Population Structure in Puget Sound Rockfishes (*Sebastes* spp.): Using Genetics To Inform Management Of A Vulnerable Species Complex

Anita Wray

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2022

Committee:

Lorenz Hauser, Chair

Eleni Petrou

Krista Nichols

Tim Essington

Program Authorized to Offer Degree:

School of Aquatic and Fishery Sciences

University of Washington

Abstract

Population Structure in Puget Sound Rockfishes (*Sebastes* spp.): Using Genetics To Inform Management Of A Vulnerable Species Complex

Anita Wray

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

Successful management relies on the correct identification of management units. Quantifying the connectivity between populations is a critical factor in defining management units and improving fishery plans. Despite stock recoveries along the US West Coast, rockfish populations in Puget Sound continue to decline, and three species were listed under the Endangered Species Act. These management measures were primarily based on data from a hybridizing species complex (Brown-Copper-Quillback Rockfish), which identified Puget Sound as a distinct population from the Washington Coast. It is unknown whether the population structure identified in previous studies is due to extensive hybridization in the region. Additionally, recent studies have suggested that there is species specific population structure for rockfish in Puget Sound. Population structure in the remaining species, and the factors determining population differentiation within Puget Sound are therefore unknown. Therefore, the overall goal of this thesis was to investigate population structure and the extent of hybridization of Puget Sound rockfishes. To accomplish this goal, fin clips from eight rockfish species (Brown (*Sebastes caurinus*), Copper (*S. auriculatus*), Quillback (*S. maliger*), Black (*S. melanops*), Yellowtail (*S. flavidus*), Redstripe (*S. proriger*), Greenstriped (*S. elongatus*), and Puget Sound (*S. emphaeus*)) were sequenced using reduced representation sequencing (RADseq), resulting in over 10,000 loci per species. First, we identified the geographic and genetic extent of hybridization for the Copper-Quillback-Brown species complex. We found that hybridization is low-level and asymmetric from Quillback into Copper and Brown, and localized in south Puget Sound. Hybridization inflated estimates of species-specific population structure in Brown and Copper Rockfish. Second, we estimated population structure and connectivity for five common species of rockfish. We identified no structure in Black and Puget Sound Rockfish, temporal structure in Redstripe Rockfish and geographic structure in Yellowtail and Greenstriped Rockfish. There was no clear effect of life history on geographic structure, but oceanography and temporal variation were evident in our results. Of the eight species analyzed, no species followed the current DPS boundaries that they are managed by. Finally, we discuss the management implications of these studies and suggest future directions for management of this species complex.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTAL FIGURES

LIST OF SUPPLEMENTAL TABLES

ACKNOWLEDGEMENTS

Scientific research is highly collaborative, and many, many people helped me get to where I am today. First, I'd like to thank my advisor, Lorenz Hauser, who provided countless hours of bioinformatics support as we trudged through heaps of initially strange looking data. Taking on a graduate student in the midst of a global pandemic is quite a challenge, and it has been a privilege to work alongside him. I am grateful for his editing skills which significantly improved my scientific writing along the way.

I'd like to thank all of my collaborators and committee members, whom this project would've never been completed without their work. To Bob Pacunski and the entire WDFW groundfish team, I thank you for your deep passion for rockfish conservation and allowing me to join in on a handful of sampling trips. To Krista Nichols, Eleni Petrou and Tim Essington who served on my committee and provided countless edits to every part of this project. To the entire MERlab, but in particular Eleni Petrou, Carolyn Tarpey and Bryan Briones Ortiz who were invaluable in data preparation and analysis and provided steadfast support through many open doors and Zoom calls. Finally, to SAFS for providing a community of passionate scientists who I continue to learn from.

I am eternally grateful to my community at Washington Sea Grant, in particular Maile Sullivan who not only trusted me to lead educational programming for two years but continued to open doors for me and connect me with some of the greatest coworkers I could've ever asked for. I have grown so much as an educator and person through working with her and cannot wait to see where our paths meet up next.

I'd be nowhere without the everlasting support of my family and friends, in particular my parents who not only provided emotional support but edits at every point of my graduate degree from my application letter to my final thesis. I am truly so grateful to have parents like them.

Chapter 1. CONTRASTING EFFECT OF HYBRIDIZATION ON GENETIC DIFFERENTIATION IN THREE ROCKFISH SPECIES WITH SIMILAR LIFE HISTORY

Authors: Anita Wray, Eleni Petrou, Krista Nichols, Robert Pacunski, Larry LeClair, Kelly Andrews, Lorenz Hauser

1.1 ABSTRACT

Hybridization can provide evolutionary benefits (e.g., population resilience to climate change) through the introduction of adaptive alleles and increase of genetic diversity. Nevertheless, management strategies may be designed based only on the parental species within a hybrid zone, without considering the hybrids. This can lead to ineffective spatial management of species, which can directly harm population diversity and negatively impact food webs. Three species of rockfish (Brown (*Sebastes caurinus*), Copper (*S. auriculatus*), and Quillback Rockfish (*S. maliger*)) are known to hybridize within Puget Sound Washington but are used to infer population structure in the entire genus, including species that do not hybridize. The goal of this project was to estimate the hybridization rates within the region and untangle the relative effect of hybridization on population genetic structure. We sequenced 290 Brown, Copper and Quillback Rockfish using restriction-site associated DNA sequencing (RADseq) from four regions within and outside Puget Sound, Washington. We showed that (i) hybridization within Puget Sound is unidirectional, ancient, widespread, and relatively low level, (ii) hybridization affected population structure in Copper and Brown Rockfish, but not in Quillback Rockfish and (iii) after taking

hybridization into account, Quillback had two distinct populations, Copper had one, and Brown had three. Our results suggested that rockfish population structure is species-specific and cannot be inferred from one species to another, especially in species that hybridize.

1.2 INTRODUCTION

Hybridization between closely related species is known to play an important role in population structure and evolution (Arnold, 1992). In terrestrial species, natural hybridization often occurs at the periphery of a species range, in areas with empty niches or low species richness, or where there is a biogeographical barrier (Arnold & Fogarty, 2009). These hybrid hotspots are often regions where individuals of mixed ancestry can persist for multiple generations. On one hand, hybridization can have detrimental effects through outbreeding depression, gene swapping, and genetic assimilation (Hails & Morley, 2005). On the other hand, hybridization can be an important evolutionary process that may increase overall genetic diversity (Hamilton & Miller, 2016), transfer adaptive alleles among species (Hedrick, 2013) and may be instrumental in the evolution of species flocks (McCartney et al., 2003; Salzburger et al., 2002). Hybridization may also increase adaptive potential in the face of changing environments (Arnold et al., 2008; Hamilton & Miller, 2016). Hybrid individuals can provide a space to maintain genetic diversity, especially where parental species are at risk of extinction (Soulé, 1985).

Despite the evolutionary benefits of natural hybridization, management regarding hybrids still remains uncertain (Allendorf et al., 2001). In the United States, there is no robust policy regarding the conservation of hybrids and their protection is still assessed on a case-by-case basis. Additionally, management of the parental species can become complex, as hybrid zones contain individuals with alleles from both (or multiple) parental species. Therefore, localized hybridization may inflate estimates of genetic differentiation of the parental species. This overestimate of genetic differentiation may, in turn, lead to the underestimation of connectivity and misconstrue population boundaries. If the extent of hybridization in a geographic region is unknown or underestimated, management units for the parental species may be misidentified. Untangling the relative contributions of hybridization and within-species connectivity is therefore critically important for the accurate identification of management units.

Most research regarding natural hybridization has been focused on terrestrial plants and animals, and in species where hybrids are easily distinguishable. Although originally thought to occur less often in the marine environment, hybrids have now been documented in a wide range of marine plant and animal taxa (Arnold & Fogarty, 2009). To date, there are over 111 examples of hybridization in 173 marine fish species (Montanari et al., 2016). Three instances of marine hybridization have been identified in rockfishes (genus *Sebastes*) (Muto et al., 2013; Roques et al., 2001; Seeb, 1998), a highly diverse genus of over 110 described species, 90 of which are found on the eastern Pacific Ocean (Love et al., 2002). Originating in the late Miocene, the genus *Sebastes* underwent a rapid radiation event creating many sister and cryptic species (Hyde & Vetter, 2007). One well known example of hybridization within *Sebastes* was identified between Copper, Brown, and Quillback Rockfish (*S. caurinus*, *S. auriculatus,* and *S. maliger*) collected from Puget Sound, Washington (Seeb, 1998). Buonaccorsi et al. (2002, 2005) and Schwenke et al. (2018) found that introgression, i.e., the persistent gene flow of one species into another through repeated backcrossing, occurs within the Copper-Brown-Quillback species complex. However, introgression appears to be asymmetric, from Quillback into both Brown and Copper Rockfishes (Schwenke et al., 2018). Additionally, this hybridization is ancient, as there is little evidence of early generation hybrids from studies using allozyme, microsatellite, or few nuclear and mitochondrial loci (Buonaccorsi et al., 2002, 2005; Schwenke et al., 2018; Seeb, 1998). As these studies identified hybrids from a small number of loci, the extent of introgression and frequency of hybrids is unknown.

In addition to detecting hybridization among Puget Sound rockfishes, previous studies also found evidence of within-species population structure; specifically, Brown, Copper, and Quillback Rockfish from the Puget Sound estuary were found to be genetically distinct from populations on the Pacific coast of Washington State (Buonaccorsi et al., 2002, 2005; Seeb, 1998). Based in part on this study, two distinct population segments (DPS) within the Puget Sound/Georgia Basin ecosystem for each of the three species were identified (Stout et al., 2001). Based entirely on this determination, the same DPSs were identified for five other rockfish species, three of which were listed under the Endangered Species Act as threatened or endangered (Drake, Berntson, et al., 2010). However, a recent genomic study (Andrews et al. 2018) revealed a lack of population structure in one of those species (Canary Rockfish, *S. pinniger*) which was delisted as a result. It is therefore possible that genetic population structure inflated by localized hybridization in Copper, Brown and Quillback leads to the false inference of management units in other rockfish species. The goal of this study was to investigate the effect of interspecific hybridization and introgression in Puget Sound on genetic population structure in Brown, Copper, and Quillback Rockfishes. Specifically, we 1) quantified the extent of hybridization between species throughout Puget Sound, 2) estimated within-species population structure and connectivity to identify distinct population segments, and 3) evaluated the effect of hybridization on population structure.

1.3 METHODS

1.3.1 Sampling Procedure

We used 290 samples from three species of rockfish (Brown, Copper, and Quillback) that were previously collected by Washington Department of Fish and Wildlife (WDFW), The Northwest Fisheries Science Center of the National Oceanographic and Atmospheric Administration, National Marine Fisheries Service (NOAA, NMFS), and Department of Fisheries and Oceans (DFO Canada) from 1999-2021 (Figure 1.1). Tissue was clipped from individual fins and either preserved in 95% ethanol or dried. Samples were collected from four different regions: 1) southern Puget Sound (Puget Sound proper, south of Admiralty Inlet, SPS), 2) British Columbia (Canadian Salish Sea north of US/Canada border, BC), 3) northern Puget Sound (US Salish Sea north of Admiralty Inlet, south of the US/Canada border and east of the Victoria Sill, NPS) and 4) the US west coast (US Pacific Coast west of Victoria sill, WC) (Figure 1.1). We also re-sampled 24 individuals from a study on rockfish hybridization that were identified as 'pure' parental species, including seven Brown, five Copper, and twelve Quillback Rockfish (Schwenke et al., 2018). Due to differences in the abundance and distribution of species across this geographic range, Brown Rockfish could only be sampled in three of these locations and the WC samples were collected in Mexico and California.

1.3.2 DNA Extraction, Library Preparation, and Sequencing

Genomic DNA was extracted using the Nexttec DNA isolation kit (Nexttec Incorporated, Middlebury, VT, USA) following manufacturer's protocol and quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA, USA). DNA concentration was normalized to 125ng in 10 µL of molecular grade water. Restriction site-associated DNA sequencing

(RADseq) libraries were prepared using a version of the Ali et al. (2016) protocol without the targeted bait capture step, referred to in the literature as BestRAD (https://github.com/merlab-uw/Protocols/blob/main/bestRAD). Briefly, genomic DNA was digested using the *Sbf*I enzyme. An adapter (P1) containing a forward amplification primer site, an Illumina sequencing primer site, and an individual 6bp barcode was ligated to each fragment at the restriction site end. Fragments were then randomly sheared using sonication and size-selected to 300-500 bp in length. Subsequently, P2 adapters were ligated to the reverse end and libraries were amplified by PCR. Each library was assessed for quality on a 1% agarose gel and a Bioanalyzer DNA 1000 kit (Agilent Technologies, Santa Clara, CA). Libraries were pooled in equimolar amounts and sequenced on a NovaSeq (paired end, 116 bp) at the University of Oregon. Samples were sequenced, paired end, on either a S4 or SP run type. Individuals were assigned to one of six RADseq libraries randomly to avoid any lane effect. The libraries included 96 individuals each from eight species of rockfish (three species are included in this chapter, five are included in Chapter 2).

1.3.3 Initial Filtering

Raw sequence data were quality checked using fastQC v0.11.9 (Andrews, 2010) and visualized in multiQC (Ewels et al., 2016). Prior to SNP calling and genome alignment, the raw sequences were demultiplexed using *process_radtags* in STACKS v2.60 (Catchen et al., 2011; Rochette et al., 2019). Here, sequences were trimmed to 104 bases and filtered for quality. Individuals with fewer than 250,000 total reads were excluded from downstream analysis (Krohn et al., 2018). Our paired-end sequences were then aligned to the Honeycomb Rockfish (*Sebastes umbrosus*) genome from GenBank (NCBI Accession Number: PRJNA562243) with Bowtie2 v2.4 using the ‘very-sensitive’ option (Langmead & Salzberg, 2012). At the time of data analysis for this study, the Honeycomb Rockfish genome was one of only two annotated full genomes and was chosen due to its closer phylogenetic relationship to our species (Hyde & Vetter, 2007). Following genome alignment, SNP calling, and basic population genetics statistics were calculated using the *gstacks* and *populations* modules from the STACKS pipeline. SNPs were called if they had a minimum mapping quality of 40. SNPs were filtered following published recommendations (O'Leary et al., 2018) requiring that loci meet the following criteria: minimum genotype depth ≥ 5, mean minimum read depth ≥ 15, genotype call rate ≥ 80%.

For the **intraspecific analyses**, we chose the SNP on each RADtag with the highest minor allele frequency. Choosing one SNP per RADtag was done after the above filtering, to ensure we were choosing high quality SNPs. SNPs with genotype frequencies that were significantly different than expectations under Hardy-Weinberg Equilibrium (HWE) were removed using the following procedure: P-values were calculated across samples for each population using the R package pegas v1.1 (Paradis, 2010). P-values were then combined for each locus using Fisher's combination of probabilities. Values were then adjusted to q-values for the false discovery rate (Benjamini & Hochberg, 1995). Loci with q-values below 0.05 were considered significantly out of HWE and removed from downstream analysis. For the **interspecific analyses**, we intended to avoid SNPs with fixed differences between species, because this would likely find differences between only two species. Due to this strategy, most of the SNPs are different between the two datasets. To accomplish this, we chose the first SNP on each RADtag using the –write-single-snp option in populations. Additionally, we did not filter for HWE as the Wahlund effect (a reduction of heterozygosity due to species specific (subpopulation) structure) would likely influence HWE p-values. For both datasets, genetic relatedness was calculated using an identity by descent estimate on all pairs of individuals within PLINK v1.07 (Purcell et al., 2007) to evaluate if our dataset included highly related individuals. F_{IS} was also calculated using vcftools v0.1.16 (Danecek et al., 2011). Final datasets were converted from vcf to other formats (GenePop and STRUCTURE) using PGDSpider v2.1.1.5 (Lischer & Excoffier, 2012).

1.3.4 Analyses – Interspecific

To visualize interspecific population structure patterns and identify individuals with mixed ancestry, we used principal components analyses (PCA) and STRUCTURE analysis, the two most commonly used approaches to describe population structure (Liu et al., 2020). First, to identify evidence of recent hybridization, we conducted PCA using the R package adegenet v2.1.8 (Jombart, 2008) and visualized the results using ggplot2 v3.3.6 (Wickham, 2016). Second, we used STRUCTURE v2.3.4 to identify admixture (Pritchard et al., 2000). Two replicates were run for 1-10 K clusters with a burn-in period of 10,000 iterations and 100,000 MCMC repetitions. STRUCTURE was run without a priori population knowledge and using the admixture model. We identified the range of likely K groups with Structure Harvester (Earl & vonHoldt, 2012), using the ΔK statistic (Evanno et al., 2005) and using the mean L(K).

1.3.5 Analyses – Intraspecific

We identified intraspecific population structure using the above two methods (PCA and STRUCTURE) in addition to pairwise and overall F_{ST} calculations. Pairwise F_{ST} values (Weir & Cockerham, 1984) were estimated between all four sampling locations with 1,000 bootstraps using the R package hierfstat v0.5-11 (Goudet, 2005). Values were considered significant if the lower limit of the 95% bootstrap confidence interval did not overlap with zero. Overall F_{ST} values (Nei, 1987) were estimated using the R package hierfstat v0.5-11 (Goudet, 2005). To determine the effect of hybridization on population structure, we re-ran the PCA and F_{ST} analyses with 'pure' individuals from each species. Individuals were determined ‘pure’ from STRUCTURE analyses if they had 0-1% ancestry from another species. We also grouped admixed individuals in three categories, high (>6% mixed ancestry), medium (1-6%), and low (>1%). Pairwise F_{ST} values were calculated between each group and a pure population, to estimate the correlation between percent admixture and genetic divergence. Additionally, we correlated percent admixture ancestry with each of the genetic groups identified in STRUCTURE in the intraspecific analysis to determine if there was a correlation between hybrid ancestry and intraspecific STRUCTURE clustering. Finally, to rule out the effect of linkage on any identified population structure, we estimated linkage disequilibrium (r^2) within each chromosome using PLINK v1.07 (Purcell et al., 2007). R^2 values were then mapped on each chromosome to identify blocks of highly linked loci using the R function LDheatmap v1.0-6 (Shin et al., 2006). Chromosomes with loci in strong LD ($r^2 > 0.5$) blocks were analyzed separately used PCAs in adegenet v2.1.8 (Jombart, 2008) to identify whether individuals clustered in the three-stripe patterns consistent with chromosomal inversions.

1.4 RESULTS

1.4.1 Interspecific Analysis

We retained, on average, 11.7 million, 18.0 million, and 18.7 million reads per individual for Brown, Copper, and Quillback Rockfish, respectively. From these retained reads, we identified 12,708 SNPs after filtering that were genotyped in more than 80% of all individuals across the three species ($N_{Quillback}$ = 87; N_{Brown} = 37, N_{Copper} = 90). Twenty individuals differed in morphological and genetic species identification, suggesting misidentification in the field; they were thus removed from later analyses. Visualization of the misidentified samples using a PCA suggested they were not hybrid individuals. Admixture analysis with STRUCTURE revealed low levels of introgression from Quillback Rockfish into both Brown and Copper Rockfish (Figure 1.2). All Brown Rockfish (100%) collected from SPS had low (~5%) Quillback ancestry. We identified fewer Brown Rockfish with Quillback ancestry from NPS, and none from the WC. Copper Rockfish had higher degrees of Quillback ancestry, with 92% of samples in SPS showing >10% Quillback ancestry. Although less common, Copper Rockfish with mixed ancestry were also found in NPS, WC and BC collections (50%, 28%, 8% of samples, respectively). One Copper Rockfish sample from BC showed evidence of recent hybridization (48% Quillback, 51% Copper, Figure 1.2), and was an outlier in the PCA plot. We also identified three individuals with mixed ancestry patterns (1 Brown and 2 Copper) that were identified as 'pure' in a previous study (Schwenke et al., 2018). None of our Quillback Rockfish samples were identified as having >5% hybrid ancestry. Our principal components analysis of the three species showed three distinct clusters of species, and the suspected hybrid intermediate located between the Quillback and Copper cluster (Figure 1.2).

1.4.2 Intraspecific Analysis

In **Copper Rockfish**, 516,618 SNPs were identified in STACKS and 21,150 SNPs were retained after filtering. Out of the 12,708 SNPs in the interspecific dataset, 17% of SNPs were shared in this dataset. We removed 24 individuals from downstream analyses, ten as a result of low read count and fourteen as a result of disagreement between genetic and morphological identification. In Copper Rockfish, principal components analysis revealed four clusters; the majority of individuals collected from BC and SPS formed distinct groups, while individuals collected from the WC and NPS were distributed across all four clusters (Figure 1.3). STRUCTURE plots followed similar patterns; BC and SPS individuals mostly formed two populations, while NPS and WC shared similar mixed ancestry patterns. F_{ST} values ranged from

0.002 from the NPS-WC comparison to 0.085 from the SPS-BC comparison (Table 1.1). All pairwise F_{ST} values were significant for all population comparisons.

We identified similar patterns between the intra and interspecific STRUCTURE plots (Figure 1.2B and 1.3B). To identify if this pattern was significant, we correlated admixture from Figure 1.2B with the two non-dominant clusters in Figure 1.3B. We found a strong correlation ($r^2 = 0.93$, Figure 1.4) between percent admixture and divergence from the first K group, suggesting that hybridization influences the observed structuring pattern. To determine the effect of hybrid individuals on population structure, we reanalyzed the data after removing the individuals with mixed ancestry >5%. From these analyses, F_{ST} values were not significant for the WC-NPS, NPS-BC, and WC-BC comparisons. Our correlation between admixture and F_{ST} revealed a weak positive but non-significant correlation between the degree of interspecific admixture and population divergence for Copper Rockfish ($r^2 = 0.201$, $p = 0.16$, Supplemental Figure 1.3) for all populations.

In **Brown Rockfish**, 279,876 SNPs were identified in STACKS and 10,055 were retained after filtering. Out of the 12,708 SNPs in the interspecific dataset, 27% of SNPs were shared in this dataset. We removed fourteen individuals from downstream analyses, eight because of low read count and six as a result of disagreement between genetic and morphological identification. Principal components analysis revealed three clusters: SPS, WC, and most of NPS formed three separate clusters. One NPS individual clustered with each of the SPS and WC groups (Figure 1.5). PCAs using axes 3 and 4 showed no additional structure (Supplemental Figure 1.2). Analysis using STRUCTURE suggested that there were two genetic clusters (K=2, Supplemental Figure 1.1), which identified SPS and WC as separate populations that mixed in NPS. This suggests weak population boundaries between NPS and SPS, and NPS and WC but little to no mixing between

SPS and WC (Figure 1.8). Pairwise population F_{ST} values were significant for all population comparisons and ranged from 0.05 in the NPS-SPS comparison to 0.18 in the SPS-WC comparison (Table 1.1). Population based summary statistics are described in Table 1.2, which identified a high F_{IS} value for NPS. This high F_{IS} value could imply the Wahlund effect, which suggests two genetically differentiated populations are present in the NPS collection.

When only **Quillback Rockfish** were used for SNP discovery, 607,854 SNPs were identified in STACKS and 8,525 SNPs were retained after filtering. Out of the 12,708 SNPs in the interspecific dataset, 32% of SNPs were shared in this dataset. We removed 20 individuals from our dataset as a result of low read count. No Quillback Rockfish were removed due to misidentification. Principal components analysis revealed that SPS and WC formed a single cluster, and NPS and BC formed a second cluster (Figure 1.6). Analysis using STRUCTURE suggested that there were two genetic clusters (K=2, Supplemental Figure 1.1), which followed the same patterns as the principal components analysis. Evidence for dispersal was present, as four individuals from WC and two individuals from NPS were in the opposite cluster. Despite these migratory individuals, limited evidence for mixed ancestry between populations was identified. F_{ST} values ranged from 0 for the SPS-WC comparison to 0.01 for the SPS-BC comparison (Table 1.1). F_{ST} values were significant for all population comparisons except the SPS/WC and NPS/BC comparisons.

1.4.3 Chromosomal Inversion Analysis

To rule out alternative causes for population structuring, we investigated linkage disequilibrium which identified 22 total LD blocks (Supplemental Figures 1.5-1.18). We identified eleven in Brown Rockfish, ten in Copper Rockfish, and one in Quillback Rockfish. No two species shared the same LD block. Principal components analysis of two LD blocks, one in Brown and one in Quillback, revealed a three-banding pattern (Figure 1.7) consistent with chromosomal inversions, where the three bands show the three genotypes for the inversion. We identified no change in the PCA clustering patterns if the inversions were incorporated into the analyses or not (data not shown). Additionally, we found no geographic patterns in inversion phenotypes.

1.5 DISCUSSION

Determining the extent of hybridization in Puget Sound rockfishes is a key step towards quantifying the relationship between hybridization and population structure. This information is also critical for the accurate conservation and management of these species. Our results suggest three independent impacts of hybridization on population structure. The interspecific analysis suggested that 1) hybridization is unidirectional and ancient, 2) hybrids largely remain in south Puget Sound, and 3) hybridization is more common but less intensive than previously reported. Population structure was completely different for the three rockfish species. For Brown Rockfish, we found three distinct clusters separating WC, NPS and SPS. For Copper Rockfish, we found four distinct clusters with similar ancestry patterns in WC and NPS and evidence of hybridization influencing population structure. Finally, we found two distinct clusters (BC/NPS and SPS/WC) for Quillback Rockfish. This difference in population structure may in part be attributable to hybridization but may also have other causes.

1.5.1 Interspecific Hybridization

Our results provided evidence for unidirectional introgression of Quillback Rockfish into Brown and Copper Rockfish. Previous studies investigating introgressive hybridization among Brown, Copper, and Quillback Rockfish were able to identify hybrid Quillback individuals, suggesting that introgression is asymmetric instead of unidirectional (Schwenke et al., 2018; Seeb, 1998). The apparent lack of Quillback hybrids in our dataset could be due to sampling differences in our study. In Schwenke et al (2018), Quillback hybrids were identified south of the Tacoma narrows (20% hybrid individuals) and in the Whidbey Basin (19% hybrid individuals). Seeb (1998) identified Quillback hybrids in south Puget Sound, over 50 km south of our south Puget Sound sampling site. Of our samples for Quillback, only one came from south of the Tacoma narrows and our Whidbey Basin fish were identified as 'pure' individuals by Schwenke et al (2018). The lack of Quillback hybrids in our dataset further argues that the proportion of Quillback hybrids increases with geographic isolation from the outer coast (Schwenke et al., 2018). Two separate shallow sills separate Whidbey Basin and south Puget Sound (Moore et al., 2008), which have the potential to increase frequency and retain hybrids within these isolated bays. More expansive sampling of Quillback Rockfish within southern Puget Sound can help determine if Quillback hybridization is localized to specific regions of the Sound.

Consistent with Schwenke et al (2018), we identified uneven introgression rates, as Copper Rockfish had a higher degree of admixture when compared to Brown Rockfish. Work prior to Schwenke et al (2018) using microsatellites detected high degrees of hybridization in Brown Rockfish (Buonaccorsi et al., 2005) but little evidence for introgressed alleles in Copper Rockfish (Buonaccorsi et al., 2002). Despite the wide applicability of microsatellites to determine population structure, some studies suggest that microsatellites cannot distinguish between introgressed individuals and their parental species (Melville et al., 2017; Nielsen et al., 2014) due to extensive homoplasy (Henriques et al., 2016). For example, a study between two sister species of groupers found complete isolation in one microsatellite locus, and incomplete linage sorting in the other (van Herwerden et al., 2006). This could potentially lead to the identification of false hybrids, and/or the misidentification of pure individuals which would drastically alter the degree of hybridization reported in a study.

The widespread but low level of Quillback introgression we detected in Brown and Copper Rockfish contrasts with previous reports. This discrepancy in our study could be due to the better genomic coverage in our dataset, given that ancient hybridization is unlikely to have significant temporal variation. Hybrid identification has been shown to depend on the number and type of markers used (Henriques et al., 2016). In addition, previous results using fewer loci have wide ranges in the degree of hybridization. For example, studies have reported either 0% (Buonaccorsi et al., 2002), 43% (Schwenke et al., 2018), or 96% (this study) hybrid Copper individuals within the same geographic region (south of Admiralty Inlet, north of Tacoma Narrows), and varying degrees of admixture between each study. Utilizing genomic data with thousands of loci has been shown to be more powerful in detecting low levels of introgressive hybridization, especially in systems with ancient introgression (Szatmári et al., 2021). Additionally, by using many more loci our results depend less on whether a locus is within an introgressed segment. This increase in loci number can be leveraged to calculate a much higher resolution to the degree of admixture across individuals and geographic regions.

Another explanation for the higher degree of hybridization in previous studies could be adaptive introgression. For example, the two loci (one mitochondrial and one nuclear) in the Schwenke et al (2018) study that detected hybridization could be preferentially selected for in the introgressed individuals. Introgression has long been identified as a source of adaptive genetic variation driving pesticide resistance in mice (Song et al., 2011) and beak traits in Darwin’s finches (Grant & Grant, 2010). More research is needed to identify if adaptive introgression is occurring in Brown and Copper Rockfish.

Results from our study identified that hybridization is concentrated with south Puget Sound, with evidence of limited dispersal into the north Puget Sound and the Washington Coast. These results add to the growing evidence that hybridization events are isolated in south Puget Sound (Schwenke et al., 2018; Seeb, 1998). Three primary potential explanations have been hypothesized for the presence of introgression in Puget Sound for Brown, Copper, and Quillback Rockfish: habitat availability, anoxic conditions, and differences in population size (Schwenke et al., 2018). First, there is much less suitable rocky habitat in south Puget Sound compared to the northern and coastal areas (Miller & Borton, 1980). As Brown, Copper, and Quillback Rockfish rely on these habitats as adults, this limited habitat availability increases overlap between species, thus increasing hybridization potential. In addition to habitat reduction, the Puget Sound experiences sporadic hypoxic conditions (Walt Deppe et al., 2013). This depletion of available oxygen forces species into shallower habitats, where oxygen concentrations are higher (Eby & Crowder, 2002). Since Quillback and Copper Rockfish are typically separated by depth (Love et al., 2002), these anoxic periods may force Quillback Rockfish into Copper habitat further increasing species overlap. These periods of hypoxia have occurred for centuries in Hood Canal, as indicated by sediment cores (Brandenberger et al., 2011), corresponding to the ancient age of hybridization. Lastly, introgression may occur when a rare species fails to find suitable mates and thus hybridizes with a closely related common species (Hubbs, 1955). Quillback Rockfish are thought to be more abundant in Puget Sound than Brown and Copper Rockfish, in both current and historical estimates (Schwenke et al., 2018). Periods of hypoxia can also cause severe rockfish die-offs (Grantham et al., 2004), further reducing available mates. This difference in population size suggests that the more common species, Quillback, would hybridize with the less common species, Brown and Copper, leading to directional introgression.

1.5.2 Intraspecific Population Structure

Despite similar ecology and life history of Brown, Copper and Quillback Rockfish, patterns of population structure differed considerably. Our results from Copper Rockfish inter- and intraspecific analyses showed similar patterns (Figure 1.4), suggesting that hybrid ancestry is driving genetic differentiation within Copper Rockfish. When hybrids were removed from analysis, there was no genetic differentiation between all pure populations within Puget Sound (Supplemental Figure 1.4). Our findings are similar to those found in other marine taxa in this region, such as harbor porpoises (Crossman et al., 2014). As was true in our study, genetic differentiation in these taxa depended on hybridization across distinct populations. Similarly, we suggest that the original genetic differentiation found between populations in Copper Rockfish (Buonaccorsi et al., 2002; Seeb, 1998) may have been inflated due to hybridization with Quillback Rockfish.

In addition to identifying the influence of hybridization on genetic differentiation in Copper Rockfish, our results provide evidence for limited dispersal between populations. The majority of Copper hybrid individuals (68%) were found in south Puget Sound, with some evidence of hybrids in north Puget Sound (20%), the Washington Coast (10%) and British Columbia (2%). The lower frequency of hybrids in the basins north of Admiralty Inlet suggests this shallow sill acts as a barrier to dispersal for Copper Rockfish. Isolation of south Puget Sound populations is likely due to circulatory features around Admiralty Inlet that restricts egg and larval dispersal (Khangaonkar et al., 2017b; Maccready et al., 2021) in addition to limited adult migration (Hannah & Rankin, 2011). Shallow sills in fjord-like marine environments are observed to act as a barrier to gene flow in Copper Rockfish along Vancouver Island (Dick et al., 2014), and Norwegian fjords for Atlantic cod (*Gadus morhua*) (Knutsen et al., 2007), blue whiting (*Micromesistius poutassou*) (Giæver &

Stien, 1998), and pearlside (*Maurolicus muelleri)* (Suneetha & Nævdal, 2001), contributing to the evolution of isolated populations. Additionally, the lack of pure Copper Rockfish in south Puget Sound suggests that this dispersal is highly directional, as pure Copper Rockfish are not dispersing from the north of Admiralty Inlet (Figure 1.9). Limited dispersal at the larval stage for both Copper and Brown Rockfish has been reported within Puget Sound and along the Washington Coast (Buonaccorsi et al., 2002, 2005). In addition, directional dispersal from south Puget Sound into the Washington Coast has been identified in Yelloweye Rockfish (Andrews et al., 2018) and Pacific cod (Drinan et al., 2018). In conclusion, the spatial variation seen in Copper Rockfish hybrids suggests limited dispersal from south Puget Sound. This suggests that the dispersal of hybrids, instead of traditional population genetic connectivity, can be used to infer migration and population connectivity in Copper Rockfish, as seen in Gilg & Hilbish (2003).

Our results from Brown Rockfish are inconclusive to the influence of hybridization on genetic differentiation. Our sample size of pure Brown Rockfish from Puget Sound is too small (n=3) to quantify any trends similar to our Copper Rockfish analyses. Future studies with a significantly larger sample size for Brown Rockfish should attempt to identify pure Brown individuals, to see if the genetic differences seen in our study are due to hybridization in south Puget Sound. When analyzing genetic differences between populations for Brown Rockfish, our results provide evidence for significant population differentiation between NPS, WC, and SPS. Previous studies on Brown Rockfish using microsatellites estimated an average F_{ST} value of 0.109 for south Puget Sound and Mexico populations, which closely resemble our F_{ST} values of 0.181. F_{ST} values are typically higher in SNP analyses due to the lower heterozygosity in a bi-allelic locus compared to a multiallelic locus (Hedrick, 2005).

We additionally suggest that the NPS population contains SPS and WC ancestry and that north Puget Sound may function as a mixing zone for coastal and south Puget Sound fish without much interbreeding. Similar patterns have been seen in the flatfish turbot (*Scophthalmus maximus*) and Atlantic Cod (*Gadus morhua*) between the Atlantic Ocean/North Sea and Baltic sea (Nielsen et al., 2003, 2004). There are many postulations as to how mixing zones are maintained, including both intrinsic and extrinsic barriers (Nielsen et al., 2003, 2004) such as geography, habitat unsuitability, and local adaptation. Extrinsically, Brown Rockfish have very small home ranges within Puget Sound (Matthews, 1990) (~30 m^2), suggesting that any movement occurs in the larval or juvenile stage. As previously mentioned, Admiralty Inlet is documented to restrict larval dispersal out of the south Puget Sound basin. After settlement, Brown Rockfish inhabit shallow water, natural, and complex rocky habitats along Puget Sound proper (Stout et al., 2001). Due to the patchy habitat available in northern Puget Sound (Palsson et al., 2009), it is possible that there is insufficient suitable habitat to support a population. This could explain the high F_{IS} value, which would further suggest the NPS acts as a mixing zone between two separate populations, also known as the Wahlund effect.

