Introgression and subtle population structure in Copper and Quillback rockfishes

by

Nathan Sykes

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in the Department of Biology

University of Victoria

Introgression and subtle population structure
in Copper and Quillback rockfishes

by

Nathan Sykes

Supervisory committee

Dr. Gregory Owens, Supervisor
Department of Biology

Dr. John Taylor, Departmental Member
Department of Biology

Dr. Ben Koop, Departmental Member
Department of Biology

Abstract

Genomic methods are increasingly being applied in fisheries to promote effective management and sustainability. Pacific rockfishes, genus *Sebastes*, inhabit inshore, shelf, and slope habitats along the North American west coast. Among these, Copper and Quillback rockfishes (abbreviated to Copper and Quillback) are closely related species known to hybridize, particularly within the Salish Sea in North America's Pacific Northwest. Here, I investigate genetic population structure and introgression patterns in Copper and Quillback rockfishes from Alaska to California. Using low-coverage whole-genome resequencing (lcWGS) across a broad geographic range, I seek to (1) describe genetic differentiation between the rockfish species, (2) assess population structure within each species, (3) identify regions of the genome with unique patterns of differentiation between species, and (4) look for signatures of introgression in the genomes of both species. My analyses reveal that Copper exhibit higher levels of population differentiation compared to Quillback, especially between coastal and Salish Sea populations. In contrast, Quillback populations appear to be more panmictic, with lower overall differentiation. Several large haploblocks are found to be segregating between the species, with introgression patterns varying across genomic regions. These findings provide novel insights into the range-wide genetic structure of these species and highlight the role of genomic architecture in local introgression.

Table of Contents

List of Figures and Tables

Chapter 1: Trends in marine genomics and an introduction to *Sebastes*

For more than a decade, genomic methods have been applied to answer evolutionary questions in model and natural systems. Accessible whole genome resequencing and powerful computational tools provide robust and high-resolution insights into the history of species and populations as well as the link between genetics and individual traits. Along with the potential to detect and describe big-picture evolutionary questions and outcomes (Nielsen et al., 2009), genomic analyses are quickly becoming instrumental in the fields of conservation (Allendorf et al., 2010) and management of natural resources (Willi et al., 2021).

One of the primary conservation concerns for fisheries managers is the ability to assess and monitor genetic diversity within and among fish populations. Genomic tools can provide insight into the number of mating adults within a population, their gene pool, and degree of connectivity with other populations (Hu et al., 2023; Siegle et al., 2013; Zhang et al., 2020). These are important metrics for fisheries managers, as low genetic diversity and small population sizes can leave exploited species vulnerable to extinction (Fagan & Holmes, 2006). Moreover, accurate characterization of conservation units (e.g. populations which do not freely interbreed) is essential for implementation of effective management strategies (Bernatchez et al., 2017). Characterization of the genetic diversity of harvested species, both within and between populations, is critical for setting catch limits, establishing marine protected areas, and assessing species' recovery.

The Pacific rockfishes (genus *Sebastes*) are a diverse clade of economically, ecologically, and culturally important fishes (Love et al., 2002). Because of their variation in morphology, niche preference, range and lifespan, they are the subject of emerging studies on the factors that drive speciation, as well as variability in life span. Additionally, a history of exploitation has led to the

assessment of some *Sebastes* as threatened or endangered within Canada, and many species have experienced reduced stocks as a result of overharvest (COSEWIC, 2009, 2020). Amid ongoing (albeit reduced) commercial and recreational fishing pressures, few range-wide assessments of genetic variation have been conducted within the genus to date. There is therefore significant impetus to quantify their genetic diversity at this stage of exploitation, to better make proactive management decisions which ensure the sustainability of the fishery.

In this chapter, I will outline the advancements and applicability of genomics in natural systems, with a focus on marine fishes. I will describe some of the observed trends in recent marine genomic studies, which provide some null expectations in this current and future studies. I will examine the concept of a species continuum and describe methods for detecting signatures of hybridization in the genomes of closely related species. I will explain the special role of structural variation in local adaptation and the speciation process, and will provide background on my study species, the genus *Sebastes*, and their adaptive radiation in the Pacific Ocean.

1.10 The state of the art in marine genomics

Advances in genomic technologies have provided researchers with powerful tools to study non-model systems. Increased computing power and the availability of high-resolution genomic data allow biologists to develop new analytical techniques and examine patterns of inheritance at their most granular. Pre-genomic methods, which leverage variation in allozymes or microsatellites, are comparatively labour intensive and limited in their ability to detect genome-wide variation. Allozyme analyses examine variation in orthologous enzymes, which exhibit differential migration through a gel. This method captures variation at the protein level but is limited to the region of the genome that codes for the allozyme protein. Microsatellites are short, repetitive

DNA sequences found throughout the genome and may be highly variable in length across individuals. Analysis of microsatellite variation requires extraction of DNA and amplification through polymerase chain reaction (PCR) before amplicons are compared through gel electrophoresis. Because of the increased variation in microsatellite loci between individuals, these can detect finer scale variation than allozymes. However, variation in microsatellite markers is generally neutral and may not capture small-scale variation such as single nucleotide polymorphisms, which would not affect migration through a gel. By contrast, next-generation sequencing (NGS) methods provide a much more comprehensive picture of the genome. In addition to allozyme coding regions and microsatellite loci, NGS methods enable sequencing of most or all of the genome. Reduced representation methods, like GBS or RADseq, digest DNA based on enzyme cut-site specificity, and preferentially cover portions of the genome proximal to the cut-sites (Baird et al., 2008). Whole genome resequencing methods use fragmented DNA from throughout the genome at equal rates (Li et al., 2009). Genotyping can then take place at individual base-pair loci along the bulk or entirety of the genome with more or less equal coverage.

Genomic methods present an opportunity to study high-resolution population differentiation (and its drivers and consequences) in marine fishes, but this is not a simple task (Hemmer-Hansen et al., 2014). Using genome data, population sizes and migration rates can be estimated using demographic models, but these models are often simplified and may not adequately account for natural selection. It is important that these challenges are met, as accurate inference of population dynamics is critical for the long-term health of fish stocks, which comprise the only remaining commercially harvested wild food stocks on Earth. This section briefly outlines the applicability

of modern genomics to marine systems, with emphasis on conservation, fisheries management, and evolution.

1.11 Conservation

The application of genomic technologies in marine conservation is a rapidly expanding area of research, with significant potential to protect biodiversity (McMahon et al., 2014). Among the primary goals of conservation biology are (1) accurate delineation of evolutionarily distinct units within species, (2) preservation of genetic diversity to ensure the long-term viability of populations. Leveraging genomics can significantly enhance the effectiveness of these goals; when compared to pre-genomic approaches, accurate genotyping of entire genomes gives conservationists more accurate estimates of population genetic parameters. Given ongoing debate about which parts of the genome are important for fundamental evolutionary processes, like local adaptation and speciation, it is crucial to examine variation throughout the genome which was not possible in pre-genomic methods.

The higher resolution conferred by whole genome sequencing enables more fine-scale delineation of conservation units within species. What constitutes a population is oft disagreed-upon (Waples & Gaggiotti, 2006) but can be most generally described as a group of conspecific individuals which freely interbreed. It is important in any conservation effort to identify unique genetic variation within populations, but also to understand what, if any, barriers exist to migration between populations. Under water, the latter is a much more challenging task; barriers to dispersal and migration are less conspicuous. By leveraging genomics, researchers can use genetic relatedness to infer historical migration, also known as historical gene flow. In so doing, genetic analyses can identify populations and, in turn, barriers to migration within species (Alexander et al., 2009; Ma & Amos, 2012). Identification of genetically distinct populations can

lead to more effective conservation strategies by enabling managers to specifically protect isolated lineages (Funk et al., 2012). One recent study identified one genetically distinct population of grey parrotfish, a threatened species who are otherwise admixed throughout their range (Tovar Verba et al., 2023). This was important because without these genomic data, a more spatially homogenous management strategy may have fallen short of protecting important genetic variation within the species. Genomic analyses can even reveal cryptic species, which can be defined as species that are phenotypically difficult to distinguish but are genetically distinct. These species may be separated geographically (e.g. Deacon rockfish were redescribed from a northern population of Blue rockfish) or in parapatry, such as in Sunset and Vermillion rockfishes, previously thought to be a single species (Frable et al., 2015; Hyde et al., 2008).

Genetic diversity serves as a reservoir of evolutionary potential that promotes resilience to present and future selective pressures. An important part of understanding how species and genomes adapt to change is getting a clear picture of present genetic diversity within species, and how that variation is organized in space. This baseline allows conservationists to assess the current genetic health of populations and identify those at risk (Beger et al., 2014; Nielsen et al., 2022). Regular characterization of populations' genetic health enables managers to more actively and effectively ensure sustainable harvest; stability or an increase in genetic diversity may suggest that management regimes are working to ensure the long-term health of the population. Conversely, populations continuing to experience declines in genetic diversity may require intervention. Interventions, such as genetic rescue, which seek to supplement gene pools through translocation of individuals with different genetic variation, can be quite successful in restoring the genetic health of depressed populations (Johnson et al., 2010; Nedoluzhko, 2023). In fisheries, aquaculture can play an important role in this process. Rearing of genetically diverse

stock in controlled environments can help conservationists know precisely what genetic variation they stand to introduce to the depauperate population and provide a stable and protected source of diverse individuals (Bernatchez et al., 2017; Yáñez et al., 2015).

Species' genetic diversity can also help managers understand how exploitation and other anthropogenic pressures impact gene pools. In the Eastern Rock Lobster, researchers found evidence that overexploitation has reduced contemporary genetic diversity and led to a single, genetically homogenized population throughout their range (Woodings et al., 2021). Indeed, conservationists argue that prolonged population declines in harvested species tend to erode genetic diversity, undermining their potential to adapt to future change.

1.12 Fisheries Management

As mentioned above, genomics help clarify the genetic structure of fish populations, and to monitor their genetic health, enhancing our ability to manage fishery stocks more sustainably (Bernatchez et al., 2017; Funk et al., 2012). Historically, fisheries managers have based delineation of catch areas based on a combination of geographical features, catch and effort data, and stock assessment models. However, genomic analyses have revealed mismatches between existing fisheries management units and biological ones (Longo et al., 2020; Mullins et al., 2018; Xuereb et al., 2022). To date, genomic methods have been applied to several non-model systems to help refine management area boundaries at small and large scales. For example, northern and southern lingcod represent two evolutionary lineages, and overlap only in a small portion of their range, which spans from the Gulf of Alaska to Baja California (Longo et al., 2020). Researchers posit a break in gene flow in northern California, whereby bathymetric and hydrologic features act as dispersal barriers. Importantly, this population differentiation was driven by only a few small regions of the genome, which would likely have gone undetected by pre-genomic methods

of genetic stock assignment. A similar break was observed on the coast of South Africa; two discrete populations of Yellowfin Tuna were found to be separated by patterns of strong coastal upwelling. Even in Pacific salmon, whose genetic population structure tend to reflect spawning site fidelity, genomic approaches have led to refinement of an existing management structure (Xuereb et al., 2022).

The capacity of genomic methods to identify genetic breaks when they represent a minority of the genome has enabled the development of more accurate stock assignment methods. These methods rely on diagnostic single nucleotide polymorphisms to determine the origin of caught fish. While some diagnostic tools have already been developed using pre-genomic genetic markers, genomics makes these methods cheaper and more effective (Baetscher et al., 2023; Longo et al., 2022). Stock identification methods are particularly useful in mixed stock fisheries, such as Pacific salmon, when catch in the open ocean may be composed of several distinct spawning site lineages. This can help conservation and management in multiple ways: it can infer dispersal and help ensure adherence of commercial and recreational fishing statutes by testing of landed samples and identifying their genetic stock.

Given the penchant of historical fisheries to exhaust their stocks, it is important for managers to take a proactive approach to stock assessment. Catch and effort data are certainly useful; raw fish biomass per catch effort has declined precipitously for many fish taxa, resulting in more stringent management regimes (Bernatchez et al., 2017; COSEWIC, 2009, 2020; Pauly et al., 2002). However, this reactive approach may be too late to mitigate the loss of genetic diversity in an overfished population. Genomic data can be combined with climate models and genotype-environment associations to enable managers to better predict future population declines (Tigano et al., 2024) and can be used to infer the genetic consequences of overharvest. This is important

because, over time, genomic data can be used together with catch and effort data to better understand if and how populations may recover. As discussed earlier, genomic data provide insights into overall genetic diversity and population connectivity (Ma & Amos, 2012) but can also infer effective population sizes in the absence of census data. Though it should be noted that effective population size inferences have been shown to overestimate the actual number of individuals in marine mammals (Peart et al., 2020) but can significantly underestimate census size in marine fishes (Hauser et al., 2002).

1.13 Ecology and evolution

Advances in genomic technologies have revolutionized our ability to explore fundamental processes in ecology and evolution. The resolution of whole genome data provides researchers with the full breadth of genetic variation within and between species. By providing insight into the genetic architecture underlying traits, genomics can be leveraged to address key questions in the field such as: (1) how do species adapt to their environment? (2) what are the processes driving speciation and maintaining species boundaries? and (3) how do historical processes influence present-day genetic diversity?

Genomic methods allow researchers to identify the genetic basis of adaptive traits and track how populations evolve in response to environmental change. Genome-wide association studies (GWA) test for associations between individual genetic markers and measurable traits – even highly polygenic ones. For example, researchers have begun to characterize the complexity of genomic variation underlying climate adaptation in commercially important tree species (De La Torre et al., 2019; Rellstab et al., 2016). For GWA, larger effect and more common loci are easiest to detect, and power is increased with increasing sample size or lower environmental

variation (for example, by phenotyping samples in a common environment) (Korte & Farlow, 2013). This can be neatly illustrated with two examples. One study in salmon sought to elucidate genomic correlates with successful migration beyond a steep gradient, but found weak signal spread across the genome (Horn et al., 2020). It is possible that, with higher sample numbers, the complex genetic architecture underlying successful migration could be described, or that in this case the trait is largely controlled by non-genetic factors, like body condition. Conversely, the genetic basis for premature timing in Pacific salmon (an important mechanism of reproductive isolation) appears to be controlled at a single locus (Prince et al., 2017). In this case, the premature timing allele is both relatively common and has a large effect size, making its detection readily apparent compared to the genome background.

Genomics can reveal the genomic consequences and mechanisms of reproductive isolation and speciation. Ecological speciation proceeds by the evolution of reproductive isolation between populations as they adapt to their unique environments (Schluter, 2001). By focusing on incipient species (those in the early stages of differentiation), research can reveal the genomic variation associated with the evolution of reproductive isolation, rather than divergence that accumulates as a consequence of that isolation. In the early stages of speciation, strong selection at adaptive loci can result in the fixation of new mutations in one of the divergent lineages (Tigano & Friesen, 2016; Wolf & Ellegren, 2017). While recombination and ongoing gene flow between the incipient species act to homogenize the bulk of the genome, differentiation at these key loci may be quite high. High-resolution genome scans comparing the divergent lineages will then show a relatively small number of regions in the genome with unusually high levels of genetic differentiation, termed genomic islands of divergence. Indeed, these islands have been associated with geographic and ecotypic differentiation, in multiple species of marine fish

(Bradbury et al., 2013; Larson et al., 2017; Longo et al., 2020; Weist et al., 2022). Notably, these regions may be very small, even contained to a single locus. This highlights the applicability of whole genome scans to uncover such adaptive divergence when compared to pre-genomic methods. A recent study on local adaptation in sockeye salmon highlights the utility of genomic methods in characterizing these islands of divergence. In their study, Euclide et al. (2023) compared the genomes of multiple sockeye salmon ecotypes from three separate populations to uncover several small, shared islands of divergence within the species. A related study identified three genomic islands of divergence separating migratory and non-migratory ecotypes of sockeye from a different watershed (Tigano & Russello, 2022). In some of these cases, the genomic islands of divergence were associated with genome structure variation. The potential role of structural variation in local adaptation and speciation is discussed in a later section.

By analyzing genome-wide patterns of variation, researchers can infer past population dynamics, such as bottlenecks, expansions, and migrations. Genomic data can uncover the evolutionary history of populations, helping to reconstruct demographic changes and understand the impact of historical events (like founder effects, bottlenecks) on current genetic diversity and population structure. These methods are derived on the coalescent theory posited by John Kingman (Kingman, 1982) and describe the genealogical relationships between genomic sequences (Marchi et al., 2021). Coalescent theory hinges on the idea that the time (before present) to the most recent common ancestor for a set of orthologous sequences in inversely proportional to population size. This allows researchers to use genomic data from contemporary samples to estimate current and past population sizes, as well as historical admixture events (Harris & Nielsen, 2013). These analyses are dependent on high-resolution genome data to identify tracts of DNA which are identical by descent from a common ancestor. For example, North American

Drosophila melanogaster were found to be descended from a mixture of African and European ancestors, with a smaller proportion of their ancestry drawn from Africa (Duchen et al., 2013). This study also revisits the historical demography of one African population to identify a historical bottleneck, whereby only a small portion of a larger population contributes to future generations, thereby narrowing the gene pool. This last point is of particular relevance to fisheries, many of whom have experienced significant harvest pressure at some point in their history, and likewise have suffered small population sizes. A recent effort to characterize population structure for improved management of Eastern Rock Lobster used neutral variation in whole genome data to attribute low genetic diversity to past overfishing (Woodings et al., 2021). It is important to note that because many coalescent models assume neutral variation, accurate demographic inference may be compromised by selection, which can affect allele frequencies on much shorter time scales (Marsh & Johri, 2024).

1.20 Observed trends in genomics of marine organisms

Marine systems and their inhabitants have many unique characteristics which affect natural selection. On land, barriers to gene flow tend to be quite conspicuous; rivers, mountain ranges, or patches of unsuitable habitat are readily apparent. Under water, these types of geological or habitat-driven barriers do exist, but other hydrological phenomena, such as currents and upwelling, are superimposed on the seascape (Grummer et al., 2019). This can lead to unexpected patterns in individual dispersal, the basis for gene flow between populations. The continuity of habitat can represent very broad, easily traversable ranges for many species. The species themselves often have a planktonic larval phase which may passively disperse over large distances. Additionally, water is host to a multitude of physiological stressors that are not present on land, such as variation in salinity and dissolved oxygen. Here, I present some of the observed

trends in the population genomics of marine fishes with consideration for the special context provided by ocean habitat.

1.21 Local adaptation in a marine environment

Local adaptation requires variation in the environment. When contrasted with terrestrial systems, marine systems contain less small-scale variation in habitat type and environmental variables (Nielsen et al., 2009). This results in more contiguous habitat, representing a selective gradient over very large spatial scales. Further, seasonal variation in environmental variables such as solute concentration and temperature may outweigh spatial variation in the same, depending on the range and habitat of the species in question (Denny & Dowd, 2022). High gene flow throughout an environmental gradient can undermine the effect of selection to favour locally adaptive alleles, through regular migration and the shuffling of genes conferred by recombination (Tigano & Friesen, 2016). The segregation of putatively adaptive alleles in continuously distributed population of marine fish, then, would not be dissimilar from neutral variation. This is what is often observed in marine systems; divergent lineages may be differentiated, as discussed earlier, by genomic islands of divergence, however within-species variation much more often reflects a paradigm of low-level differentiation throughout the genome. Exceptions may exist where environmental gradients are more severe. For example, the Baltic Sea exhibits a very steep salinity gradient from its inner gulfs to its mouth at the North Sea. Here, researchers have identified spatial variation in allele frequencies in Atlantic herring for genes associated with salinity and osmoregulation (Lamichhaney et al., 2012). While these alleles are not fixed at salinity extremes, the significant shift in allele frequencies combined with the prior knowledge of gene function enabled convincing detection of local adaptation. It is more likely, though, given the gradient in environment and high gene flow exhibited by most marine species, that a gradual

shift in allele frequencies over large spatial scales (i.e. a genomic cline) is the prevailing paradigm in marine genomics, rather than adaptation to local conditions.

1.22 Population structure and habitat connectivity

Marine fishes often exhibit low isolation by distance (IBD). Coupled with the high dispersal capacity for marine larvae, this can lead to a highly homogenous gene pool, even over large distances (Nielsen et al., 2009). Empirical evidence for panmixia in marine fishes is abundant (Díaz-Arce et al., 2024; Hemmer-Hansen et al., 2014; Nielsen et al., 2009; Tovar Verba et al., 2022) and most authors argue that high habitat connectivity coupled with the high dispersal of planktonic larvae explains the genetic homogeneity. While these facts no doubt hold true, there are nuances in the species' life history, habitat, and ecological niche that can result in more population structure.

Under water, it's not that the physical barriers to gene flow do not exist, only that they can be harder to detect (Nielsen et al., 2009). High resolution genomic data provide an opportunity to identify these breaks through inference; if geographically separated populations have higher than expected divergence, there must be some barriers to gene flow between the two populations, or locations. This can take place over smaller scales in the case of patchy habitat, such as for fishes who occupy coral reefs (Gaither et al., 2015) or on in major seas, such as the Mediterranean and Baltic, where narrow entry points and hydrological directionality lead to unique genetic variation and ecotypes (Lamichhaney et al., 2012; Puncher et al., 2018).

These various lines of evidence point to a highly complex and individual patterns of gene flow within taxa and across space. Ecology, life history, and habitat are not the only factors to consider when crafting null expectations for marine fish population genomics. Tides, currents,

bathymetry, and community also play their role in gene flow dynamics, depending on the species or population of interest. For example, Yellowfin Tuna is a highly migratory ocean fish but have a clear genetic break across a seam of coastal upwelling on Africa's west coast (Mullins et al., 2018). Currents have been shown to have a significant effect on patterns of differentiation in Gray Parrotfish (Tovar Verba et al., 2023), and Pacific Hake are genetically homogenous along the west coast of North America, but for the populations in the Salish Sea, inside of Vancouver Island (Longo et al., 2024). These three examples are illustrative of the potential for unique population structure patterns in marine fishes. It is clear that, while fishes tend to differentiate over long distances, the marine continuum is home to its own barriers to gene flow. Studies which seek to fully characterize population structure among marine fishes should aim to sample not only spatially disparate locations, but also ecologically diverse ones. Therein lies some difficulty; sampling for commercially important species is commonplace, but is largely performed during trawl or net surveys, and may lack the resolution and diversity of habitat that is required to reflect the biological reality. Targeted sampling at specific depths and locations may be better able to identify ecological barriers in marine fish.

1.23 The genomic effects of anthropogenic disturbance

Anthropogenic disturbance can affect fish populations in many ways. The most obvious and well-documented among these is overharvest, here referring to the excessive commercial exploitation of fish populations. This phenomenon is driven by intensive fishing practices that, by definition, deplete fish populations more quickly than they can be naturally restored, and has profound impacts on the genetic diversity of overfished populations (Pinsky & Palumbi, 2014; Woodings et al., 2021). Recent studies have also described the effects of urbanization and ecological disturbance on genetic diversity in marine fishes (Frei et al., 2024; Karachaliou et al.,

2024). Combined, this suggests that the direct negative effect of fishing pressures on the genetic diversity within a population can be compounded by the indirect effects of anthropogenic development.

Even over one human generation, overexploitation of fisheries can quickly result in severe population size declines and genetic bottlenecks among the targeted species (Hauser et al., 2002; Pinsky & Palumbi, 2014; Therkildsen et al., 2019). This is detrimental to the fish population; lower genetic diversity results in lower adaptive potential, and lower population sizes then leave populations at risk of further loss to their gene pool through genetic drift. Allendorf et al. (2008) suggest that lower genetic diversity is not the only potential negative outcome; overfishing can also lead to reduced population structure and directional selective changes (Allendorf et al., 2008). Fishers tend to remove a large proportion of mature, reproductively active individuals, which leads to strong selection against larger fish. A recent study demonstrated loss of genetic diversity and significant divergence in functional regions related to size, growth, and age of maturity in only five zebrafish generations (Sadler et al., 2024). Fishing practices which target a specific phenotype for harvest may in fact more significantly reduce the genetic diversity within a population by selectively removing its underlying alleles (Frankham, 2012). In response to fishing pressure, fish populations may evolve to mature at smaller sizes and younger ages to reproduce before being caught, and can be slow to recover, if they do at all (Conover et al., 2009). This is exemplified in British Columbia by the declining size and age-at-maturity of Yelloweye and Quillback rockfishes, once assessed as endangered and threatened, respectively, in their Canadian range (McGreer & Frid, 2017).

