Genetic Analyses of Central Valley Trout Populations
1999-2003

by

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Genetic variation found at 11 microsatellite loci was used to describe population structure for steelhead and rainbow trout (*Oncorhynchus mykiss*) in the Central Valley, California, looking at both spatial and temporal genetic variation as well as relationships among hatchery and wild populations. We analyzed genetic diversity at two scales: within drainage spatial allelic diversity analyzed for five trout populations sampled in Clear Creek; between and among drainage genetic diversity analyzed for 23 population of trout found in the Central Valley. DNA was amplified and analyzed for 1570 trout samples. Significant regional spatial structuring of populations was apparent, both within Clear Creek and among trout populations in the Central Valley. Significant differences in allelic frequencies were found among most river or drainage systems containing wild trout. However, less than 1% of the molecular variance could be attributed to differences between trout populations found within the Sacramento River and samples from the San Joaquin River drainage. Hatchery populations were shown to be similar in genetic diversity to geographically proximate local wild populations. Overall classification accuracy of single individuals to their stream of origin using these 11 microsatellite loci was 83%. Garza and Williamson’s (2001) $M$ over all populations of trout in the Central Valley was $M = 0.626$, below the published threshold ($M \leq 0.68$), supporting recent population reductions for steelhead within the Central Valley. Average estimated effective population size for Central Valley steelhead populations, however, was relatively high ($Ne = 5066$). Significant allelic differences were found in trout collected above and below impassable dams on the American, Yuba, Stanislaus and Tuolumne rivers. Trout sampled in Spring Creek were found to be extremely bottlenecked with genetic variation found at only two loci and an effective population size of 62. These data suggest that significant genetic population structure remains for steelhead populations within the Central Valley, and careful consideration of this genetic diversity should be part of future conservation and restoration efforts.
INTRODUCTION

Historically, anadromous steelhead (*Onchorhynchus mykiss*) were broadly distributed throughout the Sacramento and San Joaquin River drainages (McEwan 2001). There has been a substantial decline of Central Valley steelhead over the last 150 years, due primarily to lost spawning and rearing habitats, changes in water quality, and within-basin dams and diversions (Busby et al. 1996; McEwan 2001; May and Brown 2002). Natural anadromous spawning populations of winter-run steelhead still exist at low levels in the Sacramento and San Joaquin River drainages. *O. mykiss* expresses a range of variations in life history strategies, from strongly migratory to non-migratory, throughout the species’ range. Individual runs or stocks of *O. mykiss* found within the same drainage cannot be separated taxonomically based on migration timing or the distribution of anadromony (Behnke 1992; Allendorf and Utter 1979). Highly flexible life history strategies in *O. mykiss* (Shapovalov and Taft 1954), otolith microchemistry (Rybock et al. 1975; Zimmerman and Reeves 2000), and genetic studies (Gall et al. 1990; Nielsen et al. 1997) suggest that freshwater habitats may contain relic, non-anadromous components of the *O. mykiss* gene pool found in geographically proximate anadromous populations.

There has been considerable manipulation of rainbow trout in California in the hatchery environment since the early 1800’s (Busack and Gall 1980). Impacts of hatchery propagation of *O. mykiss* on wild stocks in streams and reservoirs throughout North America over the last 200 years has been the subject of many studies (see reviews in Reisenbichler and McIntyre 1977, Waples and Do 1994, Campton 1995, and Nielsen 1999). The early findings of Gall et al. (1990) suggested that anadromous steelhead populations have residualized as freshwater fish behind man-made structures and dams throughout California. Using allozyme analyses, this study argued that residual freshwater populations of *O. mykiss* reflect genetic population structure similar to their putative anadromous progenitors. Within the Central Valley there are numerous populations of non-anadromous rainbow trout upstream of both natural long-standing and artificial barriers (see Figures 1 & 2). Many of these populations have had extensive opportunity to interbreed with hatchery trout used to supplement streams and reservoirs.

Recent studies of land-locked trout populations throughout California have demonstrated genetic relationships between landlocked trout and geographically proximate
anadromous steelhead populations. Rainbow trout found in Alameda Creek above a man-made barrier were most closely related genetically to fish collected below the dam and known steelhead found in Lagunitas Creek, Marin County (Nielsen and Fountain 1999b; Nielsen 2003). Similar reports have demonstrated genetic population structure (mtDNA and microsatellite loci) for California’s resident trout and steelhead above and below natural or man-made barriers on Mokelumne River (Nielsen 1997a), Clavey River (Nielsen 1997b), Pinole Creek (Nielsen and Fountain 1999a), Stanislaus River (Nielsen et al. 1999), San Francisquito Creek (Nielsen 2000), San Mateo Creek (Nielsen and Sage 2002) and the Santa Ynez River (Nielsen et al. 2003).

This study represents genetic analyses of a diversity of samples of *O. mykiss*, i.e. fish collected above and below dams, putative natural spawning anadromous populations and hatchery trout strains found in the Central Valley, California. The California Department of Fish and Game (CDFG) and the US Fish and Wildlife Service (USFWS) collected samples, 1999 – 2003. Trout samples were analyzed for microsatellite allelic diversity at the USGS Alaska Science Center’s Conservation Genetics Laboratory. Genetic diversity was analyzed within and among samples and groups of samples at several spatial and temporal scales: 1) large river drainages; 2) year-to-year genetic diversity within selected trout populations where different year-class samples where available; 3) variation among localities where more than one locality was used as a collection source (especially in Clear Creek); 4) within sample genetic diversity was used for pairwise population genetic comparisons across broad spatial scales. We compared genotype and allelic frequencies for Clear Creek trout populations to data for a limited number of overlapping microsatellite loci from two hatchery trout strains (Mount Shasta and Crystal hatchery rainbow trout) with a history of stocking in the Central Valley. These hatchery samples were available from earlier genetic studies in the Nielsen laboratory.

This study used multiple sample locations within one drainage, Clear Creek, to test questions about fine-scale population structure for Central Valley trout populations. Spring Creek samples were collected by USFWS in an effort to provide inference about the genetic structure of native *O. mykiss* in the upper Sacramento River system. Spring Creek is a tributary to the upper Sacramento River that may have supported anadromous steelhead, but has been isolated from the influence of anadromous fish for a long period of time as a result of
Figure 1. Central Valley rivers and streams showing distribution of *O. mykiss* sample locations in relationship to impassable dams.
Figure 2. Map showing Clear Creek trout sample locations in relationship to impassable dams.
Table 1. Sample location, number = N (number in parenthesis is number sent to lab by collecting agency), collection year, and collecting agency for samples used in this study.

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<th>Year</th>
<th>Collector</th>
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<td>CDFG</td>
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</tr>
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<td>JLN</td>
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<td>Total Analyzed</td>
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mining pollution, and more recently, Keswick Dam. Additionally, stocking records do not indicate hatchery planting of domesticated rainbow trout into Spring Creek.

We compared genetic population structure derived from several sampling locations within two large river drainages in the Central Valley, the Sacramento and San Joaquin rivers. Finally, we looked at the genetic population structure for Central Valley trout as a whole, looking at relationships among and between all trout populations, and between hatchery and wild populations.

