Susitna-Watana Hydroelectric Project
(FERC No. 14241)

Genetic Baseline Study for Selected Fish Species
Study Plan Section 9.14

Initial Study Report
Part B: Supplemental Information (and Errata) to Part A (February 3, 2014 Draft Initial Study Report)

Prepared for

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

Prepared by

Gene Conservation Laboratory
Commercial Fisheries Division
Alaska Department of Fish and Game

June 2014

PART B: SUPPLEMENTAL INFORMATION (AND ERRATA) TO PART A (FEBRUARY 3, 2014 DRAFT INITIAL STUDY REPORT)

Part A Reference	Description
Section 1	ADF&G developed a draft 2014 Implementation Plan. In preparation of developing the plan, ADF&G and AEA met with NMFS and USFWS on March 12, 2014 to review preliminary results. In response to the input received during the meeting, a draft Implementation Plan was developed incorporating the comments received during the meeting and was provided to the Services on April 2, 2014 for further review and comment. The USFWS and NMFS provided comments on May 13, 2014 and May 12, 2014, respectively. These comments were considered in developing the final 2014 Implementation Plan, which is included herein as Attachment 1. The 2014 Implementation Plan includes a table indicating the comments received from the Services and how they were addressed.

PART B – ATTACHMENT 1: FINAL 2014 IMPLEMENTATION PLAN FOR THE GENETIC BASELINE STUDY FOR SELECTED FISH SPECIES IN THE SUSITNA RIVER, ALASKA

Susitna-Watana Hydroelectric Project
(FERC No. 14241)

Genetic Baseline Study for Selected Fish Species
Study Plan Section 9.14

Initial Study Report
Part B - Attachment 1
Final 2014 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.

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REGIONAL OPERATIONAL PLAN

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

June 2014

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/.

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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, 2014 – March 31, 2015
Field Dates:	May 1, 2014 – October 30, 2014
Plan Type:	Category III

Approval

Title	Name	Signature	Date
Project Leader	Andrew W. Barclay		
Biometrician	Jim Jasper		
Research Coordinator	Chris Habicht		
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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	ft3/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.	Unite d States Code
U.S. state two-letter abbreviations (e.g., AK, WA)	use

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	HA
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, $\chi 2$, 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
Hardy-Weinberg Equilibrium	HWE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	log2, etc.
minute (angular)	'
not significant	NS
null hypothesis	HO
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 Project River Mile (PRM) 187.1, approximately 34 miles upstream of Devils Canyon (Figure 1). Construction and operation of the Susitna-Watana Hydroelectric Project, FERC Project No. 14241 (Project) could impact the composition and distribution of fish populations.

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

On February 1, 2013, the Commission issued its Study Plan Determination (SPD) for 44 of the 58 proposed individual studies in the AEA RSP for the Project. In the SPD, the Commission approved the Genetic Baseline Study with certain recommended modifications.

The Genetic Baseline Study (Study 9.14) requires AEA to develop and file detailed annual project operational plans with the Commission. These operational plans 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.

On March 12, 2014, ADF&G and AEA consulted with the USFWS and NMFS regarding the scope and development of the project operational plans for the planned work in 2014. Following this consultation meeting, AEA prepared a draft 2014 Implementation Plan. This final 2014 Genetics Implementation Plan establishes details for field sampling efforts including relative priorities, temporal and spatial sampling considerations, and statistical analysis methods that take into account the experience from the 2013 field season and consultation with USFWS and NMFS(Table 1). Changes from the 2013 Genetics Implementation Plan that resulted from consultation with USFWS and NMFS are also identified.

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 represent 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 to 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 among populations 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 that are large enough to detect using methods widely used by programs that conduct population genetics and mixed-stock analyses (MSA). 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 Implementation Plan describes the study activities necessary for the application of genetic information and methods to evaluate Project effects on fish in the Susitna River. The genetic baseline study began 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 is developed to examine possible changes in population structure associated with the Project. The emphasis of tissue collection is 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 ~ PRM 153, 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 (~ PRM 98.6 – 187.1) and Upper River (> PRM 187.1; Figure 1)), 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 AEA, 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 are being used to create a tissue repository for resident and anadromous fishes in the Susitna River with particular emphasis on developing the genetic baseline for Susitna River salmon populations. Previous tissue collections and genetic analyses for resident species were limited within the Susitna River. There were few samples in the tissue archive from resident, non-salmon fish species, because these samples had only been collected opportunistically. Some genetic/phenotypic analyses had been completed on three-spine sticklebacks from the Matanuska/Susitna drainages (Cresko et al. 2004), but no population-structure analyses were available. Population analyses of Bering Cisco indicated that the Susitna River supports a single population (Brown et al. 2012).

In 2013, samples from some resident fish from some reaches in the Upper and Middle Susitna River were collected opportunistically (Table 2) while implementing this study as well as other fish distribution and abundance studies conducted by AEA.

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.

In 2013, samples from all Pacific salmon species from reaches in the Upper and Middle Susitna River were collected and samples from Chinook salmon were collected from reaches throughout Upper Cook Inlet (Tables 3 - 7).

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 if the Project created new barriers to fish movement, thereby potentially reducing the fitness of some stocks. Breeding isolation of stocks may be a sign of traits adapted for particular features of the habitats; such information would alter the impact assessment, and possibly the design of any proposed protection, mitigation, or enhancement 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, 12 adult Chinook salmon tagged downstream of Devils Canyon ascended through all impediments in Devils Canyon. Of these, seven remained above Devils Canyon during the spawning season (in Kosina, Tsisi, and Devil creeks) while five migrated back down below Devils Canyon (Appendix A). These fish appeared to spawn in a tributary just below the Canyon (Portage Creek; four fish) or a tributary within the Canyon (Chinook Creek; one fish). These observations led to questions about whether these fish 1) represented a self-sustaining,

genetically isolated, and potentially locally-adapted population (Hypothesis 1a; Figure 2), 2) were individuals originating from other geographic spawning aggregates below Devils Canyon (Hypothesis 2; e.g., Portage Creek), or 3) were 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 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 2).

In 2013, only three adult Chinook salmon tagged downstream of Devils Canyon ascended through all impediments in Devils Canyon. Of these, two remained above Devils Canyon during the spawning season (in Devil and Tsusena creeks) while one fish migrated back down below Devils Canyon and was last observed in the Lower River (Appendix A). Although access was limited, the Project collected tissues suitable for genetic analyses from both adult and juvenile Chinook salmon from waters above Devils Canyon (Table 3). Only four adults were collected. However, the success in collection of juvenile Chinook salmon was particularly noteworthy (186 fish collected from the Oshetna River and Kosina Creek) given the limited area accessible and methods used (minnow traps).

Genetic analysis can help to distinguish among hypotheses regarding population structure (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 largely be from the large populations below the canyon to the small population(s) above the canyon, based solely 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 or repeated 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 one or two 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, a deficit of heterozygotes from Hardy-Weinberg equilibrium (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 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 a few years (three to four) to assess temporal stability in allele frequencies from fish above Devils Canyon may limit the ability to conclusively distinguish among Hypotheses 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 and the proportion of the population sampled. 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 or four calendar years may represent Chinook salmon from as many as six or seven brood years given the multiple ages of maturity in any given year. If large numbers of fish can be sampled in each year, 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 will dictate the level of support for the existence of 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 each year.
- 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 Implementation Plan describes dedicated sampling effort by field crews for three months in 2014 during the spawning period of adult Chinook salmon, sufficient to collect tissue samples over a representative proportion of the entire run. Additional samples will be collected from other Project studies, as described in the Initial Study Reports (ISR) for Study 9.5 Fish Distribution and Abundance in the Upper River, Study 9.6 Fish Distribution and Abundance in the Middle and Lower River, and Study 9.7 Salmon Escapement.

To ensure that data sources (and hypotheses) are rigorously examined, AEA will work closely with geneticists from state and federal (NMFS and USFWS) genetics laboratories. ADF&G's Gene Conservation Laboratory (GCL) will be contracted to do the study. Collaboration with federal services will occur through regular updates to the Technical Working Group (TWG).

In December 2013, preliminary data analyses were conducted on Chinook salmon samples from the Susitna River drainage that were delivered to the laboratory through September 1, 2013. Results from these analyses were provided to the USFWS and NMFS in preparation for consultation (Appendix B). Consultation with USFWS and NMFS representatives with population genetic expertise was held in Anchorage on March 12, 2014. This consultation resulted in recommendations for 2014 field, laboratory, and statistical methods.

A draft of this Implementation Plan was provided to the USFWS and NMFS on April 2, 2014 for their input prior to developing this final plan. Input from these federal services is documented, evaluated and addressed in Table 1 and throughout this final Implementation Plan for 2014. This 2014 Genetics Implementation Plan establishes details for field sampling efforts including relative priorities, temporal and spatial sampling considerations, and statistical analysis methods that take into account the experience from the 2013 field season and consultation with USFWS and NMFS.

2.2. Study Area

The study area encompasses the Susitna River and its tributaries from Cook Inlet upstream to the Oshetna River confluence (PRM 235.1; Figure 1). 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 (PRM 98.6 – 187.1) and Upper Susitna River (PRM 187.1 – 235.1), tissue from juvenile Chinook salmon will be collected in the Lower River (< PRM 98.6).

3. OBJECTIVES

The goals of the FERC approved study are (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.

As described in the Study Plan, the objectives of this study are to:

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.

AEA expects that each of these goals and objectives will be met through the complete implementation of the study program, which will include data collection and analysis in both 2014 and 2015. Data collection toward achieving these objectives during the 2014 study season will be limited to the following:

- Collect juvenile and adult Chinook salmon from above Devils Canyon.
- Collect adult Chinook salmon from upper Cook Inlet tributaries.
- Opportunistically collect other salmon and non-salmon species from the Susitna River.
- Genotype Chinook salmon for Single nucleotide polymorphism (SNPs) and microsatellite (μSAT).

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 3–7). 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 August. During the three 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 on Monday of each week and Yentna River survey flights on Tuesday of each week.

During survey flights, global positioning system (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, all-terrain vehicle, 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

The 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. 2012). 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 simulations cannot be performed. Therefore, 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., single nucleotide polymorphism (SNPs)) were selected. 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. 2012; Hale et al. 2012). Without any genetic error, and under the worst-case scenario of two stocks present in a mixture at 50 percent each, sampling error will produce estimates within 7 and 10 percent of the true estimate 95 percent of the time with sample sizes of 200 and 100 fish, respectively (Thompson 1987). Genetic error will increase this uncertainty and deviations from the worst-case scenario will decrease this uncertainty.

For this study, fish populations are defined using Waples and Gaggiotti's (2006) definition: 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. Functionally, populations will be represented by single or pooled collections following the "Pooling Collections into Populations" methods below.

Possible spawning sites of each target Pacific salmon species were selected based on field sampling from previous years (Tables 3–7), 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), other recent Project studies, and talking with local biologists. The sample sites with idealized sample sizes for each are indicated in Tables 3–7. AEA will opportunistically collect samples as outlined in the sections below. However, it is unlikely that the idealized sample size for all of these sites will be obtained due to uncontrolled variables (i.e., numbers of fish at a spawning location, number of fish returning, 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, a column was added to Tables 3–7 labeled "Expected" that shows the number of fish that can be reasonably sampled at each site (or group of sites), based or previous efforts (and results) and on 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 for genetic tissues in other program studies.

4.2.1. Sample Collection Targets

- Collect tissue samples from 50 representative individuals from each of the resident fish species listed in Table 2, with an emphasis on fish collected in the Lower, Middle, and Upper Susitna River (Objective 1). These collection targets will continue to be pursued on an opportunistic-sampling level.

- Collect tissue samples from 100 individuals (total archived and new samples) from at least three spawning aggregates of chum, coho, pink, and sockeye 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 4–7; Figures 3–6; Objective 2). Collections will be pursued opportunistically.

- Collect sufficient tissue samples from Chinook salmon spawning in Knik Arm and northwestern Cook Inlet rivers (excluding Susitna River) so that at least two additional rivers in each region are represented in the baseline by up to 200 Chinook salmon (total archived and new samples; Table 3; Objective 3).

- 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 3; Figure 1; Objectives 3 and 4).

- Collect tissue samples from a target of 200 juvenile Chinook salmon at each of the following: Cheechako Creek, Fog Creek, Kosina Creek, and the Oshetna River (Table 3; Objectives 3 and 4).

4.2.2. Adult Chinook Salmon Collections

Weekly survey flights will be conducted from June 8–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 14 – August 8. Because Chinook salmon are generally spread out in streams and in lower abundance compared to other salmon species, single- and multi-day sampling trips will be required to get an adequate sample from each location (Table 3; Figure 1). During this time period, each of up to two sampling crews will attempt to collect samples from at least two locations per week. Staffing will be adequate to 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, one crew of three people will be dedicated to sampling in the Susitna River. During one of these weeks, another crew of two people 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, the crew 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 be Talkeetna. 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 C1). 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 five 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, two rows above the lateral line. Length, sex, and GPS information will be recorded on Rite-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.

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 2). Collection trips before and after the three-week intensive sampling period will be performed by one crew, but trip lengths will be longer (approximately four days – one trip per crew per week) due to the lower anticipated availability of helicopter charters. Helicopter (Enstrom F28F) flights will be chartered, as needed, through Talaheim Lodge, mainly to access sites above Devils Canyon and a jet boat will be used mainly to access sites below Devils Canyon in the Middle Susitna River.

4.2.3. Other Adult Salmon Collections

Other adult salmon will be collected opportunistically in 2014. During the Chinook salmon collection period, collections from adult pink, sockeye, chum, and coho salmon will be conducted by the Susitna River crew on an opportunistic basis. Capture and sampling of salmon will follow methods used for adult Chinook salmon.

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 locations: Cheechako Creek, Fog Creek, Kosina Creek, and the Oshetna River. 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 will 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 per day.

OmniSwab samples will be collected from each juvenile Chinook salmon captured and will be placed into individual 2 ml vials (Appendix C2). Total length (snout-to-fork) will be recorded for each sampled juvenile.

4.2.4.2. Middle River Collections below Devils Canyon

In 2014, collection of Chinook salmon juveniles in the Middle River below Devils Canyon will occur on an opportunistic basis. Samples of juvenile Chinook salmon collected in the Middle River will be classified by habitat type to examine the potential for stock-specific variation in

habitat type use. Habitat classifications will follow the macrohabitat categories as defined for Study 9.9 Characterization and Mapping of Aquatic Habitats (Appendix D): single main channel, split main channel, multiple main channel, side channel, tributary mouth, side slough, upland slough, single channel, split channel, and channel complex. Up to three locations will be sampled for each habitat type in 2014. Crews will begin juvenile collections as early as the first week of June and continue through early July. Sampling locations will be accessed by river boat.

Juvenile Chinook salmon in the Middle 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 per 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.

Pelvic fin tissue will be collected from each juvenile Chinook salmon captured and place in an individual 2 ml vial (Appendix C3). Samples will be taken from the same side of each fish to help prevent resampling of individuals.

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 will be confirmed post season using DNA.

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. 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 C1). Samplers will record on each bottle, or on datasheets for vial collections, from 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 collection location.

4.2.6. Coordination with Other Project Studies

As described in the RSP, tissue samples will also be collected by other fish studies conducted for the Project. Specific 2014 data collection efforts are described in the ISRs for Study 9.5 Fish Distribution and Abundance in the Upper River, Study 9.6 Fish Distribution and Abundance in the Middle and Lower River, Study 9.7 Salmon Escapement, and Study 9.8 River Productivity. 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 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 ml bulk sample bottles or individual 2 ml vials and slime samples will be preserved onto OmniSwabs in 2 ml vials (Appendices C1 – C3). After samples are received by the GCL, collection information will be recorded in LOKI. Tissue samples will be freeze-dried and stored at room temperature for long-term storage. Slime samples will remain on OmniSwabs for long-term storage.

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 five 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 for population genetic structure. As a result of consultation with USFWS and NMFS in March 2014, an additional 190 SNP markers and 12 microsatellite markers will be analyzed for all adult and juvenile Chinook salmon captured in the Middle and Upper River to test among hypotheses for fish spawning above Devils Canyon.

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 four microliters (µl) of assay mix (1x DA Assay Loading Buffer [Fluidigm], 10x TaqMan SNP Genotyping Assay [Applied Biosystems], and 2.5x ROX [Invitrogen]) and 5 µl of sample mix (1x TaqMan Universal Buffer [Applied Biosystems], 0.05x AmpliTaq Gold DNA Polymerase [Applied

Biosystems], 1x GT Sample Loading Reagent [Fluidigm], and 60-400ng/µl 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 one 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 reactionplates. Each reaction is conducted in a 5 µL volume consisting of 5–40 nanograms 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 one second, and annealing/extension temperature 1 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 10 µl 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. PCR product (0.5 µl) will be loaded into a 96-well reaction plate along with 0.5 µl of GS500LIZ (AB) internal lane size standard and 9.0 µl of Hi-Di (AB). PCR bands will be visualized and separated into bin sets using AB GeneMapper software version 4.0.

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

Scales from Chinook salmon 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, four 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 quality control (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.

4.6. Genetic Baseline Development

4.6.1. Consultation with Other Services Regarding Appropriate Statistical Analyses

On March 12, 2014, AEA met with representatives from USFWS and NMFS with population genetic expertise in Anchorage. At the meeting, preliminary data analyses from samples delivered to the laboratory through September 1, 2013 were presented to engender consultation regarding the future statistical methods. The new methods described in this plan incorporate recommendations to increase the numbers of markers used to screen Chinook salmon collections from the Middle and Upper River and to use statistical packages designed to detect related individuals.

Statistical analyses that can be performed to examine population structure and to develop a baseline for use as a tool in MSA are outlined below. 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. Prior to commencing field work each year, AEA and ADF&G will work in consultation with other Services (USFWS and NMFS) 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. As of the end of 2013 sampling season, an inadequate number of adult samples have been collected above Devils Canyon to characterize these spawning aggregates. As a result, juvenile collections were incorporated into the preliminary data analyses presented to USFWS and NMFS.

The Services recommended further evaluation of these juvenile collections before they can be incorporated into the baseline. Recommendations included testing for sibling relationships, comparing adult and juvenile collections from the same tributaries, and testing for temporal stability in allele frequencies within tributaries. The Services recommended using programs such as ML-Relate and FRANz to test for sibling relationships. USFWS offered to help with interpretation of results from these analyses to determine how to incorporate juveniles into the

baseline. Since then, comments from NMFS provided on May 12, 2014 recommended against removal of putative siblings (Section 8, Table 1). Therefore, this analysis will not be pursued.

If adequate numbers of adults are collected in 2014, the Services recommended testing for differences in allele frequency estimates between the adult collections and juvenile collections (see methods under "Pooling Collections into Populations," below), and examining the HWE of pooled adult/juvenile collections (see methods in "Pooling Collections into Populations," below). Since then, comments from NMFS provided on May 12, 2014 recommended against exclusion of juvenile collections based on these tests (Section 8, Table 1). Therefore, this analysis will not be pursued.

Testing for stability in allele frequencies within tributaries will depend on successful collection of adequate numbers of juvenile Chinook salmon in 2014 and will follow methods described below under "Temporal Variation."

4.6.3. Hardy-Weinberg Expectations

For each locus within each collection, tests for conformance to HWE expectations will be performed using Monte Carlo simulation with 10,000 iterations in the Adegenet package (Jombart 2008). Starting with unadjusted tests, we will evaluate what fraction are significant for each locus (across all populations) and for each population (across all loci). If the resulting proportions do not deviate much from the expected proportion (dictated by the significance level of the test), conformance to HWE expectations will not be rejected. Loci or populations that are outliers will be examined separately after correcting for multiple tests with Bonferroni's method ($\alpha = 0.05$ per number of collections). As a final set of tests, probabilities will be combined for each collection across loci and for each locus across collections using Fisher's method (Sokal and Rohlf 1995).

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 ($P > 0.05$). 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 FST estimates (corrected for sample size using Weir and Cockerham's (1984) theta calculated in FSTAT (Goudet, J. 2002)) 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 FST 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 2, 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 2, 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, the program MIGRATE (Beerli and Felsenstein 2001) will

be used to estimate migration rates and direction of migration. All tests will use a significance level of $\alpha = 0.05$, adjusted for multiple tests.

For each non-significant hypothesis test, we will conduct a power analysis to determine what level of difference would have been needed to register in a significant result (power analysis) using methods similar to those described in Ryman and Palm (2006). This power analysis will be used to determine the level of migration needed to register a significant result. These power analyses will provide the reader with guidance on the level of certainty to attribute to "non-significant" results.

4.7. Mixed-Stock Analysis

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

In response to FERC's February 1, 2013 SPD recommendations, 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 USFWS indicated 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. Therefore, this analysis will not be conducted until there is adequate sample size.

Preliminary analyses on a limited number of samples presented in consultation with USFWS and NMFS in March 2014 indicate that the collections above Devils Canyon are genetically divergent from populations below Devils Canyon within the Susitna and Yentna River drainages (Appendix B). However, these results are not adequate to determine if MSA is likely to be useful in distinguishing between populations above Devils Canyon in mixtures of fish from the Lower River. Most of the collections are of juvenile salmon, so results might be affected by the sampling of many related individuals that represent only a fraction of the spawning fish within each tributary. In addition, the data do not support extrapolation beyond Kosina Creek and the Black River. If the preliminary results hold up after additional sampling and analyses, it is possible that populations from above Devils Canyon may be identifiable using MSA.

One result from the preliminary analyses is the observation that Chinook salmon from the Middle River, below Devils Canyon (Portage and Indian creeks; Figure B3 in Appendix B), appear to lack adequate genetic distinction from other collections of fish spawning in tributaries from the Lower River for identification using MSA. This lack of genetic distinctiveness does not bode well for using MSA to identify all Chinook salmon originating in the Middle River in samples collected in the Lower River. However, if the genetic divergence observed between populations above and below Devils Canyon is temporally and spatially stable, MSA to distinguish between these population groups will likely succeed. MSA will therefore more likely be useful in the Middle River, where populations originating from above Devils Canyon are likely to be at higher proportions within mixtures.

A comprehensive analysis will be conducted when 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, 400 individuals will be sampled without replacement from reporting groups in proportions similar to those expected in the Lower River juvenile samples. The stock compositions of these mixed composition proof tests will be estimated following the BAYES protocol described below and compare these estimates to the true proportions. To account for sampling error, this procedure will be replicated 10 times in a manner similar to Habicht and Dann (2012).

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 percent 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 the Services' (NMFS and USFWS) geneticists and will be based on the reporting groups of interest and risk tolerance. For an example of this process, see Habicht et al. (2012).

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 four 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. All analyses will incorporate a flat prior.

Five independent Markov Chain Monte Carlo (MCMC) chains of 40,000 iterations will be run with different starting values and the first 20,000 iterations will be discarded to remove the influences of the initial start values. The starting values for the first chain will be defined 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. The second halves of these chains will be combined to form the posterior distribution and tabulate mean estimates, 90 percent 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 six significant digits.

The within- and among-chain convergence of these estimates will also be assessed 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, the mixture will be reanalyzed 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, the mixture will be analyzed 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. This information will be incorporated 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 Middle River by Chinook Salmon Progeny Originating in the Middle and Upper Susitna River

If the results of the Chinook salmon genetics studies conducted between 2012 and 2014 are sufficient to indicate that there is adequate genetic diversity and that this diversity is temporally and geographically stable 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 above Devils Canyon in selected Middle River habitats. Over the course of the study, 100 juvenile Chinook salmon total from each of 16 mainstem locations (across up to 10 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 fish originating from above Devils Canyon.

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 considerable 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

- Adult and juvenile Chinook salmon baseline sample collection: June through August 2014 (in collaboration with other AEA field studies).
- Other species sample collection: May through October 2014 (in conjunction with other AEA field studies).
- Juvenile Chinook salmon mixture sample collection from the Middle River: May through October 2014.
- Laboratory analysis of adult Chinook salmon baseline and juvenile mixture samples: October through November 2014.

- Statistical analysis of Chinook salmon baseline collections to examine population structure and potential application of MSA: December 2014.
- Assuming adequate genetic variation for MSA, statistical analysis of juvenile mixture samples: February 2015.
- Consultation with the Services (NMFS and USFWS) to review cumulative sample collection and genetic analysis and determine if adequate genetic variation exists for MSA of juvenile Chinook salmon mixture samples: February/March 2015
- Deliverables:
    - February/March 2015. Interim results delivered to services in preparation for consultation. 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, 2015. Draft Implementation Plan for 2015 to NMFS and USFWS for review.
    - April 30, 2015. Final 2015 Implementation Plan filed with FERC.
    - February 2016. Updated 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 implementation plans and prioritize resources among laboratory projects to meet deadlines.

Jim Jasper, Biometrician III

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

Vacant, Fishery Biologist I (four 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.

Vacant, Fish and Wildlife Technicians (two positions)

Duties: Coordinate and send sampling supplies to coordinating projects. Provide logistical support to field biologists: research, purchase and ship field gear, assemble and ship sampling supplies, coordinate communication, and receive and archive tissue samples. Extract DNA in preparation for analysis.

7. LITERATURE CITED

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8. TABLES

Table 1. Agency comments on the draft Implementation Plan, AEA's responses to agency comments and the page number(s) in this document where each comment is addressed.

