

ARTICLE

Describing Fine-Scale Patterns of Genetic Structure and Introgression of Redband Trout in a Complex River System

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Abstract

The conservation status of Redband Trout *Oncorhynchus mykiss gairdneri* has been an increasing concern of fish managers. Effective fish management first requires an understanding of the spatial distribution of distinct populations and the processes influencing gene flow. We performed a genetic analysis of Redband Trout from the Deschutes River basin in central Oregon to discern population genetic structure and the genetic impacts of an extensive hatchery stocking program and several potential barriers to dispersal. Conducting surveys in lateral habitats, we sampled over 1,400 young-of-the-year Redband Trout and genotyped them at a panel of 269 SNPs using genotyping-in-thousands by sequencing. We found that within this section of the Deschutes River basin there were multiple distinct genetic groups of Redband Trout, with an irrigation diversion dam and only one of eight waterfalls in the study area acting as complete barriers to gene flow. Within these distinct genetic groups there was a strong signal of isolation by distance. Despite the extensive stocking of large numbers out-of-basin hatchery Rainbow Trout *Oncorhynchus mykiss*, our results indicated that introgression of wild fish occurred only with a locally derived hatchery strain of Redband Trout. Hatchery influence was greatest in Fall River and in neighboring portions of the Deschutes River. The combination of spatially explicit sampling in lateral habitat with genotyping via high-throughput sequencing provided an effective sampling design for this large river and its tributaries. Such an approach may be useful elsewhere for identifying genetic management units of Redband Trout and other widespread freshwater fishes.

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Resident Rainbow Trout *Oncorhynchus mykiss* that occur east of the Cascade Mountain range, commonly known as Redband Trout *O. mykiss gairdneri* (Behnke 1992; Currens et al. 2009), have received increasing interest from fish managers regarding their conservation status (Muylfeld et al. 2015). Although still widely distributed, this species has declined to an estimated 58% of its historical range and there are varying levels of protection for remaining populations (Muylfeld et al. 2015). Questions have been raised in relation to effective long-term management regarding Redband Trout distribution, abundance, and interaction with hatchery-stocked salmonids, as well as the effects of human activities on habitat and how projected climate warming is likely to affect habitat (Muylfeld et al. 2015; Penaluna et al. 2016).

Given their extensive range, Redband Trout naturally occur in a variety of riverine systems possessing diverse physical and biological properties. To effectively manage natural populations and monitor the influence of human activity on species distribution and abundance, population genetic structure first needs to be determined and the appropriate management units identified (Ryman 1991). Previous research on Redband Trout has shown a high degree of population genetic structure between tributaries and a tendency for isolation by distance (Wishard et al. 1984; Knudsen et al. 2002; Small et al. 2007; Kozfkay et al. 2011). Past studies have primarily consisted of between-tributary comparisons, usually collecting genetic samples in lower-order streams and treating samples collected from individual streams as a single homogenous unit. However, larger rivers compose an important component of the species' overall distribution, including areas important for spawning and rearing. Large rivers are often heterogeneous and possess attributes, both of natural and anthropogenic origin, that can drive genetic structure. These attributes include fish passage barriers (Griffiths et al. 2009; Gouskov et al. 2016), habitat heterogeneity (Kanno et al. 2011; Daugherty et al. 2017; Pilger et al. 2017), differential hatchery stocking patterns (Hindar et al. 1991; Eldridge and Naish 2007; Hansen et al. 2009), and extensive river length that can generate isolation by distance (Griffiths et al. 2009; Gouskov et al. 2016; Pilger et al. 2017).

The Deschutes River, located in central Oregon, is one such large river system that supports Redband Trout throughout much of its watershed. Fish in the middle and upper segments of the basin are entirely freshwater residents, blocked by natural and anthropogenic barriers that prevent anadromy. This portion of the basin is heterogeneous and contains several natural features that could drive genetic structure (Fies et al. 1996). There is also concern regarding a number of anthropogenic impacts on Redband Trout in this area. Dams have altered water temperatures and the natural flow of wood and sediment

and blocked fish dispersal and migration corridors, and the annual discharge regime has been shifted from its renowned natural stability (Gannett et al. 2003; O'Connor and Grant 2003) to managed seasonal extremes for agricultural irrigation. The fish assemblage has been altered by the extirpation of native Bull Trout *Salvelinus confluentus*, the introduction of nonnative fish species (Zimmerman and Ratliff 2003), including invasive Brown Trout *Salmo trutta*, a marked decline in the Redband Trout recreational fishery (Fies et al. 1996), and an extensive hatchery salmonid stocking program to compensate for the decline of the fishery (Table 1; Matala et al. 2008). Redband Trout spawn extensively in the main stem of this large river, as well as in its major tributaries (Starcevich and Bailey 2017), but the presence and pattern of hatchery influence and overall genetic structure of Redband Trout in this basin segment are unknown, and this lack of knowledge hinders informed conservation and management.

In this case study, we examined hatchery introgression and the genetic structure of Redband Trout from this section of the Deschutes River basin, which includes four tributaries, 154 km of main-stem river, nine waterfalls, and two dams. Previous research in the Deschutes River basin has observed genetic structuring among specific local populations of Redband Trout (Currens et al. 1990; Matala et al. 2008; Adams et al. 2015), but genetic structure of this species across this section of the basin, particularly within the main stem of the Deschutes River itself, has not been studied. Understanding these patterns has implications for management of Redband Trout in this basin, including hatchery stocking practices and assessing status and effects of management actions.

Our objectives were to (1) assess the extent of the genetic influence of the three hatchery strains of *O. mykiss* stocked in this part of the basin, (2) characterize the genetic structure of Redband Trout inhabiting the Deschutes River and its tributaries, and (3) evaluate the influence of river distance on genetic relatedness. Based on previous research in the upper Deschutes River basin (Matala et al. 2008) and general findings that hatchery-reared *O. mykiss* tend to have reduced fitness in natural environments (Araki et al. 2007, 2009), we hypothesized that it would be unlikely for out-of-basin hatchery strains to produce offspring or introgress with wild fish. We also anticipated that major breaks in genetic structure would correspond to significant barriers to fish passage, such as waterfalls and dams. Since there are multiple barriers along this segment of the Deschutes River, we expected to identify multiple genetically distinct units of Redband Trout occupying discrete stretches of the river. Across the overall system we hypothesized that any gene flow that does occur should follow a linear stepping-stone model, with gene flow highest between neighboring populations.

TABLE 1. Summary of selected hatchery stocking activities conducted by the state of Oregon in the Deschutes River study area from 1981 through 2015. The summary includes stocking information for out-of-basin strains of Rainbow Trout from the Cape Cod Hatchery (CCH) and Oak Springs Hatchery (OSH) and for a locally derived strain of Redband Trout from Wizard Falls Hatchery (WFH), both diploid and triploid (T) hatchery fish. The summary also includes the total number of fish released and the count of individual years in which releases occurred during the reported time span. Data are from the Hatchery Management System database managed by the Oregon Department of Fisheries and Wildlife.

