

**Population genetic structure of *Oncorhynchus mykiss* in the Santa Ynez River,  
California**

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## **ABSTRACT**

Population genetic structure of the polytypic species *Oncorhynchus mykiss*, steelhead/rainbow trout, was studied in the Santa Ynez River, California. Data from 18 microsatellite marker loci were analyzed at multiple scales to investigate ancestry, migration and effective population sizes. Population samples from Salsipuedes and Hilton Creeks below Bradbury Dam were available for multiple consecutive years and allowed evaluation of temporal genetic variation and estimation of effective population size. Substantial temporal stability was evident from multiple analyses in both populations and effective sizes were low and consistent with census size estimates. In addition, several analyses indicated the presence of large numbers of siblings in both populations, and they were particularly evident in Salsipuedes Creek. Population samples from Santa Cruz and Juncal Creeks above barriers to anadromy were also analyzed to understand their ancestry and interactions with other fish in the basin. The data revealed significant differentiation between all four of these primary population samples in all analyses. However, migration was evident between Salsipuedes and Hilton Creeks, as well as from Santa Cruz Creek to both of these below barrier populations. Analyses using data from the same genetic markers in other coastal California *O. mykiss* populations provided geographic context and demonstrated the coastal steelhead ancestry of all Santa Ynez River populations. Additional analyses including data from all current Fillmore Hatchery trout strains demonstrated very little presence of these fish in any of these four population locations, although several hatchery trout were identified in Hilton Creek in multiple years. However, it is unclear whether these hatchery trout reproduce or hybridize with native fish in Hilton Creek and a signal of introgression of hatchery fish in the Santa Ynez River appears to be largely absent.

## INTRODUCTION

A critical first step in addressing the conservation and management of fish and wildlife populations is appropriately defining the population genetic structure of the target species or taxon. With enactment of the federal and state Endangered Species Acts (ESA), it has become important for managers to accurately understand and define biologically meaningful subdivision at a finer scale than traditional species-level considerations, since such spatial and temporal variability can influence population dynamics, health and risk of extinction. This task can be further complicated for populations and species that have experienced historical human movement.

The species *O. mykiss* exhibits diverse phenotypic strategies, ranging from migratory (steelhead) to non-migratory (rainbow trout) life histories. A number of variants of these strategies, including some with truncated or limited marine stages (“half-pounders”, estuarine migrations, etc.) have also been described (Shapovolov and Taft, 1954). Some of this broad array of life history strategy in *O. mykiss* is due to phenotypic plasticity; the ability to change strategy/form in response to environmental or genetic cues, and some of it appears to be heritable (Thrower et al. 2004). For example, Zimmerman and Reeves (2000) documented both resident mothers of anadromous fish and anadromous mothers of resident fish in a British Columbia drainage. In Argentina, a population of anadromous steelhead was established from resident rainbow trout broodstock, although some contribution from an anadromous founding stock cannot be ruled out (Pascual et al. 2001, Riva-Rossi et al. 2004). Genetic studies in coastal California report that resident and anadromous forms from the same river are generally more similar than the same form in adjacent drainages and are generally descended from coastal steelhead lineages (Girman and Garza 2006, Clemento 2007, Deiner et al. 2007).

Steelhead in southern California were listed as endangered under the US Endangered Species Act (ESA) in 1997 (NOAA 1997), as part of a series of listings that encompassed most steelhead populations in California. The ESA listing designated *O. mykiss* below barriers to anadromy from the Santa Maria River in the north to Malibu Creek in the south as the Southern California Steelhead Evolutionarily Significant Unit (ESU), although it is now referred to as a Distinct Population Segment (DPS). In addition, the ESU/DPS boundary was extended to the border with Mexico, after

anadromous fish were discovered in several creeks as far south as San Diego County (NOAA 2002). In this region, many steelhead populations have been extirpated or drastically reduced due to habitat loss and alteration, water diversions, overfishing and stochastic environmental variability. Although critical habitat has been designated for the Southern California Steelhead DPS, habitat loss, water extraction and non-native introductions continue to threaten the remaining native steelhead and trout populations.

The Santa Ynez River (SYR) is located north of the city of Santa Barbara, draining the Santa Ynez and San Rafael Mountains. The region is mainly arid, and flows in parts of the basin are intermittent and dependent on the transient rain events that are concentrated in the winter months (November-April). Three large dams were constructed in the 1900s to supply water and flood control to the growing populace of Santa Barbara County (see map, Figure 1). In 1920, Gibraltar Dam was erected, blocking anadromous access by steelhead to approximately the upper third of the watershed. Upstream of that, in 1930, Juncal Dam was built, creating Jameson Lake in the headwaters of the drainage. Finally in 1950, the construction of Bradbury Dam created Lake Cachuma. The three dams blocked access to about 67% of the highest quality steelhead spawning habitat in the basin. Although extensive fishery mitigation measures, including fish passage facilities, were recommended upon construction (US Dept. of Interior 1948), none were implemented with the construction of Bradbury Dam, as the SYR steelhead numbers were predicted to be reduced by “only” about 50% (Edmondson 2003).

Early reports describe the SYR as historically maintaining the largest steelhead population in southern California. California Department of Fish and Game records (CDFG 1940, 1944, 1945) estimate that before the dams, steelhead runs numbered 10,000 to 30,000 adult spawners, while juveniles in the river numbered in the millions. Less than 100 steelhead now return to the Santa Ynez River annually. Population declines were already evident in the mid-1900s, and were suspected to be due to impacts from drought and the first two dams. By 1940, mitigation measures were already being undertaken, with steelhead raised at Fillmore Hatchery planted in the Santa Ynez estuary to supplement these declining populations and the transfer of juvenile fish rescued from drying reaches of the lower SYR into perennial habitat. Recent efforts to improve habitat and accessibility have included repair of low-flow barriers to migration and increased

access to additional spawning and rearing habitat on Salsipuedes and Hilton Creeks. However, significant challenges to steelhead in the Santa Ynez River continue, as water temperatures in many parts of the basin often exceed typical thermal limits for *O. mykiss* (Busby et al. 1996) and the reservoirs (and lower river reaches) also contain warm-water species, such as largemouth and smallmouth bass, catfish, crappie and sunfish, that may be both predators and competitors of steelhead. Population subdivision, inbreeding from small population sizes, and a lack of appropriate habitat may also be obstacles to the recovery of Santa Ynez steelhead populations.

Here genetic analyses are employed to describe fine-scale population structure and inference regarding ancestry and origin of both individuals and populations of *Oncorhynchus mykiss*, steelhead/rainbow trout, from the Santa Ynez River, California, near the southern limit of the species' range. The genetic analyses were designed to provide information on specific population biology issues facing resource managers in the SYR basin and region. Spatially and temporally distributed sampling allowed evaluation of fine-scale genetic structure of some of the most important remaining *O. mykiss* populations in the Southern California Steelhead DPS/ESU. Temporal variability was evaluated and used to estimate effective population sizes ( $N_e$ ) in Salsipuedes and Hilton Creeks, the two primary *O. mykiss* populations below Bradbury Dam, the first definitive barrier to anadromy in the basin. Genetic composition and structure of several naturally-spawning populations above this dam, in Santa Cruz and Juncal Creeks, were also evaluated, and relationships and migration between these four primary populations estimated. Data from a number of other *O. mykiss* populations in the central and southern California region were used in comparative analyses to provide greater geographic perspective to within-basin population genetic structure and to identify ancestry of SYR populations. Finally, population genetic similarity of Santa Ynez River *O. mykiss* to strains of hatchery trout raised at Fillmore Hatchery on the Santa Clara River, and widely used in stocking activities in the basin and throughout the region, was assessed using individual- and population-based analyses to determine the level of interaction of these trout with native steelhead/rainbow trout in the SYR basin.

## **METHODS**

### Sampling

The majority of the sampling for this study was performed by Cachuma Project Biology Staff. Upstream and downstream migrant traps were operated on Salsipuedes Creek and Hilton Creek from January to May 1998 to 2006. Tissue samples collected from fish in the SYR lagoon, SYR mainstem and the Santa Cruz Creek drainage were obtained by hook-and-line fishing over the same time period. Biologists from the Southwest Fisheries Science Center and the University of California Santa Cruz sampled Juncal Creek and Santa Cruz Creek, the comparison populations from central and southern California and the Fillmore Hatchery trout strains (Coleman, Mt. Whitney, Wyoming, Virginia) in 2003 for a project on steelhead population structure south of Monterey Bay. For the purposes of this report, all samples of fish from a specific location, or in a particular year, are referred to as population samples, without additional assumptions about the biological details underlying this designation. A detailed summary of the sampling in the Santa Ynez River basin is found in Table 1.

### Genotyping

A total of 1581 *O. mykiss* from the SYR drainage were genotyped at 18 microsatellite marker loci. The loci were chosen from published sources and were originally described in a variety of salmonid species. Detailed information on each locus, including the original reference, primer sequences, thermocycler routine and diversity statistics, appears in Table 2. DNA was extracted from dried fin samples using a semi-automated filter system, polymerase chain reaction (PCR) amplifications were carried out, and products gel-electrophoresed on ABI 377 automated DNA sequencers. Alleles were visualized and calls made using Applied Biosystems' Genotyper software. Two people called each gel independently and resolved allele calls together when they differed. PCR was repeated for loci that failed in the first run or could not be resolved. Allele calls with discrepancies that persisted through the second run were discarded. In addition, 45 individual fish were removed prior to analysis due to missing data at more than 8 loci (average of 14 loci missing). Sixty-one percent of the dropped samples were from the 2001 Salsipuedes Creek collection, while the remainder was distributed among

the other collections. Analysis failure when sampling live fish is generally attributed to DNA degradation when samples are not quickly and adequately dried.

### Dataset finalization

Prior to analysis, all fish were compared for matching genotypes. One hundred and forty-four pairs were found to match at more than 75% of their gene copies, although some individuals were involved in more than one pair. In most cases, the observed effect was not due to missing data (i.e. the genotypes did not match because of a reduced number of loci). Given that samples from both Hilton and Salsipuedes Creeks were obtained from upstream and downstream migrant trapping over a nine-year span, the possibility of recaptures seemed quite likely. Alternatively, in an inbred population, such as one that has gone through a severe bottleneck, matching genotypes are expected due to limited genetic diversity in the parental generation(s). Distinguishing between these two possibilities is important, as including recaptures more than once could affect results. In order to address this latter concern, the probability of observing a matching genotype was examined for each pair of individuals, given the population allele frequencies (Jamieson and Taylor 1997). For full-siblings, match probabilities ranged from  $10^{-3}$  to  $10^{-6}$ , indicating that 1:1,000 to 1:1,000,000 offspring could be expected to have matching genotypes due to chance alone. Since there are over 1 million pairs of genotype comparisons in the dataset, these probabilities do not exclude the possibility of matching genotypes in populations of this size, particularly if genetic diversity has been reduced due to inbreeding. In the interest of conservatively removing individuals that may be actual recaptures, some simple rules were devised for rejecting apparent matches. First, only 100% matches were considered. Based on length measurements, matches were also rejected if fish showed negative growth over time (with 3% measurement error), if fish showed no growth over intervals of a year or more, or if fish exhibited unrealistic growth (defined as more than 1mm/day) over the recapture period. Of the remaining pairs or groups, individuals were preferentially removed from the collection with the larger sample size or to minimize the total number of individuals excluded. In total, 29 individuals were removed following application of this protocol, leaving 1507 samples for further analysis (Table 1).

## Data Analysis

Individuals were organized into groups based on the location and year from which samples were obtained. Population samples of less than 28 individuals were not included in many analyses, as small sample size can significantly alter allele frequency estimates and bias results. Most analyses were then conducted on 16 population sample groups with sample sizes ranging from 28 to 208, for a total of 1350 fish (those with a “Combined Pop ID” in Table 1). Combined groups of all individuals from each of three tributaries of the Santa Ynez River with multiple samples (Salsipuedes, Hilton and Santa Cruz Creeks) were also created, as some analyses benefit from having all potential alleles and genotypes in the sub-basin represented.

Mean observed and mean expected heterozygosity over all loci was calculated using the Microsatellite Toolkit (Park 2001). Allelic richness, which is a measure of the number of alleles that accounts for differences in sample size, was calculated for each group using FSTAT (Goudet 1995). Individual population samples and sub-basin groups were examined for Hardy-Weinberg (HWE) and linkage (LD) equilibria using the exact tests implemented in GENEPOP 3.4 (Raymond & Rousset 1995). Calculation of HWE provides an indication of how closely samples represent a population at mutation-drift equilibrium with random mating, while LD quantifies associations between loci.

Population samples were also examined for kin relationships among individuals. Related individuals and family structure can skew allele frequencies, cause significant tests for HWE and LD, and hinder accurate inference if undetected. The pairwise coefficient of relatedness,  $r_{xy}$ , was calculated for all pairs of individuals using the software KINSHIP and the estimator of Queller and Goodnight (1989). This estimator is intended to estimate kin relationships between pairs of individuals (e.g.  $r_{xy} = 0.5$  for full siblings) by quantifying the degree of allele sharing between individuals, although  $r_{xy}$  can also be affected by the number of alleles, the number of loci and the number of individuals sampled. Analyses considered both the entire pairwise relatedness matrix between all individuals from the 4 primary sub-basin populations and the mean pairwise relatedness for each individual to all other individuals, in addition to inter- and intra-drainage distributions for each of the 4 primary populations. A more direct investigation



of sibling-level relatedness was also undertaken using the program COLONY (Wang 2004), which uses a maximum likelihood approach to determine the distribution in a sample of full-sibling families nested within half-sibling families. Allele frequencies were estimated directly from each of the 4 primary populations and were dynamically updated taking the reconstructed sib-ships into account. Typing error was liberally estimated at 2% per locus.

Some phenotypic data were available for the fish sampled from Salsipuedes and Hilton Creeks. Length data were sorted into thirty 20mm wide bins, ranging from 0-600mm, with a final bin for fish greater than 600mm. Length frequency histograms for all fish from these two drainages were plotted. Interpretation of these data are complicated by the fact that fish were sampled in both the upstream and downstream direction.

The annual collections from Salsipuedes and Hilton Creeks allowed for estimates of effective population size ( $N_e$ ) within these sub-basin drainages. Effective population size can be roughly understood as an estimate of the number of breeders that takes into account variation in population size, sex ratios, the number of offspring per individual and the type of reproduction (Hedrick 1985). A temporal method using F-statistics and allele frequency data to estimate  $N_e$  (Waples 1989) is implemented in the software NeESTIMATOR 1.3 (Peel 2004). Although the method assumes non-overlapping generations and performs better when the number of generations between samples is large, the SYR sampling provides an adequate opportunity for estimation of  $N_e$ . Due to the overlapping age structure in *O. mykiss*, a single generation was defined as 2 years. Multiple estimates of  $N_e$  were made for each sub-basin drainage using all pairwise comparisons between population samples separated by at least three years. An additional  $N_e$  estimate assuming two generations was made for pairs of temporal population samples between which four or more years had passed. Admixture within samples may violate the assumption of this method of a single, randomly-mating population.

In order to assess whether individuals from separate sampled populations are actually interbreeding (as opposed to simply migrating), we attempted to uncover possible parent-offspring relationships using the software PARENTE (Cercueil 2002). Using the entire SYR data set (both large and small population samples), an assumed genotyping error rate of 2%, an assumed sampling rate of 30%, and a maximum of two

allelic incompatibilities, single parent/offspring pairs and their associated probabilities were identified. Given the difficulty of back-calculating birth year from length data, we did not use age data and simply assumed that all individuals die at some point in the distant future. Gender information was also omitted, as these data were available for only a small percentage of individuals. Previous analyses in our lab on both salmonids and harbor seals with known pedigrees have shown that only probabilities greater than 0.45 are generally indicative of true parent/offspring relationships.

Population differentiation was evaluated with  $F_{ST}$ , the variance in allele frequencies between populations, using the estimator theta ( $\theta$ ) of Weir and Cockerham (1984), and was calculated for all pairwise comparisons of sampled populations in GENETIX (Belkhir 2004), with significance of values assessed with 1000 permutations of the dataset. Initial comparisons were between the 4 large population samples within the Santa Ynez River drainage. Subsequent comparisons utilized the SYR grouped sub-basin samples, Monterey and San Luis Obispo County coastal steelhead population samples, and multiple Fillmore Hatchery strains (the source of trout planted in the SYR reservoirs).

