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Population genetic structure of *Oncorhynchus mykiss* in the California Central Valley

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Abstract

Steelhead/rainbow trout of the species *Oncorhynchus mykiss* are found in all of the major drainages of the Central Valley, which includes rivers and streams that drain into both the Sacramento and San Joaquin sub-basins. Most of the tributary rivers in this area have dams or other impoundments and many of the resulting reservoirs have been stocked with hatchery rainbow trout. Genotype data was collected from 18 highly variable microsatellite molecular markers in more than 1600 fish from the Central Valley region sampled by California Department of Fish and Game biologists, as well as a sample of adult steelhead from Battle Creek sampled by the US Fish and Wildlife Service. Analyses of these data examined population structure within the region, relationships between populations above and below barriers to anadromy, relationships of Central Valley populations with coastal steelhead populations, and population genetic diversity. Analysis focused on 17 initial "population" samples, comprised of fish sampled from the Kings, Tuolumne, Stanislaus, Calaveras, American, Yuba, Feather, Butte, Deer, Battle and McCloud River sub-basins. Additional analyses were conducted with data from the same microsatellite markers in rainbow trout hatchery stocks and steelhead from coastal and California Central Valley populations. These analyses looked at whether specific fish are, or are descended, from hatchery strains used in local stocking efforts, as well as providing biogeographic context for the Central Valley regional results.

In general, although structure was found, all naturally-spawned populations within the Central Valley basin were closely related, regardless of whether they were sampled above or below a known barrier to anadromy. This is due to some combination of pre-impoundment historic shared ancestry, downstream migration and, possibly, limited, anthropogenic, upstream migration. However, lower genetic diversity in above-barrier populations indicates a lack of substantial genetic input upstream and highlights lower effective population sizes for above-barrier populations.

In contrast to coastal steelhead, we did not find close relationships between populations above and below barriers within the same sub-basin. Instead, above-barrier populations clustered with one another and below-barrier populations clustered with one another in all tree analyses. Analysis using data from coastal steelhead populations found

that the above-barrier populations enter the California-wide trees next to the San Francisco Bay populations, whereas the below-barrier populations are most closely related to populations in far northern California, specifically the genetic groups that include the Eel and Klamath Rivers. Since Eel River origin broodstock were used for many years at Nimbus Hatchery on the American River, it is likely that Eel River genes persist there and have also spread to other basins by migration, and that this is responsible for the clustering of the below-barrier populations with northern California ones. This, in combination with the observation of large numbers of hatchery rainbow trout entering Nimbus Hatchery and potentially spawning as steelhead, suggest that the below-barrier populations in this region appear to have been widely introgressed by hatchery fish from out of basin broodstock sources. The consistent clustering of the above-barrier populations with one another, and their position in the California-wide trees, indicate that they are likely to most accurately represent the ancestral population genetic structure of steelhead in the Central Valley.

Introduction

The Central Valley of California has numerous populations of salmonid, including Chinook salmon and fish from the species (*Oncorhynchus mykiss*), commonly known as steelhead (anadromous life history) or rainbow trout (resident life history). The Central Valley is also the catchment basin for most of the precipitation run-off of the Sierra Nevada mountain range. The many tributary rivers converge into the north-flowing San Joaquin and the south-flowing Sacramento, before exiting to the ocean through the San Francisco Bay/Delta region. Much of this water is diverted for agricultural and domestic uses through a large number of levees, aqueducts and dams. While salmon have been extirpated above these dams, *O. mykiss* populations are still present.

Steelhead populations in California are divided into six Distinct Population Segments (DPSs), formerly Evolutionarily Significant Units (ESUs); five on the coast and one in the Central Valley. While Chinook salmon populations in San Francisco Bay streams are genetically related to Central Valley fall run salmon, steelhead populations in the San Francisco Bay region are more closely related to coastal populations, and are therefore included in the Central California Coast Steelhead DPS.

The California Central Valley Steelhead DPS was listed as "Threatened" under the US Endangered Species Act by the National Oceanic and Atmospheric Administration in 1998 and the status was reaffirmed in 2006. All anadromous *O. mykiss* found below impassable barriers to migration and that spawn naturally in streams that drain the Central Valley are included in the DPS, as are the fish produced by artificial propagation programs at the Feather River Hatchery and Coleman National Fish Hatchery.

Previous genetic work on population structure of steelhead in California has relied

primarily on mitochondrial DNA (e.g. Berg and Gall 1988; Nielsen et al. 1997), which is a single gene that is often not reflective of population history or true relationships (Chan and Levin 2005). Microsatellites, also known as simple sequence repeat loci, have been used in numerous studies of salmonids and have proven to be a valuable tool for elucidating population genetic structure. Recent work on *O. mykiss* in California using microsatellite loci has demonstrated that genetic structure can be easily identified with such data, both at larger scales (Aguilar and Garza, 2006; Garza et al. in review; Clemento et al. in prep) and at relatively fine ones (Deiner et al. 2007; Pearse et al. 2007a). For example, *O. mykiss* populations in the Russian River separated by waterfalls were highly genetically distinct, whereas those found above and below the two major dams (Warm Springs and Coyote) were found to show little genetic distinction (Deiner et al. 2007). In the Klamath River, genetic relationships of *O. mykiss* populations below waterfalls vary with geographic distance (Pearse et al. 2007a), a pattern referred to as isolation by distance, whereas genetic relationships of trout populations above barriers do not.

In this project, populations of *O. mykiss*, steelhead/rainbow trout, were studied in basins of California's Central Valley using molecular genetic techniques to provide insight into population structure in this region. Data were collected from 18 nuclear microsatellite loci and variation analyzed to trace ancestry and evaluate genetic distinction among populations. The goals of the study were to use population genetic analyses of the data to assess origins and ancestry of *O. mykiss* populations above and below dams in Central Valley area tributary rivers, to better understand the relationship of these populations to others in California, and to provide information on genetic

diversity and population structure of these populations. Genotypes were collected from over 1600 individual fish from 17 population samples and five hatchery rainbow trout strains. Fish populations from rivers and creeks that flow to both the Sacramento and San Joaquin Rivers were evaluated, including the McCloud River, Battle Creek, Deer Creek, Butte Creek, Feather River, Yuba River, American River, Calaveras River, Stanislaus River and Tuolumne River sub-basins (Figure 1). Analyses included fish collected both above and below barriers to anadromy in some of the study basins.

There are a number of dams in the study basins and hatchery-raised trout of a variety of strain origins have been planted in nearly all of the reservoirs above them over the last 100+ years. Many of these trout were likely of diverse geographic and phylogenetic origin, as movements of salmonids from basin to basin and from state to state was common until recently. Microsatellite data from five strains of trout commonly raised at Central Valley hatcheries – Mt. Shasta, Coleman, Eagle, Moccasin & Junction Kamloops – were also included in most analyses to detect reproduction from hatchery trout and to determine if any of the populations had a large degree of ancestry from these fish.

In addition to the hatchery trout and Central Valley steelhead, data from the same genes have been collected in over 100 other populations of steelhead from California (Aguilar and Garza 2006; Pearse et al. 2007a; Garza et al. in review; Clemento et al. in prep), covering the entire range of coastal steelhead in the state. Data from many of these populations, and for 14 of the 18 microsatellite genes, were combined with those from the Central Valley populations, to identify relationships of Central Valley populations to those from other parts of California. This combined dataset was used to construct phylogeographic trees that depict summarized genetic relationships.

Methods

Sampling

Most of the fish analyzed in this study were sampled during the course of monitoring activities by California Department of Fish and Game biologists. Tissue was sampled as small pieces of dried caudal fin preserved through desiccation on blotter paper. A population sample (N=180) of adult fish collected by the US Fish and Wildlife Service at a weir on Battle Creek was also included in the study. Upon receipt in Santa Cruz, all samples were catalogued and transferred to tubes for DNA extraction.

DNA Extraction

Total nucleic acids were then extracted from approximately $2mm^2$ of each tissue sample using Qiagen DNeasy Tissue Kits, following the manufacturer's recommended protocol for animal tissues and using a BioRobot 3000 (Qiagen, Inc.) for all liquid handling. Extracted DNA was kept frozen at 20°C until it was diluted (10:1 with autoclaved, distilled water) and distributed to 96 well plates for microsatellite amplification via polymerase chain reaction (PCR).

Although a total of 1,603 fish were initially genotyped, 44 were removed as they were discovered to be duplicate samples. A total of 1,559 fish were then included in most analyses, following removal of the duplicate genotypes from these fish. The data set was then initially divided into 17 "population" samples for analysis (Table 1), although the Kings River sample was then subdivided into two population samples for reasons described below. The primary basis for division into population samples was basin of

collection and samples taken from populations above barriers were always separated from those taken from below barriers. Fish sampled in multiple years in the same location were combined for analysis. All of these groups of fish are referred to as populations for convenience and without additional assumptions about the biological details underlying this designation.

Genotyping

Genotypic data at 18 microsatellite loci (Table 2) were collected for fish in all population samples. PCR was carried out in 15µL aliquots containing 4µL purified and diluted template DNA, 6.35µL H_2O, 1.5µL ABI 10X II PCR buffer, 0.9µL $MgCl_2$, 1.2µL dNTPs, 0.05µL DNA polymerase (Amplitaq, Applied Biosystems), and 1µL fluorescent-labeled oligonucleotide primers. Variable thermal cycling regimes were employed for different loci to maximize PCR product. PCR products were pooled to equalize peak heights and then electrophoresed on ABI 377 DNA sequencers. Allele size determination used GENOTYPER software (version 2.1; Applied Biosystems Inc.). At least two people performed all size scoring independently, discrepancies were identified and discrepant samples were rerun. If a discrepancy persisted through the second analysis, the fish was not scored at that locus. A representative fraction was re-genotyped as a control for data quality.

Data Analysis

Expected heterozygosity (Nei 1987), observed heterozygosity and number of alleles were calculated for each sample population. In order to compensate for variation in

sample sizes, genetic diversity was also assessed using allelic richness as estimated with the rarefaction method in FSTAT (version 2.9.3.2; Goudet 2001). Linkage (gametic phase) disequilibrium (LD) was evaluated to examine segregation independence of the 18 microsatellite loci in each of the population samples and using the Markov Chain Monte Carlo (MCMC) approximation of an exact test implemented in the GENEPOP program (version 3.4; Raymond and Rousset 1995). Markov chain parameters of 10000 (dememorization), 1000 (batches) and 1000 (iterations per batch) were used. Disequilibrium results were summarized as the percentage of locus pairs that were in LD.

Pairwise differentiation between all pairs of populations was also quantified using F_{ST}, as estimated by Weir and Cockerham's (1984) Θ estimator, and significance (> 0) assessed by the permutation algorithm in the Genetix software package (Belkhir et al. 2004) with 1,000 replicates.

Individual-based assignment tests were used to further evaluate the degree of recent gene flow between the population samples, as well as introgression by the hatchery rainbow trout strains. This analysis assigns each individual fish to its most likely population of origin, using its genotype alone and through comparison to a collection of potential source populations. The semi-Bayesian allele frequency estimation algorithm of Rannala and Mountain (1997) and the leave-one-out procedure implemented in GeneClass2 (Piry et al. 2004) were utilized. Patterns of misassigned fish highlight similarities in genetic composition (allele frequencies) between sample populations/locations. A Bayesian, model-based, clustering method implemented in the program *structure* (version 2.2; Pritchard et al. 2000) was also used to assign individual fish to population of origin and to identify population structure. This analysis uses a prior

hypothesis about the number of genetic "clusters" to fractionally assign the ancestry of each individual fish to each of the clusters without regard to geographic location of origin.

Phylogeographic trees were constructed using matrices of Cavalli-Sforza & Edwards' (1967) chord distance, using the software package PHYLIP (version 3.57c; Felsenstein 1993). This genetic distance was chosen because of its statistical properties (Felsenstein 2003) and because it most reliably recovers the correct topology (branching pattern) for phylogeographic trees (Takezaki and Nei 1996). The neighbor-joining algorithm (Saitou and Nei 1987) was used to determine tree topology and a consensus tree was assembled from 1,000 bootstraps of the distance matrix with the CONSENSE program of the PHYLIP software package. Internal branch lengths on the consensus tree are scaled by the number of times that relationship was found in the neighbor-joining trees constructed with the bootstrap samples. Only bootstrap values above 50% are reported.

These phylogeographic tree-building analyses were carried out with several different datasets. First, all of the populations genotyped for this study (Central Valley region and hatchery stock samples) were analyzed and the most probable tree reported. This was repeated with the Kings River sample split (see Results) and with hatchery trout removed from the Stanislaus-Upper population and both the most probable and the bootstrap consensus trees are reported.

Several additional analyses of this dataset combined with data from steelhead populations and trout strains that were analyzed in other studies were then also carried out. These subsequent analyses utilized only the 14 microsatellite loci with data available for all population samples. The first such analysis combined the Central Valley data with

that from the Fillmore Hatchery trout strains and with population samples from coastal steelhead populations from southern California (Ventura County) to the Rogue River in southern Oregon (Aguilar and Garza 2006; Garza et al. in review; Clemento et al. in prep). This analysis was repeated by successively omitting all of the hatchery stocks, the above-barrier populations only and the below-barrier populations only. Only the most probable tree is reported for these analyses.

Factorial correspondence analysis (FCA), as implemented in the Genetix software program (Belkhir et al. 2004), was also used to qualitatively explore the distribution of genotypes in the data. This analysis helps to identify outlying individual fish and to visualize overlap in the distribution of individual genotypes from different populations. The FCA method was only conducted on the dataset of the naturally-spawning Central Valley populations, as well as this dataset with all of hatchery strains.

Results

Population groupings

Linkage disequilibrium (LD) was found to be high in a number of the population samples, including the Kings River, the McCloud River, Nimbus Hatchery and the Tuolumne-Lower groups (Table 1). LD can be caused by physical linkage of loci (which is known not to be the case for these microsatellites), sampling of related individuals/family structure, and by the sampling of more than one genetically distinct group within a population sample, which is commonly referred to as admixture. For these four outlier population samples, model-based clustering was used to evaluate if this LD was due to the sampling of genetically distinct groups of fish. The resulting analyses (Figure 2) identified two genetically distinct groups in the Kings River that corresponded to the two tributaries of sampling (Mill Flat and Deer Cove Creeks) in this sub-basin. This analysis also identified some fish from the Nimbus Hatchery sample that are of hatchery rainbow trout origin (see below). However, the McCloud River and Tuolumne-Lower population samples did not break up into distinct genetic clusters, even with a high number of hypothesized genetic groups. The Kings River sample was therefore split up into its constituent tributary samples for subsequent analyses, whereas the others were not. These analyses also identified possible heterogeneity between samples from different tributaries of the upper Yuba and Feather Rivers, although LD was lower in these populations.

