

**Population Genetic Structure of Santa Ynez
Rainbow Trout – 2001
Based on Microsatellite and mtDNA Analyses**

By

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Summary

Microsatellite allelic and mitochondrial DNA (mtDNA) haplotype diversity are analyzed in eight rainbow trout (*Oncorhynchus mykiss*) collections: two from tributaries flowing into the upper Santa Ynez River watershed at Gibraltar Reservoir (Camuesa and Gidney creeks); three from tributaries between Gibraltar and Jameson reservoirs (Fox, Blue Canyon, and Alder creeks); one from a tributary above Jameson Reservoir (Juncal Creek); Jameson Reservoir; and one from the mainstem Santa Ynez River above the Jameson Reservoir. Both analyses reveal a high degree of population structure.

Thirteen microsatellite loci are amplified from 376 fish. Population pairwise comparisons show significant differences in allelic frequency among all populations with the exception of Juncal Creek and Jameson Reservoir ($p = 0.4$). Pairwise F_{st} values range from 0.001 (Juncal Creek and Jameson Reservoir) to 0.17 (Camuesa and Juncal creeks) with an overall value of 0.021. Regression analyses (Slatkin 1993) supports an isolation-by-distance model in the five populations below Jameson Reservoir (intercept = 1.187, slope = -0.41, $r^2 = 0.67$). A neighbor-joining bootstrap value of 100% (based on 2000 replicate trees) separates the populations sampled above and below Juncal Dam.

Composite haplotypes from 321 fish generated using mtDNA sequence data (D-loop) reveal four previously described haplotypes (MYS1, MYS3, MYS5 and MYS8; Nielsen et al. 1994a), and one (MYS5) was found in all populations. Mean haplotype diversity is 0.48. Pairwise F_{st} values from mtDNA range from -0.019 to 0.530 (0.177 over all populations) and are larger than those for microsatellites in 26 of 28 pairwise comparisons. In addition, the mtDNA and microsatellites provide contrasting evidence of the relationship of Fox and Alder creeks to the other six populations. Discrepancies between the two markers are likely due to the unique properties of the two marker types and their value in revealing historic (mtDNA) versus contemporary (microsatellites) genetic relationships. The contrasting results may indicate how relationships among the upper Santa Ynez River populations have changed since the installation of Juncal Dam.

Comparisons of mtDNA haplotype frequencies from fish collected for this study with samples analyzed previously in JLN's laboratory (1993) reveal significant differences in mtDNA haplotypes for Fox and Alder creeks. In the 2001 samples from this study, there is a loss of three haplotypes despite larger sample sizes. AMOVA

analysis of what we term “upper” (Alder, Fox, Blue Canyon, Camuesa, Gidney creeks and the upper Santa Ynez mainstem) and “lower” (Hilton, Salsipuedes and the lower mainstem Santa Ynez River) Santa Ynez River populations (1993-2001) reveal that 11% of the variance in haplotypes is found between the upper and lower drainage. A comparison of the mtDNA data from this study with those available for southern California coastal and California hatchery *O. mykiss* populations yields F_{st} values of 0.15 and 0.47, respectively. Differentiation of mtDNA haplotypes for population pairs of Santa Ynez River and hatchery fish show no significant differentiation between wild and at least one hatchery strain in Cachuma Reservoir, Hilton Creek, and the Lower Santa Ynez River.

Introduction

Anadromous *O. mykiss* (steelhead) at the southern extent of their range occurred historically in streams flowing from San Francisco Bay (Gall et al. 1990) to the Baja California peninsula (Behnke 2002). Urban development and habitat modifications due to high freshwater demands for human consumption have greatly limited the distribution of anadromous steelhead throughout this area. Central and southern California *O. mykiss* are characterized by highly flexible life history strategies (Shapovalov and Taft 1954), and previous genetic studies (Gall et al. 1990; Nielsen et al. 1997a) suggest that isolated freshwater habitats may contain relic, non-anadromous components of the *O. mykiss* gene pool found in geographically proximate anadromous populations.

There has been considerable manipulation of *O. mykiss* in California hatcheries since the early 1800's (Busack and Gall 1980). Impacts of hatchery supplementation of *O. mykiss* on wild stocks in streams and reservoirs throughout North America over the last 200 years has been the subject of many studies (see reviews in Reisenbichler and McIntyre 1977, Waples and Do 1994, Campton 1995, and Nielsen 1999). The early findings of Gall et al. (1990) suggest that anadromous *O. mykiss* populations have residualized as freshwater fish behind man-made structures and dams throughout California. This study argues that residual freshwater populations of *O. mykiss* reflect genetic population structure similar to their putative anadromous progenitors.

There is significant public and scientific concern over what fragments of the freshwater component of this species are part of the evolutionary legacy of the species. This issue has previously been examined in other *O. mykiss* populations isolated by dams in California. For example, land-locked populations of *O. mykiss* on Alameda Creek were most closely related genetically to putative anadromous fish collected below the dam and known steelhead found in Lagunitas Creek, Marin County (Nielsen and Fountain 1999b; Nielsen 2003). Similar studies demonstrated genetic associations between freshwater resident and anadromous *O. mykiss* above and below man-made barriers on Pinole Creek (Nielsen and Fountain 1999a), San Francisquito Creek (Nielsen 2000), and San Mateo Creek (Nielsen and Sage 2002).

Three dams divide the Santa Ynez River – Gibraltar Dam and Reservoir (completed in 1920), Juncal Dam and Jameson Reservoir (1930), and Bradbury Dam and Cachuma Reservoir (1953). This study represents genetic analyses of samples of land-locked populations from the upper Santa Ynez River drainage, collected in 2001, and analyzed in 2002. Samples were analyzed for microsatellite allelic diversity at the USGS Alaska Science Center's Molecular Conservation Genetics Laboratory, and for mtDNA diversity at the USFWS Conservation Genetics Laboratory, Anchorage, Alaska. We compared Santa Ynez River mtDNA haplotype frequencies to data from hatchery and wild southern California *O. mykiss*, both anadromous and resident life histories, available from previous studies in the Nielsen laboratory.

