Assessing Effects of Supplementation on Fitness of Sockeye Salmon in Auke Creek, AK

Final Report for Phase 2, Year 2
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prepared by

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Executive Summary

Three years of experimental hatchery supplementation were conducted in 2011-2013 in order to quantify fitness differences between wild and hatchery-origin sockeye salmon within the US-Canada transboundary region subject to the Pacific Salmon Treaty. All adult salmon ascending the weir at Auke Creek, Juneau, Alaska, have been sampled for genetic tissue since 2008. A panel of SNP and single-tandem repeat (STR) loci are used to assign adult offspring back to parents, allowing for a full enumeration of fitness, defined as the number of returning adult offspring per parent. In this year of the project (designated Phase 2, Year 2), we genotyped the remaining adult offspring that returned in 2016 (n = 600), and genotyped ~35% (n = 1,296) of the adult offspring that returned in 2017. We assigned these offspring to candidate parents from relevant brood years spanning 2009 - 2014, and produced estimates of relative adult returns per spawner for the dominant offspring ages (4 and 5 years old) between hatchery and wild adults. For brood year 2011, for which we have near-complete accounting of adult offspring, hatchery parents produced on average 9.6 adult offspring per spawner, while hatchery parents produced 0.58 adult offspring per spawner, yielding a relative return rate (hatchery:wild) of 16.6. The accounting for 2012 is not yet complete, with only 35% of the age-5 offspring genotyped, but thus far hatchery parents produced substantially more offspring per spawner than did wild parents. Final quantification of returns per spawner, as well as the distribution of individual fitness values between hatchery and wild parents, will be completed for the 2011 brood year after the completion of the 2017 adult offspring genotypes (scheduled for 2019-2020) and for all brood years following genotyping of 2018 and 2019 adult offspring (to be proposed for 2020-2021).

Project Rationale and Relevance to Pacific Salmon Commission

The overarching goal of this project is *to quantify differences in fitness between wild and hatchery-origin sockeye salmon* in Auke Creek, Alaska, using genotypic data and parentage assignment over three generations of experimental hatchery supplementation. Secondary goals of this research are to quantify changes in genetic diversity and population structure in the wild sockeye salmon population as a result of three generations of hatchery supplementation. Results of this study will provide information critical for assessing the relative costs and benefits of hatchery supplementation in managing sockeye salmon populations subject to the Pacific Salmon Treaty (PST).

Evaluating the relative fitness (defined as lifetime survival and mating success) of hatchery and wild salmon is important for several reasons. First, if hatchery individuals have low fitness, hatchery supplementation has a low return on investment and represents fishery management resources that might be better directed elsewhere. Furthermore, in cases where hatchery-origin fish are allowed to interbreed with wild members of the supplemented population, low relative fitness of hatchery-wild hybrid fish can reduce the mean fitness of the supplemented population (Araki et al. 2009). High relative fitness of hatchery fish can increase the variance in reproductive success,

thereby reducing the genetically effective size of the supplemented population (Ryman and Laikre 1991, Wang and Ryman 2001). Consequently, adequate monitoring of the effectiveness of supplementation programs requires quantification of the relative fitness of hatchery and wild individuals (Fraser 2008).

This project addresses Strategic Objective 3 of the Northern Fund, "recognizing that a carefully designed enhancement program would contribute significantly to the restoration of depressed natural stocks and assist the Parties in achieving optimum production" (PSC). This project has direct relevance to the Comprehensive Salmon Enhancement Plan for Southeast Alaska, which states that the purpose of the state's enhancement program is "to benefit the public by providing additional harvest opportunities to regional salmon fisheries *without adversely affecting natural stocks*" (ADF&G 2004, p. 1; italics added). Alaska's Genetics Policy for fishes acknowledges that the policy is constrained by the "limited amount of information available on the genetic impacts of salmon enhancement on wild stocks" (Davis et al. 1985, p. 1). Similarly, Canada's Policy for Conservation of Wild Pacific Salmon (Fisheries and Oceans Canada, 2005) calls for a biological risk framework for assessing impacts of enhancement on wild stocks, but little data exist to fully implement such assessments. The Auke Creek sockeye project is providing crucial empirical data to inform implementation of policies in both Alaska and British Columbia. Successful implementation of each proponent's conservation and management policies is essential for the success of the PST; in addition, effective enhancement and supplementation activities are directly related to harvest and harvest allocation under the PST.

