

How Much Does Inbreeding Contribute to the Reduced Fitness of Hatchery-Born Steelhead (*Oncorhynchus mykiss*) in the Wild?

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Abstract

Many declining populations are supplemented with captive-born individuals that are released directly into the wild. Because captive-born individuals can have lower fitness in the wild than their wild-born counterparts, a comprehensive understanding of the mechanisms responsible for the reduced fitness of these individuals is required for appropriate conservation and management decisions. Inbreeding among captive-born individuals is one plausible mechanism because captive breeding programs frequently use small numbers of breeders to create large numbers of siblings that are subsequently released together into the wild. We tested this hypothesis in a supplementation program for steelhead (*Oncorhynchus mykiss*) from the Hood River, Oregon, for which first-generation hatchery fish were demonstrated to have lower fitness in the wild than their wild-born counterparts. To determine the contribution of inbreeding to this fitness decline, we first assigned 11 run-years of hatchery steelhead (3005 fish) back to their broodstock parents (462 fish) using 8 polymorphic microsatellite loci. By combining pedigree analyses with species-specific estimates of genetic load, we found that inbreeding could at most account for a 1–4% reduction in the fitness of hatchery fish relative to wild fish. Thus, inbreeding alone cannot adequately explain the 15% average fitness decline observed in first-generation hatchery fish from this population.

Key words: captive breeding, hatcheries, inbreeding depression, parentage, relatedness, salmon

Large numbers of captive-born individuals are often released directly into the wild with little to no subsequent monitoring (Laikre et al. 2010). Such large-scale releases could have a number of unintended consequences, particularly when captive-born individuals are released directly into populations that continue to reproduce successfully in the wild. Because wild populations of Pacific salmonids have declined sharply over the past century (Chapman 1986; Yoshiyama et al. 1998; Gresh et al. 2000; Quinn 2005; Naish et al. 2008), supplementation programs have been implemented throughout the northern Pacific. Here, we use “supplementation” to refer to a program in which hatchery-raised juveniles are released at or near the wild adult spawning grounds with the expectation that they will one day return to breed in the wild and provide a demographic boost to the population. Such programs usually use wild fish as broodstock in order to minimize genetic differences between the wild and hatchery-reared fish. However, despite best practices, it has been well documented that hatchery fish from such supplementation programs can

have substantially lower fitness in the wild than their wild-born counterparts (Araki, Cooper et al. 2007; Williamson et al. 2010; Berntson et al. 2011; Thériault et al. 2011; Milot et al. 2013). One potential consequence of these findings is that hatchery supplementation could actually reduce the fitness of the wild populations rather than providing the intended long-term benefits. Thus, understanding the causes of reduced fitness in hatchery fish is vital for the successful conservation and management of threatened salmonids.

To date, several studies have speculated over the cause of the rapid fitness decline observed in hatchery fish from supplementation programs (e.g., McClure et al. 2008; Araki et al. 2008), but empirical evidence remains sparse. Possible explanations include environmental effects, unintentional domestication selection, and relaxed natural selection associated with the captive environment. Recent work has documented that unintentional domestication selection (selection for trait values associated with success in a captive environment) can rapidly produce fish that are subsequently

maladapted when released into the wild (Christie, Marine, French, Blouin 2012). Although unintentional domestication remains a likely explanation, multiple factors may act simultaneously to reduce the fitness of hatchery fish in the wild. One compelling explanation for the rapid reduction in fitness is inbreeding (Wang et al. 2001; Thrower and Hard 2009). Supplementation programs often use a small percentage of the returning wild population as broodstock to create large numbers of returning hatchery fish. Thus, many of the returning adult hatchery fish are siblings (Hedrick and Hedgecock 1994; Olsen et al. 2001; Christie, Marine, French, Waples et al. 2012). If these siblings mate with each other in the wild, then their offspring could have lower survivorship owing to inbreeding depression, and the adults would exhibit reduced reproductive success relative to wild adults. Furthermore, in some cases, hatchery fish have been documented to preferentially mate with other hatchery fish rather than with wild-born fish (Hansen and Mensberg 2009), which could further increase the possibility of matings between siblings.

