The influence of family-correlated survival on N_b/N for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*)

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Abstract: There exist surprisingly few data on the final variance and mean of family sizes for hatchery-born fish at the adult stage. Thus, it is difficult to predict, for a conservation hatchery operation that minimizes the variance in progeny number, how much lower the true effective population size (N_e) of a cohort of hatchery-born adults will be than N_e predicted simply by the number of parents that produced them. We used parentage analysis to estimate the survival and N_e for two integrated stocks of hatchery coho salmon (*Oncorhynchus kisutch*). One hatchery is a multigeneration stock obtained by spawning 70% hatchery with 30% naturally reproducing fish, whereas the second is a single-generation stock derived from naturally reproducing coho. There was no significant difference in average overall survival between stocks, but observed N_e was significantly less than expected for each stock. Family-correlated survival contributed to roughly a 20% reduction in N_e over the freshwater and marine life stages. This reduction is similar to previous estimates and suggests a value that can be used when estimating the effective number of hatchery parents in applications of the Ryman–Laikre formula (at least for programs such as ours that attempt to equalize sex ratios and family sizes).

Résumé : Il existe étonnamment peu de données sur la variance et la moyenne finales des tailles des familles au stade adulte chez les poissons nés en pisciculture. Il est ainsi difficile, dans une pisciculture de conservation qui minimise la variance des nombres dans la progéniture, de prédire de combien inférieure sera la véritable taille effective de la population (N_e) d'une cohorte d'adultes nés en pisciculture par rapport au N_e prédit simplement par le nombre des parents qui les ont produits. Nous utilisons une analyse de parenté pour estimer la survie et le N_e chez deux stocks intégrés de saumons coho (*Oncorhynchus kisutch*) de pisciculture. Une des piscicultures utilise un stock de plusieurs générations obtenu en croisant 70 % de poissons de pisciculture avec 30 % de poissons à reproduction naturelle, alors que l'autre est formé d'un seul stock de saumons coho à reproduction naturelle. Il n'y a pas de différence de survie globale entre les stocks, mais le N_e observé est significativement inférieur à la valeur attendue pour chacun des stocks. La survie en fonction de la famille contribue grosso modo 20 % de la réduction de N_e au cours des stades du cycle en eau douce et en mer. Cette réduction est semblable à celle des estimations antérieures, ce qui fournit une valeur qui peut être utilisée pour calculer le nombre effectif de parents de pisciculture lors de l'application de la formule de Ryman–Laikre (au moins dans les programmes comme le nôtre qui cherchent à égaliser les rapports mâles–femelles et les tailles des familles).

[Traduit par la Rédaction]

Introduction

Fish hatcheries occupy various roles ranging from fish production for commercial harvest to augmentation for recreational purposes. Recently there has been a shift in hatchery programs from merely supplying fish for harvest to incorporating conservation objectives as a means to revive threatened wild populations (Hedrick et al. 2000; Miller and Kapuscinski 2003; Brannon et al. 2004). The goal of a conservation hatchery is to boost the existing adult census size of a wild population by breeding a fraction of the wild population in captivity and releasing their offspring into the natural habitat (also known as supplementation). Although there may be a gain in total production of offspring, one cost associated with such a gain may be a reduction in the effective population size (N_e) of the total population (termed the Ryman–Laikre effect; Ryman and Laikre 1991). The simple formula

$$(1) \qquad \frac{1}{N_e} = \frac{x^2}{N_h} + \frac{(1-x)^2}{N_w}$$

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(Ryman and Laikre 1991) describes the change in the inbreeding effective size of a population that results when a large fraction of the breeders in a population descend from a small number of founders, where N_h is the effective size of the hatchery fish breeding in the wild and N_w is the effective size of the wild fish breeding in the wild. Thus, for application of the Ryman–Laikre equation, it is important to know the effective size of each group of fish. Estimating N_w for wild salmon populations can be quite difficult and has been the subject of much study (Waples 1990, 2002*a*; Shrimpton and Heath 2003). Here we focus on how accurately one can predict the effective size of the hatchery component of a supplemented population, N_h. It is often assumed that the effective size of the hatchery group can be estimated simply from the numbers of males and females used as brood stock to produce that hatchery group. However, few data exist on the realized effective size of a cohort of hatchery fish relative to the effective size as predicted by the number of brood stock that produced them. For example, if 200 male–female pairs are spawned in a hatchery and if variance in family sizes is random through all stages of the life cycle of their offspring, then the inbreeding effective size of that cohort of offspring would equal the census size, $N = 400$. Nonrandom (family-correlated) survival during any stage of the life cycle would cause the N_e of that cohort of returning adults to be less than 400. How much less can be expected for typical conservation hatchery operations remains an open question. Family-correlated survival can occur at any stage of the life cycle but may be particularly high in captivity (Allendorf 1993). Thus, some estimates of the mean and variance in family sizes, and true realized N_e of hatchery cohorts, would be very useful for application of the Ryman–Laikre formula in practice.