In addition to the direct selective and population size pressures imposed by fisheries, anthropogenic development can drastically alter local environmental conditions and result in

similar loss in genetic diversity. A metanalysis examining 73 species at 1143 samples sites around the world found significantly lower genetic diversity in marine fish populations associated with human density, agnostic of taxon or locality (Karachaliou et al., 2024). Another study, which examined the link between human-caused eutrophication and genetic diversity in freshwater whitefish, found up to a 30% decrease in genetic diversity as a result of the environmental stressors imposed by human development (Frei et al., 2024). These findings suggest that all the potential impacts of anthropogenic disturbance on fish populations have yet to be described. What is certain, however, is that anthropogenic pressures, via both fishing pressures and alteration and homogenization of habitat, have led to decreased genetic diversity in marine and freshwater fishes. This is highly consequential for all of the species involved, whose reduced adaptive potential may inhibit their persistence amidst ongoing environmental change.

1.30 Hybridization / introgression

Hybridization was once considered to be rare in marine fishes (Hubbs, 1955), but an increasing number of studies suggest that it is widespread, particularly in incipient and sympatric species associated with complex habitat (Gainsford et al., 2020; Montanari et al., 2012; Muto et al., 2013; Wray et al., 2024). Hybridization is highly consequential for species genetic diversity and can play a significant role in shaping the evolutionary trajectory of the species involved. The persistence of hybrid zones can promote species genetic diversity, and even drive population genetic differentiation (Moran et al., 2021; Wray et al., 2024). Hybridization itself can lead to the formation of new hybrid species. For example, in *Heliconius* butterflies and cichlid fishes, introgression of adaptive traits resulted in the formation of new species (Olave et al., 2022; Rosser et al., 2024). In these examples, the intermediate phenotype conferred by hybrid ancestry is fine-tuned through fixation of adaptive alleles from each parent species, resulting in a unique

combination of traits. Ultimately, these systems rely on sustained reproductive barriers between the new hybrid species and both parents. Given that most researchers can only observe present day reproductive isolation in these systems, empirical evidence is difficult to obtain and studies which examine this phenomenon are rare, especially in marine fish.

1.31 Genomic consequences of hybridization

Introgression, the incorporation of genetic material from one species into the gene pool of another through repeated backcrossing, can leave distinct signatures in the genome (Moran et al., 2021). Whether any introgressed variation will be observed in the genomes of hybridizing species is dependent on the fitness consequences of the alleles being considered. Species accumulate reproductive isolation over time. Consequently, not only will some introgressed alleles be maladaptive due to ecological differences between species, but negative epistatic interactions between loci, termed Dobzhansky-Muller incompatibilities (DMIs), will also restrict gene flow by preventing introgression at incompatible loci and nearby regions (Dobzhansky, 1982). Consequently, the location of DMIs and ecologically adaptive alleles are expected to control the location of where introgression is found in the genome (Bank et al., 2012; Moran et al., 2021). In addition to the negative fitness effects conferred by specific loci, divergence in genome architecture and recombination landscape can affect ease of introgression (Baack & Rieseberg, 2007). The expected outcome of these incompatibilities would be reduced, if any, minor parent ancestry in hybrids and backcrosses at specific, incompatible loci and in broader, species-specific linked regions.

Introgression can also introduce adaptive variation to hybridizing species (Norris et al., 2015; Song et al., 2011). Assuming growing reproductive isolation between lineages, introgression is likely being purged by selection in most of the genome – adaptive introgression is therefore

occurring against the grain (Calfee et al., 2021; Edelman et al., 2019). Contrasted with the selection against introgression summarized above, adaptive introgression is therefore predicted to result in high frequency introgressed alleles against a background of low minor parent ancestry (Sankararaman et al., 2014). This also means that factors such as the relative position of adaptive alleles and deleterious ones, the strength of selection on either type, and the genomic architecture referenced above can all impact the possibility of introgression.

1.40 Inversions

Chromosomal inversions occur when a segment of the chromosome breaks off, flips 180 degrees, and reattaches at the breakpoints in the opposite orientation. This can happen across centromeres (pericentric) or outside of them (paracentric), with the former more likely to be responsible for later misalignment of homologous chromosomes during meiosis (Kirkpatrick, 2010). Here, I outline the mechanisms by which chromosomal inversions persist within populations, preserve and collate important genetic variation. Additionally, I describe the increasingly recognized adaptive significance of inversions and their role in population divergence and speciation.

1.41 Linkage and recombination

Inversions suppress recombination between haplotypes; if an individual possesses one copy of a chromosome with an inversion and another without it, recombination, barring double crossovers or conformational oddities, will result in unbalanced gametes (Kirkpatrick, 2010). Without recombination, alleles within the inversion become tightly linked and are heritable as a single block. Over time, mutations will accumulate in the inverted region that, in the presence of recombination, might have been purged by purifying selection. Most inversions are small (Feuk et al., 2005) but can have major evolutionary consequences when at a scale that encompasses one

or more functional genes. Large-scale inversions can link together groups of genes (i.e. supergenes) and, due to the recombination suppression conferred by the structural variation, result in the emergence of complex novel phenotypes. In some cases, these highly differentiated regions of the genome can be the driver of ecotypic differentiation and reproductive isolation (Todesco et al., 2020).

It is not only adaptive mutations that are safe from recombination within chromosomal inversions, as deleterious mutations can accumulate alongside them. This process, known Muller's ratchet, describes the irreversible accumulation of harmful mutations in regions with reduced recombination, which can occur more frequently within inversions (Muller, 1964). While inversions are not precluded from recombination, the rate at which deleterious mutations are purged from the region can affect fitness in the long term (Berdan et al., 2021). The same features, then, increase the evolutionary significance and detectability of inversions may, in the long term, become detrimental.

1.42 The adaptive significance of inversions

By combining a series of tightly linked mutations, inversions have the potential to have a strong effect on a combination of traits (Westram et al., 2022). Through the maintenance of co-adapted gene complexes, inversions can be the sole drivers of ecotypic differentiation within species, such as the discrete migratory phenotypes in Atlantic cod (Berg et al., 2016, 2017). The maintenance of favourable allelic combinations, even in the face of gene flow, is the basis for the significant adaptive potential conferred by these large structural variants. Ultimately, the ecotypic differentiation conferred by chromosomal inversions can result in cascading divergence throughout the genome, and total reproductive isolation – speciation. Some researchers argue that structural variation should be better acknowledged as playing a pivotal role in the molecular

basis of adaptation and speciation (Wellenreuther & Bernatchez, 2018). Intuitively, large-scale structural variation can more readily facilitate selective sweeps for adaptive traits than can individual SNPs (Westram et al., 2022).

Without causal evidence, it is harder to ascribe local adaptation to chromosomal inversions. However, local adaptation can be inferred when there is geographic variation in the frequency of an inversion, particularly when combined with environmental or phenotypic data (Kirkpatrick, 2010). Even without the inference of local adaptation, though, inversions have been found to be the primary driver of population structure in otherwise genetically homogenous fish species. Such is the case with lingcod in the northeast Pacific (Longo et al., 2020). Longo et al. found that strong differentiation between the northern and southern populations is limited to relatively small but highly divergent regions of the genome, underlain by chromosomal inversions. This suggests that inversions in lingcod house important and ecologically relevant genetic variation and may be responsible for local adaptation within the species.

1.50 Sebastes

Pacific rockfishes (genus *Sebastes*) are a highly speciose genus of fishes that have undergone an adaptive radiation on coastlines in the northeast Pacific Ocean (Hyde & Vetter, 2007; Love et al., 2002). Subject to commercial fishing, bycatch, and targeting by recreational fishers, some species have exhibited severe population declines and have been assessed as threatened or endangered by governing bodies in Canada and the United States (COSEWIC, 2009, 2020). Here, I describe the life history and ecological characteristics of the genus, recent insights into their genomic drivers of speciation and adaptation, assess the prevalence and importance of ongoing interspecific gene flow, and highlight the conservation implications of these findings.

1.51 Life history and ecological characteristics

Pacific rockfishes have undergone rapid speciation within the last eleven million years, resulting in 108 described species primarily distributed along the Pacific coasts from Japan to Baja California, as well as the South Pacific and Atlantic (Froese & Pauly, 2021; Love et al., 2002). Among fishes, *Sebastes* are rare for their internal fertilization and vivipary; they gestate young internally and give birth to live pelagic larvae. When contrasted with the broadcast spawning of most fishes, this particular trait could be consequential for promoting genetic population structure. First, this suggests that rockfishes may preferentially select mates based on some criteria. Indeed, male rockfish perform movement-based courtship behaviours to entice observing females (Helvey, 1982). Non-random mating of this type, wherein specific male phenotypes improve their fitness, is known as sexual selection plays a pivotal role in generating premating reproductive isolation and ultimately, speciation (Price, 1998). Second, vivipary itself is postulated to promote diversification of species (Helmstetter et al., 2016). Functionally, this can be attributed to the fact that a single viviparous pregnant female can colonize new, putatively isolated habitat (Meyer & Lydeard, 1997). In coastal systems, these new habitats exist in three dimensions, and depth has been identified as a parallel driver of speciation in multiple clades (Behrens et al., 2021). Indeed, the variation and complexity of coastal benthic habitat may itself promote speciation through its variety of ecological niches (Funk et al., 2006).

After being released by the mother, pelagic rockfish larvae can be found at relatively shallow depths (to 80 m) for one to two months before transitioning to the juvenile pelagic stage (Love et al., 2002). The line between these stages is blurred, but rockfish are considered juvenile when some morphological features, such as gill rakers and fin rays, reach adult numbers. Juveniles may school in the water column around rocky outcroppings or in kelp forests, again depending

on species. The juvenile stage ends when a fish becomes more demersal, spending the bulk of their time associating with the sea floor and its structures. Notably, the structures associated with the sea floor can include giant kelp and other algae in the water column.

As adults, rockfishes can be divided into five categories based on depth and its associated habitat. These are: (1) intertidal, (2) nearshore to 30 m, (3) shallow shelf to 100 m, (4) deep shelf to 200 m and (5) slope beyond 200 m. Due largely to the depth of their habitat, relatively little is known about the specific habitat requirements of most rockfishes. Generally, though, rockfishes tend to prefer rocky bottoms with complex vertical structure – full of crevices and other features (Love et al., 2002; Matthews, 1990). Individuals from many different species are often observed inhabiting the outcropping, and individuals from the same species can be found in diverse habitat. This suggests flexibility in terms of preferred habitat, but begs the question 'how are rockfishes adapting to their niches and what processes underlie their diversification?'

1.52 Adaptation and speciation in Sebastes

As outlined above, the rapid diversification of *Sebastes* can be explained in part by their life history traits, but the genus is also rife with species complexes. Lineages within the genus have evolved convergent morphology multiple times during their radiation throughout the Pacific (Ingram & Kai, 2014). These convergent morphologies are suggestive of similar patterns of ecological niche partitioning (Deville et al., 2023a), largely associated with depth in water column. The mechanisms by which these species become reproductively isolated may be explained by a combination of morphological and genetic data.

Closely related rockfishes have similar shapes, but within these species complexes, morphological variation suggests niche partitioning by depth and trophic level (Ingram, 2015).

For example, gill rakers and eye size act as proxies for trophic position and depth of habitat, respectively (Ingram & Shurin, 2009) – more gill rakers act as a sieve for smaller prey and indicate lower trophic position. Rockfish speciation is then closely associated with divergence in habitat depth (Ingram, 2010) and the resources that are available there. Morphotypes within currently recognized species of rockfish have been documented (Kai & Nakabo, 2002), which lends further credence to this theory of speciation in parapatry. Further, Ingram (2015) finds that this body shape diversification has been near constant in the evolutionary history of *Sebastes* (Ingram, 2015). This suggests a steady accumulation of divergent traits, rather than a short burst, underlies the adaptive radiation present today.

Genomic analyses which investigate the causes and consequences of speciation in *Sebastes* have mixed results. Genome-wide divergence between species can be very low, even in morphologically distinct pairs (Narum et al., 2004), while some cryptic species are significantly genetically differentiated as to be described as distinct taxa (Kai & Nakabo, 2008). An analysis of two species pairs separated by depth found shared islands of divergence between them (Behrens et al., 2021), though disentangling the genomic basis for and the genomic consequences of speciation remains a challenge.

Niche partitioning in *Sebastes* proceeds in more ways than depth and depth-associated habitat, though. Many northeast Pacific rockfishes exhibit patterns of divergence wherein populations in fjords and seas are genetically distinct from the more genetically homogenous coast (Andrews et al., 2018; Dick et al., 2014). This parallel divergence between paired fjord and coast populations suggests that more nuanced differences in habitat may underpin ecological divergence in *Sebastes*.

1.53 Hybridization and introgression dynamics in Sebastes

As in many marine fishes, hybridization is widespread in *Sebastes* (Buonaccorsi et al., 2005; Muto et al., 2013; Narum et al., 2004; Schwenke et al., 2018; Wray et al., 2024). Extensive hybridization in this genus is not surprising, given the ecological overlap and blurred reproductive barriers in their many species complexes. Where closely related species exist in sympatry, backcrossing of hybrids is commonplace and promotes many species' genetic diversity through introgression of outside alleles (Buonaccorsi et al., 2005; Wray et al., 2024). This is particularly true in inlets and seas, such as the Salish Sea inside of Vancouver Island, and even more so within Puget Sound, a complex array of narrow inlets that makes up the south end of the Salish Sea. Here, rockfish populations have complex patterns of ancestry that strongly differentiate them from coastal populations (Andrews et al., 2018). This region's unique bathymetry and ecological characteristics create conditions conducive to the development of hybrid zones. The presence of narrow, deep channels and shallow sills in Puget Sound contributes to smaller overall population sizes and in turn to shifts in hybridization dynamics (Schwenke et al., 2018). Here, population sub-structuring of Copper and Brown rockfish is driven by directional introgression from Quillback rockfish (Wray et al., 2024). This type of directional introgression is posited to result either from relatively small population sizes in the recipient species, or due to a lower prevalence of deleterious mutations in the donor species (Moran et al., 2021). As Quillback rockfish, but not Copper or Brown, are listed as threatened due to chronically declining population sizes, the reasoning behind this observed directional introgression is unclear.

Hybridization in rockfishes is not limited to inlets and seas, however. There is evidence of hybridization in *Sebastes* throughout the Pacific, including in the *S. inermis* (Deville et al.,

2023b) and *S. vulpes* (Muto et al., 2013) complexes of the Northwest Pacific. Although little is known about the mechanisms driving reproductive isolation in these species complexes, Muto et al. (2023) showed that genomic architecture plays a critical role in maintaining species boundaries through hybrid inviability. They further demonstrated species-specific variation in quantitative trait loci largely responsible for feeding morphology and colour patterns. While divergence in colour patterns could reasonably lead to reproductive isolation via assortative mating, whether these differences resulted in or from speciation is unclear.

1.54 Management and conservation of Sebastes

In some cases, one member of a species complex may be classified as endangered or otherwise threatened, while their sibling species are not. In the specific case of Pacific rockfishes, this presents something of a quandary for managers and conservationists. It is difficult to enact management regimes which encompass a group of species with largely overlapping habitat, but with significantly different patterns of distribution and abundance. Among these are Quillback and Yelloweye rockfishes, which compared to other inshore species, have exhibited precipitous population declines in past decades (COSEWIC, 2009, 2020). While catch and release for Yelloweye rockfish is mandatory for all fishers in Canada, the efficacy of descending devices to reverse barotrauma in landed *Sebastes* is debateable (Bellquist et al., 2019; Blain-Roth & Sutton, 2019; Pribyl et al., 2012; Rankin et al., 2017).

Specific spatial protections may help promote the recovery of depressed rockfish populations. The establishment of an international network of rockfish conservation areas (RCAs) along the Canadian and U.S. coastlines is considered an important protective measure (Haggarty et al., 2016; Lancaster et al., 2015; Lotterhos et al., 2014). These RCAs are numerous, their distribution meant to reflect the lowest common denominator among larval dispersal estimates in Northeast

Pacific rockfishes. However, the efficacy of these RCAs to promote population recovery remains unclear (Haggarty et al., 2016). Less than a decade has elapsed since the establishment of the first RCAs, so the lack of response in population numbers among rockfish may simply reflect an inadequate period for population recovery. It is also possible that, while RCAs may provide rockfishes with protected areas for reproduction and early life stages, that the unprotected areas act as strong enough population sinks so as to offset the capacity of RCAs to promote population growth.

Past population genetic analyses suggest that, for most Canadian rockfish species, there is significant differentiation between populations within the Salish Sea and those outside of it (Buonaccorsi et al., 2002, 2005; Siegle et al., 2013, Andrews et al., 2018). While this suggests that the current conservation units for inshore rockfishes are sufficient to reflect the population genetic structure of these species, it is possible that historical fishing pressure resulted in genetic homogeneity before a baseline of genetic diversity within the fishery could be established. In this scenario, the continued management of all fish populations outside of the Salish Sea as a single stock could overlook or exacerbate depletion of local populations, and their genetic diversity, under continued pressure from fishers.

1.60 Conclusion

Among the rockfishes of British Columbia, Copper and Quillback present an ideal natural study system. Past research has suggested that Quillback in the Salish Sea are genetically distinct from the coastal populations, in line with findings in other species (Buonaccorsi et al., 2005). Copper rockfish may exhibit a finer-scale population structure, given some differentiation between coastal and fjord-caught fish (Dick et al., 2014). Recently, modern genomic methods were used

to describe introgression within these species but focused on hybridization in only a small portion of their range (Wray et al., 2024). Thorough sampling and high-resolution whole genome sequencing of individuals across the entire species' range may uncover some previously undescribed barriers to gene flow, or genetically unique populations. In this species complex, backcrossing with hybrids will likely result in the introgression of genetic material, so there's additional opportunity to quantify the genomic consequences of hybridization in these species.

Chapter 2: Differential population structure in two species of Pacific rockfish.

2.1 Introduction

Marine fishes tend toward subtle population structure, even panmixia. This due in part to their large population sizes and high dispersal capacity, as well as the relative continuity of marine habitat, reducing barriers to gene flow (E. E. Nielsen et al., 2009; R. Waples, 1998). Some species may exhibit population differentiation reflecting their unique life history, fragmented habitat, or capacity for dispersal. Anadromous Pacific salmon, for example, have population structure reflecting their spawning site fidelity (Xuereb et al., 2022), run timing (Narum et al., 2023), or life cycle (Tarpey et al., 2018). In the absence of strong reproductive isolation based on geography, though, intraspecific differentiation is harder to detect. High rates of migration and corresponding high gene flow can lead to largely homogenized populations, both at subcontinental and oceanic scales (Díaz-Arce et al., 2024; Natasha et al., 2022; Tovar Verba et al., 2022). This presents a challenge for fisheries managers, who seek insight into patterns of dispersal to establish conservation units. Genetic analyses, such as those which leverage sequence divergence within allozymes or microsatellites, have historically been applied to answer these questions, but have met with varying success in delimiting discrete populations (Buonaccorsi et al., 2004; Gao et al., 2016; Gilbert-Horvath et al., 2006). When population structure is subtle, or limited to some genomic regions, small numbers of markers may be unable to identify it. Genomic techniques provide researchers with improved resolution to study population structure in marine fishes (Bernatchez et al., 2017; Longo et al., 2020) but have been sparsely applied to address outstanding questions in fisheries management, many of which rely on decades-old genetic analyses. Given the increasing effectiveness and affordability of genomic

resources, it is essential to close this knowledge gap, particularly for overfished and threatened species.

Eastern Pacific rockfishes, genus *Sebastes*, comprise many commercially and recreationally valuable species, inhabiting inshore, shelf, and slope habitats along the North American west coast (Love et al., 2002, Hyde & Vetter, 2007). Inshore rockfish include Quillback (*S. maliger*), Yelloweye (*S. ruberrimus*), Copper (*S. caurinus*), Tiger (*S. nigrocinctus*), China (*S. nebulosus*), Black (*S. melanops*), Brown (*S. auriculatus*), and Deacon (*S. diaconus*) rockfishes. Among these, Copper and Quillback rockfishes (abbreviated to Copper or Quillback) hybridize extensively, particularly within the Salish Sea (Seeb, 1998; Schwenke et al., 2018; Wray et al., 2024). In Canada and the United States, stocks are managed along political boundaries (between countries and states) and at regional scales whereby fish in the Salish Sea, for example, are considered a separate stock from those of the greater coast. Importantly, the latter delineation was originally made to reflect exploitation history and not any evolutionarily significant distinction between the fish occupying the waters inside and outside of Vancouver Island. There is evidence to suggest, however, that the management units reflect genomic differentiation between the outer coast and Salish Sea populations in some species. For example, Yelloweye rockfish in both the Canadian and U.S. waterways of the Salish Sea are genetically distinct from the outer coast (Siegle et al., 2013; Andrews et al., 2018).

Pacific rockfishes exhibit characteristics that can result in spatially discrete population structure. Small adult home ranges, courtship rituals, internal fertilization, parturition of live offspring, oceanographic effects on larval dispersal, and nearshore early life-history stages may all reasonably conspire to limit gene flow over large distances and promote genetic differentiation (Buonaccorsi et al., 2002, 2005; Hannah & Rankin, 2011; Helmstetter et al., 2016; Love et al.,

2002; Matthews, 1990; Tolimieri et al., 2009). Previous examinations of population structure in Copper and Quillback rockfishes have been technologically or regionally limited. Though overlapping through most of their range, Copper exhibit a more southerly distribution; Quillback are found in California but are much less abundant at this southern extent (Love et al., 2002). Genetic analyses suggest that both species exhibit significant differentiation only when Puget Sound is considered (Buonaccorsi et al., 2002, 2005; Seeb, 1998). Another study which examined differentiation at microsatellite loci in Copper rockfish found replicate divergence between pairs of inlet and coast samples on the west coast of Vancouver Island (Dick et al., 2014). Most recently, increased hybridization between Quillback, Copper, and Brown rockfishes in Puget Sound has been hypothesized to drive differentiation between coast and Salish Sea populations (Wray et al., 2024). Together, this could be taken as an indication that inlet fish harbour some unique genetic diversity, but more robust data are required to test this hypothesis.

This study seeks to leverage low-coverage whole genome resequencing (lcWGS) of Copper and Quillback rockfishes to describe their genetic diversity and population structure throughout their native range. Through this international sampling effort, I seek to: (1) describe genetic differentiation between the species, (2) assess population structure within each species, (3) identify regions of the genome with unique patterns of relatedness between species, and (4) look for signatures of introgression in the genomes of both species. Through the first range-wide characterization of genetic diversity in these species, I provide novel insight into their patterns of dispersal, differentiation, and introgression.

2.2 Methods

2.21 Sample collection:

Samples were collected by various means, including in annual longline surveys conducted by Fisheries and Oceans Canada (DFO) and the US National Oceanic and Atmospheric Administration (NOAA) and in individual rod-and-reel catch efforts conducted by authors and collaborators (Anderson et al., 2019). In total, 113 Copper (*S. caurinus*) and 200 Quillback (*S. maliger*) rockfish were collected between June 1994 and August 2022. Samples were sorted into eight regional bins: Alaska (AK), Hecate Straight (HS), Queen Charlotte Sound (QCS), Western Vancouver Island (WVI), Salish Sea (SS), Puget Sound (PS), Washington and Oregon (WA/OR), and California (CA; Fig. 1B). Additionally, as outgroups I included 5 Gopher (*S. carnatus*) and 5 Black-and-yellow (*S. chrysomelas*) collected from the same sources which, while closely related, only range from southern Oregon to Baja California (Hyde and Vetter, 2007).

2.22 Library prep and low coverage sequencing:

All whole genome resequencing library preparation was performed using methods similar to Baym et al. (2015), Therkildsen & Palumbi (2017), and Euclide et al., (2023). Genomic DNA input was normalized to 10 ng per individual. Per Euclide et al. (2023), sample purification, product normalization, and pooling were conducted with SequalPrep plates (ThermoFisher Scientific: Waltham, MA, USA) rather than AMPure XP beads (Beckman Coulter: Brea, CA, USA). Prior to sequencing, the final pooled library was visualized on a 2% agarose E-gel (ThermoFisher Scientific) and quantified with the Qubit HS dsDNA Assay Kit (ThermoFisher Scientific).

Sequencing of an initial batch of 113 Copper and 152 Quillback rockfish, as well as the Gopher and Black-and-yellow samples, occurred at Novogene (Sacramento, CA, USA) on Illumina NovaSeq S4. An additional 48 Quillback samples were sequenced by the University of Oregon's Genomic & Cell Characterization Core Facility (GC3F; Eugene, OR, USA).

