

Report to the North Pacific Fishery Management Council

**Genetic Stock Composition Analysis of Chum Salmon Bycatch Samples from the 2005
Bering Sea Groundfish Fishery**

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Introduction

The Bering Sea provides habitat for chum salmon from many populations throughout their geographic range during their residence in the marine environment (Abe et al., 2007; Friedland et al., 2001). In some years, large numbers of chum salmon are incidentally caught as bycatch in the Bering Sea trawl fishery for walleye pollock (Stram and Ianelli, 2009; Witherell et al., 2002). When escapement of chum salmon in several western Alaska areas declined in the early 1990s (Eggers, 1995), the incidental chum salmon harvest in the trawl fishery became of concern. This led to changes in the management of that fishery (Ackley, 1997) and the first genetic stock identification analyses of chum salmon bycatch. It was during this time that many fishery agencies worked to develop coastwide genetic baselines that could be used to estimate the stock contributions to mixtures of fish such as chum salmon (Seeb et al., 1995). The Auke Bay Laboratory analyzed samples from three years in the mid-1990s to estimate the regional contribution of chum salmon stocks to the bycatch. More recently, numbers of chum salmon caught as bycatch in the Bering Sea groundfish fishery have increased to a high of over 700,000 fish in 2005. This report presents preliminary genetic stock identification results for a subset of samples collected in 2005.

The first genetic analysis of chum salmon bycatch was completed for the 1994 and 1995 summer/fall B-season walleye pollock fishery (Wilmot et al., 1998). This study used a genetic baseline of 77 populations surveyed for 20 allozyme loci. Based on a sample set of 457 chum salmon caught in the 1994 B-season pollock fishery, the stock composition of the chum salmon samples was partitioned to Asia (39-55%), western Alaska (20-35%), and southeast Alaska, British Columbia, and Washington (21-29%). Based on a larger sample set of 1,853 chum salmon harvested from the 1995 "B" fishery (11% of the total bycatch), stock estimates were partitioned to Asia (13-51%), western Alaska (33-53%), and southeast Alaska, British Columbia, and Washington (9-46%). The range of estimates reflects differences in the stocks present during different time periods and areas of capture in the fishery.

The second genetic analysis was completed by the Alaska Fisheries Science Center's Auke Bay Laboratory for the 1996 groundfish fishery (Seeb et al., 2004). In this analysis, a baseline representing 356 populations assayed for 20 allozyme markers was used. Nearly 3,000 chum salmon were collected from the eastern fishing districts, where approximately half of the catch in the 1996 B-season fishery occurred. The stock composition estimates for this section of the fishery were partitioned to Asia (30%), western Alaska (16%), Alaska Peninsula, Prince William Sound, and Kodiak (12%), and southeast Alaska, British Columbia, and Washington (42%).

In addition to these genetic analyses, two studies examined scale patterns to investigate the contribution of stocks to the chum salmon bycatch. In one study, scale analysis was used to age chum salmon from the 1993 B-season bycatch (Myers et al., 1994). The proportion of ages represented were 0.2 (22%), 0.3 (65%), 0.4 (12%), and 0.5 (1%). While a specific stock composition analysis was not completed for that particular study, many characteristics showed stratification of chum stocks in the Bering Sea including (1) reduced amount of growth in the 3rd year (a characteristic of Asian fish), and (2) differences in age of the affected fish based on the month and area in which fish were collected. In the second study, a scale pattern analysis (SPA) was used to estimate the stock composition of the 1994 chum bycatch. Based on SPA of 1,204 age 0.3 fish, the stock estimation of the sample set was partitioned to Asia (50%), western and central Alaska (18%), and SE Alaska, British Columbia and

Washington (32%) (Patton et al., 1998). As in the genetic studies, the stock composition estimates from SPA varied by date and statistical area.

Presented below are stock composition estimates for a subset of chum salmon bycatch samples from the 2005 Bering Sea groundfish fishery. This is the first analysis of chum salmon bycatch samples that utilizes DNA-based genetic markers. Genetic samples of the chum salmon bycatch were collected in 2005 from the North Pacific groundfish fishery as part of a Special Project. A subset of these samples, supplemented with available scales, was used for an Arctic/Yukon/Kuskokwim Sustainable Salmon Initiative (AYKSSI) funded project to determine the spatial and temporal distribution of chum salmon in the Bering Sea. Four distinct areas in the eastern Bering Sea were identified such that samples within those areas could be pooled and stock composition estimates compared. Whereas potential sample biases within the genetic samples of the bycatch are well documented (Pella and Geiger, 2009), the samples analyzed for the 2005 study were specifically selected with regard for their spatial and temporal distributions rather than by quantity of overall bycatch. With that caveat, a subset of over a thousand 2005 samples was analyzed and the resulting stock composition estimates were similar to those produced previously using allozymes or scale pattern analysis. Despite the issues associated with sample collection bias, the analysis of the 2005 chum bycatch samples provides a rough measure of stock distribution, and at a minimum, provides an indication of the presence and/or absence of specific stocks.

The goal of this report is to present a stock composition estimate for the 2005 AYKSSI chum bycatch samples, but it is important to understand the limitations for making accurate estimates of the entire bycatch imposed by the sampling protocols and the genetic baseline. Hence, this report is divided into three main sections. First, the sampling protocols are documented and the distribution of the AYKSSI genetic samples is compared to the overall chum bycatch (designated as non-Chinook in the NMFS database as chum salmon comprise over 99.6% of the total non-Chinook bycatch (NPFMC, 2005)). Second, the efficacy of the microsatellite DNA baseline is evaluated using principal coordinate analyses based on genetic distances, simulation studies of hypothetical mixtures, and the available phylogenetic trees. Finally, stock composition estimates are provided as a composite of all available samples as well as from three distinct time periods to determine if there continues to be a temporal effect on the composition of the bycatch.

Methods

Sample collection and DNA isolation

All samples were collected by the Alaska Fisheries Science Center's (AFSC) North Pacific Observer Program as part of either a Special Project (designated "Salmon Genetic Project" in 2005) for the Auke Bay Laboratory for genetic analysis (axillary processes) or for species identification/aging purposes (scales) (Figure 1, Table 1). Axillary processes and scales for aging 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 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 (scales slightly less than axillary process tissue) and was stored at -20 °C.

Data acquisition

Genotypes were obtained for 11 microsatellite DNA markers. First, 1 uL of a 1:4 dilution of extracted DNA was transferred to 384-well plates.² Then, the microsatellite loci were polymerase chain reaction (PCR) amplified in four multiplexed panels. Each PCR reaction was conducted in a 5 ul volume containing the template DNA, QIAGEN Multiplex PCR Mastermix, 0.2 uM of each primer, and RNase-free water. Primer sequences for the 11 loci have been described in the following publications: *Oki100* (Beacham et al., 2009a), *Omm1070* (Rexroad et al., 2001), *Omy1011* (Spies et al., 2005), *One101*, *One102*, *One104*, *One114* (Olsen et al., 2000), *Ots103* (Nelson and Beacham, 1999), *Ots3* (Banks et al., 1999), *Ots68* (Williamson et al., 2002), and *Ssa419* (Cairney et al., 2000). Thermal cycling for the PCR was performed on a dual 384-well GeneAmp PCR System 9700 (Applied Biosystems, Inc.) with the following protocol: initial denaturation at 95°C for 15 minutes, then 33 cycles at 94°C for 30 seconds, 60°C for 1.5 minutes, and polymerization at 72°C for 1 minute, followed by a final polymerization step at 60°C for 30 minutes and then storage at 15°C until removal from the thermocycler.

Samples from the PCR reactions were diluted into 96-well plates for analysis with a 16-capillary, 36 cm array on the ABI 3130xl Genetic Analyzer as follows: 1 ul diluted (1:25) PCR product, 4.4 ul Hi-Di formamide, 4.4 ul ddH₂O, 0.2 ul LIZ 600 size standard (Applied Biosystems, Inc.). Samples were denatured for 3 minutes at 95°C, then cooled to 4°C and stored until analysis on the 3130xl. Genotypes were identified with GeneMapper software (Applied Biosystems, Inc.) and exported to Excel spreadsheets (Microsoft, Inc.) for further analysis.

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

Baseline allele frequencies were downloaded from the Division of Fisheries and Oceans Canada (DFO) Molecular Genetics web page (http://www-sci.pac.dfo-mpo.gc.ca/mgl/data_e.htm) and a SPAM (ADFG, 2003) baseline file was created within Excel. To generate the BAYES baseline, a program was written in C to convert the allele frequencies into allele counts for the BAYES format. For the mixture files, allele designations were converted to match those in the baseline. Compatibility of our allele designations to the DFO baseline was confirmed with a set of samples from the DFO Molecular Genetics Lab that were analyzed on the Auke Bay Laboratory's Applied Biosystems 3130xl Genetic Analyzer. Lookup tables were generated within Excel to convert our allele calls to match those in the DFO baseline. Genotypes from converted mixtures were then exported from Excel as text files and C programs were used to format the data into both SPAM and BAYES mixture files. Stock composition analysis was performed with both the SPAM and BAYES software by using previously published procedures (ADFG, 2003; Pella and Masuda, 2001).

Principal coordinate analysis (PCO) and baseline evaluation

The baseline was examined to determine major regional groupings of populations that would then be used for stock identification analyses of the chum salmon mixtures. Larger reporting groups were used to increase estimation accuracy and to compare estimates with those from previous studies. Population genetic structure was examined in three ways. First, population groupings were evaluated

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

² Most liquid handling steps were performed with a Perkin-Elmer Janus AJL8M01 Robot.

based on the published neighbor-joining dendrogram of Cavalli-Sforza and Edwards chord distances (Beacham et al., 2009b). Second, Nei's genetic distance was calculated in the software NT-SYS (Applied Biostatistics, Inc.) from the allele frequencies of the baseline populations. Population structure was examined using a principal coordinate analysis (PCO) and resulting eigenvalues were plotted in 3-dimensional graphs. Third, baseline simulation studies were performed to evaluate the effectiveness of the baseline to allocate stocks to the correct regions. Three different types of simulation tests were performed with SPAM software (Version 3.7) by using hypothetical mixtures of 400 fish containing either 100%, equal or selected stock proportions as described in the text. In these simulations, the hypothetical mixtures were derived from the appropriate regions and then re-evaluated with the baseline to determine the percentage that reallocated back to the correct region.

