Susitna-Watana Hydroelectric Project
(FERC No. 14241)

Genetic Baseline Study for Selected Fish Species
Study Plan Section 9.14

2014 Study Implementation Report

Prepared for

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

Prepared by

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LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS

Abbreviation	Definition
ADF&G	Alaska Department of Fish and Game
AEA	Alaska Energy Authority
CFR	Code of Federal Regulations
CIRWG	Cook Inlet Region Working Group
DNA	deoxyribonucleic acid
FERC	Federal Energy Regulatory Commission
GCL	Gene Conservation Laboratory
GPS	global positioning system
ILP	Integrated Licensing Process
IP	Implementation Plan
ISR	Initial Study Report
ml	milliliter
Mm	Millimeter
MSA	mixed-stock analysis
n/a	not applicable/not available
NOAA	National Oceanic and Atmospheric Administration
NMFS	National Marine Fisheries Service
oz.	ounce
PRM	Project River Mile
Project	Susitna-Watana Hydroelectric Project
RM	River Mile(s) referencing those of the 1980s Project.
RSP	Revised Study Plan
SPD	Study Plan Determination
USFWS	United States Fish and Wildlife Service

1. INTRODUCTION

This Genetic Baseline Study for Selected Fish Species, Section 9.14 of the Revised Study Plan (RSP) approved by the Federal Energy Regulatory Commission (FERC) for the Susitna-Watana Hydroelectric Project, FERC Project No. 14241, focuses on understanding the genetic structure of selected species within the Susitna River.

A summary of the development of this study, together with the Alaska Energy Authority's (AEA) implementation of it through the 2013 study season (September 2013), appears in Part A, Section 1 of the Initial Study Report (ISR) filed with FERC in June 2014. As required under FERC's regulations for the Integrated Licensing Process (ILP), the ISR describes AEA's "overall progress in implementing the study plan and schedule and the data collected, including an explanation of any variance from the study plan and schedule." (18 CFR 5.15(c)(1)).

In accordance with the intent of the FERC February 1, 2013 SPD, AEA consulted with the Services on March 12, 2014 and received comments on developing a draft 2014 Implementation Plan for the Genetic Baseline Study for Selected Fish Species in the Susitna River, Alaska (Genetics Implementation Plan [IP]. The final 2014 Genetics IP was filed on June 3, 2014 as ISR Part B Attachment 1. The 2014 Genetics IP supersedes portions of the Revised Study Plan.

Since filing the ISR in June 2014, AEA has continued to implement the FERC-approved plan for the Genetic Baseline Study. For example:

- Collection of juvenile and adult Chinook Salmon from above Devils Canyon.
- Collection of adult Chinook Salmon from upper Cook Inlet tributaries.
- Opportunistic collection other salmon and non-salmon species from the Susitna River.
- Genotyping of Chinook Salmon samples collected in the Middle and Upper Susitna River for single nucleotide polymorphism (SNP) and microsatellite (μSAT) loci.
- On October 15, 2014 AEA held an ISR meeting for the Genetic Baseline Study for Selected Fish Species in the Susitna River.

In furtherance of the next round of ISR meetings and FERC's SPD expected in 2016, this report describes AEA's overall progress in implementing the Genetic Baseline Study from September 2013 through December 2014. Rather than a comprehensive reporting of all field work, data collection, and data analysis since the beginning of AEA's study program, this report is intended to supplement and update the information presented in Part A and Part B of the ISR for the Genetic Baseline Study for Selected Fish Species in the Susitna River through the end of calendar year 2014. It describes the methods and results of the 2014 effort, and includes a discussion of the results achieved.

2. STUDY OBJECTIVES

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

As described in the 2013 Genetics IP Section 3, 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 River populations, for 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 (Figure 2-1).

AEA expects that each of these goals and objectives will be met through the complete implementation of the study program. Data collection toward achieving these objectives during the 2014 study season was limited to the activities described in the Introduction and further detailed below.

3. STUDY AREA

As established in the 2013 and 2014 Genetics IP Section 2.2, the study area encompasses the Susitna River and its tributaries from Cook Inlet upstream to the Oshetna River confluence (PRM 235.1; Figure 3-1). For baseline data related to stock-specific sampling, there was an emphasis on tributaries of the Middle and the Upper Susitna River. For assessing habitat use (juveniles) of fish originating from the Middle (PRM 102.4 – 187.1) and Upper Susitna River (above PRM 187.1 – 261.3), tissue from juvenile Chinook Salmon was collected in the Lower River (below PRM 102.4).

4. METHODS AND VARIANCES

AEA implemented the methods as described in the Study Plan with the exception of variances explained below (Section 4.5).

4.1. Sample Collection

For this study fish populations were 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 were represented by single or pooled collections following the "Pooling Collections into Populations" methods below.

Based on field sampling in previous years (Tables 4-1 to 4-5), information gathered from the Catalog of Waters Important for the Spawning, Rearing or Migration of Anadromous Fishes, ADF&G biologists selected possible sites where fish of each target Pacific salmon species might be spawning and generated idealized sample sizes for each site (Tables 4-1 to 4-5). ADF&G and AEA's contractors made an intensive effort to collect these samples as outlined in the sections below. However, AEA recognized at the inception of this project (2013 Genetics IP) that it was unlikely to obtain the idealized sample size at all sites due to uncontrolled variables (i.e., numbers of fish at a spawning location, number of fish returning in 2013 and 2014, access limitations, water conditions, and catchability of the fish). Therefore, a column was added to Tables 4-1 to 4-5 labeled "Expected" that shows the number of fish that could reasonably be sampled at each site (or group of sites) in two years.

AEA implemented the methods for sampling to achieve collection targets as described in the 2013 and 2014 Genetics IP Section 4.2, with no variances. Cumulative collection targets ranged between 0 and 200 individuals per species per location depending on the number of archived samples and prior knowledge about likely sample collection success (Tables 4-1 to 4-5). Samples were acquired from field collections performed as a part of this Study Plan (Studies 9.14), and from each of four interrelated studies: Study of Fish Distribution and Abundance in the Upper Susitna River (Study 9.5); Study of Fish Distribution and Abundance in the Middle and Lower Susitna River (Study 9.6); Salmon Escapement Study (Study 9.7); and Eulachon Run Timing, Distribution, and Spawning in the Susitna River Study (Study 9.16). All four interrelated studies provided samples from resident fish species collected in the course of their work (Table 4-6). Study 9.5 also provided samples of juvenile Chinook Salmon (Table 4-1), and Study 9.7 provided samples from adult salmon from the Indian River and from the Middle River at Curry (detailed in Section 4.1.5). Sampling methods for this Study Plan are described below. Sampling methods for the four interrelated studies are described in those respective Initial Study Reports (2013) and in the Study Implementation or Study Completion Reports (2014). Analysis of all samples will be integrated and reported in the Updated Study Report.

4.1.1. Adult Chinook Salmon collections

To address Objectives 3 and 4, tissue samples were to be collected during the study period from Chinook Salmon spawning in drainages within Knik Arm and northwestern Cook Inlet, and within the Susitna River drainage. For drainages within Knik Arm and northwest Cook Inlet, this study was to augment the existing baseline by adding collections of up to 200 Chinook Salmon from two tributaries from each area. For the Susitna River drainage, this project was to augment the existing baseline such that all tributaries were represented by at least 50 (and ideally 200) Chinook Salmon.

Understanding the population structure of Chinook Salmon collected above and below Devils Canyon will inform policymakers regarding the relatedness and isolation of spawning aggregates. Population structure of Chinook Salmon will be measured at three different levels: within the set of individuals spawning above the canyon; among the groups of individuals spawning within the Susitna River watershed (with particular emphasis on the Middle River and Upper River); and in relationship to populations from nearby drainages in Upper Cook Inlet. These higher-level analyses will anchor the results and help provide a context for interpretation.

As in 2013, Chinook Salmon were captured using either hook-and-line, seines, or gillnets depending on the size of the stream and where the fish were located. Upon capture, a single axillary process was clipped from each Chinook Salmon and placed in a bottle of ethyl alcohol for preservation. Fish were held in the water as much as possible while hooks were removed and samples were collected, and released immediately after the sample was placed in the bottle. If necessary, crews held the fish in the water to make sure they could swim before releasing them.

For Chinook Salmon sampled above Devils Canyon, additional paired samples/data were collected including scales, length (mid-eye to fork, to nearest 5 mm), sex, and GPS information (decimal degrees, to the nearest 0.001). Therefore, for these fish, an axillary process and five scale samples were sampled into individually labeled vials. Scales were sampled at a point along the diagonal line from the posterior insertion of the dorsal fin to the anterior insertion of the anal fin, two rows above the lateral line. Length, sex and GPS information was recorded on Rite-in-the-Rain® notebooks paired with the vial identifier.

The methods used in 2013 adult salmon sample collections are detailed in the Study 9.14 ISR Part A Sections 4.1.1 and 4.1.2. In 2014, an ADF&G Gene Conservation Laboratory (GCL) sampling crew of three people dedicated to collecting juvenile and adult Chinook Salmon from above Devils Canyon was employed. Additional staff were added for a day or two at a time to provide rest days for the dedicated crew or add an additional crew. During days when fish were not present above Devils Canyon or weather prevented access, crews focused on collecting Chinook Salmon in the following areas (in order of priority) 1) Middle River tributaries (including Susitna River above Talkeetna, but below Devils Canyon, Talkeetna River, and Chulitna River); 2) Lower River tributaries; 3) Yentna River tributaries; 4) other Upper Cook Inlet tributaries (Table 4-1). Crews opportunistically collected other species of both Pacific salmon and resident species within the Susitna River.

4.1.2. Other adult salmon collections

To address Objective 2, tissue samples were to be collected from 100 individuals (total archived and samples collected during the study period) from at least three spawning aggregates of Pink, Sockeye, Chum, and Coho Salmon from each of the following drainages: 1) the Susitna River upstream of the Three Rivers Confluence (Middle Susitna River), 2) the Talkeetna River, and 3) the Chulitna River (Tables 4-2 to 4-5; Figures 4-1 to 4-4). Capture and sampling of salmon followed the methods used for adult Chinook Salmon.

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

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

Details regarding adult salmon sample collection in 2013 are reported in the Study 9.14 ISR Part A Section 4.1.2. In 2014, collections from adult pink and Sockeye Salmon were conducted during the week of August 4. Collections from Coho Salmon were conducted in an unrelated project for the weeks of September 22 and 29 and October 13 (Table 4-8). Samples from these other salmon species were collected opportunistically by the Susitna River crews.

4.1.3. Juvenile Chinook Salmon collections

4.1.3.1. Above and within Devils Canyon

To address Objectives 3 and 4, tissue samples were to be collected from an ideal target of 200 juvenile Chinook Salmon during the study period at the Oshetna River and in each of Kosina, Fog, and Cheechako creeks as described in the 2013 and 2014 IP Section 4.2.1 (Table 4-1). The expected sample size from all tributaries above Devils Canyon from all years combined was a total of 70 fish (2013 and 2014 IP Table 4-1) Methods for capturing juvenile Chinook Salmon in minnow traps followed Magnus et al. (2006). Cured salmon roe was used as bait and several minnow traps were set at each location. Minnow traps were checked at least once per day. Collections occurred at the same time as adult salmon collection trips.

In 2013, caudal fin tissue was collected as detailed in ISR Part A Section 4.1.3.1. Caudal fin sampling is lethal to the fish. As described in the 2014 Implementation Plan (ISR Part B), buccal swab samples were collected in 2014 instead of caudal fin tissue to allow for non-lethal sampling (see Appendix A for sampling methods).

4.1.3.2. Lower River collections

No juvenile sampling occurred in the Lower Susitna River in 2014.

4.1.3.3. Species identification of juvenile collections

Species identification was performed in the field using phenotypic characteristics (i.e. Pollard et al. 1997). Juvenile Pacific salmon samples were collected in the Susitna River from 2012 to 2014 by the ADF&G GCL and by contractors to AEA which were provided to GCL. Field species determinations were provided for each sample.

Confirmation of species identification of juvenile samples was provided by genetic analysis of tissue samples. Samples from 20 adult fish from each of the five Pacific salmon species native to North America were used as positive controls (100 known fish). Positive controls were selected from fish collected in the Cook Inlet region. In this report, we provide results from juvenile Pacific salmon captured within and above Devils Canyon because these samples will be used to investigate population structure.

Genomic DNA was isolated from the samples using a DNeasy® 96 Blood and Tissue Kit by QIAGEN® (Valencia, CA).

Laboratory analysis for species determination was accomplished using one of two methods: 1) microsatellites screened for the population structure project were used to positively identify Chinook Salmon and 2) SNP markers were used to discriminate among all species of Pacific salmon. Results from these analyses for samples taken in related studies were distributed to project leads. Fish-specific species identification results will be included in the associated projects reports.