Phase 1 Work

Phase 1 spanned three years of work (2014 - 2017) that occurred after Megan McPhee took over lead PI role from Bill Smoker. The objectives of this phase were to demonstrate the accuracy of parentage assignment in Auke Creek sockeye salmon with existing genomic resources; identify a cost-effective genetic marker panel for parentage assignment; complete genotyping of adults from 2008-2014; partially genotype adult returns from 2015; and assign adults returning in years 2014 and 2015 to candidate parents in unsupplemented years 2008-2010 and supplemented years 2011-2012. The final report on Phase 1 was submitted to the Pacific Salmon Commission in July 2017 and is available upon request from Megan McPhee.

Phase 2 Objectives

The ultimate goal of Phase 2 is to calculate relative fitness of hatchery- versus naturally spawned sockeye salmon from the three brood years of experimental supplementation (2011-2013). To accomplish this goal, Phase 2 consists of completing the genotyping for all first-generation progeny from the three brood years of experimental supplementation; this includes all adults that returned or will return to Auke Creek from 2014 - 2019 (Figure 1). Each year of Phase 2 has two objectives: first, to genotype a set number of adults (with that number determined by budget), and

second, to assign those genotyped adults back to parents (hatchery or wild). Complete fitness accounting is not possible until we have fully completed genotyping all returning adults from a given brood year (Auke Creek sockeye salmon exhibit 7 different freshwater/marine age combinations, and return at four different ages; see Figure 1). However, for each year of the project we do quantify how many returning adults originate from wild versus hatchery parents.

The objectives for Year 1 of Phase 2 were to genotype 1,900 adults that returned in 2016, assign those individuals back to parents, and to enumerate the returns per spawner (wild vs. hatchery) to date for relevant brood years. Results of these objectives were summarized in a final report submitted in July 2018.

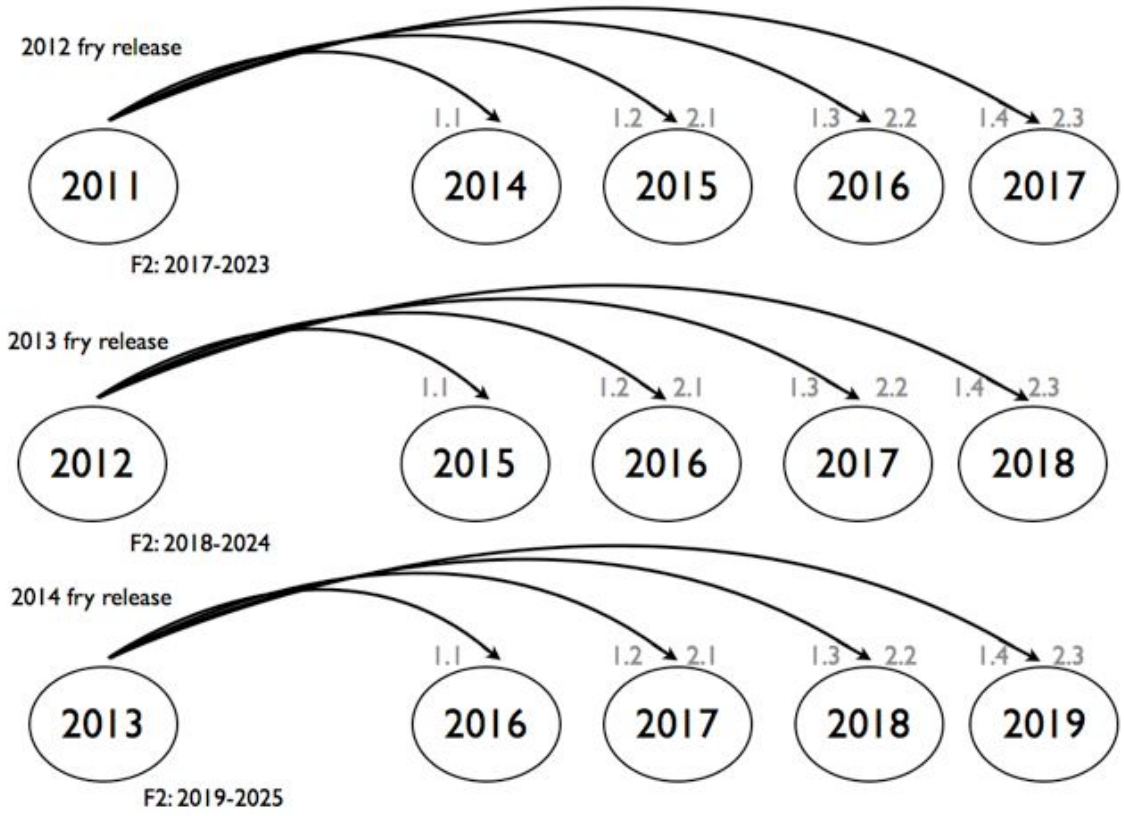

Figure 1. Schematic showing potential return years of first-generation adults resulting from brood years 2011-2013 (fry releases 2012-2014, respectively). Numbers above return-year ovals represent age of returns (e.g., 1.1 = one winter in freshwater, one winter in saltwater, age 3).

