North Pacific Research Board Final Report

Relative productivity of hatchery pink salmon in a natural stream

NPRB project 1619

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Abstract.

Private non-profit hatcheries in Alaska release 1.8B juvenile salmon annually, mostly Pink Salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and Chum Salmon (*O. keta*) in Southeast. Concerns that hatchery-produced fish may detrimentally impact the productivity and sustainability of natural Alaska salmon have been raised due to the scale of hatchery programs, documented straying of hatchery salmon, and studies of the impacts of hatchery salmon in other species and areas. We present the first study to use genetic parentage analysis to estimate the relative reproductive success (RRS) of stray hatchery- relative to natural-origin Pink Salmon in one stream (Hogan Bay) for one generation in both odd- and even-year lineages. We produced parentage assignments for 48 offspring from the odd lineage and 451 offspring from the even lineage by genotyping 7,941 fish collected in 2013–2016 at 298 single nucleotide polymorphism amplicons. Reproductive success was significantly lower for hatchery-origin relative to natural-origin fish from both lineages. Hatchery-origin females were significantly less productive than natural-origin females for both lineages, but male hatchery fish were significantly less productive only in the odd-lineage. However, the small number of offspring assignments for the odd-lineage warrants cautious interpretation. These are the first in a series of RRS analyses under the Alaska Hatchery Research Program. Future work in PWS will provide replicate analyses in four more streams, include samples from 2017–2019, investigate RRS in different mating combinations between natural- and hatchery-origin fish, and explore multi-generational effects. Important questions remain regarding the mechanisms driving the effect observed in this study.

Keywords. fitness; hatchery-wild interaction; parentage analysis; pedigree; pink salmon; relative reproductive success

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Chronology.

In December 2017, a no-cost extension was provided with the following rationale:

Given the delay in obtaining SNPs for our custom amplicon panel, we have not been able to genotype the 9,120 fish that have already been DNA extracted for this project. We are currently in the final stages of SNP selection. Following the design and ordering of primers, we will perform a few test runs to optimize the amplicon panel for full production genotyping of the fish selected for this project. Once genotypes are available, we can begin to statistically analyze samples for parentage relationships and calculate the relative reproductive success of hatchery- and natural-origin pink salmon in Prince William Sound.

We have not fully spent as much on supplies as anticipated by this date since we have not started genotyping yet. Supplies required for genotyping have relatively short shelf lives (months), so they will not be ordered until we have the SNP panel for genotyping. We anticipate having ~ $163,000 left by the original project end date. In order to successfully complete this project, we would like to request a no-cost extension through December 31, 2018.

In October 2018, Dr. Emily Lescak was added as a co-PI. She was hired by the Alaska Department of Fish and Game's Gene Conservation Lab to help with the final analyses for this project and work on the larger Alaska Hatchery Research Program.

Introduction.

Since the 1970's, private non-profit (PNP) hatcheries have been practicing extensive ocean-ranching aquaculture of Pacific salmon in Alaska to provide additional harvest opportunity to the fishing industry and local communities. 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). High hatchery proportions in natural streams in PWS have raised concerns that hatchery-produced fish may impact the productivity and sustainability of natural stocks via competition on the spawning grounds and/or genetic introgression. 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 (Christie et al., 2014; Naish et al., 2008), including for pink salmon enhancement in PWS. In light of these concerns, hatchery programs must be evaluated in the context of both the clear economic benefits of enhancement (McDowell Group, 2018a, 2018b) and the harder to quantify risks to natural stocks.

As a response to concerns regarding straying and possibilities for genetic, ecological, and behavioral interactions between hatchery- and natural-origin salmon, the Alaska Department of Fish and Game (ADF&G) and PNP hatchery corporations developed a research program to: 1) characterize the genetic stock structure of pink salmon in PWS and chum salmon in SEAK; 2) document the extent and variability in hatchery proportions in PWS and SEAK; and 3) quantify the impact on fitness of natural pink and chum salmon stocks due to straying hatchery pink and chum salmon. In 2013, a science panel comprised of representatives from government agencies, universities, and industry developed the Alaska Hatchery Research Program (AHRP) to address these questions (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main), using funds from the State of Alaska, the PNP operators, and industry representatives.

We present the first study from the AHRP to directly measure whether Pink Salmon of known hatchery ancestry have lower fitness than Pink Salmon of undocumented hatchery ancestry (natural-origin) in Alaska. We examined both odd and even year lineages to provide pseudo-replicate measures of RS within a study stream. We genotyped 7,941 individuals from Hogan Bay in two parental brood years (2013 and 2014) and two offspring years (2015 and 2016) at 298 single nucleotide polymorphism (SNP) markers and used parentage analyses to calculate RS of individual parents. We then paired the origin and sex of parents (gathered from otolith reads and field observations) to calculate the relative reproductive success (RRS) of hatchery- and natural-origin males and females. We found that hatchery-origin fish had lower RS than natural-origin in both lineages.

Objectives.

1. Genotype 8,000 pink salmon collected in 2013-2016 from odd- and even-lineages from Hogan Bay at 192 genetic markers to allow assignment of offspring to parent via parentage analysis.

   We genotyped 7,941 pink salmon collected between 2013-2016 at 298 single nucleotide polymorphism (SNP) markers. These samples represent both odd (2013-2015) and even (2014-2016) lineages.

2. Identify number of offspring attributable to each parent and calculate relative return per spawner (RRS) for hatchery- and natural-origin pink salmon by sex.

   FRANz, a Bayesian pedigree program, produced parentage assignments for 2.5% of offspring genotyped for the odd lineage and 11% for the even lineage. Offspring were assigned to both natural- and hatchery-origin parents in both lineages. In both lineages, hatchery-origin fish had significantly lower reproductive success than natural-origin fish.

3. Evaluate the power to detect differences in RRS between hatchery- and natural-origin pink salmon given final sample sizes.

   We updated the preliminary power analyses that we did prior to field sampling in 2015 and 2016 (Shedd et al., 2014) based on the final number of samples analyzed for 2015 and 2016. Given the estimated productivity of the 2013 brood year and the sampled proportion of the 2015 return, we estimated that we had limited power (< 0.2) to detect an effect of RRS < 0.5 for the odd-year Hogan Bay samples (see Box 1). For the even-year Hogan Bay samples, we had enough power (> 0.9) to detect an effect of RRS < 0.5 based on the higher productivity of brood year 2014 and the higher sampling rate of 2016 (see Box 1).

4. Communicate study findings to stakeholders, local communities and relevant management agencies and develop educational tools from them.

   We have been in regular communication with the Alaska Hatchery Research Program's science panel to discuss methods and progress. We presented to the scientific community at the Alaska Marine Science Symposium (AMSS) in January 2019, the American Fisheries Society's Alaska Chapter Meeting, as well as a public informational meeting on the AHRP in March 2019. We also co-authored an article for the Delta Sound Connection with Dr. Peter Rand from the Prince William Sound Science Center about our research and conducted two media interviews about our results. We have visited multiple schools in the Anchorage School District to talk about our research, salmon biology, and careers in STEM. Once our results have been published, we will produce a Data Nugget (datanuggets.org), which is a short lesson that can be freely accessed by educators around the world. We will also produce an interactive interpretational exhibit on our project that will be housed in a public space.

Chapters.

Relative fitness of hatchery and natural Pink Salmon in Hogan Bay, Prince William Sound, Alaska

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Abstract

Private non-profit hatcheries in Alaska release 1.8B juvenile salmon annually, mostly Pink Salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and Chum Salmon (*O. keta*) in Southeast Alaska. Concerns that hatchery-produced fish may impact the productivity and sustainability of wild Alaskan salmon have been raised due to the scale of hatchery programs, documented straying of hatchery salmon, and studies of the impacts of hatchery salmon in other species and areas. Herein we present initial results where genetic parentage analysis was used to estimate the relative reproductive success (RRS) of stray hatchery-origin versus natural-origin Pink Salmon in one stream (Hogan Bay, PWS) for one generation in both odd- and even-year lineages. We produced parentage assignments for 48 offspring from the odd lineage and 451 offspring from the even lineage by genotyping 7,941 fish collected in 2013–2016 at 298 single nucleotide polymorphism amplicons. Reproductive success, measured as return to the spawning grounds, was significantly lower for hatchery-origin versus natural-origin fish from both lineages. Hatchery-origin females were significantly less productive than natural-origin females for both lineages, but male hatchery fish were significantly less productive only in the odd-lineage. However, the small number of offspring assignments for the odd-lineage reduces robustness of interpretation. These results are the first in a series of RRS analyses under the Alaska Hatchery Research Program. Future work in PWS will include analysis of additional samples from Hogan Bay, provide replicate analyses in four more streams, include samples from 2017–2019, investigate RRS in different mating combinations between natural- and hatchery-origin fish, and explore multi-generational effects.

Keywords

fitness; hatchery-wild interaction; parentage analysis; pedigree; Pink Salmon; relative reproductive success; SNP

Introduction

Private non-profit (PNP) hatcheries have been practicing extensive ocean-ranching aquaculture of Pacific salmon in Alaska since the 1970s to provide additional harvest opportunity to common property fisheries and to support the sustainability of local salmon-dependent communities. Hatchery production plays a large role in Alaska's salmon fishing industry, contributing 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 PNP hatcheries contributed an average of 70% of the total return in PWS between 2013–2015 (Knudsen et al., 2015).