4.1.3.3.1. Microsatellites

This method was employed because GCL had already genotyped many juvenile Pacific salmon samples collected above Devils Canyon for the population structure analysis of Chinook Salmon. This method provides positive identification that a sample is a Chinook Salmon if the sample has adequate quality and quantity of DNA. However, this method cannot discriminate between a sample with poor quality DNA of Chinook Salmon or among other Pacific salmon species.

Thirteen microsatellites that are commonly used for Chinook Salmon were genotyped (Table 4-9). Samples were assayed for DNA loci developed for use in U.S-Canada Treaty fisheries (Seeb et al. 2007). Polymerase chain reaction (PCR) was carried out in 10 ul reaction volumes (10 mM Tris-HCl, 50 mM 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, MgCl2 concentrations and the corresponding annealing temperature for each primer are available in Seeb et al. (2007). PCR fragment analysis was done on an AB 3730 capillary DNA sequencer. A 96-well reaction plate was loaded with 0.5 ul PCR product along with 0.5 ul of GS500LIZ (AB) internal lane size standard and 9.0 ul of Hi-Di (AB). PCR bands were visualized and separated into bin sets using AB GeneMapper software v4.0.

Genetic data were collected as individual multilocus genotypes for the 13 microsatellite loci. Genotype data were stored as GeneMapper (*.fsa) files on a network drive that was backed up nightly. Long-term storage of the data was in an Oracle database (LOKI) on a network drive maintained by ADF&G computer services.

If at least 10 of the 13 microsatellite markers amplified, we designated the sample as a Chinook Salmon. If not, the sample was designated as "undetermined" and further analyzed using the SNP method. Undetermined samples were either not a Chinook Salmon or had inadequate quantity or quality DNA for consistent amplification using these methods.

4.1.3.3.2. Single nucleotide polymorphisms

Preamplification of the extracted DNA was conducted using unlabeled primers from Integrated DNA Technologies prior to genotyping. All primers were hydrated with TE and combined in a 10x primer mix. A Master Mix of the 10x unlabeled primers and a Multiplex PCR MMix by QIAGEN® was plated onto a 384-well plate along with the extracted DNA and loaded onto an Applied Biosystems 9700 thermocycler for preamplification. The preamplified DNA was then diluted with TE and plated into Axygen raised-well plates.

Applied BioSystems' SNP Taqman assay analysis methods were used. Preamplified DNA was diluted to a 1:10 concentration prior to genotyping and plated onto a 384-well plate along with a 10mM Tris water solution. Seven markers were used to discriminate among Pacific salmon species (Tables 4-10). Each marker was plated onto a single 384-well plate and all plates were loaded onto Applied Biosystems 9700. After PCR was complete, the plates were scanned using an Applied Biosystems Quant Studio and analyzed using the 12K Flex Software v1.2.2.

Species determination was performed by examining the clustering of positive controls and juvenile samples collected for this study (Table 4-10). Species were identified if the sample was consistent with a single species determination across all markers successfully screened and at least two markers amplified. Samples that did not amplify for at least two diagnostic markers were designated as "Failed". Samples that amplified but did not locate to a consistent species were designated as "undetermined". Samples that were not analyzed by error were designated as "N/A". Determinations were reviewed by two staff.

4.1.3.3.3. Quality control measures

Several measures were implemented to insure the quality of data produced regardless of marker type. First, each individual tissue sample was assigned a unique accession identifier. At the time DNA was extracted or analyzed from each sample, a sample sheet was created that linked each individual sample's code to a specific well number in a uniquely numbered 96-well plate. This sample sheet then followed the sample through all phases of the project, minimizing the risk of misidentification of samples through human-induced errors. Second, genotypes were assigned to individuals using a system in which two individuals score the genotype data independently. Discrepancies between the two sets of scores were then resolved with one of two possible outcomes: (1) one score was accepted and the other rejected, or (2) both scores were rejected and the score was blanked. Third, approximately 8 percent of the individuals, eight samples from each 96-well DNA extraction plate, were reanalyzed for all loci. This insured that the data were reproducible, and any errors created from the processing of individual plates were corrected.

4.1.4. Other species collections

To address Objective 1, tissue samples were to be collected from up to 50 representative individuals during the study period from each of the resident fish species listed in Table 4-6, with an emphasis on fish collected in the Lower, Middle and Upper Susitna River. Samples of resident fish species were collected opportunistically while crews were collecting adult and juvenile salmon samples. Resident fish were identified to genus or species with a field key. A small piece of fin tissue was sampled from each fish and placed into a bottle or vial of ethyl alcohol for preservation. Samplers recorded on each bottle, or on datasheets for vial collections, the areas from which the samples were collected: 1) Chulitna River, 2) Talkeetna River, 3) Upper Susitna River, 4) Middle Susitna River below Devils Canyon, 5) Middle Susitna River above Devils Canyon, or 6) Lower River. Tissues were placed in separate bottles for each species and area where they were collected.

4.1.5. Sampling coordination with other studies

Methods used in 2013 adult salmon sample collections are reported in the Study 9.14 ISR Part A Section 4.1.5. In 2014, project leads met biweekly starting on July 17 to coordinate field work. In addition, Salmon Escapement Study (Study 9.7) field crews released seven spawning survey results summarizing Chinook Salmon observations soon after the surveys were completed between July 14 and August 19, 2014. As in 2013, there was also frequent direct communication among ADF&G, the contractor liaison, and study leads designated for each interrelated study.

Samples were delivered to the GCL and were entered into ADF&G's LOKI database. Most of these samples were resident fish (Table 4-6) or juvenile Chinook Salmon from above Devils Canyon (Table 4-1) collected as part of Study 9.5. As part of the Salmon Escapement Study (Study 9.7), AEA also collected samples from salmon radio-tagged at Curry (PRM 124-126), and salmon radio-tagged in the Lower River near the confluence with the Yentna River (PRM 33-34), and in the Yentna River (RM 6). Sampling methods for all fish tissue samples provided from the interrelated studies are described in the respective ISRs and Study Implementation Reports.

4.1.6. Collection trip documentation

As in 2013, detailed notes were kept during each collection trip and then entered into the trip report database in the GCL Oracle database, LOKI, when crews returned to Anchorage (ISR Part A Appendix A). The following information was recorded for each trip: 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 were used post-season to submit Anadromous Waters Catalog nomination forms.

4.2. Tissue Storage

AEA implemented the methods for tissue storage of samples collected in ethanol as described in Section 4.3 of the 2013 and 2014 Genetics Implementation Plans, with no variances. While in the field, tissue samples were preserved in ethyl alcohol in either a 125–500 ml (4.2-16.9 oz) bulk sample bottle for each location or individual 2 ml (0.07 oz) vials. After samples were received by the GCL, collection information was recorded in LOKI. For long-term storage, samples were preserved as follows: 1) vials were pierced, ethanol removed, and sample vacuum-dried; 2) once dry, tissue samples were stored at room temperature.

Storage for tissue samples collected on buccal swabs (Appendix A) was similar: tissues were stored dry at room temperature. The only difference is that these samples were desiccated in the field (by placing the samples in desiccant beads within individual 2ml vials) rather than after they arrived at the lab.

4.3. Laboratory Analysis

Laboratory analysis began during the fourth quarter of 2013 and is continuing without variances (Figure 7.1). Methods were described in detail in the 2013 and 2014 Genetics IP Section 4.4.

The methods in the 2014 IP (ISR Part B Attachment 1) differed from those in the 2013 IP as a result of consultation with USFWS and NMFS in March 2014. The 2014 plan includes 190 SNP markers and 12 microsatellite markers to 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. Sets of markers to be screened in selected samples are in Table 4-9.

4.4. Data Retrieval and Quality Control

Data retrieval and quality control is ongoing with no variances. The Section 4.5 of both the 2013 IP and 2014 IP (ISR Part B Attachment 1) contain detailed descriptions of data retrieval and quality control methods.

4.5. Variances from Study Plan

As described in ISR Part A Section 4.5, there were no variances from the collection, storage and analysis methods described in the Genetic Baseline Study Plan in 2013. However, full access to all of the sampling sites in the 2013 Genetics IP was not available in 2013. The Study Plan for 2013 included sampling on streams that required access to Cook Inlet Regional Working Group (CIRWG) lands. Access was not granted to CIRWG lands in 2013, thereby fully or partially restricting sampling on some streams. Lack of access to CIRWG lands above or near Devils Canyon prevented potential sampling of Chinook Salmon on Cheechako, Devil, Fog, Tsusena, and Watana creeks. Lack of access to CIRWG lands also prevented potential sampling at Portage and Prairie creeks for Coho Salmon, and reduced sampling at Prairie Creek for Sockeye Salmon. The study was designed to collect the target number of samples over multiple years. In 2014, full access to the CIRWG lands was granted. Analysis of 2013 and 2014 collections will provide insight into whether there is a loss in power to test for stability in allele frequencies across years for Chinook Salmon (testing between hypotheses 1a, 1b; Figure 2-1) and, if there is, the magnitude of this loss in power.

There was one variance from the collection methods described in the Genetic Baseline Study Plan as outlined in the 2014 Implementation Plan: Associated data (latitude/longitude, length, sex) were not recorded for each individual and scales were not sampled for the 12 adult Chinook Salmon captured and sampled for genetic tissues in Fog Creek due to an oversight in the field. All the other adult Chinook Salmon captured within and above Devils Canyon were taken from within Devils Canyon where these paired data were not required. However, latitude/longitude data were recorded for the general area were the fish were collected. Lack of these data will reduce the ability to determine correlations between location and genetic relatedness and could reduce the precision of the estimation of effective population size (sex ratios affect effective population size). Lack of these data will have minimal effect on the ability to test among hypotheses for population structure.

Methods for tissue samples from Chinook Salmon juveniles collected from tributaries above Devils Canyon differed between the 2013 IP and the 2014 IP. In the 2013 ISR Part A Section 4.1.3.1, methods describe sampling caudal fin tissue. Caudal fin sampling is lethal to the fish. In the 2014 Implementation Plan (ISR Part B), methods describe sampling using buccal swabs to allow for non-lethal sampling (see Appendix A for sampling methods). Within each year, methods followed those described in their respective implementation plans.

Finally, the laboratory methods in the 2014 IP (ISR Part B Attachment 1) differed from those in the 2013 IP as a result of consultation with USFWS and NMFS in March 2014. The 2014 plan includes 190 SNP markers and 12 microsatellite markers to 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.

5. RESULTS

5.1. Sample Collection

Details regarding sample collection through September 15, 2013 are reported in ISR Part A Section 5.1. The sections below provide details regarding the 2014 sampling effort as well as a cumulative summary of all collections delivered to the GCL each year since inception of the Study, as presented in Tables 4-1 through 4-6. The genetic sampling effort through time by river area for adult salmon species and juvenile Chinook Salmon during 2013 and 2014 is provided in Table 4-8.

5.1.1. Adult Chinook Salmon collections

5.1.1.1. 2013

Details regarding the 2013 adult Chinook Salmon collections through September 15, 2013 are reported in the 9.14 ISR Section 5.1.1. Full counts for sites visited and samples collected through the end of the 2013 are reported here. AEA captured and sampled Chinook Salmon at 32 of the 35 sites surveyed. (Table 4-1). Samples from 1,405 adult Chinook Salmon were collected. Most of these samples were taken from the Susitna River drainage (1,342). Three of these samples were from above Devils Canyon (Tables 4-1 and 4-11). The remaining samples came from other drainages within upper Cook Inlet.

5.1.1.1.1. 2014

A dedicated survey flight to determine distribution and availability of Chinook Salmon for sampling occurred the week of July 1 (Table 4-8). Only the Knik Arm area was surveyed from the air. However, many of the collection flights incorporated surveys. AEA captured and sampled Chinook Salmon at 26 of the 27 sites surveyed (Table 4-1). Samples from 1,045 adult Chinook Salmon were collected. Most of these samples were taken from the Susitna River drainage (1,005). Thirteen of these samples were from above Devils Canyon (Fog and Devil creeks) and 64 were sampled within Devils Canyon from Chinook Creek and Cheechako Creek. The remaining samples came from other drainages within upper Cook Inlet.

5.1.2. Other adult salmon collections

Details regarding the collection of other adult salmon species through September 15, 2013 are provided in ISR Part A Section 5.1.2. No dedicated surveys were performed for adult salmon species other than Chinook Salmon in 2014, but samples were collected incidentally or during other ADF&G sampling efforts unrelated to the this Study.

