

Small-Scale Genetic Structure and Variation in Steelhead of the Grande Ronde River, Oregon, USA

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Abstract.—Patterns of genetic variation in summer-run steelhead *Oncorhynchus mykiss* from the Grande Ronde River, Oregon, were evaluated with 20 microsatellite loci to determine the level of fine-scale genetic structure and influences from hatchery-reared stocks. Included were temporal collections of three wild populations (Lookingglass Creek, Catherine Creek, and the upper Grande Ronde River) and one hatchery population (Wallowa Hatchery). Average gene diversity was moderate to high in all collections (range = 0.794–0.815). Genetic variance among sample locations was greater than that of temporal collections within sites, as pairwise exact tests within populations were not significant except for Lookingglass Creek ($P = 0.00005$). Structure among the four populations was evident from pairwise tests, which yielded 23 significant results from 28 comparisons (adjusted critical value = 0.0018), but Catherine Creek collections were not significantly different from Wallowa Hatchery collections. None of the eight collections had a significant probability of differing from mutation drift equilibrium (e.g., recent bottleneck) under the two-phased mutation model ($P = 0.060–0.985$) when analyzed with BOTTLENECK software. Additionally, the ratios of allele number to allele range (M values) for the eight collections (0.773–0.819) were not within the range that suggests a recent decrease in effective population size ($M < 0.68$ implies a probable bottleneck). Results from this study suggest the existence of gene flow between steelhead from Catherine Creek and Wallowa Hatchery, but no evidence for low diversity or recent bottleneck events was detected in any of the four populations. This study provides several pieces of evidence to suggest that steelhead in the Grande Ronde River

should be a conservation priority as a source of genetic diversity for the Snake River basin.

Maintenance of genetic variation within and among wild populations is central to the conservation of species, allowing the potential for local adaptation and life history evolution. Reduced genetic diversity can lead to inbreeding depression and low reproductive fitness in natural populations (e.g., Ardren and Kapuscinski 2003). Potential for reduced genetic variability in natural populations has been demonstrated in theoretical studies (e.g., Wright 1931; Nei et al. 1975) and also empirically after demographic events such as bottlenecks (Cornuet and Luikart 1996; Bellinger et al. 2003), introgression with exotic or domesticated stocks (Hindar et al. 1991; Campton 1995), and isolation of populations (Currens et al. 1990; Costello et al. 2003; Taylor et al. 2003). In salmonid fishes, anthropogenic-related factors have led to declines in abundance and genetic diversity of several natural populations. Conservation of these populations requires an understanding of genetic structure and variation in populations at both large and small geographic scales, but small-scale population genetic variation is not well understood.

As anadromous steelhead *Oncorhynchus mykiss* throughout North America decline in abundance or become extirpated (Nehlsen et al. 1991), the overall genetic variability of populations is affected (Heath et al. 2002). Endemic summer steelhead populations in the Snake River basin have experienced severe declines in abundance and were listed as threatened under the Endangered Species Act on June 17, 1998 (U.S. Office of the Federal Register 1998). In an attempt to increase

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steelhead abundance, hatchery-reared steelhead are released in many tributaries of the Snake River for mitigation of lost productivity related to hydropower development and for supplementation to help recover natural populations. Consequently, hatchery-reared steelhead comprise approximately half of the adult run in the Snake River in most years. Hatchery steelhead broodstocks in the Grande Ronde River subbasin were derived principally from fish collected at main-stem Snake River dams (a mixture of upriver stocks including that of the Grande Ronde River; Keifer et al. 1992). Because introductions of non-endemic stocks of salmonids may affect the genetic structure of local stocks (Hindar et al. 1991), NOAA Fisheries (formerly the National Marine Fisheries Service) directed elimination of the Wallowa Hatchery stock of steelhead in the Grande Ronde River subbasin by 2008 (NMFS 1999). Wallowa Hatchery steelhead have been used for harvest augmentation in the Grande Ronde River subbasin since 1984. Intervention for steelhead in the Grande Ronde River subbasin by means of hatchery supplementation, kelt (iteroparous steelhead) rejuvenation, or other techniques may be warranted, but little information exists on life histories, stock structure, and genetic differences among steelhead stocks in the Grande Ronde River subbasin.

