Shifts in Microbial Communities Based Upon Transect Habitat

By

Logan Greydanus

A PROJECT

Submitted to
Oregon State University
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the requirements for the
degree of

Honors Baccalaureate of Science in Microbiology
(Honors Scholar)

Presented May 30, 2014
Commencement June, 2014

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Abstract Approved:

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Coral reef health has been in severe decline around the globe in the past several decades, in many cases due to direct human impact. Human action, such as overfishing, habitat destruction and nutrient loading, has caused coral coverage to drop to record lows, threatening the future of these critically important biodiversity hotspots. This study investigates the changes in microbial community along reef transects on the South Pacific island of Moorea. Samples were collected at three distinct habitats along reef transects: Fringing Reef, Back Reef, and Cresting Reef. Reef habitats were compared between three regions around the island: North, East and West. Results indicate that there is a significant difference in Bacterial and Archaeal (16S) species between the inner fringing reef habitat and the outer Cresting and Back reef habitats. This difference can consistently be seen across all regions sampled around Moorea. Comparison of the 16S communities based solely on region resulted in no significant difference, suggesting that the primary driver of microbial diversity is location on transect and not region. Based on these results, it appears the Bacterial and Archaeal communities are defined by the reef habitat they belong to and that these communities are not unique to region.

Keywords: Bacteria, marine microbiome, coral reefs, DNA sequencing, 16S
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APPROVED**:**

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Mentor, representing Microbiology

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Committee Member, representing Microbiology

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Committee Member, representing Microbiology

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Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Logan Greydanus, Author

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Introduction:

Coral reefs represent the most diverse marine ecosystems on the planet. Although they make up a small percentage of the total marine habitat, the diversity of life and number of ecological roles filled is tremendous. Humans derive great benefit from reefs, as they provide shoreline protection, food, tourism and even some medicines. Coral reefs are in severe decline across the globe, in many cases due to human triggered events [17].

Corals live in a symbiotic relationship with several species of single-celled algae in the genus *Symbiodinium* [1]. These corals are the primary reef forming coral found in nature. Within each coral polyp resides a single celled algae, commonly referred to as "zooxanthellae". These algae provide oxygen and other nutrients that are used by the coral. In turn, the coral host provides a habitat and nutrients essential for *Symbiodinium* growth, namely carbon dioxide (CO_2). This symbiotic relationship is central to the reef ecosystem and its disruption often leads to reef decline. Loss of *Symbiodinium* from corals is referred to as bleaching. Although seasonal, bleaching can also be triggered by environmental stressors (i.e. increased water temperature) and results in decreased coral resilience and fitness [5]. Large bleaching events triggered by stressors are strongly associated with coral mortality [17]. The microbial community associated with coral reefs also plays a large role in shaping unique reef habitats. Corals live in oligotrophic waters and rely heavily upon microbes to respire and cycle nutrients [5]. Thus, the microbial community likely has dramatic effects upon the reef ecosystem health. Following is

a brief overview of several of the key accepted causes of coral reef decline and coral bleaching [15].

The Role of Nutrients:

Human proximity to coral reefs almost invariably leads to an increase in ambient seawater nutrient levels [5]. These increased nutrient levels are caused in part by large scale fertilization processes and find their way into the sea by a variety of methods, most notable being runoff from rivers and aeolian dust [3]. While a wide variety of both organic and inorganic nutrients play a role in altering coral habitat, the most commonly addressed are nitrogen and phosphorus. In the ocean, nitrogen is found most often as dissolved inorganic nitrogen (DIN: NH_4, NO_2, NO_3) while phosphorus is found as phosphate (PO_4^{3-}). Hence, this discussion will focus heavily upon these two inorganic nutrients, while not discrediting the role played by others. A direct link between increased nutrient levels and coral reef decline has been hard to establish in the past [2]. Nutrients, by definition being essential to coral and *Symbiodinium* growth, can often have positive effects on coral growth. In other cases, no change is observed, or results are difficult to make out. Recent studies have focused on differentiating between the direct and indirect effects of nutrient exposure, a method that can provide useful insights. Direct effects refer to those that cause a measurable change on coral physiology. Indirect effects are provoked by nutrient driven processes outside the coral [5]. By separating these two

categories, the results of nutrient exposure on corals can be more accurately measured.

Direct Effects:

In similar fashion to the overall effects of nutrient exposure, the direct effects are not entirely agreed upon as positive or detrimental. While some evidence suggests that nutrient exposure may increase coral growth [6], there are also many studies that show increased nutrients have a large negative effect. These negative effects include reduced reproductive success, increased calcification rates and increased sensitivity to light and heat stress. This last effect has recently been conclusively demonstrated [18] and represents a large challenge as the effects of climate change continue to increase in marine ecosystems. Increased nutrients also have direct effects on *Symbiodinium* growth. High levels of *Symbiodinium* caused by spikes in nutrient levels have been shown to increase the likelihood of bleaching in coral species, once again resulting in decreased reef fitness [4]. This bleaching is thought to be controlled levels of Reactive Oxygen Species (ROS) produced by the coral. Levels of ROS may also be affected by increased nutrient levels, which may increase the likelihood of bleaching. Finally, direct physiological effects on the coral skeleton can be observed due to nutrient exposure. Increases in phosphorus levels can lead to rapid rates of coral growth that comes at the expense of skeletal density [6]. This causes the corals to become more brittle and susceptible to mechanical damage.

Indirect effects:

Indirect effects encompass a broader scope and are once again difficult to classify as either positive or negative. Increased nutrient concentrations cause increases in particulate food for corals [16]. Nutrients also dramatically alter the algae levels in seawater, which can provide shielding effects from the sun, creating a cooler environment. But this increase in the levels of macro-algae can also have severe negative effects on coral health. In many places, macro-algal blooms caused by increased nutrient concentration create mats that temporarily cover corals, preventing photosynthesis. This competitive inhibition increases the likelihood of bleaching and is strongly correlated with coral mortality [16]. Nutrient exposure can also affect the growth of reef animals that in turn have an effect on coral health. Nymphs of the corallivorous starfish *Acanthaster planci* can rapidly increase in nutrient rich environments. When these nymphs reach adulthood, the mature starfish can have severe detrimental effects upon the reef [8]. In addition to this, increased plankton levels, as a result of increased nutrient concentrations, can lead to the proliferation of filter feeders and bioeroders that compete for space with corals and weaken reef structural integrity. Finally, nutrient enrichment has been shown to increase the incidence of disease in corals. This may be the result of both physiological or pathogenic diseases and will be discussed later in depth.

Nutrient effects are not localized. Plankton populations often quickly take up newly introduced inorganic nutrients [9]. Although this would seem to limit the range and effects of applicable nutrient enrichment, it often aids in propagating the effects. Heavy nutrient loads are associated with sedimentary runoff from rivers.

The sediment in the water can act to shield phytoplankton from sunlight, decreasing the rate at which they remove nutrients from the water. This can result in nutrient blooms having far-reaching spatial effects, ranging up to 50km from their point of origin. In addition to this, the increased algal blooms can have secondary effects on coral reef health. Large phytoplankton blooms can result in both increased levels of detrimental waste products and nutrient limitations. Phosphorus limitation is often observed after large algal blooms. This can lead to primary production limitation that can affect the benthic coral community.

