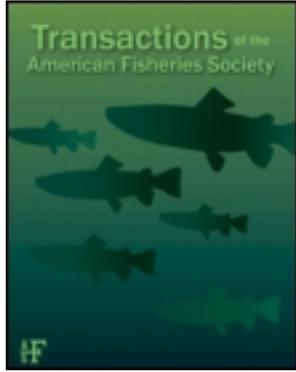


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### Diminished Reproductive Success of Steelhead from a Hatchery Supplementation Program (Little Sheep Creek, Imnaha Basin, Oregon)

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SPECIAL SECTION: GENETIC ADAPTATION

## Diminished Reproductive Success of Steelhead from a Hatchery Supplementation Program (Little Sheep Creek, Imnaha Basin, Oregon)

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### Abstract

Hatchery supplementation programs are designed to enhance natural production and maintain the fitness of the target population; however, it can be difficult to evaluate the success of these programs. Key to the success of such programs is a relatively high reproductive success of hatchery fish. This study investigated the relative reproductive success (RRS) of steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) by creating pedigrees for hatchery and natural spawning steelhead. We genotyped adult steelhead that returned to a weir and were released upstream to spawn in Little Sheep Creek, a tributary to the Imnaha River in eastern Oregon. The broodstock for this supplementation program were originally chosen from natural-origin steelhead returning to the weir and in subsequent years consisted of both natural- and hatchery-origin individuals. Microsatellite analyses showed the broodstock to be genetically similar to the natural population across years. We also genotyped adult resident rainbow trout from multiple locations upstream of the weir and determined the parentage of progeny collected at various life history stages, including returning adults in subsequent years. Analysis of progeny sampled at both the juvenile and adult life stages suggested that the RRS of hatchery-origin fish was 30–60% that of their natural-origin counterparts. Using generalized linear models to address the importance of various factors associated with reduced reproductive success, we found that the greatest effects on RRS were origin (natural versus hatchery), length, return date, and the number of same-sex competitors. Natural parents were less negatively affected by same-sex competitors. Differential survival of juveniles and the behavior of offspring and/or spawning adults may all contribute to diminished fitness in hatchery-reared salmon, although it could not be determined to what extent these effects were of a persistent, heritable nature as distinct from an environmental effect associated with hatchery rearing and release strategies.

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The decline of Pacific salmon *Oncorhynchus* spp. in western North America has increased the motivation for using artificial propagation to boost natural production and reduce demographic risk in depressed populations (NRC 1996). There

is a long history in the Pacific Northwest and across the Pacific Rim of using hatchery production to enhance fisheries and mitigate the loss of habitat; however, as the goals of many hatchery programs have expanded to include both harvest augmentation

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and conservation, there is acute interest in evaluating the reproductive performance of hatchery fish that are allowed to spawn in the wild. Existing literature from other Pacific salmon suggests that the reproductive success of hatchery fish can vary widely (see Araki et al. 2008 for a review). For example, some populations that received continuous stocking over a period of many years showed little if any genetic evidence of successful spawning by hatchery fish (McLean et al. 2004). On the other hand, some hatchery programs had significant effects on natural populations not intended to be influenced by hatchery fish (e.g., rainbow trout *O. mykiss* [Small et al. 2007], Pacific salmon [Reisenbichler and Rubin 1999], and coho salmon *O. kisutch* [Nickelson et al. 1986]). Until recently, studies of the genetic effects of hatchery propagation were limited to analysis of allele frequency shifts through time (e.g., Leider et al. 1990). Changes in allele frequencies over time are clearly limited when genetic differences are negligible to begin with. Recent efforts to develop locally derived hatchery broodstocks rather than using out-of-basin stocks require the use of alternative methods for tracking the reproductive success of hatchery fish.

Little Sheep Creek is a tributary of the Imnaha River in north-eastern Oregon with a hatchery program for steelhead (anadromous rainbow trout) developed under the Lower Snake River Compensation Plan (part of the Water Resources Development Act of 1976), the primary goals of which are mitigating the depression of salmon populations caused by hydropower development, restoring lost harvest opportunities, and supplementing and boosting natural production. Snake River steelhead populations were listed as threatened under the U.S. Endangered Species Act in 1997 and upheld in 2006.

Our study had two primary objectives: (1) to evaluate the reproductive success of steelhead from the Little Sheep Creek supplementation program spawning in the wild relative to that of natural-origin steelhead and (2) to relate fitness to specific phenotypic characters in order to understand the selective forces influencing reproductive success in this population. We addressed these objectives by using a molecular approach to create a pedigree of sampled offspring whose parents spawned in the wild.

## METHODS

**Study site and sample collections.**—Initiated in 1982, the Little Sheep supplementation program obtained broodstock from wild adult steelhead returning to a newly constructed fishweir (trap) at river kilometer 8 on Little Sheep Creek (Figure 1). This weir forms an absolute barrier at all water levels to upstream steelhead migration as anadromous adults return to spawn. Hatchery-reared juveniles were raised using an accelerated rearing program, which results in a high proportion of smoltification in 1 year rather than the 2 years that is more characteristic of the natural population. Hatchery smolts were marked by excision of the adipose fin and released from a single acclimation pond below the weir as 1-year-old smolts nearly ready for migration to the sea. From brood years 2000 through 2005, however,

20–30% of the hatchery smolts were left with adipose fins intact and were instead marked via insertion of a blank coded wire tag. When adults began to return in 1985, hatchery staff spawned both natural and hatchery fish to produce annual smolt releases. Excess fish of both hatchery and natural origin (i.e., beyond those needed for hatchery broodstock) were passed over the weir and allowed to spawn naturally.

Our study collections began in 1999 (Table 1) with adults returning to spawn between mid-March and mid-May. Before adult steelhead were passed upstream over the adult weir on Little Sheep Creek, they were marked by perforation of the operculum to avoid double-counting “fall-backs” (fish that moved back down below the weir and reentered the trap). The perforation removed a small section of tissue, and these were collected by Oregon Department of Fish and Wildlife personnel and preserved in 95% ethanol. The data associated with each tissue sample included the date of collection, the fork length and sex of the individual, and the origin (hatchery or natural). Samples of juveniles, along with resident rainbow trout beginning in 2001 (hereafter referred to as residents), were taken by electrofishing during August of each year. Fish with fork lengths of 80 mm or less were designated young of the year (age 0). Fish with fork lengths between 80 and 150 mm were considered to be 1–2 years old, and those with lengths greater than 150 mm were considered residents and potential parents. It is possible that males mature at lengths less than 150 mm and therefore could be considered potential parents, but including all juveniles except those age 0 as potential parents would make the pedigree calculations prohibitive. Our classification of resident fish also included a number of residualized hatchery fish, as determined by the presence of adipose fin clips and/or blank coded wire tags. With both natural-origin and residualized residents it was not known whether these fish were permanent residents or had yet to migrate. A stratified sampling procedure was carried out over study sites between river kilometers 8 and 39 (Figure 1). We attempted to collect genetic samples from 20 age-0 fish and 20 age-1–2 fish from each site, along with any residents captured (typically 200–250 per year).

