

## Steelhead Genetic Diversity at Multiple Spatial Scales in a Managed Basin: Snake River, Idaho

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*Abstract.*—We investigated the genetic diversity of steelhead *Oncorhynchus mykiss* in 74 wild populations and 5 hatchery stocks in Idaho's Snake River basin at the drainage, watershed, and population spatial scales using 11 microsatellite loci. We found significant genetic diversity at multiple spatial scales. Analysis of molecular variance showed that genetic diversity was greater among watersheds within drainages (3.66%) than among drainages (1.97%). Over 94% of the genetic diversity found in the Clearwater, Salmon, and Snake River drainages occurred within individual populations. Estimated effective population sizes ranged from 213 to 486.6 at the drainage scale, from 81.2 to 610.4 at the watershed scale, and from 8.4 to 4,845 at the population scale. The Middle Fork Salmon, South Fork Salmon, Lochsa, and Selway rivers—watersheds managed for wild fish—formed distinct groups in our consensus neighbor-joining (NJ) trees. At the watershed scale our analyses support differentiation of all hatchery and wild stocks. However, this was not the case for analyses at the population scale, where 236  $F_{ST}$  pairwise comparisons out of 3,081 (wild and hatchery) were not significantly different. The distribution of genetic diversity across the landscape does not appear to be organized by the A run or B run management designations for anadromous steelhead. The Dworshak hatchery stock was significantly different from all but one population (O'Hara Creek, Selway River) in pairwise  $F_{ST}$  comparisons and grouped with other Clearwater River drainage populations in our NJ trees. The Oxbow, Sawtooth, and Pahsimeroi hatchery stocks were indistinguishable from each other based on  $F_{ST}$  analysis. Currently, this study represents the most comprehensive evaluation of genetic diversity in Idaho's steelhead populations across multiple scales with different management histories.

The ability to adapt to highly variable conditions in unique environments is a critical element throughout the life cycle of anadromous salmonids. *Oncorhynchus mykiss* populations express a diversity of life history strategies from strongly anadromous (steelhead) to nonanadromous (resident or rainbow trout) throughout the species' natural range (Shapovalov and Taft 1954; Rybock et al. 1975; Taylor 1995). Many studies have shown that sympatric populations of anadromous and resident *O. mykiss* found within the same drainage cannot be separated taxonomically or genetically based on migration timing or different life histories (Allendorf and Utter 1979; Reisenbichler et al. 1992; Nielsen et al. 1994; Docker and Heath 2003; Narum et al.

2004a; Olsen et al. 2006; Heath et al. 2008). Additional studies demonstrated that steelhead isolated as resident fish behind artificial barriers and dams contain components of the *O. mykiss* gene pool formerly found in geographically proximate anadromous populations (Gall et al. 1990; Nielsen et al. 1997; Deiner et al. 2007). Variation in reproductive success and genetic compensation between different life history forms can result in changes in effective population size and subsequent patterns of genetic diversity across the landscape (Heath et al. 2001; Araki et al. 2007).

Historically, steelhead were broadly distributed throughout most of the Columbia River basin, including populations in Oregon, Washington, Idaho, and British Columbia (Behnke 1992; reviewed in Busby et al. 1996). Mallet (1974) estimated that 55% of all steelhead in the lower Columbia River were produced in the Idaho portion of the Snake River.

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Chapman (1986) estimated that as many as 554,000 steelhead entered the Columbia River during their peak abundance in the 19th century. Steelhead that spawn in Idaho are summer-run fish that migrate further from the ocean (up to 1,500 km) and spawn at higher elevations (up to 2,000 m) than other populations in the basin. There have been substantial declines in these populations over the last 150 years, primarily due to lost spawning and rearing habitats, changes in water quality, and dams and diversions (Nehlsen et al. 1991; Gustafson et al. 2007). Wild steelhead abundance in Idaho further declined relative to their historical abundance after the construction of the four lower Snake River dams: Ice Harbor in 1961, Lower Monumental in 1969, Little Goose in 1970, and Lower Granite in 1975. The construction of three Snake River dams in Hells Canyon—Brownlee in 1958, Oxbow in 1961, and Hells Canyon in 1967—prevented steelhead from upstream access because no fish passage facilities were built at these dams. Snake River basin steelhead were listed as a threatened species on August 18, 1997 (U.S. Office of the Federal Register 1997). The threatened status was reaffirmed on January 5, 2006 (U.S. Office of the Federal Register 2006). The Snake River basin steelhead distinct population segment (DPS) includes all naturally spawned steelhead populations downstream of impassable natural and artificial barriers in streams in the Snake River basin of southeastern Washington, northeastern Oregon, and Idaho. This DSP also includes fish from four artificial propagation programs: (1) the Tucannon River natural stocks, (2) the North Fork Clearwater River stock reared at Dworshak National Fish and Clearwater Fish hatcheries (hereafter referred to as the Dworshak stock), (3) the East Fork Salmon River local stock, and (4) the Little Sheep Creek–Imnaha River Hatchery steelhead hatchery programs (NOAA 2007a).

Maintenance of genetic diversity and local adaptation within and among wild fish populations is a central tenet of conservation (Waples et al. 2001; Ardren and Kapuscinski 2003). Local adaptation is most commonly regarded as a process that promotes fitness of the organism in specific environments (Gould and Lewontin 1979; Mayr 2002) and includes genotypic and phenotypic traits at both individual and population scales (Reeve and Sherman 1993). A delicate interplay among selection, mutation, and chance leads to evolutionary success through local adaptation (Brandon 1990; Rose and Lauder 1996). As part of the evolutionary history of steelhead, variation based on local adaptation and life history patterns are important legacies for conservation and restoration.

One factor thought to lead to changes in genetic diversity and local adaptation in salmonids at many

scales is the influence of artificial propagation and the distribution of hatchery offspring into natural habitats (Waples and Do 1994; Narum et al. 2006a; Heggenes et al. 2006; Eldridge and Naish 2007; Eldridge and Killebrew 2008). Large-scale artificial propagation of steelhead in the Snake and Salmon rivers began in the 1960s with the initiation of Idaho Power Company's program to relocate steelhead from the upper Snake River to the Salmon River after the construction of dams in Hells Canyon (Busby et al. 1996). Hatcheries built under the Lower Snake River Compensation Plan in the 1970s and 1980s were used to mitigate for anadromous fish losses in the Snake River basin from the construction of dams in the Snake River downstream of Lewiston, Idaho. The Idaho Department of Fish and Game manages five steelhead hatchery stocks: Dworshak, East Fork Salmon EFSB), Oxbow, Pahsimeroi, and Sawtooth (Table 1). The 1980–1999 average number of hatchery steelhead smolts released annually in Idaho from was 3.3 million in the Salmon River drainage, 2.5 million in the Clearwater River drainage, and 0.6 million in the Snake River drainage (Fish Passage Center 2008).

Introgression by domesticated *O. mykiss* stocks is thought to impose risks on genetic diversity in wild populations; that is, hatchery stocks could carry significant genetic homogeneity or have high variance in reproductive success compared with wild populations (McLean et al. 2004; Narum et al. 2006a). The impact of releasing hatchery-reared *O. mykiss* with wild fish has been the subject of many studies in other river systems (Reisenbichler and McIntyre 1977; Waples and Do 1994; Campton 1995; Nielsen 1999). Straying and introgression by hatchery fish may pose a risk to the genetic integrity of wild steelhead populations when exogenous brood stocks lack important local adaptation (Waples and Do 1994; Campton 1995; Levin and Williams 2002; Wang et al. 2002; Heggenes et al. 2006). Fine-scale gene flow among hatchery fish and wild populations of *O. mykiss* was documented in one drainage of the Snake River basin, the Grande Ronde River of Oregon (Narum et al. 2006a), but the genetic consequences of releasing hatchery fish into wild systems has not been thoroughly investigated for *O. mykiss* in Idaho. Conservation of natural genetic diversity for steelhead within a large ecosystem like the Snake River basin requires investigations of genetic structure at multiple geographic scales. Large-scale genetic analyses in managed watersheds also require a close look at the interplay of the history of hatchery stock development, hatchery release practices across the landscape, and knowledge of historical genetic diversity in wild extant populations.

TABLE 1.—Hatchery program initiation (year brood source adults were first spawned), brood source, program type, and release location for the five hatchery steelhead stocks reared in Idaho. Abbreviations are as follows: NFH = National Fish Hatchery, NF = North Fork, EF = East Fork, IPN = infectious pancreatic necrosis, IDFG = Idaho Department of Fish and Game.

Hatchery stock	Program initiation	Brood source	Program type	Release sites
Oxbow <sup>a</sup>	1966	Wild adult steelhead trapped at Hells Canyon Dam.	Segregated; all eggs taken from returning hatchery origin adults.	Snake River downstream of Hells Canyon Dam and Little Salmon River.
Pahsimeroi <sup>b</sup>	1969	Wild adult steelhead trapped at Hells Canyon Dam. Dworshak smolts were released in 1974 because the Pahsimeroi stock was lost to an outbreak of IPN.	Segregated; all eggs taken from returning hatchery origin adults.	Pahsimeroi Hatchery, Salmon River between the NF Salmon and EF Salmon rivers, Little Salmon River.
Sawtooth <sup>c</sup>	1985	Returning adults from Pahsimeroi stock smolts released at Sawtooth Fish Hatchery.	Segregated; all eggs taken from returning hatchery origin adults.	Sawtooth Fish Hatchery, Salmon River upstream of the EF Salmon River.
Dworshak <sup>d</sup>	1969	Wild adult NF Clearwater River steelhead.	Segregated; all eggs taken from returning hatchery origin adults.	Dworshak NFH, Kooskia NFH (Clear Creek), SF Clearwater River, Little Salmon River, EF Salmon River.
EF Salmon B <sup>e</sup>	1985	When the program began hatchery fish were not marked; hence, wild adults, Pahsimeroi origin adults, and Dworshak origin adults could have been incorporated into the broodstock. IDFG later attempted to establish a local, self-sustaining hatchery population of Dworshak origin by releasing marked Dworshak smolts in the EF Salmon River.	Segregated; all eggs taken from returning hatchery origin adults. However, in most years there were not enough adult returns to fill broodstock needs. First-generation Dworshak origin smolts produced from adults that returned to the Dworshak NFH were released yearly in the EF Salmon River.	EF Salmon River.

<sup>a</sup> Oxbow steelhead were reared at Magic Valley Hatchery, Niagara Springs Hatchery, and Hagerman NFH.

<sup>b</sup> Pahsimeroi steelhead were reared at Magic Valley Hatchery, Niagara Springs Hatchery, and Hagerman NFH.

<sup>c</sup> Sawtooth steelhead were reared at Magic Valley Hatchery and Hagerman NFH.

<sup>d</sup> Dworshak steelhead released in the Clearwater River drainage were reared at Dworshak National Fish Hatchery and Clearwater Fish Hatchery (beginning in 1992). Dworshak steelhead released in the Salmon River drainage were reared at Magic Valley Hatchery and Hagerman NFH.

<sup>e</sup> EF Salmon B were reared at Magic Valley Hatchery and Hagerman NFH.

