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PATTERNS OF GENETIC AND PHENOTYPIC DIFFERENCES AMONG CALIFORNIA CENTRAL VALLEY SALMONID SPECIES STEELHEAD AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) POPULATIONS

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by

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Abstract

PATTERNS OF GENETIC AND PHENOTYPIC DIFFERENCES AMONGST CALIFORNIA CENTRAL VALLEY SALMONID SPECIES STEELHEAD AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) POPULATIONS

Laura Conlin Goetz

Riverscapes and landscapes vary over spatial and temporal scales, and contribute to species' formation of phenotypic patterns in response. Landscape genetics investigates these phenomena by correlating genetic and environmental variation. Discerning lineage and environmental influences on important and complex phenotypic traits is further complicated, however, by human-driven habitat modification and fragmentation. This dissertation explores the neutral and adaptive genetic relationships of the rainbow trout and steelhead (*Oncorhynchus mykiss*) populations dispersed in California's Central Valley, a riverscape highly modified by the construction of many hydroelectric dams for agricultural and urban development. The first chapter of this dissertation established a baseline for steelhead spawned at the four Central Valley hatchery programs from 2011–2019 using parentage–based tagging to collect information on age and family sizes. This chapter confirmed significant genetic and phenotypic differences exist between CV and Nimbus hatchery programs because Nimbus was established with a population outside the CV. It also explored temporal variation in genetic and phenotypic patterns. The second chapter is a case study investigating population dynamics of *O. mykiss* in segmented watershed located between two major *O. mykiss* lineage boundaries. Utilization of a newly developed microhaplotype panel with neutral and adaptive markers enabled characterization of molecular population composition above and below an impassable barrier by comparing with over 30 reference populations. The third chapter analyzed significance of adaptive genotype and phenotype (sampling date) in *O. mykiss* trapped in the Sacramento River. The results from this chapter supported previous analyses that found significant genetic and phenotypic differences

between CV and coastal populations, and little to no association between adaptive genotype and expressed phenotype in CV-lineage individuals. Combined, the results from this dissertation inform management of *O. mykiss* genetic and phenotypic patterns amongst populations, and water use impacts on their continued viability, as well as provide a more comprehensive understanding of standing neutral and adaptive genetic variation in the CCV *O. mykiss* populations.

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about my work and different analyses that are possible - it helps shake me out of the genetics tunnel vision.

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Studying evolutionary biology within a single region has given me the space to deeply appreciate the effects of land use history on industrial and cultural uses. With this in mind, I would like to acknowledge my own personal family history of benefiting from mistreatment and displacement of others. Both sides of my family immigrated to the United States in the latter half of the 1800s: my mother's ancestors moved from Ireland to escape the Potato Famine genocide and my father's ancestors moved from Bavaria after the failed German revolutions. Both sides quickly settled in different states in the midwest: Mainly Minnesota for my Irish ancestors and Iowa for my German ancestors. These lands were cared for by tribes such as the Dakota, Ojibwe, Ioway, and Ho-Chunk. I humbly acknowledge that without the atrocities committed by the American government, my early family would not have lived the lives they did in the midwest, leading to my own existence. I was born and raised in Minneapolis, Minnesota. Though I have always deeply loved the beautiful lakes, trees, and plains of my home state, I am more acutely aware of the people who resided here before me, and what a privilege it was to live there. From Minneapolis, Minnesota, I moved to Boston, Massachusetts for my undergraduate degree, where I lived for 6 years on land taken from the Massachusett, Wampanoag (or Pokantoket), and Pawtucket (or Penacook) tribes amongst other smaller bands. Finally, for my graduate degree, I moved to Santa Cruz and Mountain View, California. Here, I have benefited professionally and personally from lands cared for originally by both the Awaswas-speaking Uypi and Ohlone Tribes. I would not be the person I am today without my time spent in these three very different regions, and I am incredibly grateful for the privilege to live on and enjoy the unique beauty that characterizes each place.

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Introduction

The increasing availability of molecular genetic tools has deepened our knowledge and understanding of conservation biology and management. Genetic data have particularly increased our ability to assess how species and populations vary genetically and phenotypically across a spatially and temporally heterogeneous landscape (Manel et al. 2003; Robinson et al. 2012; Manel & Holderegger 2013; Gray et al. 2014; Rougemont et al. 2021). Landscape genetics, the significant correlation between heterogeneous environments and the distribution of genetic variation within species and populations (Manel et al. 2003), has been widely documented across species, including mammals (California mule deer, Pease et al. 2009; white-tailed deer, Robinson et al. 2012), plants (dune sunflower, Andrew et al. 2012, prairie grass, Gray et al. 2014), and fish (electric fish, Cooke et al. 2014; brook lamprey, Rougemont et al. 2020). The connection between genotype and environmental interactions underlying phenotypic expression is often unclear, and a population's gene pool may have unknown genetic architecture for an adaptive trait because populations express the alternative ecotype based on environmental conditions (Stronen et al. 2021; Waples et al. 2022). This can be further complicated when environmental conditions vary temporally and/or spatially.

Environmental variation influences the expression of plastic traits and contributes to genetic diversity, sometimes resulting in populations developing and retaining distinct phenotypes known as "ecotypes" (Le Moan et al. 2016; Stronen et al. 2021). These alternative phenotypes provide species with additional adaptive responses to abiotic change. However, human-driven activity has influenced the expression of ecotypic traits by rapidly fragmenting habitats and altering local environmental cues (Urban 2015; Scheffer et al. 2016; Johnson & Munshi-South 2017; Reid et al. 2018; Dudgeon 2019; Brauer & Beheregaray 2020; Bury & Zajac 2020; Puckett et al. 2020). These habitat changes present unique

consequences when considering effects on the patterns of ecotypic expression because altering habitats can promote the expression of one ecotype over another (Urban 2015; Scheffers et al. 2016; Stronen et al. 2021; Zarri et al. 2022). Effective conservation management in vulnerable species hinges on correctly characterizing and tracking life history patterns and the spectrum of their distributions over heterogeneous habitats, particularly in important declining adaptive traits. However, how genetic variation contributes to conservation in practice is unclear.

One species that presents a rich case study for this discussion is rainbow trout and steelhead, or *Oncorhynchus mykiss,* a highly regulated species in its native range. This Pacific salmonid species features distinct ecotypes with different common names based on if they migrate or not. The anadromous ecotype, known as steelhead, undergoes smoltification to migrate to marine habitat, then return to spawn in freshwater after one or more years at sea (Hoar 1988). Rainbow trout reside in freshwater, and often interbreed with returning steelhead. *O. mykiss* lineages typically feature alternative migration strategies within a single population, with varying proportions in expression of anadromy or residency (Nichols et al. 2008, Olsen et al. 2006; Ohms et al. 2013, Pearse et al. 2014, Sloat and Reeves 2014, Kendall et al. 2015, Phillis et al. 2016). Additionally, there are varying proportions of other important life history traits like when individuals time their return to freshwater, known as “run timing.” Individuals from the same population may return to spawning grounds before spawning season begins as sexually immature (“early”, or “summer-run”), just before spawning season begins as sexually mature (“late”, or “winter-run”), or at an intermediate time (Quinn et al. 2016). Phenotypic expression of this complex collaboration between plastic spectrums from different life history traits in *O. mykiss* depends on complex interactions between genetic and environmental influences (Kendall et al. 2015) to maintain its persistence across populations in this species. Thus, comparison of different genetic lineages inhabiting the same environment can help to elucidate the relative effects of genetic and environmental factors on expression of life-history traits.

Different important molecular polymorphisms have been associated with underlying life history traits related to anadromy vs. residency, as well as other important traits like development rate and run timing. One locus associated with migration is located on chromosome ***Omy05***. Characterized by Pearse et al. (2019), the 55-Mb double-inversion located on chromosome Omy05 suppresses recombination between hundreds of genes, many of which significantly influence traits linked to expression of anadromy (Miller et al. 2012; Pearse et al. 2014; Pearse et al. 2019). Comparison between *O. mykiss* with varying degrees of anadromy has shown that Omy05 karyotype (ancestral (A), rearranged (R)) is significantly associated with expression of anadromy or residency, such as growth and condition factors, coloration, and production of osmoregulatory enzymes involved in smoltification (Phillips et al. 2006; Nichols et al. 2008; Miller et al. 2012; Pearse et al. 2014; Pearse et al. 2019). Previous studies have repeatedly identified genes contained in Omy05's double inversion that dramatically influence migration strategy and maturation timing, such as those involved in adiposity, circadian rhythm, and age at maturity (Nichols et al. 2008; Pearse et al. 2019). The effects of Omy05 karyotype on these traits varies in natural and hatchery trout, depending on both internal and external cues (Miller et al. 2012; Pearse et al. 2014; Arostegui et al. 2019; Pearse et al. 2019). Omy05 karyotype exhibits partial dominance by differentially influencing female and male migration strategies, with heterozygous females more likely to migrate and heterozygous males more likely to reside in freshwater within a single population (Pearse et al. 2019). Possible external cues include temperature, evidenced by a strong latitudinal cline with R haplotypes favored in colder climates (Miller et al. 2012; Pearse et al. 2019). This dissertation included Omy05 genotypes in analyses using either/both SNP and microhaplotype panels depending on Chapter and populations (Abadía-Cardoso et al. 2013; Pearse et al. 2014; Le Gall et al. 2024).

Another adaptive trait significantly associated with genetic variation is return timing in salmonids, or when an adult fish enters freshwater before spawning season (Quinn et al. 2016; Reed et al. 2017; Sinclair-Waters et al. 2020; Sinclair-Waters et al. 2022). Fish may

return earlier than most individuals (early), later than most (late), or at an intermediate time during spawning season. Individuals that return earlier are often able to utilize further inland habitat before spawning begins, but still interbreed with mid- or late-returning salmonids (Quinn et al. 2016; Prince et al. 2017). Return timing in salmonids is highly heritable (Abadía-Cardoso et al. 2013; Quinn et al. 2016; Beulke et al. 2023), and a single genomic region has been strongly associated with adults timing their entry to freshwater (Hess et al. 2016; Prince et al. 2017; Micheletti et al. 2018; Waples et al. 2022). This genomic region (herein referred to as ***GREB1L/ROCK1***), contains the genes greb1L and rock1, as well as the intergenic region between them. Variation within *GREB1L/ROCK1* has been associated with return timing (early, intermediate, late) in some Chinook salmon and some steelhead populations (Hess et al. 2016; Collins et al. 2020; Waples et al. 2022; Hugentobler et al. 2024). For this dissertation, *GREB1L/ROCK1* data was available as 16 various markers located within *GREB1L/ROCK1* including those collected across multiple studies located between 11,607,954 to 11,803,870 (Hess et al. 2016; Collins et al. 2020; Dayan et al. 2024; Le Gall et al. 2024).

Environmental characteristics such as barriers to migration, streamflow, and stream temperature also influence whether an individual will migrate or not (Sloat & Reeves 2014; Phillis et al. 2016, Apgar et al. 2017; Mattocks et al. 2019) or if they will initiate returning as a summer- or winter-run (Quinn et al. 2016; Kannry et al. 2020; Fraik et al. 2021). Increasing difficulties in a life history strategy by raising the costs in the overall fitness tradeoff that impact each individual's unique strategy. The alterations to many Pacific streams, including homogenizing stream temperature and flow profiles, have contributed significantly to decreases in frequencies of native anadromous *O. mykiss* (Lindley et al. 2006; He & Marcinkevage 2017; Eschenroeder et al. 2022). This decline has prompted the initiation of anadromous steelhead semi-captive breeding programs aimed at conserving anadromous genetic architecture and mitigating the loss of migratory fish to hydroelectric dams.

My dissertation explored the genetic relationships within and among modern CCV *O. mykiss* populations. The strength of these associations, as well as predictive value amongst populations with: varying coastal vs. inland distances, different genetic lineages, and higher frequencies of heterozygotes within the CCV populations is unclear (Willis et al. 2020; Collins et al. 2022).

In **Chapter 1**, I considered hatchery production of CCV steelhead and investigated how differences among genetic lineages and environmental variation impacted life-history traits. The SWFSC genetics team genotyped 23,670 steelhead returning to the four California Central Valley hatcheries over nine years from 2011-2019, confidently assigning parentage to 13,576 individuals to determine age and date of spawning, and rates of iteroparity and repeat-spawning within each year. I found steelhead from different genetic lineages showed significant differences in adult life-history traits despite inhabiting similar environments. Differences between coastal and Central Valley steelhead lineages contributed to significant differences in age at return, timing of spawning, and rates of iteroparity amongst programs. Adaptive genomic variation associated with anadromy also varied among hatchery programs and was associated with age of steelhead spawners. Environmental variation likely contributed to variation in phenotypic patterns observed in each hatchery population, as our study period spanned a serious drought in California. Together, these results highlight the complex interacting effects of both genetic and environmental influences on expression of life-history traits in steelhead.

In **Chapter 2**, I investigated the population structure and distribution of adaptive genetic variation in the salmonid species *O. mykiss* throughout Putah Creek, a watershed near Napa Valley, California that was transformed by the 1950's construction of multiple dams and a large artificial reservoir (Lake Berryessa) between the central coast and California Central Valley lineage ranges. In addition, hatchery-raised *O. mykiss* have been introduced throughout the watershed over many years, which may have introduced genetic variation into existing Putah Creek populations. To explore the population structure and

distribution of adaptive genetic variation in Putah Creek, I analyzed microhaplotypes from eight *O. mykiss* populations above Lake Berryessa and two populations below Lake Berryessa, and compared them with 40 reference populations from Central Valley, coastal, inland, and hatchery rainbow trout lineages. We found distinct patterns of neutral and adaptive variation between populations above Lake Berryessa and those below. Populations below Lake Berryessa resembled various Central Valley populations and hatchery rainbow trout strains, while those above were more similar to coastal *O. mykiss* populations. Additionally, Putah Creek populations below Lake Berryessa possessed significantly different proportions of adaptive variants associated with life-history compared to populations above Lake Berryessa, consistent with studies in other populations located above and below barriers to migration.

Finally in **Chapter 3**, I utilized genotyped *O. mykiss* migrating upstream in the Sacramento River (n=790) with a SNP panel for parentage-based tagging identification with Central Valley hatchery steelhead broodstock and a microhaplotype panel for adaptive genetic variation associated with run timing in other steelhead populations to explore the association between GREB1L and Pacific salmonid species *O. mykiss* run timing. I found that strength of association between GREB1L genotype and run timing phenotype depends on population ancestry, with individuals from the Central Valley exhibiting little to no association between genotype and phenotype. These results confirm significant genetic and phenotypic differences maintained between Central Valley and central coast *O. mykiss* populations in the Sacramento River, as well as the potential for differences in strength of genetic and phenotypic association to vary by lineage of a species. My results also add to the discussion of the importance of adaptive genetic variation in conservation contexts by demonstrating limitations to genetic variation and phenotype associations across lineages.

Chapter 1

Genetic Parentage Reveals the (Un)Natural History of Central Valley Hatchery Steelhead:
The Genetic Monitoring of Central Valley Hatchery Steelhead

Authors: Laura C. Goetz, Hayley Nuetzel, David L. J. Vendrami, Anne K. Beulke, Eric C. Anderson, John Carlos Garza, Devon E. Pearse

Abstract

Populations composed of individuals descended from multiple distinct genetic lineages often feature significant differences in phenotypic frequencies. Understanding how populations maintain trait variation in a novel environment can inform conservation decisions, especially when one ecotype is disproportionately threatened. We considered hatchery production of steelhead, the migratory anadromous form of the salmonid species Oncorhynchus mykiss, and investigated how differences among genetic lineages and environmental variation impacted life-history traits. We genotyped 23,670 steelhead returning to the four California Central Valley hatcheries over nine years from 2011-2019, confidently assigning parentage to 13,576 individuals to determine age and date of spawning, and rates of iteroparity and repeat-spawning within each year. We found steelhead from different genetic lineages showed significant differences in adult life-history traits despite inhabiting similar environments. Differences between coastal and Central Valley steelhead lineages contributed to significant differences in age at return, timing of spawning, and rates of iteroparity amongst programs. Adaptive genomic variation associated with anadromy also varied among hatchery programs and was associated with age of steelhead spawners. Environmental variation likely contributed to variation in phenotypic patterns observed in each hatchery population, as our study period spanned a serious drought in California. Our results highlight the complex interacting effects of both genetic and environmental influences on expression of life-history traits in steelhead.

Introduction

Species in environments with high temporal or spatial variation may express multiple potential phenotypic responses to the dynamic biotic and abiotic cues they receive from their current environment (Sommer 2020; Yamamichi 2022). In traits with conditional plasticity, environmental conditions trigger a genetically-encoded threshold that produces the more appropriate phenotype (Buoro et al. 2012; Phillis et al. 2016; Sommer 2020). In species with populations that have been transplanted by humans, how dynamic traits respond depends on the relative response of genetic variation developed in the previous environment to new environmental cues (Yamamichi 2022). Thus, understanding how species evolve and maintain trait variation can inform conservation decisions, especially when alternative phenotypes experience different ecological risks (Fox et al. 2019; Pazzaglia et al. 2021; Reid & Acker 2021). This is particularly relevant in the context of climate change and anthropogenic modifications altering environmental conditions.

Steelhead, the migratory anadromous form of the salmonid species Oncorhynchus mykiss, exhibits plasticity in numerous life-history traits. Steelhead undergo complex phenotypic, behavioral, and physiological modifications enabling migration from their natal streams to the ocean, where they mature for at least one year before returning to their natal streams to spawn. Unlike most other anadromous salmonid species that die following their first reproduction (Christie et al. 2018), O. mykiss is an iteroparous species. Individuals may survive through multiple reproductive events, and the frequency of this trait varies among populations. Populations often contain multiple life-history strategies in dynamic proportions, with alternative ecotypes frequently interbreeding (Nichols et al. 2008; Olsen et al. 2006; Satterthwaite et al. 2009; Ohms et al. 2013; Pearse et al. 2014; Sloat and Reeves 2014; Kendall et al. 2015; Phillis et al. 2016; Waples et al. 2022).

Plasticity in migration strategy, timing, and number of reproductive events increases adaptability of O. mykiss populations through sex-specific individual fitness tradeoffs related to spawn timing and growth (Fleming & Reynolds 2004; Hendry et al. 2004; Sogard et al.

2012; Kendall et al. 2015; Christie et al. 2018). The degree of plasticity and variation in traits within a single population make it challenging to determine how individual O. mykiss incorporate genetic and environmental information in life history development. Both evolutionary and ecological mechanisms influence life history expression of O. mykiss (Kendall et al. 2015; Phillis et al. 2016; Pearse et al. 2019), and important adaptive genomic variation has been identified for key life-history traits, such as migration timing (Hess et al. 2016; Waples et al. 2022). Numerous genetic loci have been identified that are associated with life-history variation in O. mykiss, including a 55-Mb double-inversion located on chromosome Omy05 associated with multiple growth and development traits and sex-specific migration strategies (Nichols et al. 2008; Miller et al. 2012; Pearse et al. 2014, 2019; Arostegui et al. 2019). Omy05 features ancestral (A) and rearranged (R) variations that have been associated with expression of anadromy and residency, respectively, in O. mykiss (Nichols et al. 2008; Miller et al. 2012; Pearse & Garza 2014; Pearse et al. 2019). Thus, comparison of different genetic lineages inhabiting the same environment can help to elucidate the relative effects of genetic and environmental factors on expression of life-history traits.

California is composed of multiple microhabitats with distinct environmental conditions, and comprises the southern extent of the native range of O. mykiss (Satterthwaite et al. 2010; Sogard et al. 2012). The construction of dams, which form impassable barriers to spawning habitat and modify natural streamflows, has contributed to the decline of anadromous O. mykiss and other native fishes. This decline prompted the creation and maintenance of hatchery populations to support anadromous fish in their native ranges. Anadromous salmonid hatcheries most often operate as semi-captive populations; juveniles are reared on-site and released to migrate and mature in the ocean before returning as adults that are manually spawned at the hatchery. In the California Central Valley (CCV), four hatchery programs rear and release steelhead in highly-regulated watersheds below dams. While regulation of CCV streams by dams homogenize stream flows throughout the year

(Sogard et al. 2012), the CCV displays higher temporal and spatial variation in stream flow and temperature, lower rainfall, higher summer temperatures, and high variation among watersheds (Satterthwaite et al. 2009; Sogard et al. 2012) as compared to coastal California habitats. Different O. mykiss life histories compositions amongst rivers have thus been predicted to be present in the CCV based on this high environmental variability (Satterthwaite et al. 2009; Sogard et al. 2012).

In this study, we focus on the genetic characterization of steelhead from the four hatchery populations in the CCV, three of which were founded from local sources, with the fourth founded with fish from a distinct genetic lineage (see "Study System," below). We collected nonlethal fin clips from every spawning steelhead from 2011-2019, including during the record-setting 2012-2016 drought. These fin clips enabled both population genetic analysis and parentage-based tagging (PBT; Anderson & Garza 2006), which has been successfully employed to understand and manage anadromous fish populations (Abadía-Cardoso et al. 2013; Evans et al. 2018; Horn et al. 2022). We reconstructed a pedigree with 13,576 parent-offspring trios and 19,043 unique adult steelhead representing virtually all steelhead spawned at four CCV hatcheries over nine years, spanning two to three generations. These data allowed us to describe patterns of iteroparity, age at spawning, and migration (straying) of hatchery steelhead over almost a decade, highlighting the interaction between genetic and environmental factors influencing important life-history traits. We also considered significance of Omy05 haplotype amongst these features. Our results highlight how the presence of multiple genetic lineages influences phenotypic patterns in CCV hatchery steelhead and how environmental variation impacts those patterns.

Methods

Study System

The CCV contains the Sacramento-San Joaquin River system, a highly impacted region that occupies the central part of California (Figure 1). This low-elevation area has warmer

seasonal temperatures compared with northern O. mykiss habitats (McEwan 2001; Eschenroeder et al. 2022). Landscape and hydrograph alterations from dams built over more than a century have reduced access to over 80% of previous salmonid spawning grounds, and homogenized temperature and flow profiles, contributing to decreased numbers of anadromous steelhead (Lindley et al. 2006; He & Marcinkevage 2017; Eschenroeder et al. 2022). Four hatchery programs produce steelhead in the CCV to mitigate these effects: Coleman National Fish Hatchery (CH), Feather River Hatchery (FRH), Mokelumne River Hatchery (MRH), and Nimbus Hatchery (NH; Figure 1). Situated on different tributaries of the Sacramento River, below major dams, these four hatcheries capture and spawn returning adult steelhead, incubate the eggs, and rear and release hundreds of thousands of marked (adipose-fin removed) hatchery-produced juveniles each year (California HSRG 2012).

The steelhead spawned at CH, FRH, and MRH were all derived from local CCV populations ('CV-lineage'), and are part of the California Central Valley Distinct Population Segment (DPS) under the Endangered Species Act (NMFS 2006, 2020). CH, FRH, and MRH are operated as 'integrated' hatchery programs that attempt to incorporate a minimum of 10% natural origin broodstock (pNOB) every generation (California HSRG 2012). Inclusion of wild-born individuals is intended to promote higher genetic diversity within broodstock programs and maintain variation underlying local adaptations (Mobrand et al. 2005). While CH is genetically distinct, FRH and MRH broodstock were previously shown to be almost genetically identical due to increased transfers of FRH eggs from 2002-2007, when steelhead returns were low at MRH (Del Real et al. 2012; Pearse & Garza 2015).

Unlike the other three hatcheries, the NH broodstock was founded by importation of eggs from coastal steelhead populations beginning in the 1950's, shortly after the construction of Nimbus Dam (California HSRG 2012). Consequently, NH steelhead are more genetically similar to coastal steelhead populations than the CV-lineage hatchery steelhead (Pearse and Garza 2015). For this reason, NH broodstock are not included in the CCV steelhead DPS, and are managed as a 'segregated' program that does not incorporate

unmarked (natural-origin) fish (McEwan 2001; NMFS 2006, 2020). However, this does not prevent NH broodstock from spawning in the wild with each other or with listed CV-lineage steelhead. It is also possible that steelhead migrants from the CCV hatcheries could be spawned at Nimbus, although they are phenotypically distinct and efforts are made to visually identify and exclude them from the broodstock.

Steelhead begin returning to CCV hatcheries in late October and continue through late March. Spawning is typically conducted between December and February, but varies among programs (Figure S1). Not all steelhead that return to a hatchery are spawned. All hatcheries attempt to exclude non-anadromous O. mykiss (freshwater resident rainbow trout) by spawning only fish larger than 16 inches (40.64 cm). Rarely, hatchery staff exclude some returning steelhead from spawning because they a phenotypically distinct (a notable case occurred in 2017 at NH when 166 returning fish were not spawned because they were phenotypically dissimilar from NH broodstock, with later genetic analyses confirming these fish were migrants from MRH). The integrated CV-lineage hatcheries (CH, FRH, MRH) include unmarked (presumed natural-origin) steelhead in their broodstock when available. At all hatcheries, eggs are stripped from females and fertilized with milt from one or two males. At hatcheries with fewer than 250 returning female steelhead on average per season (MRH and NH), each females' eggs are divided between two males. At the two larger hatcheries (CH and FRH), there are more than 250 returning females on average, each mated with a single male. This practice is intended to mitigate reductions in effective population size (Ne) at the two smaller programs. Hatcheries also differ in how long post-spawn steelhead are held before release, which can affect the frequency of repeat-spawning within a single season. At all four hatcheries, juvenile steelhead are raised on-site through a year of life before being released either at the hatchery or downstream in the same river; however, size at release varies among the hatcheries. Juvenile steelhead from all four hatcheries are marked by removal of their adipose fins shortly before release. This practice allows natural

steelhead to be visually differentiated from hatchery-produced steelhead throughout their lifespan, as adipose fins do not regenerate.