**Genetic analysis.**—DNA from fin clips was extracted and purified using the DNeasy 96-well extraction kit (Qiagen Corporation, Valencia, California). Samples were quantified using PicoGreen dye (Molecular Probes, Eugene, Oregon) with an FLx800 Microplate Reader (BioTek Corp., Winooski, Vermont). Fifteen microsatellite loci were used to construct pedigrees in the naturally spawning population: *Ocl1* (Condrey and Bentzen 1998), *Ogo4* (Olsen et al. 1998), *Oke4* (Buchholz et al. 2001), *Oki23 MMBL* (A. Spidle, Northwest Indian Fisheries Commission, unpublished; GenBank accession number AF272822), *Omy7 INRA* (K. Gharbi, French National Institute for Agriculture Research, personal communication), *Omy77* (Morris et al. 1996), *Omy1001* and *Omy1011* (Spies et al. 2005), *Ots1*, *Ots3*, and *Ots4* (Banks et al. 1999), *Ots100* (Nelson and Beacham 1999), *Ots506* (Naish and Park 2002), *p53* (P. Moran and L. Park, Northwest Fisheries Science Center, personal

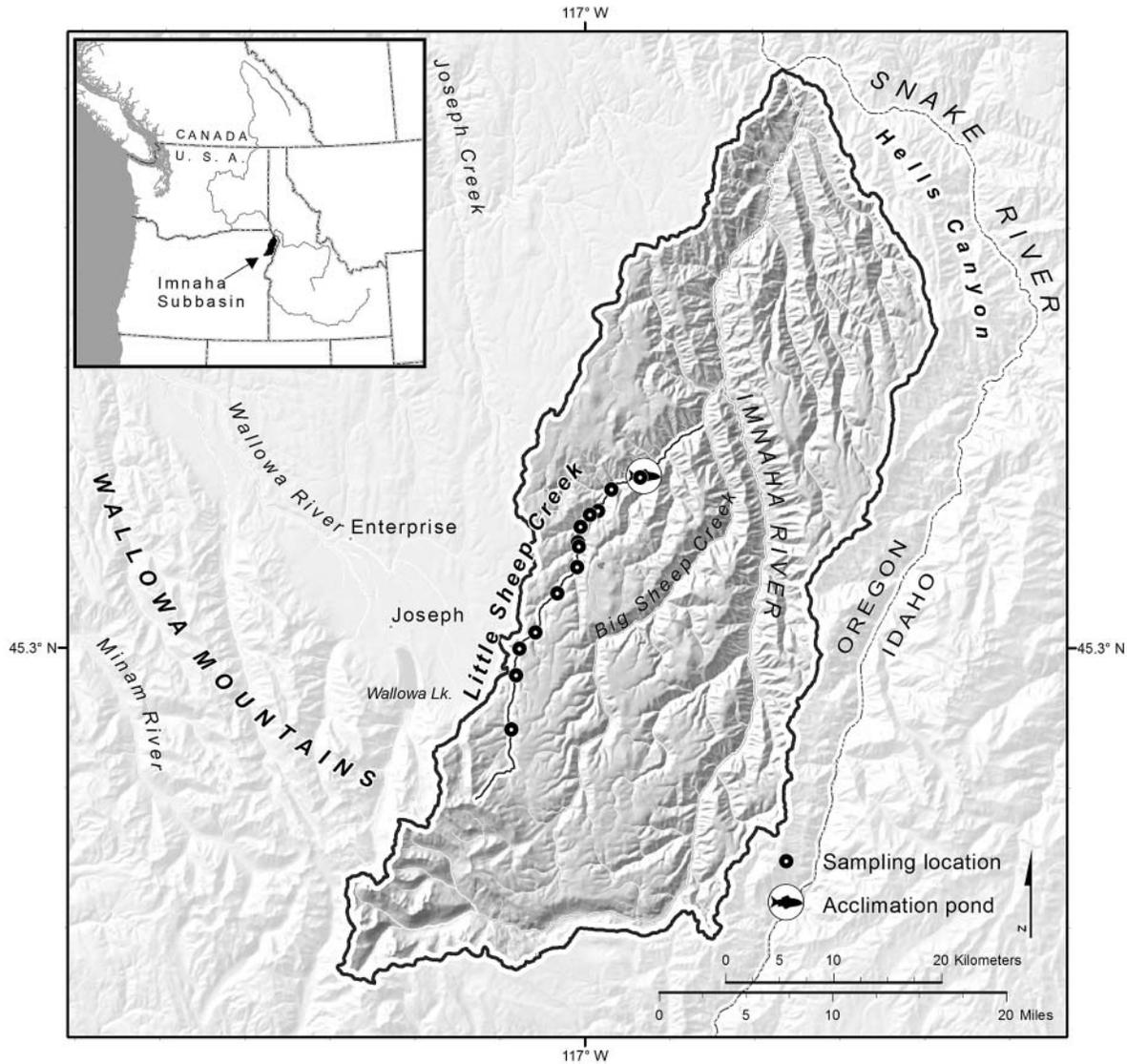


FIGURE 1. Map of Little Sheep Creek, Oregon, showing the locations of the sampled reaches.

communication), and *Ssa407* (Cairney et al. 2000). Polymerase chain reaction (PCR) amplifications were conducted using MJ Research Tetrad thermal cyclers. The PCR cocktails included the following final concentrations per 10- $\mu$ L reaction: 1 $\times$  *Taq* DNA Polymerase Buffer A, (Promega Corp., Madison, Wisconsin), 1.75 mM MgCl<sub>2</sub>, 0.2 mM of each deoxynucleotide triphosphate, 15 ng DNA, 0.6 units *Taq* DNA polymerase (Promega Corp.), and primers ranging from 0.15  $\mu$ M to 0.6  $\mu$ M each (available upon request). Fragment analysis was performed on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

**Pedigree analysis.**—Population-genetic analyses (determining conformance to Hardy–Weinberg equilibrium, heterozygosity, and the likelihood of null alleles) were performed using FS-TAT version 2.9.3.2 (Goudet 2001) and CERVUS version 3.0 (Marshall et al. 1998). Parentage determination was performed

primarily by exclusion using the programs CERVUS version 3.0 (Marshall et al. 1998) and MYKISS (S. Kalinowski, Montana State University, unpublished). MYKISS runs much more quickly than CERVUS and provides a more user-friendly output, but it has only recently become available. The adult-to-juvenile results for brood years 1999–2003 were run with CERVUS, but we reanalyzed those for brood year 2003 using MYKISS and found no differences in the parent pair–offspring matches identified by the two programs. The 2004–2008 adult-to-juvenile and adult-to-adult analyses were subsequently run with MYKISS. We chose to use exclusion primarily (rather than likelihood) because allowing any mismatches in our pedigree construction created numerous ambiguous “matches.” Exclusion is not particularly sensitive to the proportion of parents sampled if the error rate is low; any offspring with uncollected parents will simply remain unassigned (Jones et al. 2010). We assumed a genotyping

TABLE 1. Returning anadromous adults, juveniles, and resident rainbow trout analyzed for the pedigree study. Determination of the status (natural or hatchery) of returning adults was based on the presence or absence of an adipose fin, wire tag (blank or coded), visible implant fluorescent elastomer tag, or fin erosion patterns indicative of hatchery rearing.

