Genetic Stock Composition and Stock-Specific Escapement of Sockeye Salmon Past the Chignik Weir During the Transition Period in 2023

by

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Alaska Department of Fish and Game



Division of 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	@	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	K	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	ТМ	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	pH	U.S.C.	United States	population	Var
(negative log of)	•		Code	sample	var
parts per million	ppm	U.S. state	use two-letter	*	
parts per thousand	ppt,		abbreviations		
	‰		(e.g., AK, WA)		
volts	V				
watts	W				

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GENETIC STOCK COMPOSITION AND STOCK-SPECIFIC ESCAPEMENT OF SOCKEYE SALMON PAST THE CHIGNIK WEIR DURING THE TRANSITION PERIOD IN 2023

by

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> > September 2024

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ABSTRACT

The Alaska Department of Fish and Game, Division of Commercial Fisheries, and the Bristol Bay Native Association and Chignik Regional Aquaculture Association signed a cooperative agreement to use genetic tools to analyze the escapement of sockeye salmon (*Oncorhynchus nerka*) to the Chignik River in 2023. The project was designed to estimate the stock composition of escapement during the transition period between early- (Black Lake) and late-run (Chignik Lake) stocks and estimate stock-specific escapement. Analysis of the current baseline with updated methods indicate it is capable of identifying the two reporting groups with the current set of 22 genetic markers, and that producing estimates with less than the standard 190 mixture sample size still provides results within guidelines for accuracy and precision. Results from 13 collections of samples collected from June 28 through July 28, 2023, were consistent with prior years, with Black Lake comprising a majority of escapement early in the transition and Chignik Lake the majority of later escapement. Approximately 49% of the annual escapement of 888,354 was represented by genetic samples, and of that escapement of 431,905, 35% was Black Lake stock and 65% Chignik Lake stock.

Keywords: Chignik, sockeye salmon, genetic stock identification

INTRODUCTION

The Alaska Department of Fish and Game (ADF&G), Division of Commercial Fisheries, and the Bristol Bay Native Association (BBNA) and Chignik Regional Aquaculture Association (CRAA) signed a cooperative agreement to use genetic tools to analyze the escapement of sockeye salmon (*Oncorhynchus nerka*) to the Chignik River in 2023. The agreement (Appendix 1) provided funding from BBNA and CRAA for the ADF&G Gene Conservation Laboratory (GCL) to use genetic stock identification (GSI) during the migration-overlap period from about late June through July and apply the GSI data post-season to calculate escapement numbers for the two stocks. The project was designed to genotype 1,052 sockeye salmon tissue samples across appropriate temporal strata collected from the Chignik weir in 2023 for 24 genetic markers capable of distinguishing early- (Black Lake) and late- run (Chignik Lake) groups of populations, conduct GSI based upon those genotypes, and calculate stock-specific estimates of escapement past the Chignik weir. This report fulfills the agreement and provides results to BBNA, CRAA, and other interested stakeholders.

Genetic tools have been applied to delineate early- and late-run components of escapement to the Chignik River since 2010 with various sources of funding. Using funding from the Alaska Sustainable Salmon Fund (AKSSF), the GCL first analyzed samples from 2010 and 2011 post-season to provide context for samples analyzed inseason in 2012 to inform management. Results from these initial years were reported in Anderson et al. (2013). Analysis of samples from subsequent years was funded by different organizations with 2013–2014 funded jointly by CRAA and ADF&G, 2015–2017 funded by AKSSF, 2018–2019 funded by the Saltonstall-Kennedy Grant Fund, and 2020–2021 funded by cooperative agreements with CRAA.

Prior to the use of genetic data, different methods were used to estimate stock-specific escapement. Prior to 2004, scale pattern analysis (SPA) based upon consistent differences in the ages of early and late runs was used to model stock-specific escapement (Witteveen and Botz 2004). A common logistic function was used to smooth the models' outputs which were then applied to the total escapement to estimate the escapement to each run. A summary of SPA results determined that roughly equal numbers of early- and late-run fish were counted before and after July 4 and the program was discontinued. From 2004 through 2013, escapement up to July 4 was considered to be early-run sockeye salmon and escapement after July 4 was considered late-run sockeye salmon (Anderson et al. 2013). From 2014 through 2020, inseason estimates of genetic stock compositions of escapement were modeled using logistic modeling methods similar to the SPA program to estimate run timing and stock-specific escapement (Burnside and Fuerst 2023). During that time, a Bayesian hierarchical model that integrates historical and in-season GSI data to estimate a given year's stock transition function was developed and has been used by some stakeholders (DeFilippo et al. 2020). The department's current approach to estimating stock-specific escapement is based upon an Expectation-Maximization algorithm that fits mixture distributions to determine stock apportionment of Chignik early- and late-run sockeye salmon (Finkle and Power 2023).

The current genetic baseline for Chignik River sockeye salmon was constructed in 2020. It is composed of 1,691 individuals from 18 sample collections pooled into 16 populations (Table 1) and genotyped for 24 SNPs (Table 2). Due to patterns of linkage disequilibrium in 2 pairs of SNPs, the 24 SNPs are combined into 22 independent genetic markers. Four of the 16 populations are grouped into the Black Lake (early run) reporting group with the remaining 12 into the Chignik Lake (late run) reporting group (Table 1; Figure 1). The baseline was used to analyze escapement samples from 2020–2021 but had not been evaluated for its ability to identify the two reporting groups with updated GCL methods (Barclay et al. 2024). We report results of an updated baseline evaluation to assess these reporting groups. Additionally, because sample sizes were generally 80/sampling event in 2023 and differed from past genetic sampling of 190/stratum, we assessed the effect of mixture sample size on accuracy and precision of GSI with the current baseline.

OBJECTIVES

Our objectives were to:

- 1. Evaluate the baseline's ability to identify Black Lake and Chignik Lake reporting groups with updated baseline evaluation methods;
- 2. Assess the effect of mixture sample size on the accuracy and precision of GSI with the current baseline;
- 3. Genotype 1,052 sockeye salmon tissue samples across appropriate temporal strata collected from the Chignik weir in 2023 for 24 genetic markers capable of distinguishing Black Lake (early run) and Chignik Lake (late run) reporting groups,
- 4. Conduct GSI based upon those genotypes, and
- 5. Calculate stock-specific estimates of escapement past the Chignik weir.

METHODS

BASELINE EVALUATION

Reporting Group Evaluation for Genetic Stock Identification

The usefulness of the baseline for proportional GSI applications was examined with a series of evaluation tests. Genotypes of 190 individual sockeye salmon were randomly sampled from the baseline without replacement to construct test mixtures, which were then analyzed against a reduced baseline (full baseline minus the 190 individuals removed for the test mixture). To explore a range of stock compositions, up to 100 test mixtures were constructed for each group with compositions varying from 1% to 100%, and the remaining composition randomly split among the other groups. Because the removal of individuals from the baseline can reduce the accuracy of population allele frequency estimates and, consequently, the identifiability of regional groups for MSA, test mixture compositions were limited to remove no more than half of the total number of fish in a group. Random samples were selected in proportion to the number of fish in each population to avoid random sample sizes exceeding the total number of fish in a population.

