Assessing effects of supplementation on fitness of sockeye salmon in Auke Creek, AK, Year 1 of 3

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Summary
Here we report results of the first year of three of the PSC-funded study, "Assessing effects of supplementation on fitness of sockeye salmon in Auke Creek, AK." 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. 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). In this report, we summarize our progress on the tasks outlined for year one of the project. We have completed the first task, which was to genotype all adults from 2013 (SNPs + STRs) and complete STR genotyping from year 2010. Second, we used several different methods, all with real genotypic information from Auke Creek sockeye salmon, to estimate rates of parentage assignment error of two types: 'type A', where one fails to assign an offspring to a true parent that is in the parental dataset; and 'type B', where one assigns an offspring to an untrue parent whether or not the true parent is sampled. We found that we have sufficient genomic resources to achieve low error rates (0-5%). Our third task was to choose a reduced panel of loci that would allow us to optimize the trade-off between reducing assignment error versus reducing the genotyping costs of the project. We found that a panel of 48 SNP and 8 STR (or 'microsatellite') loci should be sufficient to achieve the project goals. Finally, we report on preliminary assignment of jacks from the 2011 return year to parents from the 2008 brood year. A small proportion (< 20%) of these jacks was assigned to parents, but a small proportion was expected given that most jacks returning in 2011 likely came from parents older than the 2008 brood year. We will not be able to fully quantify fitness of the first brood year of experimental supplementation (2011) until all expected offspring have returned, which will be in 2017.

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 ~2,300 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.

Objectives & Year 1 Tasks

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

Our tasks for the first year of the project (1 Jun 2014 – 31 May 2015; covered in this report) were:

Task 1: Genotype all adults from 2013 (SNPs + STRs) and complete STR genotyping from year 2010

Task 2: Estimate assignment error rates using genotypic data from return years 2008-2011

Task 3: Choose optimal marker panel

Task 4: Assign 2011 jack returns to 2008 parental genotypes

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

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.

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™ 500 LIZ® (AB) internal lane size standard and 9.0 ul of Hi-Di™ 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 analyses of parentage assignment error. A full treatment of fitness estimates 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).

Two classes of error in parentage assignment can contribute towards biases in estimates of relative fitness: 'type A', where one fails to assign an offspring to a true parent that is in the parental dataset; and 'type B', where one assigns an offspring to an untrue parent

whether or not the true parent is sampled (Araki and Blouin 2005). We estimated the rates of these two types of errors as follows. For type A errors, we genotyped 200 hatchery fry from brood year 2013 prior to release and estimated the rate at which they failed to assign to the pool of known hatchery parents from that brood year. For type B errors, we determined the rate at which offspring were assigned to a set of individuals that could not be their parents. This was conducted in two different ways. First, we attempted to assign adults returning in year 2009 as the "offspring" of adults returning in subsequent years 2011 (which could not possibly be their parents). Second, we attempted to assign the 200 hatchery fry from brood year 2013 to the wild parents that spawned in 2013 (i.e., we removed the known hatchery parents from the pool of potential parents). Although the number of fish being assigned was low, the candidate parent pool was from the same return year as the true parents, thus approximating the real analyses we will be conducting in subsequent years.

Choosing an optimal marker panel

Previous simulations (Anderson 2011 and P. Barry, UAF, unpubl.) suggested that both SNPs and STRs are required for parentage assignment in Auke Creek sockeye; however, fewer than 12-14 STR loci will be necessary when combined with sufficiently variable SNPs. Therefore, we sought to evaluate these predictions empirically, in hopes of being able to reduce the cost of the project. This was accomplished by sequentially dropping the least variable STR loci and recalculating assignment type A and B error rates (as described previously). If a marker contributed little to improved accuracy, we assessed the benefits of removing it by determining how much reduction in laboratory costs and personnel time would be gained by its omission. For example, STR loci are assayed in multiplexes (and SNPs in sets of 24), so removing a few loci might do little to reduce laboratory costs, but considerable savings might be achieved by removing an entire multiplex.

