

*Report to the North Pacific Fishery Management Council*

**Genetic Stock Composition Analysis of Chinook Salmon Bycatch Samples from the 2007  
“B” Season and 2009 Bering Sea Trawl Fisheries**

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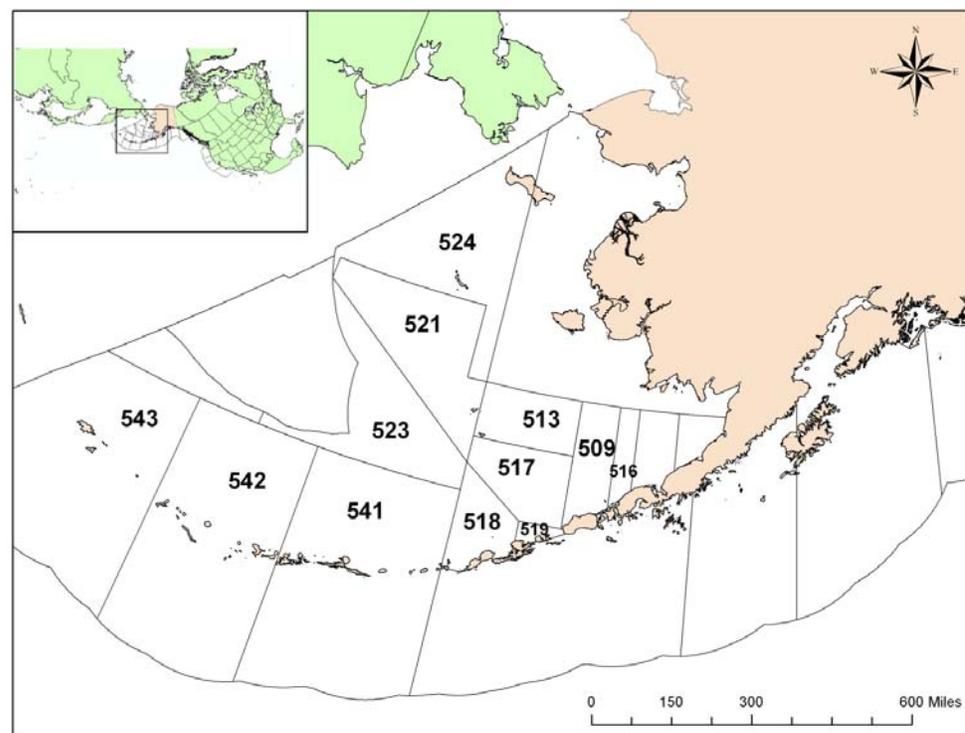
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## Introduction

The Bering Sea 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 and stock composition of salmon caught in federally managed fisheries is essential to understanding whether fisheries management could address conservation concerns. This report to the North Pacific Fishery Management Council includes preliminary genetic stock identification results for a subset of Chinook salmon bycatch samples collected from the fall 2007 “B” season and 2009 U.S. Bering Sea groundfish trawl fisheries. The final analysis will be reported in a National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum or other journal publication. National Marine Fisheries Service (NMFS) geographical statistical areas associated with the groundfish fishery are shown in Figure 1 and are used later in the report to describe the spatial distribution of the Chinook salmon bycatch and genetic samples.

