

Operational Plan: Upper Cook Inlet Commercial Eastside Set Gillnet Chinook Salmon Harvest Composition Study

by

Tony Eskelin,

and

Andrew W. Barclay

April 2015

Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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

REGIONAL OPERATIONAL PLAN SF.2A.2014.20

**UPPER COOK INLET COMMERCIAL EASTSIDE SET GILLNET
CHINOOK SALMON HARVEST COMPOSITION STUDY**

by

Tony Eskelin

Alaska Department of Fish and Game, Sport Fish Division, Soldotna
and

Andrew W. Barclay

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

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

April 2015

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/>

*Tony Eskelin,
Alaska Department of Fish and Game, Division of Sport Fish
43961 K-Beach Rd, Ste. B, Soldotna, AK 99669-8276, USA*

and

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

This document should be cited as:

Eskelin, T., and A. W. Barclay. 2014. Upper Cook Inlet commercial eastside set gillnet Chinook salmon harvest composition study. Alaska Department of Fish and Game, Regional Operational Plan ROP.2A.2014.20, Soldotna.

The Alaska Department of Fish and Game (ADF&G) administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act (ADA) of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

If you believe you have been discriminated against in any program, activity, or facility please write:

ADF&G ADA Coordinator, P.O. Box 115526, Juneau, AK 99811-5526

U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, MS 2042, Arlington, VA 22203

Office of Equal Opportunity, U.S. Department of the Interior, 1849 C Street NW MS 5230, Washington DC 20240

The department's ADA Coordinator can be reached via phone at the following numbers:

(VOICE) 907-465-6077, (Statewide Telecommunication Device for the Deaf) 1-800-478-3648,

(Juneau TDD) 907-465-3646, or (FAX) 907-465-6078

For information on alternative formats and questions on this publication, please contact:

ADF&G, Division of Sport Fish, Research and Technical Services, 333 Raspberry Rd, Anchorage AK 99518 (907) 267-2375

SIGNATURE/TITLE PAGE

Project Title: Upper Cook Inlet Commercial Eastside Set Gillnet Chinook Salmon Sampling Study

Project leader(s): Tony Eskelin

Division, Region, and Area Division of Sport Fish, Region II, Soldotna

Project Nomenclature: Chinook Salmon Research Initiative

Period Covered: 2014 Field Season

Field Dates: June 20–August 16, 2014

Plan Type: Category III

Approval

Title	Name	Signature	Date
Project Leader	Tony Eskelin		2/27/14
Co-Project Leader	Andy Barclay		3/6/14
Biometrician	Anton Antonovich		2/27/14
Regional Research Supervisor	Jack Erickson		2/11/15
Fish and Game Coordinator	Ed Jones		2.18.15
Fisheries Scientist	Robert Clark		2/29/15
Fisheries Scientist	Eric Volk		3/17/15

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	ii
LIST OF FIGURES	ii
LIST OF APPENDICES	ii
ABSTRACT	1
PURPOSE.....	1
BACKGROUND	1
OBJECTIVES.....	5
Primary Objectives	5
Secondary Objectives	5
METHODS.....	5
Study Design	5
Geographic and Temporal Stratification.....	6
Data collection.....	8
Age, Sex, and Length Sampling	8
Tissue Sampling for MSA	8
CWT Sampling	8
Laboratory Analysis	9
Assaying Genotypes	9
Laboratory Failure Rates and Quality Control.....	9
Data Reduction	9
Data Analysis.....	10
Baseline and Reporting Groups	10
Mixed Stock Analysis.....	10
Harvest of Chinook Salmon by Reporting Group.....	13
Age and Sex Composition of Chinook Salmon in ESSN Harvest	14
Coded Wire Tag Recoveries	15
BUDGET SUMMARY	16
SCHEDULE AND DELIVERABLES	17
RESPONSIBILITIES	17
REFERENCES CITED	18
APPENDIX A: PRELIMINARY ESSN CHINOOK SALMON SAMPLING SCHEDULE, 2014.....	19
APPENDIX B: ESSN CHINOOK SALMON SAMPLING FORM, 2014	21
APPENDIX C: INSTRUCTIONS FOR TISSUE SAMPLING.....	23
APPENDIX D: CODED WIRE TAG SAMPLING FORM	26

LIST OF TABLES

Table	Page
1 Proportion of Chinook salmon harvested in the ESSN fishery by reporting group, 2010, 2011, and 2013.....	5
2 Proposed strata for ASL and MSA in 2014.....	7
3 Reported Chinook salmon harvest, number and proportion sampled, and number and proportion of harvest for MSA by temporal and geographical stratum in the Upper Cook Inlet, Eastside set gillnet fishery, 2013.....	7
4 Populations of Chinook salmon in the Upper Cook Inlet genetic baseline, including the sampling location, collection years, the number of individuals sampled from each population (<i>n</i>), and the reporting groups used for mixed stock analysis of ESSN harvest.	12

LIST OF FIGURES

Figure	Page
1 Map of Upper Cook Inlet commercial fishing districts and subdistricts.	3
2 Map of Upper Cook Inlet Eastside set gillnet commercial fishing statistical areas.....	4
3 Sampling locations for Chinook salmon populations included in the genetic baseline.....	11

LIST OF APPENDICES

Appendix	Page
A1 Preliminary ESSN Chinook salmon sampling schedule, 2014.....	20
B1 ESSN Chinook salmon sampling form, 2014.....	22
C1 Nonlethal sampling of finfish tissue for DNA analysis.....	24
D1 Coded wire tag sampling form.	27

ABSTRACT

Genetic tissue and age, sex, and length information will be collected from harvested Chinook salmon in the Upper Cook Inlet Eastside set gillnet (ESSN) commercial fishery in 2014. The primary goals of the study are to estimate the proportion of Chinook salmon harvested in the ESSN commercial fishery by reporting group for 5 temporal and geographic strata. Harvest will be apportioned into the following 4 reporting groups: *Kenai River mainstem*, *Kasilof River mainstem*, *Kenai River tributaries*, and *Cook Inlet other*. Age, sex, and length composition will be estimated for each temporal and geographic stratum and for the entire 2014 season.

Keywords: Chinook salmon *Oncorhynchus tshawytscha*, Kenai River, Eastside set gillnet, commercial fishery, ESSN, Upper Cook Inlet, MSA, stock-specific harvest, Chinook Salmon Research Initiative, CSRI.

PURPOSE

Cook Inlet's Kenai River Chinook salmon stock is 1 of 12 stocks chosen statewide as indicators by the Chinook Salmon Research Initiative (CSRI). Lack of stock-specific harvest information such as age, sex, and length (ASL) composition from commercial harvests in Cook Inlet has been identified by CSRI as an information gap for this stock because these data are needed to more accurately represent harvest rates and production trends. To obtain stock-specific information, genetic samples are needed to estimate the relative contribution that genetic reporting groups such as *Kenai River mainstem* or *Kenai River tributaries* make to the ESSN commercial harvest during various times and places. This project will collect and analyze ASL data and genetic tissue samples of Chinook salmon harvested in the Upper Cook Inlet (UCI) Eastside set gillnet (ESSN) commercial fishery. The Alaska Department of Fish and Game (ADF&G) Division of Sport Fish (SF) is responsible for the collection of genetic tissue samples and ASL data. Tissue samples will be sent to the Division of Commercial Fisheries (CF) Gene Conservation Lab (GCL), which will be responsible for mixed stock analysis (MSA).

