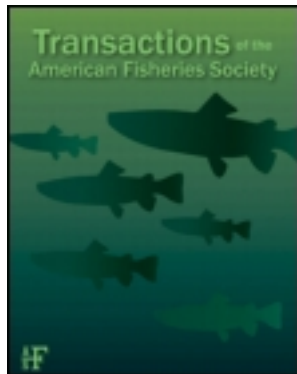


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Transactions of the American Fisheries Society

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/utaf20>

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Published online: 31 May 2011.

To cite this article: Devon E. Pearse, Stephanie L. Gunckel & Steven E. Jacobs (2011) Population Structure and Genetic Divergence of Coastal Rainbow and Redband Trout in the Upper Klamath Basin, Transactions of the American Fisheries Society, 140:3, 587-597, DOI: [10.1080/00028487.2011.583538](https://doi.org/10.1080/00028487.2011.583538)

To link to this article: <http://dx.doi.org/10.1080/00028487.2011.583538>

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ARTICLE

Population Structure and Genetic Divergence of Coastal Rainbow and Redband Trout in the Upper Klamath Basin

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Abstract

Freshwater-resident coastal rainbow trout *Oncorhynchus mykiss irideus* and the anadromous form of the subspecies, coastal steelhead (summer and winter runs), are present throughout the lower Klamath River–Trinity River system. Although coastal steelhead and other anadromous salmonids historically migrated into the Upper Klamath Basin (which encompasses the upper Klamath River and Upper Klamath Lake) and associated tributaries, the construction of Copco Dam in 1918 and Iron Gate Dam in 1962 stopped all upstream migration of fish past these barriers. In the Upper Klamath Lake basin, native Upper Klamath Lake redband trout *O. mykiss newberrii* are found along with coastal rainbow trout that were trapped above the dams or stocked from hatchery sources. However, relatively little is known about the genetic relationships among the *O. mykiss* populations within the Upper Klamath Basin. A population genetic analysis based on data from 17 variable microsatellite loci was conducted for samples collected in the Upper Klamath Basin, including rainbow trout and Upper Klamath Lake redband trout (presumably representative of the ancestral coastal and inland lineages) as well as samples of *O. mykiss* from neighboring inland lake basins. In addition, the Upper Klamath Basin samples were compared with data from *O. mykiss* populations below Iron Gate Dam. Results demonstrate the presence of distinct inland and coastal genetic lineages as well as divergent lineages represented by samples from the inland lake basins; these results have significant implications for future restoration of *O. mykiss* in the greater Klamath River–Trinity River system.

Compared with most other salmonid species, the rainbow trout *Oncorhynchus mykiss* displays an enormous amount of variation in life history pattern throughout its range (Busby et al. 1994) and even within small individual watersheds (Shapovalov and Taft 1954; Pearse et al. 2009). In addition, the extensive morphological diversity of trout in western North America led early researchers to describe as many as 50 distinct species, almost all of which have now been combined into *O. mykiss* subspecies (Behnke 1992). Because of the widespread distribution of rainbow trout and the complex evolutionary history of the various forms, phylogeographic relationships among many of them remain poorly understood. The significance of rainbow trout to

tribal cultural heritage and their popularity in recreational fisheries demand an understanding of the ecological and genetic differences among *O. mykiss* populations and subspecies. This knowledge is critical for conservation and management in the many human-impacted watersheds inhabited by rainbow trout and associated subspecies.

Redband trout constitute an enigmatic, morphologically similar group of *O. mykiss* subspecies and include the Upper Klamath Lake redband trout *O. mykiss newberrii* in Upper Klamath Lake, the Columbia River redband trout *O. mykiss gairdneri* in the Columbia and Fraser River basins, and the Sacramento golden trout *O. mykiss stonei* in the Sacramento River system

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Received March 24, 2010; accepted January 8, 2011

(Behnke 1992; Busby et al. 1994; Currens et al. 2009). Redband trout are also found in the isolated desert basins of southeast Oregon, although the taxonomic association of these geographically separate groups has long been debated (Behnke 1992; Currens et al. 2009). Several genetic studies have examined the relationships among redband trout lineages and have compared native redband trout populations with introduced hatchery rainbow trout to detect the presence of introgressive hybridization (Wishard et al. 1984; Berg 1987; Matala et al. 2008; Currens et al. 2009; DeHaan and Adams 2009; Simmons et al. 2009). Although native redband trout subspecies share some morphological similarities, genetic data indicate that they are not monophyletic with respect to other *O. mykiss* lineages (Currens et al. 2009); moreover, the relationships among the subspecies and with the coastal rainbow trout *O. mykiss irideus* remain unclear (Behnke 1992; Currens et al. 2009).

Freshwater-resident coastal rainbow trout and the anadromous form of the subspecies, coastal steelhead (summer and winter runs), are abundant in the lower Klamath River (Busby et al. 1994, 1996). However, compared with the Columbia River to the north (Knudsen et al. 2002; Kostow 2003; Brannon et al. 2004; Winans et al. 2004), relatively little is known about the relationships among *O. mykiss* populations in the Klamath River (Buchanan et al. 1994; Papa et al. 2007; Pearse et al. 2007) or between the Klamath River populations and other populations in the Klamath Mountain Province evolutionarily significant unit (Riesenbichler et al. 1992; Busby et al. 1994). Although coastal steelhead and other anadromous salmonids historically migrated into the Upper Klamath Basin (i.e., upper Klamath River and Upper Klamath Lake) and associated tributaries (Hamilton et al. 2005), the construction of Copco Dam Number 1 in 1918 and Iron Gate Dam in 1962 halted the upstream migration of fish past these barriers, effectively separating the upper and lower portions of the Klamath River basin. In addition, nonnative rainbow trout stocks have been widely planted in the upper Klamath River basin (Oregon Department of Fish and Wildlife, unpublished records); the extent of nonnative stocks' genetic impact on naturally spawning native *O. mykiss* populations is not known. Recent efforts to mitigate or remove the dams that block anadromous fish access to the Upper Klamath Basin have highlighted the importance of understanding the genetic relationships among fish populations throughout the basin to guide recovery planning efforts (Klamath Hydroelectric Settlement Agreement, Public Draft, January 8, 2010).

