacteria accounting for about 19% of the reads. Red corals showed a more even distribution between Alphaproteobacteria,

Verrucomicrobia, Gammaproteobacteria, and other bacteria, including Actinobacteria and Bacteroidetes (Neulinger et al. 2008). Most of the coral microbiome literature, for both deep and shallow corals, focuses on scleractinians, which have a rigid skeleton made of calcium carbonate. Black corals, on the other hand, have a proteinaceous skeleton that appears black, and is more flexible and less brittle. Microbiome analysis of black corals has thus far been limited to a phylum-level analysis of a single unidentified species (Penn et al 2006). Thus, there is a gap in the knowledge pertaining to deep-sea coral microbes.

Leiopathes glaberrima (Hexacorallia, Antipatharia) is a deep-water black coral commonly found on carbonate outcrops throughout the Gulf of Mexico, Mediterranean Sea, and Pacific Ocean from about 200 to 600 meters (Brooke and Schroeder 2007, Ruiz-Ramos et al. 2015). It has a variety of color morphs, the most common and visually distinct of which are red and white, the two color morphotypes targeted in this study. Previous population genetic analyses have found that there are several distinct populations of *L. glaberrima* in the Gulf of Mexico. These populations do not correlate to coral color and correlate imperfectly with site, although there is some population structure by site. Analyses on a smaller spatial scale reveals microstructure within sites by color. Maximum entropy models show that white colonies are more likely to occur at the tops of mounds, with red colonies at slightly lower points around them (Ruiz-Ramos et al. 2015). We hypothesized that the color morphs of *L. glaberrima* would host distinct microbiomes as was found in the deep-water scleractinian *L. pertusa* (Neulinger et al. 2008).

In this paper, we first address the question of whether *L. glaberrima* contains a stable and characteristic microbiome, and compare this with the microbes found in soil and water samples from their habitat and with other deep water species of coral. Next, we ask whether the microbiome population differs across space and/or among color morphotypes. Community composition is measured as the relative abundances of bacterial OTUs in each sample. Finally, we ask how the bacteria are affecting the host, what functions they are performing, and how these functions compare to the shallow-water coral microbe literature, as measured through differential gene expression of bacterial RNA.

Methods:

Sample Collection and DNA Extraction

Red and white *Leiopathes glaberrima* coral colonies were collected from three sites in the Gulf of Mexico (Table 1.1, Fig. 1.1): Viosca Knoll 826 (VK826), Viosca Knoll 906 (VK906) and Green Canyon 140 (GC140). These sites are named for the 3nm x 3nm lease blocks (defined by the US Bureau of Ocean Energy Management) where they occur. All coral collections were made between October 14 and November 4, 2010 aboard the National Oceanic and Atmospheric Administration Ship Ronald H. Brown with the remotely operated vehicle (ROV) Jason II, and were fixed in RNALater either on the seafloor or as soon as the ROV returned to the deck. They were then stored at -20°C for 24 hours before being moved to -80°C and stored until DNA extraction.

Water filters and sediment samples were collected aboard the E/V Nautilus in July 2015 with ROV Hercules at four locations in the Gulf of Mexico: VK906, Atwater Valley 357 (AT357), Green Canyon 234 (GC234), and Mississippi Canyon 751 (MC751). At each site, 330-400 L of water was pumped through a filter with 142 mm diameter and 0.2 µm pore size, using a large volume water transfer pump (McClane Labs, MA USA). Water filters were split into fourths and stored in ethanol at -20°C. Sediment samples were extracted from the top centimeter of larger push cores (7.62-cm diameter, 30.48 cm long), which were collected by the ROV at each sampling site. Push cores were collected near coral sites, in areas of flat sediment where the ROV had enough room to land. Each core was inserted straight down into the sediment and retrieved by the ROV manipulator arm. 0.5 mL fractions were extracted with a syringe and fixed overnight in 1.8% formaldehyde in 1X PBS at 4°C. Samples were then centrifuged and resuspended twice in phosphate buffered saline (PBS), centrifuged again, and stored at -20°C in 1:1 PBS/ethanol. DNA from coral samples, water filters, and soil samples was extracted using the PowerSoil DNA extraction kit (MO BIO, CA USA).

Illumina Library Preparation

In order to survey the microbial community associated with *L. glaberrima,* as well as comparing community composition between sites and hosts, 16S rRNA gene sequencing was performed. The V2 region of the 16S rRNA gene was amplified from each sample using modified 27F (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCMTGGCTCAG3') and 355R (5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCCTCCCGTAGGAGT3') primers (Rodriguez-

Lanetty et al. 2013), containing both the 16S-specific primer and the Illumina universal adapter sequence for use with the Nextera index kit (Illumina, CA USA). This produced a 350-base pair amplicon, which was cloned using *E. coli* and the Topo cloning vector (ThermoFisher, MA USA). After 8 trial Sanger sequences of this amplicon, a BLAST search revealed that the top hit for each sample was from a bacterial 16S gene. The polymerase chain reaction (PCR) mix contained 1X Promega reaction buffer, 0.3 mg/mL BSA, 1.56 mM $MgCl_2$, 250 µM of each dNTP, 0.125 µM of each primer, and 0.156 units of taq DNA polymerase. Reaction conditions were 5 minutes at 95°C, followed by 30 cycles of 95° for 40 seconds, 51° for 2 minutes, and 72° for 1 minute, followed by a final extension of 7 minutes at 72°. After this first PCR, all samples produced a strong band on a 2 percent agarose gel and were subsequently cleaned up with AMPure beads (Beckman Coulter) following the manufacturer's instructions.

A second round of PCR was performed using the Nextera index kit, resulting in completed libraries using dual index sequences. The final 50 µL PCR reaction contained 10 ng of the first-round PCR product, 5 µl each of forward and reverse Nextera barcodes (100 µM stock), 200 µM of each dNTP, 1X Roche Fast Start High Fidelity PCR buffer (which contains $MgCl_2$), and .05 units of Roche DNA polymerase. PCR conditions were 95°C for 3 minutes, followed by 8-10 cycles of 95° for 30 seconds, 55° for 30 seconds, and 72° for 30 seconds. After the cycling, there was a final extension of 5 minutes at 72° and a hold at 4°. Following this step, AMPure beads were used again to clean up the samples. Library concentration was determined using the Qubit dsDNA BR assay (ThermoFisher, MA USA). An equimolar pool of all samples was constructed and the pool concentration was confirmed using qPCR (Kapa Biosystems, MA USA). This pool was sequenced on the Illumina MiSeq with 250x250 or 300x300 paired-end sequencing, using 21-24 samples per run. On average, we recovered 566,851 reads per sample.

RNA Extraction

In order to expand the information gained through the analysis of 16S phylotypes to include bacterial genes expressed, RNA was extracted and analyzed from 4 *L. glaberrima* colonies. In 2012, 2 red and 2 white *L. glaberrima* colonies were collected on the R/V Falkor, using the ROV Global Explorer, from VK826. Colonies were kept in artificial seawater at 6°C for approximately 36 hours, preserved in RNALater, chilled at -20°C for 24 hours and then stored at -80°C. RNA was extracted using the method described in Polato et al. (2011), using Qiagen buffers RW1, RDD, and RPE (Qiagen, CA USA) and the Trizol reagent (Applied Biosystems, CA USA). After RNA extraction and DNAse treatment, RNA was

processed using the MicrobEnrich (ThermoFisher, MA USA) and RiboZero (Epicentre, WI USA) kits, following the manufacturers' instructions. The MicrobEnrich kit is essentially a reverse-poly-A selection that eliminates eukaryotic sequences with poly-A tails, and the RiboZero kit filters out ribosomal RNA (rRNA) sequences. Sequences were checked on an Agilent 2100 bioanalyzer (Agilent, CA USA) and sequenced on the HiSeq 2000 (Illumina, CA USA) platform.

Washing Experiment

In order to assess the relative abundance and importance of the loosely associated bacteria on the coral surface, an experiment was performed with 3 *L. glaberrima* colonies comparing washed and unwashed treatments. Three red *L. glaberrima* colonies from VK826 were kept alive in an aquarium at Pennsylvania State University for 15 months before the experiment. Corals were held at 4-6°C in artificial seawater (Instant ocean, 35ppm) and fed with marine snow (Two Little Fishies, FL USA). Twice a day, approximately 50mL marine snow was added, and the pumps circulating water in the aquarium were disabled for 4 hours to allow for filter feeding. These colonies were subsampled and one fragment was used directly for DNA extraction, while the other was washed in 70% ethanol followed by distilled water and then extracted using the PowerSoil kit (MO BIO, CA USA), following manufacturer's instructions.

Bioinformatics and Data Analysis

Raw DNA reads were trimmed, assembled, collapsed, and quality-controlled using Mothur (Schloss et al. 2009). Reads between 50 and 400 base pairs with no ambiguous sequences and no homopolymers longer than 8 base pairs were passed to SortMeRNA using default settings (Kopylova et al 2012) and those that did not align to 16S bacteria or archaea sequences in the SILVA database (Quast et al. 2013) were excluded from downstream analysis. Remaining reads were then classified using a modified version of the SILVA-NGS pipeline (Quast et al. 2013), run locally and using in-house python scripts to process data between steps. Reads were clustered using cdhit-est running in “accurate” mode and using a cutoff of 98% similarity (Li et al. 2006, Fu et al. 2012). Each cluster was assigned a genus using blastn against the SILVA NR99 database, release 123. Data were then analyzed using the non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) functions in the R package “vegan” (Oksanen et al. 2015). More specifically, each NMDS plot was constructed using the metaMDS function, with a wisconsin double standardization of a fourth-root transformed community by taxon

matrix, where the taxa were either genera or phyla. ANOSIM was performed on the bray-curtis dissimilarity matrix of the wisconsin-standardized fourth-root transformed matrix. ANOSIM is similar to ANOVA, but it operates on a dissimilarity matrix. The R statistic produced by this method is computed by comparing differences in community composition within and between user-defined groups. This statistic is then checked for statistical significance by randomly permuting the groups, creating a null model for comparison. A similarity percentage analysis, or "simper," was performed on the same fourth-root transformed matrix that was used to generate the ANOSIM and NMDS data. Simper outputs a table of genera that contribute most to between-group dissimilarity, calculated using a bray-curtis dissimilarity matrix. "Core" genera were assigned using CORBATA (Li et al. 2013).

In order to look for potential bacterial symbionts of *L. glaberrima,* each fasta file from the first round of cdhit was tagged by library and recombined into a new fasta file which was processed in a second cdhit run using the same settings. This generated a new list of OTUs where each sequence was a previous reference sequence, with its library ID attached as part of the sequence ID.

RNA data were analyzed using the modified pipeline described in Leimena et al. (2013). Reads were collapsed, clipped, trimmed, and quality-filtered using the fastx toolkit. They were then compared to SILVA and Rfam rRNA databases using SortMeRNA to remove rRNA sequences (Kopylova et al. 2012, as described in Leimena et al. 2013). Remaining reads were assembled de novo using Trinity (Haas et al. 2013), and compared to the NCBI nr database using BLASTX, running both locally and on the Amazon cloud. After mapping and annotation using BLAST2Go, as well as a brief analysis of gene ontology, bacterial reads were analyzed using the R package "DESeq" (Anders and Huber 2012). Genes with less than 10 sequences across all libraries were excluded from the analysis.

Species Comparisons

In order to compare between *L. glaberrima* and other coral taxa, corals from the genera *Paramuricea, Lophelia,* and *Sibopathes* were collected throughout the Gulf of Mexico (Table 1.2). The same quality control steps were used up through the SortMeRNA step. The Ribosomal Database Project (RDP) classifier was re-trained against the November 2015 database and used to classify each quality-controlled library with a confidence cutoff of 0.5 (Wang et al. 2007). The output files from RDP were then analyzed using NMDS plots and ANOSIM in R.

Results:

Phylum- and Genus-Level Comparisons

DNA analysis yielded a total of 19,272,925 raw reads for the 34 libraries included in this study, over the course of 4 separate Miseq runs. Detailed per-sample statistics are shown in Table 1.3. Phylum-level community composition of each library is shown for field-collected coral samples (Fig. 1.4), aquarium samples (Fig. 1.5), and water and sediment samples (Fig. 1.6). In general, the most abundant phyla were Proteobacteria, *Chloroplast*, Firmicutes, Actinobacteria, and Bacteroidetes.

Contrary to expectation, red and white corals showed no difference in bacterial community composition, and their polygons on the NMDS plot show significant overlap (Fig. 1.2). Likewise, using two-tailed t-tests, no significant difference in the Shannon diversity ($p=.731$, $t=2.262$) nor in the Chao1 richness ($p=.999$, $t=2.262$) was observed between red and white coral bacterial communities.

Shannon and Chao1 values were also compared between sites using two-tailed t-tests, but no significant differences were found between VK906 and GC140 (Shannon $p=0.191$, $t=3.182$; Chao1 $p=0.226$, $t=3.182$), VK826 and VK906 (Shannon $p=0.933$, $t=3.182$; Chao1 $p=0.166$, $t=3.182$), or VK826 and GC140 (Shannon $p=0.147$, $t=2.365$; Chao1 $p=0.993$, $t=2.365$). There was, however, a significant difference between the sites VK906 and GC140 in the relative abundances of bacterial genera (ANOSIM $p=0.043$, $R=0.329$). Simper (Table 1.4) indicated that the genera contributing most to this difference were *Propionibacterium* and *Halomonas,* with *Chloroplast* also appearing in the top 10*. Afipia* and *Anoxybacillus* also had fairly high ratios of contribution to standard deviation, and the higher this ratio the more reliable the data, which is important given that the method confounds within- and between-group variation (Warton et al. 2012). There was also a significant difference in genera between VK826 and GC140 (ANOSIM $p=0.041$, $R=0.177$), and an NMDS plot of these corals grouped by site is shown in Figure 1.3. Simper showed the genera *Pseudoalteromonas* and *Tumebacillus* as the largest contributors to between-group variance, and *Moraxella* had the highest ratio of contribution to standard deviation (Table 1.4). The contributions themselves were fairly low in every comparison between sites within *L. glaberrima,* however, with no value above .01. The site difference can also be seen in the core genera (see below) and Shannon diversity values (Table 1.3). GC140 has a larger collection of core genera than either of the Viosca Knoll sites and higher average Shannon diversity values, although not significantly so (mean=2.815 for VK sites, 3.684 for GC140). Throughout the paper, *Chloroplast* was included as both a

genus and a phylum (although technically it falls under Cyanobacteria in the SILVA database), because it represented such a large proportion of some libraries that it would have been too much of a simplification of the dataset to include it under any other group, or to omit it entirely.

Phylum-level community composition of each library is shown for field-collected coral samples (Fig. 1.4), aquarium samples (Fig. 1.5), and water and sediment samples (Fig. 1.6). In general, the most abundant phyla were Proteobacteria, *Chloroplast*, Firmicutes, Actinobacteria, and Bacteroidetes.

"Core" genera were calculated using CORBATA. This software produces a graph of ubiquity versus abundance for each genus in the sample set, where for each genus, abundance is the proportion of an individual library's reads that map to that genus, and ubiquity is the proportion of libraries containing that abundance. Under default settings, genera that are present at a level of at least 1% in 80% of the libraries are reported as the core (Table 1.3). This included bacteria most closely matching *Endozoicomonas* spp. and *Chloroplast* for the coral samples collected from the field. When separated by site, all corals from VK906 showed the same two core genera, whereas corals from GC140 had a more diverse core that included the genera *Streptococcus, Propionibacterium, Staphylococcus, Bacillus, Corynebacterium*, and an uncultured genus in the family Corynebacteriaceae. VK826, although not significantly different from VK906 (ANOSIM p=0.404), had only *Chloroplast* as its core, although the genus *Endozoicomonas* was just below the cutoff (present in 6/8 libraries at 1% abundance; 7 required for 80% ubiquity). There was no significant difference in community composition between washed and unwashed samples (ANOSIM p=0.7), and washed and unwashed samples were therefore combined in the CORBATA analysis, which yielded only *Chloroplast* and *Propionibacterium* spp. as core genera.

OTU Analysis

The first round of cdhit analysis yielded between 642 and 7,709 OTUs per sample for corals collected in the field, 644-1,483 OTUs per sample for aquarium-kept corals, and 4,982-13,438 for water and sediment samples (Table 1.3). In the second round, there were 87,373 clusters, which were screened for any OTUs appearing in every library. Exactly one such OTU was found, and its reference sequence matched an uncultured bacterium of the genus *Endozoicomonas* with 96.55% identity and an e-value of 5×10^{-163}. In order to get an accurate count of sequences from each library belonging to this OTU, its reference sequence was tagged as the "seed" sequence and added individually to each library.

Then clustering was re-run on each library and every sequence from the cluster with the seed sequence (which was the reference sequence for that OTU in every case) was counted as a part of the OTU. Adding in the seed sequence did not interfere with or bias the clustering; every library contained at least one other sequence that was 100% identical to the seed. Abundance in each library was recorded and is presented in Table 1.5, along with the proportion of *Endozoicomonas* spp. in each library represented by this one OTU. As with any high-throughput sequencing dataset, these relative proportions are subject to some amount of PCR bias; however, this is partially offset in two ways. First, the proportions being measured here are all from the same OTU, so all of the sequences are at least 98% identical to a reference sequence, which should at least decrease the variance of the bias associated with these sequences. Second, RDP has a built-in correction for 16S copy number that takes into account the average number of copies for each genus, along with standard deviation for this value. Sample sizes were 20 for field-collected *L. glaberrima,* 9 for *Paramuricea* spp. (6 *P. biscaya* and 3 *P. B3*), and 3 for each of the washed, unwashed, *Lophelia pertusa*, and *Sibopathes* spp. groups.

Comparing between coral samples and water/sediment samples, the proportions of quality-controlled *Endozoicomonas* spp. reads represented by the OTU were significantly higher in the field-collected coral samples than the water and sediment (one-tailed z-test, $p<0.0001$, $z=4.72$; mean 6.83% vs. 0.18%). This proportion did not differ significantly between field-collected corals and unwashed tank corals (two-tailed z-test, $p=0.484$, $z=0.700$, means 6.83% for field-collected corals, 15.56% for tank corals), nor between washed and unwashed tank corals (two-tailed z-test, $p=0.300$, $z=1.04$, means 2.58% for washed corals, 15.56% for unwashed).

