

## Naturally Spawning Hatchery Steelhead Contribute to Smolt Production but Experience Low Reproductive Success

KATHRYN E. KOSTOW\*

*Oregon Department of Fish and Wildlife,  
2501 Southwest First Avenue,  
Portland, Oregon 97207, USA*

ANNE R. MARSHALL AND STEVAN R. PHELPS<sup>1</sup>

*Washington Department of Fish and Wildlife,  
600 Capitol Way North,  
Olympia, Washington 98501, USA*

**Abstract.**—We used genetic mixture analyses to show that hatchery summer-run steelhead *Oncorhynchus mykiss*, an introduced life history in the Clackamas basin of Oregon, where only winter-run steelhead are native, contributed to the naturally produced smolts out-migrating from the basin. Hatchery-produced summer steelhead smolts were released starting in 1971, and returning adults were passed above a dam into the upper Clackamas River until 1999. In the 2 years of our study, summer steelhead adults, mostly hatchery fish, made up 60% to 82% of the natural spawners in the river. Genetic results provided evidence that interbreeding between hatchery summer and wild winter steelhead was likely minor. Hatchery summer steelhead reproductive success was relatively poor. We estimated that they produced only about one-third the number of smolts per parent that wild winter steelhead produced. However, the proportions of summer natural smolts were large (36–53% of the total naturally produced smolts in the basin) because hatchery adults predominated on the spawning grounds during our study. Very few natural-origin summer adults were observed, suggesting high mortality of the naturally produced smolts following emigration. Counts at the dam demonstrated that hatchery summer steelhead predominated on natural spawning grounds throughout the 24-year hatchery program. Our data support a conclusion that hatchery summer steelhead adults and their offspring contribute to wild winter steelhead population declines through competition for spawning and rearing habitats.

Genetic risks to wild fish populations caused by interbreeding with hatchery fish have been frequently addressed in research and have been of great concern to managers for at least the last several decades (Reisenbichler and McIntyre 1977; Hindar et al. 1991; Waples 1991; Reisenbichler and Rubin 1999; Lynch and O’Hely 2001). Most management efforts to decrease the risks of hatchery programs to wild populations have focused on decreasing interbreeding between hatchery and wild fish (Lindsay et al. 2001). Low apparent breeding success by the hatchery fish has often been assumed to indicate low risk to the wild fish (ODFW 1992b).

For many years, this premise guided a large hatchery program by the Oregon Department of Fish and Wildlife (ODFW) to produce summer-run steelhead *Oncorhynchus mykiss* for the Clackamas River, which enters the lower Willamette River in

Oregon. The Willamette River enters the lower Columbia River at river kilometer (rkm) 160, as measured from the mouth of the Columbia River. Winter-run steelhead are native to the Clackamas River, but summer steelhead were not historically present. The Skamania stock summer steelhead at the South Santiam Hatchery (henceforth, “hatchery summer” stock) were introduced into the basin in 1971 to provide a sport fishery. On average, 160,000 marked hatchery summer steelhead smolts were released into the basin annually. An average of 2.4% of them survived to adulthood, based on annual smolt releases and adult returns to North Fork Dam fish ladder at rkm 48 on the main-stem Clackamas River (ODFW, unpublished data). Substantial numbers of hatchery summer adults were passed above the dam into natural spawning grounds starting in 1975. Very few unmarked summer steelhead were ever observed in the Clackamas River, either at the dam or in the fishery, indicating poor reproduction by or survival of the hatchery fish passed upstream. It was therefore assumed that these fish did not spawn successfully. It was also assumed that life history

\* Corresponding author: kathryn.e.kostow@state.or.us  
<sup>1</sup> Deceased.

differences, such as those described by Leider et al. (1984), precluded interbreeding between summer and winter runs. Risks to wild winter steelhead from the hatchery summer steelhead program were assumed to be very low.

A decline in Clackamas River wild winter steelhead abundance occurred in the 1990s (Chilcote 1998) and caused a more careful consideration of factors affecting the population, including the hatchery summer steelhead program. We hypothesized that hatchery summer steelhead were successfully spawning and producing juvenile offspring that largely died before reaching adulthood. If this were so, hatchery summer adults and their offspring could have been occupying substantial amounts of winter steelhead spawning and rearing habitat and contributing to wild winter steelhead declines through competition. Although summer and winter steelhead show distinct differences in adult life histories, their juvenile life histories and habitat requirements are thought to be similar, which would maximize competitive interactions between them (Fraser 1969; McMichael et al. 2000; Keeley 2001). Leider et al. (1984) demonstrated that hatchery summer steelhead spawned significantly earlier than wild winter steelhead. Thus, summer steelhead may be expected to emerge earlier and occupy choice feeding territories before wild winter steelhead, which may place winter steelhead at a particular disadvantage.

To evaluate potential effects of the hatchery summer steelhead program on wild winter steelhead, we had to address two issues: (1) whether hatchery summer steelhead were spawning naturally and producing smolts and (2) whether the summer steelhead produced had any effects on the productivity of wild winter steelhead. This paper addresses the first issue by investigating the contribution by hatchery summer steelhead to naturally produced smolts out-migrating from the Clackamas River. The second issue will be addressed in a subsequent paper.

### Methods

*General approach.*—Adult fish passing upstream to natural spawning grounds in the Clackamas basin and juvenile out-migrants produced in the upper basin have been enumerated annually at North Fork Dam fish passage facilities since 1958. The ODFW has estimated that about 80% of winter steelhead spawning and rearing habitat in the Clackamas basin is above North Fork Dam (ODFW 1992a). We planned to compare the allelic composition of all steelhead populations that

might be breeding in the upper Clackamas with that of naturally produced smolts captured at the dam and use genetic methods for mixture analyses to determine the contributions of the various stocks to natural smolt production.

We assumed that three genetically distinct parental populations potentially spawned in the basin: (1) the hatchery summer steelhead stock, (2) a hatchery winter steelhead stock that was released into the lower Clackamas basin but that could stray into upstream areas, and (3) the wild Clackamas winter steelhead population. The hatchery summer steelhead stock originated from Skamania stock, which was founded in the 1950s from wild summer steelhead populations in the Klickitat and Waukegan rivers of southwest Washington (Reisenbichler et al. 1992). This stock had been transferred to ODFW South Santiam Hatchery in the mid-Willamette basin and had been reared without further stock mixing or importation since the 1970s. The winter steelhead hatchery stock, released at the U.S. Fish and Wildlife Service Eagle Creek National Hatchery in the lower Clackamas basin, was originally derived from steelhead in Big Creek, an Oregon tributary to the lower Columbia River (rkm 43). The third population, unmarked winter steelhead returning to the Clackamas River, was assumed to represent the wild native gene pool.