Similar sample divisions and comparison groups were employed for assigning individual fish to their most likely population of origin and detecting first-generation migrants between sampled populations using the software GENECLASS2 (Piry et al. 2004). The software calculates an assignment score for each individual in every population, using each individual's multi-locus genotype and the allele frequencies from each population sample. This score is the likelihood of the individual in that population divided by the sum of the likelihoods of that individual in all other populations. Individuals are then 'assigned' to the population with the highest score. Misassignment rates may indicate either ancestral similarities between groups or differences in the abundance of recent migrants. With this method, individuals from the small SYR population samples were assigned to the 16 large SYR population samples (Table 1), as well as the Fillmore Hatchery. The large SYR population samples were also self-assigned, employing a leave-one-out procedure, in which the individual to be assigned is removed from the sample before allele frequencies are calculated. Preliminary analyses revealed that 06Sals, 05Hilt and 06Hilt contained a large number of full and half siblings,

in addition to some migrants from upstream. In the context of self-assignment, a sample with significant family structure will appear unique, as the assignment algorithm removes only the single individual being assigned before recalculating the group allele frequency. Individuals drawn from this unique allele frequency distribution may tend to be assigned back to the mixture rather than to their actual source population. Self-assignment was repeated for the population samples mentioned above, with the entire population sample excluded as a potential source.

Using the SYR sub-basin groups, other coastal populations and Fillmore Hatchery strains, the probabilities that each individual fish in every population sample was a first generation migrant from each other sampled population were also calculated. This more rigorous assignment method used 1,000 simulated individuals to assess the significance of the likelihood ratio between the most likely population and the population in which the fish was sampled. Individuals were considered first-generation migrants only if they met the strict criteria of  $p < 0.01$  and a likelihood ratio greater than one. This methodology should exclude most chance misassignments to similar populations and reduce misassignment of individuals with common alleles due to historical or recent gene flow.

Construction of phylogeographic trees provided another examination of population structure. Cavalli-Sforza and Edwards chord distances (1967) were calculated and neighbor-joining trees constructed using the software package PHYLIP (Felsenstein 1993). For all phylogeographic trees, only the large SYR population samples were included. Population samples from other basins were included in some trees to provide geographic context. Chord distance/neighbor-joining trees were constructed with the SYR populations alone, with the SYR populations and the Fillmore Hatchery strains, and with the SYR populations, the Fillmore Hatchery strains and the population samples from other area basins. Majority rule consensus trees from 10,000 chord distance/neighbor-joining trees using bootstrap samples of the last two datasets were also constructed.

Bayesian, model-based clustering methods were also employed to examine population structure in the Santa Ynez River. We used the STRUCTURE program (Pritchard et al. 2000) to fractionally assign the ancestry of individuals to a number of proposed population clusters (K). Values of K, the number of proposed populations, from 1 to 7, were examined, as larger K values led to continued subdivision of population

samples, likely identifying large, extended families. Each run was repeated four times with a burn-in of 20,000 iterations and then 50,000 iterations to estimate ancestry. STRUCTURE was set to ignore prior population information, use the correlated allele frequencies model, and consider admixture. For *O. mykiss* in the SYR, historical connectivity and migration justifies the correlated allele frequencies model, while admixture remains a possibility as fish can move over the dams or migrate through the ocean into the lower basin (Salsipuedes and Hilton Creeks). Individual ancestry coefficients for various values of K were visualized in color with the program DISTRUCT (Rosenberg 2004).

Analysis of Factorial Correspondence (AFC) was also employed as a qualitative method for examining population structure by visualizing variation of individual genotypes in three-dimensional space. It is a canonical method similar to principal components analysis and was carried out using the “population” algorithm in Genetix (Belkhir 2004). Separate analyses were performed with and without the Fillmore Hatchery trout strains to evaluate the distribution of individual genotypes in relation to population differentiation.

## **RESULTS & DISCUSSION**

Table 3 shows population summary statistics. Allelic richness ranged from 5.2 in the Coche and 01Sals samples to 7.7 in 05Hilt. In general, allelic richness was comparable throughout the drainage, although slightly higher in Hilton Creek. These values are lower than what has been observed in other *O. mykiss* populations throughout California (Garza 2004). Expected heterozygosity ranged from 0.575 in 01Sals to 0.681 in 05Hilt, while observed heterozygosity ranged from 0.489 in 99Sals to 0.651 in 02Hilt. A detailed breakdown of HWE (per locus, per population) appears in Table 4. The significant deviations from HWE ( $p < 0.001$ ) found in the data are almost exclusively due to heterozygote deficiencies, which can be caused by admixture or family structure within the sample. The significant HWE disequilibrium values were found almost entirely in the Hilton and Salsipuedes population samples and not in the above barrier populations.

Linkage disequilibrium (LD, Table 3) is reported as the percentage of all pairs of loci with significant associations ( $p < 0.001$ ). It is important to note that these values do not necessarily represent physical linkage on a chromosome, as these microsatellite loci have been used to study *O. mykiss* from throughout the species range and are generally consistent with independent segregation. Significant LD tests are most likely due to admixture or the presence of family structure in the population samples. Approximately 5% of all pairs of loci are expected to be associated by chance alone, and all LD values above this expected baseline were found in population samples below Bradbury Dam. LD analysis provided strong evidence that many of the Hilton and Salsipuedes Creek population samples are composed of fish from multiple source populations and/or contain large families (mainly half- and full-siblings).

Perhaps the most striking results from the COLONY analysis were the presence of six half-sibling families composed of more than 25 individuals and the detection of 14 full-sibling families composed of more than 10 individuals (Appendix A). The Salsipuedes samples had three large half-sibling families comprising almost 20% of the entire sample and a single full-sibling family of 31 individuals. These are large enough numbers of related individuals that they should be readily detectable with the relatedness coefficient,  $r_{xy}$ , analysis.

The distribution of all pairwise relatedness values was not normal (although it is unknown if this is the true expectation for the dataset) and positively skewed (Figure 2). This may be indicative of ‘excess’ relatedness in the sample. The distribution of all mean individual relatedness values was also not normal, but was instead negatively skewed (Figure 3). Bootstrap analysis of mean, standard deviation and skew for both datasets found the observed values in the most likely area of the distribution, with the exception of the skew of the distribution of all individual  $r_{xy}$  values, which lies at the edge of the confidence interval. This provides less confidence that the skew observed in Figure 2 is predictive of an excess degree of relatedness.

The distribution of  $r_{xy}$  within each sub-basin appeared similar to the overall distribution of pairwise  $r_{xy}$  values from the dataset, but chi-square values were all significant (Figure 4). Intra- and inter-drainage distributions showed substantial overlap, limiting their diagnostic usefulness, although the means of these distributions were

generally quite different (particularly in the Juncal Creek collection). In Santa Cruz Creek and Juncal Creek the much larger number of inter-drainage observations dominated the overall distribution and masked the differences in these distributions.

The distribution of mean individual  $r_{xy}$  values within each sub-basin was significantly different from the overall distribution in all cases (Figure 5). For each individual, the mean of the within sub-basin  $r_{xy}$  values and the mean of the between sub-basin  $r_{xy}$  values were calculated, and these two distributions conveniently have the same number of data points. This made the differences between the inter/intra distributions much clearer than with the full  $r_{xy}$  dataset. The complete lack of overlap between these two distributions in the Juncal Creek population indicates the small number of founding lineages of the sample and due to the processes of genetic drift and historical isolation.

Length frequency histograms for Salsipuedes and Hilton Creeks appeared quite different (Figure 6). The distribution of fish lengths in Salsipuedes Creek is bimodal and resembles distributions observed in other coastal drainages (unpublished data) where multiple year-classes are present. The peak of smaller size fish is thought to be composed of 1 and 2 year old juveniles while the peak of larger sizes includes older fish that are pursuing a resident life history strategy or have not yet undertaken anadromous migration. Fish greater than 400mm in length have likely undergone migration to at least the estuary, where growth rates can be much higher (Bond 2006). In Hilton Creek, fish lengths were also bimodally distributed. However, the second peak was shifted substantially to larger sizes, compared with the second peak in the Salsipuedes graph, with much more frequent occurrence of fish from those larger size classes. This may reflect the larger number of upstream migrants captured in Hilton Creek. Only one upstream migrant was captured in Salsipuedes Creek in 2005. It was also noted that upstream and downstream migrants of the same size class were often captured on the same day, suggesting resident movement, in addition to potential anadromous migrations.

The annual adult escapement of steelhead to the Santa Ynez River is currently estimated to be less than 100 fish (Busby 1996). This is an estimate of census size and not the effective population size ( $N_e$ ), which takes into account differential breeding success, among other things. Using the temporal method (Waples 1989), estimates of  $N_e$  for Salsipuedes Creek ranged from 11 - 61 (mean=28.2), and for Hilton Creek 17 - 131

(mean=50.5) (Table 5). Although some of the assumptions of the method were not met, the estimates appear reasonable and are indicative of a population with census size in the vicinity of 100 adults, particularly if some residents contribute to reproduction in these populations.

The parentage analyses performed with PARENTE identified over 6,300 possible parent-offspring pairs, although probabilities ranged from 0.01 to 0.5. These are not high probabilities, indicating that there was limited power to identify parentage in this study, due to the need for large amounts of data when closely related individuals are present. Of the 648 pairs with probabilities greater than 0.45, only 30 identified a parent and offspring from different population samples. For the majority of these ‘mixed’ pairs (24 out of 30), the parent and the offspring were still located in geographically proximate river sites. For example, 57% of the ‘mixed’ pairs contained a Hilton Creek individual (either parent or offspring) together with an individual from Alisal Creek, Quiota Creek or the lower mainstem SYR. Similarly, 5 parent-offspring pairs consisted of one fish each from Grapevine Creek and East Fork Santa Cruz population samples. We also identified 4 parent-offspring pairs that contained both a Salsipuedes and a Hilton Creek fish, and 2 pairs with one Hilton Creek fish and another fish from above the dam (1 Coche Creek and 1 West Fork Santa Cruz fish). It is important to note that some of the parent-offspring pairs appeared biologically improbable based on length and date of capture information. However, as mentioned above, it is difficult to estimate actual age from length/date information. It is also very difficult to distinguish parent/offspring pairs from pairs of siblings or other close relatives, which may be the true relationships between many of these pairs. It is also likely that much of this uncertainty is due to the lack of power in the dataset, because of many similar genotypes present from past inbreeding.

In general, differentiation in the Santa Ynez River (Table 6) between population samples was significant and comparable to that observed in other California *O. mykiss* studies (Clemento et al. in review; Garza et al. 2004). Within the SYR, values of  $F_{ST}$  between sample years (mean = 0.012) were significantly smaller (t-test,  $p < 0.001$ ) than those between sample sites (mean = 0.095). In addition, the 99Sals sample was not significantly differentiated ( $p < 0.01$ ) from four other Salsipuedes Creek temporal samples. In contrast, the 06Sals sample was significantly different from all other Salsipuedes

Creek samples (mean  $F_{ST} = 0.028$ ), although differentiation values were still much smaller than those between sample sites. In Hilton Creek, samples from consecutive years generally showed a lack of differentiation, with no significant  $F_{ST}$  values for 02Hilt-03Hilt, 03Hilt-04Hilt and 05Hilt-06Hilt. The 2005 sample from Hilton Creek, like 06Sals, appeared relatively different from other Hilton Creek samples (mean  $F_{ST} = 0.016$ ). These patterns of differentiation appear to be due to the concentration of sibling groups in the 06Sals and 05-Hilt population samples (data not shown).

When compared with the Arroyo Grande (San Luis Obispo County) and Monterey County Coast populations and the Fillmore Hatchery trout strains, the sub-basin groups from the Santa Ynez River were significantly differentiated, but the values for interdrainage comparisons were smallest for the SCall group (Table 7). Differentiation between population samples within the SYR was of similar magnitude as differentiation between the SYR and populations from nearby drainages, which is a pattern that has been observed in studies of steelhead populations from throughout California (Garza et al. 2004; Girman and Garza 2006).

Individuals from the small population samples were assigned to the large population samples (Table 8) to see how fish from different sub-basins are distributed throughout the drainage. Of the 3 fish caught in the SYR estuary, 2 assigned to Salsipuedes Creek and 1 to Hilton Creek. It is difficult to be certain if these fish are anadromous, but their sizes (280, 344 and 357 mm) are consistent with the upstream migrant size classes observed in Hilton Creek. Most fish caught in the mainstem SYR below the dam assigned to Hilton Creek (19 of 31). However, all of the samples collected in the mainstem in 2003 assigned with high confidence to populations above the dam (9 to Juncal Creek and 2 to the Santa Cruz Creek sub-basin). The adult from Alisal Creek (504mm) assigned with high confidence to Hilton, while fish from Quiota Creek, a small tributary located near Hilton Creek, were assigned to Hilton, Salsipuedes and Santa Cruz Creeks. Individuals sampled within the Santa Cruz Creek sub-basin (Gvine, EFSCrz) assigned only to other Santa Cruz Creek populations.

Since fish appear capable of downstream movement over the dams, Fillmore Hatchery *O. mykiss* strains were included in most assignment analyses. Fillmore Hatchery provides the trout that are planted annually in the Santa Ynez River reservoirs



by the California Department of Fish and Game. Table 9 shows the self-assignment results of the large SYR population samples, and includes the hatchery strains as potential “populations” of origin. The large number of misassignments among temporal samples from Hilton and Salsipuedes Creeks corroborated the temporal stability suggested by differentiation estimates. The admixture indicated by misassignments to other sampling sites may be the source for some of the observed Hardy-Weinberg and linkage disequilibria, although much of it is demonstrably due to the presence of large families. Preliminary investigation revealed that admixture and family structure was particularly pronounced in the 06Sals and the 05/06Hilt population samples, so these groups were removed as potential source groups and the self-assignment repeated (Table 9). Without 06Sals as a potential source population, there were more misassignments of these fish to Hilton Creek and the Santa Cruz drainage. Without 05Hilt and 06Hilt, misassignments from these groups to Salsipuedes Creek and the hatchery groups increased. The first-generation migrant analysis found that Hilton and Salsipuedes Creeks appear to receive migrants from outside of the Santa Ynez River drainage, in addition to exchanging occasional migrants between them (Table 10). Consistent with individuals coming over the dam or down through the temporary watering system, Hilton Creek contains the most hatchery and Santa Cruz Creek migrants.

Phylogeographic trees depict genetic distances between population samples. Within the Santa Ynez River, temporal samples from Salsipuedes and Hilton Creeks clustered tightly, emphasizing the similarities between sample years (Figure 7). Population samples distributed spatially in the Santa Cruz Creek drainage also clustered together, and all were very distinct from hatchery strains (Figure 8). Bootstrap support for branches that cluster all population samples from the same sub-basin, and all of the hatchery strains, was generally very high, with only Santa Cruz Creek below 99% support (Figure 9). There was no strongly supported signal of sub-basin relationships in these trees, but there was some indication that Hilton Creek is more similar to the hatchery strains than the other populations. This is consistent with, and likely entirely due to, the presence of some hatchery fish in the Hilton Creek population samples (Table 10). There was also greater similarity between the Santa Cruz and Salsipuedes Creek population samples than any of the other sub-basins. In a wider geographic context,

Hilton Creek population samples are also slightly more similar to Fillmore Hatchery strains than other SYR population samples (Figures 10, 11), again likely due to the hatchery fish present, but all of the population samples are part of a relatively closely related and unresolved cluster of steelhead populations with little bootstrap support that is a consequence of historical or ongoing migration between basins (Girman and Garza 2006; Garza et al. 2004).

