

# Genetic Population Structure of Snake River Basin Steelhead in Idaho

by

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## ABSTRACT

Idaho Department of Fish and Game (IDFG) collected tissue samples from 74 wild juvenile steelhead *O. mykiss* populations throughout the state and five hatchery stocks in 2000. These samples were used to determine the genetic population structure of Idaho's steelhead assemblage. In this interim progress report we present results based on 31 wild populations and 5 hatchery stocks. The final analysis will include all 74 wild populations and the 5 hatchery stocks. Genetic variation found at 11 microsatellite loci was used to describe population structure for steelhead (*Oncorhynchus mykiss*) from 36 populations in the Snake River basin, Idaho. DNA was amplified and analyzed for 1905 fish samples. Significant regional spatial structuring of populations was apparent among 10 different river drainages. Many *O. mykiss* populations were most closely related genetically to other *O. mykiss* from streams within the same drainage. Significant allelic frequency differences were found in 98.5% of all pairwise comparisons for the 36 *O. mykiss* populations. AMOVA analyses showed that 2.8% of the molecular variance could be attributed to differences among 10 major river drainages (Clearwater, Middle Fork Clearwater, South Fork Clearwater, Salmon, Middle Fork Salmon, South Fork Salmon, Little Salmon, Lochsa, Selway and Snake rivers). All Idaho steelhead hatchery populations were shown to contain genetic diversity that was similar to that found in geographically proximate wild *O. mykiss*, with the exception of the East Fork Salmon "B-run" hatchery population that contained allelic structure most closely related to Ten Mile Creek *O. mykiss* in the Clearwater River drainage. Two Salmon River *O. mykiss* populations, Lemhi River and Pahsimeroi River, separated from the rest of the populations in the Snake River basin with 96% bootstrap values in an unrooted Neighbor-Joining tree based on chord genetic distance. Overall classification accuracy of single individuals to their stream of origin using these 11 microsatellite loci was 66%. Garza and Williamson's (2001)  $M$  over all populations of *O. mykiss* was  $M = 0.635$ , below the published threshold ( $M \leq 0.68$ ), supporting recent population reductions for steelhead within the Snake River drainage. Average estimated effective population size ( $N_e$  based on SMM) for Snake River steelhead populations, however, was relatively high ( $N_e = 5098$ ). These data suggest that significant genetic population structure remains for steelhead populations within the Snake River, and careful consideration of this genetic diversity should be part of future conservation and restoration efforts.

## INTRODUCTION

There are two recognized lineages of *O. mykiss* in North America – coastal (*O. m. irideus*) and inland (*O. m. gairdneri*; Behnke 1992). Historically, anadromous steelhead (*Oncorhynchus mykiss*) were broadly distributed throughout most Columbia River drainages (Busby et al. 1996). The Cascade crest is thought to separate the two *O. mykiss* lineages within the Columbia River drainage, making all up-river steelhead found in Idaho part of the inland group. The construction of dams on the Columbia River drainage has markedly changed the temperature and flow regime compared to historical patterns available to steelhead (Robards and Quinn 2002). There have been substantial declines in these populations over the last 150 years, due primarily to lost spawning and rearing habitats, changes in water quality, and within-basin dams and diversions (Busby et al. 1996). Steelhead that spawn in Idaho are summer-run fish. Steelhead in Idaho, i.e. populations in the Snake, Salmon and Clearwater rivers migrate further from the ocean (up to 1,500 km) than all other Columbia River populations and spawn at high elevations (up to 2,000 m). Anadromous steelhead in the Snake River basin found in streams of southeast Washington, northeast Oregon and Idaho, were listed under the Federal Endangered Species Act (ESA) as a threatened Evolutionarily Significant Unit (ESU) in 1997 (Federal Register Vol. 62 No. 159: 43937 - 43954).

Wild steelhead abundance in the Snake River basin declined relative to their historical abundance after the construction of the four Lower Snake River dams. Declines of wild B-run steelhead<sup>1</sup> have generated particular concern over the potential loss of life history diversity for this species. *O. mykiss* expresses a range of variations in life history strategies, from strongly migratory to non-migratory, throughout the species' range. Individual runs or stocks of *O. mykiss* found within the same drainage cannot be separated taxonomically based on migration timing or the distribution of anadromy (Behnke 1992; Allendorf and Utter 1979). Highly flexible life history strategies in *O. mykiss* (Shapovalov and Taft 1954), otolith microchemistry (Rybock et al. 1975; Zimmerman and Reeves 2000), and genetic studies (Gall et al. 1990; Nielsen et al. 1997) suggest that freshwater habitats may contain relic, non-anadromous

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<sup>1</sup> Snake River steelhead runs are commonly referred to as “A-run” or “B-run” stocks based on designations developed from the bimodal migration of adult steelhead at Bonneville Dam. Adult A-run fish migrate upstream earlier (June to August) than B-run fish (August to October); B-run fish are primarily defined as 2-ocean adults having spent two years at sea (as opposed to 1-ocean A-run fish) and are on average 75-100 mm larger than A-run fish of the same age.

components of the *O. mykiss* gene pool found in geographically proximate anadromous populations.

In recent years, over 80% of the adult steelhead passing Lower Granite Dam derived from hatchery origins (Busby et al. 1996). Large-scale artificial propagation of steelhead in the Snake and Salmon drainage began in the 1960's with the construction of Oxbow Hatchery, Niagara Springs Hatchery, and Pahsimeroi Hatchery. In the Clearwater drainage, large-scale hatchery releases began in 1970 after Dworshak National Fish Hatchery was built. The Dworshak summer steelhead stock was developed from native B-run North Fork Clearwater River steelhead in 1969 (Howell et al. 1985). Oxbow, Niagara Springs, and Pahsimeroi hatcheries were all part of Idaho Power Company's program to relocate steelhead stocks after the construction of dams in Hells Canyon. The brood source for these facilities was obtained from native steelhead trapped at the base of Hells Canyon Dam. Pahsimeroi Hatchery also incorporated some Dworshak hatchery adults into their brood source during the late 1970's (Ball 1985). Dworshak National Fish Hatchery released between 1.9 and 2.4 million smolts each year since 1997 into the Clearwater River Basin (Fish Passage Center 2004). Hatchery steelhead releases in the Salmon and Snake drainages from other Idaho hatcheries has ranged from 4 to 6 million yearly since 1997 (IDFG hatchery release database).

The impact of hatchery *O. mykiss* on wild stocks in streams and reservoirs throughout North America over the last 200 years has been the subject of many studies (see reviews in Reisenbichler and McIntyre 1977, Waples and Do 1994, Campton 1995, and Nielsen 1999). Straying and introgression by hatchery fish presents a high risk to the genetic integrity of some wild steelhead populations according to some authorities (Busby et al. 1997). Early findings of Gall et al. (1990) suggested that anadromous steelhead populations have residualized as freshwater fish behind man-made structures and dams throughout their natural range. Within Idaho there are numerous populations of non-anadromous rainbow trout upstream of both natural long-standing and artificial barriers (see Figure 1). Many of these populations have had an opportunity to interbreed with hatchery fish. The genetic integrity of locally adapted stocks of *O. mykiss* is of critical importance to issues of restoration and recovery.

