

Pacific Salmon Commission
Northern Fund

**Northern Boundary Area Sockeye Salmon Genetic Stock Identification
For Year 2009 District 101 Gillnet and District 104 Purse Seine Fisheries**

Final Report

Charles M. Guthrie III
Hanhvan Nguyen
Jeffrey R. Guyon

Auke Bay Laboratories, NMFS
Ted Stevens Marine Research Institute
17109 Pt. Lena Loop Road
Juneau, AK 99801

Submitted November 8, 2011

TABLE OF CONTENTS

Page	Contents
1	COVER PAGE
2	TABLE OF CONTENTS
3	LIST OF FIGURES
4	LIST OF TABLES
5-8	INTRODUCTION
8	OBJECTIVE
8-10	METHODS
	<i>Genetic baseline and population grouping</i>
	<i>Sample Collection</i>
	<i>DNA Extraction</i>
	<i>Single Nucleotide Polymorphism (SNP) Analysis</i>
	<i>Allele Scoring</i>
	<i>Mixture Analysis</i>
10-11	RESULTS
	<i>Stock Mixture Proportions</i>
11-12	DISCUSSION
12	CONCLUSION
13	ACKNOWLEDGEMENTS
13-15	REFERENCES
16-19	ADDITIONAL TABLES AND FIGURES

LIST OF FIGURES

Page	Figures
6	Figure 1. Geographic location of Alaska Department of Fish and Game Commercial Fishing Districts 101 and 104.
16	Figure 2. 2009 sockeye stock group proportions for each statistical week from the ADF&G District 101 gillnet (top panel) and 104 purse seine fisheries (lower panel).

LIST OF TABLES

Page	Tables
7	Table 1. Sockeye salmon baseline populations used in analysis.
8	Table 2. Regional grouping designations.
9	Table 3. 45 SNP assays used to discriminate Northern Boundary sockeye populations.
10	Table 4. Sockeye salmon harvested, genetic sample size, and genotyping success rate in each statistical week in the 2009 District 101 gillnet fishery.
10	Table 5. Sockeye salmon harvested, genetic sample size, and genotyping success rate in each statistical week in the 2009 District 104 purse seine fishery.
17	Table 6. Parameters of the posterior densities for population region proportions composing weekly mixtures of the 2009 District 101 commercial gillnet sockeye fishery.
18	Table 7. Parameters of the posterior densities for population region proportions composing weekly mixtures of the 2009 District 104 commercial purse seine sockeye fishery.
19	Table 8. Estimated numbers of sockeye salmon caught in the 2009 District 101 gillnet and 104 seine fisheries prior to statistical week 31 and throughout all statistical weeks analyzed (see Tables 4&5).

INTRODUCTION

Provisions outlined in Chapter 2 of the Pacific Salmon Treaty specify harvest sharing arrangements of Nass and Skeena River sockeye salmon between the United States and Canada. This treaty allows the United States to harvest a fixed percentage, averaged over ten years, of the annual allowable harvest (AAH) of Nass sockeye in the Alaskan District 101 gillnet fishery (GNF) and of Nass and Skeena sockeye in the District 104 purse seine fishery (PSF) prior to Statistical Week 31 (late July). There is also a District 101 PSF, but the catch in this fishery is not limited by the annex; it is used however in calculating the total return of Alaska, Nass and Skeena River stocks (along with districts 102, 103 seine and 106 gillnet). Figure 1 illustrates the locations of the Alaska Department of Fish and Game (ADF&G) commercial fishing districts in the Northern Boundary area.

Accurate estimates of the stock composition of sockeye salmon caught in boundary area gillnet and purse seine fisheries (few are caught in troll fisheries) are required to estimate the total return (catch plus escapement) of stocks subject to harvest sharing agreements. The estimated total return is then used in calculating the percentage of the AAH caught in the District 101 gillnet and District 104 purse seine fisheries. The AAH is calculated over the ten-year annex period. This approach allows for traditional fishing patterns based on stock abundance, recognizing that for some years more fish would be caught which would be compensated by other years in which less would be harvested.

It has been recognized for some time that U.S. and Canadian fishermen intercept salmon originating from the other country. Initial studies investigating the stock origins of pink and sockeye salmon caught in the Northern Boundary region between Alaska and British Columbia used mark-recapture techniques (Pella et al., 1993). These techniques involved tagging fish caught in boundary fisheries and re-capturing them at various weirs and through in-river escapement enumeration projects. This study found that a significant percent of the fish caught in districts 101 and 104 originated from Canadian stocks (Pella et al., 1993). While informative, these tagging experiments were relatively expensive and labor intensive.

A study was undertaken in 1982 to evaluate scale pattern analysis (SPA) as a means to discriminate particular stocks of fish (Marshall, 1984). This important study showed that sockeye salmon in the Alaska-British Columbia Northern Boundary area could be accurately discriminated using scales. Since then, SPA has been used by ADF&G to determine stock proportions for sockeye salmon caught in the commercial sockeye fisheries in districts 101 and 104 .

While effective, SPA requires yearly examination of source populations for each of the four major age classes (1.2, 1.3, 2.2 and 2.3) since the baseline scale patterns are strongly affected by varying environmental conditions. The requirement to reestablish or revalidate the scale pattern baseline can be expensive and burdensome. The use of more stable markers would eliminate this necessity. Like scale patterns, DNA patterns can also be used to discriminate stocks of salmon (Milner et al., 1985). Given that salmon return to their natal streams with high fidelity, they represent naturally occurring isolated populations in which genetic allele frequencies can change due to the isolation and adaptation of particular populations. These changes in allele frequencies can then be used to distinguish salmon stocks to a finer degree of resolution than SPA. For example,

scale analysis can efficiently separate 4 large stock groups (Alaska, Nass, Skeena and Fraser) whereas genetic analysis can separate 13 stock groups, adding to knowledge available to manage area fisheries.

