Article Type: Feature

Revision for Fisheries

Ancestry and Adaptation of Rainbow Trout in Yosemite National Park

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/fsh.10136

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Abstract

California's Central Valley contains an abundance of rivers with historical and potential productivity for anadromous salmonids that are currently limited by impacts such as dams, water diversions, and high temperatures. We surveyed genetic variation in Rainbow Trout *Oncorhynchus mykiss* within the upper Tuolumne and Merced Rivers in and around Yosemite National Park to evaluate both population origins (ancestry) and the evolutionary response to natural and artificial barriers to migration (adaptation). This analysis revealed that despite extensive stocking with hatchery rainbow trout strains throughout the study area, most populations retain largely indigenous ancestry. Adaptive genomic variation associated with anadromy was distributed throughout the study area, with higher frequencies observed in populations connected to reservoirs known to support adfluvial life history variants. Fish in southern Central Valley rivers experience temperatures near the upper thermal limit for salmonids and represent an important reservoir of genomic diversity for adaptation to climate change. These results highlight the importance of local adaptation as well as the potential for resident trout populations above barrier dams to contribute to the recovery of anadromous *O.*

mykiss once migratory connectivity is restored between upstream spawning and rearing habitats and the ocean.

Introduction

The Central Valley of California is both a productive agricultural region and an important ecosystem in western North America that encompasses two large river systems— the Sacramento River to the North and the San Joaquin River to the South. Together these rivers and their tributaries are home to the southernmost native populations of Chinook Salmon *Oncorhynchus tshawytscha* as well as the resident and anadromous forms of Rainbow Trout *O. mykiss*, known as ***rainbow trout*** and ***steelhead***, respectively (Fisher 1994; Busby et al. 1996; Yoshiyama et al. 1998, 2001; McEwan 2001). However, the construction of barrier dams and water diversions has greatly restricted migratory connectivity on many rivers, resulting in extremely reduced anadromous salmonid populations throughout the Central Valley (Lindley et al. 2006; May and Brown 2002; NMFS 2006, 2014; Yoshiyama et al. 1998, 2001; Katz et al. 2013). Large barrier dams in particular prevent upstream migration of adult salmonids to their spawning habitats as well as downstream juvenile migration, severely impacting anadromous species. As a result, there is an increasing focus on re-connecting migratory anadromous salmonid populations with their historical spawning grounds above impassable dams through dam removal, addition of volitional passageways, or through fish passage programs known as trap-and-haul (Anderson et al. 2014; NMFS 2014; Lusardi and Moyle 2017).

The Tuolumne and Merced Rivers are tributaries of the San Joaquin River that drain a large portion of Yosemite National Park ("Yosemite") in the central Sierra Nevada, as well as Stanislaus National Forest and other lands (Figure 1). Both spring-run Chinook Salmon and anadromous steelhead historically used these waterways to access the cool refuges of the High Sierra, where they and their offspring could escape the summertime heat and dwindling river flows of the lower elevations. Both species likely spawned in the Merced River throughout Yosemite Valley up to the bases of Half Dome and the spectacular Vernal and Yosemite Falls, and in the Tuolumne River up to Preston Falls, just downstream of the Park boundary (TAPF; Figure 1). However, the full extent of their historical migrations is unclear (Yoshiyama et al.

2001; Lindley et al. 2006). Beginning in the mid-1800's, as happened in many Central Valley rivers, a series of dams have blocked access between the ocean and the headwaters of the Tuolumne and Merced rivers for anadromous salmonids. Currently, La Grange Dam (1883) and Crocker-Huffman Dam (1906) create the upper limits to anadromous migration, and above these dams the much larger New Don Pedro and New Exchequer Dams form major reservoirs on the Tuolumne and Merced rivers, respectively, providing flood control, water storage, recreation, and power generation (Figure 1). Collectively these dams and their predecessors have prevented native salmon and steelhead from accessing these historic spawning habitats for more than a century. However, even prior to the construction of the major barrier dams, the activities of the California Gold Rush in the 1850's and subsequent development of agricultural infrastructure in the Central Valley had a huge effect on the native fauna, particularly the migratory salmonids (Yoshiyama et al. 1998, 2001), and few naturally spawning anadromous salmonids exist in the reaches below these dams today (Ford and Kirihara 2010; Cuthbert et al. 2012; NMFS 2014). This situation is further exacerbated by the poor quality of downstream habitat in the the Sacramento – San Joaquin Delta for both migratory and non-migratory native fishes (Moyle et al. 2018). Today, intensive management and hatchery supplementation maintain many salmonid populations in the Central Valley, including the California Central Valley steelhead distinct population segment (DPS)— listed as 'threatened' under the Endangered Species Act (NMFS 2006)— but inability to access more than 80% of their historical spawning habitat remains a critical issue for their recovery (Yoshiyama et al. 2001; Lindley et al. 2006). The National Marine Fisheries Service's Central Valley Recovery Plan identifies the upper Tuolumne River (UTR) and the upper Merced River (UMR) as candidate areas for reintroduction of both steelhead and spring-run Chinook Salmon to support recovery of the southern Sierra Nevada steelhead diversity group through upstream passage of adults and downstream movement of juveniles over the dams (NMFS 2014).

With few exceptions, Chinook Salmon are strictly anadromous (but see Sard et al. 2016; Brenkman et al. 2017), while self-sustaining populations of freshwater resident rainbow trout commonly persist above barrier dams that block their ability to access the ocean (Kendall et al. 2015). Individuals in above-dam populations may exhibit several life-history strategies, including a migratory adfluvial life-history utilizing a reservoir as an alternative to a fully anadromous marine migration and returning to spawn in upstream tributaries (e.g. Holecek and

Scarnecchia 2013; Leitwein et al. 2017). These populations are typically closely related to the remaining *O. mykiss* found below barriers in the same watershed (Narum et al. 2008; Clemento et al. 2009; but see Pearse and Garza 2015), although stocking of non-native hatchery rainbow trout strains into above-barrier habitats has resulted in partial or complete replacement of the indigenous ancestry in some cases (e.g. Abadía-Cardoso et al. 2016). Importantly, only the anadromous steelhead life history is listed under the ESA, while even closely related above-barrier rainbow trout populations are not protected by the ESA (NMFS 2006). Thus, in considering efforts to reconnect migratory steelhead populations below dams with their historical upriver spawning habitats, an important first step is to evaluate the genetic ancestry and adaptive potential of the rainbow trout trapped above the dams (Winans et al. 2010, 2017, 2018).

Ancestry of Yosemite Trout

There is a rich history of fish stocking in and around Yosemite that has undoubtedly influenced the distribution and genetic composition of its rainbow trout. Early visitors took a strong interest in increasing the trout populations, both for food resources and recreation (Caton 1869; Pavlik 1987). Fish planting likely began in the area in the 1870s, initially by settlers moving locally captured fish up into the previously fishless waters above waterfalls and in high alpine lakes. Stocking records of imported trout first occurred in the 1890s, and by 1895 there was a fish hatchery operating on the South Fork Merced River at Wawona that distributed both indigenous and imported trout throughout the area (Pavlik 1987). A subsequent hatchery was established in 1918 at Happy Isles on the main stem Merced River in Yosemite Valley, and the importation of eggs from other hatcheries ensured a steady supply of rainbow trout, as well as non-native species such as Lahontan Cutthroat Trout *O. clarkii henshawi*, European Brown Trout *Salmo trutta* L., and Brook Trout *Salvelinus fontinalis* (Leitritz 1970). Although most of the eggs reared at the Happy Isles hatchery were imported from outside Yosemite, some were collected at an egg-taking station on Frog Creek, a historically-fishless tributary of the UTR above Lake Eleanor in the northern part of Yosemite (Figure 1; Pavlik 1987). Thus, over the years a diverse mixture of both locally sourced rainbow trout and fish imported from throughout California have been planted within Yosemite, potentially creating admixed populations with both indigenous and hatchery ancestry. However, management of more recent stocking efforts has changed significantly, and since 2013 most trout planted in California have been sterile triploids, limiting

further naturalization and spawning by hatchery fish. The current distribution of rainbow trout within Yosemite is therefore composed of self-sustaining populations whose ancestry remains to be evaluated through genetic analysis.

