# AN ABSTRACT OF THE DISSERTATION OF

Nicholas M. Sard for the degree of Doctor of Philosophy in Fisheries Science on April 6, 2016.

Title: Investigating Demographic and Evolutionary Factors Important for Fish Reintroduction

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Michael A. Banks

Reintroduction programs are used to re-establish species back into their historical habitat. Most reintroduction programs have failed and few papers have evaluated factors that may be important to Pacific salmon. The 158 meter tall Cougar Dam has blocked Chinook salmon (*Oncorhynchus tshawytscha*) from accessing 40 kilometers of historical spawning and rearing habitat for over 50 years. Here, I evaluated a Chinook salmon trap and transport reintroduction program above Cougar Dam on the South Fork McKenzie River, Oregon from 2007 to 2015. First, I evaluated if release location and date correlated with two measures of fitness based on adult assignments to age-0 juveniles collected above the dam and adult offspring returning to the South Fork McKenzie River, respectively. I found that release location and date had little to no effect on either measure of fitness. I also evaluated if there were fitness differences between hatchery and natural origin (HOR and NOR, respectively) adults. I found consistent fitness differences between males (RRS=0.48, $p<0.001$), but not females (RRS=0.72, $p=0.81$). In addition, I found that origin ($p=0.352$) no longer explained variation in fitness after accounting for variation in fork-length, which suggests that HOR fish may be less fit, in part, because they are 2-4 cm (95% CI) smaller, or perhaps younger. I also evaluated a measure of population productivity known as cohort replacement rate (CRR) - defined as the number of future spawners produced by a spawner.

Based on genetic parentage assignments to NOR adults returning to the trap and transport facility, hereafter Cougar Trap, I found that adults reintroduced in 2007 and 2008 did not meet demographic replacement (CRR: 0.40 and 0.31, respectively). I also found a seasonal decline in the proportion of NOR adults produced above the dam that returned to the Cougar Trap. I also developed grandparentage assignment methods to determine how many precocial males and adfluvial Chinook salmon, two non-anadromous life history tactics, contributed to population productivity. I found 31 unsampled precocial males, as well as 48 age-4 and -5 probable adfluvial male and female adults contributed to the reintroduced population. My discovery of adfluvial Chinook salmon contributing to population productivity is significant because little is known about this life history tactic, and they provide resiliency to a reintroduced population. I show that adfluvial adults can be produced by anadromous mate pairs. Adfluvial adults increased CRR estimates; however, neither the 2007 or 2008 cohorts met replacement after incorporating this non-anadromous life history tactic (CRR: 0.46 and 0.35, respectively). Finally, I assessed if genetic variation in founding cohorts was maintained in their returning adult offspring returning to the South Fork McKenzie River, hereafter $F_1$ offspring. On average, 6 alleles were lost per locus between founding cohorts and their $F_1$ offspring. $N_e$ estimates were high using either demographic or genetic methods (range: 344 to 893). My dissertation research provides valuable information on factors that may be important to population productivity, as well as the maintenance of genetic variation in nascent populations established through reintroduction.

# Investigating Demographic and Evolutionary Factors Important for Fish Reintroduction

by
Nicholas M. Sard

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Nicholas M. Sard, Author

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# CONTRIBUTION OF AUTHORS

My major advisor, Michael Banks, and our lab manager Dave Jacobson, are co-authors on all four of my chapters. Kathleen O'Malley, Marc Johnson, and Mike Hogansen are co-authors on my publications "Factors influencing spawner success in a spring Chinook salmon (*Oncorhynchus tshawytscha*) reintroduction program" and "Genetic monitoring guides adaptive management of a migratory fish reintroduction program". William Ardren and Melissa Evans are co-authors on my manuscript entitled "Rare alleles are lost in a single generation following reintroduction". All co-authors provided useful comments and discussion when I was writing manuscripts. Dave Jacobson genotyped half of the fish used in my dissertation.

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Demographic and genetic considerations for a Chinook salmon reintroduction program

# General Introduction

For over a century reintroductions have been used as a conservation strategy to re-establish imperiled species back into to their historical habitat (Griffith *et al.*, 1989, IUCN/SSC, 2013). Several review papers suggest that most programs fail, which indicates more research is needed to better understand the factors important for establishing and maintaining reintroduced populations (Fischer and Lindenmayer, 2000, Wolf *et al.*, 1996). Most information published in the literature on reintroductions to date has been descriptive in nature, which can be beneficial, but may not be the most efficient way to identify limiting factors for reintroduction (Holling, 1978, Walters, 1986). To address this issue, Armstrong and Seddon (2008) suggested that researchers pose strategic questions at three different scales: 1) factors important to the reintroduced population itself; 2) how the reintroduced population interacts with other semi-isolated populations, as well as 3) the entire ecosystem.

Dams are major threat to freshwater ecosystems, and they pose a unique challenge to fish reintroductions for several reasons (Vitousek *et al.*, 1997, Wilcove *et al.*, 1998). First, there are tens of thousands of dams around the world (ICOLD, 2015). These dams are beneficial to humans for several reasons; yet, dams can negatively affect freshwater ecosystems by alternating the stream channel and temperature throughout the year (Sheer and Steel, 2006, Saunders et al. 2006). In addition, the reservoirs that dams create can be home to many invasive species that hold their own unique challenges to ecosystems (Pimentel *et al.*, 2005, Rahel and Olden, 2008). Finally, dams fragment fish populations, and can result in local extirpation for fish species that are migratory and philopatric (e.g. Morita and Yamamoto, 2002, Neraas and Spruell, 2001).

Fish ladders and dam removal are both common strategies used to restore species back into historical habitat through passive and volitional movement of the fish themselves (reviewed by Pess *et al.*, 2014, Quiñones *et al.*, 2014). Some research on these strategies has been published (e.g. Anderson *et al.*, 2015, Anderson *et al.*, 2013), which may be helpful to other current and future fish reintroduction efforts. However, not all dams are well suited for fish ladders, and some dams used for flood mitigation purposes will not be removed. In such scenarios, trap and transport reintroductions offer an alternative method for providing native fish access to their habitat, while still maintaining function (e.g. DeHaan and Bernall 2013). Until recently, there have been few papers published on fish trap and transport reintroductions (but see Baumsteiger *et al.*, 2008), and there are many uncertainties surrounding this particular type of strategy.

Genetic monitoring is a growing tool used to study several demographic and evolutionary parameters that are important to the persistence of a population over time (Schwartz *et al.*, 2007). Demographic factors such as survival and range expansion can be monitored by using genetic "fingerprints" of sampled individuals (McKelvey *et al.*, 2006, Rudnick *et al.*, 2005). In addition, measures of genetic diversity can be monitored over time to protect against the negative effects of inbreeding depression, as well as to ensure that the population is maintaining a sufficient amount of genetic variation such that individuals within a population can potentially adapt to a changing environment.

Genetic parentage methods can be used to assemble "wild pedigrees", which is an incredibly powerful and informative type of genetic monitoring (Pemberton, 2008). Wild pedigrees can be used to estimate fitness and heritability of traits (e.g. Garant *et al.*, 2003, Theriault *et al.*, 2007). Estimates of fitness for each individual can be used to determine

factors that are important to population productivity on a fine scale (Anderson *et al.*, 2015, Williamson *et al.*, 2010). In addition, when genetic pedigrees have been assembled for multiple generations, researchers can study rare or hard to sample life history tactics by using knowledge of the grandparent breeding matrix, as well as parental and grandoffspring genotypes to infer the existence of unsampled individuals (Christie *et al.*, 2011). Here I used a genetically monitored trap and transport reintroduction program for Chinook salmon to study several factors that may be important to consider on both demographic and evolutionary timescales.

Chinook salmon (*Oncorhynchus tshawytscha*) are one the seven major Pacific salmonid lineages (Neave, 1958), and are typically characterized as semelparous and anadromous (Quinn, 2011). They begin their lives in the gravel beds of tributaries that eventually flow into the northern Pacific Ocean (Healey, 1991). Life history tactics for age-0 juveniles can be incredibly diverse (e.g. Schroeder *et al.*, 2015), but have historically been classified into two general groups: ocean-type or stream-type (Healey, 1991). Stream-type juveniles spend a year or more in freshwater before migrating to the ocean, whereas ocean-type juveniles will migrate to the ocean shortly after emerging from the gravel. Chinook salmon feed in the ocean for multiple years and return back to freshwater as age-3 to age-6 sexually mature adults. Adults typically home to their natal stream, but some stray elsewhere (Ford *et al.*, 2015, Keefer and Caudill, 2013). Finally, some males forego an anadromous migration and become sexually mature in freshwater streams at age 1 or 2 (Taylor, 1989).

Spring Chinook salmon in the Willamette River basin are listed as a threatened Evolutionary Significant Unit under the United States Endangered Species Act (NMFS, 2005, NMFS, 2008). The construction and operation of several dams are a major factor contributing

to the decline in abundance of Chinook salmon within the basin. As part of a recovery plan developed by Oregon Department of Fish and Wildlife (ODFW) and the National Marine Fisheries Service (NMFS), Chinook salmon have been reintroduced above several of these dams, including the 158 meter tall Cougar Dam (ODFW and NMFS, 2010). The Cougar Dam has blocked spring Chinook salmon from accessing 40 kilometers of historical spawning and rearing habitat on South Fork McKenzie River for fifty years. Since 1996, surplus hatchery origin (HOR) adults collected at the nearby McKenzie Hatchery (44˚07'44"N, 122˚14'25"W) have been reintroduced above Cougar Dam with the initial intension of providing threatened bull trout (*Salvelinus confluentus*) with prey (in the form of juvenile Chinook salmon), as well as to restore some of the natural ecosystem functions related to nutrients released from adult carcasses (Zymonas *et al.*, 2010). However, in more recent years managers have begun to consider that possibility of a formal Chinook salmon reintroduction program with the goal of re-establishing a population above the dam, as part of the ESU.

The primary goal of my dissertation research was to study factors that may be important for reintroduction success using a genetic monitoring approach. Given that so little is known about trap and transport methods, I first evaluated if release location and date associated with individual fitness estimates for reintroduced adults. I also tested if fitness differed between hatchery and natural origin adults. My second objective was to assess population productivity by determining if cohorts were replacing themselves over time based on anadromous adult offspring. This factor is particularly important for migratory fish trap and transport reintroduction programs because the dams themselves still remain and may therefore pose substantial challenges for progeny during the smolt migration to the ocean. My third objective was to determine if/how much rare or difficult to sample life history

tactics (i.e. precocial males and adfluvial adults) contributed to the reintroduced population. As a fourth and final objective, I assessed if genetic variation initially observed in founding cohorts was maintained in $F_1$ offspring, and I estimated the effective population size using demographic and genetic approaches.

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# Factors influencing spawner success in a spring Chinook salmon (*Oncorhynchus tshawytscha*) reintroduction program

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## Abstract

Dams have contributed to the decline of migratory fishes by blocking access to historical habitat. The active transport (trap and haul) of migratory fish species above existing dams can sometimes support population recovery when the use of fish ladders or dam removal is infeasible. However, little is known about the efficacy of trap and haul conservation strategies. Here we used genetic parentage assignments to evaluate the efficacy of reintroducing adult Chinook salmon above Cougar Dam on the South Fork McKenzie River, Oregon, USA from 2008-2011. We found that mean reproductive success (RS) declined as adults were released later in the spawning season in 2009 and 2010; however release location did not affect RS. In 2010 and 2011, we tested for RS differences between hatchery and natural origin (HOR and NOR) adults. HOR males were consistently less fit than NOR males, but little evidence for fitness differences was apparent between HOR and NOR females. Interestingly, the effect of origin on RS was not significant after accounting for variation explained by body length. Our results may indicate that release date and location have inconsistent or no effect on the reproductive success of reintroduced adults when active transport strategies are employed for some migratory fishes.

## Introduction

Reintroduction programs attempt to re-establish locally extirpated species to their historical habitat (IUCN 2013) and can serve to restore a species' distribution when access to habitat has simply been blocked. However, few reintroduction programs have been successful to date (Fischer and Lindenmayer, 2000, Wolf *et al.*, 1996), and most research on reintroductions has focused on mammals and birds (Seddon *et al.*, 2005), rather than fish (but see George *et al.*, 2009). There are many migratory fish species (see Brönmark *et al.*, 2014, Lucas and Baras, 2001) whose access to critical habitat has been negatively affected by dams. Therefore, an understanding of factors that affect the success of fish reintroductions is greatly needed.

Reintroduction efforts have become an increasingly common management strategy to aid in the recovery of threatened salmonid populations, often through the decommissioning of dams or the construction of fish ladders (reviewed by Pess *et al.*, 2014). In Chinook salmon (*Oncorhynchus tshawytscha*), reintroductions following dam removal or fish ladder construction have been evaluated through studies of juvenile production and subsequent adult returns (Anderson *et al.*, 2013, Baumsteiger *et al.*, 2008). However, habitat connectivity cannot always be restored through dam removal or fish ladder construction, as with the case of high-head flood control dams (see example projects in Anderson *et al.*, 2014). In such scenarios, the collection and active transport of migratory salmonids provides an alternative approach that has received little attention in the literature (but see Evans *et al.*, 2015).

Spring Chinook salmon, in the upper Willamette River, Oregon, are listed as a threatened Evolutionary Significant Unit (ESU) under the U.S. Endangered Species Act (ESA)

(NMFS, 1999, NMFS, 2005, NMFS, 2008). Dams block migration to 32% of Chinook salmon historical habitat, and alter water temperature and flow regimes (ODFW, 2005, Sheer and Steel, 2006). In addition, it has been estimated that natural juvenile production has been reduced by up to 95% (ODFW and NMFS, 2010) as a result of dam construction and operation in most tributaries within the basin. In the mid-1990s, state and federal fisheries managers began to trap Chinook salmon for transport and release into historical spawning and rearing habitat above upper Willamette River dams to increase natural production and facilitate the species' recovery. Because habitat quality (Groves and Chandler, 1999, Kondolf and Wolman, 1993) and return timing (Anderson *et al.*, 2013, Dickerson *et al.*, 2002, Williamson *et al.*, 2010) can influence salmon reproductive success, it is important to determine whether release location and date affect the success of reintroduction efforts.

Chinook salmon often mature at various ages (Quinn, 2011), including male mini-jacks and jacks that mature prior to the youngest age class of sexually mature females (Heath *et al.*, 1994). Because matings by early maturing males can increase the effective size of a population (e.g. Araki *et al.*, 2007, Perrier *et al.*, 2014), it may be beneficial to include jacks during reintroductions. Yet this topic has received scarce attention in the literature.

Chinook salmon adults produced in hatcheries may also play an important role in reintroduction programs involving threatened or endangered ESUs. Hatchery origin (HOR) Chinook salmon may be used to found a wild-spawning population and quantify important viability parameters when little is known about the demographic risks associated with a reintroduction (e.g. juvenile survivorship through a reservoir and dam, effects from active transport stress, etc.). HOR fish may also be used to supplement reintroduction programs that use natural origin (NOR) adults, particularly when cohorts return in low numbers, which

may help to sustain natural production at a desired level. Anderson *et al.* (2013) described the reproductive success of HOR Chinook salmon naturally colonizing habitat above a dam accessible following fish ladder construction, but the active transport of HOR salmon when founding and supplementing a reintroduction has not been thoroughly evaluated.

By reintroducing both HOR and NOR adults, more may be learned about fitness differences that often exist between hatchery and wild salmon. There is growing evidence that, on average, HOR salmon are less fit in the wild than NOR salmon, and the effect is stronger among males (reviewed by Christie *et al.*, 2014). Differences between the size of eggs from HOR and NOR females may affect fitness (Heath *et al.*, 2003), and sperm competition may play a role as well (Flannery *et al.*, 2013, Lehnert *et al.*, 2012). However, no study to date has clearly identified causal mechanisms for these fitness differences (Christie *et al.*, 2014), though Theriault *et al.* (2011) hypothesized that two general processes may be relevant: sexual selection on the spawning grounds and/or natural selection early in the life cycle of salmon (i.e. before smoltification). When adult salmon are actively released during a reintroduction program, some phenotypic traits associated with greater fitness can be measured, which may be important for either natural or sexual selection (Milot *et al.*, 2013, Theriault *et al.*, 2011). Thus, recording phenotypic variation and evaluating the effects of that variation on fitness may provide insight into the mechanisms resulting in HOR and NOR fitness differences.

In this study we measured the reproductive success of adult Chinook salmon reintroduced to habitat above Cougar Dam, located on the South Fork McKenzie River, Oregon, USA. We used genetic parentage methods to test for and estimate effects from factors associated with adult reintroductions on their subsequent juvenile production (i.e.

reproductive success). Our four main objectives were to 1) test if release date and release location affected the reproductive success (RS) of reintroduced adults; 2) describe RS for jacks among reintroduced cohorts; 3) test if mean RS differed between HOR and NOR Chinook salmon reintroduced in 2010-2011 and; 4) test if adult fork length explained variation in RS.

## Methods

### *Study area*

Of all Upper Willamette River tributaries, the McKenzie River typically supports the highest proportion of NOR Chinook salmon returns (Johnson and Friesen, 2010), despite the presence of several dams, including the 158 meter tall Cougar Dam. Construction of Cougar Dam was completed in 1964, blocking access to 40 kilometers of Chinook salmon spawning habitat (Figure 1). Following construction, attempts to release Chinook salmon above the dam were terminated because of low numbers of adults returning to spawn. In 1993, HOR Chinook salmon were released above the dam, and adults have been released annually since 1995. Most or all of these HOR Chinook salmon were likely produced by the McKenzie River Hatchery (44°07’44”N, 122°14’25”W), where 99% were collected. However, in 2009, a small number (N=39) of adults were collected at the nearby Leaburg Hatchery (44°8'8.63"N, 122°36'32.32"W), which does not produce salmon. We found no evidence of fitness differences between adults collected at these two hatcheries, so we treat them equally in our analyses (data not shown). More recently, NOR Chinook salmon were reintroduced above Cougar Dam, following the construction of a trap and haul facility (hereafter Cougar Trap) completed in 2010. Adults have been collected each year throughout the duration of the spawning migration at the Cougar Trap, with the exception of July 19$^{th}$-August 6$^{th}$, 2011,

when it was closed due to repairs. Since 2007, adults were released in four locations above Cougar Reservoir. However, the number of release locations (range: 2-4), and number of release dates varied (range: 11-32) between years. (Figure 1). In addition, the sex ratio (number of males / number of females) was male skewed in all reintroduction years evaluated (Table 1).

## *Sample collection*

The number of adults Chinook salmon reintroduced each year ranged from 731 to 1,386 (Table 1). From 2008-2011, tissue samples were collected from nearly all adults (99%) passed above Cougar Dam (Table 1). All tissue samples were stored in 95% ethanol. Release location, date, and origin (HOR or NOR) were recorded for all adults reintroduced above the dam. Sex was determined by visual assessment of secondary sexual characteristics and recorded. Males were classified as jacks if they were less than 610 millimeters in total length in accordance with Oregon Department of Fish and Wildlife policy. In addition, the sex of jacks was verified using a sex linked marker (see below, 98% concordance Table S1). From 2008-2011, 131 jacks were reintroduced. The number of jacks each year ranged from 5-107 jacks (Table 2). Fork length was measured for all adults reintroduced above Cougar Dam in 2011.

Reintroduced adults spawned naturally above Cougar Dam each fall, and subyearling juveniles, i.e. fish that were less than one year in age, were sampled with a screw trap at the head of the reservoir in each following year (2009-2012). For each sampling day, the date and total number of juveniles collected in the screw trap were recorded. Overall, the number of juveniles collected (96 ± 197, mean ± 1 standard deviation), tissue sampled (44 ± 52), and genotyped (15 ± 21) varied by day. On average, juveniles were collected on 132 ± 22 days per

year. In all four years, we evaluated the proportion of juveniles genotyped per month and found no difference from the portion of juveniles collected in the screw trap (paired Wilcoxon signed-rank tests, $p > 0.05$). Mark-recapture studies indicated that the screw trap operated at a mean efficiency of 5% (Zymonas *et al.*, 2010).

## *Genotyping*

We isolated total genomic DNA (Ivanova *et al.*, 2006) from reintroduced adults and a sample of juveniles (range: 1,826-2,220) collected in each year of our study (Table 1). We used the polymerase chain reaction (PCR) to amplify 11 highly polymorphic microsatellite loci from each fish: *Ots201*, *Ots208b*, *Ots209*, *Ots211*, *Ots212*, *Ots215*, *Ots249*, *Ots253*, *Ots311*, *Ots409*, and *Ots515* (Banks *et al.*, 1999, Greig *et al.*, 2003, Naish and Park, 2002, Williamson *et al.*, 2002). We also amplified *Oty3* to determine the genotypic sex of reintroduced adults because accuracy has been proven 100% correct among Chinook salmon sampled in the Willamette River (Brunelli *et al.*, 2008). We visualized PCR products on an ABI 3730XL DNA analyzer (Applied Biosystems, Inc., Foster City, CA) and scored genotypes using GeneMapper software (Applied Biosystems, Inc.).

We estimated genotyping error by repeating the genotyping procedure described above on a random sample (n=159, 1% of total dataset) of adults and juveniles collected each year. We compared re-processed genotypes with originals and estimated error by dividing the number of discordant allele calls by the total number scored. Genetic analyses were performed at the Coastal Oregon Marine Experiment Station, Marine Fisheries Genetics Laboratory, Hatfield Marine Science Center in Newport, Oregon.

## *Microsatellite characterization and assignment power*

We calculated observed and expected heterozygosity (Nei, 1987) for each adult cohort and sample of juveniles to characterize genetic variation among loci genotyped. We then tested for deviations from Hardy-Weinberg Equilibrium (HWE), and Linkage Disequilibrium (LD) using GENEPOP (Raymond and Rousset, 1995, Rousset, 2008) for adult and juvenile year classes separately. This informed our choice of parent-offspring assignment program (see below: *Parent-offspring assignments*). We assessed the power of our suite of microsatellite loci by calculating average non-exclusionary probabilities for a random single parent, a second parent, and a parent-pair to assign to any given offspring by chance (Jamieson and Taylor, 1997). In addition, we calculated the expected number of false parent-offspring pairs mismatching at 0, 1, or 2 loci (Christie, 2010).

## *Parent-offspring assignments*

We assigned reintroduced adults from 2008-2011 as parents of subyearling juveniles captured in the screw trap the following year (e.g. 2008 adults were assigned to 2009 juveniles), because most juveniles typically emigrate from natal streams as subyearlings in this system (Romer *et al.*, 2011). Deviations from HWE may occur when sampling large numbers of juveniles because some families, and therefore genotypes, are overrepresented in the sample, known as the Allendorf-Phelps Effect (Allendorf and Phelps, 1981, Waples, 1998). We accounted for these deviations by using SOLOMON's Bayes method for no known parents, hereafter referred to as SOLOMON, because it carries no assumption with regard to HWE (Christie *et al.*, 2013).

We assigned potential mothers and fathers to juveniles separately to reduce the number of pairwise comparisons. For each run we used the default SOLOMON settings – 1000 simulated data sets and 50,000,000 genotypes because the posterior probabilities for

each parent-offspring pair differed by < 0.001 when running the analysis a second time with the same dataset, as recommended by the SOLOMON manual. We accepted all parent-offspring pairs with ≤1 genotypic mismatch that had posterior probabilities <0.05 based on our power analysis (Table S2). On average 76 ± 4% of juveniles assigned to one parent of each sex after parsing the data based on criteria described above. However, on average 12 ± 6% of juveniles were assigned to multiple parents of the same sex. We simulated comparable data (e.g. 800 parents, 2,000 offspring, and 11 microsatellites with 35 alleles per locus) using SOLOMON, and found that in 80% of these cases the true parent could be identified by choosing the parent-offspring pair with the lowest posterior probability. We therefore used this criterion when parsing SOLOMON output. However, SOLOMON assigned a single parent to an offspring without accounting for the second parent's genotypes. Thus, we could not initially account for both of the putative parents' genotypes at the same time. We verified that the genotypes of the offspring assigning to both a mother and father were in accordance with Mendelian inheritance using a function developed by N.S. in R.

## *Assignment rates*

For each juvenile cohort, we calculated the proportion of offspring that assigned to 1) both a mother and a father, 2) only a mother, 3) only a father, and 4) no parent. The reintroduction of Chinook salmon above Cougar Dam began over a decade before the first tissue samples were collected from adults in 2007. Accordingly, we expected that unsampled, residualized or precocial males could be present in the system. We expected that female Chinook salmon would be less likely to residualize because this life history has rarely been observed in small freshwater systems (but see Romer and Monzyk, 2014). Therefore,

we expected that some juveniles would have "missing" fathers and few or no juveniles would have missing mothers in our genetic pedigrees.

## *Release effects*

We estimated RS by counting the number of subyearling juveniles within a population sample that were assigned to adults released above Cougar Dam in the previous year. From 2008-2011, we examined factors associated with RS using a negative binomial generalized linear mixed-effect model (GLMM) using the R library *lme4* (Bates *et al.*, 2013). We evaluated each year separately because of the variability in release location and release date, as well as variation in the number of juveniles used in parentage assignment among years. For each year, we included *sex* as a factor, with females reported as the reference. We also included *release date* as a covariate. We were unable to evaluate the effect of *release location* across years on RS, as adults were not consistently released in the same locations in different years. In 2011, adults were released at a single location. Thus, we tested for *release location* effects only in 2008 to 2010. We accounted for similarities among Chinook salmon released on the same date and location with a *release group* random effects variable. For each reintroduction year, all variables and first order interaction terms were tested individually to determine if they significantly explained variation in RS. We included all significant variables in a single GLMM and used backwards AIC model selection to determine an adequate model to explain variation in RS.

Since 2010, both HOR and NOR Chinook salmon were reintroduced above Cougar Dam. We tested for a difference in mean RS between HOR and NOR Chinook salmon by including an *origin* (HOR or NOR) predictor, whereby HOR was the reference, when repeating the same GLMM approach described above. In addition, 2011 was the only reintroduction

year that had length measurements for all reintroduced Chinook salmon. Thus, we included length as a covariate in 2011. Finally, we did not include the *release group* random effects variable in 2011 because adults were released in a single location. We therefore applied a generalized linear model (GLM) with a negative binomial distribution using the R package MASS (Venables and Ripley, 2002).

Spawner length has been shown to be positively related to greater fitness. We therefore tested if *sex*, *origin*, or *sex*origin* significantly explained variation in *length* in 2011 using an ANOVA.

## *Relative reproductive success*

As an alternative to our GLMM approach, we also tested for potential RS differences between HOR and NOR adults using methods described in Araki and Blouin (2005), because these methods account for biases associated with Type A and B error (see Araki and Blouin (2005) for more detail). Briefly, we permutated 10,000 random relative reproductive success (RRS) values using our estimates of individual RS for HOR and NOR Chinook salmon in R (Supplemental file 1). Equation 14 in Araki and Blouin (2005) requires that Type B error is calculated, which is defined as the rate at which a false parent assigns to an offspring when the true parent is not in the dataset. We estimated Type B error by assigning subyearling juveniles to adults reintroduced after the juveniles were spawned – e.g. 2010 adults assigned to juveniles emigrating from head waters in 2010. We calculated observed RRS, after accounting for errors in assignments and potential missing parents (Araki and Blouin, 2005). We determined significance by calculating the frequency of random RRS values less than our observed value. Within each year we compared NOR and HOR Chinook salmon RS differences

for the sexes separately, as well as combined years for each sex individually. All critical values were false discovery rate corrected (Benjamini and Hochberg, 1995).