*Field sampling.*—We sampled the three stocks to form our genetic baseline data set. We sampled 68 unmarked winter-run adult steelhead over 3 years (25 in 1995, 16 in 1996, and 27 in 1997) at North Fork Dam in April and May, their peak runtime. We sampled juvenile hatchery summer steelhead ( $N = 51$ ) at South Santiam Hatchery in 1995 and juvenile hatchery winter steelhead ( $N = 50$ ) at Eagle Creek Hatchery in 1996. We collected out-migrating smolts, identified as naturally produced based on lack of adipose fin clip marks, at the North Fork Dam downstream migrant trap during the peak of steelhead out-migration from 12 to 15 May 1995 ( $N = 50$ ) and 29 April to 5 May 1996 ( $N = 42$ ). All samples were delivered frozen on dry ice to the Washington Department of Fish and Wildlife (WDFW) Genetics Laboratory in Olympia Washington, where they were stored at  $-80^{\circ}\text{C}$  until dissection. Approximately  $1\text{ cm}^3$  each of skeletal muscle, heart, liver, and retina was extracted from each fish and put in individually labeled plastic culture tubes ( $12 \times 17\text{ mm}$ ). Tissues were kept frozen on dry ice during dissection and subsequently were stored at  $-80^{\circ}\text{C}$  until analysis.

We used annual adult and juvenile counts at

North Fork Dam to estimate the sizes of parent, smolt, and adult offspring populations so that we could calculate the relative production of offspring by our three parent populations. Lower Columbia steelhead populations largely produce 2-year-old smolts and 4-year-old adults (Busby et al. 1996). Therefore, the 1995 smolt sample corresponded to the 1993 parent year and the 1997 adult offspring return year, and the 1996 smolt sample corresponded to the 1994 parent year and the 1998 adult offspring return year. The count of summer steelhead parents included both natural and hatchery summer steelhead passed above North Fork Dam, which was reduced by 15% to adjust for mortality during the long prespawning holding period. Hatchery and wild winter steelhead parents were the counts of marked and unmarked winter steelhead, respectively, passed above the dam. Smolt offspring were the counts of unmarked smolts passed below the dam. Adult offspring were the counts of unmarked winter and summer steelhead at the dam plus the estimated number of fish harvested before reaching the dam (from Chilcote 1998).

*Electrophoresis.*—All allozyme analyses for this study were conducted at WDFW Genetics Laboratory. We used horizontal starch-gel electrophoresis following the general methods of Aebersold et al. (1987) to assay genetic variation at 60 enzyme-coding loci in steelhead (Table 1). Locus and allele nomenclature followed Shaklee et al. (1990a). Additional description of laboratory methods can be found in Phelps et al. (1994a). We reported allele mobilities in accordance with the coastwide genetic stock identification consortium, a group of west coast agencies and universities that has coordinated and standardized allozyme methodologies for *Oncorhynchus* species. We used computer-based quality control procedures for genotype scoring as described in Phelps et al. (1994b). This protocol served as a check for field sampling errors and gel loading errors and provided multiple independent scores for many loci, as recommended by Shaklee and Phelps (1990).

*Genetic data analyses.*—We used the computer program BIOSYS-1 (Swofford and Selander 1989) for calculating allele frequencies, average heterozygosities, percentages of polymorphic loci, average number of alleles per locus, Hardy-Weinberg (HW) genotypic equilibrium tests per sample, and pairwise genetic distances. We used chi-square goodness-of-fit tests to compare observed genotype proportions with those expected under HW equilibrium conditions in each parent baseline and

smolt sample. For baseline sample tests, we included only loci that had at least five variant individuals, and when more than two alleles were observed at a locus, we pooled genotypes into three classes (homozygotes for the most common allele, heterozygotes for the most common allele and one of the other alleles, and all other genotypes) because chi-square values can be inflated when expected frequencies of some classes are low (Sokal and Rohlf 1981). We tested for allele frequency homogeneity between samples with a log-likelihood ratio test and *G*-statistic (Sokal and Rohlf 1981). To evaluate relationships among samples, we used Cavalli-Sforza and Edwards (1967) chord genetic distances and the NTSYS-pc computer program (Rohlf 1994) to conduct multidimensional scaling analyses (Lessa 1990).

Gametic phase (linkage) disequilibrium may be present in populations where recent introgression or hybridization has occurred (see Campton 1987) or in samples containing a mixture of individuals from noninterbreeding, well-differentiated gene pools. We evaluated gametic disequilibrium in the two wild smolt samples and in a sample formed by combining the 1995 wild winter adult sample and the hatchery summer stock sample. We assumed this combined sample would act as a simple mixture of individuals from the two populations with no hybrids present and would provide an estimate of gametic disequilibrium expected in such a sample. Using only polymorphic loci with variant alleles present at rates of 5% or higher, we calculated Burrows composite gametic disequilibrium coefficients (Weir 1979) with a computer program supplied by Jon Brodziak (National Marine Fisheries Service, Woods Hole, Massachusetts) and modified by Craig Busack (WDFW).

We used two models to evaluate genetic contributors to smolt production. Our first approach assumed smolts were a genetic admixture produced by all possible matings of the parent stocks. We used methods described by Long (1991) and his computer program (ADMIX). This program uses a weighted least-squares estimation method to calculate contributions from parental sources to an introgressed or hybridized population. Both sampling error and genetic drift are accounted for in computing standard errors (Long 1991). Given program input data requirements, we used frequencies for the common allele ( $*100$  or  $*a$ ) at 17 loci (Table 1). We tested each smolt sample separately.

