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Genetic diversity in the Snake River sockeye salmon captive broodstock program as estimated from broodstock records

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RESEARCH ARTICLE

Genetic diversity in the Snake River sockeye salmon captive broodstock program as estimated from broodstock records

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Abstract Snake River sockeye salmon spawning in Redfish Lake, Idaho are one of the most endangered taxa of Pacific salmon. The wild population nearly went extinct in the 1990s, and all surviving fish were incorporated into a captive broodstock program at that time. We used pedigree analysis to evaluate the effectiveness of the breeding program in retaining genetic variation from 1991 through 2008. Broodstock records document which males were crossed with which females, but fish from multiple crosses were frequently raised in the same tank so the exact pedigree of the population is unknown. Therefore, a simulation-based approach was used to estimate how much genetic diversity was retained by this breeding program. Results indicate that in 2008, after 5.5 generations of breeding, the average inbreeding coefficient was probably about 0.056. We estimated the inbreeding effective population size to be 41 over the entire program and 115 for the most recent generation. This amount of inbreeding is substantially less than has occurred in many high-profile captive breeding programs. Our results depend on several assumptions regarding the relatedness of fish in the breeding program, but simulations suggest our main results are relatively insensitive to these assumptions.

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Introduction

Captive propagation has been widely used to manage small populations (see Fraser 2008 for a review focusing on salmonid fishes). These propagation programs can take many forms, and can have different objectives. Common objectives for captive breeding programs include maintaining gene pools until factors limiting survival can be alleviated, speeding recovery in the wild, translocating individuals for genetic rescue, and reseeding vacant habitat (Waples and Drake 2004). One of the most high-profile applications of captive breeding has been with critically endangered species—those for which extinction in the near future is a realistic possibility. In these situations, the short-term goals of captive breeding are generally to (1) avoid complete extinction of the gene pool; (2) conserve as much genetic diversity as possible; and (3) accomplish objectives (1) and (2) without compromising prospects for long-term survival of the population/species. This is a tall order, and accomplishing all three goals requires carefully designed breeding/husbandry protocols, substantial financial and other resources, dedication and lots of hard work, and more than a little good luck.

Sockeye salmon (*Oncorhynchus nerka*) from Redfish Lake in central Idaho are the only population of sockeye salmon in the Snake River. They live farther south, migrate farther in freshwater (1,500 km), and spawn at higher elevation (2,000 m) than any other population of sockeye in the world (Benke 2002; Waples et al. 1991). The population has also been critically endangered by any reasonable criterion (Waples et al. 1991). In 1990, no anadromous adults

returned to Redfish Lake to spawn. A year later, four adults returned and that year Redfish Lake sockeye became the first population of Pacific salmon to be listed as an endangered species under the U.S. Endangered Species Act (see Good et al. 2005 for a review). The population in the wild continued to teeter on the brink of extinction and in 1992, only a single adult returned to Redfish Lake. Multiple age classes and life histories helped the population escape extinction, but only a small trickle of adults returned to Redfish Lake in the next few years (Box 1).

Captive propagation of Redfish Lake sockeye began in the spring of 1991, and during the next 7 years, 99 wild-born *O. nerka* were captured in Redfish Lake or Redfish Lake Creek and spawned in captivity. These 99 founders included 16 anadromous adults that returned to Redfish Lake from the ocean, 65 juvenile "outmigrants" that were captured while leaving Redfish Lake on their way to sea, 17 "residual" adult sockeye that lived in the lake, and one fish that was either a residual or an outmigrant (see below) (Table 1; Box 1). As we discuss below, some of these fish

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Box 1 Timeline for the Redfish Lake captive broodstock program