A total of 376 Sockeye Salmon adult samples have been collected. Samples from 336 Sockeye Salmon were collected from 11 streams and 2 sloughs (Table 4-2) in 2013. During 2014, Sockeye Salmon were sampled from Spink Creek (n=19) and Larson Lake - outlet stream (n=21; Table 4-2). Samples from 659 Chum Salmon were collected from 13 streams and several sloughs above the Three Rivers Confluence (Table 4-3) in 2013; no additional Chum Salmon samples have been collected. A total of 1,101 Coho Salmon samples have been collected, with 541 collected from 10 streams in 2013 and another 560 sampled during 2014 for another ADF&G study from 12 locations within this study area (Table 4-4). Odd-year Pink Salmon were collected from 11 streams and 1 slough during 2013 (n=1,049; Table 4-5) and even-year Pink Salmon were sampled in 2014 from Spink Creek (n=116; Table 4-5).

5.1.3. Juvenile Chinook Salmon collections

5.1.3.1. Above Devils Canyon

A total of 134 juvenile Chinook Salmon have been collected from tributaries within Devils Canyon, below the uppermost impediment to fish migration (i.e., Impediment 3; Table 4-1). Archived tissues collected in 2012 are available from Cheechako Creek, a tributary within Devils Canyon (n=35). In 2014, samples from 120 juvenile Chinook Salmon were collected in 2014 from two tributaries within Devils Canyon (Cheechako Creek n=58; Chinook Creek n=62).

A total of 226 juvenile Chinook Salmon have been collected from above Devils Canyon Impediment 3 (Table 4-1). Samples from 189 juvenile Chinook Salmon were collected in 2013 from 2 tributaries above Devils Canyon (Kosina Creek n=130; Oshetna River n=59). In 2014, samples from 54 juvenile Chinook Salmon were collected from five tributaries and the mainstem above Devils Canyon (Devil Creek n=14; Unnamed Tributary 184 n=1; Tsusena Creek n=1; Kosina Creek n=3; Oshetna River n=3; Susitna River mainstem n= 32).

5.1.3.2. Lower River collections

No sampling of the Lower River occurred in 2014. As described in ISR Part A Section 5.1.3.2, sampling during 2013 resulted in the collection of eight juvenile Chinook Salmon from the Lower River (Table 4-1).

5.1.3.3. Species identification of juvenile collections

A total of 797 juvenile Pacific salmon samples from this and other studies collected in the Susitna River from 2012 to 2014 were analyzed in the lab to determine species. Samples were collected throughout the Susitna River but most came from the Middle and Upper River. Results for species identification for collections made by AEA contractors below Devils Canyon will be reported by Study 9.5 and 9.6. Of the 398 juveniles captured within and above Devils Canyon, species were determined on 386 fish (96.5%). Unsuccessful analysis was determined to be due to 1) no DNA amplification (3 fish), loss of the sample, paired data discrepancies, or lab error (10 fish), or 3) equivocal results likely due to low amplification (1 fish). All successfully analyzed samples collected within and above Devils Canyon were identified as Chinook Salmon. Among these samples, there were no discrepancies in species identification between field identification and DNA analysis.

5.1.4. Other species collections

Results through 9/15/2013 were reported in the 9.14 ISR Section 5.1.4. Full counts for sites visited and samples collected through all of 2013 and 2014 are reported here. In total, samples were collected from 2,402 fish (1,652 in 2013 and 750 during 2014), with sampling sites further broken into one of six potential strata (Table 4-6). The target sample size of 50 total fish per species was reached for nine species (Burbot, Dolly Varden, Eulachon, Arctic Grayling, Slimy Sculpin, Three-spine Stickleback, Longnose Sucker, Rainbow Trout, and Round Whitefish). No samples were collected for six species (Alaska Blackfish, Pacific Lamprey, Coastrange Sculpin, Pacific Staghorn Sculpin, Prickly Sculpin, and Lake Whitefish).

5.1.5. Pacific salmon sampling coordination with other studies

As part of the Salmon Escapement Study (Study 9.7) effort, Pacific salmon genetic samples were collected by AEA from salmon radio-tagged at Curry and at Lower Susitna River and Yentna River fish wheel sites administered by the ADF&G Division of Sport Fish. A total of 3,361 Chinook, 394 Chum, 445 Coho, 400 Pink, 335 Sockeye Salmon, and 230 non-salmon species were sampled.

The samples from radio-tagged fish were intended to be used to supplement the data in areas where genetic sample targets were not achieved and final spawning destination could be confirmed. Final spawning locations have now been determined from telemetry and 12 Chinook Salmon with final spawning locations above or within Devils Canyon will be used in this study (Table 4-1). In 2013, seven radio-tagged Chinook Salmon were confirmed as spawning in two tributaries within the Devils Canyon below Impediment 3 (Chinook Creek n=1; and Cheechako Creek n=6) and another four were confirmed spawning in Cheechako Creek in 2014. One fish radio-tagged in 2013 with a final spawning destination above Devils Canyon Impediment 3 (i.e., Devil Creek) will also be included in the analysis.

5.1.6. Collection trip documentation

In 2014, one entry was made into the GCL Oracle database to document a survey trip and 15 entries were made to document Chinook Salmon collection trips.

5.2. Tissue Storage

As in 2013, the 2014 samples were placed into bottles (multiple fish per container) for most of the collections (75 percent) and for the remaining collections, samples were placed into vials (one fish per container).

5.3. Laboratory Analysis

Laboratory and statistical analyses are ongoing. Laboratory analysis progress is presented for three sets of markers in Table 4-13. Laboratory analyses of SNP markers to augment the microsatellite data have been screened on all juvenile and adult Chinook Salmon collected within and above Devils Canyon. For most of the adults, the 188 SNP panel has been successfully screened. However low DNA amplification in juvenile fish have yielded unreliable results for

many samples. The small initial tissue quantity resulted in low concentration DNA yields. Methods to concentrate DNA of remaining samples are being investigated, but remaining volumes are low and some samples have been exhausted. Analysis of SNP markers for Chinook Salmon collected from Susitna River drainages below Devils Canyon (Objective 3) is largely complete for 48 markers (Table 4-13). On the outset of this study, 30 locations were identified as location groupings to collect samples that might represent individual populations. This study has SNP data collected from at least 50 fish from 38 locations and from at least 100 fish from 24 locations.

The panel of 48 SNP markers has been successfully screened on at least 100 fish from three locations and at least 50 fish in four locations within West Side of Cook Inlet and from at least 100 fish from three locations and at least 50 fish from 5 collections within Knik Arm (Table 4-13). These samples will also be used to accomplish Objective 3.

5.4. Data Retrieval and Quality Control

Data retrieval and quality control are ongoing and no results are available for inclusion in this Study Implementation Report.

6. DISCUSSION

The study was designed to achieve the target number of samples over the course of multiple years. With a few exceptions, sample collections of Pacific salmon species delivered to GCL were sufficient to maintain progress toward study objectives ("Expected" columns in Tables 4-1 to 4-5).

6.1. Chinook Salmon Adults and Juveniles Above Devils Canyon

Section 4.2 of the 2013 Genetics IP outlined sample collection in locations across the Susitna River basin. In 2013, Alaska Energy Authority was not granted access to Cook Inlet Regional Working Group (CIRWG) lands to collect samples associated with this study. As a result AEA did not collect samples of adult salmon where they were observed on CIRWG lands from the air or through radio-tag tracking. For adult Chinook Salmon, tributaries not accessed included Watana, Tsusena, Fog, Devil, and Cheechako creeks (all above or within Devils Canyon on the Susitna River). In addition, Portage Creek (below Devils Canyon on the Susitna River) and Prairie Creek (a Talkeetna River tributary) received a reduced sampling intensity due to lack of land access.

Access to these lands was obtained for the 2014 field season and progress was made in collecting samples from these areas. AEA now has exceeded sampling expectations for both adult and juvenile Chinook Salmon within and above Devils Canyon (Table 4-1). However, the lack of large numbers of samples from the same locations over multiple years may provide challenges in testing among hypotheses that explain population structure. Samples collected by AEA in field studies 9.5 and 9.6 in additional years may mitigate for this shortcoming.

Laboratory analyses is complete for microsatellites but incomplete for SNPs for these collections and for adult collections within the Middle River below Devils Canyon (Table 4-13). Of the 395 juvenile Chinook Salmon screened for microsatellites, 392 individuals have genotypes for at least 11 of the 13 markers. All 107 adults have genotypes for at least 11 of 13 markers. In addition, 93 and 98 adult samples from Portage Creek and Indian River (all adult samples in the Middle River of the Susitna River below Devils Canyon), respectively, have genotypes from at least 11 of 13 markers. Of all the marker types, microsatellites offer the most useful information to satisfy Objective 3 for collections within and above Devils Canyon. These markers have large numbers of alleles providing information that will be useful in testing hypotheses about population structure as outlined in Figure 2-1.

6.2. Chinook Salmon Adults in the Susitna River Below Devils Canyon

Total number of fish collected in 2013 and 2014 (2,252 fish from 27 sites; Table 4-1) exceeded expected numbers for the full project (1,032 fish from 20 to 29 sites), although the geographic distribution of these collections differs from the anticipated distribution. In the Yentna River, 249 fish were collected from 5 sites (project expected 390 fish from 9 sites). In the Chulitna River, 776 fish were collected from 9 sites; (project expected 218 fish from 2 to 8 sites). In the Middle River, below Devils Canyon, 151 fish were collected from 3 sites (project expected 134 fish from 2 to 6 sites). In Talkeetna River, 410 fish were collected from 6 sites (project expected 196 fish from 3 to 7 sites). Finally, from the Lower River, 666 fish were collected from 4 sites (project expected 159 fish from 5 to 9 sites). Nevertheless, the collections should provide adequate representation of Chinook Salmon populations to meet objective 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." Despite exceeding the *expected* numbers of samples collected in the study area, *ideal* sample sizes were only met for 6 of the 45 sites originally targeted. Statistical analyses of these data are in progress and should provide adequate resolution to accomplish Objective 3.

6.3. Chinook Salmon Adults Outside of the Susitna Basin

Progress was made toward collecting Chinook Salmon in the other drainages from Upper Cook Inlet in 2013 and 2014. In the western side of Cook Inlet, 47 fish were collected from one site (study expected 235 fish from 4 sites). In the Knik Arm, 56 fish were collected from 3 sites (study expected 181 fish from 5 to 6 sites). Ideal sample sizes have been met for one of the 11 sites originally targeted. Nonetheless, by including collections already in archive, there should be adequate representation of Chinook Salmon populations to meet Objective 3. Statistical analyses of these data are in progress and should provide adequate resolution to accomplish Objective 3.

6.4. Other Fish Species

Sample collections were sufficient to attain or make progress towards study objectives for some resident species (Objective 1). Although the target sample size in the 2013 and 2014 IPs for each species was 50 fish for the entire Susitna River drainage, ADF&G also wanted to know roughly what part of the drainage the samples came from, and so asked field crews to reference each

collection to one of six location "strata" in the drainage (Table 4-6). Because crews worked concurrently in different strata, this meant 50 samples were able to be collected in multiple places for some species, and caused the drainage-wide target to be exceeded (see results section; Table 4-6).

The approved study methods include only opportunistic collection of resident fish species. Targets of 50 fish per species may not be met for those species that were not present during sampling, or were not susceptible to the sampling gear. No laboratory or statistical analyses are planned for genetic samples from resident species in this study. The objective for taking these samples was to develop a repository of genetic samples for target resident fish species captured within the Lower, Middle, and Upper Susitna River drainage. This was always planned as an opportunistic activity to occur while sampling for other Susitna-Watana Hydro Project licensing studies or other non-related projects.

7. CONCLUSION

During the last two years, progress was made toward all but one objective. In 2014, progress was made in collecting juvenile and adult Chinook Salmon from above Devils Canyon, collecting adult Chinook Salmon from upper Cook Inlet tributaries, opportunistically collecting other salmon and non-salmon species from the Susitna River and genotyping Chinook Salmon for SNPs and µSATs. Progress on sample collection to date exceeds expectations for Objectives 1, 2, and 3 but has not met all study goals.

We anticipated that assessing temporal stability in allele frequencies for Chinook Salmon captured above Devils Canyon was going to be challenging even with samples taken over three or four years (2014 IP; ISR Part B Attachment 1, section 2.1.1). With accessibility issues to CIRWG lands in 2013 and only one full year of sampling (2014), challenges in testing of temporal stability within locations both within and above Devils Canyon are anticipated. This testing is a key to elucidate population structure (Objective 3) and determine the MSA potential for identifying Chinook Salmon originating from tributaries within and above Devils Canyon captured below Devils Canyon (Objective 4). However, these samples may be adequate to detect high temporal variation in allele frequencies among years. Samples from additional years will be required to examine temporal stability in allele frequencies among years within specific locations in tributaries within and above Devils Canyon and to provide more precise estimates of variation in allele frequencies among years.

Laboratory analysis has begun and data have been collected to start examining Objectives 3 and 4. The panel of 48 SNP markers has been successfully screened from at least 50 fish from 38 locations and from at least 100 fish from 24 locations within the Susitna River drainage; from at least 100 fish from three locations and at least 50 fish in four locations within West Side of Cook Inlet; and from at least 100 fish from three locations and at least 50 fish from 5 collections within Knik Arm (Table 4-13). These data should be adequate to characterize the genetic population structure of Chinook Salmon from Upper Cook Inlet (Objective 3).