Collection year	Sample	Natural	Hatchery	Total	% Natural
1999	Adults	5	74	79	6.3
	Juveniles	300	0	300	100
2000	Adults	58	141	199	29.1
	Juveniles	239	0	239	100
2001	Adults	112	667	779	14.4
	Residents	9	35	44	20.5
	Juveniles	287	0	287	100
2002	Adults	191	1,179	1,370	13.9
	Residents	52	35	87	59.8
	Juveniles	349	0	349	100
2003	Adults	93	316	409	22.7
	Residents	68	54	122	55.7
	Juveniles	399	0	399	100
2004	Adults	136	683	819	16.6
	Juveniles	323	0	323	100
2005	Adults	176	288	464	37.9
	Juveniles	488	18	506	96.4
	Smolts	291	0	291	100
2006	Adults	106	248	354	29.9
	Juveniles	562	9	571	98.4
	Smolts	281	1	282	99.6
2007	Adults	82	158	240	34.2
	Juveniles	492	2	494	99.6
	Smolts	293	3	296	99
2008	Adults	144	147	291	49.5
	Juveniles	455	11	466	97.6
	Smolts	279	1	280	99.6
2009	Adults	169	114	283	59.7

error rate of 0.01 in CERVUS but used exclusion rather than likelihood to identify parent–offspring pairings. Residents were also included in the parentage analyses from collection years 2001–2003, considered both as potential parents and as potential offspring. The resulting matings (parent pair–offspring triplets plus single-parent matches) were checked to eliminate matches that were deemed physically impossible (see below for further discussion of exclusionary power). Pedigrees were constructed for a single generation only, and no “grandparentage” analyses were performed.

Relative reproductive success (RRS) was calculated from the combined data sets of parent pair–offspring triplets plus single-parent matches. We estimated the number of offspring expected based on the number of adults of each origin and sex passed over the weir (potential parents) divided by the number of offspring observed. Reproductive success was normalized to 1 for natural-origin adults. Significant deviations from expectations were noted by pairwise contingency testing using a simulated Fisher’s exact test (RxC program; M. Miller, U.S. Geological Survey).

*Analysis of phenotypic traits.*—To evaluate which covariates affected relative reproductive success, we analyzed the adult-to-juvenile and adult-to-adult data separately and for this portion considered only offspring whose parents were both known. Juvenile collections for phenotypic trait analysis spanned 5 brood years (1999–2003); the adult-to-adult data included 7 brood years (1999–2005). Later brood years were excluded because not all adult progeny had returned, making reproductive success data for some individuals incomplete. For each data set, we explored how relative reproductive success was affected by origin (hatchery or natural), sex, return timing (ordinal day), size, and density. Because the response variable (number of offspring contributed by a given parent) was an integer, we used generalized linear models (GLMs) to link the response to covariates. Our initial models treated the response as either a Poisson or a negative binomial random variable. As expected, the negative binomial distribution was strongly supported over the Poisson (difference in Akaike information criterion [AIC] values > 100). The negative binomial GLM uses a log-link to relate the mean

of the  $i$ th parent to covariates, that is,

$$\log_e = B_0 + B_1 \cdot \text{sex}_i + B_2 \cdot \text{origin}_i \dots$$

In these models, we attempted to fit return time and length as either linear or quadratic predictors.

Given multiple years of adult return data, we were also interested in examining the degree of support for the effects of density, competition, hatchery influence, and time trends. Each variable was collected on an annual scale. To avoid redundancy, no more than one of these variables was considered in the same model. The effects of year and density (in this case the total number of spawners passed over the weir) cannot be disentangled, for instance, but a model with year as a predictor can be examined relative to a model with density as a predictor. Alternative annual predictors that we considered included the number of same-sex spawners (used to evaluate the effects of competition), the number of opposite-sex spawners (used to evaluate the support for mate limitation), and the fraction of total spawners that were of hatchery origin (see Table 1). Finally, to evaluate possible temporal trends, we considered year as either a numeric or factor variable. To account for simple nonlinear relationships, each of these variables was considered as a predictor in log-space, which assumes that the effects of each are exponential. Other analyses with more years could also consider more complex functions.

To account for the unequal sizes of spawner populations and the unequal numbers of sampled progeny across years, all GLMs in our analysis included an offset term,

$$\text{offset}_i = \log_e [(p_i \cdot o_{\text{year}}) / o_{\text{max}}],$$

where  $p_i$  is the number of parents of the same origin, sex, and brood year as individual  $i$ ,  $o_{\text{year}}$  is the number of offspring sampled in the same brood year as individual  $i$ , and  $o_{\text{max}}$  is the maximum number of offspring sampled in any year of the study. Our initial list of models was pared down to 32 by removing models whose coefficients did not make biological sense. The remaining models were ranked based on their AIC scores; we included models within 10 log-likelihood units of the best model (Burnham and Anderson 2002).

*Analysis of uncollected parents.*—Although the focus of this paper was examining the factors that determine relative reproductive success, we could not use our entire adult-to-juvenile data set to examine differences in the relative reproductive success of individual parents. In the full data set (parent pair–offspring triplets as well as single-parent matches), there are a number of missing (uncollected) parents. The frequency of this result required us to estimate the number of missing parents and investigate some of the potential causes. Because the majority of uncollected parents were male, comparisons of relative reproductive success between sexes could be biased. Ultimately, offspring with one or more uncollected parents were removed from the analysis of phenotypic traits. A parent's being uncollected might be the result of sampling location (e.g., residents

would not be collected at the weir) or be explained by covariates (brood year, spawning location, etc.). To explore the latter possibility, we assumed that the probabilities of sires and dams being uncollected were independent and used logistic regression to analyze the probability of sires being uncollected. Because the sample size of uncollected dams was small (154 of 2,207 total single-parent assignments), we were not able to analyze the effect of covariates. For sires, the response variable is binary, and the probability of being uncollected can be written as

$$\text{logit}(p_i) = B_0 + B_1 \cdot \text{location}_i + B_2 \cdot \text{year}_i \dots$$

We evaluated the degree of support for offspring-specific covariates, the phenotypic characteristics of dams, and population-level covariates. The phenotypic characteristics of sires (origin and length) could not be considered because this information is missing for uncollected parents. Offspring-specific covariates included length and sampling location. The mate-specific covariates of dams included return time (ordinal day), length, and origin. Population-level characteristics included the brood year, total density, total number of males, number of hatchery males, number of natural males, and proportion of total male spawners that were of hatchery origin. We considered 25 candidate models consisting of one to four predictors and intercepts. To evaluate data support, we used the AIC as a model selection tool, the best model being the one with the lowest AIC score (Burnham and Anderson 2002).

## RESULTS

### Pedigree Analysis and RRS

Our analysis included 5,313 individual juveniles and residents collected from 1999 to 2008 and 5,292 anadromous adults returning from 1999 to 2009. Of the juveniles, 171 fish were recaptured one or more times in subsequent years (i.e., were sampled in one year at 40 mm and age 0 and then again in the following year at 75 mm) as determined by the identification of identical genotypes (the probability of identity was  $6.2 \times 10^{-19}$ , as calculated using the computer program GenAlEx version 6.4 [Peakall and Smouse 2006]). Individuals sampled multiple times were only counted once in the RRS calculations.