In this study, we used 20 microsatellite loci to examine the patterns of genetic diversity in wild and hatchery-reared steelhead populations in the Grande Ronde River. We estimated genetic diversity, structure, and probability of recent reductions in effective population size (N_e) of steelhead in the Grande Ronde River relative to other populations in the threatened Snake River evolutionarily significant unit (ESU). We primarily sought to determine (1) whether significant genetic structure exists among steelhead populations in geographically proximate tributaries of the Grande Ronde River subbasin; (2) whether hatchery-reared steelhead have reduced the genetic structure or variation of natural populations; and (3) whether patterns of genetic variation in Grande Ronde River steelhead are similar to those of populations in other subbasins in the Snake River ESU.

Methods

Sample collections.—Tissue samples (opercle punches) were collected from wild adult summer steelhead trapped at three weir sites (Lookingglass Creek, Catherine Creek, and the upper main-stem Grande Ronde River) within the Grande Ronde River subbasin during March–June of 2002 and 2003 (Figure 1). Tissue samples of adult summer steelhead were also collected from fish returning to Wallowa Hatchery in 2002 and 2003. Tissue samples were immediately

stored in 95% ethanol or lysis buffer (0.5 M EDTA, pH 8.0; 2 M tris, pH 7.5; 5 M NaCl, 20% sodium dodecyl sulfate).

Laboratory analysis.—Tissue samples were digested and DNA was extracted with Qiagen DNeasy in conjunction with a Qiagen 3000 robot. Genomic DNA was quantified and arrayed into 96-well plates for high-throughput genotyping.

The polymerase chain reaction (PCR) was used to amplify 20 microsatellite loci (Appendix 1). Amplifications were performed with 1.75 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0.5 units of *Taq* polymerase (Promega), and approximately 2–25 ng of genomic DNA in a total volume of 7–15 μL . Primer concentrations and annealing or extension temperatures for each of the 20 loci are listed in Appendix 1. Thermal cycling was performed with MJ Research instruments by use of a standard program of 2 min at 95°C, 32 cycles of 40 s at 94°C, 40 s at the locus-specific annealing temperature, 40 s at 72°C, and a 5 min to 1 h extension at the final extension temperature (Appendix 1). The forward primer was fluorescently labeled, and PCR products were genotyped by use of the manufacturer's recommended protocols with an Applied Biosystems Model 3100 genetic analyzer.

Statistical analysis.—Exact-significance testing methods (GENEPOP version 3.3; Raymond and Rousset 1995) with sequential Bonferroni corrections (Rice 1989) were used to evaluate deviations from Hardy–Weinberg equilibrium for all individual collections. Linkage disequilibrium among loci and populations was calculated in GENEPOP with sequential Bonferroni corrections (Rice 1989). To estimate the level of within-population genetic diversity, the observed heterozygosity (H_o), unbiased gene diversity (Nei 1987), and average number of alleles per locus were estimated with Microsatellite Toolkit (Park 2001). Significance of diversity measures among sites was tested with one-way analysis of variance in the Statistical Analysis System, version 8.2 (SAS Institute, Cary, North Carolina). The pairwise genetic differentiation index (F_{ST} ; temporal and geographic) was estimated from allele frequencies (Weir and Cockerham 1984) in GENEPOP. Exact tests were performed in GENEPOP to determine the significance of pairwise F_{ST} values. Significance levels were adjusted for multiple tests by use of the sequential Bonferroni method (Rice 1989).

Effective population size was estimated with two methods: the linkage disequilibrium method (Hill 1981) to estimate short-term N_e and Nei's (1987) method to estimate long-term N_e . The linkage disequilibrium method was invoked in the program NeEstimator (Bartley et al. 1992; Peel et al. 2004) to estimate

