Genetic Analyses of Central Valley Trout Populations 1999-2003

by

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ABSTRACT

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 (*Oncorhynchus 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

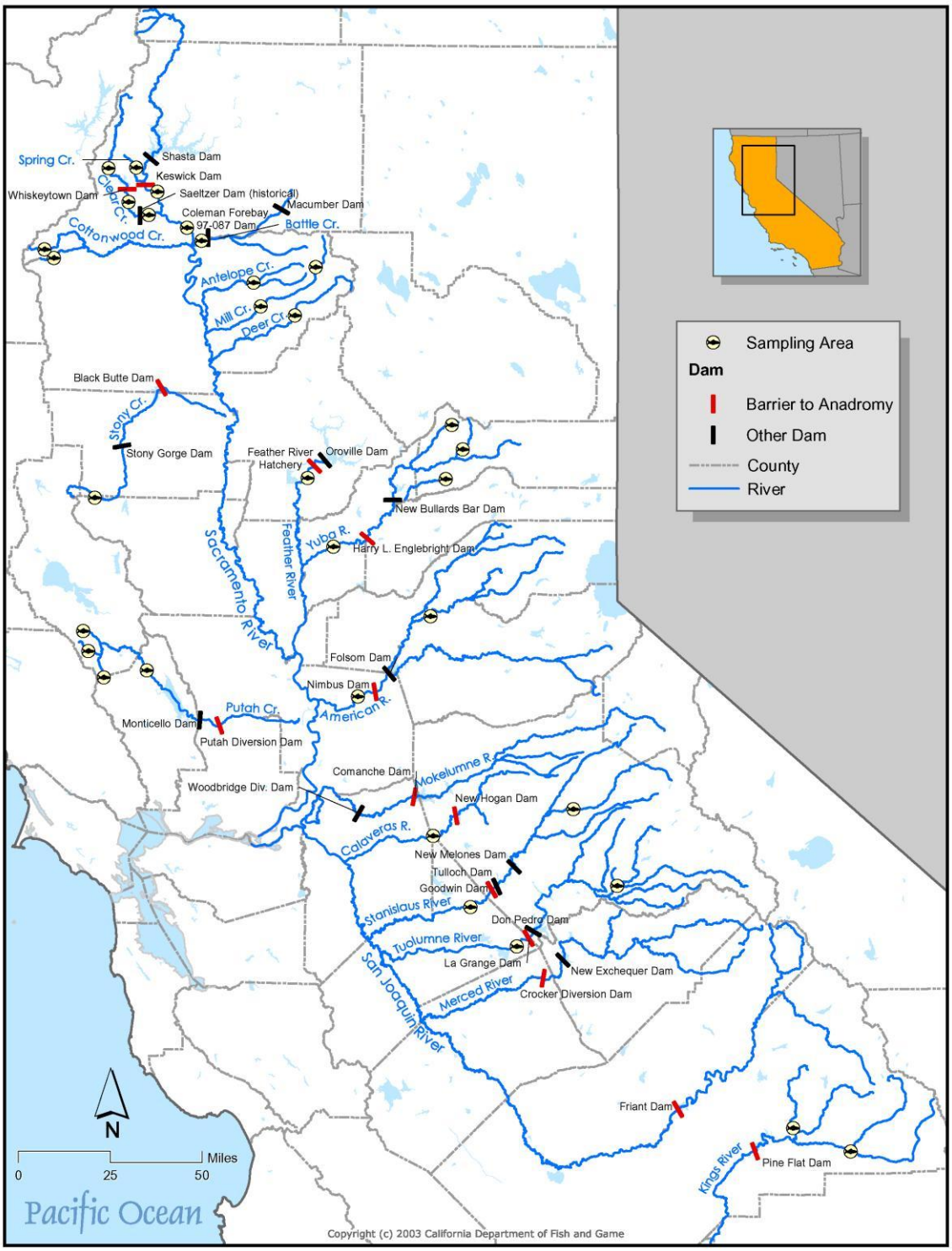

Figure 1. Central Valley rivers and streams showing distribution of *O. mykiss* sample locations in relationship to impassable dams.