Agency #	Comment	AEA Response	Page
USFWS 1	[USFWS is] comfortable with the sampling plan for this summer and we are willing to comment in the future regarding data analysis and interpretation.	No changes made.	NA
NMFS 1	Page 3: NMFS agrees that departures from HWE could support hypothesis 1b (fish above Devils Canyon are derived from spawners above and below), but only if the departures are in the direction of a deficit of heterozygotes, as expected under the Wahlund effect (population mixture). However, Hypothesis 2 would not necessarily produce any such departures if all the fish above the canyon were derived from a single lower population.	We agree. We clarified that we will test for a deficit of heterozygotes rather than any deviation from HWE. As for Hypothesis 2, we will test for allele frequency differences between all collections above Devils Canyon and populations below Devils Canyon (first step of shown in Figure 2), so we will test for this possibility.	5
NMFS 2	Page 3: "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)." This conclusion cannot be supported simply from failing to find a difference. It would be necessary to conduct a power analysis to determine how large a difference (e.g., Fst value) could exist and not be detected as statistically significant. Then, it would be necessary to translate the genetic data into estimates of gene flow to evaluate what levels of connectivity are consistent with the observed data.	We agree. We recognized that this signal may be difficult to detect using three years of data. We added a power analysis section to quantify the level of divergence (and hence level of gene flow) necessary to detect a difference (last paragraph in Section 4.6.9).	18
NMFS 3	NMFS concurs that samples from multiple years are essential to be able to make sense of the relative magnitude of spatial and temporal differences. Three years of samples may be inadequate for this purpose, especially considering that Chinook and perhaps some of the other species have generation lengths much longer than three years.	We agree that additional years may be needed, especially if we do not detect differences due to a lack of statistical power (small samples sizes, small number of years). However, if we are able to collect an adequate number of samples, we may have adequate power to distinguish among hypotheses with adequate certainty. The added power analysis section (4.6.9) will provide some guidance.	18
NMFS 4	The required sample sizes depend on the particular objective, as well as the (unknown) differences among populations. In general the numbers proposed seem reasonable. However, the logic for requiring larger samples for msat analyses is inadequately explained. This may be based on the idea that larger samples are required to provide precise estimates of all the low frequency alleles involved with msats. However, that is not the objective; the objective is to use all the data to draw biological conclusions about the species of interest. From this perspective, each msat locus is	This study has 5 objectives. Objective 3 is to draw biological conclusions about the species of interest, but Objectives 4 and 5 are to examine potential for and apply MSA to mixtures of juvenile Chinook salmon captured in different habitat types of the Lower River. For 2014, we are not proposing to pursue Objectives 3 and 4. However, Objectives 3 and 4 will be pursued in future years, so we will maintain this language. We agree that for Objective 3, sample sizes do not need to be larger for msats.	NA

	worth several SNP loci in terms of information content, as a large number of empirical studies have demonstrated.		
NMFS 5	Page 12-13: NMFS strongly recommends that the PIs NOT remove putative siblings as proposed. This is a dangerous idea that has somehow come into favor. The fact is that siblings occur in all finite populations, which is to say, all real populations in nature. Siblings, in fact, contribute part of the signal in genetic analyses that provides insights into biological processes. Purging them from the sample universe scrubs the data of this biological signal, particularly for small populations where siblings are common. What effects this purging has on subsequent analyses is impossible to determine without a great deal of empirical evaluation, but these effects could be substantial. One thing is certain: this purging makes the remaining individuals more similar to what would be expected from populations that are infinite in size and hence have no relatives. The only reason that might justify purging like this is if a particular sample is thought to have been collected non-randomly (that is, if it is thought to represent progeny from only a few families). In that case, however, the proper amount of purging could only be determined if one knew exactly how non-random the collection was, but this will seldom if ever be known in practice. Furthermore, even if this was known and relatives were removed, the result still would not be a representative collection from the population as a whole. The solution to non-random sampling, therefore, is not purging relatives but to going back into the field and collecting a representative sample.	We agree. Removal of putative siblings was added based on recommendations from USFWS and NMFS. Collection of juveniles will be made from many traps and/or seine sets over many locations within streams, in multiple streams, over many days and at least three years. This sampling design will yield close to a "random sample". We are pleased that NMFS recommends against removal of putative siblings. Section 4.6.2 reflects this change.	16
NMFS 6	Page 13: "We will 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." Care is needed here. Age structure creates mini-Wahlund effects that could cause HW departures even in mixed-age adult samples, and the same thing could happen if you combine juveniles and adults produced by different cohorts. That does not mean that combining them won't produce a more robust overall estimate of population allele frequencies.	We agree. Removal of juvenile collections based on comparisons to adults was added based on recommendations from USFWS and NMFS. We are pleased that NMFS recommends keeping all samples to provide the most robust overall estimate of population allele frequencies. Section 4.6.2 reflects this change.	16
NMFS 7	NMFS does not agree with using the Bonferroni correction for HWE tests; there are too many overall tests and thus the criterion become too conservative. Bonferroni correction controls the probability of false positives only and the correction ordinarily comes at the cost of increasing the probability of producing false negatives, consequently reducing the statistical power of the HWE tests. Instead we suggest starting with	We agree. We re-wrote section 4.6.3 to reflect this recommendation.	16

	unadjusted tests and evaluating what fraction are significant for each locus (across all pops) and for each pop (across all loci). If the resulting proportions do not deviate much from the expected proportion (dictated by the significance level of the test), there is no reason to reject HWE. Loci or pops that are outliers can be singled out for more detailed analysis, perhaps using Bonferroni or FDR.		
NMFS 8	Page 1: The project "will modify the flow, thermal, and sediment regimes of the Susitna River ...". The project will also affect migration and fish passage, among a host of other important effects. The description of project effects should be written to comprehensively describe all major project effects.	We agree that the description of potential project effects may be incomplete, and has therefore been removed from this Implementation Plan. A comprehensive description of potential Project effects will be developed within the License Application for the proposed Project.	2
NMFS 9	Page 1: "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." Genetic drift will always result in some differences unless there is complete panmixia.	We agree. Although genetic drift will always result in some differences unless there is complete panmixia, drift needs to be large enough to be detectable. We added the following phrase to clarify: "that are large enough to detect using methods widely used by programs that conduct population genetics and mixed-stock analyses (MSA)."	3
NMFS 10	Analyses of genetic distance: it is fine to use Fst as an index of genetic distance, but it must include a correction for sample size (like W&C theta). Otherwise, small samples will tend to look like outliers.	We agree. We added this change to the methods (4.6.8).	17
NMFS 11	Page 6: "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)." That might have been true for the particular study cited, but how large a sample is required will depend on the number of markers and the magnitude of divergence among populations, so this general statement is not valid.	We agree. This error is assuming no genetic error – only sampling error and is assuming the worst-case scenario of a 2-stock mixture at 50 percent for each stock. Genetic error will increase this uncertainty and deviations from the worst-case scenario will decrease this uncertainty. We added language to section 4.2 to clarify.	9
NMFS 12	Page 8, the numbering is off under "Sample Collection Targets"	Added text to captions in Tables 2, 4, 5, 6, and 7 to clarify and match target language in 4.2.1.	30, 34-40
NMFS 13	Page 9, under "Sample Collection Targets" item #9, we understand the issues regarding sample numbers, but an adequate adult Chinook salmon sample set from above the proposed dam is needed at the end of the study to make the necessary conclusions. What happens if the goal of 100 adult Chinook salmon is not realized? This should be addressed in advance.	An adequate sample size will be required to determine with confidence that the hypotheses should not be rejected (i.e. differences between populations above and below Devils Canyon). If the differences are large, detection of a difference will be possible with small sample sizes. As outlined in response to the last comment under "Hypotheses for Chinook salmon", we will perform a power analysis to determine the level of effect we could detect given the sample sizes. This power analysis will provide the information needed to determine if the sample sizes are adequate.	18

NMFS 14	Page 10, Section 4.2.4.1, identifies a sample target of 200 juvenile Chinook salmon from 4 systems in or above Devils Canyon, but later in the report under section 4.5 "Data Retrieval and Quality Control" it mentions that software will be used to identify siblings and exclude all but one individual in the baseline for every set of siblings identified. As such, given the likely small population sizes above the proposed dam site, will 200 juveniles from each system is unlikely to be sufficient.	This issue is addressed by retaining putative siblings, as outlined in our response to the first comment under "Analyses".	16
NMFS 15	Page 16, Section 4.6.5, where it says "Collections will be pooled when tests indicate no difference between collections (P>0.01)." While we agree that it is difficult to prove there is no difference between collections, we recommend though using a p value greater than 0.05 as more appropriate to reject the null hypothesis.	Changed the criteria to 0.05 in section 4.6.5.	17
NMFS 16	Appendix A Section 2.2 Regarding the radio telemetry studies, the potential impacts of the tag on the migration pattern of the salmon, especially for a stock that has to migrate the farthest and through a 7-mile long Class 5+ canyon must be considered and discussed. Also please address whether the tags let you know where the fish spawned (or if they spawned) or just indicate where they were when relocated, including noting the spatial accuracy of the tag signal recoveries.	Appendix A was included to provide background information and context for the hypotheses to be tested using genetic tools in the genetic baseline study. Specific information regarding the radio-tagging and tracking of adult Chinook salmon through Devils Canyon are presented in the Salmon Escapement Study (ISR Study 9.7). AEA disagrees that this Implementation Plan is the appropriate place to consider and discuss the effects of radio-tagging on Chinook salmon migration behavior through Devils Canyon or the spatial resolution of tag signal recoveries.	NA
NMFS 17	Appendix B - page 1, for the Black River: Were the Chinook that were sampled two juveniles which were collected in 2013? Please confirm and identify them as juveniles if that's true.	Yes, these are juveniles – clarified in Table 1 of Appendix B.	B1
NMFS 18	Table B5, Is there an overall HWE test for all markers for each population?	No. This test will be added to future tables of HWE.	NA

Table 2. Resident and non-salmon anadromous fish species targeted for genetic tissue sampling in the Susitna River and samples sizes collected in 2013. Total sample sizes are reported for the Gene Conservation Laboratory (GCL), other contractors (other), and the combined total (N). Sample sizes greater than the ideal per stratum (50) are shaded in grey. Totals across the entire study area are also reported (Total). All sampling is opportunistic: the collection target by species across all strata is 50 fish, however, opportunistic sampling will continue until 50 fish for each species are collected in each stratum.

Species	Ideal sample size per stratum	Collection Strata																	Total
		Upper Susitna River			Middle Susitna River above Devils Canyon			Middle Susitna River below Devils Canyon			Lower Susitna River			Talkeetna River			Chulitna River			
		GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	
Blackfish, Alaska	50			0			0			0			0			0			0	**0**
Burbot	50			0			0	4		4	2	102	104			0			0	**108**
Dolly Varden	50	1		1	3		3	5		5	4	3	7	35		35			0	**51**
Eulachon	50			0			0			0		283	283			0			0	**283**
Grayling, Arctic	50	17		17	21		21	45	14	59	7	4	11	5		5	3		3	**116**
Lamprey, Arctic*	n/a			0			0			0		9	9			0			0	**9**
Lamprey, Pacific	50			0			0			0			0			0			0	**0**
Pike, northern	50			0			0			0		16	16			0			0	**16**
Sculpin, coastrange	50			0			0			0			0			0			0	**0**
Sculpin, Pacific staghorn	50			0			0			0			0			0			0	**0**
Sculpin, prickly	50			0			0			0			0			0			0	**0**
Sculpin, slimy	50	15		15	40	100	140			0		52	52			0			0	**207**
Stickleback, ninespine	50			0			0			0		7	7			0			0	**7**
Stickleback, threespine	50			0			0			0	50	92	142			0			0	**142**
Sucker, longnose	50			0			0	5		5	1	102	103			0			0	**108**
Trout, lake	50			0			0			0			0			0			0	**0**
Trout, rainbow	50			0	1		1	40		40	40	7	47	19		19	23		23	**130**
Whitefish, Bering cisco	50			0			0			0			0			0			0	**0**
Whitefish, humpback	50	3		3			0			0			0			0			0	**3**
Whitefish, lake	50			0			0			0			0			0			0	**0**

Whitefish, round	50	2	2	0	57	9	66	0	1	1	6	6	**75**		

Table 3. Area, location, and sublocation 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 2 years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected) progress made toward sampling targets this year (2013), and the resulting total sample size after combining the amount archived with the 2013 samples (Total). An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted, a "-" indicates that no survey was performed. Some of the expected numbers are for groups of locations (in grey). Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. In the Total column, numbers in bold exceed expected and underlined numbers exceed ideal sample sizes. 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 in Figure 1. Sample sizes include samples arriving to the GCL through April 1, 2014 and were updated with physical counts in the lab.

					Sample sizes			
					This project			
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (2013-14)	2013	Total
				Adult Chinook salmon				
West Side Cook Inlet	Chulitna River		1	2008, 2009 (142), 2013	200	58	61	203
	Beluga River	Coal Creek	2	2009, 2010, 2011 (120)	200	80	-	120
	Theodore River		3	2010, 2011, 2012 (189), 2013	200	11	47	236
	Lewis River		4	2011, 2012 (86)	200	86	0	86
Yentna Drainage	Clearwater Creek		5	2012 (25)	200	50	-	25
	Red Creek		6	2012 (29), 2013	200	58	82	111
	Happy River		7	2012 (19)	200	38	S	19
	Red Salmon Creek		8	2012 (12)	200	24	S	12
	Hayes River		9	2012 (5), 2013	200	10	45	50
	Canyon Creek		10	2012 (32), 2013	200	64	61	93
	Talachulitna River		11	1995, 2008, 2010 (180)	200	20	-	180
	Lake Creek	Sunflower Creek	12	2009, 2011 (127)	200	71	S	127
	Kahiltna River	Peters Creek	13	2009, 2010, 2011, 2012 (110)	200	55	-	110
Susitna Drainage	Chulitna River	Middle Fork	14	2009, 2010, 2011 (182)	200	18	61	211
		East Fork	15	2013	200	200	64	416
		West Fork	16		200		S	
		Honolulu Creek	17	2013	200		31	
		Pass Creek	18	2013	n/a		33	
		Spink Creek	21	2013	200		56	
		Byers Creek	19	2013	200		55	

Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Sample sizes			
					This project			
					Ideal	Expected (2013-14)	2013	Total
Susitna Drainage	Chulitna River	Troublesome Creek	20	2013	200		71	
		Tokositna River (Bunco Creek)	22	2013	200		103	
		Tokositna River(Bunco Lake inlet stream)	23	2013	n/a		3	
Susitna Drainage	Above Devils Canyon in Upper River	Oshetna River	24		200	50	0	13
		Kosina Creek	25	2012 (10), 2013	200		3	
		Watana Creek	26		200		S	
	Above Devils Canyon in Middle River	Tsusena Creek	27		200		S	
		Fog Creek	28		200		0	
		Devil Creek	30		200		S	
	Middle Susitna River below Devils Canyon	Portage Creek	31	2009, 2010, 2011 (141), 2013	200	59	25	166
		Chinook Creek	32		200	75	S	82
		Indian River	33	2012 (1), 2013	200		81	
		Gold Creek	34		200		S	
		Lane Creek	35		200		S	
		Chase Creek	36		200		S	
	Talkeetna River	Prairie Creek	37	1995, 2008 (169), 2013	200	31	33	202
		no name creek #2	40	2013	n/a	100	25	217
		no name creek #1	39	2013	n/a		71	
		upper mainstem	38		200		S	
		Iron Creek	41	2013	200		57	
		Disappointment Creek	42	2013	200		64	
		Sheep River	43		200		S	
		Larson Creek	44		200		S	
		Chunilna Creek (Clear Creek)	45	2009, 2012 (130), 2013	200	65	5	135
	Lower Susitna River, upstream of Deshka River	Montana Creek	46	2008, 2009, 2010 (218)	200	0	-	218
		Birch Creek	47		200	50	S	91
		Sheep Creek	48	2013	200		24	
		North Fork Kashwitna River	49	2013	200		12	
		Little Willow Creek	50	2013	200		55	

Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Sample sizes			
					This project			
					Ideal	Expected (2013-14)	2013	Total
		Willow Creek	51	1991,1997, 2005, 2009 (309), 2013 (245)	200	0	-	554
Susitna Drainage	Deshka River	Moose Creek	52	1995, 2012 (103)	200	52	-	103
		Deshka River weir	53	2005 (200)	200	0	-	200
	Alexander Creek	Sucker Creek	54	2011, 2012 (143)	200	57	-	143
Knik Arm	Matanuska River	Kings River	55	2013	200	25	4	34
		Granite Creek	56	2013	200		30	
		Moose Creek	57	1995, 2008, 2009, 2012 (155)	200	45	-	155
	Eagle River	South Fork	58	2009, 2011, 2012 (73)	200	24	-	73
		Meadow Creek	59	2009 (6)	200	12	-	6
	Ship Creek		60	2009 (311)	200	0	-	311
	Little Susitna River		61	2009, 2010 (125)	200	75	-	125
Juvenile Chinook salmon								
Susitna Drainage	Above Devils Canyon	Oshetna River	24	2012 (35), 2013	200	70	34*	208
		Kosina Creek	25		200		139	
		Fog Creek	28		200		0	
	Within Devils Canyon	Cheechako Creek	29		200		-	
Susitna Drainage	Lower River	5 habitat types (100 fish/habitat type times 3 or 4 collections)	n/a	2013	1,600	1,600	39	39

*29 juvenile Chinook salmon samples acquired by ISR Study 9.5 Fish Distribution and Abundance in the Upper Susitna River

Table 4. Area, 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 2 years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected), progress made toward sampling targets this year (2013), and the resulting total sample size after combining the amount archived with the 2013 samples (Total). An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted, a "-" indicates that no survey was performed. Some of the expected numbers are for groups of locations (in grey). Sampling locations originally not included in the Implementation Plan have been included, and are indicated by an "n/a" ideal and expected value. In the Total column, numbers in bold exceed expected and underlined numbers exceed ideal sample sizes. Map numbers (Map No.) correspond to location numbers in Figure 3. Sample sizes include samples arriving to the GCL through April 1, 2014 and were updated with physical counts in the lab. All sampling is opportunistic: opportunistic sampling will continue until tissue samples are collected from at least 100 individuals (total archived and new samples) from at least three spawning aggregates within each of the three locations (Location column).

					Sample sizes			
					This project			
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (2013-14)	2013	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	2		100	100	0	0
		East Fork	1		100		0	
		Pass Creek	5		n/a	n/a	2	2
		Spink Creek	4	2007, 2008 (126)	100	0	0	126
		Byers Lake	3	1993, 2006, 2007 (243)	100	0	23	266
		Tokositna River (Sloughs)	7		100	100	S	0
		Tokositna River (Swan Lake)	8	2006, 2007, 2009 (109)	100	0	0	109
		no-name creek	6		n/a	n/a	6	6
	Middle Susitna River below Devils Canyon	Portage Creek	9		n/a	100	8	12
		Indian River	10				1	
		5th of July Creek	11		n/a		2	
		McKenzie Creek	12		100		0	
		Chase Creek	13		100		0	
		Whiskers Creek	14				1	
		sloughs 8A,11, and 21	15	1995, 1996, 1997 (156)	100	0	119	275
		slough 9 (RM 132)	16		n/a	n/a	66	66
	Talkeetna River	no-name creek	17		n/a	n/a	1	1
		Stephan Lake	18	1993, 1994, 2007 (346)	100	0	-	346
		Prairie Creek	19		n/a	n/a	2	2
		Iron Creek	20		100	50	0	0
		Disappointment Creek	21		n/a	n/a	11	11
		Sloughs	22	1997 (79)	100	21	0	79
		Sheep River	23	2008 (190)	100	0	S	190
		Larson Lake - Eastern shore	24	2011 (90)	100	10	S	90
		Larson Creek	25	1992, 1993 (200)	100	0	S	200

Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Sample sizes			
					This project			
					Ideal	Expected (2013-14)	2013	Total
Susitna Drainage	Talkeetna River	Larson Lake - outlet stream	26	2011 (126)	100	0	S	126
		Chunilna Creek	27		100	100	18	18
		Mama and Papa Bear Lakes	28	1997, 2007 (106)	100	0	75	181
		Fish Creek	29		n/a	n/a	3	3

Table 5. Area, 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), progress made toward sampling targets this year (2013), and the resulting total sample size after combining the amount archived with the 2013 samples (Total). An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted, a "-" indicates that no survey was performed. Some of the expected numbers are for groups of locations (in grey). Sampling locations originally not included in the Implementation Plan have been included, and are indicated by an "n/a" ideal and expected value. In the Total column, numbers in bold exceed expected and underlined numbers exceed ideal sample sizes. Map numbers (Map No.) correspond to location numbers in Figure 4. Discrepancies in sample sizes from previous reports are due to samples arriving after September 15, 2013, or updated lab counts. All sampling is opportunistic: opportunistic sampling will continue until tissue samples are collected from at least 100 individuals (total archived and new samples) from at least three spawning aggregates within each of the three locations (Location column).

					Sample sizes			
					This project			
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (2013-14)	2013	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	1		100		0	
		West Fork	2		100	200	S	46
		Byers Creek	3		100		18	
		Troublesome Creek	4		100		28	
		Spink Creek	5	2007, 2008 (45)	100	55	2	47
		Tokositna River mainstem	6		100	50	S	0
	Middle Susitna River below Devils Canyon	Portage Creek	7		100	100	147	147
		Indian River	8		100	100	136	136
		Gold Creek	9		n/a	n/a	5	5
		sloughs above three rivers confluence	10	1996 (103)	0	0	72	175
		5th of July Creek	11		n/a	n/a	34	34
		4th of July Creek	12		n/a	n/a	56	56
		Lane Creek	13		n/a	n/a	1	1
		Whiskers Creek	14		n/a	n/a	3	3
	Talkeetna River	upper mainstem	16		100		S	
		Disappointment Creek	17		100		S	
		Sheep River	18		100	200	S	1
		Larson Creek	20		100		S	
		Fish Creek	19		100		1	
		Sloughs	15	1995 (50)	100	50	20	70
		Chunilna Creek	21	1993 (87)	100	13	136	223

Table 6. Area, 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 2 years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected), progress made toward sampling targets this year (2013), and the resulting total sample size after combining the amount archived with the 2013 samples (Total). An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted, a "-" indicates that no survey was performed. Some of the expected numbers are for groups of locations (in grey). Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. In the Total column, numbers in bold exceed expected and underlined numbers exceed ideal sample sizes. Map numbers (Map No.) correspond to location numbers in Figure 5. Sample sizes include samples arriving to the GCL through April 1, 2014 and were updated with physical counts in the lab. All sampling is opportunistic: opportunistic sampling will continue until tissue samples are collected from at least 100 individuals (total archived and new samples) from at least three spawning aggregates within each of the three locations (Location column).

					Sample sizes			
					This project			
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (2013-14)	2013	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	2		100		0	
		East Fork	1		100		0	
		Honolulu Creek	3		100	200	0	92
		Byers Creek	4		100		0	
		Troublesome Creek	5		100		92	
		Spink Creek	6	2008 (38)	100	62	0	38
		Tokositna River mainstem	7		100	100	S	9
		Tokositna River (Bunco Creek)	8		100		9	
		Tokositna River (Swan Lake)	9	2009 (20)	100	80	0	20
	Middle Susitna River below Devils Canyon	Portage Creek	10		100		0	
		Indian River	11		100		105	
		Gold Creek	12		100	200	S	105
		McKenzie Creek	13		100		S	
		Lane Creek	14		100		S	
		Sloughs	15		100	75	42	42
		Chase Creek	16		100	75	S	0
		Whiskers Creek	17		100	75	79	79
Susitna River above three rivers confluence	Talkeetna River	upper mainstem	18		100	25	S	0
		Prairie Creek	19		100	75	S	0
		Iron Creek	20		n/a	n/a	28	28
		Sheep River	21		100	50	115	115
		Larson Lake - outlet	22	2011 (84)	100	16	S	84
		Chunilna Creek	23		100	75	66	66

		Fish Creek	24		n/a	n/a	1	1

Table 7. Area, 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 2 years of this project based on past sampling effort and success and information from the Anadromous Rivers Catalog and local biologists (Expected), progress made toward sampling targets this year (2013), and the resulting total sample size after combining the amount archived with the 2013 samples (Total). An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted, a "-" indicates that no survey was performed. Some of the expected numbers are for groups of locations (in grey). Sampling locations originally not included in the Implementation Plan have been included, and are indicated by an "n/a" ideal and expected value. In the Total column, numbers in bold exceed expected and underlined numbers exceed ideal sample sizes. Map numbers (Map No.) correspond to location numbers in Figure 6. Sample sizes include samples arriving to the GCL through April 1, 2014 and were updated with physical counts in the lab. All sampling is opportunistic: opportunistic sampling will continue until tissue samples are collected from at least 100 individuals (total archived and new samples) from at least three spawning aggregates within each of the three locations (Location column).

					Sample sizes			
						Expected		
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	(2013-14)	2013	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	1		100	100	0	1
		Spink Creek	3		100		0	
		Troublesome Creek	2		100		0	
		no-name creek	4		n/a		1	
	Middle Susitna River below Devils Canyon	Portage Creek	5		100	50	136	136
		Indian River	6		100	100	116	116
		Gold Creek	7		100	50	106	467
		5th of July Creek	8		n/a		2	
		4th of July Creek	9		n/a		107	
		slough 9 (RM132)	10		n/a		116	
		McKenzie Creek	11		100		0	
		Lane Creek	12		100		115	
		Chase Creek	13		100		0	
		Whiskers Creek	14		100		21	
	Talkeetna River	upper mainstem	15		100	25	0	0
		Disappointment Creek	16		n/a	n/a	127	127
		Sheep River	17		100	25	0	0
		Larson Creek	18		100	100	0	0
		Chunilna Creek	19		100	100	101	101
		Fish Creek	20		n/a	n/a	101	101

Table 8. Juvenile Chinook salmon samples in the Middle and Lower Susitna River, classified by habitat type as outlined in AEA RSP Section 9.9; Characterization and Mapping of Aquatic Habitats (Appendix D).

