Proposal to the Saltonstall-Kennedy Grant Program

Applicant Organization:	Alaska Department of Fish and Game
Project title:	Relative productivity of hatchery pink salmon in a natural stream
S-K Priority:	Priority #1 – Aquaculture
Project location:	Prince William Sound, Alaska
Requested award dates:	September 1, 2016 – June 30, 2018
Funding requested:	$250,000
Principal Investigator:	Tyler Dann – Fishery Geneticist
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	Alaska Dept. of Fish and Game/Gene Conservation Laboratory
Species:	Pink salmon (*Oncorhynchus gorbuscha*)

A. Project Summary

Extensive ocean-ranching aquaculture of Pacific salmon is practiced by private non-profit (PNP) sector corporations in Alaska with the intent to enhance the common property fisheries and provide harvest opportunity to the fishing industry and local communities when wild stocks cannot. Most of the approximately 1.8B juvenile salmon that PNP hatcheries release annually are pink salmon (*Oncorhynchus gorbuscha*) in Prince William Sound (PWS) and chum salmon (*O. keta*) in Southeast Alaska (SEAK) that provide 51-97 million adult salmon to the harvest, depending on even and odd-year returns of pink salmon (Vercessi 2014, 2015). The contribution of salmon from these programs provided an estimated $182 million, or 25%, of the exvessel value of the statewide commercial common property harvest in 2013 (Vercessi 2014).

The scale of these hatchery programs has raised concern that hatchery-produced fish may detrimentally impact the productivity and sustainability of wild stocks of Alaska salmon. While policies and management strategies have been implemented to reduce risk to wild stocks, the scale of the Alaska enhancement programs makes it likely that wild stocks will be affected by enhanced fish to some degree. Anticipated risks posed to natural populations by hatcheries include genetic (consequences of interbreeding between hatchery-bred and wild salmon); disease (introduction or amplification of pathogens); ecological (competition for resources); and harvest mortality (Naish et al. 2007, Waples 1991).

Loss of productivity caused by interbreeding of hatchery fish with wild con-specifics has been demonstrated in steelhead (*O. mykiss*), Chinook salmon (*O. tshawytscha*), and coho salmon (*O. kisutch*) throughout the Pacific Northwest (Anderson et al. 2013, Araki et al. 2008, Araki et al. 2009, Berejikian and Ford 2004, Chilcote et al. 2011, Christie et al. 2014). However, none of these studies have occurred in Alaska where the purpose and scale of hatchery programs are different and freshwater habitat is largely intact. Additionally, evidence from pink or chum salmon, which have very different freshwater ecology and shorter hatchery rearing periods, is sparse. One of the few studies examining chum salmon found a reduction in reproductive

success (fry per adult) for hatchery-origin females, but not for hatchery-origin males as compared to their natural-origin counterparts (Berejikian et al. 2009). Nevertheless, this reduction in fitness was not statistically significant, likely due to the study's limited statistical power to estimate the relative reproductive success (RRS) of hatchery and natural-origin salmonids (see Box 2 in Christie et al. 2014).

Recent studies have demonstrated large proportions of hatchery-origin salmon in some wild-spawning populations in Alaska (Brenner et al. 2012, Piston and Heinl 2012), raising two important questions: (1) Are hatchery-origin salmon interbreeding with wild salmon to the extent that fitness and productivity are being diminished?; and (2) Are assessments of wild stocks biased by the presence of hatchery salmon leading to excessive harvest or disguising interactions that diminish natural production of salmon? Faced with evidence of straying and uncertainty about its extent and effect, the Alaska Department of Fish and Game (ADF&G) must act with caution when considering requests by hatchery corporations for permit alterations. Any potential effect of these programs on wild stocks must be considered in the context of both benefits from enhancement programs and costs to wild stocks that the agency is mandated to protect.

Hatchery production is important to Alaska's fishing industry and its place in the international market, providing an important economic resource for local Alaska communities (McDowell Group 2012, 2013). Yet Alaska law requires that hatchery production be compatible with conservation of wild stocks. ADF&G and the PNP hatchery corporations developed a research program to investigate concerns about straying and genetic interactions between hatchery and wild stocks. In 2011, ADFG convened a Science Panel of current and retired scientists with broad experience in salmon enhancement, management, and wild and hatchery interactions from ADFG, University of Alaska, PNP aquaculture corporations, and NOAA-Fisheries.

The Panel formed the Alaska Hatchery Research Program (AHRP) to address these concerns (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main) in 2013 with funding from the State of Alaska, the PNP hatchery operators, and industry representatives. Efforts during the first three years of the program concentrated on questions of stock structure and straying and collecting the initial samples of hatchery- and natural-origin parents (F_0) in six populations of pink salmon in PWS and four populations of chum salmon in SEAK to address questions of fitness. In 2015, offspring (F_1) of the pink salmon parents sampled in 2013 returned to the streams and were sampled. Matching of the F_1 individuals to F_0 parents will provide the first estimates of relative reproductive success of hatchery-origin pink salmon in natural streams. This analysis is fully funded for a single stream and will be completed by fall of 2016.

Pink salmon mature, spawn and die at 2-years of age within their native range. This creates separate lineages, referred to as "odd-year" and "even-year", effectively providing for replicate measures of reproductive success within the study streams. Sufficient funds are currently available to support sampling of F_1 individuals returning to all study streams in 2016, but additional funds are necessary to complete the laboratory and statistical analyses. We request funds to genotype the F_0 and F_1 individuals from the even-year lineage for the same stream(s) analyzed from the odd-year lineage. This project will provide a replicate measure of the impacts of hatchery-origin pink salmon on wild populations and inform future resource management decisions.

B. Narrative Project Description

1. Project goals and objectives –

The goal of this project is to assess the impacts, if any, of hatchery-origin fish on natural systems and is 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 with the intent to enhance fisheries, provide economic opportunity to local communities, and reduce variation in annual harvests of salmon. In contrast to the mitigation hatcheries of the Pacific Northwest, built to replace wild production diminished by widespread habitat degradation and dams, the Alaska hatchery program was developed to supplement and enhance wild production. The protection and natural productivity of wild stocks was and remains a priority.

Alaska's salmon fishery enhancement program recognized from its onset that salmon stray, and that hatchery stocks would stray, so it adopted policies and regulations to mitigate concerns associated with straying. The potential for detrimental effects of hatchery production on wild stocks was recognized by policy makers early in the development of the State's hatchery programs (reviewed by Heard 2012, McGee 2004). In 1986 representatives from aquaculture associations and state and federal scientists formalized concerns regarding straying in the Alaska Department of Fish and Game Genetics Policy. Other pertinent policies include the Disease Policy and Fish Transport Permitting Policy.