Redband trout in Upper Klamath Lake (Behnke 1992, 2002) are presumed to co-occur with stocked hatchery rainbow trout and with coastal rainbow trout/steelhead that are trapped above the dams. Recently, an allozyme study by Currens et al. (2009) set the stage for a more detailed analysis of *O. mykiss* within the Upper Klamath Basin; Currens et al. (2009) surveyed *O. mykiss* populations over a broad geographic range and developed hypotheses about the evolution of coastal and inland lineages. We used data from 18 microsatellite loci that were previously shown to be variable and informative for population genetic analysis

of coastal rainbow trout/steelhead (Garza et al. 2004; Pearse et al. 2007) to describe fine-scale patterns of genetic variation and population structure in samples of *O. mykiss* from throughout the Upper Klamath Basin. We tested the following hypotheses: (1) divergent genetic lineages of *O. mykiss* occupy the Upper Klamath Basin, (2) coastal rainbow trout that are found above Iron Gate Dam and the other main-stem Klamath River dams are derived from stocks that are present below the dams, and (3) redband trout that are associated with Upper Klamath Lake are distinct from redband trout occupying the headwaters and are instead more closely related to coastal rainbow trout/steelhead. We place these results in context through comparison with samples from three neighboring inland basins (Goose Lake, Chewaucan Basin, and Fort Rock Basin; Figure 1) and with previously published data describing *O. mykiss* populations in the lower Klamath River (Pearse et al. 2007) to examine the relationships of Upper Klamath Basin populations to other redband trout and coastal rainbow trout lineages.

METHODS

Samples.—In summer 2000, Oregon Department of Fish and Wildlife staff used standard electrofishing procedures to collect samples from locations throughout the Upper Klamath Basin (Figure 1). After an initial analysis, supplemental samples were collected in summer 2007 to expand the sample set. Within the Upper Klamath Basin, the samples in this study can be divided into (1) upper Klamath River populations, consisting of samples collected between Link River Dam and Iron Gate Dam; and (2) Upper Klamath Lake populations, consisting of samples from all tributaries above Link River Dam (Figure 1). In addition, samples were collected from stream tributaries to Goose Lake, Chewaucan Basin, and Fort Rock Basin, which are three neighboring isolated inland lake basins in southeast Oregon. For comparison, the analysis included samples from three anadromous coastal steelhead populations in the lower Klamath River watershed: Blue Creek; Methodist Creek, a Salmon River tributary; and Horse Linto Creek, a Trinity River tributary (Pearse et al. 2007).

Extraction of DNA and genetic data collection.—The DNA was extracted from all samples with DNeasy 96 tissue extraction kits on a BioRobot 3000 (Qiagen, Inc.) in accordance with the manufacturer's protocols. Extracted DNA was diluted about 10:1 and was used for polymerase chain reaction amplification of 18 microsatellite loci that were previously optimized for use in *O. mykiss* (*Omy77*, *OtsG401*, *OtsG243*, *OtsG253b*, *One11b*, *Omy1011*, *Omy27*, *OtsG249b*, *OtsG409*, *OtsG103*, *OtsG85*, *Oki23*, *Ots1b*, *Ssa85*, *One13b*, *Ssa289*, *OtsG3*, and *OtsG43*; Garza et al. 2004; Pearse et al. 2007). Each locus was amplified individually, and polymerase chain reaction products were pooled before electrophoreses were conducted on ABI 377 sequencers (Applied Biosystems, Inc.). Microsatellite genotypes were scored by using GeneScan version 3.0 and Genotyper version 2.1 (Applied Biosystems). All genotypes were

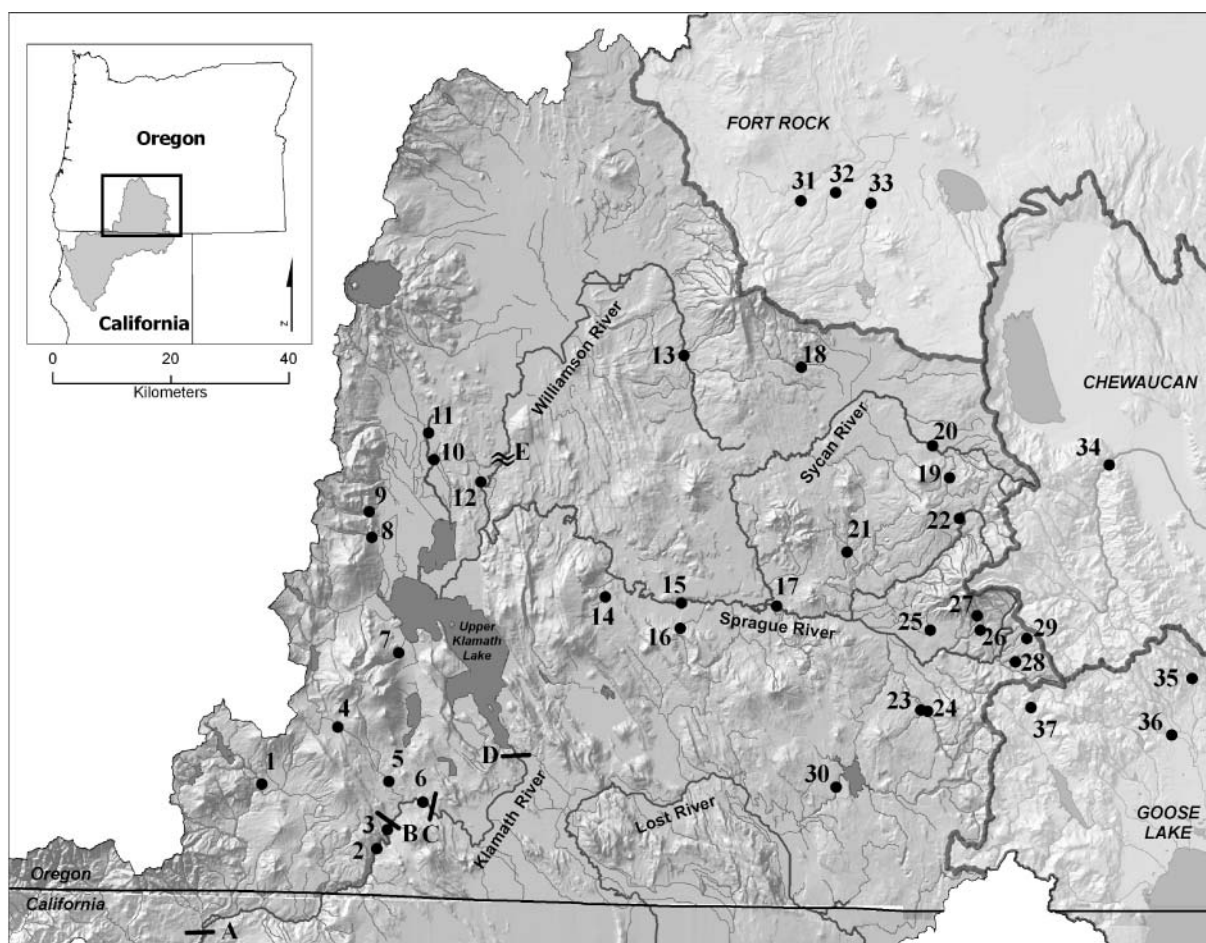


FIGURE 1. Map of the Upper Klamath Basin region, showing sample sites (site numbers are defined in Table 1) and names of major rivers and basins (A = Iron Gate Dam; B = J. C. Boyle Dam; C = Keno Dam; D = Link River Dam; E = barrier waterfall on the Williamson River). Inset depicts study area location in southern Oregon and northern California.

