

**Susitna-Watana Hydroelectric Project**  
**(FERC No. 14241)**

**Regional Operational Plan DF.#R.13-XX**

**DRAFT**

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

Prepared for

Alaska Energy Authority



Prepared by

Andrew W. Barclay  
Alaska Department of Fish and Game

March 29, 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

March 2013

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

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Project leader(s): Andrew W. Barclay Fishery Biologist III

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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.

<b>Weights and measures (metric)</b>		<b>General</b>		<b>Mathematics, statistics</b>	
centimeter	cm	Alaska Administrative Code	AAC	<i>all standard mathematical signs, symbols and abbreviations</i>	
deciliter	dL	all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.	alternate hypothesis	H <sub>A</sub>
gram	g	all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.	base of natural logarithm	e
hectare	ha	at	@	catch per unit effort	CPUE
kilogram	kg	compass directions:		coefficient of variation	CV
kilometer	km	east	E	common test statistics	(F, t, $\chi^2$ , etc.)
liter	L	north	N	confidence interval	CI
meter	m	south	S	correlation coefficient (multiple)	R
milliliter	mL	west	W	correlation coefficient (simple)	r
millimeter	mm	copyright	©	covariance	cov
		corporate suffixes:		degree (angular)	°
<b>Weights and measures (English)</b>		Company	Co.	degrees of freedom	df
cubic feet per second	ft <sup>3</sup> /s	Corporation	Corp.	expected value	E
foot	ft	Incorporated	Inc.	greater than	>
gallon	gal	Limited	Ltd.	greater than or equal to	≥
inch	in	District of Columbia	D.C.	harvest per unit effort	HPUE
mile	mi	et alii (and others)	et al.	less than	<
nautical mile	nmi	et cetera (and so forth)	etc.	less than or equal to	≤
ounce	oz	exempli gratia	e.g.	logarithm (natural)	ln
pound	lb	(for example)		logarithm (base 10)	log
quart	qt	Federal Information Code	FIC	logarithm (specify base)	log <sub>2</sub> , etc.
yard	yd	id est (that is)	i.e.	minute (angular)	'
		latitude or longitude	lat. or long.	not significant	NS
<b>Time and temperature</b>		monetary symbols		null hypothesis	H <sub>0</sub>
day	d	(U.S.)	\$, ¢	percent	%
degrees Celsius	°C	months (tables and figures): first three letters	Jan, ..., Dec	probability	P
degrees Fahrenheit	°F	registered trademark	®	probability of a type I error (rejection of the null hypothesis when true)	$\alpha$
degrees kelvin	K	trademark	™	probability of a type II error (acceptance of the null hypothesis when false)	$\beta$
hour	h	United States (adjective)	U.S.	second (angular)	"
minute	min	United States of America (noun)	USA	standard deviation	SD
second	s	U.S.C.	United States Code	standard error	SE
<b>Physics and chemistry</b>		U.S. state	use two-letter abbreviations (e.g., AK, WA)	variance	
all atomic symbols				population	Var
alternating current	AC			sample	var
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				

## 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 and Upper River (>RM 98; 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 (Waples and Gaggiotti 2006). If the canyon acts as a partial barrier, support for one of the hypotheses can be obtained by comparing the allele frequencies of fish above Devils Canyon to other nearby spawning aggregates below the canyon, by measuring the stability of the allele frequencies of these fish through time (Figure 1), and by examining conformance to Hardy-Weinberg expectations (HWE; Figure 1). Allele frequencies similar to those in nearby potentially-contributing spawning aggregates from below the canyon would indicate that the fish ascending Devils Canyon are likely 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.

High genetic divergence between fish spawning above Devils Canyon and fish spawning in contributing aggregates could indicate a self-sustaining population with little genetic flow with other populations (Hypothesis 1a), or recent colonization with small numbers of successfully-contributing families (Hypothesis 1b). A recent colonizing by a small number of successfully-contributing families, along with high gene flow from straying fish each generation (Hypothesis 1a), might also be interpreted as an indication of a self-sustaining spawning aggregate (Hypothesis 1b) 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). 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 Hypothesis 1b or Hypothesis 2, if strays originate from multiple populations 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 will be 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 will be addressed in the final Implementation Plan for 2013.

An updated, detailed annual Implementation Plan will be prepared and circulated to TWG members by April 30 of 2014. This 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 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 in season 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 markers with moderate numbers of alleles (i.e. uSATs) is 200 fish per population and for markers with two alleles (i.e. SNPs) is 100 fish per collection. Ideal sample size for baseline collections for mixed stock analyses (MSA) using markers with two alleles is 100 fish per population (Allendorf and Phelps 1981; Waples 1990). Sample sizes of 50 fish per population are adequate to conduct coarse-scale population structure analyses and MSA using SNPs (Nei 1978). 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 on previous efforts (and results) and on information from the aforementioned catalog and studies.