Wild populations are likely impacted by hatcheries despite sound policy, given the scale of Alaska's enhancement programs. This likelihood raises concern that hatchery-produced fish may detrimentally impact the productivity and sustainability of wild stocks of Alaska salmon. The risks posed to natural populations by hatcheries include genetic (consequences of interbreeding between hatchery-bred and wild salmon); disease (introduction or amplification of pathogens); ecological (competition for resources); and harvest mortality (Naish et al. 2007). While potential positive effects exist, such as reducing harvest pressure on low-production stocks, the potential for detrimental effects is the greatest concern for meeting the goals of conservation and sustained yields from wild stocks.

Recent studies have demonstrated large proportions of hatchery-bred salmon in some wild-spawning populations in PWS (Brenner et al. 2012) and SEAK (Piston and Heinl 2012). These results raised important questions: (1) Are hatchery-bred 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?

Any potential effect of these programs on wild stocks must be considered in the context of both benefits from enhancement programs and costs to wild stocks that the agency is mandated to protect. It has been argued that hatchery stocks have simply replaced the productivity of wild stocks of pink salmon in PWS so that no net gain realized (Hilborn and Eggers 2000). However, Wertheimer et al. (2004) estimated that an annual average production of 24 million hatchery pink salmon was associated with a yield loss of around 1 million wild fish. Harvest and escapement indices of wild stocks in PWS and SEAK have been consistent with historical levels during 30

years of large-scale hatchery production, suggesting that enhanced production has been compatible with sustained wild stock productivity (Wertheimer et al. 2001).

Hatchery production is important to Alaska's fishing industry and its place in the international market, yet Alaska law requires that hatchery production be compatible with sustainable productivity of wild stocks. Faced with evidence of straying and uncertainty about its extent and effect, ADFG must act with caution when considering requests by PNP hatchery corporations for permit alterations.

ADFG and the PNP hatchery corporations developed a research program to investigate concerns about straying and genetic interactions between hatchery and wild stocks. In 2011, ADFG convened a Science Panel of current and retired scientists with broad experience in salmon enhancement, management, and wild and hatchery interactions from ADFG, University of Alaska, PNP Aquaculture Corporations, and NOAA-Fisheries. The Panel addressed 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*?

The Panel developed the Alaska Hatchery Research Program (AHRP) to address these questions (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main) in 2013 with funding from the State of Alaska, the PNP operators, and industry representatives. During the first three years of the program efforts concentrated on providing information to answer questions I (population structure) and II (extent of straying).

Research is needed to answer question III and evaluate potential changes in natural populations of Alaskan pink and chum salmon due to straying of hatchery-origin fish. The main concern is that hatchery-origin fish interbreeding with natural-origin fish may reduce the fitness of wild populations. Fitness is a measure of an organism's ability to survive, reproduce and pass genetic information to future generations, and can be measured as the average contribution to the next generation by an average individual of a given type, for example, hatchery-origin versus natural-origin salmon. For salmon, fitness is often measured as the number of adult offspring produced per spawner of each sex. If hatchery-origin fish are less fit and interbreed with natural-origin fish, natural-spawning populations may lose productivity as a consequence of hatchery strays in the breeding population.

Survival of both hatchery- and natural-origin fish and their adult-to-adult relative reproductive success (RRS) are needed to evaluate whether fitness differences exist between the two types of salmon spawning in the wild. Hatchery-origin pink salmon in PWS and chum salmon in SEAK spawning in the wild can be identified because their otoliths have thermal marks. At the same time, genetic analyses allow individual fish to be traced to their respective parents, so long as their parents have been sampled (similar to paternity evaluations conducted in humans). Combining origin identified by thermal marks with current genetic techniques provided the Panel the means to design a study to evaluate fitness of natural-origin versus hatchery-origin salmon spawning in the wild in streams of PWS and SEAK.

The design of the fitness study was based on:

1. six streams in PWS with pink salmon spawning populations of about 3,000 fish each, three streams that have a low portion of strays (less than 20%) and three streams that have a high proportion of strays (around 50%); and
2. four streams in SE Alaska with chum salmon spawning populations of about 3,000 fish each, two streams that have a low portion of strays and two streams that have a high proportion of strays.

In each of these 10 "fitness" streams, about 500 adult post-spawning salmon were to be collected, their otoliths sampled to determine their origin (hatchery or wild), and genetic samples taken to identify potential parents of the next generation (F_0). Sampling of returning adult offspring occurs when offspring (F_1) of the originally sampled parental salmon return to spawn. As part of the analysis, it will be determined if these fish are offspring of F_0 males or females, of known origin (either hatchery strays or natural-origin fish) or are offspring of unsampled F_0. These data were to be used to estimate survival rates and the reproductive success to the adult stage for hatchery-origin versus natural-origin fish in each stream as well as provide data for comparisons between low and high stray rates for each of the two species with replication.

Fish spawning in these streams were to be sampled for two complete generations. For pink salmon, sampling in each stream would occur in each of six years over two brood years for each brood line, and for chum salmon, sampling in each stream was planned to occur in each of 11 years over two brood years. Pink salmon sampling was scheduled to occur annually from 2013-2018 and chum salmon sampling from 2013-2023. Data and statistics obtained from this robust experiment would provide information needed to evaluate fitness of natural-origin versus hatchery-origin stray salmon spawning in the wild in streams of PWS and SE Alaska.

Preliminary power analyses of the original design called for greater sampling in all fitness study streams (Dann et al. 2014b). These analyses indicated an inability to detect differences in fitness between hatchery- and natural-origin salmon with the original design described above. The Science Panel chose to increase the frequency of sampling resulting in tens of thousands more samples than originally planned. At the same time, funding declines limit the scope of the study that can be completed and subsequently the program's ability to answer these important questions to inform future resource management decisions.

Offspring (F_1) of the pink salmon parents sampled in 2013 returned to the streams and were sampled in 2015. Matching of the F_1 individuals to F_0 parents will provide the first estimates of relative reproductive success of hatchery-origin pink salmon in natural streams. Available funds allow for analysis of samples from a single stream (Stockdale Creek) of the six sampled; this analysis will be completed by fall of 2016. We propose to complete analysis of samples from the same stream from the replicate, even-year lineage (2014 parents, 2016 offspring) and then pursue additional even-year streams depending on the number of 2016 samples. Such a replicate study will greatly improve our ability to make inferences from the research program to inform future hatchery permitting policy. Our specific objectives are to:

1. Genotype 8,000 F_0 and F_1 individuals collected in 2014 and 2016, respectively, for as many streams as possible (depending on 2016 sampling) at 192 single nucleotide polymorphism (SNP) genetic 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 the relative return per spawner (RRS) for hatchery- and natural-origin pink salmon.