METHODS

Sample Collections

Trout fin tissue was collected and analyzed for DNA from 1570 fish in this study (Table 1). CDFG collected tissues from fish throughout the Central Valley, California, 2001-2003, for a broad scale analysis of genetic population structure (Figure 1). USFWS collected trout tissues from the Clear Creek drainage, American Trout & Salmon Company, and Spring Creek, 1999-2001 (Figure 2). This fine-scale sampling regime was designed to look at trout population above and below barriers and provide inference on potential native trout populations in the upper Sacramento River. Upper Clear Creek samples were collected above Whiskeytown Dam - a barrier to salmon migration for 40 years. A natural barrier to fish migration occurs in upper Clear Creek, near the confluence of Bear Creek (Kevin Niemela, USFWS Region 1, pers. comm.), so samples were taken above and below this barrier. Middle Clear Creek samples were collected below Whiskeytown Dam and above Saeltzer Dam, an infrequently passable barrier to fish migration. The Saeltzer Dam was removed in 2000. Samples collected in lower Clear Creek were taken below Saeltzer Dam in an area still accessible to anadromous steelhead. The contractual goal of these analyses was to provide genetic information on at least 40 individual fish per population for 10 microsatellite loci.

Deer and Mill creek trout samples were collected by both agencies independently at different times and locations, 1999-2001. Archival data from standardized microsatellite analyses of hatchery trout from the Mount Shasta and Crystal hatcheries were used in the Clear Creek study (JLN unpublished data).
Microsatellite Amplification Protocols

Microsatellite loci taken from the published literature were selected for analysis based on documented variability in *O. mykiss*, ease of amplification in polymerase chain reaction (PCR), and allele scoring rigor (Table 2). Table 3 gives the number of alleles found for each locus by population. G. K. Sage (Alaska Science Center, Conservation Genetics Laboratory) developed multiplex systems using 13 loci, grouped together for amplification of rainbow trout allelic size structure. Two protocols were utilized in the lab, made up of either three or four separate multiplex systems. A four multiplex protocol was used in the Clear Creek project (Table 4a), while a three multiplex protocol was used to collect data for the Central Valley project (Table 4b).

G. K. Sage redesigned Oneu10-F and Ots3-R primers as follows in order to incorporate them into the Clear Creek four-locus multiplex protocol. Oneu10-F was renamed Oneu10.1-F (5'-GGGAACAGAAGAGGAATAGC-3'), and Ots3-R was renamed Ots3.1-R (5'-GGTGGAGAGATTGAGAATCACA-3'). Oneu10-F, Ogo4-F, Ogo4-R and Ogo3-R were redesigned as follows for incorporation into the Central Valley three multiplex protocol. Oneu10-(F) was redesigned and renamed Oneu10.2 (F) (5'-TGTTGGCACCATTGTAACAG-3'), Ogo4-(F) became Ogo4.2 (F) (5'-CAGAATGAGTAACGAACGC-3'), Ogo4-(R) was renamed Ogo4.2 (R) (5'-GAGGATAGAAGAGTTTGGC-3'), and Ogo3-(R) was renamed Ogo3.2 (R) (5'-CACAATGGAAGACCAT-3'). Ogo1a, Ogo, and Oneu10 forward primers were modified by the addition of M13R tails, and Oneu8, Oneu11, and Ots3 were modified by the addition of M13F tails. All modifications were additions onto the 5' end and were utilized by both protocols. These tails allowed for allele fragment visualization by annealing to labeled complementary tails added to the PCR mix. The remaining loci were visualized by adding directly labeled forward primer. Allele sizes (from adapted primers) were standardized to single locus products by running known standards for allelic size for each locus on all multiplex gels.

In general, PCR reactions were conducted in 10μl volumes using approximately 50ng of genomic DNA, 0.1-0.2 U of DNA polymerase (Perkin Elmer), 10mM Tris-HCl (pH 8.3), 1.5mM MgCl₂, 50mM KCl, 0.01% each of gelatin, NP-40, and Triton X-100, and 200μm each dNTP. For all loci that utilized direct labeled primers for product visualization, the total of forward (F) and reverse (R) primers per locus per reaction equaled four pmoles, with the F primer concentration being a combination of labeled and unlabeled primer. Tailed F and R primer
Table 2. List of microsatellite loci used in this study of steelhead/rainbow trout (*Oncorhynchus mykiss*). Number in parentheses is the number of alleles found in the Clear Creek watershed for this study. Mean Hz = mean observed heterozygosity for this locus in 23 populations from throughout the Central Valley drainage.

<table>
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<tr>
<th>Locus</th>
<th>Source</th>
<th>Number of Alleles</th>
<th>Allelic Size Range (bp)</th>
<th>Mean Hz</th>
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<td>Omy27</td>
<td>Heath et al. 2001</td>
<td>8 (5)</td>
<td>99 – 115</td>
<td>0.66</td>
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<tr>
<td>Omy77</td>
<td>Morris et al. 1996</td>
<td>28 (17)</td>
<td>77 – 143</td>
<td>0.80</td>
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<tr>
<td>Omy207</td>
<td>O’Connell et al. 1997</td>
<td>24 (20)</td>
<td>97 – 165</td>
<td>0.66</td>
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<tr>
<td>Omy325</td>
<td>O’Connell et al. 1997</td>
<td>33 (20)</td>
<td>83 – 167</td>
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<td>Ogo1a</td>
<td>Olsen et al. 1998</td>
<td>12 (4)</td>
<td>122 – 168</td>
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<tr>
<td>Ogo4</td>
<td>Olsen et al. 1998</td>
<td>16 (12)</td>
<td>116 – 148</td>
<td>0.76</td>
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<tr>
<td>Oneµ8</td>
<td>Scribner et al. 1996</td>
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<td>150 – 190</td>
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<tr>
<td>Oneµ10.1 &amp; 10.2</td>
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<td>11 (8)</td>
<td>113 – 139</td>
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<td>Oneµ11</td>
<td>Scribner et al. 1996</td>
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<td>Oneµ14</td>
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<td>101 – 137</td>
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Table 3. Number of alleles found for each locus given by population.

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<th>Omy27</th>
<th>Omy77</th>
<th>Omy326</th>
<th>One10</th>
<th>One11</th>
<th>Ots1</th>
<th>Ots3</th>
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Table 4. Multiplex systems used to amplify 13 microsatellite loci on two profiles for amplification of DNA from Central Valley trout on the LI-COR automatic sequencer. Additional primer modifications made to enhance these multiplexes are given in the text. The columns “700” and “800” represent different dyes used on the LI-COR platform.

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<td>Omy27</td>
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<td></td>
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<td>Omy207</td>
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concentrations for both Clear Creek and Central Valley multiplex systems were as follows: $One_{\mu}10$ (10 pmoles), $Ogo1a$, $Ogo4$, $One_{\mu}11$, $Ots3$ (5 pmoles) and $One_{\mu}8$ (1 pmole).