Hatcheries have traditionally used broodfish that have been passed for many generations through a hatchery because such stocks perform well in a hatchery environment. In contrast, conservation hatcheries have opted to produce single-generation stocks using naturally born brood stock or have integrated captive with naturally born brood stock (Mobrand et al. 2005). It is unknown whether the mean ($\bar{k}$) and variance (V) in final family size differ between the two brood-stock strategies adopted by conservation hatcheries. Differences, if large, might cause one to substantially under- or over-estimate the effective size for a cohort of single-generation stock, relative to the expectation of an integrated stock.

Here we test whether there is a significant difference in the mean and variance in number of surviving adult progeny between a single-generation and an integrated multigeneration hatchery stock. We also estimate, for two cohorts of each stock, the reduction in realized N_e over that predicted by the number of parents that produced each cohort. We found no significant difference in survival between single-generation and multigeneration stocks, with both stocks experiencing similar reductions in N_e. This reduction was significantly less than expected, indicating that nonrandom survival occurred throughout the freshwater–marine life stage. Our data suggest that the hatchery coho (*Oncorhynchus kisutch*) used in this study experienced about a 20% reduction in N_e over that predicted throughout their freshwater and marine life stages, regardless of the type of hatchery stock.

Materials and methods

Sampling design — year 2001

For at least the past decade, the North Umpqua River hatchery program has been managed as a harvest program, augmenting the recreational and commercial fisheries. The program integrates a random collection and spawning of adults throughout the run by mixing 70% hatchery fish (i.e., adipose-clipped fish) with 30% natural fish. Each fish released from the hatchery is adipose-clipped to ensure the designation "hatchery fish". Therefore, we considered adipose-clipped coho collected in the North Umpqua to be of multigeneration hatchery origin. In fall and early winter 2001, the Oregon Department of Fish and Wildlife (ODFW) collected 100 female and 100 male coho salmon having marked adipose fins (considered multigeneration brood stock; MGHS) from the North Umpqua River at Winchester Dam (Fig. 1). They also collected 94 females and 94 males having unmarked adipose fins (considered single-generation brood stock; SGHS because progeny were reared in a hatchery). Collections were performed randomly with respect to age, run time, and length. Males and females from the multigeneration brood stock were randomly paired and spawned at ODFW's Rock Creek hatchery facility using single-pair matings (i.e., each male and female was used only once). Single-generation broods were spawned following the same protocol. Eggs from each mating pair were incubated separately until the eyed-egg stage. At this stage, we attempted to equalize the variance in progeny number by randomly sampling 140–150 eyed eggs per mating pair and rearing them to the smolt stage (progeny from MGHS and SGHS were reared separately). Although 140–150 eggs per mating pair was a goal, our observed mean in eyed eggs per mating pair was less than expected (see Results) because of high egg mortality from a few mating pairs. As a result, our variance in progeny number was also greater than expected.

Sampling design — year 2002

Sampling design for the 2002 brood stock was similar to that of 2001. However, the SGHS were collected from Calapooya Creek (Fig. 1), which is a tributary of the Umpqua River and considered part of the Umpqua population complex (Ford et al. 2004). Coho inhabiting Calapooya Creek are considered naturally reproducing (i.e., there is no recognized hatchery contribution to this system); however, it is conceivable that some of these fish historically could be of hatchery origin.

In fall and early winter 2002, ODFW personnel collected 100 female and 100 male coho salmon having unmarked adipose fins (SGHS) from Calapooya Creek at Nonpareil Dam (Fig. 1). The MGHS for 2002 consisted of 100 males and 100 females collected at Winchester Dam on the North Umpqua, as before. Collection, mating, and rearing of 2002 brood stock followed the 2001 sampling design protocol.

