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[Article]

Microsatellites Reveal Population Substructure of Klickitat River Native Steelhead and Genetic Divergence from an Introduced Stock

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Abstract.—Determining fine-scale genetic diversity and structure is critical for the conservation and management of populations, especially those under heavy anthropogenic influence. We analyzed 446 individuals at nine microsatellite loci to determine the local population structure of naturally produced steelhead *Oncorhynchus mykiss* and genetic differentiation from introduced hatchery strain steelhead in the Klickitat River of the Pacific Northwest. We detected significant genetic structure among steelhead in various tributaries to the Klickitat River; the most divergent population was located above a waterfall that acts as a partial upstream migration barrier (average pairwise $F_{ST} = 0.13$; $P < 0.0001$). Analysis of mixtures indicated an estimate of six to seven genetically distinct populations of naturally reproducing steelhead in this river system. The hatchery strain appears to remain genetically distinguishable from native stocks (average pairwise F_{ST} of 0.078 with $P < 0.0001$), as only 4.0% of naturally produced steelhead had their most likely assignment to the hatchery strain. These results indicate that the genetic integrity and variation of native Klickitat River steelhead have been maintained despite repeated hatchery introductions and that the potential is high for restoring this threatened population. Further, this study suggests that hierarchical analyses of mixtures to identify distinct populations in a watershed are a valuable method for directing management of reproductively isolated populations.

As populations of steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) throughout North America decline in abundance or are extirpated (Nehlsen et al. 1991), the temporal stability of genetic variation within populations is affected (Heath et al. 2002). While limited gene flow of steelhead populations between major river systems has been demonstrated (Taylor 1995; Beacham et al. 1999, 2004; Heath et al. 2001), further research of population structure is needed for smaller river systems (e.g., Heath et al. 2001; Hendry et al. 2002). Identification of distinct populations is necessary to assist with management and conservation of unique populations that will contribute to overall persistence of the species. Demographic and environmental processes, geographic barriers to gene flow, and selection are important for structuring genetic diversity within and among natural populations and have contributed to the complexity of population structure of steelhead in North America (Beacham et al. 1999). Further, introductions of nonendemic stocks of salmonids may impact the structure of local stocks (Hindar et al. 1991). A surge of gene flow from introduced stocks into distinct native populations can lead to loss of interpopulation diversity (Miller and Kapuscinski 2003) and introgression of exogenous genes from the nonendemic stock (Campton 1995). Thus, information regarding genetic structure within native populations is essential for conservation of diversity and to reduce impacts of exogenous introductions to locally distinct population segments.

This study addresses the fine-scale genetic structure of naturally produced steelhead within the Klickitat River subbasin, Washington. Steelhead in the Klickitat River are included in the threatened mid-Columbia River evolutionarily significant unit and are protected

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under the Endangered Species Act. Identification of distinct steelhead populations in the Klickitat River is necessary to assist with conservation, as there are several potentially isolated spawning populations within the watershed (Phelps et al. 2000). Temporal reproductive isolation as a result of differential timing of adult spawning runs may exist since historical runs of both summer and winter steelhead (predating introductions) are present in the Klickitat River (Bryant 1949; Howell et al. 1985). Differential spawning timing has been shown to reproductively isolate salmonid spawners and create distinct gene pools (e.g., Olsen et al. 2000; Hendry et al. 2002) and may be one source of genetic structure in the Klickitat River. The Klickitat River also contains diverse geography that could lead to reproductive isolation. Multiple waterfalls (partial barriers) are found in this subbasin; one near the confluence of the Klickitat River and Columbia River and others upstream could lead to genetic isolation of populations (e.g., Currens et al. 1990). Another potential contributing factor to genetic structure may be the introduction of summer-run hatchery strain steelhead, referred to as Skamania steelhead, which have been heavily stocked into the Klickitat River since 1961 (Washington Department of Fish and Wildlife, unpublished data). The Skamania strain is a mixture of steelhead from the Washougal and Klickitat rivers that has been subjected to extensive selection for early spawning to produce 1-year-old smolts (Crawford 1979). Potential for temporal reproductive isolation between summer Skamania steelhead and winter steelhead is high as a result of different spawning timing (e.g., Kostow et al. 2003), but potential for isolation is lower for Skamania summer steelhead and naturally produced summer steelhead that may have overlapping spawning timing. Additionally, hatchery strains of resident steelhead (i.e., McCloud River, Goldendale, and Mt. Whitney strains) have been stocked into the Klickitat River (Washington Department of Fish and Wildlife, unpublished data) with the possibility of residents contributing to the genetic composition of anadromous runs (e.g., Zimmerman and Reeves 2000).

