

**Genetic Determination of Stock of Origin for *Oncorhynchus mykiss*  
Collected in the Upper Deschutes River Basin.**

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Prepared by:  
Brice Adams, Patrick DeHaan, and Christian Smith  
U.S. Fish and Wildlife Service  
Abernathy Fish Technology Center  
1440 Abernathy Creek Road  
Longview, WA 98632  
(360) 425-6072  
brice\_adams@fws.gov

In cooperation with:  
Mike Riehle  
U.S. Forest Service  
Deschutes National Forest  
PO Box 249  
Sisters, OR 97759  
541-549-7702  
mriehle@fs.fed.us

## **Introduction**

Understanding the interactions between hatchery and wild individuals is important for developing effective conservation and management plans for salmonids. Numerous studies have used genetic data to address questions related to hatchery and wild interactions for a number of salmonid species. Previous studies of steelhead and rainbow trout (*Oncorhynchus mykiss*) have demonstrated that sympatric hatchery and wild populations remain reproductively isolated in some streams (Small et al. 2007; Matala et al. 2008) and interbreed in others (Araki et al. 2007; Small et al. 2007). Juvenile hatchery-origin steelhead from Round Butte Hatchery have been released into tributaries in the Upper Deschutes Basin (Figure 1) for several generations, and the interaction of these fish with the wild individuals in those streams is of interest. Releases were initiated in Whychus Creek in 2007, and the upper Deschutes River, Crooked River and its tributaries McKay and Ochoco creeks in 2008-2009 (Appendix 1). In addition, in 2012 fish passage was established at the Pelton Round Butte Dam on the Deschutes River. Round Butte Hatchery steelhead released as juveniles upstream of the dam now have access to spawning habitat above the dam when they return as adults.

Previously, the Applied Program in Conservation Genetics at Abernathy Fish Technology Center (AFTC) worked with biologists from the Oregon Department of Fish and Wildlife (ODFW) and Oregon State University (OSU) to examine the relationship between Round Butte Hatchery steelhead and wild *O. mykiss* in the Crooked River, a tributary to the upper Deschutes River above Pelton Round Butte Dam (Hawkins et al. 2011). Results of this study showed that steelhead from Round Butte Hatchery were genetically distinct from *O. mykiss* in the Crooked River and that a sample of unknown origin fish collected in the Crooked River were native Crooked River fish and not from Round Butte Hatchery.

The objective of this study was to assess the genetic difference between contemporary McKay and Whychus creeks *O. mykiss* populations and measure the level of introgression from Round Butte Hatchery steelhead. Additionally, we assessed the genetic relationship between those contemporary populations and collections previously taken from the upper Deschutes Basin (Matala et al. 2008, Hawkins et al. 2011).

## **Methods**

### *Sample Collection*

Fin clips were collected by ODFW, U.S. Forest Service (USFS) and Portland General Electric (PGE) from unclipped adult *O. mykiss* from August 2013 through October 2014 (henceforth to be labeled 2013) from McKay (n=35) and Whychus (n=40) creeks in the upper Deschutes Basin. All samples were preserved individually in 100% non-denatured ethanol.

Data generated for this study were combined with previously generated genotype data collected at AFTC (Table 1; Matala et al. 2008, Hawkins et al. 2011). Whychus Creek samples collected in 2005 were previously genotyped and analyzed by Matala et al. (2008). McKay Creek samples collected in 2005 had previously been genotyped at AFTC, but were not analyzed.

### *Laboratory analysis*

We extracted DNA from the 2013 McKay Creek and Whychus Creek collections using QIAGEN DNeasy 96 Blood & Tissue Kits following manufacturer's protocols (Qiagen Inc.). We conducted polymerase chain reactions (PCR) in 10 $\mu$ L volumes containing 2 $\mu$ L of template DNA, 5 $\mu$ L of 2X QIAGEN Multiplex PCR Master Mix (final concentration of 3mM MgCl<sub>2</sub>), 2.8 $\mu$ L of water and 0.2 $\mu$ L of oligonucleotide PCR primer mix. Primer mix concentrations and annealing temperatures for each multiplex are given in Appendix 1. PCR conditions were as follows: one denaturation cycle at 95°C for 15 minutes; 29 amplification cycles of 95°C for 30 seconds, primer specific annealing temperatures for 90 seconds (Appendix 2), and 72°C for 60 seconds; with a final extension cycle of 72°C for 10 minutes. Following PCR, the 17 amplified loci were screened on an automated DNA sequencer (ABI 3130xl, Life Technologies) using an internal size standard (Genescan-500 LIZ, Life Technologies) and analyzed using GENEMAPPER software version 4.0 (Life Technologies). Genotypes were scored by two independent readers (double-scoring). Following completion of the data collection, 10% of all samples were re-analyzed as part of AFTC's quality assessment/quality control (QA/QC) protocol.

### *Statistical analysis*

Initial analyses were carried out with the eleven independent collections indicated in Table 1. GENEPOP v4.0.10 (Raymond and Rousset 1995) was used to test for departures from Hardy-Weinberg equilibrium (HWE) for each locus and collection, and to test for evidence of linkage disequilibrium (LD) between all pairs of loci. Significant deviations from HWE expectations and high levels of LD can indicate non-random mating or non-random sampling within a population. Allelic richness and heterozygosity were calculated as measures of genetic diversity within populations. Allelic richness (the number of alleles per locus corrected for unequal sample sizes among collections) was determined with FSTAT v2.9.3 (Goudet 2001). Observed and expected heterozygosity ( $H_o$  and  $H_e$ ) were calculated using GDA v1.1 (Lewis and Zaykin 2001). Estimates of pairwise  $F_{ST}$  values among all collections were calculated using GENETIX v4.05.2 (Belkhir et al. 2004) and exact tests of genotypic differentiation were carried out using GENEPOP v4.0.10 to assess differences among collections from the McKay and Whychus creeks and Round Butte Hatchery. Bonferroni corrections for multiple tests were applied to P-values when assessing statistical significance of test results. Additionally, a factorial correspondence analysis was carried out using GENETIX v4.05.2 to view the genetic variation among all individual samples. This analysis represents each individual with a square on the graph and genetically similar individuals plot near each other.

