Project title:	Relative productivity of hatchery pink salmon in a natural stream
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Executive Summary:

Private non-profit hatcheries (PNP) in Alaska practice extensive ocean-ranching aquaculture of Pacific salmon to provide additional harvest opportunity to common property fisheries. Approximately 75% of the 1.8B juvenile salmon released annually in Alaska by these hatcheries are Pink Salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and Chum Salmon (*O. keta*) in Southeast Alaska (SEAK). Because of the value of hatchery production to the fishing industry and the regulatory mandate that it be compatible with sustainable productivity of wild stocks, the Alaska Department of Fish and Game (ADF&G) and PNP hatcheries established the Alaska Hatchery Research Program (AHRP; http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main) to address potential issues associated with Alaska's hatchery program. The AHRP was designed to address three research areas: 1) wild population genetic structure, 2) hatchery straying extent and variability, and 3) the impact on fitness (productivity) of wild Pink and Chum salmon stocks due to straying hatchery Pink and Chum salmon.

To begin to address the third research area, we used funds from this award to measure the relative reproductive success (RRS) of hatchery- and natural-origin Pink Salmon in Stockdale Creek in PWS. We defined RRS as the unweighted mean number of sampled dead adult offspring (known members of the Stockdale Creek escapement) produced by hatchery-origin fish (identified by thermal otolith marks applied in the hatchery) divided by the unweighted mean number of sampled dead adult offspring produced by natural-origin fish (fish lacking a thermal otolith mark that were born in the wild and may or may not have hatchery-origin ancestry). Our unweighted estimate of RRS is unbiased if we assume that the field sampling is representative of parent and offspring escapement. This assumption needs to be tested since we were not able to sample every individual entering the stream. Extrapolating our unweighted estimate of RRS to a weighted estimate of RRS for the full escapement population to account for potential sampling biases was beyond the scope of this project and warrants future investigation.

Pink Salmon's two-year generation time results in genetically distinct odd and even lineages. To calculate estimates of RRS for an even-lineage generation, we attempted to genotype 7,986 individuals sampled in 2014 and 2016 at 298 genetic markers (5,993 fish were successfully genotyped), reconstructed pedigrees to assign offspring to parents, and calculated RRS. Our unweighted estimates of RRS were 0.42 for females (95% confidence intervals: 0.35-0.50) and 0.28 for males (0.24–0.34). These estimates represent a statistically significant reduction in first-generation reproductive success of hatchery-origin Pink Salmon compared to natural-origin counterparts. These RRS values fall within an expected range based on studies of other Pacific salmonid species, including estimates reported for Pink Salmon from Hogan Bay, PWS (Lescak et al. 2019). Unweighted relative reproductive success was also statistically significantly reduced for matings between two hatchery-origin parents (n = 15 pairs) in our samples compared to two natural-origin parents (n = 62 pairs; RRS = 0.61; 95% confidence intervals: 0.35-0.99). Generalized linear modeling (negative binomial with log link function) indicated that origin and sample date significantly influenced the reproductive success of females, while fish length, origin, and sample date influenced the reproductive success of males.

Future research will include testing, and potentially accounting for, sampling biases of parents and offspring to develop unbiased estimates of RRS for the full population, laboratory and statistical analysis of additional samples from Stockdale Creek to increase statistical power by

increasing the number of parent-offspring assignments, examination of RRS in four additional streams, and analysis of samples from 2017-2019 to explore multi-generational effects (F_2 or grandparentage analysis). Important questions remain regarding the genetic and ecological mechanisms that contribute to the differences in fitness we observed and their biological significance. This series of studies, building on the results of this project, will provide information for ADF&G to evaluate potential risks to natural stocks posed by stray hatchery-origin Pink Salmon in PWS as they exercise their regulatory responsibility for the ocean ranching aquaculture program.

Purpose:

Description of the problem

The goal of this project was to compare the reproductive success (RS) of hatchery- and natural-origin Pink Salmon in a remote stream in Prince William Sound (PWS), Alaska and was requested under *Priority #1 – Aquaculture* by providing "research on the environmental impacts of aquaculture."

The State of Alaska began salmon enhancement in the 1970's to enhance fisheries, provide economic opportunity to local communities, and reduce variation in annual salmon harvests. Hatchery production contributed an estimated $62–$182 million or 21–30% of the exvessel value between 2007 and 2016 (Stopha, 2018). Most of the approximately 1.8B juvenile salmon released annually are Pink Salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and Chum Salmon (*O. keta*) in Southeast Alaska (SEAK; Vercessi, 2015). While natural-origin Pink Salmon spawn in over 1,000 streams in PWS (Johnson & Blossom, 2018), hatchery-origin Pink Salmon produced by four private non-profit (PNP) hatcheries contributed an average of 70% of the total return in PWS between 2013–2015 (Knudsen et al., 2015). The scale of Alaska's enhancement programs and research in other Pacific salmon species showing fitness reductions in hatchery-origin fish as compared to natural-origin counterparts (e.g., (Anderson et al., 2013; Araki et al., 2008; Fleming et al., 2011; Ford et al., 2016) raised concern that hatchery-produced fish may detrimentally impact the productivity and sustainability of wild stocks. These risks may be related to genetics (consequences of interbreeding between hatchery-bred and wild salmon), disease (introduction or amplification of pathogens), ecology (competition for resources), and/or harvest mortality (reviewed by Naish et al., 2007). These risks caused the State to implement genetic and disease policies in the beginning of the Alaska's hatchery program, based on the best science of the time, that regulated the location of hatcheries with respect to significant wild stocks, the transfer of fish between biogeographic regions, broodstock collection from wild stocks, broodstock management in hatcheries, and pathogen and disease management.