Grouping	Habitat Type	Susitna River Lower (L) or Middle (M)	Location	Year(s) Collected	N
Main Channel Habitat	Single Main Channel			none	
	Split Main Channel			none	
	Multiple Split Main Channel			none	
	Side Channel	L	Sheep Creek	2013	8
		M	slough 8A (15)	2013	1
				total	9
	Tributary Mouth	M	Whiskers Creek	2013	1
		M	Indian River	2013	75
				total	76
Off-channel Habitat	Side Slough	M	Whiskers Slough 3B	2013	1
		M	Slough 8A (44)	2013	6
		M	Slough 8A (52)	2013	8
		M	Slough 11	2013	6
		M	Whiskers Slough	2013	1
				total	22
	Upland Slough	M	Whiskers Slough Bridge	2013	3
		M	Slough 17	2013	8
				total	11
Tributary Habitat	Single Channel	M	Whiskers Creek	2013	9
				total	9
	Split Channel			none	
	Channel Complex			none	

9. FIGURES

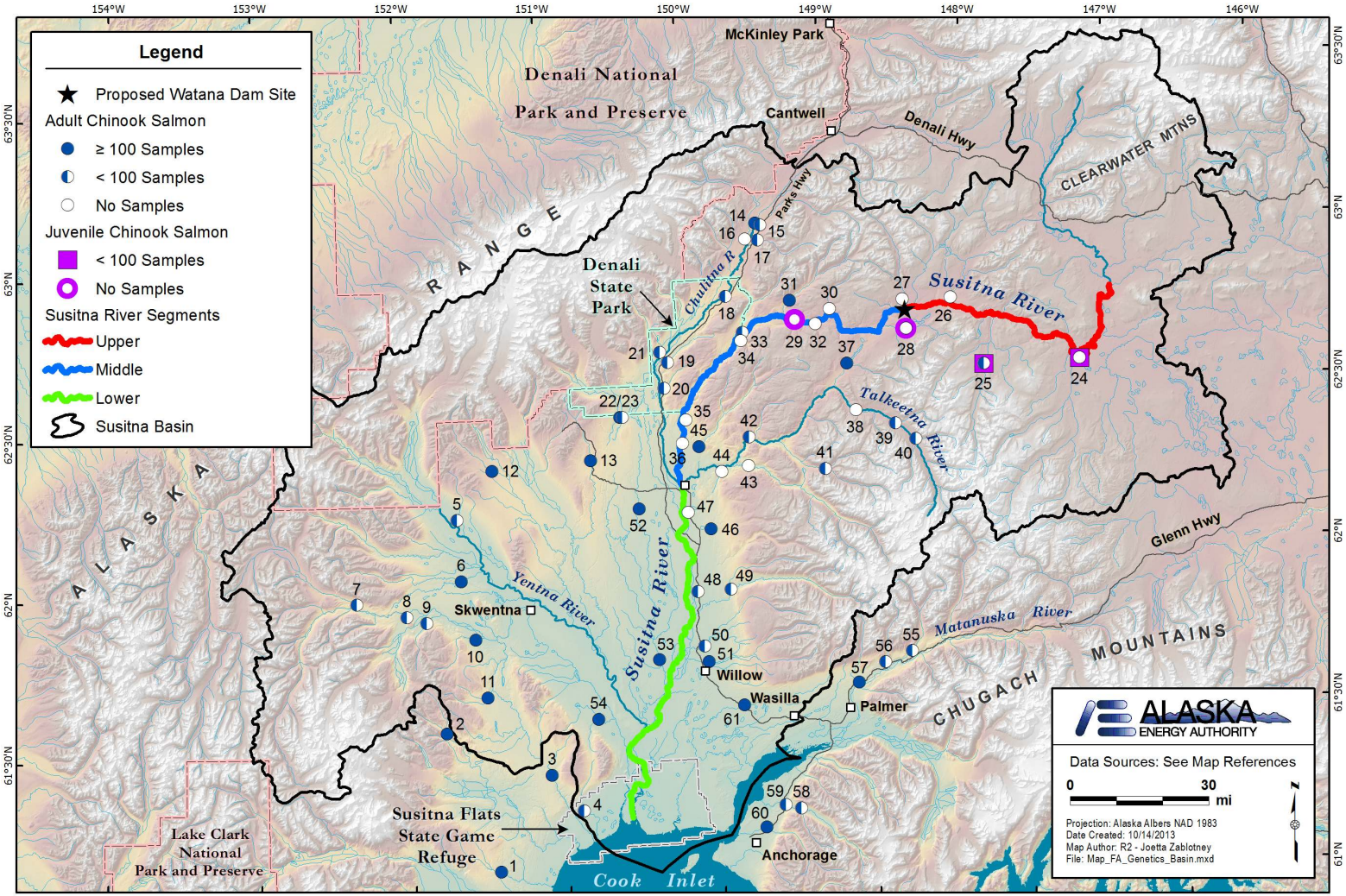

Figure 1. Potential baseline sampling locations for adult and juvenile Chinook salmon. Circles indicate the number of samples in the Gene Conservatory Laboratory archives. Numbers correspond to the map numbers in Table 3. The Lower Susitna River (below PRM 98.6), Middle River (PRM 98.6-187.1) and Upper River (PRM 187.1-235.1) segments are highlighted with the proposed dam at PRM 187.1

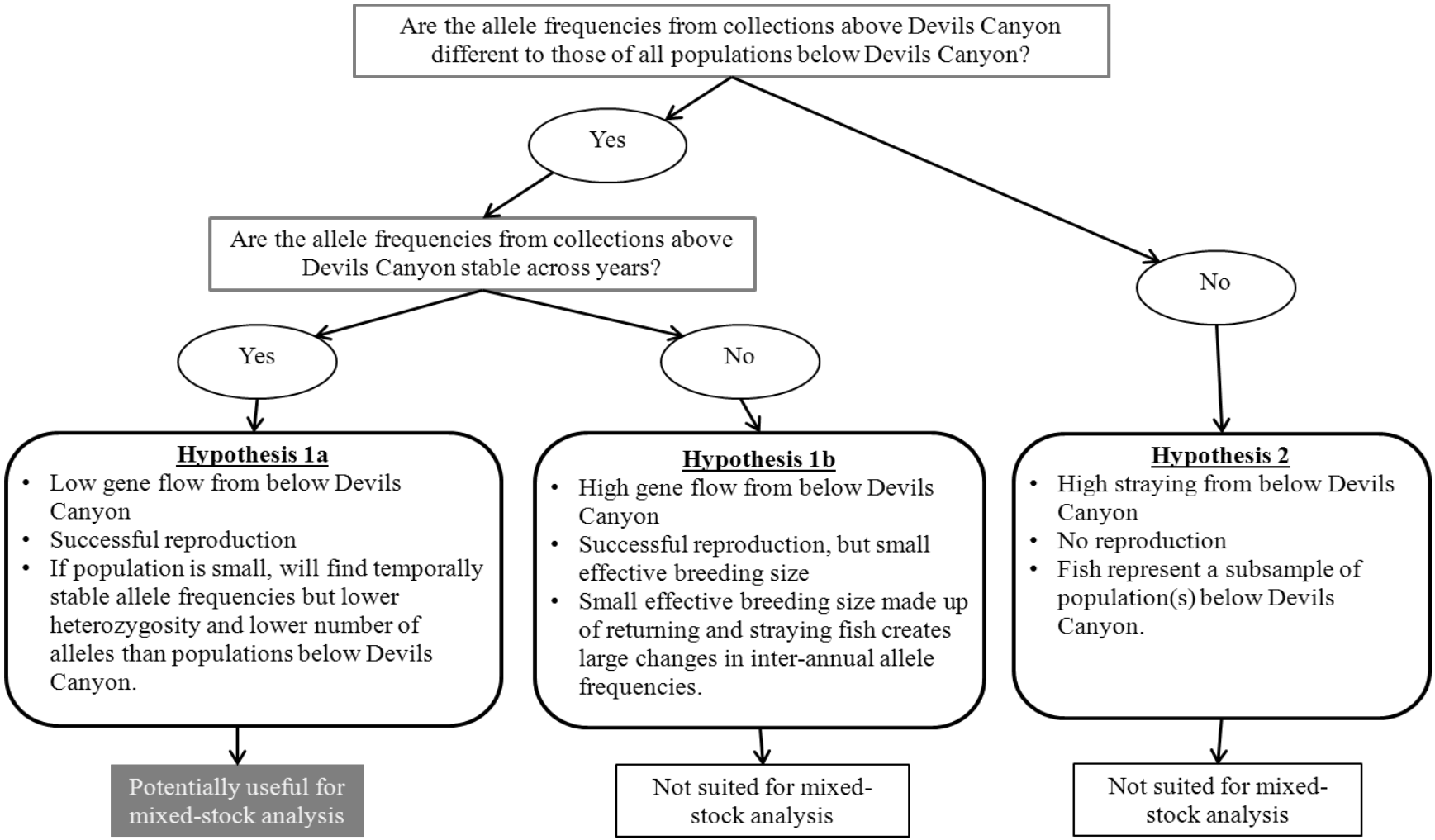

Figure 2. A generalized flow chart to distinguish among hypotheses of population structure for Chinook salmon collected over spawning habitat above Devils Canyon in the Middle and Upper Susitna River. Only a self-sustaining population (Hypothesis 1a) will potentially result in genetic variation suitable 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.

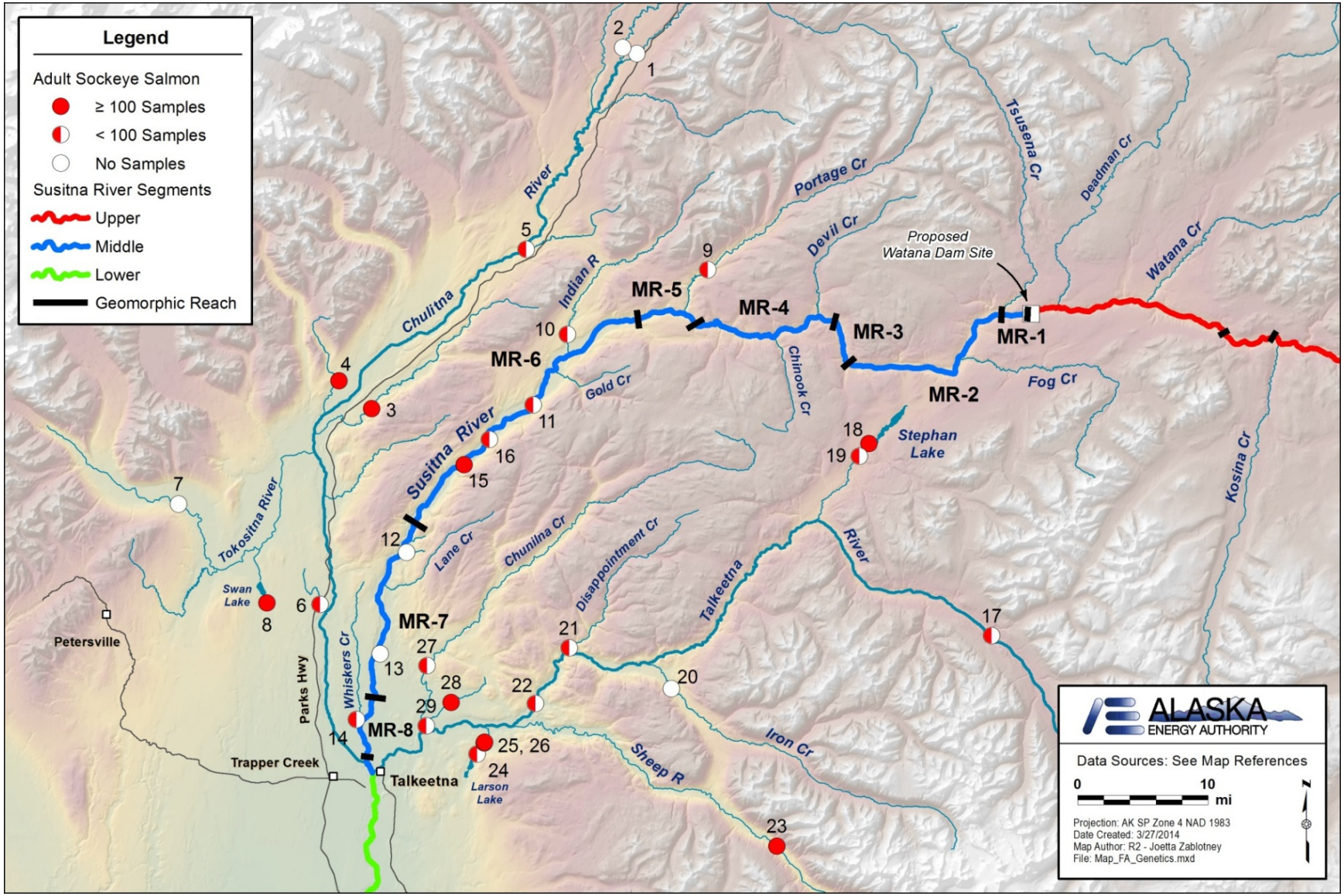

Figure 3. Potential baseline sampling locations for adult sockeye salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers in Table 4.

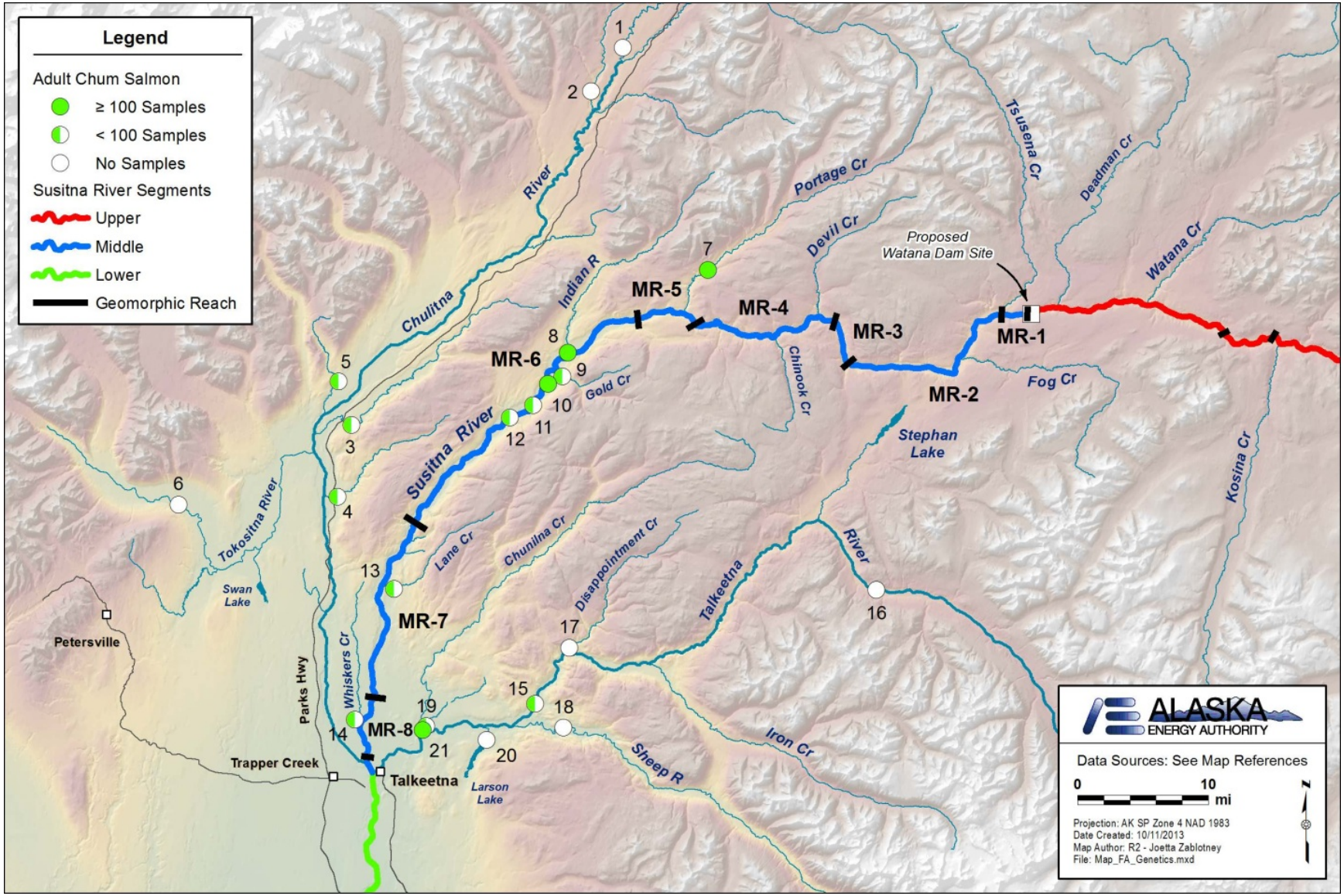

Figure 4. Potential baseline sampling locations for adult chum salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers in Table 5.

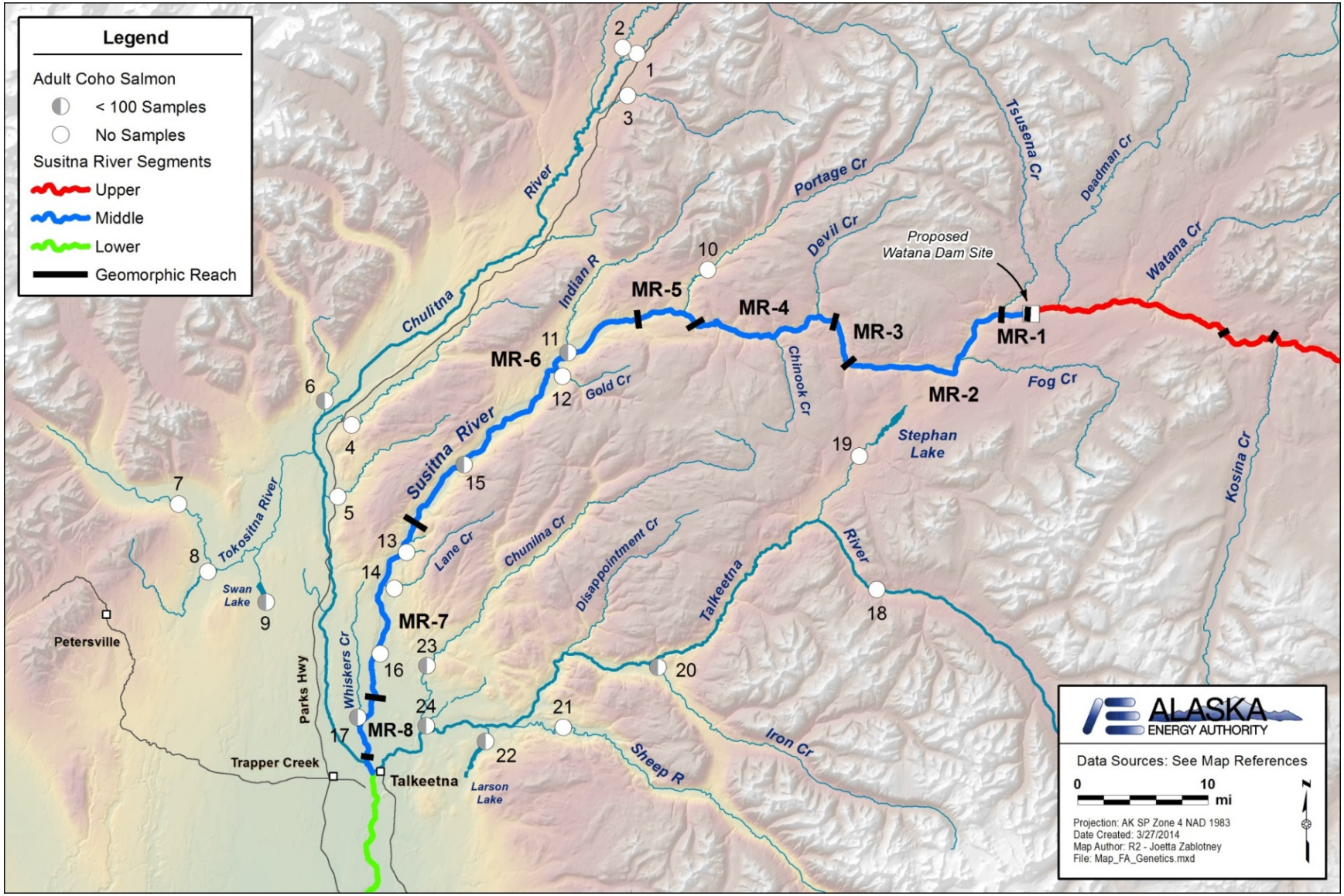

Figure 5. Potential baseline sampling locations for adult coho salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers in Table 6.

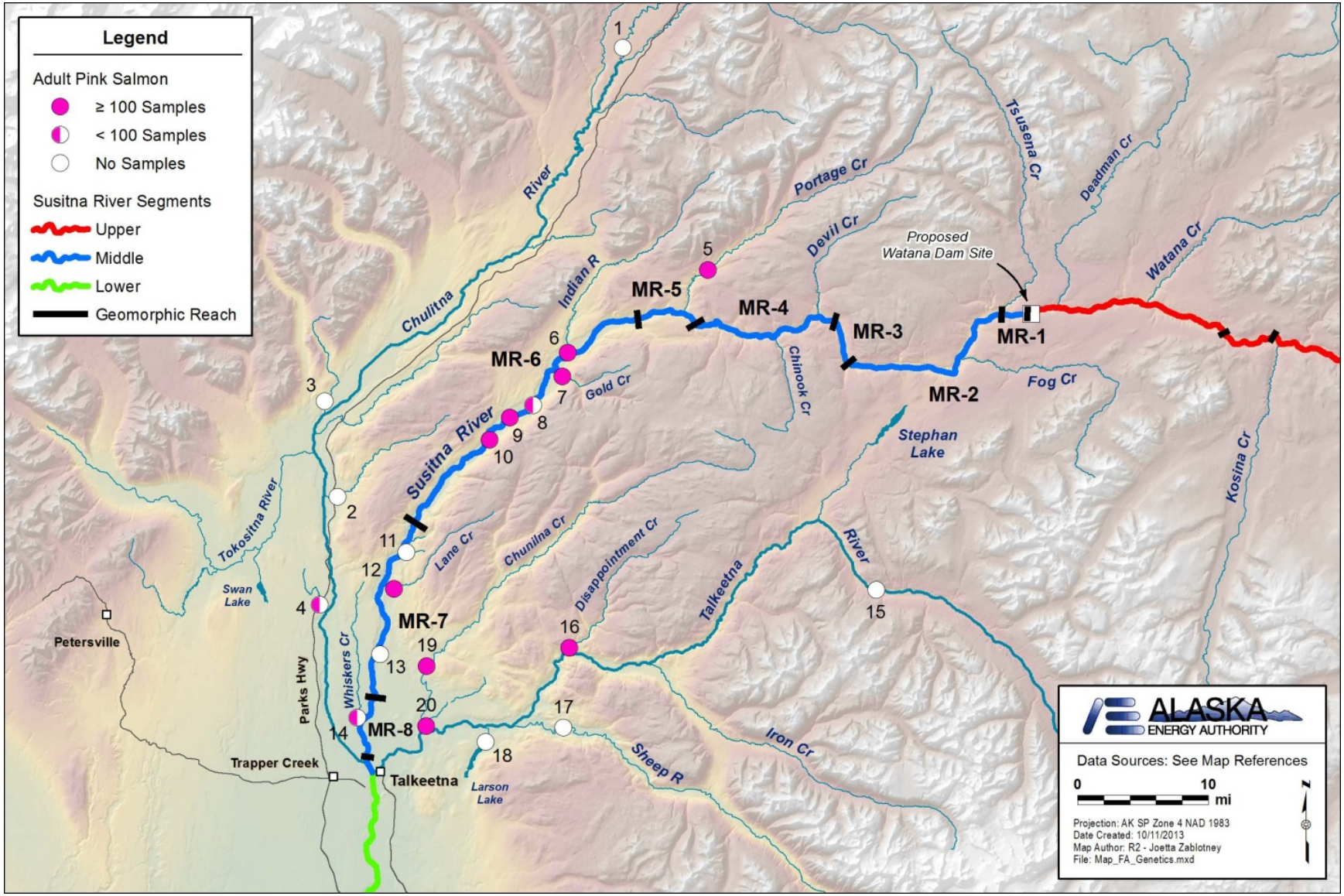

Figure 6. Potential baseline sampling locations for adult pink salmon. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers in Table 7.

PART B – ATTACHMENT 1 - APPENDIX A. SUMMARY OF SURVEYS FOR ADULT CHINOOK SALMON WITHIN AND ABOVE DEVILS CANYON

1. CUMULATIVE SUMMARY OF CHINOOK SALMON AERIAL SURVEYS (1980S, 2012 – 2013)

Prior to 1982, Devils Canyon was thought to provide a barrier to upstream migration of all salmon. Subsequent studies conducted by ADF&G, however, reported that a few Chinook salmon (peak counts of 19–46 individuals) were observed in small tributaries within and upstream of the Canyon (Table A1.1-1; ADF&G 1983, ADF&G 1984). In 1984 Chinook spawning was documented within Devils Canyon at Chinook and Cheechako Creeks and above Devils Canyon at Fog Creek (ADF&G 1985). More recently, Buckwalter (2011) observed adult Chinook salmon in Fog Creek and Tsusena Creek during 2003 and in Kosina Creek during 2011. In 2012, adult Chinook salmon were observed above Impediment 3 at Devil Creek, Fog Creek and Kosina Creek (Table A1.1-1). Extensive aerial surveys above Devils Canyon in 2013 documented adult Chinook salmon in all of the tributaries where they had been observed during previous studies (Figure A1.1-1).

Table A1.1-1. Summary of peak counts during aerial surveys for untagged adult Chinook salmon above Devils Canyon.