Sampling and DNA Extraction

Tissue samples were taken from each fish spawned at all four hatcheries from 2011-2019 and dried on blotter paper in ventilated coin envelopes. Date of spawning, phenotypically identified sex, length, and presence of an adipose fin was recorded for each sample. DNA was extracted from dried fin tissue with QIAGEN DNeasy 96 Tissue Kits following the manufacturer's animal-tissue protocol using a BioRobot 3000 (QIAGEN Inc.) for all liquid handling. DNA was then diluted 1:2 in ddH2O prior to genotyping.

SNP loci, genotyping, and basic population genetics analysis

Samples were genotyped with a panel of 96 biallelic SNP markers (Abadía-Cardoso et al. 2013), including a Y chromosome-linked marker to determine genetic sex (Brunelli et al. 2008). However, the marker composition of the panel varied slightly over time, with 92 loci genotyped across all years of the study; markers not typed across all years were removed from consideration (Table S1). All individuals were genotyped using TaqMan assays (Applied Biosystems) on 96.96 Dynamic Genotyping Arrays with the EP1 Genotyping System (Fluidigm Corporation) following the manufacturer's protocols. Two negative controls were included in each array, and genotypes were scored using SNP GENOTYPING ANALYSIS SOFTWARE V 3.1.1 (Fluidigm).

To estimate genotyping error rates for each SNP marker we inferred parent-offspring trios using parentage analysis (see below) and estimated the minimum genotyping error rate expected to produce the Mendelian incompatibilities observed at each marker across the trios. Of the 23,670 genotyped samples, 83 yielded low-quality genotypes after the initial round of genotyping (indicated primarily by large fractions of missing genotypes). These

samples were regenotyped. Any individuals missing more than 10% of loci (fewer than 82 successful genotype calls) were identified and removed.

We utilized the R package 'strataG' (version 2.0.2; Archer et al. 2017) to calculate mean expected and observed heterozygosity averaged over loci for all genotypes in recorded spawned steelhead for each hatchery. Fst values between years across the study period, both within and between hatcheries, was calculated using strataG on a random subset of 300 broodstock from each hatchery. Next, to evaluate gene flow and population structure amongst programs, this subset of 1200 individuals (300 from each hatchery) was evaluated in the model-based clustering program STRUCTURE version 2.2 (Pritchard et al. 2000; Falush et al. 2003) with a hypothesized number of populations of K = 2, 3, or 4. Finally, a principal component analysis was conducted on this subset of data to visualize relationships amongst hatchery programs.

Matching samples, repeat and iteroparous spawners

We refer to individuals that enter the hatchery and are spawned multiple times within a single year as "repeat-spawners." These can be differentiated from iteroparous individuals as the latter spawn multiple times in different seasons. Each time any fish is spawned at these hatcheries, a tissue sample is collected and assigned a unique sample ID. Therefore, the same individual may occur multiple times in our dataset with different sample IDs. In order to identify all unique sample IDs belonging to a single repeat-spawning or iteroparous individual, we searched our genotype database for samples with identical or near-identical genotypes using the close_matching_samples() function from the R package 'rubias' (Moran and Anderson 2018). A preliminary analysis with a minimum 80% of markers with matching genotypes provided a visualization of the distribution of numbers of matching genotypes (Figure S2) from which it was clear that pairs of identical samples shared at least 95% of genotypes. Thus, we identified 'clusters' of sample IDs that were from the same individual, including matches observed between different hatcheries, and determined the number of

iteroparous and repeat-spawners at each program overall, by year, and by sex. To handle cases where more than two sample IDs were from the same fish, we created a graph by defining edges between all pairs of sample IDs that were from the same individual, and then identified all the sample IDs associated with a single fish as members of a connected component in that graph using the R package 'igraph' (Csardi & Nepusz 2006). Significance of spawner sex and hatchery program on the type of multiple spawning event (iteroparity vs. repeat-spawners) was determined using Kruskal-Wallis rank sum tests.

Pedigree reconstruction

To infer the multigenerational pedigree connecting our samples over all years, we conducted parentage analyses, separately for each spawn year and hatchery program included in the study period, using our package HatchPedAgree ((https://github.com/eriqande/HatcheryPedAgree) to implement the SNP Program for Intergenerational Tagging (SNPPIT, Anderson 2012). SNPPIT assigns each offspring to the most likely parent pair—yielding a parent-offspring trio—and calculates a false discovery rate (FDR) score for each offspring assignment (to a pair of parents). Before pedigree reconstruction, we removed two linked markers (genetic sex and Omy_R04944). Based on a preliminary SNPPIT run, the loci Omy_128851-23 and Omy_131965-120 displayed an excess of Mendelian incompatibilities (over 2%) and were also removed from subsequent analyses. Final parent-offspring trios were assigned with 92 loci, assuming a genotyping error rate of 0.005 per gene copy (effectively 1% per locus). For pedigree reconstruction, the sample IDs belonging to a single fish were all re-assigned to be the same as the sample ID associated with the most complete genotype for that fish. In cases where we identified one or more loci scored as different homozygotes amongst the multiple genotypes in a cluster of genotypes from a single individual, we removed that individual from the data set. To account for potential errors in metadata (i.e. spawn date and/or sex recorded by hatchery staff), we reconstructed pedigrees by using the results of two different SNPPIT runs: one (referred to as

the “constrained” run) requiring parents to have the same recorded spawn dates and different recorded sexes, and the second (the “unconstrained” run), in which only spawning year was provided for SNPPIT to determine the possible pairings of parents. Potential parents included fish from all hatcheries, and the list of sample ID clusters was referenced while running SNPPIT to ensure iteroparous and repeat-spawners were included in the appropriate potential parent pool based on their multiple spawn dates.

Only trios for which the maximum a posteriori relationship was “parent-offspring trio” were considered and only those with FDR <= 0.01 were retained as candidate parent-offspring trios. For most of the offspring, the constrained and the unconstrained runs recovered the same parent-offspring trio assignments. Assignments that differed by SNPPIT run type were largely associated with errors in the metadata (incorrect sex or spawn date). We reconciled these disparities by inspecting the associated metadata and FDR scores of the trios in the two different SNPPIT runs as described fully in the Results section.

Pedigree based analysis

Based on the final pedigree, parentage assignments and percent of offspring assigned were determined by parent hatchery, as well as by offspring hatchery, year of spawning, and year of return. The number of offspring per spawning event for females and males was determined, then grouped by type of spawning event (single, iteroparous, repeat) to calculate both the mean observed reproductive success for each type of spawning event, and mean total observed reproductive success. Repeat and iteroparous spawners that were recorded as different sexes during different spawning events were removed.

Offspring age was calculated for all trios by subtracting offspring spawn year from parent spawn year. Age distribution was considered by hatchery program and spawn year, as well as by hatchery program and cohort year across years. Including age distribution by hatchery program and cohort year ensures identification of any cohort effects that could influence patterns observed in age distribution by spawn year. Spawn dates were binned into

five-day units to group spawning steelhead by relative spawn timing. Counts of individuals of each age were noted across recorded lengths (mm) at spawning (size-at-age) by program.

Finally, straying rates were calculated for each hatchery program across years. Strays were identified as fish that returned to spawn at a hatchery that differed from that where the parents were sampled.

Omy05

Two SNP loci in our panel (Omy_114448 and Omy_R04944) are located within the inversion complex on chromosome Omy05 (Pearse et al. 2019). Previous analyses have shown that, while Omy_R04944 is nearly perfectly associated with inversion karyotype, the association of SH114448 with the inversion in CCV steelhead is imperfect (Pearse et al. 2014; Pearse & Garza 2015). Thus, we used locus Omy_R04944 as an indicator of inversion karyotype for all analyses, and excluded it from population genetics and pedigree reconstruction. Frequencies of Omy05 karyotypes were determined for each hatchery program to assess patterns related to O. mykiss life history in relation to age, sex, and spawn date. Allele frequencies and adherence to Hardy Weinberg Equilibrium were evaluated using the R package HardyWeinberg (Graffelman 2015). Finally, the frequencies of Omy05 genotypes among returning offspring resulting from heterozygous matings (AR x AR) were determined by hatchery and by sex to test for deviations from the expected 1:2:1 genotype frequencies.

Results

Samples

Fin clips were collected from returning adult steelhead at all four hatcheries from 2011-2019, for a total of 23,670 samples (Table 1). The two largest programs, CH and FRH, yielded 9,420 and 8,218 fin clips, respectively, whereas 2,510 samples were collected from MRH, and 3,522 from NH (Table 1). However, the number of returns at each hatchery varied across years, with all hatcheries experiencing decreases in 2015 and 2016 (Table 1).

Data Preparation Overview

For samples that were re-genotyped due to low genotyping success, we retained the most complete genotype for those individuals, resulting in 23,670 unique individual genotypes at 92 loci. Setting a minimum of 82 non-missing loci in the final dataset removed 422 samples (1.78%), leaving 23,248 for further analysis. Recorded phenotypic and genotypic sex were used to determine sex, with 9 samples removed for missing both genetic and phenotypic sex. Identifying samples sharing a minimum of 95% matching genotypes revealed that 4,119 fin clip samples could be assigned to 1,925 clusters, each representing a single individual that had been sampled between two and seven times due to repeat spawning or iteroparity. The majority of these repeat-spawning/iteroparous fish were spawned at FRH (53.74%), while 19.29% were spawned at CH, 16.25% were spawned at MRH, and 10.8% were spawned at NH. In 19 of the 1,925 clusters, sex was not consistently recorded for the individual; these individuals were removed. Two samples were identified with mismatching homozygous loci in their cluster of genotypes and removed from further analysis, leaving 23,191 unique individuals for pedigree reconstruction.

Population Genetics

Estimates of heterozygosity were determined from a reduced dataset of 22,765 spawned broodstock. Rates of heterozygosity at all programs fluctuated over time, but NH had higher estimated observed and expected heterozygosities than all three CV-lineage hatcheries in all years (Table S2). Fst and analysis with the program STRUCTURE showed CH, FRH, and MRH are most similar to each other, while NH has the most genetically distinct fish, consistent with their coastal origin (Figure S3, S4, S5; Table S3). Interannual genetic divergence was lower at CH and FRH, likely due to their larger effective population sizes, (Table S3). Notably, MRH and FRH were most similar at the beginning of the study period, but became more distinct over time (Figure S5; Tables S4).

Iteroparity and Repeat-Spawning

Kruskal-Wallis rank sum tests revealed that the rate of iteroparous and repeat-spawning was significantly different across hatchery programs and between sexes (by hatchery: chi-square = 52.581, df = 4, p-value = 1.043e-10; by sex: chi-square = 27.212, df = 4, p-value = 1.801e-05). The overall rate of iteroparity was low to moderate (range =6.3-14.6%) at all three CV-lineage hatcheries, and was strongly female-biased (88.5% of iteroparous spawners were female, Table 2, S7). In contrast, the coastal-lineage NH had a low proportion of both male and female iteroparous spawners (0.2%; Table 2). Six individuals returned to and spawned at more than one hatchery on different spawn dates. Two of the six individuals were spawned within the same year at different programs: in 2017 one fish spawned at NH and FRH, and in 2018 one fish spawned at CH and FRH. Three individuals were spawned at NH in 2011 and at CH in 2012, and one fish spawned at MRH in 2018 and NH in 2019.

Repeat-spawning of the same fish multiple times within a year also varied among hatcheries and across years, and was strongly male-biased (Table 2). FRH had the highest overall rate of repeat spawning (20.0%; Table 2), with a notable reduction from 2016 onwards, consistent with changes in management practices (Table S5). The highest rate of repeat-spawning within a single season occurred at MRH in 2013 at 50.77% (Table S5). The lowest proportion of repeat-spawners was observed at CH (2.24%) in roughly equal numbers of males and females (Table 2).

Pedigree reconstruction

The pedigrees inferred from the unconstrained runs, as well as the runs constrained by sex and spawn date, were each reconstructed from 23,191 unique individuals. The number of trios selected for the final pedigree based on matching spawning metadata and statistical requirements were as follows: 13,657 trios from the constrained run had a maximum a posteriori relationship of “parent-offspring trio” and an FDR <= 0.01. Over 93% (12,774) of

these trios were also found to be statistically supported trio assignments in the unconstrained run, while 883 trios were discrepant between the constrained and unconstrained runs. Of these discrepant trios, 818 offspring did not have statistically supported assigned parents in the constrained run—likely due to errors in the metadata—but were assigned parents that met our confidence criteria in the unconstrained SNPPIT run and were therefore retained. Removing an additional 16 improbable trios with sex or spawning date conflicts left 13,576 trios with confident assignments.

Pedigree based analysis

The percentage of spawning steelhead confidently assigned to the pedigree varied by program and year. Table S6 provides details of parentage assignments, total offspring, and number of assignments by cohort and return year.

Given the filtered parentage assignments, we calculated the age distribution amongst the spawners at each program. Returning steelhead spawned at two through six years of age, with fewer numbers of older fish (Table 3). Age structure varied within programs, with CV-lineage hatcheries featuring predominantly age-two steelhead and the coastal-lineage NH dominated by age-three steelhead (chi-squared = 65.25, df = 4, p-value = 2.282e-13; Figure 2; Table 3). Notably, in 2017 NH had an increased fraction of age-two spawners return, and almost no age-three spawners (Figure 2). Age structure also varied significantly across programs by sex, with females spawning at older ages than males (chi-squared = 151.38, df = 5, p-value < 2.2e-16; Table 3), and by cohort and return year (chi-squared = 337.47, df = 5, p-value < 2.2e-16; chi-squared = 337.64, df = 5, p-value < 2.2e-16; Figure 2; Table 3).

Comparing age structure across the spawning season with spawn dates grouped into equal, 10-day, bins revealed striking differences in the spawn dates of age-two, -three, and -four spawners at NH, but not at CV-lineage hatcheries. Mean and median spawn dates by age shift earlier in the season with increasing age of spawner at NH, but not at the CV-lineage programs (chi-square = 44.673, df = 10, p-value = 2.491e-06; S8). NH showed a

clear shift in relative proportion of ages as the spawning season progressed, with older fish returning earlier than younger fish (Figure 3).

Migration among hatcheries was rare but occurred between all programs (Table 4, S9). MRH had the highest straying rate due to one significant event, when 165 steelhead that were assigned to parents at MRH in 2015 returned to NH to spawn in 2017. This single event represented 56.66% of all observed straying (Table 4).

Omy05 Associations

The marker locus for Omy05 was successfully genotyped for all steelhead sampled from 2015 onwards (N=13,090), including 10,293 offspring among the 13,576 inferred trios. Frequencies of Omy05 genotypes were estimated among these individuals overall, by hatchery program, and by hatchery and sex (Table 5) . Genotypes AA and AR are most common at all hatcheries, with RR occurring rarely, regardless of sex (Table 5). Using the Haldane Exact test for Hardy-Weinberg equilibrium on the 13,090 genotypes, CH and MRH did not deviate from expected frequencies of Omy05 genotypes, while FRH and NH both had slight, but significant, heterozygote excess (Table 5). We identified 465 returning offspring resulting from AR x AR pairings across all hatcheries, with statistically significant deviations from expected Mendelian Omy05 genotype frequencies overall and for both males and females across all programs, reflecting an excess of heterozygotes and deficit of RR homozygotes relative to expected Mendelian proportions (Table S10). Age at spawning was not associated with Omy05 genotype amongst CV-lineage broodstock, but older coastal-lineage steelhead from NH were more likely to have AA or AR genotypes, while younger fish had proportionally more RR genotypes (Figure 4, Table S11).

Discussion

We characterized patterns of variation for several important life history traits in the four steelhead hatchery populations present in the CCV from 2011-2019, which include two

genetically distinct steelhead lineages. Despite inhabiting the CCV environment since the 1950s, we found that the coastal-origin steelhead at NH maintained genetic and phenotypic distinction from CV-origin hatchery steelhead, including for key life-history traits. We included genotyping for Omy05 to characterize relative adaptive genetic variation among populations. Our sampling period included the 2012-2016 historic drought, which also likely contributed to observed phenotypic patterns.

Genetic Lineages

The initiation of NH's hatchery program with coastal-origin steelhead from Eel River imparted distinct genetic and phenotypic differences that have been maintained over more than 50 years following transplantation to the CCV (Pearse & Garza 2015; California HSRG 2012). Fst and STRUCTURE results support that NH broodstock remain genetically distinct from CV-lineage steelhead (Pearse and Garza 2015), while the similarity amongst the other programs fluctuated over time. Our subsequent analyses also confirmed striking life-history differences between NH and the other hatchery populations. Specifically, compared to CCV steelhead, NH steelhead consistently spawned at older ages, stratified spawn timing by age, typically spawned only once (semelparity), and possessed significant associations with age at spawning and Omy05 genotype.

The hatcheries established with CV-lineages feature a much greater proportion of age-two spawners, while NH broodstock spawn predominantly at age three. This likely reflects NH steelhead adaptation to coastal environments (Abadía-Cardoso et al. 2013; Pearse & Garza 2015; Eschenroeder et al. 2022). In addition, parentage analysis revealed that younger fish arrive later in the season compared to steelhead three years and older at NH, but we did not observe this pattern in the CV-lineage broodstock programs. This pattern mirrors previous population analyses conducted in coastal steelhead populations, in which fish that mature as age-two steelhead tend to spawn late in the season (Abadía-Cardoso et al. 2013). This may reflect the prevalence of alternative life-history strategies in CV-lineage

steelhead, including use of freshwater and brackish habitats in the Sacramento-San Joaquin delta rather than undergoing fully anadromous marine migrations (Olsen et al. 2006; Abadía-Cardoso et al. 2016; Leitwein et al. 2017; Pearse & Campbell 2018).

Steelhead exhibit plasticity in the number of lifetime reproductive events, with most individuals dying after first reproduction (semelparous), while some live to reproduce in multiple years (iteroparous), with both life-history strategies maintained by fitness tradeoffs involving fecundity and mortality (Seamons & Quinn 2010; Christie et al. 2018). In our pedigree, hatchery steelhead spawned either only once, more than once within a season (repeat-spawning), or in more than one spawn year (iteroparity). NH steelhead differed significantly from CV-lineage populations when comparing the average number of observed lifetime spawning events. Lower rates of iteroparity occurred in NH steelhead overall (0.2%), which is consistent with previous estimates of iteroparity in coastal populations (Abadía-Cardoso et al. 2013). By contrast, rates of iteroparity were higher in the CCV hatchery program populations, with the highest overall rate occurring at MRH (14.58%), and an even higher rate among MRH females (27.6%). Thus, despite sharing a watershed in the CCV, NH hatchery steelhead possessed low rates of iteroparity, suggesting a strong genetic influence from their coastal-lineage that has not been largely altered by current environmental conditions.

The O. mykiss chromosome Omy05 contains a chromosomal inversion with ancestral (A) and rearranged (R) variations that have been associated with expression of anadromy and residency, respectively, as well as other growth and developmental traits (Nichols et al. 2008; Miller et al. 2012; Pearse & Garza 2014; Pearse et al. 2019). The RR genotype associated with expression of residency was found at low frequency within all programs, consistent with previous observations that southern O. mykiss populations below barriers to anadromy possess high frequencies of the A haplotype at Omy05, particularly in the CCV (Abadía-Cardoso et al. 2016; Leitwein et al. 2017; Pearse & Campbell 2018; Pearse et al. 2019; Eschenroeder et al. 2022). NH steelhead were distinct from CCV fish in their

association of Omy05 genotype with age at spawning, and followed a pattern similar to that observed in coastal steelhead in the Russian River (Beulke et al. In Review). In NH steelhead, AR and RR genotypes were proportionally more frequent in age-two spawners than among older age classes. This association was not observed in CH or FRH, but genotype frequencies significantly deviated from HWE in two programs (FRH and NH). Similarly, across all programs there was a significant deviation from the expected 1:2:1 Mendelian ratio of Omy05 genotypes in returning offspring from matings between AR parents. Together these patterns suggest an important role for Omy05 in genotype-specific disassortative mating, growth, or survival, independent of genetic lineage.

Genetic variation within CV-origin hatchery steelhead reflected differences in program management strategies, past movement of eggs between programs , and accumulation of random genetic changes. Human management of spawning steelhead most strongly influenced repeat-spawning, with highest overall rates occurring in FRH. Our pedigree identified FRH steelhead with high rates of spawning multiple times within one year until 2016, after which changes in spawning protocols contributed to consistently low rates of repeat-spawning (Table S5). We also found evidence of strong population genetic similarity between FRH and MRH, reflecting previous transportation of eggs from FRH to MRH (California HSRG 2012; Pearse and Garza 2015). However, we also found that MRH became more differentiated from the CV-lineage programs over time, suggesting that genetic divergence rapidly accumulated after egg transportation stopped in 2007. In contrast, we also observed a decrease in Fst values between CH and FRH. These small changes in population structure over time suggest genetic drift acting in local differentiated pools with limited interbreeding. Random genetic changes occur over time, and rare hybridization between hatcheries introduces new variation to program broodstock.

Environmental Influence

Plasticity in life-history traits enables expression of more appropriate phenotypes based on environmental cues. The individual's response depends on the heritability of the conditional response threshold sensitivity, in addition to environmental conditions. The most optimal phenotype best balances producing the largest number of offspring possible and maximizing their probability of surviving to spawning (Satterthwaite et al. 2009; 2010). Considering return timing and age at spawning, this decision depends on growth and successfully surviving emigration. Genetically-encoded thresholds assess important environmental cues and initiate phenotypic expression to optimize survival and reproduction in the local environment (Sogard et al. 2012; Sommer 2020; Reid & Acker 2021). Environmental cues, such as the difference between relative streamflows at release and on returning to spawn (release and return streamflow differentials), smolt release location, route complexity, and water chemistry variation significantly affect both growth rates and emigration survival, thus influencing steelhead life history selection (Satterthwaite et al. 2009, 2010; Kendall et al. 2015; Sturrock et al. 2019).

Temporal variation in CCV watersheds may have influenced distribution of age at spawning in all four hatchery program populations. The record-setting 2012-2016 drought in the California Central Valley reduced streamflows by an estimated 85-90%, with an overall increase in stream temperatures (Herbold et al. 2018; Eschenroeder et al. 2022). Similarly, a strong marine heatwave affected the West coast in 2014-2016, impacting many anadromous salmonid populations (DiRienzo and Mantua 2016; Free et al. 2023). Sudden relief of the drought in 2017 coincided with higher proportions of age-two spawners across all programs (Herbold et al. 2018), seen most dramatically in the coastal-lineage steelhead at NH where age-three spawners are typically the most abundant. This resulted in two-year old NH steelhead predominating among the 2017 spawners, as well as a migration of a large number of CCV fish to NH that year, which were recognized as phenotypically distinct and not spawned. Previous research in Chinook salmon (Oncorhynchus tshawytscha), a close

steelhead relative, determined that high differentials between release and return streamflows increase straying rates between hatchery programs (Sturrock et al. 2019). The combination of extreme drought and ocean conditions when juveniles were released in 2014 and 2015 with significantly higher streamflows in 2017 may have impacted both coastal- and CV- origin steelhead, altering the observed patterns of reproductive timing and migration.

There was a single notable straying event revealed in this study, when 165 age-two fish from MRH returned to NH in 2017, a year with heavy rainfall and high streamflows after a long period of drought. These strays from MRH contributed to the increased proportion of age-two spawning at NH. However, many of the 2017 age-two spawners were also assigned to parents spawned in 2015 at NH (Table S6), indicating a significant shift from the typical three-year-old age at return for these coastal-lineage steelhead. A combination of NH's location's proximity to the San Francisco Bay and the use of downstream smolt release sites by all programs during the drought, followed by watershed-wide flooding in 2017 likely drove the high proportion of steelhead released from MRH in 2015 to return to spawn at NH in 2017 (Sturrock et al. 2019). After 2017, NH steelhead resumed predominantly returning at age three or older. Additionally, MRH steelhead returned at older ages in higher proportions in 2018 and 2019, while CH and FRH maintained high proportions of age-two spawners. These results highlight the impacts of environmental variability as well as the underlying genetic basis of life-history variation.

The strong spatial variation within CCV watersheds also likely contributed to phenotypic variation among steelhead lineages (Satterthwaite et al. 2010; Sogard et al. 2012). Steelhead life-history models comparing conditions in the American River (NH location, coastal-lineage steelhead) with Mokelumne River (MRH, CV-lineage steelhead) suggests that the Mokelumne River supports higher life-history phenotypic variation (Satterthwaite et al. 2010; Sogard et al. 2012). The American River has warmer temperatures, higher food availability, and consequently supports faster growth and smolting rates compared with Mokelumne River (Satterthwaite et al. 2010; Sogard et al. 2012).

However, because NH is the only hatchery in the CCV that supports coastal-lineage steelhead, it is unclear exactly how environmental factors impact expression of life-history traits in these populations.

Conclusions

Traits operating within a conditional threshold rely on environmental cues to trigger a phenotypic response. Populations may possess genetic variation in threshold sensitivity, leading to different phenotypic distributions amongst populations. Differences among hatchery program steelhead population responses highlight a combination of influences from genetic and environmental variation on phenotype expression, and a spectrum of responses based on genetically-encoded thresholds and strength of environmental cues. Abiotic conditions impact potential reproductive success and survival probability for individual steelhead, leading underlying genetic variation in different lineages to trigger expression of alternative phenotypes. The co-existence of multiple hatchery-managed lineages in the CCV provides the opportunity to investigate how different genetic lineages respond to similar environmental cues within a shared geographic location. Our study provides clear evidence that different steelhead genetic lineages may respond differently to novel and changing environments, maintaining strong differences in phenotypic and adaptive genetic variation and life-history traits over many generations.