BACKGROUND

All 5 species of Pacific salmon are harvested in UCI. Sockeye salmon (*Oncorhynchus nerka*) make up the majority of the harvest (Shields and Dupuis 2013) but Chinook salmon (*O. tshawytscha*) are also harvested. Recent low Chinook salmon runs in UCI have heightened interest in stock-specific harvest of Chinook salmon in these fisheries. A Chinook salmon genetic baseline that includes representative populations in UCI is available for MSA applications in fisheries using genetic stock identification (GSI) techniques (Barclay et al. 2012). Obtaining information about stock-specific harvest of Chinook salmon is needed to improve understanding of stock productivity, brood table development, and for setting and attaining escapement goals.

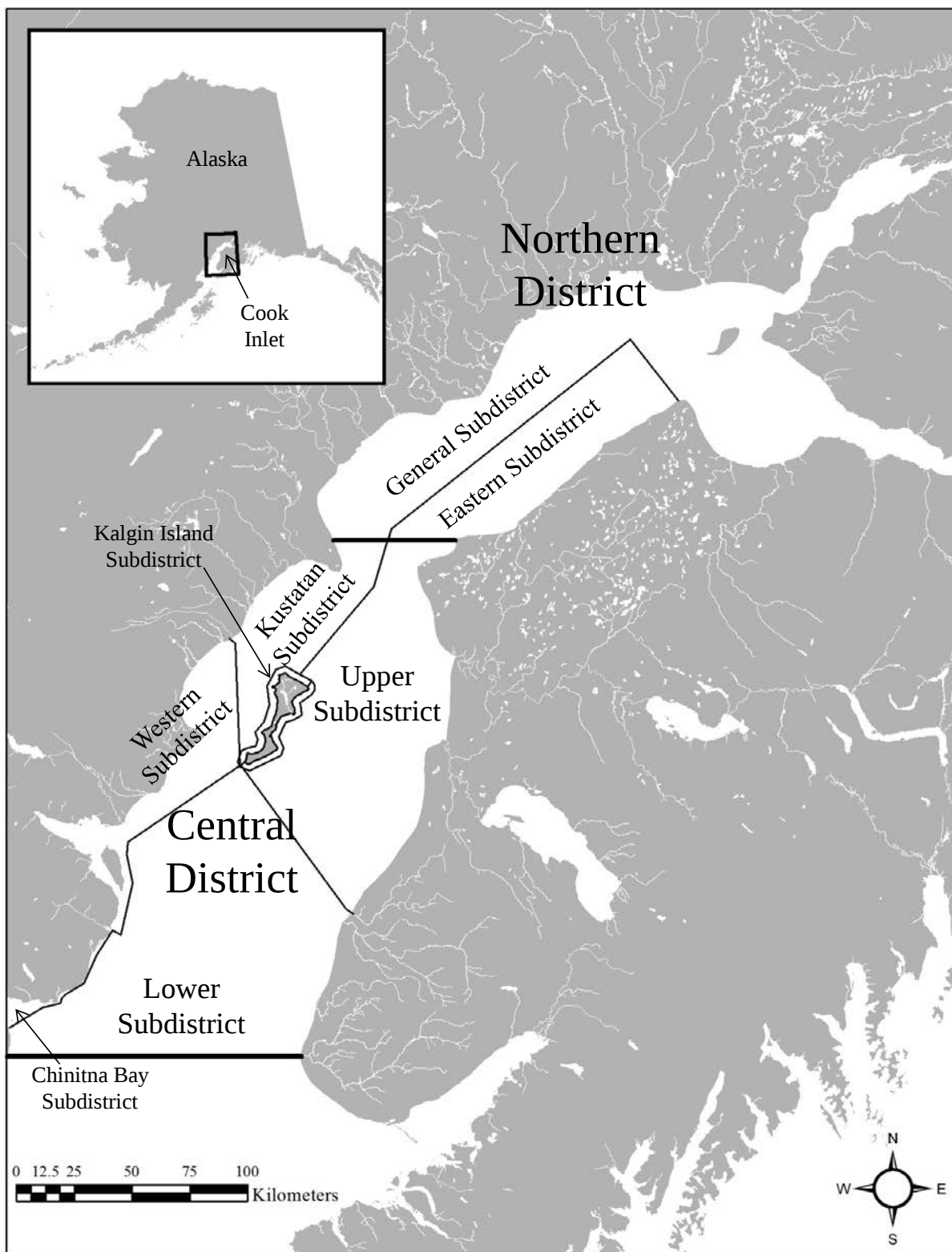
Most of the UCI Chinook salmon harvest occurs in the Upper Subdistrict set gillnet fishery of the Central District. This fishery is commonly referred to as the Eastside set gillnet (ESSN) fishery and is located along the eastern shore of Cook Inlet between Ninilchik and Boulder Point (Figure 1). Since 1966, annually on average, the ESSN fishery has accounted for 65% of all Chinook salmon harvested in UCI commercial fisheries (Eskelin et al. 2013). The ESSN fishery is composed of 3 sections (Kasilof, Kenai, and East Forelands) and 8 statistical areas (Ninilchik Beach, Cohoe Beach, Kasilof River Special Harvest area [KRSHA], South K-Beach, North K-Beach, Salamatof Beach, and East Forelands (Figure 2). The most recent 10-year (2003–2012) average ESSN Chinook salmon harvest is 9,714 fish; however, harvest has declined recently and the recent 5-year average (2009–2013) Chinook salmon harvest is 4,807 fish (Eskelin et al. 2013). The 2 lowest documented harvests of Chinook salmon in the ESSN fishery were in the past 2 years: 704 fish in 2012 and 2,988 fish in 2013. In 2013, the ESSN fishery was opened for

12 fishing periods in the Kasilof section and for 6 fishing periods in the Kenai and East Forelands sections. In addition, the KRSHA was opened during 14 days. Low Chinook salmon runs and subsequent reduced fishing time has reduced Chinook salmon harvest substantially.

The ESSN Chinook salmon harvest has been sampled by ADF&G for ASL information since 1986. In many of those years, 1 technician sampled harvested Chinook salmon at receiving sites of fish processors on regular period openings and opportunistically collected ASL samples. Beginning in 2010, genetic tissue samples were added to the collection effort. With only 1 technician assigned to collect samples, it was difficult to representatively sample all areas of the ESSN fishery during each tide or fishing period. Some areas were targeted for sampling because they were expected to have larger Chinook salmon harvests, while other areas with lower harvest were not sampled as effectively due to time constraints and because samples could not be obtained at receiving sites prior to fish being mixed from all areas at fish processing plants. The sampling effort was increased in 2013 with funding from the Chinook Salmon Research Initiative to provide temporally and geographically stratified estimates and more precise estimates by reporting group.

