



Microsatellite Analyses of San Francisquito Creek Rainbow Trout

by

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Abstract

Microsatellite genetic diversity found in San Francisquito Creek rainbow trout support a close genetic relationship with rainbow trout (*Oncorhynchus mykiss*) from another tributary of San Francisco Bay, Alameda Creek, and coastal trout found in Lagunitas Creek, Marin County, California. Fish collected for this study from San Francisquito Creek showed a closer genetic relationship to fish from the north-central California steelhead ESU than for any other listed group of *O. mykiss*. No significant genotypic or allelic frequency associations could be drawn between San Francisquito Creek trout and fish collected from the four primary rainbow trout hatchery strains in use in California, i.e. Whitney, Mount Shasta, Coleman, and Hot Creek hatchery fish. Indeed, genetic distance analyses ($\delta\mu^2$) supported separation between San Francisquito Creek trout and all hatchery trout with 68% bootstrap values in 1000 replicate neighbor-joining trees. Not surprisingly, California hatchery rainbow trout showed their closest evolutionary relationships with contemporary stocks derived from the Sacramento River. Wild collections of rainbow trout from the Sacramento-San Joaquin basin in the Central Valley were also clearly separable from San Francisquito Creek fish supporting separate, independent ESUs for two groups of *O. mykiss* (one coastal and one Central Valley) with potentially overlapping life histories in San Francisco Bay. These data support the implementation of management and conservation programs for rainbow trout in the San Francisquito Creek drainage as part of the central California coastal steelhead ESU.

Introduction

The status of freshwater rainbow trout (*Oncorhynchus mykiss*) in California's coastal drainages where anadromous steelhead have historically occurred is under considerable scrutiny following the listing of California's steelhead under the Endangered Species Act (ESA) by the National Marine Fisheries Service (NMFS) in 1997. In NMFS's proposed rule for the listing of steelhead in the Federal Register (61FR 41541, 08/09/96) stated that "it appears that resident and anadromous *O. mykiss* from the same geographic area may share a common gene pool, at least over evolutionary periods." However, the implications of life history in *O. mykiss* under this listing remain controversial. Genetic studies of the anadromous portion of coastal *O. mykiss* populations (fish demonstrating movement into salt water for maturation) have shown a great deal of biogeographic structure throughout their range in California (Nielsen et al 1994a, 1997a; Nielsen 1996a & 1999). Due to perturbations in biogeographic structure caused by anthropomorphic transfer of resident trout and extensive supplementation of hatchery trout throughout California since the turn of the century, the evolutionary status of individual populations of freshwater trout is less clear.

California's rainbow trout and steelhead appear to be different life-history morphs of the same species with a great deal of flexibility in timing and structure of anadromy in any one population (Shapovalov and Taft 1954). Both life history forms occur sympatrically in many streams systems in California and have been observed interbreeding (D. McEwan, CDFG, pers. comm.) It appears that *O. mykiss* within a specific area form a single panmictic population, rather than different life history forms comprising distinct, genetically separable populations. The anadromous component of this complex life history, however, appears to have great significance in maintaining genetic diversity for this species in California waters (Nielsen et al. 1997b, 1999b). Native rainbow trout that have residualized (i.e. exhibit no migratory behavior) from anadromous steelhead populations and remain relatively reproductively isolated will, therefore, show closer genetic relationships to their parental anadromous stocks than to geographically divergent groups of fish that may have been transferred or used for supplementation within the basin.

Table 2. List of microsatellite loci and their source publications used to amplify DNA from steelhead rainbow trout (*Oncorhynchus mykiss*) in the Nielsen laboratory. Number in parentheses is the number of alleles found in the San Francisquito Creek watershed for this study. SF Hz = heterozygosity for this locus in San Francisquito Creek drainage rainbow trout.