We used the program PHYLIP v3.69 (Felsenstein 1993) to calculate genetic distance among collection locations using Cavalli-Sforza and Edwards' (Cavalli-Sforza and Edwards 1967) chord distance. This genetic distance was used to produce a neighbor-joining tree, to estimate the support for this tree the dataset was bootstrapped with 1,000 replicates. Based on the above neighbor-joining tree we reduced the number of populations of interest for the remaining population structure analyses removing Tumalo Creek, Crane Prairie Reservoir and Oak Springs Hatchery. Next we used the Bayesian clustering program STRUCTURE v2.3.4 (Pritchard et al. 2000) to determine the proportional membership of each individual to an inferred population cluster. We conducted STRUCTURE runs for 200,000 steps preceded by a 100,000 step burn-in period. We tested a range of possible clusters ( $K=1-8$ ) and ran simulations for 100 times per  $K$ . Results were processed with the program Structure Harvester v0.6.94 (Earl and vonHoldt 2012) to determine the optimal  $K$  using the method of Evanno et al. (2005). The program CLUMPAK (Kopelman et al. 2014) was used to provide a consensus among the 100 replicates for the optimal  $K$ . Finally we analyzed the dataset using a discriminant analysis of principal components

(DAPC; Jombart et al. 2010) implemented in the adegenet package (Jombart 2008) for the R software (R Developmental Core Team 2014). This analysis allowed us to examine the differences between the collections while minimizing the variation within collections.

Genetic assignment tests were used to assess the origins for the samples collected from McKay and Whychus creeks in 2013. A genetic baseline was generated with the following eight populations (reporting groups) based on the above comparisons among collections: 1) Round Butte Hatchery steelhead, 2) Whychus Creek (collected 2005), 3) McKay Creek (collected 2005), 4) upper Crooked River (collected 2010, 2005), 5) lower Crooked River (collected 2005), 6) Tumalo Creek (collected 2006), 7) Crane Prairie Reservoir (collected 2006) and 8) Oak Springs Hatchery steelhead (collected in 2006). Baseline data were assessed using ONCOR software (Kalinowski 2008) to evaluate how well fish could be assigned to their population of origin through a jackknife, or leave-one-out test. The test was conducted by removing fish from each collection one at a time, recalculating the population allele frequencies without that individual, and then estimating the origin of the removed fish. ONCOR was also used to assign population of origin for fish collected from the McKay and Whychus creeks samples collected in 2013 based on their multilocus genotypes. Fish were assigned to the population in which their genotypes had the highest probability.

## Results

Tests of HWE indicated only two statistically significant results involving the locus *One14* in the lower Crooked River and Oak Springs Hatchery collections, and locus *Omy7i* in the Tumalo Creek collections. Tests of LD indicated that the Round Butte Hatchery steelhead collection showed four tests were statistically significant out of 136 tests, the 2013 Whychus Creek collection had three statistically significant tests out of 136 tests statistically significant, while three other collections had very few significant LD results (Table 1). Allelic richness and heterozygosity values were similar among most collections, with the exception of the Whychus Creek 2005, lower Crooked River and Oak Springs Hatchery collections having reduced values of allelic richness and heterozygosity relative to other collections (Table 1).

Comparisons of divergence among the eleven collections included in this report revealed that the 2005 and 2010 upper Crooked River collections; the 2005 and 2013 McKay Creek collections; and the 2013 Whychus Creek and Round Butte Hatchery collections were

respectively genetically similar with no statistical differences at any loci and pairwise  $F_{ST}$  values close to, or equal to, zero (Table 2). The 2005 and 2013 Whychus Creek collections were significantly different from each other (Table 2). These similarities and differences are shown graphically in Figure 2. The collection of individuals from the 2013 Whychus Creek and Round Butte Hatchery primarily clustered together while a few individuals from the 2013 Whychus Creek collection clustered with the distinct 2005 Whychus Creek collection. While the individuals from the upper Crooked River and McKay Creek tended to form a larger cluster from the remaining populations.

The neighbor-joining tree (Figure 3) showed six strongly supported clusters (>80% bootstrap values). The strongest grouping (bootstrap support=98.6%) was for the Whychus Creek 2005 and lower Crooked River collections. The Round Butte Hatchery 2005 and Whychus 2013 collections formed a group with high bootstrap support (94.7%). Additionally the McKay Creek 2005 and 2013 collections grouped together with moderate support (bootstrap support = 87.6%).

Results of the STRUCTURE analysis, with Tumalo Creek, Crane Prairie Reservoir and Oak Springs Hatchery removed, suggested that the optimal number of clusters was  $K=3$  (Figure 4). The three clusters identified represent: Whychus Creek 2013 and Round Butte Hatchery; Whychus Creek 2005 and lower Crooked River; the upper Crooked River collections (McKay Creek 2005 and 2013, upper Crooked River 2005 and 2010).

A scatterplot of the first two principal components of the DAPC, with Tumalo Creek, Crane Prairie Reservoir and Oak Springs Hatchery removed, (Figure 5) separated the four collections representing the upper Crooked River; McKay Creek 2005 and 2013, upper Crooked River 2005 and 2010, from the remaining populations. The Whychus Creek 2005 collection loosely associated with the Whychus Creek 2013 and Round Butte Hatchery collections.

