10. SOUTH FORK SKYKOMISH RIVER SUMMER STEELHEAD – (BRIEFING)

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"GREEN SHEET"

Meeting dates:	August 8-9, 2008 Commission Meeting (briefing)	**#10**
Agenda item #:	South Fork Skykomish River Summer Steelhead – briefing	
Staff Contact:	Craig Busack, Science Division Manager (Fish Program) and Denise Hawkins, Molecular Genetics Lab Project Manager (Fish Program)	
Presenter(s):	Denise Hawkins, Molecular Genetics Lab Project Manager (Fish Program) and Chad Jackson, Region 4 Fish and Wildlife Biologist (Fish Program)	

Background:

The South Fork Skykomish River, Washington, above Sunset Falls was inaccessible to anadromous fishes until 1958, when a fish collection facility was constructed. Sunset Falls is located on the South Fork Skykomish River about 3 km above the confluence with the North Fork Skykomish River and has a 145-m vertical drop; therefore, fish must be trapped, hauled above the falls, and released. Subsequently, thousands of fish, including unclipped and adipose-fin-clipped adult summer steelhead *Oncorhynchus mykiss* (anadromous form), have annually been transported above the falls during the trap operation period from mid-July to mid-December. In addition to the anadromous fish transported above the falls, rainbow trout are resident above the falls. The relationship between the anadromous and resident life history types in Puget Sound streams is unclear (Phelps et al. 1997), and was not the focus of this study.

Populations of summer steelhead in the Skykomish River basin include native fish in the North Fork Skykomish River and hatchery fish produced at Reiter Ponds Hatchery, constructed in 1974. Eggs from Skamania Hatchery in the Washougal River system, a lower Columbia River tributary, were used at Reiter Ponds Hatchery for the first few years (Crawford 1979) until sufficient returning broodstock could be gathered at the Sunset Falls fish collection facility. Reiter Ponds Hatchery is located about 10 km downstream from Sunset Falls on the main-stem Skykomish River at river kilometer 74.

Releases of hatchery summer steelhead smolts into the main-stem Skykomish River have been substantial since Reiter Ponds Hatchery began production in 1975. Releases in the North Fork Skykomish River have generally been 20,000–30,000 smolts/year, whereas releases in the South Fork Skykomish River ceased after 1992. Because hatchery steelhead are given adipose fin clips as juveniles, an adult fish with an adipose fin is considered to be from natural production. Beginning in 1998, data have been collected on the number of unclipped adult fish returning to Sunset Falls. Several hundred fish of presumed natural origin (i.e., with intact adipose fins) have been returning annually to the collection facility.

This study uses a genetic analysis to determine the source of unclipped, natural-origin summer steelhead returning to Sunset Falls. Two possible source populations are native fish from the North Fork Skykomish River and natural production of hatchery fish from Reiter Ponds Hatchery in the Skykomish River basin. Adult summer-run steelhead samples were collected from Reiter Ponds Hatchery on January 1, 2001 (representing Skamania Hatchery-origin summer steelhead) and from the Sunset Falls fish collection facility between August 16 and September 13, 2002. Steelhead smolts were collected from the North Fork Skykomish River by backpack electrofishing on one day in August 2003 and one day in August 2004 (exact collection dates are uncertain). A microsatellite DNA analysis of 10 loci was used to evaluate unmarked samples in comparison with natural-origin samples from the North Fork Skykomish River and hatchery-origin samples from Reiter Ponds Hatchery (the two most likely sources of

the unmarked fish). Results of the analyses provide evidence that the unmarked steelhead collected at Sunset Falls are more closely related to Reiter Ponds Hatchery fish than to natural-origin fish from the North Fork Skykomish River. There is evidence of some genetic influence from natural-origin North Fork Skykomish River fish to the unmarked steelhead at Sunset Falls but to a lesser degree than from Reiter Ponds Hatchery fish. This study, based on a collection of 50 samples from un-clipped steelhead returning to Sunset Falls in 2002, documents that Skamania Hatchery-origin steelhead have naturally produced offspring that are returning to spawn in a northern Puget Sound river basin. However, the results from this study do not address whether or not this is a self-sustaining population in the absence of the hatchery supplementation program.

Policy issue(s) you are bringing to the Commission for consideration:

This is a briefing of results from a genetic study. No policy issues are being brought for consideration.

Public involvement process used and what you learned:

The results of this work were internally reviewed by WDFW staff prior to submission for publication and subsequently by three external reviewers as part of the manuscript submission process. The work is published in Transactions of the American Fisheries Society 137:763-771. Todd Kassler presented this work at a meeting of the Steelhead and Cutthroat Policy Advisory Group (SCPAG) in February 2008.

Action requested:

None – briefing only.