2.23 Alignment, variant calling and quality control:

Alignment and initial quality control were done using the Snakemake pipeline *grenepipe* v0.10.0 (Czech & Exposito-Alonso, 2021). All tools were run using default settings unless specified otherwise. First, adaptor sequences were trimmed using *fastp* v0.20.0 (Chen, 2023). Reads were then aligned to a Korean rockfish (*S. schlegelii*) genome acquired from the Chinese National GeneBank Database (CNA0000824) using *bwa-mem* v2.2.1 (Vasimuddin et al., 2019). Overlapping read pairs were clipped using *BamUtil clipOverlap* v1.0.15 (Jun et al., 2015), and duplicated reads were removed using *picard MarkDuplicates* v2.23.0 (Broad Institute, 2019) with the parameters *-Xmx80g REMOVE_DUPLICATES=true VALIDATION_STRINGENCY=SILENT. FastQC* v0.11.9 (S. Andrews, 2010), *Qualimap* v2.2.2a (Okonechnikov et al., 2016), *picard CollectMultipleMetrics* v2.23.0 (Broad Institute, 2019), and *MultiQC* v1.10.1 (Ewels et al., 2016) were used to assess the quality of the data throughout the pipeline.

Variants were called using *freebayes* v1.3.6 (Garrison & Marth, 2012), for which I skipped sites with overall read depth in excess of 20x the total number of samples since average depth was 6.04 and set both minimum mapping and base quality to 20 (*-g 6260 -m 20 -q 20).* Monomorphic sites were retained in the unfiltered dataset for estimates of genetic diversity. I then used *vcftools* v0.1.16 (Danecek et al., 2011) to extract individual and site-based statistics for the remaining samples and excluded some poor-quality samples (>25% missing data). In total, 18 Copper and 6

Quillback were excluded from analysis due to missing data or suspected sample contamination. The final total number of samples retained were 95 Copper and 194 Quillback (Fig. 1B; Table S1), as well as five samples each of Gopher and Black-and-yellow.

I then filtered the data for downstream analyses using both *bcftools* v1.20 (Danecek et al., 2021) and *vcftools*; I retained only biallelic SNPs with a minor allele frequency greater than 5%, with maximum 25% missing data, minimum read depth of 5, maximum read depth of 50, and QUAL ≥ 20. I chose a minor allele frequency of threshold of 5% after testing with lower thresholds to ensure that no rare alleles were lost in the Puget Sound population. This filtering regime was applied to the whole dataset for interspecific analyses, as well as to each species separately for intraspecific analyses. After filtering, 1,047,615 SNPs were retained in the interspecific dataset, while 350,889 SNPs were retained in Copper and 1,447,179 SNPs in Quillback. The above computations and most downstream analyses were parallelized using *GNU parallel* (Tange, 2023).

2.24 Population structure, differentiation, and genetic diversity:

For both inter- and intraspecific population structure analyses, I performed principal component analyses (PCAs) in *R* v4.2.2 (R Core Team, n.d.) with the *SNPRelate* package v3.13 (Zheng et al., 2012) and assessed ancestry proportions of our samples with *admixture* v1.3.0 (Alexander et al., 2009). Sites were pruned for the ancestry analysis using *PLINK* v1.90 (Purcell et al., 2007) in 10 kbp windows, with a maximum r-squared of 0.1 between retained sites in order to reduce redundancy and avoid overrepresentation of linked regions.

To generate a phylogeny of all samples in the data set, I thinned and converted the full filtered dataset using a custom *perl* v5.32.1 (Wall et al., 2020) script, which retained 5% of sites. I then

used iqtree2 v2.3.6 (Minh et al., 2020) with 1000 *Ultrafast* bootstraps (Minh et al., 2013) to generate a consensus tree (-m "GTR+ASC" -st DNA -B 1000).

Next, I sought to characterize the genomic differentiation both between and within my study species. To achieve this, I used a custom *perl* script to calculate F_{ST} for each position in the genome and summarized the results on a per-chromosome basis in *R* (Samuk et al., 2017). This analysis was applied to both species together and separately. Intraspecific F_{ST} was first calculated between geographic population bins. Then, for more granular comparisons, sampling coordinates were rounded to the nearest degree to make bins which included at least two samples. Eight Quillback and five Copper whose coordinates remained unique after rounding were excluded from pairwise F_{ST} calculations, to ensure that any variation within a single individual did not make an outsized contribution to my result. I observed that Quillback had significantly lower population differentiation than Copper, so used *pixy* v1.2.7 (Korunes & Samuk, 2021) to estimate standing genetic diversity (pi) in both species.

Given that differentiation was highest in comparisons made between fish in the Salish Sea (including Puget Sound) and outside of it, another pairwise F_{ST} calculation was conducted between all Salish Sea samples and all others. I also subset samples inside and outside of the Salish Sea to run both principal component and admixture analyses and look for structure independent of the major axis of variation.

2.25 Tests for genomic correlates with latitude

As the samples span the bulk of the North American coast, I next sought to test for any correlation between genotype and latitude, to assess if there is evidence of genetic adaptation along a latitudinal gradient. I first subset my samples to remove those from within the Salish Sea

(including Puget Sound), as these samples were most differentiated from those on the coast and were likely to confound any correlation with latitude. I generated a RAW format genotype table with *PLINK* v1.90 (Purcell et al., 2007) from each species' filtered dataset (--*recode A*) and used a custom *python* v3.10.10 (Python Software Foundation, 2023) script to calculate Kendall's tau for ranked correlation between each locus and sample latitude. To combine individual p-values across meaningful genomic windows and reduce the likelihood of false positives, I used the weighted-Z analysis (Booker et al., 2024), where windows reflected annotated genes in the reference genome. I retained the top 1% of genes (ordered by p-value) most significantly correlated with latitude in each species, first checking for gene overlap between the two species. Next, I tested for enrichment of gene ontology (GO) terms in the same significant subset of genes in each species using the *topGO* R package v.2.5.0 (Alexa et al., 2006).

2.26 Characterization of haploblocks:

Genome-wide population differentiation tests did reveal several distinct regions with high F_{ST} in Quillback rockfish and one in Copper. Extended regions of high differentiation can be due to large structural variants causing high linkage. To test this, I sought to determine whether any of the peaks coincided with patterns of linkage disequilibrium (LD) in either species. To estimate and visualize LD within each chromosome, I again used *PLINK* v1.90 (Purcell et al., 2007). First, I thinned each species' dataset (57% of sites retained in Copper, 14% of sites retained in Quillback; --*thin*) to generate a less cumbersome list of SNPs before calculating r-squared within each chromosome. Next, to summarize the r-squared data into files of manageable size, I used a custom *python* v3.10.10 (Python Software Foundation, 2023) script to group SNPs into 500 kbp windows and extract the two highest values for each window pair, retaining the second highest.

This revealed a number of regions with high LD in both species, concentrated at chromosome ends.

Regions of high LD are often due to low recombination, but may not correspond to distinct haplotypes, like those that occur from inversions (Ishigohoka et al., 2023). To test whether the LD blocks were caused by distinct non-recombining haplotypes, I used the *lostruct* (H. Li & Ralph, 2019) *R* package. This program conducts PCAs in windows along the genome and identifies windows with significantly different population structure patterns. To improve *lostruct* performance, a new filtering regime was applied to the interspecific dataset (again using *vcftools*) to yield chromosomal variant files without missing data, as missing data can confound the individual PCAs. I ran the program on a per-chromosome basis, in windows of 100 SNPs.

Where blocks of LD coincide with abnormal interspecific population structure (i.e. *lostruct* outliers), I sometimes observed a three-column PCA pattern consistent with three genotypes (0/0, 0/1, and 1/1). To define the extent of these haploblocks, I manually investigated the *lostruct* results to identify adjacent windows within regions of high LD which had similar patterns and extracted the first and last SNP positions associated with those PCAs. Using these bounds, I subset the genomic data for each region using *bcftools* and performed new region-wide PCAs with *SNPRelate.* To visualize the distribution of genotypes within species, the top 100 SNP positions contributing to PC1 were extracted using *SNPRelate*, their relative position and genotype were plotted for each individual. To ensure that any candidate LD blocks were not centromeres, I used the R package *repeatOBserver* (Elphinstone et al., 2023) to locate the centre positions of each centromere.

2.27 Haplotype evolutionary history and geographic distribution:

Generally, I observed haplotype variation within species that was not shared between species, but in seven cases, at least one individual from either species was observed to be homozygous for the other species' common haplotype. For these regions, ten individuals from each species were selected for phylogenetic analysis, to explore the evolutionary history of each species' haplotype. I specifically chose samples that were homozygous for each haplotype in each species, when available. Ten additional samples, five each of gopher and black-and-yellow rockfishes, were added to the phylogenetic analysis. New variant calling and SNP filtering was performed for each region and sample set as above. Then, using custom *perl* scripts, I appended a sample with the Korean rockfish reference sequence to each variant file to act as outgroup, and converted the SNP table to FASTA for phylogenetic analysis. Consensus trees for each region were generated using *iqtree2* as above. Then, to determine whether introgression of the haplotypes was biased toward samples in the Salish Sea, I summarized allele counts within the haploblocks for each species in *R.*

Lastly, to better understand whether the admixture I detected was in any way driven by introgression of these large haploblocks, I tested for correlation between individual's opposite-type allele count and their proportion of mixed ancestry.

2.28 Tests for introgression:

As several haplotypes were observed to be segregating between the study species, I conducted formal tests of introgression using *Dsuite* (Malinsky et al., 2021). To accomplish this, I assigned individuals to one of four groups based on the interspecific *admixture* analysis; pure types from each species represented two of the groups, while any degree of mixed ancestry would result in

assignment to an admixed group for each species. As above, I appended a sample with the reference sequence to be used as the outgroup. Both overall and site-specific *D* were calculated for each unique combination.

2.3 Results

2.31 Interspecific analyses

Sequencing yielded an average read depth per sample of 5.25 in Copper, 6.83 in Quillback, 7.21 in Gopher and 11.38 in Black-and-yellow (Fig. S1). In the interspecific PCA, the first principal component (PC1) separates samples along species lines and explains 14.07% of the total variance (percent variance explained; PVE). PC2 (0.8% PVE), separates Copper into their Salish Sea and coastal populations, while PC3 (0.59% PVE) reveals a more continuous distribution among Quillback (Fig. 1D). An unrooted consensus tree of the full sample set reveals a pattern consistent with the PCA, whereby Quillback nodes radiate more from a central point than do the more differentiated Copper (Fig. 1C). Interspecific admixture results indicate that there are 110 Quillback with some proportion of Copper ancestry (none exceeding 7.2%), and that there are 45 admixed Copper with up to 15.7% Quillback ancestry (Fig. 1E). In both species, samples from within the Salish Sea had a higher mean minor parent ancestry; from 0.6% to 6.4% in Copper and from 0.5% to 1.4% in Quillback (Fig. S2). Overall interspecific F_{ST} was calculated to be 0.333.

2.32 Intraspecific population structure and differentiation

Intraspecific principal component analyses reveal that Copper have more defined population structure than Quillback (Fig. 2A). The first principal component in Copper (3.83% PVE) divides fish in the Salish Sea from the coastal populations. A single individual from the

Washington and Oregon region genetically grouped with the Salish Sea and Puget Sound samples. The coastal Copper samples exhibit a more continuous distribution along PC2, with the most distal samples from California and Hecate Strait group more closely together than with most Washington and Oregon samples. When excluding Salish Sea samples from these analyses, however, I found subtle population structure reflecting divergence between California, Washington/Oregon, and Hecate Strait (Fig. S3). While K = 2 was the best supported admixture result (in contrast to K = 3 or higher; Fig. S4), both the PCA and ancestry proportions do strongly reflect some degree of population differentiation along the coast.

Quillback principal components are less informative, as most samples tend toward a single dense cluster (Fig. 2A). The first PC largely separates out a subset of Salish Sea, Puget Sound and West Vancouver Island samples, with outliers concentrating in the narrow straits at the North end of the Salish Sea (Fig. S5).

I observe correlation between the first principal component in each species and their interspecific admixture proportion (Fig. 2B). This relationship was significant in both species; with an r-squared of 0.4825 in Copper ($p = 5.871e-15$) and of 0.5787 in Quillback ($p < 2.2e-16$).

Within Copper, F_{ST} between the population bins was highest in inside (e.g. Salish Sea and Puget Sound) vs outside (all others) comparisons (Fig. 2C). Values were highest between Puget Sound and Hecate Strait ($F_{ST} = 0.078$), and lowest between California and Washington/Oregon ($F_{ST} = 0.0095$). The pattern in Quillback was similar, but population differentiation was much lower – the highest F_{ST} value was between Queen Charlotte Sound and Puget Sound ($F_{ST} = 0.011$), while the lowest, highlighting the panmixia of coastal Quillback, was between California and Alaska, ($F_{ST} = 0.0003$) (Fig. 2C).

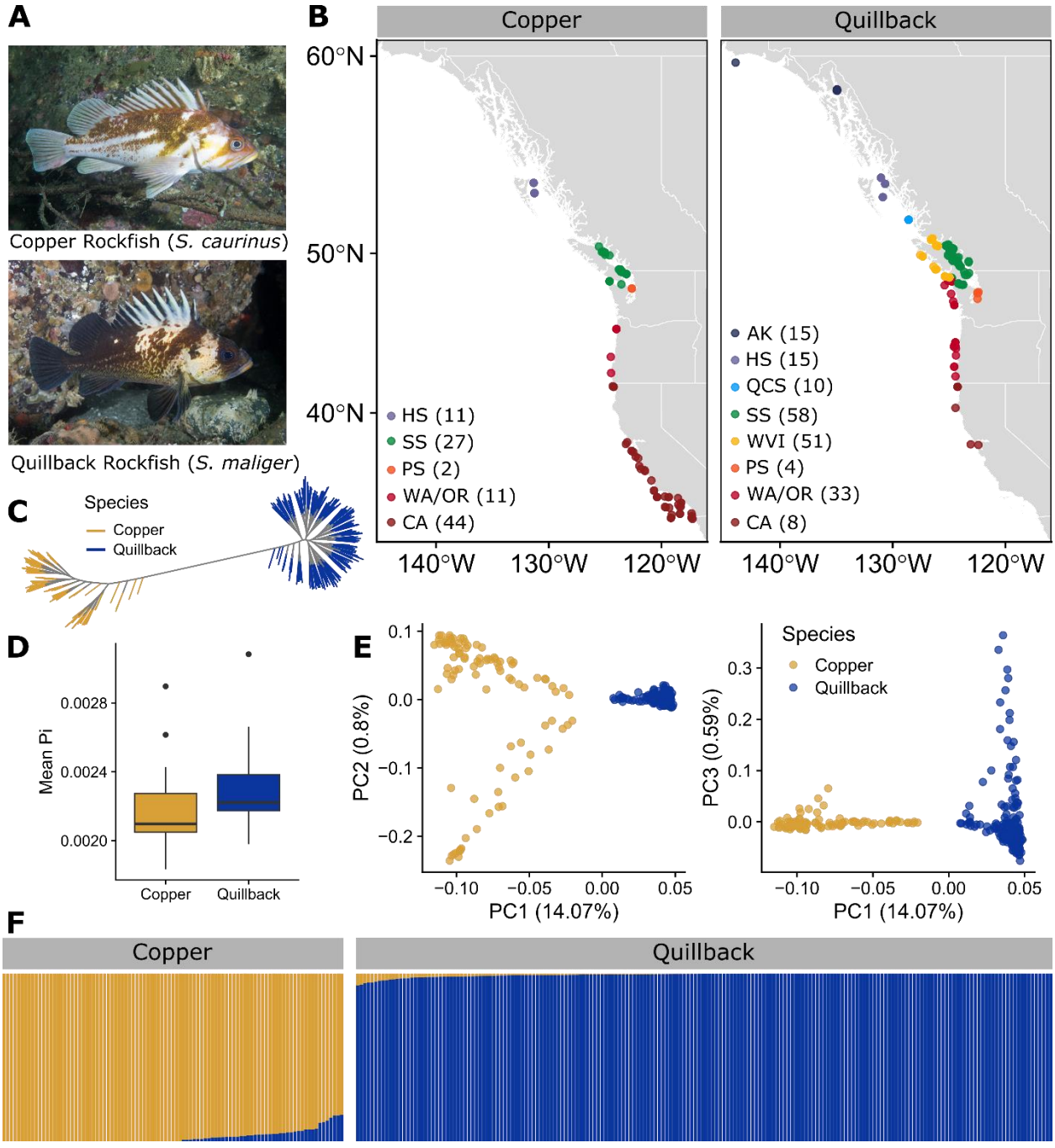

Figure 1: Sampling and interspecific differentiation. A) Underwater photographs of each species by Andy Murch at Big Fish Expeditions. **B)** Sampling distribution and geographic bins. The number in parentheses represents the samples retained after filtering from each population: Alaska (AK), Hecate Strait (HS), Queen Charlotte Sound (QCS), Salish Sea (SS), Western Vancouver Island (WVI), Puget Sound (PS), Washington and Oregon (WA/OR) and California (CA).**C)** Unrooted consensus tree of all individuals retained in the data set, coloured by species. **D)** Mean nucleotide diversity (pi) for all chromosomes in both species. **E)** Principal component analysis of all samples, coloured by species. **F)** Combined a*dmixture* analysis of all samples for K = 2.

In my analysis of pairwise differentiation between rounded coordinates, a similar trend was observed (Fig. 2D). In Copper, mean pairwise F_{ST} between coastal sampling locations was 0.008, between locations in the Salish Sea was 0.033, and in pairwise comparisons between the Salish Sea and coast was 0.058. In Quillback, these values were much lower: mean F_{ST} of comparisons on the coast was 0.003, within the Salish Sea was 0.002, and between locations on the coast and within the Salish Sea 0.004.

Admixture analyses were comparable between species (Fig. 2E); the highest confidence estimate of population structure (lowest cross-validation error; Fig. S4) was in K = 1. In Copper, cross-validation error for K = 2 was similarly low and reflective of the population structure observed in the PCA. At K = 2, Copper within Puget Sound and the Salish Sea as well as a single northern Washington coast sample appear to make up one group, while other coastal populations maintain some small proportion of that ancestry. Quillback are similar but the pattern is weaker, which is consistent with the low overall levels of differentiation observed in the species.

Across the genome, pairwise F_{ST} of geographic Copper populations was largely heterogenous, but I did observe concentrated differentiation in the form a single clear outlier peak on chromosome 20 in three comparisons (Fig. S6). The three pairwise F_{ST} calculations which possessed this peak were between the coastal populations, California, Washington/Oregon, and Hecate Strait. This recurring F_{ST} peak did not overlap with a region of high LD but may warrant further investigation in future studies. In all pairwise tests which included Salish Sea Copper, differentiation was highly heterogenous (Fig. 2F). Quillback, despite exhibiting very low levels of overall divergence in all pairwise tests, had highly differentiated peaks in some comparisons (Figs. 2F, S7, S8). While the same peaks were not shared in all comparisons (Figs. S7, S8), most correlated with regions of high LD (Fig. S9).

2.33 Correlation with latitude

Three genes met the significance threshold in both species, but none were annotated with gene information (Fig. S10, S11). Among the 40 most enriched GO terms in each species, none were shared between Copper and Quillback. However, many of the most enriched GO terms were involved either in protein binding or enzyme activity (Fig. S12).

2.34 Investigation of haploblocks

My linkage graphs revealed that 19 of the 24 chromosomes in the genome assembly contained large regions (> 1 Mbp, though often > 3 Mbp) with unusually high linkage between markers (Fig. S9). In most cases, this was limited to one region per chromosome and was located at or very near the end of chromosome. Seven of these linked regions satisfied two criteria for further investigation: (1) they coincided with regions of the genome identified by *lostruct* analysis to contain a three-column PCA pattern consistent with three genotypes, (2) at least one individual from either species was observed to be homozygous for the other species' common haplotype and (3) the regions did not overlap with centromere position (Fig. S13, S14).

For most of the genome Quillback and Copper rockfish fall together phylogenetically, but of these regions of high linkage, two exhibit an unusual phylogenetic pattern whereby one of haplotypes share a more recent common ancestor with more distantly related Black-and-yellow and Gopher rockfishes (Fig. 3D). On chromosome 12, both species exhibit high linkage within the region spanning BP 713,412-3,099,105 (Fig. 3A). Here, a single Copper individual from Oregon is homozygous for the Quillback haplotype (Fig. 3B).

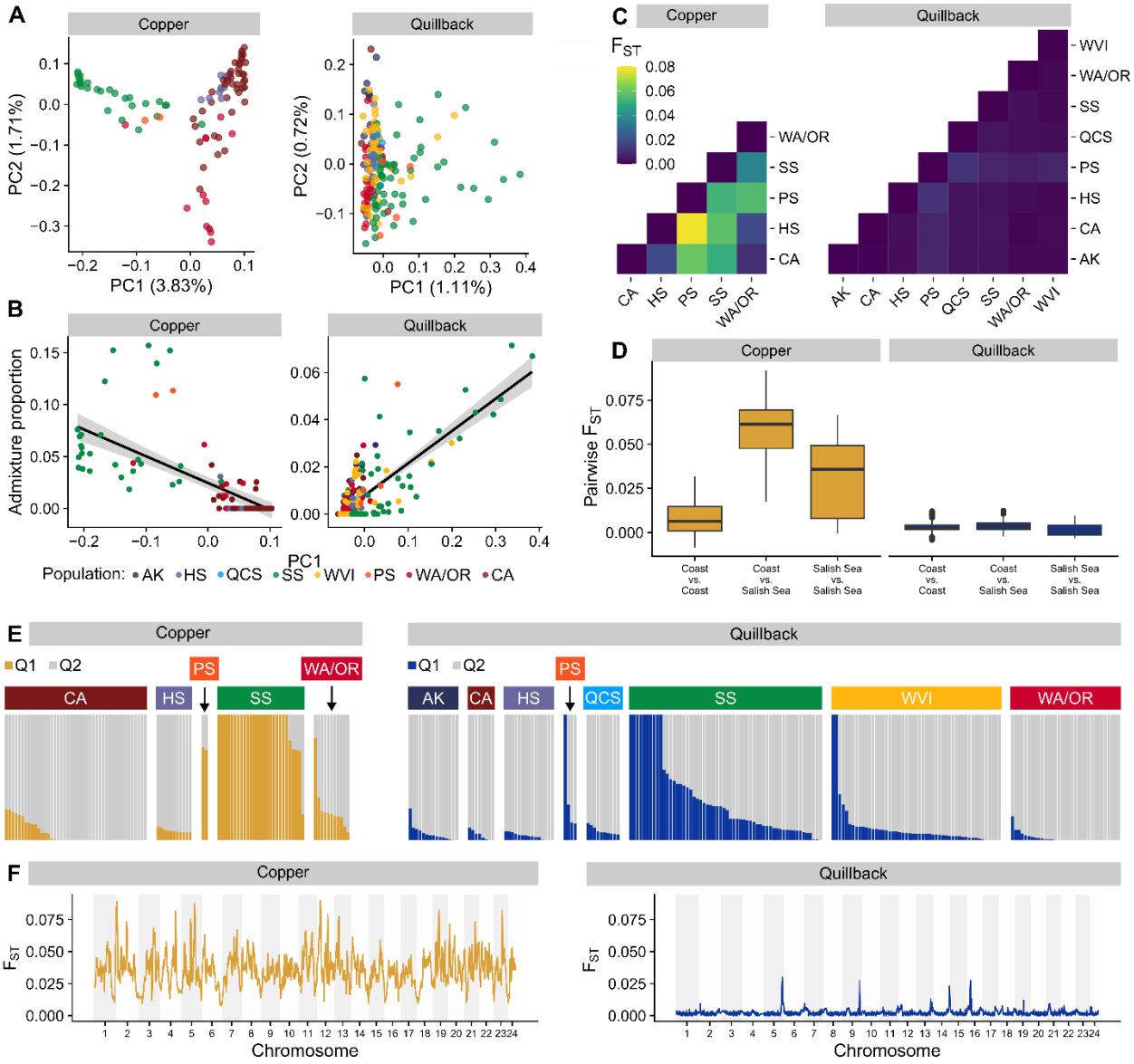

Figure 2: Intraspecific population structure and differentiation. A) Principal component analyses for both species, individual points coloured by geographic population, see legend in B. **B)** Proportion of mixed ancestry as a function of the first intraspecific principal component overlaying a linear model of the relationship, individuals coloured as in A. **C)** Heatmap of differentiation between geographic populations in both species. **D)** Boxplot showing differentiation between samples as binned by rounding of geographic coordinates. Location pairs were categorized as comparing two coastal locations, two locations within the Salish Sea, or between the coast and the Salish Sea. **E)** Intraspecific ancestry proportions as K = 2. **F)** Line plot visualizing the rolling mean of F_{ST} between all samples within the Salish Sea, and all samples from outside of it.