The stock composition of the test mixtures was estimated using the R^1 package *rubias* (Moran and Anderson 2019). Each mixture was analyzed for 1 Markov Chain Monte Carlo (MCMC) chain with 25,000 iterations and the first 5,000 iterations were discarded to remove the influence of starting values. The prior parameters for each reporting group were defined to be equal (i.e., a flat prior). Within each group, the population prior parameters were divided equally among the populations within that reporting group. Stock proportion estimates and the 90% credibility intervals for each test mixture were calculated by taking the mean and 5% and 95% quantiles of the posterior distribution from the single chain output.

The performance of each group was assessed by calculating the proportion of tests with correct allocations within 10% of the true test mixture proportion and overall bias among tests. As a guideline, we considered a group's performance to be adequate for MSA if at least 90% of tests were within 10% of the true test mixture proportion and overall bias did not exceed ± 5 %. We also calculated root mean square error (RMSE) for each set of tests with a guideline of RMSE < 0.05 for each group. These tests provided an indication of the power of the baseline for MSA when all populations from a reporting group were assumed to be represented in the baseline.

Effect of Mixture Sample Size on Accuracy and Precision

We conducted 100 cross-validation simulations using the 22-marker set genetic baseline for the Chignik River sockeye. Cross-validation analysis was done for two reporting groups: Black Lake and Chignik Lake. Procedures for each of the 100 simulations are as follows:

- Randomly select an escapement number of Chignik River sockeye from daily counts between 6/28 and 7/28 in years 2022 and 2023. Randomly set proportions for the earlyand late-run sockeye to represent true escapement proportions.² Then randomly draw 380 fish from the escapement to represent genetic tissue sample.
- 2) Randomly draw the same sample size from the reporting groups of the sockeye baseline based on the sampling proportion of tissue sample in 1).
- 3) Remove fish of the mixture in step 2) from the sockeye baseline.
- 4) Assemble a set of mixture with reduced size by randomly drawing a subset of fish from the 380 fish mixture in 2). The reduced sample sizes are 190, 95, 80, 70, 60, and 50.
- 5) Run GSI for each simulated mixture using the same baseline created in 3). Record the estimated means and the true values of the reporting group proportions.
- 6) Repeat steps 1) through 5) 100 times.

We plotted the comparisons between the estimated means and the true values of the group proportions and calculated the RMSE, deviations, and bias. The GCL guidelines for baseline evaluation aim for RMSE ≤ 0.05 , deviations within ± 0.1 of the true values $\leq 10\%$ of the time, and bias $\leq \pm 0.05$.

¹ R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <u>https://www.R-project.org</u>/ (accessed August 13, 2024).

² The escapement proportion is drawn from a beta distribution with both shape parameters set at 1.675. This setup allows the random proportion to be drawn from a diffused distribution with less concentration on the extremes (0 and 1).

GENETIC ANALYSIS

Sample Selection

We genotyped all 1,052 available samples collected from the Chignik River weir in 2023.

Laboratory Analysis

DNA Extraction and Genotyping

Genomic DNA was extracted using a NucleoSpin® 96 Tissue Kit by Macherey-Nagel (Düren, Germany). Genetic data were collected from the samples as individual multilocus genotypes for the 24 loci that were assayed (Table 2). Samples were genotyped using Taqman® assays (Applied Biosystems, Foster City, CA, USA) with multiple parallel reactions using BiomarkTM 192.24 Dynamic Arrays (Fluidigm® platform, Standard Biotools <u>https://www.standardbio.com/area-of-interest/genomics-analysis/pcr-applications/genotyping-with-microfluidics</u>). The Dynamic Arrays were read on a BioMarkTM or EP1TM System after amplification and scored using BioMarkTM Genotyping Analysis software (Standard Biotools). for quality control purposes, ~8% of tissue samples were re-extracted and genotyped to check for genotyping errors, and major genotyping errors, if any, were corrected.

Statistical Analysis

Data Retrieval and Quality Control

All subsequent analyses were performed in R, unless otherwise noted. Genotypes were retrieved from the Gene Conservation Lab database and imported into R with the *RJDBC* package.³ Two quality assurance analyses were performed to confirm the quality of the data. First, individuals missing substantial genotypic data (20% or more of loci; Dann et al. 2009) likely had poor quality DNA and were removed from further analyses. The second quality assurance analysis identified individuals with duplicate genotypes due to sampling or extracting the same individual twice. Duplicates were defined as pairs of individuals sharing the same alleles in 99% of screened loci, and the individual with the most missing genotypic data from each duplicate pair was removed from further analyses. If both had the same amount of genotypic data, the first individual was removed from further analyses.

Estimating Stock Composition

We grouped samples into temporal strata representing escapement for days within 1-2 days of when samples were taken during the overlap period from late June through late July (Table 3). The stock composition of each stratum was estimated in *rubias* using the same protocol used for evaluating reporting groups.

Stock-specific Escapement

We estimated stock-specific escapement $(C_{f,y})$ by multiplying estimates of stock composition $(p_{f,y})$ and escapement totals (C_f) for the days each stratum represented:

$$C_{f,y} = p_{f,y}C_f,$$

³ Urbanek, S. 2022. RJDBC: Provides Access to Databases Through the JDBC Interface. R package version 0.2-10. <u>https://cran.r-project.org/package=RJDBC</u> (accessed August 13, 2024).

Where f denotes the f th component stratum and y denotes the yth reporting group. In this analysis, we multiplied each realization of our stock composition from *rubias* (i.e., mixed stock analysis described above) to the escapement total:

$$C_{f,y}^{(i)} = p_{f,y}^{(i)} C_f,$$

Where *i* denotes the *i*th realization of our 20,000 posterior samples. 90% credible interval (CI) was determined by 5th and 95th quantiles of the 20,000 observations of $C_{f,y}^{(i)}$. The median, mean, and SD of the stock-specific escapement were also estimated directly from the 20,000 observations of $C_{f,y}^{(i)}$.

Note that we did not include escapement for the early and late periods of the season when representative samples were unavailable.

RESULTS

BASELINE EVALUATION

Reporting Group Evaluation for Mixed Stock Analysis

Baseline evaluation tests were constructed with proportions ranging from 1–100% for both Black Lake and Chignik Lake reporting groups. Both groups met our reporting group guidelines with this set of 22 markers. Correct allocation estimates for both reporting groups within 0.04 of the true value 90% of the time (Table 4; Figure 2). Overall bias for the Black Lake group was -0.01 and 0.01 for Chignik Lake.

Effect of Mixture Sample Size on Accuracy and Precision

A sample size of 190 or more would adequately meet the GCL's guidelines for RMSE, deviation, and bias (Table 5; Figures 3–5). A plot showed that precision remained similar for sample sizes of 380 and 190; however, dots became more diffused around the 1:1 line for sample sizes of 95 and below (Figure 6).

