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Natural Reproductive Success of First-generation Hatchery Steelhead Spawning in the Kalama River: A Progress Report

by Cameron S. Sharpe, Patrick L. Hulett, Chris W. Wagemann, Maureen P. Small and Anne R. Marshall

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Introduction

Hatchery-produced anadromous salmonids return to their rivers of release and some of them spawn, or attempt to spawn, among themselves and with native conspecifics in natural habitats. The nature and degree of the interactions between the hatchery and wild fish is of considerable concern because of the potential for those interactions to pose genetic and ecological risks to the extant wild populations (Waples 1991; Hindar et al. 1991; Busack and Currens 1995; McMichael et al. 1997; Ford 2002; Kostow et al. 2003; Araki et al. 2008).

The use of local wild-origin broodstocks in hatchery programs has received considerable attention by WDFW (e.g., 21st Century Salmon and Steelhead Initiative (WDFW 2009)) and other agencies responsible for fish management in the Pacific Northwest. Wild broodstocks are increasingly being used or recommended for use in supplementation applications (intended to increase natural production of depressed stocks) and also in harvest augmentation applications (intended to provide harvest opportunity). Current theory indicates that genetic risks to wild populations might be contained if the degree of genetic similarity between hatchery and wild stocks is high (e.g. Krueger et al. 1981; Allendorf and Ryman 1987; Fleming and Gross 1993). The presumption is that hatchery programs based upon or integrating locally adapted wild fish as broodstock would pose the least risk to wild populations. Alternatively, interbreeding between wild fish and fish domesticated for any number of generations may pose an unacceptable level of risk by causing shifts in genetically based performance traits and reducing survival in natural environments (Reisenbichler 1999, Araki et al. 2007).

The goal of the Kalama research program is to identify and empirically quantify risks imposed by hatchery programs on natural production of anadromous salmonids, and identify strategies to manage those risks. Studies of steelhead genetics, ecology, and life history have been ongoing in the Kalama River since the mid-1970's. A primary objective of Kalama research work has been to assess the relative reproductive performance and contribution of hatchery and wild steelhead spawning in the wild. For the purposes of this report, wild fish are defined as naturally produced fish, regardless of ancestry, and hatchery fish are those spawned and reared for some portion of their life in the hatchery environment. Earlier Kalama work focused on evaluating reproductive competence of highly domesticated, non-locally derived hatchery fish. The primary objective was to assess the reproductive performance of these hatchery fish relative to wild steelhead spawning in the wild. That work showed that highly domesticated steelhead of non-local origin (both summer and winter races) exhibited much lower natural reproductive success in the Kalama River than sympatric wild fish (Chilcote et al.1986; Leider et al. 1990; Hulett et al. 1996), an outcome supported by work in other watersheds (Araki et al. 2006, Mclean et al. 2003, Kostow et al. 2003).

The objective of the current Kalama research effort is focused upon estimating reproductive success of first-generation wild broodstock hatchery summer-run steelhead that were passed upstream to spawn among an approximately equal number of wild steelhead in 2003, 2004, and 2005. These brood years are our three experimental replicates. Most returning steelhead from natural production in those brood years have been, or will be, genetically sampled in 2007, 2008, and 2009. Anadromous offspring that returned in run year 2007 have been pedigreed back to their hatchery and wild parents. This document provides results of this first replicate of the experiment: comparison of production of anadromous adults from wild broodstock hatchery and wild steelhead spawning in 2003. A portion of the adult returns from the second replicate (2004) were also captured in run year 2007 and that portion of the results from the second replicate (adult returns from 2004 spawners) is also provided.

Methods

Study Area

The Kalama River, Washington is a westerly flowing tributary to the lower Columbia River entering the Columbia at river-kilometer (rkm) 117 (Figure 1). The watershed drains approximately 531 km^2 with flows ranging from 8.3 to 88.1 m^3/s mean monthly minimum and maximum, respectively (1946-74; United States Geological Survey data). Water temperatures range from 5° C (January) to 15° C (July). Two barriers (waterfalls) to anadromous adult migration exist in the system: one at the site of the Kalama Falls Hatchery (KFH) at rkm 17 and one at rkm 59. A hatchery fishway terminating in an adult trap bypasses the lower falls and provides access to virtually all anadromous adults attempting to enter the upper watershed to spawn. The upper falls is a complete barrier to upstream migration. An earthen pond in the upper watershed adjacent to Gobar Creek, a tributary to the Kalama at rkm 31, is used for final rearing and acclimation of a portion of the hatchery fish prior to release. Two endemic run forms of steelhead (*Oncorhynchus mykiss*) exist in the Kalama: winter-run (entering the river from November to June just prior to spawning) and summer-run (which enter the river from April through December, over-winter, and then spawn). Other endemic fish species include resident and anadromous cutthroat trout (*O. clarki*), mountain whitefish (*Prosopium williamsoni*), peamouth (*Mylocheilus carinus*), largescale sucker (*Catastomus macrocheilus*), Pacific lamprey (*Lampetra tridentatus*), and a number of cottids. In addition, resident rainbow trout (*O. mykiss*) are present in the system. Hatchery programs in the Kalama plant coho (*O. kisutch*) and Chinook (*O. tshawytscha*) salmon as well as non-local domesticated strains of both steelhead run types (summer-run: Skamania (Columbia Basin) stock; winter-run: Beaver Creek (Puget Sound origin) stock – see Crawford 1979).

Hatchery Practices

Broodstock Collection, Holding and Spawning

Wild summer-run steelhead were collected at KFH throughout the course of each adult return period (April through December) in 1998, 1999, and 2000 to avoid selection for differences in run-timing. Origin of the fish selected for broodstock was established by the presence of an adipose fin and absence of a stubbed dorsal fin. Most hatchery-origin steelhead returning to the Kalama have no adipose fin and a markedly eroded dorsal fin. Because the adult trap is 17 km

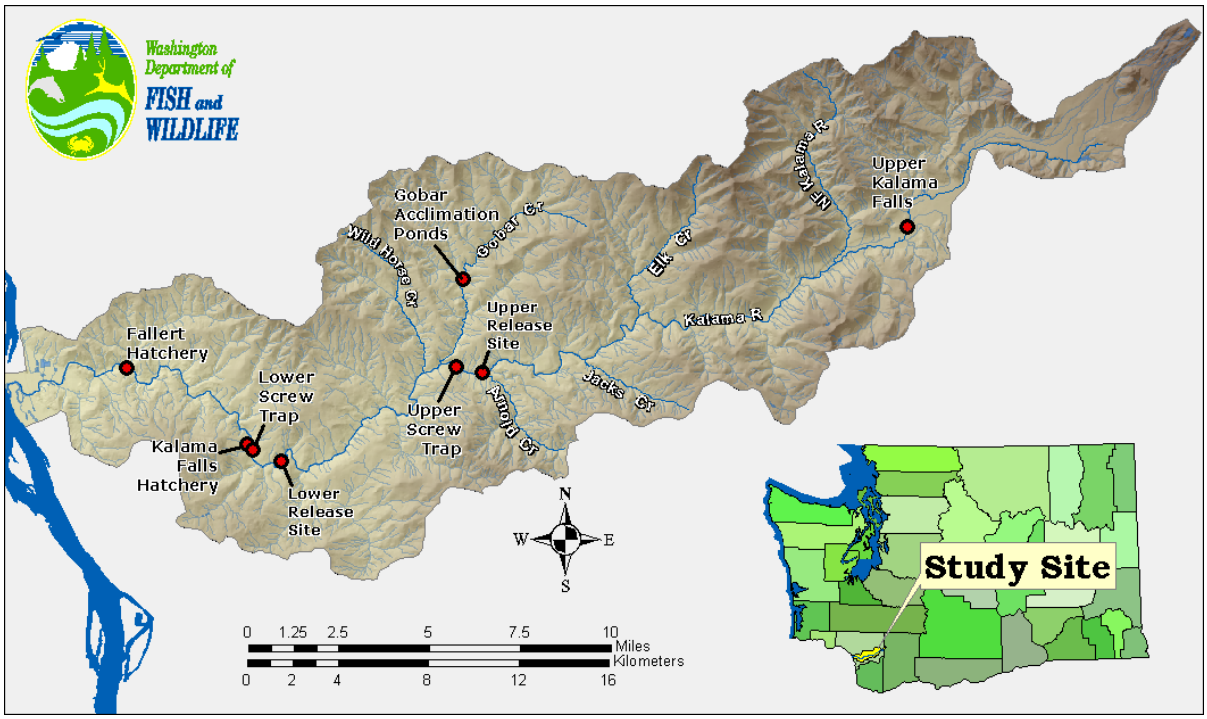


Figure 1. Map of the Kalama watershed.

upstream of the confluence with the Columbia, we assumed that all wild steelhead were of Kalama origin. Broodstock were held in a covered standard hatchery pond with formalin treatment as necessary to avoid fungus infection. Beginning in January of 1999, 2000, and 2001 the wild fish collected for broodstock were crowded in their holding pond, anaesthetized with MS222, checked for ripeness and, when judged to be ripe, spawned using a partial factorial mating design (generally 2M × 2F but, rarely, 3M × 3F and 1M × 2F).

Incubation, Rearing and Release

Eggs were incubated in incubation stacks or egg baskets in shallow troughs following normal hatchery protocols with a daily formalin treatment to control fungus growth. After absorption of the yolk sack, fry were transferred to intermediate rearing vessels (L × W × D = 4.7 × 1.0 × 0.5m). At approximately 2 gm total weight, fry were transferred to standard hatchery ponds and fed to satiation by hand several times per day. In approximately January of each year, marks were applied to the juvenile fish to permit identification when they returned as adults (Table 1). In March of 2000, 2001 and 2002 a portion (approximately 20%) of the yearling hatchery fish were transferred to Gobar Pond (Figure 1) for acclimation and volitional release beginning in April of each year and ending in late May. Also in May of each year the remainder of the juveniles were scatter-planted in the upper watershed using standard hatchery transport and planting procedures. The intent of the distributed planting was to ensure that upon return as

adults the hatchery-origin spawners would spread throughout the watershed rather than home to a single planting location.

Table 1. Marks applied to juvenile experimental brood hatchery fish to allow identification upon their return as adults. Brood Years 1999, 2000, and 2001 correspond to smolt release years 2000, 2001, and 2002, respectively. These are the wild-broodstock hatchery fish that, upon their return as adults, were passed upstream in 2002, 2003, and 2004 to spawn among themselves and with wild fish in 2003, 2004, and 2005. The estimates of numbers of smolts released were obtained from hatchery records.

Brood Year	Blank Wire Placement	Cold Brand ("letter", location)	Fin Clip	Smolts Released
1999	Snout	"S", left anterior dorsal	Adipose	69,939
2000	Snout	No brand	Adipose	39,274
2001	Right Cheek	No brand	Adipose	38,226

Enumeration, Passage and Sampling of Potential Parents of Experimental Broods

Control of the passage of adults above the KFH fish barrier and trap is an important element of the reproductive success study. It provides the opportunity to collect tissue samples for genetic identification from all potential anadromous parents above KFH, permits regulation of the proportions of wild and wild broodstock-origin adults upstream to desired 50:50 experimental level, and permits exclusion of domestic non-local origin hatchery stocks (Skamania summer-run and Beaver Creek winter-run) from upriver spawning and production areas.