Genetic diversity

Genetic diversity was relatively similar throughout the Central Valley. The two most appropriate measures for comparison are allelic richness, which scales the number of alleles by sample size, and observed heterozygosity, which is the proportion of chromosomes in the population with different microsatellite allele sizes. Allelic richness ranged from a low of 6.58 in the American-NF population to a high of 9.32 in Deer Creek (Table 1). Observed heterozygosity ranged from a low of 0.594 in the McCloud River population to 0.716 in Deer Creek. Measures of genetic diversity were compared between populations sampled above and below dams. Mean allelic richness for the above-barrier sites (7.53±0.65) was lower than the mean for the above barrier sites (8.23±0.76), although not significantly so. Observed heterozygosity was also lower in above-barrier populations (0.639±0.021) than in below-barrier populations (0.689±0.022), and the difference was marginally significant. The five hatchery trout strains had the five lowest values observed for any of the groups for both genetic diversity measures (Table 1). This is indicative of the small effective size of these hatchery strains.

Population structure

Phylogeographic trees were used to visually and quantitatively evaluate genetic relationships of Central Valley *O. mykiss* populations both with each other and with other California populations. The chord distance/neighbor-joining tree describing the relationships of the Central Valley populations in their original groupings by sub-basin is found in Figure 3a. To provide a more accurate assessment of the relationships of these populations, another tree was constructed that split the Kings River sample into the two

tributary populations. In addition, since over 10% of the fish from the Stanislaus-Upper population were identified as hatchery rainbow trout by assignment analyses (see below), hatchery fish were removed from that population in this subsequent tree (Figure 3b). Bootstrap analysis was then used to evaluate the support across loci for individual internal branches and the majority-rule bootstrap consensus tree is reported in Figure 3c.

The phylogeographic tree analysis revealed a general lack of clustering of populations by basin of origin. In addition, the short internal branch lengths and low bootstrap support values indicate the great genetic similarity of all the naturally-spawned Central Valley *O. mykiss* populations and the general lack of substantial genetic divergence between them. However, most of the populations from the above-barrier sites clustered together, exclusive of the below-barrier sites, in both the neighbor-joining and bootstrap consensus trees. The only exceptions to this are the intermingling of the Kings-Deer Cove population with the hatchery trout strains and the central position of the Kings-Mill Flat population. In contrast to the Kings River, the two population samples from the upper American River, American-NF and American-MF, were found to be very closely related in all trees. For the below-barrier populations, the American-Lower population sample and the Nimbus Hatchery sample were found to be extremely similar in all trees. The only relationship between populations in different tributary basins that was strongly supported by bootstrap analysis was that between the Battle and Deer Creek populations, although Butte Creek also consistently branches with these two. The other grouping that appeared consistently, although not with strong bootstrap support, is that between the Calaveras River population sample and the Junction Kamloops hatchery strain, possibly indicating some introgression from this strain into Calaveras River steelhead.

Phylogeographic trees constructed with coastal steelhead populations arrayed from southern California (Santa Clara River, Ventura County) to southern Oregon (Rogue River) confirm the general monophyly of Central Valley *O. mykiss* populations both above and below dams (Figure 4a). All of the hatchery strains cluster with Central Valley populations in these trees, which made it necessary to redo these analyses without the hatchery strains, to better evaluate the relationships of these populations with coastal steelhead populations. This tree (Figure 4b) also confirmed the close relationship of all Central Valley populations, and indicated that their closest relationship to coastal populations is with fish from northern California, in the group that includes Eel and Klamath River basins.

Trees were also constructed with Central Valley above-barrier and below-barrier populations separately, to evaluate whether introgression may have affected these populations differently. When only populations from above dams in the Central Valley are included in this regional analysis, the Central Valley group enters the tree closest to the central California and San Francisco Bay groups either with hatchery strains included (Figure 4c), or excluded (Figure 4d), which is what would be expected from a model of pure migration and drift determining population structure. When only populations from below barriers in the Central Valley are included in this regional analysis, they cluster within the northern California group when the hatchery strains are included (Figure 4e) or excluded (Figure 4f). Since Nimbus Hatchery on the American River used broodstock from the Eel River to produce steelhead for many years, this tree was also constructed without American River populations to see if this relationship persists and, if this large-scale importation is completely or partially the cause, to see if Eel River genes spread

beyond the American River by migration. This tree also found that the other below-barrier populations in the Central Valley cluster with northern California populations (tree not shown), indicating either broad introgression of northern California genes into Central Valley steelhead, or previously unknown shared ancestry between the two groups.

The FCA results for these populations were similar to the other analyses. This analysis produces a visual representation of individual genotypes arrayed by principal components of the allele frequency distributions of population samples. FCA results with the hatchery strains included (Figure 5b) found little overlap between the hatchery trout and those collected in all of the sub-basins, both above and below dams. The only exception to this is the Coleman trout strain, which clusters with the naturally-spawning Central Valley populations. Broad differentiation of the Junction Kamloops strain from the other rainbow trout strains is also apparent. Both this analysis and one with only the naturally-spawning populations included (Figure 5a) revealed a close relationship of all Central Valley populations, with subtle differentiation in allele frequencies mainly associated with different population samples, although moderate differentiation of the Tuolumne-Lower population from others was also apparent.

The matrix of pairwise values of F_{ST}, the standardized variance in allele frequencies between populations, was also examined for patterns of population structure (Table 3). These analyses are complicated by the dependence of F_{ST} values on effective population size, so not much inference can be drawn directly from these values, other than relative rates of recent gene flow and/or shared ancestry. The lowest value of F_{ST} observed was actually between Battle and Deer Creeks, which had less than 1% of the genetic variation

partitioned between them, slightly less than between the Nimbus Hatchery and American-Lower population samples and less than half that between the two American River populations above dams.

Individual assignment test analysis found high accuracy of assignment for Central Valley *O. mykiss* populations. The overall accuracy of assignment to population sample of origin was 86.2% (Table 4a). When misassignments to other population samples from the same basin were not considered errors, this increased only slightly to 88.1%. Assignment accuracy for individual populations ranged from 100% for the McCloud River to just below 50% for the American-Lower population, where many fish were assigned to the Nimbus sample, and for the Deer Creek population sample, where one third of misassignments were to Battle Creek, reflecting their close relationship. If probability values are used to apply a 95% confidence exclusion criterion (Table 4b), overall accuracy increases to 94.1%. When interbasin misassignments are not considered errors, this rises to 95.1%, but only 80.6% of fish are assigned. Very few hatchery fish were sampled in any of the sub-basins, with the largest number found in the Stanislaus-Upper population, where 11.5% (6 of 52) fish were identified as hatchery origin trout. Other than in the Nimbus Hatchery sample (see below), only one other fish, in the Yuba-Lower population, was identified as a hatchery fish with high confidence.

The Nimbus Hatchery sample had the highest proportion of hatchery rainbow trout identified, with 14.3% (10 of 70) of fish assigned with high confidence to one of the hatchery trout strains. Moreover, all of these hatchery trout fish were from the 31 adults sampled entering the hatchery in 2005-06. The hatchery trout were generally smaller than the steelhead, but there was significant overlap in the size distributions (Table 5).

Discussion

Population structure and genetic diversity of fish from the species *O. mykiss,* steelhead/rainbow trout, in the Central Valley region of California were analyzed using 18 microsatellite loci. These analyses help to bring population structure into focus for this group of fish, but the long history of hatchery propagation and stocking throughout the region make this a difficult task because of uncertainty about fractional ancestry of specific *O. mykiss* populations in Central Valley sub-basins. In addition, dam construction for the purposes of water diversion, hydropower production and flood control have created barriers to both migration and anadromy for many *O. mykiss* populations and have also disrupted historic patterns of gene flow, that maintained population effective size and linked population and evolutionary dynamics of different steelhead populations.

There was extensive population structure found among the steelhead /rainbow trout population studied here. The great majority of this structure was found at the level of the individual population sample, all of which were significantly differentiated, and much less of the structure was due to associations between populations, or between groups of populations. In fact, the salient characteristic of population structure for Central Valley *O. mykiss* inferred from this study is that the populations of naturally-spawning fish sampled here are all closely related, regardless of whether they are currently above or below barriers to anadromy. This indicates that hatchery rainbow trout planted above dams in the region have not replaced *O. mykiss* populations trapped upstream of dam construction, fish commonly referred to as residualized steelhead.

In phylogeographic analyses, these above-barrier populations are more similar to San Francisco Bay *O. mykiss* populations than the below-barrier populations in the Central Valley. Since this is the relationship expected for steelhead, given their extraordinary historic dependence on short distance migration events (Pearse and Garza 2007b), they may represent relatively non-introgressed historic population genetic structure for the region. Other possible explanations for this pattern that rely on complicated, widespread patterns of introgression with hatchery fish are not entirely ruled out, but are highly improbable given that the above-barrier populations also group with moderate consistency into geographically-consistent clusters (e.g. Yuba-Upper and Feather-Upper) in all analyses and also because of the low apparent reproductive success of hatchery trout in streams throughout California.

Artificial propagation of *O. mykiss* began in the Central Valley more than 125 years ago with the establishment of the Baird Station on the McCloud River, and many billions of fish have been released in Central Valley rivers, streams, lakes and reservoirs since then. This massive propagation and planting effort, much of it sparsely documented, significantly clouds efforts to disentangle residual historic structure from effects of these hatchery rainbow trout and steelhead. For this reason, we included sizable samples of major hatchery rainbow trout strains used at Central Valley hatcheries to both directly detect hatchery fish or their progeny captured in streams where natural spawning occurs, as well as to evaluate their contribution to the ancestry of these naturally-spawned populations.

In general, few hatchery trout were found amongst the population samples, with almost all hatchery trout sampled in two locations, in the Stanislaus-Upper population

and at Nimbus Hatchery. In addition, the population from the Kings River sampled at Deer Cove Creek, clusters with hatchery strains in most analyses, indicating likely hatchery trout ancestry. In addition, the modest differentiation in the Tuolumne-Lower population may be due to past rainbow trout introgression, although it could not have been recent, since there is little linkage disequilibrium and no signal of clustering with hatchery trout strains. However, it should be noted that substantial past introgression by hatchery trout into some or all of these populations can not be ruled out with these microsatellite data, because of the close evolutionary relationship of all *O. mykiss* populations and the lack of diagnostic alleles for hatchery strains. Such inference about amounts of hatchery trout introgression will be possible with novel ancestry-informative haplotype markers currently under development in the PI's laboratory.

Steelhead propagation in the Central Valley also appears to play a big role in determining population structure. Nimbus Hatchery on the American River is a substantial producer of steelhead in the Central Valley. For many years, the source of broodstock for production at Nimbus was Eel River coastal steelhead. This introgression is apparent in the phylogeographic analyses, which groups Nimbus and American-Lower populations with the Eel/Klamath River populations. The clustering together of Central Valley below-barrier populations in all analyses, and the continued clustering of Central Valley below-barrier populations with northern coastal populations when American River below-barrier populations are removed from the analyses, indicate that this out-of-basin broodstock importation appears to have spread Eel River genes to many below-barrier sites in the Central Valley through straying/migration of Nimbus Hatchery steelhead.

Another finding of importance is that over one third of the adult *O. mykiss* entering Nimbus Hatchery that were sampled were identified as hatchery rainbow trout. It is unknown whether these specific fish were actually used as broodstock for steelhead production, but they definitely could have been (G. Edwards, CDFG; pers. comm.). The origin of these trout is unknown, but they might be coming over the dam, or somehow be released from American River Hatchery, which raises the strains to which these fish are assigned. Integration of these trout into steelhead production is likely to have a number of detrimental effects, because of their reduced genetic variation, genetic predisposition against anadromy and past hatchery selection pressures. Such effects may not be restricted to the American River, since straying can, and appears to, move genes from Nimbus around the Central Valley. While these rainbow trout were primarily the smallest fish entering the hatchery, there was great overlap in length, so size alone could not be used as a criterion to restrict hatchery trout from being integrated into steelhead production. Moreover, selection of only the largest fish for breeding purposes would impose strong directional selection on these fish. Genetic broodstock management might be a possible solution to this problem.

Although all study populations were relatively closely related, some population structure was discernible. First, a clear signal of recent, ongoing migration between northern Sacramento Valley streams below barriers was evident, with extremely low and only marginally significant differentiation between Battle and Deer Creek populations. Butte Creek fish also consistently grouped with these fish. This suggests that the northern Sacramento Valley populations experience substantial gene flow between them, or have recently done so, through migration/straying, and that there is reduced gene flow with

populations to the south. Such migration is difficult to estimate directly. However, future parentage-based genetic tagging studies implemented at Coleman and Nimbus Hatcheries, as proposed by the PIs, would help to directly estimate migration rates of these hatchery steelhead to geographically proximate basins.

For the two sub-basins with multiple population samples above a barrier, the Kings and American Rivers, contrasting results were found. The Kings River samples came from two distinct sites, Deer Cove Creek, a very small tributary of the main stem near the entrance to the National Park, and Mill Flat Creek, a larger tributary that branches near Pine Flat Reservoir. The initial observation of high LD in this basin when samples from the two sites were combined was found to be due to distinct genetic composition at the two sites that was evident with multiple analyses. In general, the Deer Cove Creek population was more similar to hatchery strains than the Mill Flat Creek population. An effort was made to determine whether any recent hatchery rainbow trout plantings in the Kings River basin might be a possible explanation, but insufficient information was available.

The American River was also represented by multiple population samples. Two of these were from above Folsom Dam, in the North and Middle Fork sub-basins. These two populations were the second most genetically similar of any two in this study, with F_{ST} value of 0.02, and they also cluster with high confidence in all of the phylogeographic tree analyses, indicating recent gene flow between them. The fish sampled below Folsom Dam came from two sources, Nimbus Hatchery and the lower American River. These two samples also clustered together consistently, in spite of substantial heterogeneity within the fish sampled at Nimbus Hatchery.

The distinction between the Junction Kamloops strain and all other hatchery strains in all analyses is likely due to their ancestry from distinct evolutionary lineages of *O. mykiss*. In addition, the Central Valley and hatchery trout strains are most similar in regional phylogeographic trees, but it is unclear if this is due to the strains primarily deriving from Central Valley *O. mykiss* populations, or whether it is due to greater introgression of hatchery rainbow trout into naturally spawning populations in the Central Valley region than in coastal steelhead populations.

This high accuracy of assignment tests indicates a substantial amount of population differentiation, which is also typical of steelhead populations in the coastal California DPSs. The data from these 18 microsatellite loci and the high accuracy of individual assignment test analyses on even a small scale indicates that these genetic data can be useful as a reference baseline for genetic stock identification techniques to determine basin and tributary of origin for individual trout in management or forensic applications. Care would be required to update reference databases frequently, to account for temporal shifts in allele frequencies due to changing population and family structure that would decrease assignment power.