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N/A

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[Note]

Summer-Run Hatchery Steelhead Have Naturalized in the South Fork Skykomish River, Washington

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Abstract.—Evaluation of natural-origin, hatchery-origin, and unmarked steelhead *Oncorhynchus mykiss* from the Skykomish River drainage basin, Washington, was conducted to determine the source of unmarked steelhead that return to Sunset Falls (South Fork Skykomish River). One possible source is the large number of steelhead stocked into the Skykomish River basin from Reiter Ponds Hatchery; this hatchery stock was founded with fish from Skamania Hatchery in the Washougal River system, Washington. A microsatellite DNA analysis of 10 loci was used to evaluate unmarked samples in comparison with natural-origin samples from the North Fork Skykomish River and hatchery-origin samples from Reiter Ponds Hatchery. Results of the analyses provide evidence that the unmarked steelhead collected at Sunset Falls are more closely related to Reiter Ponds Hatchery fish than to natural-origin fish from the North Fork Skykomish River. There is evidence that unmarked steelhead at Sunset Falls are also mixing with natural-origin North Fork Skykomish River fish but to a lesser degree than with Reiter Ponds Hatchery fish. This study documents that Skamania Hatchery-origin steelhead have naturally produced offspring that are returning to spawn in a northern Puget Sound river basin.

The South Fork Skykomish River, Washington, above Sunset Falls was inaccessible to anadromous fishes until 1958, when a fish collection facility was constructed (Figure 1). Sunset Falls has a 145-km vertical drop; therefore, fish must be trapped, hauled above the falls, and released. Subsequently, thousands of fish, including unclipped and adipose-fin-clipped adult summer steelhead *Oncorhynchus mykiss* (anadromous rainbow trout), have annually been transported above the falls during the trap operation period from mid-July to mid-December. In addition to the anadromous fish transported above the falls, rainbow trout are resident above the falls. The relationship between the anadromous and resident life history types in Puget Sound streams is unclear (Phelps et al. 1997), as some streams show no indication of genetic differentiation between the two groups.

Populations of summer steelhead in the Skykomish River basin include native fish in the North Fork Skykomish River and hatchery fish produced at Reiter Ponds Hatchery, constructed in 1974. Eggs from Skamania Hatchery in the Washougal River system, a lower Columbia River tributary, were used at Reiter Ponds Hatchery for the first few years (Crawford 1979) until sufficient returning broodstock could be gathered at the Sunset Falls fish collection facility.

Releases of hatchery summer steelhead smolts into the main-stem Skykomish River have been substantial since Reiter Ponds Hatchery began production in 1975. Releases in the North Fork Skykomish River have generally been 20,000–30,000 smolts/year, whereas releases in the South Fork Skykomish River ceased after 1992 (Table 1). Because hatchery steelhead are given adipose fin clips as juveniles, an adult fish with an adipose fin is considered to be from natural production. Beginning in 1998, data have been collected on the number of unclipped adult fish returning to Sunset Falls. Several hundred fish of presumed natural origin (i.e., with intact adipose fins) have been returning annually to the collection facility.

The production of established salmonid runs by hatchery fish in areas devoid of anadromous salmonids has been documented in New Zealand (Quinn and Unwin 1993), Lake Ontario (Smith et al. 2006), and the Deschutes River, Washington (Seiler et al. 1981, 1997). The Deschutes River was planted with Chinook salmon *O. tshawytscha* from the Green River beginning in 1946 before the construction of fishways around natural barriers in 1954. Until 1954, returning Chinook salmon were netted below Tumwater Falls on the Deschutes River, transported above the falls, and released to allow natural spawning (Seiler et al. 1981). This program currently uses broodstock collected below Tumwater Falls in addition to facilitating upstream passage of unclipped fish for natural spawning above the falls. Hatchery plants of coho salmon *O. kisutch* began in 1957; since then, millions of juvenile hatchery fish have also been planted. Each year since the mid 1970s, counts of fin-clipped and unclipped coho salmon have been recorded. Documentation of hatchery-origin and natural-origin coho salmon is based on the presence and absence of fin clips and on scale analysis. However, scale analysis of

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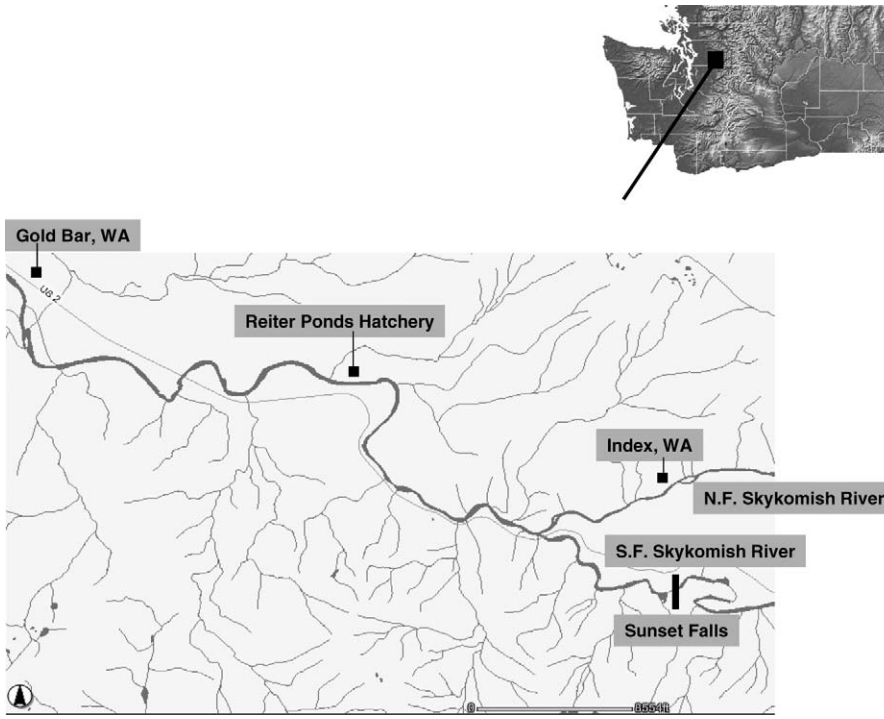