<u>Locus</u>	<u>Source</u>	<u>Number Alleles</u>	<u>Allelic Size Range (bp)</u>	<u>SF Hz</u>
Omy27	M. O'Connell pers. comm.*	11 (5)	95 – 117	0.48
Omy77	Morris et al. 1996	18 (10)	93 – 155	0.64
Omy325	M. O'Connell pers. comm.*	27 (17)	87 – 149	0.72
Oneμ2	Schribner et al. 1996	45 (17)	110 – 290	0.27
Oneμ8	Schribner et al. 1996	18 (5)	144 – 190	0.63
Oneμ14	Schribner et al. 1996	14 (5)	145 – 171	0.28
Ots1	Banks et al. 1999	33 (12)	151 – 249	0.80
Ssa14	McConnell et al. 1995	21 (6)	120 – 168	0.04
Ssa85	O'Reilly et al. 1996	14 (7)	105 – 159	0.28
Ssa289	McConnell et al. 1995	14 (8)	104 – 130	0.49

* Marine Gene Probe Laboratory, Dalhousie University, Halifax N.S.

Amplification of 10 microsatellite (Table 2) followed methods given in Nielsen and Fountain (1999a). Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard (P-E Biosystems, Foster City, CA), *O. mykiss* DNA samples of known size that were rerun on each gel, and a double-stranded reference marker developed in our laboratory showing the most common alleles available for each locus. GENESCAN (v 1.1) and GENOTYPER v 2.1 (P-E Biosystems) DNA fragment analysis software packages were used to score, bin, and output allelic (and genotypic) designations.

Analyses of heterozygosity and F_{st} (function of the probability of identity of genes within and between units) were performed using ARLEQUIN (Schneider et al. 1997). Population independence between paired comparisons of allelic frequencies were tested using Fisher's exact tests, based on a Markov chain adaptation of row-by-column

contingency tables using GENEPOP (V3.1a; Raymond & Rousset 1997). Statistical significance levels for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989).

MICROSAT was used to generate a genetic distance matrix among rainbow trout populations based on $\delta\mu^2$ genetic distance values (Goldstein et al. 1995). The NEIGHBOR and CONSENSE applications from PHYLIP v. 3.572 (Felsenstein 1995) were used to generate mean and consensus neighbor-joining trees from $\delta\mu^2$ genetic distance values.

Results

Mitochondrial DNA – Three mitochondrial haplotypes were documented in San Francisquito Creek rainbow trout, MYS1 (20%), MYS2 (10%), and MYS3 (70%; see Nielsen et al. 1994a for sequence and variation by haplotype). These haplotypes are commonly distributed in steelhead and rainbow trout throughout central and northern California (Nielsen et al 1994a & b; Nielsen and Fountain 1999a). Specifically, MYS1 is the most common haplotype for steelhead from the north coast of California to Alaska. This haplotype also dominates all of the rainbow trout hatchery strains currently in use for supplementation in streams, lakes, and reservoirs throughout California. MYS3 is found most commonly in steelhead/rainbow trout from the Russian River (Sonoma County) to Scott Creek (Santa Cruz County), and is also found in the Whitney hatchery strain of rainbow trout. MYS2 is found less frequently in California, most commonly in steelhead from the Eel River drainage. MYS2 has not been found to date in any rainbow trout hatchery strains sequenced in my laboratory.

Microsatellites - Allelic size ranges and the number of alleles for the 10 microsatellite loci tested in San Francisquito Creek rainbow trout fell within expected values for these loci in *O. mykiss* found throughout the species range (see Table 2). San Francisquito *O. mykiss* showed significant diversity, both in allelic size, 93 (Omy77) to 260 (One μ 2) base pairs (bp) and the number of alleles (mean number of alleles per locus = 9.2). Average heterozygosity for all 10 loci combined for all populations was $H_z = 0.69$, the average in San Francisquito Creek rainbow trout was $H_z = 0.47$. This loss of genetic diversity could have resulted from a small effective population size for rainbow trout in San Francisquito

Creek in recent history due to urban development and fragmentation of fish habitat. Mean F_{st} for all 10 loci combined equaled 0.12, with most genetic diversity (97%) occurring at the level of individuals within a population. GENEPOP's analysis of population independence was significant for all paired comparisons suggesting differences in population allelic structure by locality. Population bottlenecks, founder effects, and/or very low effective population size within individual streams cannot be ruled out, however. The estimated number of migrants (used as a surrogate for gene flow) among California trout populations included in this study was $Nm = 1.8$ fish per generation.

