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Genetic variation among coho salmon populations from the Kuskokwim River region and application to stock-specific harvest estimation

Final Report for Study 03-041

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FINAL REPORT SUMMARY PAGE

Title: Genetic variation among coho salmon populations from the Kuskokwim River region and application to stock-specific harvest estimation

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Geographic Area: Kuskokwim River region

Information Type: Stock Status and Trends

Issue(s) Addressed: Lack of stock structure information to support federal subsistence fishery management of coho salmon in the Kuskokwim River region.

Study Cost: $35,000

Study Duration: June 2002 to January 2003

Abstract: We conducted a pilot survey of the genetic structure of coho salmon in the Kuskokwim River region. Our objective was to determine if sufficient genetic diversity exists among coho salmon populations in this area to use genetic methods to quantify contributions of coho salmon stocks to mixed fisheries in the Kuskokwim River and estimate abundance and run timing of coho salmon stocks in the Bethel test fishery. We surveyed genetic variation at nine microsatellite loci in coho salmon samples from the Arolik, Kanektok, Kisaralik, George, Kogrukluk, Tatlawiksuk, and Takotna rivers. Low, but significant genetic population structure was detected. Mean stock contribution estimates for simulated mixtures from Arolik/Kanektok rivers, Kisaralik/George rivers, Kogrukluk/Tatlawiksuk rivers, and Takotna River ranged from 84-94%. Levels of genetic divergence should support mixed-stock analysis for at least three groups: Kuskokwim Bay, lower-mid Kuskokwim River, and upper Kuskokwim River. Increasing the number of populations and loci surveyed will improve stock composition estimates and refine stock groups that can be identified in mixtures.

Key Words: federal subsistence fisheries, genetic diversity, DNA microsatellites, coho salmon, *Oncorhynchus kisutch*, Kuskokwim River, Yukon Delta National Wildlife Refuge.

Project Data: Description – Data for this study consist of biological collections (fin tissue and DNA samples) and information (date, location, and method of capture; gender and maturity index of fish) from coho salmon. Format – Fin tissue samples stored in 90% ethanol. Sampling

and genetic data are stored in a Microsoft Access database. Custodian(s) – U.S. Fish and Wildlife Service, Conservation Genetics Laboratory, 1011 East Tudor Road, Anchorage, Alaska 99503. Availability – Access to biological samples and data is available upon request to the custodian(s).

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 subsistence fishery in the Kuskokwim River region is one of the largest and most important in Alaska, with more than 1,700 households participating (ADFG 2001; Burkey et al. 2001). The majority of the subsistence harvest is taken in the Kuskokwim River from the village of Tuluksak downstream to the village of Eek in Kuskokwim Bay (Figure 1) in waters occurring in federal conservation units (Burkey et al. 2001). Chinook salmon *Oncorhynchus tshawytscha* and chum salmon *O. keta* have historically contributed the most to subsistence catches. Years of low run strength led the Alaska Board of Fisheries to designate these species as yield concerns in 2000 (Bergstrom and Whitmore 2004). Coho salmon *O. kisutch* typically comprise 10-20% of the total subsistence harvest for salmon; the 1992-2001 average subsistence harvest for coho salmon was 34,322 (Ward et al. 2003). Coho salmon are more heavily targeted in years when other species are in low abundance (Burkey et al. 2001; Lafferty 2003); therefore reliance on coho salmon in the subsistence fishery may become increasingly important as populations of Chinook salmon and chum salmon rebuild. Coho salmon is also the dominant species in commercial catches in both numbers harvested and value (Burkey et al. 2001). The average number of coho salmon harvested in commercial fisheries from 1992-2001 was 500,961 with an average exvessel value of $1,506,322 (Ward et al. 2003). These harvests are an important source of revenue for residents to purchase fuel and supplies for subsistence fish and wildlife harvests (J. Estensen, ADFG, personal communication; Linderman et al. 2003a,b).

Coho salmon abundance and run strength are measured through a variety of assessment programs in the Kuskokwim River region including the Bethel test fishery; weirs on the Middle Fork Goodnews, Kanektok, Kwethluk, Kogrukluk, George, Tatlawiksuk, and Takotna rivers; commercial catch data; subsistence catch surveys; aerial surveys; and mark-recapture projects. An escapement goal for coho salmon has only been established for the Kogrukluk River. Other weir projects have only been operational since the mid 1990's consequently there is a lack of historic information on which to establish escapement objectives and to track long-term trends in abundance of coho salmon (ADFG 2004).

The application of genetic methods is becoming common in management and research programs for coho salmon in the Pacific Northwest. Genetic methods aid in the identification of conservation units by describing the genetic basis of population structure (Weitkamp et al. 1995; Small et al. 1998; Beacham et al. 2001; Smith et al. 2001; Olsen et al. 2003) and have been used in mixed-stock analysis (MSA) studies to estimate the contribution of stock groups to commercial and sport catches in British Columbia (Small et al. 1998; Shaklee et al. 1999; Beacham et al. 2001). Further, MSA of test fishery catches can be used to estimate stock-specific escapements. For example, Beacham et al. (2000) expanded contribution estimates of sockeye salmon *O. nerka* populations to test fisheries at the mouth of the Skeena River with known escapement to a tributary lake to the Skeena River to estimate stock escapements of sockeye salmon throughout the drainage. In Alaska, significant genetic differences have been detected for coho salmon populations within river systems (Carney et al. 1997; Smith et al. 2001) and among river systems (Olsen et al. 2003), indicating a strong potential for genetic characters to discriminate stocks within the Kuskokwim River region.

