Articles
Anadromy Redux? Genetic Analysis to Inform Development of an Indigenous American River Steelhead Broodstock

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Abstract

The construction of dams and water diversions has severely limited access to spawning habitat for anadromous fishes. To mitigate for these impacts, hatchery programs rear and release millions of juvenile salmonids, including steelhead, the anadromous ecotype of the species *Oncorhynchus mykiss*. These programs sometimes use nonindigenous broodstock sources that may have negative effects on wild populations. In California, however, only one anadromous fish hatchery program currently uses nonnative broodstock: the steelhead program at Nimbus Fish Hatchery on the American River, a tributary of the Sacramento River in the California Central Valley. The goal of this study was to determine if potentially appropriate sources to replace the broodstock for the Nimbus Hatchery steelhead program exist in the Upper American River, above Nimbus and Folsom dams. We show that all Upper American River *O. mykiss* sampled share ancestry with other populations in the Central Valley steelhead distinct population segment, with limited introgression from out-of-basin sources in some areas. Furthermore, some Upper American River populations retain adaptive genomic variation associated with a migratory life history, supporting the hypothesis that these populations display adfluvial migratory behavior. Together, these results provide insights into the evolution of trout populations above barrier dams. We conclude that some Upper American River *O. mykiss* populations represent genetically appropriate sources from which fisheries managers could potentially develop a new broodstock for the Nimbus Hatchery steelhead program to reestablish a native anadromous population in the Lower American River and contribute to recovery of the threatened Central Valley steelhead distinct population segment.

Keywords: steelhead; genetics; anadromy; fish passage; rainbow trout; evolution; adaptation

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**Introduction**

The construction of dams, water diversions, and other habitat modifications have severely reduced the available spawning habitat for Rainbow Trout *Oncorhynchus mykiss* and other anadromous fishes in virtually all rivers in California’s Central Valley (Yoshiyama et al. 1996; Lindley et al. 2006; Penaluna et al. 2016; Pearse and Campbell 2018). Unlike Chinook Salmon *Oncorhynchus tshawytscha*, which are almost exclusively anadromous (but see Brenkman et al. 2017), the species *O. mykiss* has multiple life-history forms, commonly existing as anadromous steelhead, freshwater resident Rainbow Trout, or adfluvial freshwater migrants, either sympathetically occurring or when populations are separated by waterfalls or barrier dams (Clemento et al. 2009; Pearse et al. 2009, 2014; Berejikian et al. 2014; Penaluna et al. 2016; Winans et al. 2018; Arostegui et al. 2019). Fisheries managers have conducted extensive supplementation with hatchery Rainbow Trout above dams throughout the region to support recreational fishing opportunities. These efforts include spawning, rearing, and releasing large numbers of freshwater resident Rainbow Trout from hatchery strains of highly mixed ancestry, primarily derived from source populations in the northern Central Valley of California. These hatchery Rainbow Trout strains have been distributed throughout the state of California, including throughout the Central Valley (Needham and Behnke 1962; Leitritz 1970; Busack and Gall 1980), a practice that has contributed to the homogenization and loss of genetic diversity among indigenous *O. mykiss* populations (Pearse and Garza 2015; Abadía-Cardoso et al. 2016).

Today, many watersheds contain populations of Rainbow Trout of unknown ancestry inhabiting the streams and reservoirs above large dams, with mixed populations of native and hatchery individuals in some locations (Clemento et al. 2009; Pearse and Garza 2015; Abadía-Cardoso et al. 2016; Pearse and Campbell 2018). At the same time, native steelhead populations below barriers to migration are protected under the U.S. Endangered Species Act (ESA 1973, as amended), with distinct population segments (DPSs) defined to meet the criteria for an evolutionary significant unit that is reproducibly isolated and ecologically and genetically diverse (Busby et al. 1996). Note that this definition protects only anadromous steelhead below barriers, leaving all *O. mykiss* populations above barriers to anadromy unprotected regardless of their ancestry (NOAA 2006). All anadromous steelhead populations within the Central Valley, including three of the four dedicated hatchery steelhead programs, are genetically representative of the California Central Valley steelhead DPS, with one exception—the steelhead of Nimbus Hatchery on the Lower American River (McEwan 2001; Pearse and Garza 2015).