Survey Year	Survey Type	Cheechako Cr. (PRM 155.9)	Chinook Cr. (PRM 160.4)	Impediment 3	Devil Creek (PRM 164.8)	Fog Cr. (PRM 179.3)	Fog Cr. Tributary (Fog 5.1)	Unnamed Tributary 184	Unnammed Tributary 184 (Unnamed 0.8)	Tsusena Cr. (PRM 184.5)	Proposed Watana Dam (PRM 187.1)	Deadman Cr. (PRM 189.4)	Watana Cr. (PRM 196.9)	Kosina Cr. (PRM 209.1)	Gilbert Cr. (Kosina 6.2)	Tsisi Cr. (Kosina 7.3)	Jay Cr. (PRM 211.0)	Goose Cr. (PRM 232.9)	Oshetna River (PRM 235.1)	Black River (Oshetna)	Source
1982	Helicopter	16	5		0	0	-	-	-	-		-	-	-	-	-	-	-	-	-	R2 2013
1983	Helicopter	25	8		1	0	-	-	-	-		-	-	-	-	-	-	-	-	-	R2 2013
1984	Helicopter	29	15		0	2	-	-	-	0		0	0	-	-	-	-	-	-	-	R2 2013
1985	Helicopter	18	1		0	0	-	-	-	0		-	-	-	-	-	-	-	-	-	R2 2013
2003	Helicopter	-	-		0	2	0	0	0	1		0	0	0	0	0	0	-	-	-	Buckwalter 2011
2011	Helicopter/foot	-	-		-	0	0	-	-	0		-	0	1	-	-	0	-	0	-	Buckwalter 2011
2012	Helicopter	5	4		7	1	-	0	-	0		0	0	16	-	-	0	0	0	-	HDR 2013
2013	Helicopter	40	2		25	2	0	0	0	4		0	0	3	0	0	0	0	0	0	LGL and ADF&G 2014

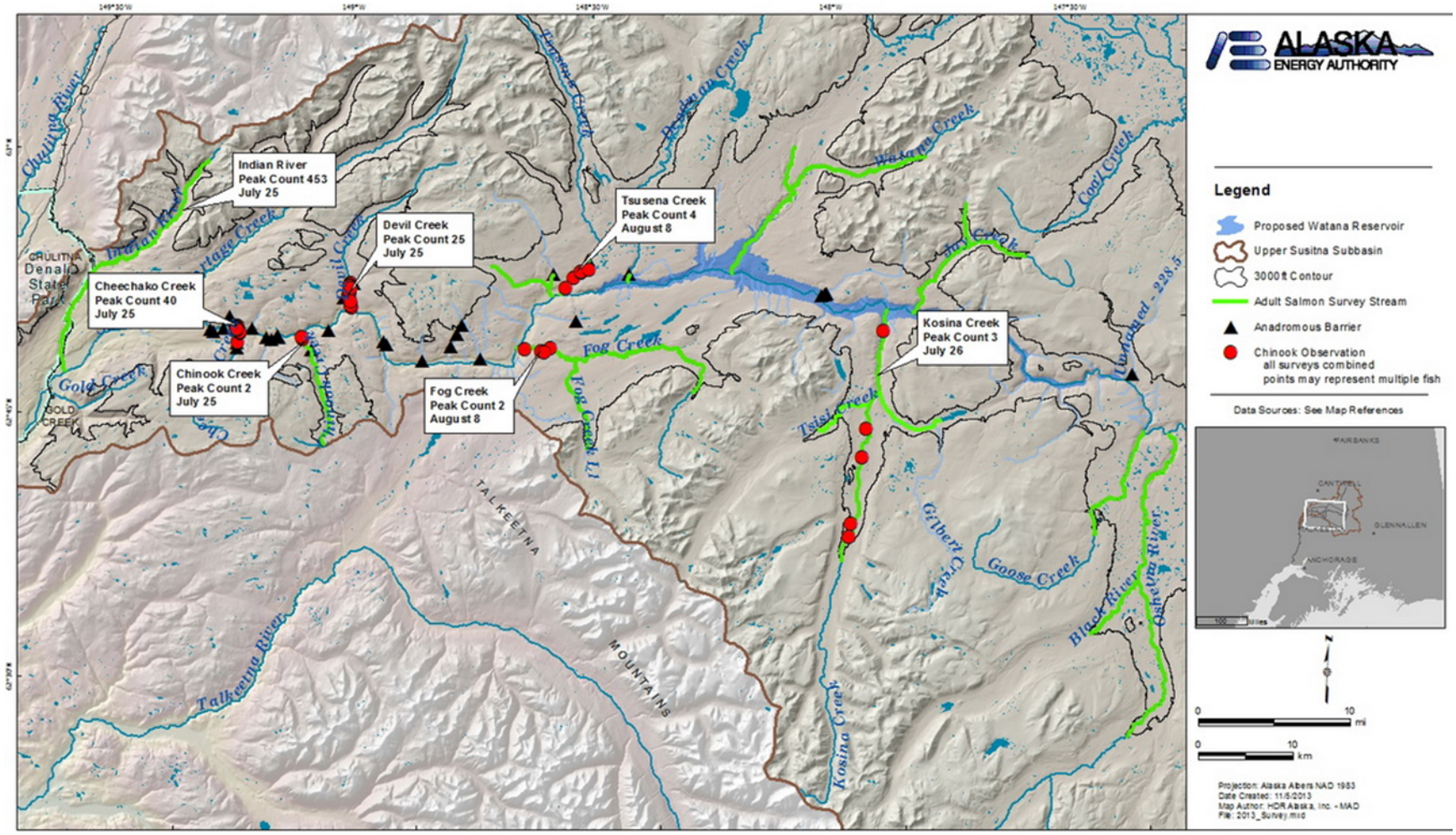

Figure A1.1-1. Summary of Chinook salmon observations during aerial spawned surveys in the Middle and Upper Susitna River segments, 2013. All survey observations were combined; and observation points may represent multiple fish.

2. CHINOOK SALMON RADIO TELEMETRY RESULTS: 2012 – 2013

Studies of adult in the Susitna River in 2012 and 2013 used radio tags to track Chinook salmon adults to their final spawning destinations (LGL 2013, LGL and ADF&G 2014). Chinook salmon were the only species identified migrating upstream of any of the three high velocity impediments in Devils Canyon (PRM 153.9 – PRM 166.1). The following sections highlight key findings of those studies as they relate to Chinook salmon passage above Impediment 3 in Devils Canyon.

2.1. Final Spawning Destinations

In 2012 and 2013 Chinook salmon were radio-tagged and tracked in the mainstem Susitna River as part of a multi-objective study to describe salmon migration behavior and identify salmon spawning locations (LGL 2013, LGL and ADF&G 2014). Radio telemetry was used to assign final destinations for Chinook salmon tagged in the Lower River (near PRM 33-34) and Chinook salmon tagged in the Middle River at Curry (PRM 123-126). Most final destinations of tagged fish were documented in tributaries (90 to 99 percent), whereas relatively few final destinations were in mainstem river habitats (Table A2.1-1).

Table A2.1-1. Number of tagged Chinook salmon with associated genetics samples and samples from untagged fish collected during surveys at spawning destinations in the Susitna River basin, 2012-2013. Juvenile collections denoted within parentheses with a "J". Rows for locations within and above Devils Canyon are highlighted in grey. Additional samples from spawning ground surveys prior to 2012 and from sites not represented by final destinations of tagged fish exist in ADF&G Gene Conservation Laboratory archives (see Table 2 in 2014 IP).

	2012			2013			
	Tagging Location		Spawning Ground Genetics Samples	Tagging Location			Spawning Ground Genetics Samples
Destination	Lower River	Middle River		Lower River	Middle River	Yentna River	
Tributary Destinations (total)	**360**	**286**	**245**	**617**	**422**	**602**	**1365**
Alexander Creek	2	0	53	2	0	0	0
Yentna River	40	0	0	72	0	596	188
Deshka River	109	1	52	155	0	0	0
Willow Creek	19	0	49	37	0	0	245
Little Willow Creek	23	0	0	22	0	0	55
Kashwitna Creek	12	0	0	21	0	0	12
Goose Creek	2	0	0	1	0	0	0
Sheep Creek	10	0	0	11	1	0	24
Montana Creek	10	3	0	12	4	1	0
Talkeetna River	53	6	79	134	30	4	255
Chulitna River	60	13	0	110	71	1	477
Lane Creek	0	0	0	0	2	0	0
4th of July Creek	0	5	0	1	4	0	0
Gold Creek	0	1	0	0	2	0	0
Indian River	7	85	1	11	88	0	81
Jack Long Creek	0	1	0	0	0	0	0
Portage Creek	11	157	1	26	213	0	25

Destination	2012 Tagging Location: Lower River	2012 Tagging Location: Middle River	2012 Spawning Ground Genetics Samples	2013 Tagging Location: Lower River	2013 Tagging Location: Middle River	2013 Tagging Location: Yentna River	2013 Spawning Ground Genetics Samples
Cheechako Creek	0	6	0	2	4	0	0
Chinook Creek	0	3	0	0	1	0	0
Devil Creek	0	1	0	0	1	0	0
Tsusena Creek	0	0	0	0	1	0	0
Kosina	2	4	10	0	0	0	3 (189J)
Mainstem Destinations (total)	**11**	**31**	**0**	**4**	**27**	**0**	**0**
Mainstem Proper	4	11	0	0	7	0	0
Downstream of Lane	3	0	0	0	2	0	0
no prior spawn location	*0*	*0*	0	*0*	*1*	*0*	0
was in Talkeetna River	*0*	*0*	0	*0*	*1*	*0*	0
Upstream of Lane	1	11	0	0	5	0	0
no prior spawn location	*0*	*0*	0	*0*	*5*	*0*	0
Tributary Mouths	2	18	0	4	15	0	0
Yentna Mouth	0	0	0	0	0	0	0
Deshka Mouth	2	0	0	3	0	0	0
Willow Mouth	0	0	0	1	0	0	0
Talkeetna Mouth	0	0	0	0	1	0	0
Lane Mouth	0	0	0	0	1	0	0
4th of July Mouth	0	1	0	0	1	0	0
no prior spawn location	*0*	*0*	0	*0*	*1*	*0*	0
Indian Mouth	0	10	0	0	6	0	0
no prior spawn location	*0*	*0*	0	*0*	*6*	*0*	0
Portage Mouth	0	7	0	0	6	0	0
no prior spawn location	*0*	*0*	0	*0*	*5*	*0*	0
was in Portage Creek	*0*	*0*	0	*0*	*1*	*0*	0
Side Channels & Sloughs	5	2	0	0	5	0	0
Slough 8A	0	0	0	0	0	0	0
Slough 9	0	0	0	0	0	0	0
Slough 11	0	0	0	0	0	0	0
Slough 21	0	0	0	0	0	0	0
Other areas	5	2	0	0	5	0	0
no prior spawn location	*0*	*0*	0	*0*	*3*	*0*	0
was in Portage Creek	*0*	*0*	0	*0*	*2*	*0*	0
Other Fates (total)	**71**	**35**	**0**	**68**	**87**	**90**	**0**
Other Mainstem	29	11	0	22	43	12	0
Max Zone downstream of Lane	29	0	0	2	43	0	0
Max Zone upstream of Lane	0	11	0	20	0	12	0
Downstream only	32	6	0	10	30	29	0
Near Release Site	6	17	0	22	14	23	0
Single or No detections	3	0	0	14	0	26	0
Tag Removed	1	1	0	0	0	0	0
Total Tags Released	**442**	**352**	**0**	**689**	**536**	**692**	**0**

2.2. Chinook Passing Above the Third Impediment of Devils Canyon

In 2012, 12 radio-tagged Chinook salmon passed upstream of Impediment 3 in Devils Canyon (Table A2.2-1) whereas in 2013, only 3 tagged fish were documented upstream of Impediment 3 (Table A2.2-1). Of all the 15 tagged Chinook salmon (2012 and 2013 combined) that migrated upstream of Impediment 3, 6 eventually migrated back downstream and were assigned to final destinations downstream. The 9 Chinook salmon that were assigned to spawning destinations above Devils Canyon were found in Kosina Creek at PRM 209.2 (5 fish), Tsisi Creek, a tributary to Kosina Creek (1 fish), Tsusena Creek at PRM 184.4 (1 fish), and Devil Creek at PRM 164.8 (2 fish). Tracking histories for each of these individual Chinook salmon are presented in Figures A2.2-1 through A2.2-15.

Table A2.2-1. Summary of radio-tagged Chinook salmon passing upstream of Impediment 3 in Devils Canyon, 2012-2013.

Tagging Site	Year	No. Large Chinnok Tagged	No. observed above I3	No. Returning Downstream	Final Spawning Desintations	No. Remaining Upstream	Final Spawning Desintations
Curry Site 1	2012	223	5	2	Portage Cr. (2)	3	Kosina Cr. (2), Devil Cr. (1)
Curry Site 2	2012	129	5	3	Portage Cr. (2) Chinook Cr. (1)	2	Kosina Cr. (1), Tsisi Cr. (1)
Lower River	2012	442	2	0	NA	2	Kosina Cr. (2)
All	***2012***	***794***	***12***	***5***		***7***	
Curry Site 1	2013	449	2	0	NA	2	Devil Creek (1), Tsusena Cr (1)
Curry Site 2	2013	81	1	1	Approached headwaters, then moved down in Tsusena Creek (1 day), in Portage Creek (3 days), then down to below Sunshine	0	NA
Curry Site 3	2013	6	0	0	NA	0	NA
Lower River	2013	698	0	0	NA	1	NA
All	***2013***	***1,234***	***3***	***1***		***2***	

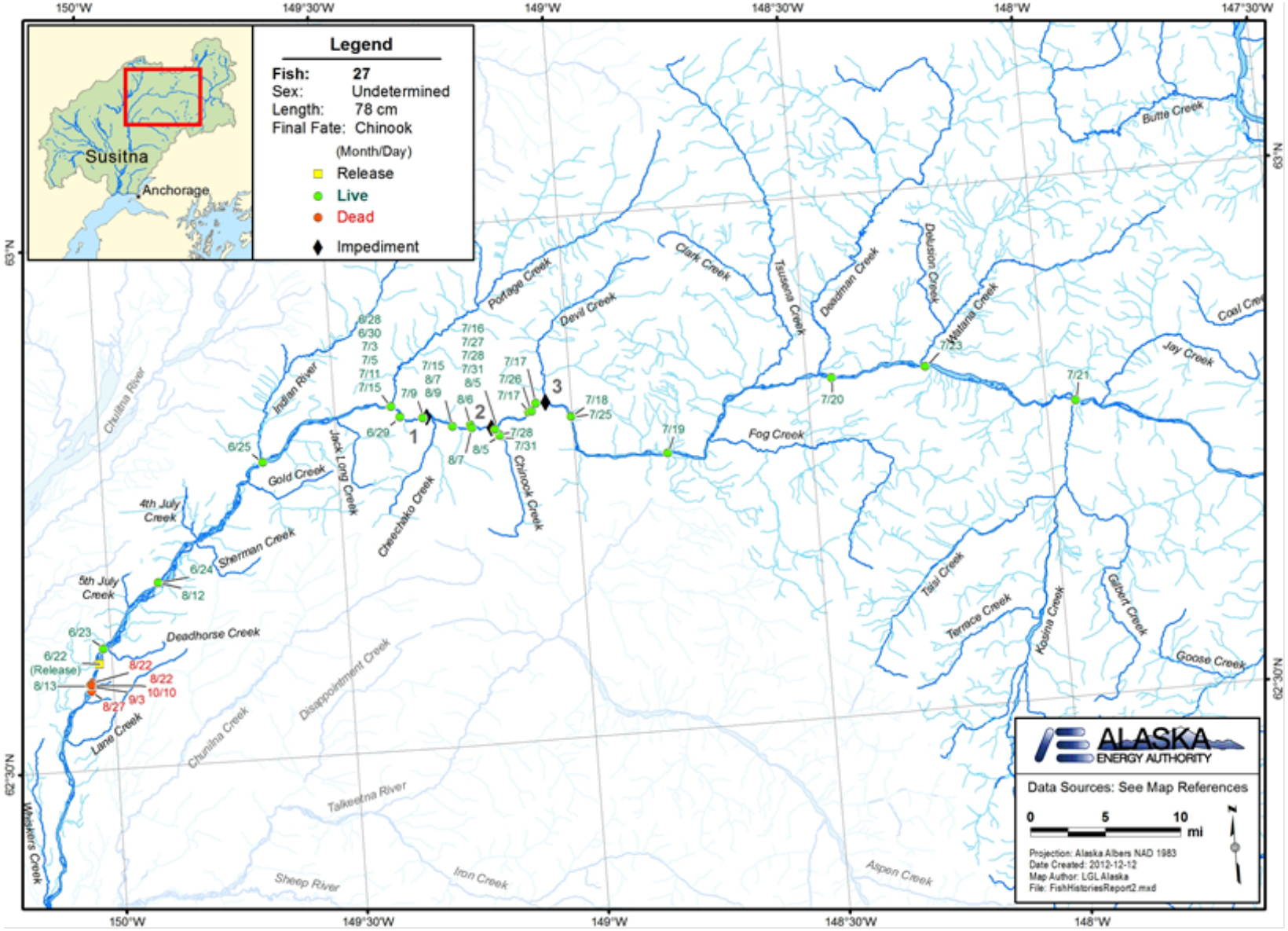

Figure A2.2-1. Tracking history of radio-tagged Fish 27 in 2012.

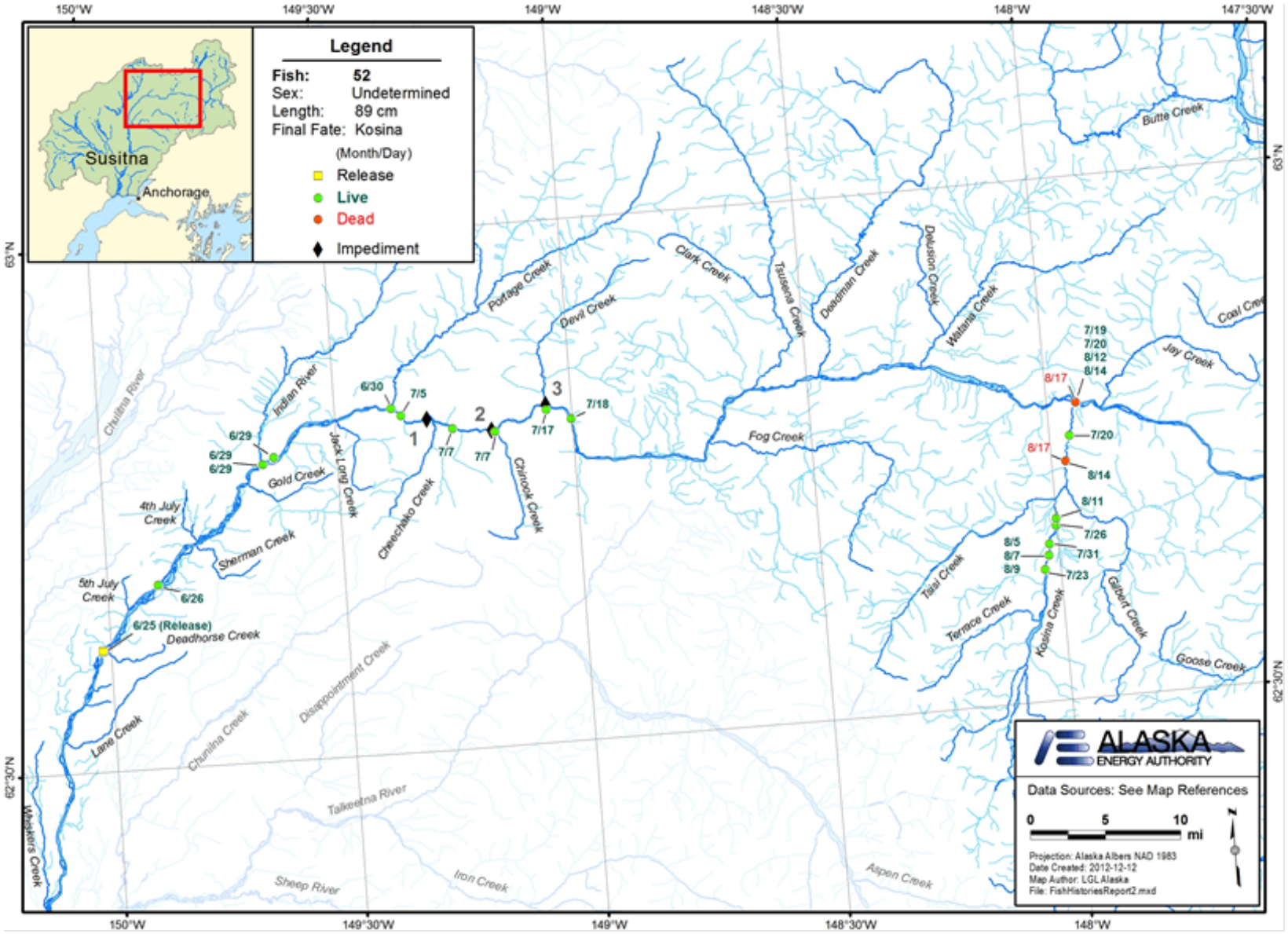

Figure A2.2-2. Tracking history of radio-tagged Fish 52 in 2012.

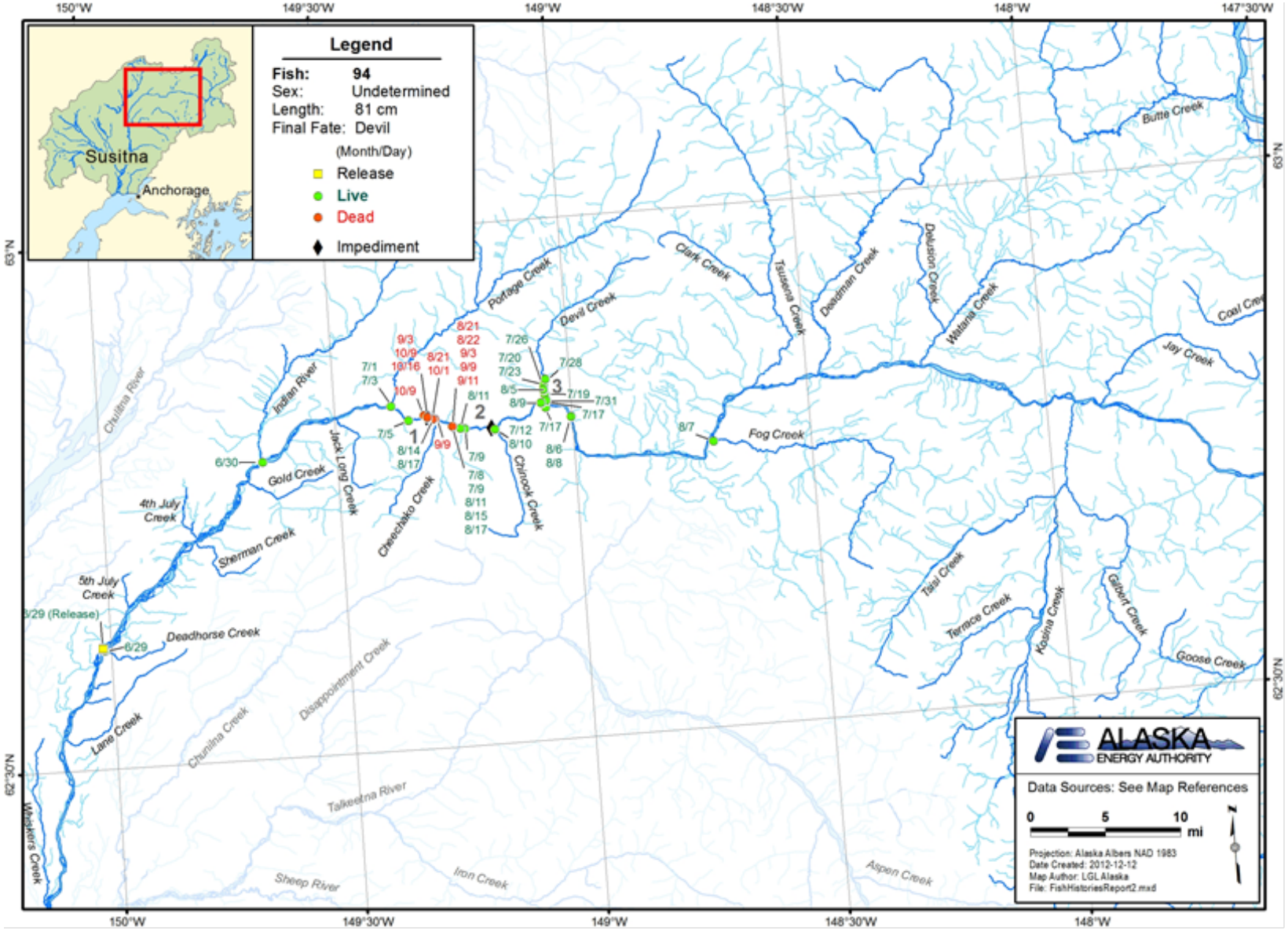

Figure A2.2-3. Tracking history of radio-tagged Fish 94 in 2012.

