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Genetic Stock Identification of Kuskokwim Area Chinook Salmon
Final Report for Study 05-305

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Final Report Summary Page

Title: Genetic stock identification of Kuskokwim Area Chinook salmon

Study Number: 05-305

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Geographic Area: Kuskokwim River

Federal Conservation System Unit: Yukon Delta National Wildlife Refuge, Togiak National Wildlife Refuge

Information Type: Stock Status and Trends

Issue(s) Addressed: In 2000, Kuskokwim River Chinook salmon were identified as a “stock of concern” due to the chronic inability to maintain expected harvest above escapement needs despite the use of specific management and conservation measures. However the efficacy of such measures is uncertain without knowing the harvest of individual Kuskokwim River populations in subsistence and commercial fisheries in the entire Kuskokwim Management Area (KMA). Previous investigation of the genetic population structure of Chinook salmon within the KMA using three different types of genetic markers found patterns of population divergence and identified four groups of populations: 1) Goodnews/Kanektok, 2) Lower Kuskokwim (from the Eek River upstream to the Hoholitna River), 3) Middle Kuskokwim (the Stony, Cheeneetnuk and Tatlawiksuk rivers), and 4) Upper Kuskokwim (the Takotna River and Pitka Fork). Genetic stock identification methods can provide information concerning the origins of fish harvested in the KMA; critically important information for an effective, sustainable management program. The existing baseline of genetic markers provides adequate coverage of the populations within the Kuskokwim River, but only moderate coverage of the populations outside of the river. We propose to refine our analyses by increasing sample sizes and geographic coverage of the genetic baseline and to begin mixed stock identification applications in KMA fisheries.

Study Cost: \$197,700

Study Duration: April 2005 – December 2007

Abstract: The subsistence fishery for Chinook salmon in the Kuskokwim Management Area is one of the largest and most significant in Alaska. Low returns in recent years have yielded shortfalls in escapements basin-wide and resulted in fishing restrictions, which directly affect local communities. Sustained productivity of salmon relies on maintenance of genetic diversity through informed management of the resource. We investigated the genetic diversity of Chinook salmon from the Kuskokwim River using two types of genetic markers, microsatellites and single nucleotide polymorphisms, to understand the population structure. Analysis of genetic data found evidence of significant structure among these Chinook salmon populations. However, much of the diversity was associated with two populations in the upper drainage.

Populations were assigned to three groups based on genetic characteristics and geographic proximity, two within the Kuskokwim River (Upper and Lower/ Middle) and the third in Kuskokwim Bay. Simulations using these groups indicate that the Lower Kuskokwim and Kuskokwim Bay groups are not sufficiently identifiable for mixed stock analysis using currently available genetic data. These genetic data provide comprehensive representation of Kuskokwim Chinook salmon in rangewide baselines that can be used in studies of Chinook salmon on the high seas.

Key Words: Chinook salmon, genetic diversity, Kuskokwim River, single nucleotide polymorphisms, microsatellites, *Oncorhynchus tshawytscha*, subsistence fishery

Project Data: Description - Data for this study consist of biological samples (tissue and DNA samples) and allele frequencies for SNP and microsatellite markers. Format - Biological samples are stored at -80 °C or in ethanol. Genetic data are stored in an *Oracle* database, LOKI, maintained by the Alaska Department of Fish and Game. Custodian - Gene Conservation Laboratory, Division of Commercial Fisheries, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518. Availability - Access to biological samples and data is available upon request to the custodians.

Report Availability: Please contact either the author(s) or Alaska Resources Library and Information Services to obtain a copy of this report.

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INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) manages the fisheries in the Kuskokwim River together with nearby coastal areas, including the Goodnews and Kanektok rivers, as the Kuskokwim Management Area (KMA; Figure 1). All six species of Pacific salmon native to North America are found in the Kuskokwim River drainage: Chinook salmon (*Oncorhynchus tshawytscha*), sockeye salmon (*O. nerka*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), pink salmon (*O. gorbuscha*), and rainbow trout (*O. mykiss*). Of these, Chinook salmon are the most intensely harvested species in the subsistence fishery followed by chum and sockeye salmon, while coho salmon are the most economically valuable species in the commercial fishery.

The Alaska State Legislature and the Alaska Board of Fisheries have designated subsistence fishing as the highest priority use of salmon in Alaska (A.S. 16.05.258). Nearly half of the annual subsistence harvest of Chinook salmon in Alaska is taken from the KMA (Fall et al. 2009). Studies by the ADF&G Division of Subsistence indicate that salmon contribute as much as 53 percent of the total pounds of fish and wildlife harvested annually in a Kuskokwim area community (Coffing 1991) and as much as 650 pounds per capita in some communities (CSIS 2007). The 38 communities in the KMA consist of 4,500 households, and approximately 1,500 of these households annually harvest salmon for subsistence use, while other households not directly involved in harvesting salmon participate by assisting with cutting, drying, smoking, and associated preservation activities. The average annual subsistence salmon harvest for 1997 through 2006 included 76,371 Chinook; 53,551 chum; 40,704 sockeye; and 33,524 coho salmon (Fall et al. 2009).

Management of KMA salmon fisheries is difficult due to harvest of mixtures of stocks and species that are several weeks and hundreds of kilometers from their spawning grounds. Limited budgets combined with the immense size and complexity of the drainage have constrained the knowledge available concerning distributions, escapement abundance, run timing, and production parameters of salmon populations. While the goal of the KMA management plan is to manage salmon runs for sustained yield, insufficient information exists to determine the escapement levels needed to produce a sustained yield. Periodic aerial surveys of the spawning grounds provide an index of escapement for Chinook salmon, but cannot provide total escapement counts. These surveys are also skewed to the lower river and coastal streams because streams in the middle and upper Kuskokwim are often cloudy with silt or stained with tannins making visibility difficult. Ground based surveys, such as weirs, provide good information on escapements to a few discreet tributaries, but vast areas remain unobserved. More recently, tracking studies of Chinook salmon using radio telemetry provided information on run timing and spawning locations farther upstream (Stuby 2007), and are aiding in completion of a total run reconstruction for Kuskokwim River Chinook salmon from 1976 – 2009 (Molyneaux et al. *in prep*).

Declines in Kuskokwim River Chinook salmon abundance were noted in the mid-1980's and led to a number of management actions restricting commercial harvest in order to better provide for subsistence needs (Francisco et al. 1988). Another series of low abundance years occurred in the late 1990's and 2000, prompting the Alaska Board of Fisheries (BOF) to classify Kuskokwim

River Chinook salmon as a “stock of concern” (Burkey et al. 2000). Low abundance led to numerous regional disaster declarations and harvest restrictions, including reduction in time allowed for subsistence harvest (Burkey et al. 2000; Estensen et al. 2009). Abundance improved from 2001-2006, prompting the BOF to rescind the stock-of-concern finding in 2007. However, since 2006 Chinook salmon run abundance has again diminished for unknown reasons (Estensen et al. 2009).

Sustained productivity of salmon has been shown to be possible only if genetic diversity and population structure are maintained (NRC 1996; Hilborn et al. 2003). This can best be accomplished through informed management of the resource. Identification of the genetic population structure of Chinook salmon within the Kuskokwim River and the development of methods for identifying groups of salmon populations within mixtures will provide vitally important information for management. A previous project funded under the U.S. Fish and Wildlife Service (USFWS), Office of Subsistence Management (Project No. 01-070; Templin et al. 2004) investigated the genetic diversity of Chinook salmon in the KMA and found evidence of population structure, with populations spawning upriver of the Holitna River drainage particularly genetically divergent. Recommendations upon completion of that project were that the performance of the genetic stock identification model could be improved with further development of the baseline, including the addition of unrepresented populations, increased sample sizes for populations already represented, and the addition of more DNA markers to improve model performance and increased resolution.

This project builds upon previous work to investigate the genetic diversity of Chinook salmon from the KMA. The existing baseline was improved by increasing sample sizes and geographic coverage of the genetic baseline, increasing the genetic markers surveyed to include Pacific Rim standardized DNA-based microsatellite loci, and increasing the genetic markers surveyed to include newly developed SNP loci. Additionally, we investigated the utility of this baseline in mixed stock analysis applications in KMA fisheries.

OBJECTIVES

This project was designed to expand the genetic baseline for Chinook salmon in the KMA and investigate its potential for application of genetic stock identification. Specific objectives of this project were:

- 1) To expand the baseline collections of Chinook salmon in the KMA by:
 - a) Increasing the collection sizes of Chinook salmon from selected populations in the existing baseline to greater than 150, with a target of 200;
 - b) Collecting samples from additional populations that have recently been identified as important but are not in the existing baseline (Table 1);
 - c) Collecting samples from Chinook salmon captured as part of tagging projects within the Kuskokwim River.
- 2) To expand the set of genetic markers in the baseline of Chinook salmon in the Kuskokwim River drainage to include a larger set of DNA-based markers by:

- a) Surveying potential microsatellite markers developed for the Yukon River and the Pacific Salmon Commission Pacific Rim baseline;
 - b) Analyzing 15 of the most informative microsatellites from this survey in the entire baseline;
 - c) Analyzing the 9 SNP markers previously surveyed and 10 SNP markers currently in development in all baseline populations.
- 3) To analyze the genetic population structure and refine mixed stock analysis of Chinook salmon in the KMA using the additional data by:
 - a) Calculating standard measures of intra- and inter-population genetic diversity such as Hardy-Weinberg equilibrium and F_{ST} ;
 - b) Investigate relationships through visual analyses (such as multidimensional scaling) and hierarchical statistical analyses;
 - c) Identification of reporting regions within the KMA through simulation studies.
 - 4) To conduct a pilot study of the application of genetic stock identification techniques to salmon harvested in mixed stock fisheries in the KMA by collecting tissue samples, assaying genotypes, and determining the relative contribution of reporting groups to:
 - a) The Bethel test fishery in 2005 and 2006;
 - b) Both the subsistence fishery in the Bethel area and the W-4 commercial fishery in 2006.

METHODS

Sample collection

Personnel from ADF&G, the USFWS, Kuskokwim Native Association, Takotna Tribal Council, and Native Village of Kwinhagak collected tissues from Chinook salmon at weir sites located near or on the spawning grounds or from sampling efforts on the spawning grounds (Table 1, Figure 1). Chinook salmon were sampled for either fin clips or axillary process tissues to assay variation at microsatellite and SNP loci. Tissues were clipped from individual salmon, placed in an individually labeled plastic tube, and preserved in denatured ethanol. Samples were transported back to the ADF&G Gene Conservation Laboratory in Anchorage for archiving and analysis. Target sample sizes were between 50 and 200 adults per collection depending on the previous sample size for the population sampled. Target sample sizes were set at 200 individuals for each baseline population.

Laboratory analysis

To insure the quality and consistency of the data, each individual was assigned a unique accession identifier. When DNA was extracted or analyzed from each individual, a sample sheet was created that linked each individual's code to a specific well in a uniquely numbered 96-well plate. This sample sheet accompanied the individual through all phases of a project, minimizing the risk of misidentification of samples. Genotypes were assigned to individuals using a double-scoring system where two researchers designated allele scores for each individual.

Approximately 8% of the individuals, eight samples from each 96-well DNA extraction plate, were reanalyzed for all markers. This insured that the data are reproducible and any errors created from the processing of individual plates were corrected. The data are permanently stored

in an *Oracle* database, *LOKI*, administered by ADF&G.

Microsatellites

Genomic DNA was extracted using a DNeasy® 96 Tissue Kit¹ by QIAGEN®, (Valencia, CA). Polymerase chain reaction (PCR) was carried out in 384-well reaction plates in 10µl reaction volumes (10mM Tris-HCl, 50mM KCl, 0.2 mM each dNTP, 0.50 units *Taq* DNA polymerase (Promega) using Dual 384-Well GeneAmp Thermal Cylers (Applied Biosystems). Polymerase chain reaction (PCR) conditions for each locus are available upon request. PCR fragment analysis was done on an Applied Biosystems 3730 capillary DNA sequencer. 0.5µl PCR product was loaded into a 96-well reaction plate along with 0.5µl of GS500LIZ internal lane size standard and 9.0µl of Hi-Di (Applied Biosystems). PCR bands were visualized and separated into bin sets using AB GeneMapper software v3.7.

Genetic data were collected as individual multi-locus genotypes for the 13 microsatellite loci currently included in the Chinook Technical Committee (CTC) baseline standardized among 11 laboratories (Table 2; Seeb et al. 2007). Alleles used in this baseline were originally defined based on holotype specimens and tested in participating laboratories. Allele sizes produced in the laboratory analysis were converted to standardized allele names prior to statistical analysis. This set of loci does not include loci surveyed previously by Templin et al. (2004) in the Kuskokwim River. Genotype data were stored as GeneMapper files on a network drive that was backed up nightly.

SNPs

Forty-four Chinook salmon SNP loci were used in this study; 1 mitochondrial and 43 nuclear DNA (Table 3). SNP genotyping was performed in 384-well reaction plates generally following the methods described in Seeb et al. (2009a). Each reaction was conducted in a 5-µL volume consisting of 0.10-µL template DNA in 0.7x TaqMan Universal Buffer (Applied Biosystems), 900 nM of each polymerase chain reaction (PCR) primer, and 200 nM of each probe. Thermal cycling was performed on a Dual 384-Well GeneAmp PCR System 9700 as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of: 92° for 15 s and annealing/extension temperature for 1 or 1.5 min. Cycling was conducted at a ramp speed of 1°C per second. The plates were read on an Applied Biosystems (AB) Prism 7900HT Sequence Detection System after amplification and scored using AB Sequence Detection software 2.2.

Data analysis

Population structure

The data were checked for duplicated multi-locus genotypes for indication of errors caused prior to extraction of the DNA. When duplicate genotypes were found, the genotype was attributed to the first individual and subsequent individuals with the same genotype were removed from the analysis to insure that any given individual did not appear more than once in the baseline. Individual genotype data were summarized as allele frequencies for all microsatellite and SNP

¹ Product names used in this report are included for scientific completeness but do not constitute product endorsement.

markers. Collections taken at the same location in different years were pooled following the recommendations of Waples (1990). Estimates of the population frequency of individual alleles for each marker were calculated from the observed frequency of the allele in the representative sample(s).

An estimate of allelic richness (El Mousadik and Petit 1996) was calculated for each population (*FSTAT* version 2.1, Goudet 1995) to account for variation in the detection of alleles at each marker caused by differences in sample size. Estimates of observed and expected heterozygosity were calculated for each marker across all populations using *GENEPOP* (version 4.011; updated version of Raymond and Rousset 1995) and for each population across all markers of a given type (*GDA*; Lewis and Zaykin 2001). Conformation of genotype frequencies to Hardy-Weinberg equilibrium (H-W) expected ratios was assessed using the exact test in *GENEPOP* and significance was determined using the sequential Bonferroni method (Rice 1989) to account for multiple tests within a marker across the 16 populations ($\alpha = 0.05/16 = 0.0031$). All pairs of SNP markers were tested for linkage disequilibrium using *GENEPOP* and the sequential Bonferroni method was used to correct for multiple tests.

Chord distances (Cavalli-Sforza and Edwards 1967) were calculated in *PHYLIP* (version 3.6, Felsenstein 2002) as a measure of population similarity and F_{ST} (Weir and Cockerham 1984) was calculated between population pairs using *GENEPOP*. Patterns of structure were visualized with multidimensional scaling (MDS) to view the inter-population chord distances in three-dimensional space. Distances between populations in this type of plot are analogous to genetic distance among populations, so patterns observed using this method reflect the genetic distinctions among the populations.

In addition to the pairwise comparison methods for investigating population relationships, a second method was used to explore the spatial clustering of populations using the Bayesian analysis of population structure developed by Corander et al. (2008) and implemented in the software *BAPS* (<http://www.rni.helsinki.fi/~mjs>). This method seeks to find the optimum partitioning of the dataset into component population clusters. Latitude and longitude for each population was included as prior information. Populations were maintained as the unit of analysis for the spatial clustering, and the entire analysis was repeated 20 times to confirm the results.

Populations were grouped hierarchically into regions based on genetic diversity, geographic features, and management needs. The initial structure of the hierarchy was based on the results of Templin et al. (2004): 1) Kuskokwim Bay (Goodnews and Kanektok rivers), 2) Lower Kuskokwim (Eek, Tuluksak, Kisaralik, Kwethluk, Aniak, George, and Kogrukluuk rivers), 3) Middle Kuskokwim (Stony, Cheeneetnuk, and Tatlawiksuk rivers), and 4) Upper Kuskokwim (Takotna river and Pitka Fork). Changes were then made to this hierarchical structure based on the results provided by the addition of populations to the baseline, larger sample sizes for each population, and the inclusion of more loci.

Patterns of genetic variation explained by the proposed hierarchy were examined by comparing allelic richness and heterozygosity using *FSTAT*. In addition, the hierarchical log likelihood ratio test (modified from Weir 1990) was used to investigate the decomposition of the overall

genetic variation into component parts at each level. However, the false positive rate provided by the log likelihood ratio statistic has been shown to increase with the number of loci (Ryman et al. 2006). For this reason, the hierarchical ANOVA implemented in *GDA* was also used to test the significance of genetic variation explained by the hierarchy.

Genetic stock identification

To evaluate the application of genetic stock identification to Chinook salmon harvests in KMA fisheries, simulations were used to assess the ability of microsatellites, SNPs, and combined marker sets to identify groups of populations (reporting regions) in hypothetical mixtures. Reporting regions were identical to the hierarchical structure defined in the population structure analysis. Because management decisions are sometimes made separately for fisheries within the Kuskokwim River, an additional set of simulations was performed that excluded Kuskokwim Bay populations to investigate the identifiability of reporting regions in mixtures that might be sampled within the Kuskokwim River.

Evaluation of SNP and microsatellite baselines for use in genetic stock identification began with simulation of mixtures of fish ($N = 400$) that originate entirely from a single region (100% simulations). This was done to estimate the ability of a marker set to correctly allocate fish to this region in mixed stock analysis. Simulations were performed using the software package *ONCOR* (Kalinowski 2008), because this package implements the unbiased simulation methods described in Anderson et al. (2008) which are especially useful when using highly polymorphic markers like microsatellites. Average estimates of mixture proportions and 90% confidence intervals were derived from 1000 simulations. Regions with mean correct estimates of 90% or better were considered identifiable in fishery applications. Each set of simulations was repeated three times with a different subset of the baseline each time: 1) microsatellites only, 2) SNPs only, and 3) microsatellites and SNPs combined.

Genetic stock identification of mixed stock fisheries in the KMA was used to estimate the composition of Chinook salmon in samples from four fisheries: the 2005 and 2006 Bethel test fisheries, the 2006 District 4 commercial fishery, and the 2006 subsistence fishery. Stock composition estimates and their 90% credibility intervals were generated using a Bayesian method for genetic stock identification (*BAYES*, Pella and Masuda 2001). Three chains were run beginning with different starting conditions and each chain was run without thinning with a Markov Chain Monte Carlo sample size of 10,000. Inference was based on the posterior distribution formed from the combined set of the last 5,000 steps of each chain. The mean of the posterior distribution was reported as the best estimate, and the central 90% of the distribution was reported as the credibility interval. The Dirichlet prior distribution parameters for all population proportions were equal (1/16).

