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Natalie Lowell

A genetic risk assessment of native shellfish aquaculture

Natalie Lowell

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Reading Committee:

Lorenz Hauser, Chair

Eric Ward

Kerry-Ann Naish

Sarah Converse

Phillip Scott Levin

Program Authorized to Offer Degree:

School of Aquatic and Fishery Sciences

University of Washington

Abstract

A genetic risk assessment of native shellfish aquaculture

Natalie Lowell

Chair of the Supervisory Committee:
Dr. Lorenz Hauser
School of Aquatic and Fishery Sciences

Global aquaculture production is expanding, as the demand for marine protein cannot be met by capture fisheries alone. Within shellfish aquaculture, growers have begun to cultivate native shellfish to limit introduction of non-native species. However, cultivation of native species poses genetic risks to wild populations if farmed and wild animals interbreed, such as loss of genetic diversity within and among populations and loss of fitness due to domestication selection. These risks threaten long-term viability of wild populations, and are thus a concern for sustainable resource management. Genetic risks of native shellfish aquaculture have received little science and policy attention, limiting effective management of aquaculture impacts to wild populations. In my first two chapters, I quantified and characterized population structure in wild populations of two native shellfish species considered for aquaculture: the Purple-hinged Rock Scallop, *Crassadoma gigantea* (Chapter 1), and the California Sea Cucumber, *Apostichopus*

californicus (Chapter 2). These results can be used by decision-makers to inform spatial management of wild shellfish species, including mitigating impacts from aquaculture. To support potential policy development regarding the genetic risks of native shellfish aquaculture, I interviewed co-managers of shellfish resources along the Pacific Coast of the United States to characterize the regulatory context for this emerging policy issue (Chapter 3). Lastly, I developed a simulation model for quantifying genetic risks of native shellfish aquaculture and used the model in a management strategy evaluation for Olympia oyster (Chapter 4), a species grown for commercial and conservation purposes.

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DEDICATION

This dissertation is dedicated to the Pacific Ocean. Hey there, Ocean, you’re so big.

Chapter 1. Subtle population structure and adaptive differentiation in the purple-hinged rock scallop, *Crassadoma gigantea*

1.1 INTRODUCTION

Over the last several decades, many studies have uncovered population structure in marine species and thus overturned the traditional paradigm of ubiquitous panmixia in marine populations (Hauser & Carvalho, 2008; Palumbi, 2004; Sanford & Kelly, 2011). Increasing evidence suggests that marine dispersal is more restricted than previously thought, due to larval behavior that results in non-random dispersal (D'Aloia et al., 2015; Gerlach et al., 2007) and oceanographic barriers such as narrow straits and gyres (Shields et al., 2010) Additionally, the increased statistical power associated with advances in sequencing technologies, including higher marker density and inclusion of markers under selection (Stapley et al., 2010), has allowed for improved detection of subtle population differentiation. Nonetheless, population differentiation in marine species remains generally smaller than in terrestrial systems (Waples, 1998), due in part to greater dispersal potential (Kinlan & Gaines, 2003), lack of physical barriers, and larger population sizes common to many marine species. These factors lead to high gene flow and minimal genetic drift, both of which act to minimize the development of population differentiation. However, in some species, adaptive differentiation can be maintained despite high gene flow (G. Anderson et al., 2019). Thus, selection may play a larger role than drift in driving subtle patterns of population differentiation in some marine species.

If populations are differentiated in an exploited species, then effective resource management will benefit from characterization of population structure. Although populations lack a singular definition, a common definition is a group of individuals that can reproduce with each other (R. S. Waples & Gaggiotti, 2006). In the context of fisheries, populations can be considered

as an important criterion for designating appropriate units for management. When management units do not match with true biological populations, fishing may lead to overexploitation (Ying et al., 2011). In the context of aquaculture, data on population structure can be used to restrict transfer of broodstock and seed to within distinct populations, such as shellfish transfer zones used in British Columbia (DFO Aquaculture Management Division, 2016), in order to minimize loss of genetic diversity among populations (Waples et al., 2012).

One species that supports recreational fisheries and aquaculture is the Purple-hinged Rock Scallop, *Crassadoma gigantea*, a marine bivalve of the Pacific Coast of North America. Due to its patchy distribution and potentially limited abundance, commercial fisheries are prohibited across the range (Leet, 2001). The lack of commercial fisheries for *C. gigantea* has led many to pursue aquaculture production as a promising method of increasing supply. However, *C. gigantea* aquaculture has been slow to develop (Leighton & Phleger, 1977; Menzel, 2018) due to challenges in identifying necessary settlement conditions (RaLonde et al., 2012). Nevertheless, there are renewed efforts in developing large-scale production methods (Catalina Sea Ranch, 2019; Culver et al., 2006). Because of their patchy distribution and potentially limited abundance, there is potential for local depletions through broodstock collection and potential for population differentiation that could be disrupted through broodstock collection and farm escape. The likely expansion of *C. gigantea* aquaculture warrants careful consideration of population structure for effective management, yet no estimates of population structure exist for the species.

Population differentiation can be shaped by life history, such as high dispersal in species with pelagic larvae leading to lower population differentiation (Selkoe & Toonen, 2011). *C. gigantea* is a broadcast spawning species with a pelagic larval duration of about four weeks (Shumway & Parsons, 2016). After settlement, young juveniles can swim in short bursts and attach

to substrates with byssal threads. Unlike most species of scallop, *C. gigantea* cement to a substrate permanently at around six months of age (Menzel, 2018; Shumway & Parsons, 2016), suggesting lower dispersal potential than other scallop species and comparable dispersal potential to other sessile, broadcast-spawning bivalves. Although life span has not been rigorously estimated, *C. gigantea* are considered relatively long-lived, with a life span of at least 20 years (MacDonald et al., 1991). Fecundity has not been quantified, although *C. gigantea* is generally considered to have high fecundity (Laurén, 1982; Llodra, 2002); for comparison, other scallop species produce hundreds of thousands to millions of eggs per spawn event (Cochard & Devauchelle, 1993). *C. gigantea* are iteroparous, and may be able to spawn multiple times per year (Laurén, 1982). Spawn timing varies by region, with spawning occurring in summer to fall in Washington (Laurén, 1982) and British Columbia (MacDonald & Bourne, 1989) and in fall to winter in California (Jacobsen, 1977), a potential signal of adaptive differentiation.

Population differentiation can also be shaped by marine biogeography (Costello & Chaudhary, 2017; Selkoe et al., 2016). *C. gigantea* is patchily distributed from Baja California, Mexico to Alaska, from subtidal habitats to depths of 80 *m* (Dijkstra, 2010). Within this region, several known oceanographic barriers to dispersal have shaped population structure in other marine species. For example, the North Pacific Current, which bifurcates as it approaches the coast of British Columbia, has been identified as an oceanographic barrier to dispersal in the California Sea Cucumber (Chapter 2; Xuereb et al., 2018) and the Bat Star (Keever et al., 2009). Point Conception in central California has also been identified as an oceanographic barrier to dispersal in several marine species with planktonic dispersal (Pelc et al., 2009). Lastly, the Salish Sea, an estuary divided into sub-basins by shallow sills, contains genetically distinct populations of Pacific

Cod (Cunningham et al., 2009) and Brown Rockfish (Buonaccorsi et al., 2005). It is unknown whether these oceanographic features shape population structure in *C. gigantea*.

The goals of this study were to quantify and describe patterns of population differentiation for *C. gigantea* to facilitate the development of guidelines for aquaculture and fisheries management. We sampled populations at a small geographic scale within the Salish Sea and at a large geographic scale across the species range along the northeastern Pacific coast to test for both fine- and broad-scale population structure. The Salish Sea was chosen as a region of focus because it is known to harbor genetically distinct populations of some marine species (Andrews et al., 2018; Buonaccorsi et al., 2005; Cunningham et al., 2009) and because it contains the majority of aquaculture production in Washington state, the largest shellfish aquaculture producer in the United States (Northern Economics, 2013). Our results were used to generate hypotheses about potential drivers of population structure, including environmental factors that may drive observed putative adaptive differentiation. Lastly, we considered the management implications of a weak signal in population structure and made recommendations for decision makers regarding designation of management units for *C. gigantea*.

1.2 Methods

1.2.1 *Sample collection*

Approximately 50 adult *C. gigantea* were collected by scuba divers from seven sites along the Pacific Coast of North America, ranging from Alaska to California (Table 1-1). For each animal, a tissue sample was excised from the mantle and stored in 100% ethanol.

1.2.2 *DNA library preparation*

DNA was extracted from tissue samples using the EZNA Mollusc DNA Kit (OMEGA Bio-tek, Norcross, GA, USA) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA quality was checked by gel electrophoresis. DNA concentration was normalized to 500 *ng* in 20 μL of PCR-grade water. We selected samples with high DNA quality for RAD sequencing, and RAD libraries were prepared following an established protocol (Etter et al., 2011) with restriction enzyme *SbfI*. Briefly, DNA samples were restricted and barcodes with a unique six-base identifier sequence and the Illumina P1 adapter were attached to the restricted DNA. Samples were then pooled into sublibraries, containing approximately 12 individuals. Sublibraries were sheared using a Bioruptor sonicator and size selected to 200-400 *bp* using a MinElute Gel Extraction Kit (Qiagen, Germantown, MD, USA). Illumina P2 adapters were ligated to DNA in sublibraries and amplified with PCR using 12-18 cycles. Finally, amplified sublibraries were combined into pools of approximately 72 individuals for sequencing. Paired-end 2 x 150-base pair sequencing was performed on an Illumina HiSeq4000 (San Diego, California, USA) at the Beijing Genomics Institute and the University of Oregon Genomics and Cell Characterization Core Facility. Only forward reads were used for analysis. To estimate genotyping error, five individuals were sequenced twice.

1.2.3 *Genotyping individuals*

Prior to locus assembly, raw sequence data were demultiplexed using the *process_radtags* module in the *STACKS* v.1.44 pipeline (Catchen et al., 2013). Individuals with a threshold of 1.25M reads were retained, thus excluding poorly sequenced individuals. The *dDocent* v.2.7.8 pipeline (Puritz et al., 2014) was then used to assemble loci. As a genome was not available for *C. gigantea* or any close relative, we conducted a *de novo* assembly of loci using an 80% clustering

similarity threshold. To be retained in the reference assembly, a read had to have a minimum depth of four within an individual and appear in a minimum of 20 individuals; these parameters were chosen following the *dDocent* user guide (Puritz, 2019). Default *dDocent* parameters were used for read mapping (matching score = 1; mismatch penalty = 4; gap open penalty = 6).

To filter variant sites for data quality, the program *vcftools* v.0.1.16 (Danecek et al., 2011) was used to remove indels and to retain only SNPs with a minimum quality score of 20. We only retained SNPs with a minimum minor allele count of 5 reads and minimum genotype depth of 10 reads, per individual. Only SNPs with a minimum minor allele frequency of 5% across all collection sites and less than 30% missing data across individuals were retained. Individuals with more than 30% missing data across SNPs were removed. In cases of multiple SNPs per RAD tag, we retained the SNP with the highest minor allele frequency (Larson et al., 2014). SNPs significantly deviating from Hardy Weinberg Equilibrium (HWE) were removed to filter out sequencing errors and poorly assembled loci from our data set, as selection and inbreeding are unlikely to cause significant deviations from HWE equilibrium at biallelic loci (R. S. Waples, 2015). We tested deviations from HWE at each SNP using the program *genepop* v.1.1.4 (Rousset, 2008). SNPs were identified as deviating from HWE if they had a q-value below 0.05 in at least two of the collection sites, after correcting for multiple tests using a false discovery rate approach (R. S. Waples, 2015).

1.2.4 *Quantifying population structure*

We quantified genetic diversity and population structure using a suite of packages in R v.3.5.0 (R Core Team, 2020) and stand-alone software. We used Weir-Cockerham F_{ST} (Weir & Cockerham, 1984) to quantify population differentiation and exact G-tests to test for significant genic and genotypic differentiation, using the R package *genepop* (Rousset, 2008). To investigate

patterns of population differentiation, we conducted discriminant analysis of principal components (DAPC) using the R package *adegenet* v.2.1.1 (Jombart, 2008). A DAPC is a multivariate method that summarizes the between-group variation (i.e., population structure), while minimizing within-group variation (Jombart et al., 2010). The built-in optimization algorithm was used to determine the number of principal components to be retained without over-fitting the model. To estimate the number of underlying ancestral populations, we used the program *ADMIXTURE* v.1.3.0 (Alexander & Lange, 2011). This program uses a maximum likelihood-based clustering analysis to estimate individual ancestries across different assumed numbers of populations, with the best fit selected by minimizing cross-validation error. We used the R package *poppr* v.2.8.1 (Kamvar et al., 2015) to conduct analyses of molecular variance (AMOVA), which summarize the partitioning of genetic variance among different hierarchical groupings. In addition to among-group and within-group variance, AMOVAs partitioned variance within individuals to allow for deviations from HWE. As a consequence, Φ_{ST} was not calculated. Specifically, we used AMOVAs to test whether patterns in population differentiation supported hypotheses of particular oceanographic barriers to dispersal: 1) whether the North Pacific Current is an oceanographic barrier to dispersal (NPC grouping) and 2) whether the Salish Sea contains a distinct genetic population (Salish Sea grouping). Collection sites were assigned to groups within each grouping in Table 1-1. Sekiu, WA was considered outside of the Salish Sea because it is west of the Victoria Sill, the outermost oceanographic barrier between the Strait of Juan de Fuca and the Salish Sea. An additional AMOVA was conducted by state (State grouping). Although not biologically meaningful, this grouping was included to evaluate how well state boundaries capture observed genetic variation. Shellfish fisheries and aquaculture generally occur within state waters, and thus are co-managed by states and tribes. To look for evidence of limited dispersal driving

continuous population differentiation (isolation-by-distance), we tested for correlation between linearized F_{ST} using all SNPs and shortest Euclidean distance through water (hereafter in-water distance) approximated in Google Maps (Google, 2019), using Mantel tests (Mantel & Valand, 1970) in R.

1.2.5 *Identifying putatively adaptive SNPs*

We used two approaches to investigate putatively adaptive SNPs: F_{ST} outlier detection and gene-environment association. F_{ST} outlier detection is used to identify loci potentially under spatial selection (Foll & Gaggiotti, 2008; Whitlock & Lotterhos, 2015), although the potential cause of selection is not investigated using this method. Gene-environment association is used to identify locus-environment associations as evidence for potential local adaptation (Günther & Coop, 2013), but does not explicitly test whether such associations are adaptive. For the purposes of this study, SNPs were classified as putatively adaptive if they were detected as F_{ST} outliers or if they were significantly correlated to environmental predictors using gene-environment association.

For F_{ST} outlier detection, we used *Bayescan* v.2.1 (Foll & Gaggiotti, 2008) and the R package *OutFLANK* v.0.2 (Whitlock & Lotterhos, 2015). *Bayescan* first applies linear regression to decompose locus-population specific coefficients into a population- and a locus-specific component. Using these as Bayesian priors, the program estimates the posterior probability of a SNP being under selection (Foll & Gaggiotti, 2008). *OutFLANK* detects F_{ST} outliers using a maximum likelihood approach. The program first infers a distribution of neutral F_{ST} from a trimmed distribution of empirically collected F_{ST} values. Then it uses this neutral model to detect outliers. This approach builds upon earlier methods (Lewontin & Krakauer, 1973) by accounting for sampling error and non-independent sampling of populations, and has lower false positive rates

compared to other F_{ST} outlier methods (Whitlock & Lotterhos, 2015). SNPs were classified as F_{ST} outliers if they were detected with either program.

For gene-environment association, we used *Bayenv2* (Günther & Coop, 2013). The program uses a Bayesian framework to test for significant correlation between allele frequencies and environmental variables, accounting for population structure using previously estimated covariance among loci. We selected a broad suite of 29 ecologically relevant variables (such as temperature, salinity, and pH; Supplemental Table 1-1) for investigation with gene-environment association. Estimates for oceanographic variables at each collection site were collected using the Bio-Oracle and Bio-Oracle 2 databases (Assis et al., 2018), through the package *sdmpredictors* v.0.2.8 in R (Bosch et al., 2017). Where possible, oceanographic variables for both sea surface and mean bottom depth were used to account for the conditions experienced by both pelagic larvae and benthic adults. Correlations with a minimum Bayes Factor of 10, or minimum "strong" support (Kass & Raftery, 1995), were retained in the analysis. Interpretation of gene-environment results can be complicated by correlation among environmental predictors and/or spatial auto-correlation of environmental predictors. Thus, we quantified correlation among environmental predictors using Pearson's correlation coefficients and spatial auto-correlation for each environmental predictor using Moran's autocorrelation *I* coefficient. Because *Bayenv2* is a univariate approach, we reported gene-association results for all environmental predictors despite correlation among some predictors, as opposed to removing correlated predictors based on variance inflation in multivariate approaches. We nonetheless report potentially confounding correlations so that results can be interpreted in light of them.

1.2.6 *Comparing putatively neutral and adaptive SNPs*

SNPs were classified as putatively adaptive if they were detected as F_{ST} outliers (with either *Bayescan* or *OutFLANK*) or if they were significantly correlated to at least one environmental predictor (*Bayenv2*). Once we identified putatively adaptive SNPs, they were used to distinguish putatively neutral SNPs. To evaluate whether selection causes different patterns of population differentiation than demographic processes alone, we used putatively adaptive (affected by demographic processes and selection) and putatively neutral SNPs (affected by demographic processes) separately in AMOVA, DAPC, and Mantel tests for isolation-by-distance, using the same methods as used for all SNPs. Putatively neutral SNPs were used in the program *NeEstimator* (Do et al., 2014) to estimate effective population size (N_e) per collection site using the linkage disequilibrium method.

We used *blastx* v.2.5.0 (Altschul et al., 1990) and the UniProt Knowledge Base (Swiss-Prot, manually annotated) (The UniProt Consortium, 2019) to identify potentially associated biological processes for putatively adaptive SNPs. Specifically, we queried the entire RAD tag containing each SNP and retained matches with a maximum *e*-value score of 10^{-10}.

1.2.7 *Simulations*

To contextualize our genetic results and their implications for population connectivity, we developed a simulation model using the Python module *simuPOP* v.1.1.10.9 (Peng & Kimmel, 2005) to evaluate population sizes, migration rates, and number of generations of drift that reproduced empirically derived pairwise F_{ST} results. Two populations of equivalent size were simulated with discrete generations, random mating, and no selection. Two parents were selected at random with replacement to produce one offspring, allowing for a random distribution of reproductive success and for census size to approximate N_e. The model was parameterized using

empirical global allele frequencies for all putatively neutral SNPs from this study. Five simulations were run for each combination of N_e (500, 2,500, 10,000) and migration rate (0.01%, 0.03%, 0.1%, 0.3%, 1%, 3%, 10%, 30%). Additionally, simulations were run for 10 (short-term), 100 (medium-term), and 1,000 (long-term) generations of drift. Pairwise F_{ST} was calculated at the end of each simulation and compared to the range of empirical pairwise F_{ST} calculated in this study.

1.3 RESULTS

1.3.1 *Sequencing*

After removing 20 (6.6% of 301 sequenced individuals) poorly sequenced individuals, the average number of reads per individual was 2.86M (standard deviation (SD) = 1.07M). The *dDocent* assembly produced 585,186 variant sites and 5,932 SNPs were retained after filtering (Supplemental Table 1-2). One individual was removed for missing data, resulting in 280 retained individuals with an average of 40 individuals per population (SD = 16.7) (Table 1-1). Genotyping error was estimated to be 0.991%. among the five replicated individuals. Of those errors, the majority were a mismatch of a single allele. Only one error (0.3% of all errors) included mismatches of two alleles. Errors were distributed fairly evenly across 261 SNPs, with one error at 93% of SNPs, two errors at 6% of SNPs, and three errors at 1% of SNPs, across replicated individuals.

We did not observe notable patterns in expected heterozygosity, observed heterozygosity, F_{IS}, or proportion of polymorphic SNPs (Table 1-1). Expected and observed heterozygosity by collection site ranged from 0.248-0.253 and 0.214-0.222, respectively. F_{IS} ranged by collection site ranged from 0.107-0.125 (Supplemental Figure 1-1). The proportion of polymorphic SNPs by collection site ranged from 0.930-0.999.

1.3.2 *Population structure*

Global population differentiation using all SNPs was small (global F_{ST} = 0.0009) but significant (genic differentiation test, $p < 0.001$, genotypic differentiation test: $p < 0.001$). Estimated pairwise F_{ST} ranged from -0.0002 to 0.0021, with the greatest values for comparisons among the Port Gamble, WA and the two California collections (Table 1-2). None of the pairwise genic or genotypic differentiation tests were significant (p > 0.05). The DAPC using all SNPs revealed differentiation between collections from inside and outside of the Salish Sea along the first discriminant function (Figure 1-1 and Supplemental Figure 1-2A), and less clear geographic patterns along the second discriminant function (Supplemental Figure 1-2B). Permutation test results from AMOVAs using all SNPs demonstrated significant population structure for Salish Sea and State groupings, with comparable among-group variation explained by these groupings (Table 1-3). Permutation tests for AMOVA demonstrated that the NPC grouping was not significant (Φ_{CT} = -0.0003, $p > 0.05$; Table 1-3), and that variation among collection sites within NPC groups was significant (NPC $\Phi_{SC} = 0.0007$, $p < 0.05$; Table 1-3). Permutation tests for AMOVA did not detect significant variation among collection sites within groups for the State or Salish Sea groupings ($p > 0.05$). The ratio of Φ_{CT} / Φ_{SC}, which increases when among-group variance is high and within group-variance is low (i.e., a proxy for strength of the grouping), was highest for the Salish Sea and State groupings. Clustering analyses with *ADMIXTURE* provided the strongest support for the model with one population (Supplemental Figure 1-3). A Mantel test for correlation between pairwise linearized F_{ST} using all SNPs and in-water distance was not significant (Mantel R = -0.517, $p > 0.05$; Supplemental Figure 1-4).

1.3.3 *Putatively adaptive differentiation*

We identified 109 (1.8% of 5,932 total SNPs) putatively adaptive SNPs: 11 using *Bayescan*, 21 using *OutFLANK*, and 90 using *Bayenv2* (Figure 1-2 and Supplemental Figure 1-5; sequences containing putatively adaptive SNPs will be available upon publication). At least one SNP was correlated to each of the 29 environmental variables (Supplemental Table 1-3). None of the putatively adaptive SNPs matched homologous genes in the UniProt Knowledge Base, and therefore it was not possible to annotate putatively adaptive loci.

SNPs identified as putatively adaptive using the gene-environment association method were correlated to an average of 2.08 environmental variables per SNP (SD = 1.68, range = 1 - 9). The five environmental variables with the most associated SNPs included minimum temperature, maximum current velocity, range in temperature, and minimum current velocity, all at mean bottom depth, and range in temperature at sea surface (Table 1-4). Roughly 48% more SNPs were correlated to environmental variables measured at mean bottom depth than at the surface, for the set of variables with both measurements: 98 SNP matches to mean bottom depth variables and 66 SNP matches to sea surface variables. We detected significant correlation among 16 environmental predictor pairs (3.9% of total pairs; Supplemental Table 1-4) and spatial autocorrelation for 5 environmental predictors: pH, mean and minimum current velocity and mean salinity at sea surface, and mean temperature at mean bottom depth (Supplemental Table 1-5).

1.3.4 *Neutral vs. putatively adaptive differentiation*

Global F_{ST}, pairwise F_{ST}, DAPC, and AMOVA revealed higher differentiation and different spatial patterns for putatively adaptive SNPs compared to putatively neutral SNPs (Table 1-3, Supplemental Tables 1-6 through 1-8, and Figure 1-1). Specifically, global F_{ST}, mean pairwise F_{ST},

and Φ_{CT} (for Salish Sea and State groupings) were over 27, 33, and 9 times greater, respectively, using putatively adaptive SNPs than putatively neutral SNPs (Supplemental Tables 1-6 through 1-8). The highest pairwise F_{ST} using putatively neutral SNPs and putatively adaptive SNPs were between the Salish Sea sites and the California sites (Supplemental Tables 1-7 and 1-8). The ratio of Φ_{CT} / Φ_{SC} was higher for neutral SNPs than putatively adaptive SNPs for the Salish Sea and State groupings, suggesting that demographic processes (e.g., limited migration) may drive differentiation among these groups to a greater extent than selection. The NPC grouping yielded significant variation among collection sites within groups using putatively neutral SNPs, suggesting that demographic processes (e.g., limited migration) cause detectable differences not captured by this grouping. We did not find significant correlation ($p > 0.05$) between in-water distance and linearized F_{ST} using putatively neutral or putatively adaptive SNPs (Supplemental Figure 1-4).

1.3.5 *Simulations*

Across all scenarios of genetic drift (10, 100, and 1,000 generations), simulated pairwise F_{ST} values were never within the range of observed pairwise F_{ST} values, due to our parameter value choices, which were few and captured a broad parameter space. However, simulated pairwise F_{ST} values within the range of observed pairwise F_{ST} values would have occurred in the parameter space between our parameter values: when the migration rate was between 10% and 30%, when N_e was small ($N_e = 500$), or when the migration rate was between 0.3% and 3% with moderate to large N_e (N_e = 2,500-10,000). Under assumptions of large populations and populations that have been stabilizing for many generations (N_e = 10,000 and 1,000 generations of drift), which may be realistic for large marine populations, results suggested that simulated pairwise F_{ST} values that fell

within the range observed in this study occurred with migration rates between 0.3% and 1% (Figure 1-3).

1.4 DISCUSSION

In this study, we quantified and characterized patterns of population differentiation in *C. gigantea,* providing the first investigation of its kind in the species. We found evidence for low but significant population structure, with results consistent with a distinct Salish Sea group. Additionally, we found subtle signatures of adaptive differentiation correlated to variation in temperature and current velocity. Putatively adaptive differentiation was greater than neutral differentiation but neutral differentiation aligned more closely with geographic patterns.

1.4.1 *Evidence for subtle population structure and adaptive differentiation*

We found limited evidence for population differentiation in *C. gigantea*. Only the global genic and genotypic differentiation tests and the AMOVA permutation tests provided evidence for population structure, and the proportions of variation explained by the site groupings with AMOVA were small and consistent with the small F_{ST} values. Overall, population structure was subtle enough that the *ADMIXTURE* clustering analysis results suggested our data are best explained by one underlying population. This result was consistent with a lack of distinct clustering by site using DAPC and no significant pairwise genic or genotypic differentiation tests between collection sites. Although these results are fairly consistent with the traditional paradigm of marine populations as large and connected populations (Hauser & Carvalho, 2008), the subtle differentiation may be surprising given the broad geographic range sampled, the lack of adult dispersal (Shumway & Parsons, 2016), and the great statistical power associated with thousands of SNPs. Many other marine bivalves demonstrated greater degrees of population differentiation

over similar or smaller geographic scales (Lal et al., 2016; K. Silliman, 2019; Van Wyngaarden et al., 2017). Although our sample sizes warrant caution in interpretation of N_e estimates (Marandel et al., 2019), it is likely that N_e estimates per collection site are not small given the infinite upper confidence limits in estimates across collection sites. Furthermore, N_e estimates using small sample sizes (~50 individuals, compared to the recommended 1% of total individuals) are generally downward biased (Marandel et al., 2019). Thus, simulation results for realistic conditions ($N_e > 500$) suggested that differentiation as low as observed here occurred when migration rates were between 0.3-3%, which is less than the 10% threshold used as a benchmark for demographic independence (R. S. Waples & Gaggiotti, 2006).

As for the factors potentially driving the observed population differentiation, we did not find evidence for isolation-by-distance shaping population structure, which is consistent with results in the Sea Scallop (Van Wyngaarden et al., 2017) but not in the Weathervane Scallop (Gaffney et al., 2010) or Great Scallop (Vendrami et al., 2017). Instead, our results were consistent with differentiation between collection sites inside and outside of the Salish Sea, suggesting that the Victoria Sill may limit dispersal between coastal waters and the Salish Sea. Differentiation among sites within and outside of the Salish Sea likely drove much of the differentiation in the State grouping, as most Washington State collection sites are situated within the Salish Sea.

The small percentage of putatively adaptive SNPs (1.8%) and the small overlap (3 SNPs) among putatively adaptive SNPs using F_{ST} outlier and gene environment association approaches suggests that our results provide only inconclusive evidence for adaptive differentiation. Subtle adaptive differentiation observed here may be due to selection related to temperature and current velocity, as these environmental predictors had the most correlated SNPs in the gene-environment association analysis. Evidence for temperature-related selection driving population differentiation

has also been observed in the Sea Scallop (Lehnert et al., 2019; Wyngaarden et al., 2018), including at small scales and with uneven breaks between environments. Additionally, sea surface temperature and dissolved oxygen were correlated to genetic divergence in the Great Scallop and its sister species the Mediterranean Scallop, (Vendrami et al., 2017). The greater proportion of SNPs significantly correlated to environmental variables at mean bottom depth than at the surface may point to a signal of selection on larval settlement site choice in *C. gigantea*. For example, Bay Barnacle larvae actively select sites with a particular flow rate that allows for optimized feeding in adult stages (Larsson & Jonsson, 2006). Additional research is needed to determine the potential of these factors as drivers of adaptive differentiation in *C. gigantea.*

Inferences on selection may be affected by spatial auto-correlation of environmental predictors and correlation among environmental predictors. Spatial auto-correlation can bias results in *Bayenv2* (Günther & Coop, 2013) when spatial population structure is present. Here, spatial population structure was subtle and pH was the only predictor with significant spatial autocorrelation included in the top ten predictors with the most correlated SNPs. Three of the top ten environmental predictors with the most correlated SNPs using gene-environment association were significantly correlated to other environmental predictors investigated here. Correlation among environmental predictors and spatial auto-correlation of predictors increase the uncertainty over which predictors are most important as selection factors in gene-environment association analyses. Nonetheless, our results can be used alongside future results to contribute to future studies on the mechanisms underlying adaptive differentiation in *C. gigantea*.

Lastly, inferences about putative adaptive differentiation should be considered within the context of the biology of the species and study design. For example, adaptive differentiation detected in adults may represent local adaptation or balanced polymorphism (Sanford & Kelly,

2011). Local adaptation occurs when resident genotypes have higher fitness in native habitats compared to distant ones, and develops when the scale of dispersal is smaller than that of the selection gradient. A similar signal of genetic differentiation can be created by balanced polymorphism, but develops when the scale of dispersal is greater than that of the selection gradient, and when purifying selection increases the frequency of adaptive genotypes. The two are distinguished by whether genetic differentiation is present at settlement or occurs via post-settlement mortality (Sanford & Kelly, 2011). Without sampling early life history stages (here, we only sampled adults) and obtaining robust estimates of dispersal, local adaptation and balanced polymorphism cannot be distinguished.

1.4.2 *Considerations for inferring population connectivity from subtle population structure*

The use of thousands of molecular markers allows for detection of quite subtle differences, which creates a multi-tiered dilemma for decision makers: Is observed subtle population differentiation signal or noise (R. S. Waples, 1998)? If it is a true signal, what can we infer from subtle spatial differentiation about population connectivity (Marko & Hart, 2011)? And when is differentiation too subtle to warrant management action (R. S. Waples, 1998)? Although there are no straightforward answers, we outline guiding principles below.

Subtle population structure can represent a false detection of population structure when there is none if assumptions of random sampling are violated. Populations may exist in broad geographic regions, yet scientists often sample in discrete locations due to limited resources. If non-random sampling leads to sampling of related individuals, results may reflect measurable differences in allele frequencies among collections that do not reflect true population differentiation (Schwartz & McKelvey, 2009; R. S. Waples, 1998). Such a false positive result could also be due to chaotic genetic patchiness, ascribed to temporally unstable genetic

heterogeneity that occurs at micro-scales in some marine species (Eldon et al., 2016). However, our collection sites within the Salish Sea served as spatial replicates given the low levels of divergence among them, and strengthened our conclusions. Because truly random sampling is hard to achieve, an alternative is to include temporal replicates in the study design. Assuming populations are in equilibrium, biologically significant population structure should be temporally stable over short time scales. Although temporal samples that reproduce results can be used to separate signal from noise (Waples, 1998), obtaining temporal samples across multiple generations is challenging for long-lived species such as *C. gigantea*, and was outside the scope of this study.

Assumptions of random sampling can also be violated in sampling the genome. RAD loci are generally assumed to be independent but in many studies thousands of RAD loci are distributed across tens of chromosomes, suggesting some RAD loci may be physically linked (Willis et al., 2017). However, linkage disequilibrium decays more rapidly with physical genomic distance in species with large N_e (Hemmer-Hansen et al., 2014) such as many marine species, increasing the likelihood that RAD loci are independent in such study systems. Although some RAD loci may be linked, they are likely few, and their bias on estimating parameters such as N_e is likely small (R. K. Waples et al., 2016). Without a reference genome, our ability to estimate linkage disequilibrium due to physical linkage and interpret results was limited. Furthermore, artifacts and biases may be introduced in library preparation, bioinformatic assembly, and filtering of loci, resulting in non-random sampling. For example, using too low a clustering threshold in *de novo* assembly can result in multiple loci being grouped as one locus, creating both an artifactual SNP and artificially increasing heterozygosity (O'Leary et al., 2018). Many of these errors can be mitigated by following best practices outlined elsewhere (O'Leary et al., 2018), many of which were followed herein.

Population genetic analyses may fail to detect true population structure if there is limited statistical power, adding uncertainty as to whether results reflect a true biological signal or sampling "noise" when only few tests yield significant results. Generally, power increases with the number and size of collections, number of loci and alleles, and magnitude of true divergence (Morin et al., 2009). Our use of nearly 6,000 SNPs suggests we had substantial statistical power for detecting divergence. Power also depends on statistical method (Ryman et al., 2006). For example, pairwise tests compare fewer individuals than global tests, resulting in reduced power. Here, only the global genic and genotypic differentiation tests and some AMOVA permutation tests yielded significant results. We suspect some of our negative test results (e.g., pairwise genic differentiation) were due to limited statistical power a small true signal. For example, the statistical power of the Mantel tests may have been limited by the relatively few pairs of sites (21) in this study. Similarly, clustering analyses such as those conducted in *ADMIXTURE* have particularly reduced power when the magnitude of true divergence is small (R. S. Waples & Gaggiotti, 2006), as was the case in this study. However, the geographic pattern in neutral differentiation is unlikely to occur by chance, suggesting it is a true, weak signal of limited dispersal shaping population structure.

Assuming a signal in subtle population structure is a true positive result, there remain limits on inferences of population connectivity from spatial differentiation alone. Differentiation can be a result of a suite of factors including mutation, gene flow, and genetic drift. To make population connectivity inferences from spatial differentiation, empiricists make assumptions that studied populations are in migration-drift equilibrium, such that the rate of differentiation decline by gene flow is equal to the rate of its increase due to drift (Marko & Hart, 2011). However, wild populations are often not in migration-drift equilibrium (Hellberg, 2009) and without estimating

the effects of these other factors, it is impossible to attribute subtle spatial differentiation to limited gene flow with confidence. For example, subtle differentiation may reflect populations with small N_e connected by high gene flow or populations with large effective populations connected by limited gene flow (Marko & Hart, 2011), each warranting different management recommendations. For example, populations with large effective populations connected by limited gene flow may be more susceptible to local over-exploitation. Here, we estimated N_e to be large and unbounded, suggesting at the least that N_e is not small, although we acknowledge that our sample size and the likely large population size of *C. gigantea* greatly limit power and likely bias our estimates of N_e downward (Marandel et al., 2019). Thus, one possible explanation for subtle population differentiation in *C. gigantea* is populations with large N_e and low levels of gene flow. Due to a lack of information on the population history this species, we cannot exclude other scenarios including recent vicariance, in which subtle population differentiation represents recently diverged populations with limited or no gene flow.

Even with certainty that population differentiation is due to limited dispersal, it remains challenging to determine when subtle differentiation is biologically meaningful (R. S. Waples, 1998). Complicating the question of the appropriate level of differentiation sufficient to differentiate populations is the ambiguity regarding what constitutes a population in the first place (Waples and Gagiotti, 2006). There are many definitions for "population" that can be categorized into ecological and genetic paradigms (Waples and Gagiotti, 2006). Specifically, the genetic framework for populations describes a group of individuals that reproduce with each other, defined by a limited number of effective migrants (N_em) among populations, whereas the ecological framework describes a group that co-occurs in space, defined by a limited migration rate (m). Approximations for operationalization suggest that within a genetic paradigm, $N_em < 25$ represents

a boundary for the lower end of population differentiation observed in natural populations (Waples & Gaggiotti, 2006). Using the approximation by Wright, $F_{ST} = \frac{1}{1+4N_e m}$ (Wright, 1984), 25 effective migrants per generation corresponds to an F_{ST} of ~0.01. Even the greatest pairwise F_{ST} estimated between populations herein (0.0021) corresponds to a number of effective migrants almost five times greater. However, this generalization for a genetic population provided by Waples & Gaggiotti (2006) is strongly affected by marker type (e.g., high mutation rate such as microsatellites versus low mutation rate such as SNPs) and assumes conditions that may not be appropriate for marine populations, such as an island model with distinct population boundaries under selective neutrality. Although, in some cases, inferences of population connectivity that assume an island model may be good approximations (Spies et al., 2018). Differentiation in marine species smaller in scale than the generalization ($F_{ST} < 0.01$ or $N_e m > 25$) provided by Waples & Gaggiotti (2006) has been considered biologically meaningful, especially when correlated to variation in life history traits such as spawn-timing (Kovach et al., 2010) or aggregation site (Jackson et al., 2014).

A central null hypothesis in population genetic analyses is panmixia, in which statistical tests are used to determine whether allele frequencies from collections are drawn from a single distribution (Rousset, 2008). This null hypothesis mirrors the traditional paradigm of marine populations as demographically "open" (Hauser & Carvalho, 2008). However, detecting subtle population structure and thus rejecting the null hypothesis of panmixia has become commonplace in studies of marine populations due to greater statistical power associated with advances in sequencing technology, including species once characterized as panmictic (Vendrami et al., 2017). The European eel is a rare example of a marine species with negligible levels of spatial differentiation assessed using whole genome sequencing, although it has a unique life history

consistent with random mating in which all adults migrate to and reproduce in the Sargasso Sea (Enbody et al., 2021). Thus, rejecting the null hypothesis of panmixia alone is not as informative for decision-makers as characterizing the spatial and/or temporal population differentiation alongside guidance for determining biological significance, particularly when signals are subtle. Overall, the level of differentiation observed in *C. gigantea* is less than the accepted definitions for genetically isolated populations (R. S. Waples & Gaggiotti, 2006). Our findings are comparable with levels of subtle differentiation considered panmictic in some species (e.g., significant pairwise F_{ST} = 0.004 – 0.008 in Skipjack Tuna (G. Anderson et al., 2020)) but representative of population genetic structure in others (Jackson et al., 2014; Kovach et al., 2010). Using our empirical and simulation results, we propose that the subtle signal of genetic differentiation in *C. gigantea* reflects genetically connected but demographically independent populations. Further fine-scale sampling, temporal replicates, and comparisons of genetic data with morphological or life history traits would facilitate estimates of population connectivity and designation of meaningful biological populations in *C. gigantea*. Inevitably, any threshold for defining minimum differentiation to warrant management action will be context specific and not always driven by genetic data alone (Waples, 1995).

1.4.3 *Conclusions and future directions*

There is risk in wrongly assuming a lack of meaningful differentiation and then losing true differentiation due to mismanagement; there is also risk in wasting limited resources by managing smaller units than are biologically meaningful (Waples, 1998). We found differentiation consistent with a distinct Salish Sea group, although finer scale sampling is needed to evaluate how distinct this genetic breaks is. Thus, we recommend that the Victoria Sill (separating the Salish Sea from the Strait of Juan de Fuca) be used as a boundary for designating management units. Using state

boundaries as well may serve as a precautionary approach, as the exact genetic breaks were not identified due to the coarse sampling scheme in our study and the State grouping captured the most among-group genetic variation. Because demographic independence may occur at a scale smaller than that suggested by genetic differentiation, managers should take precautions to prevent local exploitation from broodstock mining, as demographically independent populations take longer to be replenished by migrants than more connected populations. Because management horizons (e.g., a few years) may be much shorter than the life span of *C. gigantea* (> 20 years), local depletions may be slow to be replenished for this species.

Additionally, we recommend that future research on population connectivity in *C. gigantea* collect complementary genetic data, particularly from sites along the Pacific coast between Sekiu, WA and Monterey Bay, CA to identify potential oceanographic barriers causing genetic divergence between our northern and southern coastal sites. With evidence for the Victoria Sill as an oceanographic barrier to dispersal, we also recommend sampling within the Haro Strait and Strait of Georgia, which are also separated by sills within the Salish Sea (Davenne & Masson, 2001). If future sampling includes early life history stages, results could be used to evaluate whether putative adaptive differentiation is a signal of local adaptation or balanced polymorphism. Using whole genome sequencing in future studies would allow for detection of potential rare adaptive peaks. Lastly, complementing genetic data with other data types, including direct estimates of larval dispersal and morphological data, could facilitate separation of signal and noise in inferring population connectivity from differentiation.

1.5 TABLES AND FIGURES

Table 1-1: General information by collection site for *C. gigantea*. Columns State, NPC and SS assign collection sites to groups for AMOVA. Specifically, column State refers to state using two letter codes, column NPC refers to whether north (N) or south (S) of the North Pacific Current, column SS refers to whether inside (I) or outside (O) the Salish Sea, columns Lat and Long refer to latitude and longitude of collection sites, respectively, column I_S refers to the number of individuals sequenced, column I_R refers to the number of individuals retained in analyses, column H_E refers to mean expected heterozygosity, column H_O refers to mean observed heterozygosity, and column S_P refers to the proportion of SNPs that are polymorphic.

Site	State	NPC	SS	Lat	Long	I_S	I_R	H_E	H_O	F_{IS}	S_P
Seward	AK	N	O	60.100	-149.400	22	20	0.248	0.215	0.107	0.994
Sekiu	WA	S	O	48.160	-124.175	59	59	0.249	0.217	0.130	0.998
Port Gamble	WA	S	I	47.857	-122.580	19	15	0.248	0.216	0.122	0.997
Cypress Island	WA	S	I	48.559	-122.727	50	50	0.248	0.216	0.125	0.996
Dabob Bay	WA	S	I	47.441	-124.175	55	53	0.250	0.214	0.125	0.930
Monterey	CA	S	O	36.821	-121.969	48	44	0.249	0.217	0.124	0.999
Catalina Isl.	CA	S	O	48.332	-122.410	48	39	0.253	0.222	0.125	0.962

Table 1-2: Pairwise F_{ST} between collection sites using all SNPs for *C. gigantea*. Cells are shaded darker green for higher values. SW_AK = Seward, Alaska; SK_WA = Sekiu, Washington; PG_WA = Port Gamble, Washington, CI_WA = Cypress Island, Washington; DB_WA = Dabob Bay Washington; MB_CA = Monterey Bay, California; CI_CA = Catalina Island, California.

	SW_AK	SK_WA	PG_WA	CI_WA	DB_WA	MB_CA	CI_CA
SW_AK	-						
SK_WA	-0.0002	-					
PG_WA	-0.0001	0.0010	-				
CI_WA	0.0010	0.0007	0.0005	-			
DB_WA	0.0010	0.0010	0.0005	0.0000	-		
MB_CA	0.0010	0.0009	0.0021	0.0014	0.0018	-	
CI_CA	0.0005	0.0013	0.0021	0.0013	0.0014	0.0001	-

Table 1-3: Hierarchical population structure results (AMOVA) for *C. gigantea*. Column Grouping contains three groupings considered with AMOVA: north and south of the North Pacific Current, inside and outside of the Salish Sea, and by state (collection sites are assigned to groups within these groupings in Table 1-1). Column SNPs refers to which data set was used in the AMOVA: all SNPS (All), putatively neutral SNPs (Neutral), or putatively adaptive SNPs (Adaptive). Variations among groups (Φ_{CT}) and among collection sites within groups (Φ_{SC}) are reported, and significance of permutation tests noted with *: $p < 0.05$. The Φ_{CT} / Φ_{SC} is also reported, where negative Φ statistics were assumed to be 0.00001.

Grouping	SNPs	Φ_{CT}	Φ_{SC}	Φ_{CT} / Φ_{SC}
North Pacific Current	All	-0.000349	0.000749*	0.0134
	Neutral	-0.000375	0.000516*	0.0194
	Adaptive	0.00105	0.0129*	0.0814
Salish Sea	All	0.000847*	0.000198	4.2778
	Neutral	0.000658*	0.0000730	9.0137
	Adaptive	0.0107*	0.00680*	1.5735
State	All	0.000882*	0.000157	5.6178
	Neutral	0.000672*	0.0000490	13.7143
	Adaptive	0.0119*	0.00587*	2.0273

Table 1-4: Summary of gene-environment association results for *C. gigantea*, for five environmental variables with the highest number of correlated SNPs. Column Environmental Variable contains the name of the environmental variable in question. Column Depth refers to whether the environmental variable is measured at sea surface (S) or mean bottom depth (B). Column S_C contains the number of SNPs with evidence of correlation to the row's variable.