The second model assumed that smolts resulted largely from assortative mating, in which the three

TABLE 1.—Allele frequencies at 37 allozyme loci for samples of Clackamas River steelhead smolts and baseline samples of wild Clackamas River winter-run (CRW), hatchery summer-run (HS), and hatchery winter-run (HW) steelhead populations; *N* = number of fish successfully scored per locus. The following 23 loci had a frequency of 1.000 for the *\*a* (*\*100*) allele in all samples: *sAAT-3\**, *mAH-1,2<sup>sa</sup>\**, *mAH-4\**, *CK-A1\**, *CK-A2\**, *CK-C1\**, *FH\**, *IDDH-1\**, *mIDHP-1\**, *LDH-A1\**, *LDH-A2\**, *LDH-B1\**, *ME\**, *mMEP-1\**, *MPI\**, *PEP-LT\**, *PGM-1\**, *PGM-1r\**, *mSOD\**, *TPI-1\**, *TPI-2\**, and *TIP-4\**.

Locus, <i>N</i> , allele code (mobility), and statistic	1995 smolts	1996 smolts	CRW	HS	HW
<i>mAAT-1*</i>					
<i>N</i>	50	41	68	51	49
<i>*a</i> ( <i>*100</i> )	0.990	1.000	1.000	1.000	1.000
<i>*b</i> ( <i>*110</i> )	0.010	0.000	0.000	0.000	0.000
<i>sAAT-1,2<sup>sa,b,c</sup>*</i>					
<i>N</i>	50	41	68	51	50
<i>*a</i> ( <i>*100</i> )	0.980	0.994	0.960	0.990	1.000
<i>*b</i> ( <i>*125</i> )	0.020	0.006	0.040	0.010	0.000
<i>ADA-1*</i>					
<i>N</i>	50	42	66	51	50
<i>*a</i> ( <i>*100</i> )	0.990	1.000	0.992	1.000	1.000
<i>*b</i> ( <i>*85</i> )	0.000	0.000	0.008	0.000	0.000
<i>*c</i> ( <i>*81</i> )	0.010	0.000	0.000	0.000	0.000
<i>ADA-2<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.920	0.976	0.875	1.000	0.770
<i>*b</i> ( <i>*106</i> )	0.060	0.024	0.118	0.000	0.120
<i>*c</i> ( <i>*90</i> )	0.020	0.000	0.007	0.000	0.110
<i>ADH*</i>					
<i>N</i>	49	42	67	51	50
<i>*a</i> ( <i>*100</i> )	1.000	1.000	0.993	1.000	1.000
<i>*b</i> ( <i>*78</i> )	0.000	0.000	0.007	0.000	0.000
<i>mAH-3*</i>					
<i>N</i>	46	42	67	51	50
<i>*a</i> ( <i>*100</i> )	1.000	0.988	1.000	1.000	1.000
<i>*b</i> ( <i>*122</i> )	0.000	0.012	0.000	0.000	0.000
<i>sAH<sup>sb,c</sup>*</i>					
<i>N</i>	49	41	66	51	50
<i>*a</i> ( <i>*100</i> )	0.939	0.951	0.917	0.892	0.840
<i>*b</i> ( <i>*85</i> )	0.051	0.037	0.076	0.108	0.070
<i>*c</i> ( <i>*50</i> )	0.010	0.012	0.000	0.000	0.090
<i>*d</i> ( <i>*116</i> )	0.000	0.000	0.008	0.000	0.000
<i>ALAT<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	49
<i>*a</i> ( <i>*100</i> )	1.000	0.964	0.949	0.912	1.000
<i>*b</i> ( <i>*106</i> )	0.000	0.036	0.051	0.049	0.000
<i>*c</i> ( <i>*111</i> )	0.000	0.000	0.000	0.039	0.000
<i>CK-C2*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	1.000	1.000	0.993	1.000	0.900
<i>*b</i> ( <i>*104</i> )	0.000	0.000	0.007	0.000	0.100
<i>GAPDH-3<sup>sc</sup>*</i>					
<i>N</i>	48	42	67	51	50
<i>*a</i> ( <i>*100</i> )	0.990	1.000	0.948	1.000	1.000
<i>*b</i> ( <i>*33</i> )	0.010	0.000	0.052	0.000	0.000
<i>bGLUA<sup>sc</sup>*</i>					
<i>N</i>	49	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.980	1.000	1.000	0.980	1.000
<i>*c</i> ( <i>*11</i> )	0.020	0.000	0.000	0.020	0.000
<i>GPI-A<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.950	0.988	1.000	0.971	1.000
<i>*c</i> ( <i>*89</i> )	0.050	0.012	0.000	0.029	0.000

TABLE 1.—Continued.

