Alaska Sustainable Salmon Fund
Project Completion Report

Abstract:
The goal of this project was to develop a genetic baseline for Kenai River Chinook salmon; the
baseline genetics data will be added to the coast-wide genetic database maintained by the Pacific
Salmon Commission Chinook Technical Committee. The objectives within this goal were to:
compare samples collected from spawning aggregates within the Kenai River drainage to look
for genotype differences; and estimate and quantify overlap in the run timing of tributary and
mainstem spawning Kenai River Chinook salmon. Prior to this project (2003 and 2004), during
(2005-2007), and in subsequent years Chinook salmon in spawning condition were sampled in
10 different mainstem areas and tributaries of the Kenai River to develop a genetic baseline
database. Additionally, mixture samples for tributary versus mainstem run timing estimates were
collected via an existing netting program as they entered the lower Kenai River, during years
prior to the project (2003 and 2004), during this project (2005-2007), and in subsequent years.
The results from some of the lower river netting collections during years outside the scope of the
project are included in this report. Based on the lower river mixture sampling, most of the
Chinook salmon that enter the Kenai River prior to the middle of June are of tributary origin;
depending on the year, after the second or third week in June mainstem fish become more
predominant. Few tributary spawning Chinook salmon enter the Kenai in July. Results from the
lower river sport fishery mixture sampling demonstrate that: (1) most of the harvest in May and
June is of tributary-bound fish, and, (2) nearly all of the harvest in July is of mainstem-bound
fish. The middle river sport fishery mixture sampling results indicate that: (1) most of the
harvest in June is of tributary-bound fish; (2) the harvest in the first two weeks of July is nearly
an equal mix of tributary- and mainstem-bound fish; and, (3) nearly all of the harvest in the last
two weeks in July is of mainstem-bound fish. These results will be extremely useful in
generating estimates of escapement of tributary and mainstem Chinook, escapement goal

analyses for these stocks, as well as estimating harvest in mixed-stock fisheries outside of the Kenai River drainage.

Methods
1) Sample collection
   a) Baseline samples were collected from mainstem and tributary spawning locations. Collecting tissue from Chinook salmon for genetic analysis was non-lethal; a ½ inch sized piece of tissue from the axillary process was removed from each fish sampled, placed in a 2mL cryovial and completely covered with a Sigma Reagent Grade 95% Alcohol (Sigma Cat. # R 8382) buffer solution such that the liquid/tissue ratio was approximately 3:1. Samples were transferred to the Alaska Department of Game, Fish and Parks Gene Conservation Laboratory in Anchorage and stored at room temperature until analyzed. All Chinook salmon sampled for tissue were also sampled for age, sex and length. After sampling Chinook salmon were released alive back to the water.

   b) Mixture samples were taken from the lower river netting, lower river sport fishery, and middle river sport fishery. The tissue collected from adult Chinook salmon for genetic analysis was a ½ inch sized piece of tissue from the axillary process. Each tissue was placed in a 2mL cryovial and completely covered with a Sigma Reagent Grade 95% Alcohol (Sigma Cat. # R 8382) buffer solution such that the liquid/tissue ratio was approximately 3:1. Samples were transferred to the Gene Conservation Laboratory and stored at room temperature until analyzed. All Chinook salmon sampled for tissue were also sampled for age, sex and length and the time and location of capture was recorded.

   c) Sample size goals were determined at the beginning of the project to meet specific precision and accuracy goals. For baseline samples, the sample size goal for each spawning location was set to estimate allelic frequencies at each locus to within 5% of the true values 90% of the time under a worst-case scenario. This level of precision requires identification of 403 alleles (Thompson 1987). Given two copies of the genetic information at each locus in each diploid individual, and assuming random mating, tissue samples from a total of approximately 200 fish at each location were needed to meet the stated precision criteria. The same rationale was used to set the sample sizes for sampling the test and sport fisheries. The following sample size goals were set based on precision and accuracy goals stated in the project objectives:

      i) Lower river netting – To estimate stock composition of mainstem- and tributary-origin Chinook salmon in weekly or biweekly periods between May 16 and August 10 sample size targets were set at 30-100 samples per stratum to achieve estimates that are within 10% of the true values 90% of the time.
      ii) Lower river sport fishery – To estimate the stock composition of mainstem-origin and tributary-origin Chinook salmon caught in the sport fishery downstream of the Soldotna Bridge between May 16 and July 31 sample size targets were set at 26-50 samples per week to achieve estimates that are within 25% of the true values 90% of the time.

iii) Middle river sport fishery –To estimate stock composition of mainstem-origin and tributary-origin Chinook salmon harvested in the sport harvest between Moose River and the Soldotna Bridge in two-week intervals between approximately June 1 and July 31 sample size targets were set at 45 samples per stratum to achieve estimates that are within 15 % of the true value 90% of the time.

2) Laboratory Analysis
   a) Genotyping
      i) All Genomic DNA were extracted using a DNeasy® 96 Tissue Kit by QIAGEN® (Valencia, CA). Fifty-two SNP markers were assayed; 1 mitochondrial and 51 nuclear DNA. Genotypes for these SNPs were screened using 2 platforms.

         (1) For some of the samples, SNP genotyping was performed in 384-well reaction plates. Each reaction was conducted in a 5μL volume consisting of 5-40ng of template DNA, 1x TaqMan® Universal PCR Master Mix (Applied Biosystems) and 1x TaqMan® SNP Genotyping Assay (Applied Biosystems). Thermal cycling was performed on a Dual 384-Well GeneAmp® PCR System 9700 (Applied Biosystems) as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92°C for 1s and annealing/extension temperature for 1.0 or 1.5 min. The plates were scanned on an Applied Biosystems Prism 7900HT Sequence Detection System after amplification and scored using Applied Biosystems' Sequence Detection Software (SDS) version 2.2.