All analyses were performed using R version 3.1.1 (R Core Team, 2014). Means were reported with ± 1 standard deviation, except for *post-hoc* tests of first order interaction terms. In these cases, we report effect estimates ± standard errors. General data analysis was aided by *plyr* (Wickham, 2011) and *reshape2* (Wickham, 2007) packages in R.

## Results

### *Microsatellite characterization and assignment power*

We included a total of 11,819 Chinook salmon in our genetic parentage analysis, and we genotyped 99% (3709/3738) of adults and 100% (8081/8081) of juveniles at ≥ 10 loci (Table 1). We estimated a 2 ± 1% genotyping error rate for both adults and juveniles, and we observed variation in genotyping error among loci (2 ± 2%) likely due to mis-scoring and allele dropout. We found that all loci were out of HWE for the 2008 and 2009 adult cohorts. However, only 6 and 5 loci deviated from HWE in 2010 and 2011, respectively. Similarly, the number of locus pairs in LD was higher in 2008 and 2009, though average number of locus pairs in LD among years was low (4 ± 3). As expected, we observed all loci out of HWE and many locus pairs in LD among juvenile samples (21 ± 7). Deviations from HWE were likely due to large samples sizes because $F_{IS}$ values were small (0.005 ± 0.014, Table S3). We found that observed (0.92 ± 0.01) and expected (0.92 ± 0.01) heterozygosity, as well as the average number of alleles per locus (34 ± 1) were high and varied little among adult and juvenile datasets used in genetic parentage analyses (Table 1).

For the four reintroduction years evaluated, we assigned most (99 ± 2%) juveniles to at least one parent and an average of 79 ± 7% juveniles to both a mother and a father (Figure

S1). We found the average frequency of juveniles that were missing a mother (14 ± 5%) was nearly twice that of those missing a father (7 ± 3%).

## *Reproductive success variation*

We observed positively skewed RS distributions in all four adult cohorts (Figure S2-S5). Means and standard deviations varied between years and sexes, at least in part, due to variation in number of adults reintroduced each year and the number of juveniles genotyped (Table S4). We found evidence that jacks contributed to juvenile production in all years except 2011 (Table 2). However, because we did not genotype all juveniles produced in the system, production by jacks may simply have gone undetected by our study in that year.

## *Release effects*

The factors that were significantly associated with RS varied among the four reintroduction years we evaluated. Regression analysis suggested that in 2008 the mean RS for males was 49% that of females ($p$<0.001, Table 3). In 2009, we only found a small negative relationship between *release date* and RS ($p$<0.001, Table 3). Overall, from 2008-2010, we found that *release location* did not significantly explain variation in RS (Table S5).

In 2010, we included an *origin* term in our analysis because both NOR and HOR Chinook salmon were released in that year and thereafter. We found that *sex*, as well as the interaction terms *origin*sex* and *origin*release date* significantly explained variation of individual RS. All variables were included in the final GLMM based on AIC scores. We found that mean RS for males was 0.43 times that of females ($p$<0.001, Table 3), after accounting for variation explained by other predictors. Our results indicated that the effect of *origin* on RS was different between the sexes. Accordingly, we tested for the effect of *origin* on RS for each sex separately. We found that the mean RS for NOR males was 2.2 times that of HOR

males ($e^{0.776 \pm 0.237}$, $p$=0.001), whereas mean RS for NOR and HOR females did not differ ($p$=0.160). We also evaluated the effects that *release date* had on RS of HOR and NOR Chinook salmon separately, because the *origin*release date* term was significant ($p$=0.016, Table 3). Mean RS of NOR Chinook salmon decreased ($e^{0.018 \pm 0.006}$, $p$=0.003) each day of the release season. However, *release date* did not significantly explain variation in RS for HOR Chinook salmon ($p$=0.606).

The predictors *origin* and *length* significantly explained variation in RS in 2011. In contrast to results from 2010, we found no significant interaction between *origin* and *sex* ($p$=0.096) in 2011. We also found no significant interaction between *length* and *origin* that year ($p$=0.241). We found that mean RS for NOR Chinook salmon was 1.5 times that of HOR Chinook salmon when variation in *length* was not accounted for in the GLM ($p$=0.023). However, we found that *origin* was not a significant predictor of RS ($p$=0.352) when *length* was accounted for in the same GLM. Based on AIC scores, we included only *length* in the final GLM. We found that for each centimeter (cm) increase in *length*, mean RS increased by 6% ($p$<0.001, Table 3).

We found that mean *length* for NOR Chinook salmon was 2.0-4.0 cm (95% CI) greater than HOR Chinook salmon ($F_1$=58.4, $p$<0.001) and that males were 1.3-3.3 cm (95% CI) shorter than females ($F_1$=9.58, $p$<0.001). The interaction term *sex*origin* was not significant ($F_1$=0.04, $p$=0.788), suggesting that the effect of *origin* on *length* did not differ between the sexes.

## *Relative reproductive success*

Results from permutation tests provided corroborative evidence for RS differences between HOR and NOR male Chinook salmon. Mean RS for HOR males was significantly less

than that of NOR males in 2010 (RRS= 0.46, $p$=0.004), 2011 (RRS= 0.51, $p$=0.003), and when both years were combined (RRS= 0.48, $p$<0.001). We found no significant differences between RS of NOR and HOR females in 2010 (RRS= 1.51, $p$=0.938), 2011 (RRS= 0.89, $p$=0.434), or when both years were combined (RRS= 1.10, $p$=0.869).

## Discussion

We examined the effects of factors associated with an active trap and haul strategy on the reproductive success (RS) of Chinook salmon reintroduced above an existing dam on the South Fork McKenzie River, Oregon. We used RS as a measure of success for each Chinook salmon reintroduced above the dam, because RS is a prerequisite to total lifetime fitness, based on adult returns, in future years. Our results are consistent with findings from other studies, which have demonstrated that reintroduction of sexually mature adult salmonids can contribute to overall production of juveniles (Baumsteiger *et al.*, 2008, DeHaan and Bernall, 2013). To our knowledge, this is one of two (see Evans *et al.*, 2015) studies that provide insight on effects from release strategies associated with active transport. We found inconsistent evidence that release date affected the success of reintroduced adults and release location did not affect RS in any year evaluated. We are also the first to evaluate the RS of reintroduced jacks and found that jacks contributed to overall juvenile production, thereby mediating gene flow among generations for the reintroduced population. Overall, we consistently found fitness differences between HOR and NOR males, which corroborates findings from other HOR/NOR fitness studies. Finally, we found that length may partially explain why HOR Chinook salmon are less fit than NOR Chinook salmon, which is consistent with hypotheses that fitness differences between HOR and NOR salmon

are the result of sexual selection on the spawning ground and/or natural selection in the early life stages of juvenile salmon.

## *Estimating RS from a sample of juveniles*

For Chinook salmon, as with many fishes, estimating mean RS is difficult, particularly during early offspring life stages because hundreds of thousands of juveniles can be produced within a system. In such scenarios, sampling every juvenile is not practical and in many cases, not possible. One alternative is to wait for adult progeny to return in subsequent years, but this approach requires sampling for many years, and sometimes decades. When management action is required within a shorter time frame, sampling of early-stage juveniles can answer important questions relevant to conservation. Anderson *et al.* (2011) evaluated the accuracy of such an approach and provided some guidance when estimating mean RS in salmonids using assignments made with adults and juveniles. They noted that three important parameters to control for Type I error are: 1) the number of parents, 2) variance in RS, and 3) the number of offspring that assigned to a parent. Anderson *et al.* (2011) suggest sampling enough offspring such that estimated mean RS is >6.2. We exceeded a mean RS >6.2 in 4 of the 12 cases in our study (Table S4). Type I error rate may exceed 0.05 in the remaining 8 cases. We note, however, Ford *et al.* (2012) previously used both adult-juvenile and adult-adult assignments in a salmonid fitness study, and results were consistent between both approaches in all but one comparison. Moreover, our preliminary (unpublished) findings from parentage assignments for returning adult salmon thus far are consistent with adult-to-juvenile results presented here. Nevertheless, a conservative approach would be to interpret our results in light of potential elevated Type I error rates, which may incorrectly identify associations when no such association exists.

There are other factors that could potentially bias results. First the location of the screw trap relative to redds could potentially bias RS estimates. Indeed Anderson *et al.* (2011) noted that the probability of collecting juveniles likely decreases as the distance between a given redd and the screw trap increases. Similarly, juveniles will not likely be collected if they were produced in a redd located below the screw trap. Finally, juveniles are increasingly able to avoid a screw trap with greater size, though we do not believe this was a significant factor in our study because ongoing life history studies indicate that most juvenile Chinook salmon migrate to Cougar Reservoir as subyearlings.

## *Release effects*

Factors that explained variability in RS were inconsistent across years evaluated. Males were on average less fit than females in 2008, which was likely driven by the male-skewed sex ratio (2 males:1 female) in that year. In 2009, we only found that mean RS decreased slightly as Chinook salmon were released later in the season. Similarly, a small negative relationship between *release date* and RS was evident in 2010, though it was only for NOR Chinook salmon. Our results corroborate findings from two previous studies of Chinook salmon that reported a negative relationship between fitness and timing of entry to spawning grounds. Similar to our findings, release date effects observed in those studies were inconsistent among years (Anderson *et al.*, 2013, Williamson *et al.*, 2010). Dickerson *et al.* (2005) found that early-arriving pink salmon (*O. gorbuscha*) males were also more successful, which may be explained by a density-dependent process. Males that arrive to the spawning grounds earlier may experience less competition for mates, but as the season progresses the operational sex ratio increases. Individual mating success therefore decreases because of increased competition among males (Quinn, 2011). Results observed here and by

others suggest that; overall, *release date* is a weak and inconsistent predictor of RS, at least for spring Chinook salmon.

Water velocity and depth, as well as gravel size are important factors for Chinook salmon redd site selection (Groves and Chandler, 1999, Kondolf and Wolman, 1993). Moreover, spawning habitat quality can also affect the early survival of juvenile salmonids (Quinn, 2011). However, we found no significant effect from *release location* on RS. This is likely because Chinook salmon disperse throughout the river after release, and do not necessarily spawn near their release sites. A telemetry study of female Chinook salmon reintroduced above Cougar Dam in 2010 found that females moved a minimum distance of between 4.2 -17.1 kilometers (Zymonas *et al.*, 2010). Males were not evaluated by Zymonas *et al.* (2010) and males are likely to move more than females on spawning grounds, because they are not anchored by the construction and defense of a redd.

## *Jacks*

Our results show that the reintroduction of jacks can help contribute to juvenile production and, more importantly, contribute to gene flow among cohorts of Chinook salmon reintroduced above Cougar Dam. Gene flow among cohorts will help to increase and/or buffer effective population size of the population over time (e.g. Araki *et al.*, 2007, Perrier *et al.*, 2014). In our study system, gene flow is already achieved by overlapping age-4 and age-5 Chinook salmon adults returning to spawn. But, jacks are likely more important to gene flow between generations in coho (*Oncorhynchus kisutch*) and other migratory fishes with more constrained age at maturity.

## *HOR and NOR Fitness*

Our findings generally support the growing body of evidence that HOR salmon are less fit in the wild than NOR salmon (reviewed by Christie *et al.*, 2014). We found that male HOR Chinook salmon were less fit than NOR Chinook salmon using two different approaches: GLMM/GLM and an unbiased RRS permutation test. However, no consistent fitness differences were found between HOR and NOR females. In particular, we found no fitness differences between HOR and NOR females released in 2010 using either approach, but results from 2011 indicated that mean RS for NOR Chinook salmon (both males and females, combined) was 1.5 times greater than HOR Chinook salmon. Interestingly, this origin effect in 2011 was not significant when we accounted for RS variation explained by *length*. Indeed, NOR adults had a mean length that was 2.0-4.0 cm (95% CI) greater than HOR adults. Together, these results suggest that fitness differences observed between HOR and NOR Chinook salmon released above Cougar Dam may be explained by mean fork length, which differs significantly according to *origin*.

We did not directly test the hypotheses posited by Theriault *et al.* (2011), that fitness differences between HOR and NOR salmon are likely caused by 1) effects from sexual selection on the spawning ground and/or 2) natural selection during juvenile early life stages. However, our results are consistent with both hypotheses. First, *length* has been shown to be an important trait under sexual selection. In particular, female Chinook salmon will delay spawning with smaller males, which likely indicates that they prefer to mate with larger males (Berejikian *et al.*, 2000). Second, larger females typically dig deeper redds, are less susceptible to superimposition, and are more fecund than smaller females (Hawke, 1978, Healey and Heard, 1984, Quinn, 2011). Cumulatively, larger females may have greater fitness

because their progeny may experience less mortality associated with the location and quality of the redd where they were reared.

Some have suggested that hatchery practices relax selection for larger individuals (Hankin *et al.*, 1993, Ricker, 1981). In addition, numerous authors have reported positive relationships between body size and RS for both sexes in Pacific salmon (Berejikian *et al.*, 2001, Fleming and Gross, 1992, Schroder *et al.*, 2008). Therefore, HOR salmon are likely to be less fit compared to NOR salmon because, on average, they are smaller in size. Indeed, Ford *et al.* (2012) found that low RS for HOR Chinook salmon could be explained by a higher frequency of precocial (younger and smaller) males present among HOR spawners, relative to the NOR population. In addition, Milot *et al.* (2013) found that HOR Atlantic salmon (*Salmo salar*) returned at a younger age than NOR Atlantic salmon, and that the difference in the number of years at sea contributed to lower HOR fitness. Although precocial male life history is relatively rare in the Willamette River basin, Johnson and Friesen (2013) found that HOR Chinook salmon length has decreased over time within the basin. Our results therefore corroborate findings from Ford *et al.* (2012) and Milot *et al.* (2013) because we found that HOR Chinook salmon were on average smaller than NOR Chinook salmon, which resulted in an overall reduction in fitness. However, the relationship between size and RS that we report warrants further investigation because it is based upon data from a single reintroduction year, and we do not have age information for all reintroduced adults.

Based on our HOR/NOR findings, it may be prudent to limit the number of HOR males in the reintroduction program, because HOR males may be maladapted to the "wild" environment (e.g. Christie *et al.*, 2012) and therefore may lower the RS of NOR females. Moreover, juvenile production is not typically constrained by the number of male spawners,

in terms of gametes, such that a reduction in the number of HOR males used for reintroduction would not likely limit population productivity. However, research that evaluates potential differences in mate pair reproductive success and that manipulates the number of HOR males to determine its effect on overall productivity is warranted before such management decisions could be made (e.g. Baumsteiger et al. 2008).

Successful reintroduction programs above dams will likely prove important to the recovery of many migratory fishes, as they can increase the spawning distributions and natural production. In our study area, reintroduction provided Chinook salmon the opportunity to spawn and rear in headwater reaches of the McKenzie River for the first time in 50 years. We demonstrated that reintroduction of Chinook salmon above dams by active transport can increase natural production. This reintroduction strategy may be effective for the conservation of other migratory fishes (but see Pelicice *et al.*, 2014). Our study found that sex, origin, as well as release date can be important factors influencing the RS of reintroduced Chinook salmon and, therefore, overall population productivity. Observed fitness differences between HOR and NOR male Chinook salmon can likely be explained, at least in part, by the smaller size of HOR fish. Further research on the relationships between age at maturity, length, and fitness of HOR and NOR salmon is need to better understand HOR and NOR fitness differences. Overall, our results provide information on factors that are important for the reintroduction of a migratory fish species into historical habitat despite the continued presence of a dam.

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## Figure Legends

Figure 1. Cougar Dam (solid black horizontal line) is located on the South Fork McKenzie River, Oregon. Locations of adult Chinook salmon release sites and screw trap used to collect juveniles are indicated.

Figure S1. Proportion of juveniles that assigned to both parents (Both), were missing a Father (Father missing), were missing a Mother (Mother missing), and didn't assign to any parent (No parent) from 2008-2011 (the years when parents were reintroduced).

Figure S2. Reproductive success distribution for adults reintroduced in 2008.

Figure S3. Reproductive success distribution for adults reintroduced in 2009.

Figure S4. Reproductive success distribution for adults reintroduced in 2010.

Figure S5. Reproductive success distribution for adults reintroduced in 2011.

Figure 1.

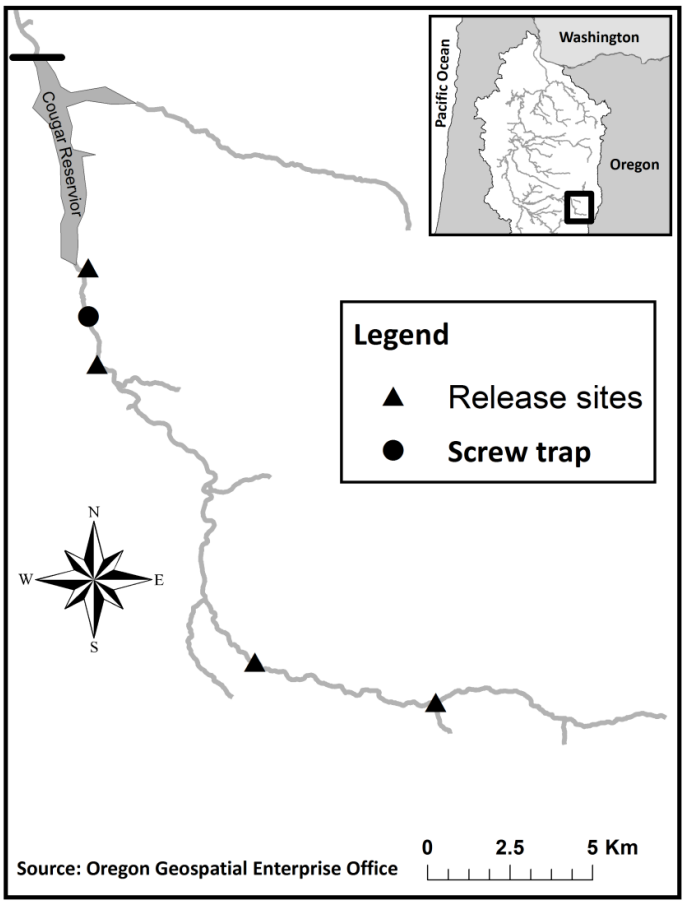
Cougar Reservior
Pacific Ocean
Washington
Oregon
Legend
Release sites
Screw trap
N
W
E
S
Source: Oregon Geospatial Enterprise Office
0
2.5
5 Km

Figure S1.

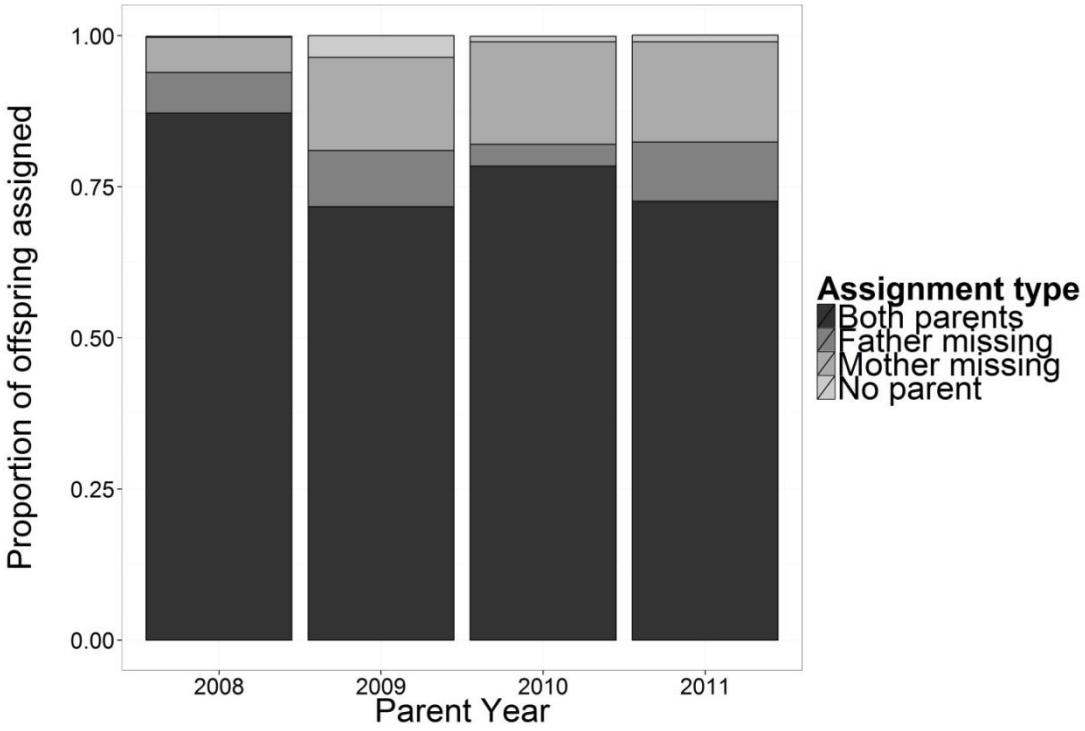
1.00
0.75
0.50
0.25
0.00
Proportion of offspring assigned
2008
2009
2010
2011
Parent Year
Assignment type
Both parents
Father missing
Mother missing
No parent

Figure S2.

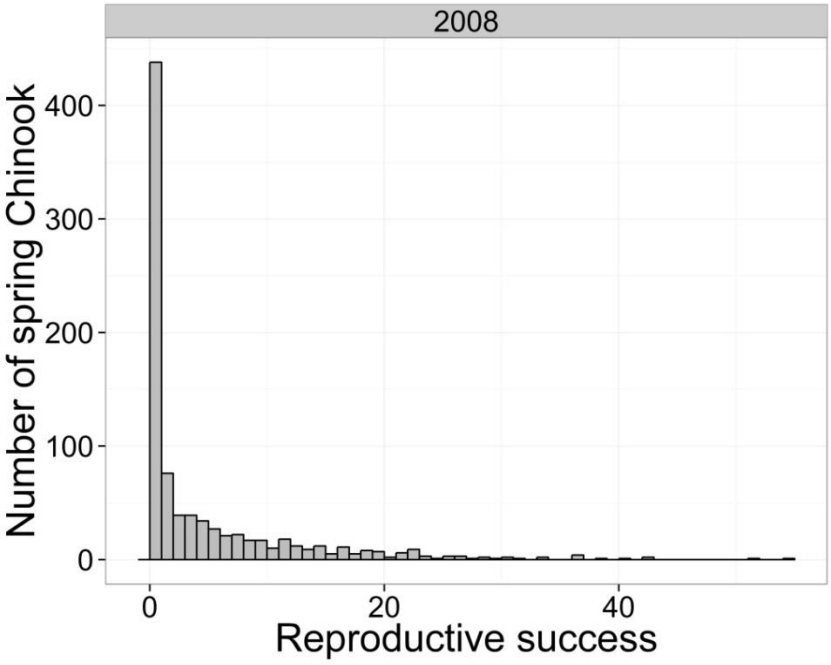
2008
Number of spring Chinook
400
300
200
100
0
0
20
40
Reproductive success

Figure S3.

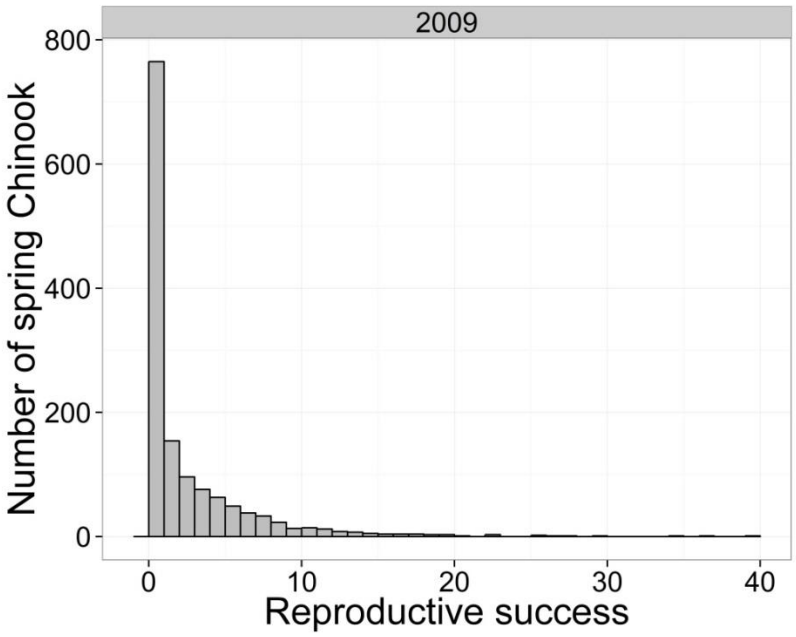
2009
800
600
400
200
0
Number of spring Chinook
0
10
20
30
40
Reproductive success

Figure S4.

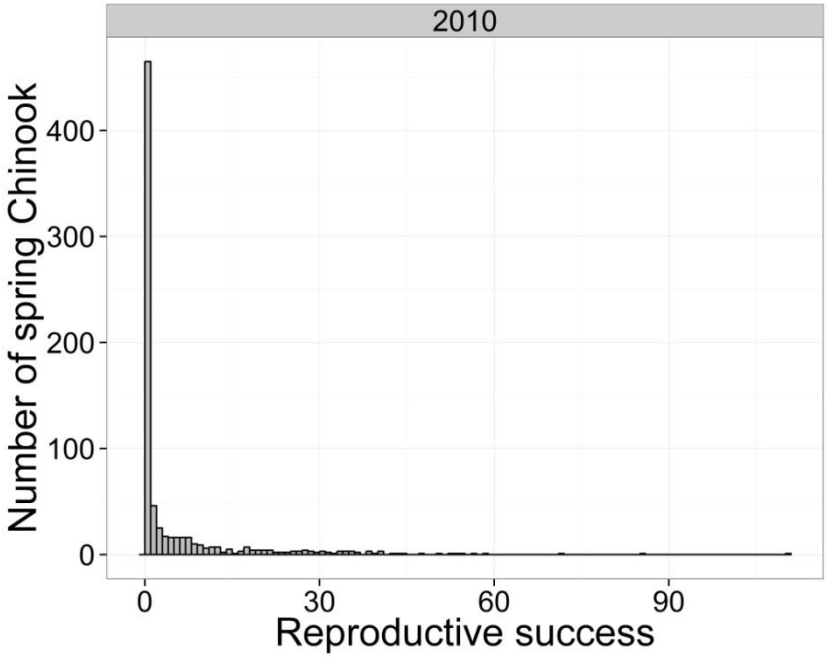
2010
Number of spring Chinook
400
300
200
100
0
0
30
60
90
Reproductive success

Figure S5.

