POPULATION GENETICS OF SHRIMP AT HYDROTHERMAL VENT COMMUNITIES AT THE MATA VOLCANOES

by

Samantha Brundige

Submitted in partial fulfillment of the requirements for graduation as an Honors Scholar with a

Bachelor of Science

at

POINT LOMA NAZARENE UNIVERSITY

April 2025

Supervised by thesis advisor

Walter Cho (Ph.D.)

and thesis committee members

Andrew Nosal (Ph.D.) and Kristopher Koudelka (Ph.D.)

Abstract

The shrimp species *Rimicaris variabilis* was collected in 2017 from deep-sea hydrothermal vent systems at the underwater Mata Volcanoes during the Underwater Fire research cruise to the Lau Basin. Nuclear DNA was extracted and analyzed from fifty eight samples using four

microsatellite markers with the intent of examining the genetic structure between the five volcano populations. Quality control analyses such as MICRO-CHECKER and a test for Hardy Weinberg equilibrium were performed to determine the usability of the markers. The population structure was measured using tests such as an AMOVA, Mantel Test, and STRUCTURE. Results showed no population structure and indicated high levels of gene flow across the sites, supporting results from previous studies. These results bring forward more information about sites that are understudied, which may give insights into the conservation of these ecosystems.

Introduction

Hydrothermal vents are one of the most unique ecosystems to exist, found only on the bottom of the ocean floor and creating extreme environments for its inhabitants (Tunnicliffe et al., 2024). Hydrothermal vents are formed at the sites of seafloor spreading at divergent plate boundaries or at interplate sites near volcanoes. These openings into the Earth's core allow for cold seawater to interact with hot magma and mix with various chemicals and minerals, which then get pushed back up to the ocean surface. As this liquid reaches the surface, it will cool down and harden to form the chimney like vent structures. These vents are categorized by their temperature, which impacts what minerals are present in the fluid.

In addition to the extreme heat and acidity, on the ocean floor these ecosystems have no exposure to sunlight and therefore are not sights of photosynthesis. Photosynthetic organisms form the basis of every, or nearly every, food chain on the planet. They are the basis of energy and biomaterial, and the biomass of photosynthetic organisms has a direct impact on higher trophic levels (Kutner et al., 2023). However, a different organism serves as the basis of this food chain, chemosynthetic bacteria. The chemosynthetic bacteria use the hydrogen sulfide found at

the vents as a way to make their own food (Van Dover et al., 2018). They do this by using chemical energy to run a process known as chemosynthesis in order to create their own food, just as plants would use solar energy to run photosynthesis. These chemosynthetic bacteria serve as the basis of the food chain for many organisms to exist at the vent, most of which are vent endemic, including gastropods and shrimp. These vents are also ephemeral in nature, meaning that they are readily created and destroyed over the course of only a few decades (Thaler et al., 2011). For its inhabitants, this means that they must be ready and able to move to a new location.

The Mata volcanoes are a series of nine underwater volcanoes located off the coast of Fiji, as seen in Figure 1. These volcanoes exist in a very small area, with only a few kilometers that separate them. The Schmidt Ocean Institute has sponsored many dives, mainly focusing on the North Matas, with the goal of learning more about our oceans in order to conserve them. Located in the Lau Basin, these active volcanoes are the site of hydrothermal vents, housing the many organisms that accompany the unique ecosystem. Two species of shrimp were found across these sites, including *Rimicaris cambonae,* recently recharacterized from *Opaepaele loihi* (Methou et al.), and the other being *Rimicaris variabilis.*