Two PNP hatchery programs exist in PWS: 1) Prince William Sound Aquaculture Corporation (PWSAC), which operates three hatcheries distributed across western and northern PWS and 2) Valdez Fisheries Development Association (VFDA), which operates one hatchery in northeastern PWS (Figure 1). All hatcheries apply unique otolith thermal marks, allowing for identification of hatchery-origin fish in the commercial harvest and in surveys of natural streams (Joyce & Evans, 1999). Hatchery-origin fish are targeted by the commercial fishery, with between 95–99% being harvested or taken as broodstock by the hatcheries from 2013–2015 (Knudsen et al., 2016). Harvest rates on natural origin Pink salmon from 2013-2015 ranged from 27% to 53% (Knudsen et al., 2016). The remaining 1–5% of hatchery fish not harvested end up straying into natural streams and potentially spawning with their natural-origin conspecifics (Knudsen et al., 2016). Hatchery proportions in natural streams in PWS range from 0–98% and are generally higher near hatchery release sites (Brenner, Moffitt, & Grant, 2012; Gorman et al., 2018). In streams near PWSAC hatcheries, hatchery proportions are known to increase throughout the season (Brenner, Moffitt, & Grant, 2012). VFDA, in contrast, intentionally selected for early run-timing, so their hatchery strays may enter natural streams earlier in the run (ADF&G Staff, 1994; Evenson et al., 2018).

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 negatively impacting natural stocks (Wertheimer et al., 2001). 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. Alaska state law requires that hatchery production be compatible with conservation of natural stocks.

Pedigree reconstructions via genetic parentage analysis allow investigators to directly measure the reproductive success (RS; see Table 1 for definition) of hatchery- and natural-origin fish to determine if there are differences in relative reproductive success (RRS; see Table 1 for definition) between the two

groups. In this case, the RRS calculations are confined to the fish that escape the fishery and are directly sampled from the spawning grounds. The RS of hatchery- and natural-origin fish may be influenced by a combination of two broad categories of mechanisms: genetic and ecological. Hatchery-origin salmonids have been shown to have lower RS than their natural-origin conspecifics in steelhead (*O. mykiss*) from Oregon and Chinook salmon (*O. tshawytscha*) from Washington (Anderson et al., 2013; Araki et al., 2008; Fleming et al., 2011; Ford et al., 2016). However, no significant differences were observed between hatchery and natural Coho salmon in Washington (Ford et al., 2006; Ford et al., 2008) or Chinook salmon in Idaho (Hess et al., 2012). An initial study found no significant difference in RS in steelhead derived from a local brood source in Oregon (Araki et al., 2007). However, a later study found an effect of broodstock on RS of hatchery steelhead in the wild (Ford et al., 2016). Berejikian et al. (2009) found no statistically significant reduction in RS for hatchery-origin Chum Salmon of either sex; however, some signal that hatchery-origin females, but not males, may have lower RRS than their natural-origin counterparts was noted (see Box 2 in Christie, Ford, & Blouin, 2014). Jasper et al. (2013) found that natural-origin Chum Salmon in PWS lost some inter-population genetic diversity due to introgression with hatchery fish.

Because of the value of hatchery production to industry's harvest and the mandate that hatchery production be compatible with sustainable productivity of wild stocks, the Alaska Department of Fish and Game (ADF&G) and PNP hatchery operators recognized the need for a research program addressing concerns about interactions between hatchery and natural stocks. In 2013, ADF&G organized a science panel comprised of current and retired scientists from ADF&G, the University of Alaska, aquaculture associations, and the National Marine Fisheries Service to develop the Alaska Hatchery Research Program (AHRP; http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main), with funding from the State of Alaska, PNP operators, and processors. The science panel of the AHRP raised three priority questions regarding hatchery-wild interactions for Pink Salmon in PWS and Chum Salmon in PWS and SEAK: 1) what is the genetic stock structure of Pink and Chum salmon in each region?; 2) what is the extent and annual variability in straying of hatchery Pink Salmon in PWS and Chum Salmon in PWS and SEAK?; and 3) what is the impact on fitness (productivity) of wild Pink and Chum salmon stocks due to straying of hatchery Pink and Chum salmon?

The AHRP study design initially called for sampling of 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 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, Dann, Habicht, Jasper, & Templin, 2014) to select those streams and years most likely to provide enough power to detect an RRS of < 0.5 (per the project study design; Taylor, 2013). This power analysis 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; Figure 1) was selected as our first study stream for genetic analysis in 2014 due to its moderate escapements (in the tens of thousands), variable hatchery proportions (21–90%), and its likely high statistical power to detect differences in RS given the samples available (Shedd et al., 2014; Box 1). Funding available limited the sample sizes for the genetic

analysis reported herein; additional samples from Hogan Bay are available and will be analyzed in the future with funding from a different source and future reporting will provide a more in-depth analysis.

This report provides the initial analysis of results from Hogan Bay concerning the RS of natural- and hatchery-origin Pink Salmon, which is the first work of this kind conducted on Pink Salmon. Pink Salmon have a strict two-year generation time that results in genetically-distinct odd and even lineages (Tarpey et al., 2017). We genotyped 7,941 individuals from Hogan Bay (Figure 1) in two parental brood years (2013 and 2014) and two offspring years (2015 and 2016) at 298 amplicons and used parentage analyses to calculate RS of individual parents. We then paired the origin and sex of parents (from otolith reads and field observations) to calculate the RRS of hatchery- and natural-origin males and females. Reproductive success was significantly lower for hatchery-origin relative to natural-origin fish from both lineages. Hatchery-origin females had a significantly lower RS than natural-origin females for both lineages, but male hatchery fish had a significantly lower RS only in the odd-lineage. However, the small number of offspring assignments for the odd-lineage warrants cautious interpretation.

Methods

Field collections

Field crews from Prince William Sound Science Center (PWSSC) collected tissue samples from five streams during the 2013–2016 field seasons (Figure 1). Due to lack of infrastructure (e.g. dams or weirs) in these remote streams, we were reliant upon in-stream sampling of carcasses, which limited the ability to collect all potential parents and their adult offspring. All available carcasses were sampled every other day (weather permitting). There were only a few sampling events in 2013, but by 2016, carcasses were sampled throughout the entire run (Figure 2). Paired otolith and heart tissue samples were collected concurrently into a cell of a 48 deep-well plate and preserved in 95% ethanol to prevent DNA degradation (Gorman et al., 2018). DNA decay in dead fish is affected by time, temperature, environment, and solar radiation (Cadet et al., 1997). We therefore chose to sample heart tissue (bulbus arteriosus) because 1) it is one of the last tissues to die, 2) it is protected from solar radiation, and 3) tests of this tissue type from carcasses yielded high genotyping success (Dann et al., 2013). Otoliths and heart tissue were separated at the Gene Conservation Laboratory (GCL) at ADF&G in Anchorage to maintain pairing integrity. Each fish's sex and sampling date were also recorded and archived in the Hatchery Wild Study database maintained by ADF&G.

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 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. 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. All reads (first, second, and supervisor over-rides) are stored in a database and final reads are reported. Error rates calculated from second reads are not used to estimate overall error rates (including otoliths not read twice).

Genetic analysis

In total, 7,941 individuals 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). We genotyped both hatchery- and natural-origin fish (determined by otolith readings) for the parental brood years (2013 and 2014) and only natural origin fish for the offspring years (2015 and 2016). The amplicons we genotyped were developed specifically for parentage analysis in PWS under contract to the University of Washington (Dann et al., In prep) 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 they provide high statistical power for resolving parent-offspring relationships (Baetscher et al., 2018). Single-SNP amplicons with high minor allele frequency in both odd- and even-year lineages and low variation in minor allele frequency among populations were selected because they maximize discriminatory power in parentage analysis and pedigree reconstruction in multiple populations (Anderson & Garza, 2006).

We randomly selected individuals to genotype that had a known origin based on otolith reads, known sex, and available tissue samples (see Tables S1 and S2 for sample sizes). We examined the sample dates of these individuals to make sure that they were collected throughout the run. Single nucleotide polymorphism genotyping 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) 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 seven minutes, it was placed in a magnetic stand for five 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 five 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 prep.). Genotypes were imported and archived in the ADF&G GCL Oracle database, LOKI.

Quality control

DNA from poor quality tissues can produce unreliable genotype data (Paetkau, 2003). Data reliability is especially important for parentage analyses given that missing or incorrect genotypes can have a large impact on parentage assignments (Harrison, Saenz-Agudelo, Planes, Jones, & Berumen, 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 in the database. Discrepancy rates were calculated as the number of conflicting genotypes divided by the total number of genotypes compared. These rates describe the difference between project data and QC data for all SNPs. These discrepancy rates are divided by two to calculate the background genotyping error rate. This estimate of error rate assumes that genotyping errors are as likely to occur during the original genotyping as during the quality control genotyping, which is a reasonable assumption given that both analyses use the same methods.

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 (Figure S1). We therefore tested two 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 (Figure S1). This 1.5 IQR cutoff was applied separately to each lineage, given the differences in allele frequencies between lineages. These cut-offs were <0.336 and >0.497 for the odd year lineage and <0.351 and >0.498 for the even year lineage. Genotype data were paired with field and otolith data from the OceanAK data warehouse (http://www.oceanak.adfg.alaska.gov), which joins field data from the PWSSC's Hatchery Wild Study database and lab data from the Cordova Otolith Lab's database into a central repository.

Parentage

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) and multilocus genotypes. 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). *FRANz* calculated cumulative exclusion probabilities (see Table 1 for definition) for first parent, second parent, and parent pair assignments. We limited final parentage assignment to those parent-offspring pairs that had a posterior probability of assignment > 90%. For both lineages, we ran sensitivity analyses using the parameters in Table S3. The genotyping error rate we used (0.5%) was derived from our QC pipeline and our N_{mmax} and N_{fmax} (maximum number of potential parents by sex; Table 1 for definition) 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 and sample dates.

Relative reproductive success

We calculated RS separately for males and females of hatchery- and natural-origin for both lineages 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 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 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 for each lineage 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).