Release location	Hatchery strain	Total released	Annual releases	Time span (year)	
				First	Last
Study reach 1–4		0	0		
Study reach 5–6	CCH	503	1	1984	1984
Tumalo Creek	CCH	39,243	27	1981	2010
(Shevlin Pond)	CCH (T)	36,190	10	2006	2015
	WFH	443	1	2007	2007
Study reach 7–10	CCH	422,594	16	1981	1996
	CCH (T)	6,510	2	2008	2009
	WFH	99,152	12	2002	2015
	WFH	12,381	1	2000	2000
Little Deschutes River	OSH	2,010,480	29	1981	2012
(Paulina Lake)	WFH	174,552	10	2003	2015
Fall River	CCH	186,738	18	1981	2009
	CCH (T)	8,757	2	2014	2015
	WFH	89,667	12	2002	2015
Wickiup and Crane Prairie reservoirs	OSH	4,627,081	35	1981	2015
	OSH (T)	264,008	7	2006	2012
	WFH	588,652	10	1999	2008
	WFH (T)	131,310	3	2013	2015
	WFH	13,647	3	2005	2013
	CCH	218,126	7	1981	2003
	CCH (T)	5,000	1	2014	2014

METHODS

Study system.—Our study focused on the Deschutes River basin (Figure 1) between Steelhead Falls (river kilometer [rkm] 206 of the Deschutes River, measured from its confluence with the Columbia River) and Wickiup Dam (rkm 365). Steelhead Falls, historically not a barrier to anadromy, is upstream from the Lake Billy Chinook impoundment created by the Round Butte Hydroelectric Dam (rkm 177), which is currently a barrier to anadromy. Wickiup Dam is a complete barrier to upstream fish

passage. Within this area, the river flows through forests of ponderosa pine *Pinus ponderosa*, high desert sagebrush steppe, and some urban areas, including the cities of Bend and Redmond. There are several notable natural waterfalls, high-velocity rapids, artificial barriers, and water diversion structures. Big Falls (rkm 213), which is located upstream of Steelhead Falls, was the historical barrier to anadromy in the Deschutes River, and Redband Trout above this waterfall are native residents. North Canal Dam (rkm 265), located in the city of Bend, was constructed in 1912 and had no upstream fish passage facilities until 2017. The Deschutes River tributaries thought to contain Redband Trout spawning in the study segment were Tumalo Creek, lower Little Deschutes River, Spring River, and Fall River. All these tributaries were surveyed, but we did not capture Redband Trout in Spring River.

Sampling.—The study area was divided into 500-m sites ($N = 418$) among 14 reaches using a geographical information system (ArcGIS; ESRI, Redlands, California) (Figure 1). These reaches corresponded to natural and artificial barriers hypothesized to influence gene flow, tributaries, and confluences with major tributaries and, hereafter, are referred to as sampling groups. Sample sites were selected using the generalized random-tessellation stratified (GRTS) design (Stevens and Olsen 2004), stratified by sampling group. The GRTS sampling design draws sites in an order that ensures a spatially balanced sample for any set of consecutively numbered sites (Stevens and Olsen 2004), which is useful when it is uncertain what proportion of the sampling frame will be surveyed during the study period. We followed the GRTS draw order for each sampling group, and sample sites were added near the ends of each reach and near the confluence of tributaries when there was poor sampling coverage for these areas in the GRTS draw. This sampling design was selected to ensure that there was a random, spatially balanced sample that was representative of the Redband Trout population within each sampling group. Within each site, we collected juvenile Redband Trout via boat or backpack electrofishing surveys (see Starcevich and Bailey 2017 for more sampling details). Targeting young-of-the-year (age 0) Redband Trout, we conducted surveys in the shallow, low-velocity lateral habitats (see Moore and Gregory 1998; Beechie et al. 2005) that are easily accessed along the channel margin. From July 8 to October 8, 2015, we surveyed 139 sites; proportionally by site, this covered 33% of the 209-km linear study area.

Redband Trout fin clips were immediately stored in 100% nondenatured ethanol for later genetic analysis. We estimated genetic metrics from a single cohort of age-0 fish, which had the advantage of minimizing potential temporal variation in allele frequencies. We collected tissue samples from 1,480 Redband Trout. Based on a length-frequency analysis (see Starcevich and Bailey 2017), 1,288 of these were classified as young of the year. Additional tissue samples

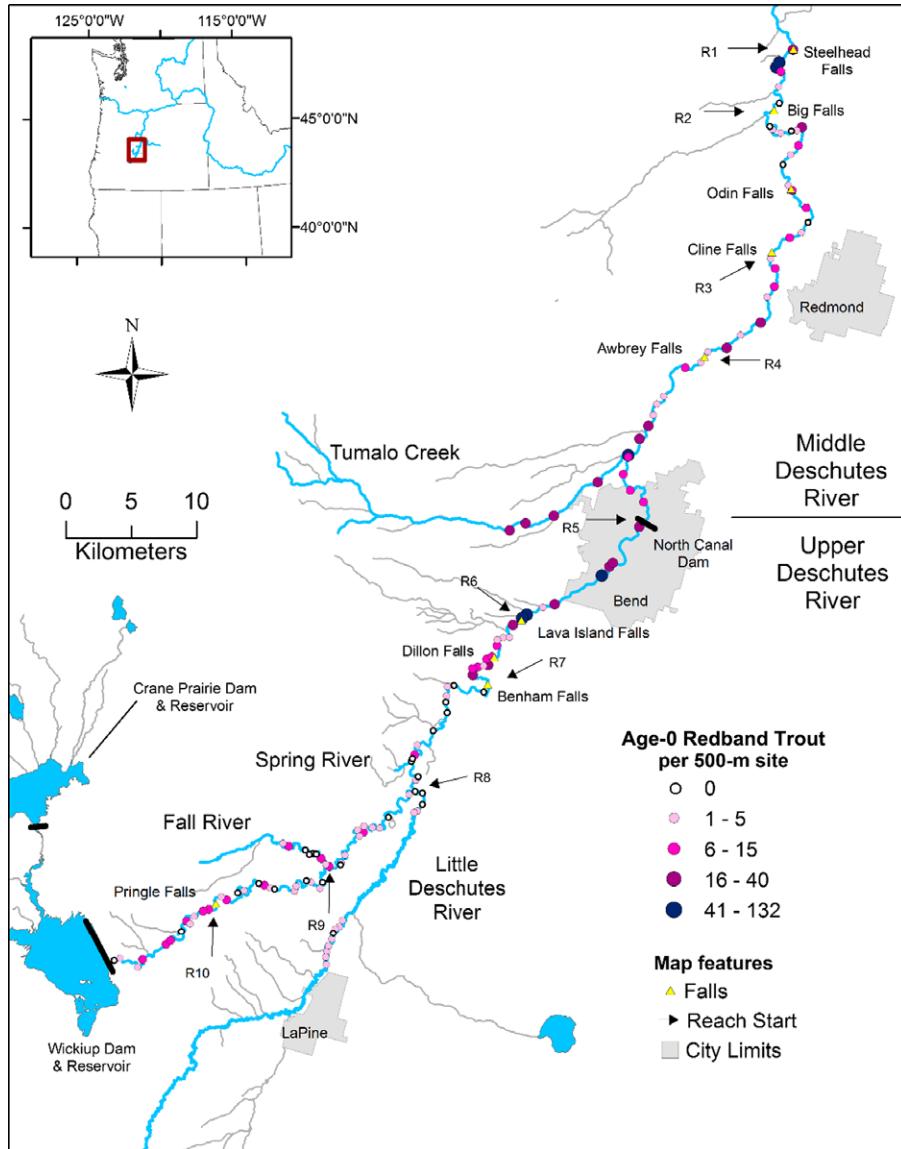


FIGURE 1. Map of the Deschutes River basin study area, study reach (i.e., sampling group, indicated by “R” followed by a number) delineations, and sample sites that indicate the number of captured age-0 Redband Trout. Tributaries of the watershed that were sampled (i.e., main-stem Deschutes River, Tumalo Creek, Fall River, and Little Deschutes River) are denoted in blue; other unsampled tributaries are in gray.