Tables

All supplementary tables are included in supplementary file “Ch1SuppTables.csv”

Table 1. Total number of samples received in 2011–2019 from all hatcheries by program and year, as well as the total number of samples removed from genetic analyses due to missing data.

	Program				
	CH	**FRH**	**MRH**	**NH**	**Total**
Samples genotyped	9,275	8,013	2,493	3,467	23,248
Missing loci	145	205	17	55	422
Total sampled	9,420	8,218	2,510	3,522	23,670
2011	930	637	207	500	2,274
2012	851	756	205	293	2,105
2013	891	1,512	130	410	2,943
2014	878	1,499	186	327	2,890
2015	1,375	580	129	87	2,171
2016	452	126	55	503	1,136
2017	989	879	647	510	3,025
2018	1,342	1,090	623	399	3,454
2019	1,567	934	311	438	3,250

Table 2. Counts for single, iteroparous, and repeat spawning for each hatchery program overall, and by sex. Percentages of uses (iteroparous, once, or repeat spawn) for each program, and by sex, were calculated from total count of individuals per program, or total females and males.

Use	Sex	CH	FRH	MRH	NH
Iteroparous	Total	588 (6.3%)	667 (8.2%)	364 (14.6%)	7 (0.2%)
	Female	532 (11.1%)	490 (13.4%)	332 (27.6%)	3 (0.2%)
	Male	56 (1.2%)	177 (4%)	32 (2.5%)	4 (0.2%)
Once	Total	8492 (91.4%)	5821 (71.7%)	1829 (73.3%)	3024 (87.3%)
	Female	4146 (86.7%)	2981 (81.5%)	871 (72.4%)	1553 (98.4%)
	Male	4346 (96.5%)	2840 (63.7%)	958 (74%)	1471 (78%)
Repeat	Total	208 (2.2%)	1626 (20%)	304 (12.2%)	433 (12.5%)
	Female	106 (2.2%)	185 (5.1%)	0 (0%)	22 (1.4%)
	Male	102 (2.3%)	1441 (32.3%)	304 (23.5%)	411 (21.8%)

Kruskal-Wallis	chi	df	p-value
use ~ program	52.581	4	1.04E-10
use ~ sex	26.215	4	2.86E-05

Table 3. Counts and percent of steelhead at age at spawning by program and sex; Kruskal-Wallis results for age-based results.

Age at spawning	Sex	CH	FRH	MRH	NH
2	Female	2249 (74.8%)	1722 (78.1%)	665 (67.4%)	90 (11.2%)
	Male	2560 (87.9%)	2109 (89.2%)	830 (88.4%)	193 (20.4%)
3	Female	660 (22%)	438 (19.9%)	297 (30.1%)	683 (85.2%)
	Male	322 (11.1%)	243 (10.3%)	100 (10.6%)	724 (76.6%)
4	Female	85 (2.8%)	41 (1.9%)	23 (2.3%)	27 (3.4%)
	Male	23 (0.8%)	12 (0.5%)	9 (1%)	28 (3%)
5	Female	11 (0.4%)	4 (0.2%)	0 (0%)	0 (0%)
	Male	5 (0.2%)	0 (0%)	0 (0%)	0 (0%)
6	Male	1 (0%)	0 (0%)	0 (0%)	0 (0%)
	Female	0 (0%)	0 (0%)	1 (0.1%)	2 (0.2%)

		chi-squared	df	p-value
Kruskal-Wallis	Age ~ program	65.248	4	2.82E-13
	Age ~ sex	151.38	5	< 2.2e-16
	Age ~ cohort year	337.47	5	< 2.2e-16
	Age ~ return year	337.64	5	< 2.2e-16
	Age ~ median spawn date	44.673	10	2.49E-06

Table 4. Count and frequency of return types in all recorded returning steelhead. Rates of return are provided for both fish returning to their origin program and those that returned to a different hatchery (strays).

		Origin Program			
		CH	**FRH**	**MRH**	**NH**
Return Program	**CH**	5880 (99.4%)	0 (0%)	2 (0.1%)	2 (0.1%)
	FRH	11 (0.2%)	4567 (100%)	37 (1.9%)	17 (1%)
	MRH	1 (0%)	0 (0%)	1704 (88.5%)	15 (0.9%)
	NH	24 (0.4%)	2 (0%)	182 (9.5%)	1713 (98.1%)
	Total	5916	4569	1925	1747

Table 5. Total counts and frequency of Omy05 genotypes by program, and by program and sex. Hardy Weinberg Equilibrium results are included.

Omy05	Sex	CH	FRH	MRH	NH
AA	Total	5030 (86.6%)	2133 (60.3%)	1259 (70.7%)	1186 (60.4%)
	Female	2558 (86.3%)	1000 (59%)	630 (71.8%)	507 (61.6%)
	Male	2472 (86.8%)	1133 (61.6%)	629 (69.7%)	679 (59.5%)
AR	Total	760 (13.1%)	1272 (36%)	476 (26.7%)	709 (36.1%)
	Female	395 (13.3%)	622 (36.7%)	227 (25.9%)	279 (33.9%)
	Male	365 (12.8%)	650 (35.3%)	249 (27.6%)	430 (37.7%)
RR	Total	20 (0.3%)	130 (3.7%)	45 (2.5%)	70 (3.6%)
	Female	10 (0.3%)	73 (4.3%)	21 (2.4%)	37 (4.5%)
	Male	10 (0.4%)	57 (3.1%)	24 (2.7%)	33 (2.9%)
HWE Results	D	7.538726	35.98472	-0.006179775	21.70496
	p-value	0.1506513	0.000352093	1	0.004105467

Figures

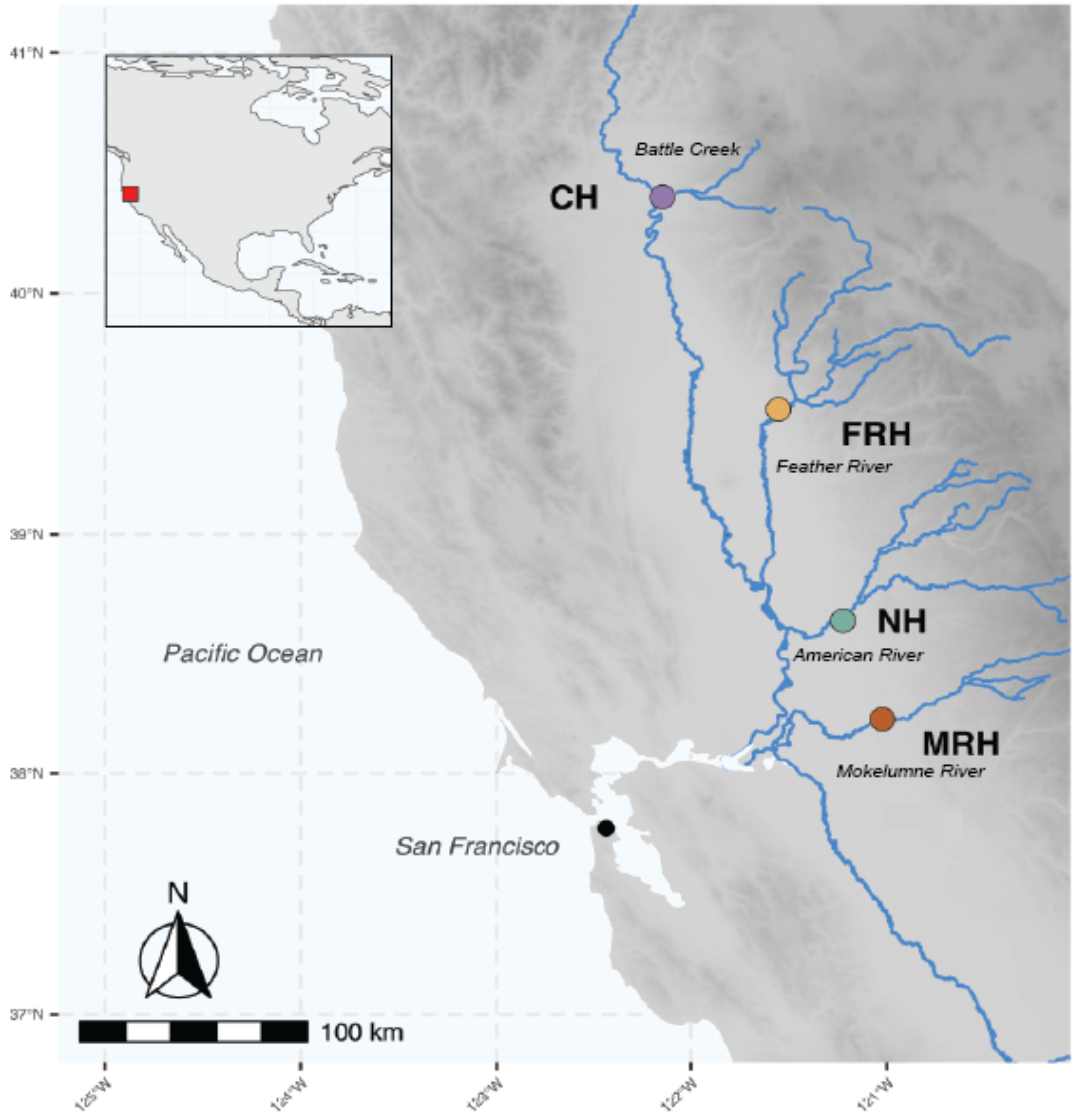

Figure 1. Map of California Central Valley showing locations of hatcheries producing steelhead in relation to San Francisco.

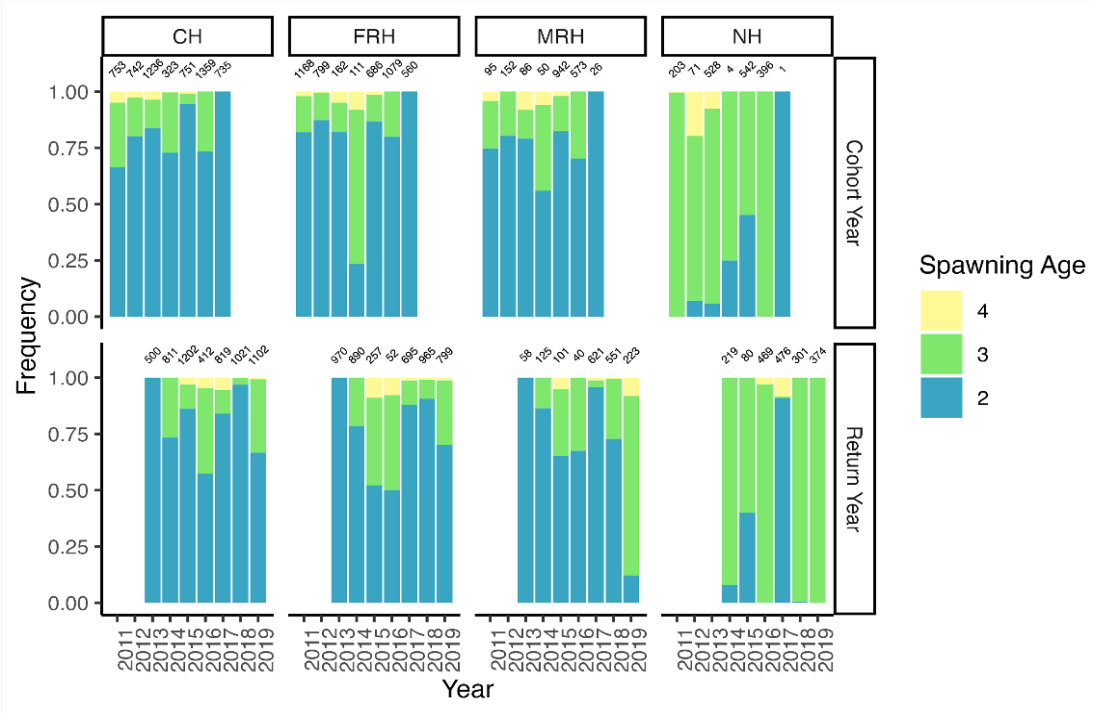

Figure 2. Age structure by program for cohort (above) and return (below) years, with counts of steelhead per year above bars. Note that all fish in return year 2013 and return year 2017 are identified as two-year-olds due to the beginning and ending of the study sampling period for parents in 2011 and offspring in 2019.

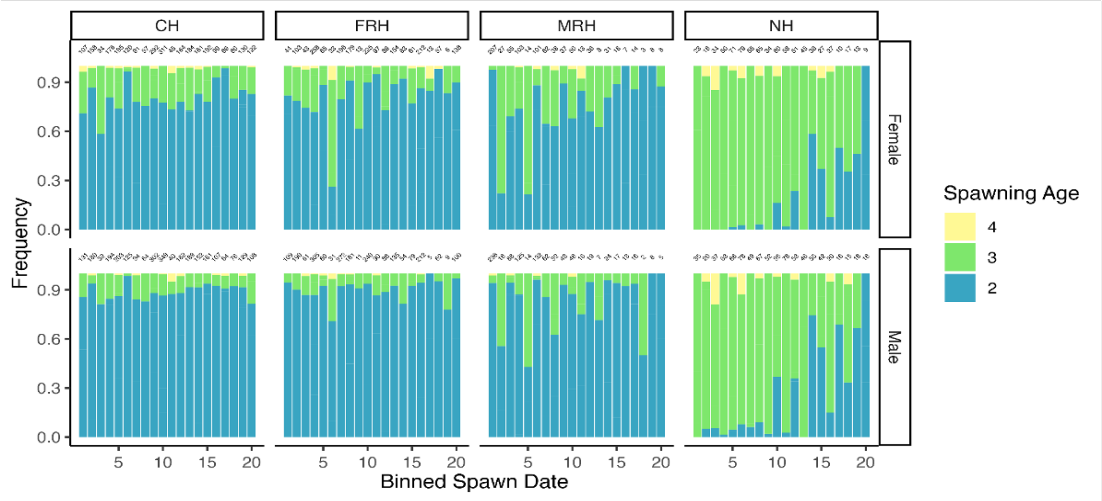

Figure 3. Spawn dates binned over 10-day intervals and separated by sex over all spawning seasons.

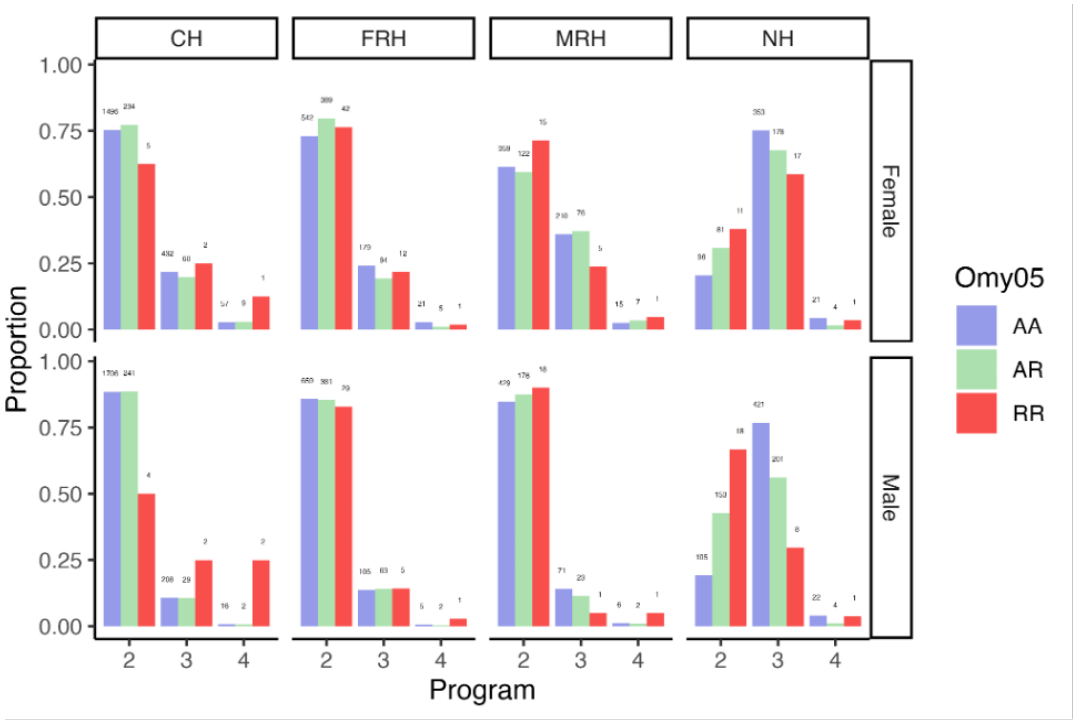

Figure 4. Frequencies of Omy05 genotypes by program, sex, and age at spawning, with counts of steelhead included above bars.

Chapter 2

Ancestry and adaptation of resident, adfluvial, and anadromous rainbow trout (*Oncorhynchus mykiss*) in Putah Creek, an ecotone crossing watershed.

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Abstract

Evaluating patterns in the distribution of genetic variation across heterogeneous landscapes provides perspectives on how environmental features contribute to molecular evolution, and how human activities influence populations. Impassable barriers, such as hydroelectric dams, and human-directed movement or propagation of individuals across riverscapes have had strong impacts on the genetic diversity observed in natural populations. We considered the population structure and distribution of adaptive genetic variation in the salmonid species *Oncorhynchus mykiss* throughout Putah Creek, a watershed near Napa Valley, California that was transformed by the 1950's construction of multiple dams and a large artificial reservoir (Lake Berryessa). In addition, hatchery-raised *O. mykiss* have been introduced throughout the watershed over many years, which may have introduced genetic variation into existing Putah Creek populations. To explore the population structure and distribution of adaptive genetic variation in Putah Creek, we analyzed microhaplotypes from eight *O. mykiss* populations above Lake Berryessa and two populations below Lake Berryessa, and compared them with 40 reference populations from Central Valley, coastal, inland, and hatchery rainbow trout lineages. We found distinct patterns of neutral and adaptive variation between populations above Lake Berryessa and those below. Populations below Lake Berryessa resembled various Central Valley populations and hatchery rainbow trout strains, while those above were more similar to coastal *O. mykiss* populations. Additionally, Putah Creek populations below Lake Berryessa possessed significantly different proportions of

adaptive variants associated with life-history compared to populations above Lake Berryessa, consistent with studies in other populations located above and below barriers to migration.

Introduction

Landscape genetics, assessing the distribution of genetic variation within and among populations in heterogeneous environments, has become a useful conservation tool with increasing accessibility of molecular techniques (Manel et al. 2003; Robinson et al. 2012; Manel & Holderegger 2013; Gray et al. 2014; Rougemont et al. 2021). Understanding how abiotic features shape population genetic dynamics provides insights into environmental influences on the adaptive evolution of populations and how human activity modifies natural patterns (Pease et al. 2009; Winans et al. 2010; Andrew et al. 2012; Cooke et al. 2014; Winans et al. 2017; Brauer & Beheregaray 2020; Stronen et al. 2022). These results can further inform management of imperiled species and/or populations by both discerning geographic distribution of underrepresented or declining adaptive genetic variation, and identifying important external influences on population genetics and phenotypic expression.

One species with strong associations between population variation and environmental features is the salmonid species *Oncorhynchus mykiss*, which possesses highly plastic life history traits that respond to both abiotic and genetic cues (Nichols et al. 2008; Sloat and Reeves 2014; Kendall et al. 2015; Christie et al. 2018). One of these traits is migration strategy, with some *O. mykiss* remaining in freshwater for their lives, while others migrate to the Pacific Ocean as juveniles before returning to their natal freshwater streams to spawn. These distinct ecotypes are known as “rainbow trout” (freshwater residents) and “steelhead” (anadromous migrants). Juvenile *O. mykiss* can mature into residents or migrants, depending on genetic and environmental interactions (Kendall et al. 2015; Phillis et al. 2016). A spectrum of migration patterns exist, including movement through rivers (“fluvial”) or within lake and reservoir systems (“adfluvial;” Holecek et al. 2012). In addition to plasticity in overall life history strategy, migrating *O. mykiss* also vary when they return to spawning

grounds as adults, a trait known as “run timing.” Individuals from the same population may return to spawning grounds before spawning season begins as sexually immature (“early”, or “summer-run”), just before spawning season begins as sexually mature (“late”, or “winter-run”), or at an intermediate time (Quinn et al. 2016). Plasticity in these traits allows for *O. mykiss* individuals to adjust their respective life history strategies based on internal (biotic) and external (abiotic) conditions, increasing chances for survival to reproduction. Environmental characteristics such as barriers to migration influence a population’s migration patterns and timing by altering life history tradeoffs (Sloat & Reeves 2014; Phillis et al. 2016; Apgar et al. 2017; Mattocks et al. 2019). Barriers like dams that block access to migration can landlock formerly anadromous populations (Arciniega et al. 2016; Leitwein et al. 2017; Pearse and Campbell 2018; Winans et al. 2018; Abadía-Cardoso et al. 2019) and negatively select against early migrating salmonids by removing access to historic spawning grounds in higher reaches (Quinn et al. 2016; Kannry et al. 2020; Fraik et al. 2021).

In recent years, genomic studies have identified key genes and adaptive genomic regions associated with important life-history traits in several salmonid fish species, providing important information about their underlying genetic basis to inform conservation and management. In particular, two regions in the *O. mykiss* genome have been specifically associated with: 1.) expression of anadromous or adfluvial migration (Omy05; Pearse et al. 2014, 2019; Arostegui et al. 2019) and 2.) summer vs. winter anadromous run timing (*GREB1L/ROCK1*; Waples et al. 2022). The strength of these associations, and predictive value amongst populations with varying coastal vs. inland migration distances, genetic lineages, and frequencies of heterozygotes remains unclear (Willis et al. 2020; Collins et al. 2020). However, surveys of Omy05 variation in *O. mykiss* have found that some *O. mykiss* populations landlocked above impassable barriers have maintained anadromous genetic architecture despite their current inability for physical movement to marine habitat (Leitwein et al. 2017; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019). This could be explained by the formation of reservoirs upstream of dams, providing habitat with sufficient capacity for

movement to preserve migratory variation (Holecek et al. 2012; Leitwein et al. 2017). Recent successes in rapid *O. mykiss* recolonization following watershed restoration provide additional support for dams negatively impacting *O. mykiss* phenotypic expression but not necessarily genetic variation (Winans et al. 2017; Fraik et al. 2021; Knoth et al. 2022).

Multiple studies have found significant genetic and phenotypic differences in life-history traits between the Central Valley (CV) and coastal lineages within the subspecies *O. mykiss irideus* that reflect regional variation in California (Sogard et al. 2012; Pearse & Garza 2015; Abadía-Cardoso et al. 2019; Goetz et al. 2024). Extensive historic movement of individuals among CV populations has partially homogenized population structure, and stocking from hatchery strains provided opportunities for introgression in wild populations (Pearse & Garza 2015; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019). Additionally, descendants of coastal steelhead propagated in CV watersheds maintain coastal genetic and phenotypic characteristics (California HSRG 2012; Goetz et al. 2024), highlighting the inherited basis of life-history traits. Other studies have found significant environmental effects on *O. mykiss* life history expression (McMillan 2012), and state-dependent models suggest that high environmental variation in the Central Valley favors altered expression of life history variation compared with the central coast (Satterthwaite et al. 2009; Satterthwaite et al. 2010; Sogard et al. 2012).

Here we evaluate and compare the population structure and distribution of adaptive genetic variation among *O. mykiss* in Putah Creek relative to reference populations from both coastal and Central Valley lineages in neighboring watersheds, as well as hatchery strains historically used to stock the Putah Creek watershed. Our results provide important insights into the complex history that has contributed to the present-day distribution of genetic variation in *O. mykiss* in Putah Creek.

Methods

Study system

Putah Creek is a watershed near Napa Valley, CA that straddles the ecotone between the California coastal and Central Valley environments (Figure 1). Located on the natural edges of coastal and CV-lineages, the genetic relationships among Putah Creek *O. mykiss irideus* and other populations in Central California are unclear, but previous genetic analyses have identified genetic similarities amongst samples from Calaveras River, Putah Creek, lower American River, and Nimbus Hatchery (Nielson et al. 2005). With a long history of human modifications, today Putah Creek is impounded in Lake Berryessa, formed by Monticello Dam in 1957 and the third largest reservoir in California. Below Monticello Dam, the creek flows through the eight miles of inter-dam reach (IDR), including Lake Solano, before passing over the smaller Putah Diversion Dam. Below this final barrier to upstream migration it continues downstream past Davis, CA and into the Yolo bypass before joining the Sacramento River and flowing out to the ocean via San Francisco Bay.

The Putah Creek watershed contains both natural and artificial barriers to freshwater migration, including the IDR, a segment of river between Monticello Dam and the Putah Diversion Dam (PDD). The watershed has an extensive history of stocking with various strains of hatchery rainbow trout by CDFW from at least 1977 until suspension in 2008 (Weaver & Mehalick 2009). Between Monticello Dam and Putah Diversion Dam the IDR possesses naturally reproducing *O. mykiss* (Weaver & Mehalick 2009; Hogan et al. 2013) and was designated as Wild Trout water by California Department of Fish and Wildlife (CDFW), ceasing stocking with hatchery trout strains in 2008 (Weaver & Mehalick 2009). However, relative contributions of native Putah Creek and hatchery rainbow trout strains to the IDR population today are unknown. While both Monticello Dam and the Putah Diversion Dam are impassable to fish, waters downstream of PDD are accessible to anadromous Central Valley steelhead, which are federally protected as threatened species under the ESA (NMFS 2006). To further our understanding of the molecular composition of Putah Creek

trout populations, we investigated the distribution of neutral and adaptive genetic variation in Putah Creek populations above and below barriers, and compared them with reference populations from various regions.

