Susitna-Watana Hydroelectric Project Document ARLIS Uniform Cover Page

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SUSITNA-WATANA HYDRO

ALASKA ENERGY AUTHORITY

April 30, 2013

Ms. Kimberly D. Bose
Secretary
Federal Energy Regulatory Commission
888 First Street, NE
Washington, DC 20426

Re: Susitna-Watana Hydroelectric Project, FERC Project No. 14241-000; Submission of Final 2013 Project Operational Plan for Fish Genetic Baseline Study (Study 9.14)

Dear Ms. Bose:

On February 1, 2013, the Federal Energy Regulatory Commission (Commission) issued its Study Plan Determination (February 1 SPD) for 44 of the 58 proposed individual studies in the Alaska Energy Authority's (AEA) Revised Study Plan (RSP) for the Susitna-Watana Hydroelectric Project, FERC Project No. 14241 (Project).[1] With regard to the Fish Genetic Baseline Study (Study 9.14), AEA proposed to develop and circulate to Technical Workgroup (TWG) members by April 30 of 2013 and 2014 detailed annual project operational plans. These operational plans are to establish additional details for field sampling efforts, including specific temporal and spatial sampling locations, to enhance the general locations for target sample collection presented in the RSP.

When approving the Fish Genetic Baseline Study, the Commission's February 1 SPD recommended that AEA consult with the U.S. Fish and Wildlife Service and National Marine Fisheries Service (collectively, Services); develop a draft project operational plan for a 15-day review and comment period by the Services; and file a final plan with the Commission by April 30.[2] The February 1 SPD provided that AEA include in its submission of the final plan "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."[3]

AEA has completed this process, and the final 2013 project operational plan for Study 9.14 appears in Attachment A. Documentation of AEA's consultation with the Services, including the Services' written comments on the draft 2013 project operational

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1 Study Plan Determination for the Susitna-Watana Hydroelectric Project, Project No. 14241-000 (issued Feb. 1, 2013) [hereinafter, "SPD"].

2 *Id.*, Appendix B, at B-43.

3 *Id.*, Appendix B, at B-43 to B-44.

plan, appears in Attachment B. AEA's response to the Services' written comments appears in Attachment C.

AEA appreciates the Services' involvement in developing the final 2013 project operational plan and looks forward to their continuing involvement during the implementation of Study 9.14. Should you have questions concerning this submission, please contact me at wdyok@aidea.org or (907) 771-3955.

Sincerely,

Wayne Dyok
Project Manager
Alaska Energy Authority

Attachments

cc: Distribution List (w/o Attachments)

Attachment A

Final 2013 Project Operational Plan, Study 9.14

Susitna-Watana Hydroelectric Project

(FERC No. 14241)

Regional Operational Plan DF.#R.13-XX

Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska

Prepared for

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

Prepared by

Andrew W. Barclay
Alaska Department of Fish and Game

April 30, 2013

REGIONAL OPERATIONAL PLAN DF.#R.13-XX

IMPLEMENTATION PLAN FOR THE GENETIC BASELINE STUDY FOR SELECTED FISH SPECIES IN THE SUSITNA RIVER, ALASKA

by

Andrew W. Barclay

Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage

Alaska Department of Fish and Game
Division of Commercial Fisheries

April 2013

The Regional Operational Plan Series was established in 2012 to archive and provide public access to operational plans for fisheries projects of the Divisions of Commercial Fisheries and Sport Fish, as per joint-divisional Operational Planning Policy. Documents in this series are planning documents that may contain raw data, preliminary data analyses and results, and describe operational aspects of fisheries projects that may not actually be implemented. All documents in this series are subject to a technical review process and receive varying degrees of regional, divisional, and biometric approval, but do not generally receive editorial review. Results from the implementation of the operational plan described in this series may be subsequently finalized and published in a different department reporting series or in the formal literature. Please contact the author if you have any questions regarding the information provided in this plan. Regional Operational Plans are available on the Internet at: http://www.adfg.alaska.gov/sf/publications/

Andrew W. Barclay,
Alaska Department of Fish and Game, Division of Commercial Fisheries,
333 Raspberry Road, Anchorage, Alaska, 99518-1599

This document should be cited as:
Andrew W. Barclay. 2013. Susitna River Genetic Baseline Study. Alaska Department of Fish and Game, Regional Operational Plan ROP.DF#R.13-XX, Anchorage.

SIGNATURE/TITLE PAGE

Project Title:	Susitna River Genetic Baseline Study
Project leader(s):	Andrew W. Barclay Fishery Biologist III
Division, Region and Area	Commercial Fisheries, Region VI, Anchorage
Project Nomenclature:	FERC Project No. 14241; Alaska Energy Authority
Period Covered	April 1, 2013 – March 31, 2015
Field Dates:	May 1, 2013 – October 30, 2013 and 2014
Plan Type:	Category III

Approval

Title	Name	Signature	Date
Project leader	Andrew W. Barclay		
Biometrician	Jim Jasper		
Research Coordinator	Chris Habicht		
Principal Geneticist	William D. Templin		
Chief Fisheries Scientist	Eric C. Volk		

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SYMBOLS AND ABBREVIATIONS

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

1. PURPOSE

The Alaska Energy Authority (AEA) has proposed a hydroelectric project on the Susitna River, which would involve construction of a dam and reservoir at river mile (RM) 184, approximately 34 miles upstream of Devils Canyon (Figure 2). Construction and operation of the Susitna-Watana Hydroelectric Project (Project) will modify the flow, thermal, and sediment regimes of the Susitna River, which may alter the composition and distribution of fish populations.

Genetic analyses can be used in two different ways to assess potential Project impacts. First, genetic analyses can describe the current genetic relationships among fish populations. These relationships will be useful in determining relatedness and isolation of spawning aggregates in the watershed and will serve as baseline for assessing potential Project impacts by species both before and after construction of the Project. For example, to determine if fish above and below the proposed dam site part of a single population. Secondly, genetic analyses can be used as tool (genetic "tag") to identify population-of-origin for rearing fish sampled in locations and at times when multiple populations are mixed. For example, this tool can be used examine habitat used by juvenile Chinook salmon populations within the Susitna River drainage. Understanding of stock-specific habitat use will provide insights into potential effects of the Project on rearing areas distant from spawning locations. For this document, a population is defined as a group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member (Waples and Gaggiotti 2006).

The usefulness of genetics as a tag depends on the degree of genetic variation among populations of interest in the Susitna watershed. Genetic variation among populations is governed by migration, genetic drift (changes in allele frequencies within loci across generations due to sampling error), and natural selection (non-random process resulting from differential reproductive fitness among alleles). If breeding isolation (lack of migration) among populations occurs over sufficient time and population sizes are small enough, genetic drift will result in variation in allele frequencies at neutral loci (loci not under natural selection) among populations. Additionally, breeding isolation coupled with differential natural selection will result in variation in allele frequencies at loci under selection among populations even in the absence of genetic drift. These variations in allele frequencies at loci among populations (from either drift or natural selection) create naturally occurring genetic "tags" that can be used to identify individual spawning populations in mixtures of several populations.

This operational plan describes the first study necessary for the application of genetic information and methods to evaluate Project effects on fish in the Susitna River. It will begin by developing a repository of fish tissues from anadromous (defined in this document as Chinook, chum, coho, pink, and sockeye salmon) and resident (defined in this document as all other species) fishes. These tissue repositories will be used for future studies necessary to characterize the genetic legacy and variation for species and populations of interest. It is important to collect tissue samples before the Project begins to examine possible changes in population structure associated with the Project. The emphasis of tissue collection will be on samples representing the five species of Pacific salmon spawning within the Susitna River watershed. Chinook salmon are a species of particular interest because they are the only anadromous species known to pass the Devils Canyon impediments, beginning at ~ RM 150, and spawn in areas below and

above the proposed dam site. Understanding the population structure of Chinook salmon collected above and below Devils Canyon will therefore inform policymakers on the relatedness and isolation of spawning aggregates. Population structure of Chinook salmon will be measured within the set of individuals spawning above the canyon, among the groups of individuals spawning within the Susitna River watershed (with particular emphasis on the Middle River (~RM 98 – 184) and Upper River (>RM 184; Figure 2)), and in relationship to populations from nearby drainages in Upper Cook Inlet. Genetic information will be assessed for its utility as a tool to investigate whether juvenile Chinook salmon originating from the Middle and Upper River rear in the Lower River; if so, these fish in the Lower River must be added to assessments of Chinook salmon production upstream.

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 fish resources, as appropriate.

2. BACKGROUND

2.1. Existing Information and Need for Additional Information

The genetics samples collected during this study will be used to create a tissue repository for resident and anadromous fishes in Susitna River with particular emphasis on developing the genetic baseline for Susitna River salmon populations. Existing tissue collections and genetic analyses for resident species are limited within the Susitna River. There are few samples in the tissue archive from resident, non-salmon fish species, because these samples have only been collected opportunistically. Some genetic/phenotypic analyses have been completed on three-spine sticklebacks from the Matanuska/Susitna drainages (Cresko et al. 2004), but no population-structure analyses are available. Population analyses of Bering Cisco indicate that Susitna River supports a single population (Brown et al. 2012).

Tissue collections and genetic analyses of Pacific salmon stocks elsewhere in Alaska are relatively well developed and are used for applied research in several watersheds. The baseline genetic data currently available for the Susitna River is comprehensive only for sockeye salmon;: data for the other four species vary from moderate (Chinook salmon) to almost non-existent (pink salmon). Ten Chinook salmon were sampled in 2012 in Kosina Creek in the Upper Susitna River for genetic analysis.

Samples obtained in this study enable the application of genetic methods in the future to assess genetic relatedness and isolation of fishes in the watershed and can be used to help determine potential impacts from the Project. For example, interbreeding by resident fish among areas might be hindered by Project-imposed barriers, thereby potentially reducing the fitness of some stocks. Breeding isolation of stocks may be a sign of 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.

2.1.1. Assessing Chinook Salmon Population Structure

In 2012, some adult Chinook salmon ascended and remained above Devils Canyon during the spawning season. This observation led to questions about whether these fish 1) represent a self-sustaining, genetically isolated, and potentially locally-adapted population (Hypothesis 1a; Figure 1), 2) are individuals originating from other geographic spawning aggregates below Devils Canyon (Hypothesis 2; e.g., Portage Creek), or 3) are individuals resulting from successful reproduction in the Upper River but with a high level of introgression from other geographic spawning aggregates below Devils Canyon (Hypothesis 1b). Identifying Chinook salmon originating from above Devils Canyon in mixtures of fish from throughout the Susitna River drainage will only be possible if these fish represent a self-sustaining population with little gene flow from populations below the canyon (Hypothesis 1a; Figure 1).

Genetic analysis can help to distinguish among these hypotheses (e.g. Waples and Gaggiotti 2006). Given the small numbers of Chinook salmon that are thought to spawn above Devils Canyon, genetic drift is expected to be the dominant mechanism for changes in allele frequencies through time. If gene flow exists, it is likely to go from large populations below the canyon to the small population(s) above the canyon, just based on demographics.

High genetic divergence between fish spawning above Devils Canyon and fish spawning in aggregates below the canyon could indicate either a self-sustaining population above the canyon with little gene flow with other populations (Hypothesis 1a), or recent colonization by small numbers of successfully-contributing families (Hypothesis 1b). A recent colonization by a small number of successfully-contributing families, along with high gene flow from straying fish each generation (Hypothesis 1b), might also be interpreted as an indication of a self-sustaining spawning aggregate (Hypothesis 1a) with data from only 1 or 2 years. The stability of allele frequencies across years (cohorts) will provide a means to distinguish between these two hypotheses (1a and 1b). Assessing stability in allele frequencies across years will need to account for effective population sizes (Waples and Teel 1990). In addition to temporally stable allele frequencies, conformance to HWE would also add support for Hypothesis 1a. Conversely, a lack of temporal stability of allele frequencies and lack of conformance to HWE would support Hypotheses 1b or 2,

On the other hand, low genetic divergence between fish spawning above Devils Canyon and fish spawning in aggregates below the canyon would indicate that a large proportion of the fish ascending Devils Canyon are strays or colonizers, and have not established 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 with samples from three return years if the samples do not represent fish from multiple cohorts and/or if the "signal" is weak, even if a large number of fish can be sampled in locations above and below Devils Canyon.

Sampling across three years (2012-14) to assess temporal stability in allele frequencies from fish above Devils Canyon may limit the ability to conclusively distinguish among Hypothesis 1a, 1b, and 2. The statistical power to detect temporal stability of allele frequencies and conformance to HWE is only possible with adequate numbers of samples obtained over multiple years and across cohorts of returning salmon. The adequacy of sample sizes across years depends on the amount

of genetic variation in the population. A small sample size may be adequate to detect large genetic deviation from populations below Devils Canyon or high inter-annual variation in samples from each area, but large sample sizes will be required to detect small genetic deviations. Samples from three calendar years may represent Chinook salmon from as many as 5 or 6 brood years given the multiple ages of maturity in any given year. If large numbers of fish can be sampled in each of the remaining calendar years (2013 and 2014), it may be possible to detect instability in allele frequencies if instability exists (some support for Hypothesis 1a). In summary, the degree of genetic divergence between fish sampled from above and below Devils Canyon and the stability of allele frequencies across years from 2012–2014 will dictate the level of support for the existence of a self-sustaining, genetically isolated, and potentially locally-adapted populations.

2.1.2. Approach to Study Design and Implementation for Chinook Salmon Above Devils Canyon

The ability to determine the level of genetic divergence of Chinook salmon captured above relative to below Devils Canyon will be a function of the following:

- Numbers of fish passing through the canyon in 2013 and 2014.
- The ages of fish sampled for genetics.
- The degree of underlying genetic divergence between fish captured above and below Devils Canyon.
- Temporal stability of allele frequencies within populations.
- Genetics baseline information on any spawning aggregates not currently included in the baseline.