The *L. glaberrima*-associated *Endozoicmonas* OTU was also compared to *Endozoicomonas* spp. found in other coral genera. The *L. glaberrima*-associated *Endozoicmonas* OTU was higher in *L. glaberrima* than in any of the other coral species: *L. glaberrima* had significantly more of the OTU than *Paramuricea* spp. (one-tailed z-test, $p<0.0001$, $z=3.972$, means 6.83% vs. 1.14%), *L. pertusa* (one-tailed z-test, $p=0.001$, $z=3.235$, mean of *L. pertusa* = 1.50%), or *Sibopathes* spp. (one-tailed z-test, $p<0.0001$, $z=4.576$, mean of *S.* spp. = 0.03%). The two *Paramuricea* species, *P. biscaya* and *P. B3* were not significantly different (two-tailed z-test, $p=0.240$, $z= 1.176$). *L. glaberrima* had significantly more of the OTU than *P.* spp. (one-tailed z-test, $p<0.0001$, $z=3.972$, means 6.83% vs. 1.14%), *L. pertusa* (one-tailed z-test, $p=0.001$, $z=3.235$, mean of *L. pertusa* = 1.50%), or *Sibopathes* spp. (one-tailed z-test, $p<0.0001$, $z=4.576$, mean of *S.* spp. = 0.03%). A one-tailed test was used only in cases where there was a prior

expectation for a difference between means. The mean was expected to be lower in water and sediment and higher in *L. glaberrima* since this OTU is the best candidate for a symbiont of *L. glaberrima.*

Coral Species Comparisons

At VK826, *L. glaberrima* and *S.* spp. microbiomes differed significantly in community composition (ANOSIM p=0.036, R=-.352), and at VK906 *L. glaberrima* microbiome differed from that of *L. pertusa* (ANOSIM p=0.029, R=0.667). Simper results showed that the genera *Anaplasma* and *Endozoicomonas* were more abundant in *L. glaberrima,* and *Mycoplasma* and *Candidatus Pelagibacter* were more abundant in *L. pertusa* (Table 1.4)*. Spirochaeta* spp. also contributed to this difference between species, and were undetected in *L. glaberrima.* Phylum spectra are shown for *Paramuricea* spp. in figure 1.7, and for *L. pertusa* and *Sibopathes* spp. in figure 1.8. Within *Paramuricea* spp.*,* 6 of the 9 colonies were *P. biscaya* from two sites, Mississippi Canyon 036 (MC36) and DeSoto Canyon 673 (DC673)*,* and the other 3 were *Paramuricea type B3* from AT357. The microbiomes associated with *P. biscaya* did not significantly differ between sites (ANOSIM p=0.333, R=0.107), so all *P. biscaya* were pooled for comparison to *P.* type *B3.* The bacterial communities associated with these two species were significantly different (ANOSIM p=0.048, R=0.556). Simper showed that the highest contributors to this difference included the closely related genera *Endozoicomonas* and *Kistimonas* (Table 1.4).

A hierarchical clustering was performed using the "pvclust" R package with 10,000 bootstrap replicates on a fourth root-transformed matrix of all coral hosts, water, and sediment. All libraries for this step were classified using RDP for consistency. The clustering is shown in Figure 1.9. The four water samples formed a single significant cluster, and the four sediment samples formed another cluster that was significant except for VK906. The overall cluster of water and sediment samples was also significant. All but two *L. glaberrima* samples grouped into one of two significant clusters. *P. biscaya* also formed a significant cluster, with the exception of one colony, which formed a significant cluster with all of the *S.* spp. and *L. pertusa*. The three *P. B3* samples formed a significant cluster (AU=93), distinct from the other *P.* spp. samples.

Gene Expression

After assembling with Trinity, there were a total of 261,296 genes, of which 79,732 (30.51%) had at least one BLAST hit to a known gene and 1,342 (0.5%) had a top BLAST hit of bacteria with e-value less

than 10^{-6}. Of these, 736 (0.3%) had at least 10 sequences across the 4 libraries and were analyzed for differential expression. The most total BLAST hits, as well as the most bacterial blast hits, were recovered during the second BLAST search, which was a BLASTX search against the Uniprot database. Out of a total 216,541 genes searched, this step yielded 30,728 genes with a top hit of eukaryotes (14.19%), 859 genes from bacteria (0.40%), 51 from archaea (0.02%), and 43 from viruses (0.02%). Within the bacterial genes, the top 5 phyla (Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes, and Firmicutes) matched the top 5 phyla found in the 16S tag sequences (and the top 3 in order) given that the Chloroplast sequences fall under Cyanobacteria in the SILVA taxonomy. While most bacterial genes could not be annotated, BLAST2GO's automated mapping and annotation pipeline revealed some broad community functions. The most abundant class of enzyme present in the bacterial genes was oxidoreductase, present in 11 sequences. At gene ontology level 4, the most highly expressed genes controlled cellular nitrogen metabolism, followed closely by macromolecule and organonitrogen compound metabolic processes.

736 (0.3%) bacterial genes had at least 10 hits across the 4 libraries and were analyzed for differential expression. Differential expression analysis using DESeq showed no significant differences between red- and white-coral-associated bacteria, even without correcting for the artificially inflated variance caused by the presence of housekeeping genes. In fact, no corrected p-value was below .611 for the white-red comparison. The colonies also clustered haphazardly in the dendrogram and not by color as expected, and the clusters were not significant (Fig. 1.10).

Discussion:

In this first foray into the microbial associates of anthipatharian corals, we show that *Leiopathes glaberrima* contains a microbiome that differs from the microbes found in the water and sediment from their deep sea habitat. This includes some stable and characteristic members, with one OTU in particular found in every sample. The microbiome differed somewhat across space but, unexpectedly, not among color morphotypes. Metabolic activities of the microbiome include participating in nitrogen metabolism and oxidative processes. The 16S amplicon sequences of *L. glaberrima*-associated microbes show similarity to those of shallow water corals at the phylum level, as well as some similarities at the genus level. For example, *Endozoicomonas* spp. were consistently recovered from *L. glaberrima* samples,

including those that were washed to remove loosely associated microbes. Similarly, Bayer et al. found 16s sequences matching *Endozoicomonas* spp. at high abundance in wide-ranging corals including *Stylophora pistillata,* a scleractinian from the Red Sea, and *Eunicella cavolini*, a Mediterranean gorgonian (Bayer et al. 2013a, 2013b).

Phylum-level community composition in *L. glaberrima* differs subjectively from that of shallower corals, which generally contain higher proportions of Cyanobacteria and Proteobacteria, particularly Gammaproteobacteria, and fewer Actinobacteria and Firmicutes (Fig. 1.4, Rodriguez-Lanetty et al. 2013, Bayer et al. 2013a,b). Gammaproteobacteria, and Proteobacteria in general, from shallow-water corals and *L. glaberrima* consist mostly of the genus *Endozoicomonas,* which is discussed in detail below. Gammaproteobacteria also contain *Vibrio* spp*.,* which have been associated with coral disease, as have Cyanobacteria and Firmicutes (Roder et al. 2014, Sweet et al. 2014, Frias-Lopez et al. 2003, Dinsdale et al. 2008). Cyanobacteria can also contribute to nitrogen fixation in shallow-water corals (Lesser et al. 2007), and nitrogen fixation may not occur at the deeper sites where *L. glaberrima* is found, which could explain the relatively lower proportions of the phylum in these corals. Actinobacteria were also found at low abundance in association with *Paragorgia* spp*.,* a gorgonian octocoral (Grey et al. 2011), and with *Lophelia pertusa* (Neulinger et al. 2008). They were undetectable in some libraries and therefore probably not obligate symbionts for the coral, but they are commonly found in association with corals (Grey et al. 2011).

As a whole, the bacterial communities varied less than expected between red and white colonies, and possibly relatedly, harbored high levels of diversity, with Shannon diversity averaging 3.16 for field-collected corals. In comparison, the shallow-water scleractinian *Porites asteroides,* which is considered to have a diverse microbiome, had no colonies with a Shannon diversity higher than 2 (Rodriguez-Lanetty et al. 2013). White and red colonies were not significantly different based on ANOSIM analysis of 16S community composition, differences in diversity indices, or gene expression levels. In site comparisons of *L. glaberrima*-associated community composition, however, GC140 was significantly different from both VK906 and VK826. The two Viosca Knoll sites, which are only 37km apart, were not significantly different from one another. Our inability to detect differences between these two sites may in part be due to the high level of variability among samples at VK826, which is clearly visible in the NMDS plot (Fig. 1.3). One potential cause of both of the high richness estimates and lack of differences between samples is the quality control of the raw sequences; Bokulich and coworkers

(2013) discovered that too lenient of a quality control threshold can artificially increase diversity indices. However, sequencing technology has improved since then, and the quality control was already quite stringent; one library had more than 3/4 of its sequences cut, and on average only 44.83% of sequences were kept, so it is unlikely that quality control was the cause. As a further test, the two libraries with the highest Chao1 richness estimates (those over 100,000 species, Table 1.3) were removed. Excluding these libraries did not change any results. Two-tailed t-tests of red versus white corals still lacked significance for either Shannon diversity (p=.741) or chao1 richness (p=.908), as did every site comparison.

Clustering provided support for the hypothesis that each coral hosts a species-specific consortium of bacteria. All of the corals clustered separately from water and sediment samples, indicating that corals host bacterial communities that are distinct from those free-living in the environment. Some caution must be ascribed to the interpretation of these results since most of the water and sediment samples were taken from sites distinct from our coral sampling sites. However, water, sediment, *L. glaberrima,* and *L. pertusa* were all examined from VK906, and both of those corals clustered with every congeneric coral before either water or sediment from the same site. Further, *P. B3* from AT357 formed a significant cluster that was separate from water and sediment samples from this site. Overall, these results cannot confirm the species-specific assemblages in any of the coral species due to the low number of samples and site as a confounding variable, but they lend support to the idea that each coral is harboring a bacterial community that differs from other corals and from its environment.

Simper analysis somewhat strengthened the argument that coral species harbor conserved assemblages of bacteria. Comparisons within the same species yielded consistently lower contribution values than comparisons between different species within a site (Table 1.4). *Endozoicomonas spp.* contributed to differences between *L. glaberrima* and *L. pertusa* and was higher in *L. glaberrima.* *Endozoicomas* was the only genus containing an OTU present in all *L. glaberrima* samples, and this OTU was also significantly more abundant in *L. glaberrima* (this study). Also of interest in this comparison is the genus *Mycoplasma,* which was absent in *L. glaberrima* and accounted for the vast majority of Tenericutes seen in the *L. pertusa* barplots (Fig. 1.8). The genera *Afipia* and *Bradyrhizobia* are closely related Alphaproteobacteria. These two genera also contributed to site variation in *L. glaberrima,* and were both more abundant at VK906, where the comparison between *L. glaberrima* and *L. pertusa* took place, so it is internally consistent that they appear at higher abundance in *L. glaberrima* here.

Bradyrhizobium is a nitrogen fixer associated with plant nodules, and could be a contaminant, although there is precedent for both Alphaproteobacteria and nitrogen fixation in shallow-water corals (Kellogg et al. 2009, Lesser et al. 2007).

Bacterial genera identified by simper in comparisons between *L. glaberrima* and both *L. pertusa* and *Sibopathes spp.* could be characteristic of the *L. glaberrima* microbiome if they are more abundant in *L. glaberrima* in both cases. The only genus that met this criterion was *Anaplasma,* which is in the order Rickettsiales*. Anaplasma* is obligately intracellular (Dumler et al. 2001), and many of the Rickettsiales are parasitic, so it could be a parasite in these corals. Alternatively, although it is not found ubiquitously in *L. glaberrima,* which would be required to be an obligate mutualist for the coral host, it could be a mutualistic symbiont whose function is either not required or easily replaced by another group of bacteria.

In the comparison between *P. biscaya* and *P. B3*, the contribution values were similar to the comparisons between *L. glaberrima* and other species. The top contributor to the differences between these species was the genus *Kistimonas.* This genus is closely related to the genus *Endozoicomonas,* which was also present, and these were the only two genera more abundant in *P. B3.* Further discussions of *Endozoicomonas spp.* as symbionts follow below, and it is possible that there is an undiscovered species forming a symbiosis with *L. glaberrima,* and a different species forming a symbiosis with *P. B3.* As noted in the results, the OTU found in every *L. glaberrima* sample was significantly more abundant in *L. glaberrima* than in *P. B3.*

An unexpected finding in these samples from the deep sea is the large number of "*Chloroplast"* sequences recovered. This group represented the second-most abundant OTU in the second round of cd-hit analysis, and matches most closely to the candidate division CAB-I. This is classified under Cyanobacteria in SILVA, but NCBI classifies it outside of the phylum, as a candidate division directly under Bacteria. The most abundant OTU from the two coral libraries showing the highest proportions of the "Chloroplast" group matched each other with 99% identity and there was only a single mismatch over a 331 base-pair overlap. The most abundant OTU from one tank coral also matched this reference sequence with 99% identity, but with a gap and a mismatch. This OTU, and group as a whole, is unlikely to be the result of contamination – "Chloroplast" sequences were present at over 90% in a library with over 400,000 quality-controlled reads, in addition to appearing in multiple other libraries at greater than

25% abundance. Furthermore, the sequencing facility had never used any of the barcodes used in this study for any plant material, no plant material had been run recently, and the cycle count was low. In the first PCR reaction where cycle counts were higher, negative controls were run with each library and no bands were ever detected even faintly in these lanes.

This "*Chloroplast*" group could represent a novel symbiont closely associated with *L. glaberrima.* A symbiont of these deep-sea corals would probably not be photosynthetic, although the majority of Cyanobacteria are. This trait could have been secondarily lost, or these symbionts could be closely related to ancestors of modern Cyanobacteria which had not yet associated with photosynthetic endosymbionts. Alternatively, it could represent a surface-derived food source of *L. glaberrima*. This would most likely be dead plant material by the time it got to this environment, but DNA from dead organisms could have still been sequenced as long as it was not degraded in the water column. [This is worth noting in a broader sense as well – especially for rare organisms in the NMDS and ANOSIM analyses, the presence of a DNA sequence in the results does not indicate that the organism it came from was ever alive while in contact with *L. glaberrima*]. Corals kept in tanks were fed a diet of marine snow, similar to what they might ingest in the wild, and "*Chloroplast*"-like sequences were recovered from these as well. A handful of chlorophyll-related sequences were recovered from the analysis of RNA from the coral samples (one annotated with "chlorophyll biosynthetic process" and 3 protochlorophyllide reductase genes). However, this analysis was not limited to bacterial RNA, and these genes did not appear in the bacterial transcripts, nor did they contain taxonomic annotations. Further, "*Chloroplast"* sequences were rare in the water and sediment samples, representing only 0.73% of the sample from VK906, which was derived from over 300L of water pumped through a filter, and 1.34% of the sediment sample. An ingested food source would be expected at high relative abundance at least in the filtered water samples if not in the sediment.

Washing had very little effect on the observed community composition of the corals. This indicates that the bacterial community is associated with coral tissue, rather than found only in mucus.

A single OTU was shared between all libraries, and it matched *Endozoicomonas* spp. with an e-value of 5×10^{-163}. *Endozoicomonas* spp. are found throughout the world's oceans, and in symbiotic associations with wide-ranging hosts including mussels, sponges, tubeworms, and shallow-water corals in the genera *Montipora* and *Porites*, among others (Rodriguez-Lanetty et al. 2013, Neave et al. 2014,

Forget and Juniper 2013). The *Endozoicomonas* sp. symbiont of at least one shallow-water coral was found in endothermal tissue, in close proximity to the coral's *Symbiodinium* spp., and further analysis at the species level revealed that *Endozoicomonas* spp. from closely related corals clustered together before other nearby *Endozoicomonas* spp., even over large geographic distances, suggesting coevolution between the genus and its coral hosts (Bayer et al. 2013a). Despite often being characterized as a symbiont of corals, this genus was not recovered from coral larvae (Rodriguez-Lanetty et al. 2013). This would imply horizontal transmission, and its prevalence in the water column and sediment supports this possibility, although Rodriguez-Lanetty and colleagues discount it as unlikely, and attribute its absence in coral larvae libraries to insufficient sequencing depth (Rodriguez-Lanetty et al. 2013). Alternatively, it could be only transiently or incidentally associated with *L. glaberrima* through its presence in the surrounding sediments and benthic water. However, *Endozoicomonas* spp. were present at significantly higher levels in *L. glaberrima* than in water and sediment. It was also the only genus (other than "*Chloroplast*") that was considered "core" in all *L. glaberrima* samples, represented mostly by a single OTU that pooled across sites more than 350 kilometers apart. No genera were considered "core" in the water and sediment samples. It is certainly plausible that this particular OTU could represent a symbiont of *L. glaberrima*, especially given its persistence across bacterial populations shown through ANOSIM to be significantly different.

Gene expression data yielded far fewer bacterial genes than expected; in fact, there were more bacterial OTUs per library than total genes recovered across all libraries. Most genes recovered were likely coral genes; of the 30,728 eukaryotic genes recovered from Uniprot, Opisthokonta, which contains the corals, accounted for 30,060 genes (97.83%). These genes matched a wide variety of eukaryotes, but this most likely represents bias in the database, since many common model organisms were abundant, including *Strongylocentrotus purpuratus* (sea urchin – 1459 sequences), *Xenopus* (frog – 343 sequences), *Mus musculus* (mouse – 740 sequences), and *Danio rerio* (zebrafish – 312 sequences), in addition to 762 human gene sequences.

There are two main reasons for the low yield of bacterial genes. First, the bacterial RNA was likely overwhelmed by coral RNA. During the 16S data collection, amplifying bacterial DNA was difficult and often required more than 28 PCR cycles, and even when PCR was successful, initial sequencing revealed that nearly all of the amplified DNA was from coral mitochondria. This suggests that either there were very few bacteria, or something in the coral was inhibiting the PCR reaction. Even if

inhibitors were present however, the relatively higher signal of mitochondria compared to bacteria suggested that few bacteria were present. Thus it is reasonable to expect that the signal of bacterial transcription could have been similarly quenched by the large amount of coral RNA, even when selecting for bacterial RNA. Second, the MicrobEnrich kit used to enrich for prokaryotic RNA had a quality check that relied on the difference in size between eukaryotic and prokaryotic rRNA. Unexpectedly, the coral rRNA was smaller (lower on the gel) than expected, causing the two normally distinct bands to overlap and become indistinguishable. This rRNA was later removed by the RiboZero kit and thus the difference in size was not inherently important to the analysis, but it was intended to be used as a proxy for the ratio of eukaryotic to prokaryotic mRNA. Without this quality check it was impossible to determine whether or not the kit was successful.