Nutrient Loading and Coral Disease:

As discussed before, one of the many effects nutrient exposure may have on coral is increased susceptibility to disease. Although the link between nutrient exposure and disease prevalence has been drawn with other animal species, such as fishes and amphibians, conclusive results are hard to obtain for coral data due to confounding factors, such as salinity and temperature. Vega-Thurber et. al. conclusively established the link between nutrient exposure and disease prevalence in their 2013 paper. In their experiment, coral reef plots were exposed to nutrients over a three-year period and then compared to controls to establish a link.

Coral reefs are in decline due to a combination of many factors, of which nutrient exposure is deemed to be the fourth most impactful [11]. Although it is not definitively known whether disease is due to physiological or pathogenic stress, disease seems to be a significant result of nutrient exposure. In the Vega-Thurber

experiment, the Florida Keys were selected as test site due to their high incidence of coral disease. Often referred to as the 'hotspot of coral disease' [12], the Caribbean reefs are threatened by factors including temperature stress, alteration of fish abundance, high coastal human populations, proximity to algae blooms, and nutrient availability. Several previous studies have established an increase in both disease and bleaching in corals exposed to nutrients, but these results are often hard to decouple from confounding factors. After measuring disease prevalence in 8 3x3 plots over the course of 3 years, Vega-Thurber et. al. were able to conclusively link nutrient exposure to disease prevalence.

Dark Spot Syndrome (DSS) was the most common disease witnessed after exposure to nutrients [17]. DSS is a broad label applied to diseased corals exhibiting dark blue/purple/black bands or spots on sections of their tissue. As with most coral diseases, it is unclear whether DSS is a physiological disease or spread by a pathogen. Because of this, speculation on its epidemiology and pathogenesis (if any) is limited. Nutrient loading may affect coral health in several ways. First, the nutrients may have a direct effect upon the coral host physiology. This could be the result of effects on unknown host pathways or uncharacterized sensitivities to certain nutrients. Secondly, a similar physiological effect may act upon the algal and microbial symbionts of the host coral. Third, either the coral or the associated symbionts may become affected by an unknown pathogen or pathogens. Fourth, competition between coral holobionts and other benthic species, such as algae, may trigger the disease. Finally, a combination of one or more of these effects may be the true cause of disease [17]. If the cause of DSS truly is a pathogen, nutrient loading

could facilitate its spread via aiding pathogen entry, evasion of host defenses or activation of virulence factors. Although is unlikely that nutrient loading directly participates in spreading a putative pathogen, it is possible that nutrient loading participates in decreasing coral resistance, making it more susceptible to infection [17].

Nutrient loading not only increases the prevalence of disease among coral species, it also increases the levels of bleaching. Although bleaching is a natural process that occurs seasonally, Vega-Thurber et. al. witnessed increased levels of bleaching in the offseason (June). The levels of bleaching were below those of normal seasonal events, but the fact that they took place outside of the normal season (July-September) suggests that these bleaching events were the result of coral exposure to nutrients. Seasonal bleaching is mainly driven by an increase in water temperature due seasonal shifts [17]. In the case of the nutrient enrichment, nutrient exposure may result in a decrease in the threshold temperature required for bleaching, making it a more common event. Increased levels of bleaching may also be due to increased Symbiodinium levels present due to removal of nutrient limitations. The production of ROS, as mentioned before, accompanies these spikes in *Symbiodinium* population and may contribute to the levels of bleaching [14].

Based on the results obtained from their experiments conducted in the Florida Keys, Vega-Thurber et. al. were able to conclusively link exposure to nutrient loading with an increase in both coral DSS and bleaching.

Coral Reef Habitats:

Coral reefs are found in tropical and subtropical waters and can usually be divided into a distinct set of zones that vary based upon coast type, temperature, geographic location and a variety of other factors. Despite the range of diversity that can be present, a "typical" reef can be divided into a few major zones [1]. The Fringing Reef is the zone closest to shore and is often part of the mainland or island shelf. Separating the Fringing Reef from the Barrier Reef habitats is a Lagoon that is characterized by calm waters. This area often has a sandy bottom with very few corals. The barrier reef can be divided into several distinct zones [1]. The Fore-Reef is the farthest from land and is subject to the highest wave forces. The Reef Crest is the highest point on the barrier reef and is also subject to strong wave forces. Often referred to as an algae ridge, this zone can become dominated by species of red algae and is often exposed during low tide. The Back Reef is the sloping zone that connects the outer barrier reef with the lagoon. This is a shallow section (< 5 m) of the reef that can be subjected to large changes in temperature and salinity. These distinct reef zones can vary dramatically in size, from a few meters to several kilometers [1]. Coral reef habitats also possess unique compositions of corals, algae and animals. These differences may lead to change in microbial community as well.

Miseq High Throughput Sequencing:

Although the majority of sequencing has traditionally been conducted on 454 sequencing machines, lately Illumina's MiSeq and HiSeq technologies have become increasingly popular. Both technologies provide several key advantages, with MiSeq being cheaper and offering slightly longer reads. Illumina-based sequencing strategies currently generate the largest amount of sequence reads per dollar. In addition to this, previous issues, such as poor performance with low genetic diversity samples, have been improved [13]. Miseq technology uses a chip-based amplification step in order to increase the amount of DNA present. DNA templates are ligated to the chip via adapters and anneal to other adapters that can be used as primers, forming bridges for amplification. Following this, sequencing occurs via synthesis using a reversible terminator dye nucleotide. The reversible terminator nucleotide is washed over the chip, incorporated into the growing DNA strand opposite its complementary base, terminates synthesis, and is read via fluorescence. The terminator portion of the dye is then removed, allowing further incorporation and sequencing to occur.

Materials and Methods:

Water Sampling:

Water samples were collected from the island of Moorea which is located in the South Pacific (Figure 1). Sampling was conducted during the dry season in August 2013. Moorea is a high basaltic island that is surrounded by a fringing reef

that encircles the island at distances between 500 m to 1500 m. Reef formation is roughly uniform around the island and can be divided into the following categories; a Fringing Reef near the shore, a shallow lagoon, a Back Reef, a Reef Crest and an ocean facing Fore-Reef **(Figure 1)**. The reef portion of these zones ranges from less than 1m to 3m deep while the lagoon portion ranges from 1-6m deep.