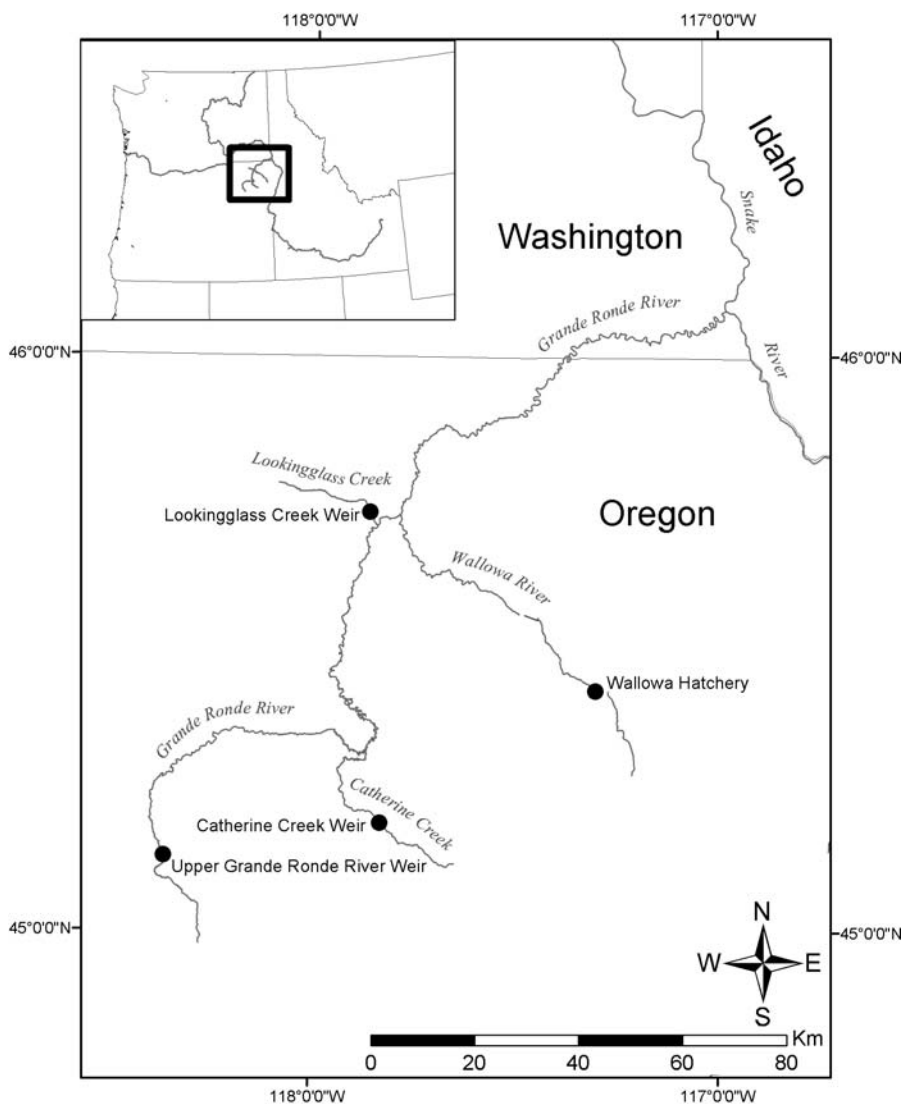


FIGURE 1.—Map of steelhead sample collection sites (circles) on the Grande Ronde River, Oregon.

the effective number of breeders (N_b) for each collection site (temporal collections were pooled to meet the minimum recommended sample size of 90). The N_b estimate was divided by 2 to correct for two sample years and then was multiplied by generation length (4 years) to estimate N_e as suggested by Waples (2002). Long-term N_e was calculated as described by Nei (1987) with both the stepwise mutation model (SMM), $N_e = \{[1/(1 - H_E)]^2 - 1\}/(8\mu)$, and the infinite allele model (IAM), $N_e = H_E/4\mu(1 - H_E)$, where H_E is expected heterozygosity and μ is the mutation rate. Nei's (1987) method uses the relationship of heterozygosity, mutation rate, and N_e to calculate long-term estimates of N_e . However, the mutation rate and model

in this method can greatly affect N_e estimates. Therefore, we generated N_e estimates for the three mutation rates (1.0×10^{-3} , 5.0×10^{-4} , and 1.0×10^{-5}) and two mutation models (SMM and IAM) available for the Nei (1987) method.

Tests for reduced population size and recent bottleneck events were estimated with Garza and Williamson's (2001) ratio of allele number to allele range, M (stepwise mutation model), and BOTTLENECK software (Cornuet and Luikart 1996). We evaluated three possible mutation models: SMM, IAM, and a two-phased model (TPM). The default parameters of 30% variance and 1,000 iterations were used for TPM estimates. The proportion of stepwise

mutations in TPM was set at 90%, as recommended by Luikart and Cornuet (1998).