The limited number of annotated bacterial genes did strengthen the 16S results. There was general agreement between taxonomic classifications based on 16s amplicons and the taxonomic assignments of expressed genes from Uniprot; the top five most abundant phyla matched between the RNA and DNA datasets, when accounting for the fact that the Chloroplast-like sequences were classified under Cyanobacteria by SILVA, and the top three were in the same order of abundance. Functional analysis of bacterial genes expressed was limited by the low number of bacterial genes recovered, despite the depletion steps to remove eukaryotic RNA. The most common functions of bacteria represented in the identified genes included cellular nitrogen processes; however, annotations lack any mention of nitrogen fixation, nitrate reduction or ammonia assimilation. There were 23 references to amino acid metabolic processes; although these could have included amino acid synthesis as well as breakdown, most were annotated as oxidases or oxidoreductases. 34 oxidase genes were recovered, and 4 reductase genes, along with 19 unspecified oxidoreductase genes. Superoxide dismutase was present but not abundant (4 genes), so this could be either a response to oxygen stress or a normal constituent of bacterial oxygen defenses expressed at background levels. As a whole, most of these processes are probably part of the basic metabolism or cellular processes of the bacteria present; in other words, housekeeping genes, which are the most common and therefore likely the majority of such a small dataset.

The dataset also contains genes matching non-Opisthokont eukaryotes, including 44 sequences matching apicomplexans. The taxonomic annotations of these genes are questionable, since *Toxoplasma* and *Plasmodium* are both well-known and abundant in the database. However, this group is

broadly parasitic, which suggests that even if the annotations can be trusted to the phylum level (as the concordance with the 16S results in bacteria suggests), there may be parasitic interactions between these deep sea corals and unicellular eukaryotes. Even if these interactions are not strictly parasitic, unicellular algae in some cases compete with corals for resources (McCook et al. 2000). This is an avenue that should be explored further; to our knowledge no papers have yet examined the eukaryotic microbes associated with corals other than *Symbiodinium* spp*.,* but if the genes they are expressing are affecting the coral host, they might be doing more harm than good.

The presence of archaeal sequences in the dataset is intriguing, especially given their absence in the 16S dataset. The primers used targeted both bacteria and archaea, but no archaeal 16S sequences were recovered. In the RNA data, there were 28 genes from Euryarchaeota, 22 from Crenarchaeota, and 1 from an uncultured, unidentified archaeon. In the Euryarchaeota and uncultured archaeon, the majority of genes were uncharacterized proteins. However, in the Crenarchaeota, only one gene was uncharacterized, and the other 21 contained ankyrin repeats. Ankyrin repeats are common in eukaryotes, but in archaea and bacteria they can facilitate interactions with eukaryotic cells, and their presence is better predicted by a symbiotic lifestyle than phylogenetic history (Jernigan and Bordenstein. 2014). Furthermore, the consensus sequences for these domains in archaea and bacteria share 73.3% identity, making it difficult to tell for certain whether the taxonomic annotations recovered here are really archaeal. An additional 51 ankyrin-containing genes were recovered from the bacterial dataset. Whether or not they come from bacteria or archaea, however, the presence of this otherwise extremely rare domain is evidence supporting symbiosis in these corals.

In conclusion, *L. glaberrima* has a characteristic microbiome that differs from surrounding corals, water, and sediment, and includes two interesting groups in particular: a putative symbiont belonging to the genus *Endozoicomonas* found in every *L. glaberrima*, and a distant relative of Cyanobacteria matching most closely to the unclassified CAB-I division of bacteria. As a whole, bacterial community composition differed between *L. glaberrima* from Viosca Knoll and GC140, and within sites it differed significantly from both *L. pertusa* and *S. spp.* Clustering and phylum-level community composition suggest that water and sediment do not differ significantly in their bacterial communities between sites, but corals do differ from water and sediment, regardless of coral taxon. Neither 16S tag sequencing nor gene expression analysis was able to differentiate between red and white *L. glaberrima*, and few bacterial genes were recovered, painting only a broad picture of the potential functions

including nitrogen metabolism and oxidoreductase activity. However, the 16S results and the taxonomic identities of the RNA indicate the presence of potential pathogens, including Firmicutes, Cyanobacteria, and apicomplexan. Ankyrin domains and an OTU that persists across a wide geographic range also suggest the possibility that symbionts are part of the microbiome of *L. glaberrima*.

References

Altschul, Stephen F., Gish, Warren, Miller, Webb, Myers, Eugene W., and Lipman, David J. (1990). Basic local alignment search tool. J. Mol. Biol. 215; 403-410.

Anders, Simon, and Wolfgang Huber. "Differential Expression of RNA-Seq Data at the Gene Level–the DESeq Package." Heidelberg, Germany: European Molecular Biology Laboratory (EMBL), 2012. http://watson.nci.nih.gov/bioc_mirror/packages/2.11/bioc/vignettes/DESeq/inst/doc/DESeq.pdf.

Bayer, T, C Arif, C Ferrier-Pagès, D Zoccola, M Aranda, and Cr Voolstra. "Bacteria of the Genus Endozoicomonas Dominate the Microbiome of the Mediterranean Gorgonian Coral Eunicella Cavolini." *Marine Ecology Progress Series* 479 (April 8, 2013): 75–84. doi:10.3354/meps10197.

Bayer, T., M. J. Neave, A. Alsheikh-Hussain, M. Aranda, L. K. Yum, T. Mincer, K. Hughen, A. Apprill, and C. R. Voolstra. "The Microbiome of the Red Sea Coral Stylophora Pistillata Is Dominated by Tissue-Associated Endozoicomonas Bacteria." *Applied and Environmental Microbiology* 79, no. 15 (May 24, 2013): 4759–62. doi:10.1128/AEM.00695-13.

Bokulich, Nicholas A, Sathish Subramanian, Jeremiah J Faith, Dirk Gevers, Jeffrey I Gordon, Rob Knight, David A Mills, and J Gregory Caporaso. "Quality-Filtering Vastly Improves Diversity Estimates from Illumina Amplicon Sequencing." *Nature Methods* 10, no. 1 (December 2, 2012): 57–59. doi:10.1038/nmeth.2276.

Brooke S., Schroeder WW (2007) State of deep coral ecosystems in the Gulf of Mexico region: Texas to the Florida Straits. in The state of deep coral ecosystems of the United States, eds Lumsden SE, Hourigan TF, Bruckner AW, & Dorr G (NOAA Silver Spring, MD), pp 233-270.

Dinsdale, Elizabeth A., Olga Pantos, Steven Smriga, Robert A. Edwards, Florent Angly, Linda Wegley, Mark Hatay, et al. "Microbial Ecology of Four Coral Atolls in the Northern Line Islands." Edited by Niyaz Ahmed. *PLoS ONE* 3, no. 2 (February 27, 2008): e1584. doi:10.1371/journal.pone.0001584.

Dumler, J. Stephen, Anthony F. Barbet, C. P. Bekker, Gregory A. Dasch, Guy H. Palmer, Stuart C. Ray, Yasuko Rikihisa, and Fred R. Rurangirwa. "Reorganization of Genera in the Families Rickettsiaceae and Anaplasmataceae in the Order Rickettsiales: Unification of Some Species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, Descriptions of Six New Species Combinations and Designation of Ehrlichia Equi and 'HGE Agent' as Subjective Synonyms of Ehrlichia Phagocytophila." *International Journal of Systematic and Evolutionary Microbiology* 51, no. 6 (2001): 2145–65.

Forget, Nathalie L., and S. Kim Juniper. "Free-Living Bacterial Communities Associated with Tubeworm (*Ridgeia Piscesae*) Aggregations in Contrasting Diffuse Flow Hydrothermal Vent Habitats at the Main Endeavour Field, Juan de Fuca Ridge." *MicrobiologyOpen* 2, no. 2 (April 2013): 259–75. doi:10.1002/mbo3.70.

Frias-Lopez, J., G. T. Bonheyo, Q. Jin, and B. W. Fouke. "Cyanobacteria Associated with Coral Black Band Disease in Caribbean and Indo-Pacific Reefs." *Applied and Environmental Microbiology* 69, no. 4 (April 1, 2003): 2409–13. doi:10.1128/AEM.69.4.2409-2413.2003.

Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. "CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data." *Bioinformatics* 28, no. 23 (December 1, 2012): 3150–52. doi:10.1093/bioinformatics/bts565.

Gray, Michael A., Robert P. Stone, Molly R. McLaughlin, and Christina A. Kellogg. “Microbial Consortia of Gorgonian Corals from the Aleutian Islands.” FEMS Microbiology Ecology 76, no. 1 (2011): 109–20. doi:10.1111/j.1574-6941.2010.01033.x.

Haas, Brian J, Alexie Papanicolaou, Moran Yassour, Manfred Grabherr, Philip D Blood, Joshua Bowden, Matthew Brian Couger, et al. “De Novo Transcript Sequence Reconstruction from RNA-Seq Using the Trinity Platform for Reference Generation and Analysis.” *Nature Protocols* 8, no. 8 (July 11, 2013): 1494–1512. doi:10.1038/nprot.2013.084.

Jernigan, Kristin K., and Seth R. Bordenstein. “Ankyrin Domains across the Tree of Life.” *PeerJ* 2 (February 6, 2014): e264. doi:10.7717/peerj.264.

Kellogg, Christina A., John T. Lisle, and Julia P. Galkiewicz. “Culture-Independent Characterization of Bacterial Communities Associated with the Cold-Water Coral *Lophelia pertusa* in the Northeastern Gulf of Mexico.” Applied and Environmental Microbiology 75, no. 8 (April 15, 2009): 2294–2303. doi:10.1128/AEM.02357-08.

Kopylova, Evguenia, Laurent Noé, and Hélène Touzet. “SortMeRNA: Fast and Accurate Filtering of Ribosomal RNAs in Metatranscriptomic Data.” Bioinformatics 28, no. 24 (December 1, 2012): 3211–17. doi:10.1093/bioinformatics/bts611.

Leimena, Milkha M., Javier Ramiro-Garcia, Mark Davids, Bartholomeus van den Bogert, Hauke Smidt, Eddy J. Smid, Jos Boekhorst, Erwin G. Zoetendal, Peter J. Schaap, and Michiel Kleerebezem. “A Comprehensive Metatranscriptome Analysis Pipeline and Its Validation Using Human Small Intestine Microbiota Datasets.” BMC Genomics 14, no. 1 (August 2, 2013): 530. doi:10.1186/1471-2164-14-530.

Lesser, Mp, Li Falcón, A Rodríguez-Román, S Enríquez, O Hoegh-Guldberg, and R Iglesias-Prieto. “Nitrogen Fixation by Symbiotic Cyanobacteria Provides a Source of Nitrogen for the Scleractinian Coral *Montastraea cavernosa*.” Marine Ecology Progress Series 346 (September 27, 2007): 143–52. doi:10.3354/meps07008.

Li, Kelvin, Monika Bihan, and Barbara A. Methé. “Analyses of the Stability and Core Taxonomic Memberships of the Human Microbiome.” Edited by Bryan A. White. *PLoS ONE* 8, no. 5 (May 6, 2013): e63139. doi:10.1371/journal.pone.0063139.

Li, W., and A. Godzik. “Cd-Hit: A Fast Program for Clustering and Comparing Large Sets of Protein or Nucleotide Sequences.” *Bioinformatics* 22, no. 13 (July 1, 2006): 1658–59. doi:10.1093/bioinformatics/btl158.

McCook, L., J. Jompa, and G. Diaz-Pulido. “Competition between Corals and Algae on Coral Reefs: A Review of Evidence and Mechanisms.” *Coral Reefs* 19, no. 4 (May 2001): 400–417. doi:10.1007/s003380000129.

Muscatine, L., P. G. Falkowski, J. W. Porter, and Z. Dubinsky. “Fate of Photosynthetic Fixed Carbon in Light- and Shade-Adapted Colonies of the Symbiotic Coral Stylophora Pistillata.” *Proceedings of the Royal Society of London B: Biological Sciences* 222, no. 1227 (August 22, 1984): 181–202. doi:10.1098/rspb.1984.0058.

Neave, M. J., C. T. Michell, A. Apprill, and C. R. Voolstra. “Whole-Genome Sequences of Three Symbiotic *Endozoicomonas* Strains.” *Genome Announcements* 2, no. 4 (August 14, 2014): e00802–14 – e00802–14. doi:10.1128/genomeA.00802-14.

Neulinger, Sven C., Johanna Järnegren, Martin Ludvigsen, Karin Lochte, and Wolf-Christian Dullo. “Phenotype-Specific Bacterial Communities in the Cold-Water Coral *Lophelia pertusa* (Scleractinia) and Their Implications for the Coral’s Nutrition, Health, and Distribution.” Applied and Environmental Microbiology 74, no. 23 (December 1, 2008): 7272–85. doi:10.1128/AEM.01777-08.

Oksanen, Jari, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2015). vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan

Olson, Nathan D., and Michael P. Lesser. "Diazotrophic Diversity in the Caribbean Coral, *Montastraea cavernosa*." Archives of Microbiology 195, no. 12 (November 12, 2013): 853–59. doi:10.1007/s00203-013-0937-z.

Penn, Kevin, Dongying Wu, Jonathan A. Eisen, and Naomi Ward. "Characterization of Bacterial Communities Associated with Deep-Sea Corals on Gulf of Alaska Seamounts." Applied and Environmental Microbiology 72, no. 2 (February 1, 2006): 1680–83. doi:10.1128/AEM.72.2.1680-1683.2006.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glockner. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." Nucleic Acids Research 41, no. D1 (January 1, 2013): D590–96. doi:10.1093/nar/gks1219.

M. L. Reaka-Kudla, in Biodiversity II, M. L. Reaka-Kudla, D. E. Wilson, E. O. Wilson, Eds. (Joseph Henry Press, Washington, DC, 1997), pp. 83–108.

Polato, Nicholas R., J. Cristobal Vera, and Iliana B. Baums. "Gene Discovery in the Threatened Elkhorn Coral: 454 Sequencing of the Acropora Palmata Transcriptome." Edited by Timothy Ravasi. *PLoS ONE* 6, no. 12 (December 28, 2011): e28634. doi:10.1371/journal.pone.0028634.

Roberts, C. M., Colin J. McClean, John E.N. Veron, Julie P. Hawkins, Gerald R. Allen, Don E. McAllister, Cristina G. Mittermeier, Frederick W. Schueler, Mark Spalding, Fred Wells, Carly Vynne, and Timothy B. Werner. "Marine Biodiversity Hotspots and Conservation Priorities for Tropical Reefs." *Science* 295, no. 5558 (February 15, 2002): 1280–84. doi:10.1126/science.1067728.

Roder, Cornelia, Chatchanit Arif, Camille Daniels, Ernesto Weil, and Christian R. Voolstra. "Bacterial Profiling of White Plague Disease across Corals and Oceans Indicates a Conserved and Distinct Disease Microbiome." Molecular Ecology 23, no. 4 (2014): 965–74. doi:10.1111/mec.12638.

Rodriguez-Lanetty, Mauricio, Camila Granados-Cifuentes, Albert Barberan, Anthony J. Bellantuono, and Carolina Bastidas. "Ecological Inferences from a Deep Screening of the Complex Bacterial Consortia Associated with the Coral, *Porites astreoides*." Molecular Ecology 22, no. 16 (August 2013): 4349–62. doi:10.1111/mec.12392.

Ruiz-Ramos, Dannise, Miles Saunders, Charles Fisher, and Iliana Baums. "Home Bodies and Wanderers: Mixed Reproductive Strategies in Sympatric Lineages of the Deep-Sea Black Coral, *Leiopathes glaberrima*." Molecular Ecology (in review).

Schloss, P.D., et al., Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol, 2009. 75(23):7537-41.

Sweet, M. J., A. Croquer, and J. C. Bythell. "Experimental Antibiotic Treatment Identifies Potential Pathogens of White Band Disease in the Endangered Caribbean Coral *Acropora cervicornis*." Proceedings of the Royal Society B: Biological Sciences 281, no. 1788 (June 18, 2014): 20140094–20140094. doi:10.1098/rspb.2014.0094.

Thompson, Janelle R., Hanny E. Rivera, Collin J. Closek, and Mónica Medina. "Microbes in the Coral Holobiont: Partners through Evolution, Development, and Ecological Interactions." *Frontiers in Cellular and Infection Microbiology* 4 (January 7, 2015). doi:10.3389/fcimb.2014.00176.

Thurber, Rebecca Vega, Dana Willner-Hall, Beltran Rodriguez-Mueller, Christelle Desnues, Robert A. Edwards, Florent Angly, Elizabeth Dinsdale, Linda Kelly, and Forest Rohwer. "Metagenomic Analysis of Stressed Coral

Holobionts." Environmental Microbiology 11, no. 8 (August 2009): 2148–63. doi:10.1111/j.1462-2920.2009.01935.x.

Tremblay, Pascale, Renaud Grover, Jean François Maguer, Louis Legendre, and Christine Ferrier-Pagès. "Autotrophic Carbon Budget in Coral Tissue: A New 13C-Based Model of Photosynthate Translocation." *The Journal of Experimental Biology* 215, no. 8 (April 15, 2012): 1384–93. doi:10.1242/jeb.065201.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. "Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy." *Applied and Environmental Microbiology* 73, no. 16 (August 15, 2007): 5261–67. doi:10.1128/AEM.00062-07.

Warton, David I., Stephen T. Wright, and Yi Wang. "Distance-Based Multivariate Analyses Confound Location and Dispersion Effects: Mean-Variance Confounding in Multivariate Analysis." *Methods in Ecology and Evolution* 3, no. 1 (February 2012): 89–101. doi:10.1111/j.2041-210X.2011.00127.x.

Chapter 2. Gene Expression and Community Shifts of Oil-Exposed Black Coral Microbiomes

Abstract:

Corals are important foundation species in both shallow and deep-water environments, but are being threatened by a variety of anthropogenic stressors, including oil spills. Short-term effects of oil exposure have been studied in the deep-sea coral *Paramuricea biscaya,* but very few studies had attempted to monitor corals for sublethal effects, and none have examined the bacterial communities associated with these corals. In this study, the black coral *Leiopathes glaberrima* was exposed to various concentrations of oil and dispersed oil in 3 experiments. Bacterial community composition was measured using non-metric multidimensional scaling (NMDS) and examined for changes in the abundance of known oil-degrading bacterial genera. There were no significant changes in community composition in any experiment and the oil-degrading genera did not change in relative abundance, and in fact their proportion was slightly higher on average in control than in oil samples.