Anadromous Adults

Anadromous adults were sampled at KFH as they attempted to ascend the watershed to the holding and spawning grounds above the hatchery. A modified barrier falls just below KFH is intended to block essentially all passage of summer-run steelhead and force the fish to use a ladder leading to an adult trap. Modification of the falls was necessary because earlier work showed that approximately half of the summer-run returning to the Kalama were able to jump the unmodified falls at lower flows (Bradford et al. 1996). Hanging a mesh curtain from a cable spanning the falls increased barrier efficiency (Sharpe et al. 2000).

All wild summer-run steelhead that returned to KFH in 2002, 2003, and 2004 (and expected to spawn in 2003, 2004, and 2005, respectively) were passed upstream except for wild fish sequestered as broodstock for the continuing hatchery program (Table 2). An approximately equal number of wild broodstock hatchery fish were also passed upstream (Table 2). For both hatchery and wild fish a tissue sample (fin clip) was obtained and run timing, size (fork length [FL], mm) and apparent gender were noted.

All adults either passed upstream or sequestered as broodstock received a colored floy tag (Floy Tag and Mfg. Co., Ltd., Seattle, WA). Snorkel surveys of the entire mainstem above KFH were conducted in September of each year to count tagged and untagged adults. We did not simply assume that untagged adults upstream of the hatchery were unsampled fish. Untagged adults upstream of KFH were fish that either got past the falls without being sampled or were adults that lost their tags. We calculated a tag loss rate from the number of tagged and untagged broodstock fish held at KFH in each year and used this rate as a correction factor to decrease the estimate of the number of unscreened adults.

Table 2. Wild broodstock (WB) hatchery and wild summer-run steelhead returns to KFH and numbers passed upstream to spawn in 2003, 2004, and 2005.

Category	Brood Year		
	2003	2004	2005
WB hatchery upstream	878	464	391
WB hatchery total to KFH	2,615	700	905
Wild upstream	921	530	427
Wild total to KFH	991	588	481

Resident Trout as Possible Parents

Resident trout can produce anadromous offspring (Olsen et al. 2006; Zimmerman and Reeves 2002; Marshall et al. 2006). Because naturally produced steelhead smolts from the Kalama larger than FL = 240 mm are exceedingly rare we think that any fish in the upper river larger than 240 mm were likely to be resident trout. We sampled the putative resident trout by electroshocking and angling from 1998 through 2005, generally in late summer through early fall, after the smolt migration had ended in each year, taking fin clips and recording biological data. We included as potential parents resident trout sampled as early as 1998 because sampling was non-lethal and, if the fish survived to spawn in subsequent years, they could have

contributed to anadromous production in the years we intensively screened for anadromous offspring (2007, 2008, and, eventually, 2009).

Residual Hatchery Steelhead as Potential Parents

Large numbers of residuals, defined here as yearling hatchery fish that failed to migrate with the rest of their cohort, were noted after each of the hatchery plants in 2000, 2001 and 2002 (Sharpe et al. 2007) and after all other plants (unpublished WDFW/KRT data). Because many of the residuals were mature or maturing males we thought some spawning with anadromous fish might have occurred either in the year the juveniles were released or in subsequent years if the juveniles over-wintered and survived to the next spawning season(s). We sampled residuals using electroshocking and angling in 2001, 2002, and 2005, obtaining DNA samples (fin clips), length, and noting sex and degree of sexual maturation. Importantly, sampling was lethal for the residuals so the only potential contribution to production of anadromous offspring had to occur prior to sampling. The implication is that a relatively small number of the residuals that we sampled actually had the opportunity to spawn and produce anadromous offspring but larger numbers might have over-wintered and had the opportunity to spawn in later years. If the residuals sampled in 2001 and 2002 did spawn and produce anadromous offspring, only 5- or 6-year-old offspring would be detected in our investigations. If the residuals sampled in 2005 produced anadromous offspring we are more likely to detect them because most of the offspring would return in the last two years of extensive sampling to pedigree anadromous fish (2008, and 2009). In 2007, only the very rare 2 year-old (1-fresh: 1-salt) anadromous fish would have been sampled.

Sampling of Anadromous Offspring of Experimental Broods

The wild broodstock hatchery- and wild-origin adults that spawned in 2003, 2004, and 2005 produced offspring that returned as anadromous adults beginning in 2005 (Table 3). The last anadromous adult from our experimental broods is expected to return in 2011. Most (85%) of the anadromous adult offspring from our experimental broods returned in 2007 and 2008 or will return in 2009 and samples from those fish are the focus of this report. Tissue samples from fish returning in 2005 and 2006 were obtained but have not been genotyped because of the expense in relation to the experimental data obtained (see Table 3). For example, in 2006 almost all samples (N = 448) would have to be processed but a relatively small percentage would be offspring of our experimental broods since the majority of the experimental offspring began to return in 2007.

Microsatellite DNA analyses

Laboratory Procedures

Fin clips collected from all fish in the study were stored in 100% ethanol at room temperature (Table 4). Genomic DNA was extracted from tissue samples using silica membrane kits (Macherey-Nagel). Microsatellite alleles were PCR-amplified using fluorescently labeled primers (see Appendix Table 1 for detailed PCR (polymerase chain reaction) information). Data processed prior to 2007 was PCR-amplified following protocols detailed in Small et al. (2007). For data processed after 2007 DNA primers had a poly-a tail added to reverse primers (indicated by "+a" after primer name) to stabilize the reaction. One plate of samples was run with both primer types to standardize allele nomenclature between different primer types. Several primers were fluorescently labeled with a vector tail in our lab (identified in Appendix Table 1 by the label "V") and the concentration for the primer and the vector are given in Appendix Table 1. The other primers were labeled at the factory when primers were constructed (no concentration given for vector). PCRs were combined in multiplexes and conducted in 384 well plates in 5 µl volumes employing 1 µl template with final concentrations of 1.5 mM MgCl2, 200µM of each dNTP, 0.05 µl Promega GoTaq and 1× Promega PCR buffer. For all multiplexes (except OmyU, see Appendix Table 1), we used a "touch-down" cycling protocol as follows: after initial two minute denature at 94°, there were 3 cycles consisting of denature at 94° for 30 seconds, annealing at 60° for 30 seconds (dropping annealing temperature one degree each cycle for three cycles) and extension at 72° for 60 seconds. These were followed by 36 cycles with the same parameters except the annealing temperature remained 50°. The final cycle was followed by a 10-minute extension at 72°. For OmyU, after initial two minute denature at 94°, there were 39 cycles with an annealing temperature of 49° for 30 seconds and extension at 72° for 60 seconds followed by a final 10-minute extension at 72°. Microsatellites were detected using an ABI 3730 automated DNA Analyzer, and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GeneMapper software (Applied Biosystems).

Table 3. Predicted age structure and relative abundance of returning anadromous adults from experimental broods of wild broodstock hatchery and wild fish spawning in 2003, 2004, and 2005. Shading represents core sampling years.

Offspring Returns by Fresh Water/Salt Water Age (n.n) & Calendar Year								
	2005	2006	2007	2008	2009	2010	2011	
2003 Spawners	1.1	2.1	3.1					1-salt
		1.2	2.2	3.2				2-salts
			1.3	2.3	3.3			3-salts
2004 Spawners		1.1	2.1	3.1				1-salt
			1.2	2.2	3.2			2-salts
				1.3	2.3	3.3		3-salts
2005 Spawners			1.1	2.1	3.1			1-salt
				1.2	2.2	3.2		2-salts
					1.3	2.3	3.3	3-salts
Offspring Returns by Expected Proportional Abundance (EPA)								
	2005	2006	2007	2008	2009	2010	2011	
2003 Spawners	0.016	0.088	0.014					1-salt
		0.163	0.542	0.046				2-salts
			0.027	0.097	0.007			3-salts
2004 Spawners		0.016	0.088	0.014				1-salt
			0.163	0.542	0.046			2-salts
				0.027	0.097	0.007		3-salts
2005 Spawners			0.016	0.088	0.014			1-salt
				0.163	0.542	0.046		2-salts
					0.027	0.097	0.007	3-salts
Importance of return year (= EPA/3)	**0.005**	**0.089**	**0.283**	**0.326**	**0.244**	**0.050**	**0.002**	

Pedigree Procedures

The 2007 returning offspring were tested for assignments to several groups of potential parents (Table 4). Since the returning offspring were nearly all ages 3 and 4, the most likely parents were wild- or hatchery-origin steelhead that had been passed upstream to spawn naturally in 2002-2003 and 2003-2004 (see Table 3). However, resident rainbow and some hatchery-origin residuals were present upstream during this time as well and may have generated anadromous offspring. We tested all groups (anadromous, resident, and residual) as potential parents. Offspring were assigned to possible parents using a maximum likelihood method implemented in CERVUS 3.0.3 (Marshall et al. 1998; Kalinowski et al. 2007). Assignments were conducted by first assigning both parents without knowledge of parental sex using the steelhead parental pool and the residents and residuals parent pool. We also made assignments with consideration to apparent gender of the potential parents by assigning offspring first to dam and then to sire and then first to sire and then to dam. We cross-checked parental assignments with the three methods and evaluated logarithm of odds (LOD) scores and delta values. LOD score is the natural logarithm of the likelihood ratio at each locus multiplied together and indicates whether the assigned parent is more likely than a randomly selected parent or parent pair. The delta value is the ratio of the highest likelihood LOD over the next most likely LOD. Simulations set with 75% of parents in the parent pool, 90% of loci scored and 5% of loci mistyped indicated a mean LOD score of 48.4 (7.39 SD) for a parent pair with neither parent known, and a mean LOD score of 20.5 (4.36 SD) for one parent assigned. We also evaluated the number of loci with allele mismatches in the putative parent-offspring pair and in the trio of putative parents and offspring. Mismatches arise from multiple sources (see Araki and Blouin 2005): mutation in offspring, scoring errors (artifact is scored rather than true allele or true allele is missed because the amplification is weak or allele is outside scoring range) and PCR problems (locus does not amplify or an error occurs during amplification). Given these sources of error, we accepted three or fewer mismatches in a parent offspring trio.

Table 4. Kalama summer steelhead collections of potential parents (anadromous adults, resident trout, and residuals) and adult anadromous offspring. Only fish with 12 or more loci in their genotype were included in analysis. WB=wild broodstock.