Given that this information is currently unknown, we propose 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 the type and number of samples to analyze. This Operational Plan describes dedicated sampling effort by field crews for 4 months each year during the spawning period of adult salmon, sufficient to collect tissue samples over a representative proportion of the entire run of each salmon species. Additional samples will be collected from other studies, as described Sections 9.5, 9.6, and 9.7 of the Revised Study Plan (RSP).

To ensure that data sources (and hypotheses) are rigorously examined, AEA will work closely with geneticists from State and Federal (NOAA and FWS) genetics laboratories. ADF&G's Gene Conservation Laboratory (GCL) will be contracted to do the study. Collaboration with Federal agencies will occur through regular updates to the quarterly Technical Working Group (TWG) meetings in 2013 and 2014. A draft of this Implementation Plan was provided to the USFWS and NOAA on 31 March 2013 for their input prior to filing the plan with FERC. Input from these federal agencies has been addressed in this final Implementation Plan for 2013.

An updated, detailed annual Implementation Plan will be prepared and circulated to TWG members by April 30 of 2014. The 2014 Genetics Implementation Plan will establish details for field sampling efforts (including relative priorities, and temporal and spatial sampling considerations, that take into account the experience from the 2013 field season) and statistical

analysis methods that take into account the success of sampling from the 2013 field season. FERC's February 1, 2013 recommendations, which were based on agency consultations and comments on the RSP are documented, evaluated, and addressed in Table 1 and throughout this Operational Plan.

2.2. Study Area

The study area encompasses the Susitna River and its tributaries from Cook Inlet upstream to the Oshetna River confluence (RM 233.4; Figure 2). For baseline data related to stock-specific sampling, there is an emphasis on tributaries of the Middle and the Upper Susitna River. For assessing habitat use (juveniles) of fish originating from the Middle (RM 98 – 184) and Upper Susitna River (RM 184 – 233.4), tissue from juvenile Chinook salmon will be collected in the Lower River (< RM 98).

3. 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 target resident fish species captured within the Lower, Middle, and Upper Susitna River drainage.
2. Contribute to the development of genetic baselines for chum, coho, pink, and sockeye salmon spawning in the Middle and Upper Susitna River drainage.
3. Characterize the genetic population structure of Chinook salmon from Upper Cook Inlet, with emphasis on spawning aggregates in the Middle and Upper Susitna River.
4. Examine the genetic variation among Chinook salmon populations from the Susitna River drainage, with emphasis on Middle and Upper Susitna River populations, for use in mixed-stock analyses (MSA).
5. If sufficient genetic variation is found for MSA, 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.

4. METHODS

4.1. Survey Flights

Prior to sample collection trips, aerial surveys will be conducted to determine presence and assess relative abundance of adult salmon at potential sampling locations (Tables 2–6). Chinook salmon in upper Cook Inlet generally reach spawning grounds between mid-July and early-August. Each year, survey flights in the Susitna River drainage above the Yentna River confluence (Susitna River) will begin the first week of July and continue through September.

During the 3 week period of July 15 – August 4, when Chinook salmon are usually on their spawning grounds, additional weekly survey flights will be conducted in the Yentna River drainage. When conditions allow, Susitna River survey flights will be conducted Monday of each week and Yentna River survey flights on Tuesday of each week. Populations sampled elsewhere in Cook Inlet (see Purpose section, above) will be surveyed from the road system or by separate studies conducted by ADF&G Sport Fish Division.

During survey flights, GPS waypoints will record locations where salmon are present along with indication of the number of each species observed. In addition, survey flights will be used to determine potential access to sampling locations (e.g., helicopter, fixed-wing, ATV, boat, etc.). Information from the survey flights will be recorded in the ADF&G Gene Conservation Laboratory (GCL) Oracle database, LOKI, and will be used inseason to determine locations and logistics for directing sampling crew efforts.

4.2. Samples to Collect

Ideal sample size for baseline collections to investigate population structure using genetic markers is affected by many variables including the generating process, whether the populations are in equilibrium or not, and the number of markers and alleles associated with them (Landguth et al. 2010). The upper end of an adequate sample size is 500 individuals, but some researchers have proposed as few as 20 to 30 individuals (Hale et al. 2012). With information on some of these variables, a simulation program is available to assess the statistical power of different sample sizes (Ryman and Palm 2006). However, without the information on these variables, we cannot perform useful simulations so we propose an idealized sample size of 200 fish per population for markers with moderate numbers of alleles (i.e. uSATs), and an idealized sample size of 100 fish per population for markers with two alleles (i.e. SNPs). Small sample sizes of 50 fish per population may be adequate to conduct coarse-scale population structure analyses and MSA depending on the values of the variables listed above (Landguth et al. 2010; Hale et al. 2012). For mixed stock collections, sample sizes of 200 fish or 100 fish per collection are adequate to provide stock composition estimates that are within 7% or 10% of the true estimate 95% of the time, respectively (Thompson 1987).

A population is defined as a group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member (Waples and Gaggiotti 2006). Functionally, populations will be represented by single or pooled collections following the "Pooling Collections into Populations" methods below. Based on field sampling from previous years (Tables 2–6), information gathered from the Catalog of Waters Important for the Spawning, Rearing or Migration of Anadromous Fishes (http://www.adfg.alaska.gov/sf/SARR/AWC/), the Susitna Hydro Aquatic Studies (Thompson et al 1986), and talking with local biologists, we selected possible sites where fish of each target Pacific salmon species might be spawning. We provide a list of these sites with idealized sample sizes for each (Tables 2-6). We will make an intensive effort to collect these samples as outlined in the sections below. However, we are unlikely to obtain the idealized sample size for all of these sites due to uncontrolled variables (i.e., numbers of fish at a spawning location, number of fish returning in 2013 and 2014, access issues associated with weather conditions and mechanical problems, water conditions, and stream characteristics and fish behavior affecting the catchability of the fish). To reflect the uncertainty in sample collection success, we added a

column to Tables 2–6 labeled "Expected" that shows the number of fish we reasonably think can be sampled at each site (or group of sites) in two years, based or previous efforts (and results) and on and information from the aforementioned catalog and studies. The following sample collection targets apply only to collections targeted in this study. Some of these samples may be collected in other program studies, but sample sites that are not targeted in this study are not listed even if they are proposed to be sampled in other program studies for genetic tissues.

4.2.1. Sample collection targets

1. Collect tissue samples from 50 representative individuals from each of the resident fish species listed in Table 7, with an emphasis on fish collected in the Lower, Middle and Upper Susitna River (Objective 1).
2. Collect tissue samples from 100 individuals (total archived and new samples) from at least 3 spawning aggregates of pink, sockeye, chum, and coho salmon from each of the following drainages: 1) the Susitna River upstream of the Three Rivers Confluence (Middle Susitna River), 2) the Talkeetna River, and 3) the Chulitna River (Tables 3–6; Figures 3–6; Objective 2).
3. Collect sufficient tissue samples from Chinook salmon spawning in Knik Arm and northwestern Cook Inlet rivers so that at least 2 additional rivers in each region are represented in the baseline by up to 200 Chinook salmon (total archived and new samples) (Table 2; Objective 3).
4. Collect sufficient tissue samples from Chinook salmon spawning in Susitna River tributaries so that each tributary is represented in the baseline by at least 50, but ideally 200 Chinook salmon (total archived and new samples; Table 2; Figure 2; Objectives 3 and 4).
5. Collect tissue samples from a target of 200 juvenile Chinook salmon at each of the following: Cheechako Creek, Fog Creek, Kosina Creek, Oshetna River (Table 2; Objectives 3 and 4).
6. Collect tissue samples from 100 juvenile Chinook salmon from 16 sites across 5 mainstem habitat types in the Lower Susitna River (1,600 fish; Objective 5).

4.2.2. Adult Chinook salmon collections

Weekly survey flights will be conducted from June 8 to September 23 to determine the timing and locations for sampling. Sampling crews will be dispatched when and where Chinook salmon are observed over spawning habitat. The most intensive sampling of adult Chinook salmon will occur July 15 – August 4. Because Chinook salmon are generally spread out in streams and in lower abundance compared to other salmon species, multi-day sampling trips will be required to get an adequate sample from each location (Table 2; Figure 2). During this time period, each of the three sampling crews will attempt to collect samples from at least two locations per week with an average of 2.5 days per trip. The two extra days each week will allow crews to be relocated and resupplied with sampling gear, food, and other camping supplies, and acquire information from GCL staff for their next sampling location(s).

During the intensive Chinook salmon sampling period, two crews will be dedicated to sampling in the Susitna River and one crew will be dedicated for sampling the Yentna River and northwestern Cook Inlet. Additional GCL staff will collect Chinook salmon samples from

locations on the road system in the Susitna River and Knik Arm. Because of the large area to be sampled and short window of opportunity each year to collect Chinook salmon samples, crews in the Susitna River will have a helicopter (Robinson R-44 II; operated by Alpine Air Alaska, Inc.) on call for transport to and from sampling locations. Base of operations for the Alpine Air helicopter will depend on the areas where crews will be sampling and will be determined in season. The Yentna River crew will charter helicopter (Enstrom F28F) flights, as needed, through Talaheim Lodge, based on the Talachulitna River.

Chinook salmon will be captured using either hook-and-line, seines, gillnets, or dipnets depending on the size of the stream and where the fish are located. Upon capture, a single axillary process will be clipped from each Chinook salmon and placed in a bottle of ethyl alcohol for preservation (Appendix A1). For Chinook salmon sampled above Devils Canyon, additional paired samples/data will be collected including scales, length (mid-eye to fork, to nearest 5 mm), sex, and GPS information (decimal, to the nearest 0.001). Therefore, for these fish, axillary process and 5 scale samples will be sampled into individually-labeled vials. Scales will be sampled at a point along the diagonal line from the posterior insertion of the dorsal fin to the anterior insertion of the anal fish, 2 rows above the lateral line. Length, sex and GPS information will be recorded on write-in-the-rain notebooks paired with the vial identifier. Fish will be held in the water as much as possible while hooks are removed and samples are collected, and released immediately after the sample has been placed in the bottle. If necessary, crews will hold the fish in the water to make sure they can swim before releasing them.

Chinook salmon collections will not be limited to the three-week intensive sampling period and may occur as early as the first week of July and as late as the last week of August. In addition to sampling adult Chinook salmon on these trips, crews may opportunistically collect samples from juvenile Chinook salmon, other salmon species, and other fish species (Table 7). Collection trips before and after the three-week intensive sampling period will be performed by two crews, but trip lengths will be longer (approximately 4 days – one trip per crew per week) due to the lower anticipated availability of helicopter charters. We will charter helicopter (Enstrom F28F) flights, as needed, through Talaheim Lodge, mainly to access sites above Devils Canyon and use a jet boat mainly to access sites below Devils Canyon in the Upper and Middle Susitna River.

4.2.3. Other adult salmon collections

Collections from adult pink, sockeye, chum, and coho salmon will begin in late July and continue through the end of the field season in late-September. During the Chinook salmon collection period, collections from these species will be conducted by the 2 Susitna River crews on an opportunistic basis. After August 4th, each of the 3 sampling crews will be assigned to one of the following drainages to collect samples from at least 3 locations for each species: 1) the Middle and Upper Susitna River, 2) the Talkeetna River, and 3) the Chulitna River. Collection locations and method of transport to sampling locations will be determined after weekly survey flights (Tables 2–6; Figures 3–6). Capture and sampling of salmon will follow methods used for adult Chinook salmon.

Previously documented spawning time periods for each species in the Middle Susitna River, indicated below, will be used as the general time periods for sampling trips (Thompson et al. 1986).

- Pink salmon – last week of July to third week of August
- Chum salmon – late-August to mid-September
- Sockeye salmon – late-August to mid-September
- Coho salmon – late-August to late-September

4.2.4. Juvenile Chinook salmon collections

4.2.4.1. Above Devils Canyon

Tissue samples from a target (ideal) of 200 juvenile Chinook salmon will be collected at each of the following: Cheechako Creek, Fog Creek, Kosina Creek, and Oshetna River. We expect to collect fewer than the ideal sample size per site (Table 2). When possible, these collections will occur at the same time as adult salmon collection trips.

Methods for capturing juvenile Chinook salmon in minnow traps and seines follow those suggested by Magnus et al. (2006). Cured salmon roe will be used as bait and several minnow traps will be set at each location. Minnow traps will be checked at least once a day.

Pelvic fin tissue will be collected from each juvenile Chinook salmon captured and place in an individual 2ml vial (Appendix A2). Samples will be taken from the same side of each fish to help prevent resampling of individuals. Total length (snout-to-fork) will be recorded for each sampled juvenile.

4.2.4.2. Lower River collections

Samples of juvenile Chinook salmon collected in the Lower River will be classified by habitat type to examine the potential for stock-specific variation in habitat type use. Habitat classifications will either follow those proposed in Study 9.9 (see Table 9.9-4 of the RSP), or those used by Murphy et al. 1989; main channels, backwaters, braids, channel edges, and sloughs). At least 3 locations will be sampled for each habitat type over the 2-year study period. Crews will begin juvenile collections as early as the first week of May and continue through early-July. Additional collections may occur between mid-August and the end of September to meet the yearly sampling goal. Sampling locations will be determined each year and will be accessed by river boat.

Juvenile Chinook salmon in the Lower River will be captured using the same methods as described for the juvenile Chinook collections above the Three Rivers Confluence. Minnow traps will be checked at least once a day and will be reset until the sampling objective (100 samples per location) has been met or few new fish are captured between checks. If the sampling objective cannot be met at a location, a new one will be selected.

Tissue samples will be collected using the same methods as described for the juvenile Chinook collections above the Three Rivers Confluence.

4.2.4.3. Species identification of juvenile collections

Species identification will be performed in the field using phenotypic characteristics (i.e. Pollard et al. 1997). A subset of juvenile putative Chinook salmon collected below Devils Canyon will

be selected during the season from each collection team and analyzed with DNA markers to verify correct field species identification. All Pacific salmon captured above Devils Canyon will be sampled and species will be identified in the field. Species identification using DNA will be confirmed post season.