Introduction:

Corals, and the 3-dimensional structures they form, create the basis for complex ecosystems that can host hundreds of thousands of animal and plant species (Reaka-Kudla 1997). These foundation species are being threatened by a variety of anthropogenic impacts including temperature and pH shifts (IPCC 2014). Oil spills, such as the 2010 Deep Water Horizon blowout, present another potential anthropogenic impact to corals, especially in the Gulf of Mexico, where drilling is common. This particular disaster involved the release of a 100-m-thick underwater plume from the Macondo well that travelled for kilometers before dispersing (White et al. 2012), and such widespread coverage merits careful investigation for impacts to coral communities.

Some of this work has been done already; many corals near the well-head were found with a brown, flocculent material covering their branches, and this material was chemically fingerprinted and traced back to the Macondo well (White et al. 2012). Subsequently, at least two more sites were found with the same pattern of impact, up to 22km from the well head, painting a broader picture of what an impacted coral looks like months, and years, after an oil spill (Fisher et al. 2014). However, this work was

focused on a single genus of octocoral, *Paramuricea,* which was the numerically dominant colonial coral at all sites examined in this study. *Paramuricea* spp. lends itself well to this kind of work because its colonies are planar and show clear signs of stress and damage that are easily analyzed through photographs, but other genera and classes of corals may react differently. Furthermore, photo-based analysis is conservative by nature, showing only clear signs of acute damage and missing any effects not readily apparent visually. As we move beyond simply identifying acutely impacted corals, other tools are needed to detect and understand the full range of responses of corals to oil and dispersant exposure.

L. glaberrima is one of the most common black corals in the Gulf of Mexico, and it occurs in a variety of color morphotypes, ranging from red to white with several intermediate color morphotypes. It is also common globally, occurring throughout the Mediterranean Sea and Pacific Ocean with a depth range of about 200 to 600 meters (Brooke and Schroeder 2007, Ruiz-Ramos et al. 2015). At this depth there is little to no light, and therefore these corals do not harbor the symbiotic unicellular alga *Symbiodinium* spp. found in many shallower corals. However, they do have a species-rich assemblage of bacteria (see Chapter 2). The bacterial organisms which are important in shallow-water corals for reasons including stress response, pathogen resistance, and nitrogen fixation (Lesser et al. 2007, Olson and Lesser 2013, Sweet et al. 2014, Roder et al. 2014, Rodriguez-Lanetty et al. 2013) may also be important in deep-sea corals. The microbiome could be the key to understanding how corals deal with oil stress. Understanding how this stress affects the bacterial communities, both in community composition and changes in bacterial gene expression, is the first step toward unraveling this complex web of interactions.

In this chapter, I ask how the microbial communities shift after oil exposure. Community composition is measured as the relative abundances of bacterial operational taxonomic units (OTUs) in each sample, where OTUs are defined by the similarity of their 16s phylotypes. Next I ask whether the bacterial community as a whole functions differently under oil stress, as measured by differential gene expression over the entire bacterial metatranscriptome. With these two sources of information we can determine if oil stress affects the bacteria associated with corals, and if so how.

Methods:

Sample Collection and Oil Exposure Experiments

Three separate experiments were performed from 2012 to 2014 on red and white *Leiopathes glaberrima* coral colonies collected from the Gulf of Mexico. In the 2012 experiment, 2 red and 2 white colonies were collected aboard the R/V Falkor using the remotely operated vehicle (ROV) Global Explorer. In 2013, 4 red colonies were collected aboard the E/V Nautilus using ROV Hercules. In 2014, 3 red and 3 white colonies were collected aboard the R/V Atlantis using deep submergence vehicle (DSV) Alvin. The basic setup for each experiment was the same: corals were collected from Viosca Knoll 826 (VK826), one of the largest known coral sites known in the deep northern Gulf (Brooke and Schroeder 2007), named after the Bureau of Ocean Energy Management 3NM by 3NM lease block where the community is found. The corals were brought to the surface by the ROV in a sealed, incubated box. At the surface, each colony was placed in a separate 5-gallon bucket filled with artificial seawater (Instant Ocean, 35 ppm) and kept in a cold room at 6°C for a maximum of 24 hours before experimentation began. Each colony was fragmented with ethanol-sterilized forceps and scissors, and each fragment was placed in a tube of 30mL artificial seawater (control group) or seawater + oil (treatment group). Oil treatments were prepared by the serial dilution of either bulk oil (2012) or water-accommodated fractions (2013, 2014) of MASS oil collected from the Macondo well (provided by M. Joye; 2012, 2014) or surrogate oil (2013). In 2012, the treatment group was exposed to 25 ppm oil – about 22.66 mg/L – for 24 hours. 50 µL oil was added to 199.95 mL artificial seawater (ASW; Instant Ocean, 35 ppm), mixed on an orbital shaker at high speed for 24 hours, and then diluted 1:10 in ASW to form the oil stock. Additionally, corals were exposed to dispersed oil, which was prepared by adding 25ppm – about 23.70 mg/L – of Corexit 9500A dispersant (Nalco, TX USA) to a separate oil stock solution. In 2013, corals were exposed to 250 µM oil – about 95.25 mg/L – for 24 hours. In 2014 corals were treated with 200 mg/L oil for 48 hours. 9.5 mL oil was added to 475 mL ASW in a baked glass beaker, mixed for 48 hours with a magnetic stir bar, and after separation in a baked glass separatory funnel, the bottom (aqueous) fraction was collected. 218.72 mL of this stock solution was added to 281.28 mL ASW to reach the final oil concentration of 200 mg/L. At the end of the exposure period, corals were preserved in RNALater (Ambion, CA USA) and kept at -20°C for 24 hours before long-term storage at -80°C. DNA was extracted using the PowerSoil DNA extraction kit (MO BIO, CA USA).

Illumina Library Preparation

The 16S rRNA gene was sequenced in order to examine differences in community composition between the oil and control coral-associated microbial assemblages. Ribosomal DNA in each library was amplified using modified 27F (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCMTGGCTCAG3') and 355R (5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCCTCCCGTAGGAGT3') primers (Rodriguez-Lanetty et al. 2013), which target the V2 region of the 16S gene. These primers also contained the universal barcode for use with the Nextera index kit (Illumina, CA USA). The product of this first polymerase chain reaction (PCR) was a 350-base-pair amplicon, which was cloned into *E. coli* using the Topo cloning vector (ThermoFisher, MA USA). A BLAST search revealed that the top hit for each of 8 trial Sanger sequences was from a bacterial 16S gene. The PCR mix consisted of 1X Promega reaction buffer, 0.3 mg/mL BSA, 1.56 mM $MgCl_2$, 250 µM of each dNTP, 0.125 µM of each primer, and 0.156 units of taq DNA polymerase. Reaction conditions were 5 minutes at 95°C, followed by 30 cycles of 95° for 40 seconds, 51° for 2 minutes, and 72° for 1 minute, followed by a final extension of 7 minutes at 72°. PCR products were checked on a 2 percent agarose gel and the remainder of each sample was cleaned up with AMPure beads (Beckman Coulter) following the manufacturer's instructions.

A second round of PCR was performed using the Nextera index kit, resulting in completed libraries, each with a unique pair of forward and reverse indices. The final 50 µL PCR reaction contained 100 ng of first round PCR product, 5 µL each of forward and reverse Nextera barcodes (100 µM stock), 200 µM of each dNTP, 1X Roche Fast Start High Fidelity PCR buffer (which contains $MgCl_2$), and .05 units of Roche DNA polymerase. PCR conditions were 95°C for 3 minutes, followed by 8-10 cycles of 95° for 30 seconds, 55° for 30 seconds, and 72° for 30 seconds. After the cycling was complete, there was a final extension of 5 minutes at 72° and a hold at 4°. Samples were again cleaned with AMPure beads. The Qubit dsDNA BR assay (ThermoFisher, MA USA) was used to determine library concentration. From this, an equimolar pool of all samples was constructed and concentrations were confirmed via qPCR (Kapa Biosystems, MA USA). This pool was then sequenced using 250x250 or 300x300 paired-end sequencing with the Illumina MiSeq platform, with 23-24 samples per run.

RNA Extraction

In order to examine the functional differences in the microbiome between oil and control samples, RNA was extracted using the extraction protocol described in Polato et al. (2011). The eluted RNA was processed using the MicrobEnrich (ThermoFisher, MA USA) and RiboZero (Epicentre, WI USA) kits, following the manufacturers' instructions. The MicrobEnrich kit eliminates eukaryotic sequences with poly-A tails, selecting for prokaryotes which lack them, and the RiboZero kit filters out ribosomal RNA (rRNA) sequences. Sequences were checked on an Agilent 2100 bioanalyzer (Agilent, CA USA) and sequenced on the HiSeq 2000 (Illumina, CA USA) platform.

Bioinformatics and Data Analysis

Quality control of the raw DNA reads, including trimming and assembly, was performed in Mothur (Schloss et al. 2009). Reads with homopolymers longer than 8, reads with ambiguous sequences as determined by Mothur, and reads shorter than 50 or longer than 400 nucleotides were eliminated. Remaining reads were then filtered with SortMeRNA to remove sequences that did not align to the SILVA 16S database (Kopylova et al 2012). Sequences that aligned to 16S were classified using the RDP classifier version 2.11, retrained with the November 2015 dataset and using a confidence cutoff of .5 (Wang et al. 2007). Data were then clustered to 98% similarity using cdhit-est running in accurate mode (Li et al. 2006, Fu et al. 2012), and analyzed using the NMDS and ANOSIM functions in the R package "vegan" (Oksanen et al. 2015). Oil and control samples from each of the three experiments were also compared for the proportions of classified sequences that had 16S tag sequences matching known oil-degrading bacterial genera, including both alkane and PAH degraders (Whyte et al. 2002, Cunliffe and Kertesz 2006, Guo et al. 2010). These genera were *Colwellia, Marinobacter, Pseudomonas, Acinetobacter, Rhodococcus, Sphingobium, Sphingomonas, Mycobacterium,* and *Paracoccus.*

RNA data were analyzed using a modified version of the pipeline described in Leimena et al. (2013). The RNA reads were collapsed, clipped, trimmed, and quality-filtered using the fastx toolkit. They were then compared to Silva and Rfam rRNA databases using SortMeRNA and only reads that did not align to rRNA were kept (Kopylova et al. 2012, as described in Leimena et al. 2013). BLAST was then used to the remaining reads against a larger database using a 3-tiered approach. First, dc-megablast was used to compare reads against the nt database. Reads with no hits with e-values below 10^{-6} were identified and re-run using BLASTX against the uniprot database. Reads with no hits were again collected

and compared a third time using BLASTX against the nr database. Reads from all 3 blast runs with hits were combined, mapped and annotated using BLAST2Go, and analyzed using the R package "DESeq" (Anders and Huber 2012). Genes with less than 10 sequences in either the control or oil group were eliminated from the differential expression analysis.

Results:

None of the 3 oil experiments showed significant differences in community composition between oil and control corals, and in 2012 the community composition of dispersed oil-treated corals also showed no significant differences from either oil (ANOSIM p=0.229) or control (ANOSIM p=0.829). The 2012 experiment NMDS plot for oil versus control is shown in Figure 2.1, and the ANOSIM had a p-value of 0.343 and an R value of 0.063. The NMDS plot with oil, control, and dispersed oil treatments is shown in Figure 2.2. The NMDS plot for the 2013 experiment is shown in Figure 2.3, and the ANOSIM had a p-value of 0.943 and an R value of -0.219. Two samples, control 4 and oil 1, were so far to the right in the NMDS plot that displaying them along with the other colonies distorted the graph, showing the other 6 colonies almost as a single point. Therefore these two colonies were removed from the NMDS analysis. ANOSIM without these two had a p value of 0.8 and an R value of -0.185. The NMDS plot for the 2014 experiment is shown in Figure 2.4, and the ANOSIM had a p-value of 0.474 and an R value of 0.

The proportion of classified reads represented by oil-degrading bacteria did not differ using a paired two-tailed z-test for any of the 3 experiments (2012: z=0.016, p=0.987; 2013: z=0.309, p=0.757; 2014: z=0.051, p=0.959). Furthermore, in all three experiments, the proportion of classified reads was higher for control than for oil. On average, in the 2012 experiment, control treatments had 1.22% oil-degrading genera and oil treatments had 1.09%. In 2013, control treatments had 6.24% and oil treatments had 1.91%. In 2014, control treatments had 16.63% and oil treatments had 15.30%.

RNA results were only obtained for the 2012 experiment, but they showed no differences between oil and control corals. Of the 1,342 bacterial genes analyzed, 701 had 10 or more sequences in both the oil and control groups, and were analyzed for differential expression using DESeq. Of these, the lowest uncorrected p-value was .08, and every corrected p-value was 1. A heatmap of the top 75 most differentially expressed genes between oil and control corals is shown in Figure 2.5. Samples were also

grouped by color, but this also did not identify any genes that were significantly different in expression level.

Figures 2.6-2.8 show the phyla represented in each of the 3 experiments for both oil and control colonies. Figure 2.7 shows that the two outliers in the NMDS plot were lower in Proteobacteria and higher in Tenericutes. From the genus-level analysis, these samples were reduced in *Endozoicomonas* spp. and higher in unclassified sequences; most of the Tenericutes could not be classified below phylum level.

Discussion:

To our knowledge this is the first experiment to measure differences in bacterial community composition in oil-exposed corals, and one of the first studies in general to examine the effects of oil on deep-sea corals. Therefore there was no precedent for deciding how long to expose the corals before sampling for bacteria. In the first two experiments, corals were not fed, and therefore the 24 hour time-point was chosen because corals were unlikely to undergo food stress in that time. In the third experiment, corals were fed with autoclaved marine snow (Two Little Fishies, FL USA) in an attempt to prolong the experiment without affecting the microbial communities. Even at the 48 hour time point, however, oil and control corals were completely overlapping in the NMDS plot. One bacterial genus of particular interest was *Colwellia*, which is known as an oil-eating bacterium and could have accounted for some of the oil-exposed corals surviving longer than the controls in the unfed experiment. However, this genus was found only sporadically in the first two experiments, and although it was more ubiquitous in the 2014 experiment, it was not significantly more abundant (% of total quality-controlled reads represented by *Colwellia*) in oil corals compared to control, and in fact the mean was slightly higher in the control group (11.05% in control corals, 7.40% in oil-exposed corals).

The failure to differentiate treatment groups by bacterial community composition indicates one of two things. Either the bacterial communities are playing an insignificant or negligible role in the host response to oil stress, or the bacterial communities react with changes in gene expression rather than shifts in taxonomic groups over the time periods tested. This is logical given that gene expression measures what's actually happening on a relatively short time scale, whereas community composition represents the identity of the bacteria present, so bacterial gene expression could theoretically change

more drastically and on a shorter time scale than relative abundance of 16S tag sequences. I surveyed for both changes in bacterial community composition and gene expression, but with only 1,342 bacterial genes (.5% of the total genes recovered), it is not surprising that no significant changes in gene expression were detectable. These genes probably represent only the most commonly occurring genes in the population, which are likely tied to essential functions of basic metabolism or other housekeeping genes. The levels of expression of these genes is unlikely to change with the stress seen in these experiments, given that the stress wasn't sufficient to alter the community composition.

All this is based on the assumption that there is a response to oil stress from the host or bacteria after 24 hours, and this may not be the case. Ruiz-Ramos et al. analyzed the same corals from the 2012 and 2013 experiment and found that the declines in health in oil-exposed corals over 96 hours was 7.5 times faster than the control samples in 2012, but only 0.22 times faster than control samples in 2013 (Ruiz-Ramos et al. in prep).

In conclusion, there were no significant differences between oil and control corals in either bacterial community composition or gene expression detected in any of the three experiments performed. It is unlikely that significant changes in community composition existed but were not detectable, given the high-throughput methods used, but changes in gene expression could have been missed due to the extremely small number of bacterial genes recovered. It would be beneficial to examine more long-range effects of oil exposure in the future for both community composition and bacterial gene expression, perhaps expanding the focus to include unicellular eukaryotes.

References

Altschul, Stephen F., Gish, Warren, Miller, Webb, Myers, Eugene W., and Lipman, David J. (1990). Basic local alignment search tool. J. Mol. Biol. 215; 403-410.

Anders, Simon, and Wolfgang Huber. "Differential Expression of RNA-Seq Data at the Gene Level–the DESeq Package." Heidelberg, Germany: European Molecular Biology Laboratory (EMBL), 2012. http://watson.nci.nih.gov/bioc_mirror/packages/2.11/bioc/vignettes/DESeq/inst/doc/DESeq.pdf.

Brooke S., Schroeder WW (2007) State of deep coral ecosystems in the Gulf of Mexico region: Texas to the Florida Straits. in The state of deep coral ecosystems of the United States, eds Lumsden SE, Hourigan TF, Bruckner AW, & Dorr G (NOAA Silver Spring, MD), pp 233-270.

Cunliffe, Michael, and Michael A. Kertesz. "Effect of Sphingobium Yanoikuyae B1 Inoculation on Bacterial Community Dynamics and Polycyclic Aromatic Hydrocarbon Degradation in Aged and Freshly PAH-Contaminated Soils." *Environmental Pollution* 144, no. 1 (November 2006): 228–37. doi:10.1016/j.envpol.2005.12.026.

Fisher, Charles R., Pen-Yuan Hsing, Carl L. Kaiser, Dana R. Yoerger, Harry H. Roberts, William W. Shedd, Erik E. Cordes, et al. "Footprint of Deepwater Horizon Blowout Impact to Deep-Water Coral Communities." Proceedings of the National Academy of Sciences 111, no. 32 (August 12, 2014): 11744–49. doi:10.1073/pnas.1403492111.

Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. "CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data." *Bioinformatics* 28, no. 23 (December 1, 2012): 3150–52. doi:10.1093/bioinformatics/bts565.

Guo, Chuling, Zhi Dang, Yukshan Wong, and Nora Fungyee Tam. "Biodegradation Ability and Dioxgenase Genes of PAH-Degrading Sphingomonas and Mycobacterium Strains Isolated from Mangrove Sediments." *International Biodeterioration & Biodegradation* 64, no. 6 (September 2010): 419–26. doi:10.1016/j.ibiod.2010.04.008.

Intergovernmental Panel on Climate Change (IPCC). 2014. Fifth assessment report. Cambridge, United Kingdom: Cambridge University Press. http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml.