Life history variables have been used to differentiate steelhead populations in Idaho for management and conservation. The A run and B run anadromous steelhead management designations are based on adult fish size and adult arrival date at Bonneville Dam (Busby et al. 1996). The Interior Columbia Basin Technical Recovery Team used the A and B run management designations as life history strategy classes for steelhead populations in Idaho (NOAA 2007b). In general, A run fish enter the Columbia River earlier in the summer after spending 1 year in the ocean before returning to spawn, whereas B run steelhead enter freshwater later in the summer after spending 2 years in the ocean. Samples collected for this study allowed us to compare genetic diversity among populations designated as A or B run steelhead in Idaho's Snake River basin. Steelhead populations in the Lochsa, Selway, and South Fork (SF) Clearwater rivers are classified B run, but the Lower Clearwater River is A run, except for Clear Creek. The North Fork (NF) Clearwater, currently blocked by Dworshak Dam, historically contained B run steelhead. In the Salmon and Snake drainages, all populations are classified as A

run except the populations in the Middle Fork (MF) and SF Salmon rivers.

This study reports the genetic structure found in 79 steelhead populations and represents the largest genetic study completed to date for steelhead in Idaho. We assessed the genetic diversity for *O. mykiss* with different states of local adaptation: A versus B run life histories, upstream versus downstream of dams and waterfalls, natural spawning anadromous populations, previously anadromous populations with no known current anadromous contribution, and hatchery-propagated stocks. The objectives of this study included (1) comparing genetic diversity among and between hatchery stocks and wild populations at various spatial scales, (2) testing genetic relationships found between nonanadromous populations and geographically adjacent anadromous populations, including those separated by waterfall barriers, (3) investigating genetic relationships between steelhead classified as A or B run. This data set addresses genetic variation of steelhead throughout Idaho's Snake River basin and provides details on the scale of diversity that currently exists within this segment of their natural distribution.

## Methods

*Designations for drainage, watershed, and population scales.*—We defined populations in this study as the sample collection locations listed in Table 2 and shown in Figure 1. Steelhead populations were grouped using the natural hierarchical stream structure that exists within Idaho, including three major river drainages (Clearwater, Salmon, and Snake rivers) and 11 watersheds (Table 2). In the Clearwater drainage there were five watersheds: Lower Clearwater (including Clear Creek), Lochsa, Selway, NF Clearwater, and SF Clearwater rivers. The Salmon River drainage contained four watersheds: the Little Salmon, SF Salmon, MF Salmon, and the main-stem Salmon rivers. The Snake River drainage was divided into two watersheds: Lower Snake (downstream of Hells Canyon Dam) and Upper Snake rivers (upstream of Brownlee Dam). The Lochsa, Selway, MF Salmon, and SF Salmon watersheds are managed as refugia for wild steelhead (IDFG 2007).

*Sample collections.*—We collected tissue samples from 3,982 fish from 79 locations that included 68 streams known to be accessible to anadromous salmonids, 6 streams with nonanadromous populations (upstream of waterfalls or currently blocked by impassable dams), and 5 hatchery stocks (Table 2). Throughout this study we refer to a population as a sample of fish from a stream or river, assuming this sample reflects the genetic diversity of *O. mykiss* at the finest scale. We included populations from watersheds managed for wild fish (MF Salmon, SF Salmon, Lochsa, and Selway rivers) and watersheds where hatchery steelhead have been released (Little Salmon, Lower Clearwater, SF Clearwater, Main Salmon, and Lower Snake rivers). We included spatially separated samples from Big Creek in the MF Salmon watershed (one near the mouth and one about 50 km upstream of the mouth) and the SF Salmon watershed (Poverty Flat and a second location about 30 km upstream at Knox Bridge). We sampled populations upstream of waterfalls presumed to be barriers to anadromous fish in upper Lick Creek and Little Salmon River near the town of New Meadows. Three formerly anadromous populations isolated upstream of Hells Canyon Dam were sampled in the Snake River drainage (Big Smoky Creek, MF Payette River, and Little Weiser River) and one population isolated upstream of Dworshak Dam was collected in the Clearwater drainage (Collins Creek).

Two nonanadromous populations were sampled from streams where there are no records of stocked hatchery *O. mykiss*: Upper Lick and Collins creeks. Little Weiser River was last stocked with hatchery

rainbow trout in 1989. Nonanadromous populations from Big Smoky Creek and the MF Payette River were sampled in roadless areas several kilometers upstream of the nearest hatchery stocking site. The Little Salmon River was stocked in May and June each year with 1,000–2,000 hatchery rainbow trout. In an effort to avoid hatchery trout at this location, we sampled in August in an area several kilometers upstream from the stocking location. We obtained samples of the Dworshak, Oxbow, Pahsimeroi, Sawtooth, and EFSB hatchery stocks by randomly netting fish from raceways in all hatcheries that rear these stocks proportionally to the distribution of each stock in all rearing hatcheries. Samples from all hatchery stocks were collected in September 2000.

Nonlethal fin clips were taken from juveniles (100–200 mm fork length) and stored in 95% ethanol. Samples were collected by fly-fishing for wild fish in July and August 2000, with few exceptions. Samples were taken from wild fish that were captured in rotary screw traps in the Pahsimeroi, Lemhi, SF Salmon (Knox Bridge), and Red rivers. We used electrofishing to collect samples from wild fish in Big Canyon (obtained in March 2001), Jacks, Mission, Little Bear and Valley creeks. Two temporally segregated collections were made in Fish Creek, first by fly-fishing in July about 5 km upstream of the mouth (summer; Table 2) and the second in September and October (fall) of the same year from a rotary screw trap located 1 km upstream of the mouth.

*Extraction and genotyping of DNA.*—We extracted DNA from fin clips using the Puregene DNA Isolation kit (Gentra Systems, Inc.). Products were amplified using the polymerase chain reaction (PCR). Microsatellite loci were selected from the published literature based on variability in *O. mykiss*, ease of PCR amplification, and a history of allele scoring rigor in our laboratory (Scribner et al. 1996; O'Connell et al. 1997; Olsen et al. 1998; Banks et al. 1999; Heath et al. 2001). Eleven microsatellite loci were used to assess genetic variation (Table 3). Several primers were redesigned for use in this study to optimize visualization and scoring of multiple loci on one gel: *Oneμ10* forward (F), 5'-TGTTGGCACCATTGTAACAG-3'; *Ogo4* (F), 5'-CAGAATCAGTAACGAACGC-3'; *Ogo4* reverse (R), 5'-GAGGATAGAAGAGTTTGGC-3'; and *Ots3* (R), 5'-CACAAATGGAAGACCAT-3'). *Ogola*, *Ogo4*, *Oneμ10*, and *Ots3* forward primers were modified by the addition of M13(R) tails, and *Oneμ8* and *Oneμ11* forward primers were modified with M13(F) tails. All M13 tails were added by the vendor to the 5' ends and allowed for allele fragment visualization by annealing to labeled complementary M13 tails added to the PCR

TABLE 2.—Diversity statistics for 79 Idaho *O. mykiss* populations, by drainage or hatchery source and watershed. *P*-values for populations significantly out of Hardy–Weinberg equilibrium (HWE) for all loci combined are indicated in bold; *P* = 0.0000 signifies *P* < 0.000016 in all cases. Abbreviations: SF = South Fork, MF = Middle Fork, EF = East Fork, N/A = not applicable, I = infinite, LDN<sub>e</sub> = linkage disequilibrium method, CI = confidence interval, H<sub>O</sub> = observed heterozygosity, H<sub>E</sub> = expected heterozygosity, N<sub>A</sub> = number of alleles, and A<sub>R</sub> = allelic richness.

Watershed and population	Code	Map	<i>N</i>	H <sub>E</sub>	H <sub>O</sub>	N <sub>A</sub>	A <sub>R</sub>	LDN <sub>e</sub>	N <sub>e</sub> 95% CI jackknife	<i>M</i>	HWE <i>P</i> -value
<b>Clearwater drainage</b>											
Lochsa River watershed											
Boulder Creek	BLDK	1	21	0.605	0.639	5.3	4.78	128.4	39.9–I	N/A	0.7916
Brushy Fork	BRUS	2	55	0.549	0.546	6.0	4.56	48.6	33.2–79.3	0.678	0.6759
Canyon Creek	CANY	3	53	0.594	0.588	6.3	5.10	58.0	40.0–94.7	0.680	0.2937
Colt Creek	COLT	4	54	0.586	0.554	6.1	4.59	29.7	22.1–41.6	0.632	0.1481
Crooked Fork Creek	CFCK	5	56	0.572	0.576	5.8	4.73	42.4	29.4–66.5	0.664	0.1695
Deadman Creek	DEAD	6	56	0.596	0.578	7.3	5.16	85.0	52.3–180.7	0.709	0.4306
Fish Creek, summer	FISH	7	56	0.614	0.594	7.2	5.20	226.1	83.7–I	0.669	<b>0.0002</b>
Fish Creek, fall	FSCT	8	56	0.583	0.565	6.5	4.86	–374.5	294.3–I	0.713	0.6136
Hungry Creek	HUNC	9	55	0.590	0.558	6.0	4.62	54.3	33.2–110.0	0.608	0.0241
Lake Creek	LAKE	10	56	0.571	0.592	5.5	4.43	122.0	56.6–1,899.9	0.596	0.1225
Papoose Creek	PAPO	11	41	0.566	0.548	6.5	4.96	14.8	10.8–20.5	0.628	<b>0.0007</b>
Storm Creek	STRM	12	54	0.569	0.598	5.8	4.46	93.3	47.5–406.4	0.646	0.5205
Warm Springs Creek	WARM	13	52	0.590	0.607	5.8	4.48	47.3	29.9–88.1	0.670	0.1399
Weir Creek	WEIR	14	49	0.608	0.634	6.5	4.97	55.8	37.1–97.4	0.631	0.1075
Lower Clearwater River watershed <sup>a</sup>											
Big Canyon Creek	BCAN	15	60	0.660	0.677	8.4	5.83	124.8	81.3–239.0	0.700	0.3717
EF Potlatch River	EPOT	16	51	0.641	0.664	6.9	5.15	77.0	49.7–146.3	0.668	0.8230
Jacks Creek	JACK	17	37	0.657	0.689	5.7	4.74	14.8	11.1–20.0	0.709	0.0227
Little Bear Creek	LBRC	18	55	0.652	0.662	6.6	5.03	15.5	12.5–19.4	0.585	<b>0.0000</b>
Mission Creek	MISS	19	49	0.632	0.622	7.4	5.44	59.3	42.8–89.9	0.643	0.2263
Clear Creek	CLRC	20	55	0.589	0.593	6.4	4.87	61.5	39.9–112.3	0.730	0.9283
NF Clearwater River watershed											
Collins Creek <sup>b</sup>	CNLC	21	50	0.579	0.611	6.5	4.87	190.2	80.7–I	0.712	0.1136
Selway River watershed											
Bear Creek	BEAR	25	41	0.603	0.614	6.1	4.90	72.7	38.7–260.0	0.603	<b>0.0007</b>
EF Moose Creek	EMOS	26	54	0.621	0.626	6.6	5.07	58.7	37.1–113.9	0.656	0.6504
Gedney Creek	GEDC	27	56	0.606	0.603	6.8	5.13	147.2	83.6–449.5	0.691	0.3439
Meadow Creek	MEDC	28	50	0.610	0.598	6.4	4.84	150.5	64.4–I	0.623	0.0991
Mink Creek	MINK	29	52	0.573	0.575	5.8	4.40	20.3	14.3–29.8	0.601	<b>0.0001</b>
NF Moose Creek	NFMO	30	52	0.608	0.583	6.5	4.98	37.8	27.0–56.6	0.669	0.0054
O'Hara Creek	OHAR	31	51	0.626	0.604	7.4	5.42	745.2	146.0–I	0.690	0.2920
Pettibone Creek	PETB	32	39	0.596	0.593	6.5	4.93	51.0	31.2–106.1	0.604	0.4451
Three Links Creek	3LNK	33	54	0.599	0.599	5.8	4.66	65.3	42.6–118.5	0.567	0.0054
SF Clearwater River watershed <sup>a</sup>											
Johns Creek	JOHN	22	45	0.604	0.566	7.3	5.53	167.3	82.0–2,000.8	0.713	0.0082
Tenmile Creek	MILE	23	49	0.601	0.617	6.0	4.73	47.7	31.6–82.9	0.757	0.2092
Red River	REDR	24	57	0.632	0.624	8.1	5.80	64.9	44.7–107.1	0.634	<b>0.0003</b>
<b>Salmon River drainage</b>											
Little Salmon River watershed <sup>a</sup>											
Boulder Creek	BOUL	67	51	0.651	0.606	8.1	5.94	95.8	53.1–298.6	0.715	0.3310
Hazard Creek	HAZC	68	51	0.671	0.663	9.4	6.26	79.2	49.8–160.8	0.791	0.0071
Little Salmon, New Meadows <sup>c</sup>	ILSR	69	42	0.654	0.638	6.5	5.40	77.2	46.4–182.1	0.640	0.3232
Little Salmon, Pinehurst	2LSR	70	51	0.670	0.628	8.8	6.22	279.3	87.8–I	0.791	<b>&lt;0.0000</b>
Rapid River	RAPR	71	51	0.636	0.626	6.8	5.30	108.3	55.8–525.2	0.658	0.0957
Main Salmon River watershed <sup>a</sup>											
Bargamin Creek	BAR	37	50	0.652	0.635	7.4	5.49	56.4	40.4–86.5	0.683	<b>&lt;0.0000</b>
Basin Creek	BASC	38	53	0.706	0.663	8.2	5.94	68.2	42.8–137.1	0.717	<b>&lt;0.0000</b>
Chamberlain Creek	HAM	39	46	0.644	0.615	7.5	5.34	371.0	63.2–I	0.706	0.1078
Horse Creek	HRSC	40	58	0.675	0.617	7.6	5.70	54.3	37.2–89.5	0.665	0.1499
Lemhi River	LEMRI	41	49	0.727	0.668	7.8	6.05	75.5	49.6–138.8	0.707	<b>0.0000</b>
Morgan Creek	MORG	42	48	0.688	0.660	8.4	6.05	78.8	51.6–146.5	0.734	0.1184
Owl Creek	OWLC	43	58	0.676	0.631	8.3	5.88	279.9	116.1–I	0.751	<b>0.0010</b>
Pahsimeroi River	PAHR	49	49	0.702	0.669	8.2	6.10	74.5	46.5–154.8	0.703	<b>&lt;0.0000</b>
Sheep Creek	EPEC	44	19	0.596	0.629	5.5	4.99	19.0	12.7–31.8	N/A	0.4685
Slate Creek	SLAT	45	55	0.662	0.612	8.1	5.85	42.0	30.0–63.0	0.779	0.0523
Valley Creek	VALC	46	49	0.696	0.657	7.4	5.64	38.9	29.3–54.2	0.678	0.3365
Warm Springs Creek	WSCK	47	45	0.689	0.656	7.5	5.68	48.8	36.3–70.2	0.682	<b>&lt;0.0000</b>
White Bird Creek	WHBC	48	56	0.653	0.648	7.5	5.41	55.3	36.1–98.5	0.699	<b>0.0011</b>
WF Yankee Fork	WFYK	50	55	0.675	0.656	7.0	5.51	31.2	24.0–41.9	0.660	0.0515
MF Salmon River watershed											
Bear Valley Creek	BVAC	58	55	0.553	0.552	5.5	4.28	41.6	28.9–65.2	0.626	0.0567