Finally, these results indicate smaller effective size in above-barrier populations, which is consistent with the expectation of decreased upstream migration and the lost influx of new genes through migration. This situation will lead to gradual genetic erosion, which can contribute to eventual population extirpation (Srikwan and Woodruff 2000). Facilitating upstream migration might help to alleviate such eventual genetic effects, but may also counteract the potential adaptation of above-barrier populations that is expected because of the strong selection against downstream migration in such populations.

Conversely, efforts to integrate above-barrier populations with those below dams to increase overall effective size of steelhead populations and reestablish historical connectivity should also proceed with great caution, as these fish have been under very strong selection against anadromy since dam construction. The consequences of such integration are not known, but could range from beneficial increases in genetic diversity and effective size, to decreased fitness of hybrids and various ecological interactions such as competition or direct predation.

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References cited

Aguilar A, Garza JC (2006) A comparison of variability and population structure for major histocompatibility complex and microsatellite loci in California coastal steelhead (*Oncorhynchus mykiss*). Molecular Ecology 15:923-937.

Banks MA, Blouin MS, Baldwin BA, Rashbrook VK, Fitzgerald HA, Blankenship SM, Hedgecock D (1999) Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281-288.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. (2004) GENETIX 4.05: logiciel sous Windows™ pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000. Université de Montpellier II, Montpellier (France).

Berg WJ, Gall GAE (1988) Gene flow and genetic differentiation among California coastal rainbow trout populations. Canadian Journal of Fisheries and Aquatic Sciences 45:122-131.

Cavalli-Sforza L, Edwards A (1967) Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Chan KMA, Levin SA (2005) Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. Evolution 59:720-729.

Deiner K, Garza JC, Coey R, Girman DJ (2007) Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and manmade-barriers in the Russian River, California. Conservation Genetics 8:437-454.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package), version 3.57c. Department of Genetics, University of Washington, Box 357360, Seattle, Washington, 98105.

Felsenstein J (2003) Inferring Phylogenies. Sinauer Associates, Sunderland MA.

Garza JC, Gilbert-Horvath E, Spence B, Williams TH, Fish H, Gough S, Anderson JH, Hamm D (in review). Population structure of steelhead in coastal California.

McConnell SK, O'Reilly P, Hamilton L, Wright JM, Bentzen P (1995) Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Canadian Journal of Fisheries and Aquatic Sciences 52:1863-1872.

Morris DB, Richard KR, Wright JM (1996) Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic studies of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53:120-126.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nielsen JL, Fountain MC, Wright JM (1997) Biogeographic analysis of Pacific trout (*Oncorhynchus mykiss*) in California and Mexico based on mitochondrial DNA and nuclear microsatellites. In: *Molecular Systematics of Fishes* (ed. Kocher TD, and C.A. Stepien), pp. 53-73. Academic Press, London.

O'Reilly PT, Hamilton LC, McConnell SK, Wright JM (1996) Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53:2292-2298.

Pearse DP, Donohoe C, Garza JC (2007a) Population genetics of steelhead (*Oncorhynchus mykiss*) in the Klamath River. Environmental Biology of Fishes 80:377-387.

Pearse DP, Garza JC (2007b) Historical Baseline for Genetic Monitoring of Coastal California Steelhead, *Oncorhynchus mykiss*. Report on Fishery Restoration Grant Program Grant# P0510530.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536-539.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA 94:9197-9221.

Raymond M, Rousset F (1995) Genepop version 1.2: Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425

Scribner KT, Gust JR, Fields RL (1996) Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:833-841.

Small MP, Beacham TD, Withler RE, Nelson RJ (1998) Discrimination of coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia using microsatellite DNA markers. Molecular Ecology 7:141-155.

Srikwan S, Woodruff DS (2000) Genetic erosion in isolated small mammal populations following rain forest fragmentation. In: Genetics, Demography and Viability of

Fragmented Populations. Young, A. & G. Clarke, eds. Cambridge University Press, Cambridge. Pp. 149-172.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389-399.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population-structure. Evolution 38:1358-1370.

Williamson, KS, JF Cordes and BP May. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2:17-19.

Figure legends:

Figure 1: Geographic information system maps of California Central Valley area stream system with sampling localities indicated. Battle Creek location not shown. Maps provided by George Edwards, California Department of Fish and Game.

Figure 2: Graphical results of model-based clustering method implemented in *structure* (Pritchard et al. 2000) for various hypotheses about the number of genetic clusters, K, and without geographic or population information. Each color represents fractional ancestry for one of the proposed clusters. Three runs of the Markov chain Monte Carlo method are shown for a large value of K (10) to show variability in population relationships inferred using such a method with closely related populations.

Figure 3: Phylogeographic trees of Central Valley *O. mykiss* populations a) neighbor-joining tree constructed with chord distances and original sub-basin groupings, b) neighbor-joining/chord distance tree with the Kings River split and hatchery trout removed from the Stanislaus-Upper population, c) majority rule bootstrap consensus tree from 1000 bootstrap replicates with same population groupings as b.

Figure 4: Phylogeographic trees of Central Valley *O. mykiss* populations with coastal steelhead populations, a) neighbor-joining tree constructed with chord distances and all Central Valley populations with hatchery trout strains, b) and without hatchery strains, c), above-barrier populations and hatchery trout only, d) above-barrier populations only, e) below-barrier populations and hatchery strains only and f) below-barrier populations only. Fourteen loci.

Figure 5: Factorial correspondence analysis of individual genotypes from 18 microsatellite loci for all Central Valley populations from this study with a), and without b), hatchery strains.

Sub-basin	Population	Age/strain	Sample size	Exp. Hz	Obs. Hz	N_a	A_r	LD
McCloud	Above-ButcherKnife/Claiborne Cks	Mixed age residents	54	0.6661	0.5939	8.78	6.67	42.5
Battle	Below-Coleman	Adults anadromous	180	0.7200	0.6991	15.44	9.00	6.6
Deer	Below-Main	Mixed age	46	0.7371	0.7162	13.06	9.32	2.6
Butte	Below-Main	Mixed age	52	0.7288	0.7064	12.39	8.63	5.2
Feather	Above-Rice/Chips Cks	Mixed age residents	52	0.7123	0.6296	11.94	8.39	9.8
Yuba	Below-Main	Mixed age	107	0.7145	0.6864	13.17	8.43	7.8
Yuba	Above-North Fork	Mixed age residents	51	0.7077	0.6657	10.94	7.79	20.3
American	Below-Nimbus	Juveniles/adults	102	0.7113	0.6607	12.82	8.36	46.3
American	Below-Main	Juveniles	19	0.6991	0.6731	7.82	7.59	3.7
American	Above-Middle Fork	Mixed age residents	60	0.7190	0.6563	11.00	7.98	8.1
American	Above-North Fork	Mixed age residents	51	0.6722	0.6473	8.83	6.58	4.1
Calaveras	Below-Main	Mixed age	48	0.7012	0.7010	9.50	7.40	3.3
Stanislaus	Below-Main	Mixed age	92	0.7226	0.7023	12.56	8.29	8.5
Stanislaus	Above-Middle Fork	Mixed age residents	52	0.6893	0.6360	11.33	7.89	10.5
Tuolumne	Below-Main	Mixed age	127	0.6980	0.6545	10.44	7.01	30.1
Tuolumne	Above-Main, Cherry Ck	Mixed age residents	47	0.7170	0.6379	10.94	7.76	5.9
Kings	Above-Deer Cove/Mill Flat Cks	Mixed age residents	59	0.6686	0.6434	9.83	7.22	66.7
American	American River Hatchery	Eagle-juveniles	50	0.5957	0.5904	5.24	4.51	13.3
American	AmericanRH/HotCreekH	Coleman-juveniles	85	0.6050	0.5860	7.22	5.31	7.5
American	American River Hatchery	Moccasin-juveniles	55	0.6122	0.5820	5.65	4.89	11.8
American	American RH/Mt.ShastaH	Mt.Shasta-juveniles	120	0.5978	0.5648	6.28	4.51	7.5
Hot Creek	Hot Creek Hatchery	Kamloops-juveniles	50	0.6112	0.5872	7.59	5.88	6.7
Total			1559					

Table 1: Sample data and summary statistics for Central Valley trout genotyped as part of this study. Population samples are classified by whether samples were taken above or below known barriers to anadromy. Exp. Hz is expected heterozygosity. Exp. Hz is expected heterozygosity.Obs. Hz is observed heterozygosity. N_a is observed number of alleles. A_r is allelic richness. LD is linkage (gametic phase) disequilibrium estimated as the proportion of locus pairs with significant non-random associations.

Locus	Primer sequences (5'-3')	No. of Alleles	Range (bp)	Reference
Oki23	F-TGTGCTATAGGGTGAATGTGC	21	118-210	Spidle et al. unpublished,
	R-AACACAGGCATCCCCACTAA			GenBank AF272822
Omy1011	F-AACTTGCTATGTGAATGTGC	26	136-260	Spies et al. unpublished,
	R-GACAAAAGTGACTGGTTGGT			GenBank AY518334
Omy27	F-TTTATGGCTGGCAACTAATGT	7	97-109	McConnell et al. 1995
	R-TTTATGTCATGTCAGCCAGTG			
Omy77	F-CGTTCTCTACTGAGTCAT	21	80-140	Morris et al. 1996
	R- GTCTTTAAGGCTTCACTGCA			
One11	F-GTTTGGATGACTCAGATGGGACT	7	114-124	Scribner et al. 1996
	R-CCTGCTGCCAACACTGTCAA*			
One13	F-TCATACCCCATGCCTCTTCTGTT	20	206-248	Scribner et al. 1996
	R-GGGTGGAGAGACAGGTATCTTGTC*			
Ots1	F-TAGCGTTCACCTGGATTCCC	13	201-293	Banks et al. 1999
	R-CATGCTATTTCCAGACGGCA*			
OtsG3	F-GGACAGGACCGTCTGCTAAATGACTG	19	139-243	Williamson et al. 2002
	R-GGATGGATTGATGAATGGGTGGG			
OtsG43	F-AACTCCCGTTGACAATTTACTGTTG	15	145-209	Williamson et al. 2002
	R-TTTTGGCAAAGTTGGCTACTCTG			
OtsG85	F-CCATGTCAGCACTGACTTAAT	35	129-285	Williamson et al. 2002
	R-GGATGTTGTTCCTAATGTTTT			
Ots103	F-AGGCTCTGGGTCCGTG	6	58-92	Small et al. 1998
	R-TGATATGGTGTGATAGCTGG			
OtsG243	F-TTATTAAACTGCACTGTCTAACTACA	5	107-117	Williamson et al. 2002
	R-GTATGCAGCAAGCCAGGTG			
OtsG249	F-ATGGCAGTTAAGAGAACAAAAGTT*	22	147-243	Williamson et al. 2002
	R-GTACAACCCCTCTCACCTACCC			
OtsG253	F-CGCTGCAGAAACATTTTCGA*	25	165-269	Williamson et al. 2002
	R-AATTGGGTCATTAAGGCTCTGTGG			
OtsG401	F-CTGCCCTGAGAAGCTGGAGTGCTC	20	165-249	Williamson et al. 2002
	R-TTGCCCCACCCTTGCATCTATCCA			
OtsG409	F-GTAGCCATTTGTGTCACCATCATT	3	86-90	Williamson et al. 2002
	R-CATTCTCCTGCCTCACAGAGTTTA			
Ssa85	F-AGGTGGGTCCTCCAAGCTAC	21	96-157	O'Reilly et al. 1996
	R-ACCCGCTCCTCACTTAATC			
Ssa289	F-CTTTACAAATAGACAGACT	10	105-125	McConnell et al. 1995
	R-TCATACAGTCACTATCATC			

Table 2: Eighteen microsatellite lociused to genotype *Oncorhynchus mykiss* in this study. Primer sequences, total number of alleles and range in allele size observed in the study populations is included, as is the reference for the original description. *Indicates primer was redesigned from original reference sequence for optimizatio purposes; Note that Banks et al. (1999) contains incorrect primer sequences.

F_{ST}	Battle	Deer	Butte	Feather-Upper	Yuba-Lower	Yuba-Upper	American-Nimbus	American-Lower	American-MiddleF	American-NorthF	Calaveras	Stanislaus-Lower	Stanislaus-Upper	Tuolumne-Lower	Tuolumne-Upper	Kings-DeerCove	Kings-UpMillFlat	Coleman	Eagle	MtShasta	Moccasin	JunctionKamloops
McCloud	0.0659	0.0561	0.0801	0.0889	0.0933	0.0780	0.0927	0.0967	0.0931	0.1097	0.1164	0.0712	0.0912	0.0916	0.0955	0.1582	0.0857	0.1172	0.1585	0.1726	0.1420	0.1918
Battle	---------	0.0099	0.0337	0.0296	0.0475	0.0418	0.0392	0.0484	0.0480	0.0663	0.0597	0.0228	0.0549	0.0457	0.0599	0.0970	0.0547	0.0551	0.1064	0.1303	0.0901	0.1274
Deer	---------	---------	0.0282	0.0344	0.0468	0.0366	0.0424	0.0480	0.0429	0.0629	0.0653	0.0271	0.0550	0.0458	0.0598	0.1081	0.0446	0.0744	0.1161	0.1332	0.0878	0.1373
Butte	---------	---------	---------	0.0441	0.0629	0.0354	0.0416	0.0452	0.0581	0.0772	0.0662	0.0436	0.0660	0.0579	0.0673	0.1241	0.0550	0.0998	0.1167	0.1379	0.1058	0.1373
Feather-Upper	---------	---------	---------	---------	0.0599	0.0501	0.0308	0.0379	0.0332	0.0521	0.0440	0.0311	0.0530	0.0488	0.0644	0.1076	0.0652	0.0693	0.1341	0.1593	0.1258	0.1370
Yuba-Lower	---------	---------	---------	---------	---------	0.0376	0.0642	0.0863	0.0415	0.0695	0.0729	0.0501	0.0802	0.0568	0.0690	0.1252	0.0763	0.1047	0.0892	0.1562	0.1037	0.1361
Yuba-Upper	---------	---------	---------	---------	---------	---------	0.0485	0.0593	0.0531	0.0679	0.0672	0.0424	0.0623	0.0569	0.0592	0.1187	0.0536	0.0963	0.0976	0.1548	0.1025	0.1257
American-Nimbus	---------	---------	---------	---------	---------	---------	---------	0.0104	0.0586	0.0713	0.0403	0.0354	0.0583	0.0506	0.0596	0.1106	0.0582	0.0789	0.1212	0.1374	0.1056	0.1211
American-Lower	---------	---------	---------	---------	---------	---------	---------	---------	0.0647	0.0829	0.0607	0.0424	0.0680	0.0705	0.0597	0.1492	0.0793	0.1054	0.1668	0.1782	0.1347	0.1593
American-MiddleF	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0255	0.0629	0.0420	0.0601	0.0600	0.0664	0.1422	0.0741	0.0990	0.1377	0.1613	0.1323	0.1513
American-NorthF	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0914	0.0565	0.0716	0.0795	0.0847	0.1602	0.0919	0.1163	0.1753	0.1814	0.1671	0.1821
Calaveras	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0546	0.0749	0.0623	0.0873	0.1317	0.0711	0.0930	0.1421	0.1773	0.1296	0.1169
Stanislaus-Lower	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0539	0.0398	0.0535	0.0957	0.0637	0.0644	0.1106	0.1456	0.0952	0.1380
Stanislaus-Upper	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0665	0.0794	0.1289	0.0690	0.0932	0.1544	0.1757	0.1422	0.1646
Tuolumne-Lower	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.0645	0.1179	0.0618	0.0886	0.1222	0.1601	0.1193	0.1322
Tuolumne-Upper	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1447	0.0958	0.1275	0.1431	0.1964	0.1335	0.1715
Kings-DeerCove	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1307	0.1214	0.1618	0.2025	0.1798	0.2156
Kings-UpMillFlat	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1086	0.1365	0.1606	0.1354	0.1441
Coleman	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1626	0.1812	0.1444	0.1765
Eagle	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1585	0.1134	0.1956
MtShasta	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1336	0.1937
Moccasin	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	0.1750

Table 3: Pairwise values of F_{ST}, the standardized variance in allele frequencies between populations, for the 18 "population" samples from this study and five hatchery rainbow trout strains.