($n = 35$) were provided from a hatchery strain of Redband Trout raised at Wizard Falls Fish Hatchery (WFH). This strain was established using native Redband Trout from Crane Prairie Reservoir in the upper Deschutes River basin as broodstock. The WFH strain is currently stocked in Fall River (Table 1). This strain was also released annually in several locations in the upper Deschutes River from Benham Falls upstream to Wickiup Dam (reaches 7–10) through 2015. We also obtained tissue samples from two common out-of-basin hatchery strains of Rainbow Trout: Cape Cod (CCH; $n = 46$) and Oak Springs (OSH; $n = 48$). Both of these strains were cultured from populations of *O. mykiss*

irideus in California. Although these strains are not currently stocked in the study area, they were stocked in the past (Table 1; Table S1 available in the Supplement provided in the online version of this article), and samples from each were included to test for introgression. During all field surveys, we followed guidelines recommended by the Use of Fishes in Research Committee (2014) for animal welfare and stress avoidance while capturing, handling, and transporting fish and for minimizing habitat disturbance and mortality.

Library preparation and genotyping.—The DNA extractions followed a modified protocol using Qiagen DNeasy Tissue Kits (Qiagen, Valencia, California). We used the

genotyping-in-thousands (GT-seq) approach (Campbell et al. 2015) to genotype our samples at a panel of 269 SNP loci using high-throughput sequencing technology. To summarize the process, extracted DNA samples were first cleaned in an ExoSAP reaction (New England Biolabs, Ipswich, Massachusetts). Then each sample was amplified in a PCR containing the forward and reverse primers for all loci and Qiagen Plus MasterMix. In a new set of 96-well PCR plates, a unique i7 index primer was added to each plate followed by an aliquot of the amplified PCR product. Each well of these plates then received one of 96 i5 index primers. The combination of the i7 and i5 primers creates a unique series of genetic barcodes to identify each individual in the library. We used SequelPrep Normalization Kits (ThermoFisher Scientific, Grand Island, New York) to normalize the PCR products. Samples from the same plate were then pooled together and subjected to a bead size selection procedure using AgencourtAMPure beads (Beckman Coulter Life Sciences, Indianapolis, Indiana). We quantified the amount of DNA product using a Kapa qPCR quantification kit (Kapa Biosystems, Wilmington, Massachusetts) using four different dilutions (1:1,000, 1:2,000, 1:4,000, 1:8,000). Based on the results of the DNA quantification, these per-plate pools were combined and normalized to a 5-nM concentration. The final pooled library was run on an Illumina NextSeq (Illumina, San Diego, California) with a 100-cycle mid-output kit. The sequencing was performed at the Columbia River Inter-Tribal Fish Commission Hagerman Genetics Laboratory. Genotyping based on the sequence reads was performed using the scripts outlined in Campbell et al. (2015).

Testing for introgression.—We tested for introgression with hatchery stocks using two methods. First, we performed a correspondence analysis based on allele frequencies to identify sampling groups that showed similarity with hatchery stocks. Our second analysis used the Bayesian clustering approach implemented by the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003) to identify admixed fish. This method simultaneously identifies genetic clusters among a group of individuals and the probability of assignment to those clusters. We used the correlated allele frequency model, inferring alpha for each population, and allowed for admixture. The program STRUCTURE was run in parallel using the package ParallelStructure (Besnier and Glover 2013) for R 3.2 (R Core Team 2015). Because STRUCTURE can be sensitive to sample sizes (Kalinowski 2011), we ran the full data set ($n = 1,377$) and a subset with a maximum of 50 randomly chosen individuals per sampling group ($n = 657$) to confirm the patterns of clustering. In the analysis we allowed K to vary from 1 to 16 with five replicates per value. We considered individual Redband Trout to be admixed between wild and hatchery stocks if they produced

ancestry coefficients (or q -values) greater than 0.2. There are no standard q -value thresholds produced by STRUCTURE for classifying individuals as hybrids (Vähä and Primmer 2006; Bohling et al. 2013). The value of $q > 0.2$ is a conservative value based on common practice in the literature and is analogous to classifying an individual as a hybrid if 20% or more of its ancestry assigns to a hatchery stock. Studies suggest that $q > 0.2$ is often indicative of true ancestry for a particular group, whereas lower levels can be due to statistical noise (Bohling et al. 2013).

Population genetics.—For each sampling group of wild-caught fish, we conducted tests of deviations from Hardy-Weinberg proportions (HWPs). Exact tests of HWPs were conducted using GenePop 4.2 (Rousset 2008). For these tests we set our P -value threshold for significant deviation of HWPs at 0.05. Because we analyzed each population at all 262 autosomal loci (see Results), we had a total of 3,406 separate tests of significance for HWPs. To minimize type I error due to multiple tests, we performed several additional analyses to compliment the raw HWP P -values. First, we used a cumulative binomial function to estimate whether the number of significant tests we observed fell outside the expected range given the number of tests and $\alpha = 0.05$ (Waples 2015). We also adjusted our P -values using the false discovery rate (FDR) procedure (Benjamini and Hochberg 1995).

To test for population effects, we compared locus-specific estimates of F_{IS} within all 13 sampling groups and the proportion of positive and negative values. A trend in F_{IS} values in either direction is indicative of population processes that can influence HWPs (Waples 2015). For the number of positive and negative values we observed, we performed a χ^2 test of equal proportions. Locus-specific effects were determined by estimating the expected number of deviant populations per locus using a binomial distribution and $\alpha = 0.05$.

Overall estimates of observed heterozygosity (H_o), expected heterozygosity (H_e), and heterozygote excess (F_{IS}) were generated for each sampling group using the R package diveRsity (Keenan et al. 2013). We also calculated the proportion of loci that were polymorphic within each group.

Genetic structure.—To assess genetic structure we performed several analyses. First, we performed pairwise estimates of genetic differentiation between sampling groups. We used the metric G_{ST} (Nei and Chesson 1983) implemented in diveRsity with 95% confidence intervals estimated using 10,000 bootstrap replicates. In addition, we estimated directional relative migration rates using the divMigrate function in diveRsity, which is based on the method described by Sundqvist et al. (2016). Relative migration does not estimate the number of migrants per generation but instead migration rates between two populations relative to others in a system. It complements G_{ST}

by providing directionality to the estimate of gene flow between populations. We estimated the relative migration rate matrix, which is scaled from 0 to 1, for pairs of populations using the G_{ST} metric. For relative migration, higher values indicate relatively greater exchange of genes between groups compared with the entire data set.

To disentangle the natural structure from the artificial groupings created by the sampling scheme, we conducted a discriminant analysis of principal components (DAPC) (Jombart et al. 2010) based on allele frequencies, which provide a model-free multivariate perspective on population structure. We used the `find.clusters` function in the R package `adegenet` (Jombart 2008) to identify the grouping with the lowest Bayesian information criterion (BIC) score based on K -means clustering. We estimated the posterior probability of individual membership to each of these identified clusters (i.e., distinct genetic groups identified among the data set), which is analogous to ancestry coefficients, for an individual Redband Trout. We did this for multiple values of K that produced similar BIC scores.