One cluster of homozygous Copper-type individuals contains the bulk of the Copper samples, while two smaller clusters of Copper represent individuals heterozygous with one of two relatively distinct Quillback haplotypes. Isolation of the 100 SNPs most contributing to PC1 score indicates that there are nearly fixed variants between the two haplotypes (Fig. 3C). Phylogenetically, the single homozygous Quillback-type Copper individual is nested within the Quillback clade (Fig. 3D). Although genome-wide Copper and Quillback are more closely related, for this region, Copper is genetically closer to Gopher and Black-and-yellow than Quillback.

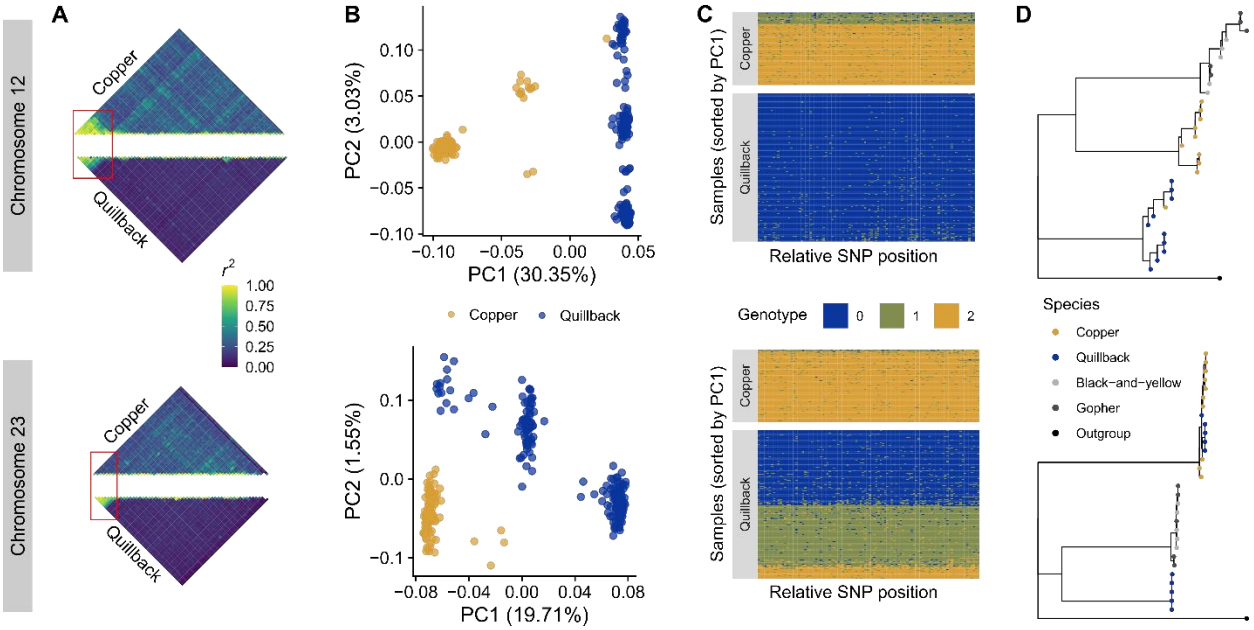

Figure 3: Characterization and distribution of two linked regions. A) Linkage disequilibrium in each chromosome; red rectangles highlight triangles of high r-squared indicating linked regions. **B)** Principal component analyses conducted within the linked regions. Points coloured by species. **C)** Genotype plots representing the 100 SNPs most associated with PC1 within each region. Rows represent individual samples. **D)** Consensus phylogenetic trees for each region. These were generated by selecting homozygous individuals from each species and five samples each of gopher and black-and-yellow rockfish, with the Korean rockfish reference as outgroup.

The chromosome 23 haploblock exhibits high linkage only within Quillback (Fig. 3A). This region spans BP 186-2,212,001. Here, Quillback cluster into three groups: homozygous for the Quillback haplotype, homozygous Copper-type, and heterozygous (Fig. 3B). Only four Copper individuals are heterozygous. The second principal component (1.55% PVE) accounts for some interspecific variation; individuals from each species do not cluster cleanly together. Phylogenetic analysis places the Copper-type Quillback within the Copper clade (Fig. 3D). Within this region, the more common Quillback haplotype is more closely related to Gopher and Black-and-yellow rockfishes than with the Copper haplotype.

2.35 Tests for introgression

Genome-wide tests for introgression indicate asymmetrical gene flow. Comparing admixed Copper to both admixed and pure Quillback yielded significantly positive D statistics (D = 0.0554; p < 2.2e-16), indicating introgression from Quillback into Copper. Assessing admixed Quillback compared to pure and admixed Copper yielded negative D statistics (D = -0.0131; p < 2.23e-16 and D = -0.0144; p < 2.2e-16, respectively), which suggests that gene flow is not occurring from Copper into Quillback. Additionally, I found that interspecific admixture proportion was correlated with haplotype count in Copper (Fig. S15, p = 0.0002) but not in Quillback (p = 0.138).

2.4 Discussion

In this study, I observe significant differences in the distribution of genetic variation between Copper and Quillback rockfishes, both within the genome and between sampling locations, despite similar levels of genetic diversity. The subtle overall population structure in both species is in line with genetic and genomic analyses in other Pacific rockfish species (An et al., 2012;

Buonaccorsi et al., 2004; Gao et al., 2016; Zhu et al., 2024). Higher differentiation between coast and inlet sampling locations is consistent with previous genetic studies in these species (Buonaccorsi et al., 2002, 2005; Dick et al., 2014), but the significant interspecific differences in range-wide population structure are here described for the first time. Additionally, I identify multiple large haplotypes independently segregating within the species complex, which contribute to signatures of directional introgression from Quillback into Copper, in support of recent findings (Wray et al., 2024). These results suggest that subtle differences in life history, while acting to reinforce reproductive isolation between the species, have resulted in differential patterns of gene flow within the species complex.

2.41 Species fidelity and directional introgression

Hybridization is ongoing between the species, but introgression is directional; genetic material primarily flows from Quillback into Copper. The low level of mixed ancestry in the sample set is consistent with long-term, low levels of hybridization purported by Schwenke et al. (2018) but as our sampling effort sought to exclude phenotypic hybrids, my analyses likely underestimate real admixture. Nonetheless, I observe an association between interspecific admixture proportion and intraspecific PC1 in both species, which suggests that the main axis of intraspecific variation is driven by introgression. However, more formal tests of introgression reveal that while admixed Copper are indeed admixed with Quillback, that the seemingly admixed Quillback do not share more derived alleles with Copper than pure Quillback do (Table S1). Therefore, the seemingly admixed Quillback house variation of a different origin.

Given the presence of a third species within this complex, it is plausible that this variation in Quillback is driven by introgression from Brown rockfish. Previous work found low but significant levels of introgression from Brown rockfish into Quillback, concentrated in Puget

Sound (Schwenke et al., 2018; Seeb, 1998; Wray et al., 2024). As the admixed Quillback are geographically concentrated within the narrow straits at the north end of the Salish Sea, the variation I observe may be due to a similar effect posited by Schwenke et al. (2018); that the environmental conditions, close quarters, low population sizes and potentially limited mate choice conferred by inlet habitat are conducive to hybridization. It is also possible that the genomic cline observed in Quillback is due to habitat-related divergence within the species, like that which is suspected to occur in Copper rockfish (Dick et al., 2014).

While, genome-wide, the average D statistics indicate gene flow from Quillback into Copper, there may still be gene flow in the other direction, albeit limited to a much lower overall fraction of the genome. In several of the haploblock regions, Quillback individuals possess Copper haplotypes, either due to introgression or simply localized incomplete lineage sorting. In Copper, the genome-wide signal of introgression correlates with the number of Quillback-type haplotypes, while in Quillback there is no such relationship. This, again, supports the interpretation that signals of admixture in Quillback do not reflect actual Copper introgression, but that individual Quillback do contain some localized signals consistent with introgression.

Overall, my findings are consistent with previous work within this species complex that describe hybridization and directional introgression within the species complex. Allele sharing appears to be driven largely by hybridization of opportunity or necessity, primarily in more complex estuarine habitats. A similar trend was observed in Japanese *S. zonatus* and *S. vulpes*, wherein hybrids are more closely related to *S. zonatus*, and the extent of their hybridization varies considerably based on locality (Muto et al., 2013). Reproductive isolation within the genus, driven by fine-scale habitat partitioning, thus appears to break down as habitat segregation and conspecific mate availability declines.

2.42 Intraspecific analyses:

Copper are significantly more differentiated throughout their range than Quillback, despite similar levels of genetic diversity. My results find support in previous work suggesting that population structure of Copper rockfish is driven by hybridization with Quillback (Schwenke et al., 2018; Wray et al., 2024). With the unique confluence of increased hybridization potential and the limitations to dispersal imposed by Salish Sea (Buonaccorsi et al., 2005), Copper rockfish exhibit a unique population structure driven primarily by directional introgression from Quillback and reinforced by the effects of genetic drift. This is not unprecedented in marine species, as local introgression has been shown to be the main cause of intraspecific differentiation in mussels (Fraïsse et al., 2016), but this finding does imply that hybrid zones may play an important role in the maintenance of genetic variation in fishes. However, this effect is asymmetric; introgression conferred by this hybridization does not result in increased population structure among Quillback. While these species do overlap significantly in their life history, niche partitioning by depth has led to reproductive isolation and speciation (Ingram, 2010). Subtle life history differences, likely associated with depth preferences and corresponding changes to habitat and resource availability, may also contribute to the observed differences in population structure.

Previous work using microsatellites found similar divergence between Salish Sea and coastal copper, but also found that Copper within Puget Sound are genetically distinct (Buonaccorsi et al., 2002). Puget Sound was not sufficiently sampled as to make a similar determination, but the agreement between earlier microsatellite analysis and the lcWGS used here is encouraging. However, given the low genetic diversity within my study species and the highly polygenic

nature of complex environmental adaptations, methods which better leverage lcWGS to target non-neutral variation could yield more informative results (Nielsen et al., 2009).

The very low levels of genetic differentiation between Quillback populations more resemble the norm among rockfishes, which tend toward subtle, if any, population structure. Given the episodic recruitment pulses characteristic of the genus (Love et al., 2002; Licandeo et al., 2020) and small home ranges of adults, such genetic homogeneity over large distances is surprising (Love et al., 2002; Tolimieri et al., 2009; Zabel et al., 2011). Reports suggest that larval dispersal may range from less than 10 km up to 120 km per generation (Buonaccorsi et al., 2005; Miller & Shanks, 2004), with generation times up to 24 years (Love et al., 2002; DFO, 2024). Together, these factors suggest that it would take several generations and hundreds of years to disperse along the entirety of the North American coast and it would be reasonable to expect strong differentiation between geographically distal sampling locations. Instead, most work suggests subtle isolation by distance effects (IBD), rather than discrete populations, are the norm within *Sebastes*.

I also did not detect population structure in Quillback within the Salish Sea or while excluding it. The coastal populations of Copper, however, do exhibit some subtle genetic clustering consistent with three groups (Fig. S4). My results suggest one break in gene flow near northern California, and another between Washington State and Hecate Strait. This is relatively consistent with the population structure of coastal lingcod (Longo et al., 2020), in which differentiation between coastal populations is driven by a few highly divergent loci. The outlier F_{ST} peak on chromosome 20 in these pairwise comparisons may similarly house important genetic variation and warrants further investigation alongside ecological data. More evidence for a genetic break in northern California can be found within *Sebastes*; early work first described two divergent populations of

Blue rockfish (Cope, 2004), from which the more northern Deacon rockfish was eventually described (Frable et al., 2015), while Yellowtail rockfish are significantly differentiated across a similar latitudinal threshold (Hess et al., 2023).

Like other inshore rockfishes, Copper and Quillback are currently managed as two separate populations in British Columbia, those inside Vancouver Island and those outside of it (DFO, 2024). My findings suggest that the current management regime is likely representative of the biological reality in Copper, but that Quillback may be more panmictic than the current regime suggests. However, the presence of some highly differentiated regions in Quillback populations suggest subtle population structure could be limited to smaller genomic regions which require further investigation.

2.43 Heterogeneity across the genome in pairwise FST calculations

The outlier F_{ST} peaks I observe are consistent with localized divergent selection, with the surrounding areas subject to the homogenizing effects of recombination and gene flow. These genomic islands of divergence may be formed by a variety of mechanisms; they may result from strong selection at loci near the centre of the peak or as a result of structural variation and/or linkage disequilibrium (Wolf & Ellegren, 2017). Outlier peaks are more likely to be observed within species exhibiting ecotypic differentiation, or in recently divergent taxa, and are theorized to play an important role in local adaptation, particularly in the presence of high gene flow (Larson et al., 2017; Quilodrán et al., 2020) but may also arise by chance in regions of low recombination (Booker et al., 2020). In other rockfish species pairs, shared islands of divergence were found in multiple pairwise comparisons of depth-separated incipient species (Behrens et al., 2021). This is consistent with divergence based on ecotype, rather than geography or chance, in speciating rockfishes. There is further support for genomic islands of divergence as underlying

ecotypes in sunflowers and other fishes, and while islands of divergence are not always associated with structural variation, they are inherently subject to reduced recombination rates (Euclide et al., 2023; Kess et al., 2021; Renaut et al., 2013; Todesco et al., 2020). This is what I observe in Copper and Quillback; while several of the peaks in the Quillback analyses coincide with regions of high LD, some do not. Notably, the peak on chromosome 20 in coastal Copper population pairs is not associated with high LD (Fig. S6, S11). This suggests that divergence is heavily influenced by the functional architecture of genomes and may be a more important driver of speciation than geographic divergence, particularly amidst ongoing gene flow.

Typical population structure analyses presume genetic differentiation based on geography, but Pacific rockfishes appear to have diversified based on partitioning of depth and depth-related differences in habitat and resource availability. Evidence for parallel radiations in body morphology and in shared islands of divergence, associated with differences in depth, suggest speciation in parapatry along environmental gradients in *Sebastes* (Behrens et al., 2021; Ingram, 2010, 2015). In Copper rockfish, for example, divergence between pairwise coast and inlet sampling locations was higher than among them (Dick et al., 2014). Those samples were taken from a narrow portion of their range, along the west coast of Vancouver Island, but were differentiated based on habitat, rather than geography. This analysis was limited to a handful of microsatellite loci, but conscientious and intentional sampling resulted in clear differentiation between the ecotypes. This may indicate that sampling regimes which prioritize large sample numbers and geographic sprawl may miss out on the more important drivers of population structure within *Sebastes* and other fishes.

2.44 Haploblocks

Population structure is typically defined as a genome-wide organization of genetic variation within species, but relatedness within and even between species is not consistent throughout the genome. I present evidence for localized variation in typical species divergence as a result of large, linked haploblocks shared between species. Regions of high LD can result from chromosomal inversions, which suppress recombination between the original and inverted haplotypes. Inversions have been shown to drive ecotypic differentiation in cod and can lead to the formation of supergenes which can result highly divergent phenotypes when introgressed from related species (Berg et al., 2016, 2017; Jay et al., 2018). In species with high gene flow, chromosomal inversions can be the primary driver of differentiation in the genome (Kess et al., 2021, Longo et al., 2020). This is of particular relevance to incipient species existing in parapatry; reproductive isolation may be promoted when alterations to genome architecture produce locally divergent haplotypes. Linkage disequilibrium can also result in strong selection on adaptive loci, whereby selective sweeps may fix not only the loci under selection, but nearby neutral variation (Kim & Nielsen, 2004; McVean, 2007). While selective sweeps and chromosomal inversions are not necessarily mutually exclusive, it is important to note that reduced recombination can arise by different means. Indeed, Ishigohoka et al. (2024) caution that reduced recombination itself can lead to divergent haplotypes, independent of selection or structural variation.

However linkage disequilibrium should arise, the reduced recombination can lead to the accumulation of differentiation between divergent haplotypes. In Copper and Quillback, genotyping of the top 100 SNPs associated with PC1 reveals the extent of genetic differentiation between haplotypes in each linked region (Figs. 3, S14). More divergent haplotypes have accumulated more divergent loci; contrast, for example, the haplotypes of chromosome 5 and

chromosome 23 (Figs 3, S14). More fixed differences between the haplotypes suggests either that the regions are evolving quickly, or more realistically, more time has elapsed since the origin of the second haplotype.

The introgression landscape in Copper and Quillback is heterogeneous in the genome, owing in part to differential genome architecture and linkage patterns. The linked regions I observe in both species are not always shared between the species. For example, the haploblock on chromosome 23 exhibits a peculiar PCA pattern, whereby PC1 separates genotypes, but PC2 divides the homozygous Copper and Quillback based on species (Fig. 3). This suggests that within each haplotype, there are Copper and Quillback versions, possibly due to recombination suppression occurring before speciation. There are multiple regions in the genome with species-specific patterns of LD, which, given the relatively recent speciation of Copper and Quillback, indicates that some parts of the genome are evolving rapidly, facilitated by local recombination suppression. The variable patterns of introgression within each of these haploblocks illustrate the variable permeability of each species' genome to foreign alleles. Ease of introgression at each of these loci is dictated by the fitness advantages (or disadvantages) conferred by the genotype (Baack & Rieseberg, 2007). This can explain the relative abundance of heterozygotes for each haplotype in the data, as well as the much more limited presence of opposite-type homozygotes; ongoing hybridization may keep these alleles in circulation for both species, but they may be maladaptive in the homozygous state when introgressed. While selection against maladaptive introgression is strong at most places in the genome, reduced recombination within these haploblocks can impede purifying selection (Martin et al., 2019).

Within these regions, phylogenetic relationships between haplotypes largely reflect the evolutionary relationship between species. The high sequence similarity of haplotypes

supersedes phylogenetic clustering based on species in all of the haploblocks (Figs. 3, S14). Combined with unambiguous genetic clustering in local PCAs, this is highly suggestive that these haplotypes are segregating between the species by introgression. One notable exception is the haploblock on chromosome 23 discussed above. Here, the separation of the species on PC2 contradicts the phylogenetic pattern and may suggest the presence of Quillback-specific haplotypes. Two of these haplotypes, however, share a more recent common ancestor with black-and-yellow and gopher haplotypes than with Copper or Quillback of the opposite haplotype (Fig. 3). This is likely the result of incomplete lineage sorting, whereby some genomic regions exhibit differential genealogies due to unique inheritance patterns of ancestral variation and rapidly evolving recombination landscapes in divergent lineages (Ishigohoka et al., 2023).

2.45 Future directions

More work is needed to fully understand the processes contributing to population structure in these and other Pacific rockfishes. The bulk of our sampling was opportunistic; more intentional sampling may elucidate the genetic basis for divergence based on depth within species. Additionally, incorporation of high-resolution environmental data may aid in disentangling genomic and environmental clines. The differentiation between inlet and coastal samples in this and previous works should be explored more fully: whole genome sequencing may yield the resolution required to unearth genomic regions involved in parallel adaptation.

Chapter 3: Broader implications

Here we observe two members of a species complex with different population structure. It remains unclear whether the observed differences are the result of divergent life history traits, species' distribution and/or abundance, larval dispersal or recruitment patterns. While these are all excellent questions for future study, this work does have some implications for the continued management of these species. This represents the first effort to characterize range-wide genomic diversity within Copper and Quillback and coincides with a recent moratorium on the Quillback fishery in California.

Management of Quillback in Canada might reasonably be reduced to just one conservation unit but given the geographic differences in fishing pressures and the necessity for further investigation of habitat-driven divergence, the more conservative structure is appropriate. It is likely especially important for Quillback, assessed as threatened in Canada since 2009 (COSEWIC, 2009), to be managed cautiously in their Canadian range. Indeed, as Copper and Quillback overlap in their habitat with endangered Yelloweye rockfish (DFO, 2024), and species identification is impossible before hooks are set, a more granular management regime which reflects the most threatened among the genus may be warranted.

Further, given the propensity of Quillback, Copper, and Brown rockfish to hybridize within the Salish Sea (and particularly Puget Sound), more importance should be placed on the health of hybrid zones as sources of genetic variation within threatened species. While recent studies have begun to characterize the extent of genetic variation within harvested species, it is not clear how historical overharvest has affected the genetic health of these populations. We suggest that regular sequencing and genetic analyses of rockfishes landed during annual surveys would be prudent to ensure that management practices may be more proactive. This can help to ensure the

long-term health of these fisheries in the face of increased environmental pressures and anthropogenic disturbance (Bernatchez et al., 2017).

The establishment and expansion of rockfish conservation areas within Canada and the U.S. are an important step toward long-term maintenance of the fishery. However, these have been organized to reflect larval dispersal based on estimates from Black rockfish, which may severely underestimate larval dispersal (Buonaccorsi et al., 2005; Lancaster et al., 2015; Lotterhos et al., 2014; Miller & Shanks, 2004). While the relative genetic homogeneity of Quillback suggested by my findings may infer high capacity for dispersal, we caution that low genetic differentiation between sampling locations is not necessarily indicative of migration. Low genetic differentiation can also result from recent population expansion following a bottleneck and/or strong homogenizing selection. Haggarty et al. (2016) found that, three to seven years after establishment, it is unclear whether RCAs are promoting recovery of demersal fishes along Canada's west coast. While it is possible that the level of protection afforded by RCA networks may be inadequate to enable recovery of these fisheries, sufficient time for recovery may not have yet elapsed. We consider the latter to be a more likely explanation, given that the well documented recruitment pulses exhibited by most rockfishes occur at the scales of years or decades (Love et al., 2002).

From an evolutionary perspective, my results represent an important step in characterizing differentiation in the genomes of Pacific rockfishes. We observe genomic islands of divergence within species exhibiting high gene flow, which may house ecologically important variation. Further, while my characterization of population structure within Copper is consistent with past studies which posit introgression from Quillback, we highlight the importance of hybridization in the promotion and maintenance of genetic variation.

Additionally, we highlight the rapid evolution of species-specific recombination landscapes. The contribution of differential genomic architecture to the process of speciation is increasingly recognized; non-recombining regions can evolve quickly and result in highly divergent haplotypes in relatively short spans of time. This can be especially important for species under panmixia, where geographic isolation cannot more easily contribute to local adaptation. Note that the divergence here does not need to be driven by selection in different habitats to accumulate, when recombination between the haplotypes is suppressed. In fact, selection is weakened against putatively deleterious mutations when haplotypes spend more generations in the heterozygous state and are unable to recombine (Kirkpatrick and Barton, 2006).

Lastly, this work represents the culmination of cooperative international and intergovernmental sampling and research. We stress that fish are themselves not beholden to political boundaries - and apparently not too many bathymetric ones, either. Healthy, cooperative relationships between fisheries managers in Canada and the US are critical in accomplishing shared long-term goals, as our fisheries are inextricably linked. This work would not have been possible without considerable effort from technicians and scientists from within both NOAA and DFO.