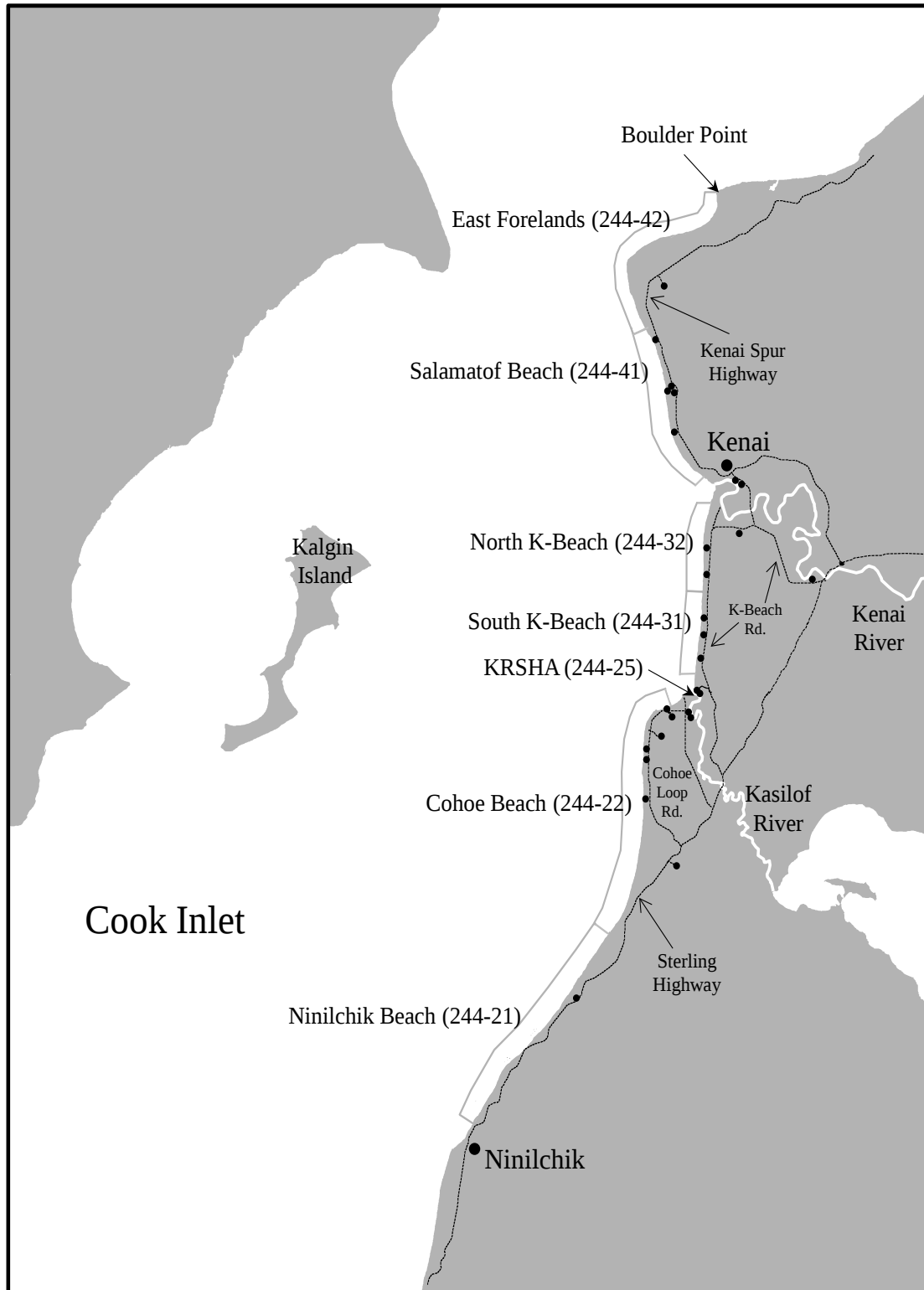
A sufficient number of representative samples were collected in 2010, 2011, and 2013 to allow for MSA, but not in 2012. For the 2010, 2011, and 2013 collections, tissues were generally subsampled postseason in proportion to the harvest within 1 or 2 fishing periods and statistical areas. The final numbers collected and successfully analyzed by GCL were 373, 343, and 708 samples in 2010, 2011, and 2013, respectively. Sample size was insufficient for temporal or geographic stratification in 2010 and 2011, so only seasonal estimates were produced. The increased sampling effort and subsequent higher sampling rate in 2013 allowed for temporal and geographic stratification for that year.

The Kenai River supports 2 genetically divergent population aggregates of Chinook salmon (Barclay et al. 2012). Those that spawn in Kenai river tributaries enter the river prior to those that spawn in the mainstem Kenai River, although there is some overlap from late-June to early July (Reimer 2013). The ESSN fishery primarily harvests Chinook salmon bound for the Kenai and Kasilof rivers. Reporting groups were defined based on 1 or more of the following criteria: 1) the genetic similarity among populations, 2) the expectation that proportional harvest would be greater than 5%, or 3) the applicability to answer fishery management questions. Reporting groups used in the MSA for each year were as follows: “*Kenai River mainstem*,” “*Kasilof River mainstem*,” “*Kenai River tributaries*,” and “*Cook Inlet other*”. The composition estimates by reporting group were similar from year to year (Table 1), with the *Kenai River mainstem* reporting group having the greatest average proportional contribution (0.692), followed by *Kasilof River mainstem* (0.290), *Cook Inlet other* (0.014), and lastly, the *Kenai River tributaries* reporting group (0.004). This project in 2014 will be similar to the 2013 sampling project which involved expanded sampling of the Chinook salmon harvest for ASL composition and genetic tissue. A total of 3 technicians will be assigned to sample the ESSN Chinook salmon harvest, which will provide coverage of the fishery during every regular period opening and also allow for sampling of some fishing periods that may be opened by Emergency Order (EO).



Note: thick lines indicate district borders and thin lines indicate subdistrict borders.

Figure 1.—Map of Upper Cook Inlet commercial fishing districts and subdistricts.



Note: Small circles represent approximate locations of processing plants or receiving sites in 2013.
K-RSHA (244-25) is Kasilof River Special Harvest Area.

Figure 2.—Map of Upper Cook Inlet Eastside set gillnet commercial fishing statistical areas.

Table 1.–Proportion of Chinook salmon harvested in the ESSN fishery by reporting group, 2010, 2011, and 2013.

Reporting group	2010		2011		2013		Average
	Proportion	SD	Proportion	SD	Proportion	SD	
Kenai River tributaries	0.011	0.010	0.001	0.004	0.001	0.004	0.004
Kenai River mainstem	0.643	0.037	0.667	0.040	0.766	0.023	0.692
Kasilof River mainstem	0.326	0.034	0.330	0.040	0.213	0.022	0.290
Cook Inlet other	0.020	0.014	0.002	0.004	0.019	0.006	0.014

OBJECTIVES

PRIMARY OBJECTIVES

- 1) Estimate the proportion of Chinook salmon harvested in the UCI ESSN commercial fishery by reporting group (*Kenai River mainstem*, *Kasilof River mainstem*, *Kenai River tributaries*, and *Cook Inlet other*) for each temporal and geographic stratum for the season such that the estimated proportions are within 13 percentage points of the true values 90% of the time.
- 2) Estimate the harvest of *Kenai River mainstem* and *Kasilof River mainstem* Chinook salmon in the UCI ESSN commercial fishery for each temporal and geographic stratum such that the estimates are within 30% of the true value, 90% of the time.
- 3) Estimate the age composition of the Chinook salmon harvested by the ESSN fishery such that the estimates are within 10 percentage points of the true values 95% of the time.