Intrinsically, there could be pre or post zygotic barriers to reproduction. Brown Rockfish are hypothesized to have colonized Puget Sound after deglaciation of the region (Buonaccorsi et al., 2005), approximately 17,000 years ago (Mosher & Hewitt, 2004). The time since divergence between coastal and Puget Sound populations may have enabled local adaptation. Any interbreeding between two locally adapted populations may induce outbreeding depression (Allendorf et al., 2001), reinforcing the isolation of those two populations (Garant et al., 2007). On the other hand, empirical evidence for outbreeding depression seems limited (McClelland & Naish, 2007). Local adaptation and adaptive differentiation may be facilitated by introgressed

Quillback alleles in southern Puget Sound. Introgression has been documented to facilitate diversification and speciation in many animal species, including cichlids and cottids (Selz et al., 2014; Stemshorn et al., 2011). Finally, rockfish are documented to have complex courting rituals (Helvey, 1982) and apparent mate choice (Johansson et al., 2012). It is possible that preference for conspecific mates for coastal Brown Rockfish might promote divergence between coastal and Puget Sound populations, as seen in swordtail (Willis et al., 2011). In northern Puget Sound where mate choice may be limited by small population sizes, choosiness might decrease and heterospecific (between hybrid and non-hybrid individuals) mating would occur (Willis et al., 2011).

Finally, Quillback Rockfish within our study system consisted of two populations: BC/NPS and SPS/WC. One potential explanation for the pattern of population structure identified in Quillback Rockfish could be limited migration patterns due to geographic barriers. Our results provided evidence of multiple migrants with limited interspecific mixed ancestry patterns. Looking at the geographic distribution of the STRUCTURE results (Figure 1.10), it is possible that the WC individuals with ancestry similar to SPS could be due to limited migration from south of Admiralty Inlet. Individuals further into the western Strait of Juan De Fuca clustered with NPS and BC samples, which could suggest gene flow in regions north of Admiralty Inlet. This limited migration could be occurring during larvae or adult stages. Limited directional migration or dispersal out of the southern Puget Sound region for Quillback Rockfish follows similar results seen in Copper Rockfish in this study and Yelloweye Rockfish in Andrews et al (2018). This could further justify the role of Admiralty Inlet in providing somewhat of a dispersal barrier for multiple rockfish species. In order to determine the age of dispersal for both Copper and Quillback Rockfish, genetic sampling of rockfish larvae is needed.

1.5.3 Implications For Fisheries Management

Current Puget Sound management practices assume three DPS in Brown, Copper, and Quillback Rockfish. Populations are divided into a 1) Puget Sound proper DPS (south of Admiralty Inlet), 2) north Puget Sound DPS (San Juan Islands) and 3) coastal DPS, which includes populations from California to Alaska (Stout et al., 2001). Results from our study suggest that Copper, Quillback, and Brown Rockfish each have a distinct population within the Puget Sound proper DPS, with evidence of limited dispersal beyond Admiralty Inlet. These results suggest that the current DPS boundaries may be inaccurate and should be re-considered. Additionally, the potential re-establishment of a recreational fishery for rockfishes in Puget Sound would benefit from immigration from the healthy Washington Coast stocks. Results from our study suggested that there is an extremely limited influx of fish from the Washington Coast. Conversely, we found evidence for directional dispersal from southern Puget Sound into the coast. Unfortunately, this directional dispersal further supports that southern Puget Sound populations are isolated from the coast and will not benefit from the population recovery seen along the Washington Coast.

As previously mentioned, introgressive hybridization can provide a source of genetic variability, which can provide essential adaptive potential in periods of rapid environmental change (Arnold et al., 2008; Hamilton & Miller, 2016). Our study found evidence for migration of hybrid individuals into coastal populations, particularly in Copper Rockfish. The introduction of introgressed Quillback alleles into the coastal Copper population may allow for more rapid adaptation in the larger Washington Coast population. As global climate change rapidly influences the marine environment along the Washington Coast (Miller et al., 2013), this genetic variation may play an important role in the persistence of rockfish species in Washington. The hybridization within Puget Sound is a natural process that likely has persisted for many generations, and as such should be conserved as a source of genetic variability for coastal populations. The results of this study indicate both a relative isolation of rockfishes in Puget Sound as well as a need to re-evaluate the population boundaries of all common rockfish in Puget Sound in order to best inform their management and potential recovery.

1.6 FIGURES

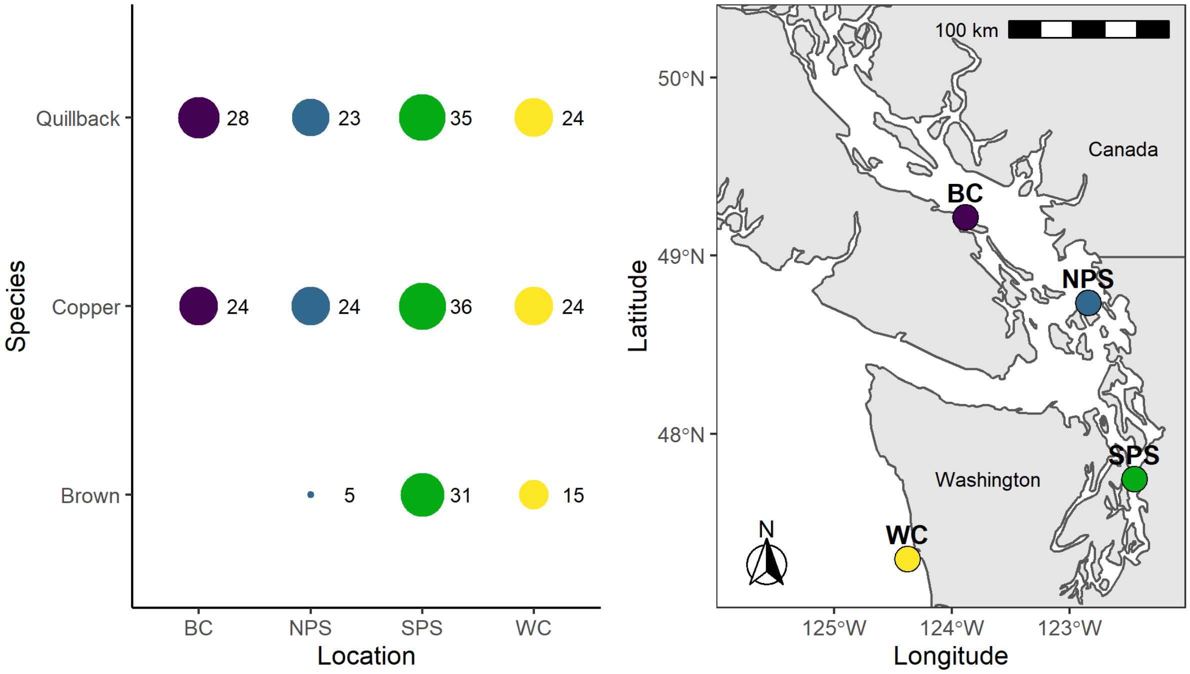


Figure 1.1 Brown, Copper, and Quillback Rockfish collection location and sample size. Sampling areas within the Salish Sea are North Puget Sound (NPS, blue), South Puget Sound (SPS, green) and British Columbia (BC, purple). The size of the circles is proportional to the size of the collection (5-36 individuals).

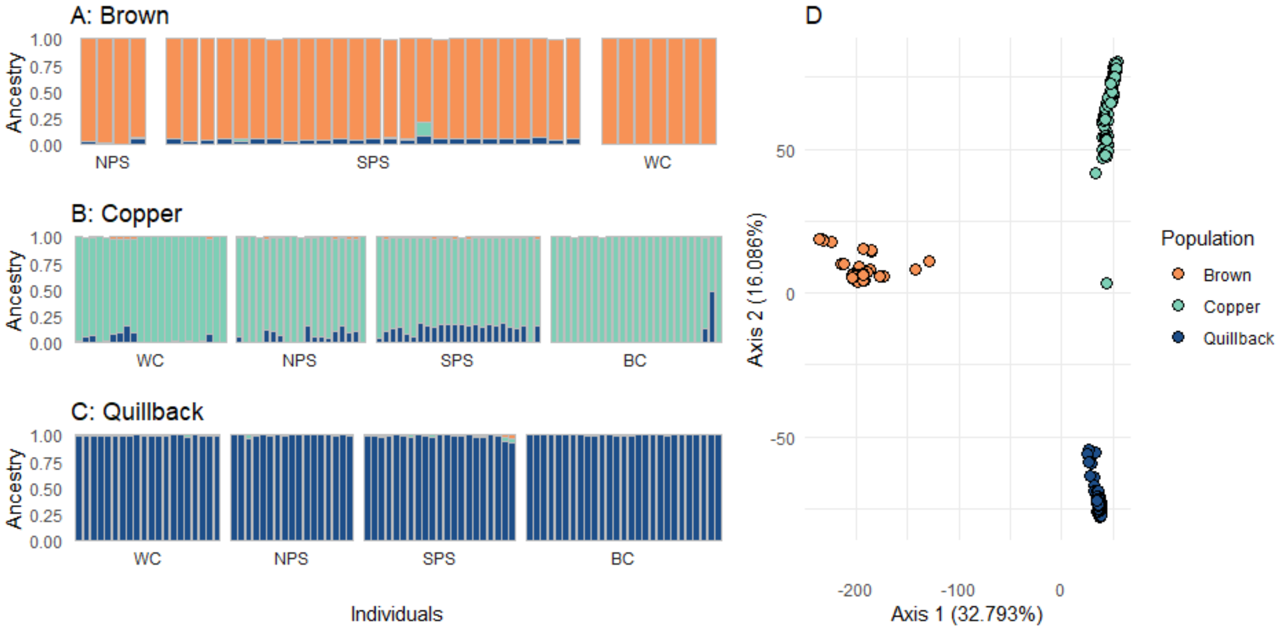


Figure 1.2 STRUCTURE analysis (A-C) and PCA (D) of Brown, Copper and Quillback rockfish separated by geographic location. A-C: Each bar represents one sample, and the colors represent the ancestry proportions for each individual. Light orange is Brown ancestry, light blue is Copper ancestry, and dark blue is Quillback ancestry. The results are split by field identification: A = Brown rockfish, B = Copper rockfish, C = Quillback rockfish. Samples within each location are ordered south to north. D: Principal Components Analysis from all samples suggests three clusters according to species. Samples are color coded according to field identification.

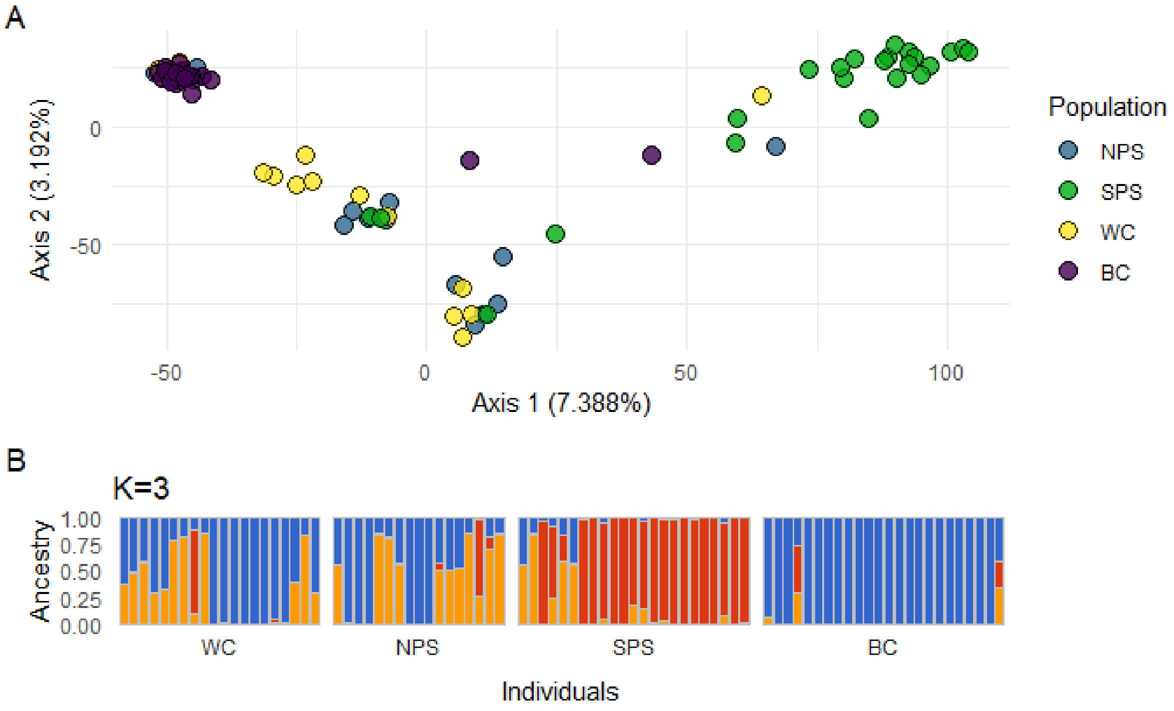


Figure 1.3 Intraspecific analysis for Copper Rockfish. (A) Each circle represents an individual fish, colored by their sampling location. (B) Plot shown is the most likely K cluster based on ΔK. Each bar represents an individual, and each color represents the genetic cluster each fish was assigned to. Populations are ordered from the coast (WC), into Puget Sound from the north (NPS) to the south (SPS), and British Columbia (BC). Samples within each location are ordered south to north.

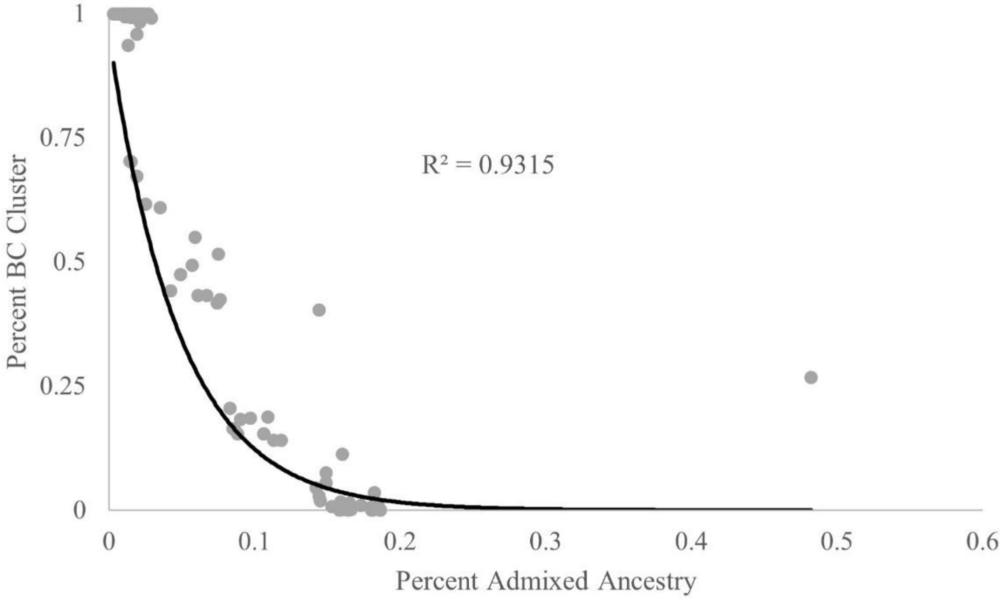


Figure 1.4 Regression between inter- and intraspecific structuring of Copper Rockfish. Percent BC cluster was defined as the percent ancestry originating from the 'blue' BC cluster (see Figure 1.2) from the STRUCTURE results of the intraspecific Copper Rockfish dataset (see Figure 1.3). Percent Admixed Ancestry was defined as the percent Quillback ancestry from the STRUCTURE results of the interspecific Brown/Copper/Quillback dataset. The x and y axis come from two separate datasets with 83% different loci. We found a significant exponential regression between the x- and y-axis (R^2 = 0.93). Each dot represents an individual.

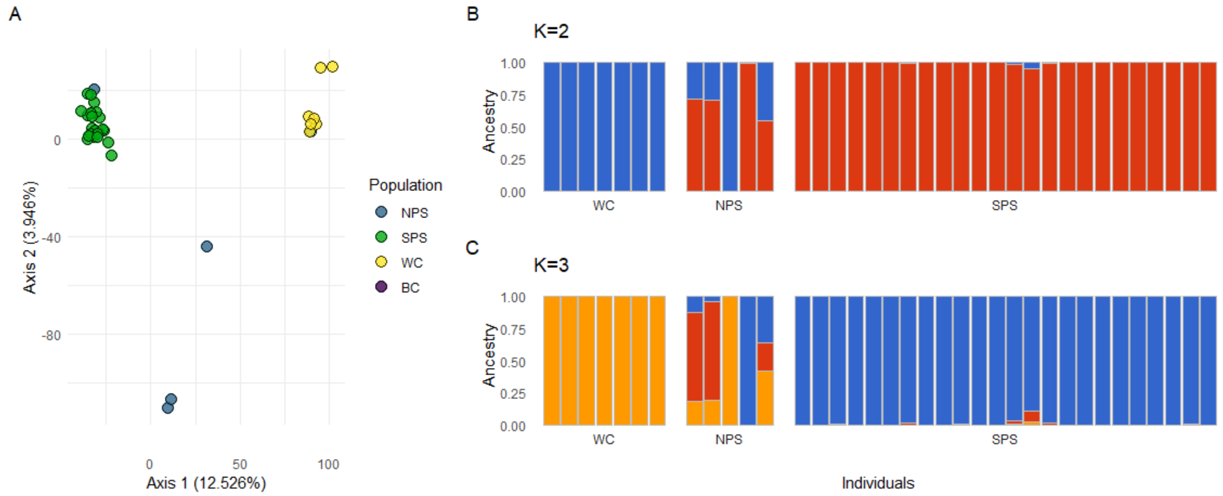


Figure 1.5 Principal components analysis and STRUCTURE plot for Brown Rockfish reveals three clusters. (A) Each circle represents an individual fish, colored by their sampling location. (B) Plot shown is the most likely K cluster based on ΔK. Each bar represents an individual, and each color represents the genetic cluster each fish was assigned to. Populations are ordered from the coast (WC), into Puget Sound from the north (NPS) to the south (SPS). Samples within each location are ordered south to north. (C) STRUCTURE plot based on K=3.

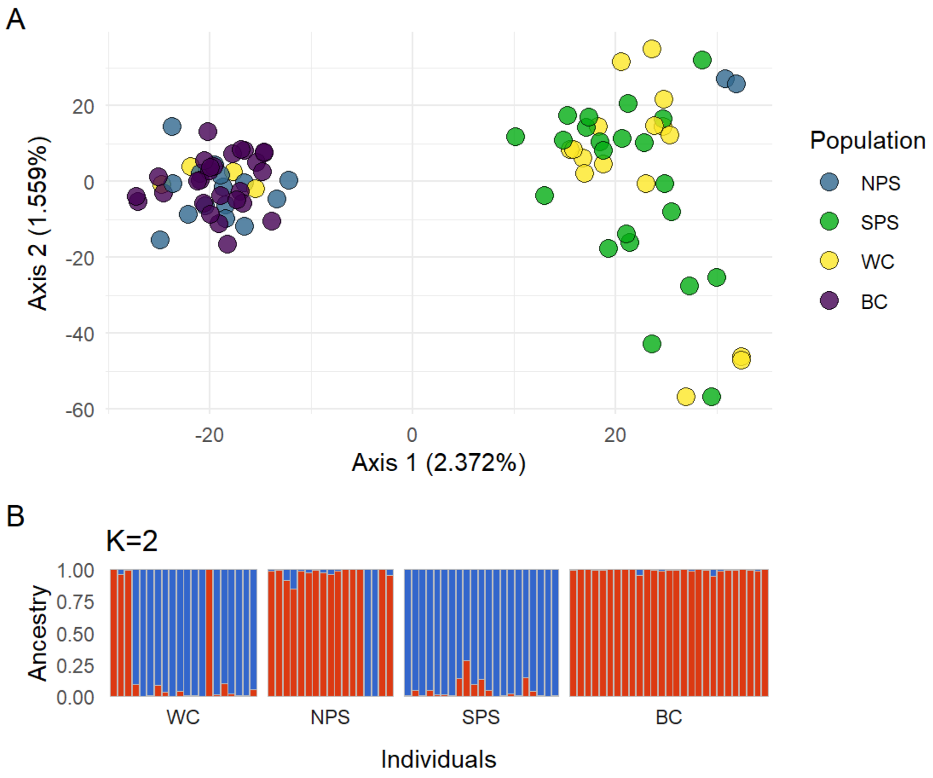


Figure 1.6 Principal components analysis and STRUCTURE plot for Quillback Rockfish reveals mixed ancestry patterns. (A) Each circle represents an individual fish, colored by their sampling location. (B) Plot shown is the most likely K cluster based on ΔK. Each bar represents an individual, and each color represents the genetic cluster each fish was assigned to. Populations are ordered from the coast (WC), into Puget Sound from the north (NPS) to the south (SPS), and British Columbia (BC). Samples within each location are ordered south to north.

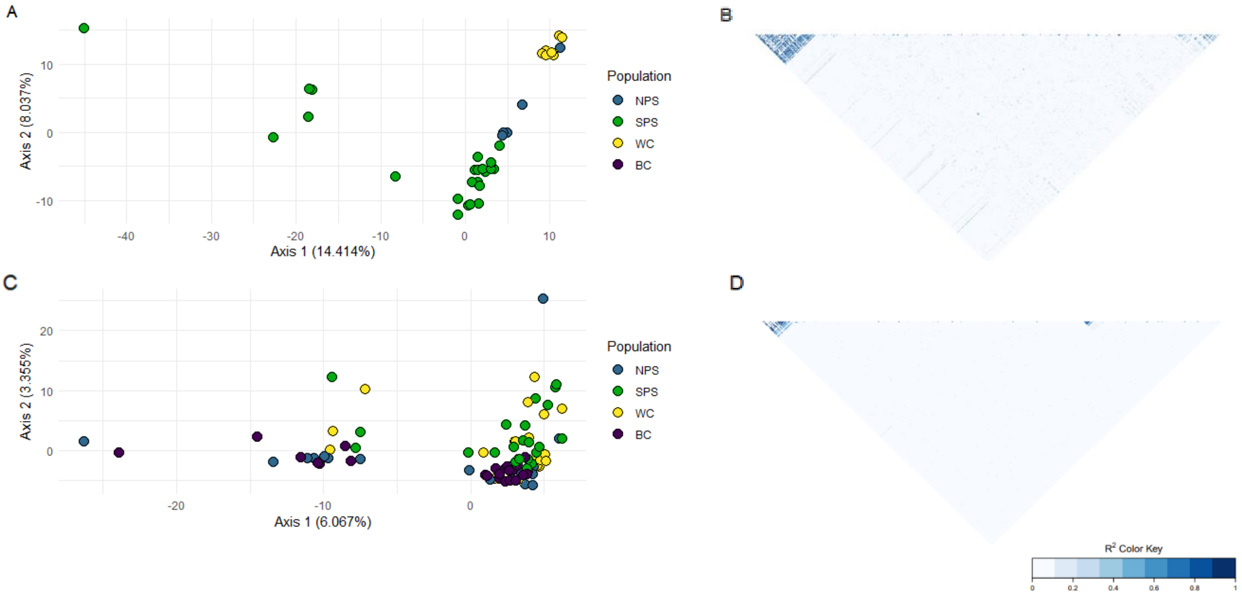


Figure 1.7 PCA (A &C) and linkage heatmap (B&D) for inversion chromosomes. A high LD block for Brown Rockfish was identified on chromosome 12 (A&B). A high LD block for Quillback Rockfish was identified on chromosome 14 (C&D).

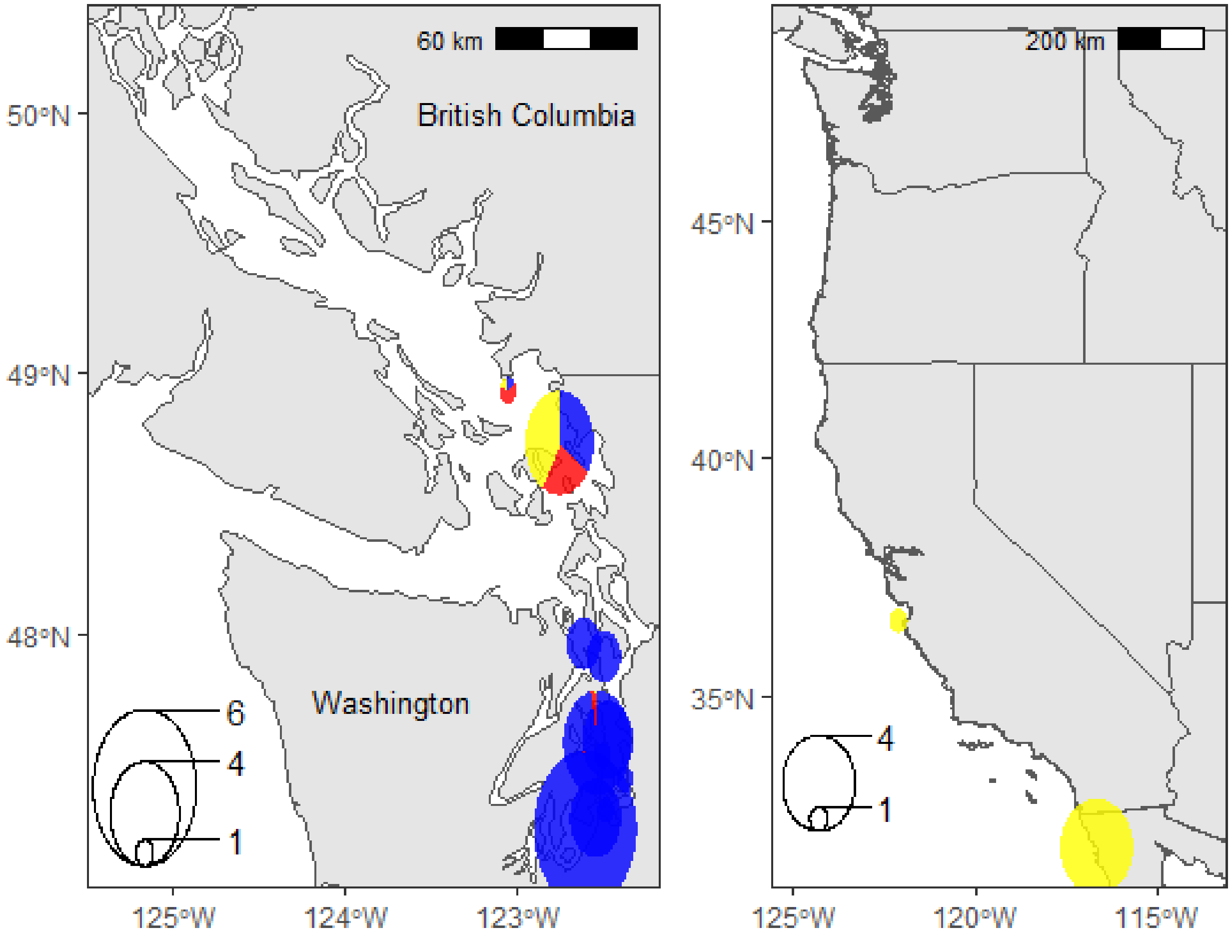


Figure 1.8 Geographic distribution of STRUCTURE results for Brown Rockfish. Pie charts are colored according to STRUCTURE plot results and adjusted for sample size (see legend in bottom left). The color of the pie corresponds to the average admixture proportions in each collection. Similar capture coordinates were pooled into the same pie.

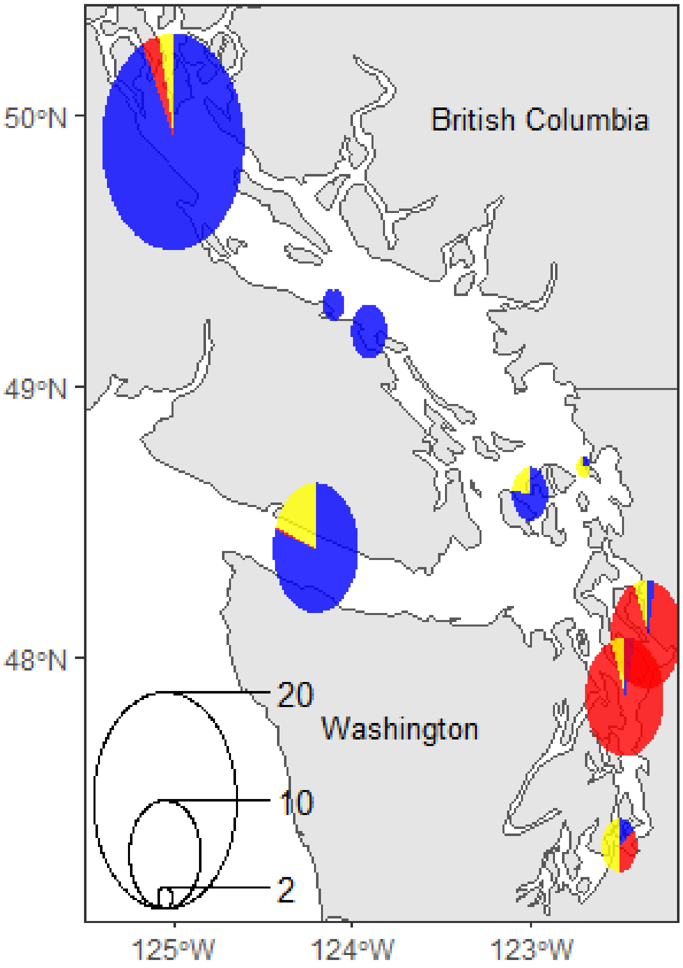


Figure 1.9 Geographic distribution of STRUCTURE results for Copper Rockfish. Pie charts are colored according to STRUCTURE plot results and adjusted for sample size (see legend in bottom left). The color of the pie corresponds to the average admixture proportions in each collection. Similar capture coordinates were pooled into the same pie.

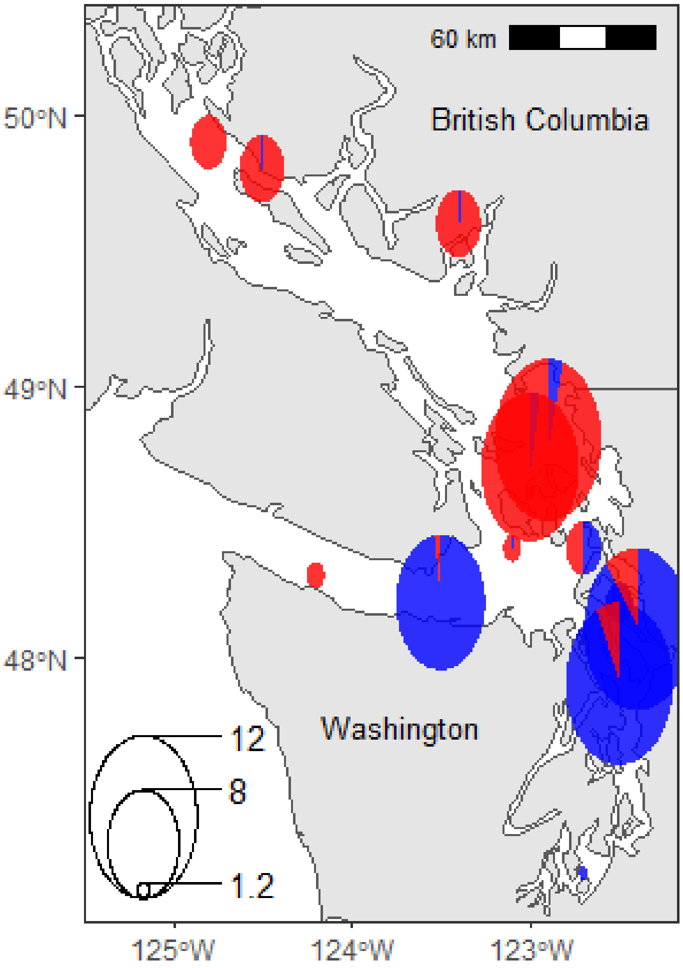


Figure 1.10 Geographic distribution of STRUCTURE results for Quillback Rockfish. Pie charts are colored according to STRUCTURE plot results and adjusted for sample size (see legend in bottom left). The color of the pie corresponds to the average admixture proportions in each collection. Similar capture coordinates were pooled into the same pie.

1.7 TABLES

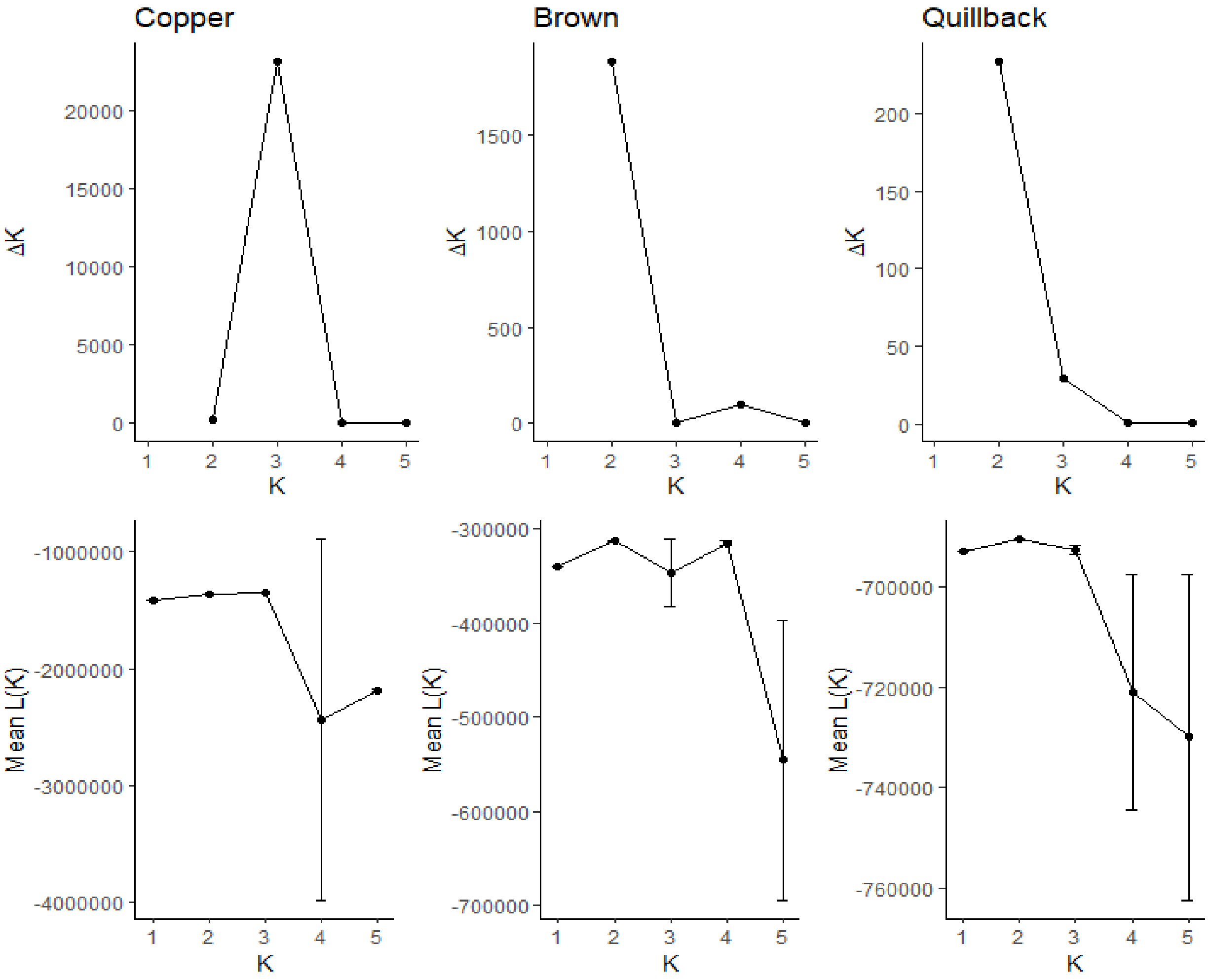
Table 1.1 Pairwise Weir and Cockerham F_{ST} estimates for three species of rockfish in Puget Sound and the Washington Coast. Bolded numbers are significantly greater than zero.

