Operational Plan: Susitna River Chinook Salmon Inriver Abundance, 2021

by Johnathon Campbell Nick A. DeCovich Adam Reimer and Andrew W. Barclay

February 2022

Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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

REGIONAL OPERATIONAL PLAN NO. ROP.SF.2A.2022.16

OPERATIONAL PLAN: SUSITNA RIVER CHINOOK SALMON INRIVER ABUNDANCE, 2021

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February 2022

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TABLE OF CONTENTS

Page

LIST OF TABLES	. iii
LIST OF FIGURES	. iii
LIST OF APPENDICES	. iii
ABSTRACT	1
INTRODUCTION	1
Purpose	1
Background	1
OBJECTIVES	2
Primary Objectives	2
Secondary Objectives	3
METHODS	3
Study Design	3
Abundance: PIT Tags	
Abundance: Genetic Mixed-Stock Analysis	
Proportion of Non-Deshka Chinook Salmon in the Deshka River Sport Fishery Distribution: Proportion of Chinook Salmon in Each of 5 Management Areas	
Sample Sizes	
Abundance of Mainstem Susitna River Chinook Salmon (Objective 1)	
Proportion of Non-Deshka River Fish in Sport Harvest (Objective 2)	
Spawning Distribution (Objective 3) Data Collection	
Marking with PIT- and Radio Tags	
Radiotelemetry	
Recapture PIT Tags at Deshka River Weir	.13
Tissue Sampling for Genetic Mixed Stock Analysis of Tagged Salmon	
Tissue Sampling of the Deshka River Sport Harvest Scale Collection	
Laboratory Analysis	
Data Reduction	
Assumptions	.16
Mark–Recapture Estimate	
Spawning Distribution	.18
Data Analysis	
PIT-Tag Abundance Estimates	
Genetic Abundance Estimates	
Proportion of non-Deshka River Chinook Salmon in Harvest Proportion of Chinook Salmon in Each of 5 Management Areas	
SCHEDULE AND DELIVERABLES	
RESPONSIBILITIES	
REFERENCES CITED	
REFERENCES CITED	.23

TABLE OF CONTENTS (Continued)

APPENDIX A: PIT TAG METHODS	25
APPENDIX B: FIELD DATA FORMS	31
APPENDIX C: BIOLOGICAL SAMPLING PROCEDURES	35
APPENDIX D: SAMPLE SIZES AND TESTS OF MARK–RECAPTURE ASSUMPTIONS	41

LIST OF TABLES

Table		Page
1	Radiotag deployment schedule for fish wheels and gillnet by date and shift (AM and PM), 2021	11

LIST OF FIGURES

Figur	re de la companya de	Page
1	Chinook salmon management zones of the Susitna River	2
2	Locations of fish wheels (open circle), fixed telemetry stations (diamonds), and Deshka weir site in	n the
	Susitna River drainage, Alaska	4
3	Locations of fish wheels and gillnetting area at the RM 34 tagging site	

LIST OF APPENDICES

Appen	ıdix	Page
Al	Passive integrated transponder (PIT) tag detection methods	26
B1	Fish wheel catch and effort field data form.	32
B2	Gillnet catch and effort field data form.	33
C1	Genetic tissue sample collection procedures.	
C2	Location of axillary process.	37
C3	Scale and length sampling procedures.	
D1	Anticipated sampling rates and sample sizes necessary to estimate mainstem Chinook salmon abundance within 25%, 90% of the time using a size-stratified Petersen model and adjusting for 25% loss of marked fish.	
D2	Detection and mitigation of selective sampling during a 2-event mark-recapture experiment	
D3	Tests for consistency of the Petersen estimator (from Seber 1982: page 438).	

ABSTRACT

The goal of this study is to estimate the abundance of Chinook salmon at river mile (RM) 34 of the mainstem Susitna River and spawning distribution among 5 management areas in 2021. A 2-event, mark-recapture experiment in combination with radiotelemetry will be used. Fish wheels and gillnets will be operated at river mile (RM) 34 to capture Chinook salmon for marking with passive integrated transponder (PIT) and radiotelemetry tags. Recapture event sampling will occur at the Deshka River weir at RM 7 where a PIT detection array will be used. Eight radiotracking stations will be strategically placed throughout the drainage to determine when radiotagged fish move in and out of the 5 management zones, allowing a determination of spawning distribution. A concurrent genetics mark-recapture study will be performed using genetics samples taken from a systematic sample of all PIT-tagged fish. The applied radio tags will also be used to estimate handling effects. In the event of a sport fishery, the proportions of non-Deshka River Chinook salmon in the sport harvest taken in 2 sections of the Deshka River will also be estimated through sampling of axillary processes from harvested fish and genetic stock identification.

INTRODUCTION

PURPOSE

In response to the recent downturns in the productivity and abundance of Chinook salmon (*Oncorhynchus tshawytscha*) stocks across Alaska and the social and economic hardships that have followed, the Alaska Department of Fish and Game (ADF&G) selected 12 indicator stocks to address knowledge gaps with studies of productivity, abundance, and other essential information needed to understand the root causes of these widespread declines (ADF&G Chinook Salmon Research Team 2013). The Susitna River was selected as one of these Chinook salmon indicator stocks and was recommended for stock assessment. In 2021, ADF&G plans to estimate the inriver abundance and distribution of Chinook salmon in 5 management areas that drain into the mainstem Susitna River (Figure 1).

BACKGROUND

Inriver abundance and spawning distribution data collected from this study will supplement similar data collected in previous studies during 2012–2017 (Cleary et al. 2015; Cleary and Campbell 2016; Yanusz et al. 2018; DeCovich et al. 2020). These abundance and spawning distribution estimates (8 years in all) along with harvest, aerial survey, and age composition data will be used in a Bayesian state-space stock-specific abundance and run-timing model that will help inform decisions on escapement goals (Reimer and DeCovich 2020). Results of the study will also help choose future assessments that are efficient and effective, advise the Alaska Board of Fisheries regulatory process, and be useful in land use planning and permitting.

A separate component of the 2021 study is to estimate the proportion of non-Deshka River Chinook salmon in the Deshka River sport harvest occurring in sections below the weir. It is unknown at this time how many of the fish harvested in this fishery would have passed the Deshka River weir and how many would have gone on to spawn in other tributaries had they not been harvested. Currently, the ADF&G statewide harvest survey (SWHS) partitions the harvest into 2 categories: above the weir and below the weir. ADF&G assumes all fish harvested below the weir are of Deshka River origin when calculating total run. The harvest sampling study will provide information regarding this assumption.

Keywords: Chinook salmon, abundance, mark-recapture, Susitna River, PIT tag, dart tag, gillnet, fish wheel, sport harvest, genetic stock identification

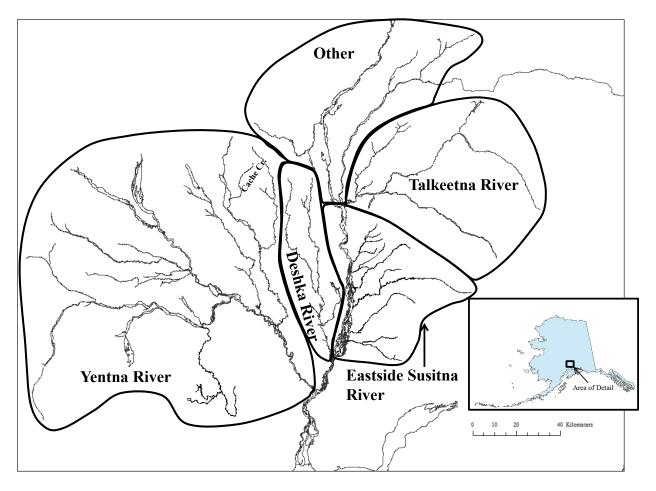


Figure 1.-Chinook salmon management zones of the Susitna River.

OBJECTIVES

PRIMARY OBJECTIVES

- Estimate the abundance of Chinook salmon ≥500 mm mid eye to tail fork (METF) length in the mainstem Susitna River upstream of the mouth of the Yentna River at river mile (RM) 34¹ using mark-recapture tagging methods such that the estimate is within 25% of the true value 90% of the time.
- 2) If the sport fishery is opened to harvest, estimate the proportion of the non-Deshka River fish in the sport harvest from each of 2 sections of the Deshka River downstream of the weir such that the estimated proportions are within 10% of the true values 90% of the time².
- 3) Estimate the distribution over 5 management areas of Chinook salmon ≥500 mm mid eye to tail fork (METF) length in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34 such that the estimate is within 15% of the true value 95% of the time using fixed telemetry stations.

¹ Defined by Alaska Energy Authority, Watana Hydroelectric Studies.

²"Within d% of the true value A% of the time" implies $P\left(p - \frac{d}{100} \le \hat{p} \le p + d/100\right) = A/100$ where p denotes the population age proportion.

SECONDARY OBJECTIVES

- Estimate the abundance of Chinook salmon ≥500 mm METF in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34 using genetic data in a mixedstock analysis (MSA) in the event there is no sport harvest allowed on the Deshka River (see Data Analysis section for explanation of this condition).
- 2) Estimate the proportion of Deshka River Chinook salmon <500 mm METF in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34 using genetic data in a mixed-stock analysis (MSA).
- ³⁾ Estimate age composition of all Chinook salmon ≥500 mm METF in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34.
- 4) Estimate age composition of all Chinook salmon <500 mm METF in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34.
- 5) Document distribution of radiotagged Chinook salmon ≥500 mm mid eye to tail fork length over the 5 management areas in the mainstem Susitna River upstream of the mouth of the Yentna River at RM 34 by collecting telemetry data while aerial Chinook salmon counts are being performed.

METHODS

STUDY DESIGN

Abundance: PIT Tags

A 2-event, capture–recapture experiment will be used to estimate the inriver abundance of Chinook salmon in the mainstem Susitna River. Fish wheels and gillnets will be used at RM 34 of the mainstem Susitna River to capture Chinook salmon for marking with PIT tags (a dart tag with an imbedded passive integrated transponder or "PIT"; Appendix A1, Figures 2 and 3). Fish will be examined for tags at a weir on the Deshka River at RM 7 (the Deshka River mouth is at Susitna RM 38.8; Figure 2) where a PIT detection array will be used. PIT tags will be detected using swim-through PIT-tag antennas at the Deshka River weir (Appendix A1). The PIT tags will allow for automated sampling of all fish at the Deshka River weir; this set-up will maximize sample size while avoiding the labor and run disruption necessary when hand-sampling fish. Radio tags will also be deployed during PIT-tagging and these will be used to quantify the proportion of tagged fish that drop out of the experiment, either through handling effects or switching drainages and heading up the Yentna River. All tagged fish will also get a secondary mark consisting of a hole punch in the left operculum so that tag loss can be assessed. Examination of fish for secondary marks will occur for all fish that are sampled for biological data at the Deshka River weir.