TABLE 2.—Continued.

Watershed and population	Code	Map	$N$	$H_E$	$H_O$	$N_A$	$A_R$	LDNe $N_e$	$N_e$ 95% CI jackknife	$M$	HWE $P$ -value
Big Creek, lower	1BIG	59	49	0.608	0.598	6.2	4.72	139.0	62.5–1	0.599	0.0071
Big Creek, upper	2BIG	60	46	0.564	0.553	5.2	4.18	28.6	19.4–45.8	0.557	0.0106
Camas Creek	CAM	61	50	0.608	0.569	6.4	4.83	135.2	68.8–794.4	0.630	0.0357
Loon Creek	LON	62	54	0.562	0.560	6.1	4.47	56.7	36.9–101.9	0.600	0.0314
Marsh Creek	MARC	63	59	0.584	0.588	5.6	4.23	43.7	29.8–70.6	0.589	0.0635
Pistol Creek	PIST	64	28	0.616	0.606	6.0	5.04	56.3	31.2–179.4	0.603	0.4441
Rapid River	RRDR	65	52	0.597	0.579	6.8	4.86	76.4	46.7–166.5	0.615	0.5792
Sulphur Creek	SULP	66	53	0.584	0.584	6.4	4.53	44.7	27.9–85.5	0.644	0.0091
SF Salmon River watershed											
EF SF Salmon River	EFSF	51	52	0.627	0.615	6.6	4.97	40.7	24.8–79.7	0.613	0.1338
Johnson Creek	JSON	52	56	0.635	0.638	6.8	5.15	228.1	80.9–1	0.667	0.0200
Lick Creek, lower	LLIK	53	52	0.616	0.578	6.8	5.14	83.4	44.9–278.5	0.680	0.1934
Lick Creek, upper <sup>c</sup>	2LIK	54	50	0.561	0.495	6.2	4.57	46.5	29.3–88.4	0.672	<0.0000
SF Salmon, Poverty Flat	POVF	55	55	0.614	0.604	6.9	5.05	48.1	31.9–82.4	0.678	0.0279
Secesh River	SECR	56	56	0.629	0.556	7.1	5.06	63.0	37.2–141.7	0.670	0.0000
SF Salmon, Knox Bridge	STOL	57	47	0.614	0.576	6.2	4.88	72.0	41.6–186.6	0.602	0.0301
Snake River drainage											
Snake River watershed <sup>a</sup>											
Captain John Creek	CAPJ	34	55	0.663	0.672	7.3	5.48	23.6	18.0–31.4	0.697	0.0000
Granite Creek	GRAN	35	49	0.681	0.677	7.9	5.87	57.2	39.4–93.9	0.749	0.2693
Sheep Creek	SHPC	36	47	0.680	0.616	7.6	5.67	67.2	45.6–114.6	0.691	0.0010
Big Smoky Creek <sup>b</sup>	BOIR	78	37	0.629	0.628	6.3	4.99	58.5	33.0–158.0	0.670	0.6987
MF Payette River <sup>b</sup>	PAYR	77	53	0.601	0.578	6.6	4.98	326.1	85.7–1	0.679	0.2929
Little Weiser River <sup>b</sup>	WEIS	79	51	0.658	0.657	7.3	5.60	4845.3	195.9–1	0.693	0.4050
Hatchery source											
Hatchery stocks											
Dworshak	DWOR	72	52	0.582	0.601	6.5	4.97	72.0	45.6–140.7	0.705	0.0646
EF Salmon B	EFRB	73	55	0.597	0.578	6.0	4.64	8.4	5.8–11.4	0.632	<0.0000
Oxbow	OXBW	74	53	0.676	0.629	7.3	5.33	126.3	70.8–398.3	0.703	0.1726
Sawtooth	SAWT	76	56	0.689	0.673	7.6	5.59	152.9	78.8–828.9	0.718	0.4137
Pahsimeroi	SIMH	75	53	0.692	0.653	7.7	5.82	211.0	80.1–1	0.684	<0.0000

<sup>a</sup> Watersheds managed with hatchery releases.

<sup>b</sup> Formerly anadromous populations blocked by impassible dams.

<sup>c</sup> Populations collected upstream of putative waterfall barriers.

mix. Remaining loci were visualized by adding directly labeled forward primers.

The PCR reactions were multiplexed into three groups: (1) *Omy325*, *Oneμ14*, *Ots1*, and *Ots4*; (2) *Ogo1a*, *Ogo4*, *Oneμ8*, and *Ots3*; and (3) *Omy27*, *Oneμ10*, and *Oneμ11*. Reactions were set up in 10–12-μL volumes using approximately 50 ng of genomic DNA, 0.06–0.1 units of Taq DNA polymerase (Promega), 10 mM tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, 200 μM each dNTP, 0.1–0.5 pmol unlabeled primers, 0.1–0.4 pmol directly labeled primers, and 0.5–1.5 pmol labeled M13 tails. The PCR reactions were carried out in MJ Research (BIORAD) or MWG thermocyclers (MWG Biotech, Inc.) with an initial denaturation time of 2 min at 94°C followed by 40 cycles at 94°C for 15 s, 52°C for 15 s, 72°C for 30 s, and a final 30-min elongation step at 72°C. The 30-min elongation step was not done for *Omy325*, *Oneμ14*, *Ots1*, and *Ots4*. Gel electrophoresis and visualization of alleles was performed using a LI-COR model IR2 automated fluorescent DNA sequenc-

er, and allele sizes were determined using GeneImagIR version 3.00 software (LI-COR). Microsatellite allele sizes were quantified in relation to the M13 single nucleotide ladder, *O. mykiss* DNA reference samples of known size, and (or) the GeneScan-350 internal size standard (Applied Biosystems). Approximately 10% of all samples were run on a second gel and scored independently for quality control.

**Statistical analyses.**—We used Microsatellite Toolkit (Park 2001) to compute basic descriptive statistics and create input files for subsequent analyses. We used FSTAT (Goudet 2001) to calculate allelic richness ( $A_R$ ) and global genetic differentiation ( $F_{ST}$ ) values and GENEPOP version 3.4 (Raymond and Rousset 1997) to calculate the observed ( $H_O$ ) and expected heterozygosity ( $H_E$ ) and to test for significant departures from Hardy–Weinberg (HW) equilibrium. A Bonferroni correction based on the number of loci ( $P = 0.05/11$ ; 0.0045) was applied to test the population-specific source of HW disequilibrium. Pairwise  $F_{ST}$  comparisons (θ; Weir and Cockerham 1984) were calculated for all population pairs using ARLEQUIN v.3.0

