Regional Operational Plan No. ROP.SF.1J.2023.02

Estimating Adult Chinook Salmon Abundance on the Chilkat and Unuk Rivers Using Transgenerational Genetic Mark–Recapture

by

Randy Peterson

Kyle Shedd

Nathan Frost

Brian W. Elliott

and

Philip Richards

March 2023

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

Symbols and Abbreviations

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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	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
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 NO. ROP.SF.1J.2023.02

ESTIMATING ADULT CHINOOK SALMON ABUNDANCE ON THE CHILKAT AND UNUK RIVERS USING TRANSGENERATIONAL GENETIC MARK–RECAPTURE

by
Randy Peterson
Alaska Department of Fish and Game, Division of Sport Fish, Juneau
and
Kyle Shedd
Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage
and
Nathan Frost
Alaska Department of Fish and Game, Division of Commercial Fisheries, Ketchikan
and
Brian W. Elliott
Alaska Department of Fish and Game, Division of Commercial Fisheries, Haines
and
Philip Richards
Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau

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

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Randy Peterson
Alaska Department of Fish and Game, Division of Sport Fish,
802 3rd Street (1st Floor), Douglas, AK 99824-5412

Kyle Shedd
Alaska Department of Fish and Game, Division of Commercial Fisheries,
333 Raspberry Rd, Anchorage, AK 99518-1599

Nathan Frost
Alaska Department of Fish and Game, Division of Commercial Fisheries,
2030 Sea Level Drive, Suite 215, Ketchikan, AK 99901-6073

Brian W. Elliott
Alaska Department of Fish and Game, Division of Commercial Fisheries,
PO Box 330, Haines, AK 99827

Philip Richards
Alaska Department of Fish and Game, Division of Commercial Fisheries
802 3rd Street (1st Floor), Douglas, AK 99824-5412

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SIGNATURE/TITLE PAGE

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Approval

Title	Name	Signature	Date
Project leader	Kyle Shedd		11-03-2022
Biometrician	Randy Peterson		10-27-2022
Fishery Scientist	Chris Habicht		10-27-2022
Research Coordinator	Steve Heinl		10-27-2022
Regional Supervisor	Lowell Fair		10-28-2022

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ABSTRACT

Transgenerational genetic mark–recapture (tGMR) methods will be used to estimate the spawning abundance of Chinook salmon *Oncorhynchus tshawytscha* at the Chilkat and Unuk Rivers in 2020 and 2021, respectively. Tissue samples from adults returning to the Chilkat River were collected in the summer of 2020 and from parr rearing in the Chilkat River in the fall of 2021. Lab work and data analysis will be completed in the winter of 2021–2022. Similarly, tissue samples from adults returning to the Unuk River were collected in the summer of 2021, and samples will be collected from parr in the fall of 2022. Lab work and data analysis will be completed in the winter of 2022–2023. These studies will be used to determine the feasibility of using tGMR to estimate escapement in Southeast Alaska by comparing tGMR abundance estimates to abundance estimates generated through existing field programs. This operational plan covers the lab and project reporting aspects of these projects. Primary data collection activities are described in the project-specific operational plans.

Keywords: Unuk River, Chilkat River, Chinook salmon, *Oncorhynchus tshawytscha*, spawning escapement, mark–recapture, transgenerational genetic mark–recapture, parentage analysis, Southeast Alaska

PURPOSE

This operational plan describes two transgenerational genetic mark–recapture (tGMR) experiments purposefully planned and conducted in Alaska. This approach (tGMR) will be used to estimate the spawning abundance of Chinook salmon *Oncorhynchus tshawytscha* returning to the Chilkat and Unuk Rivers in 2020 and 2021, respectively. The purpose of both studies is to determine the feasibility of using tGMR to estimate escapement in Southeast Alaska by comparing tGMR abundance estimates to abundance estimates generated through existing field programs. Logistics, costs, timelines, analytic tools, and the utility of the estimates generated from tGMR are all either unknown or under development at this time.

Of primary interest to ADF&G is whether escapement estimates derived from tGMR are comparable to estimates generated from existing programs. Escapement to the Chilkat River is estimated annually using a traditional mark–recapture experiment (Elliott 2022). Escapement to the Unuk River is estimated annually using a survey expansion, calibrated using previous results from traditional mark–recapture experiments (e.g., Frost et al. 2022). Although current methods and tGMR estimates for each system are expected to be similar, this has not been empirically confirmed and so this project will validate and verify the tGMR methodology.

Transgenerational genetic mark–recapture could be a cost-effective tool for estimating escapement, especially in areas where more traditional methods (weirs, traditional mark–recapture) are not possible or cost prohibitive. Of the 11 Chinook salmon escapement indicator stocks in Southeast Alaska, 6 are evaluated annually using survey expansions (Richards et al. 2022). *Survey expansions* are a correlative relationship formed between measures of absolute and relative abundance. Absolute abundance is often obtained through weir counts or estimated using traditional mark–recapture, sonars, or area-under-the-curve. Common survey methods used to measure relative abundance include helicopter surveys, foot surveys, carcass surveys, peak counts, catch per unit effort, etc. The reliability of any survey expansion relies on several, often untestable assumptions, so expansion factors are something that should be periodically re-evaluated. Though one drawback of tGMR is timeliness (results are not available for 1–2 years after), this project will help determine if tGMR can be reliably used re-calibrate some of these survey expansions.

BACKGROUND

TRANSGENERATIONAL GENETIC MARK–RECAPTURE

Transgenerational genetic mark–recapture has been used to estimate the abundance of similar-sized stocks of Pacific salmon outside of Alaska (PSC 2018). Rawding et al. (2014), the seminal paper on tGMR, used tGMR to estimate the abundance of Chinook salmon at the Coweeman River. There, intensive spawning ground surveys were conducted to sample carcasses (event 1) and later, a rotary screw trap was operated for 7 months to sample the offspring for tissue (event 2). The authors found no significant differences between tGMR estimates and estimates from three other commonly used methods (redd count expansions, area-under-the-curve, and Jolly-Seber carcass mark–recapture). Since Rawding et al. (2014), tGMR has been mostly used to estimate abundance of Chinook salmon stocks in Puget Sound (PSC 2018; CTC 2021) and coho salmon stocks in California (Whitmore 2016). A meta-analysis comparing tGMR estimates against traditional survey method estimates showed that tGMR estimates tended to be correlated (Seamons 2021); however, most of the traditional methods evaluated were based on redd counts, a methodology not widely used in Alaska.

CHILKAT RIVER

The Chilkat River is the third or fourth largest producer of Chinook salmon in Southeast Alaska (McPherson et al. 2003) and is a Pacific Salmon Commission exploitation rate and escapement indicator stock (CTC 2021). Stock assessment of Chilkat River Chinook salmon includes juvenile marking with adipose fin clips and tagging with coded wire tags, marine harvest sampling, escapement enumeration, and age, sex, length, and tag sampling of mature adults. Combined, these efforts allow for estimation of smolt abundance, marine harvest, freshwater overwinter and marine survival, fishery exploitation, escapement, and mature adult age, sex, and length composition.