**Figure 1.** Statistical areas associated with the Bering Sea Aleutian Island groundfish trawl fishery.

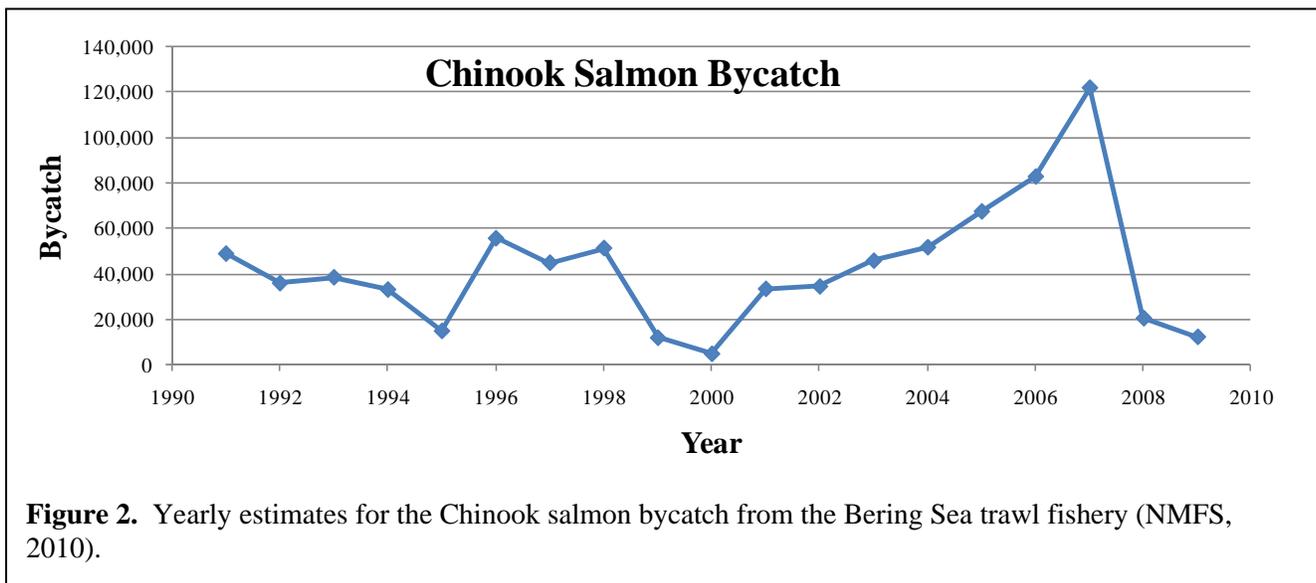


The goal of this report is to present stock composition estimates for samples collected from the bycatch of the Bering Sea trawl fishery, but it is important to understand the limitations for making accurate estimates of the entire bycatch imposed by the sampling distribution and the genetic baseline. This report is divided into the following five sections: Introduction, Sample Distribution, Genetic Stock Composition, Comparison With Previous Stock Composition Estimates, and a Summary. The analysis uses a single nucleotide polymorphism (SNP) baseline provided by the Alaska Department of Fish and Game (ADFG, unpublished) and used previously to estimate stock composition of samples from the 2005, 2006, and 2008 Chinook salmon bycatch (Guyon et al., 2010; NMFS, 2009). For additional information regarding background and methodology, this report is intended to be supplemented with the Chinook salmon bycatch report prepared previously for the 2008 Bering Sea trawl fishery (Guyon et al., 2010).

## Sample Distribution

Samples were collected by the Alaska Fisheries Science Center's (AFSC) North Pacific Observer Program as a Special Project (designated "Salmon Genetic Project") for the Auke Bay Laboratories. Samples of axillary process tissue for genetic analysis were collected opportunistically throughout the 2007 "B" season and as part of the observer's species composition analysis in 2009. Axillary process tissue was stored in coin envelopes which were labeled, frozen and shipped to the Auke Bay Laboratories. Scales for species/stock identification were collected and shipped to the Age and Growth Program of the AFSC's Fisheries Monitoring and Analysis (FMA) Division.

In 2007, an estimated 121,756 Chinook salmon were taken in the bycatch of the Bering Sea trawl fishery (NMFS, 2010), of which 52,348 were estimated from the groundfish "B" season. 2007 was the year of the highest Chinook bycatch (Figure 2). The genetic sample set for the 2007 "B" season Chinook salmon bycatch was 867 fish corresponding to an overall sampling rate of 1.7%. In contrast, during 2009, an estimated 12,416 Chinook salmon were taken in the bycatch of the Bering Sea trawl fishery (NMFS, 2010). The genetic sample set for the 2009 Chinook bycatch was 386 fish corresponding to an overall sampling rate of 3.1%.