	Brown Rockfish				**Copper Rockfish**				**Quillback Rockfish**			
	SPS	NPS	BC	WC	SPS	NPS	BC	WC	SPS	NPS	BC	WC
SPS												
NPS	**0.049**				**0.049**				**0.009**			
BC					**0.09**	**0.019**			**0.013**	0.001		
WC	**0.181**	**0.105**			**0.057**	**0.002**	**0.014**		0	**0.006**	**0.009**	

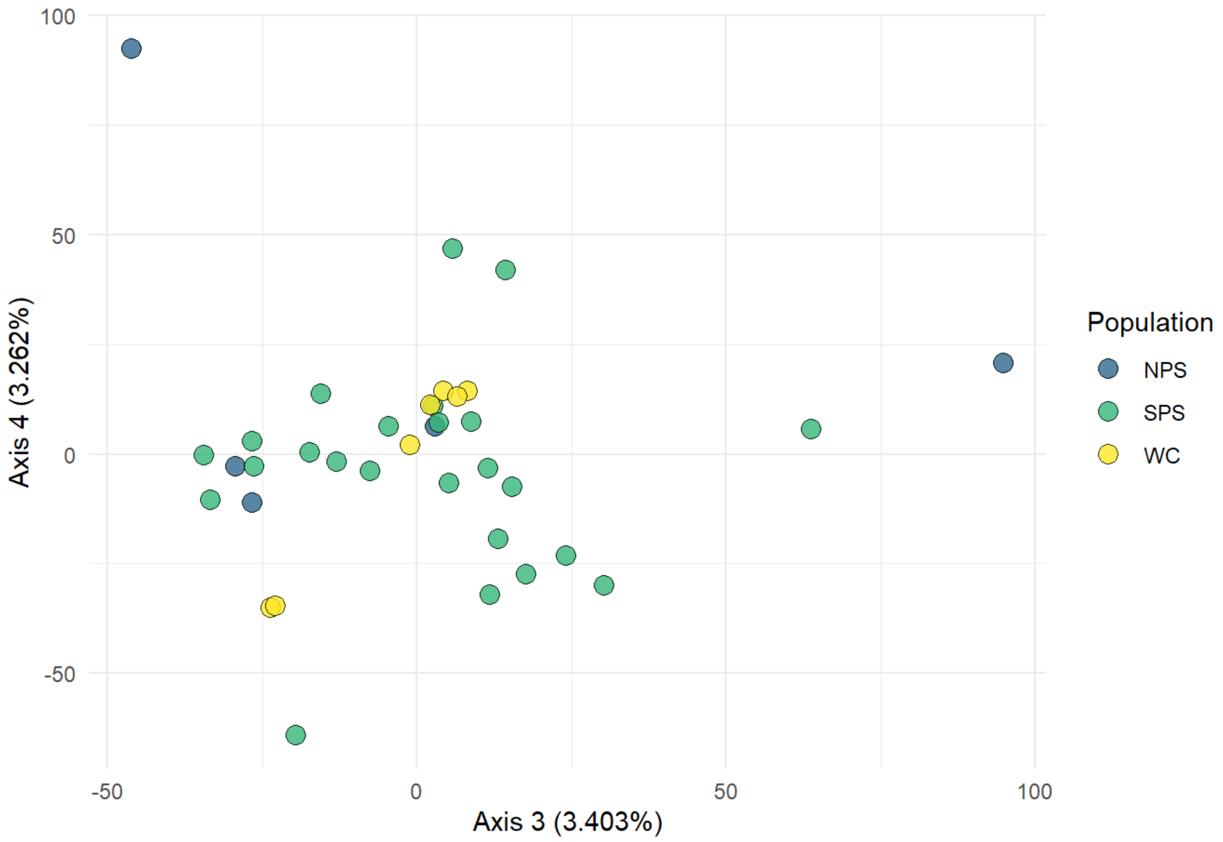
Table 1.2 Summary statistics for three species of rockfish in Puget Sound and the Washington Coast. N = number of samples per population, H_O = average observed heterozygosity, H_E = average expected heterozygosity, F_{IS} = inbreeding coefficient. All values were calculated using the R package hierfstat v0.5-11 (Goudet, 2005).

	Brown Rockfish				**Copper Rockfish**				**Quillback Rockfish**			
	N	***Ho***	***He***	F_{IS}	***N***	***Ho***	***He***	F_{IS}	***N***	***Ho***	***He***	F_{IS}
SPS	23	0.284	0.280	-0.013	23	0.273	0.286	0.045	22	0.279	0.269	-0.036
NPS	5	0.216	0.236	0.084	18	0.263	0.279	0.025	17	0.271	0.265	-0.021
BC					23	0.239	0.246	0.032	20	0.252	0.278	0.021
WC	8	0.172	0.165	-0.040	20	0.251	0.264	0.052	27	0.266	0.266	-0.002

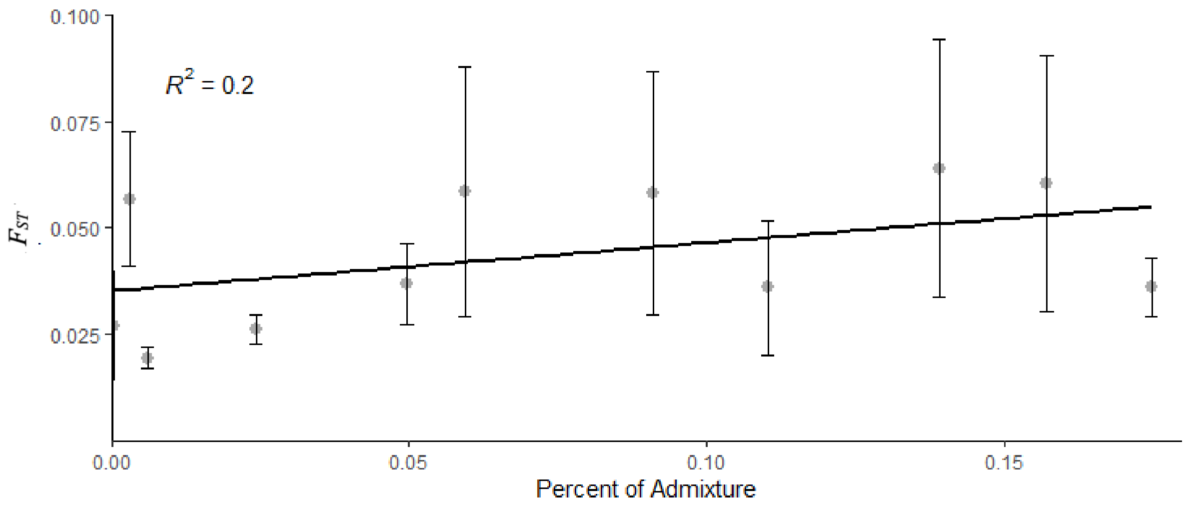
1.8 SUPPLEMENTAL FIGURES



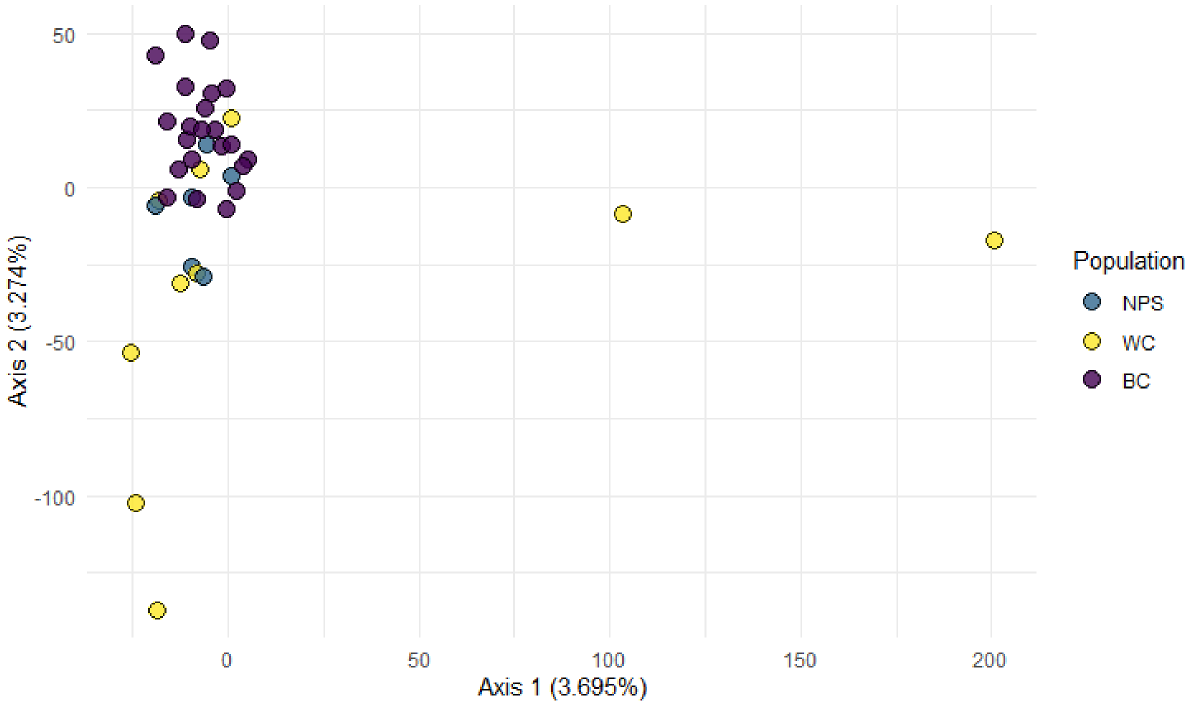
Supplemental Figure 1.1 Mean likelihood (bottom, ± 1 SD) and ΔK (top) for STRUCTURE replicate runs for Copper, Brown, and Quillback Rockfish.



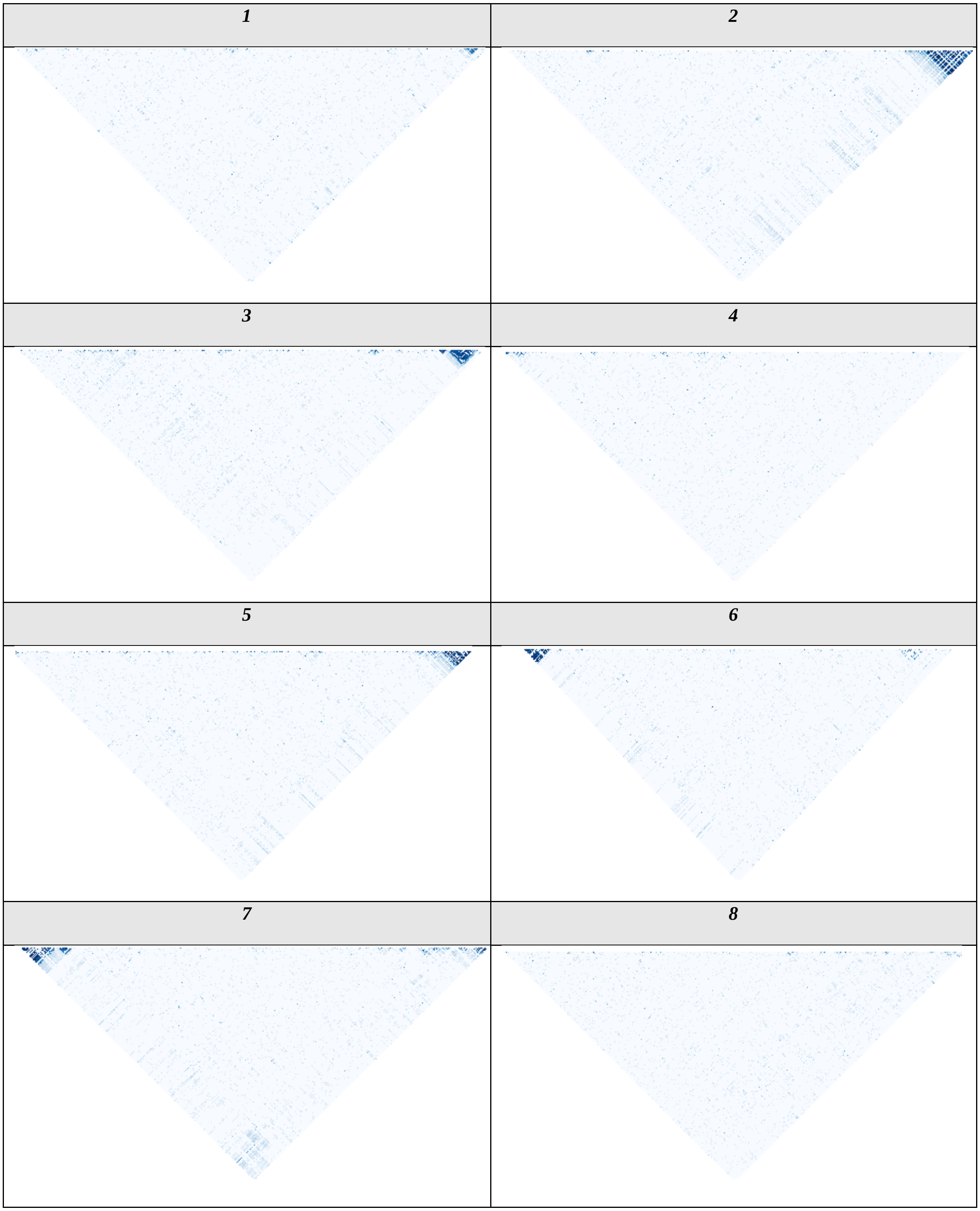
Supplemental Figure 1.2 Axis 3 and 4 from principal components analysis of Brown Rockfish. Each circle represents an individual fish, colored by sampling location.



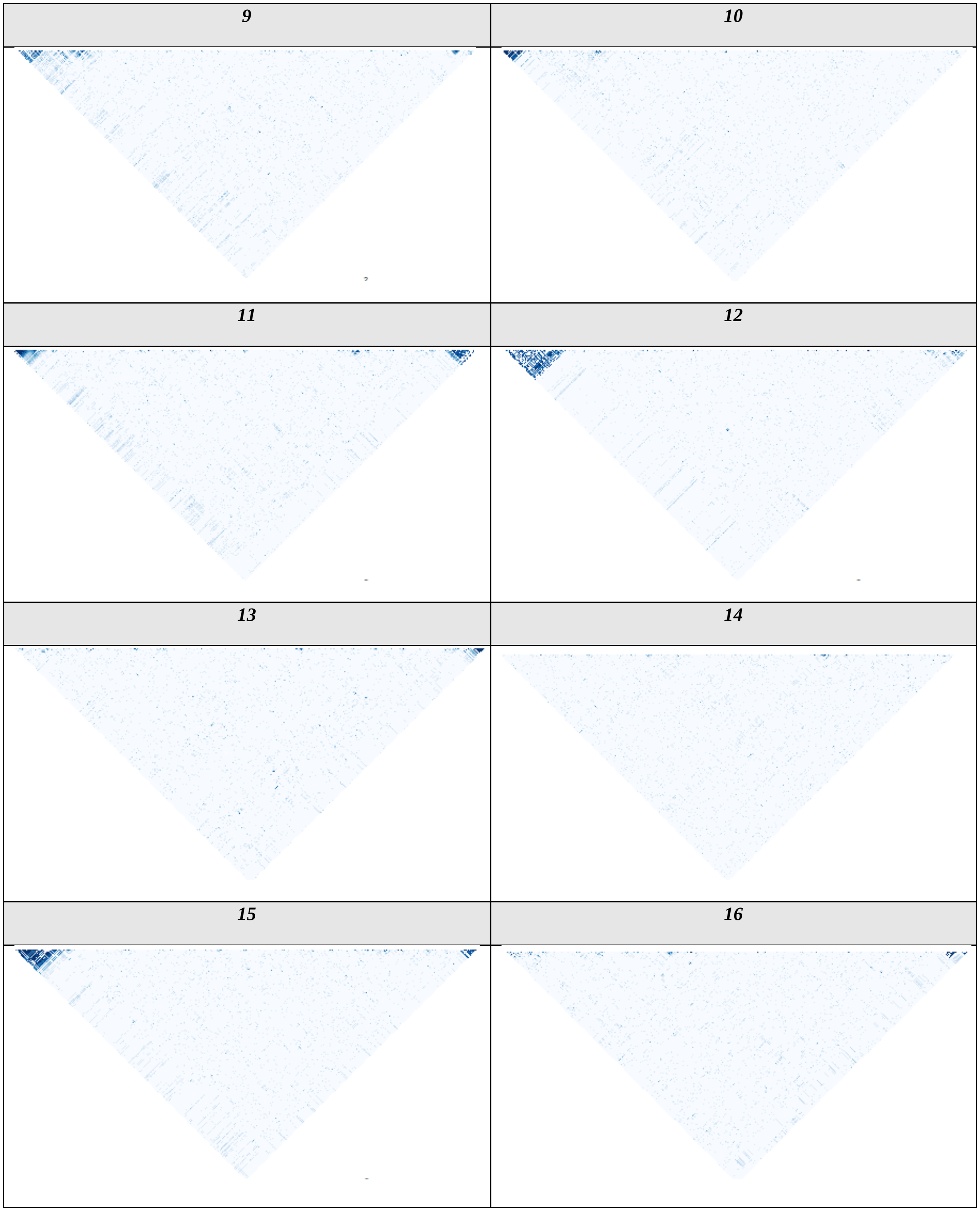
Supplemental Figure 1.3 F_{ST} increases with percent admixture in Copper Rockfish. Samples from each geographic location were grouped by percent admixture with Quillback Rockfish. Pairwise Weir and Cockerham F_{ST} was calculated between location groups and multiple British Columbia groups, which had 0% admixture.



Supplemental Figure 1.4 Principal components analysis of Copper Rockfish excluding hybrids suggests panmixia. Each circle represents an individual fish, colored by sampling location.

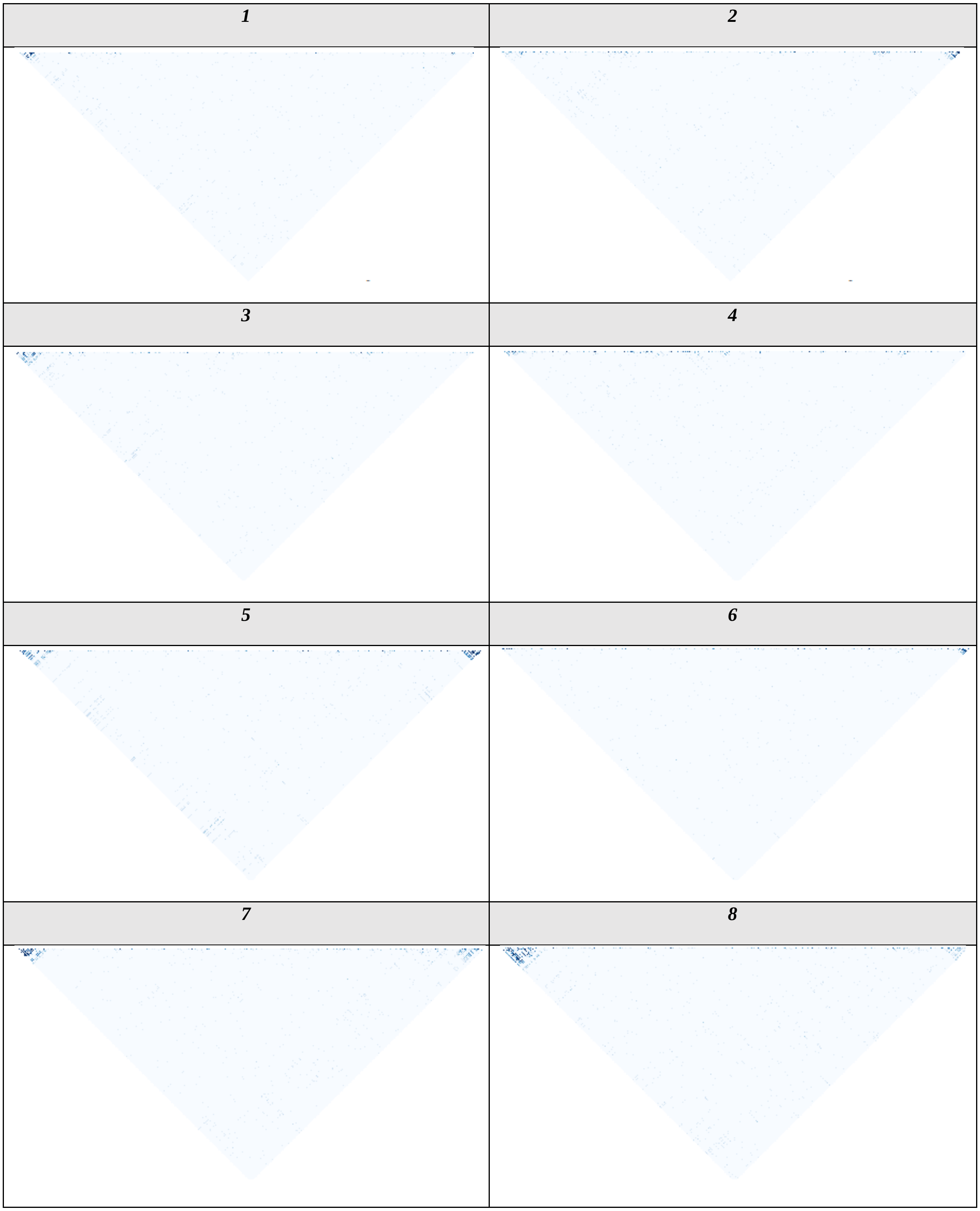


Supplemental Figure 1.5 Heat map of pairwise LD for chromosome 1-6 of Brown Rockfish. R^2 values were calculated using PLINK v1.07.

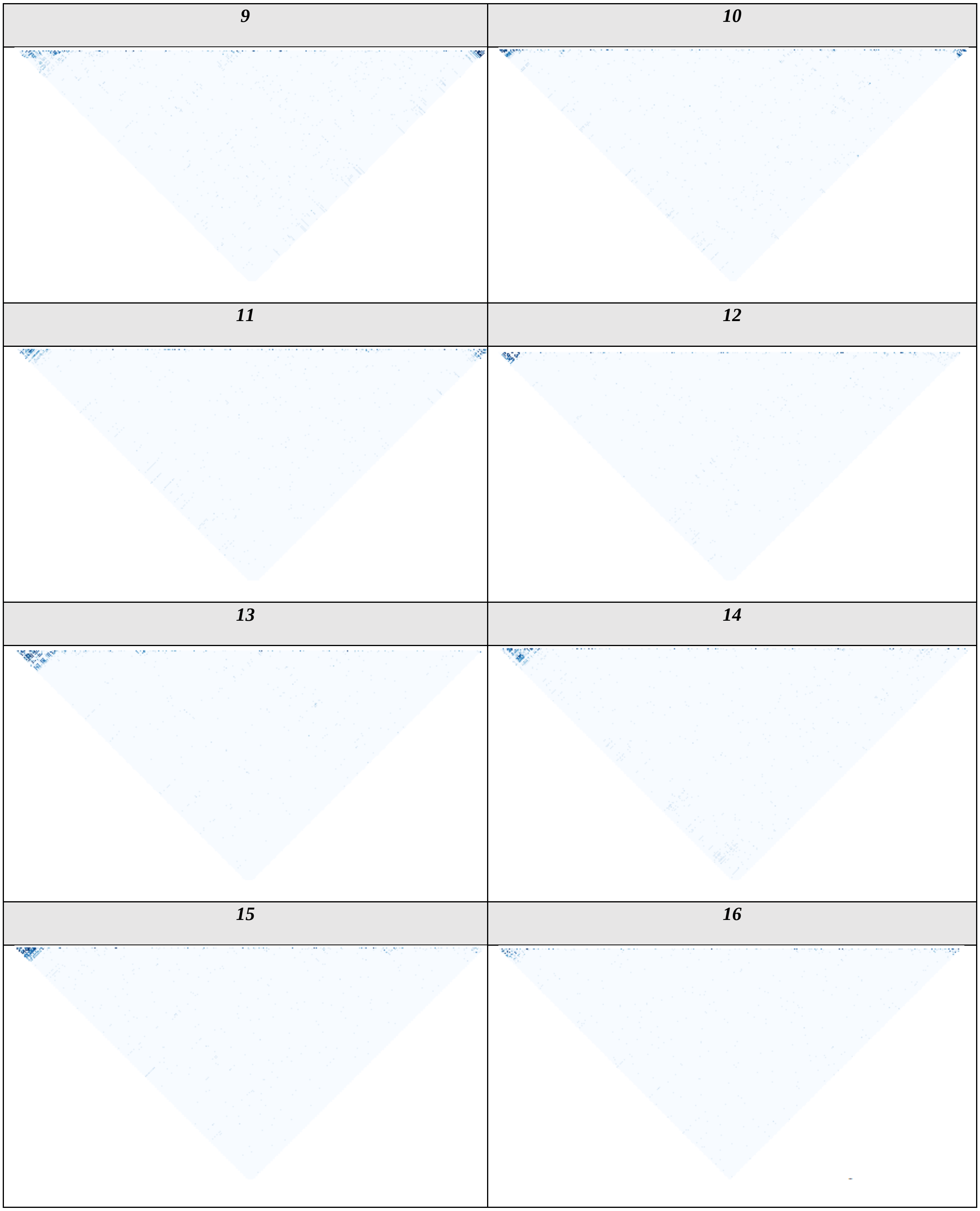


Supplemental Figure 1.6 Heat map of pairwise LD for chromosome 7-16 of Brown Rockfish. R^2 values were calculated using PLINK v1.07.

Supplemental Figure 1.7 Heat map of pairwise LD for chromosome 17-24 of Brown Rockfish. R^2 values were calculated using PLINK v1.07.



Supplemental Figure 1.8 Heat map of pairwise LD for chromosome 1-6 of Copper Rockfish. R^2 values were calculated using PLINK v1.07.



9

10

11

12

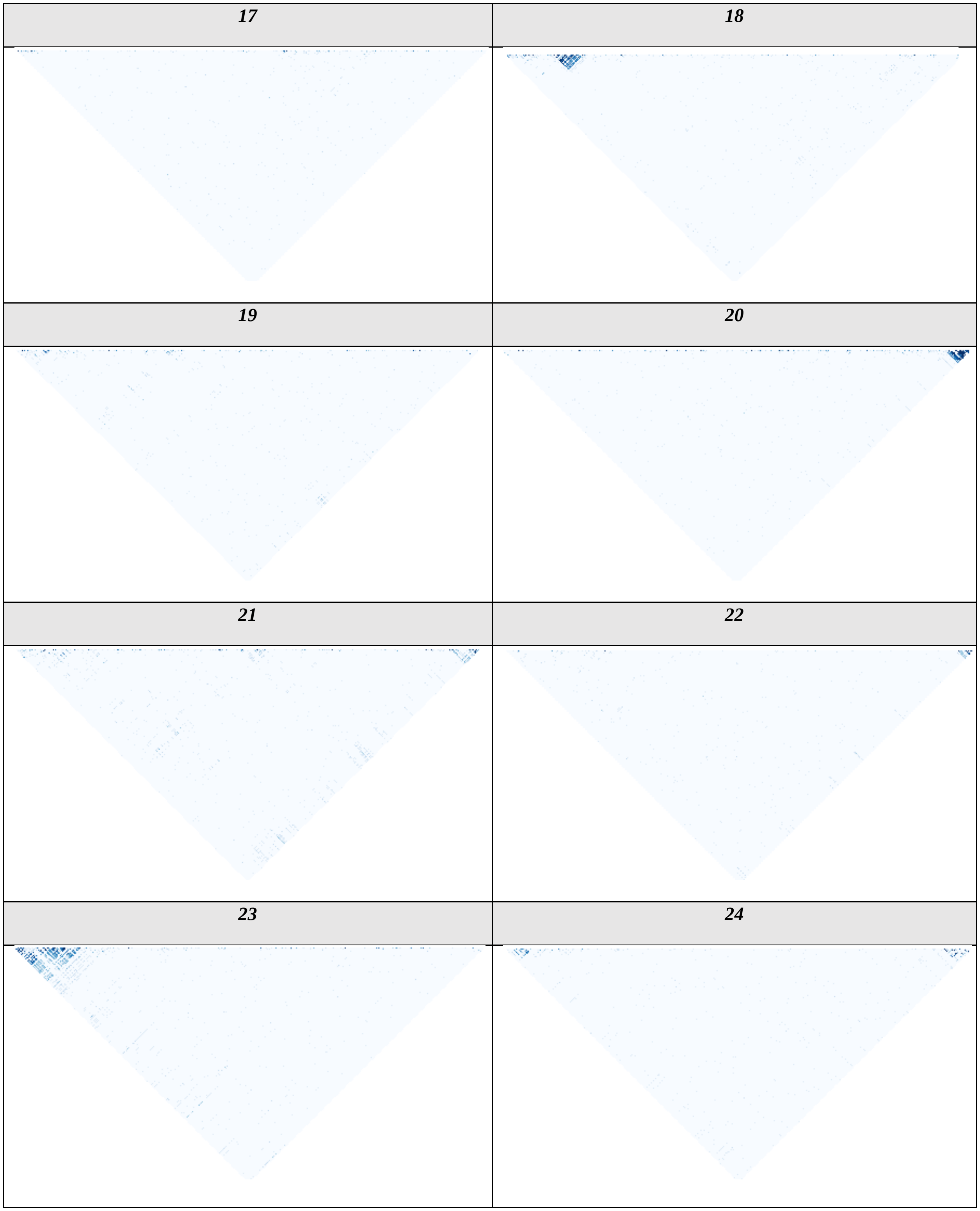
13

14

15

16

Supplemental Figure 1.9 Heat map of pairwise LD for chromosome 7-16 of Copper Rockfish. R^2 values were calculated using PLINK v1.07.



Supplemental Figure 1.10 Heat map of pairwise LD for chromosome 17-24 of Copper Rockfish. R^2 values were calculated using PLINK v1.07.

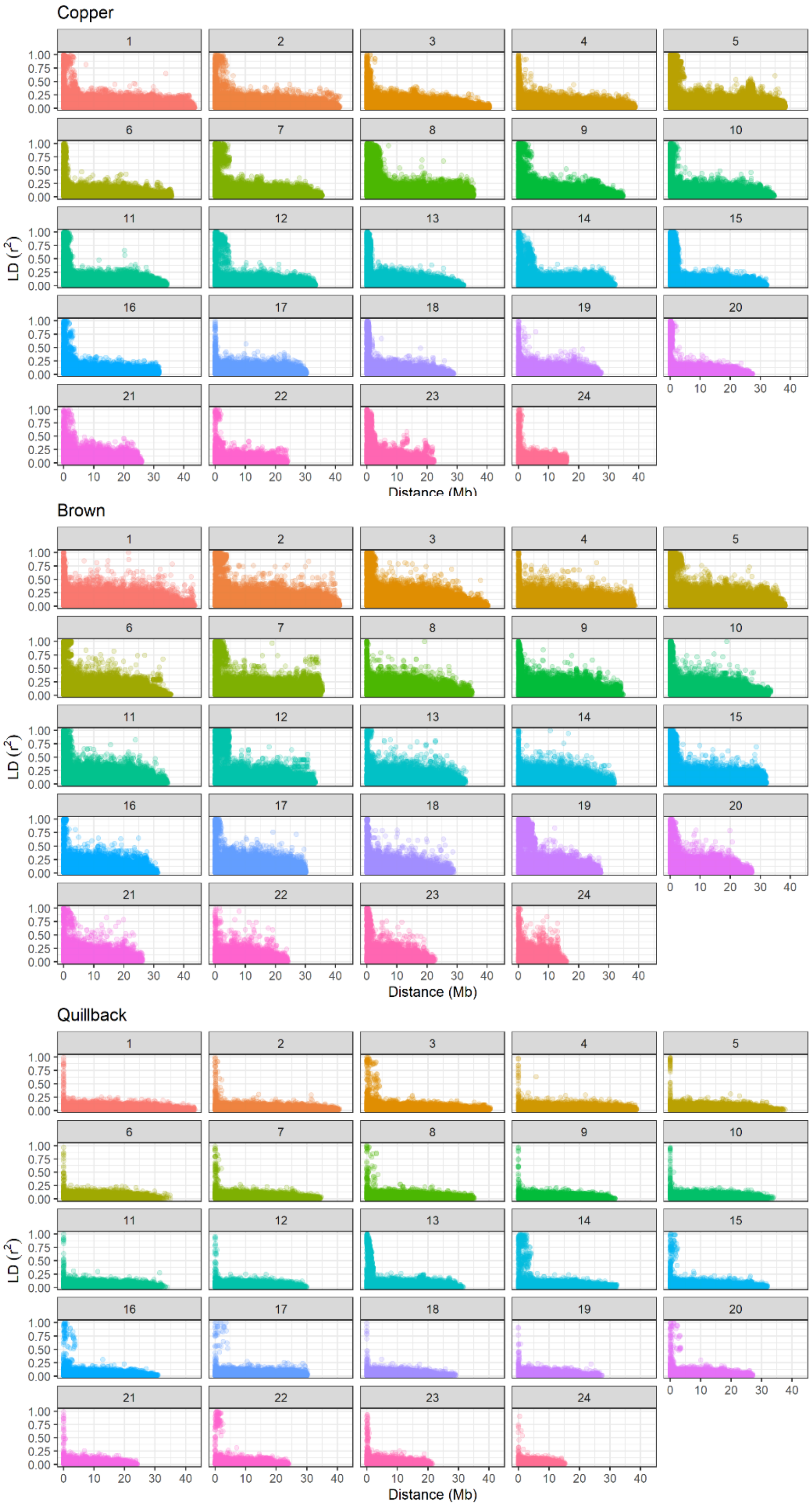
1	***2***
	
3	***4***
	
5	***6***
	
7	***8***
	

Supplemental Figure 1.11 Heat map of pairwise LD for chromosome 1-6 of Quillback Rockfish. R^2 values were calculated using PLINK v1.07.