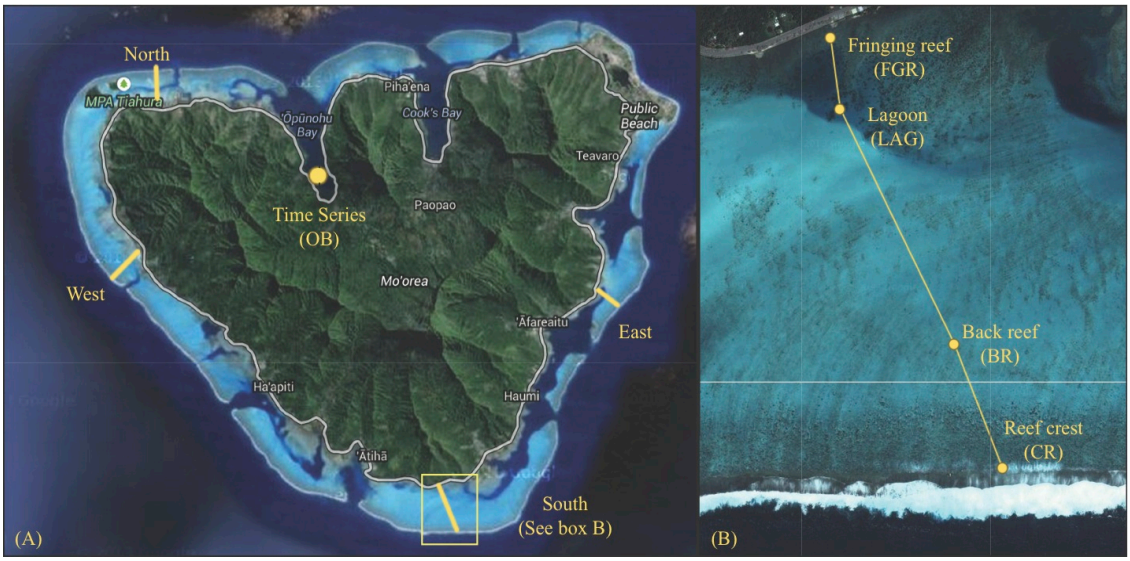

Figure 1: Moorea. On the left is an image of the island of Moorea including the location of each reef transect sampled. The right image provides a schematic of a single reef transect.

Samples were collected along 3 transects located on the north, south, east, and west of the island. In these cross-reef transects, seawater was collected at four different reef habitats moving from offshore to inshore: Reef Crest (RC), Back Reef (BR), Lagoon (LAG), and Fringing Reef (FR). Samples were collected in clean HDPE Nalgene bottles. Water samples were collected from the top 1 m of the water column.

DNA Extraction:

Seawater samples were filtered through a sequence of decreasing filter sizes to concentrate DNA for later extraction. Samples were first pre-filtered through a 5 μm Nitex filter to exclude larger particles and then run through 0.22 μm Sterivex (Millipore) filters to concentrate microbial size particles. . The Sterivex filters were stored at -80^{0}C until DNA extraction. DNA extraction was completed from all Strerivex filters using commercial DNA PowerWater extraction kits available from MoBio.

Polymerase Chain Reaction (PCR) Amplification:

PCR amplification of 16S rRNA gene was carried out using DNA extracts from all Sterivex filters using the eubacterial specific primers GTGCCAGCMGCCGCGGTAA-Forward and GGACTACHVGGGTWTCTAAT-Reverse, which yield ~500 bp. Primers were previously modified to include 8 nt barcodes, 10-nt pad sequence and 2-nt linker sequence. Amplification was preformed in triplicate for each sample along with a negative control. Therefore, four PCR reactions were carried out for each sample. Amounts and concentrations of PCR reagents can be found in Table 1. Reaction volume for each PCR reaction was 25 μl. Unique barcodes were attached to each PCR primer. Each primer consists of the following elements: the appropriate Illumina adapter, an 8-nt index sequence, a 10-nt pad sequence, a 2-nt linker and the

gene specific primer. Primers targeted the V4 region of the bacterial 16S gene. For a complete list of barcodes, see Appendix A.

Table 1: PCR Reagent Concentrations and Volumes

Reagent	Stock Concentration	Desired concentration	μl per rxn
Buffer	5x	1x	5.0
$MgCl_2$	25mM	2 mM	2.0
dNTPs	10mM	0.2 mM	0.5
Forward Primer	10μ	0.5 μM	1.3
Reverse Primer	10μM	0.5 μM	1.3
H_2O	-	-	13.8
taq Polymerase	500 μl-1	1.5 U ul-1	0.2
Template	-		1.0

Agarose Gel Electrophoresis:

PCR products were run on agarose gels to confirm success of the amplification. For each sample, 1 μl of amplicon was mixed with 4 μl of 6x loading buffer and was subsequently electrophoresed in agarose gel. Agarose gels were made by mixing 0.5g of agarose with 100ml of 1 x TAE buffer and adding 5 μl of gel red. Gels were run for ~30 minutes at 90 volts and PCR bands were photographed on a UV transilluminator with a video image processor.

Pooling and cleaning:

PCR products were first pooled together by sample. All three triplicates of each sample were combined and placed in a sterile 96 well plate. Samples were cleaned once using magnetic beads (AMPure XP by Agencourt) at beads-to-DNA ratios of 0.7 to remove PCR products smaller than 300 bp from the library. This step was preformed robotically in the Center for Genome Research and Biocomputing (CGRB). Only 35 μl of each sample was cleaned and used for subsequent steps. The other ~35 μl of each amplicon was left in reserve.

Using a Quibit® Fluorometer, the amount of DNA present in each sample was detected. The Quibit assay was carried out using two standards. Standards were made by combining 10 μl of Quibit standard solution (1 or 2) with 190 μl of master mix solution. A master mix solution was made by combining 199 μl of Quibit buffer solution with 1 μl of Quibit dye. Sample volumes were tested between 1 and 5 μl. After experimentation, it was found 2 μl was the most useful volume; therefore it was used for all subsequent assays. Thus, 2μl of sample was combined with 198μl of master mix solution.

All PCR products were then combined in equimolar amounts in one pool. This pool was then used for sequencing.

Sequencing on Miseq Platform and Analysis:

Sequencing was conducted using the Illumina MiSeq technology. Pooled amplicons were submitted to the CGRB facility. For a detailed discussion of the MiSeq technology, see the introduction. Sequence data were processed using QIIME (Quantitative Insights into Microbial Ecology). QIIME is a bioinformatics pipeline that was used to compare and summarize bacterial ass-uh between treatments (Region and Location on Reef Transect) and within samples. Operational taxonomic unit (OTU) tables were filtered to exclude host mitochondrial and chloroplast sequences before community analysis was undertaken. Statistical analysis was conducted using PRIMER 6. Bray-Curtis dissimilarity matrices of log(x+1) transformed data were used to analyze the community differences between different reef habitats. MDS plots were generated for both the comparisons of habitats and regions. 1-Way ANOVA tests were used to test differences between communities based upon region and habitat.

Results:

Analysis focused upon the transect data available for the north, south and west transects around the island of Moorea. Bacterial communities were analyzed by region in order to determine if there was a difference between the North, West and East transects. Next, comparison was preformed between regions based on habitat (Cresting, Back, Fringing Reef) in order to determine if the bacterial

communities changed along a transect. Finally, an individual transect, West, was analyzed by reef habitat. By analyzing a single transect, more sites, such as mangrove and mid-reef, could be included. These sites were excluded from the general site analysis due to lack of replicates in some transects.