Code	Sample Groups	N > 12 loci
Fish passed upstream - possible parents		
02RP	2002-03 WB hatchery summer steelhead adults	873
03OB	2002-03 wild summer steelhead adults	897
04AAY	2003-04 WB hatchery summer steelhead adults	358
03NX	2003-04 WB hatchery summer steelhead adults, 3-salt	138
04AAZ	2003-04 wild summer steelhead adults	531
Fish captured upstream - possible parents		
98MG	upper Kalama River - Resident, sampled in 1998	33
99OU	upper Kalama River - Resident, sampled in 1999	16
00AAG	upper Kalama River - Resident, sampled in 2000	8
01IW	upper Kalama River - Resident, sampled in 2001	6
02AAK	upper Kalama River - Resident, sampled in 2002	12
03AAE	upper Kalama River - Resident, sampled in 2003	53
04ABA	upper Kalama River - Resident, sampled in 2004	4
05OC	upper Kalama River - Resident, sampled in 2005	9
01IW	Juvenile hatchery summer RESIDUALS collected 2001	7
02JV	Juvenile hatchery summer RESIDUALS collected 2002	9
05IG	Juvenile hatchery summer RESIDUALS collected 2005	99
Returning offspring to be assigned to parents		
08AS	2007-08 wild summer steelhead adults	204
08EK	Wild Summer Steelhead Hatchery Broodstock Spawned 2008	42

Statistics

We tested for differences in size and run-timing between, for example, wild fish used for broodstock vs. wild fish not selected for broodstock, hatchery fish passed upstream to spawn vs. hatchery fish not passed upstream, and hatchery fish passed upstream vs. wild fish passed upstream. Our intent was to use wild fish that were representative of all the available wild fish

as broodstock in the hatchery. Also, we intended to ultimately pass upstream hatchery and wild fish that were as phenotypically similar to each other as possible. Size and run-timing were generally not normally distributed nor were variances equal so we used the non-parametric Mann-Whitney Rank Sum test to perform each comparison. We used contingency table analyses (G-tests) to test for differences in sex ratios between wild and hatchery fish passed upstream. We used SigmaStat V.3.0.1 for general data analyses and the Rank Sum tests and PopTools V. 3.0 for the G-tests. Our intent in general was to avoid non-random selection of fish for broodstock in the hatchery and ensure that experimental hatchery and wild fish passed upstream to spawn were phenotypically similar; our null hypothesis in each case was that biological parameters were equal between fish selected for some purpose vs. fish not selected. In order to reduce the risk of a type-2 statistical error (falsely accepting a null hypothesis) for those tests only we adopted a significance level of $P = 0.2$, rather than the more stringent (and less conservative) P-value of 0.05. A significance level of $P = 0.05$ was used in all other cases.

Results

Selection of wild broodstock spawned in 1999, 2000, & 2001

Wild fish selected for broodstock to begin our hatchery program were similar to all fish available to select from but some statistically significant differences were noted. In two of the three years (2000 and 2001) the fish we selected as broodstock tended to be slightly larger than wild fish not selected as broodstock (by 12.5 and 20 mm, respectively: Table 5 and Figure 2). Also, in each year, the broodstock taken into the hatchery had a median capture date earlier than the median capture date of wild fish that were not selected as broodstock (by 14, 5 and 12 d in 1999, 2000, 2001, respectively: Table 5 and Figure 2).

Selection for upstream passage of hatchery steelhead in 2002, 2003, & 2004

The returning adult offspring that we selected for passage upstream to spawn naturally as our experimental hatchery fish were similar to the fish that we did not select for upstream passage. However, as with the broodstock spawned in the first years of the study, some statistically significant differences were apparent (Table 5 and Figure 3). In two of the three years (2002 and 2004) the hatchery fish passed upstream were slightly larger (5 mm in each year). Also, in 2004, median capture date of hatchery fish passed upstream was earlier (by 20 d) than median capture date of hatchery fish not passed upstream.

Upstream passage of hatchery and wild steelhead in 2002, 2003, & 2004

Phenotypic characteristics of fish passed upstream to spawn naturally were very similar between hatchery and wild fish but we did observe statistically significant differences (Table 5 and Figure 4). In all years the hatchery fish were larger than the wild fish (by 10 – 15 mm). In 2003 and 2005 the median capture date was earlier for hatchery fish (by 3 and 6 d, respectively). We found no difference in sex ratio of hatchery and wild fish passed upstream (Table 6).

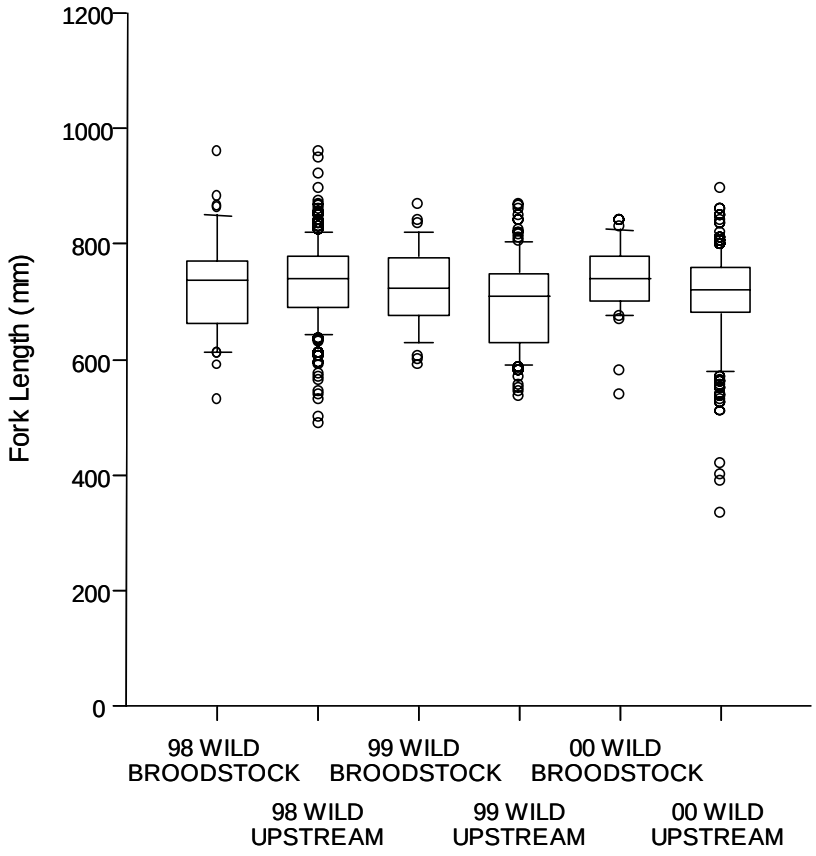
Table 5. Comparisons of size and run timing. Sample Sizes in parentheses. Significance levels less than P = 0.2 are in bold text.

Intent of Comparison	Comparison Performed	BY 1999 Median Size (FL:mm)	BY 1999 Median Capture Date	BY 2000 Median Size (FL:mm)	BY 2000 Median Capture Date	BY 2001 Median Size (FL:mm)	BY 2001 Median Capture Date
Tests for non-random selection of wild fish used for broodstock in the originating hatchery program	Wild fish used as broodstock	737.5 (44)	8/7/98 (44)	722.5 (36)	8/6/99 (36)	740 (42)	7/19/00 (44)
	Wild fish passed upstream	740 (248)	8/21/98 (249)	710 (133)	8/11/99 (134)	720 (278)	7/31/00 (278)
	Mann-Whitney *P*	0.339	**0.07**	**0.068**	0.834	**0.016**	**0.197**

Intent of Comparison	Comparison Performed	BY 2003 Median Size (FL:mm)	BY 2003 Median Capture Date	BY 2004 Median Size (FL:mm)	BY 2004 Median Capture Date	BY 2005 Median Size (FL:mm)	BY 2005 Median Capture Date
Tests for non-random selection of returning hatchery fish passed upstream to spawn among themselves and with wild fish	Hatchery fish selected for passage upstream	725 (873)	7/27/02 (877)	715 (464)	8/1/03 (463)	710 (399)	7/28/04 (398)
	Hatchery fish not selected for passage upstream	720 (1711)	7/24/02 (1720)	720 (218)	8/21/03 (217)	705 (896)	7/29/04 (895)
	Mann-Whitney *P*	**<0.001**	0.662	0.76	**<0.001**	**0.002**	0.494

Intent of Comparison	Comparison Performed	BY 2003 Median Size (FL:mm)	BY 2003 Median Capture Date	BY 2004 Median Size (FL:mm)	BY 2004 Median Capture Date	BY 2005 Median Size (FL:mm)	BY 2005 Median Capture Date
Tests for "phenotypic equality" of hatchery and wild fish passed upstream to spawn among themselves and with each other	Hatchery fish selected for passage upstream	725 (873)	7/27/02 (877)	715 (464)	8/1/03 (463)	710 (399)	7/28/04 (398)
	Wild fish passed upstream	715 (917)	7/30/02 (918)	700 (518)	8/5/03 (517)	700 (425)	8/3/04 (426)
	Mann-Whitney *P*	**<0.001**	0.46	**<0.001**	**0.026**	**<0.001**	**0.099**

Figure 2. Size and capture date of fish selected as broodstock (BROODSTOCK) and fish not selected as broodstock (UPSTREAM). The numbers 98, 99, and 00 represent run years and correspond to brood years 99, 00, and 01, respectively. Significance levels for pair-wise comparisons are provided in Table 5.



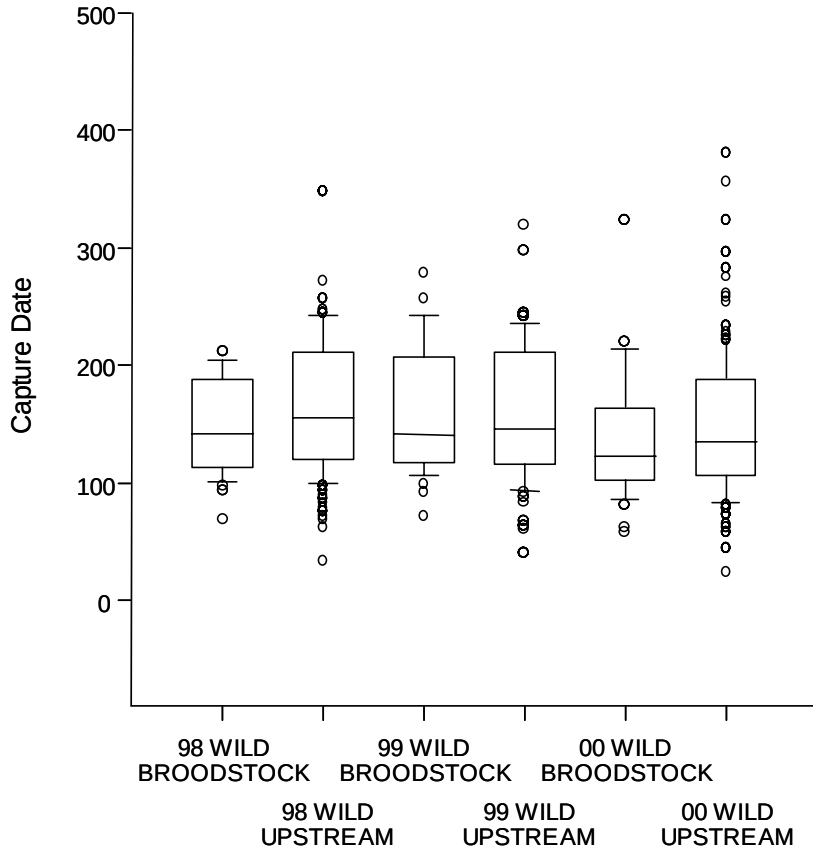
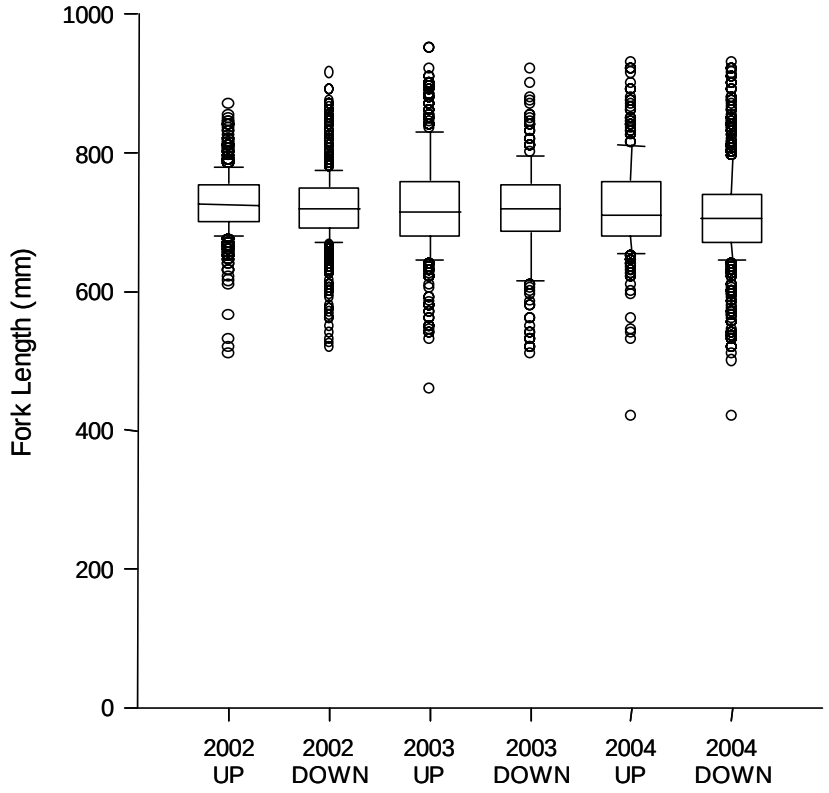