TruePopulation	McCloud	Battle	Deer	Butte	Feather-Upper	Yuba-Lower	Yuba-Upper	American-Lower	American-MiddleF	American-NorthF	American-Nimbus	Calaveras	Stanislaus-Lower	Stanislaus-Upper	Tuolumne-Lower	Tuolumne-Upper	Kings-DeerCove	Kings-UpMillFlat	Coleman	MtShasta	Eagle	JunctionKamloops	Moccasin	Sample Size	Assign. Accuracy	Number assigned
McCloud	54																							54	100.0	54
Battle	1	143	12	3	2	5					5	1	4	1	1		2							180	79.4	143
Deer	1	15	22	2	1	1			2				2											46	47.8	22
Butte		3	2	42	1	1							1		1					1				52	80.8	42
Feather-Upper		1			44	3			1		1		2											52	84.6	44
Yuba-Lower		5		1	2	86		2	1		1	2	3							1				104	82.7	86
Yuba-Upper		1	1			1	42		2				3	1										51	82.4	42
American-Lower		1				1		9			8													19	47.4	9
American-MiddleF		1	1		1	3			50	3														59	84.7	50
American-NorthF						3			5	43														51	84.3	43
American-Nimbus		1				2		11			72		4		2					7		3		102	70.6	72
Calaveras												47	1											48	97.9	47
Stanislaus-Lower		3				3			1		1	2	76		1				1					88	86.4	76
Stanislaus-Upper					1								1	44					2	1	3			52	84.6	44
Tuolumne-Lower		1									1	1	3		116									122	95.1	116
Tuolumne-Upper		1	1				1		2				1	3		38								47	80.9	38
Kings-DeerCove			1														31	1						33	93.9	31
Kings-UpMillFlat	1	3									1			1				20						26	76.9	20
Coleman		1							1									1	82					85	96.5	82
MtShasta																			1	114				115	99.1	114
Eagle																					50			50	100.0	50
JunctionKamloops																						50		50	100.0	50
Moccasin																				2			52	54	96.3	52
																			Total					1540		1327
																			Percent accuracy							**86.2**

Table 4a: Matrix of individual genotypic assignments for all fish in the study, with 5 Hatchery trout strains included as possible populations of origin. Rows represent the assigned population of origin for each fish from each populations and the columns represent all fish assigned to a given population. The most likely population of origin is always reported, even if the probability is low. Colors represent intrabasin assignments.

TruePopulation	McCloud	Battle	Deer	Butte	Feather-Upper	Yuba-Lower	Yuba-Upper	American-Lower	American-MiddleF	American-NorthF	American-Nimbus	Calaveras	Stanislaus-Lower	Stanislaus-Upper	Tuolumne-Lower	Tuolumne-Upper	Kings-DeerCove	Kings-UpMillFlat	Coleman	MtShasta	Eagle	JunctionKamloops	Moccasin	Sample Size	Assign. Accuracy	Number assigned
McCloud	54																							54	100.0	54
Battle		115	5		1						2							1						124	92.7	115
Deer	1	6	11	1					1															20	55.0	11
Butte				42																				42	100.0	42
Feather-Upper					39	1			1		1													42	92.9	39
Yuba-Lower		2				72		1	1											1				77	93.5	72
Yuba-Upper		1					33		1															35	94.3	33
American-Lower								5			3													8	62.5	5
American-MiddleF			1			1			39	1														42	92.9	39
American-NorthF									1	36														37	97.3	36
American-Nimbus		1				1		5			51		1		1					7		3		70	72.9	51
Calaveras												45												45	100.0	45
Stanislaus-Lower		1									1	1	56											59	94.9	56
Stanislaus-Upper					1								1	41					2	1	3			49	83.7	41
Tuolumne-Lower											1	1			114									116	98.3	114
Tuolumne-Upper									1					1		36								38	94.7	36
Kings-DeerCove																	30	1						31	96.8	30
Kings-UpMillFlat		1																19						20	95.0	19
Coleman		1																	73					74	98.6	73
MtShasta																			1	107				108	99.1	107
Eagle																					50			50	100.0	50
JunctionKamloops																						50		50	100.0	50
Moccasin																							51	51	100.0	51
																				Total				1242		1169
																				Percent accuracy						**94.1**

Table 4b: Matrix of individual genotypic assignments as in 4a, but with a 95% probability criterion applied. Only confident assignments reported

Fork Length (mm)	Assigned pop-1	Probability	Assigned pop-2	Probability
315	MtShasta	100		
360	MtShasta	100		
445	Moccasin	100		
505	MtShasta	100		
550	AmLo	99.232	TuolLo	0.397
550	Moccasin	100		
550	Moccasin	100		
560	StanLo	96.273	YubaUp	3.688
560	StanLo	89.765	Nimbus	9.127
565	MtShasta	100		
585	AmLo	95.29	Nimbus	4.696
590	Nimbus	99.978	Cala	0.021
590	Nimbus	61.88	YubaLo	37.664
630	MtShasta	99.999	Moccasin	0.001
630	MtShasta	100		
640	Battle	99.886	Butte	0.08
660	Nimbus	92.765	AmLo	7.229
660	Nimbus	96.109	AmLo	3.075
685	Nimbus	98.282	StanLo	1.636
690	MtShasta	97.978	Eagle	2.018
700	AmLo	69.723	Nimbus	15.561
700	Nimbus	99.999	YubaLo	0.001
710	Nimbus	99.138	AmLo	0.794
720	Nimbus	99.999	AmLo	0.001
735	YubaLo	79.1	Nimbus	20.684
740	Nimbus	90.124	AmLo	9.876
740	Nimbus	88.46	AmLo	11.54
760	Nimbus	99.999	AmLo	0.001
770	Nimbus	97.271	AmLo	2.728
800	Nimbus	99.998	StanLo	0.002
810	Nimbus	98.756	AmLo	1.232

Hatchery trout

Table 5: Size, measured by fork length, and assignment test results from 31 adult *O. mykiss* entering Nimbus Hatchery and possibly used as broodstock for steelhead production.

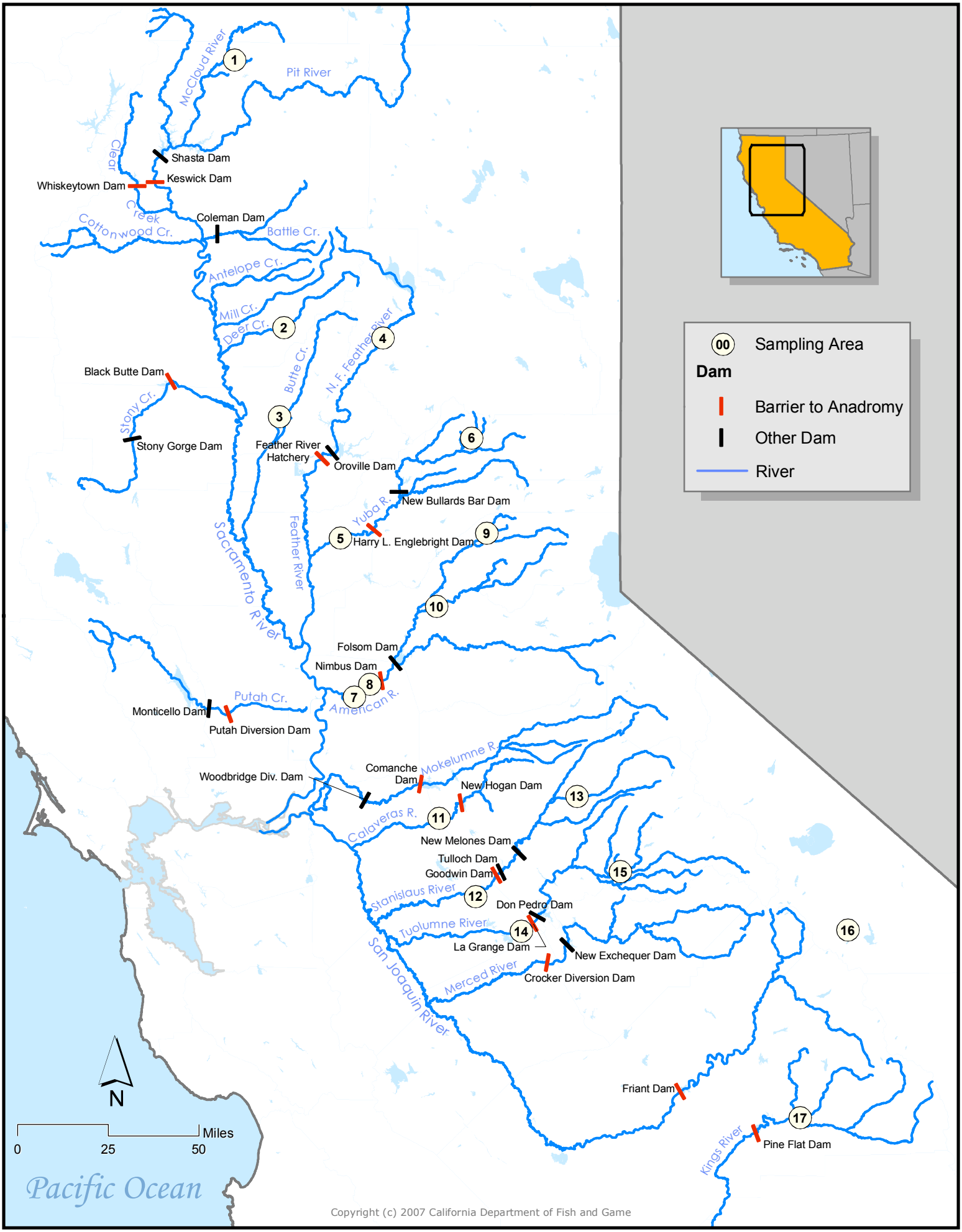
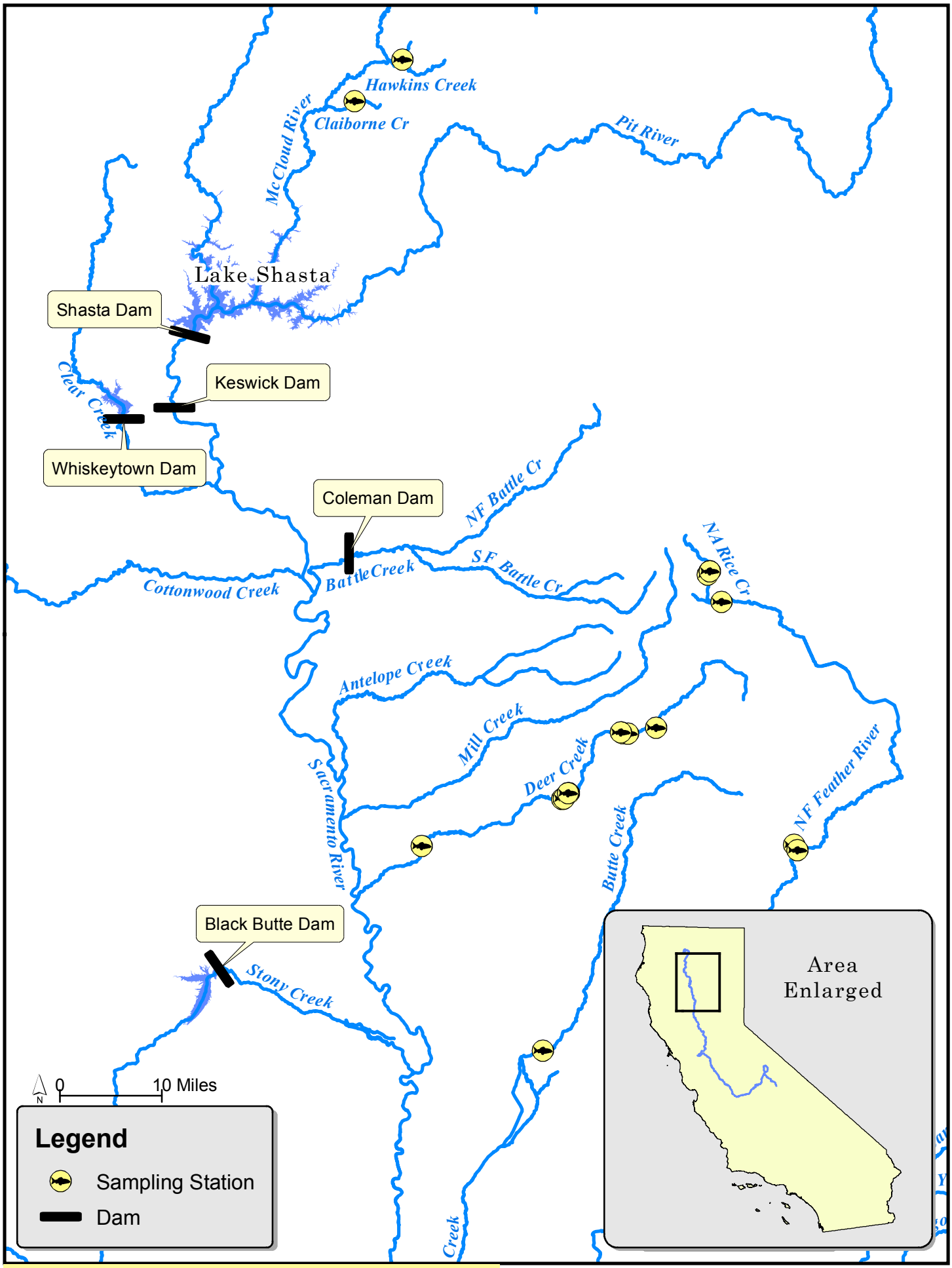
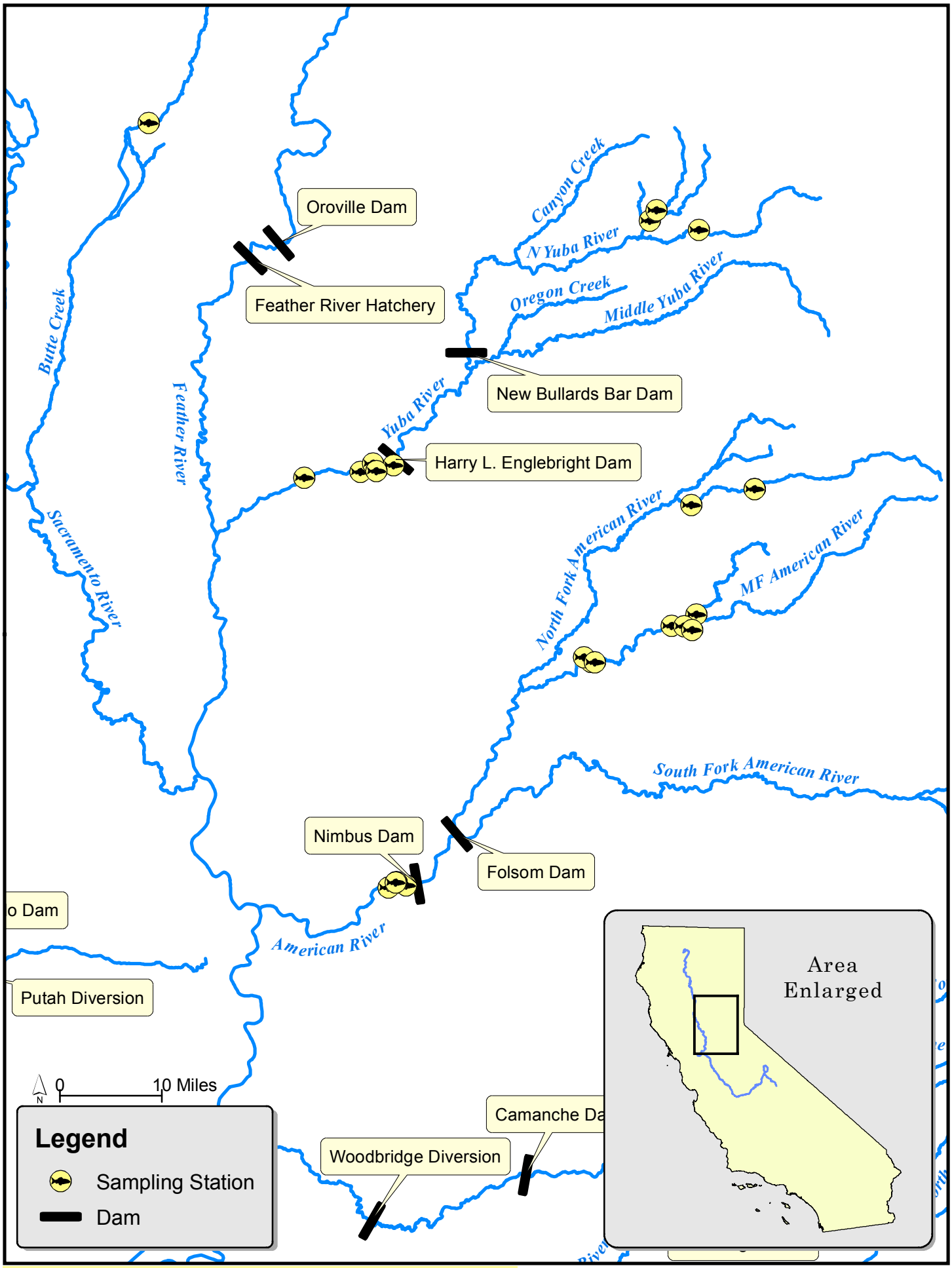