The extent to which hatchery- and natural-origin fish interact, interbreed, and influence each other's fitness has been a controversial topic in the scientific literature (e.g., Araki & Schmid, 2010; Buhle et al., 2009; Evenson et al., 2018; Hilborn & Eggers, 2000; McGee, 2004; Naish et al., 2007; Smoker & Linley, 1997; Taylor & Pearsons, 2011; Wertheimer et al., 2001), including for Pink Salmon enhancement in PWS. Some argue that hatchery Pink Salmon in PWS did not increase the overall production much above what would have been expected of natural populations without hatchery supplementation (Amoroso et al., 2017; Hilborn & Eggers, 2001; Hilborn & Eggers, 2000) and that hatchery Pink Salmon may have displaced natural fish or restricted their ecological opportunities (Amoroso et al., 2017; Heard, 2003; Hilborn & Eggers, 2000). Others, however, argue that hatchery fish complement natural stocks, increasing harvest opportunities without unduly restricting natural stocks (Wertheimer et al., 2001, 2004). Alaskan hatchery programs should be evaluated in the context of both the economic benefits of enhancement (McDowell Group, 2018a, 2018b) and the harder to quantify risks to natural stocks.

The potential for negative effects of stray hatchery-origin fish on wild stocks in Alaska has been raised by studies documenting hatchery-origin salmon in wild-spawning streams in PWS (Brenner, Moffitt, & Grant, 2012) and southeast Alaska (SEAK; Piston & Heinl, 2012). Their findings raised several important questions for managers of the Alaska enhancement program:

(1) Are hatchery-origin salmon interbreeding with wild salmon to the extent that fitness and productivity are being diminished?; (2) Is the annual assessment of wild stocks (which is largely based on visual observation) biased by the presence of hatchery salmon?; and (3) Is the presence of hatchery-origin salmon causing density interactions that diminish the productivity of wild salmon?

In 2011, ADF&G convened a Science Panel of experts either currently active in or retired from ADF&G, University of Alaska, PNP hatchery corporations, and NOAA-Fisheries with broad experience in salmon enhancement, fishery management, and wild and hatchery interactions. The Panel defined three priority questions:

I. *What is the genetic stock structure of pink and chum salmon in each region*?
II. *What is the extent and annual variability in straying of hatchery pink salmon in PWS and chum salmon in PWS and SEAK*?
III. *What is the impact on fitness (productivity) of natural pink and chum salmon stocks due to straying of hatchery pink and chum salmon*?

In 2013, the Panel developed the Alaska Hatchery Research Program (AHRP) to address these questions (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main) with funding from the State of Alaska, PNP operators, and seafood processors.

We used funds from this award to begin to address question III by measuring unweighted RRS in hatchery- and natural-origin Pink Salmon in a remote stream in PWS. Specifically, funds were used to genotype nearly 8,000 parents and offspring from an even-lineage generation in Stockdale Creek. The primary concern was that if hatchery-origin fish were less reproductively successful than natural-origin, then natural-origin stocks may lose productivity due to interbreeding with hatchery strays. This is particularly concerning if diminished RS persists in descendants of such a hybrid mating; this project addresses the RS of the initial mating (F_0.)

Project objectives

1. Genotype 8,000 F_0 and F_1 individuals collected in 2014 and 2016, respectively, at 192 single nucleotide polymorphism (SNP) markers.
    a. Genotype all F_0 parents regardless of origin from as many streams as possible.
    b. Genotype only natural-origin F_1 offspring for corresponding streams.
2. Identify the number of offspring attributable to each parent and calculate RRS for hatchery- and natural-origin Pink Salmon.

Approach:

Field collections

The AHRP fitness study design originally identified six Pink Salmon streams in PWS (three with intermediate hatchery proportions (defined as ~20%) and three with high hatchery proportions (defined as ~50%) to reconstruct pedigrees and calculate RRS of hatchery- and natural-origin fish over two complete generations (Taylor, 2013). The number of pedigree streams was later reduced to five (two streams were dropped due to low sampling of hatchery-origin fish, and one stream was added in 2014; Knudsen et al., 2015, 2016). In 2014, we performed a stream-specific statistical power analysis (Shedd, et al., 2014) to select those streams and years most likely to provide enough power to detect an RRS of < 0.5, should it exist (per the project study design; Taylor, 2013). This power analysis (Lescak et al., 2019) incorporated the number of potential parent samples collected of each origin for each brood year and the projected proportion of adult offspring to be sampled and were based on Hinrichsen (2003) and Christie et al. (2014; Box 2). Hogan Bay (60.19668N, -147.757W) and Stockdale Creek (60.31813ºN; -147.202ºW; Figure 1) were selected as the first two study streams for analysis due to their likely high statistical power to detect differences in RS given the samples available (Shedd et al., 2014). We performed an initial analysis on Hogan Bay samples collected between 2013 and 2016 using funds from the North Pacific Research Board (Lescak et al. 2019). Funding from the current award was used to analyze samples from Stockdale Creek collected in 2014 and 2016.

Due to lack of infrastructure (e.g. dams or weirs) in Stockdale Creek, we relied on in-stream sampling of carcasses, which limited the ability of field crews to collect all potential parents and their adult offspring. To strive for representative sampling, all available carcasses were sampled when crews from the Prince William Sound Science Center (PWSSC) visited the stream. This assumption of representative sampling is critical to obtain unbiased estimates of RRS for the escapement population. There were ten sampling events throughout the run in 2014 and thirty sampling events in 2016. Paired otolith and heart tissue samples were collected concurrently, each into a cell of a 48 deep-well plate and preserved in 95% ethanol to prevent DNA degradation (Gorman et al., 2018). Each fish's sex, length (mm), sampling location, and sampling date were also recorded and archived in the Hatchery Wild Study database maintained by Resource Data Inc. (formerly Finsight, LLC). Otoliths and heart tissue were separated at the Gene Conservation Laboratory (GCL) at ADF&G in Anchorage to maintain pairing integrity (Gorman et al., 2018).