Figure 12 graphically displays individual ancestry coefficients for 5 independent runs of the model-based clustering algorithm in the program STRUCTURE. These analyses use an hypothesis about the number of populations present, but no information about the origin of individual fish, to cluster individuals into their constituent genetic groups, allowing fractional ancestry. Inferred ancestry in each group is then color-coded, and fish from different population samples are grouped post-analysis for the figures. Population samples/strains are identified by the numbers alongside each plot and are defined as follows: 1-6, Monterey Coast and Arroyo Grande; 7-10, Santa Ynez River (Salsipuedes, Hilton, Santa Cruz, and Juncal Creeks, in that order); 11-14, Fillmore Hatchery Strains. Plots a) through c) include the entire data set, whereas plot d) utilizes only the 2005 population samples from Salsipuedes (7) and Hilton (8) Creeks, and plot e) uses only the 2006 population samples from these two sites. For the first three plots, the number of proposed genetic groups (K) varies and identifies the most likely subdivisions for the given collection of individuals. For K=2 (not shown), Fillmore Hatchery strains were always the first to be identified, regardless of which population samples were included in the analysis. With K=3 for the entire data set (Figure 12a), Salsipuedes Creek population samples are the first to be identified and the presence of some hatchery fish in Hilton Creek is evident. The Santa Cruz Creek population samples also appear to share some ancestry with the Salsipuedes population, as well as with other steelhead populations (in blue). With K=4 (Figure 12b), Hilton Creek was identified as a fourth distinct sub-group, even though it contains a number of hatchery fish. The pattern for K=5 (Figure 12c) is identical to K=4 with the exception of Salsipuedes Creek. Rather than inferring additional structure between the population samples, the Salsipuedes Creek individuals were further subdivided. This appears to be the result of the family structure (sibling groups) that was detected with COLONY. Subsequent runs with increasing K

continued to subdivide Salsipuedes and then Hilton Creeks, with the Juncal sample eventually falling out as distinct before hatchery groups were subdivided.

The other analyses indicated that the 2006 sample from Salsipuedes Creek had a large number of highly related individuals and that the 2005 and 2006 collections from Hilton Creek contained both family structure and migrants from upstream. So the 2005 and 2006 population samples were used to represent the two populations in separate analyses with  $K=3$ . When only the 2005 population samples were used (Figure 12d), Juncal Creek was the most distinctive of any of the population samples, aside from the hatchery strains, and all other SYR populations grouped with other coastal steelhead populations. With only the 2006 population samples included (Figure 12e), Salsipuedes Creek was again the most distinct, demonstrating the effect of family structure in distorting allele frequencies in this population sample and that extended families were sampled in multiple years in this sub-basin. In addition, all of the STRUCTURE analyses clearly identified the Santa Cruz and Juncal Creek populations as primarily descended from the same coastal steelhead lineage as populations in other basins in central and southern California, although Juncal Creek was marginally more differentiated in one analysis. This could be the result of either genetic drift, due to sustained small population size, or some low level of introgression by an unsampled *O. mykiss* population or strain. The analyses also demonstrate unambiguously that trout stocked in reservoirs in the SYR from genetically distinct strains are not widely introgressing naturally-spawning populations and may not be reproducing at all.

Analysis of Factorial Correspondence (AFC) plots provided an additional qualitative method for understanding the relationships between individual genotypes and population genetic differentiation. Analysis of only SYR basin fish, found that the Juncal Creek population was the most distinct (Figure 13). Analyses with the Fillmore Hatchery strains found clear distinction between hatchery trout and naturally-spawning fish in the SYR basin and also corroborated the other results, indicating that the moderate distinction of the Juncal Creek population is not due simply to introgression from a sampled hatchery trout strain.

## CONCLUSIONS

Population genetic analysis provides a powerful set of tools for understanding the population biology of fish and other species. Several important findings emerged from the analysis of microsatellite genetic data from populations of *O. mykiss* in the Santa Ynez River. First, the two primary steelhead (below barriers to anadromy) populations in the basin, Salsipuedes Creek and Hilton Creek, are temporally stable and genetically differentiated, although the differentiation is due at least partially to differences in the number of migrants from hatchery trout planted in the upstream reservoirs. Hatchery trout were present in Hilton Creek in multiple years, but almost completely absent from other population samples in the basin. In addition, estimation of effective population size in these two populations indicates values of about ~25-50, which is consistent with census size estimates and the generally accepted ratio of effective to census size for salmonids. While effective size is not equivalent to the number of breeders, it is similar and can be used as a rough estimate. In addition, Hilton and, in particular, Salsipuedes Creek population samples were dominated by sibling groups in some years, which can confound some inference if undetected.

Next, the Santa Ynez River basin has maintained the spatial genetic structure observed in most other coastal steelhead populations, with significant genetic differences between the four primary populations sampled: Salsipuedes, Juncal, Santa Cruz, and Hilton Creeks. In spite of this differentiation, which is moderate, analysis which combined these data with those from other *O. mykiss* populations in the region demonstrated unambiguously that all four of these populations are primarily of coastal steelhead ancestry, indicating that the *O. mykiss* populations above the dams are descended from steelhead present historically.

Analysis of migration found several important results. First, both native and hatchery fish do migrate downstream and over/around Bradbury Dam. This may be primarily due to passive transport during high flows. There was also some migration between Hilton and Salsipuedes Creeks, but it was of the same order of magnitude as migration into these populations from other *O. mykiss* populations both in the basin and in the region. Likelihood analyses indicated substantial migration between the Santa Ynez River and other regional steelhead populations. This is consistent with the results of

the tree-based analyses, which found that the Santa Ynez River population branches originate from a relatively unsupported cluster of southern and south-central California *O. mykiss* populations, indicating that these populations are all relatively closely related and connected through frequent migration.

The final significant result came from the analyses that included genotypes from all of the current rainbow trout strains from Fillmore Hatchery. These hatchery trout strains are highly distinct from all Santa Ynez River *O. mykiss* populations, in spite of their use in stocking activities in the basin, and throughout the region. Although a few hatchery fish were present below Bradbury Dam, a signal of introgression and reproduction was essentially absent from all Santa Ynez River populations. This result is consistent with what has been found for other coastal California basins (Girman and Garza 2006) and indicates that hatchery trout are different enough in life history and physiology that they do not successfully reproduce with naturally spawning fish, although they may have other detrimental ecological effects through competition and predation.

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## FIGURES

**Figure 1.** Map of the Santa Ynez River basin, with the four primary sampling areas (sub-basin drainages) indicated.

**Figure 2.** Distribution and statistics of pairwise relatedness values ( $r_{xy}$ ) between all individuals collected from the four primary sampling areas in the Santa Ynez River.

**Figure 3.** Distribution and statistics of mean pairwise relatedness ( $r_{xy}$ ) for each individual collected from the four primary sampling areas in the Santa Ynez River.

**Figure 4.** Distribution and statistics of pairwise relatedness values ( $r_{xy}$ ) for each of the four primary sampling areas. Relatedness values within and between sub-basin drainages are displayed separately in each graph. Chi-squared values statistically compare each sub-basin to the overall Santa Ynez River distribution (Figure 2).

**Figure 5.** Distribution of mean individual relatedness ( $r_{xy}$ ) for each of the four primary sampling areas. Chi-squared values statistically compare each sub-basin distribution to the overall Santa Ynez River distribution (Figure 3). Bars indicate values generated by calculating a within- (light blue) and between-sub-basin (dark blue) mean for each individual.

**Figure 6.** Length frequency histograms of all fish of known size captured in Hilton and Salsipuedes Creeks. Length data for fish from Juncal and Santa Cruz Creeks was incomplete and likely biased by sampling methodology.

**Figure 7.** Unrooted, neighbor-joining tree of Santa Ynez River population samples constructed using Cavalli-Sforza and Edwards' (1967) chord distance. Branch lengths indicate chord distance.

**Figure 8.** Unrooted, neighbor-joining/chord distance tree of Santa Ynez River population samples with Fillmore Hatchery (FH) strains included.

**Figure 9.** Majority-rule consensus tree of 10,000 neighbor-joining/chord distance trees from bootstrap replicates of the dataset including the Santa Ynez River population samples and Fillmore Hatchery (FH) strains. Internal branch lengths are scaled by percent bootstrap support, which is indicated for branches represented in over half of replicates.

**Figure 10.** Unrooted, neighbor-joining/chord distance tree of Santa Ynez River (SYR) population samples together with populations from proximate basins and Fillmore Hatchery (FH) strains. See Girman and Garza (2006) for non-SYR population information.

**Figure 11.** Majority-rule consensus tree of 10,000 neighbor-joining/chord distance trees from bootstrap replicates of the dataset with the Santa Ynez River together with populations from proximate basins and Fillmore Hatchery strains.

**Figure 12.** Results of five different analyses with the program STRUCTURE using different proposed numbers of genetic clusters (K), indicated by colors, and population samples, denoted with numbers. Population samples: 1-6, Monterey Coast and Arroyo Grande; 7-10, SYR (Salsipuedes, Hilton, Santa Cruz, and Juncal, in that order); 11-14, FH strains. Plots a) through c) include the entire data set, plot d) uses only the 05Sals (7) and 05Hilt (8), and plot e) uses only 06Sals (7) and 06Hilt (8) from those sub-basins.

**Figure 13.** Results of Analysis of Factorial Correspondence (AFC) depicting genotypes of individual Santa Ynez River fish in three-dimensional space. The top graph shows each of the 16 large population samples considered separately, while the bottom graph has all Hilton (blue) and Salsipuedes (grey) temporal samples pooled. Juncal Creek fish appear in the small group above (top graph) or below (bottom graph) the other Santa Ynez River fish.

**Figure 14.** Results of Analysis of Factorial Correspondence (AFC) depicting genotypes of individual Santa Ynez River fish, together with those from Fillmore Hatchery trout strains, in three-dimensional space. Salsipuedes (yellow), Hilton (blue), Santa Cruz (white) and Juncal (dark grey) Creeks are quite distinct from Fillmore Hatchery strains (other colors in the upper left quadrant). Inset is a side view, showing differentiation of Juncal Creek from other Santa Ynez River populations.



## TABLES

**Table 1.** Inventory of Santa Ynez River samples collected from 1998 to 2006 that were included in this study. The Combined Pop ID field indicates groups of samples that were pooled for some analyses.

**Table 2.** Summary of the loci used for genotyping, including the original reference, PCR routine, primer sequences and relevant genetic statistics calculated across all populations.

**Table 3.** Population statistics, including observed and expected heterozygosity (Hz), linkage disequilibrium (LD) and allelic richness for sample collections with at least 28 individuals. For LD, the percentage of loci pairs with significant tests ( $p < 0.001$ ) is indicated.

**Table 4.** Result of probability tests for Hardy-Weinberg equilibrium (HWE) using Monte Carlo Markov chain estimation of exact test p-values. Asterisks indicate significant deviations from HWE ( $p < 0.001$ ). These deviations are almost exclusively heterozygote deficiencies.

**Table 5.** Estimates of effective population size ( $N_e$ ) using the temporal method of Waples (1989). The method compares pairs of populations in order to estimate  $N_e$ . Within each sub-basin drainage (Sals and Hilt), differentiation ( $F$ ),  $N_e$ , and the confidence intervals were calculated for all pairwise comparisons between years. Estimates were also made assuming two generations (2gens) when four or more years had passed between samples.

**Table 6.** Matrix of pairwise population differentiation ( $F_{ST}$ ) values between large SYR population samples. All values are significantly different from zero ( $p < 0.01$ , 1000 permutations) except those in bold. Boxes denote within sub-basin comparisons.

**Table 7.** Matrix of pairwise population differentiation ( $F_{ST}$ ) values between the pooled SYR sub-basin populations, 3 Monterey County coastal steelhead populations, 3 Arroyo Grande (San Luis Obispo County) *O. mykiss* populations, and 4 Fillmore Hatchery (FH) strains. All values are significantly different from zero ( $p < 0.01$ , 1000 permutations).

**Table 8.** Assignment of individual fish from the small sample collections to the larger SYR populations and the Fillmore Hatchery strains.

**Table 9.** Self-assignment of individual fish from the population samples at left to their most likely genetic population of origin (above). Bold numbers are self-assignments back to the population of origin. Grey boxes indicate that the population was removed as a potential source and the assignment analysis repeated. See text for explanation.

**Table 10.** Analysis of first generation migrants using GeneClass2 (Piry et al. 2004) indicating the number of fish from the populations at left which appear to be first-generation migrants from the populations listed at the top. Log-likelihood scores greater

than one with p-values less than 0.01 were used to reject the null hypothesis that the individual originated in the population from which it was sampled.

**Appendix A.** Summary of COLONY results. At the top are the totals and scaled values for the number of identified half-sib (HS) and full-sib (FS) families. The graphs depict the counts of half- and full-sib families of specified sizes.

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**Table 1.** Inventory of Santa Ynez River samples collected from 1998 to 2006 that were included in this study. The Combined Pop ID field indicates groups of samples that were pooled for some analyses.

| <b>River</b>               | <b>Collection Year</b> | <b>N</b> | <b>Population ID</b> | <b>Combined Pop ID</b> | <b>Description</b>                    |
|----------------------------|------------------------|----------|----------------------|------------------------|---------------------------------------|
| Alisal Creek               | 2005                   | 1        | Alisal               |                        | Lower mainstem tributary              |
| Coche Creek                | 2004                   | 28       | Coche                | SCall                  | WF Santa Cruz Creek tributary         |
| Devils Canyon Creek        | 2003                   | 3        | DevCan               |                        | Tributary below Gibraltar Dam         |
| East Fork Santa Cruz Creek | 2004                   | 14       | EFSCz                | SCall                  | Santa Cruz Creek tributary            |
| Grapevine Creek            | 2004                   | 11       | Gvine                | SCall                  | Santa Cruz Creek tributary            |
| Hilton Creek               | 1998                   | 4        | 98Hilt               | Hilt                   | Mainstem tributary below Bradbury Dam |
|                            | 1999                   | 8        | 99Hilt               | Hilt                   |                                       |
|                            | 2000                   | 5        | 00Hilt               | Hilt                   |                                       |
|                            | 2001                   | 51       | 01Hilt               | Hilt                   |                                       |
|                            | 2002                   | 64       | 02Hilt               | Hilt                   |                                       |
|                            | 2003                   | 50       | 03Hilt               | Hilt                   |                                       |
|                            | 2004                   | 123      | 04Hilt               | Hilt                   |                                       |
|                            | 2005                   | 51       | 05Hilt               | Hilt                   |                                       |
|                            | 2006                   | 188      | 06Hilt               | Hilt                   |                                       |
| Indian Creek               | 2003                   | 2        | Indian               |                        | Tributary above Gibraltar Reservoir   |
| Los Amoles Creek           | 2002                   | 4        | LosAmol              |                        | Salsipuedes drainage tributary        |
| North Fork Juncal Creek    | 2003                   | 85       | NFJunc               |                        | Tributary to Jameson Lake             |
| Nojoqui Creek              | 1998                   | 3        | Nojo                 |                        | Lower mainstem tributary              |
| Quiota Creek               | 2002                   | 4        | 02Quiota             |                        | Lower mainstem tributary              |
|                            | 2003                   | 9        | 03Quiota             |                        |                                       |
|                            | 2004                   | 5        | 04Quiota             |                        |                                       |
| Salsipuedes Creek          | 1998                   | 16       | 98Sals               | Sals                   | Lower mainstem tributary              |
|                            | 1999                   | 45       | 99Sals               | Sals                   |                                       |
|                            | 2000                   | 13       | 00Sals               | Sals                   |                                       |
|                            | 2001                   | 140      | 01Sals               | Sals                   |                                       |
|                            | 2002                   | 21       | 02Sals               | Sals                   |                                       |
|                            | 2003                   | 134      | 03Sals               | Sals                   |                                       |
|                            | 2004                   | 52       | 04Sals               | Sals                   |                                       |
|                            | 2005                   | 55       | 05Sals               | Sals                   |                                       |
|                            | 2006                   | 208      | 06Sals               | Sals                   |                                       |
| Santa Cruz Creek           | 2003                   | 26       | SCruz                | SCall                  | Tributary to Lake Cachuma             |
|                            | 2004                   | 13       | SCruz                | SCall                  |                                       |
| Santa Ynez Estuary/Lagoon  | 1998                   | 2        | SYLag                |                        |                                       |
|                            | 1999                   | 1        | SYLag                |                        |                                       |
| Santa Ynez mainstem        | 1999                   | 2        | SYR99                |                        |                                       |
|                            | 2000                   | 2        | SYR00                |                        |                                       |
|                            | 2003                   | 11       | SYR03                |                        |                                       |
|                            | 2005                   | 1        | SYR05                |                        |                                       |
|                            | 2006                   | 15       | SYR06                |                        |                                       |
| West Fork Santa Cruz Creek | 2004                   | 37       | WFSCz                | SCall                  | Santa Cruz Creek tributary            |
|                            | Total                  | 1507     |                      |                        |                                       |

