Regional Operational Plan SF.2A.2018.10

Operational Plan: Russian River Early Run Sockeye Salmon Run Timing Study

by

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and

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May 2018

Alaska Department of Fish and Game Divisions of Sport Fish and Commercial Fisheries

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Weights and measures (metric)

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

Weights and measures (English)

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

Time and temperature

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

Physics and chemistry

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

General

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

Mathematics, statistics

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

REGIONAL OPERATIONAL PLAN SF.2A.2018.10

OPERATIONAL PLAN: RUSSIAN RIVER EARLY RUN SOCKEYE SALMON RUN TIMING STUDY

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May 2018

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SIGNATURE/TITLE PAGE

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Approval

Title	Name	Signature	Date
Project leader	Anthony Eskelin		
Project leader	Andrew W. Barclay		
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TABLE OF CONTENTS

Page

LIST OF TABLES ... ii

LIST OF APPENDICES ... ii

ABSTRACT ... 1

INTRODUCTION ... 1

Purpose ... 1

Background ... 1

Phase 1 Investigation: Migration Timing of Russian River Early-Run Sockeye Salmon ... 2

Phase 2 Investigation: Stock Composition of Early-Run Sockeye Salmon at Kenai River RM 13.7 ... 3

OBJECTIVE ... 5

METHODS ... 5

Study Design ... 5

Sockeye Salmon Capture ... 5

Tissue Sampling for Mixed Stock Analysis ... 5

Sample Selection for Mixed Stock Analysis ... 6

Data Collection ... 6

Data Analysis ... 7

Laboratory Analysis ... 7

Stock Compositions ... 8

SCHEDULE AND DELIVERABLES ... 8

RESPONSIBILITIES ... 8

REFERENCES CITED ... 9

APPENDIX A: INSTRUCTIONS FOR TISSUE SAMPLING ... 11

LIST OF TABLES

Table **Page**

1 Kenai River RM 13.7 early-run sockeye salmon-sized sonar passage, sport fishery harvest, and weir passage, 2014–2017. ... 2

2 Travel times of radiotagged sockeye salmon from Kenai River RM 13.7 sonar site to each fixed telemetry station in the Kenai and Russian River drainages, 2017. ... 3

3 Travel time (number of days) for radiotagged and spaghetti-tagged fish from the inriver gillnetting tag location (RM 8.6) to the Russian River weir, 2017. ... 3

4 Tissue collections of sockeye salmon in the Kenai River genetic baseline including year sampled and the number of individuals (*N*) analyzed from each collection and their assigned reporting groups for mixed stock analysis. ... 4

LIST OF APPENDICES

Appendix **Page**

A1. Instructions for tissue sampling. ... 12

ABSTRACT

This study will provide an estimate of the stock composition of early-run sockeye salmon in the lower Kenai River at the RM 13.7 sonar site each year for 2018–2020. A prior study estimated the mean time it takes Russian River bound early-run sockeye salmon to migrate from the Kenai River sonar at RM 13.7 to the inriver sport fishery at Kenai River RM 73.0 and to the Russian River weir near lower Russian Lake. The second phase of this early-run sockeye salmon investigation will be to estimate the stock composition of sockeye salmon passing RM 13.7 prior to July 1 in a genetic mixed-stock analysis. Based on the previous genetic baseline analysis, Kenai River sockeye salmon have sufficient genetic variation to accurately estimate the stock composition using mixed stock analysis techniques for the following 3 reporting groups: "early-run Russian River," "late-run Russian River," and "Kenai River other."

Key words: Russian River, Kenai River, early run, sockeye salmon, MSA, mixed stock analysis, genetic stock identification

INTRODUCTION

PURPOSE

This study will provide three years (2018–2020) of temporal and seasonal estimates of the stock composition of the early run of sockeye salmon in the lower Kenai River at the RM 13.7 sonar site prior to July 1 (all salmon entering prior to July 1 are defined as early run). These stock composition estimates will improve future inseason management of the Russian River sockeye salmon early run.