Environmental Variable	Depth	S_C
Minimum temperature	B	17
Maximum current velocity	B	12
Range in temperature	B	11
Minimum current velocity	B	10
Range in temperature	S	10

Table 1-5: LD N_e estimates with 95% confidence intervals per collection site for *C. gigantea*. Confidence intervals estimated by jackknifing across samples. *Pcrit* is the minimal allele frequency to retain a SNP in the analysis. Both $Pcrit_{0.05}$ (0.05%) and $Pcrit_{0.0}$ (0%) because rare alleles can affect N_e estimates.

	$Pcrit_{0.05}$		$Pcrit_{0.0}$	
Site	Estimate	95% CI	Estimate	95% CI
Seward, AK	2259.4	790.1 - Infinity	5394.2	951.1 - Infinity
Sekiu, WA	Infinity	6114.4 - Infinity	Infinity	7964.0 - Infinity
Port Gamble, WA	Infinity	1105.1 - Infinity	Infinity	107933.2 - Infinity
Cypress Island, WA	Infinity	7267.0 - Infinity	Infinity	9502.8 - Infinity
Dabob Bay, WA	23408.6	11050.1 - Infinity	24327.9	11922.0 - Infinity
Monterey, CA	Infinity	6741.6 - Infinity	Infinity	6033.6 - Infinity
Catalina Island, CA	41053.6	2898.4 - Infinity	60635.3	3135.2 - Infinity

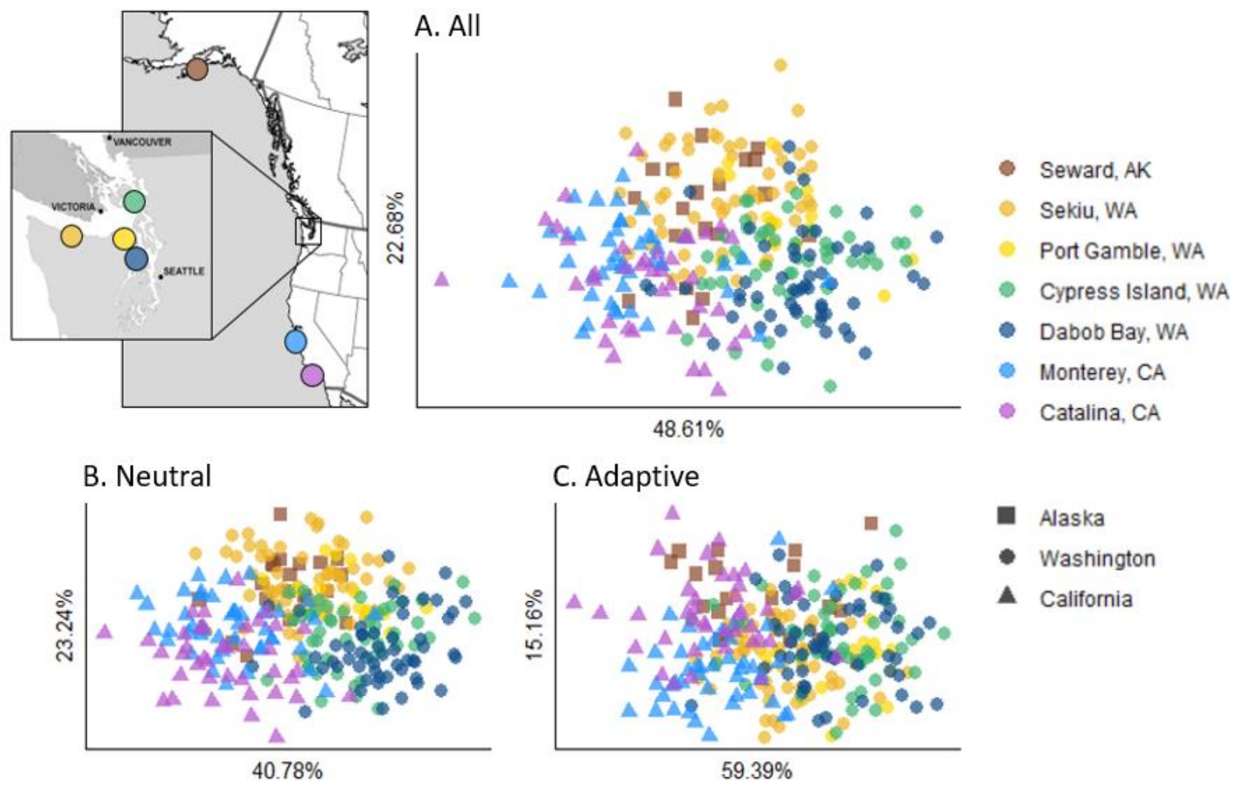


Figure 1-1: A map of collection sites and DAPC results for *C. gigantea*. The map (upper left) is color-coordinated with the DAPCs. DAPCs represent the first two discriminant functions, using all SNPs (A), putatively neutral SNPs (B), and putatively adaptive SNPs (C). For each plot, axes are labeled with the proportion of among-population variance explained by that discriminant function. Point shapes differ by state to highlight regional effects.

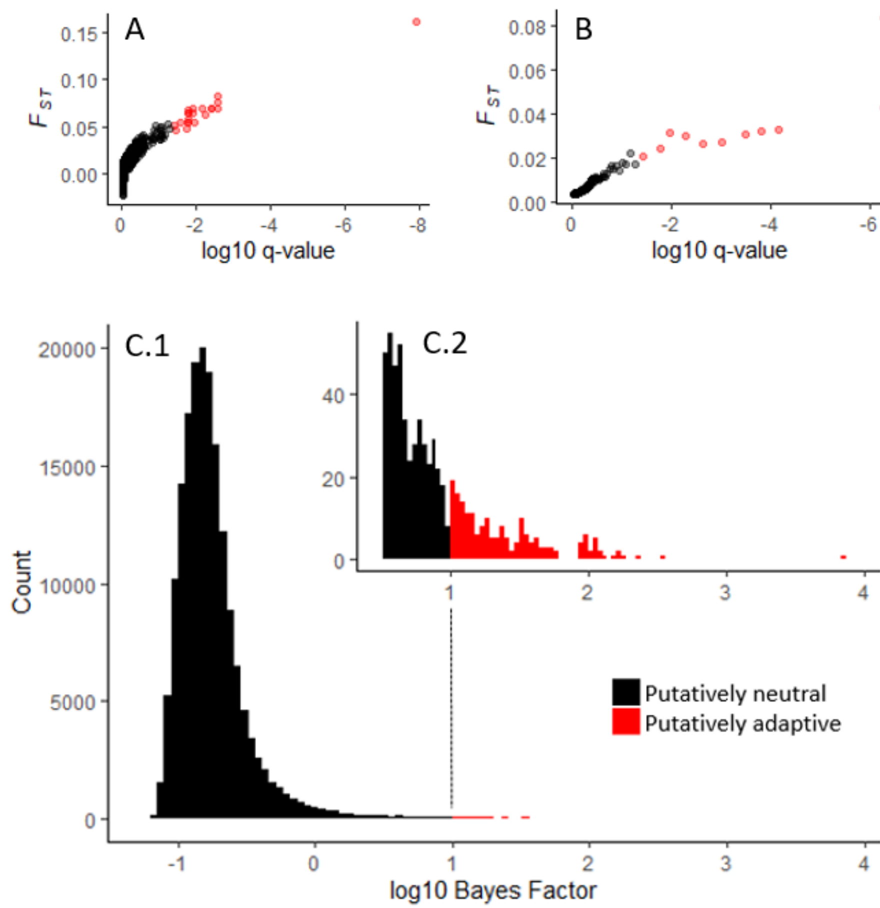


Figure 1-2: Summary of putatively adaptive loci for *C. gigantea*. Plots A and B are the $\log_{10}$ q-value by F_{ST} for outlier detection methods *OutFLANK* and *BayeScan*, respectively. Each point represents a single SNP, colored by whether it was detected as an outlier (red) or not (black). Plots C.1 and C.2 present the results from gene-environment association tests using *Bayenv2*. Plot C.1 is a histogram of $\log_{10}$ Bayes Factors for each pair of SNP and environmental predictor, and plot C2 is an inset highlighting the right tail of the distribution. The threshold for significance, $\log_{10}$ Bayes Factor = 1, is noted with a dashed line in C.1 and by coloring the significant right end of the distribution red, and the non-significant left end black.

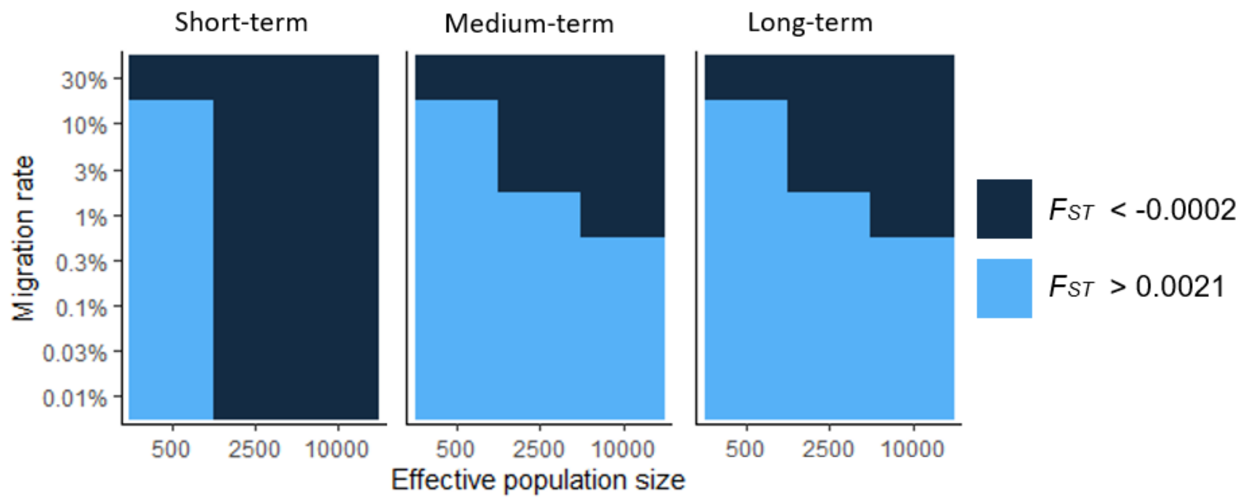


Figure 1-3: Simulation results to contextualize empirical population differentiation and estimate connectivity for *C. gigantea*. Tile maps representing mean pairwise F_{ST} across replicate simulations for each combination of effective population size, migration rate, and number of generations of drift elapsed. Short-term (left) represents results from 10 generations of drift, Medium-term (middle) from 100 generations of drift, and Long-term (right) from 1,000 generations of drift. Tile color is divided into two groups based on the scale of F_{ST}: smaller than the observed pairwise F_{ST} values between collection sites and greater than the observed pairwise F_{ST} between collection sites in this study. No simulations yielded an F_{ST} value within the range of observed F_{ST} values.

1.6 Supplementary Materials

Supplemental Table 1-1: Environmental predictor codes and descriptions from Bio-Oracle (BO) and Bio-Oracle2 (BO2) databases, used in *Bayenv2*.

Number	Code	Description
1	BO_bathymean	Average depth of the seafloor
2	BO_calcite	Calcite concentration indicates the mean concentration of calcite (CaCO3) in oceans.
3	BO_ph	Measure of acidity in the ocean.
4	BO2_curvelmax_bdmean	Maximum sea water velocity at mean bottom depth
5	BO2_curvelmax_ss	Maximum surface current velocity
6	BO2_curvelmean_bdmean	Mean sea water velocity at mean bottom depth
7	BO2_curvelmean_ss	Mean surface current velocity
8	BO2_curvelmin_bdmean	Minimum sea water velocity at mean bottom depth
9	BO2_curvelmin_ss	Minimum surface current velocity
10	BO2_curvelrange_bdmean	Range of the sea water velocity at mean bottom depth
11	BO2_curvelrange_ss	Range of surface current velocity
12	BO2_dissoxmean_bdmean	Mean mole concentration of dissolved molecular oxygen in sea water at mean bottom depth
13	BO2_dissoxmean_ss	Mean dissolved oxygen concentration
14	BO2_nitratemean_bdmean	Mean mole concentration of nitrate in sea water at mean bottom depth
15	BO2_nitratemean_ss	Mean mole concentration of nitrate at the sea surface
16	BO2_phosphatemean_bdmean	Mean mole concentration of phosphate in sea water at mean bottom depth
17	BO2_phosphatemean_ss	Mean mole concentration of phosphate at the sea surface
18	BO2_ppmean_bdmean	Mean net primary productivity of carbon at mean bottom depth
19	BO2_ppmean_ss	Mean sea surface net primary productivity of carbon
20	BO2_salinitymean_bdmean	Mean sea water salinity at the bottom at mean bottom depth
21	BO2_salinitymean_ss	Mean sea surface salinity
22	BO2_tempmax_bdmean	Maximum sea water temperature at the bottom at mean bottom depth
23	BO2_tempmax_ss	Maximum sea surface temperature
24	BO2_tempmean_bdmean	Mean sea water temperature at the bottom at mean bottom depth
25	BO2_tempmean_ss	Mean sea surface temperature

26	BO2_tempmin_bdmean	Minimum sea water temperature at the bottom at mean bottom depth
27	BO2_tempmin_ss	Minimum sea surface temperature
28	BO2_temprange_bdmean	Range of the sea water temperature at the bottom at mean bottom depth
29	BO2_temprange_ss	Range of sea surface temperature

Supplemental Table 1-2: Sites remaining after each filtering step for *C. gigantea*.

Filtering step	Sites
After *dDocent*	585,186
After removing indels	480,812
After requiring a minimum minor allele count = 5	103,373
After requiring minimum quality score = 20	102,826
After requiring minimum genotype depth = 10	102,826
After requiring minimum minor allele frequency = 0.05	35,555
After requiring maximum missing data = 30%	15,035
After removing SNPs out of Hardy Weinberg Equilibrium	11,280
After retaining SNP with highest minor allele frequency per RAD locus	5,932

Supplemental Table 1-3: Summary of *Bayenv2* results for *C. gigantea*. Number of SNPs (column Correlated SNPs) with at least strong evidence for correlation with environmental predictors (column Environmental Predictor). Column S or B refers to whether the environmental predictor was measured at sea surface (S) or mean bottom depth (B).

Environmental Predictor	S or B	Correlated SNPs
Minimum temperature	B	17
Maximum current velocity	B	12
Range in temperature	B	11
Minimum current velocity	B	10
Range in temperature	S	10
Mean current velocity	B	9
Mean bathymetry	B	8
pH	S	8
Mean nitrate	S	8
Mean calcite	S	7
Range in current velocity	B	7
Mean dissolved oxygen	S	7
Mean phosphate	S	7
Maximum current velocity	S	6
Mean primary production	B	6
Maximum temperature	B	6
Mean temperature	B	6
Minimum temperature	S	6
Maximum temperature	S	5
Mean nitrate	B	4
Mean phosphate	B	4
Mean primary production	S	4
Mean temperature	S	4
Mean dissolved oxygen	B	3
Mean salinity	B	3
Mean salinity	S	3
Mean current velocity	S	2
Minimum current velocity	S	2
Range in current velocity	S	2

Supplemental Table 1-4: Correlation among environmental predictors measured at collection sites for *C. gigantea*. Pearson's correlation coefficients (lower left) for each pair of 29 environmental predictors, shaded blue-white-red for negative-zero-positive. And asterisk in the upper right denotes a significant test ($p < 0.05$) result after Holm correction. Column and row numbers refer to a specific environmental predictor, which match those in Supplemental Table 1-1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1													*																
2	0.32																												
3	-0.68	-0.11																											
4	-0.47	-0.25	-0.23																										
5	-0.64	-0.39	0.04	0.95																									
6	0.34	0.02	0.41	-0.82	-0.68					*																*			
7	-0.77	-0.38	0.90	0.11	0.40	0.23			*																				
8	0.59	0.13	0.15	-0.85	-0.79	0.96	-0.05			*																			
9	-0.74	-0.44	0.88	0.10	0.40	0.26	0.99	-0.01																					
10	0.46	0.04	0.28	-0.77	-0.66	0.98	0.13	0.98	0.17																	*			
11	-0.44	-0.23	0.94	-0.45	-0.17	0.67	0.83	0.43	0.83	0.56																			
12	0.92	0.43	-0.53	-0.68	-0.85	0.38	-0.78	0.61	-0.76	0.44	-0.33			*		*				*		*							
13	0.98	0.38	-0.69	-0.41	-0.61	0.32	-0.79	0.57	-0.76	0.45	-0.47	0.88																	
14	-0.93	-0.43	0.64	0.58	0.78	-0.27	0.85	-0.52	0.83	-0.35	0.45	-0.99	-0.90			*				*									
15	-0.75	-0.18	0.89	-0.20	0.01	0.25	0.74	-0.01	0.70	0.08	0.80	-0.52	-0.73	0.61															
16	-0.95	-0.40	0.52	0.69	0.85	-0.46	0.74	-0.68	0.72	-0.53	0.29	-0.99	-0.91	0.98	0.54					*		*							
17	-0.56	-0.14	0.16	0.36	0.40	-0.64	0.20	-0.72	0.17	-0.73	-0.06	-0.40	-0.66	0.36	0.21	0.49													
18	0.72	0.46	-0.66	-0.44	-0.68	-0.04	-0.91	0.19	-0.91	0.00	-0.57	0.88	0.69	-0.91	-0.48	-0.82	-0.07												
19	-0.42	0.11	0.08	0.06	-0.01	-0.40	-0.11	-0.45	-0.17	-0.51	-0.09	-0.15	-0.35	0.15	0.50	0.24	0.33	0.24											
20	-0.93	-0.42	0.57	0.65	0.83	-0.37	0.79	-0.61	0.77	-0.44	0.36	-1.00	-0.90	0.99	0.55	0.99	0.42	-0.87	0.17			*							
21	-0.63	-0.31	0.97	-0.21	0.08	0.49	0.94	0.22	0.94	0.37	0.97	-0.55	-0.66	0.66	0.83	0.52	0.06	-0.74	-0.06	0.58									
22	0.95	0.42	-0.50	-0.69	-0.83	0.46	-0.71	0.68	-0.69	0.54	-0.27	0.98	0.91	-0.97	-0.56	-0.99	-0.47	0.80	-0.30	-0.99	-0.50								
23	-0.71	-0.30	0.55	-0.07	0.05	-0.08	0.43	-0.28	0.41	-0.26	0.43	-0.41	-0.72	0.46	0.83	0.48	0.47	-0.18	0.73	0.44	0.50	-0.53							
24	0.68	0.40	-0.87	-0.06	-0.34	-0.36	-0.96	-0.10	-0.96	-0.28	-0.85	0.72	0.66	-0.80	-0.73	-0.67	0.05	0.91	0.12	-0.73	-0.94	0.65	-0.37						
25	-0.82	-0.23	0.25	0.50	0.53	-0.67	0.29	-0.82	0.25	-0.79	-0.01	-0.64	-0.81	0.61	0.52	0.73	0.76	-0.23	0.74	0.66	0.16	-0.76	0.78	-0.16			*		
26	-0.41	-0.10	-0.37	0.86	0.74	-0.99	-0.16	-0.97	-0.19	-0.98	-0.63	-0.46	-0.38	0.35	-0.19	0.53	0.60	-0.05	0.42	0.45	-0.43	-0.54	0.15	0.27	0.71				
27	-0.80	-0.23	0.17	0.63	0.65	-0.78	0.27	-0.90	0.23	-0.87	-0.11	-0.68	-0.80	0.63	0.38	0.76	0.81	-0.27	0.62	0.69	0.09	-0.78	0.65	-0.12	0.98	0.81			
28	0.86	0.35	-0.22	-0.84	-0.90	0.73	-0.46	0.88	-0.43	0.78	0.05	0.90	0.81	-0.85	-0.33	-0.93	-0.58	0.60	-0.39	-0.90	-0.20	0.94	-0.44	0.37	-0.83	-0.79	-0.89		
29	0.39	0.02	0.29	-0.90	-0.81	0.95	0.04	0.93	0.07	0.91	0.55	0.51	0.38	-0.40	0.27	-0.56	-0.63	0.18	-0.14	-0.50	0.35	0.54	0.07	-0.19	-0.57	-0.93	-0.71	0.76	

Supplemental Table 1-5: Spatial autocorrelation of environmental predictors used in *Bayenv2* for *C. gigantea*. For each environmental predictor (column Environmental Predictor), we report Moran's *I* autocorrelation index (column *I*) and *p*-value (column *p*-value) from a two-sided test for each environmental predictor. Environmental predictors that are significantly ($p < 0.05$) spatially auto-correlated are shaded in red.

Environmental Predictor	*I*	*p*-value
BO_bathymean	-0.039	0.223
BO_calcite	-0.190	0.816
BO_ph	0.066	0.049
BO2_curvelmax_bdmean	-0.118	0.664
BO2_curvelmax_ss	-0.115	0.659
BO2_curvelmean_bdmean	-0.043	0.216
BO2_curvelmean_ss	0.082	0.038
BO2_curvelmin_bdmean	-0.083	0.433
BO2_curvelmin_ss	0.079	0.042
BO2_curvelrange_bdmean	-0.055	0.265
BO2_curvelrange_ss	0.069	0.052
BO2_dissoxmean_bdmean	-0.045	0.309
BO2_dissoxmean_ss	-0.038	0.215
BO2_nitratemean_bdmean	-0.019	0.210
BO2_nitratemean_ss	-0.012	0.163
BO2_phosphatemean_bdmean	-0.055	0.340
BO2_phosphatemean_ss	-0.140	0.826
BO2_ppmean_bdmean	0.029	0.115
BO2_ppmean_ss	-0.118	0.698
BO2_salinitymean_bdmean	-0.040	0.283
BO2_salinitymean_ss	0.090	0.033
BO2_tempmax_bdmean	-0.059	0.357
BO2_tempmax_ss	-0.067	0.376
BO2_tempmean_bdmean	0.088	0.039
BO2_tempmean_ss	-0.092	0.501
BO2_tempmin_bdmean	-0.058	0.285
BO2_tempmin_ss	-0.097	0.528
BO2_temprange_bdmean	-0.100	0.573
BO2_temprange_ss	-0.067	0.371

Supplemental Table 1-6: Global, mean pairwise, minimum pairwise, and maximum pairwise F_{ST} using all (n = 5,932), putatively neutral (n = 5,823), and putatively adaptive SNPs (n = 109).

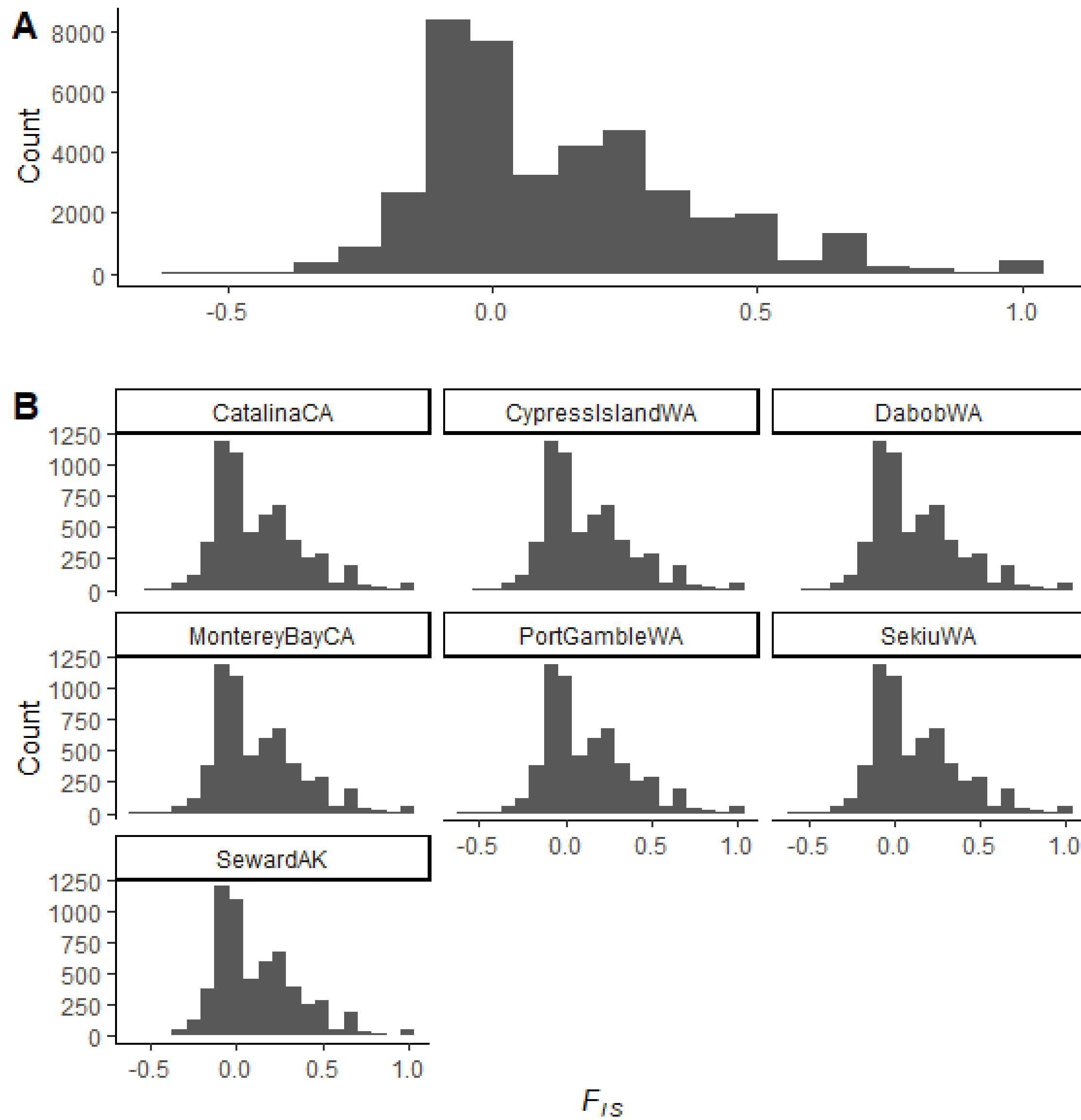
	Global F_{ST}	Mean pairwise F_{ST}	Min. pairwise F_{ST}	Max. pairwise F_{ST}
All SNPs	0.0009	0.000919	-0.0002	0.0021
Putatively neutral SNPs	0.0006	0.000567	-0.0006	0.0014
Putatively adaptive SNPs	0.0163	0.0188	0.0058	0.0409

Supplemental Table 1-7: Pairwise F_{ST} using putatively neutral SNPs. Cells are shaded darker green for greater values of F_{ST}. Site code key in Table 1-2.

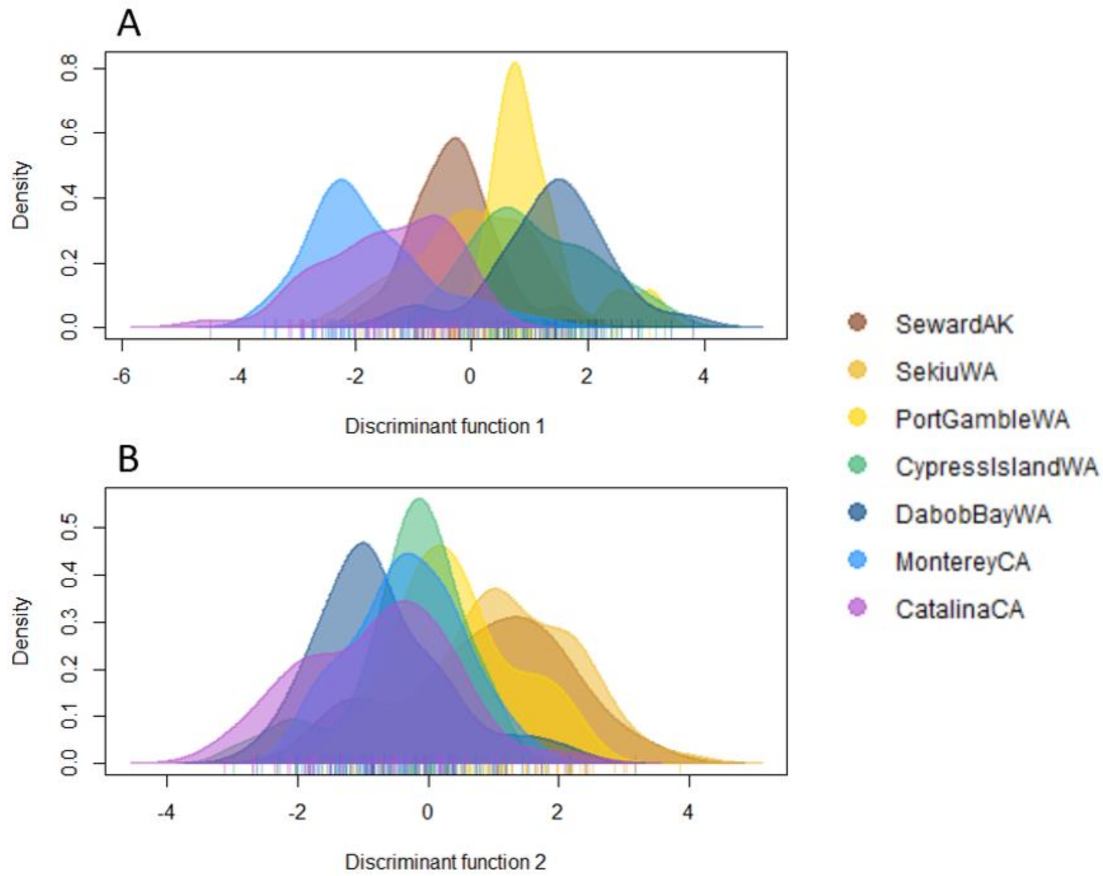
Neutral	SW_AK	SK_WA	PG_WA	CI_WA	DB_WA	MB_CA	CI_CA
SW_AK	-						
SK_WA	-0.0004	-					
PG_WA	-0.0006	0.0005	-				
CI_WA	0.0006	0.0005	0	-			
DB_WA	0.0007	0.0009	0.0002	-0.0001	-		
MB_CA	0.0006	0.0007	0.0013	0.0009	0.0014	-	
CI_CA	0.0004	0.0011	0.0013	0.0009	0.001	0	-

Supplemental Table 1-8: Pairwise F_{ST} using putatively adaptive SNPs. Cells are shaded darker green for greater values of F_{ST}. Site code key in Table 1-2.

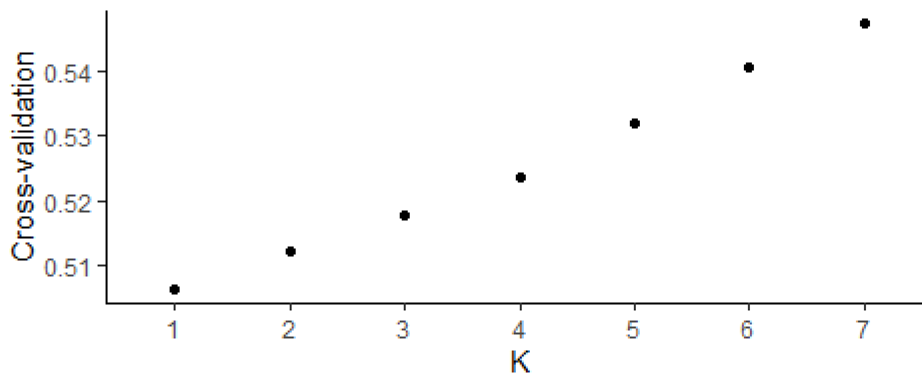
Adaptive	SW_AK	SK_WA	PG_WA	CI_WA	DB_WA	MB_CA	CI_CA
SW_AK	-						
SK_WA	0.0141	-					
PG_WA	0.0255	0.0261	-				
CI_WA	0.0177	0.0127	0.0236	-			
DB_WA	0.0135	0.0075	0.0158	0.0058	-		
MB_CA	0.0192	0.0107	0.0409	0.0259	0.0227	-	
CI_CA	0.0063	0.0134	0.0399	0.0251	0.0214	0.0077	-



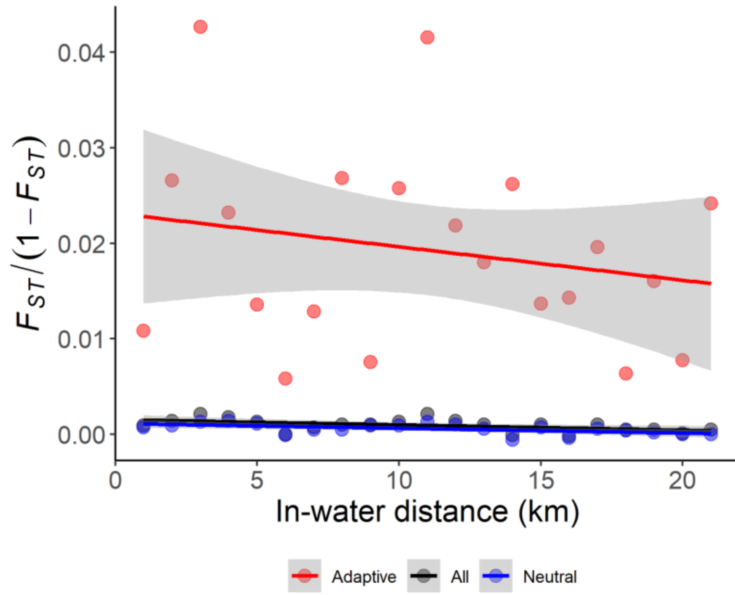
Supplemental Figure 1-1: Histograms of locus F_{IS}. Subplots A and B contain locus F_{IS} for all sites and by collection site, respectively.



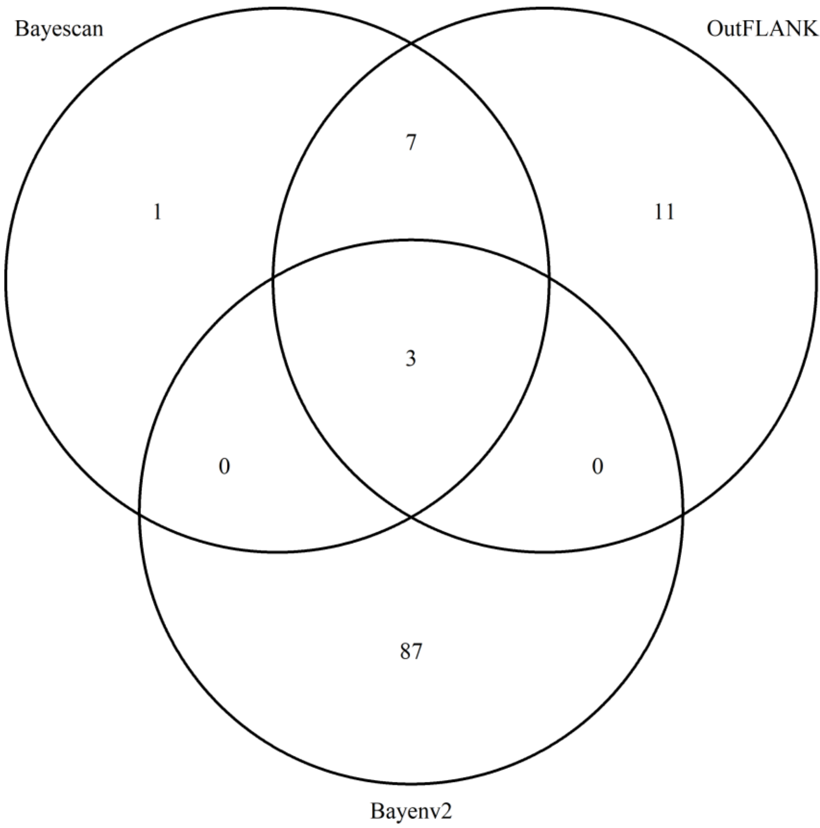
Supplemental Figure 1-2: Discriminant analysis of principal components, presented separately by discriminant function.



Supplemental Figure 1-3: Results from *ADMIXTURE* for *C. gigantea*. Cross-validation error for models with different numbers of assumed populations (K). The model with 1 underlying population had the lowest cross-validation error, and thus most support.



Supplemental Figure 1-4: Tests for isolation-by-distance for *C. gigantea*. No significant (p > 0.05) correlation between linearized F_{ST} and in-water distance using all SNPs (y = (-5.52*10-5)*x + 1.53*10-03; Mantel R = -0.517), putatively neutral SNPs (y = (-4.99*10-5)*x + 1.12*10-3; Mantel R = -0.557), or putatively adaptive SNPs (y = (3.51-*10-4)*x + 0.0232; Mantel R = -0.2114).



Supplemental Figure 1-5: Venn diagram demonstrating overlap of putatively adaptive SNPs by method for *C. gigantea*.

Chapter 2. Population structure and adaptive differentiation in the sea cucumber *Apostichopus californicus* and implications for spatial resource management

2.1 INTRODUCTION

Over the last two decades, genetic studies have provided evidence that some marine species can develop spatial population structure despite very large population sizes and planktonic larval dispersal (Hauser & Carvalho, 2008; Palumbi, 2004; Sanford & Kelly, 2011). The traditional view of marine populations as demographically panmictic followed from the hypothesis that an apparent lack of barriers to dispersal in the marine environment and long-distance planktonic larval dispersal common to many marine species would result in high gene flow across large geographic regions (Hauser & Carvalho, 2008). However, it is likely that dispersal in marine populations is more restricted than previously thought, likely due to larval behavior (D'Aloia et al., 2015; Gerlach et al., 2007) and the presence of oceanographic barriers (Costello & Chaudhary, 2017). Moreover, selection can drive population differentiation in marine populations characterized by negligible genetic drift and/or high gene flow (Lamichhaney et al., 2012; Pettersson et al., 2019). Lastly, intrinsic reproductive barriers such as chromosome inversions or genetic incompatibilities may further restrict gene flow (Barth et al., 2019).

Characterization of genetic population structure is particularly important to effective management of exploited species. Genetic population structure can be used to define the scale of management actions to best meet management objectives (Carvalho & Hauser, 1994; Reiss et al., 2009). Ignoring spatial structure in fishery management may result in overexploitation of less productive or more accessible populations (Spies & Punt, 2015). In turn, overexploitation may

reduce population diversity supporting adaptability, and thus sustainability, for current and future uses (Kenchington et al., 2003; Schindler et al., 2010). Additionally, demographic parameters such as the scale and direction of migration can be inferred from population structure (Rousset, 2004), and this information can be used to inform sustainable management practices aimed at maintaining and rebuilding wild populations, such as those in marine protected areas (Palumbi, 2003).

Estimates of genetic population structure are also useful in informing best practices for aquaculture. Sourcing wild broodstock is a common practice in aquaculture production of species that are outplanted within their native range (Davis et al., 1985; Tringali et al., 2007). If spatial population structure exists, then collecting wild broodstock from one distinct population and outplanting their seed into another can erode spatial population genetic structure and may lead to negative fitness consequences such as loss of local adaptations (R. S. Waples et al., 2012). Conversely, sourcing broodstock and outplanting their seed within a single population could help maintain both the differentiation among wild populations and fitness of released offspring in the local environment if populations are locally adapted (Ward, 2006). In recognition of such processes, the Department of Fisheries and Oceans Canada restricts translocation to within "shellfish transfer zones," in part to minimize loss of population differentiation associated with aquaculture (Miller et al., 2006; Xuereb, Benestan, et al., 2018).

The California sea cucumber, *Apostichopus californicus,* supports wild fisheries and is a novel aquaculture species, and thus would benefit from the characterization of spatial population structure. Demand for *A. californicus* has increased over the last several decades (Carson et al., 2016) as part of a global trend of growing demand for sea cucumbers (S. C. Anderson et al., 2011). Within Washington State, intense fishing pressure on this species peaked between 1988 and 1994, which reduced wild population sizes (S. C. Anderson et al., 2011; Mueller, 2016; Purcell et al.,

2013) and resulted in the closure of the Central Puget Sound fishery in 2014 (Carson et al., 2016). Despite some management intervention, including reduced quotas and a closure during peak spawning season, stocks have been slow to recover (Carson et al., 2016). Fishing pressure within Washington State is distributed differentially among six management areas (WDFW, 2020a), largely based on historic administrative boundaries and tribal fishing rights, but it is not known whether these areas represent biologically meaningful populations. Moreover, shellfish growers and scientists are developing methods for commercial aquaculture production of *A. californicus* (DeWeerdt, 2020) to supplement wild harvest, because demand remains high in overseas markets (Carson et al., 2016). Defining management units relevant to fisheries would benefit the development of aquaculture as well, as it could help to develop guidelines for broodstock sourcing and stocking practices.

Population differentiation depends in part on life history. *A. californicus* reproduce through broadcast spawning, with females producing approximately 100,000-600,000 eggs per spawning event (Whitefield & Hardy, 2019). Larvae may remain in the planktonic stage for weeks to months until they settle, while adults are motile (Cameron & Fankboner, 1989; Strathmann, 2017). Recent evidence suggests that adult sea cucumber dispersal can be facilitated through tumbling and floating behaviors, allowing them to take advantage of currents (Sheehan, 2019). Formal estimates of life span are unavailable for *A. californicus*, but have been postulated to be about 12 years (Phillips & Boutillier, 1995). Other species of sea cucumbers live to be 5 to 10 years old (Keegan & O'Connor, 1985). Thus, dispersal potential is high in *A. californicus* because all life history stages are motile and *A. californicus* are potentially long-lived, which could lead to high population connectivity. The life history strategy of *A. californicus* is that of a periodic strategist with long generation time, moderate reproductive effort, high batch fecundity and low investment per

offspring (Winemiller, 2005). This strategy is an adaptation to situations where environmental variation affecting juvenile survival is unpredictable but occurs on a large geographic scale (Winemiller & Rose, 1992), which in turn may result in high interannual variation in recruitment (Winemiller, 2005), high variance in reproductive success and low N_e/N ratios (Hedgecock, 1994). The life history of *A. californicus* may therefore suggest higher population differentiation than expected from census population sizes and high susceptibility to overfishing (Winemiller, 2005).

A. californicus is distributed from Baja California, Mexico, to Alaska, from the lower intertidal to depths of 250m (Abbott & Haderlie, 1980; Cameron & Fankboner, 1989), a region containing oceanographic barriers to dispersal that could shape population connectivity in *A. californicus*. For example, the Salish Sea, a large estuary divided into sub-basins by sills, is known to harbor genetically distinct populations in Pacific Cod (Cunningham et al., 2009) and Brown Rockfish (Buonaccorsi et al., 2005). The Victoria Sill and Admiralty Inlet are two potentially important oceanographic barriers to dispersal within the Salish Sea. The Victoria Sill is the outermost oceanographic barrier dividing the Salish Sea from the Strait of Juan de Fuca, which connects the Salish Sea to the outer coast (Davenne & Masson, 2001). The two sills at Admiralty Inlet separate the main Puget Sound basin from the San Juan Islands region and the Georgia Strait (Cannon et al., 1990). Another potential oceanographic barrier is the North Pacific Current (NPC), which bifurcates as it approaches the Pacific coast of North America into the northward Alaska and southward California currents. The NPC was already identified as an oceanographic barrier to dispersal in *A. californicus* (Xuereb, Benestan, et al., 2018) and the bat star (Keever et al., 2009).

Spatial population structure correlated with oceanographic processes was recently characterized in *A. californicus* in the region near British Columbia, Canada (Xuereb, Benestan, et al., 2018; Xuereb, Kimber, et al., 2018). These authors found evidence for local adaptation

associated with mean bottom temperature, and to a lesser extent, surface salinity and bottom current velocity (Xuereb, Kimber, et al., 2018). However, this study was restricted to British Columbia, and did not include the southern extent of the range, in particular the southern Salish Sea, where the majority of sea cucumbers are harvested in the contiguous United States (Washington Sea Grant, 2015) and where the fishery has experienced closures due to overfishing (Carson et al., 2016). Thus, estimates of spatial population structure are particularly needed in this region. Sampling the northern and southern extents of the region, beyond British Columbia, is also needed, as fisheries for *A. californicus* extend from Alaska to California.

In this study, we quantified and described patterns of population differentiation for *A. californicus* to inform effective management of wild populations and facilitate the development of management guidelines for aquaculture. We sampled populations at small and large geographic scales to test for both fine- and broad-scale population structure, particularly in regions that allow us to test hypotheses of potential oceanographic barriers to dispersal. Using restriction site-associated DNA (RAD) sequencing, we quantified genetic structure and investigated its potential drivers. Specifically, we tested whether limited dispersal and local adaptation shape population structure. We compared our results with existing estimates of spatial population structure to identify common patterns and build hypotheses about drivers of differentiation in the species. To contextualize our results for managers, we developed a two-population model to evaluate which conditions of effective population size (N_e), migration rate, number of generations of drift resulted in pairwise population differentiation within the range observed in this study.

