Title
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Journal
San Francisco Estuary and Watershed Science, 3(2)

ISSN
1546-2366

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Publication Date
2005-01-01

SAN FRANCISCO ESTUARY & WATERSHED SCIENCE
Published by the California Bay-Delta Authority Science Program and the John Muir Institute of the Environment

Genetics of Central Valley *O. mykiss* populations: drainage and watershed scale analyses

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ABSTRACT

Genetic variation at 11 microsatellite loci described population genetic structure for *Oncorhynchus mykiss* in the Central Valley, California. Spatial and temporal variation was examined as well as relationships between hatchery and putative natural spawning anadromous stocks. Genetic diversity was analyzed at two distinct spatial scales: fine-scale within drainage for five populations on Clear Creek; between and among drainage diversity for 23 populations. Significant regional spatial structure was apparent, both within Clear Creek and among rainbow trout populations throughout the Central Valley. Significant differences in allelic frequencies were found among most river or drainage systems. Less than 1% of the molecular variance could be attributed to differences found between drainages. Hatchery populations were shown to carry similar genetic diversity to geographically proximate wild populations. Central Valley M = 0.626 (below the M < 0.68 threshold) supported recent population reductions within the Central Valley. However, average estimated effective population size was relatively high (Ne = 5066). Significant allelic differences were found in rainbow trout collected above and below impassable dams on the American, Yuba, Stanislaus and Tuolumne rivers. Rainbow trout sampled in Spring Creek were extremely bottlenecked with allelic variation at only two loci and an estimated effective population size of 62, suggesting some local freshwater *O. mykiss* stocks may be declining rapidly. These data support significant genetic population structure for steelhead and rainbow trout populations within the Central Valley across multiple scales. Careful consideration of this genetic diversity and its distribution across the landscape should be part of future conservation and restoration efforts.

KEYWORDS

Genetic diversity, salmonids, steelhead, rainbow trout, Central Valley, microsatellite DNA, hatchery stocks.

INTRODUCTION

Historically, anadromous steelhead (*Oncorhynchus mykiss*) were broadly distributed throughout the Sacramento and San Joaquin River drainages (McEwan 2001). Steelhead hatcheries in the Central Valley (Coleman, Feather River, Nimbus and Mokelumne River) produce and release about 1.5 million yearlings each year (Brown 2005). Despite this abundance, 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 and others 1996; McEwan 2001; May and Brown 2002).

O. mykiss expresses a range of variations in life history strategies, from strongly migratory to non-migratory, throughout the species' range. Natural anadromous spawning populations of winter-run steelhead still exist at low levels in the Sacramento and San Joaquin river drainages. Individual runs or stocks of *O. mykiss* found within the same drainage cannot be separated taxonomically based on migration timing or the distribution of anadromy (Behnke 1992; Allendorf and Utter 1979). Highly flexible life history strategies in *O. mykiss* (Shapovalov and Taft 1954), otolith microchemistry (Rybock and others 1975; Zimmerman and Reeves 2000), and genetic studies (Gall and others 1990; Nielsen and others 1997) suggest that freshwater habitats may contain relict, non-anadromous components of the *O. mykiss* gene pool found in geographically proximate anadromous populations. Recent studies demonstrated that non-anadromous rainbow trout introduced into Argentina gave rise to anadromous fish (Pascual and others 2001), with the source of these fish derived from early Sacramento River stocks, most probably from McCloud River Hatchery fish that had been transplanted around the world at the beginning of the 20th century, including Argentina (Riva Rossi and others 2004).

Recent studies of land-locked rainbow trout populations throughout California have demonstrated genetic relationships between landlocked rainbow 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 studies have demonstrated genetic population structure (mtDNA and microsatellite loci) for California's resident rainbow 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 and others 1999), San Francisquito Creek (Nielsen 2000), San Mateo Creek (Nielsen and Sage 2002) and the Santa Ynez River (Nielsen and others 2003).

Rainbow trout in California have undergone considerable manipulation and husbandry in the hatchery environment since the early 1800s (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). Most early hatchery efforts were directed at rainbow trout, the freshwater resident life history of *O. mykiss*. Hatchery efforts for steelhead life histories were developed later by state and federal agencies and used a very different approach integrating anadromous broodstock. The early findings of Gall and others (1990) suggested that anadromous steelhead populations have residualized as freshwater fish behind man-made structures and dams throughout California. Using allozyme analyses, Gall and others (1990) argued that residual freshwater populations of *O. mykiss* reflect genetic population structure similar to their putative anadromous progenitors. A similar analysis was done for southern California *O. mykiss* populations by Nielsen and others (1997). 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 and 2).