In this pilot project, we surveyed seven coho salmon populations from the Kuskokwim River region using nine microsatellite loci to obtain a preliminary assessment of the genetic population structure of coho salmon in this area. Data were tested to assess their utility in MSA. The preliminary results indicate low, but statistically significant population subdivision and that

genetic data can be used to provide estimates of stock composition for Kuskokwim Bay, lower-mid Kuskokwim River, and Upper Kuskokwim River. In 2004, more populations and loci will be surveyed in a continuation of this project to refine the population groups that can be estimated in mixtures and increase accuracy and precision of mixture estimates.

OBJECTIVE

Conduct a pilot study to characterize the genetic diversity within and among spawning aggregates of coho salmon distributed throughout the Kuskokwim River region using six archived tissue collections and one new collection.

METHODS

Samples

Fin tissue was collected from coho salmon and stored in 90% ethanol for genetic analysis. Coho salmon were sampled using gill nets and hook and line in the Arolik and Kisaralik Rivers in 1997. Coho salmon were sampled at weir sites operating on the Kanektok, Kogrukluk, George, and Tatlawiksuk rivers in 2001 and the Takotna River in 2003 (Figure 1).

Laboratory Analysis

DNA was extracted from fin tissue from 96 coho salmon from each collection site using a standard Puregene® protocol. Genetic variation was assayed at nine microsatellite loci: *Oke-2, -3, -4* (Buchholz et al. 1999); *Oki-1*, *-3*, *-11*(Smith et al. 1998); *One-3* (Scribner et al. 1996); *Ots-3.1* (Banks et al. 1999), and *Ots-105* (Nelson 1998). For individuals from the Arolik, Kanektok, Kisaralik, Tatlawiksuk, George, and Kogrukluk rivers, PCR cycling conditions followed Olsen et al. (2003). Alleles were separated and visualized and scored also following Olsen et al. (2003) using an Hitachi FMBio II flat bed scanner and Gene Profiler (Scanalytics Inc., Fairfax, VA).

For the Takotna River, PCR amplifications of microsatellite loci were carried out in 10ul reaction volumes (approximately 30-50ng DNA, 1.5-2 mM $MgCl_2$, 0.8-1mM dNTPs, 0.01-0.05uM labeled/0.35-0.39uM unlabeled forward primer, and 0.4uM unlabeled reverse primer, and 1M betaine (for *Ots3.1*, *Oki-1*, and *Oke-2* only) using an MJResearch thermocycler. Cycling conditions were 1 cycle of 2 min at 92^o; 30 cycles of 15 sec at 92^o, 15 sec at T_a (56^o-58^o), and 30 sec at 72^o; with a final extension for 10 min at 72^o. Microsatellites were separated and visualized on 64-well denaturing polyacrylamide gels using a Li-Cor IR$^{2®}$scanner and scored with Li-Cor Saga^{TM} GT ver 2.0 software (Lincoln, NE). Li-Cor 50-350 or 50-500 base 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 2-4 alleles of predetermined size, were loaded in three lanes distributed evenly across the gels to ensure consistency of allele scores. Two researchers scored alleles independently. Samples with score discrepancies between researchers were re-amplified at the loci in question and rescored. To ensure consistency of allele scoring between the two platforms, positive controls used to score genotypes from the Arolik, Kanektok, Kisaralik, Tatlawiksuk, and

George rivers were used to score the genotypes from the Takotna River. Further, all individuals sampled from the Kisaralik River were reamplified and genotyped using both the FM-Bio II and Li-Cor platforms.

Statistical Analysis

Unless otherwise noted, analyses describing genetic variation within and among population samples were conducted using FSTAT ver 2.9.3 (Goudet 2001). Observed and expected heterozygosities calculated using BIOSYS II (Swofford and Selander 1981) and allele richness (number of alleles per locus corrected for sample size effects) were used to describe within-sample diversity. For each locus in each population, genotypic frequencies were tested for conformation to Hardy-Weinberg expectation using an exact test in GENEPOP ver3.3 (Raymond and Rousset 1995). Significance of the *p*-values was evaluated by adjusting the table-wide $\alpha = 0.05$ for 9 multiple tests using the sequential Bonferroni technique (Rice 1989).

The proportion of variability due to an among-population component (F_{ST}) was estimated according to the method of Weir and Cockerham (1984). Significance ($F_{ST}>0$) was assessed through randomization tests where alleles were permuted among samples. Pairwise tests of allele frequency differentiation between samples were conducted using a log likelihood statistic; significance was also assessed by permuting alleles among samples.

Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967) were calculated between each pair of populations using BIOSYS II. Distances were used in a multidimensional scaling analysis in S-PLUS 6.0 (Insightful, Inc.; Seattle, WA) to visualize spatial genetic relationships among populations.