Two of the loci used (*Omy77* and *Ots1*) showed strong evidence of null alleles as estimated by the program Cervus. The locus *Omy77* has previously been documented as having null alleles (Ardren et al. 1999). The presence of null alleles will eliminate a fraction of true parentage matches in a pedigree analysis, the magnitude of which depends on the frequency of those null alleles. To identify matches that might have been affected, we flagged all potential parentage groupings that had mismatches only in *Omy77* or *Ots1*, then compared genotypes to find those mismatches that would be consistent with the presence of null alleles (i.e., a mismatch of two homozygotes). We then included those matches in our final results.

Most sample sets did not deviate significantly from Hardy–Weinberg (HW) expectations, the only exceptions being four

that showed heterozygote deficits at *Omy77* and/or *Ots1*. When hatchery and natural fish were combined over all years, natural fish were in HW equilibrium for all loci except *Omy77* and hatchery fish were in HW equilibrium for all but *p53*, *Omy77*, and *Ots1*. The potential null allele issue with *Omy77* and *Ots1* could easily explain the departures from HW equilibrium for those loci. There were no significant differences in allele frequencies between hatchery and natural fish for any of the collection years, nor were there significant differences from year to year.

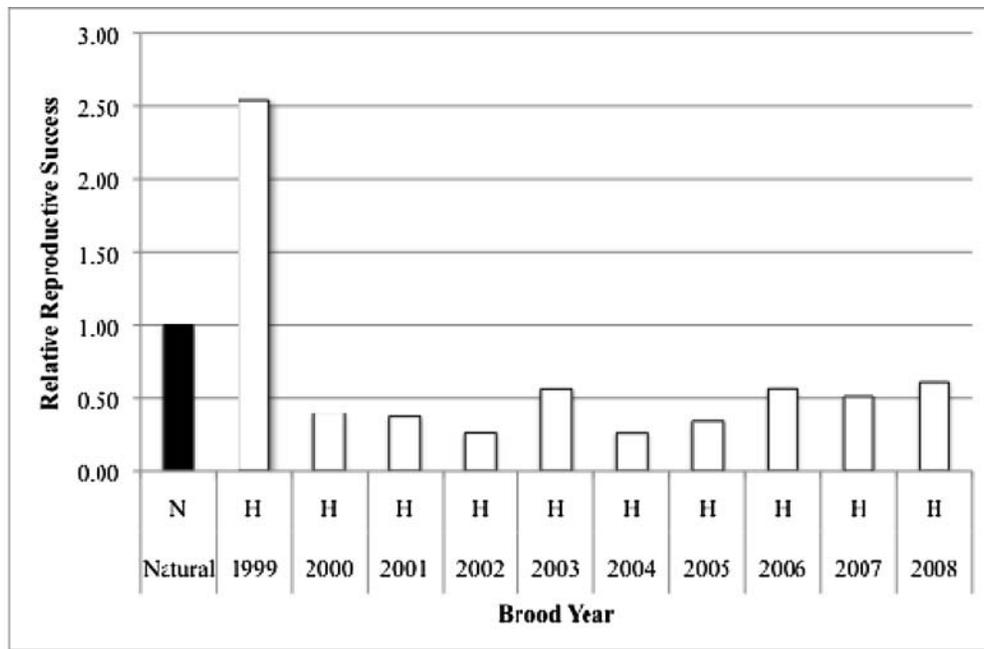
For parentage analyses, the exclusionary power of the selected loci as calculated by Cervus was 0.99966 for the first parent and 0.99998 for the second. The realized exclusionary power was lower, however, as demonstrated by the identification of multiple parent pairs for some offspring. We eliminated matings that could not logically have happened (e.g., offspring in 1999 matched to parents in 2001). When more than one parent pair was identified for a single individual, and with no other means of eliminating one of the parents, the higher-likelihood triplet was selected. We hypothesized that high relatedness in the Little Sheep Creek population might compromise assignment power. The presence of siblings as potential parents has been shown to increase the number of ambiguous assignments in parentage analyses, as compared with an analysis of unrelated parents (Double et al. 1997; Olsen et al. 2001). Ford and Williamson (2010) showed that the parentage assignment ability was lower with lower effective population sizes of the grandparent population.

A number of offspring, both juveniles and adults, could not be assigned to two parents. Approximately 40% of the juveniles in our collections were matched to two known parents, with a similar proportion being matched to a single known parent, leaving approximately 20% not matched to any known anadromous or resident parent. Similar results were found in the adult-to-adult analyses, although a smaller fraction was assigned to a single parent and a larger fraction not assigned to either parent: 36% were matched to two parents, 29% to a single parent, and 35% to neither parent. A much higher proportion (61%) of natural residents (collection years 2001–2003) were not matched to any parent. Only 12% of natural residents were matched to two parents and 27% to a single parent (79 of 292). The vast majority of the offspring (juvenile or adult) that were matched to parents were associated with anadromous adults (~99.1%), with only 15 juveniles being matched to resident parents from our collections. Four returning adults of natural origin (out of 641 possible) were matched back to a collected resident fish, which was a slightly lower percentage than was found in the adult-to-juvenile results; this result, however, was not significant ( $P = 0.65$ ).

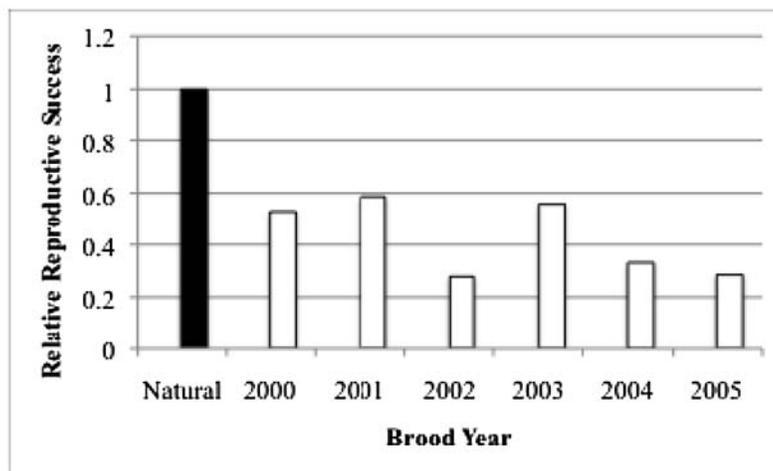
Across the study as a whole, the RRS of hatchery fish was significantly lower than that of their natural counterparts in Little Sheep Creek in both the adult-to-juvenile and adult-to-adult analyses (Table 2; Figure 2). The single exception was 1999, when hatchery fish had higher reproductive success than natural-origin fish. In that year, however, only 5 out of 78 fish put over the weir were of natural origin, and both natural males were live-spawned (i.e., a small amount of sperm was collected for

TABLE 2. Parentage assignments (numbers) for juveniles and returning anadromous adults over all brood years. In designations such as NN, the first letter indicates the origin of the female parent (N = natural, H = hatchery, and U = uncollected) and the second letter the origin of the male parent.