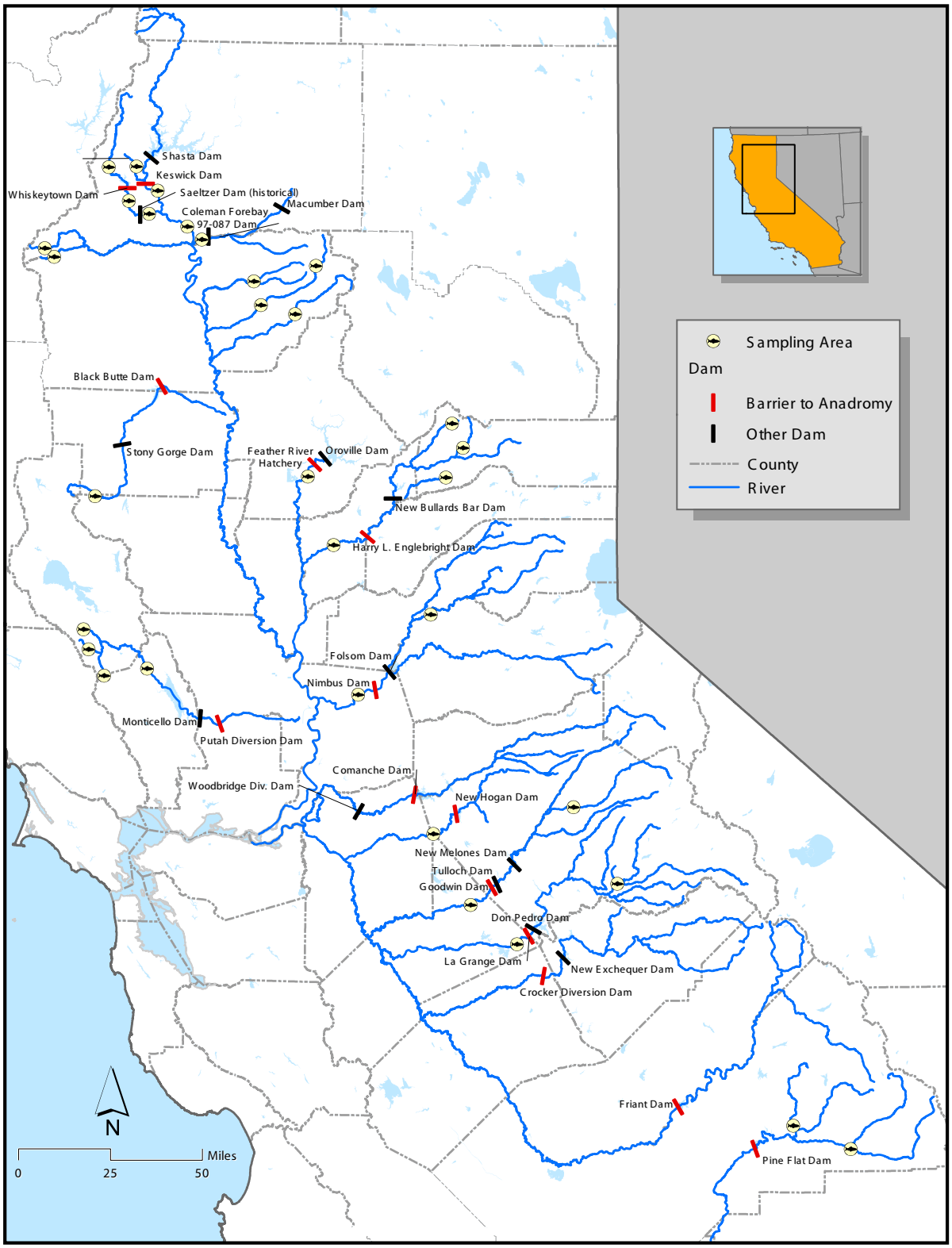

Figure 1. Central Valley Rivers and streams showing distribution of *O. mykiss* sample locations in relationship to impassable dams.

