

## The Origin and Ancestry of *Oncorhynchus mykiss* in the Wood River basin of Central Idaho

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### ABSTRACT

The origin and taxonomic identification of trout within the isolated Wood River basin of central Idaho has been in question for more than 120 years. The earliest surveys described trout specimens from the Wood River as Cutthroat Trout *Oncorhynchus clarkii*. Later surveys described them as Rainbow Trout *O. mykiss* and based on meristic examination of a single museum specimen, it was suggested they were a relict form of Redband Trout. Genetic investigations conducted over the last 30 years, using a variety of genetic markers, assumed that the native trout in the Wood River Basin were Columbia River Redband Trout *O. m. gairdneri*, but had been extensively introgressed or replaced with hatchery coastal Rainbow Trout *O. m. irideus*. In an attempt to disentangle the various hypotheses of native contemporary, native relict, non-native, or some admixture between native and non-native forms, we greatly expanded the sampling and genetic screening that had been completed in previous studies. Our results suggest that *O. mykiss* are native to the Wood River Basin, have been isolated for a long period of time, and represent a unique, old, and previously undescribed lineage of *O. mykiss*. Surprisingly, despite extensive hatchery stocking throughout the basin, introgression from non-native hatchery Rainbow Trout of coastal origin appears limited. We discuss management and conservation implications for current populations within the basin.



*Oncorhynchus mykiss* from the Wood River, Idaho (fish caught and photographed by Shawn Narum).

## INTRODUCTION

The origin and taxonomic identification of trout within the isolated Wood River basin of central Idaho has been in question for more than 120 years. The earliest surveys described trout specimens from the Wood River as Cutthroat Trout *Oncorhynchus clarkii* (Gilbert and Evermann 1894). Later surveys described them as Rainbow Trout *O. mykiss* and based on meristic examination of a single museum specimen, it was suggested they were a relict form of Redband Trout (Behnke 1979). Genetic investigations conducted over the last 30 years, using a variety of genetic markers including allozymes (Leary 2001; Williams et al. 1996), mitochondrial DNA (Williams and Shiozawa 1993), and microsatellite DNA (Kozfkay et al. 2011), assumed that the native trout in the Wood River Basin were Columbia River Redband Trout *O. m. gairdneri*, but they had been extensively introgressed or replaced with hatchery coastal Rainbow Trout *O. m. irideus*.

In an attempt to disentangle the various hypotheses of native contemporary, native relict, non-native, or some admixture between native and non-native forms, we greatly expanded the sampling and genetic screening that had been completed in previous studies. To examine the possibility of a non-native origin or intraspecific introgression we included the major lineages of Rainbow Trout and Cutthroat Trout that have been used in hatchery production and stocking purposes throughout the western United States. We included genetic markers that differentiate *O. clarkii* from these taxa from *O. mykiss* populations. Finally, in order to compare the genetic diversity and differentiation of Wood River *O. mykiss* in a broader phylogenetic context, we included samples of *O. mykiss* from throughout the Columbia River Basin.

## METHODS

### *Identifying reference populations for assessing inter- and intraspecific hybridization*

To determine the reference populations to assess intra- and interspecific hybridization/introgression, we first queried Idaho Department of Fish and Game's (IDFG) historical stocking database from 1913 to the present (<https://idfg.idaho.gov/ifwis/fishingplanner/stocking/>). We queried all stocking that had taken place in the Wood River Basin during this period and summarized all identified strains of Rainbow Trout, Cutthroat Trout, or hybrids. Strains labeled with the designation "triploid" were excluded. We identified 18 strains of Rainbow Trout stocked in the Wood River Basin as well as one designation identified as "Unspecified Rainbow". In total, more than 80 million Rainbow Trout were stocked in the basin from 1913 until 2000, when stocking switched primarily to non-fertile triploid Rainbow Trout stocks (Kozfkay et al. 2006). We also identified the stocking of steelhead (anadromous Rainbow Trout) x Cutthroat Trout hybrids, Rainbow Trout x Cutthroat Trout hybrids, Westslope Cutthroat Trout *O. c. lewisi*, Yellowstone Cutthroat Trout *O. c. bouvieri* (Henrys Lake origin), and unspecified Cutthroat Trout. These additional species/strains totaled ~3 million stocked fish over this period.