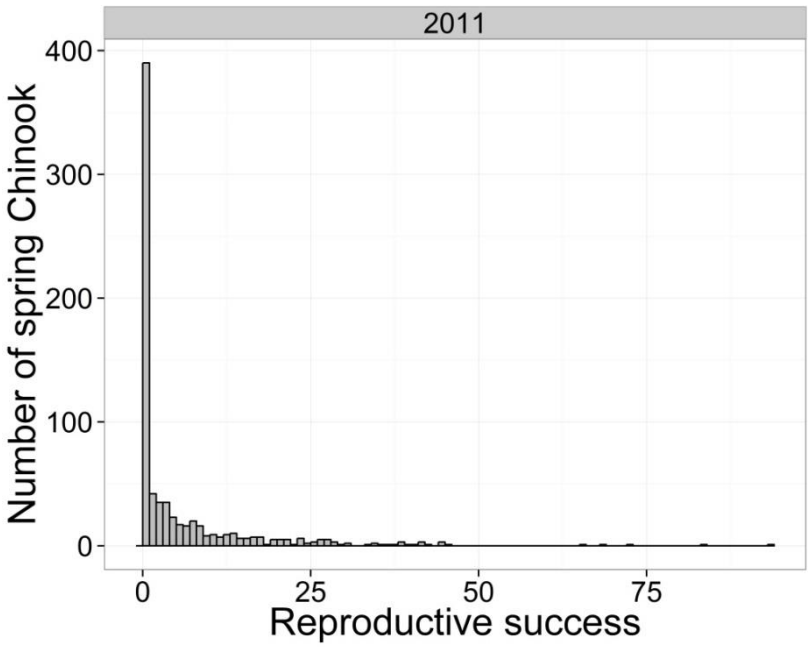
2011
Number of spring Chinook
400
300
200
100
0
0
25
50
75
Reproductive success

Table 1. Summary of the number of adult and juvenile Chinook salmon genotyped at ≥ 10 and < 10 loci, the number of adults missing a tissue sample (Missing Tissue Sample), and genetic variation observed among cohorts sampled. The sex ratio (number of males / number of females), mean number of alleles per locus (A), mean observed heterozygosity ($H_o$), and mean expected heterozygosity ($H_e$) are described.

| | | Loci genotyped | | Missing tissue | Sex | | | |
|---|---|---|---|---|---|---|---|---|
| **Year** | **Type** | **≥ 10** | **< 10** | **sample** | **ratio** | **A** | **$H_o$** | **$H_e$** |
| 2008 | Adult | 873 | 0 | 0 | 2 | 33.3 | 0.92 | 0.92 |
| 2009 | Adult | 1373 | 12 | 1 | 1.3 | 35.1 | 0.91 | 0.93 |
| 2010 | Adult | 738 | 9 | 1 | 1.8 | 34.3 | 0.93 | 0.92 |
| 2011 | Adult | 725 | 5 | 1 | 1.3 | 35.2 | 0.92 | 0.92 |
| 2009 | Juvenile | 2001 | 0 | - | - | 31.5 | 0.91 | 0.92 |
| 2010 | Juvenile | 1826 | 0 | - | - | 33.8 | 0.92 | 0.92 |
| 2011 | Juvenile | 2034 | 0 | - | - | 33.6 | 0.92 | 0.92 |
| 2012 | Juvenile | 2220 | 0 | - | - | 33.1 | 0.91 | 0.92 |

Table 2. Summary of the number (N) and mean (± one standard deviation) reproductive success (RS) estimates for Chinook salmon jacks released above Cougar Dam from 2008-2011.

| **Year** | **N** | **RS** |
|---|---|---|
| 2008 | 13 | 2.0 ± 2.6 |
| 2009 | 107 | 0.8 ± 1.6 |
| 2010 | 5 | 0.0 ± 0.0 |
| 2011 | 6 | 6.8 ± 9.1 |

Table 3. Summary of predictors for final reproductive success generalized linear mixed-effects model/generalized linear model for Chinook salmon released above Cougar Dam from 2008-2011. We used hatchery origin Chinook salmon and females as references for *origin* and *sex*, respectively. Results are described for each year separately.

| **Year** | **Predictor** | **Estimate** | **Std. Error** | **z value** | **Pr(>\|z\|)** |
|---|---|---|---|---|---|
| 2008 | *Sex* | -0.718 | 0.135 | -5.329 | < 0.001 |
| 2009 | *Release date* | -0.018 | 0.004 | -4.442 | < 0.001 |
| 2010 | *Sex* | -0.844 | 0.202 | -4.178 | < 0.001 |
| | *Origin* | 3.787 | 1.781 | 2.126 | 0.033 |
| | *Release date* | 0.001 | 0.004 | 0.240 | 0.810 |
| | *Sex*Origin* | 1.119 | 0.404 | 2.769 | 0.006 |
| | *Origin*Release date* | -0.018 | 0.008 | -2.402 | 0.016 |
| 2011 | *Length* | 0.055 | 0.012 | 4.757 | < 0.001 |

Table S1. Evaluating discordance between jacks identified using phenotypic and genotypic methods from 2008-2011. Number of jacks reintroduced (N), number of jacks that differ in phenotypic and genotypic sex call (Discordance), and number of individuals missing genotypic data (Missing data) are indicated.

| Year | N | Discordance | Missing data |
|---|---|---|---|
| 2008 | 13 | 0 | 0 |
| 2009 | 107 | 1 | 8 |
| 2010 | 5 | 1 | 0 |
| 2011 | 6 | 0 | 0 |

Table S2. Statistics describing the power to correctly resolve parent-offspring assignments are presented for each parent-offspring dataset.

| Parent[a] | Offspring[a] | NE.1P[b] | NE.2P[b] | NE.PP[b] | EFP.0[c] | EFP.1[c] | EFP.2[c] |
|---|---|---|---|---|---|---|---|
| 2008 | 2009 | $3.51E^{-07}$ | $8.55E^{-10}$ | $1.64E^{-16}$ | 0.50 | 1.68 | 5.92 |
| 2009 | 2010 | $2.43E^{-07}$ | $5.66E^{-10}$ | $7.38E^{-17}$ | 0.54 | 1.32 | 8.63 |
| 2010 | 2011 | $2.82E^{-07}$ | $6.81E^{-10}$ | $9.99E^{-17}$ | 0.30 | 1.85 | 4.20 |
| 2011 | 2012 | $3.07E^{-07}$ | $7.48E^{-10}$ | $1.21E^{-16}$ | 0.34 | 1.59 | 3.32 |

[a] The Parent and Offspring columns denote the year each was sampled

[b] Non-exclusion probabilities for a single parent (NE.1P), a second parent (NE.2P), and a parent pair (NE.PP)

[c] Expected number of false parent offspring pairs for zero (EFP.0), one (EFP.1), and two (EFP.2) genotypic mismatches

Table S3. $F_{IS}$ values for all loci genotyped for each adult and juvenile cohorts.

| Locus | Adults | | | | Juveniles | | | |
|---|---|---|---|---|---|---|---|---|
| | 2008 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2012 |
| *OT201* | -0.0074 | -0.0032 | **-0.0163** | 0.0065 | -0.0055 | 0.0064 | 0.0029 | -0.0005 |
| *OT209* | 0.0165 | 0.0342 | -0.0013 | **0.0121** | -0.0058 | 0.0119 | 0.0276 | 0.0518 |
| *OT249* | -0.0031 | 0.0101 | -0.0097 | **-0.0026** | 0.0161 | -0.0004 | 0.0012 | -0.0040 |
| *Ot253b* | 0.0085 | 0.0338 | -0.0095 | **0.0162** | 0.0310 | 0.0277 | 0.0133 | -0.0196 |
| *Ot215* | 0.0024 | -0.0043 | 0.0092 | -0.0001 | 0.0107 | 0.0144 | -0.0147 | 0.0057 |
| *Ot311* | -0.0095 | -0.0028 | **-0.0005** | -0.0043 | 0.0079 | 0.0118 | 0.0019 | -0.0058 |
| *Ot409* | -0.0164 | 0.0250 | -0.0106 | 0.0091 | -0.0085 | -0.0036 | 0.0094 | -0.0103 |
| *Ot211* | -0.0014 | -0.0162 | **0.0088** | **-0.0114** | 0.0045 | 0.0046 | 0.0062 | 0.0056 |
| *Ot208* | 0.0319 | 0.0203 | 0.0184 | 0.0287 | 0.0185 | 0.0077 | 0.0077 | 0.0255 |
| *Ot212* | -0.0011 | 0.0023 | **-0.0107** | -0.0006 | 0.0237 | 0.0246 | -0.0132 | 0.0100 |
| *Ot515* | 0.0058 | 0.0193 | **0.0025** | 0.004 | -0.0059 | -0.0214 | -0.0055 | 0.0051 |

**Boldface** values indicate non-significance after Bonferroni corrections.

Table S4. Summary of reproductive success (RS) estimates for Chinook salmon released above Cougar Dam from 2008-2011. The number of Chinook salmon reintroduced (N) and mean (± standard deviation) RS for each year based on hatchery or natural origin (HOR and NOR, respectively) and sex.

| Year | Origin | Sex | N | RS |
|---|---|---|---|---|
| 2008 | HOR | Female | 288 | 6.5 ± 8.2 |
| | | Male | 585 | 3.2 ± 6.8 |
| 2009 | HOR | Female | 605 | 2.4 ± 4.0 |
| | | Male | 781 | 2.0 ± 4.2 |
| 2010 | HOR | Female | 209 | 6.8 ± 12.7 |
| | | Male | 318 | 2.9 ± 7.5 |
| | NOR | Female | 57 | 4.5 ± 10.3 |
| | | Male | 164 | 6.3 ± 14.1 |
| 2011 | HOR | Female | 179 | 5.4 ± 9.7 |
| | | Male | 195 | 3.2 ± 6.9 |
| | NOR | Female | 145 | 6.0 ± 10.3 |
| | | Male | 212 | 6.4 ± 13.3 |

Table S5. Release location did not significantly explain variation in reproductive success from 2008-2010. Results are represented for each year separately. For all years the reference location was Bridge 1980.

| Year | Location* | Estimate | Std. Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|---|
| 2008 | Bridge 430 | 0.004 | 0.201 | 0.022 | 0.983 |
| 2009 | Slide Creek | 0.305 | 0.246 | 1.239 | 0.215 |
| | Bridge 430 | 0.018 | 0.159 | 0.114 | 0.909 |
| 2010 | Slide Creek | 0.168 | 0.585 | 0.287 | 0.774 |
| | Bridge 430 | -0.055 | 0.241 | -0.226 | 0.821 |
| | Frissell Bridge | 0.023 | 0.439 | 0.051 | 0.959 |

*River kilometers for Slide Creek, Bridge 1980, Bridge 430, and Frissell Bridge are 13, 17.2, 29.7, and 36.4 respectively

# Genetic monitoring guides adaptive management of a migratory fish reintroduction program

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Animal conservation

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## Abstract

Dams contribute to declines in fish abundance, in part, by blocking access to historical habitat. When fish ladders are infeasible, fish can be trapped below a dam and transported above to provide access to habitat. However, this conservation strategy has received little attention in the literature, and many questions regarding efficacy remain unanswered. Here we used a genetic parentage approach to evaluate cohort replacement rate (CRR), defined as the number of future spawners produced per spawner, for a spring Chinook salmon (*Oncorhynchus tshawytscha)* trap and transport reintroduction program. We used CRRs to determine if the population can sustain itself in the absence of immigration, which is important when assessing demographic viability. We also evaluated the effects of release locations and dates on the fitness of reintroduced fish. Counts of adult offspring per spawner were used to estimate fitness of salmon reintroduced in 2007 and 2008. We found that fitness decreased slightly as adults were released later in the spawning season in 2007, but not in 2008. Release location did not affect fitness in either year. We also found a seasonal decline in the likelihood that a Chinook salmon collected at the trap and transport facility was produced above the dam. Finally, 2007 and 2008 cohort replacement rates were both well below one, indicating that improvements are needed to achieve demographic stability. We demonstrate that genetic monitoring of a reintroduction program helped to inform demographic viability assessments, and suggest that our approach may be broadly applicable to other philopatric species.

## Introduction

Nearly 60,000 dams (>15 m high) have been constructed around the world (ICOLD, 2015), many of which pose a threat to migratory fish by restricting access to critical spawning, rearing, and feeding habitats (Lucas and Baras, 2001, Vannote *et al.*, 1980, Wilcove *et al.*, 1998). Ensuring that fish have access to habitat above dams may be important for increasing population productivity and persistence (e.g. McClure *et al.*, 2008, Waples *et al.*, 2008). Reintroductions have provided some fish species access to historical habitat (George *et al.*, 2009), although many fish reintroductions fail (Cochran-Biederman *et al.*, 2015). Accordingly, there is substantial interest in evaluating reintroductions to determine what factors are important when developing a program (Cochran-Biederman *et al.*, 2015). Such evaluations require clearly defined and biologically relevant benchmarks for "success" and relevant data. It is usually essential to monitor the demographic viability of a population established by reintroduction, because local extinction is inevitable if the number of sexually mature adults declines over time (e.g. Robert *et al.*, 2015).

It may be important to consider the locations and dates upon which adult fish are reintroduced, and the season in which their adult offspring return. Some salmonids, along with many other fishes, migrate to specific locations to spawn (Brönmark *et al.*, 2014, Lucas and Baras, 2001). During this migration there may be a transition from homing behavior to spawning site selection, related to temporal variability of spawning habitat quality (Cram *et al.*, 2012, Dittman *et al.*, 2010, Neville *et al.*, 2006). This transition may be relevant to a reintroduction program because adults collected during the spawning migration may not be fully comprised of fish produced by the reintroduction program, but may instead include immigrants from adjacent or distant populations. Trap and transport of immigrants could

seriously impact their source populations if migration to and from the above-dam population were unbalanced. By understanding where and when to reintroduce adults, as well as when to collect fish seasonally (E.g. Anderson *et al.*, 2013, Evans *et al.*, 2015, Sard *et al.*, 2015), managers could potentially improve the efficacy of a reintroduction program and safeguard the conservation of existing populations.

Productivity trends are also important when assessing the viability of a reintroduced population and can be readily evaluated for philopatric species (Cochran-Biederman *et al.*, 2015, Lande, 1988, Robert *et al.*, 2015). One measure of population productivity is cohort replacement rate (CRR), which is defined as the number of future spawners produced per spawner (Botsford and Brittnacher, 1998). CRR is an important metric to consider when assessing a population's demographic viability, because it provides an estimate of population productivity after controlling for immigration from neighboring populations.

In this study, we examined a reintroduction program for spring Chinook salmon (*Oncorhynchus tshawytscha*) and demonstrate how genetics-based monitoring approaches can improve demographic viability assessments and inform decisions within an adaptive management framework. Some factors important to the spawning success of reintroduced adults in this system were previously evaluated using a similar measure of fitness, reproductive success, which was based on parental assignments to age-0 juveniles captured above the dam (Sard *et al.*, 2015). However, Sard *et al.* (2015) did not evaluate factors associated with fitness estimates based on returning adult offspring produced by previously reintroduced cohorts, determine when these adult offspring return seasonally, or evaluate measures of population productivity. Primary objectives in this study were to: 1) estimate fitness of reintroduced adults, and determine if release location or date explained variation

in fitness; 2) estimate the proportion of fish entering the trap and transport facility that assign as offspring to reintroduced adults; and 3) evaluate productivity (CRR) for reintroduced cohorts.

## Methods

### *Study species and site*

Spring Chinook salmon in the Willamette River basin, Oregon are a threatened Evolutionary Significant Unit (ESU) (NMFS, 2005, NMFS, 2008) listed under the U.S. Endangered Species Act (ESA). These anadromous, semelparous fish migrate as adults from the ocean to enter the Columbia River in late-winter and early-spring, then ascend the Willamette River (a major tributary of the Columbia), with peak migration into the upper basin occurring in late May to early June. During the summer, adults hold in deep pools and thermal refugia along the mainstem and major tributary rivers, before homing to natal spawning grounds in headwater streams during the fall (Myers *et al.*, 2006). Homing rates for Pacific salmon (*Oncorhynchus* spp.) are typically high (e.g. 85-95% Ford *et al.*, 2015, Westley *et al.*, 2013). Adults in this system return to spawn at ages 3 to 6 (Johnson and Friesen, 2013), though some males forego anadromous migration and sexually mature in freshwater at age 1 or 2.

Since 1964, Cougar Dam (158 m tall) has impeded Chinook salmon from volitionally accessing historical spawning and rearing habitat on the South Fork McKenzie River, Oregon (Figure 1). Adult hatchery origin (HOR) Chinook salmon from the McKenzie River Hatchery (44°07’44"N 122°14’25"W) have been reintroduced above Cougar Dam annually since 1996. The construction of a trap and transport facility, hereafter referred to as the trap, at the base of Cougar Dam was completed in July, 2010. Since that time, this facility has collected

sexually mature, natural origin (NOR) Chinook salmon for reintroduction above Cougar Dam, while the population has continued to be supplemented with HOR salmon (Table 1). Since its construction, the trap has been operational throughout the spawning migration each year, with the exception of 19 July to 6 August, 2011, due to repairs. At both the hatchery and the trap, adults were collected using small fish ladders that end in holding tanks. Adults are placed in fish transportation trucks, driven above the dam, and released into the river to spawn. To date, there is no assisted downstream passage for juveniles produced above the dam. Instead, juvenile fish can exit the reservoir volitionally, either by passage through hydroelectric turbines or over a steep, 73 m "regulating outlet" spillway (Duncan, 2011).

## *Sample collection*

Release location and date, sex, and origin were recorded for each reintroduced adult since 2007. From 2007 until 2013, the number of Chinook salmon reintroduced above Cougar Dam ranged from 687 to 1,386 per year. In most years since 2007, the sex ratio was male biased (1.41 ± 0.38; Table 1). From 2010-2013, the number of NOR Chinook salmon reintroduced above the dam ranged from 191 to 484 annually (Table 1). Some HOR Chinook salmon collected in the trap (range: 17-30 per year) were released above the dam as well (Table 1). In total, 6,119 adult Chinook salmon were reintroduced from 2007-2013, and tissue samples were collected from 99% (n = 6,115/6,119) of adults. In an effort to determine how many adult offspring produced above Cougar Dam returned and spawned below the dam, tissue samples were collected from Chinook salmon carcasses (range: 8-60) found between the confluence of the South Fork and mainstem McKenzie rivers to the base of Cougar Dam (6.6 river kilometers) from 2011-2013. All carcass samples were collected during systematic

spawning surveys conducted annually by Oregon Department of Fish and Wildlife (Figure 1, Table 2). All tissue samples were stored in 95% ethanol for subsequent DNA isolation.

### *Genotyping and parentage assignments*

We genotyped HOR adults reintroduced from 2007-2010, as well as all NOR Chinook salmon returning to the trap from 2010-2013 at 11 microsatellite loci. We attempted to assign NOR adults collected at the trap to parents reintroduced from 2007-2010, because Chinook salmon spawn at ages 3 – 6 (Figure S1). Assignments were made using two likelihood-based programs: CERVUS (Kalinowski *et al.*, 2007, Marshall *et al.*, 1998) and COLONY (Jones and Wang, 2010). We performed separate parentage analyses for each potential pair of parent-offspring run years to reduce pairwise comparisons. We also attempted to assign the 2007-2010 reintroduced adults to potential offspring collected below the dam from 2011-2013 (for more detail, see Appendix 1).

## *Reintroduced adult fitness*

We estimated fitness by counting the number of NOR Chinook salmon returns that assigned to adults reintroduced above Cougar Dam in 2007 and 2008. We focused on these years because all of their offspring would have returned and been genotyped, with the exception of age-6 offspring produced by the 2008 cohort. However, the incorporation of age-6 adults would likely not change the results significantly, since few (<2%, Johnson and Friesen, 2013) Chinook salmon from the Willamette River mature at that age.

We evaluated factors that might explain variation in fitness using the R package *lme4* (Bates *et al.*, 2013). S*ex* and *release location* were included as factors, whereby females and Bridge 1980 (river kilometer 17.2) were treated as references, and we included *release date* as a covariate. We accounted for similarities among Chinook salmon reintroduced on the

same location and date with a random effects variable (*release group*). We tested if different release locations or variation in release dates significantly associated with fitness to determine if these factors could be used to identify ways to improve the program's efficacy. We evaluated each year separately because the number of release locations (range: 2-3) and release dates (range: 10-13) varied between reintroduction years (Table S1). Multiple release sites were chosen to broaden the distribution of spawners. We tested whether mean fitness differed among adults released at each site. Information from this analysis could be used to determine where and when future adults should be reintroduced. We thus tested each explanatory variable and all first-order interaction terms individually to determine if they significantly ($\alpha = 0.05$) explained variation in fitness using generalized linear mixed model (GLMM) regression with a negative binomial distribution. All significant variables identified were included in a full model and we used backwards Akaike Information Criterion (AIC) model selection (Akaike, 1974) to identify the best model (2 AIC units) to explain fitness variation.

## *Migration behavior*

NOR Chinook salmon appear to return to the McKenzie River and enter the trap in two "waves", one that typically occurs before and another after September 1$^{st}$ (Cannon *et al.*, 2010). One hypothesis is that these waves represent two groups of salmon; those that were produced above the dam and return early to eventually spawn in their natal reaches above the dam, and those produced below the dam that, late in the season, search for mates in areas beyond their natal spawning grounds. We, hereafter, refer to the latter group as immigrants. We used logistic regression to evaluate the seasonal likelihood that fish collected at the trap were immigrants. Managers wanted to limit the trap and transport of

immigrants because the existing population of NOR Chinook salmon in the McKenzie River, a likely source of immigrants to the trap, is considered the only "genetic legacy" population of the ESU (HSRG, 2009), and is thought to present unique genetic and life history diversity. Subjecting this legacy population to the potential risks of a novel reintroduction program, with uncertain juvenile dam passage survival (Beeman *et al.*, 2014), was unacceptable to local managers. However, managers were amenable to the trapping, transport and above-dam release of those adult salmon that had been naturally produced through the reintroduction of hatchery fish.

In 2013, managers adapted their trap and transport methods in an effort to only release offspring from past reintroductions above Cougar Dam and avoid upstream transport of immigrants. Based on our preliminary findings (see *Results* below) and knowledge that most Chinook salmon home to their natal stream, all adults that entered the trap after September 1$^{st}$ were double floy-tagged and released below the confluence of the South Fork McKenzie River with the mainstem . All floy-tagged Chinook salmon that returned to the trap were transported above the dam. Collectively, we refer to these actions as the late season downstream release (LSDR) method. We evaluated the efficacy of the LSDR method by comparing the proportion of immigrants reintroduced after September 1$^{st}$ between 2012 and 2013 using a Fisher's Exact Test.

## *Productivity of reintroduced fish*

We estimated CRR for 2007 and 2008 adults using only females in our calculations because the sex ratio was male biased in both years (Table 1) and males most likely do not limit productivity. We calculated CRR by dividing the number of female offspring that

returned to the trap from 2010-2013 by the number of females that were reintroduced in 2007 and 2008.

## Results

### *Reintroduced adult fitness*

We estimated fitness for 1,619 adults released above Cougar Dam in 2007 and 2008. Among the Chinook salmon we genotyped, 29% produced offspring that returned as adults to the trap. We observed the highest fitness means and standard deviations for both males (0.70 ± 1.56) and females (0.88 ± 1.52) in 2007 (Table 3).

We found that different factors explained fitness variation in 2007 and 2008. In 2007, there was a small negative relationship between *release date* and fitness (Table 4), whereas in 2008 mean fitness for males was less than half (0.44) that of females (Table 4). The significant *sex* effect in 2008 was likely driven in part by the 2:1 (males:females) sex ratio (Table 1). After accounting for variation explained by other predictors, *release location* did not significantly explain variation in fitness in either year (Table S2).

### *Migration behavior*

We found that 64% of NOR Chinook salmon returning to the trap in 2012 were offspring of salmon previously released above the dam, and we observed a similar result in 2013 (68%; Figure 2). We also observed a seasonal decrease in the likelihood that a NOR adult collected at the trap had been produced above the dam ($b$ = -0.046 ± 0.003, z=-13.7, $p$<0.001). Overall, *sex* did not significantly explain variation in the odds of a NOR adult being produced above the dam after accounting for variation between years ($b$ = -0.134 ± 0.162, z= -0.82, $p$=0.410).

Among the 64 Chinook salmon collected in the trap in 2013 after September 1st, 14 (22%) had been produced above Cougar Dam. We found that among the 15 floy-tagged Chinook salmon that returned to the trap, 8 (53%) were progeny from reintroduced parents. Of 49 fish that did not return, 6 (12%) were progeny from parents reintroduced above Cougar Dam. The LSDR method in 2013 resulted in the reintroduction of 7 immigrants after September 1st. This proportion of immigrants (11%) reintroduced after September 1st in 2013 was significantly less than that of 2012 (77%; Fisher's Exact Test, $p < 0.001$).

### *Salmon below the dam*

We successfully genotyped 73% (60/82) of the carcass samples collected below Cougar Dam from 2011-2013. Two samples assigned as offspring from salmon previously reintroduced above Cougar Dam (Table 2). One fish in 2011 was produced by a female reintroduced in 2007, and in 2012 one fish was produced by a male reintroduced in 2008 (Table 2). None of the samples collected below the dam in 2013 assigned as progeny of reintroduced adults.

### *Productivity of reintroduced fish*

Female Chinook salmon reintroduced above Cougar Dam in 2007 (n=318) and 2008 (n=288) spawned 126 and 89 female offspring that returned to the South Fork McKenzie River as adults from 2010-2013. However, these returns were not sufficient to meet replacement for either cohort (CRR = 0.40 and 0.31 in 2007 and 2008, respectively).

## Discussion

We evaluated the reintroduction of Chinook salmon into historical habitat above a dam to identify factors that influenced individual fitness and population productivity. Genetic

parentage assignments allowed discrimination of adults produced through the reintroduction program from immigrants, providing information that could not have been obtained through simple population census and CRR estimates. Had it been assumed that all fish collected at the trap were offspring of previously reintroduced salmon, CRR estimates would have been falsely inflated. Using only genetically identified offspring, cohorts in both 2007 and 2008 were well below (<0.5) replacement, indicating that structural, operational, management or other changes are needed for this population to achieve demographic viability without supplementation.

## *Reintroduced adult fitness*

We found that fitness decreased slightly as Chinook salmon were reintroduced later in 2007, but not in 2008. Our results are similar to other studies that have found inconsistent negative relationships between fitness and return timing (Anderson *et al.*, 2013, Sard *et al.*, 2015, Williamson *et al.*, 2010). Collectively these studies suggest that, for some philopatric fish species, any relationship between return timing and fitness may be influenced by interannual environmental variation, which may make it difficult to apply this information directly to management decisions.

Mean fitness for Chinook salmon reintroduced in 2007 and 2008 did not differ among release locations, consistent with earlier findings that release location did not affect reproductive success (Sard *et al.*, 2015). These results may be explained by movement of Chinook salmon after being released (Zymonas *et al.*, 2010) or an even distribution of spawning habitat quality. But we caution that while release location did not appear to be a significant predictor of salmon fitness in the South Fork McKenzie River, release sites could possibly influence reintroduction success in other systems and should be evaluated.

## *Migration behavior*

We observed a seasonal decline in the likelihood that Chinook salmon collected at the trap were produced by parents released above the dam. This finding is consistent with what might be expected as the result of a fine-scale temporal transition between adults homing to their natal stream and subsequent movements to find mates or suitable spawning sites, as has been observed elsewhere (Dittman *et al.*, 2010, Neville *et al.*, 2006). The pattern we observed offered an opportunity for directed adaptive management, whereby managers took action in 2013 to minimize the reintroduction of immigrants (the LSDR method). These measures were taken due to the low recruitment rate estimates (CRRs) provided by our study and interest to limit the impacts of poor juvenile passage through the dam (Beeman *et al.*, 2014) to only those salmon associated with past reintroduction. One year's results suggested that this method successfully reduced the trap and transport of immigrants. However, future monitoring will be necessary to determine if this approach remains successful. Potentially, this method may be applied to other systems with uncertain juvenile survival, if similar temporal shifts in parentage occur at collection facilities. However, we caution that the LSDR method be implemented only after be considering its effects on heritable traits such as run timing, and the importance of gene flow from immigrants.