Adding a spatial component to our evaluation, we performed a Mantel test between individual genetic distance and geographic distance using the R package `ecodist` (Goslee and Urban 2007). Genetic distance was estimated using the R package `poppr` (Kamvar et al. 2014). Geographic distance was represented as stream distance between capture locations for individual Redband Trout and was estimated using the R package `riverdist` (Tyers 2017). Reach 1 was excluded because it was highly distinct from other sampling groups (see Results). With the Mantel test we also estimated spatial autocorrelation using a simple Mantel correlogram. Because we identified North Canal Dam as a barrier to gene flow (see Results), we also conducted a Mantel test for samples downstream of the dam (reaches 2–4 and Tumalo Creek) and those upstream (reaches 5–10 and Little Deschutes River).

RESULTS

Sequencing Results

The GT-seq library produced 139.9 million DNA sequence reads that were 100 base pairs in length. Removing negative controls, the average number of reads per individual was 97,142 ($SD = 82,441$). On average 41,455 ($SD = 33,087$) of those reads per individual were on-target reads corresponding to the loci in our panel. One locus (*Omy_rbm4b-203*) did not produce usable genotypes and was removed from the data set. Across individuals, the mean proportion of loci genotyped was 93.9% (minimum = 0%, maximum = 100%, $SD = 13.5\%$). We decided to retain individuals that were genotyped at over 70% of the loci, resulting in a data set containing 1,377

individuals. Among these 1,377 individuals, the mean genotyping success rate per locus was 96% ($SD = 9.2\%$). The final data set contained 263 loci (which included a single sex-ID marker); for the subsequent analyses the sex-ID marker was not included.

Hatchery Introgression

The correspondence analysis revealed that the OSH and CCH strains were divergent from wild populations, the WFH strain was intermediate in multivariate space between wild Deschutes River populations and the other out-of-basin hatchery strains, and Fall River was the only population of Redband Trout that clustered with the WFH strain (Figure S1 available in the Supplement provided in the online version of this article).

For the STRUCTURE results, divisions were more clearly defined using the subset of 50 individuals per sampling group; thus, we report those results. The primary division was between the wild Redband Trout and the two out-of-basin hatchery strains. At $K = 2$ the WFH strain had almost equal assignment to those two groups. Wild populations formed distinct clusters at higher levels of K . At $K = 7$ the WFH strain formed a distinct cluster and Fall River had the highest proportion of ancestry assigned to the WFH strain and the highest proportion of individuals with q -values (i.e., ancestry coefficients) greater than 0.2 for that cluster (Table 2). Only reaches 7, 8, 9, 10, and Fall River had any individuals with $q > 0.2$ WFH ancestry. Excluding Fall River, only 5 out of the 25 individuals with $q > 0.2$ also produced a value greater than 0.8, suggesting individuals with moderate values ($0.2 < q < 0.8$) may be hybrids between wild Redband Trout and the WFH strain.

Population Genetics

Out of 3,406 tests of HWPs, 269 produced P -values below the 0.05 threshold. Another 299 locus–population pairings could not be tested because the sampling group was fixed (i.e., monomorphic) at that particular locus. With an error rate of 0.05 under a binomial distribution, we expected between 146 and 195 significant tests to occur by random chance. The number we observed (269) was much higher. We observed trends in the distribution of HWP deviations across populations and loci (Table S1). Most sampling groups tended to display a positive F_{IS} skew across loci; however, of the three significant χ^2 tests we observed, two displayed negative F_{IS} values. At the locus level, the number of sampling groups out of HWP for a particular locus ranged from 0 to 12. Assuming a binomial random distribution, we expected at most four sampling groups to display significant P -values per locus; however, we observed 15 loci with significant values in five or more sampling groups, suggesting some locus-level effects.

TABLE 2. Average proportion of ancestry assigned to the Wizard Falls Hatchery (WFH) strain by STRUCTURE for the wild Deschutes River populations. These are based on the mean q -value at $K = 7$ using a subset of 50 individuals per population in the analysis. Reaches and tributaries are organized from furthest downstream to farthest upstream.

Sampling group	Average WFH ancestry	Proportion of individuals with $q > 0.2$ WFH ancestry
Reach 1	0.007	0.00
Reach 2	0.010	0.00
Reach 3	0.007	0.00
Reach 4	0.009	0.00
Tumalo Creek	0.031	0.00
Reach 5	0.006	0.00
Reach 6	0.016	0.00
Reach 7	0.169	0.21
Little Deschutes River	0.008	0.00
Reach 8	0.313	0.50
Fall River	0.734	0.89
Reach 9	0.047	0.11
Reach 10	0.062	0.05
WFH	0.843	1.00

When the raw P -values were corrected for multiple tests using the FDR method, the number of significant tests dropped to 69. With this FDR correction, most sampling groups produced less than five significant deviations, except for reach 4 (11 deviations), reach 5 (15), reach 6 (8), and Tumalo Creek (13). At the locus level, most loci had deviations in three or fewer sampling groups; four (*Omy_986831*, *Omy_aromat*, *Omy_GHSR12*, *Omy_hus152*) had significant deviations in four and two (*OMS00018* and *OMS00173*) had significant deviations in five or more. Most of the deviations observed for these six loci were for the cluster of sampling groups (reaches 4–6 and Tumalo Creek) that had the most overall FDR-corrected deviations.

Levels of heterozygosity were relatively similar across all 13 sampling groups (Figure 2A): the highest values were observed in Fall River, reach 8, and reach 10 and the lowest values in the Little Deschutes River and reach 5. Every sampling group was fixed for a single allele in less than 15% of loci with the exception of the Little Deschutes River, which had over 22% of loci fixed (Figure 2B). The F_{IS} value tended to positive, with every sampling group from reach 6 downstream producing 95% confidence intervals that did not overlap zero (Figure 2C). The Little Deschutes River was the only group to produce

a highly negative F_{IS} , but its 95% confidence interval did overlap zero.

Population Genetic Structure

Average pairwise G_{ST} between sampling groups was 0.030, and no estimate produced a 95% confidence interval that overlapped zero. The highest differentiation was observed between reach 1 and all other sampling groups (G_{ST} range = 0.043–0.095; Figure 3). The Little Deschutes River (G_{ST} median = 0.033) and Fall River (median = 0.040) also produced relatively high values when compared with other sampling groups. The lowest values of differentiation were observed between adjacent reaches 2, 3, and 4 and Tumalo Creek (G_{ST} range = 0.004–0.012), between reaches 5 and 6 (0.006), between reaches 6 and 7 (0.005), and between reaches 9 and 10 (0.002). There was relatively high differentiation between reaches 4 and 5 (0.037), which were separated by North Canal Dam. For relative migration rates, the average value across all pairs of sampling groups was 0.26 with a standard deviation of 0.19. When rates were filtered to display those above 0.5, several groupings emerged (Figure 4). The following pairings showed high bidirectional relative migration: reaches 2 and 3 (≥ 0.81), reaches 3 and 4 (≥ 0.97), reach 3 and Tumalo Creek (≥ 0.56), and reach 4 and Tumalo Creek (≥ 0.65). Unidirectional relative migration was observed from Tumalo Creek to reach 2 (0.52). There was a break in relative migration over North Canal Dam, between adjacent reaches 4 and 5 (≤ 0.18). Reaches 5 and 6 showed high bidirectional relative migration (≥ 0.85) and moderate unidirectional relative migration from reach 6 into reach 7 (0.52). High bidirectional relative migration was also observed between reaches 9 and 10 (≥ 0.95). When the WFH strain was included, bidirectional relative migration was observed between the WFH strain and Fall River (≥ 0.50). These patterns were reflected in the whole network when all relative migration values were included (Figure S2).