Steelhead trout, *Oncorhynchus mykiss*, are typical of most Pacific salmonids in that their declining populations have led to the creation of numerous supplementation programs (Kostow 2009). In this study, we examined 11 run-years (i.e., the year of adult return) of winter-run steelhead from the Hood River, Oregon for which all returning anadromous fish were genotyped at eight highly polymorphic microsatellite loci. Previous work from this system revealed that hatchery fish created with two wild parents averaged 85% the reproductive success of their wild counterparts and that an additional generation in captivity reduced fitness in the wild by an additional 50% (Araki, Cooper et al. 2007). Although similar numbers of hatchery and wild fish spawned in the Hood River each year, the effective number of breeders producing the hatchery fish was much smaller than that producing the wild fish (Harmonic mean $N_b = 25$ vs. 373 fish, respectively; Christie, Marine, French, Waples et al. 2012). Thus, inbreeding is more likely to occur among returning hatchery fish (Figure 1) and thus could explain the lower reproductive success of hatchery fish relative to wild fish in the Hood River. This work differs from other Hood River studies (notably Christie, Marine, French, Blouin 2012) in that we directly quantify the extent to which inbreeding could explain the 15% average fitness reduction documented in F1 hatchery fish versus their wild-origin counterparts. To meet those ends, we first examined the potential for inbreeding among hatchery fish to cause the reported fitness declines by using parentage analyses to assign all returning adult hatchery fish back to their broodstock parents. We next used steelhead-specific estimates of the number of lethal equivalents to calculate inbreeding-associated fitness costs associated with inbred matings. Using the estimated fitness costs in combination with our pedigree, we calculated the expected reduction in fitness for hatchery fish and found that inbreeding alone cannot explain the rapid decline in fitness associated with the Hood River supplementation program.

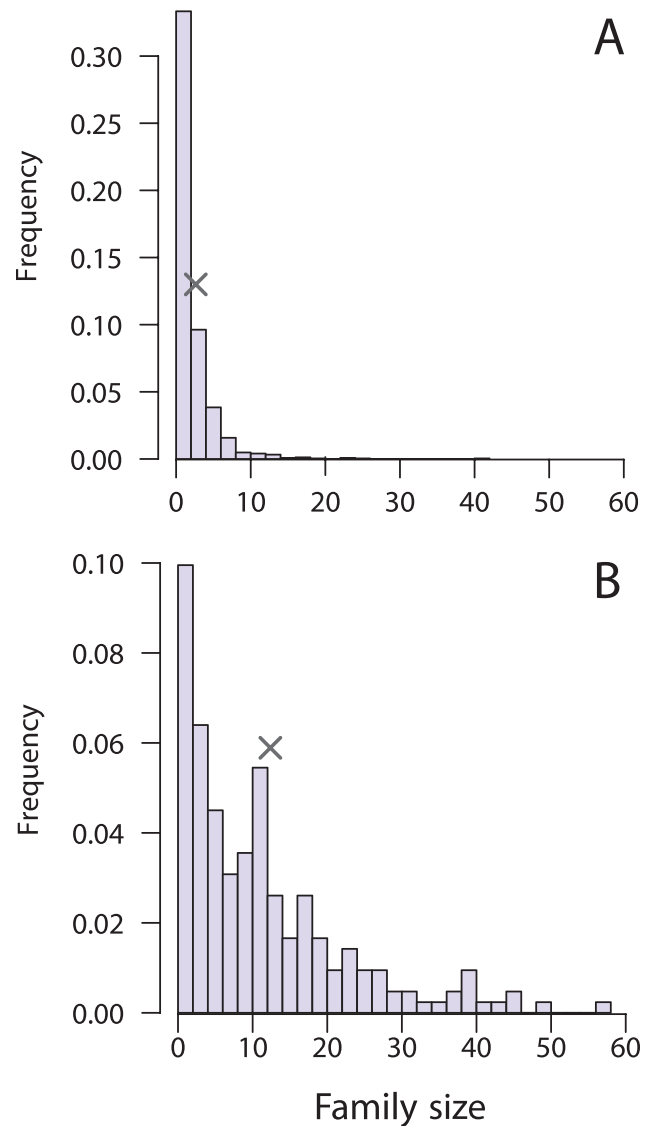


Figure 1. Frequency of family sizes for steelhead that (A) spawned in the wild or (B) were spawned in the hatchery (i.e., broodstock). The gray X in both plots denotes the average family sizes. Notice that one broodstock fish produced 60 returning F1 hatchery fish (which were all siblings), and the average hatchery family had more than 10 siblings. These data illustrate that there were many more F1 hatchery siblings on the spawning grounds than for wild-born fish, and thus inbreeding between hatchery siblings could possibly account for the reduced fitness of hatchery-born fish. Here, family sizes are calculated as the number of offspring per male and thus include both full and half siblings (results for females were nearly identical).