independently verified by two people to ensure correct and consistent scoring. Discrepancies between the two scores were resolved by consensus, re-genotyping, or deletion of that genotype from the data set.

Data analysis.—The data were analyzed with a suite of complementary analytical methods to identify concordant patterns in the distribution of genetic variation within and among individuals and populations (Pearse and Crandall 2004). The software programs GENETIX (Belkhir et al. 1996–2004) and GENEPOP (Raymond and Rousset 1995) were used to estimate basic population genetic statistics, test for Hardy–Weinberg equilibrium and linkage equilibrium, and estimate the distribution of population genetic variation as represented by F -statistics (Wright 1931). To correct for differences in sample size among populations when estimating allelic diversity, we calculated allelic richness by using rarefaction sampling based on a minimum of eight genes (HP_Rare software; Kalinowski 2005).

Genetic similarities and relationships among populations were visualized by using two approaches. First, the principal components analysis (PCA) program PCAGEN (www2.unil.ch/popgen/softwares/) was used to graphically represent the genetic variation among populations and individuals in two- and three-dimensional space. Second, PHYLIP software (Felsenstein 2004) was used to calculate Cavalli-Sforza and Edwards' (1967) chord distances and Nei's genetic distance (Nei et al. 1983) and to generate neighbor-joining networks based on these distances. Statistical support for population relationships was evaluated for both distance networks by using 10,000 bootstrap samples from the data set; the resulting trees were visualized by using TREEVIEW (Page 1996).

To complement the analyses based on population allele frequencies, genotype data were used to cluster individuals by use of the Bayesian assignment program STRUCTURE (Pritchard et al. 2000). This method is useful in evaluating genetic similarities among groups of individuals; shared genetic clustering among

individuals can be interpreted as a signal of migration, common ancestry, or both. Conversely, strong population structure is indicated when individuals have high proportional membership coefficients (Q -values) for assignment to different genetic clusters. The approach used by STRUCTURE is especially informative as an exploratory tool for data analysis because it does not require a priori designation of discrete populations. Instead, the program partitions the genotypes into a specified number of clusters (K) and assigns each individual proportionally to one or more of the clusters. Consistent patterns of division at the individual and population levels over a range of K -values provide an indication of the true population genetic relationships. Default parameters were used for the STRUCTURE analysis, including the assumption of correlated allele frequencies; 10,000 burn-in simulations were followed by 90,000 data collection simulations, and three separate runs were made for each value of K ranging from 2 to 12. Variation among runs was evaluated with the natural logarithm of the data, $\log_e P(D)$ (Pritchard et al. 2000).

RESULTS

Genetic Data

The 1,006 *O. mykiss* individuals sampled from 33 sites in the Upper Klamath Basin during summer 2000 were initially genotyped at 18 polymorphic microsatellite loci. However, in some Upper Klamath Basin populations, the *OtsG43* locus appeared to be amplifying a pseudocus in addition to the expected locus, presumably as a result of the ancestral tetraploidy common to salmonids. Because these pseudoalleles overlapped in size with the distribution of expected alleles, the genotype was impossible to determine for many individuals; hence, we dropped this locus from the analysis.

The poor quality of DNA recovered from the tissue samples of some populations meant that for 298 individuals, we were unable to obtain amplified genotypes for the minimum of nine loci (the criterion for inclusion in the final data set). The data for the remaining individuals generally were of high quality; 95% of the retained individuals had data for 10 or more microsatellite loci. To replace the individuals from sites with poor initial DNA quality and to obtain samples from more locations, we collected additional samples in summer 2007. This effort provided 186 individuals from eight sample sites, including new sites in the Fort Rock and Chewaucan basins (Figure 1; Table 1); all of these individuals were successfully genotyped. The final combined data set thus consisted of multilocus genotypes for 894 individuals from 37 populations (Table 1). For purposes of the final analysis, these data were combined with previously collected microsatellite data from three sites in the lower Klamath River (Pearse et al. 2007).

Population Genetic Analysis

Summary statistics of genetic variation for all populations are shown in Table 1. Tests for deviations from Hardy–Weinberg

equilibrium and linkage equilibrium did not uncover any systematic patterns of disequilibrium, and no population was significantly out of equilibrium for more than two loci after Bonferroni correction for multiple comparisons. Such sporadic disequilibria are common for microsatellite loci in salmonid populations, particularly when juvenile fish are sampled from relatively small populations.

All analyzed populations exhibited significant genetic differentiation as measured by pairwise values of the genetic differentiation index F_{ST} ; the exception was for the group of populations in the upper Klamath River (J. C. Boyle Reservoir and bypass, Spencer Creek, and Keno reach: populations 2–6; data not shown). Pairwise F_{ST} values among all populations were generally high relative to those typically seen in coastal steelhead populations (mean pairwise $F_{ST} = 0.09$ – 0.28), a pattern expected for populations of resident trout and in the possible presence of barriers to fish movement. As indicated by mean pairwise F_{ST} values, the most strongly differentiated populations within the Upper Klamath Basin were Moss Creek (population 7; $F_{ST} = 0.28$) and Rock Creek (population 16; $F_{ST} = 0.28$), a tributary of the Sprague River. However, because F_{ST} values are strongly influenced by factors that increase genetic drift, these results may reflect the small population sizes rather than greater isolation or divergence time for these populations. This conclusion is supported by the low allelic richness seen in the Moss Creek and Rock Creek populations (Table 1). In contrast, the high mean pairwise F_{ST} values seen for the Chewaucan River (population 34) and for the three Goose Lake populations (populations 35–37) probably do reflect evolutionary divergence since these populations do not display such reduced genetic diversity (Table 1).