With the DAPC, comparable BIC scores were observed for $K = 6$ through $K = 8$ (Figure S3). Reach 1 formed a distinct cluster (Figure 5). Reaches 9 and 10 also formed a distinct cluster across K -values as did Fall River, with individuals from both of these clusters observed in neighboring sampling groups. Redband Trout above and below North Canal Dam also formed distinct clusters with no apparent gene flow between them.

From $K = 6$ through $K = 8$, the biggest changes in groupings occurred in the middle sampling groups. At $K = 6$, reaches 2, 3, and 4 and Tumalo Creek formed a single distinct cluster. Reach 5 formed a cluster as did reaches 6 and 7, but there was substantial migration across these clusters (Figure 4). A subset of Tumalo Creek individuals clustered with reaches 6 and 7 and the Little Deschutes River clustered with the group

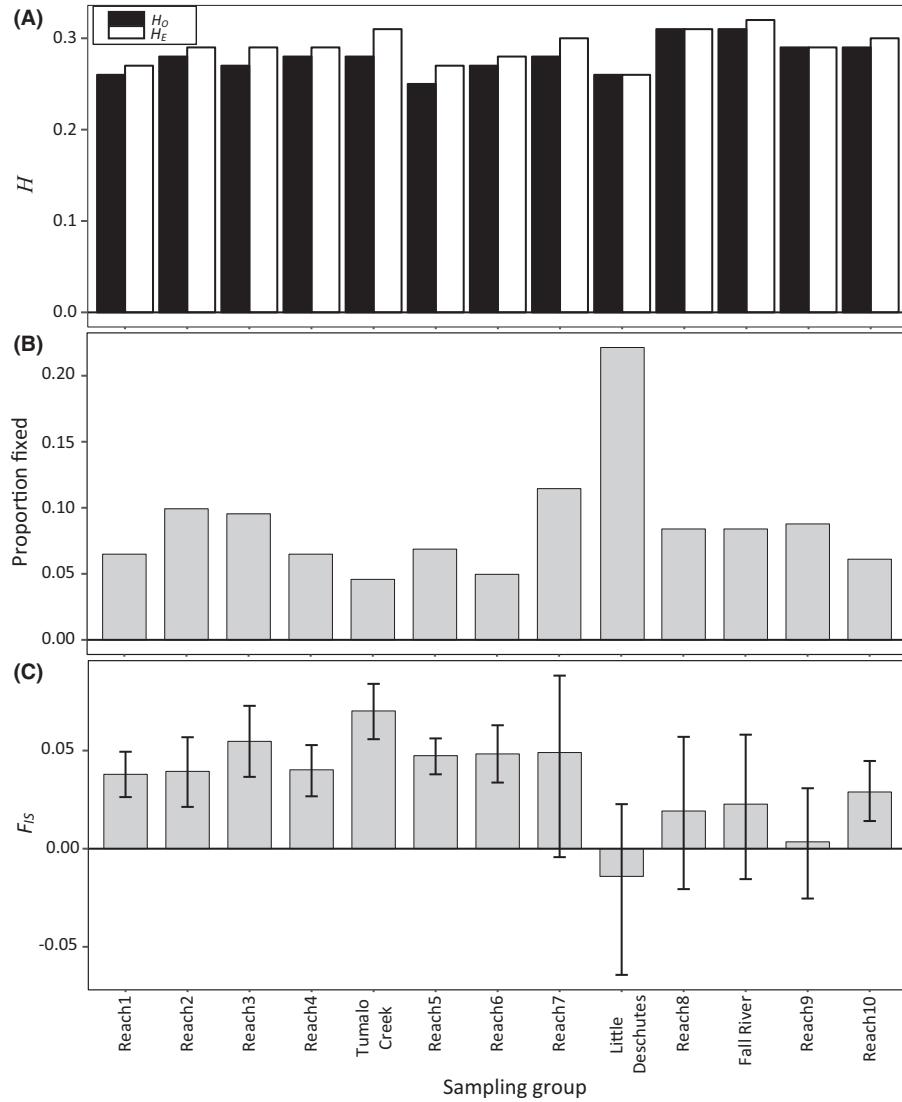


FIGURE 2. Measures of genetic diversity across the designated sampling units of Redband Trout from the Deschutes River and its tributaries, showing (A) expected (H_E) and observed (H_O) heterozygosity, (B) the proportion of loci in the data set that were fixed (i.e., monomorphic), and (C) population-specific values of F_{IS} and the associated 95% confidence intervals (error bars) based on 1,000 bootstraps.

formed by reaches 2–4. At $K = 7$ a subset of individuals from Tumalo Creek formed a distinct cluster, with some individuals from reaches 2, 3, and 4 displaying shared ancestry with this group (Figure 5). The Little Deschutes River also formed a distinct cluster, with some individuals having shared ancestry with reaches 6 and 7. When $K = 8$, a new cluster was formed by reach 4 with substantial shared ancestry with reaches 2 and 3. Little Deschutes River clustered with the reaches 2–3 group at this K -value.

The Mantel test suggested isolation by distance across the entire study area: the Mantel correlation coefficient between genetic and geographic distance was 0.369 (95%

confidence interval = 0.362–0.379, based on 1,000 bootstrap replicates) with a two-tailed P -value of 0.001. There was positive spatial autocorrelation in genetic distance for Redband Trout within 26–30 km (Figure 6), with the highest autocorrelation ($r > 0.3$) for individuals sampled within 4 km of each other. Beyond 30 km river distance there was negative autocorrelation. The correlation coefficient for all distance bins except one had an associated $P < 0.01$. When the Mantel test was restricted to samples below North Canal Dam, the correlation coefficient was 0.334 (95% confidence interval = 0.317–0.350). Upstream from the dam the correlation coefficient was 0.378 (95% confidence interval = 0.366–0.392).

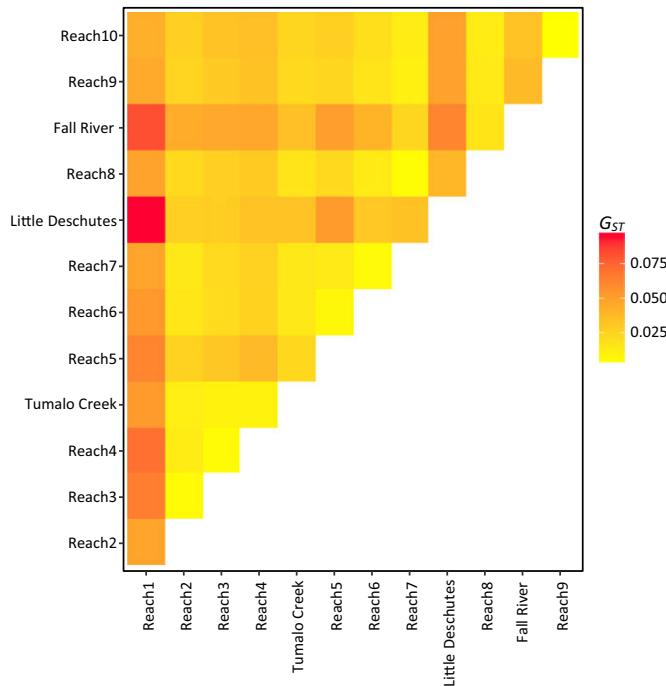


FIGURE 3. Heatmap of pairwise G_{ST} values between sampling groups. The higher values (darker colors) indicate higher genetic differentiation.