1988	Spawning surveys observed 2 males, 2 females, and 2 redds in Redfish Lake (Hall-Griswold 1990)
1989	One redd was observed in Redfish Lake, but no spawning adults (Hall-Griswold 1990)
1990	No anadromous adults or redds were observed in Redfish Lake
1991	In the spring, 856 juvenile sockeye "outmigrants" were captured leaving Redfish Lake on their way to the ocean. These outmigrants were 1–2 years old; their parents were probably a mixture of anadromous adults that spawned in 1988 and 1989 and "residual" sockeye spawning in the lake. After 1–3 years, 41 of these outmigrants matured in captivity and were either spawned, or had their milt cyropreserved
	In the fall, four anadromous adults (presumably from brood years 1986 and 1987) returned to Redfish Lake and were spawned in captivity. These were the first spawners of the broodstock program
1992	In the spring, an additional 79 juvenile outmigrants were collected from the outlet of Redfish lake. These fish probably originated from a combination of anadromous spawners in 1989 and 1990 (years for which no anadromous adults were observed but a low level of spawning could not be excluded) and residual sockeye
	In the fall, a single, male, anadromous sockeye (BY87) returned and was quickly dubbed "Lonesome Larry." His milt was frozen for use in subsequent years
	A small group of kokanee-sized *O. nerka* were observed near the sockeye spawning beach on the shore of Redfish Lake (Waples et al. 1997). This area is well separated from the kokanee spawning site in the inlet stream, Fishhook Creek; furthermore, these small fish were spawning at the same time as the sockeye (late September–October), which is about 4–6 weeks after peak kokanee spawning (mid August). Finally, these small spawners were dull green rather than bright red—a trait that is unusual in sockeye or kokanee but common in what are termed "residual" sockeye, which are progeny of anadromous *O. nerka* that never go to sea. Genetic analysis showed that these residual sockeye were genetically distinct from Fishhook Creek kokanee but closely allied to the outmigrants and to the adult sockeye (Waples et al. 1997). Three of these residuals were collected and incorporated into the program
1993	In the spring, 48 outmigrants were captured leaving Redfish Lake. There were no known anadromous returns in 1990 or 1991, so the parents of these fish were probably residual sockeye
	In the fall, eight anadromous adults (BY88 and BY89) returned and were spawned in captivity. A few more residuals were collected (6 spawned, an additional 12 males cryopreserved)
	Twenty of the outmigrants collected in 1991 are reared in captivity to maturity and then released into Redfish Lake as adults in order to spawn in the wild and supplement the wild populations
1994	One unmarked, anadromous adult (BY89) is captured. Pre-smolts are released from the hatchery. These are the first pre-smolts to be released; all fish were marked by clipping the adipose fin
1995	No anadromous returns; 4 residual males collected and spawned. Smolts are released from the hatchery. These are the first smolts to be released; all fish were marked by clipping the adipose fin
1996	One unmarked, anadromous adult is captured (BY92). Eyed-eggs are released from the hatchery. These are the first unmarked, captive-reared fish that were released
1997	No new founders
1998	One additional, unmarked, 5-year old anadromous adult is captured (BY93). This is the last founder of the captive broodstock program. This adult could have been the offspring of the 1991 outmigrants that were born in the wild, reared in captivity for 2 years, and spawned in the wild in 1993
1999	Seven captive-born, marked, anadromous adults from the broodstock program returned to Redfish Lake; the product of smolt releases from 1996. These are the first fish spawned in captivity to return to Redfish Lake
2000	257 anadromous adults return to Redfish Lake
2004	Captive broodstock program begins to use molecular instead of pedigree data to make crosses
2008	New high for number of adult returns in a single year since program began: $N = 650$

Table 1 Number of founders, spawners, and eggs produced in the Redfish Lake sockeye salmon captive broodstock program

| Year | Founders | | | | |
	Anad. returns ♀:♂	Outmigrating smolts ♀:♂	Residual adults ♀:♂	Total $N_{Spawners}$ in captivity ♀:♂	N_{Eggs} produced
1991	1:3	23:28		1:3	1,988
1992	0:1	5:4	1:2	1:2	35
1993	2:6	1:4	2:8	43:15	9,244
1994	1:0			284:175	554,995
1995			0:4	4:8	4,290
1996	1:0			470:317	493,384
1997				247:190	298,867
1998	0:1			73:69	63,134
1999				193:75	111,911
2000				287:203	346,801
2001				248:112	210,403
2002				140:172	128,492
2003				437:266	450,107
2004				350:210	257,920
2005				265:377	297,677
2006				317:318	446,632
2007				272:304	369,698
2008				237:360	354,452

The table shows the year that founders were captured. Anadromous adults were spawned (or had milt cyropreserved) the year they were captured. Outmigrating juveniles matured 1–3 years after capture

may have been full or half siblings. The first founders to be brought into captivity were outmigrating smolts captured in the spring of 1991. The origin of these fish was originally unknown, but genetic analyses later showed these fish were genetically distinct from Redfish Lake kokanee (Waples et al. 1997; Cummings et al. 1997), and genetically similar to anadromous fish returning to Redfish Lake in the fall of 1991. Residual adult salmon were captured in 1992, 1993, and 1995 and incorporated in the captive broodstock program. Residual salmon are offspring of anadromous *O. nerka* that do not go to sea. They are genetically distinct from kokanee, another form of landlocked *O. nerka* that spend their entire life cycle in fresh water and also live in Redfish Lake (Waples et al. 1997; Cummings et al. 1997). Genetic analysis showed these adults were genetically similar to the anadromous sockeye (Cummings et al. 1997).

As typically occurs with new captive propagation programs, a variety of unexpected difficulties arose that created logistical and technical challenges to program managers (Flagg et al. 1995; Johnson and Pravecek 1995). Nevertheless, because each adult sockeye female can produce several thousand eggs, the captive population rapidly expanded in size. By 1996, 5 years into the program, hundreds of adults were being spawned each fall at two hatcheries, and eggs, pre-smolts, smolts, and mature adults were being released into the wild in an effort to re-establish the wild population. The program continued to grow, and

by 2008 (the last year considered in this investigation) 650 anadromous sockeye salmon returned to Redfish Lake. This was the largest return in 50 years. Thus, the captive breeding program for Redfish Lake sockeye helped stave off extinction for a critically endangered species for almost two decades (objective 1 above).

In this paper, we evaluate the degree to which the Redfish Lake captive broodstock program accomplished objective 2—maximizing the amount of genetic diversity retained in the population. Specifically, we ask the following questions:

1. What are the levels of inbreeding in Redfish Lake sockeye and how have they changed over the course of the captive breeding program?
2. How evenly are genes of the various founders represented in the current population?
3. What is the genetic effective population size of the breeding program?

