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Genetic Diversity of Chinook Salmon from the Kuskokwim River

Final Report for Study 01-070

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Final Report Summary Page

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Geographic Area: Kuskokwim River

Information Type: Stock Status and Trends

Issue(s) Addressed: The subsistence fishery for Chinook salmon in the Kuskokwim River region is one of the largest and most significant in Alaska, but low returns in recent years have yielded shortfalls in escapements and consequently increased fishing restrictions.

Study Cost: $300,000

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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. This has 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, allozymes and microsatellites. Analysis of genetic data found evidence of significant structure in these Chinook salmon populations. Populations were assigned to four groups based on genetic characteristics and geographic proximity, three within the Kuskokwim River (Upper, Middle and Lower) and the fourth in Kuskokwim Bay. The baseline of both marker types will be useful for estimating stock composition of commercial and subsistence harvests, investigating run timing and entry patterns within the river, and examining the effectiveness of management actions for the conservation of the resource. It can also be used to aid in the identification of Kuskokwim River salmon in studies of Chinook salmon on the high seas.

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

Project Data: *Description* - Data for this study consist of biological samples (tissue and DNA samples) and allele frequencies for allozyme and microsatellite markers. *Format* - Biological samples are stored at -80 °C or in ethanol. Genetic data are stored in ASCII text files and Microsoft Excel files. *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 areas to the north and south, 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 important species for subsistence fisheries followed by chum and sockeye salmon, while coho salmon are the most important 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). The subsistence fishery for Chinook salmon in the KMA is one of the largest and most significant in Alaska. 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 (Coffing et al. 2001). 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 between 1991 and 2000 included 84,887 Chinook, 70,537 chum, 41,793 sockeye, and 36,712 coho salmon.

In September 2000, the Alaska Board of Fisheries classified Kuskokwim River Chinook salmon as a "stock of concern" (Burkey et al. 2000a). The designation was made due to the chronic inability, despite the use of specific management measures, to maintain expected harvest above escapement needs (5 AAC 39.222). A similar designation was made for chum salmon (Burkey et al. 2000b). Kuskokwim River Chinook salmon have been a conservation concern since the mid-1980's when poor escapements with low female percentages were observed (Cappiello and Burkey 1997). Inseason management of the commercial fishery did not reverse the trend, and the fishery was increasingly restricted. Since 1987, there has been no directed commercial harvest of Chinook salmon, and all commercially-taken Chinook salmon have been incidental to the commercial harvest of chum salmon. Continuing low returns in recent years have yielded shortfalls in escapements basin-wide and the further imposition of fishing restrictions, including reduction in the time allowed for subsistence harvest. The "stock of concern" designation for Chinook salmon was the culmination of a more recent string of disastrously low Chinook salmon runs that began about 1998, coupled with concern for the critical dependence on the species by local residents (Burkey et al. 2000c; Burkey et al. 2002).

Management of KMA salmon fisheries is difficult. The commercial fisheries generally harvest 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 sizes,

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 at this time to determine the escapement levels needed to produce a sustained yield. Periodic aerial surveys of the spawning grounds provide an index of escapement, 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 or stained making visibility difficult. Ground based surveys, such as towers and weirs, provide good information on escapements to systems where they exist, but vast areas remain unobserved. More recently, tracking studies of Chinook salmon using radio telemetry have begun to provide information on run timing and spawning locations farther upstream (Stuby 2003).

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 these salmon stocks within mixtures will provide vitally important information for management.

Allozymes and microsatellites are two commonly used genetic markers that are generally considered to follow Mendelian inheritance and are expressed as codominant genotypes. Allozyme analyses investigate variant forms of specific enzymes that reflect variation in the DNA at the protein-coding loci. Microsatellites are portions of DNA containing a short sequence of repeated nucleotides. Because they are susceptible to length mutations, they often have more alleles per locus than the average allozyme marker (Wright and Bentzen 1994). Microsatellites can be amplified through the polymerase chain reaction (PCR) so only small quantities of tissues are necessary, allowing use of fin-clips, scales, or even archived material.

Studies of population structure using genetic markers have revealed distinct genetic lineages of Chinook salmon within Alaska (Gharrett et al. 1987; Crane et al. 1996; Guthrie and Wilmot 2004). Within the Yukon River both allozymes and microsatellites have shown genetic subdivision of Chinook populations (Wilmot et al. 1992; Scribner et al. 1996). Studies from Alaska have also shown that these two types of nuclear markers generally provide complementary and concordant estimates of genetic differentiation among populations of salmonids (Scribner et al. 1998; Allendorf and Seeb 2000).

Genetic stock identification using allozyme data has been applied to many different salmon fisheries, including Chinook salmon fisheries in Alaska and the Pacific Northwest (Utter et al. 1987; Shaklee et al. 1999; Crane et al. 2000). A database has been developed of 48 allozyme loci for more than 250 Chinook salmon populations from California to the Kamchatka Peninsula in Russia (Teel et al. 1999). In that database, the Kuskokwim River is represented by only three populations, the Tuluksak, Kogrukluk, and Stony rivers. The remainder of the KMA is represented by samples from the Middle Fork Goodnews and Kanektok rivers.

This project investigated the genetic diversity of Chinook salmon from the KMA, increased the number of populations surveyed, and added microsatellite markers. This baseline can be used for estimating stock composition of commercial and subsistence harvests, investigating run

timing and entry patterns within the river, and ascertaining the effectiveness of management actions for the conservation of the resource. These data also contribute to the Pacific Rim baseline of Chinook salmon genetic information, aiding the identification of Kuskokwim River salmon in studies of migration patterns of juveniles and sub-adults on the high seas and also in bycatch in marine 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 are: 1) Establish a genetic baseline for Chinook salmon from the Kuskokwim River, 2) Identify genetic units for improved conservation and management, and 3) Standardize and contribute data to Pacific Rim databases.

METHODS

Sample collection

Personnel from ADF&G, the US Fish and Wildlife Service (USFWS), Kuskokwim Native Association, and subsistence users collected tissues from Chinook salmon at weir sites, from subsistence fisheries located near spawning grounds, or on the spawning grounds (Table 1, Figure 1). Chinook salmon were sampled for two tissues, fin clips and cheek muscle, to assay variation at microsatellite and allozyme loci. When lethal sampling was possible (subsistence-caught salmon or spawnouts), muscle, liver, eye, and heart tissues were sampled. Tissues were frozen in 2mL cryotubes in liquid nitrogen, remained on liquid nitrogen during field sampling and shipment to the ADF&G Gene Conservation Laboratory in Anchorage, and then stored at –80°C. ADF&G maintains archival storage of these tissues.

The target sample size was 100 adults per collection to obtain precise allele frequency estimates at low to moderately polymorphic loci (Allendorf and Phelps 1981). Allozymes may not be expressed in all tissues making it necessary to collect multiple tissues from each fish for analysis, a process that is potentially harmful or lethal to the individual. Sample sizes were kept to a level considered sufficient for statistical rigor when characterizing populations to mitigate local concern over the harm caused during the collection process. Only two of the 10 microsatellite

markers used in this study had greater than six alleles and could be considered hypervariable, potentially requiring larger sample sizes. Finally, many systems are without a means of interception (e.g. a weir) which increases the difficulty and expense of sampling large numbers of adult Chinook salmon.

Laboratory analysis

Allozyme loci

Variation at enzyme-encoding loci was assayed from protein extracts of muscle, liver, eye, heart, or fin tissues using the methods for horizontal starch gel electrophoresis described by Aebersold et al. (1987) and Van Doornik et al. (1999). We conducted a comprehensive examination for variation in the products of 24 enzymes encoded at 47 coastwide-accepted allozyme loci (Table 2). Protocols to resolve these loci follow those adopted by the collaborators supporting the Pacific Rim database for Chinook salmon (Shaklee and Phelps 1990; Teel et al. 1999). All gels were scored by two observers and conflicts were resolved by consensus. We used the enzyme nomenclature recommended by the American Fisheries Society (Shaklee et al. 1990). A photographic record of each gel was made, and the genotype data were archived electronically.

Microsatellite loci

A preliminary study of microsatellite variation in Chinook salmon identified at least nine informative microsatellite loci (ADF&G unpublished, Table 3). The ADF&G Gene Conservation Laboratory used these loci and screened additional microsatellite loci developed from Chinook, sockeye, coho, and chum salmon (Olsen et al. 1998; Banks et al. 1999; Nelson and Beacham 1999; Olsen et al. 2000) on all samples. Following this, the USFWS Conservation Genetics Laboratory examined the potential utility of screening additional markers by applying six more microsatellite loci to two populations (the Tatlawiksuk and Kwethluk rivers). No attempt was made to standardize or calibrate the data produced by USFWS with the data produced by ADF&G.

DNA Extraction The ADF&G Gene Conservation Laboratory extracted DNA from each sample using either Gentra Systems™ (Minneapolis MN) Puregene DNA isolation kit or the Chelex protocol described by Small et al. (1998).

The USFWS Conservation Genetics Laboratory extracted DNA using Qiagen DNeasy 96-well

kits. The isolated genomic DNA was quantitated on a 96-well FluoroCount™ fluorometer and diluted to a final stock concentration of 30ng/µl.

Amplification Amplification of microsatellite loci in both laboratories was conducted in 10µl volumes using 1µl template in PCR buffer purchased from Promega (Madison, WI) (10mM Tris-HCl, 50mM KCl, 0.2 mM each dNTP, 0.5 units *Taq* DNA polymerase). Thermal cycling in the ADF&G Gene Conservation Laboratory was conducted on an MJ Research PTC-225 thermocycler, while that at USF&WS was conducted on an MJ Research PTC-200 thermocycler. Specific conditions used to amplify each multiplex panel of microsatellite loci are provided in Tables 3 and 4.

Size fractionation and scoring The ADF&G Gene Conservation Laboratory fractionated microsatellite alleles using an ABI 377-96 automated DNA sequencer operated in GeneScan™ mode on a 5% denaturing polyacrylamide gel. Data were analyzed using the internal lane sizing standard and local Southern sizing algorithm in the GeneScan v.3.2. Alleles for each locus were scored, and data were tabulated for importing into statistical software with Genotyper v.2.5.

The USFWS Conservation Genetics Laboratory fractionated microsatellite alleles on 64-well denaturing polyacrylamide gels using a Li-Cor IR™ scanner and scored with Li-Cor Saga™ GT v.3.1 software (Lincoln, NE). Li-Cor 50-350bp or 50-500 size standards were loaded in the first and last lanes and at intervals of 14 lanes or less across each gel. Positive controls, consisting of 2-10 alleles of predetermined size, were loaded in three lanes distributed evenly across the gels to ensure consistency of allele scores.

In both laboratories, two researchers scored all alleles independently. Samples with score discrepancies between researchers were re-amplified at the loci in question and rescored independently.

Data analysis

Population structure

Allozyme loci Genotypes were scored from enzyme phenotypes and summarized into allele frequency estimates for all allozyme loci with the exception of the following loci: *GPI-B2*, GPIr*,* and *sMEP-2**. Heterozygote phenotypes for *GPIr** and *sMEP-2** cannot be consistently scored, so dominant and recessive phenotype frequencies were calculated for these loci (Teel et al. 1999). Similarly, the **100/*60* phenotype cannot be distinguished from the **100/*100* phenotype at *GPI-B2*.* Dominant and recessive phenotypic frequencies were calculated for

*GPI-B2*100* and *60 (*24* pooled with *100*) and reported as *GPI-B2**, and allelic frequencies were calculated for *100 and *24 (*60 pooled with *100*) and reported as *GPI-B2a** (Teel et al. 1999). Three sets of isoloci, enzymes encoded at two separate loci which cannot be distinguished by electrophoresis, were surveyed in the analysis (*sAAT-1,2**, *sMDHA-1,2**, and *sMDHB-1,2**).

Estimates of the population frequency of individual alleles for each allozyme locus were calculated from the observed frequency of the allele in the representative sample. Observed and expected heterozygosity and conformation of genotype frequencies to HWE expected ratios were assessed using a log-likelihood ratio test with a nominal significance of α=0.05, adjusted for multiple tests for loci within each population using a sequential Bonferroni correction (Rice 1989). F_{ST} was calculated between population pairs as a measure of population subdivision according to the method of Weir and Cockerham (1984) using *GENEPOP* version 3.2a (Raymond and Rousset 1995). Chord distances (Cavalli-Sforza and Edwards 1967) were calculated to summarize allele frequency differences between pairs of populations. Population structure was visualized using multidimensional scaling (MDS, Krzanowski and Marriott 1994) as implemented in *NtSYS* (Exeter Software, Setauket, NY) to reduce the interpopulation chord distances to three-dimensional space. Patterns observed using this method reflect the genetic distinctions between the populations in the analysis. Once population groups were established, two types of hierarchical gene diversity analysis were conducted to delineate the subdivision of genetic variation within and between groups of populations: log likelihood ratio statistics (Chakraborty and Liemar) and analysis of molecular variance (AMOVA Excoffier et al. 1992, implemented in *ARLEQUIN* version 2.001 Schneider et al. 1999). The log likelihood ratio method has the advantage of allowing for tests of significant variation within groups independently of other comparisons at the same hierarchical level. Significance of tests in this analysis is determined using the sequential Bonferroni correction (α=0.05; Rice 1989) for the number of tests at each level of the hierarchy. Loci scored as phenotype frequencies are not included in tests for conformation to HWE or calculations of heterozygosity and $F_{ST.}$ If not indicated otherwise, all analyses were calculated using the *S-plus* analytical software package (Mathsoft, Inc., Seattle WA).