Maximum likelihood estimation of artificial mixtures was used to determine if sufficient population subdivision exists among coho salmon populations in the Kuskokwim River region to use MSA to identify harvest components. Alleles were binned in the baseline to reduce the effects of sampling error and rare alleles using OptiBin (Bromaghin In preparation). For each locus, exact tests of homogeneity were used to test if allele pairs were similarly distributed across populations, with Monte Carlo simulation to estimate significance, to determine the binning strategy. Log-likelihood ratios were used as the test statistic and the binning procedure executed until $P<0.1$.

For the simulation analysis, for each population under study, 1000 artificial mixtures of 400 genotypes were randomly constructed using Hardy-Weinberg expectations from the baseline allele frequencies. The mean contribution estimate for the population under study should therefore approximate 100%. Mean contribution estimates of approximately 90% are generally considered robust for mixture analysis (Kondzela et al. 2002; Seeb et al. 2000; Teel et al. 1999). The program SPAM ver 3.7 (Debevec et al. 2000) was used for the simulation analysis.

RESULTS

Allele standardization

No shift in allele size was detected between the FM-Bio II and Li-Cor platforms for the loci *Oke-2* and *Oke-3*. For all other loci, positive controls and alleles sized in the Kisaralik River

samples were 2 bases smaller using Saga GT software and the Li-Cor system with two exceptions. *Ots-3.1*98* as sized on the FM-Bio II using Scanalytics software was also sized at 98 bases on the Li-Cor system. However, we redesignated this allele as **96* on the Li-Cor to follow the two base shift observed in the other alleles at this locus. *One-3*187* as sized on the FM-Bio II was sized at 183 bases on the Li-Cor. Allele sizes for allele frequency results in Table 1 are those designated using the Li-Cor and allele sizes for data collected using the FM-Bio II were adjusted accordingly.

Statistical results

Expected heterozygosity and allele richness averaged over all loci was consistent for the populations sampled (Table 1). Expected heterozygosity ranged from 0.408 in the Arolik and Tatlawiksuk rivers to 0.437 in the Kanekok River. Allele richness ranged from 3.242 in the Tatlawiksuk River to 4.075 in the George River (Table 1). After adjusting for multiple tests, genotypic frequencies for *Ots-3.1* did not conform to Hardy-Weinberg expectation in the sample from the Arolik River (Table 1).

Overall F_{ST} for the population samples was 0.011 (P=0.001). For individual loci, F_{ST} ranged from 0.001 to 0.024. F_{ST} estimates for *Oki-11* and *Ots-105* were 0.001 (P=0.171) and 0.003 (P=0.147) respectively and were not significantly greater than zero indicating that these loci will not contribute to differentiating populations in mixtures. No significant differences were detected in allele frequencies between Kogrukluk and Tatlawiksuk rivers (P=0.571) but all other pairwise comparisons were significant. The multidimensional scaling analysis indicated that the Arolik River is clearly separated from the Kanektok River and all population samples from the Kuskokwim River drainage. Within the Kuskokwim River, the Takotna River and George River were the most divergent populations.

Alleles that were similarly distributed across populations were binned prior to the simulation analysis. Over all loci, 29 alleles were binned. *Oki-11* and *Ots-105* were dropped from the simulation analysis because all alleles were binned in each of these loci, further F_{ST} estimates for these loci were not significant.

Mean contribution estimates for artificial mixtures composed of genotypes from the individual tributaries were greater than 90% for the Arolik River and Takotna River only; the remaining mean estimates ranged from 69% for the Kogrukluk River and 84% for the Kisaralik River. Tributaries were grouped into larger aggregates based on the results from the multidimensional scaling analysis and geographic proximity. Mean contribution estimates comprised of artificial genotypes from population aggregates exceeded or were close to 90%: Arolik/Kanektok River 94%, Kisaralik/George River 84%, Kogrukluk/Tatlawiksuk River 92%, and Takotna River 90% (Figure 1).

DISCUSSION

Population subdivision

Significant levels of genetic variation were detected among populations of coho salmon in the Kuskokwim River region. The Arolik River and the Takotna River were the most

divergent populations among those sampled. This pattern is similar to that observed for other species: significant allele frequency differences occur between populations of chum salmon and Chinook salmon sampled between Kuskokwim Bay and Kuskokwim River; within the Kuskokwim River, populations upstream of the Takotna River are highly divergent from populations spawning lower in the drainage (chum salmon: Seeb et al. 1997; Chinook salmon: Templin et al. 2004; coho salmon: this study).

Genetic relationships among coho salmon in the Kuskokwim River region observed in this pilot study contrast somewhat with genetic patterns seen in statewide surveys for coho salmon using data from microsatellites and mitochondrial DNA. In those studies, populations were generally not structured based on geographic proximity except for on small geographic scales (Olsen et al. 2003; Gharrett et al. 2001). Instead, variation among populations within regions was greater than variation among regions. Values for F_{ST} estimated for seven regions in Alaska using microsatellite loci were large, ranging from 0.026 to 0.172 (Olsen et al. 2003). Lack of concordance with geography and large intra-regional F_{ST} were attributed to small population sizes due to opportunistic use of marginal spawning and rearing habitat (Olsen et al. 2003).