Methods

Captive broodstock programs for salmon present opportunities and challenges that are usually not present in breeding programs for endangered birds or mammals. One advantage salmon captive breeding programs have over

their mammalian or avian counterparts is that a single female salmon can produce several thousand eggs, and in a hatchery environment, a substantial fraction of these eggs often survive to reproductive age. This often gives broodstock managers the ability to rapidly increase the size of a small population, and thereby minimize the amount of genetic diversity lost during a population bottleneck. However, this fecundity presents logistical challenges. In most broodstock programs, juvenile fish from multiple crosses are reared together, and it is impossible to identify which juveniles in a tank are the offspring of which parents. The pedigree of most salmon broodstock programs, therefore, is unknown. This is unfortunate, because there is a useful set of mathematical tools available for managing genetic diversity in populations that have a known pedigree (e.g., MacCluer et al. 1986; Lacy 1995; Ballou and Lacy 1995; Caballero and Toro 2000; Gutierrez et al. 2008). There are statistical methods for dealing with limited amounts of uncertainty in pedigrees (e.g., Pérez-Enciso and Fernando 1992; Cardoso and Tempelman 2003; Lacy 2012), but Redfish Lake captive breeding program is an extreme case: the parentage of almost all fish is unknown.

Although pedigree of the Redfish Lake sockeye salmon captive breeding program is not known, spawning records kept by Idaho Department of Fish and Game and the National Marine Fisheries Service contain a substantial amount of data. These include the number of male and females spawned each year, the specific crosses that were performed, and the number of fertilized eggs produced in each cross (hand counted at the eyed stage). Two examples illustrate the type of data available. The breeding program was initiated in 1991 when brood 1991 was created by spawning four wild fish (1 female and 3 males). The progeny of these crosses were eventually pooled together. Three years later, 55 females and 46 males from brood 1991 were crossed to create brood-lineage 1994G (which was one lineage within the 1994 brood). (Note: we are using 'brood' to refer to all the fish born in a year and 'brood-lineage' to refer to a group of fish raised together.) The 55 females and 46 males in brood-lineage 1994G shared the same mother, and some shared the same father. Therefore, the crosses that created brood-lineage 1994G were between either full or half-siblings. This ambiguity in the relationship of individuals within brood-lineages makes it impossible to identify how inbred any particular fish was in brood-lineage 1994G, and this ambiguity propagates through the breeding program. A second type of ambiguity runs through the breeding program: the brood-lineage from which parents were descended is often unknown. This is usually because fish from multiple brood-lineages were pooled together. Consider the ancestry of brood-lineage 1997Q. It was

created in 1997 by spawning fish from brood-lineages 1993A, 1993B, 1993C, 1993D, 1993E and 1993F. For any fish in brood-lineage 1997Q, it is impossible to know which brood-lineage the mother or father belonged to. For example, the mother might be from 1993B, 1993C, 1993D, or 1993E. Again, this sort of ambiguity runs through the entire breeding program.

If we make a few assumptions about the fertility of spawning adults and the survival of fertilized eggs, spawning records allow us to estimate the probability of each fish having a specific set of parents. For example, as mentioned above, three males contributed to brood-lineage 1991A. Milt from male M91-1 was used to fertilize 652 eggs; milt from male M91-2 was used to fertilize 667 eggs; and milt from male M91-3 was used to fertilize 659 eggs. If we optimistically assume that each male was equally fertile, and that eggs from each cross were equally likely to survive, the probability that a randomly chosen adult from brood-lineage 1991A was fathered by male M91-1 is equal to $652/(652 + 667 + 659) \approx 0.33$ This kind of reasoning will allow us to estimate inbreeding coefficients and other measures of genetic diversity for fish in the captive broodstock program. The assumption that each male was equally fertile and eggs from each family were equally likely to survive is unrealistically optimistic, and we will relax these assumptions later. However, first, we will introduce the general method used to deal with ambiguity in the breeding program.

Inbreeding occurs when related individuals mate (Hedrick and Kalinowski 2000). The inbreeding coefficient, f, of an individual is a measure of how much genetic diversity is present in the individual relative to a non-inbred individual in the same population. Inbreeding coefficients range from 0.0 (non-inbred; the individual has the amount of genetic diversity expected from an individual from its population) to 1.0 (completely inbred; no genetic diversity within the individual). More formally, the inbreeding coefficient of the ith individual, f_i, is equal to the probability that two alleles at a randomly chosen locus in the individual are identical by descent, that is, are descended from the same allele within the history of the breeding program.