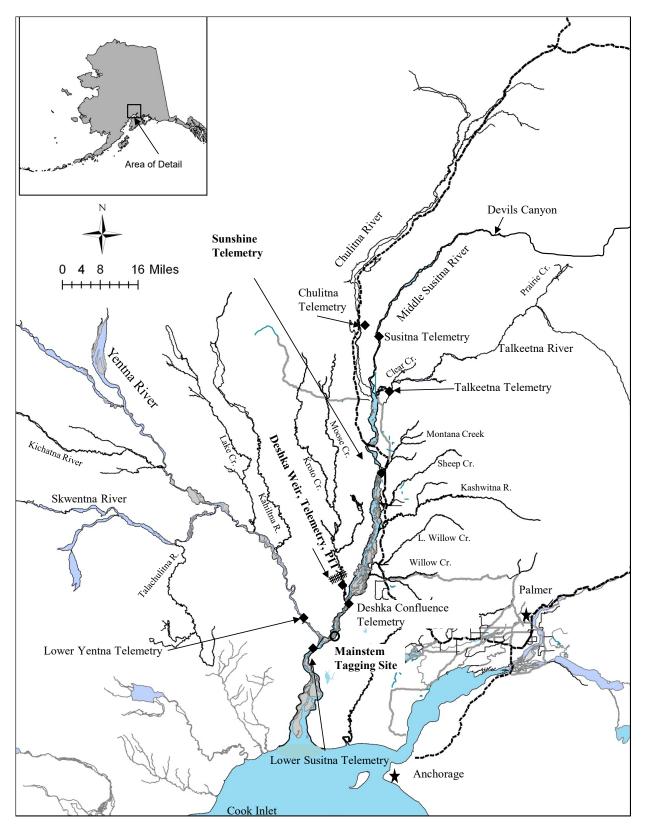


Figure 2.–Locations of fish wheels (open circle), fixed telemetry stations (diamonds), and Deshka weir site in the Susitna River drainage, Alaska.

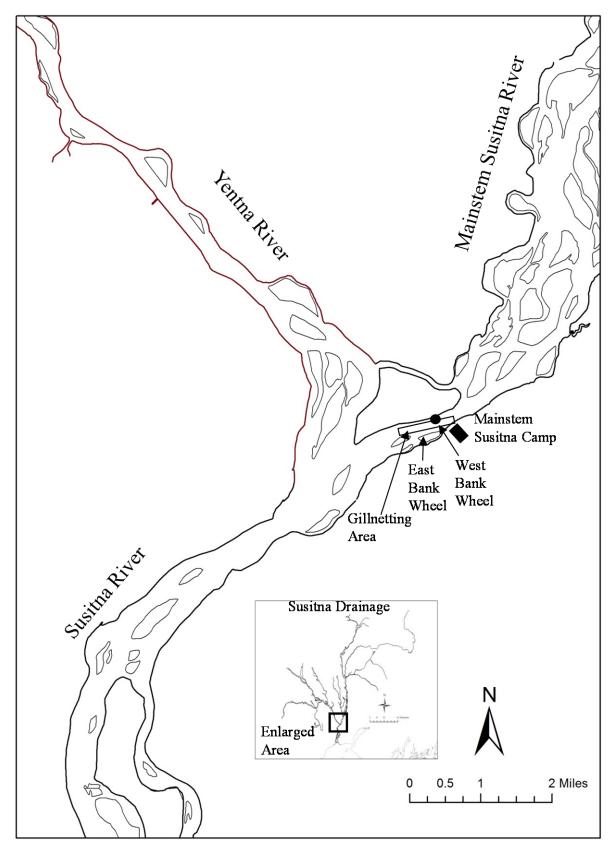


Figure 3.-Locations of fish wheels and gillnetting area at the RM 34 tagging site.

Abundance: Genetic Mixed-Stock Analysis

Genetic mixed-stock analysis will be used to produce a second mark-recapture estimate of Susitna River mainstem Chinook salmon inriver abundance in the event there is no sport harvest of Deshka River fish. A subsample of 380 PIT-tagged fish³ will be analyzed genetically to estimate the proportions of Susitna River mainstem, Deshka River, and Yentna River fish passing RM 34 of the mainstem Sustina River. These stock proportions along with Chinook salmon counts from the Deshka River weir will then be used to estimate abundance of the mainstem Susitna River stock passing RM 34.

Proportion of Non-Deshka Chinook Salmon in the Deshka River Sport Fishery

If the sport fishery is opened to harvest of Chinook salmon on the Deshka River, the harvest will be sampled for genetic tissue from 2 river sections. The first section will be from the confluence of the Deshka River and the mainstem Sustina River to an island approximately three-quarters of a mile from the confluence, and the second will be from the island to the weir. Subsamples of 95 sport harvested Chinook salmon per river section will be analyzed genetically to estimate the Deshka and non-Deshka components of the harvest from both sections.

Distribution: Proportion of Chinook Salmon in each of 5 Management Areas

Eight radio-telemetry tower stations will be set up at strategic locations (Figure 2) to record when a radio tag passes the tower going upstream or downstream. This will allow estimation of the proportion of Chinook salmon that spawned in each of 5 management zones (Figure 1). Assignments into management areas will be checked by aerial telemetry data that are collected during annual Chinook salmon aerial counts during late July.

SAMPLE SIZES

Abundance of Mainstem Susitna River Chinook Salmon (Objective 1)

Assessment of sampling effort necessary to achieve the precision criterion for Objective 1 was based largely on experience gained during the 2015–2020 experiments (Cleary et al. 2015; Cleary and Campbell 2016; Cleary et al. 2017; Campbell et al. 2018). Due to consistent capture methods, we expect sampling rate at the marking site (the proportion of the population passing the sampling site that is captured) will be similar in 2021 to that experienced in 2015–2020.

The approach of Robson and Regier (1964) was used to provide baseline sample sizes for a given population size and precision criterion under the assumption that a Petersen-type estimator will be used. These sample sizes were then interpreted in the context of probable violations of assumptions required for the Petersen estimator.

The 2015–2018 mainstem Susitna River Chinook salmon abundance estimates had to be stratified by size so we expect that size-stratified Petersen models will be needed for 2021 rather than an overall Petersen model based on pooled data. The costs of stratification to the 2015–2019 data in terms of increased CV of the abundance estimate were 1.5, 1.2, 1.2, 1.7, and 1.6 times, respectively. The following methods for determining sample size consider a size-stratified model.

³ The number of fish that can be analyzed at one time using the current laboratory platform is 380.

For the 2021 experiments, we assumed that the CVs of our final estimates of abundance using size stratification will be 1.4 times larger than if no stratification were necessary and a pooled Petersen-type model appropriate. We used the methods of Robson and Regier (1964) to calculate the necessary sample sizes to estimate the abundance of Chinook salmon in the Susitna River drainage upstream of the mouth of the Yentna River within 18% (1/1.4 = 0.71 of specified relative precision criterion of 25% stated for Objective 1) and we assumed these same sample sizes will allow us to estimate abundances of Chinook salmon within 25% of the true values 90% of the time under size stratification.

The proportion of radio tags that were censored (those that switched drainages or failed to enter the experiment) from the 2015–2020 radiotagging experiments averaged 33% and ranged from 15 to 45%, though censor rates from 2018 (43%) and 2019 (41%) are believed to be high anomalies (possibly related to unseasonably warm and smokey weather). To estimate the proportion of censored PIT-tagged fish in 2021, we excluded the two anomalies and assumed, based on the remaining years' data, we will censor 25% of our tags due to dropouts and drainage switches.

During 2015–2020, 596 (2015), 948 (2016), 624 (2017), 674 (2018), 1,048 (2019), and 667 (2020) Chinook salmon \geq 500 mm were caught in fishwheels and drift gillnets at the marking event from estimated populations of about 89,000, 66,000, 45,000, 30,604, 57,849, and 62,345 fish, respectively. These marking efforts correspond to 0.07%, 1.4%, 1.4%, 2.2%, 1.8%, and 1.1% of the population, respectively. From this, we assumed we will catch 1.3% (the average) of the Chinook salmon population \geq 500 mm at the mainstem marking site.

Using the assumptions and methods outlined above, the expected number of Chinook salmon that will be marked in the first event and the minimum sample size needed in the second event for estimation of population sizes between 20,000 and 100,000 were calculated (Appendix D1). The range of population sizes examined spans the 95% confidence intervals of the 2015–2020 mainstem Chinook salmon estimates. About 33% of the population needs to be sampled in the second event in the worst case (population of 20,000) to meet objective criteria (Appendix D1). In 2015–2020, an average of about 23% of the estimated population of Chinook salmon \geq 500 mm at RM 34 passed through the Deshka River weir. Given that the second event samples comprise all Deshka River weir fish greater than or equal to 500 mm, we are confident that for all but one of the scenarios presented in Appendix D1, sufficient sampling during the second event will occur to meet the Objective 1 criterion.

Proportion of Non-Deshka River Fish in Sport Harvest (Objective 2)

The Deshka River sport harvest area will be divided into 2 sections, one defined as the section of river from the Deshka River mouth to an island three-quarter miles upstream, and the other from the island to the Deshka River weir. It will be assumed that sampling from within each section of river will be done in a random manner.

With a sample of 95 fish from each section, the 90% relative precision of the estimate of the proportion of non-Deshka River fish in the harvest is 10%. This calculation incorporates error in GSI determination and sampling error. The precision of the estimate was derived by simulation. For each iteration of the simulation, a binomial (n = 95, P = 0.25) random variable was generated, yielding a simulated proportion p^* . We believe 0.25 is a conservative (high) estimate of the proportion of non-Deshka River Chinook salmon in the harvest. A random variable was then generated from a beta distribution with parameters determined from the mean, p^* , and the

variance dictated by the relative precision of the GSI estimate. The 90% relative precision of the GSI estimate of the proportion of non-Deshka River fish from a sample of 95 fish is 7% (A. Barclay, Fishery Biologist ADF&G, Anchorage, personal communication). Simulated relative precision was then calculated from the distribution of simulated proportions.

A minimum sample size of 95 fish from each river section will therefore allow estimates of the proportion of non-Deshka River fish in the harvest to meet the Objective 2 precision criteria.

Spawning Distribution (Objective 3)

The required sample size of radiotagged Chinook salmon in the mainstem Susitna River experiment, assuming 25% tag loss and uniform application of tags is 75, with 57 functioning tags (Objective 3; Thompson 1987). We will be applying 100 radio tags, and expect to have 75 functional tags, so Objective 3 criteria should be met.