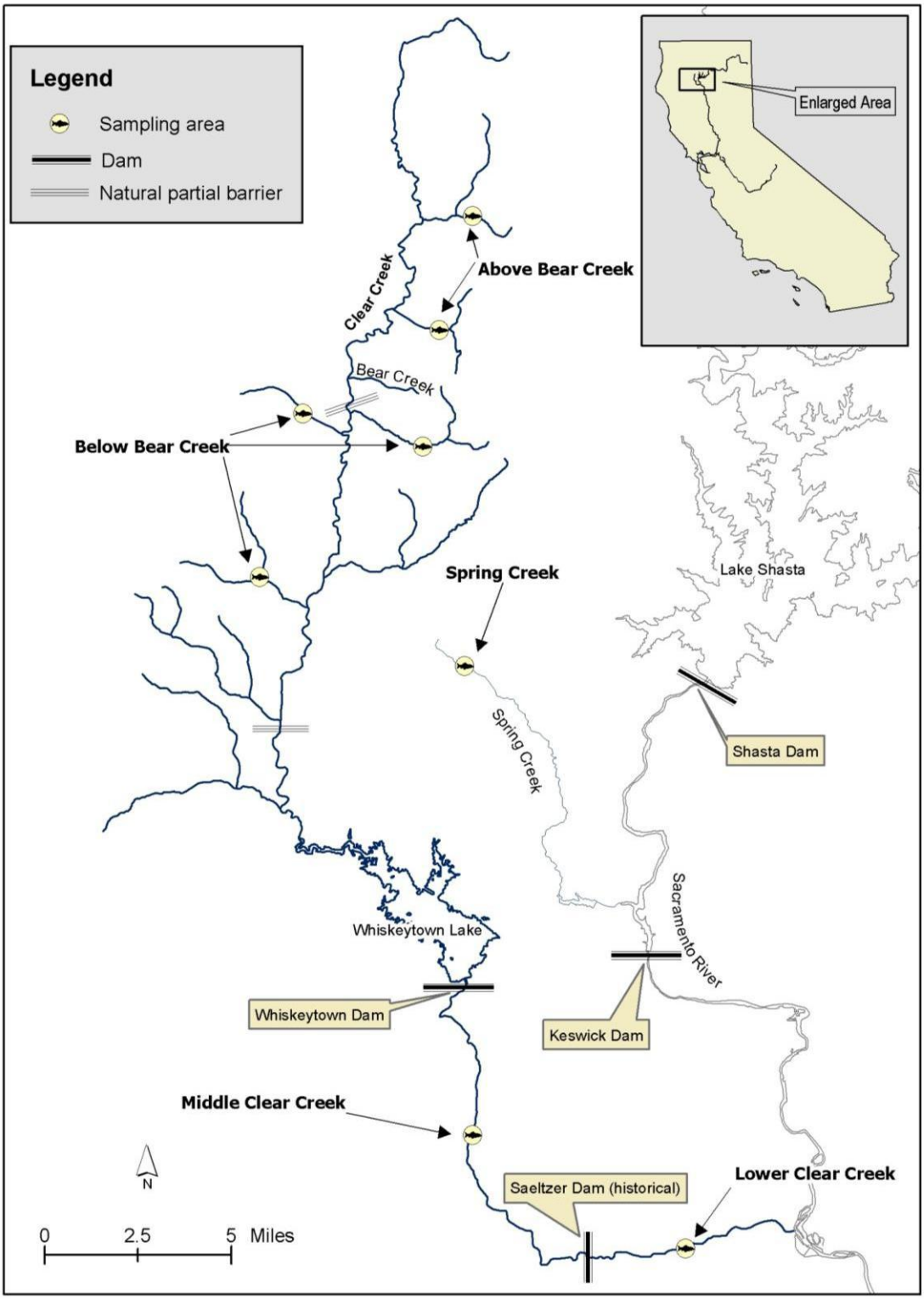

Figure 2. Map showing Clear Creek trout sample locations in relationship to impassable dams.

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.

