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

Final Report for Phase 2, Year 1
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prepared by

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Executive Summary

Three years of experimental hatchery supplementation were conducted (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 (designated the first year of Phase 2), we genotyped ~ 1,900 adult offspring that returned in 2016, assigned them to parents in the candidate parent set, and used these assignments, along with previous assignments from samples from return years 2014-2015, to estimate the relative return rate (to date) for hatchery versus wild spawners for brood year (BY) 2011. The oldest progeny of BY 2011, which returned in 2017, have yet to be genotyped. Based thus on preliminary information from the younger age classes (age 3-5 years), we calculated an average of 7.7 adult returns per hatchery spawner compared to 0.48 adult returns per wild spawner. Although these values differ substantially, we caution that these estimates are based on incomplete brood returns as well as incomplete genotyping sampling of the younger age classes thus far due to budgetary constraints. More robust quantification of returns per spawner as well as the distribution of individual fitness values between hatchery and wild parents will be completed for all three brood years once we are able to complete genotyping for returns years 2015-2019.

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." 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

give 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.

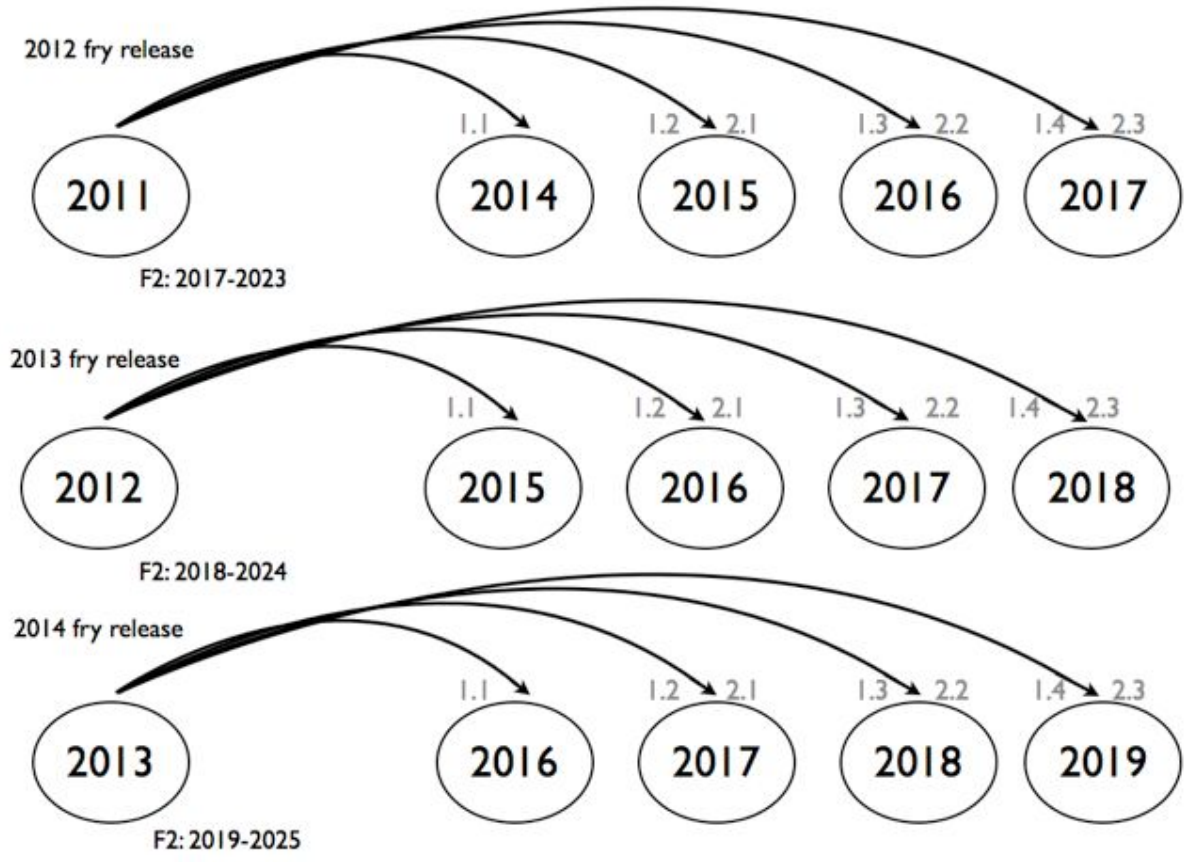

Figure 1. Schematic showing potential return years of first-generation adults resulting from brood years 2011-2013 (fry releases 2012-2014, respectively). Numbers above return-year ovals represent age of returns (e.g., 1.1 = one winter in freshwater, one winter in saltwater, age 3).

For this reporting year (Phase 2, Year 1), our specific objectives were to:

1) genotype 1,900 adult sockeye salmon that returned to Auke Creek in 2016 (~ 75% of total return);
2) assign genotyped adults from 2016 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. Detailed explanation of the methods are provided in Appendix A.

Results & Discussion

We obtained genotypes for 1,898 adults that returned in 2016 (the remaining 2 individuals were hatchery parents from 2011 that had some missing genotypes we attempted to fill in). These individuals were assigned back to a set of candidate parents from brood years 2010-2013 under the following conditions: the number of parents missing from the candidate parent set was assumed to be 5%, the genotyping error rate was assumed to be 0.005, and the maximum number of allowed allelic mismatches in a triad assignment (i.e., mother, father, and offspring) was 1.