         (2) Other samples were genotyped using a BioMark 48.48 Dynamic Array (Fluidigm http://www.fluidigm.com/biomark_ genotyping.htm). The BioMark 48.48 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame. On one side of the frame are 48 inlets to accept the sample DNA from each individual fish, and on the other are 48 inlets to accept the assays for each of the SNP markers. Once in the wells, the components are pressurized into the chip using the NanoFlex 4-IFC Controller. The 48 samples and 48 assays are then systematically combined into 2,304 parallel reactions. Each reaction was conducted in a 6.75 nL volume consisting of 1xTaqMan Universal Buffer (Applied Biosystems), 1.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems), 9 mM of each polymerase chain reaction (PCR) primer, 2 mM of each probe, 1xDA Assay Loading Buffer (Fluidigm), 12.5xROX (Invitrogen), and 0.01% Tween-20. Thermal cycling were performed on a BioMark IFC Cycler as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92° for 15 s and 60° for 1 min. The Dynamic Arrays were read on a BioMark Real-Time PCR System after amplification and scored using BioMark Genotyping Analysis software (Fluidigm).

   b) Data collection
      i) Genetic data were collected as individual multi-locus genotypes for the 52 SNP loci. Genotypes collected from both instruments were entered into the ADFG Oracle database, LOKI.

c) Laboratory failure rates and quality control
   i) The overall failure rate was calculated by dividing the number of failed single-locus genotypes by the number of assayed single-locus genotypes.

   ii) Quality control measures were instituted to identify laboratory errors and to determine the reproducibility of genotypes. The process involved the reanalysis of 8 out of every 96 fish (one row per 96-well plate; 8%) for all markers by staff not involved with the original analysis. Assuming that the inconsistencies among analyses were due equally to errors in original genotyping and errors during the quality control, error rates in the original genotyping can be estimated as ½ the rate of inconsistencies. Because baseline and mixture collections were genotyped on many projects and have been subject to many quality control analyses, we report quality control results for representative baseline and mixture projects. The baseline project consisted of 7 collections comprising 661 individuals (~ 37% of current baseline) that were genotyped as part of a recent baseline supplemental project and the mixture project consisted of 6 collections comprising 2,291 individuals (~49% of the mixture samples genotyped). These projects genotyped fish on the Fluidigm Dynamic Array platform, and were typical of our current genotyping process.

3) Data analysis
   a) Data retrieval and quality control
      i) Genotypic data were retrieved from LOKI and were imported into S-Plus (TIBCO Software Inc. 2005; Somerville, MA). Unless otherwise noted, all analyses were performed in S-Plus. Two quality control measures were conducted once genotypes were retrieved from LOKI. The first one identified and excluded duplicate fish within collections. Duplicate fish can occur as a result of sampling or extracting the same fish twice. For each pair of duplicate fish, the fish with the most number of loci scored or, if both fish have equal number of scored loci, the first fish in the collection was retained for further analyses. The second quality control analysis excluded mixture and baseline individuals with an excessive rate of unscorable markers, or dropouts. A threshold of 80% scorable markers per individual was established and all individuals that did not meet this threshold were excluded from MSA. This threshold was set to exclude individuals with poor quality DNA. Poor quality DNA leads to lower reproducibility and therefore adds error to the multi-locus genotype. The value of 80% was chosen based upon the observation that many individuals with high quality DNA had some dropouts, but generally less than 20% of markers, while those with poor-quality DNA had higher dropout rates. As a result, there was little difference in which individuals were excluded from analysis when picking the threshold as long as it was within the 70% to 90% range. This rule (referred to as the "80% rule") was used for samples from mixtures to decrease errors and estimate variances caused by poor quality DNA and missing data. This approach was an attempt to balance the benefits from better data with the loss of power to accurately and precisely estimate stock proportions due to smaller sample sizes.

      ii) Population structure

(1) After dropping invariant loci, a subset of 40 of the 52 SNP loci were selected for further analysis. Individual genotype data were summarized as allele frequencies for each SNP locus in each collection. When multiple collections were available from the same population, these collections were combined to represent the population. A minimum sample size of 50 individuals was used for inclusion of a population in the population structure analysis. Because Chinook salmon are diploid organisms, this is a minimum of 100 samples from the gene pool for determining allele frequencies at each locus. Smaller sample sizes were pooled with collections from the same tributary if the log likelihood ratio statistic (Weir 1990) detected no significant difference between the collections.

(2) Estimates of the population frequency of individual alleles for each locus were calculated from the observed frequency of the allele in the representative sample. The numbers of alleles at each locus were calculated for each population. Observed and expected heterozygosity was calculated using *FSTAT* (Goudet 1995), and conformation of genotype frequencies to Hardy-Weinberg equilibrium (HWE) expected ratios was assessed using the exact test in *GENEPOP* (Raymond and Rousset 1995). The significance of departures from HWE for each locus in each population was determined using $\alpha = 0.05$ adjusted for the number of loci (n=40) assayed in each population using the Bonferroni adjusted significance levels ($\acute{\alpha} = \alpha/n = 0.0013$).

(3) Two measures of population subdivision were calculated from allele frequency differences: Cavalli-Sforza and Edwards' chord distances (Cavalli-Sforza and Edwards 1967) and F_{ST} (Weir and Cockerham 1984). *FSTAT* was used to calculate F_{ST} values. Population structure was visualized as a tree (unweighted pair-group method, Sneath and Sokal 1973) using *PHYLIP* version 3.6, (Felsenstein 2004) to view genetic similarities between populations reflected in the interpopulation chord distances.

iii) Mixture analysis testing
(1) Simulations and proof tests were conducted to evaluate the accuracy and precision of the genetic baseline to provide compositional estimates of mixtures of Chinook salmon taken from within the Kenai River. These tests were used to help assess whether the baseline of allele frequencies at the 40 SNP loci would provide sufficient information to identify individual stocks or groups of stocks (reporting groups) in mixtures. Reporting groups for genetic stock identification of Chinook salmon in the Kenai River were defined by grouping the populations by whether they spawn in a tributary or the mainstem based on a previous report (Begich et al. 2010) and on management applications. Further separation in more fine scale reporting groups was based on a combination of genetic similarity, geographic features, and management applications.

(2) Once reporting groups were defined, simulations were performed using the Statistical Package for Analyzing Mixtures (SPAM version 3.7, Debevec et al.

2000). Baseline and mixture genotypes were randomly generated from the baseline allele frequencies assuming Hardy-Weinberg equilibrium. Each simulated mixture (N = 400) was composed 100% of the stock or reporting group under study. When a reporting group mixture was simulated, all stocks in the reporting group contributed equally to the mixture. Average estimates of mixture proportions and 90% confidence intervals were derived from 1000 simulations. Reporting groups with mean correct estimates of 90% or better are considered highly identifiable in fishery applications. Reporting groups with mean correct estimates lower than 90% can still be considered identifiable in mixtures, but sources of misallocation should be considered when interpreting the results.