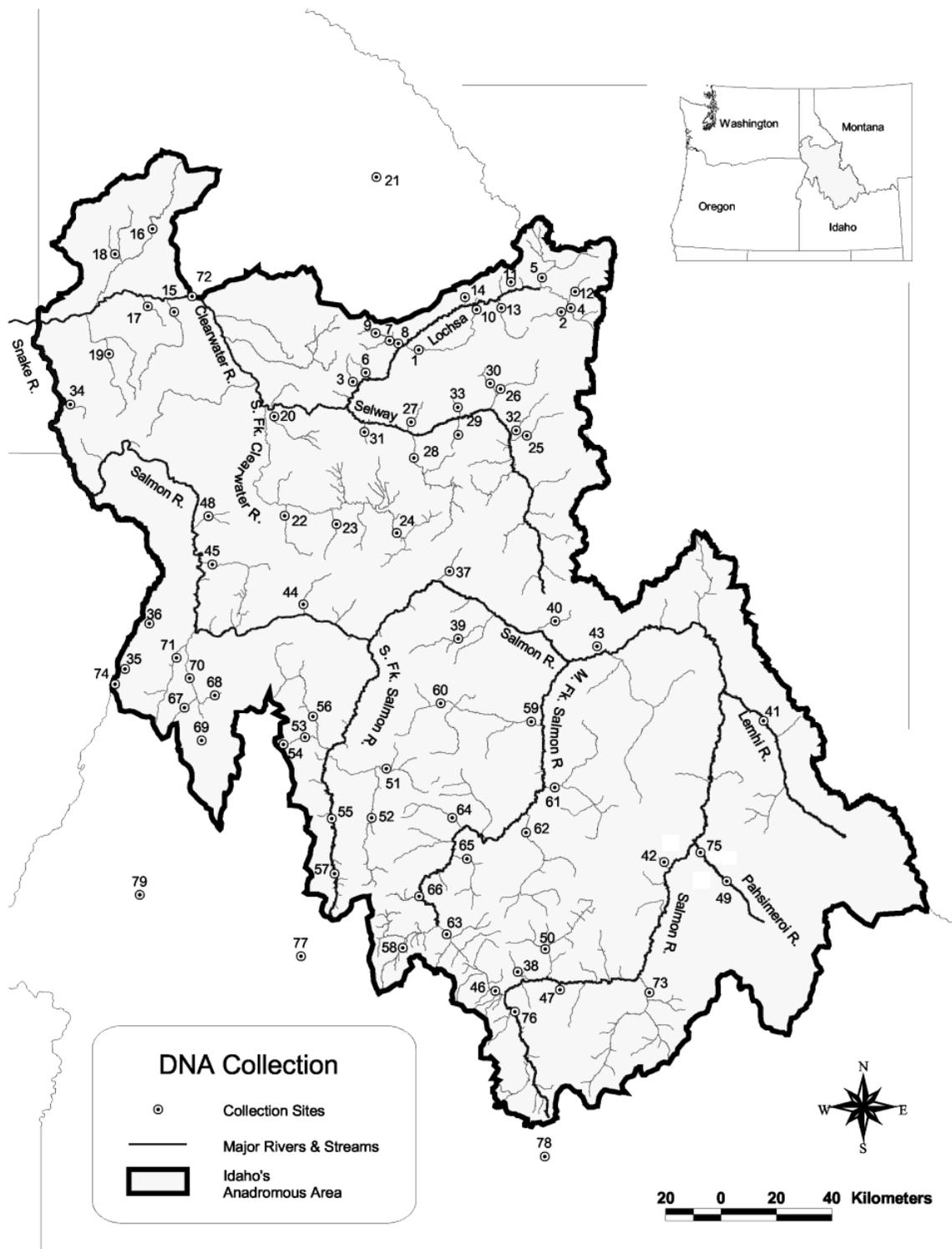


Figure 1. *O. mykiss* populations sampled for Idaho's baseline genetic database in 2000. Collection site location numbers are listed in Table 1 (this report) and Appendix I.

Table 1. Idaho drainages, populations, site locations and total number of samples (N), unbiased and observed Hz collected by the Idaho Department of Fish & Game in 2000.

Drainage	Population	Site Location		Unbiased Hz*	Observed Hz	Stream Code
		on Map	N			
Clearwater River	Big Canyon Creek	15	60	0.6601	0.6758	BCAN
	EF Potlatch River	16	51	0.6407	0.6645	EPOT
	Mission Creek	19	49	0.6317	0.6218	MISS
Middle Fork Clearwater River	Clear Creek	20	53	0.5945	0.5987	CLRC
South Fork Clearwater River	John's Creek	22	49	0.6117	0.5732	JOHN
	Red River	24	60	0.6357	0.6246	REDR
	Ten Mile Creek	23	49	0.6014	0.6168	MILE
Salmon River	Basin Creek	38	54	0.7099	0.6636	BASC
	Chamberlain Creek	39	46	0.6441	0.6148	HAM1
	Lemhi River	41	49	0.7274	0.6682	LEMR
	Pahsimeroi River	49	49	0.7019	0.6689	PAHR
	Whitebird Creek	48	56	0.6527	0.6485	WHBC
	Middle Fork Salmon River	Big Creek (lower)	59	49	0.6082	0.5983
South Fork Salmon River	Camas Creek	61	53	0.6262	0.5688	CAM1
	Loon Creek	62	54	0.5617	0.5595	LON1
	Marsh Creek	63	59	0.5836	0.5881	MARC
	East Fork SF Salmon River	51	52	0.6304	0.6151	EFSF
	Johnson Creek	52	56	0.6350	0.6379	JSON
	Poverty Flat Area	55	55	0.6142	0.6038	POVF
Little Salmon River	Secesh River	56	56	0.6285	0.5559	SECR
	Little Salmon, Pinehurst area	70	51	0.6705	0.6285	LSR2
	Rapid River	71	51	0.6363	0.6260	RAPR
Lochsa River	Brushy Fork Creek	2	55	0.5492	0.5457	BRUS
	Canyon Creek	3	53	0.5941	0.5879	CANY
	Colt Creek	4	54	0.5866	0.5544	COLT
	Fish Creek	7	56	0.6152	0.5944	FISH
Selway River	Bear Creek	25	42	0.5931	0.5860	BEAR
	East Fork Moose Creek	26	55	0.6247	0.6252	EMOS
	Gedney Creek	27	56	0.6063	0.6025	GEDC
	Three Links Creek	33	55	0.5993	0.5960	3LNK
Snake River Hatchery	Granite Creek	35	49	0.6806	0.6771	GRAN
	Dworshak Hatchery	72	52	0.5823	0.6006	DWOR
	EF Salmon "B-run"	73	55	0.5974	0.5783	EFRB
	Oxbow Hatchery	74	53	0.6758	0.6295	OXBW
	Pahsimeroi Hatchery	75	53	0.6916	0.6535	SIMH
	Sawtooth Hatchery	76	56	0.6886	0.6733	SAWT
TOTAL			1905			

\* Based on Nei's unbiased gene diversity (1987)

This study represents genetic analyses of a diversity of samples of *O. mykiss*, i.e. fish collected above and below dams, putative natural spawning anadromous populations and hatchery steelhead from Idaho. The Idaho Department of Fish and Game (IDFG) collected juvenile *O. mykiss* samples during the summer of 2000. Microsatellite allelic diversity was visualized and analyzed at the USGS Alaska Science Center's Conservation Genetics Laboratory. *O. mykiss* genetic diversity was analyzed within and among samples and groups of samples at several spatial scales: 1) allelic diversity among and between large river drainages; 2) pairwise comparisons of genetic diversity between 36 unique *O. mykiss* populations; 3) within population genetic diversity was used for comparisons across broad spatial scales. We compared genotype and allelic frequencies for *O. mykiss* populations to data for known hatchery steelhead strains with a history of stocking in Idaho, looking at relationships among and between all *O. mykiss* populations, and between hatchery and wild populations within the sampling area.

## **METHODS**

### Sample Collections

*O. mykiss* fin tissue was collected and analyzed for DNA from 1905 fish in this study (Table 1). IDFG collected tissues from fish throughout the Snake River basin, Idaho, in 2000 (Figure 1; see also Appendix I). These samples are part of a larger collection of steelhead populations taken for a broad scale analysis of genetic population structure within Idaho that have or will contribute to future technical reports and other publications (see Nielsen et al. 2003a).

### Microsatellite Amplification Protocols

Microsatellite loci taken from the published literature were selected for analysis based on documented variability in *O. mykiss*, ease of amplification in polymerase chain reaction (PCR), and allele scoring rigor. Table 2 gives the total number of alleles found for each locus across all populations.

G. K. Sage (Alaska Science Center, Conservation Genetics Laboratory) developed multiplex systems using 13 loci grouped together for amplification of steelhead allelic size structure. G. K. Sage redesigned several primers in order to establish the three multiplex

protocol used in this study, one containing 5 loci and two containing 4 loci (Table 3). *Oneμ10-(F)*, *Ogo4-(F)*, *Ogo4-(R)* and *Ogo3-(R)* were redesigned as follows: *Oneμ10-(F)* was renamed *Oneμ10.2-(F)* (5'-TGTTGGCACCATTGTAACAG-3'); *Ogo4-(F)* was renamed *Ogo4.2-(F)* (5'-CAGAATGAGTAACGAACG C-3'); *Ogo4-(R)* was renamed *Ogo4.2-(R)* (5'-GAGGATAGAAGA GTTTGGC-3'); and *Ogo3-(R)* was renamed *Ogo3.2-(R)* (5'-CACAATGGAAGACCAT-3'). *Ogo1a*, *Ogo4.2*, *Oneμ10.2* and *Ots3* forward primers were modified by the addition of M13R tails, and *Oneμ8* and *Oneμ11* forward primers were modified by the addition of M13F tails. All M13 tails were added to the primers at the 5' end. These tails allowed for allele fragment visualization by annealing to labeled complementary M13 tails added to the PCR mix. The remaining loci were visualized by adding directly labeled forward primer. Allele sizes (from adapted primers) were standardized to single locus products by running known standards for allelic size for each locus on all multiplex gels.

In general, PCR reactions were conducted in 10μl volumes using approximately 50ng of genomic DNA, 0.1-0.2 U of DNA polymerase (Perkin Elmer), 10mM Tris-HCl (pH 8.3), 1.5mM MgCl<sub>2</sub>, 50mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, and 200μM each dNTP. To visualize loci with directly labeled primers, the total of forward (F) and reverse (R) primers per locus per reaction equaled 4 pmoles, with the F primer concentration being a combination of labeled and unlabeled primer. Tailed F and R primer concentrations for the multiplex systems were as follows: *Oneμ10* (10 pmoles), *Ogo1a*, *Ogo4*, *Oneμ11*, *Ots3* (5 pmoles) and *Oneμ8* (1 pmole).

The following amounts of labeled primers were added in each of the three multiplex systems. Multiplex A had between 0.06-0.20 pmoles per reaction (*Omy325*, 0.06; *Ots1*, 0.20; *Oneμ14*, 0.40; *Ots4*, 0.06). Multiplex B was between 0.1-1.5 pmoles (*Omy77*, 0.2; M13F, 0.3; M13R, 1.5), and multiplex C had between 0.1-1.5 pmoles (M13F, 1.5; M13R, 1.5; *Omy27*, 0.1; *Omy207*, 0.2). Gel electrophoresis and visualization of microsatellite alleles was performed using LI-COR Model 4200 and IR2 automated fluorescent DNA Sequencers and sizing was performed using V3.00 Gene ImagIR (LI-COR, Lincoln, NE, USA). Microsatellite allele sizes (including the amplified primer) were determined in relation to the M13 ladder or to the GeneScan-350 internal size standard (P-E Biosystems, Foster City, CA, USA), and *O. mykiss* DNA samples of known size that were rerun on each gel. Approximately 10% of all samples were run on a second gel and scored independently to verify allelic size.