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

Stock identification results presented in this report are from a subset of samples collected from the 2005 chum bycatch that was specifically selected for an AYKSSI project to address the spatial and temporal distributions of chum salmon in the Bering Sea rather than provide an overall stock composition estimate of the bycatch. Because the sampling was not proportional with the bycatch, there may be bias in the overall stock composition estimate of the chum salmon bycatch for reasons of variable spatial and temporal sampling rates. In total, 1,084 samples were genetically analyzed from a total chum bycatch of 705,963 fish, which is an overall sample rate of 0.15%.

For the AYKSSI project, samples from four regions were selected to examine possible temporal and spatial differences (Figure 1, Table 1); however, they were not in proportion to the total catch throughout the season. Potential temporal biases in the AYKSSI sample set are observed as differences in proportionality to the catch (Figure 2). For example, the peak of the bycatch was in statistical weeks 30-32 (Figure 2, top panel), while the majority of samples analyzed for stock composition were taken in statistical weeks 25-27 and 36-38 (Figure 2, bottom panel), on the shoulders of the primary take in the bycatch. Later, we present a stock composition estimate for the entire set of genetic samples analyzed as well as composition estimates for subsets of samples taken over time to determine the significance of temporal sampling on the composition estimate.

Figure 1. The salmon bycatch areas used in the AYKSSI project to determine the spatial distribution of chum salmon in the Bering Sea. The open circle designates the area of highest chum bycatch in 2005 (NPFMC Bering Sea Chum salmon discussion paper, October 2008).

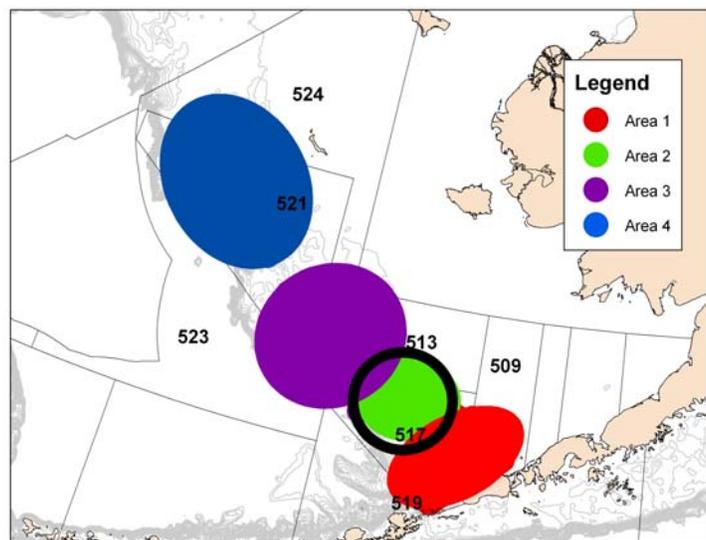
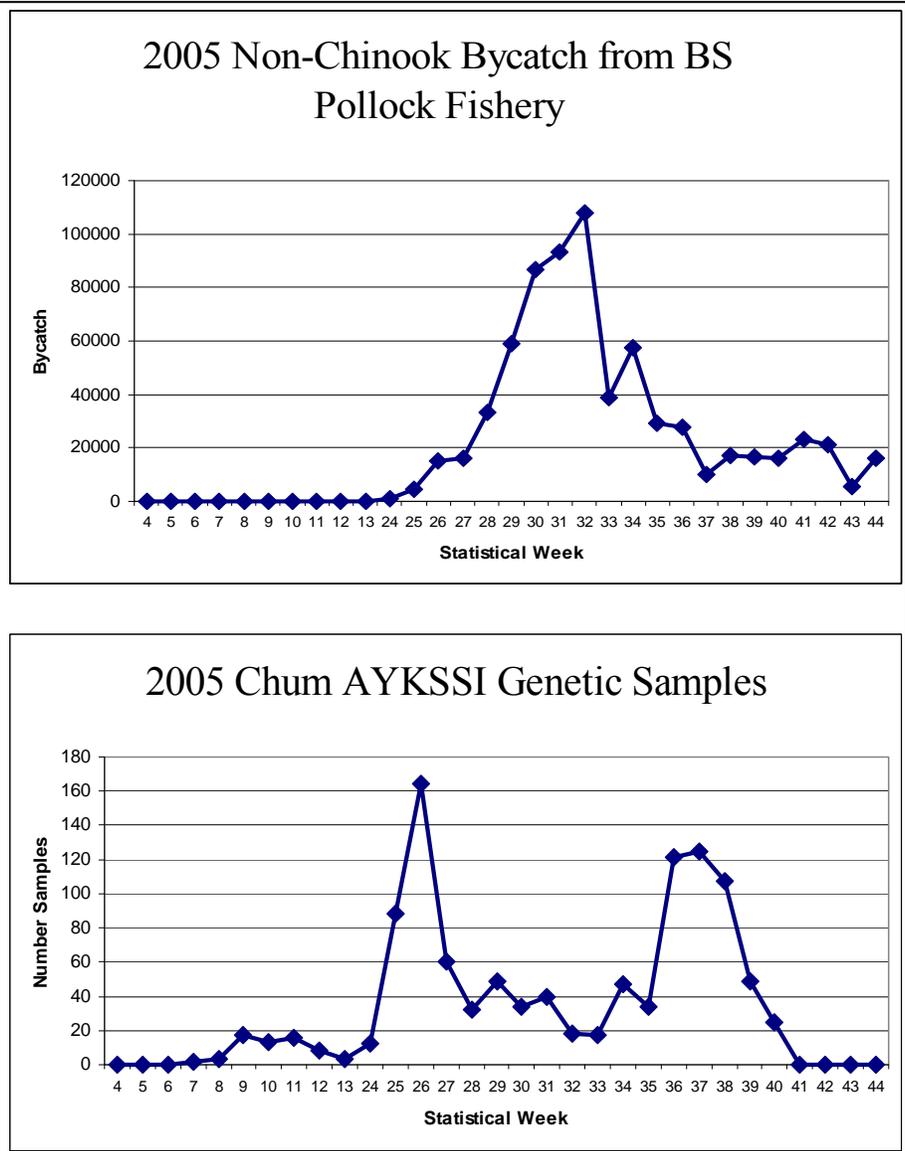


Table 1. Total number of analyzed genetic samples from the 2005 chum salmon bycatch grouped by areas designated in the AYKSSI project.

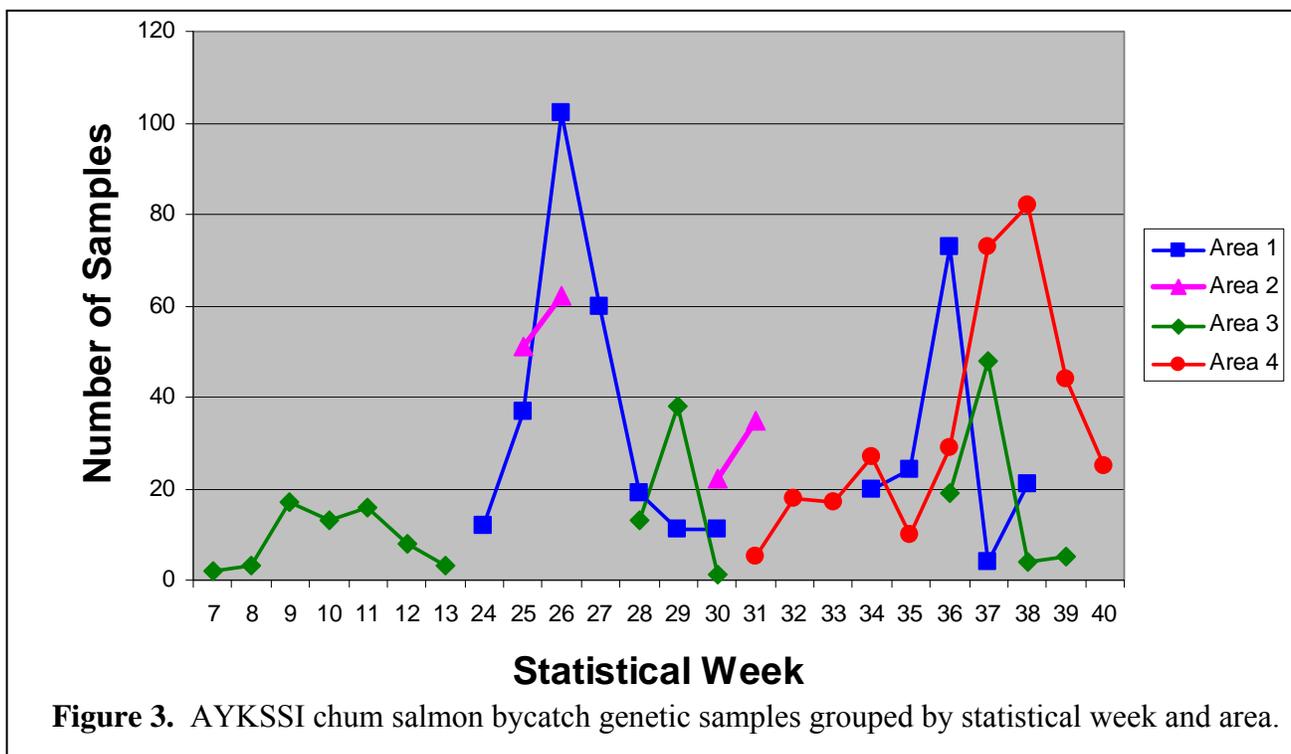
Area	Total
1	394
2	170
3	190
4	330
Total	1084

Figure 2. 2005 chum salmon bycatch and AYKSSI genetic samples graphed by statistical week. Total number of chum bycatch (top panel) compared with the AYKSSI samples genotyped from the 2005 bycatch (bottom panel).



