

ORIGINAL ARTICLE

Effective size of a wild salmonid population is greatly reduced by hatchery supplementation

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Many declining and commercially important populations are supplemented with captive-born individuals that are intentionally released into the wild. These supplementation programs often create large numbers of offspring from relatively few breeding adults, which can have substantial population-level effects. We examined the genetic effects of supplementation on a wild population of steelhead (*Oncorhynchus mykiss*) from the Hood River, Oregon, by matching 12 run-years of hatchery steelhead back to their broodstock parents. We show that the effective number of breeders producing the hatchery fish (broodstock parents; N_b) was quite small (harmonic mean $N_b = 25$ fish per brood-year vs 373 for wild fish), and was exacerbated by a high variance in broodstock reproductive success among individuals within years. The low N_b caused hatchery fish to have decreased allelic richness, increased average relatedness, more loci in linkage disequilibrium and substantial levels of genetic drift in comparison with their wild-born counterparts. We also documented a substantial Ryman–Laikre effect whereby the additional hatchery fish doubled the total number of adult fish on the spawning grounds each year, but cut the effective population size of the total population (wild and hatchery fish combined) by nearly two-thirds. We further demonstrate that the Ryman–Laikre effect is most severe in this population when (1) >10% of fish allowed onto spawning grounds are from hatcheries and (2) the hatchery fish have high reproductive success in the wild. These results emphasize the trade-offs that arise when supplementation programs attempt to balance disparate goals (increasing production while maintaining genetic diversity and fitness).

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INTRODUCTION

The large-scale release of plants and animals into the wild can have unintentional negative effects on the genetic diversity of the recipient populations (Laikre *et al.*, 2010). One widely-used strategy for creating large numbers of individuals suitable for release into the wild is to implement captive-breeding programs. Despite the large numbers of such programs, there remains a distinct lack of appropriate and effective monitoring of individuals released into the wild (Waples, 1999; Laikre *et al.*, 2010). Ideally, the genetic monitoring of populations would consist of sampling before and after the release of individuals into the wild. In practice, however, these comparisons are not feasible for many populations due to the lengthy history of anthropogenic intervention. Nevertheless, a long-term evaluation of the individual- and population-level effects of large-scale releases, particularly when combined with detailed pedigree analyses (Pemberton, 2008), can yield valuable insights into the demographic and genetic effects of population supplementation.

Wild populations of Pacific salmonids have declined sharply over the past century due to a combination of habitat destruction, hydropower and overfishing (McClure *et al.*, 2003; Quinn, 2005; Naish *et al.*, 2008). Consequently, 23% of Pacific salmon stocks are at moderate to high risk (Augerot and Foley, 2005) and 54% of evolutionarily significant units are currently listed as threatened or endangered under the US Endangered Species Act (Gustafson *et al.*,

2007; ESA salmon listings, 2009). In order to alleviate the continued population declines, supplementation programs have been commonly implemented throughout the Northern Pacific. The term ‘supplementation’ is used by programs where the main objective is to help wild populations recover, but sometimes the term is used for programs that also have a goal of harvest augmentation. Here we are considering the former. Supplementation programs generally use either hatchery fish (of various backgrounds) or returning wild fish as broodstock and release the hatchery-raised smolts at or near the wild spawning grounds. After returning from the ocean, a portion (or in many cases all) of the returning adult hatchery fish are allowed onto the spawning grounds with the wild-born fish. Wild broodstock are preferred in some supplementation programs because they can produce offspring that have much higher fitness in the wild than offspring from older, domesticated hatchery stocks (though even first generation hatchery fish can have reduced fitness in the wild; Araki *et al.*, 2007a; Araki *et al.*, 2009; Williamson *et al.*, 2010; Berntson *et al.*, 2011; Theriault *et al.*, 2011; Christie *et al.*, 2012).

Aside from creating differences in reproductive success between wild and captive-born individuals, supplementation programs may also affect important population genetic parameters (Waples and Teel, 1990). For example, genetic diversity, allelic richness and patterns of genetic drift may be altered by population supplementation. Furthermore, the effective population size for individuals produced in captive

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breeding programs can be reduced relative to their wild-born counterparts because (1) typically small numbers of individuals are used as broodstock (especially for populations or species of concern) and (2) there can be substantial variance in reproductive success among the individuals chosen as broodstock (for example, a small portion of the broodstock produce a high percentage of the surviving offspring). Supplementation practices can thus create a 'Ryman–Laikre effect', where the inbreeding effective population size of the entire population is reduced relative to that of the original wild population (Ryman and Laikre, 1991; Ryman *et al.*, 1995). Even though this effect was pointed out over 20 years ago, the problems associated with inundating a wild population with the offspring of a handful of founders have been largely ignored in current practice. In fact, the recent trend toward producing first-generation hatchery fish could exacerbate the problem because their reproductive success is usually much higher in the wild than that of domesticated stocks.