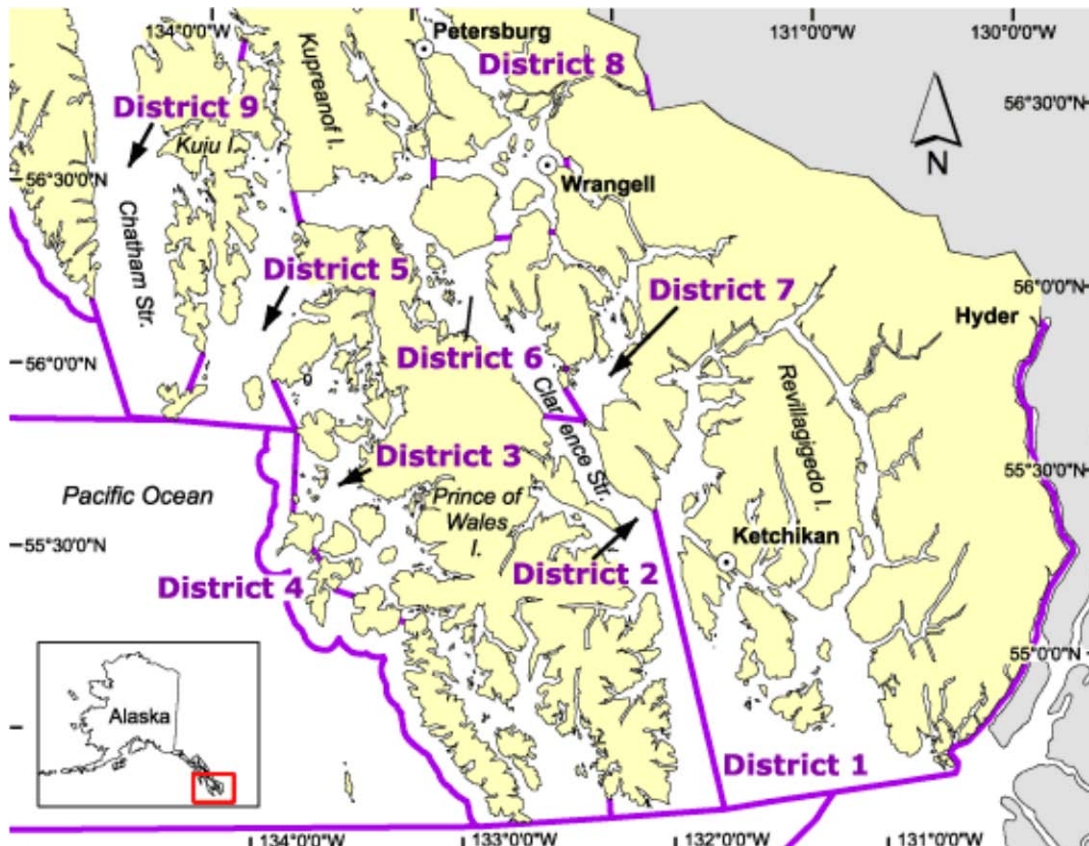


Figure 1. Geographic location of ADF&G Commercial Fishing Districts 101 (labeled District 1) and 104 (labeled District 4). Map obtained from the ADF&G web page (<http://www.cf.adfg.state.ak.us/region1/finfish/salmon/maps/ketchikan.php>).

Allozymes are naturally occurring protein variants which have been used as genetic markers. As part of a study to estimate stock composition of sockeye salmon harvested in the 1987 Northern Boundary sockeye fisheries in ADF&G Districts 104 and 106 (Pella et al., 1998), four markers were used which included two unlinked allozyme markers (*PGM-1** and *PGM-2**), freshwater age, and a brain-tissue parasite (*Myxobolus arcticus*). Freshwater age and pathogen exposure are traits that can be used in combination with other markers, to infer the stock composition of mixtures (Fournier et al., 1984; Pella and Milner, 1987). The 1987 study provided estimated proportions of 13 stock groups in the District 104 fisheries and confirmed that the majority of sockeye salmon caught were of Canadian origin (Pella et al., 1998). This analysis demonstrated that genetic markers could be effective in estimating the stock composition of sockeye salmon caught in Northern Boundary fisheries.

Although allozymes have been used in many genetic studies in salmon, it can be laborious to complete all the lab methods necessary to score them. Since then, additional genetic markers have been evaluated including microsatellite DNA repeats and single nucleotide polymorphisms (SNPs). Like allozymes, both microsatellite and SNP markers

Table 1. Sockeye salmon baseline populations used in analysis.

<i>Pop.#</i>	<i>Description</i>	<i>Region</i>	<i>Pop.#</i>	<i>Description</i>	<i>Region</i>
1	East Alsek	1	43	Hetta Lake	5
2	Alsek - Klukshu River Weir late	1	44	Kanalku Lake	5
3	Alsek - Upper Tatshenshini	1	45	Klakas Lake	5
4	Berners Bay	2	46	Sarkar	5
5	Chilkat Lake early run	2	47	Shiple Lake	5
6	Chilkat River - Mule Meadows	2	48	Three Mile Creek - Klawock	5
7	Chilkoot Lake – beaches	2	49	Hatchery Creek – McDonald Lake	6
8	Chilkoot River - Chilkoot River	2	50	Hugh Smith - Cobb Creek	7
9	Crescent Lake	2	51	Hugh Smith Lake - Bushmann Creek	7
10	Falls Lake	2	52	Nass - Bowser Lake	8
11	Sitkoh Lake	2	53	Nass - Damdochax Creek	8
12	Snettisham Hatchery/Speel Lake	2	54	Nass - Hanna Creek	8
13	Steep Creek	2	55	Nass - Meziadin Lake	8
14	Windfall Lake	2	56	Nass - Tintina Creek	8
15	Redfish Lake Beaches	2	57	Skeena - Alastair Lake	9
16	Taku - Kuthai Lake	3	58	Skeena - Four Mile Creek	9
17	Taku - Little Tatsamenie	3	59	Skeena - Fulton River	9
18	Taku - Little Trapper Lake	3	60	Skeena - Kitsumkalum Lake	9
19	Taku - Taku River Mainstem	3	61	Skeena - Lakelse Lake (Williams)	9
20	Taku – Tatsamenie	3	62	Skeena - Lower Tahlo River	9
21	Taku - Tatsamenie Lake	3	63	Skeena - McDonell Lake (Zymoetz River)	9
22	Stikine - Iskut River	4	64	Skeena – Morrison	9
23	Stikine - Little Tahltan	4	65	Skeena - Nangeese River	9
24	Stikine - Scud River	4	66	Skeena - Nanika River	9
25	Stikine - Tahltan Lake	4	67	Skeena - Pierre Creek	9
26	Kutlaku Lake	5	68	Skeena - Pinkut Creek	9
27	Hatchery Creek - Sweetwater Lake	5	69	Skeena - Slamgeesh River	9
28	Heckman Lake	5	70	Skeena - Sustut (Johanson Lake)	9
29	Helm Lake	5	71	Skeena - Swan Lake	9
30	SI – Kah Sheets Lake	5	72	Skeena - Upper Babine River	9
31	Karta	5	73	QCI - Naden River	10
32	Kegan Lake	5	74	Central - Kitlope Lake	11
33	Kunk Lake - Etoilin Island system	5	75	Fraser - Adams River (Shuswap late)	12
34	Luck Lake - P.O.W. Island	5	76	Fraser – Birkenhead	12
35	Mahoney Creek	5	77	Fraser - Chilko Lake	12
36	Mill Creek Weir - Virginia Lake	5	78	Fraser - Harrison River	12
37	Petersburg Lake	5	79	Fraser - Horsefly River	12
38	Red Bay Lake	5	80	Fraser - Raft River	12
39	Salmon Bay Lake	5	81	Fraser - Stellako River	12
40	Thoms Lake	5	82	Fraser - Weaver Creek	12
41	Unuk River - Gene's Lake	5	83	Baker Lake	13
42	Bar Creek - Essowah Lake	5	84	Cedar River	13