Material and Methods

Sample collection and DNA preparation

Samples of *O. mykiss* were collected by the USFWS from the upper Santa Ynez River watershed from May 2000 to June 2001 using electrofishing, barbless lures, or barbless flies (Figure 1; Table 1). Total genomic DNA was extracted from dried fin tissues using Chelex-100 resin (BioRad) or Purgene DNA isolation kit (Gentra Systems Inc, Minneapolis MN, USA) following methods given in Nielsen et al. (1994b) and from the manufacturer.

Microsatellites

Amplification of 13 microsatellite loci (Appendix I) followed general methods given in Nielsen et al. (1998) and Nielsen and Sage (2001). 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). Four multiplexes were developed using 13 loci that were grouped together for amplification (Table 3). One μ 10-F and Ots3-R primers were redesigned in order to incorporate them into the multiplexes used. One μ 10-F was renamed One μ 10.1-F (5'-GGGAACAGAAGAGGAATAGC-3') and Ots3-R was renamed Ots3.1-R (5'-GGTGGAGAGAGTTTGAGAATCACA-3'). Ogo1a, Ogo4, and One μ 10.1 primers were modified by the addition of universal M13R tails while One μ 8, One μ 11, and Ots3 were modified with M13F all on the forward 5' end. The remaining primers were direct labeled. In general, PCR reactions were conducted in 10 μ l volumes using approximately 50ng of genomic DNA, 0.1-0.2 U of DNA polymerase (Perkin Elmer), 10mM Tris-HCl (pH 8.3), 1.5mM MgCl₂, 50mM KCl, 0.01% each of gelatin, NP-40 and Triton X-100, and 200 μ M each dNTP. For direct labeled primers the total of forward (F) and of reverse (R) primers per locus per reaction equaled four pmoles with the F primer concentration a combination of labeled and unlabeled primer. Tailed primer concentration strength for F and R was as follows: 10 pmoles (One μ 10), 5pmoles (Ogo1a, Ogo4, One μ 11, Ots3) and 1pmole (One μ 8). The labeled primer for multiplex A was between 0.06 - 0.20pmoles per reaction (Omy325, 0.06; Ots1, 0.20; One μ 14, 0.40; Ots4, 0.06). The labeled primer for multiplex B was between 0.10-0.75pmoles (Omy77, 0.20; M13F, 0.30; M13R, 0.75). The labeled primer for multiplex C was between 0.10-1.50pmoles (Omy27, 0.10; M13F, 1.50; M13R 0.75). The labeled primer for multiplex D was between 0.30 - 2.00pmoles (Omy207, 0.30; M13F, 0.50; M13R 2.00).

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 in most cases) were determined in relation to the M13 ladder to the genescan-500 internal size standard (P-E Biosystems, Foster City, CA, USA), *O. mykiss* DNA samples of known size were rerun on each gel.

Approximately 10% of all samples were run on a second gel and scored independently to verify allelic size.

Table 1. Sample locations, distance from Jameson Reservoir, and number of samples used in the Santa Ynez River drainage *O. mykiss* analyses 2001.

Stream	Distance from		N mtDNA
	Jameson Reservoir (km)	N μsats	
Alder Creek	3.7	54	51
Blue Canyon Creek	17.1	48	45
Camuesa Creek	25.4	41	34
Fox Creek	4.9	56	42
Gidney Creek	26.3	43	38
Juncal Creek	0.0	37	34
Upper Santa Ynez River	0.0	59	42
Jameson Reservoir	0.0	38	35
TOTAL		376	321

Analyses of heterozygosity, genetic disequilibrium, and Fisher's exact tests for Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP version 3.1a (Raymond and Russet 1997). HWE tests were performed for all populations independently and combined. GENEPOP (Fisher's Exact Tests) and ARLEQUIN version 1.1 (FSTAT pairwise comparisons; Schneider et al. 1997) were used to test for differences in allelic frequencies between all possible population pairs. Statistical significance levels for allelic frequency comparisons were set using sequential Bonferroni tests (Rice 1989). We tested for a pattern of isolation-by-distance among populations sampled below Jameson Reservoir on the Santa Ynez River by regressing $^{10}\log(M)$ on $^{10}\log(\text{distance})$ according to methods given in Slatkin (1993). Collections made on Juncal Creek and the Santa Ynez River above the reservoir were made just upstream of the reservoir and, therefore, were not considered in our isolation-by-distance analyses. Comparisons among contemporary and archival southern California

populations were not possible using microsatellite loci due to a limited number of overlapping loci among studies and differences in microsatellite amplification platforms between older and current analyses.

Genetic distance values or the proportion of shared alleles between individuals and groups of individuals can be used to graphically depict genetic relationships and population genetic structure. Neighbor-Joining tree, based on Cavalli-Sforza chord distances (1967), was generated using a program written by J. Cornuet (INRA, Laboratoire de Neurobiologie comparée des invertébrés, Bures-sur-Yvette, France). Genetic relationships depicted in our consensus NJ tree were tested using bootstrap replications ($n = 2000$) to assess the reproducibility of branching patterns (Felsenstein 1985). Genetic distance relationships were 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 ($n=2000$) to assess the reproducibility of branching patterns.

mtDNA

A 314 b fragment of mtDNA (308 b of the mtDNA control region and 6 b of the tRNA phe gene), which contains the variable region described by Nielsen et al. (1994a), was amplified with the polymerase chain reaction (PCR) in an MJ Research DNA engine. The amplification profile was 92°C (1.5 min); 40 cycles of 94°C (30 sec) + 52°C (30 sec) + 72°C (45 sec); 1 cycle of 72°C (10 min). The PCR product was purified with Millipore MultiScreen-PCR Plates (Bedford MA, USA) following the manufacturers instruction. Cycle sequencing of the purified product was performed on a Stratagene Robocycler (La Jolla, CA, USA) using the following PCR profile: 92°C (2 min); 30 cycles of 94°C (30 sec) + 51°C (15 sec) + 70°C (1 min). The sequence fragments were size fractionated and visualized by electrophoresis on a 4% polyacrylamide gel using a LICOR Long Reader 4200 automatic sequencer. Gels were analyzed using e-Seq v2.0 software (Lincoln NE, USA), and the sequences were edited and exported for analysis using AlignR v2.0 Software (Lincoln NE, USA).

Table 2. List of microsatellite loci and mtDNA used previously in the Nielsen laboratory (1989-2003) to study steelhead/rainbow trout (*Oncorhynchus mykiss*). Number in parentheses is the number of alleles found in the Santa Ynez River watershed for this study. Mean Hz = mean heterozygosity for this locus in this study.

