

Genetic Analysis of Rainbow Trout (*Oncorhynchus mykiss*) in California's Putah Creek Watershed

Final Report, 2022



Figure 1. Large rainbow trout caught by an angler in the IDR. Photo: Christopher Yarnes.

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Summary

Changes to natural systems including dams and stocking of hatchery trout have created a mosaic of populations throughout the state of California. To determine the origin of rainbow trout throughout the Putah Creek watershed, we captured 173 rainbow trout from eight sites above Lake Berryessa and two sites below the impassable Monticello Dam. We conducted a population genetic analysis of these samples and compared them to samples of *O. mykiss* from throughout the state as well as hatchery rainbow trout strains commonly stocked in California. Our results show that fish downstream of Monticello and upstream of the Putah Diversion Dam (PDD), in a section known as the 'Putah Creek inter-dam Reach' (IDR), as well as those in the anadromous waters downstream of the PDD, are of native Central Valley origin. Specifically, these fish have a mixed ancestry similar to existing wild Central Valley steelhead populations and hatchery programs, with likely contributions from multiple hatchery rainbow trout strains. In contrast, all fish sampled in the upper watershed, above Lake Berryessa, share ancestry with steelhead in the Russian River and other coastal populations. There were concordant patterns in adaptive genetic variation associated with migratory life-histories throughout the watershed, with IDR fish again showing patterns similar to fish in the anadromous reach of lower Putah Creek. These results suggest that fish above Monticello Dam are remnant populations of coastal *O. mykiss* present in Putah Creek prior to the 1957 completion of Monticello Dam, after which Central Valley lineage *O. mykiss* came to predominate below the dam, likely through a combination of colonization by steelhead and stocking with hatchery rainbow trout.

Introduction

Prior to 1957, when the Monticello Dam and Putah Diversion Dam (PDD) were completed, Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) spawned in the headwaters of Putah Creek. After creation of the dams, a population of rainbow trout became established in the reach between the two dams, commonly referred to as the inter-dam reach (IDR), and in many tributaries above Lake Berryessa. The California Department of Fish and Wildlife has stocked various strains of hatchery rainbow trout in the IDR since at least 1977, and likely much earlier. Stocking ceased in 2008 after a lawsuit was filed against the Department of Fish and Wildlife over the effects of fish stocking on ESA/CESA listed species. To determine if natural production supported a wild population, the California Department of Fish and Wildlife conducted a survey in 2010 (Weaver and Mehalick, 2009) indicating trout in the IDR were likely wild and naturally produced. An additional survey was conducted in 2013 (Hogan et al., 2013), confirming the IDR met minimum qualifications for designation as a Wild Trout Water (Bloom and Weaver, 2008), and it was designated as such in 2014 ([Designated Wild and Heritage Trout Waters \(ca.gov\)](#)).

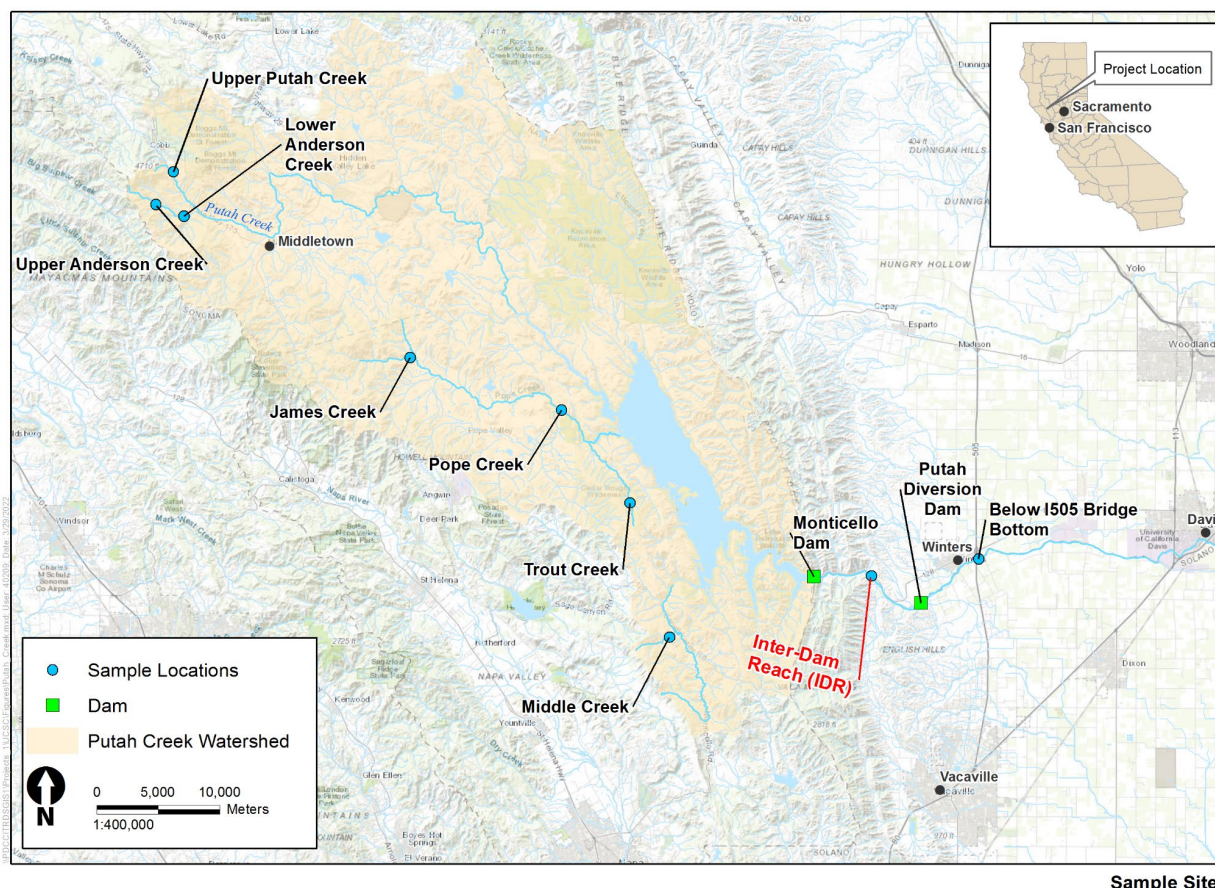
The goal of this study was to investigate the origin of rainbow trout in the IDR and other locations throughout the Putah Creek watershed and compare their genetic variation to *O. mykiss* throughout California, including Central Valley Steelhead and hatchery rainbow trout. We applied a newly developed panel of genetic markers, microhaplotypes, that offer good resolution for population genetics analysis and can be genotyped using high-throughput sequencing methods (Baestcher et al., 2019; Le Gall et al., In Prep). These markers provide similar resolution to characterize genetic diversity and population structure in California *O. mykiss* to the mixed microsatellite and single nucleotide polymorphism (SNP) panel used by Pearse and Garza (2015), and better resolution than the SNP panel used in other recent genetic studies of *O. mykiss* in the American, Tuolumne, and Merced Rivers

