Susitna-Watana Hydroelectric Project Document
ARLIS Uniform Cover Page

Title: Genetic baseline study for selected fish species, Study plan Section 9.14 : Final study plan	**SuWa 200**
Author(s) – Personal:	
Author(s) – Corporate: Alaska Energy Authority	
AEA-identified category, if specified: Final study plan	
AEA-identified series, if specified:	
Series (ARLIS-assigned report number): Susitna-Watana Hydroelectric Project document number 200	**Existing numbers on document:**
Published by: [Anchorage : Alaska Energy Authority, 2013]	**Date published:** July 2013
Published for:	**Date or date range of report:**
Volume and/or Part numbers: Study plan Section 9.14	**Final or Draft status, as indicated:**
Document type:	**Pagination:** 15 p.
Related work(s):	**Pages added/changed by ARLIS:**
Notes: A set of three attachments (A, B, and C) is cataloged separately under title: Susitna-Watana Hydroelectric Project, FERC Project no. 14241-000; Submission of final 2013 project operational plan for fish genetic baseline study (Study 9.14).	

Susitna-Watana Hydroelectric Project
(FERC No. 14241)

Genetic Baseline Study for Selected Fish Species
Study Plan Section 9.14

Final Study Plan

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

July 2013

9. FISH AND AQUATIC RESOURCES

9.14. Genetic Baseline Study for Selected Fish Species

On December 14, 2012, Alaska Energy Authority (AEA) filed with the Federal Energy Regulatory Commission (FERC or Commission) its Revised Study Plan (RSP), which included 58 individual study plans (AEA 2012). Included within the RSP was the Genetic Baseline Study for Selected Fish Species, Section 9.14. RSP Section 9.14 focuses on understanding the genetic structure of selected species within the Susitna River.

On February 1, 2013, FERC staff issued its study determination (February 1 SPD) for 44 of the 58 studies, approving 31 studies as filed and 13 with modifications. RSP Section 9.14 was one of the 13 approved with modifications. In its February 1 SPD, FERC recommended the following:

AEA consult with the FWS and NMFS prior to preparing the project operational plans; distribute draft project operational plans to the agencies by March 31 of each year of study implementation; allow 15 days for the agencies to provide comments on the draft plans; file the final plans with the Commission by April 30 of each year of study implementation; and include with the final plans, documentation of agency consultation, a description of how agency comments are incorporated into the final plans, and an explanation for why any agency comments are not incorporated into the final plans.

To the extent feasible, we recommend that AEA collect tissue samples over a representative proportion of the entire adult Chinook salmon run.

We recommend that AEA include in the 2013 project operational plan, a schedule for when the 2012 genetics studies would be available, and include provisions for filing those results with the Commission through either the initial study report, or a supplemental report in 2013. We also recommend that the report on the 2012 preliminary genetics studies clearly describe the criteria, using current scientific literature, to determine whether there is sufficient genetic uniqueness to estimate the percentage of Chinook originating from Upper and Middle River habitats in areas sampled downstream. Finally, we recommend that the report on the 2012 preliminary genetics studies clearly describe whether the study results indicate that sufficient genetic uniqueness is found to characterize the presence and relative proportion of fish originating from the Upper and Middle River in selected Lower River habitats as described in section 9.14.4.7 of the study plan.

In accordance with the February 1 SPD, AEA provided a draft Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska (Genetics IP) for review on March 31, 2013 and filed a final Genetics IP on April 30, 2013. Information in the Genetics IP supersedes relevant details within this Final Study Plan.

9.14.1. General Description of the Proposed Study

Construction and operation of the Project will modify the flow, thermal, and sediment regimes of the Susitna River, which may alter the composition and distribution of fish populations.

Genetic analysis methods can be used to address several goals associated with assessing potential Project impacts. First, there is a potential for the Project to affect genetic diversity and local adaptation of fish populations. Second, genetics can be used as a tool to assess other forms of impacts, however the usefulness of genetics as a tool to assess other impacts derives from the degree of population segregation of particular species among areas of the Susitna watershed. If breeding isolation among areas occurs over sufficient time, the unique genetic characteristics act as naturally occurring "tags" of spawning populations.

As part of the first application of genetics, this study will develop a repository of fish tissues from many resident and anadromous fish for use with future studies that may be needed to characterize the genetic legacy and variation for species and populations of interest. As a tool for assessing non-genetic impacts, this study will provide a means of assessing the degree to which Chinook salmon from the Middle and Upper River rear in areas downstream of the Middle River. If known to occur, such information alters the methods that are needed to characterize potential effects of the Project. For example, monitoring the abundance of Chinook salmon smolt leaving the Middle River to the sea would underestimate the actual contribution of the Middle and Upper River to the overall Susitna Chinook salmon population.