The American River is one of the largest tributaries of the Sacramento River, draining much of the Central Sierra Nevada to the west of Lake Tahoe and entering the main stem Sacramento River just upstream of the San Francisco Bay Delta ecosystem. Nimbus Fish Hatchery on the American River was built in 1958 to mitigate the impacts of Folsom and Nimbus hydroelectric dams on spawning and rearing habitat for salmon and steelhead. This facility spawns returning adult anadromous steelhead and Chinook Salmon and rears the juveniles for release in the Lower American River, supporting both recreational and commercial fisheries. However, the steelhead program at Nimbus Hatchery initially used eggs imported from coastal steelhead populations, particularly the Eel and Mad rivers, and these and other nonindigenous sources were propagated in the hatchery program for many years (CA HSRG 2012). As a result, the steelhead currently propagated at Nimbus are primarily descended from the coastal Northern California steelhead DPS, and are not considered to contribute to recovery of the Central Valley steelhead DPS, which is listed as “threatened” under the Endangered Species Act (NOAA 2006). Nimbus steelhead typically complete a 3–y life history before returning to the hatchery to spawn in winter as large anadromous steelhead (McEwan 2001). While these characteristics are desirable for recreational fisheries, the nonindigenous ancestry of this stock means that it is not included in the threatened California Central Valley steelhead DPS (Pearse and Garza 2015).

In 2012, the California Hatchery Scientific Review Group recommended that the steelhead broodstock currently spawned at Nimbus Hatchery be “replaced with an alternative broodstock that is appropriate for the American River” (CA HSRG 2012). However, given the lack of indigenous Central Valley steelhead in the Lower American River, the best source of a native American River steelhead broodstock could exist in the landlocked populations of *O. mykiss* in the Upper American River (UAR) above Nimbus and Folsom dams. Unfortunately, fisheries managers have extensively stocked the tributaries upstream of Folsom Dam with hatchery Rainbow Trout strains since the dam’s completion, which may have impacted the genetic composition of these populations (Pearse and Garza 2015). In addition, their isolation above the reservoir has prevented them from expressing a true anadromous life history since completion of the dam. However, populations inhabiting streams connected to lakes or reservoirs may maintain an adfluvial migratory life history (Holecek et al. 2012;
Holecek and Scarnecchia 2013; Arostegui et al. 2019), so could potentially provide phenotypic and genetic variation to support restoration of migratory anadromous ecotypes with indigenous American River ancestry (Thrower et al. 2008; Leitwein et al. 2017; Quinn et al. 2017).

The goal of this study was to evaluate the population structure and ancestry of UAR O. mykiss as potential sources of native broodstock for the Nimbus Hatchery steelhead program. This included a comparison of the ancestry of O. mykiss populations throughout the American River upstream of Nimbus and Folsom dams with other Central Valley and coastal O. mykiss populations. In addition, we surveyed variation in the inverted region of O. mykiss chromosome Omy05 that has been associated with migratory tendency (Nichols et al. 2008; Miller et al. 2012; Pearse et al. 2014; Appar et al. 2017; Leitwein et al. 2017; Pearse et al. 2018) to assess the distribution of this adaptive genomic variation in all sampled populations. Together, these data provide insight into life-history evolution in O. mykiss and will inform recommendations for the development of an indigenous broodstock for the Nimbus Hatchery steelhead program.

**Methods**

**Sampling**

We conducted sampling in the UAR using a combination of hook-and-line and electrofishing from May to August 2014. We nonlethally collected small tissue samples from the caudal fin of individual fish from 15 sites in the three major tributaries of the UAR that flow into Folsom Reservoir—the North, Middle, and South Forks—including sites in the North Fork of the North Fork, Duncan Creek, Long Canyon Creek, and the Rubicon River (Table 1; Figure 1). We dried all samples on blotting paper for future analysis.

