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Mitochondrial DNA and Nuclear Microsatellite Diversity in Hatchery and Wild *Oncorhynchus mykiss* from Freshwater Habitats in Southern California

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Abstract.—We examined mitochondrial control-region haplotype diversity and allelic frequency distributions for three polymorphic microsatellite loci in 541 coastal *Oncorhynchus mykiss* collected from six habitats associated with different levels of human activity and ocean access in southern California. Extensive urbanization, climatic unpredictability, and the accelerated rate of decline in anadromous fish suggested a probable loss of genetic diversity in this area due to habitat fragmentation, geographic isolation, and population bottlenecks. Unexpectedly high levels of genetic diversity were found in southern California populations of *O. mykiss*. Haplotype diversity (H_S) was highest in anadromous fish (H_S = 0.74) and lowest in the Whale Rock Hatchery trout (H_S = 0.32). The proportion of variation attributable to population differentiation among habitat groups (G_{ST}) was 10%. Haplotype frequencies showed a close relationship between anadromous steelhead and resident rainbow trout from closed habitats (D_{ST} = 0.03). Combined microsatellite allelic diversity (at loci *Omy77*, *Omy207*, and *Ssa289*) was highest in rainbow trout from closed habitats (88%), and lowest in Whale Rock Hatchery fish (29%). Greatest microsatellite distance ($\delta\mu$ = 17.1) was between anadromous steelhead and reservoir rainbow trout, and closest identity ($\delta\mu$ = 1.8) was among rainbow trout from closed habitats, hatchery rainbow trout, and reservoir rainbow trout. Analysis of genetic distance measures for both molecular markers showed that considerations of life history patterns and freshwater habitats that retain ocean access remain important factors in the preservation of the unique genetic diversity found in southern California coastal *O. mykiss*.

Genetic population structure in most studied species differentiates along broad-scale geographic gradients because of historic patterns of dispersal and gene flow (Avise 1994 and references therein). Such phylogenetic structure is thought to reflect the architecture of regional dispersal and genetic drift over long periods of time that relate to major geologic shifts or partitions, such as refugia and dispersal patterns during Pleistocene glaciation or species subdivision after the rise of the Sierra Nevada crest. Patterns of genotypic variation, however, can be measured on multiple scales that demonstrate different spatial and temporal subdivisions (Healey and Prince 1995; Nielsen et al. 1997). In this study, we show that microgeographic population structure in rainbow trout *Oncorhynchus mykiss* and steelhead, the species' anadromous form, can be demonstrated as fine-scale spatial groupings of genotypes that reflect patterns of recent human manipulations of habitat and population structure that have differentially impacted the unique genetic diversity documented in this species at the southern extent of its range.

Steelhead historically used coastal drainages as far south as the Rio Santo Domingo River in Baja California Norte, Mexico (Needham and Gard 1959). Today, Malibu Creek in Los Angeles County is the southernmost stream containing steelhead. Steelhead in arid, sandy streams of southern California (Santa Barbara, Ventura, and Los Angeles counties) once provided a reliable food source for Chumash Indians and early European settlers but

have recently declined to near-extirpation (Nehlsen et al. 1991; Swift et al. 1993). Of 122 streams south of San Francisco Bay with historic steelhead runs, 33% no longer support anadromous fish (McEwan and Jackson 1996). Most extirpated populations occurred at the southern extent of the range. Remaining southern steelhead populations are greatly diminished compared to historic records. Spawning runs once numbering in the tens of thousands now number less than 500 fish in all streams throughout the region (Shapolov and Taft 1954; Titus et al., in press).

This study includes populations of *O. mykiss* captured in freshwater habitats north and south of Point Conception, California. This area includes both the south-central and southern California Evolutionarily Significant Units (ESUs) proposed for listing under the Endangered Species Act (Busby et al. 1996). Risk factors outlined in the report of Busby et al. (1996) include the widespread degradation, destruction, and blocking of freshwater habitats by sedimentation and artificial water impoundments and the possible genetic effects of widespread stocking of hatchery rainbow trout.

Major river systems throughout southern California are subject to extreme variations in precipitation, frequent development of extensive sand berms that completely block river mouths, seasonally elevated water temperatures (>24°C), episodic droughts, flash floods, and fires (Swift et al. 1993; Carpanzano 1996). Numerous dams built over the last 75 years for water withdrawal and diversion in order to support urban development on most of California's southern rivers have resulted in loss of considerable steelhead freshwater habitat (McEwan and Jackson 1996). Pollution, dredging, mining, and flood control projects generated by southern California's burgeoning human population have further accelerated degradation of habitats critical to southern steelhead in both freshwater and nearshore marine environments (Nehlsen et al. 1991; Busby et al. 1996; Carpanzano 1996).

This extensive list of demographic and environmental risks suggested a probable loss of genetic diversity in remnant populations of *O. mykiss* because of habitat fragmentation, geographic isolation, and population bottlenecks. Recent developments in DNA technology allow investigations of population structure with noninvasive sampling of rare or endangered animals (Wright and Bentzen 1994; Nielsen 1996; Wenberg et al. 1996). In their recent study of California's steelhead populations, Nielsen et al. (1994b) used molecular genetics to document high levels of genetic diversity in southern *O. mykiss*, a finding recently confirmed by allozyme analyses of some of the same populations (Busby et al. 1996). In this study, we used molecular markers to address the degree and distribution of genetic diversity in southern California *O. mykiss* collected from habitats with different histories of human modification and differing access to the ocean. We combined stream populations with similar histories and manupulation patterns for molecular analyses.

Steelhead exhibit one of the most complex suites of life history traits of any Pacific salmonid (Behnke 1992). Little, however, is known, about life history patterns of steelhead from southern California rivers. The highly variable and unpredictable environmental conditions described above suggest that steelhead need dramatically flexible strategies to survive, migrate, and spawn in these arid environments. Variation in steelhead life history patterns has been well documented (Shapovalov and Taft 1954; Behnke 1992). Steelhead exhibit mixed freshwater age composition throughout their range (Burgner et al. 1992), and there have been reports of isolated populations maturing as freshwater fish (Gwartney 1983; Gall et al. 1990).

Current evidence suggests that resident coastal rainbow trout and anadromous steelhead are not taxonomically distinct but represent different life history ecotypes (Rybock et al. 1975; Parkinson et al. 1984; Wishard et al. 1984; Reisenbichler and Phelps 1985; Currens et al. 1988; Smith and Stearley 1989). The relationship between rainbow trout exhibiting anadromony and nonanadromony (i.e., freshwater residents) in coastal systems, however, remains poorly studied. When access to the ocean was effectively closed off in many streams by the construction of dams and water diversions, southern California steelhead trapped as juveniles may have matured and reproduced as freshwater resident rainbow trout.

The relationship between anadromous steelhead and coastal rainbow trout is complicated by over 100 years of introductions of hatchery-produced rainbow trout into freshwater habitats throughout southern California. The early genetic ancestry of nearly all rainbow trout strains can be traced back to egg collections made by the U.S. Fisheries Commission at Baird Station on the McCloud River in northern California at the turn of the century (Busack and Gall 1980). Eggs taken during development of the first hatchery rainbow trout strain probably contained a mixture of both coastal anadromous and resident rainbow trout (Needham and

Behnke 1962; Busack and Gall 1980; Gall and Crandell 1992). Since the 1890s, hatchery-produced rainbow trout, primarily derived from McCloud River fish, have been planted into most streams, rivers, lakes, and reservoirs in California. Hatchery strains, however, have been subject to varying degrees of hybridization among source populations, founder effects, and intensive artificial selection during a century of domestication (Busack et al. 1979; Busack and Gall 1980; Gall and Crandell 1992).

Allozyme investigations of domestic strains of rainbow trout in California have shown high levels of genetic diversity typical of that observed among local conspecific wild rainbow trout populations (Busack et al. 1979; Thompson 1985; Berg and Gall 1988). The combined effects of husbandry practices and broodstock selection during aquaculture of rainbow trout have been shown, however, to contribute to significant changes in genetic variation in hatchery fish when compared with sympatric wild stocks (Allendorf and Phelps 1980; Alexander and Hubert 1995; Reisenbichler and Brown 1995). Hatchery broodstocks derived from different geographic locales should, however, retain biogeographic genetic structure reflective of their source populations (Nielsen et al. 1994a), allowing us to compare genetic diversity in hatchery strains derived primarily from northern lineages with genetic diversity found in southern rainbow trout populations.

In this study, we examine genetic diversity for mitochondrial DNA (mtDNA) and three microsatellite loci in freshwater resident rainbow trout and steelhead collected in habitats where historic runs of southern steelhead have been virtually extirpated. We consider the effects of human manipulation of river drainages and fish stocks by comparing genetic diversity within and among rainbow trout and steelhead from six freshwater habitat groups representing different degrees of human activity: (1) streams that are closed to ocean migration of rainbow trout by dams or impassable water diversions; (2) streams that currently retain intermittent access to the ocean despite periodic dewatering of parts of the channel; (3) two southern California streams with anadromous steelhead found as migrating adults or smolts; (4) two southern California reservoirs; (5) hatcheries that hold 10 strains of rainbow trout; and (6) one hatchery (Whale Rock Hatchery) thought to retain landlocked steelhead from Morro Bay.