(Abadía-Cardoso et al., 2019; Pearse & Campbell, 2018). Using a recently genotyped baseline (Le Gall et al., In Prep), we compared the Putah Creek samples with representative populations from both coastal and Central Valley genetic lineages as well as several hatchery rainbow trout strains commonly used for stocking in lakes and rivers, which were primarily developed from Central Valley lineage source populations, although some have a complex history (Leitritz, 1970; Barngrover, 1990). An additional goal was to investigate the distribution of adaptive genetic variation, or variation due to natural selection, associated with migratory life history traits. We achieved this by genotyping genetic markers for the Omy05 genomic region associated with resident (R, rainbow trout) and anadromous (A, steelhead) life-histories (Pearse et al., 2014, 2019) and the greb1l gene region linked to summer (early, E) and winter-run (late, L) steelhead run-timing (Waples et al. 2022). This combined approach allowed us to better characterize the distribution of genetic variation among Putah Creek *O. mykiss*.

Methods

Study Area and Sampling

Upper Putah Creek originates on Cobb Mountain near Whispering Pines, CA (Figure 1). It flows southeast through the Coast Range Mountains until reaching Lake Berryessa near Winters, CA. Lake Berryessa was created when Putah Creek was impounded by the impassable Monticello Dam, below which is considered lower Putah Creek. That same year, the smaller, and also impassable, PDD was completed downstream to divert water into Putah South Canal, creating the 'inter-dam reach' (IDR). While both of these dams block upstream passage, some fish are likely able to survive when passing downstream through the Monticello Dam spillway and many, if not all, likely survive downstream movement through PDD. The remainder of Putah Creek below the PDD is open to anadromous migrants (steelhead) and eventually flows into the Sacramento River near Rio Vista, CA and eventually to the Pacific Ocean through the Golden Gate in San Francisco Bay. The roughly 6.5 miles of IDR between the two dams was the main focus of this study but other sites were important to determine the origins of trout throughout the entire Putah Creek watershed.



Sample Sites

Figure 1. Study map showing the Putah Creek watershed in tan with sampling locations above and below Lake Berryessa. Sites include the IDR between Monticello Dam and PDD as well as the anadromous water sampled from the PDD down to just below the Interstate 505 Bridge.

From September 2020 through April 2022, 173 rainbow trout were captured by hook-and-line fly fishing and backpack (Smith-Root LR-24) electrofishing (Table 1). An upper caudal clip was collected from each fish for genetic analysis, placed in Whatman grade 1 filter paper, and allowed to air dry inside a coin envelope. Fish were captured throughout the Putah Creek Watershed above and below Lake Berryessa. In addition to upper Putah Creek, samples were collected in Anderson Creek above and below the impassable Anderson Falls. Samples were also collected in Pope Creek and two of its tributaries (James Creek near Aetna Springs, CA and Trout Creek in the Cedar Roughs Wilderness Area) as well as in Middle Creek, a tributary to Capell Creek. In lower Putah Creek (below Lake Berryessa), samples were collected in the IDR and in the anadromous reach below the PDD. In addition, 23 samples collected by hook-and-line fishing within Lake Berryessa in Fall 2019 were included in the analysis (J. Rodzen (CDFW), pers. com. 2022), making a total of 196 individual samples from Putah Creek watershed. For comparative purposes, these samples were combined with genotype data from almost 2,000 *O. mykiss* samples from throughout California, including representative samples of 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).

Table 1. Summary of samples and genetic data collected for sites in the Putah Creek watershed. For each population, three measures of genetic diversity, expected (H_e) and observed (H_o) heterozygosity and allelic richness, are reported.