Adaptation to Residency

Despite dramatic differences in traits related to physiology, morphology, and behavior, the diverse life history forms of *O. mykiss* often co-exist and interbreed, forming inter-related populations in nature (Quinn 2011). Consequently, anadromous and resident fish within a drainage basin are typically closely related to each other (Olsen et al. 2006; Narum et al. 2008; Pearse et al. 2009). While offspring of a particular life history variant may take on an alternative strategy from that of their parents (Courter et al. 2013), there is a great deal of evidence pointing to heritable influences on life history strategies and associated phenotypes (*e.g.* Neave 1944; Berejikian et al. 2014; Phillis et al. 2016).

Surveys of genetic variation have found that rainbow trout in above-barrier habitats undergo specific genetic changes as populations adapt to residency. In particular, one region of chromosome Omy5 has showns a consistent association with resident (R) and anadromous (A) life-histories, although many other genomic regions are also associated with variation in this trait (*e.g.* Nichols et al. 2008; Hale et al. 2013; Hecht et al. 2013). However, unlike waterfalls, which exert knife-edge selection against downstream migration (Pearse et al. 2009; Northcote 2010), barrier dams create reservoirs above them, allowing rainbow trout trapped above them to develop an adfluvial migratory life-history by utilizing the reservoir as a rearing habitat and spawning in the tributary streams (*e.g.* Holecek et al. 2012; Holecek and Scarnecchia 2013). Importantly, despite the dramatic difference in osmotic conditions between reservoirs and the ocean, selection for an adfluvial migratory life-history appears to affect the same adaptive genomic variants on Omy5 as true anadromous migrations (Pearse et al. 2014; Leitwein et al. 2017). This suggests that adfluvial rainbow trout populations isolated above dams and reservoirs could potentially contribute to the recovery of migratory anadromous ecotypes once migratory access to the ocean is restored through dam removal or assisted fish passage (Thrower et al. 2008; Meek et al. 2014; Winans et al. 2017).

The primary goal of this study was to determine the genetic ancestry and current population structure of *O. mykiss* populations in the UTR and UMR (Figure 1). To do so we investigated the genetic relationships of *O. mykiss* in these rivers relative to i) other populations above and below barriers to anadromy in the Central Valley, ii) hatchery rainbow trout strains commonly used in California, and iii) coastal steelhead populations. In addition, we assayed adaptive genomic variation in the region of chromosome Omy5 known to be associated with anadromous and adfluvial life-history traits in *O. mykiss* to estimate the frequencies of alleles associated with migratory behavior relative to the presence of barriers to fish migration (Pearse et al. 2014; Leitwein et al. 2017; Apgar et al. 2017). We use this information to evaluate the potential for UTR and UMR populations to contribute to the recovery of anadromous steelhead below barriers in the southern Central Valley. Together, these data provide a baseline to inform future management of *O. mykiss* populations in these and other Central Valley watersheds and improve our understanding of the potential to recover anadromous steelhead populations by restoring connectivity with *O. mykiss* populations trapped in habitats upstream of the dams.

Methods

Sampling

Fish were captured in 2015 and 2016 at 37 sites throughout the UTR and UMR watersheds, including both migratory reaches (those historically accessible to migratory steelhead; Lindley et al. 2006) and those isolated above barriers that were historically fishless (Figure 1; Table 1). Because of the difficulty of accessing fish in larger rivers as well as the extremely low conductivity of Sierra Nevada streams, many sites were unsuitable to electrofishing, and most fish in the study were captured by hook-and-line. This 'Flyfishing for Science' had the added benefit of providing a mechanism to allow volunteer fly fishers to contribute to the project as citizen scientists (Williams et al. 2015). All fish were measured and fin tissue samples were taken from each individual prior to release at the site of capture. Tissue samples were dried and taken to the National Marine Fisheries Service laboratory in Santa Cruz, CA, for analysis.

Genetic Data Collection

DNA was extracted 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. All DNA extractions were diluted 2:1 with distilled water and used for polymerase chain reaction pre-amplification prior to TaqMan or SNPtype genotyping with 96.96 IFC chips. Genotypes were read and scored using Fluidigm SNP Genotyping Analysis software (Fluidigm, Inc.). All samples were genotyped at total of 92 SNPs for population genetic analysis following Abadía-Cardoso et al. (2013), a gender-ID SNP assay (Brunelli et al. 2008; Rundio et al. 2012), and three SNPs on chromosome Omy5 that have been associated with migratory life-history traits (Pearse et al. 2014; Pearse and Garza 2015; Abadía-Cardoso et al. 2016; Leitwein et al. 2017).

Data Analysis

The SNP genotype data were combined with published data from 21 representative wild coastal and Central Valley *O. mykiss* populations, three Central Valley steelhead hatcheries and five hatchery rainbow trout strains common in California (Pearse and Garza 2015). The genetic data were analyzed with the R statistical analysis program version 3.4.1 (R Development Core Team 2017). Genotypes were imported for use in R and converted to a genind object for subsequent analyses through the *pegas* package version 0.10 (Paradis 2010). Quality control of individual fish was undertaken with the "missingno" function of *poppr* version 2.5.0 (Kamvar et al. 2014) by specifying that both genotypes and loci were not allowed to have more than 5% missing data. From these filtered data, two separate approaches were implemented, (1) an individual approach and (2) a population approach, in which fish sampled along contiguous reaches without barriers to migration were combined into 'sampling units', resulting in a total of 20 discrete groups of individuals based on local geography and barriers to migration (Figure 1, Table 1).

Individual Approach

For the individual approach, prior population assignment based on collection location was not considered, and individuals were independently assigned to inferred populations. This approach was used to verify the independence or interrelatedness of sampling locations. For example, by including hatchery reference populations, do sampled individuals show genetic similarities to any hatchery population? Both Discriminant Analysis of Principle Components (DAPC) as implemented in R with the *adegenet* package version 2.0.1 (Jombart 2008; Jombart et al. 2010)

and STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) were used as complementary individual analyses.

For the individual DAPC, we limited our analysis to only new collections from the Tuolumne River and Merced River along with five hatchery trout strains as reference populations to detect hatchery introgression. DAPC is not based on a population genetic model, and relies on the conversion of SNP data into principal components to account for linkage between SNPs and allow generic methods of individual clustering to be used. As opposed to finding axes of maximal variation as with Principal Component Analysis (PCA), DAPC maximizes between-population separations and minimizes within-population variation. We identified inferred populations with DAPC by applying the "find.clusters" function of *adegenet* followed by PCA and Discriminant Analysis (DA) within the "dapc" function of *adegenet* that utilized the packages *ade4* version 2.7-8 (Chessel and Dufour 2004; Dray et al. 2007; Dray and Dufour 2007) and *MASS* version 7.3-47 (Venables and Ripley 2002).

Unlike DAPC, STRUCTURE has an explicit population genetics model and uses the individual genotype data directly. STRUCTURE assigns fractional ancestry (q – values) to K inferred populations based on descent from a common ancestral population. For each individual, the q – values sum to 1.00 and indicate what proportion of each of K inferred populations make up the individual. Migrants and individuals of mixed ancestry can be identified with STRUCTURE without *a priori* designation of defined populations (Pritchard et al. 2000). We evaluated all individuals in the quality-controlled dataset with $K = \{1,\ldots,12\}$ inferred populations with four independent runs with an initial burn-in of 100,000 steps followed by a Markov Chain Monte Carlo (MCMC) of 1,000,000 steps. For most parameters, default settings were used. Results from the STRUCTURE runs were visualized with DISTRUCT version 1.1 (Rosenberg 2004).

Population Approach

Sample units were treated as populations for identifying population genetic and phylogenetic relationships with a minimum required size of 10 individuals per sample unit (Table 1). Population genetic relationships were evaluated with DAPC using the sample unit to predefine population genetic clusters. The same sample units were also evaluated in a Neighbor-Joining population tree generated through *poppr* version 2.4.1 using a chord distance metric (Cavalli-

Sforza and Edwards 1967) and the filling of missing data by the mean of that locus. Confidence of nodes in the population tree was assessed by 1,000 bootstrap replicates.

