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GENOTYPIC DIVERSITY OF PORTIES LOBATA IN PALAU

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ABSTRACT

Reef building corals are the foundational species for coral reefs, with their calcium carbonate skeletons forming the structure of the local ecosystem. The reef building coral, *Porites lobata* grows on offshore reefs and inner bay patch reefs in Palau; two habitats that differ in local pH conditions and aragonite saturation. Low aragonite saturation state is known to cause weaker *P. lobata* skeleton that may be more prone to fragmentation. It is suspected that these differences would make *P. lobata* growing inner bays susceptible to bioerosion by skeleton-boring mussels, and therefore experience increased clonal fragmentation than on offshore reefs. To determine if this was the case, two inner bay patch reefs and two offshore reefs, were analyzed for differences in mussel boring density and genotypic diversity of the coral *Porites lobata*. While there was an increased density of mussels found in inner bay reefs, there was not a significant difference in asexual reproduction between offshore and inner bay reefs. Both habitats had mostly or solely sexual reproduction despite the differences in aragonite saturation state and mussel density. This suggests a different set of interactions between *P. lobata* in Palau and other members of the reef ecosystem than previously documented in the Eastern Pacific where mussel-hunting triggerfish caused fragmentation of *P. lobata* colonies and increased coral clonal reproduction. Indeed, the mussel-hunting triggerfish is absent in Palau. This study provides evidence for the importance of biotic interactions in determining population structure of a major reef-building coral in the Pacific.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Chapter 1

Introduction

Coral reefs are ecosystems found in tropical waters across the world that are unique in being able to maintain an incredibly high level of productivity despite being in a low nutrient environment. In a purely utilitarian context, coral reefs are a vital ecosystem, which humans rely on for an estimated $30 billion US dollars in yearly net production (Moberg et al, 1999). Fishing and seafood harvest are vital exports of coral reefs that human populations across the world rely on as a major food source. In terms quantity, upwards of 10% of all fish caught by humans are from coral reefs, and an additional percentage of fish caught use coral reefs as breeding grounds and or nurseries before returning the ocean at large. They are hotbeds of biodiversity and have provided modern medicine with numerous bioactive compounds used in everything from pharmaceuticals to cosmetics (Moberg et al, 1999). In addition, they help protect coastlines by helping to disrupt and reduce the effects of wave action (Moberg et al, 1999).

Coral animals are the foundational species of the reefs and are analogous to trees in a forest. There are many types of coral, but the focus on this paper will be on the Scleractinian, or hard coral. These corals form hard skeletons made of calcium carbonate in the form of aragonite as well as other trace elements (Chornesky et al, 1987). Since the skeleton of these animals is made of calcium carbonate, it is subject to changes in pH and fluctuations in the carbonic acid equilibrium chemistry of the surrounding water (Gattuso et al, 1999). For instance, an increase in

acidity makes it more difficult for the corals to maintain their calcium carbonate skeletons (Kleypas et al, 2006).

Corals are capable of both sexual and asexual reproduction. Sexual reproduction occurs in a timed fashion, with mature polyps releasing gametes into the water column in synchrony. This type of reproduction method is called broadcast spawning, and it generates larvae with a unique genetic composition capable of traveling longer distances to settle at new sites. Sexual reproduction is an energy intensive process, and since corals are sessile organisms, it is beholden to mates located nearby and ocean currents (Stimson, 1978).

In contrast, asexual reproduction generates a clonal fragment of the original parent colony. Asexual reproduction occurs either during budding in colonial growth, or when part of the coral colony fragments off. This fragment under most circumstances is limited to local translocation and, if the conditions are correct, the fragment will regrow in its new location (Coffroth et al, 1998). When speaking of clonal reproduction in coral there are two important terms: ramet and genet. A genet is a genotype whereas a ramet is an individual with that genotype. For instance, a unique multilocus genotype (genet) might have several individual colonies of that genotype (ramet) (Baums, 2008). There are benefits and detriments associated with each type of reproduction. Asexual reproduction is useful if there are too few mating partners nearby to allow colonies to grow; it is less energy intensive than sexual reproduction and can help preserve already existing genetic diversity. Sexual reproduction increases the genetic diversity by creating new combinations of alleles and allows for long distance colonization. Healthy coral reefs often show a balance between these two reproductive strategies (Rinkevich, 1995).

Background Information

Corals and therefore reefs as a whole currently face several threats. Rising temperatures and shifts in global CO_2 affect the ability of corals to build and maintain their skeletons, resulting in weaker structural integrity (Gattuso et al, 1999). In addition, shifts towards warmer temperature may result in bleaching events, where the coral ejects its symbiotic algae into the water column due to stress. This causes the corals to lose their color and turn white, hence the name bleaching (Porter et al, 1989). These bleaching events are often widespread, with vast swaths of reefs bleaching simultaneously across the ocean due to the increased temperature (Porter et al, 1989). Corals that bleach may recover and regain their symbionts, however if conditions do not improve they will rapidly die (Glynn et al, 2017). These long-term environmental shifts are compounded with destructive human activity, pollution, predation and infection (Kleypas et al, 2006). While coral are the foundational species of reefs, they are slow growing and can take many years to recover (Rinkevich, 1995). Large losses to reefs can decrease the productivity of reefs long term. With all of these combined stressors acting on reefs, understanding reef resilience is of the utmost importance.

While bleaching can affect entire reefs, studies have shown that within the same species, there are differences in individual corals' abilities to resist and recover from bleaching (Kleypas et al, 2006). This underpins the importance of understanding the genetic diversity of coral reefs. A reef with high levels of genetic diversity, in theory, would be more robust when faced with all types of threats from temperature to pathogens. In order to better conserve and manage reef habitats that humans interact with, it is important to understand the underlying genetic diversity of the area (Baums, 2008).

Previous work has shown bioerosion to be a key facilitator in the process of coral fragmentation and therefore asexual reproduction on a reef (Scott et al, 1988). Bioerosion is the process in which living creatures either actively or passively weaken the skeletal structure of a coral. Bioerosion will continue to gain prominence in the field of coral conservation as the rate of bioerosion is expected to increase as ocean acidification and global temperatures continue to rise (Glynn et al, 2015). Of particular prominence in the bioerosion of coral is the *Lithophaga* mussel. These mussels are small filter feeders that make their homes by boring into the skeleton of hard-bodied corals, such as *P. lobata*. The boring action alone weakens the integrity of the coral skeleton, and mussels have an easier time making boreholes when the coral skeletons are already weakened due to acidic water conditions or low concentrations of aragonite (Glynn et al, 2015). In conjunction, triggerfish also prey upon the mussels. In the process of the triggerfish extracting the mussel, the fish digs into the coral, breaking apart the weakened skeleton into fragments and creating asexual clones (Boulay et al, 2014). However, the mussel-hunting triggerfish have a geographically restricted distribution and it is unclear whether other *Lithophaga* predators also cause coral fragmentation.