Genetic distance comparisons are presented as a consensus neighbor-joining tree for a broad geographic range of rainbow trout/steelhead populations in California in Figure 1. Small sample sizes for many populations prevented NJ analyses of the genetic distance relationships among tributary and mainstem populations of trout within individual river systems. However, the inclusion of samples previously collected by CDFG for NMFS (1997) for allozyme analyses and subsequently analyzed in my Stanford University laboratory (Nielsen et al. 1999a) gave inference to broad scale biogeographic population genetic structure based on microsatellite data and allowed me to draw some conclusions on the evolutionary history of the trout currently found in San Francisquito Creek.

In these genetic distance analyses, San Francisquito Creek, Alameda Creek, and Lagunitas Creek trout were separable with high bootstrap values (>50%) from four common hatchery rainbow trout strains (Whitney, Mount Shasta, Coleman, and Hot Creek) used for supplementation in streams and reservoirs throughout California (Figure 1). These associations suggest that artificial stocking of hatchery rainbow trout in this area has not eliminated native trout in the locations sampled for these analyses. Alternatively, they may represent a historic evolutionary relationship between coastal and stream populations of *O. mykiss* in the San Francisco Bay area. The Mount Shasta Hatchery strain, most commonly used for stocking streams and creeks in the San Francisco Bay area was found to be significantly different in microsatellite allelic frequencies for all ten loci in comparisons with the San Francisquito Creek trout (Fisher's $P < 0.02$ in all comparisons).

Discussion

With the listing of California's steelhead populations as threatened or endangered evolutionarily significant units (ESUs) under the Endangered Species Act, the question of local stock integrity and historic impacts of stocking hatchery rainbow trout in the creeks and streams surrounding San Francisco Bay area has risen to the surface of public interest (Scott 1997). The California Department of Fish and Game ceased management of steelhead in S.F. Bay tributaries during the 1950's (D. McEwan, CDFG, pers. comm.) At that time urban development with its subsequent environmental damage and the construction of water barriers and dams to preserve water for human consumption prevented steelhead from migrating and spawning in many of the major streams and tributaries in this area (W. Jones, CDFG, personal communications).

Only recently have anadromous steelhead begun to appear in the mouths of these rivers again. The origins of these anadromous fish are in question. Are they part of the original evolutionary components of S. F. Bay's wild steelhead runs? Are they descendants of the landlocked populations of steelhead captured by the construction of dams and reservoirs in the 1950's that have somehow passed the gauntlet of dams and obstructions and made their way to the sea? Are they simply hatchery strays that have lost their way and ended up by chance in San Francisquito Creek? All of these hypotheses have legitimate support from the scientific literature as strong possibilities, and each creates its own set of priorities and actions in resource management for the area.

Hatchery fish could be contributing to the San Francisquito Creek stock in two different ways, as anadromous adults straying from Central Valley hatchery projects or as rainbow trout introduced into the system for sport harvest. Hatchery steelhead stocks are produced in large numbers on the Sacramento River. Strays in S. F. Bay could be derived from steelhead produced by the Coleman Hatchery, but our genetic analyses show significant allelic differentiation between trout collected on San Francisquito Creek and the Coleman Hatchery strain. The Feather River Hatchery, and to a lesser extent the Merced River Hatchery, also produce steelhead smolts for release into this system, but we currently have no genetics data on these stocks for these analyses.¹ We did sample

¹ Pending CALFED contract between my laboratory and CDFG for genetic analyses of Central Valley steelhead populations includes these populations. Expected start date is early 2000.

and have microsatellite data for wild trout from several of the major rivers in the Sacramento-San Joaquin system, including the American, Stanislaus, and Mokelumne rivers. None of the trout populations on these rivers had close haplotype or microsatellite allelic associations with the San Francisquito Creek collection made for this study (see Figure 1).

The stocking of hatchery-raised rainbow trout in streams and rivers throughout the U. S. has caused concern about potential introgressive hybridization, genetic swamping, or competitive exclusion with native trout (Reisenbichler and McIntyre 1977; Dowlings and Childs 1992; Campton 1995). The role of hatcheries in the preservation and conservation of native fish stocks remains controversial (Hindar et al. 1991; Waples and Do 1994; Busby 1996). The Sacramento River genetic origins of most of California's hatchery rainbow trout strains complicates our ability to discriminate genetically among trout of hatchery and wild origins in central California (Busack and Gall 1980; Gall 1993). Recent DNA technologies, however, give significant resolution for genetics questions at this scale that were previously not available (Nielsen 1996a, 1999; Nielsen and Fountain 1999b).