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 12 run-years of steelhead from Hood River, Oregon for which all anadromous fish were genotyped at eight highly polymorphic microsatellite loci. We first used pedigree data to calculate the effective number of broodstock breeders represented in the returning hatchery offspring. Owing to the small effective number of breeders we predicted that, in comparison to wild fish, hatchery fish would have (i) high genetic drift among years, (ii) reduced genetic diversity, (iii) increased relatedness, and (iv) substantial linkage disequilibrium (LD) among loci. We further tested for a Ryman–Laikre effect and examined how the strength of the Ryman–Laikre effect is affected by (i) the proportion of hatchery fish allowed onto spawning grounds and (ii) the reproductive success of those hatchery fish in the wild (relative to wild-born fish).

MATERIALS AND METHODS

Sample collection and typing

Samples were collected from the Hood River, Oregon, where winter-run steelhead are listed as threatened under the US Endangered Species Act (Busby *et al.*, 1996). Genetic samples for steelhead employed in this study were collected from run-years 1995–2006, which corresponds to fish born in brood-years 1993 through 2003. These run-years also encompass the initiation of the supplementation program (though programs with domesticated, non-local broodstock existed previously; see Olsen, 2003). Winter-run steelhead begin returning to their natal rivers in early December, the year of which designates the run-year, and do not spawn until spring of the next year. Thus a steelhead that spawns in May of 2000 will belong to run-year 1999 (even if it returned in March) and its offspring will belong to brood-year 2000. Because of the accelerated growth rate in hatcheries (that is, smoltification in 1 year vs a typical time of 2 years in the wild), 71% of hatchery-born steelhead return to spawn after 2.5 years, whereas 64% of wild-born steelhead return after 3.5 years (Figure 1; Araki *et al.*, 2007a). Fish from a single run-year come from multiple brood-years. Steelhead that returned to spawning grounds in the Hood River were first passed over the Poverdale dam, which was a complete barrier to migrating fishes. Every fish passed over the dam was individually handled, and samples of scales and fin tissue were collected by staff of the Oregon Department of Fisheries and Wildlife for subsequent aging and genetic analysis. Steelhead returning to the Hood River are easily categorized as hatchery or wild origin because all hatchery fish have their adipose fin removed before release.

All wild fish and an approximately equal number of hatchery fish were passed over the dam each year (wild run sizes ranged from 221 to 1027 fish). The winter-run hatchery fish were created using either two wild fish or one wild fish and a first-generation hatchery fish as parents (see Araki *et al.*, 2007a,b). Most fish were spawned with two (or occasionally more) partners, which created returning hatchery fish that were full sibs, half sibs or unrelated.

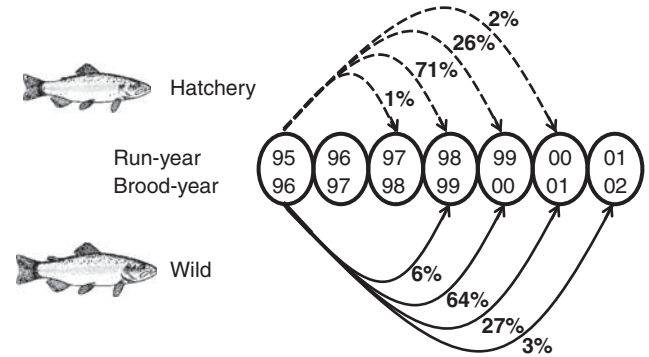


Figure 1 Distribution of run-years in which wild and hatchery fish returned. Hatchery fish born in brood-year 1996 (run-year 1995 for their parents) returned predominantly in run-year 1998, while wild fish returned predominately in run-year 1999. Notice that fish in any given run-year come from multiple brood-years.

Table 1 Number of returning adult winter-run steelhead samples

Brood-year	Hatchery		Wild	
	Female	Male	Female	Male
1993	107	121	182	120
1994	181	109	135	77
1995	119	79	201	95
1996	93	79	802	436
1997	159	131	598	386
1998	323	319	568	334
1999	309	315	392	255
2000	154	149	323	194
2001	303	250	270	192
2002	79	75	203	153
2003	95	132	219	161
Total	1922	1759	3893	2403

Numbers are reported for fish grouped by brood-year (i.e., their year of birth) and separated by sex and hatchery or wild status.

As per Araki *et al.* (2007a,b), we use 'wild' to refer to any fish spawned in the river under natural conditions, regardless of whether its parents have hatchery ancestry. Furthermore, fish used as hatchery broodstock were collected randomly from throughout the entire run period and were unlikely to be related. We have DNA samples from all broodstock, and comprehensive records on broodstock pairings in the hatchery. Extensive details on this study system, management practices, steelhead life-history and reproductive success can be found elsewhere (Olsen, 2003; Araki *et al.*, 2007a,b; Kostow, 2009).