Low DNA yields for some collections from within and above Devils Canyon may reduce the number of markers available for statistical analysis (Objectives 3 and 4). However,

microsatellites have been successfully screened for most of the collections (Table 4-13) and this marker type is likely to yield the most informative tests given the high heterozygosity and large numbers of alleles present.

Standard population structure statistical analyses will be completed on Chinook Salmon from throughout Upper Cook Inlet to understand how genetic variation is partitioned. Statistical analysis to test among hypotheses for Chinook Salmon captured within and above Devils Canyon will be determined through consultations with USFWS and NOAA.

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9. TABLES

Table 4-1. Area, location, and sublocation of baseline samples of adult and juvenile Chinook Salmon spawning aggregates for genetic analysis.

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
				Adult Chinook Salmon					
West Side Cook Inlet	Chuitna River		1	2008, 2009 (142)	200	58	-	-	142
	Beluga River	Coal Creek	2	2009, 2010, 2011 (120)	200	80	-	-	120
	Theodore River		3	2010, 2011, 2012 (189)	200	11	47	-	**236**
	Lewis River		4	2011, 2012 (86)	200	86	0	-	86
Yentna Drainage	Clearwater Creek		5	2012 (25)	200	50	-	-	25
	Nakochna River		6		n/a	-	-	22	**22**
	Red Creek		7	2012 (29)	200	58	82	-	**111**
	Happy River		8	2012 (19)	200	38	S	-	19
	Red Salmon Creek		9	2012 (12)	200	24	S	15	27
	Hayes River		10	2012 (5)	200	10	45	24	**74**
	Canyon Creek		11	2012 (32)	200	64	61	-	93
	Talachulitna River		12	1995, 2008, 2010 (180)	200	20	-	-	180
	Lake Creek	Sunflower Creek	13	2009, 2011 (127)	200	71	S	-	127
	Kahiltna River	Peters Creek	14	2009, 2010, 2011, 2012 (110)	200	55	-	-	110
Susitna Drainage	Chulitna River	Middle Fork	15	2009, 2010 (182)	200	18	61		**243**
		East Fork	16		200		64	33	
		West Fork	17		200		S	-	
		Honolulu Creek	18		200		31	75	
		Pass Creek	19		n/a		33	71	
		Spink Creek	20		200	200	56	18	**715**
		Byers Creek	21		200		55	54	
		Troublesome Creek	22		200		71	48	
		Tokositna River (Bunco Creek)	23		200		103	-	
		Tokosina River (Bunco Lake inlet stream)	24		n/a		3	-	

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
Susitna Drainage	Upper Susitna River	Oshetna River	25		200		0	-	
		Kosina Creek	26	2012 (10)	200		3	-	
		Kosina Creek (radio tag)	26	2012 (2)	n/a		-	1	
		Watana Creek	27		200		S	-	
	Middle Susitna River above Devils Canyon	Tsusena Creek	28		200		S	-	
		Tsusena Creek (radio tag)	28		n/a		1	-	
		Fog Creek	30		200		0	12	
		Susitna River mainstem	n/a		n/a	50	1	-	**107**
	Middle Susitna River within Devils Canyon	Devil Creek	31		200		S	1	
		Devil Creek (radio tag)	31		n/a		1	-	
		Chinook Creek	32		200		S	7	
		Chinook Creek (radio tag)	32		n/a		1	-	
		Cheechako Creek	33		n/a		-	57	
		Cheechako Creek (radio tag)	33		n/a		6	4	
	Middle Susitna River below Devils Canyon	Portage Creek	34	2009, 2010, 2011 (141)	200	59	25	-	166
		Indian River	35	2012 (1)	200		81	20	
		Gold Creek	36		200		S	-	
		4th of July Creek	37		n/a	75	-	25	**127**
		Lane Creek	38		200		S	-	
		Chase Creek	39		200		S	-	

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
Susitna Drainage	Talkeetna River	Prairie Creek	40	1995, 2008 (169)	200	31	32	-	**201**
		no name creek #2	41		n/a		25	28	
		no name creek #1	42		n/a		71	13	
		upper mainstem	43		200		S	-	
		Iron Creek	44		200	100	57	46	**373**
		Disappointment Creek	45		200		64	69	
		Sheep River	46		200		S	-	
		Larson Creek	47		200		S	-	
		Chunilna Creek (Clear Creek)	48	2009, 2012 (130)	200	65	5	-	135
	Lower Susitna River, upstream of Deshka	Montana Creek	49	2008, 2009, 2010 (218)	200	0	213	227	**658**
		Birch Creek	50		200		S	-	
		Sheep Creek	51		200	50	24	36	**226**
		North Fork Kashwitna River	52		200		12	50	
		Little Willow Creek	53		200		55	49	
		Willow Creek	54	1991,1997, 2005, 2009 (309)	200	0	-	-	**309**
	Deshka River	Moose Creek	55	1995, 2012 (103)	200	52	-	-	103
		Deshka River weir	56	2005 (200)	200	0	-	-	**200**
	Alexander Creek	Sucker Creek	57	2011, 2012 (143)	200	57	-	-	143
Knik Arm	Matanuska River	Kings River	58		200	25	4	S	**52**
		Granite Creek	59		200		12	36	
		Moose Creek	60	1995, 2008, 2009, 2012 (155)	200	45	-	-	155
	Eagle River	South Fork	61	2009, 2011, 2012 (73)	200	24	-	4	77
		Meadow Creek	62	2009 (6)	200	12	-	-	6
	Ship Creek		63	2009 (311)	200	0	-	-	**311**
	Little Susitna River		64	2009, 2010 (125)	200	75	-	-	125

Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Sample sizes				
					This project				
					Ideal	Expected (all years)	2013	2014	Total
Juvenile Chinook Salmon									
Susitna Drainage	Upper Susitna River	Oshetna River	25		200		59[a]	3	
		Kosina Creek	26		200		130	3	
		Upper Susitna River mainstem	n/a		n/a		-	30	
	Middle Susitna River within and above Devils Canyon	Tsusena Creek	28		n/a		-	1	
		Unnamed Tributary 184	29		n/a	70	-	1	**398**
		Fog Creek	30		200		-	-	
		Devil Creek	31		n/a		-	14	
		Chinook Creek	32		n/a		-	62[a]	
		Cheechako Creek	33	2012 (35)	200		-	58[a]	
		Middle Susitna River above	n/a		n/a		-	2	
	Lower Susitna River	5 habitat types (100 fish/habitat type times 3 or 4 collections)	n/a		1,600	1,600	8	-	8

[a] Chain-of-custody forms indicate one more fish that we received for each of these collections (duplicate entries).

Note: 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 archived sample size (Ideal), the anticipated number to be collected over the original three years of this project based on past sampling effort and success and information from the Anadromous Waters Catalog and local biologists (Expected), progress made toward sampling targets in 2013 and 2014, and the resulting total sample size after combining the amount archived with the new samples (Total). Bold and underlined values in the Total column indicate sample sizes in 2013 and 2014 met or exceeded expected numbers. An "S" in the 2013 and 2014 columns 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. Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. New locations that are now included in grouped locations are sharing the expected value for their group. Map numbers (Map No.) correspond to location numbers on Figure 4-5.

Table 4-2. Location and sublocation of baseline samples of adult Sockeye Salmon spawning aggregates for genetic analysis.

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	2		100	100	-	-	0
		East Fork	1		100		-	-	
		Pass Creek	5		n/a	n/a	2	-	**2**
		Spink Creek	4	2007, 2008 (126)	100	0	0	19	**145**
		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	-	**10**
		5th of July Creek	10		n/a		2	-	
		McKenzie Creek	11		100		0	-	
		Chase Creek	12		100		0	-	
	Mainstem sloughs above Three Rivers Confluence	sloughs 8A,11, and 21	13	1995, 1996, 1997 (156)	100	0	119		**275**
		slough 9	14		n/a	n/a	66		**66**
Susitna River above three rivers confluence	Talkeetna River	unnamed creek	15		n/a	0	1	-	**1**
		Stephan Lake	16	1993, 1994, 2007 (346)	100	0	-	-	**346**
		Prairie Creek	17		n/a	0	2	-	**2**
		Iron Creek	18		100	50	0	-	0
		Disappointment Creek	19		n/a	0	11	-	**11**

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
Susitna River above three rivers confluence	Talkeetna River	Sloughs	20	1997 (79)	100	21	0	-	79
		Sheep River	21	2008 (190)	100	0	S	-	**190**
		Larson Lake - Eastern shore	23	2011 (90)	100	10	S	-	90
		Larson Creek	22	1992, 1993 (200)	100	0	S	-	**200**
		Larson Lake - outlet stream	24	2011 (126)	100	0	S	21	**147**
		Chunilna Creek	25		100	100	18	-	18
		Mama and Papa Bear Lakes	26	1997, 2007 (106)	100	0	75	-	**181**
		Fish Creek	27		n/a	0	3	-	**3**

Note: 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 archived sample size (Ideal), the anticipated number to be collected over the original three years of this project based on past sampling effort and success and information from the Anadromous Waters Catalog and local biologists (Expected), progress made toward sampling targets in 2013 and 2014, and the resulting total sample size after combining the amount archived with the new samples (Total). Bold and underlined values in the Total column indicate sample sizes in 2013 and 2014 met or exceeded expected numbers. 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. Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. New locations that are now included in grouped locations are sharing the expected value for their group. Map numbers (Map No.) correspond to location numbers on Figure 4-1.

Table 4-3. Location and sublocation of baseline samples of adult Chum Salmon spawning aggregates for genetic analysis.

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	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**

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total
Susitna River above Three Rivers Confluence	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**

Note: 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 archived sample size (Ideal), the anticipated number to be collected over the original three years of this project based on past sampling effort and success and information from the Anadromous Waters Catalog and local biologists (Expected), progress made toward sampling targets in 2013 and 2014, and the resulting total sample size after combining the amount archived with the new samples (Total). Bold and underlined values in the Total column indicate sample sizes in 2013 and 2014 met or exceeded expected numbers. An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted.. Some of the expected numbers are for groups of locations. Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. New locations that are now included in grouped locations are sharing the expected value for their group. Map numbers (Map No.) correspond to location numbers on Figure 4-2.

Table 4-4. Location and sublocation of baseline samples of adult Coho Salmon spawning aggregates for genetic analysis.

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Ideal	Expected (all years)	2013	2014	Total[a]
Susitna River above three rivers confluence	Chulitna River	Middle Fork	2		100	200	0	0	167
		East Fork	1		100		0	0	
		Honolulu Creek	3		100		4	0	
		Byers Creek	4	2014[a] (56)	100		0	0	
		Troublesome Creek	5	2014[a] (15)	100		92	0	
		Spink Creek	6	2008 (38); 2014[a] (62)	100	62	0	0	**100**
		Tokositna River mainstem	7		100	100	S	0	65
		Tokositna River (Bunco Creek)	8	2014[a] (56)	100		9	0	
		Tokositna River (Swan Lake)	9	2009 (20)	100	80	0	0	20
	Middle Susitna River below Devils Canyon	Portage Creek	10	2014[a] (61)	100	200	0	0	**218**
		Indian River	11	2014[a] (52)	100		105	0	
		Gold Creek	12		100		S	0	
		McKenzie Creek	13		100		S	0	
		Lane Creek	14		100		S	0	
		Sloughs	15		100	75	42	0	42
		Chase Creek	16		100	75	S	0	0
		Whiskers Creek	17	2014[a] (2)	100	75	79	0	**81**

Area	Location	Sublocation	Map No.	Year(s) Collected (# archived)	Sample sizes				
					This project				
					Ideal	Expected (all years)	2013	2014[a]	Total
Susitna River above three rivers confluence	Talkeetna River	upper mainstem	18		100	25	S	0	0
		Prairie Creek	19	2014[a] (53)	100	75	S	0	53
		Iron Creek	20	2014[a] (21)	n/a	n/a	28	0	**49**
		Sheep River	21		100	50	115	0	**115**
		Larson Lake - outlet	22	2011 (84); 2014[a] (48)	100	16	S	0	**132**
		Chunilna Creek	23	2014[a] (69)	100	75	66	0	**135**
		Fish Creek	24	2014[a] (65)	n/a	n/a	1	0	**66**

[a] Includes samples collected under an unrelated general fund project in 2014.

Note: 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 archived sample size (Ideal), the anticipated number to be collected over the original three years of this project based on past sampling effort and success and information from the Anadromous Waters Catalog and local biologists (Expected), progress made toward sampling targets in 2013 and 2014, and the resulting total sample size after combining the amount archived with the new samples (Total). Bold and underlined values in the Total column indicate sample sizes in 2013 and 2014 met or exceeded expected numbers. An "S" in the 2013 column indicates that a survey was performed but sampling was not attempted. Some of the expected numbers are for groups of locations. Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. New locations that are now included in grouped locations are sharing the expected value for their group. Map numbers (Map No.) correspond to location numbers on Figure 4-3.

