Genetic Analysis of Trout (*Oncorhynchus mykiss*)
in Southern California Coastal Rivers and Streams

Sandra Jacobson[1], Jack Marshall[1], Dale Dalrymple[1], Frank Kawasaki[1],
Devon Pearse[2], Alicia Abadía-Cardoso[2], John Carlos Garza[2]

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[1] Trout Unlimited – Chapter 920 and affiliated Golden State Flycasters, San Diego, California

[2] NOAA Southwest Fisheries Science Center and University of California – Santa Cruz
110 Shaffer Road, Santa Cruz, CA 95060

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Appendix H: Individual Site Reports: San Diego and Orange Counties (120 pages; 50 MB)

River/Creek Name	Watershed	County	Pg #
Cottonwood Creek	Tijuana River	San Diego	1-3
Pine Valley Creek / Noble Canyon Creek	Tijuana River	San Diego	4-12

Kitchen Creek	Tijuana River	San Diego	13
Boulder Creek	San Diego River	San Diego	14-21
Cedar Creek	San Diego River	San Diego	22-25
Santa Ysabel Creek	San Dieguito River	San Diego	26-28
Sweetwater River	Sweetwater River	San Diego	29-55
San Luis Rey River	San Luis Rey River	San Diego	56-58
Pauma Creek / Doane Creek	San Luis Rey River	San Diego	59-81
West Fork San Luis Rey River	San Luis Rey River	San Diego	82-96
Santa Margarita River	Santa Margarita River	San Diego	97-101
San Juan Creek	San Juan Creek	Orange	102-107
Trabuco Creek / Holy Jim Creek	Trabuco Creek	Orange	108-120

Appendix I: Individual Site Reports: San Bernardino and Riverside Counties (83 pages; 15 MB**)**

River/Creek Name	Watershed	County	Pg #
Coldwater Canyon Creek	Santa Ana River	Riverside	121-145
San Antonio Creek	Santa Ana River	San Bernardino	146-171
Santa Ana River	Santa Ana River	San Bernardino	172-203

Appendix J: Individual Site Reports: Los Angeles County (155 pages; 36 MB)

River/Creek Name	Watershed	County	Pg #
Arroyo Seco Creek	Los Angeles River	Los Angeles	204-205
Santa Anita Creek / Winter Creek	Los Angeles River	Los Angeles	206-234
Roberts Canyon Creek	Los Angeles River	Los Angeles	235-239
East Fork San Gabriel River – Cattle Canyon	San Gabriel River	Los Angeles	240-262
West Fork San Gabriel River – Bear Creek	San Gabriel River	Los Angeles	263-289
West Fork San Gabriel River – Devil's Canyon	San Gabriel River	Los Angeles	290-312
West Fork San Gabriel River – upper	San Gabriel River	Los Angeles	313-335
North Fork San Gabriel River	San Gabriel River	Los Angeles	336-358

Appendix K: Individual Site Reports: Ventura County (68 pages; 17 MB)

River/Creek Name	Watershed	County	Pg #
Sespe Creek – Piedra Blanca / Lion Creek	Santa Clara River	Ventura	359-375
Sespe Creek – near Fillmore	Santa Clara River	Ventura	376-379
Piru Creek – Gold Hill	Santa Clara River	Ventura	380-400
Piru Creek – Buck Creek	Santa Clara River	Ventura	401-426

Appendix L: Tissue Log 1 (182 pages, 42 MB)

Appendix M: Tissue Log 2 (103 pages, 22 MB)

Appendix N: Tissue Log 3 (20 pages, 6 MB)

Appendix O: Tissue Log 4 (11 pages, 2MB)

Introduction

Background and Significance

Trout of the species *Oncorhynchus mykiss,* commonly known as rainbow trout, redband trout or steelhead, historically populated coastal streams and the near-shore ocean throughout California. While both forms reproduce in fresh water, steelhead migrate between the ocean and fresh water while resident rainbow trout live entirely in fresh water. Urbanization and dam construction in Southern California have inhibited or completely blocked steelhead ocean migration. As a result, many *O. mykiss* populations are isolated in freshwater streams often near remote headwaters of their native basins, and have adopted a completely resident life history. These populations are relicts of native coastal steelhead lineage and are referred to as rainbow trout. In addition to habitat degradation, poor water quality and low flows, the stocking of hatchery raised rainbow trout of different genetic backgrounds and reduced fitness into many southern California streams and reservoirs are further threats to survival of native *O. mykiss* at the southern end of their range. Understanding the ancestry of current rainbow trout populations is needed to determine which extant populations of *O. mykiss* are part of the genetic lineage of the endangered Southern California Steelhead Distinct Population Segment (DPS). The focus of this study is to document the location and genetic lineage of existing rainbow trout populations in the southernmost extent of their native range to support ongoing and future steelhead recovery efforts.

Federal ESA listing of Southern California Steelhead

A sharp decline in steelhead population started in the mid-1900s. The National Marine Fisheries Service estimates that the more robust California Central Valley steelhead runs were between 1 and 2 million prior to 1850, and now number about 3,600. Farther south, estimated annual runs of steelhead have declined from 32,000-46,000 returning adults to less than 500 today (Good et al. 2005). In the southernmost area of California, the number of Southern California steelhead has declined to the point that documented sightings are rare. The range of this DPS covers the most populated and developed areas of California, including parts of San Diego, Orange, San Bernardino, Riverside, Ventura and Los Angeles counties. The decline in Southern California Steelhead populations led to the federal listing under the Endangered Species Act (ESA) of the Southern California Coast steelhead in 1997 from the Santa Maria River at the north end to Malibu Creek at the south end. Following steelhead sightings and genetic documentation in watersheds south of Malibu Creek, the geographic boundary was extended southward to the U.S.-Mexico border in 2002. The listing status of this expanded region was reaffirmed in 2006.

Limiting factors to steelhead recovery are tightly linked to features of their life history as an anadromous species. Steelhead live in freshwater as juveniles for at least one year before migrating to the ocean where they feed and grow for several months to years before returning to freshwater to spawn. Some fish only enter the estuary or briefly enter saltwater before moving back up to tributary habitat. Many steelhead are iteroparous, surviving the first spawning run, returning to the ocean and repeating the migration cycle to fresh water in one or more future years. As such, they require passage up and down the main stem of a river, normally during periods of winter high water flow. They also need sites with appropriate gravel for spawning and year-round refuge areas for rearing. Adequate estuary habitat for growth and completion of smoltification, the process that prepares juvenile fish for the transition from fresh water to salt water, increases survival of steelhead. Nevertheless, *O. mykiss* exhibit remarkable physiological and behavioral plasticity, with life history strategy potentially switching between generations. Populations of steelhead without migratory access to and from the ocean can adopt a resident life-history, and even after generations in fresh water can produce progeny that go through smoltification, attempt to migrate to the ocean and adopt steelhead characteristics.

Federal Steelhead Recovery Strategy

To protect these steelhead from extinction, recovery efforts in watersheds throughout California have been pursued by federal and state agencies including the National Oceanic and Atmospheric Administration’s (NOAA) National Marine Fisheries Service (NMFS) and the California Department of Fish and Wildlife (CDFW). In 2012, NMFS published the Southern California Steelhead Recovery Plan as a guideline for recovery efforts. As prescribed by the ESA, an endangered species recovery plan must include a) objective, measurable criteria which when met, will allow delisting of the species; b) a description of site-specific management actions necessary for recovery; and c) an estimate of the time and cost to carry out the recommended recovery measures.

To meet these requirements, NOAA/NMFS engaged scientists and fisheries managers in Technical Review Teams to do the forward technical planning for steelhead recovery consistent with ESA requirements. The Technical Review Teams initially published a series of technical memoranda describing historic population structure in the Southern California Recovery Planning Area (Boughton et al. 2006), and defined viability criteria for anadromous *O. mykiss* (steelhead) in the Southern California and South-Central California Distinct Population Segments (Boughton et al. 2007). They subsequently used physical fish surveys as well as genetic and biochemical analyses to locate and distinguish *O. mykiss* populations in the Southern California DPSs.

As part of this effort, the NMFS Technical Recovery Team used a Threat Assessment Process to identify, prioritize and compare threats to survival among the watersheds in which the Southern California steelhead are native and to examine the possibilities for mitigation. These watersheds make up five biogeographic population groups (BPGs) that must be sustainable as the basis for recovery: the Conception Coast, Mojave Rim, Monte Arido Highlands, Santa Monica Mountains, and the Santa Catalina Gulf Coast (Figure 1 and Table 1). These BPGs were designated based on a predominance of coastal or inland habitat, whether their watersheds were ocean facing to receive incoming storms, and whether they displayed similar geographic and hydrologic characteristics (Boughton 2005, Boughton and Goslin 2006).

The Southern California Steelhead Recovery Plan distinguishes populations in the Southern California DPS based on the ideas that “population viability is more likely achievable by focusing recovery efforts on larger watersheds in each Biogeographic Population Group capable of sustaining larger populations, and DPS viability is more likely achievable by focusing on the most widely-dispersed set of such core populations capable of maintaining dispersal connectivity” (NMFS Southern California Steelhead

Recovery Plan, 2012). Populations were designated as Core 1, 2 or 3 based on known physical characteristics of the watershed and predicted characteristics of the resident trout populations. Core 1 watersheds comprise the nucleus of the recovery strategy and are highest priority. However, Core 2 and Core 3 populations are also important by virtue of supporting viability of Core 1 populations and their role in potentially supporting resident rainbow trout populations of coastal steelhead lineage.