Simulations

To estimate our Type I and Type II parentage assignment error rates (number of individuals incorrectly assigned to parents and number of assignments that were missed), we simulated 3,000 offspring genotypes for each lineage using the parental genotypes and used *FRANz* to assign them to parents using the parameters in Run 1 (Table S3). Additionally, we followed code from Baetscher et al. (2018) to use the CKMRSIM R package (Anderson, https://doi.org/10.5281/zenodo.820162) to evaluate the power of our marker set to accurately make parent-offspring and full- and half-sibling assignments using Monte Carlo simulations. CKMRSIM simulates genotypes of related and unrelated pairs of individuals from estimated allele frequencies and calculates the probabilities of those genotype pairs to compute log-likelihood ratios of true versus hypothesized relationships. These simulated distributions are then used to calculate Type I and Type II error rates.

Results

Field collections

Between 2013 and 2016, 94,607 adult Pink Salmon were sampled from all five streams (Figure 1). Of these, 25,928 were collected from Hogan Bay and the Cordova ADF&G Otolith Lab read 25,218 total

otoliths (Table S2, S4). In 2013, we did not have complete sampling throughout the spawning run, but in subsequent years, samples were collected approximately every other day (weather dependent) throughout the entire run (Figure 2). Agreements between first and second readers for designating hatchery/wild was 96–97% and distinguishing among hatcheries was 93–97% (Jenni Morella, pers. comm.). Sampled hatchery proportions for Hogan Bay each year ranged from 20% in 2016 to 91% in 2014 (see Table S2 for raw numbers of hatchery-origin fish identified each year). All hatcheries contributed fish throughout the sampling period, but most of the hatchery-origin fish were from PWSAC hatcheries (Table S4). In 2015 and 2016, 54% and 20% of the fish were hatchery-origin, respectively, and were therefore not genotyped.

Genetic analysis

Across all four years, 7,941 individuals were genotyped. The range was 751 for 2014 to 4,295 for 2016 (Tables 2, S1, S2).

Quality control

Our overall background genotyping error rate ranged from 0.30–0.70% for each year. We removed 43 fish from offspring brood year 2015 that were determined to be hatchery-origin from second reads of otoliths, because we only wanted to include natural origin fish from this year. These fish were likely originally determined to be natural-origin during the first reading of the otoliths but had their origin status updated after secondary otolith reads. For each year-origin combination, we lost 12–117 individuals who were missing >20% of genotypes, 0–12 duplicate individuals, and 1–172 individuals due to outlying levels of heterozygosity. We retained between 85¬96% of individuals of each origin collected each year. Tables 2 and S1 show our final sample sizes.

Parentage

Exclusion probabilities for both the even and odd lineages were equal to 1.00, indicating that we can be confident in the ability of our marker set to accurately assign parents to offspring. For the odd-year lineage, all three *FRANz* runs produced identical parentage assignments, while for the even-year lineage, two additional offspring were assigned parents in runs 2 and 3 (see Table S3 for run parameter values). These individuals were not included in our downstream analyses because their posterior probabilities of assignment did not meet our cut-off value of >0.90.

In the odd-year lineage, 48 offspring were assigned to 20 unique parents (three of which were hatchery-origin), for an offspring assignment rate of 2.5%. All posterior probabilities of assignment were equal to 1.00. This low assignment rate is in line with expectations due to high escapement and low sampling during the 2013 parental generation (Table 2). The three hatchery parents were from Armin F. Koernig (AFK) and Wally Noerenberg (WNH; Figure 1 and Table S5). No parent-offspring trio relationships were identified (all offspring were assigned to a single parent and the other parent was unknown).

Relative reproductive success was 0.02 for females (95% confidence intervals = 0.01 and 0.09) and 0.12 for males (95% confidence intervals = 0.02 and 0.40); this was significantly different from RRS = 1.00 for hatchery- versus natural-origin females ($p_{\text{negative binomial GLM}} = 0.002$; $p_{\text{non-parametric permutation test}} = 0.003$) and males ($p_{\text{negative binomial GLM}} = 0.013$; $p_{\text{non-parametric permutation test}} = 0.008$). Our 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 assigned to hatchery-origin parents ($\chi^2 = 46.62$, df = 1, $p < 0.001$; Table S6). Although 58% of the

potential parents genotyped were of hatchery origin, only 6% of assigned offspring had hatchery-origin parents. Female parents produced 1–16 offspring, while male parents produced 1–4 (Figure 3).

In the even-year lineage, 451 offspring were assigned to 184 unique parents (112 of which were hatchery-origin), for an offspring assignment rate of 11%. All posterior probabilities of assignment were equal to 1.00. The hatchery parents originated from AFK, Cannery Creek (CCH), and WNH (Figure 1; Table S5). *FRANz* made 22 parent-pair-offspring trio assignments, which included crosses between hatchery parents, natural parents, hatchery females and natural males, and natural females and hatchery males (Figure S2).

The RRS was 0.47 for females (95% confidence intervals = 0.37 and 0.62) and 0.87 for males (95% confidence intervals = 0.67–1.12); this was significantly different from RRS = 1.00 for hatchery- versus natural-origin females ($p_{\text{negative binomial GLM}} = 0.004$; $p_{\text{non-parametric permutation test}} = 0.002$), but not males ($p_{\text{negative binomial GLM}} = 0.610$; $p_{\text{non-parametric permutation test}} = 0.714$). Our 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 assigned to hatchery-origin parents ($\chi^2 = 13.93$, df = 1, $p < 0.001$). Although 67% of parents genotyped were of hatchery origin, only 56% of assigned offspring were matched to hatchery-origin parents (Table S6). Female parents produced 1–10 offspring, while male parents produced 1–13 (Figure 3).

Simulations

FRANz was able to correctly reconstruct parent-pair-offspring trios for all simulated offspring from both the odd and even lineages with no Type I or Type II error. Simulations performed in CKMRSIM clearly demonstrated the ability of our marker set to distinguish between potential offspring and unrelated individuals and our known age data allows us to unequivocally distinguish between parent-offspring and sibling relationships (Figure S3).

Discussion

First estimates of RRS for Pink Salmon

We present the initial direct measurements of RRS of hatchery- and natural-origin Pink Salmon in Hogan Bay and Prince William Sound. We validated the power of our SNP amplicon panel for parentage assignment and demonstrated that parentage analyses can be accomplished in remote, natural settings with sparse sampling of potential parents and offspring. Given the magnitude of hatchery production and its value to Alaska's economy, we herein provide information on the reproductive success of hatchery-origin Pink Salmon on the spawning grounds in the wild versus natural conspecifics.

RRS varies among salmonids

Estimates of RRS between hatchery- and natural-origin males and females vary across salmonids, study sites, and hatchery practices, although overall, natural-origin fish tend to have higher RS than hatchery-origin counterparts (see Araki et al., 2008 for a review). For example, hatchery male Chinook salmon from Washington and Idaho were less productive than natural males (Anderson et al., 2013; Ford, Murdoch, & Howard, 2012; Hess et al., 2012), which contrasts with our initial finding of a lack of significant difference in male RS in the even-year lineage. In hatchery steelhead, broodstock history impacted spawning success in the wild (Ford et al., 2016). One study has shown that although hatchery-origin steelheads were selected to spawn earlier than natural-origin individuals, interbreeding was not

prevented; up to 80% of natural-origin steelhead were hatchery/origin hybrids (Seamons, Hauser, Naish, & Quinn, 2012).

Origins of hatchery parents

Parents included in our study originated from all three hatcheries operated by PWSAC, but not from the Solomon Gulch hatchery (SGH) operated by the VFDA (Figure 1). However, it should be noted that a small fraction of hatchery strays in Hogan Bay was from this hatchery (406 total between 2013 and 2016; Table S4), but these individuals were not randomly selected for genotyping. The prevalence of PWSAC fish could be due to a combination of the closer proximity of these hatcheries to Hogan Bay, larger numbers of fish released, and the different brood sources, and associated run timing differences, between PWSAC and VFDA hatchery fish (Habicht et al., 2000; Seeb et al., 1999; Stopha, 2018). Consistent with previous studies in PWS, VFDA-origin fish were found to stray less throughout PWS streams than PWSAC-origin fish, despite large returns (Brenner et al., 2012; Knudsen et al., 2016). It is possible that the donor stray rates (populations that fish stray from) of PWSAC and VFDA fish are the same, but that VFDA stray fish are less common because fewer are released. It is also relevant to note that VFDA- and PWSAC-origin fish have been found in streams 300 miles away in lower Cook Inlet (Hollowell, Otis, & Ford, 2017).

Robustness of our findings

The high exclusion probabilities and posterior probabilities of assignment calculated by *FRANz*, coupled with our simulations suggest that our genetic marker panel is powerful in accurately assigning parents to offspring. Sparse sampling and high escapements in 2013 resulted in sub-optimal sampling of parents and therefore low offspring assignment rates. Given the estimated productivity of the 2013 brood year and the sampled proportion of the 2015 return, we estimated that we had limited power (< 0.2) to detect an effect of RRS < 0.5 for the odd-year Hogan Bay samples (see Box 1). Reproductive success and RRS values from our odd-year lineage should be interpreted with extreme caution since only three offspring were assigned to hatchery-origin parents. The most salient result from the odd-year lineage is the significant difference in proportion of offspring assigned to hatchery- and natural-origin parents produced by the non-parametric Chi-square test. We had enough power (> 0.9) to detect an effect of RRS < 0.5 based on the higher productivity of brood year 2014 and the higher sampling rate of 2016 (see Box 1). If the true difference in RRS for even-year males is close to RRS = 0.87, then many more parents would likely be required to have sufficient power to statistically detect that small of an effect (see Box 2 in Christie et al., 2014). Analyzing additional generations from Hogan Bay that had more complete sampling as well as additional streams will allow the reconstruction of more parent-offspring relationships and increase robustness of our findings.