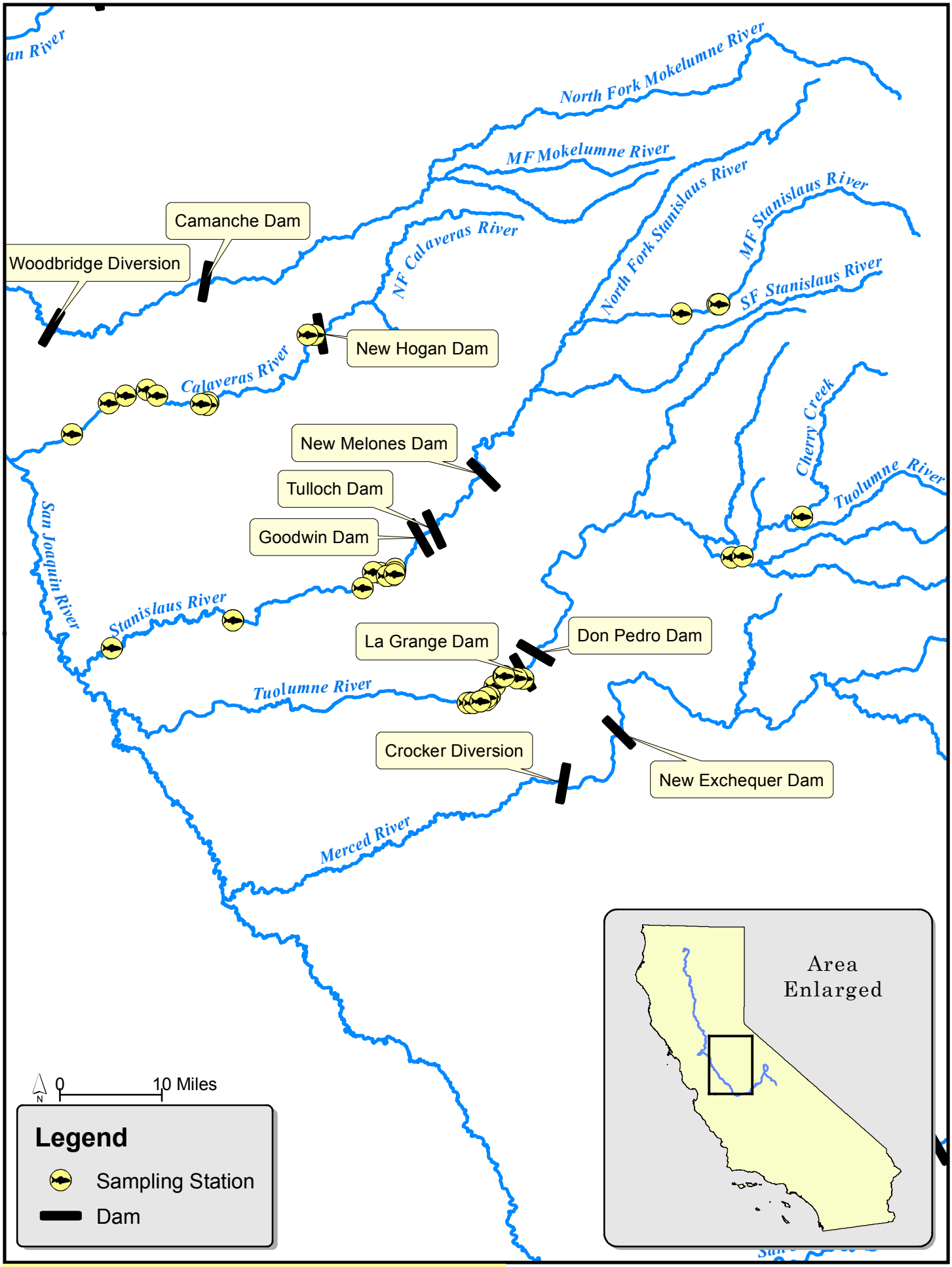
Figure 1. Central Valley Sampling Areas



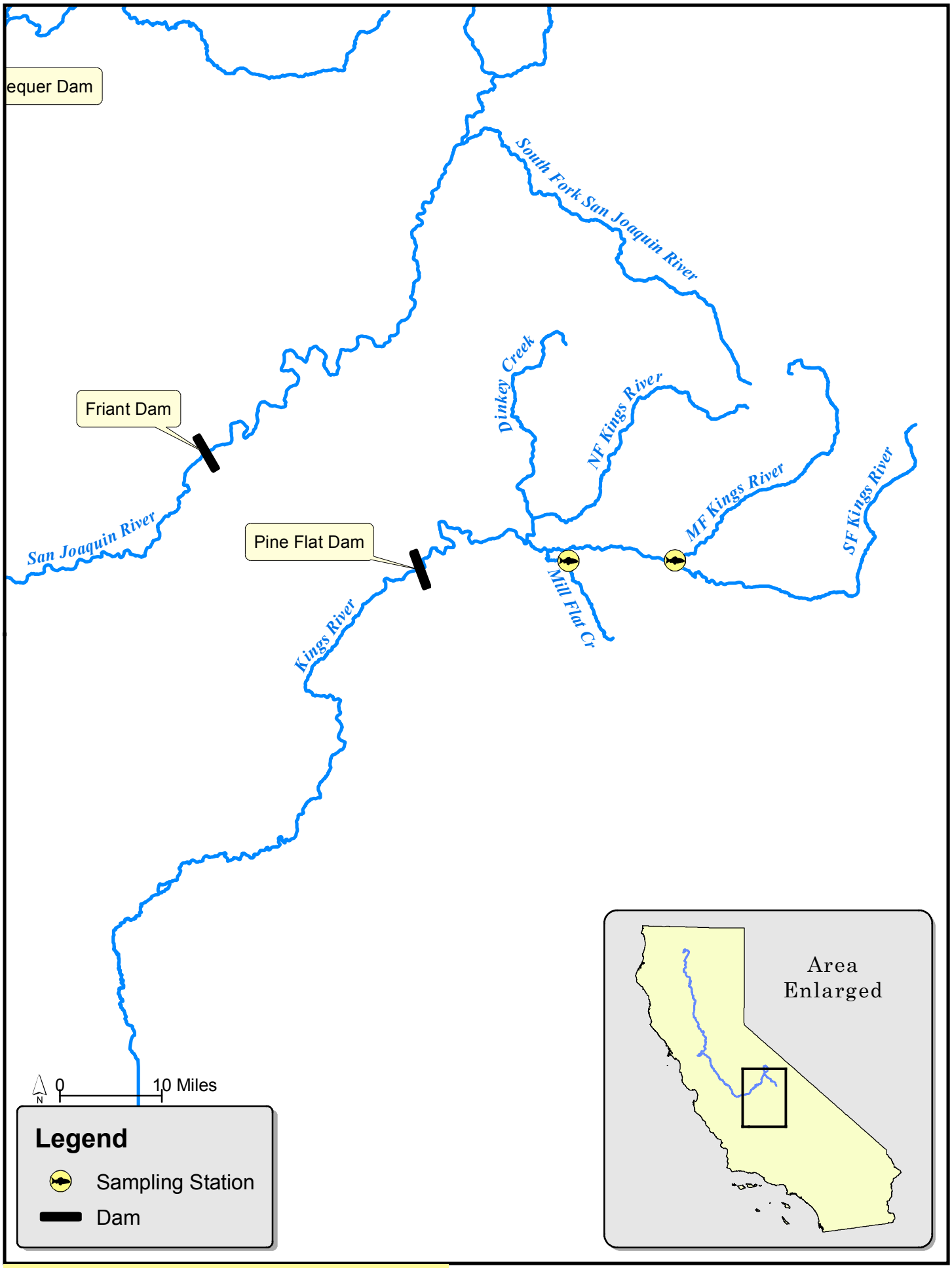
Northern section



North Central section



South Central section



Southern section

Figure 2

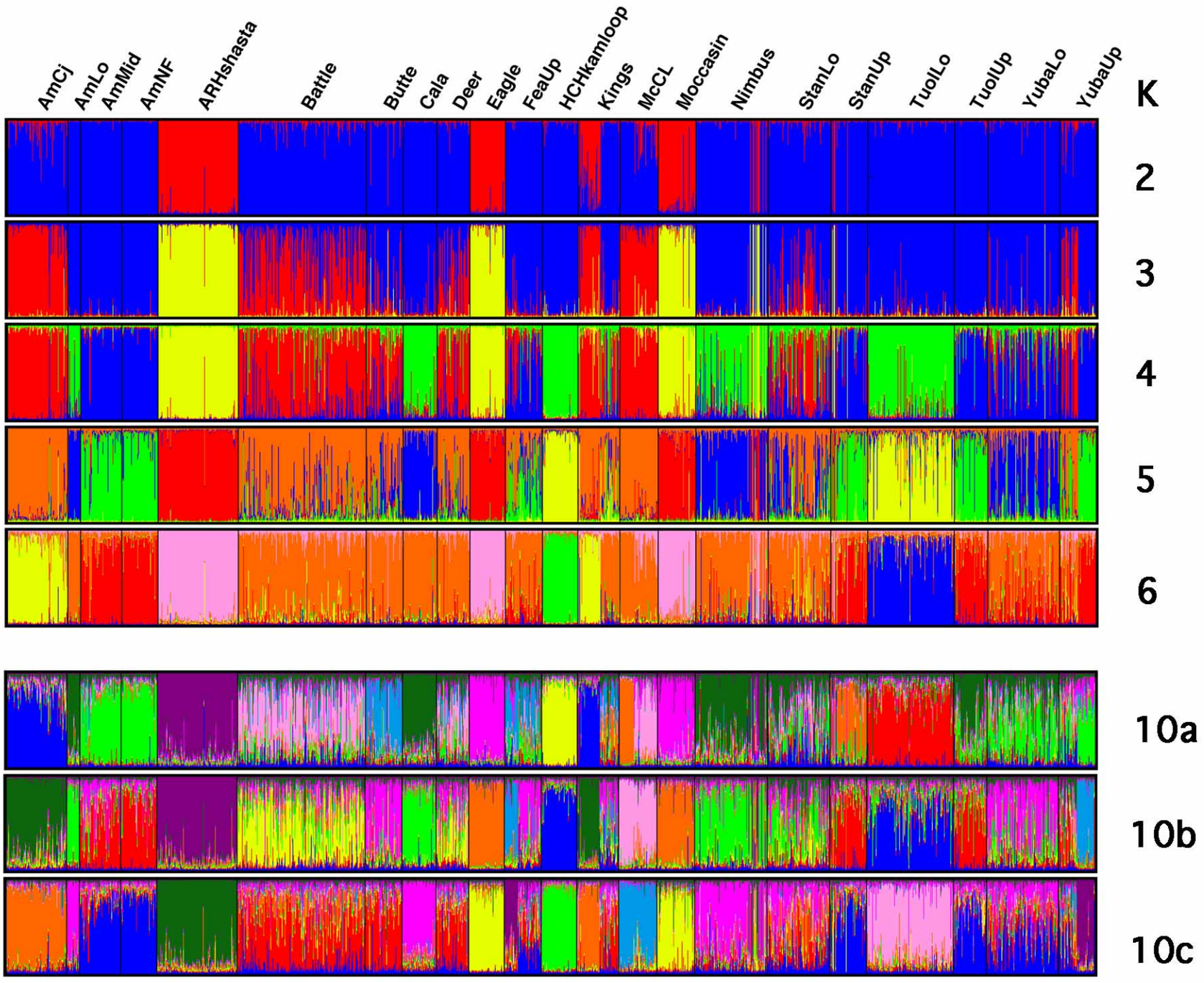

AmCj
AmLo
AmMid
AmNF
ARHshasta
Battle
Butte
Cala
Deer
Eagle
FeaUp
HCHkamloop
Kings
McCL
Moccasin
Nimbus
StanLo
StanUp
TuolLo
TuolUp
YubaLo
YubaUp
K
2
3
4
5
6
10a
10b
10c