DISCUSSION

The middle and upper Deschutes River watershed is a complex system of large river segments, tributaries, and natural and constructed barriers. Within this study area, our sampling design produced a spatially balanced and dense sample of age-0 Redband Trout in both large river segments and tributaries and provided evidence of genetic discontinuities corresponding to natural and anthropogenic features. Our findings suggest that Redband Trout in this basin are influenced by introgression with fish from a hatchery stocking program, artificial barriers preventing gene flow, and natural geologic features that may restrict gene flow without completely blocking it.

Hatchery Introgression

In the upper Deschutes River basin, the stocking of out-of-basin hatchery strains of Rainbow Trout began as early as 1913 in response to concerns about depleted salmonid populations due to overfishing (Fies et al. 1996). Since that time, dam construction and water management for agricultural irrigation have altered riverine habitat quality and connectivity and transformed the historically stable flows of the Deschutes River to one of seasonal extremes (see Starcevich et al. 2015 for a summary). These ecological

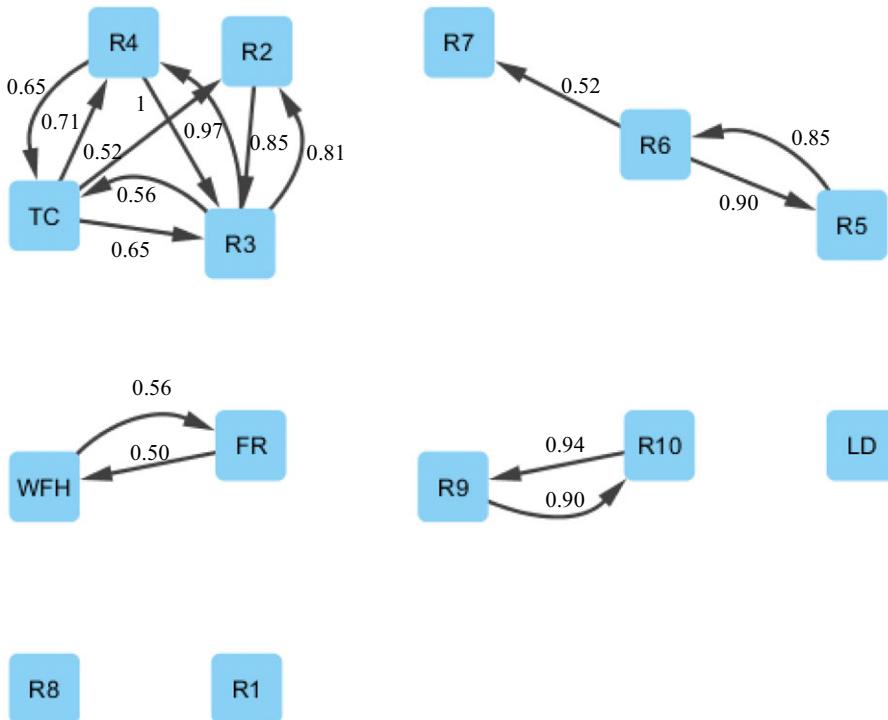


FIGURE 4. Network plots of relative migration levels between sampling groups. The arrows refer to the direction of gene flow from one group to another, and the edge values reflect the level of gene flow. Higher values mean more sharing of migrants. The overall network was trimmed to only display values with migration levels > 0.5 . Sampling groups are coded with “R” standing for reach, “TC” for Tumalo Creek, “LD” for Little Deschutes River, “FR” for Fall River, and “WFH” for Wizard Falls Hatchery.

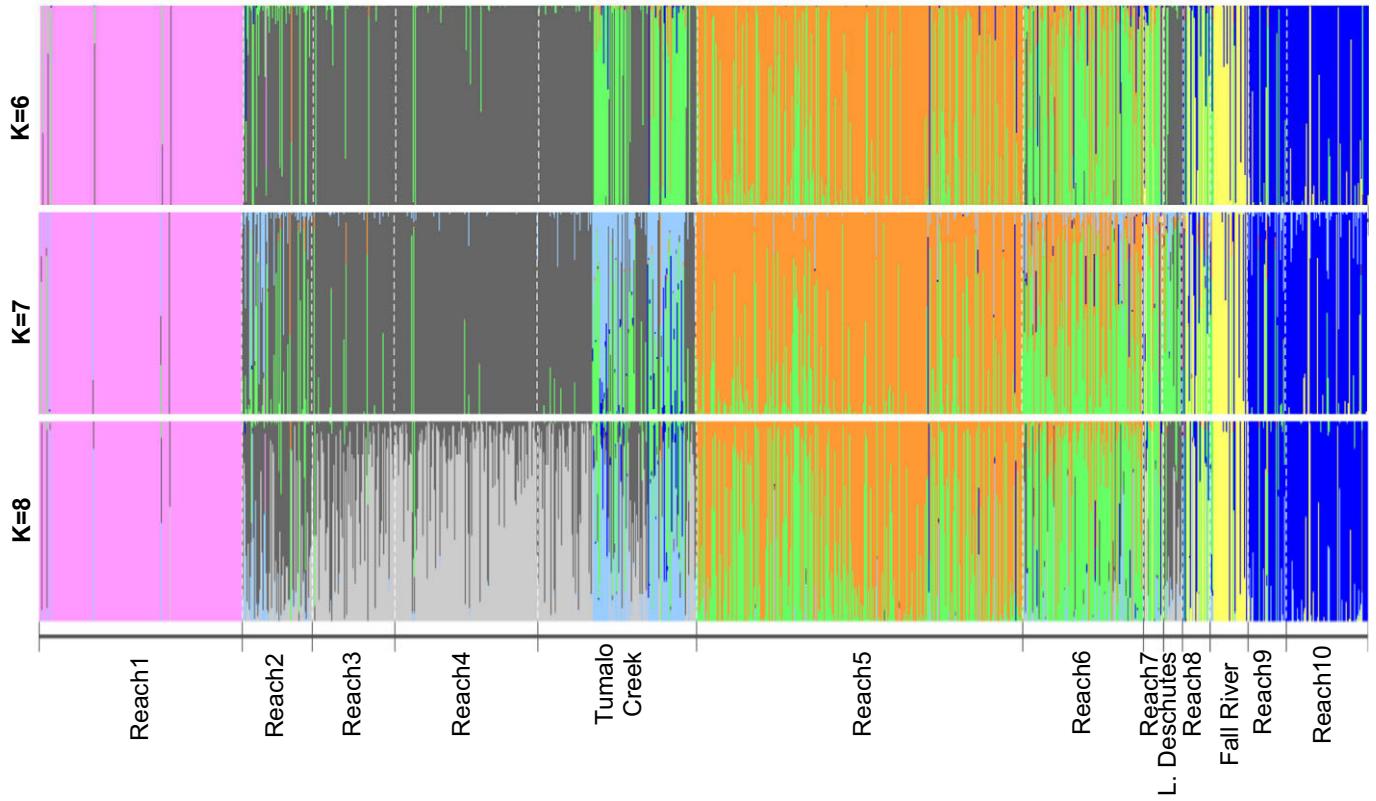


FIGURE 5. Barplots of membership probabilities for genetic groups identified via K -means clustering. The three horizontal plots reflect membership at $K = 6$, 7, and 8. The genetic groups that were identified at each K -value are denoted by distinct colors. Each vertical bar within those plots represents an individual Redband Trout grouped according to sampling groups, which are separated by vertical dotted lines.