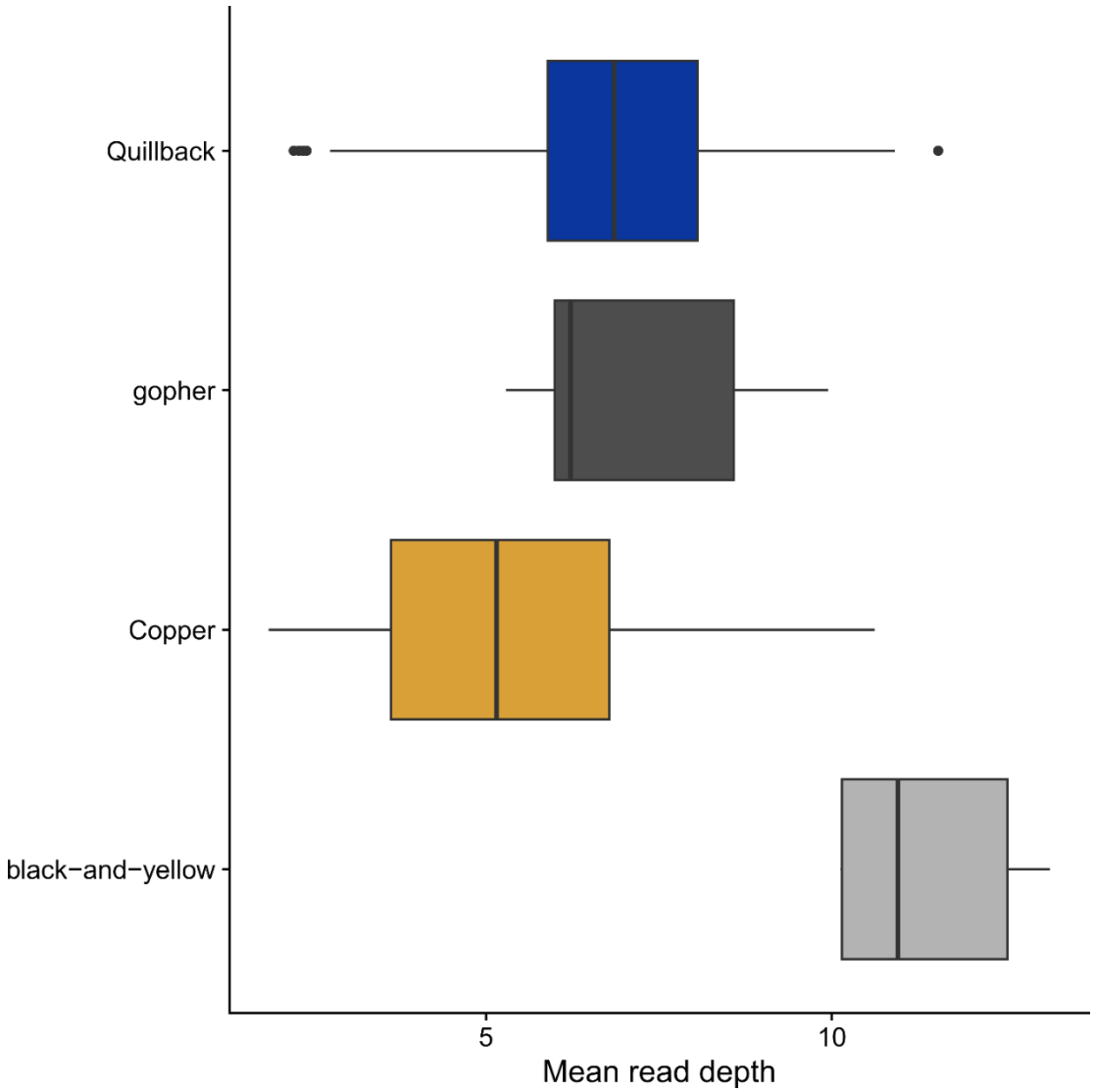

Figure S1: Mean read depth of all samples retained after quality control.

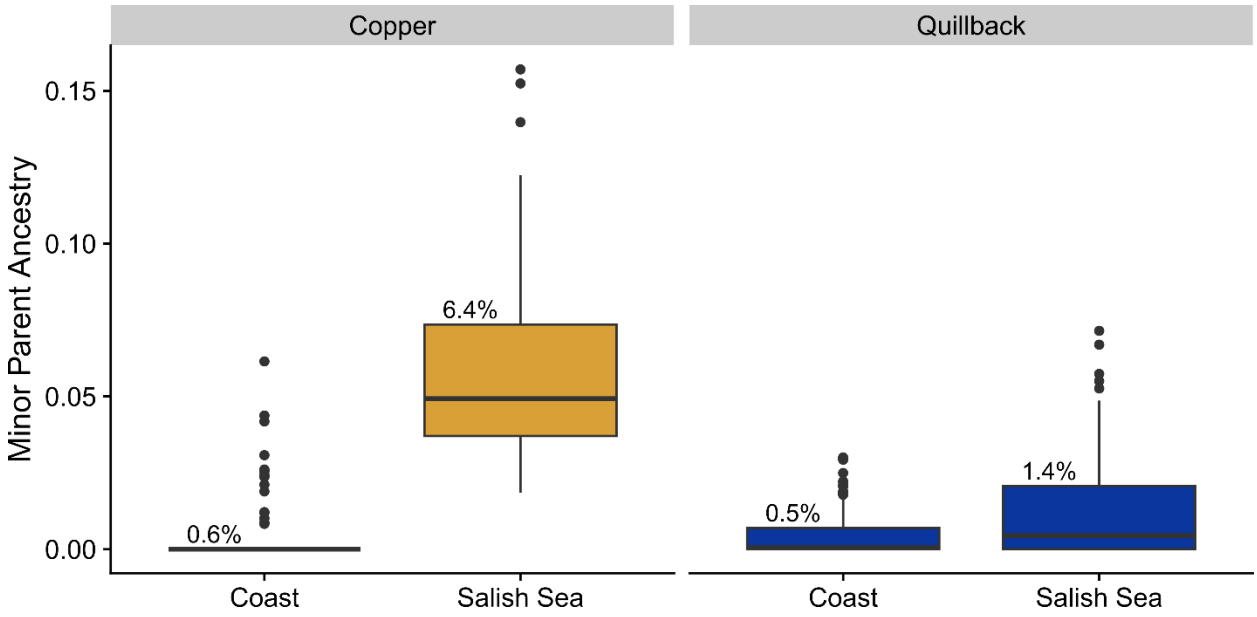

Figure S2: Boxplot of mean minor parent ancestry; Salish Sea vs. Coast.

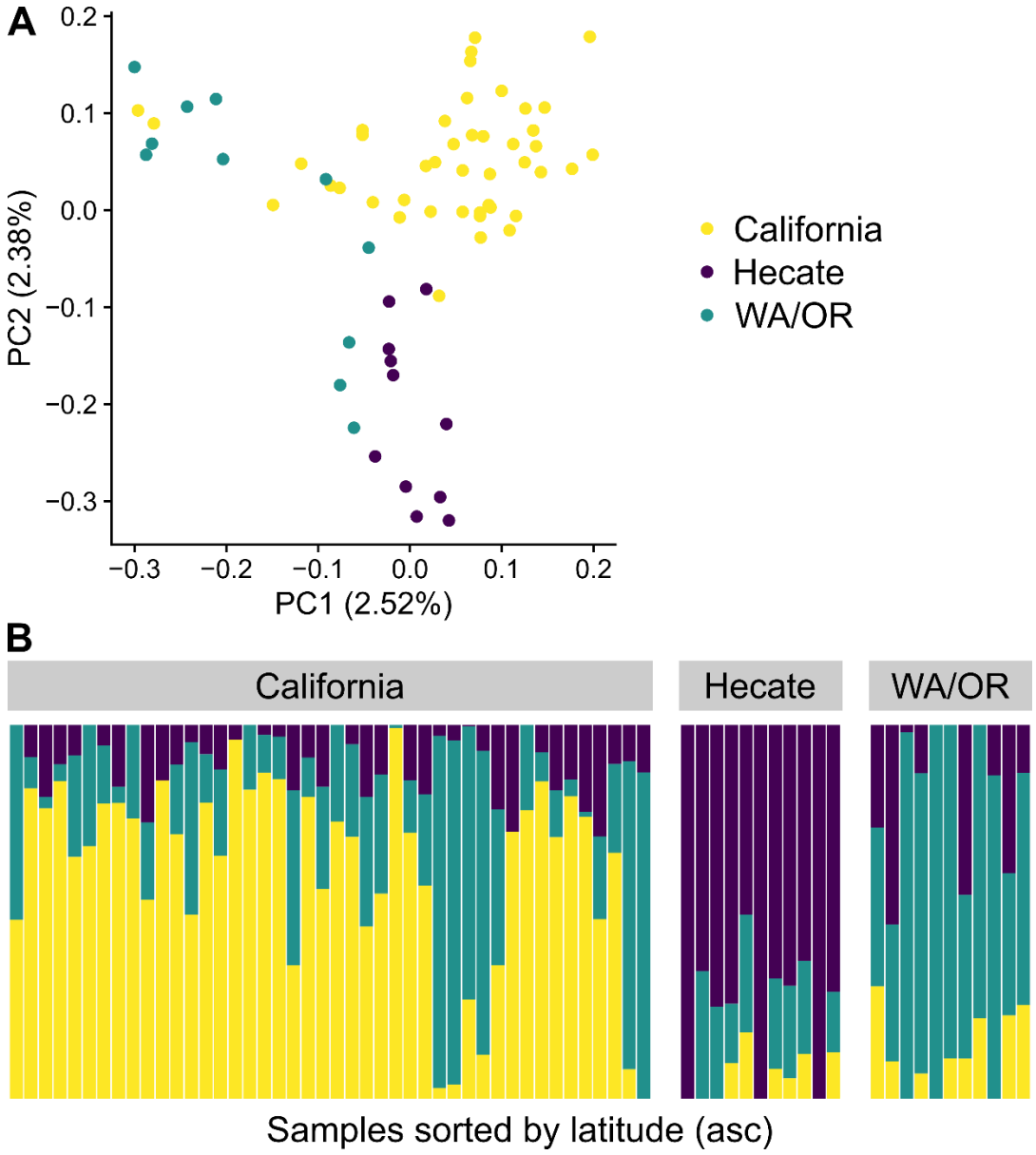

Figure S3: PCA and ancestry analysis (K = 3) for Copper Rockfish outside of the Salish Sea. Samples in the ancestry plot are ordered by increasing latitude.

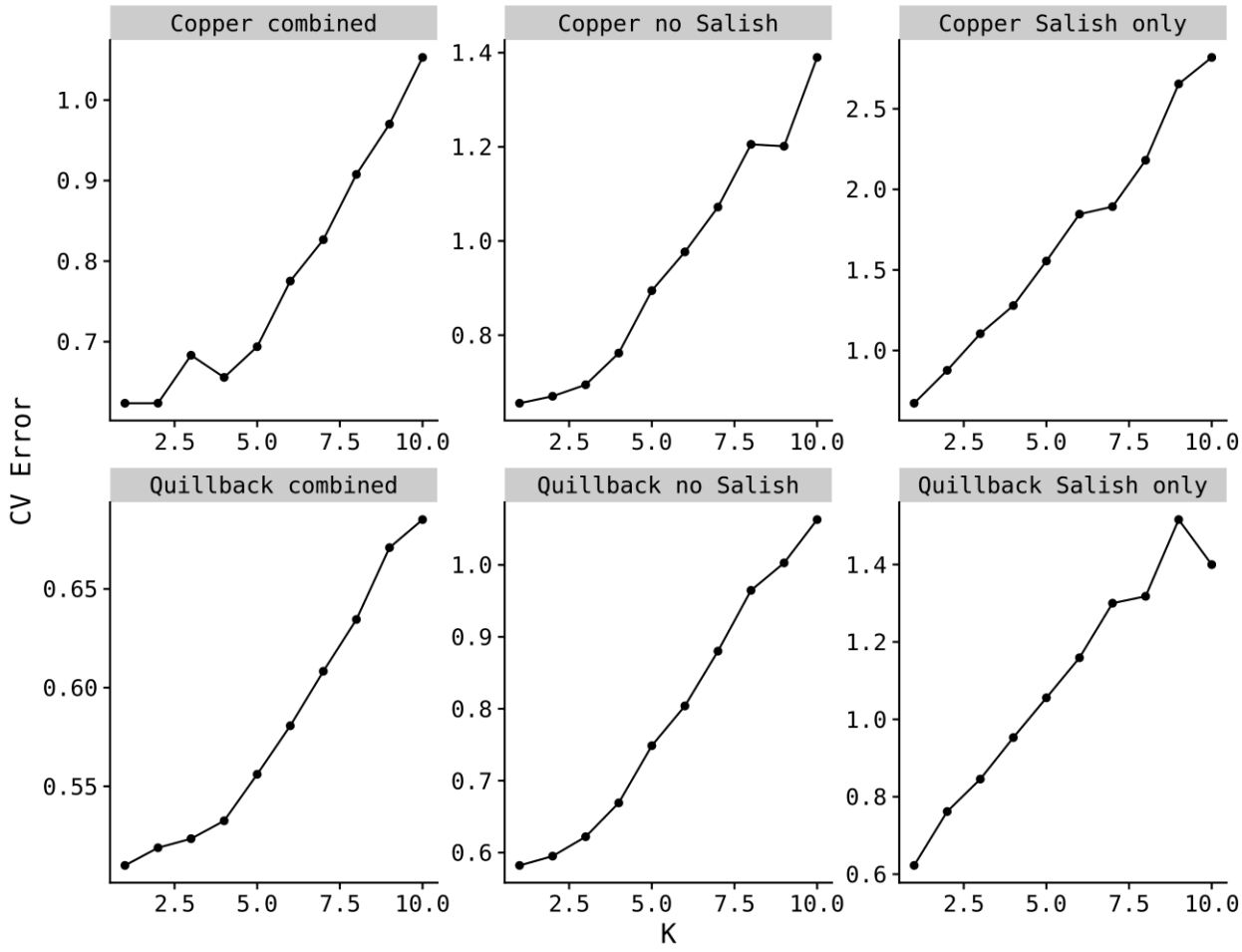

Figure S4: Cross-validation error for K=1 to K=10 in both study species. Analyses were conducted for all individuals in each species and separately for samples inside and outside of the Salish Sea.

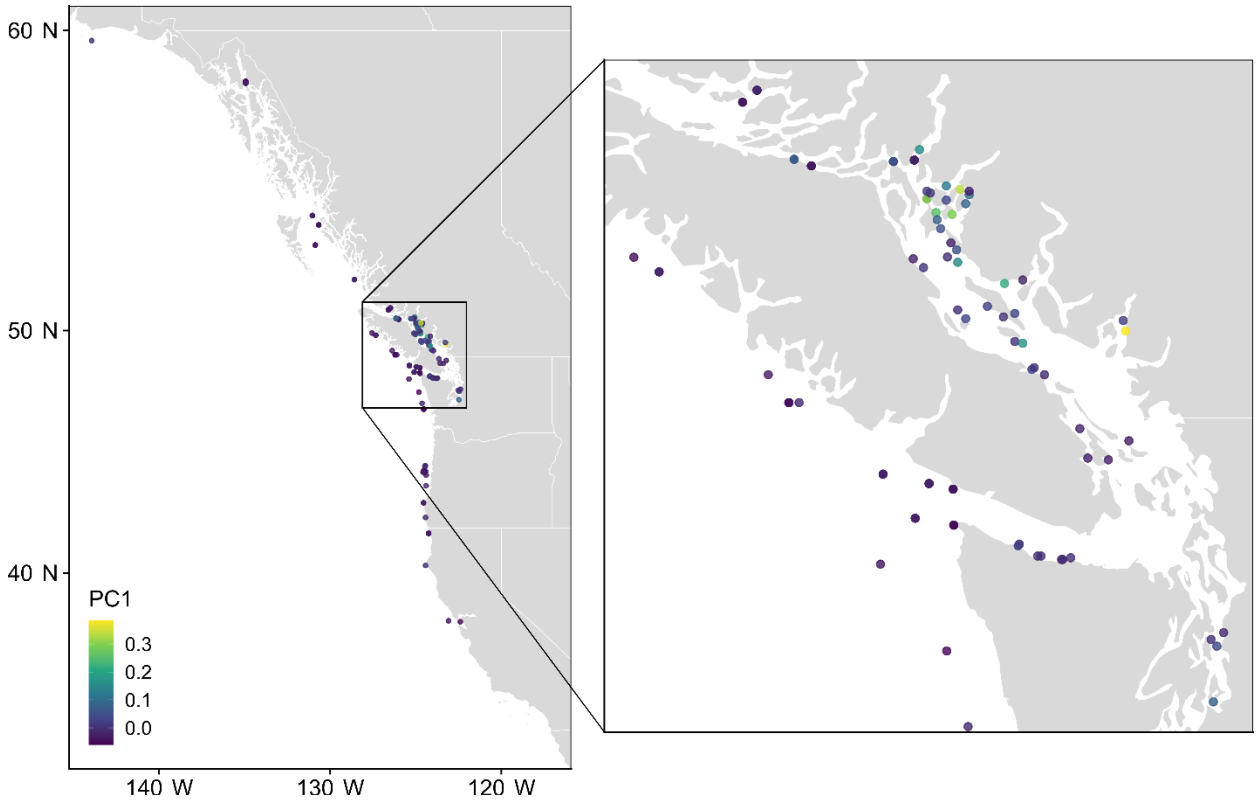

Figure S5: Sampling map of Quillback rockfish, coloured by PC1. PC1 outliers appear to be concentrated at the North end of the Strait of Georgia.

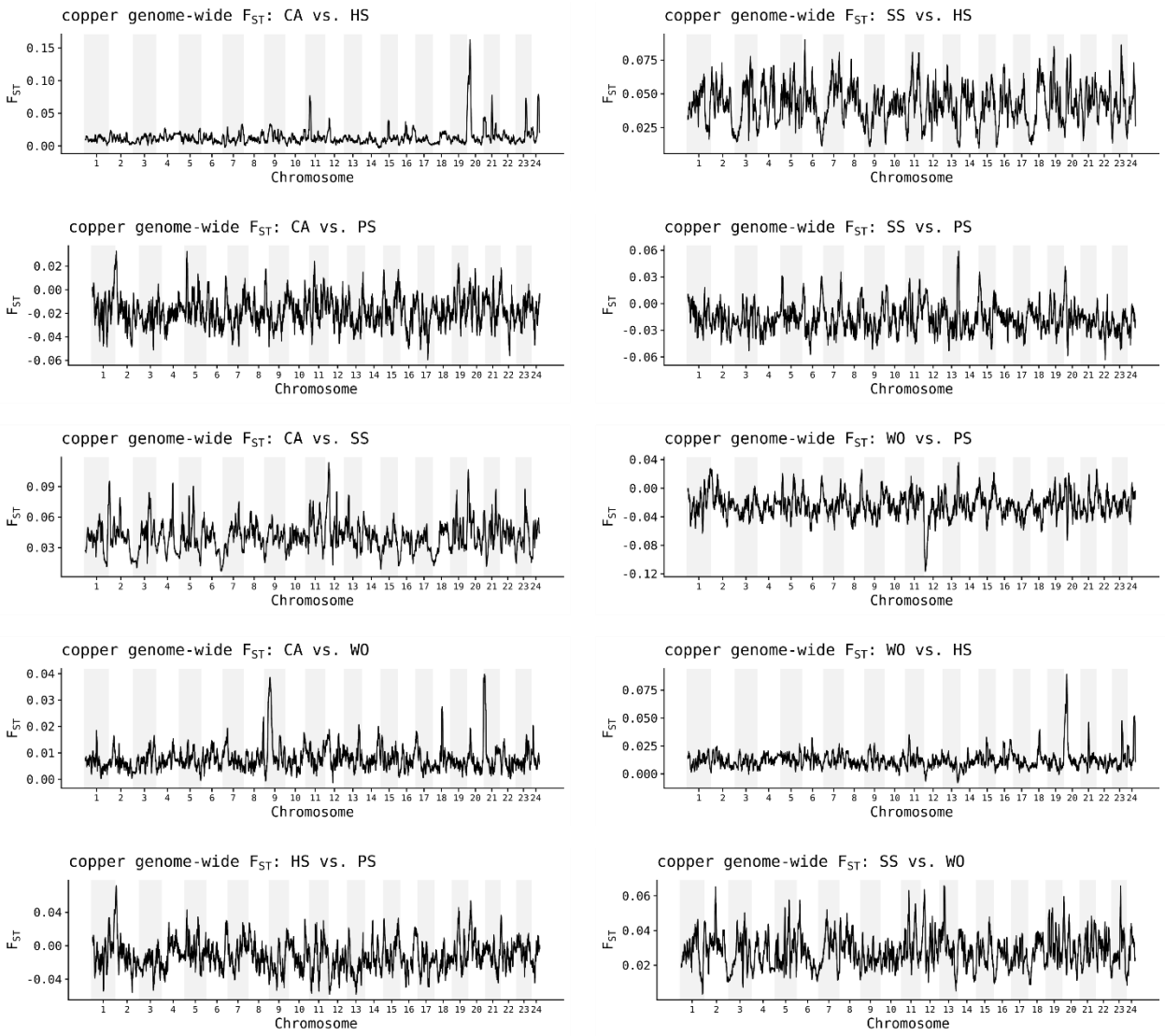

Figure S6: Rolling mean F_{ST} between geographic populations of Copper rockfish. Means were calculated in 1000 SNP windows.

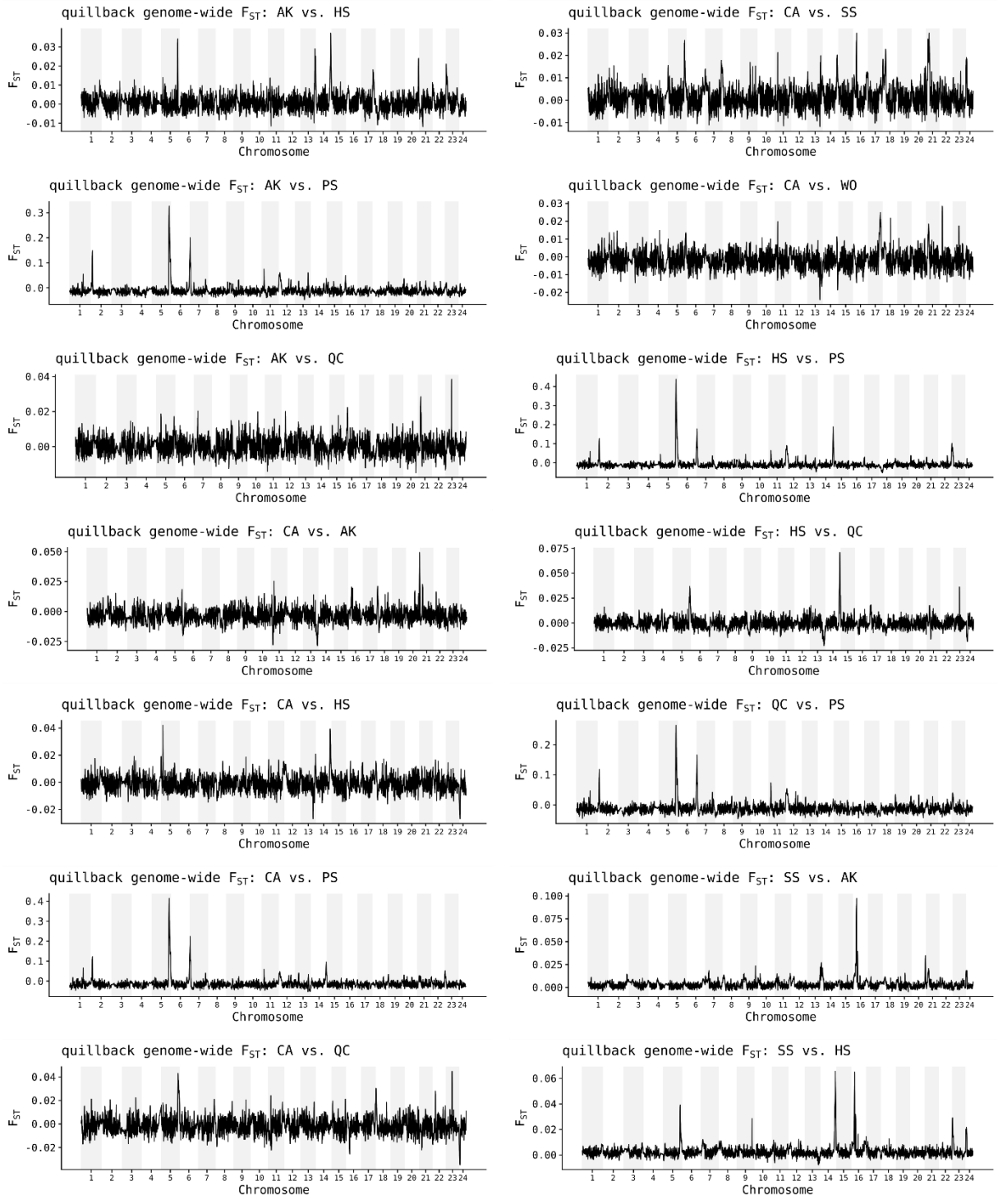

Figure S7: Rolling mean F_{ST} between geographic populations of Quillback rockfish (1). Means were calculated in 1000 SNP windows.