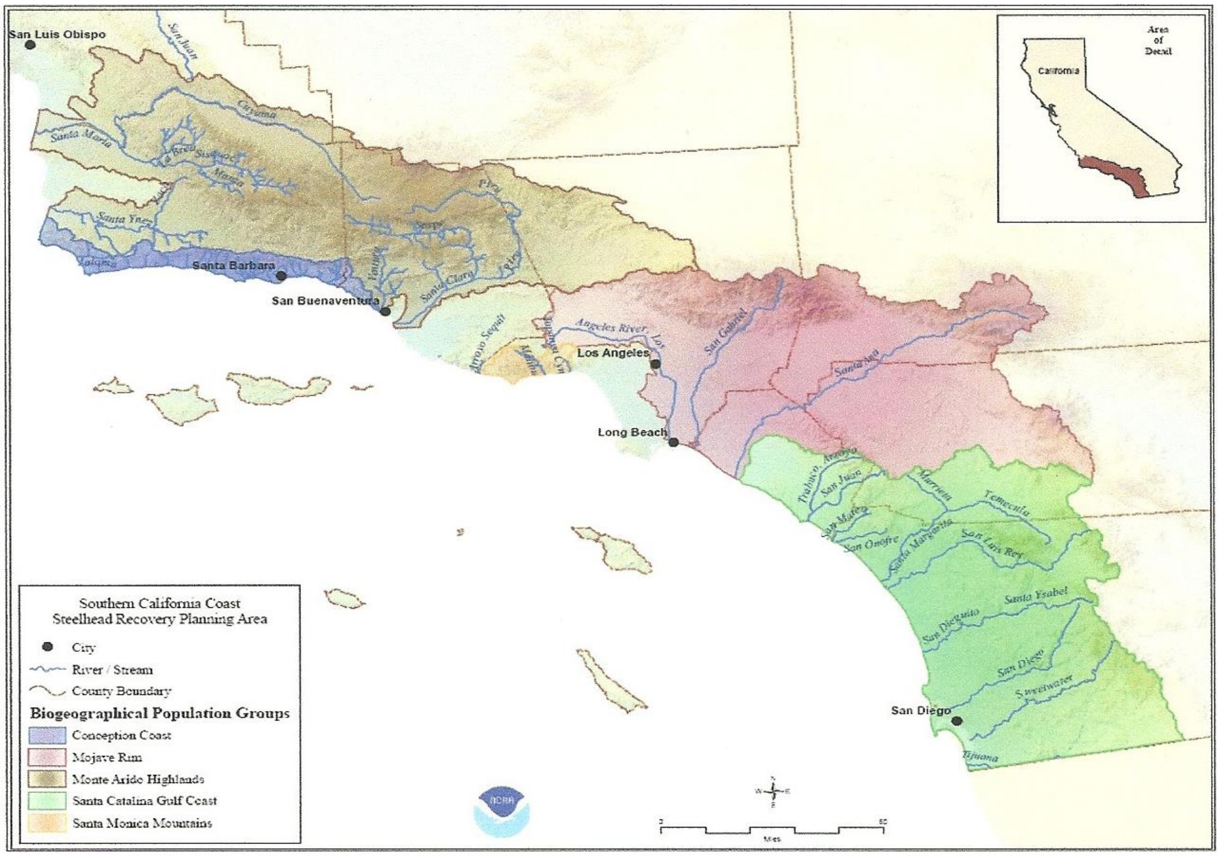


Figure 1. Biogeographical Population Groups within the Southern California Coast Steelhead planning area. From the NMFS Southern California Steelhead Recovery Plan Summary (2012).

The sampling sites for the present study are shown in bold type in Table 1. They are focused on Core 1 watersheds in addition to Core 2 and Core 3 watersheds primarily in the Mojave Rim and Santa Catalina Coast BPGs, the two southernmost BPGs in the counties of San Diego, Orange, San Bernardino, Los Angeles and Riverside (Figure 2 and Table 2). The northernmost major collection sites in this study were in Sespe and Piru creeks in the Santa Clara watershed of the Monte Arido Highlands BPG. The Santa Clara River sites were intentionally targeted in this study as a control group that overlaps with the southernmost watershed in the analysis of genetic population structure by Clemento et al (2009). Small numbers of samples (n=5) were also collected from Montecito Creek and the Ventura River tributary San Antonio Creek as part of this study.

Southern California and its coastal areas have a high degree of urban development, with over 20 million people and coastal valleys bearing agricultural crops, impervious ground cover and channelized waterways. The remaining *O. mykiss* populations in this region are primarily in remote upstream reaches of watersheds in the Los Padres, Angeles, San Bernardino and Cleveland National Forests. Collecting tissues from these trout is difficult, often requiring numerous exploratory trips to find the trout and later needing multiple sampling trips to get enough tissue samples for a population analysis.

Table 1. Watersheds and Rivers/Streams in the five Biogeographic Population Groups of the Southern California Coast Steelhead Recovery Plan. Rivers focused on for this study are in bold type.

Southern California Coast Steelhead Recovery Planning Area Component Biogeographic Population Groups			
BPG	Core #	Watershed	Rivers/Streams
Conception Coast	3	Jalama Creek	Jalama Creek
	3	Canada de Santa Anita	Canada de Santa Anita
	2	Gaviota Creek	Gaviota Creek
	3	Arroyo Hondo Creek	Arroyo Hondo Creek
	3	Tecolote Creek	Tecolote Creek
	2	Goleta Slough	San Jose, Atascadero, San Pedro,and Maria Ygnacio creeks
	1	Mission Creek	Mission Creek
	3	Montecito Creek	Monecito Creek
	1	Carpinteria Creek	Carpinteria Creek
	1	Rincon Creek	Rincon Creek
Monte Arido Highlands	1	Santa Maria River	Santa Maria River main stem, Cuyama River, Sisquoc River
	1	Santa Ynez River	Santa Ynez River main stem (lower, middle and upper reaches)
	1	Ventura River	Ventura River main stem, Coyote Creek, Matilija Creek main stem and north fork, San Antonio Creek
	1	**Santa Clara River**	Santa Clara main stem, Santa Paula Creek, Sespe Creek, Piru Creek
Santa Monica Mountai	3	Big Sycamore Canyon Creek	Big Sycamore Canyon Creek
	2	Arroyo Sequit	Arroyo Sequit
	1	Malibu Creek	Malibu Creek
	3	Solstice Creek	Solstice Creek
	1	Topanga Canyon Creek	Topanga Canyon Creek
Mojave Rim	**3**	**Los Angeles River**	Los Angeles River main stem, Arroyo Seco
	1	**San Gabriel River**	San Gabriel main stem, east fork and west fork
	2	**Santa Ana River**	Santa Ana River main stem, Lytle Creek, Mill Creek
Santa Catalina Coast	**1**	**San Juan River**	San Juan River, Trabuco Creek
	1	San Mateo Creek	San Mateo Creek
	2	San Onofre Creek	San Onofre Creek
	1	**Santa Margarita River**	Santa Margarita River
	1	**San Luis Rey River**	San Luis Rey River
	2	**San Dieguito River**	San Dieguito River
	3	**San Diego River**	San Diego River
	3	**Sweetwater River**	Sweetwater River
	3	Otay River	Otay River
	3	Tijuana River	Tijuana River

From the NMFS Southern California Steelhead Planning Area: Conservation Action Planning (CAP) Workbooks Threats Assessment report (2008) and NMFS Southern California Steelhead Recovery Plan (2012).

Genetic Analysis of Rainbow Trout Populations in Southern California

Genetic analysis has proven useful for distinguishing among populations of *O. mykiss*. Berg and Gall (1988) surveyed steelhead populations throughout California, including populations in the Southern California DPS. Their study showed evidence of high genetic variability using allele frequency analysis of 24 polymorphic loci. A status review by Busby et al. (1996) presented evidence for high genetic variability in Southern California rainbow trout populations by allozyme analysis, which was further supported by analysis of mitochondrial DNA (mtDNA) control region sequences and nuclear microsatellite genotypes (Nielsen et al. 1994; Nielsen et al. 1998a; Nielsen et al. 2003). Further molecular genetic studies to define population structure of trout in Southern California and Baja California, Mexico showed significant differentiation of trout throughout Southern California and Mexico (Nielsen, 1996; Nielsen et al 1997; Nielsen et al 1998b). However, this work did not clearly distinguish native from hatchery derived populations, leaving open the possibility that higher genetic diversity in the region was due to analysis of a combination of both native and hatchery trout coming from diverse evolutionary lineages.