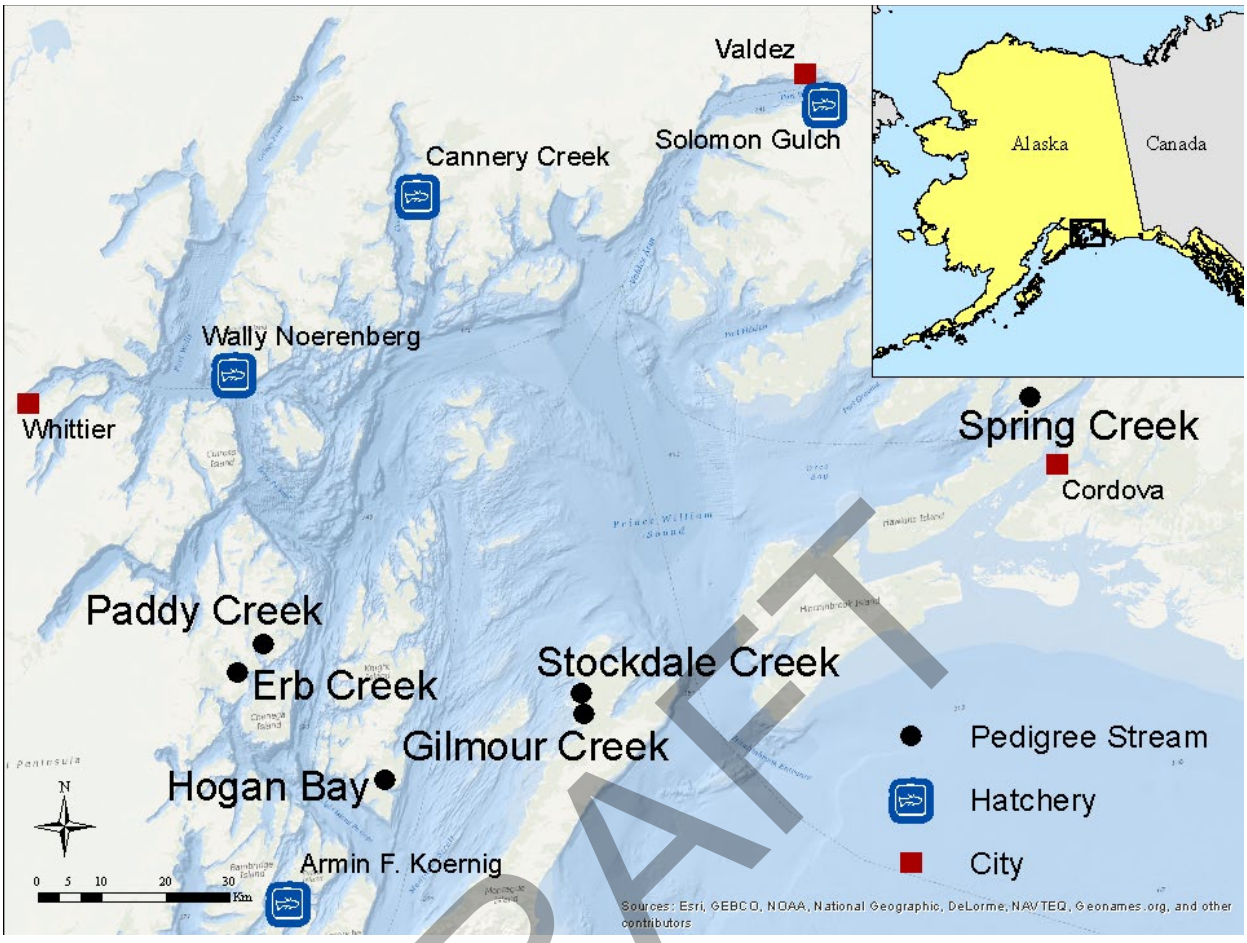

Figure 1. Map of streams identified by the Alaska Hatchery Research Program as targets for fitness studies of hatchery- and natural-origin Pink Salmon in Prince William Sound. This study focuses on Stockdale Creek, on Montague Island.

Otolith analysis

Otoliths were sent to the ADF&G Cordova Otolith Laboratory, where they were polished and inspected under a light microscope for the presence of hatchery thermal marks to determine the origin (hatchery versus natural and hatchery of origin) of each fish (Volk, Schroder, & Grimm, 2005). All trained otolith readers had previously been tested with randomized blind tests of known origin fish to assess accuracy (Joyce & Evans, 1999). Extracted otoliths (left otolith from each pair) were mounted, sulcus side up, on a petrographic glass slide with thermoplastic glue. Otoliths were wet ground at 250 rotations per minute to the mid-sagittal plane using 500-grit SiC paper until the thermal mark or wild pattern could be seen through a compound light microscope at 200X magnification. If left otoliths were missing, fragmented, or over-ground, then right otoliths were read instead. Otolith origin was determined by rings of thermal marks that were applied during the eyed egg stage and unique to each hatchery facility. Approximately 30% of otolith trays were systematically selected to be read a second time by a different reader for quality control. Any discrepancies between otolith reads were reviewed by the supervisor. As an additional level of quality control, both otoliths were read for a subset of individuals to ensure

that pairs were correctly matched during field collections. All reads (first, second, and supervisor over-rides) were stored in a database and final reads were reported. Error rates calculated from second reads were not used to estimate overall error rates (including otoliths not read twice).

Genetic analysis

A total of 7,986 Pink Salmon were genotyped at 298 single nucleotide polymorphism (SNP) amplicons (210 single [unlinked] SNPs, 88 microhaplotypes [two, linked SNPs within a single amplicon]), which is within the recommended range of markers for parentage analysis of incomplete pedigrees based on empirical studies (Lapègue, et al., 2014; Nguyen, Hayes, & Ingram, 2014; Sellars, et al., 2014; Trong et al., 2013). Single nucleotide polymorphisms were chosen because they lend themselves to high-throughput genotyping and have been successfully used for parentage analysis in salmonids (Anderson & Garza, 2006; Hauser et al., 2011). The amplicons were developed specifically for parentage analysis in PWS under contract to the University of Washington and selected from among thousands of SNPs discovered using restriction site-associated DNA sequencing (Baird et al., 2008) of PWS Pink Salmon collected in 2013 and 2014 (Dann et al., In prep.). Amplicons with microhaplotypes were prioritized as the additional alleles provide higher statistical power for resolving parent-offspring relationships (Baetscher et al., 2018). We genotyped both hatchery- and natural-origin fish (determined by otolith readings) for the parental brood year (2014) and only natural origin fish for the offspring year (2016).