Signatures of Migratory Adaptation

Of the three genotyped SNP loci located on chromosome Omy5, one (R04944) is known to accurately identify the "R" and "A" haplotypes surveyed in previous studies (Pearse et al. 2014; Leitwein et al. 2017). Based on this locus, we calculated the frequencies of the "A" allele associated with migratory behavior in all populations. These data were then considered with respect to the migratory potential of each sampling site relative to historical and current barriers and reservoirs, and populations with potential for adfluvial life history variants were identified.

Results

Sample Genotyping and Population Statistics

A total of 897 *O. mykiss* samples from the UTR and UMR were genotyped, and after filtering for missing data and loci under selection linked to the Omy5 inversion, the combined dataset of 20 sampling units and 29 reference populations consisted of 2,370 individuals and 88 bi-allelic SNP loci (Table 1). Sample sizes for the UTR and UMR populations ranged from 2-103 individuals per site; sample units smaller than 10 were used for individual-based analyses, but excluded from population-level analysis.

The distribution of neutral genetic diversity among populations showed typical patterns, with most populations isolated above barriers having reduced heterozygosity relative to downstream populations (Table 1). Similarly, most hatchery rainbow trout strains had reduced levels of variation, as did populations inferred to be of primarily hatchery origin (e.g. GROS). Conversely, larger populations connected by migration (e.g. TUOL and YOSV) tended to have high heterozygosities, similar to coastal and Central Valley steelhead populations (Table 1, Figure 1).

Individual Approach

DAPC of individuals from the 20 UTR and UMR sampling units plus five hatchery reference strains supported the inference of eight genetic groups (Figure 2). Three of these

inferred clusters are composed of fish of natural genetic origin while the other five contained fish of hatchery origin or fish in genetic composition similar to hatchery fish (Figure 2). Most fish from the UTR and UMR sampling locations were not placed in clusters with significant hatchery trout contributions. However, the Grouse Creek (GROS) sampling location is entirely placed in inferred group five along with the Kamloops Hatchery strain, while many individuals from the UNFT sample were grouped with the Coleman strain (Group 4; Figure 2). Similarly, most individuals from the Merced River Hatchery sample (MCDH) were placed in group four with the Coleman strain, while the rest were grouped with the Mt. Shasta, Eagle, and Moccasin hatchery trout lineages, supporting the mixed hatchery ancestry that had previously been inferred for lower Merced River *O. mykiss* (Pearse and Garza 2015).

STRUCTURE results showed strong convergence, verified by the highly consistent results among all four independent runs (data not shown). The distribution of STRUCTURE *q* -values in among individuals supported previous findings of relationships between coastal and Central Valley *O. mykiss*, and were similar to the individual DAPC results and population genetic analyses (see below). At low values of *K*, there were clear patterns of divergence between coastal steelhead and northern and southern Central Valley-lineage populations (*K*=4; Figure 3). These patterns remained evident at higher values of *K*, with finer patterns of differentiation consistent with those seen by Pearse and Garza (2015).

Population Approach

DAPC of sample units indicated a strong geographical component, with the first and second axes of the DAPC plot roughly encompassing East-West and North-South geography (Figure 4). This pattern of divergence is concordant with previous studies showing a primary division between coastal and Central Valley steelhead and an association between geography and genetic differentiation among *O. mykiss* populations isolated above dams within the Central Valley, but not among below-barrier Central Valley steelhead populations (Pearse and Garza 2015).

The phylogenetic tree also supported known patterns of geographic differentiation, although many nodes received less than 50% bootstrap support (Figure 5). Nonetheless, well-supported relationships among several pairs and groups of populations were consistent with previous studies, indicating that the current data set has sufficient power to resolve these

relationships (e.g. close similarity of Feather River and Mokelumne Hatchery steelhead, Nimbus Hatchery steelhead and coastal populations, and the relationships between the new UTR and UMR samples and reference samples from those locations; Pearse and Garza 2015). Among the new UTR and UMR samples, a clade of nine Tuolumne River populations (e.g. TUOL, REED, UCLV, ROOS, FROG, etc., Figure 5) was identified with moderate bootstrap support (77%), supporting their common indigenous ancestry. The South Fork Merced sample (SFMC) appears as sister to this group, but without significant support. Other UTR and UMR populations were more widely dispersed in the tree, possibly indicating more diverse sources contributing to these *O. mykiss* populations, and also reflecting the limited resolution and low bootstrap support for deeper nodes in the tree. Meaningful support (68% and 98%) was found for the relationships between the Grouse Creek (GROS) population, Kamloops Hatchery strain, and the northern Central Valley population from the McCloud River (Butcherknife Ck.), further supporting the complete hatchery origin of the isolated above-barrier GROS population (Figure 5).

Signatures of Migratory Adaptation

The frequency of Omy5 A haplotype in the sampling units within the UTR and UMR ranged from a minimum of 0.00 in GROS to maximum 0.31 in TUOL (Figure 6). Given their locations and accessibility to fish migrating from downstream reservoirs, the relatively high frequency of the A haplotype in the TUOL, FROG, and YOSV populations supports the suggestion that they sustain trout with adfluvial life histories. Conversely, the A haplotype exists at relatively low frequency in most other populations, particularly those found above barriers to migration (*e.g.* REED, JAWB, and CRAN (Figure 1, Figure 6). However, there was considerable variability among populations, likely reflecting a combination of selective factors impacting the frequency of adaptive genomic variation on chromosome Omy5 and other parts of the genome.

Discussion

Overall, the observed genetic relationships between rainbow trout in the UTR and UMR and other Central Valley *O. mykiss* populations and hatchery trout strains indicate that a mixture of lineages exists in these Yosemite watersheds. However, despite the extensive stocking with non-indigenous hatchery trout strains throughout the region, native ancestry appears to remain as the

primary component of most sampling units examined in this study, with primarily indigenous southern Central Valley - San Joaquin River ancestry in reaches that were historically accessible to migratory salmonids. This includes the Clavey River, which has been designated as Heritage and Wild Trout Waters by the California Department of Fish and Wildlife[1]. These results support the hypothesis that local adaptation has played a key role in the persistence of these lineages.

In terms of ancestry, the primary division between coastal and Central Valley *O. mykiss* that has previously been documented (Nielsen et al 2005; Pearse and Garza 2015) was also clear in multiple analyses of the present data set (Figures 3, 4, and 5). This is important because it confirms that unlike some below-barrier populations in the southern Central Valley, including *O. mykiss* sampled in the lower Tuolumne River (Pearse and Garza 2015), the trout populations in the UTR and UMR do not show evidence of introgression from the coastal-origin steelhead propagated at Nimbus Hatchery. However, the close evolutionary relationships among all Central Valley *O. mykiss*—including most hatchery rainbow trout strains commonly used in California—make precise inference of population relationships and admixture within the Central Valley difficult, and the weak signal of genetic differentiation among these populations likely reflects biological reality rather than limited resolution. Nonetheless, the relative proximities of populations shown in the DAPC revealed a clear pattern of geographic divergence among populations, with Axis 2 highlighting the North-South divergence within the Central Valley (Figure 4). This is consistent with the hypothesis that rainbow trout populations isolated above dams in the Sierra Nevada better reflect their historical geographic origins than the scrambled steelhead populations that persist below barriers to migration (Pearse and Garza 2015).

The problems with hybrids in conservation have long been recognized (Allendorf et al. 2001), and the potential conservation value of hybrid populations remains an active area of discussion (Wayne and Shaffer 2016). Within the UTR and UMR, many populations show at least some evidence of mixed ancestry, as is common in studies of *O. mykiss* above dams (e.g. Winans et al. 2017), but we did not find the complete replacement of indigenous ancestry that has been observed in some regions in California subjected to intensive hatchery trout stocking (e.g. Southern California; Abadía-Cardoso et al. 2016). Although admixed populations do not represent pure indigenous lineages, they often have high genetic diversity, and should not be

[1] wildlife.ca.gov/fishing/inland/trout-waters

entirely discounted when considering source populations for recovery efforts (Abadía-Cardoso et al. 2016).