BACKGROUND

The Kenai River Chinook salmon sonar site was recently moved from rivermile (RM) 8.6 to RM 13.7 of the Kenai River mainstem, and a new adaptive resolution imaging sonar (ARIS) was deployed at this site (Miller et al. 2016). At this new site and with the new sonar technology, nearly the entire cross section of river is ensonified and fish passage is enumerated by size. The majority of fish that pass the RM 13.7 sonar during the early run that are less than 75 cm mid eye to tail fork (MEFT) length are sockeye salmon and the majority of those are thought to be bound for the Russian River. Thus, early-run sonar counts of fish less than 75 cm METF at RM 13.7 could provide a coarse estimate of Russian River early-run sockeye salmon passage at this site. However, the actual stock composition of sockeye salmon passing the RM 13.7 sonar during the early run is not known and may include other stocks. For example, the combined total of the estimated Russian River early-run sockeye salmon sport harvest and weir passage, which is referred to as the "local return," can be 25,000 to 75,000 fish less than the total number of sockeye salmon-sized fish that pass the sonar at RM 13.7 of the Kenai River (Table 1), suggesting that the early run sockeye salmon passage at RM 13.7 is probably composed of other stocks. We know that some of those sockeye salmon-sized fish that pass RM 13.7 are probably small Chinook salmon or other species (rainbow trout, Dolly Varden), however most of the difference between the number of fish counted at the sonar and the local return of sockeye salmon to the Russian River are believed to be sockeye salmon that are not of early-run Russian River origin. In addition, there is an increasing sport harvest of sockeye salmon in the mainstem Kenai River during June; however, the number of fish harvested and the stock composition of this portion of the sockeye salmon fishery is not known.

Table 1.–Kenai River RM 13.7 early-run sockeye salmon-sized sonar passage, sport fishery harvest, and weir passage, 2014–2017.

Year	Kenai River RM 13.7 early-run sockeye salmon-sized (40–75 cm TL) sonar passage	Russian River area early-run sport fishery harvest	Russian River early-run weir passage	Russian R. local return (early-run harvest plus weir passage)	Difference between sonar passage and Russian River local return
2014	105,584	35,870	44,920	80,790	24,794
2015	156,087	29,997	50,226	80,223	75,864
2016	95,526	13,086	38,739	51,825	43,701
2017	135,970	NA	37,123	NA	NA

Note: The 2017 Russian River area early run sport fishery harvest estimate was not available (NA) when this operation plan was published.

Phase 1 Investigation: Migration Timing of Russian River Early-Run Sockeye Salmon

The first phase of this study began in 2017 when the Division of Sport Fish initiated a Russian River early-run sockeye salmon tagging study (Eskelin 2017). The goal of the first phase was to estimate the mean migration time (duration) of early-run sockeye salmon from RM 13.7 to the inriver sport fishery at RM 73.0 and the mean migration time from RM 13.7 to the lower Russian River weir. Knowing the average number of days it takes for fish to migrate from RM 13.7 to each location provides management staff with an inseason tool that can be coupled with RM 13.7 sonar counts to more effectively manage Russian River early-run sockeye salmon to meet the biological escapement goal (BEG) and provide for sustained yield (Alaska Administrative Code 5 AAC 57.150).

Sixty sockeye salmon were implanted with radio transmitters at RM 8.6 in conjunction with a separate inriver gillnetting study (Perschbacher 2017) and tracked throughout the Kenai River drainage with fixed stations, a mobile boat, and aerial surveys. Based on 22 radiotagged fish that were tracked to the Russian River area sport fishery, the mean migration time from RM 13.7 to the Russian River area sport fishery was 9.8 days (SD 2.7 days), with a range of 5.2–16.8 days (Table 2).

Additional sockeye salmon were also fitted with spaghetti tags at the RM 8.6 inriver gillnetting site to increase the sample size of tagged fish passing through the Russian River weir. Because these fish were not radiotagged, mean travel time from Kenai River RM 13.7 to the Russian River weir was estimated using the tagging location at the Kenai River RM 8.6 inriver gillnetting site as a proxy for the RM 13.7 sonar site. Data from spaghetti-tagged sockeye salmon were combined with data from radiotagged sockeye salmon to increase sample size. Mean migration time between the RM 8.6 tagging site and the RM 13.7 sonar site for radiotagged fish was less than 1 day but only the timing from the RM 8.6 tagging site was used in the combined analysis. There were 11 spaghetti-tagged fish that passed the weir with a mean migration time of 16.9 days (range: 12.2–19.4 days), and 3 radio tagged fish that passed the weir with a mean migration time of 17.7 days (range: 13.2–20.1 days) (Table 3); hence travel times were similar between spaghetti- and radiotagged fish. Overall, the 14 tagged fish (3 spaghetti, 11 radio) averaged 17.1 days from Kenai River RM 8.6 to the Russian River weir with a range of 12.2–19.2 days.