RESULTS

Sample collection

The baseline tissue collections analyzed in this study included 2,774 individual Chinook salmon from 30 collections representing 16 populations (Table 1, Figure 1). While approximately 1,500 individual samples were added, the 1993 and 1994 Takotna River samples were replaced in the baseline by the 2005 sample due to uncertainty about where they were collected. This led to a realized increase of 1,383 individuals over the previous version of the baseline. Of the 16 populations in the baseline, 10 were sampled in more than one year. Twenty-three of the collections were taken from tributaries to the Kuskokwim River, ranging from the Eek River near the river's mouth northeast to Pitka Fork in the upper Kuskokwim River drainage. In addition, seven collections from three populations were taken from three drainages that are separate from the Kuskokwim River, but which also flow into Kuskokwim Bay and are part of the KMA: Goodnews, Arolik, and Kanektok river drainages.

Laboratory analysis

Microsatellites

A total of 2,774 samples were assayed for genotypes for the 13 microsatellite markers in the CTC standardized baseline (Table 2). The average genotyping failure rate across all samples was 2.2%. Within collections the highest failure rate (6%) was from the 1992 Kanektok River collection. Within loci the highest failure rate (< 2%) was from *Ots208*. Four alleles in the dataset could not be standardized to the CTC baseline: *Omm1080*372* (two in the Arolik samples and one in the Gagarayah samples), *Omm1080*394* (one in the Takotna samples), and *Ots3M*134* (one in the Kanektok samples). Single-locus genotypes containing these alleles were removed from the analysis.

The greatest number of alleles (52) was found at *Omm1080*, and the highest F_{ST} (0.012) was found at three loci: *Ogo4*, *Oki100*, and *Ots3M* (Table 2). The highest heterozygosity was measured at *Ots208* (0.950). Over all markers and populations, 208 H-W tests were performed of which nine were significant after adjusting for multiple tests. These significant tests were spread across eight loci and four populations.

SNPs

A total of 2,774 samples were assayed for genotypes for 44 SNP markers (Table 3). The average genotyping failure rate across all samples was < 1%. Within collections the highest failure rate (6%) was from the 1994 Tuluksak River collection. Within markers the highest failure rate (7%) was from *Ots_C3N3*, a SNP in the mitochondrial DNA. Four markers (*Ots_arf-188*, *Ots_GST-375*, *Ots_HGFA-446*, and *Ots_PSMB1-197*) were monomorphic in this set of populations and were dropped from the remaining analyses.

The highest F_{ST} (0.036) was found at *Ots_MHC2*, and the highest heterozygosity was measured at *Ots_MHC1* (0.504; Table 3). Over all markers and populations, 624 H-W tests were performed of which one was significant after adjusting for multiple tests. No H-W tests were performed on *Ots_C3N3* since it is a haploid locus. No SNPs were in disequilibrium.

Data analysis

Population structure

Microsatellites: The lowest number of alleles observed in a population (Pitka Fork, 176) was only 68% of the highest number counted in a population (Goodnews River, 260; Table 4). The second lowest was another upstream population, Takotna River (206), and the second highest number of alleles was Kanektok River (256), another Kuskokwim Bay population. This pattern may be due to the effect of sample size on detection of alleles in hypervariable loci; the Pitka Fork ($N = 100$) and Takotna River ($N = 80$) were two of the smallest collections and Goodnews River ($N = 370$) and Kanektok River ($N = 245$) were two of the largest in the analysis. When allelic richness, a measure of the number of alleles that is independent of sample size, was estimated, the allele numbers were more similar among populations (range: 170-219). However, the Pitka Fork population dropped to 170 and the Tatlawiksuk population dropped to 187, both significantly below the levels found in other populations ($p = 0.012$). The heterozygosities of these populations were also significantly lower than the other populations ($p=0.012$).

The MDS based on Cavalli-Sforza and Edwards (1967) genetic distances indicated that the Pitka Fork and Tatlawiksuk rivers were distinct from the other populations in the dataset, and distinct from each other as well (Figure 2). This pattern was reflected in the pairwise F_{ST} values (Table 5) and the *BAPS* analysis (Figure 3) using geographic information. The *BAPS* analysis supported three clusters, Pitka Fork, Tatlawiksuk River, and all other populations, as the best partitioning of populations based on the microsatellite data. The Takotna and Stony rivers were also visibly separated from the main cluster of populations in the MDS, but were not separated from the group by the *BAPS* analysis.

SNPs: Four SNP loci were monomorphic within the KMA (Table 3). The number of alleles observed in each population ranged from 79 to 84 with the fewest alleles found in the Pitka Fork and Takotna River populations (Table 4). While the Pitka Fork and Takotna rivers were two of the smallest collections in the baseline ($N = 100$ and $N = 80$, respectively), only five more alleles were observed in the largest collections. When allelic richness was estimated there was very little change in the number of alleles observed.

The MDS based on genetic distances (Cavalli-Sforza and Edwards 1967) indicated that the Pitka Fork and Tatlawiksuk rivers were distinct from the other populations in the dataset (Figure 4). This pattern was reflected in the F_{ST} values (Table 6). The largest F_{ST} estimates were associated with the Pitka Fork population followed by the Tatlawiksuk River and Goodnews River populations. The Goodnews and Takotna rivers were also visibly separated from the main cluster of populations; however the *BAPS* analysis supported only three clusters, Pitka Fork, Tatlawiksuk River, and all other populations, as the best partitioning of populations based on the SNP data.

Both microsatellite and SNP data provided similar evidence of genetic population structure in Chinook salmon populations from the KMA. Populations located upstream at the northeast edge of the KMA (Pitka Fork and Tatlawiksuk River) were distinct from each other as well as from the main, central group of populations. The MDS plots (Figures 2 and 4) indicate that the Takotna River might also be separated from this group; however the *BAPS* analysis did not distinguish this population from the main group.

Based on the updated population structure and management needs, populations were grouped into three regions, including combining the Lower and Middle Kuskokwim groups used in the previous analysis (Templin et al. 2004): 1) Kuskokwim Bay (Goodnews, Arolik, and Kanektok rivers), 2) Lower/Middle Kuskokwim (Eek, Tuluksak, Kisaralik, Kwethluk, Aniak, George, Kogrukluuk, Stony, Cheeneetnuk, Gagarayah, and Takotna rivers), and 3) Upper Kuskokwim (Tatlawiksuk River and Pitka Fork). The Upper Kuskokwim region was defined based on the genetic distinctiveness of the populations from the main group, so the Takotna River population was included with the Lower/Middle Kuskokwim populations, with which it was more genetically similar.

Hierarchical gene diversity analyses based on grouping populations into these three regions showed significant genetic variation ($P < 0.001$) among these regions, as well as within regions using both microsatellites and SNPs (Table 7). The results of the log likelihood ratio tests were supported by the results of the ANOVAs, in which the among-regions and among-populations variation in both marker sets were significantly different from 0.0 (Table 8).

Genetic stock identification

The three reporting regions used for hierarchical analyses were used for simulations to examine the identifiability of the defined reporting regions in genetic stock identification (Tables 9 and 10). Two sets of 100% simulations were performed in ONCOR to investigate the precision and accuracy possible when using three regions in the entire KMA (Table 9) and two regions within the Kuskokwim River (Table 10). Mixtures in these simulations were composed entirely of individuals from a single region. Mean correct allocation to region for the Kuskokwim Bay region in these simulations was approximately 70% for each marker set with the remaining portion misallocating to the Lower/Middle Kuskokwim region. The Lower/Middle Kuskokwim region had mean correct allocations of approximately 88% for all marker sets for the entire KMA and 98-100% within the Kuskokwim River. The Upper Kuskokwim region had mean correct allocations $\geq 94\%$ across all sets of simulations, with the best performance using the combined marker set.

As an example application, stock composition estimates were made for several 2005 and 2006 KMA Chinook salmon fisheries. Sample sizes varied between 242 and 479 fish for each estimate (Tables 11 and 12). Stock composition estimates for the in-river 2005 and 2006 Bethel test fisheries and the 2006 subsistence fishery were made using only the two reporting groups within the Kuskokwim River: 1) Lower/Middle Kuskokwim and 2) Upper Kuskokwim (Table 11). In both Bethel test fishery samples, as well as the subsistence fishery sample, the Lower/Middle Kuskokwim reporting group contributed 100% to the mixture samples. Estimates for the 2006 District 4 Kuskokwim Bay commercial fishery were also made using two reporting groups: 1) Kuskokwim Bay and Lower/Middle Kuskokwim, and 2) Upper Kuskokwim (Table 12). Estimates for District 4 samples were broken into two time periods: June 15-22, and June 30-July 7. In the District 4 samples during the first period, the largest contributor to the mixture samples was the Kuskokwim Bay and Lower/Middle Kuskokwim reporting group (99%) and the Upper Kuskokwim reporting group contributed 1%. In the second period, the Kuskokwim Bay and Lower/Middle Kuskokwim reporting group made up 100% of the mixture.

DISCUSSION

This study continued investigations of the genetic diversity of Chinook salmon from the KMA by increasing sample sizes and geographic coverage of the genetic baseline, increasing the genetic markers surveyed to include Pacific Rim standardized DNA-based microsatellite loci, increasing the genetic markers surveyed to include newly developed SNP loci, and investigating mixed stock identification applications in KMA fisheries. Improved representation of the genetic population structure of Chinook salmon within the KMA and the development of methods for identifying salmon stocks within mixtures will provide important information for management of commercial and subsistence fisheries.

Population structure

In order to improve upon past investigations of genetic diversity of KMA Chinook salmon, this study increased sample sizes and geographic coverage of the genetic baseline. Two new populations, the Arolik and Gagarayah rivers, were added. Additional samples were collected in 2005 and 2006 for the Goodnews, Kanektok, Eek, Tuluksak, Kisaralik, Salmon, George, Kogrukukluk, Tatlawiksuk, and Cheeneetnuk rivers increasing the number of samples representing each of these populations by an average of 120. The Goodnews River population was originally represented by only 40 individuals collected at the weir on the Middle Fork of the Goodnews River, but is now represented by 370 from both the North and Middle forks of the river. In addition, in 2005 samples were taken closer to spawning grounds for the Takotna River population and were used to replace the 1994 collection. The newer samples were expected to be a better representation of the population in this drainage. The improved representation of the populations in the Goodnews and Takotna rivers led to more accurate understanding of the genetic similarity of these populations to the populations of the lower Kuskokwim River. In the Templin et al. (2004) analysis both of these populations were considered to be genetically distinct from the main group. Based on what is known about Chinook spawning areas in the Kuskokwim Area (e.g. Stuby 2007), the baseline represents broad geographic coverage of most major spawning areas. Notable populations still missing from the baseline include the Oskawalik River, the Hoholitna River within the Holitna River basin, and the East Fork, Middle Fork, and Little Tonzona River all within the upper Kuskokwim River basin. Collections have been made from the Necons River but were not included in this analysis.

The DNA markers chosen for this study replaced the markers surveyed in the previous study (Templin et al. 2004) which applied 47 allozyme markers and 10 microsatellite markers. The 13 microsatellite markers included in this study are the standard set included in the CTC baseline for Pacific Salmon Commission studies (Seeb et al. 2007). While most of these markers were chosen for utility in the southern part of the range of Chinook salmon, they have been successfully applied in studies conducted in the Yukon (Beacham et al. 2006), Copper (Seeb et al. 2009b), and Kenai rivers (Begich et al. 2010). The microsatellite data developed as part of this study were included in a landscape analysis of Chinook, chum, and coho salmon in western Alaska (Olsen et al. 2010). The 44 SNP markers used in this study were the same used by

ADF&G elsewhere in Alaska, allowing the inclusion of the KMA populations in a rangewide analysis of population structure in Chinook salmon (Templin et al. 2011).

The relative effect of sample size on the two separate marker types is evident from counts of the alleles observed in each collection across marker types. Microsatellites are highly polymorphic and have many potential alleles for each locus, from 6 to 52 alleles in the loci in this dataset (Table 2). For loci with three or more alleles under the worst combination of multinomial parameters a sample size of 200 individuals is needed to estimate baseline allele frequencies such that the estimates are within 5% of the true values 90% of the time (Thompson 1987). One consequence of this issue can be seen in the number of observed alleles summed across the 13 microsatellite loci within each population; more alleles are detected in populations with larger sample sizes (Table 4). When the number of alleles is adjusted for sample size (allelic richness), the number of alleles at each population is much more similar. SNP loci only have two potential alleles at each locus. Very little change is seen in the number of alleles per population in the SNP dataset.

Patterns in genetic diversity detected using new markers within Chinook salmon populations in the KMA were generally similar to past analyses (Templin et al. 2004). Populations in the northeast edge of the KMA displayed lower within-population, but greater between-population genetic diversity than populations in the rest of the KMA (Table 4). The Pitka Fork and Tatlawiksuk River populations were the most divergent populations when measured with both microsatellite and SNP marker datasets (Figures 2 and 4). These two populations had the lowest measures of allelic richness and the lowest heterozygosity for both marker types, and the largest F_{ST} values. Two Kuskokwim Bay populations, Goodnews and Kanektok rivers, had the greatest allelic richness for both microsatellites and SNPs, indicating greater genetic diversity within those populations. The Takotna and Goodnews rivers were somewhat separated from the main group in the SNP marker data, and the Takotna, Stony, and Cheeneetnuk rivers were somewhat separated from the main group for microsatellite marker data. However, while geographically located between the Tatlawiksuk and Pitka Fork rivers, the Takotna River population was more similar to the main cluster of Kuskokwim River populations than in previous analyses. As in the previous analysis, the remaining populations, from the Arolik River upstream to the Gagarayah River, are similar to one another based on the markers used in the present baseline. This may represent a limitation of the markers used in genetic analyses of Chinook salmon in this region, but likely also represents a lack of barriers to present and or historical gene flow between populations in most of the KMA. The similarity of allele frequencies may be the reason for the lower identifiability of Kuskokwim Bay and Lower/Middle Kuskokwim reporting groups in simulation analyses, where most of the misallocation was between these groups.

Improved samples sizes and the increased number of markers led to some changes to the population structure inferred from genetic information. In the original study, Templin et al. (2004) found that both allozyme and microsatellite markers indicated that the Goodnews River ($N = 40$) was one of the most distinctive populations in the KMA. However, both the SNP and microsatellite markers assayed in larger sample used in this study indicate that this population is much more similar to the Kuskokwim Bay and Lower Kuskokwim populations. On the other hand, the Tatlawiksuk River population was found to be much more divergent in this dataset than in the previous analysis, and improved samples indicate that the Takotna River is less divergent. These results demonstrate the importance of analyzing adequate baseline, both in numbers of fish

per population and distribution of populations over space and time, or risk finding anomalous results that overestimate potential for genetic stock identification.

Genetic stock identification

In simulation studies with populations from the entire KMA, three groups (Bay, Lower/Middle, and Upper) were initially used in hypothetical mixtures using microsatellite markers, SNP markers, and the combined set of markers. The greatest accuracy and precision resulted when markers were combined, yet the Kuskokwim Bay group performed at only 71% correct allocation, the Lower/Middle Kuskokwim group performed at 88% correct allocation, and the Upper Kuskokwim group performed at 97% correct allocation (Table 9). Allele frequencies between Kuskokwim Bay and lower Kuskokwim River populations are similar (Appendix A), especially when compared to upper Kuskokwim populations. This is corroborated by the BAPS analysis, in which these populations could not be partitioned. These simulation results indicated that genetic stock identification cannot currently be used to distinguish Kuskokwim River from Kuskokwim Bay populations in mixed stock applications.

An interesting finding from this analysis is the non-symmetric misallocation between the Kuskokwim Bay and the Lower/Middle Kuskokwim regions. In 100% regional simulations, the misallocation from Kuskokwim Bay to Lower/Middle Kuskokwim was approximately 30%, while misallocation from Lower/Middle Kuskokwim to Kuskokwim Bay was 12% (Table 9). This may be due in part to the fact that Lower/Middle Kuskokwim region has almost four times the number of populations as the Kuskokwim Bay region. Small misallocations from the Kuskokwim Bay 100% simulations accumulated across 11 populations in the Lower/Middle Kuskokwim region (Figure 5). However, in the Lower/Middle Kuskokwim 100% simulations, misallocation to the Kuskokwim Bay region could only accumulate over three populations.

The results of simulations performed in this study do support the identification of the Upper Kuskokwim Region composed of Tatlawiksuk and Pitka Fork populations in genetic stock identification applications. This region was consistently identifiable in mixtures from both Kuskokwim Bay and within the Kuskokwim River. However, this region is composed of two highly divergent populations, neither of which is expected to be a large contributor to lower river fisheries. During the five years (2002-2006) of radio tagging Chinook salmon in the Kuskokwim River, only 3-7% of tagged salmon were traced to spawning redds upstream of McGrath which includes Pitka Fork (Stuby 2007). During the same years, the largest proportion of tags traced to the Tatlawiksuk River was 4%. Given the relatively large amount of diversity in this portion of the drainage, further efforts should be made to obtain representative samples from other spawning locations in upper Kuskokwim River identified by radiotagging studies.

Reporting groups constructed based only on management needs and geographic location may not adequately represent the actual population structure in the KMA. In this analysis, genetic differences were more heavily weighted than geographic location in making the determination that the Takotna River population should be moved from the Upper Kuskokwim group to the Lower/Middle Kuskokwim group. At the same time, the Tatlawiksuk River population was moved out of the Lower/Middle Kuskokwim reporting group and placed in the Upper Kuskokwim group. Tests of population structure found significant differences between these

groups, despite similarities in allele frequencies between many populations. Significant genetic variation using both microsatellite and SNP markers was found among regions as well as within regions even when measured independently of other regions (Tables 6 and 8). In the previous study (Templin et al. 2004), it was suggested that larger sample sizes or additional markers might improve measures of distinctiveness and increase the ability to identify populations in mixtures in Kuskokwim Bay and the Kuskokwim River. However, despite the additions of individuals to existing collections, new populations to the analysis, and new markers, the emerging picture is that KMA population structure is shallower than previously thought. It may only be possible to improve resolution by surveying markers developed specifically for use in the KMA.