Of the 18 strains of hatchery Rainbow Trout we identified, we obtained reference genetic samples (N = 1,249) for 17 (strain and collection details available from authors). These samples came either from collections that we already had archived or from requests from other labs. Although we did not find records of McCloud River redband *O. m. stonei* strain having been stocked in the Wood River Basin, we did obtain reference samples from three populations. In doing so, we had reference samples from four of the primary California *O. mykiss* lineages (coastal, Eagle Lake, Golden Trout and McCloud River) used for hatchery strain development and stocking purposes (Behnke 1992). We felt this was important, since the vast majority (~78%) of stocking in the Wood River basin came from "Unspecified Rainbow" sources. In addition to these hatchery reference populations, we also included samples of Rainbow Trout from two populations in the Henrys Fork Basin in the upper Snake River, Idaho. As with all waters above Shoshone Falls on the Snake River, the Henrys Fork drainage historically did not contain populations of *O. mykiss* (Behnke 1992). It was stocked with hatchery Rainbow Trout during the same period as the Wood River Basin and most of the *O. mykiss* stocking came from "Unspecified Rainbow". The Henrys Fork drainage now supports robust, self-reproducing Rainbow Trout populations. We assumed, given the close geographic proximity to the Wood River basin, that the same sources of hatchery Rainbow Trout would have been stocked in both watersheds and these samples would provide good references for comparison purposes.

*O. mykiss* samples from the Wood River

Samples were collected via backpack electroshocking from throughout the Wood River Basin upstream of a barrier waterfall (Malad Gorge; Figure 1). Samples were collected between 1999 and 2021 (47 collections, 1,767 samples; Table 1). Samples were stored in 100% non-denatured ethanol or on Whatman filter sheets.

Table 1. Pedigree and collection name, sample size, total samples identified in Newhybrids as *O. mykiss*, Cutthroat Trout (CUT), F<sub>1</sub>, F<sub>2</sub>, Rainbow Backcross (RBT-BC), Cutthroat Trout Backcross (CUT-BCC), or F<sub>N</sub> hybrid (not assigning to other categories). Collection Names in bold exhibit interspecific hybridization. Also included are average “coastal” ancestries observed in each collection (Structure or DAPC). The three locations that showed evidence of coastal Rainbow Trout introgression with both analyses are shown italicized.