Results showed that the GCL's guidelines can be met with slightly relaxed sample size criteria. For example, reporting group estimates can have a RMSE close to 0.05 (Figure 3), deviations \leq 0.1 less than 10% of the time (Figure 4), and biases $\leq \pm 0.05$ (Figure 5) with a sample size of 80 to 95 fish.

GENETIC ANALYSIS

A total of 1,052 escapement samples were genotyped for 24 SNP markers. Using the 80% rule (Dann et al. 2009) for sufficiently complete genotypes, 26 individuals were removed, and 2 individuals were removed based on the criterion for detecting duplicate individuals. After data quality control, mixture sample sizes averaged 79 individuals (range 71–83; Table 3)

Estimates of Stock Composition

Black Lake comprised a majority of escapement samples in early strata, contributing 72% to the first stratum (June 27–29; Tables 6–7). The contribution of Black Lake to escapement samples declined through time, falling below 50% in the fourth stratum (July 4–6) and ending with 1.7% in the final escapement sample (July 26–29; Figure 7). Throughout all 13 strata, the Black Lake

estimate ranged from 1.6% to 72% and the range of 90% credibility intervals averaged 18% (Table 6).

Stock-specific Escapement

The escapement represented by samples in the 13 strata averaged 33,223 per stratum and ranged from 7,794 to 89,071 (Table 6). Estimates of Black Lake escapement averaged 11,737 and ranged from 376 to 64,252. It is important to note that unrepresented early and late escapement totaled 456,449, approximately 51% of the annual escapement.

Stratified Estimates of Stock-Specific Escapement for Represented Run

The total escapement past the Chignik weir in the transition period represented by 13 strata totaled 431,905. Chignik Lake comprised the majority of the escapement with an estimated 279,329 fish (64.7%) and Black Lake contributed an estimated 152,576 fish (35.3%; Table 8) during the transition period.

DISCUSSION

We applied GSI to samples of sockeye salmon escapement from the Chignik weir during the transition period between early- and late-runs to estimate the stock composition and stock-specific escapement during the transition period. We also assessed the baseline's ability to identify early- and late-run contributions in mixtures with current GCL evaluation methods and found the current baseline capable of identifying Black Lake and Chignik Lake reporting groups based upon current guidelines. We also conducted a sample size sensitivity analysis to measure the effect of sample size on accuracy and precision of GSI and found that mixture sample sizes used in 2023 satisfy current GCL guidelines.

COMPARISON OF TRANSITION WITH PREVIOUS YEARS

The transition of stock composition estimates from Black Lake (early run) to Chignik Lake (late run) in 2023 was consistent with prior years, with Black Lake comprising a majority of escapement early in the transition and Chignik Lake the majority of later escapement. When comparing 2023 estimates to those from 2010–2021, it should be noted that historical estimates were generated with slightly different methods but that results are still comparable. Namely, historical estimates sometimes used different marker sets (96 vs. 24 SNPs) and software (BAYES in 2010–2021 vs. *rubias* in 2023; Pella and Masuda 2001). However, the underlying population genetic structure of all marker sets used and the underlying model between software are nearly identical and results are comparable. While the transition from Black Lake to Chignik Lake was similar, the Black Lake estimate fell below 50% earlier in 2023 (July 4–6) than in past years (median July 13, range July 5–27; Table 9).

ESTIMATING STOCK-SPECIFIC ESCAPEMENT

As previously mentioned, different modeling approaches have been used in recent years to estimate stock-specific escapement past the Chignik weir: fitting GSI estimates to a logistic model, a Bayesian hierarchical model, and more recently an Expectation-Maximization algorithm. Because application of modeling algorithms to describe stock-specific escapement was beyond the scope of the cooperative agreement and expertise of the GCL, we did not apply any of these modeling approaches to the stock composition estimates. However, we did apply estimates to days of escapement counts that we thought were represented by the samples we analyzed (June 26–July

30) as providing estimates of stock-specific escapement was an objective of the cooperative agreement. We recommend stakeholders work with Westward Regional staff to use these data to generate annual estimates of stock-specific escapement in 2023.

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TABLES AND FIGURES

GCL Code	Location	Group	Latitude	Longitude	Date	Ν
SBROAD97	Broad Creek	Black Lake	56.4379	-158.7175	9/1/1997	93
SBSPR97	Big Spring	Black Lake	56.4316139	-158.7127639	8/8/1997	94
SBOUL97	Boulevard Creek	Black Lake	56.435	-158.7538	9/1/1997	95
SALEC97.SFAN97	Alec River	Black Lake	56.4586	-158.9362	9/1/1997	191
SCHIA08	Chiaktuak Creek	Chignik Lake	56.39	-158.916	8/29/2008	93
SCHIA97E	Chiaktuak Creek - Early	Chignik Lake	56.3898	-158.9363	8/4/1997	94
SCHIA97M	Chiaktuak Creek	Chignik Lake	56.3898	-158.9363	9/18/1997	93
SWESTF08	West Fork	Chignik Lake	56.248	-159.104	8/28/2008	94
SCUCU08	Cucumber Creek	Chignik Lake	56.276	-158.857	8/29/2008	94
SHAT96	Hatchery Beach - Chignik Lake	Chignik Lake	56.2678	-158.8627	10/18/1996	95
SHAT97E	Hatchery Beach-September	Chignik Lake	56.2678	-158.8627	9/15/1997	93
SHAT08E	Hatchery Creek - Early	Chignik Lake	56.268	-158.859	8/29/2008	92
SCLARK96	Clark River - Late	Chignik Lake	56.2294	-158.8109	10/19/1996	95
SCLRK97E	Clark River September	Chignik Lake	56.2294	-158.8109	9/16/1997	95
SCLARK08	Clark River	Chignik Lake	56.203	-158.811	8/28/2008	91
SCHIG98.SCHIG08	Chignik River	Chignik Lake	56.2721	-158.6625	8/22/1998	189

Table 1.–Populations of sockeye salmon within the Chignik River sockeye salmon baseline including GCL code, location, reporting group membership, location, sample date and sample size (N).