(3) Proof tests were conducted to examine baseline performance for MSA. In these tests, we created test mixtures by sampling approximately 200 individuals from the baseline. For tributary and mainstem reporting groups, we created two 100% mixtures by sampling 200 fish from each group and a 50% mixture by sampling 100 fish from each reporting group. For all 3 tests we rebuilt the baseline excluding the sampled fish. These tests provided an indication of the power of the baseline for MSA assuming that all the populations were represented in the baseline. The proof test mixtures were analyzed using the program BAYES (Pella and Masuda 2001). The Bayesian model implemented by BAYES places a Dirichlet distribution as the prior distribution for the stock proportions, and the parameters for this distribution must be specified. Prior parameters for each reporting group were defined to be equal (i.e., a "flat" prior) with the prior parameters for a reporting group divided equally among populations within that reporting group. We set the sum of all prior parameters to be 1 (prior weight), which is equivalent to adding 1 fish to each mixture (Pella and Masuda, 2001). We ran 5 independent Markov Chain Monte Carlo (MCMC) chains of 15,000 iterations with different starting values and discarded the first 7,500 iterations to remove the influence of the initial start values. Estimates and 90% credibility intervals from the second half of five 15,000 iteration chains were tabulated. Credibility intervals differ from confidence intervals in that they are a direct statement of probability: i.e. a 90% credibility interval has a 90% chance of containing the true answer (Gelman et al. 2000). We repeated this procedure for each reporting group. A critical level of 90% correct allocation was used to determine if the reporting group was acceptably identifiable (Seeb et al. 2000). We examined the adequacy of burn-in for each chain with the Rafferty and Lewis (1996) diagnostic. To ensure that the BAYES output was an acceptable approximation of the stationary posterior distribution and that the stock composition estimates were valid, we assessed the 5 independent (MCMC) chains for convergence among chains. We assessed among-chain convergence using the Gelman-Rubin shrink factors that are computed for all stock groups in the program BAYES. This shrink factor compared the variation within a chain to the total variation among chains (Gelman and Rubin, 1992).

iv) Mixture analyses
  (1) We estimated the stock composition of all sport fishery mixtures using the same protocol described above for the Bayesian baseline evaluation tests except for the definition of prior parameters. We used an informative Dirichlet prior distribution based upon the best available information for each mixture analysis. We believe the best available information for the prior to be the results of MSA of similar mixtures. This information was not always available, so we developed what we termed a "step-wise" prior protocol to standardize our methodology. Our protocol was as follows:
    (a) For the first time strata within the sport fishery, the prior was based upon the mean of the stock composition estimates from the first time strata of the 2005-2008 lower Kenai River netting mixtures.
    (b) For subsequent time strata within the fishery in the same year, the priors were the posterior means (i.e., the stock composition estimates) of the previous time strata.
    (c) For the first time strata in subsequent years, the prior parameters were the posterior means from the first period of same fishery from the previous year.
      (i) For the middle River sport fishery mixtures, the initial priors for 2207 and 2008 were the posterior means from lower river sport mixtures with similar dates (June 19-30, 2007 and June 17-22 , 2008, respectively).
      (ii) Priors for the initial 2008 middle river sport time stratum were chosen this way because the initial 2007 middle river sport estimates represented a later range of dates.
    (d) For all priors we defined a minimum value of 0.01 for each reporting group. Reporting groups with estimates below this value were set to 0.01 by normalizing the sum of priors for all reporting groups to 1 after adjusting the value of the small proportion stocks. For all mixtures, the prior for a reporting group was divided equally to populations within that reporting group for population prior parameters.

  (2) We estimated the stock composition of the netting data for weekly time strata for 2003 to 2008. Since weekly sample sizes were small, hierarchical Bayesian methods were used to model the prior structure. These methods provide the added benefit of making use of the temporal relationship between stock proportions in adjacent weeks to add strength to the estimates in any one week. The prior the stock proportions were modeled with a logistic normal distribution (*Okuyama and Bolker*, 2005) using time as a covariate according to the following specification:

$$y_{t,i} = \ln\left(\frac{p_{t,i}}{p_{t,C}}\right),$$

$$y_{t,i} \sim N\left(\mu_{t,i}, \sigma^2\right)$$

$$\mu_{t,i} = \alpha_i + \beta_i t,$$

$$p_{t,i} = \frac{e^{y_{t,i}}}{1 + \sum_{j=1}^{C-1} e^{y_{t,j}}},$$

$$p_{t,C} = \frac{1}{1 + \sum_{j=1}^{C-1} e^{y_{t,j}}},$$

$$\alpha_i, \beta_i \sim N(0, 1000)$$

Where C is the number of stocks and $\sigma^2 = 1$.

Results

1) Sample collection
   a) Number of individuals collected, statements about collecting, …
      i) Baseline – Tissues samples from spawning populations of Chinook salmon were collected throughout the Kenai River drainage (Table 1). Over six years (2003-2008), 22 individual collections were made with the majority of the collections (13) being made in 2005 and 2006. These collections were taken at 10 different locations; individuals from 8 of these locations were taken in multiple years. A total of 1,788 fish collected over spawning areas were analyzed for the baseline.
      ii) Lower River Netting - A total of 3,828 Chinook salmon were sampled for tissues suitable for genetic analysis from the lower Kenai River drift netting project in 2003-2008.
      iii) Lower River Sport- A total of 1,346 Chinook salmon were sampled for tissues suitable for genetic analysis from the lower Kenai River creel survey in 2006-2008.
      iv) Middle River Sport- A total of 509 Chinook salmon were sampled for tissues suitable for genetic analysis from the middle Kenai River creel survey in 2007 and 2008.
2) Laboratory analysis
   a) Genotyping and Data collection
      i) Genotypes were assayed from a total of 1,788 individuals from 22 collections representing 9 putative populations (Table 1).
      ii) A total of 5,683 individuals from 6 lower Kenai River netting collections, 3 lower Kenai River sport collections, and 2 middle Kenai River sport fishery collections were available for analysis (Table 5). From these, genotypes were assayed from 4,666 individuals.
   b) Laboratory failure rates and quality control
      i) For the baseline collections, the overall failure rate for successfully assaying genotypes was 2.87 %. Most failures occurred in the samples from Slikok Creek (success rate approximately 80%) and were due to poor tissue quality. The quality control checks employed demonstrated an error rate of 0.21%. The quality control checks also revealed pairs of individuals in some collections that had identical multi-

locus genotypes. The following populations had individuals with duplicate genotypes: Benjamin Creek (1 pair), Funny River (1 pair), Crescent Creek (1 pair), Quartz Creek (2 pairs), and Juneau Creek (4 pairs). In most cases, duplicates appear to have been the result of sampling the same fish into neighboring vials.

    ii) For the mixture collections, the overall failure rate for successfully assaying genotypes was 1.09%. The quality control checks employed demonstrated an error rate of 0.04%. Among all mixture samples, only one pair of individuals had duplicate genotypes.

3) Data analysis
   a) Population structure
     i) After correcting for multiple tests, no significant departures from HWE were found. Genetic differences between populations were measured using CSE distances calculated from allele frequencies at the 40 SNP loci. Visualizing these interpopulation distances with a UPGMA tree showed five major clusters of populations which appear to be structured largely by tributaries (Figure 2). Each of the major branches on the tree, with the exception of Juneau Creek and the mainstem, corresponds to a subdrainage within the greater Kenai River drainage (considering the mainstem spawning locations to be a subset of the whole).

   b) Mixture analysis testing
     i) To evaluate the baseline for estimating stock composition for the 2 reporting groups 100% simulations were conducted. The simulations indicated that the reporting groups can be identified with 98% and 97% accuracy for tributary and mainstem reporting groups, respectively.
     ii) When prooftests were performed on mixtures of fish composed entirely from a single reporting group (tributary or mainstem) more than 98% were correctly identified to the group of origin (Table 4). When an additional proof test was performed with a mixture comprised of 50% mainstem and 50% tributary fish, the estimates for each reporting group were within 3% of their true value.