Leave-one-out tests showed that greater than 87% of individuals assigned back to the reporting groups from which they were collected, suggesting generally accurate assignment of individuals to the eight reporting groups. Using this baseline, 19 individuals collected in McKay Creek 2013 assigned to the McKay Creek 2005 reference population, 14 to the combined upper Crooked River 2005/2010 reference population, and 2 to the Round Butte Hatchery reference population, with a probability ranging from 0.54 to 1.00 (Table 4). For samples collected in Whychus Creek 2013, 31 individuals assigned to the Round Butte Hatchery reference

population, 6 to the Whychus 2005 reference population, and 3 to the combined upper Crooked River 2005/2010 reference population, with a probability ranging from 0.57 to 1.00.

## Discussion

The results of this study indicated that collections of *O. mykiss* trout from McKay Creek have had little impact from hatchery-origin fish from Round Butte Hatchery, with only 9% of *O. mykiss* showing some level of introgression or nearly a complete match with the hatchery-origin fish. However the same cannot be said for contemporary *O. mykiss* collected from Whychus Creek, which show high levels of association with hatchery-origin fish from Round Butte Hatchery. Seventy-five percent of fish assigned either completely or nearly completely back to Round Butte Hatchery. Only 15% of fish in the contemporary Whychus collection assigned back to the reference Whychus Creek population collected in 2005. Moreover, each of the clustering methods used here (neighbor-joining, correspondence analysis, and DAPC) indicated that the 2013 Whychus Creek collection was much more similar to Round Butte Hatchery than the 2005 Whychus Creek collection. Assuming that the 2005 and 2013 collections were representative of the Whychus Creek population in those two years, our results suggest a substantial increase in the influence of hatchery-origin fish in that watershed in the past two generations.

Genetic data from the study suggested that collections were found to be genetically distinct based on sampling location. There were little differences found between temporal samples within a sampling location, except for the Whychus Creek collections in 2005 and 2013. This primarily appears to be driven by the large number of Round Butte Hatchery steelhead discussed above. Genetic differentiation in the upper Deschutes River basin follows the pattern seen in previous work by Matala et al. (2008) and Hawkins et al. (2011). Both of those studies indicated that limited gene flow occurs between the three sampled populations, Whychus Creek, Tumalo Creek and Crane Prairie Reservoir, found in the Deschutes River. Additionally the two populations found in the upper Crooked River, McKay Creek and mainstem upper Crooked River, showed marked differentiation between themselves and that of the lower Crooked River.

The neighbor-joining tree and structure plot showed a strong association between fish collected in the lower Crooked River and fish collected in 2005 from Whychus Creek, despite these populations being genetically distinct. This suggests some level of gene flow occurred

between these two populations. Evidence of this relationship supports the need for additional sampling in the lower Crooked River to investigate whether the pattern seen between temporal samples in Whychus Creek is also occurring there.

## **Conclusions**

Assuming the collections examined here are representative of the populations from which they were taken, we conclude movement of hatchery-origin *O. mykiss* from Round Butte Hatchery, either as stocking of fry or less likely adult returns over Pelton Round Butte Dam, have had a strong influence on composition of the population inhabiting Whychus Creek. Round Butte Hatchery *O. mykiss* have predominantly displaced natural-origin *O. mykiss* from Whychus Creek in the past two generations. This same pattern does not seem to occur with contemporary McKay Creek. Hatchery-origin fish may have not replaced native stocks in McKay Creek due to reduced survival, migration out of the system, or reduced reproductive success.

## **Data Management Plan**

Raw (genotype) data generated in the course of the work described here have been archived in the U.S. Fish and Wildlife Service Abernathy Fish Technology Center Progeny Database.

## **Acknowledgements**

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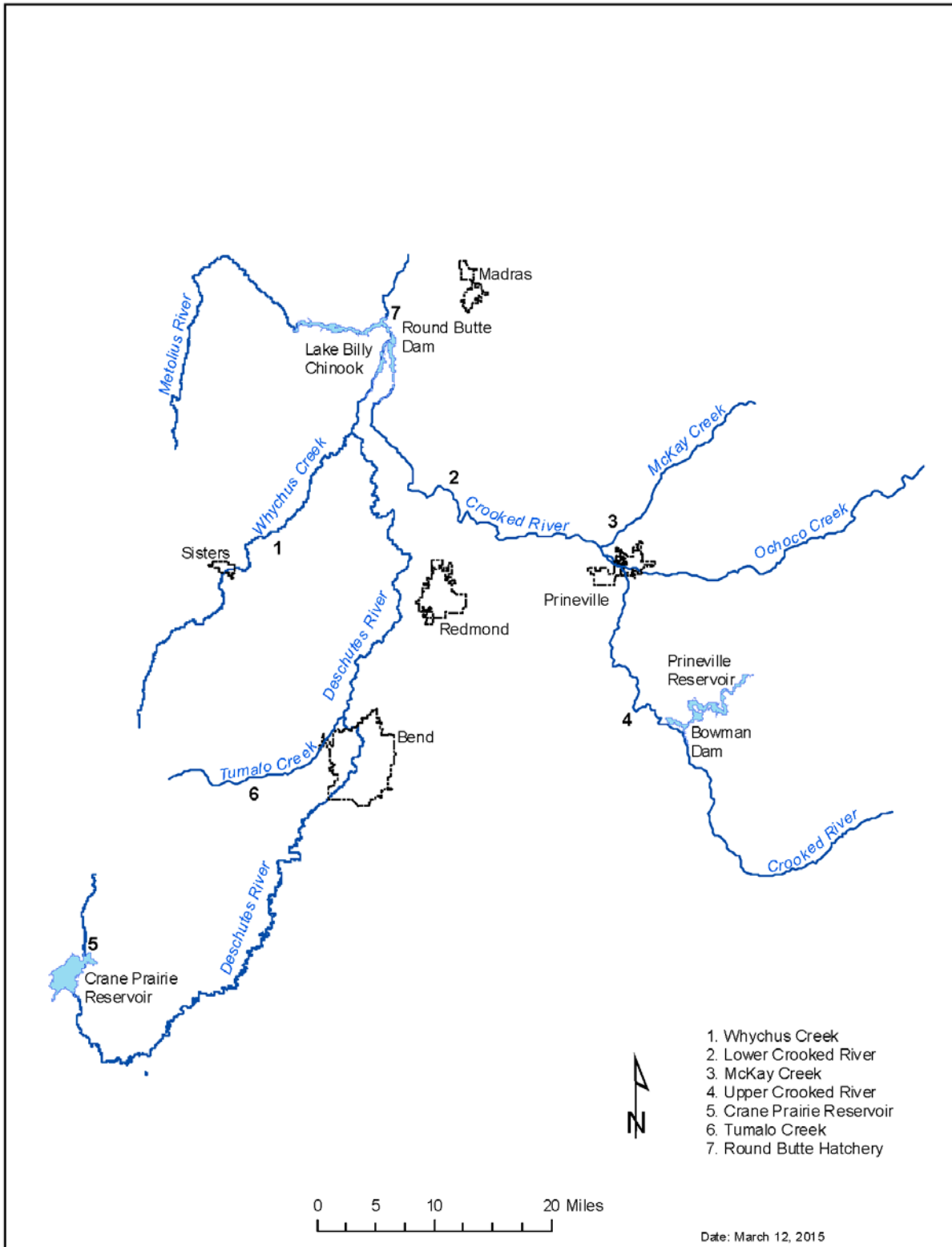