Materials and Methods

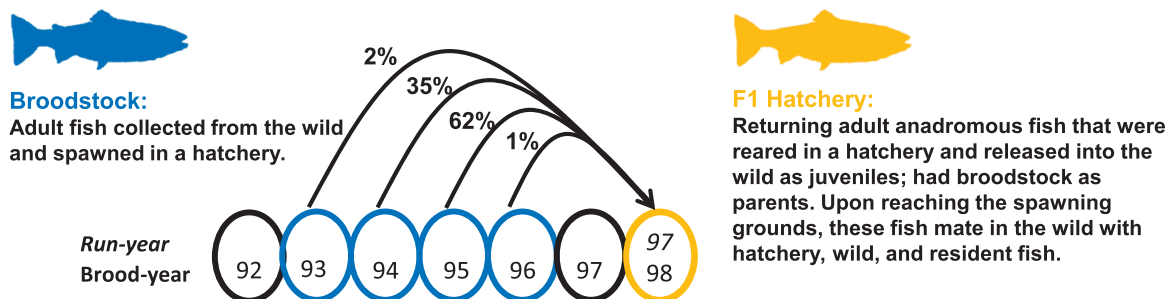
Sample Collection and Study System

Samples were collected from the Hood River, Oregon, where steelhead are listed as threatened under the Endangered Species Act (Busby et al. 1996; Good et al. 2005). All

returning adult fish en route to their spawning grounds were first required to navigate the Powerdale dam, which was located at the mouth of the Hood River and was a complete barrier to all migratory fishes. At the dam, both wild and hatchery-born steelhead were directed into an elevator leading to a fish processing room, where all fish were cataloged, measured, weighed, sexed, and had scale (for aging and DNA analysis) and fin (for DNA analysis) samples collected before being passed over the dam. Steelhead were easily categorized as hatchery or wild origin because all hatchery fish had their adipose fin removed before release. The Powerdale dam fish trap was operated by the Oregon Department of Fish and Wildlife. Genetic samples for the returning adult winter-run steelhead employed in this study were collected from run-years 1995 to 2005, which corresponds to fish born in brood-years 1993 through 2003 (Figure 2; Table 1). The number of winter-run steelhead samples analyzed in this study averaged 774 per year for a total of 8517 samples. All samples were genotyped at 8 highly polymorphic microsatellite loci (Omy1001, Omy1011, Omy1191, Omy77, One108, One2, Ssa407, and Str2), which average 36 alleles per locus.

All wild fish and an approximately equal number of hatchery fish were passed over the dam each year. The winter-run hatchery fish were created using either two wild fish or one wild fish and a first-generation hatchery fish as broodstock (see Araki, Ardren et al. 2007 for details). As per Araki, Ardren et al. (2007), we use “wild” to refer to any fish spawned in the river under natural conditions, regardless of whether its parents have hatchery ancestry. We have DNA samples from all broodstock, and detailed records on broodstock pairings in the hatchery. Most broodstock fish were spawned with two (or occasionally more) partners, which created returning hatchery fish that were full siblings (hereafter “sibs”), half sibs, or unrelated. Furthermore, the wild fish used as hatchery broodstock were collected randomly throughout the entire run period and thus were unlikely to be related. Previous work in this system has documented that first-generation hatchery fish have reduced fitness in the wild relative to wild-born fish (Araki, Cooper et al. 2007), and that those differences are probably genetically based (Araki et al. 2009; Christie, Marine, French, Blouin 2012). Extensive details on this study system, management practices, steelhead