Table 4-5. Location and sublocation of baseline samples of adult Pink Salmon spawning aggregates for genetic analysis.

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (for Archive)	Ideal	Expected (all years)	2013	2014	Total
Susitna River above three rivers confluence	Chulitna River	Middle Fork	1		100	100	0	0	**117**
		Spink Creek	3		100		0	116	
		Troublesome Creek	2		100		0	0	
		no name creek	4		n/a		1	0	
	Middle Susitna River below Devils Canyon	Portage Creek	5		100	50	136	0	**136**
		Indian River	6		100	100	116	0	**116**
		Gold Creek	7		100	50	106	0	**467**
		5th of July Creek	8		n/a		2	0	
		4th of July Creek	9		n/a		107	0	
		slough 9	10		n/a		116	0	
		McKenzie Creek	11		100		0	0	
		Lane Creek	12		100		115	0	
		Chase Creek	13		100		0	0	
		Whiskers Creek	14		100		21	0	

					Sample sizes				
					This project				
Area	Location	Sublocation	Map No.	Year(s) Collected (for Archive)	Ideal	Expected (all years)	2013	2014	Total
	Talkeetna River	upper mainstem	15		100	25	0	0	0
		Disappointment Creek	16		n/a	0	127	0	**127**
		Sheep River	17		100	25	0	0	0
		Larson Creek	18		100	100	0	0	0
		Chunilna Creek	19		100	100	101	0	**101**
		Fish Creek	20		n/a	0	101	0	**101**

Note: Sample sizes show collections already in the Gene Conservation Laboratory archives (Archived), number of samples to obtain the ideal archived sample size (Ideal), the anticipated number to be collected over the original three years of this project based on past sampling effort and success and information from the Anadromous Waters Catalog and local biologists (Expected), progress made toward sampling targets in 2013 and 2014, and the resulting total sample size after combining the amount archived with the new samples (Total). Bold and underlined values in the Total column indicate sample sizes in met or exceeded expected numbers. Sampling locations originally not included in the implementation plan have been included, and are indicated by an "n/a" ideal and expected value. New locations that are now included in grouped locations are sharing the expected value for their group. Map numbers (Map No.) correspond to location numbers on Figure 4-4.

Table 4-6. Resident and non-salmon anadromous fish species targeted for genetic tissue sampling in the Susitna River and samples sizes collected in 2013 and 2014. Sample collections are reported for the Gene Conservation Laboratory (GCL), interrelated studies (other), and the combined total (N).

Species	Target sample size (total)	Collection Strata																		Total collected
		Upper Susitna River			Middle Susitna River above Devils Canyon			Middle Susitna River below Devils Canyon			Lower Susitna River			Talkeetna River			Chulitna River			
	GCL	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
Burbot	50	1	57	58		6	6		80	80	2	51	53							197
Dolly Varden	50		183	183	3	2	5		23	23	4	4	8	35		35				254
Eulachon	50											283	283							283
Grayling, Arctic	50	20	135	155		90	90	2	76	78	7	20	27	5		5	3		3	358
Lamprey, Arctic*	n/a											10	10							10
Lamprey, Pacific	50																			0
Pike, Northern	50											19	19							19
Sculpin, Coastrange	50																			0
Sculpin, Pacific Staghorn	50																			0
Sculpin, Prickly	50																			0
Sculpin, Slimy	50	45	44	89	1	50	51		57	57	6	50	56	1		1				254
Stickleback, Ninespine	50											17	17							17
Stickleback, Threespine	50										50	63	113							113

Species	Target sample size (total)	Collection Strata																	Total collected
		Upper Susitna River			Middle Susitna River above Devils Canyon			Middle Susitna River below Devils Canyon			Lower Susitna River			Talkeetna River			Chulitna River			
	GCL	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	GCL	other studies	N	
Sucker, Longnose	50		89	89		22	22	4	84	88		51	51							250
Trout, Lake	50					1	1													1
Trout, Rainbow	50							24	68	92	44	10	54	14		14	38		38	198
Whitefish, Bering Cisco	50											2	2							2
Whitefish, Humpback	50		4	4					35	35		10	10							49
Whitefish, Lake	50																			0
Whitefish, Round	50	1	90	91		30	30	1	202	203	10	50	60	1	8	9	4		4	397

* Collected, but not on original list of target species

Table 4-7. Summary of survey flights conducted during 2013 and 2014. Surveys were performed in order to determine potential sampling locations for five salmon species (Chinook, sockeye, pink, chum, and Coho Salmon). X's indicate the occurrence of a survey flight in a given collection strata (Figure 3-1) on a certain date. Survey flight number 17 shows no X's because the survey was cancelled due to poor survey conditions.

		Collection strata								
Survey #	Date	Upper Susitna River	Middle Susitna above and within Devils Canyon	Middle Susitna below Devils Canyon	Lower Susitna River	Talkeetna River	Chulitna River	West Side Cook Inlet	Yentna Drainage	Knik Arm
1	6/11/2013	X	X	X			X			X
2	7/8/2013			X	X	X				
3	7/9/2013	X	X	X			X			
5	7/15/2013	X				X				X
6	7/16/2013			X			X			
7	7/17/2013								X	
8	7/22/2013	X	X	X		X				
9	7/23/2013				X		X			
10	7/24/2013								X	
11	7/29/2013	X	X	X		X				
12	7/30/2013				X		X			
13	8/5/2013	X				X	X			
14	8/6/2013		X	X	X	X	X			
15	8/12/2013					X				
16	8/13/2013		X	X			X			
17	8/19/2013									
18	8/26/2013				X					X
19	9/15/2013			X			X			
20	7/1/2014									X

Table 4-8. Genetic sampling effort through time by river area for adult salmon species and juvenile Chinook Salmon in 2013 and 2014. Salmon species sampled are reported by week and strata. X's indicate where sampling occurred in each week of the Project field season for all salmon species, and for sampling locations where Chinook Salmon were the only target species. Species sampled: Chinook (K), sockeye (S), pink (P), chum (Ch), and coho (Co) salmon. Sampling occurred from 6/8/2013 through 9/15/2013 and from 6/16/14 through 8/4/14.

		Collection Strata								
		Area sampled (all salmon species)			Area sampled (Chinook Salmon only)					
Week of	Species sampled	Talkeetna	Chulitna	Middle Susitna below Devils Canyon	Lower Susitna	Middle Susitna within and above Devils Canyon	Upper Susitna	Yentna	Knik	West
6/8-25/2013	K									x
6/24/2013	K				x					x
7/1/2013	K	x			x					x
7/8/2013	K				x					x
7/15/2013	K, S	x	x	x						
7/22/2013	K	x	x				x	x	x	
7/29/2013	K, S, Ch, P, Co,	x	x	x			x			
8/5/2013	K,S, Ch, P,Co	x	x	x			x			
8/12/2013	S, Ch, P, Co	x	x	x			x			
8/19/2013	S, Ch, P		x	x						
8/26/2013	*									
9/2/2013	*									
9/9/2013	*									
9/16/2013	Co		x							
9/23/2013	Co	x	x							
9/30/2013	Co	x	x							
6/16/2014	K						x		x	
6/30/2014	K	x		x	x					

		Collection Strata								
		Area sampled (all salmon species)			Area sampled (Chinook Salmon only)					
Week of	Species sampled	Talkeetna	Chulitna	Middle Susitna below Devils Canyon	Lower Susitna	Middle Susitna within and above Devils Canyon	Upper Susitna	Yentna	Knik	West
7/7/2014	K								x	
7/14/2014	K	x	x	x	x	x				
7/21/2014	K	x	x		x			x		
7/28/2014	K			x		x				
8/4/2014	K, S, P		x							

* Sampling efforts disrupted by adverse weather conditions.

Table 4-9. Marker name and source for the microsatellite (µSAT), 48 single nucleotide polymorphism (SNP), and 188 SNP marker sets.
An "x" in the marker set column indicates whether a marker is included in a marker set.

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ogo2v1	x			A
Ogo4v1	x			A
Oki100v1	x			B
Omm1080v1	x			C
Ots201bv1	x			D
Ots208bv1	x			E
Ots211v1	x			E
Ots212v2	x			E
Ots213v1	x			E
Ots3Mv1	x			F
Ots9v1	x			F
OtsG474v1	x			G
Ssa408uosv1	x			H
Ots_GTH2B-550		x	x	I
Ots_100884-287			x	J
Ots_101554-407			x	J
Ots_104569-86			x	J
Ots_105105-613			x	J
Ots_105385-421			x	J
Ots_105407-117			x	J
Ots_107074-284			x	J
Ots_108390-329			x	J
Ots_108820-336			x	J
Ots_109525-816			x	J
Ots_109693-392			x	J
Ots_110495-380			x	J
Ots_110551-64			x	J
Ots_111084b-619			x	J
Ots_112301-43			x	J
Ots_112419-131			x	J
Ots_112820-284			x	J
Ots_112876-371			x	J
Ots_113242-216			x	J
Ots_113457-40R			x	J
Ots_115987-325			x	J
Ots_117432-409			x	J

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ots_118205-61			x	J
Ots_123921-111			x	J
Ots_127236-62			x	J
Ots_128693-461			x	J
Ots_131460-584			x	J
Ots_94857-232R			x	J
Ots_94903-99R			x	J
Ots_96222-525			x	J
Ots_96500-180			x	J
Ots_96899-357R			x	J
Ots_AldB1-122			x	J
Ots_arf-188		x	x	K
Ots_AsnRS-60		x	x	K
Ots_CD59-2			x	L
Ots_cox1-241			x	M
Ots_DESMIN19-SNP1			x	J
Ots_E2-275		x	x	K
Ots_Est1363			x	N
Ots_Est740			x	N
Ots_ETIF1A		x	x	L
Ots_FARSLA-220		x	x	O
Ots_FGF6A		x	x	I
Ots_FGF6B		x	x	I
Ots_GH2		x	x	P
Ots_GnRH-271		x	x	K
Ots_GPDH-338		x	x	K
Ots_GPH-318		x	x	O
Ots_GST-207		x	x	O
Ots_HFABP-34			x	Q
Ots_HGFA-446		x	x	K
Ots_hnRNPL-533		x	x	O
Ots_hsc71-5'-453			x	R
Ots_Hsp90a			x	J
Ots_HSP90B-100		x	x	O
Ots_HSP90B-385		x	x	O
Ots_IGF-I.1-76		x	x	K
Ots_Ikaros-250		x	x	K
Ots_il13Ra2B-37			x	Q
Ots_il-1racp-166		x	x	K
Ots_ins-115		x	x	K

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ots_IsoT			x	N
Ots_LEI-292		x	x	O
Ots_LWSop-638		x	x	K
Ots_mapK-3'-309			x	S
Ots_MetA		x	x	L
Ots_MHC1		x	x	P
Ots_MHC2		x	x	P
Ots_NAML12-SNP1			x	J
Ots_nelfd-163			x	T
Ots_nkef-192			x	M
Ots_NOD1		x	x	I
Ots_ntl-255			x	S
Ots_P450		x	x	P
Ots_P450-288		x	x	U
Ots_P53		x	x	P
Ots_parp3-286			x	S
Ots_PGK-54		x	x	I
Ots_pop5-96			x	S
Ots_ppie-245			x	S
Ots_Prl2		x	x	P
Ots_PSMB1-197		x	x	O
Ots_RAD10099			x	V
Ots_RAD10252			x	V
Ots_RAD10400			x	V
Ots_RAD10583			x	V
Ots_RAD1072			x	V
Ots_RAD10807			x	V
Ots_RAD1104-38			x	Q
Ots_RAD11425			x	V
Ots_RAD11441			x	V
Ots_RAD1149			x	V
Ots_RAD11821			x	V
Ots_RAD11839			x	V
Ots_RAD1282			x	V
Ots_RAD1372			x	V
Ots_RAD14482			x	V
Ots_RAD14528			x	V
Ots_RAD14650			x	V
Ots_RAD14852			x	V
Ots_RAD1507			x	V