The following amounts of labeled primers were added in each of the four Clear Creek multiplex system. Multiplex A had between 0.06-0.20 pmoles per reaction ($Omy325$, 0.06; $Ots1$, 0.20; $One_{\mu}14$, 0.40; $Ots4$, 0.06). Multiplex B was between 0.10-0.75 pmoles ($Omy77$, 0.20; M13F, 0.30; M13R, 0.75). Multiplex C had between 0.10-1.50 pmoles ($Omy27$, 0.10; M13F, 1.50; M13R, 0.75). The labeled primer for multiplex D was between 0.30-2.00 pmoles ($Omy207$, 0.30; M13F, 0.50; M13R, 2.00). The following amounts of labeled primers were added in each of the three Central Valley multiplex systems. Multiplex A was the same as used for Clear Creek. Multiplex B was between 0.10-1.5 pmoles ($Omy77$, 0.2; M13F, 0.3; M13R, 1.5), and multiplex C had between 0.1-1.5 pmoles (M13F, 1.5; M13R, 1.5; $Omy27$, 0.1; $Omy207$, 0.2).

Gel electrophoresis and visualization of microsatellite alleles was performed using LI-COR Model 4200 and IR2 automated fluorescent DNA Sequencers and sizing was performed using V3.00 Gene ImagIR (LI-COR, Lincoln, NE, USA). Microsatellite allele sizes (including the amplified primer) were determined in relation to the M13 ladder or to the genescan-500 internal size standard (P-E Biosystems, Foster City, CA, USA), and rainbow trout DNA samples of known size that were rerun on each gel. Approximately 10% of all samples were run on a second gel and scored independently to verify allelic size.

Genetic Analyses

Genetic data were analyzed using a variety of software from different statistical packages including ARLEQUIN (Schneider et al. 2000), BOTTLENECK (Piry et al. 1999), CONENSENCE and NEIGHBOR from PHYLIP (Felsenstein 1993), and GENEPOP version 3.3 (Raymond and Rousset 1997). Heterozygosity, genetic disequilibrium, and simulated Fisher's exact tests using randomizations for Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP. Tests of HWE were performed to look at the performance of different loci among these trout populations to gain inference on population structure. It is well known that two populations that are in HWE independently may not be so when they are combined (Hartl 1988). There are several assumption built into HWE that cannot be supported without
Table 5. Hardy-Weinberg equilibrium (HWE) results for 11 loci showing populations within HWE "-", and out of HWE "+" based on exact tests performed by GENEPOP.

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<th>Ogo4</th>
<th>Omy27</th>
<th>Omy77</th>
<th>Omy325</th>
<th>Ome18</th>
<th>Ome10</th>
<th>Ome11</th>
<th>Ots1</th>
<th>Ots3</th>
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<tr>
<td>26 Stanislaus River - upper below Beardsley Dam</td>
<td></td>
<td>49</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>27 Stanislaus River - lower below Goodwin Dam</td>
<td></td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>28 Stoney Creek</td>
<td></td>
<td>63</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>29 Tuolumne River - upper above Don Pedro Reservoir</td>
<td></td>
<td>47</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>30 Tuolumne River - below La Grange Dam</td>
<td></td>
<td>45</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>31 Yuba River – Oregon, Lavazzola, Pauley and Canyon creeks</td>
<td></td>
<td>58</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>32 Yuba River - below Englebright Dam</td>
<td></td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

HWE TOTAL by Locus 25 27 29 25 22 24 25 26 24 21 24
additional knowledge of the demographics of these populations, i.e. non-overlapping populations (age class structure for these samples included adults of different age and juveniles), random mating, negligible migration (natural and artificial movement above and below dams can be undocumented or inconclusive), etc. Most importantly the assumptions that mutation can be ignored and that natural selection does not affect alleles under consideration for HWE are hard to support in studies involving microsatellite loci where we know so little about the mutation processes involved.

ARLEQUIN version 1.1 FSTAT pairwise comparisons were used to test for differences in allele frequencies between and among populations. Statistical significance levels for allelic frequency comparisons were set using sequential Bonferroni tests (Rice 1989). Partitioning of microsatellite allelic variation based on analysis of molecular variance (AMOVA) was performed using ARLEQUIN. Detection of recent reductions in population size using microsatellite data were performed on Central Valley samples using Garza and Williamson’s $M$ (2001). Effective population size ($N_e$) estimates based on microsatellite data were made under the assumption of mutation-drift equilibrium using the Single-Step Mutation Model (SSM) and the Infinite Allele Model (IAM) with a mutation rate of $2.05 \times 10^{-4}$ (Garza and Williamson 2001).

Genetic distance values reflecting the proportion of shared alleles between individuals and groups of individuals can be used to graphically depict genetic relationships and population structure. A unrooted Neighbor-Joining tree (NJ), based on Cavalli-Sforza chord genetic distances (1967), was generated using a program written by J. Cornuet (INRA, Laboratorie de Neurobiologie comparee des invertebres, Bures-sur Yvette, France). Genetic distance was determined from the NEIGHBOR application PHYLIP version 3.57c (Felsenstein 1993) using the Cavalli-Sforza and Edwards chord distance matrix. Genetic relationships depicted in our consensus NJ tree were tested using random bootstrap replications ($n = 2000$; Felsenstein 1985) to assess the reproducibility of branching patterns. The program WHICHLOCi was used to rank the
microsatellite loci used in this study based on their relative allelic differential derived from Central Valley trout populations (Banks and Eichert 2000).

RESULTS

Microsatellite Loci

GENEPOP’s analyses of expectation of HWE gave mixed results among the microsatellite loci and trout populations in this study. GENEPOP’s deviations from HWE were primarily due to heterozygote excess. Heterozygote deficiency was found at individual loci in some populations: American Trout & Salmon Co. (Ots1); lower Clear Creek both 1999 and 2000 samples (Ogo1a); Clear Creek below Bear Creek (Ots1); Cottonwood Creek (Ogo4); Nimbus Hatchery (Ogo1a); lower Stanislaus River (Ots4); upper Yuba River (Ots1). Only the sample taken below Keswick Dam on the Sacramento River (USFWS) carried one than one locus (Oneμ10, Ots1, and Ots3) with heterozygote deficiency based on GENEPOP’s analyses.

Two loci (Omy207 and Oneμ14) were found to be out of HWE in over 80% of the sample populations and were dropped from any further analyses. Two sample populations fell significantly out of HWE (p > 0.025) for the remaining 11 loci combined. Spring Creek trout samples (N = 53) were monomorphic for one allele at all but two loci (Ogo4 and Oneμ8) with only two alleles each. The upper Yuba River, including samples from Canyon, Lavezzola, Oregon, and Pauley creeks, also had only two loci in HWE (Omy27 and Oneμ11; HWE p = 0.0007), but these samples were polymorphic at the other 9 loci. We judged this variation to be informative and retained the upper Yuba River trout population in subsequent analyses. Both Deer Creek samples collected by USFWS (1999) and CDFG (2001) were found to be within HWE when analyzed independently, but fell out of HWE when these samples were combined (HWE p = 0.004).