Locus	Ho	F _{IS}	F _{ST}
One_ACBP-79	0.419	0.032	0.006
One_CO1	NA	NA	0.006
One_GPDH ^a	0.482	-0.057	0.007
One_GPDH2 ^a	0.110	0.000	0.005
One_HpaI-436	0.492	0.004	0.003
One_MHC2_190 ^b	0.345	0.005	0.078
One_MHC2_251 ^b	0.333	0.009	0.137
One_ODC1-196	0.335	0.031	0.030
One_RAD18507	0.302	0.097	0.180
One_RAD27165	0.313	0.066	0.319
One_RAG3-93	0.218	0.056	0.012
One_Tf_ex3-182	0.426	0.004	0.068
One_U1003-75	0.325	0.020	0.006
One_U1004-183	0.310	-0.021	0.242
One_U1009-91	0.350	-0.020	0.006
One_U1012-68	0.407	-0.016	0.014
One_U1016-115	0.467	0.024	0.010
One_U1209-111	0.082	0.000	0.019
One_U1212-106	0.489	-0.012	0.027
One_agt-132	0.458	0.009	0.013
One_cin-177	0.496	-0.011	0.006
One_ghsR-66	0.237	-0.009	0.004
One_redd1-414	0.454	0.008	0.011
One sys1-230	0 336	0.038	0.017

Table 2.–Single nucleotide polymorphisms surveyed in populations in the Chignik sockeye salmon genetic baseline. The locus-specific observed heterozygosities (H_0), inbreeding coefficient (F_{IS}), and genetic diversity (F_{ST}) values are given.

^a These SNPs exhibited linkage disequilibrium and were combined into a haplotype.

^b These SNPs exhibited linkage disequilibrium and were combined into a haplotype.

Table 3.–Summary of experimental design used to analyze stock composition of sockeye salmon escapement past the Chignik Weir in 2023 by temporal stratum including sample dates, dates of escapement represented by samples and numbers of fish escaped, sampled, genotyped, and included in final analyses. Note that early and late periods of escapement not represented by samples do not have stock compositions applied to escapement in subsequent tables.

Temporal Stratum	Sample Dates	Stratum Dates	Escapement	Sampled	Genotyped	Final
Early Unrepresented		June 1–26	184,340	0	0	0
1	June 28	June 27–29	89,071	80	80	80
2	June 30	June 30–July 1	39,816	80	80	80
3	July 2	July 2–3	48,122	80	80	80
4	July 5	July 4–6	25,012	80	80	80
5	July 7	July 7–8	20,142	79	79	77
6	July 10–11	July 9–12	42,047	83	83	83
7	July 14	July 13–14	26,349	80	80	80
8	July 15	July 15–16	16,036	80	80	79
9	July 17	July 17–18	22,976	79	79	79
10	July 19–21	July 19–21	26,281	71	71	71
11	July 22	July 22	7,794	80	80	78
12	July 23	July 23–25	33,801	100	100	77
13	July 28	July 26–29	34,458	80	80	80
Late Unrepresented		July 30–September 30	272,109	0	0	0
		Total	888,354	1,052	1,052	1,024

Table 4.–Summary statistics for both reporting groups in the Chignik sockeye salmon baseline. Baseline evaluation test correct allocation summary results, including the number of test mixtures (N), range of compositions tested (Range), root mean square error (RMSE), the maximum percentage points from the true proportion where 90% of point estimates occurred (Within), and mean bias (Bias) are provided for each group.

Group	Number of populations	Mean sample size (Range)	Ν	Range	RMSE	Bias	Within
Black Lake	4	118 (93–191)	100	1-100%	0.03	-0.01	0.04
Chignik Lake	12	102 (91–189)	100	1-100%	0.03	0.01	0.04

Sample size	RMSE	Deviation	Mean bias
50	0.08	0.17	-0.003
60	0.07	0.15	0.011
70	0.06	0.1	-0.005
80	0.06	0.09	-0.008
95	0.06	0.07	0.009
190	0.04	0.02	0.004
380	0.03	0	0.005

Table 5.–Results of 100 cross-validation simulation assessing accuracy and precision of different sample sizes using a 22-marker set genetic baseline for the Chignik River sockeye salmon.

Table 6.–Temporal stratum, sampling dates, represented escapement, final sample sizes (n), mean estimates of stock composition, upper and lower 90% credibility intervals, and standard deviations for samples of the sockeye salmon escapement to the Chignik River in 13 strata in 2023. Note that early and late periods of escapement not represented by samples do not have stock compositions applied to escapement (NA).

				Black Lake					Chignik Lake			
Temporal Stratum	Stratum Dates	Escapement	n	Proportion	Lower	Upper	SD	Proportion	Lower	Upper	SD	
Early Unrepresented	June 1–26	184,340	0	NA	NA	NA	NA	NA	NA	NA	NA	
1	June 27–29	89,071	80	0.721	0.602	0.835	0.070	0.279	0.165	0.398	0.070	
2	June 30–July 1	39,816	80	0.587	0.465	0.708	0.075	0.413	0.292	0.535	0.075	
3	July 2–3	48,122	80	0.610	0.487	0.731	0.075	0.390	0.269	0.513	0.075	
4	July 4–6	25,012	80	0.294	0.187	0.414	0.068	0.706	0.586	0.813	0.068	
5	July 7–8	20,142	77	0.304	0.168	0.445	0.084	0.696	0.555	0.832	0.084	
6	July 9–12	42,047	83	0.255	0.145	0.373	0.070	0.745	0.627	0.855	0.070	
7	July 13–14	26,349	80	0.084	0.030	0.155	0.039	0.916	0.845	0.970	0.039	
8	July 15–16	16,036	79	0.108	0.028	0.207	0.055	0.892	0.793	0.972	0.055	
9	July 17–18	22,976	79	0.122	0.048	0.211	0.050	0.878	0.789	0.952	0.050	
10	July 19–21	26,281	71	0.016	0.000	0.060	0.021	0.984	0.940	1.000	0.021	
11	July 22	7,794	78	0.048	0.004	0.114	0.034	0.952	0.886	0.996	0.034	
12	July 23–25	33,801	77	0.098	0.028	0.188	0.049	0.902	0.812	0.972	0.049	
13	July 26–29	34,458	80	0.017	0.000	0.062	0.022	0.983	0.938	1.000	0.022	
Late Unrepresented	July 30–September 30	272,109	0	NA	NA	NA	NA	NA	NA	NA	NA	