Brood year	NN	NH	HN	HH	NU	HU	UN	UH
<b>Adult to juvenile</b>								
1999	0	6	0	119	12	121	0	3
2000	20	9	42	20	57	82	0	2
2001	20	39	12	101	47	58	2	3
2002	24	62	36	103	113	98	5	23
2003	24	79	26	100	104	102	2	10
2004	45	108	42	169	111	166	6	28
2005	199	83	45	68	236	114	9	21
2006	62	74	16	94	205	150	3	14
2007	50	32	37	53	106	104	12	9
2008	12	8	20	8	46	21	2	0
<b>Adult to adult</b>								
1999	0	0	0	17	0	25	0	5
2000	12	5	22	20	27	53	5	3
2001	3	20	7	54	13	28	1	2
2002	1	9	16	13	14	18	1	5
2003	0	9	8	8	7	7	1	6
2004	12	25	14	34	16	21	4	5
2005	19	10	5	4	17	9	1	0



A)



B)

FIGURE 2. Relative reproductive success of steelhead in Little Sheep Creek by origin (natural [N] or hatchery [H]) in (A) adult-to-juvenile and (B) adult-to-adult analyses. Values are normalized to 1.00 for natural-origin fish. The results were combined for parent pair–offspring triplets and single-parent matches. The year 1999 is not included because no natural parents were identified in that year.

use in the hatchery before the fish was passed upstream). The geometric mean of the RRS of hatchery fish was 49% across all years in the adult-to-juvenile results, compared with 41% between 2000 and 2008, when greater percentages of natural fish were passed over the weir. The geometric mean of the RRS of hatchery fish for brood years 2000–2005 was 41% for the adult-to-adult results. No natural-origin offspring returned as adults from brood year 1999. The RRS of hatchery fish calculated from adult-to-juvenile results was very similar to that calculated from

adult-to-adult results across comparable brood years (Table 3). The number of adult offspring analyzed was lower than the number of juvenile offspring, so a few of the years were not significant for the adult-to-adult results despite their having the same relative reduction in reproductive success as the adult-to-juvenile results.

When considering both origin and sex, the natural males and females had consistently higher RRS than hatchery males and females (Figure 3). The one exception to this pattern was

TABLE 3. Comparison of relative reproductive success rates calculated from adult-to-juvenile and adult-to-adult data.

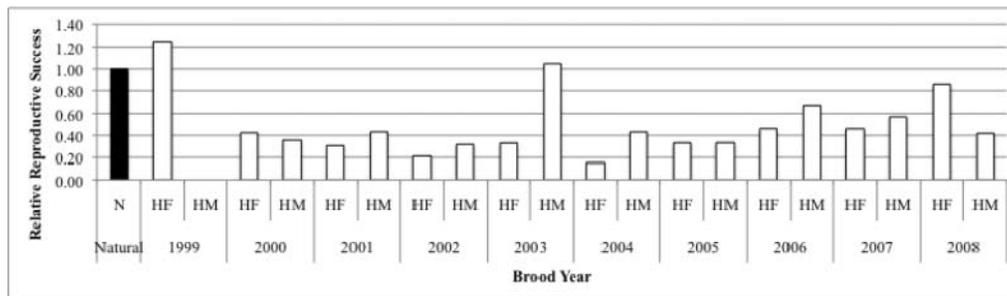
Brood year	Adult to juvenile	Adult to adult
2000	0.39	0.53
2001	0.36	0.59
2002	0.26	0.28
2003	0.56	0.56
2004	0.25	0.33
2005	0.33	0.29

2003, where hatchery males appeared to be equal to natural males. The 2003 result, however, was entirely due to a single highly successful unmarked hatchery male that produced 11 of the offspring in our sample with a single natural female. That a single fish could dominate the calculations in this way speaks to the high levels of sampling variance in this system. There was not a consistent pattern for RRS by mating type (e.g., natural females  $\times$  hatchery males versus hatchery females  $\times$  natural males, etc.), except that hatchery female  $\times$  hatchery male matings were always the least successful crosses in both the adult-to-juvenile and adult-to-adult comparisons (data not shown). Natural female  $\times$  natural male crosses were generally

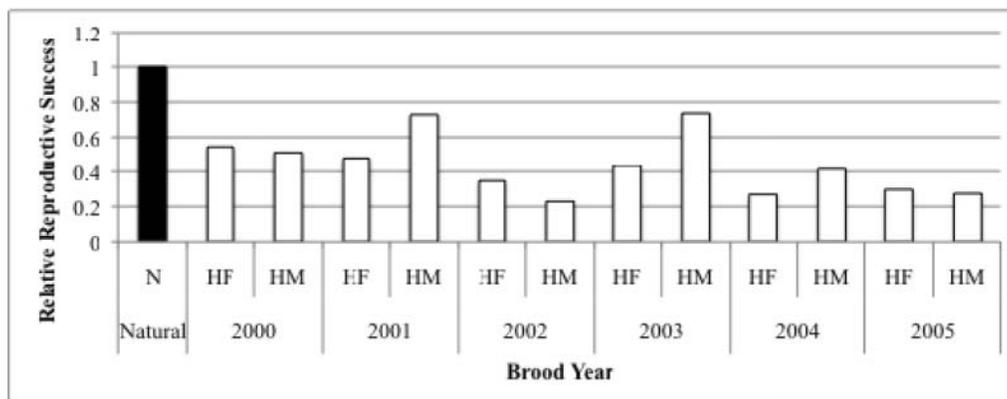
the most successful (7 out of 9 brood years for adult-to-juvenile and 4 out of 6 brood years for adult-to-adult).

Year-to-year variations in the ratio of hatchery to natural fish were high, both in those put over the weir and those used in hatchery broodstock. The number of natural adults passed over the weir in 1999 was very low (3 females and 2 males out of 78 fish altogether). In all other years, the fraction of natural fish passed over the weir ranged from 13% to 60%. The total number of fish in the hatchery broodstock each year ranged from 133 to 221, and included 3–22% natural-origin fish (data not shown).

Because of the large percentage of hatchery-origin fish in Little Sheep Creek and the demonstrated ability of those fish to reproduce, it would be reasonable to assume that some fraction of the putatively natural-origin fish brought into the hatchery broodstock were actually the offspring of hatchery parents. The adult-to-adult results allowed us to determine the origin of unmarked fish used in the hatchery broodstock. A total of 55 females and 47 males (most of which were live-spawned) were used in the hatchery broodstock from 1999 to 2005, and we determined at least one parent for 74 of them. Approximately two-thirds of the determined parental input to those unmarked fish was of hatchery origin and only one-third of natural origin. There was no statistically significant difference between males and females ( $P = 1.00$ ).



A)



B)

FIGURE 3. Relative reproductive success of steelhead in Little Sheep Creek by sex (female [F] or male [M]) and origin (natural [N] or hatchery [H]) in (A) adult-to-juvenile and (B) adult-to-adult analyses. In the adult-to-juvenile analysis, no natural males were identified as parents in 1999; in the adult-to-adult analysis, no natural parents were identified for brood year 1999. See Figure 2 for other information.

TABLE 4. Models of relative reproductive fitness of steelhead in Little Sheep Creek based on adult-to-juvenile (1999–2003) and adult-to-adult (1999–2005) data. All models were fit as negative binomial general linear models and include both intercept and offset terms. Models with interaction terms also include all fixed effects. The model most supported by the data has the lowest Akaike information criterion (AIC) score. See text for additional details.