In addition to a temporal stratification, samples were also selected for the AYKSSI project for evaluating stock differences within the four specific areas of the Bering Sea, three of which are outside the area of peak chum salmon bycatch in 2005 (Figure 1, compare circle with AYKSSI sample areas). Only AYKSSI Area 2 closely coincides with the peak bycatch location, suggesting that stock

composition estimates for that one area could be more representative of the overall bycatch than the composition estimate from all four areas. In the selected AYKSSI sample set for 2005, the smallest number of genetic samples came from Area 2 (Table 1). This combination of potential spatial and temporal biases can be visualized in the AYKSSI sample set by plotting the numbers of samples collected per area per week (Figure 3). The peaks highlight the temporal and spatial groupings that can be compared in the AYKSSI project. For example, Areas 1, 3, and 4 can be compared for weeks 35-38 to examine spatial distribution, while Area 1 can be compared for weeks 25-28 and weeks 35-37 to examine temporal distribution. Results from the AYKSSI project are anticipated in the spring of 2011. In contrast to the 2005 AYKSSI samples, genetic samples collected for the analysis of the 2006-2009 chum bycatch were not subsampled, although significant bias in those sample sets may still exist (Pella and Geiger, 2009) highlighting the need for representative sampling for future analyses. Potential biases in the 2005 AYKSSI sample set indicate that care should be taken when interpreting overall bycatch stock composition results with these samples, but, at a minimum, the presence or absence of specific stocks can be identified.

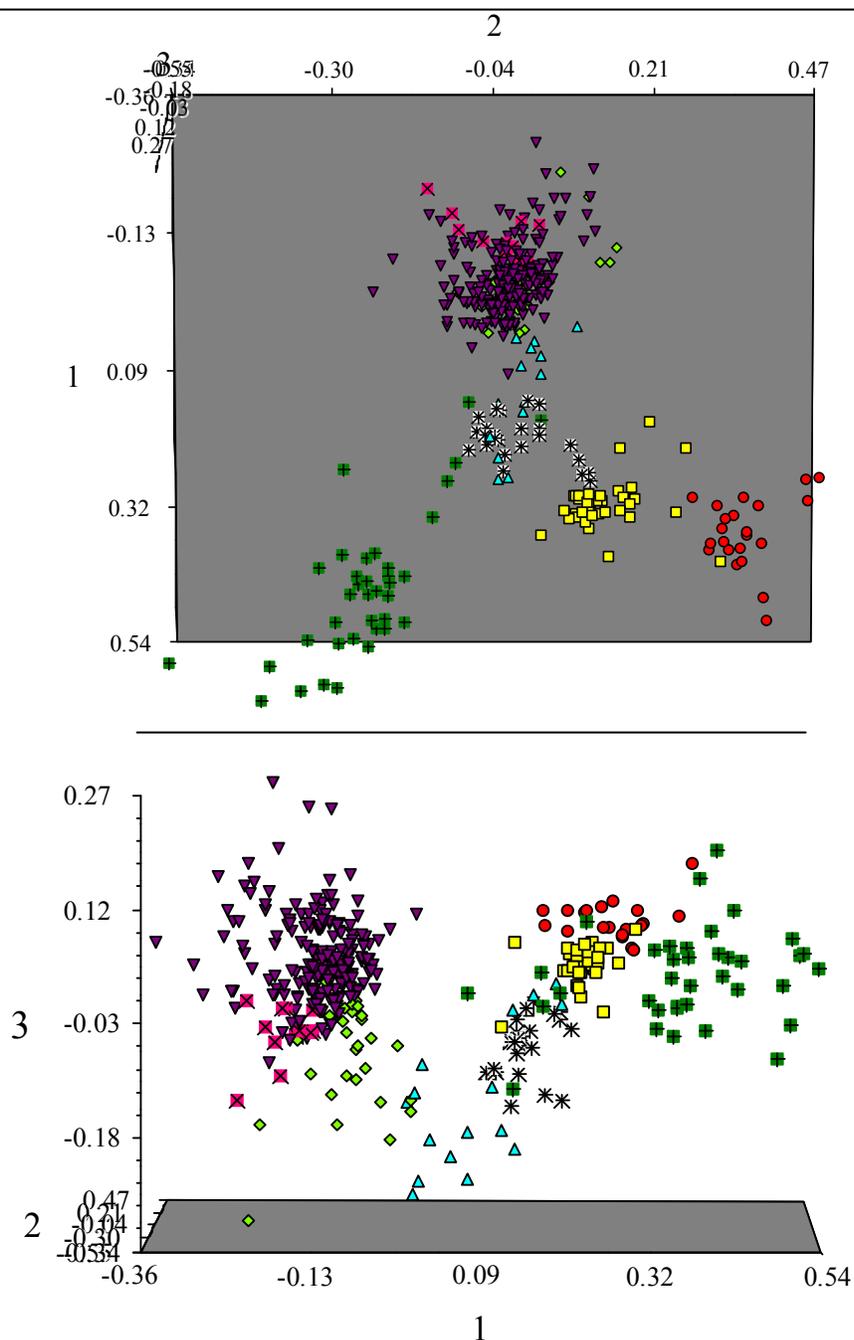


Evaluation and adequacy of the baseline

A microsatellite DNA baseline representative of chum salmon populations from throughout the entire Pacific Rim has recently been published, is available for anonymous download, and has been selected for the analysis of the 2005 AYKSSI chum bycatch samples. This baseline contains 381 populations of chum salmon (see Appendix 1 for stream origins) assayed for 14 microsatellite markers (Beacham et al., 2009b). For our analysis, 11 of the markers were used: *Oki100*, *Omm1070*, *Omy1011*, *One101*, *One102*, *One104*, *One114*, *Ots103*, *Ots3*, *Ots68*, and *Ssa419*; while *Oki2* and *One111* may be available in future analyses, pending optimization. Attempts to optimize the final loci, *Oke3*, have been unsuccessful.

To determine the ability of the 11 microsatellite markers to discriminate population structure, two different descriptive analyses were used. First, regional groupings were approximated using the published neighbor-joining dendrogram of Cavalli-Sforza and Edwards chord distances (Beacham et al., 2009b). Second, PCO was used based on Nei's genetic distance calculated from the allele frequencies of the baseline populations. By using all 381 populations in the baseline, PCO showed one population to be much different from the others (Sturgeon River on Kodiak Island) and it was excluded from further analysis to better highlight regional separations (Figure 4), although it was retained in the baseline in the Alaska Peninsula region for the stock composition analyses.

Figure 4. Principal coordinate analysis of 380 chum populations analyzed for 11 microsatellite markers. Eigenvalues were plotted in 3 dimensional space with “1” being the most informative (55.0%), “2” the second most (25.7%), and “3” the least (9.7%). Top panel shows dimensions 1 and 2 (most informative), bottom panel shows dimensions 1 and 3. Populations are designated with the following symbols: Japan/S. Russia (green plus signs), Russia (stars), Upper/Middle Yukon (red circles), Western Alaska (yellow squares), Alaska Peninsula (light blue triangles), Southeast Alaska/Northern BC (light green diamonds), British Columbia/Washington (purple down-triangles), and Skeena (magenta crosses).



From the PCO and the neighbor-joining dendrogram, the following eight regional groupings were apparent: Russia, Japan/Korea/China/S. Russia (designated “Japan/S. Russia”), Upper/Middle Yukon, coastal western Alaska (designated “Western Alaska”), Alaska Peninsula, SE Alaska/Prince William Sound/N. British Columbia (designated “SE Alaska/N. BC”), Skeena, and British Columbia/Washington (designated “BC/Washington”). Most regional groupings were clearly delineated although some were more distinct than others. For example, the Skeena region is a small group of populations that clusters on the neighbor-joining tree, but it is hard to discriminate in the PCO from the other British Columbia stocks, whereas the Upper/Middle Yukon stocks are clearly distinguishable from the Western Alaska stocks (which includes the lower Yukon). These 8 regional groupings were used for all further analyses in this report. The individual populations and the associated groupings are identified in Appendix 1.

To evaluate the ability of the 11 markers to effectively separate the 8 regional groupings in mixed-stock analyses, three simulation studies were performed in which fish from a hypothetical mixture were partitioned back to their respective regions. All simulations were performed with SPAM software. In the first simulation, an equal number of fish from each region (or 12.5% from each region) were used generate a hypothetical mixture of 400 fish. If this mixture correctly reallocates to the appropriate regions, each region would contribute 12.5% of the total. Four regions allocated to within one standard deviation of the known contribution, whereas the BC/Washington and Western Alaska regions were slightly overestimated and the Alaska Peninsula and Skeena regions were slightly underestimated (Figure 5, top panel). Previous studies have shown that many chum salmon in the Bering Sea originate from Japan/S. Russia, Russia, Western Alaska, and BC/Washington (Patton et al., 1998; Seeb et al., 2004; Wilmot et al., 1998). To test the efficacy of the baseline to distinguish between these four regional groupings, a simulated mixture was analyzed containing 25% from each of these four regions. Simulation estimates reallocate the fish back to their respective region within one standard deviation of the expected contribution for Western Alaska, BC/Washington, and Japan/S. Russia, whereas the estimate for Russia was slightly less than expected (Figure 5, bottom panel).

As a final measure of the ability of the baseline to discriminate the eight individual regions, 100% simulation studies were completed in which all samples of a hypothetical mixture were from one region and that mixture was re-evaluated against the baseline to determine the percentage reallocating back to the correct region. This analysis was completed for all 8 regions (Table 2). Upper/Middle Yukon, Western Alaska, BC/Washington, Skeena, and Japan/S. Russia all allocated back to the correct region with 87-96% accuracy whereas 83% correctly reallocated to the Russia region (5% misclassified to Western Alaska), 82% correctly reallocated to the Alaska Peninsula region, and 77% correctly reallocated back to the Southeast Alaska/N. BC region (17% misclassified to BC/Washington). These results corroborate those from the previous simulation studies (Figure 5) and suggest that stock composition estimates derived from the use of this baseline may overestimate the numbers of BC/Washington fish and underestimate the numbers of fish from the Alaska Peninsula and SE Alaska/N. BC, two areas with relatively small contributions to the overall bycatch (Patton et al., 1998; Seeb et al., 2004; Wilmot et al., 1998). The overestimation of the BC/Washington region may be due to the increased variability in that large group of populations (see PCO analysis, Figure 4).