Figure 3a

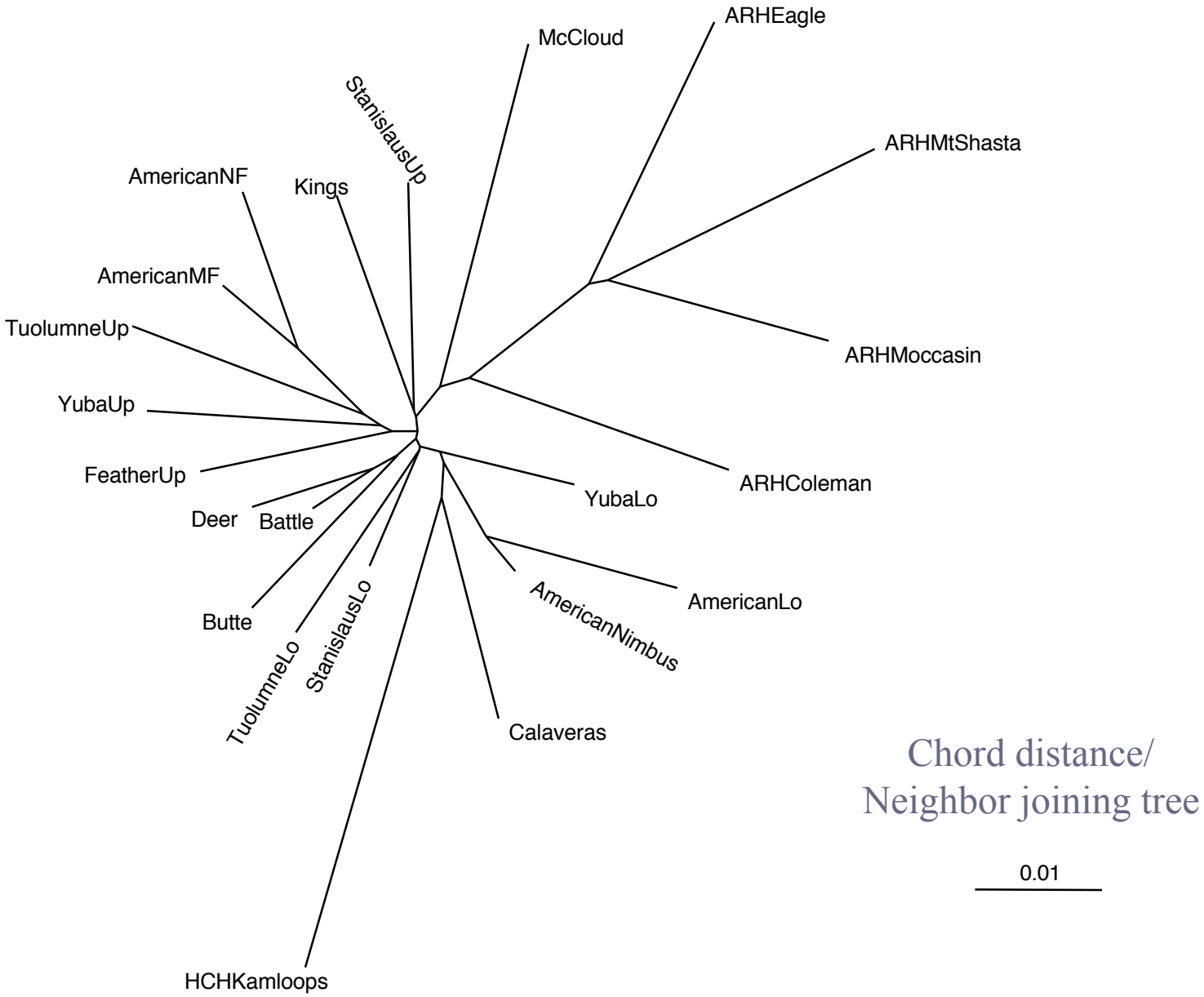

ARHEagle
McCloud
StanislausUp
ARHMtShasta
AmericanNF
Kings
AmericanMF
TuolumneUp
ARHMoccasin
YubaUp
ARHColeman
FeatherUp
YubaLo
Deer
Battle
AmericanLo
AmericanNimbus
Butte
TuolumneLo
StanislausLo
Calaveras
HCHKamloops
Chord distance/
Neighbor joining tree
0.01

Figure 3b

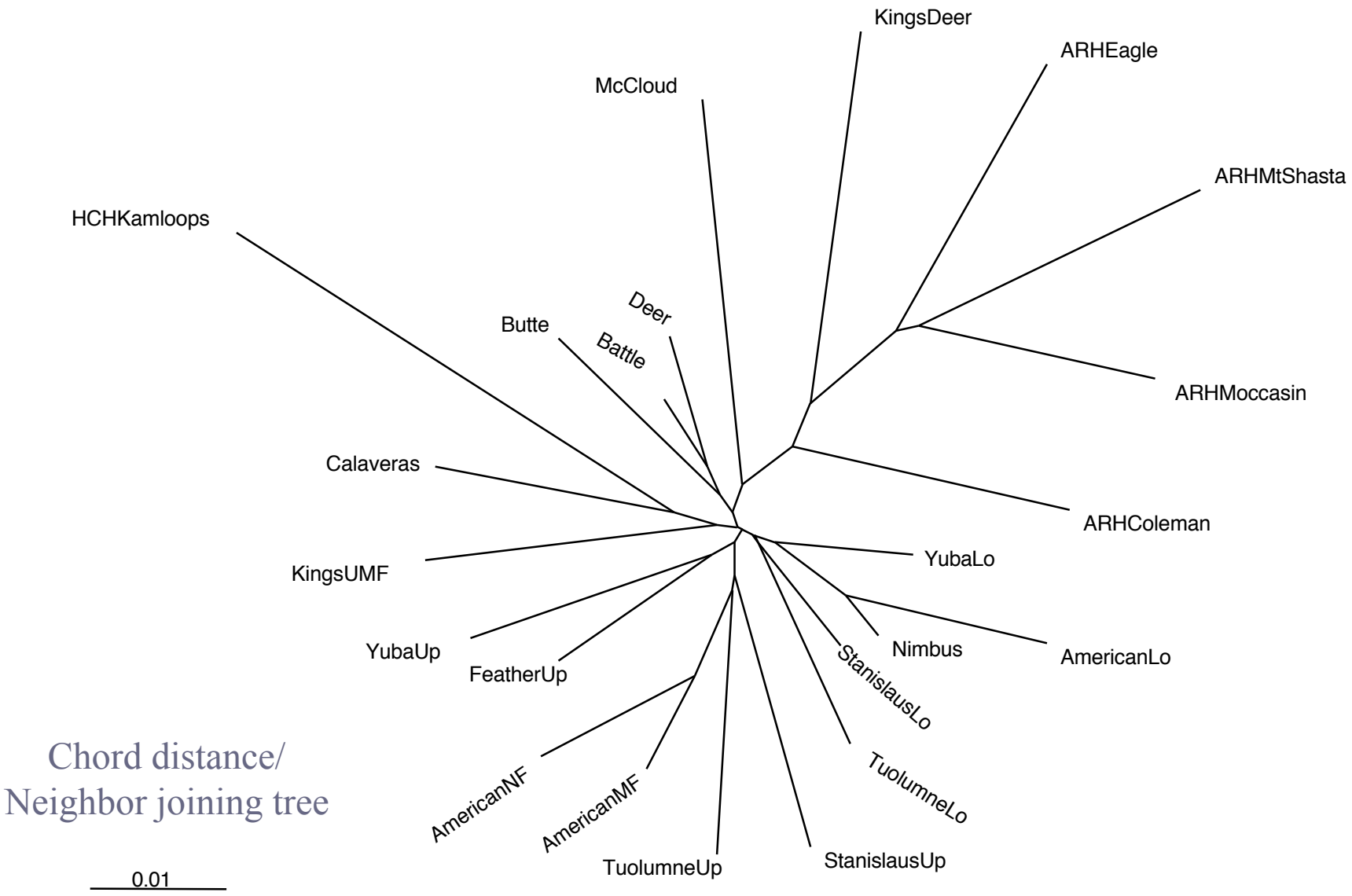
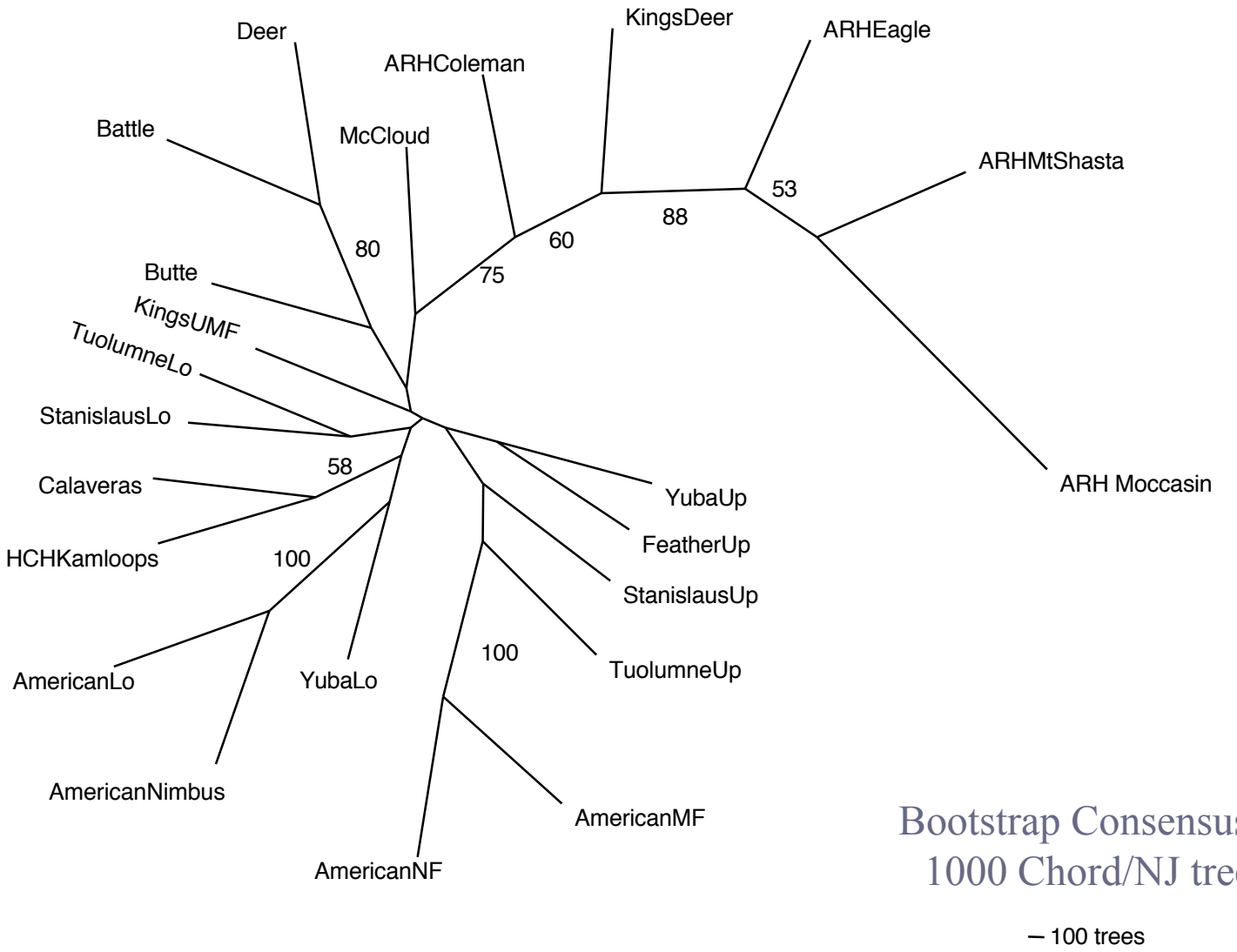

KingsDeer
ARHEagle
McCloud
ARHMtShasta
HCHKamloops
Deer
Butte
Battle
ARHMoccasin
Calaveras
ARHColeman
YubaLo
KingsUMF
YubaUp
Nimbus
AmericanLo
FeatherUp
StanislausLo
TuolumneLo
AmericanNF
AmericanMF
TuolumneUp
StanislausUp
Chord distance/
Neighbor joining tree
0.01

Figure 3c


Deer
ARHColeman
KingsDeer
ARHEagle
Battle
McCloud
ARHMtShasta
53
88
80
60
75
Butte
KingsUMF
TuolumneLo
StanislausLo
58
Calaveras
YubaUp
FeatherUp
HCHKamloops
100
StanislausUp
100
TuolumneUp
AmericanLo
YubaLo
AmericanNimbus
AmericanMF
AmericanNF
ARH Moccasin
Bootstrap Consensus of
1000 Chord/NJ trees
100 trees