Table 2. List of microsatellite loci used in this study. Mean Hz = mean observed heterozygosity per locus across 36 Idaho steelhead populations.

Locus	Source	Number Alleles	Allelic Size Range (bp)	Mean Hz
Ogo 1a	Olsen et al. 1998	14	123-168	0.58
Ogo 4.2	Olsen et al. 1998	25	118-179	0.80
Omy 27	Heath et al. 2001	10	99-117	0.52
Omy 325	O'Connell et al. 1997	32	87-149	0.88
Onem8	Scribner et al. 1996	25	144-222	0.84
Onem10.2	Scribner et al. 1996	9	113-131	0.20
Onem11	Scribner et al. 1996	3	145-149	0.44
Onem14	Scribner et al. 1996	14	143-179	0.54
Ots 1	Banks et al. 1999	32	157-279	0.81
Ots 3	Banks et al. 1999	9	77-93	0.63
Ots 4	Banks et al. 1999	8	108-130	0.68

Table 3. Multiplex systems used to amplify 13 microsatellite loci from DNA from Snake River drainage steelhead on the LI-COR automatic sequencer. Additional primer modifications made to enhance these multiplexes are given in the text. The columns "700" and "800" represent different dyes used on the LI-COR platform.

Multiplex	Anneal Temp(°C)/ Cycles	30 min. extension	Loci 700	Loci 800
A	52/40	NO	Omy 325 Ots 1	Ots 4 Oneμ14
B	52/40	YES	Omy 77 Oneμ8	Ogo 1a Ogo 4.2 Ots 3
C	52/40	YES	Omy 207 Oneμ10.2	Omy 27 Oneμ11

Table 4. Hardy-Weinberg equilibrium (HWE) results for 11 loci showing populations within HWE "-" and out of HWE "+" based on exact tests performed by ARLEQUIN 1.1.

POPULATION	N	LOCUS											Loci within HWE	
		Ogo1a	Ogo4	Omy27	Omy325	Onem8	Onem10	Onem11	Onem14	Ots1	Ots3	Ots4		
1. Big Canyon Creek	60	-	-	-	-	-	-	-	-	-	-	-	-	11
2. Clear Creek	53	-	-	-	-	-	-	-	-	-	-	-	-	11
3. EF Potlatch River	51	-	-	-	-	-	-	-	-	-	-	-	-	11
4. Johns Creek	49	+	-	-	-	-	+	-	-	-	+	-	-	8
5. Fish Creek	56	-	+	+	-	+	+	-	+	-	-	-	-	6
6. EF SF Salmon River	52	-	+	-	-	-	-	-	-	-	-	-	-	10
7. Dworshack Hatchery	52	-	-	-	-	-	-	-	+	-	-	-	-	10
8. EF Salmon "B-run" Hatchery	55	-	+	-	+	+	-	-	+	-	+	+	-	5
9. Rapid River	51	-	-	-	-	-	-	-	-	-	+	-	-	10
10. Oxbow Hatchery	53	-	-	-	-	-	-	-	-	-	-	-	-	11
11. Whitebird Creek	56	-	-	-	+	-	-	-	-	-	+	-	-	9
12. Johnson Creek	56	-	-	-	-	-	-	-	+	-	+	-	-	9
13. Pahsimeroi Hatchery	53	-	-	-	-	-	-	-	-	+	-	-	-	10
14. Brushy Fork Creek	55	-	-	-	-	-	+	-	-	-	-	-	-	10
15. Sawtooth Hatchery	56	-	-	-	-	-	-	-	-	+	-	-	-	10
16. Colt Creek	54	-	+	-	+	-	-	-	-	-	-	-	-	9
17. Poverty Flat	55	-	+	-	-	-	-	-	+	+	-	-	-	8
18. Secesh River	56	-	+	-	-	-	+	+	-	+	-	-	-	7
19. Canyon Creek	53	-	-	-	-	-	-	-	+	-	-	-	-	10
20. Camas Creek	53	-	-	-	-	-	+	-	+	+	-	-	-	8
21. Big Creek (lower)	49	-	-	-	-	-	-	-	-	+	+	-	-	9
22. Basin Creek	54	-	-	+	-	-	+	-	+	+	-	-	-	7
23. Chamberlain Creek	46	+	-	-	-	-	-	-	-	+	-	-	-	9
24. Bear Creek	42	-	+	+	+	-	+	-	-	-	-	-	-	7
25. EF Moose Creek	55	-	-	-	-	-	-	-	-	-	-	-	-	11
26. Loon Creek	54	-	-	-	-	+	-	-	-	+	-	-	-	9
27. Gedney Creek	56	-	-	-	-	-	-	-	+	-	-	-	-	10
28. Marsh Creek	59	-	-	-	-	+	-	-	-	-	-	-	-	10
29. Three Links Creek	55	+	-	+	-	-	-	-	-	-	-	+	-	8
30. Lemhi River	49	-	-	-	+	-	-	-	+	+	-	+	-	7
31. Granite Creek	49	-	-	-	-	-	-	-	-	-	-	-	-	11
32. Ten Mile Creek	49	-	-	-	-	+	-	-	-	+	-	-	-	9
33. Red River	60	-	-	-	-	+	-	-	+	-	-	-	-	9
34. Mission Creek	49	-	-	-	-	-	-	-	-	-	+	-	-	10
35. Pahsimeroi River	49	-	-	-	-	-	-	-	+	-	-	-	-	10
36. Little Salmon (Pinehurst)	51	-	-	-	-	-	-	-	+	-	-	-	-	10
Total within HWE		33	29	32	31	30	29	35	23	25	29	33		

## Genetic Analyses

Genetic data were analyzed using a variety of software from different statistical packages including ARLEQUIN version 1.1 (Schneider et al. 2000), BOTTLENECK (Piry et al. 1999), NEIGHBOR from PHYLIP (Felsenstein 1993), and GENEPOP version 3.3 (Raymond and Rousset 1997). Heterozygosity and simulated Fisher's exact tests using randomizations for Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP and ARLEQUIN.

Tests of HWE were performed to look at the performance of different loci among these *O. mykiss* populations to gain inference on population structure. It is well known that two populations that are in HWE independently may not be so when they are combined (Hartl 1988). There are several assumption built into HWE that cannot be supported without additional knowledge of the demographics of these populations, i.e. non-overlapping populations (i.e. the samples included juveniles of different ages), random mating, negligible migration (natural and artificial movement above and below dams can be undocumented or inconclusive), etc. Most importantly, the assumptions that mutation can be ignored and that natural selection does not affect alleles under consideration for HWE are hard to support in studies involving microsatellite loci where we know so little about the mutation processes involved.

ARLEQUIN  $F_{st}$  pairwise comparisons were used to test for differences in allele frequencies between and among populations. Statistical significance levels for allelic frequency comparisons were set using sequential Bonferroni tests (Rice 1989). Partitioning of microsatellite allelic variation based on analysis of molecular variance (AMOVA) was performed using ARLEQUIN. Detection of recent reductions in population size using microsatellite data were performed on 36 Idaho *O. mykiss* populations using Garza and Williamson's  $M$  (2001). Effective population size ( $N_e$ ) estimates based on microsatellite data were made under the assumption of mutation-drift equilibrium using the Single-Step Mutation Model (SSM) and the Infinite Allele Model (IAM) with a mutation rate of  $2.05E^{-4}$  (Garza and Williamson 2001 based on methods from Lehmann et al. 1998 and Rooney et al. 1999).

Genetic distance values reflecting the proportion of shared alleles between individuals and groups of individuals can be used to graphically depict genetic

relationships and population structure. A Cavalli-Sforza and Edward's chord genetic distance (1967) matrix was generated using Treemaker version 1.0 (Cornuet et al. 1999). An unrooted Neighbor-Joining tree (NJ) was generated using the NEIGHBOR application of PHYLIP and visualized with TreeView version 1.6.6 (Page 1996). To assess the reproducibility of branching patterns on the consensus tree, bootstrapping over loci ( $n = 2000$ ; Felsenstein 1985) was performed using NJBPOP (Cornuet et al. 1999). The program WHICHLOCI was used to rank the microsatellite loci used in this study based on their relative allelic differential derived from Idaho *O. mykiss* populations (Banks and Eichert 2000).

## RESULTS

### *Microsatellite Loci and HWE*

GENEPOP's analyses of expectation of HWE gave mixed results among the microsatellite loci and *O. mykiss* populations in this study (Table 4). Deviations from HWE were primarily due to heterozygote ( $H_z$ ) excess under the assumptions of the single-step mutation-drift model (SMM). Two loci (Omy77 and Omy207) were found to be out of HWE in a large portion of the Idaho sample populations and were dropped from any further analyses. Locus Omy77 (40% of the populations out of HWE) suffered from  $H_z$  deficiency in 13 steelhead populations. This locus has been shown to carry "null" alleles in other populations of *O. mykiss* (Ardren et al. 1999). Locus Omy207 had  $H_z$  excess in 18 steelhead populations. We know of no studies explaining the behavior of this locus in steelhead or *O. mykiss* populations outside of this study. This locus was dropped from further analyses because 53% of the Idaho populations did not meet HWE. All other loci conformed to HWE in over 70% of the study populations.