can efficiently be used to separate stocks of salmon (Beacham et al., 2008; Habicht et al., 2004, 2010; Smith et al., 2005a). While Canadian scientists use microsatellite markers for many of their Northern Boundary studies, ADF&G uses SNPs. Numerous studies

have been completed outlining the advantages and disadvantages of each, although both have the resolving power necessary to accurately perform stock composition studies (Smith et al., 2007).

The ADF&G has collaborated with numerous laboratories to develop a sockeye SNP baseline with 45 SNP markers (Habicht et al., 2007, 2010). This baseline was used by the ADF&G in 2004 and 2005; and by NOAA/NMFS/Alaska Fishery Science Center/Auke Bay Laboratories (ABL) in 2006, 2007, and 2008 for genetic stock composition analyses for districts 101 and 104. Currently, 84 sockeye populations are part of the SNP baseline (Table 1). As part of this process, the resolving power of the SNP baseline was evaluated using simulated mixture analyses, and this baseline was shown to be fully capable of distinguishing 13 Northern Boundary sockeye stock groups (Table 2) (Oliver 2009). The number of stock groups differs from previous years when there were 14 groups: the Klawock stock group was merged with Southern Southeast Alaska group.

Problems in accurately estimating stock proportions of catches and total returns of sockeye salmon in the early years of the Pacific Salmon Treaty resulted in an extensive investigation by the bilateral Northern Boundary Technical Committee of run reconstruction modeling. The Committee concluded that improved stock identification techniques are needed for run reconstruction models. As opposed to SPA, genetic techniques have the advantage of a relatively stable baseline (does not change yearly) and the analysis can be highly automated. The purpose of this study is to provide the sixth year of genetic data using SNP markers to compare with the scale pattern analysis. If congruence between the two techniques is evident, it is likely that genetic analysis will replace scale pattern analysis (SPA) for estimating stock composition of sockeye salmon caught in Northern Boundary fisheries. A blind testing study is now being performed to determine whether genetic markers are the viable method to replace SPA.

Table 2. Regional grouping designations

<i>Region</i>	<i>Area</i>
1	Alsek
2	Northern southeast Alaska
3	Taku
4	Stikine
5	Southern southeast Alaska
6	McDonald
7	Hugh Smith
8	Nass River
9	Skeena River
10	Queen Charlotte Island
11	Central Coast British Columbia
12	Fraser River
13	Washington

OBJECTIVE

The purpose of this study was to genetically analyze axillary process (AXP) samples from 4,402 sockeye salmon harvested in the 2009 Districts 101 gillnet and 104 purse seine sockeye fisheries to determine proportions of Canadian and U.S. fish. A SNP genetic baseline of 45 SNPs (41 markers as 3 groups of SNPs are linked) assayed in 84 sockeye populations from southeast Alaska, British Columbia, and Washington was developed by the ADF&G (Habicht et al, 2010). All the baseline SNP markers, with the exception of locus *One_Serpin* which failed during genotyping, were evaluated in the mixtures. Stock proportions were estimated using a Bayesian mixture analysis.

METHODS

Genetic baseline and population grouping

Genetic samples from 84 baseline stocks (Table 1) were collated by ADF&G in collaboration with many other laboratories including ABL and the Canadian Department of Fisheries and Oceans. The 84 populations were grouped into 13 regions (Table 2)

based on manager needs to match the scale pattern analysis groupings, geographical location and historical knowledge.

Sample Collection

Matched genetic and scale samples were collected by port samplers from ADF&G. Samples were collected from the District 101 GNF and from the District 104 PSF. Genetic samples were clipped AXP that were stored in ethanol. The genetic samples were shipped to ABL for analysis and stored at room temperature. ADF&G collected genetic and scale samples from a maximum of 269 (Table 4) fish per statistical week for each district, of which most were successfully analyzed (Table 4).

DNA Extraction

DNA was extracted from the AXP 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.). In brief, small pieces of tissue (~20 mg) were excised from ethanol-stored axillary processes. The tissue pieces were digested in a proteinase solution for 3 hours at 55°C. Protease digestions were performed in 96 well plates. After digestion, the samples were purified with a Corbett X-tractor robot producing eluted DNA which was stored at -20 °C.

Single Nucleotide Polymorphism (SNP)

Analysis

SNP genotyping was performed using Taqman chemistries (Applied Biosciences, Inc.) for 45 previously identified sockeye SNP markers (Table 3) (Elfstrom et al., 2006; Smith et al., 2005b; Habicht et al., 2007, 2010), 44 of which were assayed in this analysis. The remaining assay, *One_serp*in was excluded due to poor resolution.

Taqman reactions were performed by transferring 1 μ l of a 1:10 dilution of the eluted purified DNA to wells of a 384 well plate. Four wells were reserved for non-template controls. Each Taqman reaction was conducted in a 5 μ l volume containing the

Table 3. 45 SNP assays used to discriminate Northern Boundary sockeye populations.