Map	Collection Name	N	<i>O. mykiss</i>	CUT	F1	F2	RBT-BC	CUT-BC	F <sub>N</sub> hybrid	Structure	DAPC MP
1	<i>Little Wood R. '03</i>	31	31	0	0	0	0	0	0	<i>0.278</i>	<i>0.139</i>
2	<b>Little Wood R. '99</b>	<b>103</b>	<b>73</b>	<b>4</b>	<b>10</b>	<b>1</b>	<b>4</b>	<b>4</b>	<b>9</b>	0.039	0.000
3	Little Wood R. '21	46	46	0	0	0	0	0	0	0.082	0.000
4	<i>Silver Cr. '05</i>	43	43	0	0	0	0	0	0	<i>0.243</i>	<i>0.044</i>
5	Cold Springs Cr. '20	40	40	0	0	0	0	0	0	0.062	0.000
6	Porcupine Cr. '20	30	30	0	0	0	0	0	0	0.016	0.000
7	Grays Cr. '99	13	5	0	0	<b>1</b>	<b>7</b>	0	0	0.010	0.000
8	<b>Little Wood R. '20</b>	<b>49</b>	<b>42</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>5</b>	<b>0</b>	<b>1</b>	0.029	0.000
9	Friedman Cr. '03	14	14	0	0	0	0	0	0	0.037	0.000
10	Friedman Cr. '21	15	15	0	0	0	0	0	0	0.043	0.000
11	Muldoon Cr. '04	24	24	0	0	0	0	0	0	0.057	0.000
12	Copper Cr. '05	9	11	0	0	0	0	0	0	0.057	0.000
13	<b>Mormon Gulch '20</b>	<b>46</b>	<b>30</b>	<b>0</b>	<b>3</b>	<b>2</b>	<b>6</b>	<b>0</b>	<b>5</b>	0.023	0.000
14	N.F. Soldier Cr. '20	4	4	0	0	0	0	0	0	0.022	0.000
15	Willow Cr. '03	41	43	0	0	0	0	0	0	0.034	0.000
16	W.F. Willow Cr. '20	50	50	0	0	0	0	0	0	0.004	0.000
17	W.F. Rock Cr. '21	20	20	0	0	0	0	0	0	0.063	0.000
18	Deer Cr. '99	59	59	0	0	0	0	0	0	0.025	0.000
19	Kinsey Cr. '20	50	50	0	0	0	0	0	0	0.040	0.000
20	Deer Cr. '21	50	50	0	0	0	0	0	0	0.034	0.000
21	Deer Cr. '20	50	50	0	0	0	0	0	0	0.042	0.000
22	Big Wood R. '03	46	46	0	0	0	0	0	0	0.052	0.000
23	Big Wood R. '21	179	179	0	0	0	0	0	0	0.056	0.000
24	Big Wood R. E.F. '03	30	30	0	0	0	0	0	0	0.039	0.000
25	Greenhorn Cr. '99	29	29	0	0	0	0	0	0	0.053	0.000
26	E.F. Big Wood R. '21	80	80	0	0	0	0	0	0	0.060	0.000
27	Hyndman Cr. '20	50	50	0	0	0	0	0	0	0.080	0.000
28	Elkhorn Gulch '21	17	17	0	0	0	0	0	0	0.092	0.000
29	Elkhorn Cr. '19	8	8	0	0	0	0	0	0	0.054	0.000
30	Trail Cr. '99	36	36	0	0	0	0	0	0	0.093	0.000
31	Warm Springs Cr. '03	26	26	0	0	0	0	0	0	0.093	0.000
32	Red Warrior Cr. '99	60	60	0	0	0	0	0	0	0.047	0.000
33	Rooks Cr. '21	28	28	0	0	0	0	0	0	0.100	0.000
34	Thompson Cr. N.F. '03	24	24	0	0	0	0	0	0	0.061	0.000
35	N.F. Thompson Cr. '21	50	50	0	0	0	0	0	0	0.069	0.000
36	Castle Cr. '99	27	27	0	0	0	0	0	0	0.030	0.000
37	Placer Cr. '20	49	49	0	0	0	0	0	0	0.020	0.000
38	Castle Cr. '20	48	49	0	0	0	0	0	0	0.056	0.000
39	S.F. Warm Springs Cr. '21	50	50	0	0	0	0	0	0	0.035	0.000
40	Adams Gulch '99	40	40	0	0	0	0	0	0	0.016	0.000
41	<i>Trail Cr. '03</i>	12	12	0	0	0	0	0	0	<i>0.236</i>	<i>0.155</i>
42	Trail Cr. '21	40	40	0	0	0	0	0	0	0.073	0.000
43	Wilson Cr. '20	9	9	0	0	0	0	0	0	0.011	0.000
44	Fox Cr. '21	50	50	0	0	0	0	0	0	0.027	0.000
45	<b>Baker Cr. '21</b>	<b>27</b>	<b>24</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	0.041	0.000
46	E.F. N.F. Big Wood R. '20	45	45	0	0	0	0	0	0	0.048	0.000
47	Trail Cr. '20	17	17	0	0	0	0	0	0	0.021	0.000

### *DNA Extractions and Genetic Marker Panel*

We extracted DNA from all samples using the nexttec™ Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland). All samples were genotyped with a panel of 379 loci described in Collins et al. (2020). We used custom scripts to identify loci within the panel that exhibited additional Single Nucleotide Polymorphisms (SNPs) not described by Collins et al. (2020) and then genotyped these loci for all identified SNPs while inferring phase from the sequencing reads. In total, we identified 229 microhaplotype loci that exhibited  $\geq 3$  alleles and genotyped well in reference and study populations. This panel was genotyped following Polymerase Chain Reaction, barcoding, and library protocols described in Collins et al. (2020) and libraries were sequenced on an Illumina NextSeq 500 instrument.