**Genetic data collection**

We extracted total DNA from dried fin clips using the DNeasy 96 filter-based nucleic acid extraction system on a BioRobot 3000 (Qiagen, Inc.), following the manufacturer’s protocols. We diluted all DNA extractions 2:1 with distilled water and used them for polymerase chain reaction preamplification. We conducted genotyping of the 96 single-nucleotide polymorphism (SNP) locus panel described by Abadia-Cardoso et al. (2013) using 96.96 SNP genotyping dynamic arrays on an EP1 system (Fluidigm) with either TaqMan (Applied Biosystems) or SNP Type (Fluidigm, Inc.) assays. We included two negative controls in each array and we called genotypes using Fluidigm SNP genotyping analysis software; two people independently reviewed these to confirm correct calling. When we found discrepancies, we reran the samples and solved all discrepancies.

**Population genetic structure and ancestry**

We used genotype data to estimate standard genetic diversity statistics (observed and expected heterozygosites, the inbreeding coefficient computed according to Weir and Cockerham [1984], and deviations from Hardy–Weinberg equilibrium) using the Excel microsatellite toolkit (Park 2001) and GENEPOP 4.6 (Rousset 2008). We combined genotype data from the UAR populations with previously published SNP genotype data from 28 reference populations representing the distribution of O. mykiss in California, including the northern, middle, and southern Central Valley and northern and southern coastal steelhead populations, and five hatchery Rainbow Trout strains commonly used in California (Kamloops, Mount Shasta, Eagle Lake, Coleman, and Moccasin; Figure 1; Table S1, Supplemental Material). The combined genotype data overlapped for a total of 90 SNP loci that we included in the final dataset.

We used the clustering analyses program structure v2.0 (Pritchard et al. 2000) as an exploratory analysis to evaluate fractional ancestry of individuals and identify migrants and individuals of mixed ancestry without a priori designation of defined populations (Pritchard et al. 2000; Pearse and Crandall 2004). We did these analyses over a range of genetic clusters (K values) from 2 to 12, for all populations, with three runs of each k value with 150,000 Markov chain Monte Carlo repetitions and a burn-in period of 50,000 steps to evaluate consistency of patterns of genetic association. We compiled results from the structure runs using CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

We used a principal component analysis (PCA) to graphically investigate patterns of genetic differentiation and relationships among all populations using the R-based package adegenet 1.3-4 (Jombart 2008; R Core Team 2016). This multivariate and individual-based method uses the allele frequencies to partition variation among populations without making assumptions about an evolutionary model (Menozzi et al. 1978). Finally, we used the software package PHYLIP (Felsenstein 2005) to calculate Cavalli-Sforza and Edwards (1967) chord distances and generate a neighbor-joining phylogram of populations. Chord-distance matrices have been shown to generate the correct phylogram topology even when branch lengths are variable (Takezaki and Nei 1996; Felsenstein 2003). We evaluated the consistency of population relationships using 1,000 bootstrap datasets for statistical support. We visualized the resulting tree using Dendroscope 3 (Huson and Scornavacca 2012).

**Adaptive variation and migratory potential**

In a separate analysis, we used genotypes for three highly linked SNP loci (Omy114448, Omy121006, and R04944) to identify previously characterized haplotypes associated with either an anadromous or resident life history. These SNP loci are located in a region of the O. mykiss genome on chromosome Omy05 that is associated with migratory life-history traits, such that alternate alleles at each SNP can be used to determine which haplotypes an individual carries (Pearse et al. 2014; Leitwein et al. 2017; Pearse and Campbell 2018). Based
on this analysis, we estimated the frequencies of the two haplotypes in this region for all UAR populations to provide insight into the migratory potential of each population relative to current and historical barriers.