Locus, <i>N</i> , allele code (mobility), and statistic	1995 smolts	1996 smolts	CRW	HS	HW
<i>GPI-B1<sup>sc</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.970	0.976	0.963	0.990	0.990
<i>*b</i> ( <i>*148</i> )	0.030	0.024	0.037	0.010	0.010
<i>GPI-B2*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	1.000	1.000	0.993	1.000	1.000
<i>*b</i> ( <i>*60</i> )	0.000	0.000	0.007	0.000	0.000
<i>G3PDH-1<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.840	0.798	0.831	0.882	0.970
<i>*b</i> ( <i>*80</i> )	0.130	0.202	0.154	0.118	0.020
<i>*d</i> ( <i>*40</i> )	0.030	0.000	0.015	0.000	0.010
<i>IDDH-2*</i>					
<i>N</i>	49	41	65	51	50
<i>*a</i> ( <i>*100</i> )	1.000	1.000	0.985	1.000	1.000
<i>*c</i> ( <i>*5</i> )	0.000	0.000	0.015	0.000	0.000
<i>mIDHP-2<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.980	0.976	0.971	1.000	0.950
<i>*b</i> ( <i>*144</i> )	0.010	0.000	0.000	0.000	0.050
<i>*c</i> ( <i>*162</i> )	0.010	0.024	0.007	0.000	0.000
<i>*d</i> ( <i>*67</i> )	0.000	0.000	0.022	0.000	0.000
<i>sIDHP-1<sup>sc</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.970	0.988	0.985	1.000	0.990
<i>*c</i> ( <i>*121</i> )	0.010	0.000	0.007	0.000	0.010
<i>*j</i> ( <i>*74</i> )	0.020	0.012	0.007	0.000	0.000
<i>sIDHP-2<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	67	51	50
<i>*a</i> ( <i>*100</i> )	0.230	0.226	0.201	0.255	0.280
<i>*b</i> ( <i>*42</i> )	0.400	0.405	0.321	0.412	0.290
<i>*d</i> ( <i>*72</i> )	0.300	0.345	0.381	0.245	0.310
<i>*f</i> ( <i>*123</i> )	0.050	0.012	0.090	0.059	0.120
<i>*k</i> ( <i>*27</i> )	0.020	0.012	0.007	0.029	0.000
<i>LDH-B2<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.820	0.690	0.596	0.912	0.760
<i>*b</i> ( <i>*76</i> )	0.180	0.310	0.397	0.088	0.210
<i>*c</i> ( <i>*113</i> )	0.000	0.000	0.007	0.000	0.030
<i>LDH-C</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	1.000	1.000	1.000	1.000	0.960
<i>*b</i> ( <i>*95</i> )	0.000	0.000	0.000	0.000	0.040
<i>sMDH-A1,2<sup>sa</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	1.000	0.988	1.000	1.000	1.000
<i>*e</i> ( <i>*49</i> )	0.000	0.012	0.000	0.000	0.000
<i>sMDH-B1,2<sup>sa,b,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.885	0.905	0.926	0.897	0.890
<i>*b</i> ( <i>*78</i> )	0.095	0.089	0.063	0.103	0.110
<i>*c</i> ( <i>*116</i> )	0.005	0.006	0.007	0.000	0.000
<i>*d</i> ( <i>*83</i> )	0.015	0.000	0.000	0.000	0.000
<i>*f</i> ( <i>*120</i> )	0.000	0.000	0.004	0.000	0.000
<i>sMEP-1<sup>sb,c</sup>*</i>					
<i>N</i>	50	42	68	51	50
<i>*a</i> ( <i>*100</i> )	0.880	0.917	0.912	0.922	0.890
<i>*d</i> ( <i>*83</i> )	0.120	0.083	0.088	0.078	0.110
<i>sMEP-2<sup>sb</sup>*</i>					
<i>N</i>	47	41	68	48	50
<i>*a</i> ( <i>*100</i> )	0.968	1.000	1.000	0.948	1.000
<i>*b</i> ( <i>*80</i> )	0.032	0.000	0.000	0.052	0.000

TABLE 1.—Continued.

Locus, <i>N</i> , allele code (mobility), and statistic	1995 smolts	1996 smolts	CRW	HS	HW
<i>NTP</i> <sup>a,b,c</sup>					
<i>N</i>	50	42	65	46	50
* <i>a</i> (*100)	0.390	0.488	0.492	0.304	0.300
* <i>b</i> (*135)	0.610	0.512	0.508	0.696	0.700
<i>PEPA</i> <sup>a,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	0.970	1.000	0.963	0.980	1.000
* <i>b</i> (*111)	0.030	0.000	0.037	0.020	0.000
<i>PEPB-1</i> <sup>a,b,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	0.840	0.905	0.860	0.873	0.800
* <i>b</i> (*131)	0.000	0.000	0.007	0.000	0.030
* <i>e</i> (*-75)	0.160	0.095	0.132	0.127	0.170
<i>PEPD-1</i> <sup>a,b,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	0.960	0.952	0.985	0.902	0.990
* <i>b</i> (*94)	0.000	0.000	0.000	0.000	0.000
* <i>c</i> (*110)	0.040	0.036	0.015	0.098	0.010
* <i>d</i> (*87)	0.000	0.012	0.000	0.000	0.000
<i>PGK-2</i> <sup>a,b,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	0.610	0.583	0.449	0.735	0.360
* <i>b</i> (*115)	0.360	0.417	0.529	0.265	0.640
* <i>c</i> (*144)	0.030	0.000	0.022	0.000	0.000
<i>PGM-2</i> <sup>a</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*-100)	1.000	1.000	1.000	1.000	0.990
* <i>b</i> (*-120)	0.000	0.000	0.000	0.000	0.010
<i>PNP</i> <sup>a</sup>					
<i>N</i>	50	40	54	40	50
* <i>a</i> (*100)	1.000	0.988	0.981	1.000	1.000
* <i>b</i> (*107)	0.000	0.013	0.019	0.000	0.000
<i>sSOD-1</i> <sup>a,b,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	0.730	0.726	0.706	0.735	0.660
* <i>b</i> (*152)	0.270	0.274	0.294	0.265	0.340
<i>TPI-3</i> <sup>a,c</sup>					
<i>N</i>	50	42	68	51	50
* <i>a</i> (*100)	1.000	0.988	0.941	0.980	1.000
* <i>b</i> (*94)	0.000	0.000	0.059	0.020	0.000
* <i>c</i> (*102)	0.000	0.012	0.000	0.000	0.000

<sup>a</sup> These isolocus pairs represent two loci with common alleles having the same electrophoretic mobility and in which variant alleles cannot be assigned to either locus. Frequencies are for both loci combined (4 alleles/isolocus).

<sup>b</sup> Locus used for admixture analysis.

<sup>c</sup> Locus used for maximum likelihood estimates of mixture composition.

parent stocks bred only within their own group and did not hybridize. This may be a more realistic model for summer and winter steelhead, based on the findings of Leider et al. (1984). We also had to assume that hatchery winter steelhead were reproductively isolated from both wild winter steelhead and the summer steelhead. Using maximum likelihood estimation (MLE) methods for mixed-stock analysis, we estimated contributions from each parent stock to each smolt sample. This meth-

odology has been used to estimate stock composition in salmon fishery harvests (Shaklee et al. 1990b) and in out-migrating juvenile chinook salmon (Marshall et al. 2000). We used the MLE computer program of Milner et al. (1983) as modified by Millar (1987). Input data were allele frequencies at 22 loci that were polymorphic in at least one parent stock (Table 1) and the 22-locus genotypes for individual smolts. The MLE program would identify impossible genotypes in the smolts based on allele presence and absence in parent stocks. These genotypes may indicate a hybrid if baseline data are accurate. Hybrid genotypes could lack such distinguishing allelic combinations but might increase estimate variances.

## Results

*Genetic analyses.*—Allele frequencies for 37 variable loci in smolt and parent baseline samples are presented in Table 1. A variety of loci appeared informative for mixture analyses (e.g., *sAH*\*, *ADA-2*\*, *ALAT*\*, *GAPDH-3*\*, *LDH-B2*\*, *PGK-2*\*). Some alleles were observed in smolt samples but not in baseline samples (e.g., *sMDH-B1,2*\*83, *PEPD-1*\*87, *TPI-3*\*102; Table 1).

