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Current Status of Genetic Studies of Coho Salmon from Southcentral Alaska and Evaluations for Mixed Stock Analysis in Cook Inlet

by

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January 2017

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

FISHERY MANUSCRIPT SERIES NO. 17-01

CURRENT STATUS OF GENETIC STUDIES OF COHO SALMON FROM SOUTHCENTRAL ALASKA AND EVALUATIONS FOR MIXED STOCK ANALYSIS IN COOK INLET

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ABSTRACT

This report describes the current status of genetic studies for coho salmon in Southcentral Alaska and initial evaluations for mixed stock analysis (MSA) in Cook Inlet. Coho salmon are harvested in commercial fisheries in Upper Cook Inlet, Alaska, with an average annual harvest of 171,273 fish (2005–2014). Harvests often occur in areas where stocks intermingle, so the exploitation and productivity of individual stocks are not well known. This lack of knowledge hinders fishery management based on the sustained yield principle. Mixed stock analysis has been used to estimate stock compositions of fishery mixtures in coho salmon elsewhere in the Pacific Northwest; however, only limited baseline existed for Cook Inlet. Within Cook Inlet, we examined a baseline of 84 populations for 86 single nucleotide polymorphism markers to examine population structure and test for potential reporting groups (stocks). From the southern edge of the Kenai Peninsula, we analyzed 3 populations to investigate the genetic legacy of stocking programs in the area. Population structure indicated that populations generally cluster by drainage, with the most genetically distinct populations in the more southerly drainages and in the upper reaches of the larger drainages. Testing of potential reporting groups revealed 7 groups with adequate genetic divergence to meet the criteria for reporting groups. Due to the sparse representation of collections in the baseline for the Yentna River drainage, additional representation within this drainage would be prudent before using it as its own reporting group in MSA. The data presented in this report will allow for additional baseline evaluation tests tailored for specific MSA study objectives pertinent to Cook Inlet mixed stock fisheries in the future. The 3 populations from the southern edge of the Kenai Peninsula were most similar to each other, but divergent from Cook Inlet populations, suggesting little genetic introgression from stocking programs.

Key words: coho salmon, Cook Inlet, Resurrection River, *Oncorhynchus kisutch*, single nucleotide polymorphism, SNP, mixed stock analysis, MSA

INTRODUCTION

BACKGROUND

This report describes the current status of genetic studies for coho salmon in Southcentral Alaska and initial evaluations for mixed stock analysis (MSA) in Cook Inlet. Populations of coho salmon *Oncorhynchus kisutch* support important fisheries in the Upper Cook Inlet management area (UCI; Figure 1). Annual total harvest of coho salmon in the UCI commercial fishery averaged 171,273 fish between 2005 and 2014 (Shields and Dupuis 2016). Most harvests occur during homeward migration in the open ocean or in the lower reaches of river drainages—areas where stocks are mixed. Without stock-specific harvest and escapement information, the exploitation and productivity of any single stock cannot be estimated, limiting management for sustained yield by the Alaska Department of Fish and Game (ADF&G) under the policy for the management of sustainable salmon fisheries (5 AAC 39.222).

MSA using genetic markers has been widely applied in the Pacific Northwest to apportion coho salmon sampled from mixtures of fish harvested during the migratory portion of their life cycle to regional stock groupings. This method requires the genetic characterization of populations contributing to the mixture (baseline) as well as the fishery samples (Pella and Milner 1987). MSA has been used to estimate the migration patterns of juvenile coho salmon in marine waters off the coasts of Washington and Oregon (e.g., Teel et al. 2003; Van Doornik et al. 2007), and to estimate the stock contributions to fishery catches in the coastal waters of British Columbia and the Fraser River (e.g., Small et al. 1998; Beacham et al. 2012). In Alaska, MSA has been used for inriver applications to estimate the stock composition of test fishery catches in the Kuskokwim (Crane et al. 2007) and Yukon (Flannery and Loges 2016) rivers, but little work has been done to resolve issues in marine fisheries because these typically require larger baselines.

The genetic diversity of coho salmon has been described for both fine- and broad-scale geographic areas (e.g., Small et al. 1998; Olsen et al. 2003; Ford et al. 2004; Bucklin et al. 2007;

Johnson and Banks 2008; Beacham et al. 2011); however, to date, our understanding of coho salmon genetic diversity in Cook Inlet has been limited. Olsen et al. (2003) evaluated the genetic diversity patterns in coho salmon in Alaska that included 6 Kenai River and 2 west Cook Inlet populations. This dataset demonstrated significant genetic diversity among coho salmon populations, but weak regional structuring of populations statewide. Olsen et al. (2003) concluded that the diversifying influence of genetic drift was stronger than the homogenizing influence of gene flow in coho salmon in Alaska. For MSA within Cook Inlet, these results suggest the possibility of distinguishing among coho salmon stocks within Cook Inlet, but, given the lack of overall regional structure, relatively intensive baseline sampling may be required in order to obtain a baseline representative of the full genetic diversity of coho salmon within Cook Inlet.

In 2013, the state funded a 3-phase study to develop a Cook Inlet coho salmon baseline and apply this baseline to analyze fishery mixtures. The first phase involved an analysis using existing samples collected by the U.S. Fish and Wildlife Service (USFWS), as well as other samples opportunistically collected by ADF&G, to determine whether the genetic diversity among Cook Inlet coho salmon populations would allow for accurate MSA estimates (DeCovich et al. 2013). Statistical analysis of these data indicated that sufficient variation exists among Cook Inlet coho salmon stocks (Barclay et al. 2014), and that it was appropriate to proceed with baseline development (phase II) and sampling of the UCI commercial harvest for genetic MSA (phase III, Barclay et al. 2016b).

The development of a coho salmon baseline for Cook Inlet provides a foundation to examine the population structure of coho salmon spawning in Resurrection River and to investigate the genetic legacy of stocking programs. Coho salmon originating in the river support one of the largest marine sport fisheries for the species in the Pacific Northwest (Bosch 2011). In the early 1960s, Bear Lake (Figure 1) was chosen as a coho salmon rearing lake to increase production of coho salmon in Resurrection Bay, and was "rehabilitated" with rotenone to remove predator and competitor species in 1963, and again in 1969 and 1971 to remove stickleback *Gasterosteus aculeatus* (McHenry 1982). During this period of rehabilitation, several hatchery-reared coho salmon stocks were released into Bear Creek (outlet of Bear Lake) to increase the return to Bear Lake, including stocks from Oregon and Kodiak. Additionally, hatchery releases have been conducted in Seward Lagoon since the mid-1960s with hatchery-reared stocks from Oregon, Bear Lake, and Kodiak (McHenry 1982; Loopstra and Hansen 2015). Resurrection River, which is also at the head of Resurrection Bay, has natural spawning of coho salmon and has not been supplemented. This baseline will provide insights into possible genetic effects of these stocking events.

In this study, we present the most comprehensive analysis of Cook Inlet coho salmon population structure to date by analyzing collections using recently developed single nucleotide polymorphism (SNP) markers (Smith et al. 2006; Campbell and Narum 2011; Starks et al. 2015; Matt Smith, unpublished, University of Washington, e-mail matt_smith@fws.gov). Specific objectives were to 1) evaluate genetic variation among populations distributed throughout Cook Inlet to delineate stock groups for MSA; 2) evaluate these stock groups for their use in MSA of UCI marine fisheries; and 3) evaluate the genetic distinctiveness of coho salmon spawning in streams on the southern edge of the Kenai Peninsula, including Resurrection River, to provide insights into the genetic legacy of historical stocking programs.

DEFINITIONS

Definitions of commonly used genetic terms are provided here to better understand the methods, results, and interpretation of this study.

Allele. Alternative form of a given gene or DNA sequence.

Bootstrapping. A method of resampling data with replacement to assess the variation of parameters of interest.

F_{ST}. Fixation index is an estimate of the proportion of the variation at a locus attributable to divergence among populations.

Linkage disequilibrium. A state that exists in a population when alleles at different loci are not distributed independently in the population's gamete pool, sometimes because the loci are physically linked.

Gene flow. The introduction of genes to a population, through migration and mating from another population of the same species, thereby altering the allele frequencies of the population.

Genetic drift. The change in allele frequencies in a population through time due to random sampling at each generation. The effect of genetic drift increases with smaller population size and shorter number of generations.

Genetic marker. A known DNA sequence that can be identified by a simple assay.

Genotype. The set of alleles for 1 or more loci for a fish.

Hardy-Weinberg expectations (*HWE*). Genotype frequencies expected from a given set of allele frequencies for a locus. Fit to HWE genotypic proportions assumes random mating, no mutation (the alleles remain unchanged), no migration or emigration (no exchange of alleles between populations), infinitely large population size, and no selective pressure for or against the alleles.

Heterozygosity. The proportion of individuals in a population that have 2 different allele forms (are heterozygous) at a particular marker. Average heterozygosity can be used as measure of variability in a sample.

Locus (plural, loci). A fixed position or region on a chromosome.

Linked markers. Genetic markers showing linkage disequilibrium, or physical linkage on a chromosome.

Microsatellite. A locus made up of short repeated sequences of DNA. The number of repeats determines the allele size.

Mixed stock analysis (*MSA*). A method using allele frequencies from baseline populations and genotypes from mixture samples to estimate stock compositions of mixtures.

Population. A locally interbreeding group of spawning individuals that do not interbreed with individuals in other spawning aggregations, and that may be uniquely adapted to a particular spawning habitat. This produces isolation among populations and may lead to the appearance of unique attributes (Ricker 1958) that result in different productivity rates

(Pearcy 1992; NRC 1996). This population definition is analogous to 'spawning aggregations' described by Baker et al. (1996) and 'demes' described by the NRC (1996).

Reporting group. A group of populations in a genetic baseline to which portions of a mixture are allocated during mixed stock analysis.

Single nucleotide polymorphism (*SNP*). DNA nucleotide variation (A, T, C, or G) at a single nucleotide site. SNPs can differ among individuals or within an individual between homologous nucleotide sites on paired chromosomes.

Stock. A locally interbreeding group of salmon (population) that is distinguished by a distinct combination of genetic, phenotypic, life history, and habitat characteristics, or an aggregation of 2 or more interbreeding groups (populations) that occur within the same geographic area and are managed as a unit (from 5 AAC 39.222(f)).

METHODS

TISSUE SAMPLING

Baseline

Tissue samples suitable for genetic analyses (genetic samples) were collected and preserved in 95% ethanol (axillary process or fin; Tables 1 and 2). Tissues were either placed into individual vials or collectively into 125–500 ml containers, with 1 or more containers for each collection site for each year. Collection information including location name, latitude, longitude, and collection year were recorded for each sample.

Most baseline genetic samples were collected from spawning aggregates of coho salmon by ADF&G and USFWS personnel using fish wheels, weirs, gillnets, beach seines, or hook-and-line gear. A few baseline genetic samples were collected opportunistically by other agencies and organizations, including LGL Alaska Research Associates Inc., Cook Inlet Aquaculture Association, Redoubt Mountain Lodge, and the National Park Service. Target sample size for each baseline aggregate was 95 individuals across all years to achieve acceptable precision to estimate allele frequency (Waples 1990; Kalinowski 2004).

The baseline was augmented with genetic samples from fish captured in fish wheels, radiotagged, and successfully tracked to specific spawning sites (Merizon et al. 2010; Yanusz et al. 2011; Cleary et al. 2013, 2016a-b). Fish were tagged with F1840B coded tags that had a pulse rate of 45–47 pulses per minute in the frequency range of 150.000–152.999 megahertz, and model R4500CD radio tag receivers were used to detect the radio tags (Advanced Telemetry Systems, Isanti, Minnesota). Genetic samples were taken at the time the fish were tagged. Sites targeted for this augmentation included the Deshka and Tokositna rivers, where access to ground sampling is limited. Because final locations of radiotagged fish are recorded as the location of the aircraft during aerial surveys, quality control measures were taken to ensure that these fish were likely in the targeted drainage. Fish had to have at least one aerial location taken with a radio tag signal strength above 90 on the receiver at least 3 miles from the mouth of the river or a signal strength above 100 at least 2 miles from the mouth of the river. Additionally, fish had to show steady upstream progression towards their final location from the fish wheel where they were tagged. Flights to assess upstream progression were conducted 4 to 6 times per year, generally on a biweekly basis. Radiotagged fish meeting these quality control measures were considered baseline samples.

Known-origin Mixture

Genetic samples were collected from adult coho salmon captured within the Deshka River during their homeward migration by an ADF&G Division of Sport Fish weir project (Hayes 2013). These samples were initially collected for use in the baseline analysis; however, early analyses indicated that this collection did not meet Hardy-Weinberg expectations and, therefore, could not be considered a single baseline collection (data not shown). However, these samples were adequate to serve as a baseline test mixture.

LABORATORY ANALYSIS

Assaying Genotypes

Genomic DNA was extracted from tissue samples using the DNeasy 96 Blood and Tissue Kit by QIAGEN (Valencia, CA). Samples were genotyped for 96 SNP markers developed by various laboratories in the Pacific Northwest using 4 genotyping platforms (Table 3). The following methods were used for acquiring the SNP genotypes over the 3 years of this project.

Life Technologies OpenArray Technology was used in the initial study on 64 of the markers. A 2.5 uL sample of unnormalized DNA was loaded into an OpenArray 384-well Sample Plate. After drying the plates overnight, a reaction mix containing 2.5 uL Type I molecular grade water and 2.5 uL of 2X TaqMan OpenArray Genotyping Master Mix was added to each of the wells. The OpenArray 384-well Sample Plate was sealed and briefly centrifuged. Samples were transferred to the OpenArray Plates with the QuantStudio OpenArray AccuFill System leaving one cell without any template for a no-template control. Real-time polymerase chain reaction (PCR) data was collected using the QuantStudio 12K Flex Instrument following the standard Life Technologies protocol. Data scoring was performed using TaqMan Genotyper Software (Life Technologies).

Fluidigm SNP Genotyping Technology was employed for the rest of the study and included use of 96.96 and 192.24 Dynamic Array Integrated Fluidic Circuits (IFCs). The components were pressurized using the IFC Controllers HX and RX (Fluidigm) and reactions were conducted in 7.2 nL or 9 nL volume chambers, respectively. The first set of 96.96 IFC runs contained a mixture of 20X GT Sample Loading Reagent (Fluidigm), 2X TaqMan Universal Buffer (Applied Biosystems), 5X AmpliTaq Gold DNA Polymerase (Applied Biosystems), Custom TaqMan SNP Genotyping Assay (Applied Biosystems), 2X Assay Loading Reagent (Fluidigm), 50X ROX Reference Dye (Invitrogen), and 60–400 ng/µl DNA. One cell was not loaded with any DNA to serve as the no-template control. Thermal cycling was performed with an Eppendorf IFC Thermal Cycler as follows: 70°C for 30 min for "Hot-Mix" step, initial denaturation of 10 min at 96°C followed by 40 cycles of 96° for 15 s and 60° for 1 min. The updated methods for the 96.96 IFCs were run on the rest of the samples and consisted of a mixture of 20X Fast GT Sample Loading Reagent (Fluidigm), 2X TaqMan GTXpress Master Mix (Applied Biosystems), Custom TaqMan SNP Genotyping Assay (Applied Biosystems), 2X Assay Loading Reagent (Fluidigm), 50X ROX Reference Dye (Invitrogen), and 60–400 ng/µl DNA. Thermal cycling was performed on a Fluidigm FC1 Cycler using a Fast-PCR protocol as follows: an initial "Hot-Start" denaturation of 95ºC for 2 min followed by 40 cycles of denaturation at 95ºC for 2 s and annealing at 60ºC for 20 s, with a final "Cool-Down" at 25ºC for 10 s. The 96.96 IFC required a "Thermal-Mix" step of 70ºC for 30 min and 25ºC for 10 min prior to PCR. All IFCs were read

on a Biomark or EP1 System (Fluidigm) after amplification and scored using Fluidigm SNP Genotyping Analysis software.