Most fish sampled at sites that were historically fishless due to their positions above barriers or at high elevation represent a mixture of indigenous and imported ancestries, with some having largely largely indigenous ancestry (e.g. Frog Creek; FROG) while others appear entirely descendent from hatchery rainbow trout strains (e.g. Grouse Creek; GROS). The upper North Fork Tuolumne (UNFT) shows variable associations in different analyses, with genetic similarity to both the Coleman hatchery trout strain and coastal lineage populations. This sampling site has a long history of intensive hatchery stocking due to its location near a major road (CA Highway 108), and both it and GROS have low heterozygosities, consistent with hatchery strain ancestry. In contrast, the populations in Reed Creek (REED) and Jawbone Creek (JAWB) have high heterozygosities and are genetically similar to other nearby populations within the UTR genetic group, despite being isolated above very large natural barrier waterfalls.

Adaptive Variation and Migratory Potential

It is important to note that adaptive genomic variation like that documented on chromosome Omy5 is subject to the same factors that affect the distribution of neutral genetic variation among all natural populations, including drift due to small population sizes and introgression by non-native lineages with highly divergent patterns of variation (Pearse 2016). In the case of hatchery rainbow trout, Omy5 haplotype frequencies vary widely among strains, so their influence on introgressed wild populations is difficult to determine. However, to the extent that they reflect ongoing selection, the frequencies of alleles in this genomic region provide information about the relative fitness of alternative life-history patterns in a given set of populations.

Within the UTR and UMR, the distribution of Omy5 haplotype variation suggests that the populations most likely to express an adfluvial life history, and therefore to retain the potential to express anadromy, are those with unimpeded migratory access to Don Pedro and McClure reservoirs (e.g. TUOL, YOSV), as well as the Frog Creek population tributary to Lake Eleanor (Figures 1 and 6). Although the maximum frequency of migration-associated alleles among the UTR and UMR populations (0.31 in TUOL) is low relative to coastal anadromous and adfluvial populations (typically >0.60; Pearse et al. 2014; Leitwein et al. 2017), it is similar to that seen in

potentially adfluvial populations of *O. mykiss* in the Upper American River (0.33; Abadía-Cardoso et al.,). In addition, the genomic region of Omy5 associated with migratory life-history patterns has also been associated with differences in temperature-specific development rates (Miller et al. 2012). The additional influence of temperature could contribute to the elevated frequency of resident-associated alleles in the colder, high elevation populations, but further research is needed to better understand the factors that may influence the distribution of this adaptive genomic variation. Together these results suggest that the UTR and UMR populations that now occupy river reaches between the reservoirs and the historical barriers to upstream migration are the most likely to express migratory adfluvial behavior and retain adaptive genomic variation associated with anadromy (Holecek et al. 2012; Holecek and Scarnecchia 2013; Leitwein et al. 2017).

Conservation Implications

Efforts to restore salmonid populations and the watersheds they inhabit will require a diverse set of approaches, investment, and cooperation among stakeholders (Phillis et al. 2013; Penaluna et al. 2016; Lackey 2017; Warren et al. 2017), particularly for migratory anadromous forms like steelhead (NMFS 2014). From an evolutionary genetics perspective, there are several implications of this study for the potential restoration of connectivity between the UTR and UMR populations and the California Central Valley steelhead DPS below the dams.

First, the present study was based on a dataset with a modest number of SNPs by today's standards, and thus has relatively low power to estimate population genetic parameters—a large genomic dataset based on the thousands of loci generated by high-throughput sequencing could undoubtedly refine the results observed in the present study. For example, more than 230,000 SNP loci were recently used to accurately estimate the proportions of European, African, and Native American ancestry in admixed human populations in Colombia (Conley et al. 2017). It is also possible that hatchery trout strains that were not included in the present study have been stocked in these watersheds, so their contributions could not be specifically detected. However, the basic conclusions regarding the distribution of indigenous rainbow trout within the Tuolumne and Merced watersheds and their implications for management are unlikely to change in biologically significant ways. Similarly, further characterization of the distribution of adaptive genomic variation on chromosome Omy5 and other parts of the genome will provide insight into

the evolutionary processes affecting trout populations above dams. However, such information would not necessarily impact conservation planning because the basic principles of conservation genetic management to preserve genetic diversity remain the same (Pearse 2016). Nonetheless, as more examples of adaptive genomic variation associated with life-history traits are identified in *O. mykiss* and other salmonid species (Barson et al. 2015; Hess et al. 2016), fisheries managers will need to carefully consider the most appropriate ways to conserve and protect this important biodiversity (Pearse 2016).

Second, Pearse and Garza (2015) detected introgression by coastal-origin steelhead propagated at Nimbus Hatchery into the limited populations of *O. mykiss* that remain in the ocean-accessible river reaches below dams in the Calaveras, Tuolumne and Stanislaus rivers, and found that *O. mykiss* captured in the lower Merced River are primarily descended from hatchery trout, especially the Eagle Lake strain. The signal of hatchery ancestry observed in the sample of 59 lower Merced River fish analyzed in the present study further confirms this result. However, recent data on the physiology of steelhead in the lower Tuolumne River have shown that they have a much higher thermal tolerance than populations from northern latitudes, demonstrating their local adaptation to the high temperatures of the southern Central Valley (Verhille et al. 2016). Thus, the *O. mykiss* currently inhabiting below-barrier reaches of the Tuolumne and Merced Rivers likely represent a mixture of indigenous, hatchery, and coastal ancestry, and both admixture and local adaptation have likely influenced their current genetic composition, including the frequencies of Omy5 haplotypes and other adaptive genomic variation.

Third, although our data show that the rainbow trout trapped above these dams have both ancestry and adaptive genomic variation representative of indigenous migratory populations, the development of an anadromous steelhead population from these stocks through fish passage via two-way trap and haul or other means presents many challenges (Lusardi and Moyle 2017). Re-establishing gene flow between formerly-connected populations above and below barrier dams has many potential benefits in terms of maintenance of genetic diversity and facilitating adaptation, but these must be evaluated against the possible risks and constraints within the larger reintroduction and recovery framework (Anderson et al. 2014). Nonetheless, anadromous salmonid life-histories can emerge rapidly from formerly adfluvial populations following dam removal, demonstrating that such populations are capable of re-establishing their dormant ability to complete an ocean migration (Quinn et al. 2017). In this context, migratory adfluvial

individuals in the Tuolumne, Merced, and other Central Valley watersheds could be considered as potential contributors to future fish passage programs and re-introduction efforts (Thrower et al. 2008), provided that the logistical issues associated with reestablishing connectivity can be overcome (NMFS 2014). Thus, in considering the potential for passage of migratory fish above New Don Pedro and New Exchequer Dams, directed studies are needed to determine the potential for trapping downstream migrants, among other considerations, as has been undertaken in similar situations (e.g. Clancey et al. 2017; Winans et al. 2018).

Finally, it should be noted that the populations of steelhead in the southern Central Valley are likely among the most vulnerable to the impacts of climate change, so their continued persistence is far from certain. Therefore, in the context of protecting and restoring anadromous fish populations in California, genetic factors should be considered as secondary to the basic need for access to appropriate habitat to support all phases of the migratory life-cycle. This includes access to suitable spawning and rearing habitats, as provided via removal of large barrier dams or carefully monitored two-way trap-and-haul fish passage programs (Anderson et al. 2014), as well as modification or removal of smaller migration barriers (Apgar et al. 2017), adjustments to flow regimes, and other improvements in downstream habitats to support native fishes and restore viable migratory connectivity with the ocean for both outmigrating juveniles and returning adult salmonids (NMFS 2014). In the absence of these changes, the existence of migratory populations of salmonids in the Central Valley will continue to depend on hatchery propagation and other interventions until the dams that block their migratory paths are modified or removed (Katz et al. 2013; Quiñones et al. 2015).