The winter-run steelhead samples averaged 907 fish per brood-year for a total of 9977 samples (Table 1). We genotyped all samples at eight highly polymorphic microsatellite loci (Omy 1001, Omy 1011, Omy 1191, Omy77, One108, One2, Ssa407 and Str2), which average 36 alleles per locus (see Araki *et al.*, 2007a,b for details of microsatellite loci, Hardy–Weinberg proportions and molecular methods). These data were previously employed to determine the relative reproductive success of hatchery and wild steelhead (Araki *et al.*, 2007a,b), and of wild-born steelhead having hatchery vs wild parents (Araki *et al.*, 2009). Results from this work documented 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 *et al.*, 2007a).

Effective number of broodstock parents

To calculate the effective number of broodstock parents, we first employed parentage analysis to assign hatchery fish back to their broodstock parents.

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 ± 1 brood-year as putative offspring. Parentage analysis revealed that very few hatchery fish (<3%) had been assigned via scale ageing to the incorrect brood-year, which is not always the case with wild-born fish (for example, Seamons *et al.*, 2009). We used Mendelian exclusion to assign hatchery fish to their broodstock parents (that is, 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 fish 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 had missing data at more than two loci were not used in this study (<1%), resulting in a total of 74 unassigned fish.

After assigning hatchery fish to known broodstock pairs we calculated the mean (\bar{k}) and variance (V_k) in reproductive success for male and female broodstock from each run-year. We next estimated the inbreeding effective number of breeders (N_b) for each sex as:

$$N_b = \frac{\bar{k}N - 2}{\bar{k} - 1 + \frac{V_k}{\bar{k}}} \quad (1)$$

where N equals the number of broodstock males or females used in a run-year (Crow and Kimura, 1970; Caballero, 1994). We next combined the estimates for both sexes by setting

$$N_b = \frac{4(N_{b[\text{Female}]} \cdot N_{b[\text{Male}]})}{N_{b[\text{Female}]} + N_{b[\text{Male}]}} \quad (2)$$

Note that under some circumstances it may be necessary to adjust V_k/\bar{k} to account for errors in parentage assignment and missing parents (Araki *et al.*, 2007c). In our case, however, we had complete genotypes of all putative parents, and using assignments with or without allowing for mismatching loci did not substantially change our estimates (that is, we had very low type a and b errors using the terminology of Araki *et al.*, 2007c). Using records from the Oregon Department of Fish and Wildlife, we compared the effective number of breeders used in each year (as calculated above) to (1) the number of broodstock actually used, (2) the total number of returning offspring that the broodstock produced, and (3) the total number of wild fish passed over the dam each run-year.

Population effects of supplementation

We first calculated F_{ST} between the wild and hatchery fish grouped by brood-year using FSTAT 2.9.3 (Goudet, 1995). These results were illustrated with a principal coordinates analysis performed with the package ade4 (Thioulouse *et al.*, 1997) as implemented in R version 2.12 (R Development Core Team, 2011). Correspondence analysis on the genotype data produced a very similar pattern (data not shown). Given the high number of alleles per locus, we also calculated the multi-allele analog, G_{ST} , using RECODEDATA (Meirmans, 2006). The numbers of hatchery and wild fish in each group were both large (range: 154–1238) and roughly equal, such that the observed differentiation was not due to differences in sample sizes.

For all 11 years of data, we next calculated the allelic richness and F_{IS} for both wild and hatchery fish using FSTAT. For allelic richness, samples were rarefied to smallest sample size ($n=154$). Using 5000 permutations in FSTAT, we tested whether differences in allelic richness, within sample gene diversity, and observed heterozygosity were different between hatchery and wild fish. We also calculated the percentage of locus pairs in LD with GENEPOP 4.0 (Raymond and Rousset, 1995) using 10 000 batches and 10 000 iterations per batch. Lastly, we calculated Queller and Goodnight's (1989) pairwise measure of relatedness as implemented in GENALEX 6.41 (Peakall and Smouse, 2006). We performed 999 bootstraps and 999 permutations to determine whether the estimates were different from zero and whether estimates for hatchery and wild fish differed from one another, respectively.

Ryman–Laikre effect

To test for a Ryman–Laikre effect, we first estimated the effective number of breeders per brood-year for wild fish. We used LDNe 1.2 (Waples and Do, 2008) to estimate the effective number of wild breeders (N_w). We used a LD-based method because the presence of resident steelhead (that is, rainbow trout) in the river prevented assignment of a large portion of wild offspring to parents using pedigree methods (see Christie *et al.*, 2011 for a detailed analysis of resident fish). We selected 0.02 as the lowest allele frequency to be used in LDNe, which has been shown to generally provide a good balance between maximizing precision and minimizing bias (Waples and Do, 2008). Selecting smaller values had little effect on our estimates, whereas larger values greatly increased the variance. We calculated confidence intervals by jackknifing over loci. We next calculated the effective number of breeders for hatchery fish (N_c) using LDNe and pedigree-based methods (see the methods described above). Using the equation presented in Ryman and Laikre (1991), we calculated the effective number of breeders for hatchery and wild fish combined as:

$$\frac{1}{N_e} = \frac{x^2}{N_c} + \frac{(1-x)^2}{N_w} \quad (3)$$

where N_c and N_w are the effective number of hatchery and wild breeders, respectively. Because x theoretically equals the contribution of hatchery fish to the next generation (Ryman and Laikre, 1991), we calculated x as:

$$x = \frac{N_{\text{Hatchery}} \cdot \text{RRS}}{N_{\text{total}}} \quad (4)$$

where N_{Hatchery} equals the total number of hatchery fish passed over the dam, RRS equals the reproductive success of hatchery fish relative to wild fish and N_{total} was the total number of fish (wild and hatchery) passed over the dam for a given brood-year. We used an RRS of 0.85, which was the average reproductive success of a hatchery fish created with two wild parents (Araki *et al.*, 2007a). We took the reciprocal of Equation (3) to calculate $N_e T$, the effective number of breeders for the combined hatchery and wild components of the population.

We next calculated $N_{\text{No Hatchery}}$, which equals the best estimate of what the effective number of breeders would have been in the wild had there been no

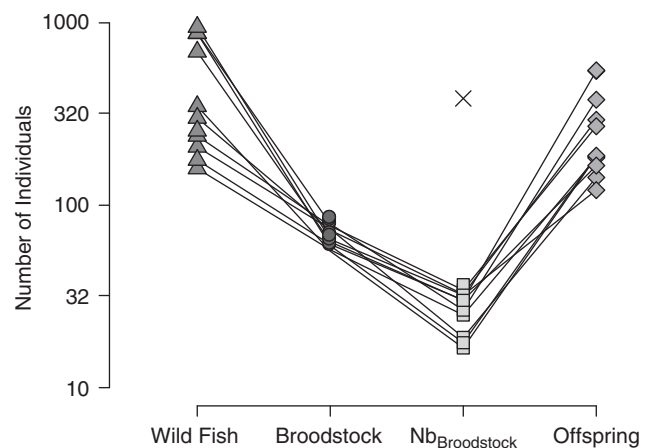


Figure 2 Genetic bottlenecks created by the supplementation program. Triangles ('Wild Fish') represent the total number of wild fish passed over the dam for 11 consecutive years. Circles ('Broodstock') are the total number of wild fish removed from the run and used in the supplementation program. Squares (' $N_{b\text{Broodstock}}$ ') are the effective number of broodstock breeders calculated using pedigree data. The \times represents the average effective number of breeders for the wild population as calculated in Araki *et al.* (2007c). Diamonds ('Offspring') represent the total number of hatchery offspring assigned to the broodstock from a given run-year. Notice that the ordinate is on a logarithmic scale and that solid lines connect years. A full color version of this figure is available at the *Heredity* journal online.

Table 2 Point estimates for the effective number of breeders estimated with LDNe and associated 95% confidence intervals (Jackknife CI) for hatchery and wild fish by brood-year (Also presented are the pedigree-based estimates for the effective number of breeders for hatchery fish (Pedigree))

Brood-year	Hatchery fish			Wild fish			
	Pedigree	LDNe	Jackknife CI	LDNe	Jackknife CI		
1993	16.5	23.6	21.7	25.7	222.4	196.9	251.6
1994	32.5	30.3	27.8	32.9	285.5	232.9	362.2
1995	25.0	22.2	20.0	24.6	180.1	157.3	208.2
1996	18.9	21.2	19.2	23.3	250.3	230.5	271.8
1997	34.3	30.7	28.1	33.6	426.6	372.7	491.6
1998	30.2	29.1	27.2	31.1	517.2	452.2	600.1
1999	36.7	38.7	35.3	42.2	588.9	500.1	710.4
2000	17.6	21.6	20.2	23.1	663.6	559.6	808.9
2001	26.6	27.8	25.3	30.5	577.3	492.0	694.4
2002	32.1	33.2	29.3	37.6	650.4	497.0	922.7
2003	30.1	27.7	24.7	30.9	866.1	604.9	1468.9

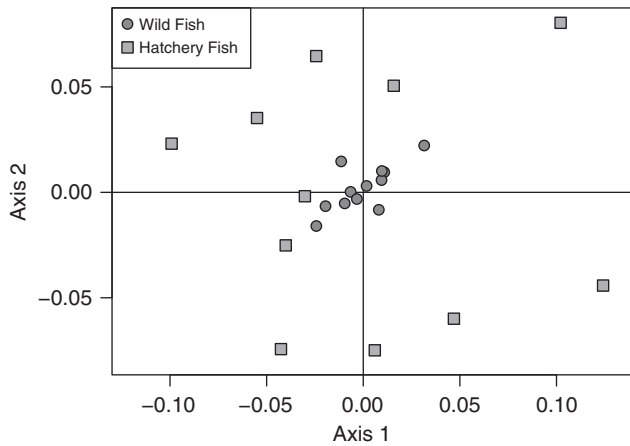


Figure 3 Principal coordinates analysis of pairwise F_{ST} between all brood-years of hatchery and wild fish. Circles represent wild fish and squares represent hatchery fish. Notice that the F_{ST} between hatchery brood-years is substantially greater than wild brood-years owing to the small effective number of breeders. A full color version of this figure is available at the *Heredity* journal online.