### *Genetic Assessments of Inter- and Intraspecific Hybridization*

We assessed interspecific hybridization (from Cutthroat Trout) using a subset of 17 loci in our panel that exhibit fixed or nearly fixed diagnostic allelic differences between *O. mykiss* and Cutthroat Trout (IDFG unpublished data). We used the software program NewHybrids (Anderson and Thompson 2002) to estimate the probability that a sample belongs to one of six *a posteriori* hybrid categories based on patterns in allelic inheritance. Fish were classified into the following categories: RBT (non-hybridized *O. mykiss*), CUT (non-hybridized *O. clarkii*), first generation hybrids ( $F_1$ ), second generation hybrids ( $F_2$ ), *O. mykiss* Trout backcrosses (RBT-BC) or Cutthroat Trout backcrosses (CUT-BC). Individuals that did not assign to a single category with a posterior probability  $\geq 99\%$  were classified as “ $F_N$  hybrid” (i.e. belonging to a hybrid class not defined by NewHybrids and are likely later stage backcrosses). For these analyses, we included reference samples from various Idaho locations including Yellowstone Cutthroat Trout from Henrys Lake, Palisades Creek, Bonneville Cutthroat Trout from the Bear River (Cottonwood Creek), Westslope Cutthroat Trout from Ball Creek, and known  $F_1$  hybrids from Henrys Lake along with all samples from Wood River Basin collections. NewHybrids was run with 50,000 burn-in iterations followed by 50,000 sample iterations, with reference individuals identified.

To assess the possibility of intraspecific hybridization/introgression, we included the baseline of reference hatchery *O. mykiss* strains discussed above and samples of *O. mykiss* from a wide geographic area of Idaho. This included sample collections of *O. mykiss* from the Boise, Salmon Falls, and Owyhee basins of southern Idaho, geographically proximate to the Wood River Basin. We also included native resident and anadromous *O. mykiss* from the Snake, Salmon, Clearwater and Kootenai rivers of Idaho. We used two methods to assess genetic population structure and to evaluate the ancestry of *O. mykiss* populations in the Wood River. We used the Bayesian model-based method implemented in the software Structure 2.33 (Pritchard et al. 2000) to identify genetic clusters and estimate ancestry coefficients (q-values) and the non-model based method of discriminate analysis of principle components (DAPC) using the R package Adegenet version 1.3-1 (Jombart et al. 2008) to provide individual membership probabilities for each sample.

To assess the genetic relationships of *O. mykiss* samples from our entire dataset (147 collections, 8,327 samples) we again used DAPC analyses using Adegenet. We also constructed an unrooted, bootstrapped ( $N = 1000$ ) Neighbor-Joining (NJ) tree based on pairwise Cavalli-Sforza Edwards chord distance (Cavalli-Sforza and Edwards 1967) calculated in GENDIST (PHYLIP v3.5; Felsenstein 1993). The tree was produced using the NEIGHBOR method in PHYLIP v3.5 and subsequently visualized and edited using FigTree v.1.4.3 (Page 1996).