Goals of the Experiment

This paper focuses specifically on the coral *Porites lobata.* This is a hard bodied, hermatypic, coral found across the Pacific Ocean and is an important reef- building coral in the Pacific. These coral are important to study as they form large colonies and often comprise the bedrock of the reef community (Cortés et al, 1997). They are found across the Pacific Ocean in various environmental contexts. The corals' range is larger than many of the other members of

the reef ecosystem and thus biotic interactions of *P. lobata* with commensals and predators change across the Pacific (Boulay et al. 2014).

This study was conducted in the waters around the island of Palau. This location provided a unique opportunity to conduct field research, as there are local variations in ecology that allow for interesting experiments and comparisons to be made (DeCarlo et al, 2015). Inner bay patch reefs are noted for having a significantly lower saturation level of aragonite in addition to a lower pH (Mollica et al, 2018) and higher wave action in comparison to offshore regions (Woesik et al, 2012). Furthermore, recent bleaching events have revealed immense variation of the local corals' abilities to resist and recover from thermal stress (Goldbuu et al, 2005). This presented a unique opportunity to investigate how local environmental conditions can play a role in the genotypic diversity of coral.

Bioerosion rates and within-species diversity of *Porites* corals from the inner bay patch reefs of Palau were compared to the offshore colonies, with mussel density being used as an approximation for relative bioerosion rates. The genotypic diversity was determined using multilocus microsatellite markers. These are tandem repeats occurring in specific locations within the genome. The microsatellites tend to be highly polymorphic, with alleles varying by how many times that specific tandem sequence is repeated (Ellegren, 2004). Microsatellites were used because they are highly polymorphic and, with enough loci, give adequate representation of the genotypic identity of each sample (Pritchard et al, 2000). It was expected that there would be higher levels of mussel boring and therefore higher levels of asexual reproduction (more clones) in *Porites* corals located in the bay region due to environmental differences when compared to the *Porites* corals located offshore.

Chapter 2

Methods

Sample Collection

The collection SCUBA dives took place in Palau over several days in March of 2017. There were four sites in total, two inner bay patch reefs named Nikko Bay 1 and Nikko Bay 2. In addition, there were two offshore sites called Offshore 1 and Offshore 2. A random sample of *Porites* colonies was chosen at each site based on distance from a central co-ordinate, with a maximum radius from each central coordinate of 15 meters. A random set of coordinates was generated within this 15 meter circle, and divers swam to each coordinate on the list. If there was a colony of *P. lobata* located at this coordinate, it was sampled. If not, divers moved on to the next coordinate. At each coral colony, a mussel sample was extracted and a piece of coral was cut off for later genetic analysis. Samples were preserved in 100% ethanol. A photograph was taken of each colony for later analysis of mussel density. Included within the frame of each photo was a colony specific tag, a ruler, and the colony itself. This process was repeated several times with different angles to give the best representation of each colony's surface area. The coordinates of each colony sampled were also recorded for later use in constructing polar plots.

Surface Area and Mussel Counts

Mussel density for each coral colony was determined using the program ImageJ (Schneider et al. 2012). For each photograph, the scale was set by first tracing the photographed

ruler with the linear tool from 0 cm to the 15 cm mark. Going into the set scale function, the number of pixels measured by the linear tool was calibrated to 15 cm. Once the scale was set, the free form tool was used to carefully outline the boundaries of each colony. Using the measure function, the surface area was calculated by converting the number of pixels into their cm^2 metric equivalent based on the calibration determined by the ruler placed in each picture.

ImageJ was also used to determine the number of mussels in each colony. The image was enlarged and split into four quadrants. The number of mussel boreholes on each colony was counted by hand for each quadrant and then added together to determine the total number of mussels in each photograph. The total surface area for each colony and total mussel count was calculated by adding all the replicates from the differentially angled photos together. For each colony, there was anywhere between 1-4 photos that were added together. Afterwards, the total number of mussel boreholes in each colony was divided by the total surface area of the colony to give the mussel density for each particular coral colony. The data for mussel densities was then categorized according to site: Nikko Bay 1, Nikko Bay 2, Offshore 1 or Offshore 2.

Statistical Analysis for Mussel Densities

The mussel density data for the different sites was compared using the latest version (3.2.5) of the free statistical tool R (R Development Core Team 2013). A preliminary Shapiro normality test and a Levene variance test confirmed that the data set was not normally distributed nor were the variances similar. Therefore, a nonparametric test was used instead of the standard one-way ANOVA to compare the sites. This is because a one-way ANOVA assumes that the

data set is normally distributed and has equal variances. Thus, the Kruskall-Wallis test was performed in order to determine whether the difference between the sites were significant.

DNA Extraction, Amplification, and Fragment Analysis

Using bone cutters, a small cross-section of the polyps were extracted from the collected samples. The extracted tissue was subsequently processed using the Qiagen DNeasy 96 Well Blood and Tissue Kit using the protocol for animal tissues (Applied Biosystems, CA). Several samples were spot-checked using NanoDrop in order to ensure that the extractions had worked and that a sufficient amount of DNA was present.

After the DNA was extracted, five multiplex polymerase chain reactions (PCRs) were run using 14 previously developed microsatellite loci (Concepcion et al, 2010). There were 3 distinct thermocycler programs used as shown, named geno-52, geno-54, and geno-56 (Table 1). All three programs were identical, except for the annealing step, which was run at 52°C, 54°C and 56°C respectively. All protocols started with a 94°C denaturation for 5 minutes, followed by 35 repeated cycles of a 94°C denaturation step for 20 seconds, a protocol specific annealing step for 20 seconds, finished off with a 72°C extension step for 30 seconds. The final step was a 30-minute extension cycle held at 72°C. Figure 1 illustrates the specific thermocycler protocol.

Once the PCR was complete, the samples were checked for successful amplification on a 2% agarose gel. PCR products with distinct bands were sent to the Penn State Genomics Core facility for fragment analysis. An ABI3730 (Applied Biosystems, CA) automated DNA sequencer with an internal size standard (Gene Scan 500-Liz, Applied Biosystems, CA) was used to visualize the products.Allele Calling and Marker Selection

The electropherograms were analyzed using GeneMapper Software 5.0 (Applied Biosystems, CA). This program was used to report which allele sizes the samples had at each microsatellite locus. Alleles were automatically called using a panel of standard bin size that corresponded to the repeat size of the microsatellite marker (e.g. dinucleotide, trinucleotide, etc.). Allele peaks that fell between bins were called by hand. Alleles falling halfway between the bins were always called up to the higher bin; otherwise the alleles were called to the closest bin. Samples that did not produce results at all markers were identified and selected to be re-run through PCR based on which ones failed at the least markers.

Of the initial fourteen microsatellite markers, eleven of the highest performing markers were chosen to conduct genotypic analysis. (Table 2) Several markers were not included in the final analysis as their success rates were too low. Samples that succeeded at a minimum of nine out of the eleven markers were included in the final genetic analysis. Polar plots were made to compare the sampling location for corals with identical genotypes. The data was exported to excel where GenAlEx was used to quantify genotypic diversity. (Peakall and Smouse, 2012)

Species Identification

Due to the morphological similarity and overlapping range of the *Porites lobata* and *Porites evermanni*. (Boulay, 2014) It was first necessary to determine that all the samples used in this study were the species P. lobata to ensure the experiment was controlled and that there was no accidental harvesting of P. evermanni. A principle coordinate axis was run using my samples and known samples of both species. After running the principle coordinate axis in GenAlEx, it was confirmed that all the samples were of P. lobata and not P. evermanni. (Peakall and Smouse,

2012) This allowed me to continue my analysis using the assumption that I was only working with one species.