Site Name	Pop	# Sampled	# Genotyped
Anderson Creek, Above Waterfalls	HAAW	33	33
James Creek	HJAC	20	20
Lake Berryessa	HLBE	23	11
Lower Anderson Creek	HLAC	4	4
Middle Creek	HMIC	25	25
Pope Creek	HPOC	6	6
Putah Creek, Anadromous Reach	HANR	28	27
Putah Creek, IDR	HIDR	27	27
Trout Creek	HTRC	16	15
Upper Upper Putah	HUUP	14	12

Genotyping and Analyses

Compiling and processing microhaplotypes

All genetic data collected for this study were obtained by sequencing ‘microhaplotype’ markers using a recently developed panel of loci (Le Gall et al., In Prep). Once collected, 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). Individuals missing genotypes for more than 60/124 loci were removed, retaining only individuals with a minimum of 64 loci. Filtering genotypes by depth and allelic balance removed a total of 213 individuals, including 12 individuals from Lake Berryessa, one from Putah Creek; Anadromous Reach, one from Trout Creek, and two from Upper Putah Creek; Upper Putah/Lower Anderson. Among the reference population samples, 961 individuals missing genotypes for more than 60 loci were removed, resulting in a final dataset containing 2,012 genotyped individuals for population genetic analysis. However, the Mokelumne River sample (BMOR) contained more than 400 individuals, so for visualization purposes 50 individuals were randomly subsampled in the structure and PCA analyses.

Population Genetic Analysis

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). We calculated mean allelic richness per population using rarefaction 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 Anderson Falls. *STRUCTURE* with a hypothesized number of populations of $K = 2, 3, 4, 6, 10$ with PCAs were conducted on only populations from the Putah Creek region, and on a subset of Putah Creek populations, Feather River, Dry Creek, and rainbow trout hatchery populations.

Adaptive genetic variation

To characterize genomic variation associated with migratory life-histories, we genotyped multiple SNPs in both the greb1/Rock genomic region and across the chromosomal inversion on Omy05 (Pearse et al., 2019; Waples et al., 2022). Among the SNPs genotyped that tag the Omy05 inversion, genotypes were highly concordant across all populations, consistent with their linkage within the inversion. We targeted a single SNP within the Omy05 chromosomal inversion (omy5_9_54854574-19; Pearse et al., 2019; Le Gall et al., In Prep) to estimate the frequencies of homozygous and heterozygous inversion haplotypes associated with anadromous (A), resident (R), and heterozygous (H) genotypes. Similarly, to characterize the distribution of variation in the greb1L genetic region, we focused on a single SNP (mhap8_71, pos. 11667915) that has been used in many recent studies of this gene region (reviewed by Waples et al., 2022). Alleles at this SNP are associated with the early- (E) and late- (L) migratory life-histories known as summer- and winter-run steelhead, respectively.

Results

Genetic diversity

Genetic diversity was relatively similar throughout Putah Creek but varied among population samples (Tables 1 & 2). Lower genetic diversity values (H_o , A_r) were observed in smaller, upstream tributaries, including Anderson Creek above waterfalls and Trout Creek. Samples from Lake Berryessa had among the highest values of allelic richness among all populations, but a lower frequency of heterozygous individuals than predicted, consistent with their apparent mixed ancestry (Tables 1 & 2).

Population structure

Below Lake Berryessa: The IDR and Anadromous Reaches

PCA and *STRUCTURE* model-based results showed that samples from the Putah Creek IDR and anadromous reach are genetically similar to each other, but distinct from upper Putah Creek populations, and consistently revealed a close relationship between the IDR and anadromous reach samples and other Central Valley lineage *O. mykiss* (Figures 2 and 3). *STRUCTURE* results for individuals sampled from these reaches show individuals with mixed genetic ancestry primarily from Central Valley steelhead and hatchery rainbow trout, with a particularly close association with the Pit River and Shasta strains (Figures 2 and 3).

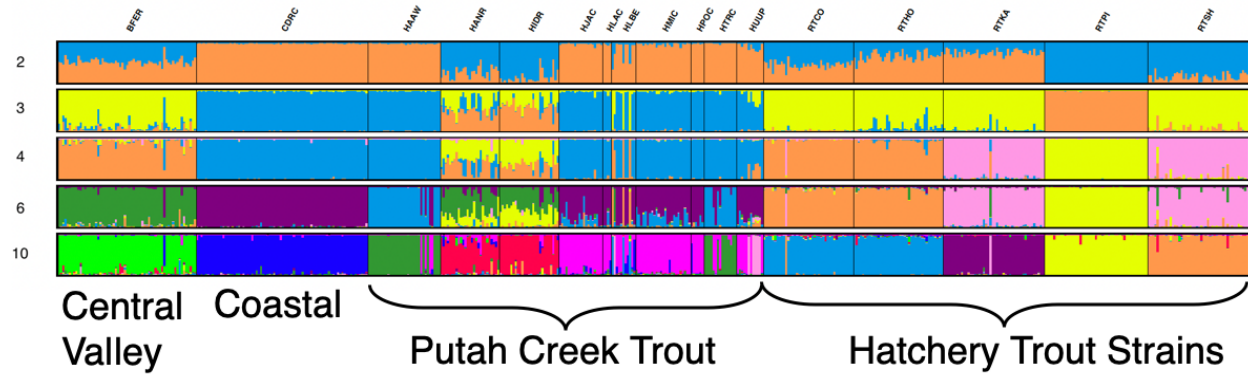


Figure 2. *STRUCTURE* plots for Putah Creek and selected representative populations. Each vertical line represents an individual sampled in a given population, with colors showing proportional assignment to k hypothesized genetic groups for at $K = 2, 3, 4, 6, 10$.

Upper Watershed

In contrast to the IDR, Upper Putah Creek *O. mykiss* share significant genetic ancestry with Coastal populations (Figures 2 and 3). Analysis using both structure and PCA revealed highly concordant patterns of relationship among individuals and populations. At low values of k , all individuals sampled in the upper Putah Creek sample sites assigned to genetic clusters shared with coastal steelhead, including the majority of fish sampled in Lake Berryessa with the exception of four likely hatchery rainbow trout individuals (Figure 2).