Qualitative examination of the allele frequency distributions across populations revealed a distinction between “coastal” and “inland” trout in the Upper Klamath Basin. For these groups, many alleles that were present at substantial frequencies ($>10\%$) in the Upper Klamath Lake populations, the Goose Lake populations, or all of these populations were absent from the upper Klamath River populations. In contrast, alleles that were present in the upper Klamath River tended to be shared with most or all of the populations in the Upper Klamath Lake and Goose Lake basins. For example, allele 96 at locus *Omy77* was observed in more than half of the populations in the Upper Klamath Lake headwaters at frequencies up to 34% and was observed in all three Goose Lake populations at frequencies greater than 50% but was absent below Link River Dam. Similarly, no allele larger than 240 base pairs was observed at locus *Omy1011* in the upper Klamath River or Goose Lake populations, but an additional 25 alleles were observed in the Upper Klamath Lake headwaters and Fort Rock Basin populations at sizes of up to 344 base pairs and at frequencies as high as 24%. This latter example, combined with the observed presence of novel pseudoalleles at the excluded locus (*OtsG43*), suggests that isolation between inland and coastal forms persisted for a sufficient period to allow substantial accumulation of de novo mutations in addition to changes in allele frequencies; this example also supports the

TABLE 1. List of collection locations for all *Oncorhynchus mykiss* samples (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) analyzed in the present study. Site numbers correspond to the sites shown in Figure 1 (four-letter codes represent lower Klamath River coastal steelhead: LKBL = Blue Creek; MSME = Methodist Creek; TRHL = Horse Linto Creek). Summary statistics are the number of samples (N), observed heterozygosity (H_o), allelic richness (A_r), mean pairwise genetic differentiation index (F_{ST}), and mean proportional membership coefficient (Q) across three STRUCTURE runs at two clusters ($K = 2$).

Site number or code	Tributary	Stream or site	N	H_o	A_r	F_{ST}	Q
Upper Klamath River							
1	Iron Gate Reservoir	Jenny Creek	39	0.65	3.9	0.15	0.03
2	Klamath River	Below J. C. Boyle Dam	14	0.65	4.2	0.10	0.05
3	Klamath River	J. C. Boyle bypass reach	19	0.66	4.1	0.09	0.12
4	Spencer Creek	Above culvert	33	0.66	3.8	0.11	0.05
5	Spencer Creek	Below culvert	35	0.68	3.9	0.11	0.07
6	Klamath River	Keno reach	10	0.74	4.1	0.11	0.02
Upper Klamath Lake							
7 ^a		Moss Creek	33	0.41	2.3	0.28	0.01
8 ^a		Rock Creek	37	0.60	3.0	0.20	0.02
9		Cherry Creek	26	0.58	3.4	0.14	0.06
10 ^a	Wood River	Fort Creek	36	0.54	3.2	0.17	0.96
11		Upper Wood River	32	0.49	2.8	0.19	0.98
12 ^a	Williamson River	Spring Creek	39	0.48	3.1	0.15	0.97
13		Deep Creek Ranch	20	0.66	4.1	0.12	0.84
14	Sprague River/Trout Creek	South Fork Trout Creek	19	0.64	3.7	0.12	0.44
15	Sprague River	Ridgeway Ranch	17	0.47	3.0	0.17	0.97
16	Sprague River	Rock Creek	10	0.30	2.0	0.28	0.04
17	Sprague River	Anderson Ranch	28	0.57	3.8	0.10	0.86
18	Sprague River/Sycan River	Long Creek	26	0.62	3.8	0.12	0.93
19	Sprague River/Sycan River	Paradise Creek	24	0.62	4.0	0.10	0.84
20	Sprague River/Sycan River	Rock Creek	36	0.65	4.0	0.10	0.83
21	North Fork Sprague River	Fivemile Creek	16	0.54	3.6	0.12	0.85
22	North Fork Sprague River	Upper North Fork Sprague River	31	0.62	3.9	0.11	0.92
23	South Fork Sprague River	Robinson Spring Creek	31	0.54	3.3	0.15	0.96
24	South Fork Sprague River	Fishhole Creek	34	0.66	3.8	0.11	0.89
25	South Fork Sprague River	Demming Creek	14	0.60	3.0	0.18	0.98
26	South Fork Sprague River	Brownsworth Creek	16	0.60	3.7	0.12	0.83
27	South Fork Sprague River	Leonard Creek	12	0.61	3.6	0.11	0.96
28	South Fork Sprague River	Pothole Creek	27	0.69	4.1	0.11	0.49
29	South Fork Sprague River	Buckboard Creek	29	0.46	3.0	0.16	0.96
30 ^b	Lost River	Miller Creek/North Canal	30	0.61	3.3	0.16	0.19
Inland basins							
31 ^a	Fort Rock Basin	Buck Creek	20	0.67	4.5	0.11	0.20
32 ^a	Fort Rock Basin	Bridge Creek	15	0.66	4.2	0.12	0.28
33 ^a	Fort Rock Basin	Silver Creek	14	0.67	4.6	0.11	0.17
34 ^a	Chewaucan Basin	Chewaucan River	15	0.57	3.7	0.19	0.02
35	Goose Lake Basin	Bauers Creek	22	0.53	3.4	0.19	0.02
36	Goose Lake Basin	Thomas Creek	19	0.48	3.4	0.19	0.03
37	Goose Lake Basin	Drews Creek	16	0.50	3.4	0.20	0.03
Lower Klamath River							
LKBL ^c		Blue Creek	48	0.73		0.10	0.02
MSME ^c	Salmon River	Methodist Creek	54	0.66		0.09	0.10
TRHL ^c	Trinity River	Horse Linto Creek	48	0.71		0.10	0.05

^aIncludes samples collected in 2007.

^bThe Lost River drainage is not a direct tributary of the Klamath River.

^cData are from Pearse et al. (2007).