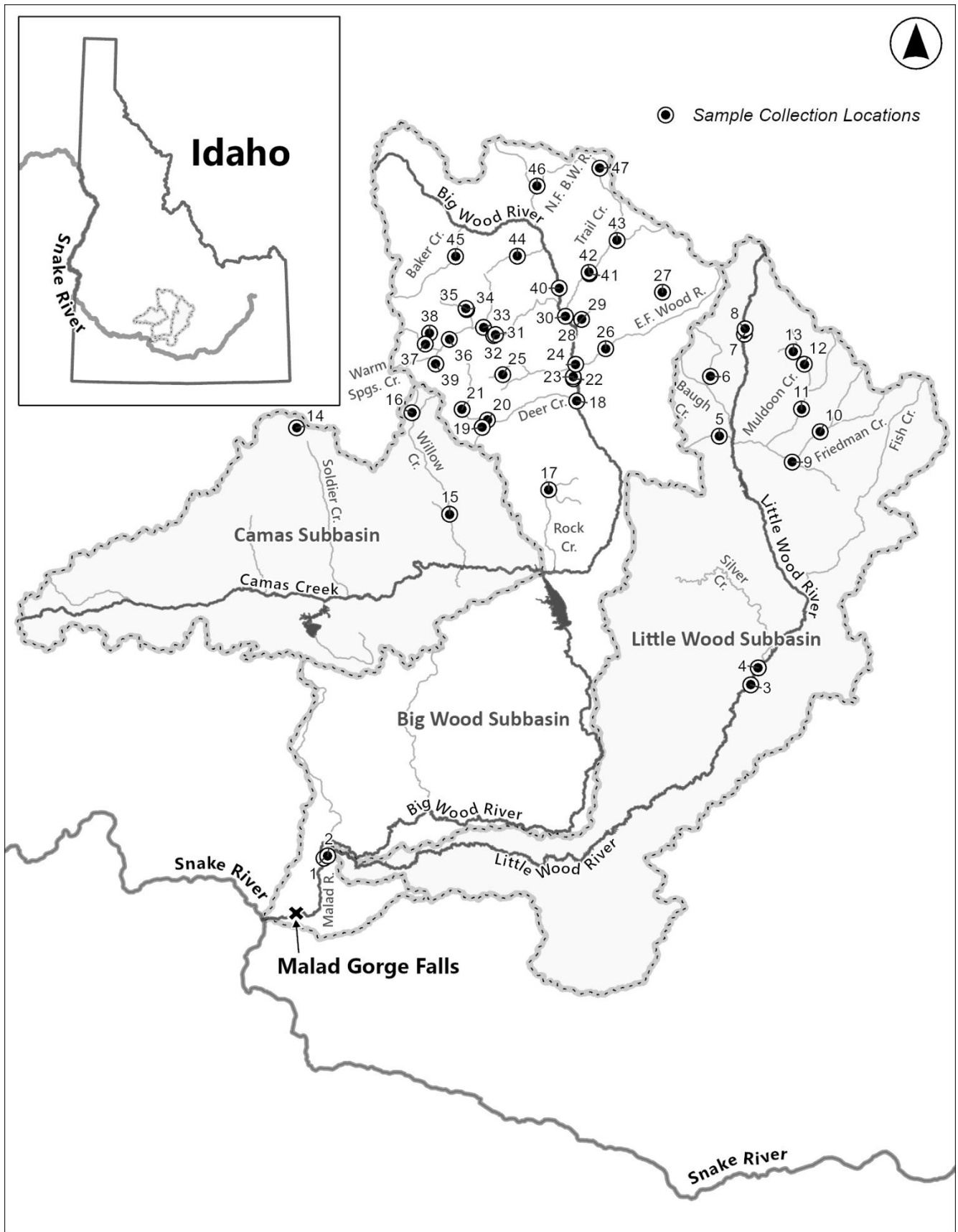


Figure 1. Map of sample collections within the Wood River Basin. Sample site numbers correspond to numbers in Table 1. Location of the Malad Gorge Falls is shown, which is an upstream migration barrier.

## RESULTS AND DISCUSSION

### *Interspecific Hybridization*

Hybrids between Cutthroat Trout and *O. mykiss* were observed in four sample locations (5 collections): Baker Creek (2021), Grays Creek (1999), Little Wood River (1999, 2020) and Morman Gulch Creek (2020; Table 1). All sample locations identified with hybrids, except Baker Creek (2021), were from the Little Wood River drainage. First generation hybrids were detected in 3 of the 4 sample collections, indicating recent hybridization events. The highest number of F<sub>1</sub> hybrids were identified in the Little Wood River-1999 sample collection (N = 10). This sample collection also contained four samples with genotypes indicative of pure Cutthroat Trout. These samples were visually identified in the field as putative Cutthroat Trout. Of the 1,831 samples from the Wood River drainage examined, 1,767 (96.5%) exhibited genotypes indicative of pure *O. mykiss*.

Intraspecific hybridization was assessed based on ancestry coefficients (q-values) reported from Structure and membership probabilities provided by DAPC (Table 1). For both methods, we used 10 genetic clusters (K) to summarize results. This was a smaller number than the most likely number of genetic clusters identified by either method but provided a sufficient number from which to efficiently summarize the data (Jombart and Collins 2015). In other words, higher numbers (15 and 20) of genetic clusters did not change ancestry levels observed in Wood River collections. Any genetic cluster that received assignments from hatchery reference populations were designated as “coastal” clusters. For Wood River sample collections we combined values observed in “coastal” clusters to provide an estimate of coastal ancestry. Both methods identified three collections as having ancestry likely from coastal origin hatchery fish. Trail Creek-2003, Little Wood River-2003 and Silver Creek exhibited q-values of 0.236, 0.278 and 0.243 respectively and membership probabilities of 0.155, 0.139 and 0.044, respectively. Bohling et al. (2013) argued that q-values observed from Structure > 0.2 usually indicate the real detection of non-native ancestry. Of the remaining sample collections, Structure identified a low level of coastal ancestry in all populations (range 0.004 - .0997; average 0.0465). These results differed from DAPC analyses, which did not identify coastal ancestry in any of the remaining sample collections.