In recent years, a higher resolution view of *O. mykiss* population genetic structure in California has been possible through more extensive analysis of microsatellite DNA and single nucleotide polymorphisms (SNPs) (Aguilar and Garza 2006; Pearse et al. 2007; Garza and Pearse 2008; Clemento et al. 2009; Pearse et al. 2011a; Limborg et al. 2011; Abadía-Cardoso 2014; Garza et al. 2014). Genetic analysis of *O. mykiss* populations in the Southern California and South Central California Coast Steelhead DPSs revealed greater genetic similarity among populations within a watershed than between proximate watersheds, even when separated by physical barriers (Clemento et al. 2009). Pearse et al. (2011b) analyzed *O. mykiss* samples from the Smithsonian archives collected from 1897-1909 to describe historical population genetic structure and compare it to that of contemporary populations in California. They observed a reduced association between genetic divergence and geographic distance separating populations in the contemporary populations. These characteristics are predicted to arise from anthropomorphic influences such as rainbow trout stocking, construction of migration barriers, and degradation of habitat (Pearse et al. 2011b).

The current study is the first large-scale population genetic analysis of *O. mykiss* at the extreme southern end of their range. It uses statistical analysis of data from high resolution microsatellite and SNP genotypes, and provides new insights into the population origins and ancestry of these populations of *O. mykiss*.

Materials and Methods

Geographic Distribution of Rainbow Trout Tissue Sample Collection

Over 600 caudal fin clips were collected for this study from trout in basins that are part of the Southern California Steelhead DPS. Tissue collection efforts for this project covered trout residing in twenty seven creeks and rivers spanning ten watersheds extending south from Montecito Creek, through the Santa Clara River watershed, to the Sweetwater River watershed in San Diego County (Figure 2 and Table 2).

Prior to this study there was little data on trout in this geographic area. The present study extends southward the geographic area focused on in Clemento et al. (2009) which included the Salinas, Arroyo Grande, Santa Ynez, Ventura and Santa Clara watersheds (Figure 2).

Exploratory and tissue collection trips were performed throughout the southern watersheds, and the tissue samples submitted by the California Department of Fish and Wildlife fisheries biologists to the NOAA Fisheries Service Southwest Fisheries Science Center in Santa Cruz, where samples were processed and analyzed by staff of the Molecular Ecology and Genetic Analysis Team.

About 50 exploratory trips were performed throughout these watersheds by volunteers from the San Diego Chapter 920 of Trout Unlimited and Golden State Flycasters. Volunteers generated the road directions and worked out the logistics. They selected the hiking trails, designated the water from which samples would be sought, and secured access from public and private entities when permissions were needed. Typically two exploratory trips were made in preparation for each tissue collection trip. On the approximately 25 tissue collection trips, the volunteers were joined by one or more biologists from the California Department of Fish and Wildlife (CDFW) who carried out the actual clipping and recording of samples. Several trips were made independently by CDFW biologists. Samples were also collected by CDFW more than a decade before the current collections in three basins: Sespe Creek, Pauma Creek, and the Sweetwater River. The two latter sample sets were resampled as part of this project.

A list of tissue collection sites and details is shown in Appendix A. Details of the individual sites for the exploratory and tissue collection trips are recorded in site reports in Appendices G-K (see List of Appendices above for details) and Tissue Collection Logs in Appendices L-0. In future years these reports may shorten the time needed for biologists and others to become familiar with the watersheds and accesses. This information will enable future researchers to see the same sites in the same way they were seen in this study and readily identify new areas to be sampled.

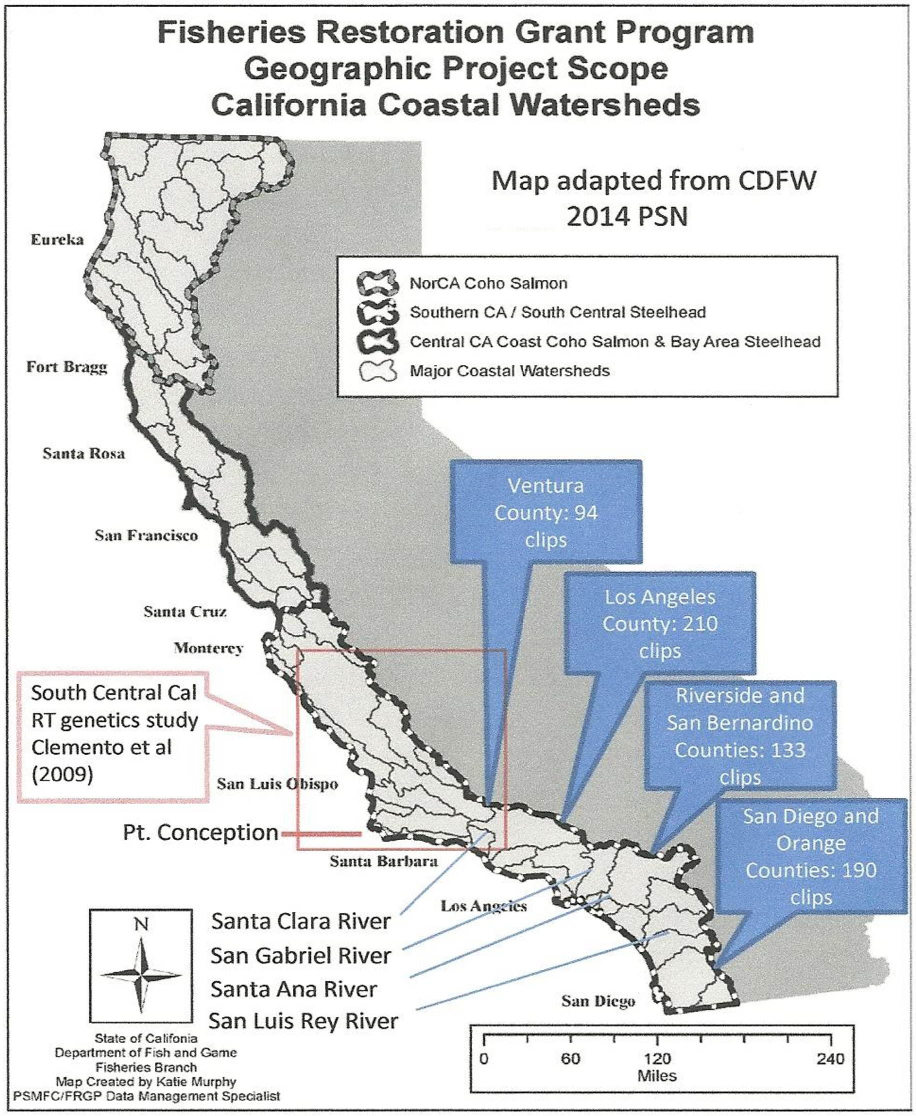


Figure 2. Illustration of trout tissue sample collection by county. Map adapted from the 2014 CDFW Fisheries Restoration Grant Program, Proposal Solicitation Notice (PSN).

Table 2. Southern California *O. mykiss* tissue collection distribution by county.

Tissue Distribution Summary		
San Diego and Orange Counties	190 samples	Trabuco Creek (14), WF San Luis Rey River (12), Pauma Creek (23+46),
		Doane Creek (10), Sweetwater River (26+48), Boulder Creek (11)
Riverside and San Bernardino Counties	133 samples	San Jacinto Creek (North Fork and Fuller Mill; (53)), Santa Ana River (23),
		San Antonio Creek (37), Coldwater Canyon Creek (20)
Los Angeles County	210 samples	North Fork San Gabriel River (17), West Fork San Gabriel-upper and lower (20),
		West Fork San Gabriel-Bear Creek (29), Devils Canyon Creek (14)
		East Fork San Gabriel (21)-Cattle Canyon Creek (17), Iron Fork (44), Fish Creek (24)
		Santa Anita Creek (24)
Ventura County	94 samples	Sespe Creek (40) -Piedra Blanca Creek (10), Piru Creek-Gold Hill (26), Buck Creek (18)

Tissue Collection Protocol

Tissue collection efforts by fly-fishing and netting started in September 2010 and finished in November 2013. Tissue recovery was designed to be non-invasive and not harmful to the trout. Captured trout were removed briefly from the river to minimize stress, placed in a bucket containing river water, and unhooked if necessary. The CDFW fisheries biologist present wrote relevant data onto both sides of the envelope used to store the tissue sample. During this procedure the assistant established the GPS coordinates, which were then written on the back of the envelope and on the collection data sheet (Appendices C and D). The assistant took a photo clearly showing the front of the envelope and the fish, preferably on a measuring board.

After resting the fish for a few moments, the CDFW biologist captured it again in one hand, holding it in or very close to the water in the bucket, and with scissors in the other hand clipped a very small triangular sample from the top lobe of the caudal fin. The sample was transferred to blotter paper which was then put into the envelope. This envelope was conserved by the biologist for later drying and storage at a CDFW facility before being sent for processing to the Southwest Fisheries Science Center lab in Santa Cruz. The fish was released into the stream, with the elapsed time out of the stream being between five and ten minutes. The full protocol for the Southern California Rainbow Trout Tissue Sampling Project in seven parts is provided in Appendix C, with comments on application in the field.

Genetic Analysis Materials and Methods

Genomic DNA was extracted from the dried fin clips using DNeasy 96 Tissue Kits (Qiagen Inc.) with a protocol modified for use on a BioRobot 3000 workstation (Qiagen). DNA was eluted in 200 uL Tris buffer for subsequent analyses.