For this reporting year (Phase 2, Year 2), our specific objectives were to:

1) genotype a total of 1,900 adult sockeye salmon - the remaining adults from 2016 (N = 619) and ~35% of the adults that returned in 2017 (N = 1,281);
2) assign genotyped adults from 2016 and 2017 back to parents;
3) enumerate adult offspring per spawner for relevant brood-year parents and return-year offspring to date.

Approach

The overall approach of the study is to sample all adult sockeye salmon ascending the weir at Auke Creek; use genotypic information from 48 SNPs and 9 STR loci to assign adult offspring back to parents; and then use this information to quantify the relative fitness of hatchery and natural-origin sockeye salmon over three experimental brood years (2011-2013). 'Fitness' is defined as the number of adult offspring returning to Auke Creek per parent. Detailed explanation of the methods are provided in Appendix A.

Results & Discussion

We obtained genotypic data for 600 individuals from 2016 and 1,296 individuals from 2017. The 2016 individuals were added to those genotyped in the previous year, allowing us to assign the full 2016 returning adults back to parents from brood years 2010 - 2013. We assigned the partial return year 2017 (those genotyped this year; ~ 35 % of total return in 2017) back to brood years 2011 - 2014. Individuals missing > 25% of their genotypic data were not included in these analyses. We accepted an assignment if it had a posterior probability (p) > 0.90. We used 'unassigned (UA)' to refer to individuals who were assigned to no parent with $p > 0.90$, and 'not assigned (NA)' to refer to assignments (either to a parent in the candidate set or to no parent) with $p < 0.90$. Assignments were made to a parental class (brood year and origin) when at least one parent was assigned with $p > 0.90$.

For the 2016 return year, 2,499 individuals were genotyped in total, 97.2% of which had genotypic data for > 25% of their loci. These individuals were assigned to candidate parents from 2010 (n = 2,062), 2011 (n = 2,422), 2012 (n = 1,554), and 2013 (n = 2,055). For the 2017 return year, 1,296 individuals were genotyped, 97.5% of which had genotypic data for > 25% of their loci. These individuals were assigned back to candidate parents from brood years 2011-2013 (same sample sizes as for return year 2016) and brood year 2014 (n = 536; note that genotypic data for brood year 2014 is incomplete due to budgetary constraints). The results of these assignments are summarized in Table 1; assignment results from previous project years (return years 2014 and 2015) are also included.

The earliest year that hatchery progeny would be expected to return was 2014 (they would be the three-year-old offspring of the 2011 brood year). Despite our efforts to focus genotyping efforts on the youngest fish returning in 2014, we did not detect any hatchery progeny until 2015. Hatchery progeny were abundant in return years 2016 and 2017, representing almost one quarter (24.8%) of the 2017 returns genotyped so far. Because we do not yet have complete returns genotyped for the focal brood years 2011-2013, we can't produce a comparative estimate of lifetime fitness of hatchery versus wild adults, but we can estimate returns per spawner of the predominant age classes of returns for 2011 and 2012; these are summarized in Table 2. Note that these estimates are based on the number of potential spawners genotyped, rather than census counts made at the weir. The relative return rate of adult offspring per hatchery parent was well above 1 (which would indicate equal reproductive success between the two spawner types), being 16.6 times the rate of returning adult per wild spawner in 2011, and 105.9 times that of wild spawners in 2012. We caution that not all of the 2017 returns have been genotyped, so return rates estimated for age 5 fish from brood year 2012 are preliminary. However, the samples chosen for genotyping would have to be heavily skewed towards hatchery parents to account for such a large relative return rate of hatchery fish from brood year 2012, and we cannot think of a mechanism leading to such dramatic skew.

It appears that the wild spawners in 2012 had particularly low reproductive success; 2018 would be the last year when their offspring were expected to return, and total returns in that year were historically low (only 923 adults were counted at the weir). Interestingly, 1 adult returning in 2016 and 12 adults returning in 2017 were assigned to a parent that was radio-tagged by USFWS (in a separate study) prior to spawning. To our knowledge, this is the first documented successful production of surviving offspring by radio-tagged salmon in the wild, which is encouraging for the use of radio-telemetry to study migration and spawning behavior in Pacific salmon.