Microsatellite loci Individual genotype data were summarized as allele frequencies for all microsatellite loci. 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 number of alleles and an estimate of allelic richness (El Mousadik and Petit 1996) were calculated for each population (*FSTAT* version 2.1, Goudet 1995). Allelic richness was calculated to account for variation in the detection of alleles at each locus caused by differences in sample size. Observed and expected heterozygosity and conformation of genotype frequencies to HWE expected ratios was assessed using the exact test in *GENEPOP*. Two measures of population subdivision were calculated from allele frequency differences: Cavalli-Sforza and Edwards' chord distances (*PHYLIP*, version 3.6, Felsenstein 2002) and F_{ST} (Weir and Cockerham 1984). *GENEPOP* was used to calculate F_{ST} values. Population structure was visualized with MDS using *NtSYS* to view the interpopulation chord distances in three-dimensional space. Patterns observed using this method reflect the genetic distinctions between the populations in the analysis. Analysis of molecular variance (AMOVA) was conducted in

ARLEQUIN version 2.001 to delineate the subdivision of genetic variation within and between groups of populations.

Genetic stock identification

Simulations were conducted to evaluate the potential application of genetic stock identification to mixtures of Chinook salmon harvested in KMA fisheries. These simulations may be used to help assess whether the baseline of allele frequencies at allozyme and microsatellite markers provides sufficient information to identify individual stocks or groups of stocks (reporting groups) in hypothetical mixtures.

Reporting groups were defined based on a combination of genetic similarity, geographic features and management applications. Simulations were performed using the Statistical Package for Analyzing Mixtures (SPAM version 3.6, 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 stock 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. Each set of simulations was repeated three times with a different subset of the baseline each time: 1) allozymes only, 2) microsatellites only, and 3) allozymes and microsatellites combined.

Simulation analyses using allozymes were also conducted with available coastwide data to determine if Kuskokwim River Chinook salmon can be detected in complex mixtures of Pacific Rim Chinook salmon for future high seas research. The available coastwide data consist of allozyme allele frequencies from 262 populations encompassing the range of Chinook salmon in the North Pacific, from California to the Yukon River including a Russian population (Kamchatka River).

Data standardization

Variation at allozyme loci was surveyed according to coastwide standards, and data were submitted to National Marine Fisheries Service, Seattle, for inclusion in the coastwide allozyme database for Chinook salmon. The two laboratories could not reach a common understanding of the standardization effort referred to in the following sentence from the project's Investigation Plan: "*The U.S. Fish and Wildlife Service's Fish Genetics Laboratory (FGL) (Bill Spearman)*

will standardize microsatellite data to allow data sharing with other agencies." In light of the large standardization effort to be conducted under OSM 02-121 "Run timing, migratory patterns, and harvest information of Chinook salmon stocks within the Yukon River," both laboratories agreed that, as an alternative to the standardization of microsatellite loci, the USFWS Conservation Genetics Laboratory would survey a subset of six loci from their microsatellite panel developed for the Yukon River (*Oke2, Oke4, Oki11, Ots3.1, OtsG409*, and *OtsG432*) for two populations, Kwethluk and Tatlawiksuk rivers.

RESULTS

Sample collection

The baseline tissue collections analyzed in this study included 1,398 individual Chinook salmon from 20 collections representing 15 populations (Table 1, Figure 1). Thirteen of the populations were collected from tributaries to the Kuskokwim River, ranging from the Eek River near the river's mouth northeast to Salmon River, a tributary of (and referred to as) Pitka Fork, in the upper Kuskokwim River drainage. In addition, two collections were taken from drainages that are separate from the Kuskokwim River, but flow into Kuskokwim Bay and are part of the KMA: the Goodnews and Kanektok river drainages. The target sample size of 100 individuals per population was met for all populations with the exception of the Hoholitna, Middle Fork Goodnews, and Kanektok rivers. Only 10 individuals could be sampled from the Hoholitna River so this collection was excluded from all analyses. Tissues were sampled for allozyme analysis, which requires multiple-tissue collections, but not all collections contain the same set of tissues. Collections sampled prior to 2001 contain muscle, liver, heart and eye tissues. Only muscle and fin tissues were sampled in 2001 and 2002 in an effort to avoid lethal sampling. All tissues were frozen in liquid nitrogen, shipped to the ADF&G Gene Conservation Laboratory in Anchorage, and stored at -80°C. The portions of these tissues used for microsatellite analysis are stored in ethanol.

Laboratory analysis

Allozyme loci

Of the 47 loci surveyed (Table 2), eight loci could not be resolved in collections containing only muscle and fin tissues: *sAAT-3*, *mAH-1*, *mAH-3*, *mAH-4*, *FDHG*, *IDDH-1*, *LDH-C* and *mSOD*. Of these, only *FDHG* was monomorphic. In addition, the variant alleles at *ADA-2*, *GAPDH-2* and *bHEX* could not be reliably resolved so these loci were dropped from the analysis. Of the remaining 36 loci, 12 had no variant alleles in the collections analyzed, nine exhibited rare polymorphism (<5%), and 15 displayed variant alleles that were more frequent (Table 5).

Microsatellite loci

Fifteen microsatellite loci (*Oke4, Oki10*, *One2, One5, One7, One9, One10, One13, One102, One103, Ots1, Ots2, Ots100*, *Ots107*, and *µSat73*) were originally surveyed (Table 3). Six of these loci (*Oke4*, *One102*, *Ots107*, *One103*, *Oki10* and *One13*) were chosen from the set of loci used at the USFWS Conservation Genetics Laboratory in an effort to use common loci between laboratories. During the course of microsatellite genotyping *Oki10*, *One2*, *One5, One10,* and *One13* were removed from the baseline due to poor resolution. Allele frequencies from ten microsatellite loci were used in this analysis (Table 6). USFWS Conservation Genetics Laboratory surveyed a subset of six loci from their microsatellite panel developed for the Yukon River (*Oke2, Oke4, Oki11, Ots3.1, OtsG409*, and *OtsG432*) for two populations, Kwethluk and Tatlawiksuk rivers.

Data analysis

Population structure

Of the 14 populations in the baseline, five were sampled in two separate years (Table 1). Only eight individuals were collected from the Cheeneetnuk River in 2001, but a full set of 100 was collected in 2002, so the 2001 collection was not included in the analysis. Allele frequencies at each locus were compared between collections within populations using log likelihood ratio statistics based on allele frequencies. Statistics were summed across loci to test for annual variation within populations. No significant differences were found between collections for any of the four populations, and collections were pooled for all remaining analyses.

Allozyme Twenty-three loci were found to be polymorphic in at least one population in the dataset. No deviation from HWE was found in the overall test for any of the populations. The number of alleles, observed and expected heterozygosities, and F_{ST} were calculated at each locus across all populations as a means to measure the genetic diversity described by individual loci

(Table 7). The greatest number of alleles (four) and highest F_{ST} (0.026) were found at *sIDHP-1*. The highest heterozygosity was measured at *ADA-1* (0.223). To measure the genetic diversity within populations, the number of polymorphic loci and number of alleles were calculated for each population (Table 8). The two populations outside the Kuskokwim River drainage, the Kanektok (19) and Middle Fork Goodnews rivers (16), showed the greatest number of polymorphic loci. Within the Kuskokwim River drainage, the number of polymorphic loci ranged from 12 to 15, with the fewest polymorphic loci found in the Pitka Fork population, the furthest upstream. A similar pattern was seen in the total number of alleles found in each population, although the fewest alleles (36) were found in the George River. Sample size did not appear to affect the number of alleles detected as more alleles were detected in the smallest collections (Middle Fork Goodnews and Kanektok rivers) than in the largest (Tuluksak River).

Genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between each pair of populations in the baseline (Table 9). These inter-population distances were then visualized in three dimensions using an MDS plot (Figure 2) where the relative distance between populations indicates the overall similarity of allele frequencies between those two populations. The MDS indicates that the Pitka Fork and Middle Fork Goodnews River are relatively distinct from the other populations in the dataset. Populations in the Takotna and Kanektok rivers are moderately distinct from the main cluster of populations. This pattern is reflected in the F_{ST} values (Table 9); the largest F_{ST} estimates are comparisons to the Pitka Fork population followed by those to the Middle Fork Goodnews River population.

The allozyme data indicate that genetic population structure does exist in Chinook salmon populations from the KMA and that this structure is associated with geographic distribution. Populations located the furthest upstream (Pitka Fork and Takotna River) and those outside the Kuskokwim River drainage (Goodnews and Kanektok rivers) are distinct from a heterogeneous grouping of populations from the middle and lower Kuskokwim River (from Eek River upstream to Tatlawiksuk River). When populations were grouped into these three regions, the AMOVA analysis indicated that the differences between regions were not significant after accounting for divergence within populations and between populations within regions. When the three geographically proximate collections from the middle Kuskokwim River (Tatlawiksuk, Cheeneetnuk and Stony rivers) were grouped separately as a fourth group, a significant amount of the genetic variation was explained by regional grouping (Table 11). A significant amount of variation was also found among populations within regions indicating that populations are diverse within these regional groups. Hierarchical gene diversity analysis of grouping into four regions was repeated using log likelihood ratio statistics (Table 12). Significant genetic variation was found among the four regions as well as within each of the regions even when measured independently of the other regions.

Microsatellite Of 140 tests of conformity to HWE using the microsatellite loci, four were significant after adjusting for the number of tests within populations. They were *Ots107* in Tuluksak, George and Cheeneetnuk rivers and *One102* in Cheeneetnuk River. The number of alleles, observed and expected heterozygosities, and F_{ST} were calculated at each locus across all populations as a means to measure the genetic diversity described by individual loci (Table 7). The greatest number of alleles (68) was found at *Ots100,* and the highest F_{ST} (0.016) was found

at *One103*. The highest heterozygosity was measured at *Ots100* (0.940). To measure the genetic diversity within populations, the number of alleles and allelic richness were calculated for each population (Table 8). With the exception of Tuluksak River (112 alleles observed), the number of alleles observed ranged from 68 to 96 with the fewest alleles found in the Pitka Fork population followed by Middle Fork Goodnews River (78). This pattern may be the effect of sample size on detection of alleles in hypervariable loci; Middle Fork Goodnews River was the smallest collection (N = 40) and Tuluksak River (N = 150) was the largest in the analysis. When allelic richness, a measure of the number of alleles independent of sample size, was estimated, similar numbers of alleles were estimated at Tuluksak and Middle Fork Goodnews rivers, but the Pitka Fork population dropped to 58, well below the level found in other populations, with the exception of Tatlawiksuk River at 64.

Genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between pairs of populations in the baseline (Table 10). These inter-population distances were then visualized in three dimensions using an MDS plot (Figure 3) where the relative distance between populations indicates the overall similarity of allelic frequencies for those two populations. The MDS indicates that the Pitka Fork, Tatlawiksuk, and Middle Fork Goodnews River are relatively distinct from the other populations in the dataset. The Takotna, Stony, Cheeneetnuk and Tuluksak rivers are moderately distinct from the main cluster of populations. The Kanektok River is associated with a tight group of populations from the lower Kuskokwim River. This pattern is reflected in the F_{ST} values (Table 10). The largest F_{ST} estimates are associated with the Tatlawiksuk population followed by the Pitka Fork and Middle Fork Goodnews River populations. It is worth noting that these three populations also have the fewest alleles and lowest allelic richness of all the populations for the microsatellite loci surveyed. Summary information for the loci analyzed by the USFWS Conservation Genetics Laboratory is found in Table 13.

Microsatellite data indicate that genetic population structure does exist in Chinook salmon populations from the KMA and that this structure may be associated with geographic distribution. Populations located at the edges of the KMA, northeast (Pitka Fork, Takotna River, and Tatlawiksuk River) and southwest (Middle Fork Goodnews River) are distinct from each other as well as from the main, central group of populations. To a lesser extent, Stony River is also separated from this group, which extends from the Kanektok River upstream to Cheeneetnuk River. Populations were grouped into four regions based on genetic diversity and geographic features: 1) Goodnews/Kanektok, 2) Lower Kuskokwim (populations between Eek and Kogrukluk rivers), 3) Middle Kuskokwim (Stony, Cheeneetnuk and Tatlawiksuk rivers), and 4) Upper Kuskokwim (Takotna River and Pitka Fork). AMOVA indicated that a significant amount of the genetic variation was explained by regional grouping (Table 11). Furthermore, the amount of variation found among populations within regions was not significant, indicating that populations are more similar to other populations within regional groups than they are to populations in other groups.