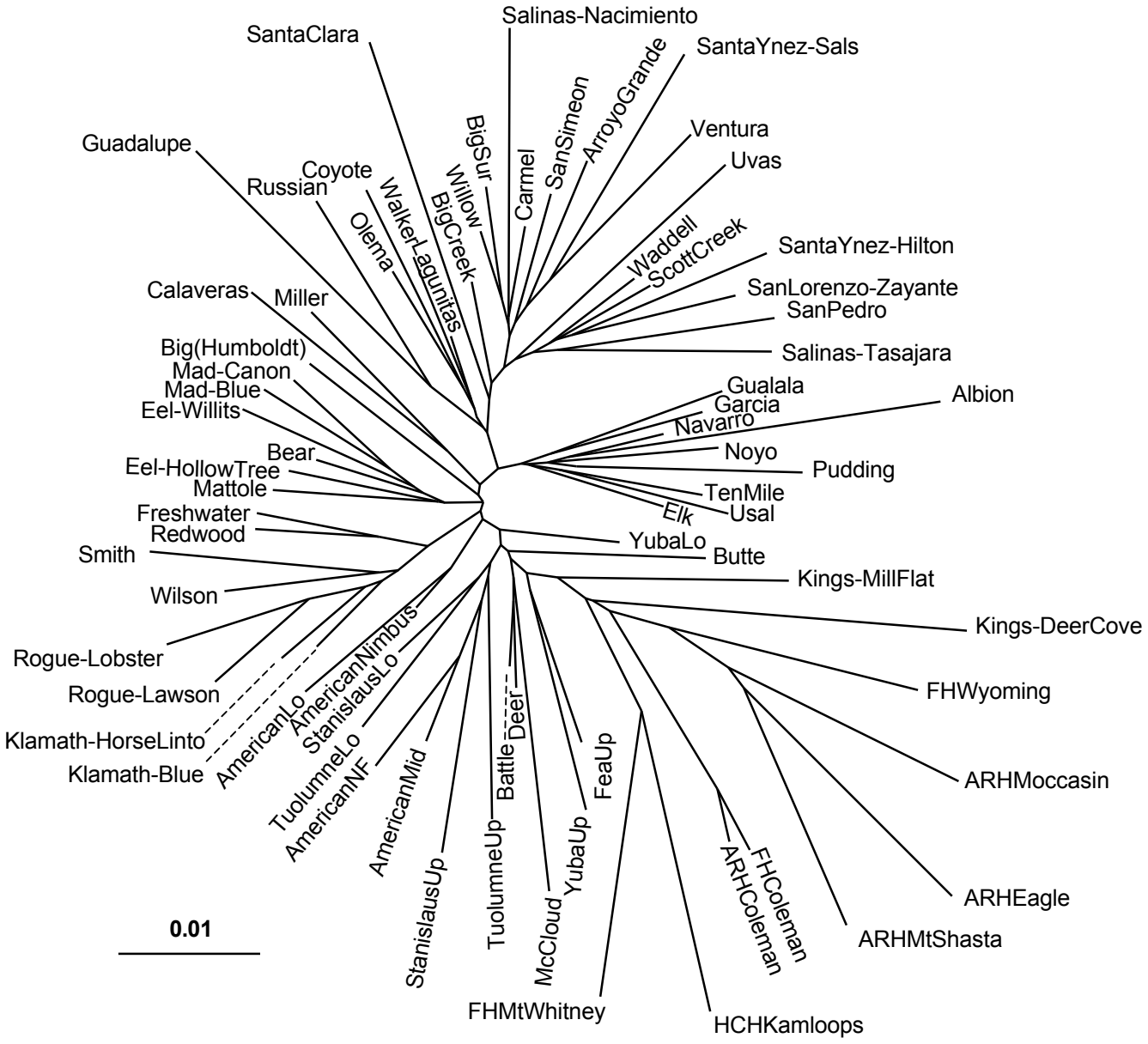


Figure 4a) All CV pops

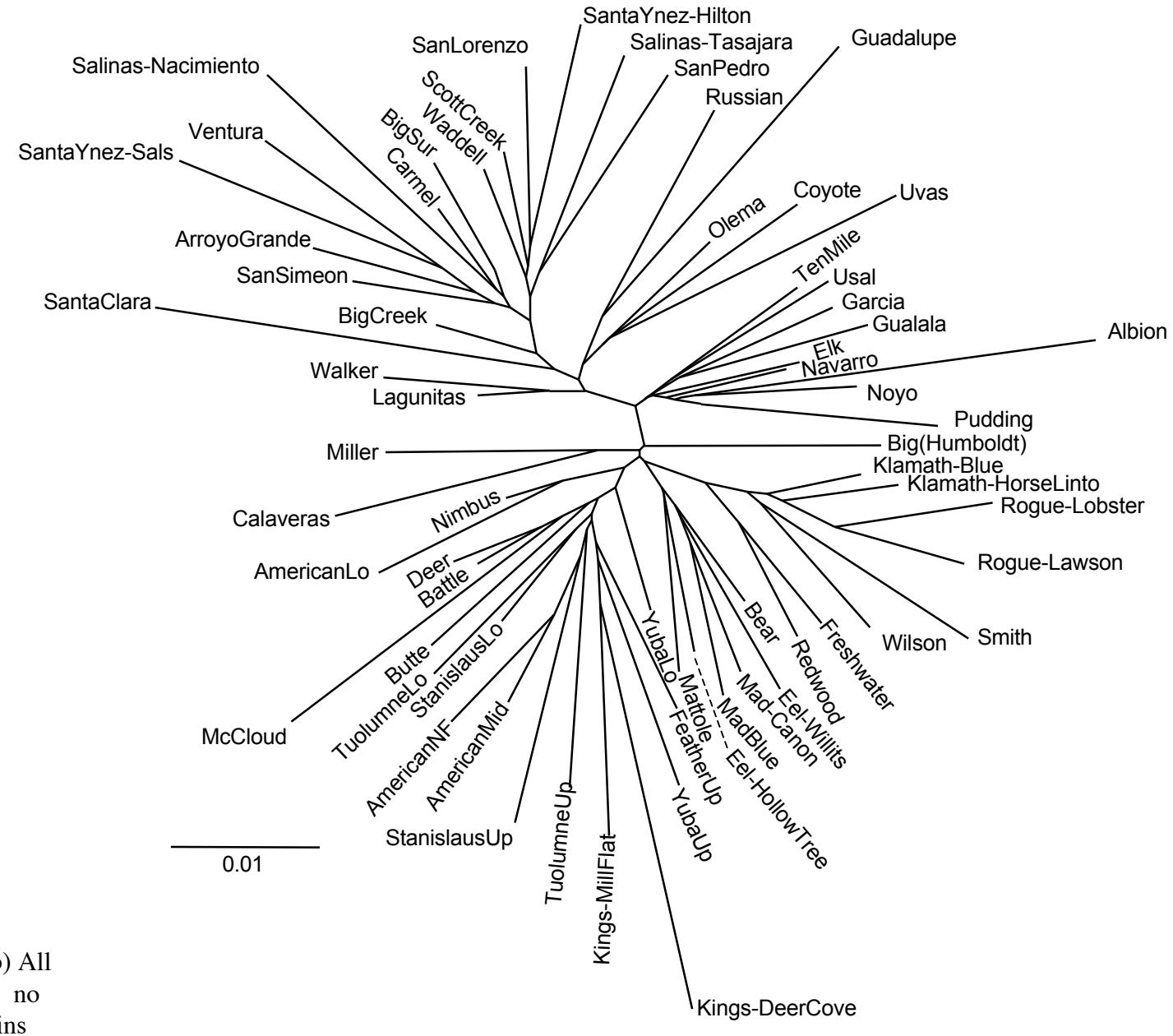


Figure 4b) All CV pops, no trout strains

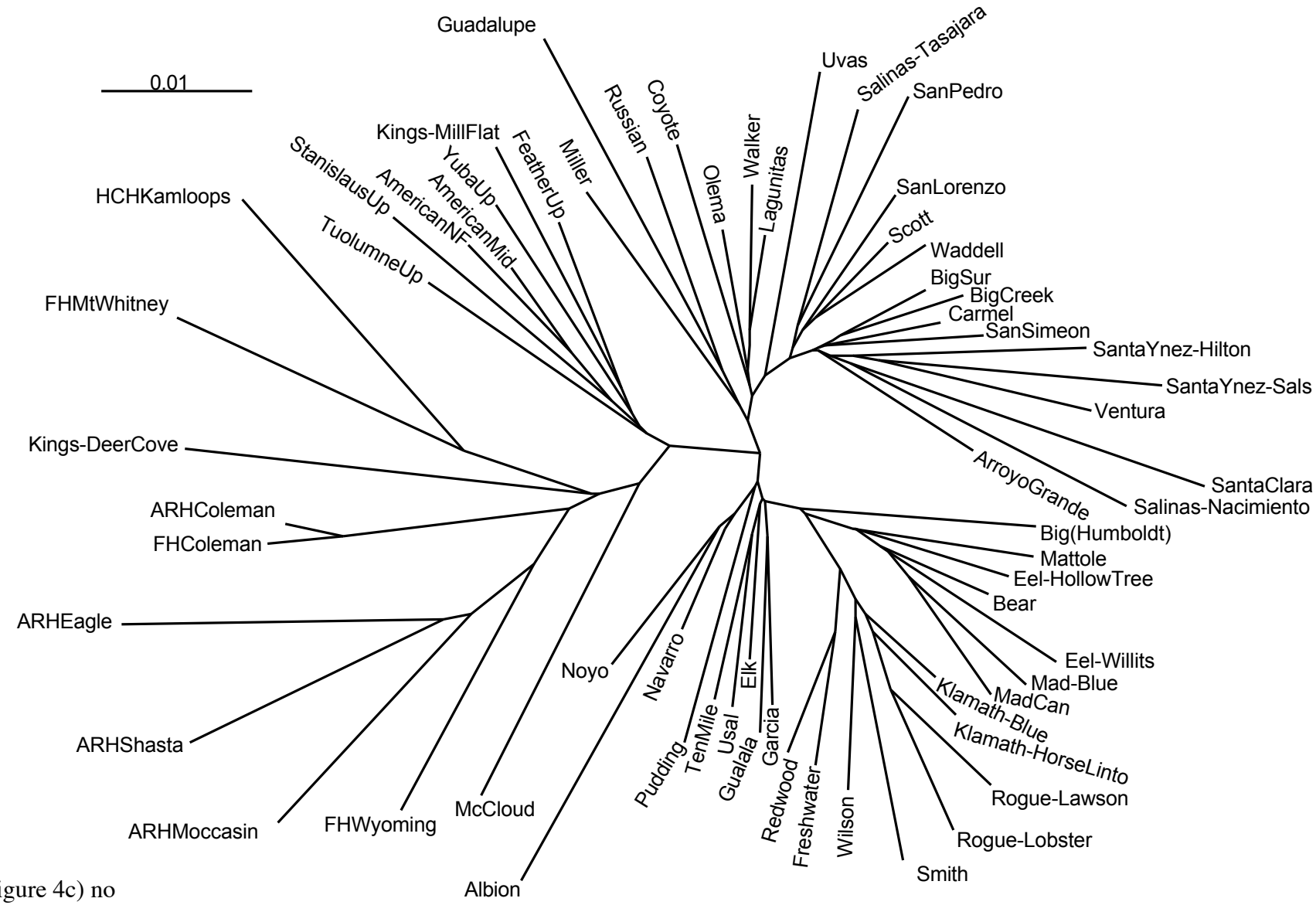


Figure 4c) no CV below-barrier pops

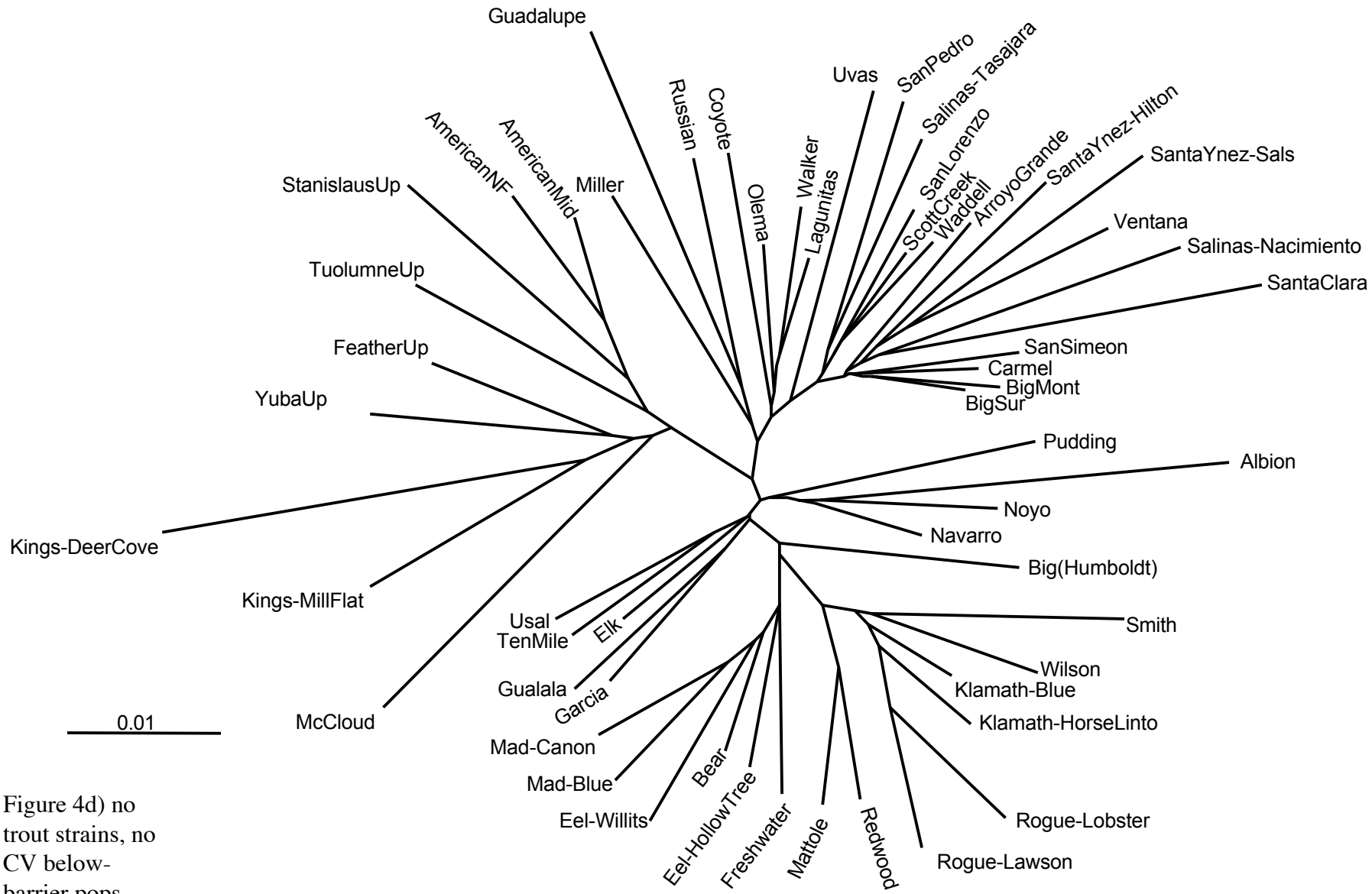


Figure 4d) no trout strains, no CV below-barrier pops

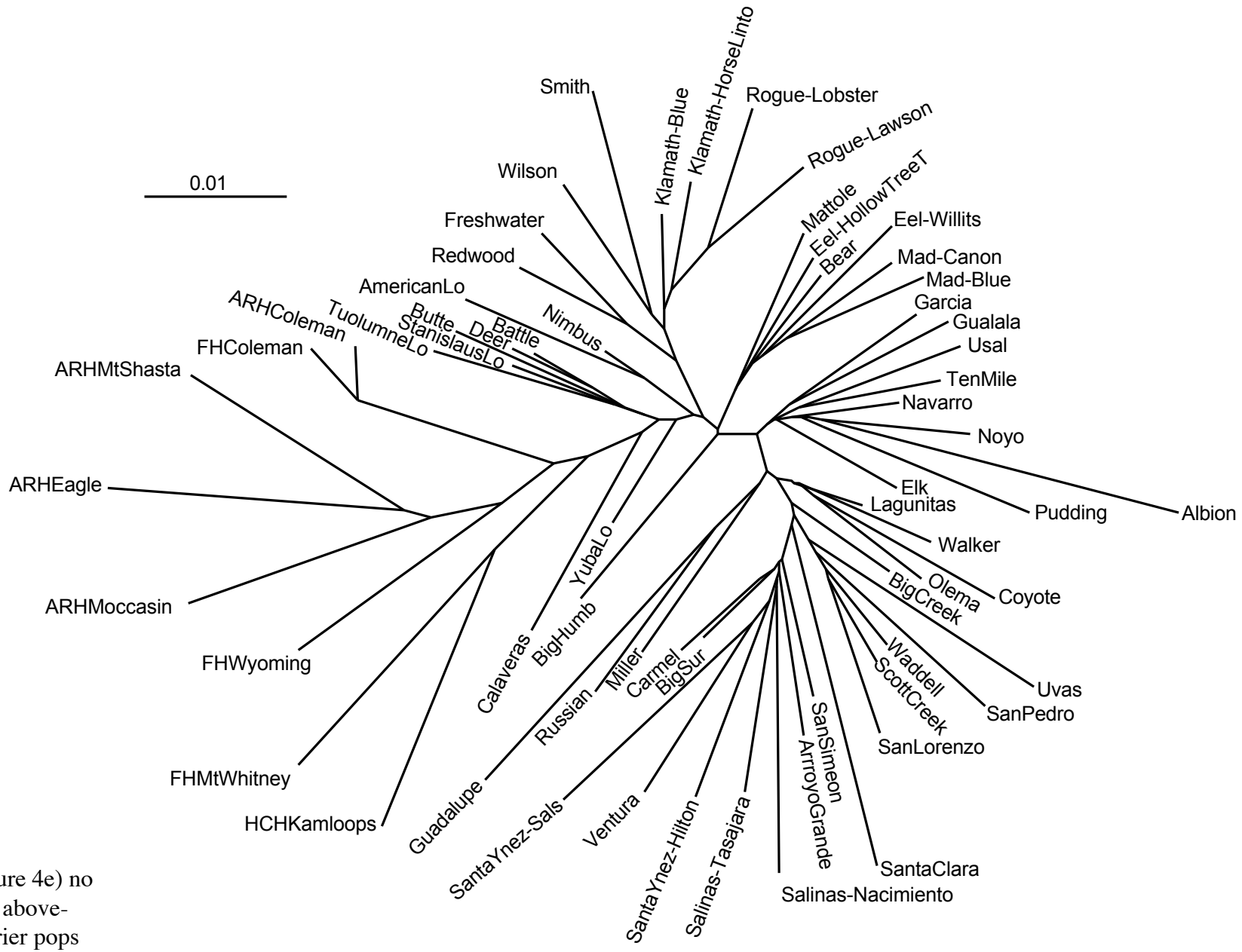


Figure 4e) no CV above-barrier pops

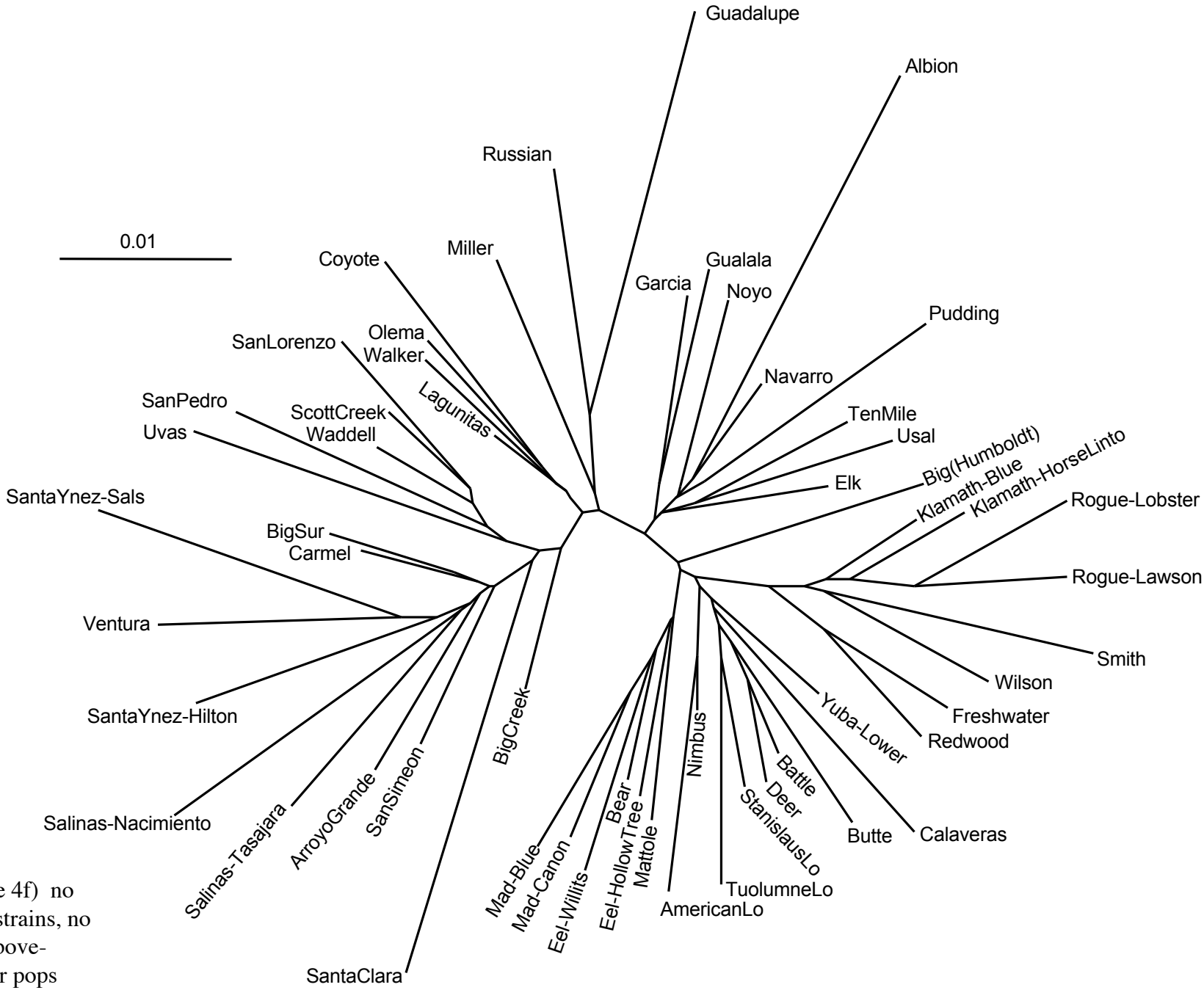