Fourteen sample populations fell significantly out of HWE for the remaining 11 loci combined: Camas Creek ( $\text{Chi}^2 = 58.9$ ;  $\text{df} = 22$ ;  $p < 0.000$ ); Lower Big Creek ( $\text{Chi}^2 = 41.7$ ;  $\text{df} = 22$ ;  $p = 0.007$ ); Johns Creek ( $\text{Chi}^2 = 40.3$ ;  $\text{df} = 22$ ;  $p = 0.01$ ); Basin Creek ( $\text{Chi}^2 = \text{Infinity}$ ;  $\text{df} = 22$ ;  $p < 0.000$ ); Fish Creek ( $\text{Chi}^2 = 56.7$ ;  $\text{df} = 22$ ;  $p < 0.001$ ); Bear Creek ( $\text{Chi}^2 = 46.4$ ;  $\text{df} = 22$ ;  $p = 0.002$ ); East Fork Salmon "B-run" ( $\text{Chi}^2 = \text{Infinity}$ ;  $\text{df} = 22$ ;  $p < 0.000$ ); Whitebird Creek ( $\text{Chi}^2 = 51.2$ ;  $\text{df} = 22$ ;  $p < 0.001$ ); Three Links Creek ( $\text{Chi}^2 = 46.2$ ;  $\text{df} = 22$ ;  $p < 0.002$ ); Lemhi River ( $\text{Chi}^2 = 61.6$ ;  $\text{df} = 22$ ;  $p < 0.000$ );

Pahsimeroi Hatchery ( $\text{Chi}^2 = \text{Infinity}$ ;  $\text{df} = 22$ ;  $p < 0.000$ ); Pahsimeroi River ( $\text{Chi}^2 = \text{Infinity}$ ;  $\text{df} = 22$ ;  $p < 0.000$ ); Red River ( $\text{Chi}^2 = 48.7$ ;  $\text{df} = 22$ ;  $p < 0.001$ ); Secesh River ( $\text{Chi}^2 = 60.8$ ;  $\text{df} = 22$ ;  $p < 0.000$ ). Sample sizes for all of these populations exceeded  $N = 42$ . We judged the allelic diversity found within these populations to be informative despite non-conformity to HWE and retained these populations in our analyses.

Optimal locus combinations provided population assignments among *O. mykiss* populations in the Snake River basin. Following the “leave-one-out” approach for reassignment, WHICHLOCI indicated that all 11 loci were needed for 66% reassignment accuracy. However, caution is advised in consideration of this value since the assignment accuracy of individuals back to their population of origin may be inflated due to the lack of alternative baseline data outside of those generated by this study. Loci were ranked according to their relative contribution to the analyses of allelic frequency differences among populations (Table 5).

### *Snake River Basin Genetic Population Structure*

#### Clearwater River

We visualized allelic diversity at 11 microsatellite loci for 160 *O. mykiss* from the Clearwater River, 53 fish from the Middle Fork Clearwater drainage, and 158 fish from the South Fork Clearwater drainage (Table 1). The average number of alleles per locus found throughout Clearwater River *O. mykiss* was 7.45. Average observed heterozygosity ( $H_z$ ) for Clearwater River *O. mykiss* populations was  $H_z = 0.63$ . Clearwater River basin  $F_{st} = 0.029$ . Clearwater River drainage  $F_{st} = 0.019$ . South Fork Clearwater drainage  $F_{st} = 0.034$ .

ARLEQUIN's population pairwise comparison found significant differences in allelic frequencies for all Clearwater River drainage *O. mykiss* populations. Mean  $M$  for the Clearwater drainage was  $M = 0.57$ . Effective population size ( $N_e$ ) for the Clearwater River drainage calculated by Garza and Williamson's (2001) program for  $M$  based on the SMM ranged from  $N_e = 2996$  (Clear Creek) to  $N_e = 4498$  (Big Canyon Creek), mean drainage  $N_e = 3629$ . AMOVA distribution of the allelic variation found within the Clearwater River drainage showed that 2.94% of the variation was found among populations and 97.06% was found within populations.

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Table 5. Microsatellite loci rank using allele frequency differential method from WHICHLOCI (Banks and Eichert 2000).

Rank	Locus	Score	% Relative Score
1	Omy325	0.1932	16.896
2	One $\mu$ 8	0.1869	16.345
3	Ots1	0.1270	11.111
4	Ots3	0.1186	10.377
5	Ogo4	0.1008	8.815
6	One $\mu$ 14	0.1008	8.815
7	Ots4	0.0740	6.474
8	Omy27	0.0730	6.382
9	Ogo1a	0.0667	5.831
10	One $\mu$ 10	0.0614	5.372
11	One $\mu$ 11	0.0409	3.581

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### Salmon River

We visualized allelic diversity at 11 microsatellite loci for 254 *O. mykiss* from the Salmon River, 215 fish from the Middle Fork Salmon River drainage, 219 fish from the South Fork Salmon River, and 102 fish from the Little Salmon River (Table 1). The average number of alleles per locus found throughout the Salmon River *O. mykiss* was 7.1. Average observed heterozygosity ( $H_z$ ) for Salmon River *O. mykiss* populations was  $H_z = 0.61$ . Salmon River basin  $F_{st} = 0.054$ . Salmon River drainage  $F_{st} = 0.065$ . South Fork Salmon drainage  $F_{st} = 0.015$  and Middle Fork Salmon drainage  $F_{st} = 0.018$ . Little Salmon River  $F_{st} = 0.025$ .

ARLEQUIN's population pairwise comparison found significant differences in allelic frequencies for all but 2% of the Salmon River pairwise population comparisons

(Table 6). Mean  $M$  for the Salmon River drainage was  $M = 0.58$ . Effective population size ( $N_e$ ) for the Salmon River drainage calculated by Garza and Williamson's (2001) program for  $M$  based on the SMM ranged from  $N_e = 2490$  (Loon Creek) to  $N_e = 7129$  (Lemhi River), mean drainage  $N_e = 4261$ . AMOVA distribution of the allelic variation found within the Salmon River drainage showed that 5.38% of the variation was found among populations and 94.62% was found within populations.

### Lochsa River

We visualized allelic diversity at 11 microsatellite loci for 218 *O. mykiss* from the Lochsa River (Table 1). The average number of alleles per locus found throughout the Lochsa River steelhead populations was 6.45. Average observed heterozygosity ( $H_z$ ) for Lochsa River *O. mykiss* populations was  $H_z = 0.57$ . Lochsa River basin  $F_{st} = 0.019$ . ARLEQUIN's population pairwise comparison found significant differences in allelic frequencies for all of the Lochsa River pairwise population comparisons. Bear Creek (Selway River) shared similar allelic structure for all 11 loci combined with three streams in the Lochsa River basin (Canyon, Colt and Fish creeks; Table 6). Mean  $M$  for the Lochsa River drainage was  $M = 0.57$ . Effective population size ( $N_e$ ) for the Lochsa River drainage calculated by Garza and Williamson's (2001) program based on the SMM ranged from  $N_e = 2315$  (Brushy Fork Creek) to  $N_e = 2986$  (Fish and Canyon creeks), mean drainage  $N_e = 2787$ . AMOVA distribution of the allelic variation found within the Lochsa River drainage showed that 1.85% of the variation was found among populations and 98.15% was found within populations.

### Selway River

We visualized allelic diversity at 11 microsatellite loci for 208 *O. mykiss* from the Selway River (Table 1). The average number of alleles per locus found throughout the Selway River steelhead populations was 6.41. Average observed heterozygosity ( $H_z$ ) for Selway River *O. mykiss* populations was  $H_z = 0.6$ . Selway River basin  $F_{st} = -0.01$ . ARLEQUIN's population pairwise comparison found no significant differences in allelic frequencies for four pairwise population comparisons (Table 6). Within-basin pairwise comparisons showed that Selway's Bear Creek shared similar allelic structure for all 11 loci combined with three streams (East Fork Moose, Gedney, and Three Links creeks;

Table 6. Pairwise *Fst* comparisons between 36 Idaho steelhead populations. Pairwise *Fst* values are given below the diagonal and the matrix of significant *Fst* P values (“+”= significant pairwise difference) is given above the diagonal. Population codes are listed in Table 1.