Optimal locus combinations provided population assignments among trout populations in the Central Valley. Following the “leave-one-out” approach for reassignment, WHICHLOCI indicated that all 11 loci were needed for 83% reassignment accuracy. However, caution is advised in consideration of this
value since the assignment accuracy of individuals back to their population of origin maybe inflated due to the lack alternative baseline data outside of those generated by this study. Loci were ranked according to their relative contribution to the analyses of allelic frequency differences among populations (Table 6).

**Year-to-Year Sample Locations**

Trout fin clips were collected for genetic analyses by both USFWS (1999) and CDFG (2001) on Deer and Mill creeks (Table 2). This allowed us to test for population differentiation within each creek for different sampling periods. Allelic frequency for the 11 microsatellite loci in Deer Creek 1999 differed significantly from the 2001 sample at only one locus – Ots1. Mill Creek 1999 differed significantly from Mill Creek 2001 at two loci – Ogo4 and Omy27. However, trout population genetic structure on both Deer Creek (Chi² = 30.36; df = 22; p = 0.11) and Mill Creek (Chi² = 36.59; df = 22; p = 0.03) did not vary significantly year-to-year over this sampling period when all loci were combined. ARLEQUIN’s population pairwise $F_{st}$ values between sample collections for Deer Creek was $F_{st}$ = -0.006 and for Mill Creek was $F_{st}$ = 0.001. Therefore, we combined these two samples for subsequent analyses.

We were also sent samples collected from the upper Sacramento River below Keswick Dam from both USFWS and CDFG. Allelic frequencies for all 11 loci were not significantly different in comparisons of these two samples (Chi² = 20.24; df = 22; p = 0.57). Therefore, we combined these two collections in subsequent analyses.

**Clear Creek Genetic Population Structure**

We visualized allelic diversity at 11 microsatellite loci for 107 trout from the upper Clear Creek drainage, 31 fish from the middle drainage below Whiskeytown Dam, and 89 fish from the lower drainage (Table 1). The average number of alleles per locus found throughout Clear Creek trout was 6.7. Average heterozygosity ($Hz$) for Clear Creek trout populations was $Hz$ = 0.63.
Trout Populations Above and Below Bear Creek

ARLEQUIN’s population pairwise comparison found significant differences in allelic frequencies for upper-basin trout above and below Bear Creek ($F_{st} = 0.106$) and GENEPOP (Fisher’s method) analysis of the same comparison was highly significant ($\chi^2 = \text{infinity}; \ df = 22$). The trout population above Bear Creek has two loci with heterozygosity deficiency and nine loci with heterozygosity excess. The trout population below Bear Creek had four loci with heterozygosity deficiency and seven loci with heterozygosity excess. However, BOTTLENECK demonstrated strong support for the assumption that both populations fit the Single-Step Mutation Model (SMM) for all 11 microsatellite loci combined (above $p = 0.02$; below $p < 0.00$). Effective population size ($Ne$) calculated by Garza and Williamson’s (2001) program for $M$ based on the SMM was $Ne = 3088$ trout above and $Ne = 3632$ trout below Bear Creek.

Trout Above and Below Whiskeytown Dam

No significant differences in allelic frequencies were found for the two years of trout samples sent from the lower Clear Creek drainage below Sealtzer Dam, 1999 and 2001 ($F_{st} = 0.016$). Significant genetic differentiation was found between trout collected in the upper Clear Creek drainage (above and below Bear Creek) and fish collected below Whiskeytown Dam and above Sealtzer Dam (i.e. Clear Creek middle; above $F_{st} = 0.102$; below $F_{st} = 0.068$). Significant frequency differences were also found comparing fish above Whiskeytown Dam and trout in the lower drainage below Sealtzer Dam (i.e., lower Clear Creek; 1999 $F_{st} = 0.145$; 2001 $F_{st} = 0.179$). Middle and lower Clear Creek trout populations were not significantly different based on population pairwise $F_{st}$ analyses ($F_{st} = 0.01$).

Clear Creek Populations and Hatchery Trout

Coleman National Fish Hatchery

Significant frequency differences across all 11 loci were found in pairwise comparisons between Coleman National Fish Hatchery (CNFH) trout and trout
collected above Bear Creek ($F_{st} = 0.12$), and CNFH and trout collected below Bear Creek ($F_{st} = 0.08$). $F_{st}$ values calculated from allelic frequencies at all 11 loci were not significantly different for comparisons among trout from CNFH and trout from lower Clear Creek ($F_{st} = 0.01$), middle Clear Creek ($F_{st} = 0.02$). Population pairwise comparisons showed no significant differences in allelic frequencies between trout from CNFH and trout from the upper Sacramento River ($F_{st} = 0.02$).

**Rainbow Trout Hatchery Strains**

Only two microsatellite loci (Omy77 and Omy27) used in this study overlapped with previous microsatellite studies of California hatchery rainbow trout (JLN unpublished data). We used these loci to compare Clear, Mill, Deer, and Spring creeks with hatchery rainbow trout from Crystal, Mount Shasta, and American Salmon and Trout Company hatchery strains (Table 7). Putatively sterile (triploid) fish from the American Trout and Salmon Company have been regularly stocked for several years into the middle reach of Upper Clear Creek as part of a put-and-take, pay-for-access sport fishery. No significant differences in allelic frequencies for these microsatellite loci were found in comparisons of hatchery trout from the American Salmon and Trout Company and the Crystal Hatchery strain ($F_{st} = 0.01$). Allelic frequencies were significantly different in comparisons made between upper Clear Creek trout and hatchery rainbow trout from the American Salmon and Trout Company, Mount Shasta and Crystal hatchery strains. CNFH, the upper Sacramento River, lower Clear Creek, and middle Clear Creek trout allelic frequencies were not significantly different using the two-locus comparison and when compared at all 11 loci combined.

**Clear Creek Analysis of Molecular Variance**

Pairwise comparisons suggested we examine one hypothesis on the distribution of genetic diversity found in Clear Creek in relationship to other local population groups. AMOVA analyses of the trout from upper Clear Creek (above and below Bear Creek; Group 1), the lower Clear Creek drainage (Clear Creek
middle, Clear Creek lower '99 and '01; Group 2), Coleman National Fish Hatchery and the mainstem upper Sacramento River (Group 3), and Deer and Mill creeks (Group 4) showed that 91.1% of the microsatellite allelic variation was found within populations; 2.5% was found among populations within the groups; 6.4% of the variation was found among the groups.