The ability to differentiate groups of Chinook salmon populations harvested in KMA fisheries would provide a valuable tool for the management of those fisheries. Commercial fisheries take place in District 1 of the lower Kuskokwim River, and in Districts 4 and 5 of Kuskokwim Bay. The directed Chinook salmon commercial fishery in District 4 of Kuskokwim Bay has an average annual harvest of 18,993 fish (ADF&G 2008). In District 5 Chinook are harvested incidental to sockeye salmon, with an average annual catch of 2,452 fish (ADF&G 2008). In the hope that a buffer zone would reduce the interception of Kuskokwim River Chinook salmon, the northern boundary of District 4 was moved south several miles in order to distance commercial fishermen from the Kuskokwim River (Figure 1). The necessity and effectiveness of this conservation measure, however, is in question because the degree to which Kuskokwim River Chinook salmon are caught in the District 4 fishery is unknown. Further development of the baseline using additional markers currently being developed for use in western Alaska Chinook salmon populations may lead to improved results from genetic stock identification applications for these fisheries (Alaska Sustainable Salmon Fund Project 44522, *SNP Panels for Chinook Salmon*).

The directed commercial fishery for Chinook salmon in the Kuskokwim River was discontinued in 1987 because of low run abundance (Whitmore et al. 2008) and was not re-established until 2008 because of the importance of this species for local subsistence use. The stock of concern finding for Chinook salmon in the Kuskokwim River between 2000 and 2007 prompted further conservation measures aimed at reducing Chinook salmon harvest and improving escapement (Whitmore et al. 2008). For example, in the Kuskokwim River, commercial chum salmon fishing was closed in June and in part or all of July in some years. Concurrent with these closures, for the first time in history subsistence fishers were placed on a four-day per week fishing schedule. Most of the Chinook salmon harvest in the Kuskokwim River is taken in the subsistence fishery, and about 80 percent of that harvest comes from the lower Kuskokwim River (Whitmore et al. 2008). For practical reasons, subsistence fishers take most of their harvest from the early half of the Chinook salmon run (Figure 6; Burkey et al. 2002, Hamazaki 2008). This practice, however, creates an added challenge to prosecuting a sustainable Chinook salmon management program because of differences in stock-specific run timings. Findings from a radio telemetry project conducted near Aniak from 2002 to 2007 suggests that earlier running Chinook salmon tend to be bound for spawning areas farthest upstream in the Kuskokwim River drainage, while later running Chinook salmon tend to spawn in tributaries progressively farther downstream (Stuby 2007). When coupled with the front-loaded subsistence harvest, this potentially causes higher harvest rates on the earlier running Chinook salmon stocks of the upper Kuskokwim River. Developing mixed stock analysis methods that work in the

KMA has potential for providing greater resolution on this issue, enabling managers to develop a more effective sustainable Chinook salmon management program.

CONCLUSIONS

- 1) Hierarchical tests indicate that significant structure exists among populations of Chinook salmon from the Kuskokwim Management Area. Although the Tatlawiksuk and Pitka Fork populations in the upper Kuskokwim River drainage are particularly genetically divergent, significant genetic variation was detected among all populations.
- 2) At the current level of resolution, genetic information can provide adequate distinction between the Upper Kuskokwim and Lower/Middle Kuskokwim groups for analysis of inriver commercial and subsistence fisheries.
- 3) At the current level of resolution, genetic stock identification does not provide adequate distinction within the KMA to estimate composition and run timing of Kuskokwim Bay separately from Lower/Middle Kuskokwim regional groups, limiting its utility for Kuskokwim Bay fisheries.

COMPLETION OF OBJECTIVES

1) To expand the baseline collections of Chinook salmon in the KMA.

The baseline of Chinook salmon was improved by the addition of 1,510 samples, more than doubling the number of individuals in the baseline. This includes the addition of Arolik and Gagarayah rivers to the baseline and the replacement of the earlier Takotna River samples with a collection of known origin. Sample sizes were increased in 10 populations and 11 of the 16 populations are represented by approximately 150 individuals or more.

2) To expand the set of genetic markers in the baseline of Chinook salmon in the Kuskokwim

The set of genetic markers available for use in studies of Kuskokwim River Chinook salmon has been increased and updated to match the current sets used in large-scale genetic analyses by laboratories in the U.S. and Canada. This will allow inclusion of Kuskokwim River populations in broadscale analyses using one of these datasets. The 13 microsatellite markers used were the same used in the CTC baseline and the alleles were standardized to make the data compatible with data collected in Norton Sound, Yukon River (Olsen et al. 2010), Kenai River (Begich et al. 2010), Copper River (Seeb et al. 2009b) and the Pacific Salmon Commission area from Southeast Alaska to California (Seeb et al. 2007). Through efficiencies available from improved technology and complementary projects, the data for 44 SNPs are presented in this report, more than twice the 19 SNPs originally proposed. These data have been included in the rangewide

SNP baseline (Templin et al. 2011) which is currently being used for Chinook salmon analyses in Alaska (DeCovich et al. 2010) and mixed stock analysis in the Bering Sea (e.g. Murphy et al. 2009; Guyon et al. 2010).

- 3) *To analyze the genetic population structure and refine mixed stock analysis of Chinook salmon in the KMA using the additional data.*

The analysis of genetic population structure and the development of mixed stock analysis applications were successfully completed. The addition of samples to the baseline and measurements at more genetic markers strengthened the signal of genetic similarity between populations of the lower Kuskokwim River and adjacent coastal rivers. At this time these populations cannot be distinguished in genetic stock identification applications. Resolution of the Upper Kuskokwim populations is now more accurately described and better resolved in genetic stock identification.

- 4) *To conduct a pilot study of the application of genetic stock identification techniques to salmon harvested in mixed stock fisheries in the KMA by collecting tissue samples, assaying genotypes, and determining the relative contribution of reporting groups.*

A pilot study was completed to investigate the application of genetic stock identification techniques to salmon harvests in fisheries in the KMA by collecting tissue samples, assaying genotypes, and determining the relative contribution of reporting groups. Samples were successfully collected from the 2005 and 2006 Bethel test fisheries, the 2006 Bethel subsistence fishery, and two periods of the District 4 commercial fishery and stock composition estimates were provided to the identifiable reporting regions.

RECOMMENDATIONS

- 1) Additional samples should be collected and analyzed from the East Fork, Middle Fork, Little Tonzona River and other locations within the upper Kuskokwim River basin where the greatest difference is found among populations. In addition, samples should be collected from the few remaining unrepresented spawning locations in the main portion of the Kuskokwim River drainage, such as the Oskawalik River, the Hoholitna River within the Holitna River basin.
- 2) An understanding of genetic population structure should be included in monitoring and assessment activities intended to sustain and maximize long-term productivity within the drainage. The limited resolution provided by genetic stock identification should not be interpreted as a lack of genetic diversity among these populations. This study supported previous measurements of significant genetic diversity of Chinook salmon from the KMA.
- 3) The performance of genetic stock identification models may be improved with further development of the baseline through the discovery and application of genetic markers chosen for specific application within western Alaska. With moderate improvement in resolution

between the lower river populations and the Kuskokwim Bay populations, genetic stock identification analyses can likely be applied to management and research applications within the KMA.

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Table 1. Chinook salmon populations included in the Kuskokwim Management Area genetic baseline analysis. Map numbers correspond to locations in Figure 1.

Map number	Populations	Sample size	Years sampled	Latitude	Longitude
1	Goodnews River	370	1993, 2005, 2006	59.254	-161.361
2	Arolik River	149	2005	59.651	-161.635
3	Kanektok River	245	1992, 1993, 2005	59.750	-161.927
4	Eek River	172	2002, 2005	60.201	-162.233
5	Tuluksak River	196	1993, 1994, 2005	61.045	-160.583
6	Kisaralik River	189	2001, 2005	60.857	-161.239
7	Kwethluk River	96	2001	60.813	-161.451
8	Salmon River	251	2002, 2006	61.065	-159.183
9	George River	190	2002, 2005	61.898	-157.714
10	Kogruklu River	150	1992, 1993, 2005	60.841	-157.846
11	Stony River	94	1994	61.769	-156.593
12	Tatlawiksuk River	190	2002, 2005	61.918	-156.245
13	Cheeneetnuk River	116	2002, 2006	61.812	-156.008
14	Gagarayah River	190	2006	61.620	-155.644
15	Takotna River	80	2005	62.969	-156.089
16	Pitka Fork	96	1995	62.888	-154.620

Table 2. Measures of genetic diversity in Chinook salmon from the Kuskokwim Management Area using microsatellite loci. Number of alleles at each locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E) and F_{ST} are shown.

Locus	Citation	A	H_O	H_E	F_{ST}
<i>Ogo2</i>	Olsen et al. 1998	14	0.758	0.754	0.003
<i>Ogo4</i>	Olsen et al. 1998	17	0.722	0.738	0.012
<i>Oki100</i>	DFO unpublished ¹	28	0.890	0.938	0.012
<i>Omm1080</i>	Rexroad et al. 2001	52	0.946	0.955	0.006
<i>Ots201</i>	Greig et al. 2003	25	0.894	0.907	0.005
<i>Ots208</i>	Greig et al. 2003	37	0.950	0.957	0.005
<i>Ots211</i>	Greig et al. 2003	29	0.928	0.936	0.005
<i>Ots212</i>	Greig et al. 2003	25	0.725	0.723	0.007
<i>Ots213</i>	Greig et al. 2003	31	0.937	0.930	0.007
<i>Ots3M</i>	Banks et al. 1999	8	0.654	0.658	0.012
<i>Ots9</i>	Banks et al. 2000	6	0.529	0.533	0.002
<i>OtsG47</i>	Williamson et al. 2002	7	0.100	0.100	0.002
<i>Ssa408</i>	Cairney et al. 2000	17	0.841	0.870	0.009

¹Personal communication, K. Miller, Department of Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada.

Table 3. Measures of genetic diversity in Chinook salmon from the Kuskokwim Management Area using SNP loci. Number of alleles at each locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E) and F_{ST} are shown. *Ots_C3N3* is a SNP in the mtDNA.

Locus	Reference	A	H_O	H_E	F_{ST}
<i>Ots_arf-188</i>	Smith et al. 2005a	1	0	0	0
<i>Ots_AsnRS-60</i>	Smith et al. 2005a	2	0.455	0.453	0.009
<i>Ots_C3N3</i>	Smith et al. 2005b	2	--	--	0.004
<i>Ots_ETIF1A</i>	unpublished	2	0.465	0.484	0.002
<i>Ots_FARSLA-220</i>	Smith et al. 2007	2	0.046	0.048	0.008
<i>Ots_FGF6A</i>	unpublished	2	0.498	0.495	0.011
<i>Ots_GH2</i>	Smith et al. 2005b	2	0.351	0.358	0.004
<i>Ots_GPDH-338</i>	Smith et al. 2005a	2	0.282	0.281	0.003
<i>Ots_GPH-318</i>	Smith et al. 2007	2	0.156	0.156	0.007
<i>Ots_GST-207</i>	Smith et al. 2007	2	0.082	0.081	0.002
<i>Ots_GST-375</i>	Smith et al. 2007	1	0	0	0
<i>Ots_GTH2B-550</i>	unpublished	2	0.412	0.414	0.010
<i>Ots_HGFA-446</i>	Smith et al. 2005a	1	0	0	0
<i>Ots_hnRNPL-533</i>	Smith et al. 2007	2	0.387	0.373	0.003
<i>Ots_HSP90B-100</i>	Smith et al. 2007	2	0.245	0.257	0.004
<i>Ots_IGF-I.1-76</i>	Smith et al. 2005a	2	0.475	0.480	0.026
<i>Ots_Ikaros-250</i>	Smith et al. 2005a	2	0.252	0.265	0.005
<i>Ots_LEI-292</i>	Smith et al. 2007	2	0.090	0.091	0.004
<i>Ots_MHC1</i>	Smith et al. 2005b	2	0.504	0.499	-0.001
<i>Ots_MHC2</i>	Smith et al. 2005b	2	0.156	0.157	0.036
<i>Ots_NOD1</i>	unpublished	2	0.476	0.473	0.008
<i>Ots_ZNF330-181</i>	Smith et al. 2005a	2	0.018	0.017	0.003
<i>Ots_LWSop-638</i>	Smith et al. 2005a	2	0.078	0.079	0.003
<i>Ots_SWS1op-182</i>	Smith et al. 2005a	2	0.465	0.440	0.020
<i>Ots_P450</i>	Smith et al. 2005b	2	0.318	0.314	0.007
<i>Ots_PGK-54</i>	unpublished	2	0.001	0.001	0.001
<i>Ots_Prl2</i>	Smith et al. 2005b	2	0.427	0.433	0.002
<i>Ots_ins-115</i>	Smith et al. 2005a	2	0.032	0.032	0.001
<i>Ots_PSMB1-197</i>	Smith et al. 2007	1	0	0	0
<i>Ots_RAG3</i>	unpublished	2	0.015	0.015	0.003
<i>Ots_S7-1</i>	unpublished	2	0.292	0.304	0.008
<i>Ots_SCkF2R2-135</i>	Smith et al. 2005a	2	0.473	0.481	0.010
<i>Ots_SERPC1-209</i>	Smith et al. 2007	2	0.063	0.068	0.001
<i>Ots_SL</i>	Smith et al. 2005b	2	0.408	0.410	0.008
<i>Ots_TAPBP</i>	unpublished	2	0.188	0.185	0.005
<i>Ots_Tnsf</i>	Smith et al. 2005b	2	0.200	0.209	0.020
<i>Ots_U202-161</i>	Smith et al. 2005a	2	0.030	0.030	0.004
<i>Ots_U211-85</i>	Smith et al. 2005a	2	0.026	0.026	0.001
<i>Ots_U212-158</i>	Smith et al. 2005a	2	0.153	0.148	0.013
<i>Ots_u4-92</i>	Smith et al. 2005a	2	0.135	0.135	0.006
<i>Ots_unkn526</i>	unpublished	2	0.258	0.258	0.007
<i>Ots_u6-75</i>	Smith et al. 2005a	2	0.241	0.236	0.004
<i>Ots_E2-275</i>	Smith et al. 2005a	2	0.358	0.359	0.012
<i>Ots_zP3b-215</i>	Smith et al. 2005a	2	0.152	0.152	0.008

Table 4. Genetic diversity in 16 populations of Chinook salmon from the Kuskokwim Management Area. Sample size (N), number of alleles (A), allelic richness (R), and observed (H_O) and expected (H_E) heterozygosities for 13 microsatellite and 44 SNP loci are shown (monomorphic SNP loci were included).

Population	N	Microsatellite				SNPs			
		A	R	H_O	H_E	A	R	H_O	H_E
Goodnews	370	260	215	0.775	0.762	84	82	0.256	0.255
Arolik	149	237	218	0.780	0.761	82	81	0.253	0.252
Kanektok	245	256	219	0.775	0.769	83	82	0.252	0.251
Eek	172	240	217	0.773	0.759	82	81	0.253	0.251
Tuluksak	196	235	213	0.772	0.759	82	81	0.253	0.252
Kisaralik	189	231	210	0.765	0.755	82	81	0.245	0.248
Kwethluk	96	225	218	0.771	0.749	81	81	0.247	0.253
Salmon	251	246	213	0.771	0.769	81	80	0.247	0.245
George	190	233	211	0.776	0.781	82	81	0.254	0.255
Kogrukluuk	150	230	211	0.769	0.763	82	81	0.246	0.247
Stony	94	215	210	0.773	0.762	82	82	0.251	0.242
Tatlawiksuk	190	213	187	0.727	0.708	80	80	0.234	0.231
Cheeneetnuk	116	226	215	0.774	0.764	82	82	0.252	0.259
Gagarayah	190	224	207	0.768	0.764	82	81	0.252	0.250
Takotna	80	206	205	0.780	0.783	79	79	0.239	0.230
Pitka	96	176	170	0.760	0.740	79	79	0.242	0.242

Table 5. Genetic variation between population pairs as measured with microsatellite loci. Cavalli-Sforza and Edwards' (1967) chord distances are below the diagonal and F_{ST} estimates are above the diagonal.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Goodnews	0	0.001	0.001	0.002	0.002	0.003	0.004	0.002	0.003	0.003	0.005	0.027	0.003	0.004	0.005	0.024
2 Arolik	0.003	0	0.001	0.001	0.001	0.004	0.004	0.001	0.001	0.002	0.006	0.023	0.003	0.003	0.004	0.017
3 Kanektok	0.003	0.004	0	0.000	0.001	0.002	0.002	0.000	0.002	0.001	0.004	0.025	0.002	0.003	0.004	0.023
4 Eek	0.004	0.004	0.003	0	0.001	0.000	0.001	-0.001	0.002	0.000	0.004	0.026	0.002	0.002	0.005	0.020
5 Tuluksak	0.005	0.005	0.004	0.004	0	0.002	0.002	0.001	0.002	0.001	0.005	0.026	0.003	0.003	0.005	0.021
6 Kisaralik	0.005	0.005	0.004	0.003	0.004	0	0.001	0.001	0.003	0.001	0.005	0.031	0.005	0.003	0.005	0.024
7 Kwethluk	0.007	0.008	0.006	0.005	0.006	0.005	0	0.001	0.003	0.002	0.006	0.028	0.004	0.003	0.005	0.024
8 Salmon	0.004	0.004	0.002	0.003	0.003	0.003	0.004	0	0.002	0.000	0.005	0.025	0.002	0.002	0.005	0.020
9 George	0.005	0.005	0.004	0.004	0.005	0.005	0.007	0.003	0	0.003	0.007	0.027	0.004	0.004	0.006	0.021
10 Kogrukuk	0.005	0.006	0.004	0.004	0.004	0.004	0.006	0.003	0.005	0	0.004	0.028	0.004	0.002	0.005	0.024
11 Stony	0.009	0.009	0.007	0.007	0.008	0.008	0.009	0.007	0.009	0.007	0	0.037	0.009	0.007	0.005	0.028
12 Tatlawiksuk	0.014	0.013	0.013	0.013	0.014	0.014	0.016	0.013	0.013	0.014	0.018	0	0.021	0.028	0.031	0.036
13 Cheeneetnuk	0.007	0.007	0.006	0.006	0.007	0.007	0.008	0.006	0.007	0.008	0.011	0.014	0	0.003	0.007	0.022
14 Gagarayah	0.006	0.006	0.005	0.005	0.005	0.005	0.007	0.005	0.006	0.005	0.009	0.014	0.006	0	0.006	0.021
15 Takotna	0.009	0.009	0.008	0.008	0.008	0.008	0.010	0.008	0.009	0.009	0.010	0.017	0.010	0.010	0	0.025
16 Pitka	0.017	0.017	0.017	0.016	0.017	0.016	0.018	0.016	0.016	0.018	0.020	0.020	0.019	0.017	0.020	0

Table 6. Genetic variation between population pairs as measured with SNP loci. Cavalli-Sforza and Edwards' (1967) chord distances are below the diagonal and F_{ST} estimates are above the diagonal.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Goodnews	0	0.003	0.004	0.005	0.011	0.012	0.004	0.011	0.006	0.010	0.008	0.027	0.005	0.010	0.009	0.035
2 Arolik	0.033	0	0.001	0.001	0.004	0.004	0.000	0.003	0.003	0.003	0.007	0.017	0.002	0.004	0.006	0.033
3 Kanektok	0.032	0.030	0	0.002	0.004	0.005	0.000	0.003	0.004	0.002	0.004	0.018	0.002	0.003	0.004	0.027
4 Eek	0.040	0.033	0.033	0	0.006	0.003	0.001	0.003	0.004	0.005	0.008	0.019	0.001	0.002	0.007	0.031
5 Tuluksak	0.049	0.040	0.037	0.044	0	0.004	0.002	0.001	0.005	0.001	0.007	0.017	0.007	0.007	0.005	0.037
6 Kisaralik	0.052	0.041	0.040	0.036	0.040	0	0.002	0.002	0.006	0.002	0.008	0.015	0.006	0.003	0.007	0.041
7 Kwethluk	0.045	0.039	0.036	0.038	0.044	0.039	0	-0.001	0.002	-0.001	0.003	0.019	-0.001	0.002	0.002	0.029
8 Salmon	0.050	0.040	0.034	0.037	0.033	0.036	0.028	0	0.005	0.001	0.005	0.016	0.004	0.003	0.002	0.034
9 George	0.045	0.040	0.038	0.038	0.041	0.041	0.037	0.040	0	0.003	0.003	0.017	0.005	0.006	0.005	0.031
10 Kogrukuk	0.050	0.040	0.032	0.040	0.031	0.034	0.034	0.030	0.035	0	0.004	0.014	0.004	0.003	0.004	0.035
11 Stony	0.051	0.051	0.041	0.051	0.050	0.051	0.048	0.045	0.045	0.043	0	0.019	0.004	0.005	0.004	0.028
12 Tatlawiksuk	0.076	0.064	0.068	0.068	0.070	0.068	0.074	0.066	0.069	0.066	0.076	0	0.022	0.015	0.017	0.047
13 Cheeneetnuk	0.042	0.038	0.036	0.037	0.050	0.047	0.036	0.043	0.046	0.045	0.046	0.078	0	0.003	0.007	0.021
14 Gagarayah	0.050	0.043	0.037	0.037	0.046	0.039	0.041	0.035	0.044	0.037	0.042	0.066	0.041	0	0.005	0.029
15 Takotna	0.059	0.051	0.049	0.053	0.052	0.054	0.051	0.043	0.053	0.050	0.050	0.069	0.059	0.053	0	0.031
16 Pitka	0.100	0.096	0.089	0.095	0.101	0.103	0.091	0.093	0.096	0.098	0.089	0.117	0.083	0.092	0.094	0

Table 7. Hierarchical log likelihood ratio (G) analysis of population structure based on allele frequencies at microsatellite loci and SNPs. Populations were grouped into three broad-scale regions: 1) Kuskokwim Bay, 2) Lower/Middle Kuskokwim, and 3) Upper Kuskokwim. The probability of the statistic (P) is provided for inferring significance.

