Report to the North Pacific Fishery Management Council

Genetic Stock Composition Analysis of Chinook Salmon Bycatch Samples from the 2008 Bering Sea Pollock Trawl Fisheries

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Introduction

It is important to understand the stock composition of salmon caught in Bering Sea fisheries because this area is a known feeding habitat for multiple brood years of Chinook salmon (*Oncorhynchus tshawytscha*) from many different localities in North America and Asia. Determining the geographic origin of salmon caught in federally managed fisheries is essential to understanding whether management of federal fisheries could address conservation concerns. This report includes preliminary genetic stock identification results for a subset of Chinook salmon bycatch samples collected from the 2008 Bering Sea groundfish fishery.

To understand the possible effects of the Chinook salmon bycatch, it is important to know where the fish are from. Since 1979, three separate stock composition estimates of the Chinook salmon bycatch have been made, all of which have shown that the majority of samples were from western Alaska stocks. Scale pattern analysis (SPA) was originally used to analyze the 1979-1982 Chinook salmon bycatch and the results suggested that 60% of the fish originated from western Alaska, 17% from southcentral Alaska, 14% from Asia, and 9% from Southeast Alaska and British Columbia (Myers and Rogers, 1988). A second study, also based on SPA, showed a similar stock composition from the 1997-1999 Chinook salmon bycatch with 56% from western Alaska, 31% from Cook Inlet, 8% from Southeast Alaska-British Columbia, and 5% from Russia (Myers et al., 2004). Finally, a genetic analysis was recently completed for Chinook salmon caught as bycatch in the 2005-2007 Bering Sea pollock fishery. In this genetic analysis, the Alaska Department of Fish and Game (ADFG) used single nucleotide polymorphisms (SNPs) to estimate the stock composition of the Chinook salmon bycatch (NMFS, 2009a). Genetic samples of the Chinook salmon bycatch from the 2005 “B”, 2006 “A”, and 2006 “B” pollock fishing seasons were analyzed, whereas the 2007 “A” estimates were derived from a limited sample set of 360 salmon collected during a test of a salmon excluder device under Exempted Fishing Permit 08-02. The only complete year for which stock composition estimates were available was 2006, and when normalized to total bycatch, approximately 42% of the samples were estimated to come from western Alaska, 23% from north Alaska Peninsula, 2% from Middle Yukon, 3% from Upper Yukon, 2% from Cook Inlet, 2% from Taku River-transboundary region, 23% from Pacific Northwest, 1% from Russia, and 2% from other regions.

While these studies represent significant advances in our understanding of Chinook stock composition, the available samples used in those studies (and ours) were not originally collected in a manner that is necessarily representative of the entire bycatch, potentially leading to significant biases in the resulting stock composition estimates (Pella and Geiger, 2009). Under a proposal funded by the Alaska Sustainable Salmon Fund (AKSSF), the Alaska Fisheries Science Center (AFSC) is currently analyzing samples from the 2008 Chinook bycatch to identify sample biases and produce stock compositions estimates for a limited strata of the bycatch.

The goal of this report is to present preliminary stock composition estimates for the 2008 Chinook salmon bycatch samples. It is important to understand the limitations of the genetic stock composition estimates presented here before attempting to apply them to estimate the stock composition of the bycatch as a whole. Limitations on our ability to estimate total bycatch stock composition are imposed by the sampling protocols under which the samples were collected, as well as by the completeness of the genetic baseline. Hence, this report is divided into three main sections. First, the sampling protocols are documented and the distribution of the genetic samples is compared to the overall Chinook salmon bycatch for 2008. Second, the efficacy of the single nucleotide

polymorphism (SNP) DNA baseline is evaluated using phenetic trees, principal coordinate analyses based on genetic distances, and simulation studies of hypothetical mixtures. Finally, stock composition estimates are provided for the 2008 Chinook salmon bycatch based on available genetic samples. A yearly stock composition estimate as well as composition estimates from the groundfish "A" and "B" fishing seasons are provided to investigate possible temporal effects on the stock composition of the bycatch.

Methods

Sample collection and DNA extraction

All samples were collected by the AFSC's North Pacific Observer Program as part of either a Special Project (designated "Salmon Genetic Project" in 2008) for the Auke Bay Laboratories for genetic analysis or for species identification/ageing purposes. Axillary processes for genetic analysis and scales for ageing were collected opportunistically throughout the season and stored in coin envelopes which were labeled, frozen and shipped to the Auke Bay Laboratories. Scales for species identification were collected in coin envelopes and shipped to the AFSC's Fisheries Monitoring and Analysis (FMA) Division for storage and analysis. DNA was extracted from the axillary processes and scales into 96-well plates with either the QIAGEN DNeasy Blood and Tissue Kits or Corbett X-tractor Gene reagents as described by the manufacturer (QIAGEN, Inc.)[1]. Extracted DNA had a final concentration of approximately 10-25 ng/ul and was stored at -20 ºC.

Data acquisition

Matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) genotyping was performed using a Sequenom MassARRAY iPLEX platform (Gabriel et al., 2009) to genotype 43 SNP DNA markers represented in the Chinook salmon baseline (Table 1). MALDI-TOF genotyping is a well established protocol first introduced 12 years ago (Ross et al., 1998) that is capable of producing high quality genotypes using an efficient multiplexed platform (Tindall et al., 2007). For genotyping, all DNA samples were quantitated using a Pico Green kit (Invitrogen) and samples were generally normalized to a DNA concentration of 5-10 ng/ul for downstream applications. Each sample was assayed in four separate multiplexed-PCR and extension reactions before being combined into two panels that were analyzed on the mass spectrometer. PCR conditions were as follows: 2 mM $MgCl_2$, 1X PCR buffer, 500 uM dNTPs, 0.1 uM primer stock, and 1 unit of PCR enzyme in a total volume of 5 ul. PCR was performed with the following protocol: initial denaturation at 95ºC for 4 minutes, then 45 cycles at 94ºC for 20 seconds, 56ºC for 30 seconds, and polymerization at 72ºC for 1 minute, followed by a final polymerization step at 72ºC for 3 minutes and storage at 4ºC until removal from the thermocycler. Unincorporated nucleotides and single stranded primers were removed by treatment with shrimp alkaline phosphatase (Sequenom, Inc.). Primer extension was performed using iPLEX reagents as described by the manufacturer (Sequenom, Inc.). Extension conditions were as follows: 94ºC for 30 seconds, then 40 cycles at 94ºC for 5 seconds and extension at 52ºC for 5 seconds, then 5 cycles at 52ºC for 5 seconds and 80ºC for 5 seconds, followed by a final step at 72ºC for 3 minutes and then storage at 4ºC until removal from the thermocycler. Unincorporated nucleotides were removed using a size exclusion resin (Sequenom, Inc.). For each sample, the four PCR reactions were combined into two panels containing 30 and 13 SNPs, respectively, which were dispensed onto a

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1. SNP markers in the ADFG baseline. A listing of the 43 loci, assays names, common alleles, frequencies of the common allele in the baseline populations, and references describing the genetic marker.

Locus	Assay Name	Common Allele	Frequency Range	Reference
xKER137	Ots_E2-275	A	0.005 - 0.997	Smith et al., 2005a
ARF	Ots_arf-188	G	0.845 - 1.000	Smith et al., 2005a
AsnRS72	Ots_AsnRS-60	C	0.260 - 0.979	Smith et al., 2005a
C3N3	Ots_C3N3	G	0.000 - 1.000	Smith et al., 2005b
ETIF1A	Ots_ETIF1A	A	0.005 - 0.894	Narum et al., 2008
FARSLA220	Ots_FARSLA-220	G	0.000 - 1.000	Smith et al., 2007
FGF6A	Ots_FGF6A	G	0.000 - 0.995	Narum et al., 2008
GH2	Ots_GH2	A	0.220 - 1.000	Smith et al., 2005b
GPDH	Ots_GPDH-338	G	0.381 - 1.000	Smith et al., 2005a
GPH318	Ots_GPH-318	C	0.569 - 1.000	Smith et al., 2005a
GST207	Ots_GST-207	C	0.117 - 1.000	Smith et al., 2007
GST375	Ots_GST-375	C	0.261 - 1.000	Smith et al., 2007
GTH2B550	Ots_GTH2B-550	C	0.000 - 0.916	Narum et al., 2008
HGFA	Ots_HGFA-446	C	0.661 - 1.000	Smith et al., 2005a
hnRNPL533	Ots_hnRNPL-533	T	0.010 - 1.000	Smith et al., 2007
HSP90B100	Ots_HSP90B-385	C	0.008 - 1.000	Smith et al., 2007
IGF191	Ots_IGF-I.1-76	A	0.235 - 1.000	Smith et al., 2005a
IK1328	Ots_Ikaros-250	G	0.750 - 1.000	Smith et al., 2005a
IL1RA	Ots_il-1racp-166	T	0.014 - 1.000	Smith et al., 2005a
LEI292	Ots_LEI-292	G	0.817 - 1.000	Smith et al., 2007
MHC1	Ots_MHC1	G	0.055 - 0.979	Smith et al., 2005b
MHC2	Ots_MHC2	T	0.000 - 1.000	Smith et al., 2005b
NOD1	Ots_NOD1	G	0.170 - 1.000	Narum et al., 2008
NRP	Ots_ZNF330-181	G	0.849 - 1.000	Smith et al., 2005a
OPLW173	Ots_LWSop-638	T	0.598 - 1.000	Smith et al., 2005a
OPSW152	Ots_SWS1op-182	A	0.156 - 1.000	Smith et al., 2005a
P450	Ots_P450	T	0.004 - 0.995	Smith et al., 2005b
Prl2	Ots_Prl2	A	0.091 - 1.000	Smith et al., 2005b
PrpI120	Ots_ins-115	A	0.831 - 1.000	Smith et al., 2005a
RAG3	Ots_RAG3	T	0.069 - 1.000	Narum et al., 2008
RFC2	Ots_RFC2-558	- (deletion)	0.121 - 1.000	Smith et al., 2005a
S71	Ots_S71	C	0.134 - 0.976	Narum et al., 2008
SClkF2	Ots_SClkF2R2-135	T	0.070 - 0.899	Smith et al., 2005a
SERPC1209	Ots_SERPC1-209	T	0.681 - 1.000	Smith et al., 2007
SL	Ots_SL	A	0.000 - 0.984	Smith et al., 2005b
TAPBP	Ots_TAPBP	C	0.206 - 1.000	Narum et al., 2008
Tnsf	Ots_Tnsf	G	0.000 - 1.000	Smith et al., 2005b
U200167	Ots_u202-161	T	0.000 - 1.000	Smith et al., 2005a
U211	Ots_u211-85	C	0.000 - 1.000	Smith et al., 2005a
U212297	Ots_U212-158	G	0.468 - 1.000	Smith et al., 2005a
UNKN4150	Ots_u4-92	T	0.275 - 1.000	Smith et al., 2005a
UNKN6187	Ots_u6-75	C	0.540 - 1.000	Smith et al., 2005a
zP3b	Ots_Zp3b-215	G	0.694 - 1.000	Smith et al., 2005a

SpectroCHIP II using a RS1000 Nanodispenser (Sequenom, Inc.). Typer Chip Linker software was used to enter the sample names and to operate the iPlex MALDI-TOF platform. Genotypes were exported to Excel spreadsheets (Microsoft, Inc.) for later analysis. All MALDI-TOF chips contained 10 known controls for assay verification.