In spring 2003 and 2004, smolts from respective 2001 and 2002 brood years were released in Calapooya Creek above Nonpareil Dam (Fig. 1). Coho salmon smolts typically migrate to the Pacific Ocean a few weeks after release. Fins from released smolts were clipped adipose left maxillary or adipose right maxillary to designate progeny from MGHS or SGHS, respectively. Coho have a 3-year life cycle. Mature

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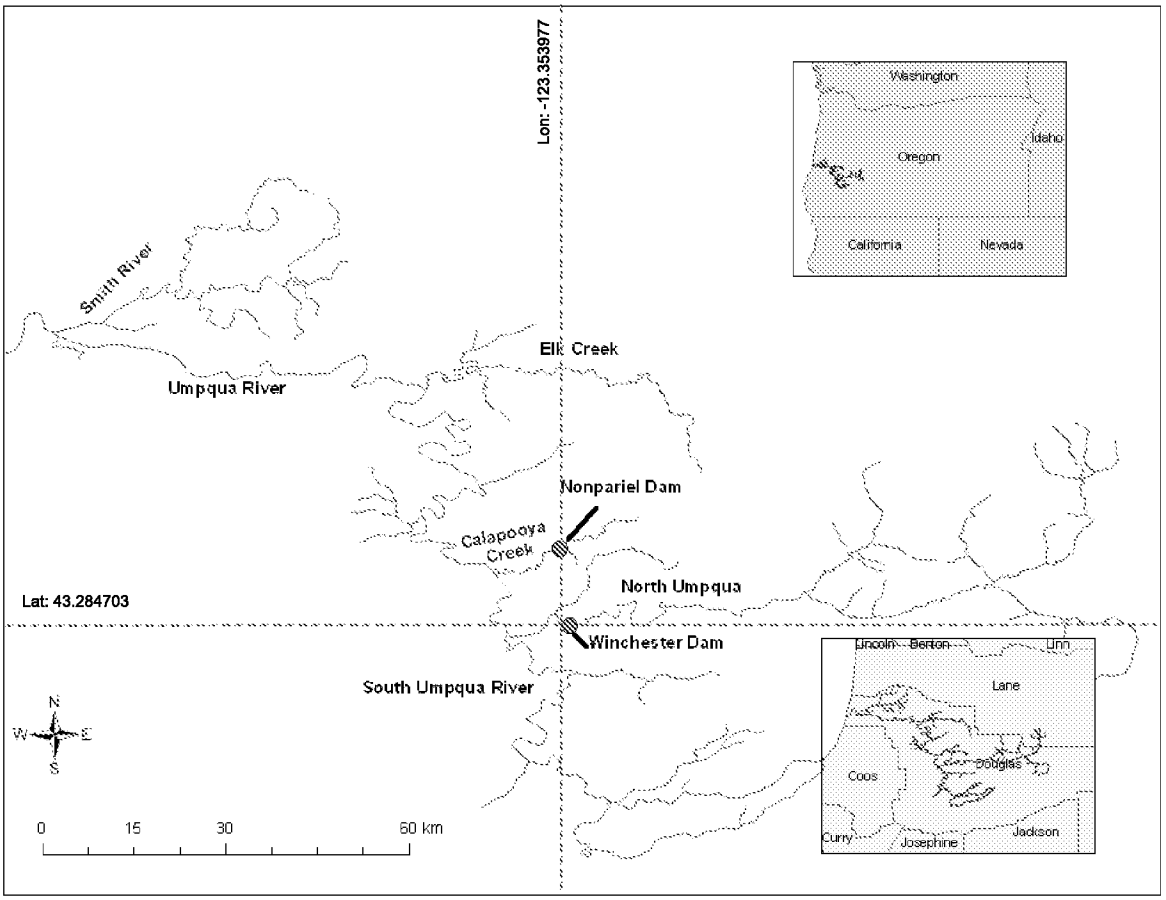
Fig. 1. Map of the Umpqua basin, Oregon, USA. Our study involved sampling natural and hatchery coho in the North Umpqua at Winchester Dam, spawning and rearing offspring at Oregon's Department of Fish and Wildlife Rock Creek Hatchery facility, and releasing smolts above Nonpareil Dam on Calapooya Creek. All returning progeny were sampled at a fish trap located at Nonpareil Dam, genotyped using 11 microsatellite loci, and assigned to most likely brood stock through parentage analysis.



adults typically return to spawn at age 3, but reproductively mature males, called jacks, can return at age 2. Thus, coho salmon released as smolts in 2001 returned to the Calapooya Creek during the fall of 2003 (jacks) and 2004 (adult males and females), and smolts released in 2002 returned in the fall of 2004 and 2005. ODFW constructed a fish trap at the base of Nonpareil Dam (Fig. 1), allowing for the capture, fin-clipping, and above-dam release of all returning adult coho salmon in this study.