DATA COLLECTION

Marking with PIT- and Radio Tags

Chinook salmon tagging will occur mid-May to 30 June 2021. Tagging will begin when water levels and debris loads allow for safe operations of fish wheels and gillnets. We believe these dates capture the majority of the run because catches are generally less than 5 fish per day when tagging begins and ends. At each site, the field crew will consist of 6 people: 2 for each 2-person fish wheel shift and 1 crew of 2 people will sample with drift gillnets in a 7.5 h split shift. At the mainstem Susitna River tagging site (Figures 2 and 3), 2 fish wheels (1 on each bank) will be operated for 12 h/d each. A 2-person crew will operate both wheels for the first 6 h shift followed by a different 2-person crew operating both wheels for the second 6 h shift. The total shift time will be 7.5 h where 1.5 h will be used for crew organization prior to and after shifts. In addition, a total of 7.5 h/d will be spent gillnetting in shifts of 3.75 h each.

Tag deployment data will be recorded on Rite-in-the-Rain data sheets and entered in Excel spreadsheets at camp. Fish wheel and gillnet catch and effort data will be recorded on the 2021 "Catch and Effort" data forms (Appendices B1 and B2). The forms will be filled with date, crew initials, total fish wheel operation time (or gillnet soak time), shift, start and stop times, crew arrival and departure time, and the total number of Chinook salmon tagged and untagged. In addition, the total number of other species captured for the shift will be recorded.

Fishwheel Operations

Both fish wheels will be operated every day of the season, except for flood events or when wheels need to be removed or repaired. Fish wheels will be aluminum, with three 6 ft wide or two 4 ft wide baskets webbed with knotless nylon 1.5-inch mesh netting (square measure). Captured fish will descend an aluminum basket chute to a fabric slide crossing above the float and exit into a live box. Live boxes will be 8 ft long, 2 ft wide, and 4 ft deep, with plywood sides with holes cut to allow water circulation. The configuration of the fish wheel axle, baskets, and floats make the fishing depth a maximum of 6.5 ft. Fish wheels will be tied to the riverbank and braced offshore with poles to position the wheels in sufficient current to make them spin. The axle height will be adjusted so that the baskets sweep as close to the river bottom as possible. A picket weir with 1.5-inch gaps between pickets will be installed between shore and the fish wheel injuries, closed-cell foam padding will be placed where appropriate to prevent injuries as fish

exit fish wheel basket chutes. The following is a set of guidelines used to direct sampling from the fish wheels:

- 1) Each fish wheel will be visited every 1 h or less. When a fish wheel has been untended for more than 1 h, all the fish in the live box shall be counted, measured, and released, but not tagged.
- 2) Fish with large, fresh injuries that are bleeding or fish that have been dropped in the boat used to access the fish wheels will be measured and released without being tagged.
- 3) No tagging will occur without first placing the fish in a water-filled tote with a cradle.
- 4) An orange PIT tag will be applied to every healthy Chinook salmon ≥500 mm METF length. The left operculum of each PIT-tagged fish will have a hole punched in it with a paper punch.
- 5) Every PIT-tagged Chinook salmon and every fifth Chinook salmon <500 mm METF length will have the distal 0.5 inches of the left axillary process removed and preserved in a uniquely numbered vial with ethanol (Appendix C1), and 4 scales will be collected and mounted on a uniquely numbered gum card (Appendix C3).
- 6) All Chinook salmon (both tagged and untagged) will be measured for METF length (Appendix C3), tallied on the data form (Appendix B1), and then released.
- 7) Other fish species will be tallied on the data form and then released.

Drift Gillnet Operations

To ensure all lengths of Chinook salmon are represented in the sample of fish \geq 500 mm METF length, drift nets will be used to supplement the catches from fish wheels. In 2013 and 2014, Chinook salmon captured in gillnets were larger on average than those captured in fish wheels (LGL and ADF&G 2014, 2015). It will be important to operate the drift gillnets as planned so that enough large fish can be tagged to provide a reasonably precise estimate of abundance in the larger size categories, should size stratification be required.

Prior to using new drift nets, old nets will be used to practice drift fishing and locate fishing sites that do not have snags. One drift gillnet mesh size (5.5 in, stretch measure) will be used. Nets will be 2 sizes: 10–12 feet deep and 15–17 feet deep. Drift locations, duration, and net depth will be changed accordingly to productive fishing site location(s) and depths or when net snags are found. One crew of 2 technicians will make as many drifts as possible during a 7.5-hour split shift. The desired capture technique will be to entangle fish by the snout to avoid injuries that gilling may cause. The net will be watched continuously until corks sink, then the net will be pulled in immediately.

Chinook salmon captured in drift gillnets will be processed as described for fish wheels above, including measurement and tally (Appendix B2).

Marking Effort

At the fish wheels, sampling will begin when the live box door is installed to hold captured fish, approximately 1 h after the crew starts its shift. The 1 h delay allows for sampling preparation and travel time. The first shift will begin at 0500 hours and will end at 1300 hours daily, and the second shift will be from 1400 to 2200 daily. After 6 h of effort during each shift, the live box door will be pulled so captured fish can escape. The fish wheel will be allowed to run in order to

prevent debris from building up on the submerged basket. The crew will spend the remainder of its shift performing data compilation and equipment maintenance.

PIT Tags

All captured healthy Chinook salmon \geq 500 mm METF length will receive an orange PIT tag (passive integrated transponder embedded dart tag, Model PDAT-PIT [HPT-12] from Hallprint, Australia) as the primary mark and a lower left operculum hole-punch as the secondary mark to allow assessment of tag loss. Each PIT tag will be associated with a unique dart tag number and unique PIT code.

To minimize handling stress, only Chinook salmon held in the live box less than 1 h will be tagged. Radiotelemetry data for coho salmon in the Kenai River (Carlon and Evans 2007) indicate that fish tagged immediately upon capture experience a mortality rate of 10% versus 20% for fish held for various times in a live box. Given that 1 crew (2 people) is tasked with sorting, tagging, and measuring fish from 2 fish wheels simultaneously, we feel a maximum 1 h holding time is a reasonable compromise. Live boxes will be checked at a maximum of 1 h intervals and the time of each check will be recorded.

Two-person crews will process selected salmon quickly to reduce handling time. Fish will be placed in a holding tank onboard a boat during tagging and a bucket will be used to frequently add water to the tank. A padded, aluminum cradle (Larson 1995) will be slipped around the fish to restrain it during tagging. One person will restrain the fish and the second will insert a PIT tag and record data. PIT tags will be inserted with stainless steel applicator needles immediately below the dorsal fin on the left side, anchored in the dorsal pterigiophores (bones). A paper punch will be used to punch a hole in the lower left operculum as a secondary mark to detect tag loss at the recapture site.

Radio Tags

One hundred of the PIT-tagged Chinook salmon \geq 500 mm METF length will also be radiotagged; 33 will be tagged per fish wheel and 34 with gillnets. Radio tags will be deployed each day in proportion to the daily average (2012–2020) historical run timing of fish \geq 500 mm METF length at the mainstem marking site (schedule given in Table 1). The first available healthy fish \geq 500 mm METF length will be radiotagged, thus avoiding selection bias by the crews. When the scheduled number of radio tags has been deployed for a particular fish wheel shift, the wheels will still run for the duration of the shift to continue with PIT-tagging. Similarly, once the scheduled number of radio tags has been deployed for a particular gillnet shift, netting will continue for the full duration of the shift to maximize the number of deployed PIT tags.

The number of radio tags scheduled to be deployed each day will be evenly split between the first and second shifts (AM vs. PM) and each fishwheel, with odd numbers of tags alternating between shifts and fishwheels (Table 1). If the scheduled number of radio tags cannot be deployed at a given wheel due to low catch during that shift, the leftover tags will be deployed by the next shift, even if it is the following day. The next shift will deploy its regularly scheduled tags first, then the leftover tags. This will continue until the leftover tags are deployed and the crew can get back on the original schedule. To increase chance that radio tags are deployed in proportion to the run, the number of tags deployed from each wheel may be adjusted depending on catch rates. Actual deployment of tags will be recorded in the "Catch and Effort" field data forms (Appendices B1 and B2).

	Fishwheel	1			Gillnet	Gillnet	
Date	AM	PM	AM	PM	AM	PN	
21 May	0	1	0	0	0		
22 May	0	0	1	0	1		
23 May	0	0	0	1	0		
24 May	1	0	0	0	0		
25 May	0	0	0	0	0		
26 May	0	1	0	1	0		
27 May	1	0	1	0	1		
28 May	0	1	0	1	0		
29 May	1	0	1	0	1		
30 May	0	1	0	1	1		
31 May	1	0	1	1	1		
1 Jun	1	1	0	1	0		
2 Jun	1	1	1	0	1		
3 Jun	0	1	1	1	1		
4 Jun	1	1	1	1	1		
5 Jun	1	0	1	1	1		
6 Jun	1	1	0	1	1		
7 Jun	1	1	1	1	0		
8 Jun	1	1	1	0	1		
9 Jun	0	1	1	1	1		
10 Jun	1	0	1	0	1		
11 Jun	0	1	0	1	0		
12 Jun	1	0	1	0	1		
13 Jun	0	1	0	1	0		
14 Jun	1	0	1	0	1		
15 Jun	0	1	0	1	0		
16 Jun	1	0	1	0	0		
17 Jun	0	0	0	0	0		
18 Jun	0	1	0	0	0		
19 Jun	0	0	1	0	1		
20 Jun	0	0	0	1	0		
21 Jun	1	0	0	0	0		
22 Jun	0	0	0	0	0		
23 Jun	0	0	0	0	0		
24 Jun	0	0	0	0	1		
25 Jun	0	0	1	0	0		
26 Jun	ů 0	1	0	0	0		
27 Jun	0	0	0	0	0		
28 Jun	ů 0	ů 0	0	0	0		
20 Jun	ů 0	ů 0	0	0	0		
Total	16	17	17	16	17	1	

Table 1.-Radiotag deployment schedule for fish wheels and gillnet by date and shift (AM and PM), 2021.

Note: Deployment indicated by "1" and "0" means not deployed.

Procedures to sample fish for radiotagging and to minimize handling stress will be identical to those described above for PIT-tagging. Radio tags will be inserted through the esophagus and into the upper stomach using a 0.38 inch (outside diameter), 12-inch long plastic tube. The antenna of the radio transmitter will be threaded through the tube and pinched by hand at the end of the tube such that the radio transmitter is held tightly against the opposite end of the tube. A paper punch will be used to punch a hole in the lower left operculum as a secondary mark. The crew will measure METF length and remove and preserve the distal 0.5 cm of the left axillary process of any radiotagged salmon.