#### 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 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 200 juvenile Chinook salmon at each of the following: Chinook Creek, Oshetna River, Indian River, Portage Creek, the mainstem Susitna River upstream of the Three Rivers Confluence, as well as Talkeetna and Chulitna rivers (1,400 fish; Objectives 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 fin, 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 4<sup>th</sup>, 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 above Three Rivers Confluence**

Tissue samples from juvenile Chinook salmon will be collected at Chinook Creek, Oshetna River, Indian River, Portage Creek, the mainstem Susitna River upstream of the Three Rivers Confluence, and the Talkeetna and Chulitna rivers. 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.

#### **4.2.5. Lower River juvenile Chinook salmon 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.

#### **4.2.6. 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).

#### **4.2.7. 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.

#### **4.2.8. 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 from the baseline collections will be extracted from axillary processes using DNeasy 96 tissue kits. Chinook salmon samples will be analyzed for 96 single nucleotide polymorphism (SNP) markers and 12 microsatellite markers.

The DNA samples will be analyzed using Fluidigm 96.96 Dynamic Arrays (<http://www.fluidigm.com>). The Fluidigm 96.96 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame. On one side of the frame there are 96 inlets to accept the sample DNA from each individual fish and on the other are 96 inlets to accept the assays for each SNP marker. Once in the wells, the components are pressurized into the chip using the IFC Controller HX (Fluidigm). The 96 samples and 96 assays are then systematically combined into 9,216 parallel reactions. Each reaction is a mixture of 4 microliters (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, three analyses will be performed to confirm the quality of the data. First, SNP markers will be identified that are invariant in all individuals or that have very few individuals with the alternate allele in only one collection. These markers will be excluded from further statistical analyses. Second, individuals will be identified that are missing substantial genotypic data because they likely have poor quality DNA. Individuals missing substantial genotypic data will be identified using the 80 percent rule (missing data at 20 percent or more of loci; Dann et al. 2009). These individuals will be removed from further analyses. The inclusion of individuals with poor quality DNA might introduce genotyping errors into the baseline and reduce the accuracies of mixed stock analyses.

The second 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. 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 *Adegene* package (Jombart 2008). Probabilities will be combined for each collection across loci and for each locus across collections using Fisher's method (Sokal and Rohlf 1995), and collections and loci that violated HWE will be excluded from subsequent analyses after correcting for multiple tests with Bonferroni's method ( $\alpha = 0.05$  per number of collections).

### **4.6.3. 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).

### **4.6.4. 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.

#### 4.6.5. 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.

#### 4.6.6. Hierarchical Log-likelihood Ratio Tests

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

#### 4.6.7. 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).

#### 4.6.8. Testing Among Hypotheses

For the first hypothesis criterion in Figure 1, we will test for panmixia (spawning aggregates belong to the same population) using Evolutionary Criterion (EV)1 (Waples and Gaggiotti 2006). Panmixia will also be examined with the Fisher’s exact test of allele frequency homogeneity. For the second hypothesis criterion in Figure 1, we will test for different levels of migration using EV2-EV4 (Waples and Gaggiotti 2006). This second hypothesis will also be tested with a hierarchical, three-level analysis of variance (ANOVA). In addition, further

support/lack of support for the hypotheses will be evaluated by calculating effective population sizes and conformation to HWE. Effective population sizes will be calculated using single-year sample (Tallmon et al. 2008), multiple-year sample (Waples 1991, Tallmon et al. 2004), linkage disequilibrium (Waples 2006), and heterozygote excess (Luikart and Cornuet 1999) methods.

## **4.7. Mixed-Stock Analysis**

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

In response to FERC from 2/1/2013, a preliminary analysis of SNP data from 42 loci using the selected pre-existing baseline and the 2012 collections will be conducted to provide some insight into the potential of genetic data to detect fish from above Devils Canyon in mixtures (SPD). We will use Fisher's exact test (Sokal and Rohlf 1995) of allele frequency homogeneity to test for differences in allele frequencies between a population spawning just below the canyon (Portage Creek; n=95) and the samples collected in Kosina Creek in 2012 (n=10). This analysis accounts for sample size, and therefore will have low statistical power. 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. However, a non-significant difference will not preclude the use of MSA: adequate genetic differentiation may be uncovered with the screening of additional SNP and microsatellite markers and more samples added to the baseline.