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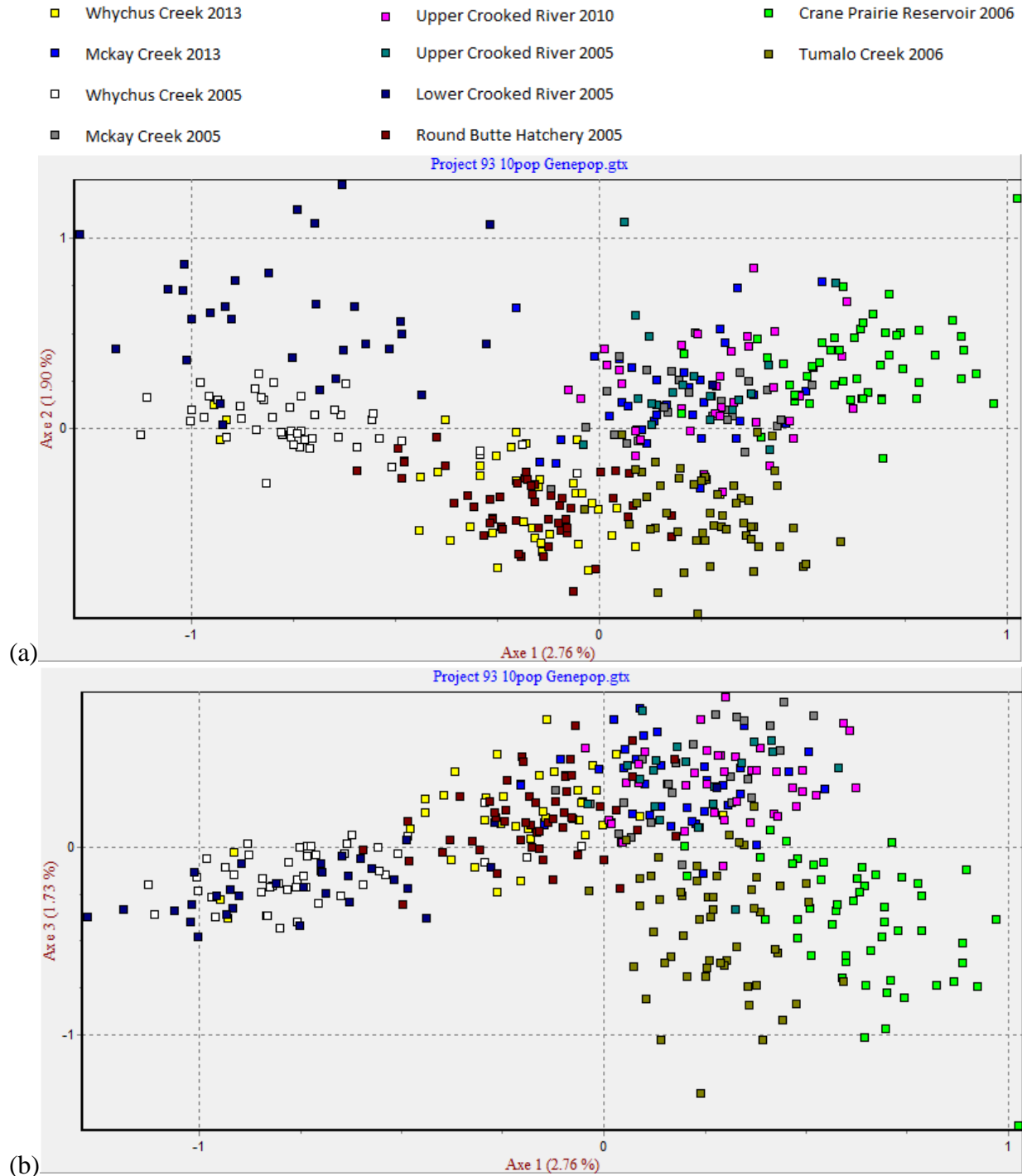
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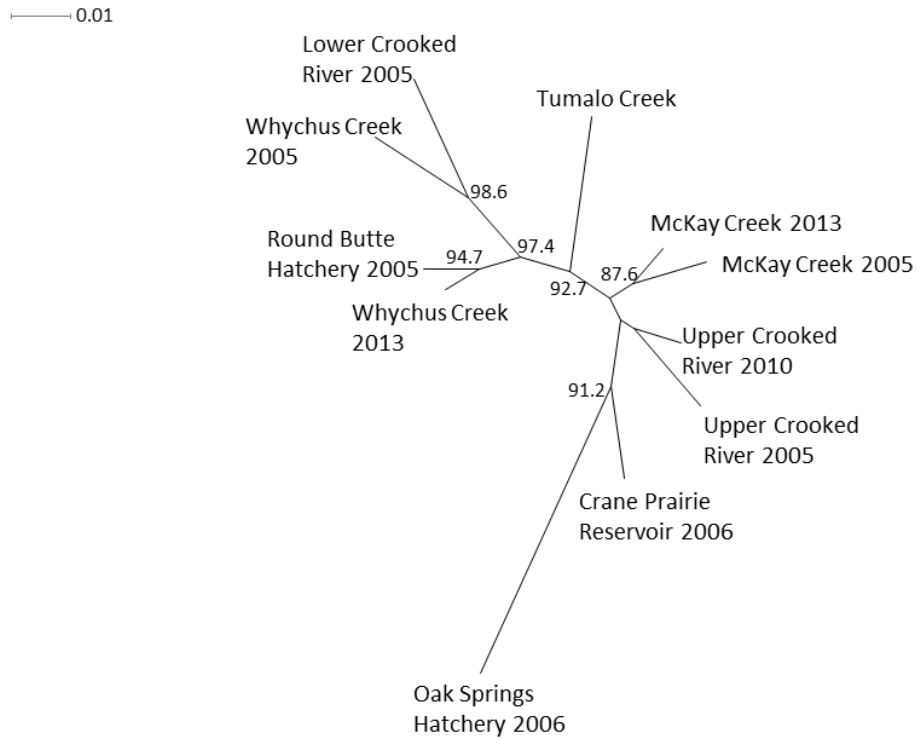
**Figure 1.** Map of upper Deschutes watershed with sample locations marked with individual numbers.