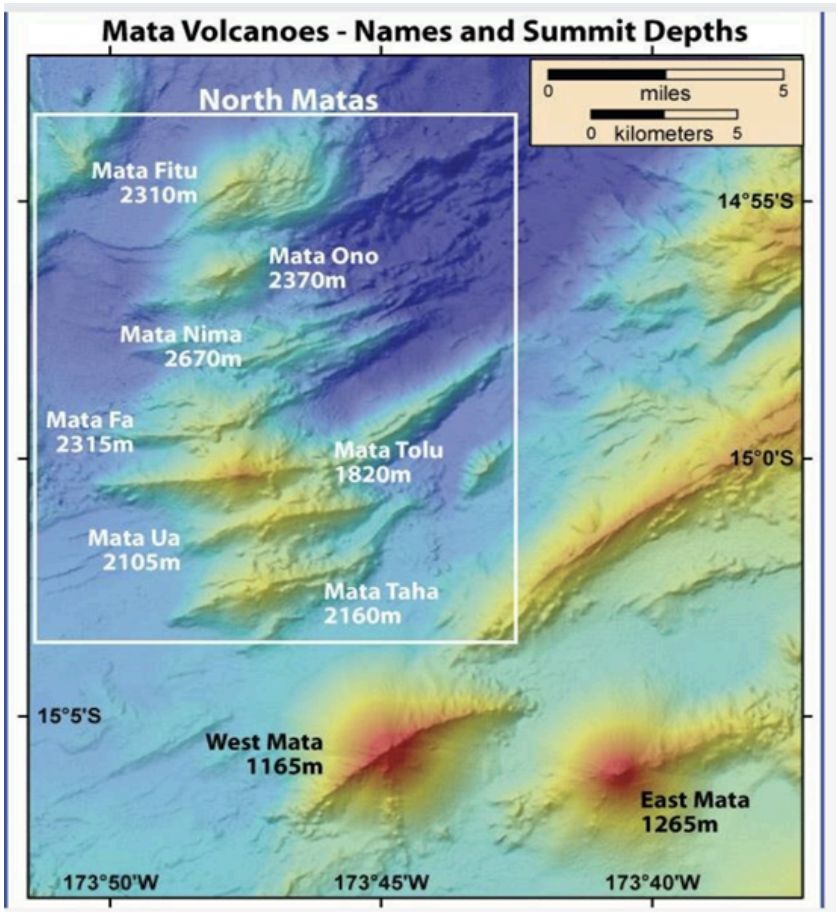

Figure 1: Map showing the depth and distance of the Mata Volcanoes.

R. variabilis is a vent endemic shrimp from the infraorder caridean (Nye & Copley, 2014). Vent shrimp experience distinct stages of development, with a larval and an adult stage. The larval stage is important for disbursement to new locations, such as other vents, especially with the periodical destruction of the ecosystems (Adams et al., 2010). As with other vent shrimp, *R. variabilis* is thought to have a reproductive strategy of brooding, which is when the mother carries the fertilized eggs under her abdomen until they hatch. Once they hatch, they will be developed enough to survive in the extreme environment. They primarily feed on the chemosynthetic bacteria that form the basis of the food chain at these vents.

In 2014, Andrew Thaler published a paper where they studied the population differentiation of the shrimp species *Chorocaris spp.* using microsatellite markers (Thaler et al., 2014). Microsatellites are small tandem repeats in the non-coding region of the genome, with less than ten base pairs in one repeat. Due to the repetition, these sites are more likely to have more mutations. Since these sites are in the non-coding region, these mutations will not have a direct impact on the morphology of the individual and are not typically under selection. This makes them good targets for identification, as the changes are able to be passed on and continue to mutate. These differences allow for the genetics to be different at an individual level, as opposed to a population level, allowing for individual identification. This is useful in determining genetic structure and gene flow within a population, as opposed to other markers which may be more useful for interpopulation analyses.

The *Chorocaris spp.* studied in the Thaler paper was later categorized as *R. variabilis,* the species of interest in this study. They sampled individuals from two basins, Manus and North Fiji, which are thousands of kilometers apart. It was found that there was a distinct genetic isolation between the Manus basin and the other basins. This means that there is likely some barrier to dispersal such as distance, or potentially a physical barrier. This was confirmed in another study from the Manus Basin that concluded that very few species extended to areas beyond their basin (Tunnicliffe et al., 2024).

This pattern has been seen with other vent endemic species as well, as shown in the study done by Xi et al. (2023), where they observed the population genetics of the polychaete *Hesiolyra bergi* across the northern and southern regions of the East Pacific Rise. This study used a mixture of nuclear and mitochondrial DNA markers to determine the connectivity. It was found that within each of the regions, there was high gene flow, but between the northern and

southern regions, there was clear differentiation for multiple markers. This supports the idea that across smaller spatial scales, the vent species are able to uphold a certain level of migration and gene flow necessary to continue their lineage in the conditions of the vent ecosystem.

Looking closer to the study site, in the Manus and North Fiji Basins and the Tonga Arc, multiple species of bythograeid crabs were studied using the CO1 gene (Lee et al., 2019). In this study, it was found that between the North Fiji Basin and the Tonga Arc, which are closer together, there is high gene flow. However, between those two sites and the Manus Basin, which are further apart, there is a distinct population structure. The study suggests that the driving factor for this distinction is the length of the larval stage and the ability to travel certain distances before a necessity to settle. The larvae are able to make it to other vent systems within a certain range, while other systems are too far for the larvae to travel.

Our study aims to determine if *R. variabilis* exhibits gene flow across the volcanoes, or if there is a barrier leading to genetic isolation, even across short distances. Using microsatellite markers, we aim to determine the connectivity of the populations across the volcanoes. This research is part of an interdisciplinary study, aiming to understand the connection between the geology, chemistry, and biology of these unique environments. This will allow us to learn more about how vent ecosystems function, as well as lead to greater insight to help conservation of these unique areas.

Materials and Methods

Sample Collection

The "Underwater Fire" research cruise FK171110 studied the Mata Volcanoes in the Lau Basin in 2017 on the R/V Falkor. Samples were collected using the ROV SuBastian at twenty-one sites across five volcanoes. This was completed in eight dives. Shrimp samples were collected via a suction sampler on the ROV. The samples were sorted on deck by freezing at -80°C or storing in 95% EtOH or RNALater before freezing. Back in the laboratory, the samples were stored long term in a -80°C degree freezer.