A more 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.
- Technical Memo regarding the 2012 collections: 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:
  - September 30, 2013. Technical memo with results from the Fisher’s exact test for differences in allele frequencies between a population spawning just below the canyon (Portage Creek) and the samples collected in Kosina Creek in 2012.
  - 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
<p>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.</p>	<p>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.</p>	<p>15-16</p>
<p>To the extent feasible, we recommend that AEA collect tissue samples over a representative proportion of the entire adult Chinook salmon run.</p>	<p>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.</p>	<p>6-8</p>
<p>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.</p>	<p>Dates for the analysis and reporting of the 2012 collections have been added to the Schedule and Deliverables section of the Implementation Plan.</p>	<p>15-16</p>
<p>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.</p>	<p>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.</p>	<p>13-14</p>
<p>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.</p>	<p>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.</p>	<p>13</p>

**Table 2. Area, location, and sub location of desired baseline samples of adult Chinook 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 2.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes		
					Archived	This project	
					Ideal	Expected	
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	
16			West Fork			200	
17			Honolulu Creek			200	
18			Byers Creek			200	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	
23			Kosina Creek	2012	10	190	
24			Watana Creek			200	50
25			Tsusena Creek			200	
26			Fog Creek			200	
27			Devil Creek			200	
29		Susitna River, above Three Rivers Confluence but below Devils Canyon	Portage Creek	2009, 2010, 2011	141	59	59
28			Chinook Creek			200	
30			Indian River	1212	1	199	
31			Gold Creek			200	75
32			Lane Creek			200	
33			Chase Creek			200	
35		Talkeetna River	Prairie Creek	1995, 2008	169	31	31
34			Upper mainstem			200	
36			Iron Creek			200	
37			Disappointment Creek			200	100
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	
43			Sheep Creek			200	50
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	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	



**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		
					Archived	This project	
					Ideal	Expected	
<b>Susitna River</b>							
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		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		Susitna River	sloughs above Three Rivers Confluence	1996	103	0	100
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.

Map No.	Area	Location	Sublocation	Year(s) Collected	Sample sizes				
					Archived	This project			
					Ideal	Expected			
1	Susitna River above Three Rivers Confluence	Chulitna River	East Fork			100	200		
2			Middle Fork			100			
3			Honolulu Creek			100			
4			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		Susitna River		Portage Creek			100	200	
11				Indian River			100		
12				Gold Creek			100		
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

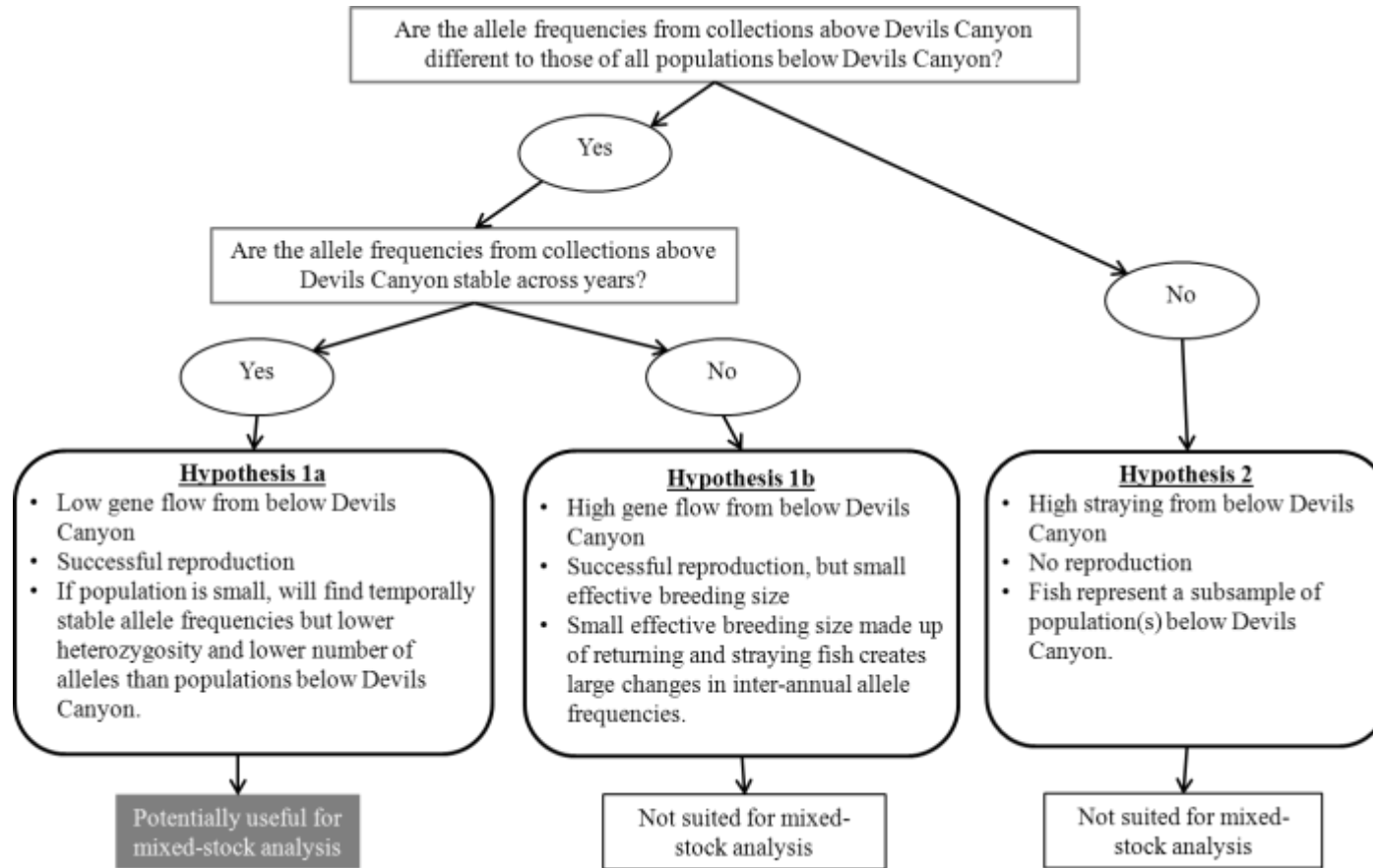
**Table 6. Location, and sublocation of desired baseline samples of adult pink salmon spawning aggregates for genetic analysis.**

