Assessing effects of supplementation on fitness of sockeye salmon in Auke Creek, Alaska, Year 2 of 3

Year 2 Report
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Summary:
This report summarizes progress made during year two of a three-year phase of a long-term project assessing the comparative fitness of wild and hatchery sockeye salmon in Auke Creek, Alaska. We report the outcome of year-two tasks, which consisted of genotyping all adult sockeye salmon returning to Auke Creek in 2012, plus a subset of adults returning in 2014 and 2015 that would be most informative for the overall project goal (i.e., jacks, which have the highest probability of originating from experimental brood years, and individuals that were sampled for age and length data). We have completed the bulk of the genotyping for the experimental brood years 2011-2013, although we are still filling in missing genotypic data. We assigned the subset of individuals returning in 2014 and 2015 (535 and 471 individuals, respectively) to parents from brood years 2008-2011 (2014 returns) and 2009-2012 (2015 returns). Of these, no individuals in 2014 were assigned to hatchery parents (which would only be expected for age 1.1 individuals), and five individuals in 2015 assigned back to hatchery parents from the 2011 brood year. For individuals returning in 2014, we compared the age as determined by scales to their age based on parent assignment and found good agreement for the dominant age classes (1.3 and 2.2; brood year 2009) and for age 1.1 individuals (brood year 2011). Poorer agreement was found for the rare age classes 1.2, 2.1 and 2.3 (brood years 2010 and 2008); we have yet to compare inconsistent cases back to original scales used in age designation, and we are still in the process of finalizing scale-parent age comparisons for the 2015 samples.

Introduction

Evaluating the relative fitness (survival and mating success) of hatchery and wild salmonids is a research priority for several reasons. First, 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 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).

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 in families of each parental pair. 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; but see Kitada et al. 2011). Conversely, a study of a supplemented population of Chinook salmon *O. tshawytscha* detected no evidence for reduced fitness of hatchery fish (Hess et al. 2012). In general, variable results may be 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). The best source of information on the effects of supplementation in transboundary 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 ~2,845 individuals, J. Joyce and S. Vulstek, 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.

Priority of Need

Enhancement is an on-going and important component of sockeye salmon production in the areas affected by the PST. In particular, sockeye salmon enhancement plays a large role in transboundary river issues relevant to the PST (e.g., TCTR 1989, 2012), and recent deliberations of the Pacific Salmon Commission attest to the concerns that biologists and managers have over the genetic effects of hatchery management. However, no study to date has adequately addressed concerns over fitness differences between hatchery and wild sockeye salmon (or in any Pacific salmon species in British Columbia and Alaska), in part because of the long-term commitment needed to fully address the question, but also because of the logistical challenges in conducting such research. The weir at Auke Creek provides an unprecedented opportunity to address this critical issue.

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. This project will provide crucial empirical data to inform implementation of these policies. 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.

Project objectives

Our specific objectives for the entire three-year study period (2014-2017) 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

Year 2 Tasks

The tasks for the second year of the project (1 Jun 2015 – 31 May 2016; covered in this report) were to:

- Genotype all adults from 2012 and the jacks from 2014, plus an additional subsample of 2014 and 2015 adults up to 2,500 individuals for both SNPs and STRs
- Assign 2012 and 2013 returning adult offspring to parents from brood years 2008-2010

- Assign the subsample of 2014 and 2015 adult offspring to parents from brood years 2008-2010

Note that these Year 2 objectives were modified slightly from those presented in the original proposal; high numbers of returning adults in 2015 necessitated a reduction in the number of non-jack adults genotyped for years 2014 and 2015 (note that the jacks in these return years are most likely result from brood years during which experimental supplementation occurred; see Appendix I).

Methods

Experimental supplementation
Supplementation of Auke Creek sockeye salmon was conducted at the Auke Creek hatchery under a separate contract to NOAA (J. Joyce, NOAA, principal investigator). Supplementation occurred for three consecutive brood years, 2011-2013. 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 were 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 for approximately one month prior to final maturation and spawning. Adults were spawned in September. Embryos were incubated over the winter, ponded in early spring (late March/early April), and released into the lake in late April/early May as young-of-the-year fry.

Actual numbers of captive parents by brood year are as follows: 30 females and 11 males in 2011; 23 females and 11 males in 2012; and 27 females and 15 males in 2013. Based on age-composition estimates from scale analysis of Auke Creek sockeye salmon (J. Joyce and S. Taylor, NOAA, unpubl. data), adults are expected to return from these three years of captive breeding during escapement years 2014-2019 (Appendix I).