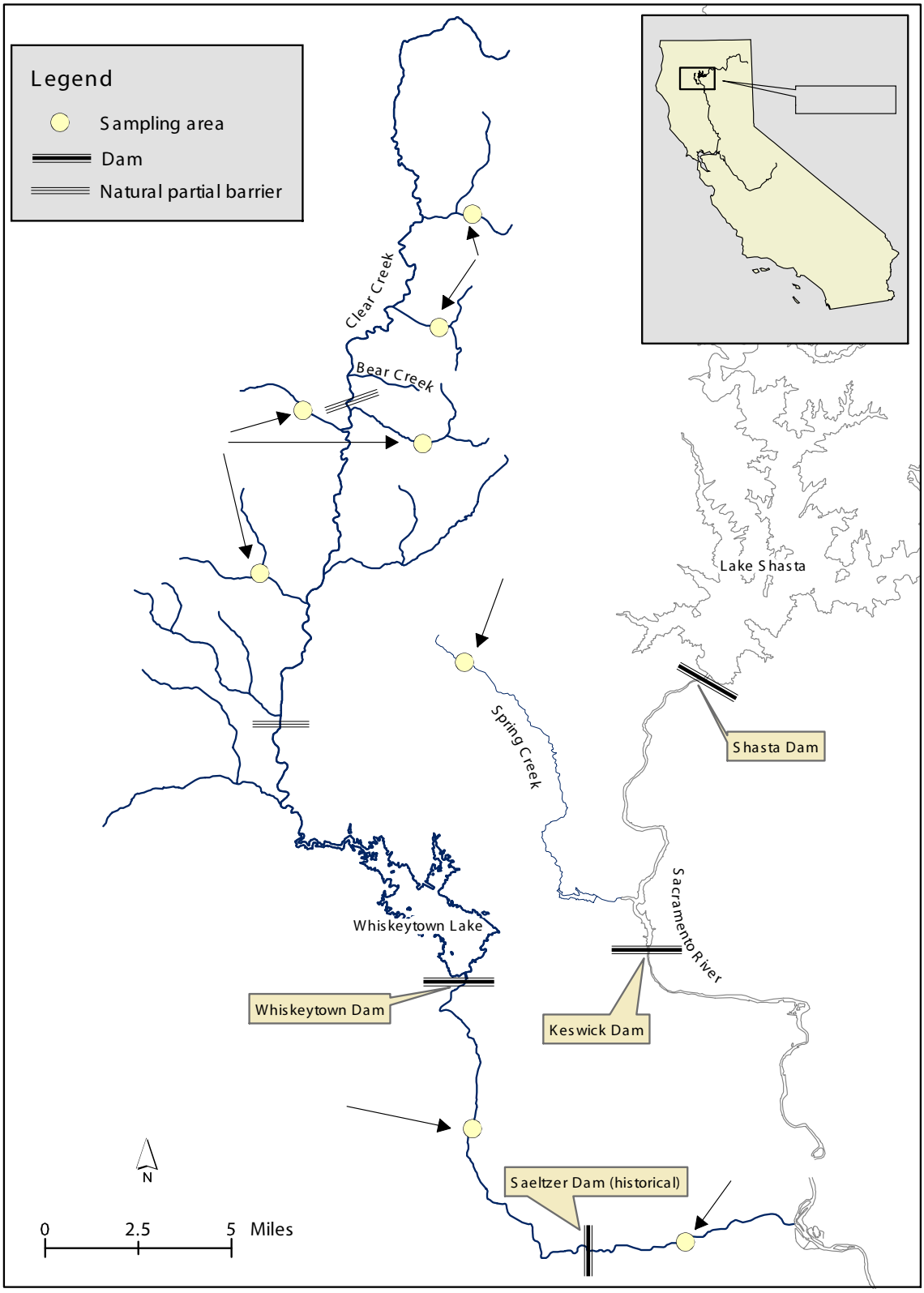

Figure 2. Map showing Clear Creek rainbow trout sample locations in relationship to impassable dams.

Spatial heterogeneity is part of an ecological architecture that occurs at various scales. Diversity in population structure in *O. mykiss* has been found in ecological and genetic studies in a wide range of contexts running from broad biogeographic structure (Okazaki 1984; Withler 1966; Nielsen and others 1994) to fine-scale drainage or basin analyses (Beacham and others 1999; Docker and Heath 2003). Depending on the scale at which collections and measurements are made, heterogeneity can affect estimates of diversity, interpretations of those differences and subsequent management implications. It is important that the scale of the measurement is congruent with the specific question being asked (Epperson and others 1999). It is also clear that critical spatial scales of genetic diversity will change with changes in ecological condition, such as climate shifts and anthropogenic manipulation of habitat, such as dam construction or urbanization of watersheds (Fuller and others 1997; Sokal and others 1998; Scribner and others 2001; Stow and others 2001).

In an attempt to demonstrate the spatial dynamics of diversity over several scales within and among watersheds, this study presents genetic analyses of multiple samples of *O. mykiss* at different life history stages; i.e., fish collected above and below dams, putative natural spawning anadromous and freshwater populations, and hatchery rainbow trout strains found in the Central Valley, California. The California Department of Fish and Game (CDFG) and the U.S. Fish and Wildlife Service (USFWS) collected samples for this study, 1999–2003. Rainbow trout samples were analyzed for microsatellite allelic diversity at the U.S. Geological Survey (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 rainbow trout populations, where different year-class samples were 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 rainbow trout populations to data for a limited number of overlapping microsatellite loci from two rainbow trout hatchery strains (Mount Shasta and Crystal hatchery rainbow trout) with a history of stocking in the Central Valley.

This study used multiple sample locations within one river drainage, Clear Creek, to test questions about fine-scale population structure. 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 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 *O. mykiss* as a whole, looking at relationships among and between all steelhead and rainbow trout populations sampled for this study.

MATERIAL AND METHODS

Sample Collections

O. mykiss fin tissue was collected and analyzed for DNA from 1,570 fish in this study (Table 1). The CDFG collected tissues from rainbow trout throughout the Central Valley, California, 2001-2003, for a broad scale analysis of genetic population structure (Figure 1). The USFWS collected rainbow trout

Table 1. Sample location, N = number of samples analyzed (number in parenthesis is number of samples 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 Cr. Upper above Bear Creek	43 (60)	1999	USFWS
Clear Cr. Upper below Bear Creek	64 (78)	1999	USFWS
Clear Cr. Middle below Whiskeytown Dam	31 (49)	1999	USFWS
Clear Cr. Lower below Sealtzer Dam	41 (50)	1999	USFWS
Clear Cr. 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
Calaveras River			
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		

tissues from the Clear Creek drainage, the American Trout & Salmon Company, and Spring Creek, 1999–2001 (Figure 2). This fine-scale sampling regime was designed to look at rainbow trout population above and below barriers and provide inference on potential native rainbow 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, a partial barrier to fish migration which is infrequently passable. The Saeltzer Dam was removed in 2000. Samples collected in lower Clear Creek were taken below Saeltzer Dam in an area that was accessible to anadromous steelhead.

Deer and Mill creek rainbow trout samples were collected by both agencies independently at different times and locations during 1999–2001. Archival data from standardized microsatellite analyses of hatchery rainbow trout from the Mount Shasta and Crystal hatcheries were used in the Clear Creek study (J. Nielsen, 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. We developed multiplex systems using 13 loci, grouped together for amplification based on 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 study (Table 4), while a three multiplex protocol was used to collect data for the Central Valley study (Table 4).

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Table 2. List of microsatellite loci used in this study of steelhead/rainbow 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 each locus in 23 populations from throughout the Central Valley drainage.

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

Table 3. Number of alleles found for each locus given by population and the total number of alleles adjusted by sample size.