Inbreeding coefficients can easily be computed from pedigrees. However, because the pedigree of the Redfish Lake breeding program is not known, we must account for this uncertainty when estimating the amount of inbreeding in a fish. In brief, we estimated inbreeding coefficients by averaging over potential pedigrees for the broodstock program. This was done as follows. Let J represent a possible pedigree for the entire broodstock program, let $P(J)$ represent the probability that this is the correct pedigree of the population, and let $f_i|J$ represent the inbreeding coefficient of the ith fish given pedigree J. With this

notation, we estimated the inbreeding coefficient of the ith fish, $\widehat{f_i}$ as

$$\widehat{f_i} = \sum_J^{\text{Pedigrees}} (f_i|J)P(J) \qquad (1)$$

where summation is taken over all possible pedigrees. Given the very large number of possible pedigrees for this population and the difficulty of estimating the probability that any pedigree was correct, the most straightforward way to calculate, $\widehat{f_i}$ was via Monte-Carlo simulation

$$\widehat{f_i} = \frac{1}{N_{\text{Pedigrees}}} \sum_J^{N_{\text{Pedigrees}}} (f_i|J) \qquad (2)$$

where $N_{\text{Pedigrees}}$ is the number of simulated pedigrees. The inbreeding coefficients of the fish in each pedigree, $f_i|J$, can be calculated using conventional analytic methods [e.g., the "additive-matrix" method as described by Ballou (1983)], and that is what we did.

We simulated pedigrees for Redfish Lake sockeye salmon by using broodstock records to randomly assign parents to all adult fish in each brood. Above, we described how this could be done if we assumed that eggs from each cross had the same probability of survival. Because this assumption is unrealistic, we simulated variation in fitness as follows. Each spawning fish was randomly assigned a normally distributed fitness, z, with mean zero and standard deviation σ_z. The fitness of the offspring of each cross, y_k was assumed to be exponentially related to sum of the fitness of the mother and father, $y_k = \exp(z_{\text{mother}} + z_{\text{father}})$. The proportion of adults growing up in a brood-lineage that were descended from the kth cross, p_k was modeled as a function of the number of eggs in the cross, and the fitness of each cross

$$p_k = \frac{y_k N_{\text{Eggs},k}}{\sum y_k N_{\text{Eggs},k}} \qquad (3)$$

where summation was taken over all crosses contributing to the brood. In this model, the standard deviation of fitness of individuals, σ_z, determines the variability of survival rates among the eggs in different crosses.

In our model, the parameter σ_z quantifies how much variation there is in fertility and juvenile survival. The value we used for this parameter could have a large influence on our results, so choosing a reasonable value may be important. We are not aware of any studies that have estimated σ_z as we have defined it, but Waples (2004) reviewed estimates of the ratio of effective population size N_e to census size (N) of hatchery populations of juvenile salmon and found that all had a N_e/N greater than 0.80, and all but one had a N_e/N ratio greater than 0.85. These estimates included variation in fecundity among females (which is not necessary in our analysis because we have data on the number of eggs fertilized from each female),

variation in survival rates among crosses, but not variation in fertility rates among males. We selected $\sigma_z = 1.0$ for most of our simulations, which produced a N_e/N ratio of approximately 0.48 within each generation (Kalinowski and Waples 2002). This value is close to the average value of 0.46 reported by Frankham (1995) for how variance in family size in natural populations reduces N_e/N. Frankham's estimate of 0.46 includes the effects of variance in fertility, fecundity, and survival rates across families. Here we are assuming that variance in fertility and survival rates alone can reduce N_e/N to 0.48, so our assumption is mildly pessimistic, but perhaps not too much.

The pedigree of the Redfish Lake captive breeding program had a few other uncertainties that we had to deal with. The most pressing of these is that the relationships among the founders is not known. It is typical to assume that founders of a breeding program are unrelated (e.g., Kalinowski et al. 1999; Rudnick and Lacy 2008), but this assumption is unrealistic for the large number of juvenile outmigrants collected in 1991, 1992, and 1993, and could cause us to underestimate the amount of inbreeding in the population. Rieman et al. (1994) examined Sr/Ca ratios in otoliths from 94 of the 1991 outmigrants and concluded that the female parents of these fish included both anadromous and residual fish, so the 1991 outmigrants were descended from at least two females. Otolith microchemistry was not performed for the 1992 and 1993 outmigrants, but they probably had few if any anadromous parents. Genetic analysis also provides some insight to the relationships of the outmigrants. Blood samples were available for 13 of the outmigrant founders collected in 1991. Kozfkay (unpublished) genotyped 13 microsatellite loci from these founders and used the computer program COLONY 2.0 (Jones and Wang 2009) to estimate that they were descended from 10 unique males and 10 unique females. Because the age which juvenile sockeye leave freshwater lakes for the sea is variable, some of the outmigrants caught in different years could have been siblings. Given all this information, we assumed in most of our analyses that the 1991 outmigrants belonged to three families (3 pairs of mating adults), and that the outmigrants of 1992 and 1993 were each descended from a single mating pair. This assumption is a rough estimate and is intended to be conservative (i.e., we are probably underestimating the amount of relatedness among the outmigrants).

A second complication that we had to deal with is that starting in 2004, genetic data were used to select mating pairs. Adult fish were genotyped at 7–13 microsatellite loci, and matings were selected that minimized the proportion of alleles shared between mates (Kozfkay et al. 2008). The goal of this analysis was to avoid mating between siblings and thereby reduce inbreeding in the next generation. We simulated this mate selection process by

randomly selecting mating pairs as described above, with the restriction that none of the crosses were between full or half siblings. This was done by checking the parentage of each cross, and randomly switching mates (among the individuals chosen to reproduce) as necessary to avoid mating between siblings.