We randomly selected a subset of sampled individuals to genotype that had a known origin based on otolith reads, known sex, and available tissue samples. We generally followed the Genotyping-in-Thousands by sequencing (GT-seq) methods described in Campbell, Harmon, & Narum (2015) other than deviations at the PCR2, purification, and quantification steps as follows. 1) During PCR2, we used 2 µL of 10 µM well-specific i5 tag primers per well, bringing the final reaction volume to 11 µL. 2) During the purification step with magnetic beads, the final elution volume was increased to 17 µL and no additional TE pH 8.0 with 1% TWEEN 20 was added. 3) To quantify libraries, quantitative PCR (qPCR) was completed using triplicate dilutions of 1:1000, 1:5000, 1:10000. Four microliters of each dilution were used as template in 10 µL reactions using 6 µL Kapa Library Quantification Kit - Illumina/ROX Low (Kapa Biosystems, Wilmington, MA.) The qPCRs were performed in 384-well plates on a QuantStudio™ 12K Flex Real-Time PCR System (Life Technologies). Final dilutions of each plate library were normalized to 4 nM. The final pooled library went through an additional purification step via magnetic beads, which involved adding 46.4 µL of Agencourt AMPure XP magnetic beads to 58 µL of pooled library. After incubation at room temperature for 7 minutes, it was placed in a magnetic stand for 5 minutes and the supernatant was discarded. A double wash of 80% ethanol (ETOH) was performed, for 30 seconds each. The tube incubated at room temperature for 5 minutes to dry off any residual ETOH. The elution was performed with 30 µL of 1X Low-EDTA TE, pH 8.0, incubated for 5 minutes before final transfer to a new 1.5 mL tube. The elution product was quantified for DNA yield via the manufacturer's direction for the Qubit 3.0 (Thermo Fisher Scientific). The final pooled library was sequenced at a concentration of 3.5 pM on an Illumina NextSeq 500 with single-end read flow cells using 150 cycles. Post-sequencing, we split reads from individual samples based on their barcodes and called genotypes according to counts of amplicon-specific alleles (Campbell et al, 2015) using GTScore (McKinney et al., In review.). Genotypes were imported and archived in the ADF&G GCL Oracle database, LOKI.

Quality control

DNA from poor quality tissues can produce unreliable genotypes (Paetkau, 2003). Data reliability is especially important for parentage analyses given that missing or incorrect genotypes can impact parentage assignments (Harrison, et al., 2013). A quality control (QC) analysis was conducted by staff not involved in the original genotyping to identify laboratory errors and measure the background error rate of the genotyping process (Dann et al., 2012). The method consisted of re-extracting DNA from 8% of fish and genotyping them for the same SNPs assayed in the original genotyping process following the same methods. Human errors introduced during the extraction and genotyping process were resolved through additional extractions and genotyping and the corrected data were retained.

Genotypes in the LOKI database were imported into *R* (R Core Team, 2018) for three additional quality assurance (QA) analyses. First, we removed individuals missing more than 20% of genotypes because they likely had poor-quality DNA. Second, we removed individuals with duplicate genotypes, as the paired field data (sex, otolith-origin, etc.) was uncertain. Duplicate genotypes can occur as a result of sampling or extracting the same individual twice and were defined as pairs of individuals sharing the same genotype in at least 95% of markers. Third, our QC analysis revealed that tissue degradation and/or contamination from multiple individuals resulted in outlying multilocus heterozygous genotypes that were not filtered out in the GTscore genotyping pipeline. We tested two individual heterozygosity filters: a +/- 3 standard deviation (SD) cutoff (Pettersson et al., 2011) and a cutoff of 1.5 interquartile range (IQR; Zar, 2010). We decided that the +/- 3 SD cutoff was not restrictive enough and inappropriate given our right-skewed distribution. We therefore decided to implement the 1.5 IQR cutoff to remove individuals with outlier heterozygosity values. Genotype data were paired with field and otolith data from the ADF&G OceanAK data warehouse (http://www.oceanak.adfg.alaska.gov), which joins field data from the Hatchery Wild Study database and lab data from the ADF&G Cordova Otolith Lab's database into a central repository.

Parentage analysis

We combined individual genotypes with collection year and sex to create input files for the pedigree reconstruction program *FRANz* (Riester, Stadler, & Klemm, 2009). This program uses a Bayesian framework and a Metropolis-Hastings coupled Markov Chain Monte Carlo algorithm to assign parentage based on life history data (birth year, death year, and sex), multilocus genotypes, an estimated genotyping error rate, and an estimated maximum number of parents that could have produced the offspring in the sample. We used *FRANz* because likelihood- and Bayesian-based parentage analyses have been shown to perform better than exclusion-only techniques (Anderson & Ng, 2014; Harrison et al., 2013; Hauser et al., 2011; Jones et al., 2010; Steele et al., 2013). Additionally, a full-probability Bayesian model for pedigree reconstruction is better suited for studies that are not able to sample all potential parents and offspring, because the model accounts for unsampled parents and can use sibships and other close relationships among sampled individuals to infer parental genotypes from progeny to fill out sparse pedigrees (Jones et al., 2010; Riester et al., 2009). We limited final parentage assignment to those parent-offspring pairs that had a posterior probability of assignment > 90%. We ran sensitivity analyses to test the robustness of parent-offspring assignments with different maximum numbers of parents and genotyping error rates. The genotyping error rate we used (0.6%) was derived from our QC pipeline and our N_{mmax} and N_{fmax} (maximum number of potential parents of each sex) values were based on escapement estimates (Russell et al., 2016). We joined the parentage

results with individual metadata to extract information about parent origin and both parent and offspring sex, length, and sample dates.