## *Salmon below the dam*

We found little evidence that Chinook salmon produced above the dam spawned in the 6.6 river kilometers of habitat below Cougar Dam. Our results could potentially be biased low because our sample sizes were small and offspring could spawn in unsampled areas. However, we expect that most adult offspring would return to spawn above Cougar Dam

because homing rates of NOR Chinook salmon are reported to exceed 85% (Ford *et al.*, 2015).

## *Productivity of reintroduced fish*

Our CRR estimates indicate that Chinook salmon reintroduced above Cougar Dam did not meet demographic replacement and are low compared to similar studies recently published (Anderson *et al.*, 2015, Evans *et al.*, 2015). Because we cannot account for adult offspring that spawned outside our sampling area, we cannot rule out that low observed CRRs might be explained by some fish spawning below the dam, termed the "spill-over" effect by Anderson *et al.* (2015). However, the philopatric behavior of salmon makes it unlikely that more than the 50% of returns necessary to exceed replacement would have spawned elsewhere. Subsequent research on the extent of the possible "spill-over" effects is needed to determine how much productivity estimates may increase. The low CRRs may also be due to the low survival rate to sexual maturity for Chinook salmon (e.g. Smolt-to-adult ratios (range: 0-2.2) Claiborne *et al.*, 2011) because there are many sources of mortality, such as poor passage through dams (Beeman *et al.*, 2014), predation (Collis *et al.*, 2001, Sinclair and Zeppelin, 2002), starvation during outmigration to the ocean (Kareiva, 2000) or while foraging in the open seas (Parker, 1962), and mortality prior to spawning (Keefer et al. 2010). CRR estimates may increase in subsequent years because the mean and variance in reproductive success estimates were highest in those years with both HOR and NOR Chinook salmon released above Cougar Dam (Sard *et al.*, 2015).

Long-term evaluations of productivity informed by CRR estimates are essential when considering the success of a reintroduction program (Anderson *et al.*, 2014, Robert *et al.*, 2015). Cochran-Biederman *et al.* (2015) summarized three common measures of success for

fish reintroduction programs: 1) survival; 2) development to sexual maturity; and 3) recruitment. Using these measures of success, this reintroduction would have been falsely deemed successful without genetic parentage analyses. We clearly show that this population is not viable without immigration, and therefore advocate for continued CRR monitoring and judicious trap and transport protocols until demographic stability can be demonstrated.

### *Conclusions*

Our genetics-based approach provided insights on several factors important to the reintroduction of McKenzie River salmon and, perhaps, other migratory fishes. Research of other migratory fish species will help to determine which of our findings are population- and site-specific or more generally applicable to other species. The genetic monitoring techniques we have described could be broadly applied to other taxa and used to calculate CRRs, as well as estimate immigration rates for philopatric species. By better understanding those factors that affect fitness, the efficacy of rare species reintroductions may be improved and help, in some measure, to counter the steady decline of global biodiversity.

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## Figure Legends

Figure 1. Cougar Dam, indicated by the solid black horizontal line, is located on the South Fork of the McKenzie River. Release sites for reintroduced adults are indicated by solid triangles.

Figure 2. The number of Chinook salmon collected at the trap from 2012-2013 that were (grey) or were not (black) produced by reintroduced parents. Black vertical dashed lines denote September 1$^{st}$.

Figure S1. Reintroduced Chinook salmon from 2007-2010 were assigned as parents to possible age-3 to -6 NOR adult offspring returning to the Cougar Trap. Return ages are indicated.

Figure 1.

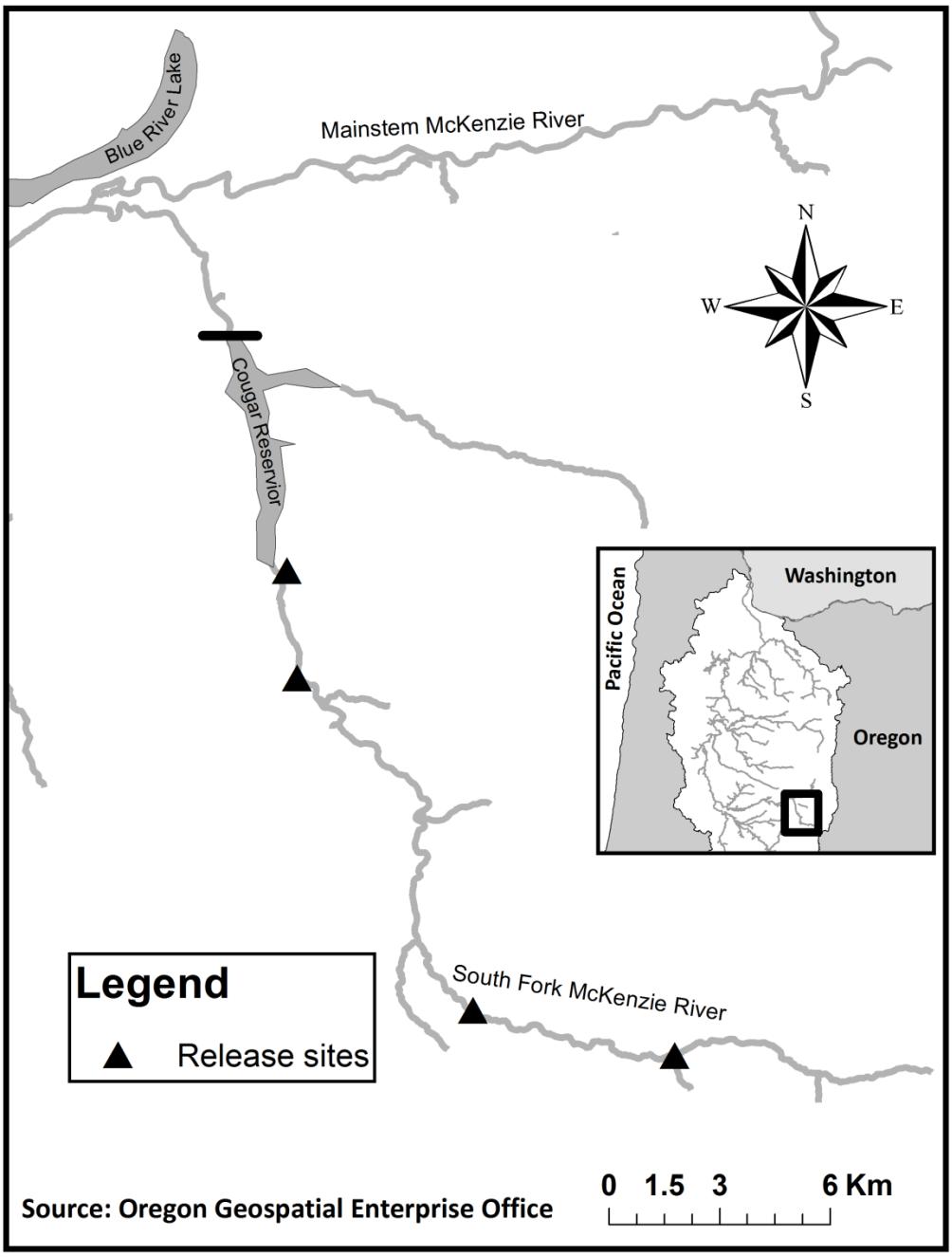
Blue River Lake
Mainstem McKenzie River
N
W
E
S
Cougar Reservior
Pacific Ocean
Washington
Oregon
Legend
Release sites
South Fork McKenzie River
Source: Oregon Geospatial Enterprise Office
0 1.5 3 6 Km

Figure 2.

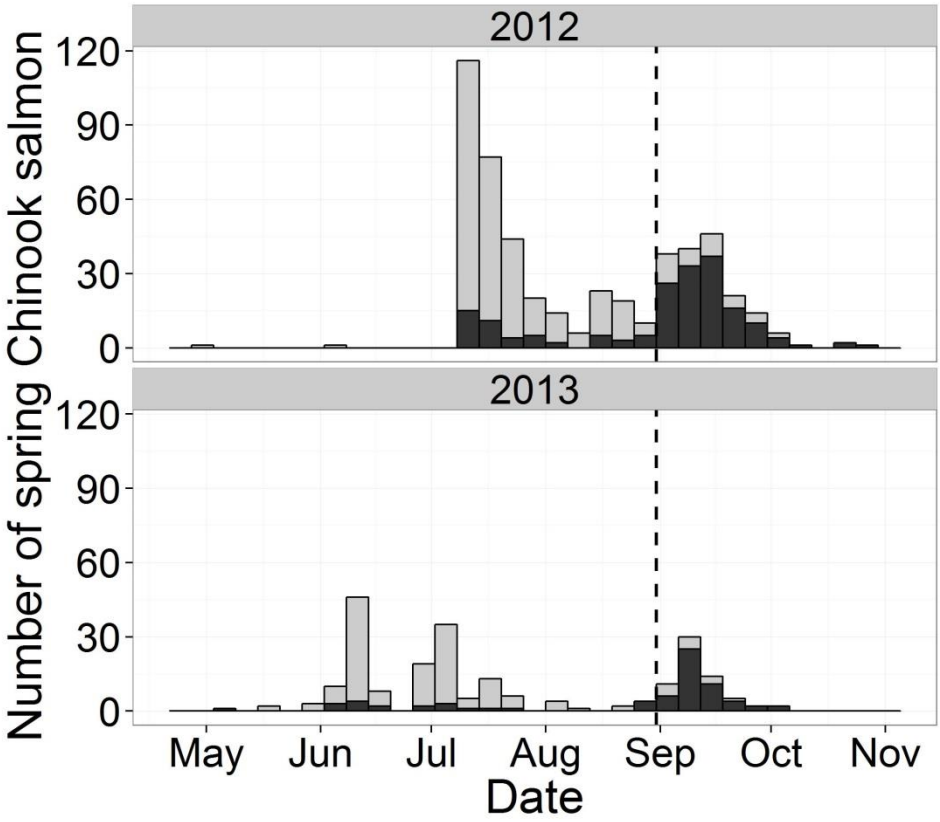
2012
2013
Number of spring Chinook salmon
120
90
60
30
0
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

Figure S1.

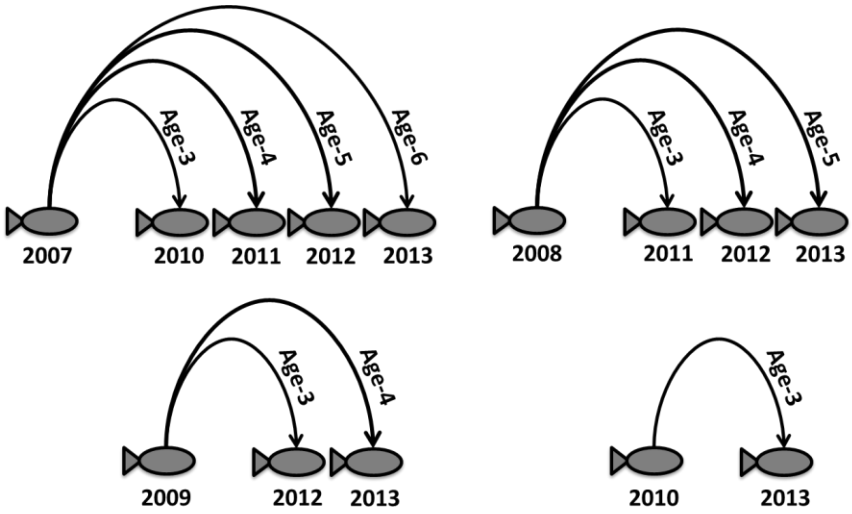
Age-3
Age-4
Age-5
Age-6
2007
2010
2011
2012
2013
Age-3
Age-4
Age-5
2008
2011
2012
2013
Age-3
Age-4
2009
2012
2013
Age-3
2010
2013

Table 1. Summary of the number of hatchery and natural origin Chinook salmon that were collected at the McKenzie Hatchery or the trap and released above Cougar Dam, 2007-2013. Hatchery origin fish that were collected at the trap and the sex ratio (male/female) of reintroduced Chinook salmon cohorts are indicated.

| Year | McKenzie Hatchery | Trap | | Total | Sex ratio |
|---|---|---|---|---|---|
| | | Hatchery | Natural | | |
| 2007 | 746 | 0 | 0 | 746 | 1.35 |
| 2008 | 873 | 0 | 0 | 873 | 2.03 |
| 2009 | 1347 | 0 | 0 | 1386 | 1.29 |
| 2010 | 497 | 30 | 221 | 748 | 1.81 |
| 2011 | 344 | 30 | 357 | 731 | 1.26 |
| 2012 | 430 | 17 | 501 | 948 | 1.16 |
| 2013 | 442 | 22 | 223 | 687 | 0.96 |

Table 2. Summary of the number of Chinook salmon sampled below Cougar Dam and the number that assigned to a mother only, father only or both parents in from 2011-2013.

| Year | Sampled | Genotyped | Assigned | | |
|---|---|---|---|---|---|
| | | | Mother Only | Father Only | Both Parents |
| 2011 | 60 | 45 | 1 | 0 | 0 |
| 2012 | 14 | 10 | 0 | 1 | 0 |
| 2013 | 8 | 5 | 0 | 0 | 0 |

Table 3. Summary of the total lifetime fitness variation observed in reintroduction years 2007 and 2008 for each sex. Number of males and females (N), percentage that assigned to adult offspring that returned back to the trap (Percent Assignment), their mean and standard deviation (SD).

| Year | Sex | N | Percent Assignment | Mean | SD |
|---|---|---|---|---|---|
| 2007 | Female | 318 | 0.39 | 0.88 | 1.52 |
| 2007 | Male | 428 | 0.30 | 0.70 | 1.56 |
| 2008 | Female | 288 | 0.38 | 0.81 | 1.46 |
| 2008 | Male | 585 | 0.20 | 0.39 | 1.03 |

Table 4. Summary of the predictors included in the final generalized linear mixed-effect model used to identify factors that significantly explained total lifetime fitness in 2007 and 2008, evaluated separately. Female was used as the reference for the predictor *sex*.

| Year | Predictors | Estimate | Std. Error | t value | Pr(>\|z\|) |
|---|---|---|---|---|---|
| 2007 | Release Date | -0.010 | 0.002 | -5.223 | < 0.001 |
| 2008 | Sex - Male | -0.815 | 0.169 | -4.831 | < 0.001 |

Table S1. Summary of the number of Chinook salmon (count) released at each location (river kilometer) and date (Julian date) for 2007 and 2008.

| Year | River kilometer | Julian date | Count |
|---|---|---|---|
| 2007 | 13 | 183 | 74 |
| 2007 | 17.2 | 179 | 70 |
| 2007 | 17.2 | 184 | 67 |
| 2007 | 17.2 | 199 | 47 |
| 2007 | 17.2 | 257 | 148 |
| 2007 | 17.2 | 276 | 69 |
| 2007 | 29.7 | 180 | 74 |
| 2007 | 29.7 | 199 | 74 |
| 2007 | 29.7 | 211 | 41 |
| 2007 | 29.7 | 276 | 82 |
| 2008 | 17.2 | 198 | 76 |
| 2008 | 17.2 | 206 | 79 |
| 2008 | 29.7 | 198 | 74 |
| 2008 | 29.7 | 200 | 77 |
| 2008 | 29.7 | 206 | 77 |
| 2008 | 29.7 | 217 | 40 |
| 2008 | 29.7 | 228 | 21 |
| 2008 | 29.7 | 235 | 63 |
| 2008 | 29.7 | 249 | 73 |
| 2008 | 29.7 | 255 | 89 |
| 2008 | 29.7 | 262 | 71 |
| 2008 | 29.7 | 280 | 74 |
| 2008 | 29.7 | 282 | 59 |

Table S2. Release location effects (by river kilometer (rkm)) after accounting for variation explained by other significant predictors for 2007 and 2008 reintroduced adults.

| Year | Predictor | Estimate | Std. Error | t value | Pr(>\|z\|) |
|---|---|---|---|---|---|
| 2007 | Date | -0.009 | 0.002 | -4.426 | < 0.001 |
| 2007 | Location: rkm13 | 0.421 | 0.236 | 1.779 | 0.075 |
| 2007 | Location: rkm 29.7 | 0.031 | 0.153 | 0.205 | 0.838 |
| 2008 | Location: rkm 29.7 | -0.458 | 0.374 | -1.225 | 0.220 |

Table S3. Statistics describing the genetic variation among reintroduced adult Chinook salmon: observed and expected heterozygosity ($H_O$ and $H_E$ respectively), and average number of alleles (K). In addition, statistics describing the power to resolve parent-offspring assignments correctly are represented for each parent-offspring dataset.

| Parent[a] | Offspring[a] | $H_O$ | $H_E$ | K | NE.1P[b] | NE.2P[b] | NE.PP[b] | EFP.0[c] | EFP.1[c] | EFP.2[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| 2007 | 2010 – 2013 | 0.92 | 0.92 | 38 | $2.33E^{-07}$ | $5.48E^{-10}$ | $6.85E^{-17}$ | 0.21 | 0.49 | 2.60 |
| 2008 | 2011 – 2013 | 0.92 | 0.92 | 38 | $2.22E^{-07}$ | $5.13E^{-10}$ | $6.27E^{-17}$ | 0.19 | 0.94 | 5.20 |
| 2009 | 2012 – 2013 | 0.91 | 0.93 | 38 | $2.29E^{-07}$ | $5.29E^{-10}$ | $6.47E^{-17}$ | 0.19 | 0.66 | 2.34 |
| 2010 | 2013 | 0.92 | 0.93 | 36 | $1.85E^{-07}$ | $4.21E^{-10}$ | $4.27E^{-17}$ | 0.02 | 0.02 | 0.21 |

[a] The Parent and Offspring columns denote the year each was sampled
[b] Non-exclusion probabilities for a single parent (NE.1P), a second parent (NE.2P), and a parent pair (NE.PP)
[c] Expected number of false parent-offspring pairs for zero (EFP.0), one (EFP.1), and two (EFP.2) genotypic mismatches

# Grandparentage assignments identify unexpected adfluvial life history tactic contributing offspring to a reintroduced population

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## Abstract

Diversity in life history tactics among individuals contributes to the persistence of a population because it helps to protect against stochastic environments by varying individuals in space and time. However, some life history tactics may not be accounted for when assessing the demographic viability of a population. One important factor in demographic viability assessments is cohort replacement rate (CRR), which is defined as the number of spawners produced by a spawner. Our objective was to determine if precocial males and adfluvial Chinook salmon (*Oncorhynchus tshawytscha*), adults that reside in freshwater their entire lives, contributed offspring to a reintroduced population from 2008-2013. Using grandparentage assignments we identified 31 precocial males and 48 probable adfluvial Chinook salmon produced by anadromous mate pairs from 2007-2012. Previously published CRR estimates for the 2007 and 2008 reintroduced adults, based on only anadromous returning adult offspring, were 0.40 and 0.31, respectively. By incorporating adfluvial adults we found CRR estimates increased by 17% (CRR: 0.46) and 13% (CRR: 0.35) for the 2007 or 2008 cohorts, respectively. As genetic monitoring practices become more common, the grandparentage resources we provide may help to determine the demographic importance of unaccounted or difficult to sample life histories.

## Introduction

Diverse portfolios in both economics and ecosystems help to increase 'additive returns' in stochastic environments (Figge, 2004, Koellner and Schmitz, 2006). Similarly, many plants and animals population have a better chance at persistence (Boer, 1968, Fox, 2005) if individuals display a diversity of life history tactics that vary them in space and time (e.g. dormancy and dispersal, Rubio de Casas *et al.*, 2015, Weaver *et al.*, 1996). Life history diversity may help populations established through reintroduction persist into the future (e.g. Greene *et al.*, 2009), however this topic has received little attention in the literature. Variation in anadromy and age-at-maturity among individuals within salmonid populations (e.g. precocial males, partial migration, and freshwater lake populations, Ricker, 1938, Rohde *et al.*, 2014, Taylor, 1989) offer an excellent opportunity to study how life history diversity contributes to the persistence of reintroduced populations because there are several programs currently on going throughout the Pacific Northwestern United States (Anderson *et al.*, 2014).

The persistence of a reintroduced population, in part, rests on its demographic and evolutionary viability through time (Anderson *et al.*, 2014, Robert *et al.*, 2015). One important factor when evaluating the demographic viability of a population is cohort replacement rate (CRR), defined as the number of spawners produced by a spawner (Botsford and Brittnacher, 1998), because estimates help determine if a population will persist into the future after controlling for immigration. Understanding how variation in life history tactics contribute to a reintroduced population can improve estimates of CRR by accounting for all forms of sexually mature adults. Here we test if life history diversity as expressed by unsampled parents that never migrated to the ocean contribute offspring to a

population of spring Chinook salmon (*Oncorhynchus tshawytscha*) from the upper Willamette River Evolutionary Significant Unit (ESU) (NMFS, 2005, NMFS, 2008), a group listed as threatened under the U.S. Endangered Species Act (ESA).

Oregon Department of Fish and Wildlife and the National Marine Fisheries Service have developed a recovery plan for Chinook salmon in the Willamette River basin (ODFW and NMFS, 2010). As part of this plan, anadromous Chinook salmon adults are being reintroduced above several dams in the Willamette River basin, including the high-head Cougar Dam, to provide access to historical spawning habitat. For the Cougar Dam system adults have been collected at a nearby hatchery and reintroduced to historical habitat above the dam via fish transportation trucks annually since 1996 (Zymonas *et al.*, 2010). In addition, natural origin Chinook salmon adults have also been collected at a trap and haul facility built at the base of Cougar Dam and reintroduced above the dam since 2010. This trap and transport reintroduction approach has enabled the near complete tissue sampling of all the potential anadromous adult parents, thereby allowing for estimates of CRR. Contrary to recently published studies for other Chinook and coho (*O. kisutch*) salmon reintroductions (Anderson *et al.*, 2015, Evans *et al.*, 2015), current CRR estimates suggest that the population above Cougar Dam is not replacing itself (Sard *et al.*, *In press*).

Excluding difficult to sample or unanticipated life histories limits our ability to have a more complete understanding of CRRs for reintroduced populations. Sard *et al.* (*In press*) used genetic parentage assignments to estimate CRR for the population established above Cougar Dam by the reintroduction program. This approach identified anadromous adult offspring returning in subsequent years; however it does not account for any adults that

sexually matured above Cougar Dam because these individuals were never encountered and therefore were not sampled.

There are two types of unsampled sexually mature adult Chinook salmon possible above Cougar Dam. First, some precocial males become sexually mature in freshwater at age-1 or age-2 and avoid ocean migration altogether (Taylor, 1989). Second, some male and female salmon may become sexually mature, presumably in the freshwater reservoir, and migrate upstream to spawn as adults older than age-2, hereafter adfluvial Chinook salmon. There is a paucity of information on adfluvial Chinook salmon in the literature outside the Great Lakes, however they have been recently studied in another reservoir-river system in the Willamette River basin (Romer and Monzyk, 2014). Perales *et al.* (2015) provided additional evidence that adfluvial Chinook salmon can survive and reproduce in several reservoir-river systems above dams in California, USA. These studies suggest that the existence of adfluvial Chinook salmon may be more common than once thought (Perales *et al.*, 2015). We sought to determine if adfluvial Chinook salmon were present, and if so, how much they increased estimates of CRR estimates for adults reintroduced above Cougar Dam.

Grandparentage assignment methods have recently arisen as a way to identify unsampled adults contributing to a population (Letcher and King, 2001). To our knowledge this approach has only been applied to salmonid populations, however grandparentage assignments could potentially be applied to any taxa that have genetic pedigrees assembled over multiple generations. Grandparentage assignment methods take advantage of that fact that grandparent pairs share one in four of their alleles at a given locus with their grandchildren, just as each parent shares one of its two alleles with its offspring (assuming diploid loci). Extending the exclusionary concept in parentage assignments to grandparent

pairs has brought insights into gene flow between resident and anadromous life histories (Christie *et al.*, 2011), and the spawning success of resident hatchery origin Chinook salmon (Ford *et al.*, 2015).

The Chinook salmon reintroduction above Cougar Dam has also been intensively sampled for age-0 juveniles produced and collected above the dam, which has informed managers on factors related to the spawning success of reintroduced adults (Sard *et al.*, 2015). Given this study system, one expectation is that most age-0 juveniles should assign to both parents because the offspring were collected above the dam and nearly all anadromous adults that could have produced them were sampled (see *Sample collection* below). However, as reported in Sard *et al.* (2015), among four genetic pedigrees only 79% ± 7% (mean ± s.d.) of juveniles assigned to both parents reintroduced from 2008-2011. There are a few explanations for this observation, and therefore it is important to test multiple working hypotheses to avoid false or biased conclusions (Chamberlin, 1965). Four hypotheses that may explain offspring with unassigned parents in each adult-juvenile genetic pedigree include: 1) genotyping error; 2) some anadromous adults lacked genotypes because of missing tissue samples or poor DNA quality; 3) incorrect sex identification of some anadromous adults that resulted in unintentional exclusion of a parent based on the Bayesian likelihood approach taken; and 4) the existence of unsampled parents residing above Cougar Dam. Here we systematically tested each of these hypotheses using a combination of parent assignment, grandparentage assignment, and simulation to determine the relative contribution of each hypothesis to the percent of juveniles that had one unassigned parent. Our findings suggest that precocial males and adfluvial Chinook salmon reside above the dam. We provide information on the inferred ages of sexually mature male

and female adfluvial Chinook salmon, the number spawning each year, as well as their spawning success with anadromous mates.

## Methods

### *Sample collection*

Since 1996, adult Chinook salmon have been reintroduced annually above the 158 meter tall Cougar Dam, which is located on the South Fork of the McKenzie River, Oregon, USA (Figure S1). Between 2007 and 2013 nearly all anadromous adults (99%, n = 6,115/6,119) reintroduced above the dam have been tissue sampled for subsequent DNA extraction (Table 1). The number of anadromous adults reintroduced above Cougar Dam each year ranged from 687 to 1,386 (Table 1). In addition, tissue was collected from a subsample of age-0 juvenile Chinook salmon captured in a screw trap above the dam from 2009 to 2014, of which 2,160 ± 335 were used for genetic analysis annually (Table S1). Each year the number of juveniles used for genetic analysis was proportional to the total number sampled in the screw trap each month (Sard *et al.*, 2015). We isolated DNA from adult and juvenile tissue samples using a protocol developed by Ivanova *et al.* (2006). We genotyped all samples at 11 microsatellite loci: *Ots201*, *Ots208b*, *Ots209*, *Ots211*, *Ots212*, *Ots215*, *Ots249*, *Ots253*, *Ots311*, *Ots409*, and *Ots515* (Banks *et al.*, 1999, Greig *et al.*, 2003, Naish and Park, 2002, Williamson *et al.*, 2002). Polymerase chain reaction products were visualized on an ABI 3730XL DNA analyzer and size scored using Genemapper software (Applied Biosystems, Inc., Foster City, CA). We estimated genotyping error rate by randomly sampling 1% of genotyped individuals and re-genotyping them at the 11 microsatellite loci described above. Genotyping error rate was calculated by dividing the number of discordant alleles scored by the total number of alleles scored. We calculated genotyping error rate for each genetic pedigree

separately so that estimates could be used in simulations (see *Hypothesis 1: Genotyping error*, Table S2).