Sampling

To investigate population structure and distribution of adaptive variation of *O. mykiss* in the Putah Creek watershed, we collected 196 samples from ten sites throughout the watershed above and below Monticello Dam and within Lake Berryessa. Fish were captured using hook-and-line fly fishing or backpack (Smith-Root LR-24) electrofishing. A nonlethal upper caudal clip was collected from each *O. mykiss* and desiccated on blotter paper prior to DNA extraction. We extracted DNA using a BioRobot 3000 with QIAGEN DNeasy 96 Tissue Kits (QIAGEN Inc.), then diluted DNA 1:2 in ddH_2O before genotyping.

In addition to the Putah Creek samples, we used previously collected genotype data from 2,075 fish from multiple reference populations in California, as well as hatchery rainbow trout strains, as a baseline for comparison (Le Gall et al., 2024). Ten Californian regions were considered for this study, with at least two *O. mykiss* populations represented per region (Table 1; Table S1; Figure 1), including coastal and Central Valley lineages, anadromous steelhead, rainbow and redband trout populations, and five strains of hatchery rainbow trout commonly used for stocking in California (Coleman, Shasta, Pit, Kamloops, and Hot Creek).

Genotyping

All genetic data collected for this study were obtained by sequencing a panel of 'microhaplotype' loci (Le Gall et al. 2024), each containing one or more single nucleotide polymorphisms (SNPs). Microhaplotypes from 2,075 individuals at 124 putatively neutral markers were processed using R package 'microhaplotopia' which utilizes 'microhaplot' (Ng 2022). We removed 14 loci located on chromosome Omy05 from the population genetic dataset, keeping 110 putatively neutral markers for analysis (Table S2). An additional 18

neutral loci with more than 20% problematic genotypes (genotypes that either did not call confidently or contained deletions compared to the reference, i.e. NX genotypes) were also identified and removed. After filtering loci for genotyping success, microhaplotype sequences for all populations were first batch filtered by a minimum haplotype depth of five reads, a minimum total depth of 20 reads, and a 0.3 ratio of second haplotype read depth to first haplotype read depth (allelic balance). This removed 13 individuals, reducing the initial 2,075 individuals to 2,061 (Table S3). Seven of these removed individuals were samples from Putah Creek. 13 individuals missing genotypes for more than 7/92 loci were also removed, retaining only individuals with a minimum of 85 loci (Table S3). Of the 13 removed individuals, two were from Putah Creek. The final dataset contained 2,048 genotyped individuals for population genetic analysis, including 187 individuals from the Putah Creek watershed (Table S3).

Population genetics

Heterozygosity (observed (H_O) and expected (H_E)) were calculated amongst individuals for each population using the R package 'strataG' (version 2.0.2; Archer et al. 2017). Pairwise F_{ST} between each population pair was also calculated using strataG, with separate analyses on the neutral and adaptive loci (Omy05 and *GREB1L/ROCK1*, see below and Table S2). We calculated mean allelic richness (A_R) per population using rarefication with the R package 'PopGenReport' (version 3.0.7; Adamack & Gruber 2014). Population structure was then visualized using the modeling-based program *STRUCTURE* (version 2.2; Pritchard et al. 2000) with a hypothesized number of populations of K = 2, 3, 4, 6, 8, 10, 12, 14, 16. Population structure visualization utilized the subsampled dataset. A principal component analysis (PCA) was also conducted to determine population structure in the IDR, as well as above and below the impassable Anderson Creek Falls. *STRUCTURE*, with a hypothesized number of populations of K = 2, 3, 4, 6, 10, and accompanying PCAs were also conducted on only populations from the Putah Creek region, and on a subset including all Putah Creek

populations, Feather River, Dry Creek, and rainbow trout hatchery populations. *STRUCTURE* figures were constructed using CLUMPAK (Kopelman et al. 2015).

Adaptive genetic variation

To characterize genomic variation associated with migratory life-histories, the Le Gall et al (2024) genotyping panel includes multiple microhaplotypes in both the *GREB1L/ROCK1* genomic region and across the chromosomal inversion on Omy05. To characterize frequencies of the inversion haplotypes on Omy05, we targeted a single SNP within the Omy05 chromosomal inversion (omy5_9_54854574-19) and estimated the frequencies of homozygous and heterozygous inversion haplotypes based on the anadromous (AA), resident (RR), and heterozygous (AR) genotypes at that locus. Similarly, to characterize the distribution of variation in the *GREB1L/ROCK1* genetic region, we focused on a single SNP (mhap8_71, pos. 11667915) that has been used in many recent studies of this gene region (e.g. Collins et al. 2020; reviewed by Waples et al. 2022). Genotypes at this SNP are associated with the early- (EE) and late- (LL) migratory life-histories known as summer- and winter-run steelhead, respectively, and individuals can also be heterozygous (EL). Adherence to Hardy Weinberg equilibrium for these loci was calculated using the R package *HardyWeinberg* (Graffelman 2015).

Results

Population genetics

Genetic diversity at the putatively neutral loci was relatively consistent throughout Putah Creek, but some variation was observed among populations (Table 1). Samples collected from smaller, upstream reaches, such as Upper Anderson Creek Above the Falls (UAC) and Trout Creek (TRC), had lower genetic diversity values (H_O, A_R). Lake Berryessa (LBE) *O. mykiss* had among the highest allelic richness and expected heterozygosity among all

populations, but a lower observed heterozygosity than predicted (Table 1). These results suggest mixed ancestry amongst Lake Berryessa individuals.

Samples from below Lake Berryessa in the IDR and anadromous reach were genetically similar to each other, but distinct from Putah Creek populations above Lake Berryessa. F_{ST} was low between these populations (0.009; Table S4). PCA and *STRUCTURE* model-based results consistently supported close relationships between the IDR and anadromous reach, and between these reaches and Central Valley steelhead and hatchery rainbow trout (Figures 2 and 3; Supp. Figures S3 and S5). However, most Central Valley *O. mykiss* below barriers had high values of H_O and A_R, while the Putah Creek anadromous reach had values similar to the above-barrier IDR, suggesting that few anadromous Central Valley steelhead are actually spawning in the anadromous reach. *STRUCTURE* results showed mixed genetic ancestry from multiple Central Valley sources within individuals from the IDR and anadromous reach (Figure 3, K=3). F_{ST} values between these CV populations and the IDR and anadromous reach were also low (Table S4).

In contrast, *O. mykiss* in the Putah Creek watershed above Lake Berryessa were more genetically similar to each other and coastal populations (Figures 2 and 3; Table S4). Smaller hypothesized numbers of populations in *STRUCTURE* (K) assigned individuals sampled from the upper Putah Creek watershed to genetic clusters shared with coastal steelhead (Figures 2 and 3; Figures S2 and S4). This pattern was also apparent in the low pairwise F_{ST} values between coastal populations and samples from the upper Putah Creek watershed (Table S4). The strong differentiation between coastal-lineage populations above Monticello Dam and Central Valley-lineages below is maintained at higher values of K, but subtle differences amongst Putah populations were observed (Figures 2 and 3). For example, some individuals in upper Anderson Creek (UAC), and Trout Creek (TRC) assign to a distinct genetic cluster at higher values of K, likely indicating further subtle population or family substructure in these small isolated populations (Figure 3, K = 6 & 10). This is consistent with

the low allelic richness and observed heterozygosity in the Upper Anderson Creek and Trout Creek samples relative to most upper Putah Creek populations (Table 1).

Adaptive genetic variation

We also genotyped microhaplotype markers targeting adaptive genetic variation associated with two life history traits: residency vs anadromy (Omy05), and summer- vs. winter-run timing in steelhead (*GREB1L/ROCK1*). An Fst analysis among Putah Creek population pairs based on these adaptive genotypes enabled comparison of neutral and adaptive Fst values amongst populations (Tables S5 and S6). We found higher Fst values in the adaptive microhaplotypes, with the highest values consistently found between populations above and below Monticello Dam (Table S6).

Among the SNPs genotyped that tag the Omy05 inversion, genotypes were highly concordant across all populations, consistent with their known linkage and locations within the inversion (Pearse et al. 2019; Le Gall et al. 2024). There was a significant difference in Omy05 genotype frequencies associated with residency between populations above and below Monticello Dam (RR; Kruskal-Wallis: chi-squared = 18.659, df = 3, p-value = 3.216e-4; Figure 4; Table 2). Putah Creek samples from the upper watershed, such as Upper Anderson Creek (UAC), Trout Creek (TRC), and Upper Upper Putah Creek (UUP), had the highest frequencies of Omy05 resident genotypes (Figure 4; Table 2). In contrast, populations with access to Lake Berryessa through adfluvial movement maintained variable frequencies of all three Omy05 genotypes (Figure 4; Table 2). Omy05 genetic variation associated with anadromy were even more frequent in the IDR and anadromous reach populations below Monticello Dam (chi-squared = 18.659, df = 3, p-value = 3.216e-4; Figure 4; Table 2).

There were also significant differences in distribution of *GREB1L/ROCK1* genotypes in Putah populations above and below Monticello Dam. We found that below Monticello Dam, both the IDR and ANR had significantly higher proportions of early-migrating (EE) genotypes (i.e., summer-run) compared to samples collected from above Monticello Dam, which

featured predominantly late-migrating (i.e., winter-run) and heterozygous genotypes (Kruskal-Wallis: chi-squared = 21.713, df = 3, p-value = 7.483e-5; Figure 5; Table 2).

Compared with reference populations, the distribution of adaptive genetic variation aligned with the inferred ancestry of Putah Creek populations. Samples from the IDR and anadromous reach, which were genetically similar to CV wild populations and hatchery strains in the population structure analyses, had Omy05 and *GREB1L/ROCK1* genotype distributions similar to CV populations like Feather River Fish Hatchery (FER; Figures 4 and 5; Table 2; Table S1). Conversely, populations above Monticello Dam in Putah Creek that are genetically similar to coastal populations had *GREB1L/ROCK1* genotype frequencies similar to coastal winter-run steelhead populations (Figure 5; Table 2; Table S1).

Discussion

Human-directed alterations to population composition and landscapes cause discernible changes to population structure and distribution of adaptive genetic variation. These downstream impacts can further affect management by complicating population and lineage identification, as well as disrupting established abiotic patterns that influence important phenotypic traits. This is particularly evident among Californian salmonid populations, including rainbow trout, *Oncorhynchus mykiss irideus*. Historic transplanting of individuals between watersheds and the operation of hatcheries have significantly impacted population structure, and construction of hydroelectric dams has residualized *O. mykiss* populations in upper watershed habitats previously accessible by anadromous steelhead (Nielson et al. 2005; Pearse & Garza 2015; Phillis et al. 2016).

Population genetics

Overall, we found that genetic relationships between *O. mykiss* populations inhabiting the Putah Creek watershed reflect larger genetic patterns and environmental influences in major California *O. mykiss* lineages. Our results show that prolonged stocking has provided

opportunities for introgression by hatchery rainbow trout strains, with individuals sampled downstream of Monticello Dam possessing mixed genetic ancestry primarily from Central Valley steelhead and hatchery rainbow trout. There was a particularly close association with Pit River and Shasta strains that is consistent with past stocking practices.

In contrast, we identified a strong signal of coastal ancestry in the populations above Monticello Dam, consistent with a previous study that noted a “curious and difficult to explain” genetic relationship between samples from Putah Creek above Lake Berryessa and coastal ancestry in populations from the Calaveras River, the lower American River, and Nimbus Hatchery (Nielsen et al. 2005). Studies using historical samples have shown that, despite decades of extensive stocking with hatchery rainbow trout strains, most modern coastal *O. mykiss* populations are genetically similar to historical samples from the same watersheds (Pearse et al. 2011; Sharo et al. 2023). Additionally, previous genetic studies have identified coastal ancestry in San Francisco Bay populations as far inland as the Napa River (Leitwein et al. 2017), which is adjacent to Putah Creek to the West. Thus, one hypothesis is that the coastal-ancestry populations above Monticello are descendants of the trout that were trapped above the dam, suggesting that prior to dam construction the Putah Creek watershed was inhabited primarily by steelhead of the coastal lineage rather than from the lineage that now dominates the Central Valley. Patterns of adaptive variation also support this hypothesis, with above dam populations containing adaptive variant genotype frequencies similar to coastal populations.

An alternative hypothesis is that the entire Putah Creek was historically inhabited by Central Valley lineage *O. mykiss* and that the coastal ancestry we identified above Monticello Dam arrived via stocking with some undocumented coastal lineage hatchery trout strains after dam construction. However, it is important to emphasize that all major hatchery rainbow trout strains propagated in California and stocked into Putah Creek over the last century have been developed primarily from inland and Central Valley *O. mykiss* source populations (Leitritz 1970; Barngrover 1988), which would not impart the signals of coastal ancestry and

adaptive variation we observed. Thus, evidence suggest that the coastal lineage above Monticello Dam likely reflects native Putah Creek ancestry, potentially introgressed by coastal lineage sources from early stocking efforts (e.g. the Silverado Fisheries Base and East Side Rearing Facility in Napa County, Leitritz 1970).

We identified population substructure amongst the above dam populations and mixed ancestry within individuals from the upper Putah Creek watershed. At higher hypothesized K values in our *STRUCTURE* plots, Upper Anderson Creek (UAC) and Trout Creek (TRC) diverge from the other upper Putah populations, consistent with our analyses suggesting higher genetic structure amongst these two populations. Additionally, some individuals sampled above Monticello Dam had mixed ancestry, particularly in Upper Putah Creek (UUP), suggesting some introgression from hatchery trout strains. These details could further support historical stocking above Monticello Dam, but only in certain streams, or it could be evidence of stocking from multiple lineages in different Putah watersheds. Regardless of the origins of the coastal ancestry in populations above Lake Berryessa, our findings are consistent with previous studies that describe distinct genetic differences between *O. mykiss* lineages, particularly between the central coast and Central Valley (Nielsen et al. 2005; Pearse & Garza 2015; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019; Goetz et al. 2024).

Adaptive genetic variation

Hydroelectric dams fragment freshwater habitats and disrupt migration routes, landlocking formerly anadromous populations (Phillis et al. 2016; Leitwein et al. 2017; Pearse and Campbell 2018; Winans et al. 2018; Abadía-Cardoso et al. 2019). Before the construction of Monticello Dam and Putah Diversion Dam in 1957, migratory *O. mykiss* spawned throughout the Putah Creek watershed (Shapovalov 1947). Establishing these dams segmented both the watershed and local populations above, between, or below these barriers. Introducing barriers to a watershed can quickly residualize populations above barriers and consequently

select for higher frequencies of genetic variation associated with residency (Leitwein et al. 2017; Pearse and Campbell 2018; Abadía-Cardoso et al. 2019). Additionally, the distribution of Omy05 haplotypes varies geographically, with evidence of a latitudinal cline: the frequency of Omy05 variation associated with residency increases with latitude (Arostegui et al. 2019; Pearse et al. 2019).

We found that populations below Monticello Dam possessed high frequencies of anadromous-associated Omy05 haplotypes compared with populations above, which is consistent with other studies investigating *O. mykiss* populations above and below barriers (Leitwein et al. 2017; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019; Kannry et al. 2020). Although most populations above Monticello Dam had higher proportions of genotypes associated with residency (AR and RR), we observed variation amongst Omy05 microhaplotype frequencies in these populations, with some above-barrier populations possessing genetic variation associated with anadromy, particularly those with migratory connectivity to Lake Barryessa. These patterns could reflect Omy05 patterns observed in other southern *O. mykiss* populations (i.e. a latitudinal cline with anadromous genetic variation more frequent in southern populations; Pearse et al. 2019), or they could reflect watershed connectivity differences and the potential for adfluvial movement. Previous studies investigating the distribution of Omy05 haplotypes above and below barriers across watersheds found variation in the frequency of genetic variation associated with anadromy above barriers that suggests increased freshwater connectivity can contribute to maintenance of the anadromous variation (Leitwein et al. 2017; Fraik et al. 2021). The presence of genetic variation associated with anadromy above barriers may be a reflection on differences amongst Putah Creek watersheds' accessibility to alternative freshwater habitats, particularly large reservoirs like Lake Berryessa.

By disrupting migration routes, hydroelectric dams also select against early-returning steelhead by blocking access to upstream spawning habitats, decreasing stream flows, and increasing temperatures (Quinn et al. 2016), thus changing the tradeoffs underlying migration

timing. Return timing in salmonids is highly heritable (Quinn et al. 2016; Waples et al. 2022), with salmonid species and populations often responding differently to altered watershed characteristics in a shared environment (Kovach et al. 2013). This suggests that molecular and lineage-specific effects contribute significantly to patterns in the distribution of adaptive genetic variation and return timing. Prior to major dam construction, many of the major central valley rivers had habitat that supported early-run salmonids, including spring run chinook and summer steelhead. In Putah Creek, we found higher frequencies of the late-returning, winter-run, allele in populations above Monticello Dam, and higher frequencies of the early-returning summer-run allele in populations below Monticello Dam. This pattern could reflect population structure before the introduction of barriers, or it could reflect environmental variation within the Putah Creek watershed, as well as the proximity of Monticello Dam to the Pacific Ocean. The distribution of *GREB1L/ROCK1* haplotypes below Monticello Dam suggests higher representation of early-returning individuals, which may be consistent with previous observations suggesting that trout in the IDR spawn in early December (Moyle 2002; Salamunovich 2009). However, further work is needed to better understand the associations between the specific alleles at this locus and run-timing phenotypes in the central valley lineage.

Conclusions

Our results show distinct genetic patterns between Putah Creek populations above Lake Berryessa and those below Monticello Dam, and that these above-barrier populations have coastal lineage ancestry despite extensive history of stocking with Central Valley origin hatchery rainbow trout strains. In addition, the striking contrast in the distribution of adaptive genotype frequencies among populations above and below Monticello Dam is consistent with their distinct genetic ancestry and the contrasting selective environments in the upper and lower parts of the Putah Creek watershed. Our results based on adaptive genomic variants in the Omy05 and *GREB1L/ROCK1* regions are concordant with the patterns of ancestry

identified in the population genetic analyses and the potential for expression of life-history variation (presence or absence of barriers). We found that *O. mykiss* below Monticello Dam showed evidence of introgression from stocking, with most individuals possessing genetic variation from multiple Central Valley-lineage sources. The combination of coastal ancestry and adaptive genetic variation suggests that Putah Creek populations above Monticello Dam are mainly residents with some adfluvial individuals connecting to other freshwater habitats. Additionally, the identification of individuals possessing anadromous genetic architecture above Monticello Dam contributes support to previous observations of the ability for heterogeneous habitat fragments to support maintenance of genetic diversity, particularly when habitat fragmentation is unavoidable (Fahrig 2018). Similar relationships between fish populations divided by dams have been found in other studies like in pygmy perch (Brauer & Beheregaray 2020) and brook lamprey (Rougemont et al. 2021). Together, these results highlight the complexity of *O. mykiss* adaptation to habitat fragmentation (Pearse & Campbell 2018; Winans et al. 2018; Fraik et al. 2021), and genetic considerations for restoring migratory connectivity in fish populations impacted by dams (Meek et al. 2014; Lusardi & Moyle 2017). Results from Putah Creek overall reflect the dynamic nature of these populations and their genomes, which continuously adapt to changing environmental factors and human-driven management practices through migration, drift, and natural selection.

Tables

All supplementary tables are included in supplementary file “Ch2SuppTables.csv”

Table 1. Putah Creek sample populations with sample sizes and population genetics results. Populations are specified as above or below the barrier Monticello Dam. Population genetics statistics included expected (H_E) and observed heterozygosity (H_O), and allelic richness (A_R).

Population	Monticello Dam	Sampled	H_E	H_O	A_R
Anderson Creek, Above Waterfalls (UAC)	**Above**	33	0.282	0.250	1.777
Upper Upper Putah Creek (UUP)	**Above**	14	0.368	0.313	2.100
Lower Anderson Creek (LAC)	**Above**	4	0.345	0.361	1.692
James Creek (JAC)	**Above**	20	0.357	0.347	2.002
Pope Creek (POC)	**Above**	6	0.363	0.317	1.911
Trout Creek (TRC)	**Above**	16	0.200	0.186	1.584
Middle Creek (MIC)	**Above**	25	0.380	0.357	2.108
Lake Berryessa (LBE)	**Above**	23	0.383	0.294	2.241
Putah Creek, IDR (IDR)	**Below**	27	0.301	0.274	1.865
Putah Creek, Anadromous Reach (ANR)	**Below**	28	0.332	0.311	1.976

Table 2. Putah Creek populations and adaptive genotype results for Omy05 and GREB1L/ROCK1. Omy05 genotype frequencies (AA = anadromous, RR = resident, AR = heterozygous) amongst Putah Creek populations, and total for above and below Monticello Dam. GREB1L/ROCK1 genotype frequencies (EE = early–migrant, LL = late–migrant, EL = heterozygous) in Putah Creek populations, and above and below Monticello Dam. Kruskal-wallis results are provided for significance in both Omy05 and GREB1L genotypes above and below Monticello Dam. Additional details and adherence to Hardy Weinberg equilibrium are provided in Table S1.

			Omy05			GREB1L/ROCK1		
	Population		AA	AR	RR	EE	EL	LL
Above Monticello Dam	Anderson Creek, Above Waterfalls	UAC	2	2	29	0	0	33
	Upper Upper Putah Creek	UUP	0	6	8	1	4	7
	Lower Anderson Creek	LAC	3	1	0	0	1	3
	James Creek	JAC	5	10	5	0	0	20
	Pope Creek	POC	3	3	0	0	2	4
	Trout Creek	TRC	0	5	11	0	1	14
	Middle Creek	MIC	4	11	10	0	2	23
Below Monticello Dam	Putah Creek, IDR	IDR	24	3	0	21	6	0
	Putah Creek, Anadromous Reach	ANR	24	4	0	14	12	1
	Kruskal-Wallis: Adaptive genotype~Above/Below		chi-squared = 18.659, df = 3, p-value = 0.0003216			chi-squared = 21.713, df = 3, p-value = 7.483e-05		
	Total count of each genotype	Above Monticello Dam	17	38	63	1	10	104
		Below Monticello Dam	48	7	0	35	18	1

Figures

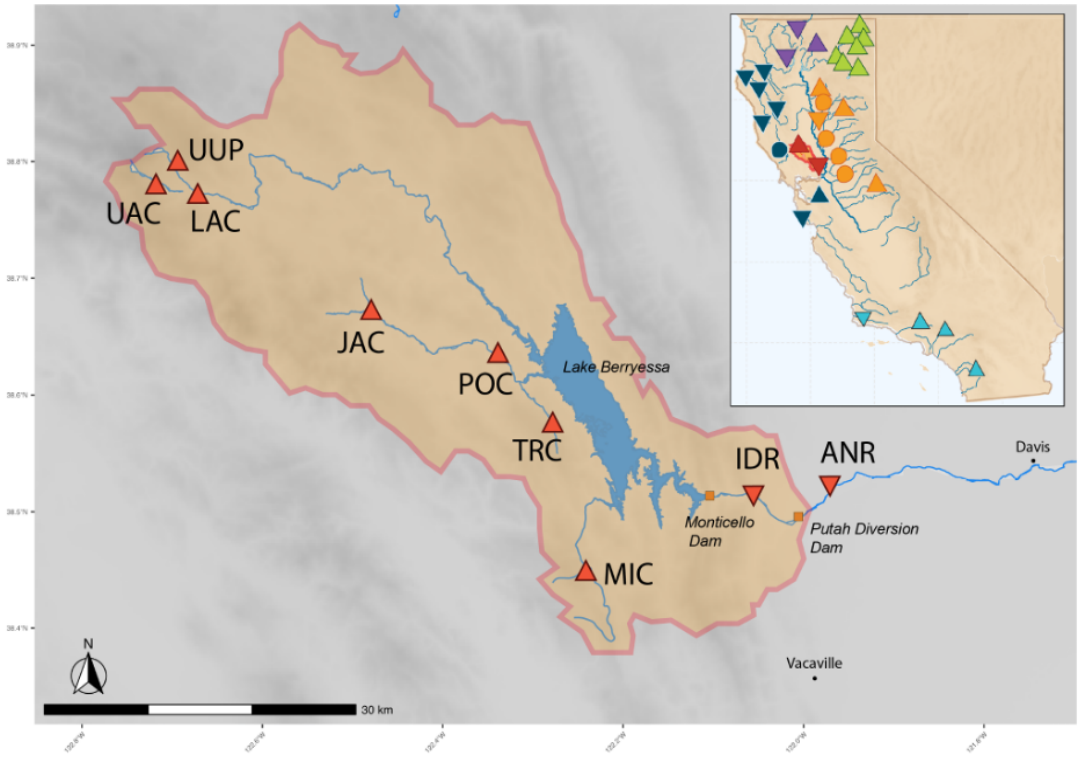


Figure 1. Map of Putah Creek watershed and populations sampled in relation to Davis, California, with triangles indicating if the population is above (∇) or below (Δ) Monticello Dam. Inset: A map of California with the reference populations shown with colors corresponding to locations listed in Table S1 and the location of Putah Creek highlighted.