Baseline and mixture conversion to SPAM and BAYES formats/stock composition analysis

Both SPAM and BAYES baseline files for 172 Chinook salmon populations surveyed for 43 SNP markers were obtained from the Alaska Department of Fish and Game (ADFG, unpublished). Compatibility of our allele designations to those found in the ADFG baseline was confirmed with a set of samples from the ADFG Gene Conservation Laboratory that were analyzed using both TaqMan (Applied Biosystems) and MALDI-TOF chemistries. Genotypes from the bycatch mixtures were exported from Excel as text files and C programs were used to format the data into SPAM and BAYES mixture files. Stock composition analysis was performed with both the SPAM and BAYES software using previously published procedures (ADFG, 2003; Pella and Masuda, 2001).

Baseline evaluation

The ADFG Chinook salmon SNP baseline used in our analysis is the same as that used in the genetic analysis of the 2005-2007 Chinook salmon bycatch (NMFS, 2009a). As a means to evaluate the regional groupings, population genetic structure was examined in three ways. First, population groupings were made using a UPGMA phenogram of Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards, 1967) as calculated using NT-SYS statistical software (Applied Biostatistics, Inc.). Second, Nei's standard genetic distance was calculated from the allele frequencies of the baseline populations (Nei, 1972) and population groupings was examined using a principal coordinate analysis (PCO). Third, baseline simulation studies were performed to evaluate the effectiveness of the baseline to allocate stocks to the correct regions. Three different simulation tests (43 SNPs, 36 SNPs, and 30 SNPs) were performed with SPAM software (Version 3.7) by using hypothetical mixtures of 400 fish containing 100% stock proportions as described in the text. In these simulations, fifteen hypothetical mixtures were derived, each containing 100% of the fish from each of the 15 different reporting regions. The simulated mixtures were then re-evaluated with the baseline to determine the percentage that allocated back to the correct region. Simulations were performed for all 43 markers, 36 markers, and 30 markers to evaluate how the baseline might be affected by missing genotype data.

Understanding the quality of the samples for the purpose of determining stock composition

Potential biases associated with the collection of genetic samples from the bycatch are well documented, and have the potential to affect resulting stock composition estimates (Pella and Geiger, 2009). Methods to collect representative samples are now being reviewed by the Alaska Fisheries Science Center and, when implemented, will reduce biases and improve defensibility of overall stock composition estimates. There are many different sources of potential bias in the current sample set. For example, due to the opportunistic nature of the sampling protocol employed, some observers likely collected samples whereas others did not (observer bias). Sources of bias derived from missing samples are not possible to correct, although other potential sources of bias such as temporal and spatial bias) can potentially be reduced using subsampling protocols. Despite these issues, the analysis of the 2008 Chinook bycatch samples has been completed providing a rough measure of stock distribution, and at a minimum, an indication of the presence and/or absence of specific stocks.

Potential biases associated with the 2008 Chinook salmon sample set were evaluated by comparing the genetic sample distributions with the overall bycatch estimates. First, the effect of temporal bias was evaluated by comparing the distribution of the 2008 Chinook bycatch with the 2008 genetic sample set. The bycatch estimates and genetic samples were graphed by statistical week (week ending on Sunday) and a visual comparison of the two distributions showed similar trends (Figure 1). As a means to evaluate the spatial distributions of the samples, the total Chinook bycatch was also compared with the bycatch samples by statistical area over time (Figure 2). While positions are known for samples taken from specific hauls, they were estimated from offloads as the first associated haul. The sample set generally correlated with the overall bycatch, however, differences were noted. For example, high levels of both bycatch and genetic samples were available from statistical area 517 during weeks 4 through 8, whereas differences were apparent late in the season when the bycatch showed a peak from statistical area 517 while the sample set had a peak from statistical area 521.

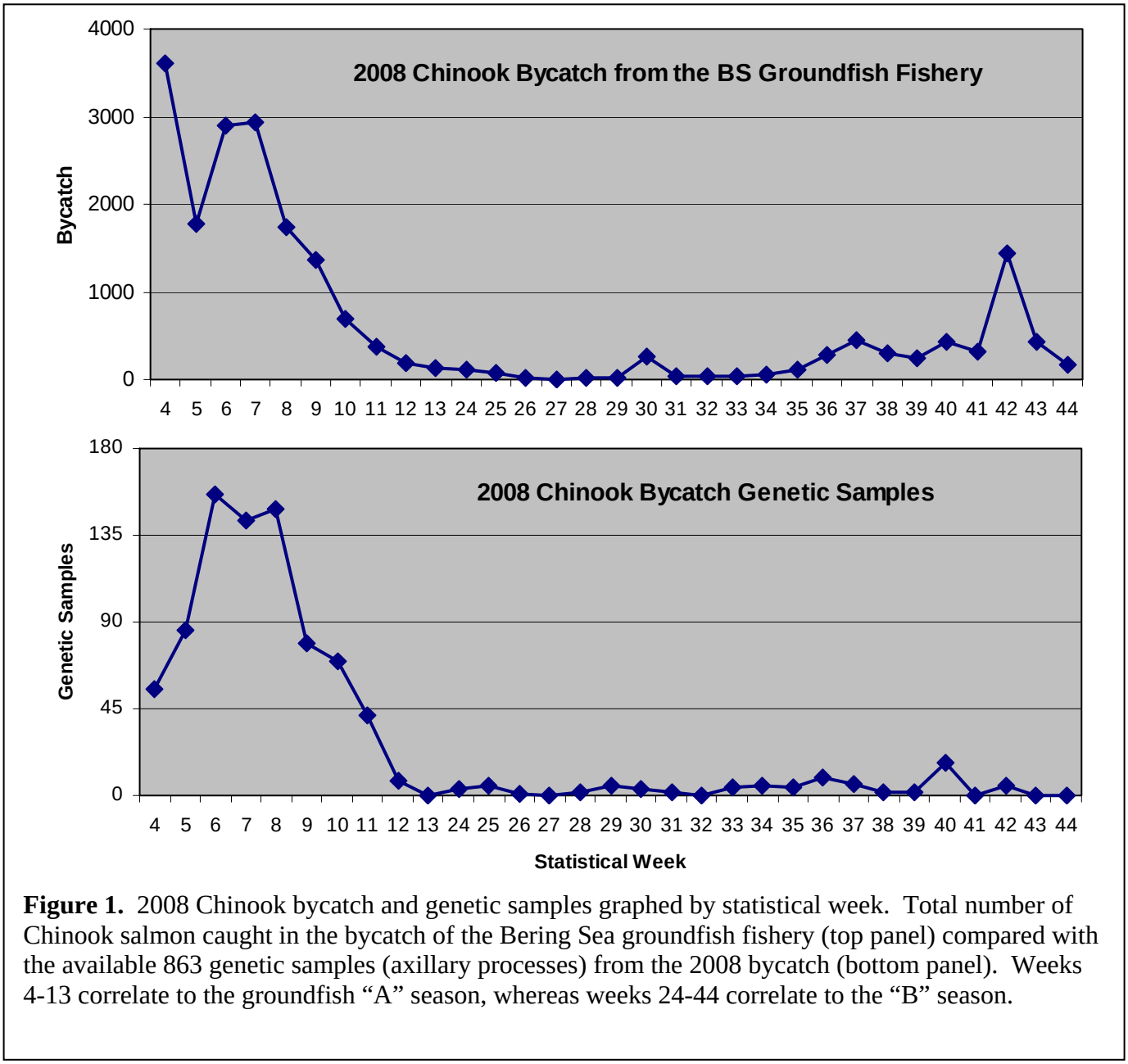

Figure 1. 2008 Chinook bycatch and genetic samples graphed by statistical week. Total number of Chinook salmon caught in the bycatch of the Bering Sea groundfish fishery (top panel) compared with the available 863 genetic samples (axillary processes) from the 2008 bycatch (bottom panel). Weeks 4-13 correlate to the groundfish "A" season, whereas weeks 24-44 correlate to the "B" season.

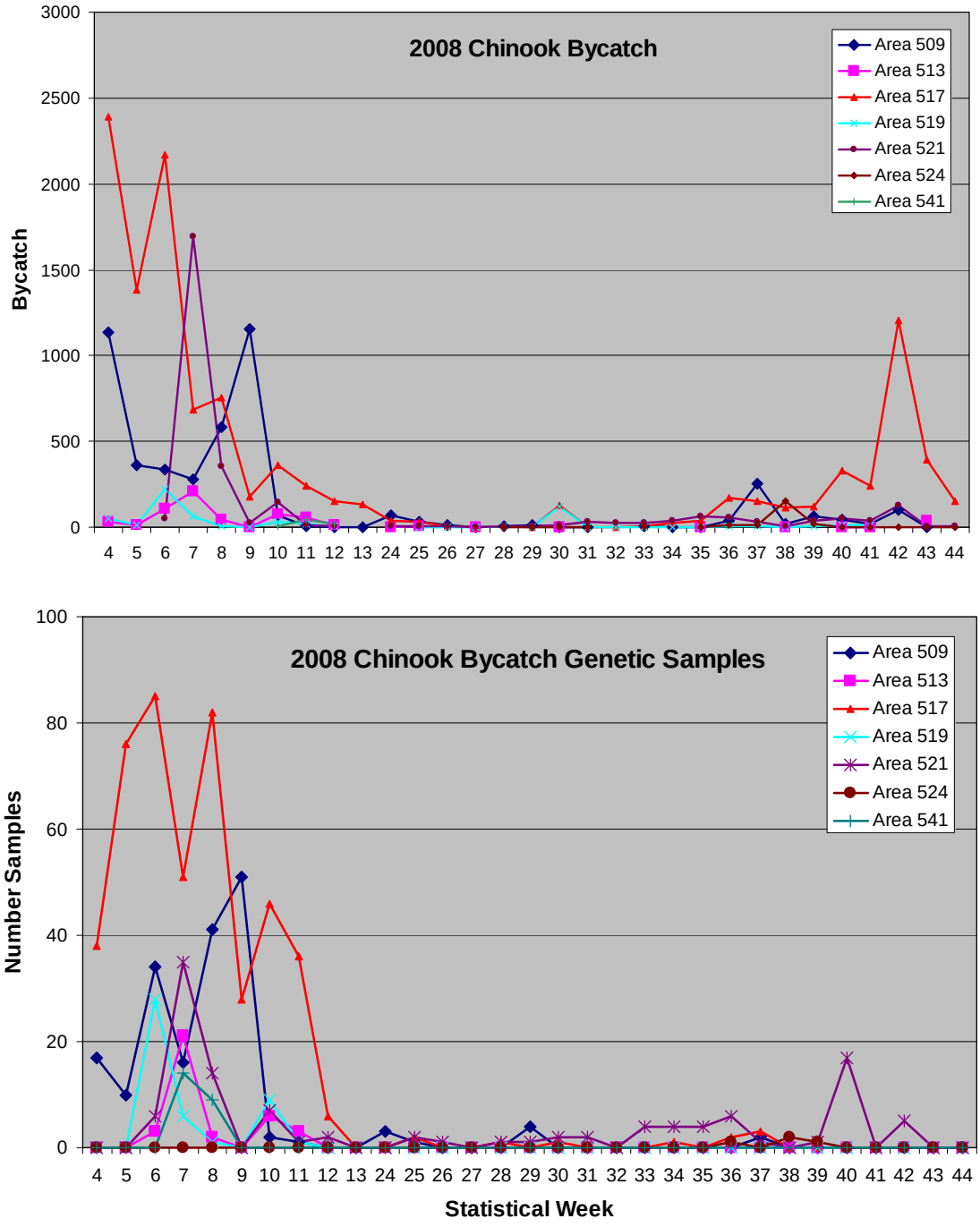

Figure 2. Comparison of the Chinook salmon bycatch by time and area with the distribution of available genetic samples. Not shown are areas 516 with an estimated 0.5 Chinook salmon taken and area 523 with an estimated 9 Chinook salmon samples taken in the total bycatch. No genetic samples were available from areas 516 and 523. Weeks 4-13 correlate to the groundfish "A" season, whereas weeks 24-44 correlate to the "B" season.