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ots_RAD1510			x	V
Ots_RAD15440			x	V
Ots_RAD1609			x	V
Ots_RAD161			x	V
Ots_RAD16976			x	V
Ots_RAD17721			x	V
Ots_RAD17873			x	V
Ots_RAD1832-39			x	Q
Ots_RAD21143			x	V
Ots_RAD21978			x	V
Ots_RAD22318			x	V
Ots_RAD2234			x	V
Ots_RAD2357			x	V
Ots_RAD2442			x	V
Ots_RAD249			x	V
Ots_RAD2687			x	V
Ots_RAD3123			x	V
Ots_RAD3391			x	V
Ots_RAD3470			x	V
Ots_RAD3513-49			x	Q
Ots_RAD3635			x	V
Ots_RAD3703			x	V
Ots_RAD3737			x	V
Ots_RAD3752			x	V
Ots_RAD3766			x	V
Ots_RAD3769			x	V
Ots_RAD3858			x	V
Ots_RAD3925			x	V
Ots_RAD4043			x	V
Ots_RAD4185			x	V
Ots_RAD4438			x	V
Ots_RAD4486			x	V
Ots_RAD4778			x	V
Ots_RAD4999			x	V
Ots_RAD5189			x	V
Ots_RAD5426-36			x	V
Ots_RAD5429			x	V
Ots_RAD6097			x	V
Ots_RAD6121			x	V
Ots_RAD6184			x	V

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ots_RAD6688			x	V
Ots_RAD7145			x	V
Ots_RAD7165			x	V
Ots_RAD7695			x	V
Ots_RAD7936-50			x	Q
Ots_RAD8200-45			x	Q
Ots_RAD8354			x	V
Ots_RAD8442			x	V
Ots_RAD856			x	V
Ots_RAD8560			x	V
Ots_RAD9039			x	V
Ots_RAD9480-51			x	Q
Ots_RAD9536			x	V
Ots_RAD962-35			x	V
Ots_RAD9704			x	V
Ots_RAD995			x	V
Ots_RAG3		x	x	I
Ots_redd1-187			x	S
Ots_S7-1		x	x	I
Ots_SClkF2R2-135		x	x	K
Ots_SERPC1-209		x	x	O
Ots_SL		x	x	P
Ots_SWS1op-182		x	x	K
Ots_TAPBP		x	x	L
Ots_TF1-SNP1			x	J
Ots_Tf-3545			x	U
Ots_TGFB			x	L
Ots_TLR3			x	L
Ots_Tnsf		x	x	P
Ots_tpx2-125			x	T
Ots_txnip-321			x	S
Ots_u07-07.161			x	W
Ots_u07-17.135			x	W
Ots_u07-25.325			x	W
Ots_u07-53.133			x	W
Ots_u202-161		x	x	K
Ots_u211-85		x	x	K
Ots_U212-158		x	x	K
Ots_U2446-123			x	Q
Ots_u4-92		x	x	K

	Marker Set			
Name	µSAT	48 SNPs	188 SNPs	Source[1]
Ots_u6-75		x	x	K
Ots_unk526		x	x	I
Ots_vatf-251			x	S
Ots_zn593-346			x	T
Ots_Zp3b-215		x	x	K

[1] A) Olsen et al. 1998, B) Department of Fisheries and Oceans Canada, unpublished, C) Rexroad and Coleman 2001, D) Oregon State University, unpublished, E) Greig et al. 2003, F) Banks et al. 1999, G) Williamson et al. 2002, H) Cairney et al. 2000, I) Northwest Fisheries Science Center, unpublished, J) Clemento et al. 2011, K) Smith et al. 2005a, L) Washington State University, unpublished, M) Campbell and Narum 2008, N) Miller et al. 2008, O) Smith et al. 2007, P) Smith et al. 2005b, Q) University of Washington, unpublished, R) Campbell and Narum 2009, S) Columbia Rivier Inter-Tribal Fish Commission, unpublished, T) University of Washington and Washington Department of Fish and Wildlife, unpublished, U) Alaska Department of Fish and Game, unpublished, V) Larson et al. 2014, W) Washington Department of Fish and Wildlife, unpublished.

Table 4-10. Species clustering in scatter plots for each of the seven standard markers used for discriminating among Pacific and Atlantic salmon at the Gene Conservation Laboratory.

Locus	Salmon/trout species in cluster	Cluster #
OKESSA1-OKE	Chinook	1
	Chum, Atlantic	2
	Sockeye	3
	Pink, Coho, Rainbow	4
OKI1-OKI	Chinook, Rainbow	1
	Coho	2
	Chum	3
	Sockeye, Atlantic, Pink	4
ONEOGO1-ONE	Pink	1
	Sockeye	2
	Coho, Rainbow, Chinook, Chum, Atlantic	3
OTSOKI1-OKI	Sockeye, Rainbow	1
	Coho, Chinook	2
	Pink	3
	Chum	4
	Atlantic	5
OTSSSA1-OTS	Chinook	1
	Coho, Rainbow, Chum, Atlantic, Sockeye, Pink	2
SSA1-SSA	Atlantic	1
	Coho, Rainbow, Chinook, Chum, Sockeye, Pink	2
	Atlantic	1
	Rainbow	2
	Sockeye	3
	Coho, Chinook, Chum, Pink	4
SSA4-SSA	Atlantic	1
	Pink, Rainbow	2
	Coho, Chinook, Chum, Sockeye	3

Table 4-11. Metadata and location of Chinook Salmon samples collected above Devils Canyon. Length (in millimeters) and sex were determined for Chinook Salmon sampled above Devils Canyon on August 6, 2013. Creek name, latitude, and longitude are also reported for these data.

Fish #	Date	Length	Sex	Creek	Latitude/Longitude
1	8/6/2013	980	M	Kosina	62.701/ -147.986
2	8/6/2013	575	F	Kosina	62.633/ -148.031
3	8/13/2013	570	M	Kosina	62.756/ -147.955

Table 4-12. Area, sampling location, sublocation, and number of samples successfully analyzed at greater than or equal to 80% of markers for the 13 microsatellite (uSATs), 48 single nucleotide polymorphism (SNP), and 188 SNP marker sets.
Map numbers (Map No.) correspond to location numbers on Figure 4-5.

Area	Location	Sublocation	Map No.	# Samples Successfully Analyzed			Samples collected
				13 uSATs	48 SNPs	188 SNPs	
			Adult Chinook Salmon				
West Side Cook Inlet	Chuitna River		1	0	138	95	142
	Beluga River	Coal Creek	2	0	118	41	120
	Theodore River		3	0	191	84	236
	Lewis River		4	0	87	0	86
Yentna Drainage	Clearwater Creek		5	25	25	25	25
	Nakochna River		6	0	0	0	22
	Red Creek		7	0	111	82	111
	Happy River		8	0	18	0	19
	Red Salmon Creek		9	0	27	0	27
	Hayes River		10	26	74	26	74
	Canyon Creek		11	0	92	0	93
	Talachulitna River		12	57	178	0	180
	Lake Creek	Sunflower Creek	13	0	126	74	127
	Kahiltna River	Peters Creek	14	0	107	0	110

Area	Location	Sublocation	Map No.	# Samples Successfully Analyzed			Samples collected
				13 uSATs	48 SNPs	188 SNPs	
Susitna Drainage	Chulitna River	Middle Fork	15	0	229	95	243
		East Fork	16	0	96	0	97
		West Fork	17	0	0	0	0
		Honolulu Creek	18	0	104	0	106
		Pass Creek	19	0	102	0	104
		Spink Creek	20	0	74	8	74
		Byers Creek	21	0	101	0	109
		Troublesome Creek	22	0	119	0	119
		Tokositna River (Bunco Creek)	23	0	100	94	103
		Tokosina River (Bunco Lake inlet stream)	24	0	0	0	3
	Upper Susitna River	Oshetna River	25	0	0	0	0
		Kosina Creek	26	13	13	11	13
		Kosina Creek (radio tag)	26	3			3
		Watana Creek	27	0	0	0	0
	Middle Susitna River above Devils Canyon	Tsusena Creek	28	0	0	0	0
		Tsusena Creek (radio tag)	28	1	1	1	1
		Fog Creek	29	12	12	12	12
		Susitna River mainstem		1	1	1	1

Area	Location	Sublocation	Map No.	# Samples Successfully Analyzed 13 uSATs	48 SNPs	188 SNPs	Samples collected
Susitna Drainage	Middle Susitna River wiithin Devils Canyon	Devil Creek	30	1	1	1	1
		Devil Creek (radio tag)	30	1	1	1	1
		Chinook Creek	31	7	7	7	7
		Chinook Creek (radio tag)	31	1			1
		Cheechako Creek	32	57	57	57	57
		Cheechako Creek (radio tag)	32	10	10	10	10
	Middle Susitna River below Devils Canyon	Portage Creek	33	93	161	97	166
		Indian River	34	98	99	97	101
		Gold Creek	35	0	0	0	0
		4th of July Creek	36	0	0	0	25
		Lane Creek	37	0	0	0	0
		Chase Creek	38	0	0	0	0
	Talkeetna River	Prairie Creek	39	48	194	95	201
		no name creek #2	40	0	53	0	53
		no name creek #1	41	0	83	0	84
		upper mainstem	42	0	0	0	0
		Iron Creek	43	57	103	57	103
		Disappointment Creek	44	0	131	0	133
		Sheep River	45	0	0	0	0
		Larson Creek	46	0	0	0	0
		Chunilna Creek (Clear Creek)	47	0	130	52	135

				# Samples Successfully Analyzed			Samples

Area	Location	Sublocation	Map No.	13 uSATs	48 SNPs	188 SNPs	collected
	Lower Susitna River, upstream of Deshka River						
Susitna Drainage		Montana Creek	48	0	213	0	658
		Birch Creek	49	0	0	0	0
		Sheep Creek	50	0	59	0	60
		North Fork Kashwitna River	51	12	61	12	62
		Little Willow Creek	52	13	103	13	104
		Willow Creek	53	69	212	49	309
	Deshka River	Moose Creek	54	0	52	35	103
		Deshka River weir	55	200	200	0	200
	Alexander Creek	Sucker Creek	56	0	144	125	143
Knik Arm	Matanuska River	Kings River	57	0	0	0	4
		Granite Creek	58	0	50	0	48
		Moose Creek	59	0	128	59	155
	Eagle River	South Fork	60	0	71	0	77
		Meadow Creek	61	0	6	0	6
	Ship Creek		62	0	59	0	311
	Little Susitna River		63	0	124	95	125

Area	Location	Sublocation	Map No.	# Samples Successfully Analyzed 13 uSATs	48 SNPs	188 SNPs	Samples collected
			Juvenile Chinook Salmon				
Susitna Drainage	Upper Susitna River	Oshetna River	25	62	52	0	62
		Kosina Creek	26	130	129	0	130
		Upper Susitna River mainstem	n/a	29	0	0	30
	Middle Susitna River within and above Devils Canyon	Tsusena Creek	n/a	1	0	0	1
		Fog Creek	29	0	0	0	0
		Devil Creek	30	14	0	0	14
		Chinook Creek	31	61	0	0	62
		Cheechako Creek	32	92	35	35	93
		Unnamed Tributary 184	n/a	1	0	0	1
		Middle Susitna River above	n/a	2	2	2	2
Susitna Drainage	Lower Susitna River	5 habitat types (100 fish/habitat type times 3 or 4 collections)	n/a	0	0	0	8

10. FIGURES

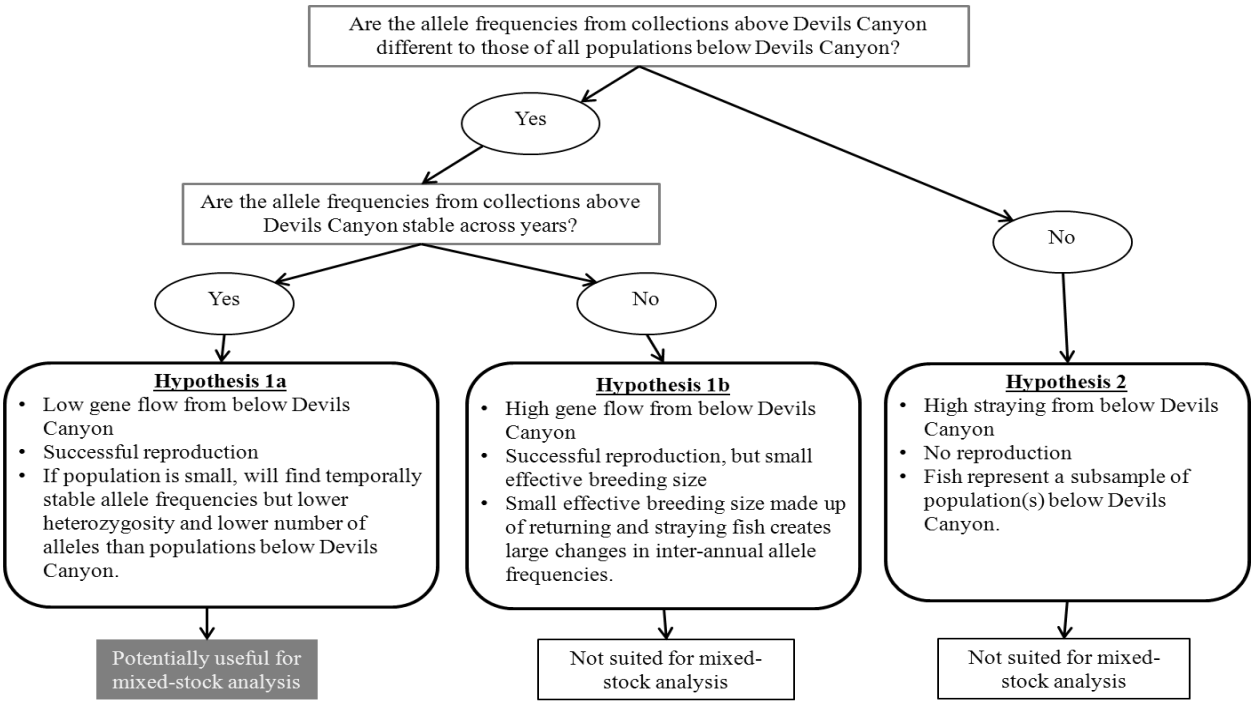

Figure 2-1. A generalized flow chart to distinguish among hypotheses of population structure for Chinook Salmon collected over spawning habitat above Devils Canyon in the Middle and Upper Susitna River. Only a self-sustaining population (Hypothesis 1a) will potentially result in genetic variation suitable for mixed-stock analysis for estimating the proportion of juvenile Chinook Salmon mixtures collected in the Middle and Lower Susitna River that originate from above Devils Canyon.