		Stock Composition						Stock-specific Escapement				
	Stratum			909	% CI				90%	CI		
#	Dates	Reporting Group	Median	5%	95%	Mean	SD	 Median	5%	95%	Mean	SD
1	June 27–29	Black Lake	72.4	60.2	83.5	72.1	7.0	64,475	53,594	74,414	64,252	6,254
1	June 27–29	Chignik Lake	27.6	16.5	39.8	27.9	7.0	24,596	14,657	35,477	24,819	6,254
2	June 30–July 1	Black Lake	58.8	46.5	70.8	58.7	7.5	23,393	18,507	28,193	23,384	2,983
2	June 30–July 1	Chignik Lake	41.2	29.2	53.5	41.3	7.5	16,423	11,623	21,309	16,432	2,983
3	July 2–3	Black Lake	61.3	48.7	73.1	61.0	7.5	29,500	23,451	35,200	29,333	3,624
3	July 2–3	Chignik Lake	38.7	26.9	51.3	39.0	7.5	18,622	12,922	24,671	18,789	3,624
4	July 4–6	Black Lake	29.0	18.7	41.4	29.4	6.8	7,244	4,674	10,346	7,342	1,711
4	July 4–6	Chignik Lake	71.0	58.6	81.3	70.6	6.8	17,768	14,666	20,338	17,670	1,711
5	July 7–8	Black Lake	30.1	16.8	44.5	30.4	8.4	6,069	3,385	8,970	6,115	1,686
5	July 7–8	Chignik Lake	69.9	55.5	83.2	69.6	8.4	14,073	11,172	16,757	14,027	1,686
6	July 9–12	Black Lake	25.3	14.5	37.3	25.5	7.0	10,632	6,084	15,668	10,718	2,938
6	July 9–12	Chignik Lake	74.7	62.7	85.5	74.5	7.0	31,415	26,379	35,963	31,329	2,938
7	July 13-14	Black Lake	7.9	3.0	15.5	8.4	3.9	2,085	792	4,096	2,210	1,032
7	July 13-14	Chignik Lake	92.1	84.5	97.0	91.6	3.9	24,264	22,253	25,557	24,139	1,032
8	July 15-16	Black Lake	10.3	2.8	20.7	10.8	5.5	1,646	456	3,313	1,738	876
8	July 15–16	Chignik Lake	89.7	79.3	97.2	89.2	5.5	14,390	12,723	15,580	14,298	876
9	July 17–18	Black Lake	11.5	4.8	21.1	12.2	5.0	2,648	1,099	4,851	2,793	1,150
9	July 17–18	Chignik Lake	88.5	78.9	95.2	87.8	5.0	20,328	18,125	21,877	20,183	1,150
10	July 19–21	Black Lake	0.7	0.0	6.0	1.6	2.1	196	0	1,587	421	549
10	July 19–21	Chignik Lake	99.3	94.0	100.0	98.4	2.1	26,085	24,694	26,281	25,860	549
11	July 22	Black Lake	4.2	0.4	11.4	4.8	3.4	330	32	890	376	265
11	July 22	Chignik Lake	95.8	88.6	99.6	95.2	3.4	7,464	6,904	7,762	7,418	265
12	July 23-25	Black Lake	9.0	2.8	18.8	9.8	4.9	3,049	933	6,361	3,302	1,657
12	July 23-25	Chignik Lake	91.0	81.2	97.2	90.2	4.9	30,752	27,440	32,868	30,499	1,657
13	July 26–29	Black Lake	0.8	0.0	6.2	1.7	2.2	260	0	2,123	592	772
13	July 26–29	Chignik Lake	99.2	93.8	100.0	98.3	2.2	34,198	32,335	34,458	33,866	772

Table 7.-Estimates of stock composition (%) and stock-specific escapement including median, 90% credibility interval, mean and standard deviation (SD).

	Stock Composition						Stock-specific Escapement (13 strata)					
	90% CI						90% CI					
Reporting Group	Median	5%	95%	Mean	SD	Median	5%	95%	Mean	SD		
Black Lake	35.4	31.7	38.7	35.3	2.1	152,869	136,876	167,223	152,576	9,208		
Chignik Lake	64.6	61.3	68.3	64.7	2.1	279,036	264,682	295,029	279,329	9,208		
							-	Fotal	431,905			

Table 8.–Stratified estimates of stock composition (%) and stock-specific escapement including median, 90% credibility interval, mean and standard deviation (SD) for the2023 Chignik escapement period represented by genetic samples (June 27–July 29).

				Black La	ake			Chignik L	Lake	
Year	Stratum	n	Proportion	Lower	Upper	SD	Proportion	Lower	Upper	SD
	June 14	190	0.959	0.894	1.000	0.036	0.041	0.000	0.106	0.036
	June 21	189	0.995	0.966	1.000	0.014	0.005	0.000	0.034	0.014
	June 27	189	0.924	0.794	1.000	0.075	0.076	0.000	0.206	0.075
	July 1	189	0.823	0.724	0.912	0.057	0.177	0.088	0.276	0.057
	July 5	190	0.788	0.699	0.871	0.052	0.212	0.129	0.301	0.052
2010	July 8–9	190	0.784	0.687	0.870	0.056	0.216	0.130	0.313	0.056
	July 11	190	0.519	0.409	0.625	0.066	0.481	0.375	0.591	0.066
	July 14	188	0.227	0.154	0.306	0.046	0.773	0.694	0.846	0.046
	July 18-19	188	0.293	0.214	0.377	0.050	0.707	0.623	0.786	0.050
	July 23	186	0.108	0.052	0.173	0.037	0.892	0.827	0.948	0.037
	July 30	190	0.013	0.000	0.062	0.022	0.987	0.938	1.000	0.022
	June 10	188	0.998	0.988	1.000	0.005	0.002	0.000	0.012	0.005
	June 17	188	1.000	1.000	1.000	0.002	0.000	0.000	0.000	0.002
	June 24	188	0.976	0.888	1.000	0.040	0.024	0.000	0.112	0.040
	June 28	190	0.832	0.744	0.918	0.054	0.168	0.082	0.256	0.054
	July 2	190	0.953	0.886	1.000	0.036	0.047	0.000	0.114	0.036
2011	July 5	190	0.785	0.696	0.866	0.052	0.215	0.134	0.304	0.052
	July 9–10	187	0.719	0.625	0.807	0.055	0.281	0.193	0.375	0.055
	July 12-13	190	0.297	0.214	0.384	0.052	0.703	0.616	0.786	0.052
	July 14	190	0.308	0.217	0.402	0.056	0.692	0.598	0.783	0.056
	July 21	186	0.123	0.062	0.192	0.039	0.877	0.808	0.938	0.039
	July 28	189	0.036	0.000	0.088	0.029	0.964	0.912	1.000	0.029
	June 11	188	0.976	0.904	1.000	0.034	0.024	0.000	0.096	0.034
	June 18	190	0.964	0.882	1.000	0.042	0.036	0.000	0.118	0.042
	June 25	189	0.993	0.955	1.000	0.017	0.007	0.000	0.045	0.017
	July 1	190	0.644	0.544	0.733	0.058	0.356	0.267	0.456	0.058
	July 5	187	0.485	0.396	0.574	0.054	0.515	0.426	0.604	0.054
2012	July 8–9ª	187	0.099	0.005	0.235	0.071	0.901	0.765	0.995	0.071
	July 11	189	0.225	0.147	0.306	0.048	0.775	0.694	0.853	0.048
	July 14	190	0.070	0.011	0.132	0.036	0.930	0.868	0.989	0.036
	July 17	189	0.003	0.000	0.020	0.009	0.997	0.980	1.000	0.009
	July 21	190	0.006	0.000	0.049	0.018	0.994	0.951	1.000	0.018
	July 28	170	0.000	0.000	0.000	0.001	1.000	1.000	1.000	0.001
	June 27	188	0.911	0.838	1.000	0.045	0.089	0.000	0.162	0.024
	July 1	189	0.858	0.761	0.942	0.055	0.142	0.058	0.239	0.055
2013	July 5	169	0.612	0.515	0.705	0.058	0.388	0.295	0.485	0.058
	July 8–9	187	0.429	0.338	0.519	0.055	0.571	0.481	0.662	0.055
	July 14	190	0.288	0.196	0.384	0.057	0.712	0.616	0.804	0.057

Table 9.–Sampling dates, final sample sizes, mean estimates of stock composition, upper and lower 90% credibility intervals, and standard deviations for samples of the escapement to the Chignik River in multiple strata in 2010–2021.