Genetic variability per sample differed slightly among parent baseline samples (Table 2). Wild winter steelhead had higher average alleles per locus and higher percentages of polymorphic loci, but sample size was also larger. Both smolt samples had a relatively high percentages of loci polymorphic at the 1% criterion level. Differences between observed and expected mean heterozygosities were not significant in any sample.

found significant deviations from expected HW proportions in baseline samples at the following loci among the number of tested loci (*N*): hatchery summer stock at *ALAT*\* (12), wild winter stock at *GAPDH-3*\* (15), and hatchery winter stock at *sIDHP-2*\* (12). These proportions of significant tests per sample were close to those expected by chance (0.05). In the 1995 smolt sample we found significant HW test results for three loci (*ADA-2*\*, *bGLUA*\*, and *PEPD-1*\*), all due to heterozygote deficiencies. At *bGLUA*\* we observed only a homozygote for a variant allele (\**c/c*). Note that *bGLUA*\**c* allele frequencies were too low in baseline samples to expect homozygous genotypes (Table 1). We did not detect any significant deviations from HW equilibria in the 1996 smolt sample.

Overall allele frequencies were significantly different ( $P < 0.001$ ) in all possible pairwise comparisons among the three parent samples and between each parent sample and the smolt samples.

TABLE 2.—Measures of genetic variability, based on 56 loci, in baseline samples of wild steelhead smolts and parent stocks. Standard deviations are shown in parentheses.

Group or stock	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic <sup>a</sup> at		Mean heterozygosity	
			>5%	>1%	Actual	Expected
1995 smolts	48.6 (0.9)	1.6 (0.1)	21.4	42.9	0.072 (0.019)	0.079 (0.020)
1996 smolts	41.8 (0.1)	1.5 (0.1)	16.1	39.3	0.073 (0.021)	0.073 (0.021)
South Santiam Hatchery Skamania summer run	49.9 (0.7)	1.4 (0.1)	23.2	32.1	0.066 (0.018)	0.069 (0.019)
Eagle Creek Hatchery Big Creek winter run	50.0 (0.0)	1.4 (0.1)	21.4	32.1	0.081 (0.022)	0.080 (0.022)
Wild Clackamas winter run	67.1 (0.4)	1.7 (0.1)	26.8	39.3	0.086 (0.021)	0.086 (0.021)

<sup>a</sup> The two levels of polymorphism indicate the frequency of alleles other than the \*a allele.

Allele frequencies of the two smolt samples were significantly different from each other ( $P = 0.03$ ). Tests included 20–22 loci, depending on sample variability. Genetic distances (22 loci) among all samples plotted in three-dimensional space (Figure 1) showed both smolt samples to be nearly equally distant from hatchery summer and wild winter steelhead samples and most divergent from the hatchery winter steelhead sample. Genetic distance between wild winter steelhead and each hatchery stock was nearly equal.

In our test sample for gametic disequilibrium (wild winter and hatchery summer samples combined), we found significant ( $P < 0.05$ ) disequilibrium between two locus pairs (*ALAT*\* and *PGK-2*\*; and *LDH-B2*\* and *PGK-2*\*). Some of the largest allele frequency differences between wild winter and hatchery summer samples occurred at *LDH-B2*\* and *PGK-2*\* (Table 1). In the 1995 smolt sample we found significant gametic disequilibrium between two locus pairs (*ADA-2*\* and *NTP*\*; and *LDHB-2*\* and *NTP*\*). In the 1996 smolt sample we found significant disequilibrium between three locus pairs (*ADA-2*\* and *sAH*\*; *ADA-2*\* and *sSOD-1*\*; and *sMEP-1*\* and *sSOD-1*\*).

*Mixture analyses of smolts.*—Estimated contributions of potential parent stocks to each smolt sample using each model are shown in Table 3. In general, we found high contributions from the hatchery summer stock using either model, particularly in the 1995 smolt sample. The wild winter stock was the other major contributor. Contributions to smolts from the hatchery winter stock were minor and imprecise according to both models.

Admixture analysis, which assumed interbreeding among stocks, indicated that hatchery summer steelhead made the largest contribution to 1995

smolts, followed by wild winter steelhead (Table 3). Admixture analysis also indicated that hatchery winter steelhead made a moderate contribution to 1995 smolts, but this estimate was quite imprecise. The mixed-stock MLE analysis, which assumed noninterbreeding, showed large proportions of wild winter and hatchery summer steelhead progeny among the 1995 smolts and only a very small proportion due to hatchery winter steelhead. The MLE program also found two 1995 smolts with impossible genotypes given parent baseline data, and these were excluded from stock composition analysis.

Wild winter steelhead were the major contributors to 1996 smolts in ADMIX results, and hatchery summer steelhead contributions were substantial (Table 3). Initial admixture analysis of 1996 smolts gave a negative contribution estimate for

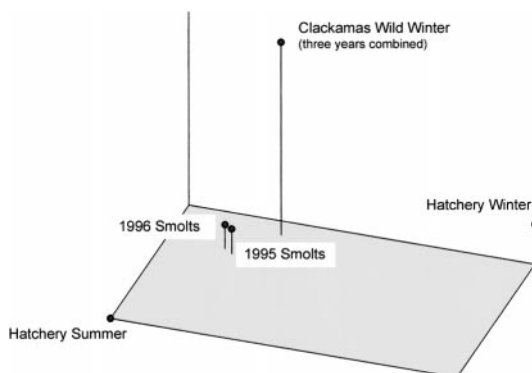


FIGURE 1.—Three-dimensional scaling plot of Cavalli-Sforza and Edwards (1967) chord genetic distances among potential parent stocks and smolt samples for wild winter and hatchery steelhead in the Clackamas River.

TABLE 3.—Parent stock contribution estimates from admixture analysis and composition estimates from mixed stock maximum likelihood (MLE) analysis for 1995 and 1996 Clackamas steelhead smolt samples and composition of 1993–1994 parents and 1997–1998 adult offspring. All values are percent; SDs are given in parentheses.