Acknowledgements

We thank John Wooster and Steve Edmondson of the NMFS/WCR/CCVO Hydro team and the members of the NMFS SWFSC Molecular Ecology and Genetic Analysis Team for their support on this project, particularly Cassie Columbus, Ellen Campbell, Elena Correa, and Mary Ables Ray. Diana Baetscher, William Foster, John Carlos Garza, Cyril Michel, and Erin Strange provided valuable comments that improved the manuscript. We also thank Rob Grasso for facilitating access and assisting with sampling in Yosemite National Park. Samples were collected under CDFW scientific collecting permit #13029. Special thanks to all of the fly fishers who contributed their time and skills to obtain samples for this project, including Ben Burford, Rob Grasso, Sean Hayes, Tom Holley, Steve Lindley, Michael Martin, Alex McHuron, Cyril Michel, Jeremy Notch, David Swank, and Larry Thompson.

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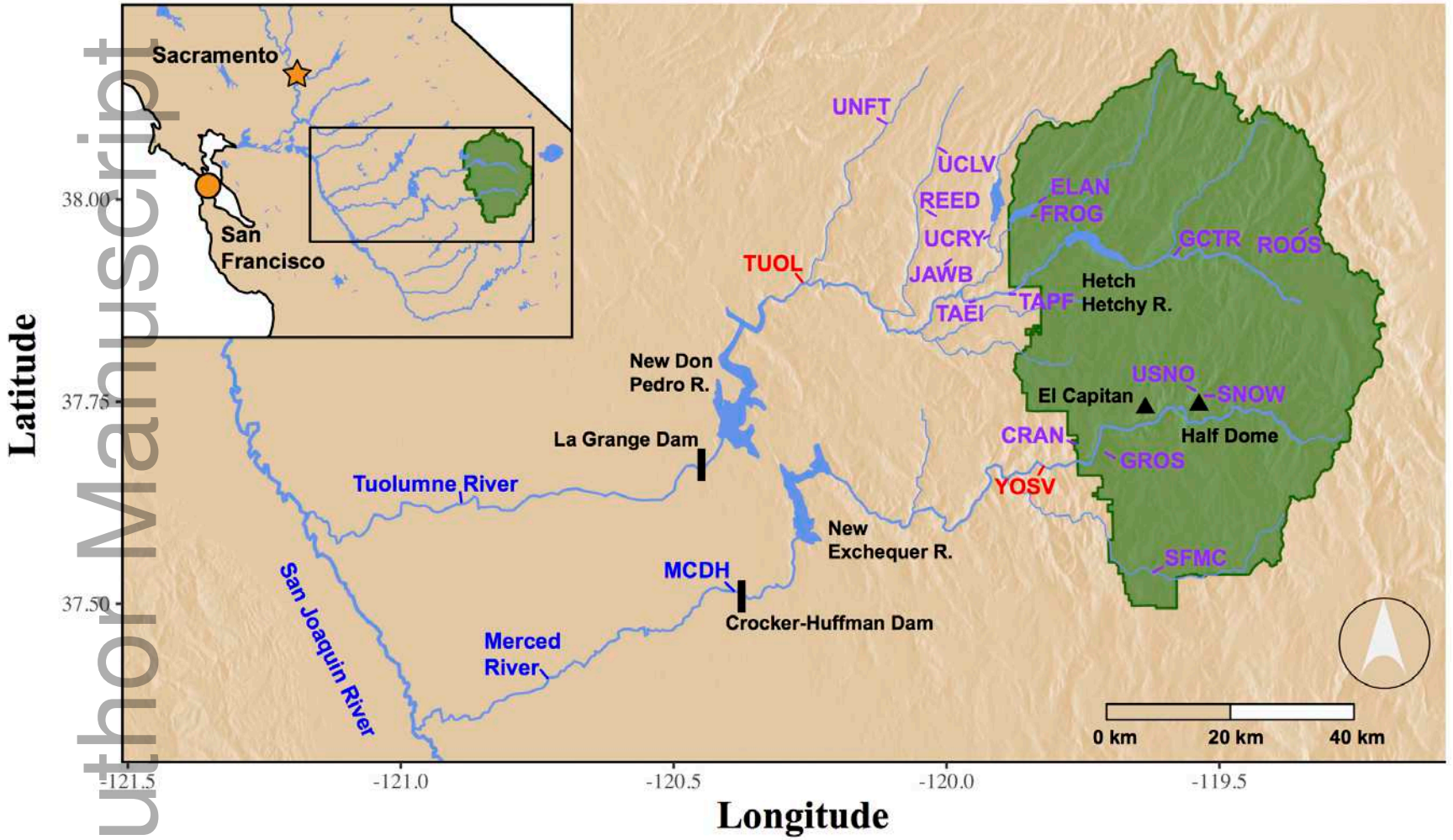

Figure 1: Map of Tuolumne and Merced River sampling locations investigated in this study showing existing dams and reservoirs. Sampling units as described in the text are labeled following the codes in Table 1, with two Pearse and Garza (2015) reference populations indicated by “Tuolumne River” and “Merced River.” Yosemite National Park is shaded green with El Capitan, and Half Dome indicated by solid black triangles. Population migratory potentials are indicated by color as potentially anadromous (blue), potentially adfluvial (red) and, resident rainbow trout (purple). La Grange Dam and Crocker-Huffman Dam are the upper limits to anadromy in the Tuolumne River and Merced River respectively and are indicated with black bars. Inset: Central California region showing the Sacramento – San Joaquin River system draining to the Pacific Ocean through San Franscico Bay. The inner box in the inset indicates the geographic extent of the main map.