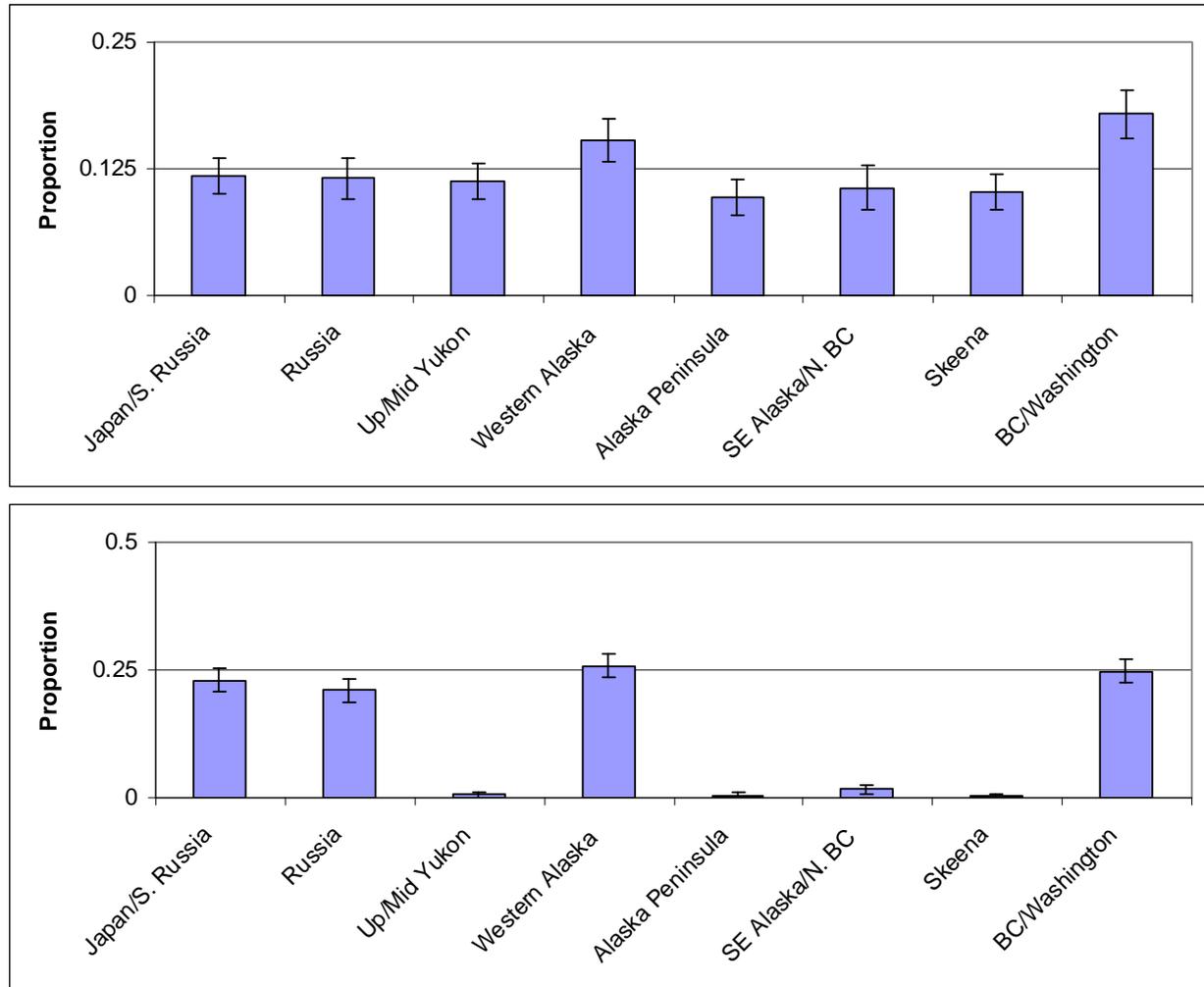


Figure 5. Baseline evaluation - results from mixed stock simulation experiments. Mixtures of fish were derived from equal proportions of all regions (top panel) and for only the Western Alaska, BC/Washington, Japan/S. Russia, and Russia regions (bottom panel). Stock compositions of the hypothetical mixtures were allocated back to the region with SPAM software based on the characteristics in the genetic baseline. Standard deviations are shown for all estimates.

The simulation results indicate that the characteristics in the 11 marker microsatellite genetic baseline describe relatively strong population structure suitable for use in performing stock composition estimates from stock mixtures, especially those that contain chum salmon originating from regions encompassing the entire Pacific Rim. In addition to the eleven microsatellite markers used in our study, the published microsatellite baseline contains an additional three loci that, if optimized, may improve estimation accuracies. Additionally, at least two other genetic baselines are currently being developed, both of which utilize single nucleotide polymorphism (SNP) markers. Once reviewed, published, and made publicly available, those baselines may be used in future analyses if found to be more effective in identifying stock origins.

Table 2. Results from simulation studies in which 100% of a hypothetical mixture of 400 fish was derived from one region (columns) and reallocated back to the region (rows) with SPAM software. The fraction of fish from each region is designated.

Region	<i>Japan</i>	<i>Russia</i>	<i>U. Yukon</i>	<i>W. AK</i>	<i>AK Penn</i>	<i>SE AK</i>	<i>Skeena</i>	<i>BC/Wash</i>
<i>Japan/S.Russia</i>	0.873	0.041	0.001	0.003	0.015	0.005	0.001	0.002
<i>Russia</i>	0.036	0.835	0.002	0.009	0.047	0.019	0.002	0.005
<i>Upper/Middle Yukon</i>	0.000	0.002	0.934	0.010	0.001	0.000	0.000	0.000
<i>Western Alaska</i>	0.008	0.055	0.059	0.960	0.040	0.005	0.001	0.002
<i>Alaska Peninsula</i>	0.002	0.007	0.000	0.004	0.819	0.010	0.002	0.002
<i>SE Alaska/N. BC</i>	0.003	0.010	0.000	0.002	0.029	0.770	0.039	0.038
<i>Skeena</i>	0.000	0.001	0.000	0.000	0.002	0.009	0.874	0.007
<i>BC/Washington</i>	0.013	0.034	0.001	0.008	0.040	0.173	0.078	0.936

Stock composition analyses, including temporal trends

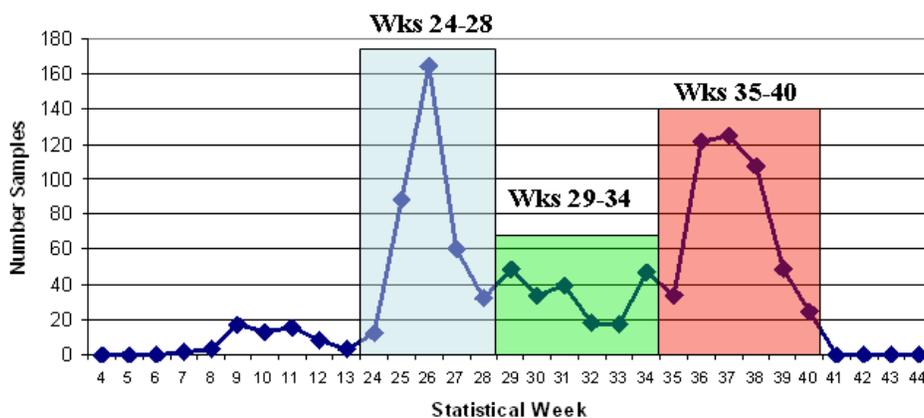
Stock composition analysis of all samples

Stock origin of the 1,084 genetic samples (genotyped for 11 microsatellite markers) was determined to be primarily of Asian origin, while the most represented fish from North America were primarily from Western Alaska and British Columbia/Washington (Table 3). The samples had relatively complete data with 787 samples missing no data, 44 missing one marker, 246 missing two markers, 2 missing three markers, and 5 missing four markers. Stock composition estimates were derived by using both the SPAM and BAYES software and yielded almost identical stock composition estimates (Table 3). BAYES software uses a Bayesian algorithm to produce stock composition estimates and can account for missing alleles in the baseline (Pella and Masuda, 2001), something considered critical for microsatellite baselines with loci containing multiple alleles derived from a limited number of samples from each baseline population. 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

Table 3. Regional SPAM and BAYES stock composition estimates for the 1,084 chum salmon samples from the 2005 AYKSSI sample set. SE is the SPAM standard error. SD is the BAYES standard deviation. The 95% credible interval is provided for all BAYES estimates.

Region	SPAM		BAYES				
	Estimate	SE	Mean	SD	2.50%	Median	97.50%
Japan/S. Russia	0.281	0.009	0.292	0.015	0.264	0.292	0.322
Russia	0.253	0.008	0.289	0.018	0.255	0.289	0.325
Upper/Middle Yukon	0.057	0.002	0.052	0.010	0.034	0.051	0.074
Western Alaska	0.166	0.005	0.162	0.015	0.132	0.161	0.192
Alaska Peninsula	0.022	0.001	0.015	0.005	0.007	0.014	0.026
SE Alaska/N. BC	0.037	0.001	0.032	0.009	0.015	0.031	0.052
Skeena	0.000	0.000	0.001	0.002	0.000	0.000	0.006
BC/Washington	0.172	0.005	0.158	0.014	0.131	0.158	0.185

options were not utilized for the stock composition analyses. For each BAYES analysis, eight Monte Carlo chains starting at disparate starting 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. 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 the Gelman and Rubin shrink statistics which were all less than 1.15 conveying strong convergence to a single posterior distribution (Pella and Masuda, 2001).



Mixtures	2005 Dates	Number of Fish
Week 24-28	June 11 - July 6	356
Week 29-34	July 12 - August 18	205
Week 35-40	August 23 - September 29	461
Total		1022

Figure 6. Genetic samples identified by early, middle, and late temporal groupings. Top panel, graph showing the 3 temporal groupings. Bottom panel, dates corresponding to the statistical week groupings and the number of fish in each temporal group.

Temporal changes in stock contributions

There was a shift in the regional contributions of the stock composition estimate over time, with western Alaska more dominant in the early sampling and Asian fish more dominant in the later. The AYKSSI sample set has the potential for both temporal and spatial biases (Figure 1 and 2) for determining overall bycatch stock composition estimates. These sample strata will be fully evaluated in the report for the AYKSSI project, but an analysis of the overall temporal effects is presented below. The large number of samples (1,084) allowed the temporal splitting of the sample set into three time segments: early peak, middle, late peak with sample sizes of 356 (early), 205 (middle) to 461 (late) (Figure 6). The goal of the analysis was to determine if regional contributions to the bycatch changed over time.