	BASC	BCAN	BEAR	BIG1	BRUS	CAMI	CANY	CLRC	COLT	DWOR	EFRB	EFSE	EMOS	EPOT	FISH	GEDC	GRAN	HAMI	JOHN	JSON	LEMR	LON1	LSR2	MARC	MILE	MISS	OXBW	PAHR	POVF	RAPR	REDR	SAWT	SECR	SIMH	WHBC	3LNK					
BASC		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
BCAN	0.026		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
BEAR	0.044	-0.009		+	+	+	-	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-				
BIG1	0.056	0.042	0.029		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
BRUS	0.081	0.040	0.013	0.100		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
CAMI	0.044	0.037	0.028	0.013	0.082		+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
CANY	0.060	0.017	-0.001	0.076	0.015	0.061		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
CLRC	0.050	0.017	-0.012	0.049	0.039	0.026	0.028		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
COLT	0.063	0.037	0.002	0.088	0.024	0.064	0.031	0.030		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
DWOR	0.060	0.023	0.007	0.071	0.066	0.048	0.047	0.015	0.056		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
EFRB	0.050	0.038	0.043	0.076	0.058	0.078	0.045	0.045	0.047	0.076		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
EFSE	0.058	0.045	0.029	0.026	0.094	0.035	0.073	0.048	0.074	0.075	0.074		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
EMOS	0.052	0.017	-0.040	0.049	0.033	0.030	0.030	0.016	0.011	0.031	0.039	0.034		+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
EPOT	0.050	0.024	0.013	0.055	0.068	0.051	0.051	0.033	0.049	0.049	0.044	0.050	0.019		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
FISH	0.051	0.028	-0.007	0.066	0.011	0.045	0.020	0.021	0.011	0.031	0.045	0.063	0.006	0.040		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
GEDC	0.063	0.027	-0.024	0.065	0.031	0.056	0.029	0.025	0.013	0.044	0.035	0.055	-0.001	0.029	0.016		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
GRAN	0.015	0.017	0.029	0.033	0.074	0.025	0.053	0.038	0.050	0.048	0.061	0.030	0.025	0.027	0.038	0.047		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
HAMI	0.021	0.000	0.007	0.012	0.054	0.001	0.028	0.010	0.051	0.022	0.041	0.012	0.017	0.017	0.018	0.031	0.011		+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+				
JOHN	0.050	0.010	-0.009	0.065	0.026	0.048	0.010	0.019	0.046	0.023	0.059	0.065	0.020	0.042	0.021	0.032	0.042	0.010		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
JSON	0.039	0.015	0.020	0.021	0.052	0.022	0.030	0.024	0.050	0.042	0.057	0.011	0.014	0.032	0.035	0.036	0.024	-0.004	0.023		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
LEMR	0.058	0.074	0.087	0.112	0.137	0.107	0.118	0.107	0.112	0.123	0.115	0.100	0.107	0.079	0.106	0.113	0.059	0.084	0.105	0.090		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
LON1	0.069	0.049	0.043	0.015	0.093	0.010	0.072	0.039	0.084	0.057	0.098	0.042	0.043	0.071	0.056	0.072	0.046	0.018	0.063	0.030	0.140		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
LSR2	0.013	0.025	0.041	0.048	0.094	0.035	0.062	0.036	0.076	0.046	0.052	0.048	0.049	0.041	0.061	0.069	0.019	0.016	0.050	0.036	0.074	0.059		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
MARC	0.061	0.052	0.056	0.025	0.097	0.013	0.077	0.036	0.073	0.062	0.079	0.037	0.047	0.057	0.051	0.064	0.038	0.021	0.068	0.035	0.123	0.028	0.056		+	+	+	+	+	+	+	+	+	+	+	+	+	+			
MILE	0.055	0.023	0.042	0.089	0.064	0.067	0.041	0.032	0.041	0.040	0.061	0.076	0.037	0.057	0.040	0.044	0.055	0.044	0.038	0.053	0.110	0.091	0.061	0.079		+	+	+	+	+	+	+	+	+	+	+	+	+			
MISS	0.041	0.017	0.011	0.044	0.063	0.046	0.051	0.036	0.045	0.054	0.034	0.035	0.024	0.013	0.039	0.021	0.021	0.027	0.045	0.025	0.075	0.064	0.039	0.048	0.062		+	+	+	+	+	+	+	+	+	+	+	+			
OXBW	0.014	0.013	0.033	0.040	0.074	0.043	0.052	0.038	0.056	0.042	0.058	0.044	0.036	0.031	0.046	0.050	0.010	0.010	0.038	0.033	0.046	0.061	0.021	0.059	0.049	0.024		+	+	+	+	+	+	+	+	+	+				
PAHR	0.078	0.108	0.153	0.166	0.178	0.163	0.151	0.154	0.155	0.169	0.148	0.157	0.159	0.130	0.154	0.164	0.104	0.135	0.139	0.143	0.024	0.203	0.101	0.183	0.144	0.130	0.081		+	+	+	+	+	+	+	+	+	+			
POVF	0.053	0.027	0.033	0.027	0.079	0.026	0.055	0.034	0.069	0.046	0.082	0.020	0.031	0.039	0.049	0.051	0.025	0.009	0.038	0.011	0.088	0.032	0.044	0.033	0.075	0.029	0.039	0.150		+	+	+	+	+	+	+	+	+			
RAPR	0.046	0.034	0.044	0.026	0.099	0.028	0.063	0.039	0.078	0.059	0.067	0.020	0.039	0.030	0.058	0.056	0.022	0.012	0.054	0.027	0.095	0.044	0.025	0.034	0.087	0.024	0.038	0.156	0.018		+	+	+	+	+	+	+	+			
REDR	0.050	0.014	0.020	0.062	0.065	0.042	0.039	0.019	0.056	0.011	0.071	0.061	0.030	0.031	0.030	0.043	0.033	0.023	0.018	0.035	0.084	0.061	0.029	0.064	0.048	0.042	0.031	0.134	0.035	0.042		+	+	+	+	+	+	+			
SAWT	0.012	0.019	0.014	0.052	0.059	0.047	0.047	0.041	0.044	0.054	0.037	0.039	0.033	0.027	0.035	0.039	0.013	0.016	0.038	0.024	0.053	0.069	0.028	0.055	0.045	0.024	0.002	0.084	0.039	0.037	0.036		+	-	+	+	+	+			
SECR	0.046	0.033	0.030	0.031	0.077	0.028	0.056	0.033	0.061	0.051	0.059	0.021	0.032	0.034	0.050	0.044	0.024	0.013	0.050	0.013	0.095	0.037	0.040	0.037	0.071	0.027	0.038	0.160	0.013	0.017	0.041	0.033		+	+	+	+	+	+		
SIMH	0.013	0.007	0.011	0.044	0.060	0.039	0.038	0.033	0.040	0.050	0.040	0.036	0.028	0.023	0.035	0.038	0.005	0.001	0.031	0.021	0.044	0.061	0.023	0.051	0.036	0.021	0.002	0.075	0.035	0.031	0.034	-0.003	0.028		+	+	+	+	+		
WHBC	0.027	0.014	0.004	0.038	0.041	0.032	0.027	0.031	0.036	0.043	0.052	0.037	0.015	0.042	0.023	0.032	0.013	-0.002	0.028	0.020	0.079	0.035	0.036	0.044	0.043	0.026	0.019	0.129	0.030	0.031	0.034	0.018	0.032	0.013		+	+	+	+	+	
3LNK	0.070	0.031	-0.026	0.071	0.031	0.059	0.027	0.031	0.017	0.058	0.043	0.058	0.006	0.047	0.030	0.007	0.049	0.036	0.034	0.036	0.118	0.076	0.071	0.076	0.040	0.036	0.058	0.160	0.059	0.063	0.050	0.049	0.053	0.041	0.034		+	+	+	+	+

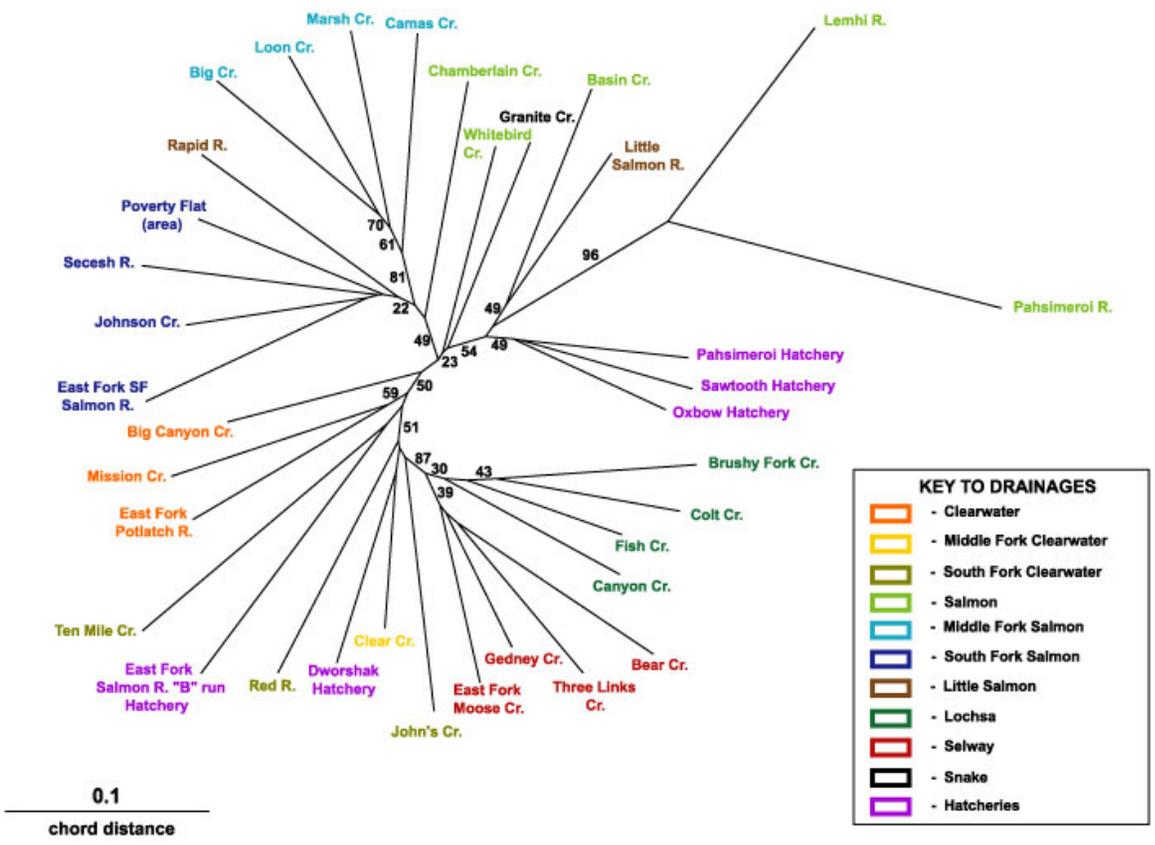


Figure 2. Unrooted Neighbor-Joining tree based on Cavalli-Sforza and Edwards (1968) chord distance for steelhead populations sampled in the Snake River drainage, Idaho. Bootstrap values (% 2000 replicate trees) are given for major branches.

Table 7. Estimations of recent reductions in population size (Garza and Williamson's  $M$ ) and effective population size ( $N_e$ ) based on the Infinite Allele (IAM) and Single Mutation (SMM) models .