2. Project impacts -

This project will yield benefits in two primary areas by: 1) allowing for more informed management and permitting, and by 2) allowing for better assessment of fishery sustainability by third party certifying bodies.

First, this project will benefit commercial fishers and aquaculture operators in the Prince William Sound fishery management area and ADFG by reducing uncertainty regarding the effect of hatchery operations on wild production. Faced with evidence of straying and uncertainty about its extent and effect, the ADFG must act with caution when considering requests by PNP hatchery corporations for permit alterations. Any potential effect of these programs on wild stocks must be considered in the context of both benefits from enhancement programs and costs to wild stocks that the agency is mandated to protect. This project will provide information that will allow ADFG to manage the fishery 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) 5.40.005.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."
3) 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."
4) 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;"
5) 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."
6) 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."

7) 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.
8) 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."
9) 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."

Second, these results will reduce uncertainty and better allow certifying bodies to assess the sustainability of the Prince William Sound pink salmon fishery. Certification of these fisheries benefits Prince William Sound communities, commercial fishers, and aquaculture corporations by adding value to fishery products and providing access to additional markets, thereby boosting profitability and the local economy.

3. Evaluation of project -

Success will be defined by estimating the impact of hatchery pink salmon on the fitness of natural pink salmon in Prince William Sound, Alaska. We will estimate the impact by calculating the RRS of natural- and hatchery-origin pink salmon that spawned in Stockdale Creek in 2014, and other streams as funding allows. Their offspring will be collected as spawning adults in the summer and fall of 2016, assigned to parent via genetic techniques, and used to estimate RRS.

Estimating RRS will require the successful completion of multiple sample collection and analytical procedures that can also be used to monitor and evaluate the relative success of the project. For example, paired otolith-tissue samples will need to be separated and processed in the laboratory to ensure analytical success; the number of samples successfully archived is a useful metric of success. Similarly, a successful project requires quality reading of otoliths to determine origin and extraction of DNA for genotyping. Construction of DNA libraries is required for successful amplicon sequencing; success of this procedure can be measured by the depth of coverage for each targeted locus (192 SNPs) for each individual selected for analysis (8,000). Importantly, the accurate genotyping of individuals will also be measured following robust quality control of laboratory processes. A representative sample of individuals (typically 8%; 640 for this project) will be reanalyzed through the entire genotyping process (DNA extraction, library construction, genotyping and analysis); reanalyzed individuals will provide a measure of our background genotyping error rate.

Accurate estimates of RRS require accurate parentage assignments. Success of this pedigree reconstruction can be measured by the proportion of offspring assigned to parent with a posterior probability > 95%. Furthermore, given the known issues with statistical power in RRS studies (see Box 2 in Christie et al. 2014) we will provide estimates of the power to detect differences in RRS, given the level of sampling in this project in order to put our results in context of the statistical limitations of this ambitious research.

Timely reporting of project results to key stakeholders will serve as a final measure of success. We expect that the information generated by this project will substantially inform hatchery policy in Alaska for decades to come, and we intend to publish results of this study in Alaska Department of Fish and Game report series as well as peer-reviewed literature, such as *Evolutionary Applications*.

4. Need for government financial assistance –

The broader research project associated with this proposal was first funded by $4.5 million in capital funds provided by the State of Alaska in 2012 as well as funding from a consortium of Alaska salmon seafood processors. A non-profit hatchery corporation subsequently provided $2 million from surplus cost-recovery revenues. While this is a considerable sum of money, the overall project is quite ambitious in scale with close to $2 million being spent each of the initial three years largely for field work and data collection. The more pivotal results of the data collection will be the genetic pedigree work described in this proposal. This scale of genetic pedigree reconstruction in a natural system will be a seminal work and different than anything done before with pink salmon. There is no longer any direct funding available from the State of Alaska with severe budget cutting taking place this year and expected in the future.

The Science Panel is seeking funds to continue important tissue and data collection for the overall research program for the next 5 to 7 years that will augment the work of this proposal and complete the necessary field work for pink and chum salmon research in PWS and SEAK.

5. Federal, state, and local government activities and permits -

No permits are required and there are no expected environmental impacts from this project. No field work is included in this project. This project will utilize samples collected by a contractor to the Alaska Hatchery Research Program. This contractor has been, and will continue to be, responsible for obtaining the appropriate permits.

6. Project work plan -

a) What is the project design?

Study Design

This study will utilize samples collected under AHRP. The original experimental design for field collections is detailed in the AHRP Request for Proposals (http://www.adfg.alaska.gov/static/fishing/PDFs/hatcheries/research/rfp_hatchery_fish_interaction.pdf) and in the Prince William Science Center Proposal (http://www.adfg.alaska.gov/static/fishing/PDFs/hatcheries/research/pwssc_h-w_proposal_6-29-12.pdf).

In order to investigate the potential for fitness impacts of hatchery-origin strays on natural-origin pink salmon in PWS, we need to know the origin and pedigree of each fish captured in select streams across multiple generations. Origin refers to the type of early life-history habitat (hatchery or natural) that a fish experienced. Pedigree refers to the family relationship among parents and offspring. 'Ancestral origin' refers to the origin of an individual's ancestors (e.g., two parents of a single origin [hatchery/hatchery or natural/natural] or two parents of mixed origin [hatchery/natural]). These ancestral origins can be determined by combining information from two sources: identification of hatchery origin from otolith marks (all hatchery salmon in PWS have thermally marked otoliths) and pedigree reconstruction from genetic data. By pairing these data within fish and across generations, we can estimate reproductive success (RS) among origin groups. The AHRP is using the relative reproductive success (RRS) of hatchery-origin fish to natural-origin fish as the measure of fitness in this study

By September 2016, the AHRP will have sampled parents (F_0) and offspring (F_1) from a complete generation of even-year pink salmon in PWS. This will be the first opportunity to reconstruct genetic pedigrees in order to directly compare the RRS of hatchery-origin spawners to natural-origin spawners in natural streams for even-year pink salmon in PWS. This information will help determine: 1) if hatchery-origin salmon spawn with natural-origin salmon and 2) if hatchery-origin salmon result in a change in fitness of wild populations (i.e. a decline in natural-origin productivity).

The original target sample size for each of the 6 pink salmon pedigree streams in PWS under this study (Figure 1) was 500 adults (F_0) and 500 offspring (F_1) from a stream with an average escapement ~3,000 salmon, with the goal to have adequate statistical power to detect a 50% reduction in fitness of hatchery-origin spawners as compared to natural-origin spawners (i.e.