This work will be conducted through collaboration among Alaska Energy Authority (AEA), Alaska Department of Fish and Game (ADF&G), and other licensing participants. Information developed in this study may also assist in the development of protection, mitigation, or enhancement measures to address potential adverse Project impacts to salmonid resources, as appropriate.

Study Goals and Objectives

The goals of this study are to (1) acquire genetic material from samples of selected fish species within the Susitna River drainage, (2) characterize the genetic structure of Chinook salmon in the Susitna River watershed and (3) assess the use of Lower and Middle River habitat by juvenile Chinook salmon originating in the Middle and Upper Susitna River.

Objectives:

1. Develop a repository of genetic samples for fish species captured within the entire Susitna River drainage, with an emphasis on those species found in the Middle and Upper Susitna River.
2. Contribute to the development of genetic baselines for each of the five species of Pacific salmon spawning in the Susitna River drainage.
3. Characterize the genetic structure of Chinook salmon in the Susitna River watershed, including determining the effective population size of fish spawning above Devils Canyon.
4. For 2013 and 2014, quantify the genetic variation among Upper and Middle River Chinook salmon for use in mixed-stock analyses, including analyses of Lower River samples of the entire Susitna Chinook salmon population.
5. If sufficient genetic uniqueness is found, estimate the annual percent of juvenile Chinook salmon in selected Lower River habitats that originated in the Middle and Upper Susitna River in 2013 and 2014.

9.14.2. Existing Information and Need for Additional Information

The baseline genetics data in the Susitna River is limited to the five Pacific salmon species. Assessing genetic relatedness and isolation of fishes in the watershed can be used to help determine potential impacts from the Project. Interbreeding among areas might be hindered by the Project, thereby potentially reducing the fitness of some stocks of resident fishes. Breeding isolation of stocks may be a sign of uniquely adapted traits for particular features of the habitats; such information would alter the impact assessment, and possibly the design of any proposed mitigation measures. To characterize relatedness and any isolation of particular resident fishes, tissue samples for genetic analysis must be collected from a range of locations.

Tissue collections and genetic analyses of Pacific salmon stocks in Alaska are relatively well developed and are used for applied research in several watersheds. The Susitna River salmon stocks are not well represented in the state's tissue repository, and samples obtained here will enable the application of genetic methods to address the four objectives listed above. The genetics samples collected during this study will be used to create a genetic repository for Susitna River fishes and will provide additional data to characterize the genetic baseline for Susitna River salmon populations. Furthermore, this genetic information will help increase our knowledge of the population structure and life history expression of Susitna River Chinook salmon.

9.1.1.1. Uncertainty in Assessing Chinook Population Structure

During the 2012 season, Chinook salmon ascended and remained above Devils Canyon during the spawning season. Ten of these fish from Kosina Creek were sampled for genetics. This observation leads to questions about whether these fish represent self-sustaining population(s) (Hypothesis 1), or, they are individuals originating from other geographic spawning aggregates (Hypothesis 2; e.g., Portage Creek), or are some combination of a local population and a nearby stock (s).

Genetic analysis can help to distinguish between these hypotheses, especially when combined with radio-tagging results from the Salmon Escapement Study, (Section 9.7). Distinguishing can be done by examining the genetic profile of fish above Devils Canyon (and their behavior) relative to other nearby spawning aggregates within the drainage, and by examining how stable the genetic profiles of these fish are through time. Non-divergent (i.e., similar) genetic profiles from those in nearby potentially-contributing spawning aggregates would indicate that the fish ascending Devils Canyon are likely strays or colonizers, but have not established into a self-sustaining population (support for Hypothesis 2). It may be possible to sample sufficient numbers of fish from the three years of this study to address Hypothesis 2 (i.e., no divergence seen from a sufficiently large sample). However, providing evidence for Hypothesis 1 may be difficult in three years, even if a large number of fish can be sampled in locations above and below Devils Canyon if the samples do not represent fish from multiple cohorts and/or our ability to detect a trend is weak.