Allele Calling and Marker Selection

The electropherograms were analyzed using GeneMapper Software 5.0 (Applied Biosystems, CA). This program was used to report which allele sizes the samples had at each microsatellite locus. Alleles were automatically called using a panel of standard bin sizes that corresponded to the repeat size of the microsatellite marker (e.g. dinucleotide, trinucleotide, etc.). Allele peaks that fell between bins were called by hand. Alleles falling halfway between the bins were always called up to the higher bin, otherwise the alleles were called to the closest bin. Samples that did not produce results at all markers were identified and selected to be re-run through PCR based on which ones failed at the least markers.

Of the initial fourteen microsatellite markers, eleven of the highest performing markers were chosen to assign genotypes (Table 2). Several markers were not included in the final analysis as their success rates were too low. Samples that succeeded at a minimum of nine out of the eleven markers were included in the final diversity analysis. Polar plots were made to compare the sampling location for corals with identical genotypes using Excel. The data was exported to excel where GenAlEx was used to quantify genotypic diversity (Peakall and Smouse, 2012).

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Due to the morphological similarity and overlapping range of *Porites lobata* and *Porites evermanni* (Boulay, 2014), it was first necessary to determine that all the samples used in this study were the species *P. lobata* to ensure the experiment was not biased by accidental harvesting of *P. evermanni*. A principle coordinate analysis was run using my samples and known samples of both species (Boulay, 2014). After running the principle coordinate analysis in GenAlEx, it was confirmed that all the samples were *P. lobata* and not *P. evermanni* (Peakall and Smouse, 2012). This allowed me to continue my analysis using the assumption that I was only working with one species.

Genotypic Diversity Indices

Using the multilocus marker data from the previous steps, the sites were characterized based on genotypic diversity, which is the number of unique multilocus genotypes present within a population. Whereas genetic diversity focuses on the variation of individual alleles within a population, genotypic diversity varies on the level of whole organisms (Baums, 2006). The indices of genotypic richness (N_G/N), genotypic diversity (G_O/G_E) and genotypic evenness (G_O/N_G) were used to quantify differences between the populations.

Genotypic diversity was calculated by dividing the observed genotypic diversity (G_O) by the expected genotypic diversity (G_E). Observed genotypic diversity is calculated by dividing the inverse of the sum of the square of the number of individual genotypes sampled (Ni) by the total number of samples (N). Expected genotypic diversity is the same is the total number of colonies sampled, given a low probability of identity value ($PID < 1.0 \times 10^{-7}$) (Baums, 2006). Probability

of identity is the probability of choosing two individuals at random from a population with the same genotype by chance rather than by decent. In other words, it is the probability of misidentifying clones. Using GenAlEx, the PID for all sites was below this threshold value, so G_E was simply the number of colonies sampled. Genotypic diversity is a useful quantification related to the relative contribution of asexual and sexual recruitment. A solely sexual population will have a diversity value of one, whereas a solely clonal population will have a diversity value approaching zero (Boulay, 2014).

Genotypic richness (N_G/N) was calculated by dividing the total number of unique genotypes (N_G) by the total number of colonies sampled (N). Genotypic richness is proportional to the frequency of sexual recruitment (Baums, 2006).

Genotypic evenness was calculated (G_O/N_G) by dividing observed genotypic diversity (G_O) by the number of unique genotypes (N_G). Populations with an equal distribution of genotypes will have an evenness approaching one, whereas a population skewed toward a single genotype will have an evenness value approaching zero. Whereas the previous two metrics were useful in quantifying recruitment, evenness is related to genet longevity (Baums, 2006).

Hardy-Weingberg and Heterozygosity

The loci were tested for accordance with Hardy-Weinberg equilibrium using the GenAlEx add-on in excel. This was done in order to determine whether they were out of expected equilibrium. In addition to characterizing the diversity through genotypes, the diversity was also characterized through the heterozygosity of the populations. Heterozygosity is another metric of diversity, which can indicate populations with low levels of genetic shuffling, or low

base diversity. It can also be used to calculate levels of population structuring and allele fixation for a population. Heterozygosity was calculated using the GenAlEx add-on in excel.

STRUCTURE Analysis

Patterns of connectivity at the population level were assessed using the program STRUCTURE version 2.3.4, (Falush et al. 2003) which uses a Bayesian framework to determine the population structure of the species in question. It analyzes the unique multilocus genotypes by assigning individuals to populations based on allele frequencies, and is useful in determining patterns of gene flow and population admixture. It sorts the samples into an *a priori* K number of populations. Samples from Indonesia, Marshalls Islands, Fiji, Samoa, Phoenix Islands and Lau were included in the analysis in order to assess how Palau is connected to the population of the Western Pacific previously described in the literature (Baums, 2012). The program was run with the null hypothesis of K=1 and included up to a K of 6. Five replicate runs at each K value were conducted with a burnin of 100,000 and 1,000,000 Markov chain Monte Carlo (MCMC) replicates. The null hypothesis of K=1 corresponds to one assumed population, which would happen in a completely admixed population. The STRUCTURE model assumed admixture and correlated allele frequencies.

Chapter 3

Results

Mussel Density

The density of mussels for each colony served as a reasonable estimate to approximate the relative amount of bioerosion at each site. Comparing offshore sites against the Nikko Bay sites using a Kruskall-Wallis non-parametric test, there was a significant difference in mussel densities between the two ecosystems ($p<0.05$). Comparing the four sites separately, there was not a significant difference between the two Nikko Bay sites ($p>0.05$) nor was there a significant difference between the two offshore sites ($p>0.05$). The Nikko Bay sites had noticeably higher levels of mussel density when compared to the offshore sites (Figure 1). In addition, the offshore cites had a significantly higher average surface area when compared to the Nikko bay sites ($p>0.05$) (Figure 3).

Diversity Data

The three diversity indices of genotypic diversity, richness and evenness were used to characterize the reproductive history of the population (Table 3). Genotypic diversity (G_O/G_E) measures the relative amount of sexual recruitment vs asexual cloning. The diversity value for the offshore sites and Nikko bay sites were very high at one and 0.96 respectively. There was no significant difference in the genotypic diversity between the two sites. In addition, only samples from Nikko Bay showed any clonality. Of the samples taken, there were two genetically identical individuals from two different genets for a total of four samples (Figure 5).

Genotypic richness (N_G/N) is proportional to the frequency of sexual recruitment in the population. In both regions, the genotypic richness was very high. Nikko bay had a richness of 0.93 whereas the offshore sites had a richness of one. Likewise, there was no significant difference between the genotypic richness between the two regions.