In the Kuskokwim River region, genetic relationships among populations are concordant with geography and F_{ST} was 0.01, considerably smaller than estimates for seven regions in Alaska using the same set of loci. Olsen et al. (2003) found that genetic structure following geographic proximity was strongest for populations in the Kenai River as may be expected in linear (riverine) habitats. Smaller F_{ST} in the Kuskokwim River region may be due to several factors. The Kuskokwim River is one of the largest producers of coho salmon in Alaska; relatively larger populations of coho salmon may occur in coastal western Alaska than in other regions in Alaska, possibly due to less patchy and more abundant spawning and rearing habitat. Alternatively, F_{ST} may have been underestimated in this study because samples were collected at weir sites and may have been population mixtures. Coho salmon radio-tagged at Kalskag and Aniak were far more likely to migrate back downstream and pass through weirs located below Kalskag and Aniak than were Chinook salmon, chum salmon, or sockeye salmon (personal communication; C. Kerkvliet, Alaska Department of Fish and Game, Commercial Fisheries Division, Anchorage), suggesting that coho salmon move within the Kuskokwim River before spawning to a larger extent than other species. However, the genotypic distributions of the samples from the Kuskokwim River used in this study all conformed to Hardy-Weinberg expectation, an indication that only single, panmictic units were sampled at each weir.

Similar run and spawning timing of the populations sampled may also play a role in the small F_{ST} observed. Differences in run and spawning timing can contribute substantially to reproductive isolation among spawning aggregates of salmonids in river systems and therefore is an important component of genetic variability among populations; examples include coho salmon in the Kenai River (Olsen et al. 2003); Chinook salmon in the Columbia River (Waples et al. in press); and chum salmon in the Yukon River (Wilmot et al. 1994; Seeb and Crane 1999) and Kuskokwim River (Seeb et al. 1997). Data on run timing of salmon stocks in the Kuskokwim River are currently being generated through mark-recapture (e.g., Kerkvliet and Hamazaki 2002) and weir projects and indicate a slightly earlier timing for Takotna River coho salmon than other stocks. The 50% cumulative passage by date of coho salmon marked near Kalskag and recovered in the Takotna River was approximately 10 days to two weeks earlier than for coho salmon recovered in the Kogrukluk, Tatlawiksuk, George, and Aniak rivers in 2002 (Linderman et al. 2003a, 2003b). Coho salmon from the Takotna River were the most

distinct in this population survey, possibly due to earlier run timing as well as geographic isolation. However, run timing in the Kuskokwim River is brief compared to the Kenai River where coho salmon return to spawn from July through late winter; accordingly among Kenai River populations, F_{ST} is much greater, 0.05.

Mixed-stock analysis

Genetic differences among populations are a prerequisite of successful mixed-stock analysis (Shaklee and Phelps 1990). Though F_{ST} was small for the Kuskokwim River region, it was significant. Further, significant allele frequency differences were detected among most of the pairwise combinations of populations. Composition of simulated mixtures was estimated for each individual population, and populations were aggregated into larger stock groups until the mean estimates were approximately 90%. For individual populations, only the Arolik and Takotna rivers had a mean estimate of 90% or greater. However, when populations were aggregated into four groups, Arolik/Kanektok, Kisaralik/George, Kogrukluk/Tatlawiksuk, and Takotna rivers, mean mixture estimates for each aggregate ranged from 84-94%.

Better-defined population aggregates and more accurate and precise estimates may be possible by surveying more populations and adding new loci. It is important to sample the full range of genetic diversity present within the Kuskokwim River for accurate MSA. Additional populations, particularly from the middle and upper Kuskokwim River, should be added to those included in this pilot survey. Genetic diversity often increases among populations in the upper portions of a drainage. For example, more among population diversity is present in the middle and upper portions than the lower portion of the Yukon River drainage for both chum and Chinook salmon (Wilmot et al. 1992). Further, increasing sample sizes and better representation of tributaries in the lower Kuskokwim River may aid in reducing bias among populations in the lower and middle portion of the drainage.

Single-locus F_{ST} estimates for two loci, *Oki-11* and *Ots-105,* were not significantly different from zero and alleles were similarly distributed within populations as shown through the allele binning procedure. Therefore, these loci were deleted from the mixture model. The number of loci used in maximum likelihood estimation of mixture composition affects the accuracy and bias of mixture estimates; increasing the number of loci used increases the potential amount of information available for discrimination of populations or population aggregates (Wood et al. 1987). Many microsatellite loci other than those surveyed in this study have been successfully used in separating coho salmon populations on a fine geographic scale in British Columbia and the Pacific Northwest; these could be screened and used in place of *Oki-11* and *Ots-105*.

Coho salmon are an important resource to residents of the Kuskokwim River region both as a subsistence resource and as cash source to provide money for other subsistence activities. Coho salmon, particularly in the middle and upper portion of the Kuskokwim River are difficult to enumerate and manage because of the preponderance of stock assessment programs in the lower portion of the drainage and a later return timing of this species. Genetic data may be useful in defining units for conservation and for estimating the stock composition of test fishery catches for a stock-specific index of run strength and timing. This may provide a cost-effective method to obtain relative abundance and timing information in the event that weir and other

stock assessment projects are discontinued in the coho salmon season due to logistical or budget constraints.