We analyzed nine microsatellite loci from samples of naturally produced steelhead from the Klickitat River as well as samples of introduced hatchery-reared steelhead (Skamania strain). We address two questions: (1) What are the population components contributing to the genetic structure of steelhead in the Klickitat River? (2) Has the introduced Skamania strain impacted the genetic composition of naturally produced Klickitat River steelhead populations?

Methods

Sample collections.—Caudal fin clips of juvenile steelhead were collected by electrofishing from three tributaries of the Klickitat River (Figure 1): Swale Creek (2001, $n = 19$), Summit Creek (2001, $n = 15$), and White Creek (2001, $n = 28$). Additional samples were collected from smolt traps to capture unknown-origin individuals representative of all upstream steelhead populations (potentially mixtures of resident, anadromous, winter-run, and summer-run steelhead). Smolt traps were placed near the mouth of the Klickitat River (river kilometer [rkm] 0) at rkm 9.6 (Lyle Falls Trap: 2000, $n = 57$; 2001, $n = 141$), 33.6 km below a natural rapids at rkm 68.8 (Hatchery Trap: 2001, $n = 43$), and 3.2 km above the natural waterfall (partial barrier) at rkm 105.6 (Upper Castile Trap: 2002, $n = 48$). Juvenile samples were also taken from Skamania strain steelhead from Washougal Hatchery (2003, $n = 95$) to determine genetic composition of the stock used in introductions to the Klickitat River. All hatchery fish introduced to the Klickitat River are fin-clipped (adipose fin) to distinguish them from naturally produced steelhead. Using this adipose fin clip as a distinct marker of hatchery or natural origin, only individuals possessing an adipose fin were sampled, and by presence of an adipose fin these samples were assumed to be progeny of natural steelhead spawners in the Klickitat River (this may include progeny of hatchery fish spawning naturally). Temporal samples over 2 years (2000 and 2001) were taken for steelhead collections at the Lyle Falls Trap location to represent year-to-year temporal genetic diversity in the Klickitat River. All fin clips were immediately placed in 95% ethanol until examined in the laboratory.

Laboratory analysis.—Fin clips were digested and DNA was extracted following standard manufacturer's protocols from QIAGEN (Valencia, California) 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 nine microsatellite loci: *OMM1007*, *OMM1019*, *OMM1020*, *OMM1036*, *OMM1046*, *OMM1050* (Rexroad et al. 2002), *Ots1* (Banks et al. 1999), *Ocl1* (Condrey and Bentzen 1998), and *Ogo4* (Olsen et al. 1996). Five loci were dinucleotide repeats (*OMM1019*, *OMM1020*, *Ots1*, *Ocl1*, and *Ogo4*), one was a trinucleotide repeat (*OMM1007*), and three were tetranucleotide repeats (*OMM1036*, *OMM1046*, and *OMM1050*). Amplifications were performed with the AmpliTaq Reagent System (Applied Biosystems, Foster City, California) in an MJ Research PTC-100 thermal cycler (Bio-Rad Laboratories, Inc., Waltham,