Results and Discussion: Year 1

Task 1: Genotype all adults from 2013 (SNPs + STRs) and complete STR genotyping from 2010

The task for year 1 of the project was to complete the catch-up genotyping at 12 STR loci for the 2010 samples, which were already genotyped for SNPs. This task has been accomplished (Table 2). We also completed the genotyping of the 2013 adults at 12 STRs and 96 SNPs; however a small number (<30 individuals) need to be rerun at either STR or SNP panels.

Table 2. Progress on genotyping for adult return years 2008 – 2015.

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
2015	TBD	Proposed – Y3	Proposed – Y3	Proposed – Y3	Proposed – Y3

Task 2: Estimate assignment error rates using genotypic data from return years 2008-2011;
Task 3: Choose an optimal marker panel

As tasks 2 and 3 are interrelated, we have chosen to report results of both in the same section. Previous analyses (see progress report appended to our 2015-16 proposal) indicated that a panel of 48 SNPs was acceptable for type A and B errors; 24 SNPs was too few. Hence for the Type A analyses, we focused on comparing panels of 93 and 48 SNPs (i.e., the 48 loci with the highest minor allele frequencies of the 93 nuclear SNPs from ADF&G's full 96-SNP panel) and varying combinations of STR loci, based on their multi- and pseudo-plex status (shown in Appendix II). For the Type B comparisons, we reported only results from 48-SNP panels, as return years 2011 and 2009 were not genotyped at the full 96-SNP panel (although return year 2013 as well as the resulting hatchery fry were).

To analyze the effect of dropping STR loci on Type A error rates (using the 2013 hatchery fry data), we pessimistically assumed that ~10% of the true parents had gone unsampled. Dropping all 12 STR loci resulted in an unacceptably high Type A error rate (19.4% with 93 SNPs) or lack of convergence (with 48 SNPs). Adding only the most variable STR locus (*Ssa419*) to the SNPs resulted in a Type A error rate of 1.5% and 3%, with 93 and 48 SNPs, respectively. Any other combination of STR loci we tested had a Type A error rate of 0% (Table 3).

Type B error rates were expected to be more sensitive to the proportion of parents unsampled, because assuming a high proportion of parents are in the data set will increase the probability that an individual is assigned to an incorrect parent rather than going unassigned. Therefore, we initially compared Type B error rates (using 2009 "offspring" assigned to 2011 "parents") over three different percentages of missing parents in the candidate set: 10%, 25%, and 50%. Preliminary analysis showed no change in rank order of Type B error rate of STR combinations over the three percentages (Table 4; data not shown for 25% missing parents), so for the 2013 fry analysis we only reported

results for 10% missing parents. For these analyses we used an empirically derived genotyping error rate of 0.0025, which was based on the average of the SNP genotyping error rate over four return years and a pessimistic estimate of the STR genotyping error rate (Appendix III).

Type B error rates in the 2009 to 2011 tests (Table 4) ranged from 5.3% (48 SNPs and all 12 STRs) to 12.8% (48 SNPs and only *Ssa419*, the most variable STR). These error rates were considered quite pessimistic, as no true parents were included in the data set, and siblings were possibly present in 2009-2011 comparisons, increasing the likelihood of false assignment. In the 2013 hatchery fry tests, error rates ranged from 0% to 6% depending on locus combination (Table 5). Results of the 2013 fry tests were considered somewhat more optimistic than the 2009-2011 tests, because siblings were unlikely to complicate the analyses. Also, we did not include the more realistic situation of attempting to assign these individuals to potential parents from multiple brood years. However, it is worth noting that the LOD scores for correct assignments in the Type A tests (Table 3) exceeded those from the comparable Type B tests (Table 5) by an order of magnitude, suggesting that if the correct parents are indeed in the dataset (which is highly likely, as discussed in the Methods; Adult sampling section) then the correct assignment is far more likely.

Based on the results described above and summarized in tables 3-5, it appears that the locus panel that best optimizes the trade-offs between the benefits of more information from more loci and the costs of additional time and materials required to gain that information consists of 48 SNPs and the STR panel without M3 (a multiplex reaction of *Oki16*, *Omy77*, and *Ots103*) and *One8* (a locus that is pseudoplexed with three other loci for electrophoresis but must be amplified alone). Dropping four of the 12 loci will reduce the costs of the project going forward.