4.2.5. Other species collections

Samples of resident fish species will be opportunistically collected while crews are collecting adult and juvenile salmon samples. Resident fish will be identified to genus or species with a field key and a picture will be taken. A small piece of fin tissue will be sampled from each fish and placed into a bottle or vial of ethyl alcohol for preservation (Appendix A1). Samplers will record on each bottle, or on datasheets for vial collections, which of the following areas the samples were collected: 1) Chulitna River, 2) Talkeetna River, 3) Upper Susitna River, 4) Middle Susitna River below Devils Canyon, and 5) Middle Susitna River above Devils Canyon. Tissues will be placed in separate bottles for each species and the area where they were collected.

4.2.6. Coordination with other Project studies

As described in the RSP, tissue samples will also be collected by four other studies conducted for the Project in 2013 and 2014: 9.5 (Upper River Fish Distribution), 9.6 (Middle and Lower River Fish Distribution), 9.7 (Salmon Escapement); and 9.16 (Eulachon Run Timing, Distribution, and Spawning). Sampling kits and collection protocols will be distributed to study leads in advance of the field season, and a weekly communication protocol will be developed to maximize collections. Collection progress will be updated using a database accessible to all study leads.

4.2.7. Collection trip documentation

Detailed notes will be kept during each collection trip and then entered into the trip report database in the GCL Oracle database, LOKI, when crews return to Anchorage. The information that will be recorded for each trip will be: 1) trip logistical information, 2) GPS waypoints where fish were collected, 3) number of fish and species collected at each location, 4) notes on other fish species present, 5) life stage of observed fish, 6) fish habitat information, and 7) recommendations for future collection trips. Collection trip records will be used postseason to submit Anadromous Waters Catalog nomination forms.

4.3. Tissue Storage

While in the field, tissue samples will be preserved in ethyl alcohol in either a 125–500 milliliter (ml) bulk sample bottle for each location or individual 2 ml vials (Appendices A1 and A2). After samples are received by the GCL, collection information will be recorded in LOKI. For long-term storage, samples will be preserved as follows: 1) sample will be placed into plastic plates and freeze-dried; 2) once dry, moisture-indicating desiccant beads will be added and the plate sealed completely with aluminum foil heat-activated tape; and 3) tissue samples will then be stored at room temperature.

4.4. Laboratory Analysis

DNA will be extracted from axillary processes using DNeasy 96 tissue kits. Two panels of SNP markers will be assayed: one to determine species identification for juvenile collections and the other to genotype Chinook salmon.

For juvenile Chinook salmon samples, species identification will be made by genotyping 5 single nucleotide polymorphism (SNP) markers (*OKESSA1-OKE, OTSSSA1-OTS, ONEOGO1-ONE, OKI1-OKI, OTSOKI1-OKI)* using Applied BioSystems' SNP Taqman assay analysis methods described below. These five markers differentiate between Pacific salmon species and rainbow trout. Positive controls for all species will be analyzed along with the unknown fish.

Both adult and juvenile Chinook salmon samples will be analyzed for 96 SNP markers and 12 microsatellite markers for population genetic structure or MSA.

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 (ul) of assay mix (1x DA Assay Loading Buffer [Fluidigm], 10x TaqMan SNP Genotyping Assay [Applied Biosystems], and 2.5x ROX [Invitrogen]) and 5 ul 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.

Scales from Chinook sampled above Devils Canyon will be mounted on gum cards at the GCL and impressions will be made in cellulose acetates and aged at the ADF&G, should age information be required.

4.5. 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, 4 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 third 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 individual 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.

The final QC analysis will identify individuals from the juvenile collections that appear to be full or half siblings. Inclusion of siblings provides inappropriately precise estimates of allele frequencies. We will use the program *ml-relate* (Kalinowski et al. 2006) to detect siblings and exclude from the baseline all but one individual from every set of siblings identified.

4.6. Genetic Baseline Development

4.6.1. Consultation with other Agencies regarding appropriate statistical analyses

Below we outline statistical analyses that can be performed to examine population structure and to develop a baseline for use as a tool in MSA. However, many of these analyses are dependent on sample sizes and the results from preceding analysis. As this information becomes available, other analyses may be more appropriate. In January of 2014 and 2015, we will work in consultation with other Agencies (NOAA and FWS) to fine-tune analyses that are most appropriate for this genetics project.

4.6.2. Adult and Juvenile collections

Adult collections from all areas will be used for baseline development. However, if inadequate numbers of adult samples are collected above Devils canyon, juvenile collections may also be incorporated into the baseline for this area. Before juvenile collections are incorporated into the baseline, we will test for sibling relationships (see methods in QC, above), test for differences in allele frequency estimates between the adult collections and juvenile collections (see methods under pooling collections, below), and examine Hardy-Weinberg equilibrium (HWE) of pooled adult/juvenile collections (see methods in pooling collections, below). We will delete all but 1 individual from every sibling group, and exclude juvenile collections from the baseline if they show significant allele frequency differences from adult collections or show deviations from HWE when pooled with adult collections.

4.6.3. 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 (α = 0.05 per number of collections).

4.6.4. 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).

4.6.5. 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 tributary (or river for mainstem spawners). 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 within the same tributary, 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.

4.6.6. 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.

4.6.7. Hierarchical Log-likelihood Ratio Tests

Genetic diversity will be examined with a hierarchical log-likelihood ratio (G) analysis with the package *hierfstat* (Goudet 2006).

4.6.8. 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*. 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).

4.6.9. Testing Among Hypotheses

For the first hypothesis criterion in Figure 1, we will test for panmixia (spawning aggregates belong to the same population) using Fisher's exact test of allele frequency homogeneity. For

the second hypothesis criterion in Figure 1, we will test for temporal stability in allele frequencies using a three-level analysis of variance (ANOVA). The three levels of the hierarchy include variation within collections, variation within location among years, and variation among locations. In addition, we will test between hypotheses 1a and 1b by investigating conformation to HWE and calculation of effective population sizes and migration rates. Conformance to HWE across markers will be tested using Fisher's exact test. Effective population sizes will be estimated using juvenile collections within cohorts. Juveniles will be binned into cohorts by total length (snout-to-fork). Finally, we will use the program MIGRATE (Beerli and Felsenstein 2001) to estimate migration rates and direction of migration. All tests will use a significance level of $\alpha = 0.05$, adjusted for multiple tests.

4.7. Mixed-Stock Analysis

4.7.1. Assessing Reporting Groups (including above Devils Canyon) for MSA

In response to FERC comments from 2/1/2013, a preliminary analysis of SNP data from 42 loci using the selected pre-existing baseline and the 2012 collections was proposed to provide some insight into the potential of genetic data to detect fish from above Devils Canyon in mixtures (SPD). Subsequent comments from both NMFS and FWS both recommended that such an analysis was inappropriate given the small sample sizes, and that testing for genetic differentiation among Chinook salmon above and below Devils Canyon for use in MSA should wait until more samples are available. We therefore will not analyze these samples until more samples collected and we can do a more comprehensive analysis.

A comprehensive analysis will be conducted when microsatellite and SNP data are available from baseline collections sampled through 2014. We will use two methods to assess the utility of reporting groups for MSA once these data are available: anticipated mixture proof tests and ONCOR leave-one-out method (Anderson et al. 2008). For the anticipated-mixture proof tests, we will sample without replacement 400 individuals from reporting groups in proportions similar to those expected in the Lower River juvenile samples. We will estimate the stock compositions of these mixed composition proof tests following the BAYES protocol described below and compare these estimates to the true proportions. To account for sampling error, we replicate this procedure 10 times in a manner similar to Habicht and Dann (2012a).

For the leave-one-out method, we will use ONCOR, a Windows-based program available at http://www.montana.edu/kalinowski, to implement the simulations. This program handles only diploid markers, so we will exclude linked and mtDNA loci from the analysis. The output from this analysis produces stock proportion point estimates for each population by reporting group.

These two analyses will determine whether the population structure is adequate for MSA to produce useful results. Generally, correct assignments of 90% to reporting groups are considered adequate for MSA, but this criterion is dependent on the purpose of the analysis. Adequate MSA performance will be determined in consultation with Agency (NOAA/FWS) geneticists and will be based on the reporting groups of interest to and risk tolerance. For an example of this process, see Habicht et al. (2012b).

4.7.2. Mixed Stock Analysis of juvenile Chinook salmon

The stock compositions of juvenile Chinook salmon will be estimated using a Bayesian approach to genetic MSA, the Pella-Masuda Model (BAYES; Pella and Masuda 2001). The Bayesian method of MSA estimates the proportion of stocks caught within each sample using 4 pieces of information: 1) a baseline of allele frequencies for each population, 2) the grouping of populations into the reporting groups desired for MSA, 3) prior information about the stock proportions of the fishery, and 4) the genotypes of fish sampled from the fishery. We will use a flat prior for all analyses.

We will run 5 independent Markov Chain Monte Carlo (MCMC) chains of 40,000 iterations with different starting values and discard the first 20,000 iterations to remove the influences of the initial start values. We will define the starting values for the first chain such that the first 1/5 of the baseline populations sum to 0.9 and the remaining populations sum to 0.1. Each chain will have a different combination of 1/5 of baseline populations summing to 0.9. We will combine the second halves of these chains to form the posterior distribution and tabulate mean estimates, 90% credibility intervals, the probability of an estimate being equal to zero, and standard deviations from a total of 100,000 iterations. For each tabulated measure, summary statistics will be based upon the raw posterior, which will be calculated out to 6 significant digits.

We will also assess the within- and among-chain convergence of these estimates using the Raftery-Lewis (within-chain) and Gelman-Rubin (among-chain) diagnostics. These values measure the convergence of each chain to stable estimates (Raftery and Lewis 1996), as well as measure the variation of estimates within a chain to the total variation among chains (Gelman and Rubin 1992), respectively. If the Gelman-Rubin diagnostic for any stock group estimate is greater than 1.2 we will reanalyze the mixture with 80,000-iteration chains following the same protocol. If the Gelman-Rubin diagnostic for any stock group estimate is greater than 1.2 after this reanalysis, we will analyze the mixture with the program HWLER (Pella and Masuda 2006). HWLER is similar to BAYES in that it estimates stock compositions based upon a Bayesian model, but differs in that it incorporates information about the effect of assigning mixture individuals to baseline populations with respect to the Hardy-Weinberg and linkage equilibria conditions observed in the baseline populations. In doing so it allows for the identification of extra-baseline individuals that contravene these equilibria conditions, but contribute to the mixture in question. We will incorporate this information into the definition of the posterior for those mixtures that failed to converge after reanalysis with 80,000-iteration chains in BAYES.

4.7.3. 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 there is adequate genetic diversity between the Chinook salmon spawning upstream of Devils Canyon and in the Middle River and its tributaries, ADF&G will characterize the presence and relative proportion of fish originating from the Upper and Middle River in selected Lower River habitats. In 2013 and 2014, 100 juvenile Chinook salmon total from each of 16 mainstem locations (across five habitat types) will be collected and preserved as outlined above. These 1,600 tissue samples 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).

4.8. 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. GCL is located in Anchorage, Alaska, has a lot of experience with applied fish genetics and 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.

5. SCHEDULE AND DELIVERABLES

- Laboratory analysis of 2012 collections: March to September, 2013.
- Adult Chinook salmon baseline sample collection: May through October 2013 and 2014 (in collaboration with other AEA field studies).
- Other species sample collection: May through October 2013 and 2014 (in conjunction with other AEA field studies).
- Juvenile Chinook salmon mixture sample collection from the Lower River: May through October 2013 and 2014.
- Consultation with agencies (NMFS/FWS) to review sample collection results from 2013 in preparation for 2014 field season and project statistical analyses: January 2014
- Preparation of Interim Study Report (ISR). September 2013 – January 2014
- Laboratory analysis of adult Chinook salmon baseline and juvenile mixture samples: October 2013 to November 2014.
- Statistical analysis of Chinook salmon baseline collections to examine population structure and potential application of MSA: December 2014
- Consultation with agencies (NMFS/FWS) to review genetic analysis and determine if adequate genetic variation exists for MSA of juvenile Chinook salmon mixture samples: January 2015
- Assuming adequate genetic variation for MSA, statistical analysis of juvenile mixture samples: February 2015.
- Prepare Updated ISR: December – January 2015

- Deliverables:

    - February 1, 2014. Interim Study Report delivered to FERC. Report describes field effort and collection results. Report will include tables of collections with associated metadata: Sampling locations, GPS coordinates, sampling dates, sample species, and sample sizes.

    - March 31, 2014. Draft Operational Plan for 2014 Fieldwork to AEA, NMFS and FWS for review.

    - April 30, 2014. Final Operational Plan for 2014 filed with FERC.

    - February 1, 2015. Updated Interim Study Report providing analysis results for population structure and MSA potential. If MSA is useful, MSA results for juvenile mixtures.

6. RESPONSIBILITIES

Andrew Barclay, Fishery Biologist III

Duties: Coordinate field and laboratory aspects of genetics project. Perform analysis of genetic structure and mixed-stock analysis. Write initial and updated study reports to AEA. Track budgets.

Chris Habicht, Fisheries Geneticist III

Duties: Coordinate with AEA and its contractors to produce genetics project deliverables on time. Review operational plans and prioritize resources among laboratory projects to meet deadlines.

Jim Jasper, Biometrician III

Duties: Biometric support. Assist in report writing. Also reviews operational plan and final report.

Vacant, Fishery Biologist I (3 positions)

Duties: Sampling trip logistics, lead sampling crews, capture spawning adult salmon, juvenile Chinook salmon, and non-salmon fish species to collect tissue samples for genetic analysis, write trip reports, and Anadromous Wasters Catalog nominations.

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Table 1. FERC recommendations from their Study Plan Determination on 2/1/2013, AEA's responses to FERC recommendations, and page number(s) in this document where each recommendation is addressed (Pages).