Comparison between regions:

Data from the three regions (North, East, West) around Moorea was analyzed in order to determine if a significant difference exists between the 16S communities by region. In this analysis, only samples taken from the Cresting Reef, Back Reef and Fringing Reef sites were used in order to maintain consistency. QIIME was used to generate taxa summaries at the family level (**Figure 2**). Looking at Figure 2, it appears as if there is a difference in the "West Region", while the North and East Regions appear similar at the family level. This difference is not significant however. Statistical analysis of data by regions was conducted using Primer 6. Analysis was preformed at both the "Class" and "Genus" levels in order to correct for possible biases present in the QIIME pipeline at low taxonomic levels. Using a 1-Way ANOSIM statistical test, it was determined that there are no significant differences in the bacterial communities based on region

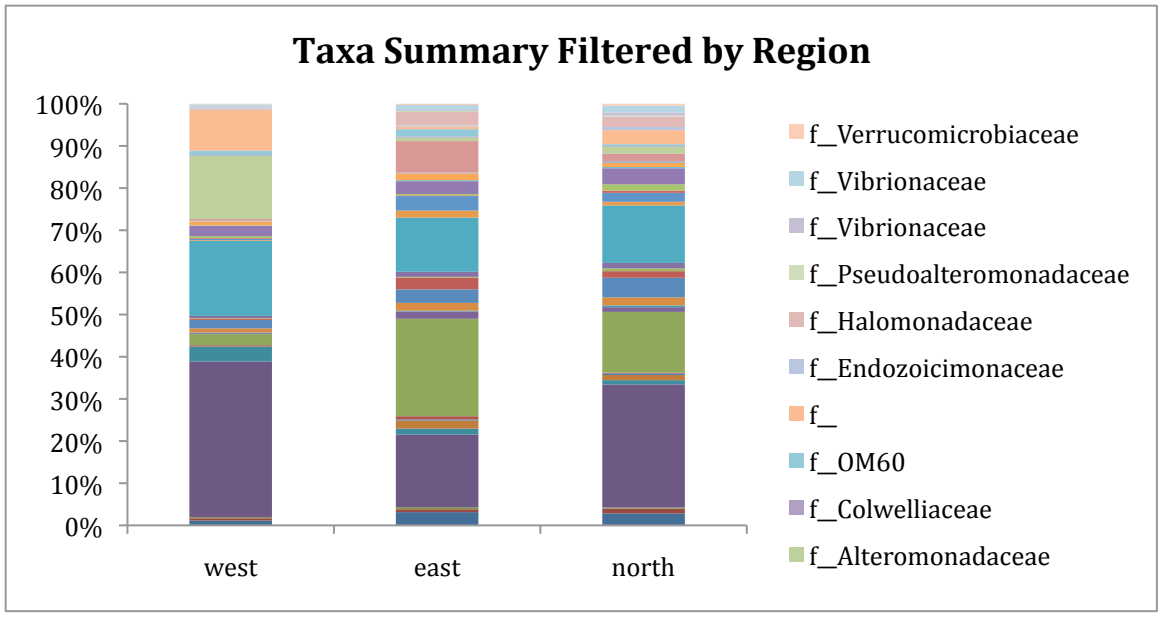

Figure 2: Taxa Summary Filtered by Region at the Family Level. No definitive conclusions can be draw from these plots, they are only meant to display trends.

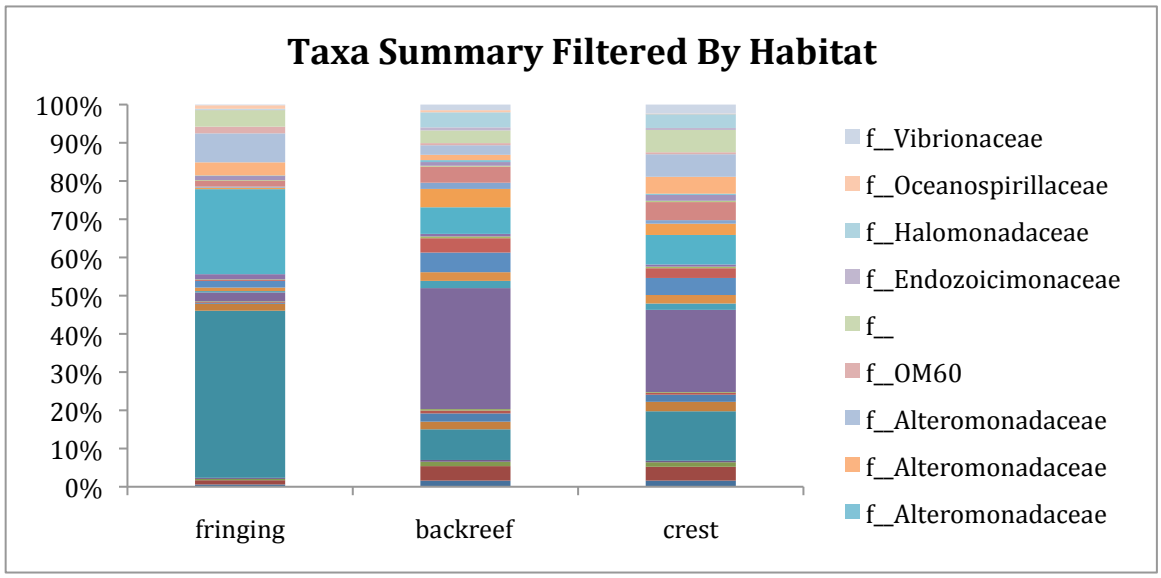

Figure 3: Taxa Summary Filtered by Reef Habitat at the Family Level. No definitive conclusions can be draw from these plots. Note similarity between the cresting and back reef transects and the relative difference in to the fringing samples.

at the class or genus level. The significance level between the north and east regions was 0.314 while between north and west it was 0.457. The two regions with the most difference were East and West, but the difference still was not significant with a significance level of 0.20. ANOSIM analysis at the genus level produced similar results with no significant differences present. Species totals and species evenness (Pielou's evenness) were consistent between regions, with an average species total of 63.27 and an average species evenness of 0.439 for all regions and similar values for each individual region.

Comparison between Sites:

Comparison between sites based on region resulted in a significant difference between barrier reef sites (Cresting and Back Reef) versus inner reef sites (Fringing Reef). This significant difference was seen across all regions analyzed off the island of Moorea. QIIME was used to generate OTU tables and taxa summaries at the family level **(Figure 3)**. Figure 3 shows a distinct difference in the Fringing Reef site, while the Back and Cresting Reef sites appear similar at the family level. The Fringing Reef sample seems to have a less diverse microbial community that is dominated by *Bacteridotes* (40%) and *Proteobacteria* (53%). A 1-Way ANOSIM test was used to test whether the differences present in the communities were significant or not. In order to compare the inner and barrier reef communities against one