DRAINAGE	POPULATION	Garza & Williamson's		
		$M$	IAM	$N_e$ SMM
Clearwater River	Big Canyon Creek	0.597	2310	4498
	EF Potlatch River	0.607	2114	3947
	Mission Creek	0.570	2030	3721
MF Clearwater River	Clear Creek	0.496	1746	2996
SF Clearwater River	John's Creek	0.482	1871	3305
	Red River	0.580	2074	3837
	Ten Mile Creek	0.577	1789	3101
Salmon River	Basin Creek	0.589	2883	6290
	Chamberlain Creek	0.604	2129	3988
	Lemhi River	0.633	3125	7129
	Pahsimeroi River	0.622	2766	5903
	Whitebird Creek	0.630	2232	4274
SF Salmon	EF SF Salmon River	0.538	2026	3709
	Johnson Creek	0.577	2067	3819
	Poverty Flat Area	0.555	1894	3364
	Secesh River	0.532	2012	3672
MF Salmon River	Big Creek (lower)	0.575	1828	3198
	Camas Creek	0.577	1990	3614
	Loon Creek	0.577	1530	2490
	Marsh Creek	0.588	1673	2821
Little Salmon River	Little Salmon, Pinehurst area	0.660	2404	4774
	Rapid River	0.533	2072	3833
Lochsa River	Brushy Fork Creek	0.578	1451	2315
	Canyon Creek	0.586	1742	2986
	Colt Creek	0.565	1691	2862
	Fish Creek	0.573	1904	3391
Selway River	Bear Creek	0.553	1709	2906
	EF Moose Creek	0.549	1977	3579
	Gedney Creek	0.617	1834	3214
	Three Links Creek	0.522	1782	3085
Snake River Hatcheries	Granite Creek	0.635	2512	5098
	Dworshak Hatchery	0.610	1660	2790
	EF Salmon "B-run"	0.522	1767	3048
	Oxbow Hatchery	0.599	2465	4956
	Pahsimeroi Hatchery	0.627	2646	5518
	Sawtooth Hatchery	0.609	2617	5425

Table 6). East Fork Moose and Gedney creeks also carried similar allelic diversity for all 11 microsatellite loci combined ( $F_{st} = -0.001$ ). Mean  $M$  for the Selway River drainage was  $M = 0.56$ . Effective population size ( $N_e$ ) for the Selway River drainage calculated by Garza and Williamson's (2001) program for  $M$  based on the SMM ranged from  $N_e = 2906$  (Bear Creek) to  $N_e = 3579$  (East Fork Moose Creek), mean drainage  $N_e = 3196$ .

### Snake River

One population of steelhead from Granite Creek on the Snake River was analyzed in this study ( $N = 49$  fish). Observed  $H_Z$  for Granite Creek was 0.68. The mean number of alleles for all 11 loci in this sample was 7.91. Population  $M = 0.64$  and Garza and Williamson's (2001) SMM  $N_e = 5098$  fish.

### *Total Basin AMOVA*

AMOVA for all Snake River drainages combined gave the following Fixation Indices:  $F_{sc} = 0.025$ ;  $F_{st} = 0.052$ ;  $F_{ct} = 0.028$ . Allelic variation was partitioned across the Snake River drainage as 2.79% among river basins, 2.44% among populations within river basins, and 94.77% within populations.

### *Idaho Steelhead Hatchery Populations*

Population pairwise comparisons showed no significant differences in allelic frequencies for Oxbow, Sawtooth, and Pahsimeroi hatcheries. Pahsimeroi Hatchery was the only hatchery population in this study with allelic frequencies that were not significantly different from wild *O. mykiss* populations. Pahsimeroi Hatchery steelhead and fish from Chamberlain Creek in the Salmon River drainage were not significantly different in allelic frequencies for the 11 microsatellite loci combined ( $F_{st} = 0.001$ ;  $P = 0.265$ ; Table 6). The average observed  $H_Z$  within the hatchery populations was  $H_Z = 0.63$  and the average number of alleles per locus was 7.22. Hatchery  $M$  ranged from  $M = 0.522$  (East Fork Salmon "B-run") to  $M = 0.627$  (Pahsimeroi Hatchery), mean  $M = 0.59$ . Dworshak Hatchery  $N_e = 2790$ ; East Fork Salmon "B-run"  $N_e = 3048$ ; Oxbow

Table 8. BOTTLENECK's mutation drift equilibrium probabilities under the heterozygote deficient (HZD), heterozygote excess (HZE), and two-tailed deficiency and excess (TTM) models for Idaho steelhead populations based on all 11 microsatellite loci combined under SMM and IAM mutation-drift equilibrium assumptions using Wilcoxon tests.

Population	SMM	SMM	SMM	IAM	IAM	IAM
	Model	Model	Model	Model	Model	Model
	HZD	HZE	TTM	HZD	HZE	TTM
Big Canyon Creek	0.01	0.99	0.02	0.97	0.03	0.07
East Fork Potlatch River	0.16	0.86	0.32	0.99	0.01	0.02
Mission Creek	0.03	0.99	0.05	0.99	0.01	0.02
Clear Creek	0.01	0.99	0.02	0.88	0.14	0.28
John's Creek	0.01	0.99	0.01	0.88	0.14	0.28
Red River	0.00	1.00	0.01	0.91	0.10	0.21
Ten Mile River	0.21	0.82	0.41	0.99	0.01	0.02
Basin Creek	0.03	0.99	0.05	0.99	0.00	0.00
Chamberlain Creek	0.05	0.96	0.10	0.97	0.04	0.08
Lemhi River	0.52	0.52	1.00	1.00	0.00	0.01
Pahsimeroi River	0.01	0.99	0.02	0.99	0.01	0.02
Whitebird Creek	0.02	0.99	0.05	1.00	0.00	0.01
East Fork South Fork Salmon River	0.09	0.93	0.18	0.99	0.01	0.02
Johnson Creek	0.14	0.88	0.27	1.00	0.00	0.01
Poverty Flat area	0.01	0.99	0.02	1.00	0.00	0.01
Secesh River	0.01	0.99	0.02	0.99	0.01	0.02
Big Creek (lower)	0.10	0.91	0.21	0.97	0.04	0.08
Camas Creek	0.00	1.00	0.01	0.91	0.10	0.21
Loon Creek	0.03	0.97	0.07	0.97	0.03	0.07
Marsh Creek	0.42	0.62	0.83	0.94	0.07	0.15
Little Salmon River Pinehurst area	0.03	0.99	0.05	0.99	0.01	0.02
Rapid River	0.23	0.79	0.47	1.00	0.00	0.01
Brushy Fork Creek	0.10	0.91	0.21	0.97	0.04	0.08
Canyon Creek	0.23	0.79	0.47	0.99	0.01	0.02
Colt Creek	0.16	0.86	0.32	0.99	0.01	0.01
Fish Creek	0.01	0.99	0.01	0.91	0.10	0.21
Bear Creek	0.32	0.71	0.64	0.99	0.03	0.05
East Fork Moose Creek	0.04	0.97	0.08	0.97	0.04	0.08
Gedney Creek	0.14	0.88	0.28	0.97	0.04	0.08
Three Links Creek	0.38	0.65	0.77	0.99	0.01	0.02
Granite Creek	0.03	0.97	0.07	0.99	0.01	0.02
Dworshak Hatchery	0.09	0.93	0.18	0.99	0.01	0.02
East Fork Salmon "B-run"	0.32	0.71	0.64	0.96	0.05	0.10
Oxbow Hatchery	0.35	0.68	0.70	1.00	0.00	0.00
Pahsimeroi Hatchery	0.35	0.68	0.70	1.00	0.00	0.01
Sawtooth Hatchery	0.10	0.91	0.21	1.00	0.00	0.00

Hatchery  $N_e = 4956$ ; Pahsimeroi Hatchery  $N_e = 5518$ ; Sawtooth Hatchery  $N_e = 5425$ .

### *Snake River $N_e$ and Bottleneck Analyses*

Garza and Williamson's (2001)  $M$  demonstrates a recent reduction in population, i.e. a population bottleneck, when  $M \leq 0.68$ . In tests of Snake River steelhead populations mean  $M$  across all 11 microsatellite loci was less than 0.68 in all populations. Garza and Williamson's (2001)  $M$  estimates of effective population size assuming mutation-drift equilibrium and a mutation rate of  $2.05E^{-4}$  for both SMM and IAM are given for steelhead populations in the Snake River drainage in Table 7. Probabilities calculated under the assumption that all loci meet expectations for mutation-drift equilibrium using three models (heterozygote ( $H_z$ ) deficiency (one tailed);  $H_z$  excess (one tailed); two tails  $H_z$  excess and deficiency) using the program BOTTLENECK are given for the Snake River steelhead populations in Table 8.

### *Snake River Genetic Distance*

A consensus Neighbor-Joining tree based on Cavalli-Sforza and Edwards chord distance for the entire set of samples analyzed in this report from the Snake River system is presented in Figure 2. Branch bootstrap values (% of 2000 replicate trees) are given for major branches. NJ analyses demonstrated inferential clustering of populations within drainages, especially the Middle Fork Salmon, South Fork Salmon, Lochsa, and Selway rivers. Ninety-six percent bootstraps supported unique allelic population structure in the Pahsimeroi and Lemhi populations. Microsatellite distance analyses of the Dworshak Hatchery population, although distinct to some degree from many other *O. mykiss* populations, does not show significantly different allelic variation as would have been predicted from previous allozyme analyses.