Details regarding the genetic parentage assignment of reintroduced adults to returning anadromous adult offspring, as well as age-0 offspring collected above the dam have previously been published (See Chapter 1 and 2 for details). In short, adults were assigned to returning adult offspring (adult-adult genetic pedigree) using both CERVUS (Kalinowski *et al.*, 2007, Marshall *et al.*, 1998) and COLONY (Jones and Wang, 2010). Anadromous adults were assigned to age-0 juveniles (adult-juvenile genetic pedigrees) using SOLOMON (Christie *et al.*, 2013) because of uncertainty surrounding Hardy-Weinberg portions violations, which are typical when genotyping juveniles. All parent-offspring pairs were allowed to mismatch at no more than one locus. Among the 11 loci used, a high number of alleles (A = 34 ± 1) and high heterozygosity ($H_o$ = 0.92 ± 0.01) were observed annually, which in turn resulted in low non-exclusionary probabilities and low expected numbers of false parent-offspring assignments (see Sard *et al.*, *In press,* Sard *et al.*, 2015). Collectively, we had sufficient power to correctly resolve parent-offspring relationships, if present.

To further investigate why only 79% ± 7% of juveniles had both parents assign, we used methods described in Sard *et al.* (2015) to assemble two additional adult-juvenile genetic pedigrees with the 2012 and 2013 reintroduced anadromous adults and samples of age-0 offspring collected above the dam in 2013 (n = 2,792) and 2014 (n = 2,087), respectively (Table 1, Table S1). For each of the six adult-juvenile genetic pedigrees, we calculated the percent of juveniles with no parent assigned, unassigned mothers, unassigned fathers, and both parents assigned. We predicted that more juveniles would have unassigned

fathers compared to unassigned mothers if only precocial male Chinook salmon (< age-3) were present above Cougar Dam. Therefore, we tested if the median percent of offspring with unassigned mothers differed compared to those with unassigned fathers using a Wilcoxon rank-sum test. Throughout this manuscript we focus our analysis on juvenile offspring with one unassigned parent because it enabled us to compare results from the genotyping error, missing anadromous adult genotypes, and incorrect sex identification of anadromous adults hypotheses with the fourth hypothesis, missing unsampled parents above the dam, which were identified by grandparentage assignments with one parent known (see Hypothesis 4: Unsampled adults above the dam and Christie *et al.*, 2011).

## *Hypothesis 1: Genotyping error*

We estimated the percent of juveniles with one unassigned parent due to genotyping error using simulations. First, we used SOLOMON (Christie *et al.*, 2013) to simulate microsatellite genotypes for parents and offspring using allele frequencies estimated from our study population. The number of parents, as well the genotyping error rate used for each simulation were specific to each adult-juvenile pedigree we assembled, since each parameter varied annually (Table 1, Table S2). SOLOMON uses a uniform reproductive success distribution, which prevented us in simulating the exact number of offspring used in each adult-juvenile genetic pedigree. We chose a number of offspring produced by each parent that resulted in a simulated number of offspring genotypes as close as possible to that used in each of the actual genetic pedigree (Table S1). However, our choice of the number of offspring produced by each parent did not substantially change our estimates for the percent of offspring with one unassigned parent explained by genotyping error (unpublished data). We assigned simulated parents to offspring with SOLOMON's no

known parent Bayesian likelihood approach using methods described in Sard *et al.* (2015). For each simulated genetic pedigree we calculated the expected percent of offspring missing one parent due to genotyping error alone. We tested if the observed median percent of juveniles with one unassigned parent differed to that expected by genotyping error using a Wilcoxon rank-sum test to determine if Hypothesis 1 alone could explain why offspring had one unassigned parent.

## *Hypothesis 2: Missing anadromous parents*

We used the same approach in Hypothesis 1 to test Hypothesis 2, except that we randomly removed a number of known simulated adult samples before making parent-offspring assignments and assembling each simulated adult-juvenile genetic pedigree instead of incorporating genotyping error. The numbers of females (2 ± 2) and males (3 ± 4) removed were based on the number of known anadromous adults missing a genotype at more than one locus, as well as the number of adults missing a tissue sample (Table 1). We randomly assigned a sex to the adults that lacked tissue samples (n=3) because we were unable to genotypically sex the fish and lacked their phenotypic sex, based on secondary sexual characteristics (Table 1). Following each of the six simulations, one for each adult-juvenile genetic pedigree, we calculated the expected percent of juveniles with one unassigned parent due to missing anadromous parent genotypes each year. We tested if the observed median percent of juveniles with one unassigned parent differed to that expected by missing anadromous parent genotypes using a Wilcoxon rank-sum test to determine if Hypothesis 2 alone could explain why offspring had one unassigned parent.

## *Hypothesis 3: Incorrect sex identification*

With the genetic pedigree data we had, we tested if some adults were incorrectly sexed using the exclusion one-parent known option in SOLOMON. Here we identified all offspring with one parent assigned and evaluated them as a known parent-offspring pair. We attempted to assign each parent-offspring pair to all adults that were the same sex as the known parent. We included only adults that did not assign to other juveniles in the genetic pedigree that year for two reasons. First, we had strong evidence that most of the sex designations we have made were correct because both Banks *et al.* (2014) and Brunelli *et al.* (2008) found high concordance between genotypic and phenotypic sex in this system (90% and 100%, respectively). It is possible that both genotyping error and the lack of secondary sexual phenotypes among early returning adults caused some of the discordance observed in Banks *et al.* (2014). Regardless, adults that had assigned to one or more offspring likely had the correct sex designation. Secondly, we wanted to limit the number of pairwise comparisons made, thereby limiting the chance of false assignment. We note that the one-parent known option in SOLOMON requires that all parent-offspring must match at all loci and there must be no missing data. As a result, we could not include 32 ± 25 parent-offspring assignments for this analysis (Table 2). We accepted assignments that matched at all loci and consider these assignments as evidence that we incorrectly identified the sex of the assigned parent. We calculated the percent of offspring explained by incorrect sex identification by dividing the number of assignments made by the total number of offspring used in each adult-juvenile pedigree. We tested if the observed median percent of juveniles with one unassigned parent differed to that expected by incorrect sex identification using a Wilcoxon rank-sum test to determine if Hypothesis 3 alone could explain why offspring had one unassigned parent.

## *Hypothesis 4: Unsampled parents above the dam*

We identified 218 (55 ± 80, annually) and 2,616 (435 ± 145, annually) unique mate pairs present in the adult-adult and adult-juvenile genetic pedigrees, respectively (Table S3). We used these mate pairs as our grandparent pairs because we have strong genetic evidence that these individuals mated in the wild based on low non-exclusionary parent pair ($9.8 \times 10^{-17} \pm 3.9 \times 10^{-17}$) probabilities (see Sard *et al.*, *In press,* Sard *et al.*, 2015). Our approach reduced the chance of false-positive grandparent pair-grandoffspring assignments by limiting pairwise comparisons to presumed grandparent pairs for which there was no evidence for their existence. We calculated the expected number of false grandparent-grandoffspring trios using the genotypes of grandparent pairs and potential grandoffspring with either an unassigned mother or father in each of our six adult-juvenile genetic pedigrees (Christie *et al.*, 2011). We focused our analysis on grandoffspring that had one parent assigned because it reduced false-positive assignments by excluding alleles in the offspring explained by the assigned anadromous parent (Christie *et al.*, 2011). We initially accepted grandparent pair-grandoffspring trios (Gtrios) that matched at all 11 loci. We further tested this hypothesis by genotyping all putative Gtrios identified at an additional four loci: *Ogo2, Ogo4, Ssa408, OtsG474 (Cairney et al., 2000, Olsen et al., 1998, Williamson et al., 2002)*. We accepted Gtrios that matched at all 15 loci for further analyses. We tested if the observed median percent of juveniles with one unassigned parent differed from that explained by unsampled Chinook salmon identified by grandparentage assignments using a Wilcoxon rank-sum test to determine if Hypothesis 4 alone could explain why offspring had one unassigned parent.

We conservatively assumed each unique grandparent pair that assigned to at least one grandoffspring only produced one unsampled adult. This assumption enabled us to

provide estimates for the number of unsampled adults produced by anadromous mate pairs that contributed offspring to the population each year, as well as the number of unsampled male and females that were successful at spawning from age-1 to age-6. We tested for differences in age at reproduction among the unsampled males and females identified by grandparentage assignments using a Wilcoxon rank-sum test. Any unsampled females identified using grandparent pairs that mated in 2007 or 2008 were incorporated into female CRR estimates for each cohort. All analyses were conducted in R version 3.1.1 (R Core Team, 2014). All p-values associated with testing why offspring had one unassigned parent were false discovery rate corrected (Benjamini and Hochberg, 1995). We provide three resources for use in future grandparentage studies, which include two R scripts (modified from SOLOMON, Christie *et al.*, 2013) to simulate the expected number of offspring with unassigned parents due to genotyping error and missing anadromous parents, as well as an R script (created by NMS) to identify Gtrios after accounting for one parent's genotypes on Github (https://github.com/nicksard/grandparentage).

## Results

### *Genetic pedigrees*

We found that 15% of the offspring in the 2012-2013 adult-juvenile genetic pedigree had one unassigned parent, which was comparable to our four previously published adult-juvenile genetic pedigrees (range: 13% - 26%). However, we observed the lowest percent of unassigned parents in the 2013-2014 genetic pedigree (8%, Figure 1). Overall, 18% ± 7% of offspring had one unassigned parent among the adult-juvenile genetic pedigrees assembled. We found the percent of offspring with an unassigned father (6% ± 3%) compared to those with an unassigned mother (11% ± 7%) did not differ statistically ($V = 28$, $p = 0.132$).

## *Hypothesis testing*

Hypotheses associated with human error could not explain all the offspring with one unassigned parent (Figure 2). We found that the estimated percent of offspring with one unassigned parent due to genotyping error (7% ± 6%, Table S2), missing anadromous parent genotypes (1% ± 1%, Table S2), and incorrect sex identification (0.3% ± 0.3%, Table S2) differed significantly to that observed in our adult-juvenile genetic pedigrees (W = 33, p = 0.02; W = 36, p = 0.01; W = 36, p = 0.01, respectively). Genotyping error accounted for most of the unassigned parents in our six genetic pedigrees (39% ± 22%), whereas missing anadromous parents (5% ± 5%) and incorrect sex identification (2% ± 1%) accounted for few unassigned parents (Figure 2, Table S2). We still found evidence that some offspring remained unexplained (9% ± 5%, Table S2 ) after testing hypotheses 1-3 in aggregate (W = 31, p = 0.049).

We found genetic evidence that 2% ± 2% of the offspring with one unassigned parent were due to existence of precocial males and adfluvial Chinook salmon residing above Cougar Dam (Table 3). A total of 244 grandoffspring were assigned to grandparent pairs based on the original 11 loci. After incorporating genotypes at an additional four loci (A = 16 ± 5 and $H_o$ = 0.83 ± 0.07), a total of 227 Gtrios matched at all 15 loci (Table 3). The percent of Gtrios that identified unsampled males versus females were similar after including the additional loci (41% and 59%, respectively, Table 3). Most (15/17) of the exclusions were observed because we were unable to successfully genotype at least one individual involved in the Gtrio at the additional loci. Our difficulty in amplifying the additional loci for these individuals was likely due to poor DNA quality in the tissue samples because we were unable to amplify the loci even after attempting to re-extract DNA from the original samples. In

addition, there were 18 juvenile offspring that assigned to two (n = 16) or three (n = 2) grandparent pairs. We excluded these assignments from all analyses because we were unable to identify a single grandparent pair after including additional loci.

We observed no Gtrios in several of the 21 possible grandparent pair-grandoffspring year class comparisons made for juveniles with unassigned fathers (10/21, 48%) and unassigned females (16/21, 76%, Table 3). For example, we did not identify a single Gtrio assignment when we compared the 509 grandparent pairs identified in 2008 to the 115 offspring with unassigned fathers in the 2009-2010 adult-juvenile genetic pedigree, which is significant because there were more than 50,000 pairwise comparisons of genotypes made. Initially we found three Gtrios for offspring with unassigned mothers that would have been younger than age-3, however after further inspection, these assignments were due genotyping error at the sex linked marker *Oty3* and therefore they were unassigned fathers (Table 3). For comparisons where we did observe at least one Gtrio, we found most (69%, 11/16) had more Gtrios observed than were expected by chance (Table 3). The percent of offspring explained by the missing adfluvial adults hypothesis differed to that of the observed data after Gtrios were genotyped at all 15 loci (W = 36, p = 0.01). We found the percent of offspring with one unassigned parent observed did not statistically differ from that expected when all four hypotheses were combined (W = 30, 0.065). However, 7% ± 4% of offspring with one unassigned parent remained unexplained after accounting for all four hypotheses.

We found 79 unsampled sexually mature Chinook salmon spawned from 2008-2013 (Table 4). In total, we identified 31 precocial males (age-1 and age-2), as well as 48 male and female adfluvial Chinook salmon that reproduced at age-4 or age-5. Unsampled males (median = 2) were younger than unsampled females (median = 4) identified by

grandparentage assignment ($W = 1339$, $p < 0.001$). The number of unsampled Chinook salmon adults identified in each adult-juvenile pedigree ranged from 1 to 34 annually (Table 4). The number of juveniles that assigned to a single grandparent pair ranged from 1-27, though this variation was in part due to the number of offspring used in assignments. We found that 34 unique grandparent pairs in 2007 produced at least one unsampled parent above the dam; whereas in 2008, 25 unique grandparent pairs were observed (Table 4). The inclusion of the adfluvial female Chinook salmon increased the CRR in 2007 from 0.40 to 0.46 (17%). Similarly, the CRR estimate for 2008 (0.31) increased by 13% (CRR = 0.35).

## Discussion

We tested four hypotheses to explain unassigned parents in our genetic pedigrees using a combination of parentage assignment, grandparentage assignment, and simulations. Our approach systematically quantified various sources of human error and found that after accounting for all of these errors some unassigned parents remained unexplained. As a final hypothesis we tested for the existence of precocial males and adfluvial Chinook salmon. We provide genetic evidence that unsampled adults resided above Cougar Dam and contribute offspring to the population. The probable existence of these adfluvial adults was corroborated by genotyping Gtrios at an additional four loci (15 total), and for some cases, unsampled parents are supported by several offspring assignments (up to 27). The incorporation of non-anadromous life history tactics only marginally increased CRR estimates. We contribute to the growing body of evidence that adfluvial male and female Chinook salmon can survive above existing dams for their entire lives and provide important insights into how many are actively spawning each year and at what age, as well as their spawning success with anadromous mates. Given our data and other recently published data

from Romer and Monzyk (2014) and Perales *et al.* (2015), we think it is possible that adfluvial Chinook salmon may contribute to other Chinook salmon reintroduction programs.

Salmon fisheries established in the Great Lakes in the 1900s demonstrate that male and female Chinook salmon can survive in freshwater environments for their entire lives (Emery, 1985). However, the expression of adfluvial Chinook salmon life history in smaller reservoirs has been doubted because of assumed limited productivity compared to that in the Great Lakes. Despite this restraint, recent reports document adfluvial Chinook salmon residing in reservoirs that ranged from 0.56 $km^3$ to 19.5 $km^3$ in total water capacity (Perales *et al.*, 2015 and references within each, see Romer and Monzyk, 2014). The reservoir created by Cougar Dam considered here is the smallest reservoir (0.27 $km^3$) in which evidence for adfluvial Chinook salmon has emerged thus far. Perhaps it is therefore not surprising that the actual number of adfluvial Chinook salmon we provide evidence for is small. We reiterate calls from both Romer and Monzyk (2014) and Perales *et al.* (2015) for more research on adfluvial Chinook salmon biology, because additional information on this life history could improve our understanding of the role these fish may have in reintroductions of Chinook salmon above dams.

We provide important information on the biology of the adfluvial Chinook salmon that collectively contributes toward improving our understanding of this rare life history. We found evidence that unsampled males contributing to the population were significantly younger than females (Table 4). The Chinook salmon male precocial life history is well known, and it is therefore not surprising that we observed several age-2 unsampled males successfully reproduced. In addition, we did not observe any age-1 to -3 adfluvial female Chinook salmon in our grandparentage assignments, which is consistent with laboratory

experiments that suggest females cannot mature prior to age-3 (Taylor, 1989). The lack of assignments identifying adfluvial females younger than age-4 also suggests we have a low false positive assignment rate. We did not observe any age-3 adfluvial Chinook salmon, however few adults typically spawn at that age within the basin (Johnson and Friesen, 2010). Unlike Romer and Monzyk (2014), we did not observe any age-6 adults. Complete age and reproductive success distributions for male and female adfluvial Chinook salmon in this system remain unknown because we did not have knowledge of all the mate pairs that occurred above the dam, we did not we sample every single juvenile produced in the system, and we were unable to identify any production by potential adfluvial-adfluvial grandparent pairs. This lack of information may help to explain why 7% ± 4% of offspring in adult-juvenile pedigrees with one unassigned parent remained unexplained after testing all four hypotheses. Regardless, our genetic evidence that both adfluvial male and female Chinook salmon were successful at contributing to offspring improves our assessment of productivity for the reintroduced population above Cougar Dam.

We found that non-anadromous life history tactics slightly increase estimates of CRR, assuming each unique grandparent parent pair that assigned to grandoffspring only produced one unsampled adult. The observed increases in CRR were not enough to meet replacement for either the 2007 or 2008 cohorts. Even with the incorporation of adfluvial Chinook salmon offspring, CRR for adults reintroduced above Cougar Dam are low compared to other recently published studies (Anderson *et al.*, 2015, Evans *et al.*, 2015). Although we note that, just as in parentage assignments, grandparentage methods are limited by genotyping error and further exacerbated by our conservative exclusion criterion, which could negatively bias our estimates. Our estimates for adfluvial Chinook salmon could also be

negatively biased because our grandparentage assignments do not account for any unsampled adults that were not successful at reproduction and there may have been other grandparent pairs that we did not include in our analysis, as noted above. Alternatively, our estimates of adfluvial Chinook salmon may be positively biased because some of the unique Gtrios identified may be incorrect based on the expected number of false Gtrios we calculated (Table 3). We think that our results are more likely negatively biased because we would expect many Gtrios assignments for unsampled mothers younger than age-3 if we had high false positive assignment rates, and this is not the case in our study. Despite these limitations with grandparentage assignment, our work is a first step towards understanding the degree adfluvial adults contribute to the demographic viability of Chinook salmon populations created by reintroduction programs.

Our results are a testament to the remarkable life history diversity in salmonids because all the unsampled adults we identified were produced by anadromous mate pairs. Considering that anadromy is a heritable trait (Thériault *et al.*, 2007, Thrower *et al.*, 2004), it is notable that we identified age-4 and age-5 male and female adfluvial Chinook salmon because one would expect adults would have migrated to the ocean rather than matured above a dam. Both phenotypic plasticity and/or selection could give rise to adfluvial Chinook salmon. Future research on how adfluvial Chinook salmon exist in reservoirs may help elucidate the interplay between these two important evolutionary concepts.

Our approaches for testing hypotheses to explain unassigned parents are subject to some biases. SOLOMON only simulates uniform reproductive success distributions for parents, which does not realistically reflect Chinook salmon biology. The uniform reproductive success distribution could potentially bias estimation of the percent of offspring

explained by genotyping error. This bias may be positive or negative depending on if genotyping errors occurred in unsuccessful or highly fit adults, respectively; whereas genotyping error that occurred in an offspring are limited to that assignment. In addition, the current model for genotyping error in SOLOMON is random, rather than locus specific, which may also bias assignment rates. The direction of the bias likely depends on variation among locus specific genotyping error rates. Furthermore, our estimates of the percent of unassigned parents explained by incorrect sex identification of adults is also biased low because some parent-offspring pairs, as well as putative incorrectly sexed adults were excluded from the analyses due to the stringent requirements for the one-parent known option in SOLOMON (Table 2). Our overall conclusions would not likely change with their inclusion because the excluded assignments represent a low proportion of the total number that could have been included in the analysis (Table 2). Collectively, our methods for testing the hypotheses may be negatively biased because 7% ± 4% of offspring in the adult-juvenile genetics pedigrees remain unexplained. However, our conclusions regarding the existence of the adfluvial life history is not affected by the bias because we often observed more Gtrios than expected by chance (Table 3).

Our research contributes grandparentage resources (i.e. R scripts on Github) that can be used to determine if unsampled life history tactics contribute to any population with genetic pedigrees assembled over multiple generations. Our approach complements currently available grandparentage methods (Christie *et al.*, 2011) by offering a script that accounts for one parent known before making grandparentage assignments. The resources we provide can help researchers assess the quality of genetic pedigrees, as well as identify

contributions from unsampled parents. Rare or difficult to sample life history tactics may help to buffer other reintroduced populations against stochastic environments.

## Acknowledgments

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## Figure Legends

Figure 1. Percent of offspring that had both parents assigned, an unassigned mother or father, or no parent assigned from 2008-2013.

Figure 2. Summary of the percent of juvenile offspring with one parent unassigned explained by our four hypotheses.

Figure S1. Cougar Dam, indicated by the solid black horizontal line, is located on the South Fork of the McKenzie River. Juveniles were collected in a screw trap located above the dam.

Figure 1.

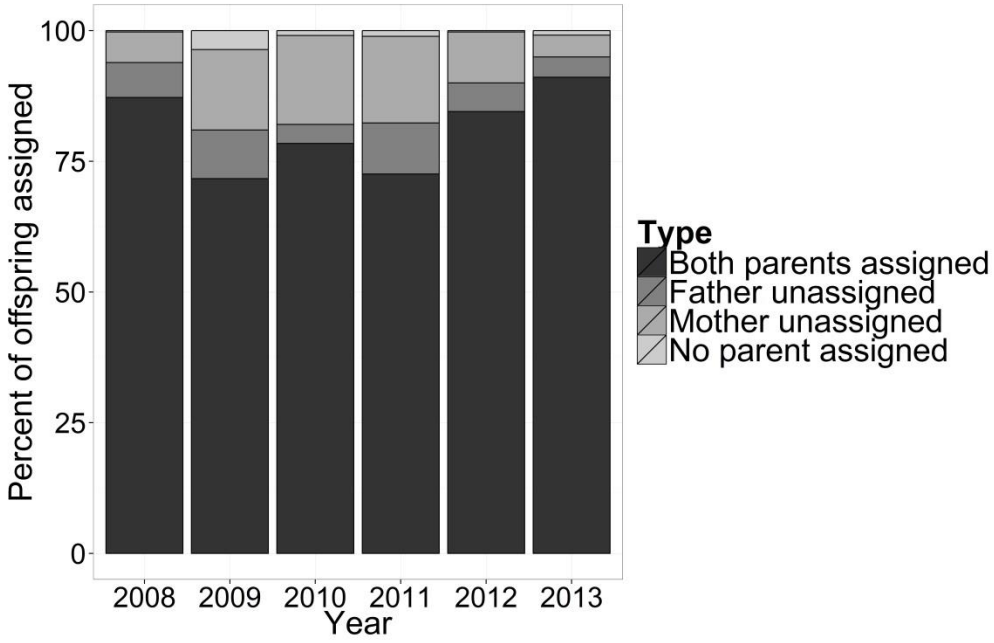
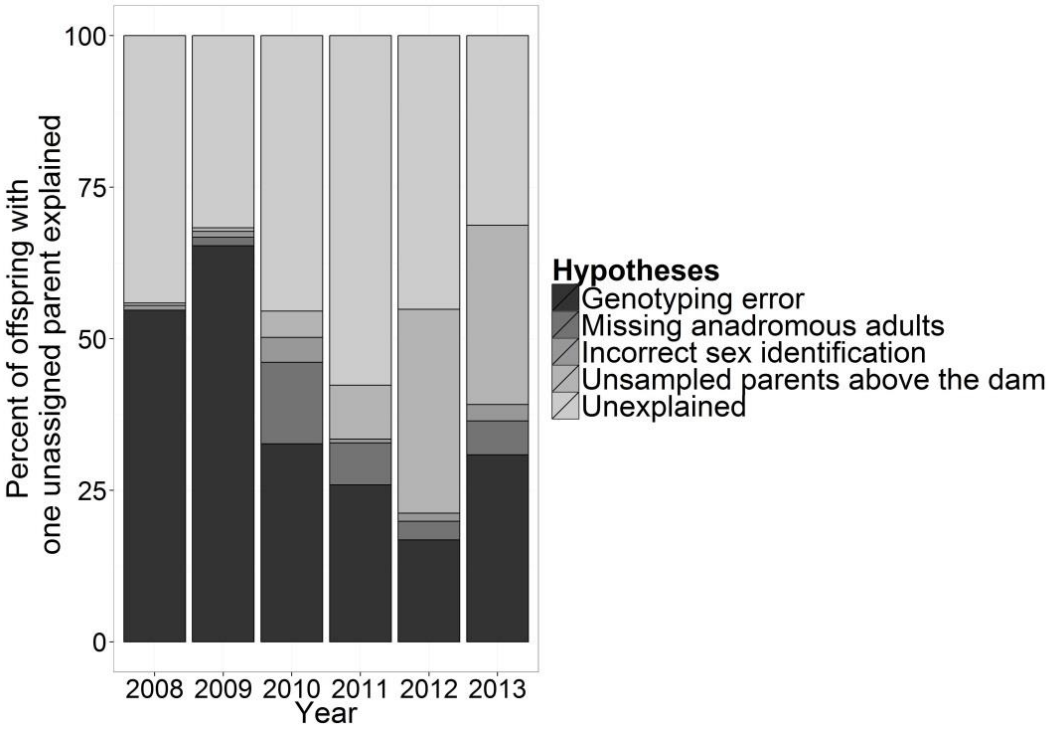

100
75
50
25
0
Percent of offspring assigned
2008
2009
2010
2011
2012
2013
Year
Type
Both parents assigned
Father unassigned
Mother unassigned
No parent assigned

Figure 2.