FERC Recommendation	AEA Response	Pages
We recommend the study plan be modified to include 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, 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.	For each year of the study, AEA will submit a draft operational plan to NMFS, and USFWS for review by March 31 and agency comments will be returned by April 15. The final draft will be submitted to FERC by April 30.	15-16
To the extent feasible, we recommend that AEA collect tissue samples over a representative proportion of the entire adult Chinook salmon run.	The field season for this study has been extended to 4 months (June - September), which will include weekly aerial surveys to confirm the presence or abundance of adult salmon at potential sampling locations. These surveys will be used to inform sampling crews where to focus their efforts.	6-8
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.	Dates for the analysis and reporting of the 2012 collections have been added to the Schedule and Deliverables section of the Implementation Plan.	15-16
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.	Criteria for determining if there is sufficient genetic diversity to estimate the percentage of Chinook salmon originating from Upper and Middle River habitats has been added the methods section of the Implementation Plan.	13-14
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.	The report on the 2012 preliminary genetics studies will include a test to determine if the allele frequencies of Chinook salmon collected from Kosina Creek are significantly different from Chinook salmon populations spawning below Devils Canyon. A significant difference in allele frequencies will bode well, but not guarantee, the usefulness of MSA to separate populations of juvenile Chinook salmon from the Middle and Lower River, as proposed. Note that this analysis has been removed in respose to NMFS and FWS comments received 4/15/2013.	13

Table 2. Area, location, and sub location of desired baseline samples of adult and juvenile Chinook salmon for genetic analysis.

Sample sizes show number of samples and sample years for collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal sample size (Ideal), and the anticipated number to be collected over the two years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected). Some of the expected numbers are for groups of locations. Sample collection targets apply only to collections targeted in this study. Some of these samples may be collected in other program studies, but sample sites that are not targeted in this study are not listed, even if they are proposed to be sampled in other program studies for genetic tissues. Map numbers (Map No.) correspond to location numbers on Figure 2.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
					Archived	This project	
						Ideal	Expected
			Adult Chinook salmon				
	West Cook Inlet						
1		Chuitna River		2008, 2009	142	58	58
2		Beluga River	Coal Creek	2009, 2010, 2011	120	80	80
3		Theodore River		2010, 2011, 2012	189	11	11
4		Lewis River		2011, 2012	86	114	86
	Yentna Drainage						
5		Clearwater Creek		2012	25	175	50
6		Red Creek		2012	29	171	58
7		Happy River		2012	19	181	38
8		Red Salmon Creek		2012	12	188	24
9		Hayes River		2012	5	195	10
10		Canyon Creek		2012	32	168	64
11		Talachulitna River		1995, 2008, 2010	180	20	20
12		Lake Creek	Sunflower Creek	2009, 2011	127	71	71
13		Kahiltna River	Peters Creek	2009, 2010, 2011, 2012	110	90	55
	Susitna Drainage						
15		Chulitna River	Middle Fork	2009, 2010, 2011	182	18	18
14			East Fork			200	200
16			West Fork			200	
17			Honolulu Creek			200	
18			Byers Creek			200	
19			Troublesome Creek			200	
20			Spink Creek			200	

21			Tokositna River (Bunco Creek)		200	

-continued-

Table 2. Page 2 of 3.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
					Archived	This project	
						Ideal	Expected
22		Above Devils Canyon	Oshetna River			200	50
23			Kosina Creek	2012	10	190	
24			Watana Creek			200	
25			Tsusena Creek			200	
26			Fog Creek			200	
27			Devil Creek			200	
29		Middle Susitna River below Devils Canyon	Portage Creek	2009, 2010, 2011	141	59	59
28			Chinook Creek			200	75
30			Indian River	1212	1	199	
31			Gold Creek			200	
32			Lane Creek			200	
33			Chase Creek			200	
35		Talkeetna River	Prairie Creek	1995, 2008	169	31	31
34			Upper mainstem			200	100
36			Iron Creek			200	
37			Disappointment Creek			200	
38			Sheep River			200	
39			Larson Creek			200	
40			Chunilna Creek (Clear Creek)	2009, 2012	130	70	65
42		Lower Susitna River, upstream of Deshka	Montana Creek	2008, 2009, 2010	218	0	0
41			Birch Creek			200	50
43			Sheep Creek			200	
44			North Fork Kashwitna River			200	
45			Little Willow Creek			200	
46			Willow Creek	1991,1997, 2005, 2009	309	0	0

47		Deshka River	Moose Creek	1995, 2012	103	97	52
48			Deshka River weir	2005	200	0	0

-continued-

Table 2. Page 3 of 3.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes Archived	This project Ideal	This project Expected
49	Knik Arm	Alexander Creek	Sucker Creek	2011, 2012	143	57	57
50		Matanuska River	Kings River			200	25
51			Granite Creek			200	
52			Moose Creek	1995, 2008, 2009, 2012	155	45	45
53		Eagle River	South Fork	2009, 2011, 2012	73	127	24
54			Meadow Creek	2009	6	194	12
55		Ship Creek		2009	311	0	0
56		Little Susitna River		2009, 2010	125	75	75

Juvenile Chinook salmon

22	Susitna Drainage	Above Devils Canyon	Oshetna River	2012	35	200	70
23			Kosina Creek			200	
24			Fog Creek			200	
25			Cheechako Creek			200	
	Susistna Draniage	Lower River	5 habitat types (100 fish/habitat type times 3 or 4 collections)			1,600	1,600

Table 3.- Location, and sublocation of desired baseline samples of adult sockeye salmon spawning aggregates for genetic analysis.

Sample sizes show number of samples and sample years for collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal sample size (Ideal), and the anticipated number to be collected over the two years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected). Some of the expected numbers are for groups of locations. Map numbers (Map No.) correspond to location numbers on Figure 3.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
						This project	
					Archived	Ideal	Expected
	Susitna River						
1		Chulitna River	East Fork			100	100
2			Middle Fork			100	
3			Byers Lake	1993, 2006, 2007	243	0	0
4			Spink Creek	2007, 2008	126	0	0
5		Tokositna River	Sloughs			100	100
6			Swan Lake	2006, 2007, 2009	109	0	0
7		Middle Susitna River below Devils Canyon	McKenzie Creek			100	100
8			Chase Creek			100	
9		Mainstem sloughs above Three Rivers Confluence	sloughs 8A,11, and 21	1995, 1996, 1997	156	0	0
10		Talkeetna River	Sheep River	2008	190	0	0
11			Stephan Lake	1993, 1994, 2007	346	0	0
12			Iron Creek			100	50
13			Sloughs	1997	79	21	21
14			Larson Creek	1992, 1993	200	0	0
15			Larson Lake - Eastern shore	2011	90	10	10
16			Larson Lake - outlet stream	2011	126	0	0
17			Chunilna Creek			100	100
18			Mama and Papa Bear Lakes	1997, 2007	106	0	0

Table 4. Location, and sublocation of desired baseline samples of adult chum salmon spawning aggregates for genetic analysis.

Sample sizes show number of samples and sample years for collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal sample size (Ideal), and the anticipated number to be collected over the two years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected). Some of the expected numbers are for groups of locations. Map numbers (Map No.) correspond to location numbers on Figure 4.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
					Archived	This project	
						Ideal	Expected
1	Susitna River above Three Rivers Confluence	Chulitna River	Middle Fork			100	200
2			West Fork			100	
3			Byers Creek			100	
4			Troublesome Creek			100	
5			Spink Creek	2007, 2008	45	55	55
6			Tokositna River mainstem			100	50
7		Middle Susitna River below Devils Canyon	sloughs above Three Rivers Confluence	1996	103	0	0
8			Indian River			100	100
9			Portage Creek			100	100
10		Talkeetna River	Sloughs	1995	50	50	50
11			Upper mainstem			100	200
12			Disappointment Creek			100	
13			Sheep River			100	
14			Larson Creek			100	
15			Chunilna Creek	1993	87	13	13

Table 5. Location, and sublocation of desired baseline samples of adult coho salmon spawning aggregates for genetic analysis.

Sample sizes show number of samples and sample years for collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal sample size (Ideal), and the anticipated number to be collected over the two years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected). Some of the expected numbers are for groups of locations. Map numbers (Map No.) correspond to location numbers on Figure 5.

					Sample sizes		
						This project	
Map No.	Area	Location	Sublocation	Year(s) Collected	Archived	Ideal	Expected
1	Susitna River	Chulitna River	East Fork			100	
2	above Three		Middle Fork			100	
3	Rivers		Honolulu Creek			100	200
4	Confluence		Byers Creek			100	
5			Troublesome Creek			100	
6			Spink Creek	2008	38	62	62
7			Tokositna River mainstem			100	100
8			Tokositna River (Bunco Creek)			100	
9			Tokositna River (Swan Lake)	2009	20	80	80
10		Middle Susitna River	Portage Creek			100	
11		below Devils Canyon	Indian River			100	
12			Gold Creek			100	200
13			McKenzie Creek			100	
14			Lane Creek			100	
15			Chase Creek			100	75
16			Whiskers Creek			100	75
17			Sloughs			100	75
18		Talkeetna River	upper mainstem			100	25
19			Prairie Creek			100	75
20			Sheep River			100	50
21			Larson Lake - outlet	2011	84	16	16
22			Chunilna Creek			100	75

Table 6. Location, and sublocation of desired baseline samples of adult pink salmon spawning aggregates for genetic analysis.

Sample sizes show number of samples and sample years for collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal sample size (Ideal), and the anticipated number to be collected over the two years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected). Some of the expected numbers are for groups of locations. Map numbers (Map No.) correspond to location numbers on Figure 6.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
						This project	
					Archived	Ideal	Expected
1	Susitna River	Chulitna River	Middle Fork			100	
2	above Three		Troublesome Creek			100	100
3	Rivers		Spink Creek			100	
4	Confluence	Middle Susitna River	Portage Creek			100	50
5		below Devils Canyon	Indian River			100	100
6			Gold Creek			100	
7			McKenzie Creek			100	
8			Lane Creek			100	50
9			Chase Creek			100	
10			Whiskers Creek			100	
11		Talkeetna River	upper mainstem			100	25
12			Sheep River			100	25
13			Larson Creek			100	100
14			Chunilna Creek			100	100

Table 7. Potential resident and non-salmon anadromous fish species targeted for genetic tissue sampling in the Susitna River.

Common Name	**Scientific Name**
Rainbow trout	*Oncorhynchus mykiss*
Humpback whitefish	*Coregonus pidschian*
Round whitefish	*Prosopium cylindraceum*
Lake whitefish	*Coregonus clupeaformes*
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*

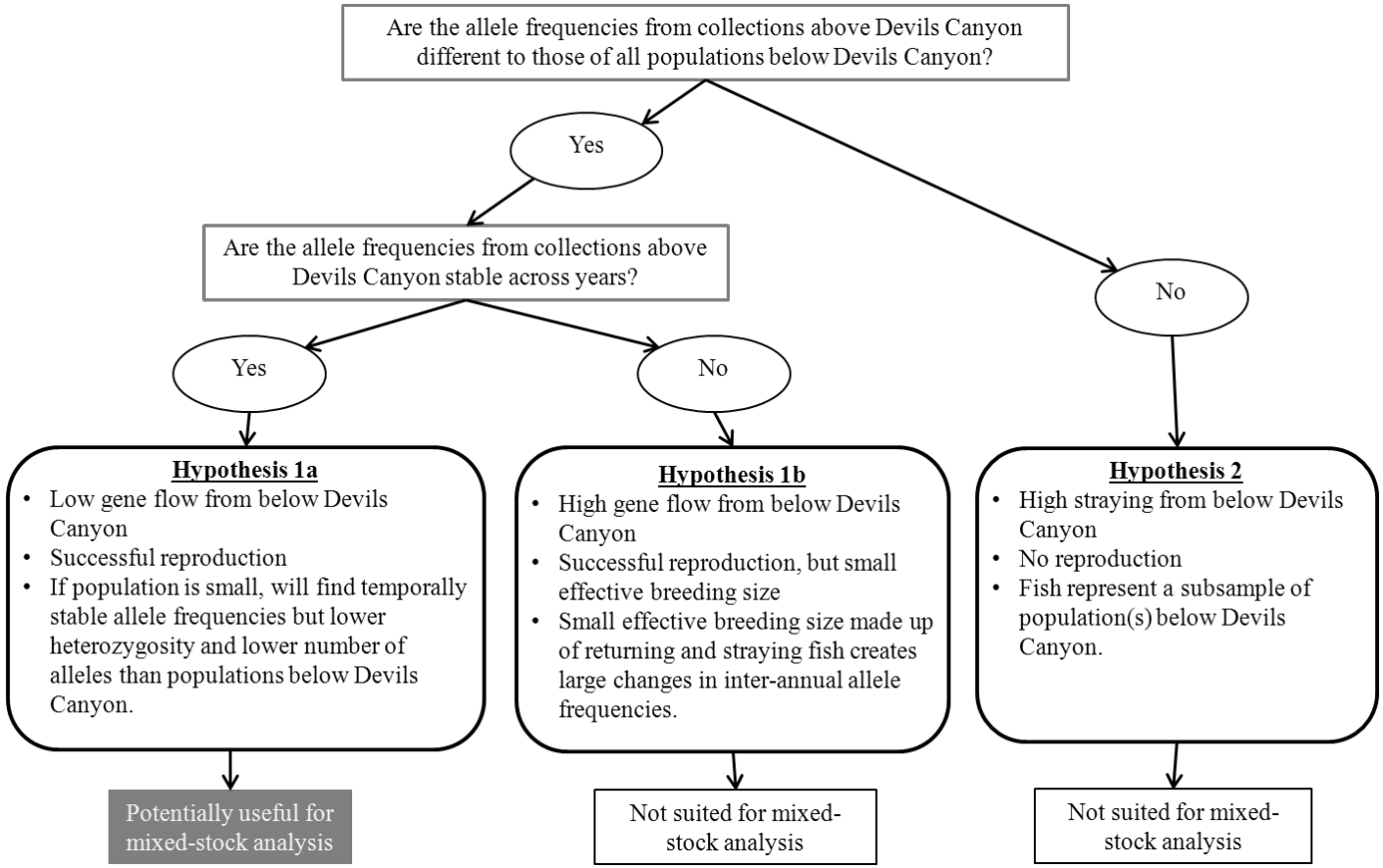


Figure 1. A generalized flow chart to distinguish among hypotheses of population structure for Chinook salmon collected over spawning habitat above Devils Canyon in the Middle and Upper Susitna River.