**Results**

**Sample genotyping and population statistics**

We generated genotype data for the total of 543 *O. mykiss* samples from the 15 UAR populations, with sample sizes ranging from 15 to 65 individuals (Table 1). We did not include in the analysis samples from four sites (French Meadows, Horseshoe Bar, Oxbow, and Ford’s Bar) where fewer than five fish were caught. Among the UAR populations, only five SNP loci deviated from Hardy–Weinberg equilibrium and none of them deviated in more than three populations. The observed and expected heterozygosities or the 90 SNP loci used were highly concordant, and distinguished individuals sampled from various sites in the UAR from other Central Valley populations and confirmed previous results on Central Valley *O. mykiss* (Nielsen et al. 2005; Pearse and Garza 2015), including the close and well-supported relationship of the existing Nimbus and Lower American River steelhead to the coastal populations from which they were derived (e.g., Eel, Noyo, and San Lorenzo Rivers; Figures 2 and 3). In contrast, all of the UAR populations cluster within the Central Valley lineage, clearly indicating their indigenous Central Valley ancestry. In all of the genetic data analysis approaches, the UAR populations further subdivided into two partially distinct clusters, here defined as group A and group B (Table 1; Figures 2 and 3; Figure S1, Supplemental Material). The group B populations were similar to previously analyzed samples from the same areas of the North Fork and Middle Fork American River (Figures 2 and 3; Pearse and Garza 2015). Mean observed heterozygosity (*H*₀) of group A was significantly lower than that of group B (mean *H*₀ in group A vs. B, 0.29 vs. 0.34; *t*-test, *t* = −6.8148, *df* = 13; *P* < 0.001), consistent with their locations as isolated above-barrier populations and also similar to the low diversity seen among hatchery trout strains (*H*₀ = 0.27), while the higher diversity of group B populations is similar to below-barrier coastal and Central Valley steelhead (Pearse and Garza 2015).

**Population genetic structure and ancestry**

We used genotypes comparable across all UAR and reference populations (*N* = 90 SNP loci; Data S1, Supplemental Material) to explore individual fractional assignment using the program *structure* (Figure S1, Supplemental Material), construct an unrooted neighbor-joining network using PHYLIP (Figure 2), and conduct a PCA (Figure 3). Results from all three analyses were highly concordant, and distinguished individuals sampled from various sites in the UAR from other Central Valley populations and confirmed previous results on Central Valley *O. mykiss* (Nielsen et al. 2005; Pearse and Garza 2015), including the close and well-supported relationship of the existing Nimbus and Lower American River steelhead to the coastal populations from which they were derived (e.g., Eel, Noyo, and San Lorenzo Rivers; Figures 2 and 3). In contrast, all of the UAR populations cluster within the Central Valley lineage, clearly indicating their indigenous Central Valley ancestry. In all of the genetic data analysis approaches, the UAR populations further subdivided into two partially distinct clusters, here defined as group A and group B (Table 1; Figures 2 and 3; Figure S1, Supplemental Material). The group B populations were similar to previously analyzed samples from the same areas of the North Fork and Middle Fork American River (Figures 2 and 3; Pearse and Garza 2015). Mean observed heterozygosity (*H*₀) of group A was significantly lower than that of group B (mean *H*₀ in group A vs. B, 0.29 vs. 0.34; *t*-test, *t* = −6.8148, *df* = 13; *P* < 0.001), consistent with their locations as isolated above-barrier populations and also similar to the low diversity seen among hatchery trout strains (*H*₀ = 0.27), while the higher diversity of group B populations is similar to below-barrier coastal and Central Valley steelhead (Pearse and Garza 2015).

**Adaptive variation and migratory potential**

The frequencies of chromosome Omy05 inversion haplotypes associated with migratory life history variation were largely consistent with expectations based on previous studies, with headwaters populations above
natural migration barriers (group A) generally having low frequencies of the migration-associated haplotype A, while those lower in the watershed and close to Folsom Reservoir had intermediate frequencies of this haplotype (group B; Table 1; mean frequency of Omy05 haplotype A in group A vs. B, 0.078 vs. 0.156; t-test, t = -1.663, df = 13, P = 0.1202). While this difference was not significant, this pattern is consistent with the biological expectation that the UAR populations most likely to express adfluvial migratory behavior are those located lower in the watershed, between Folsom Reservoir and the historical barrier to upstream migration in each tributary. However, the maximum frequency of migration-associated alleles in any UAR population (0.33 in Cache Rock) was low relative to both coastal anadromous and adfluvial populations (≥ 0.60; Pearse et al. 2014; Leitwein et al. 2017) and typical Central Valley hatchery steelhead (0.76–0.94, Pearse et al. 2018). Finally, the length of fish sampled varied significantly among sites in the UAR (ANOVA, P < 0.001), with larger fish primarily found in the lower watershed below natural barriers to migration but above Folsom Lake. Although biases in hook-and-line sampling may influence the length distributions among sites, these results further support the hypothesis that the group B populations likely express migratory adfluvial behavior, allowing them to obtain larger sizes than the group A individuals isolated above barriers to migration (Holecek et al. 2012; Holecek and Scarneccchia 2013; Leitwein et al. 2017).