4) Mixture analyses
   c) Results from lower river mixture sampling (Tables 6-11) show the majority of Chinook salmon that enter the Kenai River prior to the middle of June are of tributary origin; depending on the year, after the second or third week in June mainstem fish become more predominant. Very few tributary fish enter the Kenai in July. Results from the lower river sport fishery mixture sampling (Tables 12-14) demonstrate that most of the harvest in May and June is of tributary-bound fish, and that nearly all of the harvest in July is of mainstem-bound fish. Results from the middle river sport fishery mixture sampling (Tables 15 and 16) demonstrate that most of the harvest in June is of tributary-bound fish, the harvest in the first two weeks of July is a somewhat equal mix of tributary- and mainstem-bound fish, and that nearly all of the harvest in the last two weeks in July is of mainstem-bound fish.

Evaluation

Project objectives were addressed and exceeded. Strong genetic separation between tributary and mainstem spawning aggregates within the Kenai River drainage were found and will be useful in future examinations in mixed-stock fisheries within the Kenai River and outside. As initial lab results showed separation between spawning aggregates, additional aggregates were sampled, such as Benjamin Creek within the Killey River drainage. The results from the mixture samples collected from the lower river netting and sport sampling will be very useful in future examinations of escapement goals and escapement estimates for the early (tributary) and late (mainstem) Kenai River stocks.

Project Products
Project results are ongoing as more baselines are sampled and analyzed as well as additional mixture samples collected. Results through 2009 will be reported in an FDS report with a draft by the Spring of 2011.

Key Words
Kenai River, Chinook salmon, GSI, baseline, mixture, SNP's.

Table 1. Collections of Chinook salmon from the Kenai River used in the genetic baseline for mixed stock analysis.

Population Number	Location	Sample Year(s)	N
1	Slikok Creek	2003, 2004, 2008	200
2	Funny River	2005, 2006	220
3	Kenai Mainstem Site 1	2003, 2004	119
4	Kenai Mainstem Site 2	2006	183
5	Killey River	2005, 2006	266
6	Benjamin Creek	2005, 2006	206
7	Russian River	2005, 2006, 2007, 2008	214
8	Juneau Creek	2005, 2006, 2007	147
9	Quartz Creek	2006, 2008	68
10	Crescent Creek	2006	165
		Total	1,788

Table 2. Background information, observed heterozygosity (H_o), expected heterozygosity (H_e), and F_{ST} for each of the 40 SNP loci in the analysis of Kenai River Chinook salmon.

Assay Name	Range of Common Allele	Heterozygosity Observed (H_o)	Expected (H_s)	F_{ST}
Ots_GTH2B-550	(0.609 - 0.796)	0.453	0.432	0.015
Ots_NOD1	(0.275 - 0.770)	0.446	0.452	0.113
Ots_E2-275	(0.569 - 0.871)	0.325	0.322	0.042
Ots_AsnRS-60	(0.543 - 0.766)	0.403	0.43	0.03
Ots_ETIF1A	(0.363 - 0.678)	0.472	0.479	0.055
Ots_FARSLA-220	(0.605 - 0.891)	0.337	0.333	0.057
Ots_FGF6A	(0.373 - 0.804)	0.382	0.387	0.098
Ots_GH2	(0.755 - 0.888)	0.292	0.299	0.01
Ots_GPDH-338	(0.832 - 0.985)	0.106	0.106	0.034
Ots_GPH-318	(0.873 - 0.985)	0.107	0.112	0.027
Ots_GST-207	(0.877 - 1.000)	0.048	0.051	0.054
Ots_hnRNPL-533	(0.737 - 0.929)	0.294	0.301	0.015
Ots_HSP90B-385	(0.868 - 1.000)	0.058	0.058	0.051
Ots_IGF-I.1-76	(0.363 - 0.716)	0.496	0.472	0.051
Ots_Ikaros-250	(0.838 - 0.993)	0.136	0.134	0.058
Ots_il-1racp-166	(0.577 - 0.811)	0.448	0.419	0.034
Ots_LEI-292	(0.932 - 0.988)	0.075	0.077	0.008
Ots_MHC1	(0.519 - 0.780)	0.407	0.42	0.055
Ots_MHC2	(0.968 - 1.000)	0.025	0.026	0.008
Ots_LWSop-638	(0.901 - 1.000)	0.069	0.065	0.022
Ots_SWS1op-182	(0.560 - 0.738)	0.464	0.447	0.014
Ots_P450	(0.687 - 0.848)	0.336	0.346	0.017
Ots_P53	(0.559 - 0.779)	0.408	0.433	0.025
Ots_Prl2	(0.425 - 0.667)	0.501	0.49	0.02
Ots_ins-115	(0.950 - 1.000)	0.044	0.042	0.017
Ots_SClkF2R2-135	(0.450 - 0.834)	0.399	0.402	0.068
Ots_SERPC1-209	(0.831 - 0.998)	0.13	0.145	0.049
Ots_SL	(0.532 - 0.863)	0.407	0.379	0.042
Ots_TAPBP	(0.784 - 0.963)	0.223	0.225	0.025
Ots_Tnsf	(0.846 - 0.948)	0.18	0.174	0.008
Ots_u202-161	(0.917 - 1.000)	0.045	0.044	0.037
Ots_u211-85	(0.767 - 0.939)	0.201	0.192	0.024
Ots_U212-158	(0.857 - 1.000)	0.05	0.052	0.067
Ots_u4-92	(0.653 - 0.914)	0.281	0.29	0.041
Ots_u6-75	(0.875 - 0.964)	0.139	0.135	0.016
Ots_Zp3b-215	(0.914 - 0.995)	0.074	0.075	0.016
Ots_PGK-54	(0.975 - 1.000)	0.007	0.007	0.015
Ots_RAG3	(0.683 - 0.991)	0.243	0.255	0.063
Ots_S7-1	(0.807 - 0.909)	0.212	0.225	0.009
Ots_unkn-526	(0.797 - 0.995)	0.193	0.199	0.044

Table 3. Mean reporting group allocations of simulated mixtures of Kenai River Chinook salmon from the baseline of 40 SNPs. Each set of mixtures (N=400) was created from a single reporting region based on allelic frequencies for that region. The results reported are the mean and bounds of the middle 90% (CI) of correct allocations from 1,000 bootstrap iterations.