Relative reproductive success calculations

We calculated RS separately for males and females of hatchery- and natural-origin since most of our parent-offspring assignments were only to a single parent (parent-offspring dyads), due to incomplete sampling of potential parents. We calculated unweighted RRS separately for males and females, including all sampled potential parents (even those not assigned offspring, RS = 0). We calculated 95% confidence intervals around our unweighted RRS estimate following the methods of Kalinowski & Taper (2005). We tested for significant differences in RS between natural- and hatchery-origin fish using a non-parametric one sample permutation test ("oneway.test" function in the "coin" package in R; Hothorn, Hornik, A van de Wiel, & Zeileis, 2006), as testing for differences in RS is equivalent to testing if RRS < 1 (Araki & Blouin, 2005). We also used a parametric general linear model to test for significance (GLM; negative binomial distribution with a log link function; "glm.nb" in "MASS" package in R; Venables & Ripley, 2002). For offspring assigned to two parents (parent-offspring trios), we calculated RS separately for the four types of crosses: hatchery-hatchery, natural-natural, hatchery-natural (hatchery dam and natural sire), and natural-hatchery (natural dam and hatchery sire). Finally, we performed a Chi-square test to compare the proportions of offspring assigned to hatchery and natural-origin parents to the proportions of potential parents sampled, regardless of sex. This approach has previously been used to compare breeding success among sub-groups of individuals (see Anderson & Pearse, 2013; Chelini, Palme, & Otta, 2011). Following Ford, Murdoch, & Howard (2012) and Janowitz-Koch et al. (2019), we used a negative binomial distribution GLM and log-linked function to evaluate the association between RS and sample date, fish length, and origin separately for males and females.

Project Management:

Tyler Dann (ADF&G Fisheries Geneticist II) was an administrative point of contact and shared responsibility for reporting, budget requirements, and disseminating information to fisheries managers, commercial fishing groups, and the public.

Kyle Shedd (ADF&G Fisheries Geneticist II) was a technical point of contact and led sample selection. He supervised statistical analyses of parentage and RRS estimation, reporting and budgeting. He performed quality control of genotypes.

Emily Lescak (ADF&G Fisheries Geneticist I) was an additional technical point of contact. She performed statistical analyses of parentage and RRS estimation, shares responsibility for dissemination of work, and led report writing.

Heather Hoyt (ADF&G Fishery Biologist III) is the laboratory coordinator for all projects in the ADF&G Gene Conservation Laboratory and ensured that samples were extracted, genotyped, scored, and quality-controlled in a timely manner with minimal errors.

Nick Ellison (Fish and Wildlife Technician III/IV), Zach Pechacek (Fishery Biologist I), Paul Kuriscak (Fishery Biologist I), Mariel Terry (Fishery Biologist I), Erica Chenoweth (Fishery Biologist II), Zac Grauvogel (Fishery Biologist II), and Chase Jalbert (Fisheries Geneticist I) performed DNA extractions and genotyped individuals.

Judy Berger (Fishery Biologist III) coordinated sample collections and archiving.

Eric Lardizabal (Analyst/Programmer II) coordinated importing and archiving genotypes.

Findings:

Actual accomplishments and findings

We genotyped 7,986 Pink Salmon from Stockdale Creek. After accounting for fish filtered out during the QA process (Table 1), we were able to include 5,993 individuals in parentage analyses (Table 2). Most of the fish lost in the QA process were missing a substantial number of genotypes, which may have been due to poor tissue quality (Table 1).

Table 1. Numbers of Pink Salmon from Stockdale Creek initially genotyped and removed as a result of our quality assurance filters (Missing [missing more than 20% of genotypes], Duplicate [more than 95% identical genotypes to another fish], and Heterozygosity. [outlying individual heterozygosity]). Final represents the number of individuals that passed the QA filters and were used in parentage assignment.

Sample Year and Origin	Genotyped	Missing	Duplicate	Heterozygosity	Final
2014 Natural	436	65	4	9	358
2014 Hatchery	512	66	0	10	436
2016 Natural	7,038	1,560	46	233	5,199
Total	7,986	1,691	50	252	5,993

Table 2. Sample sizes for Pink Salmon collected in Stockdale Creek by year, sex, and origin that passed QA analysis and were used to assign parents to offspring.

Sample Year	Sex	Natural-origin	Hatchery-origin
2014	Female	221	230
2014	Male	137	206
2016	Female	2,842	0
2016	Male	2,357	0
Total		5,557	436

Our sensitivity analysis in *FRANz* revealed that increasing the maximum number of parents and genotyping error rate led to one additional parent-offspring assignment, which did not substantially change our estimate of RRS. We report results with the more conservative escapement estimate (4,038) and genotyping error rate (0.006). The cumulative exclusion probabilities for first parents, second parents, and parent pairs were all equal to 1.00. Therefore, the probability of a random pair of individuals in the population having a genotype pair compatible to an offspring genotype was equal to 0.00, which reflects the power of our marker set to accurately assign offspring to parents. All of our parentage assignments had a posterior probability of 1.00, with the exception of four individuals whose assignments were split among multiple potential parents. Of the 5,199 offspring genotyped, 1,054 were assigned to parents for a rate of 20.3%; this includes 183 two-parent assignments and 871 single parent-offspring assignments.

A chi-square test revealed a significant difference in the proportions of offspring assigned to hatchery- and natural-origin parents relative to the proportions of potential parents sampled, indicating an under-representation of offspring assigning to hatchery-origin parents ($\chi^2 = 122.96$, df = 1, p <0.001). Although 55% of parents genotyped were of hatchery-origin, only 30% of offspring assigned to hatchery-origin parents.

Mean RS of natural-origin fish was higher than that of hatchery-origin fish for both females and males (Table 3). Unweighted relative reproductive success was 0.42 (95% confidence intervals: 0.35-0.50) for females and 0.28 (95% confidence intervals: 0.24-0.34) for males, which represent statistically significant reductions in RS for hatchery-origin females and males based on negative binomial general linear models and permutation tests (all $p < 0.001$; Figure 2). For females, RS was significantly associated with sample date ($p = 0.013$), with earlier returns associated with higher RS, and origin ($p < 0.001$), but not length ($p = 0.508$). For males, RS was significantly associated with sample date, length, and origin (all $p < 0.001$; Figure 3).

Table 2. Mean unweighted reproductive success for female and male Pink Salmon from Stockdale Creek by origin for the 2014 parent brood year.