Genotypic Evenness (G_O/N_G) is proportional to the genet longevity of the population. Populations skewed toward a single genet approach zero, whereas a more even distribution of genets approaches one. The genotypic evenness of Nikko Bay was 0.97, and the evenness of the offshore sites was one. There was no significant difference between the evenness of the two regions.

Heterozygosity was also measured to give another dimension to the diversity within the population. The observed level of heterozygosity was lower than the expected level of heterozygosity based on the number of alleles present within the population for all sites. The fixation index was found to be above 0.2 for all sites, indicating that *Porites lobata* at these locations undergoes moderate to high levels of inbreeding.

Clonal Structure Related to Geography

Since the site Nikko Bay 2 was the only location to have clones, it was the only polar plot made. The graph plots the sampling location to better visualize the spatial distance between clones. As can be seen from the polar plots (Figure 5), ramets in the same genet were located in close proximity. In addition, both genets were also located adjacent to each other.

STRUCTURE and Hardy-Weinberg Results

STRUCTURE assumes Hardy-Weinberg equilibrium, which was tested to determine any outlier markers within sampling locations (Table 4). According to the cluster analysis results, the most likely K for *Porites lobata* was K=3 (Figure 5). As seen in the figure, there is evidence for some level of population structure between the offshore and the Nikko Bay *P. lobata.* However, fewer offshore samples were included in the STRUCTURE analysis compared to Nikko Bay samples due to differential marker failure between sites. The population of Nikko Bay seems to be all one population. The orange cluster that includes most of Palau becomes less prevalent from west to east across the Pacific. Contrastingly, the blue cluster is more prevalent towards the Phoenix Islands and Society Islands in the Central Pacific. Thus, there appears to be an isolation by distance pattern from the Western to Central Pacific. The purple cluster is found throughout all of the Pacific Island groups included in this analysis, and is sympatrically separated from the orange and blue clusters. It is possible that this purple cluster represents a cryptic species.

Chapter 4

Discussion

The hypothesis that Nikko Bay would have a higher concentration of mussels was accepted. The analysis of mussel density between the two sites shows that Nikko Bay densities were in fact significantly higher than the offshore sites. The set of ecological conditions present in Nikko Bay allowed mussels to accumulate to densities much higher than they accumulated in offshore areas (Woesik et al, 2012). As shown, there were specimens in Nikko Bay where mussel boreholes covered over 20% of the surface of the colony. Morphologically, the two populations also exhibited a significant difference in surface area as well. Nikko Bay colonies, on average, had less surface area than their offshore counterparts did.

Previous studies have shown that *Porites lobata* populations tend to have high levels of diversity (Schweinsberg and Lampert, 2016). The important difference between those studies and this study is that previous studies did not consider the potential effects of bioerosion on diversity in *P. lobata*. The results found are discordant with the hypothesis that greater mussel density would correlate to a decrease in genotypic diversity. With respect to genotypic diversity, there was no significant difference in any of the indices between the two ecosystems. All of the indices for the offshore populations were at one, suggesting a solely sexual population. Nikko Bay had almost entirely sexual reproduction, with diversity indices above .9 for every metric. Out of all the colonies samples, all genets were only represented once except for two genets composed of two ramets each. Due to the physical proximity of ramets of the same genet, it is probable that clones were produced from fragmentation and not from asexual larvae (Chornesky et al. 1987). These results suggest that despite the significantly higher mussel density found in the Nikko Bay sites, there was not a corresponding decrease in genotypic diversity as expected.

In fact, recent studies have shown that *Porites* growing in the inner bay patch reefs of Palau actually have a greater degree of resilience against bleaching events when compared to other locations (Shamberger et al, 2014.) These findings go against what one might consider detrimental to coral health, as the combination of higher mussel erosion and adverse water conditions would lead one to consider these corals to be the most vulnerable to bleaching and slowest to recover (Shamberger et al, 2014).

One possible way of understanding the potential interaction between *Porites lobata* and *Lithophaga* mussels in Nikko Bay is through a coevolution analysis. According to Thompson's geographic mosaic model of coevolution, coevolution between species varies on a geographic and environmental continuum (Thompson, 2005). It has been shown previously in the Eastern Pacific that an evolutionary relationship between boring mussels and coral exists (Boulay et al. 2014). Species of *Lithophaga* have been shown to prefer certain coral species to others and have adapted unique strategies to boring into hard-bodied coral depending on density, shape, and size of the coral skeletons (Owada, 2007).

Just between the two environments of Palau, there are clearly two distinct relationships between *Porites lobata* and *Lithophaga*. The Nikko Bay region has a higher density of mussel boring, thus the coral in this area must respond to the ecological conditions presented by the oceanic conditions and bioerosion pressure in a way that the offshore coral do not. Furthermore, since offshore mussels do not occur at the same density, there might be a different relationship between these species. The exact relationship between the two has yet to be determined, and whether mussels influence *Porites* in some way other than genotypic diversity is a potential avenue for future research. In addition, it would be interesting to see exactly why the two interactions differ. For instance, unsuitable environment could be the culprit for lower mussel

infestation in *P. lobata* offshore. While such an evolutionary arms race has yet to be described between corals and bioeroders, coevolutionary pressure between the coral and mussel could be vital in understanding reef dynamics across the Pacific, as they co-occur across a wide range (Scott, 1988).

This concept taken in a different context can help explain why asexual reproduction was not as much of a driving force as hypothesized for the Nikko Bay *P. lobata*. As previously mentioned, the coevolution between mussels and *Porites* varies over their geographic range due to the local conditions. Reefs in the Eastern Pacific are noted for having excavating triggerfish, which dig out mussels from the coral skeleton, thereby inducing fragmentation. Since Palau lacks the necessary triggerfish species to fragment coral as it hunts the boring mussels, there is a missing link in the chain for asexual reproduction (Boulay et al, 2014). While previous research described how triggerfish preying on *Lithophaga* mussels lead to fragmentation of the colony, endemic triggerfish in Palau have different feeding strategies. Instead of excavating mussels from the coral, the triggerfish in Palau dig through sediment to find prey or directly consume small parts of branching corals. This type of feeding strategy does not stimulate the fragmentation of non-branching corals such as massive *Porites lobata* (Kitalong et al, 1994). Therefore, increased asexual reproduction in *P. lobata* related to increased mussel densities is not observed in Palau.

Co-evolution and population structure of corals in the context of the Pacific is particularly interesting, as geographically isolated reefs would experience different selective pressure based on the local biota and environmental conditions. Therefore, gene flow is an important factor to consider when assessing the population structure of a reef in a broader context. Looking to the cluster data, it appears as if there is some structure present between the

offshore sites and Nikko Bay sites. While the sample size is still too small to make solid conclusions, the samples we did run from offshore colonies clustered differently than the Nikko Bay sites. This suggests a level of population structuring which may be due to distance, ecological/physical barriers, or even local adaptation generating distinction (Shamberger et al. 2014) Recent findings have shown Nikko Bay corals to be more resilient to bleaching events than offshore corals. Thus, population structure between these areas might limit the adaptive potential of offshore corals in the face of ongoing environmental change (Goldbuu, 2016). Furthermore, Palau exists relatively isolated from the rest of the Western Pacific, showing low levels of gene flow between the other islands included in the structure analysis.