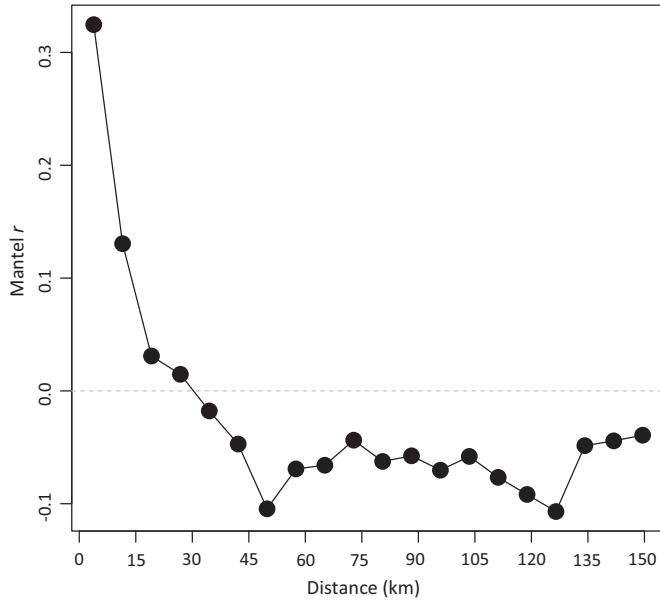


FIGURE 6. Plot of spatial autocorrelation in genetic distance among Redband Trout from the portion of the Deschutes River basin that was sampled. The distance bins reflect actual river distance between capture locations.

changes have contributed to a decline in Redband Trout abundance, and more recent out-of-basin hatchery stocking has been conducted in response to an associated decline in the recreational fishery (Fies et al. 1996). Since 1981 the main out-of-basin strain stocked in the upper segment of our study area was CCH Rainbow Trout. Immediately upstream of our study area, in Paulina Lake and Wickiup and Crane Prairie reservoirs, there have been large releases of OSH and CCH Rainbow Trout. Despite this long and extensive history of stocking, we found no evidence of these two out-of-basin strains producing offspring or introgressing with wild Redband Trout. These results are surprising given that out-of-basin strains of Rainbow Trout have introgressed with native Redband Trout in other watersheds (e.g., Small et al. 2007; Simmons et al. 2010; Kozfkay et al. 2011). However, our results were consistent with another upper Deschutes River basin study, which found that gene flow between native Redband Trout and OSH Rainbow Trout in Crane Prairie Reservoir was highly restricted (Matala et al. 2008). Introgression between Redband Trout and the OSH strain in Crane Prairie Reservoir was likely restricted by differences in spawning timing and possibly by a decrease in fitness

associated with domestication (Matala et al. 2008). The CCH strain also spawns much earlier than local Redband Trout (ODFW 2018), which likely has prevented their introgression with wild fish in our study area.

More recently, a locally derived Redband Trout strain (i.e., the WFH strain) has been stocked annually in several locations in the upper Deschutes River basin. This hatchery stocking program began in 1999 and sought to provide locally adapted hatchery fish for recreational anglers and to minimize the genetic risk to the native population (Matala et al. 2008). However, unlike the out-of-basin strains, our study suggests that the WFH strain is impacting the genetic composition of the Redband Trout population. We identified individuals that were of full and admixed WFH ancestry. Since these individuals were young of year (<120 mm) and this strain is typically released at a much larger size (total length > 205 mm), this study shows that the WFH strain is both reproducing in the wild and interbreeding with wild-origin individuals. These results support a previously noted concern that maintaining a recreational fishery by stocking a hatchery strain derived from a local population, with a spawning timing and genetic background similar to the wild population, can increase the chance of introgression with a wild population and pose a greater genetic risk relative to out-of-basin hatchery strains (Matala et al. 2008). Additional research is needed to understand the environmental and biological factors that influence the distribution and extent of gene flow from hatchery strains in this system, which would help guide stocking practices to minimize impacts on native populations. The results of this study could be used as a baseline for monitoring the genetic response of wild fish to changes in the hatchery stocking program.

Genetic Structuring among Native Redband Trout

Across the entire study area gene flow was restricted by both natural and artificial barriers. In the Deschutes River downstream of Big Falls, reach 1 was the most genetically distinct Redband Trout population sampled. Big Falls was the historical limit of steelhead (anadromous Rainbow Trout) and continues to serve as a barrier to gene flow. With fish passage facilities recently installed at Round Butte Dam, managers have released hundreds of thousands of steelhead smolts and adults upstream from the dam to establish migratory populations (Adams et al. 2015). However, no smolts have been released upstream from Steelhead Falls and no radio-tracked adult has been documented in this river reach (Becky Burchell, Portland General Electric, personal communication). Based on this information, it is unlikely any returning steelhead contributed progeny to this Deschutes River reach and suggests that these Redband Trout are a highly distinct population isolated from their conspecifics upstream from Big Falls. Further comparison between the reach 1

samples and the steelhead stock being released upstream of Round Butte Dam would confirm this question. Long-term genetic monitoring would determine whether these steelhead stocks manage to colonize reach 1 and have a genetic impact on the Redband Trout.

In the main-stem Deschutes River upstream from Big Falls, there was hierarchical structuring of Redband Trout into multiple distinct genetic clusters that spanned across our sampling groups. This complexity did not completely conform to our hypothesis of multiple distinct genetic clusters corresponding to the boundaries of our sampling groups. Instead, only a few of these boundaries limited gene flow. One was North Canal Dam, located in the city of Bend and forming the boundary between reaches 4 and 5, which appeared to be a complete barrier to gene flow. This dam has likely blocked gene flow upstream ever since it was constructed in 1912 without fish passage facilities. However, it was surprising that reaches 4 and 5 were not connected by gene flow in the downstream direction. Reaches 5 and 6, immediately upstream of the dam, had relatively high densities of age-0 Redband Trout and all the samples sites in these reaches were occupied, suggesting there was an ample source of potential dispersers. Along with the physical barrier, gene flow may be limited due to the managed flow regime. During the irrigation season (i.e., March–October), about 95% of the river's discharge is diverted into irrigation canals upstream of North Canal Dam. Whatever the cause, with the North Canal Dam creating a two-way barrier effect (see Meeuwig et al. 2010), Redband Trout in the middle and upper Deschutes River segments each formed genetically distinct but heterogeneous units.

In contrast, the sampling groups in the Deschutes River segment between Big Falls and North Canal Dam (i.e., reaches 2–4) and Tumalo Creek formed an interconnected population. When samples were grouped at the sampling group level, this population appeared to follow a linear stepping-stone model, with neighboring sampling groups having higher relative gene flow than more distant groups (Slatkin 1993). However, incorporating spatially explicitly location data and estimating genetic autocorrelation suggest that this population followed a continuous isolation-by-distance pattern. In other words, *a priori* grouping of individuals for data analysis based on hypothesized barriers provided an incomplete picture of genetic structure. This was also revealed by the clustering analysis. In this Deschutes River segment, gene flow between adjacent reaches occurred in both the upstream and downstream direction despite three waterfalls that were hypothesized to act as barriers. Gene flow also occurred from Tumalo Creek to reaches 3 and 2, predominantly in the downstream direction, which suggests that Tumalo Creek may act as a source population for this segment and that upstream gene flow may be restricted.