A panel of 15 microsatellite loci previously used to study *O. mykiss* throughout California (Garza et al. 2014) and in Southern California (a subset of those in Clemento et al. 2009) was then genotyped on all samples for which such genotypes were not already available. DNA was diluted 1:20 in ddH$_2$O and used as a template for polymerase chain reaction (PCR) amplification. PCR was carried out in 96 well microplates and all DNA was transferred from extraction plates to PCR plates with multi-channel pipets to eliminate potential sample mix-ups. The PCR conditions were as reported by Clemento et al. (2009). PCR products were electrophoresed and detected on an ABI 3730XL automated sequencer (Applied Biosystems, Inc.) with the use of fluorescently-labeled primers.

Genotypes were called using GeneMapper (Applied Biosystems, Inc.) and all allele calls checked for consistency with previously genotyped reference samples. To reduce error rate and control for bias in scoring alleles, all genotypes were derived twice independently and any discrepancies between first and second scores were resolved by two people.

A total of 96 single nucleotide polymorphism (SNP) loci were genotyped on all samples evaluated in the current study. These SNP markers are the same panel used by Abadía-Cardoso et al. (2013) and include loci from Aguilar and Garza (2008), Campbell et al. (2009), and Abadía-Cardoso et al. (2011). They have been validated in many steelhead and rainbow trout populations from California, Oregon and

Washington, as well as introduced populations in other parts of the world (unpublished data). A PCR pre-amplification was carried out in 5.4µL aliquots containing 2.5µL of 2X Master Mix (QIAGEN Inc.), 1.3µM pooled oligonucleotide primers, and 1.6µL template DNA. Pre-amplification thermal cycling conditions included an initial denaturation of 15 min at 95°C, and 13 cycles of 15s at 95°C, 4 min at 60°C (+ 1°C/cycle). Pre-amplification PCR products were diluted 1:3 in 2 mM Tris.

The SNP genotyping method was the 5' nuclease allelic discrimination or TaqMan assay (Applied Biosystems) for high-throughput genotyping. Genotyping was carried out in 96.96 Dynamic SNP Genotyping Arrays which use nanofluidic circuitry to combine 96 loci with 96 samples in 9216 reaction chambers, with thermocycling and fluorescence detection done on the EP1 Genotyping System (Fluidigm Corporation) using the manufacturer's specifications.

The combined genetic data was analyzed using standard population genetic methods employed at the Southwest Fisheries Science Center for evaluating population structure and historical ancestry. First, the model-based clustering method employed in the software STRUCTURE (Pritchard et al. 2000) was used to investigate patterns of ancestry in all *O. mykiss* populations and in individual fish. This Bayesian analysis is based on individual multilocus genotypes and uses hypotheses about the number of clusters (e.g. populations or lineages), K , represented by the dataset to infer ancestry without reference to any prior information about the geographic location of sampling or the population affiliation of any of the constituent samples.

This analysis provides estimates of individual ancestry on a fractional basis for each of the genetic clusters inferred by the method, thereby indicating the origin of individuals and the level of interbreeding within and between groups. We investigated values of K = 2 – 7 and 3 iterations were executed for each K value, with a burn-in period of 50,000 steps and 150,000 Monte Carlo Markov Chain replicates. Multiple analyses with STRUCTURE were used to evaluate repeatability and further assess individual relationships. The results from these STRUCTURE runs were reordered and visualized using the software CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004). Visual inspection of DISTRUCT plots allowed identification of regions where genetic affiliation shifts abruptly.

The patterns of clustering (Figure 3) at low K values first indicate whether *O. mykiss* populations and individuals are primarily of native coastal steelhead or hatchery rainbow trout lineage. At higher values of K, the population structure within and between basins becomes evident, as does the distinctiveness of many of the populations studied here.

Phylogeographic trees, or dendrograms, are another way to summarize relationships between populations, albeit with the constraint that they have to be strictly branching ones. Phylogeographic trees were constructed using Cavalli-Sforza and Edwards (1967) chord distances and the neighbor-joining method (Takezaki and Nei 1996), with the software PHYLIP (Felsenstein 2005). For this analysis, populations with mixed ancestry evident in the individual STRUCTURE results were first sub-divided to reflect differences among sampling locations and times. Allele frequencies were bootstrapped 10,000 times to evaluate statistical support for branching patterns.

Results

Genotypes from the 610 individual trout sampled from focal populations were combined with those from previously genotyped *O. mykiss* populations throughout California, including seven hatchery rainbow trout strains and *O. m. nelsoni*, a subspecies of trout from Baja California, Mexico, for a total of 2,180 fish analyzed (Table 3).

All individual genotype data were combined into a single dataset with 111-114 loci per population, and individuals which failed to provide scorable genotypes for 9 or more microsatellite loci and/or 10 or more SNP loci were removed from all subsequent analyses. This combined analysis provides increased resolution relative to single marker-type approaches or those based on fewer loci. Following initial analysis using the program STRUCTURE, that provided genotypic assignments for individuals, populations were split into subgroups based on geographic or temporal sampling effects.

Table 3: Summary of all populations analyzed, with "X" indicating focal populations sampled for the present study. County indicates location where a given watershed enters the ocean, not the specific collecting location within the watershed. N = Number of samples genotyped for the genetic analysis. ID = Population ID used in figures.

	County	Watershed	Tributary	Site	N	ID
	Del Norte	Klamath River		Blue Creek	32	BlKlaRi
	Humboldt	Mattole River	Bear Creek	South Fork Bear Creek	31	Matto
	Sonoma	Gualala River	South Fork Gualala River	Fuller Creek	63	Guala
	San Francisco	Sacramento River	American River	North Fork American River	49	AmRNF
	San Francisco	Sacramento River	Feather River	Yuba River	27	UpYuL
	San Francisco	Sacramento River	McCloud River	Claiborne Creek	33	McClC
	San Francisco	Sacramento River		Battle Creek	94	Battl
	San Francisco	Sacramento River		Deer Creek	45	DeerC
	San Mateo	San Francisquito Creek		Los Trancos Creek	24	LosTran
	Santa Cruz	Waddell Creek		Waddell Creek	31	Wadd
	Monterey	Carmel River		Carmel River	32	Carmel
	Monterey	Salinas River	Arroyo Seco Creek	Tassajara Creek	46	Tassaja
	Monterey	Big Sur River		Big Sur River	31	BigSuRi
	Monterey	Willow Creek		Willow Creek	31	WilloCk
	San Luis Obispo	Chorro Creek		Pennington Creek	32	PenChor
	San Luis Obispo	San Simeon Creek		San Simeon Creek	31	Simeon
	San Luis Obispo	Santa Maria River	Cuyama River	Reyes Creek	47	CuyRey
	San Luis Obispo	Santa Maria River	Sisquoc River	Manzana Creek	47	SisqMnz
	San Luis Obispo	Santa Maria River		Sisquoc River	47	Sisq
X	Santa Barbara	Montecito Creek		Montecito Creek	5	MnteCr
	Santa Barbara	Santa Ynez River	Quiota Creek	Quiota Creek	38	Quiota
	Santa Barbara	Santa Ynez River		Santa Cruz Creek	37	SCrz
	Santa Barbara	Santa Ynez River		Hilton Creek	47	Hilt
	Santa Barbara	Santa Ynez River		North Fork Juncal Creek	85	Junc
	Santa Barbara	Santa Ynez River		Salsipuedes Creek	47	Sals
X	Ventura	Ventura River		San Antonio Creek	5	VeSAnt
	Ventura	Ventura River	Matilija Creek	North Fork Matilija Creek	46	NFMati
	Ventura	Ventura River	North Fork Matilija Creek	Bear Creek	23	BearVen
	Ventura	Santa Clara River		Santa Paula Creek	47	SPau
X	Ventura	Santa Clara River	Sespe Creek	Piedra Blanca Creek	10	Piedra
	Ventura	Santa Clara River	Sespe Creek	Lion Canyon Creek	47	LionCyn
X	Ventura	Santa Clara River		Sespe Creek	40	Sespe
X	Ventura	Santa Clara River	Piru Creek	Buck Creek	17	BuckCr