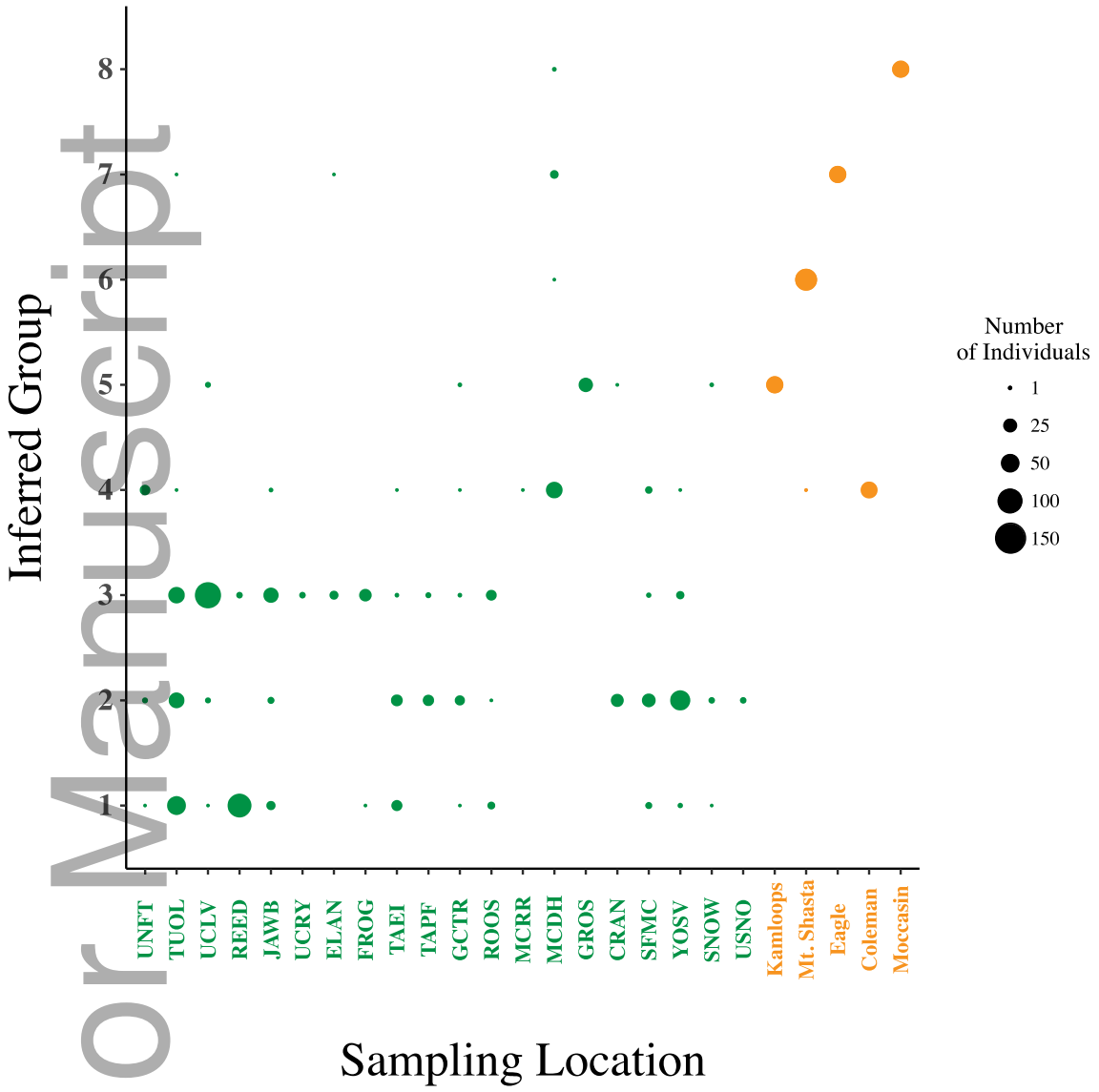

Figure 2: Individual group assignments from Discriminant Analysis of Principal Components (DAPC) of individuals from the 20 Upper Tuolumne and Merced River sample units and five hatchery reference strains. The sampling unit is indicated on the *x* – axis as in Table 1, and the hypothesized eight genetic groups of individuals are on the *y* – axis. Yosemite sampling units are colored green and hatchery trout strains are colored orange. Circle sizes indicate the number of individuals from each sampling location assigned to a particular group.

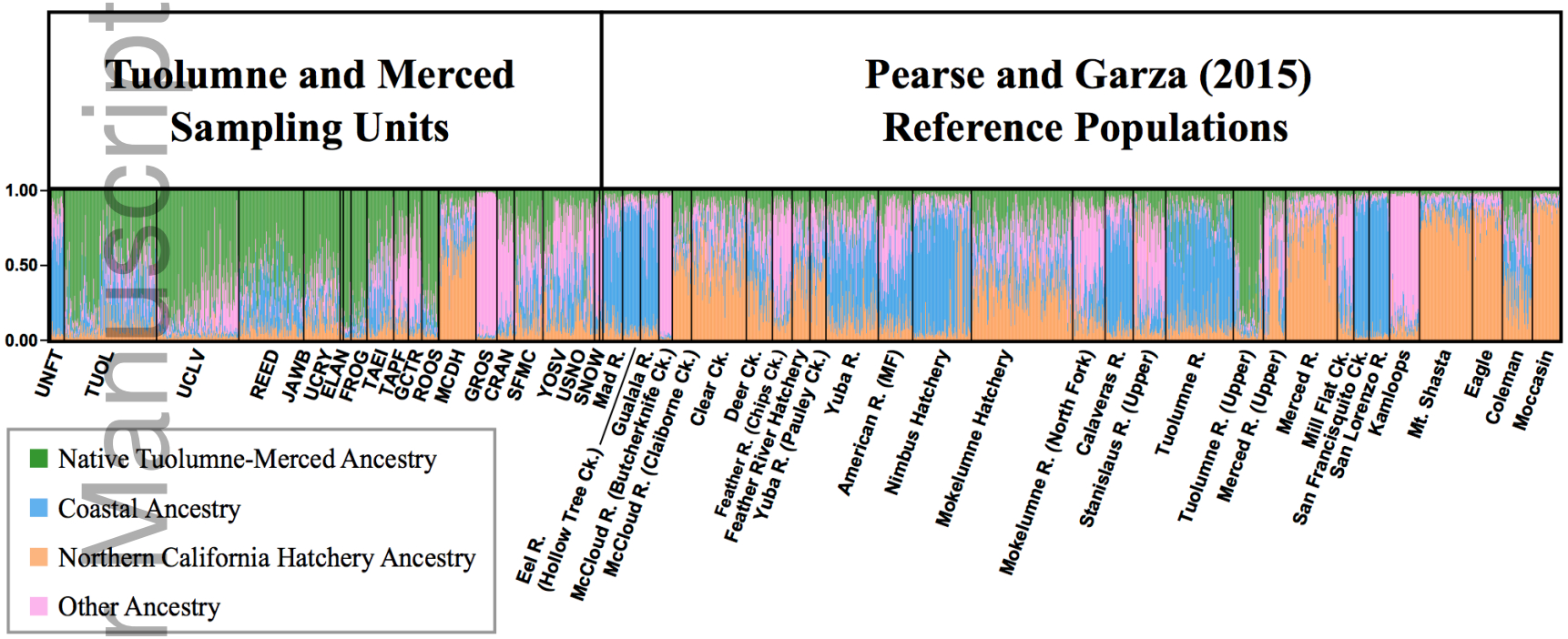

Figure 3: Individual-based plot of fractional ancestry from a hypothesized number of K = 4 distinct genetic groups. Sampling units and reference populations as described and ordered in Table 1 are indicated along the bottom of the plot. Each individual is represented by a vertical line, with the proportion of estimated ancestry from each of the hypothetical genetic groups colored proportionately within the vertical column. Here, inferred 'Yosemite' ancestry is shown in green, 'coastal' ancestry in blue, 'Northern Central Valley hatchery' ancestry in orange, and other groups are pink. See text for details.

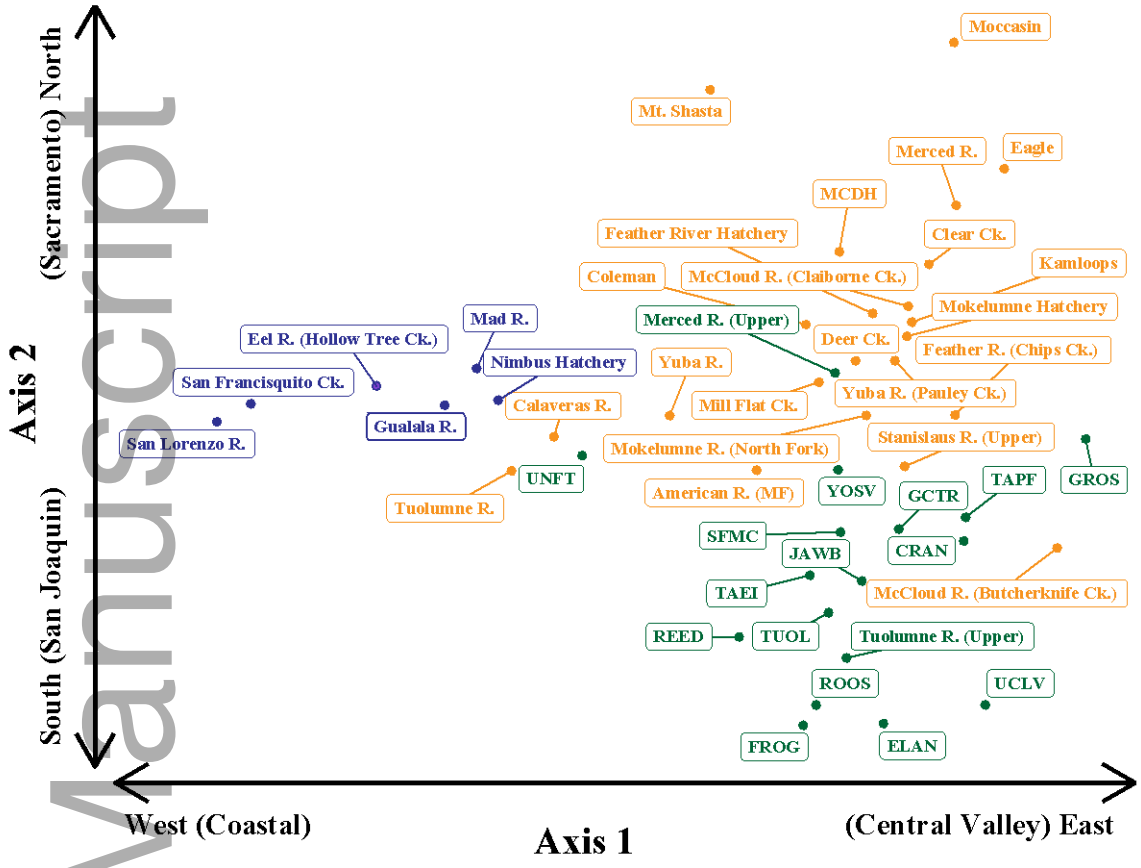

Figure 4: Population Discriminant Analysis of Principal Components (DAPC) plot showing genetic relationships among upper Tuolumne River (UTR) and upper Merced River (UMR) sampling units (green) relative to coastal (blue) and Central Valley reference populations and hatchery trout strain (orange). The central value for each population is indicated and with colors as in Figures 2 and 5.