**Spring Creek**

Spring Creek heterozygosity for the 11 microsatellite loci was $Hz = 0.048$. Spring Creek trout carried on average only 1.18 alleles per locus for the 11 loci. Garza and Williamson’s (2001) $M$ for Spring Creek trout was $M = 1.00$ and this population was monomorphic at 9 of the 11 loci. More than 1 allele was found only at loci Ogo4 and One$_\mu$8. Spring Creek $F_{st}$ population pairwise comparisons

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**Table 6. Microsatellite loci rank using allele frequency differential method from WHICHLOCi (Banks and Eichert 2000).**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Locus</th>
<th>Score</th>
<th>% Relative Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Omy325</td>
<td>139.474</td>
<td>14.165</td>
</tr>
<tr>
<td>2</td>
<td>Omy77</td>
<td>114.071</td>
<td>11.585</td>
</tr>
<tr>
<td>3</td>
<td>Ots1</td>
<td>109.722</td>
<td>11.143</td>
</tr>
<tr>
<td>4</td>
<td>Ots4</td>
<td>98.694</td>
<td>10.023</td>
</tr>
<tr>
<td>5</td>
<td>Ogo4</td>
<td>89.510</td>
<td>9.09</td>
</tr>
<tr>
<td>6</td>
<td>One$_\mu$8</td>
<td>87.920</td>
<td>8.929</td>
</tr>
<tr>
<td>7</td>
<td>Ogo1</td>
<td>83.481</td>
<td>8.478</td>
</tr>
<tr>
<td>8</td>
<td>One$_\mu$10</td>
<td>75.921</td>
<td>7.71</td>
</tr>
<tr>
<td>9</td>
<td>Ots3</td>
<td>75.768</td>
<td>7.695</td>
</tr>
<tr>
<td>10</td>
<td>Omy27</td>
<td>67.291</td>
<td>6.834</td>
</tr>
<tr>
<td>11</td>
<td>One$_\mu$11</td>
<td>42.805</td>
<td>4.347</td>
</tr>
</tbody>
</table>
Table 7. Pairwise $F_{st}$ comparisons between rainbow trout hatchery populations and Clear Creek trout collections. Pairwise $F_{st}$ values are given below the diagonal and the matrix of significant $F_{st}$ $P$ values ("*" = significant pairwise $F_{st}$ values) is given above the diagonal.

<table>
<thead>
<tr>
<th>Population</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Crystal Hatchery</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 Mount Shasta Hatchery</td>
<td>0.018</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3 Deer Creek</td>
<td>0.101</td>
<td>0.118</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4 Mill Creek</td>
<td>0.069</td>
<td>0.064</td>
<td>0.025</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 American Salmon &amp; Trout Co.</td>
<td>-0.005</td>
<td>0.022</td>
<td>0.139</td>
<td>0.091</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6 Upper Sacramento River</td>
<td>0.083</td>
<td>0.098</td>
<td>0.096</td>
<td>0.049</td>
<td>0.127</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7 Coleman National Fish Hatchery</td>
<td>0.072</td>
<td>0.090</td>
<td>0.093</td>
<td>0.046</td>
<td>0.109</td>
<td>-0.015</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8 Clear Creek - lower below Sealtzer Dam</td>
<td>0.110</td>
<td>0.127</td>
<td>0.144</td>
<td>0.078</td>
<td>0.141</td>
<td>-0.006</td>
<td>0.002</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9 Clear Creek - middle below Wiskeytown Dam</td>
<td>0.045</td>
<td>0.093</td>
<td>0.041</td>
<td>0.039</td>
<td>0.090</td>
<td>0.017</td>
<td>0.013</td>
<td>0.043</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 Clear Creek - upper above &amp; below Bear Cr.</td>
<td>0.160</td>
<td>0.131</td>
<td>0.092</td>
<td>0.080</td>
<td>0.194</td>
<td>0.096</td>
<td>0.121</td>
<td>0.169</td>
<td>0.131</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11 Spring Creek</td>
<td>0.617</td>
<td>0.509</td>
<td>0.532</td>
<td>0.551</td>
<td>0.645</td>
<td>0.709</td>
<td>0.554</td>
<td>0.622</td>
<td>0.672</td>
<td>0.374</td>
<td>+</td>
</tr>
</tbody>
</table>


Figure 3. Unrooted Neighbor–Joining tree based on Cavalli-Sforza and Edwards chord distance for the Clear Creek drainage trout populations. Branch bootstrap values (2000 replicate trees) are provided.
ranged from $F_{st} = 0.37$ (Spring Creek and upper Clear Creek) to $F_{st} = 0.71$ (Spring Creek and the upper Sacramento River trout population). Effective population size ($Ne$; Garza and Williamson 2001) based on the SMM was $Ne = 62$ trout in Spring Creek (IAM $Ne = 61$). Because of the highly bottlenecked condition of this population we excluded this group from subsequent analyses of Central Valley populations.

_Clear Creek Genetic Distance_

An unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the Clear Creek drainage is presented in Figure 3. Branch bootstrap values (2000 replicate trees) are provided in this figure. Genetic distance values demonstrate the clear distinction between upper Clear Creek trout (collected above both dams in the vicinity of Bear Creek) and trout collected from the lower and middle sections of this drainage below one or two impassable dams. Genetic distance analysis also supported the close genetic association found among fish from Coleman National Fish Hatchery, upper Sacramento River, and the middle and lower Clear Creek drainage where branch bootstrap values ranged between 12% and 39%.

_Central Valley Genetic Population Structure_

We visualized allelic diversity at 11 microsatellite loci for trout collected from 13 rivers and streams in the Sacramento River drainage, four rivers in the San Joaquin River drainage, one rainbow trout hatchery strain (the American Trout & Salmon Company), and three Central Valley hatchery populations for our basin-wide genetic analyses (Table 1). Due to the demonstrated population genetic differences found on Clear Creek (see above), we included trout from upper Clear Creek (above and below Bear Creek samples combined) and trout from lower Clear Creek (below Whiskeytown Dam) as two independent samples in our basin-wide analyses. The mean number of alleles per locus ranged from 5.6 (upper Clear Creek) to 10.5 (Deer Creek). The mean number of alleles per
locus over all populations was 7.9. Average heterozygosity for the 11 microsatellite loci in Central Valley steelhead was $Hz = 0.68$.

*Trout Collected at Two Locations On the Same River*

Samples were collected for genetic analyses at two locations (upper and lower) on the American, Yuba, Stanislaus, and Tuolumne rivers within the Central Valley. Pairwise comparisons of allelic frequencies within each of these rivers were significant: American River $F_{st} = 0.109$; Yuba River $F_{st} = 0.048$; Stanislaus River $F_{st} = 0.081$; Tuolumne River $F_{st} = 0.0476$, suggesting some degree of genetic separation within these rivers, however, no significant differences were found for $N_e$ or $M$ among these populations.

*Central Valley Pairwise Population Comparisons*

Pairwise $F_{st}$ values indicating no significant genetic differentiation ($F_{st} \ P \geq 0.05$) between populations are given in Table 8. All other pairwise comparisons supported significant allelic frequency differentiation between pairs of Central Valley trout populations.

*Central Valley Ne and Bottleneck Analyses*

Garza and Williamson’s (2001) $M$ demonstrates a recent reduction in population, i.e. a population bottleneck, when $M \leq 0.68$. In tests of Central Valley trout populations mean $M$ across all 11 microsatellite loci was less than 0.68 in all populations with three exceptions, Coleman National Fish Hatchery ($M = 0.682$), Deer Creek ($M = 0.682$), and the upper Sacramento River ($M = 0.703$; Table 9). Garza and Williamson’s (2001) $M$ estimates of effective population size assuming mutation-drift equilibrium and a mutation rate of $2.05E^{-4}$ for both SSM) and IAM are given for trout populations in the Central Valley in Table 9. Probabilities calculated under the assumption that all loci meet expectations for mutation-drift equilibrium using three models (heterozygote ($Hz$) deficiency (one tailed); $Hz$ excess (one tailed); two tails $Hz$ excess and deficiency) using the program BOTTLENECK are given for the Central Valley trout populations in Table 10.
Table 8. Fst pairwise comparisons indicating no significant genetic
differentiation (P >0.05) between trout populations within the Central
Valley based on allelic frequencies for 11 microsatellite loci.