DNA Extraction

DNA was extracted from the shrimp using the DNeasy Blood & Tissue Kit from Qiagen (Hilden, Germany). Tissue was taken primarily from the abdomen in order to obtain the highest DNA concentration. The tissue was placed in a 1.5 mL centrifuge tube with 180 µL of Buffer ATL and 20 ul of proteinase K. They were incubated at 56 ° C for three to five hours, once the tissue appeared to be dissolved. Once they were taken out of the incubator, they were mixed by vortexing, and 200 µL of Buffer AL was added immediately and mixed again. The samples were incubated again at 56 ° C for 10 minutes. Then 200 µL of molecular grade ethanol was added to the mixture. A DNeasy Mini spin column was placed into a 2 mL collection tube, then the mixture was piped into the column. These were centrifuged at 8000 rpm for one minute and the flow through was discarded. The column was placed into a new 2 mL collection tube, then 500 µL of Buffer AW1 was added, centrifuged for one minute, and the flow through was discarded. This was repeated with 500 µL of AW2 and centrifuged at 14,000 rpm. The spin column was transferred to a 1.5 mL microcentrifuge tube. The DNA was eluted using 100 µL of Buffer AE,

which incubated for one minute at room temperature and then centrifuged at 8,000 rpm. This step was repeated after one minute. The mixture in the tube contains the DNA and was used for future tests.

DNA Amplification

Concentrations of the DNA in the solutions were assessed using the Nanodrop 2000 or the Nanodrop Lite. Based on these values, dilutions were made in water with 2 μL of the DNA solution, with a goal of a final concentration of 10-20 μL of DNA. Polymerase chain reactions were performed, primarily using the Bio-Rad Reliance One-Step Multiplex RT-PCR Supermix (Hercules, CA). The microsatellite primers, retrieved from Zelnio et al. (2014) as seen in Table 1, were made into a stock with a concentration of 10 μM. The most common technique used was a multiplex PCR, where two to three markers were amplified in one reaction. The forward primer is also tagged with a fluorescent dye, which means that it is light sensitive. Anything containing the primer was wrapped in foil, and all work was done in a drawer or a covered ice bucket. If more precision was needed, the Promega GoTaq kit (Madison, WI) was used including a 5x buffer, MgCl2, forward and reverse primers, dNTP's, taq, water, and DNA in order to tune the reaction to a specific primer or shrimp individual. Cases also occurred where the Bio-Rad procedure was altered by increasing the amount of DNA used to 3 μL and decreasing the water. The program used most often was 95° for 10 minutes, 95° for 10 seconds, 60° for 30 seconds, repeated steps two and three 35 times, then a 4° hold, although the protocol varied depending on sample optimization requirements (Table 2).

Locus	**Base Pair Range**	**Dye**
Cho30	150-200	6-FAM

Cho36	129-189	HEX
Cho63	121-179	6-FAM
Cho91	180-212	HEX
Cho99	188-232	NED

Table 1: Table of microsatellite primers with their expected base pair range and fluorescent dye attached.

Stage	**Temperature**	**Length**
Denaturation	95°	10:00
Annealing	95°	0:30
Extension	52°-60°	0:30

Table 2: Table showing the steps of the Polymerase Chain Reactions used for the microsatellite primers. The denaturation and annealing temperature stayed constant, while the extension stage varied from 52˚ to 60˚.

Fragment Analysis

Once the PCR had finished running, 5 µL of the product was loaded into a 1.5x agarose gel with the intercalating agent ethidium bromide in both the gel and the 1x TBE running buffer. These were run at 120 volts for 30 minutes to an hour in order to view any separation of the banding, particularly in multiplex PCRs. These were then viewed in a gel imager compared to a 100 base pair ladder. Any samples that showed banding in the correct location were then prepared to be sent out for fragment analysis. This was done by making dilutions based on the brightness of the bands, with faint bands having a dilution factor of 1 µL of PCR product to 3 µL

of water, medium bands with a dilution factor of 1 μL PCR product to 5 μL water, and the brightest bands having a dilution of 1 μL PCR product to 10 μL water. These samples were loaded into 0.5 mL tubes and sent out to Eton Biosciences, Inc. (San Diego, CA) where they were run with the LIZ-500 size standard on a 3730xl DNA Analyzer (Applied Biosystems).

Sporadic contamination issues occurred in the PCRs so any samples that showed contamination in the negative control were run again. The contamination was narrowed down to a select few primers, and once new ones were ordered, the contamination issue was resolved.

Data Analysis

The chromatograms received from Eton Biosciences were imported into the STRand Analysis Software by the University of California, Davis' Veterinary Genetics Lab for analysis (Toonen, 2001). For each primer, data including the primer name, dye, and expected base pair range were entered into the program. The standards were set manually by deselecting outliers based on peak size, using the GeneScan Liz 500 standard. The chromatograms were then analyzed to determine peak sizes, which were used to assign genotypes to individual samples. These genotypes were compiled into various data files. Files specific to Arlequin and FSTAT were generated using GENEPOP ON THE WEB (Rousset, 2008).