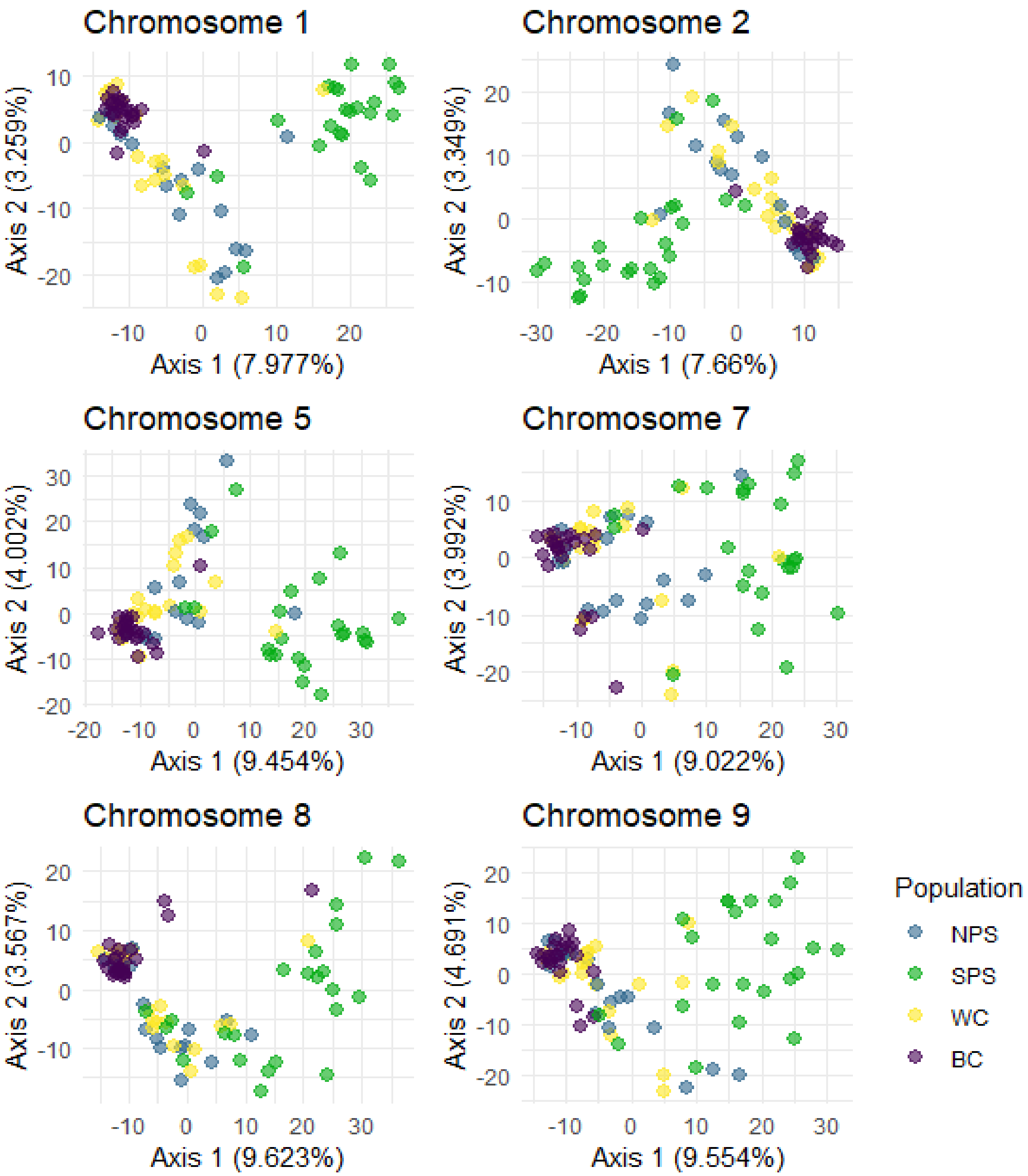
9	*10*
	
11	***12***
	
13	***14***
	
15	***16***
	

Supplemental Figure 1.12 Heat map of pairwise LD for chromosome 7-16 of Quillback Rockfish. R^2 values were calculated using PLINK v1.07.

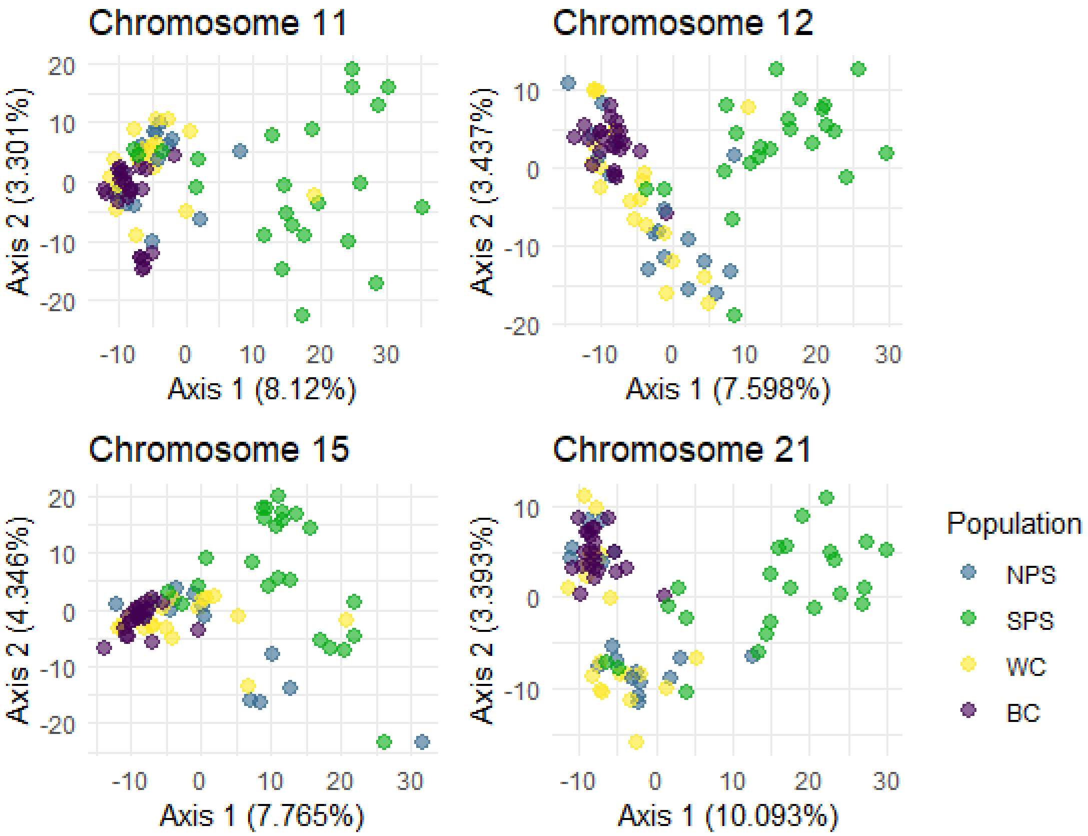
Supplemental Figure 1.13 Heat map of pairwise LD for chromosome 17-24 of Quillback Rockfish. R^2 values were calculated using PLINK v1.07.



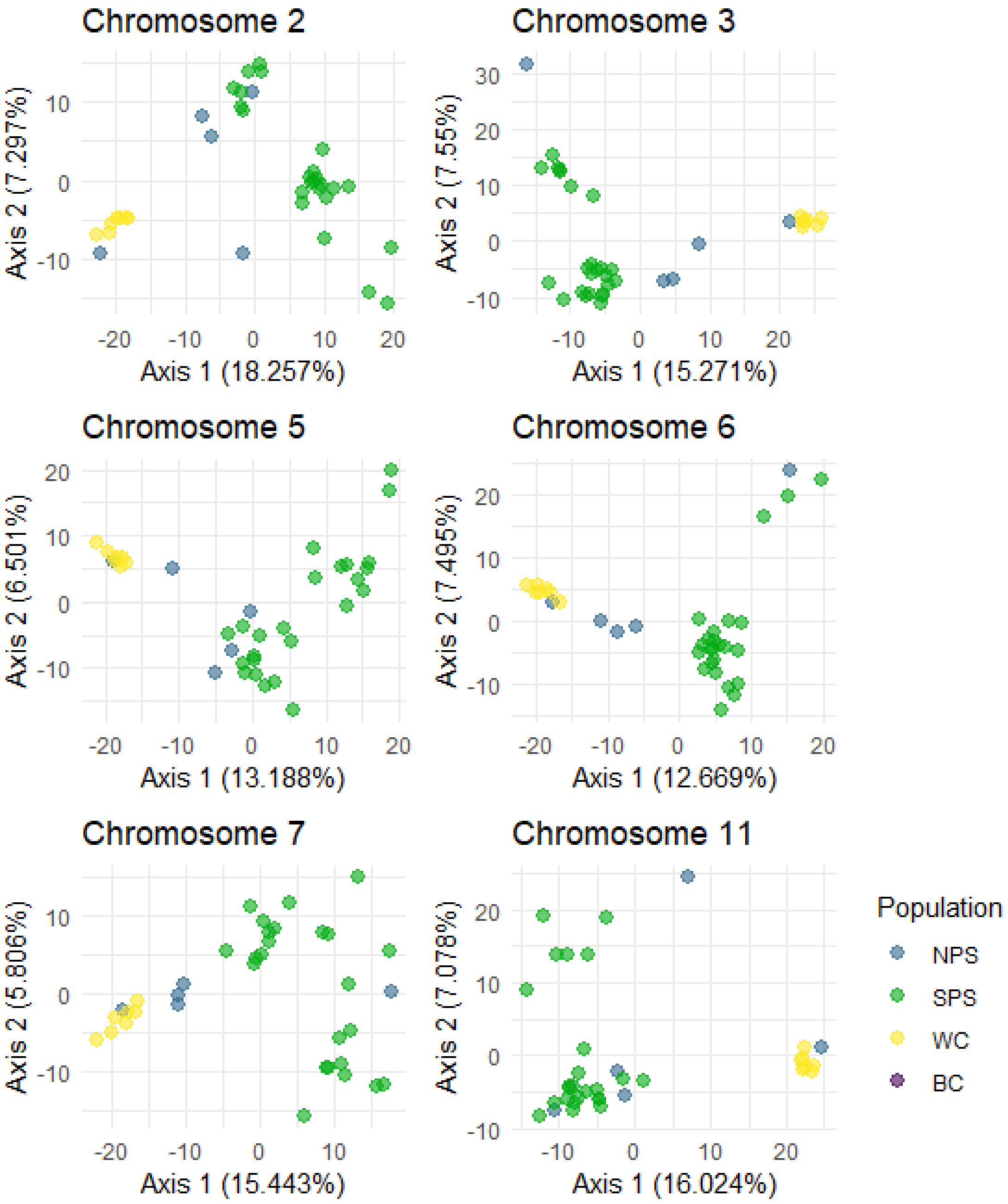
Supplemental Figure 1.14 Linkage decay plots for five species of rockfish. R^2 values were calculated using PLINK v1.07. R^2 estimates are typically higher with smaller sample sizes, as seen in our Brown Rockfish dataset.



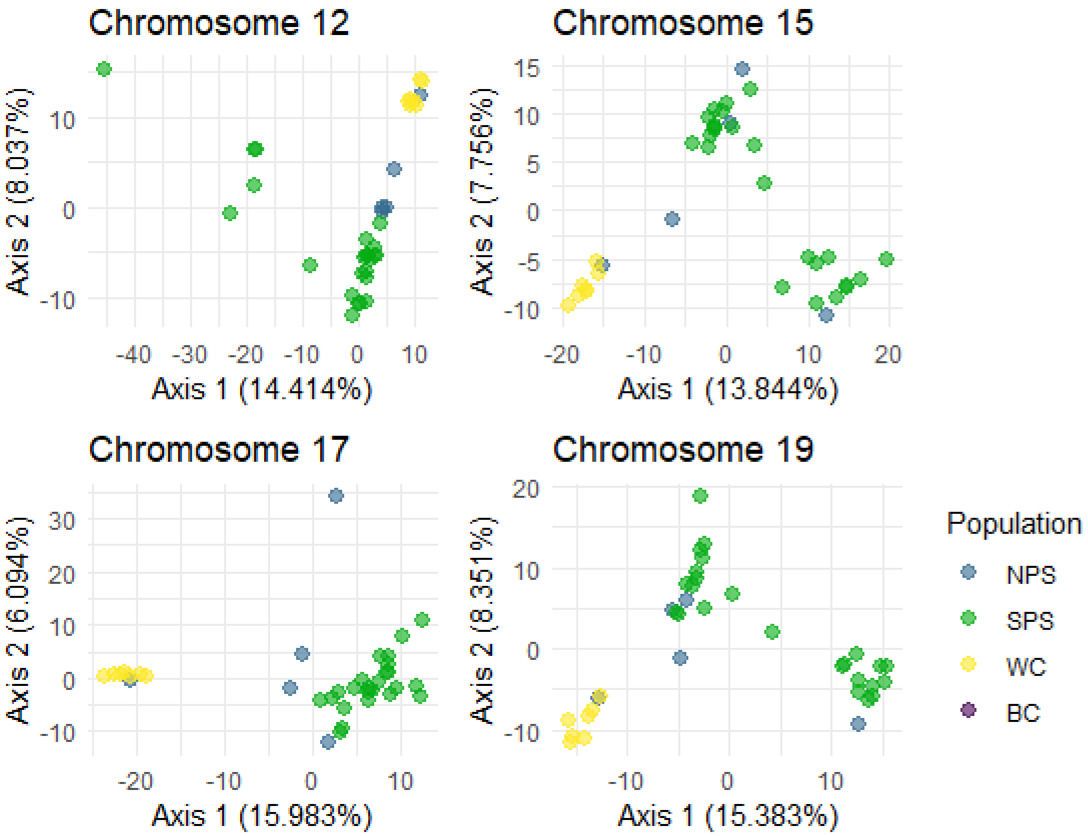
Supplemental Figure 1.15 Principal components analysis of chromosomes with high LD blocks in Copper Rockfish, chromosomes 1-9. Each circle represents an individual fish, colored by their sampling location.



Supplemental Figure 1.16 Principal components analysis of chromosomes with high LD blocks in Copper Rockfish, chromosomes 11-21. Each circle represents an individual fish, colored by their sampling location.



Supplemental Figure 1.17 Principal components analysis of chromosomes with high LD blocks in Brown Rockfish, chromosomes 2-11. Each circle represents an individual fish, colored by their sampling location.



Supplemental Figure 1.18 Principal components analysis of chromosomes with high LD blocks in Brown Rockfish, chromosomes 12-19. Each circle represents an individual fish, colored by their sampling location.

1.9 REFERENCES

Abecasis, D., Afonso, P., & Erzini, K. (2014). Combining multispecies home range and distribution models aids assessment of MPA effectiveness. *Marine Ecology Progress Series*, *513*, 155–169. https://doi.org/10.3354/meps10987

Ali, O. A., Jeffres, C., & Miller, M. R. (2016). RAD Capture (Rapture): Flexible and Efficient Sequence-Based Genotyping. *Genetics*, *202*, 389–400. https://doi.org/10.1534/genetics.115.183665

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: Setting conservation guidelines. *Trends in Ecology & Evolution*, *16*(11), 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X

Andrews, K. R., & Luikart, G. (2014). Recent novel approaches for population genomics data analysis. *Molecular Ecology*, *23*(7), 1661–1667. https://doi.org/10.1111/mec.12686

Andrews, K. S., Bartos, B., Harvey, C., Tonnes, D., Bhuthimethee, M., & Maccready, P. (2021). Testing the potential for larval dispersal to explain connectivity and population structure of threatened rockfish species in Puget Sound. *Marine Ecology Progress Series*, *677*, 95–113. https://doi.org/10.3354/meps13858

Andrews, K. S., Nichols, K. M., Elz, A., Tolimieri, N., Harvey, C. J., Pacunski, R., Lowry, D., Yamanaka, K. L., & Tonnes, D. M. (2018). Cooperative research sheds light on population structure and listing status of threatened and endangered rockfish species. *Conservation Genetics*, *19*(4), 865–878. https://doi.org/10.1007/s10592-018-1060-0

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data* (0.11.9).

Arnold, M. (1992). Natural Hybridization as an Evolutionary Process. *Annual Review of Ecology and Systematics*, *23*, 237–261. JSTOR.

Arnold, M., & Fogarty, N. (2009). Reticulate Evolution and Marine Organisms: The Final Frontier? *International Journal of Molecular Sciences*, *10*(9), 3836–3860. https://doi.org/10.3390/ijms10093836

Arnold, M., Sapir, Y., & Martin, N. H. (2008). Genetic exchange and the origin of adaptations: Prokaryotes to primates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1505), 2813–2820. https://doi.org/10.1098/rstb.2008.0021

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A., & Johnson, E. A. (2008). Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLoS ONE*, *3*(10), e3376. https://doi.org/10.1371/journal.pone.0003376

Beaudreau, A. H., Levin, P. S., & Norman, K. C. (2011). Using folk taxonomies to understand stakeholder perceptions for species conservation. *Conservation Letters*, *4*(6), 451–463. https://doi.org/10.1111/j.1755-263x.2011.00199.x

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bernatchez, L., & Wilson, C. C. (1998). Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, *7*(4), 431–452. https://doi.org/10.1046/j.1365-294x.1998.00319.x

Brandenberger, J., Louchouarn, P., & Crecelius, E. (2011). Natural and Post-Urbanization Signatures of Hypoxia in Two Basins of Puget Sound: Historical Reconstruction of Redox Sensitive Metals and Organic Matter Inputs. *Aquatic Geochemistry*, *17*, 645–670. https://doi.org/10.1007/s10498-011-9129-0

Buonaccorsi, V. P., Kimbrell, C. A., Lynn, E. A., & Vetter, R. D. (2002). Population structure of copper rockfish (*Sebastes caurinus*) reflects postglacial colonization and contemporary patterns of larval dispersal. *Canadian Journal of Fisheries and Aquatic Sciences, 59*(8), 1374–1384. https://doi.org/10.1139/f02-101

Buonaccorsi, V. P., Kimbrell, C. A., Lynn, E. A., & Vetter, R. D. (2005). Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. *Conservation Genetics*, *6*(5), 697–713. https://doi.org/10.1007/s10592-005-9029-1

Buonaccorsi, V. P., Westerman, M., Stannard, J., Kimbrell, C., Lynn, E., & Vetter, R. D. (2003). Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. *Marine Biology*, *1*(1), 1–1. https://doi.org/10.1007/s00227-004-1362-2

Burford, M. O., & Larson, R. J. (2007). Genetic heterogeneity in a single year-class from a panmictic population of adult blue rockfish (*Sebastes mystinus*). *Marine Biology*, *151*(2), 451–465. https://doi.org/10.1007/s00227-006-0475-1

Burford Reiskind, M., Carr, M., & Bernardi, G. (2011). Age-structured genetic analysis reveals temporal and geographic variation within and between two cryptic rockfish species. *Marine Ecology Progress Series*, *442*, 201–215. https://doi.org/10.3354/meps09329

Canino, M. F., Spies, I. B., Cunningham, K. M., Hauser, L., & Grant, W. S. (2010). Multiple ice-age refugia in Pacific cod, *Gadus macrocephalus*. *Molecular Ecology*, *19*(19), 4339–4351. https://doi.org/10.1111/j.1365-294x.2010.04815.x

Carvalho, G. R., & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries,* *4*(3), 326–350. https://doi.org/10.1007/BF00042908

Caselle, J. E., Wilson, J. R., Carr, M. H., Malone, D. P., & Wendt, D. E. (2010). Can we predict interannual and regional variation in delivery of pelagic juveniles to nearshore populations of rockfishes (genus *Sebastes*) using simple proxies of ocean conditions? *Reports of California Cooperative Oceanic Fisheries Investigations*, *51*, 91–105.

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

Cayuela, H., Rougemont, Q., Laporte, M., Mérot, C., Normandeau, E., Dorant, Y., Tørresen, O. K., Hoff, S. N. K., Jentoft, S., Sirois, P., Castonguay, M., Jansen, T., Praebel, K., Clément, M., & Bernatchez, L. (2020). Shared ancestral polymorphisms and chromosomal rearrangements as potential drivers of local adaptation in a marine fish. *Molecular Ecology*, *29*(13), 2379–2398. https://doi.org/10.1111/mec.15499

Crossman, C., Barrett-Lennard, L., & Taylor, E. (2014). Population structure and intergeneric hybridization in harbour porpoises *Phocoena phocoena* in British Columbia, Canada. *Endangered Species Research*, *26*(1), 1–12. https://doi.org/10.3354/esr00624

Cunningham, K. M., Canino, M. F., Spies, I. B., & Hauser, L. (2009). Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): Limited effective dispersal in the northeastern Pacific Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(1), 153–166. https://doi.org/10.1139/f08-199

Cusa, M., St John Glew, K., Trueman, C., Mariani, S., Buckley, L., Neat, F., & Longo, C. (2022). A future for seafood point-of-origin testing using DNA and stable isotope signatures. *Reviews in Fish Biology and Fisheries*, *32*(2), 597–621. https://doi.org/10.1007/s11160-021-09680-w

Dahle, G., Johansen, T., Westgaard, J. I., Aglen, A., & Glover, K. A. (2018). Genetic management of mixed-stock fisheries “real-time”: The case of the largest remaining cod fishery operating in the Atlantic in 2007–2017. *Fisheries Research*, *205*(September 2017), 77–85. https://doi.org/10.1016/j.fishres.2018.04.006

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dannewitz, J., Maes, G. E., Johansson, L., Wickström, H., Volckaert, F. A. M., & Järvi, T. (2005). Panmixia in the European eel: A matter of time…. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1568), 1129–1137. https://doi.org/10.1098/rspb.2005.3064

Dick, S., Shurin, J. B., & Taylor, E. B. (2014). Replicate divergence between and within sounds in a marine fish: The copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, *23*(3), 575–590. https://doi.org/10.1111/mec.12630

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimatorv2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources, 14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Doherty, P. J., Planes, S., & Mather, P. (1995). Gene Flow and Larval Duration in Seven Species of Fish from the Great Barrier Reef. *Ecology*, *76*(8), 2373–2391. https://doi.org/10.2307/2265814

Drake, J. S., Bernston, E. A., Cope, J. M., Gustafson, R. G., Holmes, E. E., Levin, P. S., Tolimieri, N., Waples, R. S., Sogard, S. M., & Williams, G. D. (2010). Status review of five rockfish species in Puget Sound, Washington: Bocaccio (*Sebastes paucispinis*), canary rockfish (*S. pinniger*), yelloweye rockfish (*S. ruberrimus*), greenstriped rockfish (*S. elongatus*), and redstripe rockfish (*S. proriger*) U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-108, 234 p.

Drinan, D. P., Gruenthal, K. M., Canino, M. F., Lowry, D., Fisher, M. C., & Hauser, L. (2018). Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (Gadus macrocephalus). *Evolutionary Applications*, *11*(8), 1448–1464. https://doi.org/10.1111/eva.12639

Duforet-Frebourg, N., Luu, K., Laval, G., Bazin, E., & Blum, M. G. B. (2016). Detecting Genomic Signatures of Natural Selection with Principal Component Analysis: Application to the 1000 Genomes Data. *Molecular Biology and Evolution*, *33*(4), 1082–1093. https://doi.org/10.1093/molbev/msv334

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eby, L. A., & Crowder, L. B. (2002). Hypoxia-based habitat compression in the Neuse River Estuary: Context-dependent shifts in behavioral avoidance thresholds. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(6), 952–965. https://doi.org/10.1139/f02-067

Engie, K., & Klinger, T. (2007). Modeling passive dispersal through a large estuarine system to evaluate marine reserve network connections. *Estuaries and Coasts*, *30*(2), 201–213. https://doi.org/10.1007/BF02700164

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294x.2005.02553.x

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Faria, R., & Navarro, A. (2010). Chromosomal speciation revisited: Rearranging theory with pieces of evidence. *Trends in Ecology & Evolution*, *25*(11), 660–669. https://doi.org/10.1016/j.tree.2010.07.008

Fay, J. J., & Nammack, M. (1996). Policy Regarding the Recognition of Distinct Vertebrate Population Segments Under the Endangered Species Act. *Fedral Register*, *61*.

Garant, D., Forde, S. E., & Hendry, A. P. (2007). The Multifarious Effects of Dispersal and Gene Flow on Contemporary Adaptation. *Functional Ecology*, *21*(3), 434–443. JSTOR. https://doi.org/10.1111/j.1365-2435.2006.01228.x

García-De León, F. J., Galván-Tirado, C., Sánchez Velasco, L., Silva-Segundo, C. A., Hernández-Guzmán, R., Barriga-Sosa, I. D. L. A., Díaz Jaimes, P., Canino, M., & Cruz-Hernández, P. (2018). Role of oceanography in shaping the genetic structure in the North Pacific hake *Merluccius productus*. *PLOS ONE*, *13*(3), e0194646. https://doi.org/10.1371/journal.pone.0194646

Giæver, M., & Stien, J. (1998). Population genetic substructure in blue whiting based on allozyme data. *Journal of Fish Biology*, *52*(4), 782–795. https://doi.org/10.1111/j.1095-8649.1998.tb00820.x

Gilbert-Horvath, E. A., Larson, R. J., & Garza, J. C. (2006). Temporal recruitment patterns and gene flow in kelp rockfish (*Sebastes atrovirens*). *Molecular Ecology*, *15*(12), 3801–3815. https://doi.org/10.1111/j.1365-294x.2006.03033.x

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: Coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, *5*(1), 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Grant, P. R., & Grant, B. R. (2010). Conspecific versus heterospecific gene exchange between populations of Darwin’s finches. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1543), 1065–1076. https://doi.org/10.1098/rstb.2009.0283

Grantham, B. A., Chan, F., Nielsen, K. J., Fox, D. S., Barth, J. A., Huyer, A., Lubchenco, J., & Menge, B. A. (2004). Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature*, *429*(6993), 749–754. https://doi.org/10.1038/nature02605

Haigh, R., & Starr, P. (2021). Redstripe Rockfish (*Sebastes proriger*) stock assessment for British Columbia in 2018. Fisheries and Oceans Canada.

Hails, R. S., & Morley, K. (2005). Genes invading new populations: A risk assessment perspective. *Trends in Ecology & Evolution*, *20*(5), 245–252.

Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology*, *30*(1), 33–41. https://doi.org/10.1111/cobi.12574

Hannah, R. W., & Rankin, P. S. (2011). Site Fidelity and Movement of Eight Species of Pacific Rockfish at a High-Relief Rocky Reef on the Oregon Coast. *North American Journal of Fisheries Management*, *31*(3), 483–494. https://doi.org/10.1080/02755947.2011.591239

Hedgecock, D. (1994). Does variance in reproductive success limit effective population sizes of marine organisms. In *Genetics and Evolutionn of Aquatic Organisms* (pp. 122–134). Chapman & Hall.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*(8), 1633–1638. https://doi.org/10.1111/j.0014-3820.2005.tb01814.x

Hedrick, P. W. (2013). Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*, *22*(18), 4606–4618. https://doi.org/10.1111/mec.12415

Helvey, M. (1982). First Observations of Courtship Behavior in Rockfish, Genus *Sebastes*. *Copeia*, *1982*(4), 763–770. JSTOR. https://doi.org/10.2307/1444084

Henriques, R., Von der Heyden, S., & Matthee, C. A. (2016). When homoplasy mimics hybridization: A case study of Cape hakes (*Merluccius capensis* and *M. paradoxus*). *PeerJ*, *4*, e1827. https://doi.org/10.7717/peerj.1827

Hess, J. E., Vetter, R. D., & Moran, P. (2011). A steep genetic cline in yellowtail rockfish, *Sebastes flavidus*, suggests regional isolation across the Cape Mendocino faunal break. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(1), 89–104. https://doi.org/10.1139/f10-131

Hoffmann, A. A., & Rieseberg, L. H. (2008). Revisiting the Impact of Inversions in Evolution: From Population Genetic Markers to Drivers of Adaptive Shifts and Speciation? *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 21–42. https://doi.org/10.1146/annurev.ecolsys.39.110707.173532

Hubbs, C. L. (1955). Hybridization between Fish Species in Nature. *Systematic Biology*, *4*(1), 1–20. https://doi.org/10.2307/sysbio/4.1.1

Hyde, J. R., & Vetter, R. D. (2007a). The origin, evolution, and diversification of rockfishes of the genus Sebastes (Cuvier). *Molecular Phylogenetics and Evolution*, *44*(2), 790–811. https://doi.org/10.1016/j.ympev.2006.12.026

Hyde, J. R., & Vetter, R. D. (2007b). The origin, evolution, and diversification of rockfishes of the genus *Sebastes*. *Molecular Phylogenetics and Evolution*, *44*(2), 790–811. https://doi.org/10.1016/j.ympev.2006.12.026

Iwamoto, E., Ford, M. J., & Gustafson, R. G. (2004). Genetic population structure of Pacific Hake, *Merluccius productus*, in the Pacific Northwest. In A. J. Gharrett, R. G. Gustafson, J. L. Nielsen, J. E. Seeb, L. W. Seeb, W. W. Smoker, G. H. Thorgaard, & R. L. Wilmot (Eds.), *Genetics of Subpolar Fish and Invertebrates* (pp. 187–199). Springer Netherlands. https://doi.org/10.1007/978-94-007-0983-6_15

Jackson, T. M., & O'Malley, K. G. (2017). Comparing genetic connectivity among Dungeness crab (*Cancer magister*) inhabiting Puget Sound and coastal Washington. *Marine Biology*, *164*(6). https://doi.org/10.1007/s00227-017-3152-7

Jackson, T. M., Roegner, G. C., & O'Malley, K. G. (2018). Evidence for interannual variation in genetic structure of Dungeness crab (*Cancer magister*) along the California Current System. *Molecular Ecology*, *27*(2), 352–368. https://doi.org/10.1111/mec.14443

Johansson, M. L., Banks, M. A., Glunt, K. D., Hassel-Finnegan, H. M., & Buonaccorsi, V. P. (2008). Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, *17*(13), 3051–3061. https://doi.org/10.1111/j.1365-294x.2008.03814.x

Johansson, M. L., Clifford K, Fodness B, Vazquez NA, & Banks MA. (2012). Mate selection in captive-breeding rockfishes *Sebastes spp.*: Inference from parentage analysis and the major histocompatibility complex (MHC). *Marine Ecology Progress Series*, *460*, 195–206. https://doi.org/10.3354/meps09803

Johnson, M. S., & Black, R. (1982). Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. *Marine Biology*, *70*(2), 157–164. https://doi.org/10.1007/BF00397680

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jorde, P., Knutsen, H., Espeland, S., & Stenseth, N. (2007). Spatial scale of genetic structuring in coastal cod Gadus morhua and geographic extent of local populations. *Marine Ecology Progress Series*, *343*, 229–237. https://doi.org/10.3354/meps06922

Kelly, R. P., & Palumbi, S. R. (2010). Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community. *PLoS ONE*, *5*(1), e8594. https://doi.org/10.1371/journal.pone.0008594

Khangaonkar, T., Long, W., & Xu, W. (2017). Assessment of circulation and inter-basin transport in the Salish Sea including Johnstone Strait and Discovery Islands pathways. *Ocean Modelling*, *109*, 11–32. https://doi.org/10.1016/j.ocemod.2016.11.004

Knutsen, H., Moland Olsen, E., Ciannelli, L., Espeland, S., Knutsen, J., Simonsen, J., Skreslet, S., & Stenseth, N. (2007). Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. *Marine Ecology Progress Series*, *333*, 249–255. https://doi.org/10.3354/meps333249

Krohn, A. R., Conroy, C. J., Pesapane, R., Bi, K., Foley, J. E., & Rosenblum, E. B. (2018). Conservation genomics of desert dwelling California voles (*Microtus californicus*) and implications for management of endangered Amargosa voles (*Microtus californicus scirpensis*). *Conservation Genetics*, *19*(2), 383–395. https://doi.org/10.1007/s10592-017-1010-2

Laidig, T. E. (2010). Influence of ocean conditions on the timing of early life history events for blue rockfish (*Sebastes mystinus*) off California. *Fishery Bulletin*, *108*(4).

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Larson, R. J., & Julian, R. M. (1999). Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. *California Cooperative Oceanic Fisheries Investigations Report*, 94–99.

Leigh, D. M., Lischer, H. E. L., Grossen, C., & Keller, L. F. (2018). Batch effects in a multiyear sequencing study: False biological trends due to changes in read lengths. *Molecular Ecology Resources*, *18*(4), 778–788. https://doi.org/10.1111/1755-0998.12779

Levin, P. S., Tolimieri, N., Nicklin, M., & Sale, P. F. (2000). Integrating individual behavior and population ecology: The potential for habitat-dependent population regulation in a reef fish. *Behavioral Ecology*, *11*(5), 565–571. https://doi.org/10.1093/beheco/11.5.565

Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*(2), 298–299. https://doi.org/10.1093/bioinformatics/btr642

Liu, C.-C., Shringarpure, S., Lange, K., & Novembre, J. (2020). Exploring population structure with admixture models and principal component analysis. In *Statistical population genomics* (pp. 67–86). Humana, New York, NY.

Longo, G. C., Harms, J., Hyde, J. R., Craig, M. T., Ramón-Laca, A., & Nichols, K. M. (2022). Genome-wide markers reveal differentiation between and within the cryptic sister species, sunset and vermilion rockfish. *Conservation Genetics*, *23*(1), 75–89. https://doi.org/10.1007/s10592-021-01397-4

Longo, G. C., Lam, L., Basnett, B., Samhouri, J., Hamilton, S., Andrews, K., Williams, G., Goetz, G., Mcclure, M., & Nichols, K. M. (2020). Strong population differentiation in lingcod (*Ophiodon elongatus*) is driven by a small portion of the genome. *Evolutionary Applications*, *13*(10), 2536–2554. https://doi.org/10.1111/eva.13037

Love, M. S., Morris, P., McCrae, M., & Collins, R. (1990). Life History Aspects of 19 Rockfish Species (Scorpaenidae: Sebastes) from the Southern California Bight. *NOAA Technical Memorandum NMFS*, *87*.

Love, M. S., Yoklavich, M. M., & Thorsteinson, L. (2002). *Rockfishes of the Northeast Pacific*. University of California Press.

Maccready, P., Mccabe, R. M., Siedlecki, S. A., Lorenz, M., Giddings, S. N., Bos, J., Albertson, S., Banas, N. S., & Garnier, S. (2021). Estuarine Circulation, Mixing, and Residence Times in the Salish Sea. *Journal of Geophysical Research: Oceans*, *126*(2). https://doi.org/10.1029/2020jc016738

Masson, D., & Cummins, P. F. (2000). Fortnightly modulation of the estuarine circulation in Juan de Fuca Strait. *Journal of Marine Research*, *58*(3), 439–463. https://doi.org/10.1357/002224000321511106

Masson, D., & Peña, A. (2009). Chlorophyll distribution in a temperate estuary: The Strait of Georgia and Juan de Fuca Strait. *Estuarine, Coastal and Shelf Science*, *82*(1), 19–28. https://doi.org/10.1016/j.ecss.2008.12.022

Matthews, K. (1990). An experimental study of the habitat preferences and movement patterns of copper, quillback, and brown rockfishes (*Sebastes* spp.). *Environmental Biology of Fishes*, *29*, 161–178. https://doi.org/10.1007/BF00002217

McCartney, M. A., Acevedo, J., Heredia, C., Rico, C., Quenoville, B., Bermingham, E., & Mcmillan, W. O. (2003). Genetic mosaic in a marine species flock. *Molecular Ecology*, *12*(11), 2963–2973. https://doi.org/10.1046/j.1365-294x.2003.01946.x

McClelland, E. K., & Naish, K. A. (2007). What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. *Conservation Genetics*, *8*(2), 397–416. https://doi.org/10.1007/s10592-006-9178-x

Melville, J., Haines, M. L., Boysen, K., Hodkinson, L., Kilian, A., Smith Date, K. L., Potvin, D. A., & Parris, K. M. (2017). Identifying hybridization and admixture using SNPs: Application of the DArTseq platform in phylogeographic research on vertebrates. *Royal Society Open Science, 4*(7), 161061. https://doi.org/10.1098/rsos.161061

Miller, B., & Borton, S. (1980). *Geographical Distribution of Puget Sound fishes: Maps and data source sheets.* (Vol. 2). University of Washington Fisheries Research Institute.