It has been shown that bringing a species into artificial propagation in the hatchery environment can reduce overall effective population size and lead to reduction in genetic diversity (Waples 1990; Waples and Do 1994; Hedrick et al. 1995). Small population size with subsequent inbreeding among closely related individuals increases the likelihood that genetic variation will be lost over time due to genetic drift (Tajima 1989). This appears to be the case in California's hatchery rainbow trout strains (Nielsen 1996a & b; Nielsen et al. 1997b). The concern is that if a supplementation of hatchery rainbow trout has been successful, a large fraction of the individuals in the post-supplementation population will be derived from a relatively small number of "founders" from the initial population and result in a further erosion of genetic diversity (called the Ryman-Laikre effect). It is questionable if a population subject to the Ryman-Laikre effect can actually be considered as part of an evolutionary significant unit for the conservation of the species.

I currently have no direct records of stocking of hatchery trout into San Francisquito Creek, but it seems unlikely that this stream escaped CDFG's broad scale

distribution of hatchery trout into streams and rivers for recreational purposes. Previous stocking practices may have influenced the genetic profile for trout in this area creating greater genetic distance between this collection and the other collections made on San Francisco Bay. Genetic analyses done for this study, however, showed no significant relationships among trout populations collected on San Francisquito Creek and fish collected from the four primary rainbow trout hatchery strains used for supplementation in California by CDFG suggesting a low probability of the Ryman-Laikre effect on this stream. These data support the inclusion of San Francisquito Creek trout within the same ESU as Lagunitas Creek, listed as part of the north coastal ESU for steelhead. However, at this time NMFS's ESA listings only afford protection to populations of *O. mykiss* found below man-made barriers.

These data support two steelhead/rainbow trout populations that use the streams and tributaries of San Francisco Bay (coastal and Central Valley), with the Central Valley stocks providing the founding populations for the hatchery strains in use in this area today. In any case, genetics analyses using microsatellites do not support the inclusion of trout from the smaller streams in this area in the same ESU as Central Valley populations simply because trout in these streams can be found below dams or barriers and they both share potentially overlapping habitat in San Francisco Bay.

One important factor thought to derive from the loss of natural genetic diversity is a drop in "fitness" resulting from the loss of locally adaptive genetic variation not found in any other group or population (Allendorf and Leary 1988). The markers used in this study, i.e. microsatellites, are considered genetically neutral and are fixed within a population by migration or drift over long periods of time (Jarne and Logda 1996). However, markers with no selective adaptive function can "hitchhike" with markers linked to selective forces when they are closely linked to a beneficial allele and are, therefore, carried along as the gene that is selected for increases in frequency (Kaplan et al. 1989). If the trout in small tributaries of San Francisco Bay have lost adaptive fitness for important life-history traits, such as the propensity for anadromy, cannot be predicted from population genetics data such as those presented here.

In our rush to conserve genetic signatures across the landscape, it is also important to take into account the sustainability of increased abundance that may result

from any conservation activities. Genotypic and allelic frequencies in a population do reflect a significant contribution from the past that may hold important implications for the future of the species and locally adaptive stocks. To save the fish genotypes and not fix the habitat, however, is a fallacy on the order of magnitude equal to that of the construction of "compensation hatcheries" on the Columbia River. At best, a successful conservation program will have little permanent genetic impacts on the natural diversity remaining within an ESU and its evolution through time; at worst, it will once again shift the balance of nature toward our own illusion of genetic "purity" and retain genotypes in isolation from the vagaries of evolution.

Because of the complex nature of the life history of *O. mykiss*, it seems incredibly naïve to not afford protection to a portion of the population simply because anthropomorphic artifacts such as dams and water diversions block ocean access. On the other hand, protection for all systems with rainbow trout below such artifacts is equally unjust. Despite our limited knowledge of the mechanisms of evolution, if we are to protect "evolutionarily significant units" they should be considered and defined based on informed judgment and the best scientific information available. These data make just such a contribution to our understanding of genetic population structure of rainbow trout in San Francisquito Creek.