Table 3. Results of type A error rate estimation by comparing known hatchery fry in 2014 (N=200) to potential parents (hatchery, N = 42 and wild, N = 1,985) from the 2013 brood year, using 93 SNPs and varying combinations of STR (microsatellite) loci. We set the proportion of missing parents to be 10%. Mean posterior probability is for triad assignments (offspring to both parents), averaged over all assignments.

STR combination (N loci)	Number of STR loci	N incorrect: 1 parent	N incorrect: 2 parents	Mean LOD (SD)	Mean posterior probability	Assign-ment error rate
93 SNPs						
All STRs	12	0	0	72.7 (11.5)	1.000	0
No *Ssa419*	11	0	0	67.5 (10.8)	0.999	0
Only *Ssa419*	1	2	1	25.1 (6.4)	0.976	1.5%
No M3[a]	8	0	0	56.1 (9.6)	0.997	0
No M1[b]	5	0	0	51.4 (9.5)	0.998	0
M2 only[c]	4	0	0	34.9 (7.8)	0.995	0
M1 + *Ssa419*	6	0	0	46.3 (8.5)	0.999	0
No M3, no *One8*	8	0	0	51.1 (9.2)	0.998	0
No STRs	0	26	13	20.4 (5.2)	0.866	19.4%
48 SNPs						
All 12 STRs	12	0	0	68.5 (9.9)	1.000	0
No *Ssa419*	11	0	0	63.3 (9.3)	0.998	0
Only *Ssa419*	1	6	0	20.9 (4.5)	0.944	3.0%
No M3[a]	8	0	0	52.0 (8.0)	0.996	0
No M1[b]	5	0	0	47.3 (7.8)	0.996	0
M2 only[c]	4	0	0	30.7 (6.2)	0.985	0
M1 + *Ssa419*	6	0	0	42.1 (6.7)	0.996	0
No M3, no *One8*	8	0	0	46.9 (7.7)	0.993	0
No STRs	0	Did not converge				

[a]M3 is reaction multiplex of *Oki16*, *Omy77*, and *Ots103*
[b]M1 is reaction multiplex of *Oki100*, *One102*, *One109*, and *Ok1a,b*
[c]M2 is pseudoplex of *Ssa419*, *One8*, and *Oki10* and *One114*; only *Oki10* and *One114* are amplified together

Table 4. Results of Type B error rate estimates for N = 400 "offspring" from 2009 and N = 400 "parents" from 2011 with two different assumptions regarding the proportion of candidate parents (10% and 50%) missing from the dataset. A panel of 48 SNPs was used in both cases along with various combinations of STR loci.

% missing parents	STR combination	N incorrect: one parent	N incorrect: two parents	Mean LOD (SD)	Posterior probability averaged over all assignments	Assignment error rate
10%	All 12 STRs	20	1	5.97 (4.30)	0.945	5.3%
	No *Ssa419*	35	1	5.89 (3.17)	0.909	9.0%
	Only *Ssa419*	45	6	6.34 (2.64)	0.714	12.8%
	No M3[a]	28	1	5.96 (3.67)	0.924	7.3%
	No M1[b]	44	1	4.92 (1.81)	0.888	11.3%
	M2 only[c]	41	2	5.56 (1.70)	0.859	10.8%
	M1 + *Ssa419*	48	2	6.15 (3.11)	0.831	12.5%
	No M3, no *One8*	32	0	5.51 (2.21)	0.925	8%
	No STRs	20	11	7.65 (2.91)	0.617	7.8%
50%	All 12 STRs	21	1	5.97 (4.30)	0.945	5.5%
	No *Ssa419*	35	1	5.89 (3.17)	0.909	9.0%
	Only *Ssa419*	44	6	6.37 (2.66)	0.714	12.5%
	No M3[a]	28	1	5.96 (3.67)	0.924	7.3%
	No M1[b]	42	1	5.02 (1.80)	0.888	10.8%
	M2 only[c]	41	2	5.56 (1.70)	0.858	10.8%
	M1 + *Ssa419*	47	2	6.19 (3.12)	0.829	12.3%
	No M3, no *One8*	32	0	5.51 (2.21)	0.925	8%
	No STRs	19	11	7.80 (2.83)	0.619	7.5%