Assays that failed to amplify with either the Fluidigm or OpenArray methods were reanalyzed on 1 of 2 platforms, either the Applied Biosystems Prism 7900HT Sequence Detection System or Life Technologies QuantStudio 12K Flex Real-Time PCR System. The samples that were reanalyzed on the Applied Biosystems Prism 7900HT Sequence Detection System were genotyped in 384-well reaction plates in a 5 µL volume consisting of 6–40 ng/µl of DNA, 2X TaqMan Universal PCR Master Mix (Applied Biosystems), and Custom TaqMan SNP Genotyping Assay (Applied Biosystems). One cell was not loaded with any DNA to serve as the no-template control. Thermal cycling was performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92°C for 1 s and annealing/extension temperature for 1 min. The plates were scanned on the Applied Biosystems Prism 7900HT Sequence Detection System after amplification and scored using Applied Biosystems Sequence Detection Software (SDS) version 2.2.

After August 2014, samples were reanalyzed on the QuantStudio 12K Flex Real-Time PCR System (Life Technologies). Each reaction was performed in 384-well plates in a 5 µL volume consisting of 6–40 ng/µl of DNA, 2X TaqMan GTXpress Master Mix (Applied Biosystems), and Custom TaqMan SNP Genotyping Assay (Applied Biosystems). One cell was not loaded with any DNA to serve as the no-template control. Thermal cycling was performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) as follows: an initial "Hot-Start" denaturation of 95°C for 10 min followed by 40 cycles of denaturation at 92°C for 1 s and annealing at 60°C for 1 min, with a final "Cool-Down" hold at 10°C. The plates were scanned on the system after amplification and scored using the Life Technologies QuantStudio 12K Flex Software.

Regardless of method, a genotype for a given locus and DNA sample was considered a failure if the sample appeared as an outlier to the heterozygous or homozygous clusters. Failures could be due to low quantity or low quality DNA or to sample contamination. Genotypes produced on all platforms were imported and archived in the Gene Conservation Laboratory (GCL) Oracle database, LOKI.

Laboratory Failure Rates and Quality Control

Quality control (QC) analyses were conducted to identify laboratory errors and to measure the background discrepancy rate of the genotyping process. These analyses were performed as a separate genotyping event from the original genotyping, with staff duties altered to reduce the likelihood of repeated human errors. The QC protocol consisted of re-extracting 8% of project fish and genotyping them for the same SNPs assayed in the original project. Laboratory errors found during the QC process were corrected, and genotypes were corrected in the database. Inconsistencies not attributable to laboratory error were recorded, but original genotype scores were retained in the database. Discrepancy rates were calculated as the number of conflicting genotypes divided by the total number of genotypes compared. These rates describe the difference between original project data and QC data for all SNPs, and are capable of identifying extraction, assay plate, and genotyping errors. The overall failure rate was calculated by dividing the number of failed single-locus genotypes by the number of assayed single-locus genotypes. Assuming that the discrepancies among analyses were due equally to errors during original

genotyping and during QC genotyping and that these analyses are unbiased, the error rate in the original genotyping was estimated as half the overall rate of discrepancies. This QC method is the best representation of the error rate of the GCL's current genotype production.

STATISTICAL ANALYSIS

Data Retrieval and Quality Control

We retrieved genotypes from LOKI and imported them into *R*[1] with the *RJDBC* package (Urbanek 2014).[2] All subsequent analyses were performed in *R*, unless otherwise noted.

Prior to statistical analysis, we performed 3 analyses to confirm the quality of the data. First, we identified SNP markers that had only 1 allele in all baseline individuals, or that had an alternate allele occurring in fewer than 1% of all genotypes in the baseline for the given marker. We considered these markers invariant and excluded them from further statistical analyses. Second, we identified individuals that were missing substantial genotypic data because they likely had poor quality DNA. We used the 80% rule (missing data at 20% or more of loci; Dann et al. 2009) to identify individuals missing substantial genotypic data. We removed these individuals from further analyses. The inclusion of individuals with poor quality DNA might introduce genotyping errors into the baseline and reduce the accuracies of MSA.

The final QC analysis identified individuals with duplicate genotypes and removed them from further analyses. Duplicate genotypes can occur as a result of sampling or extracting the same individual twice, and were defined as pairs of individuals sharing the same alleles in 100% of screened loci with genotypic data. The sample with the most missing genotypic data from each duplicate pair was removed from further analyses. If both samples had the same amount of genotypic data, the first sample was removed from further analyses.

Baseline Development

Hardy-Weinberg expectations

For each locus within each collection, we tested for conformance to Hardy-Weinberg expectations (HWE) using the program *Genepop* version 4.1.4 (Rousset 2008). We combined probabilities for each collection across loci and for each locus across collections using Fisher's method (Sokal and Rohlf 1995) and removed collections and loci that violated HWE from subsequent analyses after correcting for multiple tests with Bonferroni's method (Rice 1989; α = 0.05 / # of collections or loci).

Pooling collections into populations

When appropriate, we pooled some collections to obtain better estimates of allele frequencies following a stepwise protocol. First, we pooled collections from the same geographic location, sampled at similar calendar dates but in different years, as suggested by Waples (1990). We then tested for differences in allele frequencies between pairs of collections sampled on similar calendar dates at different locations that might represent the same population. We used Fisher's

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[1] The R project for statistical computing, Vienna, Austria. Available from https://www.R-project.org/.

[2] Urbanek, S. 2014. RJDBC: Provides access to databases through the JDBC interface. R package version 0.2-5. Available from http://CRAN.R-project.org/package=RJDBC.

exact test (Sokal and Rohlf 1995) of allele frequency homogeneity and based our decisions on a summary across loci using Fisher's method. When these tests indicated no difference between collections ($P > 0.01$), we pooled them. After this pooling protocol, any collection with roughly 50 samples or more was retained for subsequent analysis. Though not meeting the sample goal of 95, sample sizes close to 50 are adequate to estimate allele frequencies given the heterozygosities observed at the loci assayed (Table 3; Gregorius 1980) and to use in mixture analysis (Wood et al. 1987; Waples 1990). Finally, we tested populations for conformance to HWE following the same protocol described above to ensure that our pooling was appropriate, and that tests for linkage disequilibrium would not result in falsely positive results due to departure from HWE. Populations that conformed to HWE were used in subsequent analyses.

Removal of loci from the baseline

When testing populations for conformance to HWE we combined probabilities for each locus across populations using Fisher's method (Sokal and Rolf 1995) and examined the frequency of departures from HWE to identify loci that exhibited substantially more departures than others. We removed loci with significant departures from HWE across populations after correcting for multiple tests with Bonferroni's method ($\alpha = 0.05$ / # loci).

Removal of collections from the baseline

We removed some collections from further analysis for other reasons as per other GCL regional baselines. Collections that did not pool with other collections from the same location were removed because they either lacked reliable collection data to discern their exact sample date and location or they were juvenile collections and we had adult collections with sufficient sample size from the same location. Juvenile collections were selected for removal instead of adult collections because they more likely contain a high proportion of related individuals with similar genotypes and therefore do not have representative baseline allele frequencies for the population.

Linkage disequilibrium

We tested for linkage disequilibrium between each pair of nuclear markers in each population to ensure that subsequent analyses would be based on independent markers. We used the program *Genepop* version 4.1.4 (Rousset 2008) with 100 batches of 5,000 iterations for these tests and summarized the frequency of significant linkage disequilibrium between pairs of SNPs ($P < 0.05$). We considered pairs to be linked if they exhibited linkage in more than half of all populations. When SNP pairs were found to be linked, we either removed 1 locus of the linked pair or combined the genotypes of the pair into a composite, haploid marker for further analyses if the pattern of linkage provided information useful for MSA. We followed the methods in Barclay and Habicht (2012) for assessing whether the single or the haploid marker was more informative for MSA.

Analysis of Genetic Structure

Temporal variation

We examined the temporal variation of allele frequencies with a hierarchical, 3-level analysis of variance (ANOVA). We treated the temporal samples as subpopulations based on the method described in Weir (1996). This method allowed the quantification of the sources of total allelic variation and permitted the calculation of the among-years component of variance and the assessment of its magnitude relative to the among-population component of variance. This

analysis was conducted using the software package *GDA*[3] (Lewis and Zaykin 2001). For this test, only temporal collections with greater than 50 samples were used to maximize power and retain relatively balanced sample sizes (Ryman et al. 2006).

Visualization of genetic distances

To visualize genetic distances among populations, pairwise F_{ST} (Weir and Cockerham 1984) estimates were calculated from the final set of independent markers with the package *hierfstat*.[4] Using the pairwise F_{ST} estimates, 1,000 bootstrapped neighbor-joining (NJ) trees were constructed by resampling loci with replacement to assess the stability of tree nodes. We plotted the consensus tree with the *APE* package (Paradis et al. 2004). These trees provided insight into the variability of the genetic structure of these collections, and assisted in the selection of reporting groups used in baseline evaluation tests for MSA.

Baseline Evaluation for Mixed Stock Analysis

We used the results from the NJ consensus tree and the geographic distribution of populations to delineate reporting groups that might perform adequately for MSA within Cook Inlet. We assessed the accuracy and precision for MSA of these reporting groups using 100% proof tests and a test mixture of known-origin fish. Methods for these tests followed those used by Habicht et al. (2012). Populations from outside of Cook Inlet were not included in the baseline evaluation tests for MSA.

Proof tests

For 100% proof tests, mixtures were created by randomly sampling 200 fish from the baseline for a single reporting group and then rebuilding the baseline without the sampled fish (for *Yentna*, only 100 fish were sampled for mixtures to allow adequate baseline sample size of N>200 [Templin et al. 2011]). These tests provide a measure of the potential accuracy and precision possible for designated reporting groups, as well as a means to understand the direction of bias when estimating stock proportions.

The stock composition of the proof test mixtures was estimated using the software package BAYES (Pella and Masuda 2001). BAYES employs a Bayesian algorithm to estimate the most probable contribution of the baseline populations to explain the combination of genotypes in the mixture sample. We followed the same BAYES protocol as reported in Barclay and Habicht (2015). We ran 1 Markov Chain Monte Carlo chain with 40,000 iterations and discarded the first 20,000 iterations for each proof test to form the posterior distribution. The prior parameters for each reporting group were defined to be equal (i.e., a *flat* prior). Within each reporting 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 proof test mixture were calculated by taking the mean and 5% and 95% quantiles of the posterior distribution from the single chain output.

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[3] Genetic data analysis: computer program for the analysis of allelic data. Version 1.0. http://lewis.eeb.uconn.edu/lewishome/software.html (Accessed March 10, 2009; site currently discontinued).

[4] A package for the statistical software R. HIERFSTAT: the latest version is available at http://www.unil.ch/popgen/softwares/hierfstat.htm

Proof tests were repeated 10 times for each reporting group using a different mixture and baseline each time. These tests provided an indication of the power of the baseline for MSA, assuming that all populations were represented in the baseline.

When the mean correct allocation for repeated tests for a given reporting group fell below 90% in 3 or more repeats, we considered this reporting group a failure and not appropriate for MSA.

When a reporting group was considered a failure in the proof tests, we identified the reporting group that it most commonly misallocated to in these tests. We created a new reporting group by combining the failed reporting group with the reporting group it most commonly misallocated to. We then performed a 100% proof test to determine if this new reporting group was appropriate for MSA.

Known-origin mixture

Using a set of individuals sampled from the Deshka River weir in 2013 (Hayes 2013), we estimated the stock composition of this mixture using the same reporting groups used in the proof tests (see the Methods section *Tissue Sampling*, *Test Mixture*; Figure 1; Table 2). This mixture allowed for an additional test of the *Susitna* reporting group for MSA while not drawing samples from the baseline to construct a mixture, albeit for likely only a subset of *Susitna* reporting group populations.

RESULTS

TISSUE SAMPLING

Baseline

A total of 13,366 genetic samples were collected from spawning populations of coho salmon throughout Cook Inlet and the eastern Kenai Peninsula (Table 1; Figure 1). These samples were collected at 115 locations throughout Cook Inlet drainages and 3 locations on the eastern Kenai Peninsula. Target sample sizes of 95 fish were met at 61 locations.

A total of 1,406 genetic samples were collected from radiotagged coho salmon at the Sunshine fish wheels in 2008 and the Flathorn fish wheels from 2009 to 2012 (Table 2). A total of 170 radiotagged fish were chosen as potential baseline samples for the Deshka and Tokositna rivers. Of those fish, 121 met the quality control criteria (Table 1). Hereafter, when referring to baseline collections, the 4 years of fish wheel samples for Deshka River and the 5 years of fish wheel samples for Tokositna River are considered separate baseline collections.

Known-origin Mixture

A total of 95 genetic samples were collected from adult coho salmon at the Deshka River weir in 2013 (Hayes 2013; Figure 1; Table 2).

LABORATORY ANALYSIS

Assaying Genotypes

A total of 8,941 fish collected over spawning areas, fish wheels, and weirs were selected for analysis and assayed for 96 SNP markers (Tables 1 and 2). Baseline samples not included in the analysis were from locations with a total sample size fewer than 47 individuals, or locations where a subset of fish were chosen for analysis.

Laboratory Failure Rates and Quality Control

For all samples selected for analysis, the overall failure rate for genotypes at the 96 SNP markers was 1.88%. A subset of 113 baseline collections (80% of selected baseline collections) and the Deshka weir collection were included in the QC analysis, the overall discrepancy rate was 0.37%; therefore the overall estimated error rate was 0.19%.

STATISTICAL ANALYSIS

Data Retrieval and Quality Control

For all baseline collections, no SNPs had only 1 allele among all individuals, and 10 SNPs had alternate alleles that occurred in fewer than 1% of genotypes (Table 3). These 10 markers were considered invariant and removed; the remaining 86 SNPs were kept for subsequent analyses. Using the 80% rule for sufficiently complete genotypes, 154 individuals were removed from the baseline collections and 2 individuals were removed from the Deshka River weir collection. Based on the criterion for detecting duplicate individuals, 23 individuals were removed from baseline collections as duplicate individuals; no duplicate individuals were detected in the Deshka weir collection.

Baseline Development

Hardy-Weinberg expectations within collections

Over the remaining 86 SNPs and 142 collections, 97 of 12,212 tests deviated significantly from HWE ($P < 0.01$) without adjusting for multiple tests. These were spread over 54 SNPs, and no SNPs were out of HWE in more than 5 of the collections. No collections departed HWE at more than 5 SNPs. After adjusting for multiple tests, all collections conformed to HWE.

Pooling collections into populations and HWE within populations

A total of 87 populations (84 Cook Inlet and 3 eastern Kenai Peninsula) were identified after dropping collections with insufficient samples and pooling collections (pooled collections and collections taken at different sites are referred to as *populations*; Table 1). Collections pooled from different sampling locations included pairs of collections from the Chuitna and Tokositna rivers. Over all variant SNPs and populations, 93 of 7,482 tests did not conform to HWE ($P < 0.01$) without adjusting for multiple tests. These were spread over 52 SNPs, and no SNPs were out of HWE in more than 6 of the 87 populations. No population was out of HWE at more than 5 of 86 SNPs. After adjusting for multiple tests, all populations conformed to HWE.

Removal of loci from the baseline

After combining the HWE *p*-values across populations and adjusting for multiple tests, all 86 SNPs conformed to HWE; therefore, no additional loci were removed from the baseline.

Removal of collections from the baseline

A total of 4 collections were dropped from further analysis because they could not be pooled with other collections from the same location.

Linkage disequilibrium

In the tests for linkage disequilibrium, no SNP pairs showed significant linkage ($P < 0.05$) in greater than 50% of populations. Therefore, no SNPs were considered linked and no further linkage disequilibrium analyses were performed.