A.) Relative contributions to a typical run-year



B.) Relative contributions to the first run-year

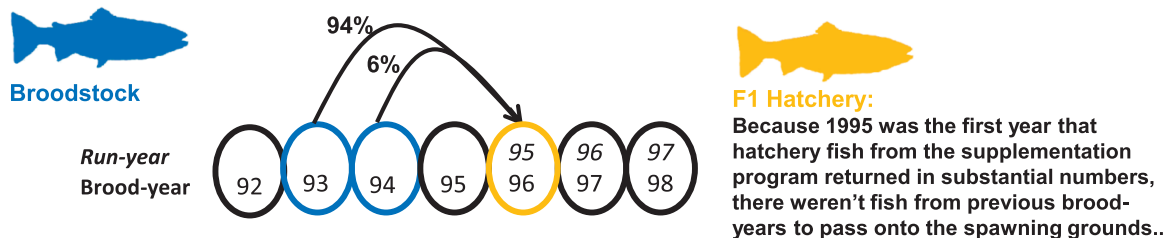


Figure 2. Illustration of return times for hatchery-born fish (“F1 hatchery fish”). Broodstock were collected as adults from the wild and spawned in a hatchery. (A) In most run-years, the returning hatchery fish that were passed over the dam came from multiple brood-years because return time varies among individuals from the same cohort. (B) However, in the first year that hatchery fish from the supplementation program were passed over the dam, the majority of fish came from a single brood-year because there were no earlier brood-years to buffer the contribution. Consequently, the greatest proportion of hatchery siblings allowed on to the spawning grounds occurred in run-year 1995. Here, the percentages are directly from the Hood River, winter-run dataset and illustrate that the greatest threat from inbreeding-associated fitness declines may occur in the first year that hatchery fish are allowed on to the spawning grounds.

Table 1 Sample sizes for the winter-run steelhead used in this study

Run-year	Broodstock		Hatchery		Wild	
	Female	Male	Female	Male	Female	Male
1995	18	15	64	89	132	79
1996	35	35	152	107	148	94
1997	35	40	110	58	113	70
1998	43	45	85	91	167	92
1999	45	48	109	108	539	339
2000	50	52	257	244	579	304
2001	41	47	273	305	608	354
2002	42	45	145	95	438	221
2003	37	39	166	170	347	201
2004	38	41	101	82	174	126
2005	34	37	94	100	239	148
Total	418	444	1556	1449	3484	2028

Numbers are reported for fish grouped by the year that they returned as adults (run-year) and separated by sex and hatchery or wild status. For each run-year, the total number of adult broodstock that were identified as parents to all the hatchery fish that were passed over the dam are also reported (broodstock). For example, in 1995, a total of 18 mothers and 15 fathers produced 64 and 89 returning adult F1 female and male hatchery fish, respectively. Note that because some broodstock contributed to multiple run-years, the total number of unique broodstock individuals ($N = 462$) is less than the total numbers reported in the table.

life history, genetic markers, and reproductive success can be found elsewhere (Olsen 2003; Araki, Ardren et al. 2007; Araki, Cooper et al. 2007; Kostov 2009; Christie et al. 2011; Christie, Marine, French, Blouin 2012).

Parentage Analysis

We first employed parentage analysis to assign returning adult hatchery fish back to their broodstock parents (Figure 2). We used genotypes of the known broodstock pairs sorted by the year in which they were spawned as the putative parents. Genotypes of the hatchery fish, sorted and grouped by brood-year, were employed as the putative offspring. Because there can be some error associated with the aging of scales, we also used hatchery fish ± 2 brood-years as putative offspring. We first used Mendelian exclusion to assign hatchery fish to their broodstock parents (i.e., each allele in an identified offspring matched at least one allele in both parents). To allow for genotyping errors, we allowed an offspring to mismatch to one allele in both parents (Christie 2010), although 81% of assignments contained no mismatches. No hatchery offspring matched to more than one broodstock pair because we had an average of 36 alleles per locus and because we knew the hatchery broodstock pairings, which reduced the required number of pairwise comparisons. Broodstock fish (potential parents) had genotype data at all loci. Hatchery fish that were missing data at more than two loci ($n = 74$, $<1\%$ of all samples) were not included in these analyses. All Mendelian assignments were independently confirmed by using SOLOMON (Christie et al. 2013) to identify parent-offspring trios and to calculate the posterior probability that the trios were false, given the frequencies of shared alleles. Using 1000 simulated data sets and 50 million simulated genotypes, all assignments identified with exclusion had less than a 0.2% probability of being false. Furthermore, there were almost no discrepancies ($<1\%$) between the assignments made with exclusion and those made with SOLOMON.