### *Intraspecific Hybridization*

To assess overall genetic relationships among *O. mykiss* collections throughout the Columbia River Basin, Idaho, Great Basin Redband Trout, and California hatchery Rainbow Trout stocks, we included all collections/samples (147 collections, 8,327 samples) in DAPC and Phylip NJ tree analyses (Figure 2). In the DAPC analyses, the first discriminant separated Wood River *O. mykiss* collections (Cluster 9) and hatchery coastal Rainbow Trout strains *O. m. irideus* (Cluster 10) from all Columbia River Redband Trout *O. m. irideus* collections (Clusters 1,2,4,5, and 7). Known non-native Rainbow Trout from the Henrys Fork drainage in Idaho all assigned to Cluster 10 with hatchery coastal Rainbow Trout strains. The second discriminant separated all Wood River collections from all the hatchery coastal Rainbow Trout strains. Several collections of Great Basin Redband Trout and collections from the lower Columbia River, while showing strong individual clustering, yielded intermediate positions in the DAPC plot.

The NJ Tree topology and bootstrap support results were consistent with the relationships observed in the DAPC plot. For example, all collections of Columbia River Redband Trout clustered together (749 support). This included all collections in Idaho outside the Wood River Basin, including samples from the Kootenai River Basin, steelhead from the Clearwater, Grande Ronde, and Salmon River basins, and resident Redband Trout populations from the Dry Creek, Boise, Salmon Falls, and Owyhee River drainages. Collections of *O. mykiss* from the Wood River drainage all clustered together (768 support) and were diverged from hatchery coastal Rainbow Trout collections. All hatchery coastal Rainbow Trout strains clustered together (953 support) and were most similar to collections of *O. mykiss* from the Chewaucan, Goose, and Warner Lake drainages. Similar phylogenetic results were previously reported by Currens et al. (2009) using allozyme analyses. Also similar to Currens et al. (2009) study, we observed that collections from Malheur Lake appeared to be more genetically similar to Columbia Basin Redband Trout than to other Great Basin Redband Trout populations.