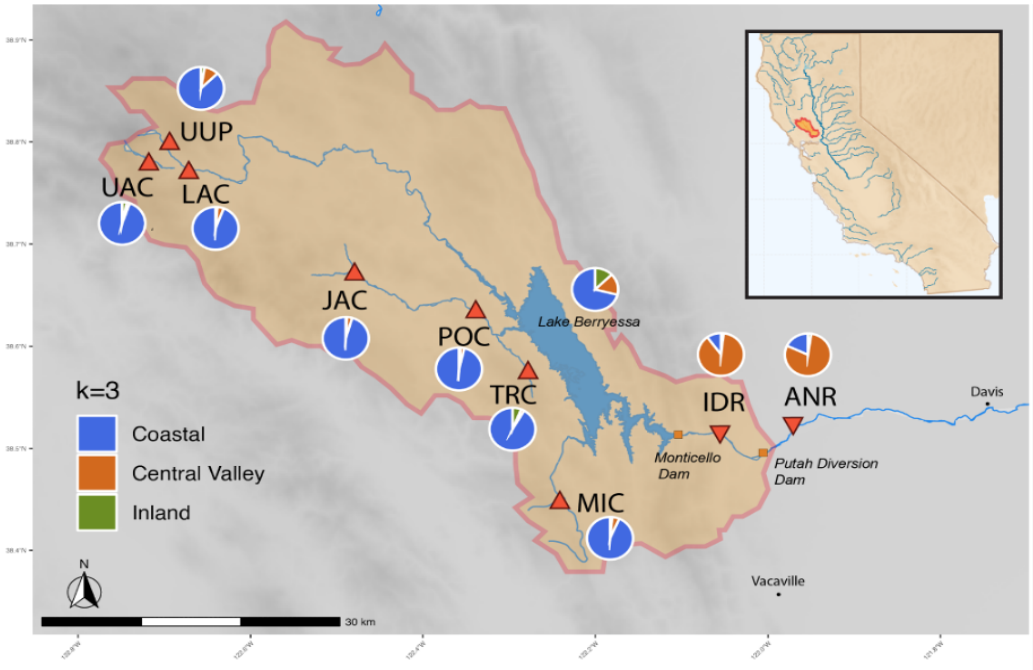
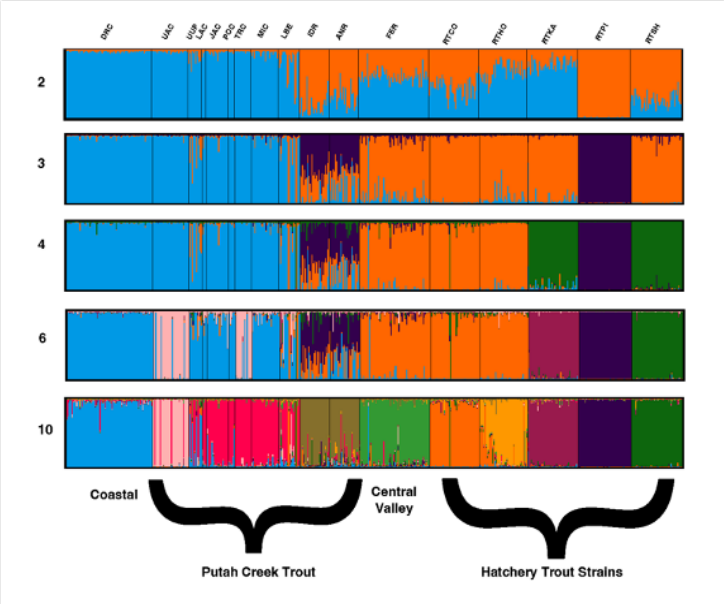


Figure 2. K=3 *STRUCTURE* results for Putah populations above and below Monticello Dam divided by Coastal, Central Valley, and inland assignments.

A.



B.

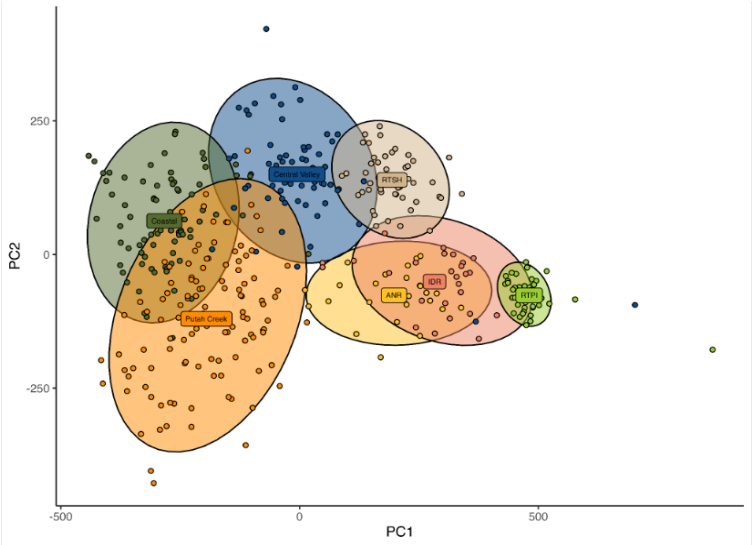


Figure 3. A.) *STRUCTURE* (K = 2, 3, 4, 6, & 10) and B.) PCA results from Putah Creek and subset of reference populations representing Central Valley (Feather River) and coastal (Dry Creek) lineages, and hatchery rainbow trout strains (Pit River (RTPI) and Shasta (RTSH)).

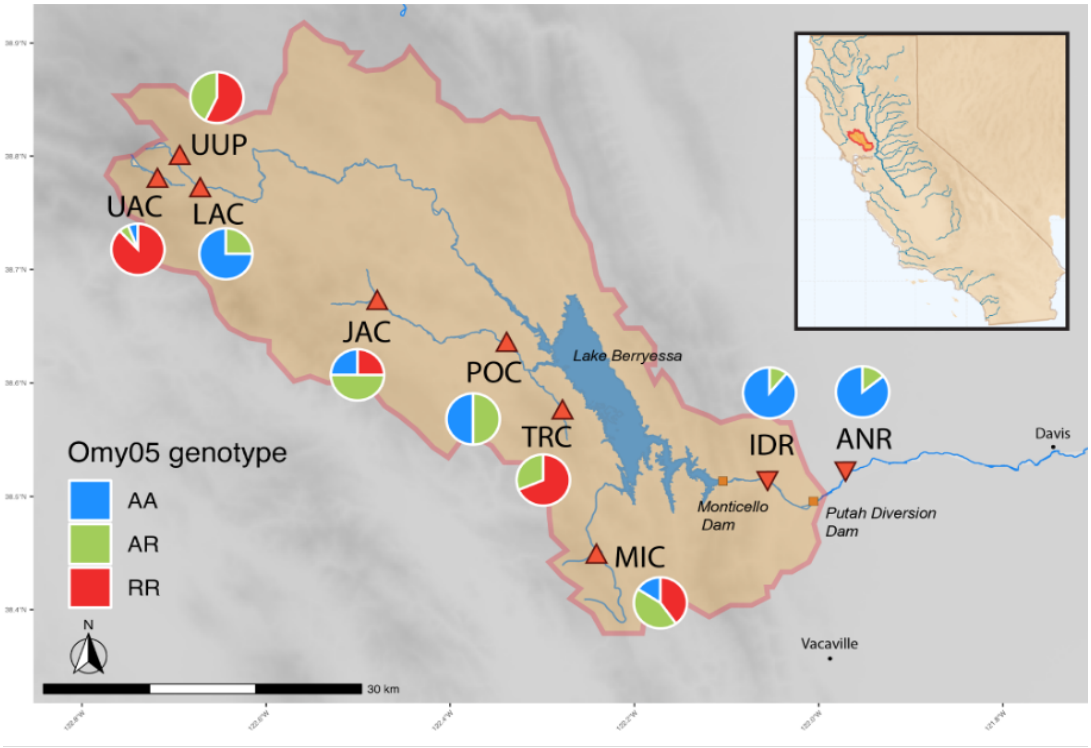


Figure 4. Frequencies of Omy05 genotypes (AA = anadromous, RR = resident, AR = heterozygous) across Putah Creek populations.

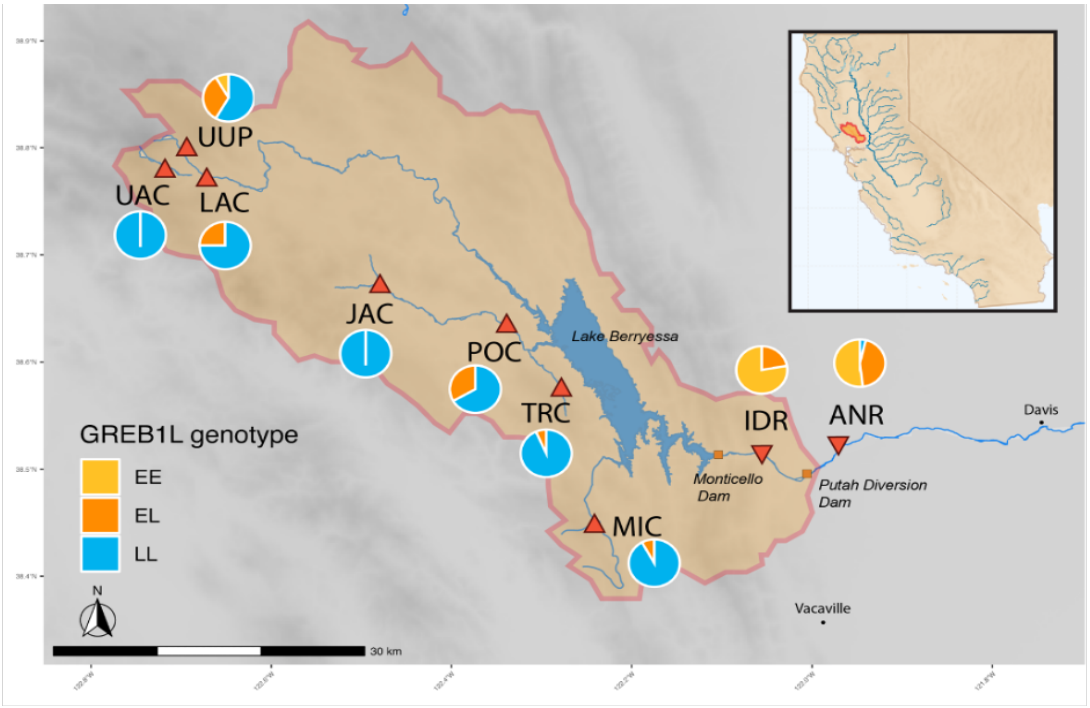


Figure 5. Frequencies of *GREB1L/ROCK1* genotypes (EE = early–migrant, LL = late–migrant, EL = heterozygous) across Putah Creek populations.

Chapter 3

Variable associations between genotype and migration timing in coastal versus Sacramento lineage steelhead

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Abstract

Understanding the influence of evolutionary genetics on behavioral life history traits is an important aim of conservation genetics. This objective can be complicated when the strength of association between genetic variation and phenotypic traits varies by lineage. To explore the association between *GREB1L/ROCK1* and Pacific salmonid species *Oncorhynchus mykiss* run timing, we genotyped *O. mykiss* migrating upstream in the Sacramento River (n=790) with a standardized SNP panel for parentage-based tagging identification with Central Valley hatchery steelhead broodstock and a newly-developed panel of microhaplotype loci targeting adaptive genetic variation associated with run timing in other steelhead populations. We found that strength of association between *GREB1L/ROCK1* genotype and run timing phenotype depended on population ancestry, with individuals from the Central Valley exhibiting little, or no, association between genotype and phenotype. These results confirm that significant genetic and phenotypic differences were maintained between Central Valley and central coast *O. mykiss* populations in the Sacramento River and, more importantly, that differences in strength of genetic and phenotypic association varies between lineages of a species. Our results further our understanding of adaptive genetic variation in conservation efforts by demonstrating limitations between genetic variation and phenotype associations across lineages.

Introduction

Recent studies have greatly improved our understanding of the associations between genetic variation and important phenotypic traits (Funk et al. 2012; Akagi et al. 2020; Koch et al. 2021; Waples et al. 2022) and the characterization of genetic and phenotypic patterns across landscapes (Manel et al. 2003; Pearse et al. 2019; Kebede et al. 2024). However, the strength of the association between genotype and phenotype often varies between populations of a single species (Willis et al. 2020; Collins et al. 2020; Narum et al. 2024; Willis et al. 2024), which complicates dissecting heritable from observed variation. These differences could result from a combination of factors, such as inherited genetic variation (Landy et al. 2020), local adaptation (Smiley-Walters et al. 2017; Kebede et al. 2024), differences in genetic buffering or thresholds (Rutherford 2000) and/or the influence of genetic and environmental interactions (Orogozo et al. 2015; Hughes et al. 2017).

In salmonid fishes, one adaptive trait significantly associated with genetic variation is migration timing when adult fish leave the ocean and enter freshwater before spawning season (Quinn et al. 2016; Reed et al. 2017; Sinclair-Waters et al. 2020; Sinclair-Waters et al. 2022). Fish may return earlier than most individuals (early), later than most (late), or at an intermediate time during the spawning season. Individuals that return earlier are often able to utilize further inland habitat before spawning begins, but still interbreed with mid- or late-returning individuals (Quinn et al. 2016; Prince et al. 2017). Return timing in salmonids is highly heritable (Abadía-Cardoso et al. 2013; Quinn et al. 2016; Beulke et al. 2023), and a single genomic region has been strongly associated with timing of adult entry into freshwater (Hess et al. 2016; Prince et al. 2017; Micheletti et al. 2018; Waples et al. 2022). This genomic region (herein referred to as *GREB1L/ROCK1*), which contains the genes *greb1L* and *rock1*, and the intergenic region between them, has been associated with return timing in some Pacific salmon and trout species, including *Oncorhynchus mykiss*, known colloquially as ocean-migrant steelhead and freshwater-resident rainbow trout (Hess et al. 2016; Collins et al. 2020; Waples et al. 2022; Hugentobler et al. 2024). Variation in the *GREB1L/ROCK1*

region has been significantly associated with run timing (early-run homozygous, heterozygous; late-run homozygous) in multiple Chinook and steelhead populations (Hess et al. 2016; Collins et al. 2022; Dayan et al. 2024). Several studies have found that *GREB1L/ROCK1* genotype described over 80% of observed variation in the time spent in freshwater (Thompson et al. 2020). Significance of *GREB1L/ROCK1* genotype association with migration timing also differs by population/lineage and watersheds within these species, particularly when comparing coastal and inland populations (Willis et al. 2020; Collins et al. 2022; Narum et al. 2024; Willis et al. 2024). It is currently unclear why the significance of association between *GREB1L/ROCK1* genotype and return timing phenotype differs amongst lineages and populations: It could reflect risks involved in increased geographic migration distances, differences in selective pressures from thermal tolerance, predation variation between habitats, or a combination of these effects (Reed et al. 2017; Micheletti et al. 2018; Willis et al. 2020; Collins et al. 2022).

In central California, *O. mykiss* populations are dispersed throughout the coast and within the Central Valley (CV). These populations, particularly those in the CV, were subjected to historic transportation between populations, the introduction of additional genetic variation from hatchery stocks, changes to the distribution of genetic variation, and spawning habitat lost to the construction of hydroelectric dams (McEwan 2001; Lindley et al. 2006; Pearse & Garza 2015; Kannry et al. 2020). These stressors contributed to the dramatic reductions in CV anadromous *O. mykiss* populations, as well as homogenized population structure (Pearse & Garza 2015). The decline of these anadromous populations prompted the construction of four hatcheries that produce steelhead in the CV. One of these hatchery programs, Nimbus Hatchery (NH), was initiated with broodstock sourced from central Californian coastal populations, including the Eel and Mad Rivers, which imparted a distinct coastal genetic signature that NH broodstock still maintains (Nielson et al. 2005; NMFS 2006; Goetz et al. 2024). Like other coastal populations (Abadía-Cardoso et al. 2013; Beulke et al. 2023), NH steelhead return at older ages and have significant associations between within-

season spawn timing and age (older fish spawn earlier; Beulke et al. 2023). They also exhibit adaptive genetic variation for the age at spawning compared with CV hatchery populations, which display higher phenotypic variation (Goetz et al. 2024). Though NH broodstock differ in age and other phenotypic traits, spawning for all CV *O. mykiss* hatchery populations occurs in winter from December through March (McEwan 2001; Eschenroeder et al. 2024; Goetz et al. 2024). CV adults have historically initiated entry to freshwater in August, with return migration peaking in September and October, but are still considered winter-run (McEwan 2001). Central coastal populations are predominantly winter-run and fixed for late maturing genetic variation, with the exception of certain populations that feature predominantly early maturing alleles, such as the upper forks of the Eel River (Kannry et al. 2020; Collins et al. 2020; Waples et al. 2022; Le Gall et al. 2024).

To further investigate the association of *GREB1L/ROCK1* with return timing in the CV, we genotyped migrating *O. mykiss* trapped in the lower Sacramento River using two different genotyping panels. We employed a SNP panel for genetic lineage and age determination and a newly developed microhaplotype genotyping panel to investigate effects of 16 markers located within *GREB1L/ROCK1* with markers collected across multiple studies located between 11,607,954 to 11,803,870 on Chromosome 28 (Omy28; Hess et al. 2016; Collins et al. 2020; Dayan et al. 2024). *GREB1L/ROCK1* genotype for each marker and individual sampling date was determined for each genetic lineage to identify any significant associations. We tested for statistically significant interactions between each *GREB1L/ROCK1* marker genotype and sampling date, as well as sex and age, using four linear mixed models (LMMs) on each inferred genetic lineage. Finally, we investigated the genetic architecture of *GREB1L/ROCK1* by creating linkage disequilibrium (LD) heatmaps and comparing heatmaps between CV and coastal lineages to determine differences in molecular structure. Our results provide further insight into the effects of adaptive genetic variation in the *GREB1L/ROCK1* region on phenotypic variation in return timing observed in Central Valley steelhead.

Methods

Sample collection

We obtained tissue samples (caudal fin clips) from 790 returning CV *O. mykiss* that were captured in 2016 through 2021 (Table 1). For this study, fish were captured using 20 feet long by 12 foot diameter fyke traps and sampled as part of a project operated by CDFW in the lower Sacramento River (Eilers et al. 2010). Four or more of these traps were placed facing downstream August through October upstream of the Sacramento–San Joaquin River Delta in the lower Sacramento River between River Miles ~42 and 93 to capture *O. mykiss* entering freshwater from the San Francisco Bay (Eilers et al. 2010; Table 1). Forklength of captured *O. mykiss* was measured in millimeters and the individual's sex, any detected tag identification numbers, and presence or absence of an adipose fin (regulatory agencies dictate hatcheries attempt to fin clip all released juveniles to mark hatchery-origin fish; NMFS 2006) was recorded in addition to collecting fin clips for genetic analyses (Eilers et al. 2010).

Hatchery parent samples

Tissue samples are taken from all steelhead spawned at Central Valley hatcheries as part of a long-term project to monitor the genetic relationships among Central Valley hatchery steelhead programs (Goetz et al. 2024). From broodyears (BY) 2011–2019, 23,670 samples were genetically analyzed to construct nearly complete pedigrees using a standardized SNP genotyping panel employed for parentage analysis of steelhead at all four Central Valley steelhead programs (Coleman, Feather River, Nimbus, and Mokelumne; Goetz et al. 2024). This effort established a baseline history of the genetic relationships among all CV hatchery steelhead for that time period and annual variation in straying and age structure of the spawning populations (Goetz et al. 2024). We used this genetic baseline to assign fish

captured in the fyke traps to their sampled hatchery parents to determine their hatchery of origin and genetic lineage.

Genotyping

Using a panel of 96 biallelic SNP markers, including a marker for sex (Abadía-Cardoso *et al.* 2013), all 790 samples were genotyped from desiccated fin clips (Table 1) with TaqMan assays (Applied Biosystems) on 96.96 Dynamic Genotyping Arrays with the EP1 Genotyping System (Fluidigm Corporation) following manufacturing protocols. Every array included two negative controls. Genotypes were scored using SNP GENOTYPING ANALYSIS SOFTWARE V 3.1.1 (Fluidigm). The composition of our SNP panel varied over the years, with 89 SNPs used through all study years, including the 2011–2019 pedigree. Only individuals possessing more than 82 genotypes out of 89 total loci were retained for analysis, leaving N=790 individuals to be assigned using PBT.

In addition to SNP genotyping, we re-genotyped 790 fyke trap samples using an adaptive microhaplot panel in the *GREB1L/ROCK1* region to associate migratory return timing with adaptive genetic variation. This microhaplotype genotyping panel was developed by the Southwest Fisheries Science Center (SWFSC) genetic laboratory to consolidate existing markers, as well as to expand the number of microhaplotypes available for neutral and adaptive molecular analyses (Hess et al. 2016; Collins et al. 2020; Dayans et al. 2024; Le Gall et al. 2024). This panel includes 16 microhaplotypes (24 total SNP positions) in the *GREB1L/ROCK1* region, with 11 newly developed markers (15 SNP positions) that were used for this study (Table 2). Located from new genome positions 11,607,954 to 11,803,870 in total, 12 markers were included from previous *GREB1L/ROCK1* studies (Hess et al. 2016; Collins et al. 2020; Table 2).

We processed microhaplotypes from 790 individuals with R package ‘microhaplotopia’ which utilizes ‘microhaplot’ (Ng 2022). Microhaplotypes were then filtered by a minimum five reads, a minimum total depth of 20, and minimum allele balance of 0.3,

removing nine individuals. Genotypes that were not confidently called, contained deletions compared to the reference genome, or contained extra alleles were also removed. We then filtered out 12 additional individuals that had genotypes for fewer than 25 markers, leaving a total of 769 genotyped individuals for further analysis (Table S2).

Analysis

Using our 790 SNP genotypes, we reconstructed two pedigrees by incorporating the fyke trap samples into the previous 2011–2019 pedigree to determine the origin and age of our samples following the methods outlined in Goetz et al. (2024). One pedigree analysis included sex and spawning date as priors for potential parent pairs and the other did not include sex and date. The two pedigrees were compared to select for statistically confident trios that were shared between analyses. Age at spawning for assigned offspring was calculated by subtracting parent spawn year from offspring spawn year. To infer the origin of individuals not assigned to hatchery parents, we used R package 'rubias' (Moran & Anderson 2018) and a subset of known hatchery individuals from the 2011–2019 pedigree for genetic stock identification (GSI) to determine potential origin with our SNP genotypes. GSI results based on SNP data were used to determine CV or coastal lineage, but were not accurate enough for assignment to specific CV populations. Pedigree assignments were used for age- and program-specific analyses, and GSI assignments were used for lineage-specific analyses.

To test for an association between genotype and sampling date, we grouped samples by GSI-predicted lineage, sex, and age at spawning. In groups with the same lineage, age at spawning, and possessing over 10 samples per each genotype (Table 3), we used R package lme4 (Bates et al. 2015) to implement four linear mixed models (LMMs). We coded *GREB1L/ROCK1* microhaplotypes to also test for multiallelic effects following Yang and Álvarez-Castro (2008). To standardize trap location, we centered trap locations by subtracting the mean kilometer distance from each trap location. The four models specifically

tested for statistically significant effects from: *GREB1L/ROCK1* marker genotype and return date overall (model 1), genotype, sex, and sample date (model 2), genotype, standardized fyke trap location, and sample date (model 3), and genotype, sex, standardized fyke trap location, and sample date (model 4) for each marker. Some markers did not have all three genotypes represented and did not include dominant effects in that specific model run (Table 3). Year of sampling was included as a random effect and trap location was included as a fixed effect.

- Model 1: mo_da ~ I(d_add) + I(d_dom_with_s) + (1|year)
- Model 2: mo_da ~ I(d_add) + I(d_dom_with_s) + sex + (1|year)
- Model 3: mo_da ~ I(d_add) + I(d_dom_with_s) + river_mi + (1|year)
- Model 4: mo_da ~ I(d_add) + I(d_dom_with_s) + sex + river_mi + (1|year)

We also investigated the distribution of *GREB1L/ROCK1* genotype and time spent in freshwater by calculating length of time between fyke trap sample dates and hatchery spawn dates in *O. mykiss* that assigned to hatchery parents with genotype.

Linkage disequilibrium (LD) heatmaps were generated for the *GREB1L/ROCK1* microhaplotypes using the R package genetics (Warnes 2012) and LDheatmap (Shin et al. 2006) for all loci with variation using r^2, with 1 indicating complete linkage disequilibrium. We generated heatmaps for all fyke samples grouped together, and for each genetic lineage and hatchery program.

Results

Genetic assignments

We reconstructed two pedigrees to assign 790 fyke samples to the 2011–2019 CV hatchery steelhead broodstock using SNP genotypes. 584 fyke samples were confidently assigned to a pair of hatchery parents: 389 to CH, 89 to FRH, 17 to MRH, and 89 to NH (Table S1). We also used GSI to infer potential origin for unassigned ad-clipped individuals by comparing their genotypes to known samples from the 2011–2019 pedigree. Including assigned fyke

samples enabled calculation of success in determining a potential hatchery of origin by comparing assigned programs. Of the 790 individuals, 58 had mismatches between assignments, all amongst the three CV-lineage hatchery programs (Table S1). NH-assigned fyke samples had the highest frequency of age-3 spawning, while CV-assigned individuals most frequently spawned at age-2 (Table S2). Age-3 fish assigned to NH were also sampled earlier in the season than age-2 NH fish (Figure S1).

We processed 790 fyke samples with the 16 *GREB1L/ROCK1* microhaplotype markers (24 total SNP positions) developed by the SWFSC MEGA lab, with 769 individuals successfully genotyped). Of the 24 total SNP locations, six markers were monomorphic and removed from further analysis (1622532_B, 11623412_B, 11629294_D, 11641623_D, 11658853_F, 11609795_mhap2_position3; Table 2; Figure S2).