X	Ventura	Santa Clara River		Piru Creek	26	Piru	
X	Los Angeles	Los Angeles River	Rio Hondo	Santa Anita Creek	24	SAnita	
X	Los Angeles	San Gabriel River		North Fork San Gabriel River	16	NFSGab	
X	Los Angeles	San Gabriel River	West Fork San Gabriel River	Bear Creek	26	SGaBear	
X	Los Angeles	San Gabriel River	West Fork San Gabriel River	Devil's Canyon Creek	19	DevSG	
X	Los Angeles	San Gabriel River		West Fork San Gabriel River	22	WFSGab	
X	Los Angeles	San Gabriel River	East Fork San Gabriel River	Cattle Creek	17	Cattle	
X	Los Angeles	San Gabriel River	East Fork San Gabriel River	Fish Fork	11	FishFk	
X	Los Angeles	San Gabriel River	East Fork San Gabriel River	Iron Fork	43	IronFk	
X	Los Angeles	San Gabriel River		East Fork San Gabriel River	19	EFSGab	
X	Los Angeles	San Gabriel River		Fish Canyon Creek	5	FishCyn	
X	Orange	San Juan Creek		Arroyo Trabuco Creek	14	TrabCr	
X	Orange	Santa Ana River	Chino Creek	San Antonio Creek	36	OraSnAn	
X	Orange	Santa Ana River		Santa Ana River-Bear Creek	23	StaAna	
X	Orange	Santa Ana River	Temescal Wash	Coldwater Canyon Creek	19	ColdCr	
X	Orange	Santa Ana River	Temescal Wash	Fuller Mill Creek	16	FulMil	
X	Orange	Santa Ana River	Temescal Wash	North Fork San Jacinto Creek	36	NFSnJa	1
X	San Diego	San Luis Rey River		Doane Creek	3	Doane	
X	San Diego	San Luis Rey River		Pauma Creek	75	Pauma	2
X	San Diego	San Luis Rey River		West Fork San Luis Rey	12	WFSLuis	
X	San Diego	San Diego River		Boulder Creek	11	SDiego	
X	San Diego	Sweetwater River		Sweetwater River	63	Sweet	3
	Baja California	Río Santo Domingo	Río San Antonio	Río San Antonio/Aroyo La Grulla	41	nelsoni	
	Ventura	Santa Clara River		Fillmore Hatchery/Coleman	49	FHCol	4
	Ventura	Santa Clara River		Fillmore Hatchery/Virginia	49	FHVirg	5
	Ventura	Santa Clara River		Fillmore Hatchery/Whitney	49	FHWhi	6
	Ventura	Santa Clara River		Fillmore Hatchery/Wyoming	49	FHWyo	7
	San Francisco	Sacramento River		American River Hatchery/Eagle	47	ARHEag	8
	San Francisco	Sacramento River		American River Hatchery/Shasta	47	ARHShas	9
	Inyo	Owens Lake Basin	Owens River	Hot Creek Hatchery	47	HCKmlp	10
					2180		

1) Split into NFSnJaB (2009) and NFSnJaE (2012), 2) Split into PaumaR (recent 2009-11) and PaumaO (old 1997), 3) Split into SweetR (2010 and 13) and SweetO (1997), 4) Fillmore Hatchery, Coleman Strain 2003 (Jan), 5) Fillmore Hatchery, Hot Creek/Virginia Strain 2002, 6) Fillmore Hatchery, Hot Creek/Wyoming Strain 2002 (Dec), 7) Fillmore Hatchery, Mount Whitney Strain, early/late 2003, 8) American River Hatchery, Eagle Lake Strain, 9) American River Hatchery, Mount Shasta Strain, 10) Hot Creek Hatchery,Kamloop strain.

Population Structure

Populations of coastal California steelhead ancestry were clearly differentiated from hatchery rainbow trout and Central Valley *O. mykiss*, while many of the southern California focal populations were of mixed ancestry (Fig. 3)

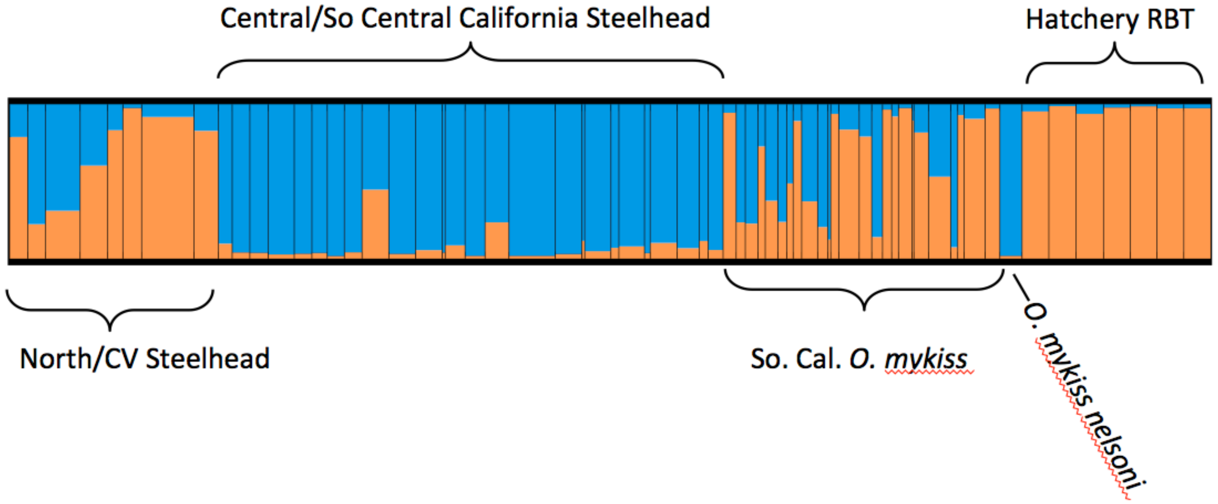


Figure 3: Population Q-values (fractional ancestry) estimated in the program STRUCTURE with the hypothesis of K=2 genetic clusters in the data. This analysis evaluates average individual-based genetic relationships among all populations and each column represents the average fractional ancestry of the population. Populations represented here in orange are derived primarily from hatchery rainbow trout lineages. Populations with more blue represent ancestry of coastal steelhead lineage, while intermediate values indicate populations with some introgression and shared ancestry from both lineages. Populations are ordered from North to South, with major groupings shown.

Examination of the focal Southern California populations at K=7 using individual Q-value (fractional ancestry) estimates clearly identified both native and introgressed samples among the focal populations (Figure 4). In addition, differences among some hatchery trout strains were visible, with apparent widespread stocking of the Fillmore Hatchery-raised Mount Whitney strain of rainbow trout (Figure 4). Furthermore, trout from Baja California, Mexico were consistently identified as distinct from all other population samples.

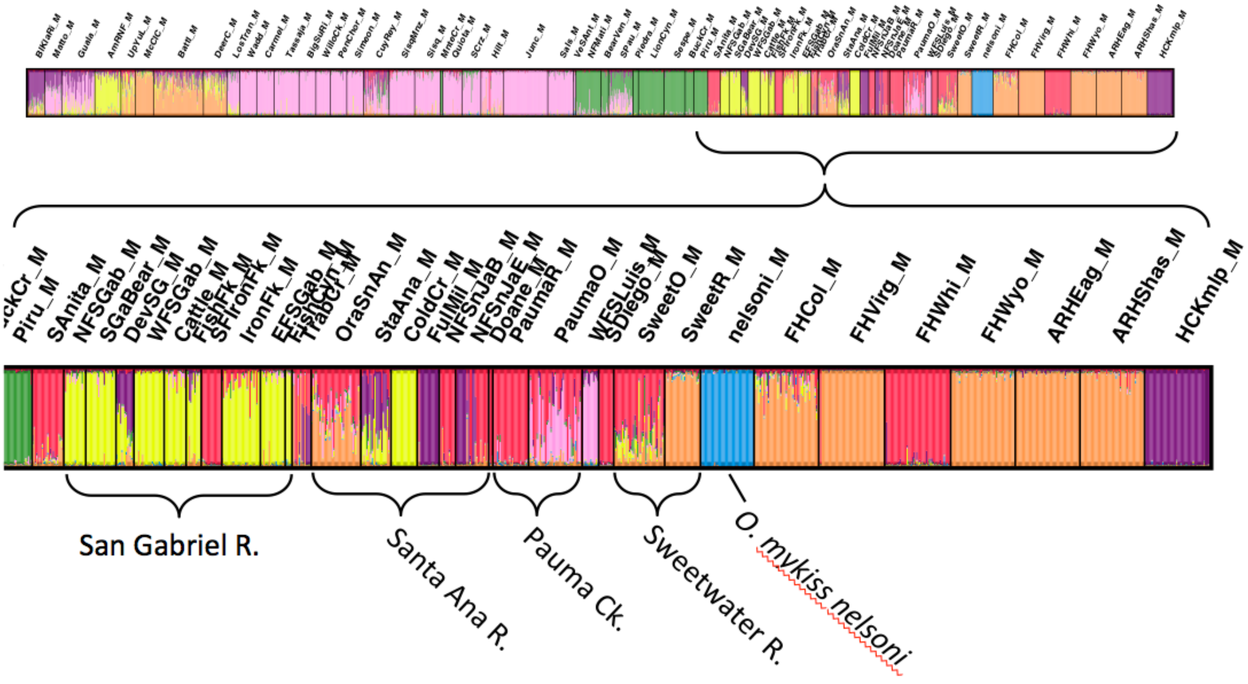


Figure 4: Individual Q-values (fractional ancestry) estimated by the program STRUCTURE for K=7 genetic clusters. This analysis shows individual-based genetic relationships without regard to location of origin. Each vertical column represents an individual's fractional assignment to each of the genetic lineages, and the higher value of K allows individuals to be clustered at a finer scale. Here hatchery rainbow trout ancestry is divided into purple, orange, and red genetic clusters, while native southern California *O. mykiss* ancestry is represented as yellow and pink. Populations are ordered as in Fig. 5.

Finally, population-level phylogenetic relationships were reconstructed using a neighbor-joining tree, and strongly confirmed the individual-based clustering results (Figure 5). In particular, the division between native coastal steelhead lineage populations and hatchery trout/Central Valley populations is clear (Fig. 5). This division identifies some Southern California trout populations as closely related to the hatchery trout/Central Valley lineage, while others appear to have primarily native coastal steelhead ancestry. These results are also concordant with the results from the individual-based structure analysis with two genetic clusters, representing populations with significant hatchery introgression and those with primarily native coastal steelhead ancestry (Figure 3).