Reporting Group	Estimate	SD	90% CI
Tributaries	0.983	0.016	(0.953 - 1.000)
Mainstem	0.972	0.019	(0.936 - 0.998)

Table 4. Mixture sample size (N), allocation proportions (P), standard deviation (SD), coefficient of variation (CV), and BAYES; 90% credibility interval (CI) for mixtures of known fish removed from the baseline populations that contribute to each reporting group.

N	Group	P	SD	CV	CI
		100% Tributary			
201	Tributary	0.981	0.022	0.022	(0.936 - 1.000)
	Mainstem	0.019	0.022	1.139	(0.000 - 0.064)
		100% Mainstem			
200	Tributary	0.014	0.016	1.173	(0.000 - 0.048)
	Mainstem	0.986	0.016	0.017	(0.952 - 1.000)
		50% Tributary /50% Mainstem			
200	Tributary	0.529	0.068	0.128	(0.416 - 0.639)
	Mainstem	0.471	0.068	0.144	(0.361 - 0.584)

Table 5. Collection year, number of samples collected , and number of samples genotyped for tissue collections of Chinook salmon sampled for genetic studies taken from fish captured in the Kenai River lower river netting program and lower and middle sport fisheries.

| Collection | Year | Samples | |
		Collected	Genotyped
Lower River Netting	2003	1,004	554
	2004	740	488
	2005	504	504
	2006	478	478
	2007	370	370
	2008	732	480
Lower River Sport	2006	516	516
	2007	388	388
	2008	442	379
Middle River Sport	2007	147	147
	2008	362	362
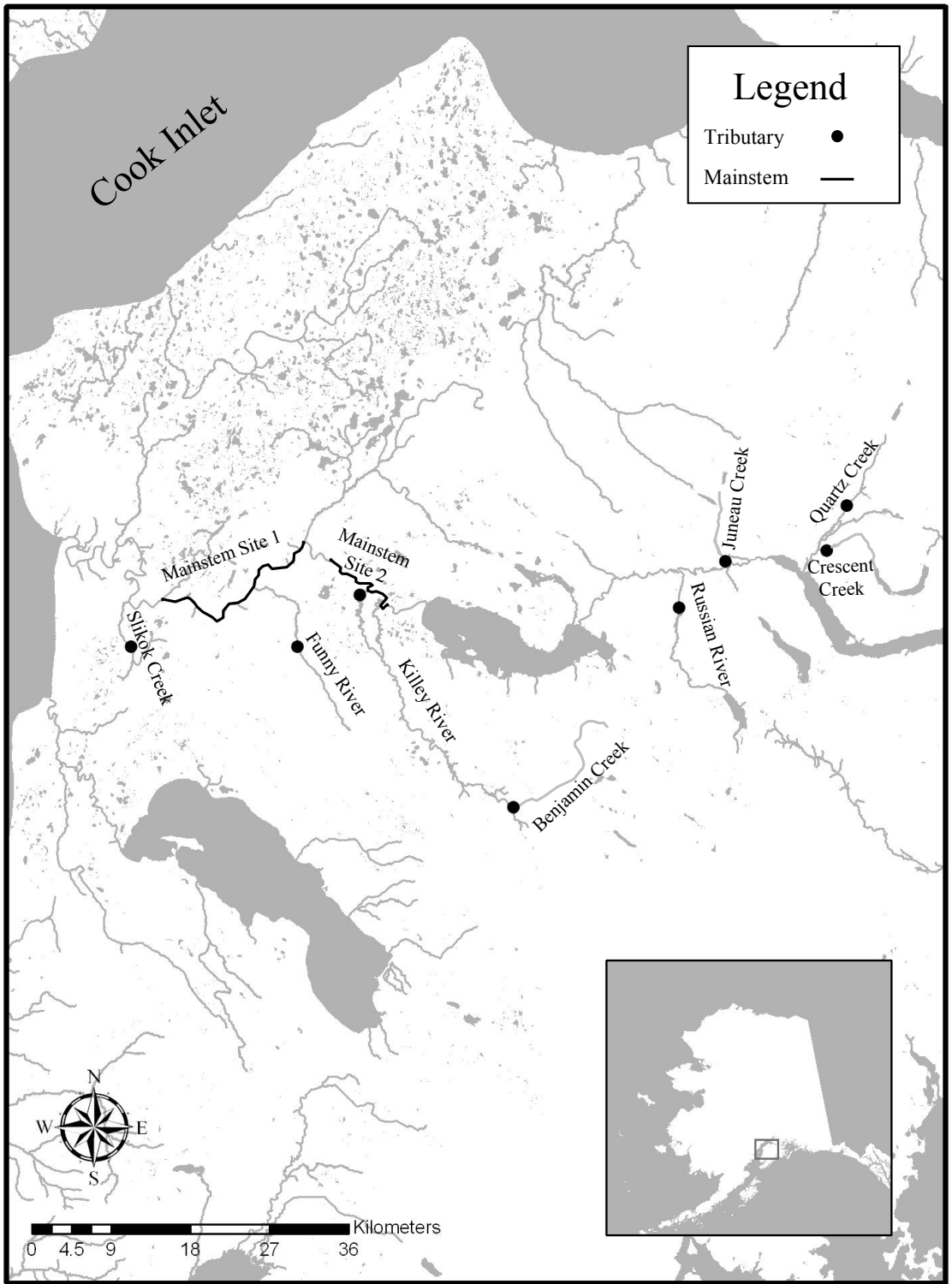
Figure 1.- Sampling locations for Chinook salmon in the Kenai River drainage used to compile a genetic baseline.
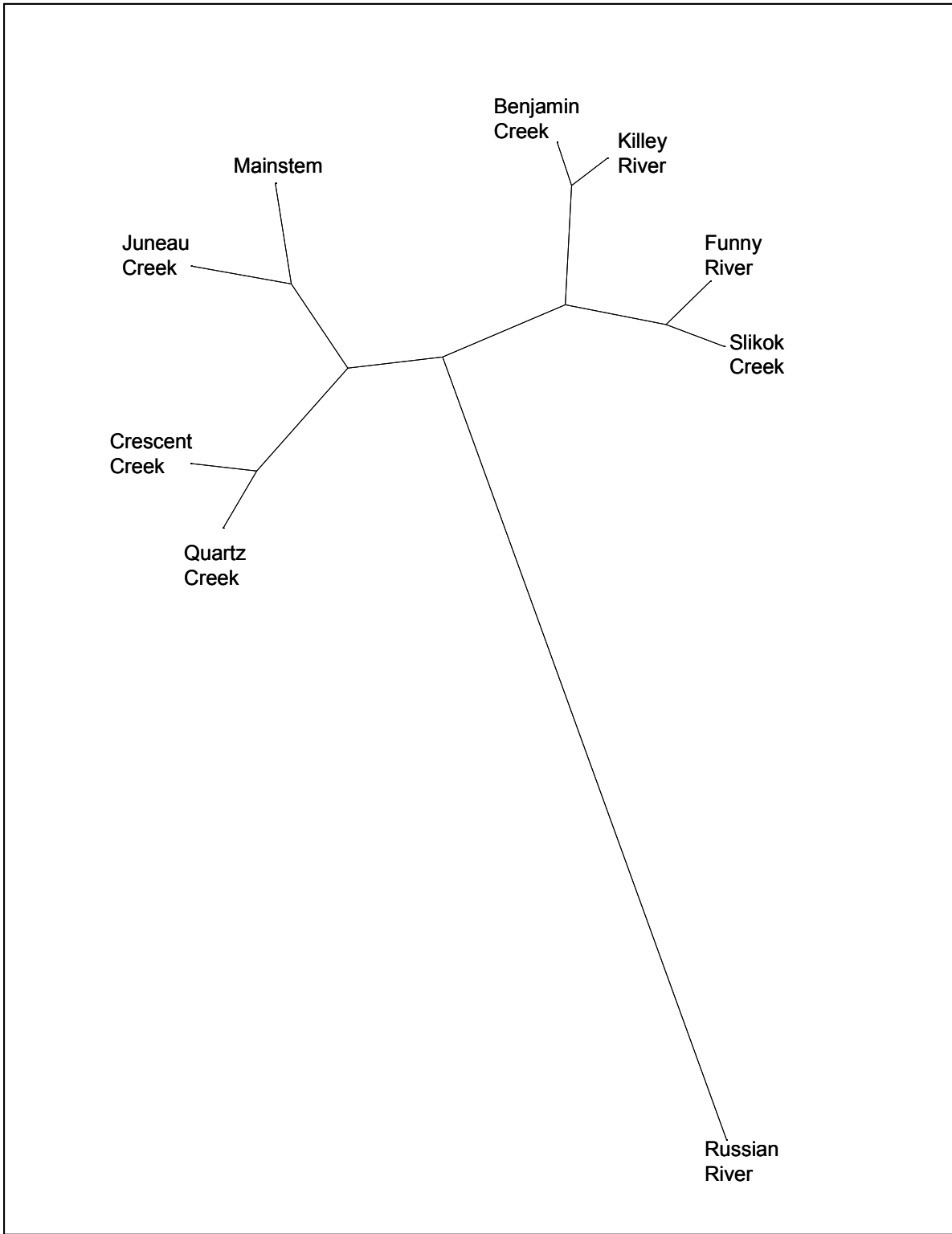
Figure 2.- UPGMA tree based on Cavalli-Sforza and Edwards (1967) chord distances between Chinook salmon populations sampled from spawning locations in the Kenai River drainage.