CONCLUSIONS

1. Significant genetic population structure was detected among seven coho salmon populations sampled from the Kuskokwim River region: Arolik River, Kanektok River, Kisaralik River, George River, Kogrukluk River, Tatlawiksuk River, and Takotna River.
2. Levels of genetic variation for coho salmon in the Kuskokwim River region are somewhat less than expected when compared to other regions in Alaska. Possible reasons include more abundant spawning habitat and therefore larger population sizes in the Kuskokwim River region than in other areas of Alaska or higher stray rates of coho salmon in coastal western Alaska.
3. Mean stock contribution estimates for simulated mixtures from Arolik/Kanektok River, Kisaralik/George River, Kogrukluk/Tatlawiksuk River, and Takotna River ranged from 84-94%. Levels of genetic divergence should support mixed-stock analysis for at least three groups: Kuskokwim Bay, lower-mid Kuskokwim River, and upper Kuskokwim River.

RECOMMENDATIONS

1.Refine population aggregates to be estimated in mixtures by surveying more population samples and replacing *Oki-11* and *Ots-105* with other loci. Suggested populations are: Goodnews River, Kwethluk River, Tuluksak River, Aniak River, Stony River, South Fork Kuskokwim River, and Salmon River.
2. Resample coho salmon from the George and Tatlawiksuk rivers from the spawning grounds.

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Table 1. Allele frequency estimates, observed and expected heterozygosity, conformation of genotypic frequencies to Hardy-Weinberg expectations, and F_{ST} for coho salmon sampled from the Arolik, Kanektok, Kisaralik, George, Kogrukluk, Tatlawiksuk, and Takotna rivers.

Locus	Arolik River	Kanektok River	Kisaralik River	George River	Kogrukluk River	Tatlawiksuk River	Takotna River	Overall
Oke-2								
N	86	96	81	95	96	96	161	
170	0.977	0.943	0.914	0.916	0.885	0.891	0.829	
172	0.023	0.057	0.086	0.084	0.115	0.109	0.171	
H_e	0.045	0.108	0.158	0.154	0.203	0.195	0.283	
H_o	0.047	0.115	0.173	0.147	0.229	0.177	0.255	
P(HW)	1	1	1	0.498	0.604	0.302	0.260	
F_{ST}								0.023
Oke-3								
N	93	96	73	96	95	96	160	
250	0.011	0.026	0.062	0.031	0.079	0.062	0.034	
256	0	0.005	0.048	0.021	0.011	0.005	0.041	
264	0.339	0.266	0.123	0.224	0.2	0.255	0.203	
274	0	0	0	0.026	0	0	0	
276	0.651	0.615	0.568	0.547	0.642	0.583	0.587	
278	0	0.089	0.199	0.151	0.068	0.094	0.134	
H_e	0.462	0.543	0.616	0.626	0.537	0.582	0.593	
H_o	0.548	0.583	0.699	0.667	0.547	0.563	0.625	
P(HW)	0.132	0.179	0.061	0.644	0.737	0.445	0.568	
F_{ST}								0.012
Oke-4								
N	87	95	81	90	96	93	155	
234	0.006	0	0	0.006	0	0.005	0	
238	0.046	0.063	0.012	0.044	0.036	0.016	0.023	
242	0.948	0.937	0.988	0.939	0.964	0.978	0.977	
244	0	0	0	0.011	0	0	0	
H_e	0.099	0.118	0.024	0.116	0.07	0.042	0.044	
H_o	0.092	0.126	0.025	0.122	0.073	0.043	0.045	
P(HW)	0.053	1	1	1	1	1	1	
F_{ST}								0.005

Table 1. Continued.

Locus	Arolik River	Kanektok River	Kisaralik River	George River	Kogrukluk River	Tatlawiksuk River	Takotna River	Overall
Oki-1								
N	88	94	87	96	96	91	158	
92	0.006	0.011	0	0.026	0	0	0	
96	0.017	0.021	0.011	0.047	0.026	0.033	0.013	
100	0.426	0.436	0.494	0.5	0.453	0.423	0.519	
104	0.142	0.154	0.08	0.135	0.089	0.143	0.127	
108	0.08	0.101	0.201	0.109	0.135	0.132	0.133	
112	0.295	0.261	0.201	0.177	0.281	0.269	0.203	
116	0.017	0.016	0.006	0.005	0.016	0	0.006	
120	0	0	0.006	0	0	0	0	
124	0.017	0	0	0	0	0	0	
H_e	0.704	0.707	0.668	0.685	0.688	0.71	0.656	
H_o	0.75	0.745	0.759	0.708	0.729	0.824	0.69	
P(HW)	0.089	0.708	0.383	0.832	0.607	0.131	0.865	
F_{ST}								0.004
Oki-3								
N	87	94	85	84	96	96	153	
70	0.132	0.223	0.306	0.25	0.328	0.276	0.281	
73	0.868	0.777	0.688	0.75	0.672	0.724	0.719	
76	0	0	0.006	0	0	0	0	
H_e	0.229	0.347	0.433	0.375	0.441	0.4	0.404	
H_o	0.241	0.362	0.412	0.381	0.365	0.49	0.444	
P(HW)	1	1	0.733	1	0.104	0.040	0.316	
F_{ST}								0.015
Oki-11								
N	90	92	89	96	96	96	162	
82	0	0	0	0	0	0	0.003	
84	0.8	0.772	0.831	0.839	0.849	0.839	0.827	
88	0.194	0.228	0.169	0.146	0.135	0.161	0.167	
90	0.006	0	0	0.016	0.016	0	0.003	
H_e	0.322	0.352	0.28	0.275	0.261	0.271	0.288	
H_o	0.3	0.283	0.292	0.302	0.281	0.302	0.333	
P(HW)	0.172	0.072	1	0.808	1	0.452	0.173	
F_{ST}								0.001

Table 1. Continued.