Drainage	Sample location	N	Year	Collector
Sacramento River				
	American River - Middle Fork	44 (47)	2002	CDFG
	American River - lower	41 (49)	2002	CDFG
	Antelope Creek	57 (70)	2001-02	CDFG
	Battle Creek	41 (216)	2003	CDFG
	Clear Creek			
	Upper above Bear Creek	43 (60)	1999	USFWS
	Upper below Bear Creek	64 (78)	1999	USFWS
	Middle below Whiskeytown Dam	31 (49)	1999	USFWS
	Lower below Sealtzer Dam	41 (50)	1999	USFWS
	Lower below Sealtzer Dam	48 (50)	2001	USFWS
	Cottonwood Creek	34 (50)	2001-02	CDFG
	Deer Creek	46 (50)	1999	USFWS
	Deer Creek	34 (40)	2001	CDFG
	Feather River	54 (86)	2001-02	CDFG
	Mill Creek	36 (40)	1999	USFWS
	Mill Creek	39 (42)	2001	CDFG
	Putah Creek	62 (64)	2002	CDFG
	Sacramento River - upper	32 (40)	2001	USFWS
	Sacramento River - upper	50 (74)	2001-02	CDFG
	Spring Creek	53 (56)	1999	USFWS
	Stoney Creek	63 (66)	2001-02	CDFG
	Yuba River - upper	58 (69)	2001-02	CDFG
	Yuba River - lower	40 (67)	2002	CDFG
San Joaquin River				
	Calaveras River	60 (98)	2002	CDFG
	Kings River	33 (36)	2002	CDFG
	Lower Stanislaus	45 (57)	2001-02	CDFG
	Upper Stanislaus	49 (63)	2002	CDFG
	Lower Tuolumne	45 (62)	2000-01	CDFG
	Upper Tuolumne	47 (80)	2002	CDFG
Hatchery	American Trout & Salmon Co.	47 (50)	1999	USFWS
	Coleman National Fish Hatchery	92 (150)	2001	USFWS
	Crystal Hatchery strain	25 (25)	1996	JLN
	Feather River Hatchery	30 (40)	2001-02	CDFG
	Mount Shasta Hatchery strain	39 (40)	1996	JLN
	Nimbus Hatchery	47 (51)	2002	CDFG
	Total Analyzed	1570		

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 *Oneμ10*-F and *Ots3*-R primers as follows in order to incorporate them into the Clear Creek four-locus multiplex protocol. *Oneμ10*-F was renamed *Oneμ10.1*-F (5′-GGGAACAGAAGAGGAATAGC-3′), and *Ots3*-R was renamed *Ots3.1*-R (5′-GGTGGAGAGAGTTTGAGAATCACA-3′). *Oneμ10-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 $MgCl_2$, 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.

Locus	Source	Number Alleles	Allelic Size Range (bp)	Mean Hz
Omy27	Heath et al. 2001	8 (5)	99 – 115	0.66
Omy77	Morris et al. 1996	28 (17)	77 – 143	0.80
Omy207	O'Connell et al. 1997	24 (20)	97 - 165	0.66
Omy325	O'Connell et al. 1997	33 (20)	83 – 167	0.86
Ogo1a	Olsen et al. 1998	12 (4)	122 – 168	0.64
Ogo4	Olsen et al. 1998	16 (12)	116 – 148	0.76
Oneμ8	Scribner et al. 1996	19 (13)	150 – 190	0.60
Oneμ10.1 & 10.2	Scribner et al. 1996	11 (8)	113 – 139	0.70
Oneμ11	Scribner et al. 1996	5 (3)	142 – 154	0.51
Oneμ14	Scribner et al. 1996	12 (8)	145 - 171	0.45
Ots1	Banks et al. 1999	30 (10)	151 – 243	0.81
Ots3	Banks et al. 1999	10 (8)	73 – 95	0.57
Ots4	Banks et al. 1999	13 (15)	101 – 137	0.56

Table 3. Number of alleles found for each locus given by population.