Miller, Ian M, Shishido, Caitlin, Antrim, Liam, & Bowlby, C. Edward. (2013). Climate Change and the Olympic Coast National Marine Sanctuary: Interpreting Potential Futures. *Marine Sanctuaries Conservation Series ONMS-13-01. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, MD. 238 pp.*

Montanari, S. R., Hobbs, J.-P. A., Pratchett, M. S., & Van Herwerden, L. (2016). The importance of ecological and behavioural data in studies of hybridisation among marine fishes.

Reviews in Fish Biology and Fisheries, *26*(2), 181–198. https://doi.org/10.1007/s11160-016-9420-7

Moore, S. K., Mantua, N. J., Newton, J. A., Kawase, M., Warner, M. J., & Kellogg, J. P. (2008). A descriptive analysis of temporal and spatial patterns of variability in Puget Sound oceanographic properties. *Estuarine, Coastal and Shelf Science*, *80*(4), 545–554. https://doi.org/10.1016/j.ecss.2008.09.016

Mosher, D. C., & Hewitt, A. T. (2004). Late Quaternary deglaciation and sea-level history of eastern Juan de Fuca Strait, Cascadia. *Quaternary International*, *121*(1), 23–39.

Muto, N., Kai, Y., Noda, T., & Nakabo, T. (2013). Extensive hybridization and associated geographic trends between two rockfishes *Sebastes vulpes* and *S. zonatus* (Teleostei: Scorpaeniformes: Sebastidae). *Journal of Evolutionary Biology*, *26*(8), 1750–1762. https://doi.org/10.1111/jeb.12175

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia university press.

Nielsen, E. E., Hansen, M. M., Ruzzante, D. E., Meldrup, D., & Grønkjær, P. (2003). Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology*, *12*(6), 1497–1508. https://doi.org/10.1046/j.1365-294x.2003.01819.x

Nielsen, E. E., Nielsen, P. H., Meldrup, D., & Hansen, M. M. (2004). Genetic population structure of turbot (*Scophthalmus maximus L.*) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology*, *13*(3), 585–595. https://doi.org/10.1046/j.1365-294x.2004.02097.x

Nielsen, T. G., Kjellerup, S., Smolina, I., Hoarau, G., & Lindeque, P. (2014). Live discrimination of *Calanus glacialis* and *C. finmarchicus* females: Can we trust phenological differences? *Marine Biology*, *161*(6), 1299–1306. https://doi.org/10.1007/s00227-014-2419-5

O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. *Molecular Ecology*, *27*(16), 3193–3206. https://doi.org/10.1111/mec.14792

Pacific Fishery Management Council. (2020). Status of the Pacific Coast Groundfish Fishery: Stock Assessment and Fishery Evaluation. *THE PACIFIC FISHERY MANAGEMENT COUNCIL*, 1–15.

Palsson, W. A., Tsou, T.-S., Bargmann, G. G., Buckley, R. M., West, J. E., Mills, M. L., Cheng, Y. W., & Pacunski, R. E. (2009). The biology and assessment of rockfishes in Puget Sound. *Fish Management Division, Washington Department of Fish and Wildlife*.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics*, *26*(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Parker, S. J., Berkeley, S. A., Golden, J. T., Gunderson, D. R., Heifetz, J., Hixon, M. A., Larson, R., Leaman, B. M., Love, M. S., Musick, J. A., O'Connell, V. M., Ralston, S., Weeks, H. J., & Yoklavich, M. M. (2000). Management of Pacific Rockfish. *Fisheries*, *25*(3), 22–30. https://doi.org/10.1577/1548-8446(2000)025<0022:MOPR>2.0.CO;2

Pawlowicz, R., Hannah, C., & Rosenberger, A. (2019). Lagrangian observations of estuarine residence times, dispersion, and trapping in the Salish Sea. *Estuarine, Coastal and Shelf Science*, *225*, 106246. https://doi.org/10.1016/j.ecss.2019.106246

Petrou, E. L., Fuentes-Pardo, A. P., Rogers, L. A., Orobko, M., Tarpey, C., Jiménez-Hidalgo, I., Moss, M. L., Yang, D., Pitcher, T. J., Sandell, T., Lowry, D., Ruzzante, D. E., & Hauser,

L. (2021). Functional genetic diversity in an exploited marine species and its relevance to fisheries management. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1945), 20202398. https://doi.org/10.1098/rspb.2020.2398

Porter, S. C. (1977). Present and Past Glaciation Threshold in the Cascade Range, Washington, U.S.A.: Topographic and Climatic Controls, and Paleoclimatic Implications. *Journal of Glaciology*, *18*(78), 101–116. https://doi.org/10.3189/s0022143000021559

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics, 155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics, 81*(3), 559–575. https://doi.org/10.1086/519795

R. Walt Deppe, J. Thomson, B. Polagye, & C. Krembs. (2013). Hypoxic intrusions to Puget Sound from the ocean. *2013 OCEANS - San Diego*, 1–9. https://doi.org/10.23919/OCEANS.2013.6741234

Reiss, H., Hoarau, G., Dickey-Collas, M., & Wolff, W. J. (2009). Genetic population structure of marine fish: Mismatch between biological and fisheries management units. *Fish and Fisheries*, *10*(4), 361–395. https://doi.org/10.1111/j.1467-2979.2008.00324.x

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

Roques, S., Sevigny, J.-M., & Bernatchez, L. (2001). Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: A rare marine example. *Molecular Ecology*, *10*(1), 149–165. https://doi.org/10.1046/j.1365-294x.2001.01195.x

Ryan, S., Wohlgeschaffen, G., Jahan, N., Niu, H., Ortmann, A., Brown, T., King, T., & Clyburne, J. (2019). State of Knowledge on Fate and Behaviour of Ship-Source Petroleum Product Spills: Volume 5, Strait of Georgia and the Juan de Fuca Strait. *British Columbia Canadian Manuscript Report of Fisheries and Aquatic Sciences 3176 Canadian Manuscript Report of Fisheries and Aquatic Sciences*.

Salzburger, W., Meyer, A., Baric, S., Verheyen, E., & Sturmbauer, C. (2002). Phylogeny of the Lake Tanganyika Cichlid Species Flock and Its Relationship to the Central and East African Haplochromine Cichlid Fish Faunas. *Systematic Biology*, *51*(1), 113–135. https://doi.org/10.1080/106351502753475907

Schwenke, P. L., Park, L. K., & Hauser, L. (2018). Introgression among three rockfish species (*Sebastes* spp.) in the Salish Sea, northeast Pacific Ocean. *PLOS ONE*, *13*(3), e0194068. https://doi.org/10.1371/journal.pone.0194068

Seeb, L. W. (1998). Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. *Journal of Heredity*, *89*(5), 393–403. https://doi.org/10.1093/jhered/89.5.393

Selwyn, J. D., Hogan, J. D., Downey-Wall, A. M., Gurski, L. M., Portnoy, D. S., & Heath, D. D. (2016). Kin-Aggregations Explain Chaotic Genetic Patchiness, a Commonly Observed Genetic Pattern, in a Marine Fish. *PLOS ONE*, *11*(4), e0153381. https://doi.org/10.1371/journal.pone.0153381

Selz, O. M., Thommen, R., Maan, M. E., & Seehausen, O. (2014). Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *Journal of Evolutionary Biology*, *27*(2), 275–289. https://doi.org/10.1111/jeb.12287

Serrao, N. R., Reid, S. M., & Wilson, C. C. (2018). Conservation genetics of redside dace (*Clinostomus elongatus*): Phylogeography and contemporary spatial structure. *Conservation Genetics*, *19*(2), 409–424. https://doi.org/10.1007/s10592-017-1012-0

Shafer, A. B. A., Cullingham, C. I., Côté, S. D., & Coltman, D. W. (2010). Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology*, *19*(21), 4589–4621. https://doi.org/10.1111/j.1365-294x.2010.04828.x

Shanks, A. L., & Eckert, G. L. (2005). Population persistence of California current fishes and benthic crustaceans: A marine drift paradox. *Ecological Monographs*, *75*(4), 505–524. https://doi.org/10.1890/05-0309

Shin, J.-H., Blay, S., McNeney, B., & Graham, J. (2006). LDheatmap: An R Function for Graphical Display of Pairwise Linkage Disequilibria Between Single Nucleotide Polymorphisms. *Journal of Statistical Software, Code Snippets*, *16*(3), 1–9. https://doi.org/10.18637/jss.v016.c03

Sivasundar, A., & Palumbi, S. R. (2010). Life history, ecology and the biogeography of strong genetic breaks among 15 species of Pacific rockfish, *Sebastes*. *Marine Biology*, *157*(7), 1433–1452. https://doi.org/10.1007/s00227-010-1419-3

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.-H., Michael, & Michael. (2011). Adaptive Introgression of Anticoagulant Rodent Poison Resistance by

Hybridization between Old World Mice. *Current Biology*, *21*(15), 1296–1301. https://doi.org/10.1016/j.cub.2011.06.043

Soulé, M. E. (1985). What Is Conservation Biology? *BioScience*, *35*(11), 727–734. JSTOR. https://doi.org/10.2307/1310054

Stemshorn, K. C., Reed, F. A., Nolte, A. W., & Tautz, D. (2011). Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus sp.*). *Molecular Ecology*, *20*(7), 1475–1491. https://doi.org/10.1111/j.1365-294x.2010.04997.x

Stout, H.A., B.B. McCain, R.D. Vetter, T.L. Builder, W.H. Lenarz, L.L. Johnson, and R.D. Methot. 2001. Status review of Copper Rockfish, Quillback Rockfish, and Brown Rockfish in Puget Sound, Washington.U.S. Dept. Commer., NOAA Tech. Memo. NMFS- NWFSC-46, 158 p.

Suneetha, K. B., & Nævdal, G. (2001). Genetic and morphological stock structure of the pearlside, *Maurolicus muelleri* (Pisces, Sternoptychidae), among Norwegian fjords and offshore area. *Sarsia*, *86*(3), 191–201. https://doi.org/10.1080/00364827.2001.10420475

Szatmári, L., Cserkész, T., Laczkó, L., Lanszki, J., Pertoldi, C., Abramov, A. V., Elmeros, M., Ottlecz, B., Hegyeli, Z., & Sramkó, G. (2021). A comparison of microsatellites and genome-wide SNPs for the detection of admixture brings the first molecular evidence for hybridization between *Mustela eversmanii* and *M. putorius* (Mustelidae, Carnivora). *Evolutionary Applications*, *14*(9), 2286–2304. https://doi.org/10.1111/eva.13291

van Herwerden, L., Choat, J. H., Dudgeon, C. L., Carlos, G., Newman, S. J., Frisch, A., & van Oppen, M. (2006). Contrasting patterns of genetic structure in two species of the coral trout Plectropomus (Serranidae) from east and west Australia: Introgressive hybridisation or ancestral polymorphisms. *Molecular Phylogenetics and Evolution*, *41*(2), 420–435. https://doi.org/10.1016/j.ympev.2006.04.024

Wallace, R. K., & Fletcher, K. M. (1996). *A Manual for understanding the Federal Fisheries Management Process, Including Analysis of the 1996 Sustainable Fisheries Act*. 62.

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*(4), 233–240. https://doi.org/10.1038/hdy.2016.60

Waples, R. S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*(5), 438–450. https://doi.org/10.1093/jhered/89.5.438

Washington State Department of Fish and Wildlife. (2011). Final Environmental Impact Statement for the Puget Sound Rockfish Conservation Plan Including Preferred Range of Actions. *Washington Department of Fish and Wildlife Fish Program*, 223.

Washington State Department of Fish and Wildlife. (2019). Washington State Threatened and Endangered Species List. *Washington Administrative Code 220-610-010, June*, 2.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, *38*(6), 1358–1370. JSTOR. https://doi.org/10.2307/2408641

Wellenreuther, M., & Bernatchez, L. (2018). Eco-Evolutionary Genomics of Chromosomal Inversions. *Trends in Ecology & Evolution*, *33*(6), 427–440. https://doi.org/10.1016/j.tree.2018.04.002

Wennerström, L., Laikre, L., Ryman, N., Utter, F. M., Ab Ghani, N. I., André, C., Defaveri, J., Johansson, D., Kautsky, L., Merilä, J., Mikhailova, N., Pereyra, R., Sandström, A., Teacher, A. G. F., Wenne, R., Vasemägi, A., Zbawicka, M., Johannesson, K., & Primmer,

C. R. (2013). Genetic biodiversity in the Baltic Sea: Species-specific patterns challenge management. *Biodiversity and Conservation*, *22*(13–14), 3045–3065. https://doi.org/10.1007/s10531-013-0570-9

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (2nd ed. 2016). Springer International Publishing : Imprint: Springer. https://doi.org/10.1007/978-3-319-24277-4

Willis, P. M., Ryan, M. J., & Rosenthal, G. G. (2011). Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish. *Behavioral Ecology*, *22*(6), 1234–1240. https://doi.org/10.1093/beheco/arr119

Zier, J., & Gaydos, J. K. (2016). The growing number of species of concern in the Salish Sea suggests ecosystem decay is outpacing recovery. *Proceedings of the 2016 Salish Sea Ecosystem Conference*, 17.

Chapter 2. DIVERGENT POPULATION STRUCTURE IN FIVE COMMON ROCKFISH SPECIES OF PUGET SOUND, WA SUGGESTS THE NEED FOR SPECIES-SPECIFIC MANAGEMENT

Authors: Anita Wray, Eleni Petrou, Krista Nichols, Robert Pacunski, Larry LeClair, Kelly Andrews, Lorenz Hauser

2.1 ABSTRACT

Quantifying the connectivity between populations of endangered or threatened marine populations is critical information for scientifically sound management. Of the 86 species managed by the Pacific Fishery Management Council (PFMC) on the West Coast of the United States, over 75% are rockfishes, and of those species, 27 were once deemed ‘at risk’. Although most stocks have been successfully rebuilt along the Washington Coast, Puget Sound stocks have yet to recover despite fisheries closures. Their connectivity to coastal populations is relatively unknown, despite the potential of recruitment subsidies from the coast and considerable interest in reopening many fisheries for recreational use. The importance of accurate connectivity estimates was demonstrated by recent research on three Endangered Species Act (ESA) listed species in Puget Sound, which demonstrated that only two species were sufficiently distinct to warrant listing as separate Puget Sound distinct population segments (DPS). The common assumption of isolation of Puget Sound populations, which stems primarily from research on three hybridizing species, may therefore be erroneous. This study aimed to provide an analysis of the connectivity of five Puget Sound rockfish populations and identify distinct population segments where appropriate. Samples from five species (Black (*Sebastes melanops*), Yellowtail (*S. flavidus*), Redstripe (*S. proriger*), Greenstriped (*S. elongatus*), and Puget Sound (*S. emphaeus*)) were collected in three areas within and one outside of Puget Sound and analyzed at over 12,000 restriction-site associated DNA sequencing (RADseq) loci. We show unique species-specific patterns of genetic diversity, which are attributed to multiple extrinsic and intrinsic factors. In particular, Black and Puget Sound Rockfish show no genetic differentiation, Yellowtail and Greenstriped were structured according to known geographic barriers, and Redstripe Rockfish revealed evidence for temporal genetic differentiation, suggesting that irregular recruitment influences population structure. None of the species followed the DPS boundaries generally assumed for rockfish, further emphasizing the importance of species-specific management for the effective recovery of these rockfish populations.

2.2 INTRODUCTION

Effective management of wild species in both conservation and economic contexts relies on the identification of distinct population units. In the United States, populations of endangered vertebrate species can be protected under the Endangered Species Act if they can be defined as distinct population segments (DPS). Such population segments need to be significant to the species as a whole and discrete from other populations (Waples, 1998). As a result, DPSs are often quite large and may include several sub-populations that may need independent management (Wallace & Fletcher, 1996). Identifying populations and DPSs can help play a critical role in the maintenance of species’ genetic diversity.

On a smaller scale, management units (MUs) are a critical component of fishery management for sustainable harvest (Reiss et al., 2009), and can be defined as populations with identifiable patterns of recruitment and mortality (Carvalho & Hauser, 1994). They can be identified based on demographic, genetic, or morphological characters. Many management units can be clustered within a DPS and are therefore a less stringent entity often connected by some exchange with other units. However, for many marine species with wide spatial distributions and high dispersal potential, defining management units can be difficult because of a lack of obvious geographic barriers and weak signals of genetic population structure (Waples, 1998).

Quantifying the connectivity between populations is a critical component for defining MU and DPS for a species of interest. The rapid advance in genomic tools has improved our ability to detect subtle population structure typical for marine species, patterns of connectivity, and complex patterns of hybridization in harvested or endangered species (Dahle et al., 2018). Current genomic technologies enable the identification of both DPSs and MUs that can inform species conservation and fishery management plans. This research has been implemented for complex species or species groups, such as the Pacific Ocean rockfishes (*Sebastes* spp.) (Andrews et al., 2018).

Pacific rockfishes are part of a diverse and hybridizing species complex with widely variable harvest intensities and conservation status, yet little information is available on species-specific patterns of population structure (Love et al. 1990; Drake et al. 2010). Despite being a diverse genus with numerous species, rockfishes display common life history characteristics important for evaluating management goals and setting any future harvest quotas. Rockfishes have ranging but relatively long life spans, late maturity, slow growth, and limited movements as adults; they are also viviparous, with high fecundity, extensive larval dispersal but high larval mortality resulting in sporadic recruitment (Love et al. 2002). These characteristics make rockfish especially vulnerable to overfishing. Despite a generally similar life history, rockfish differ in some characteristics that could affect population structure (such as adult site fidelity, habitat preferences of adults, pelagic larval timing and duration, and adult depth) but the effect of this life history variation on connectivity has not been identified. Unfortunately, rockfish management remains difficult due to the lack of resources to assess these species on a regular basis. The gap in knowledge provided by irregular stock assessments for these species is particularly concerning in regions that have not experienced a significant recovery in population size after management strategies have been established.

Of the 64 species of rockfishes managed by the Pacific Fishery Management Council (PFMC), over 83% are considered moderately or highly vulnerable to overfishing (Pacific Fishery Management Council, 2020). Differences in demographic responses between species and populations of rockfishes to management measures are particularly noteworthy along the Pacific coast of the US and Canada. While many rockfish stocks along the US West Coast have been rebuilt (Pacific Fishery Management Council, 2020), Puget Sound rockfishes, which may be isolated from coastal populations, are ‘in trouble’ (Washington State Department of Fish and Wildlife, 2011; Zier & Gaydos, 2016). Puget Sound is a 6,039 square km glacier-carved inlet in northern Washington, USA, that is the second largest estuary in the United States and is relatively isolated from the open coast. The larger Puget Sound - Georgia Basin region is comprised of several discrete basins connected to the Pacific Ocean by two oceanic straits, although the northern Johnstone Strait has little impact on circulation (Khangaonkar et al., 2017; Moore et al., 2008). Currently, two major sills define the boundaries of rockfish management units and DPSs. The Victoria Sill separates the north sound from the coast, and the Admiralty Inlet sill separates southern and northern Puget Sound. These unique oceanographic conditions allow for a strong zoogeographic break between the coast and in-shore populations (Andrews et al., 2018; Drinan et al., 2018). The circulation patterns in the region also limit the dispersal of planktonic organisms and influence genetic connectivity among populations (Engie & Klinger, 2007). As a result of these bathymetric and current conditions, many species may have distinct coastal and Puget Sound populations, e.g., Yelloweye Rockfish (*Sebastes ruberrimus*) (Andrews et al., 2018) and Pacific Cod (*Gadus macrocephalus*) (Drinan et al., 2018). Identifying the species that are bound by these oceanographic conditions, as it pertains to life history characteristics, is critical for effective management.

Several early studies investigated the genetic population structure in three Puget Sound rockfish species (Brown (*S. auriculatus*), Copper (*S. caurinus*), and Quillback (*S. maliger*)) (Buonaccorsi et al., 2002, 2005; Seeb, 1998). These studies detected significant population differentiation in Brown and Copper Rockfish (Buonaccorsi et al., 2002, 2005), leading to the assumption that all rockfish species within Puget Sound were likely isolated from populations along the coast, and were therefore DPSs (Drake et al., 2010). These three species, however, are known to hybridize within this region (Buonaccorsi et al., 2002, 2005; Schwenke et al., 2018; Seeb, 1998). There is no evidence that other rockfish species hybridize within the Washington coast and Puget Sound. Insufficient information exists to determine if the population structure identified was entirely due to localized extensive hybridization within Puget Sound, or limited connectivity within single species.

Based on the prior declaration that all Puget Sound rockfishes are a DPS, paired with precipitous population declines caused by intense fishing pressure from 1970-1990, the Puget Sound DPS of three species of rockfish were listed under the US Endangered Species Act (Drake et al., 2010). Bocaccio (*S. paucispinis*) was listed as endangered, while Yelloweye (*S. ruberrimus*) and Canary (*S. pinniger*) Rockfish were listed as threatened (Drake et al., 2010). In addition, 10 species of rockfish have been listed as candidates for state protection as DPSs within Puget Sound

(Washington State Department of Fish and Wildlife, 2019). Subsequent studies on the three ESA listed species revealed differentiation between Puget Sound and coastal populations in Yelloweye Rockfish, but not in Canary Rockfish (Andrews et al., 2018), which resulted in the delisting of Canary Rockfish. Follow up studies suggested that this population differentiation cannot be explained by larval retention (Andrews et al. 2021). Current explanations for this species specific population structure suggest that life history characteristics, such as adult site fidelity and adult depth preference, could play a large role in population structure within Puget Sound (Andrews et al., 2018). However, more research is needed to correlate life history characteristics to Puget Sound rockfish population structure.

Since rockfish are a diverse genus, additional species-specific information will be critical to the development of effective management and conservation plans. Additionally, identifying any correlation between life history patterns and genetic isolation can help broaden conclusions for other species. Further clarifying the population structure of rockfishes in Puget Sound on a species-specific level has been recommended by the American Fisheries Society (Parker et al., 2000), Washington Department of Fish and Wildlife (WDFW) (Palsson et al., 2009), and the Northwest Fisheries Science Center of the National Oceanographic and Atmospheric Administration (NOAA-NMFS) (Drake et al., 2010). Current reestablishment of a recreational fishery for Puget Sound rockfishes depend to a large extent on the level of connectivity to recovered coastal populations, so information on the genetic differentiation and isolation of Puget Sound populations is of vital importance to the management of rockfishes. This is especially important for the recreational fishery in Puget Sound, which has the potential to reopen after a decade-long closure if subsidies on from the coast are sufficient to maintain stocks.

The broad objective of this study is to provide a comprehensive survey of population structure of several common rockfishes in Puget Sound and the Washington coast, with aim to inform fisheries management and conservation. More specifically, we estimated population structure and connectivity from restriction site associated DNA (RAD) sequencing and compared population differentiation between species as it relates to life history parameters. Using these results, we evaluated the potential for supplementation of Puget Sound populations from the Washington Coast and compared population connectivity with current DPS boundaries within Puget Sound.

2.3 METHODS

2.3.1 Sampling Procedure

We used 279 samples from five species of rockfish (Black (*S. melanops*), Yellowtail (*S. flavidus*), Redstripe (*S. proriger*), Greenstriped (*S. elongatus*), and Puget Sound (*S. emphaeus*), Figure 2.1) that were collected in 1999-2021 in previous surveys of WDFW, NOAA-NMFS, and the Department of Fisheries and Oceans (DFO Canada). Individual fin clips were preserved in 95% ethanol or dried on filter paper. Samples were collected from multiple locations (see Figure 2.3), grouped into four different regions: 1) southern Puget Sound (Puget Sound proper, south of Admiralty Inlet, SPS), 2) British Columbia (Canadian Salish Sea north of the US/Canada border, BC), 3) northern Puget Sound (US Salish Sea north of Admiralty Inlet, NPS and east of the Victoria Sill) and 4) the US west coast (US Pacific Coast west of Victoria Sill, WC). Due to differences in the abundance and distribution of species across this geographic range, we have no Puget Sound Rockfish from WC and one Greenstriped Rockfish from NPS.

2.3.2 DNA Extraction, Library Preparation, and Sequencing

Genomic DNA was extracted using the Nexttec DNA isolation kit (Nexttec Incorporated, Middlebury, VT, USA) following manufacturer's protocol and quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA, USA). DNA concentration was normalized to 125ng in 10 µL of molecular grade water. Restriction site-associated DNA sequencing (RADseq) libraries were prepared using a version of the Ali et al. (2016) protocol without the targeted bait capture step, referred to in the literature as BestRAD (https://github.com/merlab-uw/Protocols/blob/main/bestRAD). Briefly, genomic DNA was digested using the *Sbf*I enzyme. An adapter (P1) containing a forward amplification primer site, an Illumina sequencing primer site, and an individual 6 bp barcode was ligated to each fragment at the restriction site end. Fragments were then randomly sheared using sonication and size-selected to 300-500 bp in length. Subsequently, P2 adapters were ligated to the reverse end and libraries were amplified by PCR. Each library was assessed for quality on a 1% agarose gel and a Bioanalyzer DNA 1000 kit (Agilent Technologies, Santa Clara, CA). Libraries were pooled in equimolar amounts and sequenced on a NovaSeq (paired end, 116 bp or 150 bp) at the University of Oregon, either a S4 or SP run type. Ninety-six individuals were randomly included in one of six RADseq libraries to avoid any lane effect (Leigh et al., 2018).

2.3.3 Initial Filtering

Raw sequence data were quality checked using multiQC (Ewels et al., 2016). Prior to SNP calling and genome alignment, raw sequences were demultiplexed using *process_radtags* in the STACKS v2.60 pipeline (Catchen et al., 2011; Rochette et al., 2019). Sequences were trimmed to 104 bases and filtered for quality. Individuals with fewer than 250,000 total reads were excluded from downstream analysis (Krohn et al., 2018). Our paired-end sequences were then aligned to the Honeycomb Rockfish (*Sebastes umbrosus*) genome from GenBank (NCBI Accession Number: PRJNA562243) with Bowtie2 v2.4. using the 'very-sensitive' option (Langmead & Salzberg, 2012). The Honeycomb Rockfish genome is one of only two annotated full genomes and was chosen due to its closer phylogenetic relationship to our species (Hyde & Vetter, 2007a). Following genome alignment, SNP calling, and basic population genetics statistics were calculated using the *gstacks* and *populations* modules from the STACKS pipeline. SNPs were called if they had a minimum mapping quality of 40. SNPs were filtered following published recommendations (O'Leary et al., 2018) requiring that loci meet the following criteria: minimum genotype depth ≥ 5, mean minimum read depth ≥ 15, genotype call rate ≥ 80%. We chose one SNP on each RADtag with the highest minor allele frequency. Genotype frequencies that were significantly different than expectations under Hardy-Weinberg Equilibrium (HWE) were removed using the following procedure: p-values were calculated across samples for each population using the r package pegas v1.1 (Paradis, 2010). P-values were then combined across samples for each locus using Fisher's combination of probabilities, and adjusted to q-values for the false discovery rate (Benjamini & Hochberg, 1995). Loci with q-values below 0.05 were considered significantly out of HWE and removed from downstream analysis.

2.3.4 Misidentification Analysis

To identify any possible cases of misidentification or interspecific hybridization in our dataset, raw sequences for all individuals analyzed together for all eight species (five from this chapter, three from previous chapter) immediately after genome alignment. SNP calling, and basic population genetics statistics were calculated using the *gstacks* and *populations* modules from the STACKS pipeline. SNP filtering followed the same protocol as in the species-specific analyses

(O’Leary et al., 2018). Different from the species-specific analysis however, we intended to avoid SNPs with fixed differences between species, because this would likely find differences between only two species. Therefore, we chose the first SNP on each RADtag using the –write-single-snp option in *populations*. Additionally, we did not filter for HWE because a reduction of heterozygosity due to species-specific (subpopulation) structure would likely influence HWE p-values (Wahlund effect). We plotted all eight species together in a principal components analysis (PCA). Any individuals that visually grouped with a species different than its field identification was removed from any downstream analysis.

2.3.5 Species Specific Analyses

We identified patterns of population structure with principal components analyses (PCAs), STRUCTURE analyses, pairwise F_{ST}, and linkage disequilibrium. After removing misidentified individuals, and to explore relationships among populations, we used the R package adegenet v2.1.8 (Jombart, 2008) which computes a principal components analysis to summarize the multivariable dataset into ten dimensions. Second, we used STRUCTURE v2.3.4 which estimates the most likely clustering pattern for our individuals (Pritchard et al., 2000). STRUCTURE was run without a priori population knowledge and using the admixture model. Two replicates were run for K clusters 1-10 with a burn-in of 10,000 iterations and 100,000 MCMC reps. We chose the range of likely K groups with Structure Harvester (Earl & vonHoldt, 2012), using both the ΔK statistic (Evanno et al., 2005) and the mean L(K). Overall and pairwise F_{ST} values (Weir & Cockerham, 1984) were estimated with 1,000 bootstraps using the R package hierfstat v0.5-11 (Goudet, 2005). F_{ST} values were considered significant if the lower limit of the 95% confidence interval did not overlap with zero.

To investigate potential causes of genetic differentiation for our species, we performed linkage analysis, individual genetic summary statistics (observed heterozygosity and read depth), population-based summary statistics (H_O, H_E, F_{IS}), individual relatedness estimates, and correlated patterns of genetic structure with various life history characteristics. We estimated linkage disequilibrium within each chromosome using PLINK v1.07 (Purcell et al., 2007). R^2 values were then mapped on each chromosome to identify blocks of highly linked loci using the R function LDheatmap v1.0-6 (Shin et al., 2006). Chromosomes with loci in strong LD ($r^2 > 0.5$) blocks were analyzed used PCAs in adegenet v2.1.8 (Jombart, 2008) to determine whether individuals clustered in the three-stripe patterns consistent with chromosomal inversions (Hoffmann & Rieseberg, 2008; Petrou et al., 2021). Summary statistics were calculated using VCFtools v0.1.13 (individual heterozygosity, individual read depth) (Danecek et al., 2011), and hierfstat v0.5-11 (H_O, H_E, F_{IS}) (Goudet, 2005). To investigate any patterns unexplained by geographic region, we attempted to correlate patterns with both genetic and life history characteristics, including size, sampling date, sequencing run and well, overall diversity, relatedness, and read depth. This was conducted by relabeling the color of each individual within the PCA graph according to the above characteristics to visualize patterns. Genetic relatedness was calculated on all pairs of individuals with PLINK v1.07 (Purcell et al., 2007) to evaluate if our dataset included related individuals (percent IBD > 10%). Finally, effective population size was estimated using the random mating, linkage disequilibrium model in NeEstimator (Do et al., 2014) with a minor allele frequency (MAF) cutoff of 0.05. Values were adjusted using Waples et al. (2016) which adjusts for the likelihood that some loci are linked in a sample set of many loci and relatively few chromosomes.

2.4 RESULTS

For **Black Rockfish,** 19,700 loci were retained after filtering. In addition, seven individuals were removed from analysis due to low read count. Six individuals were outliers based on high scores on PC1 and very low heterozygosity (Supplemental Figure 2.1 & 2.2). These low-heterozygosity individuals were not characterized by unusual patterns in body size, DNA degradation, or sequencing depth. Additionally, DNA libraries were prepared for these individuals on different days and sequencing runs, limiting the possibility of batch effects. We were unable to attribute any genetic or biological association (size, sampling date, sequencing run and well, overall diversity, relatedness, and read depth) to the four outliers along PC2 so we kept them in the dataset. Once the six outlier individuals with low heterozygosity were removed from the analysis, we found limited evidence for geographic structure (Figure 2.2). Pairwise F_{ST} estimates were small (0.001 for all comparisons), but significant (Table 2.2). On the other hand, STRUCTURE results suggested that there was one genetic cluster (K=1), which agreed with the prior PCA analyses (Supplemental Figure 2.3). We found no chromosomes with highly linked regions, suggesting that there were no chromosomal inversions.

For **Redstripe Rockfish**, 12,275 loci were retained after filtering. Nine individuals were removed from analysis, six individuals due to low read count and three due to field misidentification. Of the three misidentified individuals, one clustered with Greenstriped Rockfish and two clustered with Puget Sound Rockfish (Supplemental Figure 2.4). We found evidence for two genetic clusters for Redstripe Rockfish (K=2), but there was no clear geographic pattern (Figure 2.2). STRUCTURE results agreed with the prior PCA analyses. One of the clusters identified in the PCA and STRUCTURE analyses was primarily (20/24 individuals, or 83%) sampled in 2014 (Supplemental Figure 2.5). Samples from 2014 were collected in multiple geographic locations and at multiple dates throughout the year and had low pairwise relatedness value (Supplemental Figure 2.6) which suggests these samples are not from the same spawning pair. The average pairwise relatedness value for the 2014 sample group was not significantly higher than the samples collected in other years ($p = 0.68$). Additionally, samples from 2014 were larger in size (256 mm and 231 mm for 2014 and other year samples, respectively) but had a much smaller variance (1,653 mm^2 and 3,066 mm^2 for 2014 and other year samples, respectively). The small variance in length suggests these individuals are of similar age (Supplemental Figure 2.7). N_e estimates were 1.7 times larger for the 2014 cluster (1,229 ± 4.4) than the other cluster (729 ± 1.7) (Supplemental Table 2.2). We found no chromosomes with highly linked regions, suggesting that there were no large chromosomal inversions.

Our **Greenstriped Rockfish** dataset retained 14,439 loci after filtering. Four individuals were removed from analysis, two because of low read count and two because of field misidentification. Both misidentified individuals clustered with Redstripe Rockfish (Supplemental Figure 2.4). Only one individual from NPS was included in our final dataset, limiting our ability to make conclusions about connectivity throughout this geographic region. The PCA separated individuals collected in British Columbia from all other samples along Axis 1, while Axis 2 showed some separation of WC individuals from SPS individuals (Figure 2.2). All British Columbia samples were collected from 2021 (Supplemental Figure 2.10). STRUCTURE results suggest similar ancestry for the WC, NPS and SPS populations, and separate ancestry for the BC population. Similar patterns were seen in the F_{ST} analysis. Pairwise F_{ST} estimates between BC and all other populations (WC, NPS, SPS) were large and significant (0.025, 0.024, 0.021 for SPS, NPS and WC, respectively) (Table 2.2). The WC sample collection was significantly different from the SPS sample ($F_{ST} = 0.003$) but approximately ten times smaller compared to the BC pairwise estimates. Pairwise F_{ST} estimates for the NPS-SPS and NPS-WC were non-significant (0 and 0.003, respectively), but the NPS collection contained only one single individual. Our linkage disequilibrium results located one block of highly linked loci on Chromosome 16 (Supplemental Figure 2.8). The principal components analysis of Chromosome 16 was not consistent with either (a) the pattern of genetic structure seen in the whole chromosome PCA or (b) a three banding pattern consistent with inversions (Supplemental Figure 2.9).