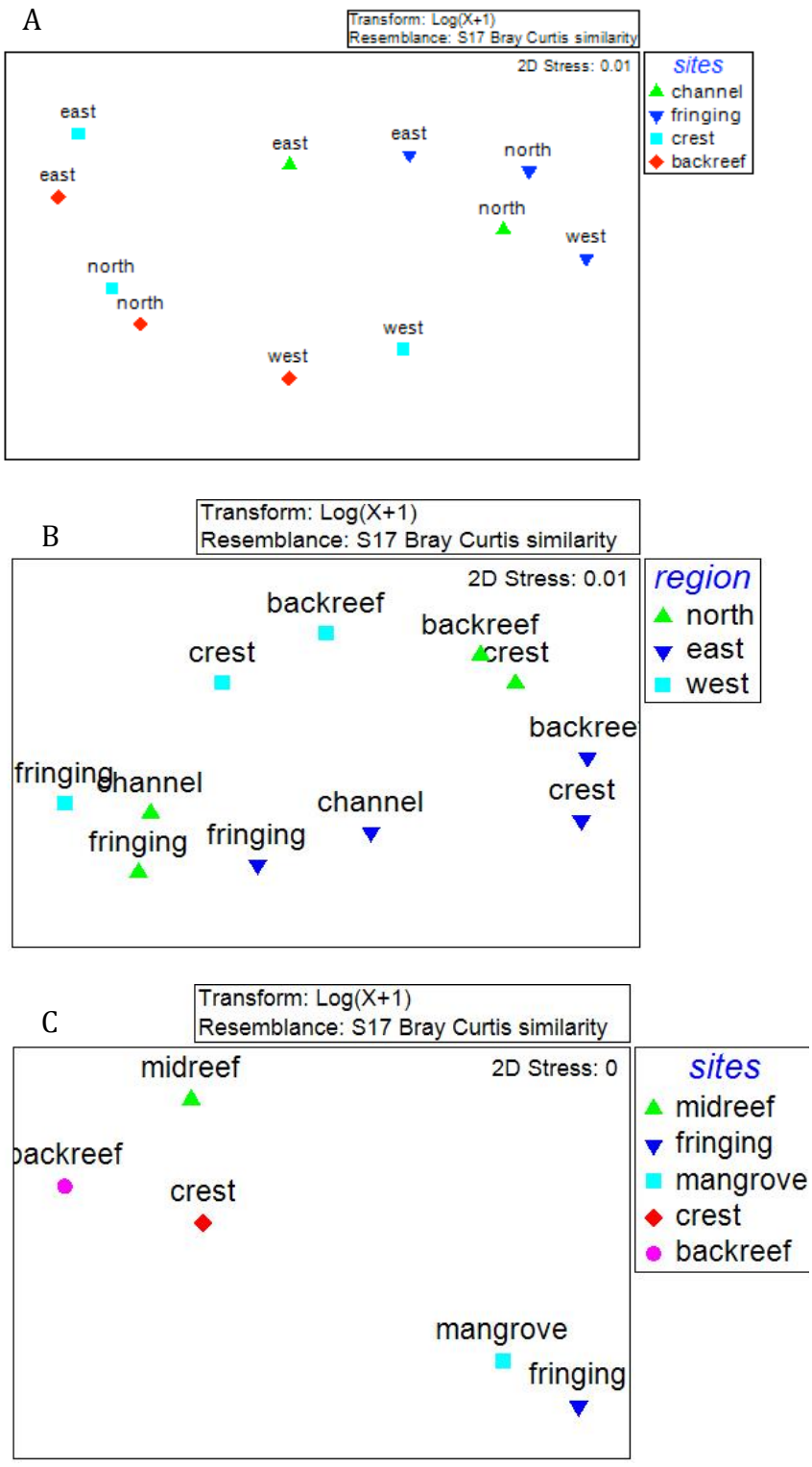

Figure 4: MDS Plots. A) MDS plot of 16S communities based upon region. B) MDS plot of communities based upon reef habitat. C) MDS plot of communities within a single reef transect, Transect West.

another, Cresting Reef and Back Reef samples were binned together. Channel samples were included in order to have a third category to compare. While there was no significant difference between the Channel and Fringing Reef samples ($p=0.60$) or the Channel and Outer Reef Samples ($p=0.107$), the ANOSIM test did produce a significant difference between the Inner and Barrier Reef Communities ($p=0.012$). These same results were replicated at the Genus level, with a significant difference between the Inner and Barrier Reef samples ($p= 0.012$). These results are further supported by an MDS plot of Cresting, Back and Fringing Reef communities (**Figure 4B)**. Based on this plot, it is clear to see that the Fringing Reef communities are significantly different than both the cresting and back reef communities. Furthermore, it appears that the Back Reef and Cresting communities are similar to one another and match up well based upon region. The communities had an average total species count of 269.75 and an average Pielou's evenness score of 0.561 at the genus level. Based upon a SIMPR test, the top three species driving the difference between Inner and Barrier Reef sites are a species of *Flavobacteria*, a species of *Prochlorococcus* and a species of *Rhodobacteria*. The Inner and Barrier Reef Communities had an average dissimilarity of 61.52%. The *Flavobacteria* and *Rhodobacteria* were more abundant in the Inner Reef Communities while the *Prochlorococcus* was most abundant in the Barrier Reef Communities.

Top Five Taxa Present at Each Site

The top five genera present in each habitat were compared with one another to observe how the representative taxa changed. The five genera compared were *Cryomorphaceae*, *Prochlorococcus*, *Rhodobacteraceae*, *Oceanospirllales* and *Glaciecola*. Figure 5 shows a comparison of these five genera based on relative abundance (%) between Cresting, Back and Fringing Reef samples **(Figure 5)**. Looking at Figure 5, it appears the dominant species of bacteria is different between the inner and barrier reef habitats. While *Cryomorphacea* seems to be the dominant species in the Fringing Reef habitat, *Proclorococcus* is the dominant species in the Cresting and Back Reef communities. Differences between genera were compared using a 1-way ANOVA test. All test were run on raw data except for the *Glaciecola* genus, which required a $\log_{10}$ transformation. The relative abundance of *Cryomorphaceae* and *Rhodobacteraceae* genera were statistically significant based upon habitat ($p=0.009$ and $p=0.001$, respectively). Other samples did not show a statistically significant difference. Cryomorphaceae abundance was significantly different between the Fringing and Back Reef habitats ($p= 0.013$) and the Fringing and Cresting Reef Habitats ($p=0.018$). There was no difference between Cresting and Back Reef habitats however ($p=0.939$). *Rhodobacteraceae* samples followed the exact same trend, with differences between Fringing-Back Reef ($p=0.001$) and Fringing-Cresting Reef ($p=0.001$), but no difference between Cresting-Back Reef ($p=0.193$). It is interesting to note that *Prochlorococcus* did not have a