Genetic stock identification

Simulations of genetic stock identification using the baseline developed in this project began at the population level. Each population was considered a reporting group, and mixtures were generated entirely from the population being tested. The average portion of these mixtures that are correctly reallocated to the originating population is a measure of the identifiability of the population in baseline. Mean correct allocations for the 14 populations ranged from 56% to 96% for the allozyme markers, 63% to 94% for microsatellite markers, and 65% to 94% for the combined set of markers. This level of identifiability was not considered adequate, so larger reporting groups were constructed.

Reporting groups composed of multiple populations were defined based on genetic similarity, geographic proximity, and management applications. We defined three reporting regions within the Kuskokwim River: 1) Lower Kuskokwim (populations between Eek and Kogrukluk rivers), 2) Middle Kuskokwim (Stony, Cheeneetnuk and Tatlawiksuk rivers), and 3) Upper Kuskokwim (Takotna River and Pitka Fork). The Middle Kuskokwim region, the most genetically diverse group of the four, was defined by the geographic proximity of the populations and the desire to segregate the upstream and downstream populations in the fishery. We performed a set of simulations that examined the identifiability of these three reporting regions when the simulated mixtures were composed of individuals drawn entirely from the populations in a single region. These simulations investigate the identifiability of the reporting regions in mixtures of fish that might be sampled within the Kuskokwim River (Table 14). The Lower and Upper reporting groups are correctly allocated 93% of the mixture and the Middle Kuskokwim received 86% of the mixture on average. Combination of the Middle and Upper Kuskokwim regions in a simulation showed that both the Lower Kuskokwim (93%) and Upper Kuskokwim (95%) regions are identifiable in mixtures taken from Chinook salmon in the Kuskokwim River (Table 15).

We performed a second set of simulations including an additional region from Kuskokwim Bay, Goodnews/Kanektok (Table 16). For these simulations, the Middle and Upper Kuskokwim regions were grouped to investigate the ability to identify upstream from downstream Chinook salmon in a regional fishery. Correct allocations in these simulations were 72%, 92% and 89% for the Goodnews/Kanektok, Lower Kuskokwim and Upper Kuskokwim regions, respectively. Another set of simulations was performed using all four reporting groups (not shown) and the results for each region were not different than regional results reported in Tables 14 and 16.

A simulation using the entire Pacific Rim allozyme baseline indicates that the Kuskokwim River populations are moderately identifiable (85% correct allocation). In this simulation, an additional 5% of the mixture was allocated to other populations from Western Alaska, 4% was allocated to Susitna River populations, and less than 1% was allocated to the Russian population.

DISCUSSION

Genetic population structure

The results of this study reveal substantial genetic diversity among Chinook salmon populations in the KMA. Populations outside the Kuskokwim River drainage displayed greater population genetic diversity than populations within the river when measured by the number of polymorphic loci and number of alleles in allozymes (Table 8), and Kanektok River had the greatest allelic richness in the microsatellite loci. In addition, genetic distances indicate that these populations had different allele frequencies at allozyme loci (Figure 2). Middle Fork Goodnews River had quite different microsatellite allele frequencies, but Kanektok River could not be distinguished from populations from the lower Kuskokwim River using the microsatellite loci surveyed in this study (Figure 3). This similarity may be the reason for the low identifiability of the Goodnews/Kanektok reporting group in simulation analyses (Table 16), where most of the misallocation is to the Lower Kuskokwim River.

Within the Kuskokwim River drainage, the greatest differences were found between populations spawning in the upper river, while little divergence was found between populations spawning lower in the river; a pattern that was found using both marker types. The Pitka Fork population was the most divergent population when measured with the allozyme markers and the second most divergent using microsatellite markers, it also expressed the fewest polymorphic allozyme loci, a low number of allozyme alleles, and the fewest alleles at microsatellite loci (Table 8). At Takotna River, the next population downriver, these measures were more similar to other Kuskokwim River populations, but allele frequencies at both marker types were still different between this population and others in the study, including Pitka Fork. The populations located in the middle river (Tatlawiksuk, Cheeneetnuk and Stony rivers) are significantly divergent from each other and are moderately different from the cluster of populations from the lower river. These distinctions are amplified using the microsatellite markers (Figure 3); Tatlawiksuk River has the largest F_{ST} values for microsatellite markers The remaining populations, from Eek River upstream to the Kogrukluk River, are similar to one another based on the markers used in the present baseline. This may represent a limitation of the markers in the present baseline, but likely also represents a lack of barriers to present and or historical gene flow between the lower and the upper Kuskokwim drainage. Allele frequency differences between the Kwethluk and Tatlawiksuk rivers in the loci run in the USFWS Conservation Genetics Laboratory (Table 6) suggest that increasing the number of microsatellite loci in the baseline could improve the ability to distinguish these clusters of populations from each other.

Tests of population structure (Tables 11 and 12) found significant genetic differences between groups when populations were assigned to four groups based on genetic characteristics and geographic proximity; supporting the combining of populations into these groups. Furthermore, AMOVA did not detect significant differences between populations within groups in microsatellite loci. However significant differentiation was detected at this level in the allozyme data, indicating that heterogeneity exists between populations within groups in the allozyme baseline. This may be the result of the allozyme markers being less neutral than microsatellites , ascertainment bias due to the number of loci surveyed for the two marker types or it may indicate

the need for larger sample sizes with more polymorphic markers such as microsatellites. This may be resolved through the addition of individuals to the baseline or by surveying more microsatellite markers.

Genetic stock identification

Although simulation studies were based on all marker types, both individually and combined, we recommend that future genetic stock identification projects within KMA be based on DNA markers to both simplify sampling and control costs. Bycatch or other high seas applications requiring a complete species-wide baseline will need to rely on allozymes until a comprehensive and standardized Pacific Rim DNA baseline can be completed.

Sufficient genetic structure exists among populations of Chinook salmon from the Kuskokwim River for the successful application of genetic stock identification to harvests of inriver fisheries using the microsatellite baseline. In simulation studies, three groups (Lower, Middle and Upper) could be identified in hypothetical mixtures, although the Middle Kuskokwim region performed only at 85% correct allocation. Analyses of population structure show that the three populations in the Middle Kuskokwim River region are a genetically diverse group and they misallocate to both the Lower and Upper Kuskokwim regions. However, when the Middle and Upper Kuskokwim River regions were combined for simulations, the combined group was identifiable above the 90% threshold, with the majority of the misallocation coming from the Cheeneetnuk River to the Lower Kuskokwim reporting group.

Simulation studies indicate that the application of genetic stock identification to fisheries occurring in Kuskokwim Bay using the current baseline will not adequately distinguish Chinook salmon from the Goodnews and Kanektok Rivers from those returning to the Kuskokwim River. The highest mean correct allocation for this group was 74% when considered in the whole baseline using only microsatellite loci (Table 16). Much of this misallocation was probably caused by the similarity of microsatellite allele frequencies between the Kanektok River and the Lower Kuskokwim River reporting region (Figure 3). This low level of identifiability is surprising given the genetic distinctiveness of the Middle Fork Goodnews River population, but it may be the result of the small sample sizes from these populations in the baseline. The fact that both of these populations show evidence of greater within-population genetic diversity than populations within the Kuskokwim River, despite the small sample sizes indicates that representation of these populations with larger sample sizes will improve measures of their distinctiveness and increase the ability to identify these populations in mixtures from Kuskokwim Bay.

A target sample size of 100 adults per collection was set as a compromise between precision, cost, and sensitivity to local sentiment concerning sampling and handling spawning Chinook salmon. A sample size of 100 is adequate to obtain precise allele frequency estimates at low to moderately polymorphic loci such as allozymes. Furthermore, eight of the ten 10 microsatellite

markers used in this study exhibited six or fewer alleles. Many systems are without a means of interception (e.g. a weir) increasing the difficulty and expense of sampling large numbers of adult Chinook salmon. We recommend a multi-year sampling program to minimize impacts on any spawning run with the ultimate goal of achieving large sample sizes to accurately and precisely characterize variation. Additional markers may also improve the performance of the baseline for these fisheries.

Simulations of hypothetical mixtures drawn entirely from the KMA indicate that these populations are moderately identifiable in the coastwide allozyme baseline. Most of the misallocation goes to the Western Alaska region with an undetectable or zero misallocation to Russia. If the results from the work with the baseline from Chinook salmon from the KMA are an indication, greater distinction will be attained for identifying KMA Chinook salmon using DNA-based genetic markers than is currently possible using the allozyme data. This will provide valuable information for identification of these populations in ongoing studies on the high seas.

The ability to differentiate groups of Chinook salmon populations harvested in KMA fisheries provides a valuable tool for the management of those fisheries. Commercial fisheries take place in Districts 1 and 2 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 20,210 fish. In District 5 Chinook are harvested incidental to sockeye salmon, with an average annual catch of 2,547 fish. 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 fishers 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 by increasing the representation of stocks from Kuskokwim Bay and by surveying additional markers should lead to improved results from genetic stock identification applications for these fisheries.

The directed commercial fishery for Chinook salmon in the Kuskokwim River was discontinued in 1987 because of low run abundance (Burkey et al 2002) and has not been re-established because of the importance of this species for local subsistence use. The stock of concern finding for Chinook salmon in the Kuskokwim River prompted further conservation measures aimed at reducing Chinook salmon harvest and improving escapement (Burkey et al. 2002). For example, in the Kuskokwim River, commercial chum salmon fishing was closed in June and in part or all of July during 2001, 2002, and 2003. Concurrent with these closures, subsistence fishers were, for the first time in history, 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 (Burkey et al. 2002). For practical reasons, subsistence fishers take most of their harvest from the early half of the Chinook salmon run (Figure 4). This practice, however, creates an added challenge to prosecuting a sustainable Chinook salmon management program because of differences in stock-specific run timings. Preliminary findings from a radio telemetry project initiated near Aniak in 2002 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 2003). 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. The genetic stock identification capabilities developed in this project have potential for providing greater resolution on this issue, enabling managers to develop a more effective sustainable Chinook salmon management program for the KMA.

CONCLUSIONS

1) Significant population structure exists among populations of Chinook salmon from the Kuskokwim Management Area. In particular, populations spawning upriver of the confluence with the Holitna River are particularly genetically divergent, both within and between populations.

2) Genetic analyses provide adequate distinction within the Kuskokwim River to estimate composition and run timing of regional groups from the inriver fisheries in districts W-1 and W-2.

3) Genetic analyses based on allozyme markers can be used in high seas or bycatch identification of Kuskokwim River Chinook salmon. Once a coastwide baseline is complete, DNA markers will allow a similar or improved level of resolution.

RECOMMENDATIONS

1) This study provides the first comprehensive analysis of the genetic diversity of Chinook salmon from the Kuskokwim Management Area. Management actions should recognize and include consideration of this diversity, attributes which should be conserved to sustain and maximize longterm productivity within the drainage. Applications of the genetic data can now begin to address questions concerning stock composition and run timing in fisheries within the Kuskokwim River.

2) The performance of genetic stock identification models could be improved with further development of the baseline. The addition of unrepresented populations coupled with increased sample sizes for those populations already present and addition of more DNA markers will improve model performance and increase resolution. With improved

performance, genetic stock identification analyses can likely be expanded to include the entire Kuskokwim Management Area (Districts W-4 and W-5). This could be efficiently achieved by using existing platforms for expanding the current population baseline. For example, sampling tissues from the Chinook radio telemetry projects operated in the Kuskokwim River when the radio receivers are implanted, and then matching those fish to the spawning locations.

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Table 1. Chinook salmon collections included in the Kuskokwim Area baseline. Tissue abbreviations are M – muscle, L – liver, H – heart, E – eye, and F – fin. Collection numbers correspond to locations in Figure 1. The Hoholitna River sample (#15) was dropped from all analyses.