Methods

Sampling protocol.—The study area included streams and reservoirs from Big Pico Creek, 3.8 km south of San Simeon Point, to the San Luis Rey River, 64 km south of Los Angeles, in southern California. All sampling sites were within 120 km north and 205 km south of the Point Conception species barrier (Figure 1). Fin clips were collected from sample fish in streams with documented runs of anadromous steelhead but where negligible numbers of spawning steelhead (<30 fish/year) have been reported over the last 50 years (Titus et al., in press). Fin samples were taken from 306 wild rainbow trout collected by electrofishing, angling, live trapping, and dipnetting (Table 1). Sampled southern steelhead consisted of 6 migrating adults and 24 smolts captured in Malibu Creek and the lower Santa Ynez river, 1992–1993. For historical comparisons, DNA was amplified from three steelhead specimens preserved by toxidermy caught by angling in the Ventura River in the early 1940s by Ben Henke (E. Henke, Ashland, Oregon, personal communication).

Hatchery rainbow trout were obtained from the Filmore, Whitney, San Joaquin, Hot Creek, and Mount Shasta state fish hatcheries in California. The Federal 6F2 rainbow trout strain (currently discontinued as a hatchery strain in California) was sent to our laboratory from the Finger Rock (Colorado) National Fish Hatchery. Rainbow trout from the Arlee strain (also discontinued in California) were sent to us by R. Snyder of the Montana Department of Fish, Wildlife and Parks (Missoula).

Hatchery collections contained samples from 10 rainbow trout strains that have been stocked in streams and reservoirs in California (California Department of Fish and Game [CDFG], unpublished data). These collections consisted of multiple samples, representing different year-classes (1992–1995) for any one strain, that were sent to our laboratory from different hatcheries. Strains stocked in the last 10 years in southern California include Mount Shasta, Whitney, Hot Creek, Coleman, Wyoming–Hot Creek, Arlee, and Federal 6F2 strains (Filmore and San Joaquin state hatcheries, unpublished records). Because early hatchery stocking records for steelhead and rainbow trout in southern California streams remain incomplete or unverified (R. Ducey, CDFG, Dry Creek Hatchery, personal communication), it was not possible to verify hatchery strains with known stocking histories specific only to southern California.

Fish from the Whale Rock Hatchery near Morro

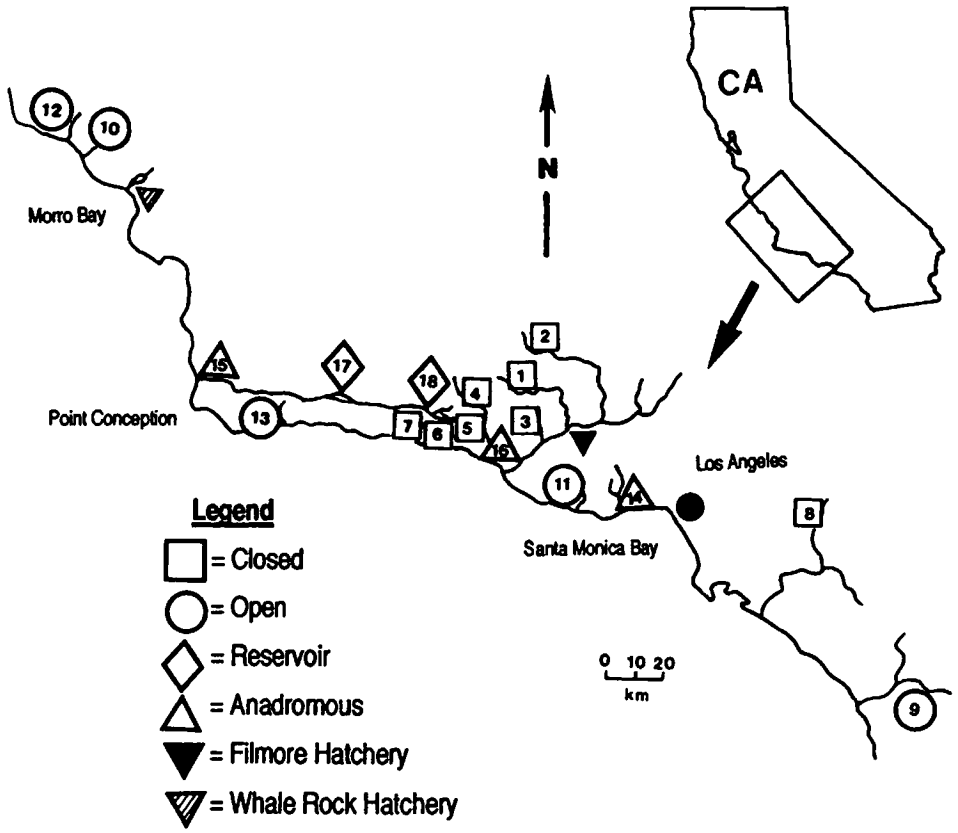

FIGURE 1.—Southern California streams and reservoirs where *Oncorhynchus mykiss* were sampled. Numbers represent specific geographic or sample locations: (1) Sespe Creek, (2) Piru Creek, (3) Santa Paula Creek, (4) Matilija Creek, (5) Fox Creek, (6) Franklin Creek, (7) Alder Creek, (8) San Antonio Creek, (9) Pauma Creek, (10) Santa Rosa Creek, (11) Arroyo Sequit, (12) Big Pico Creek, (13) Gaviota Creek, (14) Malibu Creek, (15) Santa Ynez River, (16) Ventura River, (17) Cachuma Reservoir, and (18) Jameson Reservoir. The Filmore and Whale Rock hatcheries are indicated on the map; several hatchery rainbow trout strains were from populations currently propagated in hatcheries found outside of the map area.

Bay were sent to our laboratory by B. Cox, CDFG. These samples were collected from fish used for pathology analyses at the hatchery during July 1992. This hatchery stock is thought to represent a landlocked population of steelhead. Before the impoundment of Whale Rock Reservoir in 1961, Old and Cottontail creeks supported a large steelhead run. Following dam construction, CDFG created Whale Rock Hatchery to maintain the steelhead population and provide stock for enhancement efforts in other San Luis Obisbo County reservoirs. The original Whale Rock broodstock (40 fish) were collected at a temporary weir placed at Old Creek Cove (P. Cleveland, Central Coast Enhancement, Inc., personal communication). This hatchery currently supplements fish within Whale Rock Reservoir for recreational angling. There are no records of fish released from the hatchery into waters below the dam (K. R. Anderson, CDFG, personal communication).

Caudal fin tissues (2 mm^2) collected from live fish were air-dried in the field and shipped directly to our laboratory. Skin tissue was extracted from the back of caudal fins preserved by taxidermy. Salmonid DNA was extracted from a small portion of dried fin or skin tissue using Chelex 100 resin (BioRad), following the methods given in Nielsen et al. (1994a).

Mitochondrial DNA.—We used conserved primers (S-phe and P2) to amplify a highly variable segment of salmonid mtDNA, including 188 base pairs (bp) of the control region and 5 bp of the adjacent phenylalanine tRNA gene. Double- and single-stranded amplifications were performed

TABLE 1.—Sampling areas for landlocked (closed to ocean), limited ocean-access (open to ocean), anadromous, reservoir, hatchery rainbow trout, and landlocked steelhead populations. The numbers of individuals tested for mtDNA and microsatellite genetic diversity are given by population. Ventura River adults were archived fish collected in the 1940s and were excluded from some analyses. Hatchery abbreviations are WH = Whitney Hatchery; FH = Filmore Hatchery; SJH = San Joaquin Hatchery; HCH = Hot Creek Hatchery; CH = Crystal Hatchery; MSH = Mount Shasta Hatchery; MSTH = Montana State Fish Hatchery; FRNFH = Finger Rock (Colorado) National Fish Hatchery; and WHR = Whale Rock Hatchery.