Traditional mark–recapture projects have been conducted annually in the Chilkat River drainage since 1991 (Elliott 2022). These studies handle over 20% of the inriver abundance annually and escapements estimates have on average a CV of less than 15%.

UNUK RIVER

The Unuk River produces the largest wild stock run of Chinook salmon in southern Southeast Alaska and is a Pacific Salmon Commission exploitation rate and escapement indicator stock. Stock assessment of Unuk River Chinook salmon includes juvenile marking with adipose fin clips and tagging with coded wire tags, marine harvest sampling, escapement enumeration, and age, sex, and length sampling of mature adults. Combined, these efforts allow for estimation of smolt abundance, marine harvest, freshwater overwinter and marine survival, fishery exploitation, escapement, and mature adult age, sex, and length composition.

Traditional mark–recapture projects were successfully conducted on the Unuk River in 1997–2009 and 2011 (e.g., Jones et al. 1998); however, the traditional mark–recapture project was discontinued in 2015 due to the loss of the event 1 sampling site, which resulted in too few fish being captured during event 1 in 2010, 2012–2014. Standardized spawning escapement surveys have taken place on the Unuk River since 1975 (Richards 2017). Peak aerial and foot spawning escapement survey counts are expanded to total escapement of large fish by using an established expansion factor developed from concurrent mark–recapture experiments and peak spawning

surveys (e.g., Weller and Evans 2009). Aerial and foot surveys were conducted in 2021 (Frost et al. 2022).

OBJECTIVES

The objectives of this project are to estimate the spawning abundance of Chinook salmon in the Chilkat River in 2020 and in the Unuk River in 2021 using tGMR experiments. Specifically, the objectives are:

1. Genotype up to 1,140 individuals (adults + parr) from the Chilkat River for genetic markers.
2. Estimate the spawning abundance of Chinook salmon in the Chilkat River in 2020 such that the estimate is within 30% of the true value 95% of the time (CV $\leq$ 0.15).
3. Genotype up to 1,140 individuals (adults + parr) from the Unuk River for genetic markers.
4. Estimate the spawning abundance of Chinook salmon in the Unuk River in 2021 such that the estimate is within 30% of the true value 95% of the time (CV $\leq$ 0.15).

Primary data collection is described in the project-specific operational plans. Tissue samples from adults were collected during the 2020 traditional mark–recapture project at the Chilkat River using the methods described in Elliott (2022). Juvenile tissue samples at the Chilkat River were collected during the 2021 fall coded wire tagging project using the methods described in Elliott and Peterson (2022). Tissue samples from adults at the Unuk River in 2021 were collected during annual spawning ground surveys using the methods described in Frost et al. (2022) and juvenile tissue samples will be collected during the 2022 fall coded wire tagging project using the methods described herein.

METHODS

TRANSGENERATIONAL GENETIC MARK–RECAPTURE

Study Design

The planned tGMR experiments are a variation of a traditional two-event mark–recapture study (PSC 2018). A tGMR experiment has two sampling events: returning adults are randomly sampled during event 1, and later during event 2 the surviving progeny are sampled. Marks are an individual's genotype and therefore passive, meaning that no animals are physically marked in event 1. Marks are transferred from parent to offspring. An example tGMR experiment is depicted in Figure 1.

The number of captures and recaptures counted during event 2 depends on whether the so called *binomial* or *hypergeometric* tGMR model is used (see Rawding et al. 2014). Briefly, the binomial tGMR model is somewhat akin to sampling with replacement, allowing multiple offspring from the same parent to be counted as separate recaptures. Conversely, the hypergeometric tGMR model is somewhat akin to sampling without replacement, where multiple offspring from the same parent are to be counted only once. See Figure 1 for a graphic explanation.

There are few limitations on the length of time between sampling events in a tGMR experiment, so offspring can be defined as eggs, parr, smolt, or even adults. For the planned experiments, event 2 samples will be collected from parr, though it could be easily re-defined as smolt if a problem arises during parr sampling. In addition, sampling during event 2 need not be random (see

Assumptions); however, crucially, event 1 samples must be collected randomly such that all returning adults have an equal probability of being captured.

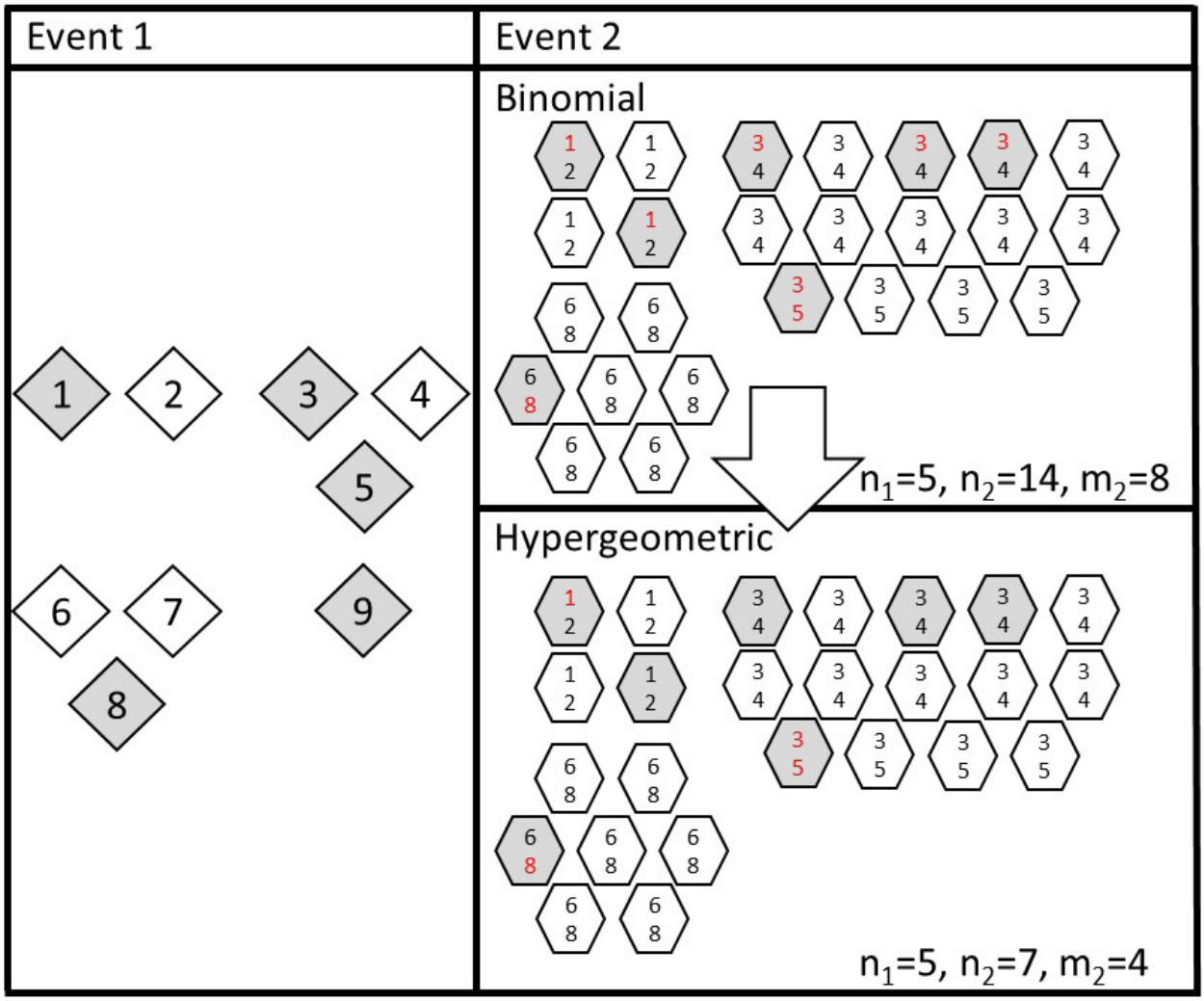

Figure 1.–Diagram depicting a simple two-event transgenerational genetic mark–recapture (tGMR) from a parent population of 9 animals (diamonds) and their progeny (hexagons).