[a]M3 is reaction multiplex of *Oki16*, *Omy77*, and *Ots103*
[b]M1 is reaction multiplex of *Oki100*, *One102*, *One109*, and *Ok1a,b*
[c]M2 is pseudoplex of *Ssa419*, *One8*, and *Oki10* and *One114*; only *Oki10* and *One114* are amplified together

Table 5. Results of Type B error rate estimates for N = 200 hatchery fry from 2013 and N = 1,985 wild parents from 2013, assuming that 10% of the candidate parents are missing from the dataset. Mean LOD is for assigned individuals only; mean posterior probability is for triad assignments (offspring to both parents), averaged over all assignments. 48 SNPs were used along with various STR combinations.

STR combination	N incorrect: 1 parent	N incorrect: 2 parents	Mean LOD (SD) of assigned	Mean posterior probability	Assignment error rate
All 12 STRs	0	0	--	1.000	0%
No *Ssa419*	0	0	--	1.000	0%
Only *Ssa419*	0	12	8.56 (2.99)	0.524	6.0%
No M3[a]	0	0	--	1.00	0%
No M1[b]	0	0	--	0.999	0%
M2 only[c]	18	4	4.94 (1.82)	0.903	0%
M1 + *Ssa419*	1	0	3.45 (--)	0.995	0.5%
No M3, no *One8*	0	0	--	0.989	0%
No STRs	0	8	1.45 (2.94)	0.514	2%

[a]M3 is reaction multiplex of *Oki16*, *Omy77*, and *Ots103*

[b]M1 is reaction multiplex of *Oki100*, *One102*, *One109*, and *Ok1a,b*

[c]M2 is pseudoplex of *Ssa419*, *One8*, and *Oki10* and *One114*; only *Oki10* and *One114* are amplified together

Task 4: Assign 2011 jack returns to 2008 parental genotypes

We genotyped 111 jacks that returned in 2011 and attempted to assign them to parents from RY 2008. Using a default genotyping error rate of 0.01, 20 jacks were fully assigned to two parents, with an average LOD score of 12.8. Increasing the genotyping error rate to 0.02 led to fewer parents being excluded based on Mendelian incompatibilities, with 52 jacks being assigned to parents, but our confidence in these assignments was lower (LOD score averaged 9.0). Many jacks returning in 2011 could have been age 2.1 rather than 1.1 and thus would have come from parents that returned in 2007 (prior to the inception of this study). In 2010, it was estimated that ~8% of smolts emigrating from Auke Creek had spent one year in fresh water (J. Joyce, NOAA, unpubl. data), so it is not surprising to observe a large number of unassigned jacks returning in 2011.

Future work

In the coming year, we will continue to collect genotypic information and perform parentage assignment. We will genotype all returning adults from 2012 (N = 1,569) and from return year 2014, we will genotype all jacks (N = 98) and up to 500 of the older adults. The 2014 jacks are important because they can be assigned back to brood years for which we have full genotypic information (2008 and 2009); the older adults could assign back to brood years prior to this. They are important for understanding changes in genetic diversity and structure accompanying supplementation but are less critical for parentage assignment. By year three we will be completely caught up on genotyping, which will be facilitated by the more streamlined panel of loci.