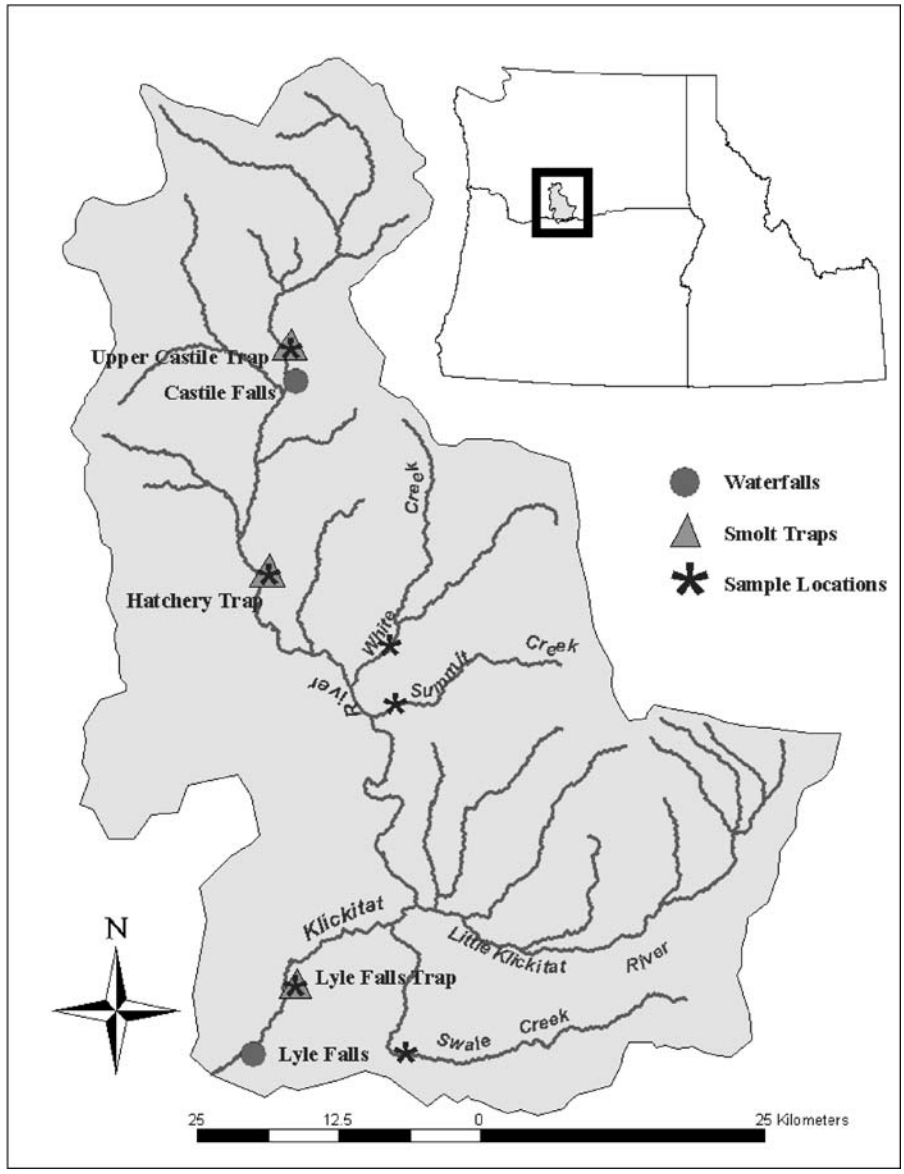

FIGURE 1.—Map of the Klickitat River subbasin and sampling locations for determining the population substructure of steelhead populations. Smolt traps were located at river kilometers 9.6, 68.8, and 105.6.

Massachusetts) following manufacturer's protocols with approximately 25 ng of genomic DNA in 15 μL total volume. Typical cycling conditions included an initial denaturation of 2 min at 92°C, followed by 30 cycles of 30 s at 92°C, 30 s at 50–62°C, and 30 s at 72°C. A final extension was carried out for 10 min at 72°C. Annealing temperatures were adjusted to optimize PCR conditions (*OMM1007* = 58°C, *OMM1019* = 58°C, *OMM1020* = 58°C, *OMM1036* = 60°C, *OMM1046* = 60°C, *OMM1050* = 60°C, *Ots1* = 54°C, *Ocl1* = 60°C, and *Ogo4* = 54°C). Forward primers were fluorescently labeled (Applied Biosystems), and PCR products were genotyped following manufacturer's protocols with an Applied Biosystems Model 3100 genetic analyzer. Alleles were scored with GeneScan and Genotyper software from Applied Biosystems.

Statistical analysis.—Exact-significance testing methods were used to evaluate each locus and population for departures from Hardy–Weinberg equilibrium with a Markov chain–Monte Carlo (MCMC)

algorithm as implemented in GENEPOP (Raymond and Rousset 1995). Corrections were made against type I error in exact tests with the sequential Bonferroni method (Rice 1989). Tests for linkage disequilibrium between all pairs of loci were also performed with the MCMC method in GENEPOP.