Screening, optimization, and identification of microsatellite markers

DNA was extracted using Qiagen DNA extraction kits. DNA concentration (~4–24 ng·μL^{-1}) was quantified using a Victor^{3}V 1420 multilabel counter (PerkinElmer Inc., Boston. Mass.).

Choosing appropriate markers for accurate parentage assignment is a function of population size, number of loci, and number and distribution of alleles per locus (Bernatchez and Duchesne 2000). We took the following strategy for choosing appropriate markers for this study. First, we screened 96 candidate loci known to amplify in salmon species. Of these loci, we successfully amplified 41 candidates for further evaluation. Next, loci were selected based on repeat motif (tetranucleotides were chosen over dinucleotide repeats because of increased scoring error for dinucleotides), allelic diversity, and allelic distribution (Blouin et al. 1996; O'Reilly et al. 1998; Bernatchez and Duchesne 2000). Using these criteria, we narrowed the pool of potential markers to 21 and screened the 2001 pair matings (n = 388) using these markers. Because of inconsistent scoring, presence of null alleles, and deviation from Hardy–Weinberg expectations (HWE), 10 of these loci where excluded from subsequent analyses. Primer information, including range of allele sizes, repeat motif, annealing temperature, and buffer pH, for the remaining 11 loci used in this study are listed in Table 1. Single-locus polymerase chain reaction (PCR) amplifications were performed in 5 μL reactions using 0.175 mmol·L^{-1} each of dNTP, 0.15 μmol·L^{-1} each primer, and 0.025 U *Taq* polymerase (1 U = 16.67 nkat; Promega Corp., Madison, Wis.) (see Table 1 for buffer and $MgCl_2$ concentrations). PCR conditions were an initial denaturation at 94 °C (3 min), followed by a touchdown procedure involving four cycles of denaturing (94 °C), annealing, and extension (74 °C), where the initial annealing temperature was decreased by 1 °C·cycle^{-1} (see Table 1 for initial and final annealing temperatures). After initial cycles, reactions were run for 30 cycles at the final annealing temperature.

Before electrophoresis, 0.8 μL PCR product from three to five separate reactions were combined (Table 1) and mixed

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Table 1. Description and polymerase chain reaction (PCR) conditions of microsatellite loci used for this study.

Marker	k	H	Range of alleles	Exclusion probability	Annealing temperature (°C)	$MgCl_2$ (mmol·L^{-1})	Buffer pH	Reference
*OTS*519[A]	8	0.72	286–310	0.35	58–54	1.0	9.0*	Naish and Park 2002
*OTS*520[A]	24	0.87	190–252	0.72	58–54	1.0	8.5†	Naish and Park 2002
*ONE*111[A]	6	0.84	180–190	0.32	52–48	2.5	9.0*	Olsen et al. 2000
*P*53[A]	19	0.91	165–197	0.73	58–54	1.0	9.0*	de Fromentel et al. 1992
*OTS*3[B]	10	0.89	142–162	0.53	58–54	1.0	9.0*	Banks et al. 1999
*ONE*μ2[B]	25	0.89	196–257	0.72	63–59	1.0	9.0*	Currens et al. 1997
*OCL*8[B]	20	0.88	90–121	0.65	50–46	1.5	9.0*	Condrey and Bentzen 1998
*OTS*215[C]	8	0.72	155–160	0.35	60–56	1.0	9.0*	M. Banks (unpublished data)
*ONE*μ13[C]	15	0.81	194–236	0.57	60–56	1.5	9.0*	Scribner et al. 1996
*OMY*1011[C]	11	0.84	178–212	0.58	50–46	1.5	9.0*	P. Bentzen (unpublished data)‡
*OKI*23[D]	20	0.80	120–180	0.74	60–56	1.5	8.5†	A. Spidle (unpublished data)§

Note: The abbreviation k represents the number of alleles per locus, and H is the observed heterozygosity. The exclusion probability is the average probability of excluding a single unrelated candidate parent from parentage of a given offspring. Loci with the same letter designate combined genotyping runs. The first annealing temperature number is the initial annealing temperature and the latter is the final annealing temperature.

*1× *Taq* reaction buffer (Promega).

†20 mmol·L^{-1} Tris, pH 8.5, and 50 mmol·L^{-1} KCl (Williamson et al. 2002).

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with a 4 μL solution containing 98% formamide and 2% Genescan LIZ 500 size standard (Applied Biosystems, Foster City, Calif.). Microsatellite reactions were visualized with an ABI 3730xl Prism (Applied Biosystems) using fluorescently labeled forward primers and analyzed using GeneMapper Software v3.7 (Applied Biosystems). To minimize potential pipetting error (PCR and genotyping were performed using 384-welled plates), DNA and PCR products were transferred using a PlateMate Plus pippetting robot (Matrix Technologies, Hudson, N.H.).