SECONDARY OBJECTIVES

- 1) Estimate the harvest of Chinook salmon for the reporting groups *Kenai River tributaries* and *Cook Inlet other* in the UCI ESSN commercial fishery for each temporal and geographic stratum¹.
- 2) Sample 35% of the Chinook salmon harvested in the UCI ESSN commercial fishery for tissue, coded wire tags, scales, sex, and mid eye to tail fork (METF) length.
- 3) Estimate the sex and length compositions of Chinook salmon harvested in the UCI ESSN commercial fishery overall and for each temporal and geographic stratum.

METHODS

STUDY DESIGN

Regular period openings in the ESSN fishery are from 7:00 AM to 7:00 PM on Mondays and Thursdays. The first scheduled regular period in the Kasilof section (statistical areas 244-21, 244-22, and 244-31) for the 2014 season is Thursday, 26 June. The fishery could be opened as early as 20 June dependent on cumulative sockeye salmon passage in the Kasilof River. The first

¹ Based on MSA results from 2010, 2011, and 2013, it is anticipated that Chinook salmon harvest of the reporting groups *Kenai River tributaries* and *Cook Inlet other* will be low (<150 fish) so no precision criteria are set for estimation of these reporting groups. Sample size is driven by Primary Objectives 1 and 2.

Kasilof section regular period or EO opening and subsequent openings will be sampled. The first scheduled regular period in the Kenai and East Forelands sections (statistical areas 244-32, 244-41, and 244-42) for the 2014 season is Thursday, 10 July. All regular fishing periods and up to 2 additional fishing periods opened by EO per week are budgeted to be sampled. If it is foreseen that there will be more than 2 openings by EO in a given week, harvest rates and insight from commercial fishery managers based on likely scenarios of future openings will be used to choose which openings to sample. The fishery is scheduled to end 15 August, with only regular fishing periods allowed after 10 August. The regular period sampling schedule is in Appendix A1.

As many Chinook salmon as possible will be sampled while distributing sampling effort to allow for collection of a representative sample of the harvest. Each technician will be assigned 1 of 3 areas to sample: 1) receiving sites for Ninilchik/Cohoe beaches, 2) receiving sites for K-Beach (north and south), and 3) receiving sites for Salamatof Beach and East Forelands (Figure 1). There will likely be overlap in sampling areas among technicians and modifications to assigned areas may occur during the season. Inseason analyses of the proportion of the Chinook salmon harvest sampled by beach will be conducted and modifications will be made to the sampling strategy as necessary. Technicians will begin sampling on the southern end of their sampling area after the first round of deliveries to each buying station. Sampling at the southern end first and moving northward will follow the fish deliveries as they occur to maximize the number of samples collected. This sampling strategy should not introduce bias. If technicians started at the northern beaches and moved south, they would miss samples and have a lower sampling rate due to the later timing of northern deliveries. There will likely be over 20 receiving sites where Chinook salmon are delivered that are spread throughout each statistical area. Technicians will sample during each opening at receiving sites until the fish are transported to processing plants. The day following each fishing period, additional Chinook salmon samples will be collected at a fish processing plant paying premium prices, where fish from all areas are delivered the day following the fishery. A technician will be stationed at that plant to get samples as they are delivered so that location of harvest by statistical area can be determined. KRSHA openings will be sampled if opened by EO.

Because the number and location of receiving sites changes each year, prior to the 2014 season, the project biologist will develop a list with contact information and a map showing locations to sample, which will be distributed to each technician. Technicians will be instructed to sample at receiving sites on their way north up the beach. There will be no set schedule for times to sample at each location. Schedules will depend on tides and the times of fishing periods.

GEOGRAPHIC AND TEMPORAL STRATIFICATION

Proposed temporal and geographic stratification was determined by management criteria and MSA results from 2010, 2011, and 2013 tissue samples. Depending on how the fishery is prosecuted and how many samples are collected in 2014, chosen temporal and geographic strata could change, but for planning purposes, 5 strata were chosen (Table 2).

Table 2.—Proposed strata for ASL and MSA in 2014.

Stratum number	Temporal stratum	Geographic area
1	26 June–9 July	Kasilof Section
2	10–31 July	Kasilof Section
3	10–31 July	Kenai and East Forelands Sections
4	1–15 August	All Sections
5	2014 season	KRSHA

In Stratum 4, the geographic area is “All sections” because sample size in that stratum is likely to be low. There may be more than 5 strata chosen if Chinook salmon harvest and the number of samples collected are sufficient to produce statistically valid results.

Sampling results and chosen temporal and geographic strata from 2013 were used to determine expected harvests and also expected sampling and selection rates for 2014 (Table 3). Samples must be collected to represent the harvest, which is seldom possible, so subsampling of collections is required postseason to ensure equivalent representation of the harvest. In 2013, technicians were able to collect 1,043 tissue samples representing 35% of the harvest. After subsampling representatively when possible, 708 samples (24% of the harvest) were used in MSA. The goal for 2014 will be to sample and select the same proportions that were sampled in 2013: tissue will be collected from 35% of the harvest and samples will be selected from 24% of the harvest for MSA after subsampling representatively by date and statistical area. Length will be incorporated into the subsample selection such that the length distribution of subsampled fish will be equivalent to the length distribution of fish not selected for subsampling, within each grouping. A grouping will usually be 1–2 days of samples within each geographic stratum.

Table 3.—Reported Chinook salmon harvest, number and proportion sampled, and number and proportion of harvest for MSA by temporal and geographical stratum in the Upper Cook Inlet, Eastside set gillnet fishery, 2013.

Dates	Geographic area	Reported harvest	Number sampled	Proportion sampled	Number selected for MSA	Proportion of harvest selected for MSA
27 June–6 July	Kasilof Section	404	162	0.40	162	0.40
8–23 July	Kasilof Section	871	298	0.34	195	0.22
8–23 July	Kenai/East Forelands	1,355	525	0.39	293	0.22
17 July–2 August	KRSHA	358	58	0.16	58	0.16
27 June–2 August	All areas	2,988	1,043	0.35	708	0.24

Proof tests conducted by the GCL demonstrated that with a fishery mixture of 100 samples, we can estimate stock composition for the 4 reporting groups (*Kenai tributary*, *Kenai mainstem*, *Kasilof mainstem*, and *Cook Inlet other*) within 0.13 of the true values 90% of the time (Barclay et al. *In prep*²). These tests followed the same protocol as reported in Eskelin et al. (2013) for baseline evaluation tests; however, instead of using test mixtures with 100% of one reporting

²Barclay, A. W., C. Habicht, T. McKinley, and R. J. Yanusz. *In prep*. Chinook salmon genetic baseline for Mixed Stock Analysis in Upper Cook Inlet. Alaska Department of Fish and Game, Fishery Data Series, Anchorage.

group, test mixtures were created with proportions from each reporting group that represented a realistic scenario for what might be expected in these fisheries. Given that there are 3 years of reporting group proportion estimates for the ESSN Chinook salmon harvest, tests were conducted under a realistic scenario for expected reporting group proportions in ESSN fishery mixtures: 0.02 for *Kenai River tributaries*, 0.58 for *Kenai River mainstem*, 0.38 for *Kasilof River mainstem*, and 0.02 for *Cook Inlet other*.

With this precision of stock composition estimates and an anticipated sample size of 100 fish, we will be able to estimate the harvest of *Kenai River mainstem* and *Kasilof River mainstem* Chinook salmon in the UCI ESSN commercial fishery in each stratum within 30% of the true values 90% of the time.

