

Pacific Salmon Commission
Northern Fund

**Northern Boundary Area Sockeye Salmon Genetic Stock Identification
For Year 2013 District 101 Gillnet and District 104 Purse Seine Fisheries**

Final Report

Charles M. Guthrie III
Hanhvan Nguyen
Jeffrey R. Guyon

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

Submitted June 1, 2015

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
8-11	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>
11-12	RESULTS
	<i>Stock Mixture Proportions</i>
12-13	DISCUSSION
13	CONCLUSION
13	ACKNOWLEDGEMENTS
13-16	REFERENCES
17-20	ADDITIONAL TABLES AND FIGURES

LIST OF FIGURES

Page	Figures
6	Figure 1. Geographic location of ADF&G fishing districts 101 and 104.
17	Figure 2. 2013 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 mixed stock analysis.
8	Table 2. Regional grouping of populations for stock composition analysis.
9	Table 3. 45 SNP assays used to discriminate Northern Boundary sockeye populations.
10	Table 4. Sockeye salmon harvested, genetic sample size, genotyping success rate, and percent catch sampled in each statistical week in the 2013 District 101 gillnet fishery.
10	Table 5. Sockeye salmon harvested, genetic sample size, genotyping success rate, and percent catch analyzed in each statistical week in the 2013 District 104 purse seine fishery.
18	Table 6. Stock composition estimates of weekly mixtures of sockeye salmon in the 2013 District 101 commercial gillnet sockeye fishery.
19	Table 7. Stock composition estimates of weekly mixtures of sockeye salmon in the 2013 District 104 commercial purse seine sockeye fishery.
10	Table 8. Estimated numbers of sockeye salmon caught in the 2013 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 (*Oncorhynchus nerka*) 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 (*O. gorbuscha*) 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 recapturing them at various weirs and other 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. SPA was used by ADF&G to determine stock proportions for sockeye salmon caught in the commercial sockeye fisheries in districts 101 and 104 until 2012.

While effective, SPA required yearly examination of source populations for each of the four major age classes (1.2, 1.3, 2.2 and 2.3) since the scale baseline patterns are strongly affected by varying environmental conditions. The requirement to reestablish or revalidate the scale pattern baseline was expensive and burdensome. The use of more stable markers has eliminated 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 the knowledge available to manage area fisheries.

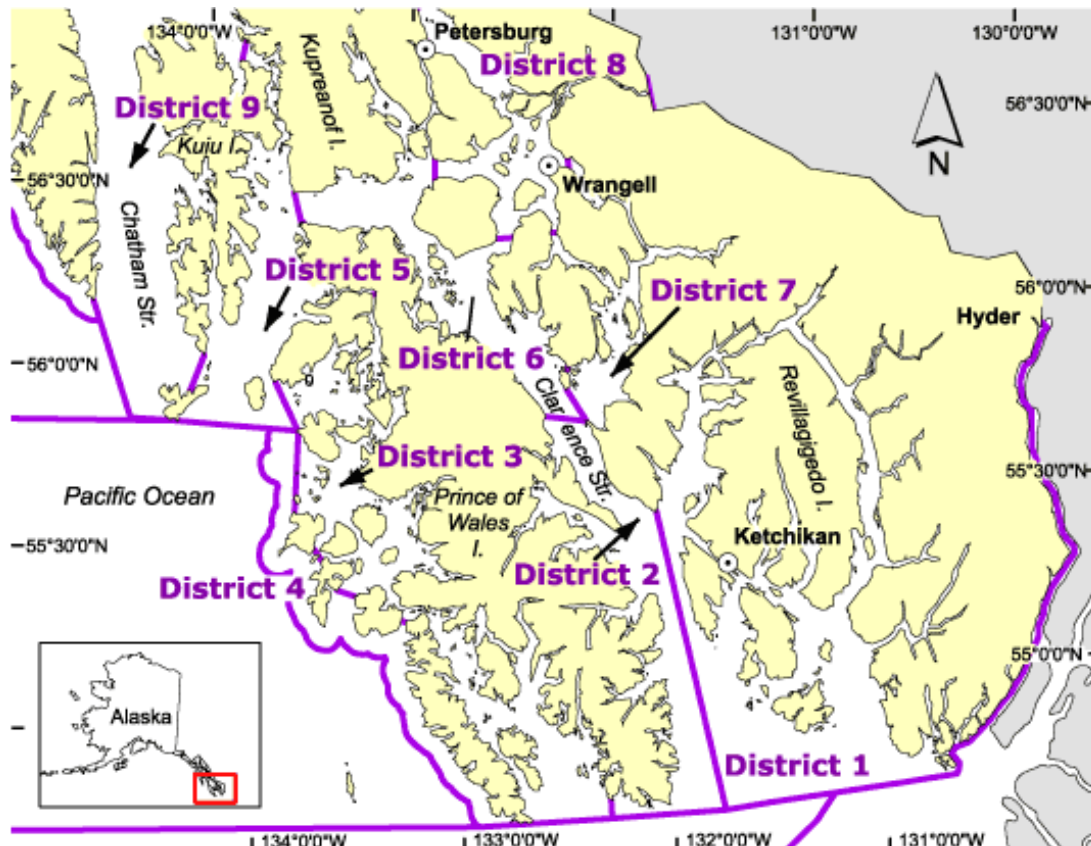


Figure 1. Geographic location of ADF&G commercial fishing districts 101 (labeled District 1) and 104 (labeled District 4). Map obtained from the ADF&G web page (<http://www.cf.adfg.state.ak.us/region1/finfish/salmon/maps/ketchikan.php>).