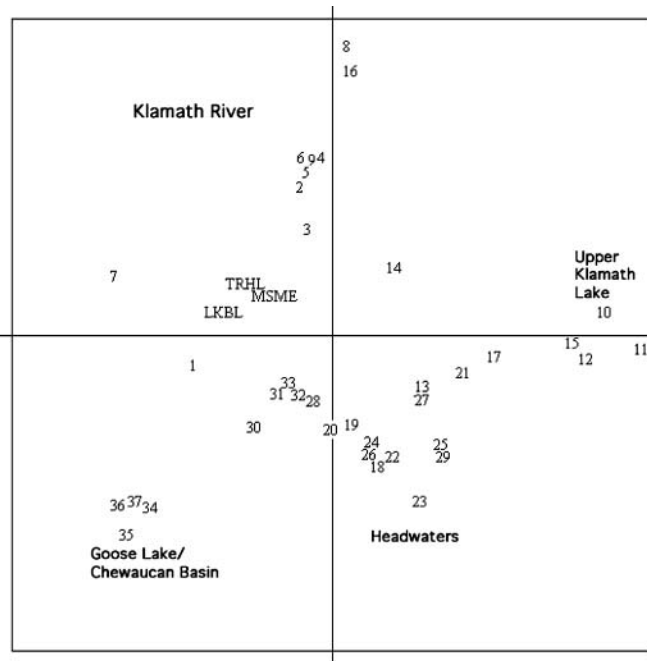


FIGURE 2. Principal components analysis results, showing all upper Klamath River and interior basin samples of *Oncorhynchus mykiss* (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) plus lower Klamath River coastal steelhead samples (site numbers and four-letter codes are defined in Table 1). The group of Goose Lake and Chewaucan River populations is highlighted in the lower left.

hypothesis that little ongoing gene flow is maintained among these populations.

Use of PCA for statistical evaluation of population genetic similarity not only supported a division between the inland and coastal lineages but also clearly differentiated the Goose Lake and Chewaucan Basin populations from all other samples included in the study. Overall patterns of genetic similarity among populations can be seen in the PCA depicted in Figure 2; the primary division between coastal and inland populations can be envisioned as a diagonal from the lower left to the upper right. Above and to the left of this line, the upper Klamath River populations 2–6 cluster tightly together with the three lower Klamath River populations and the sample from Jenny Creek (population 1). Samples from tributaries to the west side of Upper Klamath Lake (populations 7–9) and a sample from the lower Sprague River (population 16) also clustered above the diagonal. Below and to the right of the diagonal, the Upper Klamath Lake headwaters populations are grouped much more loosely, indicative of increased genetic divergence among populations as would be expected of resident trout with reduced migratory behaviors. The Fort Rock Basin populations (populations 31–33) are associated with these populations, whereas Goose Lake and Chewaucan Basin populations form a distinct cluster (Figure 2). Notably, samples from the Wood, lower Williamson, and lower Sprague rivers (populations 10–12 and 15) are clustered together in the far right of Figure 2 and are separate from the main group

of Upper Klamath Lake headwaters populations. Trout Creek (population 14) occupies a central position that is intermediate between the inland and coastal population groupings.

The population relationships identified by PHYLIP in unrooted neighbor-joining networks (Figures 3, 4) were largely concordant with the patterns seen in the PCA. Strong bootstrap support was obtained for a group of populations associated with Upper Klamath Lake (populations 10–12 and 15) and for the separation of the Chewaucan River and the three Goose Lake populations from all other samples (Figures 3, 4). More inclusive groups, such as the upper Klamath River populations that were grouped with Rock Creek (population 8) and Cherry Creek (population 9), were only weakly supported; support for substructure within the Upper Klamath Lake headwaters populations was relatively low except for the two upper Sycan River populations (populations 19 and 20). Interestingly, for the network based on Nei's genetic distance (Figure 4), the population sample from Trout Creek (population 14) is unstable in its position in the network. Although this population appears with the Upper Klamath Lake headwaters populations in the neighbor-joining network (Figure 4), it clusters with the upper Klamath River populations in the bootstrap consensus tree, as it does in the network based on Cavalli-Sforza and Edwards' chord distances.

Individual Genotypic Analysis

The program STRUCTURE proportionally divides each individual among a specified number of genetic clusters (i.e., K) and estimates the likelihood of each K -value. The distribution of Q for all individuals in a population then provides an indication of genetic associations among populations and the amount of admixture present. When a large number of populations is analyzed, however, the method may fail to provide consistent results over multiple runs with a single K -value and may tend to overestimate K . This effect can be seen in the variation among the three runs at each value of K (Figure 5). Thus, the most informative interpretation is based on the identification of consistent, biologically reasonable patterns across a range of K -values (Pritchard et al. 2000).

Examination of STRUCTURE results for the present data set indicates considerable variation both across a range of K -values ($K = 2$ –9) and among different simulation runs at the same value of K . Nonetheless, some clear and consistent patterns are seen across runs. First, population genetic differentiation is clear; highly skewed Q -values for almost all individuals led to the highly skewed population Q -values seen at the K of 2 for almost all samples ($Q < 0.2$ or $Q > 0.8$; Table 1). The only exceptions to this were samples from Trout Creek (population 14) and Pot-hole Creek (population 28), which each consisted of individuals exhibiting a range of intermediate Q -values; this result suggests ongoing hybridization between divergent lineages in these populations. Second, the same clear separation between samples from Upper Klamath Lake and headwaters populations and all other samples was identified with the individual-based STRUCTURE

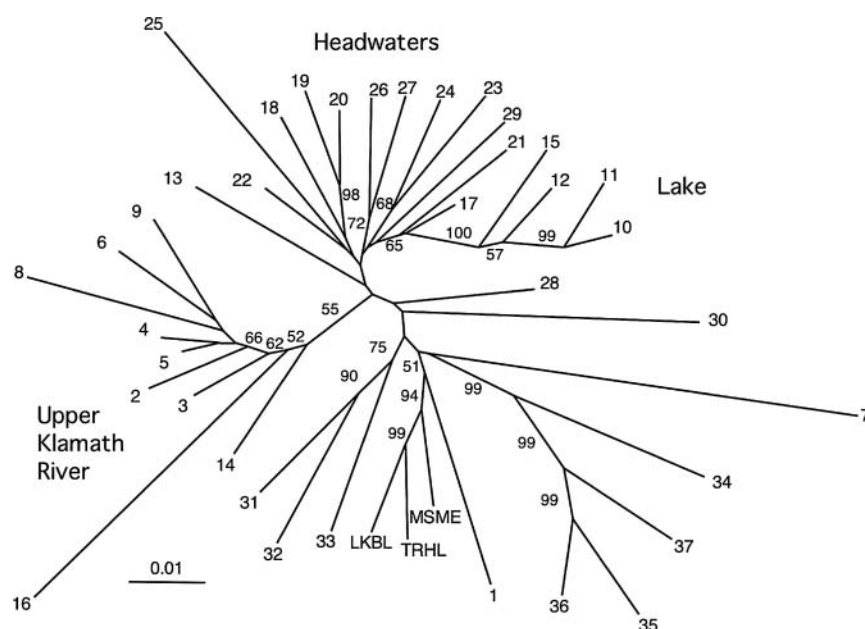


FIGURE 3. Unrooted neighbor-joining network, showing relationships among Upper Klamath Basin populations of *Oncorhynchus mykiss* (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) based on Cavalli-Sforza and Edwards' (1967) chord distances. Numbers or four-letter codes at branch ends indicate populations (site numbers and codes are defined in Table 1). Numbers on internal branches indicate the percentage of resampled data sets in which the indicated clade was present out of 10,000 bootstrapped resamplings of the data. Values greater than 70% are generally interpreted as indicating strong support for a grouping.