The objective criterion (± 0.10 with 95% confidence level) for estimating the age composition of Chinook salmon harvested in the ESSN fishery should be achieved with approximately 170 scale samples. To arrive at this sample size we assumed a worst-case scenario of 25% scale regeneration rate with multinomial proportions of equality among ages (Thompson 1987). Because we plan to collect substantially more samples in 2014, we are likely to achieve higher precision for the age composition estimates.

DATA COLLECTION

Age, Sex, and Length Sampling

Three scales will be removed from the preferred area of each fish and placed on an adhesive-coated card (Clutter and Whitesel 1956; Welander 1940). Acetate impressions will be made of the scales on the card using a press under 25,000 pounds per square inch (PSI) and the scale growth patterns will be viewed with a 40 $\times$ power microfiche reader to determine freshwater and marine residence times. Sex will be identified from external morphometric characteristics (i.e., protruding ovipositor on females or a developing kype on males). METF length will be measured to the nearest half-centimeter. Chinook salmon will be sampled for ASL composition without regard to size, sex, length, or location. ASL composition data will be recorded on data sheets (Appendix B1).

Tissue Sampling for MSA

All fish sampled for ASL will also be sampled for tissue suitable for genetic analysis. A 1½ cm (half-inch) piece of the axillary process will be removed from each fish and placed in a 2 ml plastic vial filled until the tissue samples are completely submerged with a Sigma³ reagent grade 95% alcohol buffer solution such that the liquid-to-tissue ratio is approximately 3:1. Sampling instructions are found in Appendix C1. Each plastic vial will be sequentially numbered and vial numbers recorded on data sheets (Appendix B1.). All vials will be stored at the Soldotna office until the end of the season then sent to the GCL for analysis.

CWT Sampling

All sampled Chinook salmon will be examined for an adipose finclip. Technicians will remove the head of all adipose finclipped Chinook salmon encountered. A cinch strap will be attached to the head, which will be returned to the office for storage in a freezer. All data, including the number of Chinook salmon examined and the number observed missing the adipose fin, will be

³ Product names used in this publication are included for completeness but do not constitute product endorsement.

recorded on a tag recovery form (Appendix D1). The cinch strap number will also be recorded alongside ASL data (Appendix B1) to enable cross-referencing between datasets. Collected data will be returned to the Project Leader (Anthony Eskelin). CWT forms and heads of all adipose finclipped fish will be shipped at the end of the season to the ADF&G Mark, Tag, and Age Laboratory for CWT recovery, determination of stock of origin, and for archiving data.

LABORATORY ANALYSIS

Assaying Genotypes

DNA extraction and genotyping will generally follow the methods described in detail in Barclay et al. (2012). Briefly, genomic DNA will be extracted from tissue samples using a DNeasy 96 Tissue Kit by QIAGEN (Valencia, CA). Fluidigm 192.24 Dynamic Arrays (<http://www.fluidigm.com>) will be used to screen 40 SNP markers; this differs from the methods of Barclay et al. (2012) where 96.96 Dynamic Arrays were used. The Dynamic Arrays will be read on a Fluidigm EP1 System or BioMark System after amplification and scored using Fluidigm SNP Genotyping Analysis software. Assays that fail to amplify on the Fluidigm system will be reanalyzed on the Applied Biosystems platform. The plates will be scanned on an Applied Biosystems Prism 7900HT Sequence Detection System after amplification and scored using Applied Biosystems' Sequence Detection Software version 2.2.

Genotypes produced on both platforms will be imported and archived in the Gene Conservation Laboratory (GCL) Oracle database, LOKI.

Laboratory Failure Rates and Quality Control

Overall failure rate will be calculated by dividing the number of failed single-locus genotypes by the number of assayed single-locus genotypes. An individual genotype will be considered a failure when a locus for a fish cannot be satisfactorily scored.

Quality control (QC) measures will be used to identify laboratory errors and to determine the reproducibility of genotypes. In this process, 8 of every 96 fish (1 row per 96-well plate) will be re-extracted and reanalyzed for all markers by staff not involved with the original analysis. Laboratory errors found during the QC process will be corrected, and genotypes will be corrected in the database. Inconsistencies not attributable to laboratory error will be recorded, but original genotype scores will be retained in the database.

DATA REDUCTION

Technicians will return their genetic vial boxes, scale cards, and field data to the Soldotna office daily and will be responsible for ensuring the recorded data are legible and accurate. The project biologist will ensure all data are returned, legible, and entered correctly. All data will be keypunched directly into a master electronic data file. Age data will be entered upon scale reading. CWT forms will be edited to ensure accuracy and mailed to Juneau ADF&G for data entry. A final edited copy of all data files along with a data map will be sent to ADF&G Research and Technical Services (RTS) for archiving.

DATA ANALYSIS

Baseline and Reporting Groups

The current UCI Chinook salmon genetic baseline used for MSA applications is an update of the baseline reported in Barclay et al. (2012) and includes 62 additional collections and 25 new populations (Barclay et al. *In prep*⁴; Table 4). The updated baseline includes the same set of SNP markers except that locus *Ots_FGF6B* was excluded because of its association with locus *Ots_FGF6A*.

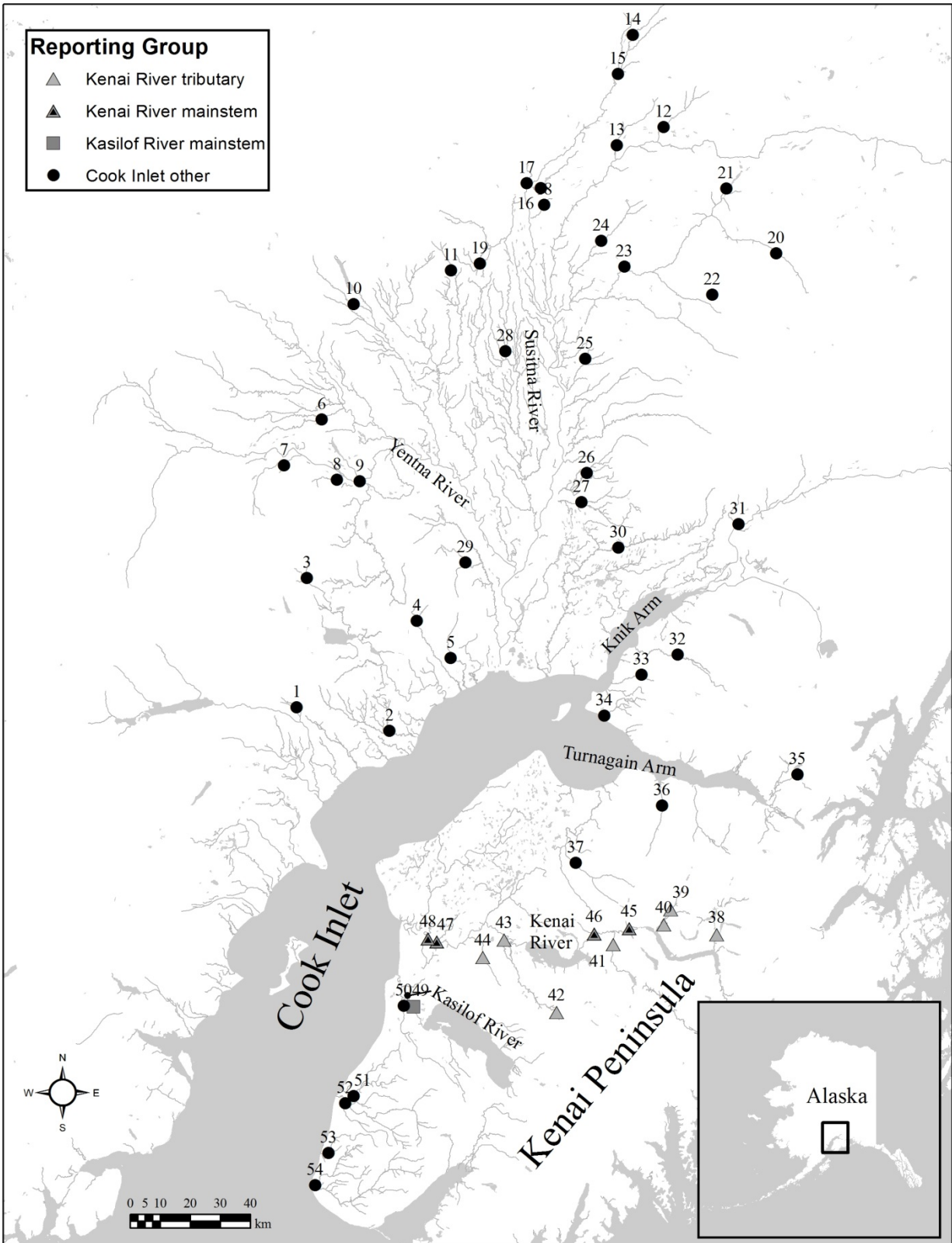
Reporting groups are defined based on 1 or more of the following criteria: 1) the genetic similarity among populations, 2) the expectation that proportional harvest would be greater than 5%, or 3) the applicability to answer fishery management questions. Based on these criteria, reporting groups chosen to apportion the harvest for this study are as follows: “*Kenai River mainstem*”, “*Kenai River tributaries*,” “*Kasilof River mainstem*,” and “*Cook Inlet other*.” The *Cook Inlet other* reporting group represents all remaining Cook Inlet Chinook salmon baseline populations not included in the 3 other reporting groups (Table 4 and Figure 3)

