Population Structure and Diversity of Polynoids Associated with Tubeworms at Axial Seamount

Andrew Paley
University of Washington, Regional Cabled Array, Molecular Ecology Research Lab
apaley@uw.edu
May 28, 2023

Abstract:

Polynoid worms at Axial Seamount represent a sizable portion of the already annelid dominant environment but have comparatively little work examining the population structure of the taxa. This study examined polynoid diversity and population structuring specifically associated with *Ridgeia piscesae* within the Caldera. Individual worms were sampled from three sites across the seamount, including the Inferno Chimney and a site of diffuse flow within the ASHES vent field and from the El Guapo Chimney within the International District field. DNA was extracted from the elytra of the Polynoids and sequenced using Restriction Site-Associated DNA sequencing to subsample a significant portion of the genome. Analysis of heterozygosity and Fis, (the inbreeding coefficient), revealed that high diversity and a low degree of inbreeding were present within the sample groups in contrast to what is observed in other deep-sea species. In addition, paired Fst analysis, Admixture, and DAPC all revealed little to no genetic differentiation between groups and that a high degree of shared ancestry was present across sites. This shared ancestry suggests that populations of Polynoids within the Caldera are panmictic and can be considered a single genetic population. This finding aligns with hypotheses of pseudo-copulatory reproduction in other deep-sea species as it allows for the copulatory mating behavior and internal fertilization proposed to still be present within the species dwelling in the Caldera while also allowing sufficient flexibility for the dispersal of propagules. This holds important implications for conserving deep-sea polynoids and presents a need for further research into their reproductive characteristics.

Plain Language Summary:

Hydrothermal systems are a unique environment within the world's oceans, acting like oases in the abyss where animals congregate forming complex, multi-layered communities. Here, tiny, fuzzy worms called Polynoids, or scaleworms dwell, feeding on the bright-red plumes of the tubeworms and acting as voracious predators. Compared to the other inhabitants of the vents, little is known about scaleworm populations and how they reproduce. This study aimed to examine the interconnectivity of scale worm populations at hydrothermal systems, also known as their population structure. This study employed genetic techniques, specifically looking at differences in recurring small mutations known as SNPs, that look for differences between the genetic material of groups and tease out the structure of populations. Individual Scaleworms

were sampled from Axial Seamount and subsequent results found that there was a high degree of connectivity between the groups and little genetic differentiation, meaning there was little population structuring at play and all groups were behaving as one large population. This result is significant because it suggests that previously held ideas about how these worms reproduce may not be complete, which has essential complications for conserving deep-sea animals. Mining operations for example may drastically reduce population size, resulting in a population bottleneck and the accumulation accumulation of harmful mutations to their genetic code through inbreeding. It is thus irresponsible to pursue such destructive operations unless more research is conducted on not only the population characteristics and behavior of different species of scale worms but also the potential impacts of mining.

Introduction:

Dissolved sulfides within vent fluids on the seafloor serve as a basis for many biological communities near hydrothermal vents. In turn, it represents an oasis in the deep ocean for some of Earth's most complex biological communities (Tunnicliffe, 1991). Tubeworms, such as *Riftia sp*. and *Ridgeia sp.*, constitute one of the main trophic layers above microbial producers. Their symbiotic, chemosynthetic bacteria utilize available hydrogen sulfide to produce sugars and other compounds that sustain the host. This relationship also facilitates the export of nutrients to higher trophic levels through predation, forming complex assemblages that function as a physical substrate for large invertebrate communities with many trophic complexes and high biodiversity (Leliévre et al, 2018; Tunnicliffe 1991). Within these assemblages, charismatic tube worms, and Alvinellidae worms, with their stunning plumes and chemosynthetic symbionts, often garner more attention from researchers than other organisms. As a result, there is a gap in the literature regarding other organismal components of tubeworm assemblages.

Polynoids, or scale worms, represent one of these overlooked organisms and require further study to understand their role in this complex environment. Polynoids are small predatory worms believed to graze on the brachial plumes of tubeworms and other microbes in various environments, including hydrothermal vents (Bonifácio and Menot, 2019). Depending on the size of the Polynoid, a strike of the predatory worm's eversible pharynx can result in a range of outcomes, from subtotal removal of small portions of the plume to amputation of the entire anterior segment of the prey animal. Polynoids are one of the most diverse families in their class and occupy a variety of ecological niches throughout hydrothermal vents, from parasites to large motile predators. Despite this, little is known about these organisms' population structure and diversity within the deep ocean (Kinberg, 1856). Furthermore, few studies aim to specifically examine Polynoids in a hydrothermal environment—choosing instead to focus only on their role in relation to other taxa, like the microbiota of the better-known *Ridgeia sp*. (Lelievere et al, 2018). Axial Seamount has some of the greatest numbers of observed tubeworm assemblages within a hydrothermal environment, and this proposed study aims to expand our understanding of Polynoid population characteristics and diversity (Tunnicliffe, 1991).

Trophic analysis of community aggregates of *Ridgeia piscesae* within the Juan de Fuca Ridge revealed that the four most common predatory species in the benthic environment were Polynoids and that *R. piscesae* constituted 83% of their diet, by biomass, in the area (Bergquist et

al, 2007; Levin et al, 2009). This suggests that Polynoids serve as a significant control on populations of tubeworms in hydrothermal environments. However, outside of a predatory role, Polynoids demonstrate one of the highest diversities among polychaete-dominated venting systems and have been observed to exhibit a high degree of specialization—both of which contribute to a lagging recognition of many species with respect to their greater role in the ecosystem (Wu et al, 2019; Bergquist et al, 2007).

The literature recognizes several unique aspects of these organisms. One study of the reproductive anatomy of a commensal Polynoid species living in sulfide clams suggests brooding in which the mother carries the developing offspring as a possible key aspect of their reproductive life history (Van Dover et al, 1991). This reproductive life history contrasts with the typical broadcast spawning of other hydrothermal organisms. It may limit the ability of Polynoids to disperse progeny across a large area and expand to new habitats in the seafloor, a key aspect in the mixing of deep ocean populations. Other groups, like tubeworms, use bottom currents to disperse their propagules; however, little research has been conducted on the larval dispersal of deep-sea Polynoids by comparison making it challenging to understand the mechanisms at play (Hilario et al, 2015; Tyler and Young 2003). The expansion of hydrothermal species is also limited not only by their reproductive life history but by the relative paucity of the specific conditions they need to survive. The appearance of new diffuse flow, venting sites, or whale falls which provide the necessary conditions for sulfide-reliant organisms to survive, are sporadic events and yet are thought to be major routes of expansion for these highly specialized species between venting sites (Feldman et al, 1998). Thus, due to a reliance on short-range reproduction and the rarity of opportunities to expand across the seafloor between venting sites, I hypothesize that polynoid worms within sites such as the Axial Caldera may exist as genetically distinct populations rather than a single homogenous population.

The biology of deep ocean organisms is notoriously challenging to study, given the remoteness of these animals' environments. During the field's infancy, biologists were often limited to specimens dredged up in the nets of anglers or, in more recent years, the few samples that could be brought up in submersibles or remotely operated vehicles (Gage and Tyler, 1991). However, the development of molecular tools has genuinely revolutionized the field of deep research. With only a comparatively small number of organisms, researchers can now answer questions regarding diversity, population structuring, and evolution, offering unprecedented

insights into the lives of these isolated populations and how they might relate to other parts of the world's oceans (Harden-Davies, 2017). Molecular methods allow researchers to ask population-level questions, which can serve as a great starting point to truly begin to understand these populations, with molecular data providing information about both the population and individual, diversity and population structure, and other metrics across a range of spatial scales (Hauser et al, 2011). Restriction site-associated DNA sequencing, among other methods, represents a unique avenue for studying Polynoids, opting to subsample the entire genome. In addition, "RAD-seq" can be a fantastic tool for organisms that lack a large-genetic database and references (such as hydrothermal Polynoids), as it yields a variety of sequences from across an organism's genome that would require significant time and investment to capture in equivalent resolution with other molecular methods (Ali et al, 2016). For these reasons, RAD-seq was selected as the core approach for this study. This study aims to examine the population structure and diversity of polynoid worms associated with bushes of *Ridgeia piscesae* within the Axial Caldera, providing preliminary insights into how these populations function on a small-spatial scale. In addition, determining the population structuring of hydrothermal polynoids may offer insights into their reproductive life history to inform future intensive studies of reproductive biology and into mechanisms of population dispersal.