Parentage analysis and estimating mean and variance in progeny number

We assessed the statistical power of our 11 loci for successful parentage analysis (α = 0.20 and 0.05) via simulations as implemented by Cervus v3.0 (Marshall et al. 1998). Cervus uses known allele frequency data to generate a pair of parental genotypes, plus a series of random genotypes representing unrelated candidate parents of one sex. Offspring are then produced by Mendelian sampling of the true parents' alleles. Once simulated parent and offspring genotypes were generated, we obtained an estimate of the number of loci needed for accurate parentage assignment assuming all candidate parents had been accurately genotyped. We estimated the proportion of loci typed (95%) from the known 2001 candidate parents and designated a genotyping error rate of 1.5%. For simulations, we based the genotyping error rate on previous published data sets (Bonin et al. 2004). We confirmed this value by matching known hatchery parents to hatchery returns (n = 384) and assessing the proportion of mistyped loci for each correct assignment. Cervus simulations were run for 10 000 cycles.

Parentage analyses were performed using exclusion and categorical allocation methods (Jones and Ardren 2003). Exclusion was implemented using WhichParent v1.0 (W. Eichert, available at http://www.bml.ucdavis.edu/facresearch/salmonsw.html or by request) where the number of mismatches was set to two. This setting was chosen because it produced the greatest number of true assignments based on preliminary runs that assigned known progeny to known hatchery brood stock. Categorical allocation, which involves calculating a logarithm of the likelihood ratio (LOD score) for any parentage relationship, was implemented using FAMOZ (Gerber et al. 2003). Simulations to calculate the LOD score threshold value for parentage assignment were implemented in FAMOZ as described by Gerber et al. (2003) (the intersection of the distributions was chosen as the threshold value). Simulations and actual parental assignments were conducted assuming a genotyping error rate of 1.5% per locus and an analysis error rate of 0.01% per locus (see Sancristobal and Chevalet (1997) and Gerber et al. (2000) for details about analysis error rate).

Once parent–offspring assignments were confirmed, we estimated the mean ($\bar{k}$) and variance (V) in progeny number per family for MGHS and SGHS (separately for both brood years). We tested the hypothesis that there was no difference in mean progeny number per parent between SGHS and MGHS (each year was analyzed separately) using a two-tailed t test (Sokal and Rohlf 1995). A test for homogeneity of variances between groups was performed using the F test (Sokal and Rohlf 1995).

Assessment of nonrandom survival and realized vs. expected N_e

Crow and Morton 1955) referenced $V/\bar{k}$ as the index of variability (R; Geiger et al. 1997; Waples 2002*b*). To assess whether survival is completely random from eyed-egg to adult life stage in hatchery coho salmon, we scaled R at the eyed-egg stage to the expected value assuming random survival in a population of constant size (i.e., $\bar{k}$ = 2) using the equation

$$(2) \qquad R^* \approx 1 + \bar{k}_2 \frac{(R-1)}{\bar{k}_1}$$

where R^* is the scaled index of variability, $\bar{k}_1$ represents the observed mean progeny number per family, and $\bar{k}_2$ is the expected mean progeny number per family (i.e., we assumed $\bar{k}_2$ = 2). If survival was random from egg to adult stage with

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Table 2. Count of hatchery coho passed above Nonpareil Dam (Calapooya Creek) for 2004–2005 cohorts.

	Male	Jack	Female	Unresolved	Dropped	Total
2004 cohort						
SGHS	69	23	71	2 (1 M, 1 J)	2 (2 M)	167
MGHS	98	38	78	2 (1 M, 1 F)	2 (2 M)	218
2005 cohort						
SGHS	121	38	169	20 (4 M, 1 J, 15 F)	0	348
MGHS	174	28	189	9 (2 M, 1 J, 6 F)	7 (2 M, 5 F)	407

Note: M, male; J, jack; F, female. Comparisons are between returns from single- and multi-generation hatchery stocks (SGHS and MGHS, respectively). Individuals excluded from analyses are as follows: progeny that were unassigned to a known parental array (Unresolved), and progeny having fewer than eight genotyped loci or were returns from incorrect hatchery matings (Dropped; see text for details).

$\bar{k}_2 = 2$, R^* at the eyed-egg stage (R_e^*) should be similar to the observed value of R at the adult stage (R_a). Therefore, a deviation between these two values (R_e^* and R_a) is an indication of nonrandom survival among families.