To infer the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations by use of GENDIST in PHYLIP version 3.5 (Felsenstein 1993). Genetic chord distances were then used to construct a neighbor-joining tree of sample populations with NEIGHBOR (PHYLIP). Bootstrap replicates of 1,000 iterations were attained in SEQBOOT, and a consensus tree was made with CONSENSE in PHYLIP.

Results and Discussion

Of the 160 tests used to evaluate deviations from Hardy-Weinberg equilibrium, 158 were nonsignificant based on sequential Bonferroni corrections (adjusted critical value = 0.05/160 tests = 0.0003). The two exceptions were the Catherine Creek (2002) and upper Grande Ronde River collections (2002) at *Omy77*, which has been reported to have low-frequency null alleles in some populations (Ardren et al. 1999). While heterozygote deficiency was possible in Wallowa Hatchery samples because of their historically admixed broodstock (Keifer et al. 1992), we did not observe such a result in this study, since a single generation of random mating will return allele frequencies to Hardy-Weinberg proportions. Of 1,520 pairwise tests of linkage disequilibrium among loci and collections, 13 were significant but results were not consistent within loci or populations.

Estimates in Table 1 indicate that genetic diversity was similar temporally and among sample collection sites. Among all collections, average H_O across loci ranged from 0.758 to 0.812, average gene diversity ranged from 0.791 to 0.815, and the average number of alleles per locus ranged from 11.2 to 12.4. None of

these measures of diversity were significantly different among sites after corrections for multiple tests. A total of 331 alleles were detected from all collections across loci.

Exact tests and pairwise F_{ST} values revealed that 23 of 28 pairwise tests among collections over all loci were significant (Table 2). Temporal collections within sites accounted for three of the five nonsignificant tests. However, exact tests of temporal collections from Lookingglass Creek were significant ($P = 0.00005$). A neighbor-joining dendrogram provided concordant results, as temporal samples within populations clustered together with moderate to high bootstrap support (Figure 2). The other two nonsignificant exact tests were pairwise tests of the Wallowa Hatchery 2002 collection with both collections from Catherine Creek (2002 and 2003). The pairwise tests and neighbor-joining dendrogram suggested that statistically significant population structure was of greater magnitude among collection sites than within sites. In addition, the genetic similarity of the Wallowa Hatchery collection with Catherine Creek collections suggested either interbreeding or shared ancestry of steelhead in Catherine Creek and endemic Wallowa River stocks used as hatchery broodstock. Given that the Wallowa Hatchery stock is derived from a mixture of Snake River populations and given the fact that our data set of 20 loci has high power to distinguish populations on small scales, the occurrence of gene flow between steelhead at Wallowa Hatchery and Catherine Creek is the more likely explanation.

Higher levels of differentiation occur in steelhead populations across the Columbia River basin than within subbasins (Winans et al. 2004), and very low temporal variation has been observed within sample sites (Waples et al. 1993; Beacham et al. 1999). Our estimates of differentiation within sites ($F_{ST} = -0.002$ –

TABLE 1.—Summary statistics (\pm SE, as applicable) for eight Grande Ronde River, Oregon, steelhead collections averaged over 20 microsatellite loci. Unbiased heterozygosity (H_E ; Nei 1987) is presented along with observed heterozygosity (H_O). Ratios of allele number to allele range (M ; Garza and Williamson 2001) are reported in addition to P -values for rejecting mutation drift equilibrium in BOTTLENECK software (Cornuet and Luikart 1996). The P -values are the probability of two-tailed Wilcoxon tests for heterozygote excess (TPM = two-phased model; IAM = infinite allele model; SMM = stepwise mutation model).