Note: n_1 samples (diamonds with grey shading) are randomly drawn from the adult population during event 1. The offspring population is randomly sampled during event 2, with data being tabulated in two different ways. Under the tGMR binomial model, n_2 is the number of offspring sampled (hexagons with grey shading) times two, and m_2 is the number of marks recovered (red text). Under the tGMR hypergeometric model, n_2 is the number of unique parents identified through the offspring sample, and m_2 is the number of unique marks recovered.

Laboratory Methods

Genomic DNA will be extracted from adult tissue samples following established methods (Shedd et al. 2021). Genomic DNA will be extracted from parr Omni swab (Whatman FTA Product No. WHAWB100035; Sigma-Aldrich, Inc., St. Louis, MO) samples using the NucleoSpin® 96 Tissue Kit by Macherey-Nagel with an adjusted protocol based on buccal swab methods.

DNA from each sample will be genotyped for two different marker sets to ensure adequate statistical power for parentage analysis: 1) a 299 single nucleotide polymorphism (SNP) marker GT-seq (Campbell et al. 2015) panel developed by the Columbia River Inter-tribal Fish Commission Hagerman Genetics Laboratory (CRITFC; Hasselman et al. 2018), and 2) the 13 microsatellite (uSAT) GAPS panel (Seeb et al. 2007; Moran et al. 2013).

GT-seq

DNA from each sample will be genotyped by enriching the DNA of each sample for specific markers via polymerase chain reaction (PCR). Resulting reactions from each sample are then "barcoded" with two 6 base-pair sequences specific to the DNA plate and DNA plate position for each individual. This DNA barcoding allows for all samples to be pooled together, or "multiplexed", and sequenced together in a single run.

Once sequenced, a bioinformatic pipeline (i.e., a series of software scripts which manipulate text to extract desired information from sequence data) will be used to parse through the millions of 150 base-pair sequence reads and assign each read back to its sample. This "de-multiplexing" relies on the known DNA barcodes that were added onto each sample prior to pooling. Next, the pipeline will be able to identify which marker any specific sequence read came from based on the known PCR primer sequence. Finally, counts of each allele of each marker for each fish will be quantified, and genotype will be inferred from these base call counts and imported into a database for long-term storage.

Specifically, GT-seq libraries will be prepared as described by Campbell et al. (2015) using the CRITFC 299 GT-seq primer panel. Primers designed to amplify the 299 target SNP markers will be used in PCR reactions containing each sample as a template prior to index-based-barcoding, NEBNext Ultra Illumina Prep, and Sequencing on an Illumina NextSeq 500 with single-end 150 base-pair reads. Sequence will be assigned to individuals by requiring perfect sequence matches of both six-base DNA barcode indices and to markers by requiring a perfect match to the first 15 base-pairs of the forward primer. Individual genotyping will also be carried out using GTscore (Garrett McKinney, Research Scientist, Washington Department of Fish and Wildlife, Olympia , personal communication), a custom GT-seq genotype calling pipeline that uses sequence matching to quantify allelic count and ratios for each marker to infer genotype. Data will be imported in the ADFG Gene Conservation Laboratory (GCL) database LOKI.

uSAT

The uSAT genotyping will follow methods described in detail in Shedd et al. (2021); however, we will only genotype a subset of 5 of the 13 uSATs in the GAPS baseline, specifically those in the 1st multi-plex reaction: Omm1080, Ots213, Ots201b, Ssa408uos, and Ots9.

Parentage Analysis

We will determine pedigree relationships among adults and parr by analyzing the genetic data with the parentage analysis software FRANz, which implements a maximum likelihood algorithm to resolve parent-offspring relationships (Riester et al. 2009). As a complimentary method for parentage analysis, we will also use COLONY, a full probability pedigree reconstruction software (Wang and Santure 2009). As a full probability pedigree reconstruction model, COLONY uses sibling relationships among the juveniles to reconstruct the genotypes of unsampled parents, allowing for the estimation of the total number of sampled and unsampled parents, which is

necessary for the hypergeometric implementation of tGMR and transgenerational rarefaction curve (tGRC) methods.

Data Analysis

Using the parentage assignments and estimates of unsampled parents, we will be able to implement both the binomial and hypergeometric tGMR models and the tGRC model as outlined in Rawding et al. (2014). Escapement, $\widehat{N}$, will be estimated using the Chapman modified Petersen estimator:

$$\widehat{N} = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} - 1 \quad (1)$$

where n_1 is the number of adults sampled during event 1, n_2 defined either as (a) the number of offspring sampled times two or (b) the number of unique parents identified from the offspring sampled during event 2, and m_2 defined either as (a) any parent-offspring relationship detected, including parents identified more than once (i.e., siblings) or (b) the number of unique parent-offspring relationships detected. Choice of (a) or (b) depends on whether the binomial or hypergeometric tGMR model is used.

Though Rawding et al. (2014) used a Petersen estimator, we use the Chapman modified Petersen estimator. We conducted a simulation study to compare the two estimators and found that the Chapman modified Petersen estimator's sampling distribution had less variability than the Petersen estimator. Results from one of the simulations is presented in Figure 2.

Variance of $\widehat{N}$ will be estimated as (Seber 1982):

$$Var(\widehat{N}) = \frac{(n_1 + 1)(n_2 + 1)(n_1 - n_2)(n_2 - m_2)}{(m_2 + 1)^2(m_2 + 2)} \quad (2)$$

The tGRC model uses a rarefaction curve to estimate the number of total breeders based on inferred parent offspring relationships. See Rawding et al. (2014) for further details.