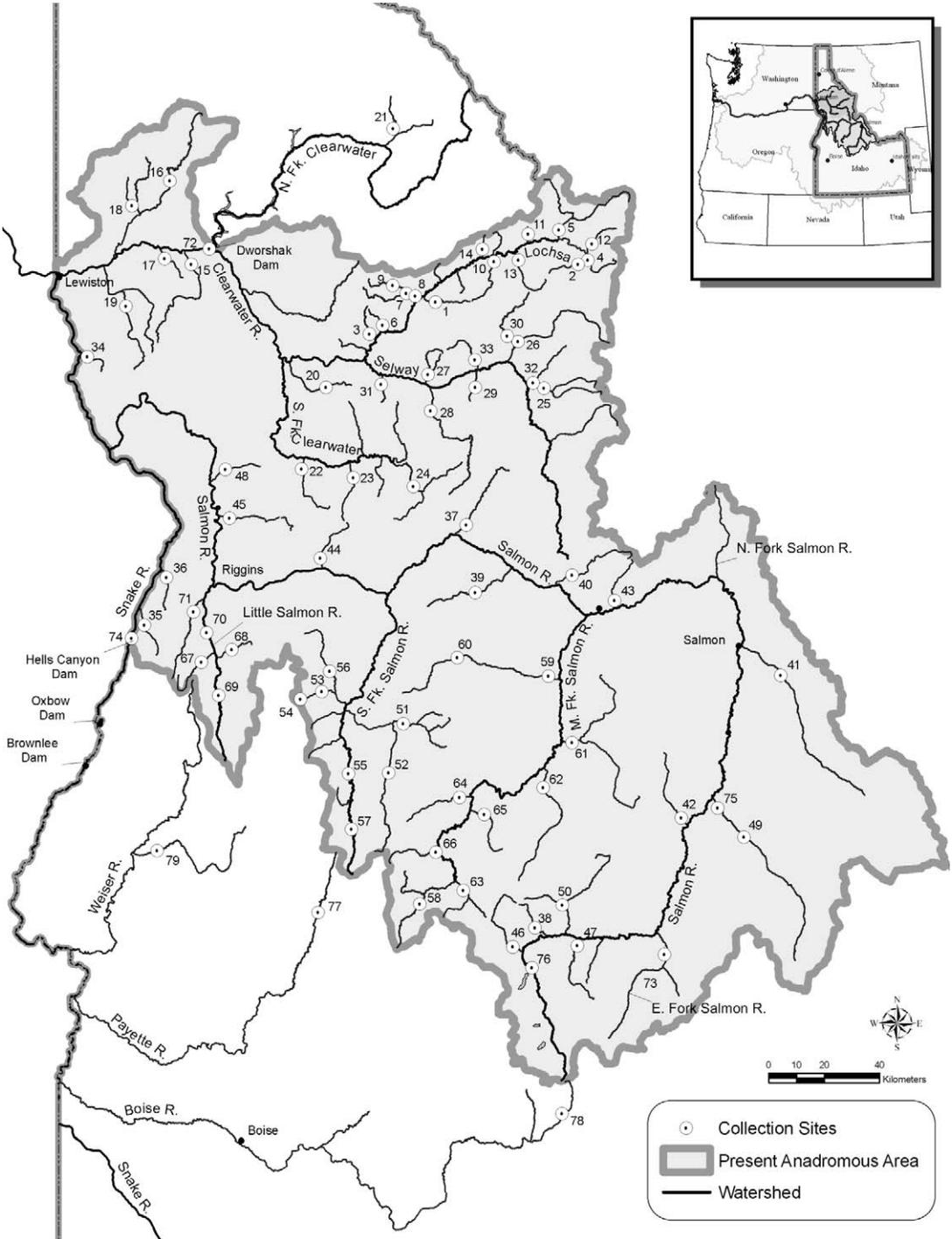


FIGURE 1.—Locations of the collection sites for *O. mykiss* sampled for genetic analysis. The location numbers correspond to the population given in Table 2.

TABLE 3.—The number of alleles ( $N_A$ ), allelic size range, and average observed heterozygosity ( $H_O$ ) for microsatellite loci used to evaluate 79 *O. mykiss* populations in Idaho.

Locus	Source	$N_A$	Allelic size range (bp)	Average $H_O$
<i>Ogola</i>	Olsen et al. (1998)	10	124–168	0.580
<i>Ogo4</i>	Olsen et al. (1998)	12	118–142	0.788
<i>Omy27</i>	Heath et al. (2001)	11	99–117	0.520
<i>Omy325</i>	O'Connell et al. (1997)	33	87–153	0.878
<i>Oneu8</i>	Scribner et al. (1996)	20	144–188	0.837
<i>Oneu10</i>	Scribner et al. (1996)	10	113–131	0.182
<i>Oneu11</i>	Scribner et al. (1996)	4	143–149	0.436
<i>Oneu14</i>	Scribner et al. (1996)	11	143–163	0.521
<i>Ots1</i>	Banks et al. (1999)	27	157–247	0.803
<i>Ots3</i>	Banks et al. (1999)	9	77–93	0.639
<i>Ots4</i>	Banks et al. (1999)	9	108–130	0.683

(Excoffier et al. 2005). The significance of individual pairwise  $F_{ST}$  comparisons was evaluated after applying a Bonferroni correction based on the number of tests ( $P = 0.05/3,081 = 0.000016$ ; Rice 1989).

We used AGARST (Harley 2001) to estimate  $M$ , the mean ratio of the number of alleles to the range of allele size across multiple loci. The published threshold of  $M < 0.68$  was used to infer whether populations had undergone recent reductions in population size (Garza and Williamson 2001). The appropriate application of  $M$  requires at least five polymorphic microsatellite loci, but the literature gives no specific selection criteria for polymorphism. We conservatively dropped 5 of our 11 loci from  $M$  analyses. Three loci (*Omy27*, *Ogola*, and *Ots1*) were removed because of the presence of single base pair variants that do not conform to the mutational model upon which this test is based. We removed two other loci (*Oneu10* and *Oneu11*) because the underlying allelic distribution did not conform to our expectations of polymorphism. *Oneu10* had 10 alleles, but one of the alleles dominated across all 79 populations, occurring at 91% or more of them. The other dropped locus (*Oneu11*) had four alleles. Two of the alleles contributed over 99% of the allelic frequency distribution across all populations (i.e., essentially a biallelic locus with very low polymorphism). Population sample sizes for calculations of  $M$  ranged from 28 to 60, exceeding the suggested size criteria ( $N \geq 25$ ; Garza and Williamson 2001), with two exceptions: Sheep Creek (Main Salmon,  $N = 19$ ) and Boulder Creek (Lochsa,  $N = 21$ ). Both were excluded from this analysis.

Effective population size ( $N_e$ ) was calculated using the linkage disequilibrium method implemented by LDNe version 1.31 (Waples and Do 2008). This method requires only one sample collection in contrast to the temporal method and incorporates a bias correction for sample sizes smaller than true  $N_e$  (Waples 2006). We reported  $N_e$  via the 0.02 threshold

frequency and jackknife 95% confidence intervals, as recommended in Waples and Do (2008).

The following statistics were also calculated at the drainage and watershed scales: observed heterozygosity ( $H_O$ ), global  $F_{ST}$ , pairwise  $F_{ST}$  comparisons,  $M$ , and  $N_e$ . Oxbow, Pahsimeroi and Sawtooth hatchery stocks share a common source population based on unpublished hatchery records (Table 1). Watershed-scale pairwise  $F_{ST}$  and neighbor-joining (NJ) analyses combined the Oxbow, Pahsimeroi and Sawtooth hatchery stocks as a single group (hatchery group) after preliminary analyses indicated no significant differences in allelic structure among these populations. The Dworshak and EFSB hatchery stocks were analyzed independently. A paired  $t$ -test was used to compare mean allelic richness ( $A_R$ ) and  $N_e$  between watersheds managed with hatchery releases and those managed for wild fish.

Genetic distance values reflecting the proportion of shared alleles among 79 populations were used to graphically depict genetic relationships and population structure. Because the common management unit for Idaho steelhead is based on watershed designations, this analysis was done at the population and watershed spatial scales. Software from PHYLIP (Felsenstein 2005) was used to create an unrooted NJ consensus tree. We used SEQBOOT to bootstrap allele frequencies 2,000 times and created distance matrices (based on Cavalli-Sforza and Edward's 1967 genetic chord distance) via the subprogram GENEDIST from PHYLIP. We generated 2,000 NJ trees using NEIGHBOR, and determined the consensus tree using CONSENSE. We used TreeView version 1.6.6 to visualize the consensus NJ trees with bootstrap values (Page 1996).

Microsatellite allelic variation was partitioned using the analysis of molecular variance (AMOVA) calculated by ARLEQUIN. The percent of variation found among the three drainages (Clearwater, Salmon and Snake), among populations within these drainages, and within populations in each drainage were assessed. An

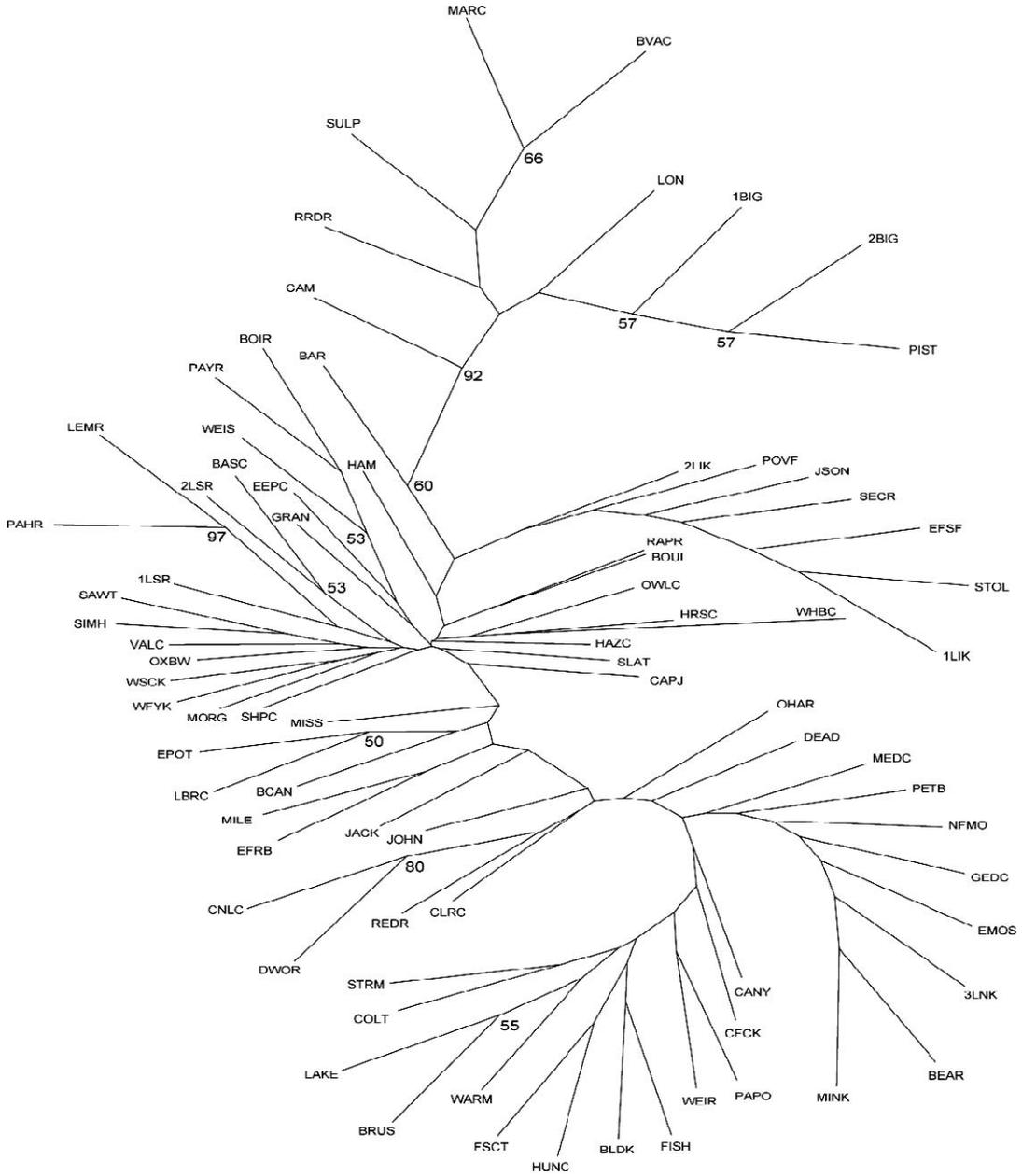


FIGURE 2.—Population-scale unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) genetic distance. Bootstrap values (percent of 2,000 trees) are given for each branch with 50% or more bootstrap support. Population codes are listed in Table 2.