To estimate the genetic diversity of each collection, expected heterozygosity (H_e), observed heterozygosity (H_o), the total number of alleles for all loci, and allelic richness (average alleles per locus corrected for sample size) were calculated for all microsatellite loci in FSTAT (Goudet 2001). Unique alleles found only in one collection and alleles found in all collections but one (hereafter referred to as "missing alleles") were tabulated for each locus from allele frequencies.

Since all samples were juveniles, collections were tested for kinship bias with the software IDENTIX (Belkhir et al. 2002). The permutation calculation in IDENTIX (options: Queller and Goodnight 1989; means, genotypes, 1,000 permutations) was performed to determine whether average relatedness within each of the eight collections departed from its random expectation.

Pairwise genetic variance (temporal and geographic) was calculated from allele frequencies (genetic differentiation index, F_{ST}; Weir and Cockerham 1984) with GENEPOP to estimate the pairwise genetic divergence among all collections. Exact tests were performed in GENEPOP to determine significance of pairwise genetic variance. Significance levels were adjusted for multiple tests with the sequential Bonferroni method (Rice 1989).

Assignment tests calculate the probability that an individual's multilocus genotype derives from alternative groups (species or populations) and assign membership to the most likely group (Paetkau et al. 1995). Assignment tests were performed with the Bayesian method (option = "leave one out") in GeneClass (Cornuet et al. 1999) and individuals were self-classified as either native steelhead or Skamania steelhead.

To determine the number of distinct populations (k) in the Klickitat River, the program STRUCTURE (Pritchard et al. 2000) was used. All 446 individuals were included without a priori population identity. The number of distinct populations (k) was determined by averaging three iterations of k between 1 and 15 at a "burn-in" of 50,000 iterations and run of 500,000 iterations and selecting the k value with the highest likelihood. STRUCTURE was also used hierarchically to determine k for three sections of the Klickitat River from smolt trap samples located at rkm 9.6, 68.8, and 105.6.

Results

The number of alleles per locus observed across all populations ranged from 9 (*OMM1020*) to 38 (*OMM1050*), and a total of 178 alleles was observed from all nine loci (average = 19.8 alleles/locus). We found that H_e averaged 0.805 with a range of 0.622 (*OMM1020*) to 0.929 (*OMM1050*) and H_o averaged 0.727 with a range of 0.422 (*OMM1020*) to 0.897 (*OMM1050*). The Upper Castile Trap collection had the lowest H_e (0.681) and H_o (0.557) followed by the Skamania collection (0.756 and 0.722, respectively; Table 1).

The collections from Swale Creek, Summit Creek, and White Creek were in Hardy–Weinberg equilibrium (individual locus P-values ranged from 0.0008 to 0.9888) after sequential Bonferroni corrections ($\alpha = 0.0007 = 0.05/72$ tests), but power to detect departures was limited in these collections because of small sample sizes. Collections from smolt traps (putatively mixed population samples) were not in equilibrium, nor was the Skamania collection. Multiple loci were out of equilibrium (sequential Bonferroni corrected $\alpha = 0.0007 = 0.05/72$ tests) for Lyle Falls Trap 2000 (two heterozygote-deficient loci), Lyle Falls Trap 2001 (four heterozygote-deficient loci), Hatchery Trap (two heterozygote-deficient loci), Upper Castile Trap (three heterozygote-deficient loci), and Skamania (two heterozygote-deficient loci and one locus with heterozygote excess). These results are not unexpected since collections from smolt traps are probably a mixture of all upstream populations and may be subject to the Wahlund effect (heterozygote deficiency caused by pooling distinct spawning aggregates). Also, results from the Skamania collection may be the result of artificial selection and domestication of this stock (Crawford 1979) or low effective population size (Luikart and Cornuet 1999). Exact tests of linkage disequilibrium resulted in 3 out of 288 significant tests (sequential Bonferroni corrected $\alpha = 0.0002 = 0.05/288$ tests), but all three were among different pairs of loci.