Sample location	Locus Ogo1a	Ogo4	Omy27	Omy77	Omy325	Oneμ8	Oneμ10	Oneμ11	Ots1	Ots3	Ots4	Total
American River - Middle Fork	6	9	4	14	16	11	5	2	11	6	5	89
American River - lower	5	8	6	14	17	9	7	4	11	6	6	93
Antelope Creek	6	11	6	16	17	11	5	3	12	7	5	99
Battle Creek	5	11	5	14	14	8	6	4	12	4	5	88
Clear Creek												
Upper above Bear Creek	5	4	2	11	10	6	6	2	7	5	4	62
Upper below Bear Creek	5	7	4	13	12	6	6	2	7	7	6	75
Middle below Whiskeytown Dam	4	9	4	9	12	6	4	3	10	6	5	72
Lower below Sealtzer Dam (1999)	7	9	4	10	13	6	4	3	10	5	5	76
Lower below Sealtzer Dam (2001	5	9	4	9	14	7	6	3	13	10	5	85
Cottonwood Creek	4	11	5	13	15	7	4	3	15	5	5	87
Deer Creek (USFWS)	4	12	5	16	22	13	6	3	15	8	11	115
Deer Creek (CDFG)	4	11	5	13	18	10	5	3	14	5	6	94
Feather River	5	11	5	14	12	10	5	3	11	4	5	85
Mill Creek (USFWS)	4	11	6	17	21	9	6	3	10	7	6	100
Mill Creek (CDFG)	4	11	6	13	17	8	5	3	9	6	6	88
Putah Creek	6	8	5	10	15	6	4	3	8	4	5	74
Sacramento River - upper (USFWS)	5	8	4	4	11	6	4	2	8	4	4	60
Sacramento River - upper (CDFG)	6	9	5	14	17	8	4	3	11	5	5	87
Spring Creek	1	2	1	1	1	2	1	1	1	1	1	13
Stoney Creek	5	8	6	16	20	12	6	3	15	7	6	104
Yuba River - upper	5	10	6	12	15	8	4	3	12	4	5	84
Yuba River - lower	6	9	5	15	18	8	5	3	11	6	5	91
Calaveras River	4	9	7	10	15	5	6	2	10	5	4	77
Kings River	3	9	5	15	12	10	4	3	11	7	6	85
Lower Stanislaus	6	11	7	17	18	10	6	4	14	7	7	107
Upper Stanislaus	4	10	5	14	16	8	6	4	9	5	5	86
Lower Tuolumne	4	8	5	9	12	4	4	3	9	3	5	66
Upper Tuolumne	5	10	5	11	16	9	6	3	10	6	4	85
American Trout & Salmon Co.	4	7	4	8	12	6	4	3	9	6	4	67
Coleman National Fish Hatchery	6	10	5	18	15	10	7	3	15	5	5	99
Crystal Hatchery strain			4	8								12
Feather River Hatchery	4	10	4	12	11	9	6	3	10	5	4	78
Mount Shasta Hatchery strain			5	12								17
Nimbus Hatchery	6	9	5	13	19	10	6	3	9	5	5	90
AVERAGE	4.78	9.09	4.82	12.21	14.78	8.06	5.09	2.91	10.59	5.50	5.16	79.12

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.

	Multiplex	Anneal Temp. °C/cycles	30 min. extension	Loci 700	Loci 800
A Clear Creek	A	52/40	NO	Omy325 Ots1	Ots4 Oneμ14
	B	50/40	YES	Omy77 Oneμ8	Ogo1a
	C	52/40	YES	Ogo4	Omy27 Oneμ11
	D	52/40	NO	Omy207	Oneμ10 Ots3
B Central Valley	A	52/40	NO	Omy325 Ots1	Ots4 Oneμ14
	B	50/40	YES	Omy77 Ots3	Ogo4 Ogo1a Oneμ8
	C	52/40	YES	Omy207 Oneμ10	Omy27 Oneμ11

concentrations for both Clear Creek and Central Valley multiplex systems were as follows: *Oneμ10* (10 pmoles), *Ogo1a*, *Ogo4*, *Oneμ11*, *Ots3* (5 pmoles) and *Oneμ8* (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; *Oneμ14*, 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), CONSENSE 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.