Figure 3. Size and capture date of returning hatchery fish passed upstream (UP) to spawn with wild fish and not passed upstream (DOWN) for run years 2002, 2003, and 2004 corresponding to brood years 2003, 2004, and 2005, respectively. Significance levels for pair-wise comparisons are provided in Table 5.



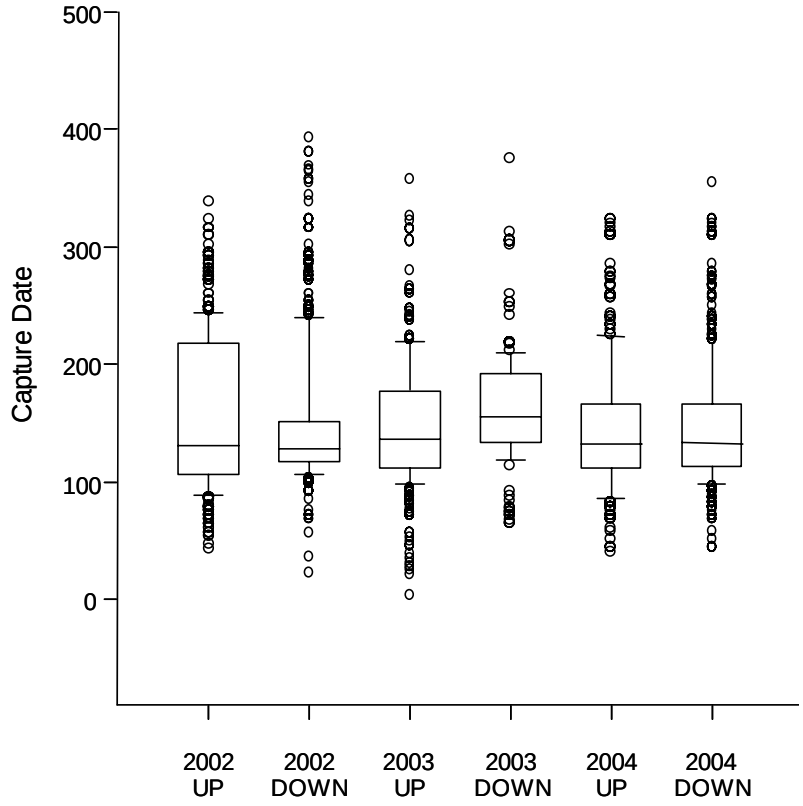


Figure 4. Size and capture date of hatchery (H) and wild (W) fish passed upstream to spawn in run years 2002, 2003, and 2005, corresponding to brood years 2003, 2004, and 2005, respectively. Significance levels for pair-wise comparisons are provided in Table 5.

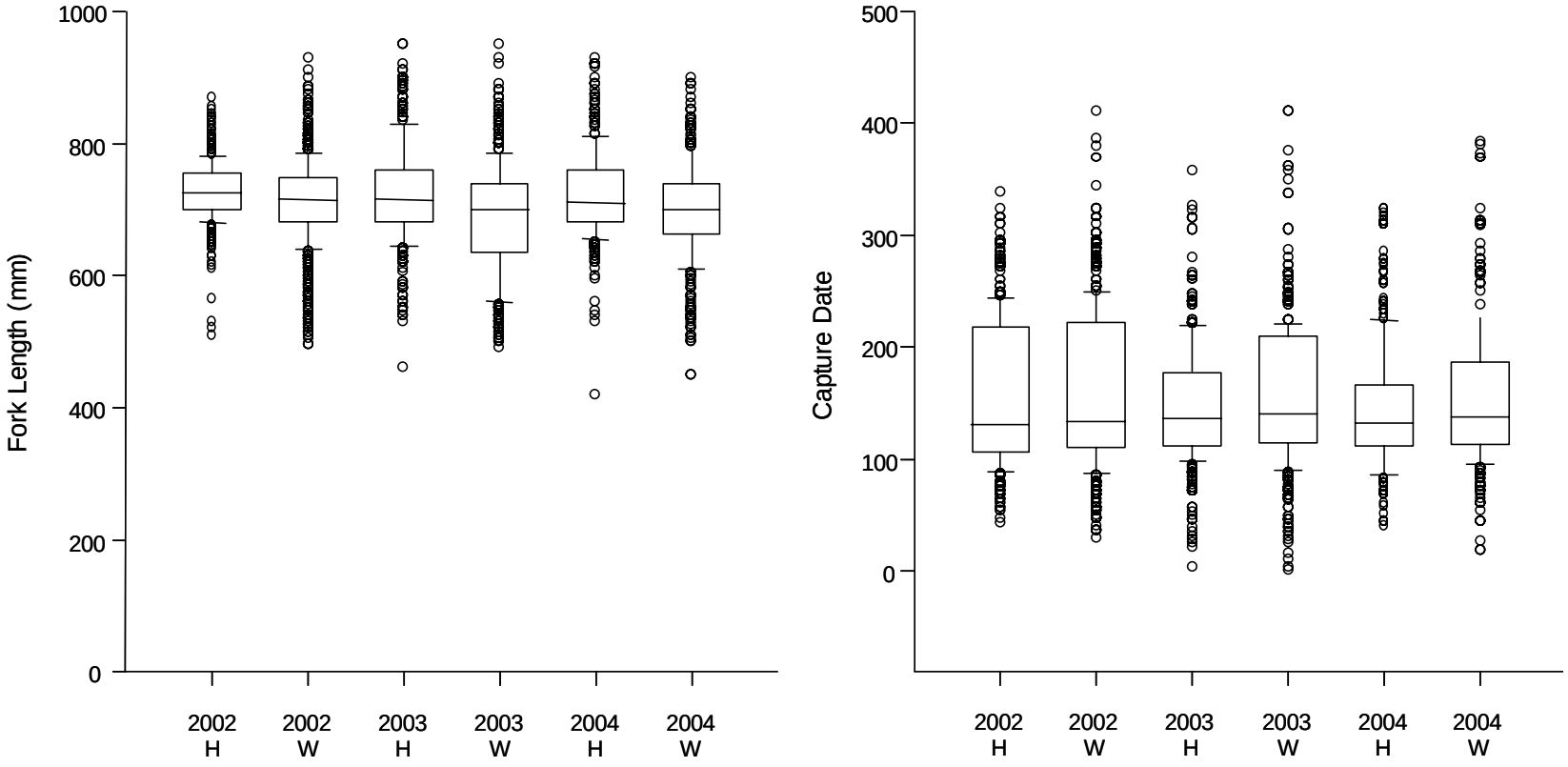


Table 6. Sex ratio comparisons between hatchery and wild fish passed upstream in 2002, 2003, and 2004.

		Males	Females	G (adj)	df	*P*-value
Run-year 2002 (2003 Spawners)	Wild Brood Hatchery Fish Passed Upstream	271	606	0.001	1	0.973
	Wild Fish Passed Upstream	283	635			
Run-year 2003 (2004 Spawners)	Wild Brood Hatchery Fish Passed Upstream	159	304	0.051	1	0.821
	Wild Fish Passed Upstream	174	343			
Run-year 2004 (2005 Spawners)	Wild Brood Hatchery Fish Passed Upstream	100	298	0.223	1	0.637
	Wild Fish Passed Upstream	101	325			

Characteristics of other potential parents

Resident Trout

A total of 154 putative resident trout were sampled between 1998 and 2005 (Table 7). We think that any of these fish are potential parents of naturally produced anadromous fish that have been or will be screened for this study. However, the resident trout samples obtained in 2003, 2004, and 2005 represent potential spawners whose offspring are most likely to be detected because of the intensity of anadromous fish sampling in 2007, 2008, and 2009. There is some possibility that a few of the resident trout captured were actually unusually large pre-smolts. Figure 5 shows that there is some overlap in size distribution between the resident trout below approximately 300mm FL and the upper size range of smolts captured in smolt trapping operations conducted between 2001 and 2005. However, the relative abundance of wild smolts larger than 250 mm is so low (21fish/13,960 smolts = 0.15%) we thought it prudent to include fish larger than 250 mm FL in the resident trout sample.

Table 7. Putative resident trout sampled from the Kalama River from 1998 through 2005.

Sample Year	N	Mean Size (FL, mm)	SE
1998	36	321.4	7.5
1999	16	341.7	8.5
2000	16	338.4	11.4
2001	6	314.8	15.9
2002	12	360.8	15.5
2003	54	365.2	5.9
2004	4	357.0	15.6
2005	10	357.1	18.7

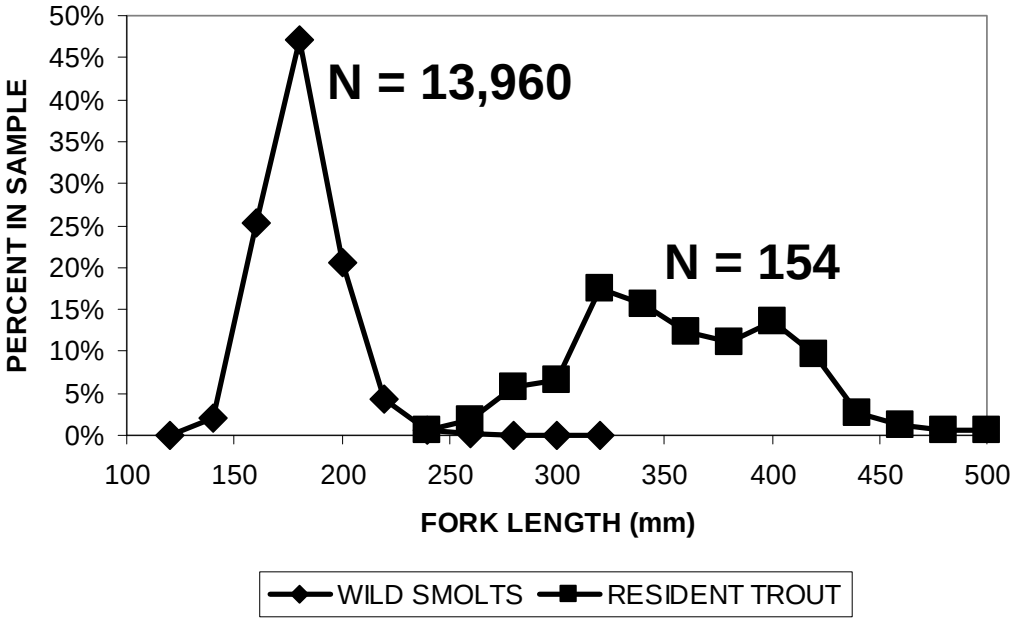


Figure 5. Size distributions of wild smolts and putative resident trout from the Kalama. Wild smolt size distribution from unpublished WDFW/KRT smolt trapping data from 2001 through 2005.