Kopylova, Evguenia, Laurent Noé, and Hélène Touzet. "SortMeRNA: Fast and Accurate Filtering of Ribosomal RNAs in Metatranscriptomic Data." Bioinformatics 28, no. 24 (December 1, 2012): 3211–17. doi:10.1093/bioinformatics/bts611.

Leimena, Milkha M., Javier Ramiro-Garcia, Mark Davids, Bartholomeus van den Bogert, Hauke Smidt, Eddy J. Smid, Jos Boekhorst, Erwin G. Zoetendal, Peter J. Schaap, and Michiel Kleerebezem. "A Comprehensive Metatranscriptome Analysis Pipeline and Its Validation Using Human Small Intestine Microbiota Datasets." BMC Genomics 14, no. 1 (August 2, 2013): 530. doi:10.1186/1471-2164-14-530.

Lesser, Mp, Li Falcón, A Rodríguez-Román, S Enríquez, O Hoegh-Guldberg, and R Iglesias-Prieto. "Nitrogen Fixation by Symbiotic Cyanobacteria Provides a Source of Nitrogen for the Scleractinian Coral *Montastraea cavernosa*." Marine Ecology Progress Series 346 (September 27, 2007): 143–52. doi:10.3354/meps07008.

Li, W., and A. Godzik. "Cd-Hit: A Fast Program for Clustering and Comparing Large Sets of Protein or Nucleotide Sequences." *Bioinformatics* 22, no. 13 (July 1, 2006): 1658–59. doi:10.1093/bioinformatics/btl158.

Neulinger, Sven C., Johanna Järnegren, Martin Ludvigsen, Karin Lochte, and Wolf-Christian Dullo. "Phenotype-Specific Bacterial Communities in the Cold-Water Coral *Lophelia pertusa* (Scleractinia) and Their Implications for

the Coral's Nutrition, Health, and Distribution." Applied and Environmental Microbiology 74, no. 23 (December 1, 2008): 7272–85. doi:10.1128/AEM.01777-08.

Oksanen, Jari, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2015). Vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan

Olson, Nathan D., and Michael P. Lesser. "Diazotrophic Diversity in the Caribbean Coral, *Montastraea cavernosa*." Archives of Microbiology 195, no. 12 (November 12, 2013): 853–59. doi:10.1007/s00203-013-0937-z.

M. L. Reaka-Kudla, in Biodiversity II, M. L. Reaka-Kudla, D. E. Wilson, E. O. Wilson, Eds. (Joseph Henry Press, Washington, DC, 1997), pp. 83–108.

Polato, Nicholas R., J. Cristobal Vera, and Iliana B. Baums. "Gene Discovery in the Threatened Elkhorn Coral: 454 Sequencing of the Acropora Palmata Transcriptome." Edited by Timothy Ravasi. *PLoS ONE* 6, no. 12 (December 28, 2011): e28634. doi:10.1371/journal.pone.0028634.

Roder, Cornelia, Chatchanit Arif, Camille Daniels, Ernesto Weil, and Christian R. Voolstra. "Bacterial Profiling of White Plague Disease across Corals and Oceans Indicates a Conserved and Distinct Disease Microbiome." Molecular Ecology 23, no. 4 (2014): 965–74. doi:10.1111/mec.12638.

Rodriguez-Lanetty, Mauricio, Camila Granados-Cifuentes, Albert Barberan, Anthony J. Bellantuono, and Carolina Bastidas. "Ecological Inferences from a Deep Screening of the Complex Bacterial Consortia Associated with the Coral, *Porites astreoides*." Molecular Ecology 22, no. 16 (August 2013): 4349–62. doi:10.1111/mec.12392.

Ruiz-Ramos, Dannise V., Miles Saunders, Charles R. Fisher, and Iliana B. Baums. "Home Bodies and Wanderers: Sympatric Lineages of the Deep-Sea Black Coral Leiopathes Glaberrima." Edited by Mikhail V. Matz. *PLOS ONE* 10, no. 10 (October 21, 2015): e0138989. doi:10.1371/journal.pone.0138989.

Ruiz-Ramos, Dannise V., Charles. R. Fisher and Iliana B. Baums. "Stress-response of the black coral Leiopathes glaberrima to the exposure of crude oil and dispersant." In prep.

Schloss, P.D., et al., "Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities." Appl Environ Microbiol, 2009. 75(23):7537-41.

Sweet, M. J., A. Croquer, and J. C. Bythell. "Experimental Antibiotic Treatment Identifies Potential Pathogens of White Band Disease in the Endangered Caribbean Coral *Acropora cervicornis*." Proceedings of the Royal Society B: Biological Sciences 281, no. 1788 (June 18, 2014): 20140094–20140094. doi:10.1098/rspb.2014.0094.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. "Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy." *Applied and Environmental Microbiology* 73, no. 16 (August 15, 2007): 5261–67. doi:10.1128/AEM.00062-07.

White, Helen K., Pen-Yuan Hsing, Walter Cho, Timothy M. Shank, Erik E. Cordes, Andrea M. Quattrini, Robert K. Nelson, et al. "Impact of the Deepwater Horizon Oil Spill on a Deep-Water Coral Community in the Gulf of Mexico." Proceedings of the National Academy of Sciences 109, no. 50 (December 11, 2012): 20303–8. doi:10.1073/pnas.1118029109.

Whyte, L. G., T. H. M. Smits, D. Labbe, B. Witholt, C. W. Greer, and J. B. van Beilen. "Gene Cloning and Characterization of Multiple Alkane Hydroxylase Systems in Rhodococcus Strains Q15 and NRRL B-16531." *Applied and Environmental Microbiology* 68, no. 12 (December 1, 2002): 5933–42. doi:10.1128/AEM.68.12.5933-5942.2002.

APPENDIX

TABLES AND FIGURES

Table 1.1: *L. glaberrima* Samples for DNA Analysis

# of Samples (Sample ID)	Lease Block	Color	Depth (m)	Latitude DD (WGS84)	Longitude DD (WGS84)
4 (645, 667, 674, 675)	VK826	Red	450-475	29.16	-88.015
4 (676, 677, 655, 671)	VK826	White	450-476	29.16	-88.015
2 (528, 533)	VK906	Red	403	29.069	-88.38
2 (546, 560)	VK906	White	393-399	29.069	-88.38
4 (475, 478. 484, 489)	GC140	Red	250-289	27.81	-91.54
4 (472, 485, 503, 510)	GC140	White	249-284	27.81	-91.54

Table 1.2: Corals Collected for Coral Species Comparisons

# of Samples (Sample ID)	Coral Species	Lease Block	Depth (m)	Latitude DD (WGS84)	Longitude DD (WGS84)
4 (786, 785, 789, NA028-133)	*Paramuricea biscaya*	DC673	2223-2388	28.31	-87.31
2 (779, 777)	*Paramuricea biscaya*	MC036	1094-1100	28.94	-88.20
3 (819, 821, 825)	*Paramuricea B3*	AT357	1052-1098	27.59	-89.70
3 (315, 641, 647	*Sibopathes* spp.	VK826	451-494	29.16	-88.02
3 (178, 179, 180)	*Lophelia pertusa*	VK906	394-396	29.069	-88.38

Table 1.3: DNA Sample Statistics

Sample Name	Raw Reads	Quality-controlled Reads	#OTUs	Shannon Diversity Index	Core Genera	Chao1 Richness
VK826 White 1 (676)	633,365	443,044	1412	0.18	Chloroplast	2475
VK826 White 2 (677)	834,662	526,142	2273	2.71	Chloroplast	5109
VK826 White 3 (655)	965,558	369,010	7709	4.04	Chloroplast	128,466
VK826 White 4 (671)	637,711	303,305	2224	4.02	Chloroplast	7720
VK826 Red 1 (645)	712,737	441,949	1078	3.13	Chloroplast	6810
VK826 Red 2 (667)	664,696	430,047	745	2.53	Chloroplast	2136
VK826 Red 3 (674)	660,092	315,763	1880	3.94	Chloroplast	5520
VK826 Red 4 (675)	685,071	382,114	1081	2.82	Chloroplast	2076
VK906 White 1 (546)	180,032	50,350	717	3.86	Chloroplast, *Endozoicomonas*	3573
VK906 White 2 (560)	689,334	435,320	943	3.20	Chloroplast, *Endozoicomonas*	3244
VK906 Red 1 (528)	707,457	471,193	642	1.02	Chloroplast, *Endozoicomonas*	1919
VK906 Red 2 (533)	755,572	500,217	953	2.33	Chloroplast, *Endozoicomonas*	2172
GC140 White 1	877,232	468,757	1718	3.63	*Streptococcus*, *Propionibacterium*,	4514

(472)					Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	
GC140 White 2 (485)	758,430	423,405	962	3.49	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	2705
GC140 White 3 (503)	1,318,223	627,984	1248	3.42	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	3141
GC140 White 4 (510)	661,385	331,519	1448	3.91	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	5815
GC140 Red 1 (475)	591,716	327,885	2531	4.36	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	5472
GC140 Red 2 (478)	778,164	449,063	1482	3.68	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	3084
GC140 Red 3 (484)	718,947	327,891	1766	3.07	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*,	10,157

					Endozoicomonas, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	
GC140 Red 4 (489)	796,971	159,363	5311	3.91	*Streptococcus*, *Propionibacterium*, Chloroplast, *Corynebacterium*, *Endozoicomonas*, *Staphylococcus*, *Bacillus*, unknown Corynebacteriaceae	127,175
VK906 Water	395,764	143,875	5483	6.18	None	13,080
VK906 Sediment	280,132	85,756	4982	6.97	None	11,642
AT357 Water	261,640	88,613	5028	6.35	None	11,071
AT357 Sediment	428,140	150,607	12,013	7.71	None	36,561
MC751 Water	351,093	127,326	6209	6.44	None	11,602
MC751 Sediment	467,002	158,522	11,321	7.90	None	26,543
GC234 Water	417,747	149,715	13,438	6.94	None	26,293
GC234 Sediment	369,666	126,605	11,788	7.58	None	23,244
Red 1 Washed (VK826)	235,732	80,907	644	1.88	Chloroplast, *Propionibacterium*	3316
Red 1 Unwashed (VK826)	270719	89,187	904	3.88	Chloroplast, *Propionibacterium*	4604
Red 2 Washed (VK826	393,505	93,909	1483	3.87	Chloroplast, *Propionibacterium*	7192
Red 2 Unwashed	189,786	62,454	699	2.81	Chloroplast, *Propionibacterium*	4217

(VK826)						
Red 3 Washed (VK826)	303,074	105,644	737	3.37	Chloroplast, *Propionibacterium*	3122
Red 3 Unwashed (VK826)	281,620	85,530	1292	3.36	Chloroplast, *Propionibacterium*	5938

Table 1.4: Similarity Percentage (Simper) Analysis of Top 10 Genera Contributing to Variance between Groups. Contribution is the contribution of each genus to the bray-curtis dissimilarity between groups, sd is the standard deviation of the contribution, ratio is the ratio of contribution to standard deviation, and av.a and av.b are the average abundances of the genus in the two groups, where the group on the left in each case is a.

***Leiopathes glaberrima* VK906 vs GC140**	contribution	sd	ratio	av.a	av.b
Propionibacterium	0.0094	0.0045	2.067	8.9119	15.7164
Halomonas	0.0093	0.0013	6.9133	7.0842	0.125
No Relative	0.0091	0.0058	1.5654	15.5997	12.0572
Afipia	0.0089	0.0028	3.1809	6.9247	0.3763
Anoxybacillus	0.0089	0.0019	4.7801	6.7629	0.125
Chloroplast	0.0082	0.0063	1.2976	17.6878	13.3776
Massilia	0.0077	0.0073	1.0499	0.25	6.0133
Bradyrhizobium	0.0076	0.0039	1.9705	6.5971	2.081
Streptococcus	0.0072	0.0039	1.8292	6.0383	11.1007
Sphingomonas	0.0072	0.0041	1.7262	1.0524	5.9154
***Leiopathes glaberrima* VK826 vs GC140**	contribution	sd	ratio	av.a	av.b
Pseudoalteromonas	0.0081	0.0064	1.2581	9.5952	4.0586
Tumebacillus	0.0078	0.0064	1.2158	1.7569	8.2022
Propionibacterium	0.0075	0.0059	1.2667	11.1455	15.7164

No Relative	0.0068	0.0057	1.1858	10.8014	12.0572
Moraxella	0.0061	0.0031	1.9545	0.2895	5.4666
Streptococcus	0.0056	0.0043	1.3113	7.9362	11.1007
Methylobacterium	0.0056	0.0037	1.51	3.7966	6.0306
Massilia	0.0055	0.0058	0.945	2.0772	6.0133
unknown Corynebacteriaceae	0.0052	0.0042	1.2609	6.4645	9.5281
Rothia	0.0052	0.0039	1.3524	5.0789	2.6045
Leiopathes glaberrima* vs *Lophelia pertusa	contribution	sd	ratio	av.a	av.b
Anaplasma	0.0244	0.0115	2.1129	15.1609	3.0904
Mycoplasma	0.0237	0.0168	1.4107	0	10.659
Bradyrhizobium	0.0150	0.0029	5.2321	7.2769	0
Halomonas	0.0132	0.002	6.6159	7.0819	0.5606
Afipia	0.0131	0.0023	5.6992	6.2994	0
Candidatus Pelagibacter	0.0127	0.0049	2.5825	0	6.5226
Endozoicomonas	0.0110	0.0074	1.4941	12.5228	7.0926
Anoxybacillus	0.0102	0.0034	3.007	5.3114	0.4387
Tumebacillus	0.0092	0.0020	4.6657	4.4294	0
Geobacillus	0.0083	0.0057	1.4654	5.2535	1.4489
Leiopathes glaberrima* vs *Sibopathes spp.	contribution	sd	ratio	av.a	av.b
Propionibacterium	0.0139	0.0119	1.1755	10.9742	3.2994
Pseudoalteromonas	0.0123	0.0107	1.1447	9.4735	3.1505
Staphylococcus	0.0117	0.0094	1.2524	10.0843	3.523
Bacillus	0.0098	0.0063	1.5521	8.2918	2.7851
Streptococcus	0.0097	0.0083	1.1696	7.9329	2.5374
Anaplasma	0.0094	0.0131	0.7186	5.8682	1.6448
Corynebacterium	0.0092	0.0071	1.2919	8.4658	3.5173

Spirochaeta	0.0090	0.0047	1.9053	0	4.6338
Vibrio	0.0088	0.0088	1.0054	6.3005	2.0473
Rothia	0.0080	0.0070	1.1476	5.0791	0.6448
Paramuricea biscaya* vs *P. B3	contribution	sd	ratio	av.a	av.b
Kistimonas	0.0267	0.0090	2.982	1.6264	16.2162
Propionibacterium	0.0216	0.0100	2.158	15.7023	3.8296
Streptococcus	0.0171	0.0086	1.9987	12.1946	2.678
Staphylococcus	0.0148	0.0071	2.0817	11.3169	3.2053
Rothia	0.0136	0.0068	1.9982	8.2203	0.9391
Haemophilus	0.0110	0.0055	2.0032	6.403	0.3333
Endozoicomonas	0.0104	0.0081	1.2888	12.2411	18.0173
Neisseria	0.0101	0.0040	2.5419	5.9806	0.3333
Anaerococcus	0.0099	0.0043	2.2708	6.4029	0.8049
Actinomyces	0.0099	0.0039	2.5534	5.496	0

Table 1.5: Absolute and relative abundance of a single *Endozoicomonas* OTU found in every sample for *L. glaberrima,* water filter, and sediment samples

Sample Name	Quality-controlled Reads	Proportion of library represented by OTU	Proportion of SILVA-assigned *Endozoicomonas* spp. represented by OTU
VK826 White 1 (676)	443,044	0.0006	0.1695
VK826 White 2 (677)	526,142	0.0040	0.7173
VK826 White 3 (655)	369,010	0.0515	0.8425
VK826 White 4 (671)	303,305	0.0361	0.9911
VK826 Red 1 (645)	441,949	0.2252	0.5277
VK826 Red 2 (667)	430,047	0.0624	0.9601
VK826 Red 3 (674)	315,763	0.0359	0.9382

VK826 Red 4 (675)	382,114	0.0056	0.4036
VK906 White 1 (546)	50,350	0.0869	0.9104
VK906 White 2 (560)	435,320	0.1773	0.9830
VK906 Red 1 (528)	471,193	0.0165	0.7893
VK906 Red 2 (533)	500,217	0.0169	0.1651
GC140 White 1 (472)	468,757	0.0633	0.6744
GC140 White 2 (485)	423,405	0.1022	0.4439
GC140 White 3 (503)	627,984	0.0301	0.3734
GC140 White 4 (510)	331,519	0.1605	0.3844
GC140 Red 1 (475)	327,885	0.0685	0.8132
GC140 Red 2 (478)	449,063	0.0556	0.9907
GC140 Red 3 (484)	327,891	0.0270	0.5621
GC140 Red 4 (489)	159,363	0.1409	0.9539
VK906 Water	143,875	0.0007	0.9099
VK906 Sediment	85,756	0.0019	0.6880
AT357 Water	88,613	0.0010	0.72
AT357 Sediment	150,607	0.0050	0.9282
MC751 Water	127,326	0.0004	0.8571
MC751 Sediment	158,522	0.0033	0.9452
GC234 Water	149,715	0.0001	0.9231
GC234 Sediment	126,605	0.0022	0.9103
Red 1 Washed (VK826)	80,907	0.0015	0.0097

Red 1 Unwashed (VK826)	89,187	0.0608	1.0186
Red 2 Washed (VK826	93,909	0.0619	0.2579
Red 2 Unwashed (VK826)	62,454	0.0048	0.0271
Red 3 Washed (VK826)	105,644	0.0141	0.0540
Red 3 Unwashed (VK826)	85,530	0.4012	1.3604

Figure 1.1: Map of the Gulf of Mexico, showing coral collection sites (red triangles) and water/sediment collection sites (yellow circles).