In 2008, an estimated 20,559 Chinook salmon were harvested as bycatch in the Bering Sea groundfish fishery (NMFS, 2009b). The genetic sample set for the 2008 Chinook bycatch was 863 fish corresponding to an overall sampling rate of 4.2%. This sample set was used to generate the estimate over the entire year (Figure 1).

Evaluation and adequacy of the baseline

A SNP DNA baseline representative of Chinook salmon populations from throughout the entire Pacific Rim has been developed by the Alaska Department of Fish and Game Gene Conservation Laboratory (ADFG, unpublished). This baseline contains 172 populations of Chinook salmon assayed for 43 SNP markers and grouped into 15 regional groups previously identified by the Alaska Department of Fish and Game (see Appendix 1 for stream origins). To determine the ability of the 43 SNP markers to discriminate population structure, two different descriptive analyses were used. First, regional groupings were identified based on a UPGMA phenogram of Cavalli-Sforza and Edwards

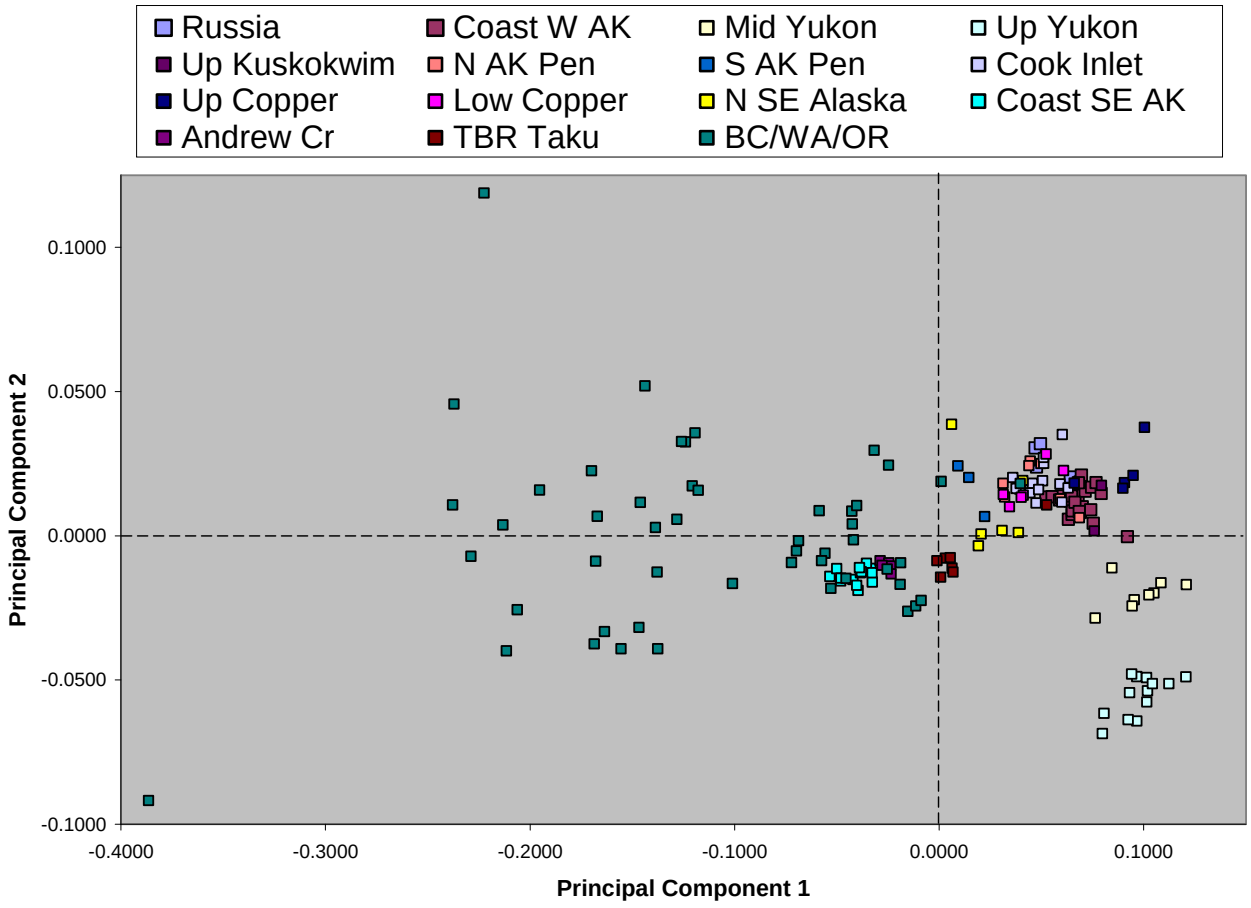

Figure 3. Principal coordinate analysis of 172 Chinook salmon populations analyzed for 43 SNP markers. Eigenvalues were plotted in two dimensional space with "1" being the most informative and "2" the second most. The 15 regional groupings are designated with population specific symbols.

chord distances (Appendix 2). Based on this analysis, regional groupings were apparent with most, but not all populations genetically grouping based on geographic distance and/or management priorities. Second, principal coordinate analysis (PCO), based on Nei's genetic distance calculated from the allele frequencies of the baseline populations, was used to separate the populations in three dimensional space. From this analysis, strong regional groupings were apparent for most populations as indicated by the groupings of the similar population symbols (Figure 3). In this analysis, the BC/Washington/Oregon grouping was relatively diverse, effectively clustering the remaining regional groups although significant structure remained.

From the PCO and the UPGMA dendrogram, the following fifteen regional groupings were apparent: Russia, coastal western Alaska (designated "Coast W AK"), middle Yukon (designated "Mid Yukon", upper Yukon (designated "Up Yukon"), upper Kuskokwim (designated "Up Kuskokwim"), north Alaska Peninsula (designated "N AK Pen"), south Alaska Peninsula (designated "S AK Pen"), Cook Inlet, upper Copper River (designated "Up Copper"), lower Copper River (designated "Low Copper"), northern Southeast Alaska (designated "N SE Alaska"), coastal Southeast Alaska (designated "Coast SE AK"), Andrew Cr, Transboundary region (designated "TBR Taku"), and British Columbia/Washington/Oregon (designated "BC/WA/OR"). These 15 regional groupings were used for all analyses in this report. The individual populations and the associated groupings are identified in Appendix 1.

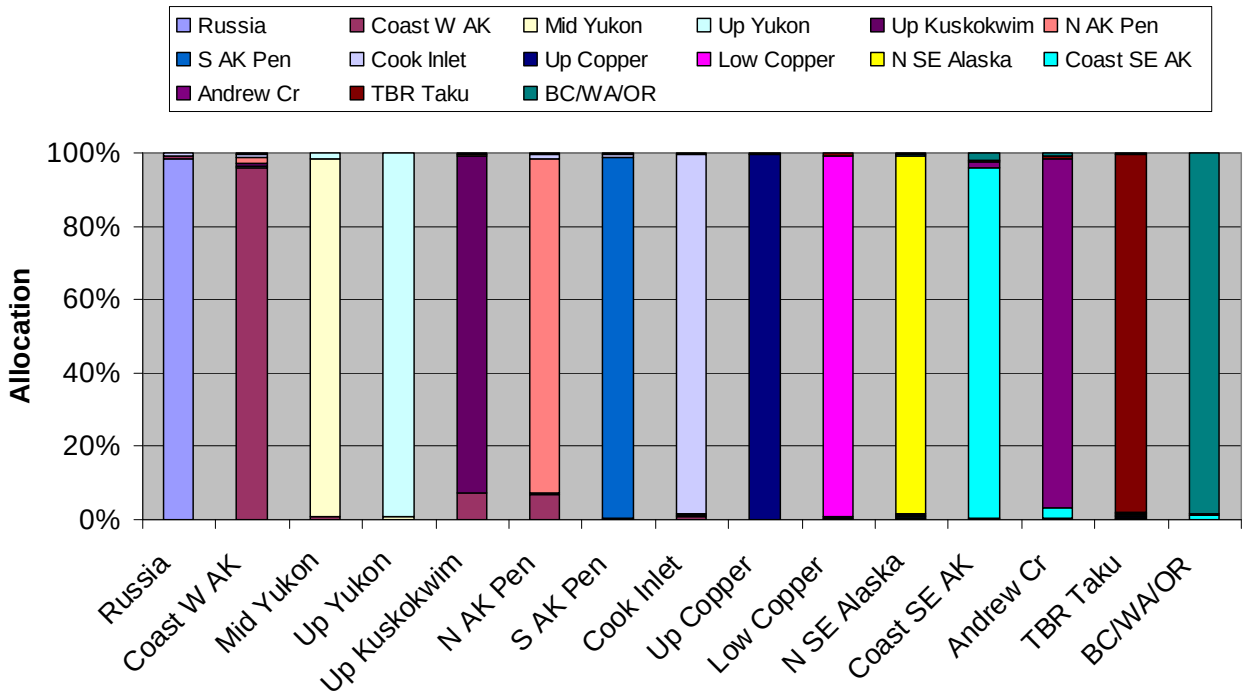

Figure 4. Results from 100% simulation experiments for simulated mixtures containing information for 43 SNP markers. In each column, 100% of the theoretical mixture of 400 fish were derived from the region identified at the bottom of the graph and analyzed against the Chinook baseline for 43 SNP markers.