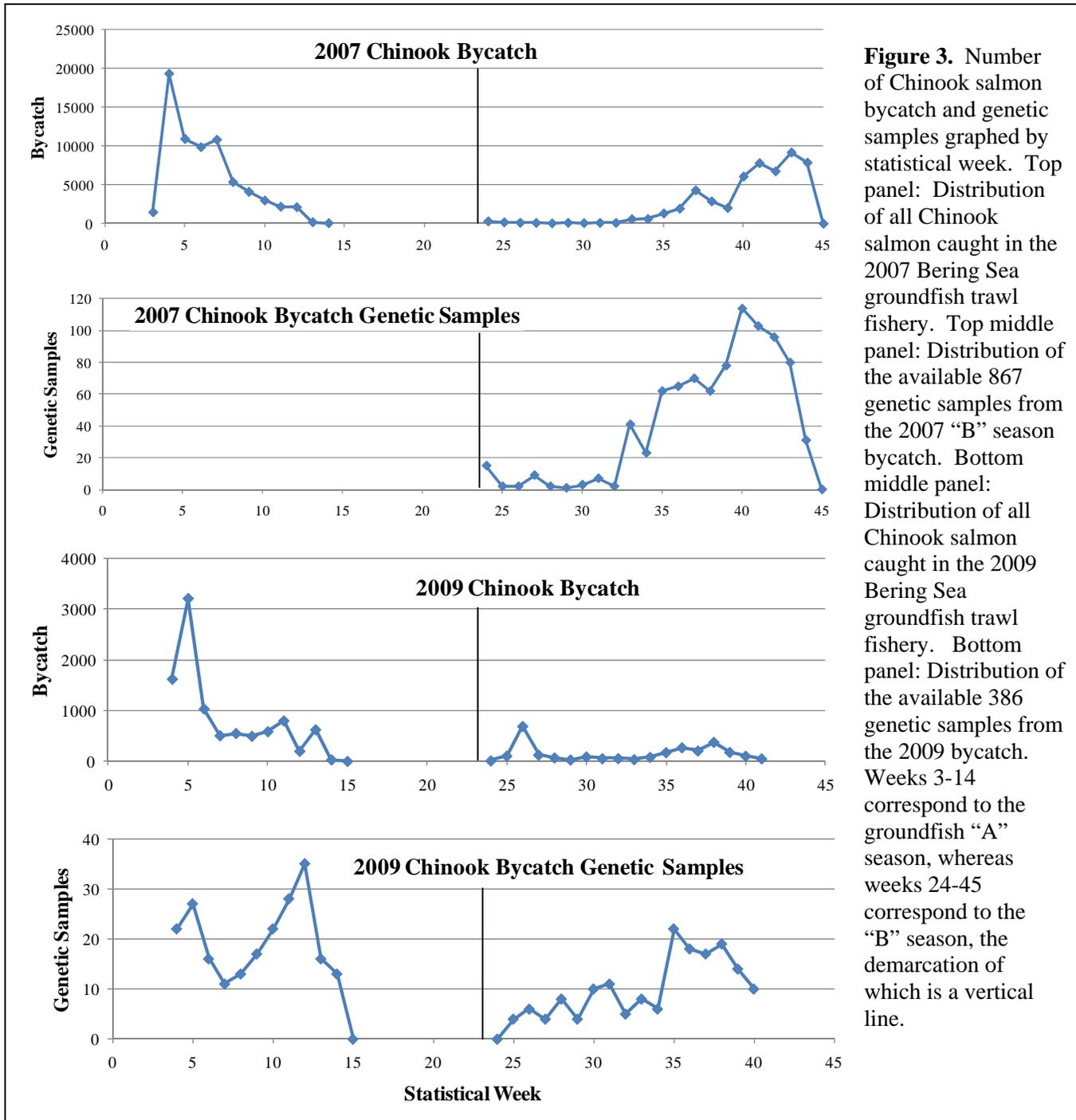


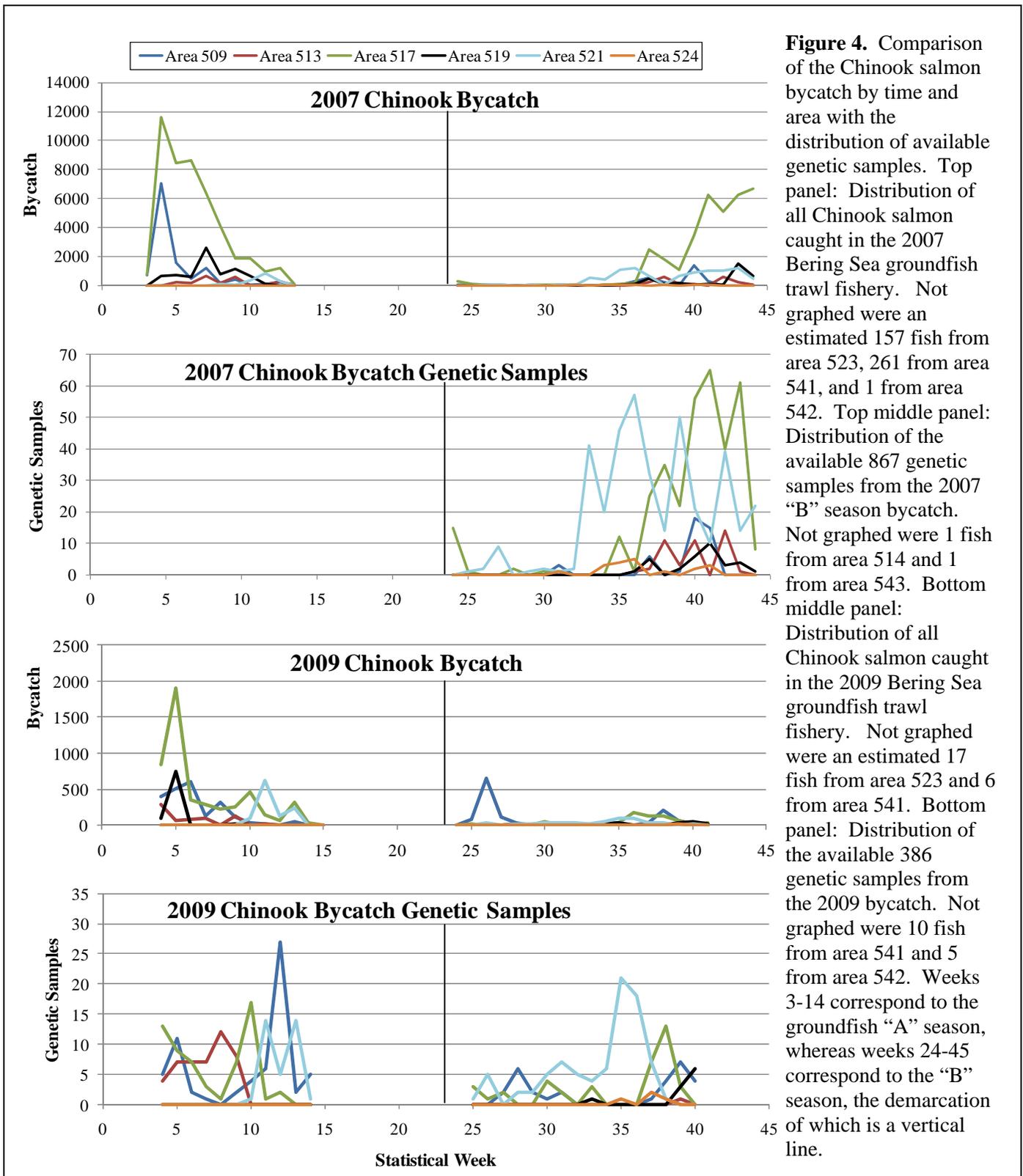
**Figure 2.** Yearly estimates for the Chinook salmon bycatch from the Bering Sea trawl fishery (NMFS, 2010).