Populations	Microsatellite			SNP		
	DF	G	P	DF	G	P
Kuskokwim	4,350	9,797.0	0.000	630	2,036.0	0.000
Among regions	580	3,315.1	0.000	84	638.8	0.000
Within regions	3,770	6,482.0	0.000	546	1,397.0	0.000
Kuskokwim Bay	580	838.0	0.000	84	147.4	0.000
Lower/Middle Kuskokwim	2,900	4,385.0	0.000	420	765.0	0.000
Upper Kuskokwim	290	1,259.0	0.000	42	484.5	0.000

Table 8. Hierarchical analysis of variance (ANOVA) of genetic variation based on microsatellite and SNP loci. Populations were grouped into three broad-scale regions: 1) Kuskokwim Bay, 2) Lower/Middle Kuskokwim, and 3) Upper Kuskokwim.

Source of variation	Microsatellite		SNP	
		90% CI		90% CI
Total	F = 0.022	(0.013-0.031)	F = 0.013	(0.007-0.020)
Among regions	θ_p = 0.005	(0.003-0.007)	θ_p = 0.004	(0.002-0.007)
Among populations	θ_s = 0.009	(0.007-0.012)	θ_s = 0.010	(0.007-0.014)

Table 9. Results of mixed stock analysis simulations in which individual regions from the Kuskokwim Management Area comprised 100% of the hypothetical mixture. Simulations were performed in ONCOR using microsatellites, SNPs, and combined marker sets. Estimates may not sum to 1.0 due to rounding errors. Entries in bold type indicate correct allocation to region of origin.

		True Composition					
		Kuskokwim Bay		Lower/Middle Kuskokwim		Upper Kuskokwim	
Estimated Composition		Est	90% CI	Est	90% CI	Est	90% CI
Microsatellites							
Kuskokwim Bay	0.67	(0.59-0.74)	0.12	(0.04-0.20)	0.01	(0.00-0.05)	
Lower/Middle Kuskokwim	0.33	(0.25-0.41)	0.88	(0.79-0.96)	0.04	(0.07-0.17)	
Upper Kuskokwim	0.00	(0.00-0.01)	0.00	(0.00-0.01)	0.95	(0.81-0.91)	
SNPs							
Kuskokwim Bay	0.71	(0.55-0.89)	0.12	(0.00-0.27)	0.01	(0.00-0.05)	
Lower/Middle Kuskokwim	0.27	(0.10-0.44)	0.87	(0.72-0.99)	0.05	(0.00-0.11)	
Upper Kuskokwim	0.02	(0.00-0.06)	0.01	(0.00-0.05)	0.94	(0.88-0.99)	
Microsatellites and SNPs							
Kuskokwim Bay	0.71	(0.64-0.80)	0.12	(0.04-0.19)	0.01	(0.00-0.02)	
Lower/Middle Kuskokwim	0.28	(0.20-0.36)	0.88	(0.81-0.95)	0.02	(0.00-0.04)	
Upper Kuskokwim	0.00	(0.00-0.00)	0.00	(0.00-0.00)	0.97	(0.95-0.99)	

Table 10. Results of mixed stock analysis simulations in which individual regions from the Kuskokwim River comprised 100% of the hypothetical mixture. Simulations were performed in ONCOR using microsatellites, SNPs, and combined marker sets. Entries in bold type indicate correct allocation to region of origin.

Estimated Composition	True Composition			
	Lower/Middle Kuskokwim		Upper Kuskokwim	
	Est	90% CI	Est	90% CI
Microsatellites				
Lower/Middle Kuskokwim	1.00 (0.99-1.00)		0.05	(0.02-0.07)
Upper Kuskokwim	0.00	(0.00-0.01)	0.95 (0.93-0.98)	
SNPs				
Lower/Middle Kuskokwim	0.98 (0.95-1.00)		0.06	(0.01-0.12)
Upper Kuskokwim	0.02	(0.00-0.05)	0.94 (0.88-0.99)	
Microsatellites and SNPs				
Lower/Middle Kuskokwim	1.00 (0.99-1.00)		0.03	(0.01-0.05)
Upper Kuskokwim	0.00	(0.00-0.01)	0.97 (0.95-0.99)	

Table 11. Sample size (N), mean estimated composition (Est), and credibility interval (90% CI) for fishery samples from two Kuskokwim River fisheries.

Reporting Group	Bethel Test Fishery				Subsistence Fishery	
	2005		2006		2006	
	June 3 - July 14		June 5 - July 26		June 17 - July 19	
	N =	374	N =	242	N =	328
Est	90%CI	Est	90%CI	Est	90%CI	
Lower/ Middle Kuskokwim	1.00	(1.00-1.00)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
Upper Kuskokwim	0.00	(0.00-0.00)	0.00	(0.00-0.00)	0.00	(0.00-0.00)

Table 12. Sample size (N), mean estimated composition (Est), and credibility interval (90% CI) for fishery samples from the District 4 commercial fishery.

Reporting Group	2006 District 4 commercial fishery			
	Period 1		Period 2	
	June 15 - 22		June 30 - July 7	
	N =	416	N =	479
Est	90%CI	Est	90%CI	
Kuskokwim Bay & Middle/Lower Kuskokwim	0.99	(0.96-1.00)	1.00	(0.98-1.00)
Upper Kuskokwim	0.01	(0.00-0.04)	0.00	(0.00-0.02)

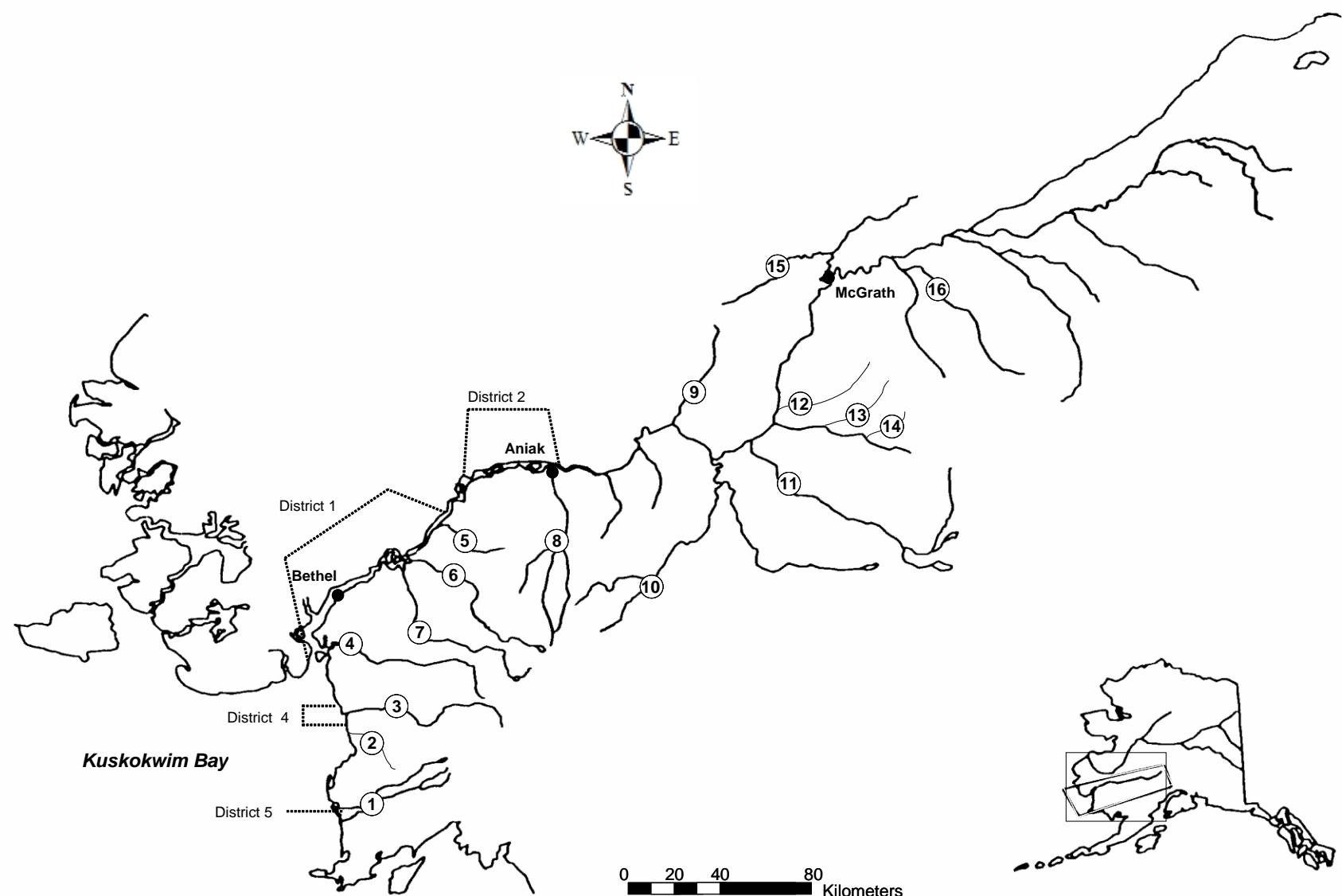


Figure 1. Map of the Kuskokwim Management Area. Numbers correspond to populations listed in Table 1.

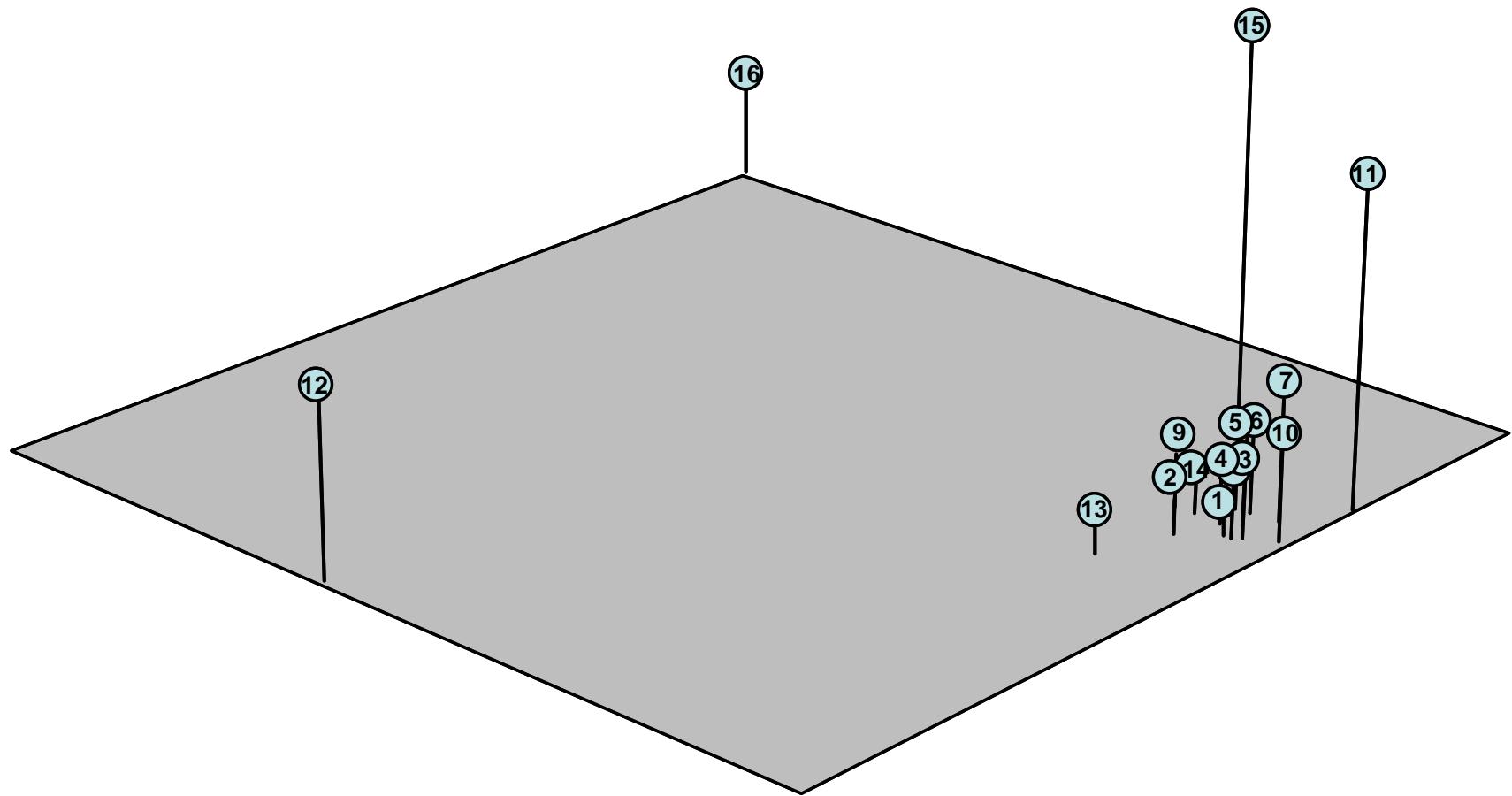


Figure 2. Multidimensional scaling of pairwise genetic distances (Cavalli-Sforza and Edwards 1967) among Chinook salmon populations in the Kuskokwim Management Area calculated from allele frequencies at microsatellite loci. Numbers correspond to populations in Table 1 and Figure 1.

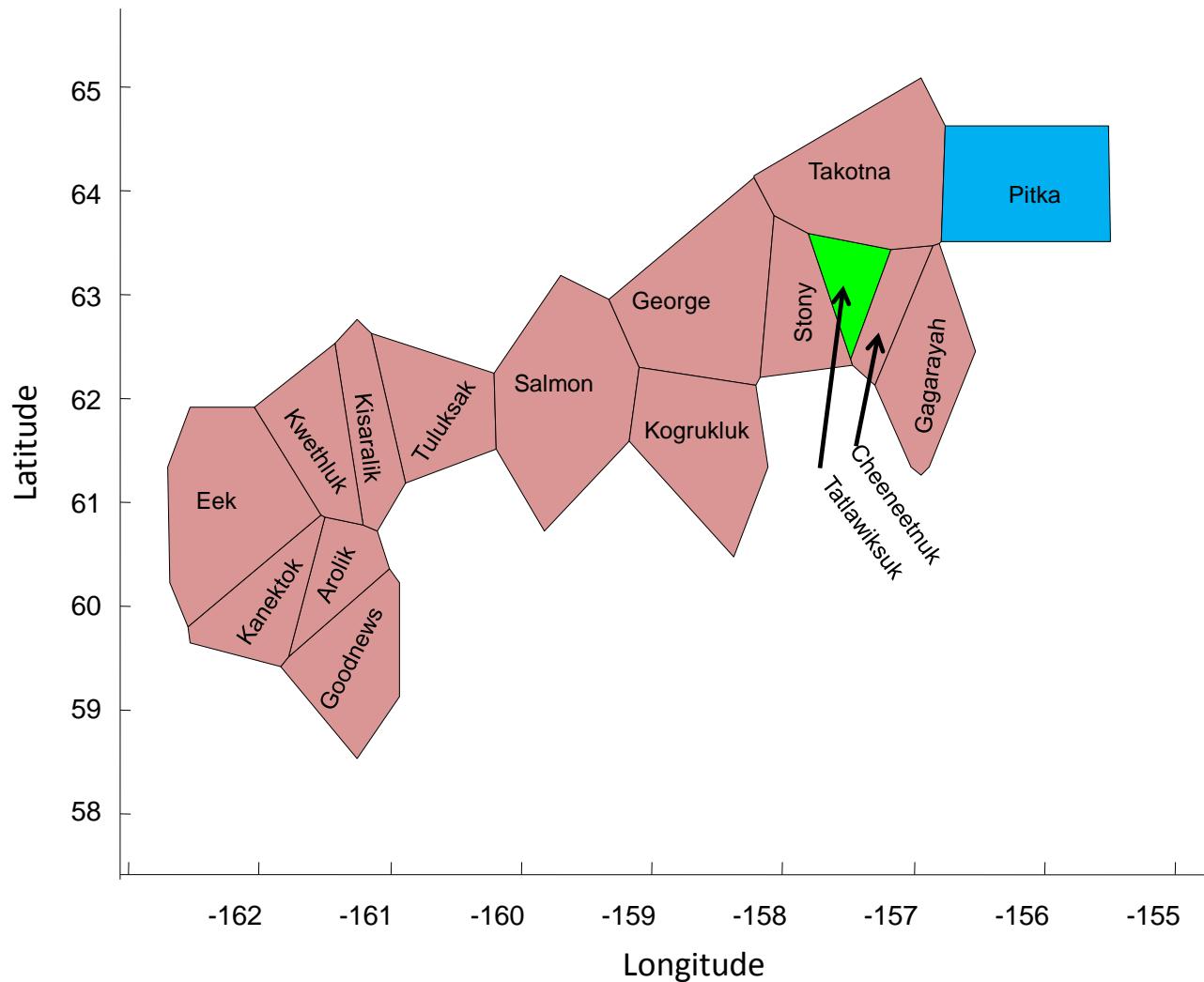


Figure 3. Voronoi tessellation describing clustering of populations based on genetic differences measured with microsatellite loci and geographic location from the program BAPS. Polygons of the same color belong to the same group. Identical clusters were produced using SNP loci.