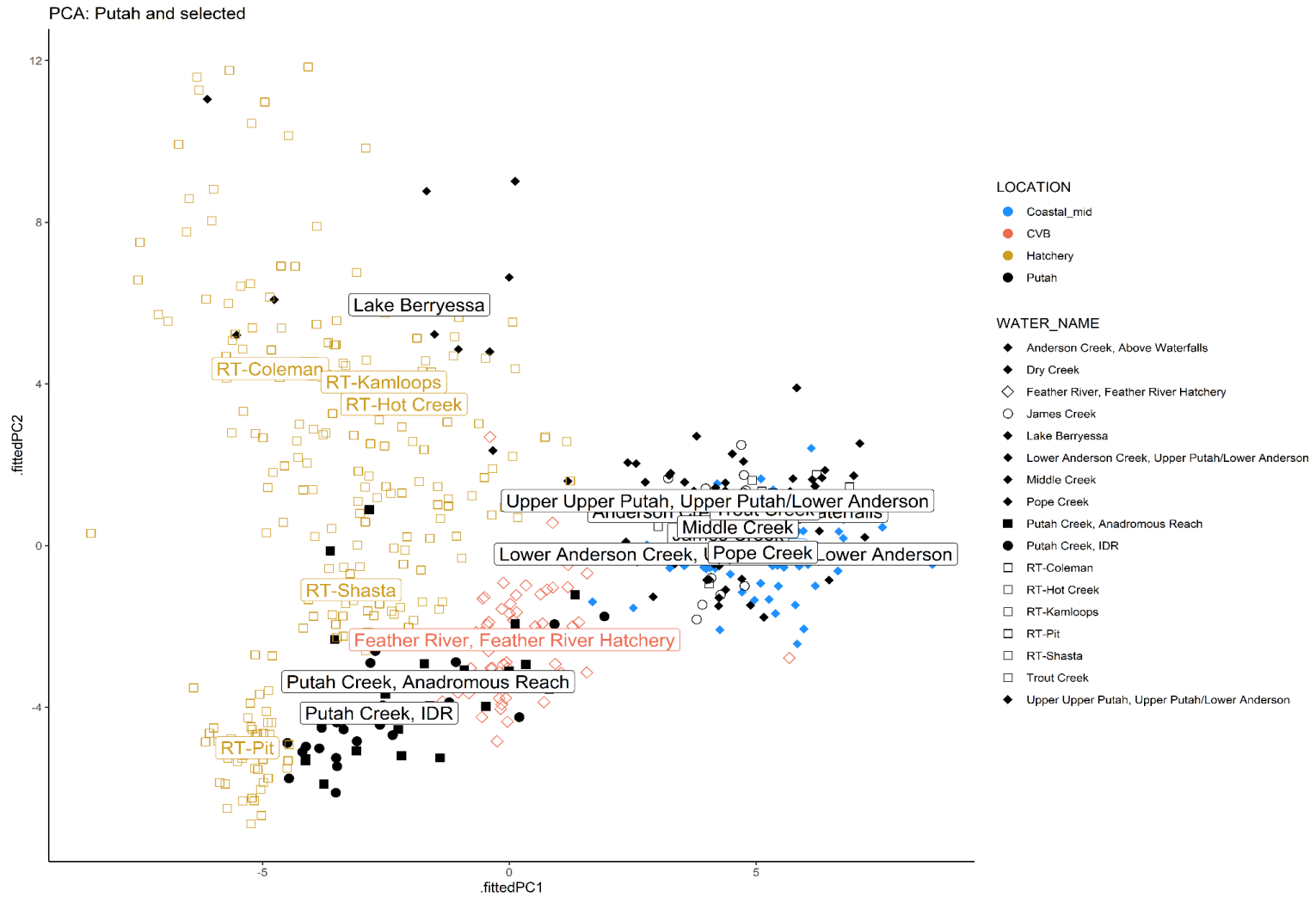


Figure 3: Principal component analysis (PCA) plots showing individual and population genetic relationships for Putah Creek and selected representative populations.

At higher values of k , the strong differentiation between coastal-lineage populations above and Central Valley-lineage below Monticello Dam remains clear, but more subtle substructure within the Putah Creek watershed also becomes apparent. For example, samples from near the source of Putah Creek on Cobb Mountain separate from the more downstream upper Putah Creek samples at high values of k , suggesting population subdivision, family structure, or possible hatchery introgression. Similarly, some individuals in upper Anderson Creek (HAAW), and Trout Creek (HTRC) assign to a distinct genetic cluster at higher values of k , likely indicating further subtle population or family substructure (Figure 2, $k=6, 10$). This is consistent with the low allelic richness and observed heterozygosity in the above Anderson Falls and Trout Creek samples relative to most upper Putah Creek populations (Table 2).

Table 2. Population names, locations, Number of samples collected, genotyped, and genetic diversity statistics for all populations included in the analysis, including reference populations.