For **Yellowtail Rockfish**, 18,979 loci were retained after filtering. Three individuals were removed from analysis, two because of low read count and one because of field misidentification. The misidentified individual was either a Brown, Copper, or Quillback Rockfish (Supplemental Figure 2.4). PCA and STRUCTURE plots revealed WC as a separate population from NPS and SPS (Figure 2.2) and identified two individuals with WC ancestry in Puget Sound and six individuals with ancestry from both Puget Sound and the Washington Coast. F_{ST} estimates were non-significant for the NPS-SPS comparison, but small and significant for the SPS-WC and NPS-WC comparisons (i.e., between WC and all of Puget Sound) (Table 2.2). Results suggest the presence of a geographic barrier between Puget Sound and the Washington Coast, somewhere in the easternmost portion of the Strait of Juan de Fuca, near the Victoria Sill (Figure 2.3). We found no chromosomes with highly linked regions, suggesting that there were no chromosomal inversions.

For **Puget Sound Rockfish**, 15,200 loci were retained after filtering. Eight individuals were misidentified in the field and were thus removed from analysis, all of which were genetically identified as Redstripe Rockfish (Supplemental Figure 2.4). Two individuals were removed due to low read count. We found no evidence of population structure in Puget Sound Rockfish. The PCA showed no distinct clustering of individuals based on geographic structure (Figure 2.2).

STRUCTURE analysis showed that K=1 using the greatest L(K), since the Evanno method does not evaluate K=1 (Supplemental Figure 2.3). All pairwise F_{ST} estimates were non-significant (Table 2.2). We found no chromosomes with highly linked regions, suggesting that there were no chromosomal inversions.

2.5 DISCUSSION

In this study, we assessed the population structure of five common rockfishes of the Salish Sea, to verify currently used DPS delineations and to inform fisheries management. Despite similar life history characteristics, we found species-specific patterns of population structure. This suggests that no life history characteristic or physical barrier can be used to infer population structure between rockfish species. For example, Yellowtail and Greenstriped Rockfish were structured by known geographic or oceanographic barriers. In contrast, Puget Sound, Black and Redstripe Rockfish showed no geographic population structure. Redstripe Rockfish, however, showed population structure likely caused by an irregular recruitment event. There are many possible mechanisms that may cause population structure in marine species, including geographic boundaries (Johansson et al., 2008), population history (Canino et al., 2010), life history characteristics such as depth preference (Sivasundar & Palumbi, 2010), and the behavior of juveniles (Levin et al., 2000), sweepstake recruitment (Burford & Larson, 2007), and chromosomal inversions (Longo et al., 2020). Here we discuss the potential causes for population structure for our study species.

2.5.1 Bathymetry and Oceanography

Oceanographic conditions and bathymetry influence population structure in many marine species, including rockfishes throughout their geographic range (Andrews et al., 2021; Hess et al., 2011; Sivasundar & Palumbi, 2010). For example, Point Conception in California is known as a biogeographic barrier for Vermilion (Longo et al., 2022), Brown (Buonaccorsi et al., 2005b), and Grass Rockfish (Buonaccorsi et al., 2003). Similar biogeographic boundaries are known in the Salish Sea, a unique glacier-carved inlet that is comprised of several discrete basins with only one large connection to the ocean (S. J. Moore et al., 2008). These bathymetric conditions allow for a strong zoogeographic break between coastal and in-shore populations. The circulation driven by the unique bathymetry in the region further restrict dispersal of planktonic organisms, which in turn influences genetic connectivity among populations (Engie & Klinger, 2007). As a result of the combined bathymetric and circulation conditions, many species may have distinct coastal and Puget Sound populations, including Pacific Cod (Drinan et al., 2018), Yelloweye Rockfish (Andrews et al., 2018), Dungeness Crab (Jackson & O'Malley, 2017) and Pacific Hake (Iwamoto et al., 2004). Results from Yellowtail and Greenstriped Rockfish follow this pattern and suggest that similar geographic and oceanographic conditions may influence population structure in these rockfish species.

Our study suggests that oceanographic conditions in British Columbia drive limited dispersal, which contribute to population isolation in Greenstriped Rockfish. Recent studies modeling the impact of oceanographic conditions on larval dispersal of Yelloweye and Canary Rockfish suggest that larvae within the British Columbia part of the Salish Sea are likely to remain within the basin north of the San Juan Islands (Andrews et al., 2021) due to current conditions in the area, which restricts nearly half of bottom layer inflow from the Juan de Fuca Strait and south/central Puget Sound (Khangaonkar et al., 2017). These conditions facilitate a high residence time (160 days) within the Strait of Georgia, which is 70 days higher than any other basin in Puget Sound (Pawlowicz et al., 2019). Thus, regional larval retention could be a mechanism contributing to limited gene flow and population structure in Greenstriped Rockfish. In addition, there is only one deep water passage between the deeper British Columbia waters and the Strait of Juan de Fuca (Khangaonkar et al., 2017). Shallow regions within San Juan Islands could restrict adult movement of deep water species, such as the Greenstriped Rockfish (Love et al., 2002). As this was the only species collected from British Columbia, this suggests that international collaboration is required to properly assess the genetic structure of rockfishes in the Salish Sea.

Another known geographic barrier to gene flow in the Salish Sea is the Victoria Sill, a shallow sill (~55 m, (Ryan et al., 2019)) that divides the Puget Sound from the Strait of Juan de Fuca and the Washington Coast. For example Yelloweye Rockfish (Andrews et al., 2018) and Pacific Cod (Drinan et al., 2018) show genetic differentiation across the Victoria Sill. The Victoria Sill is a shallow, sandy sill which causes considerable vertical mixing in the Strait of Juan De Fuca (Khangaonkar et al., 2017), influencing salinity (Masson & Cummins, 2000) and primary productivity (Masson & Peña, 2009) and larval retention times (Engie & Klinger, 2007). Thus, the combination of shallow water and a shift in current patterns could act as a barrier limiting both adult and larval dispersal. This shallow sill was also modeled to restrict larval dispersal for Canary Rockfish (K. Andrews, personal communication). Our study identified this geographic area as a possible barrier to dispersal, as we identified two distinct populations of Yellowtail Rockfish to either side of this shallow sill. This species is the only one in our study whose patterns of genetic population structure align with the previous assumption that all rockfish species are isolated within Puget Sound.

Despite the ubiquity of geographic barriers to gene flow and dispersal, genetic breaks are often species-specific, as seen in our study. Species-specific geographic barriers to gene flow have been identified in complex marine habitats such as the Baltic Sea (Wennerström et al., 2013), as well as along large coastlines for rockfish (Longo et al., 2022; Sivasundar & Palumbi, 2010) and intertidal organisms (Kelly & Palumbi, 2010). Many marine barriers are permeable/soft barriers, and as such their effectiveness depends on a species life history characteristics such as larval behavior, settlement behavior (Sivasundar & Palumbi, 2010) and parturition timing (Shanks & Eckert, 2005), which will be discussed below (section 2.5.3).

2.5.2 Population History

Current population structure and genetic diversity also depend on historical demographic events and changes in global climate patterns. In particular, the impact of past ice ages on genetic population structure is highly species-specific and likely influenced by a species' particular life history characteristics and physiological tolerances (Bernatchez & Wilson, 1998). In the North Pacific, the Pleistocene ice-ages glaciated most of Canada and parts of northern Washington (Porter, 1977) but populations may have persisted in isolated glacial refugia (Shafer et al., 2010).

In some instances, refugial populations are now in panmixia (such as in the catadromous European eel (Dannewitz et al., 2005)), likely due to high gene flow in the marine environment. In contrast, recolonization of the Salish Sea and the Washington Coast from different refugia may partly be the cause for the high genetic differentiation found in Pacific cod (Canino et al., 2010). Despite evidence for dispersal between the two populations of Pacific cod, the long time span since divergence between these two populations was enough to show genetic differentiation, suggesting these populations may not yet be in migration-drift equilibrium. Although not explicitly investigated in our study, differences in population structure among our five species of rockfish could be caused by species-specific responses to glaciation. Refugial populations for some species might have persisted where others did not. Secondary contact between multiple refugia populations could in part explain the genetic differentiation in Yellowtail and Greenstriped Rockfish, despite evidence for dispersal between the populations in both species. Additionally, populations originating from separate glacial refugia could represent a significant evolutionary legacy of the species (Serrao et al., 2018), which could provide increased adaptive potential. This increase in adaptive potential is significant for the recovery of a population, and can be the ‘significance’ criteria needed to list populations as a DPS under the ESA (Fay & Nammack, 1996).

2.5.3 Life History Characteristics

Explicit and quantitative analyses to compare differences in life history characteristics require many species and traits. For example, one study using 74 marine finfish correlated various life history characteristics and biological traits with population genetic structure (Cusa et al., 2022). Due to our limited sample size, we could not make such quantitative comparisons. Nevertheless, we provide the following qualitative comparisons based on known life history characteristics summarized in Table 2.3.

One correlation between our results and life history characteristics is with adult depth preference. Adult depth preferences may explain higher gene flow in Black Rockfish when compared to Yellowtail Rockfish (Hess et al., 2011, Hess *in prep*) along the US West Coast. In our study, the shallower water species (Black and Puget Sound) lacked genetic differentiation, while deep-water species all had some degree of genetic structure (Redstripe, Greenstriped, Yellowtail) (Table 2.3). The geographic pattern of genetic structure is different between our deep-water species, suggesting adult depth is not the only driving factor for population structure. Furthermore, the deep-water Canary Rockfish do not show genetic population structure (Andrews et al., 2018). This ambiguity of depth preference on population structure is also evident in Sivasundar & Palumbi (2010) for 15 species of rockfish along the coast of Oregon and California. However, the effect of bathymetry on genetic population structure may be very different along the open coast and the estuarine environment such as Puget Sound. The existence of population structure in fjords of deeper water species has been reported in other marine species such as Copper Rockfish (Dick et al., 2014), Pacific hake (García-De León et al., 2018), Atlantic (Jorde et al., 2007), and Pacific cod (Cunningham et al., 2009). As this research is expanded to include other fish in Puget Sound, including other rockfish species, more conclusions can be made to the role of fjord-like systems influencing population structure for deep-water species.

Timing of larval release and pelagic larval duration may also be important to explain differences in genetic structure in our species (Doherty et al., 1995). Although there is little variation in larval duration for *Sebastes* species (Caselle et al., 2010), we found no association between length of larval duration and presence of genetic structure (Table 2.3). However, it is possible that timing of larval release could drive population connectivity within the Salish Sea. Within the Puget Sound estuary, there are drastic seasonal differences in oceanographic properties including salinity, temperature, dissolved oxygen, and water density (S. J. Moore et al., 2008). In Californian fish, such seasonal differences in oceanographic conditions have been shown to impact gene flow between populations within a single species (Jackson et al., 2018), and explain differences in population connectivity between multiple species of fish with similar life history characteristics (Shanks & Eckert, 2005). Unfortunately, little is known about the specific parturition timing for rockfishes. As parturition timing is correlated with latitude and environmental conditions (Laidig, 2010), more information on rockfish parturition in Puget Sound is necessary.

2.5.4 Temporal Genetic Variation

Small scale genetic differentiation in populations with presumed high gene flow, or chaotic genetic patchiness (Johnson & Black, 1982), has been frequently observed in marine fish species

(Burford Reiskind et al., 2011; Gilbert-Horvath et al., 2006; Larson & Julian, 1999; Selwyn et al., 2016). In Redstripe Rockfish, patterns of chaotic genetic patchiness were largely explained by the likely presence of different cohorts in our sample collection, as individuals clustered in the PCA by the year of sample collection. In particular, samples collected in 2014 were genetically distinct from all other year classes. Samples collected in 2014 were primarily between 25 and 30 cm in length, close to the size at first sexual maturity (Love et al., 2002). Additionally, variance in body size in the 2014 samples was 2.5 times smaller than in the mixed year cluster, suggesting that 2014 samples were all from the same year-class (Supplemental Figure 2.7). Other *Sebastes* species show multiple genetically different larval clusters in a homogeneous adult population in northern California, specifically for juveniles of the 2000 year-class (Burford & Larson, 2007; Burford Reiskind et al., 2011). Additionally, Restripe Rockfish at the western coast of Vancouver Island show high variation in year-class strength, with a surge of recruits in 2000 and 2007 (Haigh & Starr, 2021). There are three hypotheses that may explain small-scale genetic variation in large homogeneous marine populations: (a) variation in currents introducing novel genotypes to new areas, (b) natural selection acting on larvae prior to settlement, or (c) variation in reproductive success among adults (Larson & Julian, 1999). The introduction of novel genotypes hypothesis would assume that there is an undetected population of genetically divergent Redstripe Rockfish that periodically invades the Puget Sound region. It is possible that a genetically divergent population could occur either along the coast either north or south of the Strait of Juan de Fuca or in the British Columbia waters of the Salish Sea as seen in Greenstriped Rockfish. Natural selection in the larval stage would likely result in outlier loci defining the majority of variation seen between the year classes (Duforet-Frebourg et al., 2016). Although not thoroughly examined in our study, preliminary results suggest there are a high number of loci explaining the divergence between our two samples. Finally, variation in reproductive success, commonly referred to as sweepstakes recruitment (Hedgecock, 1994), would likely result in a very low effective population size estimate since very few parents successfully produced offspring. Our results suggest that the 2014 samples come from a large parental population, reducing the possibility of sweepstakes recruitment. As such, we hypothesize this temporal variation is due to a wave of immigration from a single year class introducing novel genotypes. The lack of gene flow between individuals collected in 2014 and other year classes can be explained by the age of the 2014 fish, which likely just had reached maturity and their offspring were too small to be sampled by hook and line.

For Greenstriped Rockfish, all of the British Columbia samples were caught in the same year and are similar sizes (Supplemental Figure 2.10). Similar to Redstripe Rockfish, it is therefore possible that the proposed geographic structure is also due to year class patterns. Due to the presence of a well-cited biogeographic barrier to dispersal for this population, we do believe the structure seen in Greenstriped Rockfish is due to geographic structure instead of temporal structure. There were no distinct year class patterns in the other species. More studies to investigate temporal genetic variation in other rockfish species are also highly recommended, as it is possible that our sampling effort was unable to detect this subtle structure.

2.5.5 Barriers to Gene Flow

Despite the presence of geographic population structure for Yellowtail and Greenstriped Rockfish, both species showed evidence of migration between populations. The lack of gene flow between these populations despite the presence of multiple migrants suggests that there may be a barrier to reproduction. Multiple intrinsic and extrinsic barriers to reproduction could exist which would reinforce the genetic divergence between two populations. Outbreeding depression may be caused by local adaptation of different populations, especially if these populations originated from separate, highly divergent, glacial refugia populations (Allendorf et al., 2001). On the other hand, empirical evidence for outbreeding depression seems limited (McClelland & Naish, 2007). In addition, rockfish have complex mating rituals (Helvey, 1982) and apparent mate choice (Johansson, M. L. et al., 2012). It is possible that separate mating rituals in the two populations could influence mate choice and reinforce population differentiation.

Chromosomal inversions may act as an intrinsic barrier to reproduction between populations by inducing hybrid sterility or facilitating the maintenance of co-adapted gene complexes (Faria & Navarro, 2010). This in turn can promote local adaptation (Wellenreuther & Bernatchez, 2018). Chromosomal inversions contribute to population structure in Pacific Herring (Petrou et al., 2021), Capelin (Cayuela et al., 2020), and Lingcod (Longo et al., 2020). We did not detect any large chromosomal inversions in the five rockfish species studied (Supplemental Figure 2.8). Although it is possible that whole genome sequencing may reveal smaller chromosomal inversions not detected in our RADseq dataset (Andrews & Luikart, 2014), most previous structural variants affecting population structure were large enough to be detectable by RAD sequencing (Cayuela et al., 2020; Longo et al., 2020; Petrou et al., 2021). The absence of such differentiation in genome structure in multiple species of the rapidly speciating *Sebastes* genus demonstrates the potential of genetic population structure and adaptive radiation in high gene flow species even without chromosomal inversions.

2.5.6 Implications For Fisheries Management

Species-specific patterns of population structure pose special challenges for fisheries management by requiring independent strategies when resources are limited. The *Sebastes* species complex in Puget Sound exemplifies this challenge. Our study suggests that none of the five species of rockfish studied here corresponded to the current DPS designation, and patterns of population structure were highly species-specific. The current DPS designation also does not fit Quillback (Chapter 1), Yelloweye and Canary Rockfish (Andrews et al. (2018)). The prior DPS determination for all species of rockfish was inferred from three species known to hybridize within Puget Sound (Brown, Copper, and Quillback Rockfish) (Buonaccorsi et al., 2002a, 2005b; Seeb, 1998a). Our study suggests this inference cannot be made widely across the *Sebastes* genus, and future management measures should reflect the differences between species.

Species-specific geographic barriers pose a special challenge for spatial conservation measures such as marine protected areas (MPAs). For example, if multiple species show similar patterns of population connectivity, one MPA could be established (Abecasis et al., 2014). Given that we have identified multiple patterns of population connectivity, multiple MPAs may be needed to conserve the maximum DPS diversity for all species. For example, one large MPA for Black and three smaller MPAs for Greenstriped would effectively conserve each species' genetic diversity. As such, multiple MPAs across Puget Sound and the Washington Coast may be necessary for the conservation of all rockfish species.

A particular issue impacting the effective management of these species is the high rate of misidentification of rockfishes within Puget Sound (Beaudreau et al., 2011). Approximately 8% of our samples were misidentified, even though they were collected by professional samplers, who have a significantly lower rate of misidentification compared to recreational fishers (Beaudreau et al., 2011). Both management and conservation of species rely heavily on accurate species identifications. Due to the species-specific population structure in Puget Sound rockfishes revealed our study, there is an urgent need for accurate identifications of these species to properly assess and manage each species.

Potential reestablishment of a recreational fishery for and recovery of Puget Sound rockfishes may benefit from subsidies from the Washington Coast restocking the depleted Puget Sound populations, even if local recruitment improves. Results from our study suggested multiple different connectivity patterns. First, there was no genetic differentiation between coastal and Puget Sound and Black Rockfish, which may indicate demographic connectivity between these two areas. On the other hand, in Yellowtail Rockfish, we detected only three potential Washington coast dispersers (out of 23) in the Puget Sound population, which translates to 13% of the population. Whether this is sufficient to support a fishery requires additional stock assessment efforts.

Finally, our study identified the potential for sporadic large subsidies from genetically distinct populations in Redstripe Rockfish. Such sporadic and unpredictable recruitment pulses pose their own challenges for stock assessments, which should be addressed in conjunction with genetic identification of immigrants. The genetic markers developed here provide a reliable method to identify species, populations, and dispersers, and thus may prove to be a useful tool for future research.

2.6 FIGURES

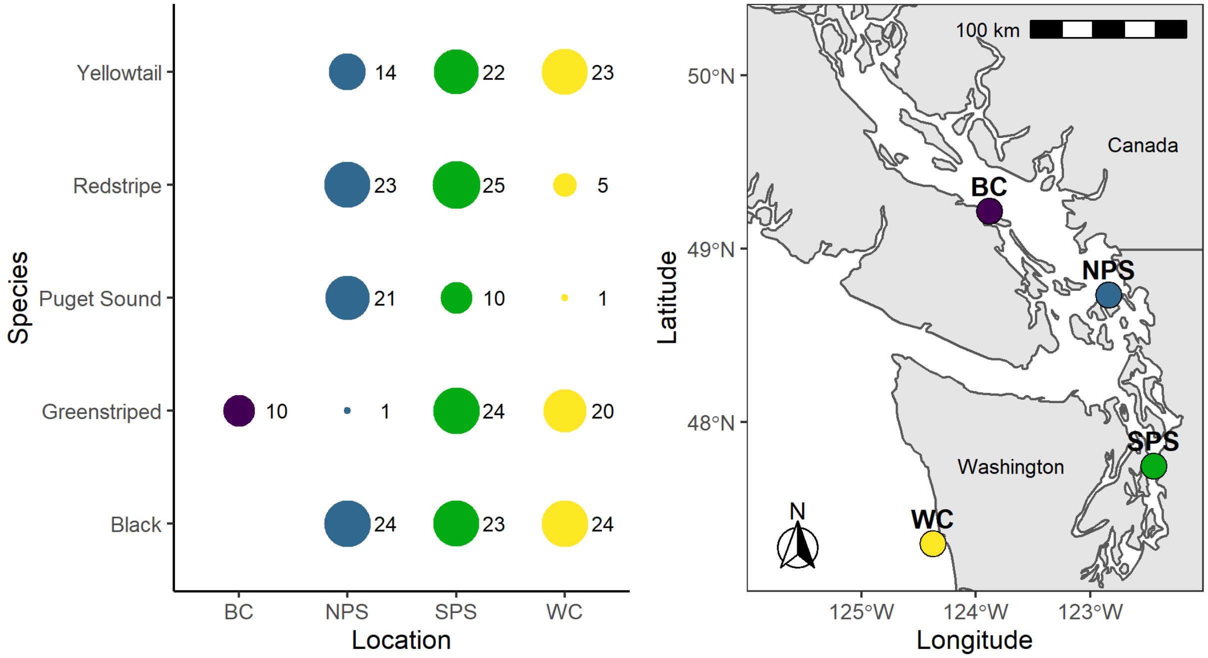


Figure 2.2 Yellowtail, Redstripe, Puget Sound, Greenstriped, and Black Rockfish collection location and sample size. Sampling areas within the Salish Sea are north Puget Sound (NPS, blue), south Puget Sound (SPS, green) and British Columbia (BC, purple). The size of the circles is proportional to the size of the collection (1-25 individuals).

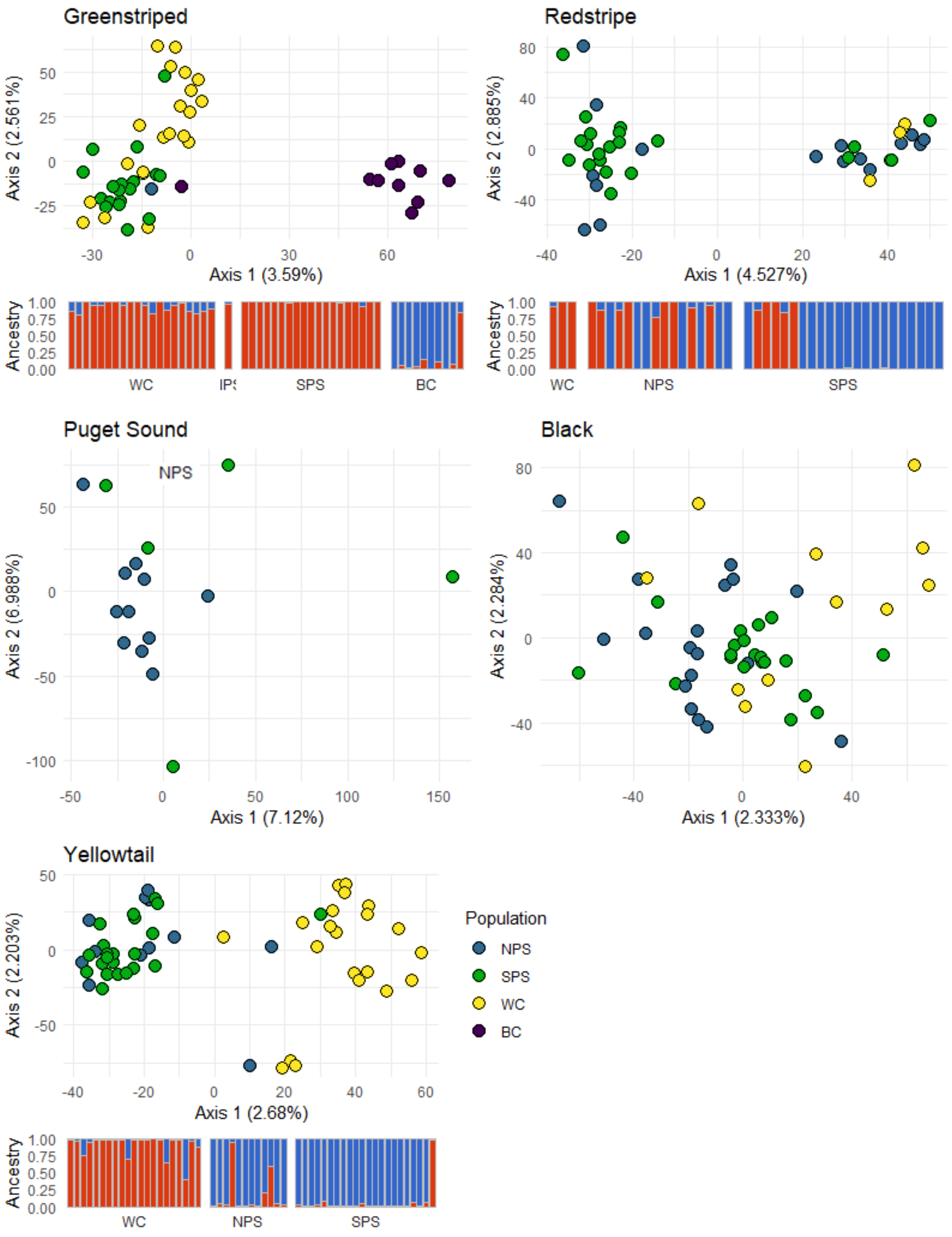


Figure 3.2 PCA and STRUCTURE plots of five rockfish species suggest different structuring patterns. For the STRUCTURE plots, each bar represents an individual, and color represents the genetic cluster to which each fish was assigned. Populations are ordered from the coast (WC), into Puget Sound from the north (NPS) to the south (SPS), and British Columbia (BC). Samples within each location are ordered south to north. For the PCAs, each point represents an individual fish, colored by their sampling location.

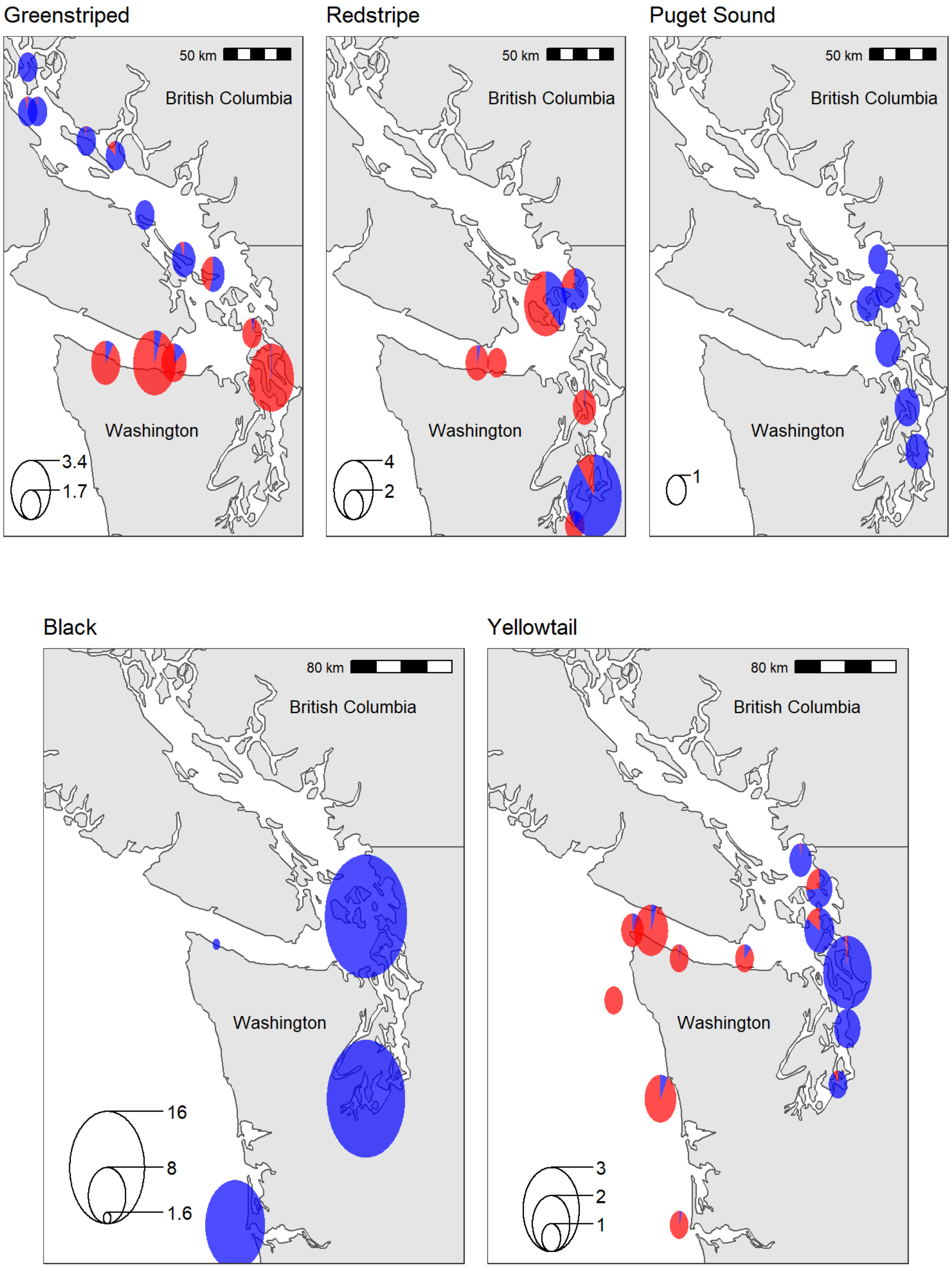


Figure 2.3 Geographic distribution of STRUCTURE clustering for five species of rockfish. Pie charts are colored according to STRUCTURE plot results (see Figure 2.2) and adjusted for sample size. The color of the pie corresponds to the average admixture proportions in each collection. Similar capture coordinates were pooled into the same pie.

2.7 TABLES

Table 2.1 Summary statistics for five species of rockfish in Puget Sound, the Washington Coast, and British Columbia. N = number of samples per population, H_O = average observed heterozygosity, H_E = average expected heterozygosity, F_{IS} = inbreeding coefficient. All values were calculated using the R package hierfstat v0.5-11 (Goudet, 2005).

		SPS	**NPS**	**WC**	**BC**
Yellowtail	N	22	12	21	
	H_O	0.27	0.27	0.27	
	H_E	0.28	0.28	0.28	
	F_{IS}	0.026	0.045	0.038	
Black	N	21	22	18	
	H_O	0.28	0.26	0.25	
	H_E	0.27	0.27	0.27	
	F_{IS}	-0.03	0.02	0.06	
Redstripe	N	22	18	3	
	H_O	0.30	0.29	0.29	
	H_E	0.31	0.31	0.30	
	F_{IS}	0.032	0.039	0.05	
Greenstriped	N	19	1	20	23
	H_O	0.28	0.28	0.28	0.26
	H_E	0.29	-	0.29	0.28
	F_{IS}	0.022	-	0.022	0.045
Puget Sound	N	5	12		
	H_O	0.26	0.26		
	H_E	0.27	0.26		
	F_{IS}	0.025	0.017		

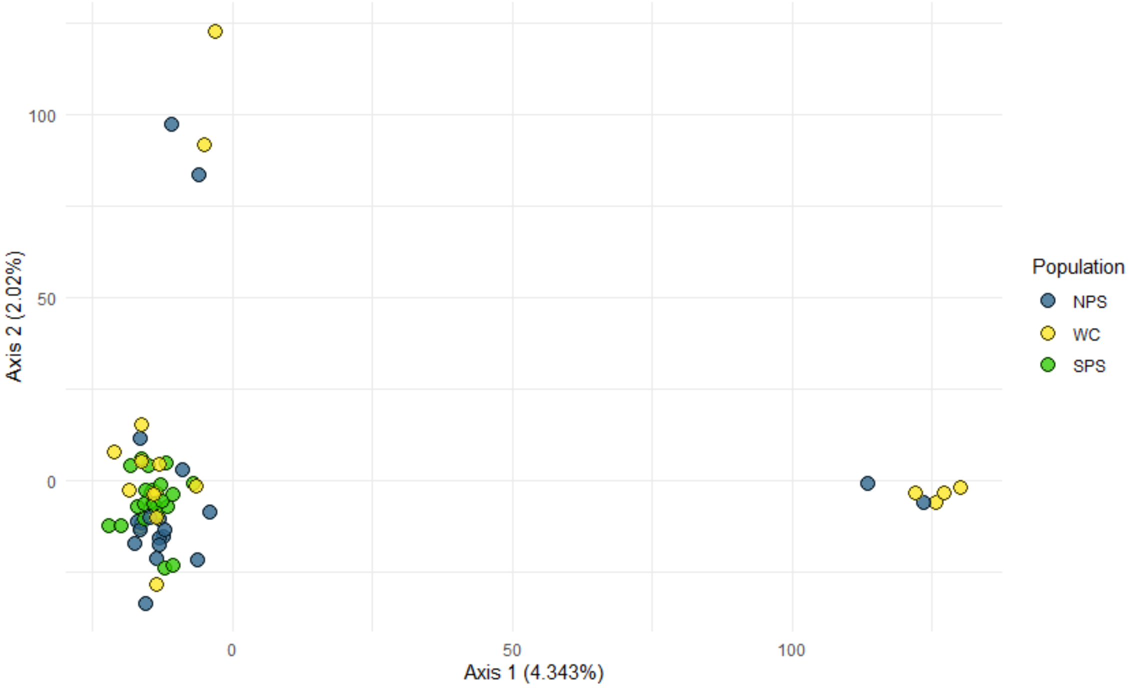
Table 2.2 Pairwise Weir and Cockerham F_{ST} estimates for five species of rockfish in Puget Sound and the Washington Coast. Bolded numbers are significantly greater than zero based on a permutation test. Italicized numbers are pairwise comparisons involving a single Greenstriped Rockfish from NPS.