Population	N	Locus Ogo1a	Ogo4	Omy27	Omy77	Omy325	Oneμ8	Oneμ10	Oneμ11	Ots1	Ots3	Ots4	HWE TOTAL
1 American River - Middle Fork below Rubicon R.	44	-	-	-	-	+	-	-	-	-	+	-	9
2 American River - lower below Nimbus Dam	41	-	-	-	-	-	-	-	-	-	-	-	11
3 American Trout & Salmon Co.	47	-	-	-	-	-	-	-	-	+	-	-	10
4 Antelope Creek below confluence	57	-	+	-	-	-	+	-	-	+	-	-	8
5 Battle Creek	41	-	-	-	-	-	-	-	-	-	-	-	11
6 Calaveras River - below New Hogan Dam	60	-	-	-	-	-	+	-	-	-	-	-	10
7 Clear Creek - upper above Bear Cr.	43	+	-	-	+	+	-	-	+	-	+	-	6
8 Clear Creek - upper below Bear Cr.	64	-	-	-	+	+	+	-	+	+	+	+	4
9 Clear Creek - middle below Wiskeytown Dam	31	-	-	-	-	+	+	-	-	-	+	-	8
10 Clear Creek - lower below Sealtzer Dam 1999	41	+	-	-	+	-	-	-	-	-	+	-	8
11 Clear Creek - lower below Sealtzer Dam 2001	48	+	-	-	-	-	-	+	+	-	-	-	8
12 Coleman National Fish Hatchery	92	-	-	-	-	+	-	-	+	-	-	-	9
13 Cottonwood Creek- Middle Fork & Beegum Cr.	34	-	+	-	-	-	+	-	-	-	-	-	9
14 Deer Creek 1999	46	+	-	-	+	-	-	+	-	-	+	+	6
15 Deer Creek 2001	34	-	-	-	-	-	-	-	-	-	-	-	11
16 Feather River - low flow channel	54	-	-	-	-	-	-	-	-	-	-	-	11
17 Feather River Hatchery	30	-	-	-	-	-	-	-	-	-	-	-	11
18 Kings River	33	-	-	-	-	+	+	+	-	+	-	-	7
19 Mill Creek 1999	36	-	-	+	-	+	-	-	-	-	+	+	7
20 Mill Creek 2001	39	-	-	+	-	-	-	-	-	-	+	-	9
21 Nimbus Hatchery	47	+	-	-	-	-	-	-	-	-	-	-	10
22 Putah Creek above Lake Berryessa	62	-	-	-	-	-	-	-	-	-	-	-	11
23 Sacramento River below Keswick Dam (USFWS)	32	-	-	-	-	-	-	+	-	+	+	-	8
24 Sacramento River below Keswick Dam (CDFG)	50	-	-	-	-	-	-	-	-	-	-	-	11
25 Spring Creek	53	+	-	+	+	+	-	+	+	+	+	+	2
26 Stanislaus River - upper below Beardsley Dam	49	-	-	-	-	-	-	-	-	-	-	+	10
27 Stanislaus River - lower below Goodwin Dam	45	-	-	-	-	-	+	+	+	-	-	+	7
28 Stoney Creek	63	-	+	-	-	-	-	-	-	-	-	-	10
29 Tuolumne River - upper above Don Pedro Reservoir	47	-	+	-	-	+	-	-	-	+	-	-	8
30 Tuolumne River - below La Grange Dam	45	-	-	-	+	-	-	-	-	-	-	-	10
31 Yuba River – Oregon, Lavazzola, Pauley and Canyon creeks	58	+	+	-	+	+	+	+	-	+	+	+	2
32 Yuba River - below Englebright Dam	40	-	-	-	-	+	-	-	-	-	-	+	9
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 (*Ne*) 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 $2.05E^{-4}$ (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 ($Chi^2 = 30.36$; df = 22; p = 0.11) and Mill Creek ($Chi^2 = 36.59$; 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 ($Chi^2 = 20.24$; 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 = 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 Oneμ8. Spring Creek F_{st} population pairwise comparisons

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

Rank	Locus	Score	% Relative Score
1	Omy325	139.474	14.165
2	Omy77	114.071	11.585
3	Ots1	109.722	11.143
4	Ots4	98.694	10.023
5	Ogo4	89.510	9.09
6	Oneμ8	87.920	8.929
7	Ogo1	83.481	8.478
8	Oneμ10	75.921	7.71
9	Ots3	75.768	7.695
10	Omy27	67.291	6.834
11	Oneμ11	42.805	4.347

Table 7. Pairwise *Fst* comparisons between rainbow trout hatchery populations and Clear Creek trout collections. Pairwise *Fst* values are given below the diagonal and the matrix of significant *Fst* P values ("+" = significant pairwise *Fst* values) is given above the diagonal.