Adult sampling
An important quality of this study is that we are able to sample nearly 100% of the potential parents, given that all adults returning to Auke Creek must be passed manually over the weir. Since 2008, all adult sockeye salmon returning to Auke Creek have been sampled at the weir for axillary process tissue for genotyping. Each year, adults are identified as male or female, axillary tissue samples are removed and stored in ethanol, and the fishes are released to volitionally move into Auke Lake and eventually spawn in the tributary creeks and possibly the shore of the lake. A variable subset of adults has also been sampled each year for length and age, and these data are linked to individual tissue samples. The annual number of adults (including jacks) passing the weir has ranged from 1,264 (in 2008) to 4,048 (in 2009), averaging 2,413 fish/year (Table 1).

Table 1. Number of females, males, and jacks (young males) returning to Auke Creek by return year

Return Year	Females	Males	Jacks	Total
2008	unspecified	unspecified	37	1,280
2009	2,207	1,794	47	4,048
2010	1,050	968	45	2,063
2011	1,299	1,010	118	2,427
2012	905	611	53	1,569
2013	1,043	910	107	2,060
2014	1,762	1,583	98	3,443
2015	2,507	2,082	131	4,720

There are three possible reasons why we would not have all parental genotypes in our samples: 1) occasionally a fish escapes over the weir prior to having its axillary process sampled; 2) some males in the population might "residualize", or become sexually mature without leaving Auke Lake; or 3) sockeye salmon that were spawned elsewhere could stray into Auke Creek and spawn in the Auke system. In cases 1 and 2, offspring should assign to only one parent, and in case 3 offspring should not assign to any parent in the dataset. In some cases, tissue degradation could also contribute to inability to obtain genotypes for some of the sampled individuals.

Genotyping

DNA extraction, SNP genotyping and scoring, and STR genotyping took place at the Gene Conservation Lab of the Alaska Department of Fish & Game, in Anchorage. Allele scoring for STRs was conducted at the University of Alaska Fairbanks.

DNA was extracted using a DNeasy® 96 Tissue Kit by Qiagen® (Valencia, CA). For SNP genotypes, extracted DNA was loaded into two Fluidigm® 192.24 Dynamic Arrays. 192 samples and 24 assays were then 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 under the following conditions: 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 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.

Amplification of STR markers 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 (Appendix I). PCR fragment analysis (electrophoresis) 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 GeneScan^{TM} 500 LIZ® (AB) internal lane size standard and 9.0 ul of Hi-Di^{TM} formamide (AB). PCR bands were visualized and separated into bin sets using AB GeneMapper® software v4.0. Scoring alleles consisted of visual confirmation or correction of alleles automatically binned by the software.

Genotyping error rate estimation
We implemented ADF&G's standard protocol for quality control of genotypic data, which consisted of re-extracting 8% of the sample individuals and repeating both SNP and STR assays and scoring, following the methods described above. The number of conflicting genotypes divided by the number of genotypes compared gave the discrepancy rate, which was then divided by two to give the genotyping error rate.

Parentage analysis
Parentage analysis consists of attempting to assign offspring to their true parents from a pool of all candidate parents based on genotypic information. This analysis is fundamental to quantifying fitness differences between hatchery and wild sockeye salmon, as individual fitness is defined in this study as the number of returning adult offspring (over all potential return years) produced by the focal individual. The earliest we will be able to estimate individual fitness is after the 2017 adult return year (for brood year 2011; see Appendix I) so for this report, methods are limited to preliminary analyses of parentage assignment. A full treatment of methods for estimating relative fitness can be found in the study proposal.

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).

Results and Discussion, Year 2
Task 1: Genotype all adults from 2012 and the jacks from 2014, plus an additional subsample of 2014 and 2015 adults up to 2,500 individuals for both SNPs and STRs
All returning adults in 2012, and all jacks returning in 2014, were genotyped at 48 SNPs and nine SNPs. In addition, we genotyped all adults sampled for age-sex-length data in 2014 and

2015, plus an additional 180 adults returning in 2015. There are some gaps in these data that we are still in the process of confirming for potential re-genotyping.

Task 2: Assign 2012 and 2013 returning offspring to parents from brood years 2008-2009 (2012 adults) and 2008 - 2010 (2013 adults).