Table 6. 2003 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16 - 6/4	42	T	1.000	0.000	0.000	(0.999 - 1.000)
		M	0.000	0.000	2.037	(0.000 - 0.001)
5/27-6/2	43	T	0.998	0.002	0.002	(0.995 - 0.999)
		M	0.002	0.002	1.382	(0.001 - 0.005)
6/3 - 6/9	48	T	0.989	0.010	0.010	(0.973 - 0.994)
		M	0.011	0.010	0.849	(0.006 - 0.027)
6/10 - 6/16	50	T	0.923	0.022	0.024	(0.886 - 0.946)
		M	0.077	0.022	0.286	(0.054 - 0.114)
6/17 - 6/23	48	T	0.599	0.045	0.075	(0.523 - 0.664)
		M	0.401	0.045	0.112	(0.336 - 0.477)
6/24 - 6/30	50	T	0.172	0.039	0.229	(0.130 - 0.226)
		M	0.828	0.039	0.048	(0.774 - 0.870)
7/1 - 7/7	46	T	0.034	0.016	0.487	(0.022 - 0.051)
		M	0.966	0.016	0.017	(0.949 - 0.978)
7/8 - 7/14	47	T	0.009	0.009	0.973	(0.005 - 0.018)
		M	0.991	0.009	0.009	(0.982 - 0.995)
7/15 - 7/21	49	T	0.004	0.006	1.559	(0.002 - 0.006)
		M	0.996	0.006	0.006	(0.994 - 0.998)
7/22 - 7/28	49	T	0.002	0.002	1.172	(0.001 - 0.002)
		M	0.998	0.002	0.002	(0.998 - 0.999)
7/29 - 8/4	47	T	0.001	0.001	0.753	(0.000 - 0.002)
		M	0.999	0.001	0.001	(0.998 - 1.000)
8/5-8/10	18	T	0.001	0.001	0.565	(0.000 - 0.002)
		M	0.999	0.001	0.001	(0.998 - 1.000)

Table 7. 2004 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16 - 6/4	13	T	1.000	0.001	0.001	(1.000 - 1.000)
		M	0.000	0.001	5.376	(0.000 - 0.000)
5/27-6/2	11	T	0.999	0.004	0.004	(0.997 - 1.000)
		M	0.001	0.004	3.155	(0.000 - 0.003)
6/3 - 6/9	43	T	0.986	0.015	0.016	(0.970 - 0.994)
		M	0.014	0.015	1.102	(0.006 - 0.030)
6/10-6/16	28	T	0.831	0.044	0.053	(0.759 - 0.895)
		M	0.169	0.044	0.262	(0.105 - 0.241)
6/17-6/23	42	T	0.317	0.050	0.156	(0.256 - 0.396)
		M	0.683	0.050	0.072	(0.604 - 0.744)
6/24-6/30	50	T	0.117	0.026	0.224	(0.089 - 0.158)
		M	0.883	0.026	0.030	(0.842 - 0.911)
7/1 - 7/7	50	T	0.066	0.015	0.227	(0.049 - 0.085)
		M	0.934	0.015	0.016	(0.915 - 0.951)
7/8 - 7/14	50	T	0.044	0.011	0.256	(0.031 - 0.060)
		M	0.956	0.011	0.012	(0.940 - 0.969)
7/15-7/21	48	T	0.033	0.010	0.310	(0.022 - 0.046)
		M	0.967	0.010	0.010	(0.955 - 0.978)
7/22-7/28	50	T	0.029	0.008	0.289	(0.019 - 0.042)
		M	0.971	0.008	0.009	(0.958 - 0.981)
7/29-8/4	49	T	0.039	0.014	0.368	(0.022 - 0.061)
		M	0.961	0.014	0.015	(0.939 - 0.978)
8/5-8/10	47	T	0.066	0.024	0.371	(0.030 - 0.110)
		M	0.934	0.024	0.026	(0.890 - 0.971)