Literature cited

- Allendorf, F. W. and R.F. Leary (1988). Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2:170-184.
- Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock (1999). Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* 90:281-288.
- Busack, C. A. and G. A. E. Gall (1980). Ancestry of artificial propagated California rainbow trout strains. *California Fish and Game* 66:17-24.
- Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz & I. V. Lagomarsino. 1996. Status review of west coast steelhead from

- Washington, Idaho, Oregon, and California. National Marine Fisheries Technical Memorandum NMFS-NWFSC-27. Seattle, Washington. 261 pp.
- Campton, D. E. 1995. Genetic effects of hatchery fish on wild populations of Pacific salmon and steelhead: what do we really know? American Fisheries Society Symposium 15: 337-353.
- Dowling, T. E. and M. R. Childs (1992). Impact of hybridization on a threatened trout of the southwestern United States. *Conservation Biology* 6:355-364.
- Felsenstein, J. (1995). PHYLIP version 3.572 - phylogeny inference package distributed by author. Seattle: Department of Genetics SK-10, University of Washington.
- Gall, G. A. E. (1993). Genetic changes in hatchery populations. Pages 81-91 in Genetic Conservation of Salmonid Fishes, I. G. Cloud and G. H. Thorgaard (eds.) Plenum Press, N. Y.
- Goldstein, D. B., A. R. Linares, L. L. Cavalli-Sforza, and M. W. Feldman. 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139:463-471.
- Hagar, J. and P. Salop (1999). An assessment of the feasibility of restoring a viable steelhead population in the Alameda Creek watershed. Draft Report available from Applied Marine Sciences, Inc. Livermore, CA.
- Hindar, K. N. Ryman, and F. M. Utter (1991). Genetic effects of cultured fish on natural populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48:945-957.
- Jarne, P. and P. J. L. Lagoda (1996). Microsatellites, from molecules to populations and back. *TREE* 11: 424-429.
- Kaplan, N. L., R. R. Hudson and C. H. Langley (1989). The "hitchhiking effect" revisited. *Genetics* 123: 887-889.
- McConnell, S. K., P. O'Reilly, L. Hamilton, J. M. Wright, P. Bentzen. (1995). Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): Genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Science* 52: 1863-1872.
- McEwan, D. and T. A. Jackson (1996). Steelhead restoration and management Plan for California. California Department of Fish and Game report.

- Morris, D. B., K. R. Richard, J. M. Wright (1996). Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. *Canadian Journal Fisheries Aquatic Sciences* 53: 120-126.
- Nielsen, J. L. (1996a). Molecular genetics and conservation of salmonid biodiversity: *Oncorhynchus* at the edge of their range. In: *Molecular Genetics in Conservation* (eds. Smith TB, Wayne RK), pp. 383-398. Oxford University Press.
- Nielsen, J. L. (1996b). Using mitochondrial and nuclear DNA to separate hatchery and wild stocks of rainbow trout. Pages 139-147 in *Aquaculture Biotechnology Symposium Proceedings*, International Congress on the Biology of Fishes, San Francisco State University, July 14-18, 1996.
- Nielsen, J. L. (1999). The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: developing a conservation strategy using genetic diversity. *ICES Journal of Marine Science* 56: 449-458.
- Nielsen, J. L. and M. C. Fountain (1999a). Microsatellite diversity in sympatric reproductive ecotypes of Pacific steelhead (*Oncorhynchus mykiss*) from the Middle Fork Eel River, California. *Ecology of Freshwater Fish* 8: 159-168.
- Nielsen, J. L. and M. C. Fountain (1999b). Microsatellite analyses of Alameda Creek rainbow/steelhead trout. Technical report submitted to Applied Marine Sciences, Inc. 10/22/99.
- Nielsen, J. L., C. A. Gan, J. M. Wright and W. K. Thomas (1994a). Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead. *Molecular Marine Biology and Biotechnology* 3: 281-293.
- Nielsen, J. L., C. A. Gan and W. K. Thomas (1994b). Differences in genetic diversity for mtDNA between hatchery and wild populations of *Oncorhynchus*. *Canadian Journal Fisheries Aquatic Sciences* 51(Suppl. 1): 290-297.
- Nielsen, J. L., M. C. Fountain and J. M. Wright JM (1997a). Biogeographic analysis of Pacific trout (*Oncorhynchus mykiss*) in California and Mexico based on mtDNA and nuclear microsatellites. In: *Molecular Systematics of Fishes* (eds. Kocher T, and Stepien C), pp. 53-73. Academic Press, San Diego.
- Nielsen, J. L., C. Carpanzano, M. C. Fountain and C. A. Gan (1997b). Mitochondrial DNA and nuclear microsatellite diversity in hatchery and wild *Oncorhynchus*