AMOVA was also conducted within drainages independently to assess the proportion of variation among the watersheds, among populations within each watershed, and within populations. The AMOVA analysis of the Clearwater drainage excluded the NF Clearwater watershed because it contained only one population, Collins Creek. We also used AMOVA to

assess the allelic variation among the five hatchery stocks and a separate AMOVA analysis was done to evaluate the Oxbow, Pahsimeroi, and Sawtooth stocks (Hatchery Group). We assessed isolation by distance (IBD) via the Mantel test with 10,000 randomizations for statistical significance (Bohonak 2002) and by regressing  $F_{ST}$

TABLE 4.—Significant  $F_{ST}$  comparisons between Idaho steelhead population pairs involving hatchery stocks ( $P \geq 0.000016$ ).

Hatchery stock	Population (watershed)	$F_{ST}$	$P$ -value
Dworshak Oxbow	O'Hara Creek (Selway)	0.009	0.003
	Pahsimeroi Hatchery	0.002	0.165
	Sawtooth Hatchery	0.002	0.202
	Chamberlain Creek (Main Salmon)	0.010	0.005
	Granite Creek (Lower Snake)	0.010	0.004
	Hazard Creek (Little Salmon)	0.011	0.001
	Horse Creek (Main Salmon)	0.009	0.007
	Morgan Creek (Main Salmon)	0.008	0.007
	Owl Creek (Main Salmon)	0.008	0.005
	Sheep Creek (Lower Snake)	0.009	0.008
	Sheep Creek (Main Salmon)	0.027	0.002
	Slate Creek (Main Salmon)	0.017	0.001
	Valley Creek (Main Salmon)	0.000	0.449
	Warm Springs Creek (Main Salmon)	0.011	0.002
Pahsimeroi	Sawtooth Hatchery	-0.003	0.834
	Big Canyon Creek (Lower Clearwater)	0.007	0.003
	Boulder Creek (Little Salmon)	0.012	0.002
	Captain John Creek (Lower Snake)	0.005	0.022
	Chamberlain Creek (Main Salmon)	0.001	0.287
	Granite Creek (Lower Snake)	0.005	0.040
	Hazard Creek (Little Salmon)	0.005	0.027
	Horse Creek (Main Salmon)	0.004	0.096
	Jacks Creek (Lower Clearwater)	0.014	0.001
	Morgan Creek (Main Salmon)	0.006	0.033
	Owl Creek (Main Salmon)	0.000	0.574
	Sheep Creek (Lower Snake)	0.001	0.310
	Valley Creek (Main Salmon)	-0.001	0.556
	Warm Springs Creek (Main Salmon)	0.007	0.021
Sawtooth	Hazard Creek (Little Salmon)	0.009	0.003
	Horse Creek (Main Salmon)	0.004	0.088
	Morgan Creek (Main Salmon)	0.009	0.007
	Owl Creek (Main Salmon)	0.007	0.014
	Sheep Creek (Lower Snake)	0.007	0.026
	Sheep Creek (Main Salmon)	0.018	0.003
	Valley Creek (Main Salmon)	0.002	0.277

distance and geographic distance on the population and drainage scales. We calculated the fluvial distance between streams from the beginning of the downstream sampling boundary in each stream by using ARCMAP 9.1 software (ESRI, Redlands, California). We assessed IBD for all 74 wild populations: the 35 wild populations in the Salmon drainage, 33 wild populations in the Clearwater drainage, and the 6 wild populations in the Snake drainage.

*A and B run life history strategies.*—Pairwise  $F_{ST}$  comparisons and AMOVA analyses were used to assess genetic differentiation between A and B run life history strategies for the 68 anadromous populations. Using pairwise  $F_{ST}$  and AMOVA, we also assessed genetic differentiation between A and B run life history strategies at the drainage scale in the Salmon and Clearwater drainages, where both runs occur.

## Results

### Hatchery and Wild Population Structure

The average  $H_O$  for all 79 populations was 0.611. The mean number of alleles by population was 6.8. Mean allelic richness ( $A_R$ ) for all populations was 5.15.

Twenty populations were significantly out of HW equilibrium for all loci combined (Table 2). The global  $F_{ST}$  was 0.051 for the 74 wild steelhead populations and 0.038 for the 5 hatchery stocks. Pairwise  $F_{ST}$  analysis among the 79 populations showed that 236 of the 3,081 comparisons (7.7%) were not significantly different; 97 nonsignificant pairs were within watersheds and 104 were between watersheds ( $F_{ST}$  data for all nonsignificant pairs available on request). Isolation by distance for all 74 wild populations in the Snake River basin was significant ( $r = 0.49$ ,  $P \leq 0.001$ ).

For 78 populations, we obtained positive values of  $N_e$  that ranged from 8.4 (EFSB stock) to 4,845.3 (Little Weiser River). The  $N_e$  for the Fish Creek fall collection was negative ( $N_e = -374.5$ ). Upper confidence intervals (CIs) around  $N_e$  estimates reached infinity for 14 populations; excluding all populations with an upper confidence level of infinity, the median  $N_e = 57.2$ .

The strongest bootstrap support (97%) in our population-scale NJ consensus tree clustered the Lemhi and Pahsimeroi rivers (Figure 2). This tree also grouped populations from the MF Salmon River with

92% bootstrap support. The Dworshak stock was paired with Collins Creek in this tree with 80% bootstrap support. These were the only strongly supported relationships inferred from our population-scale NJ tree. Other inferred relationships were weakly supported (<80% bootstrap support). Population substructuring within the MF Salmon watershed was apparent in our NJ tree. There was weak support (44%) for the separation of samples collected from the upper and lower subbasins of the MF Salmon River watershed, based on a previously published geographic delineation used for a genetic analysis of spring Chinook salmon *O. tshawytscha* (Neville et al. 2007). The branch containing Marsh and Bear Valley creeks in the upper subbasin was supported with a 66% bootstrap value. There was 57% bootstrap support for the branch containing Pistol Creek, Big Creek lower and Big Creek upper from the lower subbasin. The SF Salmon River populations grouped with 45% bootstrap support. The only branch with bootstrap support over 50% for Lochsa populations was the branch containing Brushy Fork and Lake creeks (55%). Boulder Creek and Rapid River were the only Little Salmon populations that grouped together in the consensus NJ tree (50% bootstrap support). Main Salmon, Snake, and Little Salmon populations did not form independent groups in this tree. The SF Clearwater and Lower Clearwater populations did not form independent groups but loosely grouped with each other and the EFSB stock, Dworshak stock, and Collins Creek.

#### Population-Scale Relationships

*Hatchery stocks and wild populations.*—The Oxbow, Pahsimeroi, and Sawtooth hatchery stocks were not significantly different from each other ( $F_{ST}$  range = -0.003 to 0.002,  $P \geq 0.165$ ). Of the 370 (8.6%) pairwise  $F_{ST}$  comparisons involving hatchery stocks and wild populations, 32 were not significantly different. The majority ( $N = 29$ ) of the nonsignificant pairwise  $F_{ST}$  comparisons were between the Oxbow, Pahsimeroi, or Sawtooth stocks and wild populations from the Main Salmon, Lower Snake, or Little Salmon watersheds (Table 4). Two lower Clearwater populations (Big Canyon and Jacks creeks) were not significantly different from the Pahsimeroi stock. The Dworshak stock was significantly different from all other hatchery and wild populations, except O'Hara Creek in the Selway watershed (pairwise  $F_{ST} = 0.009$ ,  $P = 0.003$ ). The EFSB stock was significantly different from all other hatchery and wild populations based on pairwise  $F_{ST}$  comparisons, and it had the lowest  $N_e$  estimate (8.4) among all sample populations (the average for all hatchery stocks was  $N_e = 114.1$ ).

*Nonanadromous and anadromous populations.*—Nonanadromous populations upstream of the Hells Canyon Dam complex in the Upper Snake watershed (Big Smoky Creek, Little Weiser River and MF Payette River) were significantly different from all other populations in this study based on pairwise  $F_{ST}$  comparisons, except for the Little Weiser River population, which was not significantly different from the anadromous population in Sheep Creek of the Lower Snake watershed (pairwise  $F_{ST} = 0.011$ ,  $P = 0.001$ ). The NJ results supported a cluster containing these nonanadromous populations in 53% of the trees (Figure 2). Three formally anadromous populations blocked by impassable dams had some of the highest  $N_e$  estimates: Collins Creek in the North Fork Clearwater River ( $N_e = 190.2$ ), Middle Fork Payette River ( $N_e = 326.1$ ), and Little Weiser River ( $N_e = 4,845.3$ ). All three of these formally anadromous populations had upper confidence limits of  $N_e$  equal to infinity. In general, diversity statistics for formally anadromous populations upstream of the Hells Canyon Dam complex were lower than populations downstream of the dams. The exception, Little Weiser River, had a higher  $H_O$  than Sheep Creek (Lower Snake River) and was the only nonanadromous population in the Snake drainage with  $M > 0.68$  (Table 2). In the Clearwater drainage, Collins Creek was significantly different from all other wild and hatchery steelhead populations, based on  $F_{ST}$  pairwise comparisons. Nonsignificant genetic differentiation between Collins Creek and the Dworshak stock (pairwise  $F_{ST} = 0.015$ ,  $P = 0.001$ ) and our population-scale NJ tree (80% bootstrap support) both inferred a close relationship between these populations.

The nonanadromous wild population found upstream of a waterfall barrier on Lick Creek (SF Salmon watershed) was significantly different from the population sampled downstream of this barrier (pairwise  $F_{ST} = 0.051$ ,  $P = 0.000$ ). Upper Lick Creek had lower genetic diversity statistics ( $H_O$ ,  $H_E$ ,  $N_A$ ,  $A_R$ , and  $N_e$ ) than did the downstream population, and it was predicted to have undergone a recent population decline ( $M = 0.672$ ). This population was out of HW equilibrium for all loci combined because of a heterozygote deficit ( $F_{IS} = 0.119$ ).

The population upstream of a waterfall on the Little Salmon River was also significantly different from the downstream population (pairwise  $F_{ST} = 0.035$ ;  $P = 0.000$ ). Heterozygosity and  $N_e$  estimates were similar between these two populations, but the downstream population had a higher number of alleles and allelic richness than the upstream population (Table 2). The upstream population was predicted to have undergone a recent reduction in population size ( $M = 0.64$ ). The downstream population carried the highest  $M$  value in

TABLE 5.—Pairwise  $F_{ST}$  values among 10 watersheds and hatchery stocks in Idaho. Fish from the Oxbow, Pahsimeroi and Sawtooth hatcheries were analyzed as a single group (hatchery group); Dworshak and EF Salmon B run hatchery stocks were independently analyzed. All comparisons were significantly different ( $P < 0.0001$  in all cases).

Watershed or hatchery	Dworshak stock	EF Salmon B run	Hatchery group	Little Salmon	Lochsa	Lower Clearwater	Lower Snake	Main Salmon	MF Salmon	Selway	SF Clearwater	SF Salmon
EF Salmon B run	0.076											
Hatchery Group	0.047	0.043										
Little Salmon	0.037	0.050	0.015									
Lochsa	0.035	0.046	0.044	0.049								
Lower Clearwater	0.028	0.028	0.015	0.015	0.025							
Lower Snake	0.041	0.042	0.006	0.011	0.039	0.010						
Main Salmon	0.041	0.041	0.003	0.009	0.038	0.013	0.005					
MF Salmon	0.053	0.078	0.049	0.030	0.057	0.039	0.034	0.035				
Selway	0.035	0.041	0.034	0.037	0.010	0.015	0.025	0.027	0.051			
SF Clearwater	0.012	0.054	0.027	0.023	0.020	0.013	0.023	0.022	0.049	0.020		
SF Salmon	0.051	0.061	0.031	0.022	0.044	0.022	0.020	0.022	0.024	0.031	0.039	
Upper Snake	0.057	0.061	0.027	0.021	0.060	0.025	0.018	0.027	0.045	0.045	0.045	0.031

this study ( $M = 0.791$ ). The downstream population was out of HW equilibrium for all loci combined because of a heterozygote deficit ( $F_{IS} = 0.063$ ).