Data	Covariates	AIC
Adult to juvenile	Length, $\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup>	4,525.3
	Length, $\log_e(\text{competitors})$ , $\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup> , sex	4,526.8
	Length, $\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup> , $\log_e(\text{competitors}) \times \text{sex}$	4,527.8
	Length, $\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup> , $\log_e(\text{competitors}) \times \text{sex}$ , sex $\times$ origin	4,529.6
	Length, $\log_e(\text{competitors}) \times \text{origin} \times \text{sex}$ , date, (date) <sup>2</sup>	4,530.7
	Length, $\log_e(\text{competitors}) \times \text{origin}$	4,538.4
	Origin, length, $\log_e(\text{competitors})$ , date, (date) <sup>2</sup>	4,544.5
	$\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup>	4,545.7
	Null model	5,543.4
Adult to adult	Length, $\log_e(\text{competitors}) \times \text{origin}$ , $\log_e(\text{competitors}) \times \text{sex}$ , date, (date) <sup>2</sup>	4,245.7
	Length, $\log_e(\text{competitors}) \times \text{origin}$ , $\log_e(\text{competitors}) \times \text{sex}$ , date, (date) <sup>2</sup> , origin $\times$ sex	4,246.7
	$\log_e(\text{competitors}) \times \text{origin}$ , $\log_e(\text{competitors}) \times \text{sex}$ , date, (date) <sup>2</sup>	4,247.2
	Length, $\log_e(\text{competitors}) \times \text{origin} \times \text{sex}$ , date, (date) <sup>2</sup>	4,248.5
	Length, $\log_e(\text{competitors}) \times \text{origin}$ , $\log_e(\text{competitors}) \times \text{sex}$	4,253.3
	$\log_e(\text{competitors}) \times \text{origin}$ , $\log_e(\text{competitors}) \times \text{sex}$	4,256.8
	Length, $\log_e(\text{competitors}) \times \text{origin}$ , date, (date) <sup>2</sup>	4,276.6
	Null model	5,679.3

High levels of promiscuity were observed in both males and females, which is consistent with results obtained for both Atlantic salmon *Salmo salar* (Garant et al. 2001) and other Pacific salmon (Berejikian et al. 2000; Seamons et al. 2004; Kuligowski et al. 2005). During the study period, more than 24% of the males and 17% of the females that contributed offspring to our sample achieved multiple matings. Individual females had up to three mates and contributed up to 48 offspring to our sample. Males had up to four mates and contributed up to 31 offspring. There were no significant differences between males and females, regardless of origin, in either the number of mates or the number of offspring found.

**Phenotypic Traits**

In our examination of the covariates affecting the RRS of individuals in Little Sheep Creek, we found that the best model of adult-to-juvenile and adult-to-adult data included origin, length, return date, and the number of same-sex competitors (Tables 4, 5). Origin had by far the strongest effect, with natural fish having higher estimates of RRS. The effect of origin is complicated, however; whereas the coefficients associated with natural fish are negative (Table 5), the interaction between competitors and origin is positive. Across the range of densities observed in Little Sheep Creek, the cumulative benefit of being natural then becomes positive. As expected, length had

TABLE 5. Coefficients of the best models of relative reproductive success for Little Sheep Creek steelhead based on adult-to-juvenile (1999–2003) and adult-to-adult (1999–2004) data. Because of the log-link used with the negative binomial general linear model, these coefficients are in log space. For the juvenile analysis the intercept refers to hatchery fish, and for the adult analysis it refers to hatchery females;  $P < 0.05^*$ ,  $P < 0.000001^{**}$ .

Covariate	Juveniles		Adults	
	Estimate	SE	Estimate	SE
Intercept	-3.84**	2.09	11.55**	0.49
Origin (natural)	-2.04*	0.93	-0.98**	0.82
$\log_e(\text{competitors})$	-2.07*	0.07	-3.04**	0.08
Length	0.0018*	0.00089	0.09*	0.04
Origin $\times$ $\log_e(\text{competitors})$	0.74**	0.16	0.57	0.14
Date	0.17**	0.04	-0.13*	0.05
(Date) <sup>2</sup>	-0.000821**	0.00019	-0.09*	0.04
Sex (male)			-2.1	0.71
Sex $\times$ $\log_e(\text{competitors})$			0.27*	0.12

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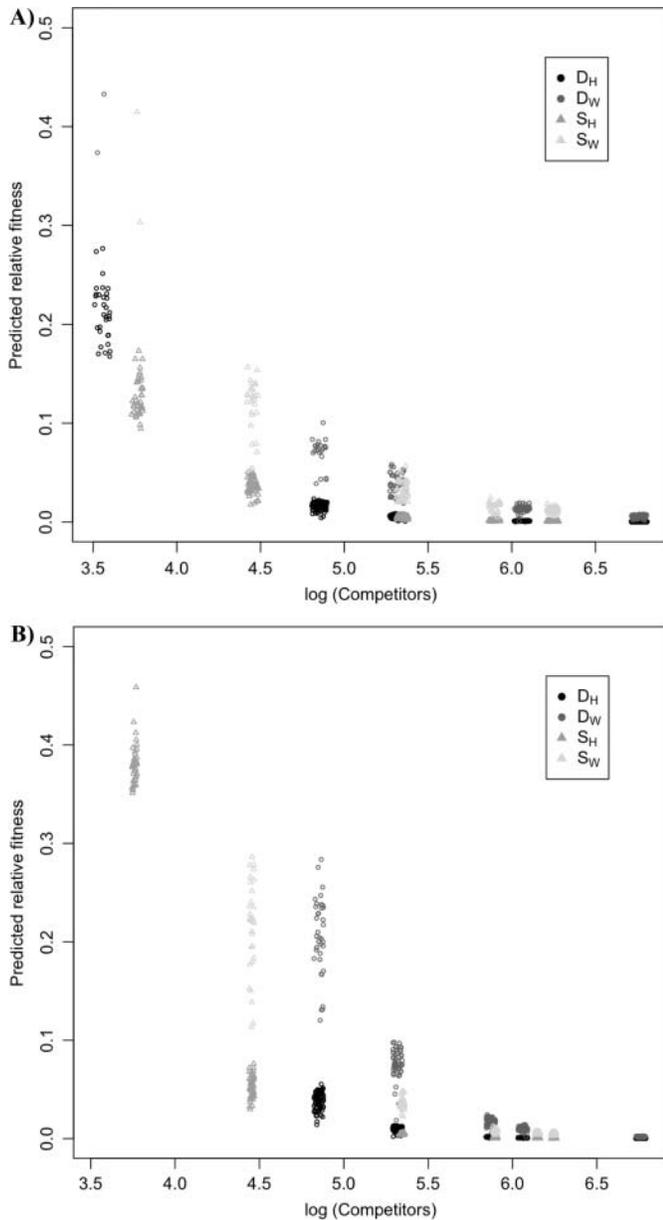


FIGURE 4. Relationship between the estimated mean relative reproductive success and density of steelhead in Little Sheep Creek in (A) adult-to-juvenile (1999–2003) and (B) adult-to-adult (1999–2009) analyses. Abbreviations are as follows:  $D_H$  = hatchery dams,  $D_W$  = natural dams,  $S_H$  = hatchery sires, and  $S_W$  = natural sires). The best model (Table 3) predicts decay in fitness as a function of the number of same-sex competitors. The predicted values have been scaled to represent 1 fish because the number of offspring sampled is not constant over time.

a positive effect, with larger fish having higher RRS for both males and females. We observed stabilizing selection for return date. The optimum return date was relatively early in the year (April 1 for adults to adults and April 12 for adults to juveniles), with lower average success for fish returning before or after that date. There was little support for including the effects of total density, or mate limitation (Table 5; Figure 4). The significant

interaction between origin and competitors was included in the best model for both data sets; the value of this coefficient was positive, suggesting natural spawners were less impacted by density than hatchery spawners (Figure 4). Sex was not included as a predictor for the adult-to-juvenile data but was for the adult-to-adult data; females were more negatively impacted than males by increasing the number of same-sex competitors, which is consistent with space-limiting density dependence.