	Locus											
Sample Location	*Ogo1a*	*Ogo4*	*Omy27*	*Omy77*	*Omy325*	*Oneμ8*	*Oneμ10*	*Oneμ11*	*Ots1*	*Ots3*	*Ots4*	*Adjusted # Alleles*
American River - Middle Fork	6	9	4	14	16	11	5	2	11	6	5	2.02
American River - Lower	5	8	6	14	17	9	7	4	11	6	6	2.27
Antelope Creek	6	11	6	16	17	11	5	3	12	7	5	1.74
Battle Creek	5	11	5	14	14	8	6	4	12	4	5	2.15
Clear Creek												
Upper above Bear Creek	5	4	2	11	10	6	6	2	7	5	4	1.44
Upper below Bear Creek	5	7	4	13	12	6	6	2	7	7	6	1.17
Middle below Whiskeytown Dam	4	9	4	9	12	6	4	3	10	6	5	2.32
Lower below Sealtzer Dam (1999)	7	9	4	10	13	6	4	3	10	5	5	1.85
Lower below Sealtzer Dam (2001)	5	9	4	9	14	7	6	3	13	10	5	1.77
Cottonwood Creek	4	11	5	13	15	7	4	3	15	5	5	2.56
Deer Creek (USFWS - 1999)	4	12	5	16	22	13	6	3	15	8	11	2.50
Deer Creek (CDFG 2001)	4	11	5	13	18	10	5	3	14	5	6	2.76
Feather River	5	11	5	14	12	10	5	3	11	4	5	1.57
Mill Creek (USFWS - 1998)	4	11	6	17	21	9	6	3	10	7	6	2.78
Mill Creek (CDFG - 2001)	4	11	6	13	17	8	5	3	9	6	6	2.26
Putah Creek	6	8	5	10	15	6	4	3	8	4	5	1.19
Sacramento River - upper (USFWS - 2001)	5	8	4	4	11	6	4	2	8	4	4	1.88
Sacramento River - upper (CDFG - 2001)	6	9	5	14	17	8	4	3	11	5	5	1.74
Spring Creek	1	2	1	1	1	2	1	1	1	1	1	0.25
Stoney Creek	5	8	6	16	20	12	6	3	15	7	6	1.65
Yuba River - upper	5	10	6	12	15	8	4	3	12	4	5	1.45
Yuba River - lower	6	9	5	15	18	8	5	3	11	6	5	2.28
Calaveras River	4	9	7	10	15	5	6	2	10	5	4	1.28
Kings River	3	9	5	15	12	10	4	3	11	7	6	2.58
Lower Stanislaus	6	11	7	17	18	10	6	4	14	7	7	2.38
Upper Stanislaus	4	10	5	14	16	8	6	4	9	5	5	1.76
Lower Tuolumne	4	8	5	9	12	4	4	3	9	3	5	1.47
Upper Tuolumne	5	10	5	11	16	9	6	3	10	6	4	1.81
American Trout & Salmon Co.	4	7	4	8	12	6	4	3	9	6	4	1.43
Coleman National Fish Hatchery	6	10	5	18	15	10	7	3	15	5	5	0.98
Crystal Hatchery strain (2 loci only)			4	8								0.48
Feather River Hatchery	4	10	4	12	11	9	6	3	10	5	4	2.60
Mount Shasta Hatchery strain (2 loci only)			5	12								0.44
Nimbus Hatchery	6	9	5	13	19	10	6	3	9	5	5	1.91
Average	4.78	9.09	4.82	12.21	14.78	8.06	5.09	2.91	10.59	5.50	5.16	

Table 4. Multiplex systems used to amplify 13 microsatellite loci on two profiles for amplification of DNA from Central Valley rainbow 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.

Location	*Multiplex*	*Annual Temp.°C/ Cycles*	*30 min. Extension*	*Loci 700*	*Loci 800*
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
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

Primers were redesigned to fit in post-PCR multiplex systems. Oneμ10-F and Ots3-R primers were redesigned 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 for incorporation into the Central Valley three multiplex protocol: Oneμ10-(F) was redesigned and renamed Oneμ10.2 (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, Ogo4.2, and Oneμ10 forward primers were modified by the addition of M13R tails, and Oneμ8, Oneμ11, and Ots3 were modified by the addition of M13F tails. All modifications were additions onto primer 5′ ends. Allele fragment visualization was facilitated by annealing to labeled complementary tails added to the PCR mix. The remaining loci were visualized by adding directly labeled forward primers. 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 10-μL volumes using approximately 50 ng of genomic DNA, 0.1 to 0.2 U of DNA polymerase (Perkin Elmer), 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% each of gelatin, NP-40, and Triton X-100, and 200 μm each dNTP. The total of forward (F) and reverse (R) primers per locus per reaction equaled four pmoles for all loci that utilized direct labeled primers for product visualization, with the F primer concentration being a combination of labeled and unlabeled primer. Tailed F and R primer 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 to 0.20 pmoles per reaction (Omy325, 0.06; Ots1, 0.20; One 14, 0.40; Ots4, 0.06). Multiplex B was between 0.10 to 0.75 pmoles (Omy77, 0.20; M13F, 0.30; M13R, 0.75). Multiplex C had between 0.10 to 1.50 pmoles (Omy27, 0.10; M13F, 1.50; M13R, 0.75). The labeled primer for multiplex D was between 0.30 to 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 to 1.5 pmoles (Omy77, 0.2; M13F, 0.3; M13R, 1.5), and multiplex C had between 0.1 to 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.

Statistical Analyses

Genetic data were analyzed using a variety of software from different statistical packages including ARLEQUIN (Schneider and others 2000), 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 rainbow trout populations to gain inference on population structure.

ARLEQUIN version 1.1 F_{st} 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 (SMM) and the Infinite Allele Model (IAM) with a mutation rate of $2.05E^{-4}$ using AGARst (Harley 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. An 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 assess locus-specific assignment

power based on the allelic frequency differential method (Banks and Eichert 2000).

RESULTS

Microsatellite Loci

GENEPOP's analyses of expectation of HWE gave mixed results among the microsatellite loci and rainbow 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 Company (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 more 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 statistical analyses. Two sample populations fell significantly out of HWE ($p > 0.025$) for the remaining 11 loci combined (Table 5). Spring Creek rainbow 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, 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 rainbow trout population in subsequent analyses. 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 $p = 0.004$). 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 population equilibrium for HWE that cannot be supported without 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 (no data available), and negligible migration (natural and artificial movement above and below dams can be undocumented or inconclusive). 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.

Assignment tests by WHICHLOCI provided information on the proportional distribution of individual fish assignments back to their population of origin. 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 may be inflated due to the lack of alternative baseline data outside of those generated by this study (Manel and others 2005). Loci were ranked according to their relative contribution to these analyses in Table 6.

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.