The pedigree contained one final uncertainty that we needed to deal with. As mentioned above, the ancestry of one of the 99 founders, M93-9, is unknown. Spawning records indicate that M93-9 fertilized 38 eggs in 1993, but do not indicate whether this male was a residual or an outmigrant (it was not an anadromous fish). We have assumed that this fish was unrelated to other founders of the population. This may not be true, but our analysis of founder contributions (see below) showed that this founder contributed only 0.2 % of the genes to fish born after 2005, so our results are not likely to be sensitive to this assumption.

After we made the above assumptions, the first analysis we performed was to estimate the average number of generations that genes were in the breeding program. This was done by simulating pedigrees and randomly choosing a gene from a brood year, and then tracing it backwards through the pedigree until a wild-born parent was reached. Results were averaged for one-million randomly generated pedigrees (one gene per pedigree). This approach is comparable to the analytic approach described by Gutierrez et al. (2008) for describing pedigree depth.

Next, we estimated the inbreeding coefficient for each brood-lineage and for each brood year. When we calculated results for each brood year, we weighted by the number of eggs in each brood-lineage. Confidence intervals were constructed by observing how much results varied across 10,000 simulated pedigrees.

In addition to estimating the inbreeding coefficients for each brood year and brood-lineage, we estimated the contributions of each founder to fish born in 2006, 2007, and 2008 (the last year of this study). Ideally, each founder should contribute equally, but this is difficult to achieve. Founder contributions were obtained from the kinship matrix of 10,000 simulated pedigrees; and these values were averaged across pedigrees.

Lastly, we estimated the effective population size of the breeding program. We used two methods to do this: the effective number of breeders and the inbreeding effective population size. We calculated the effective number of breeders, N_b each year from the number of male and female spawners each year and the number of eggs in each cross (Table 1). This calculation was done in two steps. First, we calculated the inbreeding effective size for each sex, $N_{b,\text{males}}$ and $N_{b,\text{females}}$. The effective number of breeding males was calculated (Crow and Kimura 1970):

$$N_{b,\text{males}} = \frac{\overline{k}_{\text{males}} N_{\text{males}} - 2}{\overline{k}_{\text{males}} - 1 + \frac{V_{k,\text{males}}}{\overline{k}_{\text{males}}}} \tag{4}$$

where N_{males} is the number of males spawned in a year, $\overline{k}_{\text{males}}$ is the average number of eggs fertilized by males that year, and $V_{k,\text{males}}$ is the variance in number of eggs fertilized by each male. The effective number of female breeders is calculated using the same relationship. We next calculated the effective breeding number of both sexes considered simultaneously

$$N_b = \frac{4N_{b,\text{males}} N_{b,\text{females}}}{N_{b,\text{males}} N_{b,\text{females}}} \tag{5}$$

(Crow and Kimura 1970). The resulting estimate applies to the effective number of parents of the fertilized eggs and can be directly compared with the total number of spawners to provide an estimate of the ratio N_b/N each year. N_b calculated this way can be thought of as a measure of the contribution of a given year of spawners to future levels of inbreeding in the population. Because reproductive success is evaluated at an early life stage, this estimate of N_b only accounts for sex ratio and variation among individuals in egg production. If the probability of juvenile survival after this point was the same for each cross (as assumed in some scenarios here), then N_b over a full life cycle (adult to adult) would also be given by Eq. 5 (Waples 2002). As some degree of family correlated mortality is likely between egg and adult stage, the value obtained from Eq. 5 is probably an overestimate of the actual N_b. Nevertheless, it can be useful as an index of how effective the program has been in maximizing retention of genetic diversity during the life stage over which there is the greatest opportunity for control.

We also estimated the inbreeding effective population size, N_e, for the entire broodstock program—which is equal to the harmonic mean N_e of the population for each generation. We did this using the method of Gutierrez et al. (2008, 2009), which estimates N_e from the average increase of inbreeding per generation, $\overline{\Delta f}$

$$\text{Ne} = \frac{1}{2\overline{\Delta f}} \tag{6}$$

$\overline{\Delta f}$ was calculated from the average value of Δf_i the per generation increase in inbreeding for the ith individual

$$\Delta f_i = 1 - \sqrt[t-1]{1 - f_i} \tag{7}$$

where t is the average number of generations that an individual's genes have been in the breeding program. $\overline{\Delta f}$ was estimated from ten thousand simulated pedigrees.

Results

Analysis of the pedigree showed that, on average, genes in the fish fertilized in 2008 have been in captivity for 5.5 generations. The minimum length of time in captivity was four generations, and the maximum was eight generations.

Estimates of inbreeding coefficients showed that the amount of inbreeding in the breeding program fluctuated substantially during the early years of the breeding program, and then gradually stabilized into a pattern of slow growth typical of a medium-sized population (Table 2; Fig. 1). By 2008, the average inbreeding coefficient for the fish spawned that year was 0.056. The unknown pedigree of the population did not create a substantial amount of uncertainty for the average amount of inbreeding each year. For example, the 95 % confidence interval for the average inbreeding coefficient in 2008, as obtained from 10,000 simulated pedigrees, was [0.049, 0.066].