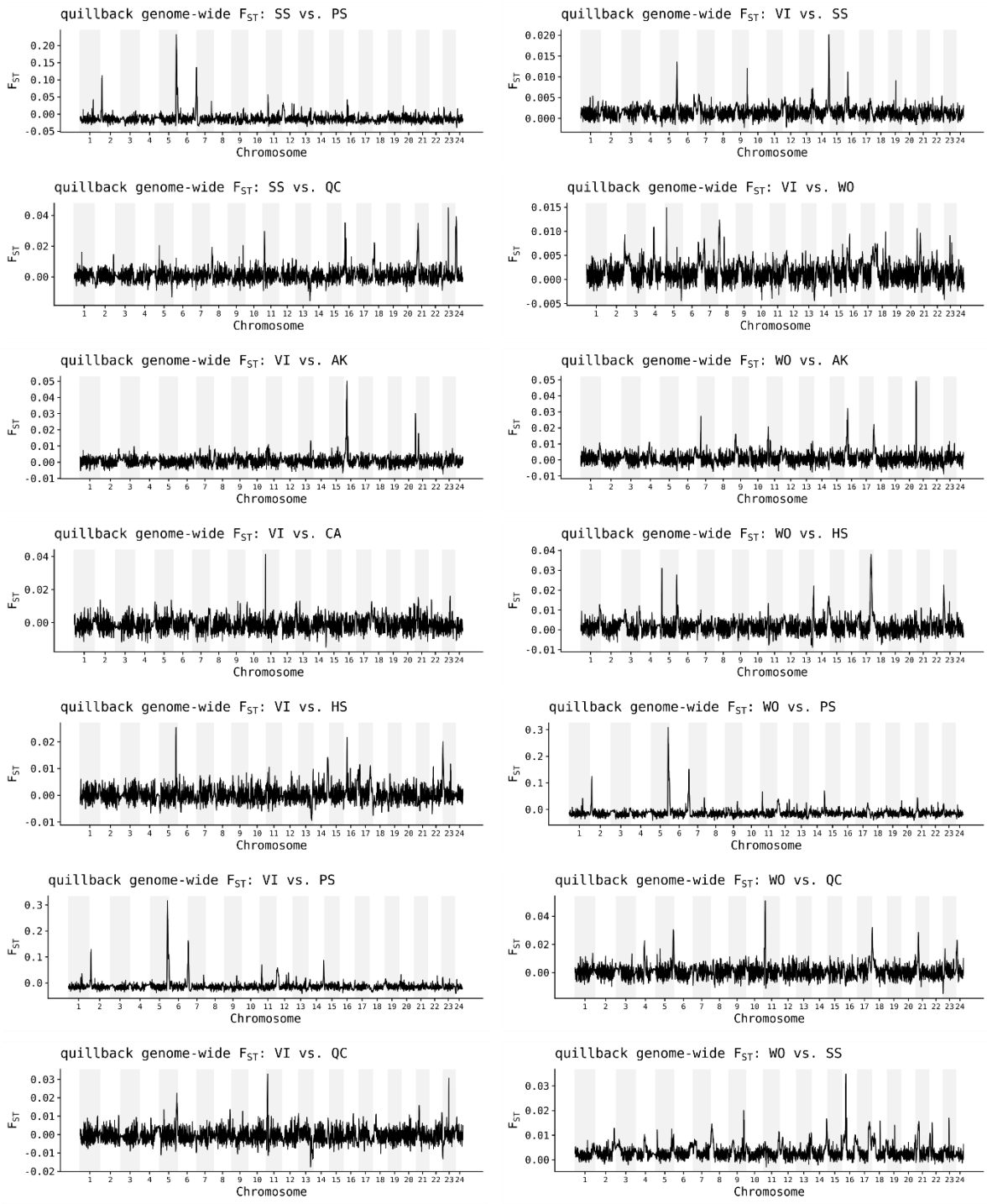

Figure S8: Rolling mean F_{ST} between geographic populations of Quillback rockfish (2). Means were calculated in 1000 SNP windows.

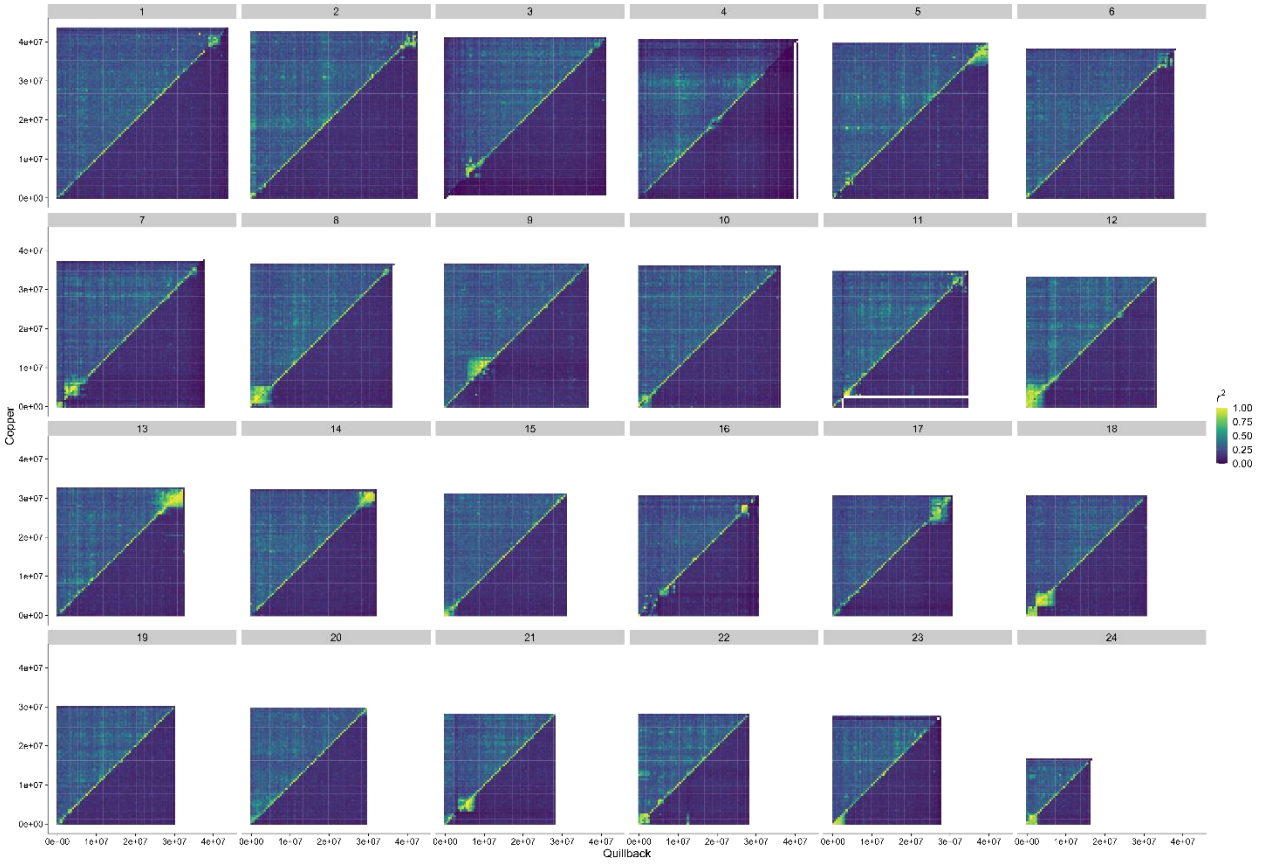

Figure S9: Linkage disequilibrium plotted for both species. Some linkage patterns were species-specific; see chromosomes 5, 9,13, 18, 23.

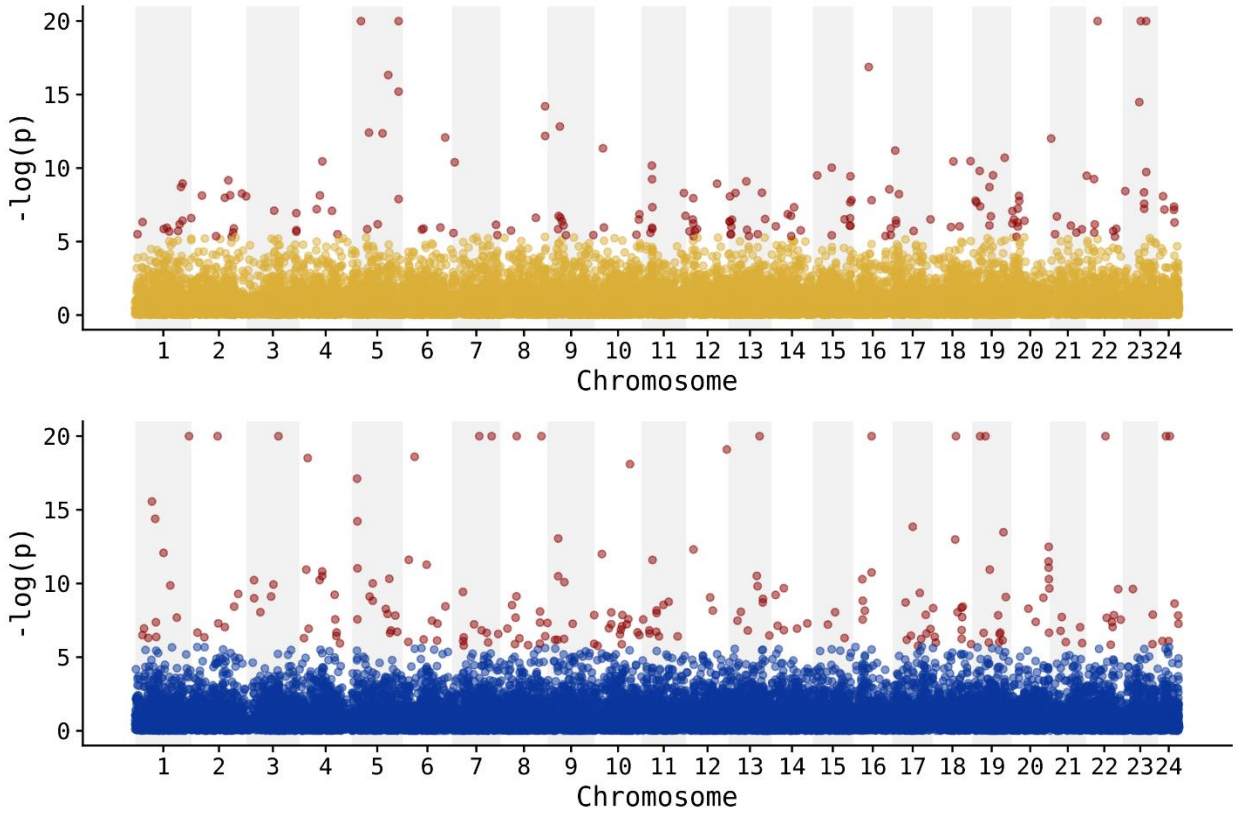

Figure S10: The weighted-Z analysis reveals genes most associated with latitude in each species. Copper (yellow) had less strongly associated genes than did Quillback (blue). The 1% of genes most significantly associated with latitude are coloured red.

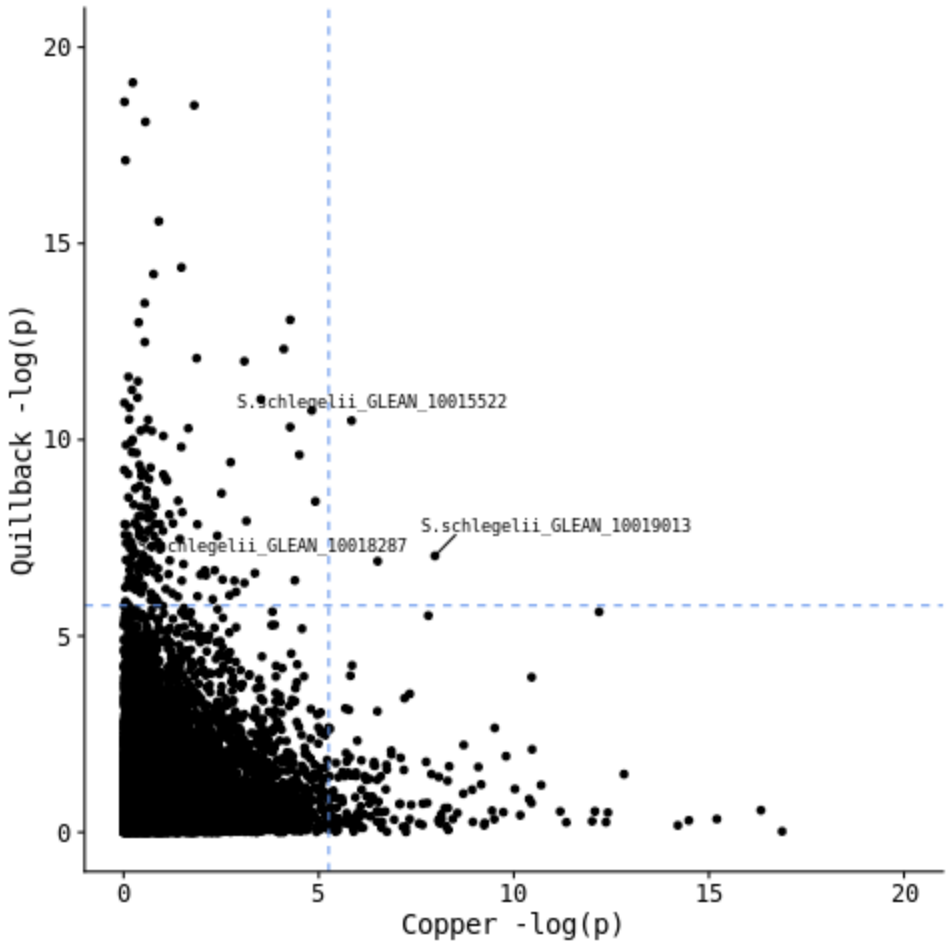

Figure S11: Comparison of the top 1% of genes most associated with latitude in each species. Blue lines represent the significance threshold, set to the top 1% of genes in each species. Only three genes achieved significance in both species.

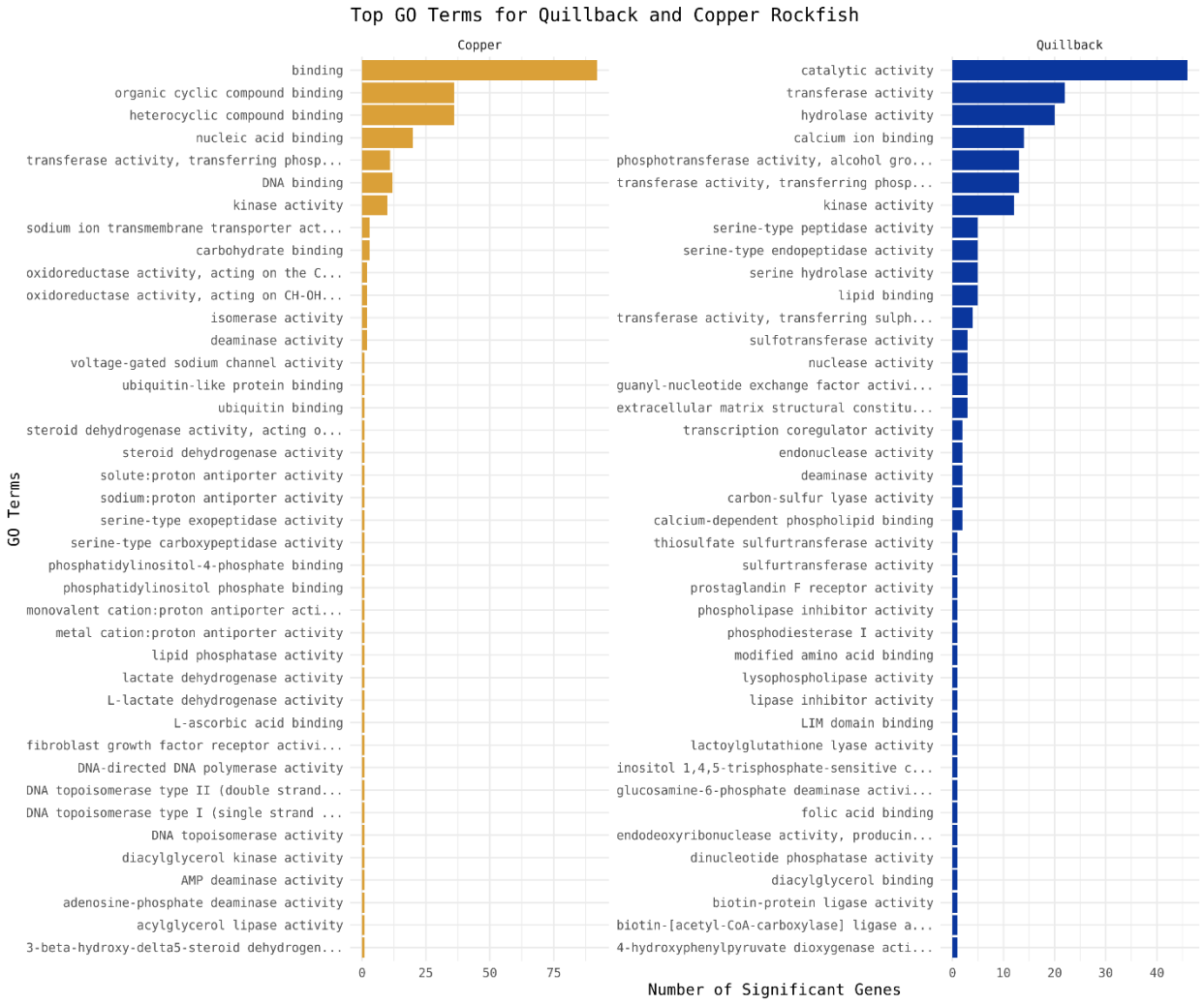

Figure S12: GO term enrichment analyses reveal the 40 most enriched terms associated with latitude in each species. Beyond the top 13 terms in Copper and 21 in Quillback, only one gene was representative of each GO term. Only the top 25 terms in Copper and 38 in Quillback achieved statistical significance ($p < 0.05$).

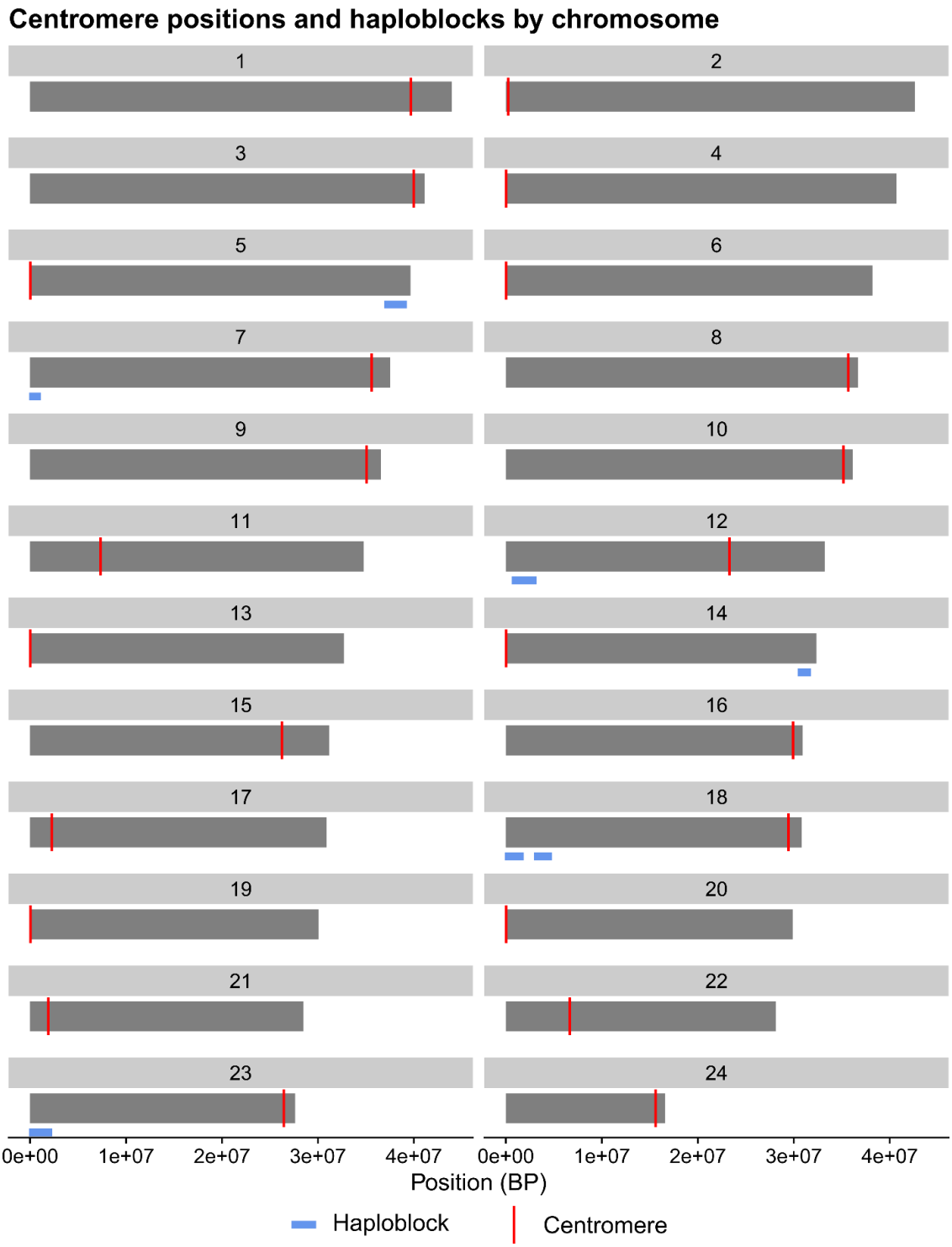

Figure S13: Centromere positions for each chromosome. The red lines represent the centre of each centromere. Blue bars are the regions spanning the shared haploblocks.

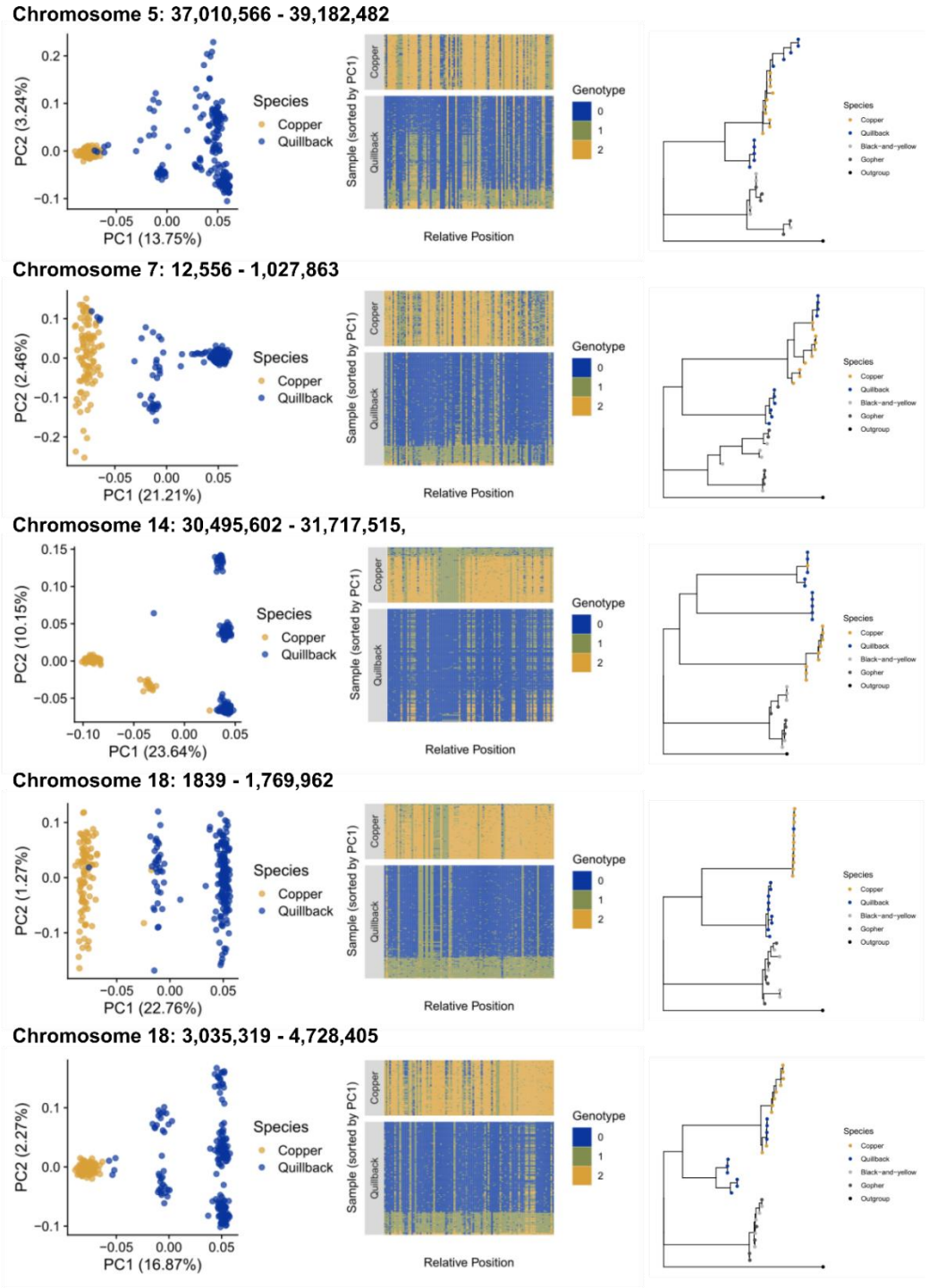

Figure S14: Characterization of the five haploblocks not pictured in Fig. 3. Principal component analyses were conducted within the linked regions, points coloured by species. Genotype plots representing the 100 SNPs most associated with PC1 within each region, rows represent individual samples. Consensus phylogenetic trees for each region were generated by selecting homozygous individuals from each species and five samples each of Gopher and Black-and-yellow rockfish, with the Korean rockfish reference as outgroup.