Location	Water Name	Pop Code	No. sampled	No. genotyped	He	Ho	Ar
Coastal_mid	Alameda Creek	CALA	45	43	0.40	0.34	2.20
Coastal_mid	Big Creek	CBIC	142	49	0.44	0.35	2.36
Coastal_mid	Dry Creek	CDRC	78	78	0.40	0.38	2.17
Coastal_mid	Eel River	CEER	48	48	0.42	0.35	2.24
Coastal_mid	Freshwater Creek	CFRC	47	47	0.36	0.32	2.03
Coastal_mid	Little North Fork Ten Mile River	CTMR	46	46	0.41	0.34	2.23
Coastal_mid	Mad River	CMAR	16	16	0.39	0.39	2.02
Coastal_mid	Middle Fork Eel River	CMFE	47	46	0.38	0.36	2.04
Coastal_north	McKenzie River	DMCR	26	25	0.32	0.28	1.95
Coastal_north	South Santiam River	DSSR	30	30	0.29	0.27	1.80
Coastal_north	Willamette River	DWIR	12	12	0.25	0.25	1.66
Coastal_socal	Coldwater Canyon Creek	SCOC	36	36	0.15	0.11	1.36
Coastal_socal	Hilton Creek	SHIC	16	16	0.36	0.34	1.95
Coastal_socal	Piru Creek	SPIC	16	16	0.35	0.32	1.93
Coastal_socal	West Fork San Luis Rey	SWSL	10	10	0.31	0.22	1.83
CVA	Frog Creek	AFRC	21	20	0.31	0.28	1.87
CVA	Middle Fork American River	AMFA	48	48	0.44	0.35	2.36

Location	Water Name	Pop Code	No. sampled	No. genotyped	He	Ho	Ar
CVA	North Arm Rice Creek	ANRI	30	29	0.30	0.27	1.87
CVA_redband	Moosehead Creek	NMOC	15	14	0.12	0.10	1.30
CVA_redband	Sheephaven Creek	NSHC	15	15	0.17	0.06	1.43
CVB	American River, Nimbus Hatchery	BNIH	48	47	0.41	0.38	2.20
CVB	Battle Creek, Coleman Hatchery	BCOH	77	76	0.40	0.38	2.16
CVB	Deer Creek	BDEC	40	28	0.46	0.33	2.42
CVB	Feather River Hatchery	BFER	64	63	0.41	0.39	2.20
CVB	Mokelumne River Hatchery	BMOR	415	412	0.40	0.40	2.17
Hatchery	RT-Coleman	RTCO	46	41	0.42	0.31	2.16
Hatchery	RT-Hot Creek	RTHO	48	41	0.41	0.30	2.17
Hatchery	RT-Kamloops	RTKA	48	46	0.37	0.30	2.10
Hatchery	RT-Pit	RTPI	48	47	0.20	0.18	1.48
Hatchery	RT-Shasta	RTSH	46	46	0.29	0.24	1.74
Inland	Buck Creek	IBUC	30	29	0.33	0.22	1.92
Inland	Eagle Lake	IEAL	124	123	0.29	0.28	1.74
Inland	North Fork Clicks Creek	INCL	30	30	0.15	0.13	1.40
Inland	North Fork Deep Creek	INDE	15	15	0.30	0.24	1.80
Inland	North Fork Shields Creek	INSH	15	15	0.27	0.23	1.71
Inland	South Fork Deep Creek	ISDE	15	15	0.22	0.19	1.53
Inland	South Fork Shields Creek	ISSH	15	14	0.25	0.22	1.63
Klamath	Horse Linto Creek	KHLC	48	48	0.40	0.34	2.21
Klamath	Shasta River	KSHR	39	36	0.37	0.33	2.07
Klamath	Spring Creek	KSPC	16	16	0.17	0.16	1.45

Location	Water Name	Pop Code	No. sampled	No. genotyped	He	Ho	Ar
Putah	Anderson Creek, Above Waterfalls	HAAW	33	33	0.30	0.25	1.79
Putah	James Creek	HJAC	20	20	0.38	0.35	2.02
Putah	Lake Berryessa	HLBE	23	11	0.47	0.27	2.40
Putah	Lower Anderson Creek	HLAC	4	4	0.36	0.36	1.72
Putah	Middle Creek	HMIC	25	25	0.39	0.35	2.10
Putah	Pope Creek	HPOC	6	6	0.39	0.33	1.94
Putah	Putah Creek, Anadromous Reach	HANR	28	27	0.36	0.31	2.02
Putah	Putah Creek, IDR	HIDR	27	27	0.31	0.27	1.87
Putah	Trout Creek	HTRC	16	15	0.22	0.19	1.60
Putah	Upper Upper Putah	HUUP	14	12	0.39	0.31	2.10

Adaptive genetic variation

In addition to evaluating population genetic relationships, we genotyped SNP markers targeting adaptive genetic variation associated with two distinct migratory traits, residency vs anadromy, and summer- and winter-run timing in steelhead. This is an active area of research, and the results are part of a larger analysis of the distribution of adaptive genome variation in Central Valley *O. mykiss*. However, preliminary results on the Putah Creek samples based on targeted SNP variation in the Omy5 and greb1L regions showed concordant patterns of variation consistent with both the ancestry of the populations and their life-history potential. For example, Omy5 resident (R) genotypes were present at high frequencies only in the upper watershed tributary populations Anderson Creek, Above Waterfalls, Trout Creek, and Upper Upper Putah Creek (0.88, 0.73, and 0.58, respectively; Figure 4a). Conversely, alleles associated with anadromy were present at the high frequencies only in the IDR and anadromous reaches, with anadromous (A) genotype frequencies of 0.89 and 0.85, respectively, while populations above Monticello Dam that had potential adfluvial migratory access to Lake Berryessa contained variable frequencies of all three Omy5 genotypes, supporting the presence of diverse life-history variation.

The distribution of greb1L SNP variation among Putah Creek samples was also variable, with a strong division between the coastal ancestry populations in the upper watershed compared with the Central Valley ancestry in IDR and anadromous reaches. Upper watershed individuals had predominantly late-migrating (L) genotypes (i.e., winter-run), with moderate frequencies of heterozygous also present in most populations (Figure 4b). The IDR and anadromous reaches, in contrast, contained almost

exclusively early-migrating homozygous (i.e., summer-run) and heterozygous individuals, similar to other Central Valley *O. mykiss* populations (Figure 4b).