The analysis of founder contributions showed a rather uneven contribution among founders to fish born in the period 2006–2008 (Fig. 2). In this analysis, we assumed the 65 outmigrants collected between 1991 and 1993 belonged to five families. Given this assumption, there were 44 founders: 16 anadromous fish (36 %), 10

Fig. 1 Average estimates of inbreeding coefficients in the Redfish Lake captive broodstock program by year. These results assume outmigrants belonged to five families and that the N_e/N ratio for fertilization and juvenile survival was 0.48

Fig. 2 Histogram of the contribution of founders to broods born 2006–2008. These results assume the outmigrants belonged to five unrelated families

Table 2 Estimated inbreeding coefficients and 95 % confidence intervals for each brood year in the Redfish Lake sockeye salmon captive broodstock program

Year	Average expected f for all the fish born each year and 95 % confidence interval for that average
1991	0.000 [0.000, 0.000]
1992	0.000 [0.000, 0.000]
1993	0.147 [0.105, 0.196]
1994	0.103 [0.087, 0.127]
1995	0.000 [0.000, 0.000]
1996	0.025 [0.016, 0.037]
1997	0.003 [0.000, 0.006]
1998	0.032 [0.026, 0.040]
1999	0.031 [0.024, 0.041]
2000	0.080 [0.070, 0.095]
2001	0.025 [0.022, 0.029]
2002	0.033 [0.025, 0.040]
2003	0.037 [0.031, 0.045]
2004	0.060 [0.051, 0.072]
2005	0.054 [0.047, 0.063]
2006	0.043 [0.037, 0.049]
2007	0.063 [0.055, 0.074]
2008	0.056 [0.049, 0.066]

These results assume that the outmigrants are descended from 10 unrelated individuals, and that the standard deviation of fitness in the breeding program was 1.0. Confidence intervals were estimated from 10,000 simulated pedigrees
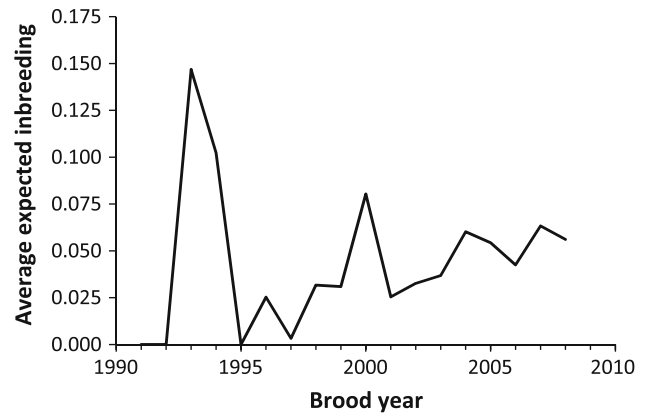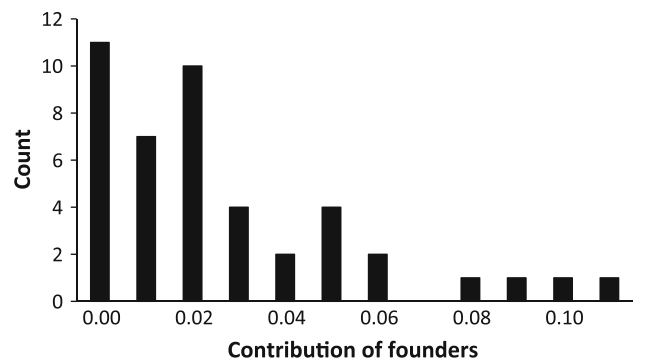
outmigrants (23 %), 17 residual fish (39 %), and one fish that was either a residual or an outmigrant. Approximately half of the genes of the fish born in 2006, 2007, and 2008 were descended from seven founders, and 90 % of the genes in these brood years are descended from 20 founders. Eleven founders made no contribution to the living population and seven had less than 1 % contribution each. 77 % of the genes in the salmon born in 2006, 2007, and 2008 were descended from anadromous fish, 22 % were descended from outmigrants, and <1 % from residuals. Therefore, genes from the anadromous fish were overrepresented in the population. Some of this imbalance was deliberate. Managers deliberately minimized the contribution of residual fish to the breeding program—out of a concern that this was a heritable trait.

The annual number of effective breeders increased as the size of the captive population grew (Fig. 3). On average the effective number of breeders, which is lowered by an uneven sex ratio and variance in the number of eggs produced or fertilized, was 64 % of the number of spawning
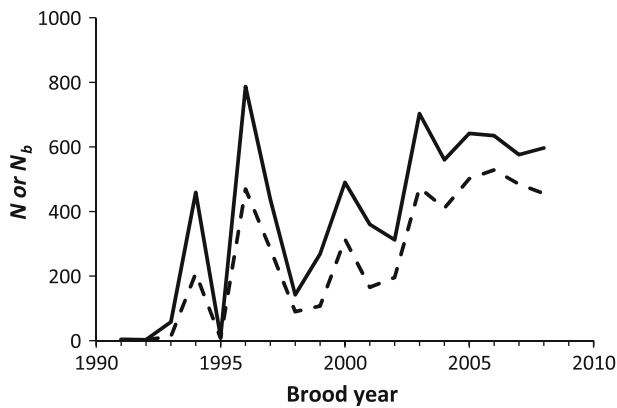
Fig. 3 Number of spawners each year (*solid line*) and effective number of breeders, N_b (*dashed line*)

adults. We estimated the per-generation effective population size of the breeding program to be approximately 41 over the entire course of the breeding program and 115 for the most recent generation.