Origin	Female	Male
Natural	2.03	3.04
Hatchery	0.85	0.86

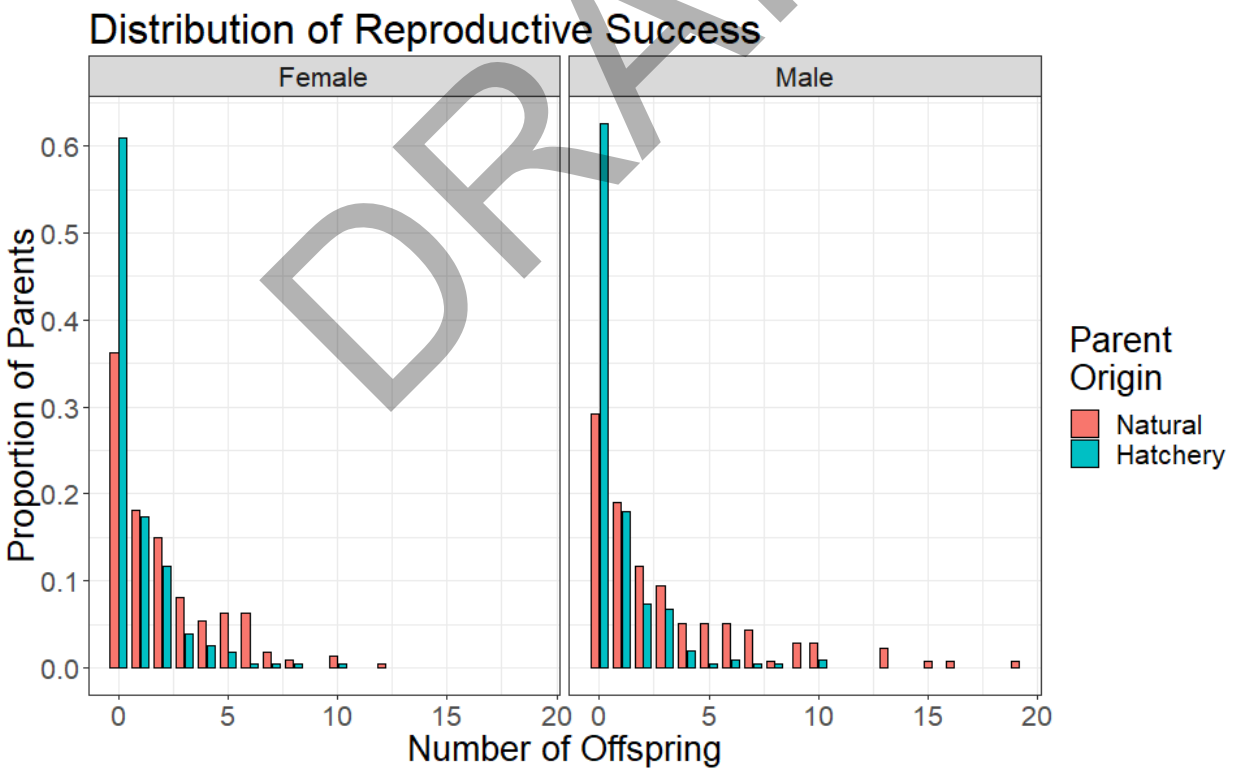

Figure 2. Distribution of unweighted reproductive success for female and male hatchery- and natural-origin Pink Salmon from Stockdale Creek for the 2014 parent brood year and 2016 offspring year.