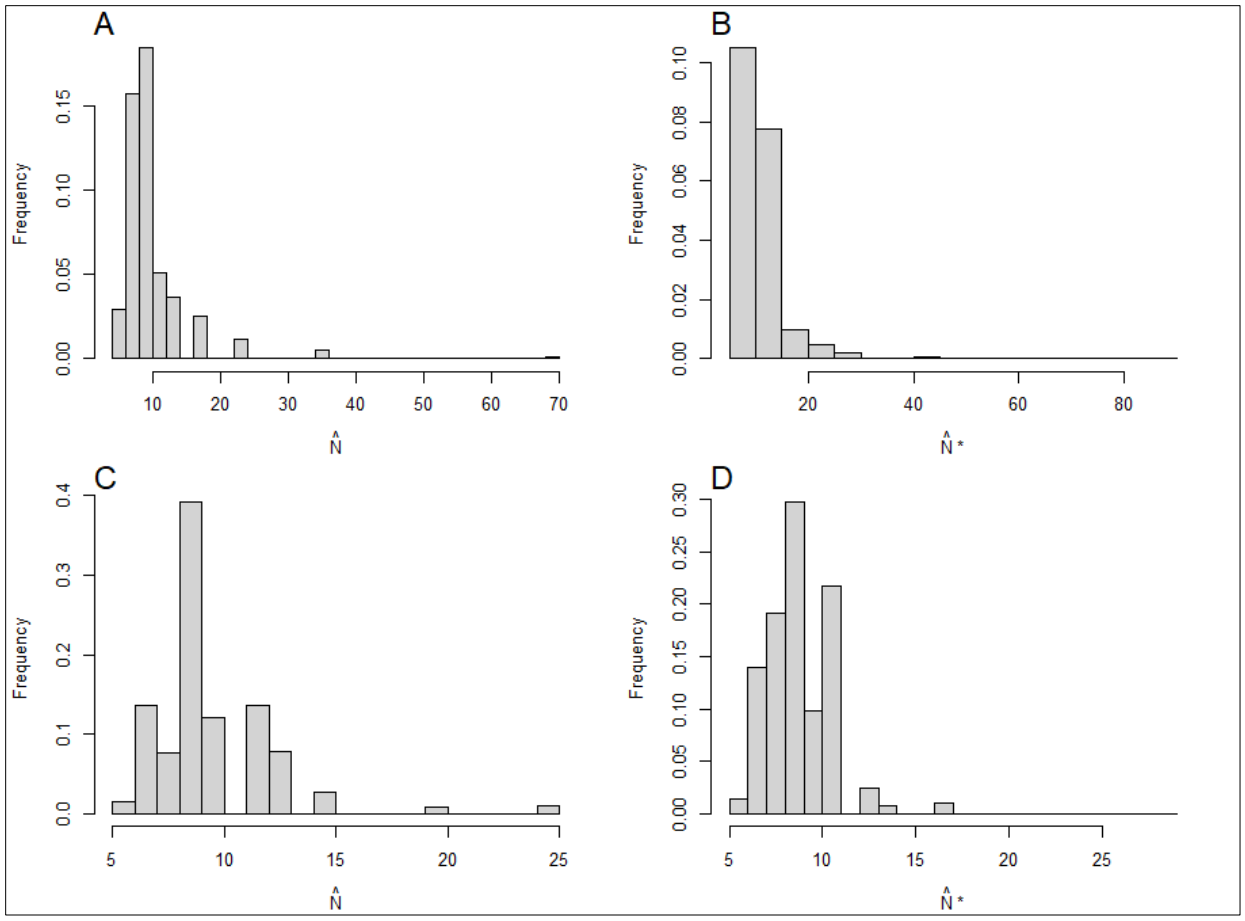

Figure 2.–Sampling distributions of the Petersen estimator, $\widehat{N}$, and Chapman modified Petersen estimator, $\widehat{N}^*$, for both transgenerational genetic mark–recapture (tGMR) models using the data depicted in Figure 1.

Note: Figure 1: (a sample of 5 from a parent population of 9 and a sample of 7 from an offspring population of 25, for a total of $\binom{9}{5}\binom{25}{7} = 60{,}568{,}200$ combinations). Panels A and B are the Petersen and Chapman modified Petersen estimates of $\widehat{N}$ and $\widehat{N}^*$, respectively from the binomial tGMR model. Panels C and D are the Petersen and Chapman modified Petersen estimates of $\widehat{N}$ and $\widehat{N}^*$, respectively, from the binomial tGMR model. Note the difference in scales among plots.

Assumptions

Transgenerational genetic mark–recapture is a modification of the so called "Petersen method" used to estimate abundance in a closed population (PSC 2018). Because of this, many of the assumptions, as well as the means to detect and mitigate deviances from these assumptions, are the same. Seber (1982) provided a list of assumptions needed for a "Petersen estimate" to be appropriate:

a) the population is closed,
b) all animals have the same probability of being captured in the first sampling event,
c) marking does not affect catchability,
d) the second sampling event is a simple random sample,
e) animals do not lose their marks in the time between sampling events, and
f) all marks are reported.

We know certain assumptions will be met and others will not be met *a priori*. Assumption (a) will not be met since the population is not closed (i.e., 100% mortality between sampling events and not all parents will produce offspring). This is not a problem provided that 1) we assume the marked and unmarked populations have the same average reproductive success and 2) a random sample of the parent population is obtained (see assumption b). Seber (1982) explains "when the first sample is random, the tags are distributed randomly through every possible subgroup or category existing in the population and therefore throughout any portion of the population subsequently removed for investigation." It is thought that both Unuk and Chilkat adult projects obtain a random, representative sampling of the returning adult populations, except for jacks.

The equal probability of capture assumptions (b, d) will, to the extent feasible, be evaluated by time, area, sex, and age. The procedures to determine if capture probabilities change in time or area will be done using contingency table analyses as recommended by Seber (1982) and are described in Appendix C1. The procedures to determine if sex- or age-selective sampling occurred are described in Appendix C2. Owing to the nature of tGMR data, we will not be able to conduct all tests described in Appendix C1 and C2. We do expect to conduct some of the tests used to evaluate assumption (b); however, we do not expect to be able to test or evaluate assumption d since event 2 is comprised of progeny only (e.g., the age of an individual not sampled in event 1 but sampled during event 2 is unknown). Not being able to test for, or even meet (d), will not be an issue provided assumption (b) is met (see assumption a).

We do not expect that the mark itself will affect catchability, assumption (c), but are aware that the handling of fish during event 1 could alter catchability. We will attempt to mitigate this assumption by 1) minimizing the holding and handling time of all captured fish, and 2) sampling as many post-spawn fish or carcasses as possible. Specific to the Chilkat project, using all tissue samples collected during the traditional mark–recapture project (event 1, lower river and event 2, spawning ground samples) could be an issue if catchability differs between the two sampling events. We will evaluate this assumption by using the procedures described in Appendix C1 and C2.

We do not anticipate any mark loss or non-reporting (assumptions e, f) because the parent-offspring mark cannot be lost, and marks are always reported so long as the genetic markers have sufficient power to detect all parent-offspring relationships. Though we anticipate meeting assumption (e), it is important to note that parent-offspring assignments are not a perfect science. Erroneous parent-offspring assignments could affect our model; however, an *a priori* power analysis using the R package *CKMRsim* (described in Baetscher et al. 2018) indicate that using both the GT-seq panel and the GAPS uSAT panel should have sufficient power to detect all parent-offspring relationships (FNR < 0.0001 for a FDR = 100 times the reciprocal of the number of anticipated pairwise comparisons of parents and offspring; Sam Rosenbaum, Graduate Student, University of Alaska Fairbanks, Juneau, personal communication).