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		
					Archived	This project	
					Ideal	Expected	
1	Susitna River above Three Rivers Confluence	Chulitna River	Middle Fork		100		
2			Troublesome Creek		100		100
3			Spink Creek		100		
4		Susitna River	Portage Creek		100		50
5			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

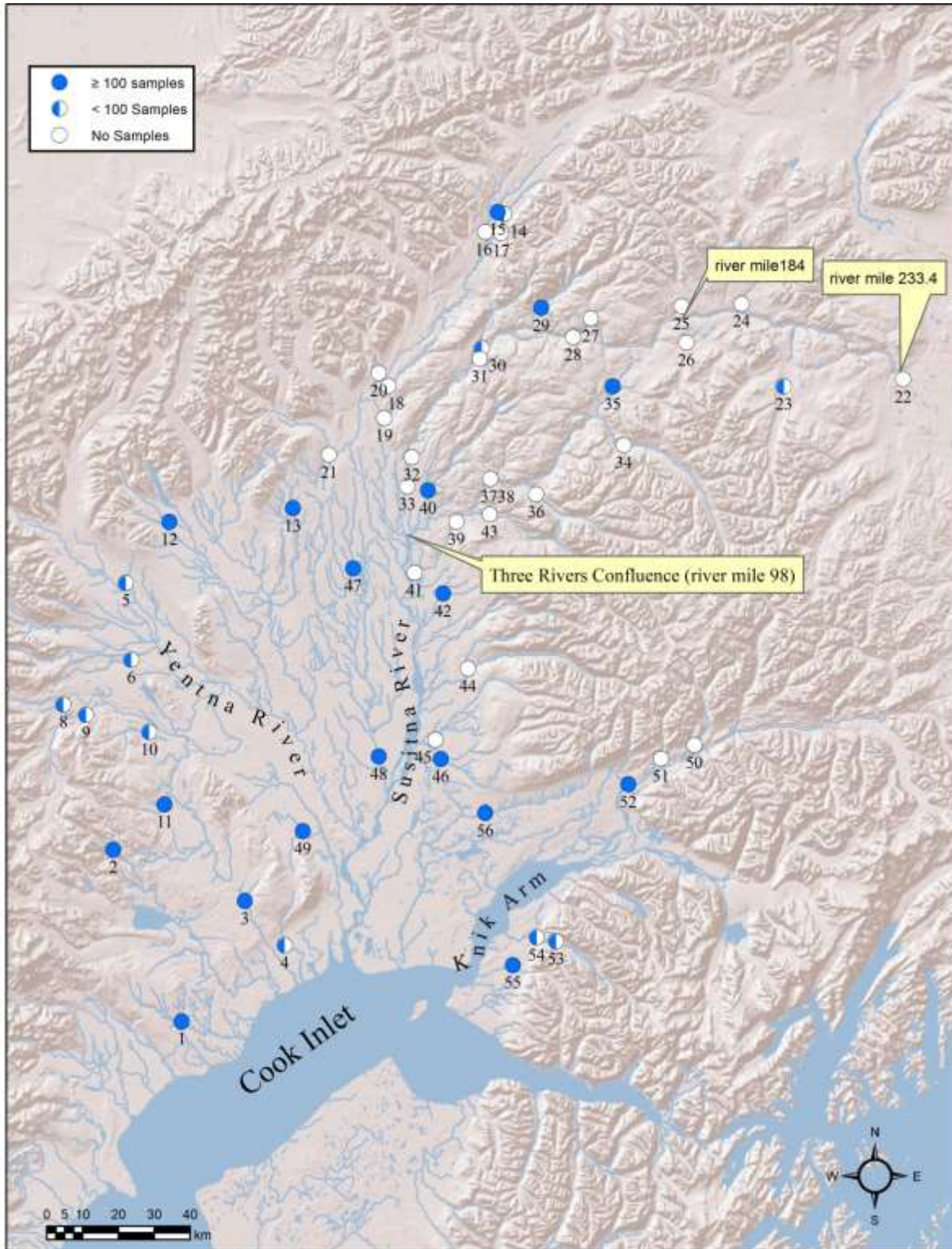
**Table 7. Potential resident and non-salmon anadromous fish species targeted for genetic tissue sampling in the Susitna River.**