Collection	Sample size	Unbiased H_E	H_O	Average number of alleles/locus	M	BOTTLENECK P -values		
						TPM	IAM	SMM
Wallowa Hatchery, 2002	49	0.805 \pm 0.003	0.785 \pm 0.002	11.2 \pm 0.7	0.814	0.08969	0.00008	0.98544
Wallowa Hatchery, 2003	50	0.807 \pm 0.003	0.798 \pm 0.002	11.3 \pm 0.7	0.791	0.29425	0.00007	0.52167
Catherine Creek Weir, 2002	50	0.799 \pm 0.004	0.758 \pm 0.002	11.8 \pm 0.7	0.799	0.26110	0.00008	0.86949
Catherine Creek Weir, 2003	50	0.815 \pm 0.004	0.812 \pm 0.002	12.0 \pm 0.7	0.806	0.08969	0.00059	0.81236
Upper Grande Ronde River, 2002	50	0.794 \pm 0.005	0.762 \pm 0.002	12.3 \pm 0.8	0.808	0.82878	0.00071	0.06021
Upper Grande Ronde River, 2003	50	0.802 \pm 0.004	0.767 \pm 0.002	12.4 \pm 0.8	0.819	0.21617	0.00004	0.29425
Lookingglass Creek, 2002	50	0.791 \pm 0.005	0.763 \pm 0.002	11.3 \pm 0.8	0.773	0.23051	0.00001	0.78413
Lookingglass Creek, 2003	50	0.797 \pm 0.004	0.773 \pm 0.002	12.0 \pm 0.8	0.805	0.47491	0.00016	0.43043

TABLE 2.—Pairwise genetic differentiation index (F_{ST}) values of eight collections of steelhead from the Grande Ronde River based on Oregon microsatellite loci. Asterisks indicate tests that were not significant at the adjusted critical level (0.05/28 tests = 0.0018).

Collection number	Collection	Collection number							
		1	2	3	4	5	6	7	8
1	Wallowa Hatchery, 2002								
2	Wallowa Hatchery, 2003	0.003*							
3	Catherine Creek, 2002	-0.001*	0.004						
4	Catherine Creek, 2003	0.001*	0.003	-0.002*					
5	Upper Grande Ronde River, 2002	0.009	0.014	0.005	0.006				
6	Upper Grande Ronde River, 2003	0.008	0.010	0.006	0.005	0.001*			
7	Lookingglass Creek, 2002	0.012	0.012	0.011	0.008	0.016	0.009		
8	Lookingglass Creek, 2003	0.010	0.012	0.010	0.006	0.014	0.012	0.005	

0.005; negative values are not surprising in an unbiased estimate) and among sites ($F_{ST} = 0.005-0.016$) support previously reported levels of hierarchical genetic variation in the Columbia River basin. However, within the Snake River drainage, differentiation among tributary populations in the Grande Ronde River subbasin was much less than that previously observed in other subbasins (i.e., Lochsa and Middle Fork Salmon rivers; J. L. Nielsen, U.S. Fish and Wildlife Service, personal communication). This may indicate

that gene flow is higher among tributary populations of the Grande Ronde River subbasin, genetic drift is greater in other Snake River subbasins, or some combination of the two.

Comparisons of long-term N_e (Nei 1987 method; $\mu = 5.0 \times 10^{-4}$) showed that steelhead in Grande Ronde River tributaries have a range of N_e (SMM range = 5,641–6,462) that may be higher than that of tributaries from any other Snake River subbasin (J. Nielsen, personal communication). Estimates of N_e based on