An approximate expression for the ratio of expected N_e/N was calculated using the equation

$$(3) \qquad \frac{N_e}{N} \approx \left[\frac{\bar{k}_2}{1+R^*}\right]$$

where $\bar{k}_2$ is the expected mean progeny number per family (we assumed $\bar{k}_2 = 2$) and N is the census size of the parent population (Waples 2002*b*). Note that N is not the census size of the cohort of adult offspring. Rather it is the census size of the parents that produced them (Waples 2005), and because the number of females equals the number of males, it is also the expected N_e of the offspring cohort given random family survival. Also note that estimates of N_e associated with family size data should be interpreted as estimates of the effective number of breeders (N_b) per year (Waples and Teel 1990; Waples 2005). Depending on whether N_b/N was estimated for egg or adult stages, the parameter R^* was calculated as above using the respective value of $\bar{k}_1$. We assessed confidence in parameters R_e^*, R_a, R_a^*, and N_b/N by bootstrap resampling (Sokal and Rohlf 1995). Randomization tests (Sokal and Rohlf 1995) were used to investigate whether estimates of R_e^*, R_a, and N_b/N were significantly different between SGHS and MGHS (each cohort was analyzed separately).

Results

Parentage simulations using Cervus concluded that our predicted success rate for the 11 loci used in this study (when sexes are known a priori) was 100% at $\alpha = 0.05$. We obtained similar estimates for the number of progeny per parent using exclusion and categorical assignment methods (the LOD threshold value for parentage assignment using FAMOZ was 8.0); therefore, only results from WhichParent are presented.

In 2001, the mean number of eggs per mating pair ($\bar{k}_e$) for SGHS cohort collected for smolt production was 147 ($V = 433.47$), and the mean number of eggs per mating pair sampled for smolt production for MGHS matings was 136 ($V = 397.34$). In April 2003, 12 016 adipose left maxillary clipped (progeny from 2001 MGHS cohort) and 12 357 adipose right maxillary clipped (progeny from 2001 SGHS cohort) smolts were released in the Calapooya Creek above Nonpareil Dam (Fig. 1).

The returning 2004 cohort was comprised of 62 marked jacks (one of which was dropped from subsequent analyses; see below) and 323 marked adults (seven of which were excluded from subsequent analyses) (Table 2). Four of eight fish were deemed dropped and excluded from further analyses. Three were probably due to spilling of gametes during hatchery spawning (WhichParent assigned returns to a male or female but not to a known mating pair), and one had less than eight genotyped loci scored. The last four fish, deemed unresolved, were deleted from further analyses because they could not be assigned to a specific mating pair, indicating that they may be age 4 (but see below) or stray marked fish from another system.

The mean number of eggs per mating pair for 2002 SGHS cohort reared for smolt production was 138 ($V = 276.66$) vs. 139 ($V = 108.16$) for MGHS. In April 2004, 11 018 adipose left maxillary clipped (progeny from MGHS) and 10 979 adipose right maxillary clipped (progeny from SGHS) smolts were released in the Calapooya Creek above Nonpareil Dam. The returning 2005 cohort was comprised of 68 marked jacks (two of which were eliminated from further analyses) and 687 marked adults (35 of which were excluded from subsequent analyses) (Table 2). Seven returns were dropped from subsequent analyses, all a consequence of having mating strategies where WhichParent assigned returns to a male of one mating pair with a female of the next mating pair. Twenty-nine fish could not be assigned to a specific mating pair and were unassigned to the 2001 parental data set, indicating they where not age-4 fish; therefore, we suspect that these fish were marked strays for reasons previously addressed.

The number of returning progeny assigned to the 2004 cohort was 214 MGHS and 163 SGHS. The 2005 cohort contained 391 and 328 assigned progeny, respectively. Calculated $\bar{k}$, V, R^*, and N_b/N for egg and adult stages are reported in Table 3. A statistically greater variance was found in MGHS than in SGHS for the adult 2004 cohort ($V_{MGHS} = 3.62$, $V_{SGHS} = 2.52$; $F_{0.05(1)99,93} = 1.43$, $P = 0.04$), but comparison between MGHS and SGHS was nonsignificant for the adult 2005 cohort ($V_{MGHS} = 6.67$, $V_{SGHS} = 5.58$; $F_{0.05(1)99,99} = 1.39$, $P = 0.18$). Despite significant vari-

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Table 3. Estimated demographic parameters for juvenile and adult coho salmon from 2001 and 2002 hatchery stocks.