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

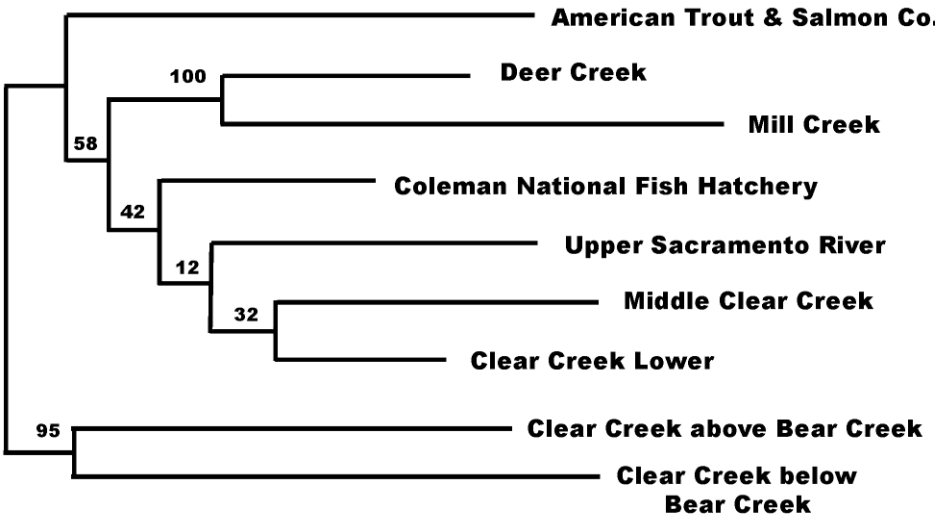

Figure 3. Unrooted Neighbor – Joining tree based on Cavalli-Sforza and Edwards chord distance for the Clear Creek drainage trout populations. Branch bootstrap values (2000 replicate trees) are provided.

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 *Ne* 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 $\geq$0.05) between trout populations within the Central Valley based on allelic frequencies for 11 microsatellite loci.

Population	Population	Pairwise Fst	Fst P
American River lower	Nimbus Hatchery	0.009	0.065
Antelope Creek	Clear Creek lower	0.014	0.051
Antelope Creek	Cottonwood Creek	0.011	0.079
Battle Creek	Cottonwood Creek	0.003	0.250
Clear Creek lower	Cottonwood Creek	0.002	0.268
Clear Creek lower	Sacramento River upper	0.011	0.078
Coleman Fish Hatchery	Sacramento River upper	0.007	0.092
Feather River	Feather River Hatchery	-0.007	0.882
Kings River	Stoney Creek	0.015	0.059
Stanislaus R. upper	Middle Fork American R.	0.001	0.345
Stanislaus R. lower	Battle Creek	0.006	0.113
Stanislaus R. lower	Feather River	0.009	0.055
Yuba River lower	Battle Creek	0.016	0.052
Yuba River lower	Cottonwood Creek	0.017	0.050
Yuba River lower	Stanislaus R. lower	0.011	0.064

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.

Drainage	Population	SSM *Ne*	IAM *Ne*	*M*
Sacramento River	American River Middle Fork	5844	2748	0.641
	American River lower	4380	2269	0.587
	Antelope Creek	5459	2628	0.658
	Battle Creek	5004	2481	0.648
	Clear Creek upper	3632	1997	0.526
	Clear Creek lower	5136	2524	0.589
	Coleman National Fish Hatchery	5225	2553	0.682
	Cottonwood Creek	5029	2489	0.656
	Deer Creek	5577	2665	0.682
	Feather River	5381	2554	0.649
	Feather River Hatchery	5983	2790	0.664
	Mill Creek	4587	2341	0.610
	Nimbus Hatchery	4023	2142	0.591
	Putah Creek	4946	2462	0.531
	Sacramento River upper	3670	2011	0.703
	Stoney Creek	7237	3155	0.647
	Yuba River upper	5920	2771	0.618
	Yuba River lower	5732	2713	0.617
San Joaquin River	Calaveras River	4087	2165	0.636
	Kings River	5927	2773	0.629
	Stanislaus River upper	4771	2403	0.612
	Stanislaus River lower	5697	2703	0.660
	Tuolumne River upper	3677	2014	0.625
	Tuolumne River lower	4669	2369	0.558
Overall estimate		5066	2488	0.626

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.