**Table 2.** Summary of the loci used for genotyping, including the original reference, PCR routine, primer sequences and relevant genetic statistics calculated across all populations.

| <b>Locus</b>    | <b>Reference</b>                | <b>Label</b> | <b>PCR routine<sup>a</sup></b> | <b>Primer sequences (5'-3')</b>                           | <b>Number of alleles</b> | <b>Size range (bp)</b> | <b>Gene diversity (H<sub>r</sub>)</b> |
|-----------------|---------------------------------|--------------|--------------------------------|---|--------------------------|------------------------|---------------------------------------|
| <i>Oki23</i>    | Smith <i>et al.</i> (1998)      | FAM          | 53(10) 55(25)                  | F-TGTGCTATAGGGTGAATGTGC<br>R-AACACAGGCATCCCCACTAA         | 21                       | 118-198                | 0.874                                 |
| <i>Omy1011</i>  | Bentzen (pers. comm.)           | HEX          | 53(10) 55(25)                  | F-AACTTGCTATGTGAATGTGC<br>R-GACAAAAGTGACTGGTTGGT          | 21                       | 132-256                | 0.824                                 |
| <i>Omy27</i>    | McConnell <i>et al.</i> (1997)  | FAM          | 53(10) 55(25)                  | F-TTTATGGCTGGCAACTAATGT<br>R-TTTATGTCATGTCAGCCAGTG        | 6                        | 97-109                 | 0.565                                 |
| <i>Omy77</i>    | Morris <i>et al.</i> (1996)     | FAM          | 53(10) 55(25)                  | F-CGTTCTCTACTGAGTCAT<br>R-CCAAGAATTTTCTGATCCGGG           | 21                       | 80-144                 | 0.902                                 |
| <i>One11b</i>   | Scribner <i>et al.</i> (1996)   | HEX          | 53(10) 55(25)                  | F-GTTTGGATGACTCAGATGGGACT<br>R-CCTGCTGCCAACACTGTCAA*      | 6                        | 114-124                | 0.662                                 |
| <i>One13b</i>   | Scribner <i>et al.</i> (1996)   | TET          | 53(10) 55(25)                  | F-TCATACCCCATGCCTCTTCTGTT<br>R-GGGTGGAGAGACAGGTATCTTGTC*  | 18                       | 206-248                | 0.853                                 |
| <i>Ots103</i>   | Beacham <i>et al.</i> (1998)    | HEX          | 53(10) 55(25)                  | F-AGGCTCTGGGTCCGTG<br>R-TGATATGGTGTGATAGCTGG              | 5                        | 58-88                  | 0.442                                 |
| <i>Ots1b</i>    | Banks <i>et al.</i> (1999)      | FAM          | 53(10) 55(25)                  | F-GGAAAGAGCAGATGTTGTAA<br>R-CATGCTATTTCCAGACGGCA          | 16                       | 201-295                | 0.713                                 |
| <i>OtsG243</i>  | Williamson <i>et al.</i> (2002) | TET          | 53(10) 55(25)                  | F-TTATTAAACTGCACTGTCTAACTACA<br>R-GTATGCAGCAAGCCAGGTG     | 8                        | 103-125                | 0.568                                 |
| <i>OtsG253b</i> | Williamson <i>et al.</i> (2002) | TET          | 53(10) 55(25)                  | F-CGCTGCAGAAACATTTTCGA*<br>R-AATTGGGTCATTAAGGCTCTGTGG     | 26                       | 132-301                | 0.896                                 |
| <i>OtsG249b</i> | Williamson <i>et al.</i> (2002) | FAM          | 53(10) 55(25)                  | F-ATGGCAGTTAAGAGAACAAAAGTT<br>R-GTACAACCCCTCTCACCTACCC    | 21                       | 147-267                | 0.875                                 |
| <i>OtsG3</i>    | Williamson <i>et al.</i> (2002) | HEX          | 53(10) 55(25)                  | F-GGACAGGACCGTCTGCTAAATGACTG<br>R-GGATGGATTGATGAATGGGTGGG | 13                       | 139-215                | 0.518                                 |
| <i>OtsG401</i>  | Williamson <i>et al.</i> (2002) | FAM          | 60(10) 60(25)                  | F-CTGCCCTGAGAAGCTGGAGTGCTC<br>R-TTGCCCCACCCTTGTCATCTATCCA | 23                       | 165-241                | 0.849                                 |
| <i>OtsG43</i>   | Williamson <i>et al.</i> (2002) | TET          | 55(10) 57(25)                  | F-AACTCCCGTTGACAATTTACTGTTG<br>R-TTTTGGCAAAGTTGGCTACTCTG  | 17                       | 141-201                | 0.787                                 |
| <i>OtsG409</i>  | Williamson <i>et al.</i> (2002) | TET          | 53(10) 55(25)                  | F-GTAGCCATTTGTGTCACCATCATT<br>R-CATTCTCCTGCCTCACAGAGTTTA  | 3                        | 86-90                  | 0.020                                 |
| <i>OtsG85</i>   | Williamson <i>et al.</i> (2002) | HEX          | 53(10) 55(25)                  | F-CCATGTCAGCACTGACTTAAT<br>R-GGATGTTGTTCCCTAATGTTTT       | 35                       | 125-337                | 0.938                                 |
| <i>Ssa289</i>   | McConnell <i>et al.</i> (1995)  | HEX          | 45(10) 48(25)                  | F-CTTTACAAATAGACAGACT<br>R-TCATACAGTCACTATCATC            | 6                        | 107-121                | 0.682                                 |
| <i>Ssa85</i>    | O'Reilly <i>et al.</i> (1996)   | TET          | 53(10) 55(25)                  | F-AGGTGGGTCCCTCCAAGCTAC<br>R-ACCCGCTCCTCACTTAATC          | 27                       | 102-167                | 0.108                                 |

<sup>a</sup> Annealing temperatures (°C) and number of cycles (in parentheses) for two-stage PCR thermal cycling protocols.



**Table 3.** Population statistics, including observed and expected heterozygosity (Hz), linkage disequilibrium (LD) and allelic richness for sample collections with at least 28 individuals. For LD, the percentage of loci pairs with significant tests ( $p < 0.001$ ) is indicated.

| <b>Population</b> | <b>Sample size</b> | <b>Expected Hz</b> | <b>Observed Hz</b> | <b>LD % of Pairs (<math>p &lt; 0.001</math>)</b> | <b>Allelic Richness</b> |
|-------------------|--------------------|--------------------|--------------------|--|-------------------------|
| 99Sals            | 45                 | 0.620              | 0.489              | 2.9%   | 5.5                     |
| 01Sals            | 140                | 0.575              | 0.508              | 15.2%  | 5.2                     |
| 03Sals            | 134                | 0.615              | 0.588              | 12.3%  | 6.1                     |
| 04Sals            | 52                 | 0.603              | 0.569              | 12.3%  | 5.4                     |
| 05Sals            | 55                 | 0.604              | 0.603              | 7.6%   | 5.8                     |
| 06Sals            | 208                | 0.626              | 0.624              | 62.0%  | 6.2                     |
| 01Hilt            | 51                 | 0.659              | 0.625              | 29.8%  | 7.2                     |
| 02Hilt            | 64                 | 0.650              | 0.651              | 26.9%  | 6.6                     |
| 03Hilt            | 50                 | 0.634              | 0.595              | 12.9%  | 6.8                     |
| 04Hilt            | 123                | 0.630              | 0.636              | 10.5%  | 6.7                     |
| 05Hilt            | 51                 | 0.681              | 0.625              | 4.7%   | 7.7                     |
| 06Hilt            | 188                | 0.659              | 0.634              | 31.0%  | 7.5                     |
| Coche             | 28                 | 0.605              | 0.593              | 1.2%   | 5.2                     |
| SCruz             | 39                 | 0.645              | 0.630              | 0.0%   | 6.6                     |
| WFSCz             | 37                 | 0.632              | 0.611              | 0.0%   | 6.5                     |
| NFJunc            | 85                 | 0.585              | 0.611              | 3.5%   | 5.4                     |
|                   | Mean               | 0.627              | 0.599              | 14.5%  | 6.3                     |

**Table 4.** Result of probability tests for Hardy-Weinberg equilibrium (HWE) using Monte Carlo Markov chain estimation of exact test p-values. Asterisks indicate significant deviations ( $p < 0.001$ ) from HWE. These deviations are almost exclusively heterozygote deficiencies.

|        | Omy1011 | Omy77 | One11b | OtsG243 | OtsG253b | OtsG401 | Omy27 | OtsG103 | OtsG249b | OtsG409 | OtsG43 | OtsG85 | Oki23 | One13b | Ots1b | OtsG3 | Ssa289 | Ssa85 |
|--------|---------|-------|--------|---------|----------|---------|-------|---------|----------|---------|--------|--------|-------|--------|-------|-------|--------|-------|
| 99Sals |         | *     |        |         | *        |         |       |         | *        |         |        | *      |       |        |       |       |        |       |
| 01Sals | *       | *     |        | *       | *        | *       |       |         |          |         | *      | *      |       |        |       |       |        |       |
| 03Sals |         | *     |        |         |          |         |       |         |          |         | *      | *      |       | *      |       |       |        |       |
| 04Sals |         | *     |        |         | *        | *       |       |         |          |         |        | *      |       |        | *     |       |        |       |
| 05Sals |         |       |        |         |          | *       |       |         |          |         |        |        | *     |        |       |       |        |       |
| 06Sals | *       | *     | *      |         | *        | *       |       |         | *        |         |        | *      | *     |        | *     | *     | *      |       |
| 01Hilt |         | *     |        |         |          |         |       |         | *        |         |        |        | *     | *      |       |       |        | *     |
| 02Hilt |         | *     |        |         | *        |         |       |         | *        |         | *      | *      |       | *      |       |       |        |       |
| 03Hilt |         |       |        |         | *        |         |       |         |          |         | *      | *      | *     | *      |       |       |        |       |
| 04Hilt |         |       |        |         |          |         |       |         |          |         |        |        |       |        |       |       |        |       |
| 05Hilt |         | *     |        |         |          |         |       |         | *        |         |        |        |       |        |       |       |        | *     |
| 06Hilt |         | *     |        |         | *        |         |       | *       |          |         |        | *      |       |        |       |       |        | *     |
| Coche  |         |       |        |         |          |         |       |         |          |         |        |        |       |        |       |       |        |       |
| SCruz  |         |       |        |         |          |         |       |         |          |         |        |        |       |        | *     |       |        |       |
| WFSCz  |         |       |        |         |          |         |       |         |          |         |        |        |       |        |       |       |        |       |
| NFJunc |         |       |        |         |          |         |       |         |          |         |        |        |       | *      |       |       |        |       |

**Table 5.** Estimates of effective population size ( $N_e$ ) using the temporal method of Waples (1989). The method compares pairs of populations in order to estimate  $N_e$ . Within each sub-basin drainage (Sals and Hilt), differentiation (F),  $N_e$  and the confidence intervals were calculated for all pairwise comparisons between years. Estimates were also made assuming two generations (2gens) when four or more years had passed between samples.

|               | <b>03Hilt</b>         |           |              | <b>04Hilt</b> |           |              | <b>05Hilt</b>         |           |             | <b>06Hilt</b> |           |              |
|---------------|-----------------------|-----------|--------------|---------------|-----------|--------------|-----------------------|-----------|-------------|---------------|-----------|--------------|
|               | <b>F</b>              | <b>Ne</b> | <b>Conf</b>  | <b>F</b>      | <b>Ne</b> | <b>Conf</b>  | <b>F</b>              | <b>Ne</b> | <b>Conf</b> | <b>F</b>      | <b>Ne</b> | <b>Conf</b>  |
| <b>01Hilt</b> | 0.022                 | 22.7      | [15.0 35.8]  | 0.016         | 30.5      | [20.5 47.1]  | 0.029                 | 17.3      | [11.9 25.6] | 0.016         | 31.6      | [22.0 46.7]  |
| <b>02Hilt</b> |                       |           |              | 0.007         | 70.2      | [40.6 146.4] | 0.019                 | 26.2      | [17.1 41.9] | 0.008         | 65.4      | [41.3 114.3] |
| <b>03Hilt</b> |                       |           |              |               |           |              | 0.026                 | 19.5      | [13.1 29.8] | 0.010         | 45.9      | [30.3 73.5]  |
| <b>04Hilt</b> |                       |           |              |               |           |              |                       |           |             | 0.008         | 62.3      | [42.7 93.9]  |
|               | <b>06Hilt (2gens)</b> |           |              |               |           |              |                       |           |             |               |           |              |
|               | <b>F</b>              | <b>Ne</b> | <b>Conf</b>  |               |           |              |                       |           |             |               |           |              |
| <b>01Hilt</b> | 0.158                 | 63.3      | [43.9 93.4]  |               |           |              |                       |           |             |               |           |              |
| <b>02Hilt</b> | 0.008                 | 130.8     | [82.6 228.7] |               |           |              |                       |           |             |               |           |              |
| <b>03Hilt</b> |                       |           |              |               |           |              |                       |           |             |               |           |              |
| <b>04Hilt</b> |                       |           |              |               |           |              |                       |           |             |               |           |              |
|               | <b>01Sals</b>         |           |              | <b>03Sals</b> |           |              | <b>04Sals</b>         |           |             | <b>05Sals</b> |           |              |
|               | <b>F</b>              | <b>Ne</b> | <b>Conf</b>  | <b>F</b>      | <b>Ne</b> | <b>Conf</b>  | <b>F</b>              | <b>Ne</b> | <b>Conf</b> | <b>F</b>      | <b>Ne</b> | <b>Conf</b>  |
| <b>99Sals</b> | 0.017                 | 28.6      | [18.3 46.9]  | 0.017         | 29.7      | [19.2 48.1]  | 0.024                 | 20.9      | [12.9 35.9] | 0.019         | 26.8      | [16.3 48.4]  |
| <b>01Sals</b> |                       |           |              | 0.015         | 32.6      | [22.9 46.5]  | 0.022                 | 22.7      | [14.8 35.1] | 0.016         | 30.2      | [19.5 48.5]  |
| <b>03Sals</b> |                       |           |              |               |           |              |                       |           |             | 0.013         | 39.3      | [24.6 67.3]  |
| <b>04Sals</b> |                       |           |              |               |           |              |                       |           |             |               |           |              |
|               | <b>05Sals (2gens)</b> |           |              | <b>06Sals</b> |           |              | <b>06Sals (2gens)</b> |           |             |               |           |              |
|               | <b>F</b>              | <b>Ne</b> | <b>Conf</b>  | <b>F</b>      | <b>Ne</b> | <b>Conf</b>  | <b>F</b>              | <b>Ne</b> | <b>Conf</b> |               |           |              |
| <b>99Sals</b> | 0.019                 | 53.7      | [32.5 96.9]  | 0.041         | 12.1      | [8.8 16.5]   | 0.041                 | 24.2      | [17.5 33.1] |               |           |              |
| <b>01Sals</b> | 0.016                 | 60.5      | [39.0 97.0]  | 0.045         | 11.0      | [8.3 14.3]   | 0.034                 | 29.2      | [22.1 37.8] |               |           |              |
| <b>03Sals</b> |                       |           |              | 0.033         | 15.2      | [11.3 19.9]  |                       |           |             |               |           |              |
| <b>04Sals</b> |                       |           |              | 0.035         | 14.2      | [9.9 19.9]   |                       |           |             |               |           |              |