Radiotelemetry

Towers

Radio receivers (ATS Model R4500C) at each stationary tracking site (Figure 1) will be visited and downloaded twice per month. Each record will contain the following fields in ASCII text format: year, Julian day, hour, minute, antenna, frequency, pulse code, signal strength, and duplicate counts. A laptop computer will be connected to the radio receiver with a serial cable and ATS software will be used to transfer the data file to the laptop. A logbook will be maintained at each station to note the date, staff, settings, and battery voltage for each visit. A checklist with radio receiver settings and the download steps will be at each site. Each downloaded file will be transferred to the Palmer local area network (LAN), uploaded to Docushare for ADF&G Region II (http://docushare.sf.adfg.state.ak.us/ dsweb/Homepage) and eventually appended into an SQL telemetry database.

Stations are positioned to bracket the downstream boundary of Yentna River, Deshka River, Eastside Susitna River, and Talkeetna River stocks, and the upstream boundary of the Eastside Susitna River stock. The current tracking protocol using fixed stations is designed to assign fish to the spatial area of the Eastside Susitna River and Talkeetna River stocks. Assignment is based on 1) detecting a fish passing a station at the lower (downstream) boundary of a stock area, and 2) not detecting it passing either below the downstream boundary station or above the upstream boundary station.

Aerial Data

In conjunction with aerial Chinook salmon counts (Oslund 2016), radio telemetry data will be collected while the aerial surveys are being performed in the mainstem Susitna River drainage. The purpose of this step is to verify the spawning location of radiotagged fish with respect to stationary tracking data. Aerial surveys are conducted as a separate project and the additional effort and expense of adding operating tracking equipment will be minimal.

A single Yagi 4-element antenna will be mounted to the helicopter and connected to a telemetry receiver with a coaxial cable. An external GPS receiver will also be connected to the receiver to get the GPS coordinates of each location where detections are made. Receivers will scan for all radiotag frequencies that have been deployed and record date, time, latitude, longitude, frequency, pulse code, mortality code, and signal strength any time it detects a radiotag. During each survey, a handheld GPS will be used to record a track of that day's flight path.

At the end of each survey, the receiver and handheld GPS will be connected to a computer to download the data for that day. All files will be stored on the local network. Postseason analysis of the information collected during these surveys will be used to determine likely spawning

locations of detected radiotagged Chinook salmon and verify management area assignments made using fixed telemetry stations.

Recapture PIT Tags at Deshka River Weir

A floating resistance board weir will be operated at RM 7 of the Deshka River from approximately 21 May to 3 September 2021. Sampling at the Deshka River weir will be conducted by an independent project and will follow a separate operational plan (Lescanec *In prep*). A dual-antenna, PIT-tag detection array will be attached to the upstream exit of the weir's sampling cage (Appendix A1). Crew members will regularly test the PIT array's detection rate. A biologist will be on call to troubleshoot issues over the phone and in person if required. The biologist will also make twice weekly visits to download the data file and make more in-depth checks of the system. System checks of the PIT-tag array are described in Appendix A1.

Tasks associated with the independent weir project will be as follows (see Lescanec *In prep* for more detail):

- 1) Clean and maintain the weir as needed to ensure its integrity.
- 2) Count and record all salmon through the weir by species.
- 3) For Chinook salmon, measure 325 proportionally sampled fish (sampling rate of 1 in 30 fish based on an inriver run size of 9,000 fish) for METF length (to the nearest 5 mm).
- 4) Look for a left operculum punch on all fish measured to assess any potential tag loss.
- 5) Note the presence of PIT-tagged fish as they are counted passing the weir and at what time they are seen. Capturing a tagged fish and reading the dart tag number may be done only if it does not disrupt movement of other fish.
- 6) Record water level and temperature.

The PIT-tag recapture technology involves a PIT-tag detection system deployed upstream of the weir trap to record PIT-tagged fish as they swim through the antennas (Appendix A1). Two antennas will be operated to increase the probability of detection. Two tests will be run daily to verify proper operation of the PIT-tag detection array (Appendix A1).

A trap incorporated into the weir will allow capture of fish for sampling. METF length will be measured on a subsample of the Chinook salmon. Fish sampled for METF length will also be examined for secondary marks to assess tag loss, although the test will be very weak given only about 325 fish will be sampled for ASL at the weir. Other species counted through the weir will be tallied.

Tissue Sampling for Genetic Mixed Stock Analysis of Tagged Salmon

A 1¹/₃ cm (half-inch) piece of the axillary process will be removed from each Chinook salmon that is tagged and from every fifth Chinook salmon <500 mm METF length captured in fish wheels and gillnets. Each sample will be placed in denatured ethanol in an individually labeled 2 ml vial (Appendix C1). All salmon samples and relevant collection data will be shipped to the ADF&G Division of Commercial Fisheries Gene Conservation Lab in Anchorage at the end of the season. All genetics sample processing, data storage, and data analysis will be the responsibility of the ADF&G Gene Conservation Lab.

For each of the 2 size strata (<500 m and \geq 500 mm), the genetic tissue samples collected throughout the season will be subsampled postseason to form a single mixture sample of 380 Chinook salmon (190 for each stratum) for genetic mixed stock analysis. Samples will be selected in proportion to the daily Chinook salmon counts to represent the seasonal catch.

Tissue Sampling of the Deshka River Sport Harvest

If harvest of Chinook salmon is allowed in the 2021 Deshka River sport fishery, a single crew member will be stationed at the Deshka Landing boat launch by 8:00 AM on days that are open to harvest of Chinook salmon. The majority of boat anglers fishing the Deshka River launch their boats from the Deshka Landing so this strategy will provide the maximum opportunity for interaction with anglers. The sampling crew will remain at the Deshka Landing until noon, or the number of boats returning falls to 1 per hour or less. Anglers in each returning boat will be approached by project staff, and if it is determined they have harvested Chinook salmon from the Deshka River, they will be asked for permission to collect a tissue sample from each harvested fish. Each tissue sample will be stored according to whether the fish was harvested in the section from the Deshka River mouth to an island three-quarter miles upstream, or from the island to the Deshka River weir. A map of the area will be provided to each angler to help them accurately identify where their harvest occurred. The number of Chinook salmon harvested per boat will also be recorded. In addition to ADF&G staff, 2 local fishing guides will also collect samples. Each guide will be supplied with two 250 ml bottles filled with ethanol; one labeled "mouth to island" and one labeled "between island and weir."

The genetic tissue samples collected throughout the season will be subsampled postseason to form a mixture sample of 95 Chinook salmon for genetic mixed stock analysis for each section of river (190 samples total). Samples will be selected in proportion to the daily Chinook salmon harvest encountered at the Deshka Landing to represent the seasonal harvest from each section of river.

Scale Collection

For every fish that had a genetic tissue sample collected by ADF&G staff (includes both fish at the marking event and fish from the harvest), 4 scales will be taken from the preferred location on the left side of the body at a point on a diagonal line from the posterior insertion of the dorsal fin to the anterior insertion of the anal fin and 2 rows above the lateral line (Welander 1940; Scarnecchia 1979; Appendix C3). If the preferred scales cannot be obtained, another scale will be taken from as close to the preferred scale as possible, always from the first or second row above the lateral line, in order to capture the early life history portion of the age. If no scales are available in the preferred area on the left side of the fish, scales will be collected from the preferred area on the right side of the fish. If scales are not obtainable from a given fish, that fish will not be sampled and sampling will continue with the next available fish.

Only scales from fish \geq 500 mm selected for genetic analysis will be aged; however, all scales collected from Chinook salmon under 500 mm will be aged to verify that Chinook salmon in this size category are all age 1.1.

Laboratory Analysis

Assaying Genotypes

Tissue samples will be genotyped following methods reported in Barclay and Habicht (*In prep*). Briefly, genomic DNA will be extracted from tissue samples using NucleoSpin 96 Tissue Kits by Macherey-Nagel (Düren, Germany). DNA will be screened for the 83 variant SNP markers reported in Barclay and Habicht (*In prep*) using Fluidigm 96.96 Integrated Fluidic Circuits. The Integrated Fluidic Circuits 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 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 genotyped.

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 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.

Reporting Groups

Three reporting groups that perform adequately for MSA within the Susitna River drainage were chosen for this study:

- 1) *Yentna* (Yentna River populations)
- 2) Susitna (Susitna River mainstem populations excluding Alexander Creek and Deshka River)
- 3) Deshka (Deshka River population)

Genetic Baseline

To estimate the proportion of *Yentna*, *Susitna*, and *Deshka* reporting groups in the fish wheel mixture, a baseline will be used containing the 30 populations from the Susitna and Yentna Rivers and 83 variant SNPs reported in Barclay and Habicht (*In prep*).

DATA REDUCTION

Crew from each sampling site will provide a daily summary of catch, effort, tags deployed or recovered, weather and water data, and any operational changes, to a lead project biologist at the Palmer Division of Sport Fish office via telephone 5 days per week.

All data collected by tagging crews (Appendices B1–B2) will be entered into Excel spreadsheets as they become available inseason and consolidated into a master Excel workbook file (Master_Susitna_2021_Chinook_ Abundance_mm_dd_yy.xlsx) with separate worksheets for each data type (e.g., tagging, recovery, fishing effort, etc.), and then stored in a dedicated subdirectory on the Palmer ADF&G LAN and uploaded to Docushare at the ADF&G Region II office (http://docushare.sf.adfg.state.ak.us/ dsweb/HomePage). A documentation spreadsheet will

also be incorporated into the workbook that describes the variables in each sheet. Only the project leader (N. Decovich) will have editing rights to the master workbook; when edited, the current date will be incorporated into the filename of the master workbook. All data files (.csv format) that are used in analyses by the R software package (R Core Team 2017) will be directly created from the latest master Excel file.

The master Excel file will serve as the basis for all data analysis required to achieve the study objectives. After all data are edited and analyzed, a final copy of the master Excel workbook and R analysis code will be emailed, along with a data map, to Research and Technical Services (RTS) in the Anchorage ADF&G office for archiving on the SF intranet site.

ASSUMPTIONS

Mark–Recapture Estimate

Chinook salmon abundance will be estimated with a Petersen-type estimator. For Petersen estimates of abundance to be unbiased, certain assumptions must be met (Seber 1982). These assumptions, expressed in the circumstances of this study, along with their respective design considerations and test procedures are as follows:

Assumption I: The population is closed to births, deaths, immigration, and emigration.

Considering the life history of Chinook salmon, there should be no recruitment (births, immigration) between sampling events. First event sampling (marking) will begin prior to any significant passage of fish past the tagging sites and will continue through the run until passage has dropped to near zero. With respect to emigration, some fish marked at the mainstem Susitna River marking sites will leave the system and migrate to the Yentna River. Also, some marked fish may fail to enter the experiment due to handling stress. Losses of fish due to either reason will be estimated from a sample of marked fish that are also instrumented with radio tags. Some fish may be harvested between the first and second events, but assuming the harvest rate on marked and unmarked fish is equal, then the PIT-tag abundance estimate at the marking site should remain unbiased.