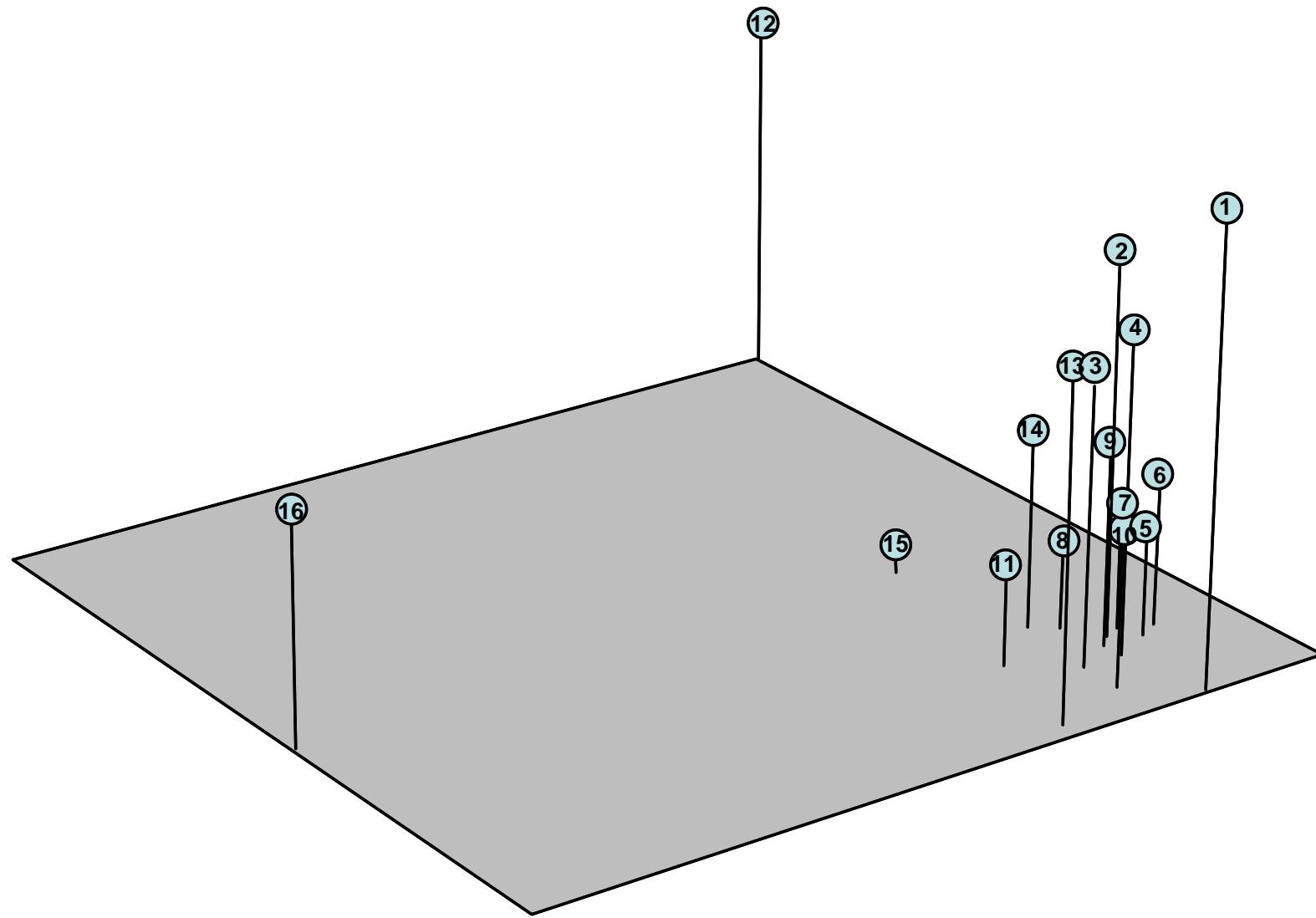


Figure 4. Multidimensional scaling of pairwise genetic distances (Cavalli-Sforza and Edwards 1967) among Chinook salmon populations in the Kuskokwim Management Area calculated from allele frequencies at SNP loci. Numbers correspond to populations in Table 1 and Figure 1.

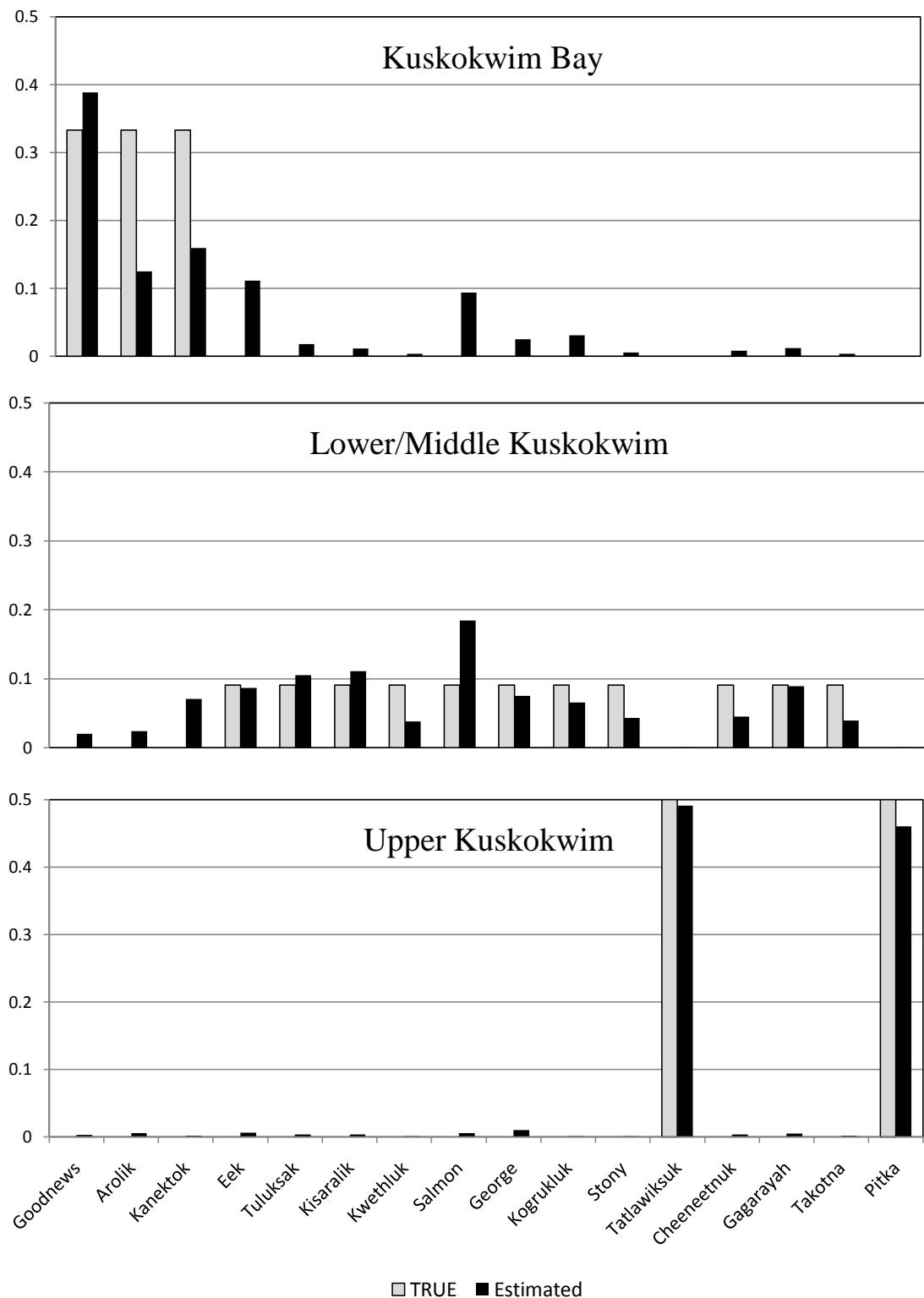


Figure 5. Population-specific estimates of proportional contribution to three single-region mixtures in 100% simulations using the combined SNP and microsatellite marker sets. Estimates were made using the ONCOR software package. Gray bars indicate true proportions in the mixture and black bars indicate estimated population proportions.

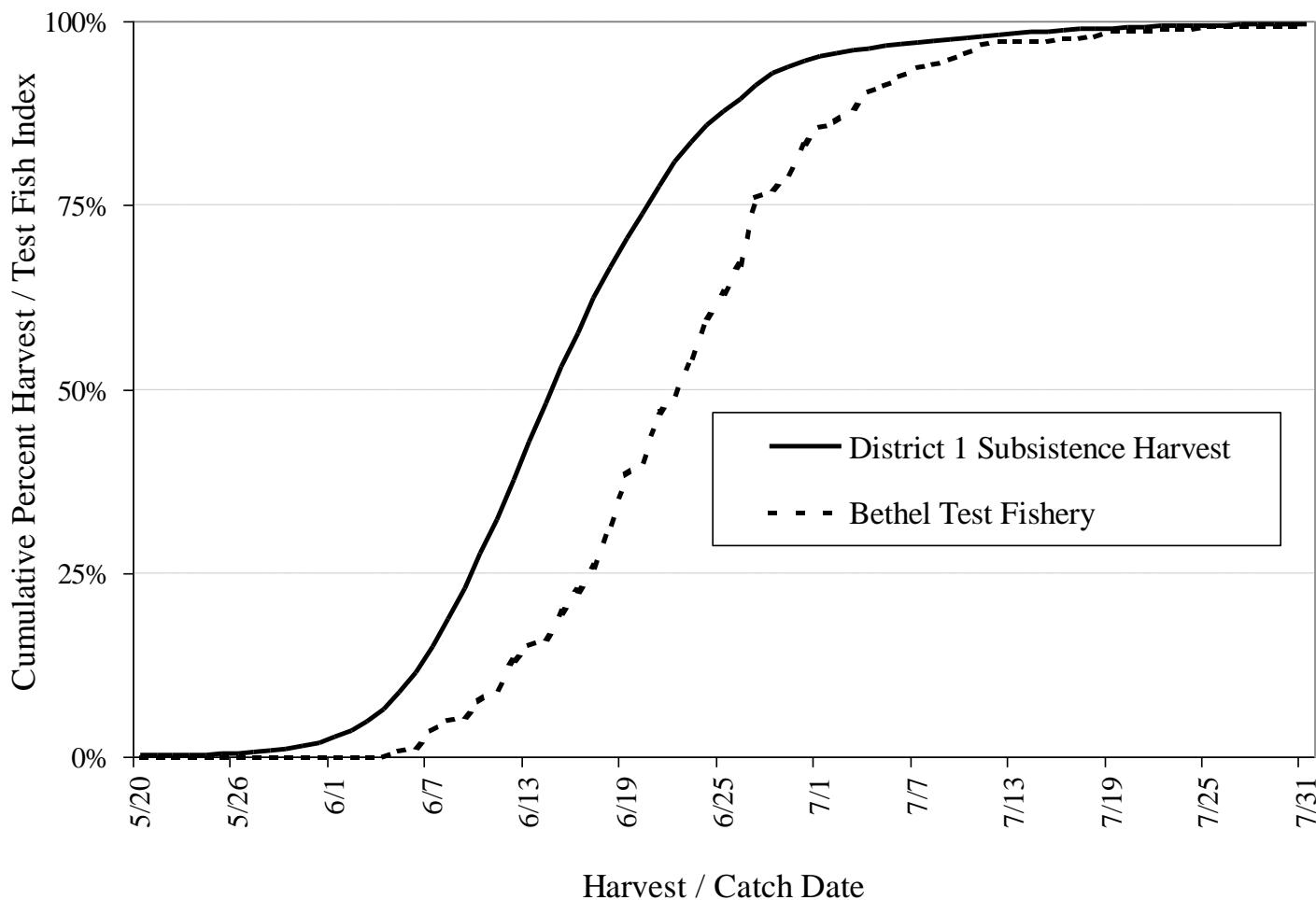


Figure 6. Average run timing of the subsistence Chinook salmon harvest in the lower Kuskokwim River (District 1), compared to the average run timing observed in the Bethel test fishery (mid-point of District 1), 1984 to 1999.

APPENDICES

Appendix A. Frequencies of alleles at 13 microsatellite loci for Chinook salmon populations from the Kuskokwim Management Area. Populations are designated as follows: Goodnews (Goo), Arolik (Aro), Kanektok (Kan), Eek, Kwethluk (Kwe), Kisaralik (Kis), Tuluksak (Tul), Salmon (Sal), George (Geo), Kogrukluuk (Kog), Stony (Sto), Tatlawiksuk (Tat), Cheeneetnuk (Che), Gagarayah (Gag), and Takotna (Tak) rivers and Pitka Fork (Pit).

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population									
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit	
<i>Ogo2</i>	N	367	146	240	169	195	189	94	245	188	149	92	189	113	190	80	96
	200	0.015	0.003	0.017	0.006	0.003	0.008	0.000	0.018	0.011	0.013	0.005	0.008	0.000	0.008	0.006	0.063
	202	0.001	0.007	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	212	0.000	0.000	0.002	0.000	0.003	0.000	0.000	0.002	0.003	0.003	0.000	0.000	0.018	0.029	0.006	0.000
	216	0.204	0.233	0.177	0.192	0.208	0.172	0.154	0.182	0.189	0.185	0.217	0.159	0.208	0.200	0.238	0.271
	218	0.019	0.034	0.021	0.038	0.018	0.026	0.032	0.027	0.021	0.000	0.027	0.016	0.009	0.008	0.013	0.010
	220	0.268	0.281	0.300	0.284	0.274	0.249	0.298	0.306	0.221	0.336	0.277	0.394	0.257	0.284	0.269	0.349
	222	0.054	0.051	0.048	0.062	0.087	0.095	0.085	0.059	0.059	0.067	0.049	0.058	0.075	0.076	0.100	0.063
	224	0.022	0.010	0.027	0.053	0.023	0.034	0.021	0.024	0.024	0.037	0.016	0.000	0.018	0.021	0.031	0.000
	226	0.373	0.312	0.354	0.317	0.346	0.362	0.356	0.341	0.423	0.299	0.326	0.333	0.372	0.342	0.288	0.240
	228	0.003	0.000	0.002	0.012	0.005	0.008	0.005	0.004	0.005	0.003	0.011	0.011	0.009	0.005	0.006	0.000
	230	0.022	0.045	0.023	0.036	0.023	0.040	0.043	0.027	0.027	0.023	0.049	0.021	0.022	0.013	0.025	0.005
	232	0.001	0.000	0.006	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
	234	0.014	0.024	0.021	0.000	0.008	0.005	0.005	0.010	0.019	0.034	0.022	0.000	0.013	0.013	0.013	0.000
	238	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Ogo4</i>	N	367	147	238	170	195	189	94	248	187	149	92	187	114	190	80	96
	136	0.277	0.310	0.298	0.271	0.331	0.267	0.324	0.284	0.337	0.305	0.201	0.345	0.346	0.363	0.219	0.375
	138	0.000	0.003	0.002	0.000	0.000	0.003	0.000	0.004	0.000	0.003	0.000	0.000	0.004	0.000	0.006	0.000
	140	0.041	0.024	0.019	0.021	0.010	0.024	0.027	0.016	0.021	0.013	0.000	0.005	0.035	0.026	0.013	0.010
	144	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	146	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
	148	0.007	0.007	0.008	0.009	0.005	0.005	0.000	0.008	0.011	0.000	0.016	0.005	0.009	0.005	0.006	0.000
	150	0.004	0.003	0.000	0.006	0.005	0.000	0.005	0.002	0.003	0.003	0.000	0.000	0.004	0.000	0.013	0.000
	152	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.004	0.005	0.000	0.000	0.000	0.004	0.000	0.006	0.000
	154	0.035	0.048	0.023	0.038	0.062	0.021	0.048	0.030	0.035	0.030	0.071	0.048	0.013	0.026	0.063	0.010
	156	0.069	0.051	0.044	0.038	0.023	0.034	0.048	0.036	0.037	0.023	0.016	0.155	0.048	0.047	0.025	0.094
	158	0.037	0.068	0.059	0.056	0.059	0.058	0.059	0.067	0.045	0.067	0.065	0.035	0.035	0.024	0.063	0.000
	160	0.409	0.340	0.433	0.421	0.372	0.434	0.372	0.401	0.356	0.403	0.516	0.209	0.351	0.379	0.438	0.172