supplementation program. We calculated this value per brood-year as:

$$N_{No\ Hatchery} = N_w \cdot \left(1 + \frac{N_{brood}}{N_{total}} \right) \quad (5)$$

where N_{brood} equals the number of fish brought into the hatchery and N_{total} was the total number of fish allowed to spawn in the wild per brood-year. It should be noted that this correction to N_w yielded only slight qualitative differences for the population in this study, but it would be particularly important for hatchery programs that use a larger percentage of the returning fish as broodstock. Lastly, we divided $N_e T$ by $N_{No\ Hatchery}$ to measure the N_b of the entire population (hatchery and wild combined) relative to the N_b in the wild had there been no supplementation program. Thus, a ratio = 1 indicates that there is no decrease in N_b owing to the hatchery program, whereas a ratio < 1 indicates a Ryman–Laikre effect. We next plotted these results as a response to x (the contribution of hatchery fish to the next generation), where $RRS = 0.85$ (see Equation (4)). We also varied RRS from 0 to 1 in the calculation of x (Equation (4)) and divided the harmonic mean of $N_e T$ and the

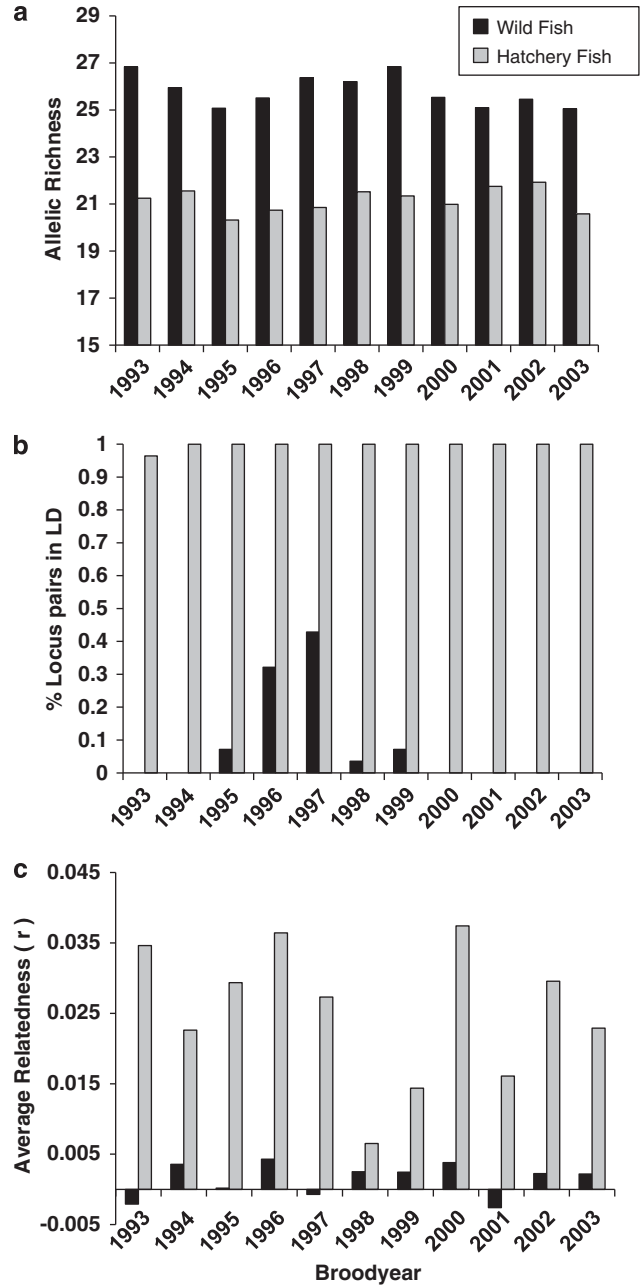


Figure 4 Differences between wild (black bars) and hatchery (gray bars) fish as a consequence of the low effective number of breeders used to create hatchery fish. (a) Allelic richness (averaged across loci) in hatchery fish was lower than wild fish. Hatchery fish also had a much greater percentage of locus pairs in LD than wild fish (b). (c) illustrates that the average relatedness of hatchery fish was substantially greater than wild fish.

harmonic mean of $N_{No\ Hatchery}$ (for the 11 brood-years) to illustrate the effect of RRS on the magnitude of the Ryman–Laikre effect.