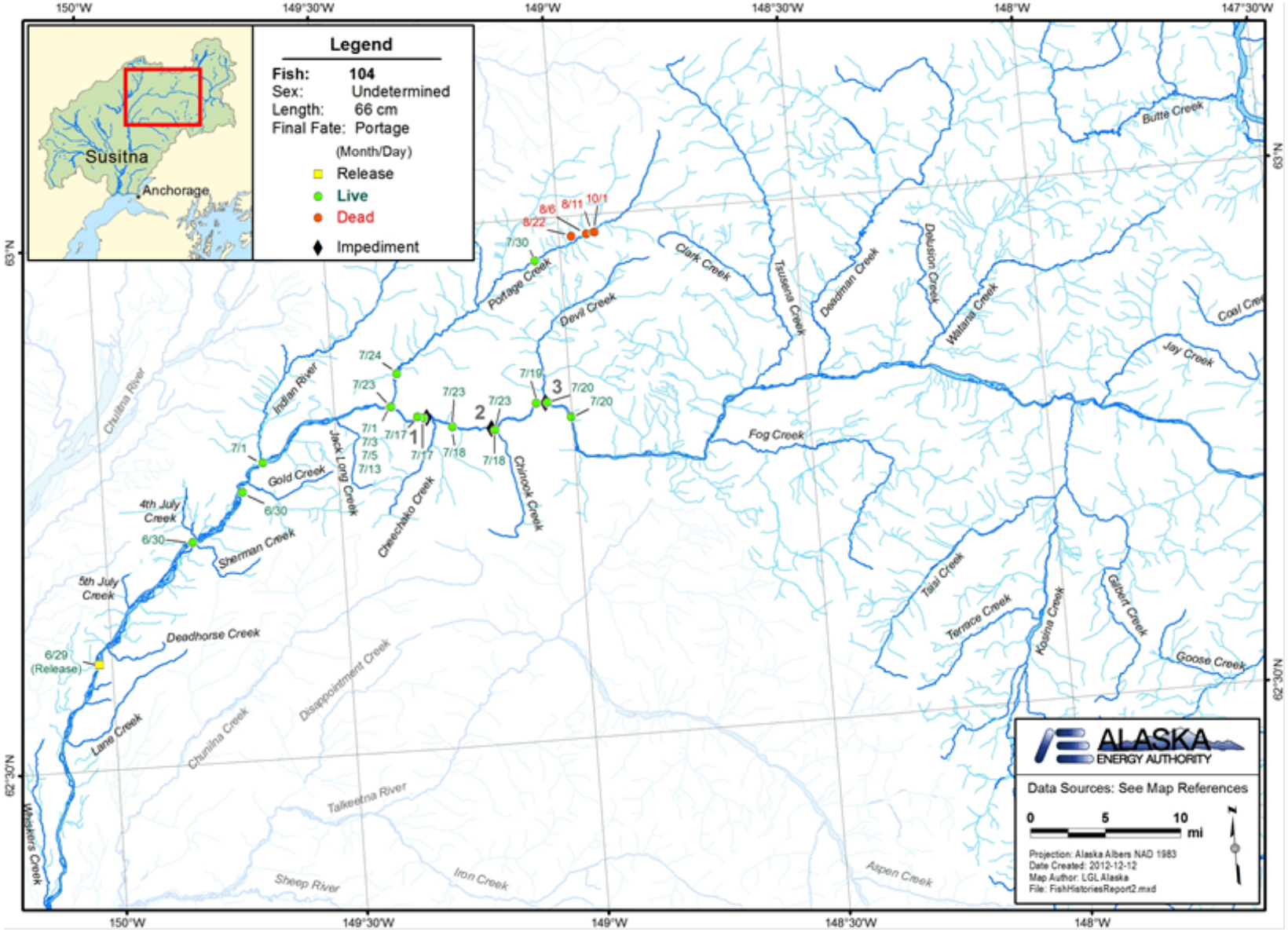

Figure A2.2-4. Tracking history of radio-tagged Fish 104 in 2012.

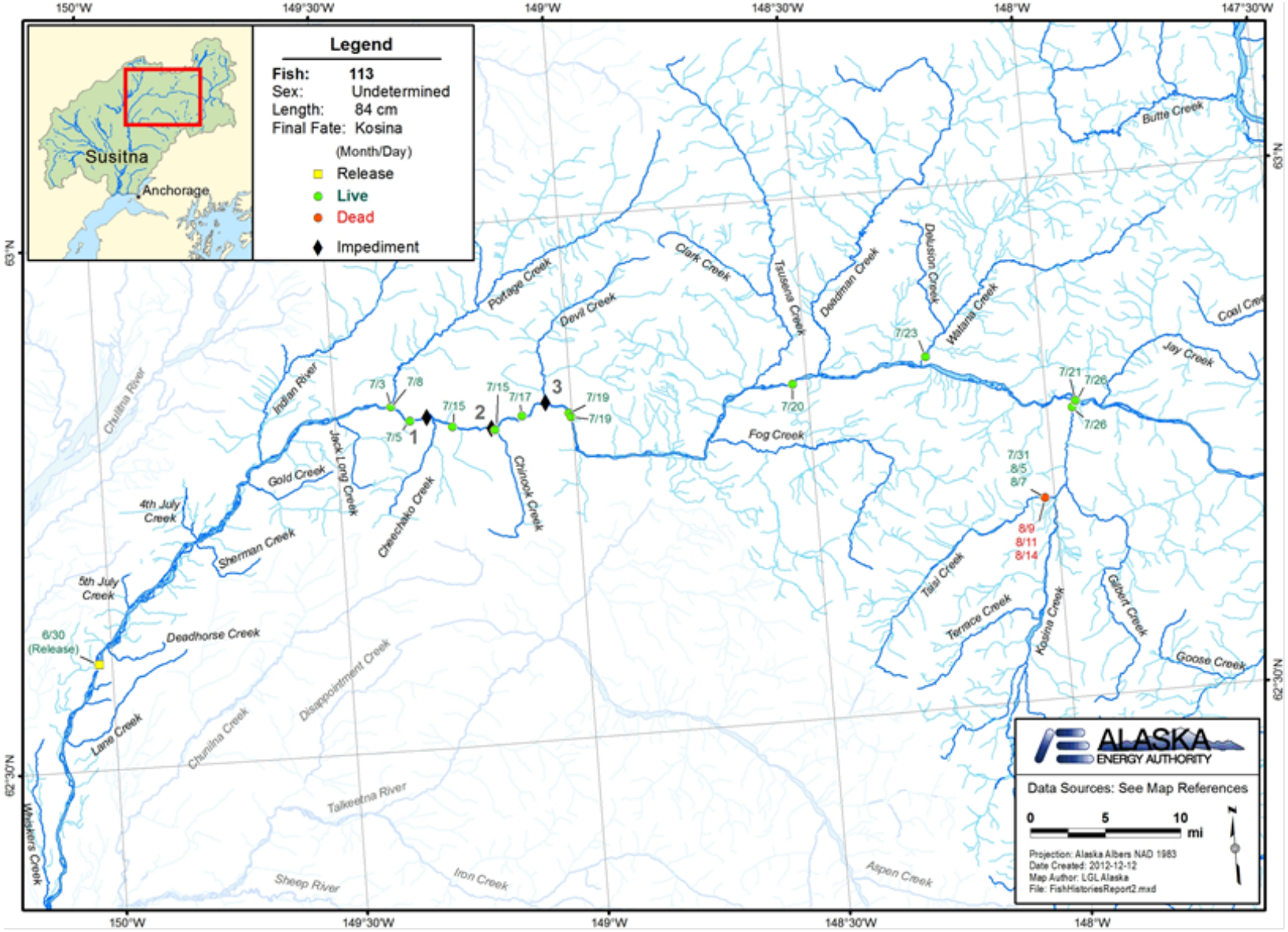

Figure A2.2-5. Tracking history of radio-tagged Fish 113 in 2012.

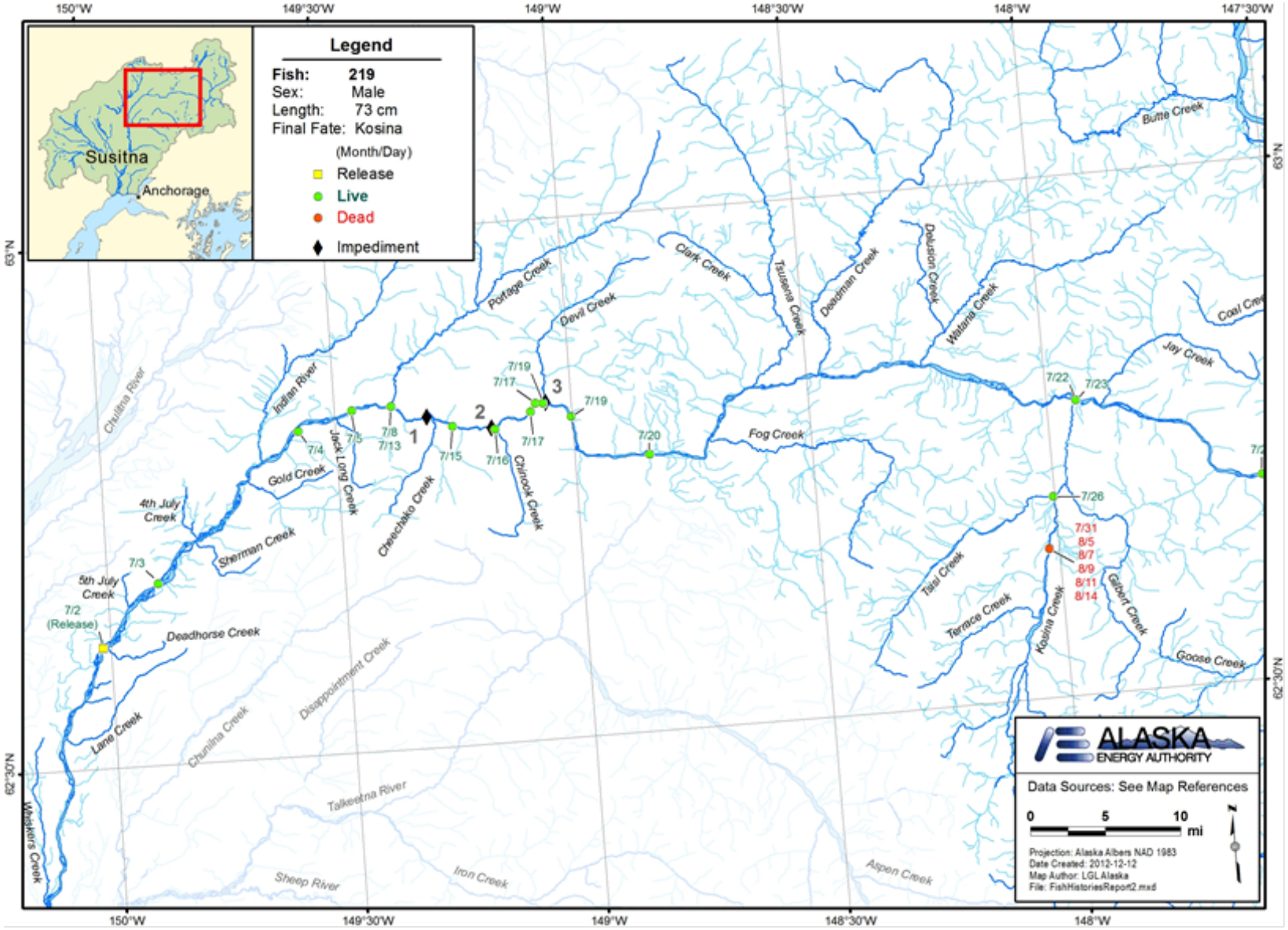

Figure A2.2-6. Tracking history of radio-tagged Fish 219 in 2012.

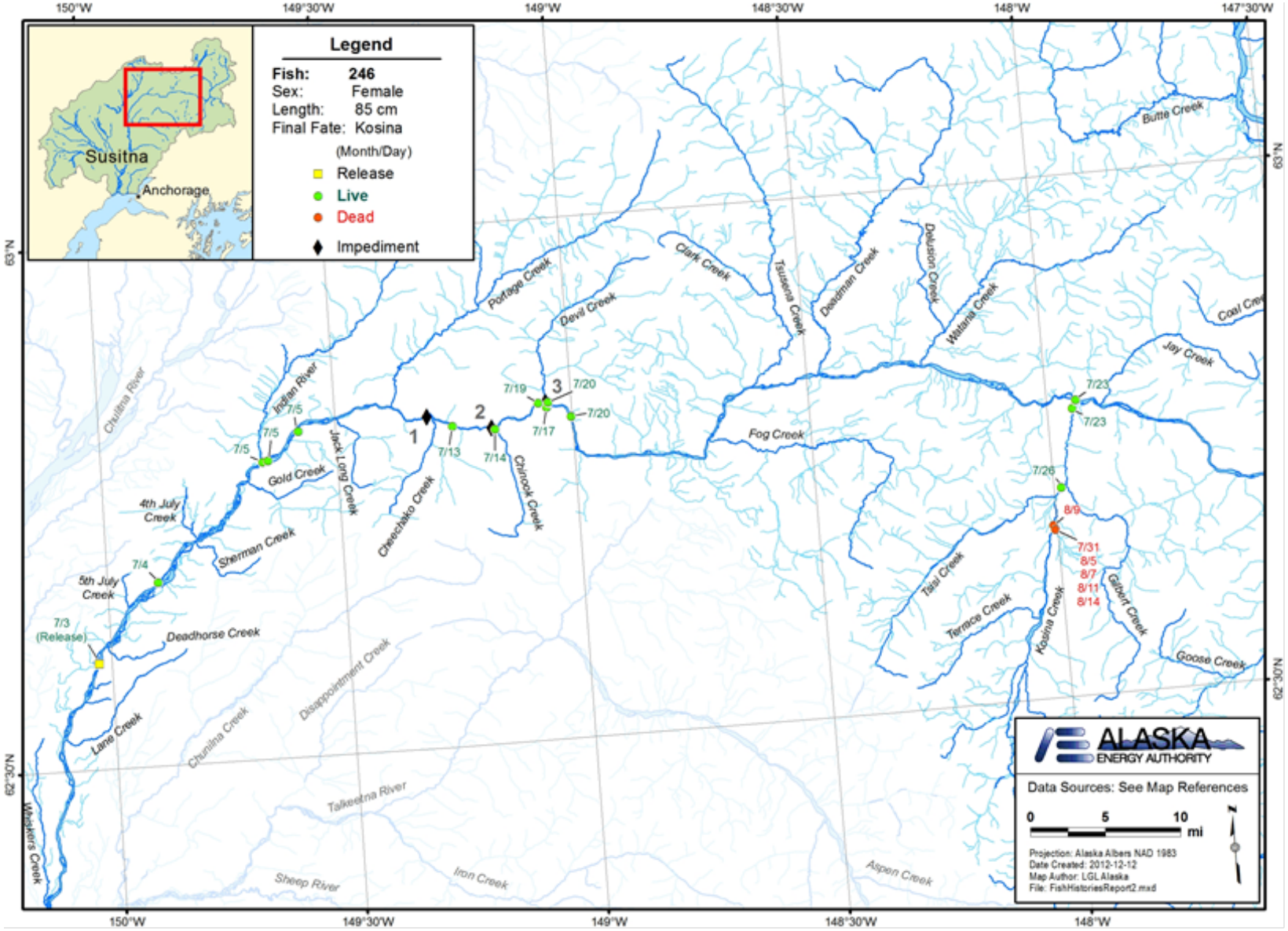

Figure A2.2-7. Tracking history of radio-tagged Fish 246 in 2012.

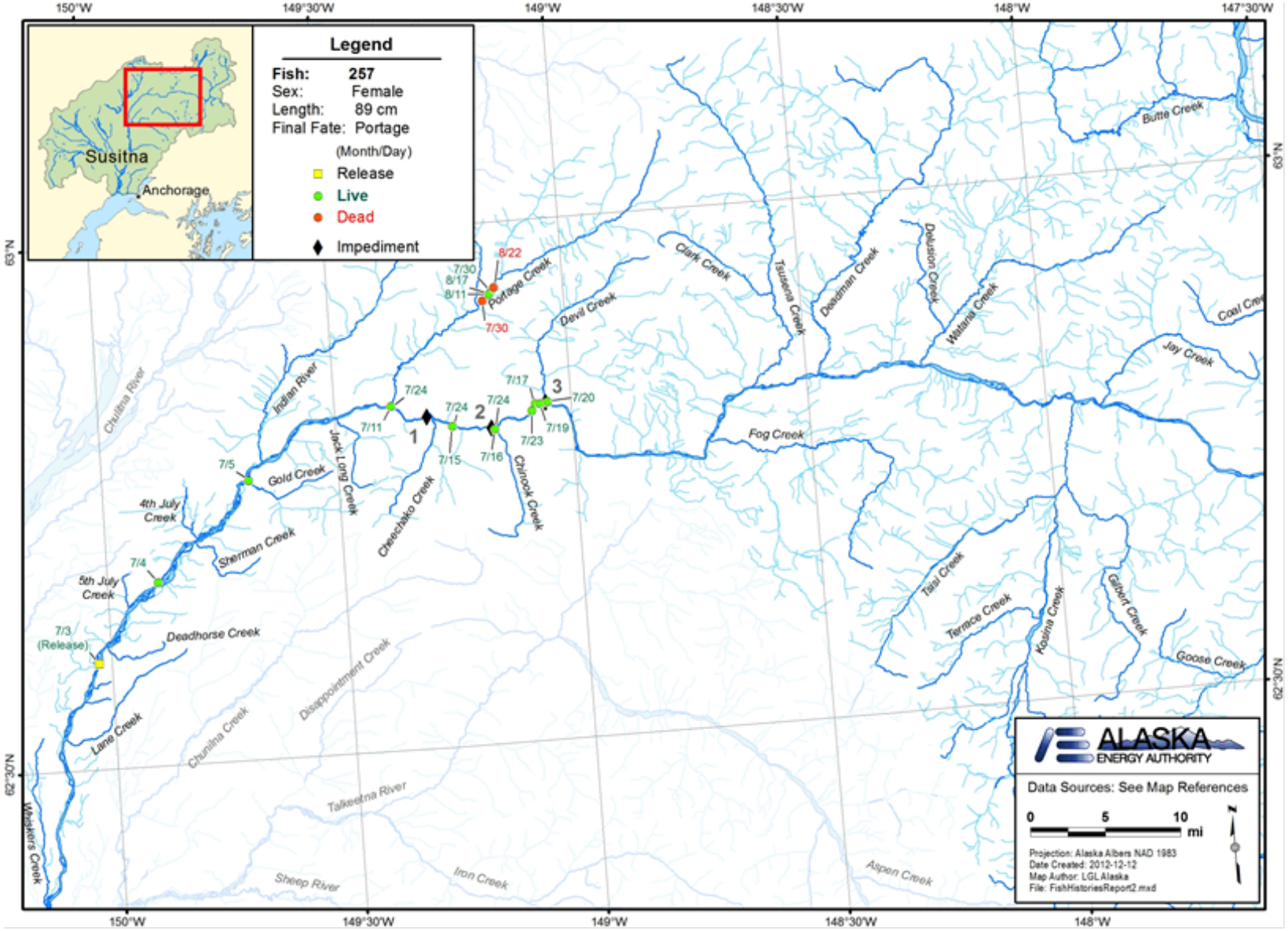

Figure A2.2-8. Tracking history of radio-tagged Fish 257 in 2012.

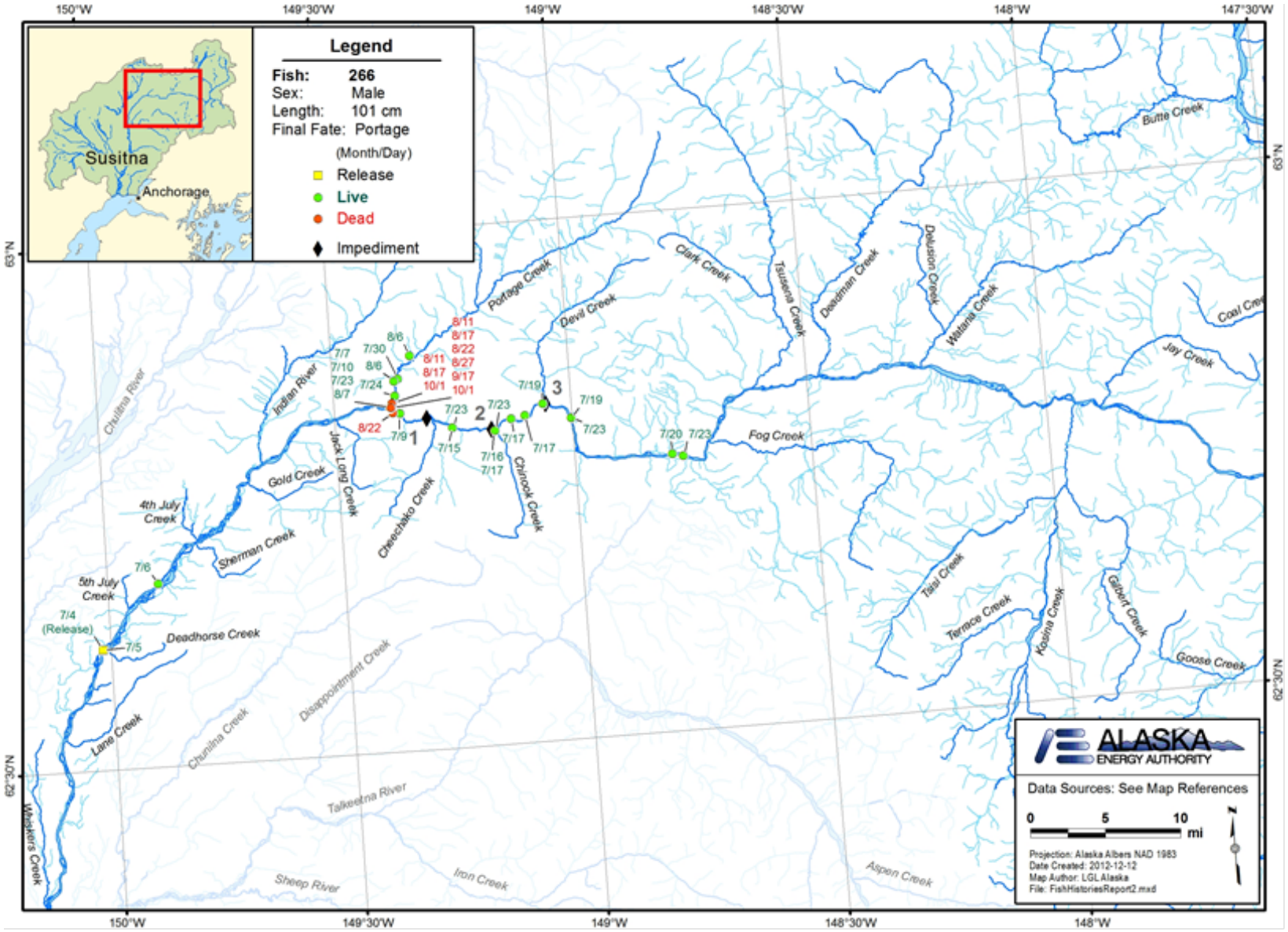

Figure A2.2-9. Tracking history of radio-tagged Fish 266 in 2012.

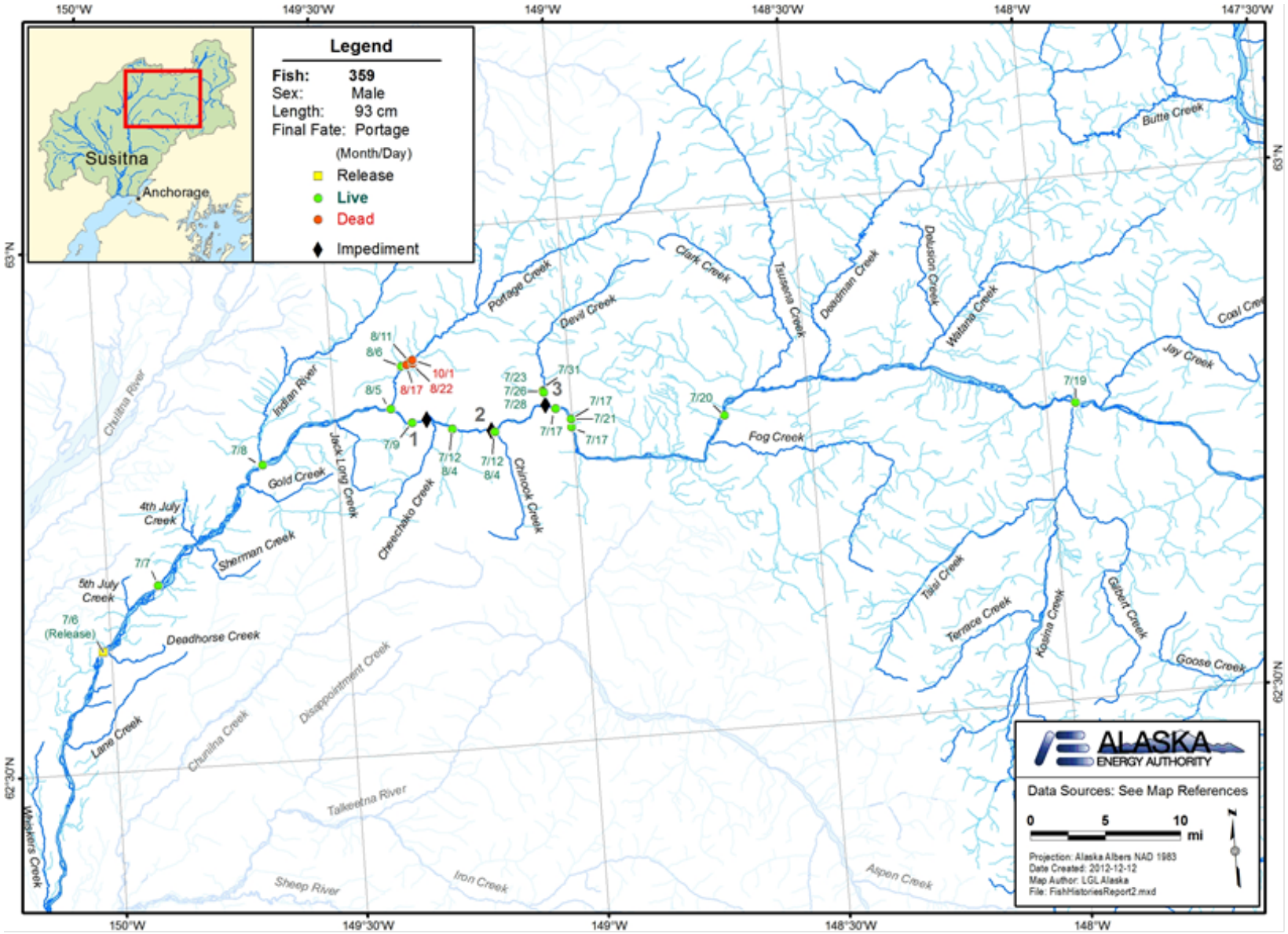

Figure A2.2-10. Tracking history of radio-tagged Fish 359 in 2012.