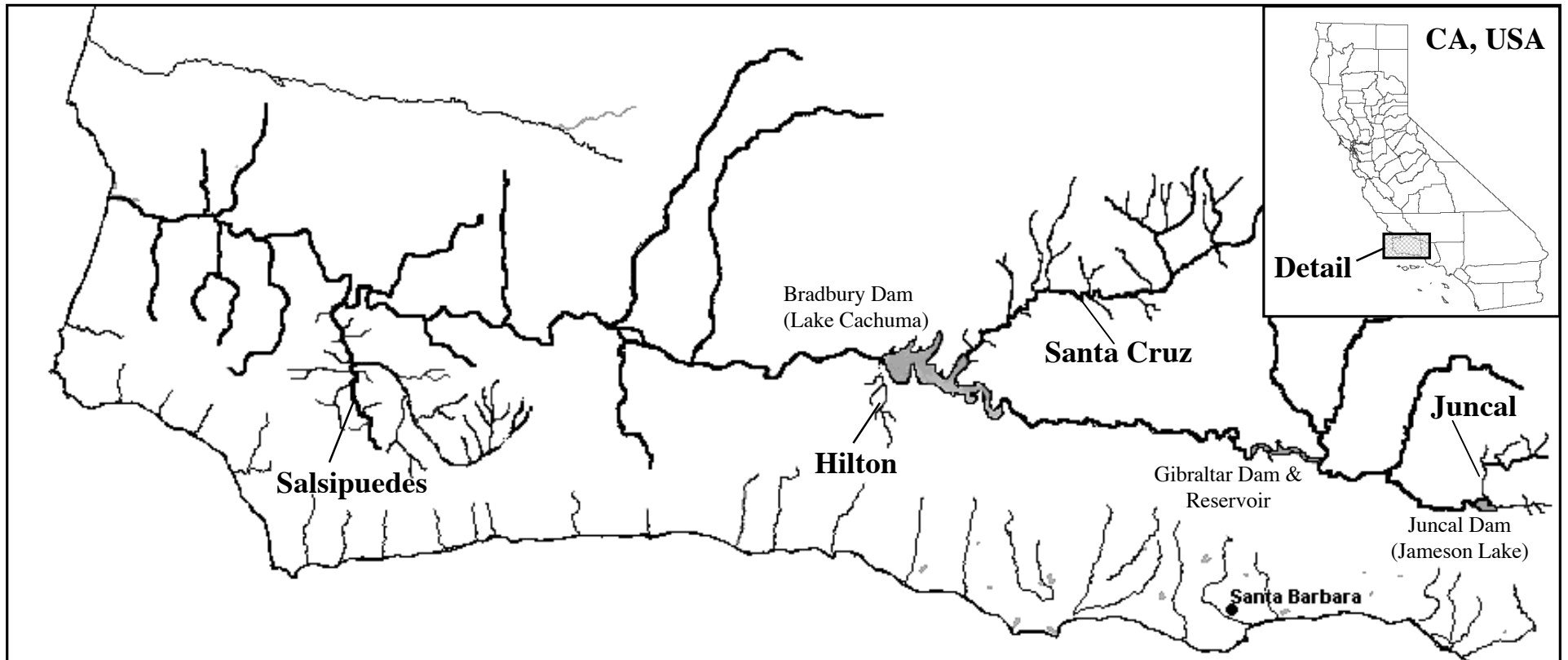
**Table 8.** Assignment of individual fish from the small sample collections to the larger SYR populations and the Fillmore Hatchery strains.

|          | Sals | Hilt | Coche | SCruz | WFSCrz | NFJunc | Fillmore |
|----------|------|------|-------|-------|--------|--------|----------|
| SYLag    | 2    | 1    |       |       |        |        |          |
| SYR99    |      | 2    |       |       |        |        |          |
| SYR00    |      | 1    |       | 1     |        |        |          |
| SYR03    |      |      |       |       | 2      | 9      |          |
| SYR05    |      |      |       |       |        |        | 1        |
| SYR06    |      | 15   |       |       |        |        |          |
| 98Sals   | 15   |      |       |       | 1      |        |          |
| 00Sals   | 12   | 1    |       |       |        |        |          |
| 02Sals   | 21   |      |       |       |        |        |          |
| LosAmol  | 4    |      |       |       |        |        |          |
| Nojo     | 2    | 1    |       |       |        |        |          |
| Alisal   |      | 1    |       |       |        |        |          |
| 02Quiota | 1    | 3    |       |       |        |        |          |
| 03Quiota | 2    | 4    |       | 3     |        |        |          |
| 04Quiota |      | 4    |       | 1     |        |        |          |
| 98Hilt   |      | 2    |       | 1     |        |        | 1        |
| 99Hilt   |      | 8    |       |       |        |        |          |
| 00Hilt   |      | 5    |       |       |        |        |          |
| EFSCz    |      |      |       | 1     | 4      |        |          |
| Gvine    |      |      |       | 11    |        |        |          |
| DevCan   |      | 2    |       | 1     |        |        |          |
| Indian   | 2    |      |       |       |        |        |          |

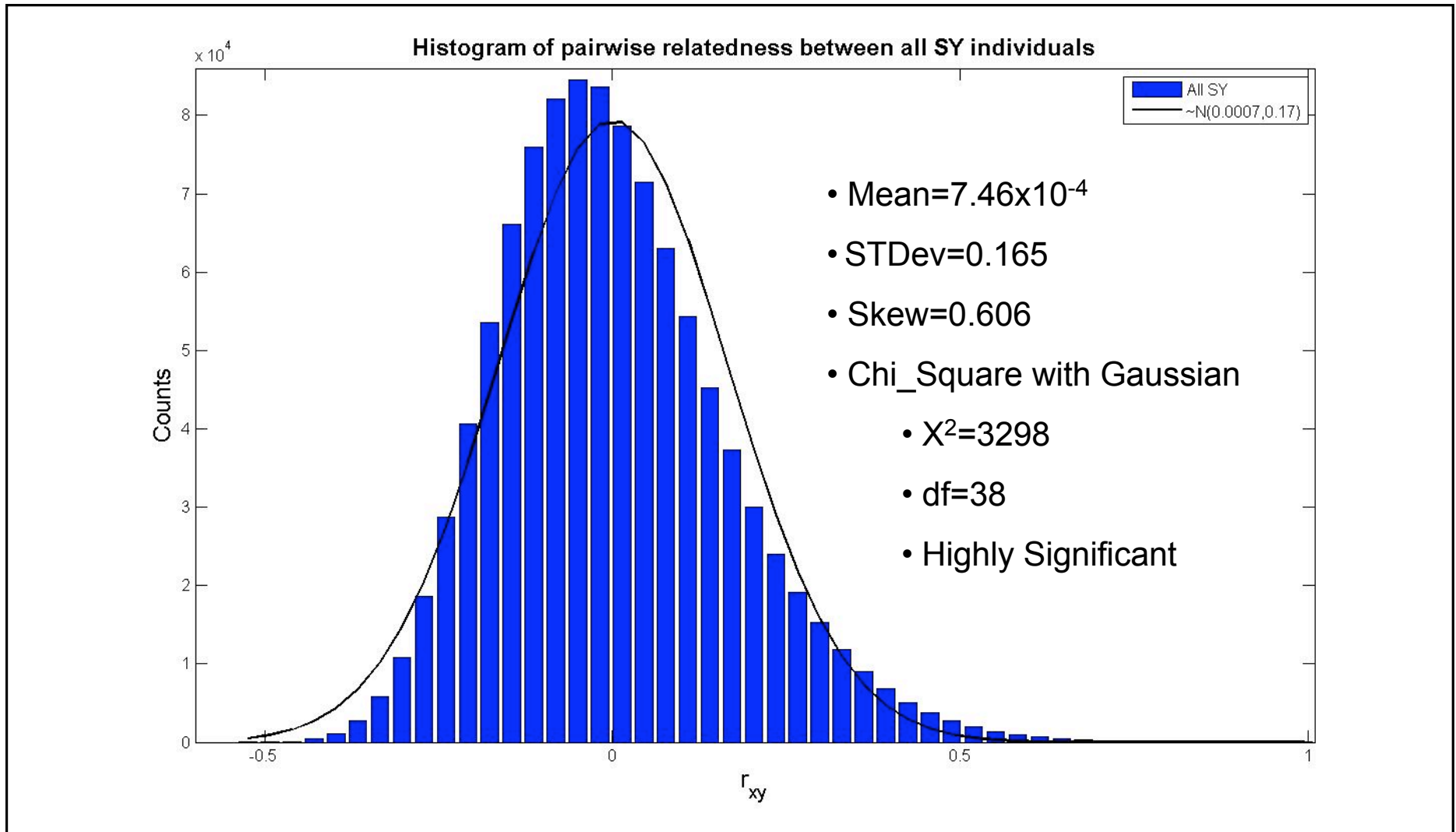




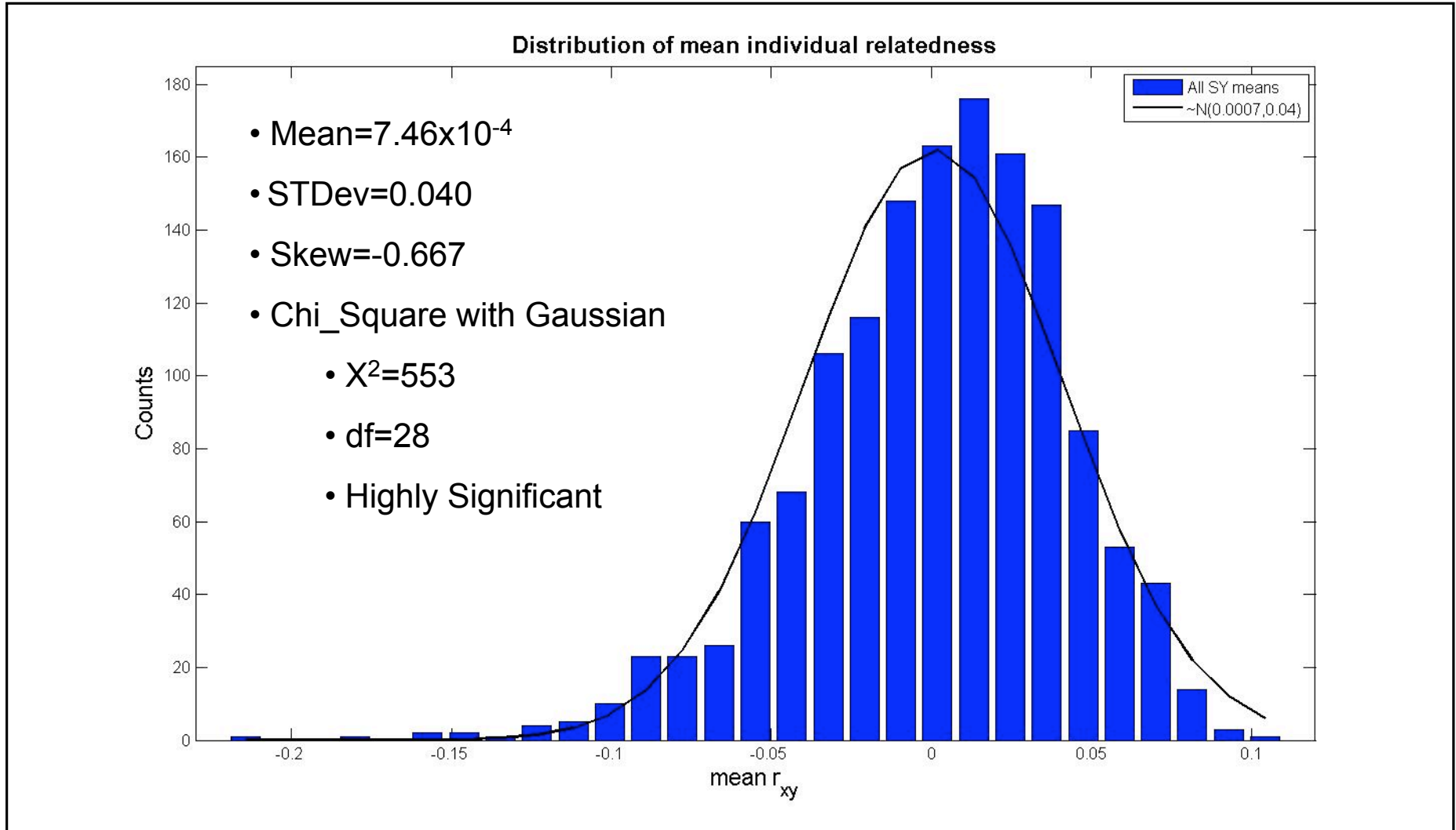




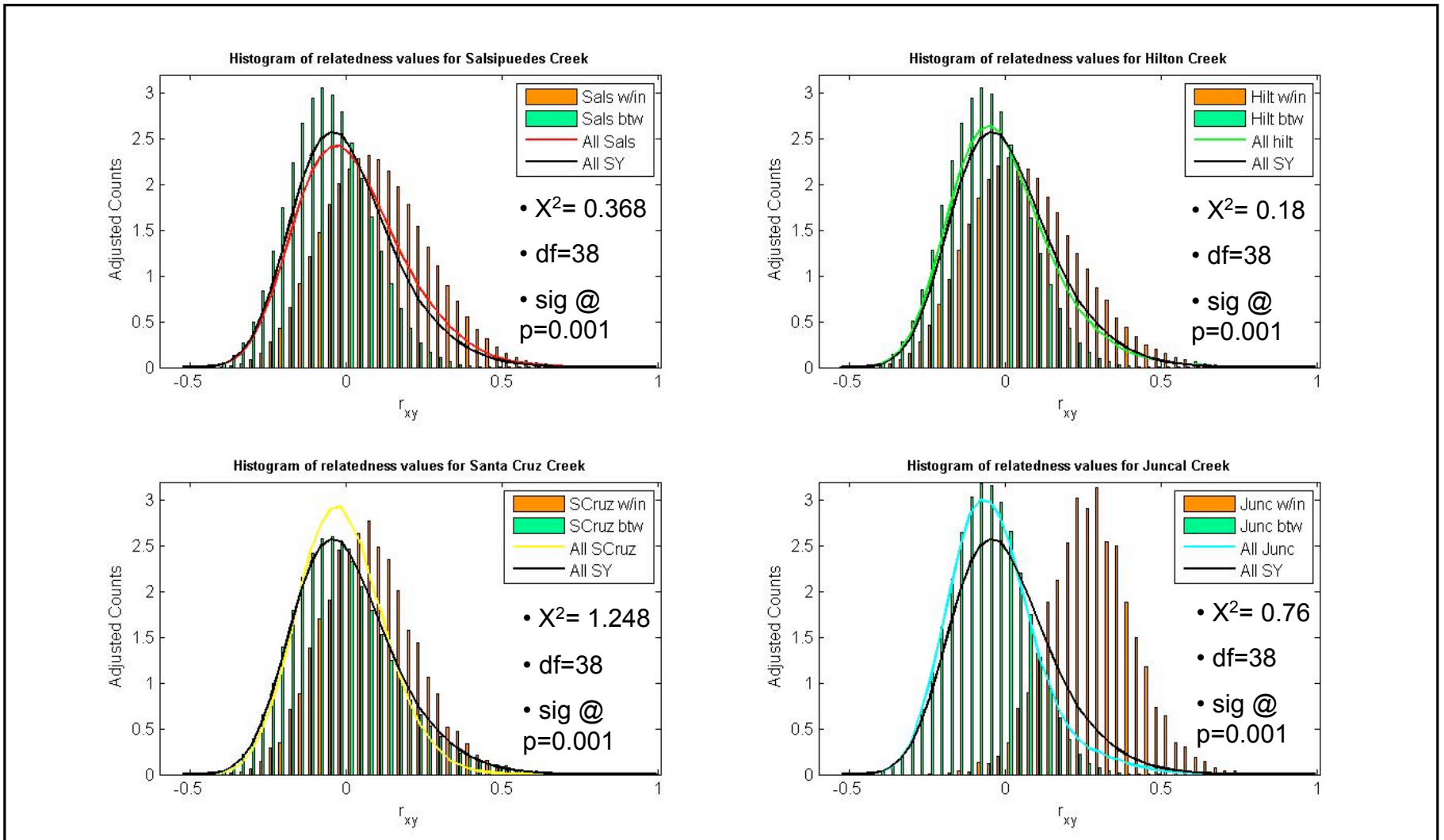
**Figure 1.** Map of the Santa Ynez River basin, with the four primary sampling areas (sub-basin drainages) indicated.