High divergence from likely contributing spawning aggregates could mean either: a self-sustaining population with little genetic flow with other populations (Hypothesis 1a) or a recent colonization event(s) with small numbers of successfully contributing families (Hypothesis 1b). (A recent colonizing attempt with small number of successfully contributing families is likely an indication of a non-self-sustaining spawning aggregate.) The stability of genetic profiles through

time (i.e., temporal stability of allele frequencies) can provide a means to start to distinguish between these two hypotheses (1a and 1b). Stable genetic profiles from aggregates above Devils Canyon would support Hypothesis 1a. A lack of temporal stability of allele frequencies would support Hypothesis 1b. The statistical power to detect temporal stability of allele frequencies is only possible with samples obtained over multiple years and across cohorts of returning salmon. Therefore, sampling across three years (2012-14) to assess temporal stability in allele frequencies from fish above Devils Canyon, given potentially low numbers of fish available for sampling, may limit the ability to conclusively distinguish between Hypothesis 1a and 1b. Finally, samples from three calendar years may represent fish from as many as 5 or 6 brood years given the multiple ages of maturity in any given year. If large numbers of fish could be sampled in each of the remaining calendar years (2013 and 2014), it may be possible to detect instability in allele frequencies if it existed (some support for Hypothesis 1a).

In summary, the degree of genetic divergence between fish sampled from above and below Devils Canyon in 2012–2014 will dictate the ability to rule out or support local adaptation. A lack of divergence from a sufficiently large set of samples would provide an indication that these are not different spawning aggregates; high divergence would likely require more years to answer the question than with the new information from 2013/2014.

9.1.1.2. Approach to Study Design and Implementation, Chinook Salmon above Devils Canyon

The sample sizes, and geographic and temporal scope of sampling from the Upper, Middle, and Lower River required to address the issue of the genetic divergence/uniqueness of Chinook salmon above Devils Canyon will be a function of the following:

- Numbers of fish passing through the canyon in 2013 and 2014.
- The age structure of fish sampled for genetics.
- The degree of genetic divergence and temporal stability between fish above and below Devils Canyon.
- The behavior of radio-tagged salmon below and above Devils Canyon.
- Genetics baseline information on the distribution of any spawning aggregates not currently known.

Given the outcome of each of these is unknown, AEA proposes a comprehensive sampling effort to help answer as many or all possible hypotheses about the genetic structure of Chinook salmon in the Middle and Upper River. Some outcomes may preclude or significantly affect which and what numbers of samples to analyze. In addition to many sources of opportunistic sample collection (Sections 9.5, 9.6, and 9.7), this study plan includes dedicated sampling effort by a field crew for 2 months each year during the spawning period of adult salmon.

To ensure that data sources (and hypotheses) are rigorously examined, AEA will work closely with geneticists from State and Federal genetics laboratories. This collaboration will come in the form of formal contracting to ADF&G's Gene Conservation Lab, and through regular updates to the agency Technical Workgroup (TWG) over the course of 2013 and 2014, when federal and other geneticists can provide input to the developing information set that includes results from the genetic analyses and the radio telemetry study. Detailed annual project operational plans will be prepared and circulated to TWG members by April 30 of 2013 and 2014. These plans will

establish additional details for field sampling efforts, including relative priorities, and temporal and spatial sampling considerations, beyond those presented in this document.

9.14.3. Study Area

The study area encompasses the Susitna River and its tributaries from Cook Inlet upstream to the Oshetna River confluence (river mile [RM] 233.4). For baseline data related to stock-specific sampling, there is an emphasis on tributaries of the Middle River and the Upper River. For assessing habitat use (juveniles) of fish originating in the Middle and Upper River, Chinook salmon tissues from juveniles will be collected in the Lower River (< RM 98).

9.14.4. Study Methods

9.1.1.3. Samples to Collect

The annual targets for data collection to meet the study objectives are indicated below. The sample sizes associated with each collection listed below represent a target rather than a sample size requirement because the abundance of each species or sub-stock is currently unknown. For baselines used for stock composition estimates, we used a sample size of 100 fish per population (Allendorf and Phelps 1981; Waples 1990). For mixtures used in stock composition estimates, we used a sample size of 200 fish or 100 fish per collection to provide stock composition estimates that are within 7% and 10%, of the true estimate 95% of the time, respectively (Thompson 1987). Sample sizes of 50 fish for other species are adequate to estimate allele frequencies for coarse-scale genetic population structure (Nei 1978). Details regarding the fish tissue collection efforts have been refined and are presented in the FERC approved Genetics Implementation Plan (IP). The descriptions in the Genetics IP supercede those bulleted below.