FIGURE 1.—Map of the Skykomish River basin, Washington, indicating locations of Reiter Ponds Hatchery, the North Fork Skykomish River, and Sunset Falls (South Fork Skykomish River), where summer steelhead were sampled to determine the genetic origin of unmarked fish returning to the falls. Inset shows study area location within the state.

unclipped coho salmon has revealed that some are of hatchery origin. Unclipped coho salmon have been found in the Deschutes River, but a genetic evaluation of their ancestral origin has not been conducted to determine whether they constitute a self-sustaining population. Anadromous sockeye salmon *O. nerka* from a hatchery source also became established in the presence of an existing native population (Hendry et al. 1996). Sockeye salmon in Lake Washington were to have a genetic affinity to Baker Lake sockeye salmon. However, other potential source populations described by Hendry et al. (1996) were stocked into Lake Washington and may have contributed to the overall population of sockeye salmon.

This study uses a genetic analysis to determine the ancestral origin of unclipped, natural-origin summer steelhead returning to Sunset Falls. Two possible source populations are native fish from the North Fork Skykomish River and natural production of hatchery fish from Reiter Ponds Hatchery in the Skykomish River basin.

Methods

The South Fork Skykomish River originates in the Cascade Mountains and flows about 51 km to its confluence with the North Fork Skykomish River near the town of Index (Figure 1). Sunset Falls is located on the South Fork Skykomish River about 3 km above the confluence with the North Fork Skykomish River. The main-stem Skykomish River continues for about 48 km to its junction with the Snoqualmie River, which then becomes the Snohomish River for 24 km before entering Puget Sound near the city of Everett. Reiter Ponds Hatchery is located about 10 km downstream from Sunset Falls on the main-stem Skykomish River at river kilometer 74.

Adult summer-run steelhead samples were collected from Reiter Ponds Hatchery on January 1, 2001 (representing Skamania Hatchery-origin summer steelhead) and from the Sunset Falls fish collection facility between August 16 and September 13, 2002. Steelhead smolts were collected from the North Fork Skykomish River by backpack electrofishing on one date in August 2003 and one date in August 2004 (exact collection dates are uncertain).

Genomic DNA was extracted for all samples by digesting a small piece of fin tissue by using silica membrane-based kits (Macherey-Nagel, Bethlehem, Pennsylvania) according to the manufacturer's recommendations. Ten microsatellite loci combined into five

TABLE 1.—Number of hatchery summer steelhead released in the Skykomish River (SR), North Fork Skykomish River (NF), and South Fork Skykomish River (SF), Washington, and the total number of returning adults counted at the Sunset Falls fish collection facility (on the SF) from 1962 to 2006 (NA = no data available). Starting in 1998, Sunset Falls counts distinguished between adipose-fin-clipped and unclipped fish; these data are reported as total counts with the counts of unclipped fish in parentheses.

	Release site			
Year	SR	NF	SF	Sunset Falls count
1962	44,820	0	0	NA
1963	0	0	0	NA
1964	0	29,230	30,293	NA
1965	24,350	26,100	0	NA
1966	11,520	24,223	0	NA
1967	0	32,860	0	NA
1968	0	40,005	0	192
1969	0	39,322	0	106
1970	0	50,078	0	303
1971	0	37,183	0	459
1972	9,240	28,870	0	420
1973	0	57,045	0	395
1974	0	66,720	0	485
1975	76,374	20,662	11,121	465
1976	74,975	10,923	10,278	292
1977	62,629	42,460	10,236	843
1978	69,237	32,080	0	598
1979	74,873	32,500	0	412
1980	59,409	41,290	18,469	1,196
1981	24,960	0	25,485	1,137
1982	49,008	13,342	15,000	636
1983	65,760	19,147	14,024	442
1984	112,063	26,835	17,132	1,579
1985	35,008	16,322	11,665	1,565
1986	95,235	18,092	20,287	1,245
1987	63,059	0	0	1,414
1988	76,400	24,708	19,851	2,048
1989	101,619	0	0	502
1990	91,758	14,571	15,929	1,208
1991	104,489	15,276	15,179	936
1992	111,547	20,360	20,023	NA
1993	72,774	7,638	0	492
1994	146,232	19,568	0	791
1995	120,043	6,741	0	600
1996	127,383	15,775	0	1,096
1997	93,745	14,610	0	791
1998	175,000	23,337	0	1,145 (840)
1999	176,320	17,812	0	715 (453)
2000	117,363	4,600	0	2,052 (1,012)[a]
2001	136,492	0	0	1,569 (503)
2002	0	154,776	0	1,491 (948)
2003	107,217	15,282	0	1,176 (303)
2004	165,000	20,150	0	1,158 (344)
2005	168,800	20,160	0	518 (318)
2006	149,440	13,398	0	701 (498)