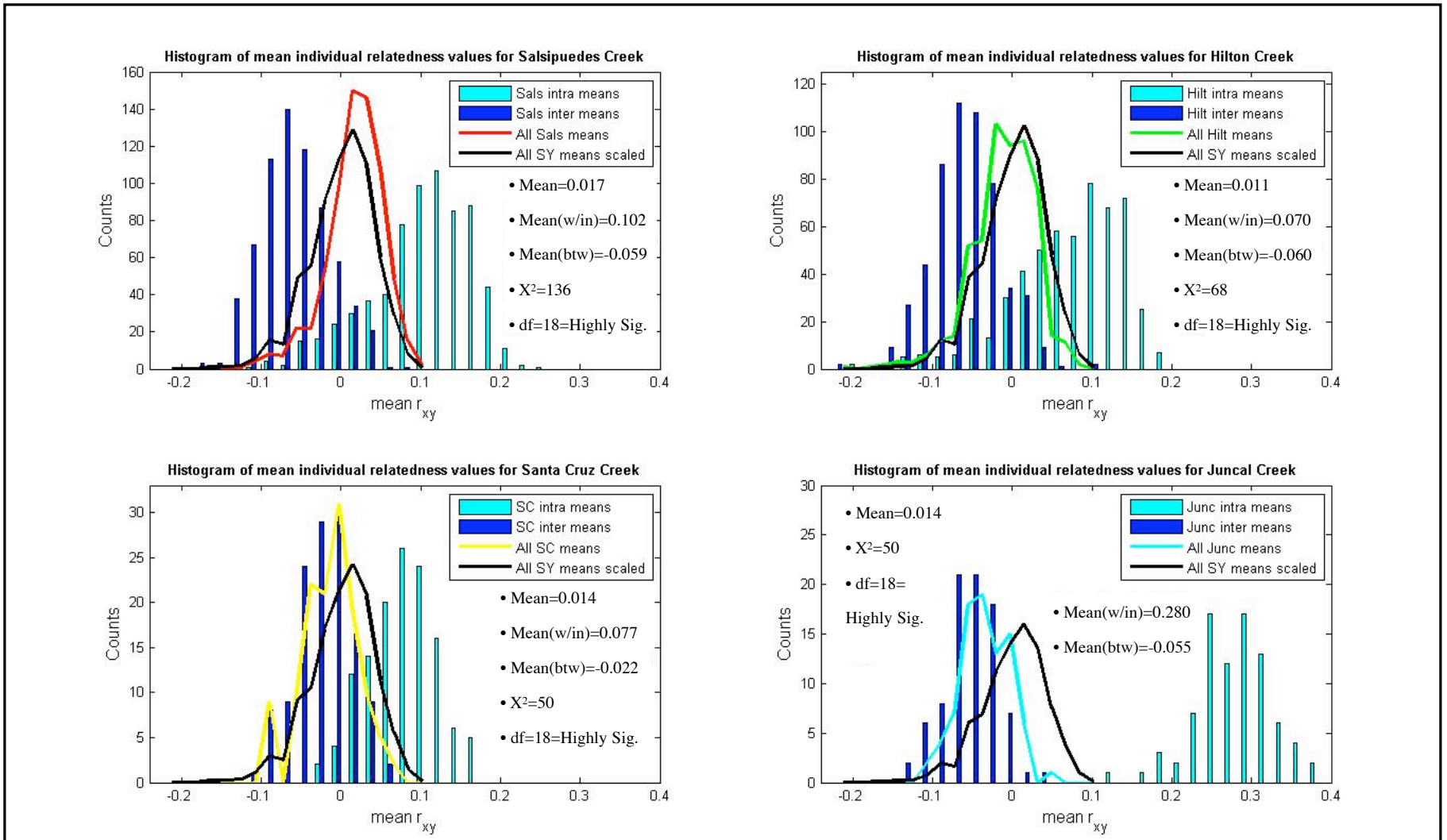
**Figure 2.** Distribution and statistics of pairwise relatedness values ( $r_{xy}$ ) between all individuals collected from the four primary sampling areas in the Santa Ynez River.



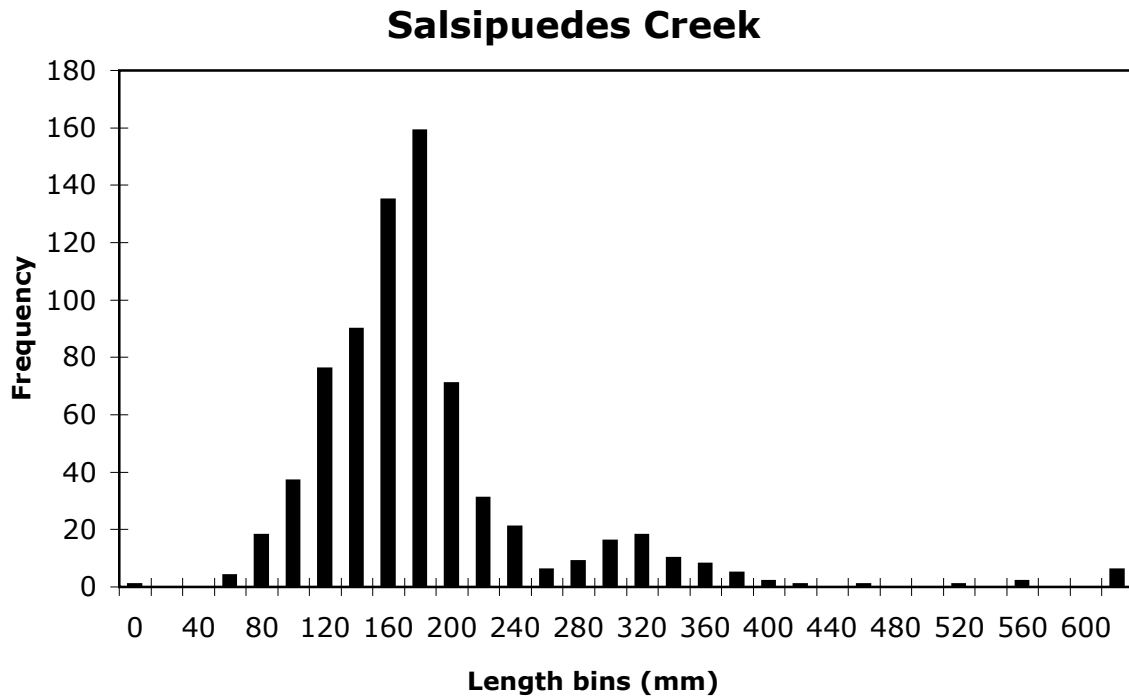
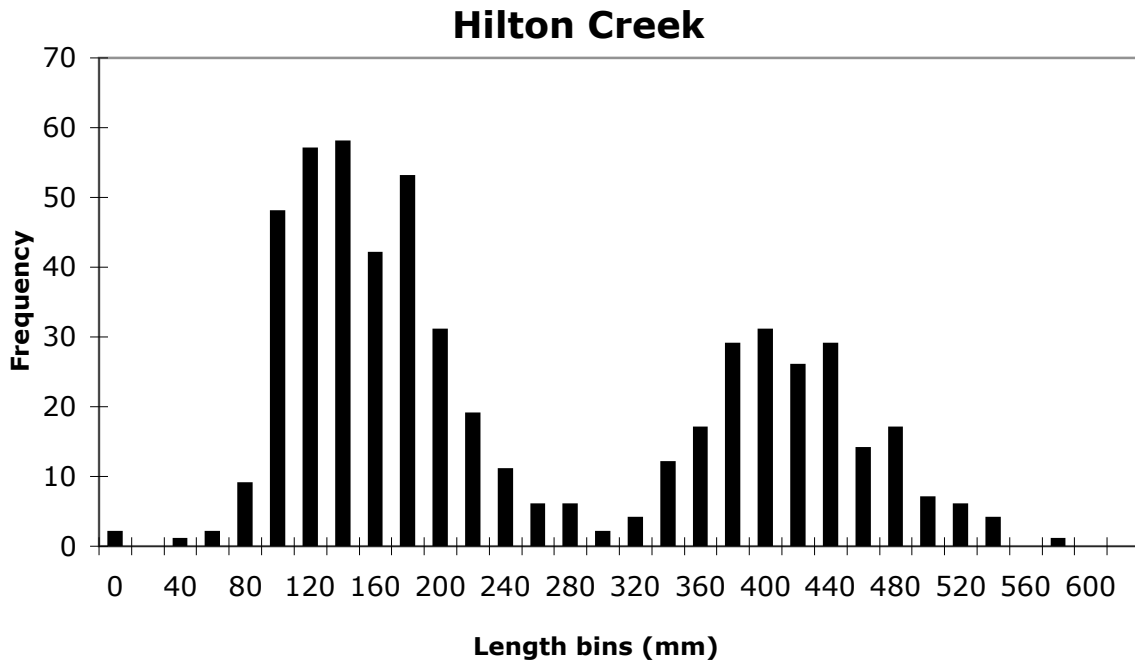
**Figure 3.** Distribution and statistics of mean pairwise relatedness ( $r_{xy}$ ) for each individual collected from the four primary sampling areas in the Santa Ynez River.



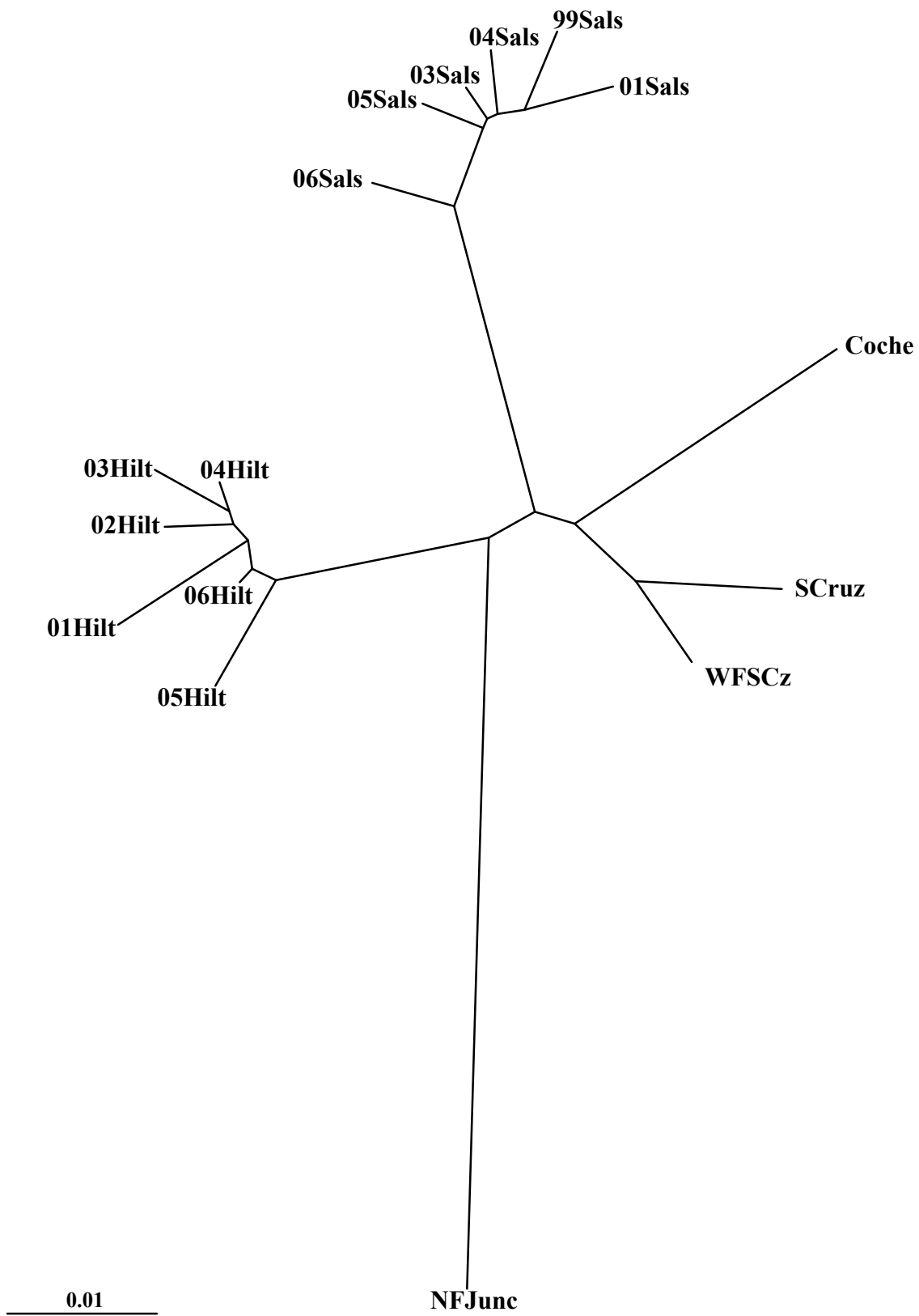
**Figure 4.** Distribution and statistics of pairwise relatedness values ( $r_{xy}$ ) for each of the four primary sampling areas. Relatedness values within and between sub-basin drainages are displayed separately in each graph. Chi-squared values statistically compare each sub-basin to the overall Santa Ynez River distribution (Figure 2).



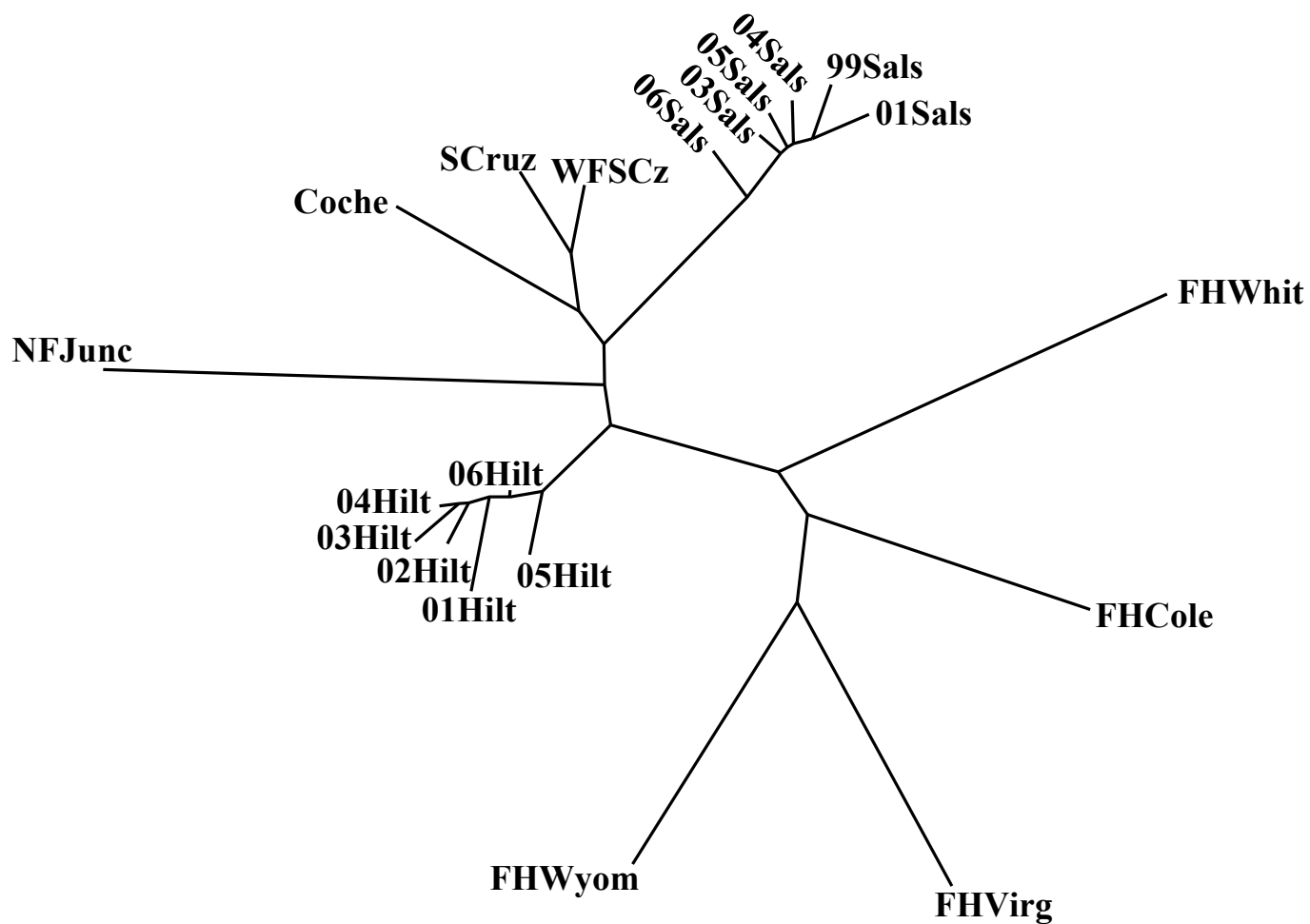
**Figure 5.** Distribution of mean individual relatedness ( $r_{xy}$ ) for each of the four primary sampling areas. Chi-squared values statistically compare each sub-basin distribution to the overall Santa Ynez River distribution (Figure 3). Bars indicate values generated by calculating a within- (light blue) and between-sub-basin (dark blue) mean for each individual.