We were able to obtain genotypes for a sufficient number of loci to attempt assignment for 1,889 of these individuals. Of these, 1,673 individuals (88.6%) assigned with a triad (mother, father, offspring) posterior probability > 0.9. A remaining 162 individuals (8.5%) assigned to one parent with a posterior probability > 0.9. Nine individuals were identified as likely immigrant strays (i.e., assigned with posterior probability > 0.9 for both parental assignments to no parents in the candidate set). We had DNA quality issues with a small portion of the samples, which prevented us from obtaining genotypes at the STR loci for 103 individuals; this contributed to the relatively high number of individuals classified as 'not assigned' (NA), or assignments made with a posterior probability < 0.9 for one or both parents. Assignments of the 2016 returning offspring by brood year, parental type (e.g., wild versus hatchery parents), and confidence (posterior probability > 0.90) are summarized in Table 1.

We detected hatchery-origin returning adults in 2016 from brood years 2011 (n = 357) and 2012 (n = 79). This represents a high relative return rate for hatchery fish. When combined with parentage information from Phase 1 (return years 2014-2015), we thus far see approximately 7.7 adult returns per hatchery spawner compared to 0.48 adult returns per wild spawner from the 2011 brood year. We caution, however, that these preliminary results are based on return-adult sampling that is thus far incomplete (i.e., we have not received sufficient funds to genotype every returning adult for all possible return years) and not representative with respect to age composition. Differences in age composition between hatchery and wild adults might be expected (e.g., McConnell et al. 2018). It is too soon to estimate these return rates for the 2012 brood year, but we do note the prevalence of hatchery-origin fish in the 2012 brood-year fish that did return in 2016.

Table 1. Distribution of assignments of the 2016 returning offspring (n = 1,889) by brood year, parent spawning type, and assignment confidence. UA ('unassigned') indicates a parental assignment with high confidence ($p > 0.9$) to no parent in the candidate set. NA indicates an assignment with low confidence ($p < 0.9$), either to a parent or to no parent in the candidate set.