Polynoid populations within Axial Seamount represent a valuable opportunity to expand our current understanding of these organisms and develop a new sentinel organism for these fragile ecosystems during anthropogenic pressure. However, international firms' growing interest in the abundance of resources in these hydrothermal systems via deep sea mining represents a grave new anthropogenic threat. A comprehensive understanding of these natural systems will prove crucial to successfully managing such endeavors. Despite an inherent degree of resilience in active vent systems, and although a comprehensive understanding of the impact of mining is yet to be determined, deep sea mining poses a potentially existential risk secondary to its ability to easily clear entire chimney systems and degrade these ecosystems in a brief period (Van Dover 2014). Accordingly, a better understanding of abundant polychaete taxa such as Polynoids may solidify their potential as a sentinel species—the value of which has been well documented in other environments (Dean 2008; Giangrande et al, 2005). Their high biodiversity and abundance within benthic communities make them excellent candidates as natural indicators of environmental health, given their sensitivity to water quality disturbances and sediment

contaminants (Dean 2008). Their high degree of specialization makes them excellent indicators of biological diversity within an ecosystem, given that small-scale perturbations that disrupt the ecosystem can drastically reduce their population in a brief time (Dean 2008). As a result, an improved understanding of the natural characteristics of these organisms, including how their genetics behave between populations, may be critical to future management efforts within hydrothermal systems.

Methods:

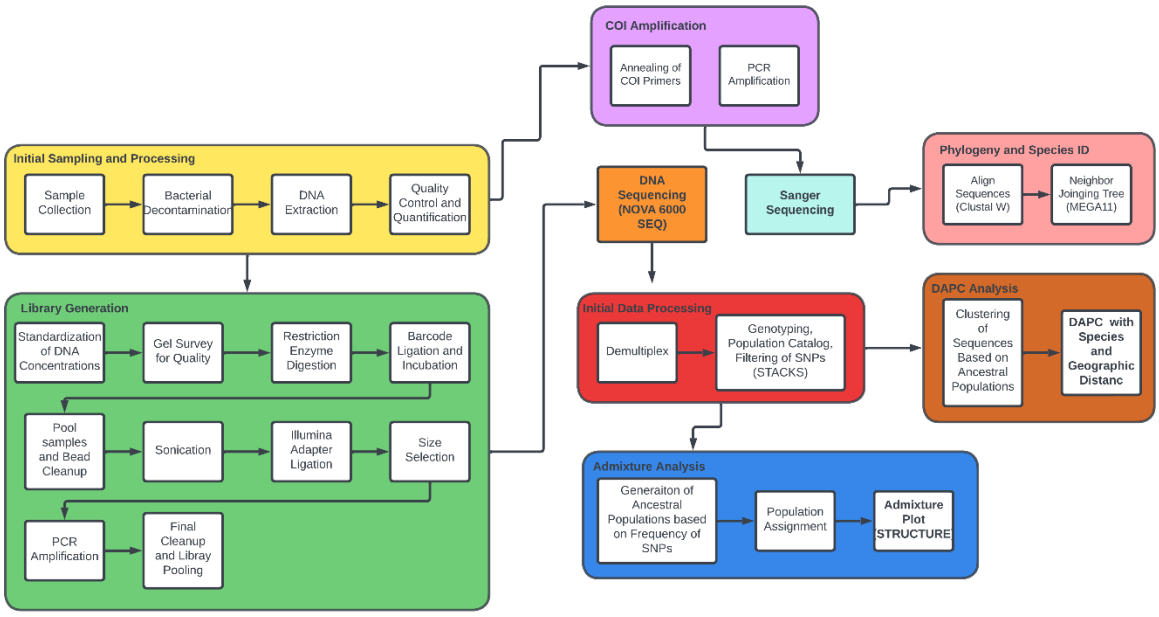

Figure 1. *Flowchart of workflow and analysis pipeline for RAD-Seq data from an initial sampling of worms to sequencing and final analysis of population structure with different colored boxes denoting critical components of the process. Not illustrated here are steps involving sample storage and additional quality control checks between main processes.*

Initial Sampling, Preservation, and DNA Extraction:

Preliminary sampling of tubeworm bushes (Figure 1 – Yellow Box) was conducted across multiple dives at two specific vent sites visited during VISIONS 22: International District and ASHES Vent Field. For each dive, samples were collected via the remotely operated vehicle ROPOS equipped with two bio-boxes on the vehicle's porch. Two "bushes" of *Ridgeia piscesae* were collected from each site (diffuse flow/chimney), limited by bio box capacity. The first sampling occurred at the surface of an actively venting chimney, Inferno, before proceeding approximately ten meters to the nearest diffuse flow site (Figure 2). Samples were placed carefully in separate bio-boxes to prevent contamination, and the coordinates of exact sampling were recorded via ROPOS's Integrated Real-Time Logging System (IRLS) for later reference.

This process was repeated approximately one week later at the adjacent vent field International District approximately two nautical miles east of ASHES, with the vent El Guapo sampled similarly. Once the ROV was on deck, samples from the bio-boxes were retrieved as soon as possible and transported to the wet lab, where processing began immediately.

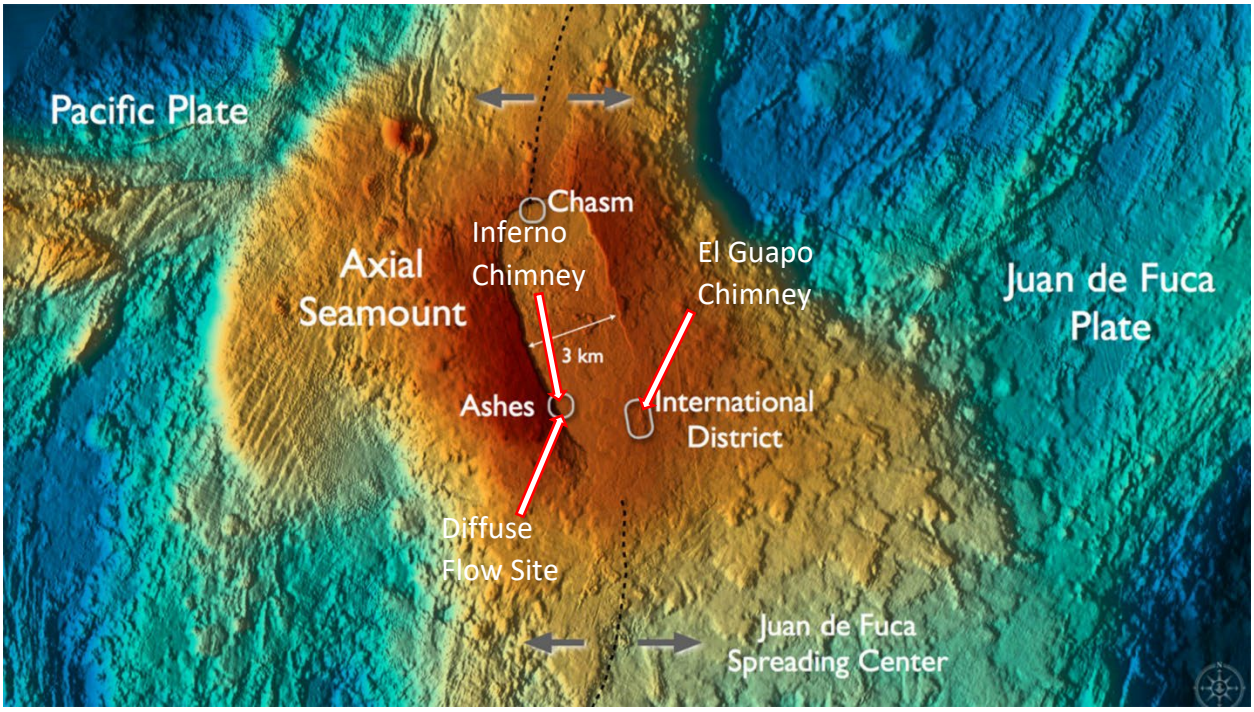

***Figure 2**. Map of three sampling sites within the Axial Caldera during VISIONS 22. Two sites were in close proximity within the ASHES vent field while the third site was approximately two kilometers east in the International District vent field. Map is courtesy of the University of Washington, Regional Cabled Array.*

Each bush was delicately washed, pulled apart, and placed into a bin to separate Polynoids from other worms before they were collected with tweezers and stored in separate gamma-sterilized falcon tubes with enough 100% ethanol to cover the sample (Rouse, 2022). Polynoids were removed, additional material, including tube worms, sulfide, palm worms, and pycnogonids, were preserved in separate jars when possible, and any remainder was disposed of. Twenty-four hours later, the ethanol for all polynoid samples was changed to allow for complete fixation (Rouse et al, 2022). In total, 61 Polynoids were collected across all three Caldera sites and processed for genetic study.