100
75
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Percent of offspring with one unassigned parent explained
2008
2009
2010
2011
2012
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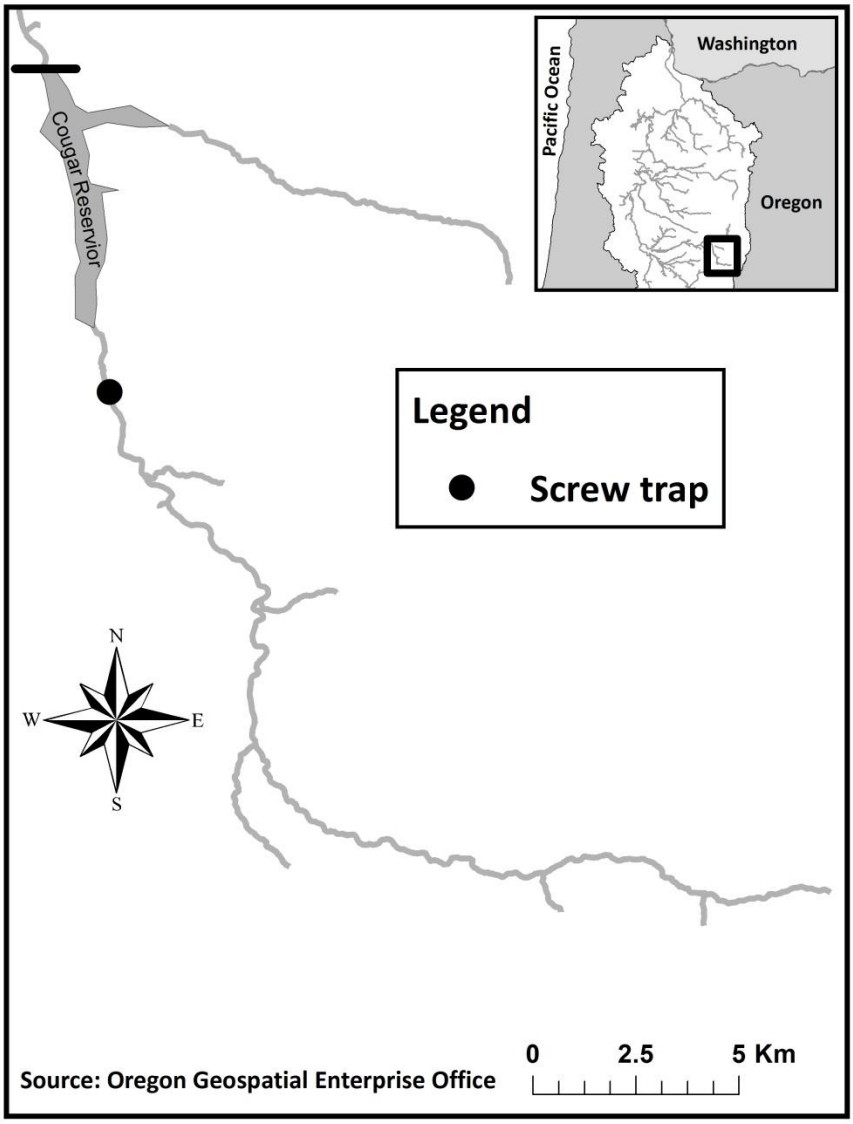
Year
Hypotheses
Genotyping error
Missing anadromous adults
Incorrect sex identification
Unsampled parents above the dam
Unexplained

Figure S1.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Screw trap
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Source: Oregon Geospatial Enterprise Office
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2.5
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Table 1. Summary of the number of anadromous adults reintroduced above the dam, number of missing tissue samples, and the number of individuals missing more than one genotype are described for each sex from 2007-2013.

| Year | Sex | N | Missing | |
|---|---|---|---|---|
| | | | Tissue | >1 GT |
| 2007 | Female | 318 | 0 | 0 |
| | Male | 428 | 0 | 3 |
| 2008 | Female | 288 | 0 | 0 |
| | Male | 585 | 0 | 0 |
| 2009 | Female | 604 | 0 | 3 |
| | Male | 782 | 1 | 10 |
| 2010 | Female | 264 | 0 | 4 |
| | Male | 484 | 1[a] | 6 |
| 2011 | Female | 324 | 1[a] | 5 |
| | Male | 407 | 0 | 1 |
| 2012 | Female | 439 | 0 | 1 |
| | Male | 509 | 0 | 1 |
| 2013 | Female | 337 | 0 | 2 |
| | Male | 350 | 1[a] | 0 |

[a] Adults were randomly assigned a sex because we lacked their tissue sample.

Table 2. Number of parent-offspring pairs included/excluded for offspring only assigned to a father or mother for each adult-juvenile genetic pedigree, as well as the number of assignments explained by incorrect sex identification from 2008-2013.

| Year | Included | | Excluded | | Assignments | |
|---|---|---|---|---|---|---|
| | Father Only | Mother Only | Father Only | Mother Only | Father Only | Mother Only |
| 2008 | 117 | 134 | 0 | 0 | 2 | 0 |
| 2009 | 193 | 109 | 88 | 61 | 5 | 0 |
| 2010 | 319 | 57 | 27 | 17 | 18 | 0 |
| 2011 | 319 | 188 | 49 | 29 | 1 | 3 |
| 2012 | 301 | 131 | 26 | 44 | 7 | 0 |
| 2013 | 71 | 57 | 16 | 24 | 5 | 0 |

Table 3. Summary of the grandparent-grandoffspring assignments (Gtrios) in the adult-juvenile genetic pedigrees. The expected (Exp.) number of false Gtrios, as well as the number of observed (Obs.) that shared alleles at 11 and 15 loci are described. We also include the unsampled parent age based on the year the grandparent pair mated (GP year) and the year the one anadromous adult assigned to the age-0 juvenile was reintroduced.

| Parent year | GP Year | Unsampled parent age | Unassigned father | | | Unassigned mother | | |
|---|---|---|---|---|---|---|---|---|
| | | | Exp. False Gtrios | Obs. Gtrios | | Exp. False GTrios | Obs. Gtrios | |
| | | | | 11 loci | 15 loci | | 11 loci | 15 loci |
| 2008 | 2007 | 1 | 4 | 1 | 1 | 4 | 0 | 0 |
| 2009 | 2007 | 2 | 4 | 3 | 2[a] | 4 | 0 | 0 |
| 2009 | 2008 | 1 | 9 | 0 | 0 | 13 | 0 | 0 |
| 2010 | 2007 | 3 | 2 | 0 | 0 | 10 | 0 | 0 |
| 2010 | 2008 | 2 | 4 | 17 | 17 | 20 | 0 | 0 |
| 2010 | 2009 | 1 | 3 | 2 | 2 | 18 | 0 | 0 |
| 2011 | 2007 | 4 | 6 | 9 | 9 | 9 | 27 | 25[a] |
| 2011 | 2008 | 3 | 12 | 0 | 0 | 18 | 0 | 0 |
| 2011 | 2009 | 2 | 11 | 12 | 9[ab] | 19 | 0 | 0 |
| 2011 | 2010 | 1 | 7 | 0 | 0 | 11 | 0 | 0 |
| 2012 | 2007 | 5 | 4 | 7 | 6[a] | 8 | 46 | 45 |
| 2012 | 2008 | 4 | 10 | 22 | 22 | 18 | 37 | 36[a] |
| 2012 | 2009 | 3 | 9 | 0 | 0 | 17 | 0 | 0 |
| 2012 | 2010 | 2 | 5 | 8 | 8[b] | 9 | 0 | 0 |
| 2012 | 2011 | 1 | 5 | 0 | 0 | 10 | 0 | 0 |
| 2013 | 2007 | 6 | 1 | 0 | 0 | 2 | 0 | 0 |
| 2013 | 2008 | 5 | 3 | 0 | 0 | 4 | 2 | 2 |
| 2013 | 2009 | 4 | 3 | 1 | 1 | 3 | 28 | 27[a] |
| 2013 | 2010 | 3 | 2 | 0 | 0 | 2 | 0 | 0 |
| 2013 | 2011 | 2 | 1 | 22 | 15[a] | 2 | 0 | 0 |
| 2013 | 2012 | 1 | 3 | 0 | 0 | 3 | 0 | 0 |

[a] Some Gtrios were excluded because we could not amplify at least on individual in the assignment at the additional loci
[b] Four Gtrios were moved from the unassigned mother to the unassigned father category after correcting for genotyping errors

Table 4. Summary of the number of unsampled Chinook salmon by age and sex identified by grandparentage assignments. Fish that had not reached an age to be sampled are indicated with *NA*.

| Year produced | Sex | Age | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| 2007 | Female | 0 | 0 | 0 | 8 | 13 | 0 | 21 |
| | Male | 1 | 2 | 0 | 7 | 3 | 0 | 13 |
| 2008 | Female | 0 | 0 | 0 | 11 | 1 | NA | 12 |
| | Male | 0 | 10 | 0 | 3 | 0 | NA | 13 |
| 2009 | Female | 0 | 0 | 0 | 1 | NA | NA | 1 |
| | Male | 1 | 4 | 0 | 1 | NA | NA | 6 |
| 2010 | Female | 0 | 0 | 0 | NA | NA | NA | 0 |
| | Male | 0 | 4 | 0 | NA | NA | NA | 4 |
| 2011 | Female | 0 | 0 | NA | NA | NA | NA | 0 |
| | Male | 0 | 9 | NA | NA | NA | NA | 9 |
| 2012 | Female | 0 | NA | NA | NA | NA | NA | 0 |
| | Male | 0 | NA | NA | NA | NA | NA | 0 |

Table S1. Summary of the number of juveniles collected in the screw trap, tissue sampled, and genotyped from 2009-2013.

| Year | Screw trap | Tissue sampled | Genotyped |
|---|---|---|---|
| 2009 | 11024 | 5916 | 2000 |
| 2010 | 28150 | 7961 | 1826 |
| 2011 | 4439 | 4141 | 2035 |
| 2012 | 6754 | 4995 | 2220 |
| 2013 | 20212 | 8485 | 2792 |
| 2014 | 11438 | 5878 | 2087 |

Table S2: Summary of the number of juveniles used in the adult-juvenile genetic pedigrees from 2008-2013, genotyping error rate, the percent of offspring with one unassigned parent, the percent of offspring with one unassigned parent explained by four hypotheses (1) genotyping error, (2) missing anadromous adults, (3) incorrect sex identification of adults, and (4) missing adfluvial adults.

| Parent year | Genotyping error (%) | Percent offspring with one unassigned parent | Hypotheses (% explained) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | Total |
| 2008 | 2 | 12.5 | 7.2 | 0.0 | 0.1 | 0.0 | 7.3 |
| 2009 | 4 | 24.7 | 19 | 0.4 | 0.3 | 0.2 | 19.3 |
| 2010 | 2 | 20.6 | 6.8 | 2.9 | 0.9 | 0.9 | 11.5 |
| 2011 | 2 | 26.4 | 6.6 | 1.9 | 0.2 | 2.4 | 11.1 |
| 2012 | 1 | 15.2 | 2.8 | 0.5 | 0.2 | 5.5 | 9.0 |
| 2013 | 1 | 8.0 | 2.1 | 0.5 | 0.2 | 2.6 | 5.5 |

Table S3. Summary of the number of mate pairs identified in adult-adult and adult-juvenile genetic pedigrees assembled from 2007-2013.

| Year | Adult-Juvenile | Adult-Adult |
|---|---|---|
| 2007 | 0 | 173 |
| 2008 | 476 | 33 |
| 2009 | 576 | 11 |
| 2010 | 258 | 1 |
| 2011 | 300 | 0 |
| 2012 | 615 | 0 |
| 2013 | 391 | 0 |

# Demographic and genetic considerations for a Chinook salmon reintroduction program

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## Abstract

Like other populations, the persistence of those established through reintroduction rest on the interplay between demographic and genetic factors over time. When reintroduced populations are established demographic parameters are typically monitored first, and in subsequent generations, genetic factors are evaluated to assess the adaptive potential of the population. However, rare genetic variation within the founding cohorts may be important to establishing a population. Here we evaluated both a measure of population productivity, defined as the number of adults produced by an adult, as well as genetic variation within a genetic pedigreed population of spring Chinook salmon (*Oncorhynchus tshawytscha*) a single generation after initial reintroduction. Productivity estimates indicated that cohorts were not replacing themselves over time (range: 0.08 - 0.43). We estimated that the population will become locally extinct within 3 ± 1 generations without continued supplementation by captively bred adults. However, estimates of effective population size were relatively high (range: 344 to 893), and 84% (253/300) of alleles were maintained in the next generation. Skewed sex ratios and variance in fitness among reintroduced adults both likely contributed to the reduction in genetic variation observed. In subsequent generations, individuals within the population may adapt to the above-dam reintroduction site thereby enabling its persistence past our estimate of 3 ± 1 generations. However, the thoughtful use of captively bred adults will likely be required to maintain genetic variation that may be important to future adaption.

## Introduction

Reintroductions attempt to restore imperiled species back into historical habitat in an effort to counteract the negative effects associated with habitat loss (IUCN/SSC, 2013, Wilcove *et al.*, 1998). Many reintroductions that occurred over the past century have failed, indicating we poorly understand factors important to success (Fischer and Lindenmayer, 2000, Griffith *et al.*, 1989). Armstrong and Seddon (2008) suggested that reintroduction efforts initially be divided into two phases: 1) establishment and 2) persistence of a population. Programs first focus on establishing a stable or growing population by monitoring demographic measures of population productivity and viability (e.g. Robert *et al.*, 2015). Once the population is established, measures of genetic variation can then be assessed to evaluate the likelihood a population will persist over longer evolutionary time scales (e.g. Franklin, 1980). This approach reflects the idea that the success of any population is based on the interplay between demographic and genetic factors over time (Lande, 1988). However, the genetic diversity present during initial founding events may be important to the immediate persistence of a population (Agashe *et al.*, 2011, Hufbauer *et al.*, 2013). This point is especially salient when captively or supportively bred individuals are used because rapid adaptation to captivity (e.g. Christie *et al.*, 2012) may negatively affect reintroduced populations (Frankham, 2008). Yet, many studies to date have only evaluated genetic diversity several generations following initial reintroduction efforts (e.g. Drag and Cizek, 2014, Huff *et al.*, 2010).

Thousands of dams around the world likely threaten native fishes by fragmenting and extirpating local populations, and pose a unique challenge for fish reintroductions (ICOLD, 2015). Restricting fish access from above-dam environments may have long term

consequences for native fishes, especially those that are migratory and philopatric, such as the Pacific salmonids (Anderson *et al.*, 2014, McClure *et al.*, 2008). Several recent salmonid reintroduction studies have focused on demographic factors associated with productivity (Anderson *et al.*, 2015, Evans *et al.*, 2015a, Sard *et al.*, *In press*), but there are few studies that have estimated the effective population size ($N_e$) and evaluated the maintenance of genetic variation in the context of $N_e$ following reintroduction (but see Evans *et al.*, 2015b, Stockwell *et al.*, 1996).

$N_e$ is an essential parameter in population genetics used to predict the decline in measures of genetic variation, as well as the effectiveness of migration and selection over time. $N_e$ is often less than the census size ($N$) of the population because of changes in population size over time, non-random variance in reproductive success, unequal sex ratios, and overlapping generations (Wright, 1931, Wright, 1938); all of which could influence how genetic variation is distributed among individuals within a reintroduced population over time. In addition, for species that do not have discrete generation times, $N_e$ is influenced by the effective number of breeders ($N_b$) within a generation (Waples, 1990). Accurately estimating both $N_b$ and $N_e$, as well as understanding the relationship between these parameters and the factors driving reductions in $N_b$ can help guide the adaptive management of reintroduced populations.

In recent years, the use of single sample estimators of $N_e$ and $N_b$ has exploded in the literature. The Waples and Do (2008) method ($LDN_e$) has been particularly popular, in part, because of several simulation-based papers that have evaluated its performance for a wide variety of species that vary in an array of life history traits (Palstra and Fraser, 2012, Robinson and Moyer, 2013, Waples *et al.*, 2014). $LDN_e$ has also been empirically evaluated for three

genetic pedigreed populations (Araki *et al.*, 2007, Duong *et al.*, 2013, Serbezov *et al.*, 2012); thereby enabling comparisons of demographic estimators $N_e$ and $N_b$ calculated using measures of fitness. In the context of reintroduction, genetic pedigreed populations provide estimates of both $N_e$ and $N_b$ using a variety of methods, which can inform managers on the number of adults to reintroduce annually through the use of $N_b/N$ ratios, as well as predicting the rates of decline in genetic variation through time by understanding the relationship between $N_b$ and $N_e$.

Here we continue an evaluation of a reintroduced spring Chinook salmon (*Oncorhynchus tshawytscha*) population that has been genetically monitored on the South Fork McKenzie River, Oregon, USA since 2007. First, we extend previous estimates of population productivity (see below) conducted by Sard *et al.* (*In press*) and estimated the number of generations the current population could persist into the future in the absence of continued supplementation with captively bred adults. In addition, we estimated $N_b$ and $N_e$ using two demographic methods based on fitness, as well as the commonly used single-sample genetic approach originally implemented in $LDN_e$. We compare genetic variation observed in founding cohorts and their $F_1$ returning adult offspring, hereafter $F_1$ offspring, to determine if the population is maintaining genetic diversity over time. Finally, we calculate the expected reduction in $N_b$ due to skewed sex ratios and variance fitness to determine their relative contribution to declines in genetic variation over time.

## Methods

### *Study species and site*

Chinook salmon are an anadromous and semelparous fish species. In the upper Willamette River, Oregon, USA, Chinook salmon are considered a threatened Evolutionary

Significant Unit (ESU) listed under the United States Endangered Species Act (ESA) (NMFS, 2005, NMFS, 2008). In the late-winter and early-spring adults enter the Columbia River and travel up the Willamette River. Adults arrive in the upper basin from late-May to early-June and hold in deep pools and thermal refugia in the mainstem Willamette River and its major tributaries (Myers *et al.*, 2006). Most adults typically return to their natal headwater streams during the fall to spawn. Adults in this system usually spawn from ages 3 to 6 (Johnson and Friesen, 2013a), though some males forego an anadromous migration and sexually mature in freshwater at ages 1 or 2.

Oregon Department of Fish and Wildlife (ODFW) and the National Marine Fisheries Service (NMFS) have developed a recovery plan for the upper Willamette River ESU (ODFW and NMFS, 2010). As part of the plan, adult Chinook salmon have been reintroduced annually above several dams within the Willamette River basin. We focused our analysis on the Chinook salmon reintroduction program above Cougar Dam, located on the South Fork McKenzie River. Only captively bred adults, collected at a nearby supplementation hatchery, were reintroduced above Cougar Dam from 1996 to 2009 (Zymonas *et al.*, 2010). Previous research indicates that the supplementation program maintains a high level of genetic variation represented in the population, which is important for establishing a reintroduced population (Johnson and Friesen, 2013b). Given that Chinook salmon typically home to their natal streams to spawn (e.g. > 85% Ford *et al.*, 2015), a trap and haul facility was built at the base of Cougar Dam in 2010 to collect returning natural origin (NOR) adult offspring produced by previously reintroduced cohorts.

As part of a genetic monitoring program that began in 2007, Sard *et al.* (*In press*) recently used genetic parentage assignments to study demographic factors associated with

the reintroduction program from 2007 to 2013. The number of adults reintroduced above Cougar Dam since 2007 ranged from 687 to 1,386 (Table S1). Here we consider adults reintroduced from 2007 to 2010 as founding cohorts for this reintroduction program and include two additional years of data (2014-2015), which enabled the identification of $F_1$ offspring produced by the 2009 and 2010 cohorts.

### *Genotyping and parentage assignments*

Tissue samples were collected from 99% (n=7,856/7,860) of adults reintroduced from 2007 to 2015. With the exception of the captively bred adults collected at a nearby supplementation hatchery in 2014 and 2015, we isolated total genomic DNA from each tissue sample using a glass-fiber protocol developed by Ivanova *et al.* (2006). Captively bred adults from 2014 and 2015 were not included in the analysis because of fiscal and time constraints, though their inclusion would not alter our conclusions (see *Results* section below). DNA samples were amplified at 11 microsatellites loci using the polymerase chain reaction (PCR): *Ots201, Ots208b, Ots209, Ots211, Ots212, Ots215, Ots249, Ots253, Ots311, Ots409,* and *Ots515* (Banks *et al.*, 1999, Greig *et al.*, 2003, Naish and Park, 2002, Williamson *et al.*, 2002). All PCR products were visualized on an ABI 3730XL DNA analyzer and size scored with GeneMapper software (Applied Biosystems, Inc., Foster City, CA).

Multilocus genotypes were used to identify parent-offspring relationships among founding cohorts and their $F_1$ offspring returning to the trap and haul facility below the Cougar Dam, hereafter Cougar Trap, from 2010-2015. Parents were assigned to $F_1$ offspring using well established parentage assignment methods implemented in CERVUS (Kalinowski *et al.*, 2007, Marshall *et al.*, 1998) and COLONY (Jones and Wang, 2010). Age 6 offspring produced by adults reintroduced in 2010 were not included in the analysis because they had

not returned to spawn in 2015; however their inclusion would likely not meaningfully alter our results because few adults return at that age in this system (<2% Johnson and Friesen, 2013a). More details regarding the genetic parentage assignments methods used can be found in Sard *et al.* (*In press*).

### *Estimating time to local extinction*

We calculated population productivity (λ), defined as the number of adults produced by a adult, for both the 2009 and 2010 cohorts (Botsford and Brittnacher, 1998). Sard *et al.* (*In press*) reported λ estimates for the 2007 and 2008 based on females only, in part, because of skewed sex ratios among reintroduced cohorts. However, we sought to apply methods in Waples (2002) that used λ estimates, regardless of sex, when estimating $N_e$. Therefore, we report overall estimates of λ for all four cohorts (2007-2010). Using λ estimates from the four cohorts, we conducted a simple simulation 10,000 times to estimate the mean number of generations it would take the reintroduced population to reach local extinction (equation 1).

Equation 1 $$N_i = N_{i-1}\lambda$$

The simulation assumed that the population was not supplemented by captively bred adults in future generations and no migration occurred. In each simulation the initial number of adults in the population was 3,753 (the same as the actual reintroduced population in this study), and similar to Waples (2002), λ was randomly drawn from a log-normal distribution based on λ estimates. Finally, each $N_i$ was rounded to the nearest integer. (See supplemental R script for more details).

### *Estimating $N_b$ and $N_e$*

The number of $F_1$ offspring that assigned to a given adult reintroduced from 2007 to 2010 was used as an estimate of individual fitness. We calculated mean ($\bar{k}$) and variance ($V_{\bar{k}}$)

in fitness for males and females in each reintroduced cohort. We followed methods implemented in Araki *et al.* (2007) and Duong *et al.* (2013) when calculating demographic measures of inbreeding and variance effective number of breeders ($N_{bI}$ and $N_{bV}$, respectively) with fitness estimates. Briefly, $N_{bI}$ was calculated for male ($N_{bm}$) and females ($N_{bf}$) in each cohort separately using equation 2 (Crow and Kimura, 1970).

Equation 2 $$N_{bI} = \frac{\bar{k}N-2}{\bar{k}-1+\frac{V_{\bar{k}}}{\bar{k}}}$$

Estimates of $N_b$ for males ($N_{bm}$) and females ($N_{bf}$) were then used to calculate $N_b$ for each cohort using equation 3 (Wright, 1938).

Equation 3 $$N_b = \frac{4N_{bm}N_{bf}}{N_{bm}+N_{bf}}$$

We also estimated $N_{bV}$ for each sex separately using equation 4 (Crow and Denniston, 1988), and we subsequently used equation 3 to calculate $N_{bV}$ for each cohort.

Equation 4 $$N_{bV} = \frac{\bar{k}(2N-1)}{2(1+\frac{V_{\bar{k}}}{\bar{k}})}$$

In addition to our demographic estimates of $N_b$, we used multilocus genotypes from $F_1$ offspring from each cohort (2007-2010) to estimate the effective number of breeders ($N_{bD}$, following nomenclature in Duong *et al.* 2013) with methods originally developed in $LDN_e$ and implemented in NeEstimator (Do *et al.*, 2014, Waples and Do, 2008). We applied an allele frequency critical value of 0.02 and report 95% jack-knifed confidence intervals (CIs) as recommended by Waples and Do (2008). Waples *et al.* (2014) showed the $LDN_e$ estimates of $N_b$ are biased, and the direction of the bias is associated with the $N_b/N_e$ ratio. We adjusted $N_b$ estimates by first estimating the $N_b/N_e$ ratio using the equation in Figure 3A in Waples *et al.* (2013), and applying the estimate (0.26) in Waples *et al.* (2014) equation 8. Finally, we report $N_b/N$ ratios using all three estimates of $N_b$ ($N_{bI}$, $N_{bV}$, and $N_{bD}$).

Following Waples (2002), we estimated $N_e$ for the reintroduced population using three different approaches (equations 5-7), where $\overline{N}_b$ and $\widetilde{N}_b$ represent the arithmetic and harmonic means of $N_b$, respectively. $X_i$ is the proportional contribution of $F_1$ offspring (λ) from a given cohort (i) to the entire generation. The generation time ($g$) for Chinook salmon in this study system is 4 years (Johnson and Friesen, 2013a).

Equation 5 $$N_e = g\overline{N}_b$$

Equation 6 $$N_e = g\widetilde{N}_b$$

Equation 7 $$N_e = \frac{1}{\Sigma\left(\frac{X_i^2}{N_{bi}}\right)}$$

## *Evaluating genetic variation*

We counted the number of alleles ($A$) and calculated expected heterozygosity ($H_e$) for each microsatellite locus genotyped from 2007 to 2015 to determine if genetic variation in the population was maintained between founding cohorts and their $F_1$ offspring. We chose to use $A$ rather than allelic richness because we have a near complete census of the population (see above) and genotyping error rate was low (i.e. 2%, Sard *et al.*, *In press*). We tested for allelic dropout in each of the 11 loci using methods developed by Brookfield (1996) and implemented in PopGenReport (Adamack and Gruber, 2014). We excluded *Ots209*, *Ots249*, and *Ots211* from further analysis because these loci showed evidence of allelic dropout and therefore may have biased our evaluation of genetic variation. We also tested each locus to determine if they were in Hardy-Weinberg Proportions (HWP), and if any loci pairs were in linkage disequilibrium (LD) using Genepop (Rousset, 2008).

We first assessed genetic variation for each locus among founding cohorts reintroduced from 2007 to 2010 , as well as in each cohort's $F_1$ offspring (Figure 1A) using

hierfstat (de Meeus and Goudet, 2007). We tested if either the *A* or $H_e$ per locus declined between the founding cohorts and their $F_1$ offspring individually using paired Wilcoxon rank sum tests. We hereafter refer to this analysis as the founding cohort approach.

Overlapping generations could help to maintain some genetic variation in the population. Therefore as a second approach, we compared *A* and $H_e$ per locus among the founding cohorts from 2007 to 2010 to all of their $F_1$ offspring, in aggregate (Figure 1B). For example, if an allele observed in adults reintroduced in 2007 was not present in any of their $F_1$ offspring, we would consider it lost based on the founding cohort approach described above. However, using this second approach we can account for overlapping generations by determining if that same allele happened to be observed in another founding cohort and was also observed in at least one of their $F_1$ offspring. We tested if either *A* or $H_e$ per locus declined between the founding cohorts and their $F_1$ offspring using paired Wilcoxon rank sum tests. We hereafter refer to this analysis as the overlapping generations approach.

To further investigate the nature of the genetic variation within reintroduced population, we calculated pairwise $F_{st}$ values among the founding cohorts and $F_1$ offspring in FSTAT and tested for significance following 1000 permutations (Goudet, 2001).

### $Factors\ affecting\ N_b$

Two factors that could potentially drive $N_b < N$ in a given cohort are the sex ratio for each reintroduced cohort, as well as variance in fitness among reintroduced adults within each cohort. Here we again use methods implemented in Araki et al. (2007) to calculate the expected reduction in $N_b$ compared to *N* to determine the relative influence of the sex ratio and variance in fitness on the maintenance of genetic diversity within the population. To determine the expected reduction in $N_e$ due to sex ratio we used the actual number of males

and females reintroduced in each cohort in equation 2. To determine the expected reduction in $N_b$ due to variance in fitness, we first standardized $V_{\bar{k}}$ based on $\bar{k} = 2$ (see Araki *et al.*, 2007 , Duong *et al.*, 2013 and references therein for more detail). Standardized measures of $V_{\bar{k}}$ were then used in equation 4 to estimates $N_{bv}$ for each sex, which were in turn used in equation 3 to estimate $N_{bV}$ for each cohort.

We used version 3.2.3 for all analyses conducted in R (R Core Team, 2015). Critical values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995). We report mean ± standard deviation where applicable.

## Results

### *Microsatellites*

Most adults (99%, n=6,542/6,593) reintroduced from 2007 to 2015 were genotyped at all loci. We found that between 0 and all 8 of the loci statistically deviated from HWP for founding cohorts and their $F_1$ offspring using either the founding cohort or overlapping generation approach (Table 1). However, most deviations observed were likely not biologically significant because most $F_{is}$ values were small (0.3% ± 2.1%, Table 1). We found that *Ot208* had consistently large positive $F_{is}$ values in the founding cohorts ($F_{is}$: 4.1% ± 3.2%, Table 1). Our general conclusions remained the same with or without the inclusion of *Ots*208; we therefore included the locus in the remainder of our analysis. We found from 0 to 12 locus pairs (out of 28 possible combinations) were in LD among founding cohorts and $F_1$ offspring. No locus pair was consistently in LD across all four groups (Table S2). We think that most locus pairs observed to be in LD were likely due to the large numbers of individuals sampled, which provided high statistical power to detect small deviations from theoretical expectations. In addition, we found higher numbers of locus pairs in LD in the founding

cohorts when they were evaluated in aggregate to account for overlapping generations, which likely reflects both high statistical power and a Wahlund-like effect.