- mykiss* from freshwater habitats in southern California. *Transactions of the American Fisheries Society* 126: 397-417.
- Nielsen, J. L. M. C. Fountain, J. C. Favela, K. Cobble and B. L. Jensen (1998). *Oncorhynchus* at the southern extent of their range: a study of mtDNA control-region sequence with special reference to an undescribed subspecies of *O. mykiss* from Mexico. *Environmental Biology of Fishes* 51:7-23.
- Nielsen, J. L., M. C. Fountain, V. L. Kirby, and D. A. Powers (1999a). Microsatellite diversity in California steelhead (*Oncorhynchus mykiss*). SKNA76FD0047 technical report submitted to National Marine Fisheries Service, Long Beach, CA 6/14/99.
- Nielsen, J. L., K. D. Crow, and M. C. Fountain (1999b). Microsatellite diversity and conservation of a relic trout population: McCloud River Redband Trout. *Molecular Ecology* 000-000.
- O'Reilly, P. T., L. C. Hamilton, S. K. McConnell, J. M. Wright (1996). Rapid detection of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 2292-2298.
- Raymond, M. and F. Rousset (1997). GENEPOP version 3.1a, August 1997. Univ. Montpellier II, 34095 Montpellier cedex 05, France (available through anonymous login at <ftp.cefe.cnrs-mop.fr> or e-mail Raymond@isem.univ-montp2.fr), 30 pp.
- Reisenbichler, R. R. and J. D. McIntyre (1977). Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* 34:123-128.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Schneider S., J.-M. Kueffer, D. Roessli and L. Excoffier (1997). ARLEQUIN: An exploratory population genetics software environment. Available: <http://anthropologie.unige.ch/arlequin>.
- Scribner, K. T., J. R. Gust, R. L. Fields (1996). Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 685-693.

- Scott, T. C. (1997). Trout's reappearance spawns controversy: significance of fish found in San Francisquito Creek is disputed. San Francisco Chronicle 12/26/97.
- Shapovalov, L. and A. C. Taft (1954). The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California and recommendations for their management. State of California Department of Fish and Game Fish Bulletin No. 98.
- Tajima, F. (1989). The effect of change in population size on DNA polymorphism. *Genetics* 123:597-601.
- Waples, R. S. and C. Do (1994). Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. Canadian Journal Fisheries and Aquatic Sciences 51(Suppl. 1): 310-329.