[a] 49.3% of the 2,052 sampled fish were unclipped.

multiplexes were screened for this study. Descriptions of the loci and polymerase chain reaction (PCR) conditions are provided in Table 2. The PCR analyses were conducted on an PTC-200 thermal cycler (M-J Research, Waltham, Massachusetts) with a simple thermal profile as follows: an initial denaturation step of 3 min at 95°C; 25–28 cycles of denaturation at 95°C for 15 s, 30 s at the appropriate temperature for each multiplex, and 1 min at 72°C; and a final extension at 72°C for 30 min. Genotypes were visualized using an ABI-3730 DNA Analyzer (Applied Biosystems, Inc., Foster City, California) with internal size standards (GS500LIZ 3730) and GENEMAPPER version 3.0 software. Allele binning and naming were accomplished using MicrosatelliteBinner version 1h (Molecular Genetics Laboratory, Washington Department of Fish and Wildlife [WDFW], Olympia).

Tests for deviation from Hardy–Weinberg equilibrium at each locus and over all loci were conducted using GENEPOP version 3.4 (Raymond and Rousset 1995) with 100 batches and 1,000 iterations. Allele frequencies were calculated in CONVERT version 1.3 (Glaubitz 2003).

Observed and expected values of heterozygosity were calculated using Genetic Data Analysis software (Lewis and Zaykin 2001). Allelic richness and the inbreeding coefficient (F_{IS}) were computed for each subpopulation with FSTAT version 2.9.3.2 (Goudet 1995). Linkage disequilibrium was compared between each locus for each collection using GENEPOP (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). A Bonferroni correction was used to adjust the significance level (α) for multiple tests (Rice 1989).

Within a group, the coefficient of identity was calculated between each pair of samples in all collections using the Queller and Goodnight (1989) estimator of relatedness in IDENTIX version 1.1 (Belkhir et al. 2002). Using this measure of relatedness, a value of 0.5 is expected for a full-sibling relationship (individuals sharing the same mother and father) between two individuals.

Population structure among the three collections was determined with an examination of pairwise genetic differentiation index (F_{ST}) values and pairwise genotypic population differentiation tests calculated in GENEPOP. Statistical significance for the population differentiation tests was evaluated using a Bonferroni correction (Rice 1989).

Ancestry for all individuals was evaluated with Bayesian analysis implemented in STRUCTURE version 2.1 (Pritchard et al. 2000). Five independent runs allowing admixture were computed with 50,000 burn-ins and 500,000 iterations. Analyses were conducted using all individuals, and the number of possible populations (K) was set at 1–3 depending on the particular test. When K was set at 1, we tested for a single ancestral group of steelhead. When K was set at 2, we tested for differences between hatchery- and natural-origin groups. For a K of 3, we tested the possibility of a novel origin of Sunset Falls samples.

TABLE 2.—Annealing temperature, number of cyles used in polymerase chain reaction (PCR) analyses, number of alleles per locus, allele size range (base pairs [bp]), observed heterozygosity (H_o), and expected heterozygosity (H_e) for multiplexed microsatellite loci that were used to determine genetic relationships among summer steelhead collections from the Skykomish River basin, Washington.

Multiplex	Locus	Annealing temperature (°C)	PCR cycles	Alleles per locus	Allele size range (bp)	H_o	H_e
OmyB2	*One102*	55	26	22	184–289	0.884	0.909
	One114	55	26	20	185–276	0.865	0.903
	Ots100	55	26	21	168–217	0.843	0.834
OmyC2	*One108*	55	28	23	160–267	0.865	0.901
	Ots103	55	28	5	57–86	0.250	0.262
	One101	55	28	7	119–242	0.498	0.519
OmyF2	*Omy1001*	52	25	19	171–224	0.847	0.855
	Omm1128	52	25	29	219–353	0.836	0.939
	Oki10	52	25	16	96–159	0.834	0.869
	One18	52	25	9	169–186	0.775	0.805

Genetic distance between pairs of subpopulations was estimated using the Cavalli-Sforza and Edwards (1967) chord distance in PHYLIP version 3.5c (Felsenstein 1993). Genetic distance among the three collection groups was calculated using GENDIST.

Maximum likelihood jackknife assignments for each fish in the baseline collections from Reiter Ponds Hatchery and the North Fork Skykomish River were performed in GENECLASS2 version 2.0g (Piry et al. 2004) using the Bayesian method described by Rannala and Mountain (1997). In the jackknife procedure, each individual fish was removed from the dataset, the allele frequencies of the baseline subpopulations were recalculated, and the fish was assigned to the most likely group. Jackknife assignments were used to evaluate the reliability of the assignments of the temporal collections and to determine the relationships among subpopulations. Correct jackknife assignment relies upon genetically distinct populations. The origin of Sunset Falls steelhead was explored by assigning them as unknown fish to either Reiter Ponds Hatchery or the North Fork Skykomish River.