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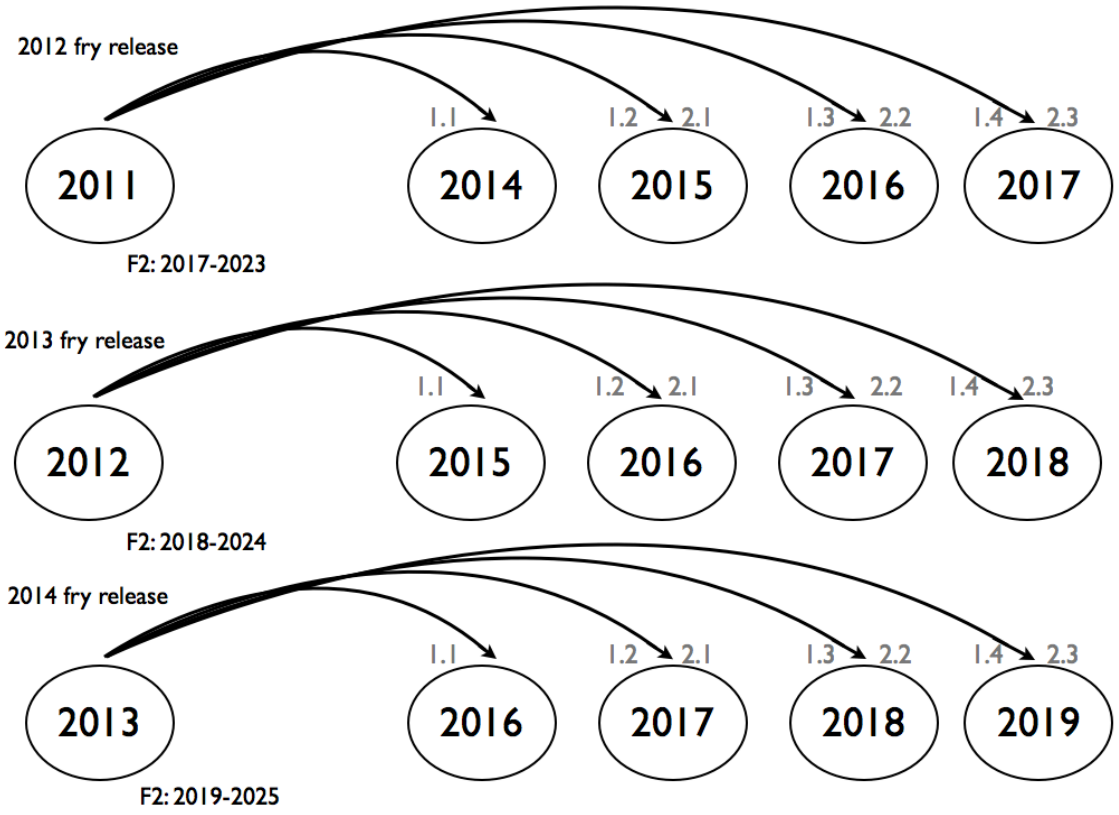
Appendix I. Schematic showing expected return years of first-generation adults from captive brood years 2011-2013. Numbers of return-year ovals represent age of returns (e.g., 1.1 = one winter in freshwater, one winter in saltwater, age 3). One more cycle would be required to compare fitness between wild-spawning individuals of hatchery and wild origin, as denoted by F2 return years.



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

PCR Multiplex	Annealing temp. (^{o}C)	Number cycles	Locus	[primer] (µM)	Capillary multiplex
1	55	35	*Oki100*	0.20	1
			One102	0.10	
			One109	0.10	
			Oki1a	0.06	
			Oki1b	0.06	
2	56	35	*Oki10*	0.20	2
			One114	0.35	
3	57	36	*Ssa419*	0.20	
4	60	35	*One8*	0.20	
5	46	35	*Oki16*	0.20	3
			Omy77	0.20	
			Ots103	0.20	

Appendix III. Estimates of genotyping error rates for STR loci from the 8% reruns for return year 2013. Variation in the number of genotypes compared resulted from different assay failure rate across multi-/pseudo-plexes.

Locus	N genotypes	Error rate
One109	127	0
One102	127	0.39%
Oki100	117	0
Oki1a	127	0
Oki1b	127	0
One8	127	0
*Ssa419**	--	--
Oki10	156	0.32%
One114	155	0
Ots103	144	0
Omy77	144	0
Oki16	144	0

*We are still in the process of verifying error rate of *Ssa419* scoring across individual scorers; we arrived at the project-wide genotyping error rate for analyses in this report pessimistically assuming an STR genotyping error rate of 0.4%.