The next phase of the project (currently funded 1 July 2019 - 30 June 2020) will finalize genotyping of the 2017 samples (n = 2,385), allowing us to produce a full accounting of the relative fitness of hatchery and wild fish from brood year 2011, and allowing us to estimate relative return rate for the predominant age classes from the 2012 brood year. Given the low adult returns in 2018 (< 1,000) and the anticipated low returns in 2019, we envision proposing one final year of work to the Northern Fund (year 2020) to genotype all returning adults in 2018 and 2019, and finalize fitness accounting for brood years 2012-2013.

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Table 1. Number (and percentage on a return-year basis) assigned to each parental class for offspring return years 2014 - 2017. Predominant age classes (4 & 5 years) highlighted in blue.

	2014*	2015	2016	2017*
2008 (all wild)	37 (6.9)	---	---	---
2009 (all wild)	379 (70.7)	2,308 (65.9)	---	---
2010 (all wild)	39 (7.3)	731 (20.9)	643 (25.7)	---
2011 hatchery	**0** **(0)**	**64** **(1.8)**	**328** **(13.1)**	**59** **(4.6)**
2011 wild	44 (8.2)	200 (5.7)	1,189 (47.6)	755 (58.3)
2012 hatchery	**---**	**1** **(0.03)**	**103** **(4.1)**	**236** **(18.2)**
2012 wild	---	6 (0.17)	15 (0.6)	138 (10.6)
2013 hatchery	**---**	**---**	**0** **(0)**	**27** **(2.1)**
2013 wild	---	---	8 (0.3)	8 (0.6)
2014 (all wild)*	---	---	---	1 (0.08)
UA/UA	4 (0.8)	9 (0.26)	7 (0.3)	3 (0.15)
UA/NA	26 (4.9)	150 (4.3)	120 (4.8)	26 (2.6)
NA/NA	6 (1.1)	23 (0.66)	18 (0.7)	3 (0.2)
Missing > 25% genotype data	1 (0.2)	11 (0.3)	67 (2.7)	33 (2.5)
Total	536	3,503	2,499	1,296

* incomplete genotyping

Table 2. Estimated adult returns per spawner, hatchery vs. wild, for the two predominant offspring age classes (4 and 5). No attempt was made to account for sex ratio as field-called sex of wild fish was uncertain. Relative return rate (RRR) was the return rate of age-4 and -5 offspring for hatchery spawners divided by that of wild spawners. Note that only ~35% of the 2017 returns have been genotyped, so return rates including age-5 fish are not comparable between brood years.

	Return Rate by Offspring Age			
Brood year	Age 4	Age 5	Ages 4 & 5	$RRR_{4\&5}$
2011 hatchery	64/41 = 1.56	328/41 = 8	392/41 = 9.6	
2011 wild	200/2381 = 0.08	1189/2381 = 0.50	1389/2381 = 0.58	16.6
2012 hatchery	103/32 = 3.22	236/32 = 7.38	339/32 = 10.59	
2012 wild	15/1522 = 0.010	138/1522 = 0.09	153/1522 = 0.10	105.9

Appendix A. Detailed Methods

Hatchery Supplementation

Experimental supplementation of sockeye salmon took place in Auke Creek over three successive brood years (2011-2013), and was conducted under a separate contract with the Northern Fund (J. Joyce, NOAA, principal investigator). During each brood year, ~30 females and ~15 males were taken from the returning wild sockeye salmon adult population for use in hatchery crosses. These adults were sampled for genetic tissue (by removing an axillary process) and then held in tanks at the weir facility in water from a deep lake intake, mimicking their natural tendency to hold deep in the lake prior to final maturation and spawning. Adults were spawned in September. Embryos were incubated over the winter, ponded in early spring, and released into the lake as young-of-the-year fry. Based on scale analysis of Auke Creek sockeye salmon samples (J. Joyce and S. Taylor, NOAA, unpubl. data), adults are expected to return 3-6 years after being spawned. Therefore, first-generation hatchery individuals are expected to return during the years 2014-2019.

Adult sampling and genotyping

All adult sockeye salmon were visually identified as male or female (although field identification is not 100% accurate; J. Joyce, NOAA, unpubl.) and sampled for genetic tissues as they were passed upstream through the Auke Creek weir. Tissue samples were sent to the ADF&G Gene Conservation Laboratory (GCL) for genotyping. Genomic DNA was extracted using DNeasy ® 96 Tissue Kit (Qiagen). Samples were genotyped at 9 short tandem repeat (STR; also known as microsatellites) and 48 single nucleotide polymorphism markers (SNPs).