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 ensure the collection of representative samples are now being reviewed by the Alaska Fisheries Science Center and, when implemented, will reduce biases and improve defensibility of resulting stock composition estimates. Potential spatial and temporal biases associated with the 2007 "B" Season and 2009 Chinook salmon bycatch sample sets were evaluated by comparing the genetic sample distribution with the overall bycatch estimate distribution (Figure 3). During the 2007 "B" season, the overall bycatch and genetic samples were generally comparable in their temporal distribution. In contrast, during 2009, the collection of genetic samples appeared to be disproportionate within the "A" season and overrepresentative of the fall "B" season.

To evaluate the sample spatial distribution, the Chinook bycatch was compared with the bycatch samples by statistical area over time (Figure 4). Spatial and temporal sample biases can become more apparent at these higher resolution scales. For example, during the 2007 "B" season, high levels of both

bycatch and genetic samples were available from statistical area 517, but statistical area 521 was overrepresented in the genetic sample set. In 2009, the genetic sample set was relatively small such that small changes in sampling efforts can result in significant sample biases. For example, statistical areas 509 and 513 were overrepresented in the 2009 “A” season genetic sample set, and area 521 was overrepresented in the “B” season sample set. In addition, spatial bias may be further exacerbated by the uncertainty of catch location for samples collected from shoreside deliveries in which the hauls are mixed and the location of the catch was taken from the first haul of a fishing trip.





**Figure 4.** Comparison of the Chinook salmon bycatch by time and area with the distribution of available genetic samples. Top panel: Distribution of all Chinook salmon caught in the 2007 Bering Sea groundfish trawl fishery. Not graphed were an estimated 157 fish from area 523, 261 from area 541, and 1 from area 542. Top middle panel: Distribution of the available 867 genetic samples from the 2007 “B” season bycatch. Not graphed were 1 fish from area 514 and 1 from area 543. Bottom middle panel: Distribution of all Chinook salmon caught in the 2009 Bering Sea groundfish trawl fishery. Not graphed were an estimated 17 fish from area 523 and 6 from area 541. Bottom panel: Distribution of the available 386 genetic samples from the 2009 bycatch. Not graphed were 10 fish from area 541 and 5 from area 542. Weeks 3-14 correspond to the groundfish “A” season, whereas weeks 24-45 correspond to the “B” season, the demarcation of which is a vertical line.

## Genetic Stock Composition

DNA was extracted from axillary process tissue and matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) genotyping was performed as described previously (Guyon et al., 2010) using a Sequenom MassARRAY iPLEX platform (Gabriel et al., 2009) to genotype 43 single nucleotide polymorphism (SNP) DNA markers represented in the Chinook salmon baseline (ADFG, unpublished). The SNP baseline contains genetic information for 172 populations of Chinook salmon grouped into 15 geographic regions (ADFG, unpublished). This baseline was used previously for the genetic analysis of the 2005, 2006, and 2008 Bering Sea Chinook bycatch (NMFS, 2009; Guyon et al, 2010). In addition to internal MALDI-TOF chip controls, 10 previously genotyped samples were included during the analyses and resulting genotypes were compared to those from the Alaska Department of Fish and Game, which used TaqMan chemistries (Applied Biosystems). 99.73% and 99.87% concordance rates between the two chemistries for the 2007 and 2009 controls, respectively, confirmed the utility and compatibility of both genotyping methods.