Analysis of Genetic Structure

Temporal variation

A total of 10 populations had temporal samples collected from 50 or more fish, and were included in the analysis of temporal variation of allele frequencies. Within populations, 7 pairs of collections were 2–4 years apart, and 3 were 15–18 years apart (Table 1). The 3-level ANOVA indicated that the ratio of variation among temporal collections to the variation among populations was 12.5%.

Visualization of genetic distances

Overall F_{ST} was 0.06 (Table 3), and pairwise F_{ST} varied from 0.00 to 0.17 (Appendix A). The NJ tree shows that populations generally cluster by drainage and coastal proximity (Figure 2). Within drainages, the most genetically divergent populations were generally those furthest upstream. The least genetically divergent populations were concentrated in the most northwestern portion of Cook Inlet. These included those from the Chuitna River and northwest, including other coastal populations and populations from the Susitna and Yentna river drainages.

Eight reporting groups (italics) were identified to test for MSA performance (Table 1; Figure 1):

1) *Southwest CI* (West side populations south of Little Jack Creek)
2) *Northwest CI* (West side populations from Little Jack Creek north to the Susitna River and Alexander Creek)
3) *Susitna* (Susitna River mainstem populations)
4) *Yentna* (Yentna River populations)
5) *Knik* (Knik Arm populations and Campbell Creek)
6) *Turnagain/Northeast CI* (Turnagain Arm and northeast Cook Inlet populations)
7) *Kenai/Kasilof* (Kenai and Kasilof river populations)
8) *Southeast CI* (Kenai Peninsula populations south of the Kasilof River)

Populations from outside of Cook Inlet were incorporated into a 9th group:

9) Outside CI (Eastern Kenai Peninsula populations)

The outside of Cook Inlet populations were not intended for inclusion in the baseline evaluation tests; therefore, the Outside CI group is not italicized.

Populations from the *Kenai/Kasilof* reporting group and *Southwest CI*, *Southeast CI*, and Outside CI groups formed the 2 most distinct clusters on the tree (Figure 2). In general, populations in the *Kenai/Kasilof* cluster were more genetically distinct with increasing river distance from Cook Inlet. Within this group, all Kasilof River populations formed a single cluster. Among populations from the *Southwest CI*, *Southeast CI*, and Outside CI cluster, genetic distinction generally increased from northern to southern populations. On an inletwide scale, there appears to be affinity among northern populations and among southern populations (i.e. *Susitna*, *Northwest CI*, and *Yentna* are more basal while *Southeast CI* and *Southwest CI* share a cluster). Populations from the outside Cook Inlet group cluster with *Southeast CI* and *Southwest CI*

populations and are most genetically similar to each other. On the NJ tree, the 2 Resurrection Bay populations, Resurrection River and Bear Creek, cluster beyond a significant node, indicating that they are most genetically similar to each other. Delight Creek is the next most similar population genetically and it is the closest population to Resurrection River by water distance.

Several populations appeared to be more genetically distinct (on longer genetic branches): Russian River, East Fork Moose River, Chester Creek, and Fox River. Of the 23 well supported nodes (50% of bootstrap trees), none occurred in the *Northwest CI*, *Yentna*, or *Susitna* reporting groups, 11 occurred within the *Kenai/Kasilof* reporting group, 4 occurred within the *Knik* reporting group, 3 occurred in the *Turnagain/Northeast CI* group, 3 occurred in the *Southwest CI* reporting group, and the *Southeast CI* reporting group and Outside Cook Inlet group had 1 each.

Baseline Evaluation for Mixed Stock Analysis

Proof tests

Correct allocation means for all 80 repeated proof tests ranged from 75.9% to 99.3% (Figure 3). The *Southeast CI*, *Southwest CI*, and *Kenai/Kasilof* reporting groups had the highest correct allocation means (>96%) and the least variation among repeats. *Turnagain/Northeast CI* had the next highest correct allocation means with repeats ranging from 93.1% to 98.3%. The *Susitna* and *Knik* reporting groups each had little variation in their correct allocation means in 9 of 10 repeats, with relatively high correct allocation means (ranging from 93.5% to 97.6% for the *Susitna* group and from 94.3% to 97.8% for the *Knik* group); however, the correct allocation mean for 1 repeat dropped considerably for *Knik* (83.5%). *Northwest CI* and *Yentna* reporting groups had the most variable results, with correct allocation means ranging from 77.2% to 96.9% for the *Northwest CI* group and from 75.9% to 95.5% for the *Yentna* group.

The *Northwest CI* and *Yentna* reporting groups had correct allocation means below 90% in more than half of the repeated tests, with misallocation occurring largely between these 2 reporting groups (data not shown). Therefore, *Northwest CI* and *Yentna* populations were combined to form a new reporting group, *Northwest CI/Yentna*, and repeated proof tests were conducted to evaluate this reporting group for MSA (Figure 4). In these tests, correct allocation means ranged from 80.6% to 98.3%, where only 1 test fell below 90% correct allocation. The 9 tests above 90% correct allocation ranged from 90.6% to 98.3%.

Known-origin mixture

In the analysis of the mixture from the Deshka River weir, the correct allocation to the *Susitna* reporting group was 96.1%, with 1.6% misallocation to the *Northwest* reporting group. Each of the remaining 6 reporting groups had allocations fewer than 1%.

DISCUSSION

COMPARISONS TO PREVIOUS STUDIES

Among Population Variation

This study builds upon an earlier, statewide survey of genetic variation of coho salmon in Alaska based on microsatellite loci (Olsen et al. 2003) that included 6 populations from the Kenai River and 2 populations from the west side of Cook Inlet. Although the Olsen et al. (2003) baseline used a different marker type than we use here, we found concordant patterns of genetic variation

among the Cook Inlet populations included in both data sets. Olsen et al. (2003) reported an overall F_{ST} of 0.05 among Cook Inlet populations; our estimate of F_{ST} = 0.06 (Table 3) was similar. Also similar was the pattern of weak regional substructuring. Though populations from the same drainage tended to cluster together in the NJ consensus tree, bootstrap support for nodes grouping populations by drainage were often fewer than 50%. Two exceptions were Kasilof River and Kenai River populations, especially those upstream of the outlet of Skilak Lake. One factor that may contribute to the strong regional structuring of these populations is their arrangement within large river systems. Olsen et al. (2003) speculated that after the deglaciation of the Kenai River drainage, coho salmon populations were founded from a single colonization event, leading to the grouping of these populations in a NJ tree. Lack of clustering of populations within the Susitna River may be in part due to the greater complexity of this watershed, multiple colonization events within the watershed, or more recent colonization of the Susitna River valley.

Within Population Variation

In the analysis of temporal variation of allele frequencies, the ratio of the variation between subpopulations to variation among populations was relatively high (12.5%) compared to other species of salmon in Cook Inlet (sockeye salmon *O. nerka*, 1.6%, Barclay and Habicht 2012; Chinook salmon *O. tshawytscha*, 5.3%; Barclay and Habicht 2015). However, our observed value was similar to a value reported for coho salmon in the Kuskokwim River (14%, Crane et al. 2007), and is much lower than values reported for coho salmon in the southern portion of its range. For example, in Oregon, the temporal component (among years) has accounted for 21% to over 40% of the overall variation among collections (Van Doornik et al. 2002; Ford et al. 2004).

Highly unimodal age-at-maturity distributions for coho salmon, especially in the southern portion of its range, may explain why temporal (interannual) variation accounts for so much of the among-collection variation compared to other Pacific salmon species (Waples 1990). The majority of coho salmon spawning in British Columbia and further south return to spawn at age 1.1 (95–98% of females, though some males spawn at age 2; Sandercock 1991). Therefore, coho salmon are intermediate to pink salmon *O. gorbuscha*, with virtually no overlap among year classes and other Pacific salmon species with greater overlap in year classes (Waples 1990). Further, regional variation in age-at-maturity distributions may partially explain why temporal variation is not as pronounced in Cook Inlet (and Kuskokwim River) populations. In Alaska, coho salmon primarily spawn at age 2.1, but at least in Cook Inlet, the age-at-maturity is less unimodally distributed. From 2009 to 2011, age-2.1 fish made up only 77–85% of the Cook Inlet commercial harvest (Tobias and Willette 2012a, 2012b; Tobias et al. 2013). This lower level of unimodality allows for more gene flow among year classes which might explain the lower proportion of variation by year in Cook Inlet than in Oregon.

EVALUATION OF GENETIC LEGACY OF STOCKING PROGRAMS

One of the goals of fisheries supplementation is to increase the number of fish available for harvest through hatchery releases, while maintaining the genetic profile of local wild stocks (e.g., Naish et al. 2007). The development of the coho salmon baseline for Cook Inlet has provided an ability to assess the influence of stocking programs in the Resurrection Bay area and northern Cook Inlet streams on contemporary populations.

Resurrection Bay

Coho salmon spawn in many tributaries at the head of Resurrection Bay (http://extra.sf.adfg.state.ak.us/FishResourceMonitor/?mode=awc). High year-to-year variation in escapement has been observed in drainages used as index streams for escapement (Airport, Box Canyon, Clear, Dairy, Grouse, Japanese, Mayor, and Salmon creeks; Vincent-Lang 1987). This high variation has been attributed to changes in productivity and to events affecting counting, including flooding events. Straying may also account for some of this variation but limited data have been collected to examine straying among tributaries. For example, 27 strays from the Bear Lake stocking were found in "local tributaries" in 1980 (McHenry 1981).

The genetic profile of local wild stocks of coho salmon in Resurrection Bay may have been disrupted by multiple fish eradication efforts, seeding with nonlocal coho salmon broodstock, and many years of enhancement in Bear Lake, Bear Creek, Grouse Lake (all of which drain into Resurrection River), and Seward Lagoon, which drains directly into Resurrection Bay. Bear Lake was treated with rotenone (a chemical used to kill fish) in 1963, and subsequently stocked with coho salmon originating from Swanson River (Cook Inlet, 1963–1966), Kodiak (unknown location, 1966), and Oregon (unknown location, 1967; Vincent-Lang 1987). Permeability of the weir and the 1964 earthquake resulted in recolonization of the lake by three-spine stickleback and Dolly Varden *Salvelinus malma*. A second rotenone treatment in 1971 was accompanied by a fish-tight weir. Subsequent coho salmon broodstock for Bear Lake came from Lake Rose Tead (Kodiak, 1972) and Upper Station (Kodiak, 1973) and fish returning to Bear Lake (1971–1972 and 1974–present; Vincent-Lang 1987; Cherry 2014). Nonlocal stocks were also stocked into Bear Creek from Oregon (unknown location, 1967), Kodiak (unknown location, 1970), and into Seward Lagoon from Oregon (unknown location, 1966–1967) and Kodiak (unknown location, 1970 and 1972). These locations, along with Grouse Lake, were subsequently stocked with returns to Bear Lake and/or Seward Lagoon. From 1986 to 2013, an average of 371,000 hatchery fry were released into Bear Lake, and from 1980 to 2013, an average of 87,000 smolt emigrated from Bear Lake (Cherry 2014). There is the potential that hatchery-origin fish released in Seward Lagoon, Bear Lake, Bear Creek, or Grouse Lake strayed into other Resurrection River tributaries and disrupted the native genetic profile.

An ideal evaluation of the genetic effects of these management actions would incorporate wild stock samples collected prior to supplementation and samples from all hatchery broodstocks. Unfortunately, no preperturbation samples exist for wild stocks, and documentation for the specific Oregon and Kodiak progenitor stocks for hatcheries is lacking (McHenry 1981; Vincent-Lang 1987). However, the baseline contains populations geographically close to the first nonlocal hatchery broodstock used (Swanson River, Sucker and Gruska creeks; Table 1) and provides an opportunity to evaluate the effects of these actions on coho salmon stocks within Resurrection Bay.

Three divergent hypotheses could be postulated regarding the effects of previous eradication and stocking programs:

1) Nonlocal stocks replaced native populations through stocking programs within Bear Creek, but did not replace native populations in the mainstem of the Resurrection River.
2) Nonlocal stocks replaced native populations throughout the Resurrection drainage.
3) Nonlocal stocks did not replace native populations in Resurrection Bay.

Each of these hypotheses predicts different relationships among and between Resurrection and outside-Resurrection populations:

1) Large genetic differences between Bear Creek and Resurrection River populations and no evidence of common ancestry (populations would not share a common node).
2) Small genetic differences between Bear Creek and Resurrection River populations and this pair would not share a common node with most geographically proximate populations (i.e., Delight Creek).
3) Small or large genetic differences between Bear Creek and Resurrection River populations with evidence of common ancestry between these 2 populations and between these two populations and geographically proximate populations.

Data from this analysis best supports Hypothesis number 3 (Figure 2). Bear Creek and Resurrection River are genetically divergent (F_{ST} = 0.03; Appendix A), but they share a common node (Figure 2), indicating that they have a common ancestry. The large F_{ST} supports a more distant common ancestry and little genetic flow between these populations. The nesting of these populations within geographically proximate populations (i.e., Delight Creek, *Southeast CI*, *Southwest CI*) and the lack of similarity to the populations near Swanson River (populations 58–60; Figures 1 and 2) support the conclusion that they have local common ancestry.

Cook Inlet

Records for stocking of coho salmon in Cook Inlet date back to 1966 and include multiple incubation facilities, stocks ancestries, and release locations (http://mtalab.adfg.alaska.gov/CWT/reports/hatcheryrelease.aspx). A full examination of how these stocking activities may have affected contemporary population structure of coho salmon in Cook Inlet was not feasible in this report given time constraints. However, this examination would be useful to gain insights into the effects of stocking programs on wild stocks.

EVALUATION FOR MSA

Delineation and Performance of Reporting Groups

Delineating reporting groups for MSA is dictated by the fishery management question at hand, the expected composition of the mixture, and the genetic structure of the underlying populations (Pella and Milner 1987; Koljonen et al. 2005; Habicht et al. 2012). In this report, we only incorporated the population structure and geographic distribution in delineating reporting groups that might perform well in MSA applications within marine waters of Cook Inlet. These results can be used to address management concerns with genetic analyses. These proof tests and the underlying population structure identified in this report can be used to provide insights into alternative reporting groups that might perform well, and to answer stakeholder questions. Alternate reporting groups will need to be tested on a case-by-case basis, depending on study objectives and the potential composition of the mixed stock sample being analyzed (e.g., within rivers).

The proof tests using reporting groups delineated using population structure and geographic distribution show promise for using MSA for coho salmon to resolve management questions in Cook Inlet fisheries (Figure 3). The consistency in performance of the proof tests across replicates was likely due to genetic similarities among populations within the reporting group, as each replicate consists of a random set of individuals within the reporting group. For example, *Kenai/Kasilof, Southeast CI,* and *Southwest CI* all allocated above 96% in every proof test

replicate (Figure 3). Many of the populations within these reporting groups clustered together above a significant node in the NJ consensus tree (Figure 2). The *Northwest CI* and *Yentna* reporting groups, on the other hand, showed high variation among replicates (77.2–96.9%, Figure 3). The populations within these clusters had comparatively shallow population structure, with few populations joined by significant nodes in the NJ tree (pairwise $F_{ST} < 0.04$; Appendix A; Figure 2). Sample size may also have been a consideration; *Yentna* was represented by approximately 300 fish from only 3 populations (see below). The proof tests used here may be optimistic because mixture samples constructed for these tests were made up of populations from single reporting groups. Proof tests performed using mixtures composed of a single reporting group often produce much more optimistic results due to the way the Bayesian algorithm is informed by the composition of the mixture. For example, if the majority of fish in a sample come from a single reporting group, the likelihood of BAYES assigning a fish to that reporting group increases. Once stakeholder issues are identified, proof tests can be done with mixture samples composed of samples from multiple reporting groups in proportion to the expected composition of a mixed stock sample from a given fishery and time.