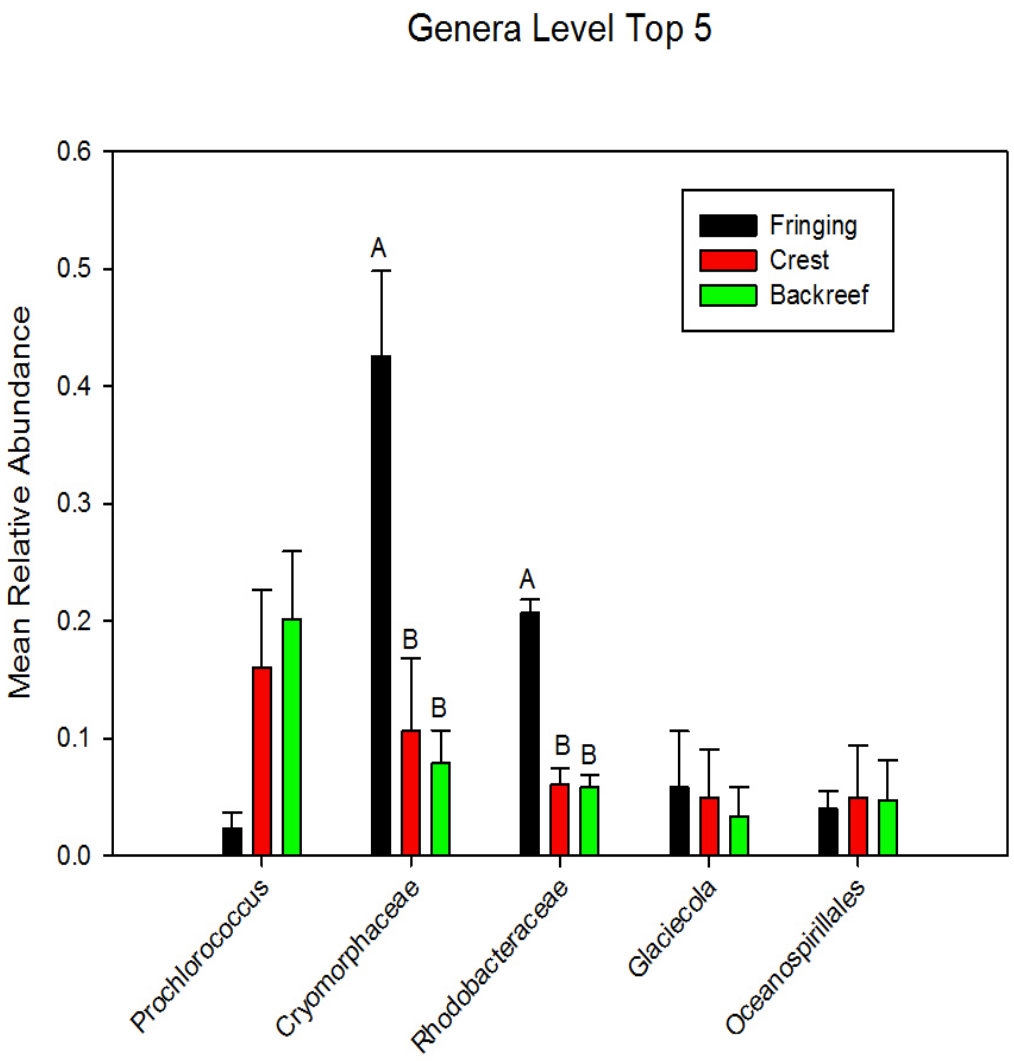

Figure 5: Comparison of the 5 most abundant taxa by reef habitat. Notice the significant difference present between in the *Cryomorphaceae* and *Rhodobacteraceae* communities.

statistically significant difference between any habitats, contrary to what can be observed in Figure 5. The large standard errors present in these samples may have led to the report of no significant difference. Repeating this analysis at the Family level led to exactly similar results.

Analysis of Single Reef Transect:

In order to investigate the diversity by sites within a region, the West transect was analyzed individually. An MDS plot was generated using the Cresting, Back and Fringing reef samples, along with Mangrove and Mid-reef samples that were excluded from previous analysis due to lack of replication in other regions. As can be seen in the MDS plot, it appears the Mangrove and Fringing Reef samples share similarity while the Cresting, Back and Mid Reef communities share similarity (**Figure 4C)**. Furthermore, the barrier reef sites (Cresting, Back and Mid reef) share 74.71% taxonomic similarity with one another based upon a SIMPR analysis. This suggests that there is a distinct difference in the Outer reef 16S communities when compared to Fringing Reef and Lagoon communities.

Discussion:

Analysis of transect data shows that there is a significant difference between the Cresting-Back Reef and Fringing Reef Communities. This difference is present at both the phylum and genus levels and at all regions sampled. It is also present in an analysis of the five most abundant taxa in each habitat. It is therefore reasonable to conclude that the microbial community changes along a reef transect.

The fact that the two barrier reef sites, Cresting and Back Reef, match up closely with one another suggests that the difference between Inner and Outer reef communities is delineated by the Lagoon that separates them. Furthermore, the channel samples that were included in the habitat analysis show similarity to both

Barrier and Inner reef communities. While the Inner and Barrier Reef communities were distinctly different from one another, the Channel samples were not. This suggests that the microbial communities exist as a gradient, gradually changing along a transect based upon environmental conditions. It is also important to note that the Channel serves as a connection between the open ocean and the lagoon, and therefore is likely to have characteristics of both the Fringing and Barrier Reef microbial communities.

No significant difference in the 16S communities based upon region suggests that the habitat on the reef, and not the cartographic location, is the primary determinate of community diversity. This difference is most likely due to the spatial factors that are associated with the Fringing, Back, and Cresting Reef communities. The outer Cresting and Back Reef communities receive a constant influx of nutrients and biodiversity from the open ocean. This can have profound effects and influence the community composition based on both top down and bottom up effects. The inner reef community is subject to a variety of nutrient conditions originating in a large part from land. Factors such as rainfall, agricultural runoff, and human byproducts can have a large effect on the close-in fringing reef.

Analysis of the top five taxa present at the genus and family level also shows that there is a significant difference between Fringing and Barrier Reef communities. Based solely on comparing the five most abundant taxa, it seems that the *Cryomorphaceae* and *Rhodobacteraceae* genera are the primary drivers of this difference. This same trend can be seen in the results of a SIMPER tests, which lists both these genera as the primary drivers of difference between Fringing-Cresting

and Fringing-Back reef communities. It is therefore reasonable to conclude that the unique distribution of these two species of bacteria creates much of the diversity seen between the inner and barrier reef communities.

A variety of factors could contribute to creating unique habitats along reef transects. First, environmental factors, such as temperature, salinity and depth play a large role in determining the microbial composition of a particular site. It is also likely that temporal factors, such as lunar cycles, tides, and seasonal changes, affect the microbial communities of these habitats. Perhaps more importantly however, human derived factors may heavily influence the compositions of these shoreline habitats. The differences between the fringing reef habitat and other, farther offshore, reef habitats could be in part influenced by human factors such as nutrient loading or habitat destruction. The aim of this analysis was not to correlate changes in microbial community with nutrient or temporal factors. The fact that a significant difference exists along reef transects does not preclude the possibility however. The fringing reef may be more susceptible to man-made nutrient loads (the result of agricultural runoff, ect.) due to its proximity to the shore. These altered nutrient loads could dramatically alter habitats, creating conditions more favorable for new dominant species. Lack of nutrient data prevented conclusive answers for these questions in this study. Future studies should focus on how microbial communities change based upon nutrient levels and temporal cycles. Interesting questions could also be examined regarding the health state and diversity of corals along reef transects, perhaps correlating them with changes in the microbial community.