2.2 Methods

2.2.1 *Sample collection*

Approximately 50 adult *A. californicus* were collected by scuba divers from nine collection sites along the Pacific Coast of North America, ranging from Alaska to Oregon, including three collection sites within the southern Salish Sea (Table 2-1, Figure 2-1). For each animal, a tissue sample was excised from a radial muscle band and stored in 100% ethanol.

2.2.2 *DNA library preparation*

DNA was extracted from tissue samples using the EZNA Mollusc DNA Kit (OMEGA Bio-tek, Norcross, GA, USA) and the Qiagen DNeasy Kit (Qiagen, Germantown, MD, USA). DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and DNA quality was checked by gel electrophoresis. DNA concentration was normalized to 500 *ng* in 20 μL of PCR-grade water. We selected samples with high DNA quality for restriction site-associated DNA (RAD) sequencing and RAD libraries were prepared following standard protocols (Etter et al., 2011). Briefly, DNA samples were barcoded with an individual six-base identifier sequence attached to an Illumina P1 adapter. Samples were then pooled into sub-libraries, containing approximately 12 individuals. Sub-libraries were sheared using a Bioruptor sonicator and size selected to 200-400 *bp* using a MinElute Gel Extraction Kit (Qiagen, Germantown, MD, USA). P2 adapters were ligated to DNA in sub-libraries and amplified with PCR using 12-18 cycles as in Etter et al. (2011). Finally, amplified sub-libraries were combined into pools of approximately 72 individuals. Paired-end 2 x 150-base pair sequencing was performed on an Illumina HiSeq4000 (San Diego, California, USA) at the Beijing Genomics Institute and the University of Oregon Genomics and Cell Characterization Core Facility. Only

forward reads were used for analysis. To estimate genotyping error, 14 individuals were sequenced twice.

2.2.3 *Genotyping individuals*

Raw RAD sequencing data were demultiplexed using the *process_radtags* module in the pipeline *STACKS* v.1.44 (Catchen et al., 2013). A threshold of 800,000 reads was used to exclude poorly sequenced individuals. Because a genome was not available for *A. californicus*, we aligned individual sequences to the genome of a closely related species, *A. parvimensis* (GenBank accession number = GCA_000934455.1). The *A. parvimensis* genome was 760,654,621 *bp*, with 21,559 scaffolds and an N50 size of 9,587. We retained reads with a minimum mapping quality score of 20. Then, we used *dDocent* v.2.7.8 to perform a reference-guided locus assembly using the filtered reads and default parameters (J. B. Puritz et al., 2014). Additionally, a parallel *de novo* assembly was performed, which produced nearly identical results for population structure (Supplemental Tables 2-1 and 2-2, and Supplemental Figure 2-1) and 1.8-2.8% lower mean expected heterozygosity, 0.9-1.8% higher mean observed heterozygosity, and 1.2-3.3% higher proportions of polymorphic SNPs than in the with-reference assembly, although with similar patterns across collection sites. The reference-guided assembly was retained for further analyses due to decreased confidence in identifying genotyping errors in the *de novo* assembly (Shafer et al., 2017).

We used *vcftools* v.0.1.16 (Danecek et al., 2011) to remove indels and to retain only single nucleotide polymorphisms (SNPs) with a minimum quality score of 20, minimum minor allele frequency of 0.05 and maximum missing data per locus of 30% across collection sites. Individuals with more than 30% missing data across SNPs were removed. In cases of multiple SNPs per RAD tag, we retained the SNP with the highest minor allele frequency (Larson et al., 2014). SNPs that

were not in Hardy Weinberg Equilibrium (HWE) were considered sequencing errors or poorly assembled loci and were removed from our data set, as selection and inbreeding are unlikely to cause significant deviations from HWE equilibrium at biallelic loci (R. S. Waples, 2015). We tested SNPs for deviations from HWE using the R package *genepop* v.1.1.4 (Rousset, 2008). SNPs were identified as being out of HWE if they had a q-value below 0.05 in at least 2 of the collection sites after correcting for false discovery rate, following R. S. Waples (2015).

2.2.4 *Population genetic structure analyses*

We used a suite of R packages, stand-alone software, and custom scripts in the programming language R v.3.5.0 (R Core Team, 2020) to quantify genetic diversity and population structure. Mean expected heterozygosity, observed heterozygosity, and the inbreeding coefficient (F_{IS}) per SNP were calculated using the R package *genepop*. The proportion of polymorphic SNPs per collection site was calculated using a custom R script.

To investigate population structure, we first calculated Weir-Cockerham fixation index (F_{ST}) (Weir & Cockerham, 1984) to quantify population differentiation using the R packages *genepop* and *hierfstat* v.0.5.7. Exact G-tests (Goudet et al., 1996) were used to test for significant genic differentiation using the R package *genepop*. To investigate patterns of spatial differentiation among collection sites, the R package *adegenet* v.2.1.1 (Jombart, 2008) was used to conduct discriminant analysis of principal components (DAPC), a multivariate method that summarizes the between-group variation (i.e., population structure), while minimizing within-group variation (Jombart et al., 2010). The built-in optimization algorithm was used to retain the number of principal components that minimized over-fitting and under-fitting of the model. To determine the potential number of underlying populations, the program *ADMIXTURE* v.1.3.0 was used to conduct a clustering analysis (Alexander & Lange, 2011). Specifically, *ADMIXTURE* uses a

maximum likelihood-based approach to estimate individual ancestries across different assumed numbers of populations, with the best fit selected using cross-validation. To examine the presence of hierarchical population structure, we conducted analyses of molecular variance (AMOVA) using the *ade4* method of the R package *poppr* v.2.8.1 (Kamvar et al., 2015). Significance of AMOVAs was determined using permutation tests with 1,000 iterations. Using AMOVA, we investigated whether the following oceanographic barriers limit dispersal: 1) the Victoria Sill (Salish Sea grouping), 2) Admiralty Inlet (Puget Sound grouping), and 3) the North Pacific Current (NPC grouping). Additionally, we conducted an AMOVA by state or province (State grouping). Although not biologically meaningful, we included the State grouping to determine how much genetic variation is captured by regional management boundaries.

2.2.5 *Identifying putative adaptive differentiation*

Adaptive differentiation was investigated using two approaches: F_{ST} outlier detection and gene-environment association. F_{ST} outlier detection methods identify statistical outliers in distributions of F_{ST} per SNP, as evidence for potential spatially-divergent selection. For F_{ST} outlier detection, two methods were used: the program *Bayescan* v.2.1 (Foll & Gaggiotti, 2008) and the R package *OutFLANK* v.0.2 (Whitlock & Lotterhos, 2015). *Bayescan* first applies linear regression to decompose F_{ST} into a population- and a locus-specific component. Using these components as Bayesian priors, the program estimates the posterior probability that a locus is under selection (Foll & Gaggiotti, 2008). *OutFLANK* detects F_{ST} outliers using a maximum likelihood approach. The program first infers a distribution of neutral F_{ST} from a trimmed distribution of empirically collected F_{ST} values and uses this neutral distribution to identify outliers. *OutFLANK* advances earlier F_{ST} outlier methods (Lewontin & Krakauer, 1973) by accounting for sampling error and

non-independent sampling of populations, and has lower false positive rates compared to other F_{ST} outlier methods (Whitlock & Lotterhos, 2015). We used default parameters in both programs, including a false discovery rate of 0.05. SNPs were classified as F_{ST} outliers if they were detected with either program, to include SNPs under weak selection, which are likely the majority of SNPs under selection (Lotterhos & Whitlock, 2015).

Prior to investigating gene-environment association, we gathered estimates for oceanographic variables at each collection site using the Bio-Oracle and Bio-Oracle 2 databases (Assis et al., 2018), which contain geophysical, biotic, and environmental data layers for marine realms, through the R package *sdmpredictors* v.0.2.8 (Bosch et al., 2017). We selected a broad suite of 29 oceanographic variables (including temperature, current velocity, salinity, and pH; complete list in Supplemental Table 1-1), including those used by Xuereb, Kimber, et al. (2018) for comparison. Where possible, oceanographic variables for both sea surface and mean bottom depth (near-bottom) were used to account for the conditions experienced by pelagic larvae and benthic adults respectively. The Eld Inlet, Washington collection site was removed from these analyses because environmental predictor data were not available. We also calculated correlation among predictor variables (Supplemental Table 2-3) and interpreted results in light of these correlations.

To investigate genomic evidence for local adaptation, gene-environment associations were explored using a univariate association method, *Bayenv2* (Günther & Coop, 2013) and a multivariate method, redundancy analysis (RDA). We used both methods as each has an advantage: the interpretation of results for univariate methods like *Bayenv2* can be clearer than multivariate methods for gene-environment association, and multivariate methods such as RDA

have lower false positive rates and greater sensitivity for detecting weak and multi-locus selection (Forester et al., 2018).

The program *Bayenv2* (Günther & Coop, 2013) uses a univariate Bayesian framework to test for significant correlation between allele frequencies and environmental predictor variables, accounting for population structure by first estimating covariance among loci. Correlations with a minimum Bayes Factor of 10, or minimum "strong" support (Kass & Raftery, 1995), were retained in the analysis.

Redundancy analyses were performed in the R package *vegan* v.2.5-6 (Oksanen et al., 2007). Redundancy analysis summarizes the variation in a set of response variables (here, the allele frequencies) due to a set of explanatory variables (here, the oceanographic variables), using an extension of multiple linear regression that allows regression of multiple response variables on multiple explanatory variables. Here, only biallelic SNPs were retained, and allele frequencies were Hellinger-transformed prior to RDA (Legendre & Gallagher, 2001). To avoid overdetermination of RDA models with many environmental predictors (Supplemental Figure 2-2), we conducted multiple RDAs on subsets of environmental predictors and reduced the dimensionality of our environmental predictors within sets by first combining them into orthogonal principal components (Supplemental Tables 2-4 through 2-7). Specifically, environmental predictors were grouped into four *a priori* sets: (1) all, (2) sea surface, (3) near-bottom and (4) current velocity and temperature predictors (measured at either sea surface or near-bottom). Sets 2 and 3 were chosen to investigate differences in putative adaptation at pelagic and benthic life history stages. Environmental predictor set 4 was chosen for comparison with the existing study on *A. californicus* in a different part of the species range, as these variables were strongly correlated with genetic population structure (Xuereb, Kimber, et al., 2018). Predictors were standardized to

a mean of 0 and standard deviation of 1 prior to PCA. PCA was performed on each set of predictors, and principal components were retained if their corresponding eigenvalue was above the mean eigenvalue across principal components.

To account for the potential confounding factor of neutral population structure in our RDA, we conducted a complementary partial RDA for each set of predictor variables, in which the effects of spatial variation were partialled out. We used Euclidean distances among collection sites to compute distance-based Morgan's eigenvector maps (MEMs) using the R package *codep* v.0.9-1 (Guénard et al., 2010), to be used as conditioning variables in partial RDAs (Dray et al., 2006). We report the variance inflation factors (VIF) to identify collinearity among spatial variables and environmental predictors, although we note that the effects of such collinearity are addressed in the removal of genetic variation explained by spatial variables in partial RDAs. ANOVA permutation tests for full models and per axis were used to assess the significance of RDA results. For significant models, we identified SNPs putatively involved in local adaption based on the loadings of SNPs in ordination space for significant axes. Specifically, SNPs were classified as putatively adaptive if their loading score was outside of 3 standard deviations of the mean (Forester et al., 2018).

2.2.6 *Comparing putatively neutral and adaptive differentiation*

SNPs were classified as putatively adaptive if they were detected as F_{ST} outliers using either *BayeScan* or *OutFLANK*, or if they were significantly correlated to environmental predictors using *Bayenv2* or RDA. Once putatively adaptive SNPs were identified, they were used to distinguish a putatively neutral SNP set and a putatively adaptive SNP set.

We used the putatively neutral and putatively adaptive data sets to address questions related to demographic and selective processes, respectively. We estimated effective population size (N_e)

per collection site from putatively neutral SNPs using *NeEstimator* v.2.1 with default settings (Do et al., 2014). Using Mantel tests (Mantel & Valand, 1970) in R, we tested for correlation between linearized F_{ST} (Rousset, 1997), using all, putatively neutral, and putatively adaptive SNPs, and shortest Euclidean distance through water (in-water distance hereafter), approximated in Google Maps (Google, 2019).

To build hypotheses for the mechanisms underlying adaptive differentiation, potential biological processes associated with putatively adaptive SNPs were identified using *blastx* v.2.5.0 (Altschul et al., 1990) and the UniProt Knowledge Base (Swiss-Prot, manually annotated) (The UniProt Consortium, 2019). We queried the 2,000 *bp* region flanking the SNP, following alignment against the reference genome of *A. parvimensis*. Matches with a maximum *e*-value score of 10^{-10} were retained. Gene ontology slim terms for biological processes were retrieved using an adaptation of the Mouse Genome Informatics database, as developed in Gavery & Roberts (2012). Gene ontology slim terms "other biological processes" and "other metabolic processes" were excluded. A particular locus could be associated with multiple gene ontologies, and a particular gene ontology could be associated with multiple gene ontology slim terms for biological processes. All matches were retained.

2.2.7 *Simulations*

To contextualize genetic connectivity in terms of migration rate for management, we developed a simulation model using *simuPOP* v.1.1.10.9 (Peng & Kimmel, 2005) in Python v.3.7.3 to determine which population sizes, migration rates, and number of generations of drift reproduced our empirically derived pairwise F_{ST} results. The model simulated two populations of equivalent size, with discrete generations, random mating, and no selection. Within each population, two parents were selected at random with replacement to produce one offspring,

leading to a random distribution of reproductive success, and allowing for census size to approximate N_e. The model was parameterized using empirical global allele frequencies for putatively neutral SNPs and simulations were run for each combination of N_e (500, 2500, 10,000) and migration rate (0.01%, 0.03%, 0.1%, 0.3%, 1%, 3%, 10%, 30%). Additionally, simulations were run for 10 (short-term), 100 (medium-term), and 1,000 (long-term) generations of drift. The long-term option was chosen to approximate equilibrium conditions, although true equilibrium depends on population history and may not be reached in some wild populations within 1,000 generations. Pairwise F_{ST} was calculated after the pre-determined number of generations of drift had elapsed and averaged across 5 replicates for each parameter combination.

2.3 RESULTS

2.3.1 *Sequencing*

After removing 33 (9% of 358 sequenced individuals) poorly sequenced individuals, the average number of reads per individual was 1.76M (standard deviation (SD) = 0.787M). The *dDocent* assembly produced 6,738,423 variant sites, and 2,075 SNPs were ultimately retained after filtering (Supplemental Table 2-8). All individuals included in the *dDocent* assembly were retained after filtering for missing data across SNPs, resulting in an average of 36 individuals per population (SD = 13.7) (Table 2-1). Genotyping error was estimated to be 1.4% among the 14 replicated individuals. All errors were mismatches of a single allele. Errors were distributed fairly evenly across SNPs, with one error at 246 SNPs, two errors at 19 SNPs, three errors at 7 SNPs, and four errors at one SNP, across replicated individuals.

2.3.2 *Population structure*

Global population differentiation was significant (global $F_{ST} = 0.0068$, 95% CI [0.005, 0.0105]; global genic differentiation test, $p < 0.001$). For the DAPC using all SNPs, we retained 53 PCs based on the optimization algorithm and observed clustering by collection site (Figure 2-1). The first PC explained 52.1% of the variance and separated sites longitudinally. The second PC explained 22.2% of the variance and separated sites latitudinally. The DAPC showed evidence for separation of Alaska, British Columbia, Washington, and Oregon sites, with some evidence for separation between sites inside and outside of the Salish Sea and between sites north and south of Admiralty Inlet within the Salish Sea. Permutation test results from AMOVAs demonstrated significant population structure for the NPC grouping using all SNPs (Table 2-2), suggesting that the North Pacific Current is an oceanographic barrier to dispersal in *A. californicus*. Significant population structure for the State grouping was also detected using permutation test results from AMOVAs using all SNPs. The State grouping also had the highest Φ_{CT} / Φ_{SC}, a ratio that is higher when the among-group variation is greater than the within-group variation. Clustering analyses with *ADMIXTURE* provided the strongest support for the model with one underlying population (Supplemental Figure 2-3). A Mantel test revealed significant positive correlations between pairwise linearized F_{ST} using all SNPs and in-water distance (Mantel $R = 0.758$, $p < 0.001$; Figure 2-2).

Of all 36 pairwise site comparisons (Table 2-3), 24 revealed significant genic differentiation ($p < 0.05$), with 8 of the 12 insignificant tests corresponding to collection site pairings with the small collection from Auke Bay, AK (Table 2-1). Only one pairwise site comparison (Keyport and Eld Inlet, WA) yielded a pairwise F_{ST} value that contained 0 in the 95% confidence interval (Supplemental Table 2-9). The largest pairwise F_{ST} values were found among

collection sites from the northern (Alaska and British Columbia) and southern (Washington and Oregon) parts of the species range (F_{ST} = 0.005 – 0.015). There was greater differentiation among pairs of Washington collection sites (pairwise F_{ST} = 0.001-0.006) compared to Alaska collection sites (pairwise F_{ST} = 0.004).

Mean expected heterozygosity and proportion of polymorphic SNPs did not vary substantially across collection sites, apart from a reduced proportion of polymorphic SNPs in Auke Bay and Chiniak Bay, AK (Table 2-1). Observed heterozygosity decreased and F_{IS} increased from North to South (Table 2-1). All collection site N_e estimates were large or infinite, with infinite upper confidence limits, using both all putatively neutral SNPs and only those with a minor allele frequency of at least 5% (Table 2-4).

2.3.3 *Adaptive differentiation*

The F_{ST} outlier SNP detection methods identified 60 (2.9% of 2,075) SNPs as putatively adaptive (Figure 2-3), with 50 identified by *BayeScan*, 46 identified by *OutFLANK,* and 36 SNPs overlapping across both methods.

Using *Bayenv2*, 181 SNPs (8.7% of 2,075) were identified as putatively adaptive. Each of these SNPs was correlated with an average of 2.5 environmental predictors per SNP and at least one SNP was correlated with each of the 29 environmental predictors (Supplemental Table 2-10). The five environmental variables with the most associated SNPs included mean salinity, mean nitrate, temperature range, and pH, all at the sea surface, and mean temperature at near-bottom (Table 2-5). Over 60% more SNP-predictor correlations represented environmental variables measured at the surface than at near-bottom, for variables with both measurements: 397 and 615 SNP-predictor correlations with near-bottom and sea surface variables, respectively.

After removing 10 multiallelic SNPs, we retained 2,065 biallelic SNPs for use in redundancy analyses (RDA). We retained two RDA models (out of 8) for statistical significance: the model using sea surface predictors was marginally significant at the full model level (ANOVA, $p = 0.063$) and for the first axis (ANOVA, $p = 0.052$), and the model using temperature and current velocity predictors (at sea surface or near-bottom) was significant at the full model level (ANOVA, $p = 0.034$) and for the first axis (ANOVA, $p = 0.035$). For the RDA using sea surface predictors, the first RDA axis explained 24.8% of the variance, and PC 2 had the greatest loading on the first RDA axis (Supplemental Table 2-11). The three predictors with the greatest loadings on PC 2 included pH, mean salinity, and temperature range (Supplemental Table 2-5). For the RDA using temperature and current velocity predictors, the first RDA axis explained 26.8% of the variance, and PC 2 had the greatest loading on the first RDA axis (Supplemental Table 2-12). The three predictors with the greatest loadings on PC 2 included temperature range at the sea surface and near-bottom, and minimum temperature at the near-bottom (Supplemental Table 2-7). Collection site clustering patterns were similar among RDA biplots (Figure 2-4), including the Salish Sea collection sites clustering tightly together and North-South separation driven by the first RDA axis in each model. Charleston, Oregon clustered more closely with the Salish Sea collection sites in the model using temperature and current velocity predictors (Figure 2-4). We identified 32 putatively adaptive SNPs based on loadings of significant (and marginally significant) RDA axes, with 24 SNPs identified from the RDA using sea surface predictors and 29 from the RDA using temperature and current velocity predictors. No partial RDAs were significant. Spatial variables used in partial RDAs covaried with predictor variables in most models, measured with variance inflation factors (Table 2-6). The environmental predictor loadings for retained PCs in all models

and a correlation matrix of environmental predictors can be found in the Supplementary Information (Supplemental Tables 2-3 through 2-7).

In total, we identified 211 (10.2% of 2,075 total SNPs) putatively adaptive SNPs, of which 41 (19.4% of 211 putatively adaptive SNPs) were detected with either F_{ST} outlier approaches and at least one gene-environment association approach and 134 (63.5% of 211) were only identified by *Bayenv2* (Supplemental Figure 2-4). Sequences of RAD loci containing putatively adaptive SNPs will be available upon publication.

Only 19 (9% of 211) putatively adaptive SNPs matched to gene ontologies, and 15 (79% of 19) matched to more than one gene ontology. Of the SNPs identified as putatively adaptive using *Bayenv2*, 15 SNPs matched to gene ontologies (Table 2-5, Supplemental Table 2-10). Of these 15 SNPs, three were also identified using *Bayescan* and *OutFLANK* and one was also identified using *Bayescan*, *OutFLANK*, and RDA. An additional four SNPs were also identified as putatively adaptive using only F_{ST} outlier methods, with the most represented biological processes including DNA metabolism, cell organization and biogenesis, and developmental processes. For comparison, the top five most frequent biological processes in the database in descending order are 1) developmental processes, 2) cell organization and biogenesis, 3) transport, 4) protein metabolism, and 5) stress response (Supplemental Table 2-13).

2.3.4 *Neutral vs. putatively adaptive differentiation*

DAPC and AMOVA revealed higher differentiation but similar spatial patterns for putatively adaptive SNPs compared to putatively neutral SNPs (Table 2-2, Figure 2-1). We retained 53 and 26 PCs for the DAPC using putatively neutral SNPs and putatively adaptive SNPs, respectively, based on the optimization algorithm. Clustering patterns using putatively neutral and putatively adaptive SNPs reflected those using all SNPs, with more distinct clustering using

putatively adaptive SNPs (Figure 2-1). Permutation test results from AMOVAs demonstrate significant population structure for NPC and State groupings, using putatively neutral and adaptive SNPs (Table 2-2). The ratio of among-group variation to within-group variation (Φ_{CT} / Φ_{SC}) was higher using putatively adaptive SNPs for the State and NPC groupings, and higher using putatively neutral SNPs for the Salish Sea and Puget Sound groupings. Mantel tests for correlation among linearized F_{ST} and in-water distance were significant using putatively neutral SNPs (Mantel $R = 0.4891$, $p < 0.01$) and putatively adaptive SNPs (Mantel $R = 0.7855$, $p < 0.0001$) (Figure 2-2). Correlation among linearized F_{ST} and in-water distance was strongest and the slope of the linear regression was greatest using putatively adaptive SNPs: the slope of the linear regression using putatively adaptive SNPs was nearly 7 and 20 times greater than the slopes using all SNPs and putatively neutral SNPs, respectively.

2.3.5 *Simulations*

Simulations revealed that in an idealized two-population system, simulated pairwise F_{ST} within the range observed in this study occurred in the long-term (1,000 generations since divergence) with small N_e ($N_e = 500$) and high migration ($m \geq 3\%$), with moderate N_e ($N_e = 2{,}500$) and moderate migration ($1\% \geq m \geq 3\%$), and with large N_e ($N_e = 10{,}000$) and small to moderate migration ($0.1\% \geq m \geq 1\%$). Most simulations that produced pairwise F_{ST} estimates within the range observed in this study were cases with moderate to large N_e ($N_e \geq 2{,}500$) and migration rates below 10%. Simulated pairwise F_{ST} remained below the lowest empirically observed pairwise F_{ST} in this study in the long-term (1,000 generations) so long as N_e was at least moderate ($N_e \geq 2{,}500$) and migration rate was high ($m \geq 10\%$). Under realistic conditions (e.g., $N_e = 10{,}000$ and 1,000 generations since divergence; our N_e estimates were large and unbounded), simulated pairwise F_{ST} was within the range of empirical pairwise F_{ST} when migration rates were 0.1-1.0% (Figure 2-5).

2.4 DISCUSSION

In this study, we quantified patterns of population differentiation in *A. californicus* and investigated potential drivers of differentiation from Alaska to Oregon, representing a significant portion of the species range. We observed population genetic structure at both fine- and broad-scales, likely driven by limited dispersal and local adaptation. Notably, we found detectable differentiation at small scales within the Salish Sea, co-varying signals of adaptive and neutral differentiation, and a latitudinal pattern in genetic diversity. Estimates of N_e were large. Using simulations, we found that migration rates may be as low as 0.1% if populations of *A. californicus* are large (N_e = 10,000) and have undergone many (1,000) generations of drift.

2.4.1 *Broad- and fine-scale population genetic structure and potential drivers*

Broad-scale population structure that we observed can be described by two patterns: isolation-by-distance and differentiation across the bifurcation zone of the North Pacific Current (NPC). Isolation-by-distance was also detected in *A. californicus* along the coast of British Columbia (Xuereb, Benestan, et al., 2018), as well as in other species of sea cucumbers including *Holothuria edulis* (Soliman et al., 2016), *H. scabra* (Uthicke & Purcell, 2004), and *H. nobilis* (Uthicke & Benzie, 2003). Many species of marine invertebrate exhibit population differentiation along a latitudinal gradient, with the region between Alaska and Oregon as a known region of divergence (Kelly & Palumbi, 2010; Kyle & Boulding, 2000). Xuereb, Benestan, et al. (2018) posit that the genetic break observed in *A. californicus* in British Colombia is due to limited dispersal across the bifurcation zone of the NPC. The NPC has also been identified as an oceanographic barrier to gene flow in the Bat Star, *Patiria miniata* (Keever et al., 2009; Sunday et al., 2014), and the Rosethorn Rockfish, *Sebastes helvomaculatus* (Rocha-Olivares & Vetter, 1999).

Our results did not provide evidence for limited dispersal across the Victoria Sill or the sills at Admiralty Inlet, despite their potential roles in shaping population structure in other marine species (Andrews et al., 2018; Buonaccorsi et al., 2005; Cunningham et al., 2009; Iwamoto et al., 2004). Although not biologically meaningful, the State grouping yielded the greatest among-group variation, likely because it captured the effects of isolation-by-distance and the divergence across the NPC bifurcation zone.

Additionally, we found a subtle pattern of decreasing observed heterozygosity from north to south (this pattern can also be observed in the data of Xuereb, Benestan, et al. (2018) following re-estimation; North, mean H_O = 0.113; South, mean H_O = 0.108). In the Northern Hemisphere, latitudinal patterns of genetic diversity are typically opposite (higher diversity in the south, lower diversity in the north), as these northerly regions were more recently colonized after the Pleistocene ice ages (Hampe & Petit, 2005). However, patterns of glaciation in the northeast Pacific are complex (Darvill et al., 2018). Since there were multiple glacial refugia along the Pacific Northwest Coast (Darvill et al., 2018), it is possible that *A. californicus* populations remained established in the northern extent of the range throughout the Last Glacial Maximum. Alternatively, this latitudinal pattern in genetic diversity may also be explained by differences in N_e. Here, our estimates of N_e all had infinite upper confidence limits, limiting possible inferences about relative population size across collection sites. Alaska supports larger sea cucumber fisheries compared to southern regions (Bruckner, 2005), which could be evidence for larger N_e, reduced genetic drift, and greater heterozygosity in the northern part of the species range.

Notably, we found that geographic distance was more strongly correlated to genetic distance among collection sites using putatively adaptive SNPs than using putatively neutral SNPs. This suggests that selection gradients may reduce effective dispersal distances, leading to stronger

signals in isolation-by-distance from neutral processes alone (Orsini et al., 2013). One exception may be the Charleston, Oregon collection site, which clusters more distinctly from other collection sites using putatively adaptive SNPs and is positioned more closely to the Salish Sea collection sites in the RDA biplot for temperature and current velocity predictors than the biplot for sea surface predictors. The difference in RDA biplots suggests different patterns in adaptive differentiation, dependent on environmental predictors. Lastly, co-variation between neutral and adaptive differentiation could also represent a false positive result for gene-environment association. Isolation-by-distance, as we found in this study, can increase the chance of a false negative result for gene-environment association if the environmental predictor is spatially auto-correlated (Nadeau et al., 2016).

At finer scales, we found detectable differences among most collection site pairs and between collection sites as close together as 110 km (in-water distance) apart, consistent with earlier findings on differentiation as close as 60 km in *A. californicus* (Xuereb, Benestan, et al., 2018) and 100 km apart in the Tar-spot Sea Cucumber, *Cucumaria pseudocurata* (Arndt & Smith, 1998). The sample from Auke Bay, Alaska behaved as an outlier across multiple analyses, likely due to stochasticity associated with smaller sample size, and leading to most insignificant pairwise genic differentiation tests and a lower proportion of polymorphic SNPs. Notably, genetic differentiation among collection sites within Washington was broader in range and at times greater than that within Alaska, despite substantially smaller in-water distances (mean = 162 km, SD = 62 km in WA and mean = 731km, SD = 339 km in AK). Lower differentiation between populations in Alaska compared to the Salish Sea may be due to colder temperatures in Alaska slowing larval growth, as colder temperatures led to slow larval growth in laboratory experiments in the sister species, the Japanese sea cucumber, *A. japonicus* (Yang et al., 2015). Slower larval growth may

result in longer pelagic larval phases and increased potential dispersal distances. Alternatively, greater genetic differentiation among populations within Washington compared to Alaska may point to underlying oceanographic barriers that can shape dispersal in complex and asymmetrical ways. In future work, a denser sampling scheme paired with an oceanographic model could be used to illuminate the potential for seascape features to influence dispersal, particularly within the Salish Sea.

We found evidence for putatively adaptive differentiation, corroborated by multiple lines of investigation. The majority of SNPs identified as putatively adaptive using F_{ST} outlier detection were identified using both methods and were also identified as putatively adaptive using at least one gene-environment association method. Using *Bayenv2*, we detected many SNPs that were not identified as putatively adaptive compared to redundancy analysis, consistent with expectations that *Bayenv2* may produce more false positives compared to redundancy analysis (Forester et al., 2018). The five environmental predictors with the most significantly correlated SNPs were salinity, nitrate, temperature range, and pH at sea surface and mean temperature at near-bottom. Of these, salinity at the sea surface and temperature at the sea bottom were also identified as potential drivers of local adaptation in the same species in British Columbia (Xuereb, Kimber, et al., 2018). Temperature and salinity are significant factors shaping growth and survival in many marine organisms (Kinne, 1964) and have been shown to affect growth and survival in *A. japonicus* juveniles (Dong et al., 2008) and growth, survival, locomotory speed, and metamorphosis in *A. japonicus* larvae (Yang et al., 2015). The greater number of SNPs significantly correlated to sea surface predictor variables using *Bayenv2* and the occurrence of temperature variables as top predictor variables is consistent with RDA results: the significant RDA models were those with environmental predictors at the sea surface and environmental predictors related to temperature

and current velocity. Thus, we hypothesize that selection at early life history stages, when larvae are at the sea surface, drives adaptive differentiation in *A. californicus*, with salinity, nitrate, pH, and temperature as possible selection factors.

We note that we cannot exclude balanced polymorphism as an alternative explanation to observed putative adaptive differentiation: it is possible that selective mortality at early life stages leads to observed patterns of putative adaptive differentiation in adults (as were sampled here), but that genetic variation is maintained across time through high gene flow (Sanford & Kelly, 2011). However, increasing evidence suggests that selection for locally adapted alleles can occur despite high gene flow (Fitzpatrick et al., 2020; Lamichhaney et al., 2012), particularly in species with large N_e.

In generating hypotheses about which environmental predictors are potential drivers of selection, it is prudent to be cautious and highlight 1) spatial auto-correlation of predictors, particularly in light of correlation between geographic distance and genetic distance using putatively adaptive SNPs as observed here, and 2) correlation among environmental predictors. Almost all environmental predictor sets contained spatially auto-correlated variables, evidenced by high variance inflation factors for spatial variables and environmental predictor variables in partial RDAs (Table 2-6). Additionally, the correlation among environmental variables adds uncertainty about which predictors are driving patterns in adaptive differentiation. These correlations highlight the limits of these analyses. However, our results are fairly consistent with those of Xuereb, Kimber, et al. (2018), even though they sampled genetic and environmental data from different collection sites. Confidence in gene-environment association results may increase if results are further corroborated in future studies, particularly if using alternative methods.

For the environmental variables with the most correlated SNPs, and for such SNPs with associated biological processes, signal transduction appeared among the most common biological processes. Future research investigating the connections between genotype, phenotype, and observed patterns in adaptive differentiation may start with the potential connection between signal transduction pathways and salinity, nitrate, temperature, and pH. Others have identified signal transduction genes as part of an adaptive response to salinity adaptation and stress in sea cucumbers (Zhang et al., 2018). In *A. japonicus,* high salinity conditions were correlated with downregulation of acetylcholinesterase, the enzyme that terminates signal transduction. Inhibition of this enzyme can lead to excessive stimulation of nerve and muscle tissue, ultimately leading to paralysis and even death in some species (Kirby et al., 2000). Transcriptomic approaches can be used in future studies of wild populations of *A. californicus* across environmental gradients or in common garden experiments with varying environmental treatments to build cases for which environmental factors shape adaptive differentiation and through which physiological pathways.

2.4.2 *Spatial considerations for sustainable management of* A. californicus

Simulation results suggest limited dispersal among collection sites: if N_e per collection site was at least 10,000 and separated at least 1,000 generations ago, genetic differentiation suggested migration rates of 0.1-1% (Figure 2-5). Here, our estimates of N_e were large with infinite upper confidence limits, demonstrating that populations are likely not small (Hare et al., 2011). This level of migration (0.1-1%) may be large enough to prevent genetic isolation, but small enough to lead to demographic independence (R. S. Waples & Gaggiotti, 2006). This has implications for spatial management of wild *A. californicus*, though those implications may differ depending on the management aim. For example, marine protected areas for *A. californicus* may need to operate at smaller scales than expected from signals of genetic differentiation at broad scales, to maintain

demographic connectivity and prevent overexploitation of local populations. Designation of management units for fisheries may need to occur at similar scales, because fishery management units are used to prevent overexploitation of local populations (Spies & Punt, 2015), largely a demographic concern. On the other hand, management units designed to protect wild populations as a genetic resource for aquaculture aim to prevent the loss of genetic diversity and fitness of wild populations due to broodstock collection and farm escapees, and may thus be better defined at a larger geographic scale than demographic units for fisheries management.

Gradual differentiation associated with the isolation-by-distance (IBD) pattern of population structure poses a unique challenge in determining the spatial scale of management units for fisheries and aquaculture. Without distinct boundaries between populations, it is difficult to delineate management units. However, in species with IBD, genetic variation can be preserved even if arbitrary boundaries are chosen (Spies et al., 2015). In *A. californicus*, it is likely that IBD and oceanographic barriers such as the NPC shape population structure, such that variation may be gradual in many parts of the range at fine and broad scales, but that oceanographic barriers may create distinct boundaries at fine scales (Xuereb, Benestan, et al., 2018). Assessing population structure at finer scales in areas of interest may illuminate oceanographic barriers to dispersal not yet detected and facilitate delineation of management units. In the absence of further sampling, it may be assumed from a precautionary standpoint that distances over which detectable variation can occur (~100 km) may be a useful starting point for defining boundaries in delineation of management units, particularly for fishery management units. As populations are further subdivided for management based on genetic data, decision-makers must also consider the risk of reduced precision associated with the need to complete stock assessments for more stocks with less data (R. S. Waples et al., 2008). From a less precautionary standpoint, state and provincial

boundaries may also be used in the absence of further data, as clustering patterns and partitioning of variance using AMOVAs suggested that this hierarchical grouping explained the most genetic variation of considered groupings. Due to observed isolation-by-distance, it is likely that additional samples will uncover differentiation at smaller scales than states and provinces.

2.5 TABLES AND FIGURES

Table 2-1: General information by collection site for *A. californicus*. Column Site, State refers to the collection site name, followed by the state or province using two letter codes, column SS refers to whether inside (I) or outside (O) the Salish Sea, column NPC refers to whether north (N) or south (S) of the North Pacific Current (NPC), column Code contains the collection site code used in tables and figures, columns Lat and Long refer to latitude and longitude of collection sites, respectively, column I_S refers to number of individuals sequenced (excluding replicates), I_R refers to number of individuals retained in analyses (excluding replicates), column H_E refers to mean expected heterozygosity, column H_O refers to mean observed heterozygosity, column F_{IS} refers to mean locus F_{IS}, and column S_P refers to the proportion of SNPs that are polymorphic.

Site, State	SS	PS	NPC	Code	Lat	Long	I_S	I_R	H_E	H_O	F_{IS}	S_P
Chiniak Bay, AK	O	O	N	CB_AK	57.712	-152.357	27	19	0.28	0.25	0.07	0.93
Yakutat Bay, AK	O	O	N	YB_AK	59.716	-139.846	41	39	0.28	0.25	0.09	0.97
Auke Bay, AK	O	O	N	AB_AK	58.367	-134.668	12	10	0.28	0.24	0.09	0.84
Bella Bella, BC	O	O	N	BB_BC	52.152	-128.139	41	32	0.29	0.23	0.12	0.95
Sekiu, WA	O	O	S	SK_WA	48.160	-124.175	47	47	0.28	0.24	0.15	0.98
James Island, WA	I	O	S	JI_WA	48.513	-122.774	53	50	0.28	0.23	0.15	0.97
Keyport, WA	I	I	S	KP_WA	47.701	-122.634	44	42	0.28	0.23	0.16	0.96
Eld Inlet, WA	I	I	S	EI_WA	47.147	-122.935	53	48	0.28	0.23	0.15	0.97
Charleston, OR	O	O	S	CH_OR	43.340	-124.378	39	38	0.28	0.23	0.16	0.97

Table 2-2: Hierarchical population structure results (AMOVA) for *A. californicus*. Column Grouping contains four groupings (column Grouping) considered with AMOVA: North Pacific Current (NPC), Salish Sea, Puget Sound, and State. Column SNPs refers to which data set was used in the AMOVA: all SNPS (All), putatively neutral SNPs (Neutral), or putatively adaptive SNPs (Adaptive). Variations among groups (column Φ_{CT}) and among collection sites within groups (column Φ_{SC}) are reported, and significance of permutation tests noted with *: $p < 0.05$. The ratio of among-group variation to within-group variation is also reported (column Φ_{CT} / Φ_{SC}).

Grouping	SNPs	Φ_{CT}	Φ_{SC}	Φ_{CT} / Φ_{SC}
NPC	All	0.0040*	0.0043*	0.944
	Neutral	0.0016*	0.0020*	0.804
	Adaptive	0.0253*	0.0247*	1.024
Salish Sea	All	0.0012	0.0056*	0.207
	Neutral	0.0006	0.0025*	0.234
	Adaptive	0.0063	0.0334*	0.187
Puget Sound	All	0.0007	0.0059	0.123
	Neutral	0.0005	0.0026	0.197
	Adaptive	0.0026	0.0357	0.074
State	All	0.0045*	0.0032*	1.424
	Neutral	0.0015*	0.0018*	0.833
	Adaptive	0.0313*	0.0154*	2.032

Table 2-3: Pairwise F_{ST} and genic differentiation test results for *A. californicus*. Pairwise F_{ST} in the lower left and pairwise genic differentiation test results in the upper right of the table (*: $p < 0.05$). Cells with higher F_{ST} values are shaded darker green. Site abbreviations provided in Table 2-1.

	CB_AK	YB_AK	AB_AK	BB_BC	SK_WA	JI_WA	KP_WA	EI_WA	CH_OR
CB_AK	-				*	*	*	*	*
YB_AK	0.004	-		*	*	*	*	*	*
AB_AK	0.004	0.004	-						
BB_BC	0.008	0.005	0.005	-	*	*	*	*	*
SK_WA	0.011	0.009	0.008	0.006	-		*	*	*
JI_WA	0.015	0.012	0.012	0.007	0.002	-	*	*	*
KP_WA	0.010	0.009	0.008	0.007	0.004	0.006	-		*
EI_WA	0.011	0.008	0.011	0.006	0.005	0.005	0.001	-	*
CH_OR	0.009	0.008	0.007	0.009	0.008	0.010	0.004	0.005	-

Table 2-4: LD effective population size (N_e) estimates with 95% confidence intervals per collection site for *A. californicus*. Confidence intervals estimated by jackknifing across samples. *Pcrit* is the minimal allele frequency to retain a locus in the analysis. Both $Pcrit_{0.05}$ (0.05%) and $Pcrit_{0.0}$ (0%) were included because rare alleles can affect N_e estimates.

	$Pcrit_{0.05}$		$Pcrit_{0.0}$	
Site	Estimate	95% CI	Estimate	95% CI
Chiniak Bay, AK	1170.4	319.6 - Infinity	40375.1	389.6 - Infinity
Yakutat Bay, AK	6368.8	1088.9 - Infinity	21798.0	1223.1 - Infinity
Auke Bay, AK	Infinity	312.6 - Infinity	Infinity	312.6 - Infinity
Bella Bella, BC	1063.5	238.1 - Infinity	1453.5	248.2 - Infinity
Sekiu, WA	16057.9	2365.8 - Infinity	21545.6	2550.8 - Infinity
James Island, WA	181563.8	2407.7 - Infinity	25852.1	2949.4 - Infinity
Keyport, WA	Infinity	4596.7 - Infinity	Infinity	5719.8 - Infinity
Eld Inlet, WA	5117.3	1605.0 - Infinity	5683.6	2055.4 - Infinity
Charleston, OR	Infinity	2645.8 - Infinity	Infinity	3272.6 - Infinity

Table 2-5: Summary of results from Bayenv2 for *A. californicus*, for five environmental predictors with the most correlated SNPs. Column Environmental Predictor contains the environmental predictor variable with correlated SNPs. Column Depth refers to whether the environmental variable is measured at sea surface (S) or mean bottom depth (B). Column S_C contains the number of SNPs with evidence of correlation to the row's variable. Column S_P contains the number of correlated SNPs that also had matching gene ontology slim terms for biological processes. Column Biological Processes contains the associated biological processes from matching GO slim terms in order of decreasing frequency, with the proportion of matches per process noted in parentheses.

Environmental Predictor	Depth	S_C	S_P	Biological Processes
Mean salinity	S	38	4	signal transduction (0.259), cell organization and biogenesis (0.207), protein metabolism (0.207), cell cycle and proliferation (0.138), RNA metabolism (0.086), transport (0.069), DNA metabolism (0.034)
Mean nitrate	S	34	4	signal transduction (0.294), cell organization and biogenesis (0.235), developmental processes (0.216), cell cycle and proliferation (0.078), transport (0.078), protein metabolism (0.059), DNA metabolism (0.039)
Temperature range	S	31	2	cell organization and biogenesis (0.387), signal transduction (0.29), RNA metabolism (0.097), developmental processes (0.065), DNA metabolism (0.065), protein metabolism (0.065), stress response (0.032)
pH	S	30	3	cell organization and biogenesis (0.353), developmental processes (0.324), signal transduction (0.265), DNA metabolism (0.059)
Mean temperature	B	27	1	signal transduction (0.5), transport (0.5)

Table 2-6: Results of the redundancy analyses (RDA). Column RDA assigns a number to each RDA for reference; column Predictors refers to the set of environmental predictors used in the RDA, where all refers to all 29 predictors, surface refers to sea surface, bottom refers to mean bottom depth, and cv & temp refers to current velocity and temperature predictors. Column Partial refers to whether spatial variables were conditioned in a partial RDA. Column R^2_{adj} refers to the adjusted R^2 value; columns p_{full} and p_{axis} refer to the p-value from the ANOVA on the full model and for axes, respectively. Column VIF > 10 refers to whether any variance inflation factors (VIF) were over ten, suggesting substantial co-variance among explanatory variables, where MEM refers to spatial variables (Morgan's eigenvector maps) and PC refers to principal components.