Population[a]	Age-class	Source	mtDNA	Microsatellite
Closed to ocean				
1 Sespe Creek (Santa Clara River)	Fry	Wild capture	35	10
2 Piru Creek (Santa Clara River)	Fry	Wild capture	23	11
3 Santa Paula (Santa Clara River)	Fry	Wild capture	41	16
4 Matilija Creek (Ventura River)	Fry	Wild capture	32	15
5 Fox Creek (Santa Ynez River)	Fry	Wild capture	19	14
6 Franklin Creek (Santa Ynez River)	Fry	Wild capture	11	8
7 Alder Creek (Santa Ynez River)	Fry	Wild capture	25	11
8 San Antonio Creek (Santa Ana River)	Fry	Wild capture	9	9
Total			195	94
Open to ocean				
9 Pauma Creek (San Luis Rey River)	Fry	Wild capture	23	15
10 Santa Rosa Creek	Fry	Wild capture	7	7
11 Arroyo Sequit	Fry	Wild capture	8	8
12 Big Pico Creek	Fry	Wild capture	12	7
13 Gaviota Creek	Fry	Wild capture	27	17
Total			77	54
Anadromous: steelhead				
14 Malibu Creek	Adult	Wild capture	5	5
	Smolt	Wild capture	8	8
15 Santa Ynez River	Adult	Wild capture	1	1
	Smolt	Wild capture	16	15
16 Ventura River	Adult	Wild capture	3	0
Total			33	29
Reservoir: rainbow trout				
17 Cachuma Reservoir	Fry	Wild capture	8	7
18 Jameson Reservoir	Fry	Wild capture	26	25
Total			34	32
Hatchery: rainbow trout (rbt) and steelhead (sth)				
19 Whitney strain (rbt)	Fry	WH, FH, SJH	27	15
20 Hot Creek strain (rbt)	Fry	HCH, FH, SJH	21	24
21 Wyoming/Hot Creek strain (rbt)	Fry	WH, FH	11	16
22 Shasta/Pit strain (rbt)	Fry	CH	12	30
23 Erwin strain (rbt)	Fry	HCH	7	10
24 Coleman strain (rbt)	Fry	HCH, FH	7	13
25 Mount Shasta strain (rbt)	Fry	MSH	26	39
26 Eagle Lake Hatchery strain (rbt)	Fry	MSH, SJH	32	14
27 Arlee strain (rbt)	Fry	MSTH	10	10
28 Federal 6F2 strain (rbt)	Fry	FRNFH	21	21
29 Whale Rock Hatchery (sth)	Fry	WRH	22	13
Total			196	205

[a] Numbers for stream and reservoir fish refer to map numbers on Figure 1.

with polymerase chain reaction (PCR). Single-stranded product was sequenced directly and the DNA visualized on x-ray film. The DNA protocols, sequence for specific primers, and the complete control region segment amplified in *O. mykiss* are given in Nielsen et al. (1994a).

Estimates of nucleotide diversity (π) and mitochondrial haplotype diversity within (H_S) and between (D_{ST}) our six habitat groups (closed stream, open stream, reservoir, hatchery rainbow trout, hatchery landlocked steelhead, and anadromous steelhead) were calculated with the methods given in Nei (1987). The proportion of genetic variation attributable to population differentiation (G_{ST}) among the six habitat groups was calculated according to methods in Nei (1987, formula 8.27).

Pairwise haplotype diversity measures (D_{ST}) were plotted as an unrooted consensus neighbor-joining (NJ) tree based on the NEIGHBOR81 and CONSENSE procedures from PHYLIP (Felsenstein 1993). One hundred replicate NJ trees were generated with resampled versions of the input data. The CONSENSE program from PHYLIP was used to obtain bootstrap estimates and assess reproducibility of branching patterns found in our consensus mtDNA tree.

We used an unbiased estimate of the Fisher's exact test (Raymond and Rousset 1995a), based on a Markov chain adaptation of row-by-column contingency tables, to analyze independence between habitat groups. This test provides the probability of being wrong when H_0 (i.e., rows and columns are independent) is rejected. Fisher's exact tests (GENEPOP) give equivalent probability values to the iterative chi-square probability test (Roff and Bentzen 1989) but have been shown to have several computational advantages in multiloci analyses (Raymond and Rousset 1995b). Fisher's exact tests were run on all possible pairs of habitat groups with four random-number seed generators and the dememorization number set to 1,000. Fifty batches were run for each test with 1,000 iterations/batch.

Microsatellites.—Three nuclear microsatellite loci developed at Dalhousie University (*Omy77, Omy207,* and *Ssa289*) were chosen based on their high level of polymorphism in rainbow trout and steelhead. *Omy77* and *Omy207* were developed specifically for *O. mykiss. Ssa289* was developed for Atlantic salmon *Salmo salar.* For each locus, primer B was labeled according to protocols given in Nielsen et al. (1994a). To amplify microsatellites, we followed the methods adapted from Nielsen et al. (1994a) and given in Nielsen et al. (1997). The size reported here for each microsatellite allele was equal to the size of the total product amplified (including amplified primer sequence). Allelic size was determined by three methods: (1) reference to the cloning vector M13mp18 (bp 6161–6326) used as a marker ladder on all gels, (2) known DNA samples of *O. mykiss* that were rerun on each gel, and (3) a double-stranded reference marker that showed the common alleles available for each microsatellite locus.

Only unambiguous bands were scored, and in the case of multiple (shadow) bands, the darkest band was scored as the allele. The appearance of stutter bands that overlap between alleles was resolved by comparing the intensity and number of stutter bands for each individual at each locus (O'Reilly and Wright 1995). To insure consistency in both PCR reactions and the scoring of microsatellites, 7.8% of all samples were rerun on different gels and scored independently.

TABLE 2.—Nucleotide[a] variation found in the mtDNA control region for rainbow trout and steelhead in southern California. Haplotypes MYS11 and MYS15 were not found in these samples. Sequence variation in these haplotypes are published in Nielsen et al. (1997). Base pair numbers are equivalent to those given in Digby et al. (1992). Single base mutation events relative to haplotype MYS1 are highlighted in bold.

mtDNA haplotype	Base pair number: 1021	1050	1052	1086	1103	1106	1109	1147	1149
MYS1	T	T	T	T	A	A	G	G	C
MYS2	**C**	T	T	T	A	A	G	G	C
MYS3	T	T	T	T	A	A	**A**	G	C
MYS4	T	T	T	**C**	**G**	A	G	G	C
MYS5	T	T	T	**C**	**G**	**C**	G	**A**	C
MYS6	T	**C**	T	**C**	**G**	**C**	G	**A**	C
MYS7	T	T	T	**C**	A	A	G	**A**	C
MYS8	T	T	T	**C**	A	**C**	G	**A**	C
MYS9	T	T	T	T	A	A	G	**A**	C
MYS10	T	T	**C**	T	A	A	**A**	G	C
MYS12	T	T	T	**C**	A	**C**	G	G	C
MYS13	T	T	T	**C**	**G**	**C**	G	G	C
MYS14	**C**	T	T	T	A	A	**A**	G	C
MYS16	T	T	T	T	A	A	G	**A**	**T**

[a] A = adenine, C = cytosine, G = guanine, T = thymine.

Alleles found in less than 5% of total population were considered rare, based on the frequency distribution of each allele in the total number of fish studied for each locus. Estimates of allelic diversity based on rare alleles were corrected by sample size, based on the number of fish tested for that locus within each habitat group. Fisher's exact tests on microsatellite allelic frequency data were run on all possible pairs of habitat groupings for each locus and for all loci combined. Statistical significance levels (initial $\alpha = 0.025$) for Fisher's exact analyses were set with sequential Bonferroni tests (Rice 1989).

A pairwise genetic distance matrix was calculated for allelic diversity according to the methods of Goldstein et al. (1995) for the three microsatellite loci combined. Delta mu ($\delta\mu$) distance measure (Goldstein et al. 1995) was calculated with MICROSAT (Minch 1997). This distance measure assumes a linear expectation of the average squared distance for each locus (assuming no correlation between mutation rate and repeat score) and uses the arithmetic average of mutation rates across loci. Delta mu ($\delta\mu$) statistics of Goldstein et al. (1995) are equivalent to general analysis of variance with an average sum of squares of dif-

TABLE 3.—Numbers of southern California *Oncorhynchus mykiss* from landlocked populations (closed to ocean), limited ocean-access streams (open to ocean), reservoirs, and anadromous and hatchery fish listed by mtDNA haplotype. Haplotypes are numbered according to base substitutions in a highly variable segment of the mtDNA control region given in Table 2. Populations are identified more fully in Table 1.

Population	mtDNA haplotype														Total
	1	2	3	4	5	6	7	8	9	10	12	13	14	16	
Closed to ocean															
1 Sespe	0	0	24	0	0	0	0	10	0	1	0	0	0	0	35
2 Piru	15	0	5	0	0	0	0	3	0	0	0	0	0	0	23
3 Santa Paula	36	0	3	0	1	0	0	0	1	0	0	0	0	0	41
4 Matilija	7	0	19	0	4	0	0	2	0	0	0	0	0	0	32
5 Fox	0	0	0	0	5	0	0	10	0	0	4	0	0	0	19
6 Franklin	2	0	1	0	4	4	0	0	0	0	0	0	0	0	11
7 Alder	2	0	1	0	6	0	0	6	4	0	0	6	0	0	25
8 San Antonio	5	0	3	0	0	0	0	0	0	1	0	0	0	0	9
Total	67	0	56	0	20	4	0	31	5	2	4	6	0	0	195
Open to ocean															
9 Pauma	11	0	3	0	8	0	0	0	0	0	0	1	0	0	23
10 Santa Rosa	1	1	0	0	4	1	0	0	0	0	0	0	0	0	7
11 Sequit	0	0	0	0	0	0	0	5	0	0	3	0	0	0	8
12 Big Pico	0	1	0	0	7	2	0	0	0	0	0	2	0	0	12
13 Gaviota	0	0	0	0	11	0	1	15	0	0	0	0	0	0	27
Total	12	2	3	0	30	3	1	20	0	0	3	3	0	0	77
Anadromous: steelhead															
14 Malibu	1	1	0	3	4	0	0	4	0	0	0	0	0	0	13
15 Santa Ynez	6	0	5	0	0	0	0	2	0	1	0	0	3	0	17
16 Ventura	1	0	1	0	1	0	0	0	0	0	0	0	0	0	3
Total	8	1	6	3	5	0	0	6	0	1	0	0	3	0	33
Reservoir: rainbow trout															
17 Cachuma	3	0	3	0	1	0	0	1	0	0	0	0	0	0	8
18 Jameson	4	0	7	0	12	0	0	3	0	0	0	0	0	0	26
Total	7	0	10	0	13	0	0	4	0	0	0	0	0	0	34
Hatchery															
19 Whitney	15	0	9	0	0	0	0	0	0	3	0	0	0	0	27
20 Hot Creek	11	0	9	0	0	0	0	0	0	1	0	0	0	0	21
21 Wyoming–Hot Creek	3	0	5	0	0	0	0	0	2	1	0	0	0	0	11
22 Shasta–Pit	12	0	0	0	0	0	0	0	0	0	0	0	0	0	12
23 Erwin	1	0	4	0	0	0	0	0	0	2	0	0	0	0	7
24 Coleman	4	0	3	0	0	0	0	0	0	0	0	0	0	0	7
25 Shasta	26	0	0	0	0	0	0	0	0	0	0	0	0	0	26
26 Eagle	0	0	0	0	0	0	0	0	0	0	0	0	0	32	32
27 Arlee	2	0	8	0	0	0	0	0	0	0	0	0	0	0	10
28 6F2	18	0	3	0	0	0	0	0	0	0	0	0	0	0	21
29 Whale Rock	1	0	0	0	1	2	0	18	0	0	0	0	0	0	22
Total	93	0	41	0	1	2	0	18	2	7	0	0	0	32	196
Overall total	187	3	116	3	69	9	1	79	7	10	7	9	3	32	535