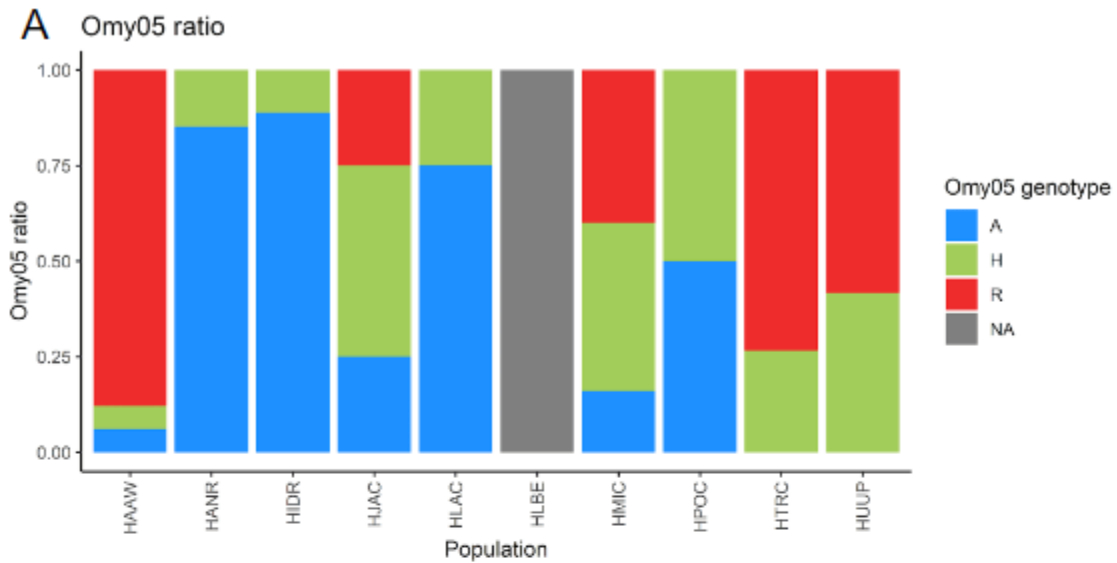


Figure 4a: Omy05 genotype ratio for Putah Creek populations. Lake Berryessa samples were not genotyped using the adaptive loci panel.

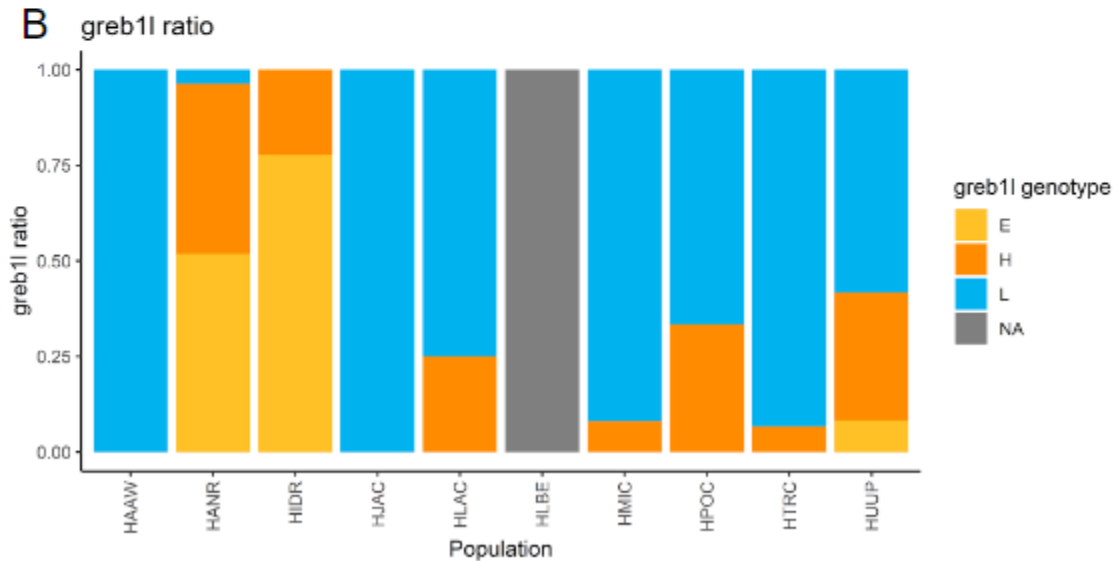


Figure 4b: greb1l genotype ratio for Putah Creek populations. Lake Berryessa samples were not genotyped using the adaptive loci panel.

Figure 4: Genotype frequencies at marker loci associated with adaptive genetic variation for migratory life-history traits a) Omy05, associated with resident (R) and anadromous (A) migration, and b) greb1L, which is associated with early (E) and late (L) run-timing variation in anadromous steelhead.

Discussion

We found that the self-sustaining population in the IDR is derived from Central Valley lineage fish similar to current steelhead broodstock at Coleman, Feather, and Mokelumne River hatcheries as well as wild steelhead populations in the Central Valley, while also sharing some ancestry with multiple Central Valley lineage hatchery trout strains. *O. mykiss* sampled above Monticello Dam are distinct from those in the IDR and anadromous reach below the dam and are more genetically similar to coastal steelhead populations than to Central Valley lineage *O. mykiss*.