From the 2007 “B” season Chinook salmon bycatch, a total of 1,090 samples were analyzed of which 868 samples were successfully genotyped for 35 or more of the 43 SNP loci. These genotypes were analyzed in GenAIEx (Peakall and Smouse, 2006) for data integrity and one duplicate genotype was removed. The remaining 867 samples used in this analysis had genetic information for an average of 40.5 markers. The loss of samples during the genotyping phase is likely a result of sample quality which was highly variable even within collections from individual observers. The sample distributions shown in Figures 3 and 4 are from the 867 sample set, although their distribution did not differ substantially from the entire 1,090 set (data not shown). Stock composition estimates were derived by using both SPAM and BAYES software and both methods yielded almost identical stock composition estimates (Table 1). Results suggest that over 90% of the samples originated from river systems flowing into the Bering Sea with the coastal western Alaska stock contributing the most (79%), followed by the upper/middle Yukon (10%) and the north Alaska Peninsula (4%).

**Table 1.** Regional SPAM and BAYES stock composition estimates for the 867 Chinook salmon samples from the bycatch of the 2007 “B” season Bering Sea groundfish trawl fishery. Standard deviations for the SPAM estimates were determined by the analysis of 1000 bootstrapping resamplings of the mixture. The BAYES mean estimates are provided with standard deviations (SD), 95% credible intervals, and the median estimate.

Region	SPAM		BAYES				
	Estimate	SD	Mean	SD	2.5%	Median	97.5%
Russia	<b>0.019</b>	0.006	<b>0.016</b>	0.005	0.008	0.016	0.029
Coast W AK	<b>0.742</b>	0.027	<b>0.785</b>	0.018	0.748	0.786	0.819
Mid Yukon	<b>0.046</b>	0.010	<b>0.046</b>	0.011	0.027	0.045	0.068
Up Yukon	<b>0.059</b>	0.010	<b>0.059</b>	0.010	0.039	0.058	0.080
Up Kuskokwim	<b>0.016</b>	0.015	<b>0.000</b>	0.002	0.000	0.000	0.003
N AK Pen	<b>0.043</b>	0.015	<b>0.040</b>	0.011	0.021	0.039	0.065
S AK Pen	<b>0.003</b>	0.003	<b>0.000</b>	0.001	0.000	0.000	0.005
Cook Inlet	<b>0.025</b>	0.010	<b>0.008</b>	0.008	0.000	0.006	0.027
Up Copper	<b>0.001</b>	0.001	<b>0.000</b>	0.001	0.000	0.000	0.003
Low Copper	<b>0.002</b>	0.002	<b>0.001</b>	0.001	0.000	0.000	0.005
N SE Alaska	<b>0.000</b>	0.001	<b>0.000</b>	0.001	0.000	0.000	0.002
Coast SE AK	<b>0.000</b>	0.001	<b>0.002</b>	0.003	0.000	0.001	0.010
Andrew Cr	<b>0.001</b>	0.002	<b>0.002</b>	0.003	0.000	0.000	0.010
TBR Taku	<b>0.004</b>	0.003	<b>0.003</b>	0.003	0.000	0.002	0.012
BC-WA-OR	<b>0.039</b>	0.007	<b>0.038</b>	0.007	0.026	0.037	0.052

From the 2009 Chinook salmon bycatch, a total of 466 samples were analyzed of which 386 samples were successfully genotyped for 35 or more of the 43 SNP loci. These genotypes were analyzed in GenAlEx (Peakall and Smouse, 2006) for data integrity and no duplicate genotypes were identified. The resulting 386 samples had genetic information for an average of 40 markers. The sample distributions shown in Figures 3 and 4 are from the 386 sample set, although their distribution did not differ substantially from the entire 466 set (data not shown). Stock composition estimates were derived by using both SPAM and BAYES software and both yielded almost identical stock composition estimates (Table 2). Results indicated that over 90% of the samples originated from river systems flowing into the Bering Sea with the coastal western Alaska region contributing the most (57%), followed by the upper/middle Yukon (21%) and the north Alaska Peninsula (14%).

**Table 2.** Regional SPAM and BAYES stock composition estimates for the 386 Chinook salmon samples from the bycatch of the 2009 Bering Sea groundfish trawl fishery. Standard deviations for the SPAM estimates were determined by the analysis of 1000 bootstrapping resamplings of the mixture. The BAYES mean estimates are also provided with standard deviations (SD), 95% credible intervals, and the median estimate.