Table 2.–Travel times of radiotagged sockeye salmon from Kenai River RM 13.7 sonar site to each fixed telemetry station in the Kenai and Russian River drainages, 2017.

	Fixed telemetry site location				
Parameter	Skilak Lake outlet (RM 49.1)	Skilak Lake inlet (RM 65.7)	Russian River area sport fishery (RM 73.0)	Russian River confluence (RM.73.7)	Russian River weir
No. of tagged fish	32	26	22	18	3
Average travel time	5.2	7.7	9.8	11.2	16.6
SD travel time	2.4	2.1	2.7	3.2	3.6
Minimum travel time	2.3	4.4	5.2	6.0	12.4
Maximum travel time	13.7	13.2	16.8	17.2	19.2
Range of travel times	11.3	8.8	11.6	11.2	6.7

Note: All travel times are given in number of days.

Table 3.–Travel time (number of days) for radiotagged and spaghetti-tagged fish from the inriver gillnetting tag location (RM 8.6) to the Russian River weir, 2017.

	Tag type		
Parameter	Spaghetti	Radio	All
No. of tagged fish	11	3	14
Average travel time	16.9	17.7	17.1
SD travel time	12.2	13.2	12.2
Minimum travel time	19.4	20.1	19.4
Maximum travel time	7.2	6.9	7.2

Note: All travel times are given in number of days.

Understanding the migration timing for Russian River sockeye salmon allows better assessment of when pulses of sockeye salmon observed at the RM 13.7 sonar may enter the Russian River area sport fishery and then pass the weir. However, we do not know the stock composition of early run sockeye salmon that pass the Kenai River RM 13.7 sonar site during the early run nor do we know how the stock composition varies annually or temporally within each year.

Phase 2 Investigation: Stock Composition of Early-Run Sockeye Salmon at Kenai River RM 13.7

The next phase of this Russian River early run sockeye salmon study detailed below will investigate the stock composition of early-run sockeye salmon at Kenai River RM 13.7 annually and temporally each season for 2018–2020.

Baseline and Reporting Groups

The current sockeye salmon genetic baseline for Upper Cook Inlet includes 69 populations analyzed for 96 genetically variant single nucleotide polymorphism (SNP) loci (Barclay and Habicht 2012). For the mixed stock analysis (MSA) of fish captured at the RM 8.6 netting project, the same baseline will be used; however, it will be reduced to 14 Kenai River populations in the baseline (Table 4). Previous analyses of sockeye salmon population structure in Cook Inlet (Barclay and Habicht 2012) have demonstrated sufficient variation for MSA of the following 3 reporting groups: 1) *Russian River early run* (Upper Russian River early-run spawning populations); 2) *Russian River late run* (Upper Russian River late-run spawning populations), and 3) *Kenai River other* (the remaining populations within the Kenai River

drainage). These reporting groups will be used in the MSA to apportion samples of fish captured by the RM 8.6 gillnetting project.

Table 4.– Tissue collections of sockeye salmon in the Kenai River genetic baseline including year sampled and the number of individuals (*N*) analyzed from each collection and their assigned reporting groups for mixed stock analysis.

Pop. no.	Reporting group	Location	Sample year	*N*
1	*Kenai River other*	Railroad Creek	1997	48
1		Johnson Creek	1997	88
2		Moose Creek	1993	47
2			1994	95
3		Ptarmigan Creek	1992	47
3			1993	95
4		Tern Lake	1992	48
4			1993	48
5		Quartz Creek	1993	94
		Kenai River, between Skilak and Kenai lakes		
6		site 1	1994	47
6		site 2	1994	48
6		site 3	1994	143
7		site 4	1993	95
7		site 5	1994	48
7		site 6	1994	95
7		Lower Russian River	1993	95
		Upper Russian River		
8	*Russian River early run*	Goat Creek	1992	96
8			1997	95
8			2009	95
9	*Russian River late run*	Goat Creek	2009	95
10		Bear Creek	2009	95
11		Upper Lake south shore	1999	95
11			2009	95
12		Upper Lake outlet	1999	95
12			2009	95
13	*Kenai River other*	Hidden Lake	1993	95
13			2008	95
14		Skilak Lake outlet	1992	96
14			1994	95
14			1995	48

Management Support

Historically, inseason management actions for early-run Russian River sockeye salmon have been supported by counts of sockeye salmon at a weir located above the fishery at the outlet of lower Russian Lake and foot survey counts of the numbers of sockeye salmon in the Russian River below the weir (Pawluk 2015). Together the 2 counts provide managers information to estimate run strength and determine if inseason management actions are necessary to achieve the escapement goal.