<table>
<thead>
<tr>
<th>Population</th>
<th>Population</th>
<th>Pairwise Fst</th>
<th>Fst P</th>
</tr>
</thead>
<tbody>
<tr>
<td>American River lower</td>
<td>Nimbus Hatchery</td>
<td>0.009</td>
<td>0.065</td>
</tr>
<tr>
<td>Antelope Creek</td>
<td>Clear Creek lower</td>
<td>0.014</td>
<td>0.051</td>
</tr>
<tr>
<td>Antelope Creek</td>
<td>Cottonwood Creek</td>
<td>0.011</td>
<td>0.079</td>
</tr>
<tr>
<td>Battle Creek</td>
<td>Cottonwood Creek</td>
<td>0.003</td>
<td>0.250</td>
</tr>
<tr>
<td>Clear Creek lower</td>
<td>Cottonwood Creek</td>
<td>0.002</td>
<td>0.268</td>
</tr>
<tr>
<td>Clear Creek lower</td>
<td>Sacramento River upper</td>
<td>0.011</td>
<td>0.078</td>
</tr>
<tr>
<td>Coleman Fish Hatchery</td>
<td>Sacramento River upper</td>
<td>0.007</td>
<td>0.092</td>
</tr>
<tr>
<td>Feather River</td>
<td>Feather River Hatchery</td>
<td>-0.007</td>
<td>0.882</td>
</tr>
<tr>
<td>Kings River</td>
<td>Stoney Creek</td>
<td>0.015</td>
<td>0.059</td>
</tr>
<tr>
<td>Stanislaus R. upper</td>
<td>Middle Fork American R.</td>
<td>0.001</td>
<td>0.345</td>
</tr>
<tr>
<td>Stanislaus R. lower</td>
<td>Battle Creek</td>
<td>0.006</td>
<td>0.113</td>
</tr>
<tr>
<td>Stanislaus R. lower</td>
<td>Feather River</td>
<td>0.009</td>
<td>0.055</td>
</tr>
<tr>
<td>Yuba River lower</td>
<td>Battle Creek</td>
<td>0.016</td>
<td>0.052</td>
</tr>
<tr>
<td>Yuba River lower</td>
<td>Cottonwood Creek</td>
<td>0.017</td>
<td>0.050</td>
</tr>
<tr>
<td>Yuba River lower</td>
<td>Stanislaus R. lower</td>
<td>0.011</td>
<td>0.064</td>
</tr>
</tbody>
</table>
Table 9. Effective population size ($Ne$) based on the SSM and IAM models and Garza and Williamson's (2001) $M$ calculated for Central Valley trout populations across all loci.

<table>
<thead>
<tr>
<th>Drainage</th>
<th>Population</th>
<th>SSM</th>
<th>IAM</th>
<th>$M$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ne</td>
<td>Ne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacramento River</td>
<td>American River Middle Fork</td>
<td>5844</td>
<td>2748</td>
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<td>American River lower</td>
<td>4380</td>
<td>2269</td>
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<td>2628</td>
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<td>2481</td>
<td>0.648</td>
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<td>1997</td>
<td>0.526</td>
</tr>
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<td>2524</td>
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<td></td>
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<td>3670</td>
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</tr>
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<td></td>
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<td>Stanislaus River upper</td>
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<tr>
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<td>2703</td>
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<td></td>
<td>Tuolumne River upper</td>
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</tr>
<tr>
<td></td>
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<td>4669</td>
<td>2369</td>
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<td>Overall estimate</td>
<td>5066</td>
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Table 10. BOTTLENECK's mutation drift equilibrium probabilities under the heterozygote deficient (HZD), heterozygote excess (HZE), and two-tailed deficiency and excess (TTM) models for Central Valley trout populations based on all 11 microsatellite loci combined.

<table>
<thead>
<tr>
<th>Population</th>
<th>Model</th>
<th>Model</th>
<th>Model</th>
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<tbody>
<tr>
<td></td>
<td>HZD</td>
<td>HZE</td>
<td>TTM</td>
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<td>American River - Middle Fork</td>
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<td>0.82</td>
<td>0.41</td>
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<td>0.01</td>
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<td>0.01</td>
</tr>
<tr>
<td>Antelope Creek</td>
<td>0.10</td>
<td>0.91</td>
<td>0.21</td>
</tr>
<tr>
<td>Battle Creek</td>
<td>0.23</td>
<td>0.79</td>
<td>0.46</td>
</tr>
<tr>
<td>Clear Creek upper</td>
<td>0.62</td>
<td>0.42</td>
<td>0.83</td>
</tr>
<tr>
<td>Clear Creek lower</td>
<td>0.74</td>
<td>0.29</td>
<td>0.58</td>
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<td>Coleman National Fish Hatchery</td>
<td>0.68</td>
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<td>0.53</td>
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<tr>
<td>Cottonwood Creek</td>
<td>0.12</td>
<td>0.90</td>
<td>0.24</td>
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<tr>
<td>Calaveras River</td>
<td>0.18</td>
<td>0.84</td>
<td>0.36</td>
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<td>Deer Creek</td>
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<td>0.23</td>
<td>0.46</td>
</tr>
<tr>
<td>Mill Creek</td>
<td>0.10</td>
<td>0.91</td>
<td>0.21</td>
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<td>Nimbus Hatchery</td>
<td>0.07</td>
<td>0.93</td>
<td>0.15</td>
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<td>Putah Creek</td>
<td>0.79</td>
<td>0.23</td>
<td>0.46</td>
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<tr>
<td>Sacramento River - upper</td>
<td>0.10</td>
<td>0.91</td>
<td>0.21</td>
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<tr>
<td>Stanislaus River - upper</td>
<td>0.09</td>
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<td>Stoney Creek</td>
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<tr>
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<td>Yuba River - upper</td>
<td>0.71</td>
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<td>0.64</td>
</tr>
<tr>
<td>Yuba River - lower</td>
<td>0.12</td>
<td>0.90</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Figure 4. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the Central Valley system derived from allelic variation at 11 microsatellite loci. Branches with bootstrap values (2000 replicate trees) are provided.
Only one population, Feather River Hatchery, showed a balanced, two-tailed $Hz$ distribution.

**Central Valley Analysis of Molecular Variance**

Analysis of molecular variance (AMOVA) of microsatellite diversity for the entire Central Valley collection partitioned allelic variance into 11.33% among populations and 88.67% within populations. The same analyses of the Central Valley divided into its two primary drainages, i.e. the Sacramento and San Joaquin rivers, distributed the allelic variance into 0.13% between the drainages, 7.48% among populations within the drainages, and 92.39% of the variance was found within individuals within populations.