#	Name	Comments
1	<i>One_ACBP-79</i>	
2	<i>One_ALDOB-135</i>	
3	<i>One_CO1 (mitochondrial)</i>	linked with 5&6
4	<i>One_ctgf-301</i>	
5	<i>One_Cytb_17 (mitochondrial)</i>	linked with 3&6
6	<i>One_Cytb_26 (mitochondrial)</i>	linked with 3&5
7	<i>One_E2-65</i>	
8	<i>One_GHII-2165</i>	
9	<i>One_GPDH-201</i>	linked with 10
10	<i>One_GPDH2-187</i>	linked with 9
11	<i>One_GPH-414</i>	
12	<i>One_hsc71-220</i>	
13	<i>One_HGFA-49</i>	
14	<i>One_Hpal-71</i>	
15	<i>One_Hpal-99</i>	
16	<i>One_IL8r-362</i>	
17	<i>One_KPNA-422</i>	
18	<i>One_LEI-87</i>	
19	<i>One_MARCKS-241</i>	
20	<i>One_MHC2_190</i>	linked with 21
21	<i>One_MHC2_251</i>	linked with 20
22	<i>One_Ots213-181</i>	
23	<i>One_p53-534</i>	
24	<i>One_ins-107</i>	
25	<i>One_Prl2</i>	
26	<i>One_RAG1-103</i>	
27	<i>One_RAG3-93</i>	
28	<i>One_RFC2-102</i>	
29	<i>One_RFC2-285</i>	
30	<i>One_RH2op-395</i>	
31	<i>One_serp</i> in-75	not resolved
32	<i>One_STC-410</i>	
33	<i>One_STR07</i>	
34	<i>One_Tf_ex11-750</i>	
35	<i>One_Tf_in3-182</i>	
36	<i>One_U301-92</i>	
37	<i>One_U401-224</i>	
38	<i>One_U404-229</i>	
39	<i>One_U502-167</i>	
40	<i>One_U503-170</i>	
41	<i>One_U504-141</i>	
42	<i>One_U508-533</i>	
43	<i>One_VIM-569</i>	
44	<i>One_ZNF-61</i>	
45	<i>One_Zp3b-49</i>	

template DNA, Taqman Universal PCR Mastermix, No AmpErase UNG (ABI), 900 nm of each PCR primer, and 200 nm probe. Thermal cycling was performed on a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems, Inc.) using the protocol from Habicht et al. (2010).

Allele Scoring

After amplification, the Taqman genotyping reactions were assayed on an ABI PRISM 7900HT Sequence Detection System and scored using Sequence Detection Software 2.2 (Applied Biosciences, Inc.). Individual genotypes were imported into our genetic database developed with Progeny software (Progeny, Inc.).

Mixture Analysis

A mixture analysis using a Bayesian estimation method (Pella and Masuda, 2001) was implemented using Bayes software and was performed for each weekly mixture sample and each district. For each BAYES analysis, 13 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 0.011905 (calculated as 1/84) was used for all 84 populations. Convergence of chains to posterior distributions of stock proportions was determined with Gelman and Rubin shrink factors (Gelman and Rubin 1992), and the first one-half of chains was discarded as burn-in before summarizing posterior distributions.

RESULTS

In 2009, 69,834 sockeye salmon were harvested in District 101 GNF which is less than the historical average of 87,345 (Table 4). In the District 104 PSF, 109,365 fish were harvested in 2009 which is approximately a third of the historical average (Table 5). Sockeye salmon DNA was isolated (Table 4&5) and genotyped for 44 SNP markers from 4,402 fish in 2009. The data was imported into a Progeny database for analysis. Samples

Table 4. Sockeye salmon harvested, genetic sample size, and genotyping success rate in each statistical week in the 2009 District 101 gillnet fishery (<http://dungie.adfg.state.ak.us:8080/CatchByMultiYear.po>).

District 101 Gillnet						
Week	2009	Historical Avg.	Extracted	Analyzed	% Analyzed	
26	15,177	13,583	260	258	99.2	
27	9,505	17,450	260	259	99.6	
28	8,494	11,725	260	259	99.6	
29	7,999	7,752	260	258	99.2	
30	7,731	8,186	260	258	99.2	
31	10,113	6,121	260	259	99.6	
32	6,269	8,138	260	259	99.6	
33	2,186	3,772	260	258	99.2	
34	1,141	1,196	269	269	100.0	
35	710	1,127	240	240	100.0	
36	397	557	187	185	98.9	
37	121	264	66	64	97.0	
Total	69,843	87,345	2,842	2,826	99.4	

Table 5. Sockeye salmon harvested, genetic sample size, and genotyping success rate in each statistical week in the 2009 District 104 purse seine fishery (<http://dungie.adfg.state.ak.us:8080/CatchByMultiYear.po>).

District 104 Purse Seine						
Week	2009	Historical Avg.	Extracted	Analyzed	% Analyzed	
28	914	22,925	0	0	0.0	
29	3,097	31,259	260	260	100.0	
30	11,960	88,050	260	260	100.0	
31	50,177	73,559	260	259	99.6	
32	7,288	46,660	146	145	99.3	
33	18,947	51,409	260	259	99.6	
34	10,410	8,606	244	244	100.0	
35	6,578	5	130	130	100.0	
Total	109,365	322,473	1,560	1,557	99.8	

resolved for at least 38 of the 44 SNPs were included in the analyses (i.e. % analyzed in Tables 4&5).

Stock Mixture Proportions

Weekly mixture samples were analyzed with Bayes software. In all analyses, the Gelman and Rubin shrink factors were less than 1.1, indicating convergence of the chains to posterior distributions. Results from this analysis are presented in both graphical form (Figure 2) and Table form (Tables 6&7). Figure 2 graphically illustrates the estimated proportions of sockeye salmon endemic to each of the 13 regions that were harvested in each district and statistical week. Tables 6 and 7 provide the same data shown in Figure 2 for the 2009 101 GNF and 104 PSF respectively, in numerical format showing the estimated stock group proportions, standard errors, and 95% probability intervals.

Analysis of the stock proportions of sockeye caught in Districts 101 GNF and 104 PSF over varying weeks shows interesting trends. For example, the sockeye commercial fishery in the 2009 District 101 GNF predominantly harvests Nass Region fish early in the season (83%), but by week 34, this stock decreased to 26% of the catch. These fish were replaced with Skeena, Southern Southeast (SSE) Alaska and Hugh Smith fish. Skeena stocks increased from less than 2% in week 26 to 54% in week 35. Hugh Smith stocks increased from less than 1% in week 26 to 14% in week 32. The SSE Alaska and McDonald peaked at 13% and 14 % respectively in week 33.

In the 2009 District 104 PSF, Skeena region stocks predominated throughout the entire fishery ranging from a high of 55% in week 31 to a low of 23% in week 35. The SSE Alaska region was the most abundant of the rest with 30% in week 30 dipping to 7% in week 35. The Fraser proportion increased from 1% to 61% during the same time period, although week 35 only represented 6% of the total catch.

The proportion estimates were used to estimate numbers of fish caught from each region for each fishery (Table 8). The small discrepancies between total numbers of fish in Tables 5 and 8 were due to rounding errors in estimating numbers of fish caught from estimated stock group proportions. Also there were no genetic samples obtained from District 104 PSF in week 28 (Table 5); therefore this week was not represented in the regional estimates in Table 8. Table 8 also shows the estimated number of fish caught per region prior to week 31. The Pacific Salmon Treaty allows for the harvest of a fixed percentage of Nass (for District 101) and Nass/Skeena (for District 104) sockeye prior to week 31.