Future directions

We are currently genotyping additional fish from offspring years 2015 and 2016 to increase parent-offspring assignments. We will extend our analyses of RRS across multiple parent-offspring brood years and streams in PWS to determine variation in RS and potentially examine the influence of grandparent origin. We will use information collected in the field on individual sex, sample date, sample location, and individual length to begin 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 fisheries with individual sample date and RS, we hope 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.

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Box 1. Statistical power to detect a difference in relative reproductive success (RRS) with incomplete sampling

Statistical power refers to the probability of detecting a difference between sampled distributions if there is truly a difference in the underlying distributions. In RRS studies the statistical power to detect a difference in the reproductive success (RS) between groups, such as hatchery-origin and natural-origin, is affected by: 1) sample sizes of parents, 2) proportion of parents from each group (i.e. proportion of hatchery-origin spawners), 3) proportions of offspring sampled , 4) stock productivity, and 5) effect size (i.e. true value of RRS; Hinrichsen, 2003). Each of these variables can shape the sampled distributions of RS for each group and thus affect the ability to determine if the distributions are statistically different from one another.

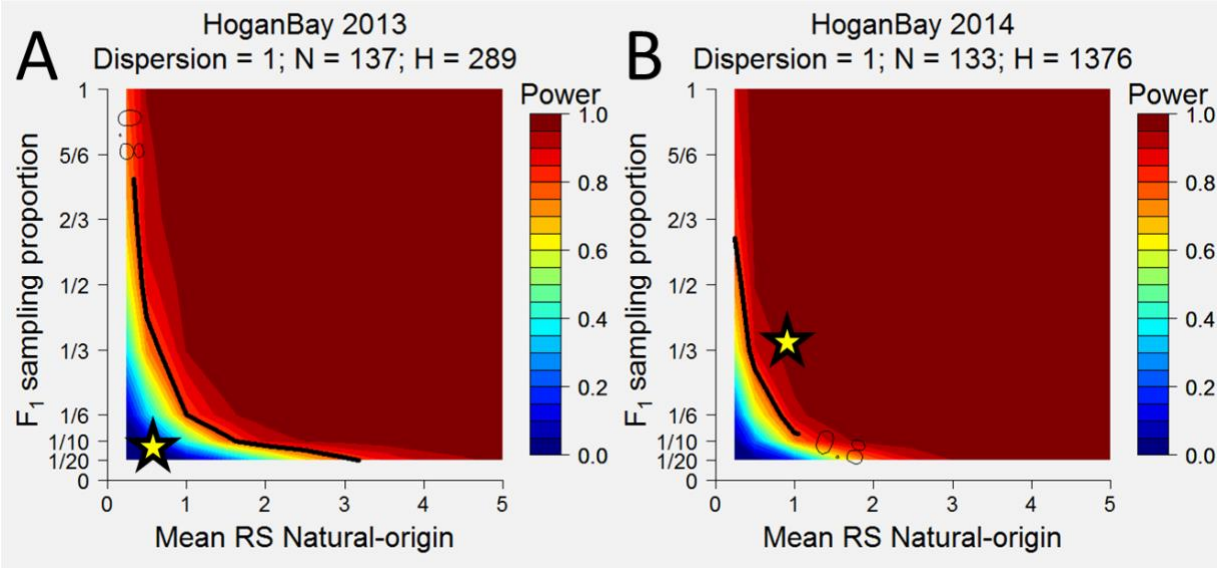
The underlying distribution of RS often approximates a negative binomial (Anderson, Faulds, Atlas, & Quinn, 2013; Christie, Ford, & Blouin, 2014). To illustrate the power relationship between the sample size of parents and the effect size (true RRS), Christie, Ford, & Blouin (2014) used a heuristic approach to simulate distributions of RS and statistically compared them for different sample sizes of parents and RRS effect sizes. This work demonstrated that at least 400 parents (equal proportion hatchery and wild) would need to be sampled to detect RRS = 0.8 at least 80% of the time (power = 0.8), given their assumed distribution of RS and complete sampling of all offspring.

Here we extend the simulation approach from Christie, Ford, & Blouin (2014) to relax the assumed distribution of RS (i.e. stock productivity, mean and variance of the negative binomial) and allow for incomplete sampling of offspring to more precisely estimate the statistical power of our RRS study in a natural pink salmon stream in PWS, Alaska. Statistical tests rely on comparing the absolute difference between sample distributions, not the relative difference. This means that anything that lowers the average RS of the sample population (i.e. incomplete sampling of offspring or low production) will inherently lower the statistical power to detect RRS < 1. Stock productivity for Pink Salmon can vary between odd- and even-year broodlines, as well as over time. In years of high production (high return per spawner), we expect that it would be easier to detect a difference in RS between hatchery- and natural-origin spawners than in years of low productivity. For example, it is easier to tell apart a distribution of RS with an average of 8 offspring per parent from one with an average of 4 offspring per parent (RRS = 0.5) than a distribution of RS with an average of 3 and 1.5 offspring per parent (RRS = 0.5), respectively. Incomplete sampling of offspring does not affect the RRS between groups, so long as sampling is unbiased. However, incomplete sampling does lower the average RS of the sampled distribution, and thus decreases the absolute difference in average RS between groups for a given effect size, making it more challenging to determine if the distributions of RS are statistically different.

For our simulations, we wanted to determine the statistical power to detect an RRS of 0.5, the level of RRS the study was designed to detect, for a given number of hatchery- and natural-origin parents sampled over a range of stock productivities (mean and variance of negative binomial) and a range of proportions of offspring sampled. We varied the mean of the negative binomial RS distribution for natural-origin from 0.25 to 5, the dispersion (variance) of that distribution from 1 to 10, and the sampled proportion of offspring from 0.05 to 1. To test for differences in mean fitness (RS), we used a non-parametric permutation (randomization) test. For each combination of negative binomial mean and dispersion and offspring sampling proportion, we assigned offspring to hatchery- and natural-origin parents assuming perfect genetic assignment and used a permutation test to determine whether the

mean RS of hatchery-origin fish was different than the mean RS of natural-origin fish (RRS = 0.5). If a parent did not have any offspring assigned to it, it had an RS value of 0 (regardless of whether we knew that the parent truly did not produce any offspring or whether its offspring were not sampled). We repeated this process 2,000 times and calculated power as the proportion of trials that had a *P*-value $\leq$ 0.05 (i.e. the proportion of times the true difference in RRS was statistically detected). Values for statistical power were interpolated between points to generate a heatmap based on the mean stock productivity and the offspring (F_1) sampling proportion.

Panels A and B show the expected statistical power for Hogan Bay brood year 2013 and 2014, respectively. Each of these plots assumed that the dispersion parameter for the underlying negative binomial defining RS was 1 and that the effect size of was RRS = 0.5. The number of natural-origin parents is denoted by N and the number of hatchery-origin parents is denoted by H, since sampling of the parental generation had already occurred when these analyses were done. Statistical power increases for both increasing productivity of the stock (mean RS) and increasing proportion of F_1 offspring sampled. The yellow stars indicate the likely stock productivity of each brood year and the sampling proportion of F_1 offspring (sampled fish/aerial survey estimates). The difference in expected power for RRS = 0.5 between these streams was demonstrated in our results.



Acknowledgments

Mark Christie graciously provided the R code for the simulations in (Christie et al., 2014). We adapted that R code for our work here.

Tables and Figures

Table 1. Definitions of *FRANz* (Riester et al., 2009) parameters and key terms for pedigree analysis.

Term	Definition
N_m- and N_{fmax}	Maximum numbers of potential male (m) and female (f) parents
Exclusion probability	Probability that a random pair of individuals in the population has a chance of having a genotype pair compatible to an offspring genotype
Posterior probability of assignment	Probability of observing the parentage when drawing a pedigree from the posterior distribution
Fitness (reproductive success; RS)	Number of returning adult offspring to a stream produced per spawner
Relative fitness (relative reproductive success; RRS)	Ratio of fitness between one type and another
Pedigree	Family relationship among parents and offspring
Ancestral origin	Origin of an individual's ancestors

Table 2. Final numbers of individual Pink Salmon from Hogan Bay, Prince William Sound, genotyped (after removing individuals during quality control and quality assurance) and estimated escapements for each brood year, within each lineage (odd and even). Escapement estimates (in thousands) come from (Russell et al., 2016).

Odd (2013-2015)						
	Year	Natural	Hatchery	Total	Escapement	Sampled
Parents	2013	321	443	764	8-47K	2-10%
Offspring	2015	1,870	0	1,870	10-19K	11-21%
Total				2,634		
Even (2014-2016)						
	Year	Natural	Hatchery	Total	Escapement	Sampled
Parents	2014	214	437	651	6-9K	8-13%
Offspring	2016	3,994	0	3,994	8K	54%
Total				4,645		
Total (2013-2016)						
Parents	2013-2014	535	880	1415		
Offspring	2014-2016	5,907	0	5,907		
Total				7,279		

