PSC Northern Fund
Final Report

Project Number: NF-2014-I-34B

Project Title: Assessing effects of supplementation on fitness of sockeye salmon in Auke Creek, AK, Year 1 (ADFG Component)

Project Manager: Sara Gilk-Baumer, Serena Rogers Olive, and Lisa Fox, Alaska Dept. of Fish and Game - Gene Conservation Laboratory, (907) 267-2535, sara.gilk-baumer@alaska.gov

Duration of Project: Start date: June 1, 2014 End Date: May 31, 2015

Abstract:
The overarching goal of this project is to use parentage-based tagging over three generations of experimental hatchery supplementation to quantify differences in fitness between wild and hatchery origin sockeye salmon in Auke Creek, Alaska. Secondary goals of this research are to test for second generation differences in fitness between wild and hatchery-origin individuals that spawn naturally, and 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. The component of the Year 1 study described herein was to complete genotyping of adults from 2010 and 2013 in the ADFG Gene Conservation Laboratory in Anchorage.

Introduction:
Evaluating the relative fitness (survival and mating success) of hatchery and wild salmonids is important for several reasons. First, very low fitness of hatchery individuals represents wasted fishery management resources (low return on investment). 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). Finally, high relative fitness of hatchery fish can increase the variance in reproductive success, thereby reducing the genetic 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).

Quantifying the relative fitness of hatchery and wild salmonids has become possible with the advent of parentage-based tagging (PBT; Anderson and Garza 2005). This method uses genotypic data to assign offspring to parents, and therefore can be used to track the relative fitness of hatchery and wild individuals by quantifying the number of offspring per each type of parental cross. PBT has rapidly become the method of choice for studying fitness differences between hatchery and wild salmonids, but the effect of hatchery breeding on fitness is highly variable among species and populations (reviewed in Araki and Schmid 2010). For example, Araki et al. (2007) detected reduced fitness after a single generation of captive breeding in the

Hood River population of steelhead trout *Oncorhynchus mykiss*, and subsequent interbreeding between hatchery and wild individuals appeared to reduce the mean fitness of the population (Araki et al. 2009). Conversely, a recent study of a supplemented population of Chinook salmon *O. tshawytscha* found no evidence for reduced fitness of hatchery fish (Hess et al. 2012). In general, variable results are probably a due to differences in broodstock provenance (local or non-local), hatchery practices, species characteristics, and length of time hatchery populations are reared prior to release (Berejikian et al. 2009). Consequently, the best source of information on the effects of supplementation in sockeye systems will be a study done in this region and on sockeye salmon.

To date, no study has attempted to quantify fitness differences between hatchery and wild sockeye salmon *O. nerka*. Auke Creek provides the ideal setting in which to measure changes in fitness resulting from hatchery supplementation of sockeye salmon for a number of reasons: 1) Auke Creek is located on the road system in Juneau, Alaska, making field sampling cost-effective; 2) the weir at Auke Creek allows researchers to sample all adults returning to spawn in the Auke Creek drainage - this is critical as it allows complete genotypic sampling of all parents and offspring; 3) the Auke Creek sockeye salmon population is relatively small (recent 5-year average is ~2,200 individuals, J. Joyce, NOAA, unpubl.), making complete enumeration of the reproductive success of hatchery and wild individuals computationally feasible; 4) a downstream smolt weir in addition to the upstream adult weir makes further exploration of potential mechanisms for fitness differences possible; and 5) results from this study are likely to be applicable to other sockeye salmon hatchery projects within the geographical region subject to the PST.

The ADFG component of Year 1 of this project aimed to genotype all adults from 2013 returns with both SNP and microsatellite markers, and to complete microsatellite genotyping for 2010 adult returns. Project partners at UAF will use this information to help estimate assignment error rates using genotypic data from return years 2008-2011, to choose optimal marker panels, and to assign 2011 jack returns to 2008 parental genotypes.

Objectives:

The primary goal of this project is *to test the null hypothesis that there is no difference in fitness (survival and reproductive success) between wild and hatchery-origin sockeye salmon* in Auke Creek. For the three-year study period, our specific objectives are:

1) To demonstrate the accuracy of parentage assignment in Auke Creek sockeye salmon with existing genomic resources;
2) To identify a cost-effective genetic marker panel for parentage assignment;
3) To complete genotyping of adults from 2012-2015 and back-fill missing genotypes from return year 2010;
4) To assign adults returning in years 2014 and 2015 to parents in brood years 2008-2011.

The ADFG component described herein relates to Objective 3: to complete genotyping of adults, and specifically to the Year 1 goal of completing genotyping of 2010 and 2013 adults for both SNP and microsatellite markers.