Parent stock	1995 Smolts				1996 Smolts			
	Composition of 1993 parents	Composition of smolts		Composition of 1997 adult offspring	Composition of 1994 parents	Composition of smolts		Composition of 1998 adult offspring
		Admixture (N = 50)	MLE (N = 48) <sup>a</sup>			Admixture (N = 42)	MLE (N = 42)	
Wild Clackamas winter run South Santiam Hatchery	15	33 (13)	54 (9)	87	37	64 (8)	59 (11)	82
Sakamania Stock summer run Eagle Creek Hatchery	82	53 (9)	44 (9)	13	60	36 (8)	36 (11)	18
Big Creek Stock winter run	3	14 (11)	2 (4)	<sup>b</sup>	3	none	5 (5)	<sup>b</sup>

<sup>a</sup> Two fish were removed from the original sample of 50 because they had genotypes that could not be allocated to a parent stock given parent baseline allele frequencies.

<sup>b</sup> All unmarked winter steelhead were presumed to be wild.

the hatchery winter stock, so we removed it from the baseline for a final estimate. Mixed-stock MLE analysis found that 1996 smolts contained a majority of wild winter steelhead progeny, a large proportion of hatchery summer steelhead progeny, and a small component of hatchery winter steelhead.

Overall, we found that stock contribution estimates varied more widely between years in admixture analyses than in mixed-stock analyses. However, it was apparent from both models that hatchery summer steelhead contributed at relatively high levels to natural production of smolts in both years.

One of the excluded genotypes in the 1995 smolt sample may indicate the presence of hatchery-origin rainbow trout *O. mykiss* because of variant alleles at *ADA-1*\*, *G3PDH-1*\*, *bGLUA*\*, *mIDHP-2*\*, and *sMDH-B1,2*\*. We based this interpretation on Roaring River hatchery stock rainbow trout allele frequencies (WDFW, unpublished data), a stock that has been planted in North Fork Reservoir. The other impossible genotype may indicate a hybrid between summer and winter stocks, based on alleles at *sAH*\*, *GPI-A*\*, and *sIDHP-1*\*. Also, two smolts in each year possessed an allele that

was absent in parent baselines, but had multilocus genotypes acceptable in MLE analyses when the single locus genotype was excluded.

*Relative production of offspring by summer and wild winter steelhead.*—The 1993 parents of 1995 smolts included 82% summer steelhead and 15% wild winter steelhead (Table 3). The 1994 parents of 1996 smolts included 60% summer steelhead and 37% wild winter steelhead. The 1997 adult offspring included 87% winter steelhead and 13% summer steelhead, and 1998 adult offspring included 82% winter steelhead and 18% summer steelhead. Winter steelhead adult offspring were believed to be primarily the progeny of the wild winter steelhead because (1) few hatchery winter parents passed the dam in 1993 and 1994 and (2) estimated hatchery winter stock contributions to 1995 and 1996 smolts were very small.

The hatchery summer stock predominated among the parents in both brood years of our study, based on numbers passing above the dam, but they produced half or less of the smolts and only a small proportion of the naturally produced adult offspring. The relatively poor reproductive success of summer steelhead is evident in the number of smolts and adult offspring produced per parent by the two brood years (Table 4). Summer steelhead produced only 18–37% of the smolts per parent that were produced by wild winter steelhead and only 4–13% of the adult offspring per parent that were produced by wild winter steelhead.

## Discussion

We found that hatchery summer steelhead contributed substantially to natural smolt production according to both of our models of stock interbreeding. The previous assumption that hatchery

TABLE 4.—Estimates of naturally produced smolt and adult offspring per parent produced by wild winter and introduced summer steelhead in the Clackamas River.

Brood year	Smolts per parent <sup>a</sup>		Adults per parent	
	Wild winter	Summer	Wild winter	Summer
1993	7.4–12.1	1.8–2.2	0.5	0.02
1994	6.1–5.7	2.1	0.5	0.06

<sup>a</sup> The range of results reflects the differences between the results of the admixture and maximum likelihood analyses.

summer steelhead did not spawn successfully had to be dismissed, which required us to evaluate which model most appropriately described the behavior of Clackamas steelhead populations. In lower Columbia basin tributaries that have native populations of both life histories, summer and winter steelhead maintain reproductive isolation through run and spawn-timing differences and seasonal migration barriers within a drainage. There are no physical barriers in the upper Clackamas and any overlap in spawn timing and location could promote interbreeding between hatchery summer and wild winter steelhead. Our genetic data provided evidence about the potential levels of interbreeding.

Baseline allele frequency data for the three Clackamas steelhead stocks demonstrated that adults classified phenotypically as wild winter steelhead were genetically divergent from both introduced summer and winter hatchery stocks. Wild winter steelhead genetic differentiation indicated that this population has not been homogenized by interbreeding with hatchery stocks. These data support the hypothesis that among-stock reproductive isolation is relatively high. However, interbreeding is not completely precluded by these results because population genetic data before the hatchery programs are not available for comparisons. Also, we would not have detected summer-winter interbreeding if such hybrids had a summer run-time phenotype.

Genetic data for smolts provided evidence that the samples contained fish from separate source populations. Heterozygote deficiencies and significant gametic disequilibria in the 1995 smolt sample indicated that it contained individuals from genetically divergent populations. Although we found no significant HW disequilibria in the 1996 sample, significant gametic disequilibria results indicated that individuals originated from separate, divergent sources. In general, estimation of gametic disequilibrium between loci is a more powerful test for nonrandom mating than single-locus HW tests (Campton 1987).

Allelic variants seen in smolts but not in baseline samples likely reflect sampling error for baselines and nonnative rainbow trout presence. Baseline sample sizes and inclusion of only one brood year from hatchery populations may underestimate variability, given steelhead allelic diversity (Phelps et al. 1994a). However, not only was one hatchery rainbow trout likely present in our smolt samples, but other genotypes with *sMDH-B1,2*\*83 alleles may indicate gene flow between nonnative

hatchery rainbow trout and native steelhead (Phelps 1991).