RRS = 0.5). Further investigation involving extensive power analyses (Shedd et al. 2014, Shedd and Habicht In prep) concluded that the best way to maximize statistical power to detect potential differences in reproductive success between natural- and hatchery-origin spawners is to sample as many parents of both origin groups as possible and a high proportion of returning adult offspring. Partly based on this information, the AHRP Science Panel modified the study design in increase the sampling numbers and proportions in 2014 and onward. Given the increase in the number of samples collected, the AHRP has decided to focus initial pedigree reconstruction to a subset of streams in order to maximize statistical power by taking a "depth" approach (intensively investigate fewer streams), rather than a "breadth" approach (limited understanding of many streams).

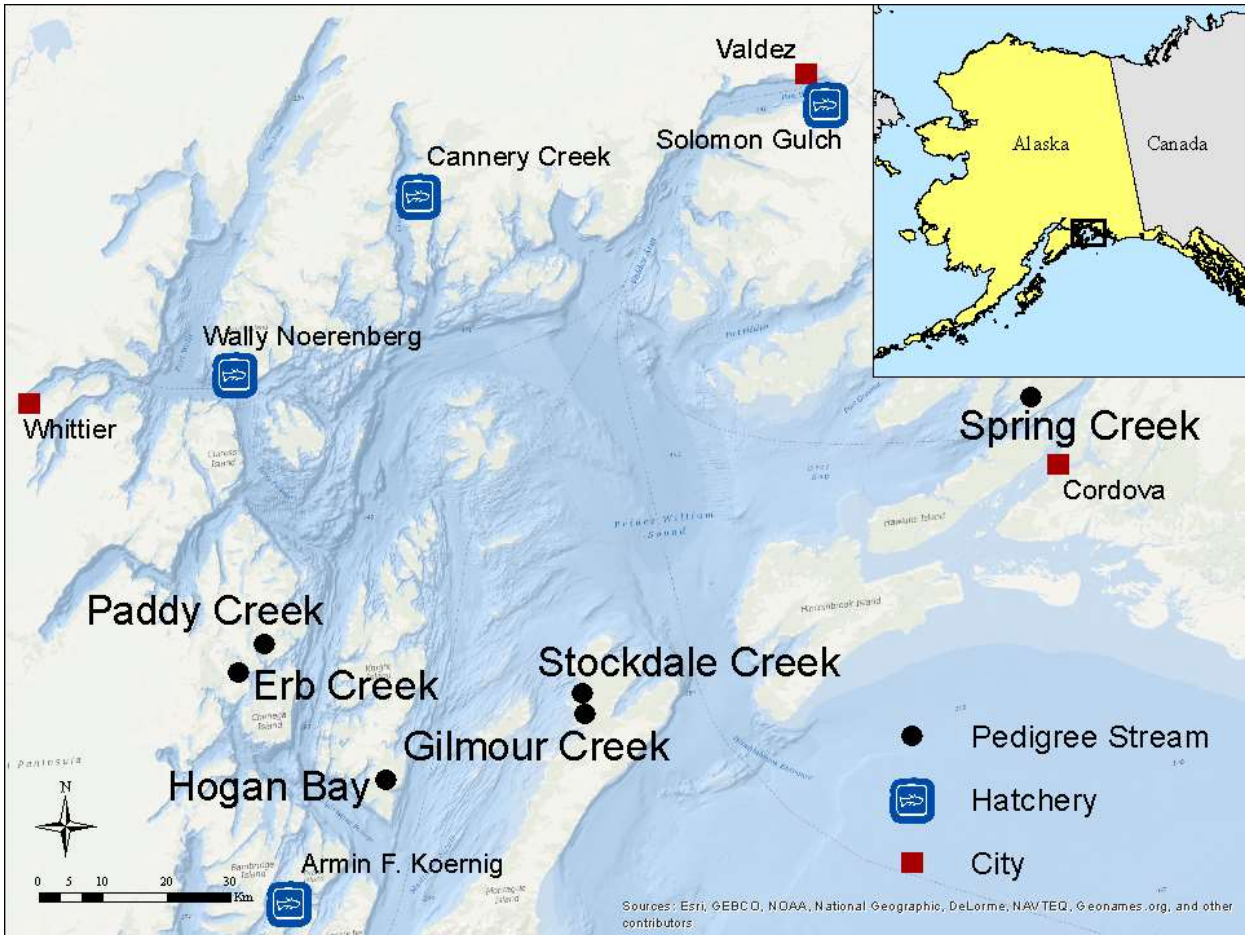


Figure 1. Location of six pink salmon pedigree streams in Prince William Sound for the Alaska Hatchery Research Program.

For this study we propose to analyze 8,000 individuals from one to three streams, depending on the number of offspring sampled in 2016. Streams have been prioritized based on anticipated power to investigate RRS < 0.5 over a wide range of potential distributions (Shedd et al. in prep; Box 1) and observed stray rates, sample sizes, and escapement estimates from 2014 (i.e., depth of sampling) to determine which streams will likely provide the most valuable information (Table 1). From the chosen stream(s) we plan to analyze all potential parents collected in 2014 and all of the potential offspring collected in 2016 (i.e. only natural-origin offspring as

determined by otolith analysis). Hatchery-origin fish collected in 2016 will not be analyzed, as they could not possibly be offspring of the previous generation that spawned in the wild systems.

Table 1. Priority list of pedigree streams to be genotyped and analyzed based on the number of parents (F_0) samples in 2014, the hatchery-origin fraction (stray rate), and statistical power (see Box 1 for details).

Priority	Stream	n 2014 Parents	2014 Stray Rate	Statistical Power	Rationale
1	Stockdale	1,551	57.7%	Very High	Prioritized for odd-year analysis and very high power
2	Erb	1,957	16.0%	Very High	Lower stray rate for even-year and very high power
3	Paddy	1,158	53.9%	High	High sampling proportion of parents in 2014 and high power
4	Hogan	2,649	89.4%	High	Prioritized for odd-year analysis and high power, but high stray rate
5	Gilmour	669	52.9%	Medium High	Similar stray rate to Stockdale and Paddy but lower power
6	Spring	151	1.6%	Very Low	Low stray rate and very low population size, not recommended

Stream Sampling

Field crews visited PWS pedigree streams at least once every three days throughout the 2014 field season to collect samples from the F_0 generation (i.e. potential parents) throughout the entire run (Knudsen et al. 2015). Paired otolith and genetic samples were collected into a cell of a 48-well deep well plate and preserved in 95% ethanol to prevent DNA degradation. Additional phenotypic data such as sex and fork length along with date and GPS location data were also collected.

Field crews plan to visit PWS pedigree streams at least every other day throughout the 2016 field season to collect samples from the F_1 generation (i.e. potential offspring) throughout the entire run following methods used in 2014 (Knudsen et al. 2015).