Population	Model HZD	Model HZE	Model TTM
American River - Middle Fork	0.21	0.82	0.41
American River - lower	0.01	0.99	0.01
Antelope Creek	0.10	0.91	0.21
Battle Creek	0.23	0.79	0.46
Clear Creek upper	0.62	0.42	0.83
Clear Creek lower	0.74	0.29	0.58
Coleman National Fish Hatchery	0.68	0.39	0.53
Cottonwood Creek	0.12	0.90	0.24
Calaveras River	0.18	0.84	0.36
Deer Creek	0.03	0.99	0.05
Feather River Hatchery	0.52	0.52	1.00
Feather River	0.42	0.62	0.83
Kings River	0.79	0.23	0.46
Mill Creek	0.10	0.91	0.21
Nimbus Hatchery	0.07	0.93	0.15
Putah Creek	0.79	0.23	0.46
Sacramento River - upper	0.10	0.91	0.21
Stanislaus River - upper	0.09	0.93	0.17
Stanislaus River - lower	0.06	0.95	0.12
Stoney Creek	0.26	0.77	0.52
Tuolumne River upper	0.01	0.99	0.02
Tuolumne River lower	0.96	0.05	0.10
Yuba River - upper	0.71	0.32	0.64
Yuba River - lower	0.12	0.90	0.24

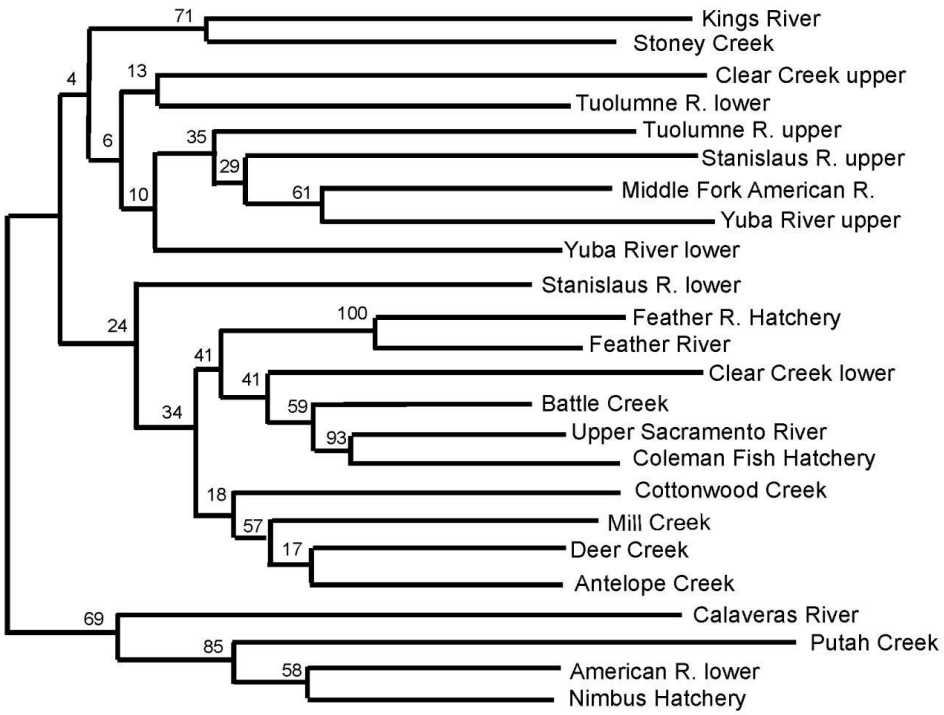

Figure 4. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the Central Valley system derived from allelic variation at 11 microsatellite loci. Branches with bootstrap values (2000 replicate trees) are provided.

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.

Clear Creek

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. myk*iss 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 *Ne* = 3632 (upper Clear Creek) to Ne = 7237 (Stoney Creek), with a mean *Ne* = 5066, excluding Spring Creek where Ne = 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 Ne 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 $M \leq 0.68$ 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.