The admixture analysis of 1995 smolts, which assumes interbreeding among parent stocks, indicated higher contributions by both the summer and winter hatchery stocks and, therefore, higher hatchery fish reproductive success than the mixed-stock MLE analysis, which assumes assortative mating. However, we believe that hatchery winter stock contributions were overestimated and very imprecise for 1995 smolts because of analytical limits of the ADMIX program. These limitations also prevented an estimate of hatchery winter stock contributions to 1996 smolts, even though *sAH*\*c allele frequencies (Table 1) implied a contribution. Because ADMIX used only the frequency of one allele (usually the most frequent) at loci that met variability criteria, the program ignored information provided by multiple alleles per locus and low-frequency alleles exclusive to one, or not present in any, parent source. Given the low proportion of hatchery winter adults (Table 3), we doubt that hatchery winter stock contributions to 1995 smolts were as large as the ADMIX point estimate suggests. We also think wild winter steelhead contributions may have been misallocated to the hatchery winter stock because of restricted data input.

Estimate accuracy was likely enhanced by the analytical advantages of the MLE program. All alleles at all variable loci were used as parent source data, which maximized differentiation among stocks. Input of multilocus genotypes of smolts allowed identification of individuals not attributable to baseline data. The very small contributions from the hatchery winter stock matched its low proportions in potential parents (Table 3). Also, all but two smolt genotypes from the 1995 and 1996 smolt samples were allocated to parent stock sources, which supports the intrastock breeding model.

This successful allocation of most smolts to a single parent source by mixed-stock MLE analysis gave us confidence that interbreeding among winter and summer steelhead was at very low levels. We recognize, however, that given the many alleles shared among stocks, interbreeding could produce genotypes more closely resembling those of a parent stock rather than a hybrid. Genotypic ambiguity may be a source of the relatively large MLE standard deviations, but this is difficult to evaluate because small sample size was likely the major contributor to large error values.

Whatever interbreeding may have occurred be-

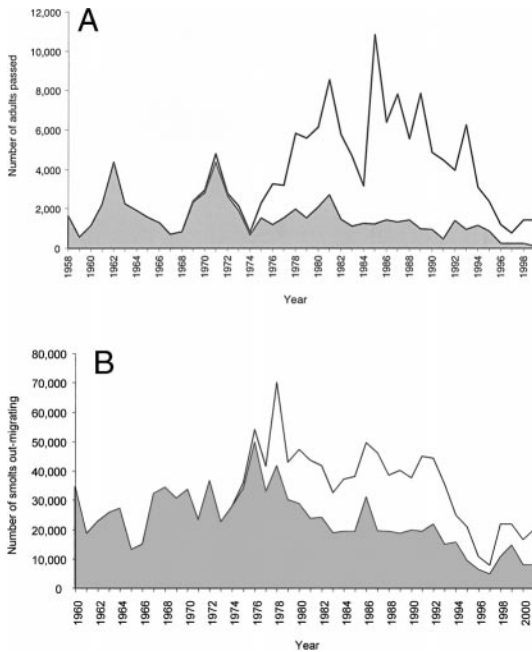


FIGURE 2.—Panel (A) shows the number of steelhead adults that passed into natural spawning areas above North Fork Dam on the Clackamas River from 1958 to 1999. The gray area shows the wild winter steelhead adults, while the line shows the total number of adults. The difference between total and wild winter adults was primarily hatchery summer steelhead, although small numbers of hatchery winter steelhead and naturally produced summer steelhead were also passed in some years. Panel (B) shows the number of naturally produced steelhead smolts out-migrating past North Fork Dam from 1960 to 2001. The gray area shows the estimated number of wild winter steelhead smolts, while the line shows the total number of smolts observed. The difference between total and the estimated wild winter smolts is estimated summer steelhead smolts, assuming that summer steelhead production over the duration of the hatchery program was similar to that in our 2 study years.

tween hatchery and wild fish, it has not diminished the genetic and biological distinctiveness of the wild winter steelhead population, and we do not believe it has had an effect on the productivity of the wild population. The decline in wild winter steelhead abundance was not likely due to diminished reproductive success of a greatly hybridized population.

Hatchery summer steelhead were able to produce smolt offspring, but they did so with much less success than wild winter steelhead. In the 2 years of this study hatchery summer steelhead produced about a third or less as many smolts per parent and about a tenth or less as many adult

offspring per parent as wild winter steelhead did. Theoretical work by Lynch and O'Hely (2001) predicted that hatchery stocks like the nonnative, mixed-origin South Santiam Hatchery summer stock, which has been in artificial production for many generations, should have substantially depressed fitness in a stream environment compared with the local wild population. Our results are consistent with Chilcote et al. (1986) and Leider et al. (1990; but see also Campton et al. 1991), who demonstrated poor reproductive success in Kalama River, Washington, of Skamania Hatchery summer steelhead, the progenitor of the South Santiam stock.

Relatively poor reproductive success by hatchery summer steelhead did not preclude these fish from producing one-third to one-half of the wild (natural origin) smolts in the Clackamas basin in the 2 years of our study. This most likely occurred because hatchery summer steelhead formed the largest proportions of spawners that produced our study smolts (Table 3). Hatchery summer steelhead also predominated in the natural spawning population in most other years of the hatchery program. From 1975 through 1999, they made up 30–88% of the steelhead passed above North Fork Dam, according to dam counts (Figure 2a). If we assume their contributions to wild smolts in other years were similar to those estimated for 1995 and 1996, we can show that annual wild winter steelhead smolt production appears to have declined over the 24-year period (Figure 2b). This apparent decline was masked by the presence of summer steelhead smolts in the total smolt counts at the dam.

We conclude that even though naturally spawning hatchery steelhead may experience poor reproductive success, they and their juvenile progeny may be abundant enough to occupy substantial portions of spawning and rearing habitat to the detriment of wild fish populations. The capacity of the Clackamas basin to produce steelhead smolts is expected to be finite (Allen 1969). Therefore, the large numbers of introduced summer steelhead would have competed heavily with wild winter steelhead for habitat resources, and this may have contributed to their decline. We will investigate potential ecological effects of the summer steelhead on the productivity of the wild winter steelhead population in a second paper.

In the Clackamas basin, smolt offspring of hatchery fish appear to have wasted the production from natural habitat because very few survived to return as adults. The summer steelhead hatchery

program was not intended to produce a natural spawning population, but the adult offspring, at least, could have contributed to fisheries. However, second-generation production failure also could be a potential risk for hatchery supplementation programs that seek to produce adult returns from naturally spawning hatchery fish and thereby boost wild population size. We caution managers about concluding that natural spawning and smolt production by hatchery fish is evidence for the success of supplementation programs. Evidence for success must also include returning adult offspring and no depression of wild fish productivity. Potential competition between hatchery and wild fish for habitat is pertinent to supplementation programs where natural reproductive success by hatchery fish is the major goal. Supplementation programs should be attuned to basin carrying capacities so that they do not reduce wild fish productivity through competition for resources.