	Sampling Location	n	Tissues Collected	Years Sampled
1	Middle Fork Goodnews River weir	40	M,L,H,E	1993
2	Kanektok River weir	78	M,L,H,E	1992, 1993
3	Eek River	100	M,F	2002
4	Kwethluk River weir	100	M,F	2001
5	Kisaralik River	100	M,F	2001
6	Tuluksak River weir	150	M,L,H,E	1993, 1994
7	Aniak River	100	M,F	2002
8	George River weir	100	M,F	2002
9	Kogrukluk River weir	100	M,L,H,E	1992, 1993
10	Stony River	100	M,L,H,E	1994
11	Tatlawiksuk River weir	102	M,F	2002
12	Cheeneetnuk River	108	M,F	2001, 2002
13	Takotna River weir	111	M,L,H,E	1993, 1994
14	Pitka Fork	100	M,L,H,E	1995
15	Hoholitna River mainstem	10	M,F	2001

Table 2. Buffers and tissues used to resolve enzyme encoding loci. Tissue abbreviations are M – muscle, L – liver, H – heart, E – eye, and F – fin. The variability of each locus was calculated as the maximum summed frequencies of variant alleles in any population.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]	Variability
Aspartate aminotransferase	2.6.1.1	*mAAT-1*	H,M	ACE6.8	invariant
		sAAT-1,2	M	TBE	invariant
		sAAT-3	E,M	TBE	≥0.05
		sAAT-4	L	TBE	≥0.05
Adenosine deaminase	3.5.4.4	*ADA-1*	E,M	TBE	≥0.05
		ADA-2	E,M	TBE	<0.05
Aconitate hydratase	4.2.1.3	*mAH-1*	E,H	ACE6.8	<0.05
		mAH-3	H,M	ACE6.8	<0.05
		mAH-4	H,M	ACE6.8	<0.05
		sAH	L,F	ACE7	≥0.05
Alanine aminotransferase	2.6.1.2	*ALAT*	M	TG	≥0.05
Formaldehyde dehydrogenase	1.2.1.1	*FDHG*	L,F	TBE	Invariant
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-2*	H	ACEN6.8	N/A
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A*	M	TBCLE	Invariant
		GPI-B2	M	TBCLE	Invariant
		GPI-B2a	M	TBCLE	<0.05
		GPIr	M	TBCLE	Invariant
Glutathione reductase	1.6.4.2	*GR*	E,M	TBCLE	Invariant
beta-N-acetylhexasaminidase	3.2.1.52	*bHEX*	L,F	TC4	N/A
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1*	L	TBCL	≥0.05
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-2*	E,M	ACE7	Invariant
		sIDHP-1	M,E,L,F	ACE6.8	≥0.05
		sIDHP-2	E,L,F	ACE6.8	≥0.05
L-lactate dehydrogenase	1.1.1.27	*LDH-B1*	H,E,F	TBCLE	Invariant
		LDH-B2	L,E,M,F	TBCLE	Invariant
		LDH-C	E	TBCLE	<0.05
Malate dehydrogenase	1.1.1.37	*mMDH-2*	M,H	ACE6.8	≥0.05
		sMDH-A1,2	M	ACE7	Invariant
		sMDH-B1,2	M	ACE7	≥0.05
Malic enzyme (NADP+)	1.1.1.40	*sMEP-1*	H,M	TC4	Invariant
		sMEP-2	M,L	TC4	≥0.05
Mannose-6-phosphate isomerase	5.3.1.8	*MPI*	E,L,F	TBE	≥0.05
Dipeptidase	3.4.-.-	*PEPA*	E,M	TBE	≥0.05
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1*	E,M	TBE,TC4	≥0.05
Proline dipeptidase	3.4.13.9	*PEPD-2*	E,M	TBE	Invariant
Peptidase-LT	3.4.-.-	*PEPLT*	E,M	TBE	<0.05
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH*	E,L,M,F	ACE7	Invariant
Phosphoglycerate kinase	2.7.2.3	*PGK-2*	E,M,L,F	ACE7	Invariant
Phosphoglucomutase	5.4.2.2	*PGM-1*	H,M	TBE	Invariant

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]	Variability
		PGM-2	L,M,F	TBCL	Invariant
Superoxide dismutase	1.15.1.1	*mSOD*	H,M	TBE	≥0.05
		sSOD-1	L,E,M,F	TBE	≥0.05
Triosephosphate isomerase	5.3.1.1	*TPI-3*	H,E,M,F	TBE	Invariant
		TPI-4	M,E,F	TBE	≥0.05

[1]Buffers are: ACE6.8 = amine-citric acid-EDTA buffer, pH 6.8; ACEN6.8 = amine-citric acid-EDTA-NAD buffer, pH 6.8 (Clayton and Tretiak 1972); TBCL = Tris-citric acid gel buffer, lithium hydroxide-boric acid electrode buffer,pH 8.5 (Ridgway et al. 1970); TBE = Tris-boric acid-EDTA buffer, pH 8.7 (Boyer et al. 1963); TC4 = Tris-citric acid buffer, pH 5.95 (Schaal and Anderson 1974).

Table 3. Microsatellite loci surveyed in Kuskokwim River Chinook salmon. Ten loci that amplified reliably and were consistently scorable (indicated by "*") were used by ADF&G to construct the baseline presented herein. Following baseline construction, six additional loci were screened in the USFWS Conservation Genetics Laboratory (indicated by "**") as a way of evaluating the potential benefit of adding loci to the baseline.

Locus	Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse	Primer (uM) [1]	Agency
*µSat73**	**F**>CCTGGAGATCCTCCAGCAGGA **R**>CTATTCTGCTTGTAACTAGACCTA	0.06	ADF&G
*Oke4**	**F>**AGGCCCAAAGTCTGTAGTGAAGG **R>**GATGAATCGAGAGAATAGGGACTGAAT	0.03	ADF&G
Oki10	**F>**GGAGTGCTGGACAGATTGG **R>**CAGCTTTTTACAAATCCTCCTG	0.40	ADF&G
One2	**F**>GGTGCCAAGGTTCAGTTTATGTT **R**>CAGGAATTTACAGGACCCTGGTT	0.04	ADF&G
One5	**F**>AACACACCAGCTGTGAAAACAAA **R**>TGTCTATCGCCAATCTCTCTGCT	0.50	ADF&G
*One7**	**F**>ACACTGCAAACACTCTGCTTACT **R**>CAAGAAGAAACCCTGTCCTCAAG	0.15	ADF&G
*One9**	**F**>CTCTCTTTGGCTCGGGGAATGTT **R**>GCATGTTCTGACAGCCTACAGCT	0.04	ADF&G
One10	**F**>ATGGGGAACAGAAGAGGAAT **R**>CTGTAGGTGTGAAATGTATTTAAA	0.30	ADF&G
One13	**F>**TCATACCCCATGCCTCTTCTGTT **R>**GATGAGTGAAAGAGAGGGAGCGA	0.25	ADF&G
*One102**	**F>**CATGGAGAAAAGACCAATCA **R>**TCACTGCCCTACAACAGAAG	0.06	ADF&G
*One103**	**F>**AATGTTGAGAGCTATTTCAATCC **R>**GATTGATGAATGGGTGGG	0.40	ADF&G
*Ots1**	**F**>GGAAAGAGCAGATGTTGTT **R**>TGAAGCAGCAGATAAAGCA	0.04	ADF&G
*Ots2**	**F**>ACACCTCACACTTAGA **R**>AATATCCTTCACACTG	0.15	ADF&G
*Ots100**	**F**>TGAACATGAGCTGTGTGAG **R**>ACGGACGTGCCAGTGAG	1.2 (0.50)	ADF&G
*Ots107**	**F>**ACAGACCAGACCTCAACA **R>**ATAGAGACCTGAATCGGTA	0.10	ADF&G

Locus	Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse	Primer (uM) [1]	Agency
*Oke2***	**F**>AGGGCCAGAGAAAAGTCTCACTAT **R**>GTCAGTCCTGCCCTCTGTGTCCTA	.006/.394 0.4	USFWS
*Ots3.1***	**F**>CAGCCCATCTGTCACTCACACT **R**>GGTGGAGAGAGTTTGAGAATCACA	.007/.393 0.4	USFWS
*Oki11***	**F**>TCTGAGACAGGCAAATGCAC **R**>GTTTTAAACCTCACCATTGAGT	.035/.365 0.4	USFWS
*Oke4***	**F**>AGGCCCAAAGTCTGTAGTGAAGG **R**>GATGAATCGAGAGAATAGGGACTGAAT	.018/.382 0.4	USFWS
*OtsG409***	**F**>GTAGCCATTTGTGTCACCATCATT **R**>CATTCTCCTGCCTCACAGAGTTTA	.02/.38 0.4	USFWS
*OtsG432***	**F**>TGAAAAGTAGGGGAAACACATACG **R**>TAAAGCCCATTGAATTGAATAGAA	.015/.385 0.4	USFWS

[1] Concentrations are for labeled and unlabeled primers respectively.

Table 4. Reaction conditions for microsatellite amplification.

Panel	Thermal cycling profile	Loci	$MgCl_2$ (mM) [1]	dNTP (mM)
53	92°C (2 min); 7 cycles of 92°C (1 min) + 53°C (2 min) + 1°C/s to 72°C + 72°C (20s); 20 cycles of 92°C (30s) + 53°C (2 min) + 1°C/s to 72°C + 72°C (20s)	*One7* *Ots2* *Ots1* *Oke4*	2.0	0.8
54	92°C (2 min); 7 cycles of 92°C (1 min) + 54°C (2 min) +1 °C/s to 72°C + 72°C (20s); 20 cycles of 92°C (30s) + 54°C (2 min) + 1°C/s to 72°C + 72°C (20s)	*Ots107* *One102* *uSat73*	2.0 (2.5)	0.8
56	92°C (2 min); 5 cycles (-1°C/cycle) of 92°C (1 min) + 61°C (30s) + 72°C (15s); 21 cycles of 92°C (1 min) + 56°C (30s) + 72°C (15s),	*Oki10* *One103* *One10*	2.5	0.8
60	92°C (2 min); 7 cycles of 92°C (1 min) + 62°C (2 min) + 1°C/s to 72°C + 72°C (20s); 20 cycles of 92°C (30s) + 60 °C (62°C if using Chelex extraction) (2 min) + 1°C/s to 72°C + 72°C (20s).	*Ots100* *One2* *One9* *One13*	2.5	0.8
A	92°C (2 min); 30 cycles of at 92°C (15 sec) + 60 °C (15 sec) + 72°C (30 sec); with a final extension for 10 min at 72°	*Oke2* *Ots3.1*	1.5	0.8
A	92°C (2 min); 30 cycles of at 92°C (15 sec) + 52 °C (15 sec) + 72°C (30 sec); with a final extension for 10 min at 72°	*Oki11* *Oke4*	1.5	1
D	92°C (2 min); 30 cycles of at 92°C (15 sec) + 54 °C (15 sec) + 72°C (30 sec); with a final extension for 10 min at 72°	*OtsG409* *OtsG432*	2.5	1

[1] Numbers in parentheses refer to modifications used when processing Chelex extractions.

Table 5. Frequencies of variant alleles at allozyme loci for Chinook salmon populations from the Kuskokwim Management Area. Populations are designated as follows: Middle Fork Goodnews (Goo), Kanektok (Kan), Eek, Kwethluk (Kwe), Tuluksak (Tul), Aniak (Ani), George (Geo), Kogrukluk (Kog), Stony (Sto), Tatlawiksuk (Tat), Cheeneetnuk (Che), and Takotna (Tak) rivers and Pitka Fork (Pit). The common allele is designated *100 (not shown); other alleles are designated by their mobility relative to the common allele.