		Locus											*HWE*
Drainage / Population	*N*	*Ogo1a*	*Ogo4*	*Omy27*	*Omy77*	*Omy325*	*Oneμ8*	*Oneμ10*	*Oneμ11*	*Ots1*	*Ots3*	*Ots4*	*Total*
1 American River Middle Fork below Rubicon River	44	–	–	–	–	+	–	–	–	–	+	–	9
2 American River lower below Nimbus Dam	41	–	–	–	–	–	–	–	–	–	–	–	11
3 American Trout & Salmon Company	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 Creek	43	+	–	–	+	+	–	–	+	–	+	–	6
8 Clear Creek upper below Bear Creek	64	–	–	–	+	+	+	–	+	+	+	+	4
9 Clear Creek middle below Whiskeytown 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 and Beegum Creek	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	

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	One 8	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

Year-to-Year Samples from One Location

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 allelic diversity and 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, rainbow 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 yearly 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 collections in subsequent analyses.

Clear Creek Drainage Results

We visualized allelic diversity at 11 microsatellite loci for 107 rainbow 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 rainbow trout was 6.7. Average heterozygosity (*Hz*) for Clear Creek rainbow trout populations was *Hz* = 0.63.

Rainbow Trout Populations Above and Below Bear Creek

ARLEQUIN's population pairwise comparison found significant differences in allelic frequencies for upper-basin rainbow 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 rainbow trout population above Bear Creek had two loci with heterozygosity deficiency and nine loci with heterozygosity excess. The rainbow trout population below Bear Creek had four loci with

heterozygosity deficiency and seven loci with heterozygosity excess. Effective population size (Ne) calculated using AGARst based on the SMM was Ne = 3088 above and Ne = 3632 below Bear Creek.

Rainbow Trout Above and Below Whiskeytown Dam

No significant differences in allelic frequencies were found for rainbow trout samples taken in two different years from the lower Clear Creek drainage below Sealtzer Dam, 1999 and 2001 (F_{st} = 0.016). Significant genetic differentiation was found between rainbow 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 rainbow 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 rainbow trout populations were not significantly different based on population pairwise F_{st} analyses (F_{st} = 0.01).

Clear Creek Populations and Hatchery Rainbow Trout

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

Rainbow trout hatchery strains. Two microsatellite loci (Omy77 and Omy27) used in this study overlapped with previous microsatellite studies of California hatchery rainbow trout (JLN unpublished data). Therefore, we used these loci to compare Clear, Mill, Deer, and Spring creeks with hatchery rainbow trout from Crystal and Mount Shasta hatcheries. The authors warn readers that they should exercise caution in drawing conclusions based on such limited data. Pairwise comparisons involving the American Trout & Company (collected for this study) and hatchery strains were done using two loci (Table 7), and all 11 loci combined. Putatively sterile (triploid) fish from the American Trout & 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 at Omy77 and Omy27 microsatellite loci were found in comparisons of hatchery rainbow trout from the American Trout & Salmon Company and the Crystal Hatchery strain (F_{st} = 0.01), but pairwise comparison of allelic frequency with the Shasta Hatchery stock was significantly different using these two loci. Allelic frequencies were significantly different in comparisons made between upper Clear Creek rainbow trout and hatchery rainbow trout from the American Trout & Salmon Company (using two as well as 11 loci combined), Mount Shasta and Crystal hatchery strains (two loci comparisons).

Table 7. Pairwise F_{st} comparisons between rainbow trout hatchery populations and Clear Creek rainbow 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.

	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 Trout & Salmon 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 Whiskeytown 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	

Clear Creek Analysis of Molecular Variance

Pairwise comparisons suggested a clear distinction in the allelic diversity found in upper Clear Creek in relationship to other local groups of fish. AMOVA analyses of the rainbow 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 rainbow trout carried on average only 1.18 alleles per locus for the 11 loci. Garza and Williamson's (2001) M for Spring Creek rainbow trout was M = 1.00 and this population was monomorphic at nine of the 11 loci. More than one allele was found only at loci Ogo4 and Oneμ8, each containing two alleles. Spring Creek F_{st} population pairwise comparisons ranged from F_{st} = 0.37 (Spring Creek and upper Clear Creek) to F_{st} = 0.71 (Spring Creek and the upper Sacramento River rainbow trout population). Effective population size (Ne) based on the SMM was Ne = 62 rainbow trout (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 (% of 2000 replicate trees) are provided in this figure. Genetic distance values demonstrate a clear distinction between upper Clear Creek rainbow trout (collected in the vicinity of Bear Creek) and rainbow trout collected from the lower and middle sections of this drainage below one or two impassable dams. Genetic distance analysis weakly supported genetic association found among fish from Coleman National Fish Hatchery, upper Sacramento River, and the middle and lower Clear Creek drainage where bootstrap values ranged between 12% and 42%.

Central Valley Watershed Results

We visualized allelic diversity at 11 microsatellite loci for rainbow 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 (American Trout & Salmon Company), and three Central Valley steelhead hatchery populations for our watershed-scale genetic analyses (Table 1). Due to the demonstrated population genetic differences found on Clear Creek (see above), we included rainbow trout from upper Clear Creek (above and below Bear Creek samples combined) and rainbow trout from lower Clear Creek (below Whiskeytown Dam) as two independent samples in our watershed 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 *O. mykiss* was Hz = 0.68.