Inbreeding and Reproductive Success

We first developed an approach to test whether the small effective number of broodstock breeders ($N_b = 25$ fish) and the large variance in family sizes created by the supplementation program (See Christie, Marine, French, Waples et al. 2012) resulted in inbreeding among returning hatchery fish that could explain the rapid fitness declines observed in these fish. We could not use the pedigree to directly calculate the fitness costs associated with inbreeding in this population for the following reasons. First, we obtained samples of adults as they were passed over the dam en route to their spawning grounds such that we could only measure the reproductive success of adults as the production of their returning adult offspring (see Sample Collection and Study System for details). Thus, on the spawning grounds, there could have been a substantial number of actual matings between relatives that we could not detect because none of their offspring survived to become reproductively mature adults. Because we could not detect matings that did not leave at least one surviving offspring, we would not be able to accurately calculate how much mortality (or decreased fitness) was due to matings between relatives. This would result in an underestimate in the fitness costs associated with inbreeding between hatchery siblings and a test with little power to detect whether inbreeding was the cause of the documented fitness decline. One could compare the success of $H \times H$ and $W \times W$ crosses that left at least one surviving offspring (where $H =$ hatchery and $W =$ wild). However, doing so could give an extremely biased estimate of relative fitness if a much higher proportion of $H \times H$ crosses left zero offspring. Second, although we could compare the reproductive success of inbred versus non-inbred matings that produced at least one surviving adult offspring, these would be unrepresentative samples for comparison because 65% of returning fish had at least one missing parent owing to matings with resident trout (see Table 4 of Araki, Ardren et al. 2007), such that we could not determine whether the mating was or

was not between relatives. Note that we could not assume that offspring with one missing parents were the product of outbred matings because steelhead are polygamous (Seamons et al. 2004; Christie et al. 2011), such that a single resident male could mate with a number of females.

Thus, in order to calculate the fitness costs associated with half- and full-sib matings, we searched the literature for estimates of the number of lethal equivalents associated with inbreeding. There are numerous estimates for vertebrates (reviewed in Keller and Waller 2002), several for rainbow trout (resident *O. mykiss*), but only one study that we are aware of where the number of lethal equivalents is estimated for anadromous steelhead (Thrower and Hard 2009). Using a full-sib breeding design, Thrower and Hard (2009) estimated that the number of lethal equivalents for anadromous steelhead is equal to 10.8. The lethal equivalents were estimated from the number of offspring that survived to return as adults and from fish that were released into the wild (as opposed to held in captivity). Next, we calculated the reduction in fitness w_f with an inbreeding level of f :

$$w_f = w_0 e^{-Bf} \quad (1)$$

where w_0 equals the fitness of the wild-born fish, and B equals one-half the number of lethal equivalents (Morton et al. 1956; Hedrick 2005). Note that here offspring survival is the only

trait affected by inbreeding that matters because the fitness of their parents is estimated as the number of offspring that survive to return as adults. Here, we set w_0 equal to 1 because we are interested in comparing the reduction in fitness of hatchery fish relative to wild-born fish. Using Equation 1, we calculated that the offspring of half and full sibs would have 0.51 and 0.26 the survival of non-inbred individuals, respectively (Supplementary Figure S1 online). Note that these values are extreme: approximately 75% of offspring from a mating between two full siblings would not survive to reproductive maturity. Because this approach results in high inbreeding-related mortality for hatchery matings, these estimates should, if anything, bias the tests in favor of finding fitness reductions due to inbreeding. We also performed a sensitivity analysis by using lethal equivalent estimates 1.5 and 2 times greater than the published estimates (Supplementary Table 1 online).

We next performed a series of simulations to determine the inbreeding-associated reduction in fitness of hatchery fish (Figure 3). We first examined an extreme scenario in which hatchery fish mated only with other hatchery fish (we know from our pedigree data that $H \times W$ matings are actually common). Using our pedigree and database records, we split returning adult hatchery fish into males and females by run-year. For each run-year, we next randomly sampled one male and one female hatchery fish (Figure 3). Because we had assigned all returning hatchery fish back to their broodstock