CDFW suspended stocking the IDR with hatchery rainbow trout in 2008 (Weaver and Mehalick, 2009). Our results are consistent with the lack of recent stocking as well as fish surveys that concluded that fish in the IDR are wild and naturally reproducing (Weaver and Mehalick, 2009; Hogan et al., 2013). Furthermore, *O. mykiss* in the IDR are genetically similar to the *O. mykiss* in the anadromous reach below Putah Diversion Dam, further justifying the ongoing efforts to improve access, habitats, and flow regime for anadromous salmonids and other native fishes in lower Putah Creek (Kiernan et al., 2012).

In contrast to the Central Valley ancestry of *O. mykiss* in the IDR and anadromous water below the Putah Diversion Dam, we found that *O. mykiss* in upper Putah Creek are derived from the coastal genetic lineage. A previous genetic study that included a sample of individuals from Putah Creek 'above Lake Berryessa' noted that "the [close] associations depicted among Calaveras River, Putah Creek, lower American River, and Nimbus Hatchery are curious and difficult to explain" (Nielsen et al., 2005). However, in light of our results, their results make sense; the context provided by our broader sampling confirms that samples from upper Putah Creek are part of the coastal lineage, as are fish from Nimbus Hatchery and the lower American River, while the Calaveras River is among the closest drainages to both the mouth of Putah Creek and the connection to the coast via the San Francisco Bay Estuary. Our results further suggest that based on the distribution of adaptive variation associated with migration, *O. mykiss* in the small tributary streams above Monticello Dam primarily exist as resident trout populations, with some adfluvial individuals connecting this metapopulation via migration upstream out of Lake Berryessa.

The coastal lineage *O. mykiss* may be well adapted to upper Putah Creek because the system shares environmental characteristics with streams flowing through the Coast Range towards the Pacific Ocean. In Alameda Creek, a watershed not dissimilar from Putah Creek that flows into southeast San Francisco Bay, *O. mykiss* from Arroyo Hondo are adfluvial and retain clear coastal ancestry (Leitwein et al., 2017). In contrast, Central Valley steelhead were historically adapted to the high elevation, snow fed, streams flowing off the west slope of the Sierra Nevada Mountains, a very different adaptive landscape.

Coastal ancestry is present in streams around the San Francisco Bay at least as far inland as the Napa River (Leitwein et al., 2017), so could have also extended into Putah Creek given its similar habitat, especially in the upper watershed that cuts through the Coast Range. Interestingly, the opposite pattern occurs in the nearby American River, where remnant populations of native Central Valley lineage *O. mykiss* persist in Sierra tributaries above the major dams, but coastal steelhead predominate in the lower American River due to their continued propagation at Nimbus Hatchery (Pearse and Garza, 2015; Abadía-Cardoso et al., 2019). Thus, our results clarify the genetic ancestry of *O. mykiss* distributed throughout Putah Creek and provide a basis for future management decisions.

We also characterized adaptive genetic variants associated with two migratory life-history traits, anadromy and run-timing. Our results based on adaptive genomic variants in the *Omy5* and *greb11*

regions are concordant with the patterns of ancestry identified in the population genetic analysis and the potential for expression of life-history variation: Individuals in the upper watershed, which are of predominantly coastal ancestry, had higher frequencies of Omy5 R resident genotypes, as well as greb1L haplotypes associated with a late-migrating, or winter-run, steelhead. In contrast, individuals sampled in the IDR and anadromous reach in lower Putah Creek have more Omy5 A genotypes, along with greater greb1L haplotype diversity, both characteristic of Central Valley lineage steelhead populations. Fish in the IDR and anadromous reaches had high frequencies of homozygous-early and heterozygous genotypes at key SNPs associated with the early, or summer-run, steelhead life-history, consistent with observations suggesting that trout in the IDR have a relatively early spawning period (Salamunovich 2009). However, it is important to emphasize that haplotype and allelic variation for both greb1L and Omy5 are imperfectly associated with life-history phenotypes, and that these effects may vary among populations and lineages (Pearse, 2016). Nonetheless, the striking contrast in the distribution of 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 drainage. Similarly complex relationships between fish populations divided by dams have been found in other studies, highlighting the complexity of adaptation to habitat fragmentation (Pearse & Campbell, 2018; Winans et al., 2018; Fraik et al., 2021). Together these observations reflect the dynamic nature of these populations and their genomes, which continuously adapt to changing environmental factors through migration, drift, and natural selection, as well as responding to human-driven management practices.