ferences in allelic size within each population (D_0), and the average squared difference between all possible pairs of populations (D_1). These values are used to obtain an estimate of variance in allele size in the total population. Delta mu maintains an estimate of the mutation process under expectation of a strict, single-step (± one repeat unit) shift for each mutation event. The F_{ST} and mean heterozygosity for the three microsatellite loci were calculated with the $\delta\mu$ program, and expected equilibrium values were developed for the stepwise mutation processes (see Rousset 1996).

Delta mu distance data were used to generate an unrooted consensus neighbor-joining tree with the NEIGHBOR81 and CONSENSE applications from PHYLIP (Felsenstein 1993) that compared microsatellite diversity among the six habitat groups and among all rainbow trout hatchery strains. One thousand replicate $\delta\mu$ trees were generated to obtain bootstrap estimates and assess re-

TABLE 4.—Mitochondrial nucleotide (π)[a] and genetic diversity (H_S)[b] found within six habitat groupings of trout and steelhead in southern California. Sample size (N) is the number of individual fish sampled by habitat group. Number of types is the number of mtDNA haplotypes found in each population. Standard errors are given in parentheses.

Habitat grouping	N	Number of types	π (SE)	H_S (SE)
Closed to ocean	195	9	0.011 (0.001)	0.55 (0.06)
Open to ocean	77	9	0.010 (0.003)	0.57 (0.03)
Anadromous	33	8	0.014 (0.013)	0.74 (0.00)
Reservoir	34	4	0.013 (0.009)	0.68 (0.00)
Hatchery (rainbow)	174	5	0.003 (0.001)	0.34 (0.07)
Whale Rock Hatchery (steelhead)	22	4	0.010 (0.017)	0.32 (0.00)

[a] Nucleotide diversity or the average number of nucleotide differences per site (Nei 1987).
[b] Genetic diversity within habitat groups according to Nei (1987).

producibility of branching patterns found in our microsatellite consensus tree.

Results

Mitochondrial DNA

Fourteen unique mtDNA haplotypes were found in southern California *O. mykiss* and hatchery rainbow trout in this study (Table 2). Among haplotypes, the average nucleotide variation was 1.3%. The two most divergent haplotypes differed by 3.1%. Three samples of adult steelhead preserved by taxidermy from the Ventura River carried mtDNA haplotypes MYS1, MYS3, and MYS5.

Average (± SE) nucleotide diversity (π) calculated among habitat groupings was 0.007 ± 0.006. Hatchery rainbow trout had the lowest nucleotide diversity found within any habitat group (0.003 ± 0.001). Greatest genetic diversity, based on rare haplotypes, was found in the anadromous population, in which 27% of the fish carried rare haplotypes (Table 3). Lowest haplotype diversity was found in hatchery rainbow trout, in which only 5% of the fish carried rare haplotypes.

Average genetic diversity (D_{ST}) between the six habitat groups was 0.07. Average genetic diversity within habitat groups (H_S) was 0.65. The proportion of genetic variation attributable to population differentiation (G_{ST}) was 10%. Genetic diversity based on population structure within each habitat group was highest in anadromous fish (H_S = 0.74) and lowest in the Whale Rock Hatchery fish (H_S = 0.32; Table 4). The greatest haplotype diversity calculated between pairs of habitat groups was found between the Whale Rock Hatchery fish and all other hatchery rainbow trout (D_{ST} = 0.56; Table 5). The D_{ST} diversity measures showed the closest haplotype relationship for hatchery rainbow trout was with resident rainbow trout from closed habitats (D_{ST} = 0.07).

Fisher's exact analyses of paired comparisons of habitat groups supported significant independence between all pairs (Fisher's $P < 0.025$), with the exception of our comparison between rainbow trout from closed habitats and reservoirs (Fisher's $P = 0.05$). Fisher's exact tests performed among the hatchery rainbow trout strains gave mixed results with 24 of the 44 possible paired comparisons (55%), showing nonindependence for mtDNA haplotype frequencies between hatchery strains (Fisher's P-values ranged from 0.30 to 1.00). Significant haplotype frequency differences were found for all comparisons between hatchery rainbow trout strains and the Whale Rock Hatchery sample (Fisher's $P < 0.01$ in all cases).

Neighbor-joining analysis of Nei's haplotype diversity measure (D_{ST}) showed a closer genetic association for steelhead with wild rainbow trout from open freshwater habitats than with either rainbow trout or Whale Rock hatchery fish (Figure 2). Bootstrap values supporting the branching pat-

TABLE 5.—Pairwise table showing the haplotype diversity between habitat groupings (D_{ST}; Nei 1987; above the diagonal) and delta mu distance measure for three microsatellite loci combined (below the diagonal). Nei's average gene diversity between habitat groups was 0.07; G_{ST}, the relative magnitude of gene differentiation among habitat groups was 0.1. Average F_{ST} for three microsatellite loci combined was 0.07, based on calculations from Goldstein et al. (1995).

Population	Closed to ocean	Open to ocean	Anadromous	Reservoir	Hatchery	White Rock Hatchery
Closed to ocean	—	0.096	0.027	0.051	0.066	0.308
Open to ocean	3.562	—	0.059	0.047	0.266	0.225
Anadromous	15.803	5.548	—	0.054	0.133	0.242
Reservoir	1.785	6.752	17.102	—	0.179	0.362
Hatchery	1.798	3.361	15.796	6.931	—	0.564
Whale Rock Hatchery	11.569	6.953	3.504	10.302	13.589	—

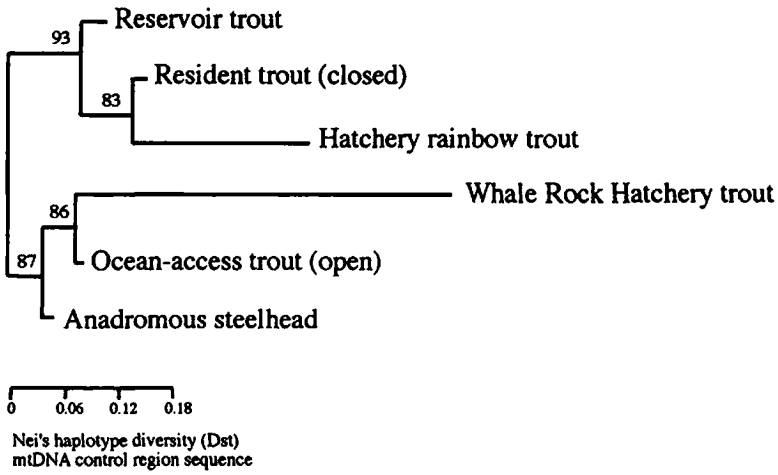

FIGURE 2.—Unrooted neighbor-joining tree showing genetic similarity (Nei's D_{ST}) based on mtDNA haplotype distributions for fish found in six southern California freshwater habitat types with different levels of human activity: (1) resident rainbow trout found above dams or impassable water diversions (closed), (2) rainbow trout collected from streams that currently retain intermittent access to the ocean despite seasonal dewatering of parts of the channel (open), (3) anadromous steelhead found as migrating adults or smolts, (4) rainbow trout collected in two southern reservoirs, (5) fish from 10 hatchery rainbow trout strains used for stocking in reservoirs and streams throughout California, and (6) one hatchery strain thought to retain landlocked steelhead captured in the Whale Rock Reservoir. Bootstrap values calculated for the branching patterns from D_{ST} analyses are based on consensus drawn from 100 replicate trees and are given as percentage values at the branching nodes.

terns in our consensus mtDNA haplotype diversity tree exceeded 80% in all cases.