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population									
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit	
162	0.067	0.078	0.048	0.065	0.077	0.056	0.048	0.056	0.083	0.070	0.071	0.072	0.057	0.037	0.069	0.042	
164	0.050	0.068	0.061	0.062	0.054	0.095	0.069	0.091	0.064	0.077	0.038	0.126	0.088	0.084	0.075	0.286	
166	0.004	0.000	0.002	0.006	0.000	0.003	0.000	0.000	0.003	0.000	0.005	0.000	0.000	0.008	0.006	0.000	
168	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.010	
170	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Oki100</i>	N	365	146	237	168	195	189	94	243	185	148	92	187	112	187	80	95
	204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000
	208	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
	212	0.005	0.014	0.011	0.003	0.013	0.005	0.005	0.012	0.000	0.003	0.011	0.000	0.000	0.003	0.025	0.000
	216	0.003	0.007	0.011	0.021	0.033	0.013	0.011	0.014	0.014	0.020	0.011	0.080	0.022	0.019	0.025	0.005
	220	0.041	0.034	0.042	0.033	0.023	0.037	0.053	0.049	0.035	0.034	0.038	0.019	0.049	0.024	0.031	0.021
	224	0.037	0.055	0.040	0.063	0.036	0.024	0.032	0.047	0.038	0.030	0.011	0.019	0.063	0.048	0.013	0.053
	228	0.063	0.027	0.072	0.042	0.064	0.087	0.037	0.070	0.043	0.044	0.038	0.040	0.027	0.053	0.075	0.016
	232	0.051	0.055	0.055	0.045	0.074	0.045	0.043	0.041	0.046	0.057	0.060	0.398	0.098	0.051	0.038	0.021
	236	0.049	0.041	0.040	0.045	0.046	0.042	0.064	0.037	0.022	0.037	0.027	0.035	0.045	0.032	0.056	0.079
	240	0.056	0.068	0.049	0.063	0.049	0.042	0.021	0.041	0.046	0.068	0.082	0.013	0.076	0.064	0.038	0.074
	244	0.030	0.048	0.053	0.048	0.013	0.034	0.069	0.047	0.027	0.037	0.076	0.011	0.040	0.086	0.069	0.132
	248	0.040	0.065	0.038	0.054	0.049	0.058	0.043	0.045	0.070	0.047	0.049	0.024	0.036	0.064	0.044	0.089
	252	0.075	0.099	0.051	0.063	0.054	0.058	0.085	0.049	0.078	0.041	0.076	0.059	0.049	0.040	0.119	0.132
	256	0.073	0.065	0.082	0.077	0.059	0.063	0.059	0.080	0.084	0.071	0.076	0.035	0.058	0.099	0.069	0.100
	260	0.059	0.045	0.080	0.074	0.064	0.066	0.059	0.068	0.035	0.135	0.087	0.019	0.098	0.072	0.038	0.026
	264	0.082	0.055	0.049	0.060	0.072	0.077	0.085	0.058	0.057	0.078	0.033	0.040	0.058	0.070	0.038	0.005
	268	0.053	0.062	0.049	0.074	0.051	0.069	0.043	0.043	0.070	0.071	0.065	0.021	0.049	0.072	0.044	0.000
	272	0.058	0.065	0.046	0.057	0.082	0.074	0.032	0.045	0.073	0.044	0.087	0.037	0.018	0.032	0.025	0.105
	275	0.040	0.062	0.049	0.030	0.059	0.050	0.048	0.068	0.065	0.041	0.016	0.011	0.049	0.027	0.050	0.016
	279	0.022	0.021	0.038	0.036	0.049	0.050	0.064	0.047	0.049	0.041	0.049	0.067	0.045	0.051	0.094	0.037
	283	0.030	0.031	0.042	0.033	0.026	0.021	0.021	0.049	0.059	0.014	0.027	0.043	0.027	0.027	0.038	0.037
	287	0.048	0.024	0.046	0.027	0.026	0.019	0.037	0.025	0.027	0.044	0.027	0.008	0.022	0.008	0.019	0.011
	290	0.037	0.021	0.019	0.018	0.015	0.021	0.048	0.031	0.016	0.024	0.038	0.005	0.009	0.013	0.025	0.005
	294	0.021	0.021	0.030	0.018	0.021	0.003	0.016	0.019	0.019	0.010	0.011	0.008	0.031	0.019	0.031	0.016
	298	0.015	0.007	0.008	0.015	0.013	0.034	0.016	0.008	0.003	0.010	0.005	0.000	0.004	0.016	0.000	0.000
	302	0.005	0.007	0.000	0.006	0.010	0.005	0.011	0.002	0.003	0.000	0.000	0.003	0.009	0.008	0.000	0.005

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population									
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit	
305	0.004	0.003	0.002	0.000	0.000	0.000	0.000	0.002	0.014	0.000	0.000	0.005	0.000	0.000	0.000	0.016	
309	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.004	0.000	0.000	0.000	
<i>Omm108</i>																	
N	364	144	240	169	195	187	94	244	186	147	91	188	113	188	79	96	
166	0.029	0.049	0.023	0.012	0.015	0.021	0.005	0.025	0.030	0.024	0.005	0.077	0.049	0.019	0.044	0.000	
170	0.010	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
186	0.000	0.000	0.002	0.003	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
190	0.014	0.014	0.006	0.003	0.013	0.016	0.011	0.008	0.003	0.010	0.044	0.000	0.040	0.021	0.032	0.000	
194	0.036	0.010	0.023	0.012	0.021	0.021	0.032	0.025	0.016	0.017	0.027	0.000	0.040	0.037	0.019	0.000	
198	0.018	0.028	0.013	0.027	0.026	0.005	0.005	0.010	0.016	0.020	0.022	0.003	0.000	0.013	0.038	0.000	
202	0.021	0.017	0.029	0.033	0.054	0.045	0.048	0.053	0.056	0.041	0.049	0.013	0.018	0.024	0.000	0.057	
206	0.036	0.049	0.046	0.074	0.046	0.053	0.037	0.061	0.022	0.092	0.060	0.029	0.013	0.066	0.013	0.010	
210	0.063	0.038	0.069	0.062	0.031	0.064	0.112	0.064	0.062	0.048	0.049	0.037	0.027	0.048	0.019	0.021	
214	0.044	0.066	0.067	0.053	0.036	0.067	0.043	0.057	0.054	0.058	0.011	0.016	0.080	0.066	0.070	0.010	
218	0.062	0.031	0.033	0.053	0.038	0.029	0.048	0.029	0.032	0.051	0.099	0.024	0.013	0.013	0.032	0.010	
222	0.032	0.035	0.040	0.044	0.054	0.019	0.005	0.037	0.022	0.031	0.038	0.008	0.035	0.053	0.019	0.000	
226	0.021	0.031	0.017	0.027	0.008	0.016	0.011	0.031	0.035	0.031	0.000	0.021	0.035	0.029	0.019	0.000	
230	0.005	0.007	0.008	0.006	0.018	0.011	0.000	0.014	0.003	0.020	0.005	0.003	0.022	0.005	0.006	0.000	
234	0.015	0.010	0.008	0.009	0.013	0.021	0.005	0.012	0.022	0.014	0.005	0.005	0.004	0.021	0.013	0.000	
238	0.025	0.007	0.015	0.009	0.010	0.003	0.005	0.008	0.022	0.020	0.011	0.016	0.018	0.011	0.038	0.005	
242	0.036	0.035	0.019	0.018	0.018	0.008	0.021	0.012	0.011	0.014	0.022	0.008	0.022	0.029	0.013	0.047	
246	0.018	0.038	0.029	0.018	0.031	0.021	0.048	0.041	0.011	0.017	0.027	0.005	0.040	0.032	0.038	0.005	
250	0.041	0.045	0.054	0.036	0.074	0.045	0.074	0.055	0.056	0.037	0.044	0.064	0.031	0.029	0.095	0.073	
254	0.045	0.049	0.060	0.050	0.038	0.045	0.043	0.047	0.040	0.034	0.082	0.117	0.053	0.085	0.025	0.115	
258	0.034	0.028	0.050	0.030	0.033	0.045	0.027	0.039	0.035	0.065	0.082	0.066	0.088	0.027	0.038	0.099	
262	0.037	0.049	0.063	0.059	0.051	0.053	0.059	0.051	0.046	0.054	0.033	0.027	0.053	0.037	0.089	0.021	
266	0.077	0.056	0.031	0.053	0.051	0.048	0.043	0.053	0.056	0.027	0.016	0.019	0.053	0.051	0.057	0.240	
270	0.048	0.059	0.054	0.030	0.059	0.056	0.037	0.041	0.046	0.068	0.115	0.056	0.053	0.027	0.089	0.099	
274	0.051	0.038	0.023	0.041	0.038	0.037	0.048	0.033	0.056	0.041	0.005	0.021	0.009	0.066	0.038	0.089	
278	0.049	0.056	0.040	0.036	0.033	0.029	0.021	0.020	0.062	0.031	0.016	0.098	0.027	0.053	0.051	0.036	
282	0.034	0.021	0.029	0.044	0.028	0.040	0.037	0.037	0.030	0.041	0.011	0.013	0.031	0.024	0.006	0.016	
286	0.004	0.010	0.013	0.024	0.033	0.024	0.059	0.025	0.048	0.010	0.011	0.013	0.027	0.005	0.032	0.021	
290	0.011	0.014	0.033	0.036	0.031	0.021	0.027	0.031	0.016	0.027	0.038	0.000	0.013	0.003	0.006	0.016	

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population									
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit	
294	0.012	0.028	0.015	0.030	0.005	0.027	0.011	0.006	0.008	0.010	0.016	0.011	0.013	0.032	0.019	0.000	
298	0.011	0.003	0.017	0.012	0.003	0.013	0.005	0.006	0.003	0.007	0.027	0.000	0.009	0.005	0.000	0.000	
302	0.005	0.003	0.004	0.003	0.005	0.029	0.011	0.008	0.011	0.000	0.000	0.000	0.009	0.000	0.000	0.000	
306	0.004	0.000	0.006	0.009	0.003	0.003	0.000	0.000	0.000	0.007	0.000	0.003	0.000	0.003	0.013	0.000	
310	0.000	0.003	0.004	0.000	0.005	0.005	0.005	0.002	0.000	0.010	0.000	0.005	0.000	0.005	0.000	0.000	
314	0.004	0.003	0.006	0.009	0.013	0.019	0.016	0.012	0.005	0.007	0.000	0.072	0.000	0.000	0.006	0.000	
318	0.005	0.010	0.006	0.006	0.008	0.005	0.000	0.006	0.008	0.000	0.005	0.008	0.000	0.019	0.000	0.000	
322	0.010	0.014	0.000	0.006	0.013	0.005	0.005	0.004	0.016	0.000	0.000	0.011	0.022	0.013	0.000	0.000	
326	0.005	0.003	0.002	0.009	0.000	0.003	0.011	0.016	0.003	0.000	0.000	0.008	0.013	0.000	0.006	0.000	
330	0.003	0.003	0.013	0.003	0.003	0.003	0.000	0.004	0.005	0.003	0.005	0.029	0.013	0.005	0.006	0.000	
334	0.008	0.014	0.010	0.003	0.008	0.003	0.005	0.002	0.019	0.003	0.000	0.032	0.009	0.011	0.000	0.000	
338	0.007	0.014	0.002	0.009	0.013	0.008	0.005	0.002	0.013	0.003	0.005	0.048	0.009	0.000	0.013	0.010	
342	0.001	0.003	0.000	0.000	0.018	0.005	0.000	0.002	0.000	0.003	0.005	0.013	0.004	0.008	0.000	0.000	
346	0.000	0.000	0.006	0.000	0.003	0.003	0.000	0.004	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	
350	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
354	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
358	0.003	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
362	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	
366	0.001	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
370	0.000	0.000	0.002	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
382	0.000	0.000	0.002	0.003	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
386	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	
402	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots201</i>																	
	N	366	147	240	170	195	188	94	247	184	148	92	188	113	190	78	96
161	0.015	0.007	0.002	0.006	0.003	0.000	0.000	0.008	0.005	0.010	0.000	0.003	0.004	0.000	0.000	0.000	0.000
165	0.015	0.000	0.006	0.003	0.005	0.011	0.005	0.002	0.003	0.000	0.000	0.005	0.000	0.003	0.000	0.005	
169	0.034	0.017	0.004	0.024	0.021	0.029	0.011	0.020	0.024	0.014	0.011	0.003	0.004	0.011	0.032	0.042	
173	0.143	0.102	0.123	0.144	0.197	0.133	0.133	0.162	0.136	0.172	0.168	0.170	0.173	0.113	0.090	0.078	
178	0.034	0.051	0.048	0.035	0.031	0.029	0.032	0.034	0.019	0.030	0.016	0.021	0.022	0.026	0.032	0.000	
182	0.053	0.051	0.040	0.032	0.051	0.035	0.064	0.047	0.060	0.064	0.027	0.005	0.049	0.037	0.103	0.042	
186	0.022	0.058	0.056	0.059	0.038	0.061	0.027	0.038	0.054	0.051	0.060	0.040	0.044	0.045	0.026	0.083	
190	0.003	0.014	0.017	0.021	0.008	0.027	0.005	0.020	0.043	0.017	0.005	0.005	0.009	0.005	0.019	0.042	

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population										
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit		
194	0.000	0.000	0.000	0.003	0.000	0.000	0.016	0.000	0.005	0.000	0.011	0.003	0.000	0.000	0.000	0.000	0.000	
198	0.001	0.010	0.006	0.006	0.008	0.000	0.000	0.002	0.003	0.014	0.000	0.003	0.000	0.000	0.000	0.000	0.000	
202	0.004	0.000	0.002	0.003	0.008	0.000	0.016	0.002	0.005	0.017	0.011	0.003	0.000	0.011	0.006	0.000	0.000	
206	0.019	0.024	0.015	0.009	0.028	0.029	0.016	0.010	0.005	0.007	0.000	0.005	0.009	0.008	0.000	0.000	0.000	
210	0.030	0.017	0.013	0.009	0.013	0.019	0.027	0.022	0.014	0.020	0.033	0.008	0.027	0.029	0.026	0.016	0.016	
214	0.044	0.041	0.058	0.050	0.044	0.085	0.074	0.063	0.068	0.054	0.087	0.040	0.018	0.063	0.019	0.089	0.089	
218	0.049	0.048	0.044	0.068	0.082	0.090	0.122	0.061	0.046	0.061	0.065	0.024	0.088	0.084	0.071	0.094	0.094	
222	0.160	0.163	0.150	0.159	0.131	0.112	0.101	0.121	0.109	0.084	0.130	0.128	0.173	0.139	0.141	0.099	0.099	
226	0.111	0.129	0.135	0.121	0.105	0.098	0.170	0.152	0.136	0.132	0.147	0.298	0.177	0.205	0.179	0.115	0.115	
230	0.057	0.092	0.100	0.085	0.103	0.106	0.048	0.095	0.122	0.115	0.120	0.085	0.071	0.095	0.071	0.130	0.130	
234	0.072	0.051	0.060	0.053	0.051	0.048	0.053	0.047	0.063	0.034	0.027	0.074	0.066	0.034	0.019	0.083	0.083	
238	0.071	0.061	0.044	0.038	0.038	0.029	0.032	0.030	0.033	0.041	0.011	0.021	0.018	0.045	0.045	0.026	0.026	
242	0.022	0.037	0.048	0.026	0.015	0.027	0.037	0.032	0.022	0.024	0.060	0.011	0.027	0.032	0.032	0.016	0.016	
246	0.026	0.010	0.017	0.032	0.010	0.021	0.005	0.012	0.011	0.034	0.011	0.043	0.013	0.016	0.013	0.042	0.042	
250	0.012	0.014	0.006	0.006	0.005	0.005	0.000	0.010	0.008	0.003	0.000	0.003	0.009	0.000	0.019	0.000	0.000	
254	0.001	0.003	0.002	0.006	0.005	0.005	0.005	0.008	0.005	0.003	0.000	0.000	0.000	0.000	0.058	0.000	0.000	
262	0.000	0.000	0.004	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots208</i>		N	366	147	240	169	192	188	93	243	184	147	92	183	112	188	80	96
150	0.001	0.003	0.004	0.003	0.003	0.000	0.000	0.004	0.003	0.003	0.000	0.000	0.004	0.016	0.000	0.000	0.000	
154	0.004	0.014	0.000	0.000	0.000	0.011	0.000	0.002	0.003	0.000	0.000	0.003	0.004	0.000	0.000	0.005	0.005	
158	0.015	0.010	0.010	0.021	0.008	0.019	0.011	0.016	0.011	0.020	0.000	0.014	0.036	0.053	0.013	0.057	0.057	
162	0.025	0.041	0.023	0.038	0.029	0.066	0.032	0.029	0.063	0.027	0.065	0.005	0.022	0.040	0.056	0.042	0.042	
166	0.034	0.071	0.063	0.074	0.039	0.056	0.054	0.066	0.065	0.078	0.054	0.090	0.027	0.021	0.006	0.036	0.036	
170	0.051	0.061	0.050	0.056	0.044	0.051	0.075	0.062	0.076	0.061	0.027	0.033	0.031	0.037	0.119	0.016	0.016	
174	0.027	0.044	0.046	0.044	0.055	0.027	0.027	0.056	0.035	0.051	0.054	0.022	0.018	0.043	0.025	0.005	0.005	
178	0.045	0.041	0.040	0.036	0.023	0.027	0.032	0.027	0.016	0.034	0.022	0.090	0.031	0.061	0.019	0.052	0.052	
182	0.046	0.037	0.044	0.027	0.039	0.037	0.022	0.045	0.041	0.054	0.027	0.038	0.027	0.064	0.050	0.063	0.063	
186	0.053	0.041	0.023	0.050	0.042	0.040	0.043	0.035	0.016	0.054	0.049	0.063	0.036	0.011	0.069	0.016	0.016	
190	0.057	0.024	0.065	0.038	0.042	0.048	0.032	0.037	0.049	0.031	0.033	0.011	0.036	0.037	0.006	0.005	0.005	
194	0.027	0.031	0.031	0.027	0.031	0.029	0.011	0.023	0.027	0.027	0.016	0.025	0.040	0.021	0.019	0.005	0.005	
198	0.059	0.034	0.035	0.012	0.021	0.005	0.032	0.031	0.014	0.024	0.038	0.027	0.031	0.032	0.044	0.010	0.010	
202	0.019	0.017	0.019	0.021	0.026	0.008	0.022	0.019	0.033	0.014	0.011	0.003	0.022	0.013	0.038	0.005	0.005	