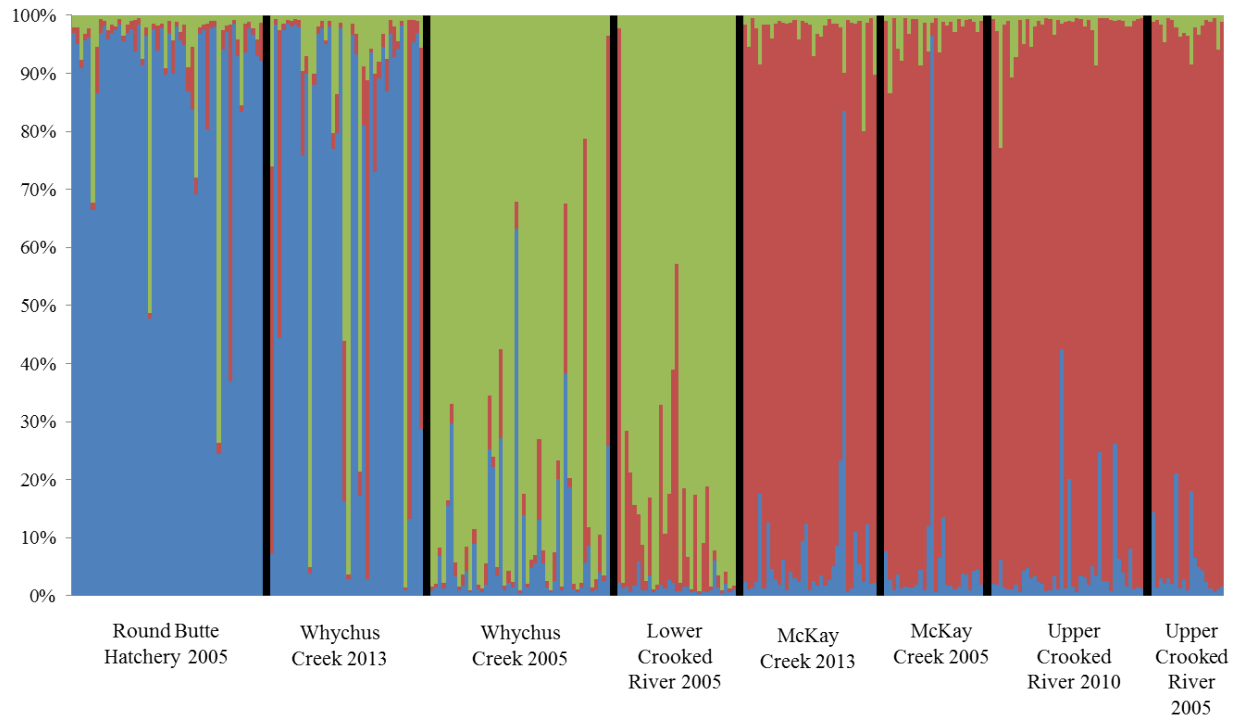
**Figure 2.** Results of the factorial correspondence analysis provide a graphical representation of genetic variation among populations of *O. mykiss* from the upper Deschutes system. Oak Springs Hatchery samples are removed to highlight samples of interest. Colors represent collection location. (a) represents factors 1 & 2 (b) represents factors 1 & 3.