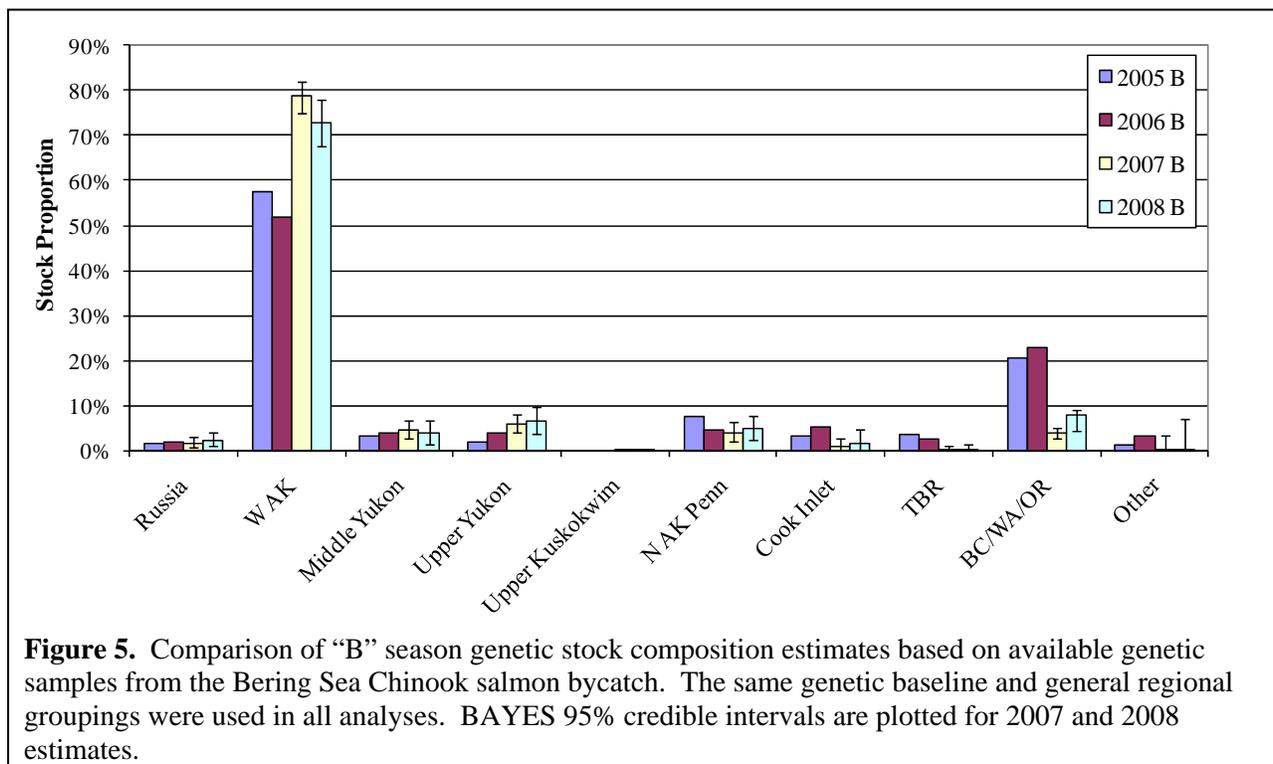
Region	SPAM		BAYES				
	Estimate	SD	Mean	SD	2.5%	Median	97.5%
Russia	<b>0.008</b>	0.005	<b>0.008</b>	0.005	0.002	0.007	0.019
Coast W AK	<b>0.526</b>	0.040	<b>0.567</b>	0.036	0.492	0.569	0.632
Mid Yukon	<b>0.066</b>	0.018	<b>0.058</b>	0.015	0.031	0.056	0.090
Up Yukon	<b>0.137</b>	0.021	<b>0.151</b>	0.021	0.112	0.150	0.194
Up Kuskokwim	<b>0.025</b>	0.017	<b>0.011</b>	0.020	0.000	0.000	0.065
N AK Pen	<b>0.146</b>	0.029	<b>0.138</b>	0.023	0.098	0.137	0.184
S AK Pen	<b>0.001</b>	0.002	<b>0.000</b>	0.001	0.000	0.000	0.001
Cook Inlet	<b>0.015</b>	0.010	<b>0.002</b>	0.004	0.000	0.000	0.015
Up Copper	<b>0.001</b>	0.002	<b>0.000</b>	0.001	0.000	0.000	0.001
Low Copper	<b>0.001</b>	0.002	<b>0.000</b>	0.001	0.000	0.000	0.002
N SE Alaska	<b>0.000</b>	0.000	<b>0.000</b>	0.001	0.000	0.000	0.002
Coast SE AK	<b>0.001</b>	0.002	<b>0.001</b>	0.002	0.000	0.000	0.007
Andrew Cr	<b>0.008</b>	0.006	<b>0.001</b>	0.003	0.000	0.000	0.010
TBR Taku	<b>0.009</b>	0.007	<b>0.001</b>	0.002	0.000	0.000	0.006
BC-WA-OR	<b>0.059</b>	0.012	<b>0.064</b>	0.013	0.041	0.063	0.091

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 (ADFG, 2003). 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 monitored with the “Percent of Maximum” value which was determined to be 91.5 (2007 “B” estimate) and 90.2 (2009 estimate), 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 baseline 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 1.05 or less for both the 2007 “B” and 2009 estimates, conveying strong convergence to a single posterior distribution (Pella and Masuda, 2001).