- Collect tissue samples to obtain samples from at least 100 (total archived and new samples) spawning Chinook salmon from any Susitna River tributary with evidence of Chinook spawning (Table 9.14-1; Objectives 1, 2,and 3).
- Collect tissue samples to obtain samples from at least 100 (total archived and new samples) spawning Chinook salmon from flanking region (Knik Arm and northwestern Cook Inlet) tributaries with evidence of Chinook spawning (Table 9.14-1; Objectives 1 and 2).
- Collect 100 tissue samples from each spawning aggregate of pink, sockeye, chum, and coho salmon from the Susitna River upstream of the Three Rivers Confluence (Objective 1 and 2).
- Collect 200 tissue samples from juvenile Chinook salmon at each of the following: Chinook Creek, Oshetna River, Indian River, Portage Creek, the mainstem Susitna River upstream of the Three Rivers Confluence, as well as Talkeetna and Chulitna rivers (1,400 fish; Objectives 1, 2, 3 and 4.
- Collect 100 juvenile Chinook salmon from 16 sites across five mainstem habitat types in the Lower Susitna River (1,600 fish; Objective 3).
- Collect 50 representative samples from each of the species listed in the Table 9.14-2, with an emphasis on fish collected in the Middle and Upper Susitna River (Objective 1.

The Project scope includes a dedicated team of two people and logistics support for two months each year to collect samples. The efforts by this study team will be in addition to the sample

collection efforts that will be done by the study teams associated with fish distribution in the Lower, Middle, and Upper River (Sections 9.5 and 9.6) and Salmon Escapement (Section 9.7).

9.1.1.4. Tissue Storage

While in the field, tissue samples will be preserved in ethyl alcohol in a 125–500 milliliter (ml) bulk sample bottle for each location. After samples are received by the Gene Conservation Laboratory (GCL), samples will be preserved as follows: At least five pieces of each sample will be placed into plastic plates and freeze-dried. Once dry, moisture-indicating desiccant beads will be added and the plate sealed completely with aluminum foil heat-activated tape. Tissue samples will then be stored at room temperature.

9.1.1.5. Laboratory Analysis

DNA from the baseline collections will be extracted from axillary processes using DNeasy 96 tissue kits. Chinook salmon samples will be analyzed for at least 96 single nucleotide polymorphism (SNP) markers and 13 microsatellite markers.

The DNA samples will be analyzed using Fluidigm 96.96 Dynamic Arrays (http://www.fluidigm.com). The Fluidigm 96.96 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame. On one side of the frame there are 96 inlets to accept the sample DNA from each individual fish and on the other are 96 inlets to accept the assays for each SNP marker. Once in the wells, the components are pressurized into the chip using the IFC Controller HX (Fluidigm). The 96 samples and 96 assays are then systematically combined into 9,216 parallel reactions. Each reaction is a mixture of 4 microliters (µl) of assay mix (1x DA Assay Loading Buffer [Fluidigm], 10x TaqMan SNP Genotyping Assay [Applied Biosystems], and 2.5x ROX [Invitrogen]) and 5 µl of sample mix (1x TaqMan Universal Buffer [Applied Biosystems], 0.05x AmpliTaq Gold DNA Polymerase [Applied Biosystems], 1x GT Sample Loading Reagent [Fluidigm], and 60-400ng/ul DNA) combined in a 6.7 nanoliter (nL) chamber. Thermal cycling is performed on an Eppendorf IFC Thermal Cycler as follows: an initial "hot mix" of 30 minutes at 70°C, and then denaturation of 10 minutes at 96°C followed by 40 cycles of 96°C for 15 seconds and 60°C for 1 minute. The Dynamic Arrays are read on a BioMark Real-Time PCR System (Fluidigm) after amplification and scored using Fluidigm SNP Genotyping Analysis software.

For some SNP markers, genotyping will be performed in 384-well reaction plates. Each reaction is conducted in a 5 µL volume consisting of 5–40 ng of template DNA, 1x TaqMan Universal PCR Master Mix (Applied Biosystems), and 1x TaqMan SNP Genotyping Assay (Applied Biosystems). Thermal cycling is performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) as follows: an initial denaturation of 10 minutes at 95°C followed by 50 cycles of 92°C for 1 second and annealing/extension temperature for 1.0 or 1.5 minutes. The plates are scanned on an Applied Biosystems Prism 7900HT Sequence Detection System after amplification and scored using Applied Biosystems' Sequence Detection Software (SDS) version 2.2.