Polynoids in hydrothermal systems are often found with epizoic bacteria growing upon the elytra or "scales" on the dorsal part of the body, so samples were cleaned adequately before any genetic work began (Sarrazin et al, 1999). From each whole Polynoid, approximately four

elytra were removed using sterile forceps and subsequently washed over multiple 8% bleach and water cycles to ensure that any extraneous microbial DNA was removed prior to extraction (Petrou et al, 2019). Using the Qiagen DNEasy Blood and Tissue extraction kit, DNA was extracted from the elytra of each sample over two days before analysis of DNA concentrations via Qubit to ensure quality.

COI and Species Identification using MEGA11:

To determine any species variation within Polynoid populations in the Axial Caldera, four samples were selected randomly from across the three sites sampled for sanger sequencing of the mitochondrial gene cytochrome c-oxidase subunit I (COI) (Figure 1 – Purple/Teal Boxes). Primers and PCR protocol were based on the methods found by Cowart et al, 2020 for Polynoid worms. Species identification (Figure 1 – Pink Box) of the four Polynoid samples sequenced for COI was conducted using the program MEGA11(https://www.megasoftware.net/). Nine reference COI sequences for a diversity of deep-sea polynoids and polychaetes were acquired from Genbank, and all sequences aligned in MEGA using the ClustalW method. A neighbor-joining tree was then constructed using the Kimura 2-parameter distance to measure divergence between sequences and bootstrapping as the phylogenetic test and 50 repetitions. Species were considered the same if they clustered with a reference on the final phylogenetic tree.

RAD-Seq, Demultiplexing, and De novo Analysis via STACKS:

Library generation for BestRAD sequencing was conducted using the methodology outlined in Ali et al, 2016 (Figure 1 – Green Box) before samples were subsequently pooled and sent to the University of Oregon Genomics Center, where NOVA 6000 SEQ was used to sequence the samples (Figure 1- Orange Box). Sequence files were demultiplexed into individuals' samples based on the genetic barcodes ligated during library generation (Figure 1 – Red Box). This process was conducted within a Linux environment using the software pipeline STACKS (https://catchenlab.life.illinois.edu/stacks/), which the University of Illinois Urbana-Champaign developed to build loci based on data from the Illumina platform and RAD-seq. The function "process radtags" was used specifically to demultiplex sequences for BestRAD data using the target of the SBF1 restriction enzyme cut site, which specifies where to divide the raw sequence files based on the enzyme used during library generation. Once samples were demultiplexed, the standard De novo analysis was used with the rest of the STACKS pipeline.

This program identifies loci in the 61 individual sequences rather than known reference sequences, employing five critical programs within the STACKS package, including ustacks, cstacks, sstacks, gstacks, and finally populations, to create a workable file for later programs. Multiple runs of the entire pipeline were used to determine the optimal parameters for de novo analysis to maximize the number of individuals and SNP variants or alleles within the final data set, both of which can be easily determined from the final population's file generated by the pipeline. Once this optimization was completed, a final run of the pipeline was used to generate the final population vcf file for analysis.

Ustacks is the first step of the pipeline and attempts to assemble loci found within the sequences of each individual; it is the root that all other stacks functions build from and was run using the parameters m = 6, M = 2, and n = 7 for three populations using a population map created with 59 of 61 samples (two sequences were dropped due to low read count). Cstacks is the next step in the pipeline. It creates a catalog for later comparison of all loci found during ustacks in sequences; it was performed using standard parameters and a population catalog of 12 samples randomly selected from the pool with a total of four from each of the sites sampled. Sstacks, stacks, and the population's program were all performed using default parameters and together match loci found in the 59 sequences against the created catalog and assemble variant sites within the populations before exporting the results as a vcf file which can be used in other programs. The exact functions of each of the components of the STACKS pipeline are explored in greater detail within the STACKS 2.64 manual (Rochette, Rivera-Colon; Catchen, 2019).

Calculation of General Population Metrics:

General population metrics, including observed heterozygosity, expected heterozygosity, and the inbreeding coefficient Fis, were all calculated using R using the vcfr, adegenet, SNPfiltR, and hierfstat packages (vcfR - https://cran.r-project.org/web/packages/vcfR/vignettes/intro_to_vcfR.html, adegenet - https://cran.r-project.org/web/packages/adegenet/index.html, SNPfiltR - https://devonderaad.github.io/SNPfiltR/, hierfstat - https://cran.r-project.org/web/packages/hierfstat/index.html). The population file was passed through a hard depth filter for a read depth of 10 and a depth filter for allele balance with a minimum ratio of 0.2 and a max of 0.8 to remove alleles caused by artificial or sequencing errors. Fixed loci were removed using the "min_mac()" function in vcfr, and samples with more than 50% missing data for SNP were

removed from the dataset. The highest frequency polymorphic SNP per rad tag was calculated for each locus and individual using the maf() function in vcfr. Individuals with missing data at that locus were filtered out using a cutoff of 20%. Finally, fixed sites were removed from the remaining sequences using the Adegenet package before the final file was exported using the gl2genepop() from Adegenet for analysis in STRUCTURE and DAPC. Calculation of observed and expected heterozygosity and the inbreeding coefficient and confidence intervals for each population was conducted in R using the hierfstat package and the previously filtered data. Specifically, the Ho(), Hs(), and boot.pp is () functions were used for all calculations.

Pairwise Fst, Admixture Analysis using STRUCTURE, and DAPC:

Pairwise Fst was used to explore the degree of genetic differentiation between populations initially and was calculated using the aerostat package in R. Using the sequence data filtered by vcfr and SNPfiltR pairwise Fst was calculated comparing the International District, ASHES diffuse flow, and ASHES Inferno Chimney populations to each other using the genet.dist() function in hierfstat and the "WC84" method argument. From there, confidence intervals were calculated using bootstrapping via the boot.post () function and 1000 replicates.

Admixture analysis (Figure 1 – Blue Box) was performed using the processed and exported gl2genepop file and the program STRUCTURE (https://web.stanford.edu/group/pritchardlab/structure.html) developed by the Pritchard Lab at Stanford University. The program uses multi-locus genotype data to determine population admixture within populations by comparing frequencies of SNPS or other markers to theoretical ancestral populations. The file was first converted to the appropriate format using PGDSpider before STRUCTURE 2.3.4 was used specifically with 28 individuals and 627 biallelic loci. Next, tests were run using a burn-in period of 5000 and 5000 MCMC repetitions after the burn-in period, with an admixture model with an initial alpha value equal to 1.0. The Burnin period allows each model to be trained on 5000 replicates of the data to avoid the erratic nature of early processing to influence the model, and then subsequently use the successive 5000 repetitions in the final analysis. A total of 40 tests were run across a range for the parameter K of 2 to 5 with ten repetitions for each value. The optimal K value for analysis was then determined by analyzing all 40 tests in Structure Harvester and the online program CLUMPAK, which use even analysis to determine similarity scores for the results of each K value.

DAPC analysis (Figure 1 – Brown Box) was performed using the magnet package in R. Filtered sequence data was used in combination with the xvalDapc() function in R to perform a discriminate analysis of principal components on the sequence data from individuals across the three sites sampled and determine the genetic relationships between both populations and individuals within and between those populations by using genetic distance as the primary metric of the DAPC. This was done using the "mean" method, and cross-validation of the principal components was performed in the same function. This method parses through SNP alleles, single nucleotide variations within a locus within the sequence dataset. It attempts to determine the optimal settings to use as principal components that predict the greatest variability in genetic structure based on their frequency.