CHILKAT RIVER

Event I Sampling

In the summer of 2020, ADF&G staff collected genetic tissues from returning adult Chinook salmon during event 1 (fish wheels) and event 2 (carcass survey) sampling for the traditional mark–recapture project. The target sample size for adults from event 1 was at least 300 fish distributed representatively throughout the run. Pelvic fin tissue was dissected from sampled fish and dried onto Whatman paper for preservation of DNA (Appendix A2). Along with each individual sampled, basic information was recorded such as size, sex, date, location, and age (from scale

samples). Each individual fish sampled was assigned a unique sample number and all associated data were saved in an ADF&G database. Pelvic fin clip samples were transported back to the ADF&G GCL, Anchorage, for genetic and statistical analysis.

Event II Sampling

In the fall of 2021, ADF&G staff collected genetic tissues from juvenile parr Chinook salmon during the existing parr sampling program. Non-lethal genetic sampling using Omni swabs was the primary method used to collect DNA from the fish mucus (slime). As a secondary DNA collection method, caudal fin tissue was dissected from incidental mortalities during the fall parr project and dried onto Whatman paper for preservation of DNA. The target sample size for juveniles was at least 500 fish. Along with each individual sampled, basic information was recorded such as date and location. Each individual fish sampled was assigned a unique sample number and all associated data were saved in an ADF&G database. Genetic tissue samples were transported back to the ADF&G GCL, Anchorage, for genetic and statistical analysis.

Sample Sizes

Expected sampling outcomes and sample size calculations were based on historical abundance estimates from the traditional adult and juvenile mark–recapture projects (Elliott 2022; Elliott and Peterson 2020) and a simulation analysis. The recent 10-year average estimated abundance of large Chinook salmon in the Chilkat River was 1,741 fish, with an average of 430 unique large (≥age-1.3) adults sampled during events 1 and 2 of the traditional adult mark–recapture adult project and an average 25,896 juveniles sampled during event 1 of the traditional juvenile mark–recapture project (Table 1). Sample sizes were calculated using a simulation analysis (assuming a spawning abundance of 1,741 fish), which begins by simulating a population of spawners and juveniles. A sample of adults and juveniles was drawn from the simulated population and adult abundance was estimated using the methods described in Rawding et al. (2014). This step was repeated 1,000 times and the average CV was calculated. These steps were repeated for different adult and juvenile sample sizes ranging from 100 to 1,000. Results suggested that the number of adults and juveniles that needed to be sampled could be as few as 550 (e.g., 300 adults and 250 juveniles). Given that we expect to handle more than this number of adults and juveniles, we anticipate that we will meet or exceed the objective criteria for Objective 2.

Table 1.–Samples sizes from spawning ground sampling and event 1 of the coded wire tag mark–recapture project for Chilkat River Chinook salmon.

	Unique adults[a]				Juveniles[b]	
Run year	Event 1	Event 2	Inriver abundance	Brood year	Parr	Smolt
2010	138	361	1,797	2010	26,360	3,175
2011	216	569	2,688	2011	26,872	5,911
2012	127	339	1,744	2012	20,282	1,875
2013	55	392	1,730	2013	18,261	2,829
2014	60	190	1,534	2014	20,449	3,574
2015	121	522	2,456	2015	40,520	3,839
2016	96	253	1,386	2016	13,937	3,473
2017	65	155	1,173	2017	12,231	3,588
2018	62	125	873			
2019	179	272	2,028			
Avg.	112	318	1,741		22,364	3,533

[a] Data from Elliott (2022).

[b] Data from Elliott and Peterson (2020).

UNUK RIVER

Event I Sampling

In the summer of 2021, ADF&G staff collected genetic tissue from returning adult Chinook salmon. Samples were collected on the spawning grounds of the main tributaries to the Unuk River (Boundary Creek, Cripple Creek, Gene's Creek, Kerr Creek, Lake Creek, Clear Creek, and the Eulachon River) in order to get a representative sample. Rod and reel snagging gear and dip nets were used to collect fish. Effort was spread equally across all sampled tributaries to promote proportional sampling. Target sample size for adults from event 1 was at least 350 fish. Pelvic fin tissue was dissected from sampled fish and dried onto Whatman paper for preservation of DNA (Appendix A3). Along with each individual sampled, basic information such as size, sex, date, location, and age (from scale samples) was recorded. Each individual fish sampled was assigned a unique sample number and all associated data were saved in an ADF&G database. Pelvic fin clip samples were transported back to the ADF&G GCL, Anchorage, for genetic and statistical analysis.

Event II Sampling

In the fall of 2022, ADF&G will collect genetic tissues from juvenile parr Chinook salmon during the existing parr sampling program. Non-lethal genetic sampling using Omni swabs will be the primary way used to collect DNA from the fish mucus (slime). As a secondary DNA collection method, caudal fin tissue will be dissected from incidental mortalities during the fall parr project and dried onto Whatman paper for preservation of DNA. The target sample size for juveniles will be at least 250 fish. Care will be taken to minimize the amount of slime removed from juveniles in order to avoid future infection, while still ensuring that enough DNA is collected onto the Omni swabs. Along with each individual sampled, basic information will be recorded such as date and

location. Each individual fish sampled will be assigned a unique sample number and all associated data will be maintained in the ADF&G database. Genetic tissue samples will be transported back to the ADF&G GCL, for genetic and statistical analysis.

Sample Sizes

Expected sampling outcomes and sample size calculations were based on historical abundance estimates from the calibrated aerial and foot surveys and juvenile mark–recapture projects (Richards et al. 2022; Frost et al. 2022) and a simulation analysis. The recent 10-year average estimated abundance of large Chinook salmon in the Unuk River was 1,849 fish, with an average of 492 adults sampled during spawning ground sampling and an average 27,460 juveniles sampled during event 1 of the traditional juvenile mark–recapture project (Table 2). Sample sizes were calculated using a simulation analysis (assuming a spawning abundance of 1,900 fish), which begins by simulating a population of spawners and juveniles. A sample of adults and juveniles was drawn from the simulated population and adult abundance was estimated using the methods described in Rawding et al. (2014). This step was repeated 1,000 times and the average CV was calculated. These steps were repeated for different adult and juvenile sample sizes ranging from 100 to 1,000. Results suggested that the number of adults and juvenile that need to be sampled could be as few as 575 (e.g., 325 adults and 250 juveniles). Given that we expect to handle more than this number of adults and juveniles, we anticipate that we will meet or exceed the objective criteria for Objective 4.

Table 2.–Samples sizes from spawning ground sampling and event 1 of the coded wire tag mark–recapture project for Unuk River Chinook salmon.

	Unique adults			Juveniles	
Run year	Sampled	Escapement	Brood year	Parr	Smolt
2011	479	3,195	2010	13,677	3,942
2012	301	956	2011	13,656	6,140
2013	284	1,135	2012	12,534	12,289
2014	280	1,691	2013	4,218	10,817
2015	474	2,623	2014	10,511	4,003
2016	356	1,463	2015	43,037	13,549
2017	403	1,203	2016	23,006	11,420
2018	678	1,971	2017	13,655	3,059
2019	1,054	3,115	2018	21,765	11,546
2020	607	1,135	2019	35,600	6,179
Avg.	492	1,849		19,166	8,294

SCHEDULE AND DELIVERABLES

Results from these projects will be documented in an ADF&G Fishery Data Series (FDS) report in 2023. Project specific timelines are described below.