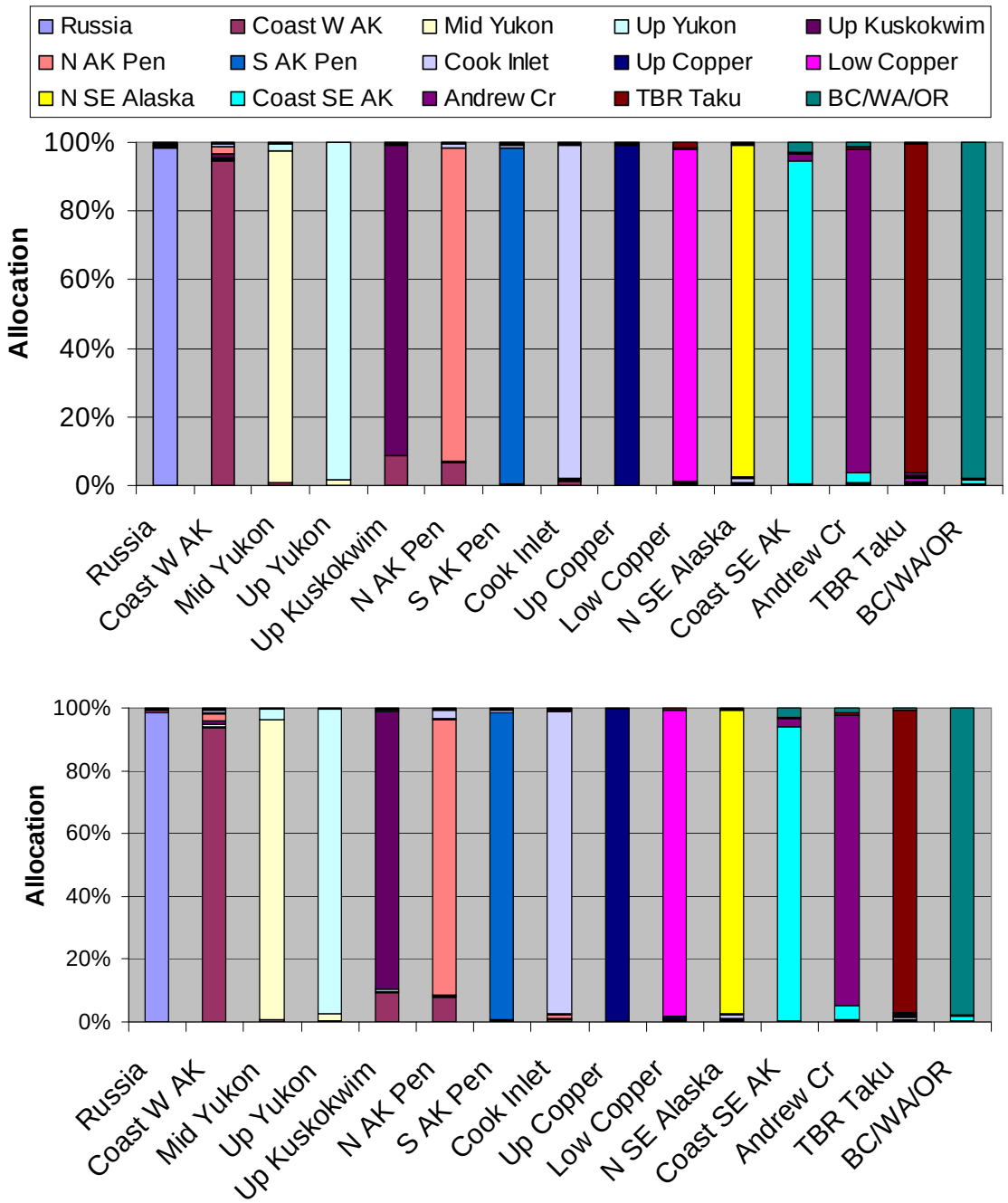

Figure 5. Results from 100% simulation experiments for simulated mixtures containing either 36 (top panel) or 30 (bottom panel) SNP markers. In each column, 100% of the theoretical mixture of 400 fish were derived from the region identified at the bottom of the graph and analyzed against the Chinook baseline.

To evaluate the ability of the 43 markers to effectively separate the 15 identified regional groupings, 100% simulation studies were performed using the SPAM software. In these simulations, 100% of a hypothetical mixture came from one of the 15 regions and was evaluated against the baseline to determine the percentage that allocated back to the correct region. This analysis was

completed for all 15 regions and all stocks partitioned back to their natal areas with greater than 91% accuracy (Figure 4).

Because not all samples were successfully genotyped for all 43 SNP markers, simulation studies were repeated using smaller loci sets of either 36 markers or 30 markers. Markers were randomly selected for this simulation analysis. The 36 marker set lacked seven loci: ETIF1A, FGF6A, GPH318, hnRNPL533, MHC2, RFC2, and SL. The 30 marker set lacked thirteen loci: GTH2B550, xKER137, C3N3, FARSLA220, GPH318, GST207, hnRNPL533, LEI292, OPSW152, TAPBP, U212297, zP3b, and S71. While simulation results are likely to differ for each loci set, the results are indicative of the power of the baseline. To evaluate the ability of the 36 marker set and the 30 marker set to effectively separate the 15 identified regional groupings, 100% simulation studies were again performed using the SPAM software for all 15 regions (Figure 5). Results for both the 36 SNP set and the 30 SNP set continued to reallocate fish back to their natal areas with high levels of accuracy (99.2% to 90.4% for the 36 SNP simulations and 99.2% to 87.8% for 30 SNP set). As a result of these simulation analyses, samples were limited to those missing information at 8 or less SNP loci.

Development and testing of MALDI-TOF genotyping assays

MALDI-TOF (Matrix Associated Laser Deionization – Time of Flight) genotyping assays were developed for genotyping Chinook salmon. The 43 SNP markers were grouped into two multiplexed panels, each containing two separate PCR and primer extension reactions. To test the accuracy of the MALDI-TOF genotyping assays, 185 Chinook samples were genotyped for 41 SNP markers (the other two markers were added later) and results compared with genotypes produced using the TaqMan assay (provided by ADFG). Out of a total possible scored 14,985 MALDI-TOF base calls there were: 14 discrepancies with the TaqMan assays (0.09%), 83 missing TaqMan haplotypes (0.55%), 56 missing MALDI-TOF haplotypes (0.37%), and 14,832 matching haplotypes (98.98%). As a separate control, all MALDI-TOF chips used during the sample analysis also contained 10 random control samples with known genotypes for comparison showing an overall accuracy rate of 99.84% (total number of matching MALDI-TOF and TaqMan genotypes/total number of MALDI-TOF genotypes). Genotyping success rate varied by chip and samples were reanalyzed as necessary to accommodate missing genotypes.

Stock composition analyses, including temporal trends

Stock composition analysis of all samples

Stock origin of the 863 genetic samples from the 2008 Chinook salmon bycatch (Figure 1 and 2) was determined to be primarily of western Alaska and north Alaska Peninsula origin (Table 2). The samples used in this analysis had genetic information for an average of 40 markers and no sample had less than 8 missing genotypes. Stock composition estimates were derived by using both the SPAM and BAYES software and both yielded almost identical stock composition estimates (Table 2). BAYES software uses a Bayesian algorithm to produce stock composition estimates and can account for missing alleles in the baseline (Pella and Masuda, 2001). In contrast, SPAM uses a maximum likelihood approach in which the mixture genotypes are compared directly with the baseline. Although Version 3.7 of the SPAM software allows Bayesian modeling of baseline allele frequencies, these options were not utilized for the stock composition analyses. Convergence of the SPAM estimates was

Table 2. Regional SPAM and BAYES stock composition estimates for the 863 Chinook salmon samples from the bycatch of the 2008 Bering Sea groundfish fishery. Standard errors for the SPAM estimates were determined by jackknifing. The BAYES mean estimates are also provided with standard deviations (SD), 95% credible intervals, and the median estimate.

	SPAM		BAYES				
Region	**Estimate**	**SE**	**Mean**	**SD**	**2.5%**	**Median**	**97.5%**
Russia	**0.011**	0.003	**0.008**	0.004	0.003	0.008	0.017
Coast W AK	**0.522**	0.017	**0.544**	0.025	0.496	0.544	0.591
Mid Yukon	**0.015**	0.002	**0.007**	0.008	0.000	0.004	0.027
Up Yukon	**0.040**	0.004	**0.046**	0.010	0.027	0.046	0.066
Up Kuskokwim	**0.071**	0.008	**0.068**	0.015	0.041	0.068	0.100
N AK Pen	**0.313**	0.014	**0.306**	0.020	0.267	0.306	0.346
S AK Pen	**0.004**	0.001	**0.000**	0.001	0.000	0.000	0.002
Cook Inlet	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.004
Up Copper	**0.000**	0.000	**0.000**	0.000	0.000	0.000	0.000
Low Copper	**0.001**	0.000	**0.000**	0.001	0.000	0.000	0.001
N SE Alaska	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.001
Coast SE AK	**0.002**	0.000	**0.005**	0.003	0.000	0.004	0.011
Andrew Cr	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.002
TBR Taku	**0.004**	0.000	**0.000**	0.002	0.000	0.000	0.005
BC/WA/OR	**0.016**	0.002	**0.015**	0.004	0.008	0.014	0.024

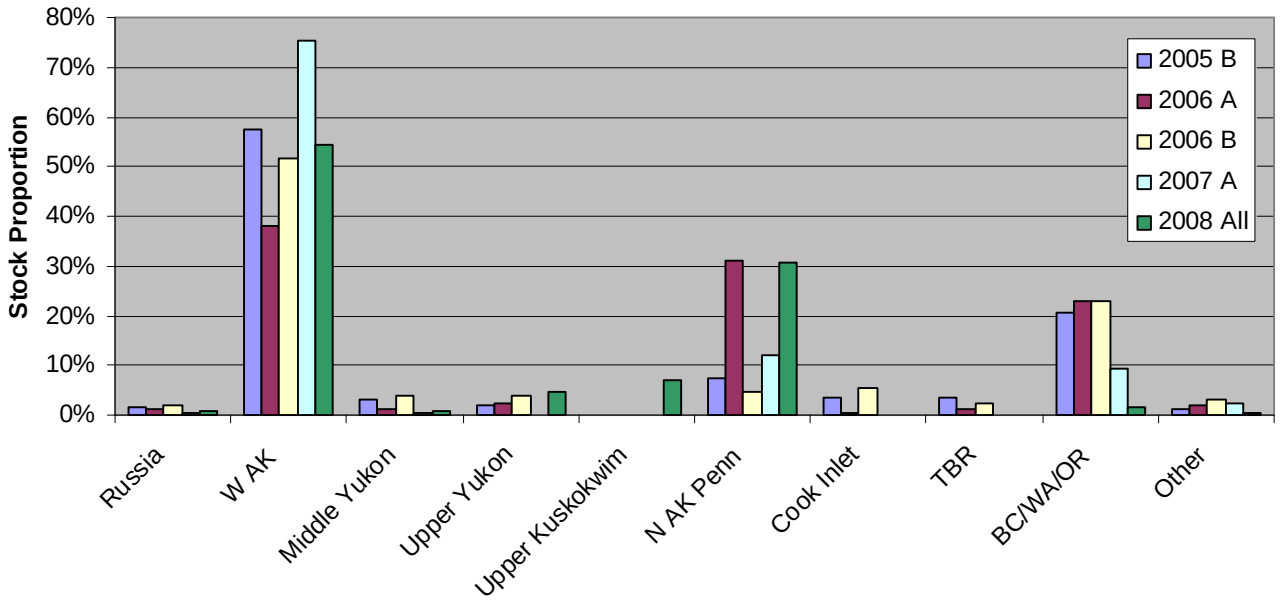

Figure 6. Comparison of the Chinook salmon bycatch stock composition estimates derived from the 2008 sample set with those shown in the Chinook Environmental Impact Statement (NMFS, 2009a). The "2008 All" are yearly estimates while 2005 "B", 2006 "A", 2006 "B", and 2007 "A" are seasonal. The 2007 "A" estimate is derived from a limited sample set collected during a test of the salmon excluder device. The same genetic baseline and general regional groupings were used in all analyses.

monitored with the "percent of Maximum" value which was determined to be 91.3, exceeding the 90% guaranteed percent achievement of the maximal likelihood. For each BAYES analysis, 15 Monte Carlo chains starting at disparate values of stock proportions were configured such that 95% of the stocks came from one designated region with weights equally distributed among the stocks of that region. The remaining 5% was equally distributed among remaining stocks from all other regions. For all estimates, a flat prior of 0.005814 (calculated as 1/172) was used for all 172 populations. The analyses were completed for a chain length of 10,000 with the first 5,000 deleted during the burn-in phase when determining overall stock compositions. Convergence of the chains to posterior distributions of stock proportions was determined with Gelman and Rubin shrink statistics which were all 1.02 or less conveying strong convergence to a single posterior distribution (Pella and Masuda, 2001).