Locus	Arolik River	Kanektok River	Kisaralik River	George River	Kogrukluk River	Tatlawiksuk River	Takotna River	Overall
One-3								
N	90	91	58	96	95	96	149	
173	0.389	0.445	0.397	0.37	0.384	0.464	0.483	
175	0.194	0.176	0.224	0.276	0.274	0.193	0.151	
177	0	0	0	0	0.005	0	0	
179	0.194	0.181	0.224	0.208	0.205	0.203	0.292	
183	0.222	0.198	0.155	0.146	0.132	0.141	0.074	
H_e	0.724	0.699	0.718	0.722	0.718	0.687	0.653	
H_o	0.689	0.714	0.741	0.708	0.705	0.729	0.638	
P(HW)	0.130	0.558	0.830	0.515	0.140	0.943	0.825	
F_{ST}								0.008
Ots-3.1								
N	90	95	95	84	70	71	162	
92	0	0	0	0.024	0	0	0	
96	0.089	0.058	0.105	0.077	0.107	0.077	0.111	
108	0	0	0	0.006	0	0	0	
114	0.006	0	0	0	0	0	0	
116	0	0	0	0	0	0	0.003	
118	0.261	0.205	0.121	0.149	0.143	0.07	0.08	
120	0	0.011	0	0	0	0	0	
122	0.072	0.053	0.084	0.089	0.136	0.113	0.111	
124	0.006	0	0	0.018	0	0	0.049	
126	0.35	0.489	0.584	0.565	0.543	0.676	0.552	
128	0	0	0	0	0	0	0.022	
130	0.211	0.184	0.105	0.071	0.071	0.063	0.071	
132	0.006	0	0	0	0	0	0	
H_e	0.752	0.678	0.615	0.638	0.65	0.515	0.656	
H_o	0.578	0.611	0.632	0.655	0.586	0.521	0.679	
P(HW)	0	0.079	0.918	0.015	0.031	0.171	0.117	
F_{ST}								0.024

Table 1. Continued.

Locus	Arolik River	Kanektok River	Kisaralik River	George River	Kogrukluk River	Tatlawiksuk River	Takotna River	Overall
Ots-105								
N	90	96	61	96	95	96	162	
129	0.789	0.745	0.852	0.802	0.842	0.839	0.806	
133	0.211	0.255	0.148	0.198	0.158	0.161	0.194	
H_e	0.333	0.38	0.252	0.317	0.266	0.271	0.313	
H_o	0.356	0.427	0.262	0.354	0.211	0.281	0.302	
P(HW)	0.753	0.291	1	0.349	0.050	1	0.620	
F_{ST}								0.003
Overall								
AR	3.63	3.47	3.43	4.07	3.48	3.24	3.60	
H_e	0.408	0.437	0.418	0.434	0.426	0.408	0.432	
H_o	0.4	0.441	0.444	0.449	0.414	0.437	0.446	
F_{ST}								0.011