Microsatellites

The three polymorphic microsatellite loci used in this study contained dimeric repeats (*Omy207* and *Ssa289*, poly[CA]-poly[GT]; *Omy77*, poly[CT]-poly[GA]; A = adenine, C = cytosine, G = guanine, T = thymine) found in tracts up to 83 repeat units long; 8–31 alleles were expressed per locus (Table 6). All three loci conformed to the expectation of a single-step allele model (SSM), with dinucleotide repeats separating alleles (Tables 7–9). Microsatellites developed specifically for *O. mykiss*, *Omy77* and *Omy207*, were more polymorphic in California rainbow trout and steelhead than the *Ssa289* locus developed for Atlantic salmon. Allelic diversity for *Omy77* and *Omy207* (measured as both the number of informative alleles and the size range of alleles found within each locus) was higher in our study of southern California rainbow trout and steelhead than results reported for the same species from more northern streams (Olsen et al. 1996; Wenburg et al. 1996). Locus *Ssa289* showed different size structure but similar allelic diversity in rainbow trout and steelhead (8 alleles) as that reported for this locus in Atlantic salmon (7 alleles; McConnell et al. 1995).

Microsatellite allelic diversity for all three loci

TABLE 6.—Microsatellite loci used to study rainbow trout and steelhead populations at the southern extent of their range in California. For primer sequence, *a* = forward, *b* = reverse (A = adenine, C = cytosine, G = guanine, T = thymine).

Primer name	Number of alleles observed	Product size (bp)	Primer sequence	Source
Omy77	27	93–153	*a*>5′-CGT-TCT-CTA-CTG-AGT-CAT *b*>5′-GTC-TTT-AAG-GCT-TCA-CTG-CA	Morris et al. (1996)
Omy207	31	98–166	*a*>5′-ACC-CTA-GTC-ATT-CAG-TCA-GG *b*>5′-GAT-CAC-TGT-GAT-AGA-CAT-CG	M. O'Connell (Dalhousie University, personal communication)
Ssa289	8	110–126	*a*>5′-CTT-TAC-AAA-TAG-ACA-GAC-T *b*>5′-TCA-TAC-AGT-CAC-TAT-CAT-C	McConnell et al. (1995)

TABLE 7.—Microsatellite allelic frequency distributions for the *Omy77* locus for rainbow trout and steelhead from populations specific to six habitats in southern California. Populations are identified more fully in Table 1.

Population	*Omy77* alleles (bp)														
	93	95	97	99	101	103	105	107	109	111	113	115	117	121	123
Closed to ocean															
1 Sespe	0	0	0	2	0	6	4	4	0	0	0	0	0	0	0
2 Piru	0	0	3	5	0	6	4	0	0	0	2	2	0	0	0
3 Paula	1	0	2	4	0	9	4	1	0	6	1	0	0	0	0
4 Matilija	0	0	7	4	0	0	10	5	1	0	0	1	0	0	0
5 Fox	0	0	3	1	0	4	1	0	5	5	0	0	2	0	0
6 Franklin	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1
7 Alder	0	0	0	0	0	7	0	0	1	3	0	0	2	2	0
8 San Antonio	0	0	0	5	0	0	0	0	0	0	0	5	0	0	0
Total	1	0	15	21	0	32	23	10	7	14	3	8	5	6	1
Open to ocean															
9 Pauma	0	0	3	1	0	2	0	0	2	15	1	0	0	0	0
10 Santa Rosa	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0
11 Sequit	0	0	0	1	0	0	0	0	0	2	0	0	2	2	0
12 Big Pico	0	0	0	0	0	0	0	0	0	5	0	0	5	1	0
13 Gaviota	0	2	3	2	0	6	0	3	6	1	0	0	0	1	0
Total	0	2	6	4	0	8	0	3	8	23	1	2	7	5	0
Anadromous															
14 Malibu	0	0	0	0	0	1	0	0	0	0	0	0	0	7	0
15 Santa Ynez	0	0	2	5	4	0	1	0	1	2	0	2	0	2	0
Total	0	0	2	5	4	1	1	0	1	2	0	2	0	9	0
Reservoir															
17 Cachuma	0	0	1	5	0	0	0	0	1	0	0	0	0	2	0
18 Jameson	0	0	1	3	0	8	5	3	3	15	14	0	0	0	0
Total	0	0	2	8	0	8	5	3	4	15	14	0	0	2	0
Hatchery															
19 Whitney	0	0	0	18	0	2	0	0	2	0	3	0	0	0	0
20 Hot Creek	0	0	0	8	0	1	1	0	0	2	1	0	0	1	0
21 Wyoming–Hot Creek	0	0	3	5	0	1	0	0	0	0	0	16	0	0	0
22 Shasta–Pit	0	0	2	25	0	0	0	0	0	4	0	0	0	14	0
23 Erwin	0	0	0	12	0	1	3	0	1	0	0	3	0	0	0
24 Coleman	0	0	0	12	0	1	0	0	0	1	0	0	0	6	0
25 Shasta	0	1	16	46	0	9	4	0	0	3	0	5	0	12	0
26 Eagle	0	0	0	9	0	0	0	0	0	13	4	0	0	0	0
27 Arlee	0	0	0	9	0	6	2	0	0	0	0	1	0	0	0
28 6F2	0	0	0	15	3	3	1	0	1	1	0	14	1	0	0
29 Whale Rock	0	0	0	10	0	0	0	0	0	0	0	0	9	0	0
Total	0	1	21	169	3	24	11	0	4	24	8	39	10	33	0

combined, based on the distribution of rare alleles, was highest in the steelhead population, in which 31% of the fish carried rare alleles. Rare-allele frequency was lowest in hatchery rainbow trout (8%); the Whale Rock Hatchery fish showed slightly more allelic diversity (14%) than hatchery rainbow trout stocks. Fisher's exact comparisons of allelic frequencies between habitat groupings showed significant independence (Fisher's $P < 0.025$) between all possible pairs for *Omy77* and *Omy207*. Allelic distributions were found to be nonindependent for *Ssa289* between hatchery and reservoir rainbow trout ($P = 0.38$) and between open and anadromous populations ($P = 0.35$). Fisher's exact comparisons among the rainbow trout hatchery strains showed significant allelic identity for all three loci combined between the following pairs of hatchery strains: Arlee and Hot Creek; Coleman and Hot Creek; Whitney and Hot Creek; Mount Shasta and Hot Creek (Fisher's P values ranged from 0.41 to 0.63).

Pairwise distance measures according to Goldstein et al. (1995) calculated between different habitat groups ranged from 1.8 to 17.1 (Table 5). Average $\delta\mu$ distance measure for the three microsatellite loci combined was 8.3. Mean F_{ST} for the three microsatellites combined was 0.07. Mean heterozygosity for the three loci combined was

TABLE 7.—Extended.

Population	*Omy77* alleles (bp)													
	125	127	129	131	133	135	137	141	145	147	149	151	153	Total
Closed to ocean														
1 Sespe	0	0	0	0	0	0	0	0	0	0	0	0	0	16
2 Piru	0	0	0	0	0	0	0	0	0	0	0	0	0	22
3 Paula	0	1	0	1	0	2	0	0	0	0	0	0	0	32
4 Matilija	0	0	1	1	0	0	0	0	0	0	0	0	0	30
5 Fox	2	0	1	0	0	0	3	0	0	1	0	0	0	28
6 Franklin	0	0	5	3	0	0	0	0	0	0	0	0	0	14
7 Alder	2	0	4	0	0	0	0	0	0	1	0	0	0	22
8 San Antonio	0	0	8	0	0	0	0	0	0	0	0	0	0	18
Total	4	1	19	5	0	2	3	0	0	2	0	0	0	182
Open to ocean														
9 Pauma	0	0	0	0	0	0	0	0	0	0	0	0	0	24
10 Santa Rosa	1	3	7	0	0	0	0	0	0	0	0	0	0	14
11 Sequit	1	0	6	0	0	1	0	0	0	0	1	0	0	16
12 Big Pico	1	0	0	0	0	0	0	0	0	0	2	0	0	14
13 Gaviota	2	0	1	0	0	0	0	0	0	3	4	0	0	34
Total	5	3	14	0	0	1	0	0	0	3	7	0	0	102
Anadromous														
14 Malibu	3	0	5	0	0	2	0	0	0	1	6	1	0	26
15 Santa Ynez	2	1	6	0	2	0	0	2	0	0	0	0	0	32
Total	5	1	11	0	2	2	0	2	0	1	6	1	0	58
Reservoir														
17 Cachuma	2	0	1	0	0	0	0	0	0	0	0	0	0	12
18 Jameson	0	0	0	0	0	0	0	0	0	0	0	0	0	52
Total	2	0	1	0	0	0	0	0	0	0	0	0	0	64
Hatchery														
19 Whitney	0	0	5	0	0	0	0	0	0	0	0	0	0	30
20 Hot Creek	0	1	1	0	0	0	0	0	0	0	0	0	0	16
21 Wyoming–Hot Creek	0	0	7	0	0	0	0	0	0	0	0	0	0	32
22 Shasta–Pit	0	0	15	0	0	0	0	0	0	0	0	0	0	60
23 Erwin	0	0	0	0	0	0	0	0	0	0	0	0	0	20
24 Coleman	5	0	0	0	0	0	0	3	0	0	0	0	0	28
25 Shasta	3	0	39	0	0	0	0	0	0	0	0	0	0	138
26 Eagle	0	0	7	0	0	0	0	1	0	0	0	0	0	34
27 Arlee	0	0	0	0	0	0	0	2	0	0	0	0	0	20
28 6F2	0	0	0	0	0	0	0	1	0	0	0	0	0	40
29 Whale Rock	0	0	0	0	0	2	0	0	0	0	2	0	3	26
Total	8	1	74	0	0	2	0	7	0	0	2	0	3	120

0.79. Mean variances in allele size by locus were 49.83 for *Omy77*; 59.03 for *Omy207*; and 5.66 for *Ssa289*. Mean variance for all three loci combined equaled 38.17. The tau of Goldstein et al. (1995, expected duration of linearity of distance for the three loci combined) equaled 105,010 ± 13,400 generations.