			Black Lake			Chignik Lake				
Year	Stratum	n	Proportion	Lower	Upper	SD	Proportion	Lower	Upper	SD
	June 28	189	0.825	0.745	0.896	0.046	0.175	0.104	0.255	0.046
	July 2	189	0.785	0.690	0.874	0.056	0.215	0.126	0.310	0.056
2014	July 6	189	0.618	0.519	0.714	0.059	0.382	0.286	0.481	0.059
2014	July 10	188	0.357	0.258	0.460	0.062	0.643	0.540	0.742	0.062
	July 14	188	0.220	0.139	0.307	0.051	0.780	0.693	0.861	0.051
	July 18	189	0.143	0.064	0.227	0.050	0.857	0.773	0.936	0.050
	June 27	190	0.905	0.815	1.000	0.054	0.095	0.000	0.185	0.054
	July 1	188	0.932	0.856	0.996	0.042	0.068	0.004	0.144	0.042
2015	July 5	187	0.864	0.775	0.944	0.051	0.136	0.056	0.225	0.051
2015	July 12	190	0.894	0.790	0.995	0.061	0.106	0.005	0.210	0.061
	July 18	182	0.363	0.253	0.476	0.068	0.637	0.524	0.747	0.068
	July 25	187	0.383	0.284	0.485	0.061	0.617	0.515	0.716	0.061
	June 27	189	0.988	0.938	1.000	0.022	0.012	0.000	0.062	0.022
	July 2	156	0.799	0.694	0.895	0.061	0.201	0.105	0.306	0.061
2016	July 7	190	0.626	0.535	0.717	0.055	0.374	0.283	0.465	0.055
2010	July 12	180	0.422	0.338	0.506	0.051	0.578	0.494	0.662	0.051
	July 17	187	0.199	0.130	0.272	0.043	0.801	0.728	0.870	0.043
	July 26–27	190	0.135	0.076	0.202	0.038	0.865	0.798	0.924	0.038
	June 25–26	189	0.986	0.917	1.000	0.029	0.014	0.000	0.083	0.029
	July 1	190	0.855	0.779	0.922	0.044	0.145	0.078	0.221	0.044
2017	July 7–8	189	0.715	0.622	0.803	0.055	0.285	0.197	0.378	0.055
2017	July 13	189	0.317	0.229	0.408	0.055	0.683	0.592	0.771	0.055
	July 18	188	0.417	0.330	0.504	0.053	0.583	0.496	0.670	0.053
	July 23	188	0.429	0.332	0.526	0.059	0.571	0.474	0.668	0.059
	June 26–27	189	0.989	0.931	1.000	0.026	0.011	0.000	0.069	0.026
	July 2	188	0.754	0.629	0.871	0.073	0.246	0.129	0.371	0.073
	July 8–12	185	0.884	0.803	0.954	0.046	0.116	0.046	0.197	0.046
2018	July 17	189	0.636	0.532	0.735	0.062	0.364	0.265	0.468	0.062
	July 22–23	189	0.559	0.453	0.659	0.063	0.441	0.341	0.547	0.063
	July 27	186	0.309	0.212	0.410	0.060	0.691	0.590	0.788	0.060
	Aug 8–9	178	0.037	0.000	0.090	0.028	0.963	0.910	1.000	0.028
	June 25	188	0.998	0.988	1.000	0.008	0.002	0.000	0.012	0.008
	July 1	188	0.984	0.892	1.000	0.037	0.016	0.000	0.108	0.037
2010	July 8	187	0.640	0.543	0.732	0.058	0.360	0.268	0.457	0.058
2019	July 13	188	0.591	0.475	0.698	0.067	0.409	0.302	0.525	0.067
	July 19	177	0.188	0.119	0.263	0.044	0.812	0.737	0.881	0.044
	July 26–29	95	0.033	0.000	0.085	0.027	0.967	0.915	1.000	0.027

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				Black La	ake			Chignik I	Lake	
Year	Stratum	n	Proportion	Lower	Upper	SD	Proportion	Lower	Upper	SD
	June 29–July 1	185	0.759	0.666	0.846	0.055	0.241	0.154	0.334	0.055
	July 6	167	0.633	0.523	0.740	0.066	0.367	0.260	0.477	0.066
2020	July 11–12	176	0.637	0.528	0.736	0.063	0.363	0.264	0.472	0.063
2020	July 17	182	0.327	0.224	0.432	0.063	0.673	0.568	0.776	0.063
	July 23	187	0.263	0.170	0.365	0.059	0.737	0.635	0.830	0.059
	August 1	189	0.162	0.096	0.234	0.042	0.838	0.766	0.904	0.042
	June 25	190	0.892	0.824	0.951	0.039	0.108	0.049	0.176	0.039
	July 1	189	0.854	0.764	0.939	0.053	0.146	0.061	0.236	0.053
	July 7	184	0.643	0.541	0.743	0.061	0.357	0.257	0.459	0.061
2021	July 13–14	185	0.342	0.258	0.428	0.052	0.658	0.572	0.742	0.052
	July 19–20	190	0.198	0.125	0.276	0.046	0.802	0.724	0.875	0.046
	July 26–27	187	0.125	0.068	0.190	0.037	0.875	0.810	0.932	0.037
	Aug 8–9	184	0.004	0.000	0.020	0.008	0.996	0.980	1.000	0.008

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^a Note these estimates were associated with a Gelman-Rubin shrink factor value of 1.42.



Figure 1.-Collection locations for populations of sockeye salmon included in the baseline.



Figure 2.–Results of baseline evaluation test mixtures with true proportion along horizontal axis and estimated proportion \pm 90% CI along vertical axis including root mean square error (RMSE), mean bias, percentage of tests with correct allocations falling within 10% of true value, and percentage of tests within 10% of true proportion.



Figure 3.-Root mean square errors (RMSE) for different sample sizes of the 100 cross-validation simulation using a 22-marker set genetic baseline for the early- and late-run of the Chignik River sockeye. RMSE shown here accounted for errors incurred during weir sampling.



Figure 4.–Deviations for different sample sizes of the 100 cross-validation simulation using a 22-marker set genetic baseline for the early- and late-run of the Chignik River sockeye. Deviations shown here accounted for sampling errors. Red dashed line marked the GCL's guideline of 10% for deviation ≥ 0.1 .



Figure 5.–Mean biases for different sample sizes of the 100 cross-validation simulation using a 22-marker set genetic baseline for the early- and late-run of the Chignik River sockeye. Biases shown here accounted for sampling errors. Red dashed lines marked the GCL's guideline of ± 0.05 for bias.