### *Estimating time to local extinction*

We found that λ was well below one in all four cohorts (0.25 ± 0.15), which indicated the population size would decline without continued supplementation or migration (Table 2). We estimated that a population of the same size and similar measures of λ would become locally extinct within 3 ± 1 generations (Figure 2).

### *Estimates of $N_b$ and $N_e$*

Based on estimates of fitness (Table S3), $N_{bI}$ ranged from 125 to 350; whereas $N_{bV}$ estimates ranged from 52 to 149 among the founding cohorts (Figure 3, Table S4). Using microsatellite genotypes, $N_{bD}$ varied little among founding cohorts (Figure 3, Table S4). We found that $N_b/N$ ratios varied little among cohorts using all three estimates of $N_b$: 0.20 ± 0.03 ($N_{bD}/N$), 0.23 ± 0.05 ($N_{bI}/N$), and 0.12 ± 0.07 ($N_{bV}/N$).

Effective population size estimates based on all three approaches ($N_{bI}$, $N_{bV}$, or $N_{bD}$) were relatively large regardless of whether they were calculated using harmonic mean (range: 344-783), arithmetic mean (range: 407-893), or based on the proportional contribution of each cohort to the next generation (range: 393-690, Table 3).

### *Evaluating genetic variation*

Using the founding cohort approach, we found the mean *A* lost per locus between founding cohorts and their $F_1$ offspring ranged from 5 to 9 (Figure 4): 2007 ($t_{(7)}$ = 3.3, p = 0.013); 2008 ($t_{(7)}$ = 5.6, p = 0.002); 2009 ($t_{(7)}$ = 5.3, p = 0.002); and 2010 ($t_{(7)}$ = 3.7, p = 0.010). We found no evidence for a reduction in $H_e$ between any founding cohort and their $F_1$ offspring (p = 0.319, p = 0.868, p = 0.319, and p = 0.303; 2007 to 2010, respectively). When

we accounted for overlapping generations, $F_1$ offspring from the reintroduction program had, on average, 6 fewer alleles per locus compared to the founding cohorts ($t_{(7)} = 9.6$, $p < 0.001$). However, we did not observe a reduction in $H_e$ ($p = 0.319$).

The probability that an allele was lost due to genetic drift declines as an allele increases in frequency - i.e. $(1-q)^{N_e}$, where $q$ is the frequency of an allele (Figure 5A). Forty seven of the total 300 alleles observed in the founding cohorts were lost in $F_1$ offspring. All of these lost alleles were observed at a low frequency (i.e. < 0.005), indicating that many had high probabilities of being lost (Figure 5B).

All but one pairwise $F_{st}$ comparisons were statistically significant, however overall values were small (range: 0.0001 to 0.0044, Table 4). Founding cohorts were most similar to their respective $F_1$ offspring (0.0007 ± 0.0005). Founding cohorts were also similar to each other (0.0016 ± 0.0004). Finally, we found higher levels of genetic differentiation among $F_1$ offspring (0.0037 ± 0.0005).

### $Factors\ affecting\ N_b$

Both skewed sex ratios and variance in fitness reduced $N_b$ compared to $N$ of each of the four cohorts. The expected reduction due to unequal sex ratios and variance in fitness were 6% ± 5% and 82% ± 9%, respectively.

## Discussion

We assessed demographic and genetic factors important to reintroduced populations over a single generation. We found the study population is declining in size, yet the population had relatively high estimates of $N_e$ - indicating that most genetic variation was maintained. Based on estimates of $N_e$, most of the alleles not observed in $F_1$ offspring had a

high probability of being lost due to genetic drift mainly associated with variance in fitness among reintroduced adults.

Productivity is an important demographic factor to consider when establishing reintroduced population. Our estimates of λ among the four cohorts evaluated suggest that the population above Cougar dam will decline in future generations, and the population may become locally extinct within 3 ± 1 generations without continued supplementation by captively bred adults and/or immigrants collected at the Cougar Trap (Sard *et al.*, *In press*). Of course, our simulation is simplistic because it does not consider the possibility for adaptation to the above dam environment, which potentially can occur in as little as a single generation (Christie *et al.*, 2012). However for adaptation to occur, the strength and direction of selection will likely need to be strong and consistent for multiple generations such that allele frequencies can adapt to the fitness landscape shaped by a stable above-dam environment.

The 50/500 rule is a common method used to assess the risk of inbreeding depression among individuals, as well as the future evolutionary potential of a given population (Franklin, 1980, Soulé, 1980). Here a population with an $N_e > 50$ will have < 1% risk of inbreeding depression, and a population of $N_e > 500$ will maintain most of the genetic variation, assuming a mutation rate of $10^3$ (Franklin, 1980, Soulé, 1980). Based on estimates of $N_b$, we found that $N_e$ was relatively high using a variety of approaches (range: 344 to 893), which suggests that most genetic variation among the founding cohorts was maintained. Our estimates of $N_b$ were calculated using estimates of fitness, as well as microsatellite genotypes. The $N_b/N$ ratios estimated in this study could potentially to be used to guide the future reintroduction of adults in the system. For instance, conservatively, no less than 105 adults should be reintroduced in a given year assuming a $g = 4$ and an $N_b/N \approx 0.12$ (based on

estimates of $N_{bv}$). Implementing such a suggestion in management would prevent the deleterious effects of inbreeding depression by keeping $N_e > 50$; however, rare genetic variation that may be important for adaptation could still potentially be lost over time if $N_e$ was not consistently larger than 500.

We found no difference between per locus $H_e$ in the founding cohorts and their $F_1$ offspring based on the paired Wilcoxon Rank Sum tests, but we did observe significant $F_{st}$ values based on permutation tests. This apparent contradiction likely reflects differences in the power for each statistical test to detect significant differences between the two groups, because the expected reduction in heterozygosity between the founding cohorts and their $F_1$ offspring is small (i.e. $\Delta h = \frac{1}{2N_e}$).

We consistently observed a reduction in alleles between the founding cohorts and $F_1$ offspring. Our results corroborate several reintroduction genetics studies that suggest genetic variation may be lost between the founding cohorts and their descendants sampled multiple generations following the initial reintroduction (Fitzsimmons *et al.*, 1997, Huff *et al.*, 2010, Miller *et al.*, 2010). Simulation work suggest that alleles can be quickly lost due to genetic drift if a bottleneck occurs, but the specific dynamics and rate of allele loss depends on the population size, as well as the number and frequencies of alleles observed at a given locus (Allendorf, 1986).

We found that 84% (253/300) of the alleles observed in the founding cohorts were also observed in $F_1$ offspring. The reduction in alleles observed was in part due to skewed sex ratio, however based on our analysis; variance in fitness was the major factor contributing to the decline in genetic diversity. Our results corroborate others studies the have provided similar evidence suggesting that variance in fitness is a major factor contributing to declines

in genetic variation (Araki *et al.*, 2007, Duong *et al.*, 2013, Serbezov *et al.*, 2012). Managers of reintroduction programs can potentially control the sex ratio of the adults reintroduced each year, and therefore mitigate against the loss of genetic variation. But, perhaps the only way to mitigate against genetic variation lost due to highly skewed fitness distributions, which are common Chinook salmon and many other species (Clutton-Brock, 1988), is to support continued gene flow into the population through immigrants.

Immigrants in reintroduced populations can potentially play an important role in restoring lost genetic variation and providing a source for new alleles. Iconic examples such as the genetic rescue of the Florida panther and Mexican wolf clearly demonstrate the value of immigrants (Hedrick and Fredrickson, 2009). The reintroduction program examined in this paper could potentially use captively bred adults or immigrants collected at the Cougar Trap to ensure the evolutionary potential of the population. However, the value of these benefits must also be carefully weighed against the risks of reintroducing individuals produced by captive and supportive breeding programs, as well as rare and highly valued native populations. First, there is evidence that HOR adults have high levels of genetic diversity (Johnson and Friesen, 2014). Yet, Sard *et al.* (2015) provided evidence that captively bred adult males are less fit compared to their NOR counter parts. Alternatively, NOR immigrants offer the potential to avoid issues associated with captive or supportive breeding programs. Yet, Chinook salmon in the McKenzie River system are considered a valuable genetic legacy population in the upper Willamette ESU (HSRG, 2009). Since 2013, managers have taken steps to limit the reintroduction of NOR immigrants due to uncertainty associated with reintroducing fish into an above-dam environment and juvenile dam passage (Beeman *et al.*, 2014). Therefore, anadromous NOR immigrants will have a limited role in contributing

genetic variation to the above dam population, at least in the immediate future. We also note that difficult to sample life histories tactics such as precocial males and adfluvial adults were not accounted for in the analysis, and there is evidence that both contribute offspring to the above dam population (Sard *et al.*, Accepted). Alternative life history tactics could act as additional sources of genetic variation and therefore bolster the population's resiliency (e.g. Perrier *et al.*, 2014).

In conclusion, we found that some genetic variation can be quickly lost after a founding event, even when $N_e$ is relatively large. Our results highlight the immediate importance that immigrants could have in contributing genetic variation to a reintroduced population. Our results conformed well to expectations based on genetic theory, and suggest others can rely on theoretical expectations when planning other reintroductions.

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## Figure legends

Figure 1. Summary of the two approaches we took to evaluate genetic variation within the reintroduced population. Panel A represents our comparison of genetic variation between a founding cohort and their $F_1$ offspring that returned to the Cougar Trap as an age-3 to age-6 adult, with the exception of age-6 adults produced by the 2010 reintroduced cohort (see Methods section). Panel B represents our overlapping generation approach, which compared genetic variation between all $F_1$ offspring returning to the Cougar Trap from 2010 to 2015 and any of the founding cohorts that could have produced them.

Figure 2. Distribution of the number of generations to local extinction for the reintroduced population above Cougar dam, assuming no migration or supplementation with captively breed individuals.

Figure 3. Estimates of the effective number of breeders in each four cohorts reintroduced from 2007 to 2010 based on three different methods. Inbreeding (NbI) and variance (NbV) effective numbers of breeders were calculated using measures of fitness. We also used microsatellite genotypes of F1 offspring for each of the reintroduced cohorts to estimate Nb using LDNe (NbD).

Figure 4. Summary of the reduction in the number of alleles observed between founding cohorts and their F1 offspring from 2007 to 2010. *Indicated a significant reduction in the number of alleles at $\alpha$=0.05 following false discovery rate corrections (Benjamini and Hochberg, 1995).

Figure 5. Summary of alleles lost (black bars) or retained (grey bars) between potential parents and F1 offspring. Panel A describes the predicted probability of an allele, based on its frequency, of being lost due to genetic drift using equations 5-7 to calculate Ne based on variance and inbreeding, as well LDNe estimates Nb. Panel B describes the proportion of alleles not observed (lost) in F1 adult offspring or were retained based on the observed allele frequencies in potential parents (x-axis).

Figure 1.

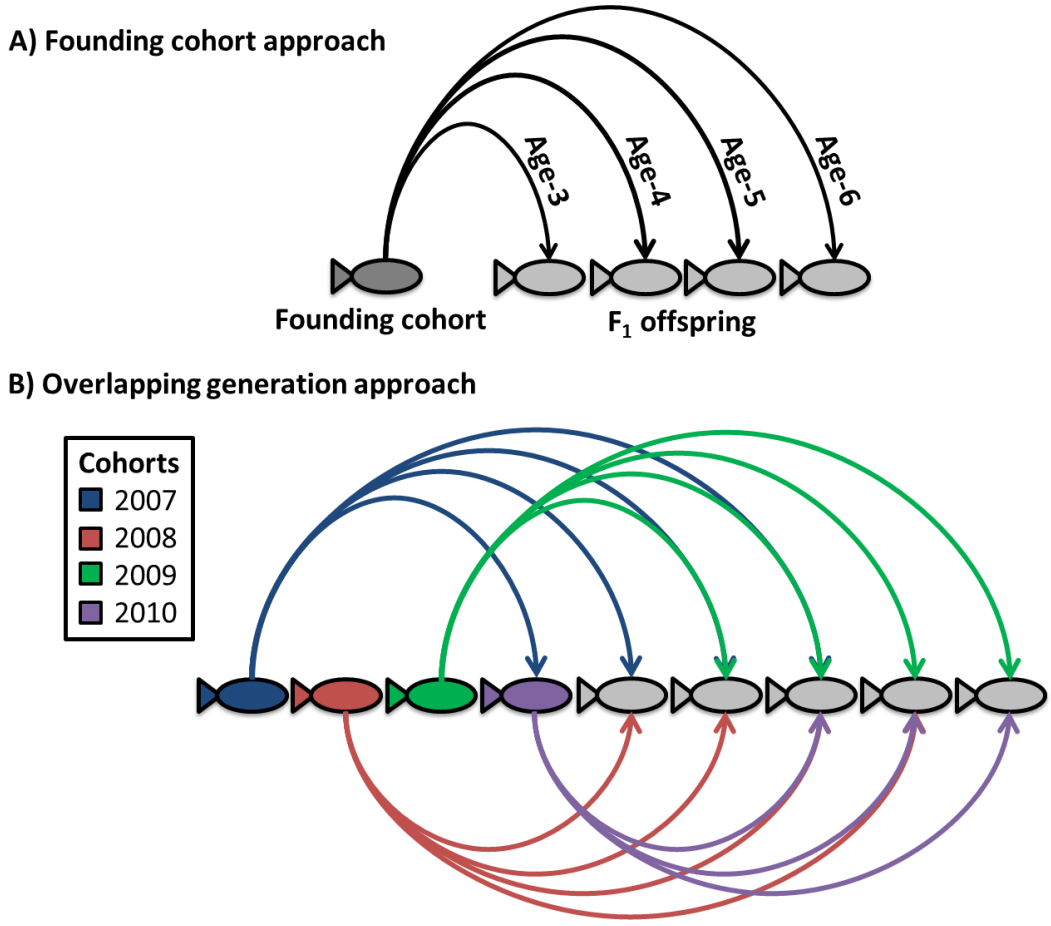
A) Founding cohort approach
Age-3
Age-4
Age-5
Age-6
Founding cohort
F1 offspring
B) Overlapping generation approach
Cohorts
2007
2008
2009
2010

Figure 2.

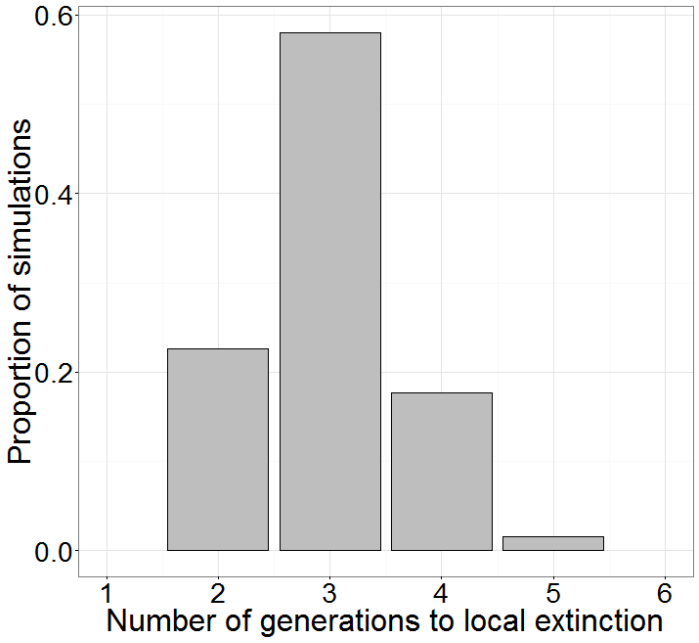
0.6
0.4
0.2
0.0
Proportion of simulations
1
2
3
4
5
6
Number of generations to local extinction

Figure 3.

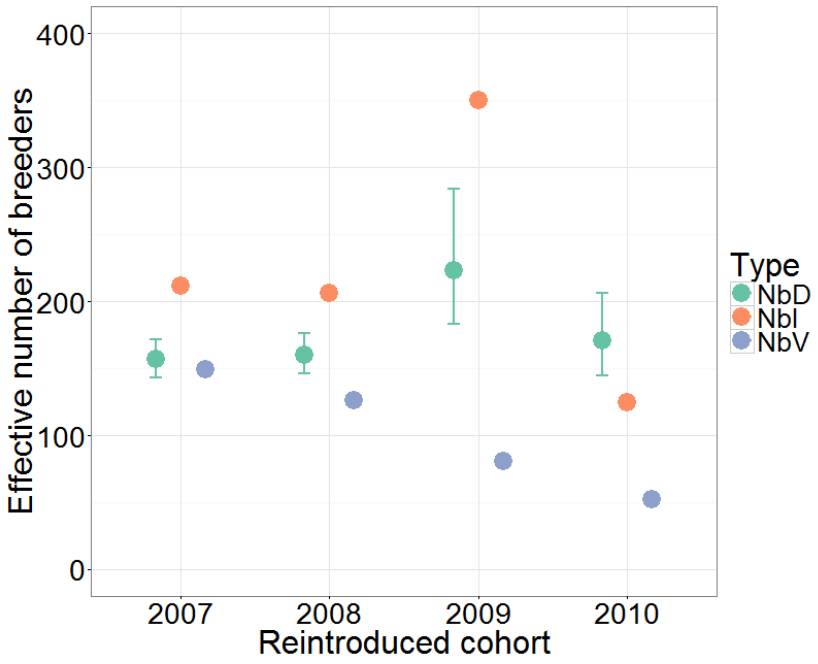
400
300
200
100
0
Effective number of breeders
2007
2008
2009
2010
Reintroduced cohort
Type
NbD
Nbl
NbV

Figure 4.

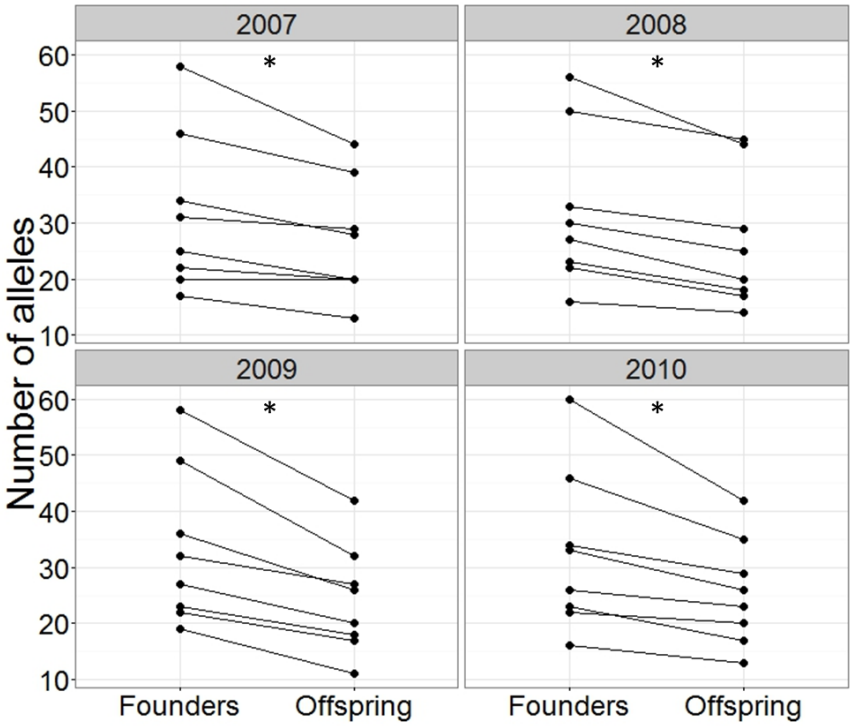
2007
2008
2009
2010
Number of alleles
60
50
40
30
20
10
*
Founders
Offspring

Figure 5.

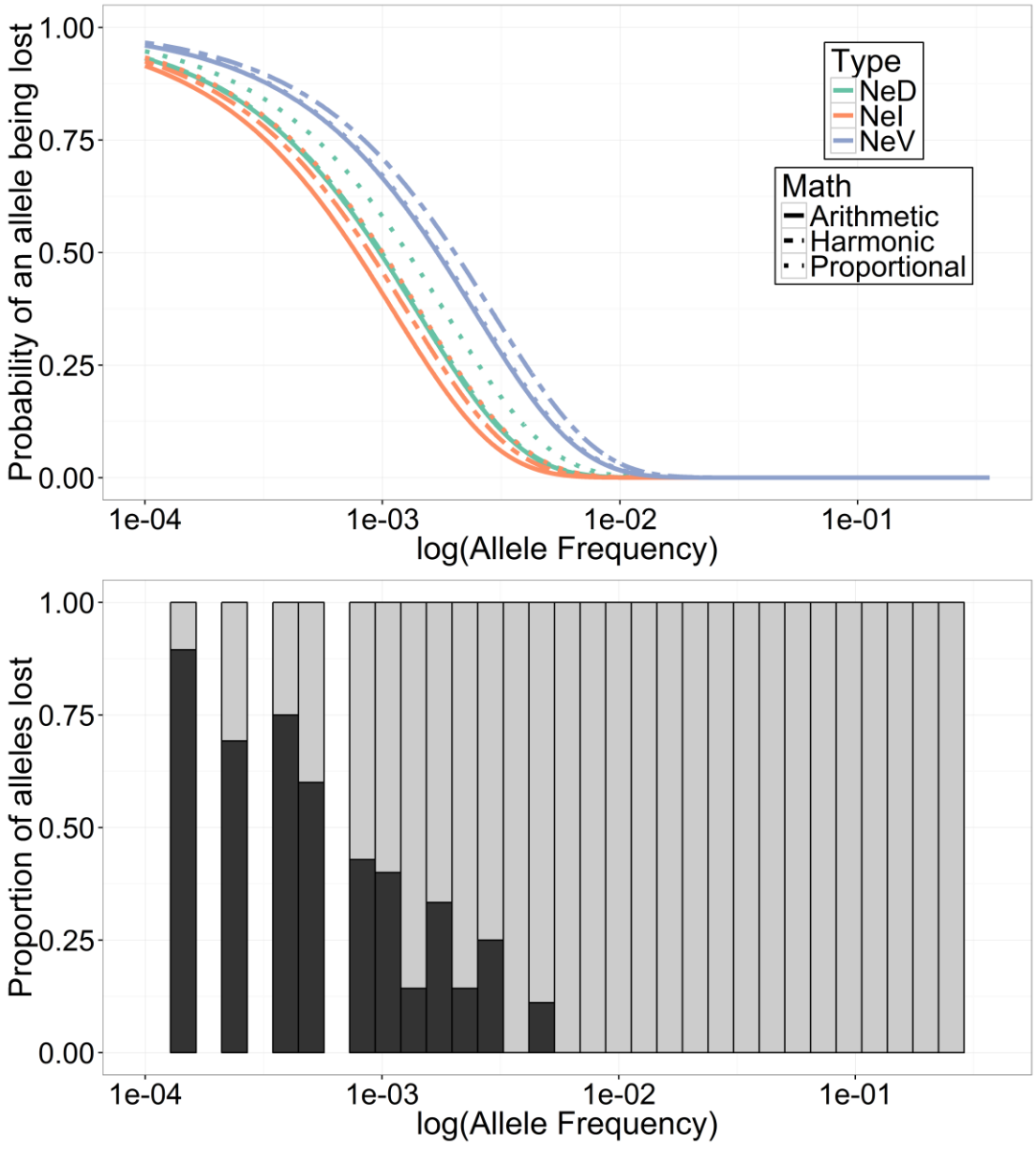
Probability of an allele being lost
1.00
0.75
0.50
0.25
0.00
1e-04
1e-03
1e-02
1e-01
log(Allele Frequency)
Type
NeD
NeI
NeV
Math
Arithmetic
Harmonic
Proportional
Proportion of alleles lost
1.00
0.75
0.50
0.25
0.00
1e-04
1e-03
1e-02
1e-01
log(Allele Frequency)

Table 1. Summary of expected heterozygosity ($H_e$), $F_{is}$, and the number of alleles (A) at 8 microsatellite loci (Locus) for both the founding cohorts (2007-2010) and overlapping generations (Combined) approaches.

| Type | Locus | 2007 | | | 2008 | | | 2009 | | | 2010 | | | Combined | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $H_e$ | $F_{is}$ | A | $H_e$ | $F_{is}$ | A | $H_e$ | $F_{is}$ | A | $H_e$ | $F_{is}$ | A | $H_e$ | $F_{is}$ | A |
| Founders | Ot201 | 0.9158 | -0.0185* | 20 | 0.9153 | -0.0074* | 23 | 0.9145 | -0.0029* | 23 | 0.9138 | -0.0160* | 23 | 0.9156 | -0.0088* | 26 |
| | Ot253b | 0.9185 | 0.0389* | 25 | 0.9219 | 0.0085* | 27 | 0.9172 | 0.0327* | 27 | 0.9199 | -0.0094* | 26 | 0.9198 | 0.0207* | 31 |
| | Ot215 | 0.9351 | 0.0111 | 31 | 0.9347 | 0.0024* | 33 | 0.9372 | -0.0043* | 32 | 0.9482 | 0.0093* | 34 | 0.9397 | 0.0045* | 37 |
| | Ot311 | 0.9523 | 0.0150* | 46 | 0.9531 | -0.0095* | 50 | 0.9509 | -0.0027* | 49 | 0.9537 | -0.0004* | 46 | 0.9528 | 0.0003* | 55 |
| | Ot409 | 0.9542 | -0.0071* | 58 | 0.9489 | -0.0164* | 56 | 0.9521 | 0.0252* | 58 | 0.9538 | -0.0103* | 60 | 0.9534 | 0.0034* | 67 |
| | Ot208 | 0.9483 | 0.0962* | 34 | 0.9395 | 0.0319* | 30 | 0.9379 | 0.0204* | 36 | 0.9451 | 0.0174* | 33 | 0.9430 | 0.0389* | 38 |
| | Ot212 | 0.8765 | -0.0074 | 22 | 0.8936 | -0.0011* | 22 | 0.8965 | 0.0027* | 22 | 0.8962 | -0.0107 | 22 | 0.8933 | -0.0012* | 25 |
| | Ot515 | 0.8576 | 0.0010 | 17 | 0.8664 | 0.0058* | 16 | 0.8739 | 0.0194* | 19 | 0.8543 | 0.0035 | 16 | 0.8656 | 0.0101* | 21 |
| Offspring | Ot201 | 0.9150 | -0.0067 | 20 | 0.9223 | -0.0040 | 18 | 0.9184 | 0.0084 | 18 | 0.9108 | 0.0273 | 17 | 0.9189 | 0.0029 | 21 |
| | Ot253b | 0.9073 | -0.0085* | 20 | 0.9209 | 0.0080 | 20 | 0.9058 | -0.0350 | 20 | 0.9163 | -0.0243* | 23 | 0.9163 | -0.0056* | 24 |
| | Ot215 | 0.9171 | -0.0424* | 29 | 0.9235 | -0.0157 | 29 | 0.9309 | 0.0121 | 27 | 0.9375 | -0.0198 | 29 | 0.9272 | -0.0196* | 32 |
| | Ot311 | 0.9467 | -0.0031 | 39 | 0.9506 | 0.0173 | 44 | 0.9521 | -0.0034 | 32 | 0.9437 | 0.0054 | 35 | 0.9496 | 0.0057* | 48 |
| | Ot409 | 0.9523 | -0.0203* | 44 | 0.9471 | -0.0124 | 44 | 0.9549 | -0.0005 | 42 | 0.9534 | -0.0028 | 42 | 0.9532 | -0.0105* | 58 |
| | Ot208 | 0.9465 | 0.0066* | 28 | 0.9375 | 0.0080 | 25 | 0.9407 | 0.0224 | 26 | 0.9403 | 0.0205 | 26 | 0.9458 | 0.0150* | 32 |
| | Ot212 | 0.8659 | -0.0096 | 20 | 0.8946 | 0.0340 | 17 | 0.8939 | 0.0511* | 17 | 0.8996 | 0.0444 | 20 | 0.8874 | 0.0245* | 21 |
| | Ot515 | 0.8670 | -0.0192 | 13 | 0.8740 | -0.0453 | 14 | 0.8533 | -0.0254 | 11 | 0.8533 | 0.0028* | 13 | 0.8655 | -0.0250* | 17 |