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
mAAT-1														
N	40	73	100	99	99	150	100	100	100	95	97	100	111	100
*-77	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
sAAT-4														
N	39	72	100	99	99	145	99	95	99	92	85	100	110	97
*63	0.013	0.062	0.000	0.000	0.000	0.034	0.000	0.000	0.020	0.022	0.000	0.000	0.018	0.000
ADA-1														
N	40	74	100	98	96	144	99	100	99	98	99	99	100	99
*83	0.162	0.095	0.160	0.117	0.109	0.111	0.111	0.180	0.197	0.128	0.131	0.152	0.130	0.010
sAH														
N	39	75	99	100	99	150	100	100	100	97	98	98	111	100
*86	0.115	0.027	0.076	0.040	0.030	0.053	0.050	0.095	0.065	0.046	0.051	0.082	0.081	0.040
*116	0.000	0.007	0.025	0.000	0.010	0.000	0.045	0.000	0.000	0.005	0.000	0.000	0.000	0.040
ALAT														
N	33	70	100	99	99	147	100	100	95	91	94	100	111	99
*90	0.015	0.064	0.050	0.091	0.061	0.082	0.095	0.075	0.089	0.099	0.176	0.125	0.041	0.025
GPIA														
N	40	73	100	100	100	150	100	100	99	96	99	100	111	99
*105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.005	0.000
*93	0.012	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-B2a														
N	40	73	100	99	100	150	100	100	99	94	98	100	111	99
*24	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sIDHP-1														
N	39	74	100	100	100	149	100	100	99	98	99	100	111	100
*94	0.000	0.000	0.005	0.005	0.000	0.007	0.010	0.000	0.000	0.000	0.005	0.000	0.014	0.075
*83	0.013	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*129	0.013	0.014	0.010	0.000	0.000	0.003	0.000	0.005	0.000	0.000	0.000	0.005	0.000	0.000
sIDHP-2														
N	39	74	100	100	100	150	100	100	100	98	99	100	110	100
*50	0.013	0.007	0.015	0.025	0.030	0.060	0.015	0.015	0.025	0.031	0.020	0.035	0.032	0.000
LDHB-2														
N	40	73	100	100	99	150	95	100	98	100	98	99	111	100
*112	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

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
mMDH-2														
N	40	74	100	99	99	150	100	100	98	96	95	100	109	100
*200	0.062	0.074	0.045	0.030	0.025	0.043	0.045	0.070	0.056	0.036	0.053	0.050	0.060	0.010
sMDHB-1,2														
N	40	74	100	99	99	150	100	100	99	100	99	100	111	100
*121	0.019	0.000	0.005	0.005	0.000	0.003	0.010	0.000	0.008	0.002	0.000	0.000	0.018	0.058
*70	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000
sMEP-1														
N	38	76	100	99	99	150	100	100	99	94	97	100	109	100
*92	1.000	0.993	0.990	0.995	0.995	1.000	0.995	0.995	0.995	0.984	1.000	1.000	0.995	1.000
sMEP-2														
N	39	72	100	99	99	150	100	100	99	93	97	100	109	100
*78	0.051	0.097	0.080	0.091	0.051	0.080	0.110	0.080	0.071	0.097	0.031	0.100	0.101	0.030
MPI														
N	40	76	100	98	100	149	100	100	100	97	99	98	110	100
*109	0.075	0.053	0.055	0.097	0.080	0.084	0.085	0.045	0.070	0.077	0.030	0.066	0.059	0.125
PEPA														
N	40	74	100	100	100	149	100	100	97	96	99	100	111	100
*90	0.038	0.041	0.095	0.050	0.070	0.057	0.025	0.045	0.052	0.052	0.020	0.060	0.041	0.015
PEPB-1														
N	36	75	100	99	99	150	100	100	97	93	98	100	109	99
*130/100	0.139	0.047	0.060	0.045	0.056	0.050	0.040	0.070	0.062	0.065	0.082	0.065	0.064	0.146
*71/100	0.069	0.013	0.005	0.005	0.000	0.000	0.000	0.000	0.005	0.011	0.005	0.045	0.032	0.040
PEPD-2														
N	40	67	100	99	99	148	100	100	97	89	99	99	110	99
*107	0.000	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
PEPLT														
N	38	69	100	99	99	146	99	100	99	90	97	100	111	100
*110	0.026	0.000	0.000	0.000	0.005	0.000	0.005	0.000	0.000	0.028	0.010	0.000	0.000	0.010
PGK-2														
N	40	71	100	100	100	150	100	100	100	100	99	100	111	99
*90	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-2														
N	40	73	100	100	100	150	100	100	99	100	99	99	109	100
*136	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sSOD-1														
N	40	76	99	100	100	150	100	100	98	98	99	100	110	100
*-260	0.038	0.059	0.056	0.060	0.075	0.053	0.075	0.065	0.020	0.041	0.051	0.020	0.000	0.000
TPI-4														
N	40	74	99	81	99	150	92	100	99	97	99	99	108	100
*104	0.150	0.088	0.126	0.031	0.091	0.060	0.092	0.160	0.086	0.119	0.131	0.035	0.069	0.045

Table 6. Allele frequencies for microsatellite loci surveyed in Chinook salmon populations from the Kuskokwim Management Area. Populations are designated as follows: Middle Fork Goodnews (Goo), Kanektok (Kan), Eek, Kwethluk (Kwe), Tuluksak (Tul), Aniak (Ani), George (Geo), Kogrukluk (Kog), Stony (Sto), Tatlawiksuk (Tat), Cheeneetnuk (Che), and Takotna (Tak) rivers and Pitka Fork (Pit). Loci marked with an asterisk (*) were assayed by the USFWS laboratory for survey purposes only and are not standardized for use in subsequent analyses.

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
uSat73														
N	40	76	87	83	94	146	81	72	99	96	75	93	94	95
138	0.000	0.000	0.000	0.012	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
140	0.750	0.717	0.805	0.711	0.755	0.767	0.753	0.694	0.758	0.667	0.873	0.677	0.766	0.774
142	0.188	0.224	0.109	0.199	0.197	0.113	0.148	0.215	0.177	0.208	0.100	0.215	0.133	0.105
144	0.025	0.026	0.029	0.030	0.011	0.041	0.019	0.035	0.005	0.021	0.000	0.038	0.016	0.000
148	0.038	0.033	0.057	0.048	0.037	0.072	0.080	0.056	0.061	0.104	0.027	0.070	0.085	0.121
Oke4														
N	40	76	93	91	94	145	86	73	97	96	95	93	93	96
243	0.275	0.257	0.253	0.297	0.223	0.214	0.215	0.226	0.247	0.260	0.216	0.263	0.215	0.172
245	0.425	0.487	0.409	0.352	0.410	0.455	0.424	0.404	0.402	0.339	0.347	0.355	0.323	0.396
247	0.225	0.204	0.242	0.236	0.223	0.241	0.221	0.212	0.242	0.276	0.337	0.280	0.312	0.203
249	0.075	0.053	0.097	0.115	0.144	0.090	0.140	0.158	0.108	0.125	0.100	0.102	0.151	0.229
One7														
N	38	71	94	93	93	145	93	95	93	95	94	94	94	94
191	0.316	0.338	0.309	0.323	0.344	0.403	0.392	0.316	0.382	0.389	0.287	0.266	0.319	0.282
193	0.684	0.662	0.691	0.677	0.656	0.597	0.608	0.684	0.618	0.611	0.713	0.734	0.681	0.718
One9														
N	40	76	89	84	94	146	89	89	99	96	96	93	96	96
165	0.050	0.033	0.056	0.036	0.043	0.058	0.062	0.056	0.076	0.063	0.036	0.038	0.026	0.010
169	0.950	0.961	0.944	0.964	0.957	0.942	0.938	0.944	0.924	0.938	0.964	0.962	0.974	0.990
172	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
One102														
N	40	76	91	85	95	146	85	92	100	96	96	94	96	96
189	0.712	0.743	0.703	0.682	0.689	0.726	0.676	0.766	0.720	0.771	0.609	0.638	0.641	0.646
193	0.287	0.257	0.297	0.318	0.311	0.274	0.324	0.234	0.280	0.229	0.391	0.362	0.359	0.354
One103														
N	40	74	86	68	72	146	84	60	100	96	71	94	79	96
104	0.287	0.385	0.349	0.434	0.319	0.414	0.369	0.292	0.454	0.349	0.296	0.452	0.323	0.255
106	0.363	0.399	0.378	0.294	0.493	0.373	0.405	0.433	0.259	0.333	0.507	0.287	0.525	0.526
110	0.350	0.216	0.273	0.272	0.188	0.212	0.226	0.275	0.287	0.307	0.197	0.261	0.152	0.219
114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
Ots1														
N	40	76	93	89	92	146	85	92	99	96	94	94	93	95
176	0.013	0.007	0.000	0.034	0.005	0.003	0.006	0.005	0.005	0.000	0.005	0.021	0.011	0.000
182	0.175	0.145	0.140	0.157	0.185	0.137	0.188	0.152	0.121	0.156	0.234	0.229	0.140	0.084
184	0.412	0.395	0.430	0.410	0.467	0.445	0.388	0.440	0.510	0.422	0.191	0.330	0.489	0.479
186	0.000	0.000	0.011	0.000	0.000	0.003	0.000	0.000	0.005	0.000	0.000	0.000	0.005	0.000
188	0.075	0.033	0.005	0.022	0.038	0.024	0.059	0.022	0.035	0.068	0.069	0.021	0.038	0.053
195	0.325	0.421	0.414	0.376	0.304	0.387	0.359	0.380	0.323	0.354	0.500	0.399	0.317	0.384

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
Ots2														
N	40	76	90	90	80	140	85	85	99	91	93	93	79	96
68	0.688	0.868	0.839	0.878	0.837	0.896	0.876	0.847	0.854	0.808	0.882	0.849	0.848	0.854
70	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
72	0.287	0.125	0.150	0.111	0.150	0.089	0.094	0.141	0.141	0.176	0.113	0.129	0.152	0.146
76	0.000	0.000	0.006	0.006	0.000	0.004	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
86	0.025	0.007	0.006	0.006	0.013	0.007	0.029	0.012	0.000	0.016	0.005	0.022	0.000	0.000
Ots100														
N	40	75	89	86	94	146	89	87	94	96	94	92	96	96
247	0.000	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
251	0.000	0.000	0.000	0.012	0.000	0.003	0.006	0.000	0.027	0.026	0.000	0.011	0.000	0.000
255	0.025	0.040	0.022	0.017	0.011	0.021	0.039	0.040	0.016	0.000	0.005	0.000	0.021	0.005
259	0.038	0.007	0.028	0.047	0.043	0.024	0.028	0.023	0.027	0.016	0.085	0.033	0.016	0.047
263	0.013	0.020	0.017	0.029	0.021	0.010	0.028	0.023	0.016	0.031	0.011	0.011	0.021	0.000
265	0.000	0.000	0.006	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
267	0.038	0.020	0.011	0.006	0.005	0.014	0.000	0.023	0.011	0.005	0.000	0.011	0.010	0.005
271	0.013	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.005	0.005	0.005	0.000	0.000
275	0.000	0.000	0.000	0.006	0.005	0.000	0.000	0.006	0.000	0.000	0.005	0.000	0.000	0.000
283	0.000	0.007	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000
287	0.000	0.000	0.006	0.000	0.005	0.007	0.000	0.000	0.000	0.000	0.000	0.005	0.010	0.000
291	0.000	0.000	0.022	0.023	0.043	0.017	0.017	0.034	0.032	0.010	0.000	0.000	0.010	0.010
293	0.000	0.007	0.011	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
295	0.075	0.020	0.022	0.017	0.027	0.021	0.011	0.011	0.016	0.021	0.016	0.016	0.021	0.000
297	0.000	0.000	0.000	0.000	0.005	0.007	0.000	0.000	0.000	0.005	0.000	0.005	0.010	0.000
299	0.025	0.047	0.039	0.029	0.021	0.031	0.028	0.034	0.043	0.021	0.027	0.033	0.005	0.005
301	0.013	0.013	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.010	0.000
303	0.100	0.040	0.017	0.047	0.032	0.021	0.034	0.023	0.032	0.042	0.043	0.022	0.042	0.010
305	0.000	0.000	0.006	0.006	0.005	0.017	0.006	0.006	0.005	0.000	0.000	0.005	0.005	0.000
307	0.050	0.067	0.073	0.047	0.069	0.079	0.034	0.115	0.043	0.057	0.202	0.060	0.089	0.094
309	0.013	0.013	0.000	0.006	0.000	0.007	0.017	0.011	0.011	0.005	0.005	0.000	0.010	0.000
311	0.063	0.060	0.118	0.081	0.101	0.075	0.067	0.109	0.085	0.073	0.160	0.054	0.083	0.203
313	0.000	0.007	0.028	0.023	0.016	0.014	0.000	0.029	0.027	0.000	0.005	0.011	0.005	0.000
315	0.087	0.080	0.056	0.093	0.080	0.082	0.056	0.080	0.096	0.151	0.090	0.103	0.109	0.161
317	0.013	0.007	0.000	0.000	0.011	0.007	0.006	0.006	0.005	0.000	0.000	0.011	0.010	0.000
319	0.038	0.107	0.101	0.087	0.048	0.058	0.107	0.092	0.085	0.104	0.096	0.109	0.115	0.104
321	0.013	0.020	0.000	0.000	0.021	0.021	0.017	0.006	0.000	0.005	0.005	0.005	0.000	0.016
323	0.038	0.080	0.067	0.058	0.059	0.079	0.067	0.057	0.074	0.057	0.064	0.130	0.078	0.073
325	0.000	0.000	0.006	0.012	0.005	0.010	0.028	0.011	0.005	0.005	0.000	0.005	0.000	0.000
327	0.025	0.027	0.096	0.058	0.059	0.038	0.090	0.052	0.037	0.026	0.043	0.043	0.042	0.104
329	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.005	0.000	0.000	0.000	0.000	0.000
331	0.050	0.053	0.022	0.047	0.048	0.051	0.079	0.011	0.027	0.036	0.016	0.082	0.036	0.010
333	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
335	0.025	0.020	0.028	0.017	0.043	0.045	0.028	0.040	0.021	0.063	0.011	0.043	0.016	0.005
337	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
340	0.013	0.013	0.011	0.023	0.027	0.014	0.034	0.017	0.027	0.021	0.016	0.022	0.026	0.010
342	0.000	0.007	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.005
344	0.025	0.040	0.028	0.012	0.016	0.038	0.006	0.029	0.021	0.016	0.005	0.005	0.010	0.026
346	0.013	0.007	0.000	0.000	0.005	0.014	0.006	0.000	0.011	0.000	0.005	0.011	0.010	0.000
348	0.013	0.020	0.017	0.029	0.016	0.014	0.011	0.011	0.016	0.057	0.005	0.060	0.031	0.052
350	0.000	0.013	0.000	0.006	0.005	0.017	0.022	0.011	0.021	0.005	0.005	0.005	0.016	0.000
352	0.025	0.007	0.017	0.012	0.011	0.003	0.006	0.006	0.043	0.036	0.000	0.011	0.021	0.016
354	0.013	0.000	0.006	0.012	0.011	0.021	0.017	0.000	0.005	0.000	0.000	0.005	0.000	0.000
356	0.000	0.027	0.011	0.012	0.021	0.014	0.006	0.011	0.016	0.005	0.016	0.005	0.000	0.000