Results

Global tests for Hardy–Weinberg equilibrium revealed two loci (*Oki10* and *Omm1128*) that deviated from expectations when the North Fork Skykomish River collections were analyzed separately and pooled. Each locus deviated from Hardy–Weinberg equilibrium for only one population; therefore, those loci and populations were included in the analyses because there was no indication of a broad-scale problem with the locus or collection. Any locus or population that deviates from equilibrium in multiple collections or loci indicates that (1) there is nonrandom mating of individuals in the population (inbreeding or assortative mating; evidenced by an increase in homozygotes), (2) the populations are small and subject to genetic drift, or (3) there have been errors in scoring of the locus (null alleles). Results of Hardy–Weinberg tests of the separate or pooled North Fork Skykomish River collections did not reveal any significant deviation from expected values; therefore, the two collections were pooled for the analysis to provide a larger sample size for this location.

The number of alleles per locus ranged from 5 to 29. Observed heterozygosity was below 50% in two loci (*Ots103* and *One101*) and over 77% for the remaining eight loci. Allele frequencies for each collection are shown in Table A.1. Observed heterozygosity for all three collections was between 0.730 and 0.771 (Table 3). Allelic richness for the Sunset Falls collection was

TABLE 3.—Description of summer steelhead collection from three locations in the Skykomish River basin, Washington (Reiter Ponds Hatchery [RPH], Sunset Falls on the South Fork Skykomish River, and North Fork Skykomish River [NF]). Number of sampled fish (N), observed heterozygosity (H_o), expected heterozygosity (H_e), observed allelic richness (A_o), inbreeding coefficient (F_{IS}), and linkage disequilibrium (LD; number of significant locus comparisons among 45 tests; presented for uncorrected α, respectively; Rice 1989) are shown.

Location	N	H_o	H_e	A_o	F_{IS} (P)[a]	LD[b]
RPH	100	0.751	0.771	11.9	0.027 (0.022)	8, 4
Sunset Falls	50	0.771	0.780	13.3	0.011 (0.253)	3, 0
NF	75	0.730	0.755	12.0	0.034 (0.027)	24, 9

[a] Bonferroni-corrected α for 30 tests: 0.05/30 = 0.0017.
[b] Bonferroni-corrected α for 45 tests: 0.05/45 = 0.0011.

TABLE 4.—Pairwise *P*-values for tests of genotypic differentiation (above diagonal; first value in each series) and the genetic differentiation index (F_{ST}; below diagonal) between summer steelhead collections from three locations in the Skykomish River basin, Washington (Reiter Ponds Hatchery [RPH], Sunset Falls on the South Fork Skykomish River, and North Fork Skykomish River [NF]). Above the diagonal, the second value in each series is the genetic distance. Bold type indicates significant genotypic differentiation tests or F_{ST} values that were significantly different from zero; comparisons were significant after Bonferonni correction of α (Rice 1989).

Location	RPH	Sunset Falls	NF
RPH		**0.0112**, 0.0182	**0.0301**, 0.0281
Sunset Falls	**0.0000**		**0.0234**, 0.0292
NF	**0.0000**	**0.0000**	

highest (13.3), while values for the Reiter Ponds Hatchery and North Fork Skykomish River collections were around 12.0. Estimates of F_{IS} were not significantly different from zero and were between 0.011 and 0.034 for all three collections; the highest values were observed for the North Fork Skykomish River (Table 3). Linkage disequilibrium between loci was high for the North Fork Skykomish River collection (24 out of 45 locus comparisons before Bonferroni correction; 9 comparisons after correction) and low for the Reiter Ponds Hatchery and Sunset Falls collections.

The analysis of relatedness among samples revealed that between 0.1% and 2.1% of the comparisons exhibited a relatedness value of 0.5 or greater, indicating a full-sibling relationship. The highest observed relatedness value occurred in the North Fork Skykomish River samples, whereas maximum relatedness observed in the Sunset Falls and Reiter Ponds Hatchery collections was below 0.5 (0.08 and 0.26, respectively).

The pairwise tests of genotypic differentiation from zero indicated that all three groups of steelhead were significantly different from each other (Table 4). The pairwise F_{ST} ranged between 0.0112 and 0.0301, and all comparisons indicated significant differences; the lowest F_{ST} was that between the Sunset Falls and Reiter Ponds Hatchery collections (0.0112).

The genetic distances among collection groups were examined to assess genetic relationships (Table 4). Genetic distances between steelhead from Reiter Ponds Hatchery and those from Sunset Falls mirrored the comparison of pairwise F_{ST} values among collections. Genetic distance was 0.0182 for the comparison of Reiter Ponds Hatchery and Sunset Falls samples and exceeded 0.0280 for the comparison of North Fork Skykomish River samples with either Sunset Falls or Reiter Ponds Hatchery fish.