Conclusions:

Analysis of the Bacterial and Archaeal communities along reef transects revealed that there is a distinct difference between the outer Cresting-Back Reef habitats and the inshore Fringing Reef habitat. This difference is present at multiple locations around the island, suggesting a common trend among all reef transects around the island. No significant difference was observed in microbial community based upon region surveyed, supporting the hypothesis that the primary driver of microbial diversity is location along the reef transect and not the cartographic location. The genera *Cryomorphaceae* and *Rhodobacteraceae* seem to be the primary drivers of the difference between inner and barrier reef communities. These differences along reef transect may be the result of a number of natural and anthropogenic environmental factors, ranging from water temperature and salinity to elevated nutrient levels. Future studies should focus on how the microbial community changes based upon nutrient loads and temporal factors along a reef transect.

References:

1. Barnes, R.S.K. and R.N. Hughes. 1999. *An Introduction to Marine Ecology*; third edition. Oxford, UK: Blackwell Science Ltd. pp. 117-141. (for bit on reef zones)

2. Bell PR, Lapointe BE, Sherwood OA, Bedford BJ: **The use of delta N-15 in assessing sewage stress on coral reefs.** *Mar Pollut Bull* 2009, **58**:793-802

3. Brodie JE, Lewis S, Bainbridge Z, Mitchell A, Waterhouse J, Kroon F: **Target setting for pollutant discharge management of rivers in the Great Barrier Reef catchment area.** *Mar Freshwater Res* 2009, **60**: 1141-1149.

4. Cunning R, Baker AC: **Excess algal symbionts increase the susceptibility of reef corals to bleaching.** *Nat Clim Change* 2013, **3**:259-262.

5. D'Angelo, Cecilia, Wiedenmann, Jorg: **Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival.** Current Op in Environmental Sustainability. **7**:82-93

6. Dunn JG, Sammarco PW, LaFleur G Jr: **Effects of phosphates on growth and skeletal density in the scleractinian coral *Acropora muricata*: a controlled experimental approach.** *J Exp Mar Biol Ecol* 2012, **411**:34-44.

7. Fabricius KE, Okaji K, De'ath G: **Three lines of evidence to link outbreaks of the crown-of-thrones seastar *Acanthaster planci* to the release of larval food limitation.** *Coral Reefs* 2010, **29:**593-605.

8. Fabricus KE: **Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis.** Mar Pollut Bull 2005, **50**:125-146

9. Furnas M, Mitchell A, Skuza M, Brodie J: **In the other 90%: phytoplankton responses to enhanced nutrient availability in the Great Barrier Reef Lagoon.** *Mar Pollut Bull* 2005, **51**:253-265.

10. Garren, M. Azam, F. **New directions in coral reef microbial ecology.** *Enviro Microb* 2012, 14, 833-844

11. Halpern BS, Walbridge S, Selkoe KA *et. al.* (2008) **A global map of human impact on marine ecosystems**. *Science,* **319,** 948-952.

12. Harvell CD, Jordan-Dahlgern E, Merkel S *et. al.* (2007) **Coral disease, environmental drivers, and the balance between coral and microbial associates**. *Oceanography,* **20,** 172-195.

13. Kozich, James J. Westcott, Sarah L. Baxter, Nielson T. Highlander, Sarah K. Schloss, Patrick D: **Development of a Duel-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform.** Journal of Environmental Microbiology. 2013, doi: 10.1128

14. Lesser MP (1996) **Elevated temperatures and ultraviolet radiation cause oxidative and inhibit photosynthesis in symbiotic dinoflagellates**. *Limnology and Oceanography,* **41,** 271-283.

15. Payet, Jérôme P. McMinds, Ryan, Burkepile, Deron E. Vega Thurber, Rebecca L.**: Short-term spatiotemporal variability in viral dynamics and lytic activities in tropical reef waters.**

16. Schaffelke B, Klumpp DW: **Short-term nutrient pulses enhance growth and photosynthesis of the coral reef macroalga *Sargassum baccularia.*** *Mar Ecol Prog Ser* 1998, **170:**95-105.

17. Vega-thurber, RL, Burkepile, DE, Fuchs, C, Shantz, AA, Mcminds, R, Zaneveld, JR: **Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching.** Global Change Biology. 2013, doi: 10.111

18. Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP: **Nutrient enrichment can increase the susceptibility of reef corals to bleaching.** *Nat Clim Change* 2013, **3**:160-164.

Appendix

Appendix A:

Illumnia Barcode Labeled Primers

Illumina 16s V4 primers - dual indexed

Generic PCR primer design — 131bp F+R

AATGATACGGCGACCACCGAGATCTACAC <i5><pad><link><16S-F>

AAGCAGAAGACGGCATACGAGAT <i7><pad><link><16S-R>

Generic read 1 primer design (sequencing primer)

<pad><link><16Sf>

Generic read 2 primer design (sequencing primer)

<pad><link><16Sr>

Generic index read primer design (sequencing primer)

Reverse complement of (<pad><link><16Sr>)

16S-F	V4: GTGCCAGCMGCCGCGGTAA	
16S-R	V4: GGACTACHVGGGTWTCTAAT	
Link	F: GT	R: CC
Pad	F: TATGGTAATT	R: AGTCAGTCAG

Sample	Date PCR	F-primer ID	F-index <i5>	R-primer ID	R-index <i7>
NN1	**10-21 1**	SA501	ATCGTACG	SA701	AACTCTCG
NN2	**10-21 2**	SA501	ATCGTACG	SA702	ACTATGTC
NN3	**10-21 3**	SA501	ATCGTACG	SA703	AGTAGCGT
NN4	**10-21 4**	SA501	ATCGTACG	SA704	CAGTGAGT
NE2 NN1	10-24 1	SA501	ATCGTACG	SA701	AACTCTCG
NE2 NN2	10-24 2	SA501	ATCGTACG	SA702	ACTATGTC
NE2 NN3	10-24 3	SA501	ATCGTACG	SA703	AGTAGCGT
NE2 NN4	10-24 4	SA501	ATCGTACG	SA704	CAGTGAGT
CC1 (NN2)	**10-28 1**	SA501	ATCGTACG	SA705	CGTACTCA
CC2	**10-28 2**	SA501	ATCGTACG	SA706	CTACGCAG
CC3	**10-28 3**	SA501	ATCGTACG	SA707	GGAGACTA
CC4	**10-28 4**	SA501	ATCGTACG	SA708	GTCGCTCG
W1	10-29 1	SA502	ACTATCTG	SA701	AACTCTCG
W2	10-29 2	SA502	ACTATCTG	SA702	ACTATGTC
W3	10-29 3	SA502	ACTATCTG	SA703	AGTAGCGT
W4	10-29 4	SA502	ACTATCTG	SA704	CAGTGAGT
W5	10-29 5	SA502	ACTATCTG	SA705	CGTACTCA
E1	10-29 6	SA502	ACTATCTG	SA706	CTACGCAG