Residual Hatchery Steelhead

Summer-run residuals were sampled in 2001 (N=7), 2002 (N=9) and 2005 (N=99). Residual hatchery steelhead were always skewed male with a large proportion of those males maturing precociously (Table 8). They are included in this report as possible parents to the naturally produced anadromous offspring. However, there is only a small chance that they could be parents of the adults that have been screened. Sample sizes in 2001 and 2002 were small and adult offspring would have to be six or seven year-old fish to show up in the samples processed for this report. More samples were obtained in 2005 but most of their offspring would not yet have returned as anadromous adults. A small number (N=14) of the 2005 residuals were from earlier hatchery plants in 2001 (N=1), 2002 (N=1), 2003 (N=4), and 2004 (N=8) and those fish would have had multiple opportunities (two or more) to contribute to anadromous production. For example, like the remaining 2005 samples, they could have spawned with late-spawning fish in the year they were planted and, additionally, in each of the subsequent years they were alive (before lethal sampling in 2005).

Table 8. Residual hatchery steelhead sampled in 2005 as potential parents of naturally produced anadromous offspring. Of the males, % Male Precocity indicates the proportion that had either fully mature testes or had enlarged (developing testes).

Brood of Origin	N	Mean FL (mm)	SE	Sex M:F	% Male Precocity
1999	1	390.0	--	1:0	100%
2001	1	442.0	--	1:0	100%
2002	4	357.8	21.7	7:1	33%
2003	8	301.8	17.1	3:2	43%
2004	85	187.0	4.4	62:23	48%

Unscreened Anadromous Steelhead

In our snorkel survey in September 2002 virtually all of the anadromous adults sighted upstream had floy tags and we were thus assured that we had sampled most of the anadromous adults. In 2003 and 2004, a partial breach of the barrier falls permitted significant numbers of summer-run adults to pass upstream without using the fishway, where they would have been tissue-sampled and given a Floy tag. Based on September snorkel survey results in those years, 40-50% of the adults observed upstream of KFH in September were not Floy-tagged. We assumed that some of the untagged fish were fish that had lost their tags but that most were unscreened wild, wild

broodstock hatchery, and Skamania-origin hatchery summer-run steelhead. Tag loss rates for fish being held for broodstock at KFH in 2003 and 2004 were 18% and 17%, respectively. We assumed that tag loss in the river was approximated by the in-hatchery tag loss so 17% – 18% of the untagged fish actually had been sampled for DNA. We categorized the remaining untagged fish as unsampled and estimated their relative abundance (Table 9) among the potential anadromous spawners by partitioning them according to the relative abundance of the spawner types (wild, wild-broodstock, and Skamania-origin) encountered in the trap at KFH in 2003 and 2004.

Table 9. Estimates for abundance of different spawner types upstream in brood years 2003, 2004, and 2005.

Brood Year	Spawner Types Upstream	DNA Sampled	Not Sampled	Total Upstream	% Not Sampled
2003	Wild Summer-run:	921	18	939	2%
	H. Wild Brood Summer-run:	878	51	929	6%
	H. Skamania Summer-run:	0	54	54	100%
	Summer-run total:	1799	123	1922	6%
2004	Wild Summer-run:	530	97	627	15%
	H. Wild Brood Summer-run:	464	94	558	17%
	H. Skamania Summer-run:	0	487	487	100%
	Summer-run total:	994	678	1672	41%
2005	Wild Summer-run:	427	70	497	14%
	H. Wild Brood Summer-run:	391	59	450	13%
	H. Skamania Summer-run:	0	238	238	100%
	Summer-run total:	818	367	1185	31%

Characteristics of msDNA loci used for pedigrees

Forty-eight microsatellite DNA loci were examined for utility in genetically identifying steelhead adults in the Kalama River. Thirty-three of the 48 loci yielded data that could be scored and with one or two alleles per individual. Of these 33 loci, 11 were out of Hardy-Weinberg equilibrium (HWE) for homozygote excess (Appendix Table 1), tested using GENEPOP3.3 (Raymond and Rousset 1995). Sources of disequilibrium were explored by testing loci with Micro-Checker (Van Oosterhout, 2004) for possible null alleles, scoring errors due to stuttering, or large allele drop-out. For the loci out of HWE Micro-Checker indicated possible null alleles. However, given the suspected small size of the breeding population, HW disequilibrium could also result from mating among close relatives. GENEPOP was used to test loci for linkage (did loci appear to be on the same chromosome or were there further clues for

non-random mating?). The loci Ots-100 and Omm-1329 had a significant linkage value, which could also result from non-equilibrium conditions such as mating among relatives. We suggested retaining both loci since they worked well and the linkage may be an artifact of previous non-random mating. Loci were assessed qualitatively on scorability (i.e. were alleles easily defined, did alleles stutter or have an unusual electropherogram morphology, or was there a plethora of 1-2 bp alleles across a large size range, making scoring difficult?). Scores ranged from bad to good. For the first round of offspring assignments we recommended keeping the 26 loci with a score of "good" or "ok" regardless of HW value. After the first round of offspring assignments, six loci were determined to have low exclusionary power (described below), and thus added little to assignment power while increasing the cost of the project. Subsequent rounds of assignments (detailed below) were conducted using 20 loci and all original assignments were redone using 20 loci for offspring and adults.

The number of alleles per locus ranged from five to 35 (Appendix Table 1). CERVUS was employed to test loci for exclusionary power (probability of excluding a random individual in the parent pool as a parent) when neither parent or when one parent was known. Exclusionary power ranged from 0.071 to 0.749 (Appendix Table 2) and was dependent upon number of alleles per locus ($r^2 = 0.75$, $F = 72$, $P < 0.001$, Figure 6). We determined that six loci had low discriminating power (in bold in Appendix Table 3). These loci were excluded from parental and offspring genotypes and the assignment test was conducted again. Initially, the female parent assignment changed for 11/1098 offspring. However, when parents with few loci were re-extracted and re-genotyped, upon re-assigned offspring the assignments changed for only two offspring. In the interest of efficiency, we decided to eliminate the loci with low exclusionary power from further genotyping and analysis.

Pedigree assignments

With sex unknown, 91 offspring were assigned two parents with a high degree of certainty (three or fewer mismatches in the trio and with LOD scores above the 95% threshold in the simulation). One trio had three mismatches but the LOD score was below the threshold. Four offspring were assigned two parents with four mismatches in the trio and LOD scores above the threshold in the simulation and four offspring were assigned two parents with four mismatches in the trio and LOD scores below the threshold. Single parents were assigned for 70 offspring with three or fewer mismatches in the pair and LOD scores above the threshold in the simulation and four offspring were assigned a single parent with two or three mismatching loci and LOD scores below the threshold. All these assignments were to steelhead parents with the exception of one fish that was assigned a single resident parent, a 305 mm (FL) trout sampled on 2 October 2003 (the next most likely parent had six loci mismatched with offspring). A total of 164/246

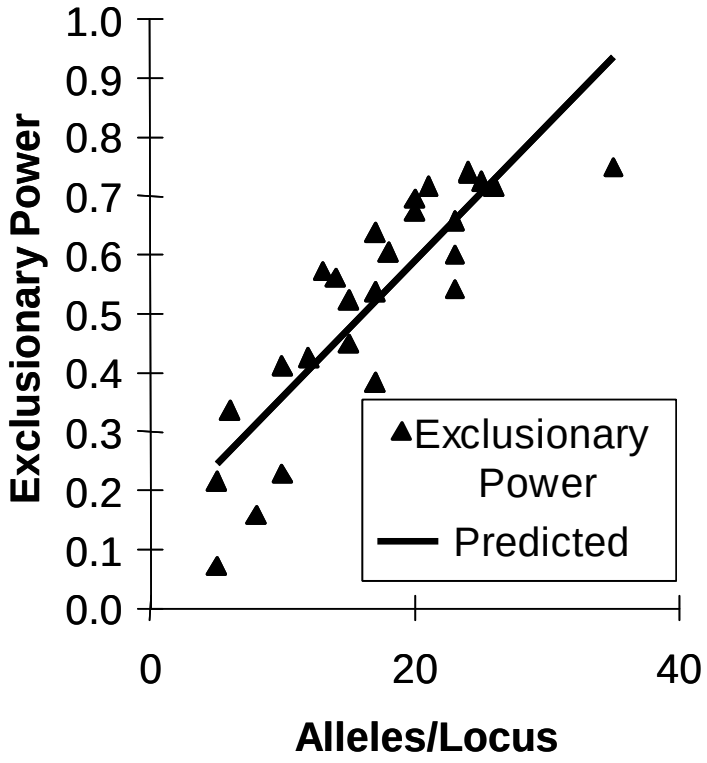


Figure 6. Relationship between exclusionary power and alleles/locus for loci used in this study.

offspring were assigned one or two parents above a 95% confidence threshold. Potential parents had been visually assessed for sex identification when they were passed upstream and we used these identities to assess parent pairs. Eighteen parent pairs appeared to be the same sex (seven male-male and 11 female-female, discussed below). The mis-assignment rate for sex identification was estimated at up to 10% for males (roughly 10% identified as males turned out to be females). This was attributed to difficulty in determining the sex of fish that were not in pre-spawning condition.

We reran the analysis first assigning dam and sire, then assigning sire and then dam. CERVUS matched the same dams and sires with offspring regardless of which parent was assigned first. Seventy-eight of the parent pairs matched the assignments with sex unknown. Differences occurred in the 16 matches assigned to same sex parents described above: when the program assigned the most likely sire (or dam), the second most likely parent was excluded from the assignment if it had been identified as a sire (or dam). In these cases, the program could only assign the second parent from the pool of putative parents of the opposite sex. In the 16 assignments that differed when sex was known, the trio mismatches were four or greater for the parent pair assigned with known sex and the LOD score was below the threshold value.

Reproductive Success

Ninety-one of the 204 sampled and genotyped anadromous adults from the 2007 run year were pedigreed back to sampled experimental steelhead that spawned in 2003 and 2004. The 91 offspring came from 73 unique pairings. We did not detect a difference in reproductive success of the wild broodstock hatchery spawners: the proportions of offspring from Hatchery × Hatchery (HH), Hatchery × Wild (HW), and Wild × Wild (WW) spawners closely approximated the proportions expected under the null hypothesis with reproductive success of hatchery spawners equal to that of wild spawners (Table 10).