Figure 6.–Estimate (posterior means) vs. true proportions from the 100 cross-validation simulations of the 22-marker set genetic baseline for the early- and late-run of the Chignik River sockeye. Red diagonal lines mark the 1:1 relationship with ± 0.1 in dashed lines.



Figure 7.–Summary of genetic stock composition estimates (mean +/- 90% CI) for each of the 13 sampling strata from the Chignik River weir in 2023.

APPENDIX A: COOPERATIVE AGREEMENT NUMBER 23-185



Alaska Department of Fish and Game Division of Administrative Services P.O. Box 115526 Juneau, AK 99811-5526 Alaska Dept. of Fish and Game Cooperative Agreement # 23-185

Cooperative Agreement Number 23-185 Title: Chignik Genetic Samples

Between:

Alaska Department of Fish and Game Division of Commercial Fisheries and (1) Bristol Bay Native Association – Chignik Fisheries Task Force & (2) Chignik Regional Aquaculture Association

I. AUTHORITY:

This agreement is entered into by and between the Alaska Department of Fish and Game, Division of Commercial Fisheries (hereinafter referred to as the "ADF&G" or the "Department") and two organizations: (1) the Bristol Bay Native Association – Chignik Fisheries Task Force (hereinafter referred to as BBNA Chignik Fisheries Task Force) and (2) the Chignik Regional Aquaculture Association (hereinafter referred to as "CRAA").

ADF&G enters into this agreement under authority AS 16.05.050(12) and AS 36.30.850.d.

II. PURPOSE OF THE AGREEMENT:

To delineate the migration timing of Chignik Lakes' two sockeye salmon runs in 2023 using Genetic Stock Identification (GSI) during the migration-overlap period from about late June through July and apply the GSI data post-season to calculate escapement and harvest numbers, timing, and age-class run numbers for the two stocks.

ADF&G has the authority, experience, and personnel to carry out the GSI project but has insufficient funds for personnel and supplies. CRAA and BBNA (Bristol Bay Native Association) have an interest in the GSI projects along with industry, Chignik Intertribal Coalition, and the Chignik Advisory Counsel, and CRAA and BBNA are funding this specific project.

III. PROJECT OBJECTIVES:

Through this project, ADF&G Gene Conservation Laboratory (GCL) staff will process samples collected in 2023 from sockeye salmon escaping through the Chignik weir. This project will genotype 1,056 sockeye salmon tissue samples across appropriate temporal stratacollected from the Chignik weir in 2023 for 24 genetic markers capable of distinguishing early- (Black Lake) and late- run (Chignik Lake) groups of populations, conduct mixed stock analysis based upon those genotypes, and calculate stock-specific estimates of escapement past the Chignik weir. Results will be made available to BBNA, CRAA and other interested parties.

IV. TERM OF THE AGREEMENT:

This Agreement shall be effective 04/15/2024 and remain in effect through 08/31/2024.

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Alaska Dept. of Fish and Game Cooperative Agreement # 23-185

V. COVENANTS OF THE ADF&G:

- a. To provide experience, personnel, and existing equipment to carry out the mutually agreed upon project.
- b. To provide management of personnel and project activities.

VI. COVENANTS OF CRAA and BBNA:

- a. To provide funding for the joint project for ADF&G personnel as identified in the Scope of Work and Financial Considerations. CRAA and BBNA will pay the State of Alaska for approved ADF&G personnel and administrative costs and will pay directly for other approved costs.
- b. To submit payment in full within 30 days of the Cooperative Agreement being signed by all parties.

VII. PROJECT POINTS OF CONTACT:

ADF&G:

ADF&G Project Lead: Tyler Dann, Fisheries Geneticist 2 Address: 333 Raspberry Rd., Anchorage, AK 99518-1599 Phone: (907) 267-2201 Email: <u>tyler.dann@alaska.gov</u>

BBNA – Chignik Fisheries Task Force:

BBNA – Chignik Fisheries Task Force Project Lead: Patrick Kosbruk, Chairman Address: PO Box 10, Perryville AK 99648 Phone: (907) 853-4021 Email: <u>patkosbruk@gmail.com</u>

CRAA:

CRAA Project Lead: Charles McCallum, Executive Director Address:2101 Cornwall Ave., Suite 201, Bellingham, WA 98225 Phone: (907) 351-9107 Email: chuckmmcallum@gmail.com

VIII. FINANCIAL CONSIDERATIONS:

Project Budget

- <u>Contractual:</u> Lab Equipment maintenance \$2/sample, 1056 samples = \$2,112
- Personnel:
 - Fisheries Geneticist 2, 0.5MM = \$5,831
 - Fishery Biologist 1, 0.5MM = \$3,871
 - Fish and Wildlife Technician 3, 0.5MM = \$3,373
- <u>Supplies:</u> Laboratory supplies (biochemicals, plastics, plates) for 1,056 samples = \$15,784

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Alaska Dept. of Fish and Game Cooperative Agreement # 23-185

- Indirect @26.99% (personnel Costs Only) = \$3,529
- Total = \$34,500

Allocation: \$25,000 BBNA Chignik Fisheries Task Force Allocation: \$9,500 CRAA Total = \$34.500

IX. GENERAL PROVISIONS

- Nothing in this agreement shall obligate any party in the expenditure of funds, or for future payments of money, in excess of appropriations authorized by law.
- Each party agrees that it will be responsible for its own acts and omissions including those of its officers, agents, and employees for damages to property or injury to persons occasioned by each party's own acts or omissions in connection with the terms of this agreement.
- Both parties agree to comply with all applicable federal or State laws regulating ethical conduct of public officers and employees.
- Each party will comply with all applicable laws, regulations, and executive orders relative to Equal Employment Opportunity.
- Nothing herein is intended to conflict with federal, state, or local laws or regulations. If there are conflicts, this agreement will be amended at the first opportunity to bring it into conformance with conflicting laws or regulations.
- Policy and position announcements relating specifically to this cooperative program may be made only by mutual consent of the agencies.
- 7. The effective date of this agreement shall be upon final signature.
- The termination date of this agreement shall be 08/31/2024. However, either party may terminate its participation in this agreement by providing to the other party notice in writing 30 days in advance of the date on which its termination becomes effective.
- A free exchange of research and assessment data among agencies is encouraged and is necessary to ensure the success of these cooperative studies.
- CRAA, BBNA and any agents or employees act in an independent capacity and not as officers, employees, or agents of the State in performance under this agreement.
- 11. This agreement may be amended by mutual written consent of the parties.
- 12. CRAA,BBNA by signing this agreement, certifies that neither it, nor its principals or subcontractors, is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from federal financial assistance programs or activities.