Discussion

Analysis of the spawning records of the Redfish Lake sockeye salmon captive broodstock program estimated that the average inbreeding coefficient among eggs fertilized in 2006, 2007 and 2008 was approximately 0.05 (Table 1). This means that we expect an egg fertilized during these years to have approximately 95 % of the genetic diversity present in the founders of the breeding program. Given the challenges faced by the early years of the breeding program, this is an unqualified success. Predicting the future rate of inbreeding in the population is difficult, but the impact of the initial population bottleneck appears to have been fully experienced, and we expect that inbreeding coefficients in the population will now slowly creep up at a rate proportional to the current effective population size, which appears to be greater than 100.

We made several assumptions in our analysis that may have affected our results. For example, we assumed the outmigrant founders collected in 1991, 1992, and 1993 belonged to five families, and we assumed that variation in fitness in crosses made at the hatchery could be modeled with Eq. 3. This model assumed that fitness among crosses was independent (i.e., that relatives didn't have similar fitnesses) and included a variance parameter for which we did not have a direct estimate. In order to better understand how much these two assumptions might have affected our results, we performed a sensitivity analysis to see how changing these assumptions affected our estimate of the amount inbreeding present in the fish born in 2006, 2007, and 2008. Our most realistic estimate of the average

inbreeding coefficient among brood years 2006–2008 was 0.054. This estimate was obtained by assuming that the 65 juvenile outmigrants belonged to five families and that σ_z was 1.0 (Eq. 3 and preceding unnumbered equations). If we assume instead that all of the outmigrants were unrelated, the average inbreeding coefficient for eggs fertilized between 2006 and 2008 drops from 0.054 to 0.052—a very modest change. If we assume that the outmigrants were unrelated *and* that all spawners had equal fitness in producing offspring that survived to reproduce, the average inbreeding coefficient decreases further to 0.049 (Table 3)—still a fairly small change given this rather extreme set of assumptions. On the other hand, if we make the more pessimistic assumptions that all the outmigrants collected each year were full siblings and that $\sigma_z = 1.412$ (which produces a juvenile N_e/N ratio of 0.20), our estimate of the average inbreeding coefficient in 2006–2008 increases only to 0.056. This suggests that our estimate of how much inbreeding has occurred in the population is robust to the assumptions that we made about relationships among the outmigrants and the variance in survivorship among crosses. Rudnick and Lacy (2008) reached similar conclusions regarding how relatedness among founders affects captive breeding programs.

We did make other assumptions that are less easy to test. For example, relatives are likely to mature at the same rate, so we may have underestimated the amount of inbreeding that occurred in the program. More importantly, we assumed that all the founders except the outmigrants were unrelated. The population of sockeye salmon in Redfish Lake was small in the years before the founders were captured, so it is likely that some of the anadromous and residual founders were related. This uncertainty affects how our estimates of inbreeding should be interpreted. Inbreeding coefficients measure how much genetic diversity there is in an individual *relative* to the ancestors of that

Table 3 Average inbreeding coefficient for fish born in 2006, 2007, and 2008 for three relationships among the outmigrants and three values of juvenile N_e/N (varied by changing σ_z from 0.0 to 1.0 to 1.1414)

N_e/N for variation in fertilization rates among males and variation in survival rates among families	Relationships among the outmigrants		
	All outmigrants are unrelated	Outmigrants belong to 5 families: 3 in 1991 1 in 1992 1 in 1993	Outmigrants belong to 3 families: 1 in 1991 1 in 1992 1 in 1993
1.00	0.049	0.050	0.052
0.48	0.052	0.054	0.055
0.20	0.054	0.056	0.056

The scenario with five families of outmigrants and a juvenile N_e/N of 0.48 is probably the most plausible

individuals for which a pedigree is available. We estimated the average inbreeding coefficient in the captive population after 2005 was 0.054. This means that fish born after 2005 have 5.4 % less genetic diversity than the founders of the breeding program. We cannot say how the captive population compares to the historic population of sockeye salmon in Redfish Lake because that depends on the unknown pedigree of the population before the captive breeding program was begun.

We estimated that sockeye salmon in the captive broodstock program born after 2005 have an inbreeding coefficient of approximately 0.054. Predicting the effect of this amount of inbreeding upon fitness in the natural environment is difficult because the effect of inbreeding varies widely across species (e.g., Ralls et al. 1988) and even across populations within species (e.g., Lacy et al. 1996). However, a review of the effects of inbreeding upon salmonid fishes (Wang et al. 2002) showed that an inbreeding coefficient of 0.10 can easily decrease weight or survival rate by 10 %. If Redfish Lake sockeye are affected by inbreeding in a similar manner, and if fitness declines linearly with inbreeding, our estimate that the inbreeding coefficient has increased by about 5 % since the beginning of the program suggests that survival rates may have declined by 5 % percent. This, of course, is a very rough estimate. As mentioned above, the impact of inbreeding upon fitness is highly variable. There is even the possibility that selection may have purged some of the deleterious genes from the captive population, and thereby reduced the impact of inbreeding. The effectiveness of such purging is controversial, perhaps because it is highly variable (e.g., Crnokrak and Barrett 2002, Leberg and Firmin 2007), so it may be just as likely that very little purging has occurred. Alternatively, it is possible that domestication selection could be a more serious problem than inbreeding. Every effort was made to minimize domestication selection in the breeding program, but this is notoriously difficult to do.