RESULTS

According to hatchery records, a total of 40 to 80 fish were used as broodstock each year. However, the effective number of broodstock parents estimated from their returning offspring ranged from 16.5 to 36.7, with a harmonic mean of 24.9 individuals (Figure 2 and Table 2). The small effective number of breeders was exacerbated by

Table 3 Estimates of the effective number of breeders with (N_eT) and without ($N_{No\ Hatchery}$) a supplementation program

Brood-year	N_c	N_w	$N_{No\ Hatchery}$	$X_{RRS=1}$	$N_eT_{RRS=1}$	$X_{RRS=0.85}$	$N_eT_{RRS=0.85}$
1993	16.5	222.4	266.5	0.43	78.9	0.37	100.9
1994	32.5	285.5	318.5	0.58	91.8	0.49	120.1
1995	25.0	180.1	208.9	0.40	118.8	0.34	141.7
1996	18.9	250.3	257.8	0.12	258.5	0.10	264.6
1997	34.3	426.6	456.4	0.23	343.6	0.19	382.1
1998	30.2	517.2	545.0	0.42	156.4	0.35	202.0
1999	36.7	588.9	618.1	0.49	142.7	0.42	187.9
2000	17.6	663.6	717.8	0.37	119.9	0.31	158.6
2001	26.6	577.3	652.9	0.54	86.8	0.46	116.8
2002	32.1	650.4	746.0	0.30	278.9	0.26	345.0
2003	30.1	866.1	973.1	0.37	196.1	0.32	256.8
H Mean	25.4	373.5	415.2	—	136.3	—	172.9

$N_{No\ Hatchery}$ equals the estimated effective number of breeders had there been no hatchery supplementation program and N_eT equals the total effective number of breeders for both wild and hatchery fish considered jointly with relative reproductive success values of 1 and 0.85 (RRS; hatchery relative to wild). Also presented is 'x', the contribution of hatchery fish to the next generation, and point estimates for the effective number of breeders for the captive (N_c [Pedigree]), and wild fish per brood-year (N_w [LDNe]). Where appropriate, we report the harmonic mean (Mean) for all brood-years.

the large variance in family size among broodstock fish (Supplementary Figure S1). In general, there was very good agreement ($r^2=0.79$) between effective number of breeders estimated with pedigree and LD-based methods (Table 2 and Supplementary Figure S2). Although hatchery and wild fish were passed above the dam in approximately equal numbers, all the hatchery fish descended from a handful of breeders, while the wild fish descended from several hundred parents (harmonic mean N_b for wild fish = 373, see also Araki *et al.*, 2007c). The low hatchery N_b created many noticeable differences between hatchery and wild fish. The point estimates for F_{ST} between hatchery fish brood-years are an order of magnitude greater than between wild fish brood-years (Figure 3). None of the qualitative results differed between F_{ST} and G_{ST} ; however, G_{ST} values were approximately an order of magnitude greater than F_{ST} (Supplementary Table S1 and Supplementary Table S2).

The average allelic richness of hatchery fish was substantially lower than wild fish across all brood-years (Figure 4a). Permutation-based tests revealed that allelic richness ($P<0.0002$) and within-sample gene diversity ($P<0.0004$) were significantly lower in hatchery fish than wild fish. Observed heterozygosity was not significantly different between the two groups ($P=0.113$). Furthermore, out of 11 brood-years examined, 99.9% of loci pairs were in LD for hatchery fish, compared with an average of 8% for wild fish (Figure 4b). In all years, there were slightly more wild fish than hatchery fish, which eliminates a potential bias for statistical tests finding greater numbers of loci pairs in LD for hatchery fish. Importantly, the brood-years for which wild fish had a noticeable percentage of loci pairs in LD corresponded with the return of the first generation of hatchery fish that mated in the wild. The large amount of LD present in hatchery fish is due to low N_b in the returning hatchery fish (Hedgecock *et al.*, 2007) and is further reflected in elevated levels of relatedness in hatchery fish compared with wild fish (Figure 4c). Results from permutation tests revealed that all hatchery estimates of relatedness were significantly greater than wild estimates. The average relatedness of all hatchery fish equaled 0.025, which is equivalent to third-cousins. F_{IS} values were not substantially different between wild and hatchery fish (≈ 0.01 in both groups).

We also documented a Ryman–Laikre effect (Table 3), in which the effective population size of the entire population is reduced due to the hatchery program. On taking the harmonic mean for 11 brood-years and setting the RRS equal to 1, the effective number of breeders for the entire population was 36.5% of the effective number of breeders