There may be other fine-scale reporting groups that will perform well, especially for questions where the baseline can be restricted and/or the composition is not expected to be complex. For example, a reporting group consisting of a single or combination of populations from the Chulitna River might perform well on a mixed stock sample of migrating fish collected in the lower Susitna River. Alternatively, the Deshka River might perform well as a reporting group for a similar mixture. Within the Kenai River, reporting groups consisting of single or combinations of populations from tributary versus mainstem spawners might perform well on a mixed stock sample of migrating fish collected in the lower Kenai River or in fisheries in salt water near the mouth of the Kenai River.

At the other extreme, this baseline may not be appropriate for fishery mixtures captured in Lower Cook Inlet. Lower Cook Inlet fishery mixtures may include fish from outside of Cook Inlet populations (for example, see Barclay et al. 2016a for Chinook salmon). Therefore, fisheries outside of Cook Inlet should include coho salmon stocks from outside of Cook Inlet.

NEED FOR FURTHER BASELINE

Adequate representation of populations is a prerequisite for applying genetic data to MSA applications (Utter and Ryman 1993). Adequate representation depends on the population structure, with lower representation needed when the structure is organized by regions (Wood 1989). In the visualization of population structure (Figure 2), Cook Inlet populations are generally structured by drainage.

The *Yentna* reporting group is currently represented by only 3 populations, which are genetically similar to each other and similar to a *Northwest CI* reporting group population (McArthur River; Figure 2). In the test for MSA (Figure 3), the *Yentna* and *Northwest CI* reporting groups had highly variable estimates and lower correct allocations than other reporting groups, which may be influenced by genetic similarities. All 3 of the Yentna River populations are from the same tributary, and additional populations within the Yentna River have either not been sampled or have insufficient samples to include in the baseline (Table 1; Figure 2). Additional samples from unsampled and undersampled areas of the Yentna River drainage will likely increase the genetic distinction of the *Yentna* reporting group and reduce its genetic similarity to the *Northwest CI*

reporting group. As funding allows, we recommend increasing sample sizes to 95 per location through the collection of additional samples and analysis of archive samples.

In fall of 2015, the Matanuska-Susitna Borough funded a 2-year baseline sampling study that seeks to increase representation within the Yentna River drainage for coho salmon. Baseline sampling will occur in 2016 and 2017. A final report of the updated baseline, including new tests for MSA, is scheduled for completion in June of 2018.

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REFERENCES CITED

Baker, T. T., A. C. Wertheimer, R. D. Burkett, R. Dunlap, D. M. Eggers, E. I. Fritts, A. J. Gharrett, R. A. Holmes, and R. L. Wilmot. 1996. Status of Pacific salmon and steelhead escapements in southern Alaska. Fisheries 21(10):6–18.

Barclay, A. W., B. J. Failor, and C. Habicht. 2016a. Report to the Alaska Board of Fisheries: Progress report on genetic and coded wire tag mixed stock analysis of Chinook salmon harvested in Cook Inlet marine sport fishery, 2014-2016. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J16-09, Anchorage.

Barclay, A. W., C. Habicht, T. M. Willette, W. Gist, and W. D. Templin. 2016b. Report to the Alaska State Legislature on status of Cook Inlet coho and sockeye salmon genetic projects, 2015. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J16-02, Anchorage.

Barclay, A. W., and C. Habicht. 2015. Genetic baseline for Upper Cook Inlet Chinook salmon: 42 SNPs and 7,917 fish. Alaska Department of Fish and Game, Fishery Manuscript Series No. 15-01, Anchorage.

Barclay, A. W., and C. Habicht. 2012. Genetic baseline for Upper Cook Inlet sockeye salmon: 96 SNPs and 10,000 fish. Alaska Department of Fish and Game, Fishery Manuscript Series No. 12-06, Anchorage.

Barclay, A. W., N. A. DeCovich, C. Habicht, T. M. Willette, W. Gist, E. C. Volk, and W. D. Templin. 2014. Report to the Alaska State Legislature on status of Cook Inlet coho and sockeye salmon genetic projects, 2014. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J14-05, Anchorage.

Beacham, T. D., J. R. Candy, C. Wallace, M. Wetklo, L. Deng, and C. MacConnachie. 2012. Microsatellite mixed-stock identification of coho salmon in British Columbia. Marine and Coastal Fisheries 4(1):85–100.

Beacham, T. D., M. Wetklo, L. Deng, and C. MacConnachie. 2011. Coho salmon population structure in North America determined from microsatellites. Transactions of the American Fisheries Society 140:253–270.

Bosch, Dan. 2011. Coho salmon thermal-marked otolith recovery, Resurrection Bay, Alaska, 2003–2005. Alaska Department of Fish and Game, Fishery Data Series No. 11-06, Anchorage.

Bucklin, K. A., M. A. Banks, and D. Hedgecock. 2007. Assessing genetic diversity of protected coho salmon (Oncorhynchus kisutch) populations in California. Canadian Journal of Fisheries and Aquatic Sciences 64:30–42.

Campbell, N. R., and S. R. Narum. 2011. Development of 54 novel single-nucleotide polymorphism (SNP) assays for sockeye and coho salmon and assessment of available SNPs to differentiate stocks in the Columbia. Molecular Ecology Resources 11(S1):20–30.

Cherry, C. 2014. Bear Lake salmon enhancement progress report 2013. Cook Inlet Aquaculture Asociation, Kenai, Alaska. http://www.ciaanet.org/Projects/BEAR%20LAKE%20RPT%202013.pdf

Cleary, P. M., R. J. Yanusz, J. W. Erickson, D. J. Reed, R. A. Neustel, J. P. Bullock, and N. J. Szarzi. 2016a. Abundance and spawning distribution of Susitna River chum Oncorhynchus keta and coho O. kisutch salmon, 2012. Alaska Department of Fish and Game, Fishery Data Series No. 16-52, Anchorage.

Cleary, P. M., R. J. Yanusz, J. W. Erickson, D. J. Reed, R. A. Neustel, and N. J. Szarzi. 2016b. Abundance and spawning distribution of Susitna River chum Oncorhynchus keta and coho O. kisutch salmon, 2011. Alaska Department of Fish and Game, Fishery Data Series No. 16-35, Anchorage.

Cleary, P. M., R. A. Merizon, R. J. Yanusz, and D. J. Reed. 2013. Abundance and spawning distribution of Susitna River chum Oncorhynchus keta and coho O. kisutch salmon, 2010. Alaska Department of Fish and Game, Fishery Data Series No. 13-05, Anchorage.

Crane, P. A., D. Molyneaux, C. Lewis, and J. Wenberg. 2007. Genetic variation among coho salmon populations from the Kuskokwim Region and application to stock-specific harvest estimation. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report 96, Anchorage.

REFERENCES CITED (Continued)

Dann, T. H., C. Habicht, J. R. Jasper, H. A. Hoyt, A. W. Barclay, W. D. Templin, T. T. Baker, F. W. West, and L. F. Fair. 2009. Genetic stock composition of the commercial harvest of sockeye salmon in Bristol Bay, Alaska, 2006-2008. Alaska Department of Fish and Game, Fishery Manuscript Series No. 09-06, Anchorage.

DeCovich, N., A. Barclay, C. Habicht, M. Willette, L. Fair. E. Volk and W. Templin. 2013. Report to the Alaska State Legislature on status of Cook Inlet coho and sockeye salmon genetic projects, 2013. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 5J13-12, Anchorage.

Flannery, B., and R. Loges. 2016. Yukon River coho salmon microsatellite baseline. Final Report for Fisheries Resource Monitoring Program Study 14-206, U. S. Fish and Wildlife Service, Conservation Genetics Lab, Anchorage. https://www.fws.gov/alaska/fisheries/genetics/pdf/41_RF_2016_FlanneryLoges_OSM%2014-206%20Final%20Report.pdf

Ford, M. J., D. Teel, D. M. Van Doornik, D. Kuligowski, and P. W. Lawson. 2004. Genetic population structure of central Oregon Coast coho salmon (*Oncorhynchus kisutch*). Conservation Genetics, 5(6):797–812.

Gregorius, H. R. 1980. The probability of losing an allele when diploid genotypes are sampled. Biometrics 36:643–652.

Habicht, C., W. D. Templin, N. A. DeCovich, and J. R. Jasper. 2012. Western Alaska Salmon Stock Identification Program Technical Document 15: Chum salmon reporting group evaluations using simulated fishery mixtures. Alaska Department of Fish and Game, Division of Commercial Fisheries Regional Information Report 5J12-22, Anchorage.

Hayes, S. 2013. Deshka River salmon weir. Alaska Department of Fish and Game, Division of Sport Fish, Regional Operational Plan ROP.SF.2A.2013.07, Anchorage.

Johnson, M. A., and M. A. Banks. 2008. Genetic structure, migration, and patterns of allelic richness among coho salmon (Oncorhynchus kisutch) populations of the Oregon coast. Canadian Journal of Fisheries and Aquatic Sciences 65:1274–1285.

Kalinowski, S. T. 2004. Genetic polymorphism and mixed-stiock fisheries analysis. Canadian Journal of Fisheries and Aquatic Sciences 61:1075–1082.

Koljonen, M., J. J. Pella, and M. Masuda. 2005. Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data. Canadian Journal of Fisheries and Aquatic Sciences 62:2143–2158.

Loopstra, D. P., and P. A. Hansen. 2015. Coho and Chinook salmon releases into Cook Inlet, Prince William Sound, and Resurrection Bay, Alaska, 2011. Alaska Department of Fish and Game, Fishery Data Series No. 15-31, Anchorage.

McHenry, E. T. 1981. Coho salmon studies in the Resurrection Bay area. Annual progress report, Alaska Department of Fish and Game. http://www.arlis.org/docs/vol2/hydropower/APA_DOC_no._657.pdf

McHenry, E. T. 1982. Coho salmon studies in the Resurrection Bay area. Alaska Department of Fish and Game. Federal Aid in Fish Restoration, Annual Performance Report, 1981-1982, Project F-9-14, 23 (G-II-A), Juneau.

Merizon, R. A., R. J. Yanusz, D. J. Reed, and T. R. Spencer. 2010. Distribution of spawning Susitna River chum Oncorhynchus keta and coho O. kisutch salmon, 2009. Alaska Department of Fish and Game, Fishery Data Series No. 10-72, Anchorage.

Naish, K. A., J. E. Taylor, P. S. Levin, T. P. Quinn, J. R. Winton, D. Huppert, and R. Hilborn. 2007. An evaluation of the effects of conservation and fishery enhancement hatcheries on wild populations of salmon. Advances in Marine Biology 53:61–194.

NRC (National Research Council). 1996. Upstream: salmon and society in the Pacific Northwest. Committee on the Protection and Management of Pacific Northwest Salmonids. National Academy Press, Washington D. C.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20(2): 289–290.

REFERENCES CITED (Continued)

Olsen, J. B., S. J. Miller, W. J. Spearman, and J. K. Wenburg. 2003. Patterns of intra- and inter-population genetic diversity in Alaskan coho slamon: Implications for conservation. Conservation Genetics 4:557–569.

Pearcy, W. G. 1992. Ocean ecology of north Pacific salmonids. University of Washington Press, Seattle.

Pella, J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99:151–167.

Pella, J. J., and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. Pages 247–276 [*In*] N. Ryman and F. Utter, editors. Population Genetics and Fishery Management, WASHU-B-87-001 C2. http://nsgl.gso.uri.edu/washu/washub87001/washub87001_full.pdf

Rice, W. R. 1989. Analyzing tables of statistical test. Evolution 43:223–225.

Ricker, W. E. 1958. Maximum sustained yields from fluctuating environments and mixed stocks. Journal of the Fisheries Research Board of Canada 15(5):991–1006.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8(1):103–106.

Ryman, N., S. Palm, C. André, G. R. Carvalho, T. G. Dahlgren, P-E. Jorde, L. Laikre, L. C. Larsson, A. Palmé, and D. E. Ruzzante. 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology 15:203–2045.

Sandercock, F. K. 1991. Life history of coho salmon (*Oncorhynchus kisutch*). Pages 395–446 [*In*] C. Groot and L. Margolis, editors. Pacific salmon life histories. University of British Columbia Press, Vancouver, BC.

Shields, P., and A. Dupuis. 2016. Upper Cook Inlet commercial fisheries annual management report, 2015. Alaska Department of Fish and Game, Fishery Management Report No. 16-14, Anchorage.

Small, M. P., R. E. Withler, and T. D. Beacham. 1998. Population structure and stock identification of British Columbia coho salmon, *Oncorhynchus kisutch*, based on microsatellite DNA variation. Fisheries Bulletin 96:843–858.

Smith, C. T., L. Park, D. Vandoornik, L. W. Seeb, and J. E. Seeb. 2006. Characterization of 19 single nucleotide polymorphism markers for coho salmon. Molecular Ecology Notes 6(3):715–720.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd edition. Freeman, San Francisco.

Starks, H. A., A. J. Clemento, and J. C. Garza. 2015. Discovery and characterization of single nucleotide polymorphisms in coho salmon, *Oncorhynchus kisutch.* Molecular Ecology Resources 16(1):277–287. doi: 10.1111/1755-0998.12430

Teel, D. J., D. M. Vandoornik, D. R. Kuligowski, and W. S. Grant. 2003. Genetic analysis of juvenile coho salmon (Oncorhynchus kisutch) off Orgeon and Washington reveals few Columbia River wild fish. Fishery Bulletin 101(3):640–652.

Templin, W. D., J. E. Seeb, J. R. Jasper, A. W. Barclay, and L. W. Seeb. 2011. Genetic differentiation of Alaska Chinook salmon: the missing link for migratory studies. Molecular Ecology Resources 11(S1):226–246.

Tobias, T. M., W. M. Gist, and T. M. Willette. 2013. Abundance, age, sex, and size of Chinook, sockeye, coho, and chum salmon returning to Upper Cook Inlet, Alaska, 2011. Alaska Department of Fish and Game, Fishery Data Series No. 13-49, Anchorage.

Tobias, T. M., and T. M. Willette. 2012a. Abundance, age, sex, and size of Chinook, sockeye, coho, and chum salmon returning to Upper Cook Inlet, Alaska, 2009. Alaska Department of Fish and Game, Fishery Data Series No. 12-11, Anchorage.

Tobias, T. M., and T. M. Willette. 2012b. Abundance, age, sex, and size of Chinook, sockeye, coho, and chum salmon returning to Upper Cook Inlet, Alaska, 2010. Alaska Department of Fish and Game, Fishery Data Series No. 12-14, Anchorage.

REFERENCES CITED (Continued)

Utter, F., and N. Ryman. 1993. Genetic markers and mixed stock fisheries. Fisheries 18(8):11–21.

Van Doornik, D. M., D. J. Teel, D. R. Kuligowski, C. A. Morgan, and E. Casillas. 2007. Genetic analyses provide insight into the early ocean stock distribution and survival of juvenile coho salmon off the coasts of Washinton and Oregon. North American Journal of Fisheries Management 27(1):220–237.

Van Doornik, D. M., M. J. Ford, and D. J. Teel. 2002. Patterns of Temporal Genetic Variation in Coho Salmon: Estimates of the Effective Proportion of 2-Year-Olds in Natural and Hatchery Populations. Transactions of the American Fisheries Society 131:1007–1019.

Vincent-Lang, D. 1987. Biological statistics for coho (*Oncorhynchus kisutch*) and sockeye (*O. nerka*) salmon in Resurrection Bay, Alaska, 1962-86. Fishery Manuscript No. 1, Juneau, AK.

Waples, R. S. 1990. Temporal changes of allele frequency in Pacific salmon: implications of mixed-stock fishery analysis. Canadian Journal of Fisheries and Aquatic Sciences 47(5):968–976.

Weir, B. 1996. Genetic data analysis. 2nd edition. Sinauer Associates, Inc, Sunderland, MA.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wood, C. C. 1989. Utility of similarity dendrograms in stock composition analysis. Canadian Journal of Fisheries and Aquatic Sciences 46:2121–2128.

Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Canadian Journal of Fisheries and Aquatic Sciences 44(4):886–881.

Yanusz, R. J., R. A Merizon, T. M. Willette, D. G. Evans, and T. R. Spencer. 2011. Inriver abundance and distribution of spawning Susitna River sockeye salmon Oncorhynchus nerka, 2008. Alaska Department of Fish and Game, Fishery Data Series No. 11-12, Anchorage.

TABLES AND FIGURES

Table 1.–Tissue collections of coho salmon throughout Cook Inlet, including the years collected, number of samples collected (N), the number of individuals analyzed from each collection included in the baseline (N_a), and source of the collection.

Map numbers correspond to sampling sites on Figure 1; unique population numbers represent all the analyzed collections that contribute to a single population. Proof tests for mixed stock analysis were performed on the 8 groups within Cook Inlet (Group).

Map No.	Pop No.	Group	Location	Collection Year	N	N_a	Source[a]
		Southwest CI					
1	1		Douglas River	2013	106	92	A
1	---			2014[b]	150	---	A
2	2		Douglas Reef River	2013	113	94	A
2	---			2014	128	---	A
3	3		Kamishak River	2013	110	92	A
3	---			2014	106	---	A
4	4		Little Kamishak River	2013	96	90	A
4	---			2014[b]	175	---	A
5	5		McNeil River	2013	41	41	A
5	5			2014[c]	183	12	A
6	---		Sunday Creek	2012[b]	7	---	A
7	---		Brown's Peak Creek	2013	9	---	A
7	---			2014	4	---	A
8	6		Knoll's Head Creek	2014[b]	200	150	A
9	---		Fitz Creek	2013	3	---	A
10	7		Silver Salmon Creek	2013	160	93	B
11	8		Tuxedni River	2012	86	81	C
12	9		Crescent River	1998	99	93	D
12	---			2012	1	---	C
12	9			2013	227	91	E
13	---		Harriet Creek	2012	1	---	C
13	10			2014	63	63	A
		Northwest CI					
14	---		Packers Creek	2013	4	---	A
14	---			2014	37	---	A
15	11		Little Jack Creek	2013	104	95	A
16	12		Montana Bill Creek	2012	101	95	C
17	---		Big River	2009	19	---	C
18	13		Kustatan River	2013	119	95	A
19	14		Farro Creek	2013	17	17	A
19	14			2014	111	78	A
		Northwest CI					
20	15		McArthur River (unnamed stream)	2014	100	95	A
21	---		Straight Creek	2014	15	---	A
22	16		Chuitna River	1992	54	53	D
23	16		Wilson Creek	2010[b]	223	94	C
24	---		Middle Creek	2008[b]	40	---	C
25	---		Lone Creek	2008[b]	70	---	C
26	17		Coal Creek	2013	41	40	A
26	17			2014	46	46	A
27	18		Theodore River	2012	19	17	C
27	18			2013	60	60	F
28	19		Lewis River	2013	57	56	F
29	20		Alexander Creek[d]	2014	101	92	F

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Table 1.–Page 2 of 5.

Map No.	Pop No.	Group	Location	Collection Year	N	N_a	Source[a]
		Susitna					
30	21		Portage Creek	2014	63	59	A
31	22		Indian River	2013	105	94	G
31	22			2014	52	50	A
32	---		Lane Creek	2014	10	---	A
33	23		Whiskers Creek	2013[c]	120	79	G
33	---			2014	2	---	A
34	---		Honolulu Creek	2013	4	---	G
35	24		Spink Creek	2008	38	32	C
35	24			2014	62	62	A
36	25		Byers Creek	2014	57	55	A
37	26		Tokositna River[e]	2008	1	1	F
37	26			2009	7	7	F
37	26			2010	11	11	F
37	26			2011	15	15	F
37	26			2012	28	28	F
38	---		Bunco Creek	2013	9	---	G
38	26			2014	56	55	A
39	---		Swan Lake	2009	20	---	C
40	27		Troublesome Creek	2013	92	88	G
40	---			2014	15	---	A
41	---		Iron Creek	2013	28	---	G
41	---			2014	12	---	A
42	28		Prairie Creek	2014	53	51	A
43	29		Sheep River	2013	115	95	G
44	30		Larson Lake outlet	2011	84	84	C
44	30			2014	48	48	A
45	31		Chunilna Creek	2013	66	64	G
45	31			2014	70	30	A
46	---		Fish Creek	2013	1	---	G
46	32			2014	65	65	A
47	---		Birch Creek	2014	2	---	A
48	---		Answer Creek	2013	7	---	A
49	33		Question Creek	2013	77	76	A
49	33			2014	76	50	A
50	---		Rabideux Creek	2014	1	---	A
51	34		Montana Creek	2013	200	87	F
52	35		Sheep Creek	2014	47	47	A
53	---		Kashwitna River	2014	24	---	A
54	---		Willow Creek	2014	27	---	A
55	36		Deshka River[f]	2009	8	8	F
55	36			2010	14	14	F
55	36			2011	17	17	F
55	36			2012	20	20	F
		Yentna					
56	---		Martin Creek	2013	36	---	A
57	---		Nakochna River	2014	8	---	A
58	---		Red Creek	2014	26	---	A
59	37		Hayes River	2014	87	84	A
60	38		Canyon Creek	2013	55	55	A
60	38			2014	105	50	A

-continued-

Table 1.–Page 3 of 5.

Map No.	Pop No.	Group	Location	Collection Year	N	N_a	Source[a]
61	39	*Yentna*	Talachulitna River	2013	74	72	A
61	39			2014	50	50	A
62	---		Sunflower Creek	2014	8	---	A
		Knik					
63	40		Little Susitna River	2013	97	94	F
63	40			2014	100	50	F
64	41		Fish Creek	2009	203	93	C
64	41			2013	94	92	F
64	---			2014	100	---	F
65	42		Cottonwood Creek	2014	94	73	A
66	43		Wasilla Creek	2013	9	9	A
66	43			2014	91	91	A
67	44		Rabbit Slough	2011	95	95	C
68	---		Matanuska River	2008	135	---	D
68	45			2009	194	94	D
68	---			2014	3	---	A
69	46		Eska Creek	2013	61	59	A
69	46			2014	65	35	A
70	47		Jim Creek	2009	68	68	C
70	47			2014	104	49	A
71	---		Jim Lake	2011	7	---	C
72	---		Eagle River	2014	24	---	A
73	48		Chester Creek	2011	54	53	C
73	48			2013	2	2	A
73	48			2014	14	22	A
74	---		Sixmile Creek	2009	46	---	C
74	---			2014	43	---	A
75	---		Ship Creek	1991	11	---	C
75	49			2012	400	93	C
75	---			2013	200	---	F
75	---			2014	189	---	F
76	---		Campbell Creek[g]	1995	5	---	C
76	50			2009	125	94	C
76	---			2010	9	---	C
		Turnagain/Northeast CI					
77	51		Rabbit Creek	2011	54	53	C
77	51			2013	2	2	C
77	51			2014	7	7	A
78	---		California Creek	2014	9	---	A
79	52		Placer Creek	2014	75	71	A
80	53		Williwaw Creek	2013	22	22	A
80	53			2014	50	49	A
81	---		Portage Creek	2013	5	---	A
81	---			2014	17	---	A
82	54		Explorer Creek	2013	95	91	A
82	54			2014	69	48	A
83	---		Placer River	2014	6	---	A
84	---		Ingram Creek	2013	7	---	A
84	---			2014	6	---	A
85	55		East Fork Sixmile Creek	2014	100[b]	90	A
86	56		Resurrection Creek	2010	96	93	C

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Table 1.–Page 4 of 5.

Map No.	Pop No.	Group	Location	Collection Year	N	N_a	Source[a]
		Turnagain/Northeast CI					
87	57		Chickaloon River/Mystery Creek	2010	104	100	C
88	58		Sucker Creek	1997	94	91	D
89	59		Gruska Creek	2013	53	53	A
89	59			2014	55	50	A
90	60		Bishop Creek	2014	62	57	A
		Kenai/Kasilof					
91	61		Trail Creek	2006	134	108	D
92	---		Moose Creek	1993	150[b]	---	C
93	62		Grant Creek	2013	100	95	H
94	63		South Fork Snow River	1998	73	71	D
94	63			2002	50	24	D
95	64		Summit Creek	2002	50	50	D
96	65		Tern Lake	2002	96	95	D
97	66		Quartz Creek	1998	75	73	D
98	---		Kenai Lake outlet	1999	56	---	D
98	---			2002	57	---	D
98	67			2014	117	95	A
99	---		Russian River	2002	31	---	D
99	68			2013	101	93	A
99	68			2014	100	47	A
100	69		Skilak River	2003	100	94	D
101	70		Skilak Lake outlet	1999	80	78	D
101	---			2002	50	---	D
101	70			2014	95	95	A
102	71		Killey River	2000	68	67	D
102	71			2002	49	25	D
103	72		East Fork Moose River	2002	100	93	D
104	73		Funny River	2006	150	92	D
105	---		Soldotna Creek	2013	8	---	A
106	74		Slikok Creek	2008	67	65	D
107	---		Beaver Creek	2013	12	---	A
108	75		Glacier Creek	2009	68	65	D
109	76		Indian Creek	2009	55	55	D
110	77		Nikolai Creek	2009	92	88	D
111	78		Tustumena Lake outlet	2009	100	90	D
		Southeast CI					
112	79		Ninilchik River	2013	108	94	A
112	---			2014	100	---	A
113	80		Deep Creek	2013	101	89	A
113	---			2014	100	---	A
114	81		Stariski Creek	2013	61	53	A
114	81			2014	100	34	A
115	82		Anchor River	2006	164	55	C
115	82			2009	40	40	C
116	83		Fox River	2013	117	109	A
116	---			2014	111	---	A
117	84		Port Graham River	2014	114	95	A

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Table 1.–Page 4 of 5.

Map No.	Pop No.	Group	Location	Collection Year	N	N_a	Source[a]
		Outside CI					
118	85		Delight Creek	2014	261	111	A
119	86		Resurrection River	2014	100	95	B
120	87		Bear Creek	2009	386	50	H
120	87			2012	67	47	C

[a] A = This Project, B = National Park Service, C = ADF&G archives, D = US Fish and Wildlife Service archives, E = Redoubt Mountain Lodge, F = ADF&G = Division of Sport Fish, G = Alaska Energy Authority, H = Cook Inlet Aquaculture Association.

[b] Juvenile collection.

[c] Collection contains juvenile and adult samples.

[d] Alexander Creek is genetically more similar to northwest Cook Inlet populations than Susitna River populations so it was included in the *Northwest CI* reporting group.

[e] Radiotagged coho samples from Sunshine fish wheels (2008) and Flathorn fish wheels (2009-2012).

[f] Radiotagged coho samples from Flathorn fish wheels (2009-2012).

[g] Campbell Creek is genetically similar to Ship Creek stock so it was grouped with Knik Arm populations.

Table 2.–Tissue collections sampled for genetic analysis from weir and fish wheel projects within the Susitna River drainage including collection location, collection years, number of fish sampled (N), and number of fish analyzed (N_a).

Map No.	Location	Collection Years	N	N_a
121	Deshka River weir	2013	95	95
122	Sunshine fish wheels	2008	28	4
123	Flathorn fish wheels	2009, 2010, 2011, 2012	1,378	176

Table 3.–Source, observed heterozygosity (H_o), and F_{ST} for 96 single nucleotide polymorphisms (SNPs) used to analyze the population genetic structure of Cook Inlet coho salmon.

These summary statistics are based upon the 84 populations within Cook Inlet detailed in Table 1.

Assay Name	Source[a]	H_o	F_{ST}[b]
Oki_arf-115	A	0.04	0.10
Oki_arp-105	B	0.45	0.06
Oki_aspAT-273	B	0.42	0.11
Oki_bcAKal-274	B	0.28	0.06
Oki_Car-353	C	0.12	0.03
Oki_carban-140	B	0.41	0.06
Oki_Cr-209[c,d]	A	-	0.04
Oki_E2-87	A	0.18	0.04
Oki_eif4ebp2-58	A	0.02	0.05
Oki_gdh-189	B	0.40	0.06
Oki_gh-183	B	0.30	0.06
Oki_GPDH-146	A	0.07	0.04
Oki_GPDH-188	A	0.06	0.04
Oki_HGFA-311	A	0.41	0.04
Oki_hsf1b-85	B	0.10	0.03
Oki_IGF-I.1-163	A	0.46	0.05
Oki_il1rac-169	C	0.02	0.09
Oki_il-1racp-176	A	0.07	0.03
Oki_ins-167	A	0.36	0.04
Oki_ins-323	A	0.03	0.06
Oki_LWSop-554	A	0.15	0.16
Oki_metA-220[d]	B	0.01	0.02
Oki_nips-159	B	0.42	0.04
Oki_p53-20	B	0.22	0.08
Oki_parp3-19	B	0.29	0.10
Oki_pigh-33	B	0.17	0.03
Oki_pop5-265	B	0.30	0.07
Oki_rpo2j-235	B	0.45	0.06
Oki_SClkF2R2-120	A	0.30	0.07
Oki_SECC22-67	B	0.32	0.03
Oki_serpin-328	A	0.22	0.05
Oki_spf30-119[d]	B	0.00	0.00
Oki_srp09-107	B	0.09	0.04
Oki_SWS1op-38	A	0.31	0.05
Oki_sys1-141	B	0.14	0.08
Oki_taf12-40	B	0.01	0.05
Oki_TniUPP-230	C	0.48	0.05
Oki_txnip-35	B	0.01	0.02
Oki_U202-136	C	0.24	0.05

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Table 3.–Page 2 of 3.

Assay Name	Source[a]	H_o	F_{ST}[b]
Oki_U216-151[d]	C	0.00	0.01
Oki_u6-257	A	0.46	0.07
Oki_vatf-363	B	0.38	0.07
Oki100974-29[d]	D	0.00	0.02
Oki101119-1006	D	0.16	0.05
Oki101419-103	D	0.46	0.06
Oki101554-35[d]	D	0.00	0.02
Oki101770-525	D	0.40	0.03
Oki102213-604	D	0.30	0.05
Oki102414-499	D	0.42	0.06
Oki102801-511	D	0.09	0.03
Oki103271-161	D	0.09	0.06
Oki103577-70	D	0.48	0.05
Oki103713-182	D	0.43	0.04
Oki104515-99	D	0.39	0.06
Oki104519-45	D	0.47	0.05
Oki104569-261[d]	D	0.00	0.00
Oki105105-245	D	0.36	0.10
Oki105115-49	D	0.18	0.08
Oki105132-169	D	0.41	0.08
Oki105235-460	D	0.41	0.08
Oki105385-521	D	0.07	0.03
Oki105407-161	D	0.46	0.06
Oki105897-298	D	0.07	0.04
Oki106172-60	D	0.45	0.05
Oki106313-353	D	0.38	0.03
Oki106419-292	D	0.27	0.04
Oki106479-278	D	0.18	0.05
Oki107336-45	D	0.27	0.04
Oki107607-213	D	0.26	0.06
Oki107974-46	D	0.46	0.04
Oki108505-331[d]	D	0.00	0.05
Oki109651-152	D	0.46	0.04
Oki109874-122	D	0.11	0.04
Oki109894-418	D	0.09	0.12
Oki110078-191	D	0.28	0.05
Oki110689-43[d]	D	0.00	0.01
Oki111681-407	D	0.05	0.02
Oki113457-324	D	0.41	0.03
Oki114448-101	D	0.23	0.05
Oki114587-309	D	0.32	0.04

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Table 3.–Page 3 of 3.