For microsatellite markers, samples will be assayed for DNA loci developed by the Genetic Analysis of Pacific Salmon group funded by the Pacific Salmon Commission for use in U.S.-Canada Treaty fisheries. Polymerase chain reaction (PCR) will be carried out in 10ul reaction volumes (10mM Tris-HCl, 50mM KCl, 0.2 mM each dNTP, 0.5 units Taq DNA polymerase

(Promega, Madison, WI)) using an Applied Biosystems (AB, Foster City CA) thermocycler. Primer concentrations, MgCl concentrations and the corresponding annealing temperature for each primer are available upon request. PCR Fragment analysis will be done on an AB 3730 capillary DNA sequencer. 0.5ul PCR product will be loaded into a 96 well reaction plate along with 0.5ul of GS500LIZ (AB) internal lane size standard and 9.0ul of Hi-Di (AB). PCR bands will be visualized and separated into bin sets using AB GeneMapper software v4.0.

All genotypes collected will be entered into the GCL Oracle database, LOKI. Quality control measures include re-extraction and re-analysis of 8 percent of each collection for all markers to ensure that genotypes are reproducible and to identify laboratory errors and rates of inconsistencies. Genotypes are assigned to individuals using a double-scoring system.

9.1.1.6. Data Retrieval and Quality Control

Genotypes will be retrieved from LOKI and imported into *R* (R Development Core Team 2011) with the *RODBC* package (Ripley 2010). All subsequent analyses will be performed in *R*, unless otherwise noted.

Prior to statistical analysis, three analyses will be performed to confirm the quality of the data. First, SNP markers will be identified that are invariant in all individuals or that have very few individuals with the alternate allele in only one collection. These markers will be excluded from further statistical analyses. Second, individuals will be identified that are missing substantial genotypic data because they likely have poor quality DNA. Individuals missing substantial genotypic data will be identified using the 80 percent rule (missing data at 20 percent or more of loci; Dann et al. 2009). These individuals will be removed from further analyses. The inclusion of individuals with poor quality DNA might introduce genotyping errors into the baseline and reduce the accuracies of mixed stock analyses.

The final QC 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 alleles in 95 percent of screened loci. The sample with the most missing genotypic data from each duplicate pair will be removed from further analyses. If both samples have the same amount of genotypic data, the first sample will be removed from further analyses.

9.1.1.7. Genetic Baseline Development

9.1.1.7.1. Hardy-Weinberg Expectations

For each locus within each collection, tests for conformance to Hardy-Weinberg expectations (HWE) will be performed using Monte Carlo simulation with 10,000 iterations in the *Adegenet* package (Jombart 2008). Probabilities will be combined for each collection across loci and for each locus across collections using Fisher's method (Sokal and Rohlf 1995), and collections and loci that violated HWE will be excluded from subsequent analyses after correcting for multiple tests with Bonferroni's method ($\alpha = 0.05$ per number of collections).

9.1.1.7.2. Pooling Collections into Populations

When appropriate, collections will be pooled to obtain better estimates of allele frequencies following a step-wise protocol. First, collections from the same geographic location, sampled at

similar calendar dates but in different years, will be pooled, as suggested by Waples (1990). Then differences in allele frequencies between pairs of geographically proximate collections that were collected at similar calendar dates and that might represent the same population will be tested. Collections will be defined as being "geographically proximate" if they were collected within the same river. Fisher's exact test (Sokal and Rohlf 1995) of allele frequency homogeneity will be used, and decisions will be based on a summary across loci using Fisher's method. Collections will be pooled when tests indicate no difference between collections ($P > 0.01$). When all individual collections within a pooled collection are geographically proximate to other collections, the same protocol will be followed until significant differences are found between the pairs of collections being tested. After this pooling protocol, these final collections will be considered to be populations. Finally, populations will be tested for conformance to HWE following the same protocol described above to ensure that pooling was appropriate, and that tests for linkage disequilibrium will not result in falsely positive results due to departure from HWE.

9.1.1.7.3. Linkage Disequilibrium

Linkage disequilibrium between each pair of nuclear markers will be tested for in each population to ensure that subsequent analyses are based on independent markers. The program *Genepop* version 4.0.11 (Rousset 2008) will be used with 100 batches of 5,000 iterations for these tests. The frequency of significant linkage disequilibrium between pairs of SNPs ($P < 0.05$) will then be summarized. Pairs will be considered linked if they exhibited linkage in more than half of all populations.

9.1.1.8. Analysis of Genetic Structure

9.1.1.8.1. Temporal Variation

Temporal variation of allele frequencies will be examined with a hierarchical, three-level analysis of variance (ANOVA). Temporal samples will be treated as sub-populations based on the method described in Weir (1996). This method will allow for the quantification of the sources of total allelic variation and permit the calculation of the among-years component of variance and the assessment of its magnitude relative to the among-population component of variance. This analysis will be conducted using the software package *GDA* (Lewis and Zaykin 2001).

9.1.1.8.2. Hierarchical Log-likelihood Tests

Genetic diversity will be examined with a hierarchical log-likelihood ratio (G) analysis.