	Yellowtail		**Black**		**Puget Sound**		**Redstripe**		**Greenstriped**		
	NPS	WC	NPS	WC	NPS	WC	NPS	WC	NPS	WC	BC
SPS	0	**0.008**	**0.001**	**0.001**	0.002		**0.002**	**0.012**	*0*	**0.003**	**0.025**
NPS		**0.006**		**0.001**				0		*0.003*	***0.024***
WC											**0.021**

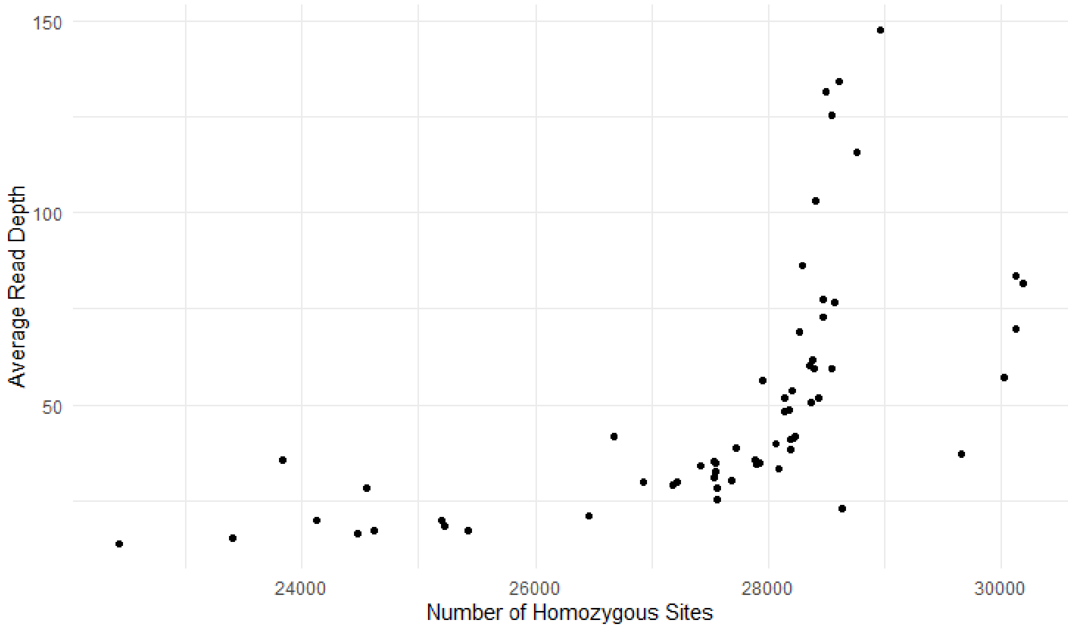
Table 2.3 Summary of life history characteristics of eight Puget Sound rockfishes. We used the following definitions to categorize the life history traits of our eight species. Depth (Shallow (S): <50m, Midwater (M): 50-100m, Deep (D): 100+ m); Population size (Endangered (E), Threatened (T), Healthy (H)) as defined by WDFW; Adult habitat (Benthic (B), Demersal (D), Pelagic (P) – as noted in the literature); Site Fidelity (High (H): >25m, Moderate (M): 15-25 m, Low (L): >15m); Larval Duration (Long (L): >2 months, Short (S): ≤2 months). Depth, juvenile and adult habitat are from Love et al. (2002)[3]. Population Size estimates are from Palsson et al. (2009) [4]. Site Fidelity estimates are from Hannah & Rankin (2011)[1] and Lowe et al. (2009)[2]. Larval duration estimates are from Carr & Syms (2006)[5]. Presence of population structure is based on results from Figure 2.2, Table 2.2, Chapter 1 and Andrews et al. (2018). Copper and Brown Rockfish are not included in the table due to the influence of hybridization on population structure (see Chapter 1).

Species	Depth[3]	Population Size[4]	Adult Habitat[3]	Site Fidelity[1,2]	Larval Duration[5]	Population Structure
Black	S	T	D	M	L	N
Puget Sound	S	T	P	-	-	N
Canary	D	E	P	D	L	N
Yellowtail	D	T	D	L	L	Y
Greenstriped	D	H	B	M	S	Y
Redstripe	D	H	D	-	L	Y
Yelloweye	D	E	P	D	L	Y
Quillback	M	E	P	D	L	Y

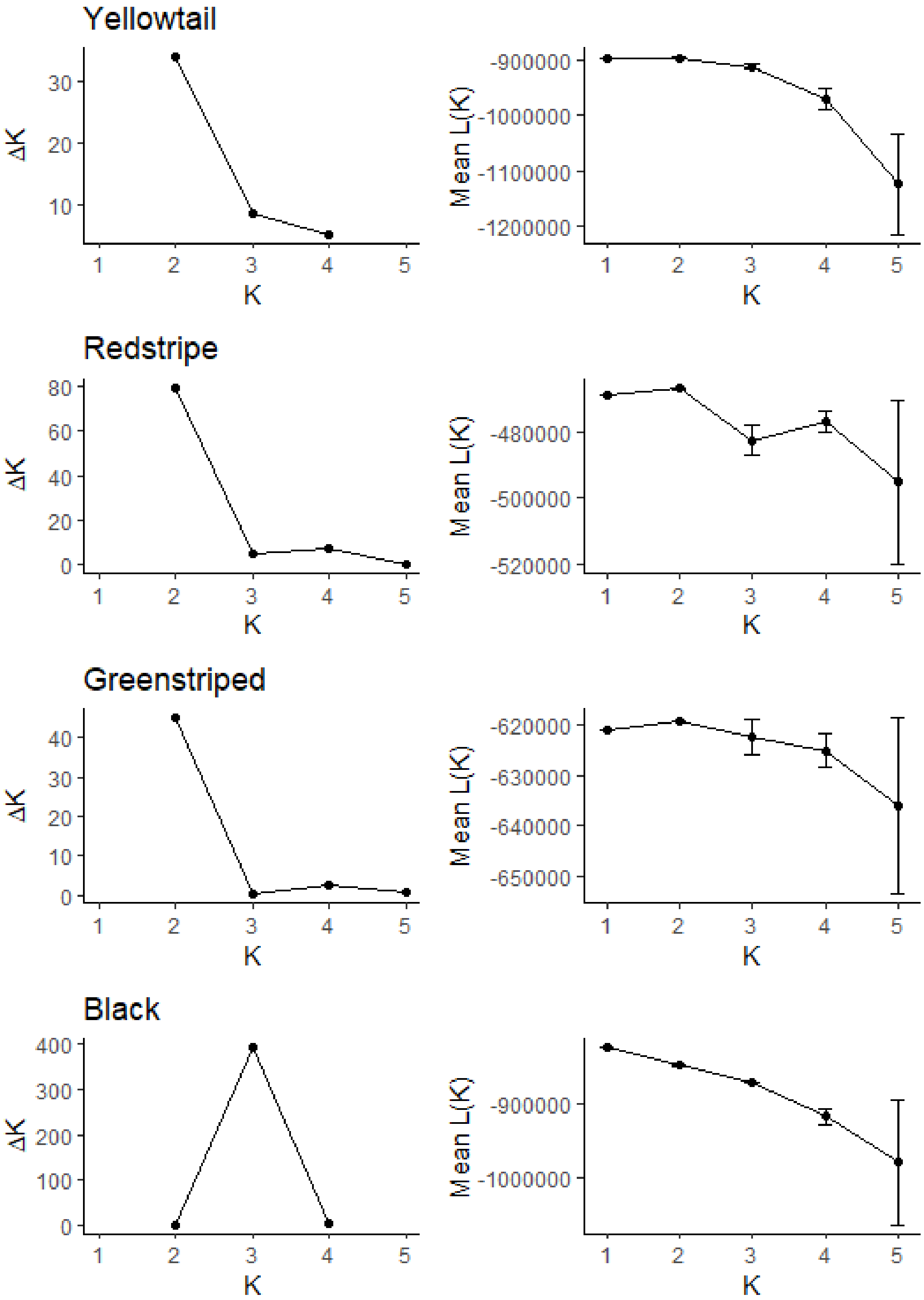
2.8 SUPPLEMENTAL FIGURES



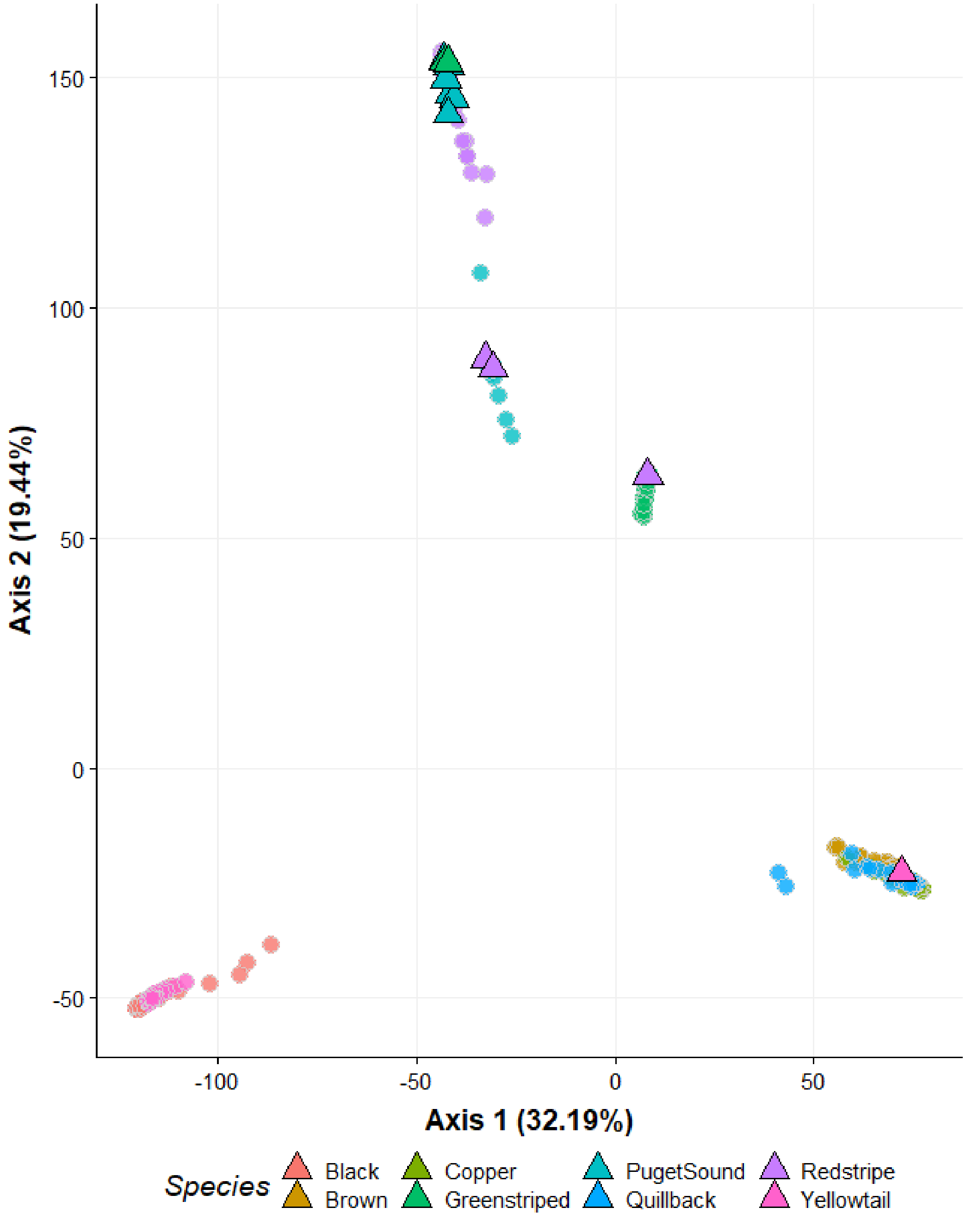
Supplemental Figure 2.1 Principal components analysis of Black Rockfish including outlier individuals with very low heterozygosity. Each point represents an individual fish, colored by sampling location.



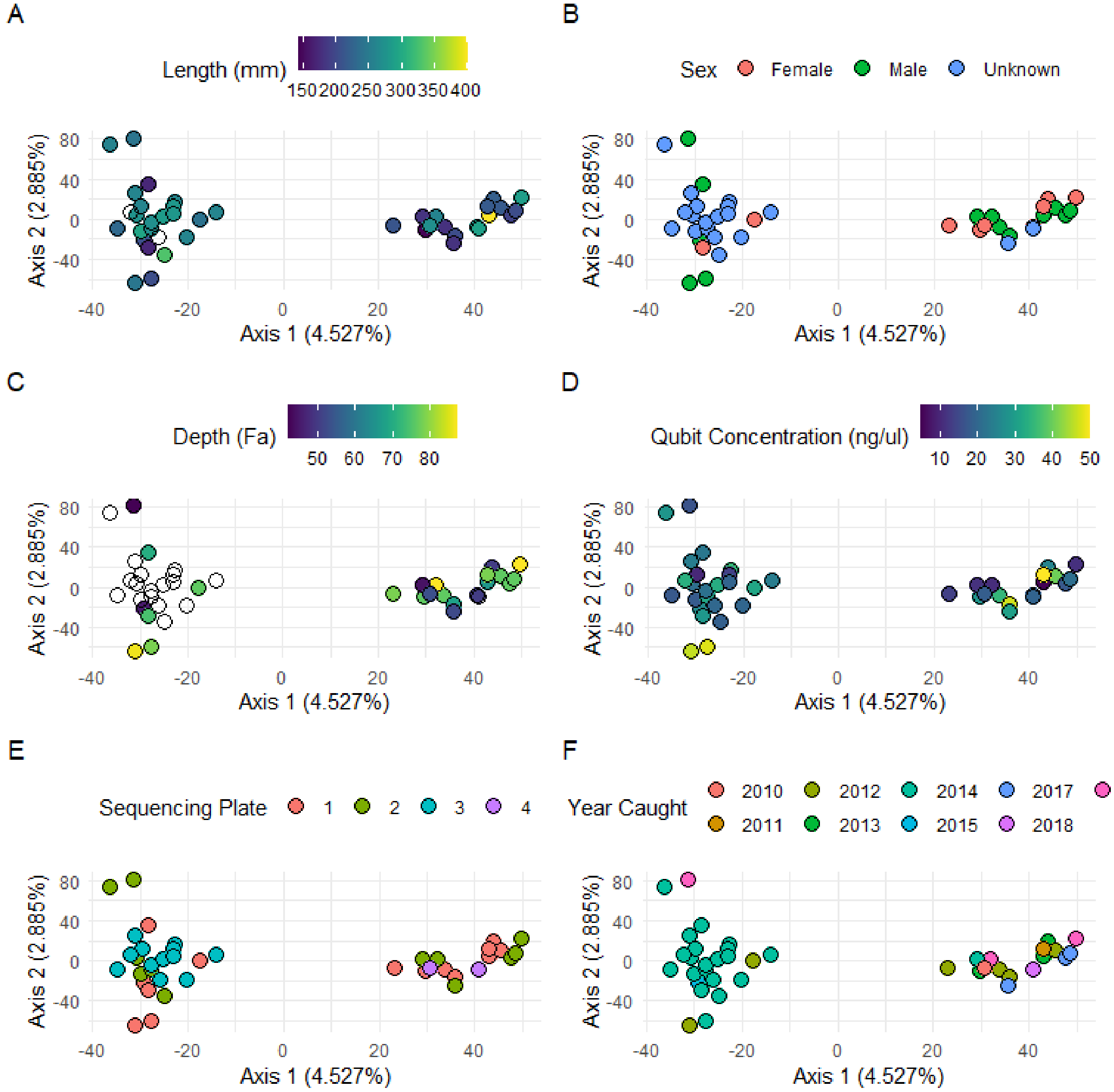
Supplemental Figure 2.2 Homozygosity excess in Black Rockfish outliers not correlated to read depth. Each point represents an individual fish. The outlying individuals removed from later analysis are on the far-right side of the plot.



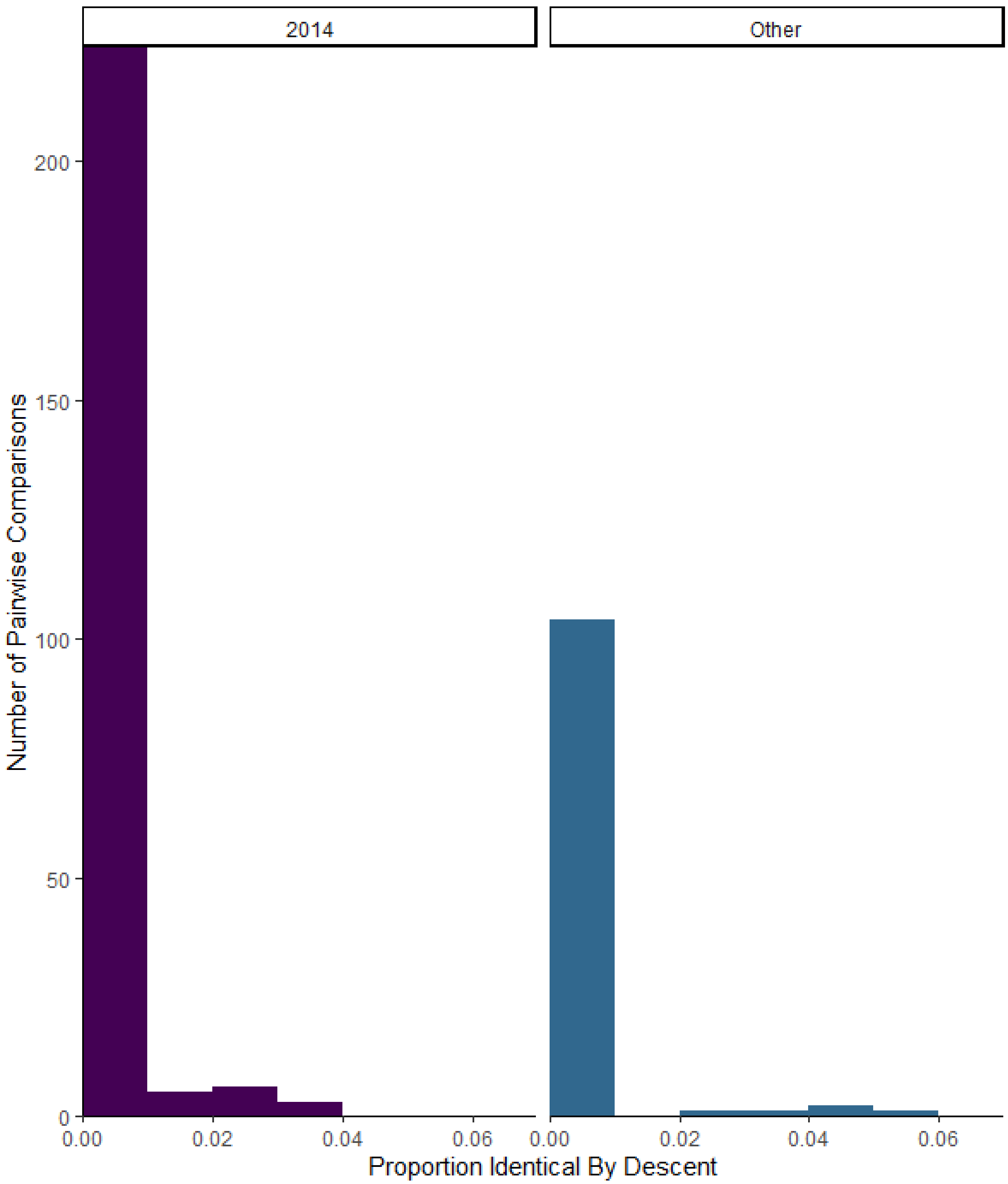
Supplemental Figure 2.3 Mean likelihood and ΔK for STRUCTURE replicate runs for Yellowtail, Redstripe, Greenstriped, and Black Rockfish.



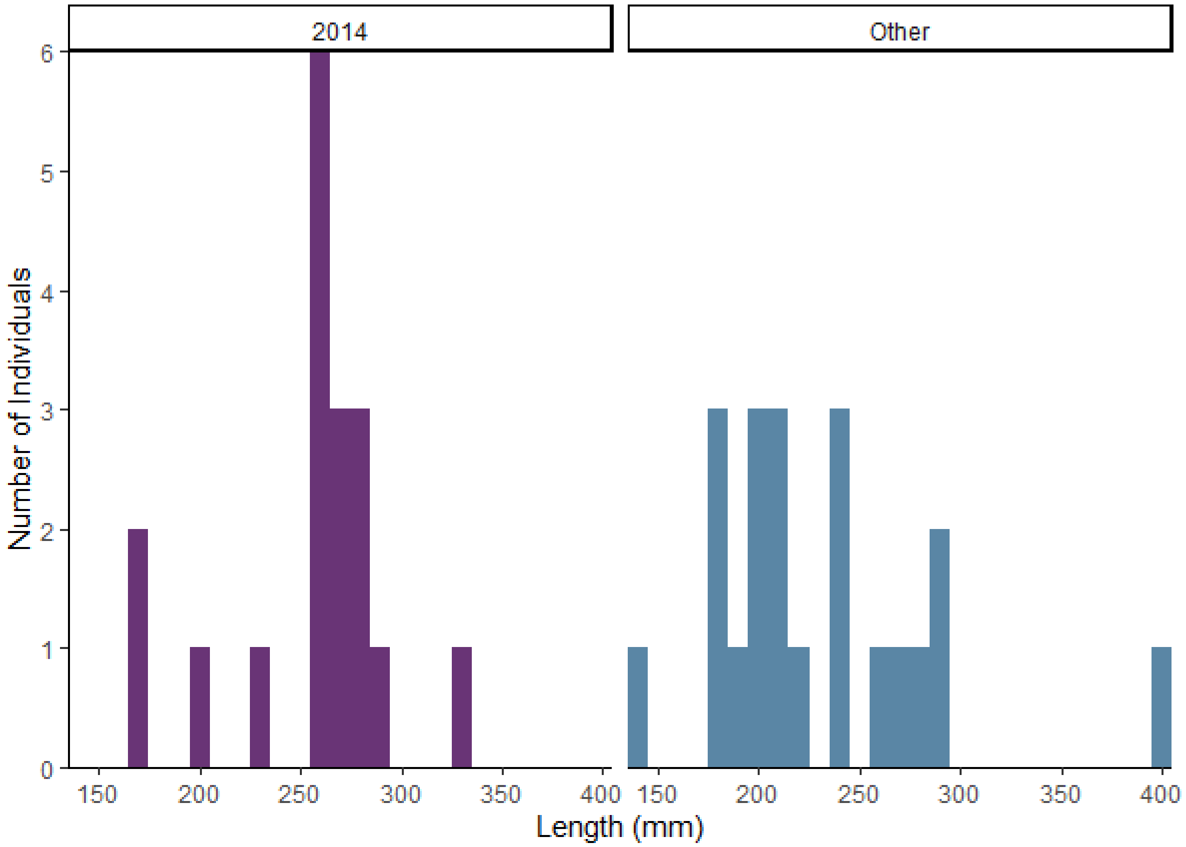
Supplemental Figure 2.4 Principal components analysis of eight species of rockfish suggests misidentified individuals. Each circle represents an individual fish, colored according to field species identification. Each triangle represents an individual where field and genetic identification disagreed.



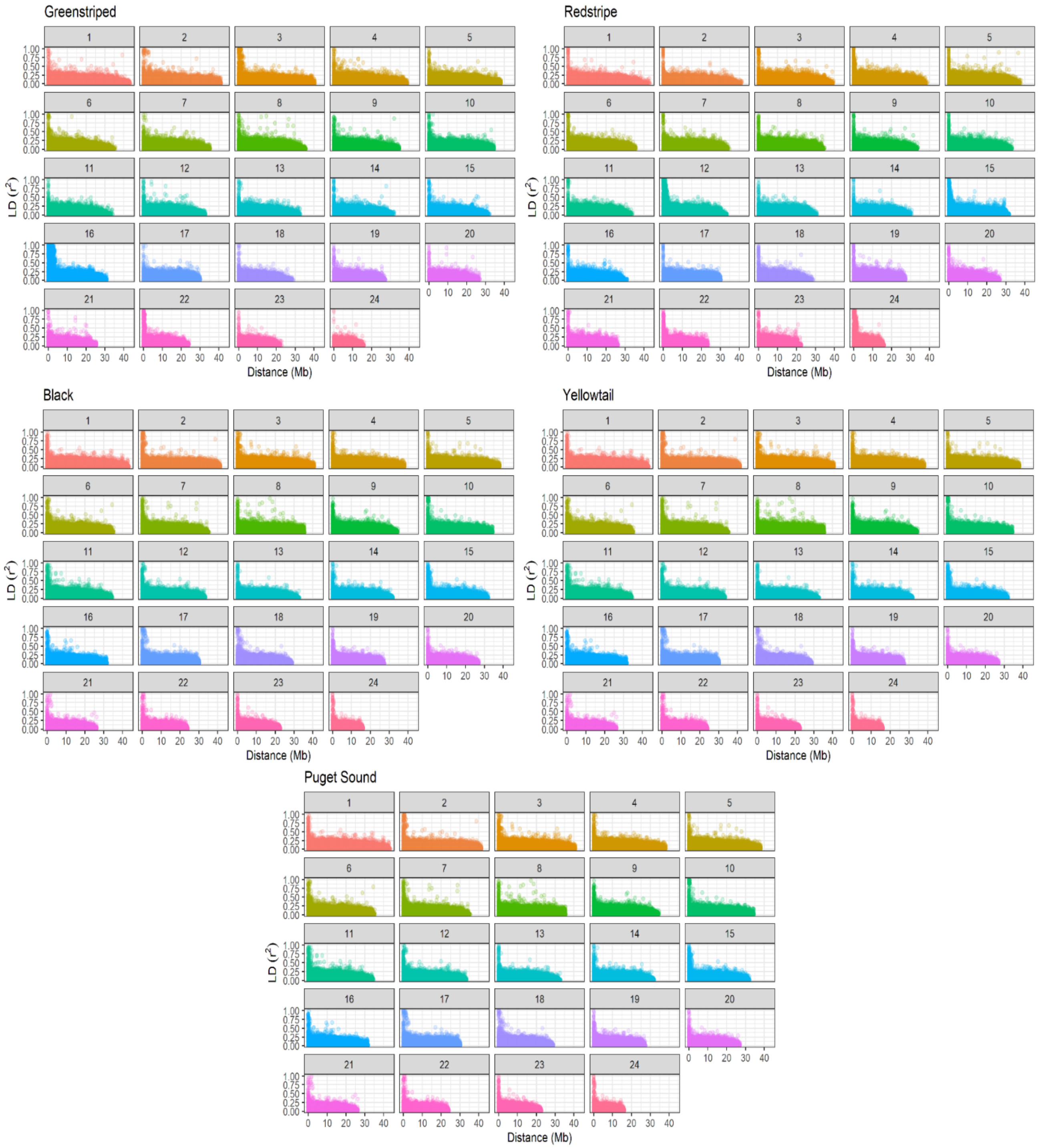
Supplemental Figure 2.5 Principal components analysis of Redstripe Rockfish labeled by life history characteristics. Each point represents an individual fish, colored by various life history and methodology characteristics. Unfilled circles show missing data. A: length of fish, B: sex, C: depth caught (fathoms), D: extraction Qubit concentration, E: sequencing plate, F: collection year.



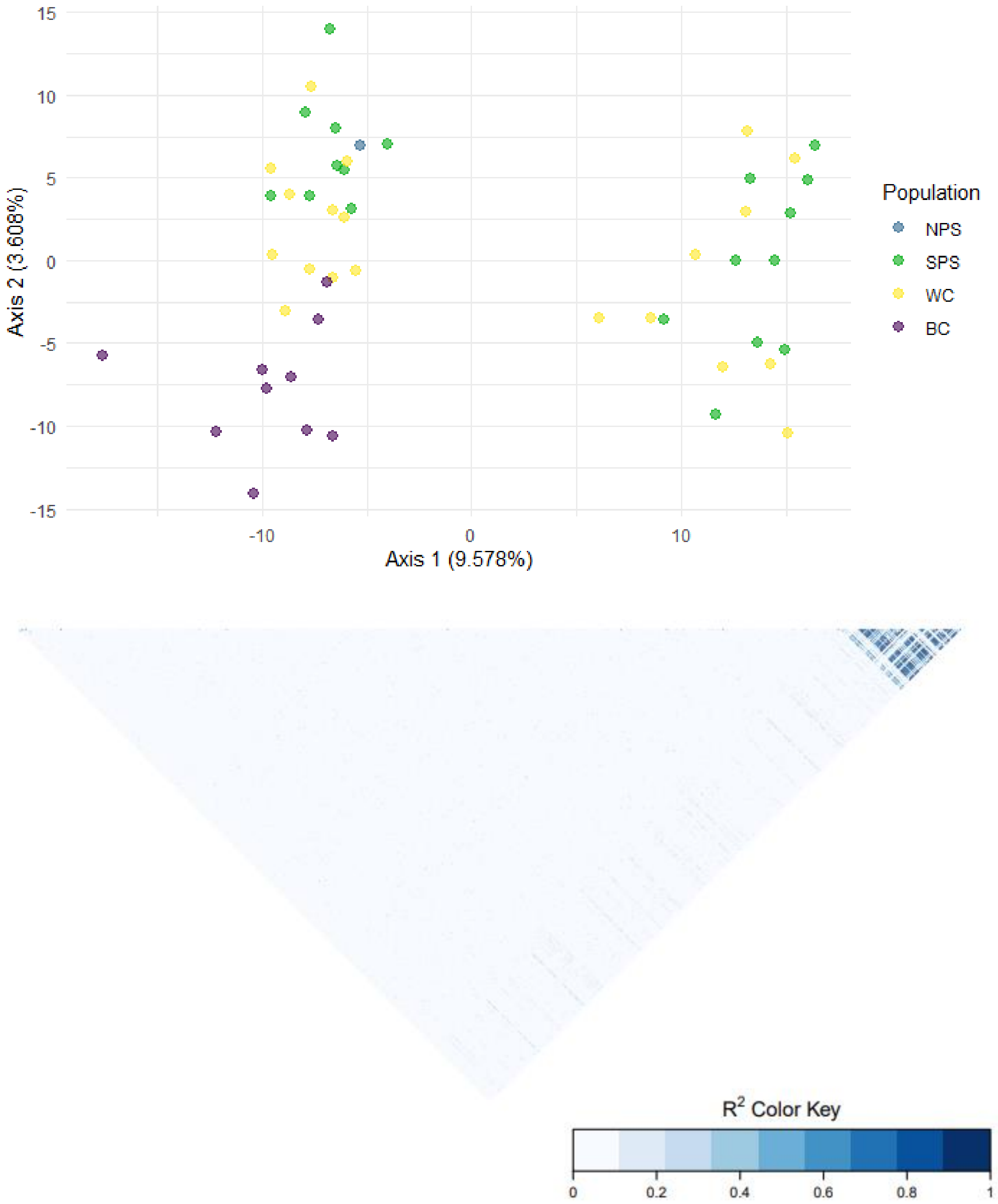
Supplemental Figure 2.6 Pairwise relatedness estimates for 2014 Redstripe Rockfish samples. Pairwise relatedness for the 2014 samples (left) was not significantly different than in any other sampling year (right, p-value = 0.69). Genetic relatedness was calculated using an identity by descent estimate on all pairs of individuals within PLINK v1.07 (Purcell et al., 2007).



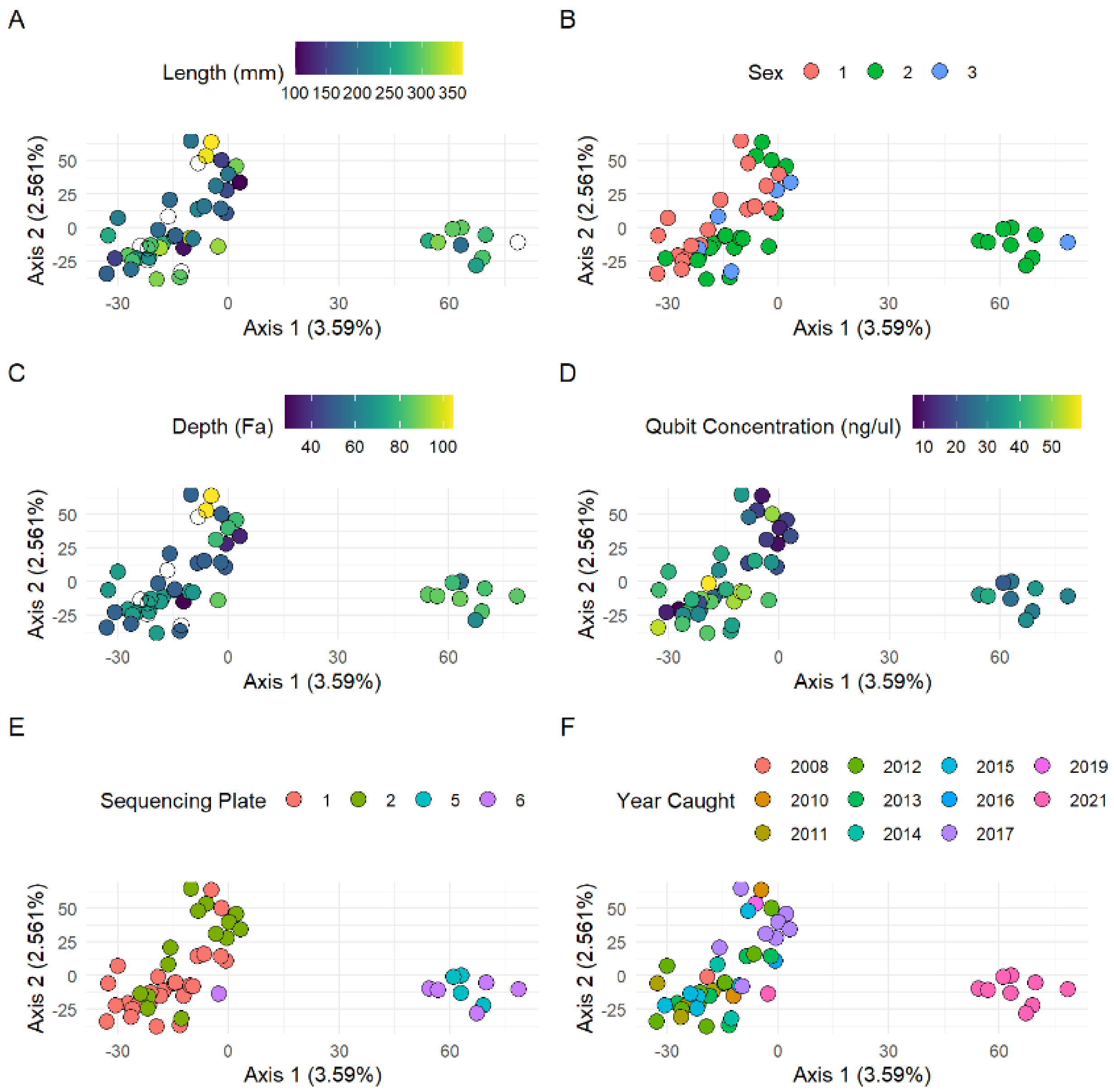
Supplemental Figure 2.7 Distribution of fish lengths for 2014 Redstripe Rockfish samples (left) compared to other sampling years (right). Samples collected in 2014 were larger on average than all other year classes combined (256 mm for 2014 and 231 mm for samples collected in other years). The variance for the 2014 samples versus the other sampling years is 1.9 times smaller.



Supplemental Figure 2.8 Linkage decay plots per chromosome for five species of rockfish. R^2 values were calculated using PLINK. One chromosome with a block of highly linked loci was identified on Chromosome 16 for Greenstriped Rockfish.



Supplemental Figure 2.9 Principal components analysis (top) and linkage heatmap (bottom) of Chromosome 16 in Greenstriped Rockfish.



Supplemental Figure 2.10 Principal components analysis of Greenstriped Rockfish labeled by life history characteristics. Each point represents an individual fish, colored by various life history and methodology characteristics. Unfilled circles show missing data. A: length of fish, B: sex, C: depth caught (fathoms), D: extraction Qubit concentration, E: sequencing plate, F: year caught.

2.9 SUPPLEMENTAL TABLES

Supplemental Table 2.1 N_e estimation based on sampling location. Effective population size was calculated using the random mating, linkage disequilibrium model using NeEstimator with a MAF cutoff of 0.05 (Do et al., 2014) and adjusted for high loci number (Waples et al. 2016). Parametric 95% confidence intervals are included in parentheses.

Population	Black	Yellowtail	Redstripe	Greenstriped	Puget Sound
NPS	1,647 (1.5)	Infinite	146 (2.4)	Infinite	Infinite
SPS	210 (1.1)	518 (1.5)	344 (2.6)	1,135 (1.4)	Infinite
WC	Infinite	224 (0.8)	Infinite	676 (2.4)	
BC				Infinite	

Supplemental Table 2.2 N_e estimation based on clustering pattern. Effective population size was calculated using the random mating, linkage disequilibrium model using NeEstimator with a MAF cutoff of 0.05 (Do et al., 2014) and adjusted for high loci number using R. K. Waples et al. (2016). Clusters were chosen based on PCA and STRUCTURE results. Parametric 95% confidence intervals are included in parentheses.