<b>Common Name</b>	<b>Scientific Name</b>
Rainbow trout	<i>Oncorhynchus mykiss</i>
Humpback whitefish	<i>Coregonus pidschian</i>
Round whitefish	<i>Prosopium cylindraceum</i>
Lake whitefish	<i>Coregonus clupeaformis</i>
Bering cisco	<i>Coregonus laurettae</i>
Eulachon	<i>Thaleichthys pacificus</i>
Pacific lamprey	<i>Lampetra tridentata</i>
Longnose sucker	<i>Catostomus catostomus</i>
Slimy sculpin	<i>Cottus cognatus</i>
Prickly sculpin	<i>Cottus asper</i>
Coastal range sculpin	<i>Cottus aleuticus</i>
Pacific staghorn sculpin	<i>Leptocottus armatus</i>
Burbot	<i>Lota lota</i>
Arctic grayling	<i>Thymallus arcticus</i>
Dolly Varden	<i>Salvelinus malma</i>
Lake trout	<i>Salvelinus namaycush</i>
Northern pike	<i>Esox lucius</i>
Threespine stickleback	<i>Gasterosteus aculeatus</i>
Ninespine stickleback	<i>Pungitius pungitius</i>
Alaska blackfish	<i>Dallia pectoralis</i>



**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.**

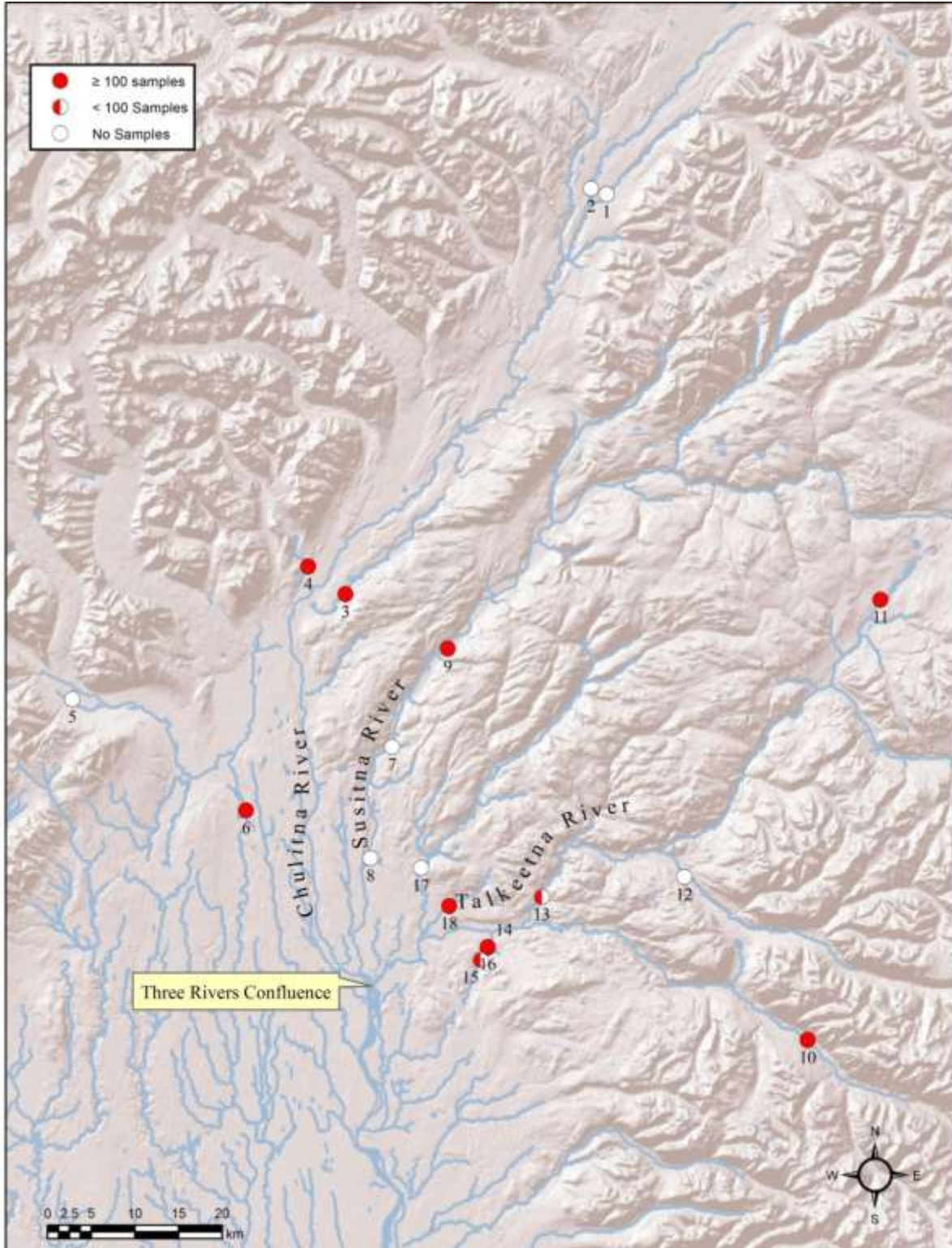
Only a self-sustaining population (Hypothesis 1a) will potentially result in genetic variation suitable for mixed-stock analysis for estimating the proportion of juvenile Chinook salmon mixtures collected in the Middle and Lower Susitna River that originate from above Devils Canyon.