analysis (cluster B at $K = 2$; Table 2). As in the population analyses, the west-side Upper Klamath Lake tributaries and lower Sprague River populations (7–9 and 16) were exceptions to this general result and clustered with the populations below Link River Dam. This division appeared in the three identical runs at the K -value of 2, was supported across the full range of K -values, and was evident from both the individual and population Q -values.

At higher values of K , variation in specific genetic clusters among runs was common, but several consistent groupings were

evident in multiple runs over a range of K -values up to 9. Table 2 shows representative results for K -values of 2, 4, 6, and 9; above a K -value of 9, the patterns were much less clear and high variance in $\log_e P(D)$ (Figure 5) and low population Q -values predominated. As at the K of 2, a primary grouping of all Upper Klamath Lake headwaters populations was consistent at all of the higher values of K . Similarly, a consistent “upper Klamath River” cluster predominated in the populations below Link River Dam (populations 2–6), in the small west-side tributaries to Upper Klamath Lake (Moss, Rock, and Cherry creeks: populations

TABLE 2. Results of analysis with STRUCTURE, showing cluster associations for population membership coefficient (Q) values greater than 0.5 at various numbers of clusters ($K = 2, 4, 6, \text{ and } 9$), as applied to *Oncorhynchus mykiss* (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) sampled in the Upper Klamath Basin. Site (population) numbers and four-letter codes are defined in Table 1. Parentheses indicate populations that did not show consistently high Q -values associated with any population group but were most strongly associated with the indicated group.

$K = 2$	$K = 4$	$K = 6$	$K = 9$	Population(s)	Population group
A	A	A	A	LKBL, MSME, TRHL	Klamath River below Iron Gate Dam
A	A	A	B	1	Jenny Creek
A	B	A	C	2, (3), 4, 5, 6, 8, 9, 16	Upper Klamath River, Upper Klamath Lake, and lower Sprague River
A	B	B	D	7	Moss Creek
A	A	C	E	30, (28)	Lost River
A	A	C	F	31, 32, 33	Fort Rock Basin
A	C	D	G	34, 35, 36, 37	Goose Lake and Chewaucan Basin
B	D	E	H	10, 11, 12, 15	Wood, lower Williamson, and lower Sprague rivers
B	D	F	I	13, (14), (17), 18–27, 29	Upper Klamath Lake headwaters

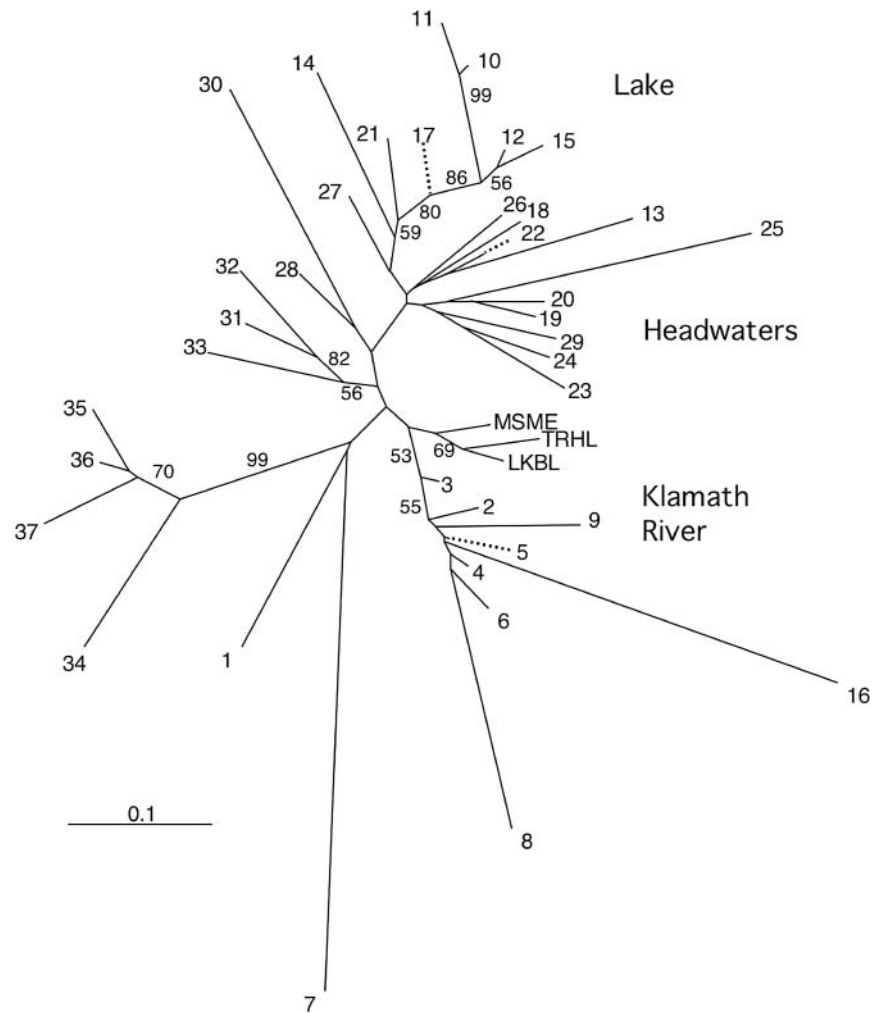


FIGURE 4. Unrooted neighbor-joining network, showing relationships among Upper Klamath Basin populations of *Oncorhynchus mykiss* (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) based on Nei's genetic distances (Nei et al. 1983). Numbers or four-letter codes at branch ends indicate populations (site numbers and codes are defined in Table 1). Numbers on internal branches indicate the percentage of resampled data sets in which the indicated clade was present out of 10,000 bootstrapped resamplings of the data. Values greater than 70% are generally interpreted as indicating strong support for a grouping.