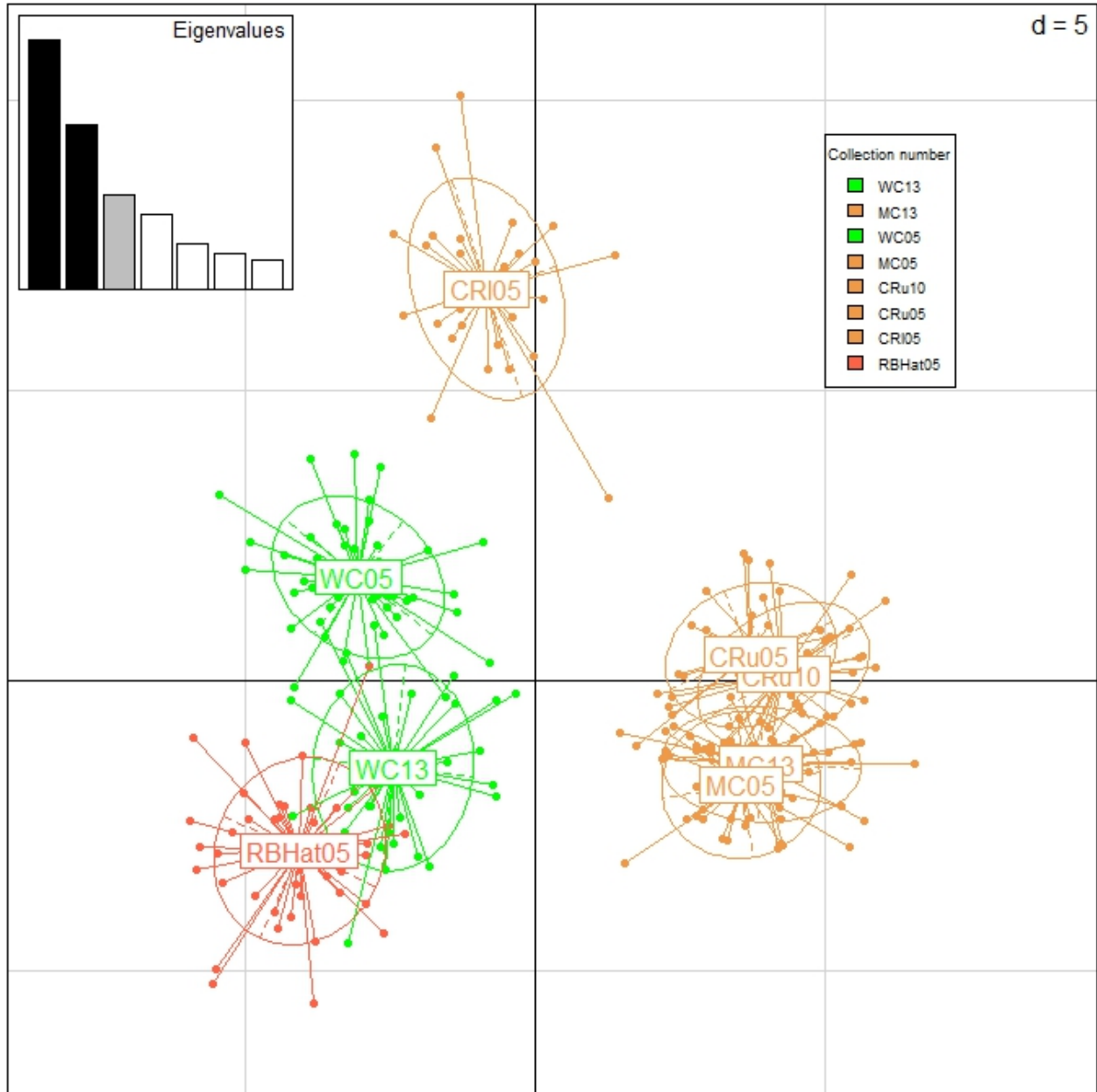
**Figure 3.** Neighbor-joining tree based on Cavalli-Sforza and Edwards' chord measure showing the relationship among populations of *O. mykiss* from the upper Deschutes system. Bootstrap values represent the percentage of time out of 1000 bootstraps that each branching pattern was observed, bootstrap values >80% are shown.



**Figure 4.** Output from the program Structure assuming  $K=3$  inferred populations. Each vertical bar on the graph represents an individual fish in the baseline dataset. The colors on each bar represent the portion of each individual's genotype that originated from each of the three inferred population clusters.



**Figure 5.** Scatterplot of individuals and collections on the first two principal components of discriminant analysis of principal components (DAPC). The figure represents individuals as dots the collections as ellipses. Tan represents individuals and collections from the Crooked River basin, green represents the Deschutes River basin and red represents Round Butte Hatchery. Tumalo Creek, Crane Prairie Reservoir and Oak Springs Hatchery collection were omitted from this analysis.



**Table 1.** Measures of within collection genetic diversity based on 17 microsatellite loci.

Collection	Population ID	N	HWE	LD	A <sub>r</sub>	H <sub>e</sub>	H <sub>o</sub>
Whychus Creek 2013	WC13	40	0	3	8.95	0.80	0.76
McKay Creek 2013	MC13	35	0	0	9.58	0.83	0.83
Whychus Creek 2005 <sup>2</sup>	WC05	47	0	2	7.23	0.69	0.70
McKay Creek 2005	MC05	26	0	0	9.02	0.81	0.80
Upper Crooked River 2010 <sup>1</sup>	Cru10	40	0	0	9.77	0.83	0.83
Upper Crooked River 2005 <sup>1</sup>	Cru05	19	0	0	9.55	0.82	0.80
Lower Crooked River 2005 <sup>1</sup>	CRI05	31	1	1	7.15	0.68	0.68
Round Butte Hatchery 2005	RBHat05	50	0	4	8.31	0.78	0.76
Crane Prairie Reservoir 2006 <sup>2</sup>	CP06	51	0	0	8.81	0.81	0.78
Tumalo Creek 2006 <sup>2</sup>	TC06	53	1	1	8.77	0.80	0.75
Oak Springs Hatchery 2006 <sup>2</sup>	OSHat06	56	1	0	5.49	0.69	0.67

HWE: Number of loci statistically out of Hardy-Weinberg Equilibrium expectations after Bonferroni correction ( $P: 0.05/17 = 0.003$ ).

LD: Number of pairs of loci out of 136 tests per population with significant linkage disequilibrium after Bonferroni correction ( $P: 0.05/136 = 0.00037$ ).

A<sub>r</sub>: Allelic richness.

H<sub>e</sub>: Expected heterozygosity.

H<sub>o</sub>: Observed heterozygosity.

<sup>1</sup> Collection from Hawkins et al. 2011

<sup>2</sup> Collection from Matala et al. 2008



**Table 2.**  $F_{ST}$  estimates (below diagonal) and P-values calculated using a permutation approach (above diagonal). Bold  $F_{ST}$  estimates are not significantly different from zero after Bonferroni correction ( $p < 0.001$ ).