Based on the data, it is unlikely that mussels play a role in shaping the genotypic diversity of *P. lobata* in Palau through the previously described method. That is not to say the interaction is completely neutral, as there are most likely other trophic interactions at work that occur when the mussel bores into the coral skeleton. These interactions are also likely to differ depending on the environmental context and can exert varying selective pressures depending on the surrounding flora and fauna.

Chapter 5

Conclusion

Based on previous research in Palau and the findings in this study, it is clear that *Porites lobata* in Palau exhibit responses to environmental stressors discordant with what we would assume for corals in these condition. While the sample size was a limiting factor in this project, there is enough evidence to suggest that mussels do not have an impact on the genotypic diversity of *Porites lobata* as previously expected. This suggests that conservation strategies in the future should consider this underlying genotypic diversity when planning how to best use asexual and sexual reproduction when managing the reef. While there might be other interactions between the two organisms worth investigating in order to construct a better plan for conservation moving forward, more research needs to be done in order to understand the coevolutionary effects. For example, there are many more locations in Palau that have yet to be sampled for mussel density or coral genotypic diversity. In addition, close attention should be paid to the relationship between stony corals and bioeroders, as changing climactic conditions are expected to increase the acidity of ocean water in the near future

Appendix A

Tables

Table 1. Multiplex reactions and microsatellite markers used. This table summarizes the standardized PCR methods used with the 14 microsatellite markers for *Porites lobata*.

Primer:			Factor:	Samples:	PLEX A
0780-vic			20	96	geno 52
0905-ned					
0340-6fam(r3)					
1551-pet					
Water				5.4	544.32
Buffer	10x	Biolase		1	100.8
MgCl	25mM	Bioline		0.8	80.64
dNTP	10mM	Bioline		0.2	20.16
0780-vic	5uM	ABI		0.25	25.2
0905-ned	5uM	ABI		0.15	15.12
0340-6fam(r3)	5uM	ABI		0.5	50.4
1551-pet	5uM	ABI		0.4	40.32
taq	5U/uL	Bioline		0.3	30.24
DNA	1:1			1	
Add:		9			
Total:				10	907.2

Primer:			Factor:	Samples:	PLEX B
2258-ned			20	96	geno 56
1556-pet					
Water				5.4	544.32
Buffer	10x	Biolase		1	100.8
MgCl	25mM	Bioline		1	100.8
dNTP	10mM	Bioline		0.2	20.16
2258-ned	5uM	ABI		0.4	40.32
1556-pet	5uM	ABI		0.6	60.48
taq	5U/uL	Bioline		0.4	40.32
DNA	1:1			1	
Add:		9			
Total:				10	907.2

Primer:			Factor:	Samples:	PLEX C
2069-pet(r2)			20	96	geno 52
					short
Water				6.5	655.2
Buffer	10x	Biolase		1	100.8
MgCl	25mM	Bioline		0.6	60.48
dNTP	10mM	Bioline		0.2	20.16
2069-pet(r2)	5uM	ABI		0.3	30.24
taq	5U/uL	Bioline		0.4	40.32
DNA	1:1			1	
Add:		9			
Total:				10	907.2

Primer:			Factor:	Samples:	PLEX D
1868-vic(r1)			20	96	geno 52
1629-6fam					
1357-pet					
Water				5.6	564.48
Buffer	10x	Biolase		1	100.8
MgCl	25mM	Bioline		0.8	80.64
dNTP	10mM	Bioline		0.2	20.16
1868-vic(r1)	5uM	ABI		0.25	25.2
1629-6fam	5uM	ABI		0.25	25.2
1357-pet	5uM	ABI		0.5	50.4
taq	5U/uL	Bioline		0.4	40.32
DNA	1:1			1	
Add:		9			
Total:				10	907.2

Primer:			Factor:	Samples:	PLEX E
1490-vic			20	96	geno 54
0072-ned					
1483-6fam (f2)					
1370-pet					
Water				4.75	478.8
Buffer	10x	Biolase		1	100.8
MgCl	25mM	Bioline		0.8	80.64
dNTP	10mM	Bioline		0.2	20.16
1490-vic	5uM	ABI PSU		0.4	40.32
0072-ned	5uM	ABI PSU		0.5	50.4
1483-6fam (f2)	5uM	ABI PSU		0.35	35.28
1370-pet	5uM	ABI PSU		0.6	60.48
taq	5U/uL	bioline		0.4	40.32
DNA	1:1			1	
		9			
Total:				10	907.2

Table 2. Final markers list. This table shows the final 11 markers chosen to use for genotyping *Porites lobata* (Concepcion et al, 2010). The suffixes for each marker is indicative of the 5' reporter die used to visualize.

Final Markers	Plex
0780-vic	
0905-ned	A
1551-pet	
2258-ned	B
1556-pet	
2069-pet(r2)	C
1868-vic(r1)	
1629-6fam	D
1357-pet	
1370-pet	E
0072-ned	

Table 3. Diversity metrics for *Porites lobata*. N is the number of samples, N_G is the number of unique genets, and G_O is the observed genotypic diversity.

Nikko Bay					
N	**N_G**	**G_O**	**Diversity (G_O/G_E)**	**Richness (N_G/N)**	**Evenness (G_O/N_G)**
52	50	48.2	0.96	0.93	0.97
Offshore					
N	**N_G**	**G_O**	**Diversity (G_O/G_E)**	**Richness (N_G/N)**	**Evenness (G_O/N_G)**
34	34	34	1	1	1

Table 4. Hardy-Weinberg equilibrium of the microsatellite loci. Significant P values for loci deviating from Hardy-Weinberg equilibrium are bolded.

Population	Locus		P value
Nikko Bay	ned	72	0.346
Nikko Bay2		**72**	**0.056**
Offshore1		**72**	**0.05**
Offshore2		**72**	**0.002**
Nikko Bay	vic	780	0.791
Nikko Bay2		**780**	**0**
Offshore1		780	0.775
Offshore2		**780**	**0.019**
Nikko Bay	ned	**905**	**0.012**
Nikko Bay2		**905**	**0**
Offshore1		905	0.559
Offshore2		905	0.189
Nikko Bay	pet	**1357**	**0**
Nikko Bay2		**1357**	**0**
Offshore1		**1357**	**0.008**
Offshore2		1357	0.345
Nikko Bay	pet	**1370**	**0.021**
Nikko Bay2		**1370**	**0.017**
Offshore1		1370	0.555
Offshore2		**1370**	**0.86**
Nikko Bay	pet	**1551**	**0.072**
Nikko Bay2		1551	0.212
Offshore1		1551	0.858
Offshore2		**1551**	**0.004**
Nikko Bay	pet	**1556**	**0**
Nikko Bay2		**1556**	**0**
Offshore1		**1556**	**0**
Offshore2		**1556**	**0**
Nikko Bay	6fam	1629	0.052
Nikko Bay2		1629	0.888
Offshore1		1629	0.999
Offshore2		1629	0.538
Nikko Bay	vic (r1)	1868	0.777
Nikko Bay2		**1868**	**0.011**
Offshore1		1868	0.994
Offshore2		1868	0.249
Nikko Bay	pet (r2)	2069	0.071
Nikko Bay2		**2069**	**0.008**
Offshore1		**2069**	**0.013**
Offshore2		**2069**	**0.019**
Nikko Bay	ned	**2258**	**0**
Nikko Bay2		**2258**	**0**
Offshore1		2258	0.178
Offshore2		**2258**	**0.002**

Table 5. Average allelic heterozygosity at each site. The average number is the average across all microsatellite loci, the effective number of alleles takes into consideration relative quantities of each of the alleles, observed heterozygosity is the average amount of heterozygotes across all loci, the expected heterozygosity is the amount of heterozygosity predicted by Hardy-Weinberg, and the fixation index quantifies the amount of inbreeding.