13. Force Majeure: The parties to this contract are not liable for the consequences of any failure to perform, or default in performing, any of their obligations under this Agreement, if that failure or default is caused by any unforeseeable Force Majeure, beyond the control of, and without the fault or negligence of, the respective party. For the purposes of this Agreement, Force Majeure will mean war (whether declared or not); revolution; invasion; insurrection; riot; civil commotion; sabotage; military or usurped power; lightning; explosion; fire; storm; drought; flood; earthquake; epidemic; quarantine; strikes; acts or restraints of governmental authorities affecting the project or directly or indirectly prohibiting or restricting the furnishing or use of materials or labor required; inability to secure materials, machinery, equipment or labor because of priority, allocation or other regulations of any governmental authorities.

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Alaska Dept. of Fish and Game Cooperative Agreement # 23-185

X. APPROVING SIGNATURES

IN WITNESS WHEREOF, the parties hereto have caused this Cooperative Agreement to be executed as of the date of last signature below.

Bristol Bay Native Association - Chignik Fisheries Task Force

Garvin Federenko, President/CEO of BBNA

4/17/2024

Date

DocuSigned by Pour Kull

Patrick Kosbruk, BBNA, Chairman of the Chignik Fisheries Task Force

4/17/2024

Date

CHIGNIK REGIONAL AQUACULTURE ASSOCIATION

DocuSigned by: Charles Mallum

Chuck McCallum, Executive Director of CRAA

4/19/2024

Date

ALASKA DEPARTMENT OF FISH AND GAME

DocuSigned by:

Britteny Cioni-Haywood Operations Manager Division of Commercial Fisheries

4/19/2024

Date

Bonnie Densen -B3CASHE704464A4

Bonnie N. Jensen, Director Division of Administrative Services

4/19/2024

Date

Page 4

Appendix A1.–Page 5 of 8.

DocuSign⁻

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Certificate Of Completion								
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Source Envelope:								
Document Pages: 4	Signatures: 4	Envelope Originator:						
Certificate Pages: 4	Initials: 0	Jennifer Messing						
AutoNav: Enabled		PO Box 110206						
EnvelopeId Stamping: Disabled		Juneau, AK 99811						
Time Zone: (UTC-09:00) Alaska		jennifer.messing@alaska.gov						
		IP Address: 158.145.15.21						
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Status: Original	Holder: Jennifer Messing	Location: DocuSign						
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Storage Appliance Status: Connected	Pool: State of Alaska	Location: DocuSign						
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Patrick Koshnik	DocuSigned by:	Sect 4/17/2024 12:22:20 PM						
patkoshuk@gmail.com	R- O.L 14	Viewed: 4/17/2024 1:25:25 PM						
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Chuck McCallum	-DocuSigned by:	Sept 4/17/2024 1-25-53 PM						
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Appendix A1.–Page 6 of 8.

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Signer Events	Signature	Timestamp		
Bonnie Jensen	DocuSigned by:	Sent: 4/19/2024 9:33:25 AM		
bonnie.jensen@alaska.gov	Bonnie Stensen	Viewed: 4/19/2024 10:18:56 AM		
Director	B3CA84E704464A4	Signed: 4/19/2024 10:19:39 AM		
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Agent Delivery Events	Status	Timestamp		
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Garven Federenko	CODIED	Sent: 4/19/2024 10:19:42 AM		
garvin.federenko@bbna.com	COPIED			
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Signing Complete	Security Checked	4/18/2024 10:19:39 AM		
Completed	Security Checked	4/18/2024 10:18:42 AM		
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Electronic Record and Signature Discl	osure			

Electronic Record and Signature Disclosure created on: 3/31/2020 10:41:05 PM Parties agreed to: Patrick Kosbruk, Chuck McCallum, Britteny Cioni-Haywood, Bonnie Jensen, Kyle Paddleford

ELECTRONIC RECORD AND SIGNATURE DISCLOSURE

Please read this Electronic Records and Signature Disclosure (ERSD). It concerns your rights regarding electronically undertaking, and the conditions under which you and the State of Alaska agree to electronically undertake, the transaction to which it relates (the "TRANSACTION").

Consent to Electronically Undertake the TRANSACTION

You can electronically undertake the TRANSACTION only if you confirm that you meet the following requirements by selecting the box next to "I agree to use electronic records and signature" (the "AGREE BOX"):

- 1. you can fully access and have read this ERSD;
- 2. you can fully access all of the information in the other TRANSACTION records;
- you can retain all of the TRANSACTION records in a form that you will be able to fully access for later reference;
- 4. you consent to undertake the TRANSACTION electronically; and
- you are authorized to undertake the TRANSACTION. (Please note that falsely undertaking the TRANSACTION may subject you to civil liabilities and penalties and/or to criminal penalties.)

If you cannot or are not willing to confirm each of these five things, do not select the AGREE BOX.

Withdrawing Consent

If you select the AGREE BOX, you can withdraw your consent to electronically undertake the TRANSACTION at any time before you complete the TRANSACTION: simply do not finalize it. The only consequence of withdrawing your consent is that you will not finalize the TRANSACTION.

If you select the AGREE BOX, your consent will apply only to this TRANSACTION. You must separately consent to electronically undertake any other transaction with the State of Alaska.

Paper Option for Undertaking the TRANSACTION

You may undertake the TRANSACTION with the State of Alaska using paper records. (State of Alaska employees who want to undertake the TRANSACTION in paper should contact the agency responsible for the TRANSACTION.) Print the paper records on the website of the State of Alaska agency responsible for the TRANSACTION, or request them from the agency. The State of Alaska homepage is at http://alaska.gov/.

Copies of TRANSACTION Records

After completing the TRANSACTION but before closing your web browser, you should download the TRANSACTION records. Or you can download the records within 30 days after completing the TRANSACTION using the link in the DocuSign email sent to the email address you used to complete the TRANSACTION. The State of Alaska will not provide a paper copy of the TRANSACTION records as part of the TRANSACTION. Under the Alaska Public Records Act (APRA), AS 40.25.100–.295, you can request a copy from the agency responsible for the TRANSACTION, but if too much time has passed, the agency may no longer have the records when you make your request. If required under the APRA, the agency will charge a fee.

Required Hardware and Software

For the minimum system requirements to electronically undertake the TRANSACTION, including accessing and thereby retaining the TRANSACTION records, visit https://support.docusign.com/guides/signer-guide-signing-system-requirements. These requirements may change. In addition, you need access to an email account.

How to Contact the State of Alaska

To ask a question on this ERSD or the DocuSign document generated after you complete the TRANSACTION or on using DocuSign to electronically undertake the TRANSACTION, contact the Alaska Department of Administration at either of the following addresses:

State of Alaska Department of Administration 550 West 7th Avenue Suite 1970 Anchorage, AK 99501 Reference: DocuSign

doa.commissioner@alaska.gov Subject: DocuSign

To ask any other question on the TRANSACTION records or to update the information for contacting you electronically, contact the State of Alaska agency responsible for the TRANSACTION using the contact information in the TRANSACTION records or, if those records contain no contact information, using the contact information on the agency's website. Again, the State of Alaska homepage is at http://alaska.gov/.