**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).

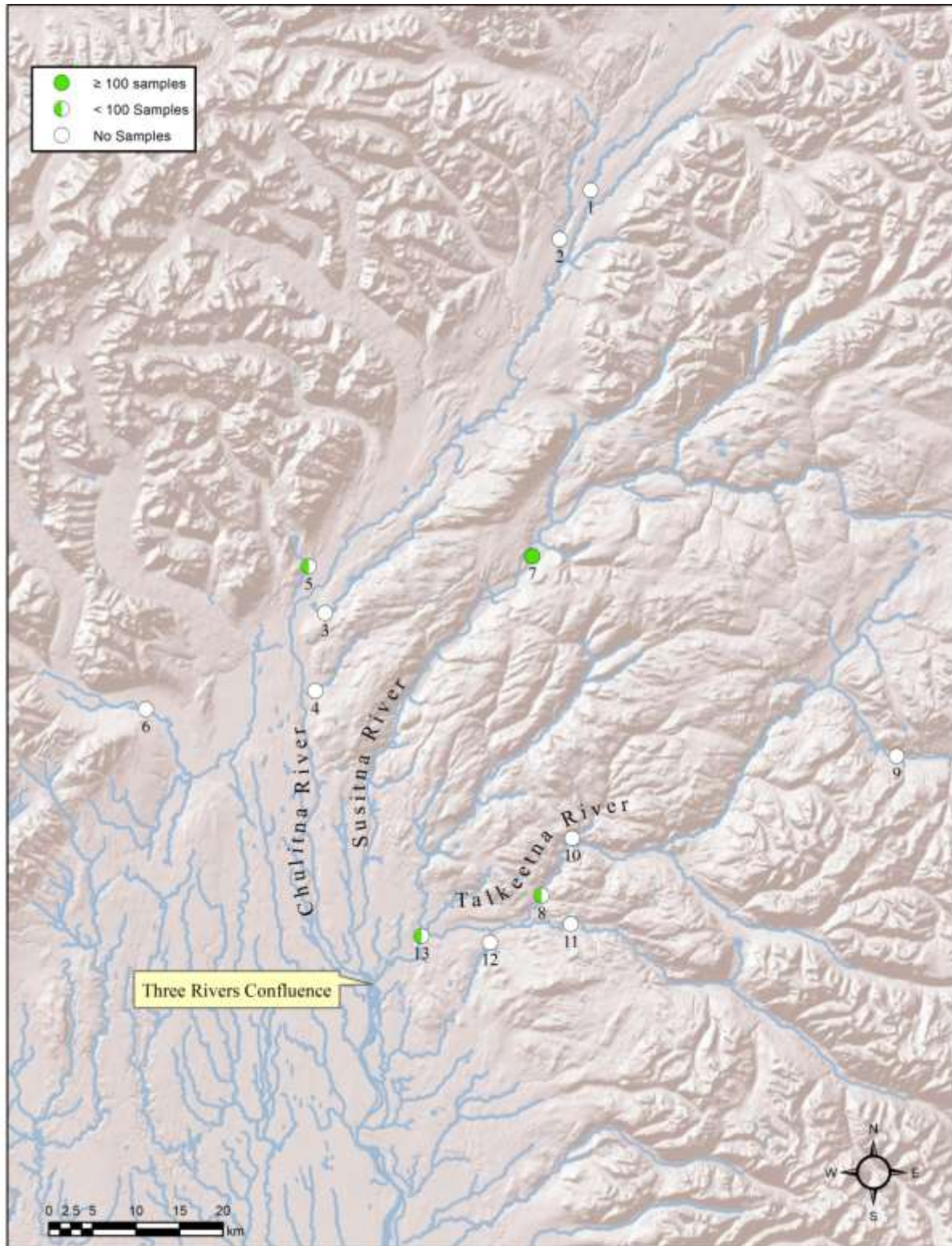




**Figure 3. Potential baseline sampling locations for adult sockeye salmon.**

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