*Spatially separated samples from the same stream.*—The populations in Big Creek (upper and lower), separated by about 50 km, were not significantly different (pairwise  $F_{ST} = 0.012$ ,  $P = 0.004$ ). Big Creek (lower) had a higher  $N_e$  estimate and diversity statistics than the upper site, but the lower Big Creek sample was out of HW equilibrium for all loci combined because of a heterozygote deficit ( $F_{IS} = 0.016$ ). Recent population declines were predicted for both Big Creek upper ( $M = 0.557$ ) and Big Creek lower ( $M = 0.599$ ). Two collections separated by about 30 km in the SF Salmon watershed (Poverty Flat and Knox Bridge) were significantly different ( $F_{ST} = 0.019$ ,  $P = 0.000$ ). These populations shared similar diversity statistics. Both had relatively low  $N_e$  estimates (48 and 72, respectively) and were predicted to have undergone recent declines in population size based on  $M$ .

*Temporally separated samples from the same stream.*—No significant allelic frequency differences were found between temporally separated samples taken from Fish Creek in the summer (56 in July) and fall (56 in October;  $F_{ST} = 0.001$ ,  $P = 0.325$ ). The summer collection had higher diversity statistics and  $N_e$  estimates than the fall collection, and the summer collection was out of HW for all loci combined because of a heterozygote deficit ( $F_{IS} = 0.034$ ). A July sample of 55 fish was collected in Hungery Creek, a tributary of Fish Creek on the Lochsa River; pairwise  $F_{ST}$  analysis indicated no significant difference between Hungery Creek and the Fish Creek summer collection ( $F_{ST} = 0.000$ ,  $P = 0.573$ ). The two summer collections were combined ( $N = 111$ ) in a subsequent analysis to further assess temporal differences within Fish Creek.

There was no significant allelic frequency differences for temporal samples from Fish Creek when the summer collections from Hungery Creek and Fish Creek were combined and compared with the Fish Creek fall collection ( $F_{ST} = -0.0003$ ,  $P = 0.534$ ).

#### Watershed-Scale Results

All pairwise  $F_{ST}$  comparisons between watersheds and hatchery stocks were significantly different (Table 5). Watershed  $N_e$  estimates ranged from 81.2 (SF Clearwater River) to 610.4 (Lochsa River; Table 6). For watersheds accessible to anadromous migrations, the median  $N_e$  was 373.2 for those managed for wild fish and was 160.3 for those managed with hatchery fish. The two lowest global  $F_{ST}$  values on the watershed scale were 0.011 for the Lower Snake and 0.018 for the Lochsa watersheds. High global  $F_{ST}$  values were calculated for three watersheds: Upper Snake (0.042), SF Clearwater (0.039), and Main Salmon (0.038). However, excluding the highly differentiated Lemhi and Pahsimeroi populations from the Main Salmon watershed reduced the global  $F_{ST}$  to 0.018. The AMOVA analysis attributed the largest proportion of allelic diversity to variation found within populations for all watersheds ( $\geq 95.8\%$ ; Table 6).

The NJ analysis grouped watersheds managed for wild steelhead with strong bootstrap support: 98% for the cluster containing the MF Salmon and SF Salmon watersheds and 99% for the Lochsa and Selway watersheds (Figure 3). There was strong bootstrap support (97%) for the branch leading to the cluster containing all Clearwater drainage watersheds, including the Dworshak and EFSB stocks. The Oxbow, Pahsimeroi and Sawtooth stocks analyzed as a single group (hatchery group) clustered with the Main Salmon watershed in 87% of the NJ trees.

*Watersheds managed for wild fish or hatchery*

TABLE 6.—Drainage- and watershed-scale descriptive statistics for Idaho steelhead (see Table 2 for abbreviations). Life history designations (in parentheses) include A run (A) and B run (B) fish. No data was reported for the NF Clearwater watershed because it contained only one population (Collins Creek).

Population	$H_O$	Global $F_{ST}$	LDNe $N_e$	$N_e$ 95% CI jackknife	Percent of populations with $M < 0.68$	
<b>Drainage</b>						
Clearwater	0.603	0.034	486.6	404.2	590.9	66
Salmon	0.612	0.046	213.0	162.8	277.4	59
Snake	0.639	0.032	240.3	182.8	332.7	33
<b>Watershed</b>						
Managed with hatchery releases						
Little Salmon River (A)	0.631	0.025	227.3	160.9	354.4	40
Lower Clearwater River (A) <sup>a</sup>	0.650	0.028	142.4	113.2	183.4	50
Main Salmon River (A)	0.644	0.038	160.3	127.6	203.1	23
SF Clearwater River (B)	0.604	0.039	81.2	64.5	105.2	33
Lower Snake River (A)	0.657	0.011	110.6	76.5	175.1	0
Managed for wild steelhead						
Lochsa River (B)	0.582	0.018	610.4	426.9	963.3	77
MF Salmon River (B)	0.576	0.025	438.9	328.3	627.8	100
Selway River (B)	0.600	0.029	307.4	221.5	457.8	78
SF Salmon River (B)	0.582	0.027	201.7	142.4	305.8	86
Upper Snake River <sup>b</sup>	0.620	0.042	274.8	170.0	604.1	67

<sup>a</sup> All populations are A run, except Clear Creek.  
<sup>b</sup> The Upper Snake River is not accessible to anadromous fish.

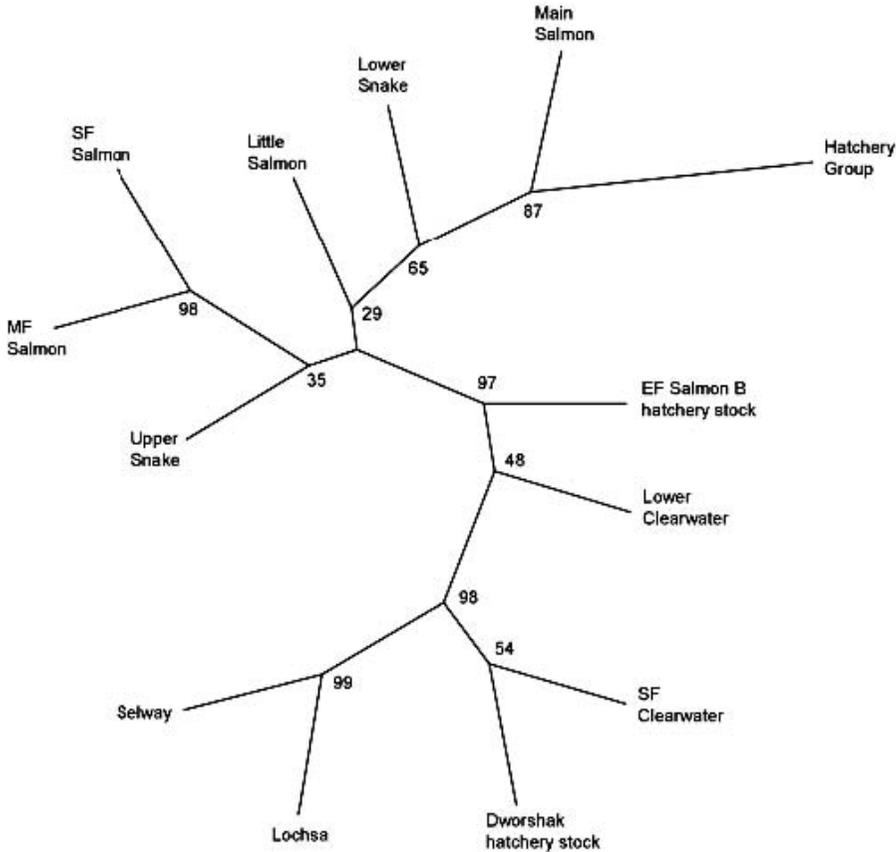


FIGURE 3.—Watershed-scale unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) genetic distance. Bootstrap values (percent of 2,000 trees) are given for each branch. The hatchery group includes fish from the Oxbow, Pahsimeroi, and Sawtooth hatcheries.

TABLE 7.—Results of analysis of molecular variance (AMOVA) of genetic diversity in Idaho steelhead populations.

Population(s)	Percentage of variation				
	Number of		Among groups	Among populations	
	Groups	Populations		Within groups	Within populations
Clearwater, Salmon, Snake drainages	3	74	1.97	3.66	94.37
Clearwater drainage <sup>a</sup>	4	32	1.39	2.11	96.50
Lochsa watershed	1	14		2.53	97.47
Lower Clearwater watershed	1	6		1.66	98.34
Selway watershed	1	9		2.33	97.67
SF Clearwater watershed	1	3		3.48	96.52
Salmon drainage	4	35	2.14	2.76	95.10
Little Salmon watershed	1	5		2.35	97.65
Main Salmon watershed	1	14		3.45	96.55
MF Salmon watershed	1	9		2.12	97.88
SF Salmon watershed	1	7		2.56	97.44
Snake drainage	2	6	1.03	2.43	96.55
Lower Snake watershed	1	3		0.96	99.04
Upper Snake watershed	1	3		4.17	95.83
Hatchery group <sup>b</sup>	1	3		0.05	99.95

<sup>a</sup> Excludes Collins Creek (NF Clearwater).

<sup>b</sup> Includes only Oxbow, Pahsimeroi, and Sawtooth hatcheries.

releases.—Watersheds managed for wild fish were genetically distinct from watersheds managed with hatchery releases, based on pairwise  $F_{ST}$  analysis (Table 5). Mean allelic richness for watersheds managed for wild fish ( $A_R = 8.38$ ,  $SD = 0.28$ ) was statistically lower than watersheds managed with hatchery releases ( $A_R = 9.76$ ,  $SD = 0.44$ ;  $t = 3.15$ ,  $df = 4$ ,  $P = 0.034$ ). Mean  $N_e$  for watersheds managed for wild fish ( $N_e = 366.6$ ,  $SD = 161.1$ ) was statistically higher than for those managed with hatchery releases ( $N_e = 144.4$ ,  $SD = 55.4$ ;  $t = 4.41$ ,  $df = 4$ ,  $P = 0.012$ ). The percentage of populations falling below the threshold of  $M < 0.68$  was greater for watersheds managed for wild fish (81.6%) than for watersheds managed with hatchery releases (29.2%;  $t = 10.9$ ,  $df = 4$ ,  $P = 0.0004$ ). Two branches containing watersheds managed for wild fish were highly supported in our watershed-scale NJ tree: MF Salmon and SF Salmon (98%) and Lochsa and Selway (99%; Figure 3).