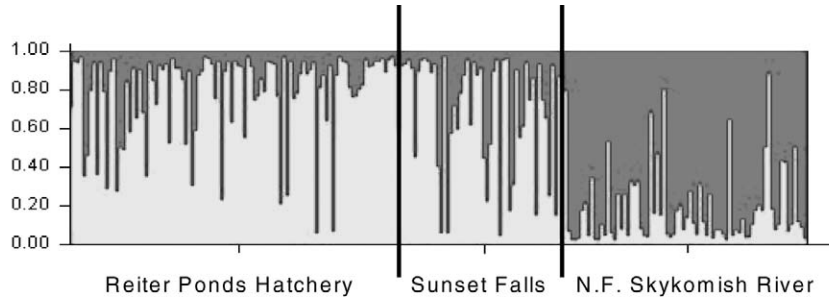
Analyses in STRUCTURE (Pritchard et al. 2000) were used to determine individual ancestry of the three collections. Average $\log_e$ score was −8,837.35 for a *K* of 1, −8,669.06 for a *K* of 2, and −8,605.1 for a *K* of 3. The point at which average $\log_e$ score reaches a plateau defines the best *K* for describing the number of ancestral groups in the data (Pritchard 2000). In our analysis, the plateau was at 2, indicating that there were two ancestral groups of steelhead in the Skykomish River (Reiter Ponds Hatchery and natural-origin fish from the North Fork Skykomish River; Figure 2). Steelhead from Sunset Falls had 69.0% ancestry in the hatchery group and 31.0% ancestry in the natural-origin group. There was no evidence that Sunset Falls steelhead were of novel origin (i.e., $K = 3$). The ancestry percentages for Sunset Falls fish thus identified a mixed sample in which hatchery origin predominated and natural origin contributed to a lesser degree.

A jackknife test of the Reiter Ponds Hatchery and North Fork Skykomish River data showed that over 93.0% of the samples were assigned back to the correct population of origin (Table 5). Assignments of the individual steelhead from the Sunset Falls collection were considered positive if the likelihood of the assignment was over 90%. For Sunset Falls, 37 individuals had positive assignments: 30 were assigned to Reiter Ponds Hatchery and 7 were assigned to the North Fork Skykomish River (Table 5).

Discussion

The presence of anadromous salmonids established from a hatchery source in the Deschutes River and Lake Washington supports a conclusion that hatchery salmonids can naturally reproduce. Unclipped steelhead collected at Sunset Falls were most likely from two origins, Reiter Ponds Hatchery fish (i.e., – Washougal River origin) and natural-origin North Fork Skykomish River fish. Waples and Gaggiotti (2006) presented criteria for concluding that two collections represent different populations. Based on those criteria, we determined that the North Fork Skykomish River and Reiter Ponds Hatchery steelhead were distinct populations. Genotypic differentiation, pairwise F_{ST}, jackknife, and STRUCTURE analyses revealed differentiation between the North Fork Skykomish River and Reiter Ponds Hatchery samples. The divergence between the two groups can be used to determine their relationships with Sunset Falls steelhead based on population-of-origin assignments for the Sunset Falls samples.

The stocking history of the Skykomish River basin



Averages over all individuals into one of two groups (K = 2) identified in the STRUCTURE analysis for the three collection locations.

	Group #1 (Black)	Group #2 (Grey)
Reiter Ponds Hatchery	21.8%	78.2%
Sunset Falls (S.F. Skykomish R.)	31.0%	69.0%
N.F. Skykomish River	81.5%	18.5%

FIGURE 2.—STRUCTURE plot showing genetic ancestry of summer steelhead in the Skykomish River basin, Washington, showing two possible populations (i.e., $K = 2$); ancestry of each individual is represented by a single bar (gray = Reiter Ponds Hatchery ancestry; black = North Fork Skykomish River natural-origin ancestry; black and gray combined = mixed ancestry). Vertical black lines delineate the collections. The ancestry percentages for each collection are given below the plot.

suggests a great potential for hatchery steelhead to become naturalized above Sunset Falls. Since 1962, annual releases in the basin have ranged from 0 to over 200,000 smolts. Adult steelhead have been observed at Sunset Falls since the late 1960s; however, their fin-clipped or unclipped status was not documented until 1998. Since 1998, the number of unclipped adult steelhead has been between 25.8% and 73.4% of the total number of steelhead counted at Sunset Falls.

Heterozygosity values and allelic richness revealed moderate to high genetic diversity in the Sunset Falls collection (Table 3). The slightly higher allelic richness values for the Sunset Falls samples may be a result of genetic contributions by both Reiter Ponds Hatchery and North Fork Skykomish River fish. Mixing of the two groups could cause an increase in the overall number of alleles that are present. Assessment of F_{IS} showed no evidence of a small population size or inbreeding among the three populations. The F_{IS} analysis also gave no indication that genetically differentiated resident rainbow trout were mixing with steelhead. Evaluation of linkage disequilibrium between the collections and loci can identify genetic drift, inclusion of family groups within collections, assortative mating, admixture, or a combination of these.

TABLE 5.—Maximum likelihood jackknife assignments to population or origin (% of fish; number of fish is in parentheses) for summer steelhead in baseline collections from Reiter Ponds Hatchery (RPH) and the North Fork Skykomish River (NF; natural-origin fish), Washington; and assignments of Sunset Falls (South Fork Skykomish River) fish to the RPH and NF populations. For Sunset Falls, only those assignments with likelihoods greater than 90% are presented.

Location (sample size)	RPH	NF
RPH ($N = 99$)	**93.9 (93)**	6.1 (6)
NF ($N = 70$)	5.7 (4)	**94.3 (66)**
Sunset Falls ($N = 37$)	81.1 (30)	18.9 (7)

The large number of locus comparisons that were significantly linked in the North Fork Skykomish River collection is the result of related individuals sampled in that collection. The analysis of relatedness revealed that over 2.0% of the individual comparisons for the North Fork Skykomish River collection had values of 0.5 or greater, whereas the relatedness values for the Sunset Falls and Reiter Ponds Hatchery collections were below 0.3.