RDA	Predictors	Partial	R^2_{adj}	p_{full}	p_{axis}	VIF > 10
1	all	Yes	0.094	>0.1	>0.1	1 MEM & 2 PCs
2	all	No	0.07	>0.1	>0.1	-
3	surface	Yes	0.16	>0.1	>0.1	2 MEMs & 2 PCs
4	surface	No	0.11	0.063	0.052-0.606	-
5	bottom	Yes	-0.081	>0.1	>0.1	-
6	bottom	No	-0.0007	>0.1	>0.1	-
7	cv & temp	Yes	0.087	>0.1	>0.1	1 MEM & 2 PCs
8	cv & temp	No	0.14	0.034	0.035-0.695	-

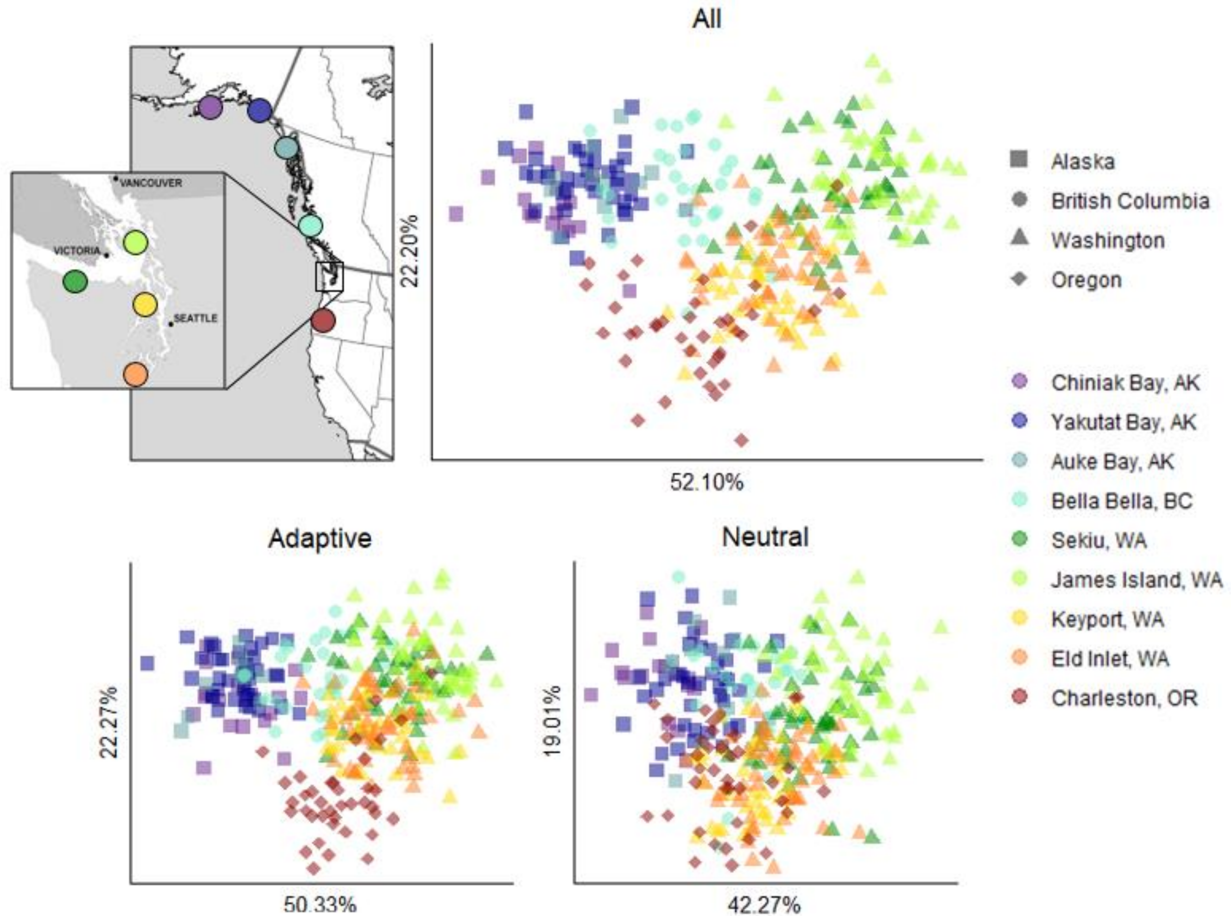


Figure 2-1: A map of collection sites and DAPC results for *A. californicus*. A map of collection sites, including an inset of the southern Salish Sea (upper left). Individuals colored by collection site plotted across the first two discriminant functions of DAPCs using all SNPs (upper right, n = 2,075), putatively adaptive SNPs (lower left, n = 211), and putatively neutral SNPs (lower right n=1,864). For each plot, axes are labeled with the proportion of among-population variance explained by that principal component. Point shapes differ by state or province to highlight regional effects.

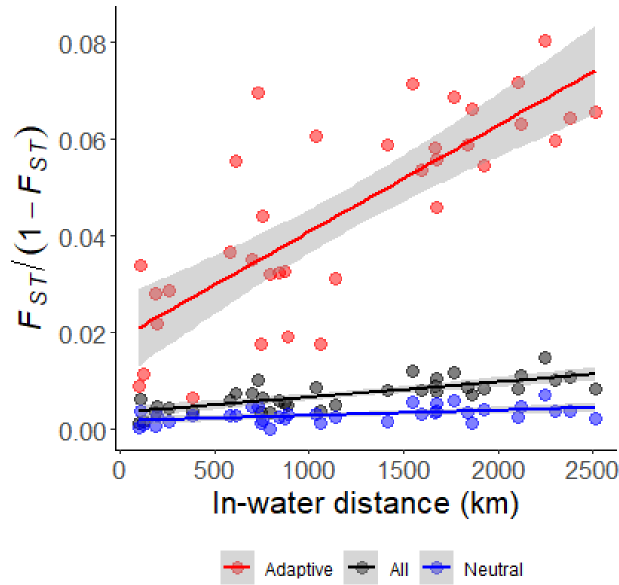


Figure 2-2: Evidence for isolation-by-distance in *A. californicus*. Correlation between in-water distance and linearized F_{ST} using all SNPs ($y = (3.2*10^{-6})*x + 3.5*10^{-3}$, adjusted $R^2 = 0.5622$, $p < 0.001$), putatively neutral SNPs ($y = (1.1*10^{-6})*x + 1.7*10^{-3}$, adjusted $R^2 = 0.2217$, $p < 0.01$), and putatively adaptive SNPs ($y = 2.2*10^{-5})*x$, adjusted $R^2 = 0.5786$, $p < 0.001$).

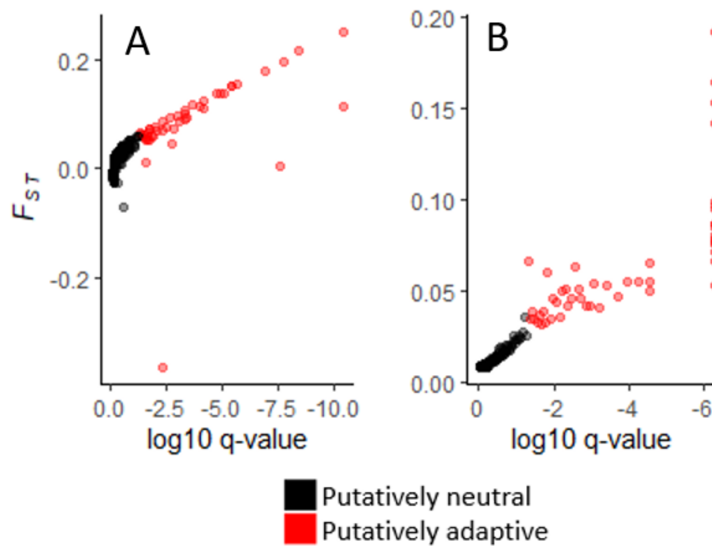


Figure 2-3: Results of F_{ST} outlier detection methods for *A. californicus*. Log_{10} *q*-value by F_{ST} for outlier detection methods, using results generated by *OutFLANK* (n = 46) in panel A and *BayeScan* (n = 50) in panel B. Each point represents a single SNP, colored by whether it was detected as an outlier (red) or not (black).

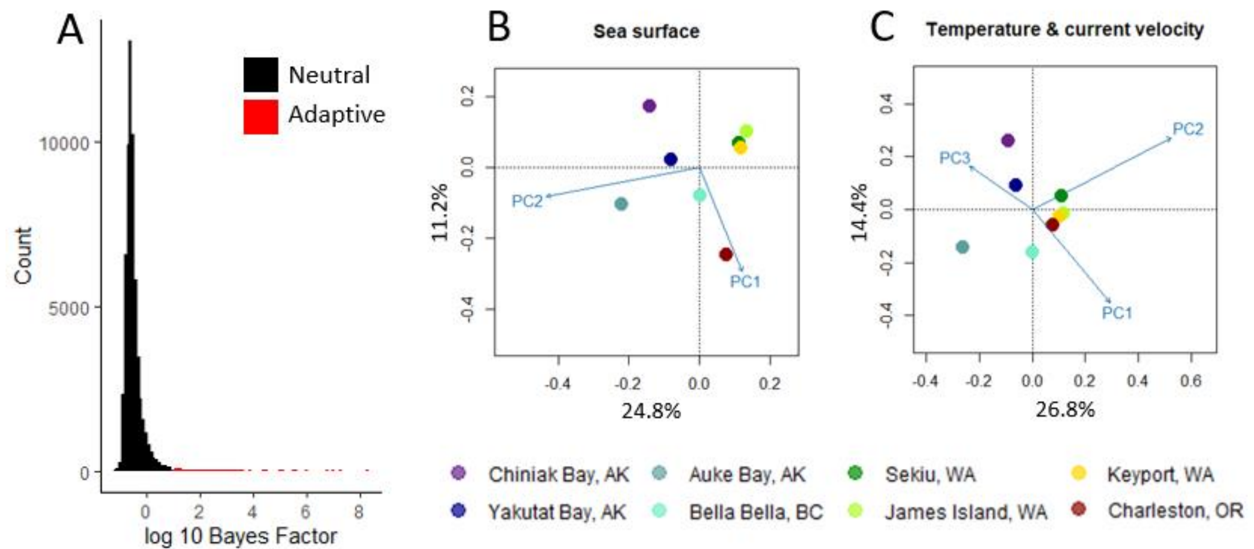


Figure 2-4: Summary of the results of univariate (*Bayenv2*) and multivariate (RDA) gene-environment associations for *A. californicus*. Panel A is a histogram of $\log_{10}$ Bayes Factors for each combination of SNP and environmental predictor. Panels B and C are RDA biplots of the first two RDA axes, with predictors as vectors and sites as points in ordination space, using Type 1 scaling, a scaling method appropriate for questions related to distance among objects. Panel B is of the retained model using sea surface predictors, and panel C is of the retained model using temperature and current velocity predictors. For each RDA biplot, the proportion of variance explained by each RDA axis is labeled on the axis.

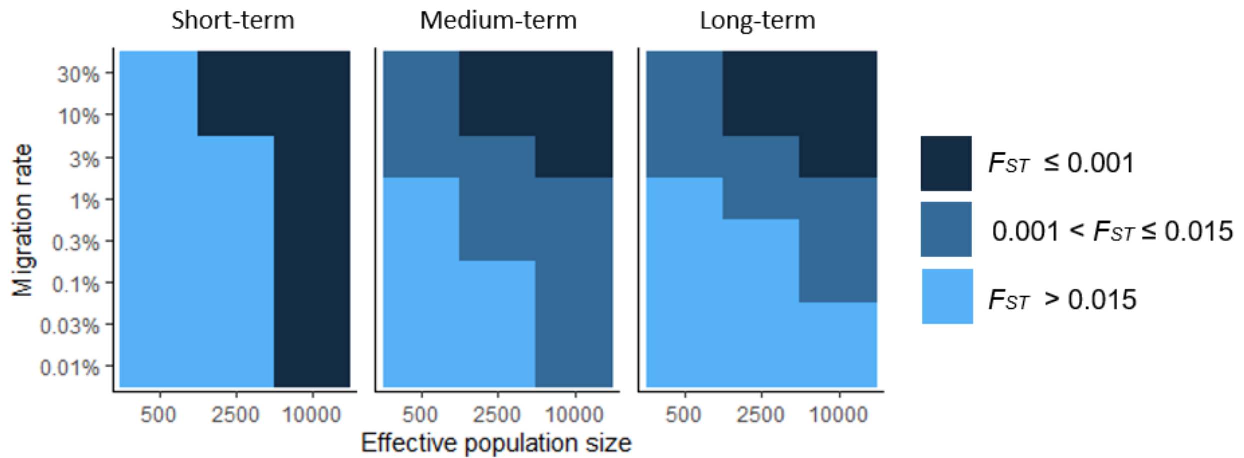


Figure 2-5: Simulation results to contextualize empirical population differentiation and estimate connectivity for *A. californicus*. Tile maps represent pairwise F_{ST} for each combination of effective population size, migration rate, and number of generations of drift elapsed. Short-term (left) represents results from 10 generations of drift, Medium-term (middle) from 100 generations of drift, and Long-term (right) from 1,000 generations of drift. Tile color is divided into three groups based on the scale of F_{ST}: smaller than the observed F_{ST} values in this study, within the range of observed pairwise F_{ST}, and greater than the observed pairwise F_{ST}.

2.6 SUPPLEMENTARY MATERIALS

Supplemental Table 2-1: Pairwise F_{ST} and genic differentiation test results, to compare *de novo* and reference-guided locus assemblies. Global F_{ST} = 0.0068 in both the *de novo* and reference-guided assembly.

De novo	CB_AK	YB_AK	AB_AK	BB_BC	SK_WA	JI_WA	KP_WA	EI_WA	CH_OR
CB_AK	-			*	*	*	*	*	*
YB_AK	0.0036	-		*	*	*	*	*	*
AB_AK	0.0043	0.0037	-						
BB_BC	0.0076	0.006	0.0038	-	*	*	*	*	*
SK_WA	0.0122	0.0094	0.0075	0.0049	-		*	*	*
JI_WA	0.0139	0.0114	0.0099	0.0061	0.0014	-	*	*	*
KP_WA	0.0101	0.0088	0.0074	0.006	0.0033	0.0056	-		*
EI_WA	0.0108	0.0098	0.0088	0.0062	0.004	0.0048	0.0006	-	*
CH_OR	0.011	0.011	0.0089	0.0093	0.0081	0.0109	0.0036	0.0045	-

Reference	CB_AK	YB_AK	AB_AK	BB_BC	SK_WA	JI_WA	KP_WA	EI_WA	CH_OR
CB_AK	-				*	*	*	*	*
YB_AK	0.004	-		*	*	*	*	*	*
AB_AK	0.004	0.004	-						
BB_BC	0.008	0.005	0.005	-	*	*	*	*	*
SK_WA	0.011	0.009	0.008	0.006	-		*	*	*
JI_WA	0.015	0.012	0.012	0.007	0.002	-	*	*	*
KP_WA	0.010	0.009	0.008	0.007	0.004	0.006	-		*
EI_WA	0.011	0.008	0.011	0.006	0.005	0.005	0.001	-	*
CH_OR	0.009	0.008	0.007	0.009	0.008	0.010	0.004	0.005	-

Supplemental Table 2-2: Expected heterozygosity, observed heterozygosity, and proportion of polymorphic SNPs by collection site, comparing *de novo* and reference-guided assemblies. Column H_E refers to mean expected heterozygosity, column H_O refers to mean observed heterozygosity, and column S_P refers to the proportion of SNPs that were polymorphic.

	De novo			Reference		
Collection site	H_E	H_O	S_P	H_E	H_O	S_P
Chiniak Bay, AK	0.273	0.256	0.946	0.278	0.251	0.932
Yakutat Bay, AK	0.274	0.253	0.985	0.279	0.250	0.973
Auke Bay, AK	0.271	0.242	0.865	0.275	0.240	0.837
Bella Bella, BC	0.278	0.235	0.969	0.286	0.232	0.951
Sekiu, WA	0.273	0.241	0.989	0.279	0.238	0.977
James Island, WA	0.271	0.235	0.987	0.277	0.233	0.968
Keyport, WA	0.271	0.237	0.987	0.278	0.233	0.964
Eld Inlet, WA	0.273	0.236	0.987	0.280	0.234	0.970
Charleston, OR	0.271	0.237	0.982	0.277	0.233	0.968

Supplemental Table 2-3: Pearson's correlation coefficients among 29 environmental predictors. Coefficients are shaded blue-white-red for negative-zero-positive. Column and row numbers refer to a specific environmental variable, noted in Supplemental Table 1-1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	1	-0.37	0.73	-0.67	-0.16	0.19	-0.61	0.12	-0.26	0.21	-0.18	0.93	-0.02	0.45	0.38	0.45	0.44	0.22	0.38	-0.15	-0.05	0.08	-0.37	-0.44	0.53	-0.32	-0.44	-0.39	0
2	-0.37	1	0.11	0.16	-0.26	-0.38	0.48	-0.35	-0.17	0.11	0.35	-0.26	-0.13	-0.05	0.22	0	0.34	0.27	0.47	0.05	0.68	0.61	0.65	0.6	-0.28	0.44	0.63	0.53	0.01
3	0.73	0.11	1	-0.29	-0.07	0.17	-0.46	0.1	-0.14	0.26	-0.13	0.67	0.02	0.08	0.2	0.35	0.5	-0.09	0.3	0.3	0.44	0	-0.3	-0.32	0.35	-0.42	-0.41	-0.31	-0.17
4	-0.67	0.16	-0.29	1	-0.07	0.05	0.23	0.1	0.47	-0.21	-0.12	-0.72	0.05	-0.55	-0.15	-0.25	-0.08	-0.34	-0.34	0.31	0.25	-0.41	0.27	0.44	-0.73	-0.02	0.27	0.39	-0.32
5	-0.16	-0.26	-0.07	-0.07	1	-0.25	0.24	-0.22	-0.2	0.47	0.14	-0.24	0.21	-0.69	-0.96	-0.74	-0.83	-0.85	-0.62	0.56	-0.41	-0.45	-0.76	-0.77	0.57	-0.25	-0.65	-0.81	0.39
6	0.19	-0.38	0.17	0.05	-0.25	1	-0.86	0.98	0.85	-0.81	-0.81	0.07	0	0.2	0.19	0.38	0.04	0.06	-0.29	0.11	-0.22	-0.55	-0.11	0	-0.34	-0.76	-0.39	0.13	-0.82
7	-0.61	0.48	-0.46	0.23	0.24	-0.86	1	-0.84	-0.59	0.52	0.85	-0.43	-0.07	-0.24	-0.3	-0.4	-0.15	-0.12	0.02	-0.1	0.29	0.45	0.23	0.19	0.07	0.82	0.53	0.06	0.73
8	0.12	-0.35	0.1	0.1	-0.22	0.98	-0.84	1	0.91	-0.86	-0.86	-0.05	-0.12	0.1	0.16	0.22	-0.04	0.04	-0.35	0.08	-0.32	-0.6	-0.07	0.06	-0.43	-0.75	-0.35	0.18	-0.85
9	-0.26	-0.17	-0.14	0.47	-0.2	0.85	-0.59	0.91	1	-0.87	-0.81	-0.44	-0.08	-0.16	0.05	0.02	-0.15	-0.06	-0.43	0.18	-0.25	-0.64	0.13	0.29	-0.7	-0.63	-0.14	0.37	-0.88
10	0.21	0.11	0.26	-0.21	0.47	-0.81	0.52	-0.86	-0.87	1	0.62	0.28	0.3	-0.29	-0.34	-0.27	-0.06	-0.33	0.25	0.26	0.22	0.31	-0.34	-0.45	0.68	0.32	-0.13	-0.56	0.73
11	-0.18	0.35	-0.13	-0.12	0.14	-0.81	0.85	-0.86	-0.81	0.62	1	0.06	-0.19	0.12	-0.13	-0.07	0.14	-0.01	0.14	-0.29	0.41	0.59	0.04	-0.04	0.4	0.83	0.39	-0.14	0.9
12	0.93	-0.26	0.67	-0.72	-0.24	0.07	-0.43	-0.05	-0.44	0.28	0.06	1	0.07	0.64	0.43	0.64	0.55	0.33	0.5	-0.23	0.15	0.31	-0.28	-0.38	0.61	-0.09	-0.29	-0.34	0.17
13	-0.02	-0.13	0.02	0.05	0.21	0	-0.07	-0.12	-0.08	0.3	-0.19	0.07	1	-0.21	-0.24	0.15	-0.23	-0.2	0.21	0.65	0.09	-0.05	-0.13	-0.21	0.2	-0.29	-0.27	-0.19	-0.13
14	0.45	-0.05	0.08	-0.55	-0.69	0.2	-0.24	0.1	-0.16	-0.29	0.12	0.64	-0.21	1	0.75	0.82	0.65	0.82	0.51	-0.73	0.15	0.57	0.32	0.24	0.08	0.3	0.32	0.31	0.05
15	0.38	0.22	0.2	-0.15	-0.96	0.19	-0.3	0.16	0.05	-0.34	-0.13	0.43	-0.24	0.75	1	0.73	0.86	0.9	0.73	-0.61	0.34	0.53	0.68	0.65	-0.39	0.23	0.57	0.69	-0.31
16	0.45	0	0.35	-0.25	-0.74	0.38	-0.4	0.22	0.02	-0.27	-0.07	0.64	0.15	0.82	0.73	1	0.77	0.62	0.54	-0.29	0.47	0.38	0.29	0.25	-0.05	0.01	0.18	0.33	-0.25
17	0.44	0.34	0.5	-0.08	-0.83	0.04	-0.15	-0.04	-0.15	-0.06	0.14	0.55	-0.23	0.65	0.86	0.77	1	0.65	0.68	-0.42	0.69	0.54	0.48	0.47	-0.19	0.26	0.46	0.47	-0.11
18	0.22	0.27	-0.09	-0.34	-0.85	0.06	-0.12	0.04	-0.06	-0.33	-0.01	0.33	-0.2	0.82	0.9	0.62	0.65	1	0.74	-0.72	0.15	0.7	0.74	0.65	-0.29	0.41	0.66	0.69	-0.14
19	0.38	0.47	0.3	-0.34	-0.62	-0.29	0.02	-0.35	-0.43	0.25	0.14	0.5	0.21	0.51	0.73	0.54	0.68	0.74	1	-0.25	0.47	0.81	0.59	0.43	0.02	0.36	0.5	0.42	0.01
20	-0.15	0.05	0.3	0.31	0.56	0.11	-0.1	0.08	0.18	0.26	-0.29	-0.23	0.65	-0.73	-0.61	-0.29	-0.42	-0.72	-0.25	1	0.14	-0.47	-0.4	-0.37	0.12	-0.6	-0.55	-0.38	-0.28
21	-0.05	0.68	0.44	0.25	-0.41	-0.22	0.29	-0.32	-0.25	0.22	0.41	0.15	0.09	0.15	0.34	0.47	0.69	0.15	0.47	0.14	1	0.48	0.39	0.38	-0.13	0.31	0.42	0.33	0.04
22	0.08	0.61	0	-0.41	-0.45	-0.55	0.45	-0.6	-0.64	0.31	0.59	0.31	-0.05	0.57	0.53	0.38	0.54	0.7	0.81	-0.47	0.48	1	0.59	0.43	0.14	0.76	0.68	0.39	0.42
23	-0.37	0.65	-0.3	0.27	-0.76	-0.11	0.23	-0.07	0.13	-0.34	0.04	-0.28	-0.13	0.32	0.68	0.29	0.48	0.74	0.59	-0.4	0.39	0.59	1	0.97	-0.71	0.5	0.92	0.96	-0.26
24	-0.44	0.6	-0.32	0.44	-0.77	0	0.19	0.06	0.29	-0.45	-0.04	-0.38	-0.21	0.24	0.65	0.25	0.47	0.65	0.43	-0.37	0.38	0.43	0.97	1	-0.84	0.42	0.89	0.99	-0.36
25	0.53	-0.28	0.35	-0.73	0.57	-0.34	0.07	-0.43	-0.7	0.68	0.4	0.61	0.2	0.08	-0.39	-0.05	-0.19	-0.29	0.02	0.12	-0.13	0.14	-0.71	-0.84	1	-0.01	-0.56	-0.85	0.65
26	-0.32	0.44	-0.42	-0.02	-0.25	-0.76	0.82	-0.75	-0.63	0.32	0.83	-0.09	-0.29	0.3	0.23	0.01	0.26	0.41	0.36	-0.6	0.31	0.76	0.5	0.42	-0.01	1	0.79	0.33	0.69
27	-0.44	0.63	-0.41	0.27	-0.65	-0.39	0.53	-0.35	-0.14	-0.13	0.39	-0.29	-0.27	0.32	0.57	0.18	0.46	0.66	0.5	-0.55	0.42	0.68	0.92	0.89	-0.56	0.79	1	0.84	0.1
28	-0.39	0.53	-0.31	0.39	-0.81	0.13	0.06	0.18	0.37	-0.56	-0.14	-0.34	-0.19	0.31	0.69	0.33	0.47	0.69	0.42	-0.38	0.33	0.39	0.96	0.99	-0.85	0.33	0.84	1	-0.45
29	0	0.01	-0.17	-0.32	0.39	-0.82	0.73	-0.85	-0.88	0.73	0.9	0.17	-0.13	0.05	-0.31	-0.25	-0.11	-0.14	0.01	-0.28	0.04	0.42	-0.26	-0.36	0.65	0.69	0.1	-0.45	1

Supplemental Table 2-4: All environmental predictor loadings on retained PCs.

Environmental Predictor	PC1	PC2	PC3
BO_bathymean	0.077	-0.321	0.041
BO_calcite	-0.081	-0.014	-0.035
BO_ph	0.047	-0.047	-0.398
BO2_curvelmax_bdmean	0.286	0.055	-0.100
BO2_curvelmax_ss	0.212	0.030	-0.234
BO2_curvelmean_bdmean	0.168	-0.076	-0.010
BO2_curvelmean_ss	0.186	0.099	-0.239
BO2_curvelmin_bdmean	0.238	-0.070	-0.155
BO2_curvelmin_ss	0.280	0.109	-0.141
BO2_curvelrange_bdmean	-0.203	0.027	0.056
BO2_curvelrange_ss	0.251	0.026	-0.177
BO2_dissoxmean_bdmean	-0.067	-0.313	-0.073
BO2_dissoxmean_ss	-0.287	-0.141	0.063
BO2_nitratemean_bdmean	-0.054	0.345	-0.044
BO2_nitratemean_ss	-0.021	0.018	-0.281
BO2_phosphatemean_bdmean	-0.042	0.338	0.122
BO2_phosphatemean_ss	-0.004	0.096	0.307
BO2_ppmean_bdmean	0.057	-0.285	0.233
BO2_ppmean_ss	0.180	-0.088	0.144
BO2_salinitymean_bdmean	-0.048	0.334	-0.092
BO2_salinitymean_ss	-0.006	0.014	-0.390
BO2_tempmax_bdmean	0.198	-0.244	0.114
BO2_tempmax_ss	0.251	-0.190	-0.064
BO2_tempmean_bdmean	0.283	-0.058	0.182
BO2_tempmean_ss	0.285	0.054	0.157
BO2_tempmin_bdmean	0.265	0.139	0.167
BO2_tempmin_ss	0.268	0.099	0.193
BO2_temprange_bdmean	-0.016	-0.338	-0.020
BO2_temprange_ss	-0.141	-0.224	-0.251

Supplemental Table 2-5: Sea surface predictor loadings for retained PCs.

Environmental Predictor	PC1	PC2
BO_calcite	-0.086	0.059
BO_ph	0.186	0.405
BO2_curvelmax_ss	0.328	0.156
BO2_curvelmean_ss	0.326	0.144
BO2_curvelmin_ss	0.395	-0.006
BO2_curvelrange_ss	0.375	0.062
BO2_dissoxmean_ss	-0.385	0.098
BO2_nitratemean_ss	0.096	0.283
BO2_phosphatemean_ss	-0.078	-0.351
BO2_ppmean_ss	0.154	-0.216
BO2_salinitymean_ss	0.131	0.401
BO2_tempmax_ss	0.276	0.004
BO2_tempmean_ss	0.285	-0.307
BO2_tempmin_ss	0.265	-0.348
BO2_temprange_ss	-0.122	0.384

Supplemental Table 2-6: Bottom depth predictor loadings for retained PCs.

Environmental Predictor	PC1	PC2
BO2_curvelmax_bdmean	0.058	-0.458
BO2_curvelmean_bdmean	0.147	-0.140
BO2_curvelmin_bdmean	0.169	-0.279
BO2_curvelrange_bdmean	-0.115	0.321
BO2_dissoxmean_bdmean	0.301	0.289
BO2_nitratemean_bdmean	-0.395	-0.097
BO2_phosphatemean_bdmean	-0.364	-0.127
BO2_ppmean_bdmean	0.343	0.029
BO2_salinitymean_bdmean	-0.387	-0.088
BO2_tempmax_bdmean	0.354	-0.197
BO2_tempmean_bdmean	0.205	-0.413
BO2_tempmin_bdmean	-0.014	-0.480
BO2_temprange_bdmean	0.345	0.182

Supplemental Table 2-7: Current velocity and temperature predictor loadings for retained PCs.

Environmental Predictor	PC1	PC2	PC3
BO2_curvelmax_bdmean	0.317	-0.066	0.098
BO2_curvelmax_ss	0.236	-0.257	0.268
BO2_curvelmean_bdmean	0.170	-0.044	0.054
BO2_curvelmean_ss	0.213	-0.118	0.454
BO2_curvelmin_bdmean	0.259	-0.167	0.116
BO2_curvelmin_ss	0.312	0.003	0.243
BO2_curvelrange_bdmean	-0.216	0.189	0.105
BO2_curvelrange_ss	0.272	-0.103	0.268
BO2_tempmax_bdmean	0.194	-0.251	-0.460
BO2_tempmax_ss	0.259	-0.299	-0.152
BO2_tempmean_bdmean	0.297	0.064	-0.338
BO2_tempmean_ss	0.308	0.202	-0.174
BO2_tempmin_bdmean	0.291	0.304	-0.091
BO2_tempmin_ss	0.291	0.286	-0.164
BO2_temprange_bdmean	-0.040	-0.471	-0.365
BO2_temprange_ss	-0.161	-0.496	0.088

Supplemental Table 2-8: Sites retained at each filtering step in *A. californicus*. All loci were SNPs after removing indels. MAF = minor allele frequency.

Filtering step	Loci
Total from *dDocent*	6738423
After removing indels	3987180
After maximum missing data per locus = 30%	94543
After minimum MAF = 0.05	9178
After minimum quality score = 20	9133
After removing SNPs out of Hardy Weinberg Equilibrium	5042
After retaining one SNP per RAD locus, with highest MAF	2075

Supplemental Table 2-9: Pairwise F_{ST} with confidence intervals and pairwise genic differentiation test results. Pairwise F_{ST} (and 95% confidence intervals) in the lower left and pairwise genic differentiation test results in the upper right of the table (*: $p < 0.05$). Confidence intervals were generated by bootstrapping across SNPs with 100 repetitions. Cells with higher F_{ST} values were shaded darker green. Site abbreviations provided in Table 2-1.

	CB_AK	YB_AK	AB_AK	BB_BC	SK_WA	JI_WA	KP_WA	EI_WA	CH_OR
CB_AK	-				*	*	*	*	*
YB_AK	**0.004** (0.001-0.005)	-		*	*	*	*	*	*
AB_AK	**0.004** (0.001-0.007)	**0.004** (0.001-0.007)	-						
BB_BC	**0.008** (0.005-0.011)	**0.005** (0.003-0.007)	**0.005** (0.001-0.008)	-	*	*	*	*	*
SK_WA	**0.011** (0.009-0.013)	**0.009** (0007-0.011)	**0.008** (0.004-0.011)	**0.006** (0.004-0.009)	-		*	*	*
JI_WA	**0.015** (0.012-0.018)	**0.012** (0.010-0.014)	**0.012** (0.008-0.017)	**0.007** (0.006-0.010)	**0.002** (0.001-0.004)	-	*	*	*
KP_WA	**0.010** (0.009-0.014)	**0.009** (0.007-0.012)	**0.008** (0.003-0.012)	**0.007** (0.005-0.010)	**0.004** (0.002-0.006)	**0.006** (0.005-0.010)	-		*
EI_WA	**0.011** (0.009-0.014)	**0.008** (0.007-0.012)	**0.011** (0.007-0.016)	**0.006** (0.005-0.010)	**0.005** (0.003-0.006)	**0.005** (0.004-0.006)	**0.001** (0.000-0.003)	-	*
CH_OR	**0.009** (0.006-0.011)	**0.008** (0.006-0.011)	**0.007** (0.003-0.010)	**0.009** (0.007-0.013)	**0.008** (0.007-0.010)	**0.010** (0.008-0.013)	**0.004** (0.001-0.005)	**0.005** (0.004-0.008)	-

Supplemental Table 2-10: *Bayenv2* results for *A. californicus*. Column Environmental Predictor refers to the environmental predictor with correlated SNPs. Column Depth refers to whether the variable was measured at sea surface (S) or mean bottom depth (B). Column S_C refers to the number of correlated SNPs. Column S_P refers to the number of correlated SNPs with associated gene ontology slim terms for biological processes. Column Biological Processes contains the associated biological processes from matching gene ontology slim terms in order of decreasing frequency, with the proportion of matches per process noted in parentheses.

Environmental Predictor	Depth	S_C	S_P	Biological Processes
Mean salinity	S	38	4	signal transduction (0.259), cell organization and biogenesis (0.207), protein metabolism (0.207), cell cycle and proliferation (0.138), RNA metabolism (0.086), transport (0.069), DNA metabolism (0.034)
Mean nitrate	S	34	4	signal transduction (0.294), cell organization and biogenesis (0.235), developmental processes (0.216), cell cycle and proliferation (0.078), transport (0.078), protein metabolism (0.059), DNA metabolism (0.039)
Temperature range	S	31	2	cell organization and biogenesis (0.387), signal transduction (0.29), RNA metabolism (0.097), developmental processes (0.065), DNA metabolism (0.065), protein metabolism (0.065), stress response (0.032)
pH	S	30	3	cell organization and biogenesis (0.353), developmental processes (0.324), signal transduction (0.265), DNA metabolism (0.059)
Mean temperature	B	27	1	signal transduction (0.5), transport (0.5)
Mean phosphate	S	26	2	cell organization and biogenesis (0.522), signal transduction (0.391), DNA metabolism (0.087)
Minimum temperature	S	24	1	signal transduction (0.5), transport (0.5)
Minimum temperature	B	22	1	signal transduction (0.5), transport (0.5)
Mean primary productivity	S	19	2	cell organization and biogenesis (0.308), protein metabolism (0.231),

				signal transduction (0.231), RNA metabolism (0.128), cell cycle and proliferation (0.103)
Mean temperature	S	18	1	signal transduction (0.5), transport (0.5)
Mean current velocity	B	14	2	cell organization and biogenesis (0.367), transport (0.245), developmental processes (0.204), signal transduction (0.184)
Mean dissolved oxygen	S	14	1	developmental processes (0.522), transport (0.217), signal transduction (0.174), RNA metabolism (0.087)
Maximum temperature	S	14	2	stress response (1)
Maximum current velocity	B	13	1	transport (0.333), developmental processes (0.167), stress response (0.148), protein metabolism (0.13), signal transduction (0.093), cell organization and biogenesis (0.037), death (0.037), RNA metabolism (0.037), cell cycle and proliferation (0.019)
Minimum current velocity	B	12	NA	NA
Minimum current velocity	S	12	1	developmental processes (0.522), transport (0.217), signal transduction (0.174), RNA metabolism (0.087)
Range in current velocity	S	12	1	DNA metabolism (1)
Maximum temperature	B	12	NA	NA
Mean primary productivity	B	11	1	DNA metabolism (1)
Mean dissolved oxygen	B	10	1	transport (0.42), cell organization and biogenesis (0.174), developmental processes (0.174), signal transduction (0.072), protein metabolism (0.058), cell adhesion (0.043), cell cycle and proliferation (0.043), RNA metabolism (0.014)
Range in current velocity	B	9	1	cell organization and biogenesis (0.571), signal transduction (0.429)
Mean phosphate	B	9	1	signal transduction (0.326), stress response (0.256), developmental processes (0.116),

				protein metabolism (0.093), transport (0.093), death (0.07), cell-cell signaling (0.023), cell cycle and proliferation (0.023)
Maximum current velocity	S	8	1	protein metabolism (1)
Mean current velocity	S	8	1	protein metabolism (1)
Mean nitrate	B	6	NA	NA
Calcite	S	5	NA	NA
Mean salinity	B	5	NA	NA
Range in temperature	B	5	NA	NA
Mean bathymetry	B	3	NA	NA

Supplemental Table 2-11: Loadings for RDA using sea surface predictors. The loadings for each PC are presented in Supplemental Table 2-5.

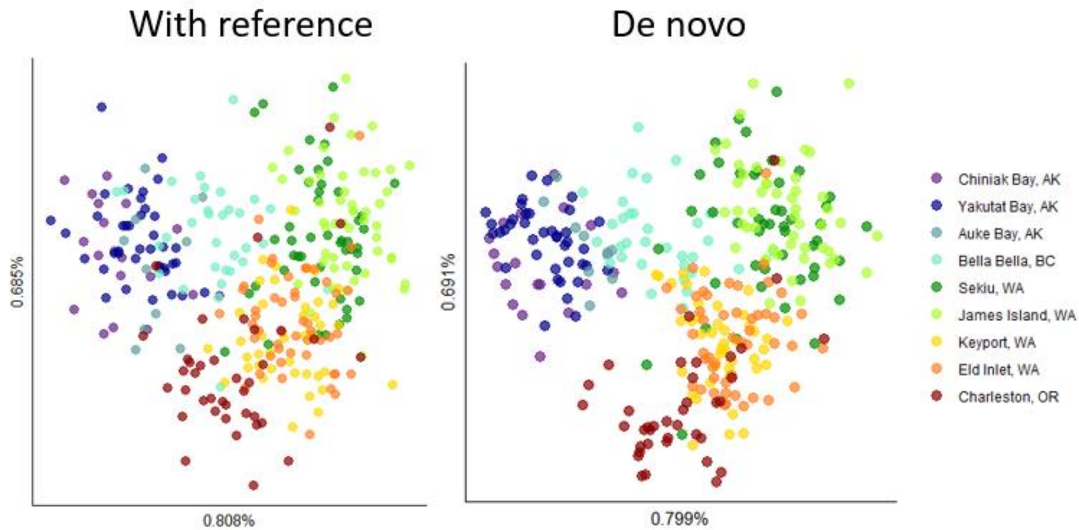
	RDA1	RDA2
PC1	0.266	-0.964
PC2	-0.964	-0.266

Supplemental Table 2-12: Loadings for RDA using current and temperature predictors. The loadings for each PC are presented in Supplemental Table 2-7.

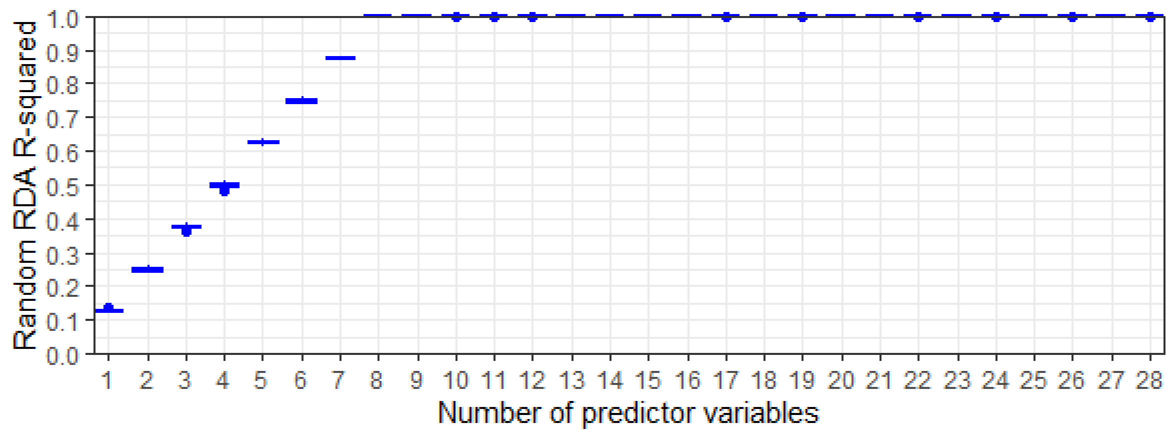
	RDA1	RDA2	RDA3
PC1	0.456	-0.742	0.492
PC2	0.810	0.574	0.116
PC3	-0.368	0.346	0.863

Supplemental Table 2-13: Counts of gene ontology slim terms in the database.

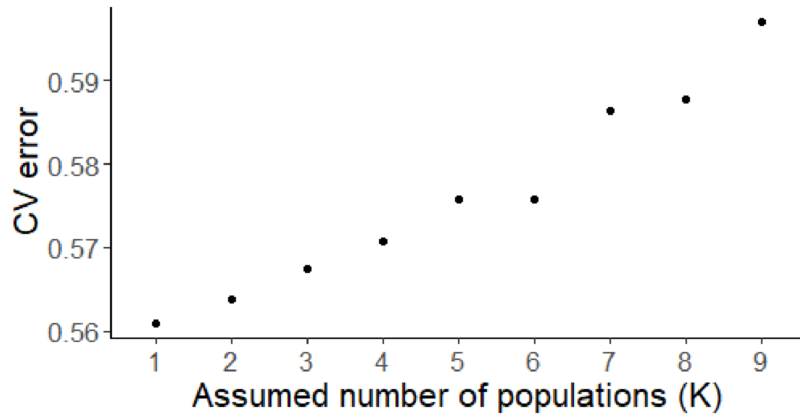
Gene ontology slim term	*n*
other metabolic processes	4102
other biological processes	3320
developmental processes	3092
cell organization and biogenesis	1712
transport	1398
protein metabolism	1261
stress response	1000
signal transduction	917
cell cycle and proliferation	675
RNA metabolism	574
cell-cell signaling	302
DNA metabolism	299
death	267
cell adhesion	107



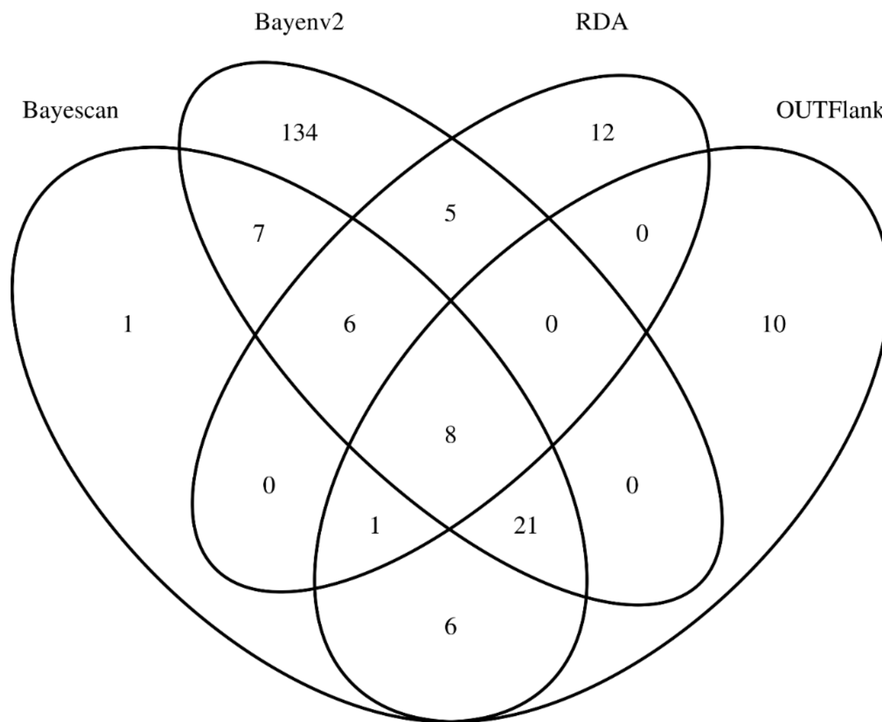
Supplemental Figure 2-1: Principal component analysis to compare reference-guided and *de novo* locus assemblies. Individuals are plotted across the first two principal components.



Supplemental Figure 2-2: Overfitting of RDA models occurred quickly with the addition of predictor variables. Using randomly generated data based on the mean and standard deviations of environmental predictor data used in this study, we demonstrated that R^2 reaches 1 with the addition of 8 predictor variables.



Supplemental Figure 2-3: Cross validation error by number of assumed underlying populations in *ADMIXTURE* clustering analysis.



Supplemental Figure 2-4: Venn diagram showing overlap among putatively adaptive SNPs by method for *A. californicus*.

Chapter 3. Informal policy and factors affecting policy change for genetic risks of native shellfish aquaculture

3.1 INTRODUCTION

Shellfish constitute the majority of marine aquaculture production in the United States (NOAA Fisheries, 2021b). Many of the cultivated shellfish species are non-native, including the most widely grown species on the West Coast, the Pacific Oyster (Pacific Shellfish Institute, 2021b). The shellfish aquaculture industry is growing (Sea Grant Association, 2016) and one facet of industry expansion is the development of new species for cultivation. Because importing non-native species poses ecological risks to native species and the broader ecosystem, many have turned to native shellfish species for commercial development. For example, on the Pacific Coast of North America, multiple native shellfish species including the Olympia Oyster, Pacific Geoduck, and Purple-hinged Rock Scallop have been identified as species to target for development or expansion of current commercial production (Sea Grant Association, 2016). However, aquaculture of native species poses genetic risks to wild populations if hatchery-produced and wild animals interbreed (Laikre et al., 2010). These risks threaten long-term population viability of both wild populations and aquaculture (R. S. Waples et al., 2012) and are thus a concern for decision makers at natural resource management agencies.