To minimize misallocation between MSA reporting groups, the Slikok Creek (a Kenai River tributary) population was removed from the baseline because it is very small and is genetically similar to the Crooked Creek (a Kasilof River tributary) population (Barclay et al. 2012). In addition, Juneau Creek, a Kenai River tributary, was grouped with the *Kenai River mainstem* reporting group due to genetic similarity (Barclay et al. 2012).

MIXED STOCK ANALYSIS

The stock composition of the commercial ESSN fishery harvest for each stratum will be estimated using the software package BAYES (Pella and Masuda 2001). BAYES employs a Bayesian algorithm to estimate the most probable contribution of the baseline populations to explain the combination of genotypes in the mixture sample. The final analysis will consist of the results from 5 separate Monte Carlo Markov chains where each chain will begin with different initial values. A random number generator will be used to create the initial values, which will sum to 1 over all reporting groups. The Dirichlet prior distribution for the composition parameters in BAYES will be based upon the best available information for each mixture analysis. We believe the best available information for the prior to be the results of MSA of similar mixtures. For the 2014 ESSN mixtures, the best available information will be the stock proportions estimates from the analysis of the 2013 ESSN Chinook salmon samples. The sum of the Dirichlet prior parameters will equal 1, thus minimizing the overall influence of the prior distribution. The chains will be run until convergence is reached (shrink factor < 1.2) for the 5 chains (Pella and Masuda 2001). The first half of each chain will be discarded in order to remove the influence of the initial values; the rest will be used to estimate the posterior distribution of stock composition proportions. The point estimates of stock composition and the variance of these estimates will be calculated from the mean and standard deviation of the posterior distributions.

⁴ Barclay, A. W., C. Habicht, T. McKinley, and R. J. Yanusz. *In prep*. Chinook salmon genetic baseline for Mixed Stock Analysis in Upper Cook Inlet. Alaska Department of Fish and Game, Fishery Data Series, Anchorage.



Note: numbers correspond to map numbers on Table 4.

Figure 3.—Sampling locations for Chinook salmon populations included in the genetic baseline.

Table 4.–Populations of Chinook salmon in the Upper Cook Inlet genetic baseline, including the sampling location, collection years, the number of individuals sampled from each population (*n*), and the reporting groups used for mixed stock analysis of ESSN harvest.

Map no.	Reporting group	Location	Added after baseline ^a	Collection year(s)	<i>n</i>
1	Cook Inlet other	Straight Creek		2010	95
2		Chuitna River		2008, 2009	134
3		Coal Creek		2009, 2010, 2011	118
4		Theodore River	X	2010, 2011, 2012	190
5		Lewis River	X	2011, 2012	87
6		Red Creek	X	2012, 2013	111
7		Hayes River	X	2012, 2013	50
8		Canyon Creek	X	2012, 2013	91
9		Talachulitna River		1995, 2008, 2010	178
10		Sunflower Creek		2009, 2011	123
11		Peters Creek	X	2009, 2010, 2011, 2012	107
12		Portage Creek	X	2009, 2010, 2011, 2013	162
13		Indian River	X	2013	79
14		Middle Fork Chulitna River	X	2009, 2010	169
15		East Fork Chulitna River	X	2009, 2010, 2011, 2013	77
16		Byers Creek	X	2013	55
17		Spink Creek	X	2013	56
18		Troublesome Creek	X	2013	71
19		Bunco Creek	X	2013	98
20		no name creek	X	2013	69
21		Prairie Creek		1995, 2008	161
22		East Fork Iron Creek	X	2013	57
23		Disappointment Creek	X	2013	64
24		Chunilna Creek		2009, 2012	123
25		Montana Creek		2008, 2009, 2010	213
26		Little Willow Creek	X	2013	54
27		Willow Creek		2005, 2009	170
28		Deshka River		1995, 2005, 2012	303
29		Sucker Creek	X	2011, 2012	143
30		Little Susitna River		2009, 2010	125

Table 4.–Part 2 of 2.

Map no.	Reporting group	Location	Added after baseline ^a	Collection year(s)	<i>n</i>
31	Kenai R. tributary	Moose Creek		1995, 2008, 2009, 2012	149
32		Eagle River	X	2009, 2011, 2012	71
33		Ship Creek		2009	261
34		Campbell Creek	X	2010, 2011, 2012	110
35		Carmen River	X	2011, 2012	50
36		Resurrection Creek	X	2010, 2011, 2012	98
37		Chickaloon River		2008, 2010, 2011	128
38		Grant Creek	X	2011, 2012	55
39		Quartz Creek		2006, 2007, 2008, 2009, 2010, 2011	131
40		Crescent Creek		2006	164
41	Kenai R. mainstem	Russian River		2005, 2006, 2007, 2008	214
42		Benjamin Creek		2005, 2006	204
43		Killey River		2005, 2006	255
44		Funny River		2005, 2006	219
45		Juneau Creek		2005, 2006, 2007	140
46		Upper Kenai R. mainstem		2009	191
47		Middle Kenai R. mainstem		2003, 2004, 2006	299
48		Lower Kenai R. mainstem	X	2010, 2011	118
49		Kasilof River mainstem		2005	321
50		Crooked Creek		2005, 2011	306
51	Cook Inlet other	Ninilchik River Weir		2006, 2010	209
52		Deep Creek		2009, 2010	196
53		Stariski Creek	X	2011	104

Note: map numbers correspond to sampling sites on Figure 3.