9.1.1.8.3. Visualization of Genetic Distances

To visualize genetic distances among collections, two approaches will be used. Both approaches are based on pairwise F_{ST} estimates from the final set of independent markers with the package *hierfstat* (Goudet 2006). The first approach is to construct 1,000 bootstrapped neighbor-joining (NJ) trees by resampling loci with replacement to assess the stability of tree nodes. The consensus tree will be plotted with the *APE* package (Paradis et al. 2004). While these trees provide insight into the variability of the genetic structure of collections, pairwise distances visualized in three dimensions are more intuitive. In a second approach, pairwise F_{ST} will be

plotted in a multidimensional scaling (MDS) plot using the package *rgl* (Adler and Murdoch 2010).

9.1.1.8.4. Estimating Effective Population Size

Effective population size will be measured using single-year sample (Tallmon et al. 2008), multiple-year sample (Waples 1991, Tallmon et al. 2004), linkage disequilibrium (Waples 2006), and heterozygote excess (Luikart and Cornuet 1999) methods. Effective population size estimates will be compared with estimates of the numbers of fish acending Devils Canyon based on tagging data to measure genetic success rate of fish acending the Canyon.

9.1.1.9. Habitat Utilization in the Lower River by Chinook Salmon Progeny Originating in the Middle and Upper Susitna River

If the results of the Chinook salmon genetics studies conducted during 2012 are sufficient to indicate that the Chinook salmon spawning upstream of Devils Canyon and in the Middle River and its tributaries are sufficiently unique, ADF&G will characterize the presence and relative proportion of fish originating from the Upper and Middle River in selected Lower River habitats.

In both 2013 and 2014, 75 juvenile Chinook salmon from each of 16 mainstem locations (across five habitat types) will be collected and preserved as outlined above. These 1,200 tissue samples collected in each year will be analyzed and the results will be pooled into a range of spatial strata to identify any Middle and Upper River fish, and where feasible, estimate the proportion of fish originating from upstream of the Three Rivers Confluence (RM 98).

9.14.5. Consistency with Generally Accepted Scientific Practice

Each method described above employs scientifically accepted principles as noted by regular citations of peer reviewed methods, where they are presented. The laboratory and analytical methods to be used for this study are widely applied in North America and Asia to characterize the origin and genetic variation in salmonid and non-salmonid fish species. ADF&G's Gene Conservation Laboratory (GCL) located in Anchorage, Alaska, is on the leading edge of applied fish genetics, and it has a long history of publishing techniques and results from its studies in the peer-reviewed literature. GCL personnel serve on many multi-national scientific work groups from around the Pacific Rim.

9.14.6. Schedule

- Baseline sample collection: June through October 2013 and 2014 (in conjunction with other AEA field studies) (see Table 9.14-3).
- Mixture sample collection from the Lower River: June through August 2013 and 2014.
- Analysis of juvenile and adult Chinook salmon tissue: November 2013 through December 2014.
- Initial and Updated Study Reports explaining actions taken and data collected to date will be issued within 1 and 2 years, respectively, of FERC's Study Plan Determination (i.e., February 1, 2013).
- An implementation plan (IP) will be developed prior to study implementation each year. The draft IP will be provided to NMFS and the USFWS each year by March 30 for a 15-

day review. The final IP will be filed with FERC each year by April 30, and will include documentation of agency consultations and how agency review comments were treated in the final IP.

9.14.7. Relationship with Other Studies

The Genetic Baseline Study for Selected Fish Species will interrelate with six other AEA Project studies (Figure 9.14-1). Four studies will provide input as predecessor studies and two that receive output from the genetic study. The four predecessor studies each provide "information" input in the form of fish tissue samples and observations to help meet the sample sizes specified in the design of the fish genetic study. Coordination among studies will be critical to meet the objectives of this study, and the predecessor studies and successor studies. The four predecessor studies providing these samples or information to help collect these samples by the dedicated sampling team are: the Upper River Fish Distribution Study (Section 9.5), the Middle and Lower River Fish Distribution Study (Section 9.6), the Salmon Escapement Study (Section 9.7), and the Eulachon Study (Section 9.16). Output from the fish genetic study will include information on the genetic structure of Chinook salmon; depending on the results, this information may be useful to the Middle and Lower River Fish Distribution Study (Section 9.6), the Salmon Escapement Study (Section 9.7) and the Fish Passage Feasibility (Section 9.11).