The data was run through MICROCHECKER in order to check for issues in the data such as null alleles, scoring errors due to stuttering, or large allele dropouts, which may impact the results of Hardy-Weinberg equilibrium and other analyses (Van Oosterhout et al.,2004). Hardy-Weinberg exact tests were performed using GENEPOP ON THE WEB with a probability test (Raymond & Rousset, 1995). This was used to determine if any of the markers are under selection in the population. Hardy-Weinberg equilibrium (HWE) is crucial for identifying if loci

are unusable in the final analysis, as any loci deviating from HWE problematic in determining overall connectivity (Guo & Thompson, 1992). The Markov chain parameters used were 5,000 dememorization steps, 500 batches, and 2,000 iterations. Linkage disequilibrium testing was used to examine the connectivity of alleles from the different loci (Ohta, 1982). This would inform us if the alleles were linked due to proximity on the chromosomes. This test was also done using GENEPOP ON THE WEB with the same Markov chain parameters.

An analysis investigating population structure was run assuming multiple populations of K=1 through K=5, where K is the population number in the program STRUCTURE version 2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007). The STRUCTURE analysis looks at population assignment, determining if there are any distinct populations seen in the data set. It determines the probability for each individual to come from a different population. The series data was filled out with a sample size of 58, two ploidy, five loci, and a missing data value of -91, although there is no missing data. We ran the data with a burnin period of 20,000 and 20,000 MCMC reps after the burnin period.

An estimation of migration rate (Nm) was performed using the rare alleles method on GENEPOP ON THE WEB (Barton & Slatkin, 1986). This estimates the number of migrants per generation, assuming diploid ploidy. For there to be considered gene flow between populations, there must be about one migrant per generation. The final test from GENEPOP ON THE WEB was a Mantel Test, testing isolation by distance using Isolde (Mantel, 1967). A pairwise table was created using the shortest distance from each location. This tests the correlation between geographic distance and genetic diversity. Finally, GENEPOP was used to convert the data files to be compatible with Arlequin and FSTAT.

An Analysis of Molecular Variance (AMOVA) was performed in Arlequin Version 3.5.2.2 to determine the population differentiation based on genetic differences from the molecular markers (Excoffier & Lischer, 2010). This was done based on different geographical scales: region, depth, and individual volcano. This analysis was performed among regions, among populations within regions, within populations, and within individuals. A similar test, population differentiation was performed for both genic and genotypic differentiation across all populations using identical parameters. This test was performed in order to determine if the genetic differences between the individuals are enough to separate into distinct populations, likely based on geographic separations.

PCA-Gen was used to perform a principal component analysis (PCA), which simplifies the dataset and finds patterns of clustering between variables (Goudet, 1999). This allows for a view of any clustering of populations. Finally, FSTAT version 2.9.3.2 was used to calculate Fst values (Goudet, 2001). Fst is the fixation index which also measures genetic differentiation between populations. Fst quantifies genetic variation within subpopulations relative to total genetic variation across the entire population.

Results

A total of 64 individuals from the species *R. variabilis* were collected from the Underwater Fire expedition, and all 64 had DNA extracted. However, only 57 of these samples were able to be completely run through the process and analyzed for all five of the markers due to time constraints and issues of contamination and precision of the markers. MICROCHECKER revealed that Cho99 had an excess of homozygotes in the population, indicating that it may not be able to be used for data analysis (Figure 2). Overall, a test for Hardy-Weinberg equilibrium

had a mean p-value of 0.251368 with five markers and 0.775714 with four markers (Table 3). However, Cho99 as an individual sample set showed a p-value of 0.015166, and was found to be significant at two populations, Mata Ua and Mata Tolu. These had p-values of 0.0081 and 0.0038 respectively. Cho30 also appeared to be significant in the population of Mata Ua with a p-value of 0.0168, although it was not significant itself. This was seen in both runs of HWE. When looking at the linkage between the markers for the four loci, there was found to be no significance for any combination of markers, as seen in Table 4.