Table 8. 2005 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16-6/4	17	T	1.000	0.000	0.000	(1.000 - 1.000)
		M	0.000	0.000	3.730	(0.000 - 0.000)
5/27-6/2	33	T	0.999	0.002	0.002	(0.998 - 1.000)
		M	0.001	0.002	2.153	(0.000 - 0.002)
6/3 - 6/9	56	T	0.992	0.006	0.006	(0.986 - 0.996)
		M	0.008	0.006	0.774	(0.004 - 0.014)
6/10-6/16	38	T	0.918	0.032	0.035	(0.864 - 0.949)
		M	0.082	0.032	0.391	(0.051 - 0.136)
6/17-6/23	29	T	0.595	0.058	0.097	(0.496 - 0.696)
		M	0.405	0.058	0.142	(0.304 - 0.504)
6/24-6/30	44	T	0.187	0.032	0.173	(0.138 - 0.243)
		M	0.813	0.032	0.040	(0.757 - 0.862)
7/1 - 7/7	54	T	0.058	0.017	0.290	(0.042 - 0.081)
		M	0.942	0.017	0.018	(0.919 - 0.958)
7/8-7/14	63	T	0.024	0.009	0.381	(0.016 - 0.036)
		M	0.976	0.009	0.009	(0.964 - 0.985)
7/15-7/21	61	T	0.014	0.005	0.384	(0.006 - 0.021)
		M	0.986	0.005	0.005	(0.979 - 0.994)
7/22-7/28	40	T	0.009	0.005	0.519	(0.002 - 0.017)
		M	0.991	0.005	0.005	(0.983 - 0.998)
7/29 - 8/4	51	T	0.007	0.004	0.595	(0.001 - 0.014)
		M	0.993	0.004	0.004	(0.986 - 0.999)
8/5-8/10	21	T	0.005	0.004	0.800	(0.000 - 0.013)
		M	0.995	0.004	0.004	(0.987 - 1.000)

Table 9. 2006 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16-6/4	2	T	1.000	0.000	0.000	(1.000 - 1.000)
		M	0.000	0.000	7.998	(0.000 - 0.000)
5/27-6/2	11	T	1.000	0.000	0.000	(1.000 - 1.000)
		M	0.000	0.000	3.191	(0.000 - 0.000)
6/3 - 6/9	20	T	0.999	0.001	0.001	(0.998 - 1.000)
		M	0.001	0.001	1.973	(0.000 - 0.002)
6/10-6/16	46	T	0.987	0.012	0.012	(0.962 - 0.995)
		M	0.013	0.012	0.908	(0.005 - 0.038)
6/17-6/23	18	T	0.793	0.055	0.069	(0.678 - 0.845)
		M	0.207	0.055	0.264	(0.155 - 0.322)
6/24-6/30	33	T	0.156	0.035	0.224	(0.096 - 0.189)
		M	0.844	0.035	0.041	(0.811 - 0.904)
7/1 - 7/7	38	T	0.012	0.010	0.823	(0.005 - 0.018)
		M	0.988	0.010	0.010	(0.982 - 0.995)
7/8 - 7/14	54	T	0.002	0.005	2.534	(0.000 - 0.003)
		M	0.998	0.005	0.005	(0.997 - 1.000)
7/15-7/21	46	T	0.000	0.002	4.725	(0.000 - 0.001)
		M	1.000	0.002	0.002	(0.999 - 1.000)
7/22-7/28	76	T	0.000	0.001	8.400	(0.000 - 0.000)
		M	1.000	0.001	0.001	(1.000 - 1.000)
7/29-8/4	55	T	0.000	0.001	5.485	(0.000 - 0.000)
		M	1.000	0.001	0.001	(1.000 - 1.000)
8/5-8/10	75	T	0.000	0.000	3.357E+07	(0.000 - 0.000)
		M	1.000	0.000	0.000	(1.000 - 1.000)

Table 10. 2007 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16-6/4	7	T	1.000	0.000	0.000	1.000 - 1.000)
		M	0.000	0.000	1.850	0.000 - 0.000)
5/27-6/2	23	T	0.999	0.001	0.001	0.999 - 1.000)
		M	0.001	0.001	1.167	0.000 - 0.001)
6/3-6/9	16	T	0.995	0.004	0.004	0.993 - 0.999)
		M	0.005	0.004	0.799	0.001 - 0.007)
6/10-6/16	34	T	0.959	0.016	0.017	0.946 - 0.991)
		M	0.041	0.016	0.401	0.009 - 0.054)
6/17 - 6/23	21	T	0.751	0.069	0.092	0.697 - 0.924)
		M	0.249	0.069	0.278	0.076 - 0.303)
6/24 - 6/30	20	T	0.323	0.109	0.338	0.252 - 0.577)
		M	0.678	0.109	0.161	0.423 - 0.748)
7/1 - 7/7	27	T	0.084	0.057	0.672	0.059 - 0.174)
		M	0.916	0.057	0.062	0.826 - 0.941)
7/8 - 7/14	55	T	0.022	0.020	0.953	0.013 - 0.032)
		M	0.979	0.020	0.021	0.968 - 0.987)
7/15 - 7/21	49	T	0.007	0.007	0.969	0.002 - 0.008)
		M	0.993	0.007	0.007	0.992 - 0.998)
7/22 - 7/28	55	T	0.003	0.003	1.128	0.001 - 0.004)
		M	0.997	0.003	0.004	0.996 - 0.999)
7/29 - 8/4	35	T	0.002	0.002	1.089	0.000 - 0.002)
		M	0.998	0.002	0.002	0.998 - 1.000)
8/5-8/10	27	T	0.001	0.001	1.193	0.000 - 0.002)
		M	0.999	0.001	0.001	0.998 - 1.000)

Table 11. 2008 Lower Kenai Net- Fitted Estimates for tributary (T) and mainstem (M)

Dates	N	Group	Mean	S.D.	CV	C.I.
5/16 - 6/4	13	T	1.000	0.000	0.000	(1.000 - 1.000)
		M	0.000	0.000	5.086	(0.000 - 0.000)
5/27-6/2	15	T	1.000	0.000	0.000	(1.000 - 1.000)
		M	0.000	0.000	4.311	(0.000 - 0.000)
6/3 - 6/9	31	T	0.999	0.002	0.002	(0.998 - 1.000)
		M	0.001	0.002	3.140	(0.000 - 0.002)
6/10 - 6/16	41	T	0.994	0.007	0.007	(0.986 - 0.997)
		M	0.006	0.007	1.230	(0.003 - 0.014)
6/17 - 6/23	45	T	0.930	0.023	0.025	(0.894 - 0.957)
		M	0.070	0.023	0.329	(0.043 - 0.106)
6/24 - 6/30	36	T	0.566	0.061	0.107	(0.474 - 0.656)
		M	0.434	0.061	0.140	(0.344 - 0.526)
7/1 - 7/7	49	T	0.194	0.045	0.231	(0.142 - 0.258)
		M	0.807	0.045	0.056	(0.742 - 0.858)
7/8 - 7/14	40	T	0.062	0.016	0.257	(0.044 - 0.085)
		M	0.939	0.016	0.017	(0.915 - 0.957)
7/15 - 7/21	49	T	0.021	0.006	0.290	(0.014 - 0.028)
		M	0.979	0.006	0.006	(0.972 - 0.986)
7/22 - 7/28	50	T	0.007	0.003	0.432	(0.005 - 0.012)
		M	0.993	0.003	0.003	(0.988 - 0.995)
7/29 - 8/4	49	T	0.003	0.001	0.507	(0.001 - 0.004)
		M	0.997	0.001	0.001	(0.996 - 0.999)
8/5-8/10	50	T	0.001	0.001	0.721	(0.000 - 0.002)
		M	0.999	0.001	0.001	(0.998 - 1.000)