The results from this study suggest that the majority of the Chinook salmon in the 863 sample set originated from western Alaska (54%), north Alaska Peninsula (31%), upper Kuskokwim (7%) and upper Yukon (5%) regions. Over 91% of the 2008 bycatch samples were collected during the "A" season and our results, when compared with the 2006 "A" season Chinook salmon bycatch estimate, were generally similar (NMFS, 2009a) (Figure 6). One difference is the presence of upper Kuskokwim fish which was identified at 7% in the 2008 sample set. While possibly a sampling artifact, it is also possible that returning Kuskokwim River salmon could have been inadvertently taken while schooling for their spring spawning migration in 2008. For example, the north Alaska Peninsula, western Alaska, and upper Kuskokwim regional groupings are located closest to the trawl fishery during the "A" season when the Chinook salmon bycatch was the highest.

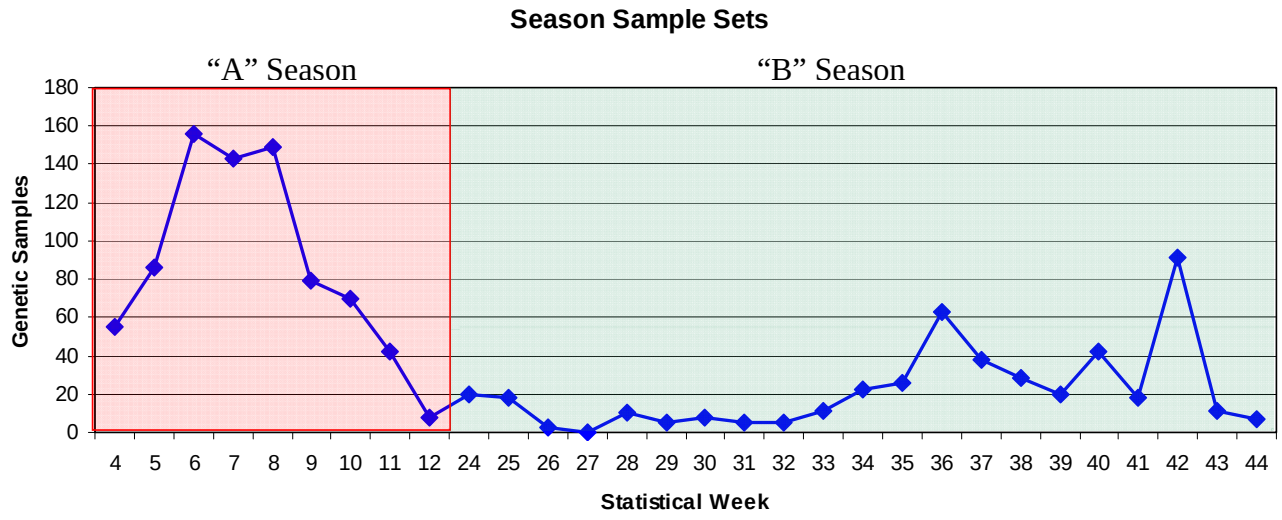

Figure 7. Sample distributions used to develop seasonal estimates from the 2008 Bering Sea groundfish fishery. There were 788 samples from the "A" season, all of which were part of the previously analyzed 863 yearly bycatch sample set. The remaining 75 samples from the "B" season were supplemented with 376 scale samples for a total of 451 genetic samples. The plot shows the distributions of the new seasonal sample sets.

Table 3. SPAM and BAYES stock composition estimates for the 2008 Chinook salmon bycatch samples from the "A" and "B" groundfish seasons. SE is the SPAM standard error. The BAYES mean estimates are also provided with standard deviations (SD), 95% credible intervals, and the median estimate.

2008 Chinook "A" Season	**SPAM**		**BAYES**				
Region	**Estimate**	**SE**	**Mean**	**SD**	**2.5%**	**Median**	**97.5%**
Russia	**0.010**	0.003	**0.008**	0.004	0.003	0.008	0.016
Coast W AK	**0.483**	0.017	**0.516**	0.027	0.463	0.517	0.568
Mid Yukon	**0.013**	0.002	**0.004**	0.006	0.000	0.001	0.019
Up Yukon	**0.039**	0.004	**0.044**	0.009	0.028	0.043	0.062
Up Kuskokwim	**0.081**	0.009	**0.079**	0.017	0.049	0.078	0.114
N AK Pen	**0.351**	0.015	**0.334**	0.022	0.293	0.334	0.378
S AK Pen	**0.005**	0.002	**0.000**	0.001	0.000	0.000	0.002
Cook Inlet	**0.001**	0.000	**0.000**	0.001	0.000	0.000	0.004
Up Copper	**0.000**	0.000	**0.000**	0.000	0.000	0.000	0.001
Low Copper	**0.001**	0.000	**0.000**	0.001	0.000	0.000	0.001
N SE Alaska	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.001
Coast SE AK	**0.002**	0.000	**0.004**	0.003	0.000	0.004	0.011
Andrew Cr	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.002
TBR Taku	**0.004**	0.000	**0.000**	0.001	0.000	0.000	0.003
BC/WA/OR	**0.010**	0.002	**0.009**	0.004	0.004	0.009	0.017
2008 Chinook "B" Season							
Russia	**0.021**	0.006	**0.023**	0.008	0.010	0.022	0.041
Coast W AK	**0.725**	0.025	**0.729**	0.026	0.676	0.730	0.779
Mid Yukon	**0.035**	0.006	**0.037**	0.014	0.014	0.036	0.068
Up Yukon	**0.063**	0.008	**0.065**	0.015	0.037	0.064	0.097
Up Kuskokwim	**0.000**	0.000	**0.000**	0.002	0.000	0.000	0.001
N AK Pen	**0.045**	0.007	**0.048**	0.014	0.024	0.046	0.078
S AK Pen	**0.006**	0.002	**0.000**	0.002	0.000	0.000	0.005
Cook Inlet	**0.020**	0.006	**0.016**	0.014	0.000	0.014	0.046
Up Copper	**0.005**	0.004	**0.003**	0.003	0.000	0.001	0.012
Low Copper	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.004
N SE Alaska	**0.000**	0.000	**0.000**	0.001	0.000	0.000	0.002
Coast SE AK	**0.016**	0.001	**0.009**	0.008	0.000	0.009	0.026
Andrew Cr	**0.000**	0.000	**0.003**	0.006	0.000	0.000	0.021
TBR Taku	**0.000**	0.000	**0.001**	0.004	0.000	0.000	0.013
BC/WA/OR	**0.063**	0.006	**0.065**	0.012	0.043	0.064	0.091

Temporal changes in stock contributions

There was a shift in regional contributions of the stock composition estimate between the samples available from the "A" and "B" groundfish seasons, with western Alaska and the north Alaska Peninsula dominant in the "A" season and western Alaska dominant in the "B" season. Genetic samples (axillary processes) from the 2008 Chinook bycatch sample set of 863 samples were predominantly collected during the Bering Sea groundfish "A" season (788 from "A" season and 75

from "B" season). The "B" sample set was then supplemented with available scale samples that were originally collected for species identification studies to increase the sample size for comparing stock composition estimates between the two seasons, therefore biases (original and induced) in the sample sets suggest caution should be used to limit the inference of the estimates to the entire fishery. While no differences were noted in genotyping efficiencies between the scale and tissue samples, scales samples have the potential to be contaminated with DNA from other fish, something that cannot be accurately measured using SNP markers for a mixed stock group. For this analysis, genetic information from 788 Chinook salmon bycatch samples were available from the "A" season and 451 Chinook salmon bycatch samples were available from the "B" season. A distribution of the sample sets used to generate the seasonal estimates is shown in Figure 7.

Understanding the temporal distribution of the salmon bycatch is important. For example, if the samples are randomly distributed or represent a distribution which can be described mathematically, temporally biased estimates could be adjusted with respect to the overall bycatch rate. Both BAYES and SPAM stock composition estimates were made from the "A" and "B" season sample sets. Convergence of the SPAM estimates was monitored with the "percent of Maximum" values which were 96 ("A" Season) and 90.8 ("B" Season), exceeding the 90% guaranteed percent achievement of the maximal likelihood. For each BAYES analysis, 15 Monte Carlo chains starting at disparate starting values of stock proportions were configured as described above. For all estimates, a flat prior of 0.005814 (calculated as 1/172) was used for all 172 populations. The analyses were completed for a chain length of 10,000 with the first 5,000 deleted during the burn-in phase when determining overall stock compositions. Convergence of the chains to posterior distributions of stock proportions was determined with Gelman and Rubin shrink statistics which were all 1.04 or less conveying strong convergence to a single posterior distribution (Pella and Masuda, 2001). The SPAM and BAYES estimates were very similar to each other; however, the stock composition estimates differed between time periods (Table 3).