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population										
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit		
206	0.014	0.014	0.021	0.009	0.018	0.021	0.065	0.027	0.005	0.027	0.027	0.011	0.049	0.040	0.006	0.005		
210	0.016	0.007	0.019	0.018	0.036	0.043	0.043	0.031	0.024	0.054	0.049	0.005	0.045	0.037	0.038	0.021		
214	0.052	0.031	0.042	0.030	0.018	0.043	0.070	0.049	0.024	0.037	0.022	0.016	0.045	0.027	0.050	0.010		
218	0.049	0.048	0.052	0.056	0.052	0.043	0.043	0.041	0.027	0.041	0.038	0.022	0.036	0.037	0.038	0.073		
222	0.055	0.044	0.054	0.059	0.073	0.056	0.070	0.056	0.060	0.041	0.038	0.104	0.063	0.066	0.038	0.115		
226	0.040	0.048	0.054	0.053	0.063	0.056	0.038	0.045	0.073	0.058	0.065	0.057	0.031	0.027	0.063	0.083		
230	0.063	0.065	0.067	0.047	0.049	0.032	0.016	0.041	0.073	0.051	0.060	0.049	0.049	0.045	0.075	0.078		
234	0.046	0.034	0.046	0.059	0.063	0.064	0.081	0.053	0.033	0.031	0.049	0.016	0.080	0.056	0.075	0.115		
238	0.033	0.024	0.046	0.036	0.047	0.059	0.065	0.053	0.052	0.037	0.016	0.022	0.063	0.056	0.031	0.063		
242	0.046	0.024	0.021	0.024	0.039	0.035	0.022	0.035	0.033	0.041	0.038	0.008	0.018	0.032	0.000	0.026		
246	0.022	0.044	0.031	0.047	0.039	0.024	0.027	0.021	0.005	0.034	0.049	0.014	0.013	0.016	0.044	0.005		
250	0.019	0.031	0.023	0.038	0.026	0.021	0.000	0.016	0.008	0.017	0.049	0.008	0.009	0.011	0.013	0.000		
254	0.012	0.024	0.021	0.015	0.018	0.021	0.011	0.010	0.041	0.027	0.011	0.005	0.018	0.019	0.000	0.000		
258	0.031	0.027	0.015	0.018	0.010	0.016	0.016	0.033	0.033	0.003	0.038	0.014	0.027	0.011	0.019	0.000		
262	0.012	0.041	0.013	0.009	0.005	0.013	0.011	0.012	0.027	0.000	0.027	0.109	0.031	0.013	0.006	0.031		
266	0.012	0.010	0.008	0.012	0.008	0.008	0.005	0.004	0.005	0.010	0.005	0.104	0.022	0.045	0.019	0.005		
270	0.008	0.010	0.010	0.009	0.003	0.005	0.005	0.004	0.003	0.003	0.016	0.008	0.018	0.003	0.000	0.005		
274	0.001	0.007	0.000	0.006	0.003	0.011	0.005	0.006	0.005	0.003	0.000	0.000	0.004	0.003	0.006	0.042		
278	0.001	0.000	0.004	0.000	0.016	0.000	0.011	0.006	0.016	0.003	0.000	0.003	0.000	0.000	0.019	0.005		
282	0.001	0.000	0.002	0.009	0.000	0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.009	0.005	0.000	0.000		
286	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
290	0.000	0.000	0.000	0.000	0.008	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.003	0.000	0.000		
294	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000		
<i>Ots211</i>																		
N	366	147	237	167	195	189	93	248	186	147	92	185	114	190	80	96		
204	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
208	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
216	0.007	0.000	0.004	0.003	0.000	0.000	0.000	0.002	0.011	0.003	0.000	0.000	0.004	0.000	0.000	0.010		
220	0.011	0.020	0.017	0.018	0.023	0.008	0.027	0.010	0.022	0.020	0.033	0.043	0.048	0.032	0.031	0.000		
224	0.060	0.037	0.032	0.030	0.051	0.050	0.048	0.036	0.022	0.041	0.082	0.154	0.031	0.016	0.063	0.021		
228	0.093	0.099	0.080	0.087	0.085	0.077	0.070	0.058	0.094	0.061	0.120	0.103	0.105	0.066	0.106	0.042		
232	0.137	0.112	0.114	0.123	0.110	0.095	0.102	0.127	0.116	0.150	0.087	0.062	0.118	0.097	0.050	0.099		
236	0.049	0.082	0.091	0.096	0.097	0.095	0.091	0.077	0.070	0.078	0.098	0.062	0.061	0.088	0.089			

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population										
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit		
240	0.042	0.048	0.040	0.030	0.028	0.050	0.038	0.038	0.032	0.037	0.049	0.030	0.031	0.034	0.050	0.016		
244	0.015	0.037	0.034	0.018	0.028	0.032	0.027	0.036	0.040	0.034	0.027	0.049	0.031	0.039	0.044	0.005		
246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000		
248	0.066	0.078	0.049	0.054	0.103	0.082	0.065	0.052	0.038	0.058	0.027	0.035	0.057	0.061	0.081	0.078		
252	0.040	0.020	0.076	0.054	0.044	0.053	0.086	0.058	0.040	0.048	0.054	0.132	0.009	0.045	0.025	0.115		
256	0.048	0.071	0.070	0.054	0.033	0.042	0.043	0.036	0.056	0.058	0.043	0.046	0.083	0.053	0.044	0.073		
260	0.061	0.054	0.051	0.054	0.054	0.058	0.065	0.079	0.065	0.041	0.060	0.057	0.079	0.118	0.050	0.016		
264	0.090	0.082	0.091	0.102	0.072	0.077	0.091	0.077	0.099	0.078	0.092	0.041	0.118	0.116	0.056	0.047		
268	0.067	0.058	0.053	0.051	0.069	0.048	0.027	0.069	0.040	0.065	0.033	0.024	0.044	0.066	0.019	0.026		
270	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000		
272	0.053	0.058	0.034	0.036	0.028	0.026	0.027	0.022	0.070	0.034	0.016	0.027	0.009	0.039	0.013	0.016		
276	0.004	0.010	0.023	0.036	0.038	0.032	0.032	0.040	0.056	0.027	0.016	0.043	0.022	0.013	0.081	0.036		
280	0.059	0.027	0.036	0.030	0.021	0.024	0.016	0.036	0.030	0.020	0.005	0.016	0.057	0.032	0.019	0.057		
284	0.027	0.037	0.032	0.033	0.018	0.013	0.048	0.040	0.024	0.048	0.033	0.011	0.035	0.026	0.000	0.036		
288	0.019	0.010	0.027	0.048	0.023	0.061	0.048	0.026	0.024	0.037	0.043	0.043	0.022	0.026	0.050	0.057		
292	0.025	0.020	0.019	0.027	0.049	0.048	0.016	0.038	0.019	0.020	0.033	0.005	0.018	0.039	0.075	0.146		
296	0.004	0.000	0.015	0.009	0.010	0.024	0.011	0.028	0.011	0.014	0.022	0.014	0.009	0.011	0.031	0.016		
300	0.018	0.027	0.006	0.003	0.015	0.005	0.011	0.010	0.013	0.020	0.005	0.000	0.004	0.008	0.025	0.000		
304	0.001	0.003	0.004	0.006	0.000	0.000	0.000	0.002	0.008	0.003	0.000	0.003	0.004	0.003	0.000	0.000		
312	0.001	0.007	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
347	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
<i>Ots212</i>		N	367	147	238	170	195	189	94	247	186	149	92	185	115	190	80	96
127	0.000	0.003	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	
131	0.000	0.007	0.004	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	
135	0.008	0.003	0.025	0.024	0.015	0.026	0.021	0.022	0.019	0.017	0.022	0.030	0.013	0.037	0.050	0.005	0.005	
139	0.146	0.119	0.107	0.109	0.105	0.116	0.059	0.103	0.094	0.094	0.065	0.186	0.104	0.124	0.081	0.047	0.047	
143	0.441	0.439	0.471	0.535	0.528	0.545	0.596	0.528	0.462	0.507	0.402	0.508	0.509	0.476	0.388	0.427	0.427	
147	0.110	0.082	0.095	0.068	0.062	0.106	0.096	0.081	0.067	0.111	0.120	0.100	0.109	0.089	0.200	0.042	0.042	
151	0.053	0.054	0.071	0.050	0.046	0.071	0.069	0.055	0.073	0.081	0.109	0.024	0.070	0.076	0.088	0.078	0.078	
155	0.061	0.119	0.074	0.068	0.074	0.058	0.064	0.059	0.067	0.057	0.130	0.068	0.017	0.066	0.056	0.214	0.214	
159	0.044	0.031	0.042	0.032	0.051	0.029	0.043	0.028	0.027	0.034	0.054	0.035	0.083	0.076	0.069	0.010	0.010	
163	0.041	0.041	0.042	0.038	0.059	0.019	0.005	0.063	0.062	0.023	0.016	0.024	0.043	0.016	0.038	0.063	0.063	

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population									
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit	
167	0.020	0.031	0.021	0.024	0.010	0.011	0.016	0.024	0.035	0.017	0.016	0.005	0.026	0.026	0.019	0.052	
171	0.005	0.020	0.013	0.012	0.013	0.008	0.000	0.014	0.065	0.013	0.027	0.008	0.013	0.008	0.013	0.047	
175	0.001	0.003	0.004	0.000	0.026	0.003	0.005	0.002	0.016	0.030	0.000	0.000	0.000	0.003	0.000	0.000	
179	0.015	0.014	0.006	0.003	0.003	0.000	0.000	0.006	0.008	0.007	0.005	0.000	0.004	0.000	0.000	0.000	
183	0.000	0.000	0.002	0.009	0.003	0.000	0.000	0.002	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	
187	0.005	0.007	0.008	0.003	0.000	0.008	0.011	0.004	0.000	0.003	0.005	0.005	0.000	0.000	0.000	0.000	
191	0.004	0.003	0.002	0.006	0.005	0.000	0.011	0.002	0.003	0.000	0.005	0.005	0.000	0.000	0.000	0.000	
195	0.018	0.007	0.002	0.009	0.000	0.000	0.005	0.004	0.003	0.003	0.005	0.000	0.000	0.003	0.000	0.000	
199	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
203	0.003	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
207	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
211	0.003	0.007	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
215	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
223	0.010	0.007	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
231	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.004	0.000	0.000	0.000	
<i>Ots213</i>	N	365	147	240	169	194	189	94	248	188	149	92	187	113	189	80	96
	214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
	218	0.004	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	222	0.003	0.000	0.010	0.003	0.003	0.003	0.005	0.006	0.003	0.007	0.005	0.000	0.022	0.005	0.000	0.000
	226	0.008	0.003	0.019	0.003	0.015	0.005	0.000	0.004	0.000	0.007	0.016	0.000	0.000	0.005	0.031	0.000
	230	0.033	0.034	0.023	0.033	0.015	0.021	0.021	0.030	0.029	0.034	0.005	0.254	0.049	0.029	0.019	0.068
	234	0.040	0.024	0.023	0.041	0.021	0.037	0.064	0.040	0.053	0.047	0.049	0.019	0.018	0.024	0.038	0.010
	238	0.147	0.102	0.115	0.107	0.103	0.098	0.053	0.119	0.090	0.107	0.103	0.184	0.159	0.077	0.075	0.094
	242	0.086	0.105	0.088	0.139	0.119	0.116	0.117	0.113	0.117	0.107	0.120	0.078	0.155	0.077	0.144	0.161
	246	0.049	0.048	0.054	0.080	0.046	0.071	0.059	0.056	0.045	0.044	0.060	0.053	0.084	0.045	0.019	0.057
	250	0.033	0.017	0.023	0.018	0.013	0.019	0.011	0.010	0.029	0.023	0.011	0.013	0.027	0.019	0.025	0.078
	254	0.003	0.017	0.013	0.015	0.010	0.013	0.021	0.002	0.016	0.000	0.000	0.000	0.027	0.029	0.006	0.000
	258	0.005	0.000	0.008	0.006	0.021	0.026	0.027	0.022	0.013	0.010	0.022	0.003	0.004	0.011	0.013	0.000
	262	0.026	0.017	0.008	0.018	0.036	0.024	0.016	0.016	0.016	0.027	0.005	0.003	0.013	0.019	0.031	0.010
	266	0.008	0.014	0.008	0.015	0.021	0.013	0.021	0.016	0.016	0.020	0.011	0.016	0.000	0.024	0.000	0.010
	270	0.023	0.010	0.010	0.009	0.005	0.005	0.016	0.006	0.005	0.017	0.011	0.003	0.000	0.008	0.019	0.005
	274	0.008	0.014	0.023	0.027	0.015	0.011	0.005	0.016	0.037	0.020	0.033	0.021	0.018	0.021	0.025	0.000

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population										
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit		
278	0.033	0.048	0.031	0.036	0.021	0.063	0.027	0.038	0.043	0.054	0.016	0.019	0.009	0.008	0.013	0.000		
282	0.051	0.065	0.027	0.021	0.057	0.034	0.021	0.040	0.029	0.047	0.054	0.016	0.027	0.034	0.038	0.031		
286	0.041	0.061	0.058	0.053	0.075	0.058	0.064	0.058	0.051	0.050	0.038	0.048	0.035	0.048	0.094	0.094		
290	0.062	0.044	0.060	0.062	0.052	0.045	0.080	0.054	0.051	0.047	0.120	0.053	0.097	0.087	0.056	0.115		
294	0.101	0.102	0.129	0.089	0.095	0.106	0.080	0.101	0.104	0.060	0.082	0.043	0.058	0.098	0.050	0.089		
298	0.055	0.078	0.069	0.089	0.080	0.074	0.112	0.052	0.080	0.074	0.027	0.043	0.049	0.079	0.063	0.073		
302	0.070	0.054	0.069	0.041	0.041	0.050	0.074	0.077	0.069	0.047	0.060	0.064	0.044	0.058	0.100	0.042		
306	0.038	0.065	0.058	0.038	0.077	0.056	0.053	0.073	0.077	0.064	0.087	0.027	0.027	0.058	0.031	0.010		
310	0.053	0.031	0.040	0.038	0.018	0.032	0.016	0.018	0.019	0.044	0.038	0.013	0.053	0.053	0.088	0.047		
314	0.011	0.044	0.017	0.015	0.028	0.011	0.011	0.018	0.003	0.020	0.022	0.024	0.013	0.050	0.025	0.005		
318	0.004	0.000	0.010	0.006	0.010	0.003	0.011	0.010	0.005	0.020	0.000	0.003	0.009	0.019	0.000	0.000		
322	0.003	0.000	0.002	0.000	0.003	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000		
326	0.001	0.003	0.000	0.000	0.000	0.005	0.011	0.002	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000		
334	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
358	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000		
<i>Ots3M</i>		N	367	147	239	169	195	189	94	248	188	149	91	189	115	189	80	96
138	0.134	0.139	0.126	0.136	0.167	0.132	0.106	0.145	0.149	0.124	0.209	0.056	0.143	0.153	0.125	0.229		
140	0.003	0.007	0.000	0.003	0.003	0.000	0.005	0.002	0.000	0.003	0.000	0.003	0.000	0.003	0.000	0.000	0.000	
142	0.031	0.024	0.031	0.018	0.031	0.008	0.027	0.024	0.035	0.020	0.011	0.008	0.009	0.026	0.025	0.005	0.005	
144	0.044	0.024	0.048	0.038	0.072	0.034	0.059	0.052	0.048	0.030	0.049	0.008	0.043	0.063	0.013	0.005	0.005	
146	0.343	0.405	0.326	0.364	0.323	0.294	0.314	0.371	0.351	0.332	0.253	0.569	0.370	0.315	0.344	0.490		
148	0.441	0.388	0.456	0.432	0.405	0.524	0.463	0.399	0.402	0.480	0.467	0.354	0.409	0.439	0.488	0.271		
150	0.004	0.007	0.010	0.009	0.000	0.008	0.027	0.006	0.016	0.010	0.005	0.003	0.026	0.000	0.006	0.000	0.000	
152	0.000	0.007	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots9</i>		N	367	147	240	170	195	189	94	247	188	149	91	189	115	190	80	96
101	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
103	0.003	0.000	0.002	0.003	0.008	0.005	0.005	0.002	0.000	0.007	0.005	0.000	0.004	0.008	0.006	0.000	0.000	
105	0.519	0.517	0.513	0.550	0.526	0.550	0.479	0.524	0.497	0.527	0.516	0.481	0.496	0.579	0.531	0.630		
107	0.456	0.452	0.456	0.415	0.451	0.392	0.457	0.429	0.415	0.433	0.451	0.505	0.461	0.397	0.388	0.359		
109	0.019	0.027	0.027	0.032	0.015	0.048	0.053	0.043	0.088	0.034	0.027	0.013	0.039	0.016	0.075	0.010		
111	0.001	0.000	0.002	0.000	0.000	0.005	0.005	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix A. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population								
								Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>OtsG47</i>																
N	367	147	238	170	195	189	94	249	187	149	92	187	115	190	80	96
156	0.947	0.959	0.939	0.938	0.941	0.968	0.931	0.960	0.965	0.953	0.951	0.973	0.913	0.961	0.938	0.932
160	0.001	0.000	0.004	0.006	0.010	0.003	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
164	0.035	0.024	0.044	0.041	0.023	0.021	0.032	0.020	0.019	0.037	0.022	0.019	0.013	0.016	0.044	0.036
168	0.000	0.003	0.000	0.000	0.010	0.000	0.016	0.004	0.005	0.003	0.011	0.000	0.043	0.016	0.006	0.000
176	0.014	0.014	0.008	0.012	0.013	0.005	0.011	0.012	0.011	0.003	0.000	0.008	0.030	0.008	0.013	0.021
180	0.003	0.000	0.004	0.003	0.003	0.003	0.005	0.004	0.000	0.003	0.005	0.000	0.000	0.000	0.000	0.000
184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
<i>Ssa408</i>																
N	366	146	240	170	195	188	94	245	187	148	92	189	113	188	79	95
188	0.040	0.055	0.042	0.041	0.038	0.027	0.021	0.037	0.021	0.030	0.033	0.019	0.022	0.019	0.025	0.005
192	0.005	0.003	0.004	0.006	0.003	0.011	0.000	0.010	0.003	0.003	0.000	0.003	0.018	0.005	0.000	0.000
196	0.022	0.024	0.040	0.035	0.046	0.051	0.048	0.043	0.064	0.034	0.109	0.085	0.022	0.051	0.057	0.179
200	0.010	0.007	0.010	0.000	0.000	0.013	0.000	0.002	0.005	0.003	0.005	0.005	0.000	0.005	0.000	0.058
204	0.010	0.003	0.013	0.012	0.018	0.016	0.011	0.002	0.003	0.007	0.005	0.003	0.013	0.000	0.006	0.000
208	0.064	0.068	0.050	0.059	0.041	0.064	0.037	0.055	0.037	0.084	0.033	0.045	0.066	0.074	0.044	0.163
212	0.034	0.048	0.025	0.018	0.015	0.013	0.016	0.022	0.013	0.010	0.022	0.008	0.022	0.011	0.006	0.021
216	0.016	0.031	0.015	0.029	0.018	0.019	0.005	0.022	0.011	0.020	0.043	0.005	0.013	0.021	0.025	0.005
220	0.074	0.045	0.052	0.038	0.054	0.053	0.032	0.045	0.059	0.034	0.027	0.024	0.053	0.045	0.070	0.042
224	0.138	0.096	0.115	0.112	0.123	0.093	0.122	0.102	0.142	0.091	0.179	0.045	0.102	0.109	0.089	0.063
228	0.135	0.161	0.144	0.165	0.154	0.197	0.154	0.184	0.152	0.206	0.152	0.103	0.181	0.237	0.152	0.105
232	0.189	0.188	0.256	0.197	0.238	0.178	0.160	0.180	0.222	0.176	0.125	0.370	0.252	0.176	0.253	0.126
236	0.172	0.168	0.119	0.124	0.164	0.146	0.234	0.143	0.158	0.193	0.163	0.085	0.071	0.152	0.196	0.089
240	0.066	0.065	0.071	0.112	0.077	0.085	0.101	0.102	0.075	0.078	0.038	0.190	0.097	0.051	0.051	0.121
244	0.020	0.031	0.040	0.041	0.010	0.029	0.043	0.031	0.029	0.027	0.027	0.008	0.049	0.032	0.013	0.021
248	0.005	0.007	0.006	0.012	0.000	0.003	0.016	0.020	0.005	0.000	0.033	0.000	0.013	0.000	0.013	0.000
252	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.003	0.005	0.003	0.004	0.013	0.000	0.000

Appendix B. Frequencies of alleles at SNP loci for Chinook salmon populations from the Kuskokwim Management Area. Populations are designated as follows: Goodnews (Goo), Arolik (Aro), Kanektok (Kan), Eek, Kwethluk (Kwe), Kisaralik (Kis), Tuluksak (Tul), Salmon (Sal), George (Geo), Kogrukluuk (Kog), Stony (Sto), Tatlawiksuk (Tat), Cheeneetnuk (Che), Gagarayah (Gag), and Takotna (Tak) rivers and Pitka Fork (Pit).