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
358	0.000	0.013	0.000	0.017	0.005	0.007	0.000	0.006	0.005	0.000	0.005	0.005	0.000	0.000
360	0.013	0.013	0.011	0.023	0.011	0.010	0.011	0.006	0.000	0.005	0.005	0.016	0.021	0.000
362	0.000	0.020	0.000	0.012	0.005	0.003	0.000	0.000	0.016	0.005	0.000	0.005	0.000	0.000
364	0.025	0.007	0.006	0.000	0.016	0.003	0.011	0.000	0.011	0.000	0.005	0.005	0.010	0.000
367	0.000	0.027	0.006	0.000	0.000	0.000	0.006	0.000	0.000	0.031	0.005	0.000	0.000	0.000
369	0.000	0.007	0.011	0.023	0.011	0.007	0.000	0.006	0.000	0.005	0.000	0.000	0.000	0.010
371	0.013	0.007	0.006	0.006	0.000	0.003	0.011	0.017	0.000	0.010	0.005	0.005	0.010	0.000
373	0.000	0.000	0.006	0.000	0.000	0.010	0.000	0.011	0.011	0.000	0.000	0.005	0.016	0.000
375	0.050	0.000	0.011	0.023	0.021	0.000	0.006	0.000	0.005	0.021	0.005	0.000	0.010	0.000
377	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	0.000
379	0.013	0.007	0.000	0.000	0.011	0.000	0.011	0.000	0.011	0.000	0.011	0.000	0.000	0.000
381	0.000	0.000	0.006	0.000	0.000	0.003	0.006	0.000	0.000	0.005	0.000	0.000	0.016	0.000
383	0.000	0.007	0.022	0.012	0.000	0.007	0.006	0.006	0.005	0.005	0.005	0.000	0.000	0.000
385	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
387	0.025	0.000	0.006	0.000	0.005	0.007	0.006	0.000	0.005	0.005	0.005	0.000	0.000	0.000
389	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
391	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
395	0.000	0.000	0.006	0.000	0.005	0.003	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000
397	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.016
399	0.000	0.000	0.000	0.000	0.005	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.005	0.000
403	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	0.000
405	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.005	0.010
407	0.013	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
409	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	0.000
Ots107														
N	40	75	90	86	96	146	85	92	99	96	96	94	96	95
196	0.000	0.000	0.006	0.000	0.000	0.003	0.006	0.000	0.005	0.000	0.000	0.000	0.000	0.000
200	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	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
208	0.050	0.013	0.011	0.006	0.000	0.014	0.006	0.011	0.005	0.010	0.005	0.000	0.042	0.111
212	0.000	0.007	0.000	0.000	0.000	0.000	0.006	0.005	0.000	0.000	0.000	0.005	0.010	0.000
216	0.000	0.000	0.000	0.000	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.020	0.000	0.006	0.010	0.003	0.018	0.000	0.015	0.016	0.016	0.005	0.000	0.000
224	0.013	0.020	0.017	0.000	0.000	0.014	0.018	0.011	0.015	0.000	0.005	0.016	0.005	0.000
228	0.000	0.013	0.006	0.006	0.005	0.014	0.024	0.022	0.005	0.021	0.021	0.000	0.000	0.000
232	0.000	0.007	0.006	0.012	0.000	0.014	0.012	0.016	0.040	0.031	0.010	0.011	0.000	0.000
236	0.050	0.047	0.033	0.023	0.031	0.045	0.059	0.016	0.040	0.094	0.073	0.027	0.036	0.016
240	0.050	0.060	0.139	0.099	0.109	0.123	0.135	0.158	0.101	0.089	0.104	0.085	0.115	0.174
244	0.063	0.113	0.161	0.122	0.188	0.106	0.129	0.136	0.126	0.099	0.052	0.090	0.083	0.037
248	0.050	0.080	0.078	0.058	0.063	0.065	0.071	0.054	0.066	0.068	0.021	0.064	0.073	0.079
252	0.063	0.047	0.039	0.070	0.047	0.045	0.029	0.027	0.071	0.052	0.005	0.074	0.010	0.016
256	0.063	0.033	0.044	0.087	0.068	0.065	0.071	0.033	0.040	0.115	0.016	0.048	0.021	0.026
260	0.075	0.067	0.072	0.081	0.094	0.096	0.071	0.098	0.071	0.089	0.094	0.090	0.068	0.053
264	0.112	0.073	0.061	0.081	0.089	0.103	0.076	0.098	0.061	0.057	0.161	0.080	0.078	0.021
268	0.100	0.060	0.039	0.023	0.068	0.038	0.029	0.038	0.076	0.036	0.078	0.074	0.125	0.116
272	0.025	0.040	0.056	0.035	0.026	0.024	0.029	0.060	0.035	0.031	0.021	0.027	0.036	0.021
276	0.000	0.047	0.022	0.029	0.021	0.003	0.018	0.033	0.015	0.000	0.000	0.027	0.021	0.005
280	0.000	0.013	0.011	0.006	0.005	0.014	0.018	0.005	0.025	0.016	0.005	0.016	0.000	0.000
284	0.000	0.007	0.000	0.012	0.005	0.000	0.006	0.038	0.005	0.005	0.010	0.005	0.010	0.005
288	0.000	0.000	0.011	0.006	0.005	0.003	0.000	0.005	0.000	0.010	0.000	0.000	0.005	0.000
292	0.000	0.027	0.006	0.035	0.010	0.021	0.006	0.005	0.005	0.000	0.036	0.021	0.042	0.095
296	0.050	0.020	0.033	0.023	0.005	0.038	0.024	0.016	0.025	0.016	0.010	0.005	0.016	0.011
300	0.038	0.007	0.017	0.006	0.005	0.014	0.006	0.011	0.020	0.010	0.000	0.005	0.010	0.005

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
304	0.038	0.060	0.022	0.047	0.005	0.034	0.029	0.005	0.015	0.016	0.010	0.021	0.010	0.053
308	0.013	0.007	0.022	0.047	0.047	0.027	0.012	0.005	0.045	0.016	0.021	0.032	0.031	0.032
312	0.025	0.027	0.044	0.023	0.031	0.024	0.035	0.054	0.025	0.036	0.010	0.048	0.021	0.032
316	0.075	0.027	0.022	0.006	0.026	0.007	0.024	0.011	0.015	0.010	0.156	0.021	0.047	0.016
320	0.038	0.027	0.011	0.017	0.016	0.021	0.018	0.016	0.020	0.010	0.036	0.027	0.026	0.016
324	0.000	0.007	0.006	0.012	0.000	0.017	0.018	0.000	0.005	0.010	0.005	0.027	0.016	0.021
328	0.013	0.013	0.006	0.012	0.010	0.003	0.000	0.011	0.005	0.021	0.016	0.021	0.010	0.000
332	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.016	0.026	0.042
336	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.005	0.000	0.000
340	0.000	0.000	0.000	0.006	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000

*Oke2**

N	93	92
177	0.000	0.011
183	0.032	0.033
185	0.097	0.065
187	0.043	0.027
189	0.204	0.092
191	0.091	0.250
193	0.199	0.212
195	0.118	0.076
197	0.038	0.016
199	0.016	0.016
201	0.043	0.027
203	0.108	0.168
209	0.011	0.005

*Oke4**

N	93	89
240	0.296	0.230
242	0.425	0.489
244	0.177	0.180
246	0.102	0.101

*Oki11**

N	93	92
78	0.134	0.120
84	0.435	0.391
86	0.430	0.489

*Ots3.1**

N	93	92
113	0.108	0.065
115	0.005	0.000
117	0.027	0.022
119	0.059	0.022
121	0.301	0.543
123	0.478	0.348
125	0.022	0.000

*OtsG409**

N	93	92
137	0.005	0.000
141	0.011	0.011

	Population													
	Goo	Kan	Eek	Kwe	Kis	Tul	Ani	Geo	Kog	Sto	Tat	Che	Tak	Pit
145				0.011							0.005			
149				0.011							0.000			
153				0.048							0.033			
157				0.043							0.016			
161				0.054							0.027			
165				0.038							0.011			
169				0.054							0.288			
173				0.070							0.103			
177				0.075							0.022			
181				0.043							0.022			
185				0.102							0.120			
189				0.043							0.049			
193				0.032							0.033			
197				0.032							0.016			
201				0.022							0.011			
205				0.011							0.011			
209				0.016							0.011			
213				0.032							0.016			
217				0.032							0.049			
221				0.054							0.027			
225				0.027							0.022			
229				0.043							0.022			
233				0.032							0.000			
237				0.027							0.016			
241				0.016							0.038			
245				0.000							0.016			
249				0.011							0.005			
253				0.005							0.000			
*OtsG432**														
N				93							92			
105				0.554							0.511			
109				0.016							0.000			
113				0.005							0.000			
125				0.011							0.000			
129				0.151							0.353			
133				0.027							0.011			
137				0.005							0.022			
141				0.005							0.011			
145				0.005							0.005			
149				0.011							0.005			
153				0.027							0.011			
157				0.011							0.011			
161				0.016							0.000			
165				0.038							0.022			
169				0.016							0.011			
173				0.032							0.000			
177				0.005							0.022			
181				0.016							0.000			
189				0.011							0.000			
193				0.005							0.000			
197				0.011							0.000			
201				0.011							0.000			
205				0.011							0.005			

Table 7. Measures of genetic diversity in Chinook salmon from the Kuskokwim Management Area from 23 allozyme and 10 microsatellite loci. Number of alleles at each locus (A), observed heterozygosity (Ho), expected heterozygosity (He) and F_{ST} are shown.

Locus	A	Ho	He	F_{ST}
Allozyme				
mAAT-1	2	0.002	0.002	0.001
sAAT-4	2	0.019	0.023	0.020
ADA-1	2	0.223	0.220	0.013
sAH	3	0.120	0.130	0.005
ALAT	2	0.135	0.140	0.015
GPI-A	3	0.004	0.004	0.002
GPI-B2a	2	0.001	0.001	0.002
sIDHP-1	4	0.025	0.026	0.026
sIDHP-2	2	0.044	0.045	0.005
LDH-B2	2	0.001	0.001	0.000
mMDH-2	2	0.094	0.089	0.002
sMDH-B1,2	3	--	--	--
sMEP-1	2	0.009	0.009	-0.001
sMEP-2	2	--	--	--
MPI	2	0.125	0.132	0.003
PEPA	2	0.084	0.089	0.004
PEPB-1	3	0.159	0.158	0.015
PEPD-2	2	0.001	0.001	0.000
PEPLT	2	0.010	0.012	0.009
PGK-2	2	0.001	0.001	0.002
PGM-2	2	0.001	0.001	0.002
sSOD-1	2	0.087	0.082	0.011
TPI-4	2	0.182	0.163	0.015
Microsatellite				
μSat73	5	0.386	0.406	0.009
Oke4	5	0.653	0.712	0.004
One7	2	0.406	0.444	0.004
One9	3	0.09	0.088	0.002
One102	2	0.409	0.422	0.006
One103	4	0.613	0.645	0.016
Ots1	6	0.671	0.654	0.012
Ots2	5	0.275	0.263	0.007
Ots100	68	0.94	0.946	0.005
Ots107	37	0.922	0.935	0.007

Table 8. Genetic diversity in 14 populations of Chinook salmon from the Kuskokwim Management Area. Sample size (N), number of polymorphic loci (P) for allozyme loci, number of alleles (A), and allelic richness (R) for microsatellite loci are shown. All microsatellite loci were polymorphic in all populations.