Locus	Source	Number Alleles ¹	Allelic Size Range (bp)	Mean Hz
Omy27	Heath et al. 2001	11 (4)	95 – 117	0.51
Omy77	Morris et al. 1996	18 (9)	93 – 155	0.70
Omy207	O'Connell et al. 1997	24 (20)	97 – 161	0.87
Omy325	O'Connell et al. 1997	27 (12)	95 – 149	0.80
Ogo1a	Olsen et al. 1998	8 (2)	124 – 162	0.35
Ogo4	Olsen et al. 1998	12 (8)	118 – 148	0.67
Oneμ8	Scribner et al. 1996	8 (7)	144 – 190	0.56
Oneμ10	Scribner et al. 1996	5 (5)	121 – 131	0.57
Oneμ11	Scribner et al. 1996	9 (4)	138 – 154	0.51
Oneμ14	Scribner et al. 1996	14 (6)	145 – 171	0.45
Ots1	Banks et al. 1999	33 (6)	151 – 249	0.78
Ots3	Banks et al. 1999	5 (4)	75 – 90	0.41
Ots4	Banks et al. 1999	7 (4)	108 – 150	0.36
mtDNA	Nielsen et al. 1994b	21 (4)	na	0.48

¹ the number of haplotypes are shown for mtDNA.

Each individual was assigned a composite haplotype as described by Nielsen et al. (1994a). Estimates of haplotype diversity and composite haplotype frequency in each population were computed using the program ARLEQUIN version 2.0 (Schneider et al. 2000). A probability test of haplotype frequency homogeneity was used to test for genetic differentiation among all population pairs. The degree of population structure over all populations and among all population pairs was estimated using conventional F-statistics from ARLEQUIN version 2.0. Samples collected from the Santa Ynez River

were compared using ARLEQUIN's F_{st} , population pairwise comparisons, and AMOVA to data available from previous studies of mitochondrial DNA in hatchery and wild *O. mykiss* (see Table 6).

A graphical depiction of the genetic relationship among populations based on the mtDNA data was generated using PHYLIP version 3.5c, as described above for microsatellites. The mtDNA haplotypes were treated as alleles of a single locus to estimate the Cavalli-Sforza & Edwards (1967) chord distance.

Results

Genetic variation within-populations

Microsatellites – Allelic size ranges and the number of alleles for the 13 microsatellite loci tested on Santa Ynez River *O. mykiss* fall within those found throughout the species range (see Table 2). Santa Ynez River *O. mykiss* show significant diversity in allelic size and the number of alleles (mean number of alleles per locus = 7). Average heterozygosity for all 13 loci combined is $H_z = 0.58$. These 13 microsatellite loci are in Hardy-Weinberg equilibrium (HWE) for all eight populations combined ($p = 0.049$ ARLEQUIN Markov chain exact tests). However, there is significant linkage disequilibrium among paired comparisons of loci (sequential Bonferroni corrections) at each sample location (range = Alder Creek 32% of all loci pairs to Juncal Creek 75% of all loci pairs). This may be due in large part to the small effective population sizes for *O. mykiss* at many of these locations.

mtDNA – Four of the 14 haplotypes described by Nielsen et al. (1994b) are observed in the Santa Ynez River samples collected for this study (Table 2). The mean haplotype diversity is 0.48, and ranges from 0.09 (Fox Creek) to 0.64 (Jameson Reservoir). Haplotype MYS5 is found in all populations and haplotypes MYS1, MYS3, and MYS8 are each found in five populations (Table 4). Haplotypes MYS1 and MYS3 are commonly found in hatchery *O. mykiss* throughout California (Nielsen et al. 1997a). Haplotypes MYS5 and MYS8 have never been found in hatchery *O. mykiss* and appear unique to southern California (Nielsen et al. 1997b & 1998).

Table 3. Microsatellite multiplex conditions used to amplify 13 microsatellite loci.

Multiplex	Anneal temperature (°C/cycles)		Locus
		30 minute extension	
A	52/40	no	Omy325 Ots4 Ots1 Oneu14
B	50/40	yes	Omy77 Ogo1a Oneu8
C	52/40	yes	Ogo4 Omy27 Oneu11
D	52/40	no	Omy207 Oneu10 Ots3

Genetic variation between-populations

Microsatellites – Mean F_{st} for all loci combined equals 0.021, indicating that most of the genetic diversity (98%) is occurring at the level of individuals within a population.

Analysis of population independence is highly significant for all paired comparisons with the exception of Juncal Creek and Jameson Reservoir ($p = 0.4$). Population pairwise F_{st} values are given in Table 5.

There are no significant differences in microsatellite allelic frequency variation for three standardized, overlapping loci (Omy27, Omy77, and Oneu11) between the collections made in 1993 (Nielsen 1998) and in this study on Fox and Alder creeks. Regression analyses (Slatkin 1993) supports an isolation-by-distance model in populations sampled between Gibraltar and Jameson reservoirs (intercept = 1.187; slope = -0.41; $r^2 = 0.67$; Table 7) in this study.

Genetic distance comparisons are presented as a consensus Neighbor-Joining (NJ) tree (Figure 2). A genetic separation of the branch containing Jameson Reservoir,

Juncal Creek and the upper Santa Ynez River above Jameson Reservoir from the rest of the populations below Juncal Dam is supported with 100% bootstrap values (based on 2000 replicate trees). Similarly, within this cluster, the upper Santa Ynez River above Jameson Reservoir is separated from the other two with 100% bootstrap values.

mtDNA – The estimate of F_{st} over all populations is relatively large (0.177) and indicative of a high degree of population structure. Estimates of F_{st} for all population pairs ranges from -0.019 to 0.530 (Table 5). Population pairs that include samples from above and below Juncal Dam have the largest F_{st} values. The F_{st} value from mtDNA is larger than the F_{st} value from microsatellites for 26 of the 28 population pairs (Table 5).

The neighbor-joining tree generated from the mtDNA data is generally concordant with the NJ tree generated from the microsatellite data (Figure 2). In both trees the three above-dam populations cluster together as do the three populations farthest downstream of the dam. The relationship of the Alder and Fox Creek populations to the two population clusters differs between the mtDNA and microsatellites.