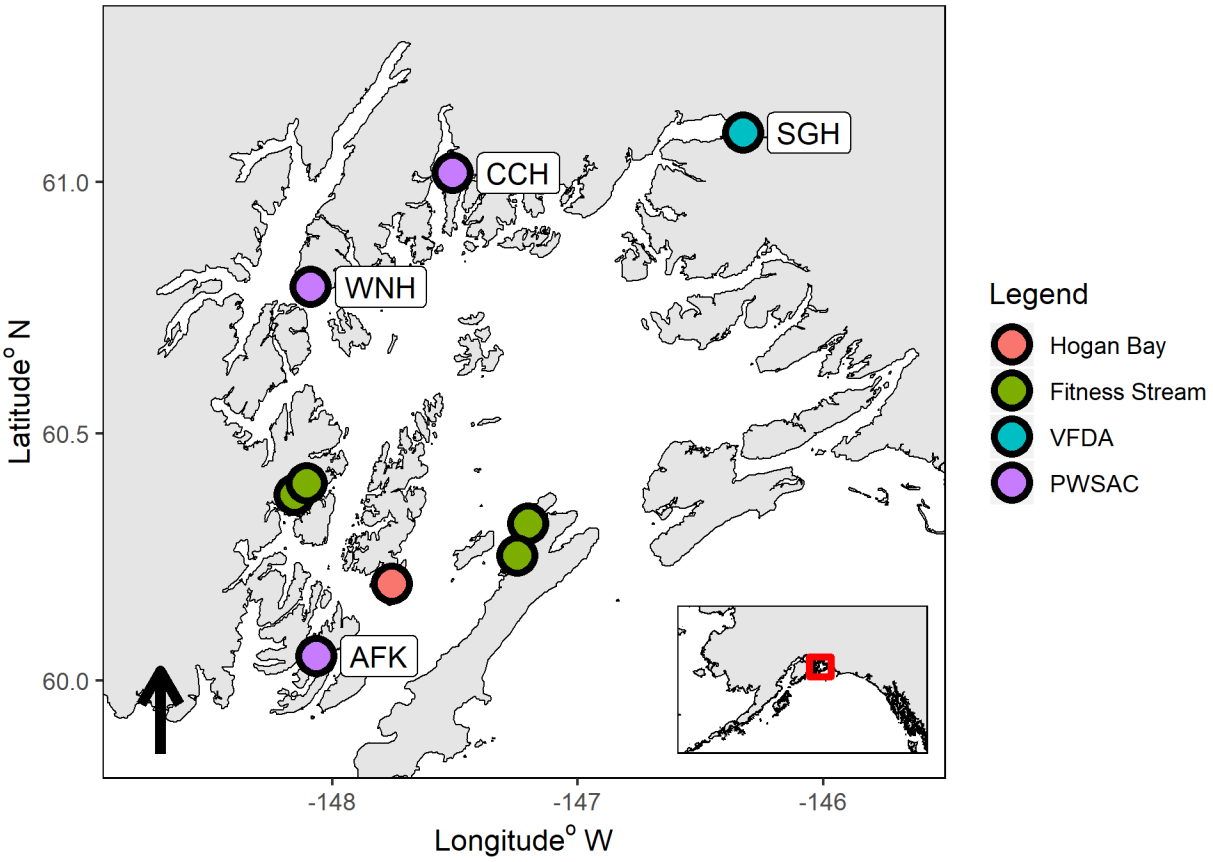


Figure 1. Map of Prince William Sound, Alaska, showing locations of Hogan Bay, additional fitness streams sampled as part of the Alaska Hatchery Research Program (AHRP), and hatcheries. Cannery Creek (CCH), Armin F. Koernig (AFK), and Wally Noerenberg (WNH) are all managed by the Prince William Sound Aquaculture Corporation (PWSAC), while Solomon Gulch (SG) is managed by the Valdez Fisheries Development Association (VFDA).

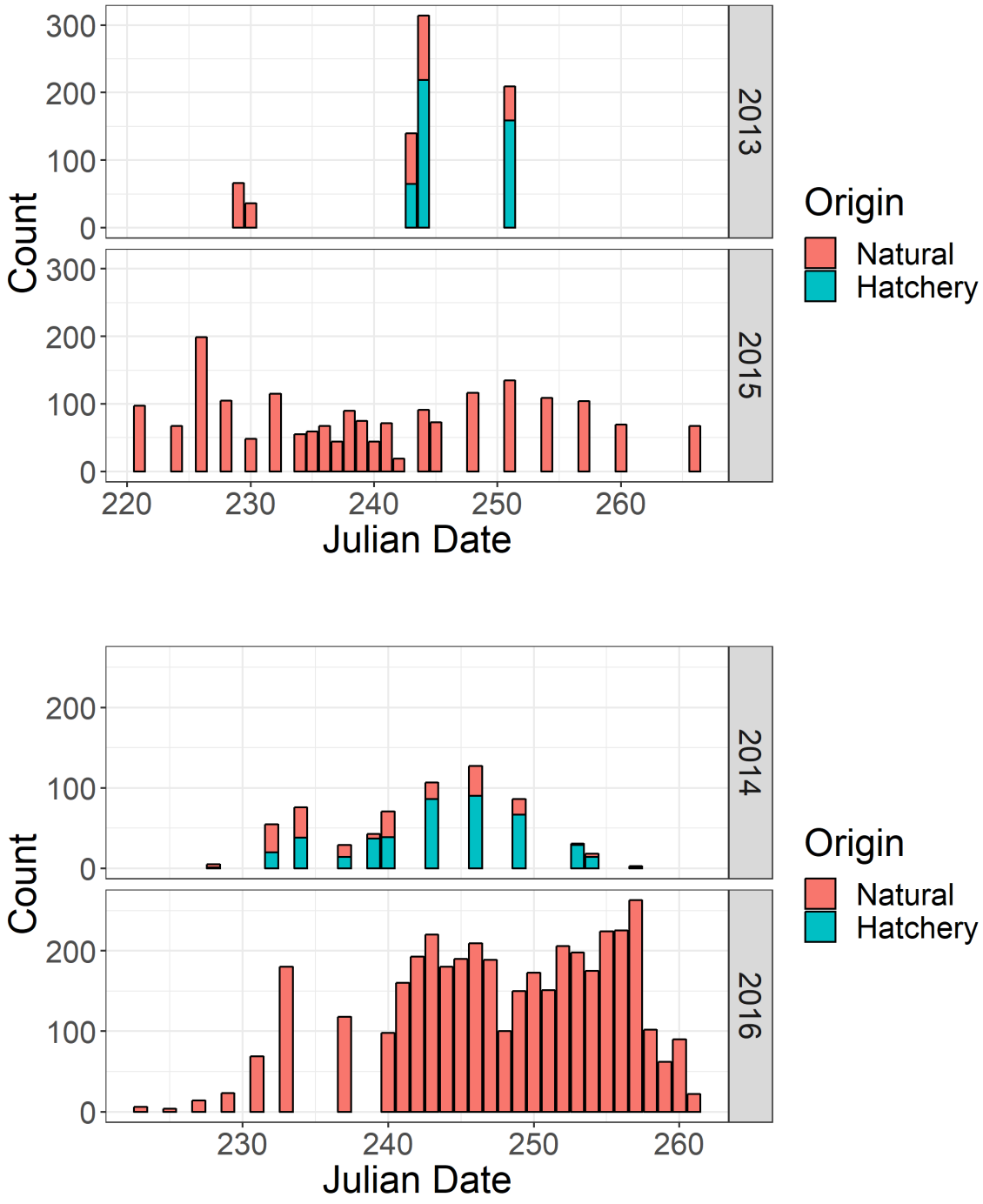


Figure 2. Number of Pink Salmon samples genotyped during the odd (top) and even (bottom) lineages to investigate relative reproductive success of hatchery- and natural-origin parents from Hogan Bay, Prince William Sound, Alaska. Within each set of lineages, the upper graph shows the parental year and the bottom graph shows the progeny year. Note that only natural origin fish were genotyped in the progeny year since only natural fish could have been the progeny from the fish spawning in this creek during the parental year.

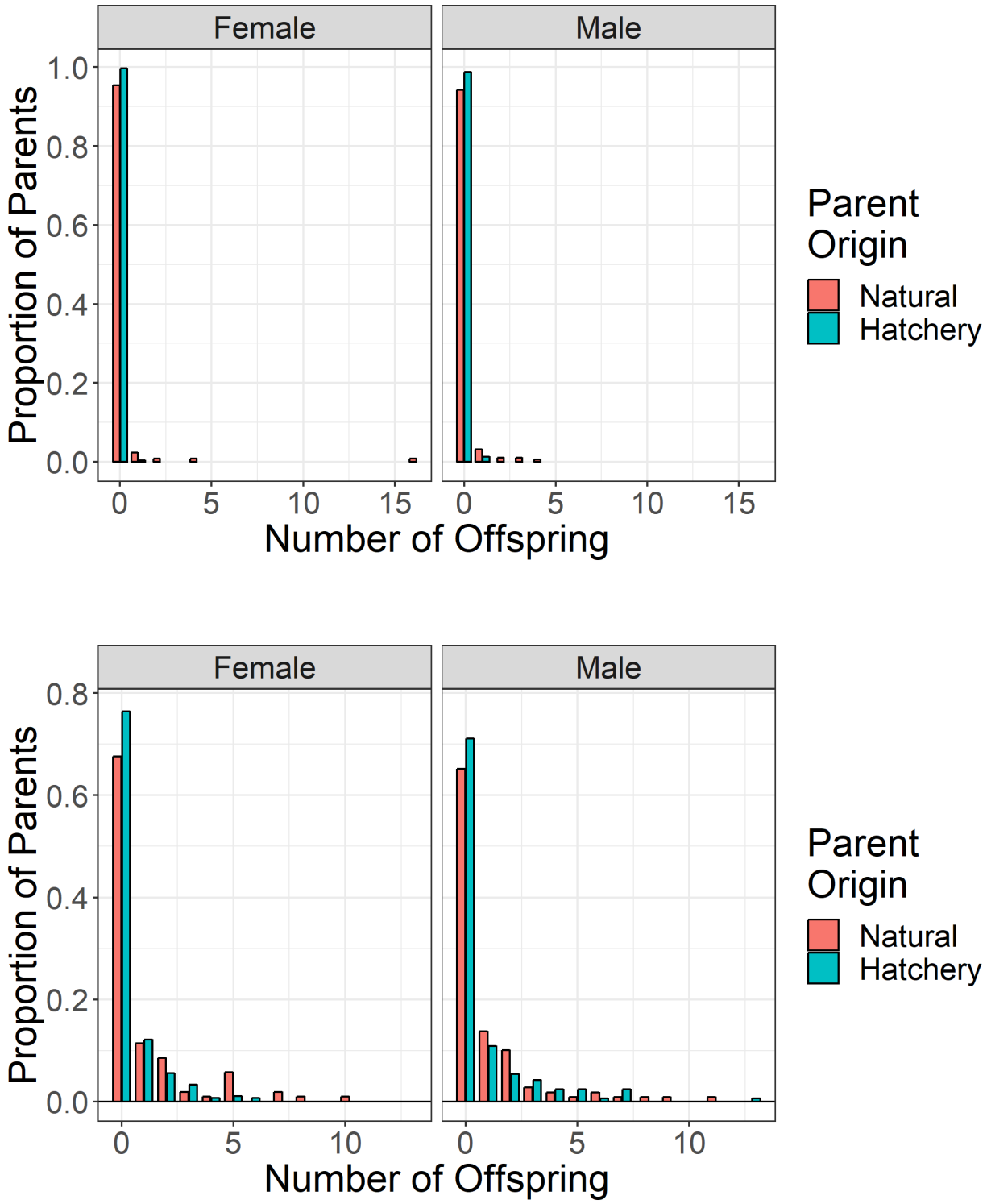


Figure 3. Reproductive success (number of adult offspring per parent) for female and male natural- and hatchery- origin Pink salmon for the odd (top) and even (bottom) lineages in Hogan Creek, Prince William Sound, Alaska.