CHILKAT

Adult genetic tissue sampling occurred in August 2020. Juvenile genetic tissue sampling occurred in September and October 2021 (parr) and will potentially be conducted again in March 2022 (smolt) if additional samples are needed (Table 3).

Table 3.–Project timeline for the Chilkat River Chinook salmon tGMR experiment.

Date	Task
February 2020	Include formal genetic-based methods in the annual Chilkat River Chinook salmon operational plan
April 2020	Send genetic sampling materials from the Gene Conservation Laboratory (GCL) in Anchorage to Haines, AK
June–September 2020	Sample adult and juvenile Chinook salmon during the traditional Chilkat River Chinook salmon stock assessment project
October 2021	Sample juvenile Chinook salmon during the traditional Chilkat River Chinook salmon fall parr stock assessment project
January–March 2022	Genotype adult and fall parr samples at the GCL
April–June 2022	Perform parentage analysis, calculate tGMR and tGRC estimates, and write a final report

UNUK

Adult genetic tissue sampling occurred in August 2021. Juvenile genetic tissue sampling will take place in September and October 2022 (parr), and potentially in March 2023 (smolt) if additional samples are needed (Table 4).

Table 4.–Project timeline for the Unuk River Chinook salmon tGMR experiment.

Date	Task
February 2021	Include formal genetic-based methods in the annual Unuk River Chinook salmon operational plan
April 2021	Send genetic sampling materials from the GCL in Anchorage to Ketchikan, AK
August 2021	Sample adult Chinook salmon during the traditional Unuk River Chinook salmon stock assessment project
September–October 2022	Sample juvenile Chinook salmon during the traditional Unuk River Chinook salmon fall parr stock assessment project
January–March 2023	Genotype adult and fall parr samples at the GCL (unless spring smolt are needed)
April–June 2023	Perform parentage analysis, calculate tGMR and tGRC estimates, and write a final report

RESPONSIBILITIES

Kyle Shedd, Fishery Geneticist, Division of Commercial Fisheries (DCF)

Shedd will oversee the shipment of genetic supplies, genotyping of samples, parentage analysis, the tGMR/tGRC analysis, and the final report.

Randy Peterson, Biometrician, Division of Sport Fish (DSF)

Peterson will assist with study design, sampling objectives, development of operational plan, the tGMR/tGRC analysis, and the final report.

Nathan Frost, Fishery Biologist, DCF

Frost will assist with sample design and the project implementation on the Unuk River, permits, equipment, personnel hiring and training, and the final report.

Brian Elliott, Fishery Biologist, DCF

Elliott will assist with sample design and the project implementation on the Chilkat River, permits, equipment, personnel hiring and training, and the final report.

Philip Richards, Fishery Biologist, DCF

Richards will assist with all aspects of the projects.

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APPENDIX A: ADULT SALMONID GENETIC SAMPLING INSTRUCTIONS

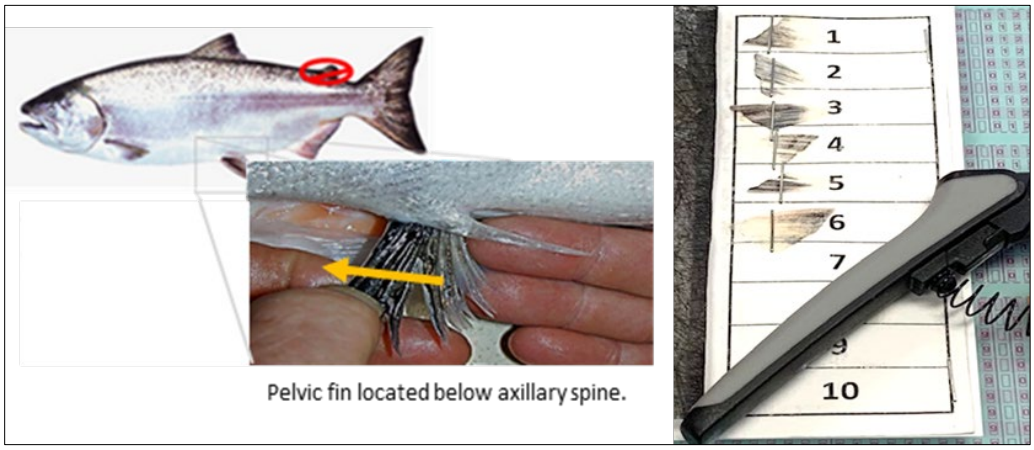
The following appendix was provided by the ADF&G Gene Conservation Laboratory, Anchorage.

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 **dry storage** (with desiccant packs) in Pelican box or watertight file box.

II. Sampling Method



III. Sampling Instructions

- Prior to sampling:
  - Set up workspace, fill out required collection information (upper left-hand corner only).
  - Place Whatman genetic card (10WGC) on mini clipboard flat for easy access. One Whatman card per scale card. Same card can be used throughout same day.

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- Sampling:
    - Wipe excess water and/or slime off the pelvic fin prior to sampling to avoid getting excess water or fish slime.
    - Fin clip will be taken from lower portion of the pelvic fin.
    - Cut off a portion of the fin clip using Fiskar scissors to get roughly a **¾– 1 inch maximum** piece and/or about the size of a small fingernail (see cutting line to left in orange).
    - Place one clipped fin tissue onto appropriate grid space. Follow sampling order 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.**
    - Fin clips will stick to the 10WGC grid card (see photo).
    - **Staple** fin clip to card; this secures the fin for handling in lab.
    - **DO NOT** staple landscape cloth to paper edge.
    - Sampling complete.
    - Periodically, wipe or rinse the scissors with water so not to cross contaminate samples.
    - Insert the 10WGC card inside Pelican case and layer with blotter cards and desiccant packs.
    - Close and secure the lid of Pelican box so drying begins.
    - Data to record: Record **each fin clip number to paired data** information (i.e., location, lat./long., sample date(s), etc.). Electronic version preferred.
- Loading the Pelican Case:
    - First card: Remove blotter papers and desiccant packs (remove vacuum pack plastic) 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 **must remain in Pelican 1400 case at all times** to dry cards flat.

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- Post-sampling storage:
    - Store dried 10WGC tissue cards in Pelican box at room temperature or below. Two-four desiccant packs fit inside Pelican 1400 case. This helps flatten the cards as they dry out over time.

- Shipping at end of the season:
    - Keep all **dried** cards layered inside Pelican box with secured lid until preparing for shipment. Pack all dry cards into photo pages and inside priority mailing box with returning sampling supplies. Tape box shut and tape return address on box.