DISCUSSION

Chapter 2 of the 1999 Pacific Salmon Treaty specifies U.S. and Canada harvest sharing arrangements of Nass and Skeena River sockeye salmon in Northern Boundary fisheries. In Alaska's District 101 and District 104 sockeye fisheries, the United States is allowed to harvest a fixed percentage of the annual allowable harvest (AAH) of Nass and Skeena River sockeye. Estimates of the stock-specific catch in these commercial fisheries are currently being provided by ADF&G using scale pattern analysis (SPA). This technique has been shown to be accurate, but requires the collection of yearly scale patterns to determine the year specific baseline for individual rivers. This is because annual fluctuations in environmental conditions can dramatically affect scale patterns.

In comparison to SPA, genetic analysis has the potential for greatly increasing the precision and accuracy of stock composition estimates in the District 101 and 104 fisheries. An additional advantage of using DNA markers is that in-season results can theoretically be provided to fishery managers because, unlike SPA, it does not require annual baseline sampling. Importantly, a SNP baseline with good coverage has already been developed by the ADF&G for Southeast Alaska and British Columbia. ADF&G and ABL are continuously updating the baseline by adding new populations and developing new markers. ADF&G made the most current sockeye baseline available to the ABL/TSMRI Genetics group for use in this analysis.

Genetic markers are more stable than scale patterns and are not normally influenced by small environmental changes in short periods of time. Differences in allele frequencies in groups of genetic markers can be used to distinguish individual stocks of fish. These allele frequency differences can be reflective of the adaptive measures taken by unique stocks of fish to thrive in different environmental conditions, although these changes can often take many generations. Genetic stock identification is a powerful technique that takes advantage of these genetic differences to discriminate stocks of fish caught in a mixed stock fishery.

Auke Bay Laboratories has completed its genetic analysis of sockeye salmon caught in Districts 101 gillnet and District 104 purse seine fisheries for 2009. It should be recognized that while a total of 45 SNPs (41 markers) are currently used in the Southeast Alaska-British Columbia baseline, not all SNPs are likely to be equally informative. A thorough analysis of the effectiveness of combinations of SNPs to resolve sockeye in southeast Alaska and British Columbia could help reduce the numbers of SNPs that need to be assayed to obtain the same resolution.

CONCLUSION

Our results indicate that a majority of sockeye salmon caught in the ADF&G District 101 gillnet and District 104 purse seine fisheries originated from Canadian stocks in 2009. Our results are in general agreement with the mark-recapture studies completed in the early 1980's (Pella et al., 1993), scale pattern analyses completed since 1982 (Marshall, 1984), allozyme/freshwater age/parasitism analyses completed in the late 1980's (Pella et al., 1998), and SNP based genetic stock composition analyses completed since 2004. These correlations strongly suggest that all stock assessment methods have produced accurate and meaningful results in the management of these Northern Boundary fisheries. Compared with other methods, SNP genotyping is the most efficient method for stock assessment since it can be partially automated and the baseline does not require annual resampling. These advantages make it possible to use SNP markers to determine stock composition in a quicker time interval, allowing for improved management of the Northern Boundary fisheries. The similarity between stock composition estimates produced using scale pattern analysis and genetic analysis helps validate both approaches for determining stock assessments (Oliver 2009, Guthrie et al. 2009).

ACKNOWLEDGMENTS

The Stock Identification and Analysis group at the Auke Bay Laboratories/Ted Stevens Marine Research Institute worked closely with many people within the Alaska Department of Fish and Game during the completion of this study. We would like to particularly thank Glen Oliver, Anne Reynolds, Jeff Rice, John Wilcox, Bill Bale, and others (ADF&G-Southeast) who coordinated the collection of the samples and Sara Gilk and Chris Habicht (ADF&G-Gene Conservation Laboratory) for providing the ADF&G SNP baseline and the group structures used in this study.

REFERENCES

- Beacham, T.D., Wetklo, M., Wallace, C., Olsen, J.B., Flannery, B.G., Wenburg, J.K., Templin, W.D., Antonovich, A., and Seeb, L.W. (2009). The Application of Microsatellites for Stock Identification of Yukon River Chinook Salmon. *N. Am. J. Fish. Manage.* 28, 283-295.
- Elfstrom, C.M., Smith, C.T., and Seeb, J.E. (2006). PRIMER NOTE: Thirty-two single nucleotide polymorphism markers for high-throughput genotyping of sockeye salmon. *Mol. Ecol. Notes.* 6, 1255-1259.
- Fournier, D.A., Beacham, T.D., Riddell, B.E., and Busack, C.A. (1984). Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. *Can. J. Fish. Aquat. Sci.* 41, 400-408.
- Guthrie, C., Masuda, M., Nguyen, H., Cheng, W., Wilmot, R.L., and Guyon, J. R., (2009). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2006 and 2007 District 101 Gillnet and District 104 Purse Seine Fisheries. Final Report to the Pacific Salmon commission Northern Fund. (Juneau, AK National Marine Fisheries Service, Alaska Fisheries Science Center, Auke Bay Laboratories), pp22.
- Guthrie, C, Nguyen, H., and Guyon, J. R., (2010). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2008 District 101 Gillnet and District 104 Purse Seine Fisheries. Final Report to the Pacific Salmon commission Northern Fund. (Juneau, AK National Marine Fisheries Service, Alaska Fisheries Science Center, Auke Bay Laboratories), pp19.
- Habicht, C., Guthrie, C., Wilmot, R., and Seeb, J. (2004). Microsatellites, Allozymes, and SNPs Describe the Population Structure and Identify Spatial Distribution of Mixture Components of Sockeye Salmon in the Bering Sea. *NPAFC Tech. Rep.* No 5, 62-64.