	WC13	MC13	WC05	MC05	CRu10	CRu05	CRI05	RBHat05	CP06	TC06	OSHat06
Whychus Creek 2013	---	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.01</b>	0.00	0.00	0.00
McKay Creek 2013	0.03	---	0.00	<b>0.09</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Whychus Creek 2005	0.05	0.09	---	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
McKay Creek 2005	0.04	<b>0.00</b>	0.10	---	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Upper Crooked River 2010	0.04	0.02	0.10	0.02	---	<b>0.10</b>	0.00	0.00	0.00	0.00	0.00
Upper Crooked River 2005	0.04	0.02	0.10	0.03	<b>0.00</b>	---	0.00	0.00	0.00	0.00	0.00
Lower Crooked River 2005	0.08	0.10	0.05	0.12	0.11	0.10	---	0.00	0.00	0.00	0.00
Round Butte Hatchery 2005	<b>0.01</b>	0.03	0.06	0.05	0.04	0.04	0.09	---	0.00	0.00	0.00
Crane Prairie Reservoir 2006	0.07	0.04	0.13	0.05	0.04	0.05	0.14	0.07	---	0.00	0.00
Tumalo Creek 2006	0.04	0.04	0.09	0.05	0.04	0.05	0.11	0.05	0.04	---	0.00
Oak Springs Hatchery 2006	0.18	0.12	0.25	0.12	0.11	0.10	0.23	0.18	0.12	0.17	---

**Table 3.** Evaluation of the power of the genetic baseline for correctly assigning individuals to population using a jackknife, or leave-one-out test. The first column represents the collection name with subsequent columns representing the number of individuals from that collection (N), the percent of individuals that correctly assigned to its original collection location (% Correct), and numbers of fish that assigned to each baseline population.

Population	N <sup>1</sup>	% Correct	WC05	MC05	CRu10/05	CR105	RBHat05	CP06	TC06	OSHat06
Whychus Creek 2005	42	88.1	37	0	2	1	2	0	0	0
Mckay Creek 2005	17	94.1	0	16	1	0	0	0	0	0
Upper Crooked River 2010/2005	55	92.7	0	4	51	0	0	0	0	0
Lower Crooked River 2005	31	87.1	3	0	1	27	0	0	0	0
Round Butte Hatchery 2005	41	97.6	0	0	1	0	40	0	0	0
Crane Prairie Reservoir 2006	40	100.0	0	0	0	0	0	40	0	0
Tumalo Creek 2006	52	100.0	0	0	0	0	0	0	52	0
Oak Springs Hatchery 2006	54	100.0	0	0	0	0	0	0	0	54

1: only samples with complete genotypes were assigned

**Table 4.** Population assignment of individuals collected from McKay and Whychus creeks in 2013 and 2014.

Individual Name	Best Estimate	Probability	Second Best	Probability
<b>McKay Creek Samples</b>				
A-095	McKay Creek 2005	1.00		
A-097	McKay Creek 2005	1.00		
A-099	Upper Crooked River 2010/2005	0.72	McKay Creek 2005	0.28
B-048	McKay Creek 2005	0.84	Upper Crooked River 2010/2005	0.16
A-062	McKay Creek 2005	0.91	Upper Crooked River 2010/2005	0.07
B-047	McKay Creek 2005	1.00		
A-100	Round Butte Hatchery 2005	0.54	Upper Crooked River 2010/2005	0.30
A-098	McKay Creek 2005	0.81	Upper Crooked River 2010/2005	0.18
B-050	Upper Crooked River 2010/2005	1.00		
B-010	McKay Creek 2005	1.00		
B-014	McKay Creek 2005	1.00		
B-037	Upper Crooked River 2010/2005	0.84	McKay Creek 2005	0.16
B-039	McKay Creek 2005	1.00		
B-019	McKay Creek 2005	1.00		
B-049	Upper Crooked River 2010/2005	1.00		
B-013	Upper Crooked River 2010/2005	0.78	McKay Creek 2005	0.22
B-015	Upper Crooked River 2010/2005	1.00		
B-040	McKay Creek 2005	0.93	Upper Crooked River 2010/2005	0.07
B-008	Upper Crooked River 2010/2005	0.66	McKay Creek 2005	0.34
B-042	Upper Crooked River 2010/2005	1.00		
B-043	Upper Crooked River 2010/2005	0.96	McKay Creek 2005	0.04
B-051	Upper Crooked River 2010/2005	0.99	McKay Creek 2005	0.01
A-061	McKay Creek 2005	1.00		
B-021	McKay Creek 2005	1.00		
A-055	Upper Crooked River 2010/2005	0.90	McKay Creek 2005	0.10
A-096	Upper Crooked River 2010/2005	1.00		
B-016	Round Butte Hatchery 2005	0.95	Upper Crooked River 2010/2005	0.05