In the genetic mark–recapture analysis, the marked fraction will be estimated from the stock proportions of *Susitna* and *Deshka* fish sampled from the population passing Sustina RM 34. Because the marked fraction is determined at the time of tagging, the estimate is not affected by handling effects, which manifest later. However, the population estimate from this analysis is sensitive to harvest between marking and recapture because harvest can change the total weir count (recaptured fraction) without changing the marked fraction in turn (traditional mark–recapture analysis assumes equal harvest rate of marked and unmarked fish such that the marked fraction is the same at both the first and second events). Thus, without accounting for how many Deshka River Chinook salmon were harvested, the population estimate at RM 34 will be biased low (see Equation 7 below).

Assumption II: There is no trap induced behavior.

There is no explicit test for this assumption because the behavior of unhandled fish cannot be observed. We will attempt to meet this assumption by minimizing holding and handling time of all captured fish. Any obviously stressed or injured fish will not be tagged. Examples include fresh seal bites that penetrate the muscle, capture injuries such as torn opercula, large skin wounds or broken snouts, or being dropped in the boat while tagging. This assumption does not apply to the genetics mark-recapture estimate; there are no physical second event recaptures for this method.

Assumption III: Tagged fish will not lose their marks between sampling events and all marks are recognizable.

We have found little evidence of tag loss in similar experiments conducted on the mainstem Susitna and Yentna Rivers in past years. We will continue to test this assumption by examining Chinook salmon sampled in the ASL sample at the Deshka River weir, although the test will be weak due to the small sample size. A fish with a secondary mark, but no PIT tag will indicate the PIT tag (primary mark) has been lost. This assumption does not apply to the genetics mark–recapture estimate.

Assumption IV: One of the following 3 conditions will be met:

- 1) Marked fish will mix completely with unmarked fish between samples.
- 2) All Chinook salmon will have the same probability of being captured in the second event.
- 3) All Chinook salmon will have the same probability of being caught in the first event.

With respect to the first condition, it is impossible that marked and unmarked fish will mix completely. Fish wheels and gillnets will be operated continuously during the run, with fish marked early in the run never having the opportunity to mix with unmarked fish from later stages of the run by the time they are sampled in the second event.

With respect to the second condition, the second event only consists of the weir at the Deshka River and therefore probability of capture cannot be uniform in the second event. All (100%) of the Deshka River population will be sampled in the second event, whereas none (0%) of the remaining stocks will be sampled in the second event.

With respect to the third condition, the marking event involves use of fish wheels and gillnets across the river and consistently through time. This design is intended to sample the population uniformly. However, fluctuations in water levels can affect the efficiency of fish wheels, resulting in variation in probability of capture over time. Also, the probabilities of capture by fish wheels may vary between banks due to differences in channel morphology and water flow (Yanusz et al. 2007). Further, uneven fishing efficiency and effort between gillnets and fish wheels may also result in uneven probability of capture between midriver and bank-oriented populations, and probability of capture may differ among size categories.

Unlike previous Susitna River mark-recapture studies, spatial diagnostic tests ("Equal Proportions Test"; Arnason et al. 1996) cannot be conducted for this study because there is only the single recapture event (Deshka River weir). In addition, temporal tests of marked fractions in the recapture event are considered unreliable due to the documented effects of tagging on sulking behavior of Chinook salmon (Bernard et al. 1999). Size-based tests of differential probability of capture are, however, still possible using length data from marked fish from the first event and captured and recaptured fish from the second event at the Deshka River weir.

The accuracy of the final abundance estimate will depend on the partially untestable assumption that the probability of capture in the first event was spatially even. It is noted, however, that all three ADF&G mark–recapture estimates of Chinook salmon abundance at RM 34 of the Susitna

River from 2015 through 2017⁴ were analyzed as simple Petersen estimates within 2 size strata (DeCovich et al. 2020). The spatial test of probability of capture was only significant in 1 of 6 instances in these estimates. In the significant case, the "Mixing Test" (Arnason et al. 1996) allowed the Petersen estimate to be used. Given our ability to continue testing and correcting for size-related probability of capture failures and our historical success at using the Petersen estimate, we believe it is likely that the mark–recapture experiment will yield unbiased results.

Length data will be analyzed for statistical bias due to gear selectivity (Appendix D2). If different probabilities of capture by size are indicated, data will be fully stratified into size groups where probability of capture is homogeneous within groups, and abundance estimates will be calculated for each size group and summed.

Contingency table analyses recommended by Seber (1982) and described in Appendix D3 will be used to determine if a Petersen estimate can be used. The "Mixing" and "Equal Proportions" tests (Tests I and II in Appendix D3) will not be performed for the reasons described above. Test III in Appendix D3 will be performed. Based prior experience, it is anticipated that a pooled Petersen estimator will be used, possibly within each of 2 size strata.

Spawning Distribution

Spawning distribution by management area will be calculated as the proportion of all radio tags achieving upstream migration that enter each area. This calculation assumes an even application of radio tags to the stocks migrating past the tagging site. Although past experiments have revealed evidence of bank orientation at the tagging site, equal application of radio tags across the river in the past has produced radio telemetry estimates of the Deshka River escapement (product of mark–recapture estimate and telemetry-based estimate of the Deshka River proportion) that are similar to the Deshka River weir count. This result is consistent with the assumption of uniform application of radio tags to stocks at the tagging site.

DATA ANALYSIS

PIT-Tag Abundance Estimates

A 2-sample mark-recapture model will be used to estimate the number of Chinook salmon passing by the first event sampling site. The appropriate abundance estimator will depend on the results of tests of size and sex selective sampling (Appendix D2). If stratification is not needed, Chapman's (1951) version of Petersen's abundance estimator for closed populations (see Seber 1982) will be used:

$$\hat{N} = \frac{(\hat{M}_U + 1)(\hat{C} + 1)}{(R+1)} - 1 \tag{1}$$

where

 \hat{N} = estimated number Chinook salmon at RM 34,

 \hat{M}_{U} = the estimated number of marked Chinook salmon moving upstream of the Susitna River mainstem tagging site and remaining in the mainstem river,

⁴ Between 2015 and 2017 we operated multiple recapture sites and could explicitly test for equal probability of capture.

 \hat{C} = the estimated number of Chinook salmon \geq 500 mm that are inspected for marks at the second event sampling sites, and

R = number of marked Chinook salmon recaptured during second event sampling.

For Chinook salmon, we will estimate $M_{_{U}}$ as follows:

$$\hat{M}_U = \hat{p}_{UP} M \tag{2}$$

where M is the total number of marked Chinook salmon, and

$$\hat{p}_{UP} = \frac{r_{up}}{r} , \qquad (3)$$

and where r is the number of radio tags applied and r_{up} is the number of r that entered the mark-recapture experiment.

We will estimate \hat{C} as follows:

$$\hat{C} = C_T \hat{p}_{500+}$$
 (4)

where

 C_{T} = total number of Chinook salmon counted past the Deshka River weir and

 \hat{p}_{500+} = estimated proportion of Chinook salmon at the Deshka River weir that were \geq 500 mm METF.

The proportion \hat{p}_{500+} is estimated from length composition data at the Deshka River weir:

$$\hat{p}_{500+} = n_{500+} / n \tag{5}$$

where

n = total number of Chinook salmon sampled for length at the Deshka River weir, and

 n_{500+} = those members of *n* that were \geq 500 mm METF.

If stratification by size or sex is required (Appendix D2), the data will be fully stratified and estimates for each stratum will be generated using Equations 1–5. Stratum estimates of abundance and variance (see below) will be summed over size strata for estimates pertinent to the entire population.

An estimate of the variance for \hat{N} within a size stratum will be obtained through simulation. The estimated number of marks continuing upstream will be simulated as a binomial variable $[\hat{M}_U^* - \operatorname{bin}(M, \hat{p}_{Up})]$, and the number of recaptures R will be modeled as a binomial variable $[R^* - \operatorname{bin}(\hat{C}, \hat{M}_U / \hat{N})]$. The number of Chinook salmon ≥ 500 mm METF length at the Deshka River weir will be modeled as binomial variables $\operatorname{bin}(C_T, \hat{p}_{500+})$, and simulated values \hat{C}^* will be calculated

using Equation 4. A large number of simulated values R^* , \hat{M}_U^* , and \hat{C}^* will be generated, and simulated samples of the abundance estimate \hat{N}^* will be calculated using Equation 1.

A minimum of 1,000,000 simulations (B) will be drawn. The approximate variance of \hat{N} will be calculated as follows:

$$\operatorname{var}(\hat{N}) = \frac{\sum_{b=1}^{B} (\hat{N}_{b}^{*} - \hat{\overline{N}}^{*})^{2}}{B - 1}$$
(6)

where $\hat{\overline{N}}^*$ is the average of the \hat{N}_b^* . Confidence intervals will be calculated from the B simulations using the percentile method.

Size stratification tests will be conducted first and the data partitioned into appropriate size classes. Test III in Appendix D3 will be conducted within each size stratum. A nonsignificant result of this test may indicate that the Chapman–Petersen model is adequate, without the need for assumptions relating to even probability of capture in the marking event. A significant result means the assumption of even marking is required.

Genetic Abundance Estimates

In the absence of sport harvest on the Deshka River, abundance at the mainstem RM 34 marking site as estimated from genetics mark–recapture will be calculated as follows. The reason for this condition is that the genetics estimate depends on recovering 100% of the Deshka River fish; a sport harvest will reduce the number measured at the weir and the estimate will be biased low.

The stock composition of fish captured at the Susitna RM 34 fish wheels will be estimated using the R package *rubias* (Pella and Masuda 2001). The *rubias* package is a Bayesian approach to the conditional genetic stock identification model based upon computationally efficient C code implemented in R. It uses cross-validation and simulation to quantify and correct for biases in reporting group estimates. The mixture samples will be analyzed with a single Markov Chain Monte Carlo chain (MCMC) with 25,000 iterations and the first 5,000 iterations will be discarded to remove the influence of starting values. The prior parameters for each reporting group will be defined to be equal and will sum to 1 (i.e., a flat prior). Within each reporting group, the population prior parameters will be divided equally among the populations within that reporting group. To correct for bias in the MCMC reporting group estimates, an additional parametric boot strapping step will be performed by simulating 100 mixtures with similar stock composition as the MCMC estimates. The degree of bias observed in the simulated mixture analyses will then be used to correct the MCMC estimates. Stock proportion estimates and the 90% credibility intervals for each proof test mixture will be calculated by taking the mean and 5% and 95% quantiles of the posterior distribution from the single chain output. The *rubias* posterior output of stock composition estimates from the genetic mixed-stock analysis of a mixture sample of 380 PIT-tagged fish will be used to estimate the number of Susitna River mainstem Chinook salmon passing RM 34. Abundance estimates will be produced for each iteration *i* of the posterior using a modified Chapman's estimator (Seber 1982):

$$\widehat{N}_{i} = \frac{(W+1)(\widehat{S}_{i}+1)}{\widehat{D}_{i}+1} - 1$$
(7)

where

- \widehat{N}_i = estimated number Susitna River mainstem Chinook salmon in MCMC iteration *i*,
- W = the number of Chinook salmon passing the Deshka River weir site (= number inspected for marks),
- \hat{S}_i = the estimated number of Chinook salmon of Susitna River mainstem origin in the 380sample mixture (= number of marked fish), and
- \widehat{D}_i = the estimated number of Chinook salmon of Deshka River origin in the 380-sample mixture.