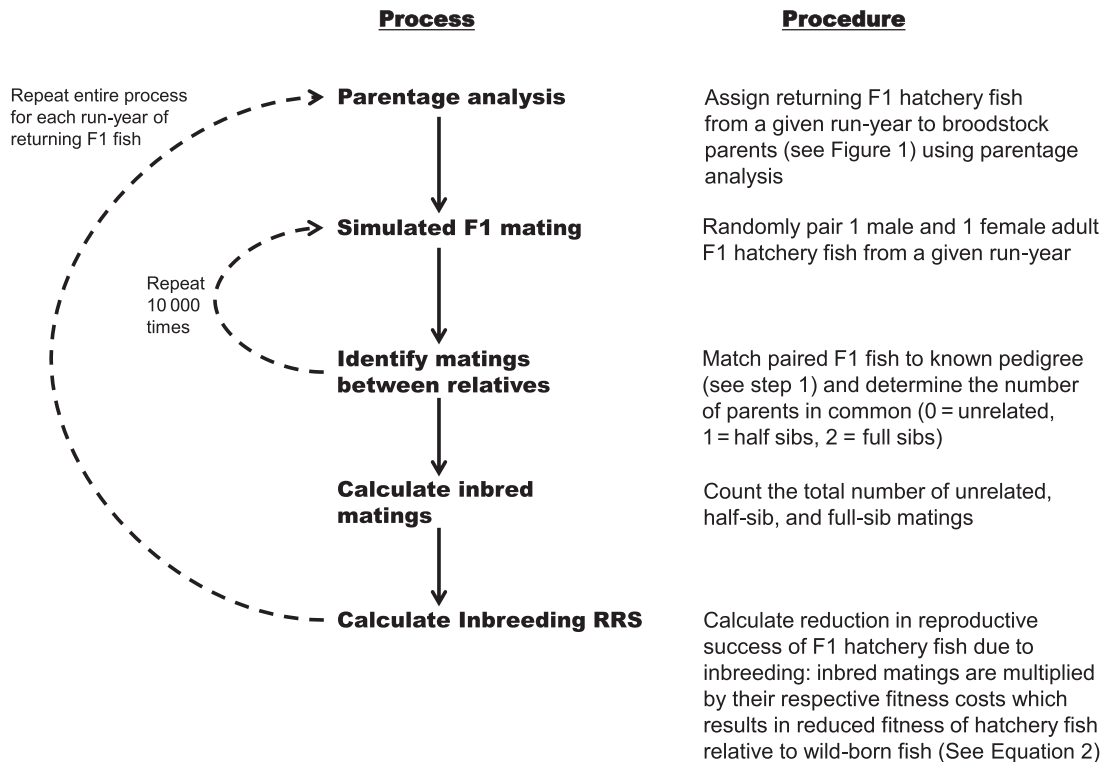


Figure 3. A flow chart illustrating the steps employed to determine whether first-generation hatchery fish had reduced reproductive success in the wild as a consequence of breeding with relatives. It is important to note that this process focuses on determining whether the observed fitness declines in hatchery fish could be explained by inbreeding, not whether inbreeding was occurring in the spawning population.

parents, we knew which hatchery fish were full siblings, half siblings, or unrelated. Using our pedigree, we matched the sampled hatchery fish to their known parents and calculated the number of unique parents. If the hatchery fish we sampled had four unique parents, then they were unrelated. If the hatchery fish had three unique parents, then they were half sibs and if the hatchery fish had two unique parents, then they were full sibs. We repeated the above procedure 10 000 times and tabulated the number of observed full-sib, half-sib, and unrelated matings. Here, we modeled monogamous matings. Steelhead in the wild often mate with multiple partners (e.g., Seamons et al. 2004), however, incorporating this mating structure into our analysis would not change these results because under random mating, the mean number of inbred offspring would be equivalent. To calculate the relative reproductive success (RRS) of hatchery fish compared to a population with no inbreeding-associated fitness declines, we used the following equation:

$$\text{RRS} = \frac{(1 \cdot N_{\text{unrelated}}) + (u_{0.125} \cdot N_{\text{half sib}}) + (u_{0.25} \cdot N_{\text{full sib}})}{N} \quad (2)$$

where N equals the total number of draws (here: 10 000), and $N_{\text{unrelated}}$, $N_{\text{half sib}}$ and $N_{\text{full sib}}$ equal the number of simulated pairings between unrelated individuals, half sibs and full sibs, respectively. The fitness costs associated with various degrees of inbreeding were calculated as described above ($w_{0.125} = 0.51$; $w_{0.25} = 0.26$). Because it is unlikely that hatchery fish mate exclusively with other hatchery fish, we also repeated the above calculations assuming that hatchery fish mate randomly with respect to all fish present on the spawning grounds. Here, we added in the number of wild males and females for each run-year (Table 1) and considered any mating between hatchery and wild fish to have a fitness value equal to 1 (wild and hatchery fish cannot share parents). All calculations were performed in R 2.12 R Core Team (2012) and scripts are available from the corresponding author upon request.