E2	10-29 7	SA502	ACTATCTG	SA707	GGAGACTA
E3	10-29 8	SA502	ACTATCTG	SA708	GTCGCTCG
E4	10-29 9	**SA503**	TAGCGAGT	SA701	AACTCTCG
T1	**11-1 1**	**SA503**	TAGCGAGT	SA702	ACTATGTC
T2	**11-1 2**	**SA503**	TAGCGAGT	SA703	AGTAGCGT
T3	**11-1 3**	**SA503**	TAGCGAGT	SA704	CAGTGAGT
T4	**11-1 4**	**SA503**	TAGCGAGT	SA705	CGTACTCA
T5	**11-1 5**	**SA503**	TAGCGAGT	SA706	CTACGCAG
North1	**11-1 6**	**SA503**	TAGCGAGT	SA707	GGAGACTA
N2	**11-1 7**	**SA503**	TAGCGAGT	SA708	GTCGCTCG
N3	**11-1 8**	SA504	CTGCGTGT	SA701	AACTCTCG
N4	**11-1 9**	SA504	CTGCGTGT	SA702	ACTATGTC
N5	**11-1 10**	SA504	CTGCGTGT	SA703	AGTAGCGT
Vc2	11-7 1	SA504	CTGCGTGT	SA704	CAGTGAGT
Vc3	11-7 2	SA504	CTGCGTGT	SA705	CGTACTCA
Vc4	11-7 3	SA504	CTGCGTGT	SA706	CTACGCAG
Vc5	11-7 4	SA504	CTGCGTGT	SA707	GGAGACTA
Vc6	11-7 5	SA504	CTGCGTGT	SA708	GTCGCTCG
Vc7	11-7 6	**SA505**	TCATCGAG	SA701	AACTCTCG
Vc8	11-7 7	**SA505**	TCATCGAG	SA702	ACTATGTC
Vc9	11-7 8	**SA505**	TCATCGAG	SA703	AGTAGCGT
Vc10	11-7 9	**SA505**	TCATCGAG	SA704	CAGTGAGT
Vc11	11-7 10	**SA505**	TCATCGAG	SA705	CGTACTCA
N1	**11-11 1**	**SA505**	TCATCGAG	SA706	CTACGCAG
N4	**11-11 2**	SA506	CGTGAGTG	SA701	AACTCTCG
C1	**11-11 3**	SA506	CGTGAGTG	SA702	ACTATGTC
C2	**11-11 4**	SA506	CGTGAGTG	SA703	AGTAGCGT
C3	**11-11 5**	SA506	CGTGAGTG	SA704	CAGTGAGT
C4	**11-11 6**	SA506	CGTGAGTG	SA705	CGTACTCA
off	**11-11 7**	SA506	CGTGAGTG	SA706	CTACGCAG
N1	11-12 1	SA506	CGTGAGTG	SA707	GGAGACTA
N2	11-12 2	SA506	CGTGAGTG	SA708	GTCGCTCG
N3	11-12 3	**SA507**	GGATATCT	SA701	AACTCTCG
N4	11-12 4	**SA507**	GGATATCT	SA702	ACTATGTC
C1	11-12 5	**SA507**	GGATATCT	SA703	AGTAGCGT
C2	11-12 6	**SA507**	GGATATCT	SA704	CAGTGAGT
C3	11-12 7	**SA507**	GGATATCT	SA705	CGTACTCA
C4	11-12 8	**SA507**	GGATATCT	SA706	CTACGCAG
off	11-12 9	**SA507**	GGATATCT	SA707	GGAGACTA
NN1 t0	**11-15 1**	**SA507**	GGATATCT	SA708	GTCGCTCG
NN2 t0	**11-15 2**	SA508	GACACCGT	SA701	AACTCTCG
NN3 t0	**11-15 3**	SA508	GACACCGT	SA702	ACTATGTC

NN4 t0	**11-15 4**	SA508	GACACCGT	SA703	AGTAGCGT
CC1 t0	**11-15 5**	SA508	GACACCGT	SA704	CAGTGAGT
CC2 t0	**11-15 6**	SA508	GACACCGT	SA705	CGTACTCA
CC3 t0	**11-15 7**	SA508	GACACCGT	SA706	CTACGCAG
CC4 t0	**11-15 8**	SA508	GACACCGT	SA707	GGAGACTA
off2 t0	**11-15 9**	SA508	GACACCGT	SA708	GTCGCTCG
NN1 tf	11-19 1	SB501	CTACTATA	SA701	AACTCTCG
NN2 tf	11-19 2	SB501	CTACTATA	SA702	ACTATGTC
NN3 tf	11-19 3	SB501	CTACTATA	SA703	AGTAGCGT
NN4 tf	11-19 4	SB501	CTACTATA	SA704	CAGTGAGT
CC1 tf	11-19 5	SB501	CTACTATA	SA705	CGTACTCA
CC2 tf	11-19 6	SB501	CTACTATA	SA706	CTACGCAG
CC3 tf	11-19 7	SB501	CTACTATA	SA707	GGAGACTA
CC4 tf	11-19 8	SB501	CTACTATA	SA708	GTCGCTCG
off2 tf	11-19 9	SB501	CTACTATA	SA709	GTCGTAGT
11-8 VP1	**11-26 1**	SB501	CTACTATA	SA710	TAGCAGAC
11-8 VP2	**11-26 2**	SB502	CGTTACTA	SA701	AACTCTCG
12-8 Vp1	**11-26 3**	SB502	CGTTACTA	SA702	ACTATGTC
12-8 VP2	**11-26 4**	SB502	CGTTACTA	SA703	AGTAGCGT
12-8 VP4	**11-26 5**	SB502	CGTTACTA	SA704	CAGTGAGT
13-8 VP1	**11-26 6**	SB502	CGTTACTA	SA705	CGTACTCA
13-8 VP2	**11-26 7**	SB502	CGTTACTA	SA706	CTACGCAG
13-8 VP4	**11-26 8**	SB502	CGTTACTA	SA707	GGAGACTA
18-8 VP1	**11-26 9**	SB502	CGTTACTA	SA708	GTCGCTCG
18-8 VP2	**11-26 10**	SB502	CGTTACTA	SA709	GTCGTAGT
18-8 VP4	**11-26 11**	SB502	CGTTACTA	SA710	TAGCAGAC
19-8 VP1	12-3 1	SA501	ATCGTACG	SA709	GTCGTAGT
19-8 VP2	12-3 2	SA502	ACTATCTG	SA709	GTCGTAGT
19-8 VP4	12-3 3	SA503	TAGCGAGT	SA709	GTCGTAGT
14-8 VP1	12-3 4	SA504	CTGCGTGT	SA709	GTCGTAGT
14-8 VP3	12-3 5	SA505	TCATCGAG	SA709	GTCGTAGT
14-8 VP5	12-3 6	SA506	CGTGAGTG	SA709	GTCGTAGT
15-8 VP1	12-3 7	SA507	GGATATCT	SA709	GTCGTAGT
15-8 VP3	12-3 8	SA508	GACACCGT	SA709	GTCGTAGT
15-8 VP5	12-3 9	SB501	CTACTATA	SA710	TAGCAGAC