Results:

Species Identification and Diversity of Polynoids:

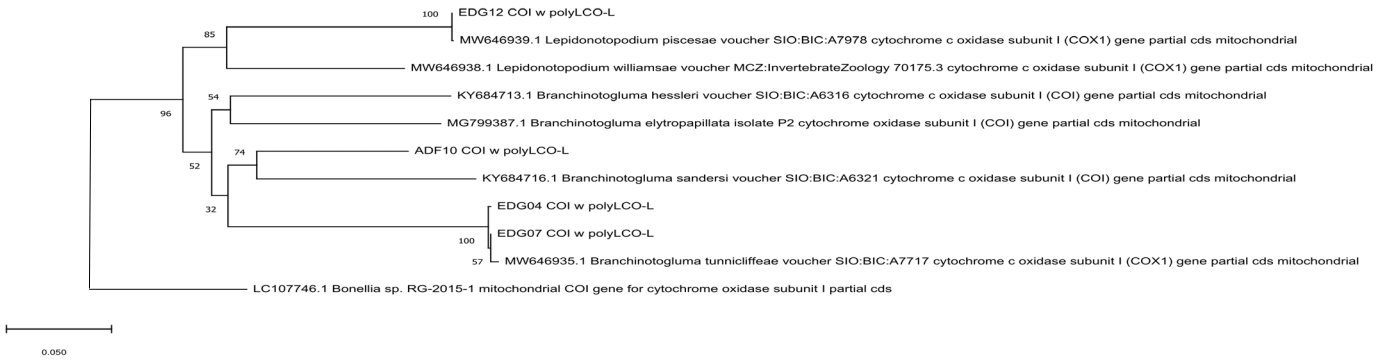

Figure 3. *Phylogenetic tree grouping unidentified Polynoid worms with reference sequences using Sanger sequencing of COI gene and the Program MEGA11. Unidentified polynoids are denoted by the subscripts EDG12, EDG04, EDG07, and ADF10 and were selected randomly for Sanger sequencing from a pool of 61 across the entire Axial Caldera. Unidentified individuals that group with a reference sequence are considered the same species. Numbers on the branch nodes represent bootstrap values and measure confidence that the phylogenetic relationship is accurate.*

Analysis of the COI gene for four Polynoids sampled across the Caldera revealed a surprising amount of diversity, with three distinct species and two genera being identified. Samples of tubeworms from the sample bushes within the Caldera were confirmed to all be the same species, *Ridgeia piscesae*, while the scale worms that inhabited them varied (Figure 3). From the site of diffuse flow in ASHES, the sampled Polynoid clustered with the reference for *Branchinotogluma sanders* with a bootstrap value of 0.74. At International District, two samples clustered well with the voucher for *Branchinotogluma tunnicliffeae* with a bootstrap value of 0.57. Other samples grouped significantly well with the voucher for another genus with the

species *Lepidonotopodium piscesae* and a bootstrap of 1.00. It is important to note that this finding explains the subsequent loss of individuals during the filtering process, (61 to 28 sequences), as STACKS will discard sequences from separate species due to the inherently high genetic differences between individuals. As a result, further analysis with admixture analysis, DAPC, and F-statistics will characterize only one of the three species observed.

Heterozygosity, Inbreeding, and Genetic Differentiation among Sample Groups

***Table 1**: General population metrics, including observed heterozygosity, expected heterozygosity, and Inbreeding coefficients for each of the three sample populations. All three parameters were calculated using the package hierfstat in R Studio with data from 28 Polynoid worms, 6 for the Inferno Chimney, 7 for International District, and 15 for the Diffuse Flow group. Confidence intervals for the inbreeding coefficient are shown in parentheses and are significant if the range falls outside zero, and the index itself is in bold and was calculated using bootstrapping.*

Sample Site	ASHES – Inferno Chimney	International District – El Guapo Chimney	ASHES – Diffuse Flow
Observed Heterozygosity (Ho)	0.239	0.231	0.215
Expected Heterozygosity (Hs)	0.233	0.234	0.223
Inbreeding Coefficient (Fis) and Confidence Intervals	**-0.026**, (-0.072 – 0.007)	**0.013**, (-0.027 – 0.052)	**0.036,** (-0.004 – 0.063)

Heterozygosity is a measure of genetic variation at a particular locus within a population and was similar across all three sample sites. Observed heterozygosity is specifically the heterozygosity for a subpopulation that is calculated from the actual number of heterozygotic genotypes present. The other parameter, expected heterozygosity, is calculated in accordance with the Hardy-Weinberg Equilibrium, which if true holds that the population is not evolving and no inbreeding is occurring. Within the ASHES – Inferno Chimney, observed heterozygosity was slightly greater than expected, while at both the International District -El Guapo Chimney and the ASHES – Diffuse Flow sites, expected heterozygosity was greater than observed (Table 1). The inbreeding coefficient, "Fis," allows us to measure the reduction in heterozygosity attributed to inbreeding within a population and, in a sense, allows for estimating the degree of inbreeding present in each population. It is measured on a scale of -1 to 1, with -1 indicating a lack of inbreeding due to the mixing of populations, 0 being an expected amount of inbreeding based on the population, and 1 being an observable degree of inbreeding within the population. In the ASHES – Inferno site, Fis was slightly negative at -0.026, while at the other two sample sites, Fis was positive at 0.013 and 0.036 at El Guapo and the diffuse flow site, respectively.

However, all three sites were not significantly different from the expected value of 0 based on the bootstrapping confidence intervals.

Table 2. *Pairwise Fst or genetic distance is shown below the dashed line in bold and was calculated using the aerostat package in R. Each value compares the genetic distance between two of the three sample populations with values ranging between 0 and 1. Values above the dashed line represent the confidence intervals for each pairwise Fst value calculated using bootstrapping.*

	ASHES -Inferno Chimney	International District – El Guapo Chimney	ASHES – Diffuse Flow
ASHES – Inferno Chimney	-	-0.0041 – 0.0196	-0.0003 – 0.0187
International District – El Guapo Chimney	**0.00697**	-	-0.0068 – 0.0091
ASHES – Diffuse Flow	**0.00894**	**0.00099**	-

Fst is a measure of genetic differentiation between two groups or subpopulations. It can range between 0 and 1, with 0 representing a high degree of alleles being shared between groups and 1 indicating the populations are fixed and there is no sharing or mixing between groups. Fst was highest between the diffuse flow site and Inferno Chimney within the ASHES vent field at 0.00894 and lowest between the El Guapo Chimney at the International District vent field and the diffuse flow site at ASHES (Table 2). The fixation index between the two chimney sites was in the middle, with a value of 0.00697. All these values were not significantly greater than 0 based on the bootstrapping confidence intervals, meaning that the fixation index comparing all three groups can be considered equal to 0.

Admixture Analysis:

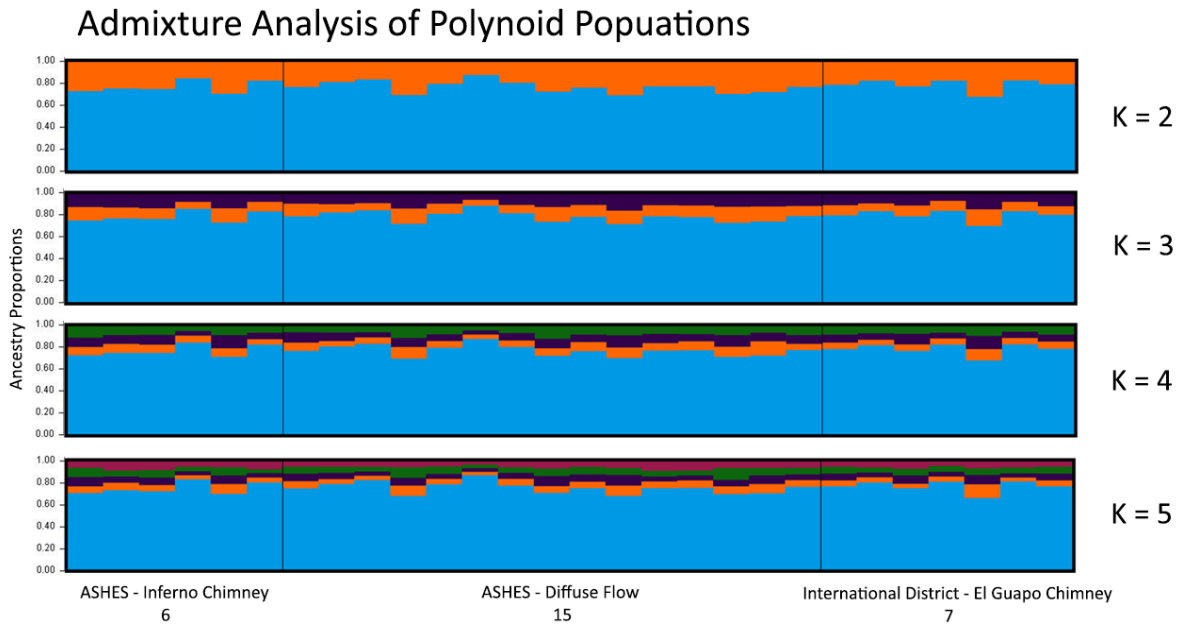

Figure 4. *Admixture Plots created using the program STRUCTURE and SNP data from RAD-Seq of 29 individual Polynoids from Axial Seamount across a range of inferred ancestral populations or K-values. Each color represents a theoretical ancestral population based on SNP frequencies found within all sequences. An individual is assigned a population based on statistical similarity to the ancestral population. The relative coloring of each bar represents the proportion of shared ancestry (genetic similarity) between an individual sequence and the theoretical population. Populations are grouped according to their sample groups labeled at the bottom, and the number below represents the number of individuals in each sample group.*