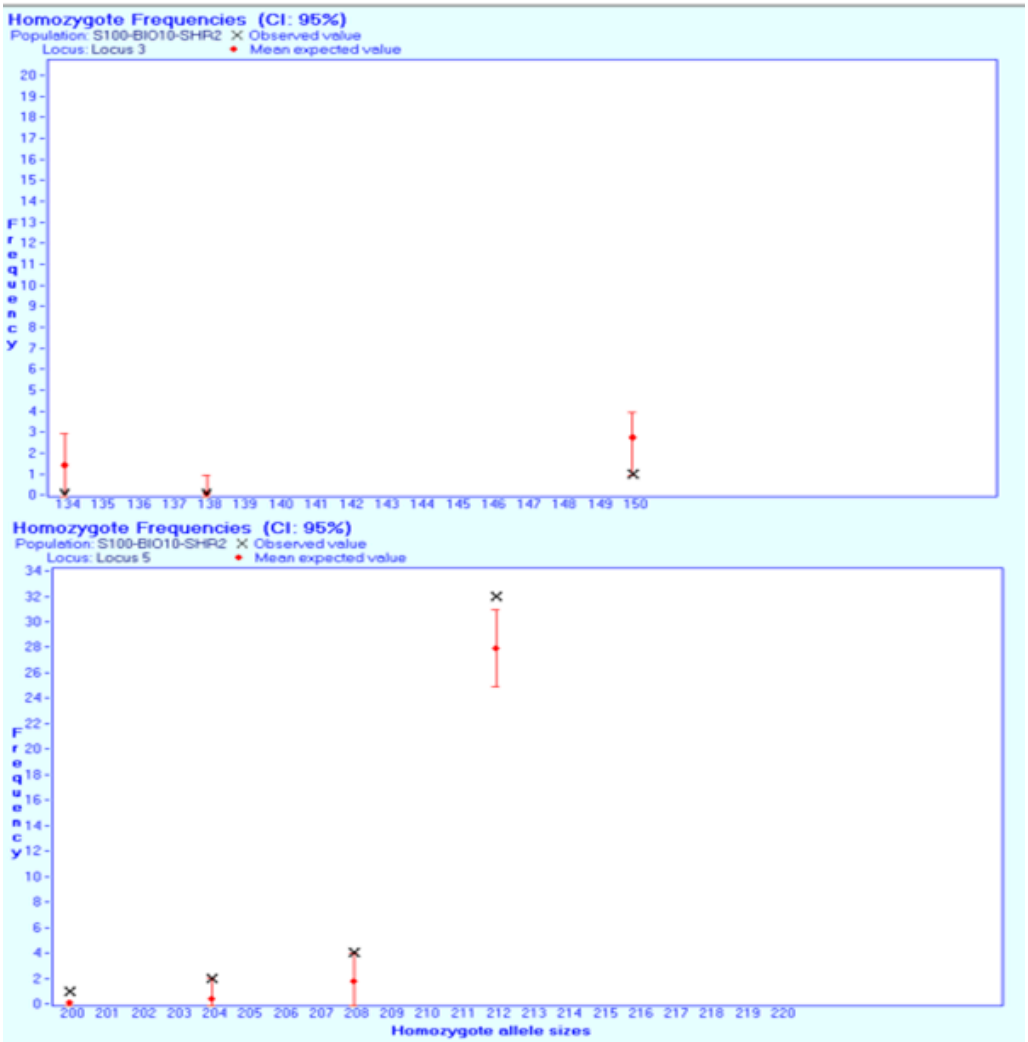

Figure 2: Figure showing the results of MICROCHECKER on two example loci. The top image is of Cho63, showing no issues, while the bottom image is of Cho99, showing a large number of one allele. This shows there is an excess of homozygotes, which may show null alleles or dropouts, which could affect the data set.

	Mata Fitu	**Mata Ono**	**West Mata**	**Mata Ua**	**Mata Tolu**
Cho30	1.000	1.000	N/A	**0.0168**	1.000
Cho91	1.000	1.000	N/A	0.1939	0.1448
Cho63	0.6300	N/A	1.000	0.1335	0.1995
Cho36	0.7509	N/A	1.000	0.4836	0.9054
Cho99	**0.0081**	1.000	1.000	**0.0038**	0.5386

Table 3: Results from Hardy-Weinberg equilibrium test with the significant values in bold. N/A indicates that no information was found, which should indicate no deviations from Hardy-Weinberg.

Locus Pair	**Chi2**	**df**	**P-Value**
Cho30 & Cho91	2.600530	8	0.956878
Cho30 & Cho63	2.690169	8	0.952273
Cho91 & Cho63	5.292134	8	0.725945
Cho30 & Cho36	0.286416	8	0.999984
Cho91 & Cho36	9.455294	8	0.305354
Cho63 & Cho36	6.714938	8	0.567678
Cho30 & Cho99	1.679519	8	0.989311
Cho91 & Cho99	5.125571	8	0.744074
Cho63 & Cho99	9.647034	8	0.290678

Cho36 & Cho99	4.801619	8	0.778554

Table 4: Results from the linkage disequilibrium test, determining if the loci are jointly inherited. All of the p-values above are high above the significance level, indicating no joint inheritance of any loci.

The results from the quality analyses indicated that Cho99 was an unfit primer for further analysis. The indication of null alleles and the deviations from Hardy-Weinberg indicate that the results from this primer are not able to be trusted. Due to this, the following analyses of population structure were completed using only the four other primers.

The STRUCTURE analysis showed there was no distinct separation into populations, no matter how many populations were designated (Figure 3). If the STRUCTURE results were to be significant, the bars would be vertical, to show individuals sorted into distinct populations. However, the bars are horizontal, indicating that there is an equal probability that each sample could have come from any population.

Figure 3: STRUCTURE results indicating population assignment for four (right) and five (left) loci assuming three and five populations for regions and volcanoes. Vertical bars would indicate distinct populations found within the samples, while the horizontal bars indicate the samples cannot be separated into distinct populations.

The number of migrants was also measured and adjusted to the population size, with an average of 2.76643 migrants for the set of four loci (Table 5). For a population to be considered to have genetic diversity, there must be at least one migrant per generation, which this population exceeded. The mantel test showed now significance with a p-value of 0.36610.