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_GTH2B550</i>																	
N	366	146	244	170	190	185	93	246	189	149	84	189	114	190	80	94	
C	0.678	0.702	0.697	0.688	0.661	0.619	0.672	0.636	0.728	0.698	0.696	0.704	0.732	0.700	0.681	0.910	
G	0.322	0.298	0.303	0.312	0.339	0.381	0.328	0.364	0.272	0.302	0.304	0.296	0.268	0.300	0.319	0.090	
<i>Ots_NOD1</i>																	
N	367	147	241	169	189	188	91	249	188	147	92	185	115	190	80	94	
C	0.520	0.622	0.583	0.592	0.693	0.633	0.593	0.643	0.630	0.643	0.668	0.673	0.574	0.574	0.594	0.553	
G	0.480	0.378	0.417	0.408	0.307	0.367	0.407	0.357	0.370	0.357	0.332	0.327	0.426	0.426	0.406	0.447	
<i>Ots_E2-275</i>																	
N	366	145	244	169	190	188	88	247	190	148	92	190	112	190	79	96	
A	0.723	0.748	0.713	0.805	0.737	0.798	0.710	0.755	0.816	0.753	0.734	0.900	0.737	0.782	0.785	0.708	
G	0.277	0.252	0.287	0.195	0.263	0.202	0.290	0.245	0.184	0.247	0.266	0.100	0.263	0.218	0.215	0.292	
<i>Ots_arf-188</i>																	
N	367	147	242	171	190	188	92	250	190	149	92	190	116	187	80	96	
G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots_AsnRS-60</i>																	
N	367	146	244	171	191	189	89	249	189	149	91	190	116	190	78	95	
C	0.676	0.575	0.660	0.617	0.691	0.563	0.663	0.643	0.624	0.648	0.637	0.608	0.612	0.584	0.724	0.800	
T	0.324	0.425	0.340	0.383	0.309	0.437	0.337	0.357	0.376	0.352	0.363	0.392	0.388	0.416	0.276	0.200	
<i>Ots_C3N3</i>																	
N	367	146	240	168	100	186	92	246	189	99	90	189	115	188	80	96	
G	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
T	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots_ETIF1A</i>																	
N	365	147	241	171	190	187	93	250	187	146	90	183	115	190	80	95	
A	0.541	0.595	0.620	0.515	0.592	0.604	0.575	0.574	0.578	0.640	0.611	0.587	0.543	0.611	0.656	0.574	
C	0.459	0.405	0.380	0.485	0.408	0.396	0.425	0.426	0.422	0.360	0.389	0.413	0.457	0.389	0.344	0.426	

Appendix B. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_FARSLA-220</i>																	
N	367	147	244	170	190	189	93	250	190	149	91	189	116	189	80	96	
A	0.035	0.054	0.018	0.024	0.018	0.011	0.027	0.014	0.024	0.007	0.011	0.061	0.047	0.011	0.013	0.021	
G	0.965	0.946	0.982	0.976	0.982	0.989	0.973	0.986	0.976	0.993	0.989	0.939	0.953	0.989	0.988	0.979	
<i>Ots_FGF6A</i>																	
N	367	147	242	171	191	188	91	250	190	149	92	189	115	190	80	96	
G	0.471	0.449	0.486	0.430	0.521	0.479	0.456	0.468	0.563	0.510	0.549	0.648	0.457	0.445	0.438	0.573	
T	0.529	0.551	0.514	0.570	0.479	0.521	0.544	0.532	0.437	0.490	0.451	0.352	0.543	0.555	0.563	0.427	
<i>Ots_GH2</i>																	
N	364	147	244	168	189	186	92	245	190	147	91	183	81	190	80	82	
A	0.820	0.789	0.766	0.762	0.799	0.798	0.777	0.747	0.763	0.779	0.714	0.760	0.802	0.705	0.744	0.720	
T	0.180	0.211	0.234	0.238	0.201	0.202	0.223	0.253	0.237	0.221	0.286	0.240	0.198	0.295	0.256	0.280	
<i>Ots_GPDH-338</i>																	
N	367	147	243	171	191	189	92	250	190	149	92	189	116	189	80	95	
A	0.151	0.173	0.156	0.120	0.168	0.143	0.185	0.170	0.163	0.195	0.185	0.251	0.164	0.159	0.150	0.184	
G	0.849	0.827	0.844	0.880	0.832	0.857	0.815	0.830	0.837	0.805	0.815	0.749	0.836	0.841	0.850	0.816	
<i>Ots_GPH-318</i>																	
N	366	147	244	170	190	189	92	251	189	149	90	189	116	190	80	96	
C	0.918	0.908	0.916	0.918	0.855	0.910	0.908	0.920	0.862	0.896	0.928	0.958	0.948	0.918	0.900	0.964	
T	0.082	0.092	0.084	0.082	0.145	0.090	0.092	0.080	0.138	0.104	0.072	0.042	0.052	0.082	0.100	0.036	
<i>Ots_GST-207</i>																	
N	364	147	241	171	189	189	93	251	189	149	91	189	115	190	80	96	
C	0.948	0.956	0.956	0.977	0.976	0.984	0.946	0.952	0.963	0.960	0.951	0.952	0.943	0.932	0.950	0.974	
T	0.052	0.044	0.044	0.023	0.024	0.016	0.054	0.048	0.037	0.040	0.049	0.048	0.057	0.068	0.050	0.026	
<i>Ots_GST-375</i>																	
N	367	147	243	171	190	189	93	251	190	149	91	189	116	190	80	96	
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots_HGFA-446</i>																	
N	367	147	244	171	191	189	91	251	189	149	92	189	116	190	80	95	
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix B. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_hnRNPL-533</i>																	
N	365	146	243	170	189	187	91	249	189	149	91	188	115	190	80	96	
A	0.271	0.226	0.267	0.241	0.249	0.217	0.269	0.225	0.238	0.245	0.269	0.207	0.300	0.253	0.175	0.344	
T	0.729	0.774	0.733	0.759	0.751	0.783	0.731	0.775	0.762	0.755	0.731	0.793	0.700	0.747	0.825	0.656	
<i>Ots_HSP90B-100</i>																	
N	367	147	244	170	190	188	93	248	190	149	91	188	116	190	80	96	
C	0.857	0.816	0.844	0.821	0.837	0.872	0.871	0.845	0.871	0.896	0.907	0.793	0.875	0.839	0.825	0.792	
T	0.143	0.184	0.156	0.179	0.163	0.128	0.129	0.155	0.129	0.104	0.093	0.207	0.125	0.161	0.175	0.208	
<i>Ots_IGF-I.1-76</i>																	
N	367	147	241	171	190	188	90	248	188	149	92	188	113	189	79	95	
A	0.574	0.520	0.421	0.439	0.434	0.378	0.439	0.379	0.497	0.389	0.446	0.258	0.460	0.376	0.392	0.363	
T	0.426	0.480	0.579	0.561	0.566	0.622	0.561	0.621	0.503	0.611	0.554	0.742	0.540	0.624	0.608	0.637	
<i>Ots_Ikaros-250</i>																	
N	365	147	242	167	189	188	92	251	190	149	92	188	115	189	80	94	
A	0.149	0.122	0.149	0.171	0.146	0.178	0.125	0.167	0.161	0.131	0.207	0.090	0.148	0.188	0.163	0.245	
G	0.851	0.878	0.851	0.829	0.854	0.822	0.875	0.833	0.839	0.869	0.793	0.910	0.852	0.812	0.838	0.755	
<i>Ots_LEI-292</i>																	
N	365	146	244	171	190	189	93	249	190	149	91	189	115	190	80	96	
A	0.036	0.065	0.053	0.050	0.066	0.040	0.038	0.044	0.076	0.057	0.033	0.082	0.022	0.024	0.050	0.031	
G	0.964	0.935	0.947	0.950	0.934	0.960	0.962	0.956	0.924	0.943	0.967	0.918	0.978	0.976	0.950	0.969	
<i>Ots_MHCl</i>																	
N	367	146	244	169	190	189	91	240	190	148	91	189	113	190	80	96	
A	0.549	0.548	0.516	0.479	0.513	0.545	0.549	0.529	0.545	0.534	0.511	0.550	0.460	0.529	0.519	0.505	
G	0.451	0.452	0.484	0.521	0.487	0.455	0.451	0.471	0.455	0.466	0.489	0.450	0.540	0.471	0.481	0.495	
<i>Ots_MHC2</i>																	
N	365	146	243	170	190	187	91	245	189	149	92	190	115	190	77	96	
G	0.167	0.127	0.101	0.085	0.103	0.080	0.033	0.059	0.048	0.070	0.060	0.250	0.052	0.087	0.091	0.010	
T	0.833	0.873	0.899	0.915	0.897	0.920	0.967	0.941	0.952	0.930	0.940	0.750	0.948	0.913	0.909	0.990	
<i>Ots_ZNF330-181</i>																	
N	367	147	244	171	191	188	93	251	190	149	92	190	116	190	80	95	
A	0.014	0.020	0.010	0.006	0.010	0.011	0.000	0.000	0.003	0.003	0.011	0.000	0.026	0.005	0.006	0.016	
G	0.986	0.980	0.990	0.994	0.990	0.989	1.000	1.000	0.997	0.997	0.989	1.000	0.974	0.995	0.994	0.984	

Appendix B. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_LWSop-638</i>																	
N	367	147	243	170	191	189	91	250	190	149	92	190	116	190	80	96	
C	0.033	0.037	0.025	0.053	0.024	0.063	0.071	0.036	0.058	0.040	0.038	0.016	0.043	0.058	0.031	0.036	
T	0.967	0.963	0.975	0.947	0.976	0.937	0.929	0.964	0.942	0.960	0.962	0.984	0.957	0.942	0.969	0.964	
<i>Ots_SWS1op-182</i>																	
N	367	147	243	170	191	189	86	251	188	149	92	190	116	190	80	96	
A	0.571	0.670	0.658	0.638	0.759	0.730	0.680	0.733	0.614	0.765	0.603	0.692	0.642	0.682	0.650	0.484	
T	0.429	0.330	0.342	0.362	0.241	0.270	0.320	0.267	0.386	0.235	0.397	0.308	0.358	0.318	0.350	0.516	
<i>Ots_P450</i>																	
N	366	147	244	171	191	187	88	246	189	149	87	186	116	188	80	96	
A	0.182	0.204	0.166	0.225	0.264	0.257	0.233	0.254	0.198	0.208	0.138	0.159	0.181	0.168	0.188	0.125	
T	0.818	0.796	0.834	0.775	0.736	0.743	0.767	0.746	0.802	0.792	0.862	0.841	0.819	0.832	0.813	0.875	
<i>Ots_Prl2</i>																	
N	365	147	241	162	191	186	85	243	185	149	92	171	115	190	80	96	
A	0.659	0.667	0.656	0.657	0.678	0.712	0.665	0.673	0.705	0.671	0.668	0.778	0.657	0.679	0.744	0.651	
G	0.341	0.333	0.344	0.343	0.322	0.288	0.335	0.327	0.295	0.329	0.332	0.222	0.343	0.321	0.256	0.349	
<i>Ots_ins-115</i>																	
N	367	147	244	170	190	189	91	251	190	149	92	190	116	190	80	96	
A	0.977	0.986	0.973	0.982	0.987	0.987	0.978	0.974	0.976	0.983	0.995	0.987	0.970	0.987	1.000	1.000	
G	0.023	0.014	0.027	0.018	0.013	0.013	0.022	0.026	0.024	0.017	0.005	0.013	0.030	0.013	0.000	0.000	
<i>Ots_PSMB1-197</i>																	
N	367	147	241	171	190	189	93	251	190	149	91	189	116	190	80	96	
T	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
<i>Ots_SClkF2R2-135</i>																	
N	365	147	240	167	190	188	91	249	187	147	91	187	115	190	80	96	
A	0.458	0.391	0.410	0.383	0.424	0.402	0.434	0.438	0.487	0.466	0.538	0.412	0.378	0.397	0.513	0.234	
T	0.542	0.609	0.590	0.617	0.576	0.598	0.566	0.562	0.513	0.534	0.462	0.588	0.622	0.603	0.488	0.766	
<i>Ots_SERPC1-209</i>																	
N	367	147	243	171	190	189	93	251	189	149	91	189	115	190	80	96	
A	0.041	0.031	0.039	0.029	0.037	0.042	0.016	0.024	0.040	0.050	0.060	0.037	0.030	0.039	0.050	0.000	
T	0.959	0.969	0.961	0.971	0.963	0.958	0.984	0.976	0.960	0.950	0.940	0.963	0.970	0.961	0.950	1.000	

Appendix B. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_SL</i>																	
N	365	147	243	170	191	186	79	246	188	149	89	190	110	190	79	96	
A	0.649	0.677	0.617	0.674	0.681	0.720	0.734	0.699	0.662	0.715	0.730	0.726	0.773	0.737	0.709	0.828	
G	0.351	0.323	0.383	0.326	0.319	0.280	0.266	0.301	0.338	0.285	0.270	0.274	0.227	0.263	0.291	0.172	
<i>Ots_TAPBP</i>																	
N	367	147	242	170	190	189	93	248	188	149	91	189	115	189	80	96	
C	0.879	0.905	0.893	0.882	0.842	0.881	0.914	0.905	0.891	0.886	0.841	0.937	0.887	0.905	0.919	0.969	
T	0.121	0.095	0.107	0.118	0.158	0.119	0.086	0.095	0.109	0.114	0.159	0.063	0.113	0.095	0.081	0.031	
<i>Ots_Tnsf</i>																	
N	364	142	242	168	190	187	89	243	189	149	91	189	114	190	79	96	
A	0.110	0.106	0.132	0.104	0.087	0.094	0.107	0.117	0.082	0.081	0.176	0.058	0.167	0.084	0.146	0.307	
G	0.890	0.894	0.868	0.896	0.913	0.906	0.893	0.883	0.918	0.919	0.824	0.942	0.833	0.916	0.854	0.693	
<i>Ots_u202-161</i>																	
N	367	147	244	171	191	188	90	251	190	149	92	190	116	190	80	96	
A	0.010	0.007	0.008	0.023	0.016	0.013	0.017	0.010	0.039	0.020	0.005	0.032	0.017	0.011	0.013	0.000	
T	0.990	0.993	0.992	0.977	0.984	0.987	0.983	0.990	0.961	0.980	0.995	0.968	0.983	0.989	0.988	1.000	
<i>Ots_u211-85</i>																	
N	367	147	243	171	191	188	92	250	189	149	92	190	115	190	80	96	
C	0.977	0.986	0.990	0.997	0.982	0.971	0.984	0.988	0.979	0.993	0.989	0.992	0.987	0.982	1.000	0.995	
T	0.023	0.014	0.010	0.003	0.018	0.029	0.016	0.012	0.021	0.007	0.011	0.008	0.013	0.018	0.000	0.005	
<i>Ots_u212-158</i>																	
N	367	146	243	171	190	186	93	251	190	148	92	189	116	189	80	95	
A	0.044	0.051	0.074	0.050	0.118	0.089	0.059	0.080	0.092	0.081	0.076	0.040	0.082	0.093	0.081	0.200	
G	0.956	0.949	0.926	0.950	0.882	0.911	0.941	0.920	0.908	0.919	0.924	0.960	0.918	0.907	0.919	0.800	
<i>Ots_u4-92</i>																	
N	366	147	241	167	191	188	86	248	189	149	91	189	113	189	80	96	
C	0.092	0.068	0.087	0.084	0.107	0.040	0.058	0.083	0.071	0.104	0.055	0.029	0.097	0.106	0.025	0.063	
T	0.908	0.932	0.913	0.916	0.893	0.960	0.942	0.917	0.929	0.896	0.945	0.971	0.903	0.894	0.975	0.938	
<i>Ots_u6-75</i>																	
N	365	147	243	169	191	188	90	249	189	149	92	190	116	189	80	95	
C	0.874	0.857	0.893	0.873	0.859	0.896	0.861	0.837	0.844	0.876	0.897	0.897	0.845	0.897	0.794	0.805	
T	0.126	0.143	0.107	0.127	0.141	0.104	0.139	0.163	0.156	0.124	0.103	0.103	0.155	0.103	0.206	0.195	

Appendix B. Continued

	Goo	Aro	Kan	Eek	Tul	Kis	Kwe	Population	Sal	Geo	Kog	Sto	Tat	Che	Gag	Tak	Pit
<i>Ots_Zp3b-215</i>																	
N	366	146	241	170	191	188	89	251	190	149	92	188	116	189	79	94	
G	0.937	0.935	0.942	0.924	0.924	0.944	0.949	0.920	0.924	0.923	0.875	0.888	0.935	0.862	0.937	0.846	
T	0.063	0.065	0.058	0.076	0.076	0.056	0.051	0.080	0.076	0.077	0.125	0.112	0.065	0.138	0.063	0.154	
<i>Ots_PGK-54</i>																	
N	367	147	244	171	190	189	92	250	190	149	91	189	116	189	80	96	
A	0.003	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
T	0.997	1.000	0.998	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>Ots_RAG3</i>																	
N	367	147	243	169	190	189	93	251	190	149	91	189	116	190	80	95	
C	0.015	0.003	0.010	0.003	0.008	0.005	0.011	0.004	0.008	0.007	0.011	0.000	0.004	0.003	0.000	0.032	
T	0.985	0.997	0.990	0.997	0.992	0.995	0.989	0.996	0.992	0.993	0.989	1.000	0.996	0.997	1.000	0.968	
<i>Ots_S7-1</i>																	
N	367	146	241	165	190	188	93	242	188	149	91	188	112	189	79	96	
C	0.800	0.784	0.782	0.770	0.858	0.771	0.823	0.857	0.782	0.775	0.863	0.854	0.799	0.844	0.886	0.729	
T	0.200	0.216	0.218	0.230	0.142	0.229	0.177	0.143	0.218	0.225	0.137	0.146	0.201	0.156	0.114	0.271	
<i>Ots_unkn526</i>																	
N	365	147	243	171	190	189	93	248	190	149	91	188	116	190	79	95	
A	0.163	0.146	0.148	0.216	0.134	0.198	0.172	0.127	0.155	0.154	0.148	0.168	0.181	0.205	0.095	0.047	
G	0.837	0.854	0.852	0.784	0.866	0.802	0.828	0.873	0.845	0.846	0.852	0.832	0.819	0.795	0.905	0.953	

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