Figure 4f) no trout strains, no CV above-barrier pops

Figure 5a

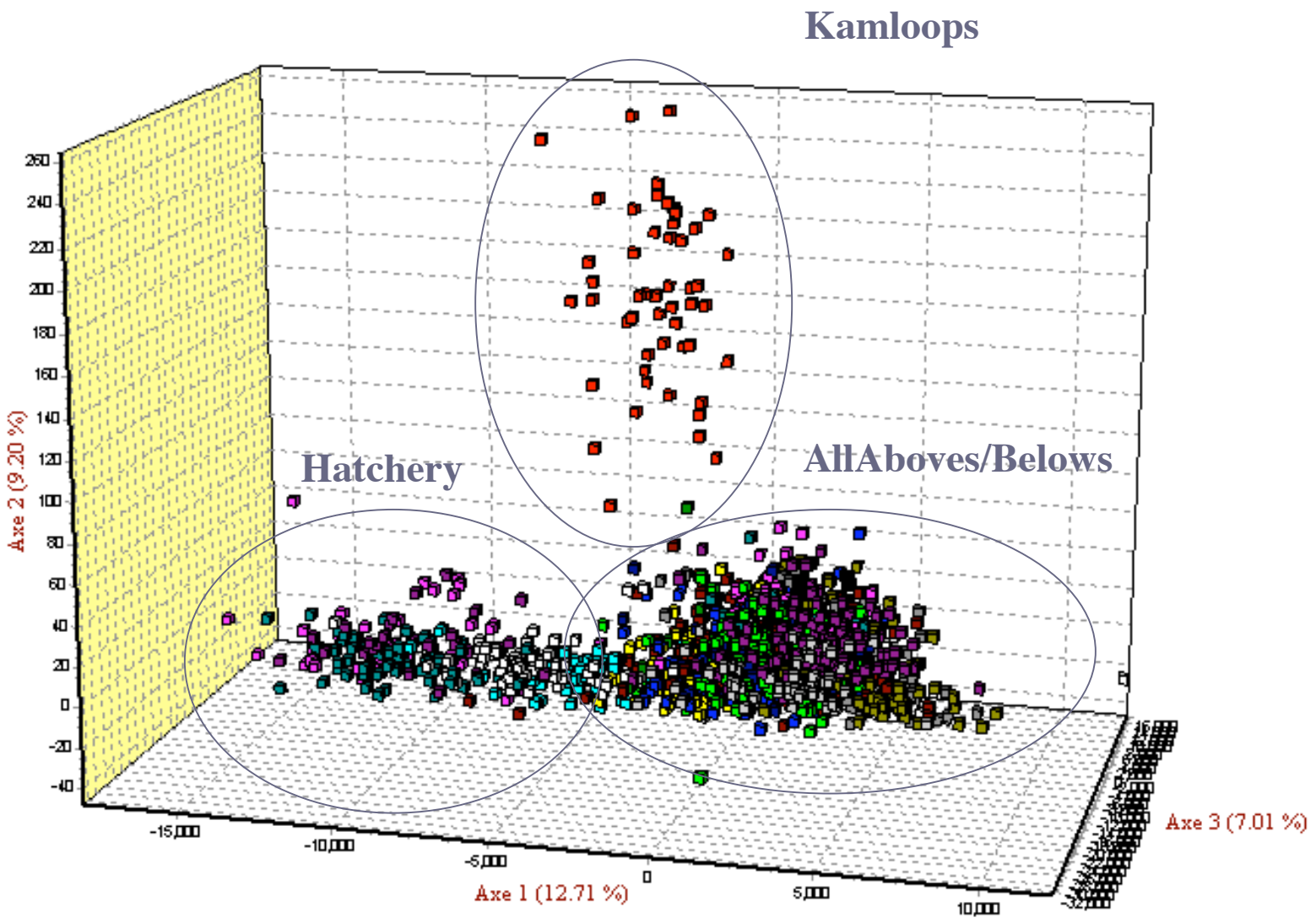
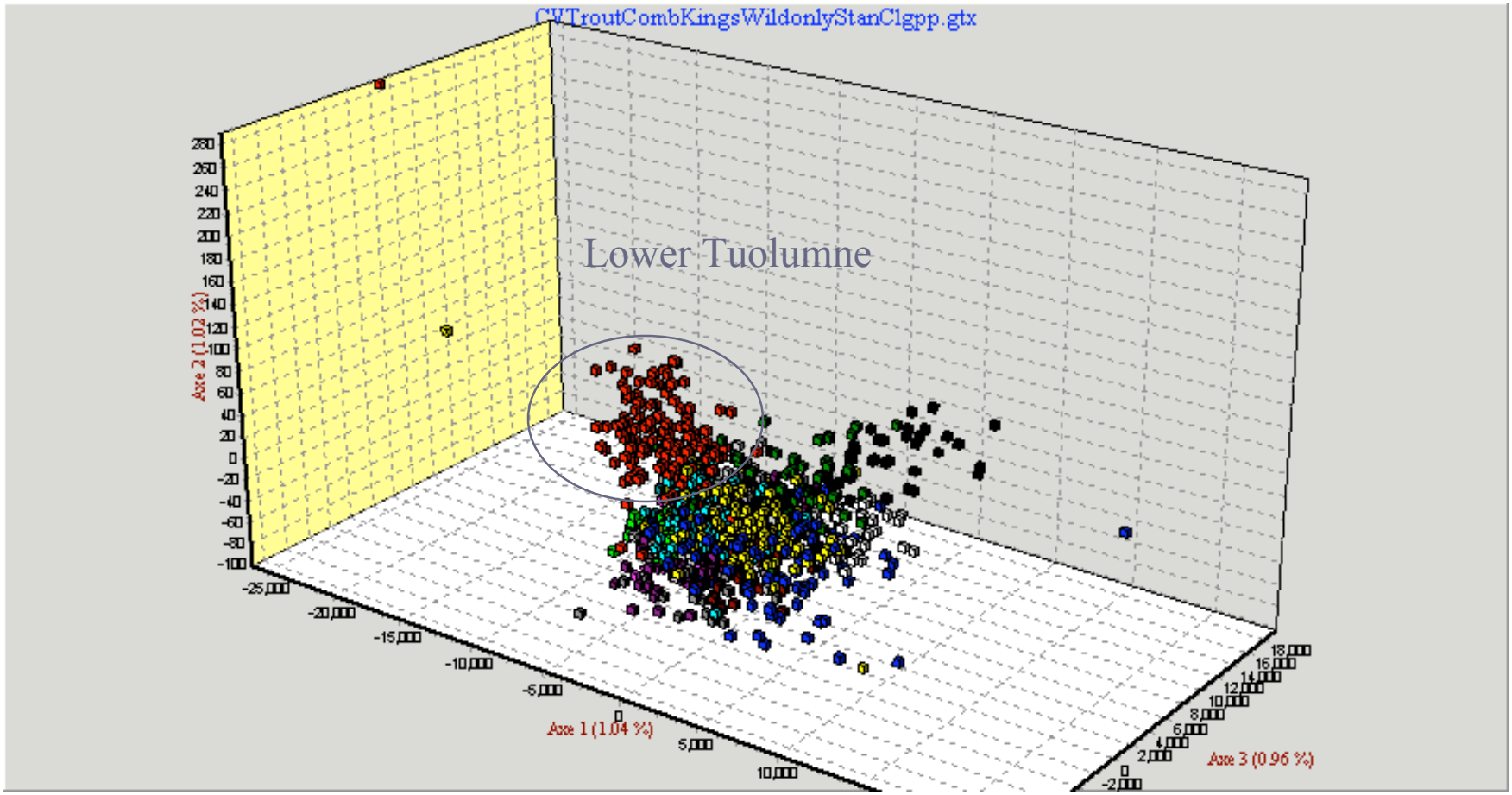

Kamloops
Hatchery
AllAboves/Belows
Axe 2 (9.20 %)
Axe 1 (12.71 %)
Axe 3 (7.01 %)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-15,000
-10,000
-5,000
0
5,000
10,000

Figure 5b


CVTroutCombKingsWildonlyStanClgpp.gtx
Lower Tuolumne
Axe 2 (1.02 %)
Axe 1 (1.04 %)
Axe 3 (0.96 %)