Number of Migrants for Mean N=10	3.20906
Number of Migrants for Mean N=25	1.42512

Number of Migrants for Mean N=50	0.958082
Number of Migrants After Correction for Size	**2.76643**

Table 5: Table showing the estimates of the number of migrants (Nm) using the rare alleles method. For a population to be considered diverse, there must be more than one migrant per generation. This shows that there are about three migrants per generation.

The results of the AMOVA showed significance in one area, which was among populations within regions when separated out by region (North vs. South vs. West Mata), as seen in Table 6. As the structure became more narrowed, there was no significance. The population differentiation test showed a significance at Cho63 and an overall significance across all loci (Table 7).

Comparison	**Among Regions**	**Among Populations within Regions**	**Within Populations**	**Within Individuals**
Volcanic Region (North vs. South vs. West Mata)	0.74813	**0.01848**	0.65448	0.43207
Depth	0.48836	0.09564	0.64799	0.4421
Volcano	0.20822	0.21516	0.65553	0.43527

Table 6: AMOVA results determining significance of each sample set from different geographical separations. The data was split up by region, depth, and by volcano. The significant p-values are highlighted in bold, indicating that there is evidence of population distinction at that level of separation.

P-Value Across all Loci (Fisher's Method)	
Locus	**P-Value**
Cho30-MSA	0.099231
Cho91-MSA	0.144275
Cho63-MSA	**0.035378**
Cho36-MSA	0.443815
Chi2=16.8007 (df=8)	P-Value=**0.032253**

Table 7: Results from the genotypic differentiation test. All significant values are bolded, with Cho63 showing significance, and there being an overall significant difference.

PCA-Gen showed no clustering of any of the populations, as shown in Figure 4. The populations at each volcano (as seen in the color matching the key) were all mixed around on the chart and did not show any evidence of clustering or separation. Fstat gave Fis values, which for the most part showed to be around zero (Table 8). However, at West Mata, Cho63 had a value of -1.00. Cho30 had a value distant from zero at Mata Ua, with a value of 0.484. Many loci had values of 0.00 at multiple populations, verifying the results of HWE. Fstat also gave allelic richness, which averaged around 2-3 alleles per locus and population (Table 9). The combination with the lowest allele size was Cho30 at West Mata with only 1.00 allele. Cho 63 had the largest number of alleles in the population, with 3.00 found at West Mata.

Figure 4: Results of the principal component analysis from PCA-Gen, showing if there is clustering of populations. The numbers indicate the fourteen subpopulations, split into the

five volcanoes as assigned by colors. No clustering of colors indicates no distinct populations.

	Mata Ua	**Mata Ono**	**West Mata**	**Mata Tolu**	**Mata Fitu**
Cho30	0.484	-0.200	N/A	-0.090	-0.163
Cho91	0.273	0.143	0.000	0.258	-0.127
Cho63	0.088	0.000	-1.000	-0.032	-0.011
Cho36	-0.116	0.000	-0.333	-0.158	-0.074

Table 8: Results of the Fis estimates from FSTAT of five (top) and four (bottom) loci. Values close to -1.000 indicated heterozygote excess while values close to 1.000 indicate heterozygote deficiency. Values close to zero show that there is a balance of homozygotes and heterozygotes, indicating gene flow.

Allelic Richness per Locus and Population						
Based on min. Sample size of: 2 diploid individuals						
Population	**Mata Ua**	**Mata Ono**	**West Mata**	**Mata Tolu**	**Mata Fitu**	**All**
Cho30	**1.978**	**1.786**	**1**	**1.52**	**1.807**	**1.692**
Cho91	**1.875**	**1.971**	**2**	**1.634**	**1.652**	**1.739**
Cho63	**2.663**	**1.5**	**2**	**2.226**	**2.996**	**2.476**
Cho36	**2.107**	**1.5**	**3**	**2.021**	**2.497**	**2.113**

Table 9: Table showing allelic richness from FSTAT with five (left) and four (right) loci. These values indicate the number of alleles in each population to determine genetic diversity. Average is around two to three alleles, which indicates diversity.

Discussion

This study allowed for a greater insight into the population structure of hydrothermal vent communities, specifically *Rimicaris variabilis* at the Mata Volcanoes. After analysing four microsatellite markers from 61 individuals of this species, we were able to further learn about the connectivity of this population. The results of this study indicate high levels of genetic connectivity among *R. variabilis* populations across the Mata volcanoes. The findings from these microsatellite analyses align with previous microsatellite and mitochondrial studies that suggested significant gene flow among the hydrothermal vent shrimp populations (Thaler et al. 2014). Although other studies have shown greater geographic distances to be a barrier to gene flow, this data suggests that *R. variabilis* is capable of maintaining gene flow across smaller spatial scales in the Lau Basin. These results do not prove to be surprising considering the ephemeral nature of the vents and the larval dispersal tactic of the shrimp.

MICROCHECKER revealed that Cho99 had an excess of homozygous individuals. This suggests possible null alleles, which may have contributed to deviations from the dataset. The Hardy-Weinberg Equilibrium analysis indicated that most loci were in equilibrium, with the exception of Cho99, which showed an excess of homozygotes in certain populations. The significance values here indicated that Cho99 may be under selection and would not be suitable for population genetic analyses. By removing Cho99 from the analysis, the results became clearer, and likely more reflective of the population structure. Furthermore, linkage disequilibrium results did not indicate significant associations between alleles. This supports the

assumptions of independent assortment among the loci. These analyses served as quality checkers for the loci and played a role in determining if the loci would be suitable for population genetic analyses.