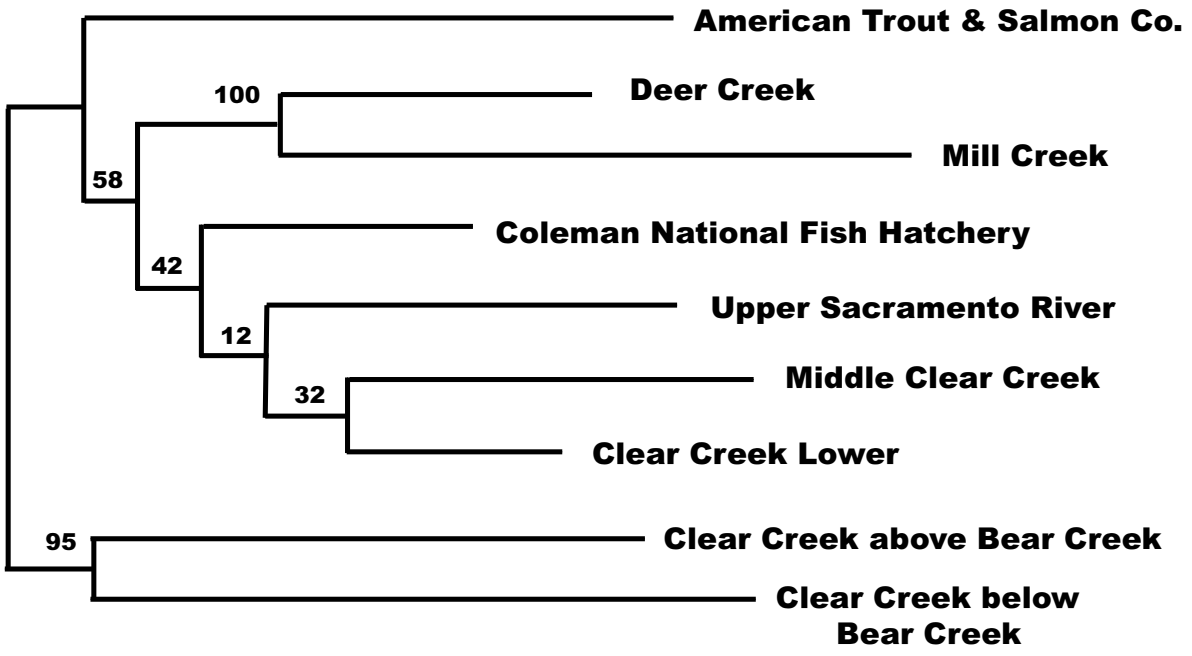

Figure 3. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the Clear Creek drainage rainbow trout populations. Branch bootstrap values (percent of 2000 replicate trees) are provided.

Rainbow 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 between the two locations 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.

Central Valley Pairwise Population Comparisons

Only 2% (N = 15) of the population pairwise F_{st} comparisons indicated no significant genetic differentiation between Central Valley populations (Table 8). All other pairwise comparisons supported significant pairwise allelic frequency differentiation.

Central Valley M and Ne

Garza and Williamson's (2001) M indicates a recent reduction in population, i.e. a population bottleneck, when M < 0.68. In tests of Central Valley rainbow trout populations mean M calculated across 11 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). Estimates of effective population size assuming mutation-drift equilibrium and a mutation rate of $2.05E^{-4}$ for both SMM and IAM are given by population in Table 9.

Table 8. F_{st} pairwise comparisons indicating no significant genetic differentiation (p > 0.05) between rainbow trout populations within the Central Valley based on allelic frequencies for 11 microsatellite loci.

Population	*Population*	*Pairwise F_{st}*	*F_{st} 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 SMM and IAM models and Garza and Williamson's (2001) M calculated for Central Valley rainbow trout populations across all loci.

Drainage / Population	*SMM 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 Estimates	5066	2488	0.626