Box 1. Stream-specific power analysis based on 2014 sampling

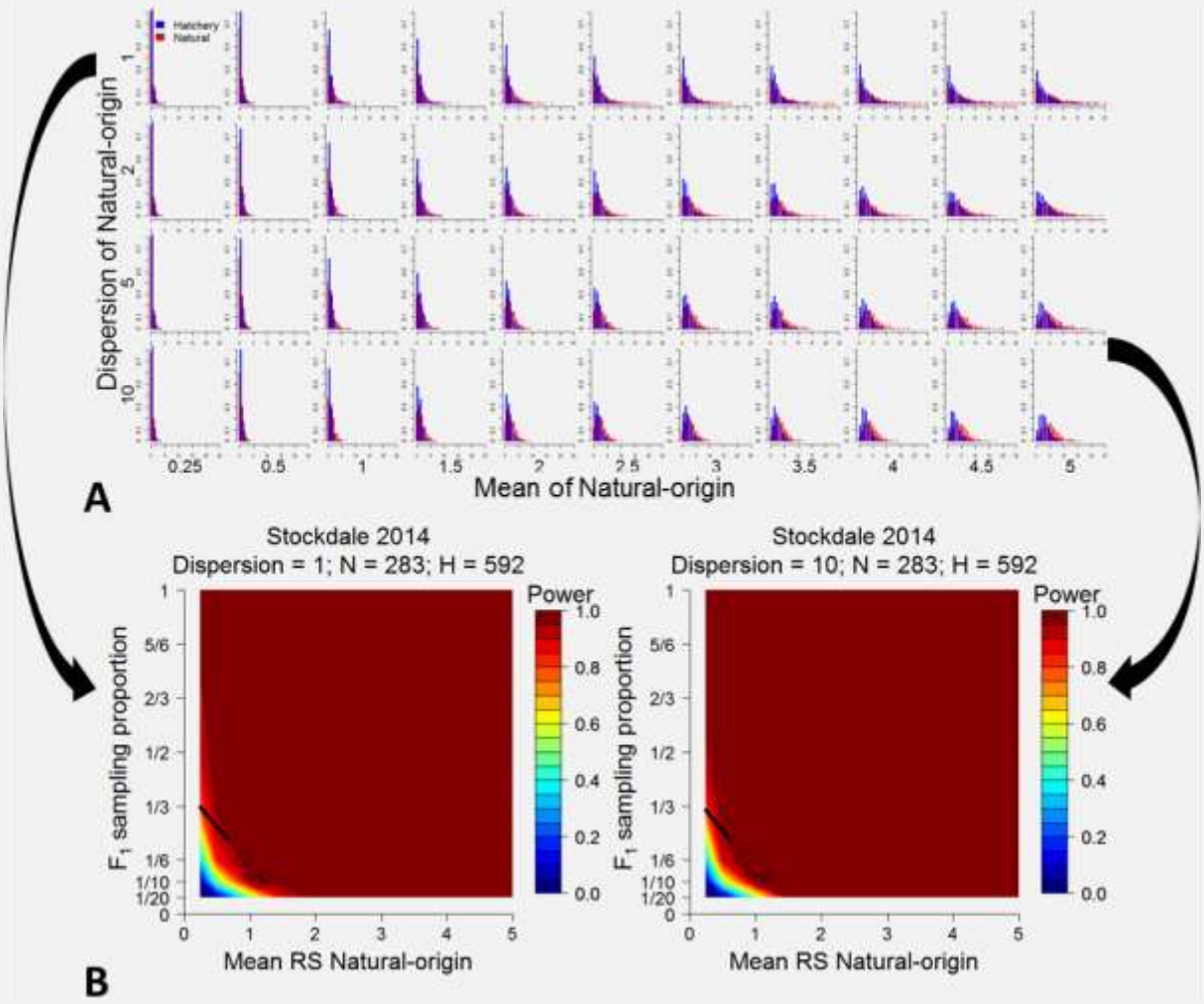


Illustration of power analysis to detect a relative reproductive success (RRS) of 0.5 (hatchery/natural) based on stream-specific numbers of parents sampled by origin and sex.

A. 44 different potential distributions of reproductive success as characterized by the negative binomial distribution for 11 values of mean reproductive success (natural-origin) and 4 values of dispersion factor (i.e. variance component). The hatchery-origin group has a distribution with equal variance, but a mean value that is half that of the natural-origin group (i.e. RRS of 0.5).

B. For each stream, offspring were generated by taking draws equal to the number of parents sampled in each origin group from one of the 44 sets of distributions of reproductive success. Offspring were then pooled and randomly subsampled without respect to origin based on different levels of F_1 sampling proportion (to represent the fact that this program does not sample all returning adult offspring). The samples of offspring were then assigned back to parents and those reproductive success values were compared with a one-way permutation test ($\alpha = 0.05$; see text for details) in order to test for a statistically significant difference in reproductive success between the origin groups (the known difference is always RRS = 0.5). This process was repeated 1,000 times for each stream, distribution, and level of F_1 sampling proportion in order to assess the power of statistical tests for these combinations (i.e. number of significant tests / 1000 trials). Example shown here is a colored contour plot for 2014 sampling of Stockdale Creek. Each of these two graphs indicate the number of natural- (N) and hatchery-origin (H) parents and depict the expected power to detect an RRS of 0.5 based on the inherent productivity of the system (x-axis), the proportion of F_1 offspring sampled (y-axis), and two different levels of variance in the distribution of reproductive success (left and right graph). The solid black line indicates the contour for power = 0.8.

Otoliths and genetic tissue are separated at the Gene Conservation Laboratory (GCL) in Anchorage maintaining pairing integrity. Otoliths are sent to the ADF&G Cordova Otolith Laboratory where they are subsequently polished and inspected under a light microscope for the presence of hatchery thermal marks (Volk et al. 2005). All trained otolith readers had previously been tested with randomized blind tests of known origin fish to assess accuracy (Joyce and Evans 1999).

Heart tissue was selected as the genetic tissue for collections and single nucleotide markers (SNPs) were selected as the genetic marker. DNA decay in dead fish is affected by time, temperature, chemical 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 the solar radiation that can damage DNA, and (3) tests of this tissue type from both live and dead (non-rotten) salmon indicated high genotyping success (Dann et al. 2014a). SNPs were chosen because they lend themselves to high-throughput genotyping and have been successfully used for parentage analysis in salmonids (Anderson and Garza 2006, Hauser et al. 2011).

Previous studies documented that DNA from poor quality tissues can produce unreliable genotypic data and questionable estimates of stock composition in mixed stock analyses (Paetkau 2003; ADF&G unpublished data). Data reliability is even more important for parentage analyses due to the large influence that missing or incorrect genotypes can have on parentage assignments relative to stock of origin assignments. Poor-quality DNA samples are excluded from analyses by implementing the "80% Rule", whereby individuals missing genotypes for 20% or more of screened markers are removed from further analysis (Dann et al. 2009).