The lack of genetic differentiation, as shown by STRUCTURE and AMOVA, suggest that barriers to dispersal are minimal within the study region. The presence of larval dispersal mechanisms likely play a crucial role in this dispersal. As vent shrimp such as *R. variabilis* undergo a planktonic larval stage, which enables them to be carried by deep-sea currents to new vent sites at different volcanoes. This mobility is essential given the ephemeral nature of hydrothermal vents, as populations must be able to recolonize newly formed vents rapidly to ensure survival.

The AMOVA results demonstrated that the majority of genetic variation is found within individuals rather than among populations or regions. This finding supports the hypothesis that *R. variabilis* is moving across the Mata volcanoes, with little evidence of structured genetic subpopulations. The lack of strong isolation by distance, as shown by the Mantel test, further suggests that dispersal across vent sites occurs at a sufficient rate to prevent genetic divergence over the scales examined in this study.

The F-statistic and allelic richness results further support the conclusion of high gene flow. The average allelic richness ranged from 2-3 alleles per locus, indicating a level of genetic diversity, which is consistent with expectations for this hydrothermal vent population. While some loci, such as Cho30 at West Mata, displayed lower allelic richness, this is likely due to sampling limitations rather than true population structure. West Mata, only having two samples, was expected to have potential significant results in some analyses. The genotypic differentiation

results showed little significance, which could indicate low diversity and gene flow. However, since this result is not consistent with other tests, it is likely not telling the whole story.

These results indicate high gene flow and no population structure across any spatial scale. These findings are supported by multiple other studies that have been completed with similar vent species. Most notably, the study by Andrew Thaler, where he looked at *R. variabilis* between different basins in the south pacific (Thaler et al., 2014). Other studies concerning the same populations and locations from this study show a consistent story. In Erman & Mahmoudi et al., 2024, mitochondrial DNA was analyzed using the CO1 gene to determine connectivity of *R. variabilis* across the Mata Volcanoes. Another study using a species of snail, *Ifremaria nautilei*, analyzed six microsatellite markers to determine connectivity. Both of these studies, with such close relation to our study, showed no population structure and high gene flow across the volcanoes. This supports our findings with the same study site and the same population.

These results appear to be supported through other species as well, indicating these results are common and expected for vent endemic species. For Xi et al., 2023 and Lee et al., 2019, both examined different species, polychaetes and crabs, respectively. Both of these studies found that across a smaller spatial scale, there was high gene flow and no population structure. With our study having a geographical range of about five to ten kilometers, it is well within reason to allow the spatial scale to support our conclusions.

While the geography is a large clue to supporting our hypothesis, the biology also provides insights into why these conclusions may hold true. As mentioned earlier, the shrimp have a brooding reproductive strategy, with the larvae being released more fully developed, allowing for higher survival and a wider range of distribution in the extreme environments

(Adams et al, 2010). The results of this study are only strengthened by looking at the study from multiple perspectives.

The findings of this study have important implications for conservation and management of hydrothermal vent ecosystems. The observed genetic connectivity suggests that vent shrimp populations can recover from localized disturbances, as long as source populations remain intact. However, given the increasing interest in deep-sea mining and hydrothermal vent exploitation, understanding the resilience and dispersal capabilities of vent-endemic species is crucial. Before any conclusions are drawn in this area, further studies should be done on other species and other populations of *R. variabilis* in different geographic locations.

Overall, our study provides evidence for high levels of gene flow among *R. variabilis* populations across the Mata volcanoes. The combination of microsatellite analysis, STRUCTURE modeling, and AMOVA suggests that genetic structure is minimal across the study sites, likely due to larval dispersal and the hydrothermal vent ecosystem's dynamic nature. While the findings align with previous mitochondrial studies, additional genomic investigations may help refine our understanding of vent shrimp population dynamics and their resilience in the face of environmental and anthropogenic changes.

Acknowledgement

I would like to deeply thank my mentor, Dr. Walter Cho, for his guidance, support, and encouragement throughout this project. I am also sincerely grateful to my committee members, Dr. Andrew Nosal and Dr. Kristopher Koudelka, for their insightful feedback and expertise, which greatly contributed to the development of this project.

I would like to acknowledge the Point Loma Nazarene University Summer Research Program and thank the Research Associates for providing the funding for the opportunity to work alongside fellow students and professors, making this project possible. Finally, I would like to thank Dr. Mark Mann for his leadership and for giving me the opportunity to share my research as a part of the Honors Program.

Adams, D. K., Mills, S. W., Shank, T. M., & Mullineaux, L. S. (2010). Expanding dispersal studies at hydrothermal vents through species identification of cryptic larval forms. *Marine Biology*, *157*(5), 1049-1062. https://doi.org/10.1007/s00227-009-1386-8

Barton, N. H. & Slatkin, M., 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 56: 409-415.