Understanding the temporal distribution of the chum 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 three sample sets (Table 4). All BAYES stock composition estimates were again performed using eight Monte Carlo chains starting at disparate starting values of stock proportions. Gelman and Rubin shrink statistics were calculated and in all cases, they were below 1.10 suggesting strong convergence to a single posterior distribution. The SPAM and BAYES estimates were very similar to each other; however, the stock composition estimates differed between time periods (Table 4).

Table 4. SPAM and BAYES stock composition estimates for the early, middle, and late time periods of the AYKSSI subset of 2005 chum salmon bycatch samples. SE is the SPAM standard error. SD is the BAYES standard deviation. The 95% credible interval is provided for all BAYES estimates.

Wk 24-28	Region	SPAM		BAYES				
		Estimate	SE	Mean	SD	2.50%	Median	97.50%
	Japan/S. Russia	0.169	0.009	0.181	0.023	0.139	0.180	0.227
	Russia	0.175	0.009	0.158	0.028	0.106	0.158	0.215
	Upper/Middle Yukon	0.112	0.006	0.115	0.023	0.072	0.114	0.160
	Western Alaska	0.257	0.014	0.274	0.033	0.213	0.273	0.344
	Alaska Peninsula	0.046	0.002	0.044	0.015	0.019	0.042	0.076
	SE Alaska/N. BC	0.028	0.002	0.023	0.017	0.000	0.021	0.061
	Skeena	0.000	0.000	0.001	0.003	0.000	0.000	0.009
	BC/Washington	0.211	0.011	0.205	0.026	0.155	0.205	0.258
Wk 29-34	Region							
	Japan/S. Russia	0.360	0.025	0.376	0.036	0.307	0.375	0.448
	Russia	0.259	0.018	0.284	0.039	0.210	0.284	0.364
	Upper/Middle Yukon	0.057	0.004	0.052	0.020	0.018	0.051	0.097
	Western Alaska	0.149	0.010	0.148	0.033	0.088	0.147	0.215
	Alaska Peninsula	0.014	0.001	0.007	0.006	0.000	0.005	0.024
	SE Alaska/N. BC	0.022	0.002	0.011	0.012	0.000	0.007	0.043
	Skeena	0.004	0.000	0.006	0.011	0.000	0.000	0.039
	BC/Washington	0.116	0.008	0.116	0.027	0.067	0.115	0.173
Wk 35-40	Region							
	Japan/S. Russia	0.357	0.016	0.369	0.024	0.323	0.369	0.416
	Russia	0.266	0.012	0.298	0.027	0.246	0.298	0.353
	Upper/Middle Yukon	0.025	0.001	0.022	0.013	0.001	0.020	0.051
	Western Alaska	0.113	0.005	0.105	0.020	0.068	0.104	0.145
	Alaska Peninsula	0.011	0.001	0.016	0.009	0.002	0.015	0.036
	SE Alaska/N. BC	0.031	0.001	0.030	0.015	0.006	0.029	0.063
	Skeena	0.000	0.000	0.001	0.002	0.000	0.000	0.006
	BC/Washington	0.176	0.008	0.160	0.020	0.122	0.159	0.200

The differences in stock structure by week were significant for both the SPAM and BAYES estimates (Figure 7, see non-overlapping differences in the plotted 95% credible intervals). For example, fish from Western Alaska and the Upper/Middle Yukon were more prevalent in the early part of the season (Weeks 24-28) than the later (Weeks 35-40) whereas the inverse relationship was apparent for stocks from Asia (Figure 7). This is similar to trends observed previously for chum salmon bycatch samples genetically analyzed from the 1994 and 1995 years (Wilmot et al., 1998).

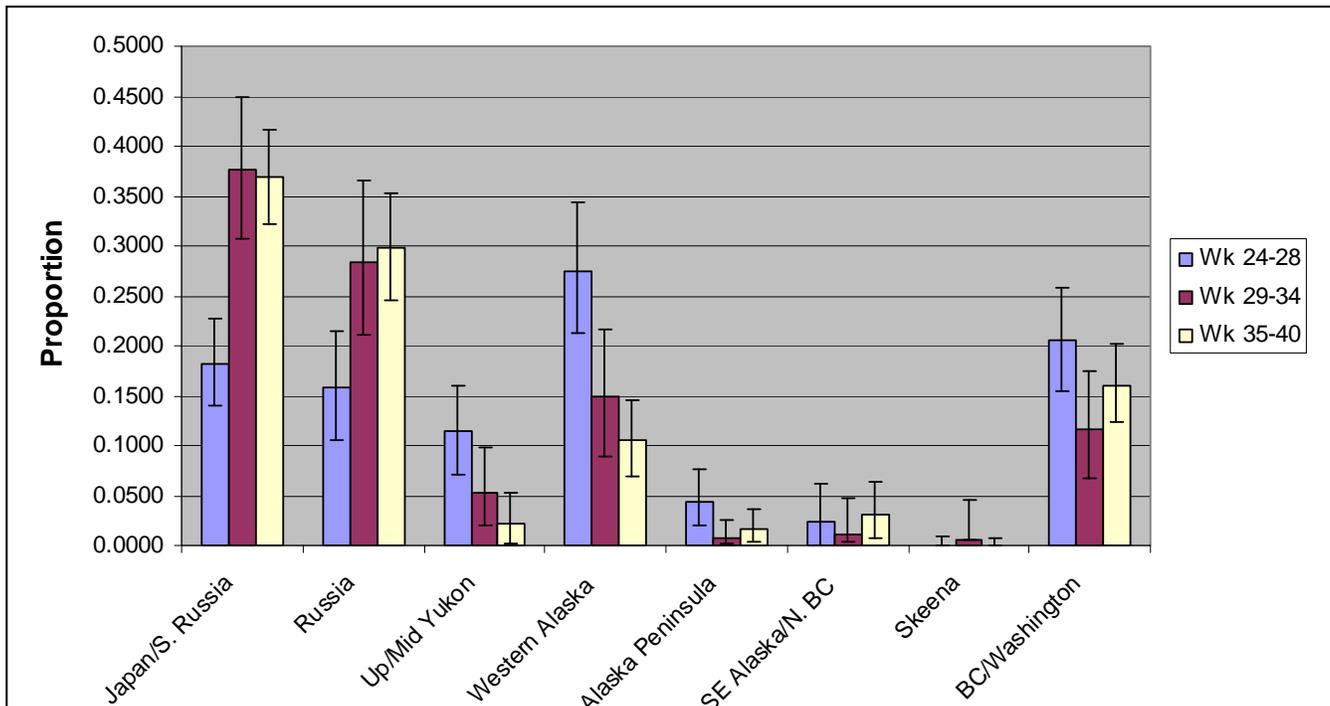
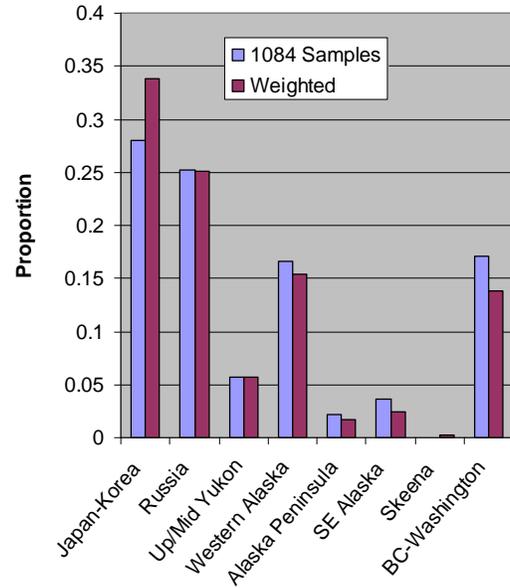


Figure 7. Early, middle, and late BAYES stock composition estimates and 95% credible intervals for the three temporal periods from the AYKSSI subset of samples from the 2005 chum salmon bycatch. Early designates weeks 24-28, middle designates 29-34, and late designates weeks 35-40.

The stock composition of the chum salmon bycatch varied during the course of the season even within closely spaced temporal groupings. For example, disregarding the changing spatial distributions of the sample set (Figure 3), estimates for weeks 24-28 and 35-40 show strong stock differences yet are only separated by 6 weeks. One way to adjust for the effects of the changing distribution is to weight the estimates by the proportion of bycatch caught in each time interval and then compare that estimate with the overall estimate determined for the 1,084 sample set (Table 3). The weighted stock composition estimates were very similar to the estimate produced from the 1,084 samples as a whole (Figure 8) suggesting the potential for a simple linear relationship in which the two sample peaks could be averaged to identify the stock composition of the entire bycatch. For example, if Western Alaska and Yukon stocks decline over time while Asia stocks increase, a weighted average (stock composition estimates for each time period expanded by the integrated total bycatch over the same time periods) between the two peaks may produce an acceptable stock composition estimate for the entire bycatch. Such an analysis would not account for the strong spatial biases in the AYKSSI sample set (Figure 1), but could account for temporal biases.

Figure 8. Comparison of the aggregated stock composition estimate produced from the available 1,084 genetic samples with a weighted estimate based on the temporal stock compositions weighted by the proportion of bycatch caught in each time interval.



Stock Composition Summary

The unweighted stock composition results from the AYKSSI chum bycatch sample set indicate that the major contributing regions were: Upper/Middle Yukon (5-6%), western Alaska (16%), BC/Washington (16-17%), Japan/S. Russia (28-29%) and Russia (25-29%). There was little contribution from southeast Alaska/Northern British Columbia, Alaska Peninsula, or Skeena. SPAM simulation studies described above indicate some potential to misallocate SE Alaska fish/N. BC to BC/Washington (Table 2), but because stock composition estimates for the SE Alaska/N. BC stocks were low, they were combined in Figure 9 with the BC/Washington region to allow comparison with previous estimates (Patton et al., 1998; Seeb et al., 2004; Wilmot et al., 1998). Although the AYKSSI genetic sample distribution is different than the overall non-Chinook bycatch distribution (Figure 2), the results derived from our study are similar to those from the 1994 bycatch (Figure 9). The 1994-1996 chum bycatch estimates were produced with allozyme data and the 2005 chum bycatch estimates were derived for the first time from DNA based microsatellite markers.