7–9), and at Rock Creek (population 16) in the lower Sprague River (cluster B at $K = 4$; Table 2). Interestingly, in most runs with K -values greater than 5, the lower Williamson River (population 12) clustered with the Wood River (populations 10 and 11) and with samples from the lower Sprague River: Ridge-way Ranch (population 15) and Anderson Ranch (population 17; $Q \approx 0.4$). This pattern parallels that seen in the PCA and neighbor-joining analyses and may indicate genetic differentiation between lacustrine redband trout in Upper Klamath Lake (as noted by Behnke 1992) and stream-dwelling redband trout in the Upper Klamath Lake headwaters. Finally, in all runs with K -values greater than 4, the Goose Lake samples were identified as being distinct from the Upper Klamath Basin populations, as evidenced by high Q -values, and consistently clustered together with the Chewaucan Basin population. Similarly, the Fort Rock

Basin populations were identified as unique and did not cluster with populations from the Upper Klamath Basin or other inland lake basins (Table 2).

DISCUSSION

With the exception of the Klamath River populations sampled between Iron Gate Dam and Link River Dam, every population sampled for the present study was genetically distinct as measured by pairwise F_{ST} values. This result was also supported by the clustering analysis with STRUCTURE, which assigned high Q -values, thereby associating most individuals and populations with single genetic clusters. The lack of significant differentiation among upper Klamath River populations is consistent with the extensive fish movements reported in these areas, despite the constraints imposed on fish migrations by the construction

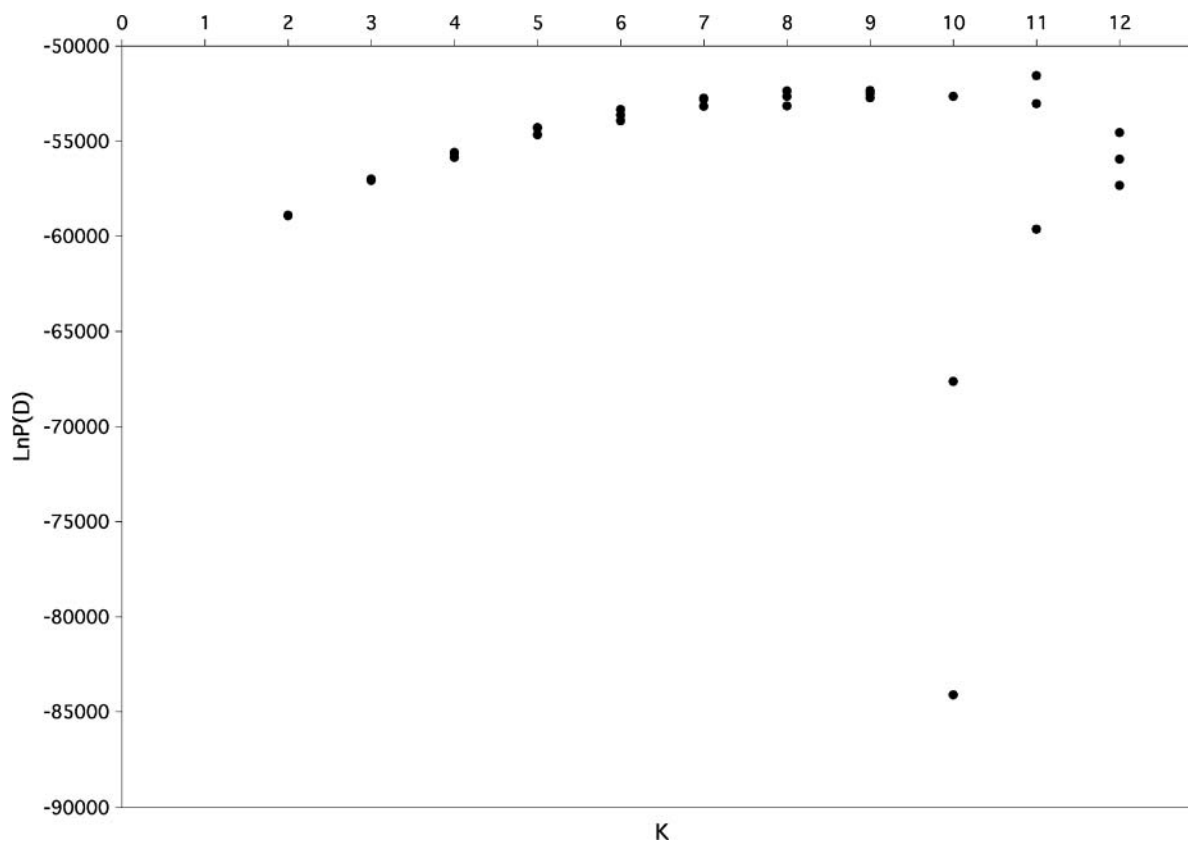


FIGURE 5. $\text{Log}_e P(D)$ results from three replicate STRUCTURE runs performed at each evaluated number of clusters ($K = 2-12$), as applied to *Oncorhynchus mykiss* (including coastal rainbow trout/steelhead and Upper Klamath Lake redband trout) sampled in the Upper Klamath Basin.

of dams and the regulation of flow in the upper Klamath River (Jacobs et al. 2008).

The patterns observed with the microsatellite data show strong similarities to the allozyme findings of Buchanan et al. (1994) and Currens et al. (2009), including the primary division between inland and coastal lineages. Analysis of the data presented here also identified a secondary division separating *O. mykiss* populations in the lower reaches of the Upper Klamath Lake system from populations farther up in the headwaters of the Williamson and Sprague rivers. This result is evident from the concordant patterns observed in multiple complementary methods of population genetic analysis and also closely parallels the allozyme allele frequency patterns noted by Currens et al. (2009).

Behnke (1992, 2002) identified redband trout found in Upper Klamath Lake (i.e., the original type source of Upper Klamath Lake redband trout) as typical of the subspecies and noted morphological differences between these fish and the stream-dwelling redband trout in the headwaters of Upper Klamath Basin. Long-term isolation of the headwaters populations is supported—at least in the case of the Williamson River—by the presence of barrier waterfalls that prevent fish movement into the upper reaches of that system. Neither of these genetic groups is closely associated with the coastal rainbow trout/steelhead pop-

ulations below Link River Dam, and our data suggest that there is substantial divergence between the redband trout present in the headwaters of the Williamson and Sprague rivers and those found in Upper Klamath Lake. The latter are known to be migratory adfluvial populations that primarily spawn in the highly spring-influenced tributaries of Upper Klamath Lake. It is worth noting that extensive stocking took place in some of these tributaries, including the Williamson and Wood rivers, beginning in 1925 and continuing as late as 1990 (Oregon Department of Fish and Wildlife, unpublished records).