Results

The low number of broodstock contributing to each run-year of hatchery fish created substantial opportunity for inbreeding. In fact, the mean family size for returning hatchery fish was roughly 10 times larger than that for wild-born fish (Figure 1). One hatchery male produced nearly 60 returning adult offspring (full and half sibs), which certainly increased the odds for between-sibling matings. By examining our pedigree, we further determined that 25% of returning hatchery fish in an average run-year descended from only two mothers and two fathers, and that 50% of hatchery fish had descended from only six mothers and seven fathers (Supplementary Figure S2 online). These results are further illustrated with Lorenz curves (Zeileis 2012), where it can be seen that 50% of the broodstock mothers produced only 17.8% of the returning offspring, and that 80% of the broodstock mothers produced only 50.2% of the returning offspring (Supplementary Figure S3 online). The greatest

proportion of siblings on the spawning grounds occurred in 1995, the year for which 94% of returning adults came from a single brood-year (Figure 2).

Despite the large number of hatchery siblings on the spawning grounds, our analyses revealed that the mean reduction in fitness due to inbreeding could not explain the large observed differences in fitness between hatchery and wild fish. In the event that all hatchery fish mated only with other hatchery fish, the inbreeding RRS of hatchery fish averaged 0.958 that of wild fish (Table 2). Thus, even given that extreme mating system, inbreeding only could account for a 4% average reduction in the fitness of hatchery fish in the wild. If we assume random mating with wild steelhead, then the inbreeding RRS of hatchery fish averaged 0.993 (i.e., less than 1% of the reduction in fitness can be explained by inbreeding). Given evidence from our own pedigree that hatchery fish mate with wild fish, we conclude that inbreeding RRS of hatchery fish compared with outbred fish is better represented with the simulations that included the wild fish. Using the actual pedigree to determine the number of matings between related hatchery fish (a problematic approach, as pointed out in Inbreeding and Reproductive Success) resulted in an even smaller proportion of the fitness reduction being explained by inbreeding (data not shown).

Increasing the number of lethal equivalents to 16 and 22 (1.5 and 2 times the original estimates) resulted in nearly identical conclusion (Supplementary Table S1 online), namely that inbreeding alone cannot explain the documented fitness decline. It is worth noting that at such a high level of genetic load, nearly all the offspring of full- and half-sib matings would die. The low effect of inbreeding occurs largely because of the overlapping generations; on average,

Table 2 Estimates of the reduction in reproductive success in hatchery fish due to inbreeding

Run-year	RRS	
	Hatchery	Hatchery + Wild
1995	0.890	0.980
1996	0.960	0.988
1997	0.967	0.992
1998	0.964	0.995
1999	0.965	0.998
2000	0.964	0.995
2001	0.970	0.995
2002	0.963	0.998
2003	0.957	0.993
2004	0.974	0.996
2005	0.962	0.996
Mean	0.958	0.993

Reduction in fitness is relative to a wild, randomly mating population with a fitness of 1. Results are presented for two scenarios, the first in which it is assumed that hatchery fish only mate with other hatchery fish ("Hatchery"), and the second in which hatchery and wild fish are assumed to mate randomly with one another ("Hatchery + Wild"). Given that the pedigree documents successful matings between hatchery and wild fish, the true inbreeding RRS is best represented by the Hatchery + Wild scenario. At most, inbreeding could account for a 4.2% average reduction in the fitness of hatchery fish.

about two-thirds of the hatchery fish in each run-year come from one brood-year and a third from the subsequent brood-year (Figure 1). This conclusion is illustrated by the fact that the first run-year examined (1995) had a substantially lower inbreeding RRS than the other years and is attributed to the first run-year consisting of individuals from a single brood-year. In 1995, most returning hatchery fish came from a single brood-year because they represented the first returns from the initiation of the supplementation program. The average probability of two randomly selected hatchery fish from that run-year being siblings equaled 0.20 (Supplementary Table S2 online), and we estimated the greatest expected decline in fitness associated with inbreeding in that run-year (Table 2).