Estimates of the number of sockeye salmon passing the sonar at RM 13.7 are available inseason and travel time to the Russian River area sport fishery and to the Russian River weir were estimated in phase 1 of this study. Estimates of the stock composition of the sockeye salmon migrating upstream past RM 13.7 prior to July 1 (early run) that are of Russian River early-run origin may allow managers to make run strength assessments and inseason management decisions earlier than they have historically occurred because it might not be necessary any longer to wait for the arrival of sockeye salmon at the Russian River. This new information about stock composition combined with run timing will improve inseason management of this valuable sport fisheries resource.

OBJECTIVE

Estimate the proportion of the inriver sockeye salmon early run at Kenai River RM 13.7 by reporting group (*early-run Russian River*, *late-run Russian River*, or *Kenai River other*) for each temporal stratum and annually for each year during 2018–2020 such that the estimated proportions are within 0.10 of the true values 90% of the time.

METHODS

STUDY DESIGN

Sockeye Salmon Capture

Sockeye salmon will be captured as part of a separate inriver netting project at RM 8.6 of the Kenai River (Perschbacher *In prep*). The primary goal of that study will be to capture a representative biological (age, sex, and length) sample of the Chinook salmon runs; however numerous sockeye salmon are captured, which can satisfy the sampling goals for the sockeye salmon tissue collection portion of this study.

As pertains to the sockeye salmon project, inriver gillnetting will be conducted every day from 16 May through 30 June. The gillnetting crew will be composed of 3 fishery technicians, with 2 technicians working each shift (6:00 AM–2:00 PM). Each technician will be scheduled 5 days per week for 8 hours per day. Inriver nets will be fished with equal frequency by location (nearshore and midriver) and mesh size. See Perschbacher (2017 and *In prep*) for more details regarding the inriver gillnetting project.

Tissue Sampling for Mixed Stock Analysis

Sockeye salmon captured in the first 8 sets per day of the inriver gillnetting study, which represents about the first third of each sampling day, will be measured for length and sampled for genetic tissue. Tissue samples will also be collected from all other sockeye salmon captured in May unless the tissue collections begin to impede the inriver gillnetting study, at which time tissue samples will only be collected from fish captured during the first 8 sets per day. In June,

tissue samples will be collected from the first 8 sets per day; however, samples may be collected from captured sockeye salmon from additional sets dependent on the number of sockeye salmon captured each day. In 2017, 417 length samples were collected from sockeye salmon, so we expect a similar number in 2018 and likely more if we collect tissue from all captured sockeye salmon in May and from additional sets in June. The goal is to analyze 400 representative tissue samples from each year, so we may assist the inriver gillnetting crew by adding another crew member in mid to late June to collect the additional samples and reduce interference with the inriver gillnetting project study design. The additional samples will allow for subsampling from the collections postseason to represent the proportion of the sockeye salmon catch of all inriver gillnetting sets by day in the MSA.

A 1⅓ cm (half-inch) piece of the axillary process will be removed from each fish and placed on a Whatman[1] paper card in its own grid space and then stapled in place. Whatman cards with tissue samples will be placed in an airtight case with desiccant beads to preserve the tissue for DNA extraction. Tissue sampling instructions are detailed in Appendix A1.

Sample Selection for Mixed Stock Analysis

Subsampling of collections is required postseason to ensure analyses accurately represent the captures by date or week. Once the number of samples required from each day is determined, samples will be selected randomly from all available tissues sampled on that date.

Stratification

Sample size will be approximately 100 tissues for each stratum. We hope to analyze 4 temporal strata each year. Dates for each stratum will be approximately each week in June but these will be subject to change dependent on tissue collections and captures by date.