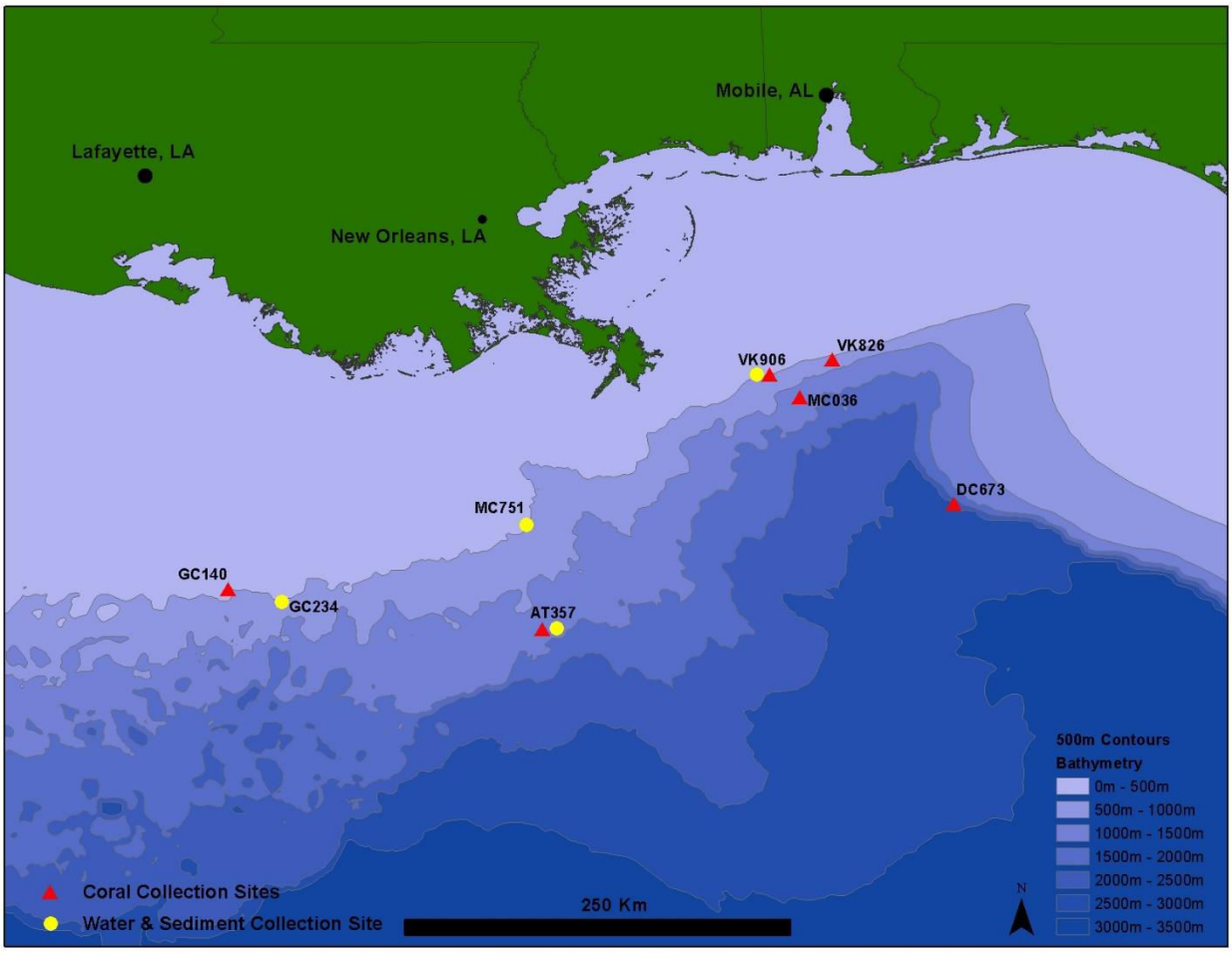

Figure 1.2: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for *Leiopathes glaberrima* in the Gulf of Mexico, grouped by coral color. Each point is a library, and polygons cover the smallest area encompassing all points in a group with straight lines. Stress=0.104.

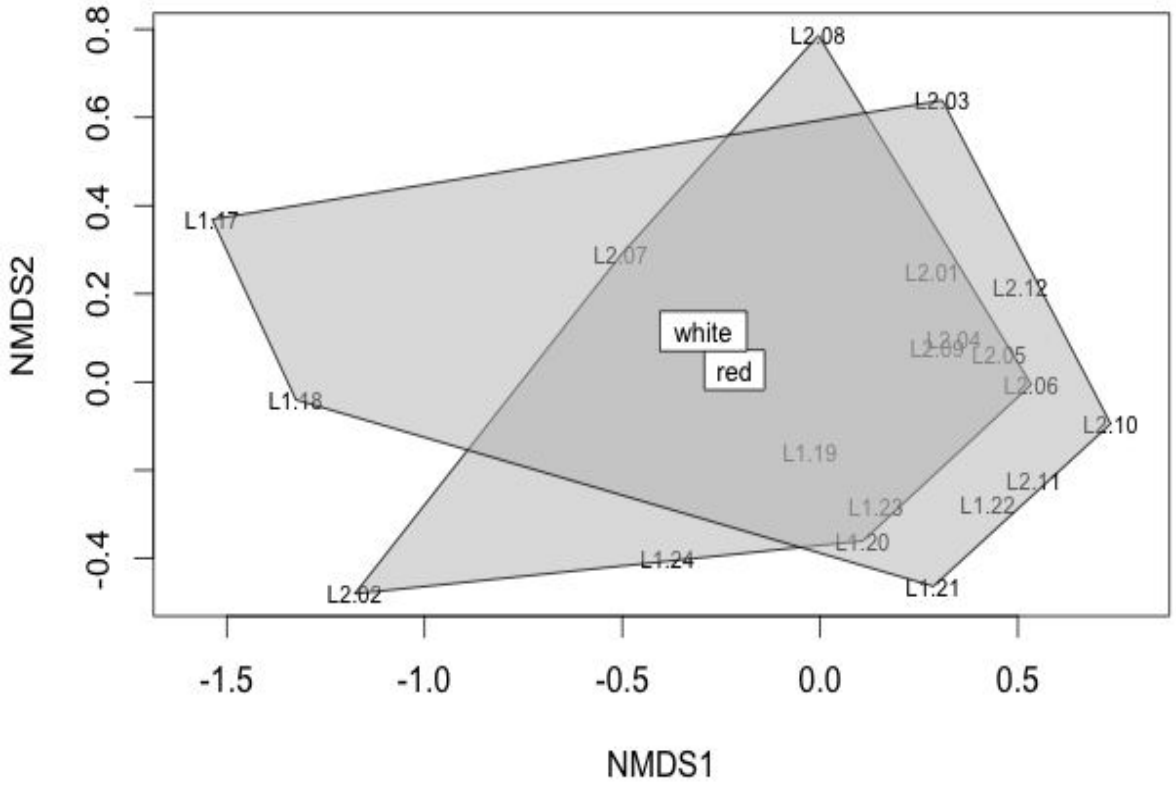

Figure 1.3: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for *Leiopathes glaberrima* in the Gulf of Mexico, grouped by site. Each point is a library generated from a distinct colony, and polygons cover the smallest area encompassing all points in a group with straight lines. Stress=0.104.

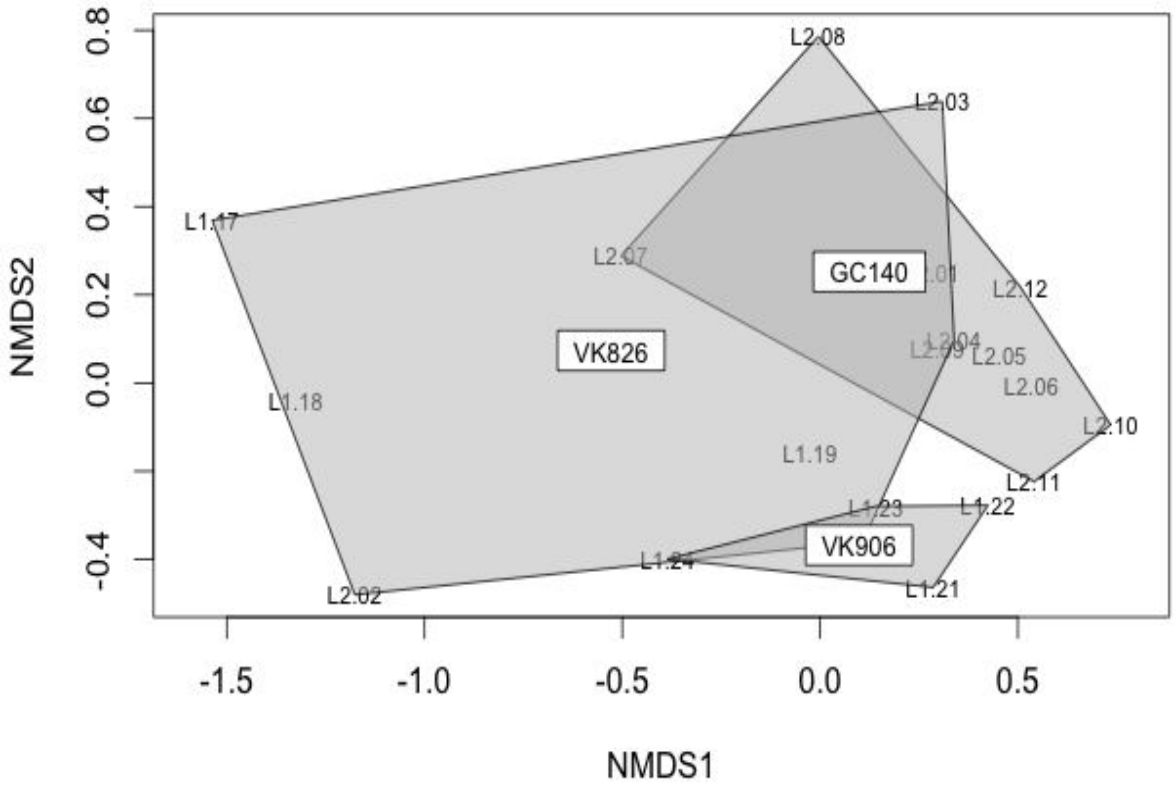

Figure 1.4: Phylum composition of bacterial OTUs by 16s library for field-collected *Leiopathes glaberrima* corals.

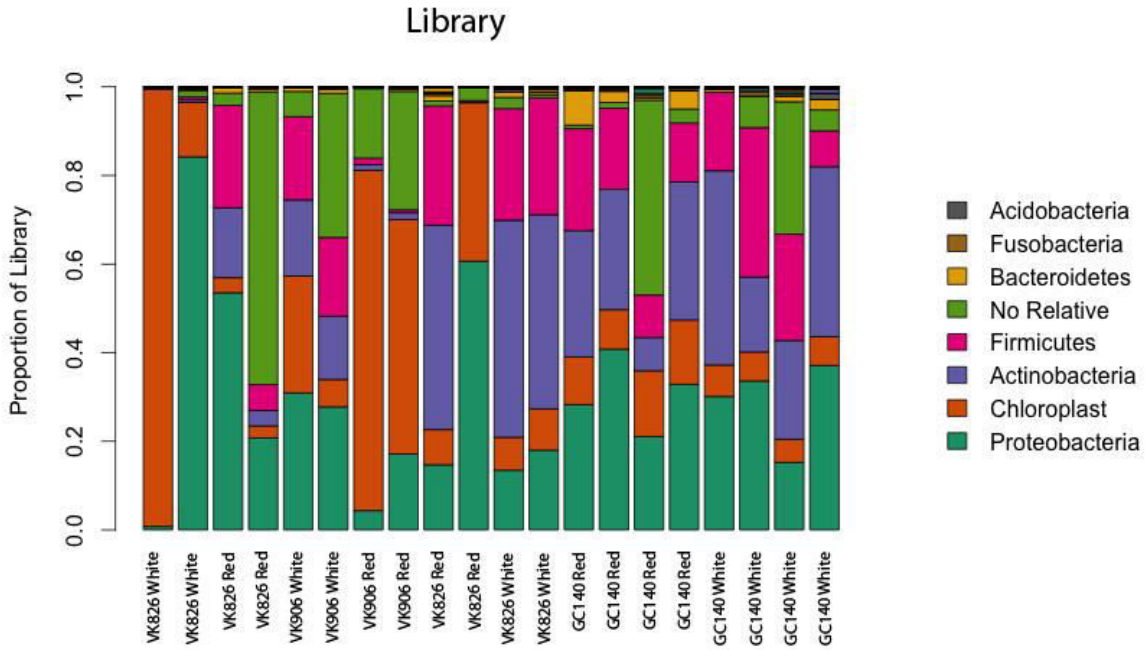

Figure 1.5: 16S bacterial phylum composition of *L. glaberrima* corals kept in a climate-controlled aquarium. “Washed” samples were rinsed vigorously in 70% ethanol followed by DI water. “Unwashed” samples were transferred directly from the tank to the DNA extraction kit.

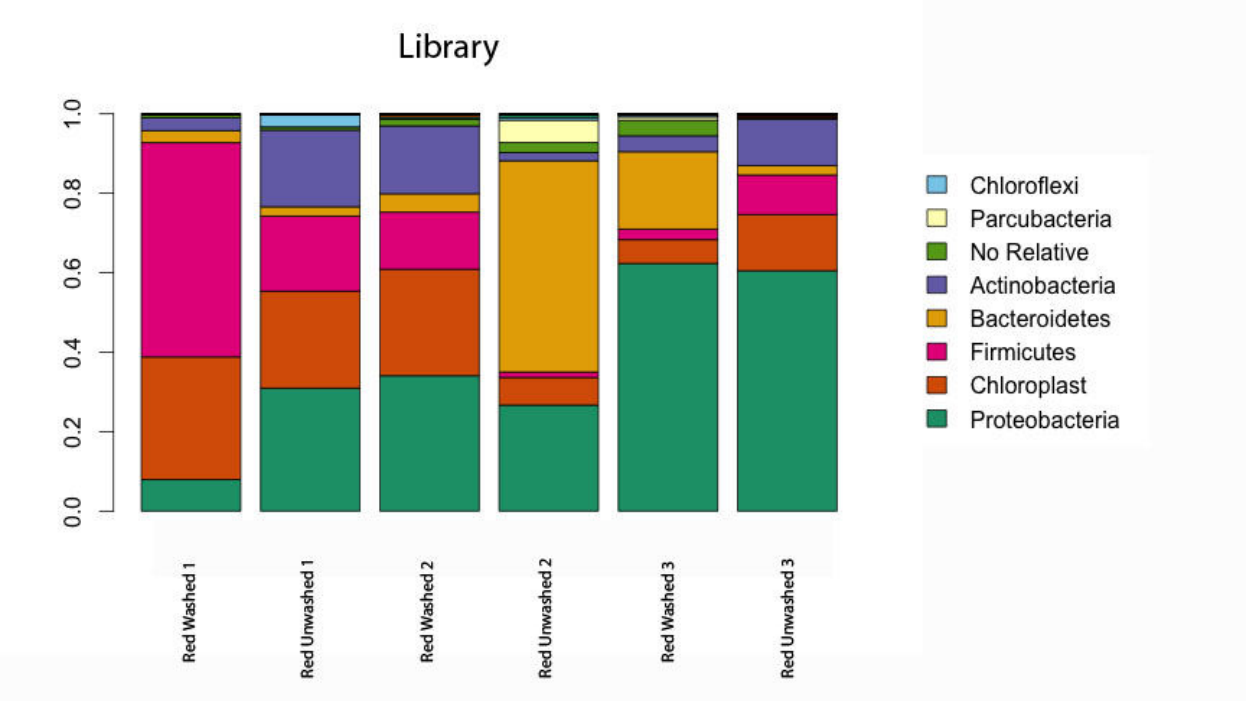

Figure 1.6: Phylum composition by 16S tag sequencing of water filter and sediment samples.

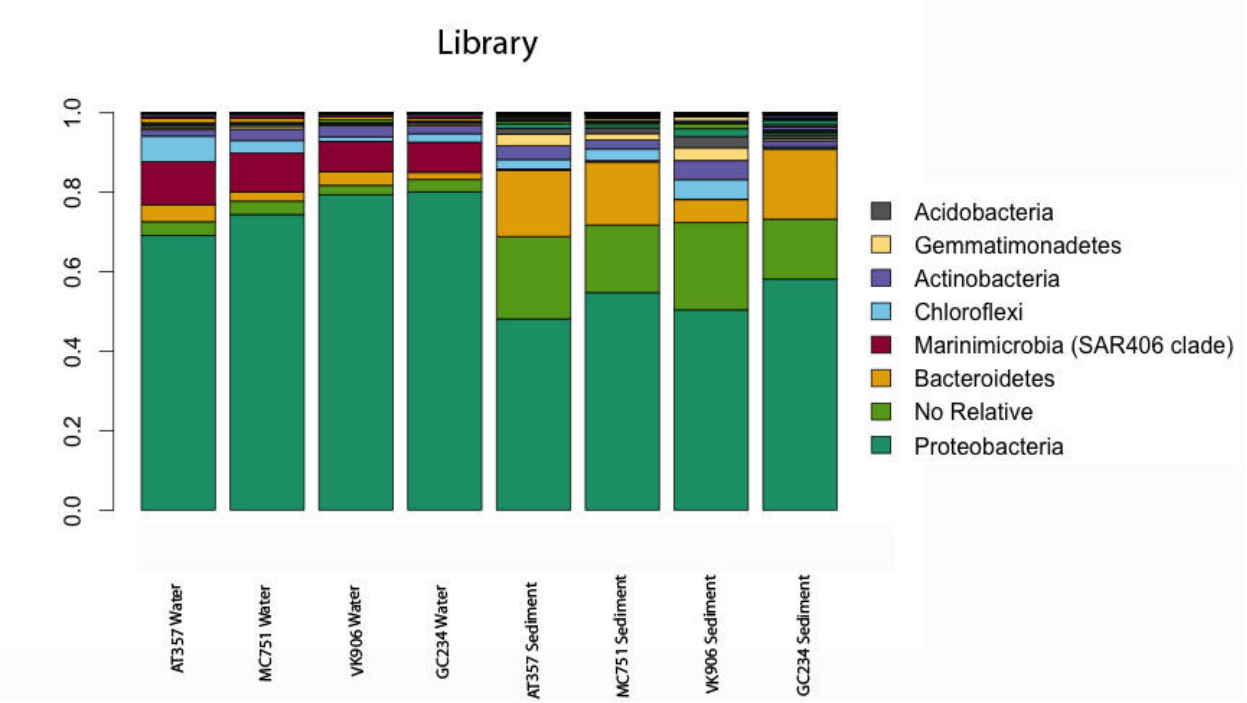

Figure 1.7: Phylum composition by 16S tag sequencing of *Paramuricea* spp. corals. Each bar is a library. Samples from DC673 were *P. biscaya* and are labeled "DC," samples from MC036 were *P. biscaya* and are labeled "MC," and samples from AT357 were *P. B3* and are labeled "AT."