### Uncollected Parents

A number of offspring (both juvenile and returning adult) could not be attributed to any candidate parents. Genotyping error would contribute to this; we estimated our genotyping error to be 0.0028 per locus (per Hoffman and Amos 2005; Johnson and Haydon 2007), which would result in approximately 12% of true parentage matches being incorrectly eliminated. Beyond genotyping error, it is likely that different factors are contributing to the adult-to-juvenile and adult-to-adult results. Some of the returning adults not matched back to either parent were probably strays from other spawning tributaries or natural adults produced from spawners below the weir in Little Sheep Creek, and therefore we would not have sampled their parents. The stray proportion does not affect the adult-to-juvenile RRS calculations, however, because we would have sampled all of the potential anadromous parents for any juveniles produced above the weir. The unsampled parents of juveniles in our study were probably resident rainbow trout, as has been seen in other studies (Sharpe et al. 2000; Zimmerman and Reeves 2002; Araki et al. 2007a).

Our sampling of anadromous adults was much more thorough than our sampling of residents, as we did not attempt to conduct sampling of the entire stream. Of the 2,207 juveniles matched to a single parent, 93% had known mothers and only 7% had known fathers. Of the 237 adults matched to a single parent, 86% had known mothers and 14% had known fathers. The overall sex ratio of female to male anadromous adults in the system was approximately 1.2–1, so the skewed results of uncollected females to uncollected males is clearly not a reflection of the sex ratio. These results are consistent with matings involving anadromous females with “sneaker” resident males, as documented in other systems (e.g., Seamons et al. 2004; Araki et al. 2007c).

In our examination of the variables that determine the probability of a sire’s being uncollected, the sampling site, origin of the dam, and total number of males were selected in the best model (Table 6). Kilometers upstream was found to have a positive effect (0.308; SE = 0.079), whereas the total number of males was found to have a negative effect (–0.315; SE = 0.063). The positive effect of site on uncollected parents would be expected if resident males reside further upstream. The negative effect of the number of males suggests that sneaking is less likely when there are more competing anadromous males. Dam origin was also estimated to have a positive effect in the best model (0.242; SE = 0.14), but this was not statistically significant

TABLE 6. The seven best-fitting logistic regression models predicting whether or not a sire will be uncollected when the dam is known. Covariates in brackets were not statistically significant at the 0.05 level.

Covariates	AIC
Site, total males, [dam origin]	1,563.7
Site, hatchery males, [dam origin]	1,564.3
Site, total males	1,564.6
Site, hatchery males	1,565.0
Site, total males, [date]	1,566.5
Site, hatchery males, [date]	1,567.0
Site, year, dam origin	1,570.7

( $P \approx 0.09$ ). Taken together, these results suggest that the uncollected parents are resident fish that contributed significantly to collected offspring.

Despite including 300 residents (collected from 2001 to 2004) as potential parents in our study, we were able to document only 15 matings involving resident parents (11 for the adult-to-juvenile data and 4 for the adult-to-adult data). Of those 15 matings, however, only one involved a hatchery resident (residualized hatchery fish). Three of the 15 matings involved natural anadromous females, 7 involved hatchery females, and the remaining 5 matings were single-parent matings with resident fish; presumably the uncollected parent in these matings was also a resident fish.

## DISCUSSION

### Pedigree Analysis

Many recent studies of reproductive success in naturally spawning populations have revealed a surprising lack of concordance between social mating systems and genetic mating systems (Primmer et al. 1995; Goossens et al. 1998). In other cases, behavioral observations are confirmed through genetic analysis (Ribble 1991; Brotherton et al. 1997; Girman et al. 1997). Comparisons of our genetic data with direct observations are limited because of the enigmatic nature of summer-run steelhead in the Snake River basin: these fish return to spawn at a time when water levels are high and the rivers are turbid. Spawning observations and redd counts are not generally feasible in this system. The current study, therefore, not only reveals differential reproductive success between hatchery- and natural-origin fish, it also provides fundamental new insights into the breeding biology of Snake River steelhead.

Our study revealed a large difference in RRS between hatchery and natural fish in Little Sheep Creek. It also demonstrated, however, that hatchery and natural fish interbreed widely in the study population. Despite the higher reproductive success for natural individuals, hatchery fish outnumbered natural ones by more than five to one, yielding an overall hatchery contribution to our offspring sample that was nearly twice that of natural fish.

Several previous studies have found similar reductions in the RRS of hatchery-reared steelhead, although often the hatchery stocks were derived from out of basin (Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Leider et al. 1990; Chilcote 1998). More recent publications have looked specifically at programs using locally derived broodstock. In a 2004 review of fourteen RRS studies of anadromous salmonid hatchery programs, half of which used locally derived broodstock, reductions in RRS were reported for all multigenerational steelhead programs examined (as well as some programs for Chinook salmon *O. tshawytscha*), despite the inclusion of local broodstocks (Araki et al. 2008). One of the studies of steelhead in the Hood River found no significant difference in RRS between hatchery and natural fish after one generation in production (Araki et al. 2007a) but a significant loss of RRS in hatchery fish after three generations (decreasing approximately 40% per generation; Araki et al. 2007b). Further, this lowered reproductive success persisted over at least one generation (i.e., reproductive success in natural-born descendants of two hatchery parents was lower than that of natural-born descendants of two natural parents; Araki et al. 2009). The lower RRS of hatchery fish in our study is intermediate to that found by Araki et al. (2009), even though the Little Sheep Creek hatchery program has been producing smolts for over six generations.

Over the brood years for which we have both adult-to-juvenile and adult-to-adult RRS calculations, the results are very similar for both life stages (Table 3). Although the calculated adult-to-adult RRS is the ultimate goal of these types of studies, adult-to-juvenile studies are often much simpler logistically. Weirs are easier to operate on smaller tributaries, and although the entire spawning population may not be represented it is more feasible to monitor multiple tributaries within a given system. In addition, larger sample sizes (and therefore greater analytical power) are possible with juvenile collections; by contrast, additional sampling effort will not yield greater numbers of adults, as those sample sizes are limited to the number of returning natural-origin adults. Our data suggest that adult-to-juvenile RRS results are extremely informative, and can provide valuable insight in systems that are not amenable to adult-to-adult analysis.