GREB1L/ROCK1 genotype and date

Individuals with CV and Nimbus Hatchery (coastal) ancestry had a strong bimodal distribution in migration timing (Figure 2). A majority of CV origin steelhead returned much earlier than fish from Nimbus, regardless of *GREB1L/ROCK1* genotype (Figure 3). Most CV origin steelhead returned between August 1 and November 1, with more Nimbus origin steelhead sampled between January 1 and April 1 (Figure 2). Despite a strong bimodal distribution in migration timing between individuals with CV and Nimbus Hatchery (coastal) ancestry, we did not find an association between *GREB1L/ROCK1* and migratory timing in the CV-lineage *O. mykiss* overall (Figure 3; Figure 4). We had sufficient sample sizes in CV-lineage age-2 *O. mykiss* to implement linear mixed models to determine the strength of association between *GREB1L/ROCK1* genotype and sampling date (Table 3; Table S3). We did not find any association between any *GREB1L/ROCK1* marker, sampling date, or other factors (Table S4). We did not have sufficient sample sizes for NH across genotypes for modeling because NH sample sizes were skewed to later in the season, and often skewed towards a single

genotype or allele for each *GREB1L/ROCK1* genomic position (Figure 3, Figure 4; Table 3, Table S3).

Of 584 PBT-assigned samples, less than 10% were re-sampled at a hatchery program as a spawning adult. When considering the time between fyke trapping and hatchery spawning dates in fish with both dates within a year, we found that CV-lineage steelhead returned over 75 days before spawning at a hatchery program, regardless of mhap8 genotype (Figure S3). NH-lineage steelhead of all three mhap8 genotypes were re-sampled within 50 days, with the exception of one EE individual that spent over 100 days in freshwater (Figure S3).

LD heat maps

Overall, there were three regions of concentrated LD values: within block D (11667578_F, mhap6, mhap8), within part of block G (Ga; 11683310_H2, 11684744_H1, H3, H4), and between these two blocks. 11625241_C had moderate r^2 values (> 0.5) with those two blocks as well (Figure 5). There were also low levels of LD in the flanking region including mhap1 and mhap2 (Figure 5). Dividing samples by lineage revealed differences in LD between NH and the CV-lineage fyke samples. NH fish showed low linkage ($r^2 = 0.2$) through 11683310_H1 that connects to the highly concentrated regions that the CV populations did not have (Figure 5). Additionally, Nimbus showed high values of LD throughout the D–Ga region with 11684744_H that was slightly reduced in CV populations (Figure 5; Figure S4). Though LD was reduced in the flanking side of block D in the CV populations overall, there were higher LD values between certain markers, particularly 11625241_C (Figure 5).

Discussion

Our analyses of *O. mykiss* returning to freshwater through San Francisco Bay enabled characterization of return migration numbers to central California, and allowed us to associate return timing with adaptive genetic variation. We found significant differences between NH

and the CV-lineage hatchery programs that likely reflect ancestral genetic divergence. NH exhibited similar patterns to coastal winter-run steelhead populations, while CV-lineage *O. mykiss* migrated significantly early regardless of *GREB1L/ROCK1* genotype. These results contribute to our understanding of the distribution of *GREB1L/ROCK1* variation across landscapes and help to untangle the effects on phenotypic expression from potential environmental influences.

Genotypic distribution

GREB1L/ROCK1 variation was differentially distributed between NH and the other CV-lineage fyke trapped samples as predicted, with CV-origin *O. mykiss* possessing a broader representation of genotypes for all polymorphic loci compared to NH, which was often skewed towards one allele per marker. Other studies in coastal *O. mykiss* similarly identified high frequencies of mature migrant variation, with a small minority of populations possessing early migration variation (Kannry et al. 2020; Waples et al. 2022; Le Gall et al. 2024). This pattern has been particularly well-documented in some Eel River populations, which may have been where Nimbus Hatchery originally obtained its *O. mykiss* broodstock (NMFS 2006; Pearse & Garza 2015). These differences most likely stemmed from pre-existing genetic variation before the construction of barriers like hydroelectric dams that limit migration and trap previously unrestricted populations above barriers in other coastal populations (Micheletti et al. 2018; Kannry et al. 2020).

Genetic variation in GREB1L, as well as the general distribution of genetic and phenotypic variation, is understudied in CV-lineage salmonids and often focused on CV Chinook salmon (*Oncorhynchus tshawytscha*). However, previous studies have described the California Central Valley as supporting a wide range of life history variation that likely also applies to the distribution of *GREB1L/ROCK1* variation (McEwan 2001; Satterthwaite et al. 2010; Sogard et al. 2012; Goetz et al. 2024). This plasticity in CV-lineage *O. mykiss* life history expression has both genetic (Goetz et al. 2024) and environmental (Satterthwaite et

al. 2010; Sogard et al. 2012) influences, but it is unclear how much watershed-specific adaptive genetic variation has been altered by historic movement of individuals between populations (NMFS 2006; Pearse & Garza 2015). Whether this life history plasticity in CV-lineage *O. mykiss* descends from ancestral or human-directed influences, or a combination of both, it can be considered a characteristic of the modern CV *O. mykiss* lineage that was also reflected in the distribution of variation amongst the *GREB1L/ROCK1* markers used in this study.

Similar to other steelhead studies comparing genetic structure of inland and coastal populations, we found significant patterns in linkage disequilibrium amongst the *GREB1L/ROCK1* markers by lineage. Previous studies characterized a single block of markers with high LD in coastal populations and two blocks in inland or interior populations (Collins et al. 2020; Waples et al. 2022; Narum et al. 2024). Here, we found that NH samples had higher LD between a smaller number of loci, and inland populations having overall weaker LD values between higher numbers of markers. The differences in LD patterns between CV and NH *O. mykiss* could contribute to observed differences in phenotypic variation, and the strength of associating adaptive genetic variation, between lineages by altering gene function underlying phenotypic expression.

Phenotypic patterns and significance of genetic variation

We observed distinct phenotypic differences that supported known distinctions between coastal-origin NH steelhead and the other CV-lineage hatchery programs: NH-assigned fyke samples had a high proportion of individuals at age-3 compared with CV-origin *O. mykiss*, and older NH fish were sampled earlier in the season (Abadía-Cardoso et al. 2013; Beulke et al. 2023; Goetz et al. 2024). Additionally, we found significant differences in sampling date timing: CV-assigned O. mykiss were predominantly sampled during late summer and early fall, while NH was sampled mostly in January and February. These differences in sampling dates likely do not reflect differences in spawn timing, however, because pedigree results

from 2011–2019 confirmed that NH steelhead generally spawn during the same period as the other CV programs (Goetz et al. 2024). The results from connecting fyke trap samples with their hatchery return dates to calculate the number of days between each date also supports significant differences in time spent in freshwater between lineages and genotypes. Overall, these results suggest that return timing and spawn timing experience different forms of selection underlying their expression, and that many CV-origin *O. mykiss* likely mature further in freshwater while NH *O. mykiss* return to freshwater shortly before spawning (Quinn et al. 2016; Narum et al. 2024).

Our study quantified returning numbers of *O. mykiss* in the Sacramento River both spatially and temporally, and confirmed that CV-lineage *O. mykiss* adopt multiple alternative life history strategies compared to coastal populations that return to natal freshwater streams shortly before spawning (Zimmerman et al. 2009; Sogard et al. 2012, Eschenroeder et al. 2024; Goetz et al. 2024). Although the Central Valley is thought to have only winter-run steelhead (McEwan 2001), we sampled many CV-origin *O. mykiss* with early migrant genetic architecture, and were able to identify some phenotypically early individuals when connecting fyke trap and hatchery sample dates in CV-lineage *O. mykiss*. Additionally, we identified three EE NH coastal steelhead with two of them constituting the only two phenotypic summer-run samples with NH ancestry. NH broodstock originated from either the Eel or Mad Rivers (Nielson et al. 2005; NMFS 2006; Goetz et al. 2024), where summer-run genotypes were detected in the Middle Fork of Eel River but not in the South Fork (Kannry et al. 2020). CV-lineage *O. mykiss* historically were sampled nearly year-round as adults returning to natal streams (McEwan 2001), which is consistent with our results. We found that CV-lineage *O. mykiss* were more likely to return when other individual were returning, regardless of adaptive genotype, suggesting the importance of influences from other sources such as other genetic variation, heritability of return timing (Abadía-Cardoso et al. 2013; Beulke et al. 2023), and environmental cues (Eschenroeder et al. 2024). Based on other observations, it is likely that

this could also result from higher rates of residency observed in some Central Valley rivers (Zimmerman et al. 2009); these individuals utilize little to no marine resources.

The recent discoveries of the association between *GREB1L/ROCK1* genotype and run timing in other Chinook and steelhead populations has prompted discussion of large-effect loci and what they can realistically contribute to conservation applications (Pearse 2016; Prince et al. 2017; Waples et al. 2022). Despite petitions to re-classify Northern California summer-run steelhead as a Distinct Population Segment (DPS) under the protection of the Endangered Species Act (ESA), NOAA fisheries determined that summer-run fish repeatedly interbreed with winter-run steelhead and should not constitute as a DPS (Waples et al. 2022). In the samples we collected from the California Central Valley, we found early-migrating individuals with all polymorphic marker genotypes, suggesting that the association between *GREB1L/ROCK1* and seasonal run timing seen in many *O. mykiss* populations does not apply to CV *O. mykiss* populations. Adaptive loci are valuable tools for identifying important populations for conservation, but effective conservation programs need to also consider each watershed's environmental capabilities to support ecotypes of interest. Early migration in steelhead has important environmental conditions that have been broadly interrupted by anthropogenic modifications of watersheds, such as the introduction of dams (Quinn et al. 2016; Prince et al. 2017), and are positively impacted by the availability of rich interior spawning habitats (Waples et al. 2022). Identifying and cultivating the relevant genetic diversity underlying declining ecotypes is as important, but reintroducing lost habitat characteristics by removing barriers to migration is critical if restoration efforts are going to be successful.

Tables

All supplementary tables are included in supplementary file “Ch3AllTables.csv”

Table 1. Location coordinates, years sampled, and counts of samples received from each location.

River mile	Location name	Latitude	Longitude	2016	2017	2018	2019	2020	2021	Years Implemented
42.91	Below American Confluence	38°25'40"N	121°31'56"W	0	19	13	21	0	2	2016-17 through 2020-21
42.94		38°25'41"N	121°31'56"W	3	30	9	18	0	1	2016-17 through 2020-21
46.27		38°27'32"N	121°30'14"W	4	12	8	35	1	0	2015-16 through 2020-21
48.5		38°28'23"N	121°31'51"W	3	27	17	32	1	5	2015-16 through 2020-21
51.32		38°30'13"N	121°33'34"W	3	12	3	0	0	0	2015-16, 2016-17, 2017-18, 2020-21
51.68		38°30'31"N	121°33'24"W	5	6	1	0	0	0	2015-16, 2016-17, 2017-18, 2020-21
51.97		38°30'43"N	121°33'18"W	1	9	5	0	0	1	2015-16, 2016-17, 2017-18, 2020-21
74.56	Below Feather Confluence	38°43'20"N	121°36'23"W	0	0	0	0	36	0	2020-21
74.75		38°43'30"N	121°36'22"W	0	0	0	0	12	0	2020-21
75.71		38°44'19"N	121°36'11"W	0	0	0	0	3	0	2020-21
75.95		38°44'31"N	121°36'06"W	0	0	0	0	44	0	2020-21
76.97		38°45'20"N	121°35'38"W	0	0	0	0	11	0	2020-21
78.13		38°46'17"N	121°35'48"W	0	0	0	0	14	0	2020-21
78.15		38°46'19"N	121°35'49"W	0	0	0	0	11	0	2020-21
78.18		38°46'20"N	121°35'50"W	0	0	0	0	2	0	2020-21
78.21		38°46'21"N	121°35'50"W	0	0	0	0	10	0	2020-21
78.46		38°46'32"N	121°35'59"W	0	0	0	0	6	0	2020-21
88.54	Above Feather Confluence	38°48'6"N	121°41'53"W	0	0	0	27	0	0	2019-20
88.65		38°48'11"N	121°41'59"W	0	18	55	16	0	0	2017-18 through 2019-20
88.76		38°48'12"N	121°42'6"W	0	0	0	10	0	0	2019-20
88.83		38°48'11"N	121°42'11"W	0	18	25	2	0	0	2017-18 through 2019-20
91.48		38°49'29"N	121°43'24"W	0	25	30	75	0	0	2017-18 through 2019-20
91.65		38°49'46"N	121°43'30"W	0	20	3	10	0	0	2017-18 through 2019-20

Table 2. List of microhaplotypes, genome position, source, and predicted genotypes.

Locus	SNP	Position	Scaffold	Fluidigm	CRITFC GTseq	Hess et al. 2016, name	Collins et al. 2020, name	Variation	GREB1L/intergenic/ROCK1	Early Allele	Late Allele	Region grouping	Early	Mid	Late
Omy_Ch28_greb1_mhap1	Omy_Ch28_greb1_mhap1	11,607,954			Omy28_11607954		Omy28_11607954, marker 1	yes	GREB1L missense variant	C	T	A	C/C	C/T	T/T
Omy_Ch28_greb1_mhap2	Omy_Ch28_greb1_mhap2	11,609,793	scaffold79929e:592595			52458_16	Omy_RAD52458-17, marker 2	yes	GREB1L stop gained	G	A		G/G	A/G	A/A
Omy_Ch28_greb1_mhap2	Omy_Ch28_greb1_mhap2	11,609,794	scaffold79929e:592596		Omy_RAD52458-17	52458_16	Omy_RAD52458-17, marker 2	yes	GREB1L synonymous variant	G	T	B	G/G	G/T	T/T
Omy_Ch28_11623532_B	Omy_Ch28_11623532_B	11,622,532						no	GREB1L missense variant	T	G	NA	T/T	G/T	G/G
Omy_Ch28_11623412_B	Omy_Ch28_11623412_B	11,623,412						no	GREB1L missense variant	C	T	NA	C/C	C/T	T/T
Omy_Ch28_11625241_C	Omy_Ch28_11625241_C	11,625,241			Omy28_11625241		Omy28_11625241, marker 4	yes	GREB1L intron variant	A	G	C	A/A	A/G	G/G
Omy_Ch28_11629294_D	Omy_Ch28_11629294_D	11,629,294						no	GREB1L intron variant	C	T	NA	C/C	C/T	T/T
Omy_Ch28_11641623_D	Omy_Ch28_11641623_D	11,641,623			Omy_GREB1_09		Omy_GREB1_09, marker 6	no	GREB1L intron variant	T	G	NA	T/T	G/T	G/G
Omy_Ch28_11658853_F	Omy_Ch28_11658853_F	11,658,853			Omy28_11658853		Omy28_11658853, marker 7	no	GREB1L upstream variant	A	C	NA	A/A	A/C	C/C
Omy_Ch28_11667578_F	Omy_Ch28_11667578_F	11,667,578			Omy28_11667578		Omy28_11667578, marker 8	yes	intergenic region	T	C		T/T	C/T	C/C
Omy_Ch28_greb1_mhap6	Omy_Ch28_greb1_mhap6	11,667,682	scaffold79929e:649195					yes	intergenic region	A	T		A/A	A/T	T/T
Omy_Ch28_greb1_mhap8	Omy_Ch28_greb1_mhap8	11,667,915	scaffold79929e:649428	Omy_RT8000200	Omy_RAD47080-54	47080_53	Omy_RAD47080-54, marker 9	yes	intergenic region	A	G		A/A	A/G	G/G
Omy_Ch28_greb1_mhap8	Omy_Ch28_greb1_mhap8	11,667,954	scaffold79929e:649467	Omy_RT652305			??? Collins et al. 2020	yes	intergenic region	G	A	D	G/G	A/G	A/A
Omy_Ch28_11676622_G	Omy_Ch28_11676622_G	11,676,622			Omy28_11676822		Omy28_11676822, marker 11	yes	intergenic region	T	G	E	T/T	G/T	G/G
Omy_Ch28_11676603_G	Omy_Ch28_11676603_G	11,676,603		Omy_RT663057			new	yes	intergenic region	C	T	F	C/C	C/T	T/T
Omy_Ch28_11683310_H	Omy_Ch28_11683310_H	11,683,310					new	yes	intergenic region	T	A		T/T	A/T	A/A
	Omy_Ch28_11683310_H	11,683,335					new	yes	intergenic region	C	T		C/C	C/T	T/T
	Omy_Ch28_11684744_H	11,684,712					new	yes	intergenic region	A	T		A/A	A/T	T/T
Omy_Ch28_11684744_H	Omy_Ch28_11684744_H	11,684,744					new	yes	intergenic region	C	G		C/C	C/G	G/G
	Omy_Ch28_11684744_H	11,684,769					new	yes	intergenic region	G	T		G/G	G/T	T/T
	Omy_Ch28_11684744_H	11,684,802					new	yes	intergenic region	G	A	G	G/G	A/G	A/A
Omy_Ch28_greb1_mhap10	Omy_Ch28_greb1_mhap10	11,803,846	scaffold79929e:780205				???	yes	ROCK1 downstream	G	T		G/G	G/T	T/T
	Omy_Ch28_greb1_mhap10	11,803,870	scaffold79929e:780229				???	yes	ROCK1 downstream	C	T	H	C/C	C/T	T/T

Table 3. Counts of Central Valley and Nimbus females and males at age-2 and age-3 for each microhaplotype and genotype.

		Central Valley lineage				Nimbus lineage			
		Female		Male		Female		Male	
Locus	genotype	age-2	age-3	age-2	age-3	age-2	age-3	age-2	age-3
11607954_mhap1_position1	C/C	191	24	173	13	8	22	22	7
	C/T	17	3	23	1	7	8	5	6
11609793_mhap2_position1	A/A	8	1	5	0	2	3	3	0
	A/G	42	7	45	2	7	10	14	5
	G/G	157	20	141	12	6	17	10	8
11609794_mhap2_position2	G/G	157	20	141	12	6	17	10	8
	G/T	42	7	45	2	7	10	14	5
	T/T	8	1	5	0	2	3	3	0
11609795_mhap2_position3	C/C	207	28	191	14	15	30	27	13
11622532_B_position1	G/G	212	28	197	14	15	30	27	13
11623412_B_position1	T/T	211	28	197	14	15	30	27	13
11625241_C_position1	A/A	112	10	105	10	0	0	0	0
	A/G	75	15	74	3	1	5	4	2
	G/G	22	3	18	1	14	25	22	11
11629294_D_position1	T/T	206	28	194	14	13	30	27	13
	G/G	211	28	197	14	15	30	27	13
	C/C	211	28	197	14	15	30	27	13
11667578_F_position1	C/C	18	5	19	2	15	27	25	12
	C/T	83	11	74	1	0	3	2	1
	T/T	111	12	103	11	0	0	0	0
11667682_mhap6_position1	A/A	111	12	102	11	0	0	0	0
	A/T	83	11	75	1	0	3	2	1
	T/T	18	5	20	2	15	27	25	12
11667915_mhap8_position1	A/A	119	13	106	11	0	1	0	0
	A/G	73	10	71	1	0	2	2	1
	G/G	17	5	20	2	14	27	23	11
11667954_mhap8_position2	A/A	17	5	20	2	14	27	23	11
	A/G	73	10	71	1	0	2	2	1
	G/G	119	13	106	11	0	1	0	0
11676622_G_position1	G/G	69	12	56	2	12	26	23	8
	G/T	65	10	78	6	0	1	0	2
	T/T	29	3	29	2	0	0	0	0
11678603_G_position1	C/C	111	12	103	11	0	0	0	0
	C/T	83	11	74	2	0	5	3	2
	T/T	18	5	20	1	15	25	24	11
11683310_H_position1	A/A	156	22	145	12	12	27	24	12
	A/T	34	5	35	1	0	3	0	1
	T/T	4	0	6	1	0	0	0	0
11683310_H_position2	C/C	102	11	102	12	0	0	0	0
	C/T	78	13	69	1	0	6	1	2
	T/T	14	3	15	1	12	24	23	11
11684744_H_position1	A/A	108	12	104	12	0	0	0	0
	A/T	81	13	73	1	0	6	3	2
	T/T	15	3	15	1	15	24	23	11
11684744_H_position2	C/C	41	6	34	3	15	27	24	12
	C/G	88	18	93	4	0	3	2	1
	G/G	75	4	65	7	0	0	0	0
11684744_H_position3	G/G	108	12	104	12	0	0	0	0
	G/T	81	13	73	1	0	6	3	2
	T/T	15	3	15	1	15	24	23	11
11684744_H_position4	A/A	15	3	15	1	15	24	23	11
	A/G	81	13	73	1	0	6	3	2
	G/G	108	12	104	12	0	0	0	0
11803846_mhap10_position1	G/G	8	1	5	0	0	2	0	2
	G/T	70	7	63	3	8	16	10	2
	T/T	134	20	129	11	7	12	17	9
11803870_mhap10_position2	C/C	82	11	87	3	2	1	3	2
	C/T	99	15	82	8	3	15	10	6
	T/T	31	2	28	3	10	14	14	5

Figures

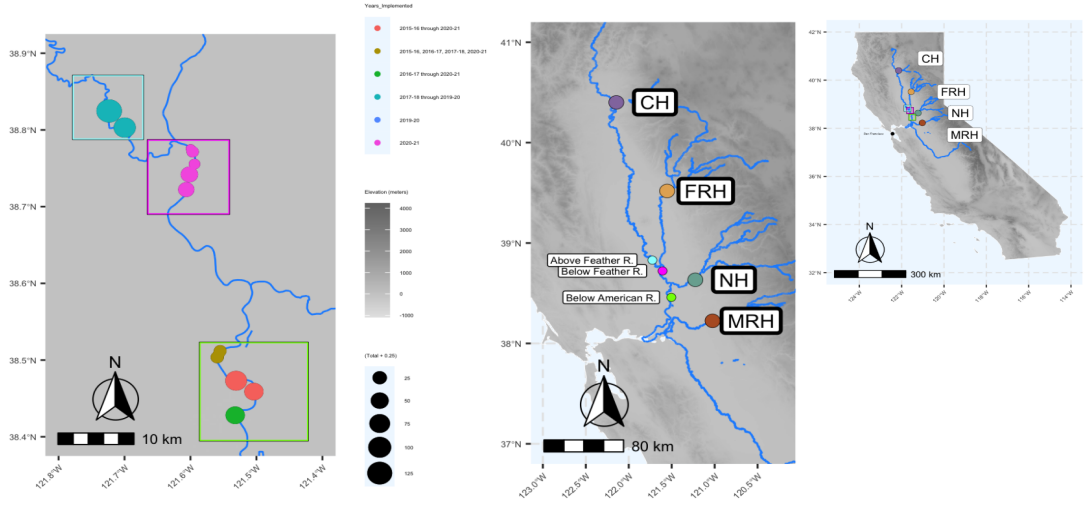


Figure 1. Map of study area with dots representing sample sizes collected per trapping site and colors representing the years each trap location was implemented. Central Valley hatchery program locations are included, and San Francisco in the inset maps.

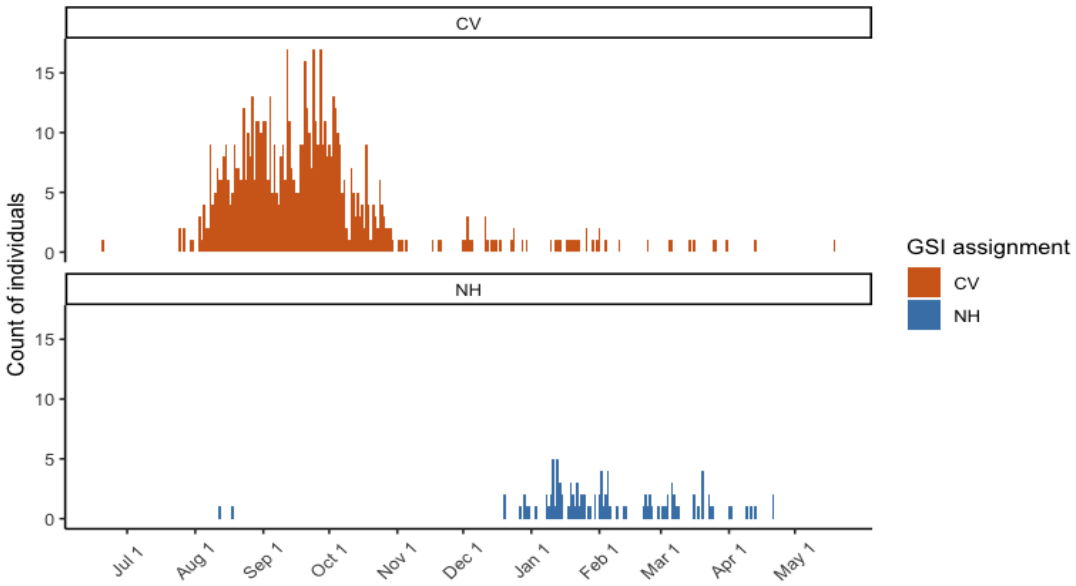


Figure 2. Counts of sample sizes per trapping date and GSI lineage assignment across years.