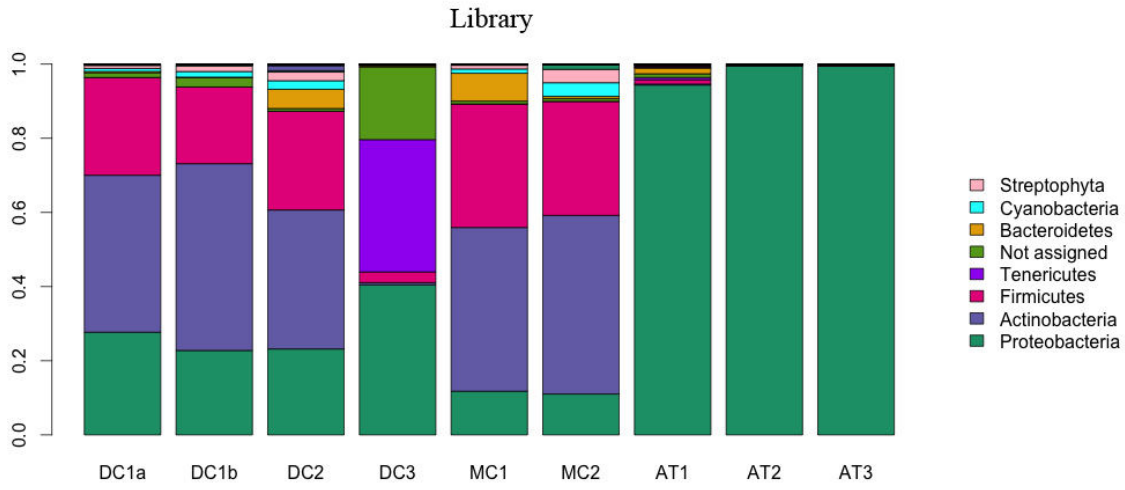

Figure 1.8: Phylum composition by 16S tag sequencing of *Lophelia pertusa* and *Sibopathes* spp. corals. Each bar is a library. *L. pertusa* samples are from VK906, and *S.* spp. samples are from VK826.

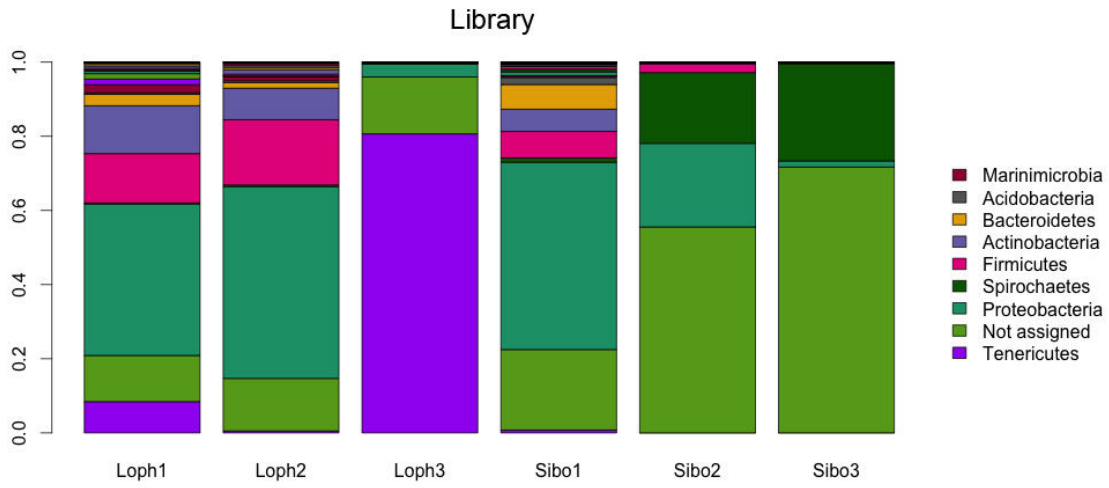

Figure 1.9: Cluster dendrogram of fourth root-transformed community-by-genus matrices for all coral species, water, and sediment samples. AU stands for Approximately Unbiased p-value, and BP stands for Bootstrap Probability value. Clusters with AU higher than 95 are deemed significant and highlighted with red rectangles. Each sample is either *Leiopathes glaberrima* (Leio), *Paramuricea biscaya* (ParaMC and ParaDC), *Paramuricea B3* (ParaAT), *Sibopathes* spp. (Sibo), or *Lophelia pertusa* (Loph). Sites are MC036 (MC), DC673 (DC), AT357 (AT), VK826 (826), VK906 (906 or VK), or GC140 (140 or GC). *L. glaberrima* are also designated red (R) or white (W). "Water" refers to water filter samples, and "Sed" refers to sediment samples. Additional numbers are colony designations; ParaDC1a and ParaDC1b were two libraries from the same colony. The dendrogram was calculated using Ward's method of hierarchical clustering with 10,000 bootstrap replicates.

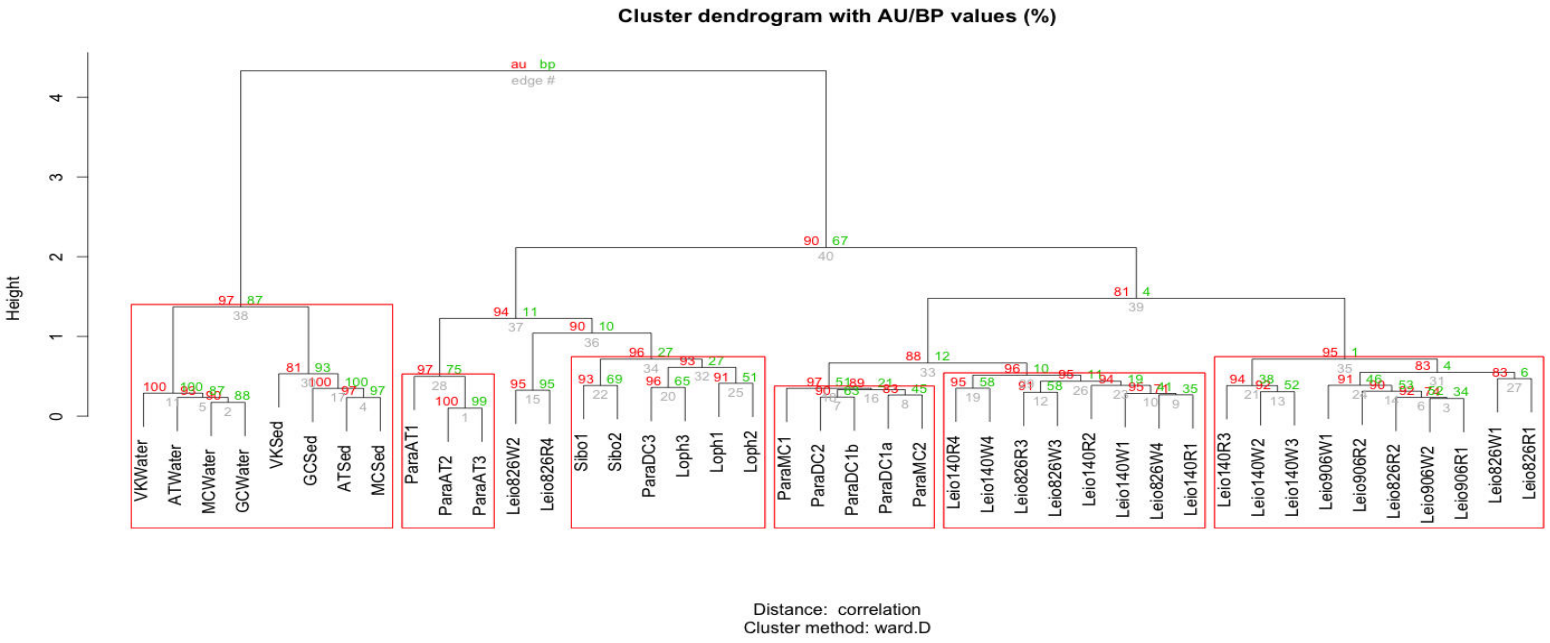

Figure 1.10: Cluster dendrogram of bacterial gene expression for red vs white *L. glaberrima* corals. AU stands for Approximately Unbiased p-value, and BP stands for Bootstrap Probability value. Clusters with AU higher than 95 are deemed significant and highlighted with red rectangles. However, none were significant here. The dendrogram was calculated with Ward's method of hierarchical clustering with 10,000 bootstrap replicates.

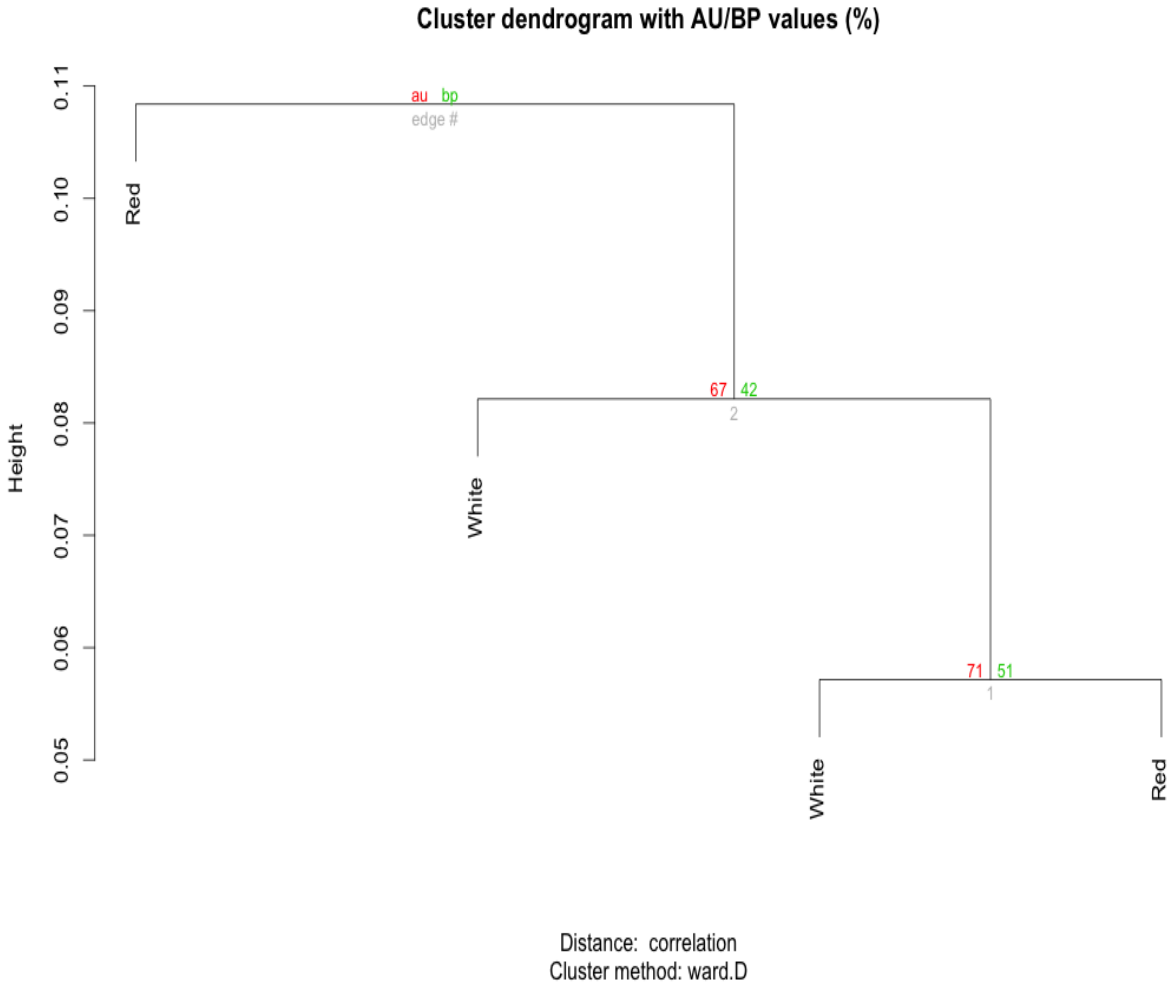

Figure 2.1: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for oil vs control treatment groups from the 2012 experiment. Points with the same number are fragments from the same colony, and the letters refer to either oil or control treatments. Each point is a library, and points with the same number are fragments from the same colony. Oil treatments are labeled "O" and control "C." Colonies 1 and 4 are red, and 2 and 3 are white. Stress=0.092.

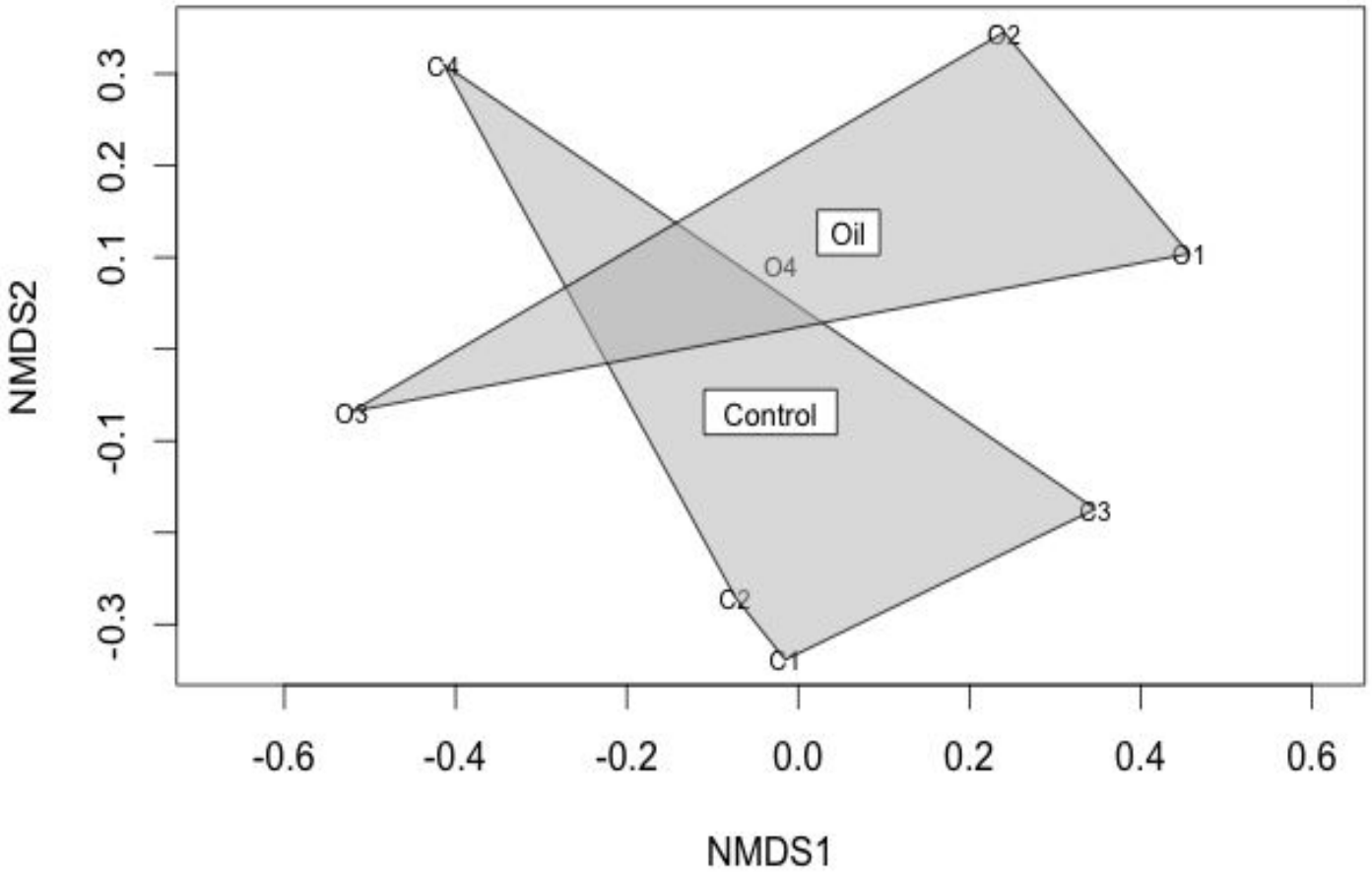

Figure 2.2: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for oil, dispersed oil, and control treatment groups from the 2012 experiment. Points with the same number are fragments from the same colony, and the labels refer to oil, dispersed oil, and control treatments. Each point is a library, and points with the same number are fragments from the same colony. Oil treatments are labeled "O," dispersed oil "OD," and control "C." Colonies 1 and 4 are red, and 2 and 3 are white. Stress=0.128.

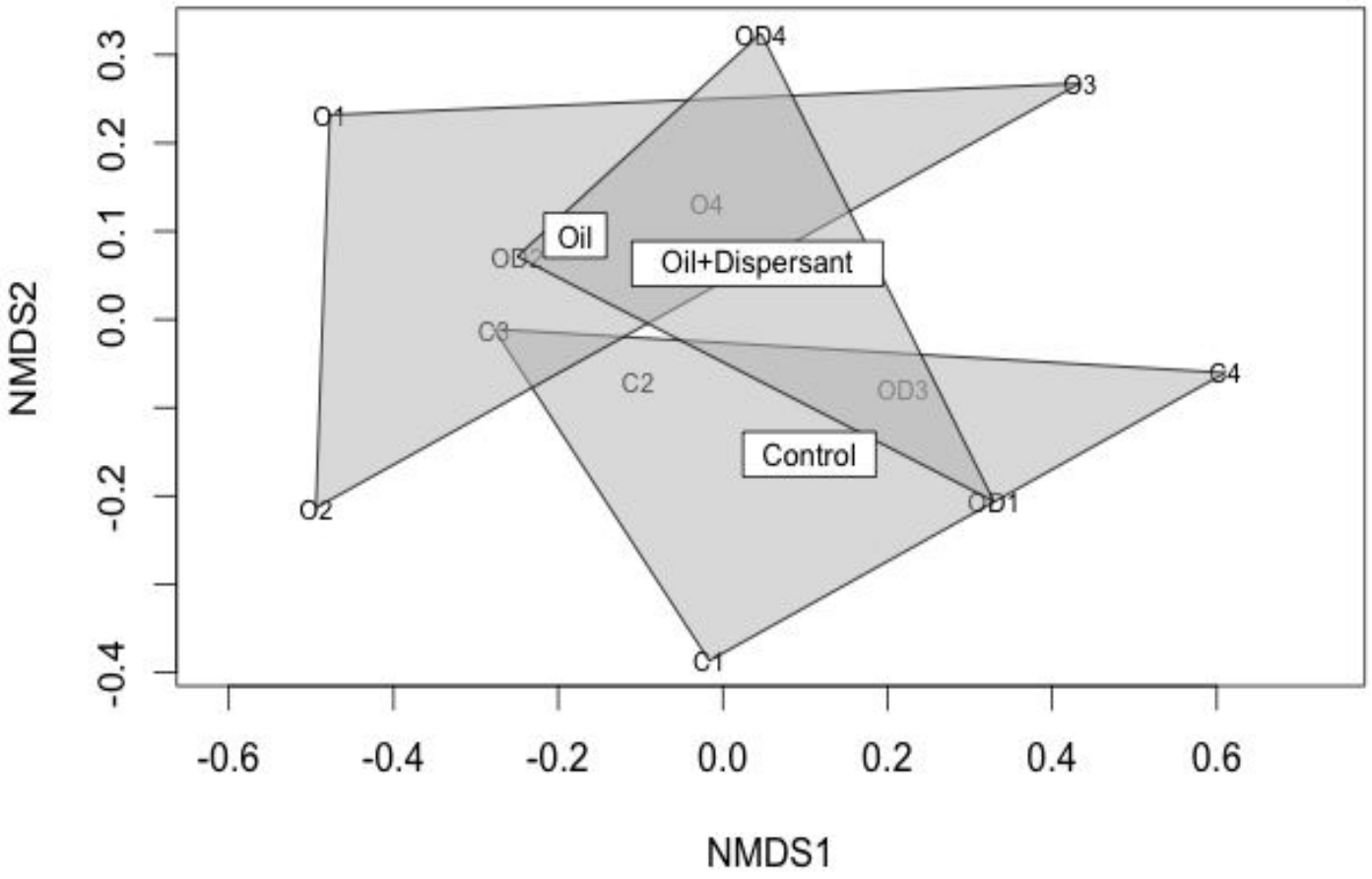

Figure 2.3: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for oil vs control treatment groups from the 2013 experiment. Points with the same number are fragments from the same colony, and the letters refer to either oil or control treatments. Each point is a library, and points with the same number are fragments from the same colony. Oil treatments are labeled "O" and control "C." The C4 and O1 points were considered outliers and were removed. All colonies are red. Stress=0.017.