Collections from the Santa Ynez River have been analyzed for mtDNA haplotype diversity since 1993 (Table 6). Year-to-year comparisons of haplotypes amplified from Fox and Alder creeks (1993 vs. 2001), show a loss of three haplotypes (MSY9, MYS12 and MYS13) despite larger sample sizes in 2001. AMOVA analysis demonstrates that 30% of the haplotype variation is explained by year-to-year differences in haplotype diversity in these two streams.

AMOVA analysis of upper (Alder, Fox, Blue Canyon, Camuesa, Gidney creeks and the upper Santa Ynez mainstem) and lower (Hilton, Salsipuedes and the lower mainstem Santa Ynez River) Santa Ynez River populations (1993-2001) reveals that only 11% of the haplotype variances is found in a comparison of the upper and lower drainage. Basin-wide population pairwise comparisons demonstrate non-discrimination for haplotype frequencies between both Gidney and Blue Canyon creeks in the upper basin and Salsipuedes Creek in the lower basin.

The F_{st} value based on haplotype frequencies comparing the data from this study with archival mtDNA data available for southern California *O. mykiss* populations is 0.15 (Table 8). The F_{st} value for Santa Ynez River *O. mykiss* and five California hatchery strains commonly used for stocking in southern California is 0.47. Pairwise comparisons

of haplotype frequencies between Santa Ynez River and five California hatchery *O. mykiss* strains are given in Table 9. Differentiation of population pairs for Santa Ynez River *O. mykiss* and hatchery fish show no significant differentiation between wild fish and at least one hatchery strain in Cachuma Reservoir, Hilton Creek, and the Lower Santa Ynez River. Based on mtDNA haplotypes two strains of hatchery *O. mykiss*, Whitney and Wyoming/Pit hatchery strains, can not be differentiated from any of the above three lower Santa Ynez River populations.

Table 4. Composite haplotype frequencies for each population in this study.

Haplotype	Alder Creek	Blue Cyn. Creek	Camuesa Creek	Fox Creek	Gidney Creek	Jameson Reservoir	Juncal Creek	Santa Ynez
MYS1	0.235	0.044	0	0	0	0.257	0.177	0.119
MYS3	0.020	0	0	0.024	0	0.229	0.206	0.095
MYS5	0.569	0.489	0.559	0.952	0.395	0.514	0.618	0.786
MYS8	0.177	0.467	0.441	0.024	0.605	0	0	0

DISCUSSION

Microsatellites and mtDNA provide complementary data, allowing for a detailed analysis of genetic population structure. In this study, the two genetic marker types provide both concordant and contrasting results, yielding insight into the population structure and demographics of Santa Ynez River *O. mykiss*. For example, the tests of allelic (microsatellites) and haplotypic (mtDNA) frequency homogeneity reveal significant differences between most population pairs. Both microsatellites and mtDNA reveal significant genetic variation between populations from opposite sides of Juncal Dam, but only the microsatellites reveal genetic variation between most populations from the same side of the dam. In general, polymorphic microsatellites are a more powerful

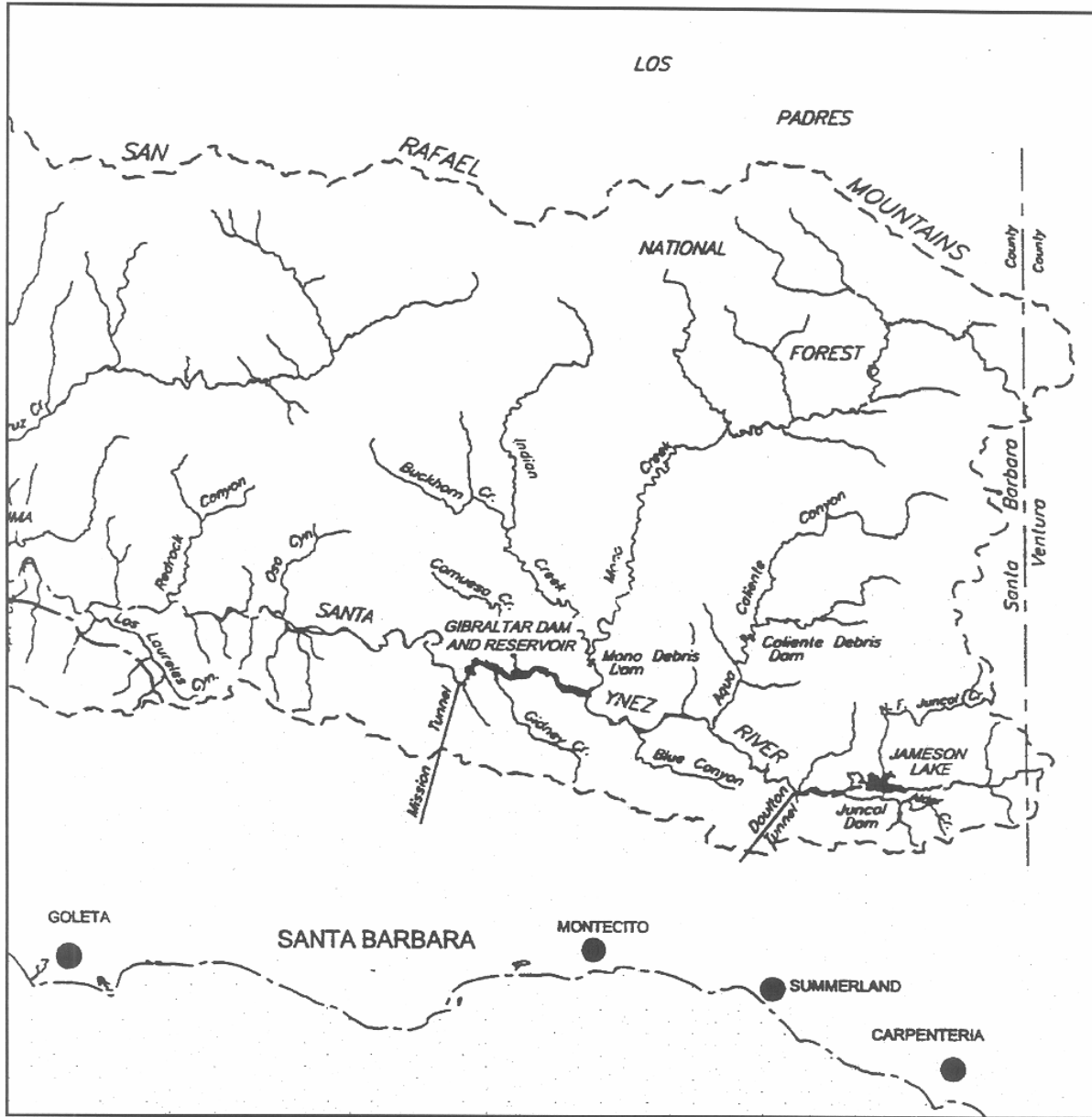


Figure 1. Map showing upper Santa Ynez River drainage with rainbow trout (*O. mykiss*) sampling locations 2001.