Appendix 1. San Francisco Creek rainbow trout genotypes

Fish #	mtDNA	Omy27	Omy325	Omy77	Oney14	Oney2	Oney8	Ols1	Ssa14	Ssa289	Ssa85										
SFM201		107	107	105	125	103	111	151	153	238	238	156	156	155	163	150	150	112	114	105	105
SFM202		107	107	127	139	93	111	151	153	240	240	158	160	155	183	148	148	118	122	105	105
SFM203		103	107	113	123	103	103	na	na	240	240	158	158	151	165	150	150	112	116	105	105
SFM204		107	107	121	121	103	109	161	161	na	na	156	174	155	241	150	150	112	112	105	105
SFM205		105	107	105	129	103	109	153	153	258	258	158	160	155	165	150	150	110	116	105	105
SFM206		103	107	123	123	103	103	157	157	256	256	156	158	155	165	150	150	112	124	105	105
SFM207		105	107	125	137	109	111	151	161	240	240	158	160	155	163	136	150	110	110	105	105
SFM208		111	111	127	137	109	111	157	157	256	256	156	174	163	165	136	136	112	112	135	139
SFM209		107	107	125	125	103	103	157	157	240	242	156	158	151	165	150	150	116	116	105	105
SFM210		103	107	123	123	103	103	157	157	240	240	156	158	155	165	148	148	116	116	105	105
SFM211		103	107	113	125	103	103	157	157	248	252	156	158	155	165	150	150	112	124	105	105
SFM212		103	107	123	123	103	103	157	157	254	258	156	158	155	165	134	134	112	116	105	105
SFM213		105	107	105	129	103	129	157	157	236	238	158	158	155	163	144	144	112	124	105	105
SFM214		105	105	105	121	103	129	161	161	232	232	156	158	155	241	148	148	112	112	105	105
SFM215		105	105	139	141	103	129	161	161	232	240	156	158	163	165	150	150	112	112	105	105
SFM216		105	109	123	131	103	109	153	153	232	232	158	160	155	155	150	150	110	116	105	105
SFM217		105	107	105	137	93	103	157	157	260	260	158	158	165	165	148	148	112	120	105	139
SFM218		105	105	103	105	103	111	153	153	236	238	158	158	155	163	152	152	114	124	105	105
SFM219		105	107	125	133	103	103	na	na	238	238	156	158	155	163	148	148	112	122	105	135
SFM220		105	107	121	133	103	103	na	na	238	238	156	158	155	163	148	148	112	122	105	135
SFM221		105	105	105	125	103	105	153	153	240	240	158	158	155	183	150	150	110	110	105	105
SFM222	MYS 3	105	105	105	105	103	109	na	na	232	232	158	158	155	155	150	150	110	112	105	105
SFM223		105	107	123	123	93	103	na	na	258	258	158	158	155	155	150	150	112	122	105	105
SFM224		105	105	105	123	103	109	153	161	248	252	158	158	155	155	144	148	110	112	105	105
SFM225	MYS 3	105	109	105	137	103	135	151	157	240	240	158	160	155	163	150	150	110	120	105	105
SFM226		107	107	105	105	105	133	151	151	230	234	156	158	155	175	150	150	112	112	105	127
SFM227		105	105	127	133	103	111	151	157	226	226	158	160	155	183	150	150	118	118	105	105
SFM228	MYS 3	107	107	123	123	103	103	157	157	240	240	154	158	155	165	134	134	116	124	105	105
SFM229		103	107	125	137	103	129	157	161	254	254	156	174	165	243	148	148	112	116	105	105
SFM230	MYS 3	105	107	113	125	111	111	153	153	240	240	158	158	155	183	150	150	110	112	105	131
SFM231		109	109	127	127	99	109	151	151	258	258	156	158	155	167	148	148	112	112	105	105
SFM232		107	107	123	123	103	109	153	153	240	240	156	158	151	155	150	150	110	112	105	105

Appendix 1 (cont.)

SFM233	MYS 1	105	105	113	129	103	129	153	157	232	232	156	158	163	163	150	150	112	112	105	141
SFM234	MYS 3	105	111	105	141	103	103	153	153	244	246	174	174	163	163	150	159	na	na	105	135
SFM235		107	109	125	135	103	129	157	157	232	232	158	158	163	165	136	136	112	112	105	105
SFM236	MYS 2	105	107	127	137	109	111	147	153	236	236	158	160	155	163	152	152	112	120	105	135
SFM237		107	107	105	133	103	103	151	157	232	232	156	160	155	241	144	144	112	112	135	137
SFM238	MYS 3	105	107	123	137	103	103	153	157	260	260	160	160	155	155	144	144	112	112	105	105
SFM239		na	na	111	123	103	103	na	na	na	na	na	na	na	na	na	na	na	na	105	105
SFM240		105	107	123	137	103	103	161	161	232	232	158	160	179	179	148	148	112	112	105	105
SFM241		105	107	105	137	103	103	161	161	232	232	156	158	155	165	148	148	112	112	105	105
SFM242	MYS 1	103	105	105	123	109	127	151	151	246	248	156	156	165	167	152	152	112	112	105	141
SFM243		103	103	105	141	103	129	na	na	250	252	158	158	155	165	150	150	112	112	105	105
SFM244		105	109	105	105	103	105	151	151	246	248	156	158	167	171	136	136	112	122	105	141
SFM245		105	105	119	123	103	129	161	161	230	230	160	174	155	241	148	148	112	112	105	105
SFM246	MYS 3	105	107	135	135	103	103	157	157	240	240	160	160	155	181	150	150	120	120	105	135
SFM247		107	107	na	na	103	105	157	157	254	258	156	156	163	165	na	na	112	120	na	na