9.14.8. Level of Effort and Cost

The total estimate for the cost of the study over two years is approximately $900,000. The estimated cost for each of the four study objectives described above is as follows:

1) $50,000 annually
2) $170,000 annually
3) $150,000 annually
4) $80,000 annually

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9.14.10. Tables

Table 9.14-1. Area, location, and sublocation of desired baseline samples of adult Chinook salmon spawning aggregates for genetic analysis. Samples (Total) and sample years for collections in the Gene Conservation Laboratory archives, desired remaining nmber (Need), and number slated for genetic analysis (To analyze) and indicated. Some systems listed may not have spawning stocks in them, including some of those noted from above Devils Canyon.

Location	Sublocation	Year(s) Collected	Total	Need	To analyze
West Cook Inlet					
Chuitna River		2008, 2009	142	0	96
Beluga River	Coal Creek	2009, 2010, 2011	120	0	96
Theodore River		2010, 2011, 2012	189	0	96
Lewis River		2011, 2012	86	10	96
Yentna Drainage					
Clearwater Creek		2012	25	71	96
Red Creek		2012	29	67	96
Happy River		2012	19	77	96
Red Salmon Creek		2012	12	84	96
Hayes River tributary		2012	5	91	96
Canyon Creek		2012	32	64	96
Talachulitna River		1995, 2008, 2010	180	0	96
Lake Creek	Sunflower Creek	2009, 2011	127	0	96
Kahiltna River	Peters Creek	2009, 2010, 2011, 2012	110	0	96
Susitna Drainage					
Chulitna River	East Fork	2009, 2010, 2011	13	83	96
	Middle Fork	2009, 2010	169	0	96
	Honolulu Creek			96	96
	Byers Creek			96	96
	Troublesome Creek			96	96
	Spink Creek			96	96
	Tokositna River (Bunco Creek)			96	96
Indian River		2012	1	95	96
Portage Creek		2009, 2010, 2011	141	0	96
Susitna above Devils Canyon	Kosina Creek	2012	10	86	96
	Chinook Creek			96	96
	Fog Creek			96	96
	Tsusena Creek			96	96
	Watana Creek			96	96
	Devils Creek			96	96
	Oshetna River			96	96
Talkeetna River	Upper mainstem			96	96
	Stephan Lake weir	2008	19	77	96
	Prairie Creek	1995, 2008	150	0	96
	Disappointment Creek			96	96
	Larson Creek			96	96
	Chunilna Creek (Clear Creek)	2009, 2012	130	0	96
Birch Creek				96	96
Montana Creek		2008, 2009, 2010	218	0	96
Sheep Creek				96	96

Location	Sublocation	Year(s) Collected	Total	Need	To analyze
North Fork Kashwitna				96	96
Little Willow Creek				96	96
Willow Creek		1991,1997, 2005, 2009	309	0	96
Deshka River	Moose Creek	1995, 2012	103	0	96
	Deshka River weir	2005	200	0	96
Alexander Creek	Sucker Creek	2011, 2012	143	0	96
Knik Arm					
Matanuska River	Kings River			96	96
	Granite Creek			96	96
	Moose Creek	1995, 2008, 2009, 2012	155	0	96
Eagle River	South Fork	2009, 2011, 2012	73	23	96
	Meadow Creek	2009	6	90	96
Ship Creek		2009	311	0	96
Little Susitna River		2009, 2010	125	0	96
Total				2838	4896

Table 9.14-2. Potential Susitna River fish species for targeted for genetic analysis sampling.

Common Name	Scientific Name
rainbow trout	*Oncorhynchus mykiss*
humpback whitefish	*Coregonus pidschian*
round whitefish	*Prosopium cylindraceum*
lake whitefish	*Coregonus clupeaformes*
Alaska whitefish	*Coregonus nelsonii*
Bering cisco	*Coregonus laurettae*
eulachon	*Thaleichthys pacificus*
Pacific lamprey	*Lampetra tridentata*
longnose sucker	*Catostomus catostomus*
slimy sculpin	*Cottus cognatus*
prickly sculpin	*Cottus asper*
coastal range sculpin	*Cottus aleuticus*
Pacific staghorn sculpin	*Leptocuttus armatus*
Burbot	*Lota lota*
Arctic grayling	*Thymallus arcticus*
Dolly Varden	*Salvelinus malma*
lake trout	*Salvelinus namaycush*
northern pike	*Esox lucius*
threespine stickleback	*Gasterosteus aculeatus*
ninespine stickleback	*Pungitius pungitius*
Alaska blackfish	*Dallia pectoralis*

Table 9.14-3. Preliminary schedule for the Genetic Baseline Study for Selected Fish Species.