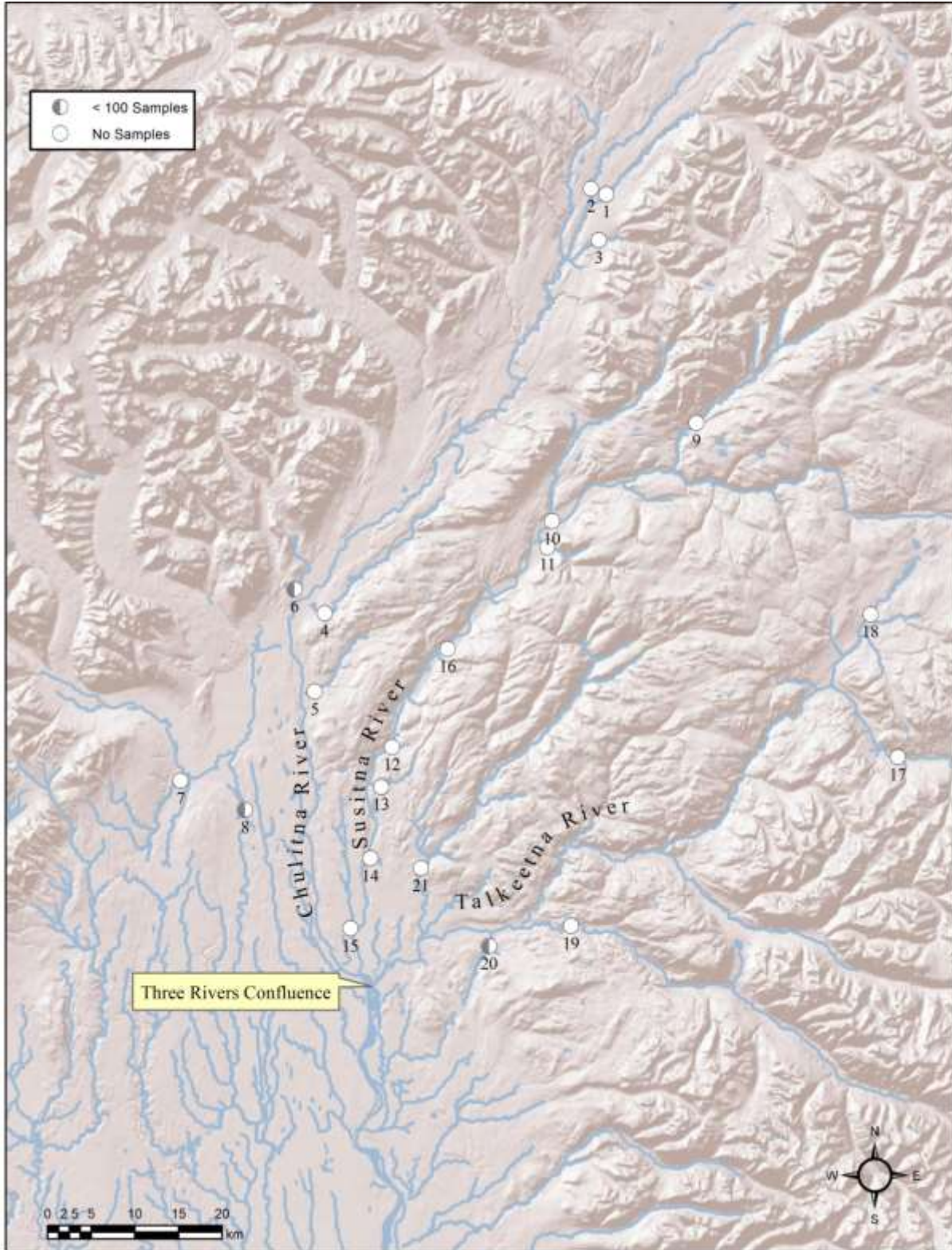




**Figure 4. Potential baseline sampling locations for adult chum salmon.**

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.