Table 12. 2006 Lower Kenai Sport Fishery Bayes Estimates for tributary
(T) and mainstem (M)

Date	N	Group	Mean	S.D.	CV	C.I.
5/16 - 6/4	22	T	0.997	0.014	0.014	(0.984 - 1.000)
		M	0.003	0.014	4.467	(0.000 - 0.016)
6/6 - 6/11	36	T	1.000	0.004	0.004	(1.000 - 1.000)
		M	0.000	0.004	9.649	(0.000 - 0.000)
6/13 - 6/18	51	T	0.995	0.023	0.023	(0.969 - 1.000)
		M	0.005	0.023	4.576	(0.000 - 0.031)
6/20 - 6/25	36	T	0.938	0.094	0.100	(0.738 - 1.000)
		M	0.062	0.094	1.525	(0.000 - 0.262)
6/27 - 6/30	29	T	0.172	0.105	0.610	(0.034 - 0.371)
		M	0.828	0.105	0.127	(0.629 - 0.966)
7/1 - 7/9	74	T	0.012	0.025	2.202	(0.000 - 0.063)
		M	0.988	0.025	0.026	(0.937 - 1.000)
7/11 - 7/16	55	T	0.002	0.013	6.198	(0.000 - 0.004)
		M	0.998	0.013	0.013	(0.996 - 1.000)
7/18 - 7/23	90	T	0.001	0.006	7.018	(0.000 - 0.001)
		M	0.999	0.006	0.006	(0.999 - 1.000)
7/25 - 7/30	119	T	0.000	0.003	8.060	(0.000 - 0.000)
		M	1.000	0.003	0.003	(1.000 - 1.000)

Table 13. 2007 Lower Kenai Sport Fishery Bayes Estimates for tributary
(T) and mainstem (M)

Date	N	Group	Mean	S.D.	CV	C.I.
5/23-6/10	30	T	0.992	0.026	0.026	(0.942 - 1.000)
		M	0.008	0.026	3.142	(0.000 - 0.058)
6/12-6/17	39	T	0.999	0.005	0.005	(1.000 - 1.000)
		M	0.001	0.005	8.840	(0.000 - 0.000)
6/19-6/30	52	T	0.745	0.126	0.169	(0.538 - 1.000)
		M	0.255	0.126	0.493	(0.000 - 0.462)
7/1-7/8	37	T	0.084	0.077	0.917	(0.003 - 0.238)
		M	0.916	0.077	0.084	(0.762 - 0.997)
7/10- 7/15	51	T	0.017	0.037	2.167	(0.000 - 0.100)
		M	0.983	0.037	0.038	(0.900 - 1.000)
7/17-7/22	88	T	0.034	0.059	1.733	(0.000 - 0.166)
		M	0.966	0.059	0.062	(0.834 - 1.000)
7/24-7/31	85	T	0.001	0.006	5.089	(0.000 - 0.006)
		M	0.999	0.006	0.006	(0.994 - 1.000)

Table 14. 2008 Lower Kenai Sport Fishery Bayes Estimates for tributary (T) and mainstem (M)

Date	N	Group	Mean	S.D.	CV	C.I.
5/17 - 6/1	26	T	0.982	0.043	0.043	(0.890 - 1.000)
		M	0.018	0.043	2.408	(0.000 - 0.110)
6/3 - 6/8	49	T	0.999	0.004	0.004	(0.999 - 1.000)
		M	0.001	0.004	7.410	(0.000 - 0.001)
6/11- 6/15	50	T	0.999	0.005	0.005	(1.000 - 1.000)
		M	0.001	0.005	9.002	(0.000 - 0.000)
6/17- 6/22	33	T	0.998	0.015	0.015	(0.999 - 1.000)
		M	0.002	0.015	7.317	(0.000 - 0.001)
6/24- 6/29	26	T	0.959	0.107	0.112	(0.688 - 1.000)
		M	0.041	0.107	2.633	(0.000 - 0.312)
7/2- 7/6	23	T	0.090	0.080	0.896	(0.005 - 0.251)
		M	0.910	0.080	0.088	(0.749 - 0.995)
7/8- 7/13	38	T	0.010	0.028	2.870	(0.000 - 0.060)
		M	0.990	0.028	0.028	(0.940 - 1.000)
7/16- 7/20	48	T	0.061	0.042	0.694	(0.009 - 0.141)
		M	0.939	0.042	0.045	(0.859 - 0.991)
7/24- 7/27	50	T	0.006	0.019	3.325	(0.000 - 0.037)
		M	0.994	0.019	0.019	(0.963 - 1.000)
7/29- 7/31	35	T	0.004	0.026	5.800	(0.000 - 0.008)
		M	0.996	0.026	0.026	(0.992 - 1.000)

Table 15. 2006 Middle Kenai Sport Fishery Bayes Estimates for tributary (T) and mainstem (M)

Date	N	Group	Mean	S.D.	CV	C.I.
6/21- 6/30	60	T	0.871	0.077	0.089	(0.734 - 0.991)
		M	0.129	0.077	0.599	(0.009 - 0.266)
7/3 - 7/14	31	T	0.406	0.165	0.406	(0.154 - 0.693)
		M	0.594	0.165	0.278	(0.307 - 0.846)
7/17-7/31	56	T	0.022	0.032	1.464	(0.000 - 0.089)
		M	0.978	0.032	0.033	(0.911 - 1.000)

Table 16. 2007 Middle Kenai Sport Fishery Bayes Estimates for tributary (T) and mainstem (M)

Date	N	Group	Mean	S.D.	CV	C.I.
6/12-6/21	59	T	0.998	0.010	0.010	(0.997 - 1.000)
		M	0.002	0.010	6.409	(0.000 - 0.003)
6/24- 6/28	84	T	0.861	0.057	0.066	(0.761 - 0.947)
		M	0.139	0.057	0.411	(0.053 - 0.239)
7/1-7/12	92	T	0.559	0.083	0.148	(0.420 - 0.694)
		M	0.441	0.083	0.188	(0.306 - 0.580)
7/15-7/31	125	T	0.051	0.031	0.612	(0.011 - 0.110)
		M	0.949	0.031	0.033	(0.890 - 0.989)