Only a self-sustaining population (Hypothesis 1a) will potentially result in genetic variation suitable for mixed-stock analysis for estimating the proportion of juvenile Chinook salmon mixtures collected in the Middle and Lower Susitna River that originate from above Devils Canyon.

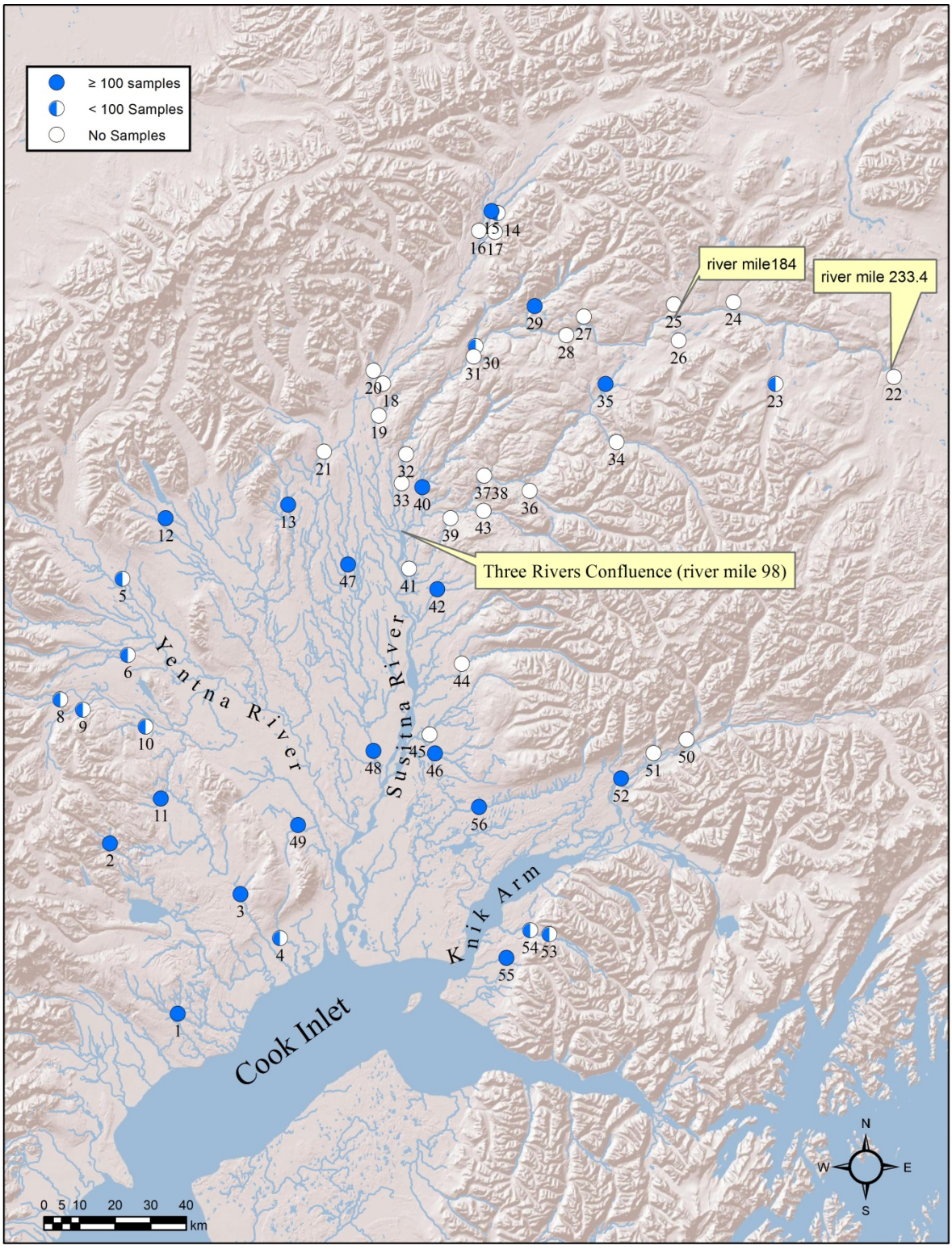


Figure 2. Potential baseline sampling locations for adult Chinook salmon.

Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 2. Call-outs point to divisions between the Lower Susitna River (below river mile (RM) 98), Middle River (RM 98-184) and Upper River (RM 184=233.4). RM 184 is the location of the proposed dam.

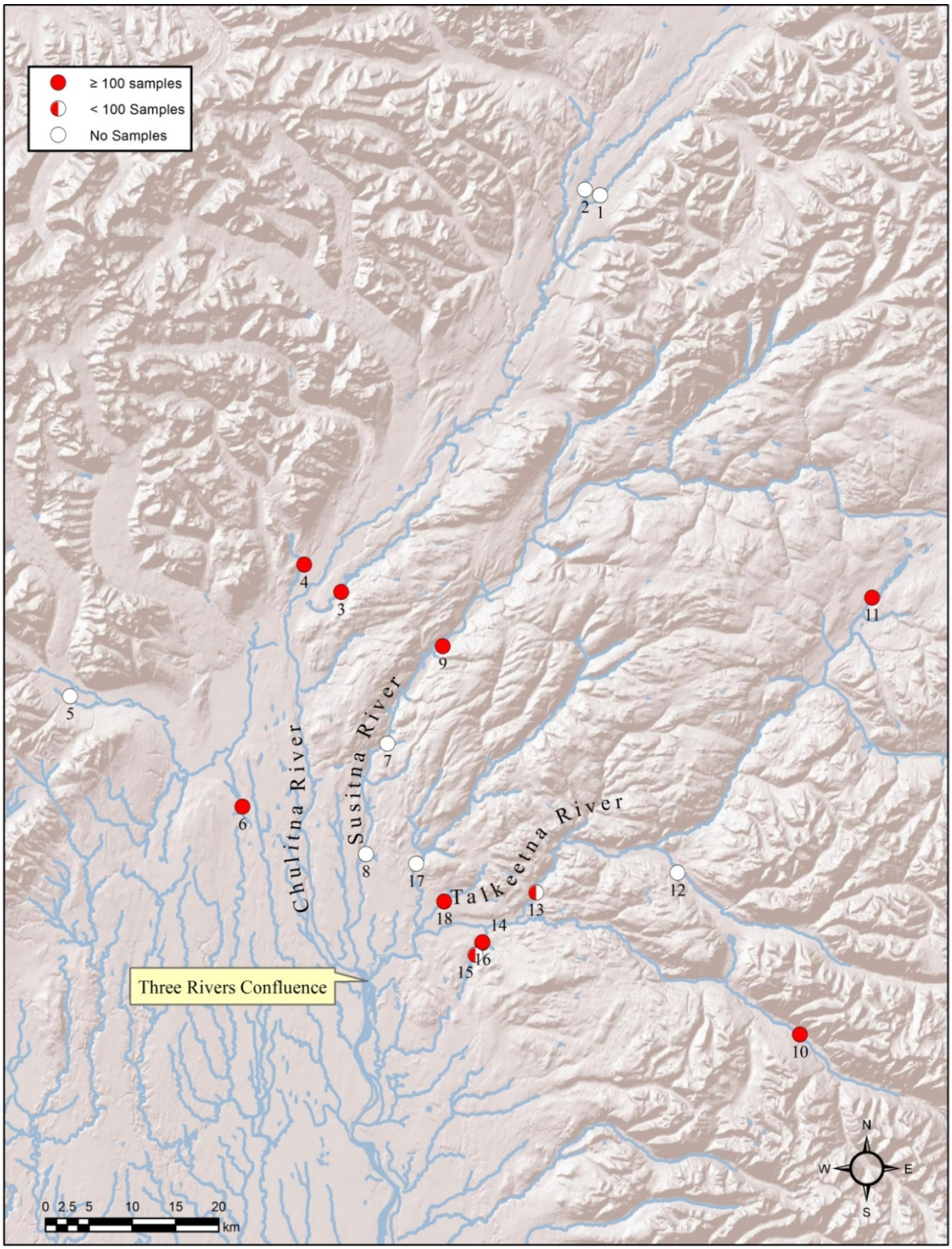


Figure 3. Potential baseline sampling locations for adult sockeye salmon.

Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 3.

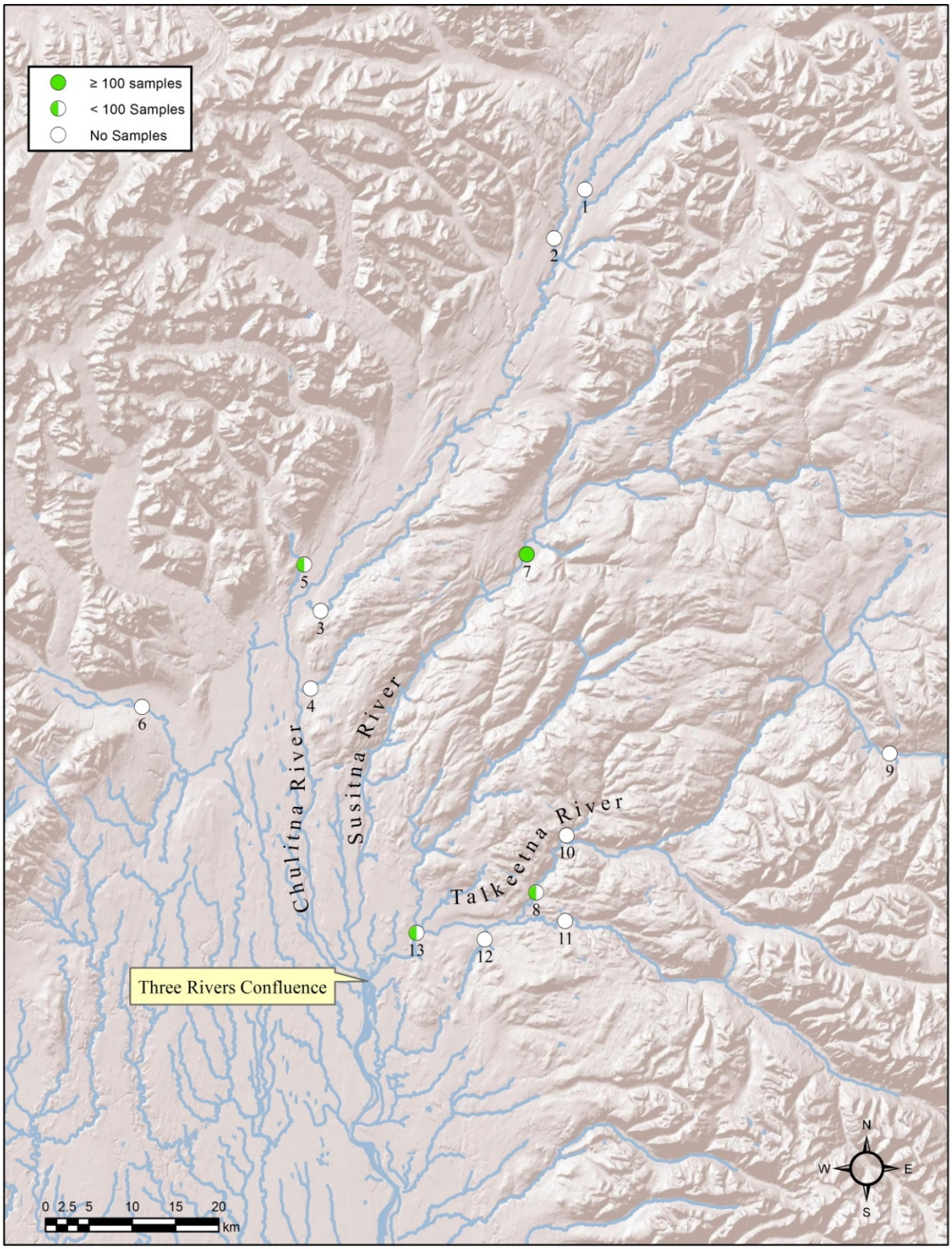


Figure 4. Potential baseline sampling locations for adult chum salmon.

Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 4.