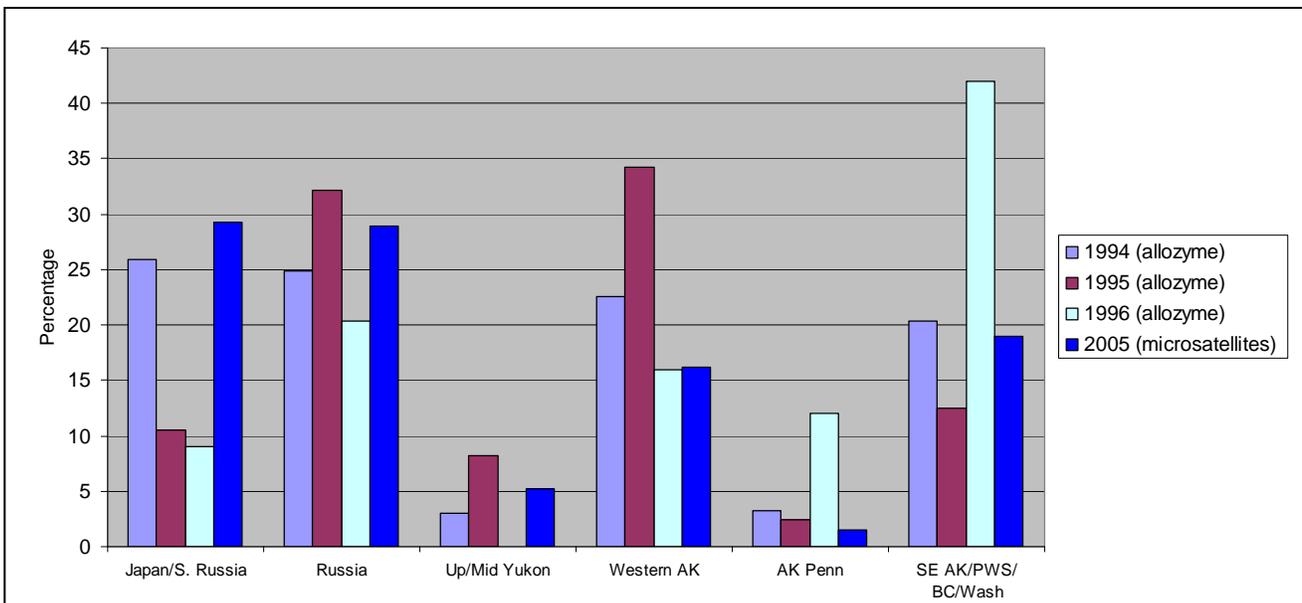
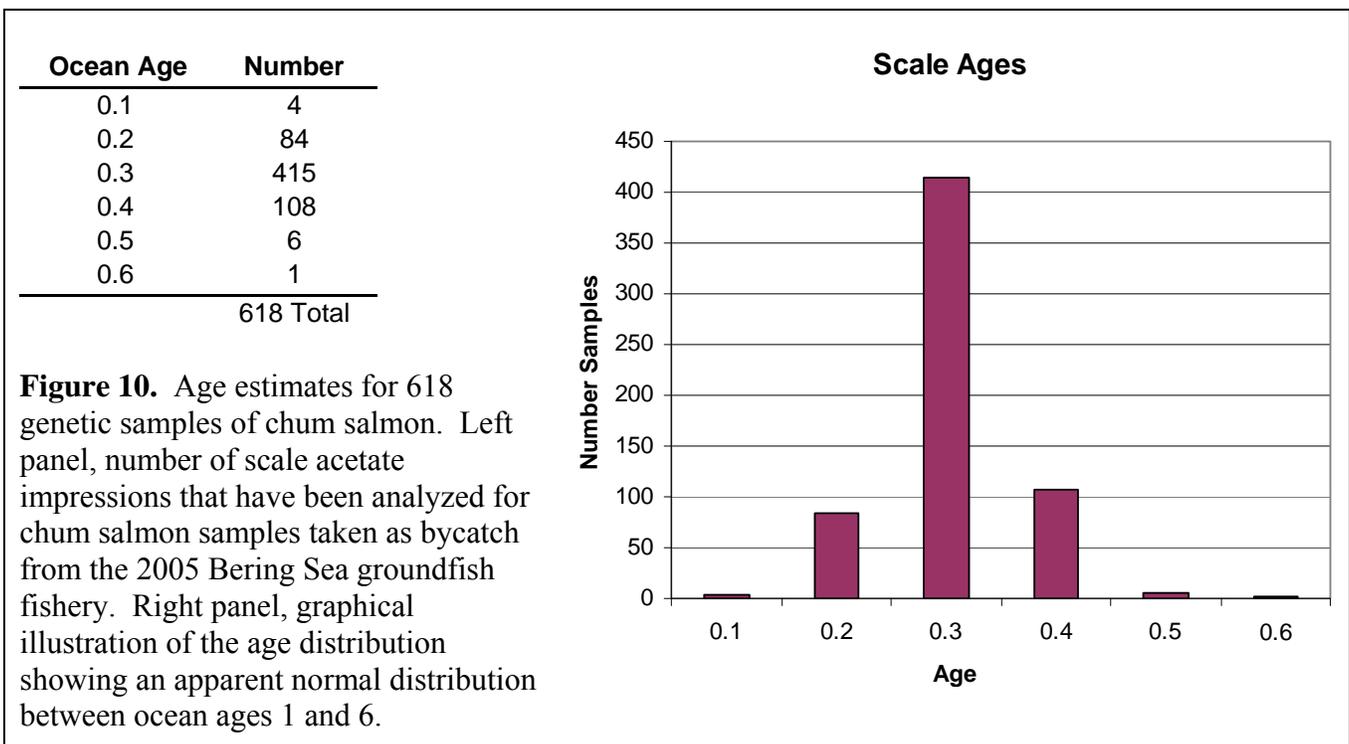


Figure 9. Comparison of 2005 stock composition estimates with those from the 1994-1996 years. The 1994-1996 estimates were derived using allozyme markers while the 2005 estimates were produced for the first time using DNA based markers. For estimates across different years, not all areas may contain the same populations as different baselines were used in producing these estimates.

Age structure of AYKSSI genetic samples

Ocean migration patterns influence the age at which salmon are caught in the trawl fisheries. As part of the AYKSSI project, spatial and temporal changes of the chum salmon distribution in the Bering Sea will be analyzed with respect to ocean age; consequently, 618 scales from the 2005 chum salmon bycatch from the Bering Sea groundfish fishery were analyzed. Acetate impressions were made and digitized into TIFF files. Scale analysis shows that the majority of samples came from ocean age 3 fish (Figure 10, left panel). Other ages appear to be part of a normal distribution centered about the mean (Figure 10, right panel).



Summary and discussion with future implications

Communities in western Alaska and elsewhere are dependent on salmon for subsistence and commercial purposes. Decreasing salmon returns to western Alaska have caused hardships in these communities. Salmon-dependent communities have expressed concerns that the Bering Sea pollock fisheries could be responsible for the decreasing salmon returns due to the inadvertent catch of salmon as bycatch. Stock composition estimates of the salmon bycatch are needed for pollock and salmon fishery managers to understand whether the pollock fisheries may be impacting salmon returns, however much work remains before such estimates can be produced. To guide the efforts to estimate the stock composition of the total bycatch, this report provides a stock composition analysis of a stratified sample set from the 2005 chum salmon bycatch. The limitations of this analysis for understanding the stock composition of the bycatch are summarized below.

Sampling issues:

Samples from the 2005 chum salmon bycatch were specifically collected for an AYKSSI funded project to determine spatial and temporal distributions of chum salmon in the Bering Sea. We highlight the inherent spatial and temporal biases in the sample set, which limits the application of the AYKSSI sample stock composition estimate to the entire 2005 chum salmon bycatch. With the need to fully understand the effects of the salmon bycatch on western Alaska salmon escapements, changes to the sample collection protocols are being reviewed and new procedures are expected to be implemented during the 2011 fishing season.

Evaluation of the baseline:

We have selected a chum salmon microsatellite baseline developed by Dr. Beacham at the Division of Fisheries and Oceans Canada (DFO) because it is the only publicly available baseline with known populations and references (Beacham et al., 2009b). This baseline represents 381 chum populations distributed throughout the Pacific Rim and is available for anonymous download through a Division of Fisheries and Oceans web portal. While only 11 of the 14 markers have been utilized, these markers provide discriminatory power to identify stock distributions for 8 regional groupings. Additional markers may be added in the future as they are standardized. In addition, at least two other chum salmon baselines are currently being developed and may be considered in future analyses after they are made publicly available, published, and evaluated. Improvements in stock composition estimates will require further baseline development with additional markers and populations, as well as periodic checks to determine if there is drift of allele frequencies or migration within the baseline.

Stock composition estimates:

Overall, Asian fish dominated the AYKSSI sample set, with Western Alaska and BC/Washington as the largest contributors from North American stocks. For this analysis, over one thousand samples were genotyped from the 2005 AYKSSI sample set. Stock composition estimates were prepared using both a Bayesian and maximum likelihood approach (SPAM), both of which provided very similar overall estimates. These results suggest that the genetic baseline provided criteria from which to confidently identify the 8 identified regional groupings of chum salmon.

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

Western Alaska fish dominated in the early part of the sampling effort; Asian fish dominated in the middle and late sampling times. As the AYKSSI genetic sample temporal distribution was dissimilar to the overall chum bycatch in 2005 (Figure 2), separate stock composition estimates were produced for samples taken at three different time periods (early, middle, and late) in the bycatch. Stock composition estimates for these three time periods differed, suggesting temporal stratification of chum salmon stocks in the Bering Sea and/or changes in fishing locations. When stock composition estimates were adjusted for sampling rate, the weighted stock composition estimate was remarkably similar to the composite stock composition estimate of the 1,084 initial samples. This suggests the potential for a simple linear relationship over time in which some stocks decrease (Western Alaska and Upper/Middle Yukon) while others increase (Asia).