Table S1. Final sample sizes and numbers of Pink Salmon from Hogan Bay removed during quality control for each brood year. Origin refers to individuals removed from the 2015 offspring year because they were not classified as natural origin after a secondary otolith read. Duplicate indicates individuals removed because they share 95% of genotypes. Heterozygosity refers to individuals removed because of outlying measures of heterozygosity, determined by 1.5 interquartile range cut-offs. Final indicates the number of individuals used in parentage analysis.

Year	Origin	Genotyped	Origin	Missing > 20% Genotypes	Duplicate	Heterozygosity	Final
2013	Natural	334		12	0	1	321
2013	Hatchery	461		12	4	2	443
2014	Natural	239		19	0	6	214
2014	Hatchery	512		51	2	22	437
2015	Natural	2,100	43	42	2	143	1,870
2016	Natural	4,295		117	12	172	3,994
Total		7,941		253	20	346	7,279

Table S2. Numbers of Pink Salmon in Hogan Bay collected each year, otoliths read, and genotyped. 'Final' numbers represent those that passed all quality control and quality assurance filters.

Year	Samples Collected	Otoliths Read	Hatchery Total	Natural Total	Hatchery Genotyped	Natural Genotyped	Final Hatchery	Final Natural
2013	829	799	465	334	461	334	443	321
2014	2,651	2,572	2,332	240	512	239	437	214
2015	9,441	9,017	4,828	4,191	0	2,100	0	1,870
2016	13,007	12,830	2,601	10,229	0	4,295	0	3,994
Total	25,928	25,218	10,226	14,994	973	6,968	880	6,399

Table S3. *FRANz* (Riester et al., 2009) parameters used for parentage analysis of Pink Salmon from Hogan Bay for odd and even-year lineages. Escapement estimates are reported in (Stopha, 2016, 2017; Vercessi, 2014; Vercessi, 2015) and the genotyping error rate reflects the project's overall error rate (0.5%). N_m – and N_{fmax} define the maximum number of potential parents (males and females).

Lineage	Run	N_m- and N_{fmax}	Source escapement estimate	Genotyping Error Rate (%)
Odd	1	23,500	Aerial survey	0.5
	2	4,300	Stream walk	0.5
	3	23,500	Aerial survey	1.0
Even	1	4,500	Aerial survey	0.5
	2	3,300	Stream walk	0.5
	3	4,500	Aerial survey	1.0

Table S4. Number of Hogan Bay Pink Salmon collected and numbers of otoliths read, assigned to hatcheries, and identified as natural-origin. See Figure 1 for hatchery locations. AFK = Armin F. Koernig, CCH = Cannery Creek, SG = Solomon Gulch, WNH = Wally Noerenberg. AFK, CCH, and WNH are all PWSAC-operated, while SG is operated by the Valdez Fisheries Development Association.

			Hatchery Origin					
Year	Fish Collected	Otoliths Read	AFK	CCH	SG	WNH	Hatchery Total	Natural Total
2013	829	799	366	13	0	86	465	334
2014	2,651	2,572	1,688	217	2	425	2,332	240
2015	9,441	9,017	2,607	1,110	324	787	4,828	4,191
2016	13,007	12,830	2,090	267	80	164	2,601	10,229
Total	25,928	25,218	6,751	1,607	406	1,462	10,226	14,994

Table S5. Number of assigned parents sampled from Hogan Bay originating from each Pink Salmon hatchery in Prince William Sound (see Figure 1 for locations).

Year	Armin F. Koernig (AFK)	Cannery Creek (CCH)	Wally Noerenberg (WNH)	Solomon Gulch (SG)	Total
2013	2	0	1	0	3
2014	202	22	41	0	265

Table S6. Numbers of potential natural- and hatchery-origin parents genotyped and number of offspring assigned to natural- and hatchery-origin parents for Hogan Bay Pink Salmon. Chi-square tests revealed significant differences in proportions of offspring assigned to hatchery- and natural-origin parents relative to the proportions of hatchery- and natural-origin parents genotyped.

Lineage	Origin	Potential Parents	Assigned Offspring
Odd	Natural	322	45
	Hatchery	445	3
Even	Natural	214	208
	Hatchery	437	265

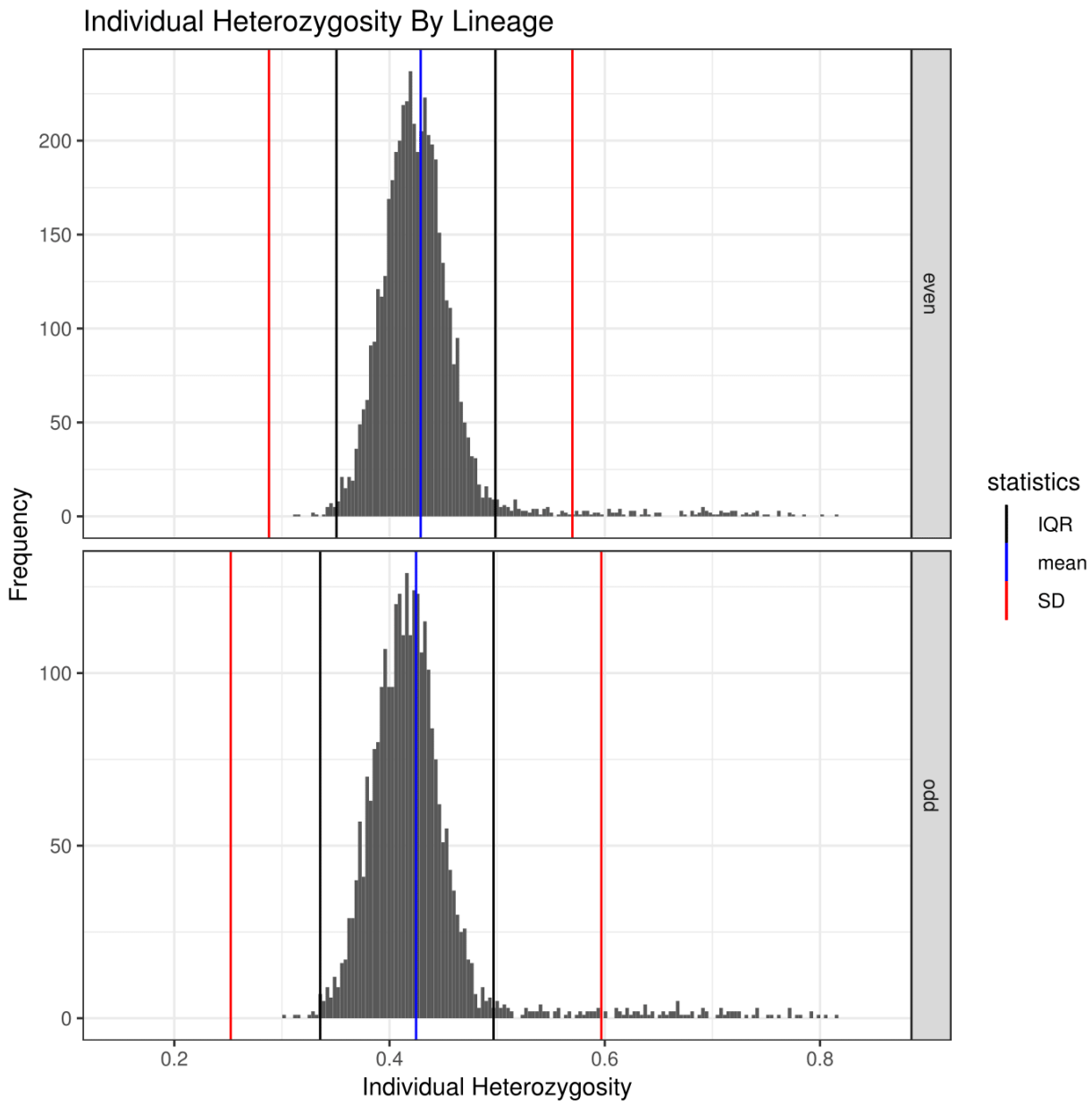


Figure S1. Histogram of individual heterozygosity showing the mean and two different cutoffs (IQR = interquartile range and SD = +/- 3 standard deviations) for Pink Salmon progeny and adults from the even (top) and odd (bottom) lineages collected from 2013 to 2016 in Hogan Bay, Prince William Sound. Individuals outside of the IQR cut-offs were excluded from further analysis.