In addition to the 91 offspring where both parents were identified, we identified a single parent for 74 offspring from 70 parents (some had multiple offspring). Of these, 26 of the parents were of hatchery origin, 43 were wild and one was a resident trout. Most of the anadromous single parents were female (M:F = 15:54) We compared the proportions of single-parent offspring hatchery- and wild-origin spawners and noted that, for offspring from the 2003 brood, significantly more wild fish were identified as the parent than hatchery fish (Table 11). None of the residual hatchery steelhead were identified as parents of any of the 2007 run-year anadromous steelhead. For both resident and residual trout it is likely that more anadromous offspring might be identified among offspring that were sampled in 2008 or will be sampled in 2009 since a higher proportion of offspring should return in those years.

Table 10. Comparison of observed (Obs) to expected (Exp) anadromous offspring returning from anadromous spawners in brood years 2003 and 2004. Hatchery (H) and wild (W) steelhead in experimental groups were passed upstream in nearly equal ratios. Observed returning offspring are those fish unambiguously pedigreed back to their anadromous hatchery and wild parents. Expected numbers of returning offspring were derived by assuming equal reproductive success of H and W fish. G-test statistics are from Sokal and Rohlf (1981: Tests for Goodness of Fit p. 705). Returns from the 2003 brood are essentially complete. Returns from the 2004 brood are partial.

	Experimental Genotyped Parents			Returning 2007 Anadromous Offspring by Parental Cross			G-test Statistics		
	H	W		HH	HW	WW	G	df	*P*
2003 Brood	873	897	Obs.	21	27	22	3.66	2	0.16
			Exp.	17	35	18			
2004 Brood	496	531	Obs.	4	9	8	0.89	2	0.64
			Exp.	5	10	6			

Table 11. Comparison of observed (Obs) to expected (Exp) anadromous offspring returning from anadromous spawners in brood years 2003 and 2004. Experimental group is the number of genotyped hatchery (H) and wild (W) steelhead passed upstream. Observed returning offspring are those fish unambiguously pedigreed back to a single hatchery or wild parent. The origin of the second parent is unknown. Expected numbers of returning offspring were derived by assuming equal reproductive success of H and W fish. G-test statistics are from Sokal and Rohlf (1981: Tests for Goodness of Fit p. 705). Returns from the 2003 brood are essentially complete. Returns from the 2004 brood are partial.

	Experimental			Returning 2007 Anadromous Offspring by Parental Type (Single-parent Offspring only)		G-test Statistics		
	H	W		H	W	G	df	*P*
2003 Brood	873	897	Obs.	23	43	6.11	1	0.01
			Exp.	33	33			
2004 Brood	496	531	Obs.	3	4	3.84	1	1
			Exp.	3	4			

Few fish have yet to be pedigreed back to the 2004 brood year so for general description of the data we combined the results for the two brood years. For both cross type (HH, HW, or WW) and spawner origin (H or W) we usually only noted a single offspring (Figures 7 and 8) but up to four offspring were identified as coming from one of the single-parents.

Problematic Genotype Scores and Pedigree Assignments

Erroneous Gender Assignments: Eighteen of the 73 crosses that were detected among the 2003 and 2004 spawners had the same gender recorded for the parental pairs (11 female:female and 7 male:male). We knew that our gender assignments were not 100% accurate because it is difficult to discriminate between male and female summer-run steelhead, especially early in the run year before sexual dimorphisms have developed. We thought, however, that the error rate was lower than was shown here. Because either gender could be misidentified, the error rate (18/73 = 24.7%) suggests that our accuracy on gender calls was about 11.1%. That estimate assumes (1) equal probability of calling a male female and a female male, and (2) a 1.5% probability that we miscalled gender on both parents in a pair.

Another independent estimate of the error rate in gender calls was available. The apparent gender of wild fish held at KFH for broodstock was recorded at the time the fish were captured and sequestered in the holding pond. In January of each year when the broodstock were first sorted and checked for ripeness we recorded the apparent gender again (after sexual dimorphisms had become

more apparent). In 1999, of the 43 fish used as broodstock, five females had originally been called male (12% error). In 2000 and 2001, zero of 36 (0%) and two of 43 (5%) females were originally called males, respectively. Therefore, the average error rate estimate was approximately 6%. We think that this is an underestimate of the true error rate because we never recorded an instance in those three years where a broodstock male was initially called a female. Since the pedigree data demonstrate that we had gender miscalls for both sexes we conclude that the true error rate per individual is likely close to that shown in the pedigree data (11.1%).

Crosses With Different Brood Years: Two of the 73 unique parental crosses were between parents sampled from the two different brood years. For that to occur one of the parents must have returned in consecutive years but not have been sampled in the year it produced the offspring that was eventually detected. Alternatively, the situation could have arisen through a sample processing error (a specimen placed in the wrong box) or possibly by misidentification of a downstream migrant kelt (sampled on the way out but not on the way upstream).

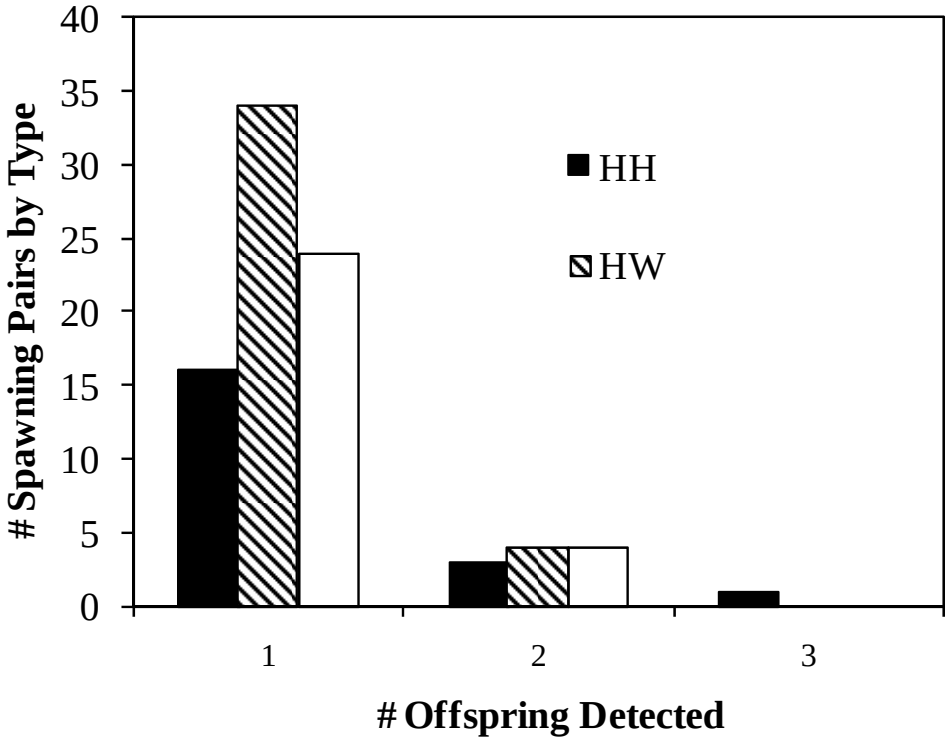


Figure 7. Offspring per cross type (2003 and 2004 broods, combined).

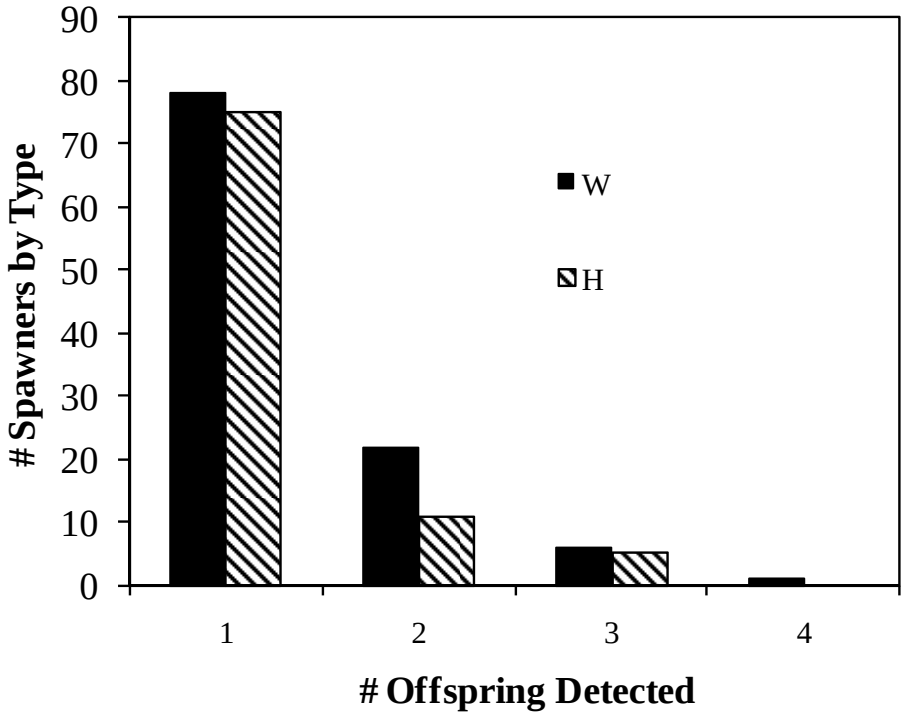


Figure 8. Offspring per parental type (brood years, single parent, and both parent, combined).

Low LOD Scores: Two of the offspring assignments, each with three allelic mismatches in the parent-offspring trio, yielded LOD scores (30.9 and 24.0) lower than the lower bound of the 95%CI for simulated LOD scores (33.9). For the purposes of this report we included those assignments in our analyses because they met the threshold for matching alleles but it may be the case that they were not correct assignments. We will continue to review this issue in future work.

Sample Matches: We encountered many instances (37) where different samples were genetically identical or nearly so (Appendix Table 4). Sample matches were identified among all potential parents, anadromous offspring, resident trout and residual hatchery steelhead. Most matches (19/37 = 51%) occurred because the same fish were sampled multiple times as repeat spawners. Eight of the 37 (22%) were resident trout that were probably sampled twice based on sample location, size, or, especially, presence of a punch scar in the caudal fin. The remaining ten matches (27%) were probably different fish with identical or nearly identical genotypes. The

significance to our study of that level of unexplained genotype matches has not yet been resolved but will be addressed in future work.

Discussion

Reproductive success of first-generation wild broodstock hatchery fish appeared to be similar to that of wild fish in the first replicate of our experiment. The outcome is in agreement with initial results from a similar reproductive success study on the Hood River, Oregon (Araki et al. 2006), where first generation wild-broodstock winter-run steelhead appeared to be as reproductively competent as the wild fish from which they were derived (but see Araki et al. 2007 and Araki et al. 2008). Because we present results from only the first of three replicates the results should be considered preliminary, especially since the results are somewhat equivocal. Considering only the "best" assignments, when a parents-offspring trio was unambiguously established, there is no statistical support for rejecting the null hypothesis that the hatchery and wild fish were equally reproductively competent. That outcome is supported by analysis of both the more complete returns from 2003 spawners and the partial returns from 2004 spawners. The confounding outcome is that when considering the assignments when only a single parent was identified, wild fish were significantly more abundant among those parents than hatchery fish. The latter observation suggests that wild fish, through some unknown mechanism, tended to spawn more with fish that were among the unscreened potential parents. For example, perhaps wild fish tended to enter tributaries more than the hatchery fish and spawn with resident trout. We included resident trout among our collections of potential parents but we think that the 154 resident trout specimens included only a fraction of the resident trout in the watershed. We expect that analysis of our radiotelementy studies of anadromous spawners (discussed below) may help us understand if there was unequal spatial distribution of hatchery and wild spawners. There are other equally speculative reasons for "single-parent" assignments, including the possibility that the phenomenon is just a sampling anomaly, and the issue will be addressed as we complete the study.