Allelic richness was lowest in the Upper Castile Trap sample (6.4) and next lowest in the Skamania collection (7.0); the highest value (9.7) was in both the Lyle Falls Trap 2000 and 2001 (Table 1). A total of 24 unique alleles (alleles found in only one collection) were detected and identified to each collection: Summit Creek (7), White Creek (2), Lyle Falls Trap 2000 (2), Lyle Falls Trap 2001 (10), and Hatchery Trap (3). However, when a minimum 2% allele frequency criteria was implemented to potentially distinguish rare alleles from unique alleles, the total number of unique alleles dropped from 24 to 7 (Summit Creek = 2, White Creek = 1, Lyle Falls Trap 2000 = 3, and Hatchery

TABLE 1.—Genetic variation and assignment results for each sample collection of steelhead from tributaries of the Klickitat River and from Washougal Hatchery (Skamania) between 2000 and 2003. Data include sample size (n), total alleles over all loci (A), allelic richness averaged over loci (AR), expected heterozygosity (H_e), observed heterozygosity (H_o), unique alleles, and missing alleles for nine microsatellite loci. "Missing alleles" are defined as alleles found in all collections but one.

Collection	n	A	AR	H_e	H_o	Unique alleles[a]	Missing alleles	Assigned Skamania
Swale 2001	19	98	9.5	0.850	0.778	0	3	5.3
Summit 2001	15	88	9.6	0.858	0.729	2	8	0.0
White 2001	28	98	8.2	0.785	0.797	1	6	7.1
Lyle Falls Trap 2000	57	137	9.7	0.844	0.772	3	0	1.8
Lyle Falls Trap 2001	141	155	9.7	0.835	0.744	0	0	5.7
Hatchery Trap 2001	43	123	9.5	0.833	0.723	1	0	4.5
Upper Castile 2002	48	83	6.4	0.681	0.557	0	7	0.0
Skamania 2003	95	92	7.0	0.756	0.722	0	4	95.8
Total	446	178	9.5[b]	0.805[b]	0.727[b]	7	28	na

[a] Only unique alleles at frequencies of >2% are listed.
[b] Indicates average rather than total.

Trap = 1; Table 1). Also, a total of 28 missing alleles were detected (all >2% average allele frequency in other collections). Missing alleles per collection were as follows: Swale Creek = 3, Summit Creek = 8, White Creek = 6, Upper Castile Trap = 7, and Skamania = 4 (Table 1).

Juvenile samples are possibly subject to sampling error associated with kinship (Beacham et al. 1999); however, significant kinship bias (sequential Bonferroni corrected $\alpha = 0.006 = 0.05/8$ tests) was not observed in any of the eight collections in this study. The observed values and significance of each collection were as follows: Lyle Falls Trap 2000 = −0.021 (P = 0.98), Lyle Falls Trap 2001 = −0.008 (P = 0.87), Hatchery Trap = −0.022 ($P = 0.05$), Upper Castile Trap = −0.015 ($p = 0.10$), Swale Creek = −0.059 ($P = 0.97$), Summit Creek = −0.073 (P = 0.58), White Creek = −0.042 ($P = 0.52$), and Skamania = −0.010 ($P = 0.50$). None of the eight collections contained clusters of highly related individuals, and relatedness within each group was homogeneously distributed among all pairs of individuals.

Pairwise F_{ST} tests revealed significant genetic structure between 9 out of 10 pairwise comparisons (Table 2; sequential Bonferroni corrected $\alpha = 0.005 = 0.05/10$ tests). The only nonsignificant pairwise test was between Swale Creek and Summit Creek, but power was limited because of small sample sizes. The collection from Upper Castile Trap was the most divergent collection from all other collections (average of four pairwise F_{ST} values = 0.130), followed by the Skamania collection (average of four pairwise F_{ST} values = 0.078; Table 2). Temporal variance between samples collected from Lyle Falls Trap in 2000 and 2001 was small ($F_{ST} = 0.005$) but still significant ($P <$ 0.0018). This suggests mixed collections may have been composed of different population proportions in different collection years (2000 and 2001).

Assignment tests between naturally produced Klickitat River steelhead and Skamania hatchery steelhead were highly accurate (96.0% correct assignment of all individuals with two a priori–defined groups; Figure 2). The proportion of misassigned individuals was similar between the two groups (4.0% of naturally produced steelhead assigned to Skamania and 4.2% of Skamania assigned to naturally produced steelhead).