We will estimate \hat{S}_i as follows:

$$\hat{S}_i = M \left(\hat{s}_i + \hat{d}_i \right) \tag{8}$$

where *M* is the number of fish in the mixture sample, \hat{s}_i is the estimated proportion of the *Susitna* reporting group in the mixture, and \hat{d}_i is the estimated proportion of the *Deshka* reporting group in the mixture.

We will estimate \widehat{D}_i as follows:

$$\widehat{D}_i = M(\widehat{d}_i). \tag{9}$$

The variability of the \hat{D}_i and \hat{S}_i over MCMC iterations incorporates both uncertainty from the mixture model estimation and sampling variability. The mean Susitna River mainstem abundance estimate and 90% credibility intervals will be calculated by taking the mean and 5% and 95% quantiles of the posterior distribution of abundance estimates.

Proportion of non-Deshka River Chinook Salmon in Harvest

If there is a sport fishery, the proportion of non-Deshka River Chinook salmon in the sport harvest from each section of the Deshka River will be estimated using the *rubias* mixture analysis protocol described above.

Proportion of Chinook Salmon in Each of 5 Management Areas

The diagnostic procedures described in Appendix D2 will be used on radiotagged Chinook salmon to investigate size-mediated variability in probability of capture and subsequent radiotagging during the marking event. The test results will guide size stratification. Size stratification points will automatically include those associated with the PIT-tag abundance analysis and may include additional size strata, depending on the results of the tests in Appendix D2.

For each marking size stratum, radiotagging data will be used to estimate spawning distribution:

$$\hat{p}_{l,s} = n_{l,s} / n_s \tag{10}$$

where $\hat{p}_{l,s}$ is the estimated proportion of salmon from stratum *s*, spawning management area *l*, n_s is the number of fish radiotagged in stratum *s* that travelled to a spawning area, and $n_{l,s}$ is the number of fish from n_s that travelled to area *l*.

The total number of salmon spawning in area *l* can be estimated as follows:

$$\hat{N}_{l} = \sum_{s=1}^{S} \hat{N}_{s} \hat{p}_{l,s}$$
(11)

where N_s is the abundance estimate for size stratum *s*, and the proportion of salmon spawning in each area is estimated as follows:

$$\hat{p}_{l} = \hat{N}_{l} / \sum_{s=1}^{S} \hat{N}_{s}$$
 (12)

Variance for these parameters will be estimated using simulation. Variation in estimates of spawning distribution parameters within each of *S* strata will be modeled using multinomial distributions and the observed data described in Equation 10.

Equations 11 and 12 will then be used to provide simulated estimates of spawning distribution proportions. Variance for each of these parameters will then be estimated using methods analogous to Equation 6.

Dates	Activity		
Approximately 22 May-30 June 2021	Marking operations at RM 34 mainstem Susitna River site		
Approximately 19 May-15 July 2021	Weir sampling at Deshka River		
15 September–31 December 2021	Data reduction and analysis		
30 March 2022	Finalized 2021 Fishery Data Series Report. Genetics results will be reported separately, to be determined by ADF&G Gene Conservation Lab		

SCHEDULE AND DELIVERABLES

RESPONSIBILITIES

John Campbell, Fishery Biologist II

Duties: Lead all radiotelemetry and PIT data recovery and tracking portions of project, and supervise project FB I. Coordinate data collection, data analysis, purchasing, reporting, crew training, radiotracking station setup and downloads. Assist with hiring and writing the operational plan. Coauthor on report.

Adam Reimer, Biometrician

Duties: Advise all portions of the biometrics including planning, sample sizes, statistical methods, and data analysis. Perform data analysis and produce final estimates. Coauthor on report.

Andy Barclay, Fishery Biologist III

Duties: Advise portions of the genetics: planning, sample sizes, statistical methods, data analysis, and reporting. Supply tissue collection materials and instructions.

Nick Decovich, Fishery Biologist III

Duties: Supervise all aspects of project (excluding data analysis): planning, budget, data collection, and reporting. Lead author on operational plan and report.

Steve Dotomain, Fishery Biologist I

Duties: Supervise the mainstem Susitna River site and assist with planning, hiring, and training field staff, data collection, data analysis, supervision, and purchasing. Assist with writing the operational plan and final report.

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APPENDIX A: PIT TAG METHODS

Appendix A1.–Passive integrated transponder (PIT) tag detection methods.

All healthy Chinook salmon \geq 500 mm mideye to tail fork (METF) length captured at the mainstem Susitna River site (RM 34) will be tagged with an orange, 14 cm long, vinyl, dart tag containing a PIT (model PDAT-PIT [HPT-12] from Hallprint, Australia). Each PIT tag (Figure A1-1) is associated with a unique number (10,000–25,000) printed twice on the dart tag (bottom and top portions), contact information for the researchers, and an embedded Biomark (Biomark Inc.) high performance FDX-B glass PIT (HPT-12).

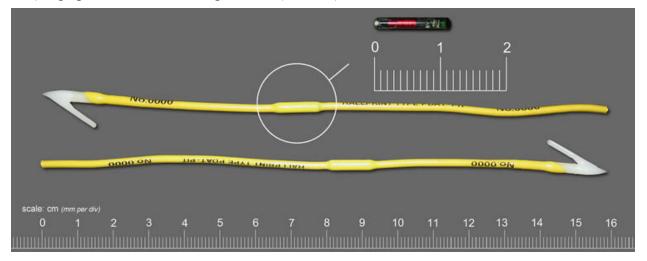


Figure A1-1.–Example of Hallprint PDAT-PIT tag.

Each tag will be applied beneath the dorsal fin with a hollow 8-gauge stainless steel applicator needle. In addition to the PIT tag, each fish will also receive a left operculum hole punch to estimate tag loss at the recapture sites. Instructions from the tag manufacturer are quoted as follows:

HALLPRINT TECHNICAL NOTES 2 APPLICATION INSTRUCTIONS FOR PLASTIC TIPPED DART TAGS - TUNA

This information is for guidance to the first-time user. Procedures will vary, depending on the species to be tagged, size of tag, fish and field conditions.

Always check correct location of the dart head etc. before engaging in actual tagging operations (see over).

Tag description

Plastic tipped dart tags are constructed from a cylindrical printed and numbered marker, moulded to a plastic barbed head. Several sizes/shapes of dart head are used in combination with various length/diameter markers.

-continued-

Appendix A1.–Page 2 of 4.

(quoted instructions continued)

Loading of applicator

It is a good idea to keep a pre-loaded one, with the tags, your measure, notes and a pen, in a handy place so that the fish does not have to wait too long for you to find them.

Load tag with only the barb exposed at the pointed end.

If the tag does not slide easily out of applicator then it is either choked with debris or bent. This must be rectified otherwise you will probably get a hung-up tag.

Loosely fitting tags can be secured by making a slight bend in the printed marker—do not alter the applicator.

Some researchers prefer to use a handle which can be made from a short length of 20 mm diameter dowel with a hole drilled in one end. Retractable and non-retractable fabricated handles are available (other than for PDX/PDXL needles) from Hallprint if needed.

Insertion of tags into fish

Minimize trauma and damage to fish. Keep it under control. If it has noticeably suffered by capture do not waste time tagging it. Release gently, or keep if legal and you intend to eat.

1) Remove a scale with the applicator point just below the base of a dorsal spine on the second dorsal fin (see over). Avoid placing the tag too deeply into muscle.

2) Hold needle with exposed tag barb in line with fish, with barb facing head. <u>Turn needle</u> so barb is on the fish side.

3) Start inserting the needle at a shallow angle under the scales until you feel it pierce the skin, then raise the needle to an angle of 45 degrees so making clearance for the barb.

4) When barb is below skin, return to a shallow angle and insert until the barb is just beyond the fin spine. A slight "click" can be felt as the barb slides over the bone and locks behind it.

5) Pause for a second then withdraw the needle smoothly. A slight tug will help "set" the tag. Particularly with small tags/small fish do not place any undue strain on either tag or fish after insertion.

6) The fish should then be gently released. Fish showing undue stress, damage or inability to swim should not be released if tagged.

(end quote)

Prior to deployment, all PIT tags will be scanned with a 134.2 kHz signal from a Biomark 601 hand-held reader to ensure that the PIT tag is operating properly and to determine its unique code. The code will be recorded along with the corresponding dart tag number. The same process will be performed independently by a different person to ensure that each code and corresponding dart tag number is correct.

-continued-

Appendix A1.–Page 3 of 4.

Antenna Set-Up

A double antenna, Biomark PIT detection system will be installed immediately upstream of the fish cage at the Deshka River floating weir (Figure A1-2). The system will consist of a 2 m long, 1 m wide, and 1.5 m tall U-shaped chute, constructed of 2.5 cm size mesh polyethylene netting, that will force fish that have passed through the weir cage to swim through two 1.2 m \times 1.5 m Biomark antennas located 1 m and 2 m upstream of the weir.



Figure A1-2.-PIT detection antennas above the Deshka River floating weir and cage, 2015.

Chest Enclosure

Antennas will have 30 m Biomark antenna exciter cables attached to them and will be routed along the stream bed to the stream bank where they will be connected to a Biomark IS1001 chest enclosure (Figure A1-3), which will contain 2 Biomark IS1001 24V control nodes, a Biomark IS1001 data logger, a Biomark IS1001 data logger board, and two 12V, 75AH, maintenance-free batteries. The system will be kept charged by a 200 W solar panel mounted 1 to 2 m above the chest enclosure in an area that receives direct sunlight. A 24 V charge controller will be used to control the voltage being supplied to the batteries and prevent the batteries from being drained during low light periods.

-continued-

Appendix A1.–Page 4 of 4.



Figure A1-3.-Biomark IS 1001 chest enclosure showing the components and the 2 antenna exciter cables.