**Figure 6.** Length frequency histograms of all fish of known size captured in Hilton and Salsipuedes Creeks. Length data for Juncal and Santa Cruz Creeks was incomplete and likely biased by sampling methodology.



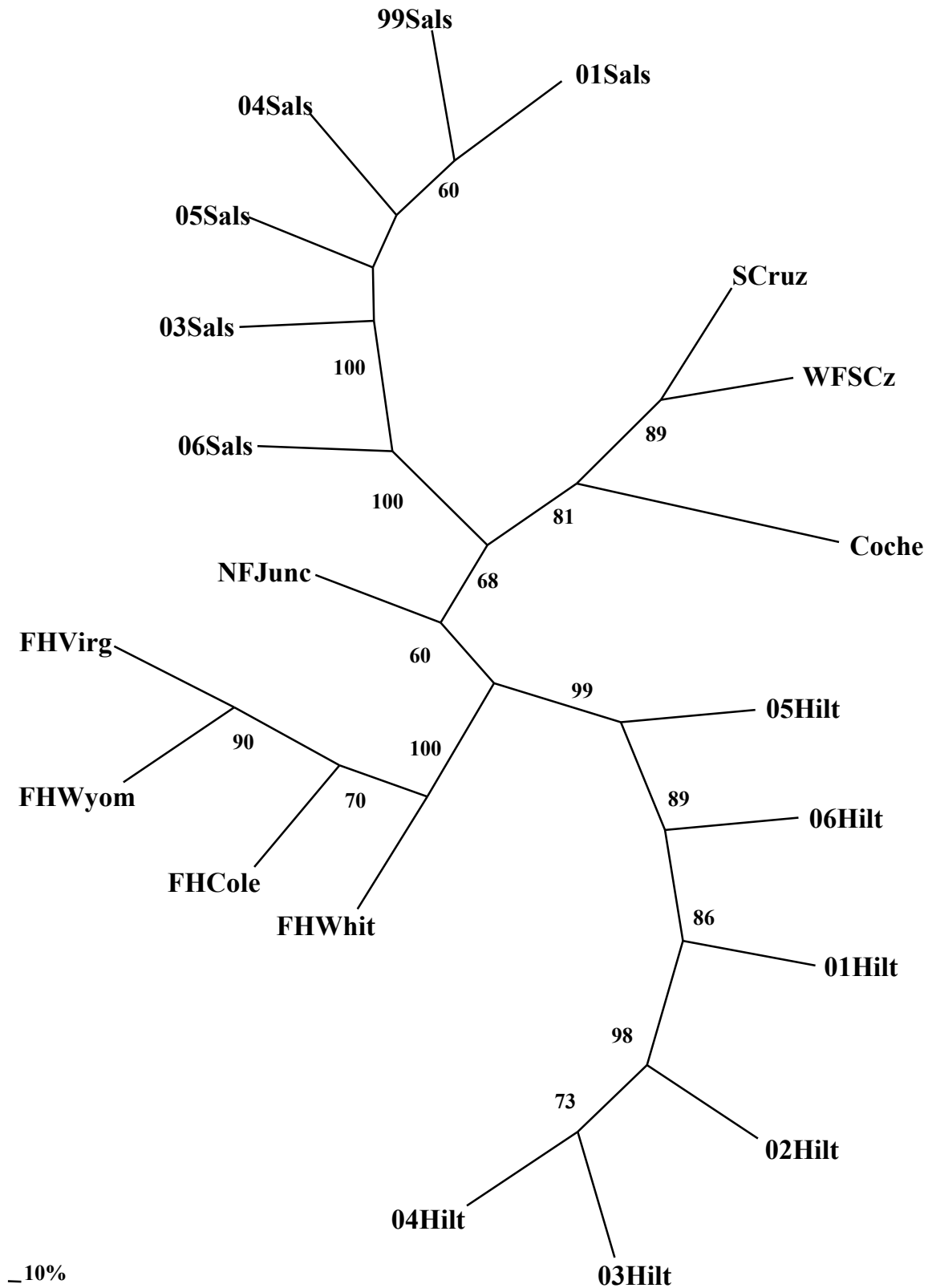
**Figure 7.** Unrooted, neighbor-joining tree of Santa Ynez River population samples constructed using Cavalli-Sforza and Edwards' (1967) chord distance. Branch lengths indicate chord distance.



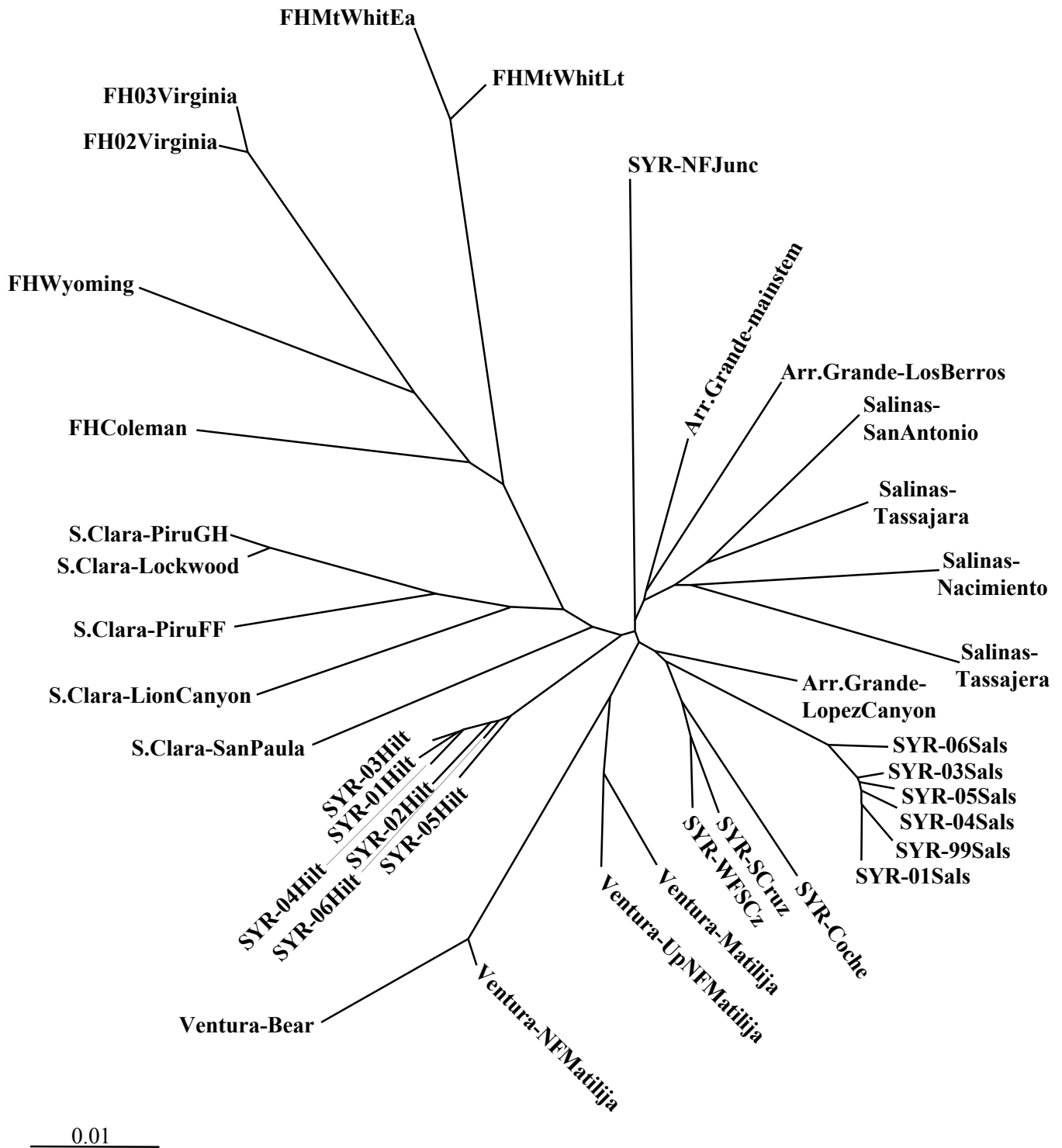
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**Figure 8.** Unrooted, neighbor-joining/chord distance tree of Santa Ynez River population samples with Fillmore Hatchery (FH) strains included.

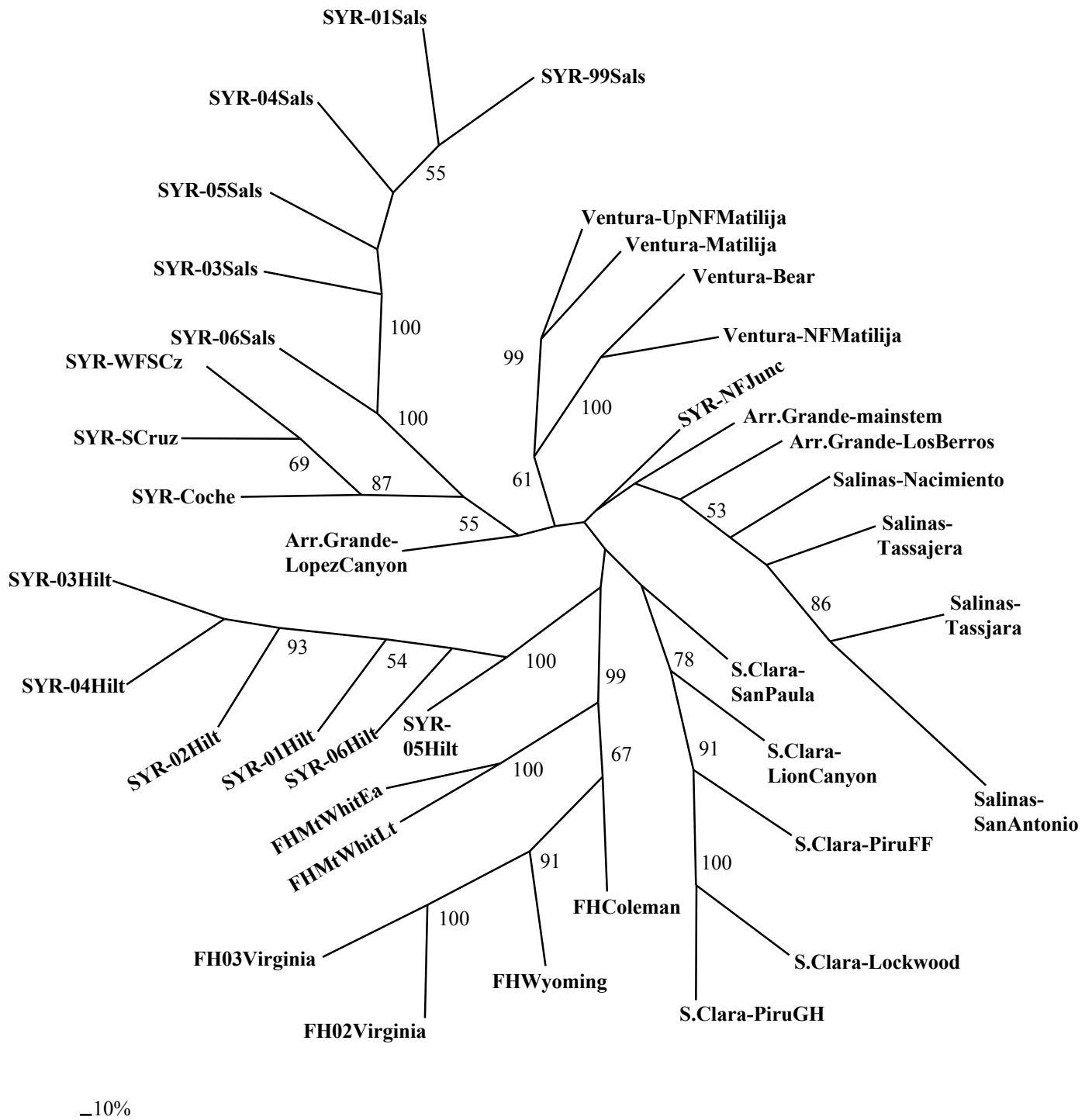




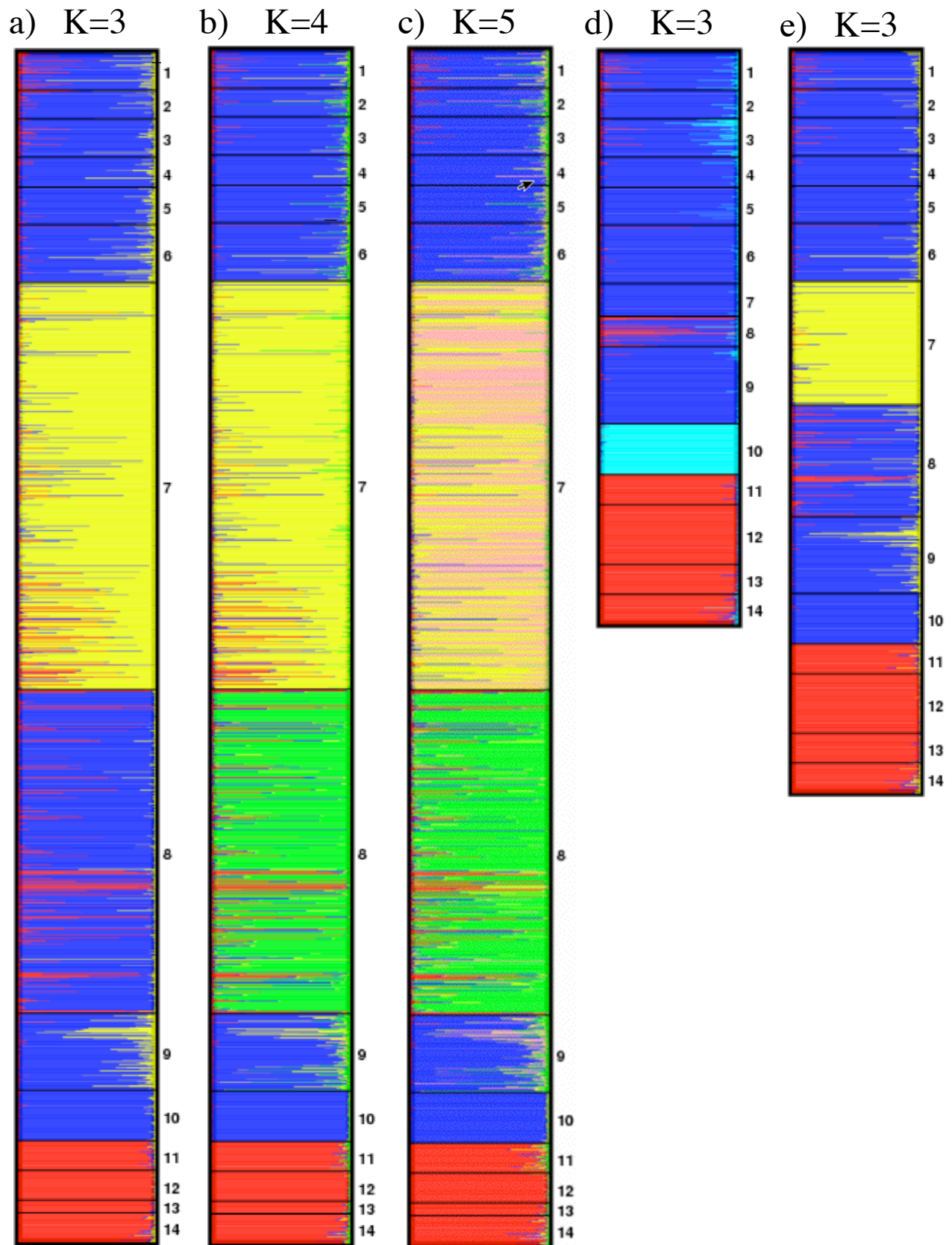
**Figure 9.** Majority-rule consensus tree of 10,000 neighbor-joining/chord distance trees from bootstrap replicates of the dataset including the Santa Ynez River population samples and Fillmore Hatchery (FH) strains. Internal branch lengths are scaled by percent bootstrap support, which is indicated for branches represented in over half of replicates.