Population	Average # of Alleles	Effective Alleles	Observed Heterozygosity	Expected Heterozygosity	Fixation index
Nikko Bay	9.818	5.482	0.573	0.801	0.275
Nikko Bay2	9.909	6.848	0.556	0.818	0.308
Offshore1	8.909	4.845	0.557	0.722	0.224
Offshore2	8.364	4.934	0.541	0.771	0.309

Table 6. GPS location for the sample sites.

Reef	Latitude(DD.ddd)	Longitude (DD.ddd)
Nikko Bay	7.334486889	134.5048642
Nikko Bay2	7.334358813	134.4933455
Offshore1	7.271427149	134.5230147
Offshore2	7.299455898	134.55847

Appendix B

Figures

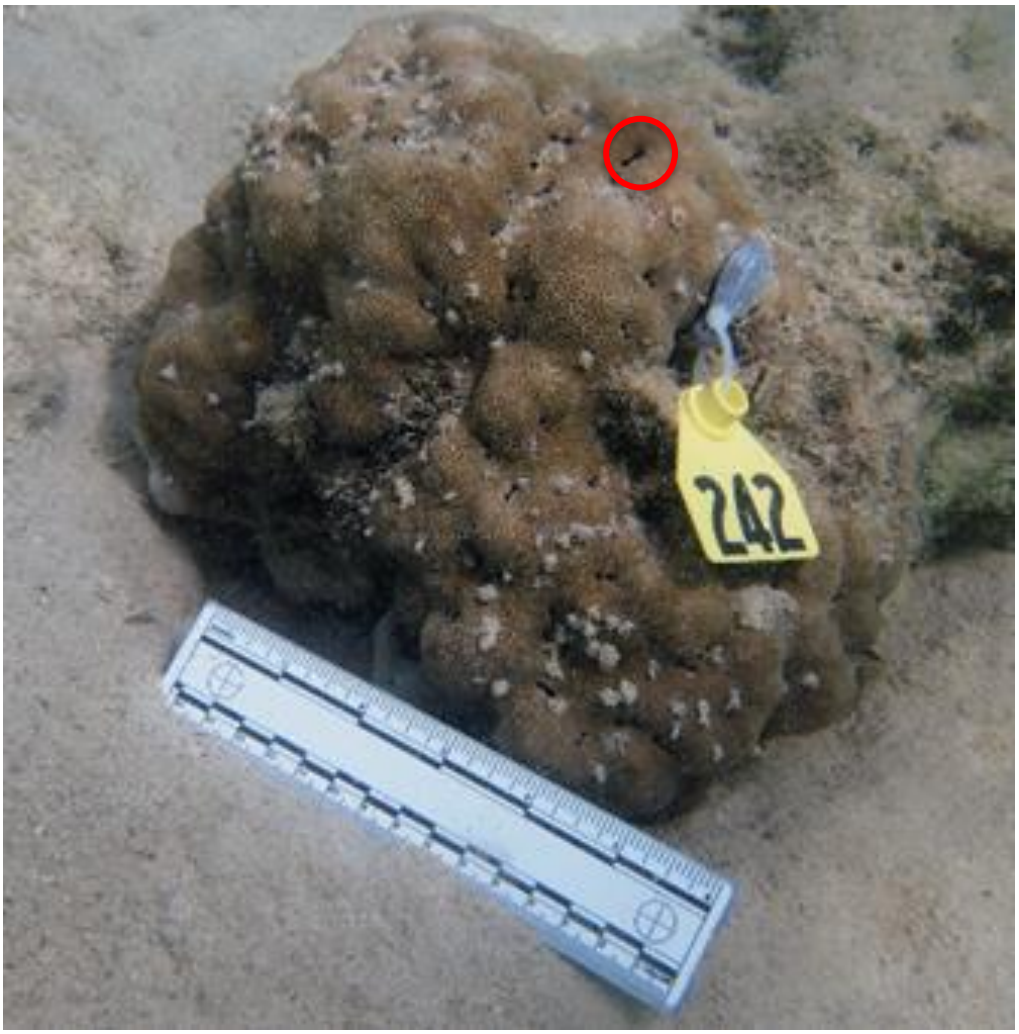

Figure 1. Example of *Porites* image analyzed for mussel density. Circled in the figure is a mussel boreholes that was counted for each colony.

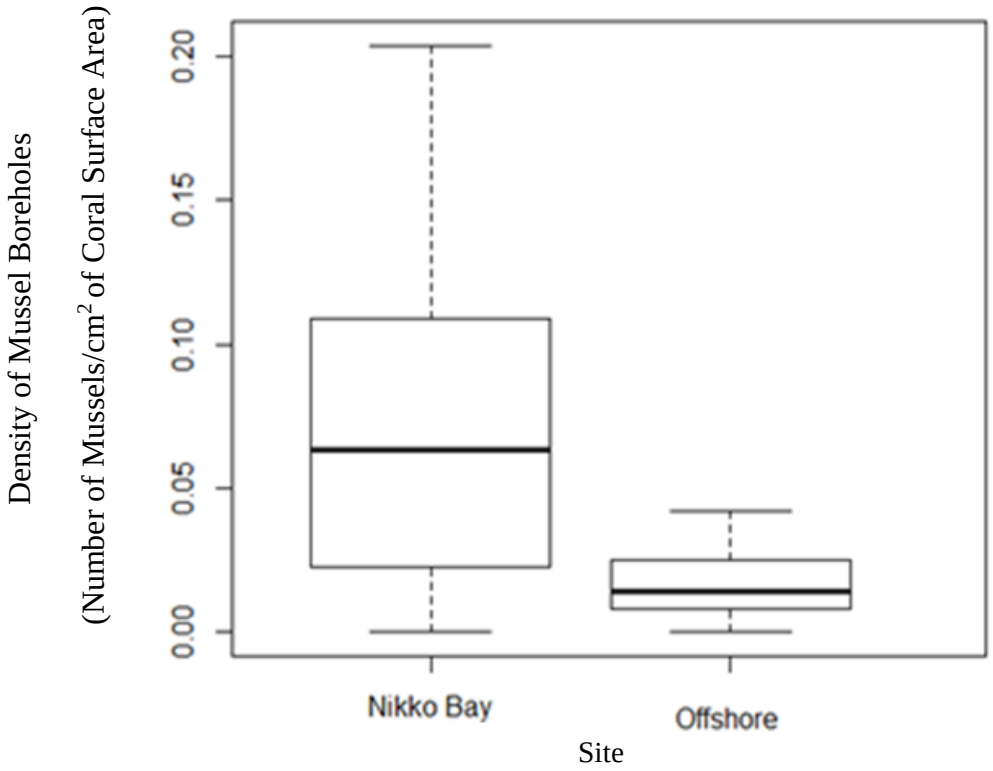

Figure 2. Box and whisker plot of Nikko Bay and offshore mussel density. This plot depicts the minimum, first quartile, median, third quartile, and maximum mussel densities.