Laboratory Analysis

DNA extraction and genotyping generally will follow the Genotyping-in-Thousands by sequencing (GT-seq) methods (Campbell et al. 2014). While 5' exonuclease genotyping methods have been efficient and cost-effective for projects involving <96 SNPs (Dann et al. 2012, Seeb et al. 2009), the newly developed GT-seq method allows for massively parallel sequencing reactions that is transformational for obtaining high quality genotypes for > 200 SNPs. Briefly, genomic DNA will be extracted from individual tissue samples using DNeasy 96 Tissue kits (QIAGEN, Valencia, CA). Extracted DNA from individuals will be combined with a PCR cocktail for multiplex PCR of ~ 200 SNP amplicons currently being developed specifically for parentage analysis under contract to the University of Washington. This amplicon panel will be selected from among thousands of SNPs discovered using restriction site associated DNA sequencing of PWS pink salmon collected in 2013 and 2014. SNPs with high minor allele frequency in both broodlines (odd- and even-year) will be selected. High minor allele frequency markers maximize discriminatory power in parentage analysis and pedigree reconstruction (Anderson and Garza 2006). Following multiplex PCR, individual samples will be labeled with unique DNA barcode adapters, normalized, and ultimately pooled into a single sequencing library for next generation sequencing (NGS). Post-sequencing, reads from individual samples will be split based on the unique barcodes and genotypes will be called according to counts of amplicon-specific alleles (Campbell et al. 2014). Genotypes will be imported and archived in the Gene Conservation Laboratory Oracle database, *LOKI*.

A quality control analysis (QC) will be conducted to identify laboratory errors and to measure the background discrepancy rate of the genotyping process. The QC analyses will be performed

by staff not involved in the original genotyping (Dann et al. 2012). Briefly, the method will consist of re-extracting 8% of project fish and genotyping them for the same SNPs assayed in the original genotyping process following the same methods. Discrepancy rates will be calculated as the number of conflicting genotypes, divided by the total number of genotypes compared. These rates will describe the difference between original project data and QC data for all SNPs and are capable of identifying extraction, assay plate, and genotyping errors. Assuming that discrepancies among analyses are due equally to errors during the original genotyping and during quality control, error rates in the original genotyping will be estimated as half the rate of discrepancies.

Statistical Analysis

Genotypes in the *LOKI* database will be imported into the statistical package *R* for analysis (R Core Team 2015). Prior to statistical analysis, two statistical quality control analyses will be performed to ensure only high-quality genotype data are used in subsequent analyses. First, individuals that are missing a substantial number of genotypes will be identified and removed from further analyses. We will use what we refer to as the 80% rule, which excludes individuals missing genotypes for 20% or more of loci, because these individuals likely have poor-quality DNA. The inclusion of individuals with poor quality DNA might introduce genotyping errors and reduce the accuracies of parentage analyses. The second statistical quality control analysis will identify individuals with duplicate genotypes and remove them from further analyses. Duplicate genotypes can occur as a result of sampling or extracting the same individual twice, and will be defined as pairs of individuals sharing the same genotype in 95% of markers screened. The individual with the most complete genotypes from each duplicate pair will be retained for further analyses.

Once statistical quality control is complete for genetic data, all other sampling data including otolith origin will be queried from ADF&G's Alaska Salmon Biological Data Warehouse and joined to individuals based on the plate barcode and individual position. Collection year and sex will be used to create input files for pedigree reconstruction program FRANz (Riester et al. 2009). Briefly, FRANz uses a Bayesian framework and a Metropolis-Hastings coupled Markov Chain Monte Carlo (MCMC) algorithm to assign parentage based on phenotypic data (brood year and sex) and multilocus genotypes. Likelihood- or Bayesian-based parentage analysis has been shown to perform better than exclusion-only techniques (Anderson and 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, as the model accounts for this and can use sibships and other close relationships among sampled individuals to infer parental genotypes from progeny to assist in filling out sparse pedigrees (Jones et al. 2010, Riester et al. 2009). Final parentage assignment will be limited to those parent-offspring pairs or parent-pair-offspring trios that have a posterior probability > 95%.

The reproductive success of each origin group (hatchery- and natural-origin) will be calculated separately for each sex, as we anticipate that most offspring will be assigned to single-parent-offspring pairs since only a proportion of potential parents are being sampled, and this provides a method to account for parents that produce zero sampled offspring (Araki and Blouin 2005, Christie et al. 2014). Reproductive success is the number of F_1 offspring assigned to F_0 parents. Differences in reproductive success will be tested using a non-parametric one sample

permutation test ("oneway.test" function in the "coin" package in R), as testing for differences in RS is equivalent to testing if RRS < 1 (Araki and Blouin 2005). Results of the permutation test will provide a mean estimate of RRS along with 90% confidence interval (based on the permutation distribution). To compliment the analysis of RS, we will also use a generalized linear model (GLM; negative binomial distribution with a log link function; "glm.nb" in "MASS" package in R) to investigate the effects of covariates such as length, timing, and origin, on individual reproductive success (Ford et al. 2012).

b) Who will be responsible for carrying out the various activities?

Tyler Dann, ADF&G Fisheries Geneticist II, will be the administrative point of contact and will supervise data collection, analyses and report preparation. Mr. Dann will be responsible for reporting, budget requirements, and disseminating information to fisheries managers, commercial fishing groups, and the public.

Kyle Shedd, ADF&G Fisheries Geneticist I, will be the technical point of contact and will lead sample selection, statistical analyses of parentage and RRS estimation, and reporting in ADF&G's Fishery Manuscript Series and peer-reviewed journal such as Evolutionary Applications.

Heather Hoyt, ADF&G Fishery Biologist II, is the laboratory coordinator for all projects in the ADF&G Gene Conservation Laboratory, and will ensure that samples are extracted, genotyped, scored, and quality-controlled in a timely manner and with minimal errors.

Wei Cheng, ADF&G Fisheries Geneticist I, will conduct quality control of laboratory procedures.

Paul Kuriscak, AD&G Fishery Biologist I, will genotype samples.

Christina Elmaleh, ADF&G Fish and Wildlife Technician IV, and Zach Pechacek, AD&G Fish and Wildlife Technician III, will separate otolith and tissue samples, extract DNA, and archive samples.

c) Data Sharing Plan:

This project will not generate any environmental data. Parentage analysis results will be stored in *LOKI* and made available within one year of the termination of this project. Associated data, including locations and dates of samples will be included with the parentage data. Progress reports and annual reports will be made available to NOAA and the Alaska Hatchery Research Program Science Panel via the standard reporting processes. A final report detailing results will be published in the ADF&G Fishery Manuscript Series upon project completion, which is available to the public on-line and by request. Finally, results will be published in a peer-reviewed genetic or fisheries journal such as Evolutionary Applications.

d) What are the major products/deliverables and how will project results be disseminated?