Admixture analysis attempted to assign individuals to ancestral populations based on the frequency of SNPs across their sequences and yielded 28 individuals with 634 unique loci found. Six individuals were assigned to the Inferno-Chimney population at ASHES, 15 to the site of diffuse flow also at ASHES, and seven to the chimney El Guapo at International District (Figure 4). A high degree of shared ancestry between groups was observed in the three groups of Polynoid samples within the Axial Caldera. When models were run using the program STRUCTURE with a range of k-values, (the number of expected ancestral populations), between 2 and 5 the program structure harvester revealed no significant variations between runs. Across all four tests, all three sites can attribute approximately 70% of their genetic ancestry to a single population, with the remaining 30% variable depending on the number of expected populations. For two populations, the entirety of the remainder can be attributed to a single population as expected; however, for k values of 3, 4, and 5, the remainder is equally attributed to a mix of ancestral populations.

Discriminate Analysis of Principal Component between Sample Locations:

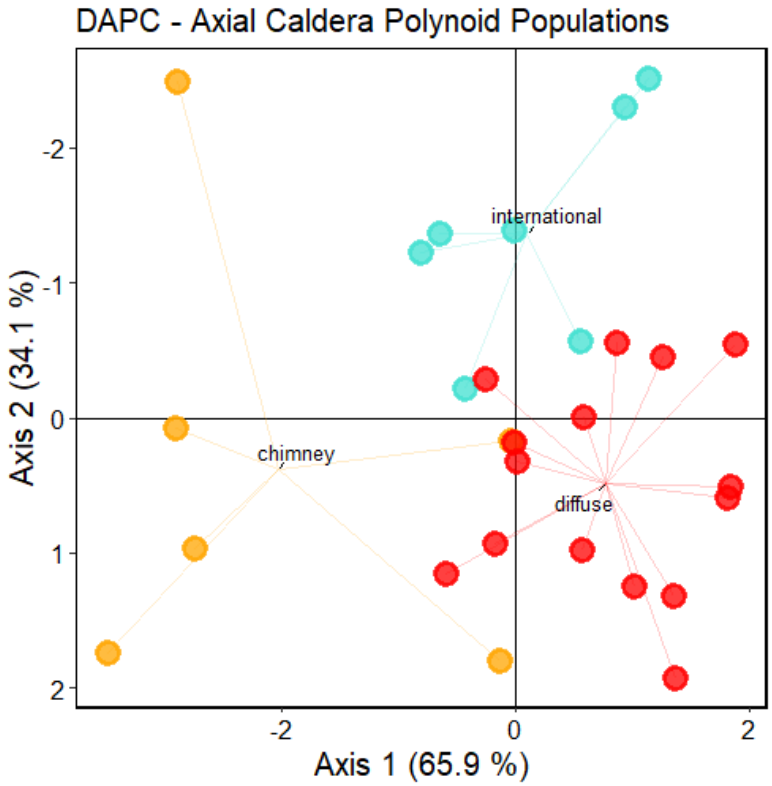

Figure 5. *DAPC plot using SNP data from RAD-Seq for three populations of polynoid worms within Axial Caldera. Each color represents a population of worms (labels in the center of each cluster), and circle an individual sequence from that population, with increasing distance between dots denoting increasing genetic distance. Each axis represents a unique combination of alleles (genotype) determined using the program VCFR in R that best explains the variability in the genetic distance seen between populations with the percent variation explained denoted in parentheses.*

DAPC analysis based on SNP data from 28 individuals across Axial Caldera separated the International District diffuse flow, ASHES Chimney, and ASHES Diffuse Flow populations from each other based on genetic relatedness (Figure 5). Three distinct clusters appeared, with seven individuals in the International population, 15 in the ASHES chimney clusters, and six in the ASHES diffuse flow cluster. Individuals within the ASHES diffuse flow site appeared to cluster more than any other of the three groups, followed by the International District group and the Chimney group showing the greatest variation and genetic differentiation. A large degree of overlap is observed across all three genetic clusters, although the international district and diffuse flow cluster appear to cluster more than either does with the Inferno Chimney.

Discussion

Species Diversity of Polynoids within Axial Caldera

Sanger sequencing of the Cytochrome C Oxidase Subunit I gene on four randomly selected individuals from the 61 Polynoids collected revealed an unexpected three distinct species within the confines of the Caldera. These included *Branchiotogluma piscesae, Branchiotogluma tunnicliffiae, and Lepitonotopodium piscesae*, all of which had been observed within the Caldera in previous expeditions (Pettibone 1988; Desbruyeres and Segonzac, 1997). Although not the primary focus of this study, this result is exciting as it reveals a great deal of species diversity across the Caldera on a small spatial scale. In addition, *L. piscesae* was sampled within the confines of a tube worm assemblage which goes against what has been observed with this species in the past. It is solitary and lower in abundance than the other two species. However, most importantly, it is typically found on the periphery of the venting systems, where it feeds on detritus and bacterial mats (Desbruyeres and Segonzac, 1997). This finding suggests, as a result, that this species may be adapted to feed on the assemblages of *R. piscesae* like the other two species and suggests interactions between all three species may be expected within the confines of the tube worm bush.

However, while exciting, these findings of an abundance of species diversity do raise potential concerns in the population structure aspect of this study. Many samples were removed from the working dataset during the STACKS pipeline, and the R-code filters with vcfR and snpfiltR, reducing the sample size from 59 initially to 28 in the final analysis. Small sample sizes can potentially skew the results of genetic studies altering the signal of genetic variation, and cannot be discounted (Puechamille 2016). In addition, the rigorous filtering within the STACKS pipeline tends to fail when exposed to populations containing multiple species, intentionally or not, because it was inherently not designed for such operations (Catchen et al, 2011). This can be potentially attributed to the high degree of genetic variability and differentiation that are seen between biological species; however, while it is essential to acknowledge, even with the reduced sample size, the results this study yielded were still significant for one species and suggest a general trend of a lack of population structuring and the panmixia of polynoids within the Axial Caldera.

Polynoid Heterozygosity, Genetic Diversity, and Inbreeding:

Populations of Polynoids within the confines of the Axial Caldera showed higher levels of heterozygosity compared with other species in the deep ocean and, as a result, showed comparatively fewer signs of substantial inbreeding as well (Plouviez et al, 2008). Observed heterozygosity for the sample sites ASHES Inferno Chimney, a site of diffuse flow within the ASHES vent field, and the El Guapo Chimney within International District only deviated slightly from the expected heterozygosity for each group, resulting in inbreeding coefficients that were not significant compared to the expected value of 0. Fis ranges from 1, where the expected heterozygosity is greater than the observed, suggesting inbreeding may be occurring in the population, and -1, where the observed heterozygosity is greater than the expected and the mixing of the population may be occurring. However, when the value is not significantly different from 0 (as seen here), it suggests normal heterozygosity levels typically seen within a single genetic population. These results contrast what has been observed among other species of deep-sea Polynoids. *Branchipolynoe seepensis* is a Polynoid worm commonly inhabiting sulfide-reliant mussels at cold seeps within the Mid-Atlantic Ridge. It has seen extensive work looking at the population genetics of its widely dispersed populations (Plouviez et al, 2008; Desbruyeres and Segonzac, 1997). One study conducted in 2008 showed that with subpopulations in a small area, there were great heterozygote deficiencies at all the loci examined, suggesting that within the populations examined, there was a high amount of inbreeding occurring (Plouviez et al, 2008). This stands in contrast to what is seen here, with individuals within Axial Caldera showing no signs of inbreeding based on the statistical insignificance of the Fis-values.