Activity	2012				2013				2014				2015
	1 Q	2 Q	3 Q	4 Q	1 Q	2 Q	3 Q	4 Q	1 Q	2 Q	3 Q	4 Q	1 Q
Implementation Plan development					——				——				
Sample Collection						——	——	——		——	——	——	
Initial Study Report								——	Δ				
Sample analysis								——	——	——	——		
Updated Study Report												——	▲

Legend:

——Planned Activity
----- Follow-up activity (as needed)
Δ Initial Study Report
▲ Updated Study Report

9.14.11. Figures

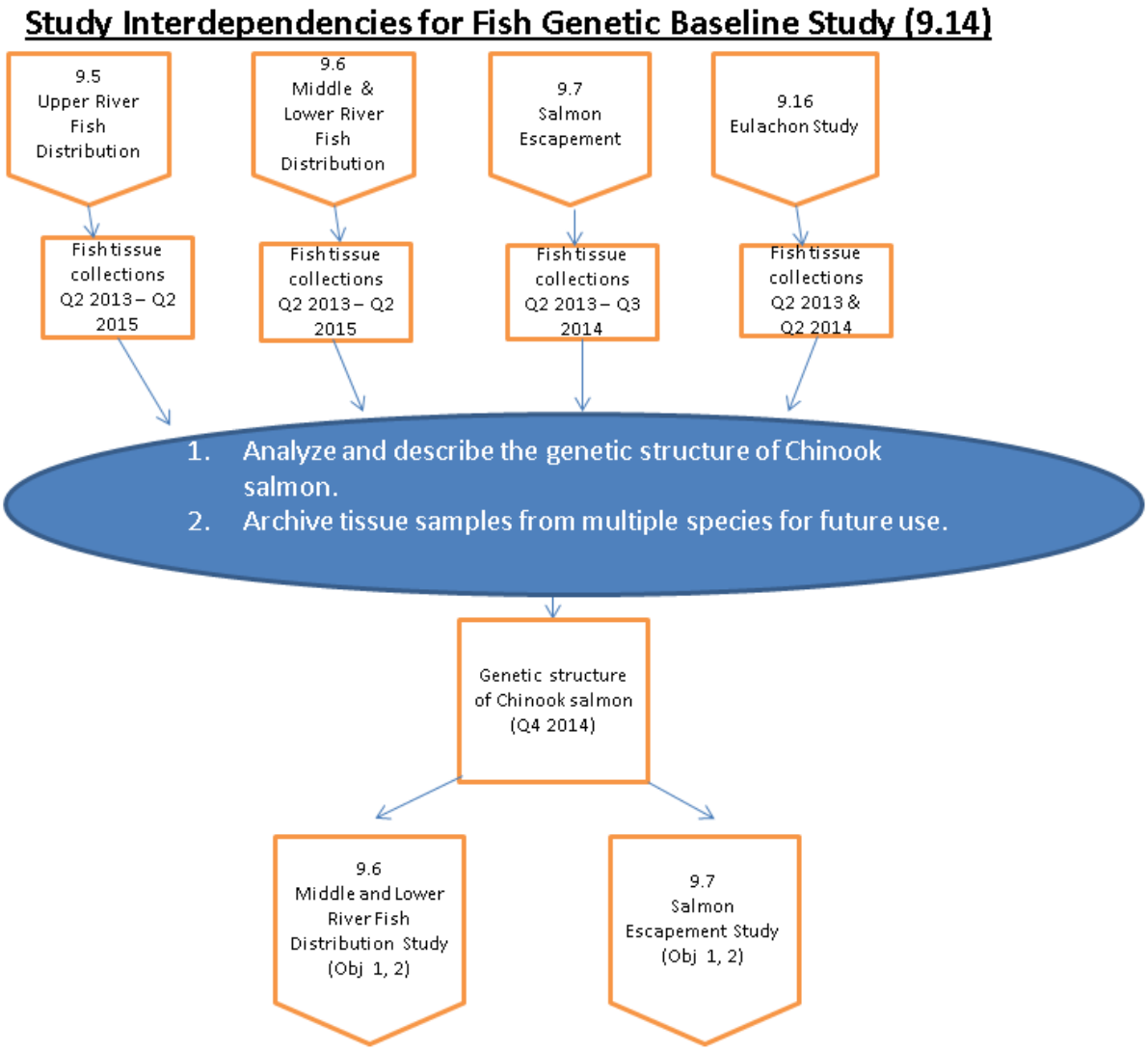

Figure 9.14-1. Study interdependencies for the Genetic Baseline Study for Selected Fish Species.