The NJ branching patterns found by analyses of microsatellite allelic diversity among habitats were similar to the associations developed from our mtDNA analyses, with the exception of the position of rainbow trout from open habitats in relation to steelhead and Whale Rock Hatchery fish (Figure 3). In 111 of the 1,000 bootstrap microsatellite trees, the reservoir and hatchery rainbow trout terminal positions were reversed. The consensus NJ tree developed from microsatellite distance data gave bootstrap values above 70% for all branches, except the branch joining reservoir and resident rainbow trout from closed habitats.

Bootstrap values calculated for a consensus NJ analysis of microsatellite allelic diversity among the different hatchery rainbow trout strains were not significant for any branching nodes (bootstrap values = 21–47%), providing no significant resolution for genetic differentiation among these strains with these loci. Three factors—the lack of consistent, supportable genetic structure among

TABLE 8.—Microsatellite allelic frequency distributions for the *Omy207* locus for rainbow trout and steelhead from populations specific to six habitats in southern California. For populations, numbers and full names are given in Table 1; bp = base pair. (*Omy* 207 was not run on the Erwin Hatchery strain.)

Population	*Omy207* alleles (bp)																
	98	100	102	104	106	108	110	112	114	116	118	120	122	124	126	128	130
Closed to ocean																	
1 Sespe	0	0	0	3	0	0	0	0	4	0	0	0	0	0	0	0	0
2 Piru	1	0	0	10	0	3	0	0	3	0	0	0	0	1	1	0	0
3 Paula	0	0	0	0	0	0	0	0	5	0	0	1	0	2	1	7	0
4 Matilija	0	1	0	12	0	0	1	0	1	2	1	0	0	0	0	0	2
5 Fox	0	0	0	2	0	0	0	0	2	0	0	0	0	0	2	2	0
6 Franklin	0	3	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0
7 Alder	0	0	2	0	0	0	0	0	0	0	3	0	0	0	3	0	0
8 San Antonio	0	0	0	2	2	0	0	4	4	0	0	0	0	6	0	0	0
Total	1	4	2	30	2	3	1	4	20	2	4	2	0	9	7	9	2
Open to ocean																	
9 Pauma	0	0	8	0	3	4	0	0	4	0	0	4	0	0	0	0	0
10 Santa Rosa	0	0	0	1	0	0	0	0	0	0	3	1	2	0	0	0	0
11 Sequit	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
12 Big Pico	0	0	0	0	0	0	3	0	5	0	0	1	0	0	0	0	0
13 Gaviota	0	0	0	6	0	1	0	0	3	0	0	2	0	0	0	0	0
Total	0	0	8	7	3	5	3	0	13	0	3	8	2	0	0	0	0
Anadromous																	
14 Malibu	0	0	0	1	0	0	0	0	3	1	2	0	2	0	0	0	1
15 Santa Ynez	0	0	0	1	0	0	0	1	5	2	2	0	0	0	3	1	0
Total	0	0	0	2	0	0	0	1	8	3	4	0	2	0	3	1	1
Reservoir																	
17 Cachuma	0	0	1	4	0	0	1	0	2	0	0	0	0	0	2	0	0
18 Jameson	0	1	0	0	0	0	0	0	4	0	8	0	0	2	10	5	0
Total	0	1	1	4	0	0	1	0	6	0	8	0	0	2	12	5	0
Hatchery																	
19 Whitney	0	0	0	0	0	0	0	0	2	0	2	0	0	2	5	0	0
20 Hot Creek	0	0	0	4	0	0	0	0	15	0	0	1	0	2	0	0	0
21 Wyoming–Hot Creek	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
22 Shasta–Pit	0	0	0	8	0	0	0	1	3	2	0	0	5	0	11	0	0
24 Coleman	0	0	0	7	0	0	0	2	2	4	0	0	2	0	2	0	0
25 Shasta	0	0	0	8	0	0	0	1	20	1	0	1	0	0	1	0	0
26 Eagle	0	0	0	2	0	0	0	12	2	1	1	2	0	0	7	0	0
27 Arlee	0	0	0	1	0	0	0	0	9	0	0	0	1	1	0	0	0
28 6F2	0	0	0	2	0	0	0	0	6	0	1	18	0	0	0	0	0
29 Whale Rock	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	1	1
Total	0	0	0	32	0	0	0	16	68	8	4	23	9	6	27	1	1

the hatchery strains (for both mtDNA and microsatellites), incomplete or unverified hatchery stocking records, and low sample size within several hatchery populations—suggested pooling of all hatchery rainbow trout strains as one population for these analyses.

Discussion

Historical Abundance

Over the last 100 years, significant alteration of freshwater habitat has occurred on southern California streams because of urban land development, reservoir construction, pollution, mining, and water diversion for agricultural and urban use. These changes have resulted in the loss of considerable steelhead habitat (McEwan and Jackson 1996). Environmental conditions in marginal habitats throughout southern California include elevated water temperatures, frequent droughts, floods, and fires (Moore 1980; Carpanzano 1996). Because of the arid climatic conditions in this area, migration and life history structure in steelhead depend strongly on erratic patterns of rainfall and subsequent stream flow, which have been made more unpredictable by numerous obstructions and other human activities (Moore 1980; Carpanzano 1996).

Steelhead in southern California are at a high

TABLE 8.—Extended.

Population	*Omy207* alleles (bp)														
	132	134	136	138	140	142	144	146	148	152	154	156	158	166	Total
Closed to ocean															
1 Sespe	0	0	0	1	2	0	0	0	0	0	2	0	0	0	12
2 Piru	0	0	0	1	0	0	0	0	0	0	0	0	0	0	20
3 Paula	0	0	0	1	3	0	0	1	0	0	1	0	0	0	22
4 Matilija	2	0	0	3	1	0	0	1	0	0	0	0	0	1	28
5 Fox	0	0	0	0	0	0	0	0	0	1	0	1	0	0	10
6 Franklin	1	1	1	0	0	1	0	0	0	0	0	0	0	0	10
7 Alder	0	0	1	5	0	0	0	0	0	0	0	0	2	0	16
8 San Antonio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
Total	3	1	2	11	6	1	0	2	0	1	3	1	2	1	136
Open to ocean															
9 Pauma	0	0	0	0	1	0	0	0	0	0	0	0	0	0	24
10 Santa Rosa	0	0	0	0	2	0	0	0	2	0	0	1	0	0	12
11 Sequit	9	0	0	0	0	0	0	0	0	0	0	0	0	0	10
12 Big Pico	1	0	0	0	4	0	0	0	0	0	0	0	0	0	14
13 Gaviota	0	0	0	0	0	0	1	0	0	1	4	0	0	0	18
Total	10	0	0	0	7	0	1	0	2	1	4	1	0	0	78
Anadromous															
14 Malibu	2	0	0	0	1	2	0	0	0	0	0	0	1	0	16
15 Santa Ynez	2	0	0	0	0	0	0	0	0	0	0	5	0	0	22
Total	4	0	0	0	1	2	0	0	0	0	5	1	0	0	38
Reservoir															
17 Cachuma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
18 Jameson	0	1	5	0	0	0	0	1	0	4	0	1	0	0	42
Total	0	1	5	0	0	0	0	1	0	4	0	1	0	0	52
Hatchery															
19 Whitney	0	0	0	0	0	0	0	0	0	0	0	1	0	0	12
20 Hot Creek	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22
21 Wyoming–Hot Creek	10	0	0	0	1	0	0	0	0	9	0	0	0	0	24
22 Shasta–Pit	0	0	9	0	1	0	0	0	0	0	0	0	0	0	40
24 Coleman	7	0	0	0	0	0	0	0	0	0	0	0	0	0	26
25 Shasta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32
26 Eagle	0	0	5	0	2	0	0	0	0	0	0	0	0	0	34
27 Arlee	3	0	0	0	2	0	0	0	0	1	0	0	0	0	18
28 6F2	0	0	0	0	2	0	0	0	0	13	0	0	0	0	42
29 Whale Rock	0	0	2	0	0	0	0	0	0	2	0	3	0	0	18
Total	20	0	16	0	8	0	0	0	0	25	0	4	0	0	268

risk of extinction; 35% of the historical runs are already extirpated, and 45% show a precipitous decline (Nehlsen et al. 1991; Busby et al. 1996; Titus et al., in press). Published estimates of historical abundance (pre-1960s) of spawning steelhead throughout southern California include 20,000–30,000 fish in the Santa Ynez River (Shapovalov and Taft 1954); 4,000–6,000 fish in the Ventura River (Titus et al., in press); 1,000 fish in Malibu Creek (Nehlsen et al. 1991); and 7,000–9,000 fish in the Santa Clara River (Moore 1980). Currently, remnant steelhead runs are thought to exist within the study area on Gaviota Creek, the lower sections of the Ventura, Santa Clara, and Santa Ynez Rivers, and on Malibu Creek. The present estimate of total run size is less than 200 adults (Carpanzano 1996; Titus et al., in press). The six adults and 24 smolts included in this study were the only known captures of wild steelhead in this area during the study period (M. Cardenas, CDFG, personal communication).