Discussion

Our results show that the UAR populations have almost exclusively Central Valley O. mykiss lineage.
ancestry, although we cannot completely rule out some influence from external hatchery strains (e.g., Kamloops). Populations from the headwater tributaries, including Duncan Creek, Long Canyon Creek, the North Fork of the North Fork, and the upper Middle Fork below French Meadows, formed a genetic cluster defined as group A, while most remaining UAR populations, including those found in the lower watershed with connectivity to Folsom Reservoir, clustered together in group B. Overall, the genetic relationships of group A and B populations relative to the hatchery Rainbow Trout strains and other Central Valley and coastal *O. mykiss* suggest that group B is most representative of the historical genetic ancestry of the American River. This conclusion is supported by both the PCA and structure analysis that show greater similarity between the group B populations and reference populations from the middle and southern Central Valley, while the group A populations show more similarity to reference populations from the northern Central Valley and to hatchery Rainbow Trout strains. The group B populations also had both phenotypic (larger size) and genomic (higher frequencies of Omy05 haplotype A) variation consistent with an adfluvial life history.

Figure 2. Unrooted neighbor-joining tree constructed with chord distances estimated from allele frequency data collected from populations of Rainbow Trout *Oncorhynchus mykiss* in the Upper American River (UAR) in the Central Valley of California between May and August 2014 to identify potentially appropriate sources of broodstock for the Nimbus Hatchery steelhead program. Populations from the UAR are listed with Group designations and numbered as in Table S1 (Supplemental Material), and Central Valley and coastal reference populations are grouped and named as in Table 1 (Supplemental Material). Edge labels are percentages from 1,000 bootstrap replicates, and the length of the internal branches is in proportion with the number of trees in which the branch was found. Only bootstrap proportions above 70% are shown.
Compared with the clear population structure of coastal steelhead populations (Garza et al. 2014; Abadía-Cardoso et al. 2016), the close relationships among all *O. mykiss* within the Central Valley do not allow complete separation of hatchery trout strains from wild populations (Pearse and Garza 2015; Leitwein et al. 2017; Pearse and Campbell 2018), reflecting the biological reality of their recent shared genetic ancestry. In addition, we based the present study on a dataset with a low number of SNPs, and thus it has relatively low power to discriminate population genetic structure. While analysis of a large number of SNP loci or multiallelic SNP microhaplotypes (Gattepaille and Jakobsson 2012; McKinney et al. 2017; Baetscher et al. 2018) or more detailed analysis of adaptive genomic variation on Omy05 (Pearse et al. 2018) and other parts of the genome (e.g., Nichols et al. 2008) would likely provide greater resolution of these issues, such an expanded analysis of Rainbow Trout within the UAR is unlikely to change the basic conclusions or management implications of the present study in biologically significant ways.