The genetic structuring in the upper Deschutes River segment above the North Canal Dam was more complex. Reaches 5 and 6 at the downstream end of the segment and reaches 9 and 10 at the upstream end each formed their own respective genetic group, with high bidirectional gene flow among reaches within them. This suggests that Lava Island Falls (i.e., the boundary between reaches 5 and 6) and Pringle Falls (i.e., the boundary between reaches 9 and 10) do not act as barriers. Even though reach 7 shared much of its ancestry with reach 6, gene flow between these reaches was relatively low, suggesting that Benham Falls and Dillon Falls have restricted, without eliminating, gene flow between reaches 6 and 7.

As described above, Fall River and reach 8 were highly influenced by the WFH strain. Reach 8, relative to the overall system, showed a mixed ancestry with reaches 7 and 9, but estimates of gene flow suggested it was relatively isolated from those reaches. This was surprising given that there are no significant physical barriers at either end of reach 8. It is not clear what factors are influencing gene flow in this reach. One factor reducing the probability of gene flow may be low relative Redband Trout abundance in reach 8, which has the lowest effective population size across this section of the Deschutes River (Bohling et al. 2017). There may be a number of factors limiting abundance in reach 8, including hatchery introgression and competition with large annual hatchery releases in Fall River and high relative densities of juvenile nonnative Brown Trout in Fall River and Spring River (Starcevich and Bailey 2017). Several studies have shown that introduced Brown Trout tend to outcompete other native salmonid species in this region (Gatz et al. 1987; Wang and White 1994; McHugh and Budy 2005). More research is needed to understand the genetic and ecological processes that may be affecting this area.

The middle and upper Deschutes River segments in this study share a similar pattern of genetic and spatial autocorrelation, with high autocorrelation at small spatial scales (<4 km) that rapidly diminished with river distance (between 4 and 30 km). This suggests that most of the gene flow occurs at relatively short distances in this study area. At least two factors may be contributing to this isolation-by-distance pattern. First, resident Redband Trout likely show spawning site fidelity similar to anadromous steelhead and other salmonids, who tend to spawn near the natal area where they were born (Quinn 2005), which limits gene flow over longer distances. Second, the extensive young-of-year distribution in this study suggests an extensive distribution of suitable spawning habitat in the main-stem Deschutes River (Starcevich and Bailey 2017), which in turn suggests that adults did not have to stray far from their natal areas to find mates or suitable spawning habitat. Research in other systems will help determine whether this pattern of genetic autocorrelation is consistent among Redband Trout.

We observed unexpected patterns of genetic variation in some of the tributaries in the study area. Tumalo Creek appeared to contain multiple genetic groups, some of which resembled neighboring groups in the Deschutes River and one that appeared to be unique to this tributary (Matala et al. 2008). Similarly, Redband Trout from the Little Deschutes River were distinct from those in adjacent reaches of the Deschutes River but clustered with individuals collected below North Canal Dam in the DAPC. Combined with the low levels of diversity, this evidence suggests that the Little Deschutes River contains a distinct population that is isolated from Deschutes River populations. We surveyed in a relatively short section of the Little Deschutes River near its confluence with the Deschutes River, and we did not attempt to identify attributes that could influence gene flow in Tumalo Creek. Further investigation in both of these tributaries is needed to understand how they contribute to Redband Trout diversity in the Deschutes River basin.

Management Implications

Interest has grown among fish managers in developing hatchery strains derived from local populations to supplement wild populations or enhance a recreational fishery. The guiding expectation for this approach is that these strains would produce hatchery fish with local adaptations and fitness similar to their wild counterparts (Christie et al. 2014). However, introgression between a locally derived hatchery strain and their wild counterpart can become a management concern, leading to a loss of genetic diversity (Christie et al. 2012) and reduced fitness in the entire population (Christie et al. 2014). Recent fitness studies found that the reproductive success, when spawning in the wild, of hatchery salmonids produced with locally derived wild-origin broodstock averaged only half that of their wild-origin counterparts (reviewed by Christie et al. 2014). This reduction in fitness is heritable, and through introgression hatchery strains can have potential negative fitness consequences for wild populations (Araki et al. 2007, 2009; Christie et al. 2012). This pattern has been demonstrated across several salmonid species, geographic locations, and hatchery practices; this consistency of outcome suggests a general phenomenon (Christie et al. 2014).

When the locally derived WFH Redband Trout strain was developed, the goals of the hatchery program were to revive the local recreational trout fishery and minimize the genetic risk to the wild Redband Trout population (Matala et al. 2008). As evidenced by the recent fitness studies, however, the locally derived strain may pose substantially greater genetic risk to wild populations than initially expected. Our study does not address whether stocking the WFH strain is reducing fitness, but it is clear that the locally derived strain is introgressing with the wild population. To minimize these risks in the upper Deschutes River basin, local fish managers ended all hatchery releases

below Wickiup Dam in 2015 and adopted a hatchery stocking program in Fall River that by 2019 will only use triploid (i.e., sterilized) hatchery Rainbow Trout and triploid WFH Redband Trout to bolster the recreational fishery in this tributary. Other hatchery programs using locally derived strains to boost a recreational fishery should evaluate the potential consequences of their practices on wild populations.

Our analysis of the genetic structure of Redband Trout in this Deschutes River basin study area identified several distinct genetic groups that managers may view appropriately as separate populations. These include four populations in the main-stem Deschutes River, each facing distinct discharge and temperature regimes and a unique array of factors potentially limiting population status (see Starcevich and Bailey 2017). Monitoring and management priorities could then be tailored to the needs of each population and should consider the spatial scales at which gene flow occurs.

The installation of a fish ladder on North Canal Dam in 2017, after our sample was collected, provided the potential for upstream fish passage for the first time since in 1912. This may break down the division between the middle segment population and the population in reaches 5 and 6. We suggest periodic sampling and genetic analysis of age-0 fish from both populations to determine the effects of the fish ladder on gene flow.

Recent reviews have emphasized the need to assess the status of Redband Trout throughout their range, in a wide variety of rivers and stream, and in a spatially explicit manner (Muhlfeld et al. 2015; Penaluna et al. 2016), both to improve our understanding of the species and inform management. Surveying large rivers can be costly, time-consuming, and logistically difficult because of their sheer length as well as greater relative depth, width, and discharge, which can restrict the use of certain sampling methods (Beechie et al. 2005). Logistical constraints that cause large survey gaps in the sampling frame may result in a sample that is not representative of the attributes of particular study area that influence genetic structuring or it may lead to detecting population differentiation when it is not really there. For example, sampling on opposite ends of a population displaying isolation by distance can give the illusion of two discrete differentiated units (Schwartz and McKelvey 2009). Our spatially explicit sampling design that focused on surveying lateral habitats believed to be important habitat for age-0 Redband Trout provided an efficient means to continuously sample this large system.

Discerning genetic structure within a continuously distributed species also requires a large sample size and sufficient number of genetic markers to provide ample statistical power. Our survey sampled over 1,400 fish, and the use of GT-seq allowed our entire sample to be run on a single high-throughput flow cell and genotyped using automated scripts, which dramatically reduced processing time

and per sample costs. Even though the SNP panel we used was originally designed for steelhead (Campbell et al. 2015), we observed a high genotyping success rate for these resident Redband Trout populations and most loci were polymorphic. This suggests that we have a robust data set for examining the population genetics of Redband Trout from this basin and that the panel could be applied to other populations in the species range. Overall, this study provides a potential methodological template for sampling in river systems the size and complexity of the Deschutes River basin and may be useful for population delineation and monitoring throughout the range of Redband Trout and that of other widely distributed salmonid species.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.