3.1.1 *What are the genetic risks of native shellfish aquaculture?*

Although hatchery production methods differ based on the goal of the program (e.g., commercial or conservation goals), they generally differ from wild reproduction, resulting in genetic impacts for offspring. These genetic impacts can affect wild populations if hatchery-produced and wild individuals interbreed, such as through escape or intentional release (Laikre et

al., 2010). Although not mutually exclusive, these genetic impacts of aquaculture can be organized into a simple framework with three genetic risks: loss of genetic diversity within populations, loss of population differentiation, and loss of fitness (R. S. Waples et al., 2012).

The first genetic risk of hatchery production is loss of genetic diversity within a population. For production facilities, collecting and maintaining large populations of parents may be cost prohibitive and logistically challenging. Thus, hatchery production often involves relatively few parents producing many offspring, compared to wild populations (Straus et al., 2015). However, small parental populations may produce offspring with less genetic diversity than a wild cohort. Upon interbreeding between hatchery-produced and wild animals, the genetic diversity of the wild population may decline (Ryman & Laikre, 1991). Moreover, interbreeding between highly related individuals can lead to inbreeding depression, in which fitness declines (Plough & Hedgecock, 2011; Taris et al., 2007; S. Wang et al., 2002).

Second, hatchery production can also reduce population differentiation among wild populations if movement of broodstock and seed occurs across distinct populations or if broodstock from distinct populations are crossed in the hatchery and seed are released into either population (R. S. Waples et al., 2012). Population differentiation may also be reduced when the proportion of hatchery-produced escapees in several wild populations is high enough that their genetic contribution overrides genetic differences among wild populations, a process known as swamping (Waples & Do, 1994) (Figure 2-1).

Third, loss of fitness can occur due to domestication selection, through relaxation of natural selection, selection for traits that provide advantages in the captive environment, or both (Reisenbichler & Rubin, 1999). The purpose of shellfish hatcheries is to increase survival of offspring, and in commercial aquaculture specifically, to maximize optimal growing conditions.

Thus, natural selection is usually relaxed (Lynch & O'Hely, 2001). Optimal growing conditions (density, ambient temperature, etc.) may differ slightly from the natural environment. Additionally, animals with traits better suited to the captive environment have higher reproductive success in the captive environment than their wild counterparts (Doyle, 1983), leading to domestication selection. Domestication selection can be intentional, such as selection for production traits in hatchery lines (Dale-Kuys et al., 2017; de Melo et al., 2016), or unintentional, through favoring specific traits, such as culling slowly developing larvae, leading to selection for faster development (Taris et al., 2007). As a result, hatchery-produced offspring that survive in the captive environment can be less fit in the wild one (Fritts et al., 2007; Pearsons et al., 2007). If hatchery-related traits are passed on to the wild population through interbreeding of hatchery-produced and wild animals, fitness of wild populations can decline (Besnier et al., 2015). Domestication selection can also result in loss of genetic diversity, as fit individuals have greater reproductive success, leading to fewer effective breeders (Currens & Busack, 1995).

A central concern to natural resource managers is the sustainability of wild resources for present and future uses (ADFG, 2019; LNRD, 2019; NNRD, 2019; WDFW, 2019). Maintaining fitness and genetic diversity, both within and among populations, can support the sustainability of wild resources because these factors contribute to adaptability and productivity (Hughes et al., 2008; Schindler et al., 2010). For these reasons, the genetic risks associated with hatchery-produced finfish, in particular salmon, have resulted in decades of research and policy development to mitigate risks to wild populations (Mobrand et al., 2005; Naish et al., 2007; Paquet et al., 2011). There is substantially less research on shellfish (Nascimento-Schulze et al., 2021; Valenzuela-Quiñonez, 2016) and formal regulation of these risks in shellfish species.

3.1.2 *What information would inform potential policy development?*

Informal policy is becoming increasingly more common (Mendelson, 2006), particularly for emerging risks (Blais & Wagner, 2007), because of the associated time and resource costs associated with formal policies. Formal policies require a public comment period and are legally binding (e.g., 85 FR 81822, a federal rule updating designation of Pacific salmon hatchery programs under the Endangered Species Act). Informal policies, often in the form of guidance documents, provide specificity and practical guidance for implementation of formal policy (Mendelson, 2006). Although designed to not be legally binding, informal policies can be deemed legally binding through litigation (Franklin, 2010). Informal policies can therefore change behavior of regulated entities, albeit not to the extent of formal policy. Given the lack of formal policy for genetic risks of native shellfish aquaculture, it remains unclear to what extent agencies use informal policy to mitigate these risks. Because informal policies do not require a public comment period, they may be less visible to agency outsiders unless they specifically request it.

Illuminating to what extent agencies use informal policy for mitigating the genetic risks of native shellfish aquaculture may be useful for many reasons. Because the genetic risks of native shellfish aquaculture is often a trans-boundary issue (involving coordination among states and provinces), characterizing patterns and variation in informal policy will benefit interagency coordination. Such activities might include identification of shared management goals and targets (Kark et al., 2015) and the definition of management units in shared waters, used for restricting movement of broodstock and seed (Fisheries and Oceans Canada, 2011). Furthermore, the multiplicity of state and tribal agencies along the Pacific Coast of the United States likely provides variation in policy approaches (Galle & Leahy, 2008), and raising awareness of such variation in

policies may serve as a starting point for comparing policy alternatives, a critical step of many policy development processes (Greco et al., 2016; Punt et al., 2016).

Potential policy development for genetic risks of native shellfish aquaculture will likely be influenced by more than top-down directives, regardless of whether policies are formal or informal (Miner, 2006). According to the punctuated equilibrium theory of policy change (PET), most policy changes are incremental except for rare, substantial changes (True et al., 1999) due to bounded rationality. Bounded rationality refers to the rational, cognitive, and institutional factors that constrain an individual's decision making within an organization, and that lead to behavior better described by satisficing rather than by strictly rational optimization (Miner, 2006). These factors may include an individual's values relating to organizational goals and knowledge of potential policy outcomes (Bell, 1985; Miner, 2006), as well as the cognitive difficulties associated with accounting for uncertainty, conceptualizing the problem, and risk perception (Jones, 2003). Institutional factors such as budgets, organizational culture, and existing relationships with interest groups also shape individual decision making (Lester, 1995; Sapat, 2004). PET posits that rare, substantial changes are due to changes in agenda setting due to limited attention spans in organizations (True et al., 1999) and that these shifts in attention are due to external perturbations that disrupt policy ideas, beliefs, and institutions (Sabatier & Jenkins-Smith, 1993; Smith, 2000; Thelen, 2003). Shedding light on these factors and how they shape the level of attention given to this issue may inform potential policy change regarding genetic risks of native shellfish aquaculture.

3.1.3 *Study aim*

The aim of this study is to describe the regulatory context regarding the genetic risks of native shellfish aquaculture (hereafter referred to as genetic risks), within the Pacific Coast region

of the United States. Specifically, we used a mixed-methods approach, which combines quantitative and qualitative methods (Johnson & Onwuegbuzie, 2004), to interview state and tribal government shellfish resource managers, in order to illuminate 1) current management practices, 2) factors that may influence decision-making, and 3) the level of policy attention given to this issue. Where possible, we provided comparisons to the same issues in the management of hatchery production of Pacific salmon. Although Pacific salmon and their hatchery production differ from their counterparts in shellfish, the management of hatchery production of Pacific salmon provides the closest example of what policies could be in place for native shellfish aquaculture. Our results will benefit decision makers considering potential policy development for the genetic risks of native shellfish aquaculture, at a time when this issue is receiving increased attention in academic (Hornick & Plough, 2019; Nascimento-Schulze et al., 2021) and policy arenas (WDFW, 2016).

3.2 METHODS

3.2.1 *Study system*

We focused our study on the Pacific Coast region of the United States, which supports the largest shellfish aquaculture industry in the country (Washington Sea Grant, 2015), with demonstrated interest in expanding native shellfish aquaculture (Culver et al., 2006; Pacific Shellfish Institute, 2021a). Shellfish aquaculture largely takes place in coastal waters where states (Alaska, Washington, Oregon, California) and tribes have jurisdiction. Though a number of tribes have land rights in this region, we only included Western Washington treaty tribes (n = 20) in our study because of their reaffirmed rights for shellfish harvest (J. W. Anderson, 1999) and status as co-managers for shellfish resources. Although there is variation in governance and policy-making among tribes and states, in general, formal policy making is directed by rule-making bodies (e.g., legislature, fish and wildlife commissions, tribal councils) and implemented by administrative

bodies (e.g., Department of Natural Resources, Department of Fish and Wildlife) with often broad agency discretion (SOSU, 2016; WDFW, 2021). We targeted shellfish resource managers at state and tribal agencies for interviews because we were interested primarily in informal policy making.

3.2.2 *Interview methodology*

For each of the four U.S. Pacific Coast states and twenty Western Washington treaty tribes, we identified potential interviewees as individuals whose work pertained to management or regulation relating to shellfish aquaculture and/or wild shellfish resources. Depending on the administration, managers with such positions worked in a variety of agencies (e.g., natural resources, fish and wildlife, and agriculture). Herein, we refer to these agencies collectively as "natural resource agencies." Individuals were identified through online research and by contacting natural resource agency offices. We incentivized participation using a raffle for an Amazon gift card. The University of Washington Human Subjects Division deemed this study "exempt" from federal human subjects regulations, including the requirement for Institutional Review Board approval and continuing review.

In the spring of 2018, we interviewed 18 shellfish resource managers from 14 state and tribal natural resource agencies, representing all four US states along the Pacific west coast (n=4) and half (n=10) of the Western Washington treaty tribes. We used semi-structured interviews (Charnley et al., 2017; Dillman, 2014; Saldaña & Omasta, 2016) to gather information regarding the regulatory context surrounding this emerging management issue. Throughout, we apply the PET framework not for the purposes of testing the theory or predicting policy change, but for illuminating relevant factors for informing potential decision making. Specifically, our objectives were 1) to characterize current management practices, 2) to illuminate factors that may influence decision-making, and 3) to assess the relative attention given to genetic risks of native shellfish

aquaculture as it relates to agenda setting. To provide context on manager and agency priorities and values, we asked managers about their responsibilities, their three highest priority tasks, and rationales for why such tasks were high priority. Below we provide an overview of the interview themes, and the full interview protocol is provided in the Supplementary Materials.

For the first objective, we asked managers about the degree to which their agency currently manages for genetic risks of native shellfish aquaculture, regardless of the program goal (commercial aquaculture, restoration aquaculture, etc.). For the second objective, we identified *a priori* factors for investigation: how managers conceptualize of genetic risks of native shellfish aquaculture, how they perceive these risks, the degree of resource limitation (i.e., available funding) at the agency, and the nature of the relationship with a primary interest group, the aquaculture industry. To shed light on how managers conceptualized the problem, we asked managers to rationalize responses to questions related to genetic risks, which encouraged them to define the problem. To gauge the level of risk perceived by managers for this issue, we used both indirect and direct lines of questioning and contextualized these results with the greatest concerns managers held for the future of wild shellfish resources. To characterize the degree of resource limitation, we asked managers what tasks they would delegate to a hypothetical new staff member and searched for responses related to resource limitation in rationales for other responses. Lastly, we asked managers to what degree the relationship between the agencies and the aquaculture industry is regulatory or advisory in nature. Throughout, we asked for the rationale behind stated priorities, concerns, and other responses to illuminate manager values and beliefs. For the third objective, we synthesized our results to characterize the level of policy attention and contextualized the level of policy attention with rationales provided by managers and paradigm shifts in the scientific literature.

We included multiple considerations in our methodology to circumvent social desirability bias, in which participants answer questions in a way that is expected to be viewed favorably, while gathering information about relative concern over genetic risks of native shellfish aquaculture. To accomplish this task we 1) maximized neutral language in the interview tool (Nederhof, 1985), 2) used triangulation by asking questions in multiple ways (Krefting, 1991), and 3) ordered questions from broad to specific, such that the direct, closed-ended question that was more likely to lead to bias in subsequent responses (Fisher, 1993) was asked last.

Closed questions were analyzed by tallying frequencies of possible answers. For open questions, we transcribed interviews and developed a coding methodology based on values coding (Miles et al., 2014) and thematic analysis (Ayres, 2008) for data condensation in ATLAS.ti v8.4.4 (Hwang, 2008). We then aggregated codes into themes and described the frequency and relationships among codes and themes within the context of our research objectives (Huberman & Miles, 2002; Miles et al., 2014). We reported the occurrence of codes and themes by number of managers and number of agencies represented by interviewed managers where appropriate. A single coder (the first author) coded all interviews.

3.3 RESULTS AND DISCUSSION

Although all 18 interviewed managers held positions with responsibilities in shellfish resource and/or shellfish aquaculture management, the professional responsibilities for interviewed managers varied (Table 3-1). Managers from most surveyed agencies (number of responding agencies (*a*) = 11 of 14) reported native shellfish aquaculture within their jurisdictions.

3.3.1 *If and how are genetic risks currently managed?*

Half of the agencies represented in interviews (*a* = 7) responded that they managed shellfish resources for genetic risks in some capacity (Figure 3-2A). As one interviewee noted:

We do manage genetic risks in the fact that that's one of our primary concerns when we get proposals for [growing] native species. Genetics is always a big question.

The most reported methods for managing genetic risks included 1) encouraging use of local broodstock in hatcheries (*a* = 4), 2) preventing aquaculture of native shellfish species (*a* = 3) either completely or for species that are not yet cultivated within their jurisdiction, and 3) encouraging use of many broodstock individuals (*a* = 2). Reported methods centered on broodstock choice (i.e., number and source of broodstock) and did not include considerations related to breeding design, captive environment conditions, or growing practices on farms.

By comparison, the genetic risks of hatchery-reared finfish, especially salmon, have resulted in more complex and formal policy at multiple levels. At a national level, the United States Congress tasked a team of scientists to comprehensively review Pacific salmon hatcheries in the Columbia River Basin and Puget Sound region (Mobrand et al., 2005), initiating an era of broad hatchery reform (California Hatchery Scientific Review Group, 2012; USFWS, 2013). This review resulted in recommended operational procedures, of which many aimed to reduce genetic risks (Mobrand et al., 2005). Moreover, hatcheries enhancing populations protected under the Endangered Species Act of 1973 (ESA) that use wild broodstock or producing animals that are likely to interact with species protected under the ESA are required to comply with the ESA. This entails development of a Hatchery and Genetic Management Plan (NOAA Fisheries, 2019), in which NOAA Fisheries works with program operators to identify growing practices that mitigate all three types of genetic risks (Table 3-2). Once permitted, hatcheries are required to submit

annual reports so that agencies can ensure compliance (NOAA Fisheries, 2012, 2021a). Many state and tribal natural resource agencies have implemented statutes, regulations, and best management practices to address genetic risks of aquaculture to wild populations in finfish (Davis et al., 1989; *NWIFC: Genetics*, 2008; WDFW, 2020b). More complex and formal policy in the management of native shellfish aquaculture should be considered, particularly without empirical data suggesting genetic risks will be less severe in shellfish than in Pacific salmon.

3.3.2 *What factors may affect policy change regarding genetic risks of native shellfish aquaculture?*

We identified potential *a priori* factors that may bound rational decision making, leading to incremental policy change (Miner, 2006). Specifically, we analyzed manager responses regarding problem conceptualization, level of risk perception, degree of resource limitation, and the relationship with a primary interest group, the aquaculture industry.

How do managers conceptualize genetic risks?

Policy change can be slow when policy problems are hard to conceptualize (Jones, 2003). Genetic concepts, when compared to other biological concepts, pose greater cognitive challenges to both students and teachers, likely due to genetic processes occurring on scales not accessible to the senses, the complex and extensive vocabulary, and central use of mathematics in defining concepts (Bahar et al., 1999). To understand to what degree genetic risks as a policy problem poses cognitive challenges, we investigated 1) whether descriptions of genetic risks were consistent with best available science and 2) the completeness of managers' perceptions of the policy problem.

Most managers demonstrated understanding consistent with best available science regarding genetic concepts, although a couple of commonly shared ideas were at odds with our scientific understanding. Firstly, some managers did not fully appreciate that many shellfish

production practices beyond broodstock choice have genetic consequences. Secondly, some managers suggested that escape can be beneficial to wild populations. It is unsurprising that the conditions under which hatchery production can provide demographic and genetic risks or benefits is unclear, as there is a history of controversy and disagreement among experts (Brannon et al., 2004; R. S. Waples, 1999). Although supplementation can provide a demographic boost (Janowitz-Koch et al., 2019), it can still be accompanied by a loss of genetic diversity (Christie et al., 2012). Hatchery production is expected to reduce genetic diversity in large wild marine populations under most circumstances (Hedgecock & Coykendall, 2007; R. S. Waples et al., 2016). However, the application of these guiding principles is made challenging by the lack of empirical data on central genetic parameters (e.g., the effective population size to census size ratio) in wild shellfish populations and generally few studies tracking genetic effects of aquaculture releases on wild shellfish populations (Hornick & Plough, 2019; Morvezen et al., 2016).

Biodiversity conservation and specifically genetic biodiversity are complex topics, and this can lead to reduced policy attention (Haig et al., 2016; R. Taylor et al., 2017; Sandström et al., 2019). This was noted by one manager:

It's more at an academic level, something we talk about at the beer pub, but it's not something that I'm getting calls from mayors or chambers of commerce about.

Biodiversity can be measured using many units of measurement and at multiple scales, from the visible (ecosystems and species) to the invisible (genetic diversity), complicating its operationalization and policy development to mitigate its loss (Lidskog, 2014). Furthermore, the effects of biodiversity loss are rarely observed by individuals, especially for genetic processes that are subcellular and cannot be observed without advanced technologies (Zaccai and Adams 2012). Perhaps as a result, efforts to conserve biodiversity rarely go as far as conserving genetic diversity

(with the exception of key agricultural and forest interest species, gene banks for imperiled species, and some hatchery programs) despite the role of genetic diversity in species persistence that can then contribute species richness and ecosystem diversity (Laikre, 2010).

Notably, of the three categories of genetic risks identified in Table 3-2, loss of genetic diversity among populations (number of managers (*m*) = 11) and within populations (*m* = 9) were discussed more frequently than loss of fitness associated with domestication selection (*m* = 2). We observed the same pattern in asking for manager recommendations for future policy development (Table 3-3). This pattern may suggest that domestication selection is not perceived as a significant component of genetic risks of aquaculture to wild shellfish populations, and is surprising given the prominence of concerns over domestication selection in the literature on genetic risks of hatchery production in salmon (Baskett & Waples, 2013; Castellani et al., 2018; Ford, 2002). The reduced attention to domestication selection in shellfish aquaculture may be due in part to the fact that broodstock movement is already regulated in many jurisdictions using permits for the mitigation of another risk, disease (*m* = 6, *a* = 4). The availability of a relevant policy instrument focused on limited mixing of distinct groups may have led to greater focus on this risk, as policy instruments may influence problem conceptualization (Simons & Voß, 2018).

How do managers perceive genetic risks?

Risk perception can affect administrator decision making (Jones, 2003). To contextualize how genetic risks are perceived among other risks, we first asked managers to report their greatest concerns for the future of wild shellfish resources and noted whether genetic risks were listed among these concerns. Managers then were asked directly to discuss their level of concern over genetic risks compared to previously named concerns.

Few managers (m = 4, a = 2) reported genetic risks as one of their greatest concerns for the future of wild shellfish resources, suggesting relatively low risk perception among managers. For comparison, most managers reported being most concerned about long-term sustainability of managed resources (m = 15, a =12), with the remaining named concerns being environmental threats to such sustainability, such as ocean change and pollution (Figure 3-3). Notably, named concerns were not mutually exclusive, and mitigating genetic risks is a means towards resilience to environmental threats named by managers.

Mirroring results from indirect questioning, most managers reported feeling “a little concerned” over genetic risks in response to direct questioning (Figure 3-4). The direct question gauging level of concern over genetic risks was the question managers declined to answer most. Because social desirability bias can lead to nonresponse (Kreuter et al., 2008), fewer responses to this question suggest that social desirability most affected this question. A greater level of concern about genetic risks was associated with managers who reported aquaculture activities within their high priority tasks (Figures 3-4B.1 and 3-4B.2) and those who reported managing for genetic risks (Figures 4C.1 and 4C.2). These patterns point to correlation between degree of agency proximity to the aquaculture industry (e.g., advising, regulation, or participation) and managers’ perception of genetic risks of native shellfish aquaculture. Although causal relationships cannot be inferred from these data, this correlation may provide evidence for either manager risk perception driving informal policy development for genetic risks or risk perception driven by organizational practices and norms. The most reported factors driving high concern were fear of local population displacement and expectation that the problem will worsen. The most reported factor driving low concern was lack of information on genetic risks (Table 3-4).

What is the nature of resource limitation?

Resource limitation can drive path dependency, in which decisions are dependent on previous decisions, bounding rational decision making and slowing policy change (Kirk et al., 2007). Resource limitation was a common theme among manager responses. Managers reported that their budgets did not increase alongside their mandated responsibilities or that new issues could not be addressed due to limited budgets ($m = 7$, $a = 7$), for example:

The scale of our capacity is not scaled appropriately to the size of ... the seriousness of the issue and what it takes to manage it.

When asked what tasks would be given to a hypothetical new staff member, more than half of managers ($m = 11$, $a = 8$) named current tasks as opposed to novel tasks, suggesting that resources were not sufficient for current goals.

Resource limitation comes as no surprise, as the last couple of decades have brought financial hardships. For example, the Great Recession of 2008 created budget deficits for many agencies with long lasting impacts (O'Sullivan, 2020), including for state wildlife agencies (Frankovich, 2019). Moreover, state wildlife management is in a period of heightened reform and budget crisis, defined in part by increasing threats to wild resources and reduced hunting- and fishing-dependent revenue (e.g., license sales) (Jacobson & Decker, 2006). The economic crisis associated with the Covid-19 pandemic will likely deepen lingering budget deficits, which may further slow policy change for low priority issues.

What is the nature of the relationship between surveyed agencies and the aquaculture industry, beyond genetic risks?

Periods of incremental policy change are expected particularly in cases with resource limitation and limited information for decision making (Kirk et al., 2007; Simon, 2013), as were reported by managers in this study (resource limitation, $m = 7$, $a = 7$; limited information on genetic

risks, $m = 6$, $a = 6$). In particular, policy instrument choice is often constrained by institutional factors such as implementation style and organizational setting (Jones, 2003; Linder & Peters, 2016; Miner, 2006). Thus, we asked managers if and how their agency advises or regulates the aquaculture industry (a primary interest group) beyond genetic risks, in order to illuminate possible policy instruments for genetic risks.

Almost all managers reported that their agency regulates or advises aquaculture in some capacity ($m = 17$, $a = 13$, Figure 3-2B), including through indirect input, such as commenting on federal permits and advising shellfish growers on growing practices (Table 3-5). A commonality among regulatory or advisory actions at most agencies was that compliance was voluntary except for a select few actions, such as registration of facilities and regulating broodstock import and transfer. In the absence of sudden policy attention to this issue, it is likely that policy development will resemble these policy actions: few formal regulations, such as regulating broodstock collection, and non-binding recommendations for production.

One factor that may slow policy development for mitigation of genetic risks is a lack of clarity around agency authority, given involvement of multiple agencies. When asked whether their agency has regulatory or advisory influence over industry growing practices, one manager responded:

Our agency ... doesn't do a lot of managing [aquaculture companies] at all... I don't know if anyone in our staff is running around inspecting anything, so, it just sort of could be, lack of clarity on which agency would do that.

Multiple agencies share regulatory authority over aspects of the aquaculture industry. For example, depending on the exact state, their departments of health, natural resources, wildlife, ecology, and agriculture all may hold some regulatory authority over aquaculture companies. This may lead to

a lack of role clarity around which agency has authority for a specific issue, slowing policy change in a manner parallel to a by-stander effect (Fischer et al., 2011).

Furthermore, natural resource agencies often have complicated relationships with powerful interest groups, including regulated industries, due to paradoxical and competing goals of preservation and use of natural resources (Cheever, 1996). This complexity may be further exacerbated by conflicts of interest, such as an agency deriving revenue from the profits of a regulated industry, as in the case with revenue from the wild geoduck fishery funding of Washington Department of Natural Resources and Washington Department of Fish and Wildlife activities (WDNR, 2008). A potential complexity for the relationship between natural resource agencies and shellfish aquaculture companies as it relates to genetic risks is the participation of agencies in hatchery production: almost half of the managers reported that their agency funds and/or operates a shellfish hatchery and/or farm ($m = 8$, $a = 7$), with purposes ranging from growing native species for restoration or commercial production to growing non-native species for beach enhancement or commercial production. Balancing mitigation of genetic risks among other goals may be complicated by the agency's institutional history of involvement in shellfish production.

3.3.3 *How much policy attention do genetic risks of native shellfish aquaculture receive and why?*

After synthesizing results across managers and agencies, genetic risks receive relatively little policy attention. Few managers ($m = 6$, $a = 3$) reported mitigating genetic risks as part of their responsibilities or highest priority tasks in their current position. Although managers from half of agencies reported managing for genetic risks in some capacity ($a = 7$), this task included small-scale and non-binding actions. This finding is mirrored by a relatively low level of concern across managers (Figure 3-4A) and low occurrence of genetic risks among the greatest concerns for the

future of wild shellfish resources (*m* = 4, *a* = 2). Only two managers included tasks related to managing genetic risks for the hypothetical new staff member. A third of managers specifically named managing genetic risks as low priority considering limited resources and higher priorities (*m* = 6, *a* = 6).

Low risk perception and little policy attention to this issue are unsurprising given some of the factors reported by managers and explored here: perceived lack of information, perceived lack of clarity on agency authority, and cognitive challenges posed by genetic concepts, to name a few. However, a match between risk perception and policy attention should not always be expected. Risk perception can be affected by the degree of power to control outcomes (Slovic, 1987), such as greater concern for risks that decision-makers have limited power to mitigate. Possible mismatch between risk perception and degree of power may explain why ocean change was among the most widely reported concerns for the future of wild shellfish resources and yet only one manager reported developing climate change policy among highest priority tasks. Furthermore, although agencies hold much discretion over policy implementation, legal mandates still constrain aspects of decision-making and agenda setting.

Multiple factors identified here as potentially driving low risk perception and little policy attention also apply to finfish systems. Why, then, has this issue received more policy attention in finfish than in shellfish? Low policy attention and limited regulation around genetic risks of aquaculture to wild shellfish populations may be associated with fewer conservation protections and resources directed towards invertebrate taxa generally. In 2012, roughly 94% of government funding for species protected under the ESA went to vertebrate species, despite the majority of listed species being invertebrates, plants, and lichens (Evans et al., 2016). Similarly, although invertebrates can be protected at the subspecies level, only vertebrate populations can be

designated as distinct population segments under the ESA (16 U.S.C. 1532(16)). Listing limitations for invertebrates suggest that vertebrates are prioritized over invertebrates for limited conservation funding.

Reduced attention to genetic risks of aquaculture to wild shellfish populations may also be due to traditional paradigms in the scientific literature, that have since shifted. Historically, many believed marine invertebrate populations could not develop population differentiation, let alone local adaptation, because of their immense population sizes, broadcast spawning behavior, and large larval dispersal distances (Sanford & Kelly, 2011). However, upon investigation and boosted by more sensitive technologies, population differentiation has been observed in marine invertebrate populations (Bible & Sanford, 2016; Sanford & Kelly, 2011; K. Silliman et al., 2018; Wendling & Wegner, 2015). Historically, many believed that the large census sizes must harbor immense levels of genetic diversity, and that reductions due to interbreeding with hatchery-produced animals would have a negligible effect. However, many species of shellfish have smaller effective population sizes (N_e) relative to the census size compared to finfish, with some more than an order of magnitude smaller (Hauser & Carvalho, 2008). This may be due in part to the high fecundity of shellfish, often on the scale of up to tens of millions of gametes per individual (Gjerde, 1986), and the vulnerability of generally smaller gametes to environmental conditions. Together, this could result in fewer parents than expected contributing to following generations, and result in less genetic diversity than expected given the census size (Hedgecock & Pudovkin, 2011).

Perhaps in part due to this paradigm shift, genetic risks have received increased attention and consideration for policy development. The impetus for this current study arose from an ad-hoc stakeholder meeting convened by Washington Department of Fish and Wildlife in 2016, in which multiple agencies, shellfish companies, and scientists called for genetic risks assessments to inform

whether policy development is necessary (Childers et al., 2016). The Puget Sound Federal Task Force Action Plan, FY2017-2021, identified the need for genetic risk assessment of shellfish aquaculture as a priority federal action (Puget Sound Federal Task Force, 2017). Genetic risks have even been cited as a reason for downgrading the sustainability label for Pacific Geoduck from "Green" to "Yellow" by the Monterey Bay Aquarium Seafood Watch program (Monterey Bay Aquarium, 2016).

3.4 Study limitations

Our study provides novel insights into the regulatory context of this emerging management issue. Nonetheless, our study has some limitations. First, we only surveyed natural resource managers at administrative agencies, and thus did not survey other potentially important policy actors including legislators. Second, we expect particular biases to have influenced our results despite our best attempts to eliminate them. We expect sampling bias because we surveyed managers from a subset of possible governments. Although we achieved a relatively high participation rate (nearly 60% of agencies), our results are likely more representative of participating managers and agencies than non-participating managers and agencies. We suspect participating agencies reported higher levels of concern than those that did not participate, which might be related to social desirability and willingness to participate. We expect social desirability bias skewed our results towards greater levels of concern for genetic risks. Our characterization of risk perception was performed with broad strokes; risk perception is a highly complex process (Slovic, 1987), for which there are many models for analysis (Jasanoff, 1998). Within the context of risk perception and political decision making, our framing of the study and interview questions may have introduced other biases, including status quo bias and loss aversion bias (Moshinsky &

Bar-Hillel, 2010). Lastly, although theories of PET and agenda setting have been widely applied to different governments including different scales of government (Liu et al., 2010; True et al., 1999), they were developed in the context of national public policy in the United States. Factors driving policy change and agenda setting may differ between nations, states, and tribes.

3.5 Conclusions

In this study, we provided the first characterization of the regulatory context surrounding genetic risks of native shellfish aquaculture on the Pacific Coast of the United States, including documenting the use of informal policy and making it visible to agency-outsiders. Notably, reported measures for managing genetic risks of native shellfish aquaculture did not include measures for mitigating domestication selection, such as requiring particular hatchery conditions or preventing use of selected hatchery stocks. This pattern was also reflected in the greater attention to loss of genetic diversity than to loss of fitness due to domestication selection in problem conceptualization and manager recommendations for mitigating genetic risks. Domestication selection may be a great risk in shellfish hatcheries due to the vulnerability of calcifying larvae to ocean acidification and the practice at some shellfish hatcheries of buffering sea water *pH* (J. C. Clements & Chopin, 2017). This practice may result in reduced resilience to ocean acidification at a critical life history stage. We hope that the identification of informal policy for genetic risks provided here can be used to inform potential policy development and can serve as an impetus to further investigate the potential for domestication selection in shellfish hatcheries.

3.6 TABLES AND FIGURES

Table 3-1: Summary of manager responsibilities. Most reported priority tasks (A) and rationale for priority tasks (B) in descending order of the number of managers (*m*), as well as species focus (C) in manager positions. Priority tasks and rationales for priority tasks were only included if they were reported by a minimum of five managers. The number of agencies represented by manager responses was also reported where applicable (*a*).

	m	*a*
A. Priority tasks		
1. Managing wild harvest	15	13
2. Aquaculture activities	8	6
B. Rationale for priority tasks		
1. Sustainability of wild resources	15	11
2. Producing economic value	15	11
3. Providing subsistence, cultural, and recreational uses	10	8
4. Exercising sovereignty	7	6
C. Species focus		
1. Shellfish only	11	7
2. Shellfish and finfish	7	7

Table 3-2: Examples of Hatchery and Genetic Management Plan requirements pertaining to all three types of genetic risks. The three genetic risks include loss of genetic diversity within populations, loss of population differentiation, and loss of fitness (Figure 3-1). The column Genetic Risk contains a specific genetic impact and the column HGMP Requirement contains requirements from the Hatchery and Genetic Management Template (NOAA Fisheries, 2019) that pertain to the genetic risk.

Genetic Risk	HGMP Requirement
Loss of genetic diversity within a population	Describe breeding design (Section 8.3)
	Describe measures used to minimize loss of genetic diversity within a population (Section 8.5)
Loss of population differentiation	Describe the origin of broodstock and their relationship to wild fish of the same species or population (Section 6.1)
	Describe measures used to minimize loss of genetic diversity among populations (Section 6.3)
Loss of fitness	Describe water source, including differences between 1) hatchery water and source water and 2) hatchery water and water used by a naturally spawning population (Section 4.1)
	For hatchery broodstock, describe any intentional or unintentional selection that changed characteristics of broodstock (Section 6.2)

Table 3-3: Manager recommendations for future policy development regarding genetic risks of native shellfish aquaculture. Recommendations (column Recommendation) listed in descending order of the number of managers (column *m*), and the number of agencies is also reported (column *a*). The associated genetic risk (column Risk) accompanies each recommendation. Recommendations included using local broodstock relative to outplant location (Local broodstock), requiring a minimum number of broodstock on spawns (Number of broodstock), monitoring population genetic parameters to inform broodstock collection (Monitoring population differentiation), shifting from informal to formal policy (Formal regulation), and reducing escape (Reducing escape).

Recommendation	*m*	*a*	Risk
1. Local broodstock	6	4	Loss of genetic diversity among populations
2. Number of broodstock	3	3	Loss of genetic diversity within populations
3. Monitoring population differentiation	3	2	Loss of genetic diversity among populations
4. Formal regulation	2	2	All genetic risks
5. Reducing escape	1	1	All genetic risks

Table 3-4: The reported factors driving concern and lack of concern for genetic risks of native shellfish aquaculture. Factors listed in descending order of the number of agencies represented by manager responses (column *a*). The number of manager responses per method is also reported (column *m*).

	Factor	*m*	*a*
Driving concern	Local population displacement	3	3
	Problem expected to worsen	3	3
	Uncertainty driving precaution	1	1
Driving lack of concern	Lack of information	6	6
	Low priority	3	3
	Hatcheries already following best practices	3	3
	Genetic risks secondary to demographic boost	3	3

Table 3-5: Reported methods of regulating or advising aquaculture. Methods (column Method) listed in descending order of the number of agencies represented by manager responses (column *a*). The number of manager responses per method is also reported (column *m*).

Method	*m*	*a*
1. Providing indirect input such as commenting on federal permits	8	8
2. Advising shellfish growers on growing practices	5	5
3. Tracking and controlling import and transport of broodstock	6	4
4. Limiting which species can be cultivated	4	4
5. Managing for disease and pest control	5	3
6. Requiring registration of facilities	4	3
7. Outreach and education, including forming stakeholder committees to develop best practices	3	3

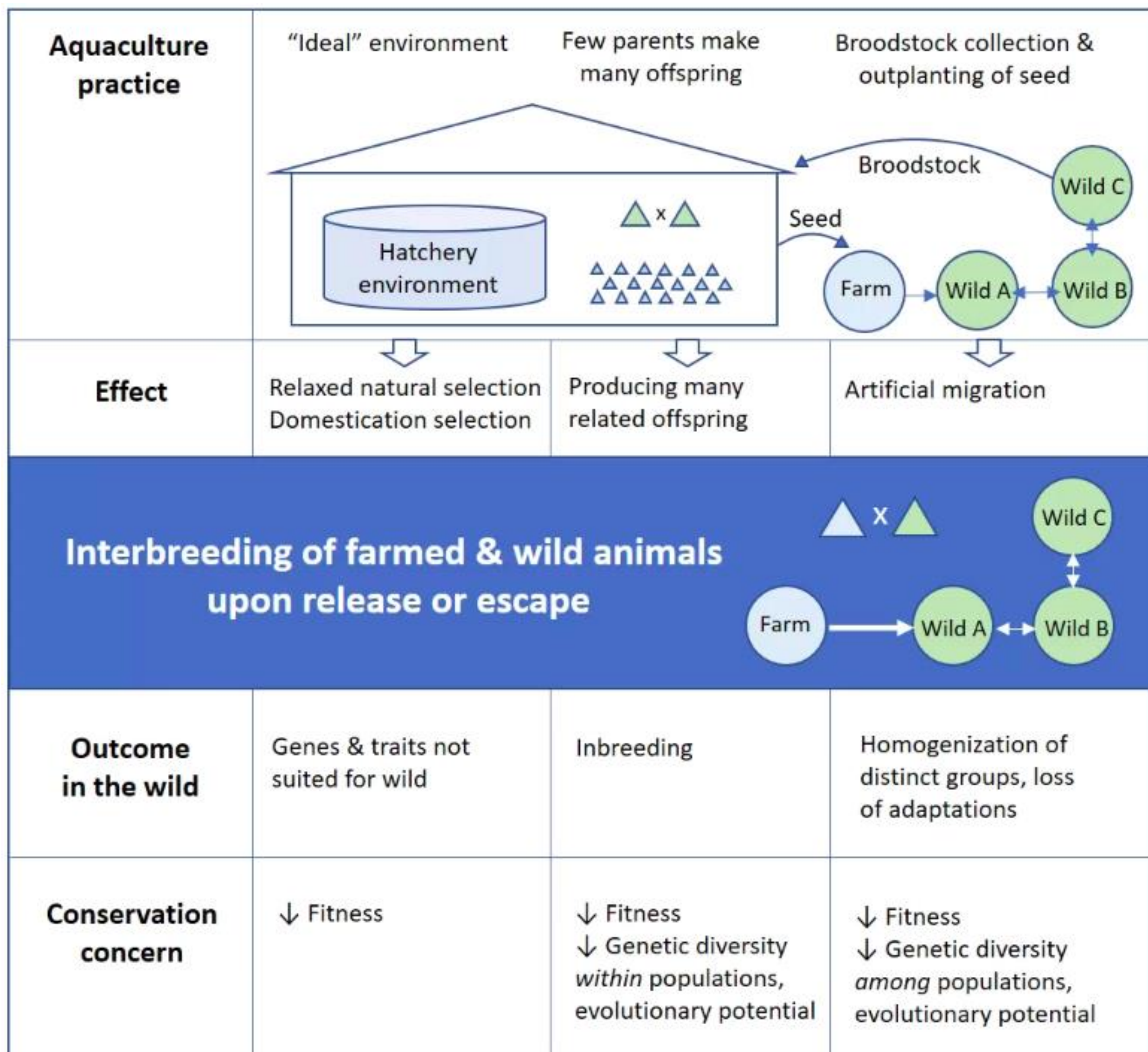


Figure 3-1: Conceptual diagram of the types of genetic risks and their relevance to conservation and sustainable resource management.

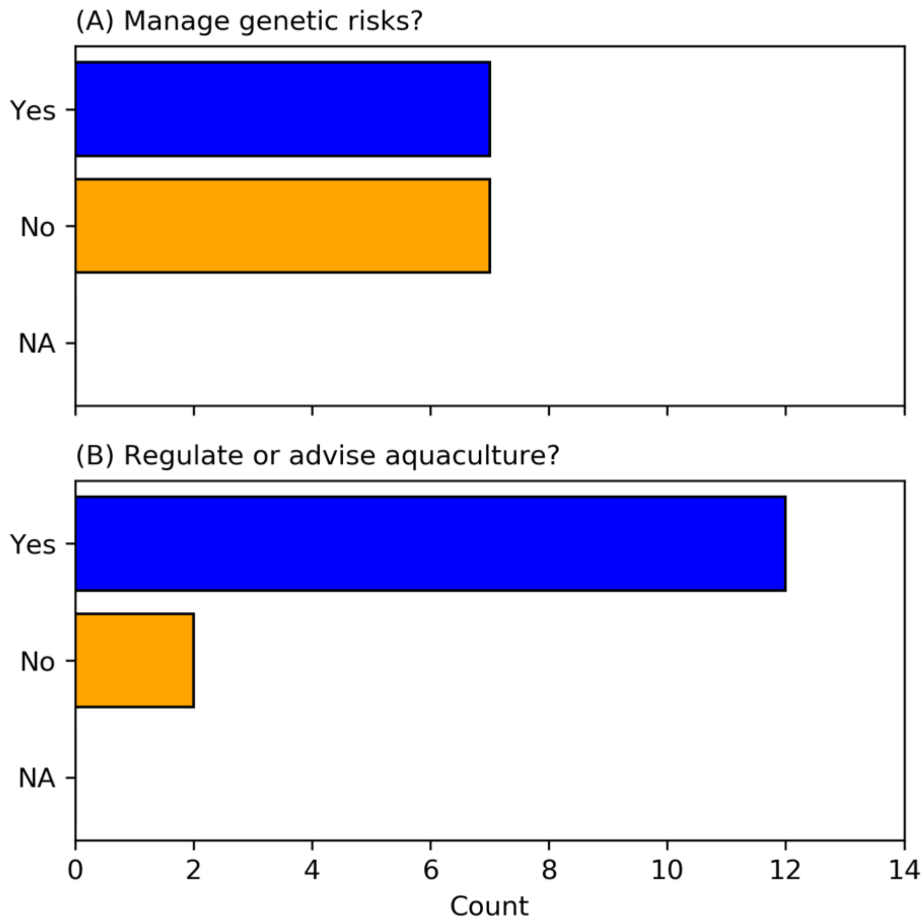


Figure 3-2: Manager responses to whether the manager's agency (A) manages for genetic risks of native shellfish aquaculture in any way and (B) regulates or advises aquaculture in any way.

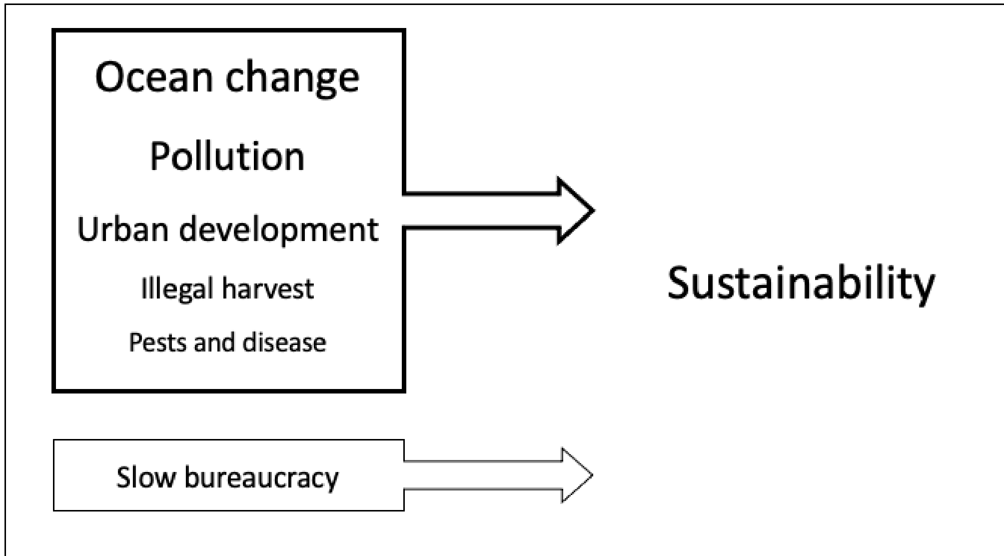


Figure 3-3: Graphical representation of the frequency and relationship of reported concerns for the future of wild shellfish resources using direct and indirect questioning. Relative frequencies of concerns were similar when looking at responses to direct questioning versus both direct and indirect questioning. Font size represents the relative frequency with which each concern was named: sustainability of wild resources, ocean change (m=14, a=12), pollution (m=11, a=10), urban development (m=9, a=7), illegal harvest (m=6, a=5), pests and disease (m=2, a=2), and slow bureaucracy (m=4, a=4). The boxes with arrows were chosen to represent different types of concerns that ultimately relate to sustainability of wild resources. The box with a thick outline and containing multiple concerns represents external impacts that threaten the sustainability of wild resources. The box containing a single concern, slow bureaucracy, represents the internal factors slowing policy change for sustainability. Concerns included in the figure were named by at least two managers and by managers representing at least two agencies.