for wild fish alone despite a near doubling of the total population size. This percentage was reduced to 32.8% when we estimated the effective number of breeders for the wild population had no wild fish been brought into the hatchery (see harmonic means for N_eT and $N_{No\ Hatchery}$ in Table 3, which were simply divided to obtain these percentages). These percentages changed to 46.3% and 41.6%, respectively, when the population-specific RRS estimate of 0.85 was used (See Table 3 for harmonic means). In brood-year 1996, there was no evidence for a Ryman–Laikre effect, which was the brood-year for which, relative to wild fish, the fewest hatchery fish were allowed onto the spawning grounds. In fact, we found a strong negative relationship ($r^2=0.65$) between the contribution of hatchery fish to the next generation and the reduction in the effective number of breeders for the combined hatchery and wild population (Figure 5a). We also demonstrated, in this population, that a higher reproductive success of hatchery fish resulted in a stronger Ryman–Laikre effect (Figure 5b). Using a wide range of theoretical values for N_c , N_w , RRS, and the proportion of hatchery fish allowed onto spawning grounds, we further illustrate that the Ryman–Laikre effect is most pronounced when (i) the effective number of broodstock breeders is low relative to the wild, (ii) the proportion of hatchery fish allowed onto spawning grounds is high, and (iii) the RRS of hatchery fish is high (Supplementary Figure S3).

DISCUSSION

For this Hood River steelhead population, we demonstrate that the effective number of breeders in the supplementation program can be surprisingly low (harmonic mean across years = 25 fish). In each cohort of hatchery fish, we also observed lower genetic diversity, higher relatedness, substantial fluctuations in allele frequencies and extensive LD in comparison with wild-born fish. Increased rates of drift could contribute to fitness declines in fish from multi-generation or conventional hatchery programs (for example, owing to random fixation of deleterious alleles). The comparatively low amount of drift among brood-years of wild fish is likely due to the much larger effective number of breeders in the wild and the wild brood-years consisting of offspring from a greater number of run-years.

We also documented a substantial Ryman–Laikre effect in 10 of 11 brood-years. This effect revealed that although the supplementation program doubled the total number of breeding adults in the river each year, it cut the effective population size to roughly one-third of what it would have been had there been no hatchery supplementation

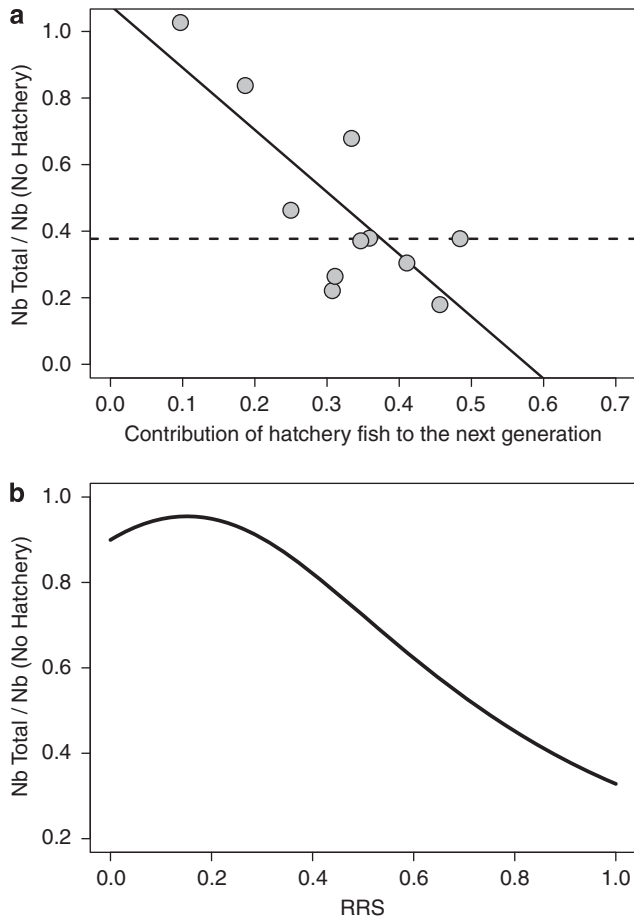


Figure 5 Illustration of a substantial Ryman–Laikre effect. The ordinate equals the total effective number of breeders (hatchery and wild fish pooled; $N_{b\text{Total}}$) divided by the effective number of breeders for the population had no hatchery program been implemented ($N_{b\text{NoHatchery}}$). Thus the ordinate equals the magnitude of the Ryman–Laikre effect (with smaller values equating to a stronger effect). (a) Relationship between the contribution of hatchery fish to the next generation (see Equation (4)) and the Ryman–Laikre effect for 11 brood-years. The dashed line represents the median ordinate value for all years. RRS was fixed at 0.85 such that the contribution of hatchery fish directly reflects the proportion of hatchery fish allowed onto the spawning grounds. (b) Relationship between relative reproductive success of hatchery fish (RRS) and the Ryman–Laikre effect. For visual clarity, we took the harmonic mean of $N_{b\text{Total}}$ and the harmonic mean of $N_{b\text{NoHatchery}}$ across all 11 brood-years to generate a single point estimate for each distinct RRS value.

program. We further illustrated that allowing more than one hatchery fish for every 10 returning wild fish onto the spawning grounds led to a substantial reduction in the overall effective number of breeders (Table 3). This result is due to the effective number of breeders for hatchery fish equaling about one-tenth of the total effective number of breeders. Clearly, if the goals of supplementation are to bolster the wild population, then allowing only one hatchery fish access to the spawning grounds per 10 wild fish will yield little demographic benefit considering that an equivalent number of wild fish were removed from the population to be used as broodstock. Allowing more hatchery fish onto the spawning grounds, however, would decrease the effective population size, which is also at odds with conservation goals. Although it often occurs, the practice of allowing all returning hatchery fish onto spawning grounds without the careful monitoring of important genetic parameters (for example, N_b) could

have large impacts on the long-term conservation of that population (for example, genetic variation important for future adaptation could be rapidly reduced).