Approach:

Genotyping

Each summer, all adult sockeye salmon were visually identified as male or female and sampled for genetic tissues as they are passed upstream through the Auke Creek weir. Tissue samples were sent to the ADFG Gene Conservation Laboratory (GCL) for genotyping. Genomic DNA was extracted using DNeasy ® 96 Tissue Kit (Qiagen). Samples were genotyped at 12 microsatellites markers and 96 single nucleotide polymorphism markers (SNPs).

Microsatellites were genotyped at GCL. Amplification was carried out in 10 ul 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 fragment analysis was done 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 ('scored') by individuals at UAF.

SNP assays were also conducted at GCL. Samples were analyzed for 96 SNP loci. Extracted DNA was loaded into a Fluidigm® 96.96 Dynamic Array in a post-PCR laboratory at GCL. The 96 samples and 96 assays were then systematically combined into 9,216 parallel reactions. 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 s and 60° for 1 min. The Dynamic Arrays were 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

GCL conducted a quality control analysis (QC) to identify laboratory errors and to quantify genotyping error rate (which is necessary for downstream parentage analysis). The QC analyses were performed by staff not involved in the original genotyping. We re-extracted 8% of sample individuals and genotyped them for the same markers assayed in the original round of genotyping. The discrepancy rate (which identifies 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.

Results/Findings:

Genotyping

The Year 1 schedule specified that ADFG-GCL would complete genotyping on all adults from 2013 (SNPs and microsatellites) and complete microsatellite genotyping from year 2010. All individuals from the 2010 adult return have been genotyped for 12 microsatellite markers (Table 1). In addition, all individuals from the 2013 return have been genotyped for 12 microsatellite markers and 96 SNP markers.

Table 1. Progress on genotyping for adult return years 2008 – 2014.

Year	No. of adults	% DNA extracted	% Genotyped for SNPs	% Genotyped for STRs	% Scored for STRs
2008	1,264	100	100	100	100
2009	4,064	100	100	100	100
2010	2,000	100	100	100	100
2011	2,427	100	100	100	100
2012	1,569	Proposed – Y2	Proposed – Y2	Proposed – Y2	Proposed – Y2
2013	1,843	100	100	100	100
2014	98 jacks + ~500 adults	Proposed – Y2	Proposed – Y2	Proposed – Y2	Proposed – Y2

Quality control of genotypic data

No catastrophic errors were found during QC procedures. A total of 316 fish were reanalyzed, for a total of 23,568 QC project genotypes. The failure rate for 2013 fish was low at 0.68%. Few inconsistencies were found (0.07% across comparisons). The failure rate for 2010 fish was higher at 2.33%, which was due primarily to poor tissue quality for some fish. Similar to 2013, few inconsistencies were found in the QC (0.81% across comparisons).

Evaluation:

We accomplished the following:

- A total of 3,843 sockeye salmon were genotyped at 12 microsatellite markers for Auke Creek adult return years 2010 and 2013.
- A total of 1,843 sockeye salmon were genotyped at 96 SNP markers for Auke Creek adult return year 2013.
- Quality control procedures revealed no catastrophic errors, and very few inconsistencies (<1% across all comparisons).
- Genotypes have been shared with UAF for completion of additional objectives associated with Year 1 of this project.

Date Prepared: March 12, 2015

References:

Anderson, E. C., and J. C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172:2567–2582.

Araki, H., and C. Schmid. 2010. Is hatchery stocking a help or harm? Aquaculture 308:S2–S11.

Araki, H., W. R. Ardren, E. Olsen, B. Cooper, and M. S. Blouin. 2007. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood river. Conservation Biology 21:181–90.

Araki, H., B. Cooper, and M. S. Blouin. 2009. Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. Biology Letters 5:621–4.

Berejikian, B. A., D. M. Van Doornik, J. A. Scheurer, and R. Bush. 2009. Reproductive behavior and relative reproductive success of natural- and hatchery-origin Hood Canal summer chum salmon (*Oncorhynchus keta*). Canadian Journal of Fisheries and Aquatic Sciences 66:781–789.

Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications 1:535-586.

Hess, M. A., C. D. Rabe, J. L. Vogel, J. J. Stephenson, D. D. Nelson, and S. R. Narum. 2012. Supportive breeding boosts natural population abundance with minimal negative impacts on fitness of a wild population of Chinook salmon. Molecular Ecology 21:5236–50.

Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325–329.

Wang, J., and N. Ryman. 2001. Genetic effects of multiple generations of supportive breeding. Conservation Biology 15:1619–1631.