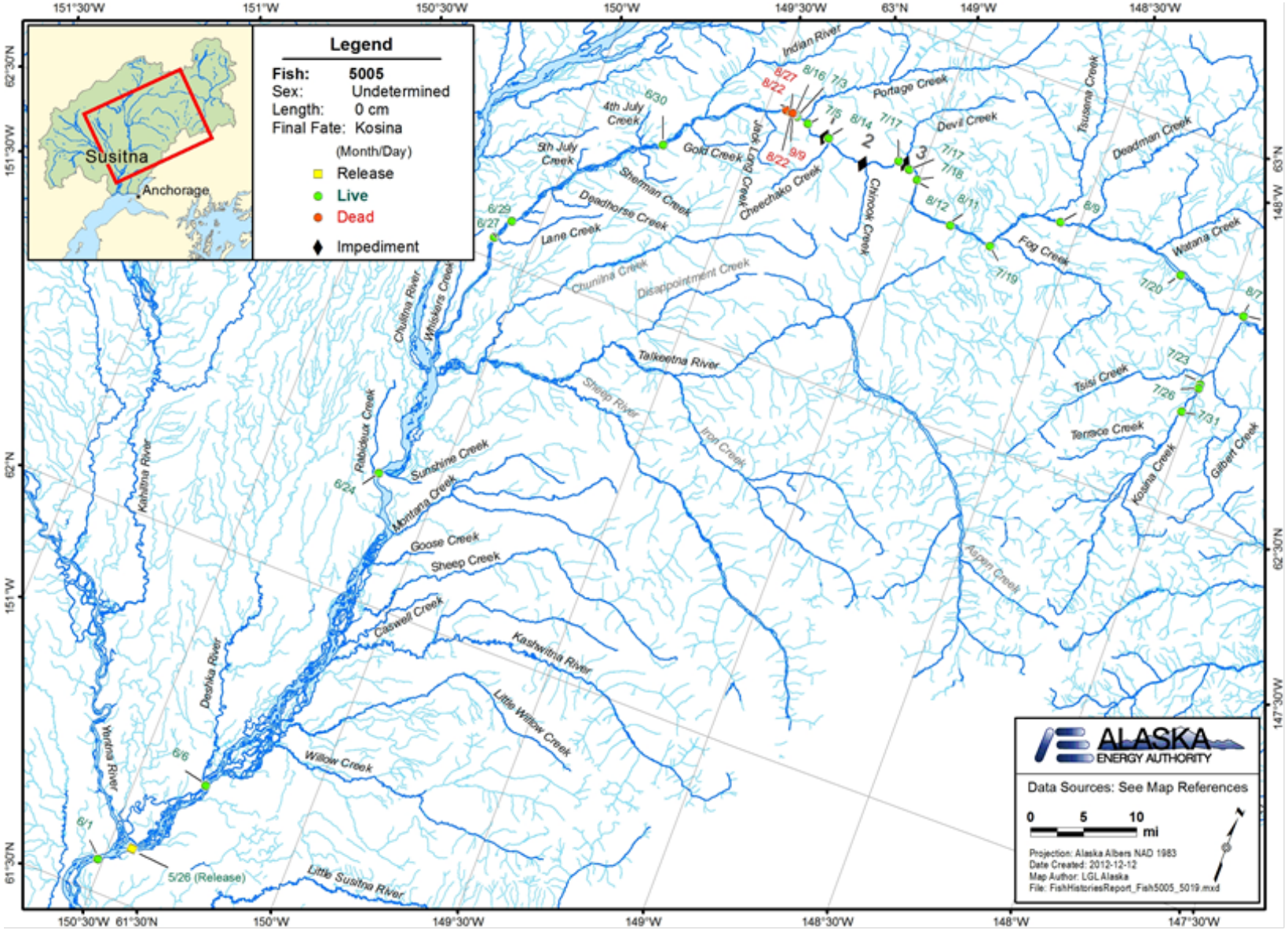

Figure A2.2-11. Tracking history of radio-tagged Fish 5005 in 2012.

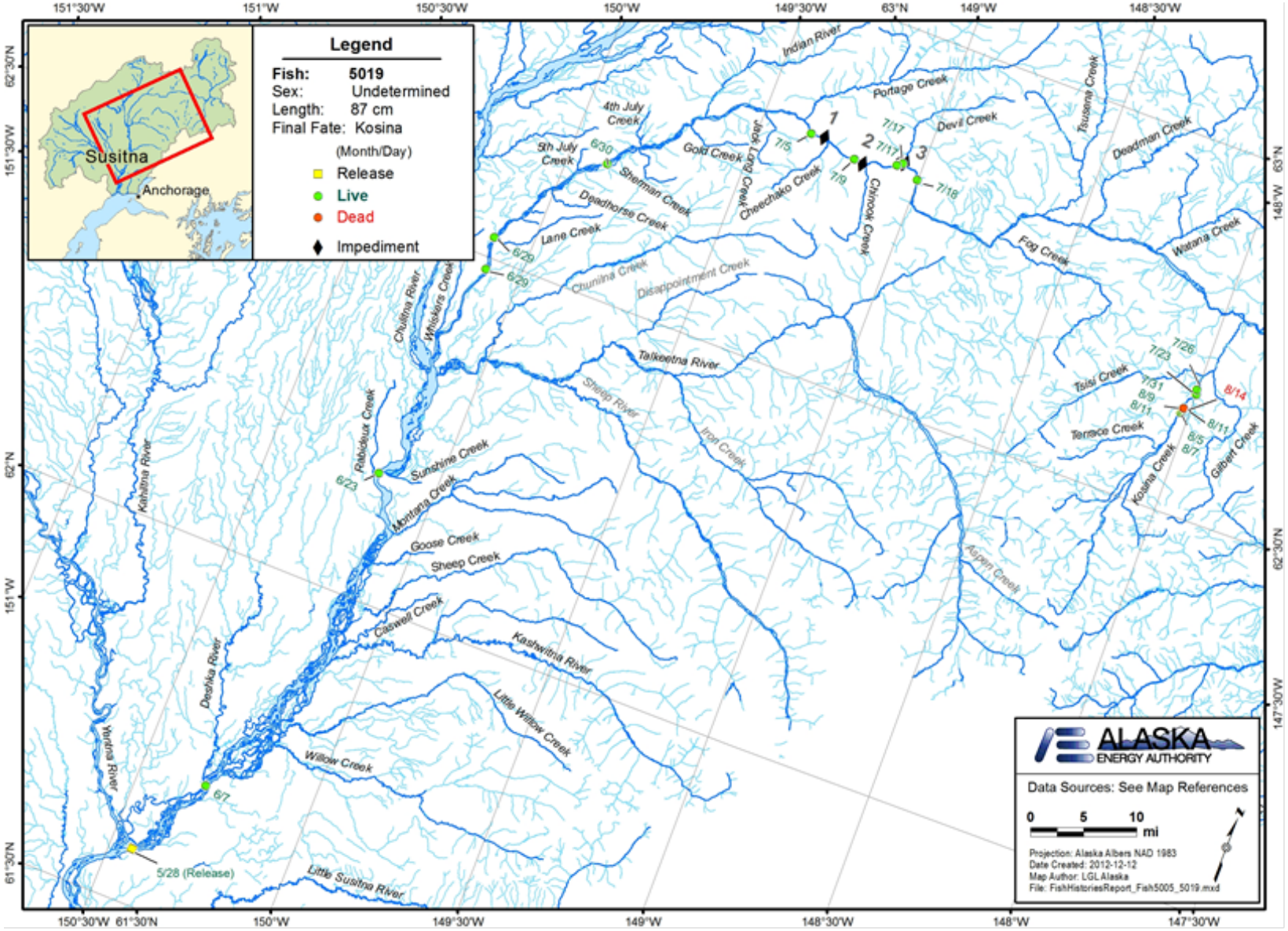

Figure A2.2-12. Tracking history of radio-tagged Fish 5019 in 2012.

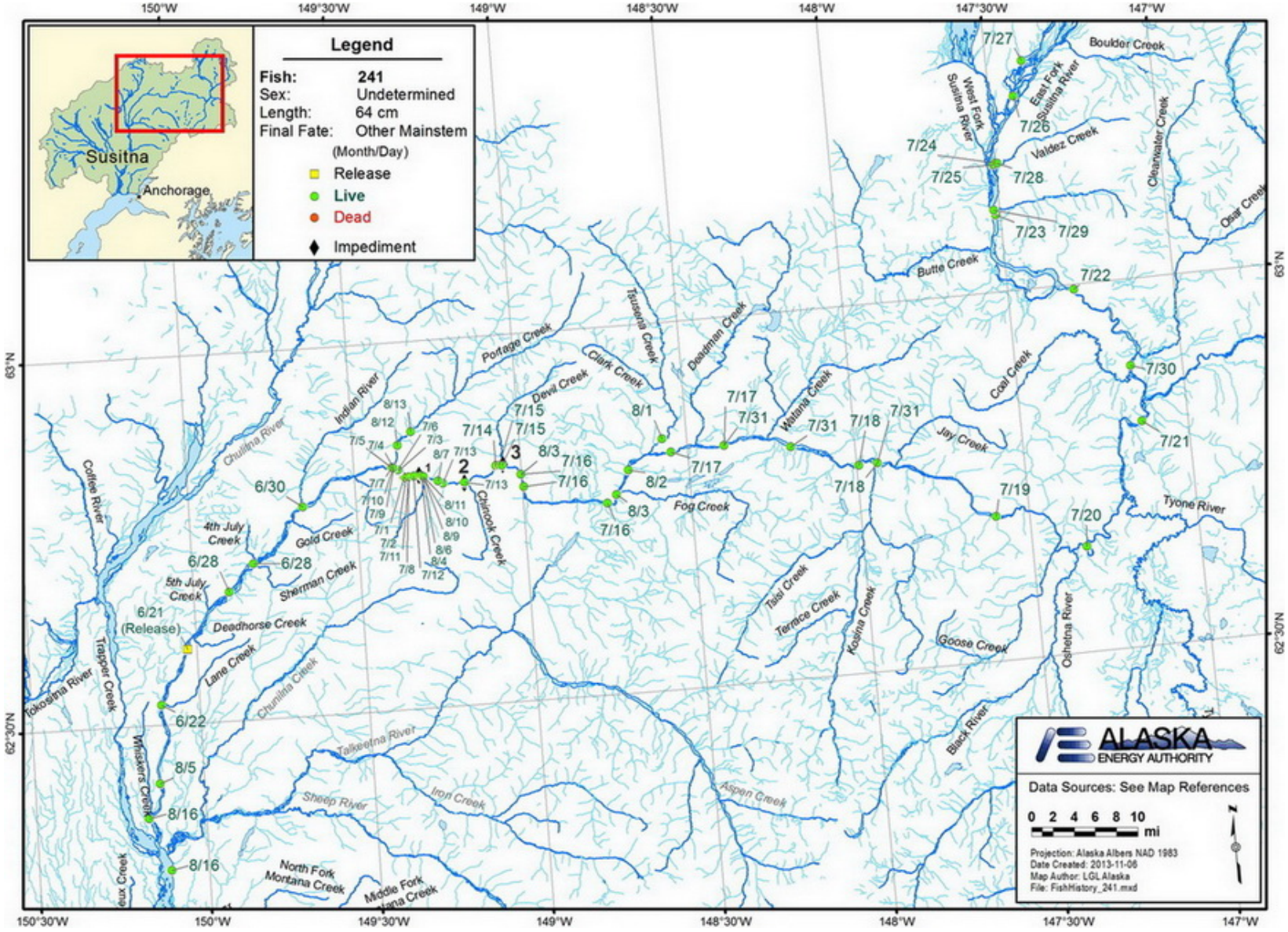

Figure A2.2-13. Tracking history of radio-tagged Fish 241 in 2013.

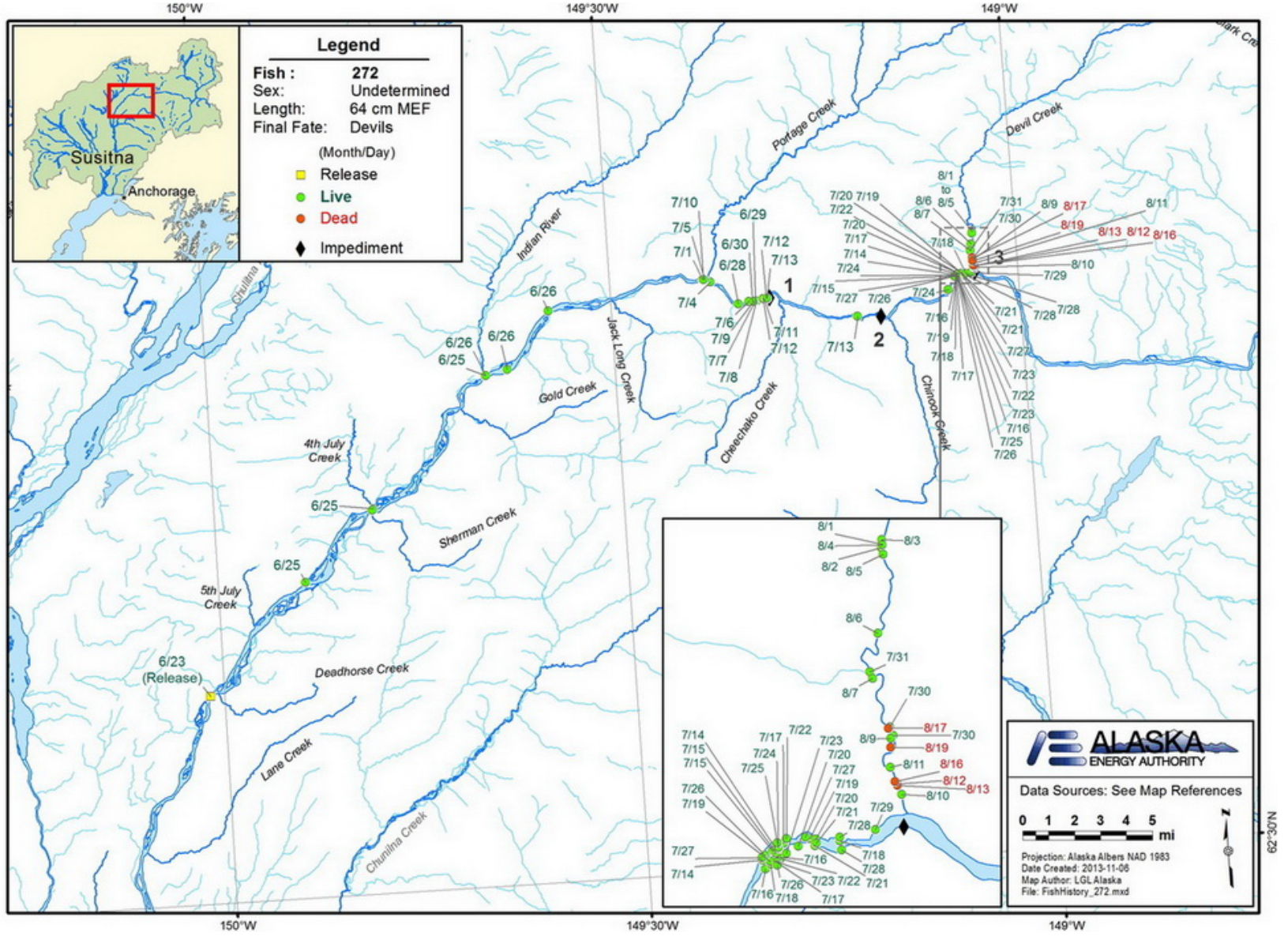

Figure A2.2-14. Tracking history of radio-tagged Fish 272 in 2013.

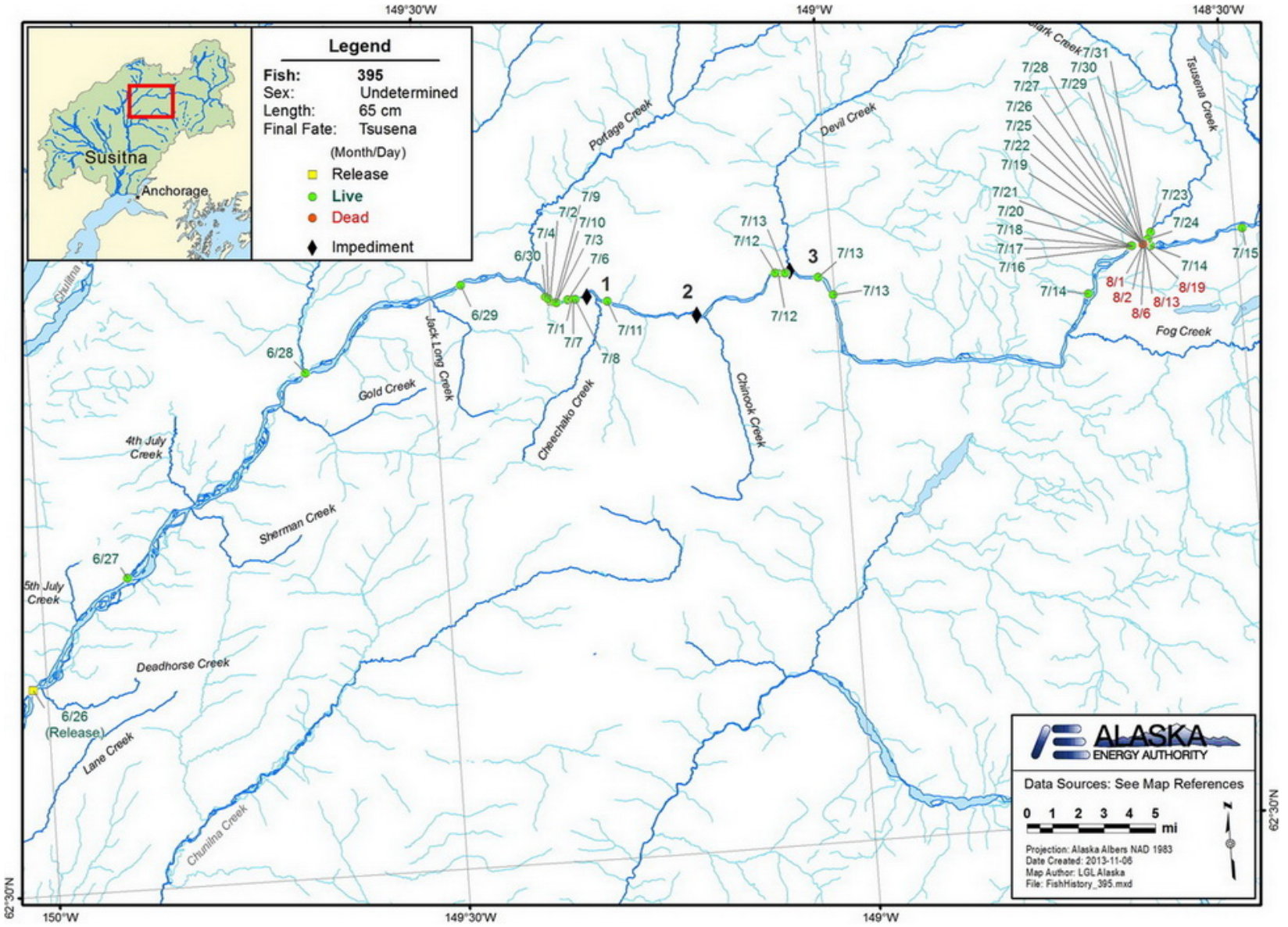

Figure A2.2-15. Tracking history of radio-tagged Fish 395 in 2013.

3. ABUNDANCE ESTIMATE FOR CHINOOK SALMON ABOVE DEVILS CANYON, 2013

As reported in the draft Initial Study Report for the Salmon Escapement Study (ISR Study 9.7), too few tagged and untagged Chinook salmon were observed above Devils Canyon in 2013 to develop statistically precise estimates of the number of Chinook salmon passing above Devils Canyon (LGL and ADF&G 2014). Two different approaches were used to estimate the abundance of Chinook salmon above Devils Canyon (i.e., above Impediment 3). The first approach involved expanding the peak aerial count by the estimated observer efficiency during the spawner surveys. In 2013, the peak count of live Chinook salmon (all sizes combined) above Devils Canyon was 29 fish (25 in Devil Creek, 1 in Fog Creek, and 3 in Kosina Creek), which was obtained during the aerial spawner survey conducted from July 25–27. If this number (29) is expanded based on an observer efficiency of 46.3 percent, which was obtained during the AEA 2013 aerial spawner survey in Indian River, then an estimated 63 live Chinook salmon were above Devils Canyon at the time of the survey. This would be considered a minimum number as the visibility of Chinook salmon during aerial surveys was better in Indian River than some of the tributaries above Devils Canyon.

The second approach involved expanding the number of radio-tagged Chinook salmon detected above Devils Canyon by the marked fraction of Chinook salmon in the Middle River. Of the Chinook salmon released in the lower river in 2013, 58 passed Lane Creek, and none were detected above Impediment 3 in Devils Canyon. Of the 445 radio-tagged large Chinook salmon that were released at Curry and entered the study area (i.e., that reached Gateway) in 2013, three (0.7 percent) were detected above Impediment 3 in Devils Canyon. If AEA expands these three radio-tagged fish by the estimated marked fraction of large Chinook salmon (6.3 percent, or each tagged fish represented at total of approximately 15.9 fish), then it can be inferred that 48 large Chinook salmon migrated above Devils Canyon in 2013. As a sensitivity analysis of an extreme and unlikely event, if four or five radio-tagged large Chinook salmon had actually migrated above Impediment 3 in 2013, then the expanded counts would have been 63 or 79 fish, respectively.

4. REFERENCES

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Buckwalter, JD. 2011. Synopsis of ADF&G's Upper Susitna Drainage Fish Inventory, August 2011. November 22, 2011. ADF&G Division of Sport Fish, Anchorage, AK. 173 pp.

HDR Alaska, Inc. 2013. 2012 Upper Susitna River Fish Distribution and Habitat Survey, Fish Distribution Report. Susitna-Watana Hydroelectric Project FERC Project No. 14241. April 2013. Prepared for the Alaska Energy Authority, Anchorage, Alaska.

LGL Alaska Research Associates, Inc. 2013. Adult Salmon Distribution and Habitat Utilization Study. Susitna-Watana Hydroelectric Project FERC Project No. 14241. January 2013. Prepared for the Alaska Energy Authority, Anchorage, Alaska.

LGL Alaska Research Associates, Inc. & Alaska Department of Fish and Game (ADF&G), Division of Sport Fish. 2014. Salmon Escapement Study (Study Plan Section 9.7) draft Initial Study Report: Susitna-Watana Hydroelectric Project FERC Project No. 14241. February 2014. Prepared for the Federal Energy Regulatory Commission on behalf of the Alaska Energy Authority, Anchorage, Alaska.

R2 Resource Consultants, Inc. 2013. Synthesis of Existing Fish Population Data. Susitna-Watana Hydroelectric Project FERC Project No. 14241. February 2013. Prepared for the Alaska Energy Authority, Anchorage, Alaska.

PART B – ATTACHMENT 1 - APPENDIX B. MATERIALS FROM A PRELIMINARY GENETIC ANALYSIS OF CHINOOK SALMON FROM THE SUSITNA AND YENTNA RIVER DRAINAGES PRESENTED ON MARCH 12, 2014 AT THE ANCHORAGE AEA BUILDING TO ENGENDER CONSULTATION WITH THE UNITED STATE FISH AND WILDLIFE SERVICE AND NATIONAL MARINE FISHERIES SERVICE.

Table B1. Tissue collections of Chinook salmon collected in the Susitna and Yentna drainages including: 1) year sampled, 2) number of samples collected (N), and 3) number of individuals successfully analyzed from each collection included in the current baseline. Matching population numbers (Pop. No.) indicate collections combined that make up the 26 baseline populations. Collection dates highlighted in red indicate collections that were sampled for this project. Collections of juveniles are designated with "(Juv)" at the end of the location name.

Pop. No.	Area/ Drainage	Location	Sublocation	Year Collected	N	Analyzed
	Above Devils Canyon, Susitna River					
1		Oshetna River (Juv)		2013	55	49
1			Black River (Juv)	2013	2	2
2		Kosina Creek		2012	10	10
2				2013	2	2
2		Kosina Creek (Juv)		2013	131	127
	Middle Susitna River, below Devils Canyon					
3		Portage Creek		2009	15	15
3				2010	10	10
3				2011	116	114
3				2013	25	23
4		Indian River		2013	81	79
	Chulitna River					
5		East Fork Chulitna River		2009	5	5
5				2010	2	2
5				2011	6	6
5				2013	64	64
6		Middle Fork Chulitna River		2009	72	72
6				2010	97	97
---				2013	61	---
---		Honolulu Creek		2013	31	---
---		Pass Creek		2013	33	---
7		Byers Creek		2013	55	55
8		Spink Creek		2013	56	56
9		Troublesome Creek		2013	71	71
10		Tokositna River	Bunco Creek	2013	103	98
	Talkeetna River					
11		Upper Talkeetna Trib #1		2013	71	69
---		Upper Talkeetna Trib #2		2013	25	---
12		Stephan Lake weir		2008	19	19
12		Prairie Creek		1995	52	52
12				2008	98	98
---				2013	33	---
13		Iron Creek		2013	57	57
14		Disappointment Creek		2013	64	64
15		Chunilna Creek		2009	50	50
15				2012	50	49
---				2013	5	---

Pop. No.	Area/ Drainage	Location	Sublocation	Year Collected	N	Analyzed
	Lower Susitna River					
---		Sheep Creek		2013	24	---
---		Kashwitna River		2013	12	---
16		Montana Creek		2008	33	33
16				2009	155	155
16				2010	30	30
17		Little Willow Creek		2013	55	54
18		Willow Creek	Willow Creek mainstem	2005	74	74
18			Deception Creek	2009	122	100
---				2013	245	---
---		Deshka River	Moose Creek	1995	51	---
19				2012	52	52
19			Deshka River weir	2005	200	200
20		Alexander Creek	Sucker Creek	2011	91	90
20				2012	53	53
---			Wolverine Creek	2011	1	---
	Yentna River					
---		Skwentna River	Happy River	2012	18	---
---			Red Salmon Creek	2012	12	---
21			Hayes River	2012	5	5
21				2013	45	45
22			Canyon Creek	2012	31	31
22				2013	61	61
23			Talachulitna River	1995	58	58
23				2008	74	72
23				2010	48	48
---		Clearwater Creek		2012	25	---
24		Red Creek		2012	29	29
24				2013	82	82
---		Lake Creek	mainstem	2008	1	---
25			Sunflower Creek	2009	53	53
25				2011	74	74
26		Kahiltna River	Peters Creek	2009	27	27
26				2010	6	6
26				2011	37	34
26				2012	40	40
			Total			2,921

Table B2. Genetic statistics by locus including measures of observed heterozygosity (Ho), Fis and Fst.