The results from STRUCTURE analyses of naturally produced steelhead samples indicated an estimate of six or seven distinct populations (k) in the Klickitat River. When all collections of naturally produced steelhead were analyzed together in STRUCTURE, the estimate of k was equal to seven. However, hierarchical tests of k within the three sections of the Klickitat River indicate that k equals 1 at the Upper Castile Trap, k

TABLE 2.—Pairwise genetic differentiation index F_{ST} among collections of steelhead from tributaries of the Klickitat River and from Washougal Hatchery (Skamania) between 2000 and 2003 (mixture samples not included). Values in bold italics on the diagonal are the average of four pairwise F_{ST} values for each collection.

Collection	1	2	3	4	5
Swale 2001	***0.053***				
Summit 2001	0.012[a]	***0.056***			
White 2001	0.030	0.030	***0.057***		
Upper Castile 2002	0.134	0.110	0.120	***0.130***	
Skamania 2003	0.037	0.072	0.046	0.156	***0.078***

[a] Not significant at Bonferroni critical value = 0.05/10 = 0.005.

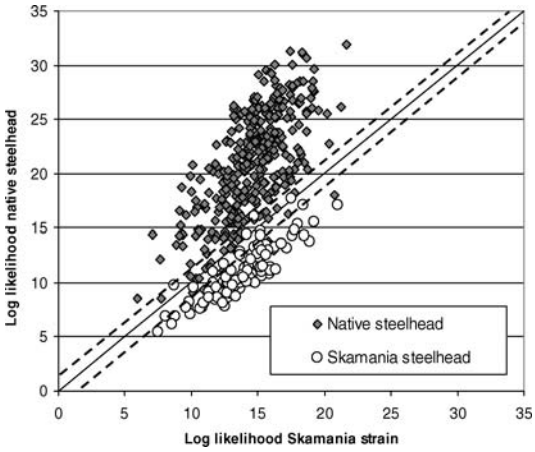

FIGURE 2.—Results of assignment tests for each individual for 351 native Klickitat River steelhead and 95 Skamania strain steelhead from nine microsatellite loci. The solid black diagonal line is the line of equal probability. The dotted lines represent the region in which assignment likelihood is within one order of magnitude of equal probability.

equals 2 at the Hatchery Trap, and k equals 6 at the Lyle Falls Trap (averaged over 2 years). Pritchard et al. (2000) warn that k is not a highly accurate measure and should only be used as an estimate for the number of distinct populations in a data set.

Discussion

Distinct Populations

Understanding the variability and adaptations of fishes in small rivers is important to maintaining and conserving genetic diversity in these systems. When the number of genetically distinct populations in a system is unknown, it is valuable to first estimate this number before collecting samples from specific locations to study population structure (e.g., Hendry et al. 2002). We used a test of multilocus genotypes with non-a priori-assigned individuals (STRUCTURE) to estimate the number of populations in our data set. An estimate of six to seven distinct groups of steelhead was determined to exist in our sample collections from the Klickitat River, suggesting multiple spawning populations with limited gene flow. Significant pairwise F_{ST} was also observed between several sampling locations, providing further evidence to support multiple spawning aggregates within the Klickitat River. While juvenile samples comprised of clusters of related individuals could resemble multiple distinct populations, kinship bias was not observed in any of our juvenile collections. The hierarchical STRUCTURE test of k within the three sections of the Klickitat River provides a basis for genetic structure and future sampling efforts. A single population was identified above Castile Falls, one population between Klickitat Hatchery and Castile Falls, and four populations between Lyle Falls and Hatchery Trap. Alternatively, it is possible that all six populations detected at Lyle Falls originated from lower river sources (downstream of Klickitat Hatchery).

Isolated populations account for a portion of the genetic structure in the Klickitat River. The most divergent population in this study was located above Castile Falls, a partial barrier to upstream migration that may restrict gene flow into this putatively resident population. Genetic drift is probably the divergent force in the upper Castile Falls population, as this collection had the lowest genetic diversity, no unique alleles, and seven missing alleles (i.e., bottleneck or founder event). Low genetic variation is expected in isolated populations above natural barriers as shown by other studies (Currens et al. 1990; Costello et al. 2003; Taylor et al. 2003). However, resident populations may contribute to the genetic composition of anadromous runs (Zimmerman and Reeves 2000) and, therefore, are included as potential components of genetic variation. Several waterfalls with varying potentials as isolating mechanisms are located in the Klickitat River subbasin and these locations require further research to identify additional distinct populations.