A Project Operational Plan will be produced by the principal investigators upon funding approval and made available to the AHRP Science Panel and published as part of the ADF&G Project Operational Plan directive.

Progress reports and annual reports will be made available to NOAA via the standard reporting processes. Progress reports and solicitations for project design and analysis will be provided to

the AHRP Science Panel using the Technical Document series (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesResearch.main).

Upon project completion a final report detailing results will be published in the ADF&G Fishery Manuscript Series, which is available to the public on-line and by request. Results will also be published in a peer-reviewed genetic or fisheries journal such as Evolutionary Applications.

Oral presentations will be provided at the:

1) AHRP Spring 2018 meeting which will be open to the public,
2) Prince William Sound Science Center in Cordova, Alaska in an advertised public meeting and,
3) American Fisheries Society Western Division annual meeting in 2018.

Locations and dates for these meeting are to be announced. Anticipated audiences at these oral presentations include representatives from: fishing industry, aquaculture industry, environmental non-governmental organizations, and scientists (including the AHRP Science Panel). NOAA will be acknowledged as the funding source for this project in all reports and presentations.

e) What are the project milestones?

Project activities will occur throughout September 1, 2016 and June 31, 2018 (22 months). Activities and expenditures occurring prior to project start date will be funded separately. All invoices will be submitted for payment in approved form within 30 days of project end date.

Major project tasks include the separation of paired otolith and tissue samples, reading of otoliths to determine origin, extraction of DNA, genotyping of samples, quality control of laboratory analyses, statistical analyses, writing of departmental reports and peer-reviewed publications, and presenting findings to stakeholders and the scientific community.

Months 1-3: Paired otolith and tissue samples will be separated at the Gene Conservation Laboratory under the direction of Mr. Shedd. Otolith and tissue samples will be separated following established protocol in Months 1-2; otoliths will be transferred to the ADF&G Cordova otolith laboratory in Month 3.

Months 4-6: Otoliths will be inventoried, prepared and read to determine origin (hatchery or natural) for all 8,000 samples identified for analysis.

Months 7-8: DNA will be extracted by Mr. Pechacek and Mrs. Elmaleh under the supervision of Ms. Hoyt following established protocol.

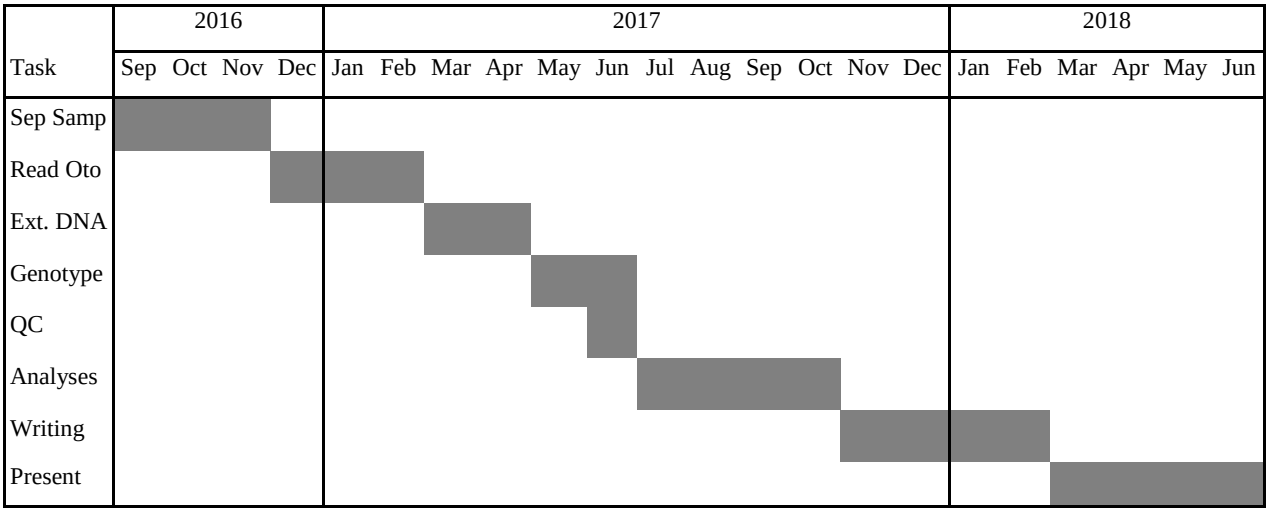
Months 9-10: Samples will be genotyped by Mr. Kuriscak and Ms. Hoyt under the supervision of Ms. Hoyt following established protocol.

Month 10: Quality control of DNA extraction and genotyping will be conducted by Ms. Cheng under the supervision of Ms. Hoyt following established protocol.

Months 11-14: Bioinformatic analyses of sequence data, statistical quality control of genotypes, parentage analyses and estimation of RRS will be conducted by Mr. Shedd under the supervision of Mr. Dann following established protocol.

Months 15-18: Mr. Dann and Mr. Shedd will prepare a report to be published in ADF&G Fishery Manuscript Series as well as peer-reviewed publication for a target journal of Evolutionary Applications.

Months 19-22: Mr. Shedd will present key findings to the fisheries science community at the annual meeting of the Western Division of the American Fisheries Society in spring of 2018 (meeting date to be determined). Mr. Dann will present key findings to ADF&G fishery managers, commercial fishing groups, seafood processors and interested non-governmental organizations at a meeting of the AHRP in spring of 2018 (meeting date to be determined).



7. Project management -

The ADF&G Gene Conservation Laboratory has many years of experience providing researchers and fishery managers with genetic information to inform fisheries management and policy. ADF&G Administrative procedures provide for immediate invoice payment and tracking of expenses. The principal investigators are funded through other sources and both have successful track records in preparing projects, participating in data collection and analysis and producing reports in a timely fashion.

Tyler Dann – ADF&G, Gene Conservation Laboratory, Fisheries Geneticist II, Southcentral and Southwest Alaska Genetics Project leader. Mr. Dann will act as the PI, budget coordinator, and will be in charge of all aspects of project implementation. Mr. Dann will ensure that work is completed and accurately reported in a timely fashion and will finalize all data reporting.

Kyle Shedd – ADF&G, Gene Conservation Laboratory, Fisheries Geneticist I, Hatchery Research and Westward Region Analyst. Mr. Shedd will oversee data collection, analysis and report preparation.