Central Valley Analysis of Molecular Variance

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

Central Valley Genetic Distance

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

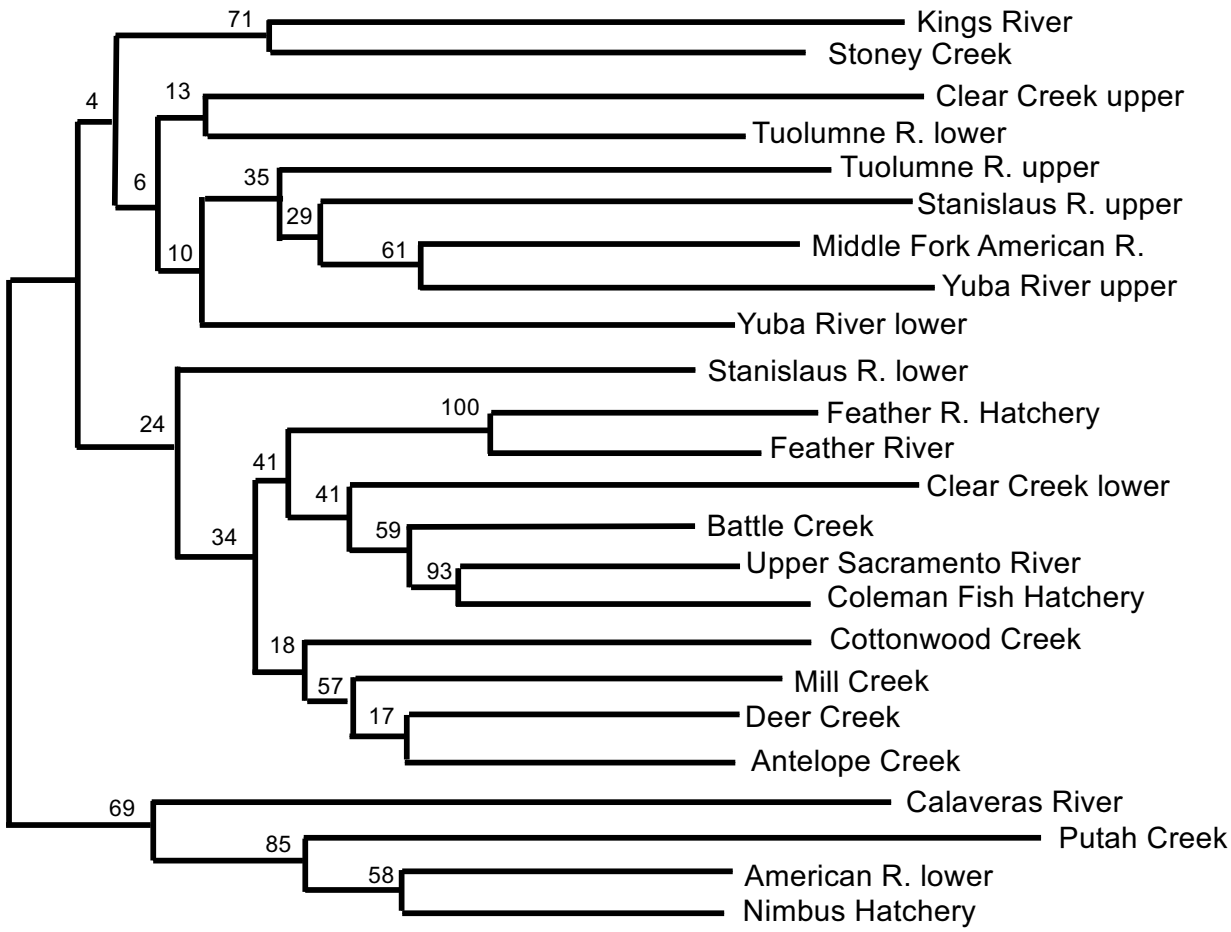

Figure 4. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the Central Valley system derived from allelic variation at 11 microsatellite loci. Branches with bootstrap values (percent of 2000 replicate trees) are provided.

DISCUSSION

This study focused on the genetic population structure of Central Valley steelhead and rainbow trout populations at two distinct scales. First, we investigated a fine-scale analysis of rainbow trout found within the Clear Creek drainage to look for potential wild rainbow trout in an upper Sacramento River tributary; analyzed drainage population structure; and tested hypotheses related to the credibility of specific localities as native strains. Secondly, we analyzed the current population genetic structure of steelhead found throughout the entire Central Valley watershed relating hatchery and putative wild populations, and populations found above and below barriers within the system. We examined implications derived from each of these scales independently and then together.

Clear Creek Drainage

Significant genetic population structure was documented within the Clear Creek drainage with these analyses. Rainbow 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 rainbow trout were also significantly differentiated from hatchery rainbow trout from the Coleman National Fish Hatchery and rainbow trout collected from the American Trout & Salmon Company.

Our analyses of hatchery rainbow trout in comparison with Clear Creek populations were less rigorous than the rest of the analyses performed in this study due to the limited overlap in standardized microsatellite loci available from past studies. Therefore, caution is advised in drawing conclusions from these limited data. Our analyses did show significant differences among Mount Shasta Hatchery rainbow trout, the Crystal hatchery rainbow trout strain, and upper Clear Creek rainbow 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 rainbow trout (Nielsen 1996a, 1996b; Nielsen and others 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 indicated that the vast majority of fish plantings 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 CDFG. 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 rainbow trout from these three hatchery strains (Nielsen and others 1997), however, no microsatellite data are currently available that are specific to Darrah Springs hatchery 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, 1996b). 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 informative in comparisons between hatchery and wild rainbow trout in the Sacramento River drainage. Genetic comparisons using mtDNA are confounded by the fact that the most common haplotypes (MYS1 and MYS3) found in rainbow trout in the Sacramento River system were the same for both hatchery and natural spawning fish. 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 rainbow trout were also significantly different from rainbow trout collected in Deer and Mill creeks suggests that putative anadromous origins for upper Clear Creek populations deserve further study. No significant genetic differences were found among several rainbow trout populations collected in the lower Clear Creek drainage, below Whiskeytown Dam. Lower Clear Creek rainbow 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, naturally or through stocking.

The Spring Creek rainbow 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 population bottleneck without further information on its history. This extreme bottleneck condition does, however, suggest caution when this population is considered as a candidate for restoration activities within the Clear Creek drainage. The potential for high levels of inbreeding in this population and potential viability problems that may incur need to be considered in future management plans involving Spring Creek. The impacts of removing any potential spawner from this population for artificial propagation must be balanced with the genetic impacts such a removal would have on the natural spawning population. Artificial propagation programs have been shown to result in significant genetic change that may lead to changes in locally adaptive traits (Unwin and Glova 1997; Reisenbichler and Rubin 1999; Waples and others 2005, in press). Consideration of genetic impacts of low effective population size in both the donor and recipient populations and the adaptive impacts of artificial culture should be included in any management decisions affecting Spring Creek fish.

However, genetic diversity measured by neutral genetic markers should not be the only criteria considered when choosing broodstock for recovery programs. Consideration must be placed on the retention of adaptive characteristics that have allowed this group of fish to survive in Spring Creek, despite greatly reduced numbers. On the other hand, the threat of out-of-basin transfers into Clear Creek and the risks of outbreeding depression may pose considerable risk to the locally adapted rainbow trout population in the Spring Creek. All of these factors should be part of the dialogue on short- and long-term implication of broodstock development and supplementation and how to best conserve and manage local endemic populations of rainbow trout throughout the Central Valley in light of the demand for increased steelhead restoration.

Central Valley Watershed

Significant steelhead genetic population structure was found throughout the Central Valley. Pairwise population comparisons showed significant differentiation in all but 2% of the population-pairwise 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 and others 1999; Heath and others 2001, 2002; Beacham and others 2004).