Discussion

Many threatened, endangered, and commercially important populations are supplemented with captive-born individuals that are released directly into the wild. For the steelhead released in the Hood River, a goal of the supplementation program is to create fish with equal reproductive success to their wild-born counterparts. Because this goal is not being met, it is important to determine the mechanism that is responsible for the fitness reductions associated with hatchery fish in the wild. Given the limited number of broodstock used in the program, inbreeding among returning relatives was one plausible mechanism. It is important to keep in mind that this study does not examine whether inbreeding occurs in the Hood River population, but rather whether inbreeding could contribute to the documented fitness reductions of hatchery-born fish. Here, we demonstrate that inbreeding can, at the very most, account for a 4% reduction in the fitness of the hatchery fish relative to their wild-born counterparts. This result suggests that inbreeding alone cannot adequately explain the 15% reduction in fitness observed in hatchery fish from this population.

We believe our estimates of the contribution of inbreeding to the fitness reduction of hatchery fish are robust and may likely be overestimates of the actual inbreeding-associated fitness reduction for several reasons. First, we considered a scenario for inbreeding whereby hatchery fish mate exclusively with other hatchery fish (which would maximize inbreeding between hatchery fish). However, from both pedigree and observational data, we know that hatchery fish interbreed with wild fish (as intended by the supplementation program). Even if the hatchery fish mated exclusively with one another, only 6% of matings, on average, would be between relatives (Supplementary Table S2 online). One caveat here is that if hatchery fish preferentially mated with their siblings, then we may have underestimated the amount of inbreeding and associated fitness reductions. Assortative mating between hatchery siblings seems unlikely, however, especially in light of other studies illustrating random mating or even inbreeding avoidance in fishes (e.g., Landry et al. 2001; Frommen and Bakker 2006). Second, we used a species-specific estimate of the number of lethal equivalents that is considerably higher than that reported for most vertebrates,

including other salmonids (Keller and Waller 2002; Thrower and Hard 2009). We also performed additional analyses using estimates of lethal equivalents 1.5 and 2 times greater than the published estimates, which would result in almost no surviving offspring associated with inbred matings, and we still documented only modest declines in fitness (Supplementary Table S1 online). Third, we did not include resident trout in our scenario of random mating between wild and hatchery fish because we do not have accurate estimates of their population size. Resident fish frequently mate with steelhead and the vast majority of resident genes passed to anadromous offspring come from wild fish (Christie et al. 2011). Thus, the total number of wild fish present in the population is probably much larger than the numbers we used in our simulations and could further decrease the probability of inbreeding between hatchery fish. Finally, we assumed that wild fish were completely outbred even though the relative reduction in fitness associated with hatchery inbreeding would be lessened if there were any inbreeding-associated fitness reductions within wild-born fish. All of the above reasons make for a test that, if anything, was biased towards finding inbreeding-associated fitness reductions. The fact that these analyses suggested only a 1–4% reduction in the fitness of the hatchery fish suggests that inbreeding played only a minor role (if any) in causing the low estimate of fitness of the hatchery fish in this system.

Under what conditions might inbreeding have contributed more to the apparent fitness decline of hatchery fish? In this system, the potential for inbreeding was substantially reduced by overlapping generations. Thus, had there been consecutive run-years similar to 1995 (perhaps owing to failed brood-years), inbreeding could have become a larger problem in this population. One way to minimize the possibility of inbreeding would be to spread the contribution of a single brood-year over more run-years. For example, it could be beneficial to prevent the first year of returning hatchery fish onto the spawning grounds, and there may be merit in letting some fish take 2 years to smolt in the hatchery. Another recommendation would be for supplementation programs to closely monitor the effective numbers of breeders and subsequent number of siblings in order to avoid unintended costs associated with inbreeding (Waples 1999). Because supplementation programs have the goal of enhancing the wild population, they deliberately take few breeders from the wild to use as broodstock. Thus, low N_b in supplementation hatchery fish might be a common problem, which, in addition to reducing the overall genetic diversity of the population, has recently been demonstrated to affect fitness-related traits (Naish et al. 2013). We, therefore, recommend that supplementation programs be carefully monitored to determine the extent to which inbreeding occurs.

In conclusion, we found that despite the large number of hatchery siblings on the spawning grounds, inbreeding cannot explain the rapid decline in fitness of hatchery fish (relative to wild fish) in the Hood River supplementation program. Here, the potential for inbreeding was largely ameliorated by overlapping generations. This result suggests that other factors, such as domestication selection (Araki et al. 2009; Christie,

Marine, French, Blouin 2012), are primarily responsible for the reduced fitness of hatchery fish in the Hood River.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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