In this population, we further documented that the effective size of the total population decreased as the reproductive success of the returning hatchery fish increased, which is due to hatchery fish with higher reproductive success having a greater contribution to subsequent generations (see Equation (3)). This result is also at odds with the goals of some supplementation programs, which aim to create fish that have reproductive success equal to their wild counterparts. Here we show that if supplementation programs meet that goal, then they may be unintentionally decreasing the effective population size. These results make it apparent that any supplementation program will involve some inherent trade-offs. Explicitly accounting for the demographic, genetic and societal costs and benefits of supplementation could pave the way for more prudent management actions.

Our results illustrate in a practical example some of the general outcomes implied by the Ryman–Laikre equation (see Equation (3) and Supplementary Figure S3), which is determined by the effective number of hatchery and wild breeders (N_b) and ‘ x ’, the contribution of hatchery fish to the next generation. Some points to keep in mind about the Ryman–Laikre effect are that: (1) if N_b/N in the hatchery is less than or equal to N_b/N in the wild, then N_eT (the combined wild and hatchery effective size) can never be larger than it would be without the program; (2) if N_b/N is higher in the hatchery than in the wild, then it may be possible to actually increase N_eT via supplementation. This could be accomplished by equalizing variance in family sizes in the hatchery. However, this benefit would only be realized if the hatchery contribution to the next generation, ‘ x ’, is fairly low; (3) ‘ x ’ should be calculated by taking the relative reproductive success of hatchery fish into account (see Equation (4)), because what matters most is the fraction of genes in the next generation that come from hatchery fish; and (4) the effect of RRS on N_eT increases as the proportion of hatchery fish relative to wild fish allowed access to spawning grounds is increased (Supplementary Figure S3). Thus, in order to balance demographic gains with the loss of genetic diversity, supplementation programs may be most useful for a quick demographic boost, when wild returns are very low, and when the programs are only implemented for a short period of time (Waples, 2004).

Our results also suggest several additional management practices that might be considered. Supplementation programs create two large bottlenecks, each corresponding to a reduction in the number of breeders (Figure 2). The first bottleneck occurs simply by choosing a limited number of individuals for broodstock. The second bottleneck is created by the large variance in reproductive success among those hatchery broodstock (Supplementary Figure S1). As mentioned above, deliberately equalizing the variance in reproductive success among broodstock could help to increase the genetic diversity of hatchery fish without taking more breeders from the wild. Equalizing family sizes should also reduce the rate of domestication (Allendorf, 1993; Christie *et al.*, 2012). Of course, any variation in survival that occurs after smolts are released will generally be beyond the control of managers (for example, Reisenbichler *et al.*, 2004). In this study, the V_k/\bar{k} was surprisingly large—similar to that observed among breeders in wild populations. Furthermore, broodstock family sizes were not correlated with eggs used per female or any other phenotypic trait of the parents that we could measure (that is, length, weight, age and run-timing; Christie *et al.*, 2012). Thus, determining the cause of the high variance in family size in each brood-year of hatchery fish would

be particularly useful. Another practice that could mitigate the Ryman–Laikre effect would be to spread the contribution of a single brood-year over multiple release years. For example, it might be beneficial to exclude the first year of returning hatchery fish onto the spawning grounds (which come from a single brood-year), and there might be merit in allowing a portion of hatchery steelhead take 2 years to smolt in the hatchery (1 year is typical hatchery practice).

In conclusion, we found that a contemporary supplementation program greatly reduced the effective size of a wild population. These results further illustrate that different conservation goals can be at odds with each other in a supplementation program. For example, the small N_b of hatchery fish created in a supplementation program can have unintended genetic consequences, but bringing more wild individuals into the breeding program can also have negative consequences for the population. Furthermore, adding more hatchery fish to the population may temporarily increase the census size, but can drastically decrease the effective population size. Thus, we recommend that (1) programs that release large numbers of captive-born individuals into the wild be rigorously monitored, and that (2) more consideration be given to balancing the competing goals of increasing the census size of the population (while minimizing domestication) and preserving the wild population's genetic diversity.

DATA ARCHIVING

R scripts for performing parentage analyses are available for download at <https://sites.google.com/site/parentagemethods/>. Hatchery genotypes and associated scale-ageing data can be found at dryad:doi:10.5061/dryad.2g257.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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