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 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

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

Table 1. Sockeye salmon baseline populations used in mixed stock 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 - Kluksu 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	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 - Etoin 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

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).

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 ADF&G in 2004 and 2005; and by NOAA/NMFS/Alaska Fishery Science Center/Auke Bay Laboratories (ABL) in 2006-12 (Guthrie et al. 2009, 2010, 2011, 2012, 2013, 2014) 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).

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 of run reconstruction modeling by the bilateral Northern Boundary Technical Committee. 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. Congruence was found between the two techniques, so genetic analysis replaced SPA for estimating stock composition of sockeye salmon caught in Northern Boundary fisheries in 2012. A blind testing study performed determined genetic markers are the viable method to replace SPA (Oliver personal communication, 2011).

Table 2. Regional grouping of populations for stock composition analysis.

<i>Region</i>	<i>Area</i>
1	Alesek
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 3,608 sockeye salmon harvested in the 2013 District 101 gillnet and District 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 and British Columbia, and Washington was developed by ADF&G (Habicht et al, 2010).

METHODS

Genetic baseline and population grouping

Genetic samples from 84 baseline stocks (Table 1) were collected 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, the SPA 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 299 (Table 4&5) fish per statistical week for each district, of which over 98% were successfully analyzed (Table 4&5).

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 at least 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 from Life Technologies for 45 previously identified sockeye SNP probes. Of the 45 sockeye SNP markers (Table 3) (Elfstrom et al., 2006; Smith et al., 2005b; Habicht et al., 2007, 2010), 44 were assayed in this analysis. The remaining assay, *One_serpin* was excluded due to poor resolution. Taqman reactions were performed by two methods: OpenArray (OA) and 384 well plate. OA Taqman reactions were performed by

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_HpaI-71</i>	
15	<i>One_HpaI-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_serpin-75</i>	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>	

transferring 2.5 μ l of the eluted purified DNA and 2.5 μ l of 2x Taqman OA Genotyping Master Mix into the wells of an OA 384 well sample plate. Forty-five through-holes on each of the 48 subarrays of the OpenArray® Digital PCR Plates were loaded with 33 nl of the template DNA using an AccuFill autoloader. Each through-hole was spotted with a different SNP assay. Thermal cycling was performed on the Life Technologies QuantStudio.

Table 4. Sockeye salmon harvested, genetic sample size, genotyping success rate, and percent catch analyzed in each statistical week in the 2013 District 101 Gillnet fishery.

<i>District 101 Gillnet</i>						
<i>Week</i>	<i>2013</i>	<i>2003-2012 Avg.</i>	<i>Extracted</i>	<i>Analyzed</i>	<i>% Analyzed</i>	<i>% Catch</i>
25	9,082	5,616	258	255	98.8	2.8
26	12,186	13,706	258	253	98.1	2.1
27	7,267	15,575	299	293	98.0	4.0
28	5,507	8,874	261	259	99.2	4.7
29	6,559	6,970	259	245	94.6	3.7
30	5,790	6,748	259	244	94.2	4.2
31	5,130	6,442	260	254	97.7	5.0
32	1,684	6,731	236	229	97.0	13.6
33	662	3,339	182	174	95.6	26.3
34	477	1,272	136	135	99.3	28.3
35	154	1,158	0	0	0.0	0.0
36	55	617	0	0	0.0	0.0
37	16	261	0	0	0.0	0.0
38	8	131	0	0	0.0	0.0
39	1	16	0	0	0.0	0.0
Total Catch	54,578	77,453				4.3
Sampled Catch	54,344	75,271	2,408	2,341	97.2	4.3

Table 5. Sockeye salmon harvested, genetic sample size, genotyping success rate, and percent catch analyzed in each statistical week in the 2013 District 104 Purse Seine fishery.