Brood Year	Hatchery/ Hatchery	Wild/Wild	Wild/(NA/UA)	UA/UA	UA/NA	NA/NA
2010	---	357	120	---	---	---
2011	248	776	127	---	---	---
2012	79	7	9	---	---	---
2013	0	2	1	---	---	---
Unknown	---	---	---	9	100	54

We had parentage assignments for 85 of the 236 fish that were sampled for age and length (most of these samples have yet to be genotyped). Of those, eight individuals (9.4%) had parentage-based ages that differed from scale-based ages, and in all of these cases the scale-based age was one year older than the parentage-based age. Seven out of these eight individuals were designated scale-age 2.3 and were from brood year 2011 based on parentage; the other individual was from brood-year 2012 based on parentage and was designated scale age 1.3.

Conclusions and Next Steps

We completed this year's objectives, which were to genotype 1,900 fish from the 2016 returns, assign them to parents, and calculate the relative returns per spawner between wild and hatchery fish. There was some missing genotypic data, particularly for STR loci which are more sensitive to DNA quality. Care is being taken going forward to maintain the correct ratio of tissue to ethanol during weir collections. There are only two more years of adult returns (2018 & 2019) left before all first-generation progeny from the three brood years of experimental supplementation have returned as adults. We still need to complete genotyping for return years 2015 and 2016 (which have only been partially genotyped to date due to budgetary constraints) as well as complete genotyping for 2017-2019. Once completed this will represent the only hatchery-wild fitness comparison for sockeye salmon across the species' range, and will be the highest-power (statistically) study of hatchery versus wild salmon fitness comparisons in the State of Alaska.

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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. Cross details are provided in Table A1. 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 was 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. Multiplexes, including specific cycling parameters and locus-specific primer concentrations, are defined in Table A2. 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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Table A1. Individuals (identified by Fish ID) involved in crosses at the Auke Creek Hatchery in brood years 2011-2013. ADFG&G species, location, and year ('SILLY') codes are provided by year; the entire identity of genetic sample is the SILLY Code plus Fish ID; e.g., SAUKE11_8312. Individuals with an asterisk are missing > 75% of genotypic data. In 2012, some females were crossed with more than one male.

Brood Year			Brood Year			Brood Year	
2011 (SAUKE11)			2012 (SAUKE12)			2013 (SAUKE13)	
Female	Male		Female	Male(s)		Female	Male
8312	8301		11001	11003		263898	263931
8313	8301		11002	11003		263899	263931
8314	8302		11004	11006		263900	263932
8315	8302		11005	11006		263901	263932
8316	8303		11007	11009		263902	263933
8317	8303		11008	11009		263903	263933
8318	8304		11010	11012		263904	263934
8319	8304		11011	11012		263905	263934
8320	8305		11013	11015		263906	263935
8321	8305		11014	11015		263907	263935
8322	8305		11016	11012/11015		263908	263936
8323	8306		11017	11012/11015		263909	263936
8324	8306		11018	11020		263910	263937
8325	8306		11019	11020		263911	263937
8326*	8307		11021	11023		263912	263938
8327	8307		11022	11023		263913	263938
8328	8307		11024	11020/11023		263914	263939
8329	8308		11025	11020/11023		263915	263939
8330	8308		11026	11028/11029		263916	263940
8331	8308		11027	11028/11029		263917	263940
8332	8309		11030	11028/11029		263918	263941
8333	8309		11031	11028/11029		263919	263941
8334	8309		11032	11029		263920	263942
8335*	8310					263921	263943
8336	8310					263929	263945
8337	8310						
8338	8311						
8339	8311						
8340	8311						
8341	8303						

Table A2. Details of STR genotyping conditions, including their PCR multiplex and electrophoresis multi- or pseudo-plex status.

PCR Multiplex	Annealing temp. (°C)	Number cycles	Locus	[primer] (μM)	Capillary multiplex
M1	55	35	*Oki100*	0.20	1
			One102	0.10	
			One109	0.10	
			Oki1a	0.06	
			Oki1b	0.06	
M2	56	35	*Oki10*	0.20	2
			One114	0.35	
M3	57	36	*Ssa419*	0.20	
M4	60	35	*One8*	0.20	