#### Acknowledgments

Funding for this study was provided by Portland General Electric (PGE). Doug Cramer, PGE, and Sharon Vendshus of ODFW provided field and sampling support. Genetic analysis was conducted at the Washington Department of Fish and Wildlife Genetics Laboratory, with special thanks to intern David Cierebiej. Also thanks to Robin Waples, Mark Chilcote, and an anonymous reviewer for valuable reviews, comments, and discussions.

#### References

- Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA Technical Report NMFS 61:1–19.
- Allen, K. R. 1969. Limitations on production in salmonid populations in streams. Pages 3–18 in T. G. Northcote, editor. Symposium on salmon and trout in streams. University of British Columbia, Vancouver.
- Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz, and I. V. Lagomarsino. 1996. Status review of West Coast steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum NMFS-NWFSC-27.
- Campton, D. E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. Pages 161–192 in N. Ryman and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.
- Campton, D. E., F. W. Allendorf, R. J. Behnke, F. M. Utter, M. W. Chilcote, S. A. Leider, and J. J. Loch. 1991. Reproductive success of hatchery and wild steelhead. Transactions of the American Fisheries Society 120:816–827.
- Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550–570.
- Chilcote, M. 1998. Conservation status of steelhead in Oregon. Oregon Department of Fish and Wildlife, Portland.
- Chilcote, M. W., S. A. Leider, and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Transactions of the American Fisheries Society 115:726–735.
- Fraser, F. J. 1969. Population density effects on survival and growth of juvenile coho salmon and steelhead trout in experimental stream-channels. Pages 253–266 in T. G. Northcote, editor. Symposium on salmon and trout in streams. University of British Columbia, Vancouver.
- Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48:945–957.
- Keeley, E. R. 2001. Demographic responses to food and space competition by juvenile steelhead trout. Ecology 82:1247–1259.
- Leider, S. A., M. W. Chilcote, and J. J. Loch. 1984. Spawning characteristics of sympatric populations of steelhead trout (*Salmo gairdneri*): evidence for partial reproductive isolation. Canadian Journal of Fisheries and Aquatic Sciences 41:1454–1462.
- Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239–252.
- Lessa, E. P. 1990. Multidimensional analysis of geographic genetic structure. Systematic Zoology 39:242–252.
- Lindsay, R. B., K. R. Kenaston, and R. Kirk Schroeder. 2001. Reducing Impacts of Hatchery Steelhead Programs. Oregon Department of Fish and Wildlife, Information Report 2001-01, Portland.
- Long, J. C. 1991. The genetic structure of admixed populations. Genetics 127:417–428.
- Lynch, M., and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2:363–378.
- Marshall, A. R., H. L. Blankenship, and W. P. Connor. 2000. Genetic characterization of naturally spawned Snake River fall-run chinook salmon. Transactions of the American Fisheries Society 129:680–698.
- McMichael, G. A., T. N. Pearsons, and S. A. Leider. 2000. Minimizing ecological impacts of hatchery-reared juvenile steelhead trout on wild salmonids in a Yakima basin watershed. Pages 365–380 in E. E. Knudsen, C. R. Steward, D. D. MacDonald, J. E. Willams and D. W. Reiser, editors. Sustainable fisheries management: Pacific salmon. Lewis Publishers, New York.
- Millar, R. B. 1987. Maximum likelihood estimation of

- mixed stock fishery composition. *Canadian Journal of Fisheries and Aquatic Sciences* 44:583–590.
- Milner, G. B., D. J. Teel, and F. M. Utter. 1983. Genetic stock identification study. National Marine Fisheries Service Report to the Bonneville Power Administration, Contract DE-A179-82BP28044-M001, Portland, Oregon.
- ODFW (Oregon Department of Fish and Wildlife). 1992a. Clackamas subbasin fish management plan. Oregon Department of Fish and Wildlife, Portland.
- ODFW (Oregon Department of Fish and Wildlife). 1992b. Santiam and Calapooia subbasin fish management plan. Oregon Department of Fish and Wildlife, Portland.
- Phelps, S. R. 1991. Genetic analysis of Yakima River steelhead: initial analysis of within-basin genetic diversity and comparison to hatchery steelhead and rainbow trout. 1991 Annual Progress Report of the Washington Department of Fisheries to the Bonneville Power Administration, Contract DE-B179-89BP00102, Portland, Oregon.
- Phelps, S. R., B. M. Baker, P. L. Hulet, and S. A. Leider. 1994a. Genetic analysis of Washington steelhead: initial electrophoretic analysis of wild and hatchery steelhead and rainbow trout. Washington Department of Fish and Wildlife, Report 94-9, Olympia.
- Phelps, S. R., L. L. LeClair, S. Young, and H. L. Blankenship. 1994b. Genetic diversity patterns of chum salmon in the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 51(Supplement 1):65–83.
- Reisenbichler, R. R., and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* 34:123–128.
- Reisenbichler, R. R., J. D. McIntyre, M. F. Solazzi, and S. W. Landino. 1992. Genetic variation in steelhead of Oregon and Northern California. *Transactions of the American Fisheries Society* 121:158–169.
- Reisenbichler, R. R., and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES Journal of Marine Science* 56:459–466.
- Rohlf, F. J. 1994. NTSYS-pc: numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, New York.
- Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990a. Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* 119:2–15.
- Shaklee, J. B., C. Busack, A. Marshall, M. Miller, and S. R. Phelps. 1990b. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon. Pages 235–265 in Z. I. Ogita and C. L. Markert, editors. *Isozymes: structure, function, and use in biology and medicine*. Progress in Clinical and Biological Research, volume 344. Wiley, New York.
- Shaklee, J. B., and S. R. Phelps. 1990. Operation of a large-scale, multi-agency program for genetic stock identification. Pages 817–830 in N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. *Fish-marking techniques*. American Fisheries Society, Symposium 7, Bethesda, Maryland.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*, 2nd edition. Freeman, San Francisco.
- Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Champaign.
- Waples, R. S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48:124–133.
- Weir, B. S. 1979. Inferences about linkage disequilibrium. *Biometrics* 35:235–254.