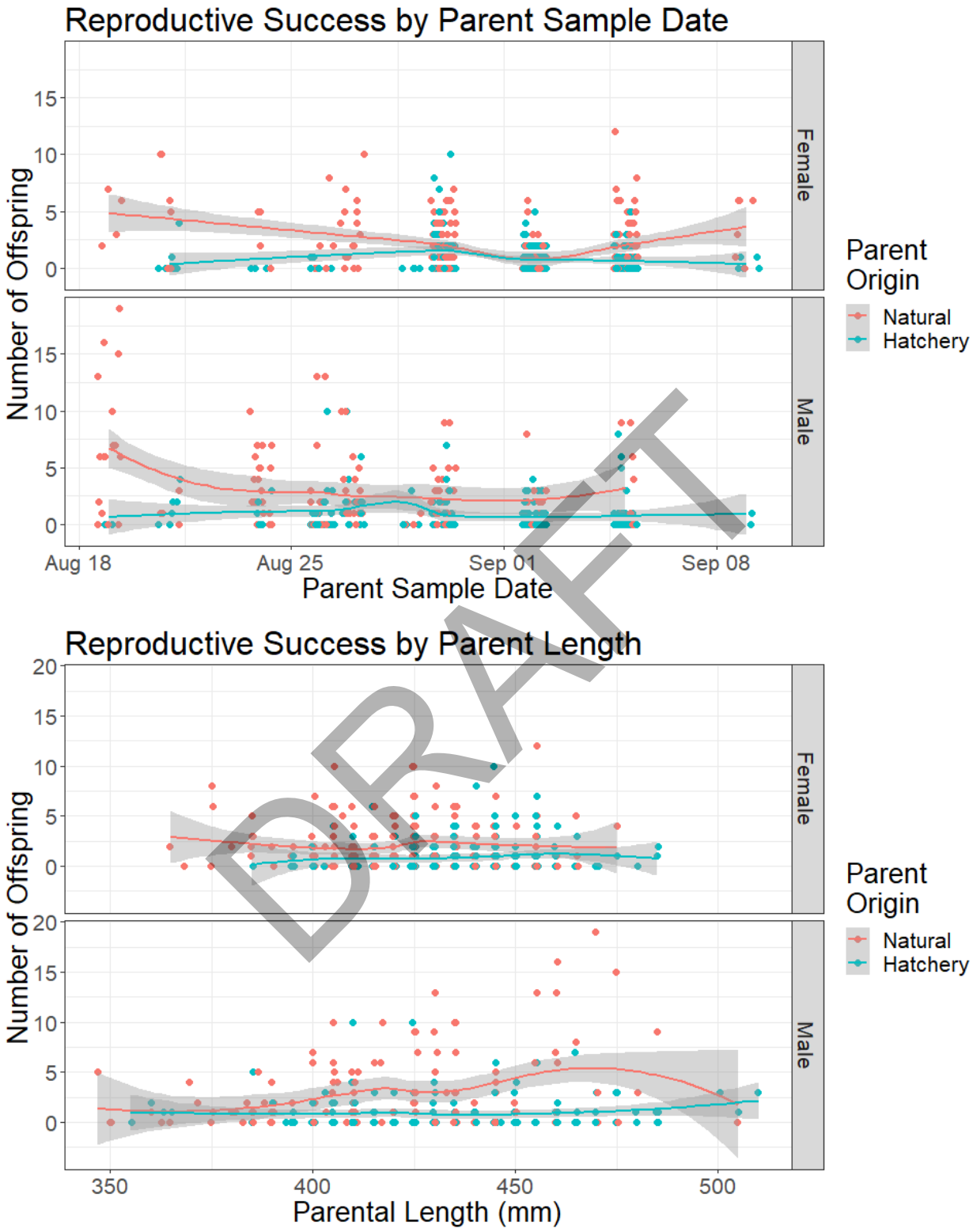

Figure 3. Sample date was significantly associated with reproductive success (RS; number of offspring) based on negative binomial general linear models (GLM) for both female (p=0.013) and male (p<0.001) Pink Salmon from Stockdale Creek (top) for the 2014 parent brood year. Length was significantly associated with RS for males (p < 0.001), but not females (bottom) based on negative binomial GLM.

Offspring from four types of crosses (two hatchery-origin parents [HH], two natural-origin parents [NN], hatchery-origin female with natural-origin male [HN], and natural-origin female with hatchery-origin male [NH]) were represented in our parent-offspring trios (Figure 4).

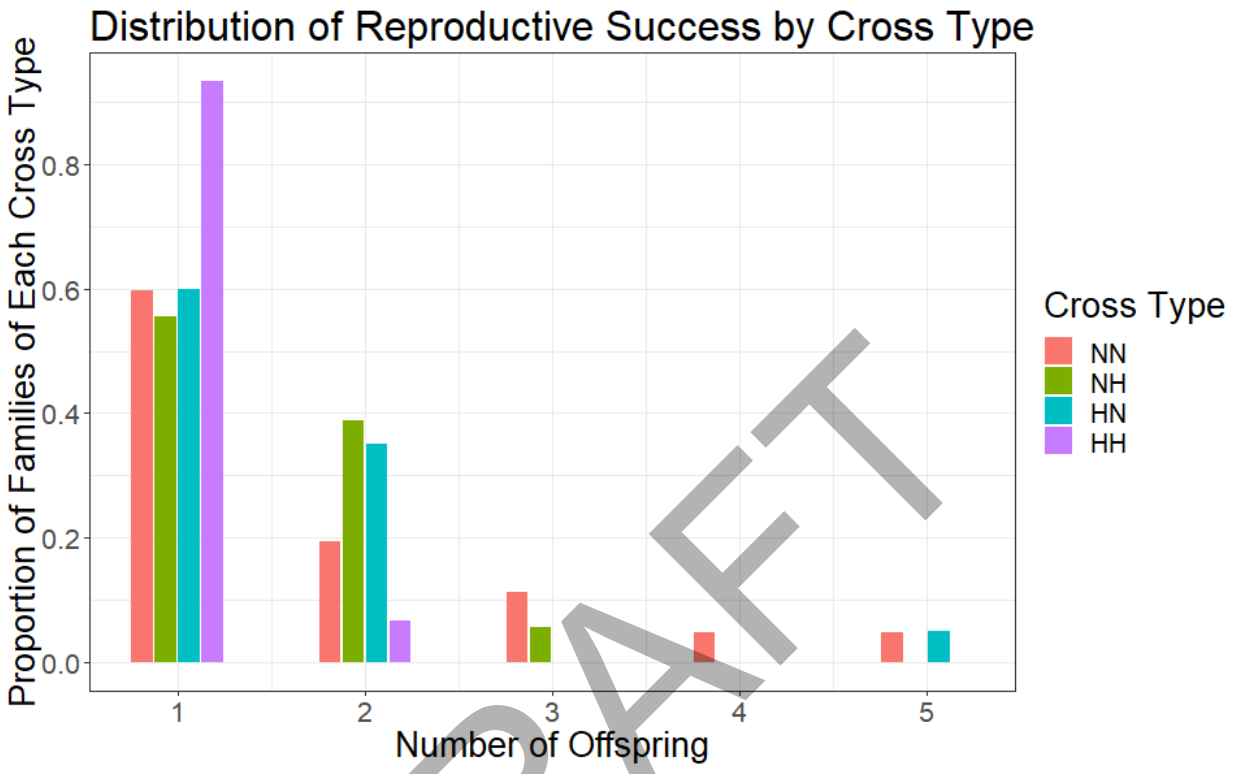

Figure 4. Distribution of unweighted reproductive success by cross type for Pink Salmon from Stockdale Creek for 2014 parent brood year. NN = two natural-origin fish; NH = natural-origin female and hatchery-origin male; HN = hatchery-origin female and natural-origin male; HH = two hatchery-origin fish. All possible cross types are present, which indicates that hatchery- and natural-origin Pink Salmon interbreed in Stockdale Creek.

Most detected matings were between two natural-origin parents (Table 4). Mean RS was highest for this cross-type, lowest for matings between two hatchery-origin parents, and intermediate for hybrid matings (Table 4). Reproductive success was significantly reduced in matings between two hatchery-origin parents as compared to two natural-origin parents (Table 5).

Table 3. Number of families and mean unweighted reproductive success (RS) for each cross type. NN = two natural-origin fish; NH = natural-origin female and hatchery-origin male; HN = hatchery-origin female and natural-origin male; HH = two hatchery-origin fish.

Cross Type	Number of Families	Mean RS
NN	62	1.76
NH	18	1.50
HN	20	1.55
HH	15	1.07

Table 4. Unweighted relative reproductive success (RRS) of different cross types detected in Pink Salmon parents sampled in Stockdale Creek in 2014. NN = two natural-origin fish; NH = natural-origin female and hatchery-origin male; HN = hatchery-origin female and natural-origin male; HH = two hatchery-origin fish.