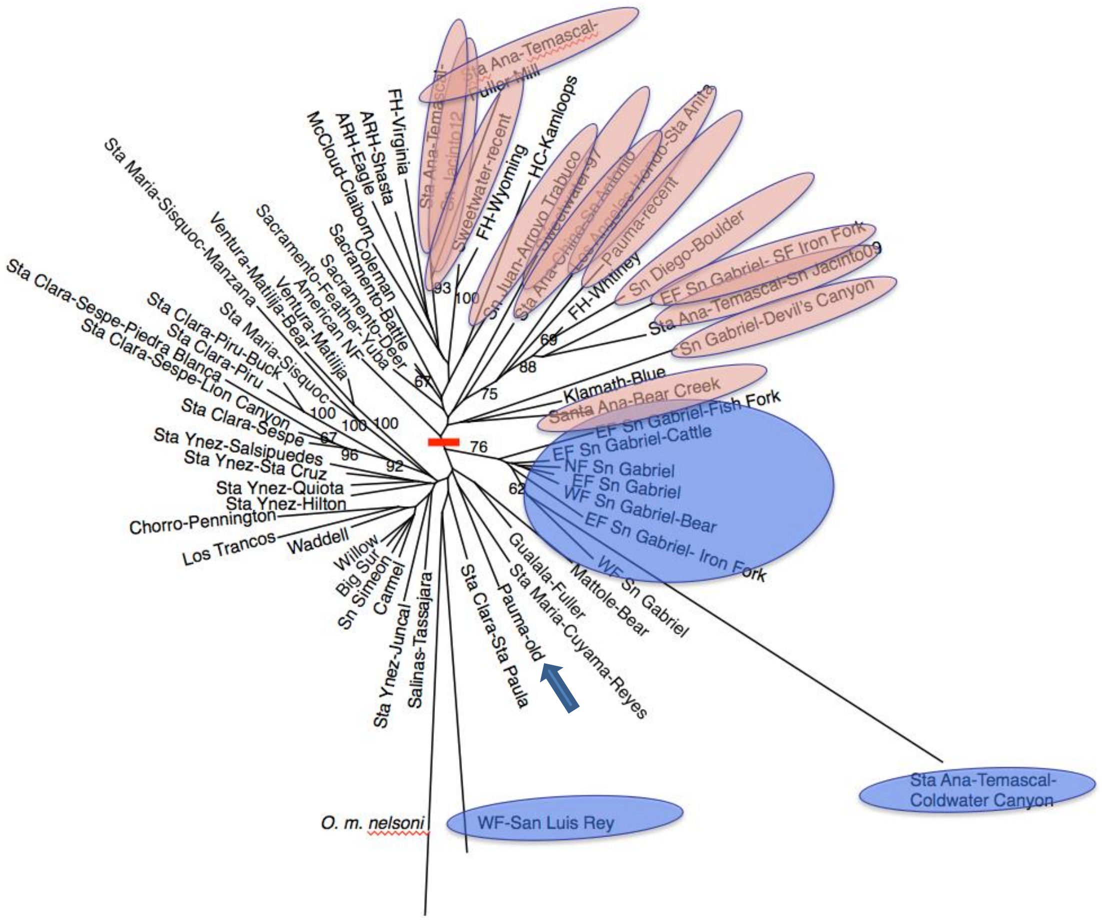
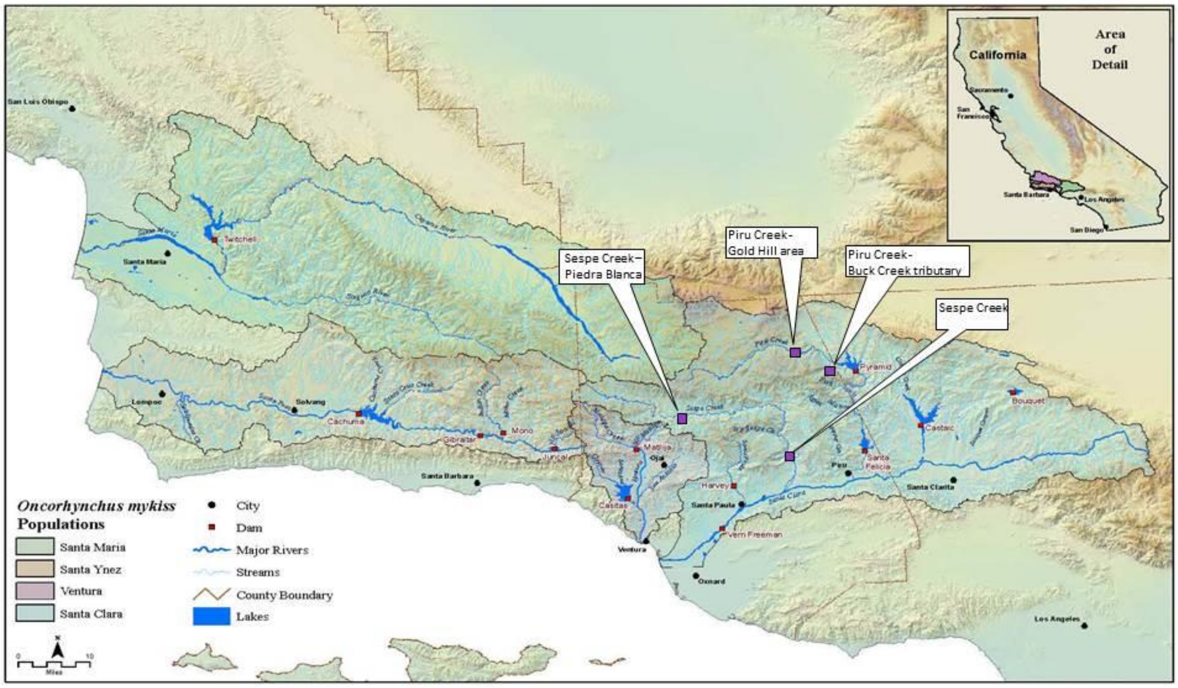


Figure 5: Neighbor-joining dendogram of all populations included in the analysis, with significant bootstrap support (>60%) for internal branches shown. Note that the branch indicated by the red mark almost perfectly divides coastal steelhead lineage populations from those with significant hatchery rainbow trout/Central Valley ancestry. The focal populations for the present study are color coded; those that cluster with the hatchery rainbow trout strains are shown in pink, while those closer to coastal *O. mykiss* populations are blue.

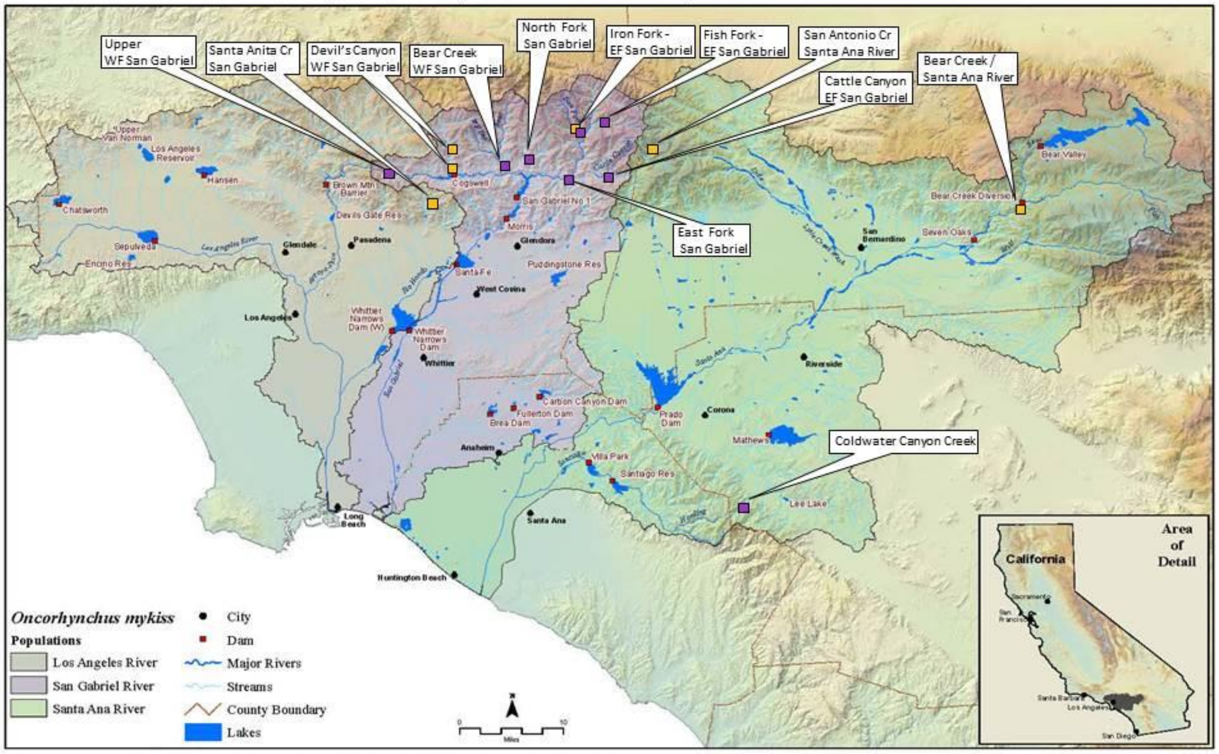
The results from Figure 5 were annotated on maps of the relevant Biogeographic Population Group designations in Figures 6-8 to illustrate relative geospatial locations of trout populations with native coastal Southern California steelhead (dark symbols; purple) or hatchery (light symbols; yellow) genetic ancestry. The northern end of the study area in the Monte Arido Highlands BPG contains Sespe Creek and Piru Creek tributaries of the Santa Clara River (Figure 6); the middle section comprises the Mojave Rim BPG with Los Angeles River, San Gabriel River and Santa Ana watersheds (Figure 7); and the southern section comprises the Santa Catalina Coast BPG covering coastal parts of Orange County and San Diego County (Figure 8).