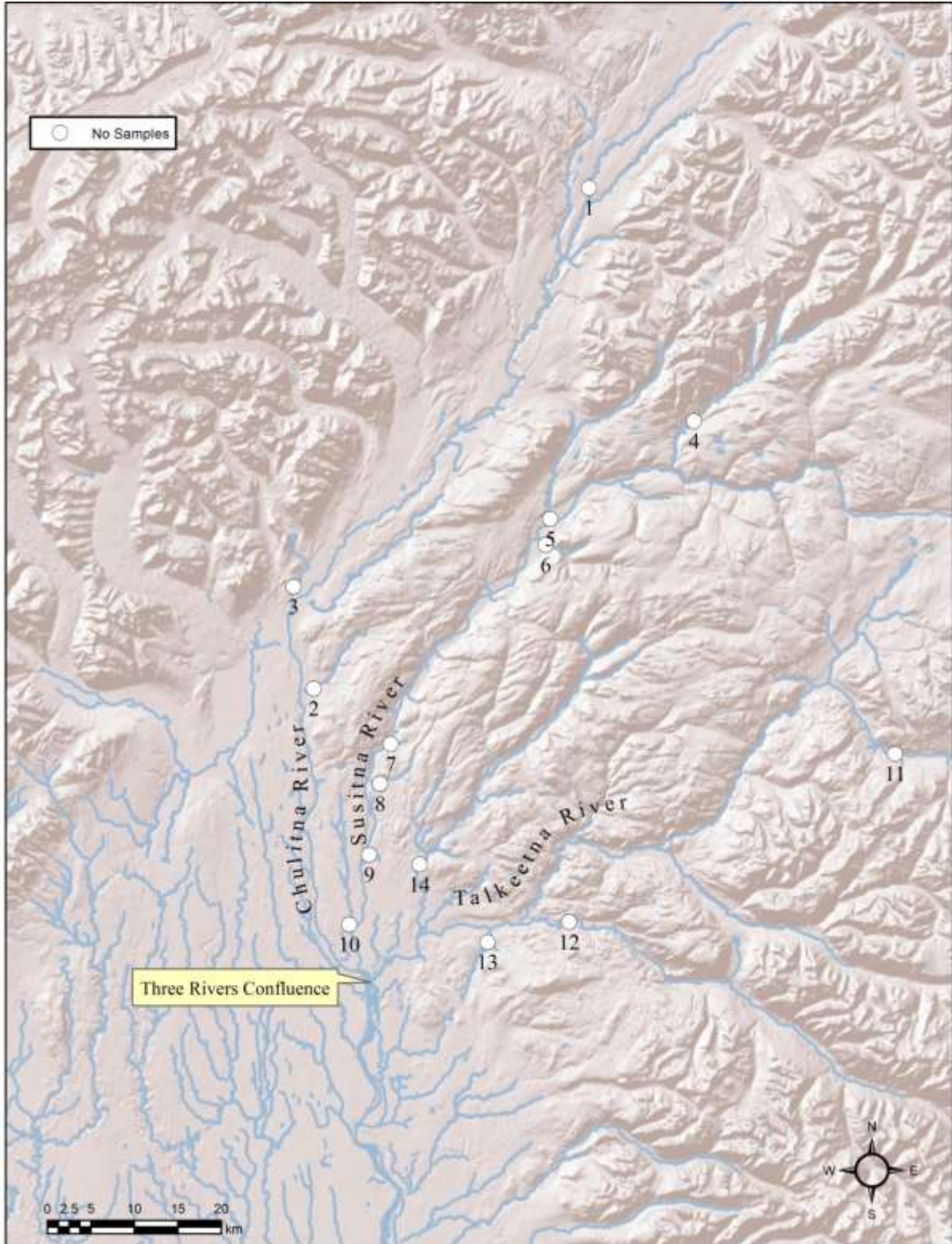


Figure 6. Potential baseline sampling locations for adult pink salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives.

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

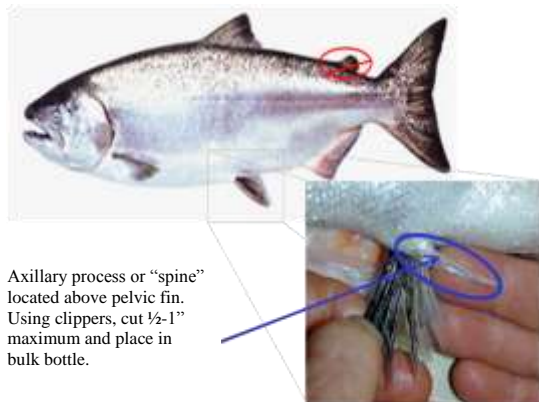
#### I. General Information

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. Steps for collecting axillary process tissues:



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



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



Supplies included in sampling kit:

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.

- 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.

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

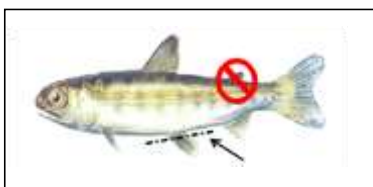
### ADF&G Gene Conservation Lab, Anchorage

#### 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.

<b>Return to ADF&amp;G Anchorage lab:</b>	ADF&G – Genetics 333 Raspberry Road Anchorage, Alaska 99518	Lab staff: 907-267-2247 Judy Berger: 907-267-2175 <b>Freight code:</b> _____
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#### Appendix A 2.–Vial sampling instructions for juvenile Chinook salmon.