We think that the comparison of reproductive success between hatchery and wild fish was "fair" in the sense that we created our hatchery program using wild fish that were generally representative of the wild stock and passed upstream hatchery adults that were phenotypically similar to wild fish passed at the same time. We did, however, find statistically significant differences suggesting our broodstock selection and fish passage protocols were not genuinely random. That result is not unique to the Kalama program. Other work with steelhead (McLean et al. 2005) showed that hatchery steelhead programs tended to select larger early-returning fish for broodstock even when the intent was not to do so. We argue, however, that the differences in adult size and run timing in the Kalama program are small and unlikely to result in a detectable bias in estimates of reproductive success. For example, the largest size bias detected was the 20mm difference between wild fish collected for broodstock and wild fish not collected for broodstock in 2000, a difference of approximately 3%. Regarding temporal bias for selecting fish for any purpose (spawning or passage) vs. not selecting fish for that purpose, the average

difference was less than 1 week (6.9d). The maximum bias was 20d for hatchery fish selected for passage upstream in 1999. Because these fish hold in freshwater for approximately 7 months and up to 10 months (WDFW/KRT records, data not shown) before spawning we think it unlikely that a difference of a few days to a few weeks influenced our estimates of reproductive success.

It is not clear how problematic the presence of unscreened spawners in 2004 and 2005 will be. Clearly we can expect that a smaller proportion of returning adult offspring will be unambiguously assigned to known parents. We cannot, however, predict how many unassigned offspring will result from the unscreened parents. Most of the unscreened steelhead were probably of Skamania origin because most of the summer-run steelhead returning in those years were of that type. Because Skamania summer-run steelhead have demonstrably much lower reproductive success than wild summer-run (Chilcote et al. 1986) and because their spawn timing is 2 to 3 months earlier than wild fish it seems likely that the effect of the unscreened Skamania spawners might be less than that suggested by their abundance.

There were a relatively small number of anomalies in the pedigree results but, given the assignment rates and small run size, we think it is prudent to attempt to resolve the uncertainties. For example, there were some unexplained genotype matches or near matches among samples that were apparently taken from different fish. The significance of that phenomenon has not yet been adequately explored in this study but will be. Also, as noted in the results section, gender does not appear to be a particularly useful parameter to limit or validate assignments when gender calls are made by visual inspection. We think that it would be useful to use the recent advancements in DNA-based gender assignments (Brunelli et al. 2008) for *O. mykiss* for at least the parents of offspring when our gender identifications was of the same sex. Finally, a relatively large number of anadromous offspring were pedigreed to a single parent or none at all. Because we sampled most of the adult summer-run that spawned in 2003 we think it would be useful to determine the identity of the unknown parents. The candidates include resident wild trout and residual hatchery steelhead and, as noted in the methods and results sections, the likelihood of detecting anadromous offspring arising from non-anadromous parents increases for 2008 run-year samples. Other candidate parents include unscreened wild summer-run, hatchery summer-run, Skamania summer-run, early-run hatchery winter-run and wild winter-run with the last being very abundant during the experimental spawning years. Some of the returning anadromous adults might have been wild strays from, for example, the Lewis River summer-run steelhead stock. We think that it might be possible to use existing data to resolve the source of the unknown parents and untyped offspring. First we could use mixed stock maximum likelihood estimation (MLE: eg. Small et al. 2009) procedures to partition likely origins of the unassigned offspring. Baseline collections for the putative donor stocks have been run by MGL for some of the loci that we used for our study. If, for example, many of the unassigned

offspring are from the early-spawning Skamania escapees in the upper watershed then it should be possible to assign the unknowns to that stock or determine if a large proportion of the untyped "offspring" were simply strays from another watershed.

A number of assumptions must be considered as this project approaches completion. For example, there is no legal harvest of wild fish anywhere in the watershed and no legal harvest of hatchery fish above KFH. It does seem likely, however, that there are non-compliant anglers fishing in the Kalama. It is probably reasonable to assume that if equal numbers of hatchery and wild fish were illegally harvested then the results of our work were not compromised. What is not known is whether poachers would discriminate between hatchery and wild fish and perhaps harvest hatchery fish at a rate less than that of wild fish. It seems unlikely that poachers would remove more wild fish than hatchery fish.

Another assumption is that hatchery and wild fish had equal opportunity to interact reproductively. In a study of wild broodstock winter-run steelhead in the Chilliwack River, B.C. Canada (Nelson et al. 2005), spatial distribution of hatchery fish during holding appeared to match that of wild fish, but during spawning hatchery fish tended to spawn closer to their hatchery of origin while the wild fish spawned throughout the watershed. We scatter-planted our hatchery juveniles to ensure that they did not home preferentially to a single release location. While we assume that our planting procedures resulted in a more dispersed distribution of the hatchery fish we do not know if the spatial and temporal distribution of hatchery and wild fish were equal during holding (possibly influencing pre-spawning mortality) and spawning (possibly influencing survival during incubation and quality of rearing habitat after emergence). To address the issue, extensive radiotelemetry surveys of adult hatchery and wild adults were conducted in the watershed in 2003, 2004, and 2005 but the data have not yet been analyzed. That work is scheduled to begin this summer.

Sampling of residual hatchery fish and resident wild fish may have been inadequate to fairly estimate their contribution to anadromous production. For residuals, most were sampled in the year that they were released and they would only have overlapped slightly with the spawning season for summer-run steelhead. We did, however, sample some older residuals that had multiple opportunities to spawn with anadromous fish and all residual trout specimens will be retained in the potential parent pool in further analyses. For wild resident trout, many apparently had not yet spawned at the time we sampled them. Not all resident trout have been aged yet but several that were show no spawning check (data not shown) on their scales. Those fish are still potential parents because they were released alive and could have spawned in subsequent spawning seasons. As with the residual trout samples, resident trout will be included in the parent pools for further analyses. We expect that all scale samples from resident trout will be analyzed by the time we near completion of the study.

Finally, the issues of statistical power and experimental bias must be addressed more thoroughly than in this document. While the literature on the topics for salmonid reproductive success research is growing (Hinrichsen 2003, Araki and Blouin 2005, Araki et al. 2008) and the analytical tools are available, we have not yet directly estimated assignment error rates, one of the key parameters needed to estimate and correct for statistical bias. We think we have the data in hand to perform those analyses because as we were developing the protocols to perform the pedigree assignments (Small et al. 2006) we pedigreed relatively large numbers of known offspring (residual hatchery steelhead) of known crosses (our hatchery broodstock). It should be possible to directly estimate locus-specific assignment error rates from those assignments and that work will be done as the project progresses. It will be important to do so.

In summary, our results are consistent with expectations based on review and ongoing consideration of the literature. However, the statistical power and precision in our first replicate was lower than desired. The run size for wild summer-run steelhead in 2007, and thus the number of adult offspring available for sampling, was relatively low. Because statistical power and precision for these types of experiments is in large part driven by the number of offspring that can be assigned, our run size, assignment rates, or both would have to be higher to achieve the desired statistical power for this single replicate. We do expect that, because approximately 15% of the offspring from the 2003 spawners were sampled in 2008, statistical power for the first replicate will increase when those samples have been added to the analyses. There is another opportunity to increase the number of offspring assigned to fish that spawned in 2003. Examination of length-frequency data has recently been completed for anadromous adults that have returned to date and it is now apparent that approximately 100 or more 1-salt fish (jacks) from 2003 spawners returned in 2006. That is an unusual and unexpectedly large number of jack returnees. Our intent was not to include 2006 returnees in the pedigree analyses but there were so many of them that they might well represent more than the expected 8.8% of the total cohort (see Table 3). Since only about 300 fish that returned in 2007 were potential offspring of 2003 spawners, the 100 or more jacks that returned in 2006 might allow us to increase assignments by about 25%. We will explore this option in the near future and we are pursuing supplemental funding to support the additional work.

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Appendix Table 1. Table of PCR conditions for loci used in Kalama steelhead parentage analysis. All reactions were conducted with 1.5mM final concentration for MgCl2, 1x Promega PCR buffer, and 0.05Units of Taq DNA polymerase. Loci were combined into multiplexes where samples were PCR-amplified at two or more loci in a single reaction. "V1, V2, V3, and V4" indicate that vector tails were added to the primers, and +a indicates the addition of a poly-a tail to the primer. All multiplexes were run with a "touch-down" protocol except OmyU. In the touchdown, initial temperature was dropped one degree each cycle for three cycles and the final annealing temperature was 50 degrees for 36 cycles.

Multiplex	Primer/Vector	Dye label	Conc [uM]	Anneal T	# Cycles
OmyI	Omm-1302 V2 +a	none	0.24	50	36
	V2	6fam	0.12		
	Omm-1306 V3 +a	none	0.28		
	V3	ned	0.14		
	Omm-1321 V4 +a	none	0.2		
	V4	pet	0.1		
OmyJ	Omm-1322 V1 +a	none	0.34	50	36
	V1	vic	0.17		
	Omm-1316 V3 +a	none	0.38		
	V3	ned	0.19		
	Omm-1329 V4 +a	none	0.22		
	V3	pet	0.11		
OmyL	One-102+a	6fam	0.13	50	36
	Ots-100 +a	ned	0.06		
OmyN	Omm-1070 +a	vic	0.09	50	36
	Omy-1011 +a	ned	0.06		
OmyO	Omy-1001 +a	6fam	0.07	50	36
OmyQ	Oki-10 +a	none	0.12	50	36
	V4	pet	0.06		
OmyS	One-108 +a	6fam	0.06	50	36
	One-114 +a	vic	0.12		
OmyT	Omm-1130 +a	6fam	0.09	50	36
	Omm-1128 +a	vic	0.07		
	Omy-77 +a	ned	0.06		
OmyU*	Sco-110 +a	6fam	0.1	47	39
	Sco-103 +a	vic	0.07		
	One-18 +a	ned	0.05		

Appendix Table 2. Locus data for Kalama summer steelhead reproductive success study. The score column has a qualitative assessment of locus scorability: good = good, ok = a few 1 bp alleles, good? = scorable but mix of 2 and 4 bp alleles or very wide range, mixed = mix.