Resident rainbow trout may provide a component of genetic structure in this study, as resident steelhead have been shown to contribute to anadromous gene pools (Zimmerman and Reeves 2000; Narum et al. 2004) and may have been present in smolt traps and tributary samples. The Klickitat River is in the transition zone between coastal and inland forms of steelhead (Behnke 2002) and both forms may be present. Further, unique hatchery strains of resident rainbow trout, such as the McCloud strain (Nielsen et al. 1999), have been stocked into the Klickitat River and may contribute to genetic structure (Phelps et al. 2000).

Differences between hatchery and naturally produced steelhead also represent an important component of the genetic structure in the Klickitat River. Despite the potential for gene flow between Skamania strain steelhead and native steelhead (especially summer run) in the Klickitat River, genetic divergence between the two groups remains substantial. A highly significant F_{ST} of 0.078 ($P < 0.0001$) and high assignment fidelity between naturally produced steelhead and Skamania steelhead suggest little interbreeding between these stocks and low reproductive ability of the Skamania stock since few of the naturally reproduced juveniles of unknown origin were assigned to the Skamania stock. This is consistent with allozyme studies that have

shown summer-run Skamania steelhead experience low reproductive success (Leider et al. 1990; Kostow et al. 2003) and are genetically differentiated from temporally isolated winter-run steelhead in the Clackamas River (Kostow et al. 2003). Evidence from our study suggests strong reproductive isolation between native and Skamania steelhead with overlapping adult spawning runs. Either gene flow is limited between summer runs of native and Skamania steelhead, or native and Skamania hybrids exhibit similarly low reproductive success as pure Skamania steelhead and have not fully introgressed with native steelhead. Additionally, a selected harvest of Skamania fish reduces the potential of this stock to contribute to reproduction in the Klickitat River, as an average of 98.1% of the sport catch in the Klickitat River from 1986 to 2003 was composed of Skamania strain steelhead (Washington Department of Fish and Wildlife, unpublished data). To fully understand gene flow between native and Skamania steelhead in the Klickitat River, studies that directly measure reproductive success through parentage analysis may be needed.

Since multiple distinct populations of steelhead are evident in the Klickitat River, further efforts to identify the components of genetic structure are needed. Results from the current study suggest future efforts should focus on the following components: summer- and winter-migrating adults, isolated populations above migration barriers, resident populations, and introduced nonnative populations.

Conservation Implications

The results of this study indicate positive signs but also challenges for restoring and managing threatened native steelhead in the Klickitat River. The genetic integrity of the native populations appears largely unaffected by the introduced Skamania stock, allowing for potentially successful restoration efforts through supplementation with locally adapted fish as broodstock (Miller and Kapuscinski 2003). However, evidence for multiple self-recruiting populations of steelhead in the Klickitat River suggests consideration for maintaining genetic diversity as supplementation occurs. Effects of supplementation can include decreased genetic variation (Waples 1994), changes to effective population size (Ryman and Laikre 1991; Ryman et al. 1995), and altering stochastic genetic drift (O'Connell et al. 1997). Yet, the threatened species status of Klickitat River steelhead warrants supplementation under guidelines suggested in the literature to reduce potentially negative impacts of supplementation (Campton 1995; Miller and Kapuscinski 2003; Reisenbichler et al. 2003). In the Sacramento River (winter-run Chinook salmon *O. tshawytscha*; Hedrick et al. 1994, 2000), Snake River (sockeye salmon *O. nerka*; Cummings et al. 1997), and Puget Sound (pink salmon *O. gorbuscha*; Olsen et al. 2000), supplementation has been a benefit to natural populations, minimizing the risk of population extinction. In these situations, careful genetic monitoring and evaluation are desirable to limit genetic risks (Olsen et al. 2000).

Management to conserve the diversity of native steelhead in small rivers should focus on identifying native genetic variation through intensive sampling and genetic analyses to identify discrete spawning aggregations (temporal spawning migrations, isolated populations, resident rainbow trout, and introduced stocks), restoration through supplementation with locally adapted fish as broodstock, and genetic monitoring and evaluation. This study provides evidence to suggest that reproductively isolated populations of steelhead with multiple life histories contain high levels of genetic variability. Consideration of population structure and life history variation are essential for management directed toward preserving native genetic diversity of threatened populations.

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