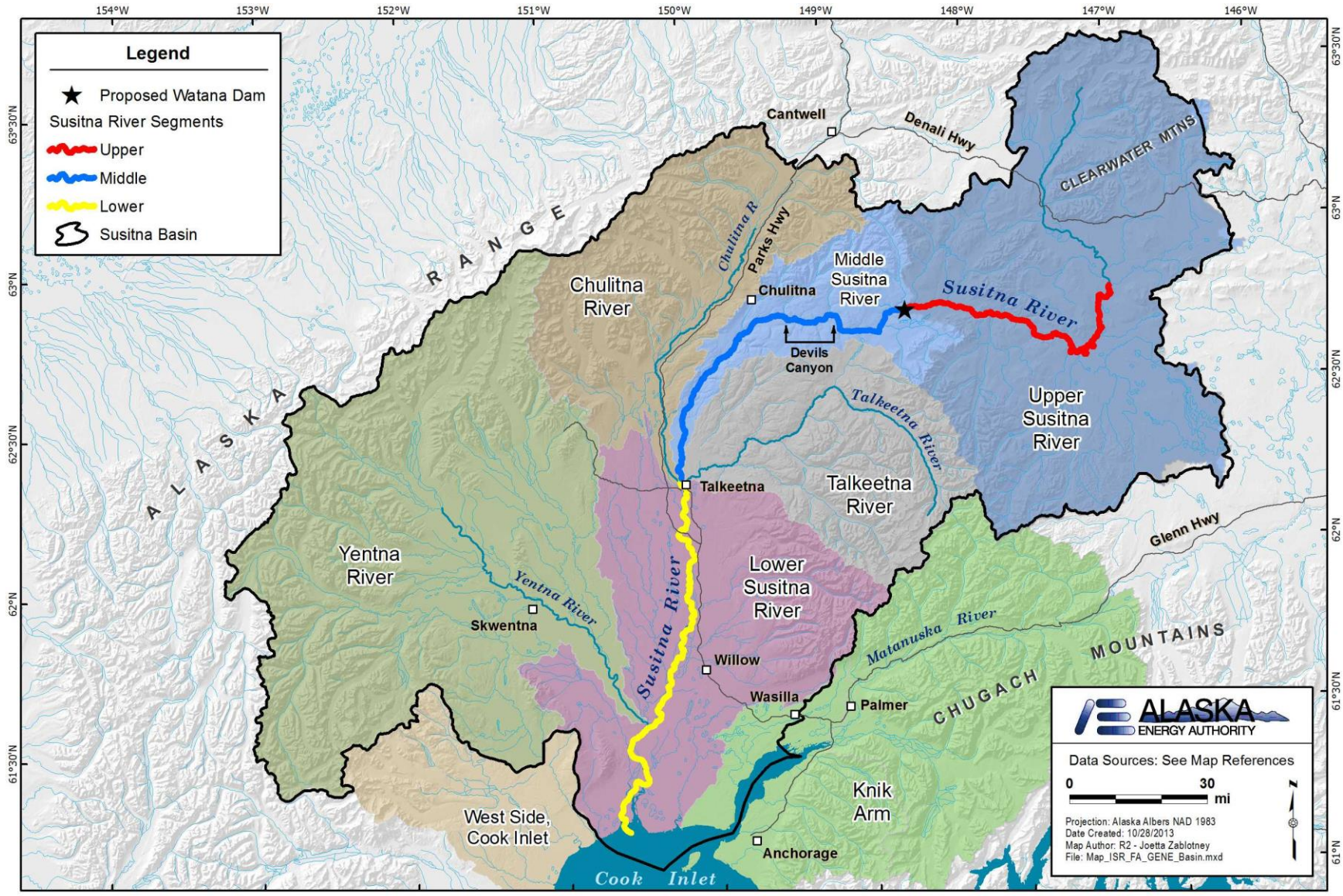

Figure 3-1. Collection strata for samples collected for genetic archive and/or analysis.

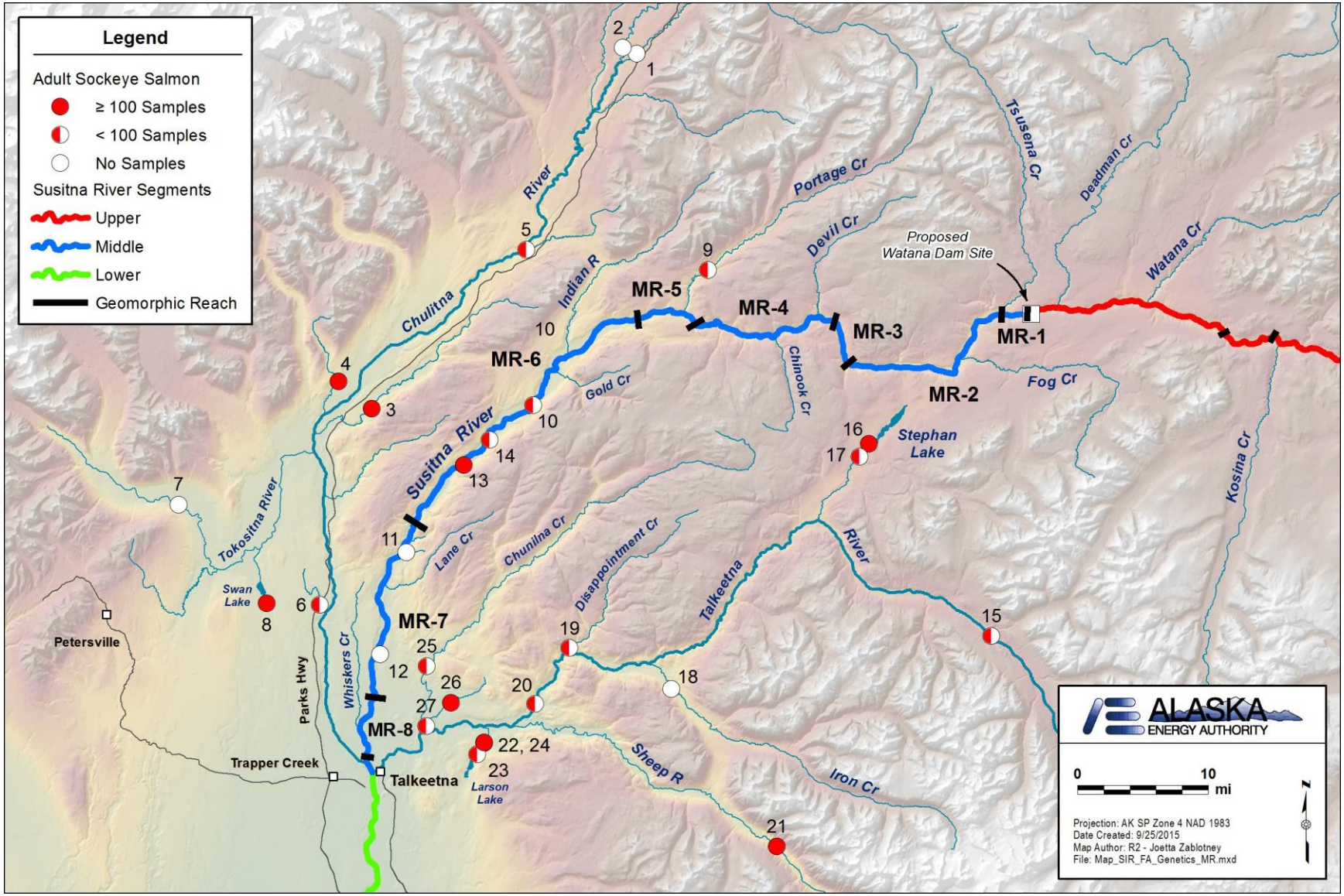

Figure 4-1. Baseline sampling locations for adult Sockeye Salmon sampled through 2014. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 4-3.

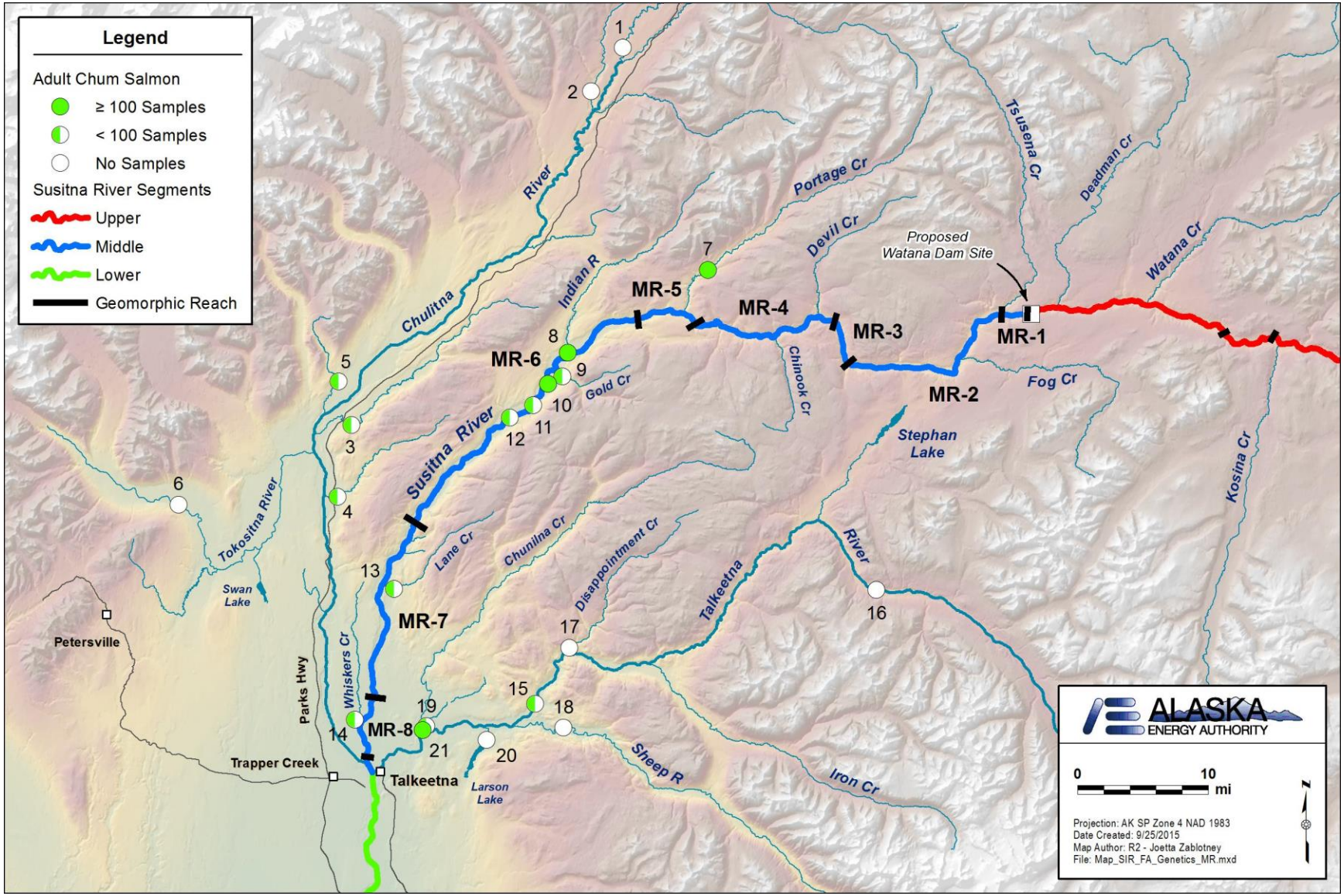

Figure 4-2. Baseline sampling locations for adult Chum Salmon sampled through 2014. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 4-4.