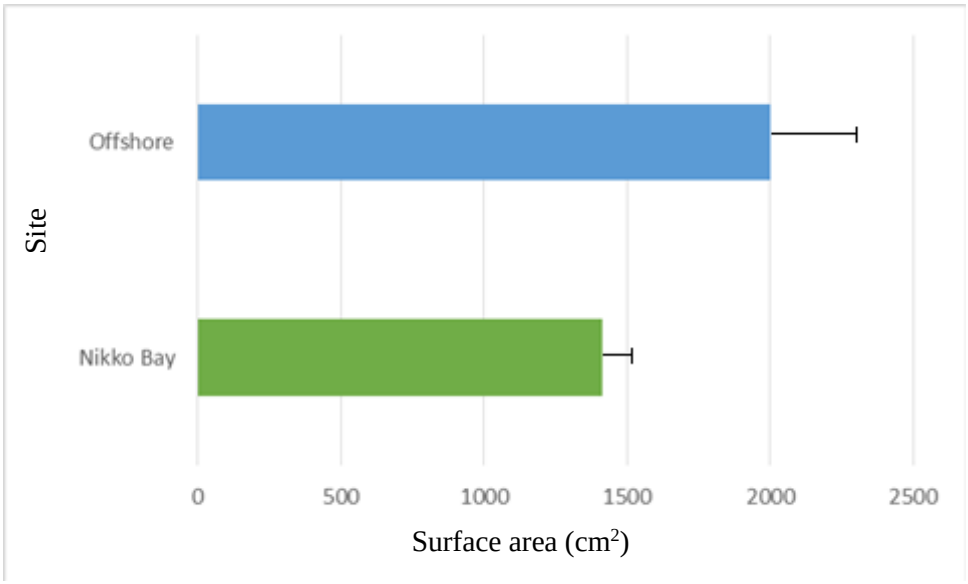

Figure 3. Surface area comparison of Nikko Bay and offshore. This figure shows the average surface of Nikko Bay colonies in comparison to offshore colonies.

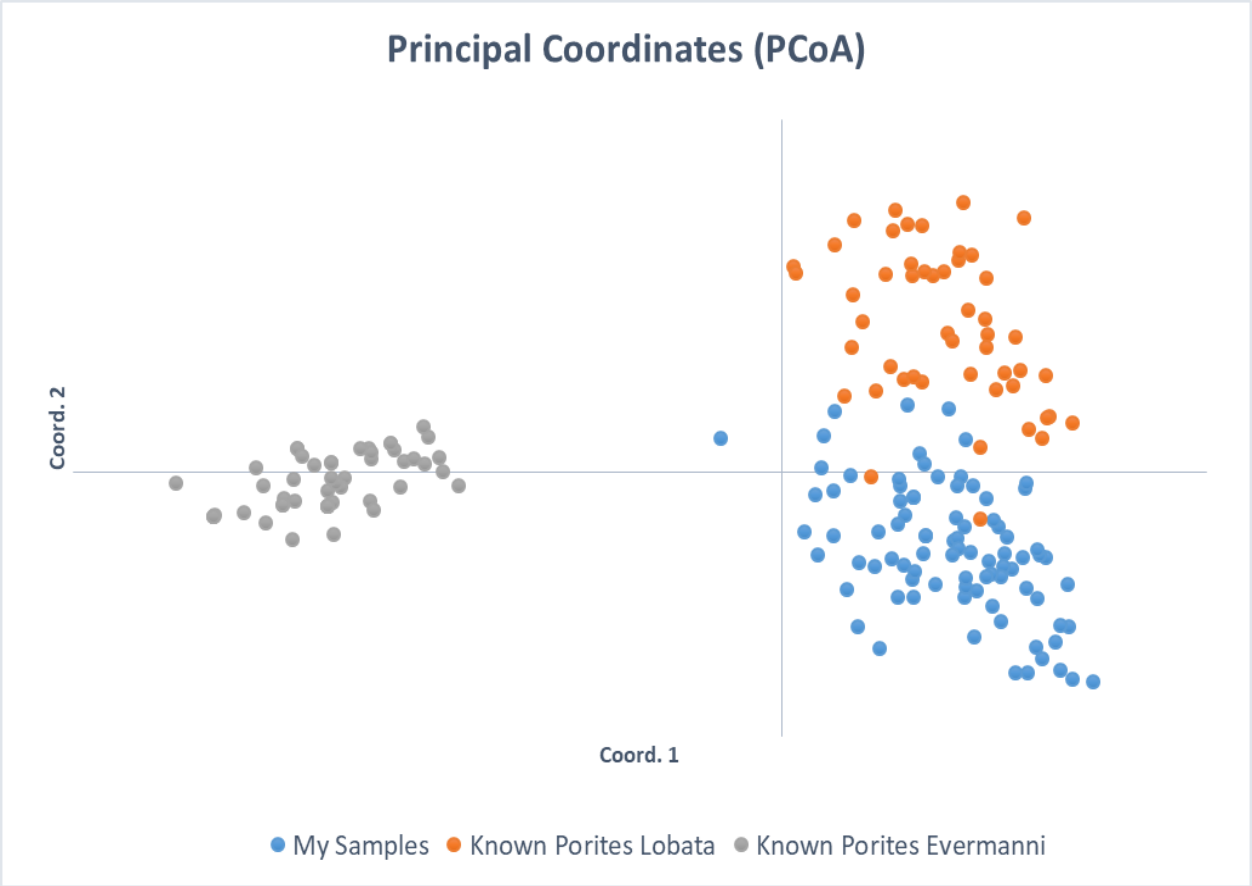

Figure 4. PCoA of my *Porites* samples clustered with known Porites samples from the Eastern Pacific (Boulay et al, 2014). This figure shows the clustering based on genetic distance for samples known to be *Porites lobata,* known to be *Porites evermanni,* and my samples. My samples are shown to cluster exclusively with *Porites lobata,* thus allowing analysis to continue assuming only one species.