*A run and B run life history strategies.*—Of the 68 anadromous steelhead populations in this study, 26 were considered as A run and 42 as B run populations, based on Idaho Department of Fish and Game management designations. Pairwise  $F_{ST}$  comparisons between all A and B run anadromous populations combined were significantly different ( $F_{ST} = 0.015$ ;  $P = 0.000$ ). Our AMOVA analysis of the 68 anadromous populations partitioned 1.33% of the variance between life histories. Pairwise  $F_{ST}$  comparisons of all watersheds containing A run steelhead (Little Salmon, Main Salmon, Lower Snake, and Lower Clearwater) were significantly different from watersheds with B run life histories (Lochsa, Selway, MF Salmon, SF Salmon and

SF Clearwater; Table 5). In general, watersheds with A run life histories exhibited greater genetic diversity ( $H_o$ ) than watersheds containing B run steelhead life histories, but median  $N_e$  was higher in B run watersheds ( $N_e = 307.4$ ) than in A run watersheds (median  $N_e = 193.8$ ; Table 6). Within the Salmon River drainage, the pairwise  $F_{ST}$  comparison between A run (Main Salmon and Little Salmon) and B run (MF Salmon and SF Salmon) steelhead were significantly different ( $F_{ST} = 0.021$ ;  $P = 0.000$ ). Our AMOVA partitioned 1.88% of the variance between life history types within the Salmon River drainage. Within the Clearwater drainage, the pairwise  $F_{ST}$  comparison between A run (Lower Clearwater) and B run (Lochsa, Selway, SF Clearwater and Clear Creek) fish was also significantly different ( $F_{ST} = 0.021$ ,  $P = 0.000$ ). The AMOVA partitioned 1.81% of the variance between life history types within the Clearwater River drainage.

#### Drainage-Scale Results

Our  $F_{ST}$  pairwise comparisons between all drainages were significantly different: Salmon–Clearwater pairwise  $F_{ST} = 0.022$ ; Clearwater–Snake pairwise  $F_{ST} = 0.027$ ; and Snake–Salmon pairwise  $F_{ST} = 0.010$  ( $P = 0.000$  in all cases). The AMOVA analysis of the three drainages indicated that diversity was greater among watersheds within individual drainages (3.66%) than among drainages (1.97%; Table 7). Mantel tests showed significant isolation by distance within each drainage: Clearwater ( $r = 0.51$ ,  $P = 0.0001$ ); Snake ( $r = 0.81$ ,  $P = 0.0017$ ); and Salmon ( $r = 0.30$ ,  $P = 0.0023$ ). Estimated  $N_e$  for the Clearwater drainage ( $N_e = 485.8$ ) was more than double that obtained for the Salmon

drainage ( $N_e = 213$ ) and the Snake drainage ( $N_e = 240.3$ ).

### Discussion

Interpreting results on different spatial scales is a critical concept in conservation (Ray 2001) and defining spatial units at which to administer conservation and recovery efforts is challenging, especially in a basin as complex as the Snake River, Idaho. In this study, we assumed each population of fish sampled in a stream or river reflected genetic diversity of *O. mykiss* at the finest scale. We analyzed genetic diversity from the population scale through a natural geomorphic hierarchy up to the basin level. Utter (2001, 2004) discussed genetic population structure in anadromous salmonids as hierarchical, low genetic exchange occurring at higher levels (e.g., groups of populations occupying drainages and watersheds) and increased exchange and diversity at lower levels (e.g., populations occupying tributaries or streams). This is generally based upon the interplay between homing and straying in salmonids (Quinn 1993; Hendry et al. 2004). Life history, geomorphology, and geologic processes can also affect genetic structure and isolation-by-distance patterns (Beacham et al. 1999; Narum et al. 2004b; Papa et al. 2007). Several genetic studies of *O. mykiss* in the Columbia River basin have previously investigated fine-scale population genetic structure (Beacham et al. 1999; Beacham et al. 2004; Winans et al. 2004; Narum et al. 2006a). To our knowledge, this is the first study to document significant genetic diversity in this species across a broad geographic area and at multiple scales.

Our AMOVA results are similar to those reported for British Columbia steelhead populations (Heath et al. 2001). However, a different trend in genetic diversity was found in two other studies—steelhead from Oregon and northern California (Reisenbichler et al. 1992) and steelhead from British Columbia and the Columbia River (Beacham et al. 2004)—that detected more variation among regions than among populations within regions. This discrepancy may result from a lack of standardization on geographic scales and the various ways drainages or regions were defined in different studies. This highlights the importance of spatial scale in evaluations and comparisons of population genetic structure. Our estimated global  $F_{ST}$  of 0.051 for all 74 wild populations of Idaho steelhead falls between two previously published estimates for steelhead: 0.038 (Heath et al. 2001) and 0.066 (Beacham et al. 2004). However, this result could reflect unique allelic variation based on different microsatellite loci, as well as differences in the spatial scales applied in each study. Population pairwise  $F_{ST}$  estimates were similar

across studies and indicated that steelhead genetic diversity was mainly partitioned at the finest scale (see also Hendry et al. 2002).

At the population level, pairwise  $F_{ST}$  estimates demonstrated a finer-scale population structure. Only 201 (7.4%) of the 2,701 population pairwise  $F_{ST}$  estimates among wild populations were nonsignificant. The percentage of pairwise  $F_{ST}$  estimates that were not significantly different was larger for comparisons of populations within the same watershed (97 of 318 or 30.5%) than comparisons of populations from different watersheds (104 of 2,383 or 4.4%). Our population-level  $F_{ST}$  results are similar to previously reported levels of genetic differentiation for other steelhead populations (Hendry et al. 2002; Pearse et al. 2007), supporting the inference that steelhead populations in the Snake River basin are genetically distinct.

Estimates of effective population size can be used to infer reductions in contemporary gene flow due to habitat fragmentations and patterns of persistence in managed populations (Palstra and Ruzzante 2008). The LDNe estimates of effective size for Idaho steelhead populations seem biologically reasonable in most cases. Several of the  $N_e$  estimates lacked strict confidence intervals. Waples and Do (2008) suggested that negative or infinite confidence interval of  $N_e$  can occur when the empirical  $r^2$  is less than expected  $r^2$  because of sampling error or when unbiased estimators of  $F_{ST}$  or genetic distance exist (i.e., no evidence of disequilibrium based on genetic drift). It is difficult with our current genetic data to differentiate among these possibilities for the steelhead populations falling outside of strict confidence interval values for  $N_e$ . Steelhead  $N_e/N$  ratios ranging from 0.17 to 0.53 have been reported (Ardren and Kapuscinski 2003; Araki et al. 2007). Using these ratio estimates and our LDNe estimates (excluding populations where  $N_e$  was negative and or had an upper confidence limit = infinity), individual Idaho steelhead populations would range in size from 16 to 316 ( $N_e/N = 0.53$ ) or 49–884 ( $N_e/N = 0.17$ ). Actual population numbers probably fall somewhere in between. The lowest positive  $N_e$  estimate was found in the EFSB hatchery stock ( $N_e = 8.4$ ). Personal observations in 2000–2002 of the number of fish spawned in this population ranged from 21 to 50 and sex ratios were not equal (C. Kozfkay, unpublished data). So a low  $N_e$  estimates for this population was not unexpected.

Using genetic data to infer fundamental management units at different geographic scales has been a topic of discussion in conservation and management (Neville et al. 2006; Narum et al. 2008). Idaho's Snake River steelhead are managed by watershed exclusively for wild fish or with hatchery additions and wild fish.

Managing demographic groups at the watershed level follows an ecological paradigm (Waples and Gaggiotti 2006). We acknowledge that combining stream populations at this scale may be in conflict with the evolutionary paradigm of reproductive cohesion. Genetic tests at the watershed scale are intended to view how operational management has affected the diversity of genetics across this landscape and are not intended to infer unique evolutionary structure at the watershed level. However, all watershed units in this study met HW equilibrium and were significantly differentiated from each other in pairwise  $F_{ST}$  estimates (range, 0.009–0.057). The Lochsa, Selway, MF Salmon, SF Salmon watersheds, which are all managed for wild fish, exhibited high levels of genetic differentiation, the MF Salmon watershed displaying the highest pairwise  $F_{ST}$  values compared with the other watersheds ( $F_{ST} \geq 0.024$  in all cases). Watershed management decisions related exclusively to wild populations of Snake River steelhead appear to be maintaining unique genetic diversity.

In our watershed NJ tree, all of the watersheds clustered according to drainage location, except for the Lower and Upper Snake watersheds. In the population NJ tree, most populations clustered according to watershed location. Steelhead populations from the Lochsa, Selway, MF Salmon, and SF Salmon watersheds formed cohesive clusters in our population NJ analysis (Figure 2), but only the branch leading to the MF Salmon populations was supported by a high bootstrap value. Populations from the Main Salmon, Lower Snake, Little Salmon, Lower Clearwater, and SF Clearwater watersheds weakly aggregated but did not form distinct clusters. Our NJ and  $F_{ST}$  analyses support Utter's (2004) premise of hierarchical genetic structure across different scales. However, Utter's hierarchical model would predict greater divergence among drainages than within them, whereas our AMOVA results indicated greater variation among watersheds within drainages than among drainages, supporting genetic diversity organized on hydrogeographic scales (Busby et al. 1996; Scribner et al. 1996; Beacham et al. 1999; Winans et al. 2004).

Natural and artificial barriers such as waterfalls and dams can lead to increased genetic differentiation and isolation and can affect geographical patterns of genetic structure (Castric et al. 2001; Manel et al. 2003; Taylor et al. 2003). It is expected that impassable waterfalls form isolated populations of *O. mykiss* with subsequent demographic factors that can impact genetic diversity (Currens et al. 1990; Beacham et al. 1999; Deiner et al. 2007). Our data support significant differentiation between sites upstream and downstream of natural migration barriers. Two sites with impassible waterfalls

(Lick Creek and the Little Salmon River) showed genetic differentiation between collections taken upstream and downstream of barriers. This suggests that upstream populations are isolated, have low population size (or low reproductive success), and are possibly less temporally stable. The upstream Lick Creek population was out of HW equilibrium, suggesting that isolation may have had an impact on population genetic structure. Even though HW equilibrium was supported in the upper Little Salmon population, genetic diversity estimates were still lower than its respective downstream population. One possible explanation could be that contemporary population size is not large enough to counter losses of genetic diversity associated with isolation in the upper Little Salmon River. A similar pattern was observed for the populations upstream of artificial barriers.

Declining genetic diversity upstream of barriers has been documented in many previous publications (Currens et al. 1990; Costello et al. 2003; Taylor et al. 2003; Wofford et al. 2005; Narum et al. 2006b). Four formerly anadromous populations upstream of dams (Big Smoky Creek, MF Payette River, Little Weiser River, and Collins Creek) were genetically differentiated from geographically proximate anadromous populations sampled downstream of the dams. Measures of genetic diversity ( $A_R$  and  $N_A$ ) were low in populations upstream of dams and recent population declines were predicted for three of these populations. Little Weiser River, however, had  $M$  above the threshold for recent declines and also had the highest  $N_e$  estimate of all 79 populations. Geographic separation in these populations is great, and the genetic structure reflects an isolation-by-distance pattern that confounds the impact of the dams on gene flow in different drainages. The SF Salmon collections were differentiated over a smaller distance and lacked significant barriers to gene flow. Patterns reflecting lower genetic diversity upstream of natural waterfalls on a smaller scale supported theoretical predictions of eroded diversity due to drift in isolated populations, whether from the isolation itself, small  $N_e$ , or a combination of such factors. The diversity of these results emphasize current management practices that foster the preservation of remaining genetic diversity on multiple scales and demonstrate the need to develop different approaches to address the effects of isolation best able to benefit populations, watersheds, and drainages.