Mitochondrial DNA

The methods and protocols for use of mtDNA in population studies are well established (Avise 1994, and literature therein). The control region of this maternally inherited molecule has been used extensively because of its rapid rate of evo-

TABLE 9.—Microsatellite allelic frequencies for the *Ssa289* locus for rainbow trout and steelhead from populations specific to six habitats in southern California. For populations, numbers and full names are given in Table 1; bp = base pair. (*Ssa* 289 was not run on the Erwin Hatchery strain.)

Population	*Ssa289* alleles (bp)										
	104	110	112	114	116	118	120	122	124	126	Total
Closed to ocean											
1 Sespe	0	8	4	0	1	0	1	4	0	0	18
2 Piru	0	0	7	0	0	0	5	0	2	0	14
3 Paula	0	0	19	1	0	0	2	0	2	0	24
4 Matilija	0	11	8	0	1	0	4	2	1	1	28
5 Fox	0	7	13	0	0	0	0	2	0	0	22
6 Franklin	0	4	6	0	0	0	2	0	2	0	14
7 Alder	0	1	12	0	0	0	0	0	1	0	14
8 San Antonio	0	5	0	2	3	0	2	0	0	0	12
Total	0	36	69	3	5	0	16	8	8	1	146
Open to ocean											
9 Pauma	0	8	8	0	3	0	3	0	2	0	24
10 Santa Rosa	0	7	4	0	1	0	0	0	0	0	12
11 Sequit	0	5	1	0	0	0	2	2	0	0	10
12 Big Pico	0	0	10	0	0	0	1	3	0	0	14
13 Gaviota	0	16	8	0	0	0	0	0	0	0	24
Total	0	36	31	0	4	0	6	5	2	0	84
Anadromous											
14 Malibu	0	10	5	1	2	0	2	0	0	0	20
15 Santa Ynez	0	11	5	1	0	0	4	2	1	0	24
Total	0	21	10	2	2	0	6	2	1	0	44
Reservoir											
17 Cachuma	0	4	2	1	0	0	5	0	0	0	12
18 Jameson	0	12	34	0	0	0	0	2	0	0	48
Total	0	16	36	1	0	0	5	2	0	0	60
Hatchery											
19 Whitney	0	7	0	9	0	0	6	3	1	0	26
20 Hot Creek	0	7	0	11	0	0	1	0	3	0	22
21 Wyoming–Hot Creek	0	13	4	3	3	0	4	1	2	0	30
22 Shasta–Pit	0	12	0	3	0	0	9	6	10	0	40
24 Coleman	0	5	0	10	0	0	10	0	3	0	28
25 Shasta	0	5	0	17	0	0	2	2	6	0	32
26 Eagle	0	20	0	4	0	0	7	0	0	1	32
27 Arlee	0	7	5	2	1	0	2	0	3	0	20
28 6F2	0	13	6	0	8	0	11	0	3	1	42
29 Whale Rock	0	12	2	0	0	0	0	3	9	0	26
Total	0	101	17	59	12	0	52	15	40	2	298

lution, the ease of extraction and amplification, and a significant literature on the theory and application of mtDNA sequence analysis that is available to researchers (Avise et al. 1987; Hillis et al. 1996). Sequence data developed from this molecule have played an important role in high-resolution analyses of population structure in many closely related vertebrate groups (Moritz et al. 1987, 1995; Stoneking et al. 1991; Avise 1994; Avise et al. 1994; Moritz 1994; Tessier et al. 1995).

Literature comparisons suggest that the range of genetic diversity calculated from our mtDNA control region sequence was consistent with intraspecific diversity expected in geographically proximate populations (Nei 1987). Mitochondrial genetic diversity within our habitat groupings ranged from 34% to 74%. These values are not greatly different from those previously reported for control region sequence from geographically proximate, but nonoverlapping, populations of kangaroo rats *Dipodomys* sp. (29–89%; Thomas et al. 1990) but are lower than values calculated for a 295-bp segment of the mtDNA control region (63–100%) sequenced from a migratory shorebird collected from Alaska to Florida by Wenink et al. (1993).

Habitat fragmentation and population declines suggest that southern California populations of *O.*

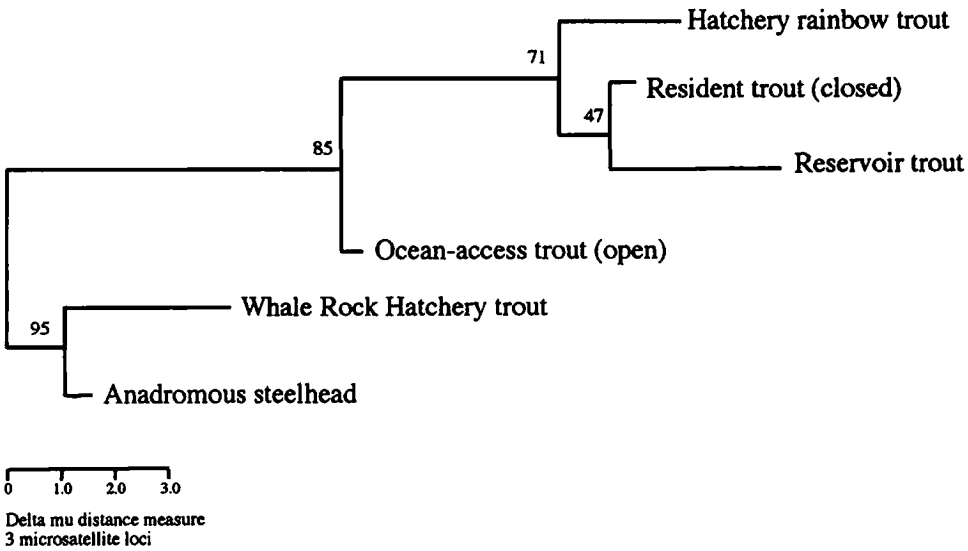

FIGURE 3.—Consensus, unrooted neighbor-joining tree showing genetic distance relationships ($\delta\mu$; Goldstein et al. 1995) based on three microsatellite loci combined calculated for populations of *Oncorhynchus mykiss* found in six southern California freshwater habitats with different levels of human activity (see Figure 2 for habitat descriptions). Bootstrap values calculated for the branching patterns from the $\delta\mu$ analyses are based on consensus drawn from 1,000 replicate trees and are given as percentage values at the branching nodes.

mykiss may be vulnerable to the effects of inbreeding, loss of rare alleles, and genetic drift. Recent studies, however, have shown significant genetic differences among steelhead populations throughout California, and high levels of genetic diversity were found in several populations (Nielsen et al. 1994b; Cramer et al. 1995; Busby et al. 1996). Our present study demonstrated significant genetic diversity in mtDNA control-region sequence in both anadromous and resident forms of *O. mykiss* in southern California.

Earlier reports on the genetics of southern California steelhead documented mtDNA haplotypes that were found to be common in southern fish but were rare (MYS8) or nonexistent (MYS5, MYS6) in northern fish (Nielsen et al. 1994b; Cramer et al. 1995). Genetic analyses of museum samples (juvenile fish) have shown that extensive mtDNA diversity was present in coastal rainbow trout from southern California collected during the first half of the 20th century before barriers were constructed on several rivers for water withdrawal projects (Nielsen 1996). This diversity included the mitochondrial DNA haplotypes found today primarily in fish from southern California habitats (MYS5, MYS6, and MYS8). In this study, we provided the first evidence of one of these haplotypes (MYS5) in an adult steelhead by means of the amplification and sequencing of mtDNA from a taxidermied specimen captured in the Ventura River in the early 1940s.

These uniquely southern haplotypes were found in numerous freshwater habitats throughout the study area, including habitats currently closed to ocean access (Carpanzano 1996). These findings suggest that these habitats may serve as refugia for residual *O. mykiss* that have recent anadromous lineage but are now nonanadromous. Such life history changes could result from the loss of ocean access because of the construction of artificial barriers, such as dams and water diversions. Mitochondrial DNA results reported here confirm our earlier findings of unique southern haplotypes within freshwater populations throughout southern California. Genetic distance analyses, however, suggests that wild freshwater rainbow trout from habitats that retain access to the Pacific Ocean show the closest genetic similarity to southern steelhead.