Black		Yellowtail		Redstripe		Greenstriped		Puget Sound	
Outliers	Infinite	Coast	323 (1.8)	2014 Year Class	1,229 (4.4)	British Columbia	Infinite	Puget Sound	Infinite
Admixed	969 (2.7)	Puget Sound	850 (1.7)	Other Year Classes	729 (1.7)	Coast and Puget Sound	1,141 (3.5)		

2.10 REFERENCES

Abecasis, D., Afonso, P., & Erzini, K. (2014). Combining multispecies home range and distribution models aids assessment of MPA effectiveness. *Marine Ecology Progress Series*, *513*, 155–169. https://doi.org/10.3354/meps10987

Ali, O. A., Jeffres, C., & Miller, M. R. (2016). RAD Capture (Rapture): Flexible and Efficient Sequence-Based Genotyping. *Genetics*, *202*, 389–400. https://doi.org/10.1534/genetics.115.183665

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: Setting conservation guidelines. *Trends in Ecology & Evolution*, *16*(11), 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X

Andrews, K. R., & Luikart, G. (2014). Recent novel approaches for population genomics data analysis. *Molecular Ecology*, *23*(7), 1661–1667. https://doi.org/10.1111/mec.12686

Andrews, K. S., Bartos, B., Harvey, C., Tonnes, D., Bhuthimethee, M., & Maccready, P. (2021). Testing the potential for larval dispersal to explain connectivity and population structure of threatened rockfish species in Puget Sound. *Marine Ecology Progress Series*, *677*, 95–113. https://doi.org/10.3354/meps13858

Andrews, K. S., Nichols, K. M., Elz, A., Tolimieri, N., Harvey, C. J., Pacunski, R., Lowry, D., Yamanaka, K. L., & Tonnes, D. M. (2018). Cooperative research sheds light on population structure and listing status of threatened and endangered rockfish species. *Conservation Genetics*, *19*(4), 865–878. https://doi.org/10.1007/s10592-018-1060-0

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data* (0.11.9).

Arnold, M. (1992). Natural Hybridization as an Evolutionary Process. *Annual Review of Ecology and Systematics*, *23*, 237–261. JSTOR.

Arnold, M., & Fogarty, N. (2009). Reticulate Evolution and Marine Organisms: The Final Frontier? *International Journal of Molecular Sciences*, *10*(9), 3836–3860. https://doi.org/10.3390/ijms10093836

Arnold, M., Sapir, Y., & Martin, N. H. (2008). Genetic exchange and the origin of adaptations: Prokaryotes to primates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1505), 2813–2820. https://doi.org/10.1098/rstb.2008.0021

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A., & Johnson, E. A. (2008). Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLoS ONE*, *3*(10), e3376. https://doi.org/10.1371/journal.pone.0003376

Beaudreau, A. H., Levin, P. S., & Norman, K. C. (2011). Using folk taxonomies to understand stakeholder perceptions for species conservation. *Conservation Letters*, *4*(6), 451–463. https://doi.org/10.1111/j.1755-263x.2011.00199.x

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bernatchez, L., & Wilson, C. C. (1998). Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, *7*(4), 431–452. https://doi.org/10.1046/j.1365-294x.1998.00319.x

Brandenberger, J., Louchouarn, P., & Crecelius, E. (2011). Natural and Post-Urbanization Signatures of Hypoxia in Two Basins of Puget Sound: Historical Reconstruction of Redox Sensitive Metals and Organic Matter Inputs. *Aquatic Geochemistry*, *17*, 645–670. https://doi.org/10.1007/s10498-011-9129-0

Buonaccorsi, V. P., Kimbrell, C. A., Lynn, E. A., & Vetter, R. D. (2002). Population structure of copper rockfish (*Sebastes caurinus)* reflects postglacial colonization and contemporary patterns of larval dispersal. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(8), 1374–1384. https://doi.org/10.1139/f02-101

Buonaccorsi, V. P., Kimbrell, C. A., Lynn, E. A., & Vetter, R. D. (2005). Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. *Conservation Genetics*, *6*(5), 697–713. https://doi.org/10.1007/s10592-005-9029-1

Buonaccorsi, V. P., Westerman, M., Stannard, J., Kimbrell, C., Lynn, E., & Vetter, R. D. (2003). Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. *Marine Biology*, *1*(1), 1–1. https://doi.org/10.1007/s00227-004-1362-2

Burford, M. O., & Larson, R. J. (2007). Genetic heterogeneity in a single year-class from a panmictic population of adult blue rockfish (*Sebastes mystinus*). *Marine Biology*, *151*(2), 451–465. https://doi.org/10.1007/s00227-006-0475-1

Burford Reiskind, M., Carr, M., & Bernardi, G. (2011). Age-structured genetic analysis reveals temporal and geographic variation within and between two cryptic rockfish species. *Marine Ecology Progress Series*, *442*, 201–215. https://doi.org/10.3354/meps09329

Canino, M. F., Spies, I. B., Cunningham, K. M., Hauser, L., & Grant, W. S. (2010). Multiple ice-age refugia in Pacific cod, *Gadus macrocephalus*. *Molecular Ecology*, *19*(19), 4339–4351. https://doi.org/10.1111/j.1365-294x.2010.04815.x

Carvalho, G. R., & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, *4*(3), 326–350. https://doi.org/10.1007/BF00042908

Caselle, J. E., Wilson, J. R., Carr, M. H., Malone, D. P., & Wendt, D. E. (2010). Can we predict interannual and regional variation in delivery of pelagic juveniles to nearshore populations of rockfishes (genus *Sebastes*) using simple proxies of ocean conditions? *Reports of California Cooperative Oceanic Fisheries Investigations*, *51*, 91–105.

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

Cayuela, H., Rougemont, Q., Laporte, M., Mérot, C., Normandeau, E., Dorant, Y., Tørresen, O. K., Hoff, S. N. K., Jentoft, S., Sirois, P., Castonguay, M., Jansen, T., Praebel, K., Clément, M., & Bernatchez, L. (2020). Shared ancestral polymorphisms and chromosomal rearrangements as potential drivers of local adaptation in a marine fish. *Molecular Ecology*, *29*(13), 2379–2398. https://doi.org/10.1111/mec.15499

Crossman, C., Barrett-Lennard, L., & Taylor, E. (2014). Population structure and intergeneric hybridization in harbour porpoises *Phocoena phocoena* in British Columbia, Canada. *Endangered Species Research*, *26*(1), 1–12. https://doi.org/10.3354/esr00624

Cunningham, K. M., Canino, M. F., Spies, I. B., & Hauser, L. (2009). Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): Limited effective dispersal in the northeastern Pacific Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(1), 153–166. https://doi.org/10.1139/f08-199

Cusa, M., St John Glew, K., Trueman, C., Mariani, S., Buckley, L., Neat, F., & Longo, C. (2022). A future for seafood point-of-origin testing using DNA and stable isotope signatures. *Reviews in Fish Biology and Fisheries*, *32*(2), 597–621. https://doi.org/10.1007/s11160-021-09680-w

Dahle, G., Johansen, T., Westgaard, J. I., Aglen, A., & Glover, K. A. (2018). Genetic management of mixed-stock fisheries “real-time”: The case of the largest remaining cod fishery operating in the Atlantic in 2007–2017. *Fisheries Research*, *205*(September 2017), 77–85. https://doi.org/10.1016/j.fishres.2018.04.006

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dannewitz, J., Maes, G. E., Johansson, L., Wickström, H., Volckaert, F. A. M., & Järvi, T. (2005). Panmixia in the European eel: A matter of time…. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1568), 1129–1137. https://doi.org/10.1098/rspb.2005.3064

Dick, S., Shurin, J. B., & Taylor, E. B. (2014). Replicate divergence between and within sounds in a marine fish: The copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, *23*(3), 575–590. https://doi.org/10.1111/mec.12630

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimatorv2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources, 14*(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Doherty, P. J., Planes, S., & Mather, P. (1995). Gene Flow and Larval Duration in Seven Species of Fish from the Great Barrier Reef. *Ecology*, *76*(8), 2373–2391. https://doi.org/10.2307/2265814

Drake, J. S., Bernston, E. A., Cope, J. M., Gustafson, R. G., Holmes, E. E., Levin, P. S., Tolimieri, N., Waples, R. S., Sogard, S. M., & Williams, G. D. (2010). Status review of five rockfish species in Puget Sound, Washington: Bocaccio (*Sebastes paucispinis*), canary rockfish (*S. pinniger*), yelloweye rockfish (*S. ruberrimus*), greenstriped rockfish (*S. elongatus*), and redstripe rockfish (*S. proriger*) U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-108, 234 p.

Drinan, D. P., Gruenthal, K. M., Canino, M. F., Lowry, D., Fisher, M. C., & Hauser, L. (2018). Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evolutionary Applications*, *11*(8), 1448–1464. https://doi.org/10.1111/eva.12639

Duforet-Frebourg, N., Luu, K., Laval, G., Bazin, E., & Blum, M. G. B. (2016). Detecting Genomic Signatures of Natural Selection with Principal Component Analysis: Application to the 1000 Genomes Data. *Molecular Biology and Evolution*, *33*(4), 1082–1093. https://doi.org/10.1093/molbev/msv334

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eby, L. A., & Crowder, L. B. (2002). Hypoxia-based habitat compression in the Neuse River Estuary: Context-dependent shifts in behavioral avoidance thresholds. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(6), 952–965. https://doi.org/10.1139/f02-067

Engie, K., & Klinger, T. (2007). Modeling passive dispersal through a large estuarine system to evaluate marine reserve network connections. *Estuaries and Coasts*, *30*(2), 201–213. https://doi.org/10.1007/BF02700164

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294x.2005.02553.x

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Faria, R., & Navarro, A. (2010). Chromosomal speciation revisited: Rearranging theory with pieces of evidence. *Trends in Ecology & Evolution*, *25*(11), 660–669. https://doi.org/10.1016/j.tree.2010.07.008

Fay, J. J., & Nammack, M. (1996). Policy Regarding the Recognition of Distinct Vertebrate Population Segments Under the Endangered Species Act. *Fedral Register*, *61*.

Garant, D., Forde, S. E., & Hendry, A. P. (2007). The Multifarious Effects of Dispersal and Gene Flow on Contemporary Adaptation. *Functional Ecology*, *21*(3), 434–443. JSTOR.

García-De León, F. J., Galván-Tirado, C., Sánchez Velasco, L., Silva-Segundo, C. A., Hernández-Guzmán, R., Barriga-Sosa, I. D. L. A., Díaz Jaimes, P., Canino, M., & Cruz-Hernández, P. (2018). Role of oceanography in shaping the genetic structure in the North Pacific hake *Merluccius productus*. *PLOS ONE*, *13*(3), e0194646. https://doi.org/10.1371/journal.pone.0194646

Giæver, M., & Stien, J. (1998). Population genetic substructure in blue whiting based on allozyme data. *Journal of Fish Biology*, *52*(4), 782–795. https://doi.org/10.1111/j.1095-8649.1998.tb00820.x

Gilbert-Horvath, E. A., Larson, R. J., & Garza, J. C. (2006). Temporal recruitment patterns and gene flow in kelp rockfish (*Sebastes atrovirens*). *Molecular Ecology*, *15*(12), 3801–3815. https://doi.org/10.1111/j.1365-294x.2006.03033.x

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: Coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, *5*(1), 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Grant, P. R., & Grant, B. R. (2010). Conspecific versus heterospecific gene exchange between populations of Darwin's finches. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1543), 1065–1076. https://doi.org/10.1098/rstb.2009.0283

Grantham, B. A., Chan, F., Nielsen, K. J., Fox, D. S., Barth, J. A., Huyer, A., Lubchenco, J., & Menge, B. A. (2004). Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature*, *429*(6993), 749–754. https://doi.org/10.1038/nature02605

Haigh, R., & Starr, P. (2021). Redstripe Rockfish (*Sebastes proriger*) stock assessment for British Columbia in 2018. *Fisheries and Oceans Canada.*

Hails, R. S., & Morley, K. (2005). Genes invading new populations: A risk assessment perspective. *Trends in Ecology & Evolution, 20*(5), 245–252. https://doi.org/10.1016/j.tree.2005.02.006

Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology, 30*(1), 33–41. https://doi.org/10.1111/cobi.12574

Hannah, R. W., & Rankin, P. S. (2011). Site Fidelity and Movement of Eight Species of Pacific Rockfish at a High-Relief Rocky Reef on the Oregon Coast. *North American Journal of Fisheries Management*, *31*(3), 483–494. https://doi.org/10.1080/02755947.2011.591239

Hedgecock, D. (1994). Does variance in reproductive success limit effective population sizes of marine organisms. In *Genetics and Evolutionn of Aquatic Organisms* (pp. 122–134). Chapman & Hall.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*(8), 1633–1638. https://doi.org/10.1111/j.0014-3820.2005.tb01814.x

Hedrick, P. W. (2013). Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*, *22*(18), 4606–4618. https://doi.org/10.1111/mec.12415

Helvey, M. (1982). First Observations of Courtship Behavior in Rockfish, Genus *Sebastes*. *Copeia*, *1982*(4), 763–770. JSTOR. https://doi.org/10.2307/1444084

Henriques, R., Von der Heyden, S., & Matthee, C. A. (2016). When homoplasy mimics hybridization: A case study of Cape hakes (*Merluccius capensis* and *M. paradoxus*). *PeerJ*, *4*, e1827.

Hess, J. E., Vetter, R. D., & Moran, P. (2011). A steep genetic cline in yellowtail rockfish, *Sebastes flavidus*, suggests regional isolation across the Cape Mendocino faunal break. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(1), 89–104. https://doi.org/10.1139/f10-131

Hoffmann, A. A., & Rieseberg, L. H. (2008). Revisiting the Impact of Inversions in Evolution: From Population Genetic Markers to Drivers of Adaptive Shifts and Speciation? *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 21–42. https://doi.org/10.1146/annurev.ecolsys.39.110707.173532

Hubbs, C. L. (1955). Hybridization between Fish Species in Nature. *Systematic Biology*, *4*(1), 1–20. https://doi.org/10.2307/sysbio/4.1.1

Hyde, J. R., & Vetter, R. D. (2007). The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). *Molecular Phylogenetics and Evolution*, *44*(2), 790–811. https://doi.org/10.1016/j.ympev.2006.12.026

Iwamoto, E., Ford, M. J., & Gustafson, R. G. (2004). Genetic population structure of Pacific Hake, *Merluccius productus*, in the Pacific Northwest. In A. J. Gharrett, R. G. Gustafson, J. L. Nielsen, J. E. Seeb, L. W. Seeb, W. W. Smoker, G. H. Thorgaard, & R. L. Wilmot (Eds.), *Genetics of Subpolar Fish and Invertebrates* (pp. 187–199). Springer Netherlands. https://doi.org/10.1007/978-94-007-0983-6_15

Jackson, T. M., & O'Malley, K. G. (2017). Comparing genetic connectivity among Dungeness crab (*Cancer magister*) inhabiting Puget Sound and coastal Washington. *Marine Biology*, *164*(6). https://doi.org/10.1007/s00227-017-3152-7

Jackson, T. M., Roegner, G. C., & O'Malley, K. G. (2018). Evidence for interannual variation in genetic structure of Dungeness crab (*Cancer magister*) along the California Current System. *Molecular Ecology*, *27*(2), 352–368. https://doi.org/10.1111/mec.14443

Johansson, M. L., Banks, M. A., Glunt, K. D., Hassel-Finnegan, H. M., & Buonaccorsi, V. P. (2008). Influence of habitat discontinuity, geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). *Molecular Ecology*, *17*(13), 3051–3061. https://doi.org/10.1111/j.1365-294x.2008.03814.x

Johansson, M. L., Clifford K, Fodness B, Vazquez NA, & Banks MA. (2012). Mate selection in captive-breeding rockfishes *Sebastes* spp.: Inference from parentage analysis and the major histocompatibility complex (MHC). *Marine Ecology Progress Series*, *460*, 195–206. https://doi.org/10.3354/meps09803

Johnson, M. S., & Black, R. (1982). Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. *Marine Biology*, *70*(2), 157–164. https://doi.org/10.1007/BF00397680

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jorde, P., Knutsen, H., Espeland, S., & Stenseth, N. (2007). Spatial scale of genetic structuring in coastal cod *Gadus morhua* and geographic extent of local populations. *Marine Ecology Progress Series*, *343*, 229–237. https://doi.org/10.3354/meps06922

Kelly, R. P., & Palumbi, S. R. (2010). Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community. *PLoS ONE*, *5*(1), e8594. https://doi.org/10.1371/journal.pone.0008594

Khangaonkar, T., Long, W., & Xu, W. (2017). Assessment of circulation and inter-basin transport in the Salish Sea including Johnstone Strait and Discovery Islands pathways. *Ocean Modelling*, *109*, 11–32. https://doi.org/10.1016/j.ocemod.2016.11.004

Knutsen, H., Moland Olsen, E., Ciannelli, L., Espeland, S., Knutsen, J., Simonsen, J., Skreslet, S., & Stenseth, N. (2007). Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. *Marine Ecology Progress Series*, *333*, 249–255. https://doi.org/10.3354/meps333249

Krohn, A. R., Conroy, C. J., Pesapane, R., Bi, K., Foley, J. E., & Rosenblum, E. B. (2018). Conservation genomics of desert dwelling California voles (*Microtus californicus*) and implications for management of endangered Amargosa voles (*Microtus californicus* *scirpensis*). *Conservation Genetics*, *19*(2), 383–395. https://doi.org/10.1007/s10592-017-1010-2

Laidig, T. E. (2010). Influence of ocean conditions on the timing of early life history events for blue rockfish (*Sebastes mystinus*) off California. *Fishery Bulletin*, *108*(4).

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Larson, R. J., & Julian, R. M. (1999). Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. *California Cooperative Oceanic Fisheries Investigations Report*, 94–99.

Leigh, D. M., Lischer, H. E. L., Grossen, C., & Keller, L. F. (2018). Batch effects in a multiyear sequencing study: False biological trends due to changes in read lengths. *Molecular Ecology Resources*, *18*(4), 778–788. https://doi.org/10.1111/1755-0998.12779

Levin, P. S., Tolimieri, N., Nicklin, M., & Sale, P. F. (2000). Integrating individual behavior and population ecology: The potential for habitat-dependent population regulation in a reef fish. *Behavioral Ecology*, *11*(5), 565–571. https://doi.org/10.1093/beheco/11.5.565

Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*(2), 298–299. https://doi.org/10.1093/bioinformatics/btr642

Liu, C.-C., Shringarpure, S., Lange, K., & Novembre, J. (2020). Exploring population structure with admixture models and principal component analysis. In *Statistical population genomics* (pp. 67–86). Humana, New York, NY.

Longo, G. C., Harms, J., Hyde, J. R., Craig, M. T., Ramón-Laca, A., & Nichols, K. M. (2022). Genome-wide markers reveal differentiation between and within the cryptic sister species, sunset and vermilion rockfish. *Conservation Genetics*, *23*(1), 75–89. https://doi.org/10.1007/s10592-021-01397-4

Longo, G. C., Lam, L., Basnett, B., Samhouri, J., Hamilton, S., Andrews, K., Williams, G., Goetz, G., Mcclure, M., & Nichols, K. M. (2020). Strong population differentiation in lingcod (*Ophiodon elongatus*) is driven by a small portion of the genome. *Evolutionary Applications*, *13*(10), 2536–2554. https://doi.org/10.1111/eva.13037

Love, M. S., Morris, P., McCrae, M., & Collins, R. (1990). Life History Aspects of 19 Rockfish Species (Scorpaenidae: Sebastes) from the Southern California Bight. *NOAA Technical Memorandum NMFS*, *87*.

Love, M. S., Yoklavich, M. M., & Thorsteinson, L. (2002). *Rockfishes of the Northeast Pacific*. University of California Press.

Maccready, P., Mccabe, R. M., Siedlecki, S. A., Lorenz, M., Giddings, S. N., Bos, J., Albertson, S., Banas, N. S., & Garnier, S. (2021). Estuarine Circulation, Mixing, and Residence Times in the Salish Sea. *Journal of Geophysical Research: Oceans*, *126*(2). https://doi.org/10.1029/2020jc016738

Masson, D., & Cummins, P. F. (2000). Fortnightly modulation of the estuarine circulation in Juan de Fuca Strait. *Journal of Marine Research*, *58*(3), 439–463. https://doi.org/10.1357/002224000321511106

Masson, D., & Peña, A. (2009). Chlorophyll distribution in a temperate estuary: The Strait of Georgia and Juan de Fuca Strait. *Estuarine, Coastal and Shelf Science*, *82*(1), 19–28. https://doi.org/10.1016/j.ecss.2008.12.022

Matthews, K. (1990). An experimental study of the habitat preferences and movement patterns of copper, quillback, and brown rockfishes (*Sebastes* spp.). *Environmental Biology of Fishes*, *29*, 161–178. https://doi.org/10.1007/BF00002217

McCartney, M. A., Acevedo, J., Heredia, C., Rico, C., Quenoville, B., Bermingham, E., & Mcmillan, W. O. (2003). Genetic mosaic in a marine species flock. *Molecular Ecology*, *12*(11), 2963–2973. https://doi.org/10.1046/j.1365-294x.2003.01946.x

McClelland, E. K., & Naish, K. A. (2007). What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. *Conservation Genetics*, *8*(2), 397–416. https://doi.org/10.1007/s10592-006-9178-x

Melville, J., Haines, M. L., Boysen, K., Hodkinson, L., Kilian, A., Smith Date, K. L., Potvin, D. A., & Parris, K. M. (2017). Identifying hybridization and admixture using SNPs: Application of the DArTseq platform in phylogeographic research on vertebrates. *Royal Society Open Science, 4*(7), 161061. https://doi.org/10.1098/rsos.161061

Miller, B., & Borton, S. (1980). *Geographical Distribution of Puget Sound fishes: Maps and data source sheets.* (Vol. 2). University of Washington Fisheries Research Institute.

Miller, Ian M, Shishido, Caitlin, Antrim, Liam, & Bowlby, C. Edward. (2013). Climate Change and the Olympic Coast National Marine Sanctuary: Interpreting Potential Futures. *Marine Sanctuaries Conservation Series ONMS-13-01. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, MD. 238 pp.*

Montanari, S. R., Hobbs, J.-P. A., Pratchett, M. S., & Van Herwerden, L. (2016). The importance of ecological and behavioural data in studies of hybridisation among marine fishes.

Reviews in Fish Biology and Fisheries, *26*(2), 181–198. https://doi.org/10.1007/s11160-016-9420-7

Moore, S. K., Mantua, N. J., Newton, J. A., Kawase, M., Warner, M. J., & Kellogg, J. P. (2008). A descriptive analysis of temporal and spatial patterns of variability in Puget Sound oceanographic properties. *Estuarine, Coastal and Shelf Science*, *80*(4), 545–554. https://doi.org/10.1016/j.ecss.2008.09.016

Mosher, D. C., & Hewitt, A. T. (2004). Late Quaternary deglaciation and sea-level history of eastern Juan de Fuca Strait, Cascadia. *Quaternary International*, *121*(1), 23–39.

Muto, N., Kai, Y., Noda, T., & Nakabo, T. (2013). Extensive hybridization and associated geographic trends between two rockfishes *Sebastes vulpes* and *S. zonatus* (Teleostei: Scorpaeniformes: Sebastidae). *Journal of Evolutionary Biology*, *26*(8), 1750–1762. https://doi.org/10.1111/jeb.12175

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia university press.

Nielsen, E. E., Hansen, M. M., Ruzzante, D. E., Meldrup, D., & Grønkjær, P. (2003). Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology*, *12*(6), 1497–1508. https://doi.org/10.1046/j.1365-294x.2003.01819.x

Nielsen, E. E., Nielsen, P. H., Meldrup, D., & Hansen, M. M. (2004). Genetic population structure of turbot (*Scophthalmus maximus L.*) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology*, *13*(3), 585–595. https://doi.org/10.1046/j.1365-294x.2004.02097.x

Nielsen, T. G., Kjellerup, S., Smolina, I., Hoarau, G., & Lindeque, P. (2014). Live discrimination of *Calanus glacialis* and *C. finmarchicus* females: Can we trust phenological differences? *Marine Biology*, *161*(6), 1299–1306. https://doi.org/10.1007/s00227-014-2419-5

O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. *Molecular Ecology*, *27*(16), 3193–3206. https://doi.org/10.1111/mec.14792

Pacific Fishery Management Council. (2020). Status of the Pacific Coast Groundfish Fishery: Stock Assessment and Fishery Evaluation. *THE PACIFIC FISHERY MANAGEMENT COUNCIL*, 1–15.

Palsson, W. A., Tsou, T.-S., Bargmann, G. G., Buckley, R. M., West, J. E., Mills, M. L., Cheng, Y. W., & Pacunski, R. E. (2009). The biology and assessment of rockfishes in Puget Sound. *Fish Management Division, Washington Department of Fish and Wildlife*.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics*, *26*(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Parker, S. J., Berkeley, S. A., Golden, J. T., Gunderson, D. R., Heifetz, J., Hixon, M. A., Larson, R., Leaman, B. M., Love, M. S., Musick, J. A., O'Connell, V. M., Ralston, S., Weeks, H. J., & Yoklavich, M. M. (2000). Management of Pacific Rockfish. *Fisheries*, *25*(3), 22–30. https://doi.org/10.1577/1548-8446(2000)025<0022:MOPR>2.0.CO;2

Pawlowicz, R., Hannah, C., & Rosenberger, A. (2019). Lagrangian observations of estuarine residence times, dispersion, and trapping in the Salish Sea. *Estuarine, Coastal and Shelf Science*, *225*, 106246. https://doi.org/10.1016/j.ecss.2019.106246

Petrou, E. L., Fuentes-Pardo, A. P., Rogers, L. A., Orobko, M., Tarpey, C., Jiménez-Hidalgo, I., Moss, M. L., Yang, D., Pitcher, T. J., Sandell, T., Lowry, D., Ruzzante, D. E., & Hauser,

L. (2021). Functional genetic diversity in an exploited marine species and its relevance to fisheries management. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1945), 20202398. https://doi.org/10.1098/rspb.2020.2398

Porter, S. C. (1977). Present and Past Glaciation Threshold in the Cascade Range, Washington, U.S.A.: Topographic and Climatic Controls, and Paleoclimatic Implications. *Journal of Glaciology*, *18*(78), 101–116. https://doi.org/10.3189/s0022143000021559

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics, 155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics, 81*(3), 559–575. https://doi.org/10.1086/519795

R. Walt Deppe, J. Thomson, B. Polagye, & C. Krembs. (2013). Hypoxic intrusions to Puget Sound from the ocean. *2013 OCEANS - San Diego*, 1–9. https://doi.org/10.23919/OCEANS.2013.6741234

Reiss, H., Hoarau, G., Dickey-Collas, M., & Wolff, W. J. (2009). Genetic population structure of marine fish: Mismatch between biological and fisheries management units. *Fish and Fisheries*, *10*(4), 361–395. https://doi.org/10.1111/j.1467-2979.2008.00324.x

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

Roques, S., Sevigny, J.-M., & Bernatchez, L. (2001). Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: A rare marine example. *Molecular Ecology*, *10*(1), 149–165. https://doi.org/10.1046/j.1365-294x.2001.01195.x

Ryan^1, S., Wohlgeschaffen, G., Jahan, N., Niu, H., Ortmann, A., Brown, T., King, T., & Clyburne, J. (2019). State of Knowledge on Fate and Behaviour of Ship-Source Petroleum Product Spills: Volume 5, Strait of Georgia and the Juan de Fuca Strait, British Columbia. *Canadian Manuscript Report of Fisheries and Aquatic Sciences 3176 Canadian Manuscript Report of Fisheries and Aquatic Sciences*.

Salzburger, W., Meyer, A., Baric, S., Verheyen, E., & Sturmbauer, C. (2002). Phylogeny of the Lake Tanganyika Cichlid Species Flock and Its Relationship to the Central and East African Haplochromine Cichlid Fish Faunas. *Systematic Biology*, *51*(1), 113–135. https://doi.org/10.1080/106351502753475907

Schwenke, P. L., Park, L. K., & Hauser, L. (2018). Introgression among three rockfish species (*Sebastes* spp.) in the Salish Sea, northeast Pacific Ocean. *PLOS ONE*, *13*(3), e0194068. https://doi.org/10.1371/journal.pone.0194068

Seeb, L. W. (1998). Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. *Journal of Heredity*, *89*(5), 393–403. https://doi.org/10.1093/jhered/89.5.393

Selwyn, J. D., Hogan, J. D., Downey-Wall, A. M., Gurski, L. M., Portnoy, D. S., & Heath, D. D. (2016). Kin-Aggregations Explain Chaotic Genetic Patchiness, a Commonly Observed Genetic Pattern, in a Marine Fish. *PLOS ONE*, *11*(4), e0153381. https://doi.org/10.1371/journal.pone.0153381

Selz, O. M., Thommen, R., Maan, M. E., & Seehausen, O. (2014). Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. *Journal of Evolutionary Biology*, *27*(2), 275–289. https://doi.org/10.1111/jeb.12287

Serrao, N. R., Reid, S. M., & Wilson, C. C. (2018). Conservation genetics of redside dace (*Clinostomus elongatus*): Phylogeography and contemporary spatial structure. *Conservation Genetics*, *19*(2), 409–424. https://doi.org/10.1007/s10592-017-1012-0

Shafer, A. B. A., Cullingham, C. I., Côté, S. D., & Coltman, D. W. (2010). Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology*, *19*(21), 4589–4621. https://doi.org/10.1111/j.1365-294x.2010.04828.x

Shanks, A. L., & Eckert, G. L. (2005). Population persistence of California current fishes and benthic crustaceans: A marine drift paradox. *Ecological Monographs*, *75*(4), 505–524. https://doi.org/10.1890/05-0309

Shin, J.-H., Blay, S., McNeney, B., & Graham, J. (2006). LDheatmap: An R Function for Graphical Display of Pairwise Linkage Disequilibria Between Single Nucleotide Polymorphisms. *Journal of Statistical Software, Code Snippets*, *16*(3), 1–9. https://doi.org/10.18637/jss.v016.c03

Sivasundar, A., & Palumbi, S. R. (2010). Life history, ecology and the biogeography of strong genetic breaks among 15 species of Pacific rockfish, *Sebastes*. *Marine Biology*, *157*(7), 1433–1452. https://doi.org/10.1007/s00227-010-1419-3

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.-H., Michael, & Michael. (2011). Adaptive Introgression of Anticoagulant Rodent Poison Resistance by

Hybridization between Old World Mice. *Current Biology*, *21*(15), 1296–1301. https://doi.org/10.1016/j.cub.2011.06.043

Soulé, M. E. (1985). What Is Conservation Biology? *BioScience*, *35*(11), 727–734. JSTOR. https://doi.org/10.2307/1310054

Stemshorn, K. C., Reed, F. A., Nolte, A. W., & Tautz, D. (2011). Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). *Molecular Ecology*, *20*(7), 1475–1491. https://doi.org/10.1111/j.1365-294x.2010.04997.x

Stout, H. A., McCain, B. B., Vetter, R. D., Builder, T. L., Lenarz, W. H., Johnson, L. L., & Methot, R. D. (2001). *Status review of copper rockfish (*Sebastes caurinus*), quillback rockfish (*S. malinger*), and brown rockfish (*S. auriculatus*) in Puget Sound, Washington* (noaa:29024). https://repository.library.noaa.gov/view/noaa/29024

Stout, H.A., B.B. McCain, R.D. Vetter, T.L. Builder, W.H. Lenarz, L.L. Johnson, and R.D. Methot. 2001. Status review of Copper Rockfish, Quillback Rockfish, and Brown Rockfish in Puget Sound, Washington. U.S. Dept. Commer., NOAA Tech. Memo. NMFS- NWFSC-46, 158 p.

Suneetha, K. B., & Nævdal, G. (2001). Genetic and morphological stock structure of the pearlside, *Maurolicus muelleri* (Pisces, Sternoptychidae), among Norwegian fjords and offshore area. *Sarsia*, *86*(3), 191–201. https://doi.org/10.1080/00364827.2001.10420475

Szatmári, L., Cserkész, T., Laczkó, L., Lanszki, J., Pertoldi, C., Abramov, A. V., Elmeros, M., Ottlecz, B., Hegyeli, Z., & Sramkó, G. (2021). A comparison of microsatellites and genome-wide SNPs for the detection of admixture brings the first molecular evidence for hybridization between *Mustela eversmanii* and *M. putorius* (Mustelidae, Carnivora). *Evolutionary Applications*, *14*(9), 2286–2304. https://doi.org/10.1111/eva.13291 van Herwerden, L., Choat, J. H., Dudgeon, C. L., Carlos, G., Newman, S. J., Frisch, A., & van Oppen, M. (2006). Contrasting patterns of genetic structure in two species of the coral trout Plectropomus (Serranidae) from east and west Australia: Introgressive hybridisation or ancestral polymorphisms. *Molecular Phylogenetics and Evolution*, *41*(2), 420–435. https://doi.org/10.1016/j.ympev.2006.04.024

Wallace, R. K., & Fletcher, K. M. (1996). *A Manual for understanding the Federal Fisheries Management Process, Including Analysis of the 1996 Sustainable Fisheries Act*. 62.

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*(4), 233–240. https://doi.org/10.1038/hdy.2016.60

Waples, R. S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*(5), 438–450. https://doi.org/10.1093/jhered/89.5.438

Washington State Department of Fish and Wildlife. (2011). Final Environmental Impact Statement for the Puget Sound Rockfish Conservation Plan Including Preferred Range of Actions. *Washington Department of Fish and Wildlife Fish Program*, 223.

Washington State Department of Fish and Wildlife. (2019). Washington State Threatened and Endangered Species List. *Washington Administrative Code 220-610-010*, *June*, 2.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, *38*(6), 1358–1370. JSTOR. https://doi.org/10.2307/2408641

Wellenreuther, M., & Bernatchez, L. (2018). Eco-Evolutionary Genomics of Chromosomal Inversions. *Trends in Ecology & Evolution*, *33*(6), 427–440. https://doi.org/10.1016/j.tree.2018.04.002

Wennerström, L., Laikre, L., Ryman, N., Utter, F. M., Ab Ghani, N. I., André, C., Defaveri, J., Johansson, D., Kautsky, L., Merilä, J., Mikhailova, N., Pereyra, R., Sandström, A., Teacher, A. G. F., Wenne, R., Vasemägi, A., Zbawicka, M., Johannesson, K., & Primmer, C. R. (2013). Genetic biodiversity in the Baltic Sea: Species-specific patterns challenge management. *Biodiversity and Conservation*, *22*(13–14), 3045–3065. https://doi.org/10.1007/s10531-013-0570-9

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (2nd ed. 2016). Springer International Publishing : Imprint: Springer. https://doi.org/10.1007/978-3-319-24277-4

Willis, P. M., Ryan, M. J., & Rosenthal, G. G. (2011). Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish. *Behavioral Ecology*, *22*(6), 1234–1240. https://doi.org/10.1093/beheco/arr119

Zier, J., & Gaydos, J. K. (2016). The growing number of species of concern in the Salish Sea suggests ecosystem decay is outpacing recovery. *Proceedings of the 2016 Salish Sea Ecosystem Conference*, 17.