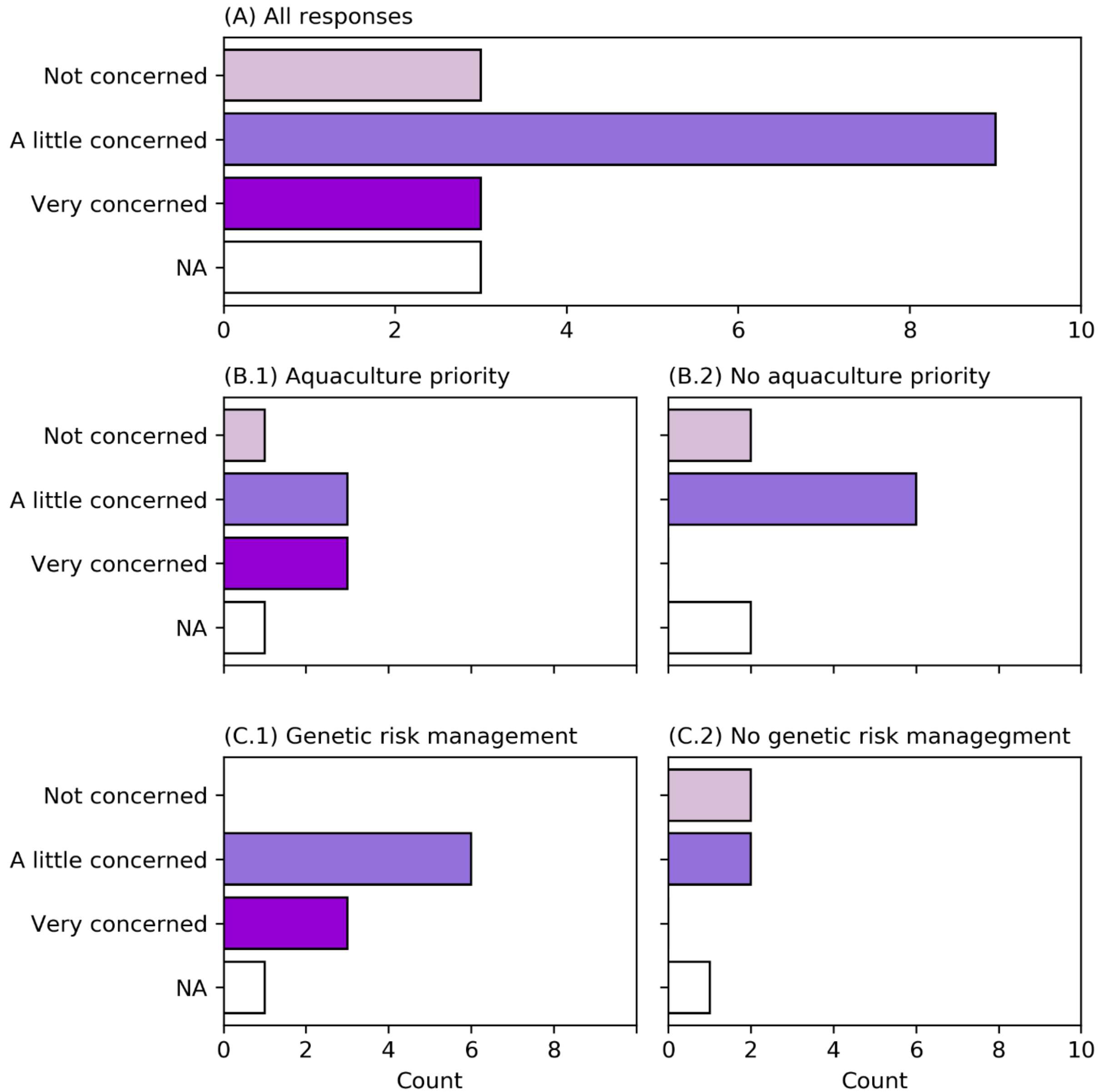


Figure 3-4: Histograms representing level of concern regarding genetic risks of native shellfish aquaculture, reported directly by managers. The first row (A) includes all responses for all managers. The second row divides responses from all managers into two groups: those that reported aquaculture activities in their priority work (B.1) and those that did not (B.2). The third row divides responses for all managers into two groups: those that reported their agency did manage genetic risks of native shellfish aquaculture (C.1) and those that did not. Greater levels of concern are associated with managers that reported aquaculture activities in high priority tasks and from agencies that manage genetic risks of native shellfish aquaculture.

Chapter 4. Genetic risk assessment model for native shellfish aquaculture

4.1 INTRODUCTION

Aquaculture is rapidly developing worldwide because the global demand for marine protein can no longer be satisfied by capture fisheries alone (Merino et al., 2012). The U.S. marine aquaculture industry is growing at an average of 8% per year (NOAA Fisheries Office of Aquaculture, 2016), with shellfish aquaculture valued at over $300 million annually (FAO, 2017; NOAA Fisheries Office of Aquaculture, 2016). Although farmed shellfish is among the more sustainable animal proteins (Hilborn et al., 2018), shellfish aquaculture is not without environmental costs, including spreading disease (Elston, 1990) and producing marine debris (Lusher et al., 2017). Additionally, aquaculture of native species poses genetic risks to wild populations when farmed and wild individuals interbreed. Understanding the genetic risks of native shellfish aquaculture has become increasingly salient as shellfish growers have begun to cultivate native species, in part to avoid introduction of non-native species.

Genetic risks of aquaculture are well studied in finfish systems, primarily salmonids, in which a robust body of literature shows that hatchery production of fish can reduce genetic diversity and fitness in wild populations (reviewed in Naish et al., 2007). Comparatively, only sparse research exists on the genetic risks of native shellfish aquaculture to wild populations (Camara & Vadopalas, 2009; Morvezen et al., 2016; Nascimento-Schulze et al., 2021). Traditionally, it was assumed that marine populations generally had immense population sizes with little to no population structure (Hauser & Carvalho, 2008), characteristics that would make marine species nearly immune to the genetic impacts of relatively small-scale shellfish farming. However, more recent studies suggest that shellfish populations can exhibit population structure

(Sanford & Kelly, 2011) and N_e can be smaller than expected based on census size (Hauser & Carvalho, 2008), likely due to high fecundity and high family-specific mortality at early life history stages (Hedgecock, 1994).

Existing models that investigate genetic risks to wild finfish populations have provided valuable insights for mitigating genetic risks of aquaculture. For example, Ford (2002) developed a model and demonstrated that reductions in fitness can occur in wild salmon population receiving escapees over short time-scales (within 20 generations) and using wild broodstock does not prevent domestication selection altogether. (Baskett & Waples, 2013) developed a model that demonstrated how highly domesticated broodstock can lead to fewer reductions in fitness in the wild salmon populations than wild broodstock, if strong purifying selection acts between release and reproduction. Volk et al. (2015) applied the Offshore Mariculture Escapees Genetic Assessment model to show that fitness reductions can lead to demographic impacts in Sablefish.

However, existing models that investigate genetic risks to wild populations make assumptions about life history and aquaculture production that are more applicable to finfish than shellfish species. For example, models quantifying genetic risks in salmonids often assume semelparity and short generation times (Baskett & Waples, 2013; Ford, 2002). Yet, many shellfish species are long-lived and iteroparous, such as the cultivated Pacific geoduck with a life span of over 100 years (Shaul & Goodwin, 1982). These characteristics of shellfish allow for potentially greater opportunity for interbreeding of farmed and wild animals and greater variation in reproductive success (R. S. Waples et al., 2011), both of which shape outcomes for genetic risks of aquaculture to wild populations.

Available models are also limited in their suitability for genetic risk assessment of native shellfish aquaculture in that they focus on a single risk, such as loss of fitness (Baskett & Waples,

2013; Castellani et al., 2015; Ford, 2002; Volk et al., 2015) or loss of genetic diversity within populations (Darden et al., 2017). Yet, hatchery production results in many genetic impacts to offspring, and through escape or release, can causes genetic impacts in wild populations if farmed and wild animals interbreed. These genetic impacts can be broadly categorized into three genetic risks (R. S. Waples et al., 2012). The first genetic risk is the loss of genetic diversity within wild populations, resulting from high reproductive success of hatchery individuals compared to their wild counterparts. Given that many marine shellfish species have high fecundity, few broodstock individuals can produce many offspring in a hatchery setting, and thus, escapees from relatively few families can make up a large proportion of wild recruitment (Ryman & Laikre, 1991). The second genetic risk is the loss of genetic diversity among populations, caused by artificially increasing gene flow between isolated populations, for example, through movement of breeding individuals (broodstock) and hatchery-produced offspring (seed) across biological populations. Population diversity contributes to population viability because it diversifies the response to environmental variability; this is known as the portfolio effect (Schindler et al., 2010). The third genetic risk is loss of fitness, which can occur due to multiple factors. One process that can lead to loss of fitness is the relaxation of wild selection pressures (e.g., food scarcity) and introduction of new selection pressures (e.g., selection for growth rate), resulting in domestication selection and production of individuals with traits that may not be well suited to the natural environment. When hatchery individuals escape and interbreed with wild individuals, these maladapted traits may reduce the fitness of wild populations (Skaala et al., 2019). Although some level of domestication selection is inevitable in hatchery production (Currens & Busack, 1995), it can be mitigated through equalizing family sizes (Allendorf, 1993) and avoiding use of domesticated broodstock lines (Ford, 2002). Another process leading to loss of fitness is inbreeding depression, when

recessive deleterious alleles are exposed to selection (Plough & Hedgecock, 2011). Inbreeding depression becomes a greater risk in small populations, such as hatchery broodstock, and can be mitigated through breeding designs that maintain genetic diversity (Fiumera et al., 2004). Finally, fitness can also be lost if the scale of movement of broodstock and seed across locally adapted populations is sufficient to erode local adaptations (Allendorf et al., 2001). Managers may be more focused on preserving fitness than neutral genetic diversity, as fitness may affect demographic changes in the short-term more than neutral genetic diversity (Ryman, 1991). However, genetic diversity is correlated to fitness outcomes (Reed & Frankham, 2003), and even neutral differentiation may become adaptive under changing environmental conditions (Barrett & Schluter, 2008). Thus, in addition to maintaining fitness of wild populations, preserving both neutral and adaptive genetic diversity is central to conservation practitioners (Kardos et al., 2021).

A simulation model quantifying all genetic risks is better suited to capturing the overall effect of aquaculture than models focused on fewer risks because genetic risks interact. For example, using few broodstock may increase the risk of loss of genetic diversity within a population, but decrease the risk of loss of fitness due to domestication selection, as drift can overpower selection in small populations (Li, 1978). Movement of broodstock and seed across isolated populations may cause loss of genetic diversity among populations as well as loss of fitness if genetic differentiation is adaptive (R. S. Waples et al., 2012) but may increase diversity within populations (Lind et al., 2007). Lastly, growing practices can lead to a reduction in fitness through multiple processes, such as inbreeding, domestication selection, and loss of local adaptations (R. S. Waples et al., 2012).

Rapid shellfish aquaculture development has produced a pressing need to quantify genetic risks so that best management practices can create a balance between a thriving coastal economy

and the protection of wild marine natural resources. To quantify these risks, we built an open-source, individual-based model using the *simuPOP* module (Peng & Kimmel, 2005) in Python (Van & Drake, 2009) that captured all three genetic risks to wild populations and reflects shellfish life history and aquaculture production. Individual-based models are particularly well suited for investigating genetic questions because they allow for individual variability and transmission of attributes across generations (Castellani et al., 2013). Our model simulated shellfish production and escape, and quantified changes in genetic diversity within populations, genetic diversity among populations, and population-level fitness. We defined a simple fitness landscape that allows for simulation of both local adaptation and domestication selection, and included dominance effects among alleles to simulate inbreeding depression. We demonstrated the model's utility by using it in a management strategy evaluation for Olympia Oyster, *Ostrea lurida*, in which we quantified each of the three genetic risks, trade-offs, and emergent effects. We selected Olympia Oyster because it is a threatened species cultivated along the Northwest Pacific Coast for both species restoration and commercial production. The model was designed for application to species cultivated in the Northeast Pacific, where the largest shellfish aquaculture sector in the United States resides (Washington Sea Grant, 2015), but can be applied to other contexts. Our results provide an important contribution to the limited literature on genetic risks of native shellfish aquaculture (Hornick & Plough, 2019; Morvezen et al., 2016) and practical management recommendations for a species of conservation concern.

4.2 METHODS

4.2.1 *Model description*

We built a novel individual-based model, with spatial structure, to explore the potential effects of aquaculture production practices and escape on neighboring wild populations. In

addition to using values from previously published studies, we gathered some parameter values from a survey of 23 native shellfish growers in 2017-2018. A complete list of default parameter values is provided in Supplemental Table 4-1. We adapted the Grimm et al. (2006) protocol to describe the model here, including sections for model description, state variables and scales, process overview and scheduling, initialization, input, and submodels. Simulation was done in Python v.3.7.3 using the module *simuPOP* v.1.1.10.9 (Peng & Kimmel, 2005).

The purpose of the model was to quantify genetic impacts to wild populations due to interbreeding with escapees from native shellfish aquaculture, for different shellfish production scenarios. The genetic impacts measured by the model included 1) loss of genetic diversity within populations, 2) loss of genetic diversity among populations, and 3) loss of fitness. We assumed three wild subpopulations (Wild 1, Wild 2, and Wild 3) and one farm subpopulation (Farm), individuals with 35 loci, and a fitness landscape capturing the effects of local adaptation and domestication selection in order to measure all genetic risks.

We measured genetic diversity within populations using two metrics: heterozygosity and allelic richness. For these metrics, we simulated a set of 10 multiallelic neutral loci initialized with entirely unique alleles per locus as in an identity-by-descent framework (J. Wang et al., 2016). Using this framework, heterozygosity (the observed proportion of heterozygous genotypes) began at 1 and allelic richness (the number of unique alleles per locus) began at 2N (where 2 represents the two alleles of each diploid individual and N = census size), and both decreased over time due to genetic drift and no mutation. After some reductions in allelic richness occurred within subpopulations, heterozygosity and allelic richness could increase in a subpopulation due to introduction of foreign alleles through migration. The identity-by-descent framework was chosen to increase sensitivity to genetic drift and to estimate N_e in future applications of the model.

Among-population genetic diversity was measured using Weir-Cockerham F_{ST} (Weir & Cockerham, 1984) at a separate set of ten biallelic neutral loci. We added this set of loci because the identity-by-descent loci inherently started at $F_{ST} = 0$, far from migration-drift equilibrium F_{ST}, making it more challenging to measure changes in F_{ST}.

Fitness was measured using mean fitness, defined here as the average individual fitness value per subpopulation. An individual's fitness value (f_i) was defined by the mean fitness across five adaptive loci:

$$f_i = \frac{\sum_{n=1}^{5} A_n}{5} \tag{4.1}$$

in which A_n represented the fitness value of the genotype at the nth (of 5) adaptive locus, which was affected by the individual's environment (e.g., Farm, Wild 1, Wild 2, or Wild 3; Table 4-1). Different sets of five adaptive loci contributed to fitness for each life history stage (larva, juvenile, adult) to reflect expected differences in particular loci affecting distinct traits across life history stages (Castellani et al., 2015). Thus, 15 adaptive loci were simulated in total and the 10 adaptive loci that did not affect fitness in a particular life history stage behaved as neutral loci during that stage. Allele fitness effects were defined using two selection coefficients, s (default $s = 0.05$) and z (default $z = 0.5$), where $s << z$, to produce both local adaptation and domestication selection dynamics (Table 4-1). Specifically, domestication selection dynamics were created by assigning opposite advantageous alleles in the wild and farm environments and by using z to define the fitness effects of disadvantageous alleles in the farm environment. Local adaptation dynamics were created by varying fitness effects for the disadvantageous allele in the wild subpopulations by a factor of s. Because inbreeding depression often occurs in development (Ehiobu et al., 1989; Hemmings et al., 2012; Taris et al., 2007; Tiira et al., 2006), simulated alleles incurred dominance

effects (the advantageous allele had complete dominance) in the larval stage and additive effects in the juvenile and adult stages. Thus, mean fitness captured the effects of local adaptation, domestication selection, and inbreeding depression. Pairwise F_{ST} at adaptive loci were also estimated, although this response variable reflected a combination of demographic and selective forces on genetic diversity among subpopulations.

4.2.2 *State variables and scales*

Our model comprised four hierarchical levels (Figure 4-1A), with unique state variables at each level. Ordered from lowest to highest, levels included individual, subpopulation, population, and the abiotic environment. State variables that we included for individuals were identity, age, sex, genotypes, fitness, mother identifier, father identifier, and a broodstock indicator denoting whether an individual was broodstock. Some of these variables were static (identity, sex, genotypes, mother identifier, father identifier), while others changed over the course of an individual's life (age, fitness, broodstock indicator). Hatchery-produced individuals and their descendants had an additional state variable, filial generation number, denoting the generation number since wild broodstock were used to produce seed.

We developed our model to address the complicated and diverse life histories of marine invertebrates, including bivalves, crustaceans, and echinoderms (McHugh & Rouse, 1998). To simplify, we divided simulated shellfish life histories into three phases: 1) fertilized eggs and larvae, which have not reached age at settlement, 2) juveniles, between settlement and first maturity, and 3) adults past the age at first maturity. These phases broadly reflect the life history of many shellfish species cultivated or considered for cultivation, including 1) Olympia Oyster, *Ostrea lurida*, 2) Pacific Geoduck, *Panopea generosa*, 3) Purple-hinged Rock Scallop, *Crassadoma gigantea*, 4) Blue Mussel, *Mytilus trossolus*, and 5) California Sea Cucumber,

Apostichopus californicus. In general, most marine invertebrate eggs and larvae are planktonic and disperse for several weeks, then settle out of the water column and metamorphose into sessile juveniles, then mature into sessile reproducing adults. There are some exceptions, for example, the motile juvenile and adult stages of California Sea Cucumber (Cameron & Fankboner, 1989) and the brooded larvae of Olympia Oyster (Baker, 1995). Our model is sufficiently flexible to accommodate stage-specific migration and escape rates for a particular species' life history.

Subpopulations in our modeling framework constituted groups of individuals that reproduced with each other and were characterized by two state variables, 1) the number and 2) list of individuals present. Subpopulations included a farm (Farm) with individuals produced in a hatchery, and three wild subpopulations (Wild 1, Wild 2, and Wild 3). The population comprised all subpopulations (including the Farm when present), which were connected through migration, broodstock collection, and escape.

The highest hierarchical level was the abiotic environment. The abiotic environment was characterized by the state variable climate effect (c_x), which affected the scale of recruitment across the entire population and represented bottom-up forcing on population dynamics, which have been observed in multiple marine invertebrate species (Menge et al., 2009, 2011; Ortega et al., 2012). The climate effect was an auto-correlated random walk

$$C_x = 0.9C_{x-1} + Y \ , \tag{4.2}$$

in which the climate effect in year x (C_x) was a function of an AR(1) autoregressive parameter (0.9), the climate effect in the previous year (C_{x-1}), and a normally distributed random variable with zero mean and unit variance (Y). The autoregressive parameter controlled the degree of mean-reversion, such that simulated values fluctuated around a common mean with a high degree of autocorrelation. Without the autoregressive parameter, the time series of C_x behaved as a random

walk. Heterogeneity in the abiotic environment was characterized by state variables for the relative fitness effects of alleles at adaptive loci by subpopulation (Table 4-1), such that subpopulations were distributed across unique local environments arranged in a gradient, and local environments were homogenous.

We divided the temporal structure of the model into three consecutive phases: pre-farm, during-farm, and post-farm, to be able to evaluate the effect of farmed escapees on wild subpopulations (Underwood, 1992). Each phase of the model was constructed to last fifty years; fifty years was chosen as a duration that represented a realistic duration of a shellfish farm, with sufficient time for genetic impacts to accrue and for wild subpopulations to begin to return to pre-disturbance conditions after farm removal. In addition to the changes across years, we built in seasonal variation with monthly time steps, allowing processes such as reproduction, seed production, migration, and escape to be parameterized to a particular species' seasonal timing. The spatial structure of the model was taken into account by the migration rates that connected subpopulations in a stepping-stone model (Figure 4-1A), in which migration was limited to neighboring subpopulations.

4.2.3 *Process overview and scheduling*

The model proceeded in monthly time steps, in which some processes occurred every month and others occurred in only some months with specified seasonality. We modeled the pre- and post-farm phases (Figure 4-1B) as identical regarding the processes and order of processes: migration, aging, natural mortality, and wild recruitment. We included three additional processes in the during-farm phase (Figure 4-1C): migration, aging, natural mortality, harvest, wild recruitment, seed production, and escape (in order).

During all phases of the model, migration, aging, and natural mortality occurred in each month (in order). During the pre- and post-farm phases, an opportunity for wild recruitment occurred after mortality, as defined by input parameters for monthly probabilities of wild recruitment (o_i for probability of wild recruitment in month i, default values in Supplemental Table 4-1). The Farm was created through collection of wild broodstock and production of seed at the beginning of the first year of the during-farm phase of the model, prior to migration, aging, and natural mortality. In each year of the during-farm phase, harvest occurred (Supplemental Figure 4-1) after natural mortality, according to a harvest control rule. After potential for harvest, wild recruitment, seed production, and escape were allowed to occur, as defined by input parameters for monthly probabilities for these processes (o_i, y_i, v_i, w_i for probabilities of wild recruitment, seed production, larval and gamete escape, and juvenile and adult escape in month i, respectively; default values in in Supplemental Table 4-1). The Farm was removed at the end of the during-farm phase.

For simplicity, response variables were reported once a year despite monthly time steps. Response variables were retrieved in December of each simulated year for simulation output. Response variables measured internally within *simuPOP* (allelic richness, heterozygosity, and pairwise F_{ST}) were measured immediately after reproduction and were not retrieved for output until December. Mean fitness was measured using a custom function and retrieved for simulation output in December of each year. Additionally, the model output subpopulation size and, optionally, 1) allele frequencies at biallelic loci per subpopulation, per year and 2) individual information (age, sex, identification number, parent identification numbers) per subpopulation, per month.

4.2.4 *Initialization*

Each wild subpopulation was initialized with 3,000 individuals (equal sex probabilities for each animal). Because stable age distributions were skewed, we initialized the subpopulations with a right-skewed age distribution. Specifically, the probability of initializing a particular age for an individual (P_a) was defined by

$$P_a = \frac{e^{-0.1a}}{\sum_{n=1}^{12u_m} e^{-0.1\left(\frac{n}{12}\right)}}, \tag{4.3}$$

in which e represented Euler's number, a represented the age in years, and u_m represented the maximum age in years (default $u_m = 10$). Advantageous alleles at adaptive loci (depending on subpopulation) were initialized at a frequency of 0.9. Neutral alleles at multiallelic loci (for measuring heterozygosity and allelic richness) were initialized in an identity-by-descent framework, with two unique alleles in each individual of the entire population for a given locus. Neutral alleles at biallelic loci (for measuring pairwise F_{ST}) were initialized using an input file (described in the next section) with allele frequencies that yielded an equilibrium pairwise F_{ST} near empirical values for Olympia Oyster (Silliman, 2019; Supplemental Table 4-2).

4.2.5 *Input*

Our model optionally allowed initial allele frequencies to be specified for each subpopulation, to yield desired pairwise F_{ST} among wild subpopulations. We constructed sets of these initial frequencies by initializing the model from random starting values, running the model for a large number of time steps (analogous to a 'burn in phase'), and sampling allele frequencies from years after which neutral F_{ST} stabilized at desired values. By exploring the parameter space and running simulations until allele frequencies reached migration-drift equilibrium, we generated

allele frequencies yielding equilibrium and desired pairwise F_{ST}. We initialized allele frequencies from a random year in the data file.

4.2.6 *Submodels*

Migration: We modeled individual migration rates as stage-based (m_l, m_j, and m_a for larvae, juveniles, and adults, respectively; default values in Supplemental Table 4-1). Migration (and escape) rates were treated as stage-based because dispersal is often limited to gametes and larvae in shellfish species considered for aquaculture. Migration was also asymmetric: individuals from Wild 1 and Wild 3 were only allowed to migrate to Wild 2, but individuals from Wild 2 could migrate to either Wild 1 or Wild 3, leading to approximately two times as many immigrants in Wild 2 as in Wild 1 and Wild 3 (Supplemental Table 4-3).

Natural mortality: For wild subpopulations, we modeled the mortality rate for an individual as the product of the specified stage-based mortality rate (t_l, t_j, and t_a for larvae, juveniles, and adults, respectively; default values in Supplemental Table 4-1) and an individual's fitness factor (F_i). An individual's fitness factor was defined by

$$F_i = \left(1 + v_f\right) + \frac{\left(1 - v_f\right) - \left(1 + v_f\right)}{(1-(1-z))}\left(f_i - (1-z)\right), \qquad (4.4)$$

in which parameter v_f defined the magnitude of variation in mortality rate that was attributed to differences in fitness, such that an individual's fitness value, with possible values between 1-z to 1, mapped linearly to a fitness factor value, with possible values between 1- v_f to 1+ v_f. In other words, the fitness factor increased the individual mortality rate when individual fitness was low and decreased it when individual fitness was high. In the Farm, the mortality rate was the product of the stage-based mortality rate, fitness factor, and an additional parameter (b; default b = 0.1) governing the relative mortality in the Farm as a proportion of wild mortality, as the very purpose

of hatchery production is to increase survival. Additionally, all individuals died upon reaching the maximum age.

Wild recruitment: In each recruitment event in wild subpopulations, the size of the offspring generation (R_w) was modeled as a log-normal random variable

$$R_w = re^{\left(\sigma_r C_x - \frac{\sigma_r^2}{2}\right)}, \tag{4.5}$$

in which r was a constant recruitment size parameter (default $r = 750$), σ_r was a parameter for the standard deviation in recruitment (default $\sigma_r = 0.01$), and C_x was the current year's climate effect. The product of $\sigma_r C_x$ generated a normally distributed random variable with standard deviation σ_r, and the remaining term was a bias correction to ensure the mean deviation was 0. We assumed density independent recruitment because stock-recruitment relationships are unknown in many shellfish species (Bradbury et al., 2000). We also allowed recruitment outside the hatchery in the Farm due to reproduction of hatchery-produced adults. According to grower survey data, the majority of growers reported wild populations of the native species they grew living either on their farm or within one mile (Supplemental Figure 4-1). Because younger shellfish are often less fecund and many shellfish species are sequential hermaphrodites with unequal sex ratios when young (Charnov & Bull, 1989), recruitment from hatchery-produced adults in the Farm (R_f) was reduced by a factor q (default $q = 0.25$) compared to recruitment in wild subpopulations, and otherwise mirrored recruitment in wild subpopulations:

$$R_f = q \cdot re^{\left(\sigma_r C_x - \frac{\sigma_r^2}{2}\right)}. \tag{4.6}$$

Adults with greater individual fitness values had higher probabilities of being selected as parents, such that the probability of selecting a parent was defined as the parent's fitness value divided by the sum of all potential parent fitness values. Each offspring was produced by selecting two parents

with replacement (weighting probabilities by individual fitness values) to produce a specified number of offspring (d_w, default d_w = 10) for wild families and families produced by hatchery-produced adults in the Farm. Fecundity did not vary by age.

Harvest: The harvest component of the Farm was only implemented if at least 20 non-broodstock individuals of minimum harvest age (c_a, default c_a = 2) or older were present in the Farm. We allowed harvest to occur based on a defined monthly (based on grower data, Supplemental Figure 4-2) harvest rate (h, default h = 1/12), such that animals over the maximum harvest age (c_b, default c_b = 3) were harvested first. Then, any animals older than this cutoff were harvested as well. This cutoff prevented the rare occurrence of very old animals continuing to persist on the Farm.

Seed production: In each seed production event, wild broodstock were used to produce a number of seed (K_f). We assumed broodstock were collected every f (default f = 1) years and were also collected if there were not enough remaining broodstock to spawn (at least one male and one female were needed to spawn). A number of broodstock, n (default n = 100), were collected from a source subpopulation, p (default p = Wild 1). Broodstock were either 1) selected randomly from all adults in a subpopulation, regardless of sex, with at least one male and one female or 2) an equal number of males and females were selected randomly from all adults in the source subpopulation, depending on the parameter l (default l = equal sex ratio), an option for species that can be sexed using external characteristics. Broodstock parent selection and production of seed was modeled as a log-normal variable, as in wild reproduction, except that the number of seed (k, default k = 250), standard deviation of seed production (σ_s, default σ_s = 0.01), and family sizes in the Farm (d_f, default d_f = 20) were specified separately from wild equivalents and no climate effect was included:

$$K_f = ke^{\left(\sigma_s - \frac{\sigma_s{}^2}{2}\right)}. \quad (4.7)$$

Escape: Larval, juvenile, and adult escape mirrored migration, except movement occurred only from hatchery-produced individuals and their descendants in the Farm to Wild 1 based on stage-based escape rates (j_l, j_j, and j_a for larvae, juveniles, and adults, respectively; default values in Supplemental Table 4-1). We considered gamete escape to be potentially significant in shellfish aquaculture compared to finfish aquaculture, as farms are often near wild subpopulations of conspecifics (Supplemental Figure 4-1). Because gametes were not explicitly simulated within the modeling framework, we simulated gamete escape through the production of farm-wild hybrids recruiting to Wild 1. Potential parents for gamete escape include all non-broodstock adults in the Farm and all adults in Wild 1. The scale of recruitment in Wild 1 due to gamete escape was defined by parameter g (default g = 20), and family size for families produced due to gamete escape (d_g, default d_g = 10) was specified separately from wild family size.

4.2.7 *Simulation experiment: Olympia Oyster management strategy evaluation*

To evaluate effects of escape rates, strength of selection, and broodstock collection practices on wild subpopulation genetics, we developed 12 scenarios. These 12 scenarios represented all combinations of three scenario themes and four scenario conditions (parameter combinations of selection strength and escape rate). We chose the themes broadly to represent theoretical practices in Restoration Aquaculture (RA), Commercial Aquaculture (CA), and a Worst-case Scenario (WCS) (Table 4-2), in which the Farm represented a novel wild subpopulation in the RA theme. CA and RA growing practices (e.g., number of broodstock per spawn, source location relative to outplant site, breeding design) for Olympia Oyster do not vary substantially beyond the release of broodstock and lack of harvest in RA (B. Vadopalas, R. Crim,

M. Jackson, personal communication); our scenario design reflected the similarity in practices. However, restoration of extirpated or nearly extirpated subpopulations would result in a wild subpopulation that resembles the Farm subpopulation more than Wild 1 in the model. The WCS theme was chosen as an extreme case of CA, using fewer broodstock and foreign (as opposed to local) broodstock. Scenario conditions were each combination of low ($s = 0.05$, $z = 0.5$) and high ($s = 0.09$, $z = 0.9$) selection strength and low (0.07, twice the larval migration rate) or high (0.5) escape rate. We simulated 12 replicates per scenario. A complete list of default parameter values is included in the Supplementary Materials (Supplemental Table 4-1).

We compared scenarios using the percent change in response variables due to escapees from the Farm. The percent change (M) was defined by

$$M = \frac{\frac{\sum_{y=90}^{100} x_y}{10} - \frac{\sum_{y=40}^{50} x_y}{10}}{\frac{\sum_{y=40}^{50} x_y}{10}} \cdot 100\,, \qquad (4.8)$$

in which X_y represented the response variable value in year y, and years 90 – 100 and years 40 – 50 represented the final 10 years of the during-farm and pre-farm phases, respectively. We tested for significant ($p < 0.05$) differences among percent change in response variables by scenario, scenario theme, and scenario conditions using analysis of variance (ANOVA) and Tukey tests in R 3.5.0 (R Core Team, 2020). Specifically, one-way ANOVAs were performed to compare the effect of scenario, scenario theme, and scenario conditions on percent change in each response variable. Tukey tests were then used to evaluate differences among each pair of scenarios, scenario themes, and scenario conditions. We performed separate ANOVAs and Tukey tests for 1) all wild subpopulations and subpopulation pairs and 2) only Wild 1 (for heterozygosity, allelic richness,

and mean fitness) and subpopulation pairs containing Wild 1 (for neutral and adaptive F_{ST}), the subpopulation receiving escapees, as greatest effects were expected in the subpopulation receiving escapees (Wild 1). We also noted whether response variables restored to pre-disturbance values after removal of the Farm. Because some response variables were not expected to return to pre-disturbance levels at the population level (heterozygosity and allelic richness), we compared subpopulation-level response variable values in Wild 1 to those in Wild 3, because Wild 3 was the most sheltered from the genetic impacts of escapees due to stepping-stone migration.

4.3 RESULTS

Based on the recruitment, seed production, escape, and mortality parameters used in our model (Supplemental Table 4-1), wild subpopulation sizes stabilized near N = 3,300 during the pre-farm and post-farm phase of simulations. During the farm phase of simulations, the wild subpopulation sizes stabilized slightly higher near N = 3,300-3,600 and the Farm size stabilized near N = 1,200 (Supplemental Table 4-4, Supplemental Figure 4-3). Stable Wild 1 subpopulation sizes varied by theme due to differences in broodstock origin and broodstock fate after use. Specifically, the Wild 1 subpopulation size was smaller in the CA theme than in the RA theme because broodstock were killed after use in the CA theme but returned to Wild 1 in the RA theme. The Wild 1 subpopulation size was smaller in the CA theme than the WCS theme because broodstock were not collected from Wild 1 in the WCS theme. The Farm size was comparable across themes, highest in RA, and lowest in WCS (Supplemental Table 4-5), as expected due to absence and presence of harvest in these themes, respectively.

Generation length and mean survival and survivorship per age class were estimated from a random selection of 12 simulations that covered all themes and conditions. Generation length, approximated by the average age of parents, was 3.53 years (95% confidence interval = 3.48 –

3.58 years). Mean survival and mean survivorship by year class were consistent across scenarios (Supplemental Table 4-6). Escape from the Farm produced different patterns in response variables, with variation by scenario, scenario theme, and scenario conditions (Supplemental Figures 4-4 through 4-9), with the greatest effects under High Selection and High Escape conditions (Figure 4-2). Using all wild subpopulations, one-way ANOVAs revealed statistically significant differences between at least two scenarios in percent change of all response variables, between at least two scenario themes in all response variables except mean fitness, and between at least two scenario conditions in all response variables except allelic richness (Supplemental Table 4-7). Tukey tests following one-way ANOVA revealed that the mean value of percent change per response variable was significantly different for 29.1% of scenario pairs, 53.3% of scenario theme pairs, and 33.3% of scenario conditions pairs, across response variables (Supplemental Tables 4-8 through 4-22).

Compared to the one-way ANOVA and Tukey test results using all wild subpopulations, one-way ANOVAs and Tukey tests in Wild 1 revealed nearly identical patterns in results but greater effect sizes (Supplemental Tables 4-23 through 4-38, and Figures 4-3 through 4-5), as were expected because only Wild 1 received escapees. Tukey tests using only Wild 1 revealed that the mean value of percent change per response variable was significantly different for 34.2% of scenario pairs, 53.3% of scenario theme pairs, and 33.3% of scenario conditions pairs, across response variables. The response variables that yielded the least and most significant Tukey tests across scenarios, scenario themes, and scenario conditions were neutral F_{ST} (12%) and allelic richness (52%), respectively (Supplemental Tables 4-24 through 4-38).

4.3.1 *Within-population genetic diversity*

As expected, heterozygosity and allelic richness decreased in all subpopulations over time due to genetic drift (Supplemental Figures 4-4 and 4-5), with greater reductions in allelic richness than heterozygosity (mean percent change across scenarios and subpopulations: allelic richness = -43.2%, heterozygosity = -1.51%). At the end of the post-farm phase, heterozygosity and allelic richness in Wild 1 generally restored to values near those in the relatively unaffected Wild 3 subpopulation in the CA and RA themes (Figure 4-2, Supplemental Figures 4-4 and 4-5).

Under High Selection and High Escape conditions, heterozygosity was lost faster in the CA and RA themes than in the WCS theme, suggesting that domestication selection was a greater force in reducing diversity than broodstock size, which was much smaller in the WCS theme (Figure 4-2). This interpretation was supported by smaller heterozygosity declines in the WCS theme under Low Selection and High Escape conditions (Supplemental Figure 4-4). Heterozygosity in Wild 1 followed the Farm dynamics in RA and CA themes under High Escape conditions, but not under Low Escape conditions (Supplemental Figure 4-4). In contrast, the WCS theme led to an increase in heterozygosity in Wild 1 compared to Wild 2 and Wild 3 because offspring of broodstock from Wild 3 escaped, introducing new genetic diversity. Admixture of Wild 1 and Wild 3 in the WCS theme resulted in significantly smaller reductions in heterozygosity compared to in other themes (Figure 4-4A). Even when heterozygosity was reduced considerably in Wild 1, it recovered relatively quickly to levels of the relatively unaffected Wild 3 subpopulation after the Farm was removed (Figure 4-2).

Allelic richness showed similar patterns to heterozygosity but was more sensitive to broodstock size than to selection (Figures 4-2 and 4-4B, Supplemental Figure 4-5). The highest reduction in allelic richness was observed in the Farm in the WCS theme under High Escape and

High Selection conditions, while Farm allelic richness reduction was lower in the CA and RA theme under the same conditions (Figure 4-2). Somewhat surprisingly, allelic richness in Wild 1 was relatively unaffected compared to Wild 3 in all scenarios of the CA and RA themes (Supplemental Figure 4-5). Under the WCS theme, allelic richness in Wild 1 always increased relative to Wild 2 and Wild 3 because of introduction of foreign alleles from Wild 3 via escapees. The allelic richness of Wild 2 was higher than that of Wild 1 and 3 in all scenarios, because it received more immigrants than the peripheral subpopulations Wild 1 and Wild 3 (Supplemental Figure 1-1).

4.3.2 *Among-population genetic diversity*

As expected, neutral F_{ST} was fairly stable in the pre- and post-farm phases (Supplemental Figure 4-6). The lower F_{ST} between Wild 1 and Wild 2 compared to the other pairwise comparisons was a serendipitous result of the burn-in to achieve equilibrium F_{ST}. At the end of the post-farm phase, neutral F_{ST} between wild subpopulation pairs including Wild 1 did not generally return to pre-disturbance values (Supplemental Figure 4-6).

Under High Selection and High Escape conditions, the Farm and Wild 1 diverged more quickly from other subpopulations in the RA and CA themes than in the WCS theme, due to domestication selection driving losses of genetic diversity in the Farm and in Wild 1 through escape (Supplemental Figure 4-6). This interpretation aligned with the greatest reductions in heterozygosity under the same conditions in the RA and CA themes.

Reduction in neutral differentiation among subpopulations was particularly striking in the WCS theme compared to the CA and RA themes (Supplemental Figure 4-6). Under High Escape conditions, neutral F_{ST} between Wild 1 and Wild 3 declined to almost zero, and under Low Escape conditions, neutral F_{ST} between Wild 1 and Wild 3 approximately halved (Supplemental Figure

4-6), because broodstock were collected from Wild 3 and offspring escaped into Wild 1. Importantly, this collapse of subpopulation differentiation showed little sign of recovery even 50 years after removal of the Farm.

We also observed low neutral F_{ST} between the Farm and the broodstock source subpopulation, across scenarios, as the Farm was derived from the broodstock source subpopulation (Wild 1 for the CA and RA themes, Wild 3 for the WCS theme). Lastly, we observed a decrease and then increase in neutral F_{ST} between Wild 1 and Wild 2 in the WCS theme, only under High Escape conditions (Supplemental Figures 4-6 and 4-7).

Adaptive F_{ST} generally increased in the pre- and post-farm phases, as adaptive alleles rose in frequency due to selection (Supplemental Figure 4-8). For subpopulation pairs with substantial changes in adaptive F_{ST} (e.g., Wild 1 and Wild 3 in the CA theme, under High Selection and High Escape conditions), adaptive F_{ST} did not generally restore to pre-farm values within 50 years of the Farm's removal (Supplemental Figure 4-8). Patterns in adaptive F_{ST} generally mirrored those in neutral F_{ST}, except for a greater magnitude of effects in CA and RA themes and smaller magnitudes of effect in the WCS theme (Supplemental Figure 4-8).

4.3.3 *Fitness*

As expected, mean fitness generally increased in wild subpopulations during the pre- and post-farm phases (and in wild subpopulations except Wild 1 in the during-farm phase) due to selection (Supplemental Figures 4-9 and 4-10). Mean fitness in the Farm was lower under High Selection conditions because, by definition, z was greater in these conditions, leading to greater reductions in fitness values of alleles (Table 4-1). Mean fitness generally did not return to values near those in the relatively unaffected Wild 3 subpopulation after removal of the Farm, but began to return to these values in most scenarios (Supplemental Figure 4-10).

Under High Selection and High Escape conditions, domestication selection drove the greatest increases in mean fitness in the Farm and domesticated escapees drove the greatest reductions in mean fitness in Wild 1 (Figure 4-2). Mean fitness also approached values near those in the relatively unaffected Wild 3 subpopulation more quickly under these conditions after Farm removal. The CA and RA themes caused greater reductions in mean fitness, particularly under High Selection conditions (Figure 4-3, Supplemental Figures 4-9 and 4-10), because fewer broodstock used in the WCS resulted in greater genetic drift, limiting effects of domestication selection. However, reductions in mean fitness in Wild 1 in the WCS theme were smaller but not significantly smaller than in other themes, across conditions (Figure 4-4).

4.4 DISCUSSION

We produced an open-source, individual-based simulation model for conducting genetic risk assessments of native shellfish aquaculture and demonstrated its utility in assessing a variety of genetic impacts, trade-offs among genetic impacts, and potential remedies for Olympia Oyster aquaculture. We found significant differences in percent change per response variable in combinations of scenarios, scenario themes, and scenario conditions, driven primarily by High Selection and High Escape conditions, with notable differences between the Worst-case Scenario (WCS) theme and the other themes. In general, we found results consistent with population genetic theory, including localized effects in Olympia Oyster and a rapid reduction in neutral population differentiation when using foreign broodstock. Validating the use of a complex model to investigate genetic risks, we also identified emergent and unexpected results including 1) a greater reduction in neutral heterozygosity in the Farm in Commercial (CA) and Restoration Aquaculture (RA) themes, despite their use of more broodstock, under High Selection and High Escape conditions, and 2) the same WCS theme yielding the greatest reduction in allelic richness in the

Farm and the smallest reduction in allelic richness in the wild subpopulation receiving escapees (Wild 1).

4.4.1 *Within-population genetic diversity*

Within-population genetic diversity, measured with heterozygosity and allelic richness, decreased most in the Farm across scenario conditions, as expected (Allendorf, 1987; Ryman & Laikre, 1991). Farm escapees caused localized declines in genetic diversity in wild subpopulations, specifically reducing heterozygosity in Wild 1 in the CA and RA themes under High Selection and High Escape conditions only, but not under less extreme conditions or in connected populations Wild 2 or Wild 3 (Supplemental Figures 4-4 and 4-5). The lack of reductions in genetic diversity in Wild 2 and Wild 3 suggested that limited wild migration, at rates that reproduced empirical pairwise F_{ST} in Olympia Oyster, protected Wild 2 and Wild 3 from loss of genetic diversity. Of course, the brooding life history of Olympia Oyster is unusual for most shellfish species, which are usually broadcast spawners with extended pelagic larval periods. Effects on adjacent populations may therefore be more pronounced in species with more connected populations such as rock scallops (*Crassadoma gigantea*, Chapter 1) and California Giant Sea Cucumber (*Apostichopus californicus*, Chapter 2). In scenarios with reductions in heterozygosity, heterozygosity restored or nearly restored to levels in relatively unaffected Wild 3, suggesting that even limited gene flow from neighboring subpopulations can alleviate losses in genetic diversity over short temporal scales (R. S. Waples et al., 2012). In fully isolated populations, only mutation can restore lost genetic diversity, although very slowly (R. S. Waples et al., 2012). However, mutation was not simulated in our model.

Although loss of genetic diversity in hatchery-produced populations has been observed in many shellfish species, including in the Silver-lipped Pearl Oyster (Lind et al., 2009), the New

Zealand Greenshell Mussel (Apte et al., 2003), and the Pearl Oyster (Chu, 2006), the effect of such loss on wild populations is less understood and of greater conservation interest. Genetic diversity was not significantly lower in wild populations receiving hatchery-produced animals than in only wild populations in Eastern Oyster restoration (Hornick & Plough, 2019) or Great Scallop stock enhancement (Morvezen et al., 2016). Different outcomes in our study compared to the Eastern Oyster and Great Scallop may be due to higher gene flow among populations in these species than in Olympia Oyster populations. Alternatively, although a hatchery-produced population may represent few cohorts, a wild population receiving escapees receives many diverse cohorts over time, potentially ameliorating losses in genetic diversity associated with genetic drift in hatchery production (Hornick & Plough, 2019). The mean generation length in our study was 3.5 years, suggesting that multiple cohorts from the Farm may have contributed per generation, further ameliorating losses of genetic diversity in Wild 1. Furthermore, simulation studies may have higher power in detecting small changes in genetic diversity, as all simulated individuals (~3,000 per subpopulation) were sampled here compared to a small subsample in empirical studies (e.g., 48 wild individuals in Hornick & Plough (2019)).