Locus	Score	Ho	He	FIS	*P*-value	Repeat unit (bp)	Min	Max	Total Alleles	HWE P-value
One-102	Good	0.91	0.90	-0.01	0.66	4	182	290	20	0.62
One-114	Good	0.89	0.91	0.03	0.20	4	181	281	20	0.07
Ots-100	Good	0.86	0.85	-0.02	0.79	2	160	236	23	0.05
One-101	Good	0.52	0.56	0.09	0.11	4	119	243	8	0.01
One-108	Good	0.95	0.92	-0.03	0.95	4	161	295	26	0.05
Ots-103	Good	0.36	0.37	0.03	0.36	4	60	86	5	0.30
Omy-77	Good	0.80	0.81	0.02	0.34	2	97	147	15	0.04
Ots-1	Good?	0.61	0.75	0.20	0.00	2	162	251	17	0.00
Ots-3M	Good	0.66	0.64	-0.04	0.83	2	130	152	10	0.36
Omm-1070	Good	0.92	0.93	0.01	0.32	4	164	267	24	0.49
Omm-1130	Good?	0.86	0.92	0.06	0.01	4	204	357	21	0.00
Omy-1011	Good	0.90	0.89	-0.01	0.68	4	138	252	17	0.80
Oki-10	Good	0.87	0.85	-0.02	0.83	4	99	176	17	0.82
Omm-1128	ok	0.87	0.93	0.07	0.00	4	219	377	29	0.00
Omy-1001	Good	0.83	0.87	0.05	0.04	2	171	238	22	0.04
One-18	Good	0.74	0.78	0.05	0.09	2	166	186	10	0.04
Omm-1304	Good	0.77	0.75	-0.03	0.76	4	242	262	6	0.13
Omm-1329	Good	0.82	0.87	0.05	0.05	4	162	215	13	0.19
Omm-1302	Good	0.87	0.86	-0.01	0.68	4	229	279	14	0.65
Omm-1306	Good	0.88	0.90	0.02	0.29	2	304	398	23	0.05
Omm-1321	Good	0.71	0.84	0.15	0.00	4	261	325	14	0.00
Omm-1316	Good	0.80	0.92	0.13	0.00	4	289	398	25	0.00
Omm-1322	Good	0.78	0.87	0.11	0.00	4	197	272	15	0.01
Omm-1325	Bad	0.62	0.66	0.05	0.13	4	302	329	7	0.18
Omm-1310	Bad	0.41	0.79	0.49	0.00	2	232	317	18	0.00
Omm-1029	Bad	0.19	0.41	0.53	0.00	2	203	334	15	0.00
Ogo-3	Mixed	0.63	0.72	0.12	0.00	2	182	201	10	0.00
Ots-101	Mixed	0.56	0.94	0.41	0.00	4	185	459	45	0.00
Ots-107	Bad	0.59	0.91	0.35	0.00	4	181	464	57	0.02
Sco-110	Good	0.64	0.79	0.19	0.00	4	150	263	12	0.00
Omm-1138	Good	0.62	0.63	0.01	0.44	2	142	151	5	0.57
Omy-325	Bad	0.92	0.94	0.02	0.12	1	88	155	31	0.00
Sco-103	Good	0.93	0.93	-0.01	0.65	4	199	303	24	0.73
All				0.09	0.00			Mean	18.73	

Appendix Table 3. Locus summary including number of alleles (k), number of parents genotyped per locus (NP), number of offspring genotyped per locus (NO), heterozygosity observed (Ho) and heterozygosity expected (He), exclusionary power for first (Excl(1)) and second (Excl(2)) parent, Hardy-Weinberg equilibrium (HW) and the calculated frequency of null (non-amplifying) alleles (Null). Under HW, values in equilibrium are indicated as "NS", values with too few individuals for the type of test conducted by CERVUS are indicated by "NA", values significantly out of equilibrium at the 1% level are indicated by "". Loci with lowest exclusionary power are in bold type.**

Locus	k	NP	NO	Ho	He	Excl (1)	Excl (2)	HW	Null
One-102	20	168	889	0.91	0.90	0.67	0.81	NS	-0.01
One-114	20	164	933	0.90	0.91	0.70	0.82	NA	0.01
Ots-100	23	172	969	0.87	0.85	0.54	0.71	NS	-0.02
Omm-1329	13	188	1025	0.82	0.87	0.57	0.73	NS	0.03
One-101	**8**	**176**	**937**	**0.54**	**0.56**	**0.16**	**0.27**	**NS**	**0.01**
One-108	26	177	968	0.96	0.92	0.72	0.84	NS	-0.02
Ots-103	**5**	**185**	**1013**	**0.36**	**0.37**	**0.07**	**0.20**	**NS**	**0.01**
Omy-77	15	176	1019	0.80	0.81	0.45	0.62	NS	0.01
Ots-1	**17**	**170**	**977**	**0.64**	**0.76**	**0.38**	**0.56**	******	**0.09**
Ots-3M	**10**	**178**	**998**	**0.66**	**0.64**	**0.23**	**0.39**	**NS**	**-0.03**
Omm-1070	24	171	948	0.91	0.93	0.74	0.85	NA	0.01
Omm-1130	21	165	948	0.89	0.92	0.72	0.84	NA	0.01
Omy-1011	17	164	969	0.90	0.89	0.64	0.78	NA	-0.01
Oki-10	17	180	1003	0.86	0.84	0.54	0.70	NS	-0.01
Omm-1128	35	180	941	0.91	0.93	0.75	0.86	NA	0.01
Omy-1001	23	180	986	0.82	0.87	0.60	0.75	NS	0.03
One-18	10	184	1007	0.75	0.78	0.41	0.59	NS	0.02
Omm-1304	**6**	**187**	**983**	**0.77**	**0.75**	**0.34**	**0.51**	**NS**	**-0.02**
Omm-1302	14	187	1023	0.87	0.86	0.56	0.72	NS	-0.01
Omm-1306	23	186	992	0.89	0.90	0.66	0.79	NS	0.00
Omm-1321	15	182	1009	0.78	0.84	0.52	0.69	NS	0.04
Omm-1316	25	183	994	0.80	0.92	0.73	0.84	NA	0.07
Omm-1322	18	180	976	0.80	0.88	0.61	0.75	**	0.05
Sco-110	12	179	1012	0.63	0.79	0.43	0.60	NS	0.11
Omm-1138	**5**	**178**	**997**	**0.62**	**0.63**	**0.22**	**0.39**	**NS**	**0.01**
Sco-103	24	176	979	0.93	0.93	0.74	0.85	NA	0.00

Appendix Table 4. Samples with matching or nearly matching multilocus genotypes. Table continues on subsequent pages. "HSR" indicates wild-brood hatchery summer-run and "WSR" indicates wild summer-run.

CODE MATCHES	FIRST SAMPLE	SECOND SAMPLE	SCORE	# ALLELES SCORED	# ALLELES MATCHING	EXPLAINATION
01QG0017 03NX0035	HSR 01-02 Jacks	HSR 3-salts upstream 03-04	100%	40	40	repeat spawner (sampled twice)
01QG0037 02RP0448	HSR 01-02 Jacks	HSR upstream 02-03	100%	40	40	repeat spawner (sampled twice)
02AAK0001 03AAE0042	resident	resident	98%	40	39	Probable resamp of same fish
02AAK0003 03AAE0020	resident	resident	100%	40	40	Probable resamp of same fish
02AAK0003 05OC0003	resident	resident	100%	40	40	Probable resamp of same fish
02JV0051 02JV0053	residual	residual	100%	40	40	2 diff fish
02RP0011 02RP0232	HSR upstream 02-03	HSR upstream 02-03	83%	12	10	2 diff fish
02RP0011 03NX0006	HSR upstream 02-03	HSR 3-salts upstream 03-04	89%	18	16	2 diff fish
02RP0047 03NX0076	HSR upstream 02-03	HSR 3-salts upstream 03-04	100%	36	36	2 diff fish
02RP0081 04AAY0151	HSR upstream 02-03	HSR upstream 03-04	100%	40	40	could be repeat spawner but not noted
02RP0087 03NX0041	HSR upstream 02-03	HSR 3-salts upstream 03-04	94%	34	32	repeat spawner (sampled twice)

CODE MATCHES	FIRST SAMPLE	SECOND SAMPLE	SCORE	# ALLELES SCORED	# ALLELES MATCHING	EXPLAINATION
02RP0094 02RP0656	HSR upstream 02-03	HSR upstream 02-03	98%	40	39	repeat spawner (sampled twice)
02RP0259 03NX0103	HSR upstream 02-03	HSR 3-salts upstream 03-04	100%	40	40	repeat spawner (sampled twice)
02RP0338 03NX0101	HSR upstream 02-03	HSR 3-salts upstream 03-04	100%	40	40	repeat spawner (sampled twice)
02RP0450 03NX0124	HSR upstream 02-03	HSR 3-salts upstream 03-04	97%	38	37	repeat spawner (sampled twice)
02RP0487 03NX0095	HSR upstream 02-03	HSR 3-salts upstream 03-04	98%	40	39	2 diff fish
02SP0034 04AAZ0213	wild by02 broodstock	WSR upstream 03-04	95%	38	36	Prob two diff fish
03AAE0020 05OC0003	resident	resident	100%	40	40	Probable resamp of same fish
03AAE0024 03AAE0028	resident	resident	100%	40	40	Probable resamp of same fish
03AAE0024 05OC0002	resident	resident	100%	40	40	Probable resamp of same fish
03AAE0028 05OC0002	resident	resident	100%	40	40	Probable resamp of same fish
03OB0025 04AAZ0132	WSR upstream 02-03	WSR upstream 03-04	98%	40	39	2 diff fish
03OB0059 04AAZ0269	WSR upstream 02-03	WSR upstream 03-04	100%	36	36	repeat spawner (sampled twice)
03OB0103 04AAZ0291	WSR upstream 02-03	WSR upstream 03-04	100%	38	38	repeat spawner (sampled twice)

CODE MATCHES	FIRST SAMPLE	SECOND SAMPLE	SCORE	# ALLELES SCORED	# ALLELES MATCHING	EXPLAINATION
03OB0159 04AAZ0236	WSR upstream 02-03	WSR upstream 03-04	95%	40	38	repeat spawner (sampled twice)
03OB0258 04AAZ0197	WSR upstream 02-03	WSR upstream 03-04	100%	40	40	repeat spawner (sampled twice)
03OB0274 04AAZ0285	WSR upstream 02-03	WSR upstream 03-04	100%	40	40	repeat spawner (sampled twice)
03OB0277 04AAZ0262	WSR upstream 02-03	WSR upstream 03-04	100%	40	40	repeat spawner (sampled twice)
03OB0307 04AAZ0258	WSR upstream 02-03	WSR upstream 03-04	98%	40	39	repeat spawner (sampled twice)
03OB0351 04AAZ0298	WSR upstream 02-03	WSR upstream 03-04	100%	40	40	possible repeat spawner (not noted)
03OB0378 04AAZ0267	WSR upstream 02-03	WSR upstream 03-04	100%	40	40	repeat spawner (sampled twice)
03OB0408 04AAZ0275	WSR upstream 02-03	WSR upstream 03-04	98%	40	39	repeat spawner (sampled twice)
03OB0563 04AAZ0335	WSR upstream 02-03	WSR upstream 03-04	100%	34	34	repeat spawner (sampled twice)
04AAZ0296 04AAZ0485	WSR upstream 03-04	WSR upstream 03-04	100%	40	40	2 diff fish
04AAZ0502 04AAZ0529	WSR upstream 03-04	WSR upstream 03-04	100%	40	40	2 diff fish
05IG0002 05OC0001	residual	resident	94%	36	34	2 diff fish
98MG0012 99OU0008	resident	resident	100%	40	40	Resamp of same fish

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