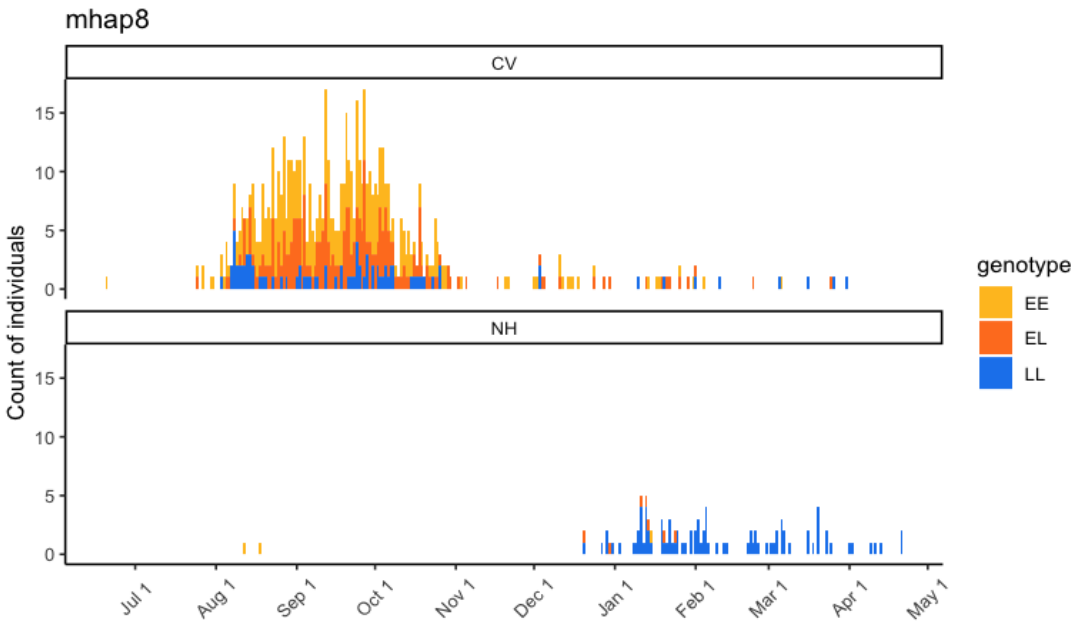


Figure 3. Counts of sample sizes per trapping date and GSI lineage assignment across years by mhap8 genotype and known phenotype.

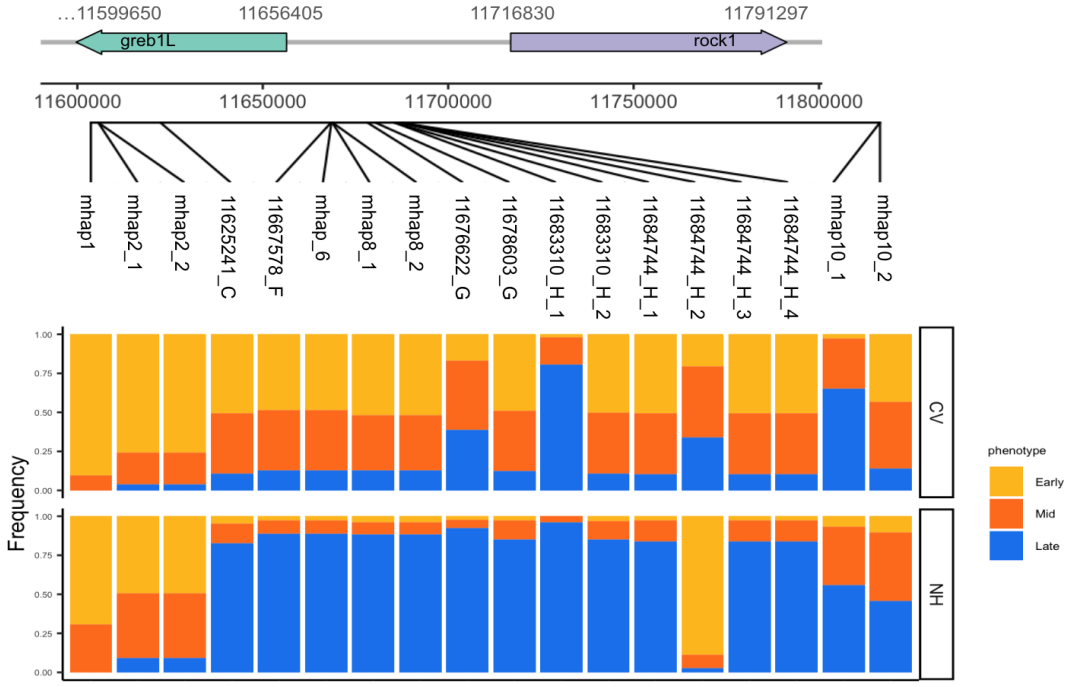


Figure 4. Frequency of predicted phenotype per microhaplotype locus for CV and NH GSI assignments.

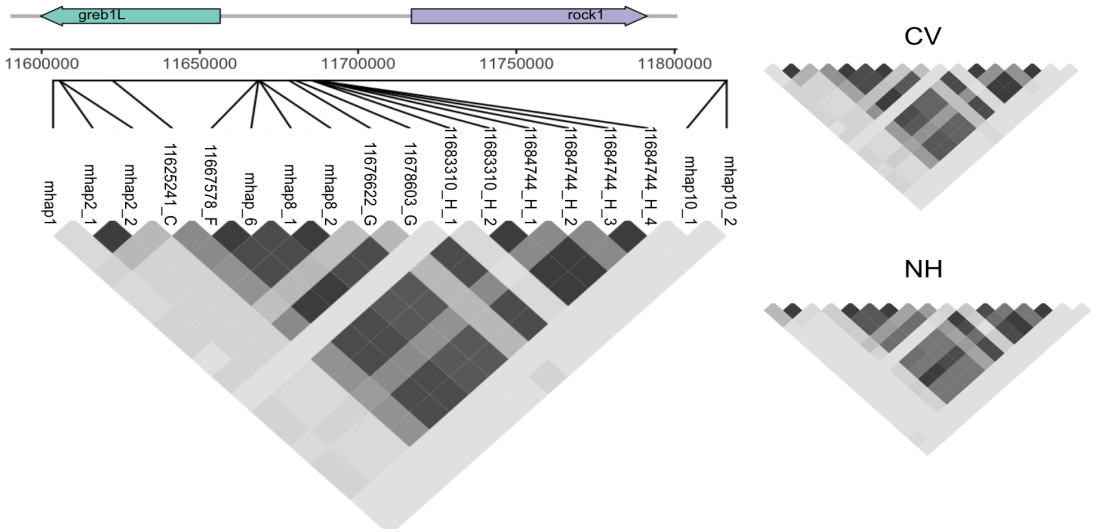


Figure 5. Linkage disequilibrium heat maps for all fyke samples with gene map, as well as by CV and NH GSI assignments.

Conclusions

My dissertation explored genetic relationships and phenotypic patterns in California Central Valley (CCV) *Oncorhynchus mykiss* populations. The CCV and its river systems were heavily transformed in the last 200 years through widespread damming of rivers and diversion of water (Bartolome & Spiegal 2020), and the population structure of its *O. mykiss* populations was 'scrambled' from transplanting individuals between watersheds (Pearse & Garza 2015). Of the four hatchery programs in the CCV that produce steelhead to mitigate losses from hydroelectric dams, only one program, Nimbus Hatchery (NH), sourced their broodstock from a central coast rather than a Central Valley *O. mykiss* population. Samples collected from 2011–2019 were genotyped using a SNP panel with both neutral and adaptive loci (Omy05) for parentage-based tagging (PBT), population genetics analyses, and investigating the distribution of adaptive genetic associated with growth and development. To expand on characterizing genetic patterns in CCV *O. mykiss* populations, I utilized genotyped *O. mykiss* populations in Putah Creek, California, a segmented watershed located between the central coast and Central Valley using neutral and adaptive microhaplotypes (Omy05 and *GREB1L/ROCK1*) for comparison with over 30 reference populations. Finally, I compared the strength of association between adaptive genetic variation underlying migration timing (*GREB1L/ROCK1*) in other populations with fyke trap sampling date in central coast and CCV-lineage *O. mykiss.* I found (1) NH broodstock maintained genetic and phenotypic distinctions that resembled patterns established in central coast populations despite inhabiting the CCV for over 75 years, (2) significant neutral and adaptive differences in *O. mykiss* populations above and below an impassable barrier, and (3) significant differences in strength of association between lineage and *GREB1L/ROCK1* genotype, with CV-lineage *O. mykiss* showing no associations between genotype and phenotype. Overall, I found that CCV *O. mykiss* population dynamics represent a mixture of native and stocked populations that reflect historic and modern riverscape uses, and are genetically and phenotypically distinct from other neighboring lineages.

To investigate neutral and adaptive genetic and phenotypic patterns in *O. mykiss*, I utilized multiple genotyping panels and samples with associated phenotypic measurements. These panels included single SNPs (Abadía-Cardoso et al. 2013) and multiallelic markers known as 'microhaplotypes' (Le Gall et al. 2024). Markers used to characterize adaptive genetic variation within these loci differ among studies, complicating comparisons amongst populations. Improving knowledge of the genomic structure underlying migration timing enables elucidation of mechanisms behind key migration characteristics that may have significant conservation implications (Waples et al. 2022). These data provide information about the distribution of adaptive genomic variation among individuals and populations, which is critical for understanding and managing migratory steelhead.

Using our SNP panel (Abadía-Cardoso et al. 2013), I reconstructed a pedigree for the CCV steelhead hatchery programs that enabled determining age at spawning and family sizes. Genotyping additional samples with this panel also enabled expanding the pedigree to assign new individuals to the pedigree. Our SNP panel was also used for genetic stock identification (GSI) analyses, which allowed for identifying unassigned fish. Omy05 (Omy_R04944) was included on the SNP panel after 2015 that determined initial genotype distributions in the CCV hatchery programs, but developing adaptive microhaplotype panels enabled utilization of multiallelic markers. The SWFSC genetics laboratory developed a microhaplotype genotyping panel for *O. mykiss* (Le Gall et al. 2024), and expanded it to include multiple targeted markers associated with life-history variation and specific genomic regions from other studies, including Omy05 (Pearse et al. 2019) and *GREB1L/ROCK1* (Hess et al. 2016; Collins et al. 2020), as well as adding additional markers for these loci. This approach enabled me to characterize genetic and associated phenotypic patterns in CCV *O. mykiss*, and how they differ from dynamics established in other lineages.

CV-lineage *O. mykiss* spawned younger and returned earlier in the season, and lacked significant associations between genetic variation and adaptive life history traits. Specifically, CV-lineage *O. mykiss* did not show significant influences between Omy05

genotype and age at spawning (Beulke et al. 2023) or between *GREB1L/ROCK1* genotype and sampling date (Hess et al. 2016; Collins et al. 2020; Kannry et al. 2020; Willis et al. 2020). Decreased association between adaptive genetic variation and life history traits in CV-lineage *O. mykiss* likely reflects a combination of influences from lineage and environmental effects, in which local adaptation to CCV environmental conditions results in differences underlying genetically-coded responses to abiotic cues. State-dependent models comparing environmental conditions of the CCV and the central coast found that the CCV supports a higher mix of life history strategies compared to central coast watersheds (Satterthwaite et al. 2009, 2010; Sogard et al. 2012). Future research considering the distribution of genetic and phenotypic diversity in CV-lineage *O. mykiss* transplanted in other locations and/or incorporating temporal environmental variation in abiotic modeling could further illuminate how much genetic and environmental factors influence phenotypic expression in *O. mykiss*, respectively. The environmental support for multiple life history strategies within a population in the CCV could also explain why the coastal broodstock population at NH overall maintained coastal phenotypic patterns in the CCV, with the exception of extreme temporal variation recorded in Chapter 1, in which the relief of severe drought conditions coincided with an increase in age-2 steelhead returning to NH (Goetz et al. 2024). It is possible that evolving in the central coast imparted strong lineage-specific effects that enable maintenance of coastal patterns with the exception of extreme temporal environmental variation. Directly comparing transplanted coastal and CV populations with those in their native region could also clarify genetic and environmental contributions to plasticity in life history traits and phenotypic expression.

Though both external and internal factors influence *O. mykiss* life history strategies, conservation of native *O.mykiss* currently focuses on: protecting populations that phenotypically express declining adaptive traits, understanding factors influencing important life history phenotypes, and identifying potential reservoir populations with anadromous and/or early–returning genetic architecture. Anadromous below-barrier steelhead populations

are organized into Distinct Population Segments (DPSs) with protection under the U.S. Endangered Species Act (ESA; Federal Register 2006). The CV steelhead DPSs is one of the six California steelhead DPSs, and is listed as “Threatened” (Federal Register 2006; NMFS 2014; Pearse & Garza 2015; NMFS 2020). This protection does not extend to include residualized populations above barriers, despite life history plasticity in *O.mykiss* (Abadía-Cardoso et al. 2019). However, substantial ecological variation in freshwater habitat fragments can support isolated *O. mykiss* populations maintaining anadromous or summer-run genetic variation (Leitwein et al. 2017; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019), which may explain why salmonid populations respond positively to dam removals (Winans et al. 2017; Fraik et al. 2021). For example, the distribution of adaptive genetic variation can unexpectedly be distributed in heterogeneous environments, similar to the identification of Omy05 anadromous variation in some above-barrier populations in Chapter 2. These results can provide data for identifying rivers that are good candidates for dam removal or increasing habitat heterogeneity (Leitwein et al. 2017; Pearse & Campbell 2018; Abadía-Cardoso et al. 2019), but that necessitates actual action for successful conservation. The maintenance of genetic architecture underlying declining adaptive traits in some *O. mykiss* populations and successes of dam removals highlight the potential for identifying reservoir populations (or habitats!) and implementing radical habitat restoration to promote expression of declining plastic phenotypes.

The ESA is a vital tool in conservation that outlines criteria for critical habitats for conservation, but application of protections in practice is inconsistent, particularly in places like the CCV. The ESA includes language that specifically covers habitat protection, and habitats in the CCV are listed as critical under the ESA, but modern human uses of the CCV are still the most prominent stressors to *O. mykiss*. In the CCV, over 80% of spawning habitat remains inaccessible behind dams (Lindley et al. 2006) and water diversion to agricultural and industrial uses has overall decreased stream flows, and increased river temperatures (Eschenroeder et al. 2022; He & Marcinkevage 2017). Molecular research, like my

dissertation, that characterizes standing neutral and adaptive genetic variation provide useful tools for conservation, but these results often highlight the strong impacts of a changing climate and anthropogenic influences on population dynamics. It has been a privilege to work with a salmonid species under federal protection, but it also exemplifies that many political problems in salmonid conservation urgently require immediate implementation of non-molecular solutions, such as the removal of dams and increasing water available to rivers for struggling native Californian salmonids. My dissertation provides a framework for understanding the genetic and phenotypic patterns in the CCV, and may help identify reservoir populations for targeted restoration efforts. I hope to participate as a researcher in dialogues on what genetics in *O. mykiss* can meaningfully contribute to conservation, but ultimately, I envision a future where critical habitats (and the species they support!) are protected through stronger and more consistent practices that incorporate knowledge from multiple sources.

Appendix 1

Supplementary Tables for Chapter 1 are available in supplemental files.

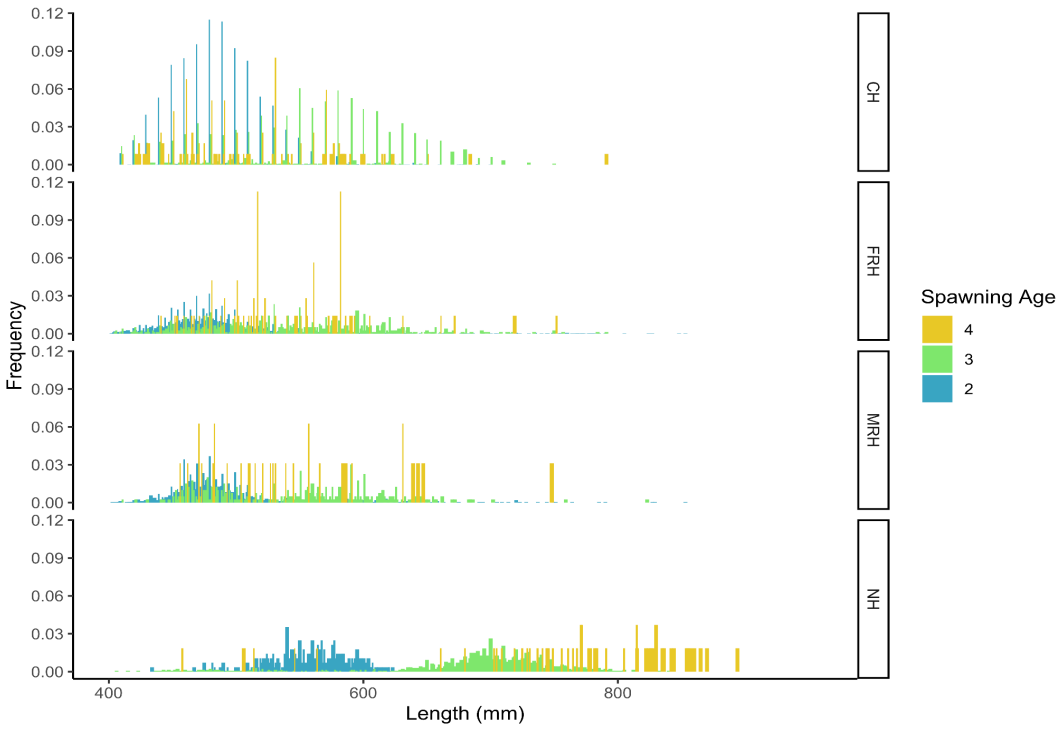


Figure A1. Frequency of measured fin tail fork length (mm) for each age at spawning by hatchery program. Steelhead at CH, FRH, and MRH had more length overlap between different spawning ages compared to Nimbus age-2 and age-3 steelhead.

Supplementary figures for Chapter 1.

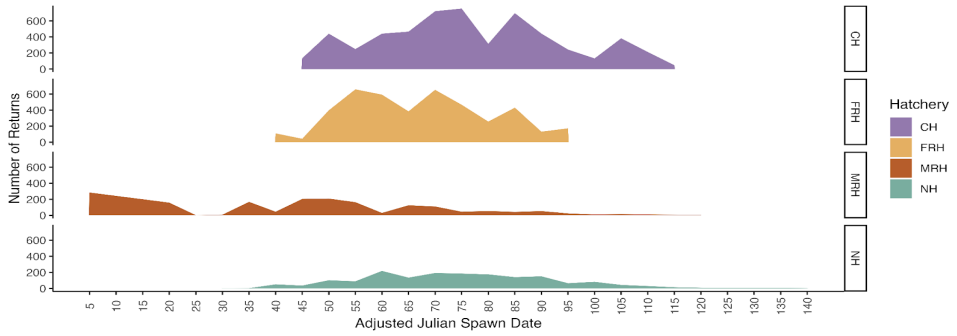


Figure S1. Histogram showing the number of spawning steelhead across programs and all spawning seasons. Spawning dates were converted to Julian dates and adjusted to sequential order by spawn season timing, with the x-axis starting at November 1st.

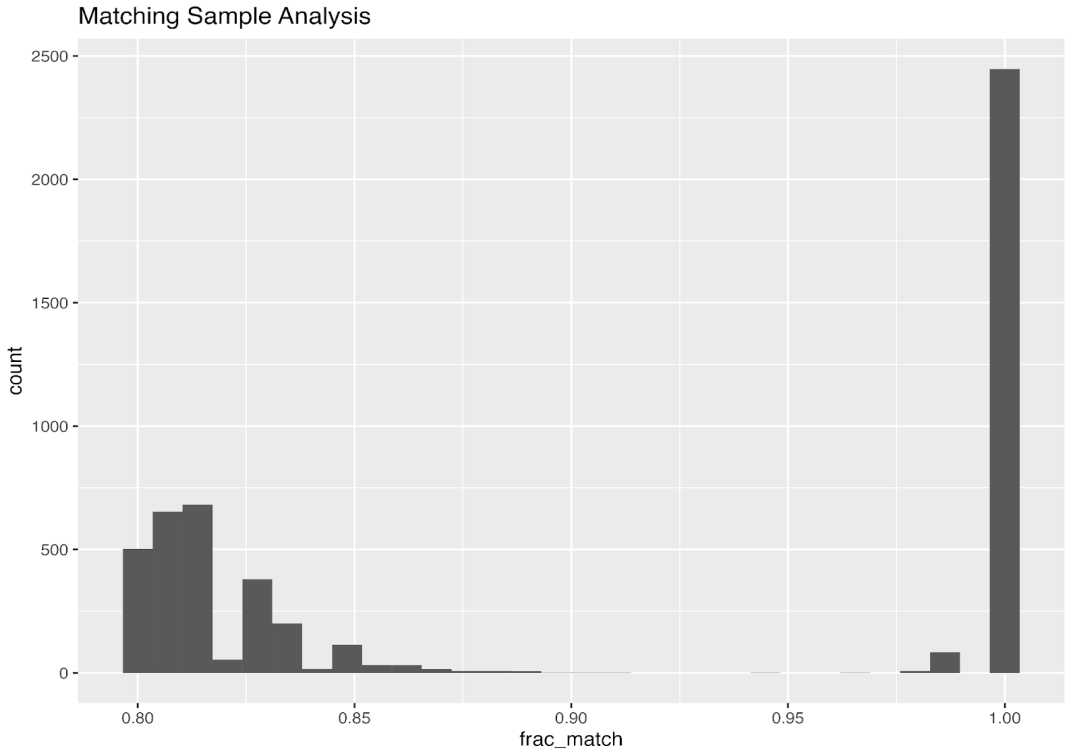


Figure S2. Histogram showing the matching sample analysis results using 'rubias' (Moran & Anderson 2018) that guided identification of individuals with multiple samples for the parentage analysis. Samples with more than 95% matching genotypes were considered to be from a single individual.

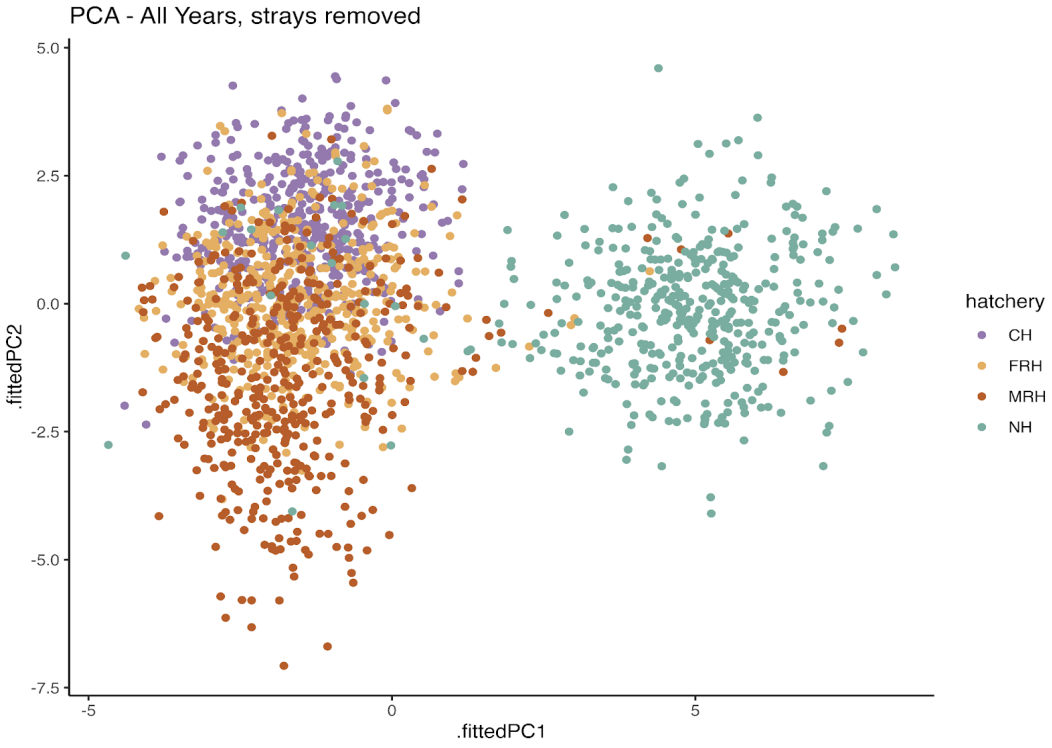


Figure S3. PCA results from STRUCTURE across all years, with hatchery programs distinguished by color.

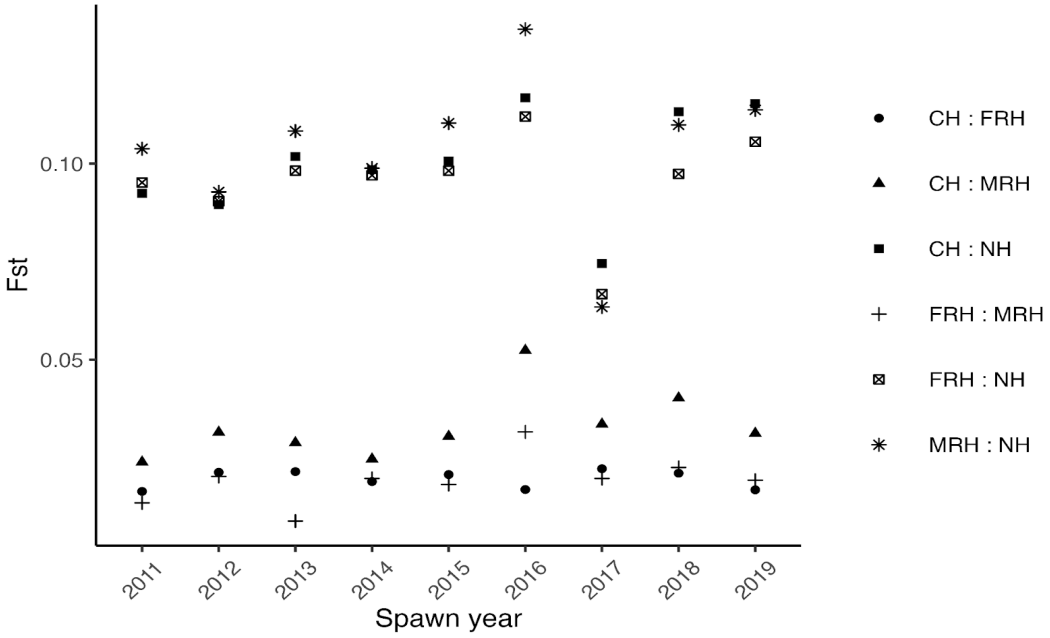
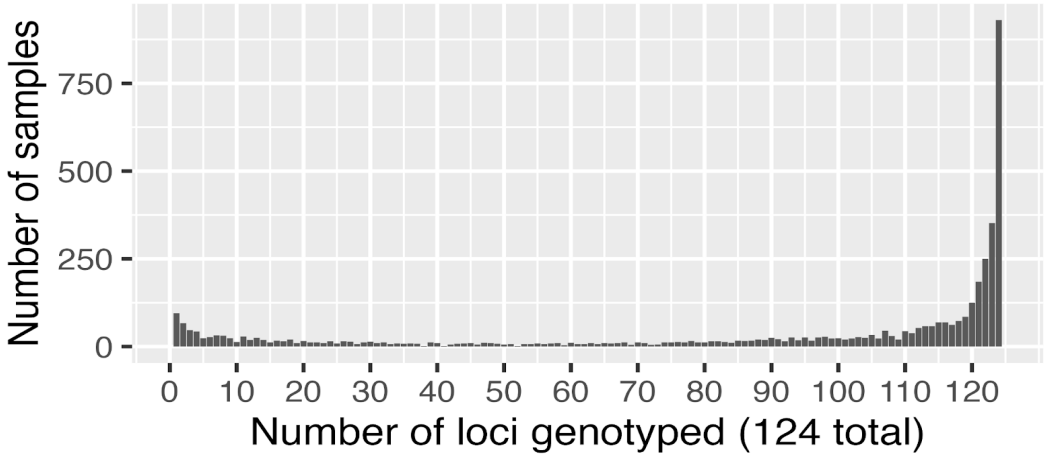


Figure S4. Fst between hatchery programs across study period calculated using 'strataG' (Archer et al. 2017).