The pairwise genotypic differentiation tests indicated that all three groups of steelhead were significantly different from each other over all loci. However, after Bonferroni correction, the number of significant locus comparisons was 4 of 10 for Sunset Falls and Reiter Ponds Hatchery, 8 of 10 for Reiter Ponds Hatchery and the North Fork Skykomish River, and 6 of 10 for Sunset Falls and the North Fork Skykomish River. This suggests that Sunset Falls steelhead are more similar to Reiter Ponds Hatchery fish than to North Fork Skykomish River fish.

The analysis of genetic distance and pairwise F_{ST}

provides additional evidence that Sunset Falls steelhead are more similar to Reiter Ponds Hatchery fish than to North Fork Skykomish River fish. There may be some influence of North Fork Skykomish River steelhead in the Sunset Falls collection; however, the overall relationship appears to suggest that the Reiter Ponds Hatchery genetic contribution is greater.

Analysis of ancestry identified two distinct groups that could be classified as hatchery and natural origin (i.e., $K = 2$). This was evidenced by a majority of the Reiter Ponds Hatchery steelhead forming one ancestral group and a majority of natural-origin North Fork Skykomish River fish forming the second ancestral group. This result supports the distinction found in the pairwise F_{ST} and genetic distance analyses. The average percent ancestry of Sunset Falls steelhead was higher in the cluster occupied by the hatchery-origin group, suggesting that these samples were derived primarily from a Reiter Ponds Hatchery source. Although segregation of the two ancestral groups was strong, there was also evidence of some mixing within each group. The evaluation of ancestry for three populations ($K = 3$) provided no evidence that the Sunset Falls group has a unique origin, but it supported the conclusion that there has been mixing between hatchery- and wild-origin steelhead in the Skykomish River basin.

The jackknife analysis suggested that steelhead from the baseline sources were genetically differentiated, as over 93.0% of individuals were assigned back to the correct population of origin. This high level of differentiation provides confidence that the assignments of Sunset Falls steelhead to population of origin were correct. Population-of-origin assignments for Sunset Falls steelhead allocated a majority (81.1%) of the individuals to Reiter Ponds Hatchery, supporting the relationship between Sunset Falls and Reiter Ponds Hatchery fish.

The results of this study indicate that the unclipped steelhead returning to Sunset Falls are most closely related to fish from Reiter Ponds Hatchery. The most likely origin of the genetically distinguishable Sunset Falls steelhead is natural reproduction of Reiter Ponds Hatchery fish in the South Fork Skykomish River above Sunset Falls. Evidence also indicates a lesser contribution by North Fork Skykomish River steelhead to the South Fork Skykomish River population above Sunset Falls. The mixed ancestry of some samples from the Sunset Falls collection indicates that hatchery steelhead have reproduced naturally in this area for multiple generations; however, it is not known whether the Sunset Falls population would be self-sustaining if the hatchery supplementation program ceased. Evaluation of whether the naturally produced Sunset Falls fish are self-sustaining will be possible if transportation of fin-clipped fish above the falls is halted. A genetic study could determine the ancestral origin of fish that are naturally reproducing; the presence of the same ancestral group after several generations would then need to be documented. A parentage analysis of all steelhead transported above Sunset Falls and their returning offspring could also document the reproductive success of naturally spawning, hatchery-origin fish.

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Appendix: Allele Frequencies of Skykomish River Steelhead

TABLE A.1.—Allele frequencies for 10 microsatellite loci examined in summer steelhead collected from three locations in the Skykomish River basin, Washington (Reiter Ponds Hatchery [RPH], Sunset Falls on the South Fork Skykomish River, and North Fork Skykomish River [NF]). Number of fish sampled at each location (RPH, Sunset Falls, and NF, respectively) is presented in parentheses for each locus.

Allele size (base pairs)	Location		
	RPH	Sunset Falls	NF
***One102* (96, 48, 71)**			
184	—	—	0.014
188	0.120	0.063	0.014
192	0.104	0.094	0.352
196	0.010	0.021	0.148
200	0.016	0.031	0.049
208	0.135	0.073	0.042
212	0.052	0.083	0.049
216	—	—	0.049
220	0.016	0.052	0.007
224	0.099	0.125	0.035
228	0.026	0.042	0.007
232	—	—	0.042
236	0.234	0.115	0.056
240	0.073	0.052	0.049
245	0.026	0.031	—
248	0.010	0.052	—
253	0.042	0.125	0.021
257	0.016	0.010	0.014
261	0.016	—	—
273	—	—	0.007
285	—	—	0.021
289	0.005	0.031	0.021
***One114* (81, 50, 62)**			
185	0.068	0.030	0.097
189	0.062	0.040	0.040
193	0.105	0.060	0.113
197	0.043	0.100	0.081
201	0.068	0.090	0.008

TABLE A.1.—Continued.