		Allozymes		Microsatellites	
Population	N	P	A	A	R
Goodnews	40	16	41	78	77
Kanektok	78	19	44	96	80
Eek	100	15	41	95	75
Kwethluk	100	14	38	95	78
Kisaralik	100	13	37	96	74
Tuluksak	150	14	39	112	79
Aniak	100	15	40	96	78
George	100	13	36	90	73
Kogrukluk	100	15	39	99	79
Stony	100	15	40	89	73
Tatlawiksuk	100	14	38	84	64
Cheeneetnuk	100	13	37	95	75
Takotna	111	15	40	93	76
Pitka Fork	100	12	37	68	58

Table 9. Genetic variation between population pairs as measured with 23 allozyme loci. Cavalli-Sforza and Edwards' chord distances are in the lower diagonal and F_{ST} is in the upper diagonal

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Goodnews	0	0.017	0.006	0.028	0.018	0.021	0.018	0.004	0.011	0.009	0.019	0.015	0.007	0.032
2	Kanektok	0.075	0	0.007	0.005	0.002	0.001	0.003	0.009	0.007	0.001	0.010	0.010	0.004	0.034
3	Eek	0.068	0.061	0	0.010	0.002	0.007	0.004	-0.001	0.002	0.002	0.013	0.009	0.005	0.043
4	Kwethluk	0.079	0.062	0.049	0	0.000	-0.001	0.001	0.016	0.006	0.003	0.014	0.003	0.007	0.035
5	Kisaralik	0.077	0.061	0.040	0.037	0	0.000	0.000	0.008	0.006	-0.001	0.012	0.009	0.006	0.034
6	Tuluksak	0.075	0.053	0.054	0.039	0.045	0	0.002	0.012	0.005	0.001	0.012	0.004	0.004	0.033
7	Aniak	0.083	0.065	0.043	0.044	0.040	0.054	0	0.006	0.006	0.000	0.007	0.007	0.007	0.034
8	George	0.068	0.062	0.038	0.051	0.044	0.052	0.052	0	0.002	0.003	0.007	0.012	0.009	0.054
9	Kogrukluk	0.065	0.055	0.048	0.042	0.048	0.038	0.056	0.042	0	0.001	0.008	0.001	0.002	0.048
10	Stony	0.064	0.052	0.049	0.047	0.040	0.046	0.047	0.050	0.038	0	0.002	0.004	0.003	0.033
11	Tatlawiksuk	0.076	0.070	0.060	0.054	0.050	0.058	0.056	0.045	0.052	0.050	0	0.008	0.016	0.051
12	Cheeneetnuk	0.071	0.065	0.056	0.042	0.058	0.053	0.065	0.055	0.045	0.054	0.056	0	0.003	0.037
13	Takotna	0.065	0.067	0.061	0.056	0.069	0.055	0.068	0.066	0.040	0.056	0.070	0.053	0	0.027
14	Pitka Fork	0.096	0.106	0.096	0.091	0.097	0.101	0.089	0.113	0.101	0.097	0.101	0.101	0.083	0

Table 10. Genetic variation between population pairs as measured with 10 microsatellite loci. Cavalli-Sforza and Edwards' (1967) chord distances are in the lower diagonal and F_{ST} is in the upper diagonal.

	Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Goodnews	0	0.004	0.004	0.005	0.004	0.010	0.007	0.004	0.006	0.002	0.022	0.008	0.009	0.018
2	Kanektok	0.011	0	0.001	0.000	0.002	0.000	0.000	0.000	0.003	0.004	0.020	0.004	0.008	0.016
3	Eek	0.012	0.008	0	0.000	0.001	0.001	0.000	-0.001	0.002	0.005	0.016	0.005	0.005	0.010
4	Kwethluk	0.013	0.008	0.006	0	0.004	0.002	0.001	0.003	0.000	0.002	0.022	-0.001	0.008	0.017
5	Kisaralik	0.011	0.009	0.007	0.006	0	0.003	0.000	0.000	0.005	0.006	0.019	0.009	0.001	0.009
6	Tuluksak	0.012	0.007	0.005	0.007	0.007	0	-0.001	0.004	0.002	0.005	0.022	0.009	0.008	0.016
7	Aniak	0.012	0.008	0.007	0.008	0.007	0.006	0	0.002	0.003	0.004	0.017	0.005	0.005	0.012
8	George	0.014	0.008	0.006	0.007	0.007	0.007	0.008	0	0.005	0.002	0.020	0.008	0.006	0.010
9	Kogrukluk	0.012	0.008	0.007	0.006	0.007	0.006	0.007	0.008	0	0.003	0.033	0.007	0.011	0.020
10	Stony	0.012	0.010	0.009	0.008	0.009	0.009	0.008	0.010	0.008	0	0.028	0.007	0.011	0.019
11	Tatlawiksuk	0.014	0.012	0.013	0.014	0.011	0.013	0.012	0.013	0.014	0.014	0	0.018	0.015	0.022
12	Cheeneetnuk	0.012	0.007	0.009	0.007	0.008	0.007	0.008	0.009	0.008	0.009	0.013	0	0.011	0.021
13	Takotna	0.012	0.010	0.009	0.010	0.008	0.009	0.010	0.009	0.011	0.012	0.012	0.008	0	0.004
14	Pitka Fork	0.020	0.016	0.014	0.016	0.015	0.015	0.017	0.014	0.017	0.016	0.015	0.016	0.009	0

Table 11. Hierarchical gene diversity analysis of Chinook salmon populations from the Kuskokwim Management Area using allozyme and microsatellite markers. Populations were grouped into four regions: 1) Goodnews/Kanektok, 2) Lower Kuskokwim, 3) Middle Kuskokwim, and 4) Upper Kuskokwim. Results of analysis of molecular variance (AMOVA), degrees of freedom (DF), variance components (σ), and the significance of each hierarchical grouping are shown (* indicates a significant test).

Marker type	Source of variation	DF	σ		% of total	F_{ST}	F_{SR}	F_{RT}
Allozymes								
	Among regions	3	0.0033	*	0.51			
	Among populations within regions	10	0.0039	*	0.61			
	Within populations	2742	0.6370	*	98.88			
	Total	2755	0.6442		100.00	0.011	0.006	0.005
Microsatellites								
	Among regions	3	0.0109	*	0.43			
	Among populations within regions	10	0.0010		0.04			
	Within populations	2518	2.5389	*	99.53			
	Total	2531	2.5508		100.00	0.005	0.000	0.004

Table 12. Hierarchical log likelihood analysis of population structure based on allele frequencies at 23 allozyme loci. Populations were grouped into four regions: 1) Goodnews/Kanektok, 2) Lower Kuskokwim, 3) Middle Kuskokwim, and 4) Upper Kuskokwim. The * indicates a significant test.

Populations	DF	Overall	P
Kuskokwim	377	859.9	0.000 *
Among regions	87	373.5	0.000 *
Within regions	290	486.5	0.000 *
Goodnews/Kanektok	29	49.0	0.012 *
Lower Kuskokwim	174	228.7	0.003 *
Middle Kuskokwim	58	91.6	0.003 *
Upper Kuskokwim	29	117.2	0.000 *

Table 13. Genetic diversity between Chinook salmon populations from the Kwethluk and Tatlawiksuk rivers as measured by the microsatellite loci from the ADF&G and USFWS laboratories.

Locus	A	Ho	He	F_{ST}
ADF&G				
μSat73	5	0.310	0.342	0.045
Oke4	4	0.683	0.718	0.006
One7	2	0.428	0.426	-0.002
One9	2	0.062	0.070	-0.006
One102	2	0.415	0.458	0.005
One103	3	0.540	0.639	0.044
Ots1	5	0.699	0.662	0.047
Ots2	4	0.197	0.215	-0.006
Ots100	45	0.935	0.931	0.014
Ots107	31	0.914	0.928	0.023
USFWS				
Oke2	13	0.865	0.860	0.021
Ots3.1	7	0.654	0.625	0.055
Oki11	3	0.562	0.604	-0.001
Oke4	4	0.614	0.682	0.000
OtsG409	30	0.881	0.920	0.030
OtsG432	23	0.616	0.642	0.030

Table 14. Percent allocation of mixed stock analysis simulations in which each of three reporting regions from the Kuskokwim River comprised 100% of the hypothetical mixture. Simulations were repeated using allozymes, microsatellites and combined marker sets. Bold type indicates correct allocation to the originating reporting region.

Population	Lower Kuskokwim	Middle Kuskokwim	Upper Kuskokwim
Allozymes			
1 Lower Kuskokwim	**90**	23	6
2 Middle Kuskokwim	7	**75**	4
3 Upper Kuskokwim	3	2	**90**
Microsatellites			
1 Lower Kuskokwim	**93**	12	6
2 Middle Kuskokwim	5	**85**	2
3 Upper Kuskokwim	2	2	**92**
Allozymes and Microsatellites			
1 Lower Kuskokwim	**93**	12	4
2 Middle Kuskokwim	5	**86**	2
3 Upper Kuskokwim	1	2	**93**

Table 15. Percent allocation of mixed stock analysis simulations in which each of two reporting regions from the Kuskokwim River comprised 100% of the hypothetical mixture. Simulations were repeated using allozymes, microsatellites and combined marker sets. Bold type indicates correct allocation to the originating reporting region.

Population	Lower Kuskokwim	Upper Kuskokwim
Allozymes		
1 Lower Kuskokwim	**90**	20
2 Upper Kuskokwim	10	**80**
Microsatellites		
1 Lower Kuskokwim	**93**	10
2 Upper Kuskokwim	7	**90**
Allozymes and Microsatellites		
1 Lower Kuskokwim	**93**	4
2 Upper Kuskokwim	6	**95**

Table 16. Percent allocation of mixed stock analysis simulations in which each of three reporting regions from the Kuskokwim Management Area comprised 100% of the hypothetical mixture. Simulations were repeated using allozymes, microsatellites and combined marker sets. Bold type indicates correct allocation to the originating reporting region.

Population	Goodnews/ Kanektok	Lower Kuskokwim	Upper Kuskokwim
Allozymes			
1 Goodnews/ Kanektok	**64**	3	3
2 Lower Kuskokwim	29	**88**	19
3 Upper Kuskokwim	7	10	**78**
Microsatellites			
1 Goodnews/ Kanektok	**74**	3	2
2 Lower Kuskokwim	23	**91**	9
3 Upper Kuskokwim	2	6	**88**
Allozymes and Microsatellites			
1 Goodnews/ Kanektok	**72**	3	2
2 Lower Kuskokwim	24	**92**	9
3 Upper Kuskokwim	3	6	**89**

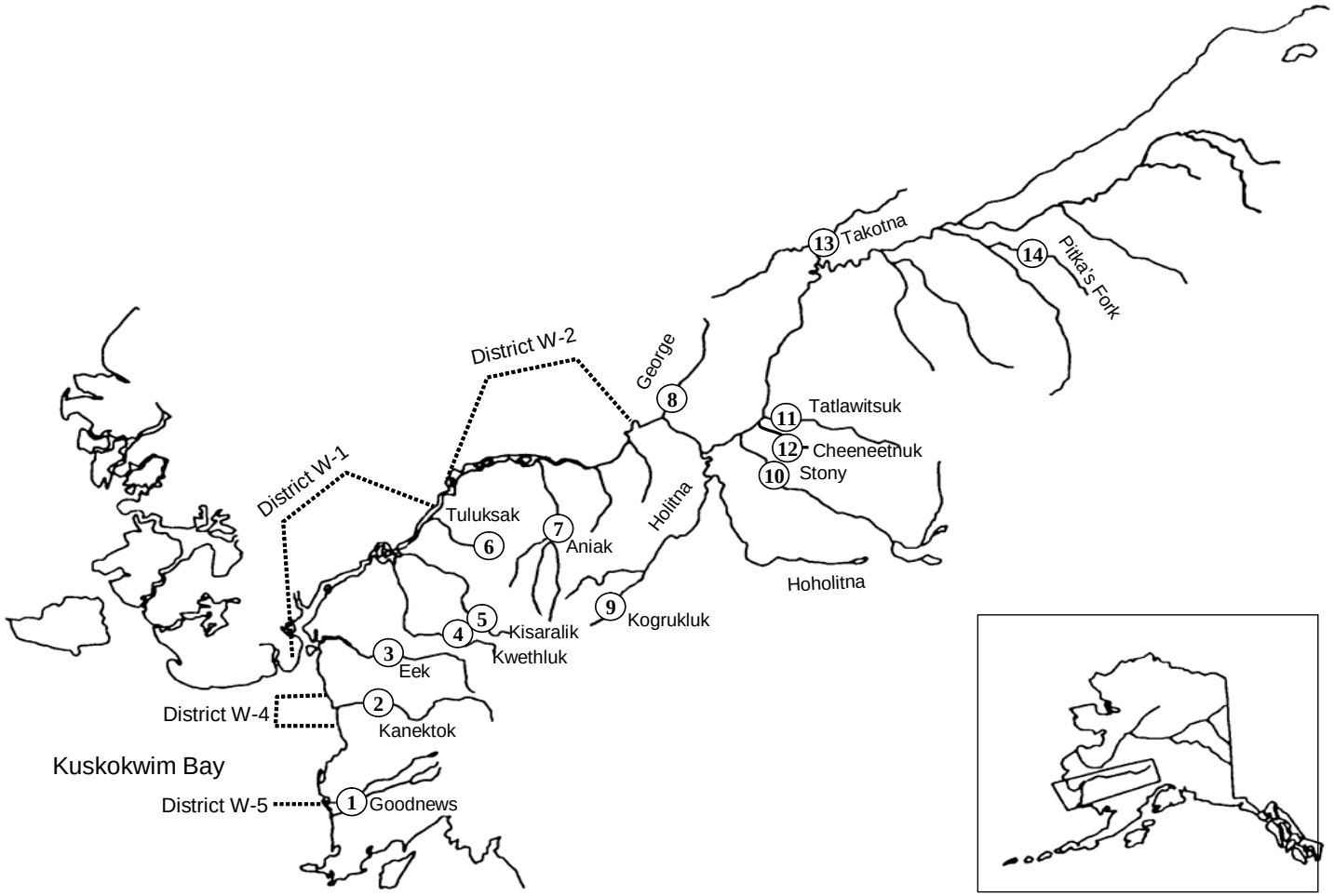

Figure 1. Map of the Kuskokwim Management Area. Numbers correspond to collections listed in Table 1.