Table 5. Population pairwise F_{st} values from microsatellites (below diagonal) and mtDNA (above diagonal) for the 2001 Santa Ynez River rainbow trout (*O. mykiss*) populations. An asterisk (*) indicates the population pairs were judged significantly different by a probability test of haplotype frequency homogeneity or microsatellite allelic frequency distributions.

Population	Alder Creek	Blue Cyn. Creek	Camuesa Creek	Fox Creek	Gidney Creek	Jameson Reservoir	Juncal Creek	Santa Ynez
Alder Creek	--	0.082*	0.080*	0.220*	0.181*	0.038*	0.035*	0.071*
Blue Cyn. Cr.	0.045*	--	-0.019	0.375*	0.004	0.195*	0.192*	0.244*
Camuesa Cr.	0.086*	0.056*	--	0.361*	0.025	0.197*	0.183*	0.223*
Fox Creek	0.038*	0.031*	0.071*	--	0.530*	0.294*	0.210*	0.073
Gidney Creek	0.081*	0.067*	0.077*	0.081*	--	0.295*	0.305*	0.379*
Jameson Res	0.038*	0.125*	0.161*	0.091*	0.149*	--	-0.014	0.080
Juncal Creek	0.039*	0.132*	0.171*	0.101*	0.143*	0.001	--	0.021
Santa Ynez	0.043*	0.101*	0.157*	0.078*	0.137*	0.029*	0.039*	--

tool for detecting the presence of inter-population genetic variation at these fine spatial scales because each locus has many characters (alleles) and multiple loci can be examined (mtDNA is effectively a single locus).

The estimates of the degree of population structure (F_{st}) between population pairs are almost always higher for the mtDNA than for microsatellites. In addition, one mtDNA haplotype (MYS8) appears geographically distinct, occurring only in populations below Juncal dam. These results likely indicate that many of the Santa Ynez River *O. mykiss* populations are small and some may have experienced a genetic bottleneck. Because the effective population size for mtDNA is $\frac{1}{4}$ that of nuclear genes (Birky et al. 1983), mtDNA is likely to exhibit a higher F_{st} than nuclear genes like microsatellites, especially when the population size is low.

Table 6. Mitochondrial DNA haplotype frequencies in Santa Ynez River, southern (NMFS southern steelhead ESU) and northern California steelhead and rainbow trout 1992-2002.

Population	Drainage	Location	Year	mtDNA haplotypes ¹														N	Source		
				1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Santa Ynez	Santa Ynez R.	Alder Creek	1993	2		1		6				6	4			6		25	c		
		Alder Creek	2001	12		1		29				9							51	a	
		Blue Canyon Creek	2001	2				22				21							45	a	
		Cachuma Reservoir	1993	3		3		1				2							9	c	
		Camuesa Creek	2001					19				15							34	a	
		Devil's Creek	1996			1						2							3	c	
		Fox Creek	1993					2				12				4	2		20	c	
		Fox Creek	2001			1		40				1							42	a	
		Franklin Creek	1993	2				4	5										11	c	
		Gidney Creek	2001					15				23							38	a	
		Hilton Creek	1993	1		1						1		1				1	5	c	
		Hilton Creek	1995	5		3						1						2	11	c	
		Hilton Creek	1999	11		15						7			2		1		36	j	
		Indian Creek	1996					1											1	c	
		Jameson Reservoir	1993	4		7		13				1							25	c	
		Jameson Reservoir	2001	9		8		18											35	a	
		Juncal Creek	2001	6		7		21											34	a	
		Lower Santa Ynez R.	1994	1				2											3	c	
		Lower Santa Ynez R.	1999	1		5						2			2				10	j	
		Peachtree Creek	1993			2													2	c	
		Salsipueses Creek	1994					12				11							23	c	
		Salsipueses Creek	1999	1		5		5				20							31	j	
		Upper Santa Ynez R.	2001	5		4		33											42	a	
		Santa Ynez R. total				65	0	64	0	243	5	0	134	4	1	4	4	9	3	536	

Table 6 (cont.)

Population	Drainage	Location	Year	mtDNA haplotypes ¹														N	Source
				1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Southern California <i>O. mykiss</i> populations																			
	Arroyo Sequit		1993									5			3		8	e	
	Gaviota Creek		1993					2		1	6	1					10	e	
	Gaviota Creek		1996								6						6	f	
	Malibu Creek		1993	1	1		1	6			4						13	e	
	Malibu Creek		1994	1				2			1						4	b	
	Marro Bay		1996	2							7						9	f	
	Pico Grande Creek		1993		1			7	2							2	12	e	
	Pico Grande Creek		1996		1			2	2								5	f	
	San Luis Rey R.	Pauma Creek	1994	11		3		8								1	23	e	
	Santa Ana River	San Antonio Creek	1996	5		3								1			9	b	
	Santa Clara R.	Sespe Creek	1994			24					10		1				36	e	
	Santa Clara R.	Piru Creek	1997	15		5					3						23	j	
	Santa Clara R.	Santa Paula Creek	1997	36		3		1					1				41	b	
	San Mateo Creek		2001					16									16	k	
	Santa Rosa Creek		1994	1	1			4	1								7	e	
	Santa Rosa Creek		1996	1				3	1								5	f	
	Ventura River	Matilija Creek	1994	6		13		3			2						24	e	
		Matilija Creek	1996	7		19		4			2						32	b	
		Matilija Creek	2002	3		17		11			2						33	d	
	S. California coastal total			89	4	87	1	69	6	1	48	2	2	0	3	3	1	316	
California hatchery strains of <i>O. mykiss</i>																			
	Coleman hatchery strain		1996	4		3											7	b	
	Hot Creek Hatchery strain		1996	11		9								1			21	b	
	Mount Shasta Hatchery strain		1992	54		14							1	4			73	g	
	Mount Shasta Hatchery strain		1993	52													52	h	
	Mount Shasta Hatchery strain		1996	120													120	j	
	Whitney Hatchery		1996	15		9								3			27	b	
	Wyoming/ Hot Creek Hatchery strain		1996	3		5							2	1			11	b	
	Hatchery total			259	0	40	0	0	0	0	0	0	3	9	0	0	0	311	

Table 6 (cont.)