- Habicht, C., Templin, W.D., Willette, T.M., Fair, L.F., Raborn, S.W., and Seeb, L.W. (2007). Post-season stock composition analysis of Upper Cook Inlet sockeye salmon harvest, 2005-2007. ADF&G Fish. Man. No 07-07.
- Habicht, C., L.W. Seeb, K. W. Myers, E. V. Farley, Jr., and J. E. Seeb. (2010). Summer fall distribution of stocks of immature sockeye salmon in the Bering Sea as revealed by single-nucleotide polymorphisms. *Trans. of the Amer. Fish. Soc.*, 139:1171 – 1191.
- Marshall, S.L., Oliver, G.T., Bernard, D.R., and McPherson, S.A. (1984). The accuracy of scale pattern analysis in separating major stocks of sockeye salmon (*Oncorhynchus nerka*) from southern Southeastern Alaska and northern British Columbia. Alaska Department of Fish and Game, Division of Commercial Fisheries, Informational Leaflet 230, Juneau.
- Milner, G.B., Teel, D.J., Utter, F.M., and Winans, G.A. (1985). A genetic method of stock identification in mixed populations of Pacific salmon, *Oncorhynchus* spp. *Marine Fish. Rev.* 47, 1-8.
- Oliver, G.T. (2009). Northern Boundary Area Genetic Stock ID -final report for northern fund cooperative agreement ADF&G IHG 05-006; NF NP1. Alaska Department of Fish and Game, Pacific Salmon Commission, Northern Fund Final Report.
- Pella, J., Hoffman, M., Hoffman, S., Masuda, M., Nelson, S., and Talley, L. (1993). Adult sockeye and pink salmon tagging experiments for separating stocks in northern British Columbia and southern Southeast Alaska, 1982-1985. NOAA Tech. Memo. NMFS-AFSC-18.
- Pella, J., and Masuda, M. (2001). Bayesian methods for analysis of stock mixtures from genetic characters. *Fish. Bull.* 99, 151-167.
- Pella, J., Masuda, M., Guthrie III, C., Kondzela, C., Gharrett, A.J., Moles, A., and Winans, G.A. (1998). Stock composition of some sockeye salmon, *Oncorhynchus nerka*, catches in southeast Alaska, based on incidence of allozyme variants, freshwater ages, and a brain-tissue parasite (NMFS). NOAA Tech. Rep. NMFS 132.
- Pella, J.J., and Milner, G.B. (1987). Use of genetic marks in stock composition analysis. *In* Population genetics and fisheries management (N. Ryman and F. Utter, eds.), p. 247-276. Univ. Washington Press, Seattle, WA.
- Smith, C.T., Templin, W.D., Seeb, J.E., and Seeb, L.W. (2005a). Single Nucleotide Polymorphisms Provide Rapid and Accurate Estimates of the Proportions of U.S. and Canadian Chinook Salmon Caught in Yukon River Fisheries. *N. Am. J. Fish. Manage.* 25, 944-953.

Smith, C.T., Elfstrom, C.M., Seeb, L.W., and Seeb, J.E. (2005b). Use of sequence data from rainbow trout and Atlantic salmon for SNP detection in Pacific salmon. *Mol. Ecol.* *14*, 4193-4203.

Smith, C.T., Antonovich, A., Templin, W.D., Elfstrom, C.M., Narum, S.R., and Seeb, L.W. (2007). Impacts of Marker Class Bias Relative to Locus-Specific Variability on Population Inferences in Chinook Salmon: A Comparison of Single-Nucleotide Polymorphisms with Short Tandem Repeats and Allozymes. *Trans. Am. Fish. Soc.* *136*, 1674-1687.

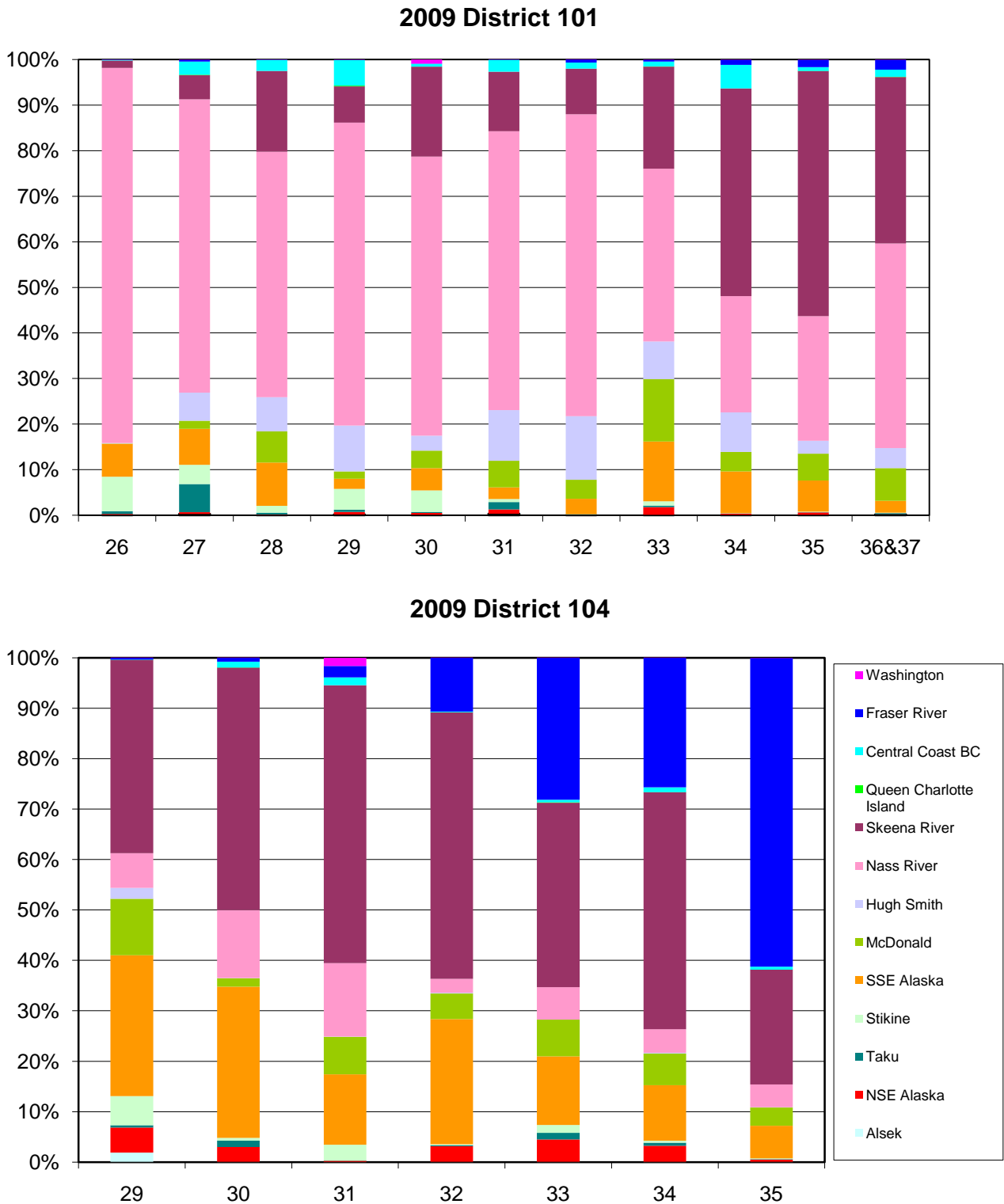


Figure 2. 2009 sockeye stock group proportions for each statistical week from the ADF&G District 101 gillnet (top panel) and 104 purse seine fisheries (lower panel).