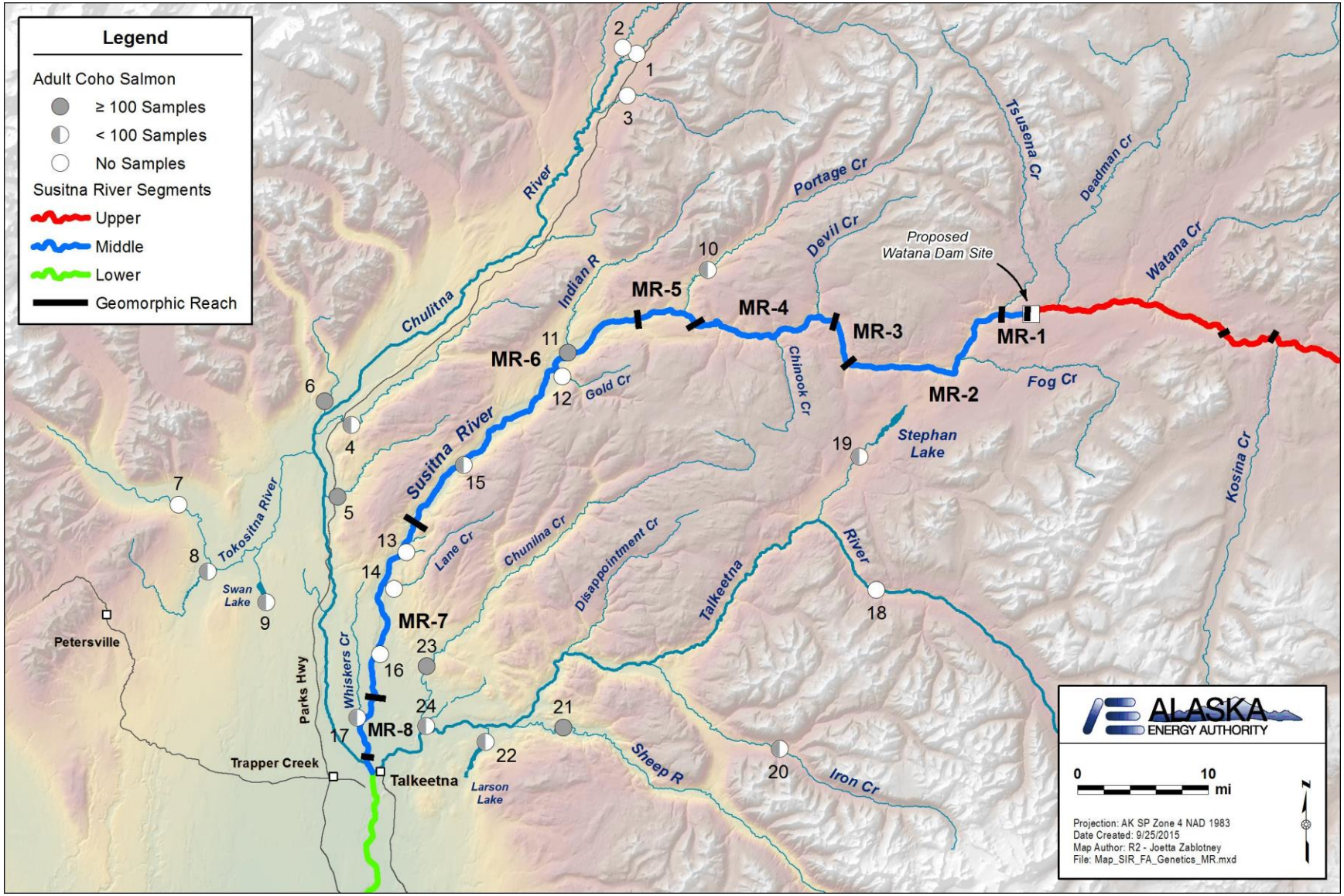

Figure 4-3. Baseline sampling locations for adult Coho Salmon sampled through 2014. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 4-5.

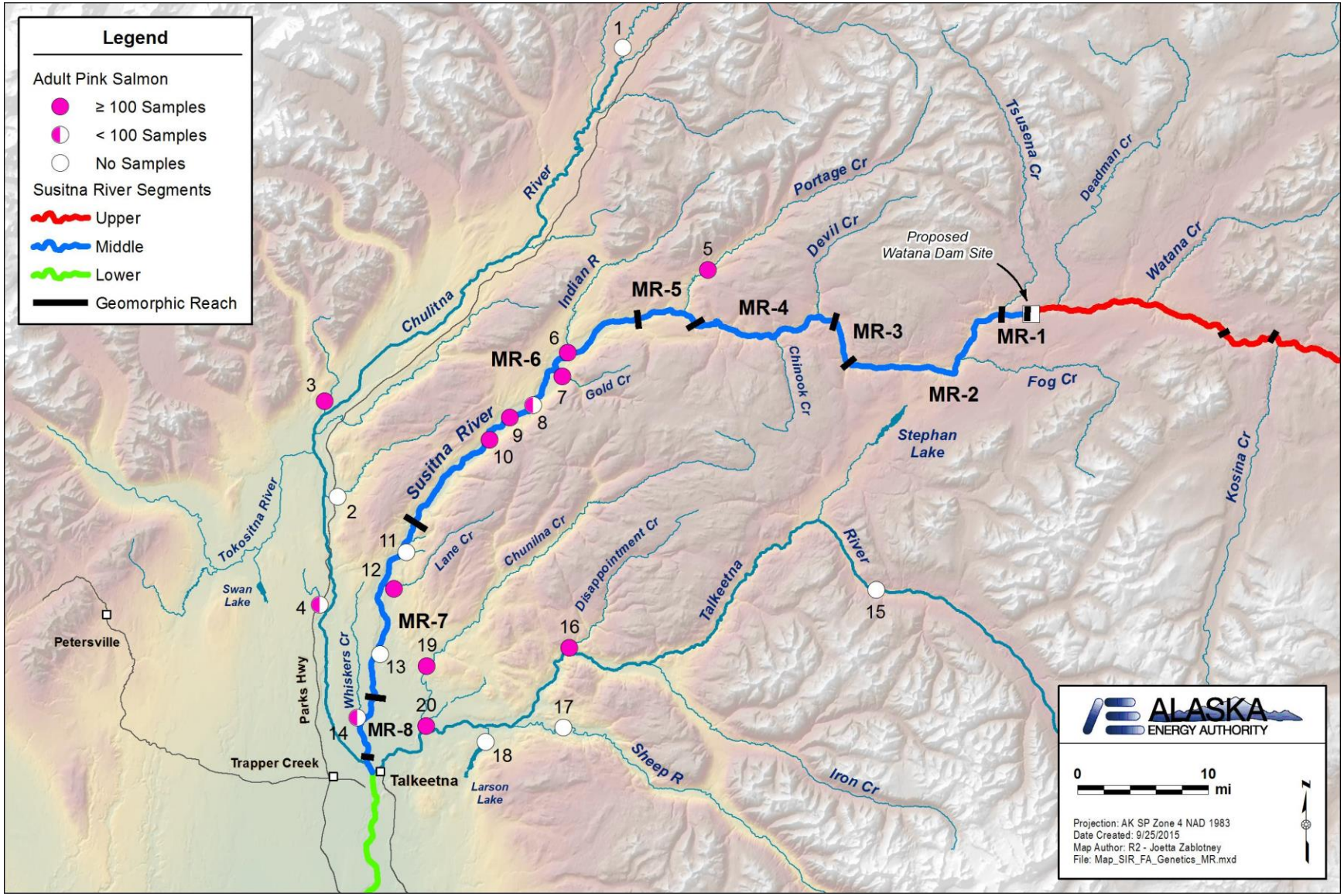

Figure 4-4. Baseline sampling locations for adult Pink Salmon sampled through 2014. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers on Table 4-6.

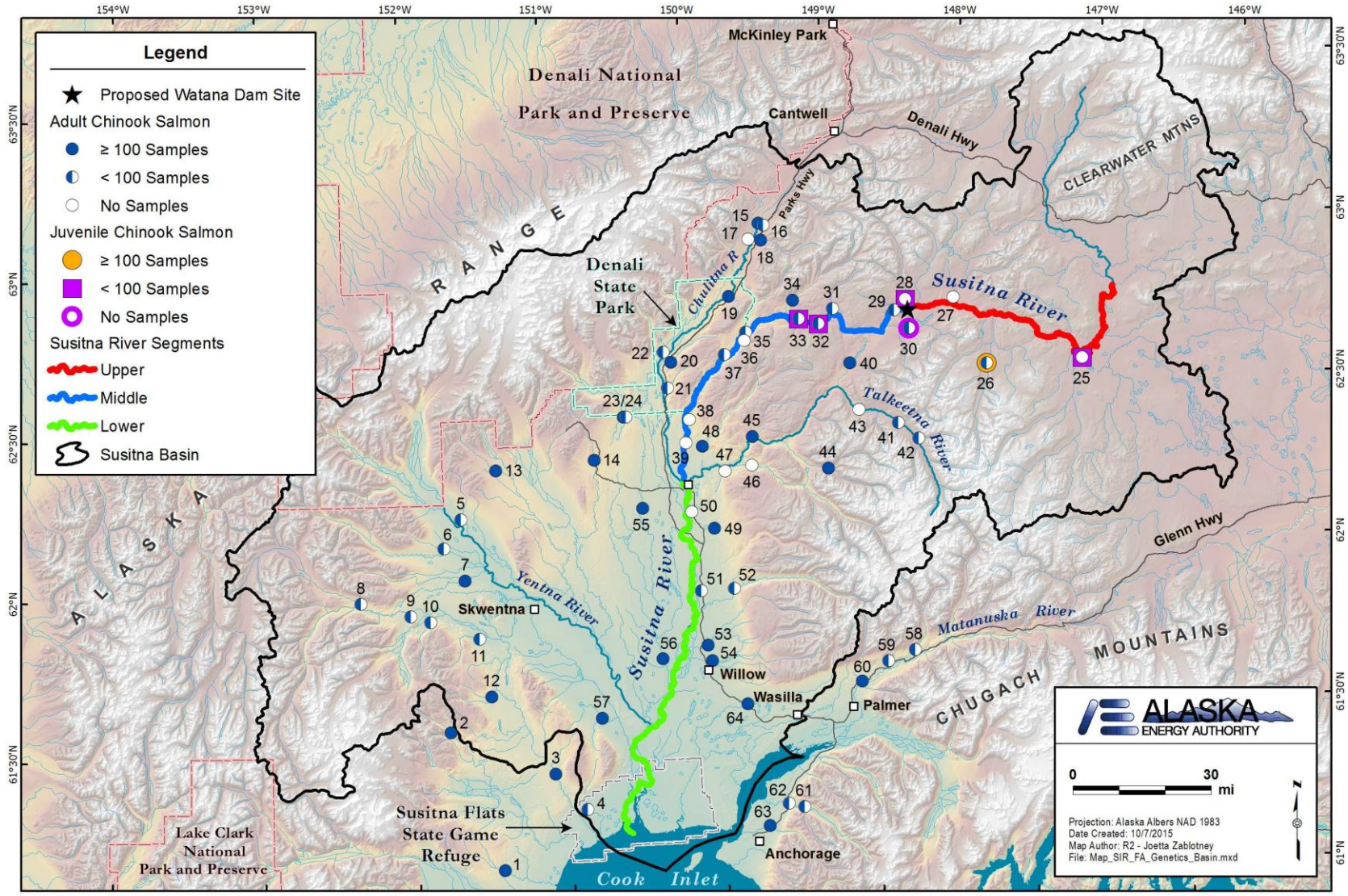

Figure 4-5. Baseline sampling locations for adult and juvenile (inset) Chinook Salmon sampled through 2014. Circles indicate the number of samples in the Gene Conservation Laboratory archives. Numbers correspond to map numbers in Table 4-2. The Lower Susitna River (below project river mile (PRM) 102.4), Middle River (RM 102.4-187.1) and Upper River (RM 187.1-235.1) segments are highlighted with the proposed dam at PRM 187.1.

APPENDIX A: NON-LETHAL JUVENILE FINFISH OMNISWAB SAMPLING FOR DNA ANALYSIS

Non-lethal Juvenile Finfish OmniSwab Sampling for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

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

Figure 1

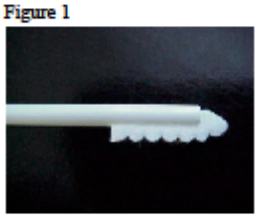

Figure 2

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.

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

Steps for taking mucus samples in 2.0ml vials:

- 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

Return to ADF&G Anchorage lab: ADF&G – Genetics
333 Raspberry Road
Anchorage, AK 99518

Lab staff: 907-267-2247
Judy Berger: 907-267-2175
Freight code: _____