	Egg					Adult				
	k_e	V_e	R_e	R_e^*	N_b/N^*	k_a	V_a	R_a	R_a^*	N_b/N^*
MGHS 2001	136	397.34	2.92	0.97 (1.00–1.08)	1.02 (0.96–1.00)	2.14	3.62	1.68 (1.25–2.00)	1.64 (1.27–197)	0.76 (0.66–0.86)
SGHS 2001	147	433.47	2.94	0.97 (1.00–1.09)	1.02 (0.95–1.00)	1.72	2.52	1.46 (1.11–1.79)	1.53 (1.15–1.90)	0.79 (0.69–0.92)
MGHS 2002	139	108.16	0.78	1.00 (n/a–1.02)	1.00 (0.99–n/a)	3.84	6.67	1.73 (1.25–2.13)	1.38 (1.16–1.57)	0.84 (0.77–0.93)
SGHS 2002	138	273.92	1.99	1.01 (n/a–1.05)	0.99 (0.97–n/a)	3.13	5.58	1.67 (1.30–2.05)	1.43 (1.17–1.63)	0.82 (0.76–0.92)

Note: Comparisons are between single- and multi-generation brood stocks (SGHS and MGHS, respectively). Subscripts represent the egg (e) and adult (a) life stages. Mean and variance in progeny number estimated from parentage analysis are designated as k and V, respectively. The parameter R is the observed index of variability (Crow and Morton 1955) and is calculated as V/k. The asterisk denotes scaled values of R and effective size to census size (N_b/N) obtained from eqs. 2 and 3, respectively. Confidence intervals are reported in parenthesis. The designation "n/a" indicates confidence values that were unascertainable via bootstrapping due to the small variance among replicates.

ances between MGHS and SGHS for the 2004 cohort, estimates of $\bar{k}_a$ between groups were nonsignificant in both cohorts (2004 cohort, $t = 1.66$, $t_{0.05(2),189} = 1.97$, $P = 0.10$; 2005 cohort, $t = 1.80$, $t_{0.05(2),198} = 1.97$, $P = 0.07$). Values of R_e^*, R_a^*, and N_b/N were nonsignificant (all $P > 0.05$) between MGHS and SGHS for each cohort; however, confidence intervals for R_e^* and R_a^* were nonoverlapping for all within-stock comparisons (Table 3), indicating that nonrandom survival occurred between egg and adult stages for the 2004 and 2005 cohorts. Likewise, confidence intervals were nonoverlapping for all egg per adult N_e/N comparisons (Table3).

The expected N_b of the 2004 cohort, given random family survival, was 200 for MGHS and 188 for SGHS. The expected N_b for the 2005 cohort was 200 for both MGHS and SGHS. In contrast, the observed N_b for each cohort was always significantly (i.e., nonoverlapping confidence intervals) less than expected (2004 cohort, $N_{b\ MGHS} = 152$, $N_{b\ SGHS} = 148$; 2005 cohort, $N_{b\ MGHS} = 168$, $N_{b\ SGHS} = 164$), indicating that on average a 20% reduction in N_b (caused by family-correlated survival) occurred in the life cycle of coho salmon during this study.

Discussion

Our study found no difference in survival between two types of integrated (i.e., managed as a component of a natural population) hatchery programs — one that integrates 30% naturally reproducing fish with 70% hatchery fish vs. one that only uses naturally produced fish as brood stock. These findings seem to contradict those of previous studies (Fleming and Gross 1993; Berejikian et al. 1997, 2001), i.e., that fish maintained in a hatchery for multiple generations generally are less fit (the component of fitness being survival) than fish that have never experienced captive conditions. However, there is quite a distinction between present and previous studies. Previous studies compared segregated hatchery brood fish (i.e., managed as if they are a distinct population relative to natural populations) with naturally reproducing ones; in contrast, our study compares two types of integrated hatchery programs. Furthermore, theoretical studies have indicated that hatchery programs with a one-way gene-flow rate of 10%–20% per generation can quickly achieve the fitness level of the donor population (Ford 2002; Lynch and O'Hely 2001). Our study provides empirical evidence indicating that relative survival of progeny from a multigeneration hatchery stock exposed to an integrated breeding program is similar to that of a naturally reproducing stock experiencing hatchery conditions for the first time. However, we urge caution when applying these findings to other such hatchery programs because conclusions are often context-specific and depend on the type of brood stock used (integrated vs. segregated), the breeding program (in this case, single matings and equalization of family size), the amount of gene flow between stocks, and brood-stock history.