OVERALL TOTAL	mtDNA haplotypes ¹														N
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	413	4	191	1	312	11	1	182	9	12	4	7	12	4	1163

¹ *O. mykiss* haplotypes were first described in Nielsen et al. 1994a

Sources:

a - This report

b - Nielsen et al. 1997a

c - Nielsen 1998

d - Nielsen et al. 2003 (submitted)

e - Nielsen et al. 1994b

f - Nielsen et al. 1997b

g - Nielsen 1996a

h - Nielsen et al. 2000

j - Nielsen 2000b

k - Nielsen and Sage 2002

Table 7. Stream distance between sample locations for rainbow trout collected in the Santa Ynez watershed 2000-2001. Calculated distances represent the shortest distance between sample sites in river kilometers (km) using Terrain Navigator 2002 (Maptech, Amesbury, MA) from digitized USGS quadrangle maps for this area. AJR = mainstem above Jameson Reservoir

	1	2	3	4	5	6	7
1. St. Ynez AJR	-						
2. Jameson Res.	0.0	-					
3. Alder Creek	5.0	3.7	-				
4. Juncal Creek	1.4	0.0	4.6	-			
5. Fox Creek	6.2	4.9	1.1	5.8	-		
6. Blue Cyn. Creek	18.4	17.1	13.4	18.0	12.2	-	
7. Camuesa Creek	26.7	25.4	21.7	26.3	20.5	8.3	-
8. Gidney Creek	27.6	26.3	22.6	27.2	21.4	9.2	2.7

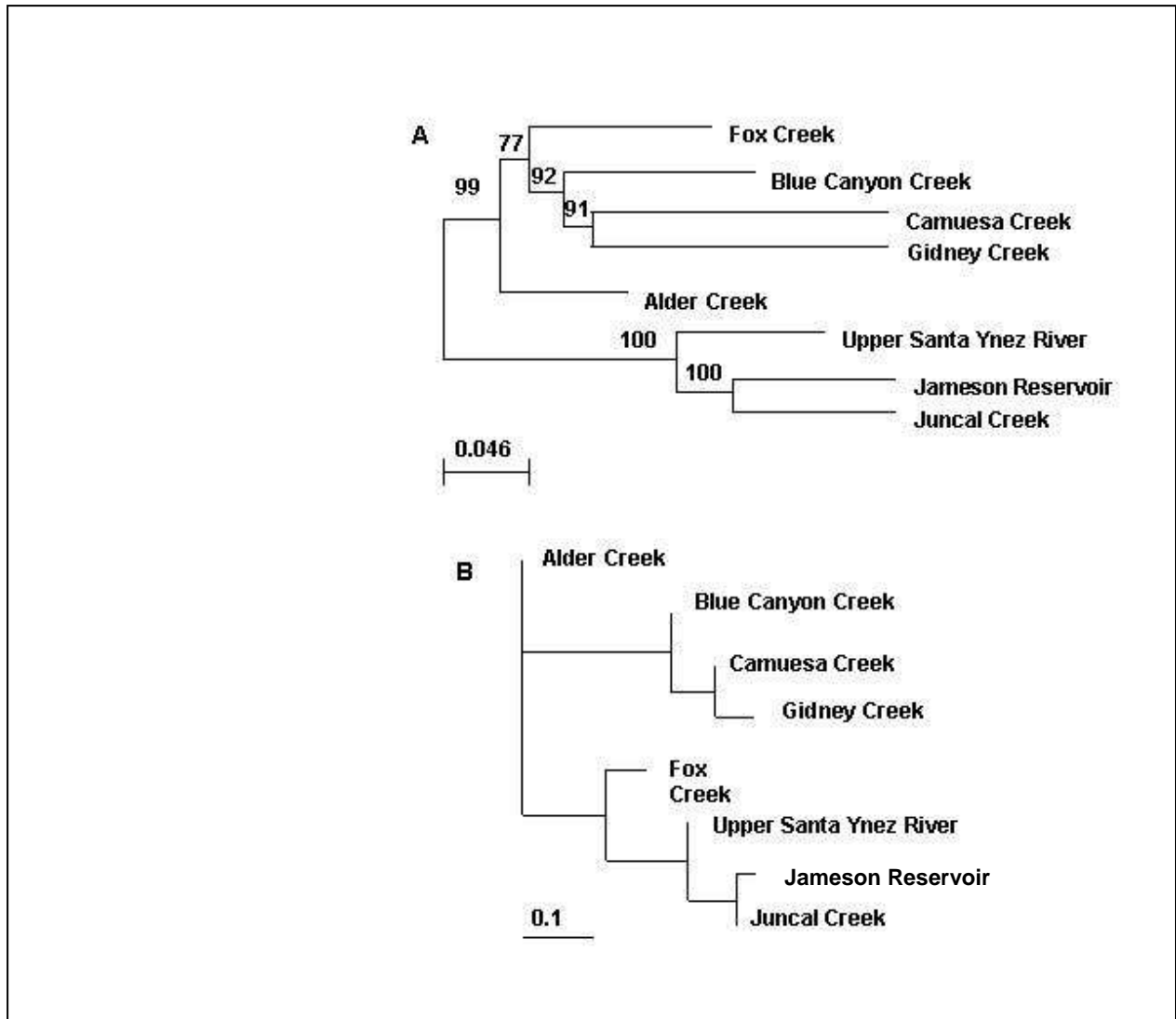


Figure 2. Neighbor-joining (NJ) trees generated using Cavalli-Sforza and Edwards (1967) chord distance from microsatellite (**A**) and mtDNA (**B**) data. Bootstrap values given are based on 2000 replicate trees.