System Checks

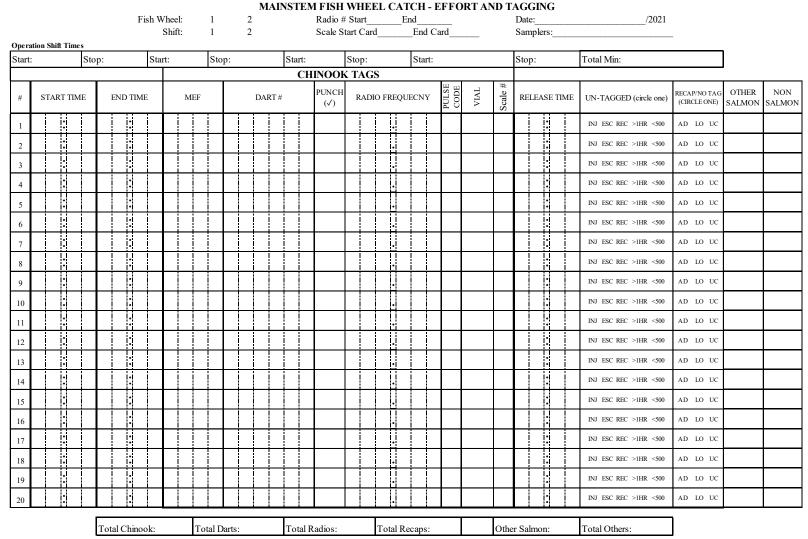
In order to ensure that the system is operational and the antennas are working properly, 2 tests will be performed daily. In addition to daily checks of the battery voltage, tests will be performed to ensure that any PIT tags passing through the antennas are being recorded.

The first test will be performed every morning and afternoon. It will consist of testing the antennas by using a test PIT tag mounted to a 2 m long, 2 cm diameter wooden dowel. During periods of time when the trap door is closed on the weir (no fish passing), the PIT tag will be moved through all areas of each antenna to ensure that there are no "dead spots" where the tag is not being detected. If dead spots are detected, the systems will be configured until there are no dead spots.

The second test will also take place as conditions allow. As the weir crew passes fish, they will record the time they observe an orange dart-tagged fish passing through the weir. Twice per week, the PIT detection data will be downloaded and the crew will assess whether a PIT tag was detected during the time period that the dart-tagged fish was observed passing through the weir. The system has real time indicators for when a tag is detected, and if circumstances allow, this test can be used to provide immediate evaluation of the system.

APPENDIX B: FIELD DATA FORMS

Appendix B1.–Fish wheel catch and effort field data form.



* AD, LO, UC = Adipose, left operculum, upper caudal

* Salmon: Chinook=King=KS, Sockeye=SO, Coho=CO, Chun=CU, Pink=P. NP=Northern Pike, B=Burbot, AG=Arctic Grayling, RT=Rainbow Trout, BC=Bering Cisco, HWF=Humpback Whitefish, RWF=Round Whitefish, LNS=Longnose Sucker, AC=Arctic Char. INJ=Injured, ESC=Escaped, REC=Recaptured

Comments:

Appendix B2.-Gillnet catch and effort field data form.

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MAINSTEM GILL NET CATCH - EFFORT - TAGGING

* AD, LO, UC = Adipose, left operculum, upper caudal

Total Min:

Total Chinook:

* Salmon: Chinook=King=KS, Sockeye=SO, Coho=CO, Chum=CU, Pink=P. Others: NP=Northern Pike, B=Burbot, AG=Arctic Grayling, RT=Rainbow Trout, BC=Bering Cisco, HWF=Humpback Whitefish, RWF=Round Whitefish, LNS=Longnose Sucker, AC=Arctic Char. INJ=Injured, ESC=Escaped, REC=Recaptured

Total Radios:

Total Recaps:

Total Others:

Total Darts:

Comments:

APPENDIX C: BIOLOGICAL SAMPLING PROCEDURES

Appendix C1.–Genetic tissue sample collection procedures.

Non-lethal Sampling of Finfish Tissue for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use axillary process samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. This is a non-lethal method of collecting tissue samples from adult fish for genetic analysis. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal fins.

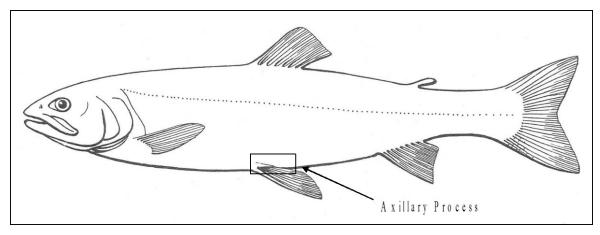
Sample preservative: Ethanol (ETOH) preserves tissues for later DNA extraction without having to store frozen tissues. Avoid extended contact with skin.

II. Sample procedure:

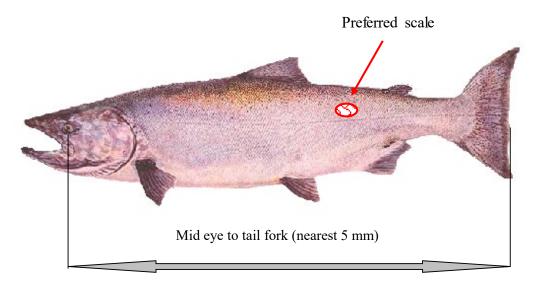
- 1. Tissue type: Axillary process, clip axillary process from each fish (Appendix C2).
- 2. Data to record: Record each vial number to paired data information.
- 3. Prior to sampling, fill the tubes half way with ETOH from the squirt bottle. Fill only the tubes that you will use for a particular sampling period.
- 4. To avoid any excess water or fish slime in the vial, wipe the axillary process dry prior to sampling. Using the dog toe nail clipper or scissors, clip off axillary process (1/2 -1" max) to fit into the cryovial.
- 5. Place axillary process into ETOH. The tissue/ethanol ratio should be **slightly less than 1:3** to thoroughly soak the tissue in the buffer.
- 6. Top up tubes with ETOH and screw cap on securely. Invert tube twice to mix ETOH and tissue. Periodically, wipe the dog toe nail clippers or scissor blade so not to cross contaminate samples.
- 7. Discard remaining ethanol from the 500ml bottle before returning samples. **Tissue samples must remain** in **2ml ethanol** after sampling. HAZ-MAT paperwork will be required for return shipment. Store vials containing tissues at cool or room temperature, away from heat in the white sample boxes provided. In the field: keep samples out of direct sun, rain and store capped vials in a dry, cool location. Freezing not required.
- III. Supplies included with sampling kit:
 - 1. (1) Dog toe nail clipper used for cutting the axillary process
 - 2. (1) Scissors can be used to cut a portion axillary process if clippers don't work for your crew
 - 3. Cryovial- a small (2ml) plastic vial, pre-labeled.
 - 4. Caps with or without gasket to prevent evaporation of ETOH.
 - 5. Cryovial rack- white plastic rack with holes for holding cryovials while sampling
 - 6. Ethanol (ETOH) in (2) 500 ml plus (1) 125 ml Nalgene bottle
 - 7. Squirt bottle to fill or "top off" each cryovial with ETOH
 - 8. Paper towels use to blot any excess water or fish slime off axillary process
 - 9. Printout of sampling instructions
 - 10. (3) three pair of lab gloves (size large)
 - 11. Laminated "return address" label

IV. Shipping: HAZMAT paperwork is required for return shipment of these samples and is included in the kit.

Ship samples to: ADF&G - G	enetics	Lab staff:	1-907-267-2247
333 Raspberr	ry Road	Judy Berger	r: 1-907-267-2175

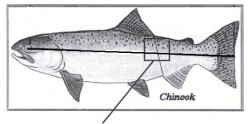


Appendix C2.–Location of axillary process.



Length measurements are taken mid eye to tail for to the nearest 5 mm.

The preferred scale is located on the left side of the fish, 2 rows above the lateral line along a diagonal line from back (posterior) of the dorsal fin to the front (anterior) of the anal fin.



Preferred Area

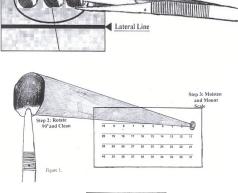
Pluck the "preferred scale" from the fish using forceps.

Pliers may be necessary to remove scales if the fish has been in freshwater for an extended period.

Remove all slime, grit, and skin from scale by moistening and rubbing between thumb and forefinger. Moisten the clean scale and mount it on the gummed card directly on top of the number "1."

A good scale has a well-rounded shape.

Hold scale up to light and examine for overall size, shape, regeneration, deformities, etc.



Step 1: Pluck Scale



-continued-

Correct scale mounting

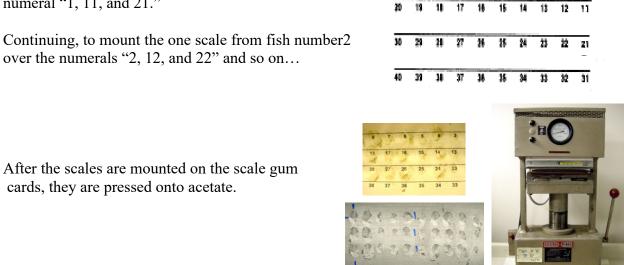
Incorrect scale mounting

After the scales are mounted on the scale gum cards, they are pressed onto acetate.

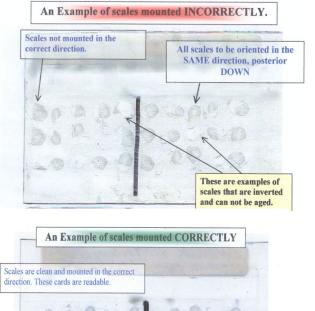
over the numerals "2, 12, and 22" and so on...







10



When sampling Chinook salmon, take 3 scales per fish. Mount scale from fish number 1 over the numeral "1, 11, and 21."

Appendix C3.–Page 2 of 3.

Appendix C3.–Page 3 of 3.

Common problems encountered with inexperienced scale collectors include torn edges, inadequate scale cleaning, selecting regenerated or distorted scales, inverted scale mounting, and dirty gum cards. Common data recording errors include recording the scale number for sample, incorrect number of scale samples collected than recorded in data, and more than 1 fish with the same collection number. The following steps should help resolve these problems:

- 1) Experienced staff must take extra measures to ensure that less experienced staff become fully proficient at sampling before the first sampling event. Before the first sampling event, take a fish and slowly walk through the sampling routine with less experienced crew. Be sure to demonstrate steps 2–6.
- 2) Locate the lateral line and preferred scale sampling area.
- 3) Identify irregular scale patterns that are the result of regenerated scales.
- 4) Remove the scales in a manner that reduces torn edges.
- 5) Properly clean and mount scale samples.
- 6) Identify inversely mounted scales.