Table 6. Parameters of the posterior densities for population region proportions composing weekly mixtures of the 2009 District 101 commercial gillnet sockeye fishery.

	Week 26			Week 27			Week 28			Week 29		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alesek	0.1	0.38	(0.0,1.3)	0.3	0.51	(0.0,1.8)	0.0	0.12	(0.0,0.3)	0.1	0.41	(0.0,1.4)
NSE Alaska	0.1	0.32	(0.0,1.1)	0.4	0.54	(0.0,1.9)	0.2	0.43	(0.0,1.5)	0.6	1.09	(0.0,3.9)
Taku	0.6	1.61	(0.0,6.0)	6.2	3.84	(0.0,13.3)	0.3	0.95	(0.0,3.5)	0.5	0.97	(0.0,3.4)
Stikine	7.6	3.09	(0.8,13.2)	4.2	2.83	(1.4,12.4)	1.5	1.33	(0.3,5.5)	4.5	2.44	(0.9,9.7)
SSE Alaska	7.2	2.17	(3.3,11.8)	7.9	2.40	(3.5,13.0)	9.5	2.40	(5.2,14.6)	2.3	1.63	(0.1,6.2)
McDonald	0.1	0.35	(0.0,0.8)	1.8	2.44	(0.0,7.8)	6.8	3.62	(0.0,13.8)	1.6	2.13	(0.0,6.8)
Hugh Smith	0.1	0.37	(0.0,1.0)	6.2	2.23	(2.2,10.9)	7.5	4.00	(0.0,16.5)	10.1	2.69	(5.0,15.4)
Nass River	82.4	2.67	(76.8,87.3)	64.4	3.27	(57.8,70.6)	53.9	5.62	(42.1,63.3)	66.5	4.49	(55.3,73.6)
Skeena River	1.6	1.35	(0.0,5.0)	5.3	1.96	(2.1,9.7)	17.7	5.23	(9.9,29.6)	8.0	3.75	(3.5,18.4)
Queen Charlotte I.	0.0	0.04	(0.0,0.0)	0.1	0.27	(0.0,0.9)	0.0	0.04	(0.0,0.0)	0.2	0.41	(0.0,1.4)
Central Coast BC	0.1	0.45	(0.0,1.6)	2.9	2.22	(0.0,7.5)	2.4	1.41	(0.0,5.6)	5.6	1.79	(2.5,9.5)
Fraser River	0.0	0.13	(0.0,0.4)	0.4	0.65	(0.0,2.2)	0.1	0.17	(0.0,0.5)	0.1	0.26	(0.0,0.9)
Washington	0.0	0.16	(0.0,0.4)	0.0	0.14	(0.0,0.3)	0.0	0.10	(0.0,0.2)	0.0	0.13	(0.0,0.2)

	Week 30			Week 31			Week 32			Week 33		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alesek	0.0	0.14	(0.0,0.3)	0.3	0.42	(0.0,1.5)	0.0	0.10	(0.0,0.2)	0.1	0.28	(0.0,0.9)
NSE Alaska	0.5	0.68	(0.0,2.4)	1.0	1.01	(0.0,3.4)	0.1	0.28	(0.0,0.9)	1.7	1.59	(0.0,5.3)
Taku	0.2	0.78	(0.0,2.7)	1.6	2.84	(0.0,9.1)	0.0	0.15	(0.0,0.4)	0.3	1.04	(0.0,3.9)
Stikine	4.7	2.41	(0.1,9.8)	0.7	1.63	(0.0,6.0)	0.0	0.16	(0.0,0.4)	1.0	1.88	(0.0,6.5)
SSE Alaska	4.9	2.29	(0.4,9.7)	2.5	1.75	(0.2,6.7)	3.4	1.95	(0.1,7.9)	13.1	3.81	(6.0,21.0)
McDonald	3.8	2.39	(0.0,9.0)	5.9	3.79	(0.0,12.8)	4.2	3.01	(0.0,10.0)	13.7	4.11	(6.3,22.2)
Hugh Smith	3.3	2.29	(0.0,8.0)	11.1	3.59	(5.1,18.9)	14.0	3.82	(7.0,21.6)	8.3	4.86	(0.0,17.7)
Nass River	61.3	3.78	(53.7,68.5)	61.2	3.25	(54.7,67.5)	66.2	3.42	(59.2,72.7)	37.9	3.13	(31.9,44.1)
Skeena River	19.7	3.19	(13.8,26.3)	13.1	2.35	(8.8,18.0)	10.0	2.53	(5.8,15.6)	22.4	2.83	(17.1,28.1)
Queen Charlotte I.	0.0	0.05	(0.0,0.0)	0.0	0.04	(0.0,0.0)	0.0	0.04	(0.0,0.0)	0.0	0.16	(0.0,0.4)
Central Coast BC	0.6	1.25	(0.0,4.4)	2.6	1.96	(0.0,6.6)	1.3	0.84	(0.2,3.4)	1.1	1.58	(0.0,5.1)
Fraser River	0.1	0.18	(0.0,0.6)	0.0	0.13	(0.0,0.4)	0.7	0.56	(0.0,2.1)	0.4	0.55	(0.0,1.9)
Washington	0.9	0.79	(0.0,2.8)	0.0	0.06	(0.0,0.1)	0.0	0.08	(0.0,0.2)	0.0	0.12	(0.0,0.2)

	Week 34			Week 35			Week 36&37		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alesek	0.1	0.41	(0.0,1.4)	0.1	0.39	(0.0,1.3)	0.1	0.53	(0.0,1.9)
NSE Alaska	0.2	0.52	(0.0,1.9)	0.5	0.76	(0.0,2.7)	0.1	0.25	(0.0,0.8)
Taku	0.1	0.21	(0.0,0.6)	0.1	0.27	(0.0,0.8)	0.2	0.63	(0.0,2.3)
Stikine	0.1	0.26	(0.0,0.6)	0.1	0.50	(0.0,1.6)	0.1	0.36	(0.0,1.0)
SSE Alaska	9.2	2.35	(5.0,14.1)	6.8	2.45	(2.6,12.1)	2.6	1.33	(0.5,5.7)
McDonald	4.3	3.53	(0.0,11.6)	5.9	3.50	(0.0,12.3)	7.2	1.97	(3.7,11.4)
Hugh Smith	8.7	3.35	(2.8,15.5)	2.8	3.03	(0.0,10.0)	4.4	1.78	(1.4,8.4)
Nass River	25.5	3.03	(19.8,31.6)	27.4	2.95	(21.8,33.4)	44.9	3.21	(38.7,51.3)
Skeena River	45.6	3.68	(38.4,52.8)	53.8	3.44	(47.0,60.5)	36.6	3.26	(30.3,43.0)
Queen Charlotte I.	0.0	0.04	(0.0,0.0)	0.0	0.05	(0.0,0.0)	0.0	0.05	(0.0,0.0)
Central Coast BC	5.1	1.56	(2.5,8.6)	0.8	1.19	(0.0,3.9)	1.6	1.37	(0.0,4.7)
Fraser River	1.2	0.75	(0.1,2.9)	1.6	0.95	(0.2,3.9)	2.2	1.04	(0.6,4.6)
Washington	0.0	0.12	(0.0,0.3)	0.0	0.14	(0.0,0.3)	0.0	0.11	(0.0,0.2)

Table 7. Parameters of the posterior densities for population region proportions composing weekly mixtures of the 2009 District 104 commercial purse seine sockeye fishery.