Assay Name	Source[a]	H_o	F_{ST}[b]
Oki116362-411	D	0.19	0.07
Oki116865-244	D	0.47	0.03
Oki117043-374	D	0.35	0.12
Oki117144-64	D	0.36	0.07
Oki117286-291[d]	D	0.00	0.01
Oki117742-259	D	0.39	0.05
Oki117815-369	D	0.33	0.04
Oki118152-314	D	0.14	0.03
Oki118175-264	D	0.41	0.04
Oki118654-330	D	0.43	0.06
Oki94903-192	D	0.36	0.04
Oki95318-100	D	0.42	0.09
Oki96127-66	D	0.43	0.05
Oki96158-278	D	0.40	0.04
Oki96376-63	D	0.33	0.05
Oki97954-228	D	0.26	0.05
Average/Overall[e]		0.28	0.06

[a] A = Smith et al. 2006; B = Campbell and Narum 2011; C = Matt Smith, unpublished, University of Washington, e-mail matt_smith@fws.gov; D = Starks et al. 2015.
[b] Weir and Cockerham (1984).
[c] Mitochondrial SNP marker.
[d] These were removed from further analysis because they were invariant.
[e] Overall statistics are based on the 86 variant SNPs used in the proof tests for mixed stock analysis.

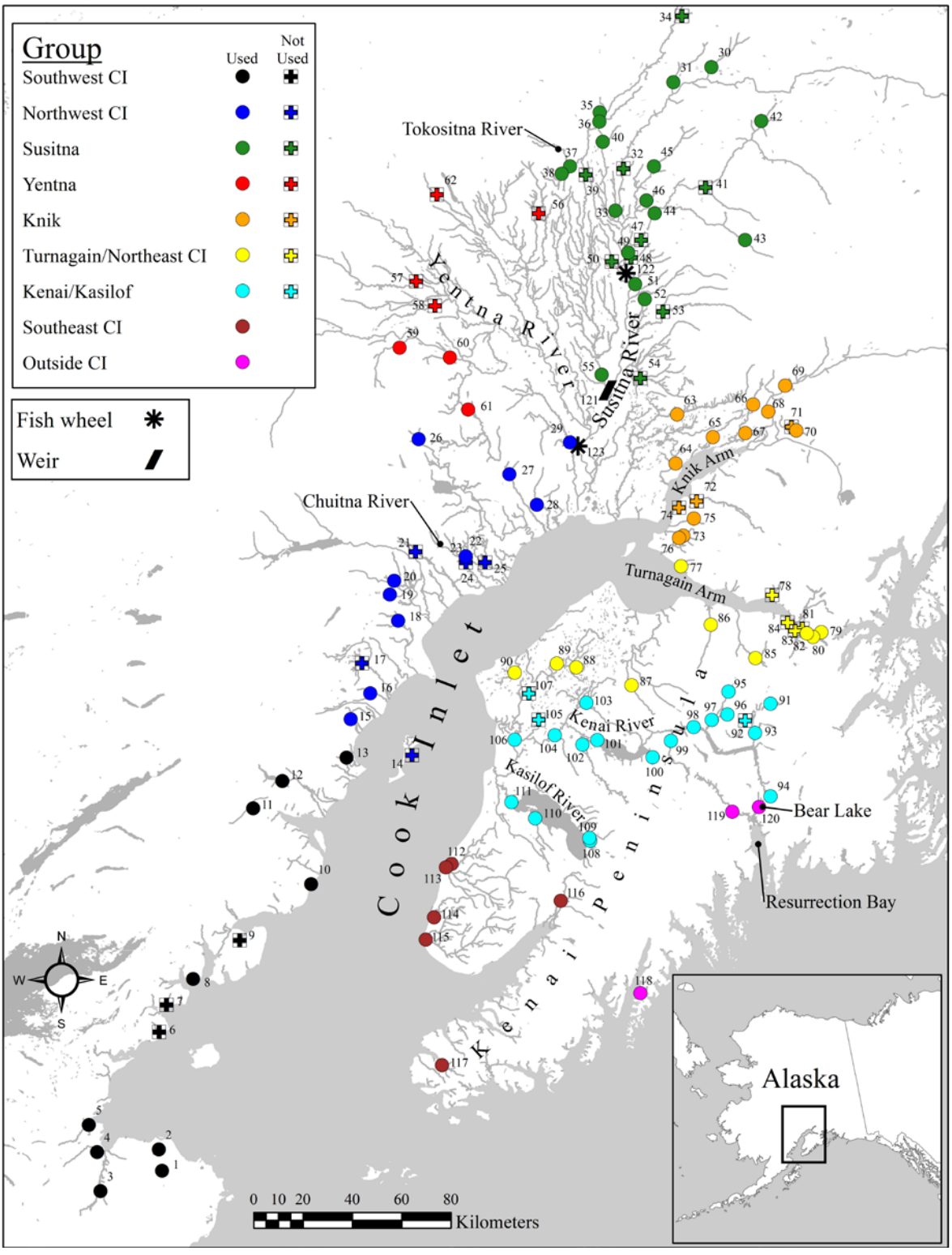

Figure 1.–Sampling locations for coho salmon originating in Cook Inlet, Alaska, 1991–2014. Numbers correspond to map numbers on Tables 1 and 2.

Note: Colored circles and crosses correspond to the 8 Cook Inlet reporting groups used in the mixed stock analysis proof tests and one outside of Cook Inlet group.

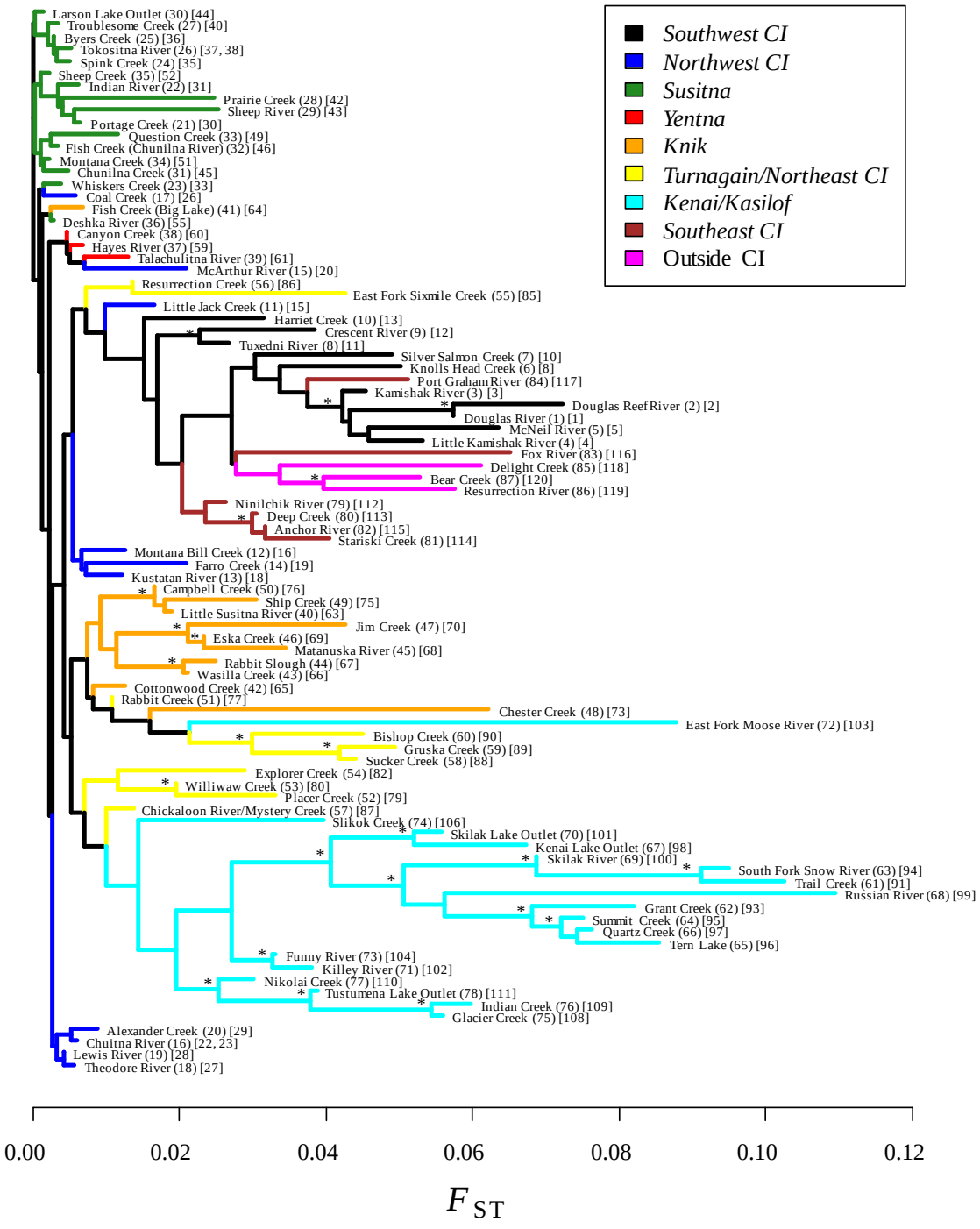

Figure 2.–Consensus neighbor-joining tree based on F_{ST} (Appendix A; Weir and Cockerham 1984) between coho salmon populations sampled from spawning areas in drainages of Cook Inlet and the eastern Kenai Peninsula, Alaska (see Table 1 for collection details).

Note: Colors denote groups as in Figures 1, 3, and 4. Numbers in parentheses correspond to unique population numbers on Table 1 and numbers in brackets correspond to map numbers on Figure 1. Bootstrap consensus nodes occurring in >50% of trees are marked with an asterisk.

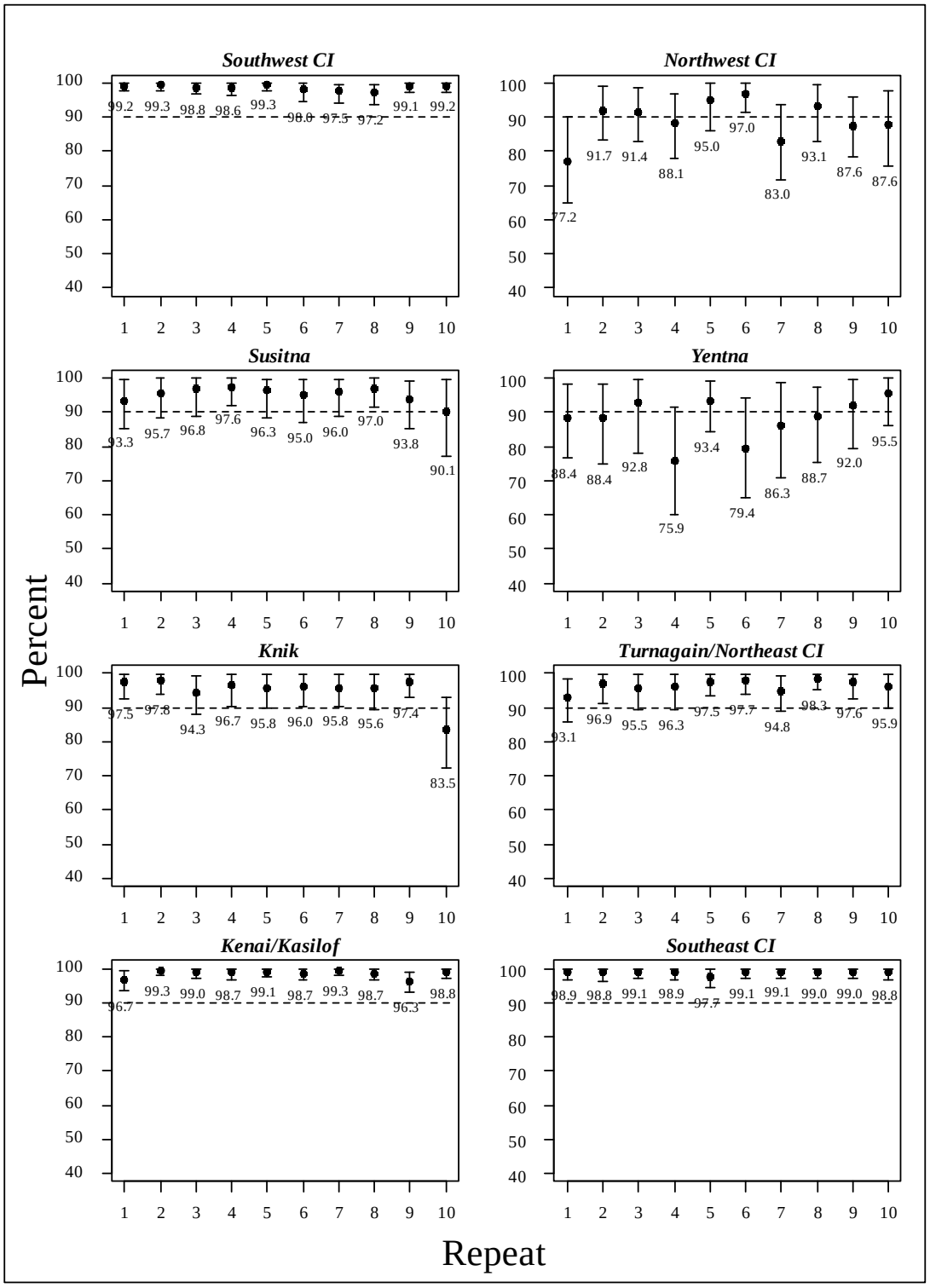

Figure 3.–Results of repeated proof testing for eight reporting groups. The points represent the correct allocation mean from each repeat with 95% credibility intervals for each point. Correct allocation means (%) for each repeat are included below the lower credibility interval.

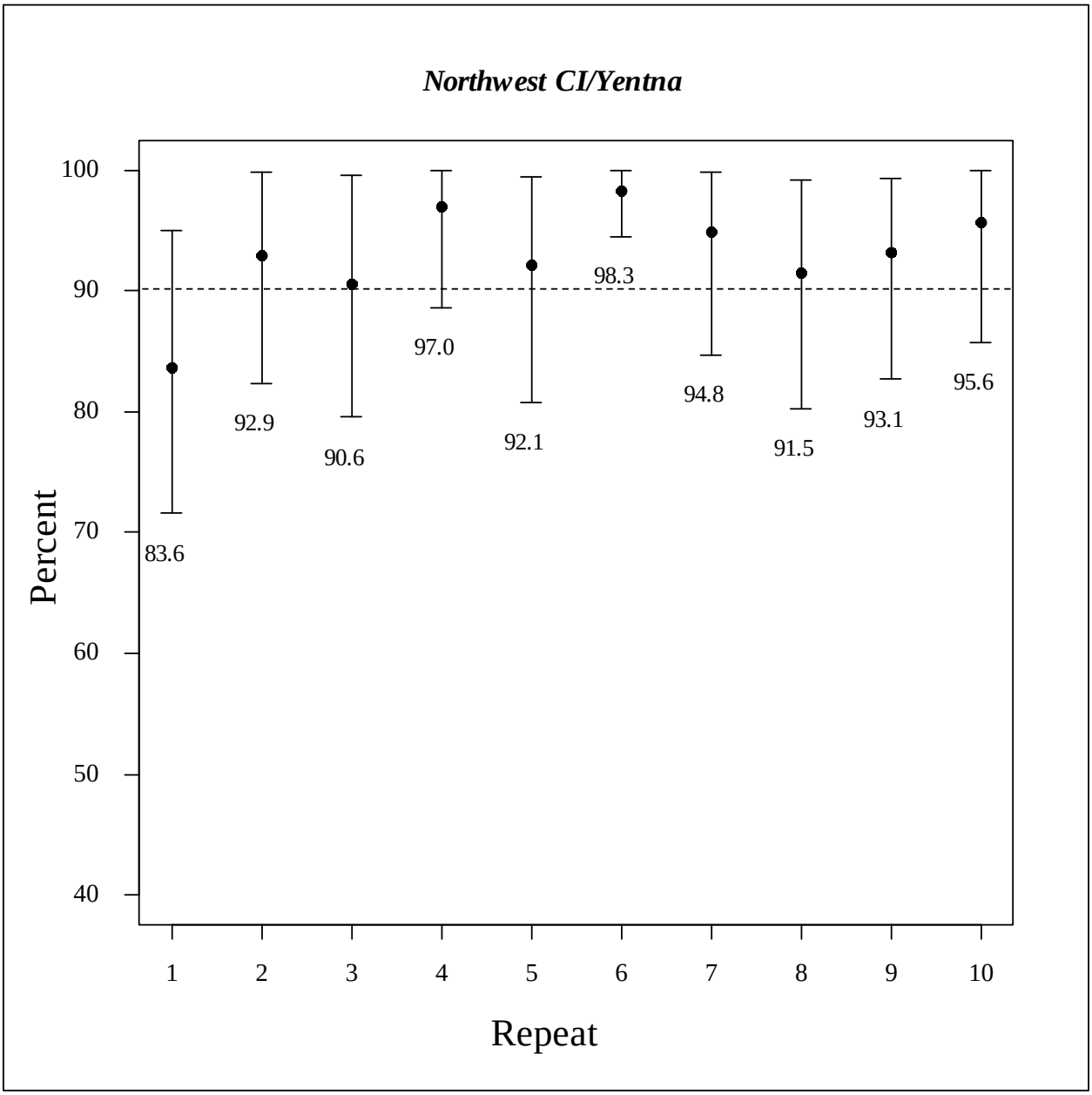

Figure 4.–Results of repeated proof testing for the *Northwest CI/Yentna* reporting group. The points represent the correct allocation mean from each repeat with 95% credibility intervals for each point. Correct allocation means (%) for each repeat are included below the lower credibility interval.