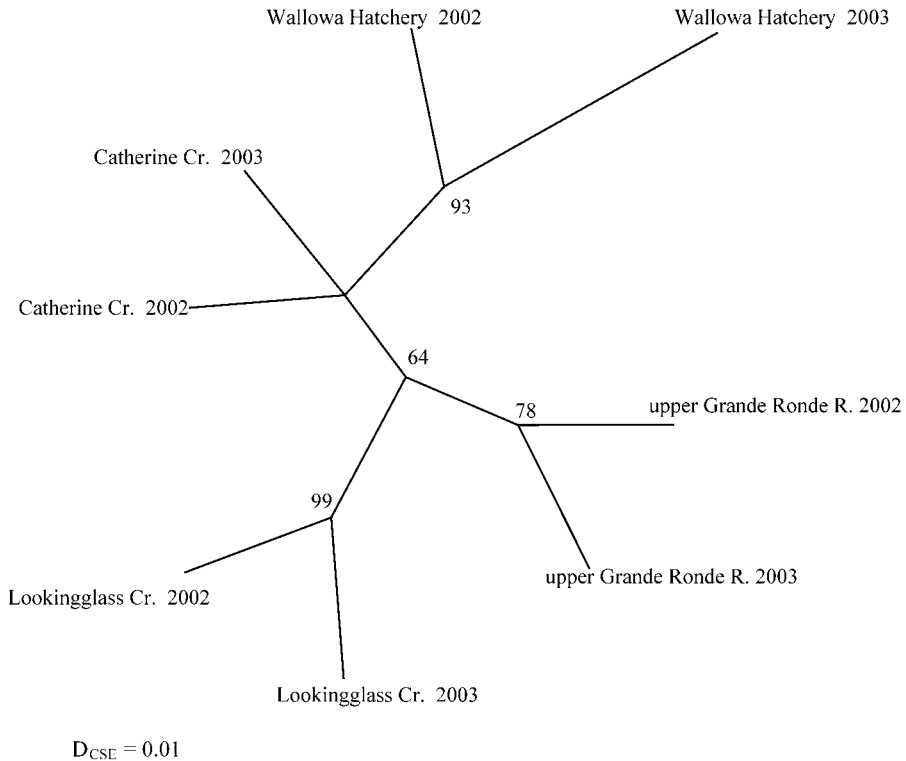


FIGURE 2.—Neighbor-joining dendrogram of genetic distances (Cavalli-Sforza and Edwards 1967) for eight steelhead collections from the Grande Ronde River based on 20 microsatellite loci (D_{CSE} = scale of Cavalli-Sforza-Edwards genetic distance). Bootstrap values greater than 50% are located at nodes and were generated from 1,000 iterations.

a range of μ (1.0×10^{-3} to 1.0×10^{-5}) for both SMM and IAM are shown in Appendix 2. Single-generation N_e estimates (linkage disequilibrium method; Hill 1981) for steelhead collections from the Grande Ronde River were 155.1 (95% confidence interval [CI] = 139.5–174.1) for Catherine Creek, 353.6 (95% CI = 295.7–437.4) for Lookingglass Creek, 226.8 (95% CI = 198.3–264.1) for the upper Grande Ronde River, and 311.0 (95% CI = 259.5–385.9) for Wallowa Hatchery. Relatively high estimates of long-term N_e in steelhead of the Grande Ronde River subbasin support the assertion that genetic drift is less pronounced in this subbasin than in Snake River subbasins with smaller N_e values.

When examined in BOTTLENECK software, none of the eight Grande Ronde River sample collections had a significant probability of differing from mutation drift equilibrium under the TPM (Wilcoxon's two-tailed P -values = 0.060–0.985; Table 1) and therefore showed no evidence of genetic bottlenecks. Estimated P -values for other mutation models (SMM and IAM) are shown in Table 1, but TPM is the most appropriate model for microsatellite loci used in this study (Di Rienzo et al. 1994; Cornuet and Luikart 1996). Additionally, M -values for the eight collections (range = 0.773–0.819) were not suggestive of a reduced N_e based on guidelines reported in the literature ($M < 0.68$ = probable bottleneck; Garza and Williamson 2001; Shrimpton and Heath 2003). These results may be in contrast to the status of steelhead from other subbasins in the Snake River basin (J. L. Nielsen, personal communication) and indicate either that Grande Ronde River steelhead have not had reduced population sizes relative to other regions in the ESU or that hatchery practices in the Grande Ronde River have increased diversity, thereby masking bottleneck signals. Ultimately, the results of these tests suggest little evidence of recent severe genetic bottlenecks of steelhead populations in the Grande Ronde River; however, historical bottlenecks cannot be ruled out.

Concerns about declining steelhead stocks (e.g., Nehlsen et al. 1991), particularly the threatened Snake River ESU, have led to cautious management practices in the Columbia River basin. However, populations of steelhead in the Grande Ronde River appear to have maintained higher genetic diversity, minimal hatchery stock influence, and a less likely occurrence of bottleneck events relative to other populations in the Snake River (J. L. Nielsen, personal communication). This is a positive indication for steelhead in the Grande Ronde River, but broader-scale studies with common loci are necessary to directly compare relative genetic diversity within and among ESUs of the Columbia River basin. The cause of these differences among

subbasins is unknown, but inconsistent management practices, historical contingency and isolation, and patchy habitat due to degradation are common in the Snake River basin. Potential for isolation only appears to exist in historical contingency events (e.g., glaciations) or in the differences among fluvial ecosystems, since the Grande Ronde River drains the Blue and Wallowa mountains to the west of the Snake River and the majority of the eastern Snake River tributaries drain the Bitterroot Mountain Range.