Allele size (base pairs)	Location		
	RPH	Sunset Falls	NF
***One114* (81, 50, 62)**			
205	0.191	0.190	0.202
209	0.062	0.100	0.129
213	0.062	0.080	0.040
217	0.198	0.160	0.057
221	0.012	0.030	0.065
225	0.031	0.020	0.040
229	0.043	0.020	0.057
232	0.006	0.010	—
236	—	0.010	—
244	0.006	—	—
248	—	0.010	0.024
252	0.006	—	—
256	0.006	—	0.032
260	0.031	0.040	0.016
276	—	0.010	—
***Ots100* (99, 50, 68)**			
168	0.096	0.160	0.125
172	0.303	0.300	0.360
174	0.030	0.010	—
176	0.086	0.230	0.132
180	0.147	0.090	0.125
182	—	—	0.015
185	0.061	0.030	0.059
186	0.126	0.110	0.059
189	—	0.010	—
191	0.005	—	0.044
195	0.040	—	—
197	0.015	—	—
199	0.081	0.020	0.052
201	0.005	—	—
203	—	0.010	—
205	—	—	0.007
209	—	—	0.015
211	0.005	—	—
213	—	—	0.007

TABLE A.1.—Continued.

Allele size (base pairs)	Location		
	RPH	Sunset Falls	NF
	***Ots100* (99, 50, 68)**		
215	—	0.010	—
217	—	0.020	—
	***One108* (100, 50, 72)**		
160	—	0.010	—
164	0.030	—	—
168	0.045	0.010	0.028
173	0.015	—	—
177	0.020	0.040	0.035
181	0.025	0.070	—
185	0.130	0.150	0.236
189	0.075	0.070	0.063
193	0.090	0.260	0.111
197	0.065	0.080	0.153
201	0.030	0.060	0.007
205	0.055	0.050	0.167
209	0.120	0.060	0.125
213	0.005	0.010	0.007
217	0.050	—	0.007
221	0.210	0.090	0.035
225	0.005	0.020	—
237	—	—	0.007
241	0.005	—	—
245	0.010	0.010	—
249	—	—	0.007
262	—	—	0.014
267	0.015	0.010	—
	***Ots103* (99, 50, 71)**		
57	0.005	—	—
74	0.066	0.040	0.028
78	0.061	0.070	0.085
82	0.813	0.890	0.887
86	0.056	—	—
	***One101* (88, 50, 71)**		
119	0.313	0.410	0.451
127	0.665	0.510	0.521
132	0.023	0.030	0.014
158	—	0.010	—
178	—	—	0.014
206	—	0.010	—
242	—	0.030	—
	***Omy1001* (95, 50, 57)**		
171	0.026	—	—
175	0.016	0.010	0.070
179	0.032	0.100	0.088
181	0.047	0.020	0.009
183	0.090	0.060	0.097
185	—	0.010	—
187	0.284	0.230	0.202
191	0.263	0.210	0.263
193	0.005	0.020	—
195	0.032	0.090	0.009
197	—	0.010	—
199	0.032	0.120	0.035
201	—	—	0.018
203	0.026	0.010	0.009
205	—	0.020	—
207	0.037	0.040	0.044
210	0.042	—	0.044
212	0.016	0.010	0.097
224	0.053	0.040	0.018

TABLE A.1.—Continued.

Allele size (base pairs)	Location		
	RPH	Sunset Falls	NF
	***Omm1128* (94, 49, 70)**		
219	0.016	0.020	0.014
223	0.032	0.020	0.079
227	0.021	0.041	0.057
231	—	0.010	0.057
235	0.037	0.051	0.007
238	—	0.010	0.057
242	0.096	0.020	0.193
251	0.144	0.051	0.014
255	0.021	0.051	0.057
258	0.090	0.174	0.057
263	0.085	0.153	0.014
266	—	0.010	0.007
271	—	—	0.014
275	0.096	0.102	0.043
279	0.027	0.061	0.029
282	0.011	0.010	—
287	0.021	—	0.014
291	0.021	0.031	0.057
295	0.080	0.020	0.036
299	0.043	0.041	0.021
302	—	0.031	—
304	0.037	0.020	0.036
308	0.005	0.010	—
311	0.096	0.041	0.114
316	0.016	0.010	—
328	—	0.010	—
333	0.005	—	—
341	—	—	0.007
353	—	—	0.014
	***Oki10* (97, 50, 70)**		
96	0.005	0.020	0.014
98	0.175	0.220	0.379
100	0.021	0.050	0.150
102	0.041	0.090	0.036
104	—	0.030	0.043
108	0.170	0.100	0.064
112	0.067	0.030	0.079
117	0.134	0.020	0.036
121	0.046	0.100	0.014
125	0.036	0.100	0.036
129	0.005	0.010	—
133	0.005	—	0.029
137	0.247	0.170	0.093
142	0.046	0.010	0.021
155	—	0.040	0.007
159	—	0.010	—
	***One18* (96, 50, 67)**		
169	0.104	0.100	0.306
172	0.031	0.030	—
174	0.365	0.320	0.291
176	0.208	0.230	0.112
178	0.130	0.160	0.060
180	0.089	0.070	0.067
182	0.010	0.040	0.052
184	0.063	0.040	0.105
186	—	0.010	0.008