STRs were amplified, electrophoresed, and scored (i.e., genotypes confirmed) at ADF&G. Amplification was carried out in 10 µl reaction volumes [10 mM Tris-HCl, 50 mM KCl, 0.2 mM each dNTP, 0.5 units Taq DNA polymerase (Promega, Madison, WI)] using an Applied Biosystems (AB, Foster City, CA) thermocycler. PCR fragments were analyzed on an AB 3730 capillary DNA sequencer. A 96-well reaction plate was loaded with 0.5 ul PCR product along with 0.5 ul of GS500LIZ (AB) internal lane size standard and 9.0 ul of Hi-Di (AB). PCR bands were visualized and separated into bin sets using AB GeneMapper software v4.0. Automated binning was subsequently confirmed or corrected manually.

SNP assays were conducted at ADF&G. Extracted DNA was loaded into two Fluidigm® 192.24 Dynamic Arrays in a post-PCR laboratory at ADF&G. Groups of 192 samples and 24 assays were systematically combined into 4,608 parallel reactions on each array. Each reaction was a mixture of 4µl of assay mix (1x DA Assay Loading Buffer (Fluidigm), 10x TaqMan® SNP Genotyping Assay (Applied Biosystems), and 2.5x ROX (Invitrogen)) and 5µl of sample mix (1x TaqMan® Universal Buffer (Applied Biosystems), 0.05x AmpliTaq® Gold DNA Polymerase (Applied Biosystems), 1x GT Sample Loading Reagent (Fluidigm) and 60-400ng/µl DNA) combined in a

7.2nL chamber. Thermal cycling was performed on an Eppendorf IFC Thermal Cycler as follows: 70°C for 30 min for "Hot-Mix" step, initial denaturation of 10 min at 96°C followed by 40 cycles of 96° for 15 sec and 60° for 1 min. The Dynamic Arrays was read on a Fluidigm® EP1™ System or BioMark™ System after amplification and scored using Fluidigm® SNP Genotyping Analysis software.

Assays that failed to amplify on the Fluidigm system were reanalyzed on the Applied Biosystems platform. Each reaction on this platform was performed in 384-well reaction plates in a 5μL volume consisting of 5-40ng/μl of template DNA, 1x TaqMan® Universal PCR Master Mix (Applied Biosystems), and 1x TaqMan® SNP Genotyping Assay (Applied Biosystems). Thermal cycling was performed on a Dual 384-Well GeneAmp® PCR System 9700 (Applied Biosystems) as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92°C for 1s and annealing/extension temperature for 1 min. The plates were scanned on an Applied Biosystems Prism 7900HT Sequence Detection System after amplification and scored using Applied Biosystems' Sequence Detection Software (SDS) version 2.2.

Quality control of genotypic data

Quality control analysis (QC) was conducted at ADF&G to identify laboratory errors and to quantify our genotyping error rate (which is necessary for downstream parentage analysis; see below). The QC analyses were performed by staff not involved in the original genotyping. ADF&G staff re-extracted 8% of sample individuals and assayed them for the same markers assayed in the original round of genotyping. The discrepancy rate (which identified DNA extraction, assay plate, and genotyping errors) was calculated as the number of conflicting genotypes divided by the total number of genotypes compared. The discrepancy rate was then divided by two to give the genotyping error rate.

Parentage analysis

Parentage analysis consists of using genotypic information to identify, from a pool of candidate parents, the true parents of a given individual. This analysis is fundamental to quantifying fitness differences between hatchery and wild sockeye salmon, as we have defined individual fitness for this study as the number of returning adult offspring (over all potential return years) produced by a focal individual. The earliest we will be able to estimate individual fitness is after the 2017 adult return year (for brood year 2011), so here we limit describe our methods for parentage assignment.

Parentage analysis relies on simple Mendelian inheritance to assign offspring to parents based on a combination of exclusion and probabilistic methods. For this study, we used the program *FRANz* (Riester et al. 2009) to assign offspring to parents. Its algorithm uses Mendelian principles in combination with prior information (e.g., genotyping error rate) to determine the maximum likelihood pedigree (i.e., set of parent-offspring triads that best fits the observed genotypic data).

FRANz was our method of choice because it is computationally feasible (Ford et al. 2012, Kodama et al. 2012), can handle both SNP and STR data, and is expected to perform well in situations such as this study, where we are able to sample a very high proportion (approaching 100%, depending on rates of straying and residualism) of potential parents (Almudevar and LaCombe 2012).

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