There are several pieces of evidence suggesting that steelhead in the Grande Ronde River should be a conservation priority as a source of genetic diversity for the Snake River basin. This study reveals (1) distinct genetic structuring of steelhead within the Grande Ronde River, (2) potential influence of the hatchery stock on the Catherine Creek population, and (3) the existence of a Snake River subbasin that contains steelhead with higher genetic variation than is present in other subbasins. Due to these observations, conservation of Grande Ronde River steelhead merits a high priority for management. This includes conservation of existing genetic diversity, habitat protection and restoration, and further monitoring and evaluation of supplementation efforts. As supplementation and harvest augmentation with hatchery-reared steelhead continues in the Grande Ronde River subbasin, monitoring and evaluation of genetic diversity and structure of natural populations will assist the preservation of steelhead in this system.

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Appendix 1: Amplification of Microsatellite Loci

TABLE A.1.1.—Polymerase chain reaction primers and conditions used to amplify and genotype steelhead microsatellite loci. Final extension time for all loci was 1 h with the exception of five loci (*OMM107*, *OMM1020*, *OMM1036*, *OMM1046*, and *OMM1050*), which had a 5-min final extension.

Locus name	Temperature (°C)		Primer concentration (µM)	Number of alleles	Reference
	Annealing	Final extension			
<i>Ocl1</i>	60	72	0.15	17	Condrey and Bentzen (1998)
<i>Oke4</i>	54	54	0.4	12	Buchholz et al. (1999)
<i>Ogo4</i>	48	72	0.6	13	Olsen et al. (1998)
<i>Oki23MMBL</i>	54	72	0.6	20	Rexroad et al. (2002)
<i>OMM1007</i>	58	72	0.2	9	Rexroad et al. (2002)
<i>OMM1020</i>	58	72	0.2	10	Rexroad et al. (2002)
<i>OMM1036</i>	60	72	0.2	34	Rexroad et al. (2002)
<i>OMM1046</i>	60	72	0.2	24	Rexroad et al. (2002)
<i>OMM1050</i>	60	72	0.2	36	Rexroad et al. (2002)
<i>Omy71NRA</i>	56	72	0.4	12	K. Gharbi, personal communication
<i>Omy77</i>	48	48	0.6	21	Morris et al. (1996)
<i>Omy1001</i>	60	60	0.4	25	Spies et al. (2005)
<i>Omy1011</i>	54	72	0.4	16	Spies et al. (2005)
<i>Oney14</i>	60	72	0.3	8	Scribner et al. (1996)
<i>Ots3</i>	47	72	0.3	7	Banks et al. (1999)
<i>Ots4</i>	60	60	0.15	7	Banks et al. (1999)
<i>Ots100</i>	54	54	0.3	16	Nelson and Beacham (1999)
<i>Ssa289</i>	54	54	0.4	8	McConnell et al. (1995)
<i>Ssa407</i>	60	60	0.4	19	Cairney et al. (2000)
<i>Ssa408</i>	54	54	0.4	17	Cairney et al. (2000)

Appendix 2: Effective Population Size Estimates

TABLE A.2.1.—Estimates of effective population size (*N_e*) for steelhead collections from multiple subbasins of the Snake River. The *N_e* estimates based on the infinite allele model (IAM) and stepwise mutation model (SMM) are shown for a range of mutation rates (µ). The asterisk indicates the mutation model and rate used for the *N_e* estimates reported in the text.

Collection	<i>n</i>	<i>H_E</i>	<i>N_e</i> (µ = 1.0 × 10 ⁻⁵)		<i>N_e</i> (µ = 5.0 × 10 ⁻⁴)		<i>N_e</i> (µ = 1.0 × 10 ⁻³)	
			IAM	SMM	IAM	SMM*	IAM	SMM
Lookingglass Creek	100	0.7940	96,359	282,061	1,927	5,641	964	2,821
Upper Grande Ronde River	100	0.7980	98,762	293,843	1,975	5,877	988	2,938
Catherine Creek	100	0.8070	104,534	323,079	2,091	6,462	1,045	3,231
Wallowa Hatchery	99	0.8060	103,866	319,629	2,077	6,393	1,039	3,196