^a “X” indicates populations that have been added since the Barclay et al. (2012) baseline.

HARVEST OF CHINOOK SALMON BY REPORTING GROUP

The number of Chinook salmon from reporting group g ($\hat{H}^g$) harvested in the commercial ESSN fishery between the first opening as early as late June and the last opening on or before 15 August will be estimated as follows:

$$\hat{H}^g = \sum_{i=1}^T \sum_{j=1}^S H_{i,j} \hat{p}_{i,j}^g \quad (1)$$

where

$\hat{p}_{i,j}^g$ = estimated proportion of ESSN harvest in time stratum i and geographic stratum j comprising Chinook salmon from reporting group g (*Kenai River mainstem, Kasilof River mainstem, Kenai River tributaries, or Cook Inlet other*) based on Bayesian mixed stock analysis as described in the previous section,

$H_{i,j}$ = ESSN Chinook salmon harvest in time stratum i and area stratum j obtained from fish ticket data,

T = number of time strata (prior to 10 July, 10–31 July, and after 31 July), and

S = number of geographic strata (Kenai/East Forelands and Kasilof sections).

$\text{var}(\hat{H}^g)$ will be estimated as follows:

$$\text{var}(\hat{H}^g) = \sum_i \sum_j (H_{i,j})^2 \text{var}(\hat{p}_{i,j}^g) \quad (2)$$

where $\text{var}(\hat{p}_{i,j}^g)$ will be available from the Bayesian mixed stock analysis (Pella and Masuda 2001).

AGE AND SEX COMPOSITION OF CHINOOK SALMON IN ESSN HARVEST

The age (or sex) proportions of Chinook salmon harvested in the commercial ESSN fishery by sampling stratum will be estimated as follows:

$$\hat{p}_{i,j}^z = \frac{n_{i,j}^z}{n_{i,j}} \quad (3)$$

where $\hat{p}_{i,j}^z$ is the estimated proportion of salmon of age (or sex) category z from sampling stratum (i, j) , $n_{i,j}^z$ equals the number of fish sampled from sampling stratum (i, j) that were classified as age (or sex) category z , and $n_{i,j}$ equals the number of Chinook salmon sampled for age (or sex) determination from sampling stratum (i, j) .

The variance of $\hat{p}_{i,j}^z$ will be estimated by

$$\text{var}[\hat{p}_{i,j}^z] = \left(1 - \frac{n_{i,j}}{H_{i,j}}\right) \frac{\hat{p}_{i,j}^z (1 - \hat{p}_{i,j}^z)}{n_{i,j} - 1} \quad (4)$$

where $H_{i,j}$ is the number of Chinook salmon harvested in a sampling stratum (i, j) .

The estimates of harvest by age (or sex) categories in each sampling stratum will be calculated by

$$\hat{H}_{i,j}^z = H_{i,j} \hat{p}_{i,j}^z \quad (5)$$

with its variance estimated as follows:

$$\text{var}[\hat{H}_{i,j}^z] = H_{i,j}^2 * \text{var}[\hat{p}_{i,j}^z] \quad (6)$$

The total harvest by age (or sex) category and its variance will then be estimated by summation:

$$\hat{H}^z = \sum_{i=1}^T \sum_{j=1}^S \hat{H}_{i,j}^z \quad (7)$$

and

$$\text{var}[\hat{H}^z] = \sum_{i=1}^T \sum_{j=1}^S \text{var}[\hat{H}_{i,j}^z]. \quad (8)$$

where $T = 3$ and $S = 2$ are the number of time and geographic strata respectively.

Finally, the total proportion of the ESSN harvest by age (or sex) category and its variance will be estimated by

$$\hat{p}^z = \frac{\hat{H}^z}{H} \quad (9)$$

and

$$\text{var}[\hat{p}^z] = \frac{\text{var}[\hat{H}^z]}{H^2}. \quad (10)$$

CODED WIRE TAG RECOVERIES

With the low numbers of CWT recoveries expected, no direct estimates of CWT recoveries by stock will be made, but the data will be archived with the Mark, Tag, and Age Laboratory in Juneau.

BUDGET SUMMARY

Proposed FY14 and FY15 costs:

Line item	Category	FY14 budget (\$K)	FY15 budget (\$K)
100	Personnel	4.7	25.5
200	Travel	–	–
300	Contractual	3.0	31.0
400	Commodities	1.0	2.0
500	Equipment	–	–
Total		8.7	58.5

Funded personnel FY14:

PCN	Name	Level	Funded man months
NP	Vacant	FWT II	0.25
NP	Vacant	FWT II	0.25
114062	Fender, Shannon	FWT II	0.33
Total			0.83

Funded personnel FY15:

PCN	Name	Level	Funded Man Months
NP	Vacant	FWT II	1.7
NP	Vacant	FWT II	1.7
114062	Fender, Shannon	FWT II	1.7
Total			5.1

SCHEDULE AND DELIVERABLES

Date	Activity
Mid to late June 2014	Hiring and preseason training (Eskelin)
Late June to mid-August 2014	ESSN Chinook salmon harvest sampling (3FWT)
September 2014	Data edited, tissue collection transferred to GCL, and CWT forms and heads mailed to Juneau Mark, Tag, and Age lab (Eskelin)
September 2014	Tissue, age, sex, and length subsamples selected and scales aged (Eskelin)
October 2014	Draft ASL composition estimates completed (Eskelin)
December 2014	Tissues analyzed by GCL and draft MSA results disseminated (Barclay)
January 2015	Harvest estimates completed by temporal, geographic strata and reporting group (Eskelin and Antonovich)
January 2015	Memo detailing 2014 ESSN Chinook MSA results (Eskelin and Barclay)

RESPONSIBILITIES

Principle Investigator

Tony Eskelin, Project Leader, Fishery Biologist II:

The project leader is responsible for writing the operational plan. This position will serve as the project biologist and will be responsible for hiring and training personnel and supervision of data collection. The project biologist will be responsible for collating data and transferring tissue samples to Anchorage for MSA and associated data, and any CWT heads and data forms to the Mark, Tag and Age lab in Juneau. This position will be responsible for all scale aging. This position will also ensure all data is in proper format and archived with RTS at the completion of the field season and will be primary author on any reporting.

Coprinciple Investigator

Andy Barclay, Fishery Biologist III:

This position is the Gene Conservation Lab representative. This position is responsible for the analysis of tissue samples for MSA and providing estimates to the project biologist and biometrician. This position will be co-author on FDS reports and memos.

Consulting Biometrician

Anton Antonovich, Biometrician III:

This position will provide guidance on sampling design and data analysis, prepare estimates of harvest of Chinook salmon by reporting group and assist with preparation of the operational plan and any reports.

Sampling Crew

Shane Fender, Fish and Wildlife Technician II, 20 June–16 August.

Fish and Wildlife Technician II (non-perm), 24 June–16 August.

Fish and Wildlife Technician II (non-perm), 24 June–16 August.

Responsibilities of these positions include operating State of Alaska vehicles, adhering to sampling schedule, sampling harvested Chinook salmon for ASL and tissue, recording data accurately, and entering data into a computerized database in a timely manner.