This discrepancy is partly due to the different size constraints within the two environments. The polynoids at Axial Seamount are not as confined as those living within the spatial confines of mussel beds. They are dispersed over a much larger area by comparison, avoiding the possible inbreeding that comes with spatially restricted populations (Van Dover et al, 1991; Ellstrand and Elam 1993). Within the context of Axial Caldera, this may mean that populations of Polynoids are not as spatially restricted as initially proposed in this study. If polynoids at Axial were as spatially constrained to the same degree as the mussel-dwelling *B. seepensis,* a large reduction in heterozygosity due to substantial inbreeding should be observed as small groups of worms are isolated to individuals chimneys or diffuse flow sites. However, these patterns are not observed in the polynoids within the Caldera suggesting they are in some manner freely moving between sites and interbreeding, unlike *B. seepiensis*. These variations in

inbreeding levels and heterozygosity may partly be attributed to differences in life history between Axial Polynoids and those found within the Lucky Strike region. *B. seepensis* is an obligatory commensal organism with the mussels it inhabits, meaning it must live near its host for most of its life history to survive (Van Dover et al, 1991; Desbruyeres and Segonzac 1997). For the species of Polynoids found at Axial Caldera, this may not be the case, as although they are often found near the tubeworms they are not obligatory predators of these organisms (Desbruyeres and Segonzac 1997; Bergquist et al, 2007). Polynoids at venting sites around the Caldera are capable scavengers and predators on other organisms, such as detritus and bacterial mats if needed, reinforcing the idea that these organisms are not as intimately tied to the tubeworm assemblages they inhabit as the commensal species is to its mussel host (Bergquist et al, 2007; Chevaldonne and Jollivet 1993). In addition, most of what is known about deep sea Polynoid reproduction and life history can be attributed to work examining *B. seepensis*. Comparatively, little literature examines these same trends in other species. Polynoidae is one of the most diverse taxa within the already abundant Polychaete class, with a considerable variation in life history strategies present at all ocean depths. Thus differences in the life histories of these two organisms may contribute to discrepancies in the observed population parameters (Rouse et al, 2022). A greater body of literature must be developed encompassing many more species of deep-sea Polynoids, specifically regarding reproductive biology and larval dispersal before further trends can be established.

Shared Ancestry and Population Structuring:

Signals of shared ancestry between the three sample sites ASHES Inferno Chimney and Diffuse Flow and the El Guapo Chimney at International District were prevalent for one species, suggesting little population structuring is occurring between groups of Polynoids within the Caldera. Admixture analysis across a range of theoretical ancestral populations revealed that while all three sample sites seemed ancestrally dominated by a particular group, the relative proportions of each ancestral population contributing to modern groups remain consistent across sample sites. This suggests a high degree of shared ancestry across the three sites. All groups are in panmixia, where all populations are mixing and can be functionally considered one genetic population. In addition, DAPC analysis reveals a similar trend and shows the complexity of genetic relatedness between individuals in and between sample sites. The "tightness" of each

cluster is comparable to the degree of genetic relatedness between individuals in each subpopulation and was variable across all three sample sites observed, with individuals from the site of diffuse flow being most genetically related and those at the ASHES – Inferno Chimney being most distant from one another on average. This high variation within groups may be a result of low sample size for the species analyzed across the three sites, as a substantial number of sequences were filtered out during DAPC analysis due to the inherent variation between species. Despite this, little genetic distance is observed between groups, once again suggesting that although a slight degree of differentiation may be present, panmixia is still freely occurring within this population. Finally, paired Fst analysis between the three sites further confirmed the results of the previous two analyses. No significant values of genetic differentiation were observed between any of the three tests, and all were equivalent to zero. Fst is a measure of genetic differentiation within a population and can range between 0 and 1, with the more significant number indicating a greater genetic distance or differentiation between two subpopulations. In the case of worms within the Caldera, a value of 0 suggests that a high degree of genetic material or alleles are being shared between groups and suggests that a high degree of mixing between groups must occur.

All these results point towards the conclusion that the three sites studied across the Axial Caldera are panmictic, meaning that a high degree of mixing between sites of Polynoids is occurring and that all can be considered one genetic population. These results stand in contrast to what was initially proposed in this study. Various barriers to reproduction would prevent interbreeding and lead to population structuring based on what is currently hypothesized about deep-sea Polynoid reproduction and life history. Population structuring and mixing are inherently tied to reproduction, and it is barriers to the reproduction between groups of individuals that are the primary drivers of genetic differentiation between groups (Hocutt, 2000). If the original hypothesis were correct, habitat restrictions or another biological mechanism might be at play in preventing mixing. For example, physically, the relative rarity of sites of venting or diffusing on the deep-sea floor may lead to a lack of suitable food sources in the form of tube worm assemblages for the worms to travel between sites of venting (Karson et al, 2015). Moreover, biologically, restrictions on reproduction imposed by life history, such as the brooding of young and the limited dispersal of propagules, may restrict the mixing of populations. If these factors were indeed at play, they would be reflected in the signals of high genetic differentiation

between sample sites, as it would suggest individuals are not intermixing. Furthermore, this original idea is supported by previous literature suggesting that deep-sea Polynoids such as *B. deepens* brood fertilized zygotes under their elytra and copulate with one another via internal fertilization (Wallace 2005; Gaudron et al, 2017). This reproductive strategy is unusual among polychaetes in the degree of parental care provided and in its potential to limit the dispersal of young compared to other broadcast spawning species or taxa (Rouse et al, 2022). However, new literature about deep sea polynoids may be vital to understanding the trends within the Axial Caldera population.

One study looking at another species of deep-sea Polynoid that inhabit the East Pacific rise found that geographic distance played little role in the population structuring of populations (Guggolz et al, 2018). They found that on a much larger spatial scale than the confines of the Axial Caldera, little genetic differentiation was present in this species across the entirety of the East Pacific Rise, a mid-ocean ridge (Hutardo et al, 2004). This suggests that single genetic populations of deep-sea Polynoid can exist on massive scales in specific locations and still actively intermix with one another. This idea helps significantly expand the understanding of how these worms reproduce in the deep ocean despite being relegated to rare hydrothermal systems. Previous evidence suggests that these organisms are not fully-fledged broadcast spawners where gametes are dispersed into the water column and fertilized externally, although this would be the most straightforward explanation for the trends observed (Gaudron et al, 2017; Wallace 2005). Instead, they may perform a pseudo-copulatory reproductive strategy, at least regarding the species on the EPR (Gaudron et al, 2017). Past studies revealed evidence of internal sperm storage and copulatory structures in other species of hydrothermal Polynoid, including the previously mentioned *B.* deepens, which pointed toward the idea that these organisms engage in copulation (Wallace 2005). This is profoundly important to the concept of population structuring as individuals from two separate groups or subpopulations would need to be near each other to engage in reproduction. In addition, current evidence suggests that deep-sea polynoids of at least some species engage in the form of parental care known as brooding, where fertilized eggs are protected under the elytra or scales of the worm, which this study previously suggested may function as a barrier to the widespread dispersal of propagules (Gaudron et al, 2017). However, new evidence suggests that while this brooding behavior may occur, it is not limiting regarding larval dispersal. It is hypothesized that the females of hydrothermal polynoids

can periodically release the brooded propagules in limited quantities in response to environmental cues, allowing larvae to drift between venting sites via hydrothermal plumes, currents, or some other physical mechanism (Gaudron et al, 2017). The exact nature of these cues and mechanisms is not entirely understood. However, the stimulus is thought to be seasonal, with possible changes in the flux of detritus from the surface ocean being one proposed mechanism. Regardless, this life history strategy would explain the lack of population structuring within the Axial Caldera while maintaining many preconceived notions about scale worm reproduction (Gaudron et al, 2017).

It is also essential to consider whenever discussing Polychaetes and Polynoidae that these taxa are known for being incredibly diverse with a vast range of reproductive strategies (Rouse et al, 2022). With what is known now, this taxon can have a wide range of life histories depending on its environment. For example, specific taxa rely on asexual fragmentation, others on epitoky which is a substantial change in body form to a reproductive morph, and now with the deep sea polynoids, some with copulation (Rouse et al, 2022). This means, in practice, that another mechanism could be at play, resulting in the lack of population structuring observed at Axial Seamount. The deep ocean, in general, is renowned for the unconventional and alien behaviors that many of its organisms display, with the chemosynthetic worms that the Polynoids rely on being examples of how the deep ocean can continue to defy our expectations of what is biologically possible (Desbruyeres and Segonzac 1997). As a result, further research into the reproductive biology and life history of the specific species that inhabit Axial Seamount may be critical to determining the exact method and behaviors by which these organisms are reproducing in the deep ocean.