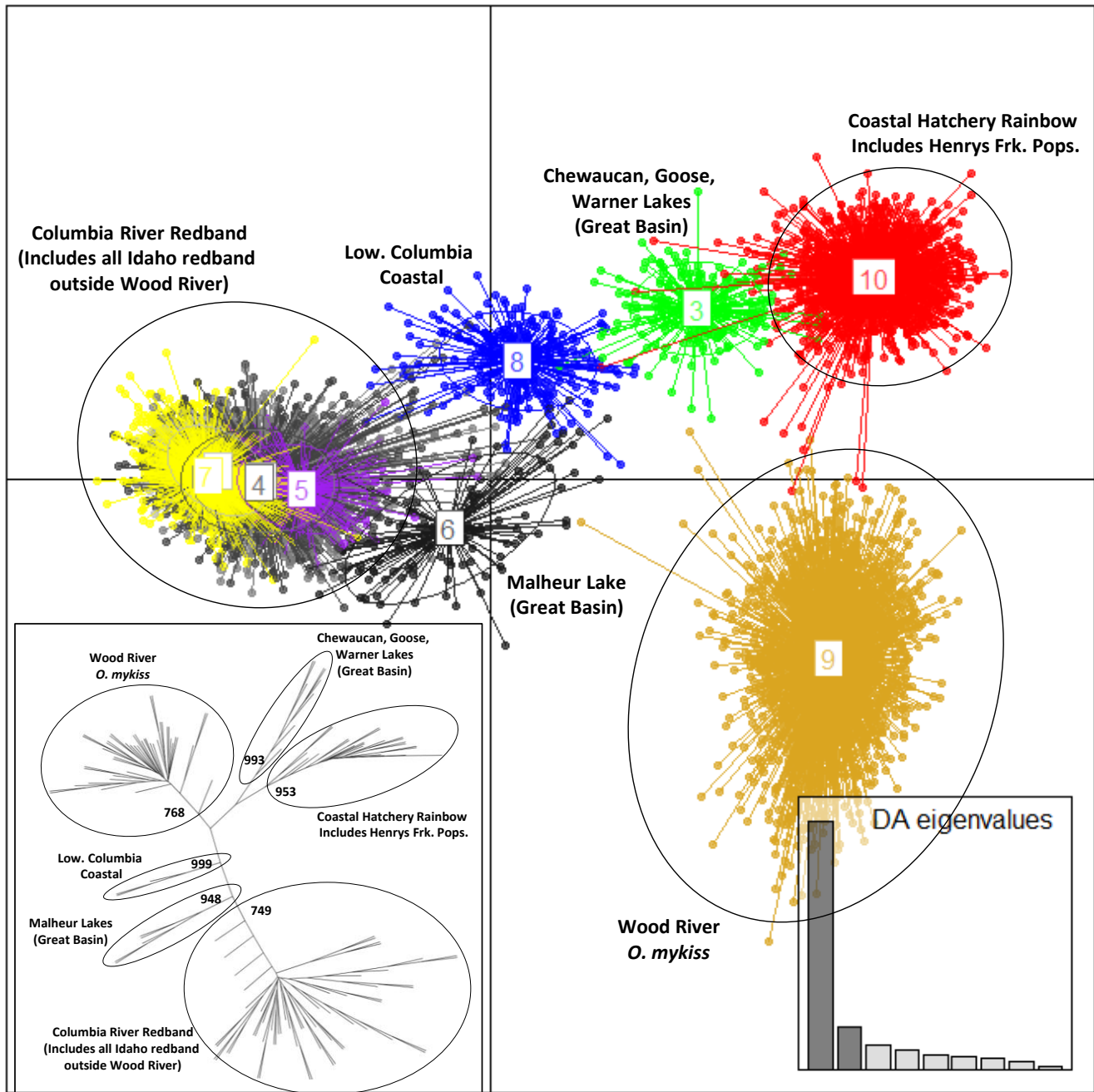


Figure 2. Scatterplot of all individuals on the two principal components of DAPC. Lower Right inset are DA eigenvalues. Left inset is a NJ tree based on pairwise Cavalli-Sforza Edwards chord distance showing similar patterns of genetic clustering among sample collections (>50 bootstrap support).

The isolated Wood River Basin has been noted previously as having “faunal peculiarities” (Hubbs and Miller 1948). The basin contains an endemic species of sculpin *Cottus leiopomus*, and genetically divergent populations of Bridgelip Sucker *Catostomus columbianus* (Smith 1966) and Mountain Whitefish *Prosopium williamsoni* (Miller 2006). Results from this study indicate that *O. mykiss* in the Wood River Basin appear genetically diverged from all other sampled *O. mykiss* populations in Idaho. This divergence is surprising given the speculation that the colonization timing of *O. mykiss* in the Snake River may be constrained by the Bonneville flood (14,500 years before present) and presumably the formation of Shoshone Falls, which marks the upstream range of *O. mykiss* in the Snake River (Behnke 1992). This divergence does not appear to be due to inter- or intraspecific hybridization despite an extensive history of stocking within the basin. We observed hybridization from Cutthroat Trout in only a limited number of sites and analyses suggest recent hybridization

(pure CUT, F<sub>1</sub> and backcrosses detected). These results can be explained by the previous stocking of diploid Westslope Cutthroat Trout in adjacent high mountain lakes to provide fishing opportunities. We ran two analyses to assess whether populations showed evidence of hatchery coastal Rainbow Trout ancestry. While both methods identified three sample collections that showed moderate levels of non-native introgression, the remaining sites showed low (avg. ~5% from Structure) or no evidence of coastal Rainbow Trout ancestry (from DAPC).

Despite the fact that *O. mykiss* in the Wood River show limited evidence of hatchery coastal Rainbow Trout introgression, they appear to share a more recent (but distant) common ancestor with *O. mykiss* lineages found outside the Columbia River Basin. The ancestral Snake River was not connected to the Columbia River until the draining of ancient Lake Idaho during the late Pliocene (~2 million years ago; Gillerman et al. 2006). Instead, the Snake River was believed either to have flowed southeast through Oregon to California into the Klamath or Sacramento Basins, or south, near Hagerman, Idaho through the Humboldt River and Lahontan Basin (reviewed in Hershler and Liu 2004). These connections provided opportunities for faunal exchange between these southern basins and the Wood River. Multiple periods of basaltic volcanism and megaflooding (Lamb et al. 2013) throughout the Pleistocene near the vicinity of the Wood River and its connection with the Snake River (Malde 1971) provided numerous opportunities to isolate the Wood River Basin and the fish species in it. We believe this is the most likely explanation for the shared pattern of divergence observed across taxa.

We argue that this study provides evidence that *O. mykiss* in the Wood River Basin represent a distinct, previously undescribed lineage, unique from populations throughout the species range. The obvious caveat to our findings, is that our study is limited to 229 nuclear DNA loci. We suggest that ongoing work examining mitochondrial DNA, morphology, and karyotyping be completed before a taxonomic assessment can be performed. In the meantime, we believe this finding is significant and deserves continued conservation and management attention.

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