The A and B run life history types are generally considered phenotypic differences, but this has not been supported with genetic data (Brannon et al. 2004). Although we found a significant difference in the pairwise  $F_{ST}$  comparison of all A run and B run

populations, AMOVA analysis partitioned only 1.33% of the variance between life history types. Watersheds managed for wild B run steelhead (MF and SF Salmon and Lochsa and Selway) clustered with high bootstrap support within their respective drainages. However, these populations did not form a cohesive B run group at the watershed scale. The watershed-scale NJ tree did not group all A run watersheds together, suggesting both types of steelhead group genetically according to drainage locality, not life history type. Furthermore, all watersheds were significantly different based on  $F_{ST}$  pairwise analysis, irrespective of life history type (Table 6). These findings suggest that steelhead genetic diversity in Idaho's Snake River Basin is distributed more geographically than by this run designation. However, considering our significant  $F_{ST}$  values, we cannot rule out the possibility that these anadromous life histories have evolved independently multiple times within different watersheds, similar to run-timing diversity found in other salmonid life histories (Waples et al. 2001; Waples and Gaggiotti 2006). New Bayesian algorithms allow integration of allelic frequency data with nongenetic data, such as environmental or climatic parameters, that may shed light on factors contributing to life history diversity and metapopulation structure (Hanski and Gaggiotti 2004). Biologically meaningful life history differences may not be reflected genetically in the same way as drainage-level geographical influences. The genomics of neural and behavioral plasticity leading to differences in life history is just beginning to be explored (see gene expression research in Atlantic salmon *Salmo salar* by Aubin-Horth et al. 2005a, 2005b). Resource managers can use these new developments relating salmonid life history diversity to incorporate relevant consideration for local adaptation when prescribing conservation decisions based on genetic structure.

Three of the five hatchery stocks in Idaho (Oxbow, Pahsimeroi and Sawtooth) were genetically indistinguishable, reflecting the common history in the development of these stocks. The Pahsimeroi and Oxbow stocks were derived primarily from wild adults trapped at Hells Canyon Dam in the mid 1960s, and the Sawtooth stock was founded from the Pahsimeroi stock. As previously stated, the EFSB stock was founded in 1985 using wild EFSB fish and returning adults derived from smolt releases of the Dworshak and Pahsimeroi stocks. After initial founding, the EFSB stock was primarily derived from the Dworshak stock. We expected the EFSB stock to be more similar to the Dworshak stock, however, this relationship was not clearly supported in our  $F_{ST}$  or NJ analyses. In pairwise  $F_{ST}$  comparisons, the EFSB stock was more differentiated from the Dworshak stock than from the hatchery

group (Table 4). The population NJ tree clustered EFSB in a group containing the Dworshak stock and all Clearwater watersheds with 97% bootstrap support. However, a subsequent branch (98% bootstrap support) separated the EFSB from the Dworshak stock and other Clearwater watersheds. In the population scale NJ tree, EFSB was associated with Tenmile Creek in the SF Clearwater River (Figure 2). The EFSB stock was out of HW equilibrium, suggesting possible admixture from the multiple populations used to establish this stock. Admixture for the EFSB stock may have resulted from contributions from wild fish of local origin, strays from the Sawtooth and Pahsimeroi stocks, as well as Dworshak stock releases. The EFSB stock also had the lowest genetic diversity and  $N_e$  of the five hatchery stocks and was the only hatchery stock to have a predicted recent decline, based on  $M$  ratios, which may be indicators of variation in effective reproductive success among the different hatchery or wild stocks contributing to this population.

The Dworshak stock was significantly different from all other hatchery stocks and all but one wild population (O'Hara Creek), based on  $F_{ST}$  pairwise analyses. O'Hara Creek is in the Clearwater drainage, but this creek is located in a watershed (Selway) managed exclusively for wild fish, and there are no records of hatchery fish released into O'Hara Creek. The Dworshak stock was originally developed from steelhead taken from the NF Clearwater River (Howell et al. 1985). Our population NJ analyses supported a Clearwater drainage origin for this stock and revealed a strong association with Collins Creek in the NF Clearwater (80% bootstrap support; Figure 2). In the watershed NJ tree, the Dworshak stock clustered with the SF Clearwater River, but this relationship could be predicted because Dworshak stock smolts (and in some years, surplus adults that returned to Dworshak National Fish Hatchery) were released into the SF Clearwater River watershed (Fish Passage Center 2008). This result contradicts previous findings that the Dworshak stock was the most divergent from all other inland populations (Busby et al. 1996). This is probably a result of the lack of inclusion of many Snake River steelhead populations in the Busby et al. (1996) regional analysis.

The genetic impacts of hatchery development and releases in the Columbia River basin have been of great concern to local resource agencies responsible for conservation (Busby et al. 1996, 1997). Stocking of hatchery-origin fish can potentially erode natural levels of genetic differentiation and genetic diversity (Waples 1994). This can occur directly by increasing the variance in reproductive success of a wild population (Ryman and Laikre 1991) or indirectly by increasing

artificial gene flow due to a higher occurrence of straying among hatchery-origin fish than wild fish (Waples 1991). In this study we were able to compare watersheds managed with hatchery additions and those managed exclusively for wild fish. Genetic diversity within watersheds managed exclusively for wild fish appeared to remain largely unaffected by hatchery stocks. Watersheds managed for wild fish had the most distinct genetic structure and differentiation at each spatial scale we analyzed. Average watershed  $N_e$  was higher in watersheds managed for wild fish, suggesting high reproductive success in wild steelhead without hatchery additions. Significant fine-scale genetic differentiation was evident among populations within these watersheds, indicating a level of genetic differentiation probably due to limited gene flow. Our data also showed significant differentiation between spatially separated samples from the same stream in the SF Salmon River (Poverty Flat and Knox Bridge). These results demonstrated fine-scale genetic structure that has not been impacted by hatchery releases.

Hatchery stocks have contributed to the genetic diversity found in watersheds managed with hatchery releases. Introgression by hatchery fish appears to have primarily occurred in populations in the Main Salmon, Little Salmon, and Lower Snake watersheds. All but two nonsignificant pairwise  $F_{ST}$  comparisons involving the Oxbow, Pahsimeroi or Sawtooth stocks included a wild population from the Main Salmon, Little Salmon, or Lower Snake watersheds. The two other nonsignificant pairwise comparisons involved the Pahsimeroi stock and wild Lower Clearwater populations in Big Canyon and Jacks creeks. Our nonsignificant  $F_{ST}$  relationships suggest some effect from hatchery releases at the population scale. In the Main Salmon watershed, eight wild populations were indistinguishable from the Oxbow stock, six were indistinguishable from the Pahsimeroi stock, and five from the Sawtooth stock. This result probably reflects introgression with hatchery fish in this watershed. The three Lower Snake wild populations (Granite, Sheep, and Captain John creeks) also shared genetic signatures similar to the Oxbow, Pahsimeroi, and Sawtooth stocks (i.e., hatchery group), which was not surprising given that the primary release site for the Oxbow stock is in the Snake River downstream of Hells Canyon Dam. All of the populations where nonsignificant  $F_{ST}$  estimates were observed occurred in close proximity to hatchery release sites, except for three populations: Owl, Horse, and Chamberlain creeks. These three streams enter the Main Salmon River downstream of hatchery release sites and may be influenced by hatchery adult strays.

At the watershed scale, all hatchery and wild pairwise  $F_{ST}$  relationships were significantly different,

indicating that hatchery releases appear to have not significantly changed genetic diversity on the watershed scale. Many populations in watersheds managed for wild fish and hatchery releases were significantly different genetically from contributing hatchery stocks. The demographics and locations of hatchery releases differed over time, and this may have helped sustain genetic diversity on the watershed scale. In the Main Salmon watershed, six of the populations were significantly differentiated from contemporary hatchery stocks, and in the Little Salmon watershed three of the populations were significantly differentiated from contributing hatchery stocks. All populations in the SF Clearwater watersheds and four populations from the Lower Clearwater watershed were significantly different from the hatchery stocks. Without knowledge of pre-hatchery genetic structure in these watersheds, it is difficult to decipher these genetic relationships. However, our results suggest that in most areas, genetic signatures for current hatchery releases do not dominate genetic diversity present in local steelhead populations.

Dramatic population declines and fluctuating population size can result in losses of genetic diversity (Waples 1990). Recent declines in Idaho wild adult steelhead escapements may have caused declines in genetic diversity compared with historical levels. We found comparatively lower proportions of  $M$  values falling below the threshold ( $<0.68$ ) estimates in watersheds managed with hatchery releases but lower average  $N_e$ . Lower average  $N_e$  estimates at the watershed scale reflect previously published theoretical expectations on the effects of supportive breeding on  $N_e$  (Ryman and Laikre 1991). Genetic contributions from hatchery fish may have masked or reduced reductions in genetic diversity after demographic declines in wild fish, leading to higher  $M$  ratios but with limited reproductive success (i.e., fewer individuals contributing to successive generations compared to watersheds exclusively managed for wild fish). Narum et al. (2006a) found that steelhead populations in the Grande Ronde River had no evidence of bottleneck signatures and suggested that this was a result of hatchery releases made throughout this watershed. However, rebounds in abundance due to hatchery releases do not necessarily result in rebounds in genetic variation or diversity. Hatchery rearing and release practices may have successfully buffered against bottlenecks in some populations associated with hatchery releases but in doing so may also have homogenized genetic diversity within these watersheds. This was especially true for stream populations where hatchery releases were from the Oxbow, Pahsimeroi and Sawtooth stocks, and no genetic

differentiation was observed. Populations within watersheds managed for wild fish may retain less genetic diversity individually due to bottlenecks and random genetic drift. However, populations managed for wild fish may be more diverse collectively at the watershed scale, based on the range of unique local adaptations found within each watershed. Documentation of genetic diversity at multiple scales for Idaho's Snake River steelhead and our discussion of factors leading to differences among them demonstrate the need to balance information at the population and broader watershed scales in efforts to understand and maximize the benefits of genetic diversity in this highly managed system.

### Concluding Remarks

Steelhead in Idaho are managed at the watershed scale, based on watersheds managed exclusively for wild fish and those managed with hatchery releases. Our results demonstrated that genetic structure and diversity in Idaho's steelhead exists at multiple spatial scales. However, results for steelhead at the population, watershed, and drainage scales were not always congruent. When hatchery stocks were compared with watersheds, all of the comparisons were significantly different. This was not the case for comparisons made at the population scale, where some wild populations were not significantly different from hatchery stocks. Some populations within watersheds managed with hatchery releases were significantly different from contributing hatchery stocks, despite large-scale releases within these watersheds. Genetic population structure influenced by hatchery fish seemed most obvious in some populations in the Main Salmon, Little Salmon, and Lower Snake watersheds. Pairwise  $F_{ST}$  relationships indicated that populations within watersheds managed for wild fish appear to be unaffected by hatchery stocks. Watersheds managed for wild fish had the most distinct genetic structure and differentiation at each spatial scale we analyzed. There was also substantial substructuring among populations within these watersheds, indicating that fine-scale diversity exists in many areas within this highly managed basin. Furthermore, management history and life history designation do not always appear to be indicative of the distribution of genetic diversity across the landscape. Despite the fact that the Snake River in Idaho is a highly managed basin with numerous hatchery releases, unique genetic diversity was apparent in this system at three different spatial scales: population, watershed, and drainage. These data will assist managers in prioritizing their efforts to conserve wild stocks, especially in light of observed genetic effects of declines at the population spatial scale. The conserva-

tion of existing levels of genetic diversity in wild steelhead described in this study can assist in the management of Snake River basin steelhead at different spatial scales. Genetic diversity in anadromous steelhead documented across this highly complex landscape with a diverse management history can contribute to integrated management decisions based on a balance of ecosystem and genetic population structure.

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