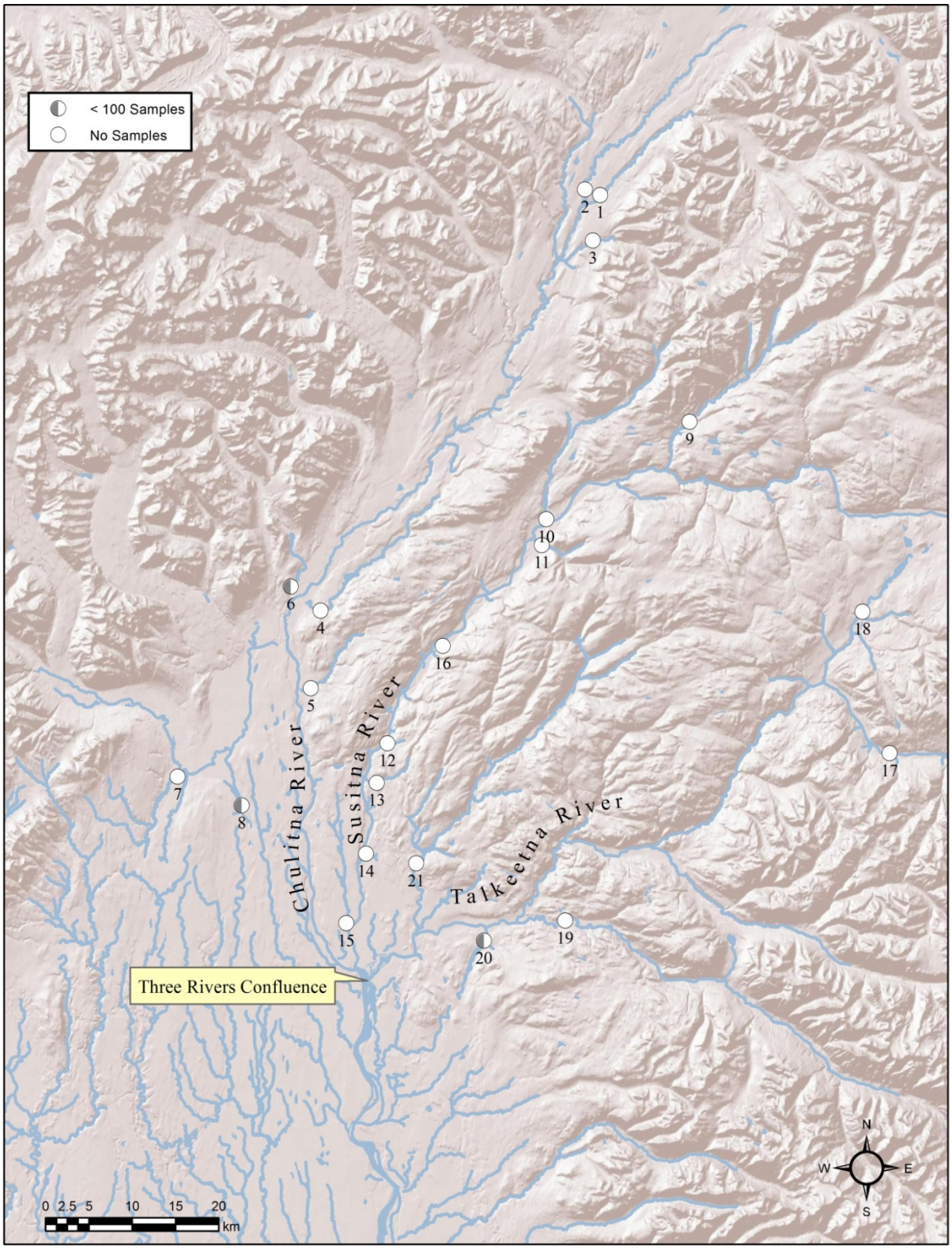


Figure 5. Potential baseline sampling locations for adult coho salmon.

Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 5.

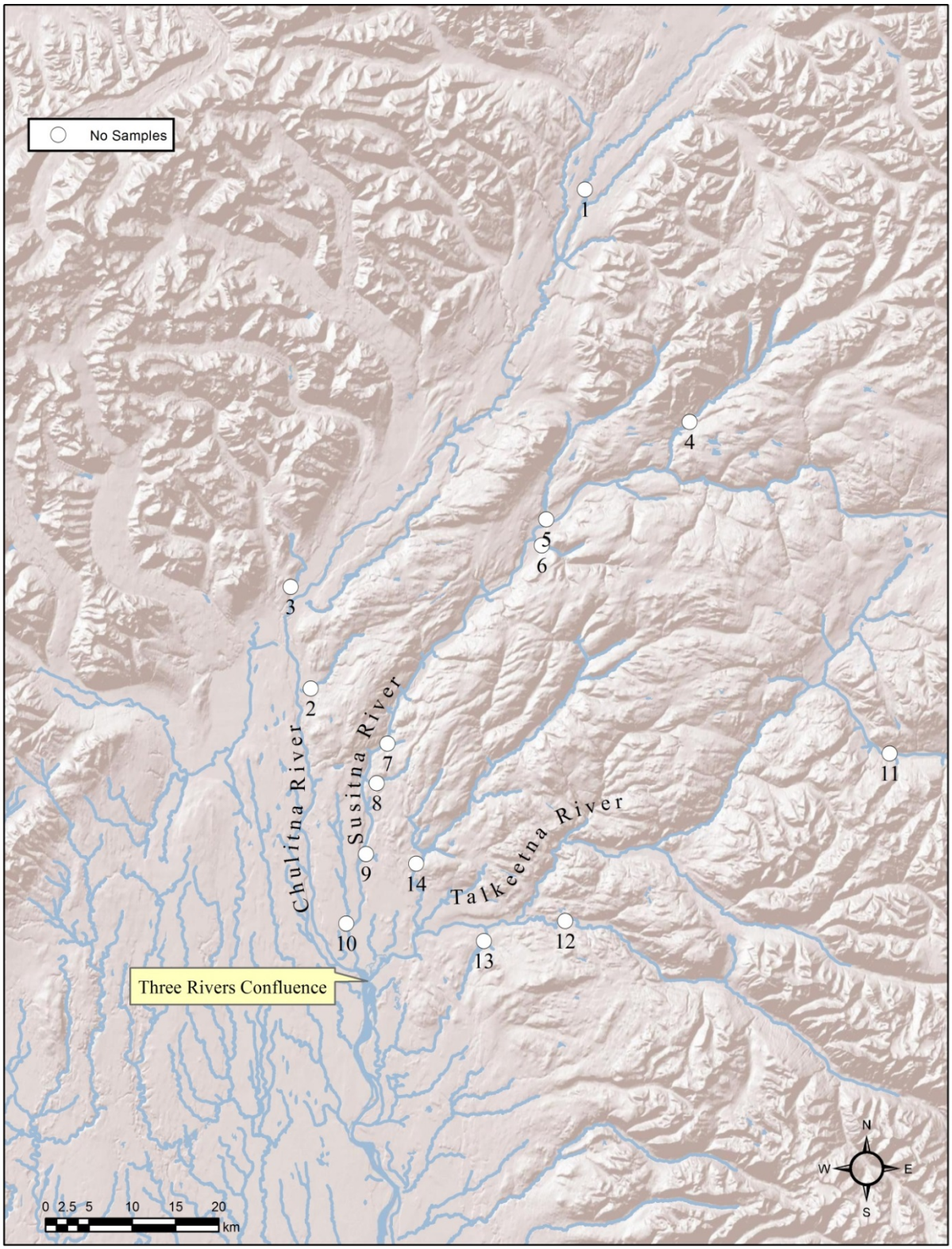


Figure 6. Potential baseline sampling locations for adult pink salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives.

Numbers correspond to map numbers on Table 6.

APPENDIX A: GENETIC SAMPLING INSTRUCTIONS

Non-lethal Bulk Sampling Finfish Tissues for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

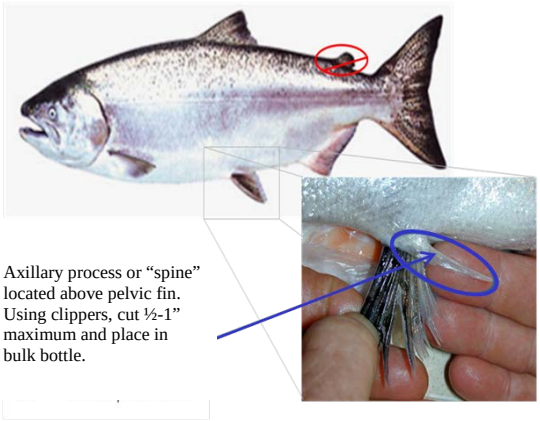
I. General Information

We use axillary process samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. This is a non-lethal method of collecting tissue samples from adult fish for genetic analysis. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal fins.

II. Sampling Method

Preservative used: Isopropanol/Methanol/Ethanol (EtOH) preserves tissues for later DNA extraction. Avoid extended contact with skin.

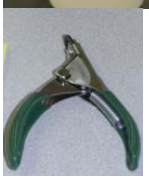
Sampling instructions are written for (N=100 fish/125ml) bulk bottle. <u>Steps for collecting axillary process tissues:</u>



Axillary process or "spine" located above pelvic fin. Using clippers, cut ½-1" maximum and place in bulk bottle.

- Wipe dry the axillary process "spine" prior to sampling to avoid getting excess water or fish slime into the 125ml bottle (see diagram).
- Clip off the axillary "spine" using dog nail clippers or scissors to get roughly a **½ - 1" inch maximum** piece and/or about the size of a small fingernail.
- Place each tissue piece into bulk bottle **(place only one piece of axillary from each fish)**.
- Repeat: **up to 100 fish /125ml bulk bottle (**into same bottle). If you don't reach this number of fish per location, that's ok. Maximum storage capacity 125ml bulk for proper preservation of axillary tissue is (N=100).
- Record on **each label:** Location, sampling date (mm/dd/yyyy), sampler's name(s), total number of fish sampled, latitude/longitude, and field notes (if any). Use pencil. This insures correct data with each collection bottle.
- If collection occurs over 4~5 day period, "refresh" EtOH at end of the collection.
- After the collection is complete and 24 hours have passed, "refresh" the axillary tissues as follows: carefully pour off ¾ EtOH and then pour fresh EtOH into sample bottle containing axillary clips. Cap and invert bottle twice mixing EtOH and tissue.
- Freezing not required, store sample bottle in upright cool location for good tissue quality.

SILLY: _____
Location: _____
Sample Date(s):___/___/___
Sampler's name:_____
Total # fish sampled:_____
Latitude:_____
Longitude:_____
Species:_____
Comments:_____
ADF&G:Preserved in EtOH



<u>Supplies included in sampling kit</u>:

1. Clipper- used to cut a portion of **one** axillary process per fish.
2. Sample target: 100 axillary clips/125ml bulk bottle.
3. Labels on bulk sample bottles: Location, Sample date, Sampler, Total # fish sampled and comments (if any).
4. **1:125ml** wide mouth bottle(s) for EtOH "refresh" step.
5. Sampling instructions.

Return to ADF&G Anchorage lab:	ADF&G – Genetics 333 Raspberry Road Anchorage, Alaska 99518	Lab staff: 907-267-2247 Judy Berger: 907-267-2175 **Freight code:** _____

Appendix A 1.–Bulk sampling instructions for adult salmon and other adult fish species. Fin tissue will be sampled when axillary process is not available.

Non-lethal Juvenile Finfish Tissue Sampling for DNA Analysis

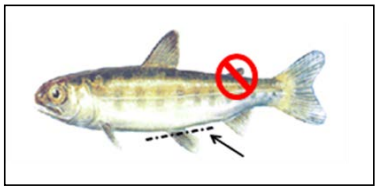
ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use a portion of one pelvic fin tissue sample from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal fins.

Preservative used: Isopropanol/Methanol/Ethanol (EtOH) preserves tissues for later DNA extraction. Avoid extended contact with skin.

II. Sampling Method



- Wipe excess water and/or slime off the pelvic fin prior to sampling to avoid getting either water or fish slime into the 2.0ml vial (see diagram on reverse side).
- Prior to sampling, fill the tubes half way with EtOH. Fill only the tubes that you will use for each sampling period. The squirt bottle is for day use only since it will leak overnight when unattended.
- Cut off only one pelvic fin/fish along dotted line (shown in diagram to left and on reverse side) using scissors to collect tissue sample from **only one** pelvic fin.
- Place one pelvic fin tissue into a 2.0ml vial pre-filled with EtOH. Ethanol/tissue ratio should be **slightly less than 3:1** to thoroughly soak the tissue in the buffer. Not a problem with juvenile samples.
- Top up vials with EtOH and screw cap on securely. Invert vial twice to mix EtOH and tissue. Periodically, wipe or rinse the scissors with water so not to cross contaminate samples with any tissue from the previous fish sampled.
- **Only one pelvic fin clip per fish into each vial/location.**
- Data to record: Record **each vial number to paired data** information (i.e. location, lat./long., sample date(s), etc.). Electronic version preferred.
- **Tissue samples must remain in 2ml EtOH**. Store vials containing tissues at room temperature but away from heat. In the field: keep samples out of direct sun, rain and store capped vials in a dry, cool location. Freezing not required.

III. Supplies included in sampling kit:

1. Scissors - for cutting one pelvic fin/fish.
2. Cryovials - 2.0ml pre-labeled plastic vials.
3. Caps – cap for each vial.
4. Bullet box- box for holding cryovials while sampling.
5. EtOH – ethanol in Nalgene bottle(s).
6. Squirt bottle – to fill and/or "top off" each cryovial with EtOH.
7. Laminated "return address" labels.
8. Sampling instructions.

IV. Shipping: "in commerce" on roadways for return shipment of these samples.

Return to ADF&G Anchorage lab:	ADF&G – Genetics 333 Raspberry Road Anchorage, Alaska 99518	Lab staff: 907-267-2247 Judy Berger: 907-267-2175 **Freight code: _____**

Appendix A 2.–Vial sampling instructions for juvenile Chinook salmon.

Attachment B

Record of Consultation, Development of 2013 Project Operational Plan, Study 9.14

March 29, 2013

To: **Sue Walker (NMFS)**
Catherine Berg (USFWS)

Subject: **Distribution of Draft 2013 Project Operational Plan for Comment, Fish Genetic Baseline Study (RSP 9.14)**

Dear Ms. Walker and Ms. Berg:

On February 1, 2013, the Federal Energy Regulatory Commission (Commission or FERC) issued its Study Plan Determination (SPD) for 44 of the 58 proposed individual studies in the Alaska Energy Authority's (AEA) Revised Study Plan (RSP) for the Susitna-Watana Hydroelectric Project, FERC Project No. 14241 (Project).[1] With regard to the Fish Genetic Baseline Study (RSP 9.14), AEA proposed to develop and circulate to Technical Workgroup (TWG) members by April 30 of 2013 and 2014 detailed annual project operational plans. These operational plans are to establish additional details for field sampling efforts, including specific temporal and spatial sampling locations, to enhance the general locations for target sample collection presented in the RSP.