Microsatellites

Microsatellites are presumed to be abundant in all eukaryotic genomes (Tautz 1989). They are currently thought to be the best available tool for studies of closely related groups of organisms (Schlötterer and Pemberton 1994; Garza et al. 1995; O'Reilly and Wright 1995; Olsen et al. 1996; Takezaki and Nei 1996). Demonstrated advantages

for the use of microsatellites in population studies include high levels of co-dominant allelic variation in repeat number, accelerated divergence times leading to polymorphism not available with other molecular markers, and relative ease of examination (Wright and Bentzen 1994; Olsen et al. 1996; O'Reilly et al. 1996; Takezaki and Nei 1996). Highly polymorphic microsatellite loci have been described in several studies of *O. mykiss* (Sakamoto et al. 1994; Nielsen et al. 1994b; Morris et al. 1996; Wenberg et al. 1996).

Many investigators using microsatellite DNA loci have proposed new genetic distance measures based on the single-step mutation model (SSM), which appears appropriate for microsatellite loci (Slatkin 1995; Goldstein et al. 1995; Shriver et al. 1995; Michalakis and Excoffier 1996; Takezaki and Nei 1996). This model differs from the infinite-allele model (IAM) used for classical genetic markers in that each addition or subtraction of a repeat unit within a microsatellite locus is considered a single mutation event (Goldstein et al. 1995; Kimmel et al. 1996). Computer simulation of data on organisms with known phylogenetic relationships have shown that under the assumptions of the IAM and the SSM, Nei's standard distance (D_S) and the $\delta\mu$ of Goldstein et al. (1995), respectively, give appropriate estimates of genetic associations (Nei 1995; Takezaki and Nei 1996).

Comparisons with Other Studies

Recent studies have shown congruence among mitochondrial and nuclear markers for biogeographic population structure in coastal steelhead and rainbow trout with allozymes, mtDNA, and nuclear microsatellites (Nielsen et al. 1994b, 1994c; Cramer et al. 1995; Busby et al. 1996). With this study, we documented congruence for molecular measures drawn from mtDNA and microsatellites in *O. mykiss* collected from different microgeographic habitats in southern California. These data also show that significant genetic identity remains between the Whale Rock Hatchery fish and wild steelhead in the Santa Ynez River and Malibu Creek. Despite a loss of genetic diversity within this hatchery stock (when compared with wild anadromous populations), our data show that this strain retains some of the unique molecular signatures found in this area, including haplotypes MYS5, MYS6, MYS8, and several microsatellite alleles found only in southern *O. mykiss* when compared with northern stocks (Nielsen et al. 1997).

The genetic association made with both mtDNA and microsatellites between the Whale Rock fish and southern steelhead are in direct contradiction to allozyme analyses done by the National Marine Fisheries Service (NMFS) on Whale Rock Hatchery fish in 1995. That study showed a closer genetic relationship between the Whale Rock strain and fish from two northern coastal populations (Ten Mile River and Lagunitas Creek) than to any southern group of fish analyzed (Busby et al. 1996). Unpublished records indicate that the Whale Rock Hatchery strain has never been supplemented with northern steelhead stocks (Anderson, personal communication). We did find one fish in our sample from the Whale Rock population that carried a mtDNA haplotype more commonly associated with northern strains of steelhead (MYS1). This haplotype, however, has been found to have a ubiquitous distribution throughout coastal California and into Baja's Rio Santo Domingo rainbow trout (Nielsen et al. 1997). It is found at significantly lower frequencies in steelhead populations from southern California (Nielsen et al. 1994b).

One possible explanation for associations between the Whale Rock Hatchery fish and northern strains of *O. mykiss* is introgression produced by undocumented introductions within the hatchery or the Whale Rock Reservoir. For three years (1983, 1986, and 1989) the Whale Rock Hatchery broodstock was developed by a commercial aquaculture venture (SilverKing Oceanic Farms, Santa Cruz, California). Progeny used for supplementation into the Whale Rock Reservoir were reared during these years at the SilverKing facility (Sampson 1993). Alternative broodstock collections containing central and northern coastal strains of steelhead were incorporated into the SilverKing operation for aquaculture purposes. Inadvertent mixing of stocks or raceway transfers at this time may have resulted in the introduction of northern genotypes into the Whale Rock Reservoir.

Adult rainbow trout taken in 1992 from the mouth of Old Creek, where it flows into the reservoir, were spawned locally at the Whale Rock Hatchery, raised on site, and released into the reservoir in June 1992 (Samson 1993). A subset of the progeny of this local collection was fin-clipped for DNA analysis for this study. Factors such as sampling error and year-to-year variation in the genetic structure of the hatchery broodstock could have contributed to the different results found with allozyme analysis in 1995 when compared with the 1992 DNA samples. The samples taken for

DNA analyses were collected at random over time from several fry tanks and represented a diversity of progeny from all matings performed that year. The 1995 samples used for allozyme analysis were collected at one point in time and may represent progeny from as few as two adults (Cleveland, personal communication).

Temporal changes in allele frequencies from year-to-year are well documented in salmonids (Waples and Teel 1990; Jorde and Ryman 1996). Therefore, samples collected with different goals in different years may not represent the same component of the total genetic diversity available at the hatchery over time. Whatever the cause, the lack of congruence among allozyme, mtDNA, and microsatellite analyses for this population leaves significant questions concerning its position within the southern steelhead ESU and its potential role in the restoration of anadromous runs.

Hatchery Practices

The influence of hatchery practices with rainbow trout on indigenous southern rainbow trout are not well documented, and evidence is lacking as to the type of hatchery fish that was planted into California habitats during the early part of this century. Therefore, it is impossible to assume that present day hatchery strains represent the total genetic material that may have influenced southern stocks. We can only assume that genetic diversity found in contemporary hatchery strains represent genotypes with a high probability of recent introductions into local streams and reservoirs. Both mtDNA and three microsatellite loci lacked sufficient statistical rigor to genetically separate the different strains identified by hatchery managers as stocks with a known history of introductions into southern streams.

Based on this lack of genetic resolution among strains and incomplete records of hatchery introductions in the area, representative fish from all hatchery strains with any probability of introduction into southern streams were considered a single sample unit (hatchery rainbow trout) for these analyses. This is not meant to detract from the fact that the unique adaptations selected for by hatchery managers within each strain are to some degree genetically based. The recent common ancestry and long management history of mixing hatchery stocks, however, suggest that quantitative genetics and adaptive loci traced through different family histories may be the best path to document actual differences among the hatchery rainbow trout strains (see Hard 1995).

Our current data did show that both mtDNA and microsatellite analyses suggest close genetic associations between hatchery rainbow trout and rainbow trout in reservoir and closed stream habitats. Unlike studies comparing inland rainbow trout populations with coastal hatchery strains (see Williams et al. 1996), the close evolutionary history of California's coastal steelhead and hatchery rainbow trout makes the study of direct introgression from hatchery fish more difficult in this geographic area. The identification of genetic patterns within ancestral groupings that have been altered through cultural and other human activity is a critical goal in defining the future role of hatcheries in conservation (Utter 1995). The threat of genetic introgression is highest when cultured fish are less divergent from wild populations (Fleming 1995).

We were able to compare genetic diversity for mtDNA control-region sequence and microsatellites in hatchery rainbow trout with wild-caught rainbow trout from southern streams and reservoirs. Hatchery rainbow trout had the lowest levels of genetic diversity for both molecular markers. For any one strain, we analyzed multiple samples representing different year-classes from several hatchery populations. Allozyme studies of hatchery rainbow trout have reported different levels of genetic diversity within different hatchery strains, depending on the stock's management history (Allendorf and Utter 1978; Busack et al. 1979; Gall and Crandell 1992). Hatchery rainbow trout have experienced extensive inbreeding, exchange of eggs among hatcheries, and mixing of stocks (Busack et al. 1979). Hatchery management practices such as these have contributed to the decline of between-population genetic diversity for hatchery strains in this species (Waples 1995).

Concluding Observations

In conclusion, population viability depends on the conservation of genetic variation over the entire species' range. These data show that *O. mykiss* carrying mtDNA haplotypes and microsatellite alleles contributing to high levels of genetic diversity remain in freshwater habitats throughout southern California, an area considered by many as "uninhabitable" for this species. Similar levels of genetic diversity were not found in our survey of hatchery rainbow trout with a history of propagation in California. Indeed, genetic diversity for both mtDNA and microsatellites were found to be lowest in all hatchery populations, including the Whale Rock Hatchery fish thought to preserve an indigenous strain of southern steelhead. These

analyses suggest that preservation of the gene pool by hatchery supplementation alone will not maintain the present level of diversity found in this species.

Rainbow trout from habitats with varying levels of human activity and ocean access proved to have significantly different measures of genetic diversity for mtDNA and microsatellites; the greatest array of rare genotypes was found in declining runs of southern steelhead. Analysis of genetic distance measures for both molecular markers, however, showed that consideration of life history patterns and freshwater habitats that retain ocean access remain important factors in the preservation of components of the unique genetic diversity found in *O. mykiss* at the southern extent of their range.

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