Northern End of Southern California RT Genetics Study Area (Monte Arido Highlands BPG):
Piru Creek and Sespe Creek, tributaries of Santa Clara River



Map generated by Hunt and Associates for the 2008 Southern California Coast Steelhead Recovery Planning Area report for NOAA / NMFS (Mark Capelli, lead) and annotated to show results of Southern California rainbow trout tissue genetic analysis (Dr. Carlos Garza lab, NOAA Southwest Fisheries Science Center) for Trout Unlimited –San Diego study funded by Cal. Dept. of Fish and Wildlife, Grant Agreement P0950015.

Figure 6. Northern End of Southern California rainbow trout genetics study area: Piru Creek and Sespe Creek, tributaries of Santa Clara River. Trout populations with native coastal (dark symbol; purple) genetic background are shown in their respective sampling locations.

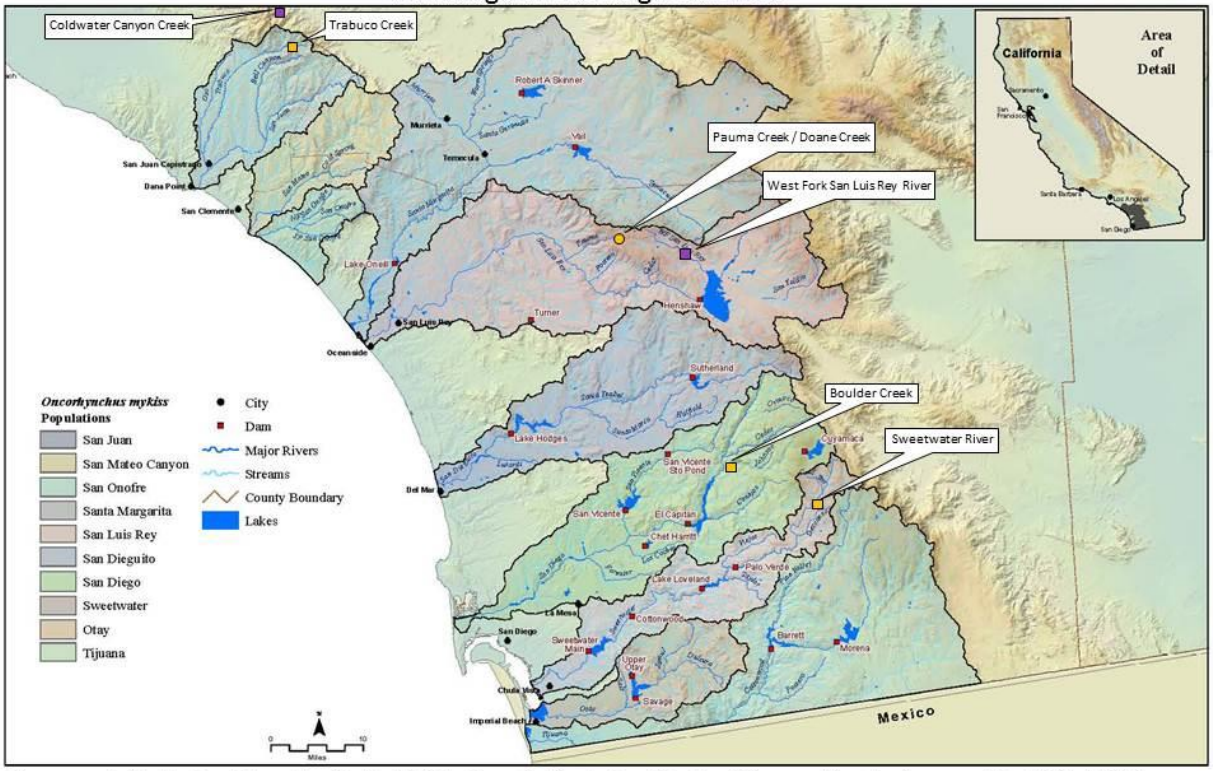


Middle Section of Southern California RT Genetics Study Area (Mojave Rim BPG):
Los Angeles River, San Gabriel River, Santa Ana River

Map generated by Hunt and Associates for the 2008 Southern California Coast Steelhead Recovery Planning Area report for NOAA / NMFS (Mark Capelli, lead) and annotated to show results of Southern California rainbow trout tissue genetic analysis (Dr. Carlos Garza lab, NOAA Southwest Fisheries Science Center) for Trout Unlimited –San Diego study funded by Cal. Dept. of Fish and Wildlife, Grant Agreement P0950015.

Figure 7. Middle Section of Southern California rainbow trout genetics study area: Los Angeles River, San Gabriel River, and Santa Ana River. Trout populations with native coastal (dark symbol, purple) or hatchery (light symbol, yellow) genetic background are shown in their respective sampling locations.

Southern End of Southern California RT Genetics Study Area (Santa Catalina Coast BPG):
San Diego and Orange Counties



Map generated by Hunt and Associates for the 2008 Southern California Coast Steelhead Recovery Planning Area report for NOAA / NMFS (Mark Capelli, lead) and annotated to show results of Southern California rainbow trout tissue genetic analysis (Dr. Carlos Garza lab, NOAA Southwest Fisheries Science Center) for Trout Unlimited –San Diego study funded by Cal. Dept. of Fish and Wildlife, Grant Agreement P0950015.

Figure 8. Southern End of Southern California rainbow trout genetics study area: San Diego and Orange Counties. Trout populations with native coastal (dark symbol; purple) or hatchery (light symbol; yellow) genetic background are shown in their respective sampling locations. Round symbol indicates lack of downstream dam.

Conclusions and Discussion

The analysis conducted here of Southern California *O. mykiss* populations identified two major classes of existing trout in this region, native coastal steelhead lineage and hatchery rainbow trout. The majority of the populations sampled fell into the second category, representing almost complete introgression or replacement of native fish by introduced hatchery rainbow trout. Apart from the populations and basins in the part of the DPS to the north of major urbanization (e.g. Santa Clara, Ventura, Santa Ynez and Santa Maria rivers), only three groups of populations contained significant evidence of native coastal steelhead ancestry 1) the San Luis Rey River, 2) Coldwater Canyon in the Santa Ana River, and 3) populations from the San Gabriel River system, with the exception of the Iron Fork and Devil's Canyon Creek populations.

These three groups with substantial native ancestry, inferred from concordant results of multiple analyses, should be the focus of conservation planning so as to ensure their persistence. It is important to note that some other populations, most notably Bear Creek in the Santa Ana River and Devil's Canyon Creek in the West Fork San Gabriel River, contained limited signals of native ancestry unfortunately overlaid with a strong signal of introgression by hatchery rainbow trout. While these populations are not "pure" native southern California trout, many are likely to be self-sustaining and adapting to the current local environment. They therefore represent potentially useful genetic resources, some of which are of native ancestry, for the continuing persistence of viable networks of *O. mykiss* populations in southern California and should not be considered as insignificant.

Moreover, hatchery rainbow trout are members of the same species so some introgression does not necessarily render a population less viable than a purely native population. In fact, the introduction of some novel genetic diversity in these relatively isolated populations may actually increase viability by providing more variation to adapt to changing environmental conditions or to avoid inbreeding depression. Nonetheless, comparison of samples from Pauma Creek collected in 1997 with those collected from 2010-2013 suggests that either competition or continued hatchery stocking has resulted in a complete replacement of that population with hatchery trout over that time period, although sampling differences cannot be ruled out as an explanation for this pattern. This highlights the ongoing risks to native *O. mykiss* populations of hatchery rainbow trout stocking.

Overall, relatively few populations in this study appear to be pure native Southern California *O. mykiss*. Thus, in order to identify any additional populations with native ancestry, future efforts should particularly expand sample collecting in watersheds where mixed and purely native stocks have been identified, as well as evaluating potentially adaptive genetic variation associated with life history diversity in southern California *O. mykiss* (Pearse et al. 2014).

Implications of the study results for Southern California Steelhead recovery efforts

The NOAA/NMFS Southern California Steelhead Recovery Plan (2012) describes specific goals and strategies for augmenting steelhead populations within the Southern California Distinct Population Segment (DPS). It articulates recovery objectives and designates high priority watersheds to “prevent the extinction of Southern California steelhead in the wild and ensure the long-term persistence of viable, self-sustaining, harvestable, interacting wild populations of steelhead distributed across the DPS.” The Recovery Plan further addresses factors limiting the species’ ability to survive and reproduce in the wild. A central tenet of the recovery plan is that a viable DPS (Figure 1 and Table 1) will consist of a sufficient number of viable discrete populations that may be spatially dispersed but nevertheless adequately connected to achieve the long-term persistence and evolutionary potential of the species.