REFERENCES CITED

- Barclay, A. W., C. Habicht, R. A. Merizon, and R. J. Yanusz. 2012. Genetic baseline for Upper Cook Inlet Chinook salmon: 46 SNPs and 5,279 fish. Alaska Department of Fish and Game, Fishery Manuscript Series No. 12-02, Anchorage. <http://www.adfg.alaska.gov/FedAidpdfs/FMS12-02>
- Clutter, R., and L. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. International Pacific Salmon Commission, Bulletin 9. Westminster, British Columbia, Canada.
- Eskelin, T., A. W. Barclay, and A. Antonovich. 2013. Mixed stock analysis and age, sex, and length composition of Chinook salmon in Upper Cook Inlet, Alaska, 2010–2013. Alaska Department of Fish and Game, Fishery Data Series No. 13-63, Anchorage. <http://www.adfg.alaska.gov/FedAidpdfs/FDS13-63>
- Pella, J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99:151-167.
- Reimer, A. M. 2013. Migratory timing and distribution of Kenai River Chinook salmon, 2010–2013, a report to the Alaska Board of Fisheries, 2014. Alaska Department of Fish and Game, Division of Sport Fish, Regional Information Report 2A13-06, Anchorage. <http://www.adfg.alaska.gov/FedAidpdfs/RIR.3A.2013.06>
- Shields, P., and A. Dupuis. 2013. Upper Cook Inlet commercial fisheries annual management report, 2013. Alaska Department of Fish and Game, Fishery Management Report No. 13-49, Anchorage. <http://www.adfg.alaska.gov/FedAidpdfs/FMR13-49>
- Thompson, S. K. 1987. Sample size for estimating multinomial proportions. The American Statistician 41(1):42-46.
- Welander, A. D. 1940. A study of the development of the scale of Chinook salmon *Oncorhynchus tshawytscha*. Master's thesis. University of Washington, Seattle.

**APPENDIX A: PRELIMINARY ESSN CHINOOK SALMON
SAMPLING SCHEDULE, 2014**

Appendix A1.–Preliminary ESSN Chinook salmon sampling schedule, 2014.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
15-Jun	16-Jun	17-Jun	18-Jun	19-Jun	20-Jun	21-Jun
22-Jun	23-Jun	24-Jun	25-Jun	26-Jun	27-Jun	28-Jun
	Training			Regular Period Kasilof section	Peninsula Processing	
29-Jun	30-Jun	1-Jul	2-Jul	3-Jul	4-Jul	5-Jul
	Regular Period Kasilof section	Peninsula Processing		Regular Period Kasilof section	Peninsula Processing	
6-Jul	7-Jul	8-Jul	9-Jul	10-Jul	11-Jul	12-Jul
	Regular Period Kasilof section	Peninsula Processing		Regular Period All Sections	Peninsula Processing	
13-Jul	14-Jul	15-Jul	16-Jul	17-Jul	18-Jul	19-Jul
	Regular Period All sections	Peninsula Processing		Regular Period All Sections	Peninsula Processing	
20-Jul	21-Jul	22-Jul	23-Jul	24-Jul	25-Jul	26-Jul
	Regular Period All sections	Peninsula Processing		Regular Period All Sections	Peninsula Processing	
27-Jul	28-Jul	29-Jul	30-Jul	31-Jul	1-Aug	2-Aug
	Regular Period All sections	Peninsula Processing		Regular Period All Sections	Peninsula Processing	
3-Aug	4-Aug	5-Aug	6-Aug	7-Aug	8-Aug	9-Aug
	Regular Period All sections	Peninsula Processing		Regular Period All Sections	Peninsula Processing	
10-Aug	11-Aug	12-Aug	13-Aug	14-Aug	15-Aug	
	Regular Period All sections	Peninsula Processing		Regular Period All Sections	Peninsula Processing	

Note: up to 2 fishing periods per week will be sampled if opened by EO.

**APPENDIX B: ESSN CHINOOK SALMON SAMPLING
FORM, 2014**

Appendix B1.-ESSN Chinook salmon sampling form, 2014.

ESSN Chinook Salmon Sampling Form								
Date: _____			Sampler(s): _____					
Start Time: _____			End Time: _____					
Card	Scale	Sex	Length	VIAL	Sample Location/Comment	Stat AREA	CWT #	Age
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

Sex: 1-male 2-female
 Length: mid eye to fork-of-tail in nearest 5 mm.

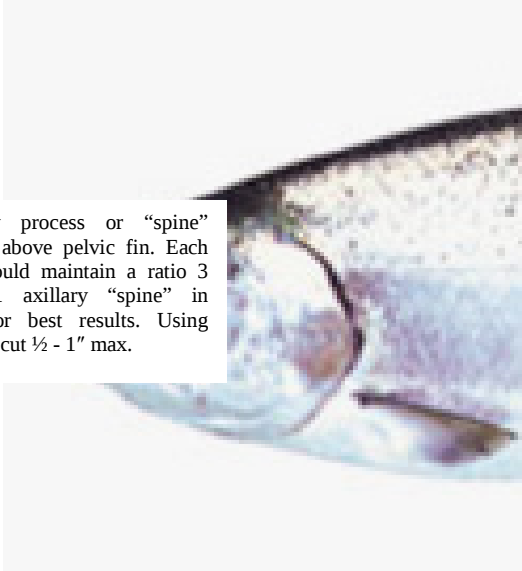
APPENDIX C: INSTRUCTIONS FOR TISSUE SAMPLING

General Information

We use axillary tissue samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish or in or reporting group proportions in mixed-stock sampling.

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

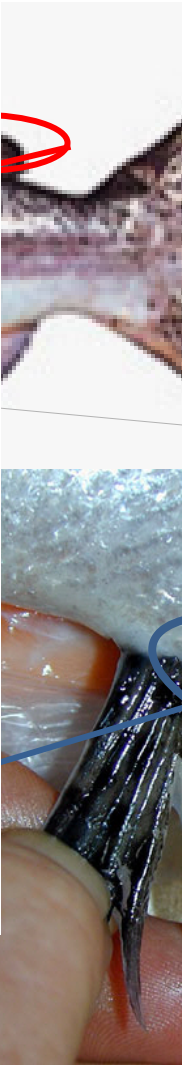
Sampling Method



Axillary process or “spine” located above pelvic fin. Each clip should maintain a ratio 3 ETOH/1 axillary “spine” in vials for best results. Using clipper; cut ½ - 1” max.

Axillary process or “spine” located above pelvic fin. Each clip should maintain a ratio 3 EtOH/1 axillary in vials for best results. Using clipper, cut ½-1” max.

- Wipe excess water and/or slime off the axillary process “spine” prior to sampling to avoid getting either water or fish slime into the 2.0 ml vial (see diagram).
- 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.
- 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 axillary process into ETOH. The ethanol/tissue ratio should be **slightly less than 3:1** to thoroughly soak the tissue in the buffer.
- Top up tubes with ETOH and screw cap on securely. Invert tube twice to mix ETOH and tissue. Periodically, wipe or rinse the clippers with water so not to cross contaminate samples.
- Data to record: Record **each vial number to paired data** information (i.e. location, lat./long., sample date(s), etc.).
- Discard remaining ethanol from the 500ml bottles before shipping. **Tissue samples must remain in 2ml ETOH**, these small quantities require





APPENDIX D: CODED WIRE TAG SAMPLING FORM