Locus	Locus #	Ho	Fis	Fst
GTH2B-550	1	0.460	0.020	0.024
NOD1	2	0.428	0.009	0.054
Ots_2KER-137	3	0.304	0.002	0.023
Ots_AsnRS-72	4	0.314	0.002	0.031
Ots_ETIF1A	5	0.494	0.005	0.004
Ots_FARSLA-220	6	0.344	-0.001	0.020
Ots_FGF6A	7	0.455	-0.014	0.025
Ots_FGF6B	8	0.437	-0.013	0.018
Ots_GH2	9	0.328	-0.034	0.101
Ots_GPDH	10	0.062	-0.020	0.024
Ots_GPH-318	11	0.111	0.000	0.011
Ots_GST-207	12	0.034	-0.023	0.006
Ots_hnRNPL-533	13	0.300	0.013	0.010
Ots_HSP90B-100	14	0.240	0.003	0.013
Ots_IGF1-91	15	0.434	0.003	0.011
Ots_IK1-328	16	0.107	0.046	0.031
Ots_IL-1RA	17	0.522	-0.109	0.019
Ots_LEI-292	18	0.002	-0.001	0.000
Ots_MHC1	19	0.423	0.001	0.012
Ots_MHC2	20	0.006	-0.015	0.012
Ots_OPLW-173	21	0.043	0.044	0.010
Ots_OPSW-152	22	0.456	-0.019	0.017
Ots_P450	23	0.266	-0.018	0.018
Ots_P53	24	0.427	0.014	0.023
Ots_Prl2	25	0.464	0.018	0.054
Ots_PrpI-120	26	0.043	-0.032	0.012
Ots_SClkF2	27	0.343	0.009	0.020
Ots_SERPC1-209	28	0.167	0.016	0.030
Ots_SL	29	0.503	-0.032	0.031
Ots_TAPBP	30	0.373	0.002	0.020
Ots_Tnsf	31	0.227	-0.001	0.040
Ots_U200-167	32	0.095	0.001	0.039
Ots_U211	33	0.246	0.003	0.019
Ots_U212-297	34	0.034	-0.033	0.016
Ots_UNKN6-187	35	0.059	-0.038	0.034
Ots_zP3b	36	0.063	0.008	0.015
PGK-54	37	0.035	0.021	0.008
RAG3	38	0.247	0.029	0.024
S7-1	39	0.134	0.016	0.002
unkn526	40	0.270	0.020	0.086
Overall		0.258	-0.004	0.027

Table B3. Pairwise Fst values between all collections. Collection numbers correspond with collections in Table 1. (Page 1 of 2).

	Pop. No.												
Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.0000	0.1572	0.0614	0.0683	0.0687	0.0630	0.0593	0.0629	0.0605	0.0524	0.0810	0.0791	0.0867
2	0.1572	0.0000	0.0634	0.0605	0.0780	0.0774	0.0644	0.0625	0.0587	0.0754	0.0639	0.0673	0.0681
3	0.0614	0.0634	0.0000	0.0020	0.0378	0.0433	0.0136	0.0178	0.0088	0.0057	0.0058	0.0079	0.0058
4	0.0683	0.0605	0.0020	0.0000	0.0389	0.0422	0.0104	0.0147	0.0067	0.0069	0.0086	0.0101	0.0098
5	0.0687	0.0780	0.0378	0.0389	0.0000	0.0014	0.0100	0.0054	0.0267	0.0291	0.0470	0.0472	0.0520
6	0.0630	0.0774	0.0433	0.0422	0.0014	0.0000	0.0143	0.0101	0.0350	0.0360	0.0553	0.0568	0.0590
7	0.0593	0.0644	0.0136	0.0104	0.0100	0.0143	0.0000	-0.0025	0.0034	0.0104	0.0248	0.0269	0.0278
8	0.0629	0.0625	0.0178	0.0147	0.0054	0.0101	-0.0025	0.0000	0.0052	0.0115	0.0296	0.0315	0.0307
9	0.0605	0.0587	0.0088	0.0067	0.0267	0.0350	0.0034	0.0052	0.0000	0.0075	0.0144	0.0180	0.0193
10	0.0524	0.0754	0.0057	0.0069	0.0291	0.0360	0.0104	0.0115	0.0075	0.0000	0.0140	0.0160	0.0175
11	0.0810	0.0639	0.0058	0.0086	0.0470	0.0553	0.0248	0.0296	0.0144	0.0140	0.0000	0.0021	-0.0013
12	0.0791	0.0673	0.0079	0.0101	0.0472	0.0568	0.0269	0.0315	0.0180	0.0160	0.0021	0.0000	0.0025
13	0.0867	0.0681	0.0058	0.0098	0.0520	0.0590	0.0278	0.0307	0.0193	0.0175	-0.0013	0.0025	0.0000
14	0.0969	0.0620	0.0029	0.0107	0.0495	0.0560	0.0276	0.0321	0.0187	0.0201	0.0057	0.0098	0.0019
15	0.0634	0.0637	-0.0002	0.0019	0.0413	0.0439	0.0173	0.0213	0.0133	0.0080	0.0070	0.0113	0.0071
16	0.0634	0.0624	-0.0005	0.0029	0.0308	0.0376	0.0113	0.0124	0.0086	0.0047	0.0067	0.0095	0.0085
17	0.0853	0.0600	0.0076	0.0053	0.0392	0.0447	0.0196	0.0206	0.0177	0.0163	0.0101	0.0142	0.0126
18	0.0940	0.0684	0.0144	0.0063	0.0436	0.0500	0.0236	0.0252	0.0248	0.0196	0.0153	0.0206	0.0202
19	0.0803	0.1209	0.0213	0.0277	0.0679	0.0755	0.0399	0.0437	0.0311	0.0216	0.0376	0.0351	0.0366
20	0.0710	0.0796	0.0061	0.0078	0.0412	0.0490	0.0147	0.0204	0.0126	0.0075	0.0156	0.0170	0.0174
21	0.0659	0.0751	0.0152	0.0109	0.0339	0.0363	0.0145	0.0198	0.0172	0.0083	0.0159	0.0235	0.0258
22	0.0592	0.0761	0.0062	0.0097	0.0264	0.0330	0.0069	0.0144	0.0081	0.0046	0.0136	0.0170	0.0186
23	0.0651	0.0671	0.0079	0.0090	0.0352	0.0416	0.0117	0.0164	0.0091	0.0055	0.0152	0.0182	0.0191
24	0.0642	0.0830	0.0139	0.0117	0.0360	0.0421	0.0151	0.0186	0.0138	0.0088	0.0214	0.0234	0.0294
25	0.0661	0.0980	0.0117	0.0162	0.0537	0.0608	0.0221	0.0291	0.0139	0.0112	0.0243	0.0251	0.0258
26	0.0728	0.0921	0.0082	0.0109	0.0441	0.0528	0.0139	0.0185	0.0097	0.0081	0.0164	0.0212	0.0206

Table B3. (continued; page 2 of 2)

Pop. No.	Pop. No.												
	14	15	16	17	18	19	20	21	22	23	24	25	26
1	0.0969	0.0634	0.0634	0.0853	0.0940	0.0803	0.0710	0.0659	0.0592	0.0651	0.0642	0.0661	0.0728
2	0.0620	0.0637	0.0624	0.0600	0.0684	0.1209	0.0796	0.0751	0.0761	0.0671	0.0830	0.0980	0.0921
3	0.0029	-0.0002	-0.0005	0.0076	0.0144	0.0213	0.0061	0.0152	0.0062	0.0079	0.0139	0.0117	0.0082
4	0.0107	0.0019	0.0029	0.0053	0.0063	0.0277	0.0078	0.0109	0.0097	0.0090	0.0117	0.0162	0.0109
5	0.0495	0.0413	0.0308	0.0392	0.0436	0.0679	0.0412	0.0339	0.0264	0.0352	0.0360	0.0537	0.0441
6	0.0560	0.0439	0.0376	0.0447	0.0500	0.0755	0.0490	0.0363	0.0330	0.0416	0.0421	0.0608	0.0528
7	0.0276	0.0173	0.0113	0.0196	0.0236	0.0399	0.0147	0.0145	0.0069	0.0117	0.0151	0.0221	0.0139
8	0.0321	0.0213	0.0124	0.0206	0.0252	0.0437	0.0204	0.0198	0.0144	0.0164	0.0186	0.0291	0.0185
9	0.0187	0.0133	0.0086	0.0177	0.0248	0.0311	0.0126	0.0172	0.0081	0.0091	0.0138	0.0139	0.0097
10	0.0201	0.0080	0.0047	0.0163	0.0196	0.0216	0.0075	0.0083	0.0046	0.0055	0.0088	0.0112	0.0081
11	0.0057	0.0070	0.0067	0.0101	0.0153	0.0376	0.0156	0.0159	0.0136	0.0152	0.0214	0.0243	0.0164
12	0.0098	0.0113	0.0095	0.0142	0.0206	0.0351	0.0170	0.0235	0.0170	0.0182	0.0234	0.0251	0.0212
13	0.0019	0.0071	0.0085	0.0126	0.0202	0.0366	0.0174	0.0258	0.0186	0.0191	0.0294	0.0258	0.0206
14	0.0000	0.0043	0.0072	0.0101	0.0242	0.0312	0.0165	0.0291	0.0158	0.0198	0.0298	0.0246	0.0220
15	0.0043	0.0000	0.0022	0.0074	0.0102	0.0276	0.0105	0.0134	0.0096	0.0113	0.0179	0.0166	0.0130
16	0.0072	0.0022	0.0000	0.0061	0.0113	0.0255	0.0086	0.0130	0.0070	0.0092	0.0124	0.0152	0.0073
17	0.0101	0.0074	0.0061	0.0000	0.0037	0.0336	0.0133	0.0163	0.0108	0.0136	0.0155	0.0256	0.0165
18	0.0242	0.0102	0.0113	0.0037	0.0000	0.0473	0.0210	0.0160	0.0200	0.0194	0.0232	0.0333	0.0210
19	0.0312	0.0276	0.0255	0.0336	0.0473	0.0000	0.0102	0.0340	0.0193	0.0194	0.0226	0.0164	0.0197
20	0.0165	0.0105	0.0086	0.0133	0.0210	0.0102	0.0000	0.0124	0.0022	0.0015	0.0061	0.0079	0.0037
21	0.0291	0.0134	0.0130	0.0163	0.0160	0.0340	0.0124	0.0000	0.0068	0.0049	0.0025	0.0237	0.0181
22	0.0158	0.0096	0.0070	0.0108	0.0200	0.0193	0.0022	0.0068	0.0000	0.0008	0.0031	0.0094	0.0069
23	0.0198	0.0113	0.0092	0.0136	0.0194	0.0194	0.0015	0.0049	0.0008	0.0000	0.0020	0.0091	0.0083
24	0.0298	0.0179	0.0124	0.0155	0.0232	0.0226	0.0061	0.0025	0.0031	0.0020	0.0000	0.0131	0.0120
25	0.0246	0.0166	0.0152	0.0256	0.0333	0.0164	0.0079	0.0237	0.0094	0.0091	0.0131	0.0000	0.0077
26	0.0220	0.0130	0.0073	0.0165	0.0210	0.0197	0.0037	0.0181	0.0069	0.0083	0.0120	0.0077	0.0000

Table B4. Inbreeding coefficient (Fis) for each locus for each population. Locus numbers match numbers in Table 2 and population numbers match numbers in Table 1. N/A indicates that the marker was fixed for 1 allele. (Page 1 of 3).

Pop.	Locus #													
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-0.038	-0.053	NA	NA	-0.137	NA	-0.316	-0.316	0.390	NA	NA	NA	0.351	-0.070
2	-0.083	0.252	-0.053	0.035	-0.175	0.172	-0.126	-0.160	-0.143	NA	-0.030	NA	0.074	0.107
3	0.038	-0.080	-0.098	-0.094	0.041	0.147	0.017	0.035	0.203	-0.042	-0.001	-0.049	0.222	-0.076
4	0.210	-0.035	0.101	0.163	0.059	-0.110	-0.097	-0.085	-0.182	-0.006	-0.114	-0.020	0.061	0.348
5	-0.049	-0.004	-0.109	0.045	0.105	-0.007	-0.109	-0.053	-0.155	-0.086	-0.086	NA	-0.109	-0.118
6	-0.062	-0.092	-0.018	0.043	-0.165	0.005	-0.057	-0.056	-0.043	-0.091	0.023	0.000	0.028	0.054
7	-0.069	0.245	-0.205	0.184	-0.112	-0.302	-0.051	-0.020	0.053	0.128	0.100	0.000	-0.096	0.027
8	0.004	0.219	0.076	-0.017	-0.101	0.189	0.155	0.162	-0.038	-0.111	-0.068	0.000	-0.134	0.162
9	0.139	0.019	-0.138	-0.160	-0.065	0.039	0.222	0.101	0.021	-0.014	-0.102	-0.007	0.145	0.092
10	0.013	0.043	-0.043	-0.134	0.003	0.102	-0.150	-0.066	-0.137	-0.043	0.117	-0.021	0.030	-0.125
11	-0.154	-0.190	-0.050	-0.130	-0.087	0.132	-0.118	-0.175	-0.162	NA	-0.007	-0.008	0.016	-0.082
12	0.120	-0.004	-0.020	-0.033	-0.042	-0.021	-0.124	-0.112	-0.075	0.000	-0.026	-0.022	0.015	0.034
13	0.185	0.008	-0.148	-0.204	0.353	0.059	-0.121	-0.188	-0.065	-0.009	-0.047	-0.009	-0.167	0.140
14	-0.211	-0.019	-0.056	-0.089	0.194	-0.228	-0.037	-0.018	-0.216	-0.024	0.066	-0.033	-0.026	0.177
15	0.276	0.209	0.320	0.015	0.274	0.050	0.263	0.301	0.047	-0.004	0.199	-0.013	0.027	0.006
16	0.055	0.024	-0.036	-0.029	0.031	-0.066	-0.019	-0.071	-0.096	-0.039	-0.082	-0.032	-0.002	0.034
17	-0.017	0.027	0.017	0.078	0.098	-0.066	-0.125	-0.089	0.043	-0.009	-0.046	-0.015	0.004	-0.087
18	0.353	0.241	0.147	0.069	-0.008	0.198	0.020	0.041	-0.051	0.000	0.303	-0.010	-0.097	0.076
19	-0.057	-0.051	0.085	0.015	0.015	0.007	0.104	0.087	0.061	-0.027	-0.039	0.000	0.068	-0.060
20	-0.031	-0.005	0.108	0.066	-0.016	-0.067	0.043	0.013	0.068	0.322	0.055	-0.011	-0.026	-0.053
21	0.060	-0.081	-0.086	0.046	-0.010	-0.039	0.057	0.094	-0.256	-0.010	-0.021	-0.010	0.121	-0.210
22	-0.210	-0.002	-0.001	0.062	0.172	0.110	0.127	0.182	-0.049	-0.011	-0.017	-0.022	0.022	-0.030
23	0.010	-0.039	0.043	-0.008	-0.026	0.006	-0.041	-0.006	-0.041	-0.003	-0.029	-0.023	-0.162	0.088
24	0.080	-0.086	0.070	-0.134	0.010	-0.098	0.182	0.166	0.108	-0.005	-0.028	-0.014	-0.077	-0.117
25	0.011	0.089	-0.040	0.093	-0.008	-0.013	-0.042	-0.123	-0.173	-0.070	0.093	-0.008	-0.127	0.081
26	0.121	-0.155	-0.059	-0.008	-0.163	-0.073	-0.103	-0.047	0.010	0.122	-0.029	0.000	0.072	-0.064

Table B4. (Continued; page 2 of 3)

Pop.	Locus #													
No.	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	-0.361	NA	-0.098	NA	0.127	NA	NA	-0.400	0.123	-0.115	0.117	NA	NA	NA
2	0.234	-0.018	-0.163	NA	-0.134	NA	NA	0.026	0.038	0.111	0.191	NA	-0.139	-0.050
3	0.008	0.156	-0.090	0.000	0.029	NA	-0.032	0.177	-0.028	-0.079	0.129	-0.006	0.027	-0.019
4	-0.013	-0.054	-0.036	NA	0.030	NA	-0.007	0.020	0.220	-0.144	-0.070	-0.020	0.071	-0.164
5	-0.081	-0.028	-0.136	NA	0.096	NA	0.180	-0.173	0.221	-0.018	-0.041	-0.027	-0.043	-0.078
6	-0.047	0.032	-0.027	NA	-0.078	0.000	-0.021	-0.131	-0.087	-0.082	-0.034	-0.006	-0.085	-0.073
7	0.100	0.244	-0.160	NA	-0.350	NA	-0.039	-0.114	-0.015	-0.115	-0.026	-0.009	-0.035	0.000
8	0.007	-0.031	-0.302	NA	-0.045	NA	-0.009	-0.005	0.315	0.319	0.102	-0.019	0.006	-0.068
9	0.182	0.278	-0.101	NA	0.077	NA	-0.022	-0.047	0.076	-0.170	-0.035	0.000	0.034	0.317
10	-0.106	0.115	-0.211	NA	-0.148	0.000	-0.011	0.166	-0.069	0.036	-0.111	-0.060	-0.220	-0.091
11	0.090	-0.030	-0.385	NA	-0.243	NA	-0.015	-0.083	0.001	0.204	-0.081	NA	0.050	-0.054
12	-0.198	-0.026	-0.104	NA	-0.058	NA	-0.013	-0.061	0.003	-0.035	-0.105	NA	-0.045	0.038
13	-0.353	-0.009	0.001	NA	0.140	NA	0.663	-0.299	0.217	0.061	0.100	NA	0.198	-0.047
14	0.016	-0.033	-0.017	NA	-0.055	NA	0.000	0.008	0.024	0.111	0.125	NA	0.012	0.066
15	0.331	-0.026	0.095	NA	-0.048	NA	1.000	0.258	-0.198	-0.066	0.061	-0.009	0.214	0.133
16	-0.051	-0.032	0.069	-0.002	0.038	NA	-0.017	0.057	-0.053	0.029	-0.046	-0.027	0.032	-0.026
17	-0.073	0.055	-0.122	NA	0.098	-0.027	-0.018	0.044	-0.048	-0.005	-0.012	-0.006	0.070	0.027
18	-0.065	-0.019	-0.128	NA	0.230	0.000	-0.039	-0.101	-0.057	-0.033	0.282	-0.071	0.107	0.498
19	-0.029	0.102	-0.122	-0.002	0.049	NA	-0.008	0.022	-0.011	0.002	0.030	-0.014	-0.060	0.043
20	0.008	-0.029	-0.106	NA	0.019	NA	-0.014	-0.017	-0.078	-0.054	-0.053	-0.040	-0.004	0.037
21	0.025	0.344	-0.224	NA	0.018	NA	0.000	0.087	0.062	-0.053	-0.017	-0.021	-0.217	-0.021
22	-0.016	-0.059	-0.233	-0.006	0.047	NA	0.263	-0.145	-0.167	0.227	0.062	-0.022	-0.007	-0.110
23	0.036	0.020	-0.206	0.000	0.081	-0.009	0.020	-0.136	-0.021	0.221	0.043	-0.017	0.089	0.049
24	0.032	-0.078	-0.059	NA	0.002	NA	-0.043	-0.098	-0.034	-0.111	0.103	-0.023	0.114	0.062
25	0.178	-0.025	-0.128	NA	-0.144	-0.012	NA	-0.100	-0.012	0.189	0.133	-0.057	0.037	-0.089
26	0.153	-0.024	-0.172	NA	0.156	0.000	-0.014	-0.020	-0.198	-0.044	-0.130	-0.019	-0.064	0.101

Table B4. (Continued; page 3 of 3).

Pop.	Locus #												
No.	29	30	31	32	33	34	35	36	37	38	39	40	Overall
1	-0.126	0.201	0.069	0.129	NA	NA	NA	NA	NA	0.150	NA	-0.324	-0.048
2	-0.093	-0.129	-0.045	NA	0.041	NA	-0.018	-0.026	NA	0.133	0.101	-0.113	-0.019
3	-0.030	0.039	0.151	0.035	0.061	-0.022	-0.046	-0.026	-0.022	-0.103	-0.090	0.161	0.022
4	-0.044	-0.050	0.149	-0.076	0.109	-0.020	-0.027	-0.048	-0.020	-0.037	-0.055	0.222	0.006
5	-0.086	-0.068	-0.058	0.000	-0.041	NA	NA	NA	NA	-0.056	-0.109	-0.032	-0.045
6	-0.078	-0.081	0.043	-0.024	-0.067	0.000	0.000	0.000	-0.003	-0.050	-0.021	-0.015	-0.047
7	-0.082	-0.120	0.039	0.000	0.038	NA	-0.029	-0.010	0.000	-0.099	0.347	0.142	-0.027
8	0.116	0.297	-0.069	-0.009	-0.078	-0.009	0.000	0.000	0.000	0.517	0.200	0.101	0.062
9	0.059	0.001	-0.123	-0.045	-0.120	NA	-0.007	-0.014	NA	0.243	-0.053	0.118	0.026
10	0.232	0.030	-0.132	-0.034	-0.041	-0.005	0.000	-0.049	-0.022	0.246	0.131	-0.016	-0.028
11	-0.075	0.127	0.211	0.174	-0.042	-0.008	0.016	-0.072	0.000	0.014	-0.097	0.118	-0.061
12	-0.049	-0.105	-0.015	-0.067	-0.056	NA	-0.056	-0.046	0.000	0.061	0.017	0.136	-0.042
13	-0.187	0.085	-0.047	0.054	-0.146	NA	-0.067	0.032	NA	0.225	0.054	0.400	0.014
14	-0.150	-0.113	-0.105	-0.024	-0.090	0.000	-0.008	-0.042	NA	-0.056	-0.077	0.088	-0.024
15	0.044	0.032	-0.099	-0.085	0.252	-0.012	-0.047	0.222	0.391	-0.030	0.105	-0.014	0.124
16	-0.049	0.001	-0.031	0.044	-0.050	-0.039	-0.029	-0.019	-0.039	0.011	-0.082	0.084	-0.007
17	0.050	0.025	-0.034	0.179	-0.084	-0.024	-0.021	-0.009	0.096	0.180	-0.037	-0.075	0.001
18	0.184	-0.057	-0.060	-0.060	0.128	-0.071	-0.082	0.303	0.000	0.188	-0.030	0.161	0.077
19	-0.044	0.055	0.094	-0.077	0.036	-0.010	-0.002	0.014	-0.014	-0.046	0.087	-0.109	0.002
20	0.065	-0.147	0.011	-0.040	0.042	-0.014	-0.014	-0.029	-0.025	0.062	-0.017	-0.028	-0.007
21	-0.057	0.012	-0.167	-0.010	0.303	-0.021	NA	-0.010	-0.032	0.063	-0.065	-0.010	-0.012
22	-0.197	-0.023	0.165	-0.011	0.302	-0.034	NA	0.000	-0.017	0.050	-0.071	0.141	0.014
23	-0.062	0.119	0.007	-0.020	-0.104	-0.063	-0.006	-0.014	-0.026	-0.142	-0.034	0.007	-0.010
24	-0.129	-0.051	-0.106	-0.023	-0.099	0.000	NA	-0.019	-0.043	-0.107	0.218	0.105	-0.005
25	-0.052	-0.136	-0.051	-0.034	-0.153	-0.008	-0.080	-0.008	-0.048	-0.136	0.069	0.093	-0.023
26	-0.061	0.323	-0.018	0.266	0.008	0.000	-0.014	-0.055	0.390	0.253	0.073	-0.017	-0.010

Table B5. Probability that a locus is out of Hardy-Weinberg equilibrium expectations for each locus/population combination. Locus numbers match numbers in Table 2 and population numbers match numbers in Table 1. Laboratory error detected during quality control measures after data were analyzed detected errors in Chunilna Creek (#15) collection. Highlighted cells have p-values < 0.05. N/A means that the marker was fixed for 1 allele. (Page 1 of 3).