IV. Supplies included in sampling kit:

1. Scissors - for cutting a portion of selected fin.
2. Whatman genetics card (10WGC) – holds 10 fish/card.
3. Bostitch stapler – staple secures fin clip to card.
4. Pelican Case - 1st stage of drying/holding card with samples.
5. Pelican 1400 case – long term dry storage for all cards
6. Desiccant packs – removes moisture from samples.
7. Pre-cut blotter paper – covers full sample card for drying.
8. Shipping box – put sealed Pelican case inside a box.
9. Clipboard – holds Whatman genetics card while sampling.
10. Zip ties – to secure the Pelican case for return shipment.
11. Laminated “return address” labels.
12. Sampling instructions.
13. Pencil

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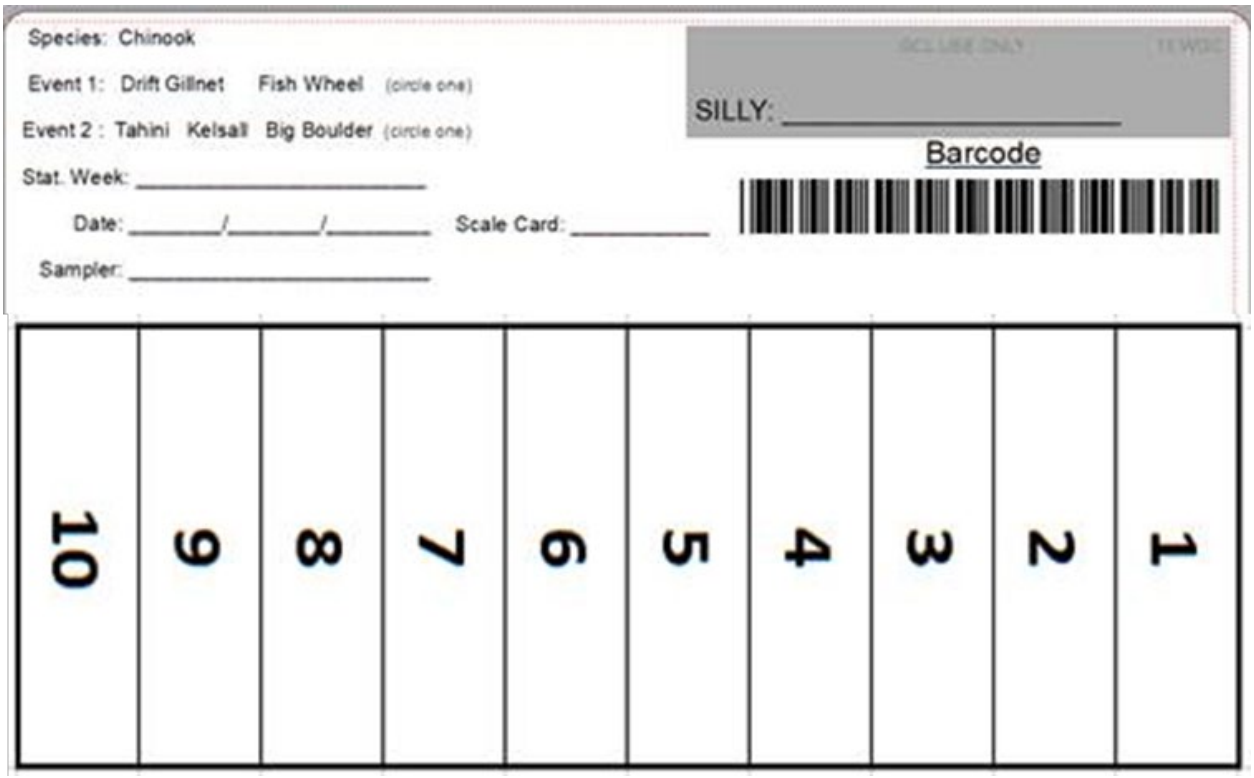
V. Shipping:

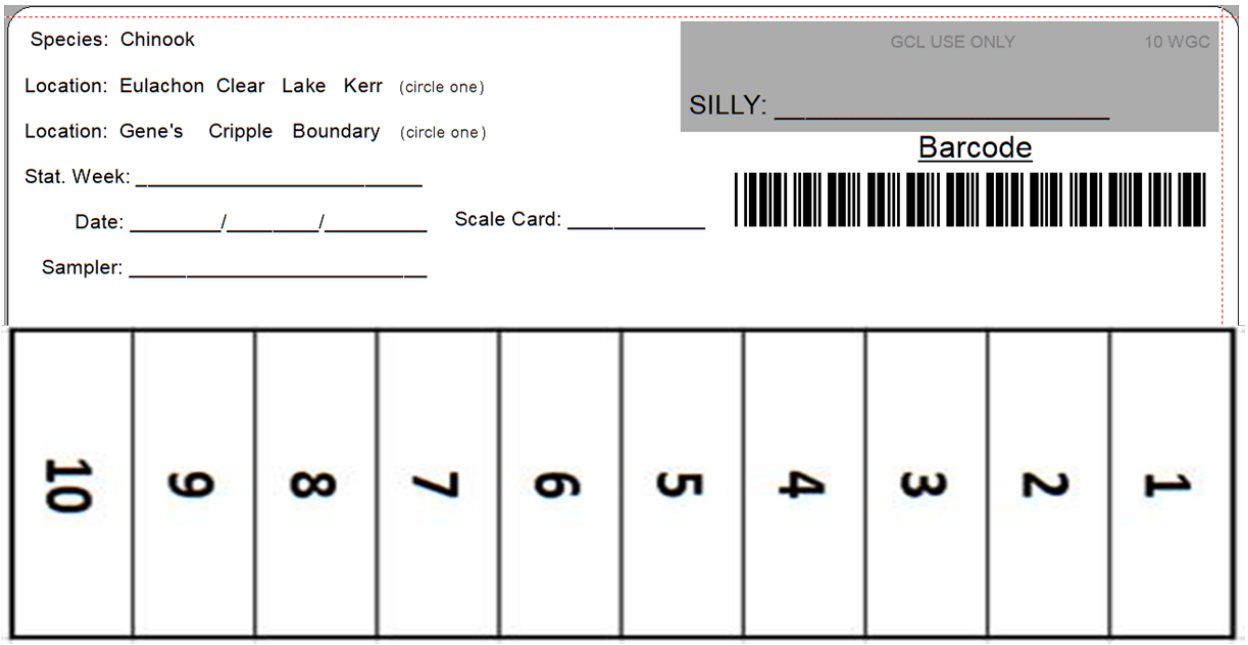
- Address the sealed mailer box for return shipment to ADF&G Genetics lab

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

Appendix A2.–Whatman genetics card for the Chilkat River.



Appendix A3.–Whatman genetics card for the Unuk River.



APPENDIX B: JUVENILE SALMONID GENETIC SAMPLING INSTRUCTIONS

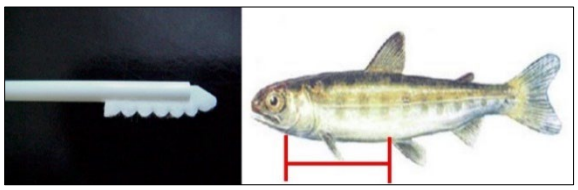
We use the mucus samples from juvenile fish using OmniSwab to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting samples is that only quality tissue samples give quality results. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal fish.

I. Sampling Method

Steps for taking mucus samples in 2.0ml vials:

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

Figure 1.



Figures 2 and 3.