There can be no doubt that fish in the Redfish Lake captive breeding program are at least modestly inbred. This is substantially less inbreeding than is present in many other captive breeding programs. For example, the average inbreeding coefficient in the Redfish Lake sockeye population (0.054) is less than that for the captive population of California condors (*Gymnogyps californianus*), which is 0.08 (Ralls and Ballou 2004). Several other captive populations have average inbreeding coefficients substantially greater than 0.05. For example, the captive population of Przewalski's horse (*Equus przewalski*) has an average inbreeding coefficient greater than 0.20 (Volf 1999). The McBride Mexican wolf captive population had an average inbreeding coefficient of 0.19 before it was crossed with two other captive populations having inbreeding coefficients of 0.61 and 0.26 (Kalinowski et al. 1999; Hedrick

and Fredrickson 2007). Black-footed ferrets (*Mustela nigripes*) lost approximately two-thirds of their genetic diversity in the thirty-years following 1972 (Wisely et al. 2002), and, therefore have inbreeding coefficients of approximately 0.66.

These comparisons should not be viewed as a measure of how well managers have raised endangered species because each species presents unique challenges and because the amount of inbreeding in a population can be highly influenced by factors that managers cannot control. For example, pandas (*Ailuropoda melanoleuca*) are notoriously reluctant to breed in captivity and usually have only one cub, which would make it difficult to grow a captive population of pandas quickly. Comparing inbreeding coefficients among captive breeding programs is further complicated by the fact that the amount of inbreeding within a breeding program is strongly correlated with the number of founders, and this number is usually dictated by circumstances outside of the control of the breeding program. Lastly, the total amount of inbreeding in a pedigree is also strongly affected by the depth of the pedigree, i.e., the number of generations animals are bred in captivity. Nonetheless, comparing the Redfish Lake captive breeding program to breeding programs for other endangered species clearly shows that the Redfish Lake sockeye population has experienced less inbreeding than many other populations that have been propagated to prevent extinction.

Our analysis of founder contributions showed that 18 out of 44 founders contributed few or no genes to the current populations. This happened despite efforts to equalize the contribution of most of the founders. As is usually the case, the founders of the captive population were collected in the early years of the breeding program, and these years were beset by difficulties experienced while figuring out how to rear and reproduce wild fish in captivity. These difficulties included high mortality rates, low fertilization rates, low eye-up rates, and asynchronous mating. The mortalities were from a number of factors but most notably bacterial kidney disease contracted from the wild and fish jump outs (Pravecek and Johnson 1997). Poor fertilization rates and pinheading also resulted from nutritional deficiencies and the use of cryo-preserved milt (Pravecek and Johnson 1997). As program managers gained experience, survival rates increased and breeding was better controlled. For example, photoperiod manipulation and hormone injections were used to stimulate proper maturation and reduce asynchronous mating.

The results of this study suggest that reasonably precise analyses of genetic diversity in captive broodstock programs can be obtained even when the parentage of most fish in the program is unknown. This is good news, because the husbandry practices and the record keeping used by the Redfish Lake captive breeding program are similar to those

used in many captive broodstock programs. This suggests that the simulation-based analysis presented here might be useful for other species. Genetic data offer alternative methods for monitoring genetic diversity and estimating effective population size (see Wang 2005 for a review), and could be used to periodically checking estimates obtained from pedigree records and for reducing the number of uncertainties in the analysis. Genetic analysis was not used in this investigation because tissue samples from most founders were not available.

Individual fitness and population level diversity are two important factors for the persistence of Redfish Lake sockeye salmon, but other factors may ultimately decide the fate of this population. Three potentially serious threats to this population include hatchery selection, existing hydropower development in the Snake and Columbia Rivers, and global climate change. The seriousness of these threats to the persistence of Snake River sockeye is highlighted by the fact that the Snake River population almost went extinct in the 1990s and many of the causes for this decline do not seem to have been mitigated (Good et al. 2005). Global climate change and hatchery selection (e.g., Araki et al. 2007; Christie et al. 2012) may make survival of the population in the future even more difficult. Assessing the magnitude of any of the future challenges to sockeye salmon in the Snake River, however, is difficult; predicting the cumulative impact of these factors (and others) is a formidable challenge. However, the results of this present investigation provide reason for optimism. Sockeye salmon in Redfish Lake captive broodstock program appear to have retained approximately 95 % of the genetic variation of the fish that founded the captive population, and we can hope that this is enough to avoid most of the harmful effects of inbreeding and to provide enough genetic variation for the population to adapt to future challenges.

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