Comparison of 2005 with earlier years:

The 2005 AYKSSI stock composition estimates were comparable to those from the 1994 bycatch samples, although it is recognized that small changes in stock composition could represent large changes in individual stocks at the escapement level. The 2005 analysis is the first year for

which DNA-based markers have been used to analyze chum salmon bycatch samples and the similarity with estimates originally derived using both scale pattern and protein markers helps validate the more cost-efficient DNA based methods.

Future estimates:

Proportionate sampling in future years will yield stock composition estimates with greater certainty in the origin of stocks and the proportion of critical stocks in the bycatch. Also, questions such as the composition in time and space, and warm versus cold years, can be tested to see if changes in harvest strategy would have less impact on critical stocks. In addition, the suitability of more refined regional reporting groups will be explored in consultation with other genetic laboratories. Such an analysis with more than 50 reporting groups has recently been reported for chum salmon collections taken from the Gulf of Alaska (Beacham et al., 2009c).

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Appendices

Appendix 1. Chum salmon populations in the DFO microsatellite baseline with regional designations used in the analyses of this report.

DFO Number	Population Name	Region Number	Region
8	Big_Creek	1	Upper/Middle Yukon
89	Big_Salt	1	Upper/Middle Yukon
86	Black_River	1	Upper/Middle Yukon
87	Chandalar	1	Upper/Middle Yukon
28	Chandindu	1	Upper/Middle Yukon
82	Cheena	1	Upper/Middle Yukon
81	Delta	1	Upper/Middle Yukon
7	Donjek	1	Upper/Middle Yukon
5	Fishing_Br	1	Upper/Middle Yukon
88	Jim_River	1	Upper/Middle Yukon
85	Kantishna	1	Upper/Middle Yukon
2	Kluane	1	Upper/Middle Yukon
59	Kluane_Lake	1	Upper/Middle Yukon
181	Koyukuk_late	1	Upper/Middle Yukon
90	Koyukuk_south	1	Upper/Middle Yukon
10	Minto	1	Upper/Middle Yukon
6	Pelly	1	Upper/Middle Yukon
439	Porcupine	1	Upper/Middle Yukon
83	Salcha	1	Upper/Middle Yukon
4	Sheenjek	1	Upper/Middle Yukon
1	Tatchun	1	Upper/Middle Yukon
9	Teslin	1	Upper/Middle Yukon
84	Toklat	1	Upper/Middle Yukon
348	Agiapuk	2	Coastal Western Alaska/Lower Yukon
376	Alagnak	2	Coastal Western Alaska/Lower Yukon
3	Andreafsky	2	Coastal Western Alaska/Lower Yukon
357	Aniak	2	Coastal Western Alaska/Lower Yukon
301	Anvik	2	Coastal Western Alaska/Lower Yukon
80	Chulinak	2	Coastal Western Alaska/Lower Yukon
347	Eldorado	2	Coastal Western Alaska/Lower Yukon
358	George	2	Coastal Western Alaska/Lower Yukon
307	Gisasa	2	Coastal Western Alaska/Lower Yukon
371	Goodnews	2	Coastal Western Alaska/Lower Yukon
288	Henshaw_Creek	2	Coastal Western Alaska/Lower Yukon
339	Imnachuk	2	Coastal Western Alaska/Lower Yukon
361	Kanektok	2	Coastal Western Alaska/Lower Yukon
362	Kasigluk	2	Coastal Western Alaska/Lower Yukon
328	Kelly_Lake	2	Coastal Western Alaska/Lower Yukon
340	Kobuk	2	Coastal Western Alaska/Lower Yukon
343	Koyuk	2	Coastal Western Alaska/Lower Yukon
363	Kwethluk	2	Coastal Western Alaska/Lower Yukon
336	Kwiniuk_River	2	Coastal Western Alaska/Lower Yukon
303	Melozitna	2	Coastal Western Alaska/Lower Yukon

373	Mulchatna	2	Coastal Western Alaska/Lower Yukon
372	Naknek	2	Coastal Western Alaska/Lower Yukon
330	Niukluk	2	Coastal Western Alaska/Lower Yukon
329	Noatak	2	Coastal Western Alaska/Lower Yukon
345	Nome	2	Coastal Western Alaska/Lower Yukon
302	Nulato	2	Coastal Western Alaska/Lower Yukon
374	Nunsatuk	2	Coastal Western Alaska/Lower Yukon
13	Peel_River	2	Coastal Western Alaska/Lower Yukon
322	Pikmiktalik	2	Coastal Western Alaska/Lower Yukon
331	Pilgrim_River	2	Coastal Western Alaska/Lower Yukon
346	Shaktoolik	2	Coastal Western Alaska/Lower Yukon
341	Snake	2	Coastal Western Alaska/Lower Yukon
368	Stuyahok_River	2	Coastal Western Alaska/Lower Yukon
375	Togiak	2	Coastal Western Alaska/Lower Yukon
154	Tozitna	2	Coastal Western Alaska/Lower Yukon
342	Unalakleet	2	Coastal Western Alaska/Lower Yukon
344	Ungalik	2	Coastal Western Alaska/Lower Yukon
323	Carroll	3	SE AK/PWS/N. BC
353	Constantine	3	SE AK/PWS/N. BC
414	Crag_Cr	3	SE AK/PWS/N. BC
210	Dipac_Hatchery	3	SE AK/PWS/N. BC
319	Disappearance	3	SE AK/PWS/N. BC
276	Ensheshese	3	SE AK/PWS/N. BC
227	Gambier	3	SE AK/PWS/N. BC
237	Greens	3	SE AK/PWS/N. BC
234	Herman_Creek	3	SE AK/PWS/N. BC
162	Kateen	3	SE AK/PWS/N. BC
238	Kennell	3	SE AK/PWS/N. BC
351	Keta_Creek	3	SE AK/PWS/N. BC
437	Klewnuggit_Cr	3	SE AK/PWS/N. BC
423	Kumealon	3	SE AK/PWS/N. BC
127	Lachmach	3	SE AK/PWS/N. BC
448	LagoonCr	3	SE AK/PWS/N. BC
444	Nakut_Su	3	SE AK/PWS/N. BC
422	Nass_River	3	SE AK/PWS/N. BC
321	Neets_Bay_early	3	SE AK/PWS/N. BC
320	Neets_Bay_late	3	SE AK/PWS/N. BC
377	Olsen_Creek	3	SE AK/PWS/N. BC
236	Sawmill	3	SE AK/PWS/N. BC
249	Shustnini	3	SE AK/PWS/N. BC
416	Stumaun_Cr	3	SE AK/PWS/N. BC
30	Taku	3	SE AK/PWS/N. BC
18	Takwahoni	3	SE AK/PWS/N. BC
247	Tuskwa	3	SE AK/PWS/N. BC
232	Wells_Bridge	3	SE AK/PWS/N. BC
352	Wells_River	3	SE AK/PWS/N. BC
248	Yellow_Bluff	3	SE AK/PWS/N. BC
360	Alogoshak	4	Alaska Peninsula
333	American_River	4	Alaska Peninsula
366	Big_River	4	Alaska Peninsula

354	Coleman_Creek	4	Alaska Peninsula
355	Delta_Creek	4	Alaska Peninsula
359	Egegik	4	Alaska Peninsula
332	Frosty_Creek	4	Alaska Peninsula
365	Gertrude_Creek	4	Alaska Peninsula
370	Joshua_Green	4	Alaska Peninsula
364	Meshik	4	Alaska Peninsula
283	Moller_Bay	4	Alaska Peninsula
369	Pumice_Creek	4	Alaska Peninsula
367	Stepovak_Bay	4	Alaska Peninsula
335	Sturgeon	4	Alaska Peninsula
350	Uganik	4	Alaska Peninsula
334	Volcano_Bay	4	Alaska Peninsula
356	Westward_Creek	4	Alaska Peninsula
239	Ahnuhati	5	BC/Washington
69	Ahta_____	5	BC/Washington
155	Ain_	5	BC/Washington
183	Algard	5	BC/Washington
58	Alouette	5	BC/Washington
325	Alouette_North	5	BC/Washington
428	Arnoup_Cr	5	BC/Washington
153	Ashlulm	5	BC/Washington
156	Awun	5	BC/Washington
133	Bag_Harbour	5	BC/Washington
164	Barnard	5	BC/Washington
16	Bella_Bell	5	BC/Washington
79	Bella_Coola	5	BC/Washington
49	Big_Qual	5	BC/Washington
201	Big_Quilcene	5	BC/Washington
281	Bish_Cr	5	BC/Washington
198	Bitter_Creek	5	BC/Washington
103	Blackrock_Creek	5	BC/Washington
390	Blaney_Creek	5	BC/Washington
138	Botany_Creek	5	BC/Washington
264	Buck_Channel	5	BC/Washington
169	Bullock_Chann	5	BC/Washington
61	Campbell_River	5	BC/Washington
78	Cascade	5	BC/Washington
76	Cayeghle	5	BC/Washington
42	Cheakamus	5	BC/Washington
398	Cheenis_Lake	5	BC/Washington
51	Chehalis	5	BC/Washington
19	Chemainus	5	BC/Washington
47	Chilliwack	5	BC/Washington
392	Chilqua_Creek	5	BC/Washington
117	Chuckwalla	5	BC/Washington
139	Clapp_Basin	5	BC/Washington
107	Clatse_Creek	5	BC/Washington
118	Clyak	5	BC/Washington
62	Cold_Creek	5	BC/Washington