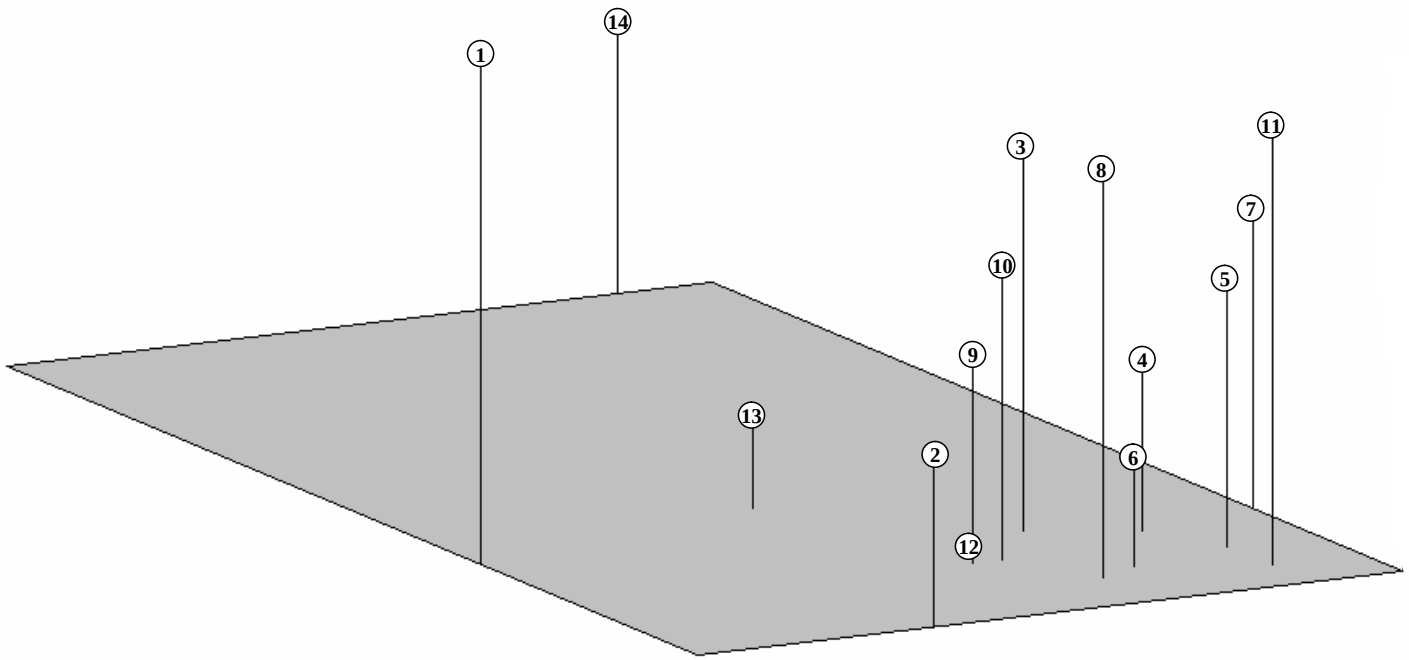

Figure 2. Multidimensional scaling of pairwise genetic distances (Cavalli-Sforza and Edwards 1967) between Chinook salmon populations in the Kuskokwim Management Area calculated from allele frequencies at 23 allozyme loci. Numbers correspond to collections in Table 1 and Figure 1.

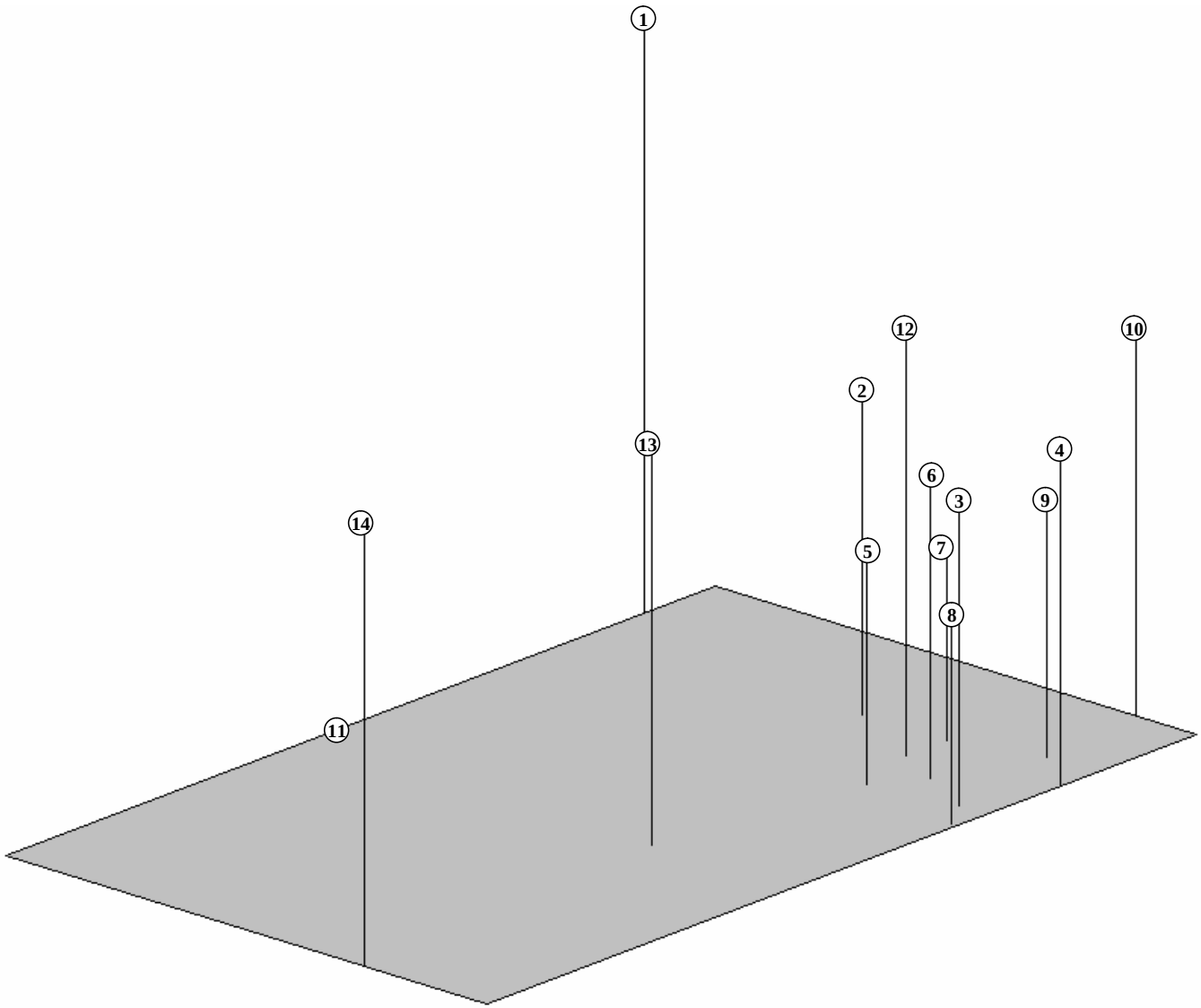

Figure 3. Multidimensional scaling of pairwise genetic distances (Cavalli-Sforza and Edwards 1967) between Chinook salmon populations in the Kuskokwim Management Area calculated from allele frequencies at 10 microsatellite loci. Numbers correspond to collections in Table 1 and Figure 1.

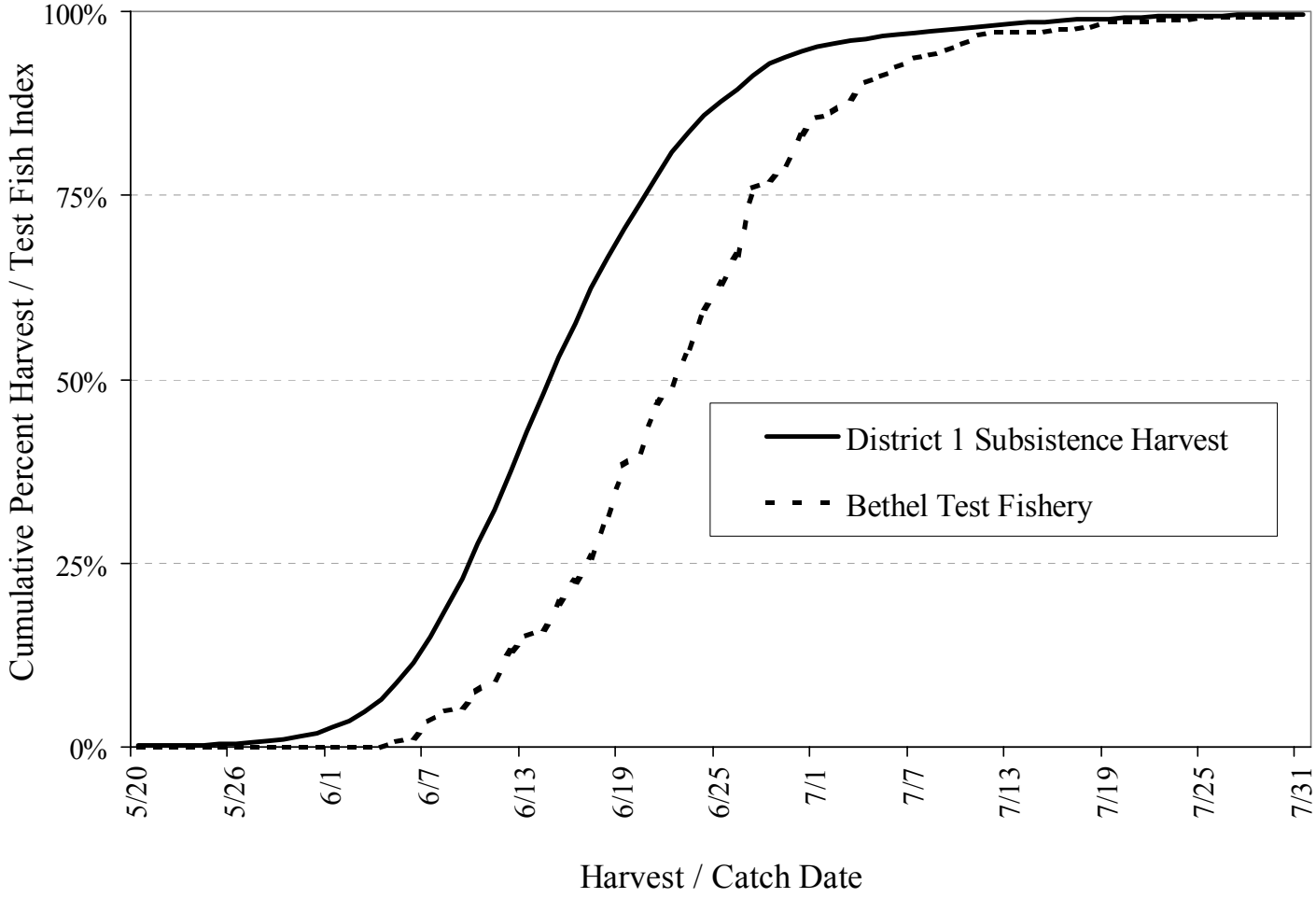

Figure 4. Average run timing of the subsistence Chinook salmon harvest in the lower Kuskokwim River (District 1), compared to the average run timing observed in the Bethel test fishery (mid-point of District 1), 1984 to 1999 (Burkey et al. 2002).

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