**Central Valley Genetic Distance**

A consensus Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the entire Central Valley system is presented in Figure 4. Bootstrap values (2000 replicate trees) are provided for all branches in this figure.

**DISCUSSION**

This study focused on the current genetic population structure of Central Valley steelhead and rainbow trout populations at two scales: a fine-scale analysis of trout found within the Clear Creek drainage to look for potential wild trout in an upper Sacramento River tributary by analyzing drainage population structure and test hypotheses related to the credibility of specific localities as native strains; and an analysis of the current population genetic structure found throughout the entire Central Valley relating hatchery and putative wild populations, and populations found above and below barriers within the system. We examine implications derived from each of these scales independently and then together.
Significant genetic population structure was documented within the Clear Creek drainage with these analyses. Trout sampled in upper Clear Creek (above and below Bear Creek) carried significantly different allelic frequencies for all 11 microsatellite loci from fish collected below Sealtzer Dam in the lower drainage. Upper Clear Creek trout were also significantly differentiated from hatchery trout from the Coleman National Fish Hatchery and trout collected from the American Trout and Salmon Company.

Our analyses of hatchery rainbow trout in comparison with Clear Creek populations were less rigorous due to the limited overlap in standardized microsatellite loci available from past studies. This was primarily due to the fact that the original microsatellite analyses done in the Nielsen laboratory for hatchery trout were performed during the development and early application of microsatellite loci to fisheries issues. The numbers of loci available for such analyses at that time were extremely limited compared to what is available now. We have learned a lot about microsatellite loci in the last 6 years and have developed complex multiplex systems that do not always overlap with our earlier efforts. Standardization of microsatellite data for individual loci across amplification and visualization platforms is time consuming and costly. Our limited analyses did show significant differences among Mount Shasta Hatchery trout, the Crystal hatchery rainbow trout strain, and upper Clear Creek trout at two microsatellite loci, Omy77 and Omy27. These two overlapping loci, however, were highly polymorphic and have demonstrated significant population structure in other hatchery/wild comparisons in trout (Nielsen 1996a, b; Nielsen et al. 1997). We recommend that new, more rigorous sampling with additional temporal replicates and more overlapping microsatellite loci be incorporated in future analyses of hatchery rainbow trout in California.

A review of stocking records in upper Clear Creek indicates that the vast majority of fish plantings have originated from the Mount Shasta Hatchery and secondarily from the Darrah Springs Hatchery (K. Niemela, USFWS Region 1 pers. comm.); both facilities are operated by the California Department of Fish
and Game. Darrah Springs Hatchery is thought to rear Mount Shasta, Eagle River and Hot Creek (Coleman) rainbow trout strains. Limited, unstandardized microsatellite data are available on three loci (Omy77, Omy207, and Omy289) for trout from these three hatchery strains (Nielsen et al. 1997), however, no microsatellite data are currently available that are specific to Darrah Springs fish. Previous comparisons of hatchery rainbow trout using mtDNA sequence data showed limited differentiation in haplotype frequencies among these three hatchery stocks (Mount Shasta, Hot Creek, and Whitney strains; Nielsen 1996a & b).

This is not unexpected since most California hatchery rainbow trout are derived from the original Mount Shasta strain (Busack and Gall 1980). Common ancestral source populations for Mount Shasta Hatchery stock from the McCloud River when it was still a tributary to the Sacramento River make mtDNA sequence even less rigorous in comparisons between hatchery and wild trout in the Sacramento River drainage. A comparison of mtDNA haplotypes within the Clear Creek drainage may add inference on the direction of gene flow from hatchery fish to naturally spawning trout populations. However, these results will be confounded by the fact that the most common haplotypes (MYS1 and MYS3) found in trout in the Sacramento River system are the same for both hatchery and wild fish. Only rare haplotypes will allow comparisons. As far as we know no rigorous molecular marker has been identified that can clearly differentiate hatchery from wild *O. mykiss* in systems where the hatchery fish were originally derived from local wild stocks despite the fact that the hatchery fish have been in husbandry for over 100 years, as in the case of the Mount Shasta Hatchery strain.

The fact that upper Clear Creek trout were also significantly different from trout collected in Deer and Mill creeks suggests that putative anadromous origins for upper Clear Creek populations deserves further study. No significant genetic difference was found among trout populations collected in the lower Clear Creek drainage, below Whiskeytown Dam. Lower Clear Creek trout populations could not be differentiated from the Coleman National Fish Hatchery stock or from fish
captured in the upper Sacramento River, suggesting significant gene flow has occurred among these populations.

The Spring Creek trout population sampled for this study was severely bottlenecked with limited allelic diversity found at only two loci and an estimated effective population size of 62. We cannot speculate on the cause of this bottleneck without further information on the history of this population. This extreme bottleneck condition does, however, suggest that this population is not a good candidate to contribute to restoration within the Clear Creek drainage due to a lack of genetic diversity. Consideration of genetic impacts of low effective population size in both the donor and recipient populations would have to be included in any management decisions to remove or transfer Spring Creek fish.

**Central Valley**

Significant steelhead genetic population structure was found throughout the Central Valley. Pairwise population comparisons showed significant differentiation in all but 2% of the population-pair comparisons. Genetic diversity and regional structuring of population genetic variation developed from the 11 microsatellite loci were in the same general range of values published in previous studies of Pacific steelhead (Beacham et al. 1999; Heath et al. 2001, 2002).

Estimates of effective population size based on SSM ranged from $N_e = 3632$ (upper Clear Creek) to $N_e = 7237$ (Stoney Creek), with a mean $N_e = 5066$, excluding Spring Creek where $N_e = 62$. Estimates of effective population size based on a single-step-mutation model for microsatellites should be viewed with caution. Immigration, as a result of hatchery propagation, will serve to depress the estimate of $M$ and inflate the estimate of effective population size (P. Moran, NMFS Seattle, WA, pers. comm.) There is no established standard for population viability based on estimates of effective population size. The true relationship between $N_e$ and actual census numbers of adult steelhead in the Central Valley is unknown. This parameter, however, has considerable relative value because it may reflect the scale of variation in reproductive success within and between systems and can give insight into the relationship between census
population size and the number of effective breeders (Frankham 1995; Heath et al. 2002). Small effective population size is expected to lead to potentially high rates of genetic drift and higher expectations of population extinction (Newman and Pilson 1997). However, recent studies suggest that the predictive value of Ne on genetic diversity is somewhat speculative since small population size coupled with increased genetic drift may actually lead to increased genetic diversity at neutral alleles through a mechanism called “founder flush” (Williamson and Slatkin 1999; Nielsen 1999; Hansen et al. 2002; Ardren and Kapuscinski 2003). A comparison of the patterns of demographic estimates for steelhead within the Central Valley and estimates of effective population size over time (using DNA analyses from archived scales) could be informative for future conservation strategies.