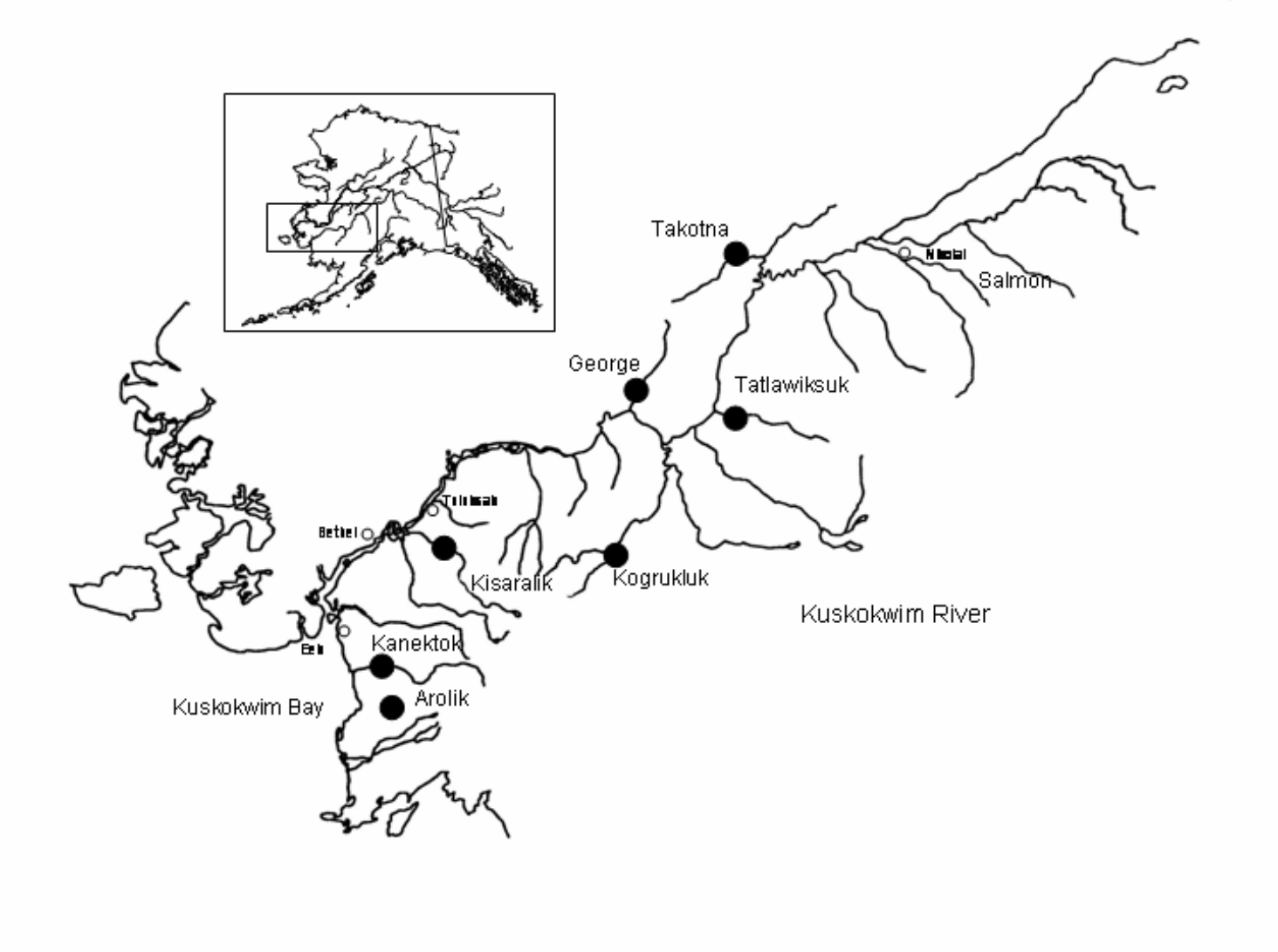

Figure 1. Sampling locations of coho salmon collected for genetic analysis in the Kuskokwim River region.