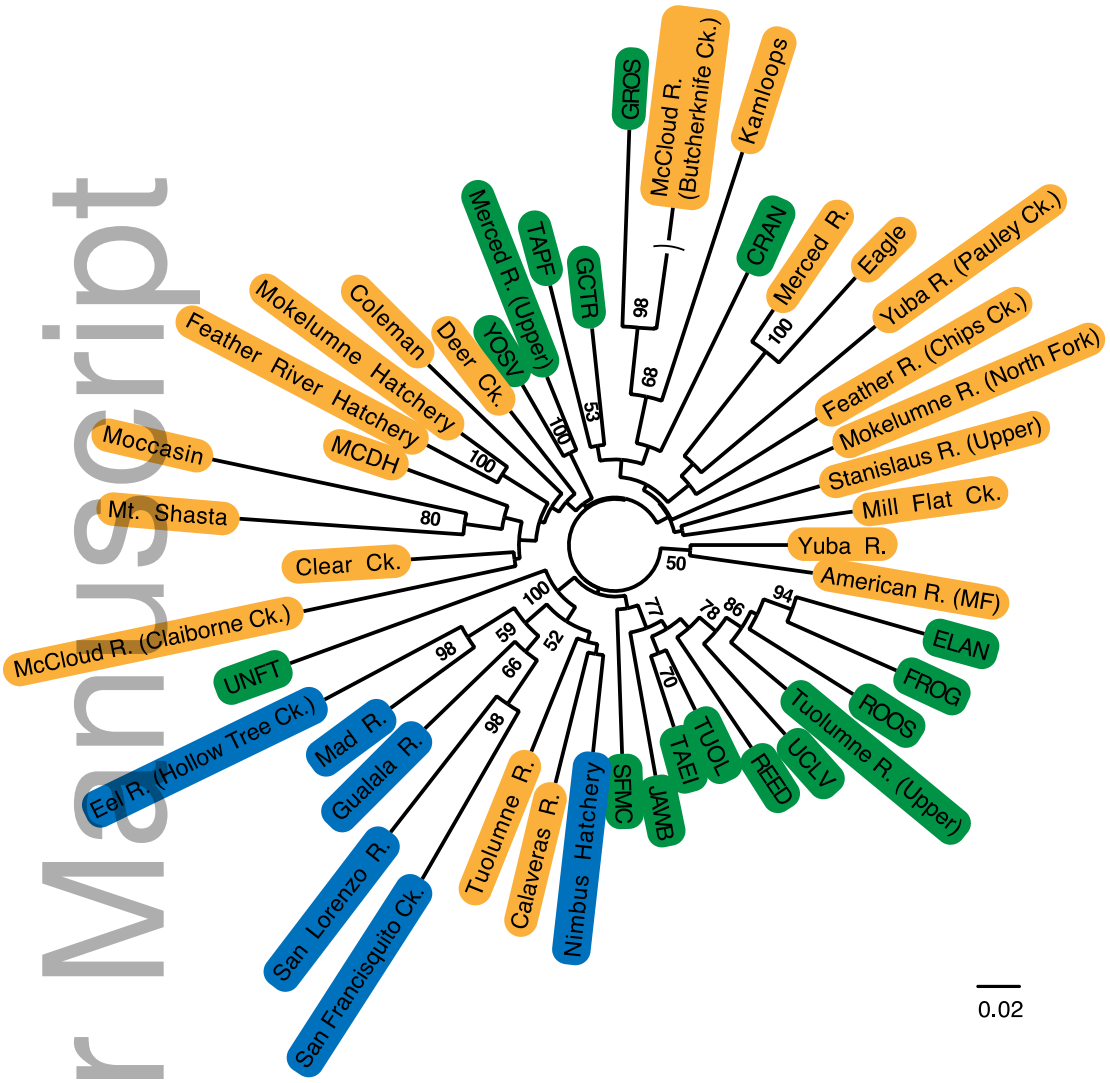

Figure 5: Neighbor Joining phylogenetic tree based on population chord distances showing relationships among sampling units within the UTR and UMR relative to other Central Valley and coastal *O. mykiss* populations. Colors highlight Yosemite (green), coastal (blue), and Central Valley reference populations and hatchery trout strains (orange). Bootstrap support, from 1,000 replicates is depicted (values of less than 50% not shown). The branch to McCloud R. (Butcherknife Ck.), indicated by a bisecting curve, has been shortened to one third of the original length for visual presentation.