Implications for Deep Sea Conservation of Polynoid Populations

To summarize, the results of this study suggest that Polynoid worms within the Axial Caldera are panmictic, interbreeding between sites of hydrothermal flow throughout the Caldera. This suggests a lack of clear population structuring or division between sites and that the entirety of the Caldera should be treated as a single biological population. These trends suggest that polynoid worms inhabiting Axial Seamount have a reproductive life history incorporating long-range larval dispersal, which would allow for the high degree of mixing seen between sites. These results have many implications for conserving Polynoid worms within hydrothermal

systems, especially in the face of growing anthropogenic pressure, such as deep-sea mining. Deep-sea mining operations have been considered potentially devastating for deep ocean populations in various environments, from abyssal plains filled with manganese nodules to complex, active hydrothermal venting sites (Van Dover and Lee 2014). Whether these impacts come from the burying of organisms via discarded sediments or direct mortality caused by the machinery used to mine, without adequate research exploring the impacts on specific populations of polynoids in terms of their reactions to deep ocean mining, it is difficult to predict the potential damage that could be caused to these populations. Large-scale disturbances to populations of polynoids that inhabit these hydrothermal systems could have potentially significant consequences for the stability of their populations. The total removal of a single chimney could result in a substantial loss of genetic diversity from the gene pool of the population, which can leave the population more susceptible to the impacts of inbreeding and the lack of adaptability associated with little genetic diversity and dangers of a modern, changing ocean even in the relatively stable conditions of the deep ocean (Hughes et al, 2008).

These are only the known potential impacts as well. The lack of concrete literature examining the exact mechanisms behind larval dispersal for deep-sea polynoids at hydrothermal systems is particularly concerning. Mining efforts could disrupt these invisible processes in some way, whether interference with the cues of larval dispersal or the recruitment factors. In addition, they could cause similar damage to the direct removal of genetic diversity. This could result in a reduction in gene flow between sites within the Caldera and the resulting inbreeding depression that could occur due to the creation and isolation of essentially many small populations that were previously connected (Keller and Waller 2002). Regardless, without further work expanding our understanding of the biology of these organisms and the potential dangers they face in response to mining operations on the deep-sea floor, it also makes it incredibly irresponsible to move forward with such potentially destructive operations without adequate preparation.

Conclusion:

Polynoid worms that inhabit the assemblages of *R. Piscesae* within the Caldera of Axial Seamount represent a comparatively understudied group of polychaetes within the field of deep-sea biology, especially as it pertains to the structure of their populations and reproduction. With the growing demand to exploit the deep ocean for its abundant resources (mainly via deep sea

mining), there is a growing need to develop a foundational understanding of how all populations in the deep ocean function to avoid irreparable damage to these ecosystems. Little literature explores the mechanics of reproduction and population structuring in deep-sea polynoids. What does exist is usually confined to only a few species, leaving a substantial gap in our understanding of reproductive biology in an incredibly diverse taxon (Wallace 2005; Rouse et al, 2022). Previous work suggested all the characteristics of short-range reproduction with structures of internal fertilization and copulation and brooding behavior cited in certain species such as *B. seepiensis*. In addition, the relative paucity of suitable habitats in the deep ocean for Polynoids, such as those found at Axial that rely on the chemosynthetic tubeworms such as *Ridgeia sp.*, may potentially serve as a barrier for the connectivity between groups at different venting sites (Tyler and Young 2003; Karson et al, 2015). This foundation of the literature suggested that a high degree of population structuring may be present between venting sites within Axial Caldera. However, this was not what was observed.

Little genetic differentiation was observed between any of the three sample sites across the Caldera, including ASHES Inferno, a site of diffuse flow within ASHES, and El Guapo within the International District. In addition, shared ancestry between all three sample sites combined with high heterozygosity and little inbreeding suggested a high degree of panmixia between all populations, further suggesting that polynoids within the Caldera behave as one genetic population. This suggests that the reproductive life history of these organisms is far more complex than originally thought, relying on a form of pseudo-copulation and larval dispersal influenced by a series of stimuli from the surface ocean (Gaudron et al, 2017). However, this study was only able to examine the specific population structuring of one species of polynoid. Population structuring and patterns in genetic parameters may drastically vary with the inherent diversity of the taxa, thus further work sequencing all 61 individuals collected from this study could reveal key differences in the relationships between different species and the true scale of species diversity at play within the caldera. This possibility, combined with the population being well-connected across the Caldera, also holds implications for the sustainable exploitation of hydrothermal resources, as small-scale disruptions to a single subpopulation could have drastic implications for the genetic diversity and gene pool of the whole.

However, as with any organism inhabiting the deep ocean, our knowledge of their biology is nowhere near complete. Future research is needed to expand our understanding of these populations and their behavior. First, continued analysis of the Polynoid populations within Axial Seamount represents a fantastic opportunity to expand our understanding of their population dynamics. Many critical species with various lifestyles are readily found within the Caldera. Repeating this study with an expanded dataset encompassing many different taxa may be valuable in developing a complete understanding of their populations (Desbruyeres and Segonzac 1997). In addition, a foundational understanding of the reproductive biology of these organisms is essential to explaining the nature of these trends in genetic relatedness and population structure. More research must be conducted examining the critical points of larval dispersal, mating, and development for these organisms at a species level to draw definitive conclusions about the genetic trends at play.

Acknowledgements:

I'd like to thank Deb, Orest, Mike, Katie, and everyone at the Regional Cabled Array for instilling a love and passion for hydrothermal systems and the deep sea, and for generously helping me with sampling and sharing dive time. Thank you to the crew of ROPOS, R.V. Thompson, and the students of VISIONS 22 for their help with all the sampling and humoring my ridiculous worm-related antics. I'd like to thank Kerry who helped develop this project and trusted me to get it done, and the MER Lab for help with all molecular work, and for generously supplying the resources and materials needed. Thank you to Bryan especially, for spending countless hours the past year teaching me everything I know about RAD-Seq, genetics, analysis, and much more. If not for you, I have no doubt I'd still be sitting in the lab with nothing but a pile of worms. And finally thank you to Ginger and Francois who read countless drafts that were too long and incoherent, but stayed with me nonetheless and helped me write something I am truly proud of.

References

Ali, O. A., O'Rourke, S. M., Amish, S. J., Meek, M. H., Luikart, G., Jeffres, C., & Miller, M. R. (2016). RAD Capture (Rapture): Flexible and Efficient Sequence-Based Genotyping. *Genetics*, *202*(2), 389–400. https://doi.org/10.1534/genetics.115.183665

Bergquist, D. C., Eckner, J. T., Urcuyo, I. A., Cordes, E. E., Hourdez, S., Macko, S. A., & Fisher, C. R. (2007). Using stable isotopes and quantitative community characteristics to determine a local hydrothermal vent food web. *Marine Ecology Progress Series*, *330*, 49–65. https://doi.org/10.3354/meps330049

Bonifácio, P., & Menot, L. (2019). New genera and species from the Equatorial Pacific provide phylogenetic insights into deep-sea Polynoidae (Annelida). *Zoological Journal of the Linnean Society*, *185*(3), 555–635. https://doi.org/10.1093/zoolinnean/zly063

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. *G3 Genes|Genomes|Genetics*, *1*(3), 171–182. https://doi.org/10.1534/g3.111.000240

Chevaldonné, P., & Jollivet, D. (1993). Videoscopic study of deep-sea hydrothermal vent alvinellid polychaete populations: biomass estimation and behavior. *Marine Ecology Progress Series*, *95*, 251–262. https://doi.org/10.3354/meps095251

Cowart, D. A., Matabos, M., Brandt, M. I., Marticorena, J., & Sarrazin, J. (2020). Exploring Environmental DNA (eDNA) to Assess Biodiversity of Hard Substratum Faunal Communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and Investigate Recolonization Dynamics After an Induced Disturbance. *Frontiers in Marine Science*, *6*. Retrieved from https://www.frontiersin.org/articles/10.3389/fmars.2019.00783

Dean, H. K. (2008). The use of polychaetes (Annelida) as indicator species of marine pollution: a review. *Rev. Biol. Trop.*, *56*.

Desbruyères, D., & Segonzac, M. (1997). *Handbook of Deep-sea Hydrothermal Vent Fauna*. Editions Quae.

Ellstrand, N. C., & Elam, D. R. (1993). Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics*, *24*(1), 217–242. https://doi.org/10.1146/annurev.es.24.110193.001245

Emlen, D. J., & Zimmer, C. (2020). *Evolution: Making Sense of Life* (Third). New York: Macmillian Learning.