Erman, L. & Mahmoudi, S., (2024). Elucidating Population Genetics of Shrimp Species on Underwater Volcanoes. [Honor's Thesis]. Point Loma Nazarene University.

Excoffier, L. and H.E. L. Lischer (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10: 564-567.

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164(4), 1567–1587. https://doi.org/10.1093/genetics/164.4.1567.

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular ecology notes, 7(4), 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x.

Guo, S. W. & Thompson, E. A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361-372.

Goudet, J. (1999) PCA-GEN for Windows, V. 1.2. Distributed by the author, Institute of Ecology, Univ. of Lausanne.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from Goudet (1995).

Kutner, D. S., Bowman, J. S., Saldanha-Corrêa, F. M. P., Chuqui, M. G., Tura, P. M., Moreira, D. L.,…Signori, C. N. (2023). Inorganic carbon assimilation by planktonic community in Santos Basin, Southwestern Atlantic Ocean. *Ocean & Coastal Research*, *71*, 1-18. https://doi.org/10.1590/2675-2824071.22085dsk

Lee, W.-K., Kim, S.-J., Hou, B. K., Van Dover, C. L., & Ju, S.-J. (2019). Population genetic differentiation of the hydrothermal vent crab Austinograea alayseae (Crustacea: Bythograeidae) in the Southwest Pacific Ocean. *PloS One*, *14*(4), e0215829. https://doi-org.pointloma.idm.oclc.org/10.1371/journal.pone.0215829

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

Methou, P., Chen, C., & Komai, T. Revision of the alvinocaridid shrimp genus Rimicaris Williams & Rona, 1986 (Decapoda: Caridea) with description of a new species from the Mariana Arc hydrothermal vents. (1175-5334 (Electronic)).

Nye, V., & Copley, J. (2014). Reproductive ecology of a hippolytid shrimp, Lebbeus virentova (Caridea: Hippolytidae) at the Von Damm Vent Field, Mid-Cayman Spreading Centre. *Marine Biology*, *161*(10), 2371-2380. https://doi.org/10.1007/s00227-014-2512-9

Ohta, T., 1982. Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proc. Natl. Acad. Sci. U. S. A*. 79: 1940-1944.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945.

Raymond M. & Rousset F, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity, 86:248-249

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resources 8: 103-106.

Thaler, A. D., Plouviez, S., Saleu, W., Alei, F., Jacobson, A., Boyle, E. A.,…Van Dover, C. L. Comparative population structure of two deep-sea hydrothermal-vent-associated decapods (Chorocaris sp. 2 and Munidopsis lauensis) from southwestern Pacific back-arc basins. (1932-6203 (Electronic)).

Thaler, A. D., Zelnio, K., Saleu, W., Schultz, T. F., Carlsson, J., Cunningham, C.,…Van Dover, C. L. (2011). The spatial scale of genetic subdivision in populations of Ifremeria nautilei, a hydrothermal-vent gastropod from the southwest Pacific. *BMC evolutionary biology*, *11*, 372. https://doi.org/10.1186/1471-2148-11-372

Toonen RJ and Hughes S, Increased Throughput for Fragment Analysis on ABI Prism 377 Automated Sequencer Using a Membrane Comb and STRand Software, Biotechniques, 31:1320-1324 (Dec 2001).

Towson, J. (2024). Patterns of Population Structure and Gene Flow of Gastropods at Hydrothermal Vents at the Mata Volcanoes. [Honor's Thesis]. Point Loma Nazarene University.

Tunnicliffe, V., Chen, C., Giguère, T., Rowden, A. A., Watanabe, H. K., & Brunner, O. (2024). Hydrothermal vent fauna of the western Pacific Ocean: Distribution patterns and biogeographic networks. *Diversity & Distributions*, *30*(2), 1-16. https://doi.org/10.1111/ddi.13794

Van Dover, C. L., Arnaud-Haond, S., Gianni, M., Helmreich, S., Huber, J. A., Jaeckel, A. L.,…Yamamoto, H. (2018). Scientific rationale and international obligations for protection of active hydrothermal vent ecosystems from deep-sea mining. *Marine Policy*, *90*, 20-28. https://doi.org/https://doi.org/10.1016/j.marpol.2018.01.020

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICROCHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Xi, L., Sun, Y., Xu, T., Wang, Z., Chiu, M. Y., Plouviez, S., Jollivet, D., & Qiu, J. (2023). Phylogenetic divergence and population genetics of the hydrothermal vent annelid genus Hesiolyra along the East Pacific Rise: Reappraisal using multi‐locus data. *Diversity & Distributions*, *29*(1), 184–198. https://doi-org.pointloma.idm.oclc.org/10.1111/ddi.13653

Zelnio, K. A., Thaler, A. D., Jones, R. E., Saleu, W., Schultz, T. F., Dover, C. L. v., & Carlsson, J. (2010). Characterization of nine polymorphic microsatellite loci in Chorocaris sp. (Crustacea, Caridea, Alvinocarididae) from deep-sea hydrothermal vents. *Conservation Genetics Resources*, *2*, 223-226.