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II. Supplies included with sampling kits:

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

III. Shipping: No special paperwork required for return shipment of these samples. Return to ADF&G Gene Conservation Laboaratory, Anchorage:

ADF&G — Genetics	Lab Staff: 907-267-2247
333 Raspberry Road	Judy Berger: 907-267-2175
Anchorage, AK 99518	Freight Code:_____

APPENDIX C: PROJECT BIOMETRIC DETAILS

Tests of consistency for Petersen Estimator

Three contingency table analyses are used to determine if the Petersen estimate can be used (). 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; $m_{\bullet j}$ and $m_{i\bullet}$ represent summation over the ith and jth 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: H_0: $\theta_{ij} = \theta_j$ for all i and j.

Area–time marking stratum (i)	Area–time recapture stratum (j)				Not recaptured
	1	2	…	T	$a_i - m_{i\bullet}$
1	m_{11}	m_{12}	…	m_{1t}	$a_1 - m_{1\bullet}$
2	m_{21}	m_{22}	…	m_{2t}	$a_2 - m_{2\bullet}$
…	…	…	…	…	…
s	m_{s1}	m_{s2}	…	m_{st}	$a_s - m_{s\bullet}$

--continued--

II. Equal Proportions Test[1] (SPAS[2] terminology)

Tests the hypothesis (condition 4) that the marked to unmarked ratio among recapture strata is constant: H_0: $\Sigma_i a_i \theta_{ij} / U_j = k$, 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 H_0 means the Petersen estimator should be used only if the degree of closure among tagging strata is constant; i.e., $\Sigma_j \theta_{ij} = \lambda$ (Schwarz and Taylor 1998). A special case of closure is when all recapture strata are sampled, such as in a fishwheel-to-fishwheel experiment where $\Sigma_j \theta_{ij} = 1.0$, otherwise biological and experimental design information should be used to assess the degree of closure.

	Area–time recapture stratum (j)			
	1	2	…	t
Recaptured ($m_{\cdot j}$)	$m_{\cdot 1}$	$m_{\cdot 2}$	…	$m_{\cdot t}$
Unmarked ($n_j - m_{\cdot j}$)	$n_1 - m_{\cdot 1}$	$n_2 - m_{\cdot 2}$	…	$n_t - m_{\cdot t}$

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: H_0: $\Sigma_j \theta_{ij} p_j = d$, 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 marking stratum (i)			
	1	2	…	s
Recaptured (m_i)	$m_{1\cdot}$	$m_{2\cdot}$	…	$m_{s\cdot}$
Not recaptured ($a_i - m_{i\cdot}$)	$a_1 - m_{1\cdot}$	$a_2 - m_{2\cdot}$	…	$a_s - m_{s\cdot}$

[1] 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 ($\Sigma j \theta ij = \lambda$) will also lead to (expected) nonsignificant Test III results.

[2] Stratified Population Analysis System (Arnason et al. 1996).

Appendix C2.–Detection of sex- or age-selective sampling.

Contingency table analysis is generally used to detect if sex- or age-selective sampling occurred during the first and/or second sampling events. Age-selective sampling is taken as a proxy for size-selective sampling. Observed counts of males to females are compared between marked (M) vs. recaptured (R), captured (C) vs. R, and M vs. C under a null hypothesis that the probability a sampled fish is male or female is independent of sample. If the proportion is estimated rather than observed (usually C), contingency table analysis will not be used and instead the proportions of females (or males) compared between samples using a two-sample test (e.g., Student t-test).

M vs. R	**C vs. R**	**M vs. C**
Case I:		
Fail to reject H_o	Fail to reject H_o	Fail to reject H_o
There is no sex selectivity detected during either sampling event.		
Case II:		
Reject H_o	Fail to reject H_o	Reject H_o
There is no sex selectivity detected during the first event but there is during the second event sampling.		
Case III:		
Fail to reject H_o	Reject H_o	Reject H_o
There is no sex selectivity detected during the second event but there is during the first event sampling.		
Case IV:		
Reject H_o	Reject H_o	Either result possible
There is sex selectivity detected during both the first and second sampling events.		
Evaluation Required:		
Fail to reject H_o	Fail to reject H_o	Reject H_o
Sample sizes and powers of tests must be considered:		

A. If sample sizes for M vs. R and C vs. R tests are not small and sample sizes for M vs. C test are very large, the M vs. C test is likely detecting small differences which have little potential to result in bias during estimation. *Case I* is appropriate.

B. If a) sample sizes for M vs. R are small, b) the M vs. R p-value is not large (~0.20 or less), and c) the C vs. R sample sizes are not small and/or the C vs. R p-value is fairly large (~0.30 or more), the rejection of the null in the M vs. C test was likely the result of size/sex selectivity during the second event which the M vs. R test was not powerful enough to detect. *Case I* may be considered but *Case II* is the recommended, conservative interpretation.

C. If a) sample sizes for C vs. R are small, b) the C vs. R p-value is not large (~0.20 or less), and c) the M vs. R sample sizes are not small and/or the M vs. R p-value is fairly large (~0.30 or more), the rejection of the null in the M vs. C test was likely the result of size/sex selectivity during the first event which the C vs. R test was not powerful enough to detect. *Case I* may be considered but *Case III* is the recommended, conservative interpretation.

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D. If a) sample sizes for C vs. R and M vs. R are both small, and b) both the C vs. R and M vs. R p-values are not large (~0.20 or less), the rejection of the null in the M vs. C test may be the result of size/sex selectivity during both events which the C vs. R and M vs. R tests were not powerful enough to detect. *Cases I, II, or III* may be considered but *Case IV* is the recommended, conservative interpretation.

Case I. Abundance is calculated using a Petersen-type model from the entire data set without stratification. Composition parameters may be estimated after pooling sex and/or age data from both sampling events.

Case II. Abundance is calculated using a Petersen-type model from the entire data set without stratification. Composition parameters may be estimated using sex and/or age data from the first sampling event without stratification. If composition is estimated from second event data or after pooling both sampling events, data must first be stratified to eliminate variability in capture probability (detected by the M vs. R test) within strata. Composition parameters are estimated within strata, and abundance for each stratum needs to be estimated using a Petersen-type formula. Overall composition parameters are estimated by combining stratum estimates weighted by estimated stratum abundance according to the formulae below.

Case III. Abundance is calculated using a Petersen-type model from the entire data set without stratification. Composition parameters may be estimated using sex and/or age data from the second sampling event without stratification. If composition is estimated from first event data or after pooling both sampling events, data must first be stratified to eliminate variability in capture probability (detected by the C vs. R test) within strata. Composition parameters are estimated within strata, and abundance for each stratum needs to be estimated using a Petersen-type type formula. Overall composition parameters are estimated by combining stratum estimates weighted by estimated stratum abundance according to the formulae below.

Case IV. Data must be stratified to eliminate variability in capture probability within strata for at least one or both sampling events. Abundance is calculated using a Petersen-type model for each stratum, and estimates are summed across strata to estimate overall abundance. Composition parameters may be estimated within the strata as determined above, but only using data from sampling events where stratification has eliminated variability in capture probabilities within strata. If data from both sampling events are to be used, further stratification may be necessary to meet the condition of capture homogeneity within strata for both events. Overall composition parameters are estimated by combining stratum estimates weighted by estimated stratum abundance.