8. Participation by persons or groups other than the applicant –

The project and interim results have been presented in a wide range of venues, including AFS Alaska chapter meetings, the 2015 National meeting, Pink and Chum workshops, and a special meeting in December 2014 in Anchorage to present draft results to the public. Interactions between project staff and the public will continue. There have also been presentations to regional plan team meetings in PWS and SEAK, Seine and Gillnet Task Force Meetings, Fisherman preseason meetings in PWS. In addition progress reports are available on the ADF&G web site (2013 and 2014 results will soon be posted).

Additional collaboration with NOAA fishery biologist and Science Panel member Dr. Jeff Hard will continue. Dr. Hard is participating a project partner, offering his expertise on the genetic effects of hatchery-wild interactions.

9. Outreach and Education -

The overarching goal of this study is to help management agencies (i.e., ADF&G) and interested stakeholders understand the impact of hatchery pink salmon on populations of wild pink salmon in PWS. As such, this project is focused on protecting the Nation's natural resources for the long-term and, importantly, is a collaborative effort that includes natural resource users, managers and the interested public (e.g., AHRP Science Panel). We have identified four primary education and outreach activities to communicate project findings.

We will publish written reports that describe in detail project findings in two outlets. The findings of this study will be relevant to resource management, future hatchery permitting, and evaluation of fishery sustainability by third-party organizations (e.g., Marine Stewardship Council); publication in ADF&G report series provides direct and accountable public access to project findings. Publication in peer-reviewed literature adds credibility to our study findings and extends outreach to the broader scientific community.

We will prepare visual presentations of project findings for general public and fishery science audience. We plan to present at forums such as annual meetings of the Alaska Hatchery Research Program, Prince William Sound Science Center, regional aquaculture associations (e.g., Prince William Sound Aquaculture Association), aquaculture Regional Planning Teams (http://www.adfg.alaska.gov/index.cfm?adfg=fishingHatcheriesPlanning.regional), the annual meeting of the American Fisheries Society's Western Division, and the Anchorage Zoo's Wildlife Wednesday lecture series. Similarly, Mr. Dann and Mr. Shedd will design an informative poster that conveys key results and implications in a format easily accessible to the public and stakeholders. All outreach products will reference NOAA's Saltonstall-Kennedy program as a source of funding for the project.

C. Budget Justification

State of Alaska Fiscal Year 2017

Budget	Total
100 Personnel*	$50,741
200 Travel	$0
300 Contractual	$8,640
400 Supplies	$114,912
500 Equipment	$0
Subtotal	$174,293
Expenses not subject to indirect cost recovery	$123,552
600 Indirect	$10,656

Grand Total:	$184,948

State of Alaska Fiscal Year 2018

Budget	Total
100 Personnel*	$50,958
200 Travel	$3,380
300 Contractual	$0
400 Supplies	$0
500 Equipment	$0
Subtotal	$54,338
Expenses not subject to indirect cost recovery	$3,380
600 Indirect	$10,701
Grand Total:	$65,039

*State of Alaska Salary Calculator used

Budget Narrative State of Alaska Fiscal Year 2017/Federal Fiscal Year 2016:

a) Personnel

SFY17/FFY16:

Line 100 – Personnel

This project will fund the following staff to manage the project, analyze samples in the lab. Total = $50,741

- Kyle Shedd, Fisheries Geneticist I (17B), PCN 11-7080 will manage the project and coordinate analyses for 1.5 months @ $4,947month salary + $3,310/month benefits = $ 12,386
- Wei Cheng, Fisheries Geneticist I (17G), PCN 11-7021 will perform quality control analyses for 1 months @ $5,696/month salary + $3,599/month benefits = $9,295
- Heather Hoyt, Fishery Biologist II (16D), PCN 11-5038 will ensure that samples are extracted, genotyped and quality-controlled for 1 month @ $4,778/month salary + $3,244/month benefits = $8,022
- Paul Kuriscak, Fishery Biologist I(14M), PCN 11-4263 will genotype for 1 month @ $5,361/month salary + $3,470/month benefits = $8,831
- Christie Elmaleh, Fish and Wildlife Technician IV (13B), PCN 11-5177 will assist with tissue separation, DNA extraction, and sample archiving for 1 month @ $3,611/month salary + $2,793/month benefits = $6,404

- Zach Pechacek, Fish and Wildlife Technician III (11B), PCN 11-7607 will assist with tissue separation, DNA extraction, and sample archiving for 1 month @ $3,178/month salary + $2,625/month benefits = $5,803

b) Travel

Line 200 – Travel

None

c) Equipment

Line 500 – Equipment

None

d) Supplies

Line 400 – Supplies

Kits to extract DNA from 8,640 fish: 8,640 @ $2/fish = $17,280

Laboratory plastics for sampling and analyzing 8,640 fish: 8,640 @ $2/fish = $17,280

Biochemical assays for analyzing 8,640 fish: 8,640 @ $7.30/fish = $63,072

Sequencing kit for genotyping 8,640 fish: 8,640 @ $2/fish = $17,280

Total = $114,912

Note that total fish count includes additional 8% (640 fish) for quality control.

e) Contractual costs

Line 300 – Contractual

Maintenance contracts for laboratory equipment: 8,640 @ $1/fish = $8,280

Total = $8,280

Note that total fish count includes additional 8% (640 fish) for quality control.

f) Other costs

Line 600 – Indirect

21% of Line 100 Personnel Costs = $10,656

Budget Narrative State of Alaska Fiscal Year 2018/Federal Fiscal Year 2017:

a) Personnel

SFY18/FFY17:

Line 100 – Personnel

This project will fund the following staff to manage the project, perform bioinformatics, test hypotheses and write reports and publications. Total = $50,958

- Kyle Shedd, Fisheries Geneticist I (17C), PCN 11-7080 will perform bioinformatics to determine parentage, analyze data to test hypotheses, and write reports and publications for 6 months @ $5,118/month salary + $3,375/month benefits = $50,958

b) Travel

Line 200 – Travel

Tyler Dann will travel from Anchorage to Cordova to present study findings to stakeholders. Total =$1,690

- Airfare: 2 tickets @ $500/ticket = $1,000
- Ground transportation: 0
- Lodging: 3 nights @ $150/night = $450
- Meals per diem: 4 days at $60/day = $240

Kyle Shedd will travel from Anchorage to AFS meeting to present study findings to fishery science community. Total =$1,690

- Airfare: 2 tickets @ $500/ticket = $1,000
- Ground transportation: 0
- Lodging: 3 nights @ $150/night = $450
- Meals per diem: 4 days at $60/day = $240

c) Equipment

Line 500 – Equipment

None

d) Supplies

Line 400 – Supplies

None

e) Contractual costs

Line 300 – Contractual

None

f) Other costs

Line 600 – Indirect

21% of Line 100 Personnel Costs = $10,701

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