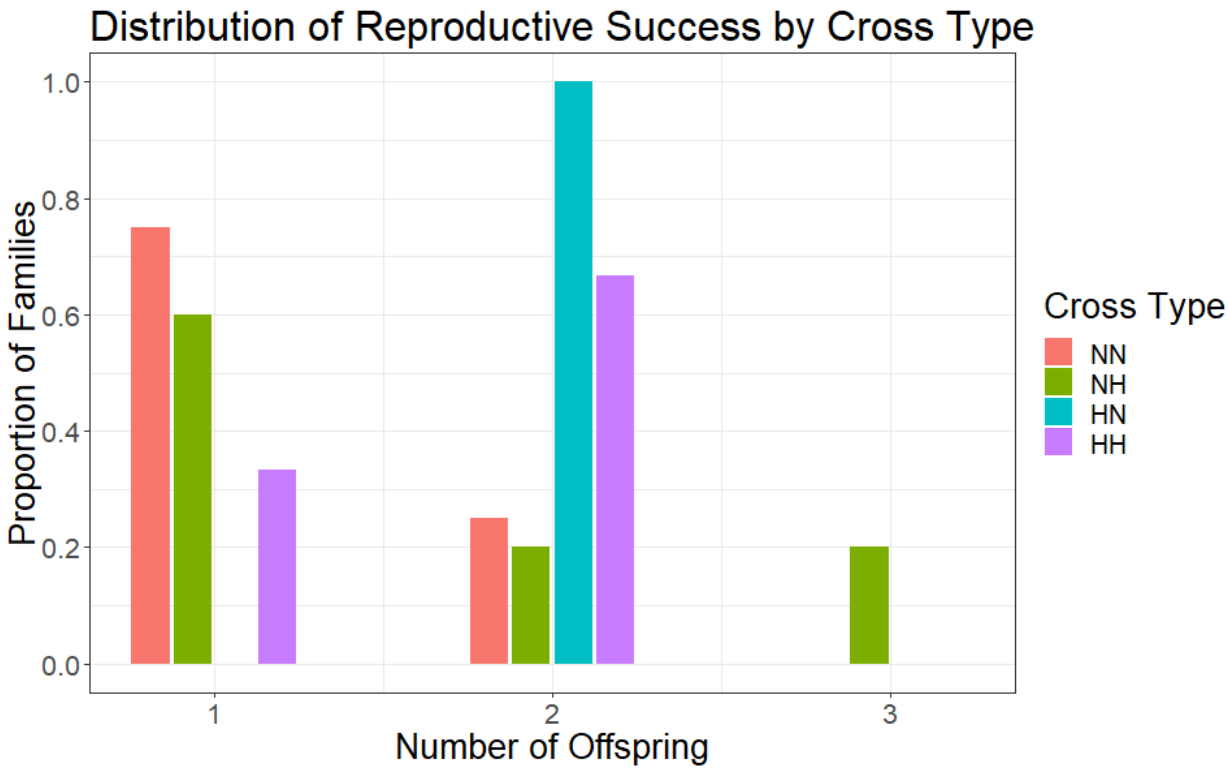


Figure S2. Distribution of family size by cross type for the even-year lineage Pink Salmon collected in 2014 (parental year) and 2016 (offspring year) from Hogan Bay, Prince William Sound. First letter in the cross designates the origin of the dam (H = hatchery, N = natural) and second letter designates the origin of the sire.

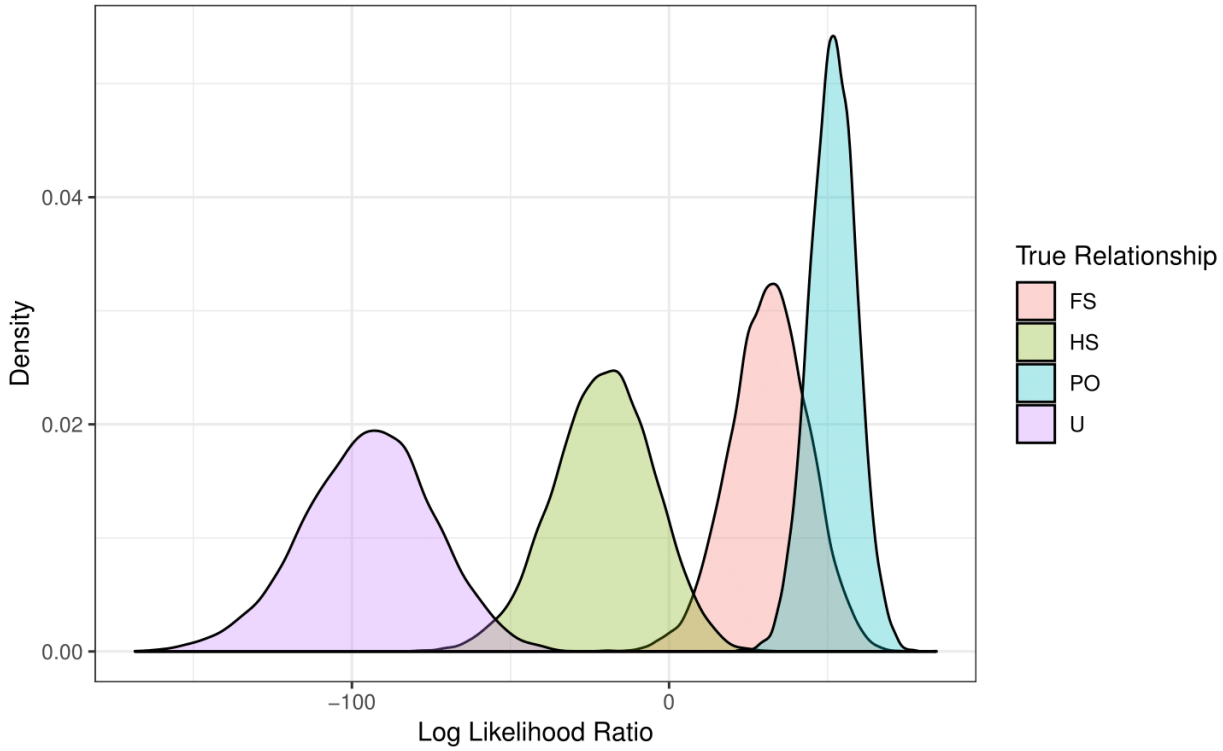


Figure S3. Log likelihood ratios of true versus hypothesized relationships, calculated using CKMRSIM (Anderson, DOI: 10.5281/zenodo.820162), implemented in R. These distributions demonstrate that our marker set has the power to distinguish between true parent-offspring relationships (PO) and unrelated individuals (U). Since there is no ambiguity about the age of our fish, we can distinguish full-siblings (FS) and half-siblings (HS) from PO.

Conclusions.

We demonstrate the capability to study RS in hatchery- and natural-origin pink salmon in remote, natural settings in PWS despite access to permanent infrastructure (weirs) to collect fish. This is the first in a series of studies focused on interactions between hatchery- and natural-origin pink salmon that will eventually allow us to examine variation in fitness across multiple streams and brood years. Despite incomplete sampling of adults in the stream, we were able to successfully assign Pink Salmon offspring to parents for both the odd and even lineages. Our results demonstrate that both natural- and hatchery-origin (stray) parents contribute progeny that return to their natal grounds to spawn. Future work will explore the relative contributions of ecological and genetic mechanisms to fitness reductions in hatchery fish, particularly females, and extend our analyses to multiple streams to determine whether our observed RRS values are consistent for Pink Salmon across streams in PWS.

Management or Policy Implications. Please address how your project outcomes directly, or indirectly, relate to resource management or policy.

This project enabled the genetic analysis of a sub-set of samples collected over four years by the Alaska Hatchery Research Program (AHRP; http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main) in one creek (Hogan Bay) located in Prince William Sound (PWS), Alaska, to provide estimates of relative reproductive success (RRS) between hatchery- and natural-origin Pink Salmon for one odd-year and one even-year. Analysis of results provides initial estimates of RRS for Pink Salmon in PWS. These data along with further RRS results from Hogan Bay and the other four streams sampled by AHRP over seven years will reduce uncertainty when extrapolating RRS results to other streams within PWS and provide further insight into the issues associated with enhancement of Pink Salmon in PWS. These data will directly inform how the Alaska Department of Fish and Game (ADF&G) manages fisheries targeting hatchery-enhanced stocks and reviews permits of hatchery operations.

This project and the overall AHRP will provide information key to assessing hatchery permit requests. Information from this research in conjunction with the AHRP will be used by ADF&G when assessing impacts from hatchery-proposed activities on wild stocks. Under Alaska Administrative Code, hatchery operators must apply for Fish Transport Permits and Permit Alteration Requests, and file Basic and Annual Management Plans. These documents are subject to department review. ADF&G must follow the precautionary principle when considering requests by PNP hatchery operations for permit alterations. Potential costs to wild stocks should be considered in weighing the benefits from enhancement programs.