## Comparison With Previous Stock Composition Estimates

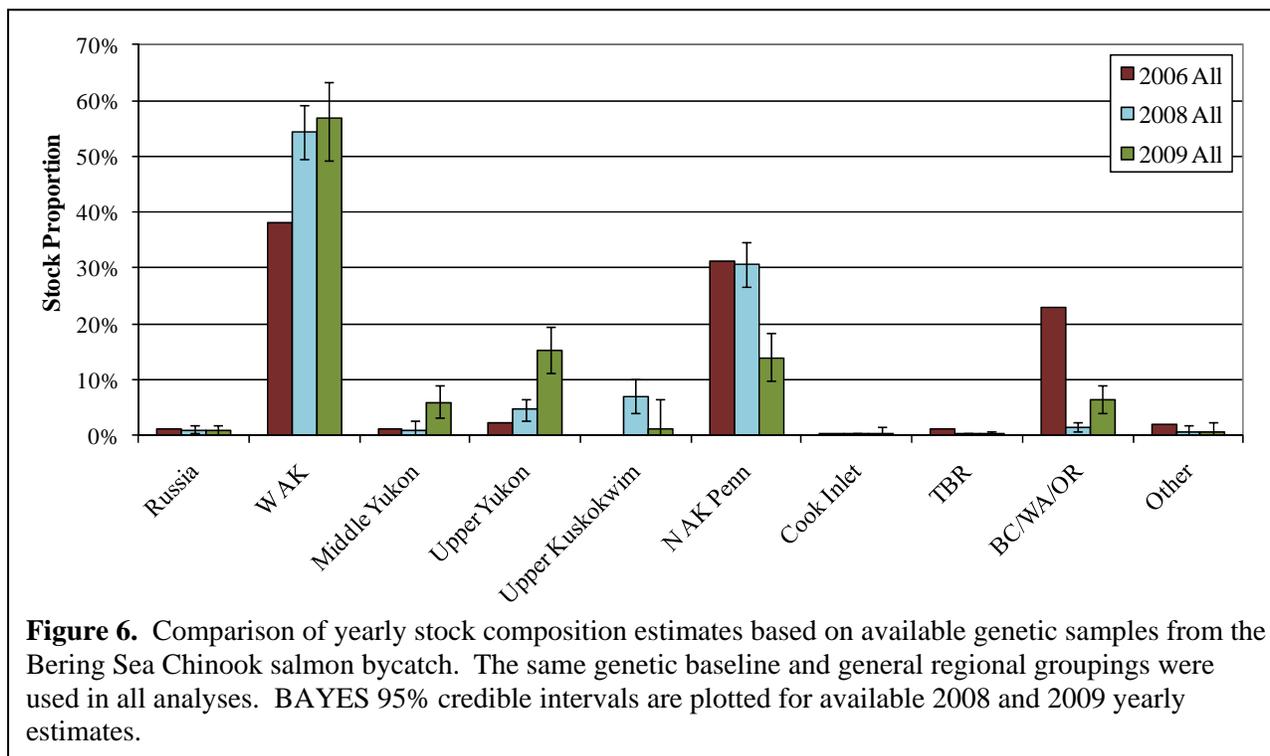
Stock composition results from the analysis of the 2007 “B” and 2009 Chinook salmon bycatch samples are in general agreement with previous estimates in that most samples were from stocks originating from river systems directly flowing into the Bering Sea. Regional stock composition estimates of the Chinook salmon bycatch between the 2007 “B” and 2008 “B” seasons were similar (Figure 5, see overlapping 95% credible intervals) (Guyon et al., 2010), although those two years appear to differ from the 2005 “B” and 2006 “B” seasons, which have a smaller proportion from western Alaska and increased number from the Pacific Northwest. However, caution must be used in comparisons across years as there are differences in where and when genetic samples were collected from year-to-year.



With regard to the 2009 stock composition estimates, substantial differences were apparent when compared to previous years (Figure 6, see non-overlapping 95% credible intervals between the 2008 and 2009 estimates). The largest differences in 2009 relative to 2008 were (1) the decrease in north Alaska Peninsula fish and (2) the increase in Yukon River fish. Previous analyses have shown that the stock composition of the north Alaska Peninsula Chinook salmon stocks is highest during the spring “A” season (Guyon et al., 2010). When the temporal distribution of the 2009 genetic sample set was compared with the total 2009 bycatch estimates (Figure 3), the 2009 genetic sample set under-represented the bycatch from the spring “A” season. For example, approximately 78% of the 2009 Chinook salmon bycatch occurred during the “A” season while only 57% of the genetic samples were collected during that same time period. In 2008, when the stock composition estimate for the north Alaska Peninsula was relatively high (30.6%), over 91% of the genetic samples were collected during the “A” season even though only 76% of the bycatch occurred during that same time period. This suggests that biases in the 2008 sample set may have led to overestimating the contribution of north Alaska Peninsula fish in 2008 and

underestimating the stock contribution in 2009. When the 2009 genetic sample set was partitioned between the “A” (220 samples) and “B” seasons (166 samples), the stock composition for the north Alaska Peninsula from the “A” season was estimated using BAYES to be 24.7% (28.6% SPAM), a number closer to that observed in previous years.