When approving the Fish Genetic Baseline Study, the Commission recommended that AEA consult with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) and, by March 31, prepare a draft project operational plan for review and comment by the agencies. Following a 15-day review and comment period, AEA will prepare and file the final plan with the Commission by April 30, which will include "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."[2]

The purpose of this letter is to distribute for comment by USFWS and NMFS, as well as other TWG members, the draft 2013 operational plan for the Fish Genetics Baseline Study. Due to the large file size of this document, AEA has uploaded the draft plan to the Project website at the following link:
http://www.susitna-watanahydro.org/wp-content/uploads/2013/03/SuWaGeneticsSPD_Draft_OP_2013_0329-FINAL.pdf

To allow sufficient time for AEA to file the final plan with the Commission by April 30 as directed, AEA requests that all comments be submitted, in writing, by Monday, April 15, 2013. Please submit all comments to:

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[1] Study Plan Determination for the Susitna-Watana Hydroelectric Project, Project No. 14241-000 (issued Feb. 1, 2013) [hereinafter, "SPD"].

[2] *Id.*, Appendix B, at B-43 to B-44.

Betsy McGregor
Environmental Manager
Alaska Energy Authority
813 West Northern Lights Blvd.
Anchorage, AK 99503
E-mail: bmcgregor@aidea.org

AEA looks forward to receiving comments on the attached draft plan, and to working with NMFS, USFWS, and TWG in implementing the Fish Genetic Baseline Study, as well as all other licensing studies for the proposed Project. If you have questions concerning this submission, please do not hesitate to contact me at (907) 771-3957.

Sincerely,

Betsy McGregor
Environmental Manager
Alaska Energy Authority

cc:
Betsy McCracken, USFWS
Michael Buntjer, USFWS
Eric Rothwell, NMFS
Brian Lance, NMFS
Marie Steele, ADNR
Stormy Haught, ADF&G
Joe Klein, ADF&G
Klaus Wuttig, ADF&G
Bill Templin, ADF&G
Chris Habicht, ADF&G
Andy Barclay, ADF&G
Eric Volk, ADF&G
Matt LaCroix, EPA
Matt Cutlip, FERC
Jeff Davis, ARRI
Dara Glass, CIRI
Jan Koningsberg
Wayne Dyok, AEA

U.S. Fish and Wildlife Service Review of the draft
"Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska" - Susitna-Watana Hydro Project
April 12, 2013

FERC's February 1, 2013 Study Determination recommended that the study plan be modified to include 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, FERC recommend that AEA collect tissue samples over a representative proportion of the entire adult Chinook salmon run.

FERC also recommended 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. The Study Determination also recommended 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, it recommended 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.

AEA did not consult with FWS or NMFS prior to preparing the 2013 project operational plan, but rather, provided the completed 2013 draft operational plan to the Services for review in March 31, 2013. Omitting the first step in the process, of consultation with FWS prior to developing the operational plan, has not allowed FWS input into the development of the plan. Thus FWS is only able to provide a review of the draft plan. This was completed by Dr. Jeff Olson, Deputy Director of the Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, Region 7.

General comments on Susitna River genetics operational plan:

This plan describes the collection of tissue samples from resident and anadromous fish in the Susitna River. The focus of the plan is on estimating the genetic population structure of Chinook salmon by assaying variation at 96 SNP and 12 microsatellite loci. The plan further proposes to perform mixed-stock analysis of juvenile Chinook salmon sampled in lower Susitna River mainstem habitats to estimate habitat use by Chinook salmon originating from the middle and upper portions of the drainage.

We recommend redrafting the proposal to correct grammatical errors and to improve clarity in the following sections: Section 2.1.1, Assessing Chinook salmon population structure (section unclear); Section 4.2, Samples to collect (section unclear, with some background information missing), and Section 4.6.8, Testing among hypotheses (more detail needed). See specific comments, below.

Specific comments:

Page 3, Section 2.1.1, Assessing Chinook salmon population structure: This section could be improved by organizing it into three paragraphs, one for a description of each of the hypotheses of population structure above Devil's Canyon. For Hypothesis 1a, temporal variation in allele frequencies may be seen in small, genetically isolated populations (Waples and Teel 1990).

Page 5, Section 3, Objectives: In the last line of the paragraph introducing the objectives, it reads "…(3) assess the use of Lower and Middle River habitat by juvenile Chinook salmon originating in the Middle and Upper Susitna River." Should this be "Lower River habitat" (delete the word Middle), to follow what is written in Objective 5, "…selected Lower River habitats…"?

Page 5, Section 3 Objectives, Objective #3: There needs to be justification on why samples outside of the Susitna River are being collected for Chinook salmon.

Page 6, Section 4.2, Samples to collect: This section about recommended sample sizes was confusing; some background information and citations seem to be missing (e.g., for the first sentence) or misplaced (e.g., Nei 1978). Sample sizes are partially dependent on the genetic divergence among stocks, the information content of the genetic markers, and adequate estimate of allele frequencies. A more thorough description or better references, for example the recent reports for chum salmon and sockeye salmon MSA, would be useful here.

Page 7, Section 4.2.1, Sample collection target #5 and Page 9, Section 4.2.4, Juvenile Chinook salmon collection above Three Rivers confluence: Why is only the Oshetna River being sampled for juveniles, since adults were collected in Kosina Creek and juveniles have been seen here? We have not checked the Anadromous Waters Catalog, but all tributaries above the Canyon should be sampled for juveniles. Chinook salmon juveniles can migrate quite some distances from their tributary of origin (e.g., Daum and Flannery 2011). There should be some justification on why juvenile samples collected below the falls that are used for "baseline" will likely not comprise a mixture of stocks (please see Specific Comment Page 13, for other suggestions on how these samples could be used.)

Page 9, Section 4.2.6, Other species collections: It sounded like resident species are going to be in bulk collections. Is that a single bulk collection for the entire Susitna River, or a bulk collection for each sampling site (recommend the latter)?

Page 12, Section 4.5, Data Retrieval and Quality Control: Elimination of siblings will only be done for juvenile collections for baseline?

Page 12, Section 4.6.2, H-W Expectation: There may be some deviations from HW expectation by chance. Is it really necessary to delete the collection(s) from further analysis? Should HW testing be conducted after temporal pooling?

Page 13, Section 4.6.8, Testing among hypotheses: This section needs to be expanded. The Evolutionary Criteria of Waples and Gaggiotti (2006) should be described, and related to Hypotheses 1a, 1b, and 2. What are the three levels of the hierarchical analysis?
Evaluating the Evolutionary Criteria/Hypotheses through estimating effective population size may not be very powerful if confidence limits are large. Also, unless large sample sizes are achieved, estimating Ne may not be very successful (Waples 1989, England et al. 2005). It may not be possible to use the temporal method, because the time span in the samples collected may not be large enough. The collections of juveniles may be useful in Ne estimation, provided they represent a single cohort and

population. It may be possible to determine if juveniles are from one cohort by measuring individual length to determine if sizes fall in a single mode.

Another possible analysis is to use the program MIGRATE to both estimate migration rates and direction and Neμ. This analysis may also be of interest for the juvenile collections above and below the canyon.

Page 14, Section 4.7.1 Assessing reporting groups (including above Devil's Canyon for MSA: Delete preliminary test using Kosina Creek 2012, N=10. Wait until more samples are collected.

Review of the draft "Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska" - Susitna-Watana Hydro Project

April 12, 2013

FERC's February 1, 2013 Study Determination recommended that the study plan be modified to include 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, FERC recommend that AEA collect tissue samples over a representative proportion of the entire adult Chinook salmon run.

FERC also recommended 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. The Study Determination also recommended 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, it recommended 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.

AEA did not consult with FWS or NMFS prior to preparing the 2013 project operational plan, but rather, provided the completed 2013 draft operational plan to the Services for review in March 31, 2013. Omitting the first step in the process, of consultation with NMFS prior to developing the operational plan, has not allowed NMFS' input into the development of the plan. Thus NMFS is only able to provide a review of the draft plan. This was completed by Dr. Jeff Guyon, Director of the Fisheries Genetics Program at NMFS Auke Bay Laboratory, part of the Alaska Fisheries Science Center.

The draft "Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska" is a proposal by the Alaska Department of Fish and Game (ADF&G) to primarily evaluate the genetic distinction of Susitna River Chinook salmon. This species has been documented to migrate past the proposed dam site at mile 184 and spawn in the upper river, although little is known about this species in the upper river watershed. The proposal also includes plans to (1) collect genetic samples from other salmon species primarily below the proposed dam site to supplement other projects and (2)

to collect non-salmonid genetic samples on an opportunistic basis. The authors then propose to analyze both adult and juvenile Chinook salmon to ascertain whether Chinook salmon spawning upstream of the proposed dam site are part of a self-sustaining, genetically isolated population. Three population hypotheses are outlined in section 2.1.1 and the proposed experiments provide a strategy how to address each of them. Throughout the proposal, the authors do an outstanding job identifying the limitations of the study. The ADF&G genetics laboratory is a preeminent laboratory with extensive experience in the genetic analysis of salmon populations.

Because of the prominence of Chinook salmon in the draft plan, this review is focused primarily on the analysis proposed for that species. While the proposal is very well written, potential suggestions for improving the study are as follows:

1. Section 4.2.1 While the proposed sampling strategy is impressive, adult Chinook salmon are inherently difficult to sample because of their large size and preferred spawning habitat, often in fast deep water. This is clearly recognized in Table 2 as the preferred sample size is identified as 200 for each of the 6 sublocations above Devil's Canyon, yet the expected cumulative total for all 6 aggregated sublocations is identified as only 50. Given anticipated sampling difficulties, it's unclear whether ADF&G will be able to collect the minimum sample set of 50 representative Chinook salmon above Devil's Canyon in just two years, especially above the proposed dam site. Even if successful, 50 appears to be a low number of samples to compare to identify genetic differences in related stocks. The authors should consider other options in case the realized sample numbers are too low to address project objectives.

2. Section 4.7.1 Regarding the proposed preliminary analysis of the 10 samples collected in Kosina Creek in 2012, there is concern regarding the validity of the test. It's possible given the small number of samples that the power of the test may not be strong enough to identify differences if they exist. It's also possible that the small sample set could be biased in some way and therefore suggest differences where they may not exist. Because of this potential for misinterpretation, the authors should consider first performing some type of power analysis with existing populations of known genetic divergence to gauge the validity of comparing 10 samples from a single aggregation. If the test can't statistically be done with 10 samples, it might be best to hold any comparison until the sample sets are strong enough for a statistically reliable test.

3. Regarding the sampling locations upstream of the proposed dam site, the authors should consider including adult and juvenile Chinook salmon sampling upstream of the Oshetna River (location 22 on Figure 2). My understanding is that the Oshetna River is the furthest upstream location that juvenile Chinook salmon were identified in the past, but it's possible those juveniles could have originated from further upstream spawning aggregates and it's not clear whether locations upstream of the Oshetna River have been surveyed for even presence or absence of salmon; no apparent barrier to their migration is noted and habitat appears suitable.

4. Given that previous studies were completed in the past regarding the proposed dam site, it would be helpful to determine whether samples such as scales are available from historical studies. DNA from historical scales might help differentiate between the 3 different hypotheses identified in 2.2.1.

5. Section 4.6 While the proposed tests will be used to differentiate between the three hypotheses, the specific level of divergence used to discriminate fish populations is unclear. This is presumably because the number of available samples will shape the utility of the potential tests and interpretation of the results will be done later in 2014 and 2015 in consultation with other laboratories.

6. Juvenile salmon species can be difficult to distinguish, thus the authors should include species ID for juveniles collected at least upstream of the proposed dam site and possibly below. Such an analysis might provide additional information regarding potential spawning success of all salmon species.

In addition, there are some minor grammatical suggestions:

1. Page 1, first paragraph: Figure 2 is referenced prior to Figure 1.
2. Page 1, second paragraph: should read "… the proposed dam site are part of a …"
3. Page 2, fourth paragraph: extra semicolon after "salmon"
4. Page 3, first paragraph: need space after "3)"
5. Page 4, last paragraph: should read "The 2014 Genetics Implementation…"
6. Page 5, under "Objectives": might consider separating into primary and secondary objectives
7. Page 7, under "Sample collection targets": might consider separating into primary and secondary sampling goals
8. Page 12, second paragraph: "…. exclude from the baseline all …" – are juveniles going to be included in the genetic baseline?
9. Page 32-36, Figures 2-6: helpful to identify the proposed dam site on the maps

SUSITNA-WATANA HYDROELECTRIC PROJECT

MEETING RECORD

AEA Team Member		Other Party	
Name:	*Chris Habicht*	**Name:**	*Betsy McCracken*
Organization:	*ADFG Genetics*	**Organization:**	*USFWS*
Study Area:	*Susitna River and Upper Cook Inlet*	**Phone Number:**	*907-271-2783*
Date:	*2012 09 19*	**Time:**	
Meeting held by: [ ] AEA Team [X] Other Party			

Others at meeting: Bill Templin

Subject: At the request of Betsy, we met to discuss genetic applications associated with the SuWa hydro project.

Discussion: We had a broad discussion regarding genetic applications for understanding population structure and genetic mixed-stock analyses. We also discussed what is known about population structure of Chinook salmon in Upper Cook Inlet, temporal stability of allele frequencies in wild populations, and our experience collecting Chinook salmon adults above Devils Canyon in 2012.

Action Item: Betsy asked for the following 4 items by email:

1. An updated Upper Cook Inlet Chinook collection list that includes all 2012 samples that our lab collected.
2. An allele frequency temporal stability paper for sockeye salmon.
3. The Chinook salmon baseline as it stood in May, 2012 – you can see from the updated collection list (above) that there are more collections to add now.
4. The trip report for the 2012 collections of Chinook salmon made by ADFG Gene Conservation Lab in 2012.