As expected (Nei et al., 1975), the loss in allelic diversity was always greater than in heterozygosity (Supplemental Figures 4-4 and 4-5). However, allelic diversity and heterozygosity showed different dynamics in different themes. For example, the Farm in the WCS theme under High Selection and High Escape conditions had a greater loss in allelic richness compared to the other themes, but a smaller loss in heterozygosity (Figure 4-2), despite much smaller broodstock sizes. Because this pattern was less prominent under High Selection and Low Escape conditions, and absent under the remaining conditions, we hypothesized that this pattern was due to an interaction between selection and escape. Themes CA and RA under High Escape and High

Selection conditions were the only scenarios where fitness in the Farm increased markedly over time (Supplemental Figure 4-9). It thus seems likely that under these conditions, relatively large broodstock size allowed strong selection in the Farm, while high escape also increased the probability of escapees being recaptured and used as broodstock, thus further increasing selective effects. Furthermore, broodstock collected from Wild 1 had progressively lower heterozygosity (Figure 4-2), further reducing heterozygosity in the Farm. In the WCS theme, domestication selection in the small broodstock was weaker, therefore allowing no increase in fitness in the Farm (Supplemental Figure 4-9). Additionally, broodstock could not be recaptured because they were collected from a different subpopulation, and thus had heterozygosity that was unaffected by escapees. The difference between the themes in the dynamics of allelic diversity and heterozygosity is therefore likely a combination between the use of differently sized broodstock from different origins, the strength of domestication selection, and the likelihood of using recaptured farm escapees as new broodstock.

However, if strong domestication selection and collection of hatchery-origin broodstock explained the greater reduction in heterozygosity in CA and RA themes than in the WCS theme under High Selection and High Escape conditions, we would have expected the same pattern in allelic richness. We hypothesize that the different patterns in allelic richness and heterozygosity were due to the distribution of allele frequencies. Although allelic richness and heterozygosity are both expected to decrease with genetic drift, heterozygosity is affected by the distribution of allele frequencies and relatively unaffected by rare alleles. Furthermore, allelic richness and heterozygosity are not always positively correlated. For example, allelic richness and heterozygosity were negatively correlated in European Beech trees due to genetic bottlenecks associated with founder events, selection during population establishment, and increased gene flow

at low population densities during post-glacial colonization (Comps et al., 2001). When alleles are lost due to a population bottleneck, allelic richness is expected to decrease, but heterozygosity may be buffered if remaining allele frequencies are fairly even (Watterson, 1984). If allele frequencies are particularly skewed, as could have happened in our simulations due to complex population dynamics and a large number of alleles, heterozygosity may have been reduced to a greater degree in the CA and RA themes than the WCS theme compared to allelic richness. Unfortunately, these hypotheses cannot be directly tested, because tracking allele frequencies at multiallelic loci (with hundreds to thousands of alleles) was computationally too intensive and thus not included in simulation output.

Another emergent result was greater levels of genetic diversity, measured by both allelic richness and heterozygosity, in Wild 1 compared to the other wild subpopulations in the WCS theme, across conditions (Figure 4-4, Supplemental Figures 4-4 and 4-5). Greater levels of within-population genetic diversity in the WCS theme suggested that the use of foreign broodstock (Wild 3) introduced novel alleles to Wild 1 through escape, and that this effect counteracted loss of genetic diversity due to the genetic bottleneck of hatchery production with few broodstock. Darden et al. (2017) also found greater levels of within-population genetic diversity and greater influxes of novel alleles in wild populations receiving escapees, associated with using broodstock from divergent wild populations. In other words, admixture through foreign broodstock collection (Wild 3) and local escape (Wild 1) buffered Wild 1 from large reductions in genetic diversity. Within our simulation framework, admixture of neutral alleles had no fitness outcomes. However, in real-world contexts, admixture of distinct populations can result in outbreeding depression if populations are locally adapted (Gilk et al., 2004). In some cases, local adaptations can persist even in small, isolated populations receiving high levels of gene flow from foreign populations

and such gene flow may provide beneficial genetic diversity to inbred populations (Fitzpatrick et al., 2020). Because artificial gene flow can create irreversible and unintended consequences (Laikre et al., 2010), it is crucial to consider potential genetic impacts of mixing potentially distinct populations through aquaculture production.

Because the Farm in the RA theme represented a newly restored wild subpopulation, the conservation implications of the CA and RA themes were strikingly different despite similar outcomes. The largest reductions in genetic diversity occurred in the Farm across scenarios, with the next largest effects in Wild 1 in few scenarios, suggesting relatively limited impacts to wild subpopulations in the short-term. However, in the RA theme, the Farm represented a novel wild subpopulation (Wild 0), suggesting that restoration aquaculture provided a demographic boost to the wild metapopulation through the addition of Wild 0, but also reduced within-population genetic diversity. Maintaining genetic diversity in a restored population is vital, particularly if the causes of initial population decline leading to restoration have not been resolved (Camara & Vadopalas, 2009).

4.4.2 *Among-population genetic diversity*

Consistent with theoretical expectations, genetic differentiation between subpopulations increased when one subpopulation diverged due to receiving Farm escapees, and decreased when gene flow increased between subpopulations due to foreign broodstock collection and local escape (R. S. Waples et al., 2012). Notably, the reduction in neutral F_{ST} between Wild 1 and Wild 3 was large in the WCS theme, particularly under High Escape conditions, due to foreign broodstock (Wild 3) and local escape (Wild 1), and did not return to pre-disturbance values after removal of the Farm (Supplemental Figure 4-6). The same effect has been measured empirically: the use of foreign broodstock contributed to loss of neutral differentiation in wild populations of Coho

Salmon (Eldridge & Naish, 2007) and potentially in Atlantic Salmon (Glover et al., 2012). Because neutral differentiation can serve as future adaptive differentiation (Kardos et al., 2021), this loss of neutral among-population genetic diversity represents a threat to population viability associated with use of foreign broodstock. Neutral F_{ST} did not even begin to restore in the WCS under most scenario conditions, consistent with expectations that neutral divergence accrues over very long time scales (Whitlock & Mccauley, 1999), highlighting the importance of using local broodstock. If N_e is large (e.g., 10^5), it could take thousands of generations for neutral divergence to restore (R. S. Waples et al., 2012).

Similar to patterns in heterozygosity, neutral F_{ST} among Wild 1 and Wild 3 in CA and RA themes was shaped by an interaction of selection and escape, as it increased fastest under High Selection and High Escape conditions, but much less under the other conditions. Domestication selection may therefore contribute to changes in neutral F_{ST} among wild subpopulations, a process that has to our knowledge not been described yet.

We observed two other unexpected patterns in neutral F_{ST}. First, neutral F_{ST} between the Farm and broodstock source subpopulation was low, despite a genetic bottleneck in hatchery production, such as reductions in allelic richness in the Farm across scenarios (Supplemental Figure 4-5). Genetic drift due to fewer broodstock resulted in a greater neutral F_{ST} between the Farm and Wild 3 in the WCS theme, compared to the Farm and Wild 1 in the CA and RA themes. Nonetheless, the level of neutral F_{ST} between the Farm and broodstock source subpopulation across themes remained surprisingly low. Broodstock sizes of 25 and 100 may therefore have been sufficient to minimize genetic divergence from the source subpopulation.

A second unexpected pattern in neutral F_{ST} was a decrease, and then increase, between Wild1 and Wild 2, in the WCS theme under High Escape conditions (Supplemental Figure 4-7).

We hypothesize that the change in dynamics in neutral F_{ST} was due to an interaction between foreign broodstock and high escape. Specifically, the initial decrease in neutral F_{ST} between Wild1 and Wild 2 may have tracked the decline among Wild 1, Wild 3, and the Farm, suggesting that Wild 1 and Wild 2 received alleles from Wild 3 through escape and migration, respectively. In Darden et al. (2017), the influx of novel alleles in the wild population also occurred quickly due to simulated net pen escapees from foreign broodstock, with additional net pen escapees providing proportionally smaller numbers of novel alleles. The following increase in neutral F_{ST} between Wild1 and Wild 2 may have been due to the accruing loss of genetic diversity in Wild 1 due to genetic drift in hatchery production (Supplemental Figures 4-4 and 4-5). This explanation would also explain why neutral F_{ST} between Wild 2 and Wild 3 did not increase, as neither subpopulation received escapees.

Because allele frequencies at adaptive loci were shaped by both demographic and selective forces, it is unsurprising that adaptive F_{ST} captured a clearer signal of domestication selection in CA and RA themes and weaker signals of genetic drift in the WCS theme, and otherwise mirrored neutral F_{ST} (Supplemental Figures 4-6 through 4-8). The conservation implications are greater in the RA theme, where the Farm represented a locally restored wild subpopulation. For example, rapidly accruing adaptive divergence between the newly restored wild subpopulation (Farm in the RA theme) and other wild subpopulations (Supplemental Figure 4-8) suggests that the restored subpopulation contained disadvantageous alleles due to domestication selection, potentially limiting the demographic benefits of restoration. Although difficult to prove empirically, selection in the hatchery was one posited explanation for minimal demographic benefits of a restored population of the Eastern Oyster (Carlsson et al., 2008).

4.4.3 *Fitness*

As expected (Li, 1978), selection drove changes in mean fitness more quickly in scenario themes with greater numbers of broodstock (CA and RA) and under High Selection and High Escape conditions (Supplemental Figures 4-9 and 4-10). Consistent with Ford (2002), using wild broodstock (all themes) was not sufficient to curtail domestication selection completely (Supplemental Figure 4-9), with fitness reductions in the wild possible over few generations. We observed the greatest reductions in the Farm, and second greatest reductions in Wild 1, as expected due to escapees. Most studies investigating fitness reductions due to domestication selection measured effects in hatchery-produced offspring (like our Farm) compared to wild counterparts (Araki et al., 2008; Christie et al., 2014; McFarland et al., 2020). Designing experiments to measure fitness reductions in wild descendants of hatchery-produced offspring is more logistically challenging but of greater conservation interest. Potential fitness reductions in wild populations could be mitigated by processes such as purifying selection, which could remove maladapted animals prior to reproduction (Baskett & Waples, 2013), or domestication selection affecting trait distributions such as spawn timing (Waters et al., 2015), which could reduce introgression.

Consistent with Castellani et al. (2018), we found only weak signals of reduced fitness in wild subpopulations receiving escapees over 50 years when escape rate was low (here, 7%; Castellani et al. (2018), intrusion rate = 5-10%), compared to large reductions in mean fitness when escape rate was high (here, 50%; Castellani et al. (2018), intrusion rate = 30-50%), suggesting that escape rates have to be high to create reductions in fitness in wild populations over small temporal scales. However, genetic impacts such as reductions in fitness due to domestication selection accrue over time (Castellani et al., 2018), thus managers should consider the generation length of species relative to the duration of an aquaculture program (Willoughby & Christie, 2019). For

example, increasing generation length, such as through using old broodstock in long-lived species, can mitigate domestication selection and loss of genetic diversity (Wildt, 2000). Extending generation length to mitigate genetic impacts of captive breeding is a technique used in genome resource banking, such as for endangered species, but has not been applied in aquaculture contexts, to the best of our knowledge. Given that some shellfish species are particularly long-lived (Shaul & Goodwin, 1982), it may be worth the effort to collect old broodstock. Lastly, 50 years of domestication selection may represent different numbers of generations depending on species life history, and may represent more generations than expected if aquaculture production results in a reduction of the generation interval (Hershberger et al., 1990), further increasing effects of genetic drift and domestication selection.

When domestication selection caused reductions in mean fitness in Wild 1, mean fitness generally did not return to levels in Wild 2 and Wild 3 (Supplemental Figure 4-10), suggesting that mean fitness did not recover at similar temporal scales in which it was lost. Similarly, fisheries-induced selection can cause rapid evolutionary change, such as selection for early maturation due to removal of large and old fish, and rates of recovery can be quite slow (Law, 2000). The rate of recovery in fitness depends on the strength of natural selection, which may be weaker than the strength of anthropogenic selection. Moreover, although fitness can be restored over time, evolution is irreversible, and the genetic integrity of the original population cannot be restored exactly (R. S. Waples et al., 2012). Delays in restoration of fitness may be of greater concern in the RA theme. In the RA theme, the Farm represented a newly restored wild subpopulation, and low fitness combined with generally low population sizes (for imperiled species targeted for aquaculture) could increase extinction risk (Lynch & O'Hely, 2001).

A central finding of our study was the potential for strong domestication selection to reduce both fitness and neutral genetic diversity, highlighting both the interactions among genetic risks and the complexity of the trade-off in mitigating loss of fitness due to domestication selection and loss of within-population genetic diversity (Fraser, 2008). Equalizing family sizes can limit domestication selection (Allendorf, 1993), a potentially important practice in shellfish hatcheries that buffer sea water pH to reduce mortality for calcifying larvae (J. Clements & Chopin, 2016). Although use of polyploid animals may prevent interbreeding of farmed and wild animals altogether (Piferrer et al., 2009), the development of polyploid strains inevitably involves severe domestication and, if polyploidy is incomplete, even limited interbreeding may cause severe reductions in fitness (R. S. Waples et al., 2012). Intermediate levels of domestication selection can result in the greatest reductions in fitness after escape (Baskett & Waples, 2013), warranting caution particularly for intentional selection, including the production of polyploid animals. However, if strong purifying selection removes maladaptive genes from a population between escape and reproduction, then polyploid animals may be a reasonable strategy for mitigating genetic risks of native shellfish aquaculture. Unfortunately, domestication selection, in particular in connection with triploidization, in shellfish aquaculture is understudied compared to in finfish aquaculture (Nascimento-Schulze et al., 2021) and future research on this topic is crucial to mitigating genetic risks of native shellfish aquaculture

4.4.4 *Evaluating model approach & future directions*

We identified several advantages and disadvantages in using a complex, spatially explicit individual-based simulation model to quantify genetic impacts of native shellfish aquaculture. Advantages included explicit consideration of trade-offs among genetic impacts, such as a reduced loss of mean fitness and greater loss of allelic richness in the WCS theme, and discovery of

emergent effects, such as unexpected patterns in heterozygosity and allelic richness. Disadvantages included computational inefficiency (e.g., each simulation took ~48 hours to complete), which prevented broad or complete coverage of parameter space, and difficulty in interpreting causes of observed patterns when multiple factors interacted. For example, the relative contributions of multiple factors (local adaptation, domestication selection, and inbreeding depression) to reductions in fitness are of conservation interest, and could not be disentangled using our results. We also included some oversimplifications that may have limited the application of our results. For example, we started some parameters at non-equilibrium levels (e.g., adaptive allele frequencies) and excluded factors such as mutation, which prevented genetic diversity from reaching mutation-drift equilibrium.

The model is sufficiently flexible to allow exploration of much larger parameter spaces than was possible in this study. For example, future simulations could be used to further inform the trade-off between mitigating loss of fitness and loss of within-population genetic diversity. Because fitness was affected by local adaptation, domestication selection, and inbreeding depression, evaluating simulations across this parameter space could help resolve the relative contributions of each.

Future developments of the model will improve assessment of the genetic risks of native shellfish aquaculture. For example, the inclusion of density dependent processes could increase sensitivity to population dynamics and potentially increase realism, particularly for dynamics in small populations like genetic drift in hatchery production and reproduction on the Farm. Density independent recruitment and mortality were two reasons why the presence and absence of harvest did not greatly affect the Farm size. Lastly, N_e, a key parameter for conservation concerns, could also be measured from loss in heterozygosity, after deciding on how to account for complex model

structure. The Ryman-Laikre effect, a reduction in the N_e in the total wild-captive system due to a substantial hatchery contributions (Ryman & Laikre, 1991), is a widely studied and important genetic impact to assess (R. S. Waples et al., 2012).

4.4.5 *Management implications and conclusions*

Multiple insights gathered from simulation results can be used to inform Olympia Oyster aquaculture management. First, most genetic impacts were limited to the local wild subpopulation, suggesting that realistic migration rates result in localized effects in Olympia Oyster, particularly for within-population genetic diversity and mean fitness. In species with less structured populations, effects may be smaller but wider reaching. Second, for scenarios with large genetic impacts (High Selection and High Escape conditions), most response variables did not return to pre-disturbance values (or comparable values in relatively unaffected Wild 3) within fifty years (Figure 4-2), suggesting long-lasting impacts of escapees and the importance of populations connectivity for recovery of genetic diversity and fitness. Third, our results suggest that restoration aquaculture can result in significant losses of fitness and genetic diversity in newly restored populations (the Farm in the RA theme), resulting in potentially negative impacts to the broader metapopulation. Comparatively, across our scenarios in the CA and WCS themes, few produced substantial genetic impacts in wild subpopulations (Wild 1, Wild 2, and Wild 3), suggesting selection strength and escape rates must be high or the number of broodstock quite few for significant genetic impacts in the short-term (50 years, ~14 generations). However, measured effects, even if small, were slow to return to pre-disturbance values. Furthermore, we explored a small portion of the total parameter space and, as with all models, made simplifying assumptions that may limit the application of our results to real-world contexts. Ultimately, empirical examples of significant genetic impacts to hatchery-produced animals (Christie et al., 2014; Laikre et al.,

2010; Lind et al., 2009) and wild populations receiving escapees (Glover et al., 2012) exist, demonstrating that greater effects than measured here are possible.

4.5 TABLES AND FIGURES

Table 4-1: Allele fitness effects. Allele fitness effects were defined by residing environment and selection coefficients *s* and *z*, such that $s << z$. Domestication selection dynamics were created by varying which allele was advantageous and by using different selection coefficients to define the fitness effects of disadvantageous alleles in the wild and farm environments. Local adaptation dynamics were created by varying fitness effects for the disadvantageous allele in the wild populations (Allele 1), by multiplying *s* by factors of 1, 2, and 3.

	Allele	
	0	*1*
Wild 1	1	$1-s$
Wild 2	1	$1-2s$
Wild 3	1	$1-3s$
Farm	$1-z$	1

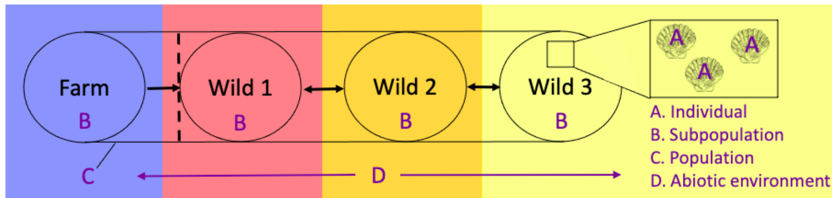
Table 4-2: Scenario theme descriptions. Scenario themes differed by broodstock source subpopulation, number of broodstock collected and how often, whether broodstock were returned to the wild after use or killed, and whether harvest occurred in the Farm subpopulation.

Theme	Source	Number	Returned	Harvest
Commercial aquaculture (CA)	Local (Wild 1)	100 / year	No	Yes
Restoration aquaculture (RA)	Local (Wild 1)	100 / year	Yes	No
Worse-case Scenario (WCS)	Foreign (Wild 3)	100 / 3 years	No	Yes

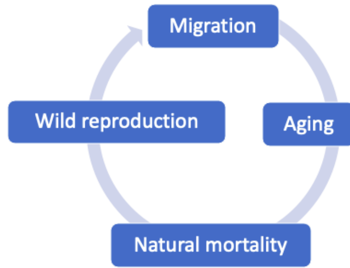
Table 4-3: Percent of significant Tukey tests per response variable.

Response variable	%
Allelic richness	42.7
Heterozygosity	32.0
Mean Fitness	28.0
Neutral F_{ST}	16.0
Adaptive F_{ST}	33.3

A. Simulated population structure & hierarchical levels



B. Wild processes



C. Farm and wild processes

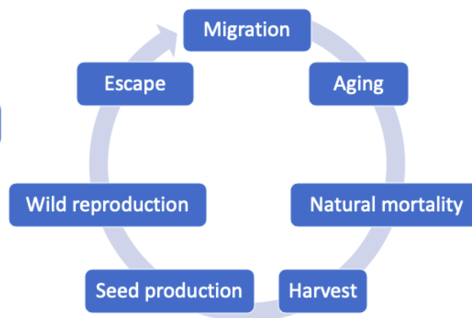


Figure 4-1: Model structure and order of processes in monthly time steps. In Panel A, black arrows represent movement of animals through escape (farm to wild) and migration (among wild). The dashed line between the farm and Wild 1 represents the distinction across the three phases of the model: the spatial structure across phases of the model is identical except for the farm is introduced in the beginning and removed at the end of the second phase. The four hierarchical levels are labeled in purple. Panels B and C represent the order of processes in each phase of the model, with B representing those in the first and last phases (wild subpopulations only) and C representing those in the middle phase (farm and wild subpopulations).

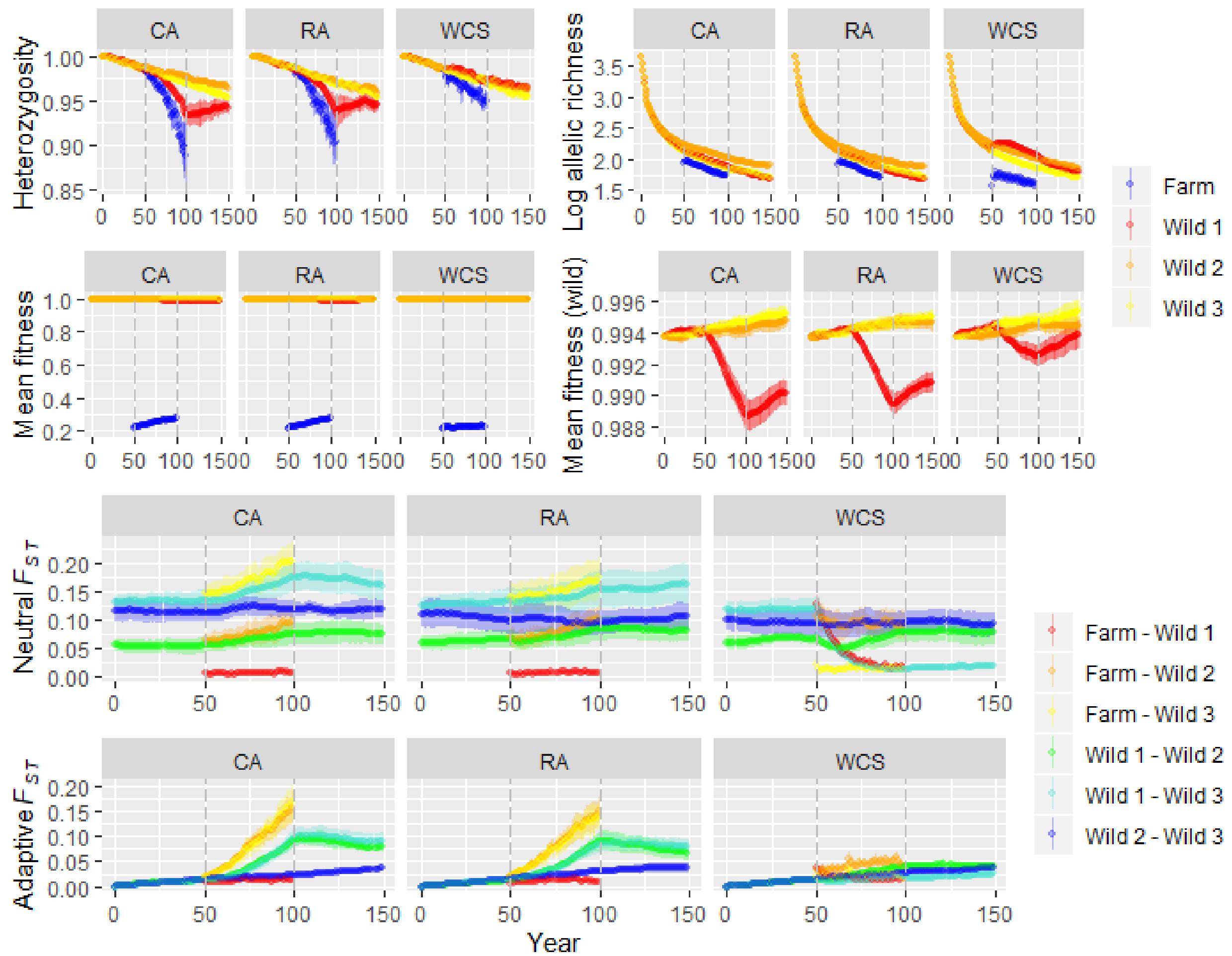


Figure 4-2: Comparing response variables by scenario theme, under High Selection and High Escape conditions. Mean (and 95% confidence intervals) for heterozygosity, $\log_{10}$ allelic richness, mean fitness in all subpopulations, mean fitness in only wild subpopulations, neutral F_{ST}, and adaptive F_{ST} by scenario theme are displayed. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).

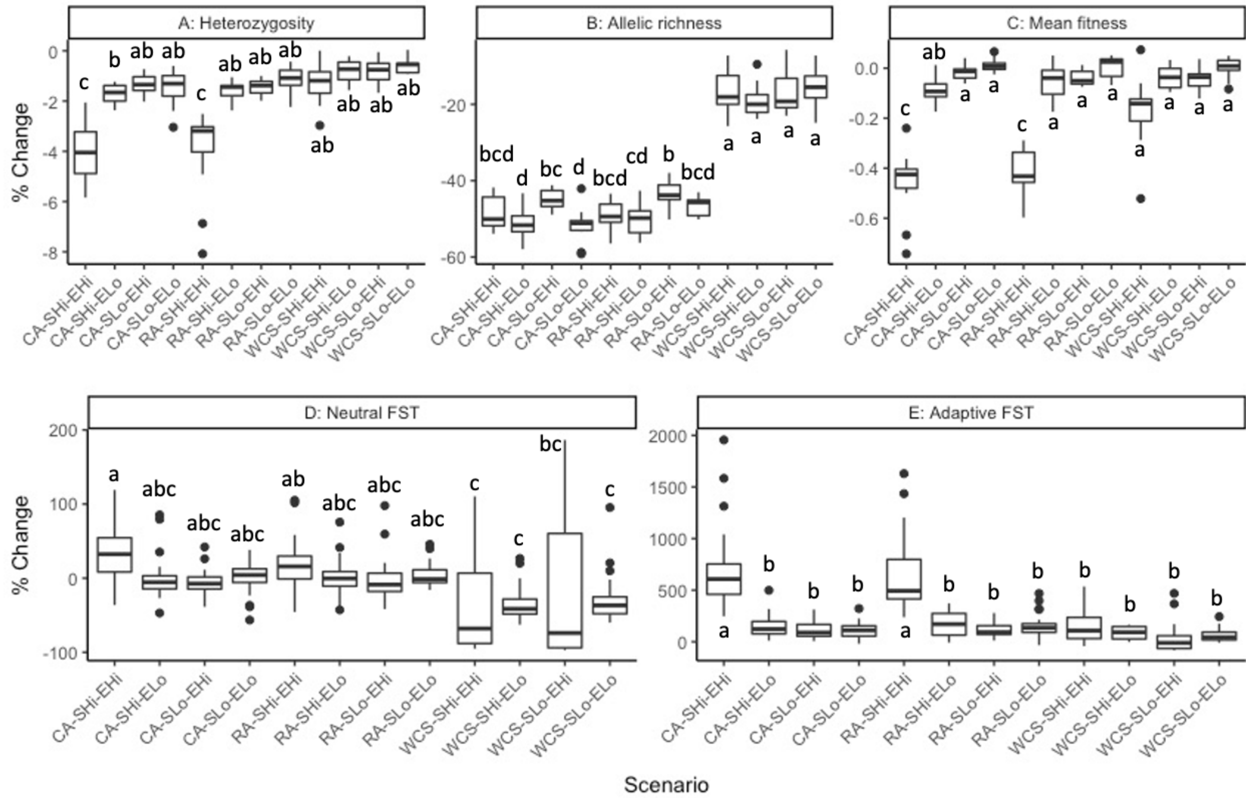


Figure 4-3: Box plots comparing percent change per response variable by scenario. Subplots A, B, and C represent response variables measured in Wild 1, and Subplots D and E represent subpopulation pairs including Wild 1. For each boxplot, the bolded middle represents the median, the hinges represent the 25% and 75% quartiles, and the whiskers extend to 1.5 times the interquartile range in both directions from the corresponding hinges. CA = Commercial Aquaculture, RA = Restoration Aquaculture, WCS = Worst-case Scenario, E = escape rate, S = selection strength, Lo = low, and Hi = high, such that CA-SLo-ELo = Commercial Aquaculture under Low Selection and Low Escape conditions. Lower case letters represent unique groups differentiated by Tukey tests.

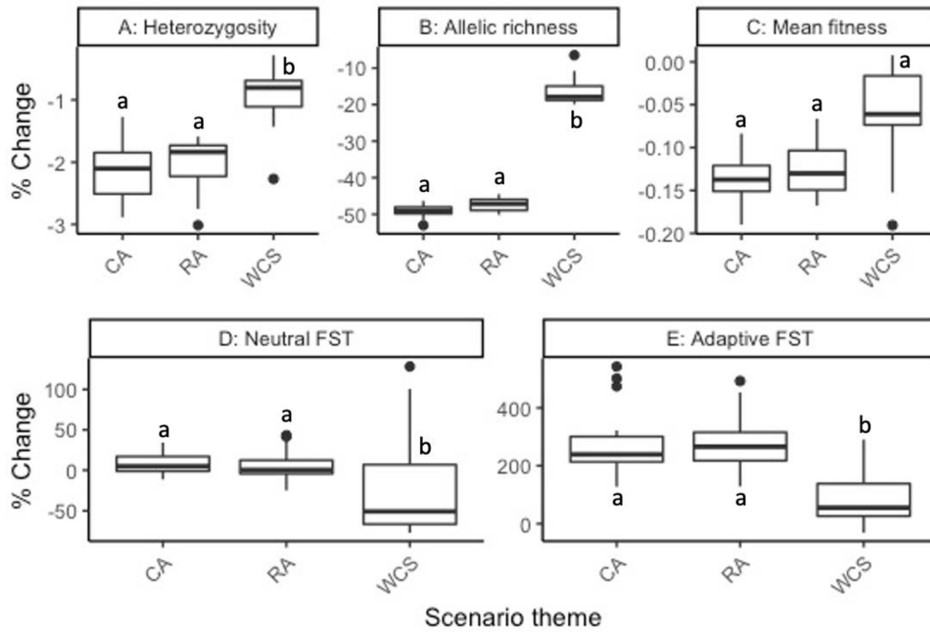


Figure 4-4: Box plots comparing percent change per response variable by scenario theme. CA = Commercial Aquaculture, RA = Restoration Aquaculture, WCS = Worst-Case Scenario. Lower case letters represent unique groups differentiated by Tukey tests.

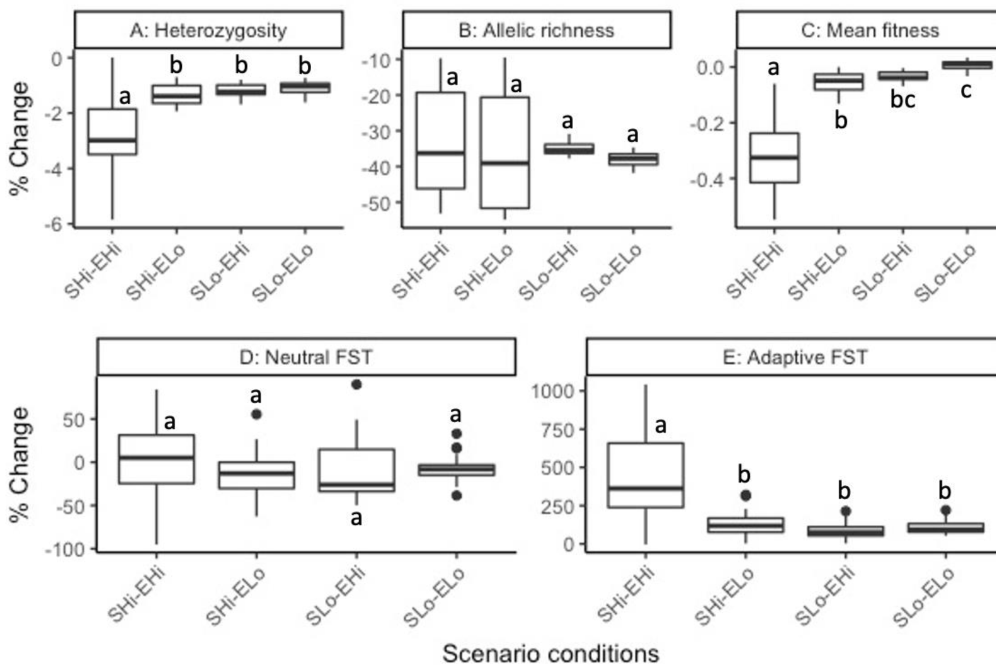


Figure 4-5: Box plots comparing percent change per response variable by scenario conditions. E = escape rate, S = selection strength, Lo = low, and Hi = high, such that SLo-ELo = Low Selection and Low Escape conditions. Lower case letters represent unique groups differentiated by Tukey tests.

4.6 SUPPLEMENTARY MATERIALS

Supplemental Table 4-1: Parameter symbols, descriptions, and default values.

Symbol	Description	Default value
s	Wild-wild selection coefficient	0.05
z	Farm-wild selection coefficient	0.5
u_s	Age at settlement	1/12
u_a	Age at maturity	1
u_m	Maximum age	10
t_l	Larval mortality rate	0.99
t_j	Juvenile mortality rate	0.3
t_a	Adult mortality rate	0.3
m_l	Monthly larval migration rate	0.035
m_j	Monthly juvenile migration rate	0
m_a	Monthly adult migration rate	0
o_i	Probability of wild reproduction event in month i (of 12 months)	O_4 = 1, rest = 0
r	Scale of wild recruitment	750
d_w	Family size per breeding event for wild subpopulations	10
σ_r	Standard deviation in scale of wild recruitment	0.01
y_i	Probability of seed production event in month i	O_1 = 1, rest = 0
k	Scale of seed production	250
σ_s	Standard deviation in scale of seed production	0.01
d_f	Family size per breeding event for the farm subpopulation	20
p	Broodstock source subpopulation	Wild 1
n	Number of broodstock collected at once	100
f	Interval in years for collecting fresh broodstock	1
l	Whether to collect equal sex ratio in broodstock	False
q	Reduced recruitment in farm subpopulation due to lower fecundity and uneven sex ratios common to young shellfish	0.25
b	Reduced farm mortality factor	0.1
v_i	Probability of larval or gamete escape event in month i	O_4 = 1, rest = 0
w_i	Monthly probability of juvenile or adult escape event	All = 0
j_l	Larval escape rate	0.07
j_j	Juvenile escape rate	0
i_a	Adult escape rate	0
g	Scale of wild1 recruitment due to gamete escape	20
d_g	Family size for farm-wild1 hybrid families, to simulate gamete escape	10
h	Monthly harvest rate	0.0833
c_a	Minimum harvest age	1
c_b	Maximum harvest age	2

Supplemental Table 4-2: Mean, minimum, and pairwise F_{ST} from simulations and the empirical values for comparison. We ran simulations with different migration rates for 750 years to find which migration rates resulted in equilibrium F_{ST} near empirical F_{ST}. Migration rates 2%, 3.5%, and 5% yielded the closest values, and 3.5% was chosen for Management Strategy Evaluation simulations. Values in the table were measured over years 400-750 in the simulations, when F_{ST} was at equilibrium. Empirical F_{ST} was estimated in Silliman (2019).

	Simulated migration rate			Empirical
	2%	3.5%	5%	
Mean pairwise F_{ST}	0.118	0.0948	0.109	0.095
Minimum pairwise F_{ST}	0.0349	0.0288	0.0289	0.00138
Maximum pairwise F_{ST}	0.183	0.175	0.202	0.177

Supplemental Table 4-3: Transition matrix for wild migration. The matrix represents migration rates from each subpopulation (rows) to each subpopulation (columns).

	Wild 1	Wild 2	Wild 3
Wild 1	$1–m$	m	0
Wild 2	$0.5m$	1-m	$0.5m$
Wild 3	0	m	1-m

Supplemental Table 4-4: Stable subpopulation sizes, by model phase. Mean (and 95% confidence interval) subpopulation size in the last 25 years of each phase.

	Pre-farm	During-farm	Post-farm
Wild 1	3,282 (3,280-3,284)	3,589 (3,577-3,601)	3,286 (3,283-3,288)
Wild 2	3,294 (3,291-3,296)	3,301 (3,298-3,303)	3,303 (3,301-3,305)
Wild 3	3,284 (3,282-3,288)	3,280 (3,278-3,283)	3,291 (3,289-3,294)
Farm	-	1,209 (1,206-1,212)	-

Supplemental Table 4-5: Stable Farm sizes, by theme. Mean, lower 95% confidence interval limit, and upper 95% confidence interval limit for Farm subpopulation size in the last 25 years of the during-farm phase, by scenario theme.

	Mean	Lower 95% CI	Upper 95% CI
Commercial Aquaculture	1,223	1,222	1,223
Restoration Aquaculture	1,257	1,257	1,258
Worst Case Scenario	1,148	1,148	1,148

Supplemental Table 4-6: Survival and survivorship estimates by age class for the population. The population includes all subpopulations combined, including the Farm. Estimates were calculated across 12 random simulations that included all scenario themes and conditions. Ages were grouped into 10 age classes, in which age 1 represents individuals age 1-11 months, age 2 represents individuals aged 12-23 months, and so forth. Columns s_x and l_x contain the mean survival and survivorship respectively, with 95% confidence intervals in parentheses.

Age	s_x	l_x
1	0.802 (0.801-0.803)	1.000 (1-1)
2	0.799 (0.796-0.801)	0.802 (0.801-0.803)
3	0.752 (0.748-0.756)	0.641 (0.639-0.643)
4	0.795 (0.793-0.796)	0.481 (0.480-0.484)
5	0.795 (0.793-0.796)	0.383 (0.380-0.385)
6	0.795 (0.793-0.796)	0.304 (0.302-0.306)
7	0.795 (0.794-0.796)	0.242 (0.240-0.244)
8	0.795 (0.794-0.796)	0.192 (0.190-0.194)
9	0.795 (0.793-0.796)	0.153 (0.151-0.154)
10	0.000 (0-0)	0.121 (0.120-0.123)

Supplemental Table 4-7: Analysis of variance (ANOVA) results, using all wild subpopulations. One-way ANOVAs were performed to compare the effect of scenario, scenario theme, and scenario conditions on percent change for each of five response variables. *F*-values are reported for each ANOVA. $p < 0.05$ = *.

	Scenario	Theme	Conditions
Allelic richness	9.975*	51.94*	1.072
Heterozygosity	8.161*	11.18*	14.11*
Mean fitness	6.503*	1.552	17.47*
Neutral F_{ST}	5.282*	17.52*	3.432*
Adaptive F_{ST}	19.44*	15.88*	36.91*

Supplemental Table 4-8: Tukey test results using all wild subpopulations for percent change in heterozygosity, for pairwise comparisons of the 12 scenarios. RA = Restoration Aquaculture, CA = Commercial Aquaculture, and WCS = Worst-case Scenario. The number represents the set of conditions, where 1 = High Selection and High Escape conditions, 2 = High Selection and Low Escape conditions, 3 Low Selection and High Escape conditions, and 4 = Low Selection and Low Escape conditions. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	0.59	-										
CA-3	0.73	0.14	-									
CA-4	0.90	0.31	0.17	-								
RA-1	0.90	0.31	0.17	-1.07	-							
RA-2	0.78	0.20	0.06	-0.12	0.95	-						
RA-3	0.84	0.25	0.11	-0.06	1.01	0.06	-					
RA-4	0.81	0.22	0.08	-0.09	0.97	0.02	-0.03	-				
WCS-1	0.91	0.32	0.18	0.01	1.07	0.13	0.07	0.10	-			
WCS-2	0.91	0.33	0.19	0.01	1.08	0.13	0.07	0.11	0.00	-		
WCS-3	1.10	0.52	0.38	0.20	1.27	0.32	0.26	0.30	0.20	0.19	-	
WCS-4	0.99	0.40	0.26	0.09	1.16	0.21	0.15	0.18	0.08	0.08	-0.11	-

Supplemental Table 4-9: Tukey test results using all wild subpopulations for percent change in mean fitness, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	0.13	-										
CA-3	0.13	0.00	-									
CA-4	0.14	0.01	0.01	-								
RA-1	0.02	-0.11	-0.11	-0.12	-							
RA-2	0.14	0.01	0.01	0.00	0.12	-						
RA-3	0.13	-0.01	-0.01	-0.02	0.11	-0.02	-					
RA-4	0.14	0.01	0.00	-0.01	0.12	0.00	0.01	-				
WCS-1	0.11	-0.03	-0.03	-0.04	0.09	-0.04	-0.02	-0.03	-			
WCS-2	0.15	0.02	0.02	0.01	0.13	0.01	0.03	0.01	0.05	-		
WCS-3	0.11	-0.03	-0.03	-0.04	0.09	-0.03	-0.02	-0.03	0.00	-0.04	-	
WCS-4	0.14	0.01	0.01	0.00	0.12	0.00	0.02	0.00	0.04	-0.01	0.03	-

Supplemental Table 4-10: Tukey test results using all wild subpopulations for percent change in adaptive F_{ST}, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-348.6	-										
CA-3	-367.7	-19.1	-									
CA-4	-370.5	-21.9	-2.8	-								
RA-1	-17.3	331.3	350.4	353.2	-							
RA-2	-332.8	15.8	34.8	37.7	-315.5	-						
RA-3	-388.7	-40.1	-21.1	-18.2	-371.4	-55.9	-					
RA-4	-347.4	1.2	20.3	23.1	-330.1	-14.6	41.4	-				
WCS-1	-365.7	-17.1	2.0	4.8	-348.4	-32.9	23.0	-18.3	-			
WCS-2	-397.8	-49.2	-30.1	-27.3	-380.5	-65.0	-9.1	-50.4	-32.1	-		
WCS-3	-443.1	-94.5	-75.5	-72.6	-425.8	-110.3	-54.4	-95.7	-77.4	-45.3	-	
WCS-4	-420.7	-72.1	-53.0	-50.2	-403.4	-87.9	-32.0	-73.3	-55.0	-22.9	22.4	-

Supplemental Table 4-11: Tukey test results using all wild subpopulations for percent change in neutral F_{ST}, for pairwise comparisons of the 12 scenarios. Scenario name in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-28.8	-										
CA-3	-31.6	-2.9	-									
CA-4	-26.8	2.0	4.9	-								
RA-1	-12.8	16.0	18.8	14.0	-							
RA-2	-27.8	1.0	3.8	-1.0	-15.0	-						
RA-3	-29.2	-0.4	2.4	-2.5	-16.4	-1.4	-					
RA-4	-21.8	7.0	9.8	5.0	-9.0	6.0	7.4	-				
WCS-1	-46.9	-18.1	-15.3	-20.1	-34.1	-19.1	-17.7	-25.1	-			
WCS-2	-52.2	-23.4	-20.5	-25.4	-39.4	-24.4	-23.0	-30.4	-5.3	-		
WCS-3	-38.9	-10.1	-7.2	-12.1	-26.1	-11.1	-9.7	-17.1	8.0	13.3	-	
WCS-4	-45.6	-16.8	-14.0	-18.9	-32.8	-17.8	-16.4	-23.8	1.3	6.6	-6.7	-

Supplemental Table 4-12: Tukey test results using all wild subpopulations for percent change in allelic richness, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-1.74	-										
CA-3	1.61	3.35	-									
CA-4	-1.92	-0.18	-3.53	-								
RA-1	-1.14	0.59	-2.75	0.78	-							
RA-2	-1.31	0.43	-2.92	0.61	-0.17	-						
RA-3	2.03	3.77	0.42	3.95	3.18	3.34	-					
RA-4	-0.15	1.59	-1.76	1.77	1.00	1.16	-2.18	-				
WCS-1	10.02	11.76	8.41	11.94	11.16	11.33	7.99	10.17	-			
WCS-2	8.73	10.46	7.12	10.65	9.87	10.04	6.70	8.87	-1.29	-		
WCS-3	9.78	11.51	8.17	11.70	10.92	11.08	7.74	9.92	-0.25	1.05	-	
WCS-4	10.39	12.13	8.78	12.31	11.53	11.70	8.36	10.54	0.37	1.66	0.62	-

Supplemental Table 4-13: Tukey test results using all wild subpopulations for percent change in heterozygosity, for pairwise comparisons of the three scenario themes. RA = Restoration Aquaculture, CA = Commercial Aquaculture, and WCS = Worst Case Scenario. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	0.013	-	
WCS	0.43	0.41	-

Supplemental Table 4-14: Tukey test results using all wild subpopulations for percent change in allelic richness, for pairwise comparisons of the three scenario themes. Scenario theme key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$

	CA	RA	WCS
CA	-		
RA	0.37	-	
WCS	10.24	9.87	-

Supplemental Table 4-15: Tukey test results using all wild subpopulations for percent change in neutral F_{ST}, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	-1.11	-	
WCS	-24.10	-22.99	-

Supplemental Table 4-16: Tukey test results using all wild subpopulations for percent change in adaptive F_{ST}, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	0.12	-	
WCS	-135.15	-135.27	-

Supplemental Table 4-17: Tukey test results using all wild subpopulations for percent change in mean fitness, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	0.004	-	
WCS	0.02	0.02	-

Supplemental Table 4-18: Tukey test results using all wild subpopulations for percent change in heterozygosity, for pairwise comparisons of the four scenario conditions. S = selection strength, E = escape rate, Hi = high, and Lo = low, such that SLo-ELo = Low Selection and Low Escape conditions. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	-0.51	-		
SLo_EHi	-0.64	-0.13	-	
SLo_ELo	-0.65	-0.14	-0.008	-

Supplemental Table 4-19: Tukey test results using all wild subpopulations for percent change in adaptive F_{ST}, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	232.10	-		
SLo_EHi	272.18	40.10	-	
SLo_ELo	251.86	19.79	-20.31	-

Supplemental Table 4-20: Tukey test results using all wild subpopulations for percent change in mean fitness, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	-0.10	-		
SLo_EHi	-0.082	0.020	-	
SLo_ELo	-0.0100	0.0010	-0.019	-

Supplemental Table 4-21: Tukey test results using all wild subpopulations for percent change in allelic richness, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	1.07	-		
SLo_EHi	-1.51	-2.58	-	
SLo_ELo	0.18	-0.88	1.70	-

Supplemental Table 4-22: Tukey test results using all wild subpopulations for percent change in neutral F_{ST}, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	16.36	-		
SLo_EHi	13.34	-3.01	-	
SLo_ELo	11.50	-4.86	-1.85	-

Supplemental Table 4-23: Analysis of variance (ANOVA) results, using only Wild 1. One-way ANOVAs were performed to compare the effect of scenario, scenario theme, and scenario conditions on percent change for each of five response variables. *F*-values are reported for each ANOVA. $p < 0.05$ = *.