The second difference is the increase in middle/upper Yukon River stocks from 5.3% in 2008 to 20.9% in 2009. When the spatial and temporal distribution of genetic samples was compared with the overall 2009 bycatch (Figure 4), the genetic sample set overrepresented area 521, an area located offshore the Yukon River. When the 2009 genetic sample set was partitioned between those in area 521 (119 samples) and those not in area 521 (267 samples), a much higher number of Chinook salmon from the upper/middle Yukon River were represented in the area 521 sample set (33.1% versus 14.9% BAYES estimates) while less were from the North Alaska Peninsula and Pacific Northwest. This analysis illustrates how biases in sample collection can impact the accuracy of resulting stock composition estimates. Subsampling protocols to minimize some spatial and temporal biases are possible, but such protocols cannot account for biases in which no samples are represented, that is, some observers collect samples and others do not, nor would they be effective when the overall sample size is limited.



## Summary

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 U. S. 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, but steadily increased to a peak of 121,756 in 2007 (NMFS, 2010). The Chinook salmon bycatch has abated in more recent years dropping to a total of 12,416 Chinook

salmon in 2009. Stock composition estimates of the Chinook salmon bycatch are needed for pollock and salmon fishery managers to understand whether the pollock fisheries may be impacting salmon returns. While stock composition estimates were developed for available bycatch sample sets, work remains before unbiased estimates of the entire bycatch can be produced. This report provides a stock composition analysis of genetic sample sets from the 2007 “B” season and 2009 Bering Sea Chinook salmon bycatch. The results and limitations of this analysis are summarized below.

#### Sampling issues:

We highlight the inherent spatial and temporal biases in the 2007 “B” and 2009 sample sets (Figures 3-4) which may limit the application of the genetic sample stock composition estimates from these samples to the entire 2007 “B” and 2009 Chinook salmon bycatch. Through a collaboration with the Alaska Department of Fish and Game, Auke Bay Laboratories is currently investigating methods for mitigating the effects of bias in a different bycatch sample set. Methods developed through that collaboration could possibly improve overall stock composition estimates for the 2007 “B” season, 2009, and future Chinook salmon bycatch.

With regard to improved sampling protocols, NMFS recently published a proposed rule and notice of availability for Amendment 91 to the Fishery Management Plan for Groundfish of the Bering Sea and Aleutian Islands Management Area (75 FR 14016, March 23, 2010). If approved, this rule would require that all salmon bycatch taken in the Bering Sea pollock fishery be sorted by species and counted to ensure compliance with the salmon bycatch caps for the pollock fishery. This may provide additional opportunity for observers to provide representative sampling of the salmon bycatch for genetic analysis, and improve the capability to characterize the origin of salmon taken as bycatch in the Bering Sea pollock fishery.

#### Stock composition estimates:

Overall, the majority (>90%) of the genetic samples collected from the 2007 “B” and 2009 Chinook salmon bycatch of the Bering Sea trawl fishery were from Alaskan stocks originating from river systems directly flowing into the Bering Sea. The Chinook salmon bycatch stock composition estimates for the 2007 “B” season were similar to those from the 2008 “B” season (Guyon et al., 2010) where about three-quarters of the samples were from coastal western Alaska with smaller contributions from other stocks including the Yukon River and the north Alaska Peninsula. In contrast, the 2009 yearly Chinook salmon stock composition estimate showed substantial deviations from the 2008 estimate specifically with regard to the north Alaska Peninsula (13.8% versus 30.6%) and the middle/upper Yukon stocks (20.9% versus 5.3%). Temporal and spatial biases in the collection of genetic samples may have contributed to these differences suggesting that stock composition estimates derived from these samples should be viewed as estimates of the sample set rather than estimates of the entire Chinook salmon bycatch.

#### Application of these estimates:

The extent to which any salmon stock is impacted by the bycatch of the Bering Sea trawl fishery is dependent on many factors including (1) the overall size of the bycatch, (2) the age of the salmon caught in the bycatch, (3) the age of the returning salmon, and (4) the total escapement of the affected stocks taking into account lag time for maturity and returning to the river. As such, a higher stock composition estimate one year does not necessarily infer greater impact than a smaller estimate the next. Efforts to better understand these relationships and their impacts are the subject of a North Pacific Research Board proposal from Drs. Criddle and Adkison for which Auke Bay Laboratories is collaborating.

## Acknowledgements

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