	Week 29			Week 30			Week 31			Week 32		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	1.9	1.08	(0.3,4.4)	0.1	0.37	(0.0,1.3)	0.0	0.14	(0.0,0.3)	0.1	0.23	(0.0,0.6)
NSE Alaska	5.0	3.57	(0.6,12.4)	2.9	1.70	(0.6,7.1)	0.2	0.66	(0.0,2.5)	3.1	1.69	(0.7,7.2)
Taku	0.5	1.57	(0.0,6.1)	1.3	1.04	(0.0,3.8)	0.0	0.19	(0.0,0.5)	0.2	0.68	(0.0,2.3)
Stikine	5.8	4.04	(0.1,13.2)	0.5	1.06	(0.0,3.9)	3.2	1.80	(0.0,7.0)	0.2	0.82	(0.0,2.8)
SSE Alaska	27.9	3.38	(21.5,34.7)	30.0	3.50	(23.2,36.9)	13.9	2.69	(9.0,19.6)	24.8	3.95	(17.4,32.8)
McDonald	11.2	3.10	(5.4,17.6)	1.7	1.97	(0.0,6.3)	7.4	2.09	(3.7,11.9)	5.1	2.21	(1.5,10.1)
Hugh Smith	2.2	2.51	(0.0,8.0)	0.0	0.23	(0.0,0.5)	0.1	0.30	(0.0,0.8)	0.1	0.64	(0.0,1.8)
Nass River	6.9	1.89	(3.6,10.9)	13.5	2.36	(9.2,18.4)	14.6	2.47	(10.0,19.7)	2.8	1.58	(0.6,6.6)
Skeena River	38.3	3.17	(32.3,44.7)	48.1	3.25	(41.7,54.5)	55.1	3.23	(48.7,61.4)	52.8	4.35	(44.3,61.4)
Queen Charlotte I.	0.0	0.05	(0.0,0.0)	0.0	0.06	(0.0,0.1)	0.0	0.04	(0.0,0.0)	0.0	0.07	(0.0,0.1)
Central Coast BC	0.0	0.15	(0.0,0.2)	1.2	1.19	(0.0,4.0)	1.6	1.03	(0.0,3.9)	0.2	0.65	(0.0,2.2)
Fraser River	0.4	0.56	(0.0,1.9)	0.7	0.67	(0.0,2.4)	2.2	1.00	(0.7,4.5)	10.6	2.77	(5.8,16.5)
Washington	0.0	0.15	(0.0,0.4)	0.0	0.07	(0.0,0.1)	1.6	0.99	(0.0,3.9)	0.0	0.20	(0.0,0.4)

	Week 33			Week 34			Week 35		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	0.0	0.14	(0.0,0.3)	0.1	0.31	(0.0,0.9)	0.0	0.22	(0.0,0.5)
NSE Alaska	4.5	1.77	(1.1,8.3)	3.2	2.02	(0.0,7.3)	0.5	0.84	(0.0,2.9)
Taku	1.3	1.97	(0.0,6.4)	0.6	1.43	(0.0,5.2)	0.1	0.44	(0.0,1.1)
Stikine	1.5	1.99	(0.0,6.3)	0.4	1.27	(0.0,4.9)	0.1	0.60	(0.0,1.4)
SSE Alaska	13.6	2.43	(9.1,18.7)	11.0	2.67	(6.2,16.6)	6.5	2.66	(2.0,12.4)
McDonald	7.3	1.99	(3.8,11.5)	6.3	2.08	(2.6,10.7)	3.7	2.27	(0.0,8.8)
Hugh Smith	0.0	0.12	(0.0,0.2)	0.2	0.56	(0.0,1.9)	0.1	0.28	(0.0,0.7)
Nass River	6.4	1.89	(3.2,10.6)	4.7	1.45	(2.2,7.9)	4.5	1.92	(1.5,8.9)
Skeena River	36.5	3.22	(30.3,43.0)	47.0	3.33	(40.5,53.5)	22.7	4.00	(15.4,31.0)
Queen Charlotte I.	0.1	0.25	(0.0,0.9)	0.0	0.06	(0.0,0.0)	0.0	0.08	(0.0,0.1)
Central Coast BC	0.5	1.00	(0.0,3.5)	0.9	1.46	(0.0,4.8)	0.6	1.60	(0.0,5.9)
Fraser River	28.1	2.99	(22.4,34.2)	25.7	2.95	(20.1,31.7)	61.2	4.53	(52.1,69.8)
Washington	0.0	0.12	(0.0,0.2)	0.0	0.12	(0.0,0.2)	0.1	0.32	(0.0,0.7)

Table 8. Estimated numbers of sockeye salmon caught in the 2009 District 101 gillnet and 104 seine fisheries prior to statistical week 31 and throughout all statistical weeks analyzed (see Tables 4&5).

<i>Region</i>	<i>Area</i>	District 101 Gillnet		District 104 Seine	
		<i>Prior to 31</i>	<i>Total</i>	<i>Prior to 31</i>	<i>Total</i>
1	Alsek	62	100	71	105
2	NSE Alaska	154	301	504	2,063
3	Taku	769	945	163	515
4	Stikine	2,406	2,501	242	2,179
5	SSE Alaska	3,214	4,136	4,454	17,407
6	McDonald	1,180	2,461	544	6,915
7	Hugh Smith	2,297	4,621	72	135
8	Nass River	33,258	45,145	1,828	11,351
9	Skeena River	4,411	7,941	6,937	51,744
10	Queen Charlotte I.	21	22	2	21
11	Central Coast BC	992	1,436	139	1,165
12	Fraser River	64	153	100	14,021
13	Washington	79	82	2	832
Totals		48,908	69,843	15,056	108,452