Table 8. Population pairwise F_{St} s for mtDNA frequencies found in the Santa Ynez drainage and 13 southern California trout populations based on analyses of F_{St} in ARLEQUIN. Above the diagonal F_{St} is given for each pair; below the diagonal "+" = significant or "-" = no significant differences in pairwise comparisons (ARLEQUIN's Markov chain analyses) of mtDNA frequencies.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1 Alder Creek		0.057	0.099	0.061	0.055	0.129	0.188	0.053	0.054	0.116	0.123	0.106	0.257	0.235	-0.014	0.258	0.066	0.358	0.227	0.335	0.247	0.391	0.216	0.021	0.208	
2 Blue Canyon	+		0.212	-0.019	0.079	0.004	0.284	0.185	0.192	0.214	0.024	0.244	0.228	0.145	-0.005	0.219	0.179	0.545	0.403	0.398	0.401	0.583	0.351	0.158	0.311	
3 Cachuma Res.	+	+		0.257	0.305	0.255	-0.053	0.099	0.177	-0.057	0.168	0.384	0.217	0.265	0.104	0.207	0.221	0.270	-0.026	0.129	0.034	0.368	0.644	0.176	0.019	
4 Camuesa Creek	+	-	+		0.048	0.025	0.316	0.183	0.183	0.243	0.052	0.223	0.284	0.202	-0.001	0.296	0.169	0.598	0.447	0.431	0.449	0.636	0.338	0.153	0.327	
5 Fox Creek	+	+	+	-		0.182	0.357	0.118	0.084	0.273	0.194	0.066	0.395	0.369	0.031	0.443	0.090	0.583	0.456	0.468	0.471	0.611	0.131	0.062	0.325	
6 Gidney Creek	+	-	+	-	+		0.306	0.278	0.305	0.258	-0.007	0.379	0.182	0.058	0.064	0.152	0.278	0.604	0.461	0.410	0.447	0.641	0.501	0.278	0.353	
7 Hilton Creek	+	+	-	+	+	+		0.179	0.244	-0.001	0.239	0.394	0.255	0.290	0.201	0.235	0.285	0.213	0.002	0.125	0.064	0.282	0.519	0.255	0.065	
8 Jameson Res.	+	+	+	+	+	+	+		-0.012	0.091	0.246	0.075	0.390	0.390	0.077	0.407	0.101	0.378	0.202	0.314	0.264	0.424	0.231	0.031	0.122	
9 Juncal Creek	+	+	+	+	+	+	+	-		0.153	0.275	0.021	0.447	0.444	0.081	0.475	0.085	0.477	0.291	0.391	0.347	0.526	0.193	0.011	0.182	
10 L. Santa Ynez	+	+	-	+	+	+	-	+	+		0.180	0.340	0.234	0.280	0.115	0.265	0.188	0.377	0.067	0.085	0.163	0.471	0.548	0.167	0.003	
11 Salsipuedes Cr.	+	+	+	+	+	-	+	+	+	+		0.359	0.130	0.035	0.063	0.098	0.261	0.531	0.380	0.313	0.377	0.572	0.464	0.258	0.286	
12 U. Santa Ynez	+	+	+	+	+	+	+	+	-	+	+		0.586	0.562	0.149	0.616	0.129	0.632	0.499	0.544	0.515	0.656	0.078	0.046	0.330	
13 Arroyo Sequit	+	+	+	+	+	+	+	+	+	+	+	+		0.088	0.219	0.103	0.380	0.651	0.411	0.388	0.418	0.698	0.815	0.400	0.375	
14 Gaviota Creek	+	+	+	+	+	+	+	+	+	+	-	+	-		0.197	0.001	0.399	0.660	0.477	0.406	0.455	0.700	0.748	0.419	0.391	
15 Malibu Creek	-	-	+	+	+	+	+	+	+	+	+	+	+	+		0.234	0.066	0.475	0.271	0.363	0.301	0.536	0.318	0.025	0.235	
16 Marro Bay	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+		0.442	0.625	0.414	0.419	0.367	0.664	0.858	0.448	0.390	
17 Big Pico Creek	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0.547	0.340	0.433	0.400	0.604	0.273	-0.034	0.277	
18 Pauma Creek	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0.100	0.566	0.034	-0.023	0.855	0.508	0.364	
19 San Antonio Cr.	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-		0.295	-0.032	0.197	0.758	0.289	0.119	
20 Sespe Creek	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0.377	0.628	0.685	0.450	0.085	
21 Piru Creek	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+		0.091	0.696	0.345	0.222
22 Santa Paula Cr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+		0.838	0.564	0.423
23 San Mateo Cr.	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+		0.262	0.467
24 Santa Rosa Cr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+		0.254
25 Matilija Creek	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Table 9. Differentiation of population pairs for mtDNA frequencies found in the Santa Ynez drainage and five California hatchery trout strains based on Markov chain analyses of F_{st} in ARLEQUIN. Above the diagonal P is given for each pair; below the diagonal "+" = significant differentiation and "-" = no significant differentiation for haplotype frequencies.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1- Alder Creek		0.001	0.011	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2 - Blue Canyon	+		0.000	0.620	0.011	0.304	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000
3 - Cachuma Reservoir	+	+		0.000	0.000	0.000	0.644	0.011	0.004	0.803	0.002	0.000	0.000	0.040	0.075	0.031	0.389
4 - Camuesa Creek	+	-	+		0.073	0.222	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000
5 - Fox Creek	+	+	+	-		0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6 - Gidney Creek	+	-	+	-	+		0.000	0.000	0.000	0.000	0.220	0.000	0.000	0.000	0.000	0.000	0.000
7 - Hilton Creek	+	+	-	+	+	+		0.000	0.000	0.135	0.000	0.000	0.000	0.049	0.171	0.047	0.079
8 - Jameson Reservoir	+	+	-	+	+	+	+		0.822	0.002	0.000	0.038	0.000	0.000	0.000	0.000	0.000
9 - Juncal Creek	+	+	+	+	+	+	+	-		0.003	0.000	0.272	0.000	0.000	0.000	0.000	0.000
10 - L. Santa Ynez R.	+	+	-	+	+	+	-	+	+		0.000	0.000	0.000	0.001	0.007	0.001	0.220
11 - Salsipuedes Creek	+	-	+	+	+	-	+	+	+	+		0.000	0.000	0.000	0.000	0.000	0.000
12 - U. Santa Ynez R.	+	+	+	+	+	+	+	+	-	+	+		0.000	0.000	0.000	0.000	0.000
13 - Shasta Hatchery	+	+	+	+	+	+	+	+	+	+	+	+		0.000	0.000	0.000	0.000
14 - Whitney Hatchery	+	+	-	+	+	+	-	+	+	-	+	+	+		0.663	0.203	0.099
15 - Hot Creek Hatchery	+	+	-	+	+	+	-	+	+	+	+	+	+	-		0.643	0.196
16 - Coleman Hatchery	+	+	-	+	+	+	-	+	+	+	+	+	+	-	-		0.052
17 - Wyoming/Pit Hatchery	+	+	-	+	+	+	-	+	+	-	+	+	+	-	-	-	