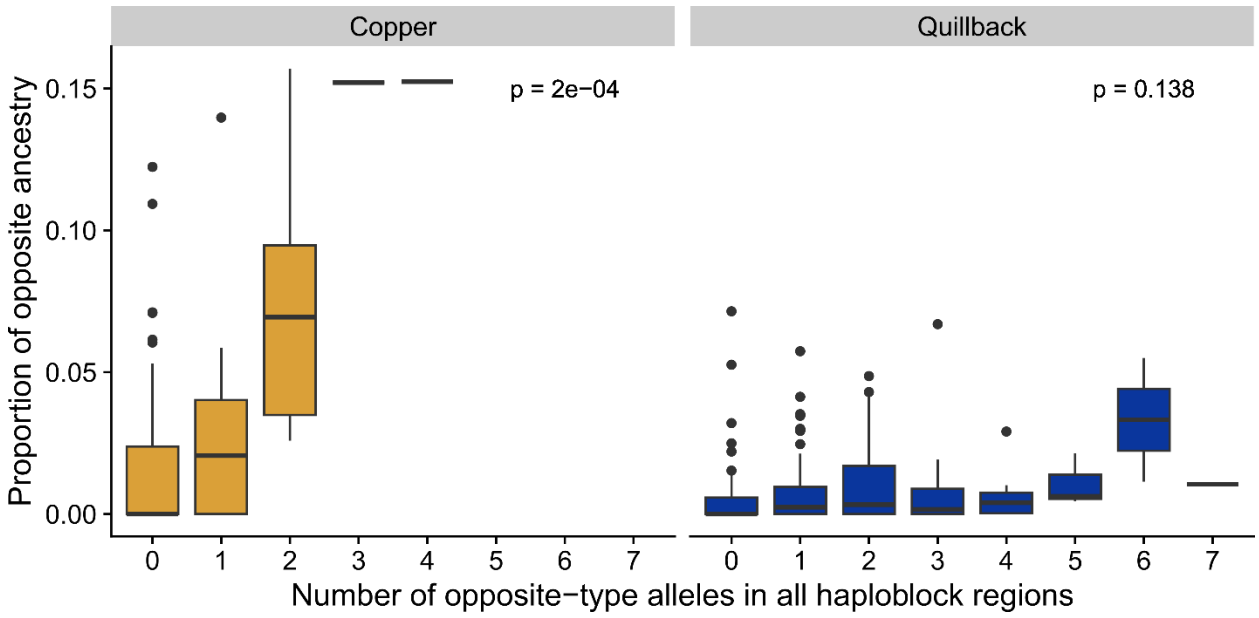

Figure S15: Proportion of admixture as a function of the total number of opposite-type alleles in each of the seven haploblocks in each individual. Correlation between Quillback ancestry and number of Quillback alleles is highly significant in Copper. Association between Copper admixture and Copper alleles at haploblock loci does not achieve significance in Quillback.

P1	P2	P3	D	p-value	BBAA	ABBA	BABA	Interpretation
Copper	**Admixed copper**	**Admixed quillback**	0.0535155	2.30E-16	97416	24093.9	21646.1	Excess of alleles shared between admixed copper and admixed quillback
Admixed quillback	**Quillback**	**Admixed copper**	0.0144827	2.30E-16	66734.4	29715.4	28866.9	Excess of alleles shared between quillback and admixed copper
Copper	**Admixed copper**	**Quillback**	0.0553873	2.30E-16	96740.1	24266.5	21719.5	Excess of alleles shared between quillback and admixed copper
Admixed quillback	**Quillback**	**Copper**	0.0131044	2.30E-16	68528	28962.1	28212.9	Excess of alleles shared between quillback and copper

Table S1: D statistics for each trio of admixture groups. Positive D indicates excess of allele sharing between bolded groups (higher ABBA than BABA). Excess of alleles shared between both Quillback types and admixed Copper indicate introgression from Quillback into Copper. More alleles shared between pure Quillback and both Copper types (rows 2 and 4) indicate that 'admixed' Quillback do not have Copper ancestry. P-values were all the lowest possible in the algorithm.

References

Alexa, A., Rahnenführer, J., & Lengauer, T. (2006). Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics*, *22*(13), 1600–1607. https://doi.org/10.1093/bioinformatics/btl140

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., & Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends in Ecology & Evolution*, *23*(6), 327–337. https://doi.org/10.1016/j.tree.2008.02.008

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*(10), Article 10. https://doi.org/10.1038/nrg2844

An, H., Kim, M. J., Park, K., Cho, K., Bae, B., Kim, J., & Myeong, J. I. (2012). Genetic Diversity and Population Structure in the Heavily Exploited Korean Rockfish, *Sebastes schlegelii*, in Korea. *Journal of the World Aquaculture Society*, *43*(1), 73–83. https://doi.org/10.1111/j.1749-7345.2011.00544.x

Anderson, S. C., Keppel, E. A., Edwards, A. M. 2019. A reproducible data synopsis for over 100 species of British Columbia groundfish. DFO Can. Sci. Advis. Sec. Res. Doc. 2019/041. 321 p.

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*. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Baack, E. J., & Rieseberg, L. H. (2007). A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development*, *17*(6), 513–518. https://doi.org/10.1016/j.gde.2007.09.001

Baetscher, D. S., Nuetzel, H. M., & Garza, J. C. (2023). Highly accurate species identification of Eastern Pacific rockfishes (*Sebastes* spp.) with high-throughput DNA sequencing. *Conservation Genetics*, *24*(5), 563–574. https://doi.org/10.1007/s10592-023-01521-6

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

Bank, C., Bürger, R., & Hermisson, J. (2012). The Limits to Parapatric Speciation: Dobzhansky–Muller Incompatibilities in a Continent–Island Model. *Genetics*, *191*(3), 845–863. https://doi.org/10.1534/genetics.111.137513

Beger, M., Selkoe, K. A., Treml, E., Barber, P. H., von der Heyden, S., Crandall, E. D., Toonen, R. J., & Riginos, C. (2014). Evolving coral reef conservation with genetic information. *Bulletin of Marine Science*, *90*(1), 159–185. https://doi.org/10.5343/bms.2012.1106

Behrens, K. A., Girasek, Q. L., Sickler, A., Hyde, J., & Buonaccorsi, V. P. (2021). Regions of genetic divergence in depth-separated *Sebastes* rockfish species pairs: Depth as a

potential driver of speciation. *Molecular Ecology*, *30*(17), 4259–4275. https://doi.org/10.1111/mec.16046

Bellquist, L., Beyer, S., Arrington, M., Maeding, J., Siddall, A., Fischer, P., Hyde, J., & Wegner, N. C. (2019). Effectiveness of descending devices to mitigate the effects of barotrauma among rockfishes (*Sebastes spp*.) in California recreational fisheries. *Fisheries Research*, *215*, 44–52. https://doi.org/10.1016/j.fishres.2019.03.003

Berdan, E. L., Blanckaert, A., Butlin, R. K., & Bank, C. (2021). Deleterious mutation accumulation and the long-term fate of chromosomal inversions. *PLOS Genetics*, *17*(3), e1009411. https://doi.org/10.1371/journal.pgen.1009411

Berg, P. R., Star, B., Pampoulie, C., Bradbury, I. R., Bentzen, P., Hutchings, J. A., Jentoft, S., & Jakobsen, K. S. (2017). Trans-oceanic genomic divergence of Atlantic cod ecotypes is associated with large inversions. *Heredity*, *119*(6), 418–428. https://doi.org/10.1038/hdy.2017.54

Berg, P. R., Star, B., Pampoulie, C., Sodeland, M., Barth, J. M. I., Knutsen, H., Jakobsen, K. S., & Jentoft, S. (2016). Three chromosomal rearrangements promote genomic divergence between migratory and stationary ecotypes of Atlantic cod. *Scientific Reports*, *6*(1), 23246. https://doi.org/10.1038/srep23246

Bernatchez, L., Wellenreuther, M., Araneda, C., Ashton, D. T., Barth, J. M. I., Beacham, T. D., Maes, G. E., Martinsohn, J. T., Miller, K. M., Naish, K. A., Ovenden, J. R., Primmer, C. R., Young Suk, H., Therkildsen, N. O., & Withler, R. E. (2017). Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution*, *32*(9), 665–680. https://doi.org/10.1016/j.tree.2017.06.010

Blain-Roth, B. J., & Sutton, T. M. (2019). Effects of barotrauma and recompression events on subsequent embryo condition of Yelloweye Rockfish. *Fisheries Research*, *211*, 212–216. https://doi.org/10.1016/j.fishres.2018.11.022

Booker, T. R., Yeaman, S., Whiting, J. R., & Whitlock, M. C. (2024). The WZA: A window-based method for characterizing genotype–environment associations. *Molecular Ecology Resources*, *24*(2), e13768. https://doi.org/10.1111/1755-0998.13768

Booker, T. R., Yeaman, S., & Whitlock, M. C. (2020). Variation in recombination rate affects detection of outliers in genome scans under neutrality. *Molecular Ecology*, *29*(22), 4274–4279. https://doi.org/10.1111/mec.15501

Bradbury, I. R., Hubert, S., Higgins, B., Bowman, S., Borza, T., Paterson, I. G., Snelgrove, P. V. R., Morris, C. J., Gregory, R. S., Hardie, D., Hutchings, J. A., Ruzzante, D. E., Taggart, C. T., & Bentzen, P. (2013). Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. *Evolutionary Applications*, *6*(3), 450–461. https://doi.org/10.1111/eva.12026

Broad Institute. (2019). Picard Toolkit. *Broad Institute, GitHub Repository*. https://github.com/broadinstitute/picard

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. (2004). Molecular genetic structure suggests limited larval dispersal in Grass Rockfish, *Sebastes rastrelliger*. *Marine Biology*, *145*(4), 779–788. https://doi.org/10.1007/s00227-004-1362-2

Calfee, E., Gates, D., Lorant, A., Perkins, M. T., Coop, G., & Ross-Ibarra, J. (2021). Selective sorting of ancestral introgression in maize and teosinte along an elevational cline (p. 2021.03.05.434040). *bioRxiv*. https://doi.org/10.1101/2021.03.05.434040

Chen, S. (2023). Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. *iMeta*, *2*(2), e107. https://doi.org/10.1002/imt2.107

Conover, D. O., Munch, S. B., & Arnott, S. A. (2009). Reversal of evolutionary downsizing caused by selective harvest of large fish. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1664), 2015–2020. https://doi.org/10.1098/rspb.2009.0003

Cope, J. M. (2004). Population genetics and phylogeography of the Blue Rockfish (*Sebastes mystinus*) from Washington to California. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(3), 332–342. https://doi.org/10.1139/f04-008

COSEWIC. (2009). *COSEWIC assessment and status report on the Quillback Rockfish* Sebastes maliger *in Canada*.

COSEWIC. (2020). *COSEWIC assessment and status report on the Yelloweye Rockfish,* Sebastes ruberrimus*, Pacfic Ocean outside waters population, Pacific Ocean inside waters population, in Canada.*

Czech, L., & Exposito-Alonso, M. (2021). grenepipe: A flexible, scalable, and reproducible pipeline to automate variant and frequency calling from sequence read*s* (arXiv:2103.15167). *arXiv*. https://doi.org/10.48550/arXiv.2103.15167

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

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2), giab008. https://doi.org/10.1093/gigascience/giab008

De La Torre, A. R., Wilhite, B., & Neale, D. B. (2019). Environmental Genome-Wide Association Reveals Climate Adaptation Is Shaped by Subtle to Moderate Allele Frequency Shifts in Loblolly Pine. *Genome Biology and Evolution*, *11*(10), 2976–2989. https://doi.org/10.1093/gbe/evz220

Denny, M. W., & Dowd, W. W. (2022). Physiological Consequences of Oceanic Environmental Variation: Life from a Pelagic Organism's Perspective. *Annual Review of Marine Science*, *14*(Volume 14, 2022), 25–48. https://doi.org/10.1146/annurev-marine-040221-115454

Deville, D., Kawai, K., Fujita, H., & Umino, T. (2023a). Ecomorphology of three closely related *Sebastes* rockfishes with sympatric occurrence in Seto Inland Sea, Japan. *Hydrobiologia*, *850*(18), 4049–4066. https://doi.org/10.1007/s10750-023-05286-4

Deville, D., Kawai, K., Fujita, H., & Umino, T. (2023b). Genetic divergences and hybridization within the *Sebastes inermis* complex. *PeerJ*, *11*, e16391. https://doi.org/10.7717/peerj.16391

Díaz-Arce, N., Gagnaire, P.-A., Richardson, D. E., Walter III, J. F., Arnaud-Haond, S., Fromentin, J.-M., Brophy, D., Lutcavage, M., Addis, P., Alemany, F., Allman, R., Deguara, S., Fraile, I., Goñi, N., Hanke, A. R., Karakulak, F. S., Pacicco, A., Quattro, J. M., Rooker, J. R., … Rodríguez-Ezpeleta, N. (2024). Unidirectional trans-Atlantic gene flow and a mixed spawning area shape the genetic connectivity of Atlantic Bluefin Tuna. *Molecular Ecology*, *33*(1), e17188. https://doi.org/10.1111/mec.17188

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

Dobzhansky, T. (1982). *Genetics and the Origin of Species*. Columbia University Press.

Duchen, P., Živković, D., Hutter, S., Stephan, W., & Laurent, S. (2013). Demographic Inference Reveals African and European Admixture in the North American *Drosophila melanogaster* Population. *Genetics*, *193*(1), 291–301. https://doi.org/10.1534/genetics.112.145912

Edelman, N. B., Frandsen, P. B., Miyagi, M., Clavijo, B., Davey, J., Dikow, R. B., García-Accinelli, G., Van Belleghem, S. M., Patterson, N., Neafsey, D. E., Challis, R., Kumar, S., Moreira, G. R. P., Salazar, C., Chouteau, M., Counterman, B. A., Papa, R., Blaxter, M., Reed, R. D., … Mallet, J. (2019). Genomic architecture and introgression shape a butterfly radiation. *Science*, *366*(6465), 594–599. https://doi.org/10.1126/science.aaw2090

Elphinstone, C., Elphinstone, R., Todesco, M., & Rieseberg, L. (2023). RepeatOBserver: Tandem repeat visualization and centromere detection (p. 2023.12.30.573697). *bioRxiv*. https://doi.org/10.1101/2023.12.30.573697

Euclide, P. T., Larson, W. A., Shi, Y., Gruenthal, K., Christensen, K. A., Seeb, J., & Seeb, L. (2023). Conserved islands of divergence associated with adaptive variation in sockeye salmon are maintained by multiple mechanisms. *Molecular Ecology*, *n/a*(n/a). https://doi.org/10.1111/mec.17126

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

Fagan, W. F., & Holmes, E. E. (2006). Quantifying the extinction vortex. *Ecology Letters*, *9*(1), 51–60. https://doi.org/10.1111/j.1461-0248.2005.00845.x

Feuk, L., MacDonald, J. R., Tang, T., Carson, A. R., Li, M., Rao, G., Khaja, R., & Scherer, S. W. (2005). Discovery of Human Inversion Polymorphisms by Comparative Analysis of Human and Chimpanzee DNA Sequence Assemblies. *PLOS Genetics*, *1*(4), e56. https://doi.org/10.1371/journal.pgen.0010056

Fisheries and Oceans Canada (DFO). (2024). Groundfish Integrated Fisheries Management Plan 2024. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/41255732.pdf

Frable, B. W., Wagman, D. W., Frierson, T. N., Aguilar, A., & Sidlauskasi, B. L. (2015). A new species of *Sebastes* (Scorpaeniformes: Sebastidae) from the northeastern Pacific, with a redescription of the Blue Rockfish, *S. mystinus* (Jordan and Gilbert, 1881). *FISHERY BULLETIN*, *113*(4), 355–377. https://doi.org/10.7755/FB.113.4.1

Fraïsse, C., Belkhir, K., Welch, J. J., & Bierne, N. (2016). Local interspecies introgression is the main cause of extreme levels of intraspecific differentiation in mussels. *Molecular Ecology*, *25*(1), 269–286. https://doi.org/10.1111/mec.13299

Frankham, R. (2012). How closely does genetic diversity in finite populations conform to predictions of neutral theory? Large deficits in regions of low recombination. *Heredity*, *108*(3), 167–178. https://doi.org/10.1038/hdy.2011.66

Frei, D., Mwaiko, S., Seehausen, O., & Feulner, P. G. D. (2024). Ecological disturbance reduces genomic diversity across an Alpine whitefish adaptive radiation. *Evolutionary Applications*, *17*(2), e13617. https://doi.org/10.1111/eva.13617

Froese, R., & Pauly, D. (2021). *Species in genus Sebastes*. FishBase. https://fishbase.de/identification/SpeciesList.php?genus=Sebastes

Funk, D. J., Nosil, P., & Etges, W. J. (2006). Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences*, *103*(9), 3209–3213. https://doi.org/10.1073/pnas.0508653103

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Gainsford, A., Jones, G. P., Hobbs, J.-P. A., Heindler, F. M., & van Herwerden, L. (2020). Species integrity, introgression, and genetic variation across a coral reef fish hybrid zone. *Ecology and Evolution*, *10*(21), 11998–12014. https://doi.org/10.1002/ece3.6769

Gaither, M. R., Bernal, M. A., Coleman, R. R., Bowen, B. W., Jones, S. A., Simison, W. B., & Rocha, L. A. (2015). Genomic signatures of geographic isolation and natural selection in coral reef fishes. *Molecular Ecology*, *24*(7), 1543–1557. https://doi.org/10.1111/mec.13129

Gao, T., Han, Z., Zhang, X., Luo, J., Yanagimoto, T., & Zhang, H. (2016). Population genetic differentiation of the Black Rockfish *Sebastes schlegelii* revealed by microsatellites. *Biochemical Systematics and Ecology*, *68*, 170–177. https://doi.org/10.1016/j.bse.2016.07.013

Garrison, E., & Marth, G. (2012). *Haplotype-based variant detection from short-read sequencing* (arXiv:1207.3907). arXiv. https://doi.org/10.48550/arXiv.1207.3907

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

Grummer, J. A., Beheregaray, L. B., Bernatchez, L., Hand, B. K., Luikart, G., Narum, S. R., & Taylor, E. B. (2019). Aquatic Landscape Genomics and Environmental Effects on Genetic Variation. *Trends in Ecology & Evolution*, *34*(7), 641–654. https://doi.org/10.1016/j.tree.2019.02.013

Haggarty, D. R., Shurin, J. B., & Yamanaka, K. L. (2016). Assessing population recovery inside British Columbia's Rockfish Conservation Areas with a remotely operated vehicle. *Fisheries Research*, *183*, 165–179. https://doi.org/10.1016/j.fishres.2016.06.001

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

Harris, K., & Nielsen, R. (2013). Inferring Demographic History from a Spectrum of Shared Haplotype Lengths. *PLOS Genetics*, *9*(6), e1003521. https://doi.org/10.1371/journal.pgen.1003521

Hauser, L., Adcock, G. J., Smith, P. J., Bernal Ramírez, J. H., & Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand Snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences*, *99*(18), 11742–11747. https://doi.org/10.1073/pnas.172242899

Helmstetter, A. J., Papadopulos, A. S. T., Igea, J., Van Dooren, T. J. M., Leroi, A. M., & Savolainen, V. (2016). Viviparity stimulates diversification in an order of fish. *Nature Communications*, *7*(1), 11271. https://doi.org/10.1038/ncomms11271

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

Hemmer-Hansen, J., Therkildsen, N. O., & Pujolar, J. M. (2014). Population Genomics of Marine Fishes: Next-Generation Prospects and Challenges. *The Biological Bulletin*, *227*(2), 117–132. https://doi.org/10.1086/BBLv227n2p117

Hess, J. E., Hyde, J. R., & Moran, P. (2023). Comparative phylogeography of a bathymetrically segregated pair of sister taxa of rockfishes (genus *Sebastes*): Black Rockfish, *Sebastes melanops*, and Yellowtail Rockfish, *Sebastes flavidus*. Marine Biology, 170(5), 62. https://doi.org/10.1007/s00227-023-04207-2

Horn, R. L., Kamphaus, C., Murdoch, K., & Narum, S. R. (2020). Detecting genomic variation underlying phenotypic characteristics of reintroduced Coho Salmon (*Oncorhynchus kisutch*). *Conservation Genetics*, *21*(6), 1011–1021. https://doi.org/10.1007/s10592-020-01307-0

Hu, G., Chen, F., Zhang, Y., Luan, P., Luo, Z., Niu, J., Zheng, P., Wang, S., Zhang, T., Shu, Y., & Ji, F. (2023). Estimates of the Effective Population Size and Genetic Structure of the

Critically Endangered Ship Sturgeon (*Acipenser nudiventris*) in the Chinese Section of the Ili River. *Fishes*, *8*(7), Article 7. https://doi.org/10.3390/fishes8070354

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., Kimbrell, C. A., Budrick, J. E., Lynn, E. A., & Vetter, R. D. (2008). Cryptic speciation in the Vermilion Rockfish (*Sebastes miniatus*) and the role of bathymetry in the speciation process. *Molecular Ecology*, *17*(4), 1122–1136. https://doi.org/10.1111/j.1365-294X.2007.03653.x

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

Ingram, T. (2010). Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1705), 613–618. https://doi.org/10.1098/rspb.2010.1127

Ingram, T. (2015). Diversification of body shape in *Sebastes* rockfishes of the north-east Pacific. *Biological Journal of the Linnean Society*, *116*(4), 805–818. https://doi.org/10.1111/bij.12635

Ingram, T., & Kai, Y. (2014). The Geography of Morphological Convergence in the Radiations of Pacific *Sebastes* Rockfishes. *The American Naturalist*, *184*(5), E115–E131. https://doi.org/10.1086/678053

Ingram, T., & Shurin, J. B. (2009). Trait-based assembly and phylogenetic structure in northeast Pacific rockfish assemblages. *Ecology*, *90*(9), 2444–2453. https://doi.org/10.1890/08-1841.1

Ishigohoka, J., Bascón-Cardozo, K., Bours, A., Fuß, J., Rhie, A., Mountcastle, J., Haase, B., Chow, W., Collins, J., Howe, K., Uliano-Silva, M., Fedrigo, O., Jarvis, E. D., Pérez-Tris, J., Illera, J. C., & Liedvogel, M. (2023). Distinct patterns of genetic variation at low-recombining genomic regions represent haplotype structure (p. 2021.12.22.473882). *bioRxiv*. https://doi.org/10.1101/2021.12.22.473882

Jay, P., Whibley, A., Frézal, L., Rodríguez de Cara, M. Á., Nowell, R. W., Mallet, J., Dasmahapatra, K. K., & Joron, M. (2018). Supergene Evolution Triggered by the Introgression of a Chromosomal Inversion. *Current Biology*, *28*(11), 1839-1845.e3. https://doi.org/10.1016/j.cub.2018.04.072

Johnson, W. E., Onorato, D. P., Roelke, M. E., Land, E. D., Cunningham, M., Belden, R. C., McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J., Wildt, D. E., Penfold, L. M., Hostetler, J. A., Oli, M. K., & O'Brien, S. J. (2010). Genetic Restoration of the Florida Panther. *Science*, *329*(5999), 1641–1645. https://doi.org/10.1126/science.1192891

Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population scale DNA sequence data. *Genome Research*, gr.176552.114. https://doi.org/10.1101/gr.176552.114

Kai, Y., & Nakabo, T. (2002). Morphological differences among three color morphotypes of *Sebastes inermis* (Scorpaenidae). *Ichthyological Research*, *49*(3), 260–266. https://doi.org/10.1007/s102280200037

Kai, Y., & Nakabo, T. (2008). Taxonomic review of the *Sebastes inermis* species complex (Scorpaeniformes: Scorpaenidae). *Ichthyological Research*, *55*(3), 238–259. https://doi.org/10.1007/s10228-007-0029-7

Karachaliou, E., Schmidt, C., Greef, E. de, Docker, M. F., & Garroway, C. J. (2024). Urbanization is associated with reduced genetic diversity in marine fish populations (p. 2024.02.20.581210). *bioRxiv*. https://doi.org/10.1101/2024.02.20.581210

Kess, T., Brachmann, M., & Boulding, E. G. (2021). Putative chromosomal rearrangements are associated primarily with ecotype divergence rather than geographic separation in an intertidal, poorly dispersing snail. *Journal of Evolutionary Biology*, *34*(1), 193–207. https://doi.org/10.1111/jeb.13724

Kim, Y., & Nielsen, R. (2004). Linkage Disequilibrium as a Signature of Selective Sweeps. *Genetics*, *167*(3), 1513–1524. https://doi.org/10.1534/genetics.103.025387

Kingman, J. F. C. (1982). On the Genealogy of Large Populations. *Journal of Applied Probability*, *19*, 27–43. https://doi.org/10.2307/3213548

Kirkpatrick, M. (2010). How and Why Chromosome Inversions Evolve. *PLOS Biology*, *8*(9), e1000501. https://doi.org/10.1371/journal.pbio.1000501

Kirkpatrick, M., & Barton, N. (2006). Chromosome Inversions, Local Adaptation and Speciation. *Genetics*, *173*(1), 419–434. https://doi.org/10.1534/genetics.105.047985

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: A review. *Plant Methods*, *9*(1), 29. https://doi.org/10.1186/1746-4811-9-29

Korunes, K. L., & Samuk, K. (2021). *pixy*: Unbiased estimation of nucleotide diversity and divergence in the presence of missing data. *Molecular Ecology Resources*, *21*(4), 1359–1368. https://doi.org/10.1111/1755-0998.13326

Lamichhaney, S., Barrio, A. M., Rafati, N., Sundström, G., Rubin, C.-J., Gilbert, E. R., Berglund, J., Wetterbom, A., Laikre, L., Webster, M. T., Grabherr, M., Ryman, N., & Andersson, L. (2012). Population-scale sequencing reveals genetic differentiation due to local

adaptation in Atlantic herring. *Proceedings of the National Academy of Sciences*, *109*(47), 19345–19350. https://doi.org/10.1073/pnas.1216128109

Lancaster, D., Haggarty, D., & Ban, N. (2015). Pacific Canada's Rockfish Conservation Areas: Using Ostrom's design principles to assess management effectiveness. *Ecology and Society*, *20*(3). https://doi.org/10.5751/ES-07815-200341

Larson, W. A., Limborg, M. T., McKinney, G. J., Schindler, D. E., Seeb, J. E., & Seeb, L. W. (2017). Genomic islands of divergence linked to ecotypic variation in Sockeye salmon. *Molecular Ecology*, *26*(2), 554–570. https://doi.org/10.1111/mec.13933

Li, H., & Ralph, P. (2019). Local PCA Shows How the Effect of Population Structure Differs Along the Genome. *Genetics*, *211*(1), 289–304. https://doi.org/10.1534/genetics.118.301747

Li, R., Li, Y., Fang, X., Yang, H., Wang, J., Kristiansen, K., & Wang, J. (2009). SNP detection for massively parallel whole-genome resequencing. *Genome Research*, *19*(6), 1124–1132. https://doi.org/10.1101/gr.088013.108

Licandeo, R., Duplisea, D.E., Senay, C., Marentette, J.R., and McAllister, M.K. 2020. Management strategies for spasmodic stocks: a Canadian Atlantic redfish fishery case study. Canadian Journal of Fisheries and Aquatic Sciences 77(4): 684-702. doi:10.1139/cjfas-2019-0210.

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., Head, M. A., Parker-Stetter, S. L., Taylor, I. G., Tuttle, V. J., Billings, A. A., Gauthier, S., McClure, M. M., & Nichols, K. M. (2024). Population genomics of coastal Pacific Hake. *North American Journal of Fisheries Management*, *44*(1), 222–234. https://doi.org/10.1002/nafm.10969

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

Lotterhos, K. E., Dick, S. J., & Haggarty, D. R. (2014). Evaluation of rockfish conservation area networks in the United States and Canada relative to the dispersal distance for black rockfish (*Sebastes melanops*). *Evolutionary Applications*, *7*(2), 238–259. https://doi.org/10.1111/eva.12115

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

Ma, J., & Amos, C. I. (2012). Principal Components Analysis of Population Admixture. *PLOS ONE*, *7*(7), e40115. https://doi.org/10.1371/journal.pone.0040115

Malinsky, M., Matschiner, M., & Svardal, H. (2021). Dsuite—Fast D-statistics and related admixture evidence from VCF files. *Molecular Ecology Resources*, *21*(2), 584–595. https://doi.org/10.1111/1755-0998.13265

Marchi, N., Schlichta, F., & Excoffier, L. (2021). Demographic inference. *Current Biology*, *31*(6), R276–R279. https://doi.org/10.1016/j.cub.2021.01.053

Marsh, J. I., & Johri, P. (2024). Biases in ARG-Based Inference of Historical Population Size in Populations Experiencing Selection. *Molecular Biology and Evolution*, *41*(7), msae118. https://doi.org/10.1093/molbev/msae118

Martin, S. H., Davey, J. W., Salazar, C., & Jiggins, C. D. (2019). Recombination rate variation shapes barriers to introgression across butterfly genomes. *PLOS Biology*, *17*(2), e2006288. https://doi.org/10.1371/journal.pbio.2006288

Matthews, K. R. (1990). An experimental study of the habitat preferences and movement patterns of Copper, Quillback, and Brown rockfishes (*Sebastes* spp.). *Environmental Biology of Fishes*, *29*(3), 161–178. https://doi.org/10.1007/BF00002217

McGreer, M., & Frid, A. (2017). Declining size and age of rockfishes (*Sebastes* spp.) inherent to Indigenous cultures of Pacific Canada. *Ocean & Coastal Management*, *145*, 14–20. https://doi.org/10.1016/j.ocecoaman.2017.04.019

McMahon, B. J., Teeling, E. C., & Höglund, J. (2014). How and why should we implement genomics into conservation? *Evolutionary Applications*, *7*(9), 999–1007. https://doi.org/10.1111/eva.12193

McVean, G. (2007). The Structure of Linkage Disequilibrium Around a Selective Sweep. *Genetics*, *175*(3), 1395–1406. https://doi.org/10.1534/genetics.106.062828

Meyer, A., & Lydeard, C. (1997). The evolution of copulatory organs, internal fertilization, placentae and viviparity in killifishes (Cyprinodontiformes) inferred from a DNA phylogeny of the tyrosine kinase gene X-src. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *254*(1340), 153–162. https://doi.org/10.1098/rspb.1993.0140

Miller, J. A., & Shanks, A. L. (2004). Evidence for limited larval dispersal in black rockfish (*Sebastes melanops*): Implications for population structure and marine-reserve design. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(9), 1723–1735. https://doi.org/10.1139/f04-111

Minh, B. Q., Nguyen, M. A. T., & von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution*, *30*(5), 1188–1195. https://doi.org/10.1093/molbev/mst024

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, *37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Montanari, S. R., van Herwerden, L., Pratchett, M. S., Hobbs, J.-P. A., & Fugedi, A. (2012). Reef fish hybridization: Lessons learnt from butterflyfishes (genus *Chaetodon*). *Ecology and Evolution*, *2*(2), 310–328. https://doi.org/10.1002/ece3.83

Moran, B. M., Payne, C., Langdon, Q., Powell, D. L., Brandvain, Y., & Schumer, M. (2021). The genomic consequences of hybridization. *eLife*, *10*, e69016. https://doi.org/10.7554/eLife.69016

Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *1*(1), 2–9. https://doi.org/10.1016/0027-5107(64)90047-8

Mullins, R. B., McKeown, N. J., Sauer, W. H. H., & Shaw, P. W. (2018). Genomic analysis reveals multiple mismatches between biological and management units in Yellowfin Tuna

(*Thunnus albacares*). *ICES Journal of Marine Science*, *75*(6), 2145–2152. https://doi.org/10.1093/icesjms/fsy102

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

Narum, S. R., Buonaccorsi, V. P., Kimbrell, C. A., & Vetter, R. D. (2004). Genetic Divergence between Gopher Rockfish (*Sebastes carnatus*) and Black and Yellow Rockfish (Sebastes chrysomelas). *Copeia*, *2004*(4), 926–931. https://doi.org/10.1643/CG-02-061R2

Narum, S. R., Horn, R., Willis, S., Koch, I., & Hess, J. (2023). Genetic variation associated with adult migration timing in lineages of Steelhead and Chinook Salmon in the Columbia River. *Evolutionary Applications*, *17*(2), e13626. https://doi.org/10.1111/eva.13626

Natasha, J., Stockwell, B. L., Marie, A. D., Hampton, J., Smith, N., Nicol, S., & Rico, C. (2022). No Population Structure of Bigeye Tunas (*Thunnus obesus*) in the Western and Central Pacific Ocean Indicated by Single Nucleotide Polymorphisms. *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.799684

Nedoluzhko, A. (2023). Sea of opportunities: Marine genomics in an era of global environmental change. *BMC Genomics*, *24*(1), 286. https://doi.org/10.1186/s12864-023-09392-4

Nielsen, E. E., Hemmer-Hansen, J., Larsen, P. F., & Bekkevold, D. (2009). Population genomics of marine fishes: Identifying adaptive variation in space and time. *Molecular Ecology*, *18*(15), 3128–3150. https://doi.org/10.1111/j.1365-294X.2009.04272.x

Nielsen, E. S., Hanson, J. O., Carvalho, S. B., Beger, M., Henriques, R., Kershaw, F., & von der Heyden, S. (2022). Molecular ecology meets systematic conservation planning. *Trends in Ecology & Evolution*, *38*(2). https://doi.org/10.1016/j.tree.2022.09.006

Norris, L. C., Main, B. J., Lee, Y., Collier, T. C., Fofana, A., Cornel, A. J., & Lanzaro, G. C. (2015). Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proceedings of the National Academy of Sciences*, *112*(3), 815–820. https://doi.org/10.1073/pnas.1418892112

Okonechnikov, K., Conesa, A., & García-Alcalde, F. (2016). Qualimap 2: Advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics*, *32*(2), 292–294. https://doi.org/10.1093/bioinformatics/btv566

Olave, M., Nater, A., Kautt, A. F., & Meyer, A. (2022). Early stages of sympatric homoploid hybrid speciation in crater lake cichlid fishes. *Nature Communications*, *13*(1), Article 1. https://doi.org/10.1038/s41467-022-33319-4

Pauly, D., Christensen, V., Guénette, S., Pitcher, T. J., Sumaila, U. R., Walters, C. J., Watson, R., & Zeller, D. (2002). Towards sustainability in world fisheries. *Nature*, *418*(6898), 689–695. https://doi.org/10.1038/nature01017

Peart, C. R., Tusso, S., Pophaly, S. D., Botero-Castro, F., Wu, C.-C., Aurioles-Gamboa, D., Baird, A. B., Bickham, J. W., Forcada, J., Galimberti, F., Gemmell, N. J., Hoffman, J. I., Kovacs, K. M., Kunnasranta, M., Lydersen, C., Nyman, T., de Oliveira, L. R., Orr, A. J., Sanvito, S., … Wolf, J. B. W. (2020). Determinants of genetic variation across eco-evolutionary scales in pinnipeds. *Nature Ecology & Evolution*, *4*(8), 1095–1104. https://doi.org/10.1038/s41559-020-1215-5

Pinsky, M. L., & Palumbi, S. R. (2014). Meta-analysis reveals lower genetic diversity in overfished populations. *Molecular Ecology*, *23*(1), 29–39. https://doi.org/10.1111/mec.12509

Pribyl, A. L., Schreck, C. B., Kent, M. L., Kelley, K. M., & Parker, S. J. (2012). Recovery potential of Black Rockfish, *Sebastes melanops* Girard, recompressed following barotrauma. *Journal of Fish Diseases*, *35*(4), 275–286. https://doi.org/10.1111/j.1365-2761.2012.01345.x

Price, T. (1998). Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *353*(1366), 251–260. https://doi.org/10.1098/rstb.1998.0207

Prince, D. J., O'Rourke, S. M., Thompson, T. Q., Ali, O. A., Lyman, H. S., Saglam, I. K., Hotaling, T. J., Spidle, A. P., & Miller, M. R. (2017). The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Science Advances*, *3*(8), e1603198. https://doi.org/10.1126/sciadv.1603198

Puncher, G. N., Cariani, A., Maes, G. E., Van Houdt, J., Herten, K., Cannas, R., Rodriguez-Ezpeleta, N., Albaina, A., Estonba, A., Lutcavage, M., Hanke, A., Rooker, J., Franks, J. S., Quattro, J. M., Basilone, G., Fraile, I., Laconcha, U., Goñi, N., Kimoto, A., … Tinti, F. (2018). Spatial dynamics and mixing of bluefin tuna in the Atlantic Ocean and Mediterranean Sea revealed using next-generation sequencing. *Molecular Ecology Resources*, *18*(3), 620–638. https://doi.org/10.1111/1755-0998.12764

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

Python Software Foundation. (2023). *Python* (Version 3.10.10). https://www.python.org

Quilodrán, C. S., Ruegg, K., Sendell-Price, A. T., Anderson, E. C., Coulson, T., & Clegg, S. M. (2020). The multiple population genetic and demographic routes to islands of genomic divergence. *Methods in Ecology and Evolution*, *11*(1), 6–21. https://doi.org/10.1111/2041-210X.13324

R Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rankin, P. S., Hannah, R. W., Blume, M. T. O., Miller-Morgan, T. J., & Heidel, J. R. (2017). Delayed effects of capture-induced barotrauma on physical condition and behavioral competency of recompressed Yelloweye Rockfish, *Sebastes ruberrimus*. *Fisheries Research*, *186*, 258–268. https://doi.org/10.1016/j.fishres.2016.09.004

Rellstab, C., Zoller, S., Walthert, L., Lesur, I., Pluess, A. R., Graf, R., Bodénès, C., Sperisen, C., Kremer, A., & Gugerli, F. (2016). Signatures of local adaptation in candidate genes of oaks (*Quercus* spp.) with respect to present and future climatic conditions. *Molecular Ecology*, *25*(23), 5907–5924. https://doi.org/10.1111/mec.13889

Renaut, S., Grassa, C. J., Yeaman, S., Moyers, B. T., Lai, Z., Kane, N. C., Bowers, J. E., Burke, J. M., & Rieseberg, L. H. (2013). Genomic islands of divergence are not affected by geography of speciation in sunflowers. *Nature Communications*, *4*(1), 1827. https://doi.org/10.1038/ncomms2833

Rosser, N., Seixas, F., Queste, L. M., Cama, B., Mori-Pezo, R., Kryvokhyzha, D., Nelson, M., Waite-Hudson, R., Goringe, M., Costa, M., Elias, M., Mendes Eleres de Figueiredo, C.,

Freitas, A. V. L., Joron, M., Kozak, K., Lamas, G., Martins, A. R. P., McMillan, W. O., Ready, J., … Dasmahapatra, K. K. (2024). Hybrid speciation driven by multilocus introgression of ecological traits. *Nature*, *628*(8009), 811–817. https://doi.org/10.1038/s41586-024-07263-w

Sadler, D. E., Sävilammi, T., van Dijk, S. N., Watts, P. C., & Uusi-Heikkilä, S. (2024). Size-selective harvesting drives genomic shifts in a harvested population. *Journal of Fish Biology*, *n/a*(n/a). https://doi.org/10.1111/jfb.15901

Samuk, K., Owens, G. L., Delmore, K. E., Miller, S. E., Rennison, D. J., & Schluter, D. (2017). Gene flow and selection interact to promote adaptive divergence in regions of low recombination. *Molecular Ecology*, *26*(17), 4378–4390. https://doi.org/10.1111/mec.14226

Sankararaman, S., Mallick, S., Dannemann, M., Prüfer, K., Kelso, J., Pääbo, S., Patterson, N., & Reich, D. (2014). The genomic landscape of Neanderthal ancestry in present-day humans. *Nature*, *507*(7492), 354–357. https://doi.org/10.1038/nature12961

Schluter, D. (2001). Ecology and the origin of species. *Trends in Ecology & Evolution*, *16*(7), 372–380. https://doi.org/10.1016/S0169-5347(01)02198-X

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. (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

Siegle, M. R., Taylor, E. B., Miller, K. M., Withler, R. E., & Yamanaka, K. L. (2013). Subtle Population Genetic Structure in Yelloweye Rockfish (*Sebastes ruberrimus*) Is Consistent with a Major Oceanographic Division in British Columbia, Canada. *PLOS ONE*, *8*(8), e71083. https://doi.org/10.1371/journal.pone.0071083

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.-H., Nachman, M. W., & Kohn, M. H. (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

Tange, O. (2023, May 22). GNU Parallel 20230522 ('Charles'). *Zenodo*, 42–47.

Tarpey, C. M., Seeb, J. E., McKinney, G. J., Templin, W. D., Bugaev, A., Sato, S., & Seeb, L. W. (2018). Single-nucleotide polymorphism data describe contemporary population structure and diversity in allochronic lineages of Pink salmon (*Oncorhynchus gorbuscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(6), 987–997. https://doi.org/10.1139/cjfas-2017-0023

Therkildsen, N. O., Wilder, A. P., Conover, D. O., Munch, S. B., Baumann, H., & Palumbi, S. R. (2019). Contrasting genomic shifts underlie parallel phenotypic evolution in response to fishing. *Science*, *365*(6452), 487–490. https://doi.org/10.1126/science.aaw7271

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Tigano, A., & Russello, M. A. (2022). The genomic basis of reproductive and migratory behaviour in a polymorphic salmonid. *Molecular Ecology*, *31*(24), 6588–6604. https://doi.org/10.1111/mec.16724

Tigano, A., Weir, T., Ward, H. G. M., Gale, M. K., Wong, C. M., Eliason, E. J., Miller, K. M., Hinch, S. G., & Russello, M. A. (2024). Genomic vulnerability of a freshwater salmonid under climate change. Evolutionary Applications, 17(2), e13602. https://doi.org/10.1111/eva.13602

Todesco, M., Owens, G. L., Bercovich, N., Légaré, J.-S., Soudi, S., Burge, D. O., Huang, K., Ostevik, K. L., Drummond, E. B. M., Imerovski, I., Lande, K., Pascual-Robles, M. A., Nanavati, M., Jahani, M., Cheung, W., Staton, S. E., Muños, S., Nielsen, R., Donovan, L. A., … Rieseberg, L. H. (2020). Massive haplotypes underlie ecotypic differentiation in sunflowers. *Nature*, *584*(7822), Article 7822. https://doi.org/10.1038/s41586-020-2467-6

Tolimieri, N., Andrews, K., Williams, G., Katz, S., & Levin, P. S. (2009). Home range size and patterns of space use by lingcod, Copper rockfish and Quillback rockfish in relation to diel and tidal cycles. *Marine Ecology Progress Series*, *380*, 229–243. https://doi.org/10.3354/meps07930

Tovar Verba, J., Ferreira, C. E. L., Pennino, M. G., Hagberg, L., Lopes, P. F. M., Padovani Ferreira, B., Maia Queiroz Lima, S., & Stow, A. (2023). Genetic structure of the threatened Gray Parrotfish (*Sparisoma axillare*) in the Southwestern Atlantic. *Coral Reefs*, *42*(1), 105–117. https://doi.org/10.1007/s00338-022-02324-w

Tovar Verba, J., Stow, A., Bein, B., Pennino, M. G., Lopes, P. F. M., Ferreira, B. P., Mortier, M., Maia Queiroz Lima, S., & Pereira, R. J. (2022). Low population genetic structure is consistent with high habitat connectivity in a commercially important fish species (*Lutjanus jocu*). *Marine Biology*, *170*(1), 5. https://doi.org/10.1007/s00227-022-04149-1

Vasimuddin, Md., Misra, S., Li, H., & Aluru, S. (2019). Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. *2019 IEEE International Parallel*

and Distributed Processing Symposium (IPDPS), 314–324. https://doi.org/10.1109/IPDPS.2019.00041

Wall, L., Christiansen, T., & Orwant, J. (2020). *Perl v5.32.1*. https://www.perl.org

Waples, R. (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

Waples, R. S., & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*(6), 1419–1439. https://doi.org/10.1111/j.1365-294X.2006.02890.x

Weist, P., Jentoft, S., Tørresen, O. K., Schade, F. M., Pampoulie, C., Krumme, U., & Hanel, R. (2022). The role of genomic signatures of directional selection and demographic history in the population structure of a marine teleost with high gene flow. *Ecology and Evolution*, *12*(12), e9602. https://doi.org/10.1002/ece3.9602

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

Westram, A. M., Faria, R., Johannesson, K., Butlin, R., & Barton, N. (2022). Inversions and parallel evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *377*(1856), 20210203. https://doi.org/10.1098/rstb.2021.0203

Willi, Y., Kristensen, T. N., Sgro, C. M., Weeks, A. R., Orsted, M., & Hoffman, A. A. (2021). Conservation genetics as a management tool: The five best-supported paradigms to assist

the management of threatened species. *PNAS*, *119*(1), e2105076119. https://doi.org/10.1073/pnas.2105076119

Wolf, J. B. W., & Ellegren, H. (2017). Making sense of genomic islands of differentiation in light of speciation. *Nature Reviews Genetics*, *18*(2), 87–100. https://doi.org/10.1038/nrg.2016.133

Woodings, L. N., Murphy, N. P., Liggins, G. W., Miller, M. E., Ballinger, G. M., Lau, S. C. Y., & Strugnell, J. M. (2021). Population genomics of the Eastern Rock Lobster, *Sagmariasus verreauxi*, during spawning stock recovery from over-exploitation. *ICES Journal of Marine Science*, *78*(7), 2448–2459. https://doi.org/10.1093/icesjms/fsab117

Wray, A., Petrou, E., Nichols, K. M., Pacunski, R., LeClair, L., Andrews, K. S., Kardos, M., & Hauser, L. (2024). Contrasting effect of hybridization on genetic differentiation in three rockfish species with similar life history. *Evolutionary Applications*, *17*(7), e13749. https://doi.org/10.1111/eva.13749

Xuereb, A., Rougemont, Q., Dallaire, X., Moore, J.-S., Normandeau, E., Bougas, B., Perreault-Payette, A., Koop, B. F., Withler, R., Beacham, T., & Bernatchez, L. (2022). Re-evaluating Coho salmon (*Oncorhynchus kisutch*) conservation units in Canada using genomic data. *Evolutionary Applications*, *15*(11), 1925–1944. https://doi.org/10.1111/eva.13489

Yáñez, J. M., Newman, S., & Houston, R. D. (2015). Genomics in aquaculture to better understand species biology and accelerate genetic progress. *Frontiers in Genetics*, *6*. https://doi.org/10.3389/fgene.2015.00128

Zabel, R. W., Levin, P. S., Tolimieri, N., & Mantua, N. J. (2011). Interactions between climate and population density in the episodic recruitment of bocaccio, *Sebastes paucispinis*, a

Pacific rockfish. *Fisheries Oceanography*, *20*(4), 294–304. https://doi.org/10.1111/j.1365-2419.2011.00584.x

Zhang, Y., Luan, P., Ren, G., Hu, G., & Yin, J. (2020). Estimating the inbreeding level and genetic relatedness in an isolated population of critically endangered Sichuan taimen (*Hucho bleekeri*) using genome-wide SNP markers. *Ecology and Evolution*, *10*(3), 1390–1400. https://doi.org/10.1002/ece3.5994

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

Zhu, B., Gao, T., He, Y., Qu, Y., & Zhang, X. (2024). Population Genomics of Commercial Fish *Sebastes schlegelii* of the Bohai and Yellow Seas (China) Using a Large SNP Panel from GBS. *Genes*, *15*(5), Article 5. https://doi.org/10.3390/genes15050534