Adults returning in 2012 should have originated from brood years 2006 – 2009, so we did not expect that a majority of the 2012 adults would assign to parents in our database. We expected that a greater proportion of individuals returning in 2013 (originating from brood years 2007 – 2010) would assign to parents in the database, and that is what we observed (results summarized in Table 2). In 2012 and 2013, over half of the individuals assigned with triad posterior probability (p) < 0.90. This could be due to having quite a few candidate parents from 2009 and 2010 that are missing genotypic information, as well as having a large number of offspring expected to have originated from brood years prior to 2008 (particularly for the 2012 offspring). Brood years 2008- 2010 are the experimental brood years of primary interest for the project (i.e., brood years 2011-2013). Therefore, we have not prioritized back-filling missing genotypes for these individuals, whereas for the brood years from which we will calculate fitness, greater effort will be given to filling in the missing data (and thus increasing assignment accuracy). Furthermore, in those assignments, we will constrain the analysis to only allow compatible assignments, thus further increasing the probability of attaining the correct assignment. We continue to refine the parameters of the FRANz analyses, as well.

Table 2 NEW. Summary of parentage assignments for adults returning 2012 -2013 (task 2) and 2014-2015 (task 3). Table indicates number of individuals we attempted to assign to parents (N), the number of assignments that occurred with a posterior triplet probability (p) > 0.95, and the number of assignments with 0.90 < p < 0.95. The remaining statistics are only for those assignments with p > 0.90.

Return Year	Brood Years	N	p > 0.95	p > 0.90	Assignments w/ p > 0.90			
					Parents			Hatchery origin?
					Two	One	Zero	
2012	2008-2009	1,480	583	240	24	36	762	N/A
2013	2008-2010	2,054	1004	177	803	118	260	N/A
2014	2008-2011	535	423	16	394	41	4	0
2015	2009-2012	471	331	25	333	21	2	5

Task 3: Assign the subsample of 2014 and 2015 adult offspring to parents from brood years 2008-2012

We attempted to assign 535 and 471 adult offspring from 2014 and 2015, respectively. A majority (~80%) assigned to 2, 1 or no parents with a triplet posterior probability >0.90. Of these, the vast majority assigned to two parents in our dataset. Interestingly, no individuals from

2014 assigned back to hatchery parents (in theory, age 1.1 jacks could have assigned back to hatchery parents from 2011), whereas five individuals returning in 2015 assigned back to hatchery parents (the progeny of four separate crosses involving four females and 3 males altogether, all from brood-year 2011). All five of these hatchery progeny were males, age 4, although one of them was identified in the field as a jack.

For individuals sampled for age-sex-length (ASL) data in 2014, we compared the ages as determined by scale analysis (J. Taylor and J. Joyce, unpubl.) to their ages as inferred from parentage assignment. In this case, a comparison was deemed consistent when at least one parent (assigned with a posterior probability > 0.90) spawned in the year that was consistent with the scale age of its offspring. A comparison was deemed inconsistent when one or both parents (assigned with a posterior probability >0.90) spawned in a year not consistent with the age of the offspring as determined by scale analysis. When no parent was assigned, or when a parent assigned with a posterior probability < 0.90, the comparison was deemed 'unknown'. The results are summarized in Table 3. We found good agreement for the dominant age classes (1.3 and 2.2; brood year 2009) and for age 1.1 individuals (brood year 2011). Much lower agreement was found for the rare age classes 1.2, 2.1 and 2.3 (brood years 2010 and 2008). We have not yet gone back to the original scales to check the ages of the individuals that had inconsistent comparisons. However, a simple linear regression between age and mid-eye-fork length found that age based on parentage assignment explained a greater amount of variation in length (R^2 = 0.280) than did age based on scales (R^2 = 0.196). Perhaps these results suggest that scale-ageing error is greater for those particular scale ages (1.2, 2.1 and 2.3), or that parentage assignment error was greater for individuals resulting from brood years 2008 and 2010. We note that these inferences so far have been based on limited samples, and inferences will be strengthened in the future by analyzing more return years.

Table 3. Comparisons of age of returning 2014 adults as determined by scale analysis versus age suggested by parent assignment.

Scale Age	Brood Year (scales)	N	Consistent	Inconsistent	Unknown
1.1	2011	5	5 (100%)	0	0
1.2	2010	8	1 (14.3%)	6	1
2.1	2010	6	0 (0%)	6	0
1.3	2009	140	127 (90.7%)	7	6
2.2	2009	117	112 (95.7%)	1	4
2.3	2008	39	21 (53.8%)	14	4

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Appendix I. 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). One more cycle would be required to compare fitness between wild-spawning individuals of hatchery and wild origin, as denoted by F2 return years.