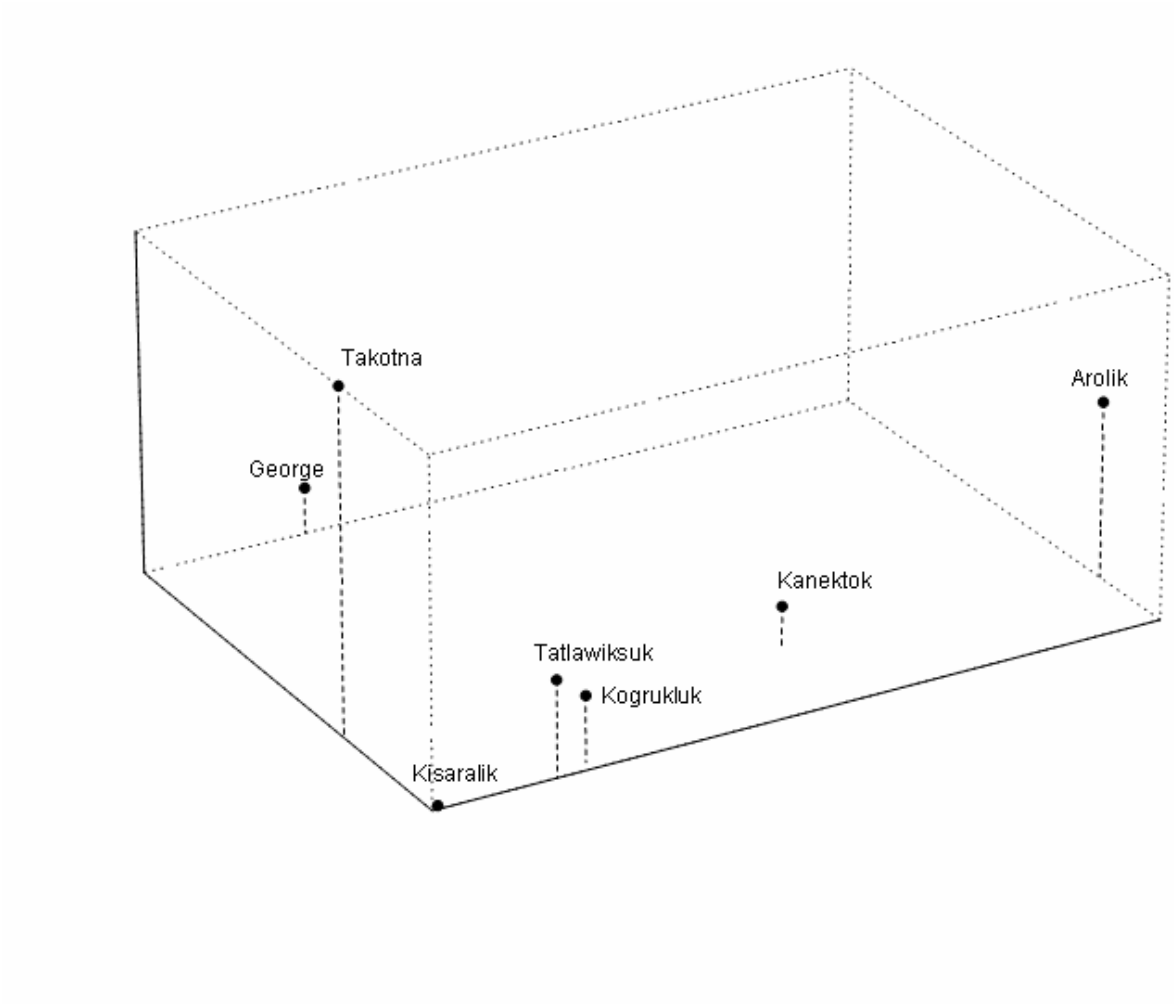

Figure 2. Multidimensional scaling analysis of Cavalli-Sforza and Edwards pairwise genetic distances. Distances were calculated from allele frequency estimates for nine microsatellite loci from seven populations of coho salmon sampled from the Kuskokwim River region.

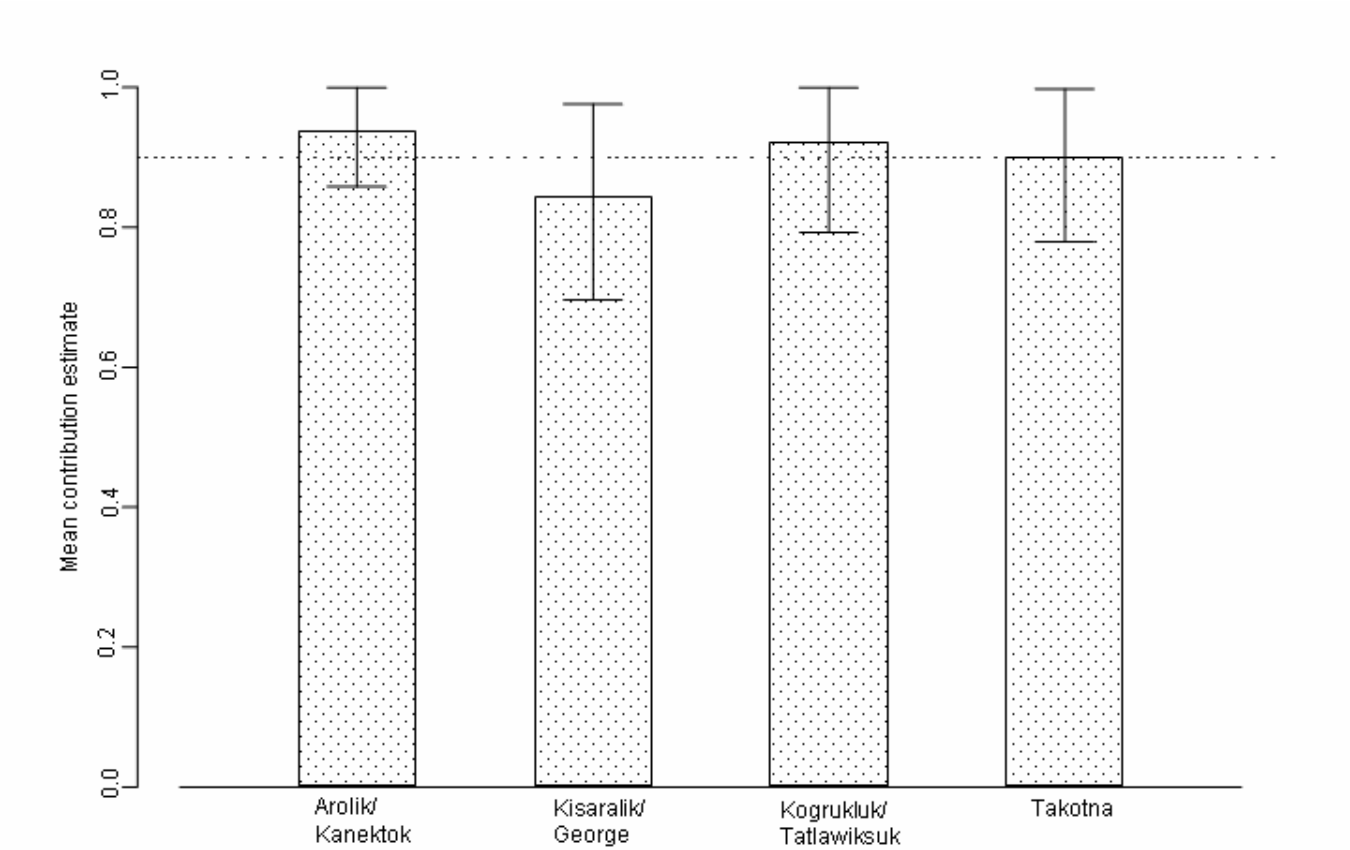

Figure 3. Mean contribution estimates of simulated mixtures composed 100% from the Arolik/Kanektok rivers, Kisaralik/George rivers, Kogrukluk/Tatlawiksuk rivers, and Takotna River. Mean contribution estimates of approximately 90% (dotted line) are considered robust for mixture analysis.