Pop. No.	Locus # 1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000	1.000	N/A	N/A	0.397	N/A	0.023	0.024	0.005	N/A	N/A	N/A	0.025
2	0.349	0.003	0.575	0.843	0.041	0.045	0.172	0.077	0.114	N/A	1.000	N/A	0.558
3	0.631	0.264	0.198	0.259	0.629	0.099	1.000	0.713	0.023	1.000	1.000	1.000	0.009
4	0.102	0.814	0.457	0.175	0.817	0.452	0.487	0.488	0.111	1.000	0.585	1.000	0.782
5	0.658	1.000	0.582	0.811	0.474	1.000	0.343	0.784	0.216	0.624	0.617	N/A	0.586
6	0.441	0.230	1.000	0.621	0.028	1.000	0.415	0.497	0.619	0.378	1.000	1.000	0.789
7	0.584	0.110	0.183	0.308	0.423	0.041	0.784	1.000	0.730	0.399	0.607	1.000	0.477
8	1.000	0.131	1.000	1.000	0.420	0.303	0.273	0.398	0.765	0.613	1.000	1.000	0.475
9	0.332	1.000	0.325	0.336	0.633	1.000	0.066	0.593	1.000	1.000	0.611	1.000	0.273
10	1.000	0.590	0.737	0.293	1.000	0.213	0.169	0.487	0.185	1.000	0.325	1.000	0.756
11	0.202	0.089	0.758	0.440	0.465	0.270	0.397	0.167	0.274	N/A	1.000	1.000	1.000
12	0.142	1.000	0.837	0.771	0.525	0.801	0.131	0.182	0.382	1.000	1.000	1.000	1.000
13	0.180	1.000	0.305	0.184	0.014	1.000	0.508	0.188	0.669	1.000	1.000	1.000	0.333
14	0.073	1.000	0.748	0.507	0.208	0.102	0.744	1.000	0.100	1.000	0.590	1.000	1.000
15	0.005	0.039	0.001	1.000	0.006	0.455	0.008	0.002	0.738	1.000	0.090	1.000	1.000
16	0.492	0.779	0.772	0.688	0.683	0.355	0.762	0.278	0.213	1.000	0.373	1.000	1.000
17	0.865	0.882	1.000	0.297	0.272	0.451	0.117	0.265	0.532	1.000	1.000	1.000	1.000
18	0.021	0.102	0.365	1.000	1.000	0.266	1.000	1.000	0.716	1.000	0.160	1.000	1.000
19	0.406	0.406	0.208	1.000	0.802	1.000	0.135	0.209	0.390	1.000	0.557	1.000	0.329
20	0.721	1.000	0.252	0.471	0.872	0.439	0.577	1.000	0.693	0.064	1.000	1.000	0.808
21	0.754	0.730	0.706	0.733	1.000	1.000	0.767	0.771	0.094	1.000	1.000	1.000	1.000
22	0.063	1.000	1.000	0.547	0.130	0.314	0.211	0.121	0.752	1.000	1.000	1.000	1.000
23	1.000	0.598	0.538	1.000	0.766	1.000	0.628	1.000	0.653	1.000	1.000	1.000	0.026
24	0.556	0.458	0.621	0.179	1.000	0.281	0.063	0.102	0.233	1.000	1.000	1.000	0.557
25	1.000	0.312	0.791	0.399	1.000	1.000	0.648	0.191	0.078	0.627	0.368	1.000	0.163
26	0.280	0.115	0.585	1.000	0.120	0.541	0.308	0.658	1.000	0.298	1.000	1.000	0.462

Table B5. (Continued; page 2 of 3).

Pop. No.	Locus # 14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	1.000	0.012	N/A	0.506	N/A	0.379	N/A	N/A	0.006	0.416	0.663	0.502	N/A	N/A
2	0.370	0.009	1.000	0.051	N/A	0.114	N/A	N/A	1.000	0.692	0.224	0.031	N/A	0.118
3	0.469	1.000	0.016	0.316	1.000	0.868	N/A	1.000	0.020	0.802	0.361	0.116	1.000	0.661
4	0.001	1.000	1.000	0.814	N/A	0.802	N/A	1.000	1.000	0.074	0.282	0.484	1.000	0.599
5	0.575	0.588	1.000	0.218	N/A	0.415	N/A	0.033	0.136	0.122	0.825	0.796	1.000	1.000
6	0.537	0.589	0.770	0.843	N/A	0.323	1.000	1.000	0.099	0.372	0.277	0.705	1.000	0.263
7	1.000	0.671	0.096	0.267	N/A	0.016	N/A	1.000	0.507	1.000	0.407	1.000	1.000	1.000
8	0.341	1.000	1.000	0.029	N/A	0.747	N/A	1.000	1.000	0.010	0.017	0.419	1.000	1.000
9	0.336	0.109	0.070	0.459	N/A	0.721	N/A	1.000	0.620	0.626	0.181	0.811	1.000	1.000
10	0.284	0.388	0.333	0.046	N/A	0.154	1.000	1.000	0.145	0.594	0.825	0.304	1.000	0.039
11	0.556	0.437	1.000	0.001	N/A	0.046	N/A	1.000	0.459	1.000	0.136	0.453	N/A	0.761
12	0.813	0.010	1.000	0.194	N/A	0.483	N/A	1.000	0.400	1.000	0.737	0.216	N/A	0.563
13	0.340	0.009	1.000	1.000	N/A	0.337	N/A	0.001	0.027	0.143	0.765	0.578	N/A	0.109
14	0.056	1.000	1.000	1.000	N/A	0.773	N/A	1.000	1.000	1.000	0.406	0.452	N/A	1.000
15	1.000	0.001	1.000	0.373	N/A	0.698	N/A	0.007	0.007	0.023	0.527	0.589	1.000	0.033
16	0.795	0.517	1.000	0.360	1.000	0.646	N/A	1.000	0.466	0.548	0.762	0.481	1.000	0.687
17	0.375	0.382	0.646	0.131	N/A	0.259	1.000	1.000	0.622	0.580	1.000	0.879	1.000	0.402
18	1.000	0.782	1.000	0.383	N/A	0.156	1.000	1.000	0.560	1.000	0.786	0.055	1.000	0.521
19	0.364	0.692	0.038	0.056	1.000	0.473	N/A	1.000	0.748	1.000	1.000	0.766	1.000	0.355
20	0.696	1.000	1.000	0.235	N/A	1.000	N/A	1.000	0.846	0.471	0.558	0.593	1.000	1.000
21	0.176	1.000	0.044	0.115	N/A	1.000	N/A	1.000	0.538	1.000	1.000	1.000	1.000	0.141
22	1.000	1.000	1.000	0.036	1.000	0.812	N/A	0.008	0.173	0.119	0.045	0.527	1.000	1.000
23	0.336	0.745	1.000	0.007	1.000	0.353	1.000	1.000	0.078	0.820	0.004	0.638	1.000	0.238
24	0.365	0.836	0.611	0.561	N/A	1.000	N/A	1.000	0.285	0.763	0.296	0.347	1.000	0.247
25	0.663	0.044	1.000	0.166	N/A	0.122	1.000	N/A	0.309	1.000	0.058	0.170	1.000	0.654
26	0.691	0.147	1.000	0.075	N/A	0.098	1.000	1.000	0.840	0.041	0.688	0.164	1.000	0.530

Table B5. (Continued; page 3 of 3).

Pop. No.	Locus # 28	29	30	31	32	33	34	35	36	37	38	39	40
1	N/A	0.402	0.140	0.767	0.329	N/A	N/A	N/A	N/A	N/A	0.328	N/A	0.039
2	1.000	0.267	0.223	0.746	N/A	0.670	N/A	1.000	1.000	N/A	0.230	0.316	0.244
3	1.000	0.746	0.819	0.051	0.610	0.608	1.000	1.000	1.000	1.000	0.202	0.365	0.087
4	0.194	0.819	0.749	0.062	1.000	0.344	1.000	1.000	1.000	1.000	1.000	1.000	0.112
5	1.000	0.502	0.621	0.560	1.000	1.000	N/A	N/A	N/A	N/A	1.000	0.590	0.814
6	0.611	0.346	0.259	0.707	1.000	0.620	1.000	1.000	1.000	1.000	1.000	1.000	0.876
7	1.000	0.578	0.481	1.000	1.000	1.000	N/A	1.000	1.000	1.000	1.000	0.062	0.349
8	1.000	0.416	0.012	0.672	1.000	1.000	1.000	1.000	1.000	1.000	0.003	0.264	0.554
9	0.008	0.626	1.000	0.434	1.000	0.584	N/A	1.000	1.000	N/A	0.086	1.000	0.450
10	0.594	0.023	0.771	0.293	1.000	0.741	1.000	1.000	1.000	1.000	0.011	0.058	1.000
11	1.000	0.610	0.355	0.221	0.044	0.720	1.000	1.000	1.000	1.000	1.000	0.599	0.085
12	0.710	0.617	0.226	1.000	0.479	0.515	N/A	0.686	1.000	1.000	0.514	1.000	0.028
13	1.000	0.133	0.630	1.000	1.000	0.345	N/A	1.000	1.000	N/A	0.131	1.000	0.003
14	0.595	0.193	0.431	0.596	1.000	0.682	1.000	1.000	1.000	N/A	0.743	1.000	1.000
15	0.190	0.710	0.810	0.388	0.591	0.005	1.000	1.000	0.008	0.001	0.769	0.254	1.000
16	0.778	0.491	1.000	0.754	1.000	0.512	1.000	1.000	1.000	1.000	1.000	0.372	0.250
17	0.794	0.639	0.816	1.000	0.147	0.322	1.000	1.000	1.000	0.051	0.012	1.000	0.478
18	0.001	0.270	0.711	1.000	1.000	0.403	1.000	1.000	0.165	1.000	0.220	1.000	0.064
19	0.506	0.559	0.372	0.173	0.374	0.473	1.000	1.000	1.000	1.000	0.558	0.053	0.148
20	0.743	0.510	0.104	1.000	1.000	0.779	1.000	1.000	1.000	1.000	0.564	1.000	0.767
21	1.000	0.770	1.000	0.326	1.000	0.019	1.000	N/A	1.000	1.000	1.000	1.000	1.000
22	0.372	0.056	0.817	0.156	1.000	0.003	1.000	N/A	1.000	1.000	0.704	1.000	0.214
23	1.000	0.446	0.142	1.000	1.000	0.172	0.629	1.000	1.000	1.000	0.087	0.715	1.000
24	1.000	0.218	0.678	0.365	1.000	0.304	1.000	N/A	1.000	1.000	0.308	0.011	0.378
25	0.595	0.588	0.129	0.699	1.000	0.121	1.000	0.610	1.000	1.000	0.161	0.354	0.390
26	0.381	0.564	0.001	1.000	0.004	1.000	1.000	1.000	1.000	0.001	0.004	0.641	1.000

Figure B1. Meeting agenda for March 12, 2014 consultation with the United State Fish and Wildlife and National Marine Fisheries Service.

SUSITNA-WATANA HYDRO

Agenda and Schedule
Susitna Chinook Salmon Genetics
03/12/2014

LOCATION:	**Alaska Energy Authority – Board Room** **813 West Northern Lights Blvd.** **Anchorage, AK 99503**
TIME:	**1:00 p.m. – 3:00 p.m. (AKST)**
SUBJECT:	**Chinook Genetics Consultation**
GoTo MEETING:	**https://www4.gotomeeting.com/register/325190927** **1-800-315-6338 CODE 3957#**
Goal	**Consultation**

Agenda Items

1:00 - 1:10	**Welcome and introductions**
1:10 - 1:20	**AEA provide context for the consultation**
1:20 - 1:45	**ADF&G and AEA review study design components and progress**
1:45 - 2:15	**ADF&G review preliminary results for Chinook salmon**
2:15 - 3:00	**Consult on study design and analyses**

This schedule is based upon the time allotted for each topic and subject to revision upon completion of topics as the day progresses. If you are interested in a specific topic, please notify AEA at the beginning of the day and AEA will attempt to notify you via email when the TWG addresses the specific topic.

Figure B2. Sampling sites for Chinook salmon analyzed in preparation for March 12, 2014 consultation with the United State Fish and Wildlife and National Marine Fisheries Service. Numbers match the "Pop. No." column in Table B1.

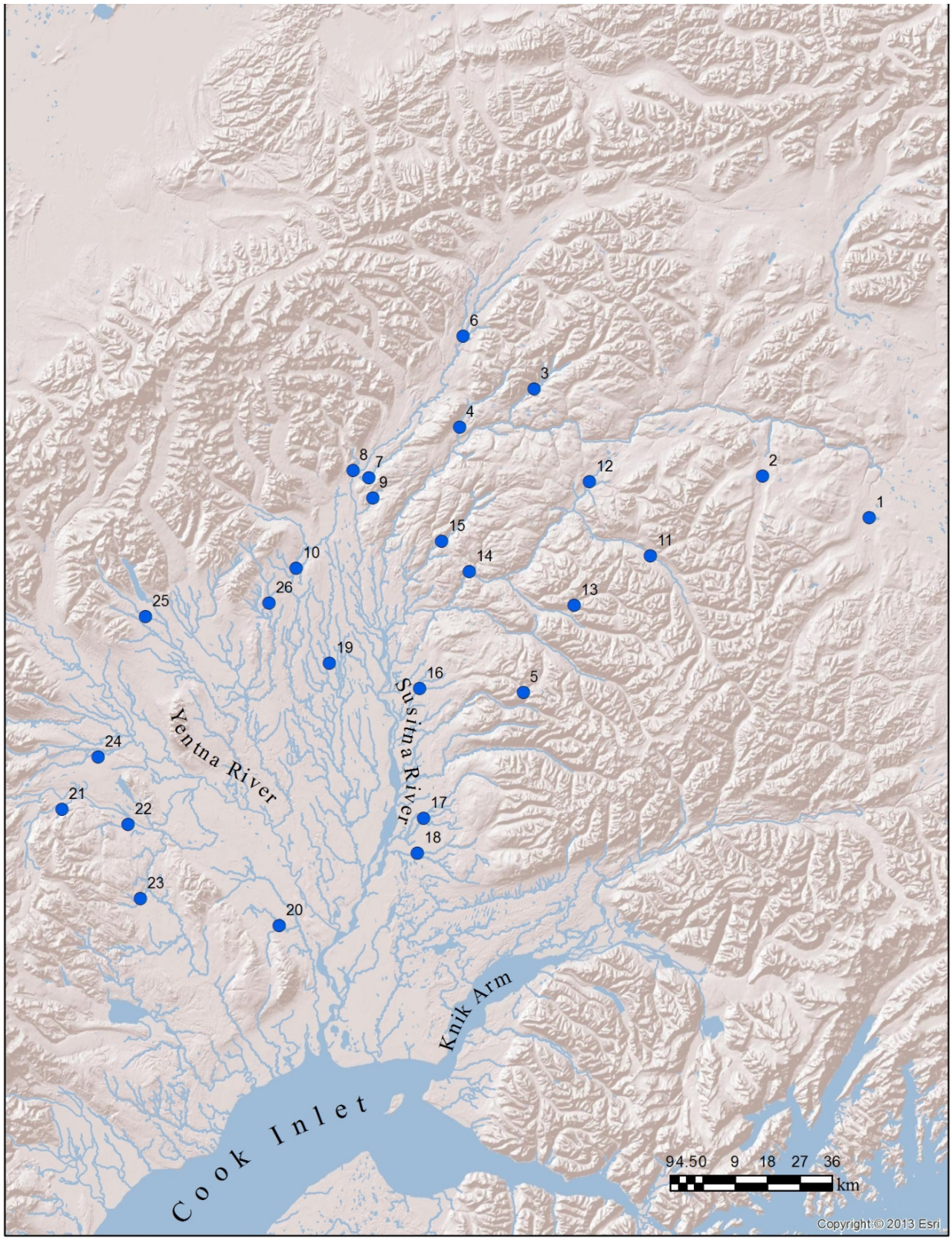
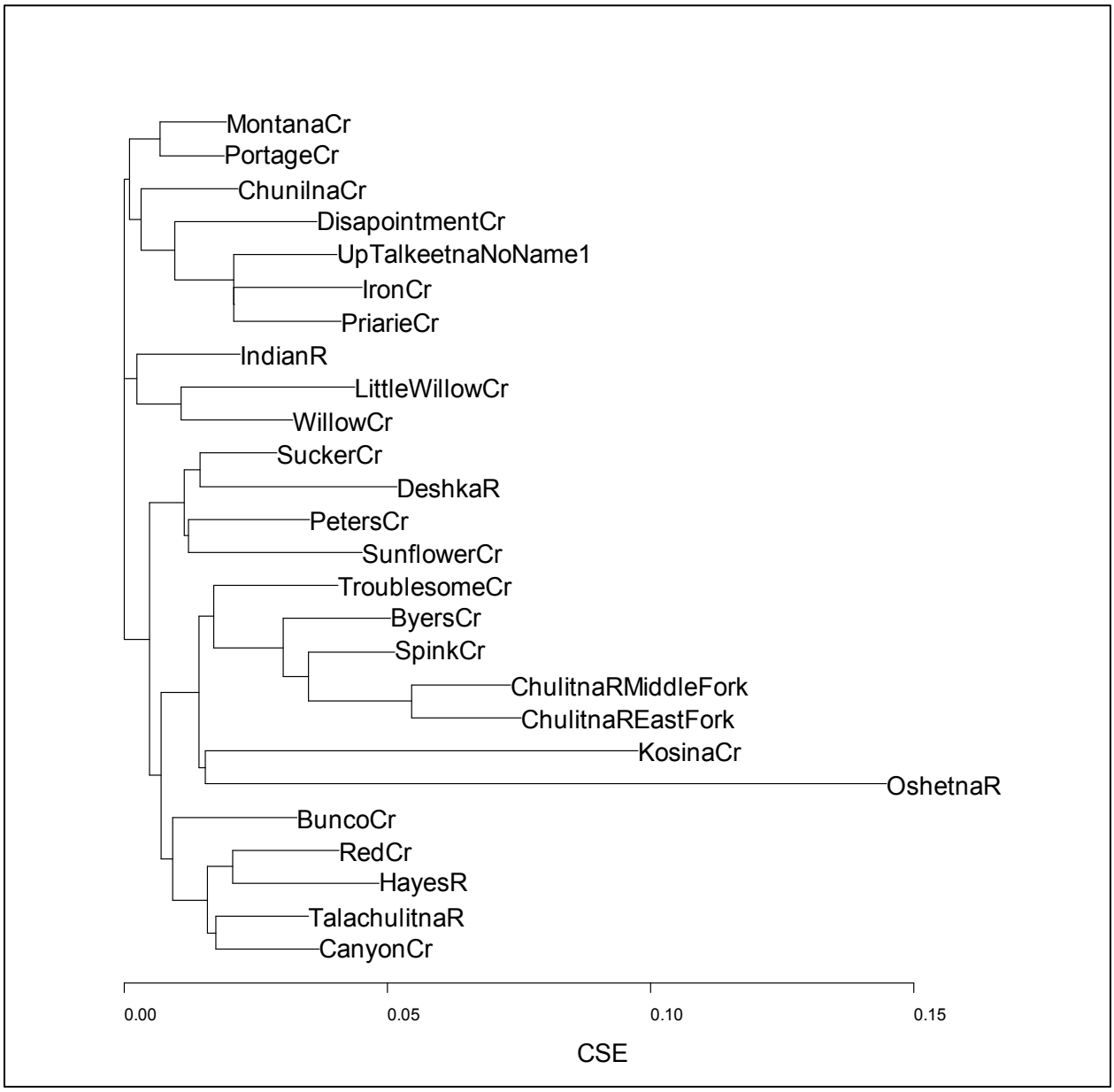


Figure B3. Neighbor-joining tree based on Cavalli-Sforza and Edwards genetic distances among collections of Chinook salmon collected from the Susitna and Yentna River drainages.



PART B – ATTACHMENT 1 - APPENDIX C: GENETIC SAMPLING INSTRUCTIONS

Appendix C1.–Bulk sampling instructions for adult salmon and other adult fish species.

Non-lethal Bulk Sampling Finfish Tissues for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

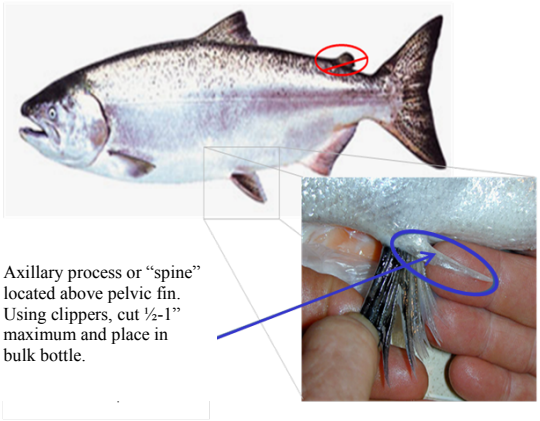
I. General Information

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.

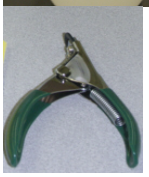
Sampling instructions are written for (N=100 fish/125ml) bulk bottle. <u>Steps for collecting axillary process tissues:</u>



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 C2.-Omniswab sampling instructions for juvenile Chinook salmon.

Non-lethal Juvenile Finfish OmniSwab Sampling for DNA Analysis
ADF&G Gene Conservation Laboratory

I. General Information

We use the mucus samples from juvenile fish using OmniSwab to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting sample 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 fish.

II. Sampling Method

2.0ml vials:

Figure 1

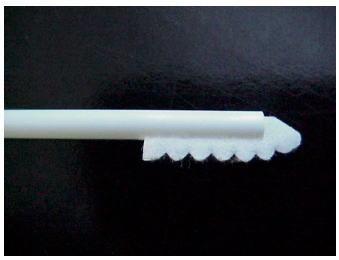


Figure 2

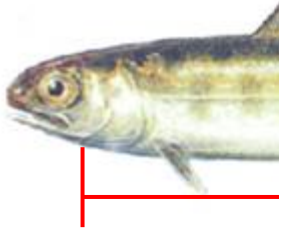


Figure 3

III. Supplies included with sampling kits:

1. OmniSwab – plastic applicator swab for collecting mucus from fish.
2. 2.0ml vials – pre-labeled individual vial and cap for sample storage.
3. Silca beads – vial pre-filled ½ silca beads/capped prior to sampling.
4. White boxes – storage for individual capped vials with silca beads.
5. Hinged plastic box – used while sampling, protects vials from rain.
6. Sampling instructions.

- Organize work area prior to sampling.
- Hinged plastic box will hold up to 50 silca pre-filled vials. Works best with 40 vials or less so hinged lid can close easily between sampling events.
- Lift lid on white box, should be marker line upper left edge of box bottom; starting vial #1,2,3… left to right.
- Load plastic box with vial #s 1,2,3… in **consecutive** order. **All vials remain capped until sampling each fish.** Do not uncap vials ahead of time since silca will begin absorbing moisture. Want to minimize exposure time to moisture.
- Cover work area (cooler, tarp, rain coat, backpack, under tree) to protect samples from rain and/or direct sunlight.
- Wipe right hand dry before opening each OmniSwab to reduce excess water dripping on swab pad applicator.
- Dry hands, open OmniSwab by peeling package open at the handle end of swab and remove carefully.
- Pick up one fish and hold in palm of left hand with belly side up (Figure 1).
- **Do not touch swab pad applicator (Figure 2)**.
- Sample location on fish is located between lower jaw and front of pelvic fin (Figure 3).
- Hold OmniSwab handle in right hand, **gently** rub the swab pad serrated edge against preferred area (Figure 3 and below):
  - **Rub swab pad back/forth 8-10 times (back/forth=1 time).**
  - **Very important to complete total 10 swab cycles on fish!**
- Be careful not to depress ejector tip while swabbing fish.
- Once sampling is complete, release fish back to the local stream or waterway.
- **Uncap vial** with dry hand after sample is taken. Tilt vial on slight angle making room for swab pad in silca beads and eject swab pad (using release button at tip) into one vial. Cap and swiftly shake capped vial to distribute silca beads around applicator pad to enhance drying process.
- **Place only one swab pad per vial!**
- Record metadata (vial #, date, location, lat/long, etc…) electronic copy preferred.
- Place each individual vial back into white storage box, working from vial #s 1,2,3…100 consecutively until the entire box of 100 vials are full.
- Swab pads will slowly dry inside capped vials and be dry by the end of the day.
- In field: store vial collection at room temperature away from heat and/or place in **dry** cooler or tote.
- In lab: Store in -20* freezer (lid on).

IV. Shipping: No special paperwork required for return shipment of these samples.

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

Appendix C3.–Vial sampling instructions for juvenile Chinook salmon.

Non-lethal Juvenile Finfish Tissue Sampling for DNA Analysis

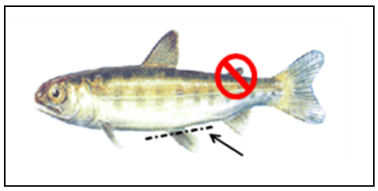
ADF&G Gene Conservation Lab, Anchorage

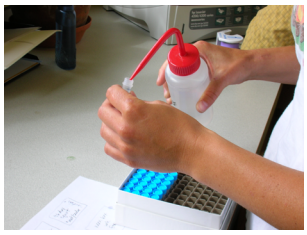
I. General Information

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:** _____

PART B – ATTACHMENT 1 - APPENDIX D. HABITAT MAPPING UNITS FROM SUSITNA-WATANA HYDROELECTRIC PROJECT "CHARACTERIZATION AND MAPPING OF AQUATIC HABITATS (9.9)" THAT ARE USED IN ASSIGNING JUVENILE CHINOOK SALMON COLLECTED IN THE MIDDLE AND LOWER RIVER FOR THIS STUDY.

Table D1. Nested and tiered habitat mapping units and categories for macrohabitats and mainstem channel mesohabitats.

Level	Unit	Grouping	Category	Definitions
1	Major Hydrologic Segment	Segments	Upper, Middle, Lower River	*Upper River* – PRM –187.1 – 261.3 (habitat mapping extended up to mainstem PRM 235.1 and included the Oshetna River. *Middle River* – PRM –102.4 – 187.1 *Lower River* – PRM 0 – 102.4
2	Geomorphic Reach	Upper River Segment	6 reaches	Geomorphic reaches that uniquely divide the Major Hydrologic Segments based on geomorphic characteristics.
		Middle River Segment	8 reaches	
		Lower River Segment[1]	6 reaches	
3	Macrohabitat	Main Channel Habitat	Single Main Channel	Single dominant main channel.
			Split Main Channel	Two dominant channels.
			Multiple Split Main Channel	Three or more distributed dominant channels.
			Side Channel	Channel that is turbid and connected to the active main channel but represents non-dominant proportion of flow[1]
			Tributary Mouth	Clear water areas that exist where tributaries flow into Susitna River main channel or side channel habitats (upstream Tributary habitat will be mapped as a separate effort).
		Off-Channel Habitat[2]	Side Slough	Overflow channel contained in the floodplain, but disconnected from the main channel.
			Upland Slough	Similar to a side slough, but contains a vegetated bar at the head that is rarely overtopped by mainstem flow. Has clear water.[1.]
		Tributary Habitat	Single Channel	Single dominant channel
			Split Channel	Two dominant channels
			Channel complex	Three or more distributed dominant channels