*Indicated significant (α=0.05) departure from expected Hardy-Weinberg proportions following false discovery rate corrections (Benjamini and Hochberg, 1995)

Table 2. Estimates of population productivity (λ) for each reintroduced cohort from 2007-2010.

| Year (i) | Recruits (R) | Spawner (S) | λ | $S_i/S_t$ | $R_i/R_t$ |
|---|---|---|---|---|---|
| 2007 | 320 | 746 | 0.43 | 0.20 | 0.40 |
| 2008 | 246 | 873 | 0.28 | 0.23 | 0.31 |
| 2009 | 115 | 1386 | 0.08 | 0.37 | 0.14 |
| 2010 | 117 | 748 | 0.16 | 0.20 | 0.15 |
| Total (t) | 798 | 3753 | 0.21 | - | - |

Table 3. Estimates of $N_e$ calculated using the arithmetic mean (Arith.), harmonic (Harm.) mean, or proportionally (Prop.) to the number of $F_1$ offspring produced of each cohort.

| Type | $N_e$ | | |
|---|---|---|---|
| | Arith. | Harm. | Prop. |
| LDNe | 711 | 697 | 544 |
| Inbreeding | 893 | 783 | 690 |
| Variance | 407 | 344 | 398 |

Table 4. Summary of pairwise $F_{st}$ values among Founders and $F_1$ offspring (Offspring) each cohort produced.

| | Founder 2007 | Founder 2008 | Founder 2009 | Founder 2010 | Offspring 2007 | Offspring 2008 | Offspring 2009 | Offspring 2010 |
|---|---|---|---|---|---|---|---|---|
| Founder 2007 | - | 0.0017* | 0.0022* | 0.0011* | 0.0009* | 0.0023* | 0.0026* | 0.0013* |
| Founder 2008 | | - | 0.0011* | 0.0017* | 0.0041* | 0.0007* | 0.0019* | 0.0033* |
| Founder 2009 | | | - | 0.0016* | 0.0043* | 0.0021* | 0.0001 | 0.0030* |
| Founder 2010 | | | | - | 0.0040* | 0.0030* | 0.0023* | 0.0012* |
| Offspring 2007 | | | | | - | 0.0040* | 0.0039* | 0.0033* |
| Offspring 2008 | | | | | | - | 0.0033* | 0.0044* |
| Offspring 2009 | | | | | | | - | 0.0030* |
| Offspring 2010 | | | | | | | | - |

*Indicated significant (α=0.05) genetic differentiation following false discovery rate corrections (Benjamini and Hochberg, 1995)

Table S1. Summary of the number of adult Chinook salmon reintroduced above Cougar Dam from 2007 to 2015 from the supplementation hatchery on the McKenzie River (Hatchery). The number of natural origin adults collected at the trap and haul facility built at the base of Cougar Dam also indicated.

| Year | Hatchery | Natural origin | Total |
|---|---|---|---|
| 2007 | 746 | 0 | 746 |
| 2008 | 873 | 0 | 873 |
| 2009 | 1386 | 0 | 1386 |
| 2010 | 527 | 221 | 748 |
| 2011 | 374 | 357 | 731 |
| 2012 | 447 | 500 | 947 |
| 2013 | 464 | 223 | 687 |
| 2014 | 689* | 193 | 882 |
| 2015 | 619* | 241 | 860 |

* Note that hatchery origin adults collected in 2014 and 2015 were not included in the analysis.

Table S2. Summary of locus pairs in linkage disequilibrium among founding cohorts and $F_1$ offspring using both the founding cohort (Found.) and overlapping generation (Combined) approaches.

| Locus 1 | Locus 2 | Founding cohorts | | | | $F_1$ offspring | | | | Combined | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2007 | 2008 | 2009 | 2010 | 2007 | 2008 | 2009 | 2010 | Found. | Off. |
| OT201 | Ot253b | **0.001** | 0.123 | 0.195 | 0.684 | 0.239 | 0.940 | 1.000 | 0.328 | **0.001** | 0.180 |
| OT201 | Ot215 | 0.177 | **0.001** | 0.178 | 0.684 | 0.681 | 0.068 | 0.187 | 0.659 | 0.457 | 0.199 |
| Ot253b | Ot215 | 0.123 | 0.187 | 0.094 | 0.246 | 0.820 | 0.820 | 1.000 | 0.314 | 0.199 | 1.000 |
| OT201 | Ot311 | 0.187 | 0.187 | 0.684 | 1.000 | 0.752 | 0.154 | 0.125 | 1.000 | 0.708 | 0.127 |
| Ot253b | Ot311 | 0.314 | 0.374 | 0.328 | 0.225 | 0.684 | 0.441 | 1.000 | 0.094 | 0.127 | **0.001** |
| Ot215 | Ot311 | 1.000 | 1.000 | 0.187 | 0.684 | 0.940 | 1.000 | 0.684 | 0.234 | 0.457 | 1.000 |
| OT201 | Ot409 | 0.330 | 0.266 | 0.080 | **0.001** | 0.262 | 0.351 | 1.000 | 0.262 | 0.430 | 1.000 |
| Ot253b | Ot409 | 0.192 | 0.264 | 0.383 | 0.802 | 0.535 | 0.262 | 1.000 | 0.177 | 0.823 | 1.000 |
| Ot215 | Ot409 | 1.000 | 1.000 | 1.000 | 0.595 | 0.187 | 0.853 | 1.000 | 0.332 | 0.439 | 0.708 |
| Ot311 | Ot409 | 1.000 | 1.000 | 1.000 | 1.000 | 0.469 | 0.751 | 1.000 | 1.000 | 0.176 | 1.000 |
| OT201 | Ot208 | 0.426 | 0.192 | **0.001** | 0.094 | 0.178 | 0.314 | 0.682 | 0.351 | **0.001** | 0.661 |
| Ot253b | Ot208 | 0.090 | 0.177 | 0.187 | 1.000 | 0.684 | 0.802 | 1.000 | 0.318 | 0.391 | 0.841 |
| Ot215 | Ot208 | 0.142 | 0.317 | 0.266 | 0.383 | 0.632 | 0.470 | 0.247 | 0.187 | 0.199 | 0.708 |
| Ot311 | Ot208 | 1.000 | 1.000 | 1.000 | 0.062 | 1.000 | 1.000 | 1.000 | 0.820 | 0.712 | 1.000 |
| Ot409 | Ot208 | 1.000 | 0.247 | 0.731 | 1.000 | 1.000 | 0.752 | 1.000 | 1.000 | 0.139 | 1.000 |
| OT201 | Ot212 | 0.131 | 0.177 | **0.001** | 0.684 | **0.001** | 0.846 | 0.386 | 0.682 | **0.001** | 0.771 |
| Ot253b | Ot212 | 0.314 | 0.087 | 0.210 | 0.328 | **0.001** | 0.564 | 0.847 | 0.090 | 0.323 | 0.199 |
| Ot215 | Ot212 | 0.262 | 0.466 | 0.314 | 0.659 | 0.195 | 0.314 | 0.432 | 0.957 | **0.001** | 0.090 |
| Ot311 | Ot212 | 0.754 | 0.177 | 0.094 | 0.752 | 0.095 | 0.432 | 0.259 | 0.535 | 0.127 | 1.000 |
| Ot409 | Ot212 | 0.605 | 0.247 | 0.225 | 0.684 | 1.000 | 1.000 | 0.266 | 1.000 | **0.001** | 0.708 |
| Ot208 | Ot212 | 0.731 | 0.060 | **0.047** | 0.317 | 0.595 | 0.566 | 0.684 | 1.000 | **0.001** | 0.432 |
| OT201 | Ot515 | 0.094 | **0.009** | **0.032** | 0.372 | 0.372 | 0.727 | 0**.001** | 0.128 | **0.001** | **0.001** |
| Ot253b | Ot515 | 0.165 | 0.094 | **0.001** | 0.090 | 0.094 | 0.095 | 0.802 | 0.094 | **0.001** | 0.391 |
| Ot215 | Ot515 | 0.137 | 0.262 | 0.187 | 0.195 | **0.032** | **0.001** | 0.113 | 0.247 | **0.001** | 0.090 |
| Ot311 | Ot515 | 0.183 | **0.012** | 0.094 | 0.684 | 0.187 | 0.684 | 0.983 | 0.189 | **0.001** | **0.030** |
| Ot409 | Ot515 | 0.143 | 0.342 | 0.211 | 0.328 | 0.247 | 0.094 | 0.639 | 0.230 | 0.098 | **0.001** |
| Ot208 | Ot515 | 0.209 | 0.088 | **0.001** | 1.000 | 0.441 | 0.247 | 0.247 | 0.211 | **0.001** | 0.391 |
| Ot212 | Ot515 | 0.732 | 0.060 | 0.195 | **0.001** | **0.001** | **0.047** | 0.802 | 0.225 | **0.020** | 0.401 |

**Boldface** values are significant after FDR corrections.

Table S3. Number (N) of male and female Chinook salmon reintroduced from 2007 to 2010. Mean ($\bar{k}$) and variance ($V_{\bar{k}}$) estimates for fitness are summarized.

| Year | Sex | N | Mean | Variance |
|---|---|---|---|---|
| 2007 | Female | 318 | 0.89 | 2.30 |
| 2007 | Male | 428 | 0.71 | 2.40 |
| 2008 | Female | 288 | 0.80 | 2.12 |
| 2008 | Male | 585 | 0.39 | 1.00 |
| 2009 | Female | 604 | 0.16 | 0.22 |
| 2009 | Male | 782 | 0.13 | 0.18 |
| 2010 | Female | 264 | 0.26 | 0.50 |
| 2010 | Male | 484 | 0.18 | 0.39 |

Table S4. Estimates of the inbreeding ($N_{bI}$) and variance ($N_{bV}$) effective number of breeders from estimates of fitness, as well as from multilocus genotypes from $F_1$ offspring in $LDN_e$ ($N_{bD}$) for each cohort reintroduced from 2007 to 2010. Estimates of $N_{bD}$ included 95% jack-knifed confidence intervals.

| Cohort | $N_{bI}$ | $N_{bV}$ | $N_{bD}$ |
|---|---|---|---|
| 2007 | 212 | 149 | 157 (143-172) |
| 2008 | 206 | 126 | 160 (146-176) |
| 2009 | 350 | 81 | 223 (183-284) |
| 2010 | 125 | 52 | 171 (145-206) |

## General discussion

Reintroduction programs are an important conservation strategy that is not well understood because many programs fail (Fischer and Lindenmayer, 2000, IUCN/SSC, 2013). This issue is of particular concern for fishes because research on species within this taxon is underrepresented in the reintroduction literature (Seddon *et al.*, 2005). My dissertation contributes to our collective knowledge regarding reintroductions, with a particular emphasis on fish, as well as migratory and philopatric species. First, I found that release location and date have little to no effect on two different measures of fitness for adults reintroduced above Cougar Dam. In addition, I found that hatchery origin (HOR) fish are, on average, less fit in the wild compared to their natural origin (NOR) counterparts, which corroborates similar findings from several other studies (reviewed by Christie *et al.*, 2014). What was particularly striking about my data was that I may have found a possible explanation as to why HOR males are less fit in this study system, in that they are less fit in the wild, in part, because they are smaller, or perhaps younger. In addition to assessing factors that associate with individual fitness, I also evaluated a measure of population productivity, cohort replacement rate (CRR). I found that cohorts reintroduced above Cougar Dam were not replacing themselves. My findings indicate that continued supplementation is necessary to ensure the viability of this population until sufficient management actions are taken to address issues causing the low numbers of $F_1$ adult offspring returning to the Cougar Trap. Given the low CRRs based on anadromous returns in the system, I assessed how much non-anadromous life history tactics contribute to population productivity. The genetic pedigrees that I assembled from my first two chapters, which encompassed multiple generations, were used to identify adult offspring produced by anadromous mate pairs. I developed a

grandparentage script that accounted for one parent's genotypes when assigning grandparent pairs (anadromous mate pairs) to grandoffspring. I found that both precocial males (age 1 or 2), as well as adfluvial adults (> age 2) contributed offspring to the reintroduced population. Adfluvial adults are a rare and little studied Chinook salmon life history tactic (but see Romer and Monzyk, 2014). Using the grandparentage assignments, I contributed new information on the age-at-maturity and fitness for adfluvial adults. Finally, I evaluated the genetic variation in the population after a single generation in the wild. I found consistent evidence that alleles were lost between founding cohorts and their $F_1$ offspring returning back to the Cougar Trap. My results suggest that both demographic and genetic factors should be considered as soon as a reintroduced population is founded, rather than initially focusing on demographic factors, and genetic variation multiple generations after the founding event.

## *Factors associated with spawner success*

Little is known about the efficacy of methods employed during trap and transport reintroduction programs. Previous research suggests that the location and timing of spawning are important factors for the fitness of adult salmonids (Dickerson *et al.*, 2002, Groves and Chandler, 1999). I tested the prediction that the date adults were released during a trap and transport reintroduction was associated with individual fitness. I found that release date had weak and inconsistent effects on the fitness of reintroduced adults, which is consistent with other studies that sampled salmonids at weirs or at fish ladders (Anderson *et al.*, 2013, Evans *et al.*, 2015, Williamson *et al.*, 2010). Collectively, these studies data indicate that release date may not be a major factor to consider for salmonid reintroductions. I also tested the prediction that the release locations were associated with fitness of reintroduced

adults. The location adults reintroduced above the dam ranged from close to Cougar Reservoir to tens of kilometers above the dam. I consistently found that mean fitness did not differ among release locations, which may suggest that each release location was equally sufficient for reintroduction. Alternatively, the location adults are released may not be a major factor to consider because fish may move from their initial release site (Zymonas *et al.*, 2010). I was the first to publish information on the effects of release location and date for trap and transport reintroductions.

## *Demographic considerations*

One important factor to the initial success of a reintroduced population is productivity. I evaluated population productivity using CRR, which is defined as the number of spawners produced by a spawner (Botsford and Brittnacher, 1998). CRRs for adults reintroduced above Cougar Dam in 2007 and 2008 were to determine if the cohorts were producing enough adult offspring to at least maintain the population's current size. However, my genetic monitoring approach indicated that neither the 2007 or 2008 cohort met replacement. A genetics-based approach was essential because I found that nearly half of the adults returning to the Cougar Trap were not produced above the dam. Estimates of CRR would have been falsely inflated had it been assumed that all adults collected at the Cougar Trap were progeny of previously reintroduced cohorts. The reintroduction above Cougar Dam is currently a source immigrants and genetic variation for the larger metapopulation because it founded by surplus HOR adults, and continues to be supplemented by HOR adults annually due to low CRRs.

All other studies on population productivity for salmonid reintroductions to date indicate that populations are replacing themselves over time (Anderson *et al.*, 2015, Evans *et*

*al.*, 2015). These studies suggest that there may be something uniquely different about the reintroduction above Cougar Dam. A hypothesis to explain the discrepancy between CRRs in my study system and others is that juvenile passage through Cougar Dam is worse compared to other dams. One potential management action would be to provide juveniles safe alternatives for passage through the dam because both of the currently available options are lethal to most juveniles that attempt to pass the dam. The first option requires that juvenile fish pass through the turbines at the base of the dam, which is dangerous due to the high probability of blade strikes and barotrauma. Alternatively, juveniles can pass the dam by swimming through the labyrinth of pipes within the regulating outlet, a structure used to make the stream temperature below the dam seasonally representative; however this route concludes with a near vertical 70 meter descent ending on a cement pad.

Anderson *et al.* (2015) suggest that CRRs were downwardly biased because not all fish home exactly to their native stream, which they termed a spill-over effect. I attempted to account for this spill-over effect by trying to assign carcasses, collected in the South Fork McKenzie River below the dam, to previously reintroduced cohorts. However, I found that few carcass samples were $F_1$ offspring produced above the dam. There could be other $F_1$ offspring that are unaccounted for in CRRs because I did not evaluate carcasses sampled outside the South Fork McKenzie River due to financial and time limitations. More research should be done to evaluate the extent of the possible spill-over effect to determine how much CRRs may increase.

The genetic monitoring approach used here revealed that most adults collected later in the spawning season were not produced above the dam. These fish were likely produced elsewhere in the McKenzie River, which is an issue because Chinook salmon in this system

are considered a genetic legacy stock. Given low CRR estimates for above dam outplants and uncertainty surrounding juvenile passage through the dam, managers took action in 2013 and implemented the Late Season Downstream Release (LSDR) method to limit the transport of NOR immigrants into the above dam population. I found that LSDR method was successful at minimizing the number of NOR immigrants, thereby protecting this genetic legacy stock to undue risk associated with reintroduction. This research highlights an example where genetic monitoring revealed important information about the reintroduced population that was immediately used to improve its management. I advocate that other reintroductions use genetic monitoring practices because they provide valuable, and sometimes unforeseen, information that can help to improve the efficacy of reintroduction programs.

I found that release location and date had little to no effect on the fitness on reintroduced adults. My results were consistent across both adult assignments to juveniles and adult offspring returning to spawn in subsequent years. This consistency is important because adult to adult genetic pedigrees take many years to assemble, which can delay management actions. The efficacy of other reintroduction programs may be improved by correlating factors associated fitness, as measured by assignments to immature life stages, because this approach could provide information to managers more quickly.

## *Contribution from rare or difficult to sample life history tactics*

Some life history tactics contribute to population resiliency by varying individuals in space and time (Greene *et al.*, 2009). A commonly known life history tactic in Chinook salmon are precocial males, who forego an anadromous migration and become sexually mature in freshwater at age 1 or 2 (Taylor, 1989). Another less well known life history tactic are adfluvial Chinook, which are adults that also live entirely in freshwater that become

sexually mature at age 3 or older. Adfluvial Chinook have rarely been documented in the literature, but two recent articles suggest they may be more common in reservoirs than previously thought (Perales *et al.*, 2015, Romer and Monzyk, 2014).

CRRs in the Cougar Dam system are biased low, in part, because they do not account for precocial males or adfluvial adults. I sought to determine if non-anadromous life history tactics were contributing to the population above Cougar Dam. Given that I have multi-generational genetic pedigrees, I searched for missing parents using a grandparentage assignment approach (Christie *et al.*, 2011). I used mate pairs identified in the genetic pedigrees as my known grandparent pairs. I then developed an R script that assigned grandparent pairs to grandoffspring after accounting for one parent's genotypes. Through this conservative approach I identified precocial males, as well as adfluvial males and females that produced juvenile offspring in the population. In fact, some grandparent pairs assigned to as many as 27 age-0 juveniles, indicating that some adfluvial Chinook salmon may be just as successful at reproduction as anadromous adults. My assignments suggest that most adfluvial adults reproduce at age 4 or 5, and that the number contributing to the population each year is low, likely less than 100. However, CRRs in the system were still well below one even after accounting for adfluvial Chinook salmon identified with the grandparentage approach.

Grandparentage assignments enabled me to contribute valuable biological information about adfluvial Chinook salmon, as well as improve estimates of population productivity. The grandparentage script I developed can be broadly applied to other populations with multi-generational genetic pedigrees to identify rare and difficult to sample life history tactics.

## *Genetic considerations*

To date, more than 19,000 Chinook salmon have been genotyped at 11 highly polymorphic microsatellite loci for the reintroduced population above Cougar Dam. I have used these genotypes to better understand factors driving individual fitness, evaluate population productivity, and to study difficult to sample life history tactics. Given this immense about of genotypic data, I wanted to focus on the genetics of the reintroduced population as a final topic for my dissertation. Typically reintroductions focus on demographic aspects of a population first and then later on the genetic variation within the population. However, the genetic variation within the population can be important when the population is being established (Agashe *et al.*, 2011, Hufbauer *et al.*, 2013). I found that rare alleles observed in the founding cohorts were lost after a single generation in the wild. Those results were consistent across cohort and overlapping generation approaches for both putatively neutral and adaptive loci. Most of the genetic variation lost was likely due to random genetic drift, yet some alleles that were lost may have been influenced by low CRRs, biased sex ratios, and variation in fitness, which all can be considered bottlenecks for genetic variation. Surprisingly, the estimate of effective population size was relatively high (833) and suggests that most of the genetic variation within the population should maintained, assuming $N_e$ is maintained at this high level.

The genetic pedigree approach applied here also enabled the study of genetic variation within both HOR and NOR immigrants to the population. I found that both could restore genetic variation that was lost between founding cohorts and their $F_1$ offspring. Both sources of immigrants have their advantages and disadvantages. HOR immigrants contained valuable genetic variation, yet I also showed that HOR adults are less fit compared to their

NOR counterparts, likely due to fork-length and/or age. Continued reintroduction of HOR adults may slow the population's adaptation back to the wild environment. However, without continued reintroduction of HOR fish, the population would not exist because CRRs are low. NOR immigrants may be adapted to wild environment, but they are considered a genetic legacy stock. As a result, managers have limited the number of NOR immigrants in the population to protect these fish from uncertainties surrounding the reintroduction.

## *General conclusion*

My research contributes to the growing body of literature on reintroductions. Several key insights throughout my PhD include: 1) Release location and date had little to no effect on the fitness of reintroduced Chinook salmon above Cougar Dam; 2) Cohorts did not replace themselves and that most adults returning later in the spawning season were not produced above the dam; 3) Adfluvial Chinook salmon contribute to the population, but CRRs are still well below one even after account this rare life history and precocial males; and 4) I observed a reduction in genetic variation between founding cohorts and $F_1$ offsping. These findings are most applicable to the study site that I evaluated, but considering their consistency with other studies, information I have described in my dissertation could potentially be applied to other salmonid reintroductions, and more generally, to other migratory, philopatric fishes. Reintroductions for many threatened or endangered species could benefit from genetic monitoring approaches. However, with the genomics age upon us, next-generation sequencing based approaches to monitoring populations will provide even more insight on factors important to the success of reintroductions. If such approaches were to be applied to the reintroduction above Cougar Dam, perhaps we could better understand

how supportively bred organisms adapt back to the “wild” and how adfluvial adults arose from anadromous mate pairs.

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## Appendix A.

### *Genotyping and parentage assignments (continued)*

We isolated total genomic DNA from tissue samples using a glass-fiber protocol developed by Ivanova et al. (2006) and amplified 11 highly polymorphic microsatellites using the polymerase chain reaction (PCR): Ots201, Ots208b, Ots209, Ots211, Ots212, Ots215, Ots249, Ots253, Ots311, Ots409, and Ots515 (Banks et al., 1999, Greig et al., 2003, Naish and Park, 2002, Williamson et al., 2002). Most reintroduced adults were genotyped at 10 or more loci (99%, n= 6,079/6,119). We also genotypically sexed all adults using the sex-linked marker Oty3 because it has been shown to accurately identify the sex of Chinook salmon in the Willamette River basin (Brunelli et al., 2008). We visualized PCR products on an ABI 3730XL DNA analyzer (Applied Biosystems, Inc., Foster City, CA) and size scored genotypes using GeneMapper software (Applied Biosystems, Inc.). We empirically estimated genotyping error rate by re-genotyping one random sample from each 96-well plate. Genotyping error was estimated by summing the number of discordant allele calls and dividing by the total number of allele calls made. We performed all genetic analyses at the Marine Fisheries Genetics Laboratory at Hatfield Marine Science Center, Newport, Oregon.

We evaluated the power of microsatellites to correctly infer parent-offspring relationships by calculating average number of alleles per locus, as well as observed and expected heterozygosity (Nei, 1987) . We then calculated non-exclusionary power for the first parent, second parent, and parent pair (Jamieson and Taylor, 1997), as well as expected number of false parent-offspring pairs when they mismatched at 0-2 loci (Christie, 2010).

We identified parent-offspring pairs using CERVUS (Kalinowski et al., 2007, Marshall et al., 1998). The likelihood approach applied in this algorithm simulated genetic data

comparable to the given system when estimating log-likelihood statistics (LOD) and Δ scores, which were then used to assess confidence in assignments. Our simulations used the default setting for offspring (n=10,000) and the number of male and female adults in the actual datasets being analyzed. We conservatively set the proportion of parents sampled for each sex to 70%, given that nearly all adults were sampled for genetic analysis. Finally, genotyping error was set to our empirical estimate of 2%. We accepted all parent-offspring pairs with ≤1 genotypic mismatches based on our power analysis (Table S3).

Several parent-offspring assignment algorithms have been developed, each with their own benefits and biases (Harrison et al., 2013). Walling et al. (2010) suggested an approach of combining results from alternate parent-offspring assignment methods as a means of increasing accuracy and completeness of genetic pedigrees. Accordingly, we also used COLONY (Jones and Wang, 2010) to assign parents to offspring because Harrison et al. (2013) noted COLONY performs well at correctly identifying parent-offspring pairs. We compared CERVUS and COLONY genetic pedigrees and found they were 96 ± 3% concordant in parent-offspring assignments. For observed differences between assignment programs, we chose the assignment used in the final genetic pedigree based on several criteria. In brief, assignments with the lowest number of mismatches between parent and offspring took precedence. In addition, offspring that assigned to both parents in a given year took precedence over assignments to a single parent in a different year, based on non-exclusionary power calculations (Table S3). If the number of mismatches were the same the COLONY based assignment took precedence (see Harrison et al., 2013). Composite genetic pedigrees following these criteria were used for all subsequent analyses.

We found that 82% (262/319) and 89% (217/243) of offspring produced by the 2007 and 2008 reintroduced cohorts were assigned to both parents, respectively. We included any adult offspring that assigned to only a single parent in our analysis, which is not unusual in a parentage study (e.g. Araki et al., 2009, Evans et al., 2015). Four reasons to expect some returning offspring may only assign to a single parent include: 1) Some anadromous adults (n=4) were not tissue sampled, two of which could have been potential parents; 2) the 1% of fish that were genotyped at fewer than 10 loci may have resulted in false parental exclusions; 3) our 2% genotyping error may have also resulted in falsely excluding some true parents; and 4) some unsampled precocial males could exist in the system. We considered all anadromous adults that returned to the trap and assigned to at least one anadromous parent previously reintroduced above the dam as offspring because our molecular markers provide high power to correctly infer the existence of a true parent (Table S3) and the four explanations for "missing" parents (see above) each likely contribute to a some offspring assigning to one parent. In addition, missing precocial males do not bias cohort replacement rate estimates because we based our calculations only on females. We also do not think our fitness estimates are substantially biased because we found similar effects for release location and date as compared to other studies that used comparable measures of fitness, and as noted above, most returning adult offspring assigned to both parents.

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