Individual Name	Best Estimate	Probability	Second Best	Probability
B-041	McKay Creek 2005	1.00		
A-056	Upper Crooked River 2010/2005	1.00		
B-022	McKay Creek 2005	0.54	Round Butte Hatchery 2005	0.39
B-011	McKay Creek 2005	1.00		
A-051	Upper Crooked River 2010/2005	1.00		
B-024	McKay Creek 2005	0.92	Upper Crooked River 2010/2005	0.08
B-038	McKay Creek 2005	1.00		
B-017	McKay Creek 2005	0.60	Upper Crooked River 2010/2005	0.40
<b>Whychus Creek Samples</b>				
1180-018	Round Butte Hatchery 2005	0.98	McKay Creek 2005	0.02
1180-019	Round Butte Hatchery 2005	1.00		
1180-020	Upper Crooked River 2010/2005	0.57	Round Butte Hatchery 2005	0.41
1180-021	Round Butte Hatchery 2005	1.00		
1180-022	Round Butte Hatchery 2005	1.00		
1180-023	Round Butte Hatchery 2005	1.00		
1180-024	Round Butte Hatchery 2005	1.00		
1180-026	Round Butte Hatchery 2005	1.00		
1180-027	Round Butte Hatchery 2005	1.00		
1180-028	Round Butte Hatchery 2005	0.98	Upper Crooked River 2010/2005	0.02
1180-029	Whychus Creek 2005	1.00		
1180-030	Round Butte Hatchery 2005	1.00		
1180-031	Round Butte Hatchery 2005	1.00		
1180-032	Round Butte Hatchery 2005	1.00		
1180-033	Round Butte Hatchery 2005	1.00		
1180-034	Round Butte Hatchery 2005	1.00		
1180-035	Round Butte Hatchery 2005	1.00		
1180-036	Round Butte Hatchery 2005	1.00		
1180-037	Round Butte Hatchery 2005	1.00		
1180-038	Whychus Creek 2005	0.94	Upper Crooked River 2010/2005	0.06

Individual Name	Best Estimate	Probability	Second Best	Probability
1180-039	Whychus Creek 2005	1.00		
1180-040	Round Butte Hatchery 2005	1.00		
1180-041	Round Butte Hatchery 2005	1.00		
1180-042	Whychus Creek 2005	1.00		
A-025	Round Butte Hatchery 2005	1.00		
A-006	Upper Crooked River 2010/2005	1.00		
A-009	Round Butte Hatchery 2005	1.00		
A-019	Round Butte Hatchery 2005	0.86	Upper Crooked River 2010/2005	0.13
A-008	Round Butte Hatchery 2005	1.00		
A-007	Round Butte Hatchery 2005	1.00		
A-011	Round Butte Hatchery 2005	1.00		
A-013	Round Butte Hatchery 2005	1.00		
A-012	Round Butte Hatchery 2005	1.00		
A-004	Round Butte Hatchery 2005	1.00		
A-010	Round Butte Hatchery 2005	1.00		
B-060	Whychus Creek 2005	1.00		
B-055	Upper Crooked River 2010/2005	0.83	McKay Creek 2005	0.17
B-061	Round Butte Hatchery 2005	0.99	Upper Crooked River 2010/2005	0.01
B-059	Round Butte Hatchery 2005	1.00		
B-057	Whychus Creek 2005	0.95	McKay Creek 2005	0.03

**Appendix 1.** Steelhead fry and smolt release data (courtesy ODFW). Values in red represent the total for each year.

<b>Year</b>	<b># Steelhead Fry Released</b>	<b>Release Site</b>
<b>2007</b>	275,000	Whychus Creek
<b>2008</b>	291,000	Whychus Creek
	40,000	Crooked River (Mainstem)
	70,000	Ochoco Creek
	124,000	McKay Creek
	<b>525,000</b>	
<b>2009</b>	91,584	Deschutes River (Mainstem)
	278,823	Whychus Creek
	239,269	Crooked River (Mainstem)
	86,172	Ochoco Creek
	136,440	McKay Creek
	<b>832,288</b>	
<b>2010</b>	17,462	Deschutes River (Mainstem)
	229,797	Whychus Creek
	212,460	Crooked River (Mainstem)
	46,566	Ochoco Creek
	105,502	McKay Creek
	<b>611,787</b>	
<b>2011</b>	43,161	Deschutes River (Mainstem)
	288,768	Whychus Creek
	172,752	Crooked River (Mainstem)
	71,999	Ochoco Creek
	129,186	McKay Creek
	<b>705,866</b>	
<b>2012</b>	27,664	Deschutes River (Mainstem)
	248,131	Whychus Creek
	161,934	Crooked River (Mainstem)
	54,660	Ochoco Creek
	116,864	McKay Creek
	<b>609,253</b>	
<b>2013</b>	38,362	Deschutes River (Mainstem)
	291,921	Whychus Creek
	125,179	Crooked River (Mainstem)
	80,240	Ochoco Creek
	88,264	McKay Creek
	<b>623,966</b>	

<b>Year</b>	<b># Steelhead Smolts Released</b>	<b>Release Site</b>
<b>2010</b>	750	Deschutes River / Lake Confluence
	3,600	Whychus Creek (FS Road 6360 Crossing)
	6,000	Crooked River (RM 38.5)
	3,000	Crooked River (Opal Springs Dam)
	<b>13,350</b>	
<b>2011</b>	795	Deschutes River / Lake Confluence
	5,456	Whychus Creek (FS Road 6360 Crossing)
	5,472	Crooked River (RM 38.5)
	2,000	Crooked River (Opal Springs Dam)
	<b>13,723</b>	
<b>2012</b>	500	Deschutes River / Lake Confluence
	4,871	Whychus Creek (FS Road 6360 Crossing)
	4,498	Crooked River (RM 38.5)
	2,000	Crooked River (Opal Springs Dam)
	<b>11,869</b>	
<b>2013</b>	780	Deschutes River / Lake Confluence
	2,209	Whychus Creek (FS Road 6360 Crossing)
	4,095	Crooked River (RM 38.5)
		Crooked River (Opal Springs Dam)
	<b>7,084</b>	

**Appendix 2.** Microsatellite loci used in this study.

Locus	Annealing Temperature (°C)	Final Primer Concentration (μM)
Ssa407	60	0.28
Omy1001UW	56	0.25
Oke4	56	2.00
Ots3	56	1.00
Ogo4	60	2.00
Omy1011UW	60	1.00
Omy7iNRA	60	0.50
Ssa289	56	0.66
Oneμ14	56	2.00
Ots100	60	0.66
Ots4	60	2.00
Ocl1	60	2.00
Oki23	60	2.00
Omy77	60	0.50
Ssa408	60	0.50
Ogo3	60	1.00
Ots1	60	1.00