The relationships among populations in the two neighbor-joining (NJ) trees are generally concordant with the exception of the Alder and Fox Creek populations. The relationship of these populations to the two population clusters differs between the mtDNA and microsatellites. This apparent discrepancy is likely due to the unique properties of the two marker types and their value in revealing historic (mtDNA) versus contemporary (microsatellites) genetic relationships. The genetic relationships revealed by the mtDNA NJ tree most likely depict the nature of population structure prior to the installation of Juncal Dam in 1930. That is, the mtDNA is less influenced by contemporary gene flow than by historic population structure. As a result, the mtDNA NJ tree reveals a genetic relationship that would be predicted under a model of isolation-by-distance prior to the installation of the dam; Alder and Fox creeks are most closely related to the populations above Juncal Dam. The installation of the dam created a migration barrier. As a result, Alder and Fox creeks are now more likely influenced by gene flow with the populations below Juncal Dam. Microsatellites are more sensitive to this contemporary gene flow, and the microsatellite NJ tree reveals a genetic relationship that would be predicted given the physical barrier to gene flow. Collectively, the two marker types indicate how relationships between the upper Santa Ynez River populations have changed since the installation of Juncal Dam.

The neighbor-joining analyses of microsatellite data support significant genetic separation between the groups of populations above and below Juncal Dam (100% bootstrap support). The populations in, and geographically adjacent to, Juncal Dam may represent populations that are introgressed with hatchery fish due to historic stocking of hatchery *O. mykiss* in the reservoir. In contrast, fish sampled below Jameson Reservoir may be derived from a local population of resident *O. mykiss* that have not associated or interbred with stocked hatchery fish. Our analysis supporting isolation-by-distance (Slatkin 1993) for streams below the Jameson Reservoir also lends inferential evidence for differentiation of *O. mykiss* populations found in tributaries below Juncal Dam.

The populations of rainbow trout in the headwaters of the Santa Ynez River represent a putative adfluvial population of rainbow trout using the headwaters

tributaries to spawn and moving downstream to rear after their first year (Dr. R. Thomas, pers. comm.). Although these reported behavioral mechanisms have not yet been documented scientifically, they could conceivably contribute to the reproductive isolation found between reservoir fish and tributary-resident *O. mykiss* populations putatively residualized from local anadromous stocks before the construction of dams and barriers on these southern rivers. Under this hypothesis, relic populations of locally adapted fish derived from anadromous ancestors still spawn in upper headwater stream habitats that provide a behavioral isolating mechanism separating them from hatchery fish that spawn in the lower reaches of the same tributaries near the reservoir where they were stocked.

Despite significant hatchery supplementation throughout the small coastal drainages of California, only three lower basin Santa Ynez River *O. mykiss* populations contain haplotype frequencies that mirrored those found in hatchery strains. Based on haplotype frequency data, none of the upper Santa Ynez populations, including the fish found in Jameson Reservoir, appear to be significantly influenced by hatchery fish. Differentiation of population pairs for Santa Ynez River and hatchery fish show no significant differentiation between wild fish and at least one hatchery strain in Cachuma Reservoir, Hilton Creek, and the Lower Santa Ynez River. Based on mtDNA haplotypes two hatchery strains, Whitney and Wyoming/Pit hatchery strains, can not be differentiated from any of the above three lower Santa Ynez River populations. It is important, however, to consider the fact that most hatchery *O. mykiss* strains around the world were founded from wild fish taken from the upper Sacramento River in the mid-1800s. This long-term aquaculture is reflected today in the Mount Shasta Hatchery *O. mykiss* strain. This hatchery strain is dominated by two mtDNA haplotypes, MYS1 and MYS3. These haplotypes are found in many wild populations throughout California and fish with these haplotypes remain ambiguous as to hatchery or wild origins. Despite unique frequency differences, 24% of all fish sequenced for mtDNA from the Santa Ynez River carry one or the other haplotype (MYS1 or MYS3) common to most hatchery strains in California. There appears to be genetic similarity between landlocked populations of *O. mykiss* throughout California and their closely related anadromous progenitors (Nielsen 2000 & 2003; Nielsen and Fountain 1999a). Phenotypic plasticity is an important characteristic that allows *O. mykiss* populations to persist in the

heterogeneous and dynamic environment of southern California. The importance of phenotypic plasticity is ignored when protection and management is prescribed differently for sympatric resident and anadromous phenotypes. One important question is whether or not the current resident stocks that show genetic isolation above dams and barriers would, or could, contribute to increased population viability for local anadromous runs, most of which have declined to the point of listings under the U.S. Endangered Species Act.

Pascual et al. (2001) have demonstrated that the anadromous population of steelhead in Argentina was founded from introductions of from resident rainbow *O. mykiss* (but see also Behnke 2002). According to Behnke (2002), the diversity of ancestral life history forms found in most *O. mykiss* strains in husbandry today could have provided the hereditary basis for the development of a steelhead-like population in Argentina. Zimmerman and Reeves (2000) used otolith microchemistry to demonstrate that a small proportion (4 – 22%) of individuals in sympatric populations of anadromous and resident *O. mykiss* appear to have maternal contribution from their opposite life history type. These arguments suggest that landlocked *O. mykiss*, derived from anadromous populations before urban development and stream blockage, may retain significant adaptive behavior for anadromy long after access to the marine environment has been lost.

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