Estimates of effective population size based on SMM 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 and considered a relative value without additional demographic information (Waples 1990; Heath and others 2002; Ardren and Kapuscinski 2003). Immigration, as a result of hatchery propagation and stocking, 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 or among stocks of hatchery and wild fish and can give insight into the relationship between census

population size and the number of effective breeders (Frankham 1995; Heath and others 2002; McLean and others 2004; Seamons and others 2004). 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 and others 2002; see also 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 rainbow trout from the Feather River. These data suggest genetic similarities among hatchery populations and geographically proximate rainbow trout populations with high levels of gene flow. There are several hypotheses about what 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 National Fish Hatchery steelhead 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.) In 1995, the USFWS started releasing hatchery steelhead above the barrier weir at Coleman hatchery to spawn naturally in an effort to reestablish a self-sustaining steelhead population. Out-of-basin steelhead eggs were introduced into the Coleman Hatchery from the Mad River Hatchery in 1978 (Campton and others 2004). The founding stock of the Feather River Hatchery appears to have been primarily from local origins, with much of the original founding stock derived from strays from the Coleman National Fish Hatchery. Nimbus Hatchery steelhead were of mixed origins, including fish collected for broodstock from the Van Arsdale Fisheries Station on the Eel River. There were, however, extensive transfers of eyed-eggs and juveniles between Nimbus and Feather River hatcheries (Campton and others 2004).

It is interesting to observe that in this study hatchery-wild gene flow was 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. Microsatellites were able to trace gene flow in hatchery steelhead introduced into Lake Michigan in another recent study (Barton and Scribner 2004). Additional molecular markers and additional fine-scale sampling may be needed to provide information on the movements of hatchery fish within the basin and estimates of reproductive success 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 rainbow 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 rainbow 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 rainbow 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 rainbow trout populations around dams and barriers within these rivers, however, the potential artifact of hatchery stocking of rainbow trout above such barriers cannot be ruled out as a potential contributing factor in these relationships. A more thorough spatial analysis at each location, such as was done on Clear Creek in this study, may allow inference on the direction and duration of such isolation between rainbow trout population pairs above and below barriers in these rivers.

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 and others 1994; McCusker and others 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 rainbow 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 Central Valley drainage populations exists for *O. mykiss*. 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 for steelhead in these two river systems and little divergence between them relative to the relatively high level of structuring that occurs among individual rivers within the sub-drainages. However, we cannot rule out potential homogenization effects of past inter-drainage transfers and out-of-basin stocking of hatchery steelhead in anadromous waters. For example, the Mokelumne River Hatchery (San Joaquin River drainage) has a history of obtaining steelhead eggs from the Feather River Hatchery (Sacramento River

drainage) and the Calaveras River was stocked with Nimbus Hatchery steelhead in brood-years 1992 and 1995. The relative impacts, both genetic and adaptive of these inter-drainage transfers are unknown.

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 rainbow 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 rainbow 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 hatchery stocking, and a depiction of the genetic diversity on a finer scale based on multiple within-drainage sampling, 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 most non-hatchery stocks currently in decline (Mills and others 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 populations have also had potentially significant effects on Central Valley steelhead with important implications for genetic diversity and restoration (McEwan 2001). The implications of intraspecific 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 stocks, is important to our understanding of the status of remaining wild populations.

This study provides important information on Central Valley steelhead genetics previously not available to the interested public and mangers. Genetic differentiation between the major drainages within the Central Valley, Sacramento and San Joaquin rivers, were not great supporting a close evolutionary relationship among steelhead populations throughout the Central Valley. However, retaining the significant genetic variation depicted in this study in pairwise comparisons between different populations appears critical to future management considerations

dedicated to the conservation of genetic diversity. The impacts of previous hatchery management practices are reflected in the current genetic relationships found between hatchery and geographically proximate naturally spawning stocks. This relationship needs to be included in future hatchery management plans and consideration for conservation. We recommend implementation of genetic protocols comparing hatchery releases and local spawning stocks to monitor the genetic impacts of continued hatchery practices throughout the Central Valley to reduce the potential for genetic divergence of hatchery steelhead from natural spawning stocks and to maintain genetic diversity and fitness in natural spawning populations not currently influenced by hatchery stocks.

Looking at rainbow 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 management questions brought to these analyses on Clear Creek were concise and the microsatellite data were efficient at answering them. The weakness 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. We highly recommend further study of California's hatchery populations to address this issue. Our analysis of the Central Valley steelhead, however, leaves us with as many questions as it does answers. We recommend additional fine-scale genetic analyses, within individual rivers, be considered as additional information in interpretation of these broader basin-wide results.

Management and conservation of genetic diversity demands quantitative values that can address differences at multiple levels or scales. The geographic distribution of genetic diversity and structuring across the landscape depends on the ecological reality of spatial heterogeneity and recent changes in ecological condition that may not reflect current management expectations or needs. Genetic implications of broodstock selection and hatchery development of local stocks need to be emphasized in current and future stock development plans. In considerations of genetic diversity in a species such as coastal *O. mykiss* with significant variation in life history facilitating local adaptation at both freshwater and marine life stages, it is important that evidence of fine scale structuring be taken into account in combination with larger basin-level analyses. Such data add inference on the potential importance of smaller groups of fish in ecosystem structure and population dynamics within watersheds under consideration for restoration. The mechanisms providing flexibility of life history in *O. mykiss* still elude genetic considerations with current marker technologies and should be the focus of future research for this species. Management considerations of the unique life history traits found in *O. mykiss* in California may hold the key to their survival and adaptation to future environmental conditions.

ACKNOWLEDGMENTS

G. Kevin Sage developed microsatellite multiplex systems used in this study. The authors wish to thank Katie Perry, Kevin Niemela, Matt Brown, Jess Newton, James Smith, Cesar Blanco, Michael Lacy, Dennis McEwan, George Edwards, Randy Benthin and Sara Graziano for their assistance and contributions to this study. We especially want to thank Paul Moran, Don Campton and three anonymous reviewed for their corrections and suggestions in review of the draft manuscript. The manuscript was improved substantially by their comments. This study was partially funded by the CALFED Bay-Delta Program

through the California Department of Fish and Game; U.S. Fish and Wildlife Service, Region 1; and the U.S. Geological Survey, Alaska Science Center.

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