SUSITNA-WATANA HYDROELECTRIC PROJECT

MEETING RECORD

AEA Team Member		Other Party	
Name:	*Chris Habicht*	**Name:**	*Betsy McCracken*
Organization:	*ADFG Genetics*	**Organization:**	*USFWS*
Study Area:	*Susitna River and Upper Cook Inlet*	**Phone Number:**	*907-271-2783*
Date:	*2012 11 02*	**Time:**	
Meeting held by: [ ] AEA Team [X] Other Party			

Others at meeting: Bill Templin, Andy Barclay

Subject: At the request of Betsy, we met to discuss AEA's study plan objectives for the Fish Genetics Proposed Study Plan

Discussion: We had a broad discussion regarding AEA's Baseline Fish Genetics Proposed Study Plan, where Betsy asked for clarification on the genetic analyses proposed and advice on what analyses might be useful in addressing the primary questions: 1) answer whether or not the Chinook above Devil's Canyon are genetically distinct, 2) determine the effective Chinook spawning population size above Devil's Canyon, and 3) if these Chinook are genetically distinct above Devil's Canyon, what proportion of the Susitna River spawning population do they contribute? We also discussed genetic structure of other Pacific salmon, basic population genetic theory, and genetic mixed-stock analyses applications.

Action Item: Betsy incorporated our responses into a document: "USFWS Comments on AEA's Baseline Fish Genetics Proposed Study Plan (PSP)" that she provided back to us the following day for review.

Attachment C

Comment/Response Table for Development of Final 2013 Project Operational Plan

Responses to Comments on Draft Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska.

Reference Number	Commenter	Comment Date	Comment	AEA's Response
NMFS-1	NMFS	4/12/2013	"Section 4.2.1 While the proposed sampling strategy is impressive, adult Chinook salmon are inherently difficult to sample because of their large size and preferred spawning habitat, often in fast deep water. This is clearly recognized in Table 2 as the preferred sample size is identified as 200 for each of the 6 sublocations above Devil's Canyon, yet the expected cumulative total for all 6 aggregated sublocations is identified as only 50. Given anticipated sampling difficulties, it's unclear whether ADF&G will be able to collect the minimum sample set of 50 representative Chinook salmon above Devil's Canyon in just two years, especially above the proposed dam site. Even if successful, 50 appears to be a low number of samples to compare to identify genetic differences in related stocks. The authors should consider other options in case the realized sample numbers are too low to address project objectives."	After considering other options, as requested, AEA has revised the Implementation Plan (IP) to include identifying tissues from juvenile Chinook as a potentially useful tool for augmenting adult collections. *See* Implementation Plan Section 4.6.2.

Reference Number	Commenter	Comment Date	Comment	AEA's Response
NMFS-2	NMFS	4/12/2013	"Section 4.7.1 Regarding the proposed preliminary analysis of the 10 samples collected in Kosina Creek in 2012, there is concern regarding the validity of the test. It's possible given the small number of samples that the power of the test may not be strong enough to identify differences if they exist. It's also possible that the small sample set could be biased in some way and therefore suggest differences where they may not exist. Because of this potential for misinterpretation, the authors should consider first performing some type of power analysis with existing populations of known genetic divergence to gauge the validity of comparing 10 samples from a single aggregation. If the test can't statistically be done with 10 samples, it might be best to hold any comparison until the sample sets are strong enough for a statistically reliable test."	Agreed. AEA will process the samples, but not test, analyze, or report until sample sizes are appropriate. AEA has revised IP to reflect this change. *See* Implementation Plan Section 4.7.1.
NMFS-3	NMFS	4/12/2013	"Regarding the sampling locations upstream of the proposed dam site, the authors should consider including adult and juvenile Chinook salmon sampling upstream of the Oshetna River (location 22 on Figure 2). My understanding is that the Oshetna River is the furthest upstream location that juvenile Chinook	No salmon have been documented in the Susitna watershed above the confluence of the Oshetna River. The salmon escapement study (Section 9.7) will apply radio tags to the salmon population to document fish distribution in the Upper River, including above the Oshetna River, in 2013 and 2014. With 10-15% of the

Reference Number	Commenter	Comment Date	Comment	AEA's Response
			salmon were identified in the past, but it's possible those juveniles could have originated from further upstream spawning aggregates and it's not clear whether locations upstream of the Oshetna River have been surveyed for even presence or absence of salmon; no apparent barrier to their migration is noted and habitat appears suitable."	fish radiotagged in the Middle River (1 in 5 to 1 in 7 fish) each year, radiotagging will detect very small aggregations of fish in the Upper River and this will provide the high-powered test to find any fish above the Oshetna River. Although AEA has not revised the IP in response to this comment, AEA acknowledges that the boundary may be reconsidered as information becomes available.
NMFS-4	NMFS	4/12/2013	"Given that previous studies were completed in the past regarding the proposed dam site, it would be helpful to determine whether samples such as scales are available from historical studies. DNA from historical scales might help differentiate between the 3 different hypotheses identified in 2.2.1."	AEA has contacted several experts and leads of historical studies, and determined that no Chinook salmon were sampled from above Devils Canyon during these studies. No change to IP based upon this comment.
NMFS-5	NMFS	4/12/2013	"Section 4.6 While the proposed tests will be used to differentiate between the three hypotheses, the specific level of divergence used to discriminate fish populations is unclear. This is presumably because the number of available samples will shape the utility of the potential tests and interpretation of the results will be done later in 2014 and 2015 in consultation with other laboratories."	Agreed. Our approach needs to be partially determined by samples and preliminary results. As proposed, we will confer with NMFS and USFWS before analysis begins.

Reference Number	Commenter	Comment Date	Comment	AEA's Response
NMFS-6	NMFS	4/12/2013	"Juvenile salmon species can be difficult to distinguish, thus the authors should include species ID for juveniles collected at least upstream of the proposed dam site and possibly below. Such an analysis might provide additional information regarding potential spawning success of all salmon species."	Agreed. Above Devils Canyon, AEA will collect tissues from all Pacific salmon captured and AEA will verify species through DNA analysis. Below Devils Canyon, field identification will be to Pacific salmon species, but DNA analysis will be used to verify that field species identification is being done correctly. AEA has revised IP in response to this comment. *See* Implementation Plan Sections 4.2.4.3 and 4.4.
NMFS-11	NMFS	4/12/2013	Comments 1-5 listed by NMFS as "minor grammatical suggestions"	Accepted. *See* various sections of the Implementation Plan.
NMFS-7	NMFS	4/12/2013	"Page 5, under "Objectives": might consider separating into primary and secondary objectives"	No change to IP. Objectives should remain as written in RSP.
NMFS-8	NMFS	4/12/2013	"Page 7, under "Sample collection targets": might consider separating into primary and secondary sampling goals"	AEA does not believe it is necessary to distinguish between primary and secondary goals. AEA has not revised IP in response to this comment.
NMFS-9	NMFS	4/12/2013	"Page 12, second paragraph: "…. exclude from the baseline all …" – are juveniles going to be included in the genetic baseline?"	Juveniles will be included in the baseline above Devils Canyon, if needed for supplementing adult collections (see response to NMFS-1). No juveniles collected below Devils Canyon will be used for baseline. *See* Implementation Plan Sections 4.2.1 and 4.6.2.
NMFS-10	NMFS	4/12/2013	"Page 32-36, Figures 2-6: helpful to identify the proposed dam site on the maps"	AEA has clarified in the caption in Figure 2 that the dam site is RM 184. Figures 3-6 do not include the area where the

Reference Number	Commenter	Comment Date	Comment	AEA's Response
				proposed dam is located. *See* Implementation Plan Figure 2.
USFWS-1	USFWS	4/12/2013	"Page 3, Section 2.1.1, Assessing Chinook salmon population structure: This section could be improved by organizing it into three paragraphs, one for a description of each of the hypotheses of population structure above Devil's Canyon. For Hypothesis 1a, temporal variation in allele frequencies may be seen in small, genetically isolated populations (Waples and Teel 1990)."	Agreed. In response to this comment, AEA has revised the IP. *See* Implementation Plan Section 2.1.1.
USFWS-2	USFWS	4/12/2013	"Page 5, Section 3, Objectives: In the last line of the paragraph introducing the objectives, it reads "…(3) assess the use of Lower and Middle River habitat by juvenile Chinook salmon originating in the Middle and Upper Susitna River." Should this be "Lower River habitat" (delete the word Middle), to follow what is written in Objective 5, "…selected Lower River habitats…"?"	AEA has not changed the IP in response to this comment. Chinook salmon contributions to the Lower and Middle River from upstream sources are of interest (Goal 3). It is in the Lower River that we will examine contributions at the level of habitat type (Objective 5). Other studies will be sampling juveniles from the Middle River opportunistically. These samples will be preserved but only analyzed if needed.
USFWS-3	USFWS	4/12/2013	"Page 5, Section 3 Objectives, Objective #3: There needs to be justification on why samples outside of the Susitna River are being collected for Chinook salmon."	AEA has not revised the IP in response to this comment. The IP includes this collection of samples because it was included in RSP, in response to FERC requests.
USFWS-4	USFWS	4/12/2013	"Page 6, Section 4.2, Samples to collect: This section about recommended sample	Agreed. AEA's approach will be partially determined by samples and preliminary

Reference Number	Commenter	Comment Date	Comment	AEA's Response
			sizes was confusing; some background information and citations seem to be missing (e.g., for the first sentence) or misplaced (e.g., Nei 1978). Sample sizes are partially dependent on the genetic divergence among stocks, the information content of the genetic markers, and adequate estimate of allele frequencies. A more thorough description or better references, for example the recent reports for chum salmon and sockeye salmon MSA, would be useful here."	results. AEA will confer with technical representatives from NMFS and USFWS before analysis begins. AEA has modified the IP to include appropriate citations and clarify the rationale for appropriate sample sizes. *See* Implementation Plan Section 4.2.
USFWS-5	USFWS	4/12/2013	"Page 7, Section 4.2.1, Sample collection target #5 and Page 9, Section 4.2.4, Juvenile Chinook salmon collection above Three Rivers confluence: Why is only the Oshetna River being sampled for juveniles, since adults were collected in Kosina Creek and juveniles have been seen here? We have not checked the Anadromous Waters Catalog, but all tributaries above the Canyon should be sampled for juveniles. Chinook salmon juveniles can migrate quite some distances from their tributary of origin (e.g., Daum and Flannery 2011)."	From Devils Canyon to the Oshetna River, 4 tributaries will be targeted for sampling of juvenile Chinook salmon (Oshetna Creek, Kosina River, Fog Creek, and Cheechako Creek). Above the Oshetna River, results from the Salmon Escapement Study (RSP Section 9.7) in 2013-2014 will determine whether additional tributaries should be surveyed - see response for NMFS-3. AEA has revised the IP and RSP to add juvenile collection sites from above Devils Canyon to the Oshetna River. *See* Implementation Plan Sections 4.2.1 and 4.2.4.1.
USFWS-6	USFWS	4/12/2013	"There should be some justification on why juvenile samples collected below the falls that are used for "baseline" will	Agreed. Juvenile collections below Devils Canyon will not be used as baseline. Adult collections below Devils

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			likely not comprise a mixture of stocks (please see Specific Comment Page 13, for other suggestions on how these samples could be used.)"	Canyon should be sufficient. AEA has revised IP in response to this comment. *See* Implementation Plan Section 4.2.1.
USFWS-7	USFWS	4/12/2013	"Page 9, Section 4.2.6, Other species collections: It sounded like resident species are going to be in bulk collections. Is that a single bulk collection for the entire Susitna River, or a bulk collection for each sampling site (recommend the latter)?"	AEA has revised IP to specify five spatial groups: Chulitna R., Talkeetna R., Upper Susitna River, and Middle Susitna River (broken into above and below Devils Canyon). *See* Implementation Plan Section 4.2.5.
USFWS-8	USFWS	4/12/2013	"Page 12, Section 4.5, Data Retrieval and Quality Control: Elimination of siblings will only be done for juvenile collections for baseline?"	Clarified to IP to indicate adult salmon will be analyzed for sibling relationships, but adult siblings will still be used in tests. *See* Implementation Plan Section 4.6.2.
USFWS-9	USFWS	4/12/2013	"Page 12, Section 4.6.2, H-W Expectation: There may be some deviations from HW expectation by chance. Is it really necessary to delete the collection(s) from further analysis? Should HW testing be conducted after temporal pooling?"	No change to IP. AEA agrees that deviations from HWE may be by chance, and will confer with technical representatives from NMFS and USFWS prior to analysis.
USFWS-10	USFWS	4/12/2013	"Page 13, Section 4.6.8, Testing among hypotheses: This section needs to be expanded. The Evolutionary Criteria of Waples and Gaggiotti (2006) should be described, and related to Hypotheses 1a, 1b, and 2. What are the three levels of the hierarchical analysis? Evaluating the	AEA has revised IP to clarify. Upon determining sample sizes and results, AEA will select an appropriate approach after seeking input from NMFS and USFWS technical representatives. *See* Implementation Plan Section 4.6.9.

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			Evolutionary Criteria/Hypotheses through estimating effective population size may not be very powerful if confidence limits are large. Also, unless large sample sizes are achieved, estimating Ne may not be very successful (Waples 1989, England et al. 2005). It may not be possible to use the temporal method, because the time span in the samples collected may not be large enough. The collections of juveniles may be useful in Ne estimation, provided they represent a single cohort and population. It may be possible to determine if juveniles are from one cohort by measuring individual length to determine if sizes fall in a single mode."	
USFWS-11	USFWS	4/12/2013	"Another possible analysis is to use the program MIGRATE to both estimate migration rates and direction and Neμ. This analysis may also be of interest for the juvenile collections above and below the canyon."	No change to text, but AEA agrees to evaluate various analytical methods and will confer with technical representatives from NMFS and USFWS.
USFWS-12	USFWS	4/12/2013	"Page 14, Section 4.7.1 Assessing reporting groups (including above Devil's Canyon for MSA: Delete preliminary test using Kosina Creek 2012, N=10. Wait until more samples are collected."	Agreed. AEA will process the samples, but not test, analyze, or report. AEA revised IP text to reflect this change. *See* Implementation Plan Section 4.7.1.