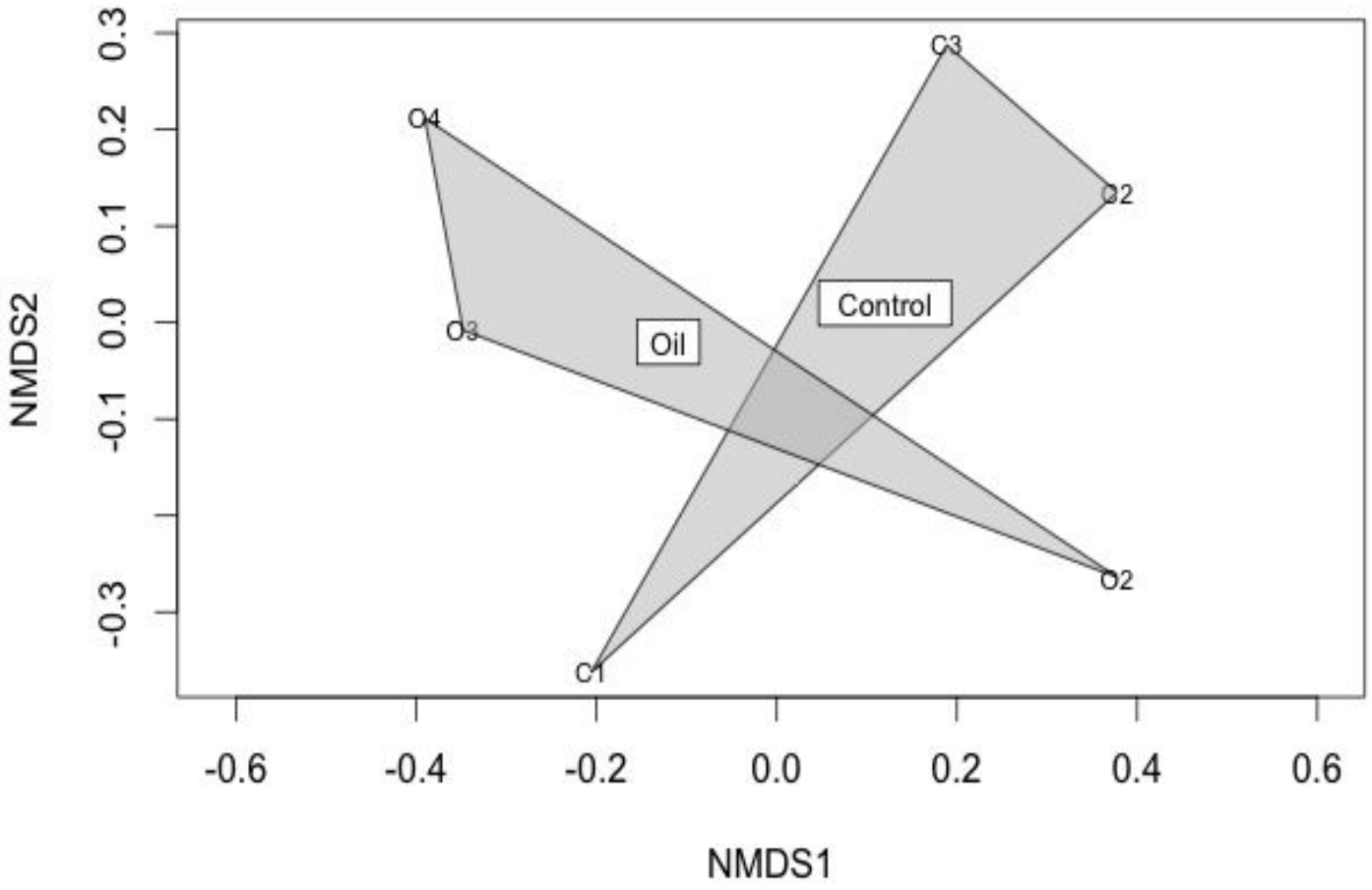

Figure 2.4: Non-metric multidimensional scaling (NMDS) plot of bacterial community composition for oil vs control treatment groups from the 2014 experiment. Points with the same number are fragments from the same colony, and the letters refer to either oil or control treatments. Each point is a library, and points with the same number are fragments from the same colony. Oil treatments are labeled "O" and control "C." Colonies 1-3 are red, and 4-6 are white. Stress=0.141.

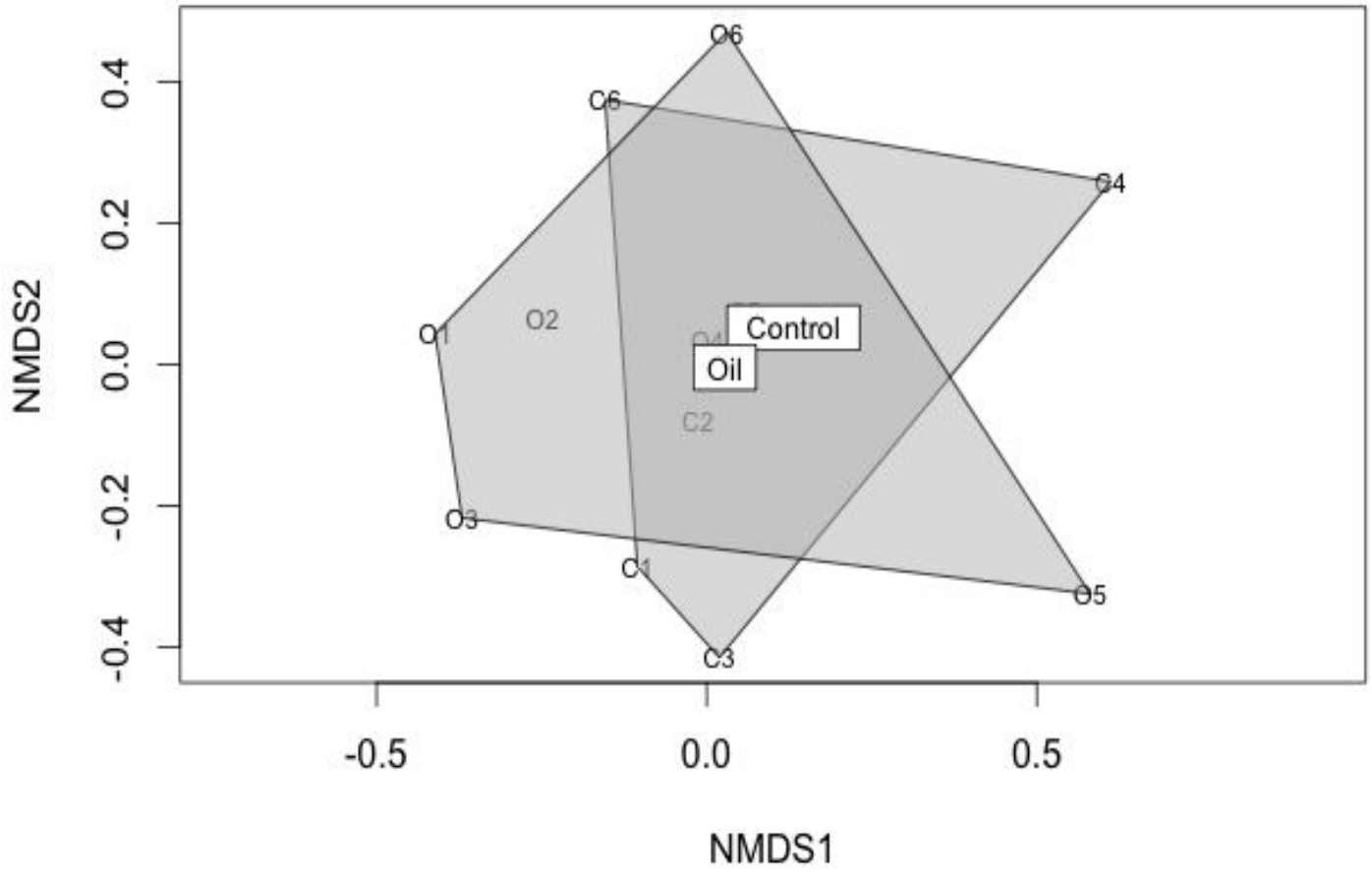

Figure 2.5: Heatmap of the top 75 most differentially expressed genes between oil and control groups. Each column is a fragment, and fragments with the same color and number are from the same colony.

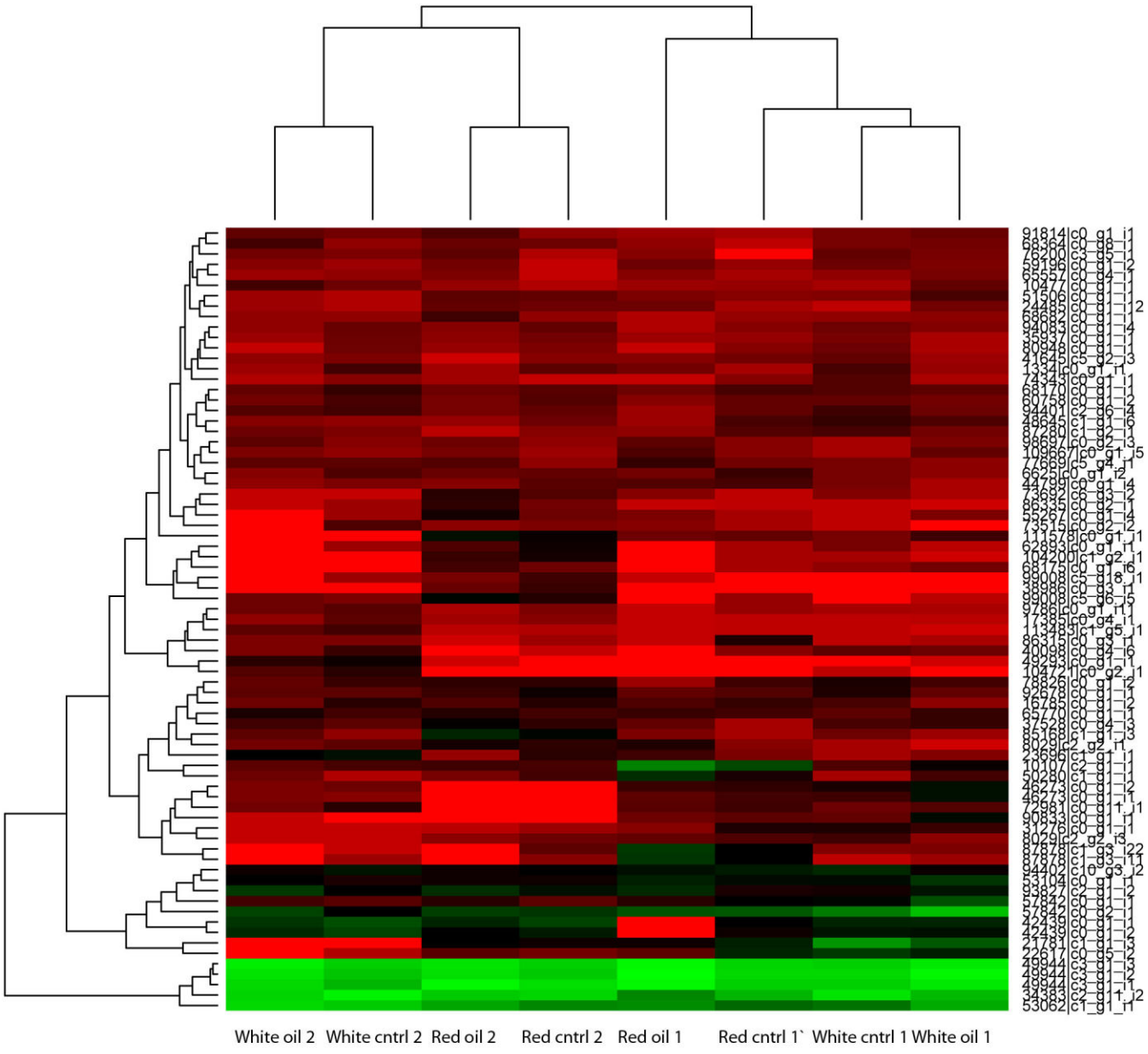

Figure 2.6: Community composition in the 2012 experiment by phylum. Each bar is a library constructed from a single coral fragment exposed to oil (O), dispersed oil (OD), or artificial seawater control (C). Samples with the same number are from the same colony. Colonies 1 and 4 are red, and 2 and 3 are white.

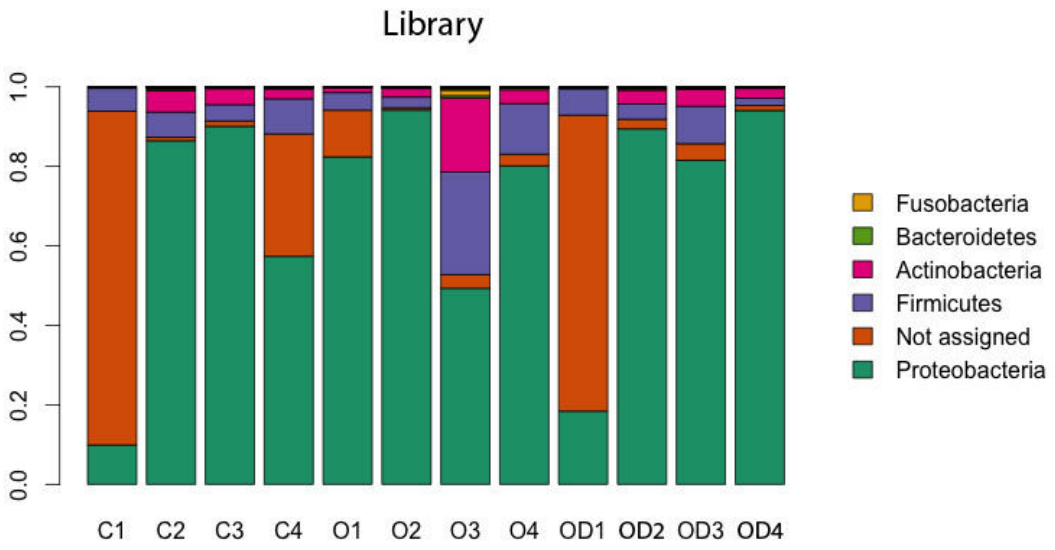

Figure 2.7: Community composition in the 2013 experiment by phylum. Each bar is a library constructed from a single coral fragment exposed to oil (O) or artificial seawater control (C). Samples with the same number are from the same colony. All 4 colonies are red.

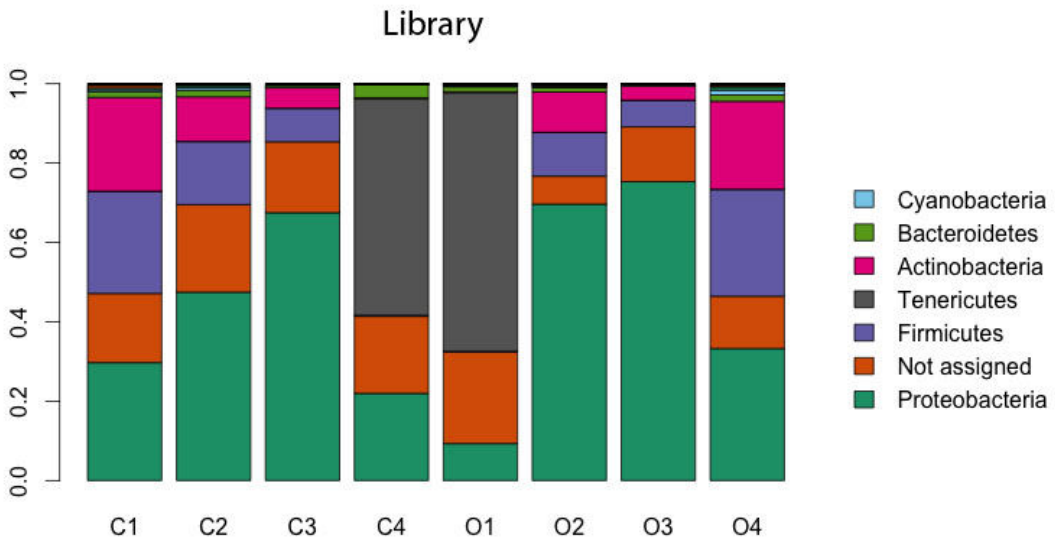

Figure 2.8: Community composition in the 2014 experiment by phylum. Each bar is a library constructed from a single coral fragment exposed to oil (O) or artificial seawater control (C). Samples with the same number are from the same colony. Colonies 1-3 are red, and 4-6 are white.