Cross Type Comparison	RRS with 95% Confidence Intervals
HH/NN	0.61 (0.35-0.99)
HN/NN	0.88 (0.58-1.30)
NH/NN	0.85 (0.55-1.28)

Need for additional work

Future research will include testing, and potentially accounting for, sampling biases of parents and offspring to develop unbiased estimates of RRS for the full population. For example, if sampling rates of parents and offspring change throughout the season, we can stratify estimates of RRS based on differences in sampling rate to calculate a weighted estimate of RRS.

We are currently genotyping additional fish from offspring year 2016 to increase parent-offspring assignments. We will extend our analyses of RRS across multiple parent-offspring years and streams in PWS to determine variation in RS and examine whether fitness costs of hatchery-origin parents carry-over to the next generation (i.e. the influence of grandparent origin), if possible. We will use information collected in the field on individual sex, sample date, sample location, and individual length to continue to test hypotheses of potential causal mechanisms for differential RRS, as well as document phenotypic differences between hatchery- and natural-origin fish across streams (e.g., Lin et al., 2008, 2016; Peterson, Hilborn, & Hauser, 2014, 2016). By associating openings of local area commercial fisheries with individual sample date and RS, we aim to determine if harvest practices impact estimates of RS, as well. Simulations and modeling may provide additional evidence for the type of mechanisms likely driving observed RRS values, their biological significance, and their historical impact on overall reproductive success in natural systems like PWS. Taken together, these future directions will provide information for policy makers evaluating both the benefits of the hatchery programs to the economic wellbeing of the fishing industry and communities relying on fishing revenues and potential risks to natural stocks.

Evaluation:

The project's objectives were to:

1. Genotype 8,000 F_0 and F_1 individuals collected in 2014 and 2016, respectively, at 192 single nucleotide polymorphism (SNP) markers.
    a. Genotype all F_0 parents regardless of origin from as many streams as possible.
    b. Genotype only natural-origin F_1 offspring for corresponding streams.
2. Identify the number of offspring attributable to each parent and calculate RRS for hatchery- and natural-origin pink salmon.

To meet these objectives, we genotyped 948 Pink Salmon collected in 2014 and 7,038 collected in 2016 from Stockdale Creek at 298 amplicons, for a total of 7,986 individuals. Of these individuals genotyped, 5,993 passed all the quality control and quality assurance filters and were used in pedigree reconstruction. Most of the excluded fish failed to genotype at more than 80% of markers likely due to poor tissue quality of carcass samples (Table 1). We focused on one stream because 1) we had adequate samples from Stockdale Creek; 2) power analyses suggested a high likelihood of being able to detect differences in RS between hatchery- and natural-origin Pink Salmon from Stockdale Creek (should they exist) based on available samples; and 3) genotyping individuals from multiple streams would not have allowed for adequate sampling depth to assign enough offspring to parents and thereby provide meaningful estimates of RS and RRS.

As planned, we genotyped both hatchery- and natural-origin fish from the parental generation (2014) and only natural-origin fish from the offspring generation (2016). Rather than the 192 SNP marker set we had originally intended to use, one of the project PI's collaborated with the Seeb Lab at the University of Washington to design a genetic marker set that was optimized for parentage analysis of Pink Salmon from PWS (Dann et al., In prep). Using sensitivity analyses and simulations, we were able to demonstrate the power of this 298-amplicon marker set to both accurately and precisely assign parents and offspring (Lescak et al. 2019).

The mean RS values we estimated are biased low due to several factors; however, our RRS and chi-square calculations account for these factors if they affected hatchery- and natural-origin offspring in the same way since they are relative measures of RS. Because we were reliant upon carcass sampling, rather than dams or weirs, we were not able to sample every potential parent in 2014 and offspring in 2016 in Stockdale Creek, raising the question of whether or not the carcasses that were collected are fully representative of the 2014 and 2016 spawning populations. In addition, poor tissue quality from carcass samples likely resulted in lower successful genotyping, further reducing sampling proportions. The RS we measured only included fish that returned and died in Stockdale Creek and, therefore, did not account for offspring that fail to home back to Stockdale Creek (i.e. fish that were harvested in the fishery or strayed to another stream in PWS). In addition, our estimates of RS are influenced by run strengths of the parent and offspring years, since these affect the proportions of fish sampled. Finally, the RS estimates are for fish that escape the terminal fisheries in PWS, where about 27% of wild (unmarked) and 98% of hatchery (marked) fish are harvested (Knudsen et al., 2015) and, therefore, cannot be included in calculations of survival to adulthood.

Lastly, we were able to robustly estimate unweighted RRS for different mating combinations of hatchery- and natural-origin fish for the first time in PWS, by assigning 183 offspring to two

parents. These results indicated that hatchery-hatchery crosses resulted in the lowest unweighted RS, hatchery-natural (regardless of direction) crosses resulted in intermediate unweighted RS, and natural-natural crosses resulted in the highest unweighted RS (Table 5). Unlike other estimates of RS reported here, these cross-specific estimates only account for crosses where at least one progeny was identified and, therefore, exclude crosses that produced no offspring.

Project results have been shared with members of the AHRP Science Panel and we co-authored an article for Delta Sound Connections about the project with Dr. Peter Rand of the Prince William Sound Science Center. We plan to disseminate this research publicly at the National American Fisheries Society Meeting in September 2019 and the 2020 American Fisheries Society Alaska Chapter Meeting. This final report will be posted on the AHRP website and results from this study will be included in peer-reviewed scientific papers. Once these results have been published, we will prepare a data nugget (datanuggets.org), a free online education tool based on our research.

We have begun procurement of materials to build an informational kiosk in Cordova, Alaska to communicate our results with the public. We plan to point the kiosk to the AHRP website and intend to expand upon the information available as results from more streams and generational replicates become available.

Lastly, Co-PI Lescak has participated in local educational outreach through the *Kids2College* program, which brings professionals into elementary school classrooms to talk about college and career preparedness, Campbell STEM Elementary School's Lunch and Learn series, and Romig Middle School's STEM Day. Through the Skype a Scientist Program, she connected with high school classes in Missouri and Virginia to talk about our research and STEM careers and she also participated in the Letters to a Pre-Scientist Program, which matches scientists with school-age pen pals.

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