Feldman, R. A., Shank, T. M., Black, M. B., Baco, A. R., Smith, C. R., & Vrijenhoek, R. C. (1998). Vestimentiferan on a Whale Fall. *The Biological Bulletin*, *194*(2), 116–119. https://doi.org/10.2307/1543041

Gage, J. D., & Tyler, P. A. (1991). *Deep-Sea Biology: A Natural History of Organisms at the Deep-Sea Floor*. Cambridge University Press.

Gaudron, S. M., Hourdez, S., & Olu, K. (2017). Aspects on gametogenesis, fertilization, and embryogenesis of two deep-sea polychaetes from Eastern Atlantic cold seeps. *Deep Sea Research Part I: Oceanographic Research Papers*, *129*, 59–68. https://doi.org/10.1016/j.dsr.2017.10.003

Giangrande, A., Licciano, M., & Musco, L. (2005). Polychaetes as environmental indicators revisited. *Marine Pollution Bulletin*, *50*(11), 1153–1162. https://doi.org/10.1016/j.marpolbul.2005.08.003

Glover, A. G., Källström, B., Smith, C. R., & Dahlgren, T. G. (2005). World-wide whale worms? A new species of Osedax from the shallow North Atlantic. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2587–2592. https://doi.org/10.1098/rspb.2005.3275

Guggolz, T., Lins, L., Meißner, K., & Brandt, A. (2018). Biodiversity and distribution of polynoid and spionid polychaetes (Annelida) in the Vema Fracture Zone, tropical North Atlantic. *Deep*

Sea Research Part II: Topical Studies in Oceanography, *148*, 54–63. https://doi.org/10.1016/j.dsr2.2017.07.013

Harden-Davies, H. (2017). Deep-sea genetic resources: New frontiers for science and stewardship in areas beyond national jurisdiction. *Deep Sea Research Part II: Topical Studies in Oceanography*, *137*, 504–513. https://doi.org/10.1016/j.dsr2.2016.05.005

Hauser, L., Baird, M., Hilborn, R., Seeb, L. W., & Seeb, J. E. (2011). An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (Oncorhynchus nerka) population. *Molecular Ecology Resources*, *11*(s1), 150–161. https://doi.org/10.1111/j.1755-0998.2010.02961.x

Hilário, A., Metaxas, A., Gaudron, S. M., Howell, K. L., Mercier, A., Mestre, N. C., et al. (2015). Estimating dispersal distance in the deep sea: challenges and applications to marine reserves. *Frontiers in Marine Science*, *2*. Retrieved from https://www.frontiersin.org/articles/10.3389/fmars.2015.00006

Hocutt, G. D. (n.d.). *Reinforcement of premating barriers to reproduction between Drosophila arizonae and Drosophila mojavensis* (Ph.D.). Arizona State University, United States -- Arizona. Retrieved from https://www.proquest.com/docview/304580934/abstract/E5F39F06576141FCPQ/1

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609–623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Hurtado, L. A., Lutz, R. A., & Vrijenhoek, R. C. (2004). Distinct patterns of genetic differentiation among annelids of eastern Pacific hydrothermal vents. *Molecular Ecology*, *13*(9), 2603–2615. https://doi.org/10.1111/j.1365-294X.2004.02287.x

Ivanenko, V., & Defaye, D. (2013, October 25). handbook corrigendum.

Karson, J. A., Kelley, D. S., Fornari, D. J., Perfit, M. R., & Shank, T. M. (2015). *Discovering the Deep: A Photographic Atlas of the Seafloor and Ocean Crust*. Cambridge University Press.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241. https://doi.org/10.1016/S0169-5347(02)02489-8

Lelièvre, Y., Sarrazin, J., Marticorena, J., Schaal, G., Day, T., Legendre, P., et al. (2018). Biodiversity and trophic ecology of hydrothermal vent fauna associated with tubeworm assemblages on the Juan de Fuca Ridge. *Biogeosciences*, *15*(9), 2629–2647. https://doi.org/10.5194/bg-15-2629-2018

Levin, L. A., Mendoza, G. F., Konotchick, T., & Lee, R. (2009). Macrobenthos community structure and trophic relationships within active and inactive Pacific hydrothermal sediments. *Deep Sea Research Part II: Topical Studies in Oceanography*, *56*(19), 1632–1648. https://doi.org/10.1016/j.dsr2.2009.05.010

Norlinder, E., Nygren, A., Wiklund, H., & Pleijel, F. (2012). Phylogeny of scale-worms (Aphroditiformia, Annelida), assessed from 18SrRNA, 28SrRNA, 16SrRNA, mitochondrial cytochrome c oxidase subunit I (COI), and morphology. *Molecular Phylogenetics and Evolution*, *65*(2), 490–500. https://doi.org/10.1016/j.ympev.2012.07.002

Petrou, E. L., Drinan, D. P., Kopperl, R., Lepofsky, D., Yang, D., Moss, M. L., & Hauser, L. (2019). Intraspecific DNA contamination distorts subtle population structure in a marine fish: Decontamination of herring samples before restriction-site associated sequencing and its effects on population genetic statistics. *Molecular Ecology Resources*, *19*(5), 1131–1143. https://doi.org/10.1111/1755-0998.12978

Pettibone, M. H. (n.d.). new species and new records of scaled polychaetes (Polychaeta: polynomial) from The Axial Seamount Caldera of the Juan De Fuca Ridge in the Northeast Pacific and the East Pacific Ocean of Northern California. P*roceedings of the Biological Society of Washington*, *103*(4), 14.

Plouviez, S., Daguin-Thiébaut, C., Hourdez, S., & Jollivet, D. (2008). Juvenile and adult scale worms Branchipolynoe seepensis in Lucky Strike hydrothermal vent mussels are genetically unrelated. *Aquatic Biology*, *3*(1), 79–87. https://doi.org/10.3354/ab00060

POLiKEY - DAWE. (n.d.). Retrieved May 10, 2022, from https://www.awe.gov.au/science-research/abrs/online-resources/polikey

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627. https://doi.org/10.1111/1755-0998.12512

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

Rouse, G., Pleijel, F., & Tilic, and E. (2022). *Annelida*. Oxford, New York: Oxford University Press.

Sarrazin, J., Juniper, S. K., Massoth, G., & Legendre, P. (1999). Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. *Marine Ecology Progress Series*, *190*, 89–112. https://doi.org/10.3354/meps190089

Tunnicliffe, V. (1991). The biology of hydrothermal vents : Ecology and evolution. *Undefined*. Retrieved from https://www.semanticscholar.org/paper/The-biology-of-hydrothermal-vents-%3A-Ecology-and-Tunnicliffe/faa4d2681f37b234fad588f26aca0ffca84b6aab

Tyler, P. A., & Young, C. M. (1999). Reproduction and dispersal at vents and cold seeps. *Journal of the Marine Biological Association of the United Kingdom*, *79*(2), 193–208. https://doi.org/10.1017/S0025315499000235

Tyler, Paul A., & Young, C. M. (2003). Dispersal at hydrothermal vents: a summary of recent progress. *Hydrobiologia*, *503*(1), 9–19. https://doi.org/10.1023/B:HYDR.0000008492.53394.6b

Van Dover, C., Trask, J., Gross, J., & Knowlton, A. (1999). Reproductive biology of free-living and commensal polynoid polychaetes at the Lucky Strike hydrothermal vent field (Mid-Atlantic Ridge). *Marine Ecology Progress Series*, *181*, 201–214. https://doi.org/10.3354/meps181201

Van Dover, C. L., Arnaud-Haond, S., Gianni, M., Helmreich, S., Huber, J. A., Jaeckel, A. L., et al. (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/10.1016/j.marpol.2018.01.020

Van Dover, Cindy Lee. (2014). Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: A review. *Marine Environmental Research*, *102*, 59–72. https://doi.org/10.1016/j.marenvres.2014.03.008

Wallace, J. (2005). Reproductive Attributes Of Polynoid Polychaetes From Hydrothermal Vents On The East Pacific Rise. https://doi.org/10.21220/S2-ZY51-8J97

WoRMS - World Register of Marine Species - Polynoidae Kinberg, 1856. (n.d.). Retrieved May 17, 2022, from https://www.marinespecies.org/aphia.php?p=taxdetails&id=939

Wu, X., Zhan, Z., & Xu, K. (2019). Two new and two rarely known species of Branchinotogluma (Annelida: Polynoidae) from deep-sea hydrothermal vents of the Manus Back-Arc basin, with remarks on the diversity and biogeography of vent polynoids. *Deep Sea Research Part I: Oceanographic Research Papers*, *149*, 103051. https://doi.org/10.1016/j.dsr.2019.05.011