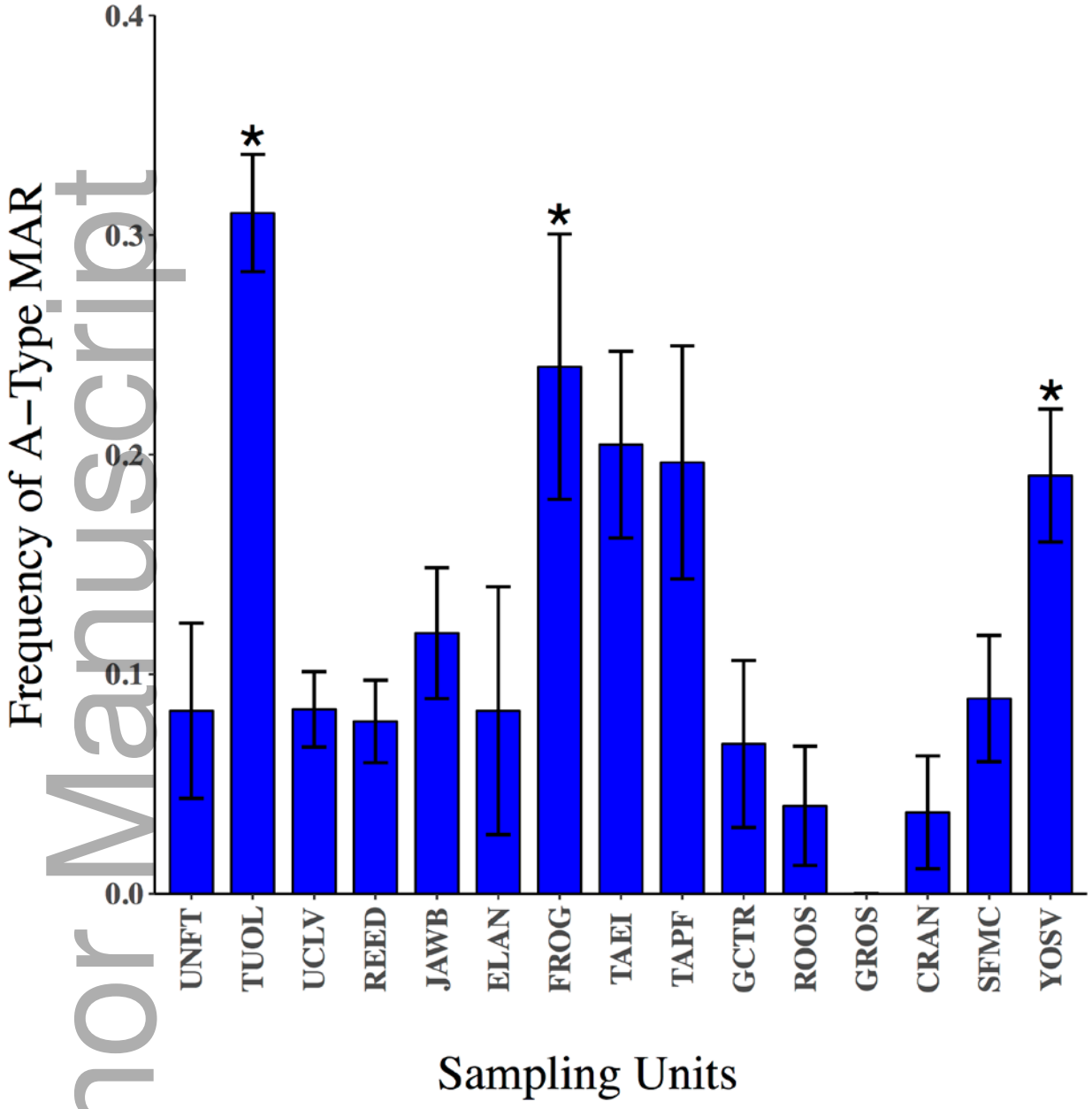

Figure 6: Frequency of the anadromous type Omy5 Migration-Associated Region haplotype (A-Type MAR) estimated from Upper Tuolumne River and Upper Merced River sampling units examined in this study. Potentially adfluvial populations with access to reservoirs are indicated by asterisks. Standard error for allele frequency estimates are shown (Hartl and Clark 1997).

Table Captions

Table 1: Sample information and summary statistics for genetic data analysis. The full name for sampling units are given along with shortened codes that are used in the manuscript. The sample size for each sampling unit and the categorization regarding migratory potential are also presented. For each population, the number of samples that were included in genetic analyses, expected heterozygosity (H_E) and the frequency of the anadromous type Omy5 Migration-Associated Region, F(A MAR) are provided. For each collection that comprised a sampling site, the major drainage basin, the dates of collection, sample size (N), and WGS 84 coordinates are indicated.

Sampling Unit	Code	Sample Size	Population Type	Samples Passing QC for Genetic Analyses	H_E	F (A MAR)	Drainage Basin	Date(s)	N	Latitude	Longitude
Upper North Folk Tuolumne River	UNFT	24	Above Barrier	21	0.27	0.08	Tuolumne River	6/8/15	24	38.1	-120.11
Tuolumne River	TUOL	150	Historically Anadromous	145	0.36	0.31	Tuolumne River	5/12/15	11	37.9	-120.07
							Tuolumne River	8/27/15	3	37.9	-120.26
							Tuolumne River	10/8/15	41	37.84	-120.06
							Tuolumne River	5/12/15	31	37.84	-120.04
							Tuolumne River	6/9/15	11	37.89	-119.97
							Tuolumne River	5/15/15	36	37.89	-119.95
							Tuolumne River	5/14/15	17	37.88	-119.97
Upper Clavey River	UCLV	131	Above Barrier	129	0.30	0.08	Tuolumne River	6/9/15	68	37.99	-120.05
							Tuolumne River	6/8/15	55	38.07	-120.01
							Tuolumne River	6/8/15	8	38.09	-120.01
Reed Creek	REED	103	Above Barrier	102	0.36	0.08	Tuolumne River	5/13/15	103	37.98	-120.02
Jawbone Creek	JAWB	59	Above Barrier	57	0.35	0.12	Tuolumne River	5/13/15	59	37.93	-119.99
Upper Cherry Creek	UCRY	5	Above Barrier	5	0.31	NA	Tuolumne River	6/9/15	5	37.96	-119.92
Eleanor Creek	ELAN	12	Above Barrier	12	0.30	0.08	Tuolumne River	6/19/16	12	38	-119.83
Frog Creek	FROG	25	Above Barrier	25	0.29	0.24	Tuolumne River	6/18/16	25	37.98	-119.84
Tuolumne River - Above Early Intake	TAEI	45	Historically Anadromous	42	0.36	0.20	Tuolumne River	5/13/15	45	37.88	-119.94
Tuolumne River - Above Preston Falls	TAPF	28	Above Barrier	23	0.32	0.20	Tuolumne River	6/10/15	23	37.88	-119.88
							Tuolumne River	6/11/15	5	37.95	-119.79
Grand Canyon Tuolumne River	GCTR	22	Above Barrier	21	0.34	0.07	Tuolumne River	7/18/15	22	37.93	-119.58
Roosevelt Lake	ROOS	26	Above Barrier	26	0.32	0.04	Tuolumne River	8/13/15	26	37.964	-119.339
Merced River Ranch	MCRR	1	Ocean Accesible	1	0.39	NA	Merced River	4/9/10	1	37.52	-120.4
Merced River Hatchery	MCDH	58	Ocean Accesible	58	0.35	0.60	Merced River	12/9/14	58	37.52	-120.37
Grouse Creek	GROS	34	Above Barrier	33	0.23	0.00	Merced River	8/11/16	34	37.69	-119.7
Crane Creek	CRAN	27	Above Barrier	27	0.32	0.04	Merced River	8/10/16	27	37.7	-119.76
South Fork Merced River	SFMC	49	Historically Anadromous	45	0.34	0.09	Merced River	8/11/16	4	37.55	-119.63
							Merced River	8/11/16	43	37.539	-119.621
							Merced River	8/11/16	2	37.517	-119.667
Yosemite Valley	YOSV	85	Historically Anadromous	81	0.35	0.19	Merced River	8/11/16	3	37.671	-119.819
							Merced River	6/30/15, 8/12/16	47	37.68	-119.74
							Merced River	6/29/16	2	37.725	-119.712
							Merced River	8/11/16	4	37.725	-119.712
							Merced River	8/11/16	15	37.723	-119.557
							Merced River	6/30/2015, 8/10/2016	14	37.75	-119.54
Snow Creek	SNOW	8	Historically Anadromous	8	0.33	NA	Merced River	8/10/16	8	37.76	-119.53
Upper Snow Creek	USNO	5	Above Barrier	5	0.34	NA	Merced River	8/10/16	5	37.77	-119.54

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Table 1, continued.

Pearse & Garza (2015) Reference Populations				
North Coast				
Mad R.	31	Ocean Accesible	31	0.35
Eel R. (Hollow Tree Ck.)	28	Ocean Accesible	28	0.34
Gualala R.	29	Ocean Accesible	29	0.40
Central Valley				
McCloud R. (Butcherknife Ck.)	21	Historically Anadromous	21	0.17
McCloud R. (Claiborne Ck.)	33	Historically Anadromous	30	0.33
Clear Ck.	94	Ocean Accessible	86	0.34
Deer Ck.	45	Ocean Accessible	41	0.37
Feather R. (Chips Ck.)	31	Historically Anadromous	31	0.32
Feather River Hatchery	30	Ocean Accessible	28	0.37
Yuba R. (Pauley Ck.)	25	Historically Anadromous	25	0.31
Yuba R.	90	Historically Anadromous	82	0.41
American R. (MF)	58	Historically Anadromous	54	0.37
Nimbus Hatchery	98	Ocean Accessible	92	0.40
Mokelumne Hatchery	162	Ocean Accessible	159	0.37
Mokelumne R. (North Fork)	51	Historically Anadromous	51	0.33
Calaveras R.	47	Ocean Accessible	44	0.37
Stanislaus R. (Upper)	52	Ocean Accessible	51	0.34
Tuolumne R.	112	Ocean Accessible	106	0.39
Tuolumne R. (Upper)	47	Historically Anadromous	47	0.34
Merced R. (Upper)	35	Historically Anadromous	35	0.35
Merced R.	83	Ocean Accessible	81	0.29
Mill Flat Ck.	26	Historically Anadromous	26	0.36
South Coast				
San Francisquito Ck.	24	Ocean Accessible	24	0.36
San Lorenzo R.	32	Ocean Accessible	32	0.37
Hatchery Trout Strains				
Kamloops	47	NA	47	0.23
Mt. Shasta	92	NA	83	0.32
Eagle	47	NA	47	0.25
Coleman	47	NA	47	0.33
Moccasin	47	NA	46	0.25