	Scenario	*Theme*	*Conditions*
Allelic richness	154.4*	683.7*	0.678
Heterozygosity	25.07*	16.15*	29.97*
Mean fitness	58.55*	2.741	102.0*
Neutral F_{ST}	5.719*	20.83*	2.289
Adaptive F_{ST}	32.95*	15.92*	52.48*

Supplemental Table 4-24: Tukey test results using only Wild 1 for percent change in heterozygosity, for pairwise comparisons of the 12 scenarios. Scenario key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	2.27	-										
CA-3	2.65	0.38	-									
CA-4	2.49	0.22	-0.16	-								
RA-1	0.00	-2.27	-2.65	-2.49	-							
RA-2	2.43	0.16	-0.22	-0.07	2.43	-						
RA-3	2.54	0.28	-0.10	0.05	2.55	0.12	-					
RA-4	2.84	0.58	0.20	0.35	2.85	0.42	0.30	-				
WCS-1	2.68	0.41	0.03	0.19	2.68	0.25	0.14	-0.16	-			
WCS-2	3.16	0.90	0.52	0.67	3.17	0.74	0.62	0.32	0.48	-		
WCS-3	3.18	0.91	0.54	0.69	3.18	0.76	0.64	0.34	0.50	0.02	-	
WCS-4	3.37	1.10	0.72	0.88	3.37	0.94	0.82	0.52	0.69	0.20	0.19	-

Supplemental Table 4-25: Tukey test results using only Wild 1 for percent change in mean fitness, for pairwise comparisons of the 12 scenarios. Scenario key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	0.37	-										
CA-3	0.44	0.07	-									
CA-4	0.47	0.10	0.03	-								
RA-1	0.04	-0.33	-0.40	-0.43	-							
RA-2	0.41	0.04	-0.03	-0.06	0.36	-						
RA-3	0.42	0.05	-0.02	-0.05	0.38	0.01	-					
RA-4	0.46	0.09	0.02	-0.01	0.42	0.06	0.04	-				
WCS-1	0.29	-0.08	-0.15	-0.18	0.25	-0.12	-0.13	-0.18	-			
WCS-2	0.42	0.05	-0.02	-0.05	0.38	0.01	0.00	-0.04	0.13	-		
WCS-3	0.41	0.04	-0.03	-0.06	0.37	0.01	-0.01	-0.05	0.13	-0.01	-	
WCS-4	0.46	0.09	0.02	-0.01	0.42	0.05	0.04	0.00	0.17	0.04	0.05	-

Supplemental Table 4-26: Tukey test results using only Wild 1 for percent change in adaptive F_{ST}, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-34.84	-										
CA-3	-40.99	-6.15	-									
CA-4	-34.28	0.56	6.71	-								
RA-1	-14.89	19.96	26.10	19.39	-							
RA-2	-34.85	-0.01	6.14	-0.57	-19.96	-						
RA-3	-37.41	-2.57	3.57	-3.14	-22.53	-2.57	-					
RA-4	-30.84	4.00	10.15	3.44	-15.96	4.01	6.57	-				
WCS-1	-69.41	-34.57	-28.42	-35.13	-54.52	-34.56	-31.99	-38.57	-			
WCS-2	-70.52	-35.68	-29.54	-36.25	-55.64	-35.68	-33.11	-39.68	-1.12	-		
WCS-3	-52.73	-17.89	-11.74	-18.45	-37.84	-17.88	-15.31	-21.88	16.68	17.80	-	
WCS-4	-63.80	-28.95	-22.81	-29.52	-48.91	-28.95	-26.38	-32.95	5.61	6.73	-11.07	-

Supplemental Table 4-27: Tukey test results using only Wild 1 for percent change in neutral F_{ST}, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-544.2	-										
CA-3	-581.0	-36.8	-									
CA-4	-584.0	-39.8	-3.0	-								
RA-1	-43.9	500.3	537.1	540.1	-							
RA-2	-525.2	19.0	55.8	58.8	-481.3	-						
RA-3	-579.9	-35.6	1.1	4.1	-535.9	-54.6	-					
RA-4	-541.1	3.2	40.0	43.0	-497.1	-15.8	38.8	-				
WCS-1	-551.5	-7.3	29.5	32.5	-507.6	-26.3	28.4	-10.4	-			
WCS-2	-608.9	-64.6	-27.9	-24.9	-565.0	-83.7	-29.0	-67.8	-57.4	-		
WCS-3	-665.7	-121.5	-84.7	-81.7	-621.8	-140.5	-85.9	-124.7	-114.2	-56.9	-	
WCS-4	-630.3	-86.1	-49.3	-46.3	-586.4	-105.1	-50.4	-89.2	-78.8	-21.4	35.4	-

Supplemental Table 4-28: Tukey test results using only Wild 1 for percent change in allelic richness, for pairwise comparisons of the 12 scenarios. Scenario name key in the caption of Supplemental Table 4-8. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA-1	CA-2	CA-3	CA-4	RA-1	RA-2	RA-3	RA-4	WCS-1	WCS-2	WCS-3	WCS-4
CA-1	-											
CA-2	-2.72	-										
CA-3	3.78	6.51	-									
CA-4	-3.02	-0.29	-6.80	-								
RA-1	-0.38	2.35	-4.16	2.64	-							
RA-2	-1.32	1.40	-5.10	1.70	-0.94	-						
RA-3	5.18	7.91	1.40	8.20	5.56	6.50	-					
RA-4	1.94	4.67	-1.84	4.96	2.32	3.26	-3.24	-				
WCS-1	31.89	34.62	28.11	34.91	32.27	33.21	26.71	29.95	-			
WCS-2	29.55	32.28	25.77	32.57	29.93	30.87	24.37	27.61	-2.34	-		
WCS-3	32.07	34.79	28.29	35.09	32.45	33.39	26.88	30.13	0.17	2.51	-	
WCS-4	32.92	35.64	29.13	35.93	33.29	34.24	27.73	30.97	1.02	3.36	0.85	-

Supplemental Table 4-29: Tukey test results using only Wild 1 for percent change in heterozygosity, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$

	CA	RA	WCS
CA	-		
RA	0.1	-	
WCS	1.25	1.15	-

Supplemental Table 4-30: Tukey test results using only Wild 1 for percent change in allelic richness, for pairwise comparisons of the three scenario themes. Scenario theme key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	1.85	-	
WCS	32.10	30.25	-

Supplemental Table 4-31: Tukey test results using only subpopulation pairs containing Wild 1 for percent change in neutral F_{ST}, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	-1.97	-	
WCS	-36.59	-34.62	-

Supplemental Table 4-32: Tukey test results using only subpopulation pairs containing Wild 1 for percent change in adaptive F_{ST}, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	4.79	-	
WCS	-186.78	-191.58	-

Supplemental Table 4-33: Tukey test results using only Wild 1 for percent change in mean fitness, for pairwise comparisons of the three scenario themes. Scenario themes key in the caption of Supplemental Table 4-13. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	CA	RA	WCS
CA	-		
RA	0.01	-	
WCS	0.07	0.06	-

Supplemental Table 4-34: Tukey test results using only Wild 1 for percent change in heterozygosity, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	-1.73	-		
SLo_EHi	-1.90	-0.17	-	
SLo_ELo	-2.01	-0.28	-0.11	-

Supplemental Table 4-35: Tukey test results using only subpopulations containing Wild 1 for percent change in adaptive F_{ST}, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	360.97	-		
SLo_EHi	410.40	49.42	-	
SLo_ELo	386.65	25.67	-23.75	-

Supplemental Table 4-36: Tukey test results using only Wild 1 for percent change in mean fitness, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

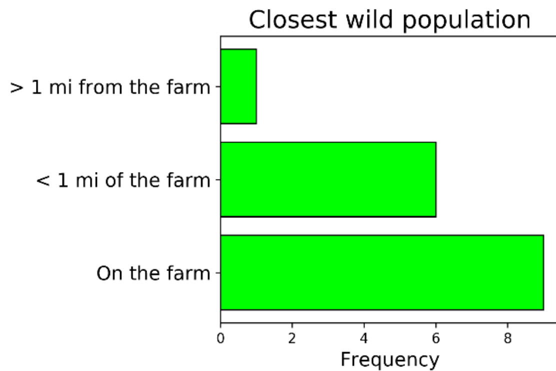
	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	-0.29	-		
SLo_EHi	-0.32	-0.03	-	
SLo_ELo	-0.36	-0.07	-0.04	-

Supplemental Table 4-37: Tukey test results using only Wild 1 for percent change in allelic richness, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

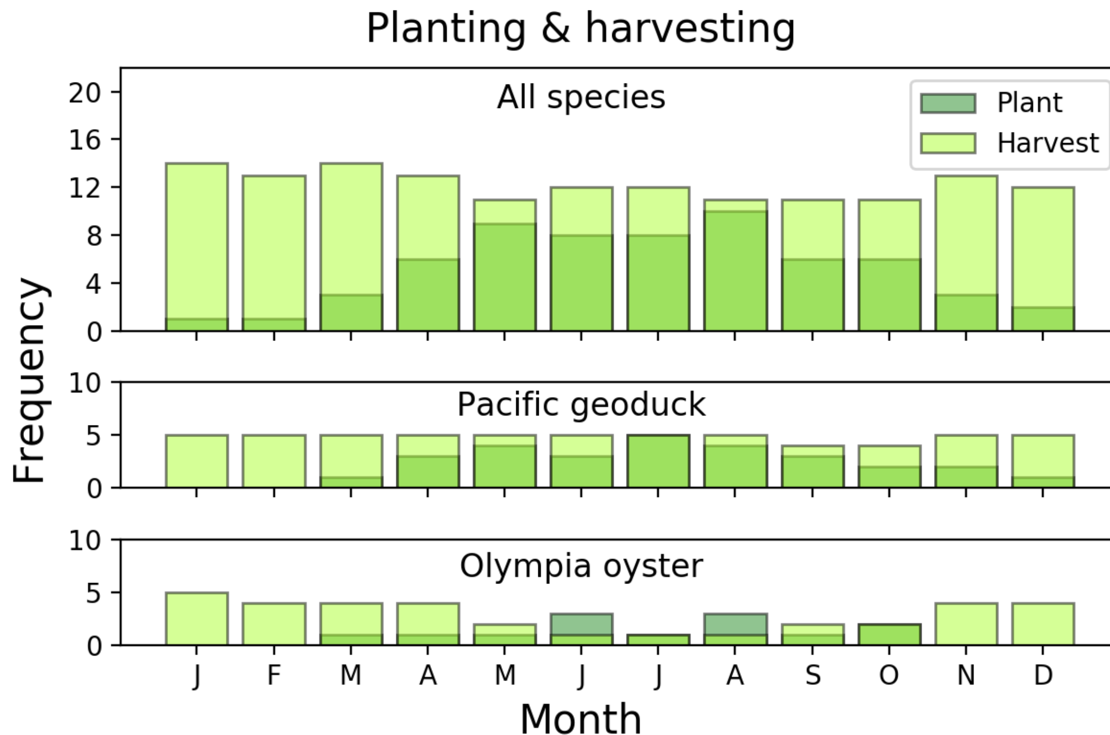
	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	2.00	-		
SLo_EHi	-3.17	-5.17	-	
SLo_ELo	-0.11	-2.11	3.06	-

Supplemental Table 4-38: Tukey test results using only Wild 1 for percent change in neutral F_{ST}, for pairwise comparisons of the four scenario conditions. Scenario conditions key in the caption of Supplemental Table 4-18. Cells contain the mean difference in percent change between percent comparisons and are highlighted gray if $p < 0.05$.

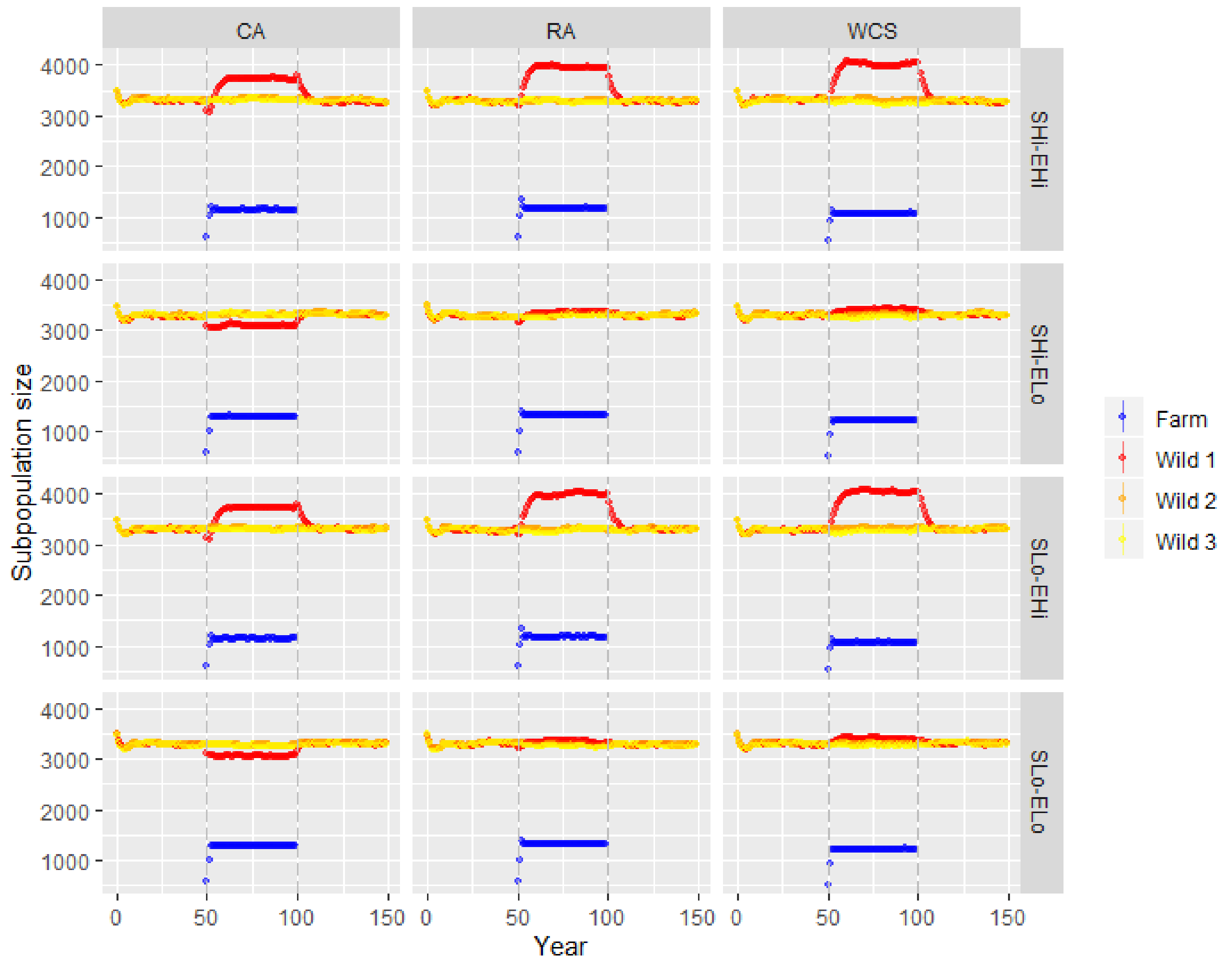
	SHi_EHi	SHi_ELo	SLo_EHi	SLo_ELo
SHi_EHi	-			
SHi_ELo	18.64	-		
SLo_EHi	15.61	-3.03	-	
SLo_ELo	14.87	-3.77	-0.74	-



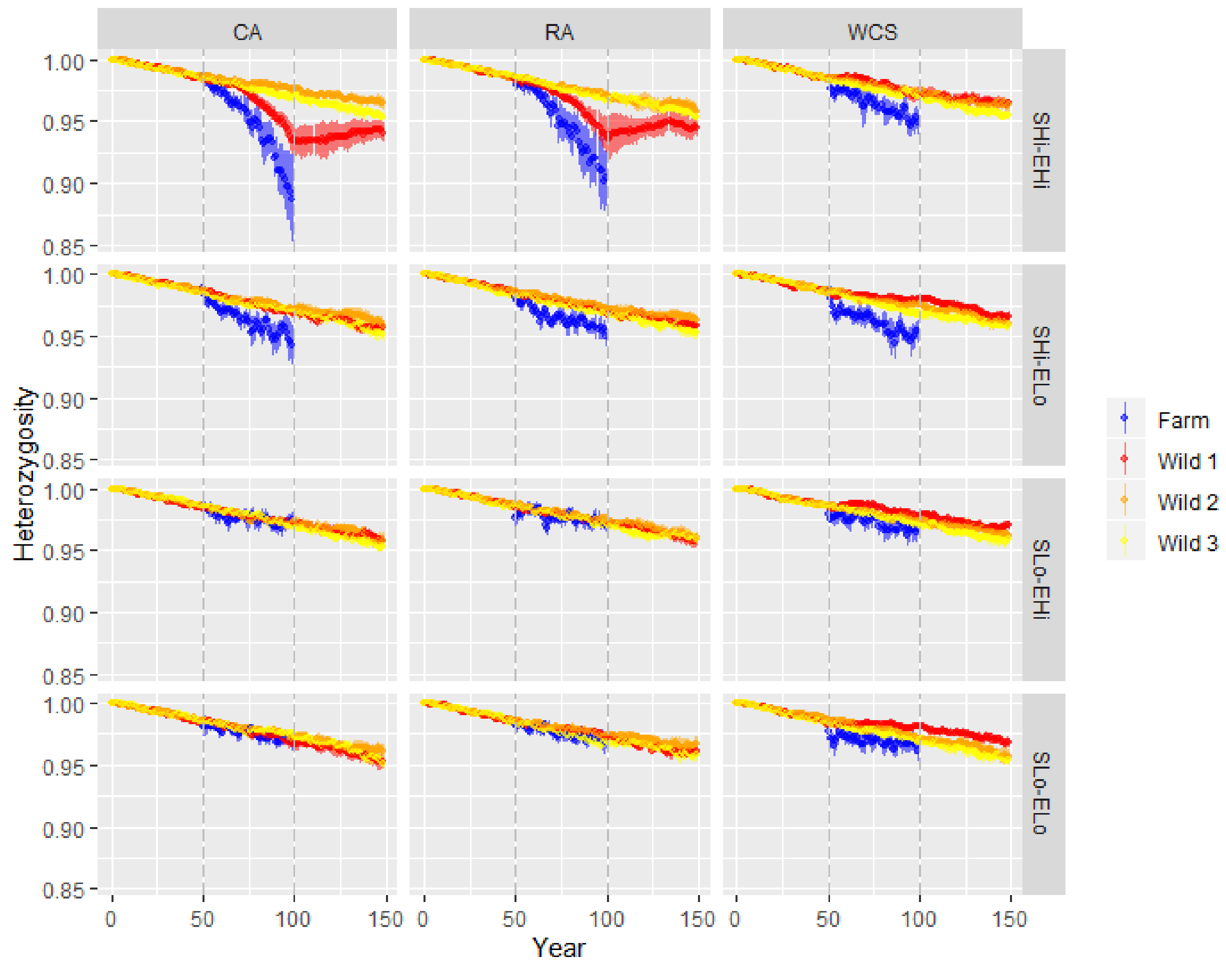
Supplemental Figure 4-1: Grower survey results, reporting distance between farm and closest wild population. We asked "How close is the closest wild population, of the same native shellfish species you grow, to your farm?" Out of 15 respondents, most reported seeing the population on their farm.



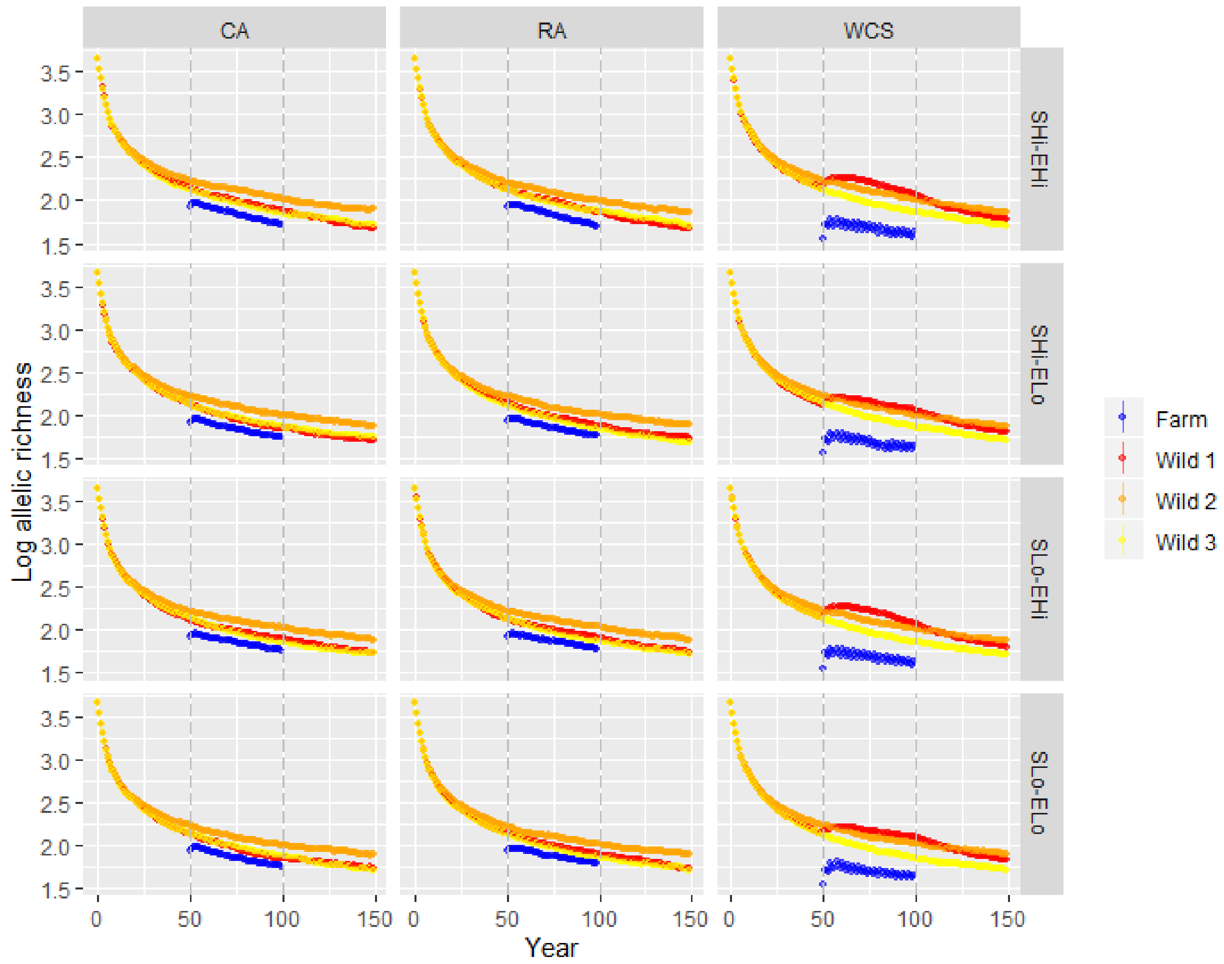
Supplemental Figure 4-2: Grower survey results for seasonality of planting and harvesting. Results reported for all species (N = 14), Pacific Geoduck (N = 5), and Olympia Oyster (N = 5).



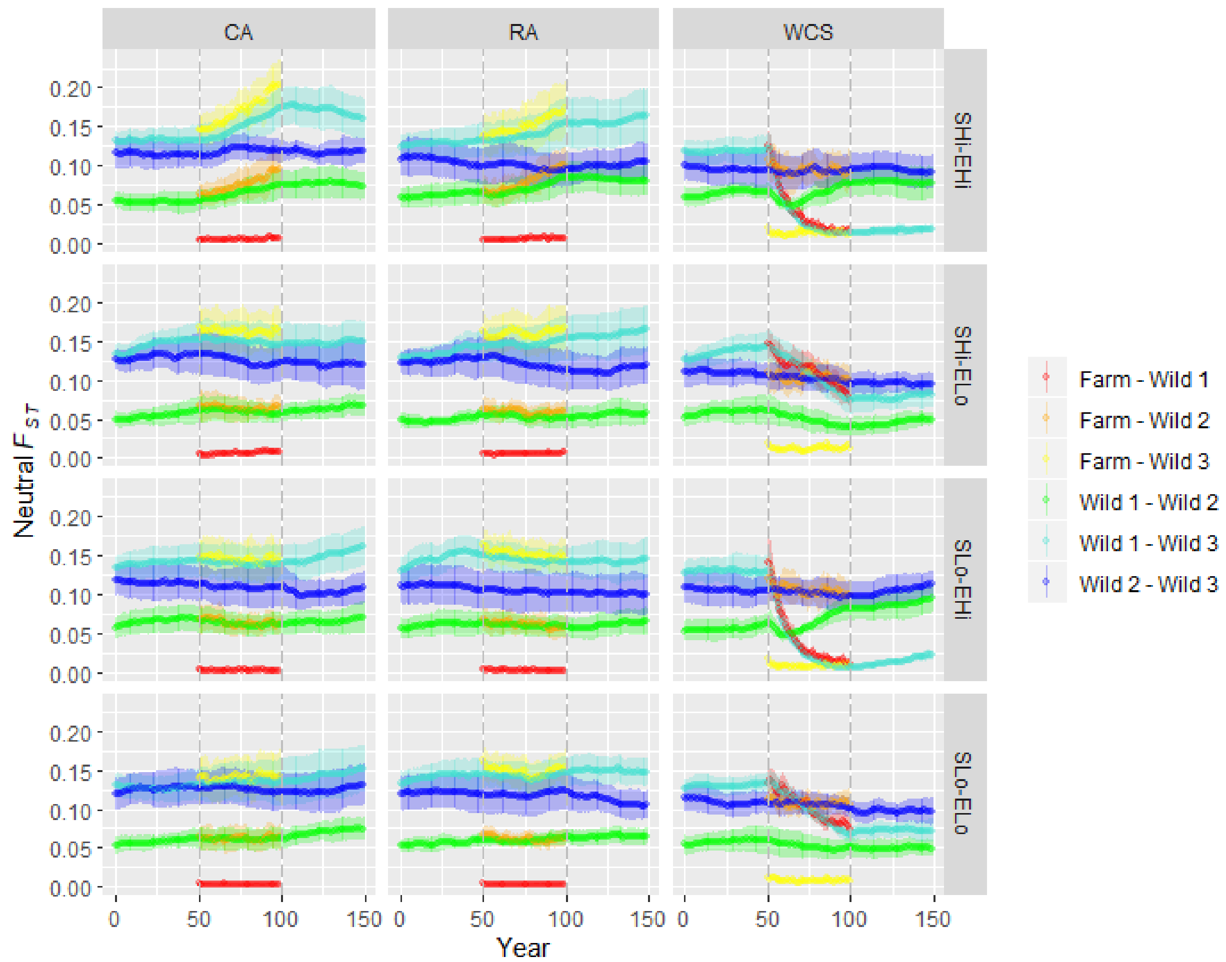
Supplemental Figure 4-3: Mean population size (and 95% confidence interval) over time, per subpopulation, faceted by scenario theme and conditions. CA = Commercial Aquaculture, RA = Restoration Aquaculture, and WCS = Worst Case Scenario. S = selection strength, E = escape rate, Hi = high, and Lo = low, such that SLo-ELo = Low Selection and Low Escape conditions. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



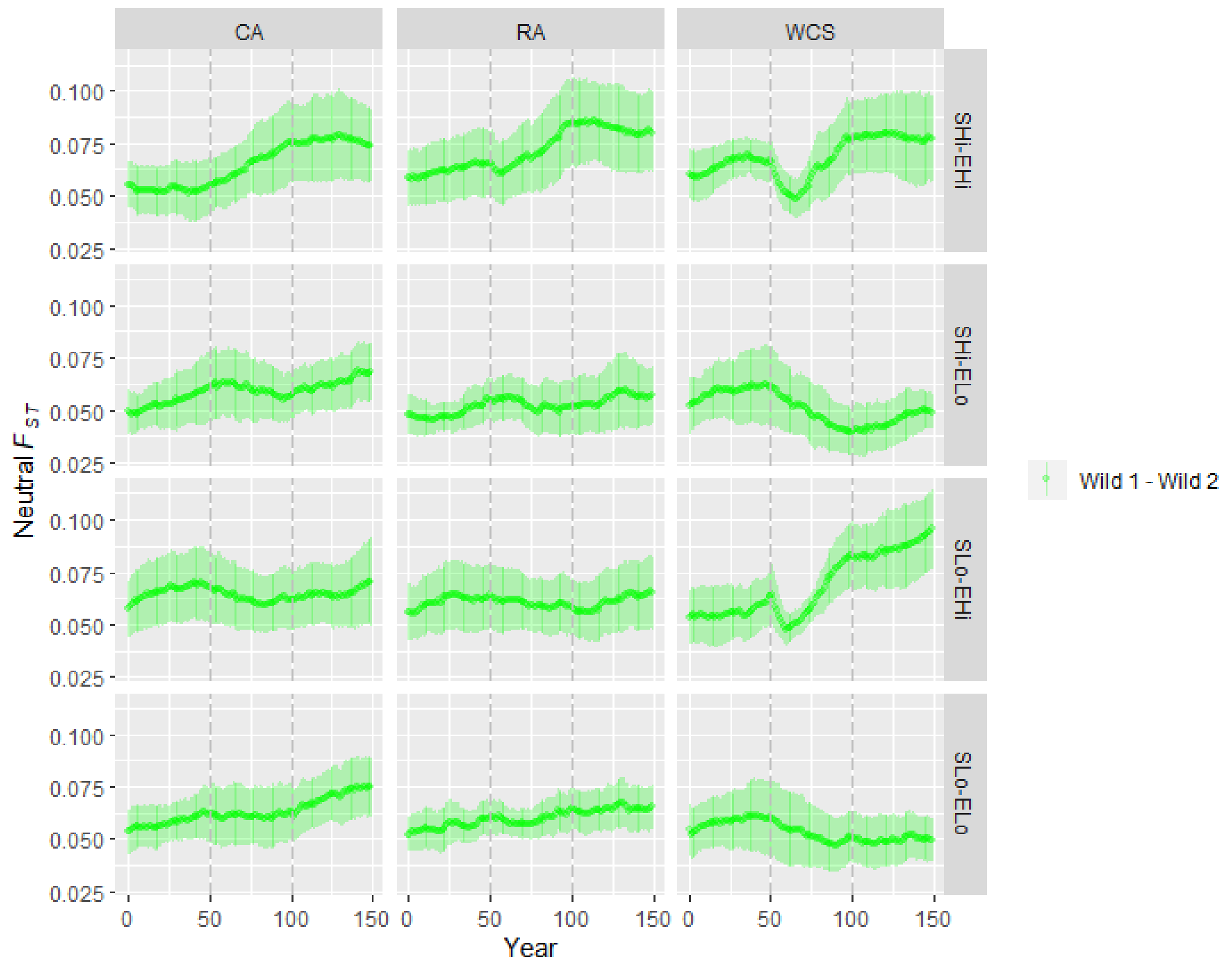
Supplemental Figure 4-4: Mean heterozygosity (and 95% confidence interval) over time, per subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



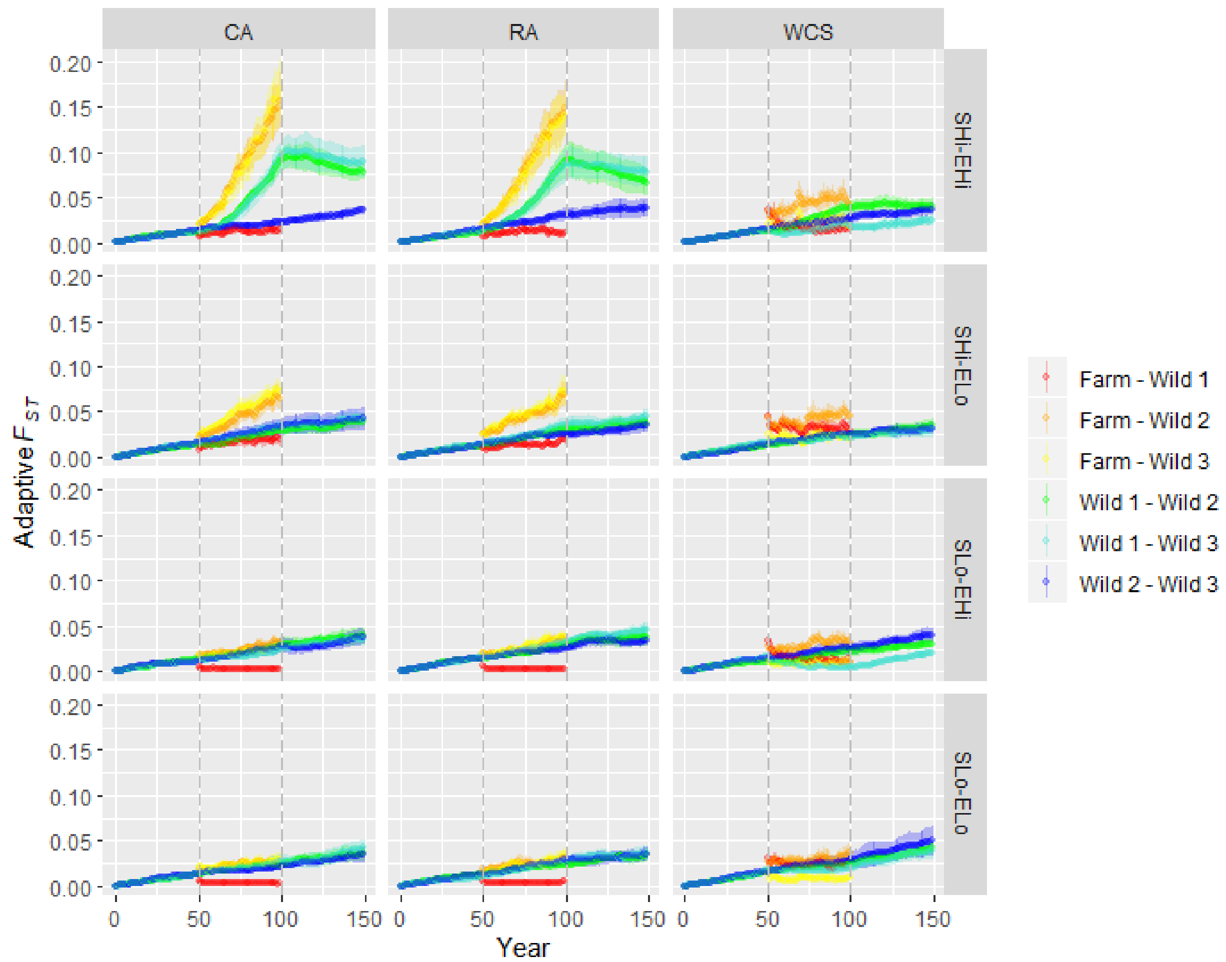
Supplemental Figure 4-5: Mean log10 allelic richness (and 95% confidence interval) over time, per subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



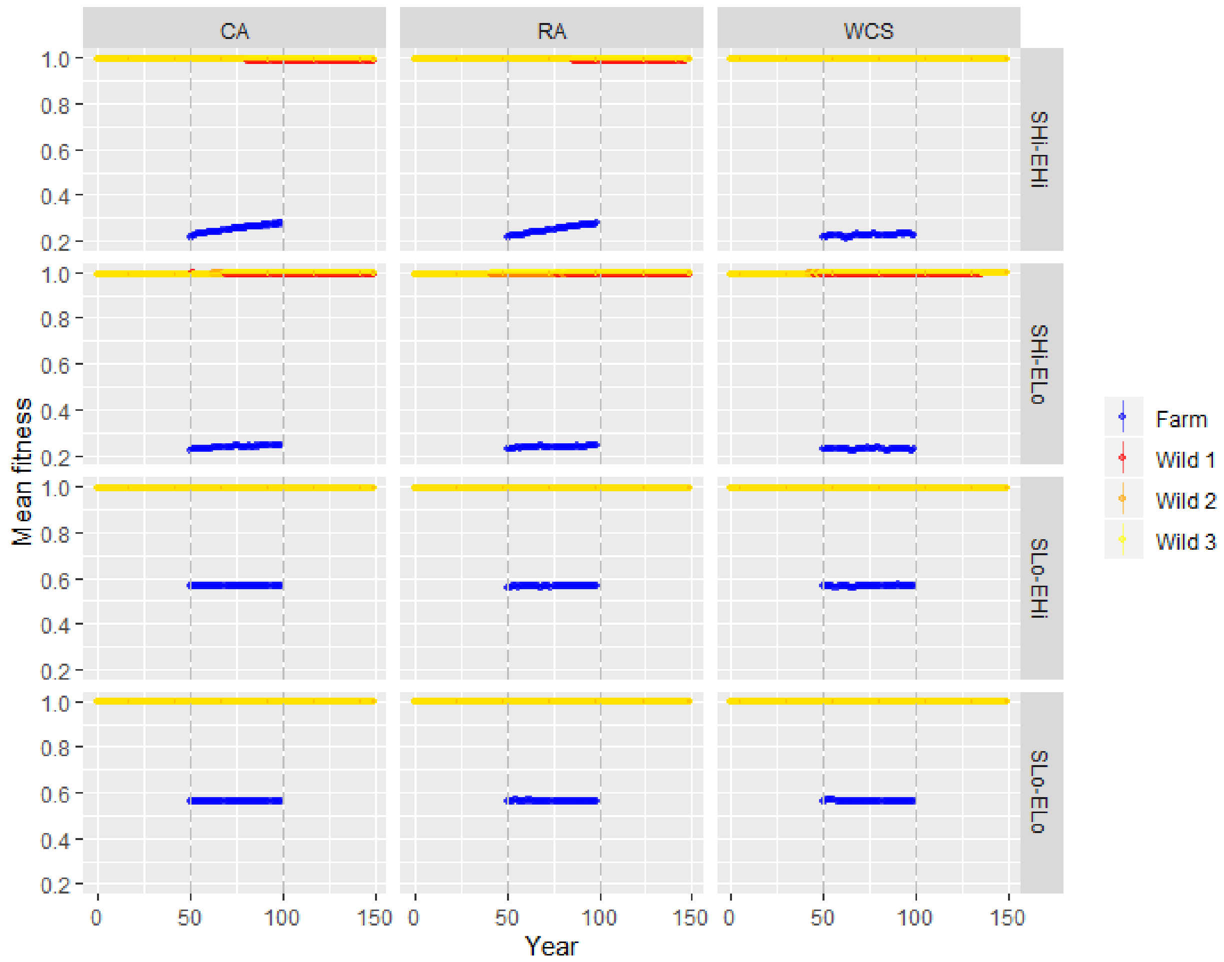
Supplemental Figure 4-6: Mean neutral F_{ST} (and 95% confidence interval) over time, per subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



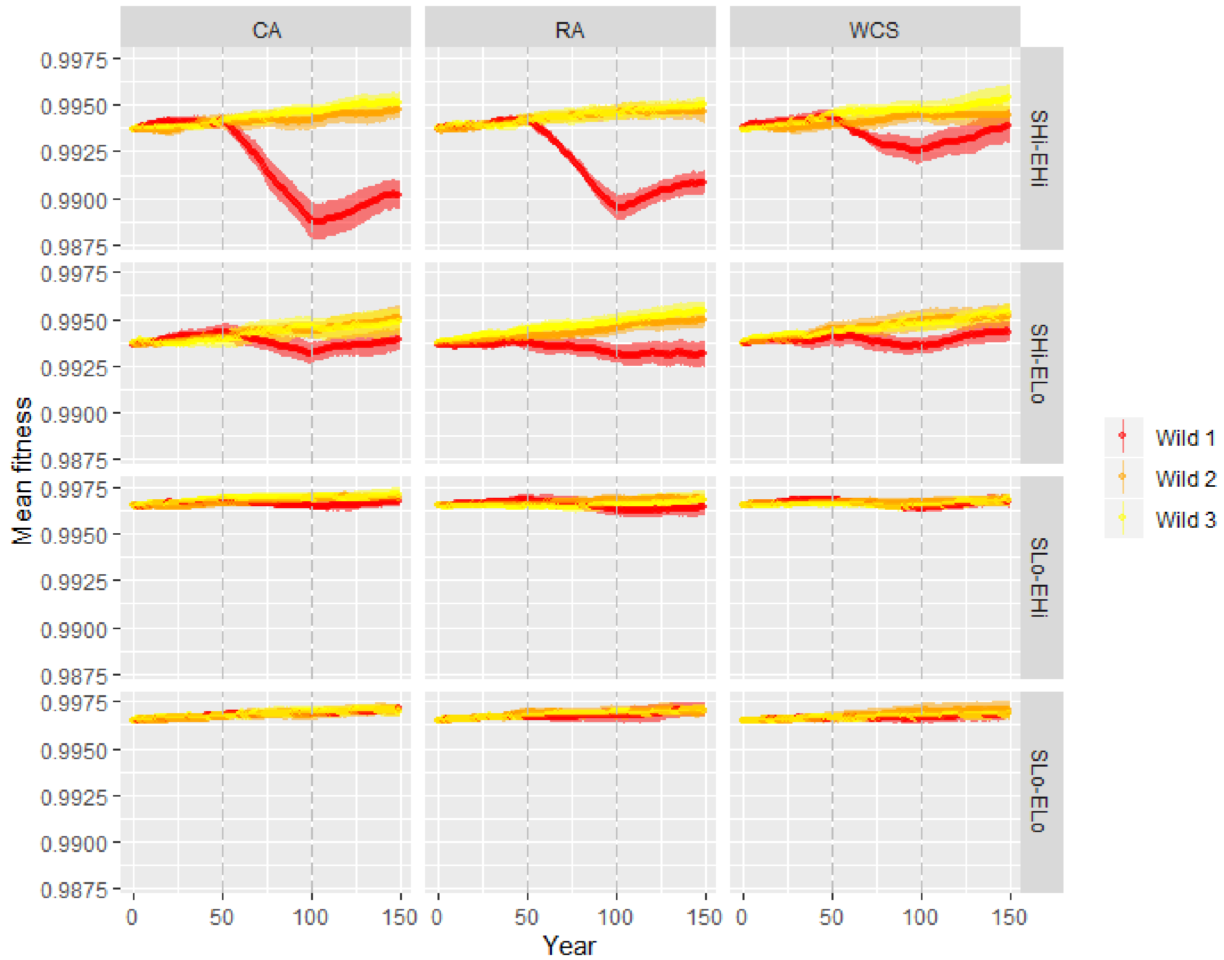
Supplemental Figure 4-7: Mean neutral F_{ST} (and 95% confidence interval) over time between Wild 1 and Wild 2, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



Supplemental Figure 4-8: Mean adaptive F_{ST} (and 95% confidence interval) over time by subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



Supplemental Figure 4-9: Mean fitness (and 95% confidence interval) over time, by subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).



Supplemental Figure 4-10: Mean fitness (and 95% confidence interval) over time, by wild subpopulation, faceted by scenario theme and conditions. Scenario theme and conditions key in the Supplemental Figure 4-3 caption. Dashed gray lines represent the introduction of the farm (year 50) and removal of the farm (year 100).

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