## DISCUSSION

This study focused on genetic population structure in 36 Snake River *O. mykiss* populations as part of a larger ongoing study of 79 Idaho *O. mykiss* populations. Completion of the second phase of this project is expected late 2004 or early 2005. Final publication of these results will be submitted at that time. Relationships among populations shown in this report may change after the completed data set is analyzed, therefore, the results in this report should be considered preliminary. A previous analysis of four steelhead populations from the Clearwater River basin was made in relationship to the Dworshak Hatchery fish (Nielsen et al. 2003a). A total of seven steelhead populations from the Clearwater River basin were included in this report, adding fish from Mission Creek, Red River and Ten Mile Creek. Big Canyon Creek carried the highest observed heterozygosity ( $H_z = 0.68$ ) and the largest estimate of effective population size (SMM  $N_e = 4498$ ). Clear Creek from the Middle Fork Clearwater drainage had the lowest estimated effective population size (SMM  $N_e = 2996$ ). John's Creek from South Fork Clearwater River carried the lowest observed  $H_z = 0.57$  in this drainage.

Populations in the lower Clearwater drainage from Big Canyon Creek, Mission Creek, and EF Potlatch River are considered A-run fish and are downstream of Dworshak NFH. Dworshak stock smolts are released annually in Clear Creek (Middle Fork Clearwater) and the South Fork Clearwater River to provide for angler harvest. Dworshak NFH adults and smolts have been stocked in many South Fork Clearwater tributaries for state and Nez Perce Tribe supplementation efforts. Our microsatellite analyses showed mixed results in genetic associations for this group. Two *O. mykiss* samples collected from streams in the Clearwater River drainage, Mission Creek and East Fork Potlatch River, separated from the rest of the Clearwater River group containing the Dworshak hatchery stock with 59% bootstrap replication in 2000 NJ trees (Mission/Dworshak  $F_{st} = 0.054$ ; EF Potlatch/Dworshak  $F_{st} = 0.049$ ). The Big Canyon Creek *O. mykiss* population was marginally distinct from Dworshak hatchery stocks based on allelic frequencies for these 11 microsatellite loci

(pairwise  $F_{st} = 0.023$ ). Ten Mile Creek in the South Fork Clearwater was weakly associated with the East Fork Salmon B-run hatchery stock in our NJ tree, with very low bootstrap replication (25%; pairwise  $F_{st} = 0.061$ ). Clear Creek, John's Creek and the Red River (SF Clearwater R.) *O. mykiss* populations were more difficult to differentiate from Dworshak hatchery fish (pairwise  $F_{st} = 0.015$ ; 0.023; 0.011; respectively). Weak bootstraps (38%) supported the NJ branch containing Dworshak Hatchery and Clear Creek.

The steelhead population from Dworshak Hatchery was the most divergent single population of inland steelhead based on genetic traits determined by protein electrophoresis (Busby et al. 1996). However, this conclusion was based on very limited sampling of Idaho steelhead populations. In this microsatellite study, Dworshak Hatchery fish were significantly different in allelic frequencies from all other Snake River basin populations, but only 1.4% of all basin-wide population pairwise comparisons were non-significant, indicating significant genetic structure throughout this system. Using microsatellite analyses Dworshak Hatchery had the lowest estimated effective population size of the five hatcheries populations we analyzed (SMM  $N_e = 2790$ ). Only wild *O. mykiss* populations from Loon Creek from the Middle Fork Salmon River ( $N_e = 2490$ ) and Brushy Fork Creek on the Lochsa River ( $N_e = 2315$ ) had lower effective population size estimates.

We found little support from the genetic distance analyses to separate the East Fork Salmon B-run hatchery stock from the Dworshak Hatchery (also considered B-run fish) and several other Clearwater River drainage *O. mykiss* populations (see Figure 2). These two hatchery populations shared their most common alleles for all but three loci (Ogo4, Omy325, and Ots3). A 50% bootstrap value supported the NJ branch pattern grouping the East Fork Salmon B-run hatchery stock with *O. mykiss* from Ten Mile Creek, East Fork Potlatch River and Mission Creek in the Clearwater River drainage in our NJ analysis (pairwise  $F_{st}$  range = 0.034 – 0.061). The EF Salmon B-hatchery stock was expected to cluster closely with Dworshak Hatchery fish, but instead we found a closer genetic association with *O. mykiss* from Ten Mile Creek. This suggests

that differential reproductive success may have biased the resulting genetic population structure away from the Dworshak hatchery contribution. However, only 25% to 51% of the NJ bootstrap trees separate the branch containing the Dworshak hatchery population from the EF Salmon B-hatchery population.

Significant genetic separation of the Lochsa and Selway River *O. mykiss* from Clearwater River drainage fish was shown in these microsatellite analyses. The differentiation was greater between Clearwater/Lochsa than between Clearwater/Selway (Clearwater/Lochsa mean pairwise  $F_{st} = 0.061$ ; Clearwater/Selway mean pairwise  $F_{st} = 0.037$ ). Genetic pairing of the Lochsa and Selway River *O. mykiss* populations was supported with a bootstrap value of 87% suggesting fine-scale population genetic structure across both drainages. Genetic substructure among streams within each of the Lochsa and Selway rivers was less significant (all bootstraps < 43%), but additional sampling within both drainages may reveal fine-scale genetic substructure. Bear Creek in the Selway River drainage was unusually common in  $F_{st}$  pairwise comparisons with no significant differences in allelic frequency relative to nine other populations (Table 6). This resulted from the fact that the Bear Creek *O. mykiss* population contained a high proportion of the most common alleles for all 11 loci and carried limited genetic diversity outside of these common alleles, making this population difficult to differentiate from many other groups of *O. mykiss* where common alleles predominated.

Salmon River drainage wild steelhead showed significant biogeographic structuring in this study (see Figure 2). Support for genetic population structure was greatest for the Middle Fork Salmon River where 81% bootstraps supported clustering within this drainage. Significant bootstrap values (61-70%) also supported genetic substructure among tributaries sampled in the Middle Fork Salmon River suggesting fine-scale population differentiation within this drainage. Middle Fork Salmon River within-drainage pairwise  $F_{st}$  values were all significant. Genetic diversity within the Middle Fork Salmon River was not as large as that found within the Clearwater River drainage (mean MF Salmon River  $F_{st} = 0.017$ ; Clearwater River  $F_{st} = 0.021$ ). Genetic samples from Big and Loon creeks were

more closely allied than either was to samples from Marsh Creek (Big/Loon  $F_{st} = 0.015$ ; Big/Marsh  $F_{st} = 0.025$ ; Loon/Marsh  $F_{st} = 0.028$ ). *O. mykiss* sampled in Camas Creek, downstream from Loon Creek were also genetically distinct from other tributaries sampled in the Middle Fork Salmon River (within-drainage comparisons mean  $F_{st} = 0.012$ ) and were more closely allied with Loon Creek *O. mykiss* ( $F_{st} = 0.010$ ). Similar genetic relationships are depicted in our NJ tree (Figure 2). Additional samples taken from sites above Loon Creek in the Middle Fork Salmon River remain to be analyzed and may add resolution to the within-drainage separation found in this system.

*O. mykiss* collected from the South Fork Salmon River, considered B-run fish, also clustered together in our NJ tree, but with less statistical support based on replicate trees. Rapid River (tributary of the Little Salmon River) *O. mykiss* fell within this group in these microsatellite analyses. A velocity barrier weir separates fish from upper Rapid River and downstream locations in the Little Salmon River where there are large hatchery releases yearly to provide angler harvest. Samples collected in Rapid River were taken upstream of the weir. Bootstrap values supporting branching patterns within this cluster were not high, ranging from 6-28%. *O. mykiss* samples collected from Secesh River were not as isolated genetically from other South Fork Salmon River populations in this study as previously reported in ICBTRT (2003). However, within-SF Salmon River drainage pairwise  $F_{st}$  values using Secesh River samples were all significant: Secesh/EF South Fork  $F_{st} = 0.021$ ; Secesh/Poverty Flat  $F_{st} = 0.013$ ; Secesh/Johnson Creek  $F_{st} = 0.013$ .

Oxbow hatchery fish were only weakly separated from four geographically proximate sample locations (Oxbow/Little Salmon  $F_{st} = 0.021$ ; Oxbow/Granite  $F_{st} = 0.010$ ; Oxbow/Whitebird  $F_{st} = 0.019$ ; Oxbow/Chamberlain  $F_{st} = 0.010$ ). The Little Salmon River *O. mykiss* weakly grouped with fish from Basin Creek from the upper Salmon River in our NJ tree (49% bootstrap). Whitebird Creek, a tributary just downstream of the mouth of the Little Salmon River was part of the larger grouping of *O. mykiss* including fish from the mainstem Snake River in Granite Creek.  $F_{st}$  analyses showed no significant differences in allelic frequency

for microsatellite loci between Whitebird Creek and Chamberlain Creek, a putative A-run population.

Three steelhead hatchery populations, Oxbow, Sawtooth and Pahsimeroi hatcheries, were statistically similar in allelic frequency for all 11 microsatellite loci and grouped together in our NJ analysis of chord genetic distance (see Figure 2 and Table 6). All of these hatchery populations were primarily derived from wild steelhead collected at the base of Hells Canyon Dam and would be expected to have similar genetic signatures. Only one wild population in the Salmon River drainage carried allelic frequencies that were not significantly different from hatchery steelhead (Chamberlain Creek and the Pahsimeroi Hatchery pairwise  $F_{st} = 0.001$ ,  $p = 0.265$ ). Our NJ analysis weakly placed Chamberlain Creek *O. mykiss* within the group containing South Fork Salmon River and Middle Fork Salmon river B-run populations with 49% bootstraps.