Assuming that the samples are representative of sockeye salmon passing RM 8.6 both sampling error and genetic error will affect estimates of the proportion of sockeye salmon by reporting group. According to sampling theory (Thompson 1987), and under a worst-case scenario of reporting groups at equal proportions, a multinomial proportion can be estimated to within 010 of the true values 90% of the time with a sample size of at least 100. Additional uncertainty will originate from the mixed stock analysis, which will be assessed using MSA proof tests in the final report following methods reported in Barclay and Habicht (2012).

DATA COLLECTION

Each Whatman tissue collection card will have a unique barcode and a numbered grid. Card barcodes (5-digit) and grid position numbers (1–48) will be recorded on Allegro handheld computers for each sample. If the computers are not working, then we will simply put the date on each Whatman card. All Whatman cards will be stored at the Soldotna office until the end of the season then sent to the GCL for analysis and archiving.

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

DATA ANALYSIS

Laboratory Analysis

Assaying Genotypes

We will extract genomic DNA from tissue samples using a NucleoSpin 96 Tissue Kit by Macherey-Nagel (Düren, Germany). DNA will be screened for 96 SNP markers. To ensure that DNA concentrations are high enough with the dry sampling method used to preserve samples, preamplification will be conducted before screening the DNA.

The concentration of template DNA from samples will be increased using a multiplexed preamplification PCR of 96 screened SNP markers. Reactions will be conducted in 10 µL volumes consisting of 4 µL of genomic DNA, 5 µL of 2X Multiplex PCR Master Mix (QIAGEN), and 1 µL each of 2 µM SNP unlabeled forward and reverse primers. Thermal cycling will be performed on a Dual 384-Well GeneAmp PCR system 9700 (Applied Biosystems) at a 95°C hold for 15 min followed by 20 cycles of 95°C for 15 s, 60°C for 4 min, and a final extension hold at 4°C.

We will screen the preamplified DNA for the 96 SNP markers using Fluidigm 96.96 Dynamic Array Integrated Fluidic Circuits (IFCs), which systematically combine up to 96 assays and 96 samples into 9,216 parallel reactions. The components are pressurized into the IFC using the IFC Controller RX (Fluidigm). Each reaction will be conducted in a 7.2 nL volume chamber consisting of a mixture of 20X Fast GT Sample Loading Reagent (Fluidigm), 2X TaqMan GTXpress Master Mix (Applied Biosystems), Custom TaqMan SNP Genotyping Assay (Applied Biosystems), 2X Assay Loading Reagent (Fluidigm), 50X ROX Reference Dye (Invitrogen), and 60-400 ng/µl DNA. Thermal cycling will be performed on a Fluidigm FC1 Cycler using a Fast-PCR protocol as follows: a "Thermal-Mix" step of 70°C for 30 min and 25°C for 10 min, an initial "Hot-Start" denaturation of 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 2 s and annealing at 60°C for 20 s, with a final "Cool-Down" at 25ºC for 10 s. The Dynamic Array IFCs will be read on a Biomark or EP1 System (Fluidigm) after amplification and scored using Fluidigm SNP Genotyping Analysis software.

Genotypes will be imported and archived in the Gene Conservation Laboratory's Oracle database, LOKI.

Laboratory Failure Rates and Quality Control

The 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 instituted 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) are 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.

Assuming that the inconsistencies among analyses (original vs. QC genotyping) are due equally to errors in original genotyping and errors during the QC genotyping, and that the analyses are

unbiased, error rates in the original genotyping will be estimated as one-half the rate of inconsistencies.

Stock Compositions

The stock composition of the inriver netting samples 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 prior parameters for each reporting group will be defined to be equal (i.e., a flat prior). Within each reporting group, the population prior parameters will be divided equally among the populations within that reporting group. 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.

SCHEDULE AND DELIVERABLES

Dates	Activity
Mid-May–July, 2018–2020	Inriver gillnetting and tissue collections (*Eskelin and Perschbacher*)
Fall 2018, 2019, 2020	Data edited, tissue collection transferred to GCL (*Eskelin*)
Winter 2020-2021	Mixed-stock analysis complete (*Barclay*)
Spring 2021	FDS report transmitted to regional staff for review (*Eskelin and Barclay*)

RESPONSIBILITIES

Principal investigator: *Tony Eskelin, Project Leader, Fishery Biologist II*

Duties: As project leader, responsible for writing the operational plan. Serves as the project biologist, who is responsible hiring and training personnel, supervising data collection, collating data, and transferring tissue samples and associated data to Anchorage for MSA. Serves as the primary author on any reporting.