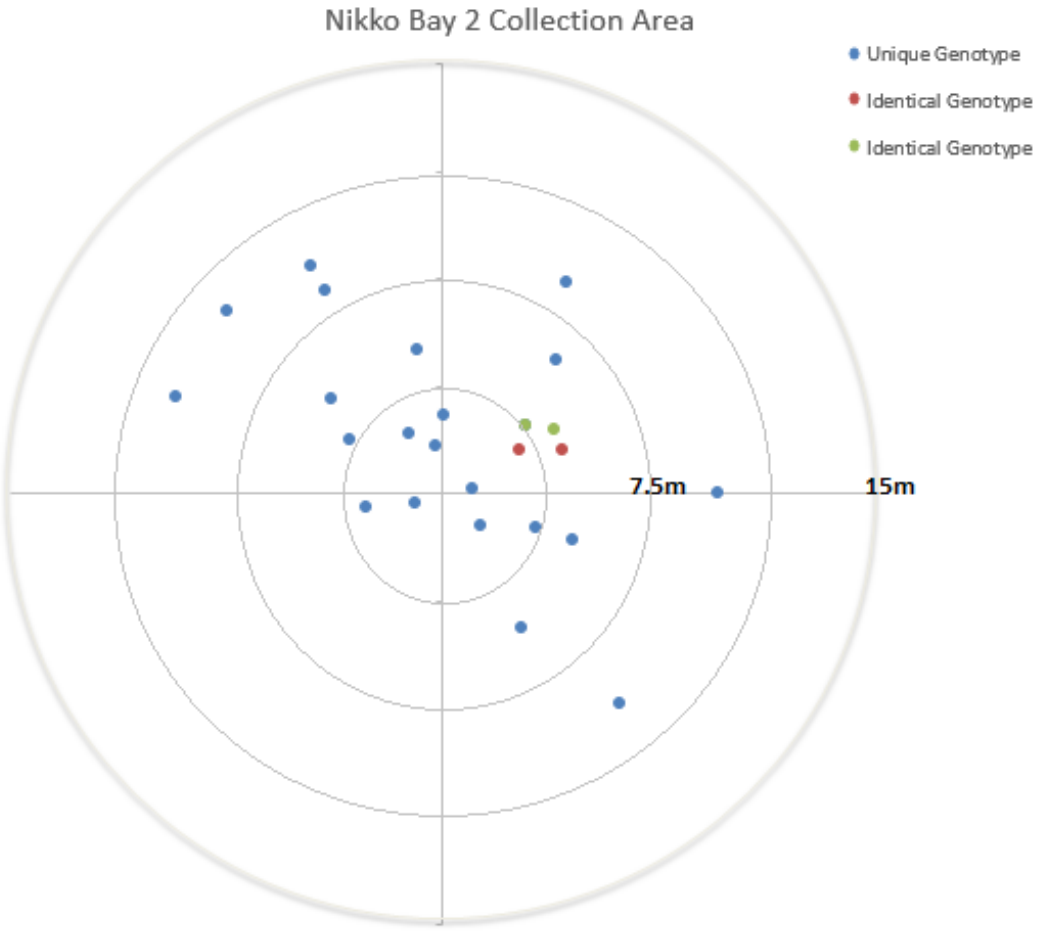

Figure 5. Polar plot of sample collection area for Nikko Bay 2 showing the spatial distribution of the colonies around a center coordinate.

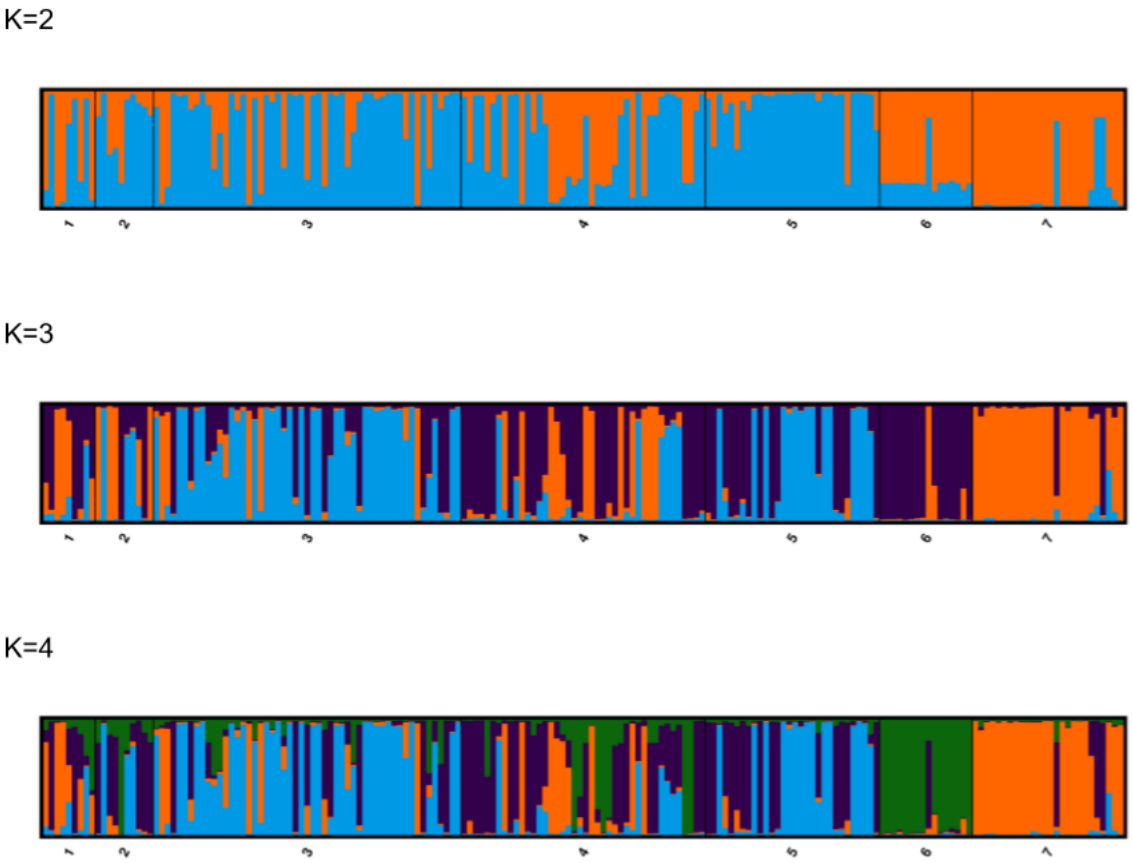

Figure 6. Structure results for *Porites lobata* across several reefs. These graphs show clustering across 5 different population assumptions, K=2 to K=4. The most likely K is K=3. The populations listed in order from 1-7 are from the locations 1=Indonesia, 2=Marshall, 3=Phoenix, 4=Samoa, 5=Society, 6=Lau, 7=Palau (Baums et al, 2012)

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ACADEMIC VITA

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Education
Major: B.S. Biology,
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Honors: Biology

Thesis Title: My Thesis
Thesis Supervisor: Iliana Baums

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Title: Volunteer for Super Utilizer Team

Description: Over the past summer I worked as a volunteer for the super utilizer team at Crozer hospital. As a volunteer, I assisted doctors with making home visits to patients with critical care needs, mobility issues, or chronic illnesses. The goal of the team was to help keep patients out of hospitals which would hopefully improve their quality of life and also help lower medical costs. Each week I would attend a meeting of an interdisciplinary team of doctors and provide feedback and notes based on the visits I attended.

Institution/Company: Crozer Keystone Health System

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Grants Received:

Eberly College of Science Undergradaute Grant

Presentations:

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