While we have demonstrated lowered reproductive success in hatchery steelhead, we don't know how much of the difference is genetic as opposed to environmental. Clearly, there is ample interbreeding between the hatchery and natural components of the Little Sheep Creek steelhead population (particularly between hatchery females and natural males), yet it is equally clear that hatchery-reared fish left fewer offspring per individual than their natural counterparts. Consistent with interbreeding, there was no significant genetic difference at neutral loci between hatchery and natural fish in this system. Adaptation can be present despite the absence of neutral allele frequency differences (e.g., Vasemagi et al. 2005), provided the selection gradient is strong enough to overcome the high levels of gene flow; however, it seems unlikely that domestication can fully explain the reduced

fitness of hatchery fish in the wild. Despite the similarities in their life cycles, there is an obvious environmental difference between hatchery and natural fish, namely, the accelerated rearing and early smoltification experienced by artificially produced steelhead in Little Sheep Creek and essentially all summer steelhead hatcheries in the interior Columbia River basin. We did not see evidence of differential RRS between natural and hatchery broodstock spawned in the hatchery (data not shown), however, we cannot completely rule out poor performance by natural fish spawned in the hatchery.

Efforts to understand the reproductive behaviors and genetic interactions of hatchery and natural fish can be confounded due to the relatively small proportion of natural fish in many years. For example, in 1999 78 adult steelhead were passed over the weir on Little Sheep Creek, yet only 5 were natural fish (3 females and 2 males). The number of natural adults returning between 2000 and 2009 was much higher both in absolute and in relative terms. Because the number of natural fish in the system in 1999 was so small and the interfamily variance in reproductive success so great, we focused our comparisons of hatchery–natural interaction on results obtained from brood year 2000–2008 juveniles and brood year 2000–2005 returning adults. However, it is worth noting that in 1999 hatchery fish actually performed better than expected, though not quite significantly so ( $P = 0.06$  for adult-to-juvenile results and  $P = 0.175$  for adult-to-adult results) and better than in all other years with larger numbers of natural fish. It is important not to dismiss the 1999 results in case they reflect density dependence that truly affects the RRS of hatchery fish spawning in the wild.

### Resident–Anadromous Interactions

The large number of juveniles in our study not attributed to any of the sampled parents were probably the offspring of residents, and our results suggest that residualized hatchery fish do not contribute in significant numbers. The large number of returning adults not matched back to parents may partially reflect this large contribution of resident fish, but it might also include strays returning to Little Sheep Creek. A number of hatchery fish returning to Little Sheep Creek are outplanted into neighboring Big Sheep Creek, and we know anecdotally that many of them move immediately out of Big Sheep Creek and return to the Little Sheep Creek weir (and are again trucked back to Big Sheep Creek). It's possible that naturally produced offspring of fish outplanted into Big Sheep Creek return to Little Sheep Creek as adults, and we plan to analyze a single year of outplanted fish as potential parents of returning Little Sheep Creek adults to test this idea.

Two problems with uncollected parents are immediately evident, relating to both power and bias. First, uncollected parents result in less statistical power whether parentage assignment is by exclusion or by likelihood methods. For either method, we cannot predict how many “matches” we might find in the unsampled group. Another way to view the problem is that a large fraction of uncollected parents introduces error into the

estimation of allele frequencies, upon which the likelihood inference depends. Second, power is diminished in evaluating fitness-associated traits. In the absence of genotyping errors, the fraction of offspring whose parents cannot be identified is proportional to the fraction of uncollected parents. Having more uncollected parents will require oversampling juveniles to obtain the same power in GLM estimation of selection gradients.

Our pedigree studies allow us to identify and begin to quantify the interactions of anadromous steelhead and resident rainbow trout. “Interbreeding” of the two life history types has been suggested as a mitigating effect for fluctuating effective population sizes in anadromous populations (Ardren and Kapuscinski 2003; Seamons et al. 2004; Araki et al. 2007c). The amount of interaction between the anadromous and resident life history types varies from system to system, perhaps depending on migration distance, spawn timing, spawning location, or annual environmental conditions. Most systems show little or no genetic difference between sympatric anadromous steelhead and resident rainbow trout (e.g., the Copper River, Alaska [Olsen et al. 2006]) and closer genetic associations with geography than with life history (e.g., California to Alaska [McCusker et al. 2000]; British Columbia [Docker and Heath 2003]; and the Walla Walla and Touchet rivers, Washington [Narum et al. 2004]).

Otolith microchemical analysis has also been used to document the interbreeding of life history types by using Sr:Ca ratios in the freshwater growth regions of the otolith to identify fish with mothers of resident or anadromous origin. Again, different river basins yielded different patterns: Zimmerman and Reeves (2000) found no crossover in life history in the Deschutes River (Oregon), but they did find both steelhead of resident origin and residents of steelhead origin in the Babine River (British Columbia). Their results showed a higher prevalence of residents with anadromous mothers (22%) than steelhead with resident mothers (4%), but their sample sizes were small, ranging from 9 to 24 individuals. Similar studies of rainbow trout in the Grande Ronde river system (neighbor to Little Sheep Creek) estimated that 30% of the migrating smolts and 20% of the returning anadromous adults tested had resident mothers (J. Ruzycki, Oregon Department of Fish and Wildlife, unpublished data). Anecdotal observations from the Grande Ronde system support this finding, as resident fish were often seen near actively spawning anadromous females (it was common to see four to five resident fish near most redds), and resident and anadromous spawn timing overlapped (Ruzycki et al. 2009). Experimental crosses of resident rainbow trout and anadromous steelhead from the Grande Ronde system showed that all four possible types of crosses would produce offspring capable of smoltification and emigration, with a significant dam effect for life history characteristics of the offspring (Ruzycki et al. 2009).

Little Sheep Creek undoubtedly has a substantial resident rainbow trout population, as evidenced by the large number of juveniles missing either a sire or both parents and the low recapture rate of resident fish. Out of 1,750 resident fish handled over

8 years, only 61 resident fish were recaptured in a subsequent year. Seven of the fish originally categorized as residents were recaptured 3 years later as anadromous adults, however. It will never be possible to collect all resident fish in this system, so our results for anadromous–resident crosses will probably remain speculative in nature, lacking sufficient numbers and power for stringent testing. With the few anadromous–resident crosses that we have been able to identify thus far, however, it appears that residualized hatchery fish do not contribute on a large scale. Given the numbers of residualized hatchery fish in the system, this is an intriguing result.

Despite our inability to completely exclude the potential genetic effects of supplementation, our results do not support them and it seems more appropriate to revisit phenotypic plasticity related to environmental factors associated with hatchery rearing practices and smolt release locations. Even nongenetic factors such as smolt release locations can have lasting effects if there is demonstrated site fidelity of offspring of hatchery-origin adults. As our study continues, we will have the opportunity to compare the RRS of second-generation offspring of hatchery- and natural-origin adults as well as to accumulate selection gradient data over a range of hatchery–natural proportions and spawner and juvenile rearing densities. For example, the 1999 results represent the performance of hatchery fish spawning at low density in the (essential) absence of natural fish. The 2000 and 2001 adult returns resulted in higher spawner densities, and in 2002 spawner density was the highest during this study (1,181 hatchery and 192 natural adults passed over the weir). In 2003–2009 the densities returned to a more intermediate level, ranging between 240 and 823 fish over the weir, and as of 2008 there has been a 1:1 ratio of hatchery to natural fish passed over the weir as well. The relatively short time series examined here limits our ability to disentangle the effects of time and density. However, fluctuations in density (and sex ratio) over longer time periods will increase our ability to separate these effects.

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