Co-principle investigator: *Andy Barclay, Fishery Biologist III*

Duties: Represents the Gene Conservation Laboratory and is responsible for the analysis of tissue samples for MSA and providing estimates to the project biologist. Serves as coauthor on Fishery Data Series (FDS) report.

Inriver Gillnetting Project Leader: *Jeff Perschbacher, Fishery Biologist I*
Duties: Responsible for RM 8.6 sockeye salmon tissue collections and conducts the existing inriver gillnetting study. Responsible for being the crew leader of the technicians that will sample sockeye salmon.

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APPENDIX A: INSTRUCTIONS FOR TISSUE SAMPLING

Adult Finfish Tissue Sampling for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use fin tissues as a source of DNA to genotype fish. Genotyped fish are used to determine the genetic characteristics of fish stocks or to determine stock compositions of fishery mixtures. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible.

Preservative used: Silica desiccant bead packet dries and preserves tissues for later DNA extraction. Quality DNA preservation requires **Evening drying** (in dehydrator 5-6 hours at 200°F – **NOT** microwave); **Dry storage** weathertight file box (desiccant packs).

II. Sampling Method

III. Sampling Instructions

- **Every morning:** before sampling, load 3 desiccant packs (2-Pelican, 1-file box) into dehydrator @ **160° F** for at least 12 hours. **(NOT SAMPLES)!**
- **Prior to sampling**: Set up work space, fill out required **red** collection information (upper left hand corner only) and place Whatman genetics card (48WGC) on clipboard, secure with rubber band; ready to sample.
- **Sampling:**
  - Wipe fin prior to sampling.
  - Briefly wipe or rinse clippers between samples reducing cross contamination.
  - Using clippers, cut one axillary fin per fish.
  - Place one clipped fin tissue onto # 1 grid space. Follow numerical sampling order (#'s 1-48) printed on card - **do not deviate**. If large tissue sample, center tissue diagonally on grid space.
  - **Only one fin clip per fish into each numbered grid space**.
  - Staple each sample to 48WGC (see photo).
  - Sampling complete, dust tissues with ½ **tsp. non-iodized salt** to promote the preservation process.
  - Fold landscape cloth "rain fly" over samples for protecting the tissue samples taken for storage and transport.
- **Loading the Pelican Case:**
  - First card: Remove blotter papers and desiccant packs from Pelican Case. Place first card in Pelican Case with tissues facing up. Next, place blotter paper directly over card and place 2 desiccant packs on top. Close and secure lid so drying begins.
  - Up to 4 cards can be added per case. Add them so the tissue samples always face the desiccant pack through blotter paper: 2nd card facing down between desiccant packs; 3rd card facing up between desiccant packs; and 4th card facing down on top of second desiccant pack. Close and secure Pelican Case after inserting each card.
  - All Whatman cards **remain in Pelican overnight** to dry flat.
- **Post-sampling storage:** Every morning, move dried tissue cards into weathertight file box at room temperature. Two desiccant packs are allocated for file box: load 3 desiccant packs (2-Pelican, 1-file box) into dehydrator @ **160° F** for at least 12 hours. **(NOT SAMPLES)!**
- **Shipping at end of the season:** Fold barcode flap under card, pack 2 **dried** cards per plastic photo page, slide in manila envelope; pack inside priority mailing box. Tape box shut and tape return address on box.

IV. Supplies included in sampling kit:

1. Clippers - for cutting a portion of selected fin.
2. Whatman genetics card – holds 48 fish/sheet.
3. Pelican Case - 1st stage of drying and holding card samples.
4. Silica packs – desiccant removes moisture from samples.
5. Pre-cut blotter paper – covers full sample card for drying.
6. Weathertight file box – dry storage prior to return shipment.
7. Plastic photo page – 2 cards per page for return shipment.
8. Manila envelope – pack dried cards in manila envelope.
9. Shipping box – put sealed manila envelope inside box.
10. Clipboard – holds Whatman genetics card while sampling.
11. Stapler – extra protection, secure sample to numbered grid.
12. Staples – only use staples provided, specific for stapler.
13. Rubber bands – secure paper to clipboard (optional).
14. Non-iodized salt – distribute ~ ½ tsp. salt over each card.
15. Laminated "return address" labels.
16. Sampling instructions.
17. Pencil

V. **Shipping**: Address the sealed mailer box for return shipment to ADF&G Genetics lab.

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