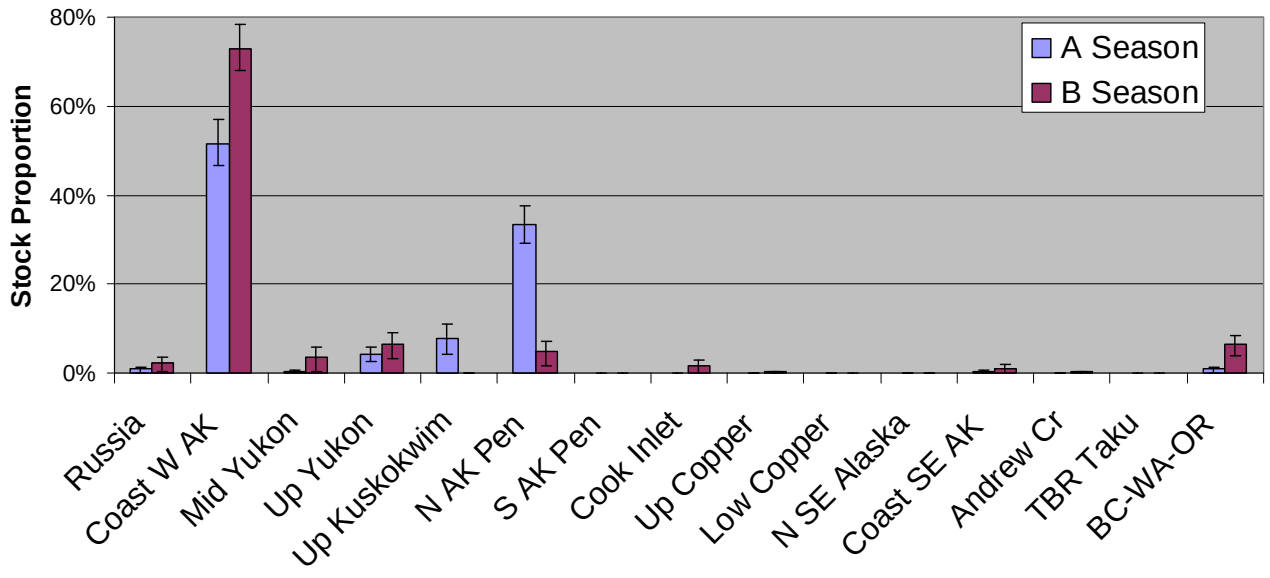

Figure 8. Chinook salmon stock composition estimates for bycatch samples taken during the "A" and "B" groundfish seasons. The ranges of the 95% BAYES credible estimates are shown. "Stock Proportion" is the estimated fraction of fish derived from the reporting region that were found in the mixture.

The differences in stock structure between seasons was significant for both the SPAM and BAYES estimates (Figure 8, see non-overlapping differences in the plotted BAYES 95% credible intervals). While most Chinook salmon are caught as bycatch in the pollock trawl "A" season, large numbers of Chinook salmon can also be caught in the "B" season fishery. Understanding the effects of the bycatch for both seasons is important and likely linked to the life history of the Chinook salmon. For example, fish from the north Alaska Peninsula and upper Kuskokwim were more prevalent in the bycatch samples from season "A" than in season "B" (Figure 8). This could represent either changes in the distribution of Chinook salmon stocks, movement of fishing effort, and/or potential biases in the sample collection protocols. Similarities between the 2006 and 2008 Chinook salmon stock estimates for north Alaska Peninsula (Figure 6) suggest some temporal stability for these observations. For the 2008 estimate, the decrease in north Alaska Peninsula and upper Kuskokwim fish during the "B" season was offset by an increase in western Alaska (52% to 73%) and BC/WA/OR (1% to 6%) fish.

Summary and discussion with future implications

Communities in western Alaska and elsewhere are dependent on Chinook salmon for subsistence and commercial purposes. Decreasing Chinook salmon returns to western Alaska rivers have caused hardships in these communities and led to the recent declaration of a fisheries disaster for Yukon River Chinook salmon by the United States Secretary of Commerce (Locke, 2010). Salmon-dependent communities have expressed concern regarding the numbers of salmon caught as bycatch in the Bering Sea pollock fishery. The incidental harvest of Chinook salmon in the Bering Sea trawl fishery averaged 48,308 salmon per year between 1992-2009 (NMFS, 2009b), but steadily increased to a peak of 121,909 in 2007 (Gisclair, 2009). The bycatch has abated in more recent years, although has coincided with a general decline in western Alaska Chinook salmon stocks. Stock composition estimates of the salmon bycatch are needed for state and federal fishery managers to understand whether the pollock fishery may be impacting salmon returns to western Alaska, however additional modifications to the sample collection protocols are needed before unbiased estimates can be produced. The results of our study and the limitations of this sample set for purposes of preparing stock composition estimates of the bycatch are summarized below.

<u>Sampling issues:</u>

Samples from the 2008 Chinook salmon bycatch were collected by the North Pacific Observer Program in an opportunistic manner as part of a "Special Project" for the Auke Bay Laboratories. Subsequently, sampling methods for the collection of genetic samples have been evaluated (Pella and Geiger, 2009) and changed for 2009. Resulting recommendations for further changes are currently being reviewed by managers at the Alaska Fisheries Science Center and additional changes are expected to be implemented in time for the 2011 fishery. *Samples collected before 2011 have the potential to be biased, the extent to which is unknown, suggesting that stock composition estimates derived from these samples should be viewed as stock composition estimates of the sample set rather than stock composition estimates of the entire Chinook salmon bycatch.*

<u>Development of efficient genotyping assays:</u>

There are many different methods used to genotype SNP markers. MALDI-TOF is a well established protocol that offers a flexible alternative for accurately genotyping samples by using

multiplexed assays (many assays performed simultaneously on one sample). MALDI-TOF is well suited for instances in which large numbers of samples are genotyped for limited numbers of SNPs (less than 200). MALDI-TOF assays have been developed for all 43 SNPs and tests show that they are highly accurate and efficient.

Evaluation of the baseline:

The ADFG Chinook salmon SNP baseline was selected for the analysis of the 2008 bycatch samples and is the same baseline previously used for the analysis of the 2005-2007 years (NMFS, 2009a). It is anticipated that this baseline will be published this year and publicly dispersed, a requirement before information derived from using this baseline is used to formulate federal public policy (NOAA Draft Data and Information Policy Directive, December 18, 2009). The ADFG SNP baseline represents 172 Chinook salmon populations distributed throughout the Pacific Rim. Our analyses suggest that this baseline can accurately discriminate the 15 reporting regions identified in this report. The reporting region for coastal western Alaska is large and efforts are underway at the Alaska Department of Fish and Game and the University of Washington to add additional markers with improved discriminatory power.

Stock composition estimates:

Western Alaska (54%) and north Alaska Peninsula (31%) Chinook salmon dominated the 2008 bycatch sample set. For this analysis, 863 samples were genotyped and stock composition estimates were prepared using both a Bayesian and maximum likelihood approach, both of which provided very similar overall estimates. Each of the BAYES estimates were derived from 15 disparate Markov chain starting points, all of which converged at the same posterior distribution. These results suggest that the genetic baseline provided criteria from which to confidently identify the 15 regional groupings of Chinook salmon.

Temporal effects on stock composition estimates of the Chinook salmon sample set:

Western Alaska fish dominated the bycatch samples derived from both the 2008 “A” (52%) and “B” (73%) groundfish seasons. In addition, a third of the fish from the “A” season were from the north Alaska Peninsula, although estimates from that region decreased significantly during the “B” season. While total Chinook salmon escapements to the north Alaska Peninsula are not fully known, they are likely less than other western Alaska river systems (e.g. Nushagak, Kuskokwim, and Yukon) suggesting the potential for higher exploitation rates on those populations.

Comparison of the 2008 Chinook salmon bycatch stock composition analysis with earlier years:

When stock estimates from the 2008 Chinook salmon bycatch samples were compared with those from previous years, they were similar in that the majority of samples were from stocks originating from river systems directly flowing into the Bering Sea with the largest estimates coming from regions located physically close to the groundfish “A” season fishery. As in previous estimates, overall contributions from the middle and upper Yukon stocks were relatively small, while lower river Yukon fish were grouped with coastal western Alaska stocks.

Future estimates:

Representative genetics sampling planned for future years should yield more precise stock composition estimates of the Chinook salmon bycatch. Also, refinements on the stock composition in time and space, and “warm” versus “cold” years, may provide information on how harvest strategies

could be changed to lessen the impact on critical stocks. In addition, other research is anticipated to test the similarity of fish taken as bycatch in individual trawls. If Chinook salmon migrate as homogeneous schools, the effects of the bycatch would be different than if all salmon are mixed and caught in proportion to the size of each population.

Acknowledgements

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Appendices

Appendix 1. Chinook salmon populations in the ADFG SNP baseline with regional designations used in the analyses of this report. The following abbreviations were used for run timing: Su (summer), Fa (fall), Wi (winter), and Sp (spring).