This project will provide information that will allow ADF&G to better manage fisheries targeting enhanced stocks and assess aquaculture permits with more certainty in striving to meet Alaska Constitution, Alaska Administrative Code, Alaska Statute, and Genetic Policy directives including:

1) Alaska Constitution Section Article 8, Section 8.4: “Fish, forests, wildlife, grasslands, and all other replenishable resources belonging to the State shall be utilized, developed, and maintained on the sustained yield principle, subject to preferences among beneficial uses.”
2) Alaska Administrative Code (AAC) 5.39.220.a: “…conservation of wild salmon stocks consistent with sustained yield shall be accorded the highest priority.”
3) Alaska Administrative Code (AAC) 5.39.222.c.1: “wild salmon stocks and the salmon's habitats should be maintained at levels of resource productivity that assure sustained yields.”
4) Alaska Administrative Code (AAC) 5.39.222.c.1.D: “effects and interactions of introduced or enhanced salmon stocks on wild salmon stocks should be assessed; wild salmon stocks and fisheries on those stocks should be protected from adverse impacts from artificial propagation and enhancement efforts”
5) Alaska Administrative Code (AAC) 5.39.222.c.3.J: “proposals for salmon fisheries development or expansion and artificial propagation and enhancement should include assessments required for sustainable management of existing salmon fisheries and wild salmon stocks”
6) Alaska Administrative Code (AAC) 5.39.222.c.5: “in the face of uncertainty, salmon stocks, fisheries, artificial propagation, and essential habitats shall be managed conservatively as follows:”
    (a) a precautionary approach, involving the application of prudent foresight that takes into account the uncertainties in salmon fisheries and habitat management, the biological, social, cultural, and economic risks, and the need to take action with incomplete

knowledge, should be applied to the regulation and control of harvest and other human-induced sources of salmon mortality

7) Alaska Administrative Code (AAC) 5.40.005.c: “Where hatchery returns enter a segregated location near the release site and can be harvested without significantly affecting wild stocks, a special harvest area may be designated by regulation adopted by the board, within the hatchery permit, or by emergency orders issued by the commissioner.”
8) AAC 5.40.220.b.1: “The physical and environmental nature of the proposed location must be suitable for enhancing runs or for establishing new runs, and must have the potential to make a reasonable contribution to the common property fishery. The proposed hatchery returns may not unreasonably or adversely affect management of natural stocks. The returns for the proposed hatchery may not require significant alterations in traditional fishery time, area, gear type, or user group allocations.”
9) AAC 5.40.860.b.4: “The commissioner will, in his or her discretion, consider a permit alteration, suspension, or revocation in accordance with AS 16.10.430 . If the commissioner decides to consider a permit alteration, suspension, or revocation, the coordinator will notify the appropriate regional planning team. The regional planning team may make a written recommendation to the commissioner on the proposed alteration, suspension, or revocation. The regional planning team shall use the following performance standards in their review, evaluation, and recommendation to the commissioner, including whether: the hatchery does not significantly impact wild stocks in a negative manner;”
10) Alaska Statute (AS) 16.05.020.2: “The commissioner shall manage, protect, maintain, improve, and extend the fish, game and aquatic plant resources of the state in the interest of the economy and general well-being of the state.”
11) AS 16.05.050.16: “The commissioner has, but not by way of limitation, the following powers and duties to permit and regulate aquatic farming in the state in a manner that ensures the protection of the state's fish and game resources and improves the economy, health, and well-being of the citizens of the state.”
12) AS 16.05.730: Management of wild and enhanced stocks of fish.
    (a) Fish stocks in the state shall be managed consistent with sustained yield of wild fish stocks and may be managed consistent with sustained yield of enhanced fish stocks.
    (b) In allocating enhanced fish stocks, the board shall consider the need of fish enhancement projects to obtain brood stock. The board may direct the department to manage fisheries in the state to achieve an adequate return of fish from enhanced stocks to enhancement projects for brood stock; however, management to achieve an adequate return of fish to enhancement projects for brood stock shall be consistent with sustained yield of wild fish stocks.
    (c) The board may consider the need of enhancement projects authorized under AS 16.10.400 and contractors who operate state-owned enhancement projects under AS 16.10.480 to harvest and sell fish produced by the enhancement project that are not needed for brood stock to obtain funds for the purposes allowed under AS 16.10.450 or 16.10.480(d). The board may exercise its authority under this title as it considers necessary to direct the department to provide a reasonable harvest of fish, in addition to the fish needed for brood stock, to an enhancement project to obtain funds for the enhancement project if the harvest is consistent with sustained yield of wild fish stocks. The board may adopt a fishery management plan to provide fish to an enhancement project to obtain funds for the purposes allowed under AS 16.10.450 or 16.10.480(d).
    (d) In this section, "enhancement project" means a project, facility, or hatchery for the enhancement of fishery resources of the state for which the department has issued a permit.

13) AS 16.10.750(a): “The legislature finds that the state is committed to maintaining and enhancing its wild stocks of salmon by careful management, by initiating a 20-year rebuilding program, and by investing in the fishing industry.”
14) Genetic Policy,
    (a) Introduction: “The genetic policy contains restrictions that will serve to protect the genetic integrity of important wild stocks. Certainly, in Alaska where wild stocks are the mainstay of the commercial fishery economy, it is necessary to protect these stocks through careful consideration of the impacts of enhancement activities.
    (b) Protection of Wild Stocks: “Gene flow from hatchery fish straying and intermingling with wild stocks may have significant detrimental effects on wild stocks. First priority will be given to protection of wild stocks from possible harmful interactions with introduced stocks. Stocks cannot be introduced to sites where the introduced stock may have significant interaction or impact on significant or unique wild stocks.”
    (c) II. Protection of Wild Stocks, C. Stock Rehabilitation and Enhancement:1. “A watershed with a significant wild stock can only be stocked with progeny from the indigenous stocks.”

Publications.

Lescak E, Shedd K, Dann T, Heather H, Prince D, Habicht C. *In prep*. Relative fitness of hatchery and natural Pink Salmon in Hogan Bay, Prince William Sound, Alaska.

Outreach.
We presented to the scientific community at the Alaska Marine Science Symposium (AMSS) in January, 2019, the American Fisheries Society's Alaska Chapter Meeting as well as a public informational meeting on the AHRP on March 7, 2019.

Results of our study will be included in a summary of hatchery research presented to a public Board of Fisheries Hatchery Committee meeting on March 8th. Meeting details can be found here: http://www.adfg.alaska.gov/index.cfm?adfg=fisheriesboard.meetinginfo&date=03-08-2019&meeting=anchorage

Public interest in hatchery policy in general and pink salmon production in Prince William Sound in particular has grown during our project period. That increased interest has resulted in media contacts with Principal Geneticist Chris Habicht resulting in one radio and one online news story including results of our project:
https://www.kbbi.org/post/adfg-study-begins-answer-whether-hatchery-salmon-produce-fewer-offspring
https://craigmedred.news/2019/02/23/hatchery-misfits/

We also co-authored an article for Delta Sound Connections about the AHRP project with Dr. Peter Rand from the Prince William Sound Science Center.

We have begun procurement of materials to build an informational kiosk communicating our results with the public. We are purchasing a standalone iPad kiosk from https://www.ipadkiosks.com and will be adding figures and narrative from our final report to a new webpage associated with the Alaska Hatchery Research Program area of the Alaska Department of Fish and Game's webpage here: http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.findings_updates

We plan to point the information kiosk to this area of the Fish and Game website and hope to house the kiosk in the Cordova Center. We have begun communications with Cordova Center staff but have not reached an agreement on long-term housing of the kiosk in their facility. As a result we have also begun communication with staff at the Prince William Sound Science Center about locating our kiosk in their library. Our intent is to locate the kiosk in a high-traffic public space in Cordova so that local stakeholders as well as visitors can learn about our results. We also intend to expand upon the information available on the kiosk as results from further stream and generational replicates become available.

Once our results have been published, we will produce a Data Nugget (datanuggets.org), which is a short lesson that can be freely accessed by educators around the world. Co-PI Lescak has participated in local educational outreach through the *I Know I Can* program, which brings professionals into second-grade classrooms in Anchorage, AK 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.

Synopsis.

Private non-profit hatcheries (PNP) practice extensive ocean-ranching aquaculture of Pacific salmon in Alaska to provide additional harvest opportunity to common property fisheries. Approximately 75% of the 1.8B juvenile salmon released annually in Alaska are Pink Salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and Chum Salmon (*O. keta*) in Southeast Alaska (SEAK). The extent to which hatchery and wild fish interact, interbreed in streams, and influence each other's fitness has been a controversial topic. Because of the value of hatchery production to the fishing industry and the mandate that hatchery production 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) focused on Pink Salmon in PWS and Chum Salmon in SEAK. This program was designed to answer questions related to 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 of hatchery Pink and Chum salmon.

To begin to address the third question, we used NPRB funds to directly measure the relative reproductive success (RRS) of hatchery- and natural-origin Pink Salmon in a remote natural stream in PWS (Hogan Bay). We define RRS as the ratio of the number of sampled dead adult offspring produced by hatchery-origin fish divided by the number of sampled dead adult offspring produced by natural-origin fish (fish born in the wild that may have hatchery-origin ancestry). Hatcheries in PWS thermally mark otoliths, which allows identification of hatchery-origin fish that stray into natural streams. Pink Salmon have a two-year generation time that results in genetically-distinct odd and even lineages. To calculate RRS in both lineages, we genotyped 7,941 individuals sampled between 2013 and 2016 at 298 genetic markers, reconstructed pedigrees to match offspring to parents, and calculated RRS.

In the even lineage, RRS was 0.47 for females (95% confidence intervals = 0.37 and 0.62) and 0.87 for males (95% confidence intervals = 0.67–1.12); these estimates represent a significant reduction in reproductive success (RS) of hatchery-origin females compared to natural-origin counterparts, but not a statistically significant reduction for hatchery-origin males. For the odd lineage, RRS was 0.02 for females (95% confidence intervals = 0.01 and 0.09) and 0.12 for males (95% confidence intervals = 0.02 and 0.40); these estimates represent significant reductions in RS for hatchery-origin females and males but were based on small sample sizes.

Future research will increase sample sizes for Hogan Bay, examine RRS in four additional streams in PWS, include samples from 2017-2019 to explore multi-generational effects, and investigate RRS in matings between natural- and hatchery-origin fish relative to matings between two natural-origin fish. Important questions remain regarding the genetic and ecological mechanisms that contribute to the observations from the overall AHRP and the biological significance of results. This series of studies from the AHRP will provide information for ADF&G to evaluate potential risks to natural stocks posed by stray hatchery-origin Pink Salmon in PWS.

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