**Management implications**

Folsom Dam was completed in 1955, but replaced a previous dam built in 1893, and North Fork Dam blocked upstream migration above the confluence with the Middle Fork after its completion in 1939. In addition to blocking upstream anadromous migration, these barriers also trapped *O. mykiss* above them, creating the residuated resident trout populations that persist there today. Our results suggest that UAR *O. mykiss* could potentially contribute to development of a broodstock more genetically appropriate than the current Nimbus Fish Hatchery steelhead broodstock for the reestablishment of an anadromous Central Valley DPS population indigenous to the American River (CA HSRG 2012). This will become a critical issue in the context of proposals to reestablish connectivity with the Central Valley DPS through two-way trap-and-haul around Nimbus and Folsom dams (NMFS 2014; Lusardi and Moyle 2017). Furthermore, while our results suggest that any of the UAR populations would be more appropriate representatives of the Central Valley steelhead DPS than the existing Nimbus broodstock when considering overall genomic ancestry, the most appropriate sites would be those low in the watershed, close to Folsom Lake, as these have both indigenous American River ancestry and genomic and phenotypic variation consistent with migratory adfluvial life history. However, regardless of the genetic ancestry of the UAR populations or the distribution of Omy05 and other adaptive genomic variation among them, genetic data alone are insufficient to make such management decisions (Pearse 2016). Additional experiments, including evaluation of disease pathology and ocean survival in UAR *O. mykiss* and experimental evaluation of growth and development of offspring of UAR trout in a hatchery setting (Phillis et al. 2018).
will need to be conducted in order to fully evaluate the potential for UAR O. mykiss to contribute to the development of an indigenous American River steelhead strain. While collecting a sufficient number of UAR trout to initiate fish passage or develop a broodstock presents a formidable challenge, one option would be to trap downstream migrants (smolts) from sites in the North Fork, Middle Fork, or both forks; these could be evaluated and used to initiate spawning and juvenile rearing experiments at the facility. These migratory individuals would represent much of the historical genetic diversity of American River steelhead, supporting recovery of indigenous Central Valley steelhead in the Lower American River.

The question of the potential for the reemergence of anadromy from UAR source populations despite the lack of a truly anadromous life history for over 100 y is not trivial, but there is increasing evidence that such a revolution is possible. The ability of resident Rainbow Trout isolated above barriers to produce anadromous offspring has been demonstrated in other watersheds (Thrower et al. 2004; Courter et al. 2013; Berejikian et al. 2014; Phillis et al. 2016), but marine survival may be low relative to offspring of true anadromous parents (Thrower and Joyce 2004). However, Quinn et al. (2017) have recently shown the spontaneous reemergence of anadromy in Bull Trout Salvelinus confluentus following dam removal on the Elwah River, Washington. In addition, expression of anadromy from otherwise primarily resident-ancestry populations of O. mykiss has been inferred in the Santa Cruz River in Patagonia, Argentina (Pascual et al. 2001; Riva-Rossi et al. 2007). This population is believed to be founded from hatchery Rainbow Trout of primarily Central Valley origin (Riva-Rossi et al. 2004), following transport via train from the western United States through Michigan and New York, and then by steamship, first to Europe and then to the Southern Hemisphere. Thus, given the ability of O. mykiss to rapidly evolve residency following isolation above dams and waterfalls (Pearse et al. 2009; Pearse et al. 2014), it is feasible that a similarly rapid return to anadromy could occur if they are provided with the appropriate opportunity and habitat (Thrower et al. 2008).

**Supplemental Material**

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**Table S1.** Oncorhynchus mykiss steelhead reference populations from northern (NC) and southern (SC) coastal California, and the northern (CVN), middle (CVM), and southern (CVS) Central Valley of California, as well as hatchery Rainbow Trout strains (HS) from these regions. The Kamloops hatchery trout strain originated in Canada. See Pearse and Garza (2015) for details.

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**Table S1.** Oncorhynchus mykiss steelhead reference populations from northern (NC) and southern (SC) coastal California, and the northern (CVN), middle (CVM), and southern (CVS) Central Valley of California, as well as hatchery Rainbow Trout strains (HS) from these regions. The Kamloops hatchery trout strain originated in Canada. See Pearse and Garza (2015) for details.

**Figure S1.** Analysis using structure software to determine potentially appropriate sources of broodstock for the Nimbus Hatchery steelhead program. We used the analysis to group individuals into different numbers of genetic groups (k), from 2 to 12, with individuals represented by single vertical lines. Groups of individuals that share colors across different values of k can be interpreted as being more genetically similar than individuals that are consistently a different color. For example, the coastal lineage, represented here by the Eel and Noyo rivers to the north and the San Lorenzo River to the south, is clearly distinguishable at all values of k, as is the shared coastal ancestry of Nimbus Hatchery and the American River below Nimbus Dam (e.g., yellow at k = 6). The Upper American River (UAR) group A populations show variable associations, including with hatchery Rainbow Trout strains, while group B populations are consistent within and among different values of k. Other relationships are more variable both within and across values of k, and highlight the close and intermingled ancestry among the Central Valley populations and hatchery trout strains. See Pearse and Garza 2015 for details.
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