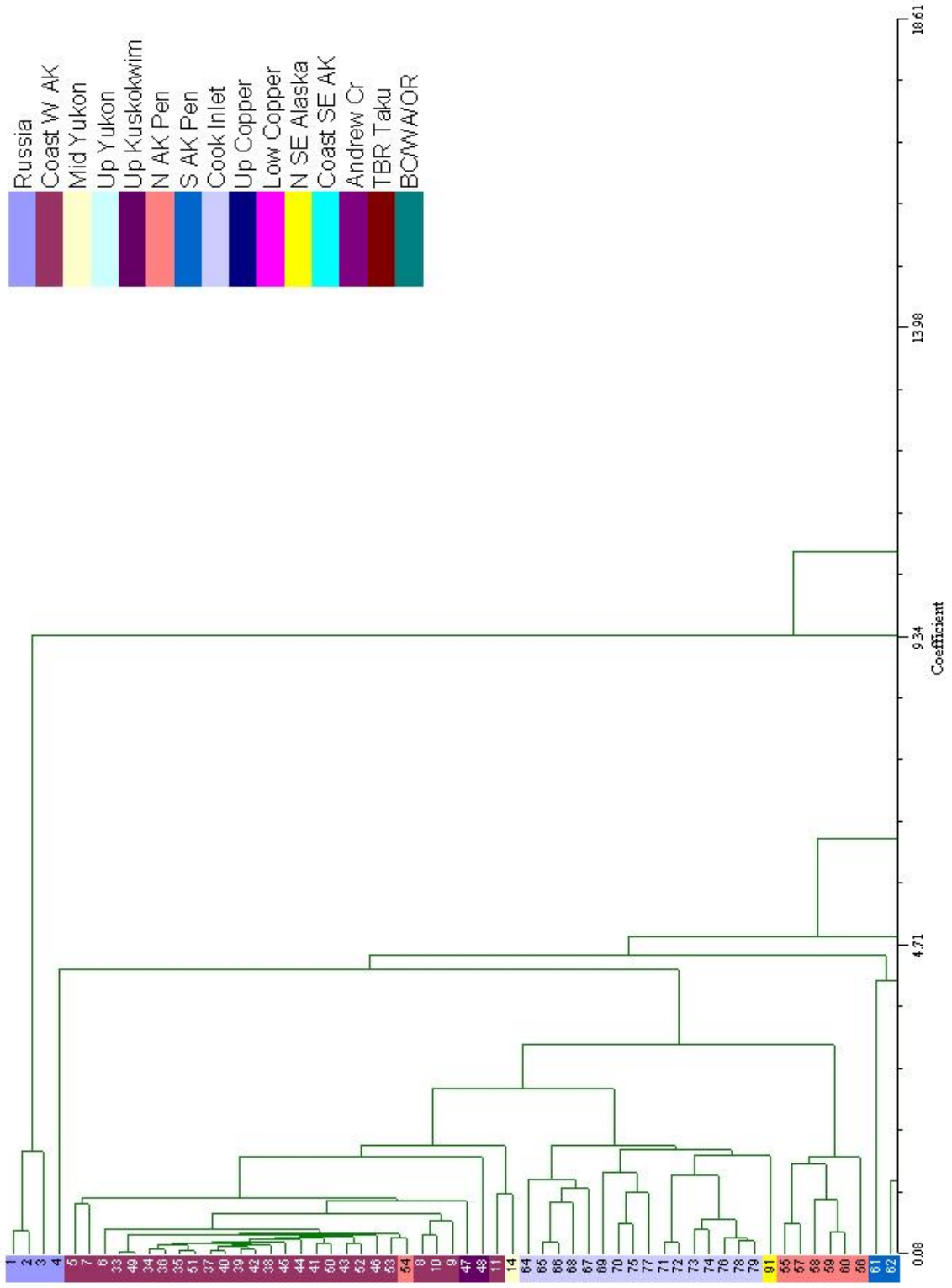
Region Number	Reporting Region	Geographic Region	Pop. No.	Location
1	Russia	Kamchatka Peninsula	1	Bistraya River
1	Russia	Kamchatka Peninsula	2	Bolshaya River
1	Russia	Kamchatka Peninsula	3	Kamchatka River late
1	Russia	Kamchatka Peninsula	4	Pakhatcha River
2	Norton Sound	Norton Sound	5	Pilgrim River
2	Norton Sound	Norton Sound	6	Unalakleet River
2	Norton Sound	Norton Sound	7	Golsovia River
2	West Coast of Alaska	Lower Yukon	8	Andreafsky River
2	West Coast of Alaska	Lower Yukon	9	Anvik River
2	West Coast of Alaska	Lower Yukon	10	Gisasa River
2	West Coast of Alaska	Lower Yukon	11	Tozitna River
2	West Coast of Alaska	Lower Kuskokwim	33	Goodnews River
2	West Coast of Alaska	Lower Kuskokwim	34	Arolik River
2	West Coast of Alaska	Lower Kuskokwim	35	Kanektok River
2	West Coast of Alaska	Lower Kuskokwim	36	Eek River
2	West Coast of Alaska	Lower Kuskokwim	37	Kwethluk River
2	West Coast of Alaska	Lower Kuskokwim	38	Kisaralik River
2	West Coast of Alaska	Lower Kuskokwim	39	Tuluksak River
2	West Coast of Alaska	Lower Kuskokwim	40	Aniak River
2	West Coast of Alaska	Lower Kuskokwim	41	George River
2	West Coast of Alaska	Lower Kuskokwim	42	Kogrukluk River
2	West Coast of Alaska	Lower Kuskokwim	43	Stony River
2	West Coast of Alaska	Lower Kuskokwim	44	Cheeneetnuk River
2	West Coast of Alaska	Lower Kuskokwim	45	Gagaryah River
2	West Coast of Alaska	Lower Kuskokwim	46	Takotna River
2	West Coast of Alaska	Bristol Bay	49	Togiak River
2	West Coast of Alaska	Bristol Bay	50	Nushagak River
2	West Coast of Alaska	Bristol Bay	51	Mulchatna River
2	West Coast of Alaska	Bristol Bay	52	Stuyahok River
2	West Coast of Alaska	Bristol Bay	53	Naknek River
3	Middle Yukon	Middle Yukon	12	Henshaw Creek
3	Middle Yukon	Middle Yukon	13	South Fork Koyukuk River
3	Middle Yukon	Middle Yukon	14	Kantishna River
3	Middle Yukon	Middle Yukon	15	Chena River
3	Middle Yukon	Middle Yukon	16	Salcha River
3	Middle Yukon	Middle Yukon	17	Beaver Creek
3	Middle Yukon	Middle Yukon	18	Chandalar River
3	Middle Yukon	Middle Yukon	19	Sheenjek River
4	Upper Yukon	Upper Yukon	20	Chandindu River
4	Upper Yukon	Upper Yukon	21	Klondike River
4	Upper Yukon	Upper Yukon	22	Stewart River

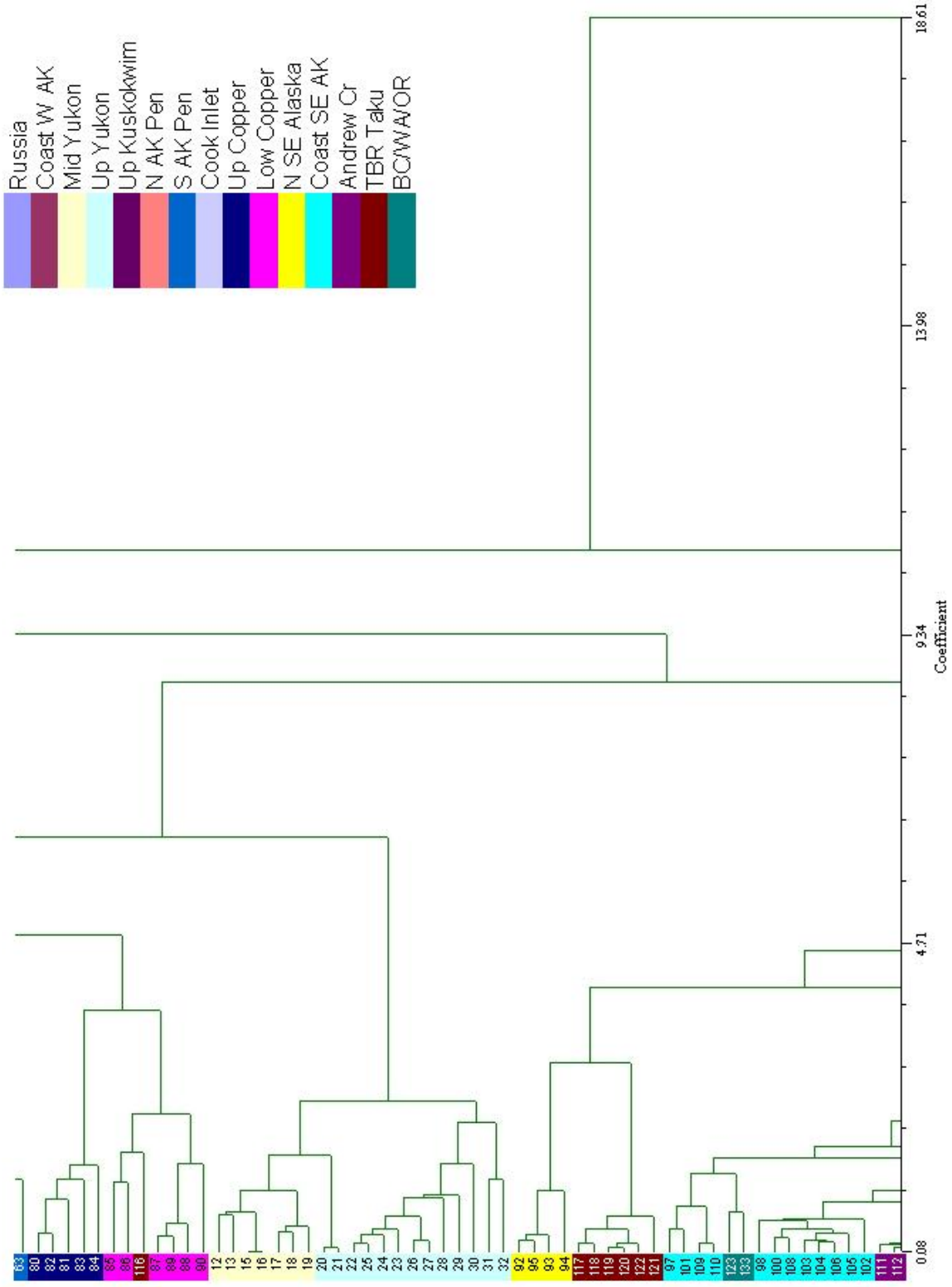
4	Upper Yukon	Upper Yukon	23	Mayo River
4	Upper Yukon	Upper Yukon	24	Blind River
4	Upper Yukon	Upper Yukon	25	Pelly River
4	Upper Yukon	Upper Yukon	26	Little Salmon River
4	Upper Yukon	Upper Yukon	27	Big Salmon River
4	Upper Yukon	Upper Yukon	28	Tatchun Creek
4	Upper Yukon	Upper Yukon	29	Nordenskiold River
4	Upper Yukon	Upper Yukon	30	Nisutlin River
4	Upper Yukon	Upper Yukon	31	Takhini River
4	Upper Yukon	Upper Yukon	32	Whitehorse Hatchery
5	Upper Kuskokwim	Upper Kuskokwim	47	Tatlawiksuk River
5	Upper Kuskokwim	Upper Kuskokwim	48	Salmon River - Pitka Fork
6	West Coast of Alaska	Bristol Bay	54	Big Creek
6	West Coast of Alaska	Bristol Bay	55	King Salmon River
6	Northern Alaska	Northern Alaska Peninsula	56	Meshik River
6	Northern Alaska	Northern Alaska Peninsula	57	Milky River
6	Northern Alaska	Northern Alaska Peninsula	58	Nelson River
6	Northern Alaska	Northern Alaska Peninsula	59	Black Hills Creek
6	Northern Alaska	Northern Alaska Peninsula	60	Steelhead Creek
7	Southern Alaska	Chignik River	61	Chignik River
7	Southern Alaska	Kodiak Island	62	Ayakulik River
7	Southern Alaska	Kodiak Island	63	Karluk River
8	Cook Inlet	Susitna River	64	Deshka River
8	Cook Inlet	Susitna River	65	Deception Creek
8	Cook Inlet	Susitna River	66	Willow Creek
8	Cook Inlet	Susitna River	67	Prairie Creek
8	Cook Inlet	Yentna River	68	Talachulitna River
8	Cook Inlet	Kenai River	69	Crescent Creek
8	Cook Inlet	Kenai River	70	Juneau Creek
8	Cook Inlet	Kenai River	71	Killey Creek
8	Cook Inlet	Kenai River	72	Benjamin Creek
8	Cook Inlet	Kenai River	73	Funny River
8	Cook Inlet	Kenai River	74	Slikok Creek
8	Cook Inlet	Kenai River	75	Kenai River mainstem
8	Cook Inlet	Kasilof River	76	Crooked Creek
8	Cook Inlet	Kasilof River	77	Kasilof River mainstem
8	Cook Inlet	Lower Kenai Peninsula	78	Anchor River
8	Cook Inlet	Lower Kenai Peninsula	79	Ninilchik River
9	Upper Copper River	Upper Copper River	80	Indian River
9	Upper Copper River	Upper Copper River	81	Bone Creek
9	Upper Copper River	Chistochina River	82	E. Fork Chistochina River
9	Upper Copper River	Upper Copper River	83	Otter Creek
9	Upper Copper River	Upper Copper River	84	Sinona Creek
10	Lower Copper River	Gulkana River	85	Gulkana River
10	Lower Copper River	Tazlina River	86	Mendeltna Creek
10	Lower Copper River	Tazlina River	87	Kiana Creek
10	Lower Copper River	Klutina River	88	Manker Creek
10	Lower Copper River	Klutina River	89	Tonsina River
10	Lower Copper River	Chitina River	90	Tebay River
11	Northern SE AK	Situk River	91	Situk River