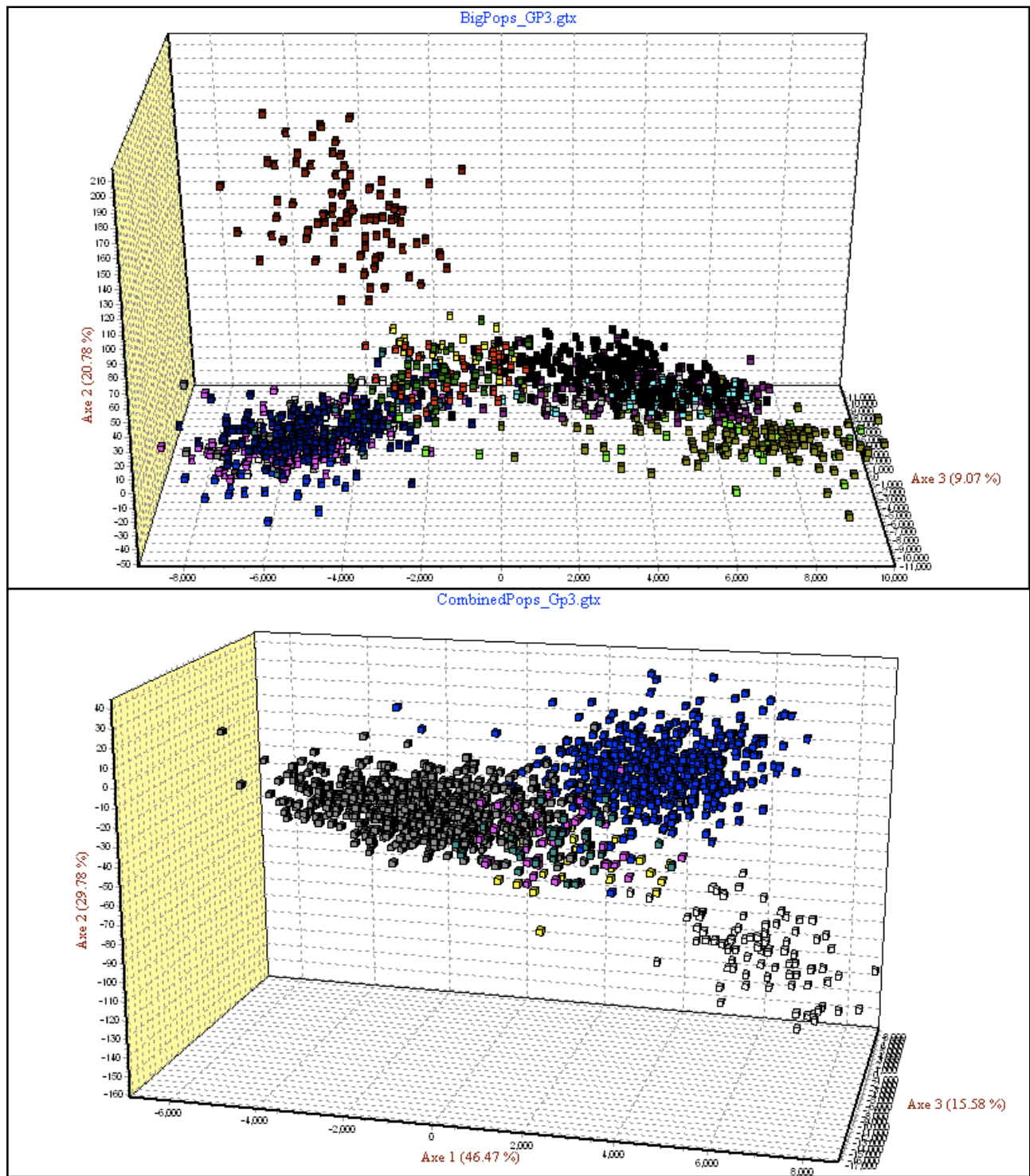
**Figure 10.** Unrooted, neighbor-joining/chord distance tree of Santa Ynez River (SYR) population samples together with populations from proximate basins and Fillmore Hatchery (FH) strains. See Girman and Garza (2006) for non-SYR population information.



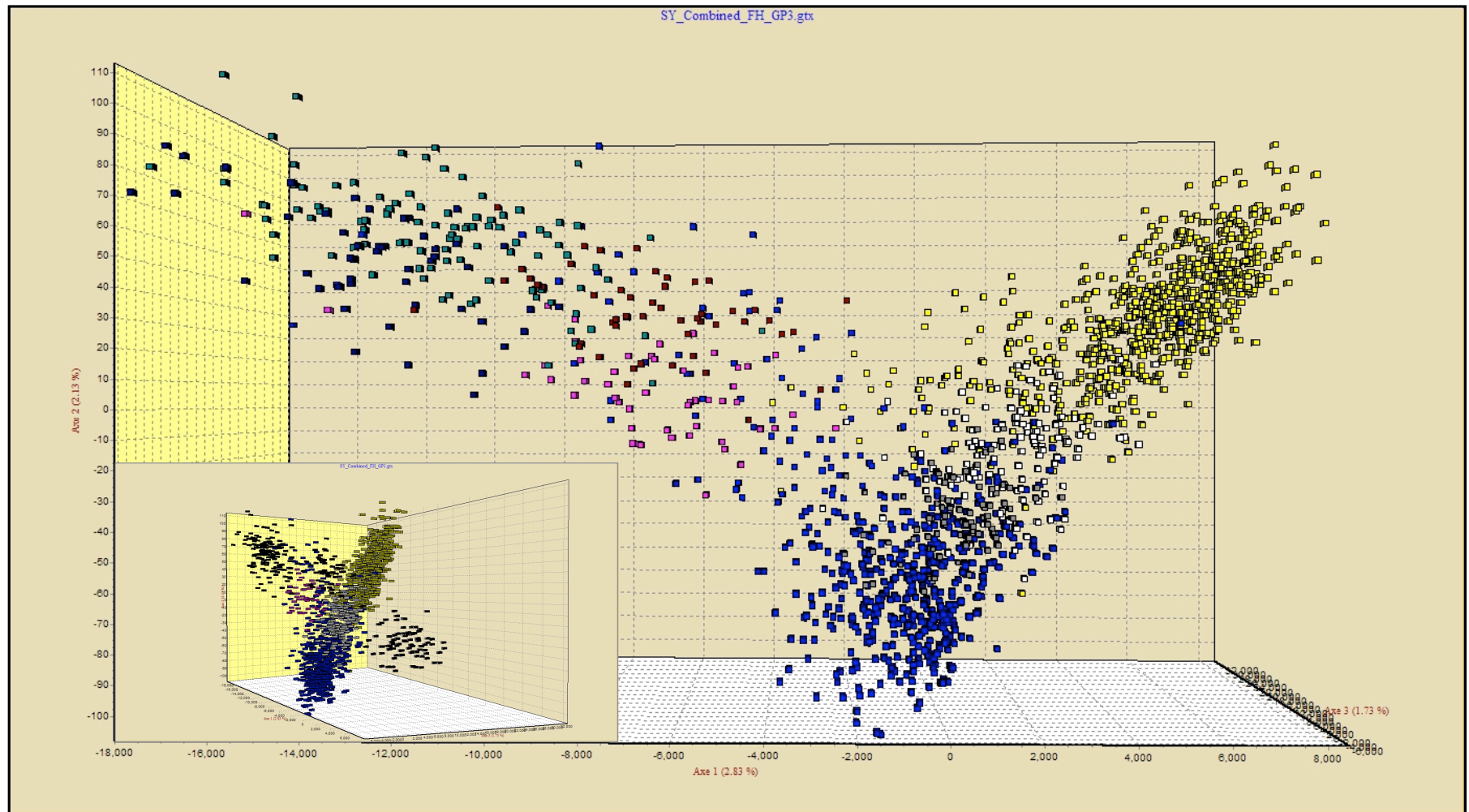
**Figure 11.** Majority-rule consensus tree of 10,000 neighbor-joining/chord distance trees from bootstrap replicates of the dataset with the Santa Ynez River together with populations from proximate basins and Fillmore Hatchery strains.



**Figure 12.** Results of five different analyses with the program STRUCTURE using different proposed numbers of genetic clusters ( $K$ ), indicated by colors, and population samples, denoted with numbers. Population samples: 1-6, Monterey Coast and Arroyo Grande; 7-10, SYR (Salsipuedes, Hilton, Santa Cruz, and Juncal, in that order); 11-14, FH strains. Plots a) through c) include the entire data set, plot d) uses only the 05Sals (7) and 05Hilt (8), and plot e) uses only 06Sals (7) and 06Hilt (8) from those sub-basins.

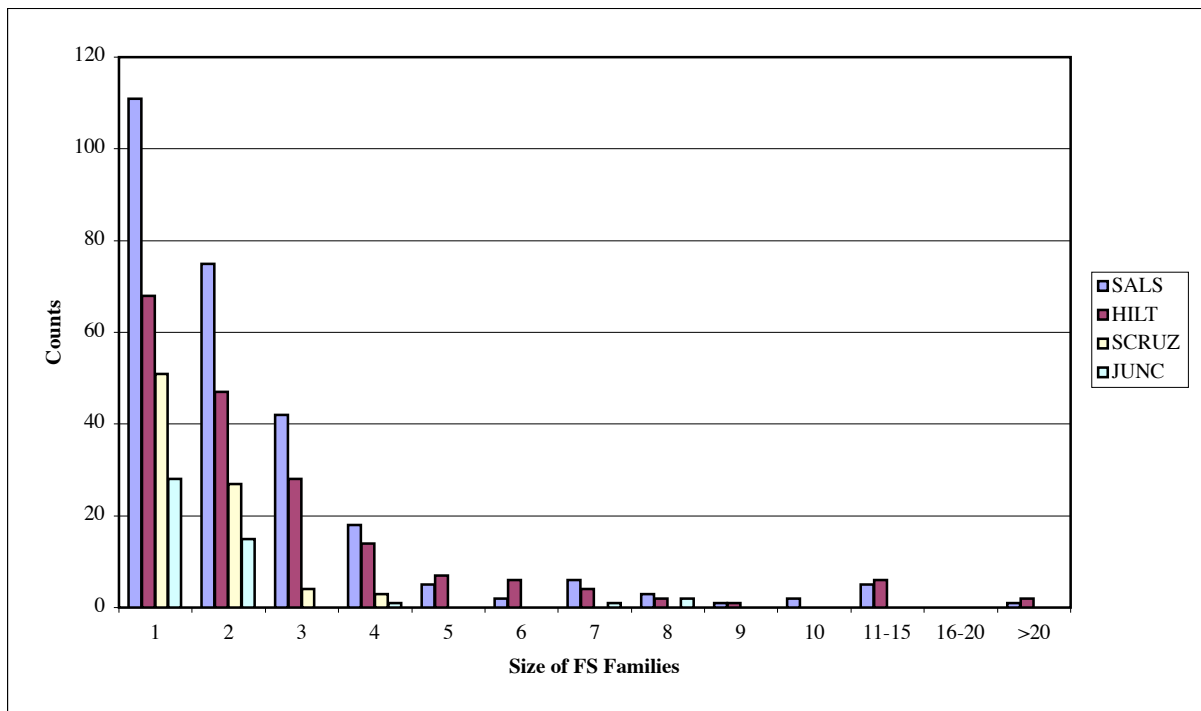
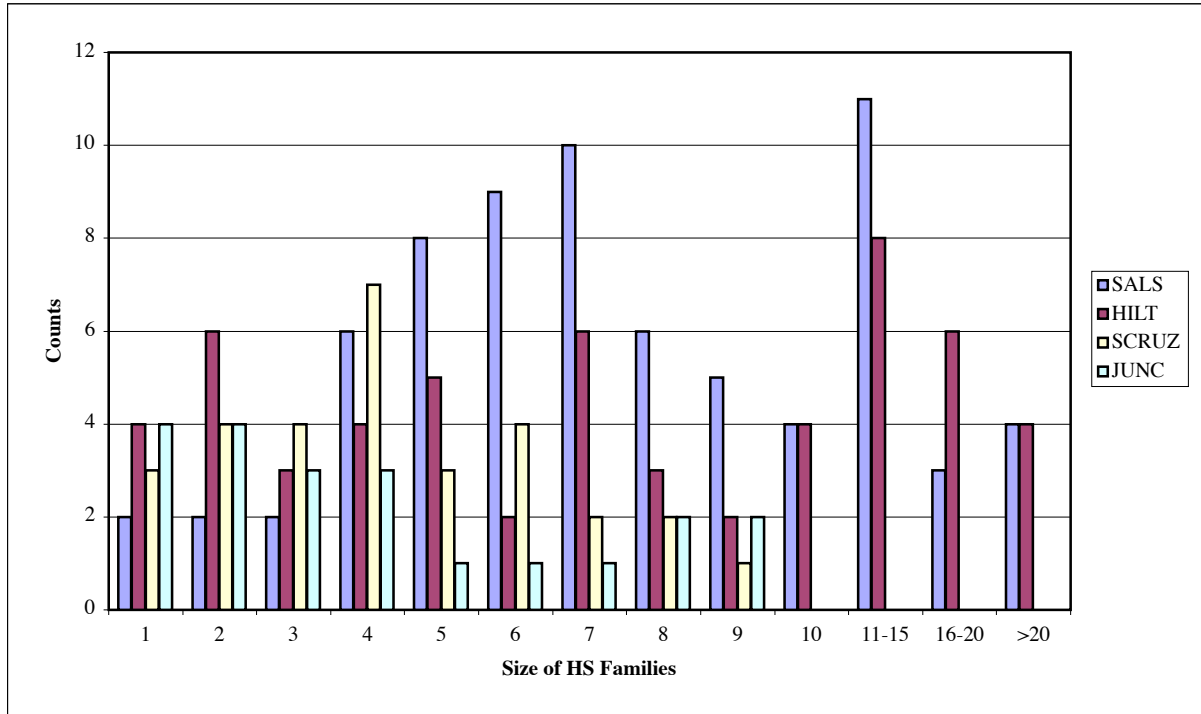


**Figure 13.** Results of Analysis of Factorial Correspondence (AFC) depicting genotypes of individual Santa Ynez River fish in three-dimensional space. The top graph shows each of the 16 large population samples considered separately, while the bottom graph has all Hilton (blue) and Salsipuedes (grey) samples pooled. Juncal Creek fish appear in the small group above (top graph) or below (bottom graph) the other Santa Ynez fish.



**Figure 14.** Results of Analysis of Factorial Correspondence (AFC) depicting genotypes of individual Santa Ynez River fish, together with those from Fillmore Hatchery trout strains, in three-dimensional space. Salsipuedes (yellow), Hilton (blue), Santa Cruz (white) and Juncal (dark grey) Creeks are quite distinct from Fillmore Hatchery strains (other colors in the upper left quadrant). Inset is a side view, showing differentiation of Juncal Creek from other Santa Ynez populations.

|        | N   | Number of HS Fams | Scaled HS Fams | Ave HS Fam Size | HS Fams with >25 indivs | Number of FS Fams | Scaled FS Fams | Ave FS Fam Size | FS Fams with >10 indivs | Ave # of FS Fams per HS Fam |
|--------|-----|-------------------|----------------|-----------------|-------------------------|-------------------|----------------|-----------------|-------------------------|-----------------------------|
| Sals   | 684 | 72                | 0.11           | 9.5             | 3                       | 271               | 0.40           | 2.5             | 6                       | 3.8                         |
| Hilt   | 544 | 57                | 0.10           | 9.5             | 3                       | 185               | 0.34           | 2.9             | 8                       | 3.2                         |
| SCall  | 129 | 30                | 0.23           | 4.3             | 0                       | 85                | 0.66           | 1.5             | 0                       | 2.8                         |
| Juncal | 85  | 21                | 0.25           | 4.0             | 0                       | 47                | 0.55           | 1.8             | 0                       | 2.2                         |



**Appendix A.** Summary of COLONY results. At the top are the totals and scaled values for the number of identified half-sib and full-sib families. The graphs depict the counts of half- and full-sib families of specified sizes.