The Snake River steelhead ESU includes both resident and anadromous *O. mykiss* (ICBTRT 2003). Differentiation of freshwater and anadromous life history components for *O. mykiss* has been controversial since the renaming of steelhead and rainbow trout to the *Oncorhynchus* genus by Smith and Stearley (1989). Early genetic studies of *O. mykiss* above and below waterfalls suggested unique evolutionary lineages for these two life history types (Currens et al. 1990). Recent studies of coastal *O. mykiss* populations have been unable to genetically differentiate anadromous and freshwater-resident types sampled from the same river drainages suggesting parallel evolution of divergent life histories within each river (Beacham et al. 1999; Nielsen 1999; Nielsen et al. 1999; McCusker et al. 2000; Docker and Heath 2003). However, studies of population structure and otolith microchemistry have been shown to support reproductive separation between anadromous and resident components of *O. mykiss* within the same river drainage (Zimmerman and Reeves 2000 and 2002).

Pascual et al. (2001) have documented the development of anadromous runs of steelhead derived from introduced rainbow trout in Patagonia, Argentina. Previous genetic studies have suggested a resident contribution to the anadromous component of the Snake River steelhead population (Moran

unpublished data; Figure IV-2 page 60 ICBTRT 2003). The source of the anadromous component of Snake River *O. mykiss* and the interplay between anadromous and resident life histories are important questions for restoration and de-listing criteria.

Two Salmon River *O. mykiss* populations in this study were found to have uniquely distinct allelic frequencies and genetic structure – Lemhi River and Pahsimeroi River. These two *O. mykiss* populations separated from the rest of our sample locations with 96% bootstraps on the NJ tree. Allele frequencies comparisons between both populations were statistically significant (pairwise  $F_{st} = 0.024$ ,  $p < 0.000$ ). Both populations shared a similar level of observed heterozygosity ( $H_z = 0.67$ ) and average number of alleles per locus (8). The Pahsimeroi River population had a lower estimated effective population size (Pahsimeroi SMM  $N_e = 5903$ ; Lemhi SMM  $N_e = 7129$ ) and Garza and Williamson's  $M$  was lower in the Lemhi River *O. mykiss* ( $M = 0.46$  versus Pahsimeroi  $M = 0.55$ ), probably reflecting different characteristics of recent population declines.

These *O. mykiss* populations were collected from high mountain valley, spring-fed streams with highly productive populations. These streams, unlike other systems in the Snake River basin contain a relatively steady flow regime with groundwater-mitigated stream temperatures that are warmer than average in the winter and cooler than average in the summer. These environmental conditions may have led to unique life history structure in these populations where large components of the population remain in fresh water throughout their life, not migrating to the Pacific Ocean.

Anadromous steelhead currently found in these rivers are presumably primarily derived from several hatchery stocking efforts (ICBTRT 2003). Consistent reproductive isolation between freshwater and anadromous *O. mykiss* based on a pattern of freshwater maturation in wild resident fish could lead to separation of resident strains despite hatchery stocking of steelhead. It is significant that these unique sample locations can be rigorously separated from the other study sites, including local hatcheries, suggesting a unique evolutionary

status for these populations. Additional sampling in putative “resident” stocks in the Snake River basin may help support genetic structure for unique evolutionary life histories for *O. mykiss* in this area.

Snake River steelhead have been genetically differentiated from other interior Columbia River steelhead populations (Busby et al. 1996) and listed under the Endangered Species Act by the Federal government. In this study, significant biogeographic population genetic structure was documented within the Snake River basin in Idaho. Wild steelhead abundance in the Snake River has declined precipitously over the last 50 years, with many stocks currently in decline (Busby et al 1996 and 1997). Habitat alterations due to water diversions, increased water demands, changes in water management strategies, dams and barriers, bank protection, dredging, sediment disposal, gravel mining, contaminant exposure, climate change and shifts in ocean conditions have clearly impacted the size and distribution of steelhead runs throughout the Columbia River (Robards and Quinn 2002). The loss of access to upriver spawning habitats, declines in once viable tributary populations, and limited productivity in large river populations have also had potentially significant effects on Snake River steelhead with important implications for genetic diversity and restoration (ICBTRT 2003).

Estimates of effective population size ( $N_e$ ) incorporate relative parameters related to demographic information. In small populations,  $N_e$  is important because it is inversely related to the rate of loss of genetic diversity. Estimates of  $N_e$ , however, are based on several assumptions (identity-by-descent, random mating, temporal stability in finite populations) that are generally difficult to support for *O. mykiss* and can often overestimate population size (Heath et al. 2002, Palm et al. 2003; Ardren and Kapuscinski 2003). The relationship between effective population size (i.e. the estimated number of individuals contributing genes to the next generation) and actual demographic population size is important in understanding the effects of artificial husbandry on the genetic composition of hatchery stocks (Waples and Do 1994). Comparisons of these two characteristics deserve attention in all hatchery populations considered for

supplementation of wild stocks. Temporal changes in genetic structure and effective population size can be used to monitor intermittent gene flow and address concerns about population stability (Frankham 1995) and size in populations of concern (Heath et al. 2002). We recommend additional temporal sampling in Snake River steelhead hatchery populations to gain inference on these issues (see Hansen et al. 2002 and Guinard et al. 2002).

Implications of intra-specific hatchery production on wild steelhead stocks within genetically distinct river drainages are also a critical concern for steelhead restoration. The degree of straying and interbreeding with hatchery fish, especially non-native derived populations, is important to our understanding of the status of remaining wild stocks and the position hatcheries can play in the restoration of steelhead in the ESU. Local gene flow to or from hatchery fish may link hatchery stocks with geographically adjacent wild populations, for example: (1) Oxbow, Sawtooth, and Pahsimeroi hatcheries with the Little Salmon River (Pinehurst), Granite Creek, Whitebird Creek and Basin Creek; (2) Dworshak Hatchery with Clear Creek, John's Creek, Red River, and Ten Mile Creek. Directionality of this gene flow is difficult to interpret from these data since hatchery stocks were, for the most part, developed from local adult steelhead populations at specific times and from different migration patterns. This relationship will also depend on the management history of the hatchery broodstock, the amount and extent of hatchery stocking into wild river systems, and the reproductive success of straying hatchery fish in the wild. To address this question genetically, a broader coverage of both spatial and temporal samples is required.

Analysis of mitochondrial DNA (mtDNA) sequence from both hatchery and wild populations may add resolution to this question. Maternally inherited mtDNA has been used to discriminate between hatchery and wild stocks based on sexually dimorphic selection or admixture from divergent gene pools that occurred in either population's recent history (Nielsen et al. 1994). However, when the hatchery stock is derived from local wild sources within the same ESU, the resolution found in mtDNA analyses is minimal unless significant artificial

selection for females has occurred within the hatchery broodstock development (Nielsen et al. 2003b).

Genetic analyses of 36 *O. mykiss* populations throughout the Snake River basin provide a better understanding of population structure in this complex system. The dynamic genetic structure of *O. mykiss* in this basin will be better understood when additional populations within individual drainages are analyzed. We also suggest testing year-to-year variation at unique sample sites within individual drainages where hatchery stocks have been stocked and in drainages with no hatchery stocking. Additional sampling of adult fish to compare hatchery and wild fish interactions will also add significant rigor to these analyses. The hint of genetic separation between different life histories, i.e. resident and anadromous fish from the Salmon River, is very intriguing and should be followed with more sample locations where separation of these life history types is suspected. The significance of the microsatellite separation found in the Lemhi and Pahsimeroi river *O. mykiss* populations make them a good candidate for molecular systematic studies using other rigorous genetic tools where time since divergence can be confidently estimated. Our analysis of the Idaho steelhead leaves us with as many new questions as it provides answers. It is clear that the addition of data from the remaining sample collections currently in analysis at ASC will add new insight and complexity to these analyses.

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Appendix I. Wild steelhead populations that were sampled in 2000 that will be analyzed and included in the final report.

<b>Drainage</b>	<b>Population</b>	<b>Site Location on Map</b>	
Clearwater River	Jacks Creek	17	
	Little Bear Creek	18	
Salmon River	Bargamin Creek	37	
	Horse Creek	40	
	Morgan Creek	42	
	Owl Creek	43	
	Sheep Creek	44	
	Slate Creek	45	
	Valley Creek	46	
	Warm Springs Creek	47	
	WF Yankee Fork	50	
	Middle Fork Salmon River	Bear Valley Creek	58
Big Creek (upper)		60	
Pistol Creek		64	
Rapid River		65	
Sulphur Creek		66	
South Fork Salmon River	Lick Creek (downstream of barrier)	53	
	Lick Creek (upstream of barrier)	54	
	Stolle Meadow	57	
Little Salmon River	Boulder Creek	67	
	Hazard Creek	68	
	Little Salmon River, upstream of falls	69	
Lochsa River	Boulder Creek	1	
	Crooked Fork Creek	5	
	Deadman Creek	6	
	Fish Creek (fall migrants)	8	
	Hungry Creek	9	
	Lake Creek	10	
	Papoose Creek	11	
	Storm Creek	12	
	Warm Springs Creek	13	
	Weir Creek	14	
	Selway River	Meadow Creek	28
		Mink Creek	29
		NF Moose Creek	30
		O'hara Creek	31
Pettibone Creek		32	
Snake River		Captain John Creek	34
	Sheep Creek	36	
Boise River	Big Smoky Creek	78	
North Fork Clearwater River	Collins Creek	21	
Payette River	MF Payette River	77	
Weiser River	Little Weiser River	79	