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References

- Abadía-Cardoso, A., Brodsky, A., Cavallo, B., Arciniega, M., Garza, J.C., Hannon, J., Pearse, D.E. 2019. Anadromy Redux? Genetic Analysis to Inform Development of an Indigenous American River Steelhead Broodstock. *Journal of Fish and Wildlife Management* 10 (1): 137–147. doi: <https://doi.org/10.3996/072018-JFWM-063>
- Adamack, A.T. and Gruber, B. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods Ecol Evol*, 5: 384–387. doi: <https://doi.org/10.1111/2041-210X.12158>
- Archer, F.I., Adams, P.E. and Schneiders, B.B. 2017. Stratag: An r package for manipulating, summarizing and analysing population genetic data. *Mol Ecol Resources*, 17: 5–11. doi: <https://doi.org/10.1111/1755-0998.12559>
- Baetscher, D.S., Clemento, A.J., Ng, T.C., Anderson, E.C., Garza, J.C. 2018. Microhaplotypes provide increased power from short-read DNA sequences for relationship inference. *Molecular Ecology Resources* 18(2). <https://doi.org/10.1111/1755-0998.12737>
- Barngrover, B.G. 1990. Rainbow trout strains in California state hatcheries. *California Fish and Game* 76(2). Spring 1990
- Bloom, R. and Weaver, J. 2008. California Heritage and Wild Trout Program handbook (Draft). State of California Natural Resources Agency. Department of Fish and Game. Rancho Cordova, CA
- Fraik A.K., McMillan J.R., Liermann M., Bennett T., McHenry M.L., McKinney G.J., Wells A.H., Winans G., Kelley J.L., Pess G.R., Nichols K.M. 2021. The Impacts of Dam Construction and Removal on the Genetics of Recovering Steelhead (*Oncorhynchus mykiss*) Populations across the Elwha River Watershed. *Genes (Basel)*, 12(1):89. doi: 10.3390/genes12010089. Erratum in: *Genes (Basel)*; 2022), 13(9): PMID: 33450806; PMCID: PMC7828262
- Hogan, S., Buchanan, C., and Zuber, C. 2013. Putah Creek Summary Report. State of California Natural Resources Agency. Department of Fish and Game. Rancho Cordova, CA
- Kiernan, J.D., Moyle, P.B., and Crain, P.K. 2012. Restoring native fish assemblages to a regulated California stream using the natural flow regime concept. *Ecol Appl*, 22(5): 1472–1482. doi: <https://doi.org/10.1890/11-0480.1>
- Le Gall, R., Barthelemy, N., Clemento, A. J., Columbus, C., Campbell, E., Correa, E., Rodzen, J., Garza, J. C., and Pearse, D. E. In Prep. Development of a novel microhaplotype panel for steelhead/rainbow trout (*Oncorhynchus mykiss*) and application for phylogenetic analysis in California.
- Leitritz, E. 1970. A history of California's fish hatcheries 1870–1960. *Fisheries Bulletin* 150. 89 pp.
- Leitwein, M., Garza, J.C. and Pearse, D.E. 2017. Ancestry and adaptive evolution of anadromous, resident, and adfluvial rainbow trout (*Oncorhynchus mykiss*) in the San Francisco bay area: application of adaptive genomic variation to conservation in a highly impacted landscape. *Evol Appl*, 10: 56–67. doi: <https://doi.org/10.1111/eva.12416>

Nielsen, J.L., Pavey, S., Wiacek, T., and Williams, I. 2005. Genetics of Central Valley *O. mykiss* Populations: drainage and watershed scale analyses San Francisco Estuary and Watershed Science 3(2). DOI: 10.15447/sfews.2005v3iss2art3

Pearse, D. E., Miller, M. R., Abadia-Cardoso, A., Garza, J. C., Abadía-Cardoso, A., Garza, J. C., Abadia-Cardoso, A., & Garza, J. C. 2014. Rapid parallel evolution of standing variation in a single, complex, genomic region is associated with life history in steelhead/rainbow trout. *Proceedings of the Royal Society B: Biological Sciences*, 281(1783), 20140012. <https://doi.org/10.1098/rspb.2014.0012>

Pearse, D. E., and Garza, J. C. 2015. You Can't Unscramble an Egg: Population Genetic Structure of *Oncorhynchus mykiss* in the California Central Valley Inferred from Combined Microsatellite and Single Nucleotide Polymorphism Data. *San Francisco Estuary and Watershed Science*, 13(4). doi:<https://doi.org/10.15447/sfews.2015v13iss4art3>

Pearse, D. E. 2016. Saving the spandrels? Adaptive genomic variation in conservation and fisheries management. *J. Fish Biol.* 89:2697–716

Pearse, D.E. and Campbell, M.A. 2018. Ancestry and Adaptation of Rainbow Trout in Yosemite National Park. *Fisheries*, 43: 472-484. <https://doi.org/10.1002/fsh.10136>

Pearse, D. E., Barson, N. J., Nome, T., Gao, G., Campbell, M. A., Abadía-Cardoso, A., Anderson, E. C., Rundio, D. E., Williams, T. H., Naish, K. A., Moen, T., Liu, S., Kent, M., Moser, M., Minkley, D. R., Rondeau, E. B., Briec, M. S. O., Sandve, S. R., Miller, M. R., ... Lien, S. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. *Nature Ecology & Evolution*, 3(12), 1731–1742. <https://doi.org/10.1038/s41559-019-1044-6>

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

Salamunovich, T. 2009. 2008-2009 Trout spawning/redd surveys in the Putah Creek Interdam Reach. 1 October 2009. Memorandum prepared for Solano County Water Agency. 17 pp

Waples, R.S., Ford, M.J., Nichols, K., Kardos, M., Myers, J., Thompson, T.Q., Anderson, E.C., Koch, I.J., McKinney, G., Miller, M.R. and Naish, K., Narum, S. R., O'Malley, K. G., Pearse, D. E., Pess, G. R., Quinn, T. P., Seamons, T. R., Spidle, A., Warheit, K. I., and Willis S. C. 2022. Implications of Large-Effect Loci for Conservation: A Review and Case Study with Pacific Salmon. *Journal of Heredity*, 113(2), pp.121-144

Weaver, J. and Mehalick, S.. 2009. Putah Creek Summary Report. State of California Natural Resources Agency. Department of Fish and Game. Rancho Cordova, CA

Winans, G. A., Allen, M.B., Baker, J., Lesko, E., Shrier, F., Strobel, B., and Myers, J. 2018. Dam trout: Genetic variability in *Oncorhynchus mykiss* above and below barriers in three Columbia River systems prior to restoring migrational access. *PLoS ONE* 13 (5): e0197571. <https://doi.org/10.1371/journal.pone.0197571>