77	Colonial	5	BC/Washington
168	Cooper_Inlet	5	BC/Washington
197	County_Line	5	BC/Washington
12	Cowichan	5	BC/Washington
161	Dak_	5	BC/Washington
259	Dana_Creek	5	BC/Washington
250	Dawson_Inlet	5	BC/Washington
91	Dean_River	5	BC/Washington
261	Deena	5	BC/Washington
170	Deer_Pass	5	BC/Washington
46	Demamiel	5	BC/Washington
177	Draney	5	BC/Washington
114	Duthie_Creek	5	BC/Washington
427	East_Arm	5	BC/Washington
94	Elcho_Creek	5	BC/Washington
193	Ellsworth_Cr	5	BC/Washington
203	Elwha	5	BC/Washington
263	Fairfax_Inlet	5	BC/Washington
32	Fish_Creek	5	BC/Washington
429	Flux_Cr	5	BC/Washington
102	Foch_Creek	5	BC/Washington
179	Frenchman	5	BC/Washington
96	Gill_Creek	5	BC/Washington
166	Gilttoyee	5	BC/Washington
145	Glendale	5	BC/Washington
135	Gold_Harbour	5	BC/Washington
11	Goldstream	5	BC/Washington
66	Goodspeed_River	5	BC/Washington
136	Government	5	BC/Washington
205	Grant_Creek	5	BC/Washington
100	Green_River	5	BC/Washington
450	GreenRrHatchery	5	BC/Washington
141	Harrison	5	BC/Washington
438	Harrison_late	5	BC/Washington
64	Hathaway_Creek	5	BC/Washington
17	Heydon_Cre	5	BC/Washington
407	Hicks_Cr	5	BC/Washington
400	Homathko	5	BC/Washington
411	Honna	5	BC/Washington
204	Hoodsport	5	BC/Washington
185	Hooknose	5	BC/Washington
406	Hopedale_Cr	5	BC/Washington
412	Hutton_Head	5	BC/Washington
278	Illiance	5	BC/Washington
152	Inch_Creek	5	BC/Washington
146	Indian_River	5	BC/Washington
92	Jenny_Bay	5	BC/Washington
115	Kainet_River	5	BC/Washington
144	Kakweiken	5	BC/Washington
395	Kanaka_Cr	5	BC/Washington

402	Kano_Inlet_Cr	5	BC/Washington
389	Kawkawa	5	BC/Washington
95	Kemano	5	BC/Washington
192	Kennedy_Creek	5	BC/Washington
101	Khutze_River	5	BC/Washington
126	Khutzeymateen	5	BC/Washington
282	Kiltuish	5	BC/Washington
93	Kimsquit	5	BC/Washington
187	Kimsquit_Bay	5	BC/Washington
419	Kincolith	5	BC/Washington
106	Kitasoo	5	BC/Washington
99	Kitimat_River	5	BC/Washington
275	Kitsault_Riv	5	BC/Washington
21	Klinaklini	5	BC/Washington
418	Ksedin	5	BC/Washington
125	Kshwan	5	BC/Washington
112	Kwakusdis_River	5	BC/Washington
436	Kxngeal_Cr	5	BC/Washington
262	Lagins	5	BC/Washington
131	Lagoon_Inlet	5	BC/Washington
167	Lard	5	BC/Washington
160	Little_Goose	5	BC/Washington
50	Little_Qua	5	BC/Washington
413	Lizard_Cr	5	BC/Washington
119	Lockhart-Gordon	5	BC/Washington
176	Lower_Lillooet	5	BC/Washington
137	Mace_Creek	5	BC/Washington
242	Mackenzie_Sound	5	BC/Washington
116	MacNair_Creek	5	BC/Washington
55	Mamquam	5	BC/Washington
121	Markle_Inlet_Cr	5	BC/Washington
27	Martin_Riv	5	BC/Washington
338	Mashiter_Creek	5	BC/Washington
109	McLoughin_Creek	5	BC/Washington
178	Milton	5	BC/Washington
194	Minter_Cr	5	BC/Washington
254	Mountain_Cr	5	BC/Washington
111	Mussel_River	5	BC/Washington
157	Naden	5	BC/Washington
337	Nahmint_River	5	BC/Washington
14	Nanaimo	5	BC/Washington
399	Necleetsconnay	5	BC/Washington
113	Neekas_Creek	5	BC/Washington
173	Nekite	5	BC/Washington
104	Nias_Creek	5	BC/Washington
143	Nimpkish	5	BC/Washington
53	Nitinat	5	BC/Washington
191	Nooksack	5	BC/Washington
186	Nooseseck	5	BC/Washington
318	NorrishWorth	5	BC/Washington

159	North_Arm	5	BC/Washington
184	Orford	5	BC/Washington
287	Pa-aat_River	5	BC/Washington
260	Pacofi	5	BC/Washington
56	Pallant	5	BC/Washington
65	Pegattum_Creek	5	BC/Washington
48	Puntledge	5	BC/Washington
98	Quaal_River	5	BC/Washington
147	Quap	5	BC/Washington
108	Quartcha_Creek	5	BC/Washington
199	Quinault	5	BC/Washington
110	Roscoe_Creek	5	BC/Washington
397	Salmon_Bay	5	BC/Washington
195	Salmon_Cr	5	BC/Washington
134	Salmon_River	5	BC/Washington
200	Satsop	5	BC/Washington
410	Seal_Inlet_Cr	5	BC/Washington
158	Security	5	BC/Washington
130	Sedgewick	5	BC/Washington
393	Serpentine_R	5	BC/Washington
317	Shovelnose_Cr	5	BC/Washington
206	Siberia_Creek	5	BC/Washington
25	Silverdale	5	BC/Washington
196	Skagit	5	BC/Washington
171	Skowquiltz	5	BC/Washington
447	SkykomishRiv	5	BC/Washington
132	Slatechuck_Cre	5	BC/Washington
43	Sliammon	5	BC/Washington
15	Smith_Cree	5	BC/Washington
54	Snootli	5	BC/Washington
180	Southgate	5	BC/Washington
26	Squakum	5	BC/Washington
142	Squamish	5	BC/Washington
128	Stagoo	5	BC/Washington
265	Stanley	5	BC/Washington
52	Stave	5	BC/Washington
396	Stawamus	5	BC/Washington
409	Steel_Cr	5	BC/Washington
424	Stewart_Cr	5	BC/Washington
327	Sugsaw	5	BC/Washington
324	Surprise	5	BC/Washington
75	Taaltz	5	BC/Washington
251	Tarundl_Creek	5	BC/Washington
149	Theodosia	5	BC/Washington
22	Thorsen	5	BC/Washington
129	Toon	5	BC/Washington
279	Tseax	5	BC/Washington
202	Tulalip	5	BC/Washington
97	Turn_Creek	5	BC/Washington
430	Turtle_Cr	5	BC/Washington

165	Tyler	5	BC/Washington
33	Tzoonie	5	BC/Washington
140	Vedder	5	BC/Washington
70	Viner_Sound	5	BC/Washington
45	Wahleach	5	BC/Washington
172	Walkum	5	BC/Washington
73	Waump	5	BC/Washington
105	West_Arm_Creek	5	BC/Washington
326	Widgeon_Slough	5	BC/Washington
277	Wilauks_Cr	5	BC/Washington
120	Wilson_Creek	5	BC/Washington
401	Worth_Creek	5	BC/Washington
60	Wortley_Creek	5	BC/Washington
270	Andesite_Cr	6	Skeena
123	Date_Creek	6	Skeena
269	Dog-tag	6	Skeena
266	Ecstall_River	6	Skeena
268	Kalum	6	Skeena
273	Kispiox	6	Skeena
163	Kitwanga	6	Skeena
271	Kleanza_Cr	6	Skeena
122	Nangeese	6	Skeena
274	Skeena	6	Skeena
124	Upper/Middle_Kitsumkal	6	Skeena
267	Whitebottom_Cr	6	Skeena
434	Zymagotitz	6	Skeena
41	Abashiri	7	Japan/Korea/China/S. Russia
218	Amur	7	Japan/Korea/China/S. Russia
215	Avakumovka	7	Japan/Korea/China/S. Russia
40	Chitose	7	Japan/Korea/China/S. Russia
315	Gakko_River	7	Japan/Korea/China/S. Russia
292	Hayatsuki	7	Japan/Korea/China/S. Russia
44	Horonai	7	Japan/Korea/China/S. Russia
213	Kalininka	7	Japan/Korea/China/S. Russia
252	Kawabukuro	7	Japan/Korea/China/S. Russia
313	Koizumi_River	7	Japan/Korea/China/S. Russia
300	Kushiro	7	Japan/Korea/China/S. Russia
37	Miomote	7	Japan/Korea/China/S. Russia
211	Naiba	7	Japan/Korea/China/S. Russia
391	Namdae_R	7	Japan/Korea/China/S. Russia
231	Narva	7	Japan/Korea/China/S. Russia
298	Nishibetsu	7	Japan/Korea/China/S. Russia
293	Ohkawa	7	Japan/Korea/China/S. Russia
297	Orikasa	7	Japan/Korea/China/S. Russia
214	Ryazanovka	7	Japan/Korea/China/S. Russia
312	Sakari_River	7	Japan/Korea/China/S. Russia
311	Shari_River	7	Japan/Korea/China/S. Russia
36	Shibetsu	7	Japan/Korea/China/S. Russia
299	Shikiu	7	Japan/Korea/China/S. Russia
253	Shiriuchia	7	Japan/Korea/China/S. Russia

310	Shizunai	7	Japan/Korea/China/S. Russia
217	Suifen	7	Japan/Korea/China/S. Russia
35	Teshio	7	Japan/Korea/China/S. Russia
39	Tokachi	7	Japan/Korea/China/S. Russia
38	Tokoro	7	Japan/Korea/China/S. Russia
314	Tokushibetsu	7	Japan/Korea/China/S. Russia
291	Toshibetsu	7	Japan/Korea/China/S. Russia
296	Tsugaruishi	7	Japan/Korea/China/S. Russia
383	Tugur_River	7	Japan/Korea/China/S. Russia
226	Tym_	7	Japan/Korea/China/S. Russia
230	Udarnitsa	7	Japan/Korea/China/S. Russia
316	Uono_River	7	Japan/Korea/China/S. Russia
309	Yurappu	7	Japan/Korea/China/S. Russia
207	Anadyr	8	Russia
384	Apuka_River	8	Russia
382	Bolshaya	8	Russia
380	Dranka	8	Russia
223	Hairusova	8	Russia
378	Ivashka	8	Russia
225	Kamchatka	8	Russia
219	Kanchalan	8	Russia
379	Karaga	8	Russia
294	Kikchik	8	Russia
209	Kol_	8	Russia
233	Magadan	8	Russia
295	Nerpichi	8	Russia
381	Okhota	8	Russia
212	Oklan	8	Russia
222	Ola_	8	Russia
386	Olutorsky_Bay	8	Russia
228	Ossora	8	Russia
224	Penzhina	8	Russia
385	Plotnikova_R	8	Russia
221	Pymta	8	Russia
220	Tauy	8	Russia
290	Utka_River	8	Russia
208	Vorovskaya	8	Russia
387	Zhypanova	8	Russia