The presence of distinct inland and coastal genetic groups of *O. mykiss* in the Upper Klamath Basin supports the hypothesis that an ancestral lineage of redband trout, which was isolated in the upper basin, was secondarily invaded by the coastal lineage when the lower Klamath River connected with Upper Klamath Lake and provided an outlet to the Pacific Ocean (Currens 1997; Currens et al. 2009). However, the distribution of populations associated with the two lineages is not clearly divided at Link River Dam but instead presents a mosaic pattern that is centered around Upper Klamath Lake. Historically, anadromous salmonids, including coastal steelhead, had access to the Upper Klamath Basin via the Link River (Hamilton et al. 2005). Today, Link River Dam is not a complete physical barrier between Upper Klamath Lake and Upper Klamath River populations

because a fish ladder allows fish passage. Although the extent to which migratory *O. mykiss* use this facility is not clear, the small creek populations along the west shore of Upper Klamath Lake (Moss, Rock, and Cherry creeks: populations 7–9) consistently grouped with the coastal-lineage upper Klamath River populations, as did the Rock Creek population in the lower Sprague River (population 16), and showed no evidence of significant genetic exchange with the redband trout populations in the Upper Klamath Lake headwaters or Upper Klamath Lake.

The samples from Trout Creek (population 14), a tributary of the lower Sprague River, also showed an association with the Klamath River coastal genetic lineage rather than with the Upper Klamath Lake and headwaters populations. However, the individuals in Trout Creek were clearly identified as a mixture of both coastal and inland lineages based on the STRUCTURE analysis (results not shown). The Trout Creek population also appeared in an intermediate position in the PCA (Figure 2) and was unstable in its position in the phylogenetic networks. These results and the close proximity of populations 14–16 despite their divergent genetic structure suggest that the lower Sprague River is an area of active hybridization. More detailed sampling and analysis of sites in the vicinity of Trout Creek in the Sprague River will be needed to resolve the fine-scale genetic structure in this watershed.

The single-site sample analyzed from Jenny Creek in the present study (population 1) did not show a strong genetic similarity to either the Upper Klamath Lake or upper Klamath River groups but instead appeared to be most similar to the lower Klamath River populations. The Jenny Creek sample was consistently pure ($Q > 0.9$) at all K -values, which is consistent with the isolation of this population. At higher values of K , the Jenny Creek population was identified as a unique population or was clustered with the upper Klamath River populations, the three lower Klamath River populations, or both groups (Table 2). In previous analyses based on allozymes (Buchanan et al. 1994; Currrens et al. 2009), genetic associations to both the coastal and inland lineages were observed in populations sampled from different tributaries and reaches of Jenny Creek. However, because only a single Jenny Creek site was sampled for the present study, it is difficult to relate the current results to these earlier studies, especially given the history of stocking of coastal-origin hatchery rainbow trout in this watershed and the presence of waterfall barriers that isolate the Jenny Creek population from the main-stem Klamath River.

Overall, the results of our detailed microsatellite analysis of *O. mykiss* in the Upper Klamath Basin are largely concordant with the hypothesized broad-scale relationships among coastal rainbow trout/steelhead and inland redband trout lineages. On the basis of morphological characteristics (Behnke 1992, 2002) and allozyme data (Currrens et al. 2009), Upper Klamath Lake redband trout in the Upper Klamath Basin appear to be a distinct native form of redband trout, whereas populations of coastal rainbow trout/steelhead predominate below Link River Dam and inhabit some tributaries of Upper Klamath Lake. Further-

more, as was suggested by Currrens et al. (2009), the redband trout in the Williamson and Sprague River headwaters are genetically distinct from those associated with lower tributaries of Upper Klamath Lake. Finally, the genetic separation between samples from Goose Lake and Chewaucan Basin and those from Fort Rock Basin is consistent with the suggestion that the former populations are ancestrally associated with Sacramento golden trout from the Sacramento and Pit rivers, whereas the latter populations are derived from Columbia River redband trout (Currrens 2009).

The findings of this study have implications for current plans to remove dams from the upper Klamath River (Klamath Basin Restoration Agreement) and to reintroduce anadromous salmonids into the Upper Klamath Basin (Hooton and Smith 2008). Our results indicate that multiple lineages of *O. mykiss* are present in the upper basin. Before dam construction, coastal- and inland-lineage fish with anadromous life histories were probably present (Hamilton et al. 2005). However, given the productive capacity of Upper Klamath Lake and the Upper Klamath Basin, it is also likely that resident life histories co-occurred with anadromous forms of *O. mykiss* in the upper basin. If connectivity of the upper basin with the lower Klamath River basin and Pacific Ocean is re-established, *O. mykiss* with anadromous life histories could be naturally re-established in the upper basin by two potential mechanisms: (1) resumption of seaward migration by populations occupying the upper basin and (2) invasion of the upper basin by anadromous forms from the lower Klamath River basin. The degree to which resident and anadromous life histories persist in a reconnected Upper Klamath Basin will depend on their long-term productive capacity. Anadromous forms may be more productive during periods of favorable seaward migration and marine productivity, whereas resident forms may have an advantage during periods when Upper Klamath Lake is productive. Because of the complexity and uncertainty surrounding the relationship between resident and anadromous forms of *O. mykiss* in the Upper Klamath Basin, the Oregon Department of Fish and Wildlife is taking a conservative approach to re-establishing coastal steelhead in the upper basin. The reintroduction plan adopted by the Oregon Fish and Wildlife Commission in 2008 (Hooton and Smith 2008) calls for no active intervention in the re-establishment of coastal steelhead in the Upper Klamath Basin.

ACKNOWLEDGMENTS

Rhine Messmer and William Tinniswood collected the majority of the fish samples. Kathryn Kostow archived the samples collected in 2000 and made them available for this study. Members of the Molecular Ecology Team at the Southwest Fisheries Science Center, especially E. Anderson, A. Clemento, and J. C. Garza, contributed comments and discussion on data analysis; A. Abadia, C. Gallardo, L. Gilbert-Horvath, A. Martinez, and E. Martinez contributed to the laboratory effort. This work was

funded in part by the Federal Aid in Sport Fish Restoration Program (U.S. Fish and Wildlife Service).

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