APPENDIX A

Appendix A.–Pairwise F_{ST} (Weir and Cockerham 1984) between coho salmon populations sampled from spawning areas in drainages of Cook Inlet and the eastern Kenai Peninsula, Alaska.

Population numbers correspond to population numbers on Table 1.

Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	36	37	38	39
1	0.00																						
2	0.01	0.00																					
3	0.02	0.03	0.00																				
4	0.02	0.04	0.01	0.00																			
5	0.04	0.05	0.03	0.03	0.00																		
6	0.04	0.05	0.03	0.04	0.05	0.00																	
7	0.04	0.06	0.03	0.04	0.04	0.05	0.00																
8	0.04	0.05	0.03	0.04	0.05	0.03	0.04	0.00															
9	0.06	0.07	0.05	0.05	0.06	0.05	0.05	0.02	0.00														
10	0.05	0.07	0.04	0.04	0.06	0.05	0.05	0.03	0.04	0.00													
11	0.06	0.08	0.05	0.06	0.06	0.05	0.05	0.02	0.03	0.03	0.00												
12	0.06	0.08	0.05	0.06	0.07	0.05	0.04	0.02	0.04	0.03	0.02	0.00											
13	0.07	0.08	0.05	0.06	0.08	0.06	0.06	0.03	0.04	0.04	0.01	0.01	0.00										
14	0.06	0.07	0.05	0.06	0.06	0.06	0.05	0.03	0.05	0.04	0.03	0.02	0.02	0.00									
15	0.08	0.09	0.06	0.08	0.08	0.06	0.06	0.04	0.06	0.05	0.03	0.03	0.02	0.03	0.00								
16	0.06	0.08	0.05	0.05	0.06	0.05	0.05	0.03	0.04	0.03	0.01	0.01	0.01	0.02	0.03	0.00							
17	0.06	0.08	0.05	0.05	0.07	0.06	0.05	0.03	0.05	0.03	0.02	0.02	0.02	0.03	0.02	0.01	0.00						
18	0.06	0.08	0.05	0.06	0.07	0.05	0.05	0.02	0.04	0.04	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.00					
19	0.06	0.08	0.05	0.05	0.06	0.05	0.05	0.03	0.04	0.03	0.02	0.01	0.01	0.02	0.02	0.00	0.00	0.00	0.00				
36	0.07	0.09	0.06	0.07	0.08	0.06	0.05	0.03	0.04	0.03	0.02	0.01	0.01	0.02	0.03	0.00	0.01	0.01	0.01	0.00			
37	0.06	0.08	0.05	0.06	0.07	0.06	0.05	0.03	0.05	0.04	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.00		
38	0.06	0.08	0.05	0.06	0.07	0.05	0.05	0.02	0.04	0.03	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.00	0.00	
39	0.08	0.09	0.07	0.09	0.09	0.07	0.07	0.04	0.06	0.04	0.02	0.02	0.02	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00

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Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	36	37	38	39
20	0.07	0.08	0.05	0.06	0.07	0.05	0.06	0.03	0.05	0.04	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02
21	0.07	0.07	0.05	0.06	0.07	0.06	0.06	0.04	0.05	0.04	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02
22	0.06	0.08	0.06	0.06	0.08	0.06	0.06	0.03	0.05	0.03	0.02	0.02	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
23	0.06	0.08	0.06	0.06	0.08	0.06	0.06	0.04	0.05	0.04	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
24	0.06	0.07	0.05	0.05	0.07	0.05	0.05	0.02	0.04	0.03	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01
25	0.07	0.08	0.06	0.07	0.08	0.06	0.06	0.03	0.05	0.04	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
26	0.07	0.09	0.06	0.07	0.08	0.06	0.06	0.04	0.05	0.04	0.02	0.02	0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
27	0.08	0.09	0.07	0.08	0.09	0.07	0.08	0.05	0.06	0.05	0.04	0.04	0.04	0.04	0.05	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.04
28	0.06	0.08	0.05	0.06	0.07	0.06	0.06	0.04	0.06	0.04	0.04	0.03	0.04	0.04	0.06	0.03	0.03	0.04	0.03	0.03	0.04	0.03	0.05
29	0.06	0.07	0.05	0.06	0.07	0.05	0.05	0.03	0.04	0.03	0.02	0.02	0.01	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
30	0.06	0.08	0.06	0.06	0.08	0.06	0.06	0.04	0.05	0.04	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
31	0.06	0.08	0.05	0.06	0.07	0.06	0.05	0.03	0.04	0.03	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
32	0.07	0.09	0.06	0.07	0.08	0.07	0.07	0.05	0.05	0.05	0.02	0.03	0.02	0.04	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
33	0.06	0.08	0.05	0.06	0.07	0.06	0.05	0.03	0.04	0.03	0.01	0.01	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
34	0.06	0.08	0.05	0.06	0.07	0.05	0.05	0.03	0.04	0.04	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
35	0.06	0.08	0.05	0.06	0.07	0.06	0.06	0.03	0.04	0.03	0.02	0.02	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
40	0.05	0.07	0.05	0.05	0.07	0.05	0.05	0.04	0.04	0.04	0.03	0.03	0.03	0.04	0.04	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.03
41	0.06	0.08	0.05	0.06	0.07	0.06	0.05	0.03	0.04	0.03	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
42	0.06	0.07	0.05	0.06	0.06	0.06	0.05	0.03	0.05	0.04	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.02
43	0.06	0.08	0.06	0.07	0.07	0.05	0.06	0.04	0.05	0.05	0.03	0.03	0.03	0.04	0.04	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.03
44	0.06	0.08	0.06	0.07	0.07	0.06	0.05	0.04	0.06	0.05	0.04	0.03	0.03	0.04	0.04	0.03	0.03	0.02	0.02	0.03	0.03	0.02	0.03
45	0.08	0.09	0.06	0.07	0.08	0.08	0.08	0.04	0.06	0.06	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.04
46	0.06	0.08	0.05	0.06	0.06	0.06	0.06	0.03	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.03

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Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	36	37	38	39
47	0.09	0.11	0.08	0.09	0.09	0.08	0.07	0.05	0.07	0.08	0.05	0.05	0.06	0.06	0.05	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.05
48	0.11	0.13	0.11	0.10	0.10	0.11	0.11	0.08	0.09	0.10	0.08	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.05	0.08	0.06	0.06	0.06
49	0.06	0.07	0.05	0.06	0.07	0.06	0.06	0.05	0.06	0.04	0.04	0.03	0.03	0.05	0.05	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.04
50	0.05	0.07	0.04	0.05	0.06	0.05	0.05	0.03	0.05	0.04	0.03	0.02	0.02	0.03	0.04	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.03
51	0.05	0.07	0.04	0.05	0.06	0.05	0.05	0.03	0.04	0.04	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02
52	0.08	0.09	0.07	0.08	0.09	0.06	0.07	0.05	0.06	0.06	0.05	0.03	0.03	0.05	0.05	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.04
53	0.07	0.09	0.06	0.07	0.08	0.06	0.06	0.04	0.05	0.05	0.03	0.02	0.02	0.03	0.04	0.01	0.03	0.02	0.01	0.01	0.02	0.02	0.02
54	0.06	0.09	0.06	0.07	0.09	0.07	0.07	0.05	0.06	0.05	0.04	0.03	0.03	0.04	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
55	0.08	0.10	0.07	0.08	0.09	0.08	0.06	0.05	0.05	0.05	0.05	0.04	0.04	0.06	0.05	0.05	0.04	0.04	0.04	0.04	0.05	0.04	0.05
56	0.05	0.07	0.04	0.05	0.06	0.05	0.04	0.03	0.04	0.03	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02
57	0.06	0.07	0.04	0.05	0.06	0.05	0.05	0.03	0.04	0.03	0.02	0.02	0.02	0.03	0.03	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02
58	0.07	0.08	0.07	0.07	0.08	0.06	0.08	0.05	0.06	0.06	0.05	0.05	0.06	0.05	0.06	0.04	0.04	0.04	0.03	0.05	0.05	0.05	0.05
59	0.08	0.10	0.08	0.08	0.09	0.07	0.09	0.06	0.08	0.07	0.06	0.06	0.06	0.05	0.07	0.05	0.04	0.05	0.04	0.06	0.06	0.05	0.06
60	0.09	0.10	0.07	0.08	0.08	0.07	0.08	0.06	0.07	0.07	0.06	0.06	0.05	0.06	0.06	0.04	0.04	0.04	0.03	0.06	0.05	0.05	0.06
61	0.11	0.14	0.10	0.11	0.11	0.10	0.13	0.11	0.12	0.10	0.10	0.10	0.11	0.11	0.12	0.10	0.11	0.10	0.11	0.11	0.11	0.10	0.10
62	0.08	0.10	0.07	0.08	0.09	0.08	0.09	0.07	0.08	0.09	0.08	0.09	0.09	0.10	0.10	0.08	0.09	0.08	0.09	0.09	0.08	0.09	0.09
63	0.10	0.13	0.10	0.11	0.11	0.09	0.13	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.11	0.09	0.10	0.10	0.10	0.11	0.09	0.09	0.09
64	0.07	0.10	0.06	0.08	0.07	0.07	0.10	0.08	0.09	0.08	0.08	0.09	0.08	0.08	0.09	0.07	0.08	0.07	0.08	0.09	0.08	0.08	0.08
65	0.09	0.12	0.08	0.09	0.09	0.08	0.10	0.09	0.09	0.09	0.08	0.09	0.09	0.10	0.10	0.09	0.09	0.08	0.09	0.10	0.09	0.09	0.09
66	0.08	0.10	0.06	0.08	0.08	0.07	0.09	0.08	0.09	0.08	0.08	0.09	0.09	0.09	0.09	0.08	0.08	0.07	0.08	0.09	0.08	0.08	0.09
67	0.08	0.10	0.07	0.09	0.09	0.06	0.08	0.07	0.09	0.07	0.06	0.07	0.07	0.08	0.10	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08
68	0.12	0.16	0.11	0.12	0.14	0.09	0.12	0.10	0.11	0.12	0.11	0.12	0.12	0.13	0.13	0.11	0.11	0.10	0.11	0.12	0.12	0.11	0.12
69	0.09	0.12	0.08	0.09	0.09	0.08	0.09	0.07	0.08	0.07	0.06	0.06	0.07	0.07	0.09	0.06	0.08	0.06	0.07	0.07	0.07	0.07	0.07

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Pop. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	36	37	38	39
70	0.08	0.10	0.07	0.08	0.09	0.06	0.08	0.06	0.08	0.07	0.05	0.06	0.06	0.06	0.08	0.05	0.06	0.05	0.05	0.06	0.06	0.06	0.07
71	0.06	0.09	0.05	0.06	0.07	0.06	0.05	0.06	0.06	0.04	0.04	0.04	0.05	0.05	0.07	0.03	0.04	0.04	0.04	0.04	0.05	0.04	0.06
72	0.11	0.15	0.10	0.11	0.12	0.10	0.11	0.10	0.09	0.11	0.09	0.10	0.10	0.10	0.11	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.10
73	0.06	0.09	0.06	0.06	0.07	0.06	0.06	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.06	0.02	0.04	0.03	0.03	0.03	0.04	0.04	0.05
74	0.08	0.11	0.07	0.09	0.10	0.08	0.06	0.06	0.07	0.06	0.04	0.04	0.04	0.05	0.06	0.03	0.05	0.04	0.04	0.03	0.05	0.04	0.04
75	0.08	0.10	0.07	0.07	0.09	0.06	0.08	0.05	0.08	0.07	0.06	0.06	0.06	0.07	0.08	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.07
76	0.08	0.10	0.07	0.08	0.10	0.06	0.07	0.06	0.08	0.07	0.07	0.06	0.06	0.07	0.08	0.06	0.06	0.05	0.06	0.07	0.06	0.06	0.08
77	0.05	0.07	0.04	0.05	0.06	0.04	0.06	0.03	0.05	0.05	0.04	0.03	0.04	0.04	0.06	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.05
78	0.06	0.08	0.05	0.06	0.07	0.05	0.05	0.05	0.06	0.05	0.04	0.04	0.05	0.05	0.06	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.05
79	0.04	0.05	0.03	0.04	0.05	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.05	0.03	0.02	0.03	0.02	0.03	0.03	0.03	0.04
80	0.03	0.05	0.02	0.03	0.04	0.04	0.04	0.03	0.04	0.03	0.03	0.04	0.04	0.04	0.05	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.05
81	0.03	0.05	0.02	0.03	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.05	0.05	0.04	0.06	0.04	0.04	0.05	0.04	0.05	0.04	0.04	0.07
82	0.03	0.05	0.02	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.05	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.05
83	0.06	0.08	0.05	0.06	0.08	0.06	0.06	0.05	0.07	0.05	0.06	0.06	0.08	0.07	0.08	0.06	0.06	0.07	0.06	0.06	0.07	0.06	0.08
84	0.03	0.05	0.02	0.03	0.04	0.03	0.03	0.03	0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.06	0.05	0.05	0.05	0.06	0.06	0.06	0.07
85	0.05	0.07	0.04	0.06	0.05	0.06	0.06	0.05	0.06	0.08	0.05	0.07	0.07	0.07	0.08	0.06	0.07	0.06	0.06	0.07	0.07	0.07	0.09
86	0.04	0.06	0.03	0.04	0.05	0.06	0.05	0.05	0.06	0.06	0.05	0.06	0.07	0.07	0.08	0.06	0.06	0.05	0.06	0.06	0.06	0.06	0.08
87	0.06	0.07	0.04	0.06	0.06	0.06	0.06	0.05	0.07	0.07	0.05	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.04	0.06	0.06	0.06	0.06

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Pop. No.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	40	41	42	43	44	45	46
20	0.00																						
21	0.00	0.00																					
22	0.01	0.01	0.00																				
23	0.01	0.01	0.01	0.00																			
24	0.01	0.01	0.00	0.00	0.00																		
25	0.01	0.01	0.01	0.00	0.00	0.00																	
26	0.01	0.01	0.01	0.00	0.00	0.00	0.00																
27	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.00															
28	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.04	0.00														
29	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.03	0.00													
30	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.03	0.01	0.00												
31	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.00	0.01	0.00											
32	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0.03	0.01	0.01	0.01	0.00										
33	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.03	0.03	0.00	0.00	0.01	0.01	0.00									
34	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.03	0.01	0.01	0.00	0.01	0.00	0.00								
35	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.03	0.03	0.00	0.01	0.00	0.01	0.00	0.01	0.00							
40	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.04	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.00						
41	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.02	0.00					
42	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.04	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.00				
43	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.05	0.04	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.00			
44	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.05	0.04	0.03	0.03	0.04	0.04	0.03	0.02	0.03	0.03	0.02	0.02	0.01	0.00		
45	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.06	0.06	0.04	0.04	0.05	0.06	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.00	
46	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.05	0.04	0.02	0.02	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.00