Predictions regarding N_b are often calculated using sex-ratio information of the organism in question (Wright 1938; Waples and Teel 1990). Estimating N_b this way assumes that variance in reproductive success among males (or females) is random. Our data clearly show that this assumption is violated for hatchery-reared coho salmon. Both SGHS and MGHS groups had significantly greater variances in family

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size than would be expected based on random survival from egg to adult (i.e., confidence intervals for R_e^* do not overlie those of R_a). Comparisons between values of R_a and R_e^* for MGHS and SGHS cohorts were not significantly different, suggesting that the extent of among-family selection, whether occurring in the hatchery or wild (see below), appears similar between MGHS and SGHS. These findings, which corroborate Waples (2002*b*), indicate that even in closely monitored hatchery operations, sex-ratio information may be a poor indicator of N_b for hatchery-reared coho salmon. Fortunately, nonrandom survival was not extreme enough to substantially reduce N_b, a finding similar to that of Waples (2002*b*), who also applied demographic data to predict N_b/N for coho salmon.

It is difficult to discern the exact stage at which family-correlated survival occurred for both groups. Mortality can occur in the hatchery at the egg–presmolt stage, in freshwater as smolts migrate to estuarine habitat, or during estuarine–ocean phases. Although this study was not intended to test where differential survival occurred, minimizing the variance in family size at the egg stage should reduce the effects of selection in captivity (Allendorf 1993). It is interesting that estimated survival from egg to released fry was approximately 89% and 80% for 2004 and 2005 cohorts, respectively (data not shown). As predicted, these estimates leave little room for family-correlated survival to occur at the hatchery stage (at least for the 2004 cohort) and suggest that differential survival among family groups transpired during smolt migration to estuarine habitat or during the ocean stage of their life cycle, a finding similar to those of Hobday and Boehlert (2001) and Linley (2001).

Few studies have examined the demographic parameters necessary to compute N_b and N_b/N for hatchery salmon populations; therefore, the reduction in N_b below N is generally unknown. Summarizing $\bar{k}$ and V in female families for five cohorts of hatchery-reared coho salmon, Waples (2002*b*) showed that N_b/N ranged from 0.59 to 0.94 (mean = 0.76). These estimates may tend to underestimate the overall variance in $\bar{k}$ because V was computed for only females; therefore, N may only approximate the expected N_b for the cohort. Nevertheless, our estimates of N_b/N, which ranged from 0.76 to 0.84 (mean = 0.8), are similar to those of Waples (2002*b*), suggesting that N_b for hatchery salmon might be generally predictable from knowledge of the number of parents used as brood stock, particularly when hatchery practices perform single-pair matings and equalize family size prior to release. In fact, when these data are averaged together (i.e., Waples (2002*b*) and current study), a 22% (±8%) reduction in N_b is predicted in the life cycle of hatchery coho salmon. Scaled adult estimates of N_b/N for other salmon species reared in captivity, which are >0.7 for most cohorts (Hedrick et al. 2000; Waples 2002*b*), are similar to those for coho salmon, suggesting that this value (22%) may be used in conjunction with census data for calculating N_h of other salmon species.

Although a 22% reduction in N_b might be predicted for coho salmon, this value, as a general predictor, should be used with caution. Any factor that causes selection among family groups can increase the variance in family size and subsequently reduce N_b. These factors can include, but are not limited to, artificial selection, size-selected predation, isolated disease outbreaks, and varying freshwater or ocean conditions, none of which is mutually exclusive. It should also be stressed that this study attempted to equalize or minimize the variance in family size at an early life stage and equalize sex ratios. Equalization of these parameters is promoted by conservation hatchery programs as a means to maintain higher N_e; therefore hatchery programs not attempting to equalize these parameters would expect on average a >22% reduction; how much greater depends on the level of variation among family groups and mating design (e.g., full factorial vs. 1:1 pair matings)

In conclusion, our data and data in Waples (2002*b*) suggest a simple calculation for estimating one of three necessary parameters for predicting the Ryman–Laikre effect, N_h. More importantly, this parameter appears stable regardless of the type of integrated hatchery breeding program used by hatchery managers. Predicting the N_e of wild populations of salmon from census data has also been the subject of much study, and a rough rule of thumb for that parameter is also emerging (e.g., Waples 2002*a*). Much fewer data exist on the third parameter, the relative reproductive success of hatchery vs. wild fish when breeding in the wild (but see Araki et al. 2006). Reliable estimates of these parameters will allow fisheries professionals to predict the loss of genetic diversity associated with supportive breeding.

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