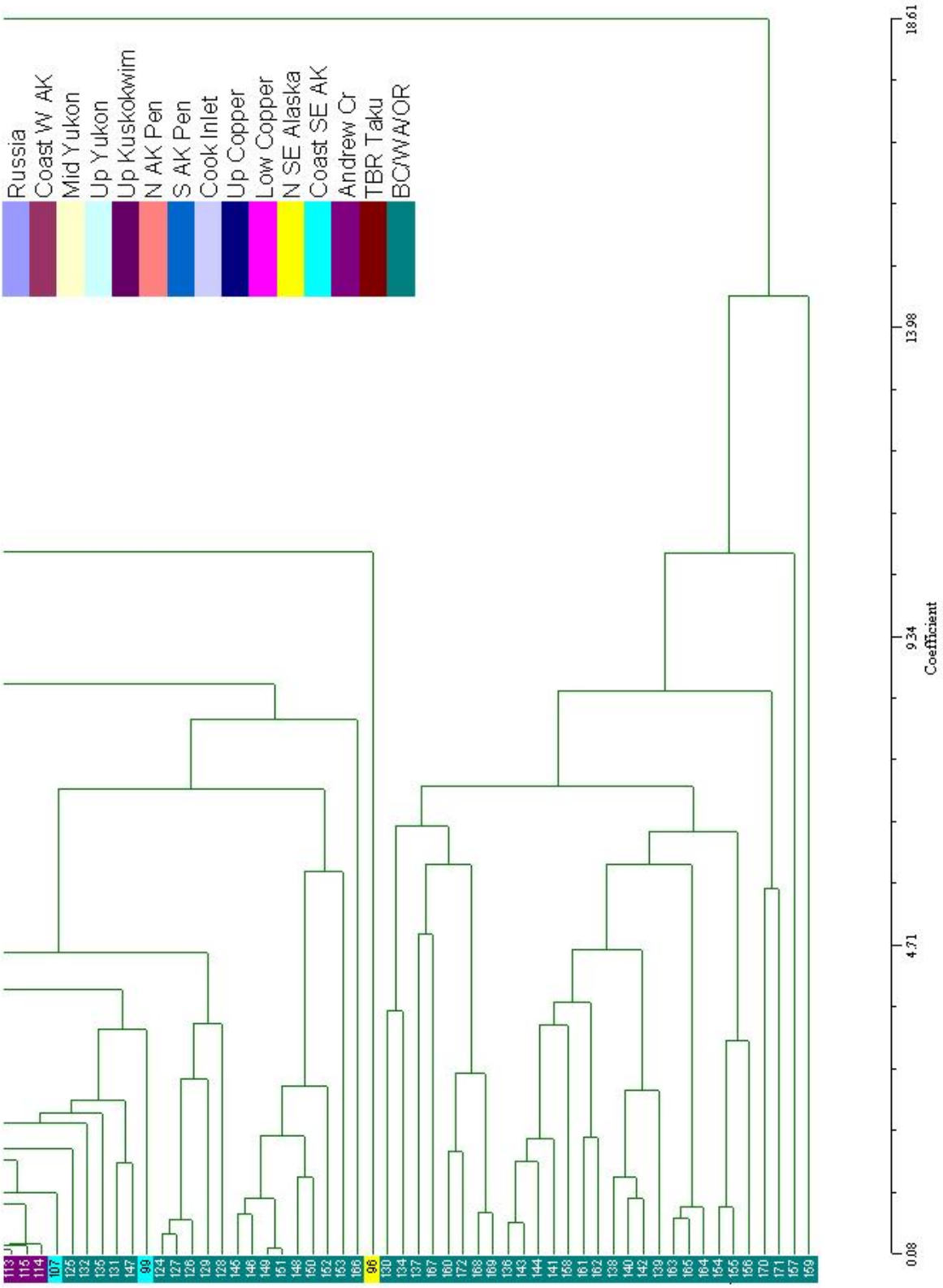
11	Northern SE AK	Chilkat River	92	Big Boulder Creek
11	Northern SE AK	Chilkat River	93	Tahini River
11	Northern SE AK	Chilkat River	94	Tahini River - Pullen Creek Hatchery
11	Northern SE AK	Chilkat River	95	Kelsall River
11	Northern SE AK	Admiralty Island	96	King Salmon River
12	Coast Southeast Alaska	Chickamin River	97	King Creek
12	Coast Southeast Alaska	Chickamin River	98	Chickamin River
12	Coast Southeast Alaska	Chickamin River	99	Chickamin River - Little Port Walter
12	Coast Southeast Alaska	Chickamin River	100	Chickamin River - Whitman Lake Hatchery
12	Coast Southeast Alaska	Chickamin River	101	Humpy Creek
12	Coast Southeast Alaska	Chickamin River	102	Butler Creek
12	Coast Southeast Alaska	Unuk River	103	Clear Creek
12	Coast Southeast Alaska	Unuk River	104	Cripple Creek
12	Coast Southeast Alaska	Unuk River	105	Genes Creek
12	Coast Southeast Alaska	Unuk River	106	Kerr Creek
12	Coast Southeast Alaska	Unuk River	107	Unuk River - Little Port Walter Hatchery
12	Coast Southeast Alaska	Unuk River	108	Unuk River - Deer Mountain Hatchery
12	Coast Southeast Alaska	Keta River	109	Keta River
12	Coast Southeast Alaska	Blossom River	110	Blossom River
13	Andrew Creek	Andrew Creek	111	Andrews Creek
13	Andrew Creek	Andrew Creek	112	Crystal Lake Hatchery
13	Andrew Creek	Andrew Creek	113	Medvejie Hatchery
13	Andrew Creek	Andrew Creek	114	Hidden Falls Hatchery
13	Andrew Creek	Andrew Creek	115	Macaulay Hatchery
14	TBR Taku	Taku River	116	Klukshu River
14	TBR Taku	Taku River	117	Kowatua River
14	TBR Taku	Taku River	118	Little Tatsemeanie River
14	TBR Taku	Taku River	119	Upper Nahlin River
14	TBR Taku	Taku River	120	Nakina River
14	TBR Taku	Taku River	121	Dudidontu River
14	TBR Taku	Stikine River	122	Tahltan River
15	BC/WA/OR/CA	North Coast BC	123	Kateen River
15	BC/WA/OR/CA	Nass River	124	Damdochax Creek
15	BC/WA/OR/CA	Nass River	125	Kincolith Creek
15	BC/WA/OR/CA	Nass River	126	Kwinageese Creek
15	BC/WA/OR/CA	Nass River	127	Oweegee Creek
15	BC/WA/OR/CA	Upper Skeena River	128	Bulkley River
15	BC/WA/OR/CA	Upper Skeena River	129	Sustut River
15	BC/WA/OR/CA	Lower Skena River	130	Ecstall River
15	BC/WA/OR/CA	Lower Skena River	131	Lower Kalum River
15	BC/WA/OR/CA	Central BC Coast	132	Lower Atnarko River
15	BC/WA/OR/CA	Central BC Coast	133	Kitimat River
15	BC/WA/OR/CA	Central BC Coast	134	Wannock River
15	BC/WA/OR/CA	South BC Mainland	135	Klinaklini River
15	BC/WA/OR/CA	South BC Mainland	137	Porteau Cove
15	BC/WA/OR/CA	West Vancouver Island	138	Conuma River
15	BC/WA/OR/CA	West Vancouver Island	139	Marble Creek

15	BC/WA/OR/CA	West Vancouver Island	140	Nitinat River
15	BC/WA/OR/CA	West Vancouver Island	141	Robertson Creek
15	BC/WA/OR/CA	West Vancouver Island	142	Sarita River
15	BC/WA/OR/CA	East Vancouver Island	143	Big Qualicum River
15	BC/WA/OR/CA	East Vancouver Island	136	Nanaimo River
15	BC/WA/OR/CA	East Vancouver Island	144	Quinsam River
15	BC/WA/OR/CA	Upper Fraser River	145	Morkill River (Su)
15	BC/WA/OR/CA	Upper Fraser River	146	Salmon River (Su)
15	BC/WA/OR/CA	Upper Fraser River	147	Torpy River (Su)
15	BC/WA/OR/CA	Middle Fraser River	148	Chilko River (Su)
15	BC/WA/OR/CA	Middle Fraser River	149	Nechako River (Su)
15	BC/WA/OR/CA	Middle Fraser River	150	Quesnel River (Su)
15	BC/WA/OR/CA	Middle Fraser River	151	Stuart River (Su)
15	BC/WA/OR/CA	North Thompson River	152	Clearwater River (Su)
15	BC/WA/OR/CA	North Thompson River	153	Louis River (Sp)
15	BC/WA/OR/CA	South Thompson River	154	Lower Adams River (Fa)
15	BC/WA/OR/CA	South Thompson River	155	Lower Thompson River (Fa)
15	BC/WA/OR/CA	South Thompson River	156	Middle Shuswap River (Su)
15	BC/WA/OR/CA	Lower Fraser River	157	Birkenhead River (Sp)
15	BC/WA/OR/CA	Lower Fraser River	158	Harrison River
15	BC/WA/OR/CA	Puget Sound	159	Makah National Fish Hatchery (Fa)
15	BC/WA/OR/CA	Puget Sound	160	Forks Creek (Fa)
15	BC/WA/OR/CA	Puget Sound	161	Upper Skagit River (Su)
15	BC/WA/OR/CA	Puget Sound	162	Soos Creek Hatchery (Fa)
15	BC/WA/OR/CA	Snake River	163	Lyons Ferry Hatchery (Su/Fa)
15	BC/WA/OR/CA	Upper Columbia	164	Hanford Reach
15	BC/WA/OR/CA	Deschutes River	165	Lower Deschutes River (Fa)
15	BC/WA/OR/CA	Mid Upper Columbia	166	Carson Hatchery (Sp)
15	BC/WA/OR/CA	Willamette River	167	McKenzie River (Sp)
15	BC/WA/OR/CA	Oregon Coast	168	Alsea River (Fa)
15	BC/WA/OR/CA	Oregon Coast	169	Siuslaw River (Fa)
15	BC/WA/OR/CA	California	170	Klamath River
15	BC/WA/OR/CA	California	171	Eel River (Fa)
15	BC/WA/OR/CA	California	172	Sacramento River (Wi)

Appendix 2. UPGMA dendrogram based on Cavalli-Sforza and Edwards chord distances for the Chinook salmon populations represented in the ADFG SNP genetic baseline (3 pages).




Russia
Coast W AK
Mid Yukon
Up Yukon
Up Kuskokwim
N AK Pen
S AK Pen
Cook Inlet
Up Copper
Low Copper
N SE Alaska
Coast SE AK
Andrew Cr
TBR Taku
BC/WA/OR
18.61
13.98
9.34
4.71
0.08
Coefficient
79 80 82 81 83 84 85 86 116 87 89 88 90 12 13 15 16 17 18 19 20 21 22 25 24 23 26 27 28 29 30 31 32 92 95 93 94 117 118 119 120 122 121 97 101 109 110 123 133 98 100 108 103 104 106 105 102 111 112


Russia
Coast W AK
Mid Yukon
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