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Pop. No.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	40	41	42	43	44	45	46
47	0.05	0.05	0.04	0.04	0.04	0.05	0.04	0.07	0.07	0.05	0.05	0.05	0.06	0.05	0.04	0.05	0.04	0.05	0.04	0.04	0.05	0.03	0.02
48	0.07	0.07	0.06	0.07	0.06	0.07	0.07	0.10	0.08	0.07	0.06	0.07	0.08	0.07	0.06	0.07	0.07	0.06	0.05	0.05	0.05	0.06	0.06
49	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.05	0.05	0.03	0.03	0.04	0.05	0.03	0.03	0.04	0.01	0.03	0.03	0.03	0.03	0.05	0.04
50	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.04	0.03	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.01	0.01	0.02	0.03	0.02
51	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.03	0.01
52	0.04	0.04	0.04	0.03	0.03	0.04	0.03	0.06	0.06	0.03	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.06	0.04
53	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.04	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.03	0.02	0.03	0.04	0.02
54	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.03	0.03	0.04	0.05	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.04	0.03
55	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.07	0.06	0.04	0.05	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.05	0.06	0.07	0.07	0.06
56	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.04	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.03	0.03	0.02
57	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.04	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.02
58	0.05	0.05	0.04	0.05	0.04	0.06	0.05	0.06	0.06	0.04	0.05	0.05	0.06	0.05	0.05	0.04	0.06	0.04	0.05	0.05	0.05	0.06	0.04
59	0.05	0.06	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.05	0.05	0.06	0.07	0.06	0.05	0.05	0.06	0.05	0.05	0.05	0.07	0.06	0.04
60	0.03	0.05	0.05	0.05	0.04	0.05	0.04	0.06	0.06	0.04	0.04	0.04	0.05	0.05	0.04	0.04	0.05	0.03	0.05	0.05	0.06	0.06	0.05
61	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.13	0.13	0.11	0.11	0.12	0.13	0.11	0.11	0.11	0.10	0.11	0.10	0.12	0.12	0.11	0.10
62	0.09	0.10	0.09	0.09	0.09	0.09	0.09	0.11	0.11	0.09	0.09	0.09	0.10	0.09	0.09	0.09	0.08	0.09	0.08	0.09	0.10	0.10	0.08
63	0.11	0.11	0.10	0.10	0.10	0.11	0.10	0.12	0.13	0.10	0.10	0.11	0.12	0.10	0.11	0.10	0.09	0.10	0.09	0.10	0.11	0.10	0.09
64	0.09	0.09	0.09	0.08	0.08	0.09	0.09	0.10	0.10	0.08	0.09	0.09	0.10	0.08	0.08	0.09	0.06	0.09	0.07	0.09	0.09	0.09	0.07
65	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.09	0.10	0.10	0.11	0.10	0.09	0.10	0.08	0.09	0.08	0.10	0.10	0.10	0.08
66	0.09	0.10	0.09	0.09	0.09	0.09	0.09	0.11	0.11	0.09	0.09	0.09	0.10	0.09	0.08	0.09	0.07	0.08	0.07	0.08	0.09	0.09	0.07
67	0.08	0.09	0.07	0.09	0.07	0.08	0.08	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.06
68	0.12	0.14	0.12	0.13	0.12	0.13	0.12	0.16	0.15	0.11	0.12	0.12	0.13	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.12	0.13	0.11
69	0.08	0.08	0.07	0.07	0.07	0.08	0.07	0.09	0.09	0.08	0.07	0.08	0.09	0.07	0.07	0.08	0.07	0.08	0.07	0.08	0.09	0.08	0.07

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Pop. No.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	40	41	42	43	44	45	46
70	0.07	0.08	0.06	0.07	0.06	0.07	0.06	0.07	0.07	0.06	0.06	0.07	0.07	0.06	0.06	0.07	0.05	0.07	0.06	0.06	0.07	0.06	0.05
71	0.05	0.05	0.04	0.05	0.04	0.05	0.05	0.06	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.05	0.03	0.05	0.05	0.04	0.05	0.06	0.05
72	0.10	0.10	0.11	0.10	0.09	0.10	0.09	0.11	0.12	0.09	0.09	0.10	0.11	0.10	0.09	0.10	0.10	0.09	0.09	0.09	0.10	0.10	0.09
73	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.05	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.03	0.04	0.04	0.04	0.05	0.05	0.04
74	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.07	0.05	0.04	0.04	0.04	0.06	0.03	0.05	0.05	0.05	0.04	0.05	0.06	0.06	0.07	0.06
75	0.07	0.07	0.05	0.06	0.05	0.06	0.07	0.09	0.07	0.05	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.08	0.06
76	0.07	0.08	0.06	0.07	0.06	0.07	0.07	0.10	0.08	0.06	0.06	0.06	0.08	0.07	0.06	0.07	0.06	0.06	0.06	0.07	0.07	0.08	0.06
77	0.04	0.04	0.03	0.04	0.03	0.04	0.03	0.06	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.04	0.04	0.04	0.05	0.03
78	0.05	0.06	0.04	0.05	0.03	0.05	0.05	0.07	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.05	0.04	0.05	0.04	0.04	0.04	0.05	0.04
79	0.02	0.03	0.03	0.03	0.02	0.03	0.03	0.04	0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.04	0.04	0.05	0.03
80	0.03	0.04	0.04	0.04	0.03	0.04	0.04	0.05	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.04	0.05	0.05	0.05	0.03
81	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.07	0.05	0.04	0.05	0.04	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.06	0.07	0.06	0.05
82	0.04	0.04	0.03	0.04	0.03	0.04	0.04	0.05	0.04	0.03	0.04	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.05	0.05	0.05	0.04
83	0.08	0.08	0.06	0.07	0.06	0.07	0.07	0.08	0.07	0.07	0.08	0.07	0.08	0.06	0.06	0.07	0.08	0.07	0.07	0.09	0.08	0.09	0.08
84	0.06	0.07	0.06	0.07	0.05	0.07	0.07	0.08	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.08	0.06
85	0.07	0.08	0.07	0.07	0.06	0.07	0.07	0.09	0.06	0.07	0.07	0.07	0.08	0.07	0.07	0.08	0.06	0.07	0.07	0.07	0.07	0.07	0.06
86	0.08	0.08	0.07	0.07	0.06	0.07	0.07	0.08	0.07	0.06	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.07	0.06	0.07	0.07	0.07	0.06
87	0.07	0.07	0.06	0.06	0.05	0.06	0.06	0.09	0.08	0.06	0.06	0.07	0.07	0.06	0.05	0.06	0.06	0.06	0.05	0.05	0.06	0.06	0.05

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Pop. No.	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
47	0.00																						
48	0.09	0.00																					
49	0.06	0.08	0.00																				
50	0.04	0.06	0.01	0.00																			
51	0.04	0.04	0.03	0.01	0.00																		
52	0.05	0.08	0.05	0.04	0.03	0.00																	
53	0.04	0.07	0.03	0.02	0.02	0.01	0.00																
54	0.06	0.07	0.04	0.03	0.03	0.04	0.03	0.00															
55	0.07	0.10	0.07	0.05	0.04	0.07	0.05	0.06	0.00														
56	0.04	0.06	0.03	0.01	0.01	0.04	0.02	0.03	0.02	0.00													
57	0.04	0.07	0.04	0.02	0.01	0.03	0.02	0.04	0.05	0.01	0.00												
58	0.08	0.07	0.07	0.05	0.04	0.07	0.06	0.07	0.08	0.04	0.06	0.00											
59	0.08	0.08	0.07	0.05	0.05	0.08	0.06	0.07	0.08	0.04	0.06	0.01	0.00										
60	0.08	0.09	0.07	0.05	0.04	0.08	0.06	0.07	0.08	0.04	0.05	0.03	0.03	0.00									
61	0.11	0.17	0.10	0.10	0.10	0.12	0.10	0.09	0.12	0.10	0.10	0.15	0.13	0.14	0.00								
62	0.09	0.16	0.10	0.08	0.08	0.10	0.09	0.09	0.10	0.08	0.08	0.12	0.13	0.11	0.07	0.00							
63	0.10	0.15	0.10	0.09	0.09	0.11	0.09	0.08	0.12	0.10	0.09	0.13	0.12	0.12	0.02	0.06	0.00						
64	0.09	0.14	0.07	0.07	0.07	0.10	0.08	0.08	0.10	0.07	0.07	0.12	0.12	0.10	0.05	0.02	0.04	0.00					
65	0.10	0.16	0.09	0.08	0.08	0.10	0.09	0.08	0.10	0.08	0.08	0.13	0.13	0.11	0.05	0.02	0.05	0.02	0.00				
66	0.09	0.14	0.08	0.07	0.07	0.10	0.09	0.08	0.09	0.07	0.07	0.12	0.12	0.09	0.06	0.02	0.04	0.01	0.01	0.00			
67	0.10	0.14	0.07	0.06	0.07	0.08	0.06	0.07	0.09	0.06	0.06	0.09	0.09	0.10	0.08	0.08	0.08	0.06	0.07	0.07	0.00		
68	0.13	0.17	0.11	0.11	0.10	0.13	0.12	0.10	0.11	0.10	0.11	0.14	0.15	0.13	0.11	0.08	0.10	0.08	0.08	0.07	0.09	0.00	
69	0.08	0.13	0.07	0.07	0.06	0.09	0.06	0.07	0.09	0.06	0.06	0.10	0.10	0.09	0.03	0.06	0.03	0.04	0.05	0.05	0.05	0.09	0.00

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Pop. No.	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
70	0.08	0.12	0.06	0.05	0.05	0.07	0.06	0.06	0.08	0.05	0.05	0.08	0.08	0.08	0.09	0.06	0.08	0.05	0.06	0.05	0.02	0.07	0.05
71	0.08	0.09	0.04	0.03	0.04	0.05	0.04	0.05	0.06	0.03	0.03	0.08	0.08	0.08	0.09	0.08	0.09	0.06	0.07	0.06	0.04	0.08	0.05
72	0.12	0.11	0.11	0.10	0.07	0.11	0.09	0.09	0.12	0.09	0.07	0.09	0.10	0.09	0.15	0.14	0.14	0.13	0.14	0.13	0.12	0.13	0.11
73	0.07	0.09	0.05	0.03	0.03	0.04	0.03	0.04	0.06	0.03	0.02	0.07	0.06	0.07	0.09	0.08	0.08	0.06	0.07	0.06	0.05	0.09	0.05
74	0.08	0.12	0.07	0.04	0.04	0.05	0.04	0.05	0.06	0.04	0.03	0.09	0.10	0.10	0.11	0.11	0.12	0.10	0.10	0.10	0.07	0.13	0.07
75	0.09	0.10	0.06	0.05	0.05	0.08	0.07	0.07	0.08	0.05	0.06	0.08	0.09	0.09	0.12	0.11	0.11	0.10	0.11	0.09	0.07	0.10	0.08
76	0.10	0.11	0.07	0.05	0.06	0.08	0.07	0.07	0.09	0.06	0.06	0.08	0.09	0.09	0.12	0.11	0.11	0.10	0.10	0.08	0.07	0.10	0.08
77	0.07	0.08	0.05	0.03	0.03	0.05	0.03	0.05	0.06	0.03	0.03	0.05	0.05	0.05	0.10	0.08	0.09	0.08	0.08	0.07	0.05	0.11	0.06
78	0.07	0.08	0.05	0.04	0.04	0.06	0.05	0.05	0.07	0.03	0.03	0.07	0.08	0.08	0.11	0.09	0.10	0.08	0.09	0.08	0.05	0.09	0.06
79	0.06	0.09	0.04	0.03	0.03	0.06	0.04	0.05	0.05	0.02	0.03	0.05	0.06	0.05	0.11	0.07	0.10	0.07	0.09	0.07	0.07	0.12	0.08
80	0.06	0.09	0.05	0.03	0.03	0.06	0.04	0.05	0.06	0.03	0.03	0.06	0.06	0.06	0.09	0.06	0.09	0.06	0.08	0.06	0.06	0.11	0.07
81	0.07	0.10	0.06	0.04	0.04	0.07	0.06	0.06	0.07	0.04	0.04	0.06	0.06	0.06	0.11	0.07	0.10	0.07	0.08	0.07	0.07	0.12	0.08
82	0.06	0.09	0.04	0.03	0.03	0.06	0.04	0.05	0.05	0.03	0.03	0.05	0.06	0.06	0.09	0.06	0.09	0.06	0.07	0.06	0.06	0.10	0.06
83	0.10	0.13	0.09	0.07	0.07	0.09	0.07	0.08	0.08	0.06	0.07	0.09	0.10	0.10	0.12	0.10	0.13	0.11	0.11	0.11	0.10	0.14	0.10
84	0.08	0.11	0.06	0.05	0.05	0.07	0.06	0.07	0.07	0.04	0.05	0.06	0.08	0.08	0.11	0.08	0.11	0.08	0.08	0.07	0.06	0.09	0.09
85	0.08	0.11	0.07	0.05	0.06	0.08	0.07	0.07	0.10	0.06	0.06	0.08	0.09	0.10	0.13	0.10	0.13	0.09	0.10	0.09	0.08	0.14	0.10
86	0.08	0.12	0.06	0.05	0.06	0.08	0.06	0.07	0.08	0.06	0.05	0.09	0.10	0.09	0.12	0.09	0.11	0.08	0.10	0.09	0.08	0.12	0.08
87	0.07	0.10	0.05	0.05	0.05	0.08	0.06	0.07	0.08	0.05	0.06	0.06	0.07	0.07	0.12	0.09	0.11	0.08	0.09	0.08	0.08	0.10	0.09

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Pop. No.	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87
70	0.00																	
71	0.03	0.00																
72	0.11	0.09	0.00															
73	0.04	0.01	0.09	0.00														
74	0.07	0.04	0.12	0.03	0.00													
75	0.07	0.06	0.12	0.06	0.07	0.00												
76	0.07	0.06	0.12	0.06	0.07	0.01	0.00											
77	0.05	0.04	0.09	0.03	0.05	0.03	0.03	0.00										
78	0.04	0.03	0.11	0.04	0.05	0.02	0.02	0.03	0.00									
79	0.06	0.04	0.10	0.04	0.06	0.06	0.07	0.03	0.04	0.00								
80	0.05	0.04	0.10	0.04	0.05	0.05	0.06	0.03	0.04	0.01	0.00							
81	0.06	0.05	0.11	0.05	0.07	0.06	0.06	0.03	0.04	0.02	0.01	0.00						
82	0.05	0.04	0.10	0.03	0.05	0.05	0.06	0.03	0.04	0.01	0.00	0.01	0.00					
83	0.09	0.08	0.13	0.08	0.08	0.10	0.11	0.06	0.08	0.06	0.05	0.06	0.05	0.00				
84	0.06	0.05	0.11	0.06	0.08	0.07	0.07	0.04	0.05	0.03	0.03	0.03	0.03	0.05	0.00			
85	0.07	0.07	0.11	0.07	0.08	0.09	0.09	0.06	0.06	0.05	0.05	0.04	0.04	0.07	0.05	0.00		
86	0.07	0.06	0.11	0.07	0.08	0.08	0.09	0.06	0.06	0.06	0.05	0.04	0.04	0.06	0.03	0.05	0.00	
87	0.07	0.07	0.10	0.07	0.09	0.07	0.08	0.05	0.05	0.05	0.04	0.04	0.04	0.07	0.04	0.05	0.03	0.00