Many of the Central Valley steelhead population pairs showing genetic similarity in microsatellite allelic frequencies were not surprising, such as Nimbus Hatchery and the lower American River, Coleman National Fish Hatchery and the upper Sacramento River, and the Feather River Hatchery and trout from the Feather River. These data suggest genetic similarities among hatchery populations and geographically proximate trout populations with high levels of gene flow. There are several hypotheses that could have contributed to this relationship which are not necessarily independent or exclusive. Gene flow among these populations may be high due to the straying of hatchery fish into adjacent wild populations. But it is equally possible that this similarity of genetic structure between wild steelhead and hatchery populations may reflect a common ancestry and the local origins of the hatchery stock.

The Coleman Hatchery stock was derived from adult steelhead collected from the upper Sacramento River in 1947, and steelhead from the upper Sacramento River were regularly incorporated as hatchery broodstock until 1984 (K. Niemela, USFWS Region 1, pers. comm.) The founding stock of the Feather River Hatchery appears to have similar local origins, but the steelhead at Nimbus Hatchery are of mixed origins and include fish collected for broodstock from the Van Arsdale Fisheries Station on the Eel River. It is interesting to observe that
hatchery-wild gene flow is only found at the local scale regardless of hatchery origins. Hatchery-wild interaction at a broader scale within the Central Valley is less clear from these analyses because hatchery stocks do not carry unique diagnostic microsatellite alleles allowing viable estimates of rates of gene flow or introgression. Other molecular markers and additional fine-scale sampling may be needed to provide information on hatchery movements within the basin and estimates of straying and introgression at distant locations.

Other pairwise population similarities were more cryptic and difficult to explain. Results from allelic frequency comparisons and genetic distance analyses among Yuba, Stanislaus, and the Middle Fork American rivers are difficult to interpret. In the case of the Yuba River, most of the associations found in this study are the result of frequencies for common alleles at a few loci (2-3), and do not represent highly significant genetic associations for the rest of the markers. Additional information on the management history of these populations may also shed some light on these findings.

Garza and Williamson’s (2001) $M$ can be used to detect recent population size reduction using microsatellite data. A value of $M < 0.68$ represents a recent bottleneck within the populations according to a survey of published studies and simulations done by Garza and Williamson (2001). There were only three trout populations within the Central Valley sampled for this study that had estimated $M$ values greater than 0.68, Coleman National Fish Hatchery ($M = 0.682$), Deer Creek ($M = 0.682$), and upper Sacramento River trout ($M = 0.703$). These data support a general recent reduction in population size for steelhead throughout the Central Valley. Differences in management strategy, conservation plans and straying may explain why the three populations with $M > 0.68$ appear to have escaped the recent population reductions shown for the rest of the Central Valley steelhead.

Significant differences in allelic frequencies were found for trout samples collected at two locations above and below impassable dams on large river systems in the Central Valley, i.e., the American, Yuba, Stanislaus, and Tuolumne rivers. This suggests some degree of genetic separation between
upper and lower trout populations around dams and barriers within these rivers. A more thorough spatial analysis at each location, such as was done on Clear Creek, may allow inference on the direction and duration of such isolation between trout population pairs above and below barriers in the Central Valley.

Genetic studies comparing freshwater resident rainbow trout and steelhead within individual river basins have consistently suggested polyphyletic origins for these two life histories resulting from parallel evolution rather than two distinct life-history lineages (Phelps et al. 1994; McCusker et al. 2000; Docker and Heath 2003). No significant differences were found for estimates of effective population size (Ne) or Garza and Williamson’s (2001) M among the upper and lower trout populations sampled within the major Central Valley drainages suggesting the differences we found in allelic frequencies do not reflect differential population bottlenecks based on life history.

Comparison of molecular variance between the two main river drainages within the Central Valley, i.e., the Sacramento and San Joaquin rivers, demonstrated that less than 1% of the allelic variance was partitioned between these two drainages, suggesting that no clear genetic division between these trout populations exists for these markers. It is important to note that we had no replicate temporal samples, or sub-basin samples from the San Joaquin basin (such as those taken from Clear Creek). The lack of divergence between the Sacramento and San Joaquin river basins most likely reflects a common ancestry in these two rivers and little divergence between them relative to the relatively high level of structuring that occurs among individual rivers within each sub-drainage.

Genetic distance analyses using Neighbor-Joining supported similar associations between hatchery and wild stocks within the Central valley as we reported using $F_{st}$ and population pairwise comparisons. Bootstrap values were low for many of the branch patterns in these analyses, but some associations depicted in our Neighbor-Joining tree are rather intuitive based on the known history of hatchery populations within the drainages. The grouping of Deer, Mill, and Antelope creeks in our NJ tree with a bootstrap value of 57% gives relatively
mild support for residual population structure for anadromous steelhead in these streams. Battle Creek trout, on the other hand, are difficult to separate genetically in any of these analyses from the upper Sacramento River and the Coleman National Fish Hatchery stocks.

Other population genetic associations depicted by these analyses are more difficult to interpret. The clustering of trout populations from the upper portions of the Tuolumne, Stanislaus, American, and Yuba rivers (35% bootstrap support) could be due to two alternative factors: (1) shared ancestry among native, ancestral populations not influenced by hatchery steelhead or other anadromous populations downstream from the four dams found on these rivers or (2) the influence of introduced rainbow trout from hatchery populations that have been stocked extensively in reservoirs throughout California. Additionally, the associations depicted among Calaveras River, Putah Creek, lower American River, and Nimbus Hatchery are curious and difficult to explain, as is the pairing of upper Yuba River with the Middle Fork American River. Without a better understanding of the history of these populations and a clearer depiction of the genetic signature on a finer scale, we cannot speculate on any meaningful biological interpretation of these associations.

Central Valley wild steelhead abundance has declined precipitously over the last 25 years, with many stocks currently in decline (Mills et al. 1997; McEwan 2001). Habitat alterations due to water diversions, increased water demands, changes in water management strategies, dams and barriers, bank protection, dredging, sediment disposal, gravel mining, contaminant exposure, and climate change and ocean conditions have clearly impacted the size and distribution of steelhead runs in the Central Valley. The loss of access to upriver spawning habitats, declines in once viable tributary populations, and limited productivity in large river source populations have also had potentially significant effects on Central Valley steelhead with important implications for genetic diversity and restoration (McEwan 2001). The implications of intra-specific hatchery production on wild steelhead stocks within the Central Valley are also critical to discussions of steelhead restoration. The degree of straying and
interbreeding with hatchery fish, especially non-native derived populations, is important to our understanding of the status of remaining wild stocks.

Looking at trout populations throughout the Central Valley and comparing these analyses with those we performed on Clear Creek leads us to suggest that to gain better understanding of population structure in this complex system sampling additional populations within individual drainages may be required. The questions brought to these analyses on Clear Creek were concise and the microsatellite data were efficient at answering them. The only failing in this part of our study was the lack of significant overlap between old microsatellite data on rainbow trout hatchery stocks and the new analyses. This is easily corrected with further study of these hatchery populations, which is highly recommended. Our analysis of the Central Valley steelhead, however, leaves us with as many questions as it does answers. Perhaps consideration of the fishery management history, unknown to the authors of this report, will help with some of these questions, but we highly recommend in-depth genetic analyses within individual rivers be considered as additional information in interpretation of these broader basin-wide results.

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APPENDIX I – databases appended electronically.