Appendix 2

Supplemental tables for Chapter 2 are available in supplemental files.



Supplemental figures for Chapter 2.

Figure S1. Count of samples successfully genotyped across the number of loci successfully genotyped. Samples successfully genotyped for more than 85 markers were kept for further filtering.

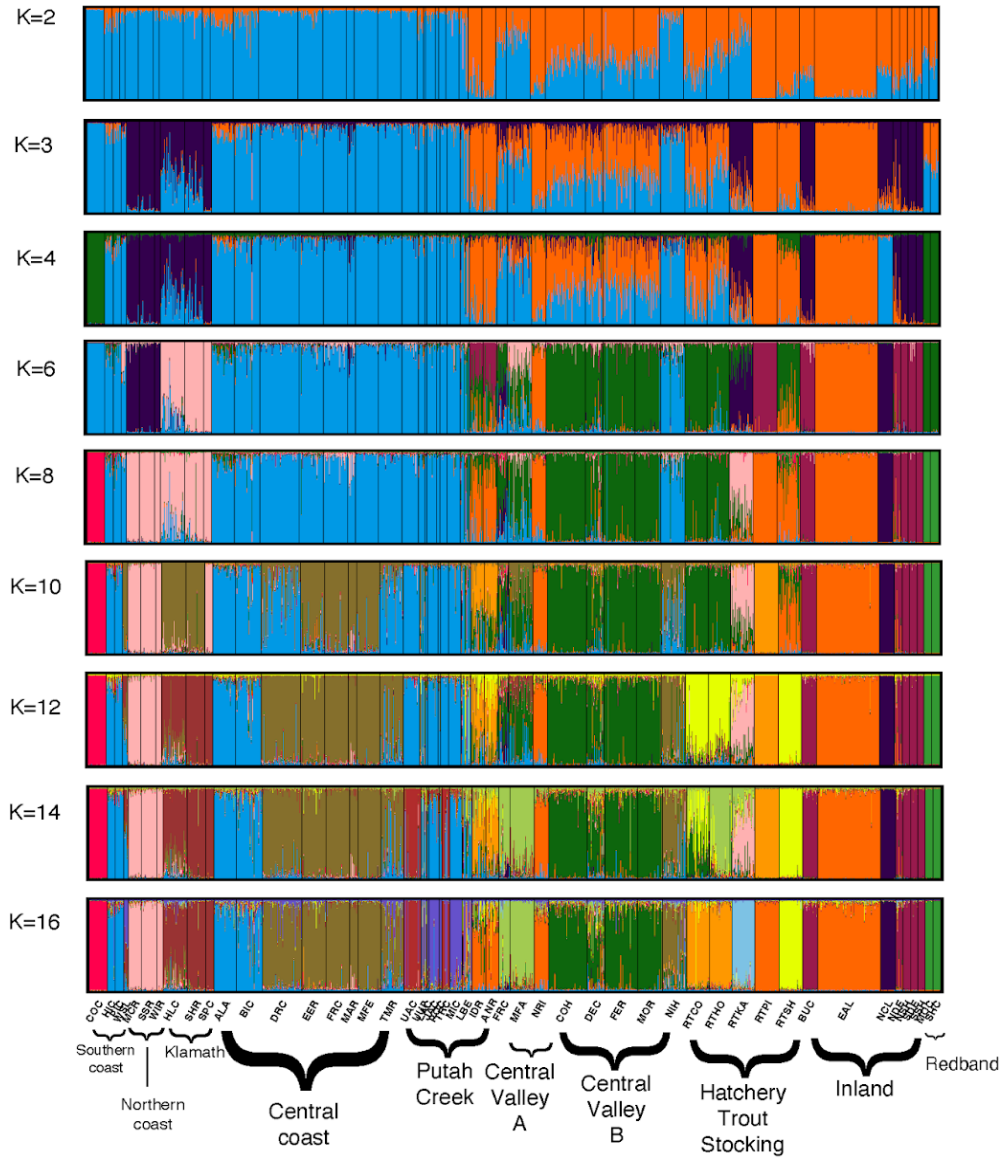


Figure S2. *STRUCTURE* figure with all populations included. Information for all reference populations is available in Table S1.

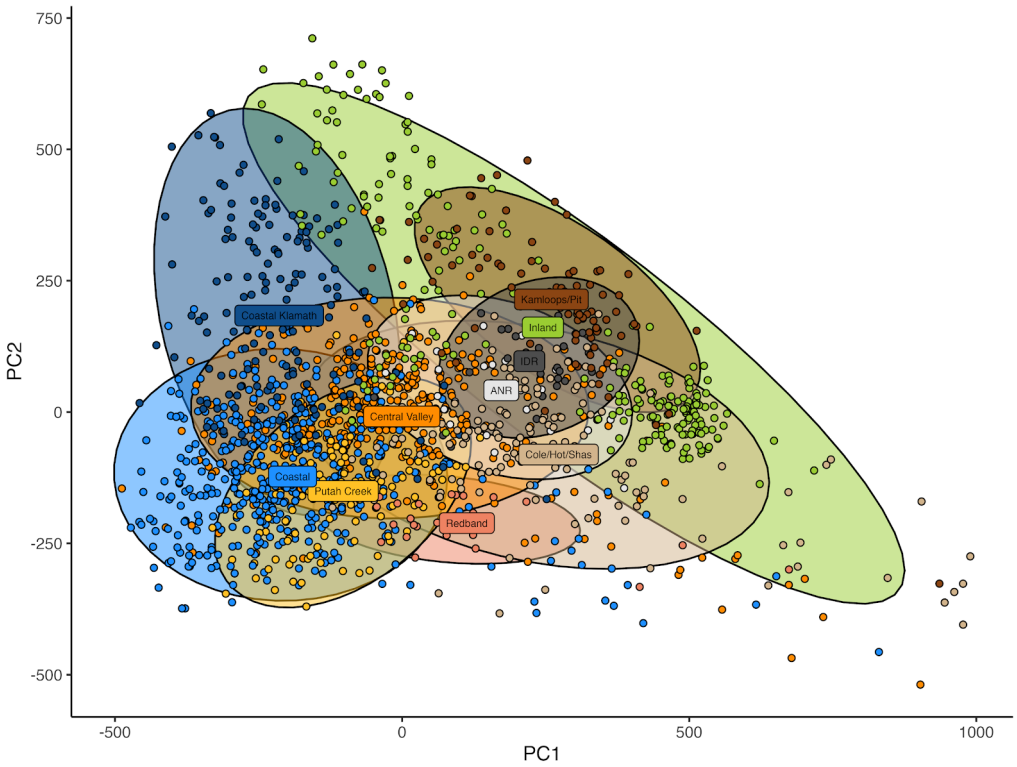


Figure S3. PCA with all populations included.

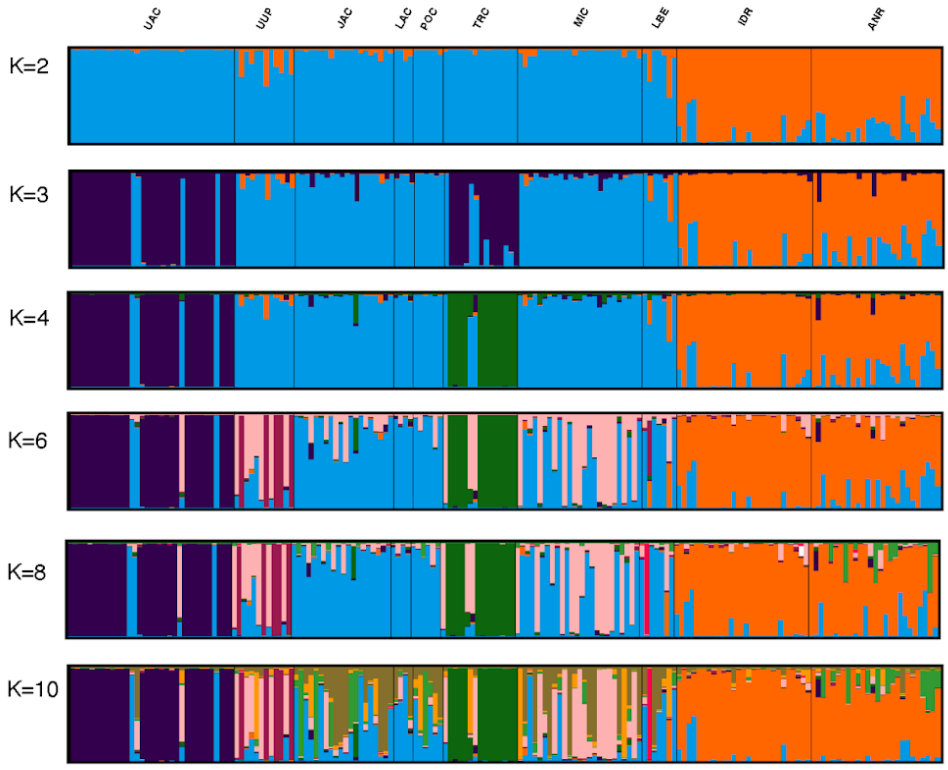


Figure S4. *STRUCTURE* figure for only the Putah Creek populations.

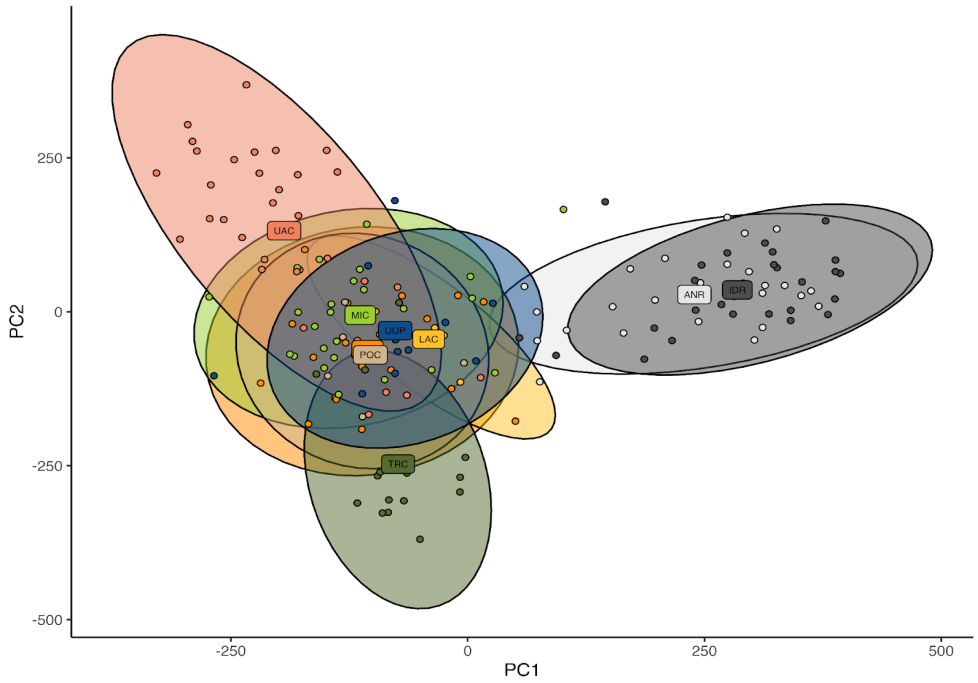


Figure S5. PCA for only the Putah Creek populations.

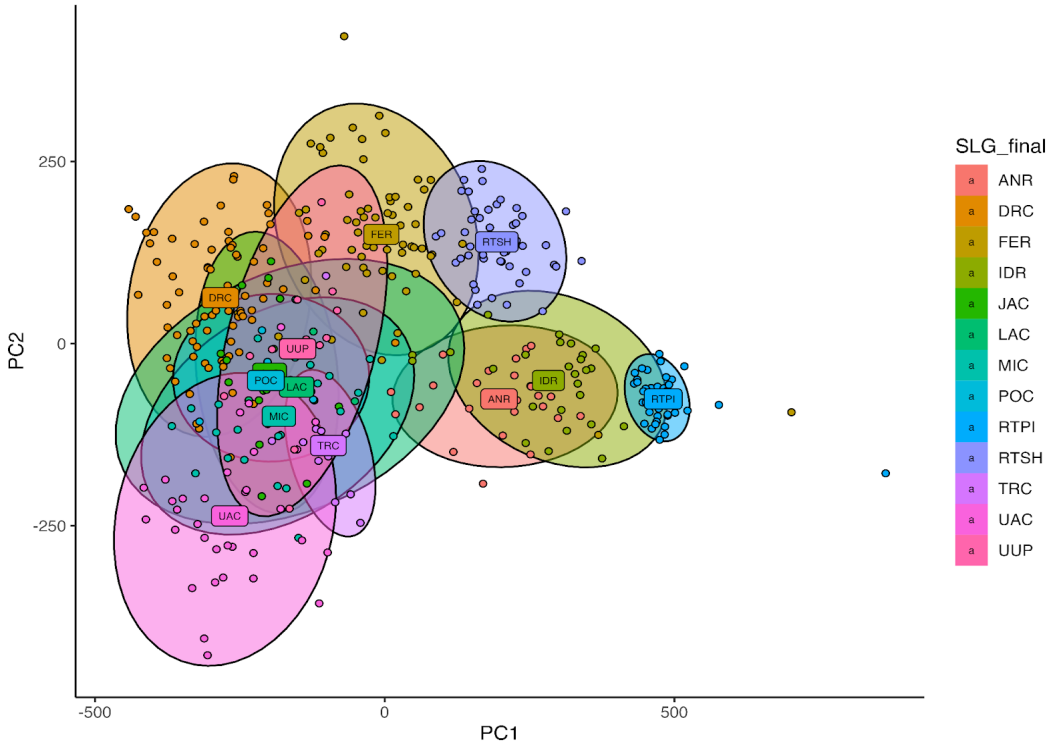


Figure S6. PCA results from Putah Creek and subset of reference populations (Central Valley (Feather River), coastal (Dry Creek), and hatchery rainbow trout strains (Pit Creek (RTPI), Shasta Creek (RTSH)) without grouping by lineage.

Appendix 3

Supplemental tables for Chapter 3 are available in supplemental files.

Supplemental tables and figures for Chapter 3.

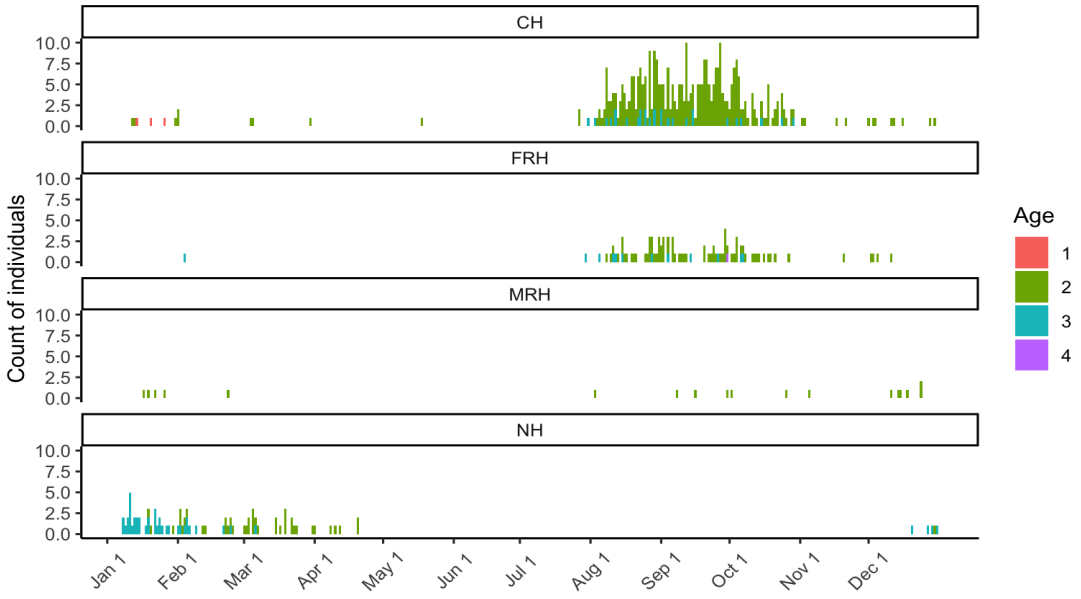


Figure S1. Distribution of age, hatchery program assignment, and date of fyke trap sampling in assigned individuals.

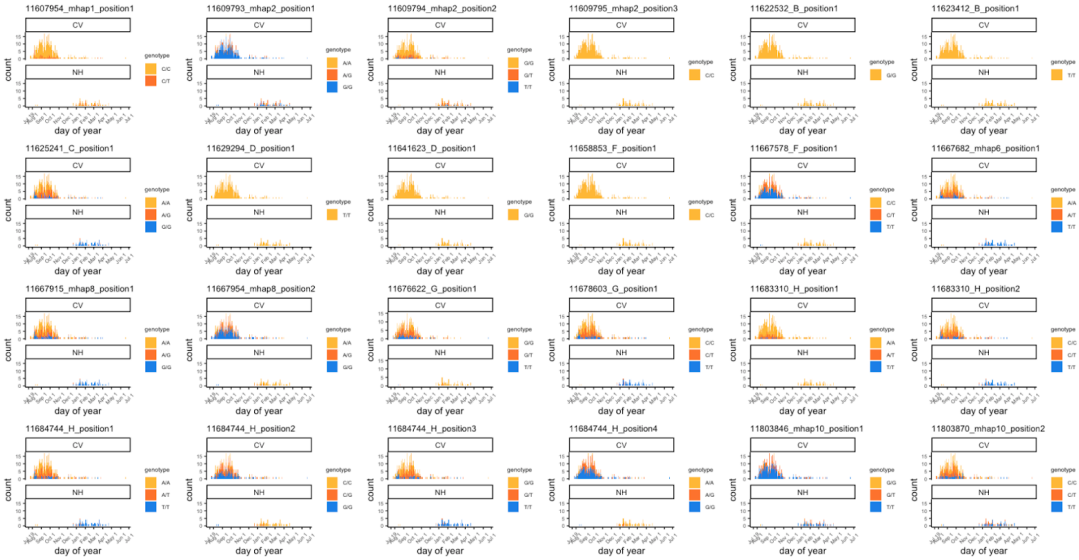


Figure S2. Count of samples and genotype distribution per trapping date and GSI lineage across years.

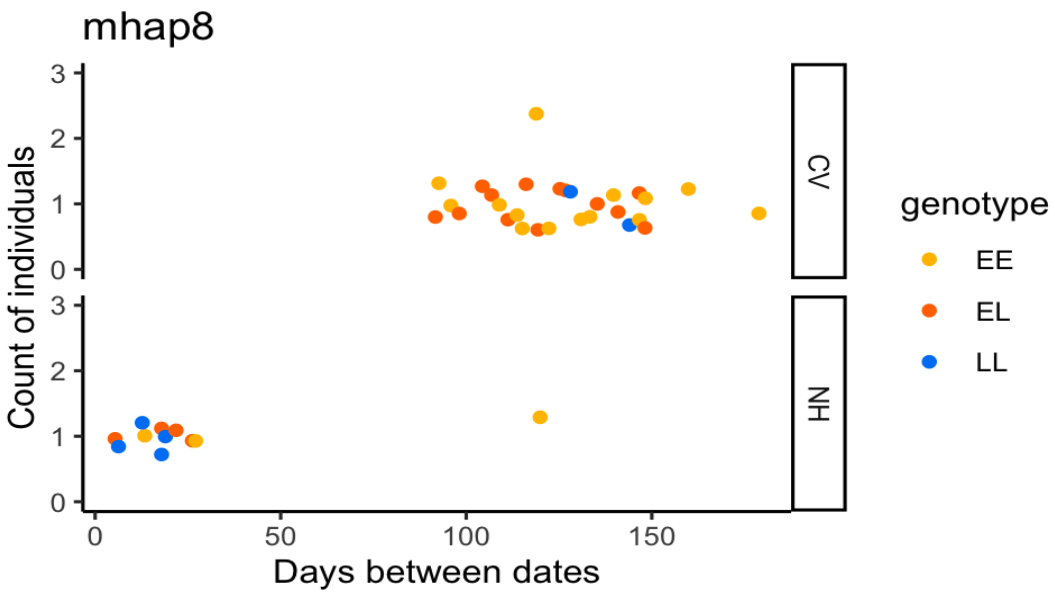


Figure S3. Number of days between fyke trap and hatchery sampling dates by GSI lineage and mhap8 genotype.

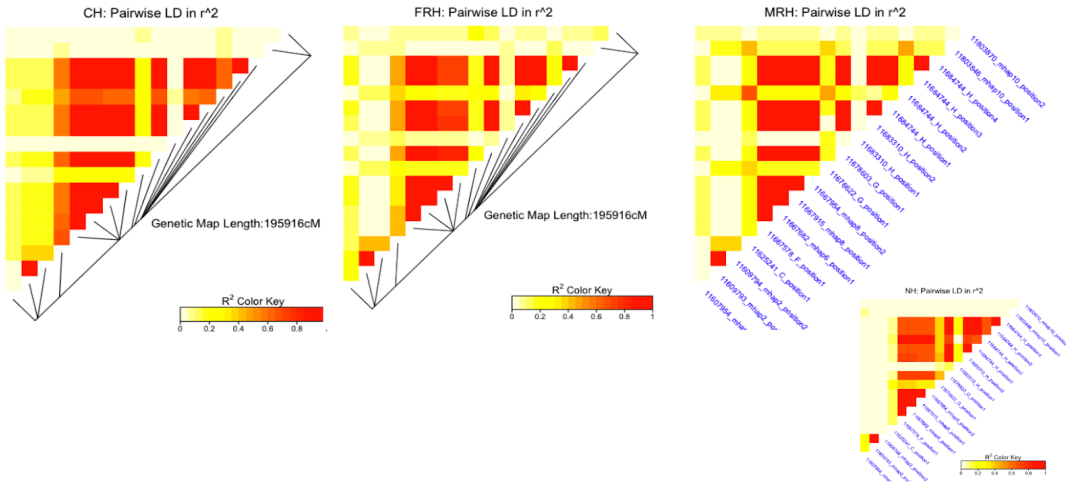


Figure S4. LD heatmaps by hatchery program.

List of Supplemental Files

1. Supplementary Table S1 from Chapter 1 (Ch1_TableS1.csv)
2. Supplementary Table S2 from Chapter 1 (Ch1_TableS2.csv)
3. Supplementary Table S3 from Chapter 1 (Ch1_TableS3.csv)
4. Supplementary Table S4 from Chapter 1 (Ch1_TableS4.csv)
5. Supplementary Table S5 from Chapter 1 (Ch1_TableS5.csv)
6. Supplementary Table S6 from Chapter 1 (Ch1_TableS6.csv)
7. Supplementary Table S7 from Chapter 1 (Ch1_TableS7.csv)
8. Supplementary Table S8 from Chapter 1 (Ch1_TableS8.csv)
9. Supplementary Table S9 from Chapter 1 (Ch1_TableS9.csv)
10. Supplementary Table S10 from Chapter 1 (Ch1_TableS10.csv)
11. Supplementary Table S11 from Chapter 1 (Ch1_TableS11.csv)
12. Supplementary Table S1 from Chapter 2 (Ch2_TableS1.csv)
13. Supplementary Table S2 from Chapter 2 (Ch2_TableS2.csv)
14. Supplementary Table S3 from Chapter 2 (Ch2_TableS3.csv)
15. Supplementary Table S4 from Chapter 2 (Ch2_TableS4.csv)
16. Supplementary Table S5 from Chapter 2 (Ch2_TableS5.csv)
17. Supplementary Table S6 from Chapter 2 (Ch2_TableS6.csv)
18. Supplementary Table S1 from Chapter 3 (Ch3_TableS1.csv)
19. Supplementary Table S2 from Chapter 3 (Ch3_TableS2.csv)
20. Supplementary Table S3 from Chapter 3 (Ch3_TableS3.csv)
21. Supplementary Table S4 from Chapter 3 (Ch3_TableS4.csv)

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