Minimize the handling of gum cards and keep them as dry as possible. Wet gum cards should be dried out slowly. Excessive heat when drying may cause the scale to become unglued from the gum card. After the gum cards are dry, they should be stored with wax paper between each gum card. Check the numbering between the Access database and the gum card.

Reading scale age

Readers will review a test set of 50 scale samples from both Chinook and coho salmon. The test set contains scale samples from 2003 through 2007 for each species from various locations. Chinook salmon scale samples will include some fish of known ages (e.g., hatchery fish). Reader's test-set ages will be compared to previously determined age estimates and known ages. Ages that do not match will be reviewed and re-read. Once the reader ages are resolved, then the reader will begin with the collected samples from this season.

To estimate scale age, at least 1 scale per sample must have all of the following:

- 1) a clean focus
- 2) little or no regeneration in the freshwater growth
- 3) minimal tearing on the edge
- 4) clearly identified annuli through winter growth periods and crossing over of rings

If none of the scales from a sample contain all of these characteristics, than the age will be recorded as "NR" not readable. Samples with differing scale age estimates (i.e., scale 1 = 2; scale 2 = 2; scale 3 = 1) will be recorded as "NR."

A large number of scales have been collected from the projects. It is better to reject a fish from the samples than to use questionable scales.

APPENDIX D: SAMPLE SIZES AND TESTS OF MARK-RECAPTURE ASSUMPTIONS

				2nd ev	vent
Population size (N)	Marks deployed	Mark loss	Valid marks	Sample size needed	Sample % of N
100,000	1,300	25%	975	8,884	8.9
80,000	1,040	25%	780	8,691	10.9
60,000	780	25%	585	8,387	14.0
40,000	520	25%	390	7,839	19.6
20,000	260	25%	195	6,555	32.8

Appendix D1.–Anticipated sampling rates and sample sizes necessary to estimate mainstem Chinook salmon abundance within 25%, 90% of the time using a size-stratified Petersen model and adjusting for 25% loss of marked fish.

Note: Marks deployed based on average estimated capture rate in 2015–2020 (0.013).

Appendix D2.-Detection and mitigation of selective sampling during a 2-event mark-recapture experiment.

Size- and sex-selective sampling may cause bias in 2-event mark-recapture estimates of abundance and size and sex composition. Kolmogorov-Smirnov (KS) 2-sample tests are used to detect size-selective sampling, and contingency table analyses (chi-square tests of independence) are used to detect evidence of sex-selective sampling.

Results of the KS and chi-square tests will dictate whether the data need to be stratified to obtain an unbiased estimate of abundance. The nature of the detected selectivity will also determine whether the first, second, or both event samples are used for estimating size and sex compositions.

Definitions

- M = the distribution of lengths or sexes of fish marked in the first event,
- C = the distribution of lengths or sexes of fish inspected for marks in the second event, and
- R = the distribution of lengths or sexes of fish marked in the first event and recaptured in the second event.

Size-selective sampling: KS tests

Three KS tests are used to test for size-selective sampling:

Test 1	C vs R	Used to detect size selectivity during the 1st sampling event. H _o : Length distributions of populations associated with C and R are equal.
Test 2	M vs R	Used to detect size selectivity during the 2nd sampling event. H _o : Length distributions of populations associated with M and R are equal.
Test 3	M vs C	Used to corroborate the results of the first two tests. H _o : Length distributions of populations associated with M and C are equal.

Sex-selective sampling: chi-square tests

Three contingency table analyses (chi-square tests on 2×2 tables) are used to test for sexselective sampling:

Test 1	C vs R	Used to detect sex selectivity during the 1st sampling event. H _o : Sex is independent of the C–R classification.
Test 2	M vs R	Used to detect sex selectivity during the 2nd sampling event. H _o : Sex is independent of the M–R classification.
Test 3	M vs C	Used to corroborate the results of the first two tests. H _o : Sex is independent of the M–C classification.

-continued-

Appendix D2.–Page 2 of 3.

There are several possible results of selectivity testing, interpretation, and prescribed action (Table D2-1).

Table D2-1.–Possible results of selectivity testing, interpretation, and action.

_		KS or chi-square tes	st	_	
Case	M vs R (2nd event test)	C vs. R (1st event test)	M vs. C (1st vs 2nd event)	Interpretation and	action
Ι	Fail to reject H _o	Fail to reject H _o	Fail to reject H _o	Interpretation:	No selectivity during either sampling event.
				Action: Abundance: Composition:	Use a Petersen-type model without stratification. Use all data from both sampling events.
II	Reject H _o	Fail to reject H _o	Reject Ho	Interpretation:	No selectivity during the 1st event but there is selectivity during the 2nd event.
				Action: Abundance: Composition:	Use a Petersen-type model without stratification. Use data from the 1st sampling event without stratification. 2nd event data only used if stratification of the abundance estimate is performed, with weighting according to Equations 1–3 below.
III	Fail to reject H _o	Reject Ho	Reject Ho	Interpretation:	No selectivity during the 2nd event but there is selectivity during the 1st event.
				Action: Abundance: Composition:	Use a Petersen-type model without stratification. Use data from the 2nd sampling event without stratification. 1st event data may be incorporated into composition estimation only after stratification of the abundance estimate and appropriate weighting according to Equations 1–3 below.
IV	Reject H _o	Reject Ho	Either result	Interpretation:	Selectivity during both 1st and 2nd events.
				Action: Abundance: Composition:	Use a stratified Petersen-type model, with estimates calculated separately for each stratum. Sum stratum estimates for overall abundance. Combine stratum estimates according to Equations 1-3 below.
V	Fail to reject H _o	Fail to reject H _o	Reject H _o	Interpretation:	The results of the 3 tests are inconsistent.
	-	-	-	Action:	Need to determine which of Cases I–IV best fits the data. Inconsistency can arise from high power of the M vs C test or low power of the tests involving R. Examine sample sizes (generally M or C from <100 fish and R from <30 are considered small), magnitude of the test statistics (D_{max}), and the <i>P</i> -values of the 3 tests to determine which of which of Cases I–IV best fits the data.

Composition estimation for stratified estimates

An estimate of the proportion of the population in the kth size or sex category for stratified data with I strata is calculated as follows:

$$\hat{p}_k = \sum_{i=1}^{I} \frac{\hat{N}_i}{\hat{N}} \hat{p}_{ik} \tag{1}$$

with variance estimated as

$$var[\hat{p}_{k}] \approx \frac{1}{\hat{N}^{2}} \sum_{i=1}^{I} \left(\hat{N}_{i}^{2} var[\hat{p}_{ik}] + \left(\hat{p}_{ik} - \hat{p}_{k} \right)^{2} var[\hat{N}_{i}] \right)$$
(2)

where

 \hat{p}_{ik} = estimated proportion of fish belonging to category k in stratum i;

 \hat{N}_i = estimated abundance in stratum *i*,

and the estimated total abundance is

$$\hat{N} = \sum_{i=1}^{I} \hat{N}_i .$$
(3)

Tests of consistency for Petersen Estimator

Three contingency table analyses are used to determine if the Petersen estimate can be used (Seber 1982). If any of the null hypotheses are not rejected, then a Petersen estimator may be used. If all three of the null hypotheses are rejected, a temporally or spatially-stratified estimator (Darroch 1961) should be used to estimate abundance.

Seber (1982) describes 4 conditions that lead to an unbiased Petersen estimate, some of which can be tested directly:

- 1) Marked fish mix completely with unmarked fish between events.
- 2) Equal probability of capture in event 1 and equal movement patterns of marked and unmarked fish.
- 3) Equal probability of capture in event 2.
- 4) The expected number of marked fish in recapture strata is proportional to the number of unmarked fish.

In the following tables, the terminology of Seber (1982) is followed, where *a* represents fish marked in the first event, *n* fish are captured in the second event, and *m* marked fish are recaptured; $m \cdot j$ and $m_i \cdot represent$ summation over the *i*th and *j*th indices, respectively.

I. Mixing Test

Tests the hypothesis (condition 1) that movement probabilities (θ_{ij}) , describing the probability that a fish moves from marking stratum *i* to recapture stratum *j*, are independent of marking stratum: H₀: $\theta_{ij} = \theta_j$ for all *i* and *j*.

Area-time		Not recaptured		
marking stratum (i)	1	2	 t	$a_i - m_{i^{\bullet}}$
1	m_{11}	<i>m</i> ₁₂	 m_{lt}	$a_1 - m_1$.
2	m_{21}	m_{22}	 m_{2t}	$a_2 - m_2$.
S	m_{sl}	m_{s2}	 m_{st}	$a_s - m_{s}$.

II. Equal Proportions Test⁵ (SPAS⁶ terminology)

Tests the hypothesis (condition 4) that the marked to unmarked ratio among recapture strata is constant: H₀: $\sum_i a_i \theta_{ij} / U_j = k$, where k is a constant, U_j is unmarked fish in stratum j at the time of 2nd event sampling, and a_i is the number of marked fish released in stratum i. Failure to reject H₀ means the Petersen estimator should be used only if the degree of closure among tagging strata is constant; i.e., $\sum_j \theta_{ij} = \lambda$ (Schwarz and Taylor 1998: page 289). A special case of closure is when all recapture strata are sampled, such as in a fishwheel-to-fishwheel experiment where $\sum_j \theta_{ij} = 1.0$, otherwise biological and experimental design information should be used to assess the degree of closure.

		Area-time reca	pture stratum (j)	
	1	2		t
Recaptured $(m_{.j})$	<i>m</i> •1	<i>m</i> •2		$m_{\bullet t}$
Unmarked $(n_j - m_{.j})$	$n_1 - m_{\bullet 1}$	$n_2 - m_{\bullet 2}$		$n_t - m_{\bullet t}$

III. Complete Mixing Test (SPAS terminology)

Tests the hypothesis that the probability of re-sighting a released animal is independent of its stratum of origin: H₀: $\Sigma_j \theta_{ij} p_j = d$, where p_j is the probability of capturing a fish in recapture stratum *j* during the second event, and *d* is a constant.

		Area-time man	rking stratum (i)	
	1	2	•••	S
Recaptured (m_i)	m_{l} .	<i>m</i> ₂ .		m_{s} .
Not recaptured $(a_i - m_i)$	$a_1 - m_1$.	$a_2 - m_2$.		$a_s - m_{s}$.

⁵ There is no 1:1 correspondence between Tests II and III and conditions 2–3 above. It is pointed out that equal probability of capture in event 1 will lead to (expected) nonsignificant Test II results, as will mixing, and that equal probability of capture in event 2 along with equal closure $(\Sigma j\theta i j = \lambda)$ will also lead to (expected) nonsignificant Test III results.

⁶ Stratified Population Analysis System (Arnason et al. 1996).