The objectives stated in the Southern California Steelhead Recovery Plan are to:

1. Prevent steelhead extinction by protecting existing populations and their habitats.
2. Maintain current distribution of steelhead and restore distribution to previously occupied areas that are essential for recovery.
3. Increase abundance of steelhead to viable population levels, including the expression of all life history forms and strategies.
4. Conserve existing genetic diversity and provide opportunities for interchange of genetic material between and within meta-populations.
5. Maintain and restore suitable habitat conditions and characteristics for all life-history stages so that viable populations can be sustained.
6. Conduct research and monitoring necessary to refine and demonstrate attainment of recovery criteria.

Previous studies of genetic structure of juvenile *O. mykiss* collected from freshwater locations above and below barriers in Core 1 watersheds (Table 1) indicated that contemporary populations are dominated by indigenous coastal steelhead lineage fish (Nielsen et al. 1997, Nielsen et al 2003; Garza and Clemento 2007; Clemento et al. 2009). Populations of *O. mykiss* downstream of introduced barriers in this region are genetically similar to populations upstream of barriers in the same basin, suggesting that the latter have value in restoring steelhead runs once barriers to migration are overcome (Boughton et al. 2006). Since anadromous fish are relatively rare in the highly disturbed watersheds studied here, these current resident trout populations may be critical resources for steelhead recovery. This study documents rainbow trout populations of coastal steelhead lineage in the most southern biogeographical population groups (BPGs) in the Southern California Steelhead DPS. These data provide information for steelhead recovery efforts that support Objectives 1-4 of the Southern California Steelhead Recovery Plan. This study provides data on existing populations that are priorities for conservation measures, and establishes the baseline for maintaining and augmenting the current distribution of rainbow trout that are of coastal steelhead lineage.

The southernmost population sampled and genotyped for this study was in the Sweetwater River, whose headwaters are in the Cuyamaca mountains of San Diego County (Figure 1). The Sweetwater

River flows into the ocean less than 30 km north of the U.S.- Mexico border. Samples were taken in 2013 near its headwaters in Cuyamaca Rancho State Park, and those fish were mainly of hatchery rainbow trout descent. However, a population of native coastal steelhead lineage fish was identified further north in San Diego County in the remote West Fork San Luis Rey River, a watershed designated as a Core 1 population in the Southern California Steelhead Recovery Plan (Table 1 and Figure 8). This population is the southernmost population of native rainbow trout in the U.S. documented in this study. A population sample of *O. mykiss nelsoni*, a subspecies of trout found only in Baja California, Mexico, was included as an additional reference population. It was also found to be derived from coastal steelhead lineage, and most closely related to the West Fork San Luis Rey River population. This further supports the native ancestry of these populations.

The case of Pauma Creek, a tributary of the San Luis Rey River near its headwaters in Palomar Mountain State Park, is one that illustrates the challenges of maintaining native coastal steelhead lineages trout populations. As noted above, comparison of samples from 1997 with those collected in 2010-2013 for the current project suggests that either competition or continued hatchery trout stocking has moved the Pauma Creek population from 'mixed' status to complete hatchery replacement over that time period. However, it is possible that the recent collections are of different subpopulations than those from 1997, as abrupt changes in ancestry were found over small spatial scales in other basins (see below).

In contrast to the West Fork San Luis Rey River, which is upstream of Lake Henshaw, Pauma Creek does not have a downstream dam as a barrier to steelhead migration. However, other challenges exist for trout in the San Luis Rey River watershed (Jacobson et al, 2010). There may also be other native trout populations in the Santa Catalina Coast BPG that were not identified in this study. Numerous exploratory trips to rivers and creeks throughout San Diego and Orange counties by members of the Trout Unlimited – San Diego Chapter/ Golden State Flycasters and colleagues (Appendix A) indicated that either stream conditions in these waterways did not look sufficient to support trout or that when trout tissue was taken, they were of hatchery ancestry (Sweetwater River, Boulder Creek, Trabuco Creek) as seen in Figure 5.

The southernmost population of predominantly coastal steelhead lineage fish in the Mojave Rim BPG was observed in Coldwater Canyon Creek. This stream lies just north of Trabuco Creek in Orange County, but drains into the Santa Ana River basin in Riverside County. It is not accessible to the public without prior permission. The documentation of a pure native rainbow trout population here will likely spur further implementation of appropriate conservation measures for this area, and evaluation of neighboring streams for native rainbow trout.

Rainbow trout populations in the San Gabriel River in the northern Mojave Rim BPG show clear evidence of native ancestry, although it varies according to geographic location. Ancestry can change over very small spatial scales in this basin, with neighboring canyons in the West Fork San Gabriel River having rainbow trout of coastal steelhead lineage interspersed with those of hatchery descent (Fig. 7). For example, samples from the East Fork, North Fork and West Fork of the San Gabriel River show native genetic ancestry, but nearby populations in the West Fork San Gabriel – Devil's Canyon and East Fork-San Gabriel – Iron Fork show hatchery introgression. The presence of hatchery ancestry in trout from

Devil’s Canyon was unexpected given its remote location upstream of Cogswell Reservoir and the rugged terrain of the canyon.

The northernmost watershed with large population samples analyzed in this study was the Santa Clara River, with tissue samples collected from Piru and Sespe creeks. These fish showed clear native coastal steelhead genetic lineage, which is concordant with the results of Clemento et al (2009). These collections were designed as a positive control for this study, since they overlapped with the southernmost population samples analyzed by Clemento et al (2009) and were processed by the same lab, using similar protocols and data analysis programs as the earlier study.

This study provides evidence that habitat conditions are indeed sufficient in southern watersheds of the Southern California Steelhead DPS to support survival of trout that are of native coastal steelhead lineage. However, the presence of physical barriers to ocean migration and other habitat characteristics increases the challenges of maintaining long-term viability of steelhead populations. The range of challenges that trout encounter in urbanized Southern California watersheds includes: population fragmentation, low population size per watershed, geographically distant and relatively little suitable spawning and rearing habitat, lack of connectivity due to low water flow, geographically distant neighboring populations both within the watershed and between adjacent basins, sections of poor water quality—particularly in estuaries, and physical migration barriers. Steelhead recovery efforts that target one or more of these challenges are described and prioritized in the Southern California Steelhead Recovery Plan (2012).

Genetic determinants of steelhead behavioral characteristics

The genetic population analysis of *O. mykiss* described here for the more southern watersheds in the Southern California DPS provides an increasingly diverse collection or “library” of tissue samples for addressing questions concerning population dynamics and responses to separation by barriers and genetic mechanisms underlying steelhead ocean migration behavior. The study by Clemento et al. (2009) demonstrated that the fish above and below the same barrier are generally a single, monophyletic group (clade), with closer genetic relationships between populations in the same basin, even when separated by physical barriers, than between adjacent populations. This was also true in the Russian River (Deiner et al. 2007) where steelhead below barriers were genetically similar to fish above dams, suggesting that they had not been isolated very long. However, genetic variation in populations at all below-barrier sites was high compared to that in populations from above-barrier sites. In addition, Garza et al. (2014) analyzed genetic variation of steelhead populations from the California-Oregon border to just north of the current study area and found a significant reduction in allelic diversity in southern populations. While most of the populations sampled in the present study were above reservoirs, the tissue collected is a valuable resource for future population genetic studies and to identify individuals of unknown ancestry with a high degree of accuracy to a particular population based on its genetic profile.

Samples collected in this study will also be important for ongoing studies of genetic determinants of migratory behavior of *O. mykiss* in California. Microsatellite and SNP genotyping of steelhead and rainbow trout in California has been instrumental in revealing population structure and phylogeographic lineages. This work, coupled with new, higher-resolution molecular genetic analysis, will help to resolve the heritable genetic basis and distinctions between resident and anadromous life histories in this species. Recent studies using quantitative trait locus mapping and other approaches suggest that there are discrete genetic loci that underlie this complex behavioral trait (Nichols, 2008; Martinez et al. 2011; Hecht et al. 2012; Miller et al. 2012; Pearse et al. 2014). In particular, Pearse et al. (2014) recently showed that one region of chromosome Omy5 is strongly associated with life-history variation in coastal California *O. mykiss* populations. However, the extent of interactions between genetic factors and environmental conditions in triggering smoltification remain unclear. It also remains to be determined which genes are important for this behavioral plasticity, and whether heritable epigenetic states such as DNA methylation and chromatin acetylation at particular regions of the genome are important for differential gene expression underlying these phenotypes.

Overall, the more extensive geographic coverage of *O. mykiss* population genetic structure in this study addresses a number of important issues in southern steelhead biology and recovery. For the first time, a large enough sample of Southern California coastal steelhead and rainbow trout populations at the extreme southern end of their range can be compared with those from northern watersheds. Statistically robust population genetic analyses have been applied to evaluate the ancestry and relationships among these critical watersheds for recovery of Southern California steelhead. This dataset also fills in gaps in understanding of the geographical distribution of native *O. mykiss* populations in watersheds in the southern part of the Southern California DPS and serves as a baseline for evaluating the impact of hatchery trout on native trout, as well as the success of ongoing and future steelhead recovery projects.

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