<i>District 104 Purse Seine</i>						
<i>Week</i>	<i>2013</i>	<i>2003-2012 Avg.</i>	<i>Extracted</i>	<i>Analyzed</i>	<i>% Analyzed</i>	<i>% Catch</i>
27	0	878	0	0	0.0	0.0
28	5,152	3,762	160	160	100.0	3.1
29	3,250	10,625	120	119	99.2	3.7
30	4,700	27,072	180	180	100.0	3.8
31	11,408	59,209	180	177	98.3	1.6
32	15,995	65,827	160	158	98.8	1.0
33	25,454	42,719	260	259	99.6	1.0
34	10,873	46,727	140	138	98.6	1.3
35	5,202	8,604	0	0	0.0	0.0
36	848	246	0	0	0.0	0.0
Total Catch	82,882	265,669				1.4
Sampled Catch	76,832	255,941	1,200	1,191	99.3	1.6

Samples not successfully scored on OA were genotyped on 384 well plates. 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 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 ABI Dual 384-Well GeneAmp PCR System 9700 using the protocol from Habicht et al. (2010).

Allele Scoring

After amplification, the Taqman genotyping reactions were assayed on a Life Technologies QuantStudio and scored using QuantStudio 12K Flex Software v1.2.2. 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. Most Monte Carlo chain lengths were 10,000; one chain length was 250,000 to obtain convergence. In one instance two reporting groups were combined to obtain convergence.

RESULTS

In 2013, 54,578 sockeye salmon were harvested in District 101 GNF which is less than the 2003 to 2012 average of 77,453 (Table 4). In the District 104 PSF 82,882 fish were harvested in 2013 which is 31% of the 2003-2012 average of 265,669 (Table 5). Sockeye salmon DNA was isolated (Table 4&5) and genotyped for 44 SNP markers from 3,608 fish in 2013. The data was imported into a Progeny database for analysis. Samples 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 of the analyses, the Gelman and Rubin shrink factors were less than 1.2, indicating convergence of the chains to posterior distributions. In one instance two reporting groups were combined to obtain convergence. 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 in numerical format showing the estimated stock group proportions, standard errors, and 95% credible intervals for the 2013 101 GNF and 104 PSF respectively.

Analysis of the stock proportions of sockeye caught in districts 101 GNF and 104 PSF over varying weeks shows interesting trends (Tables 6&7). For example, the sockeye commercial fishery in the 2013 District 101 GNF harvested a greater proportion of Nass Region fish; with a high of 82% in week 26, and a low of 31% in week 33. The two SSE Alaska highs were 18% in week 32 and 15% in week 33. Hugh Smith and Central Coast BC had peaks in week 28 at 16.4% and 15.8% respectively, while Stikine and Skeena stocks peaked at 16% and 15% in week 33 respectively. McDonald Lake's high was 17% in week 34.

The sockeye commercial fishery in the 2013 District 104 PSF harvested a greater proportion of Fraser River fish late in the season; a high of 85% in week 33, followed by 77% in week 34 and 62% in week 32. SSE Alaska and Skeena fish were abundant before week 32 with highs of 30% and 28% in week 30 respectively.

The proportion estimates were used to estimate numbers of fish caught from each region for each fishery (Table 8). The two fish discrepancy in Table 4, and the 1 fish discrepancy in Table 5 in the total numbers of fish when compared to Table 8 were due to rounding errors in estimating numbers of fish caught from estimated stock group proportions. Since there were no genetic samples obtained from District 104 PSF in weeks 35 and 36; and District 101 GNF weeks 35-39 (Table 5); those weeks were not represented in the regional estimates in Table 8. Table 8 also shows the estimated number of fish caught per region prior to Statistical 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 salmon. Estimates of the stock-specific catch in these commercial fisheries were being provided by ADF&G using scale pattern analysis (SPA). This technique was replaced by genetic analysis in 2012.

Genetic markers are more stable than scale patterns and are not normally influenced by small environmental changes in short periods of time. Allelic frequency differences of genetic markers can be used to distinguish individual stocks of fish. These allele frequency differences can be reflective of 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 2013. 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 GNF and District 104 PSF originated from Canadian stocks in 2013. Our results are in general agreement with the mark-recapture studies completed in the early 1980's (Pella et al., 1993), SPA 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 Genetics Program 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 Anne Reynolds, and others (ADF&G-Southeast) who coordinated the collection of the samples; Andy Piston for providing catch data; 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. (2008). 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.

- Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. *Stat. Sci.* 7:457–511.
- 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.
- Guthrie, C, Nguyen, H., and Guyon, J. R., (2011). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2009 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.
- Guthrie, C, Nguyen, H., and Guyon, J. R., (2012). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2010 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.
- Guthrie, C, Nguyen, H., and Guyon, J. R., (2013). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2011 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.
- Guthrie, C, Nguyen, H., and Guyon, J. R., (2014). Northern Boundary Area Sockeye Salmon Genetic Stock Identification For Year 2012 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 Technical Report 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 Fishery Manuscript 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. *Transactions of the American Fisheries Society*, 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.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.
- 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 Technical Memorandum NMFS-AFSC-18.
- 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 Technical report NMFS 132.
- Pella, J., and Masuda, M. (2001). Bayesian methods for analysis of stock mixtures from genetic characters. *Fish. Bull.* 99, 151-167.
- 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.

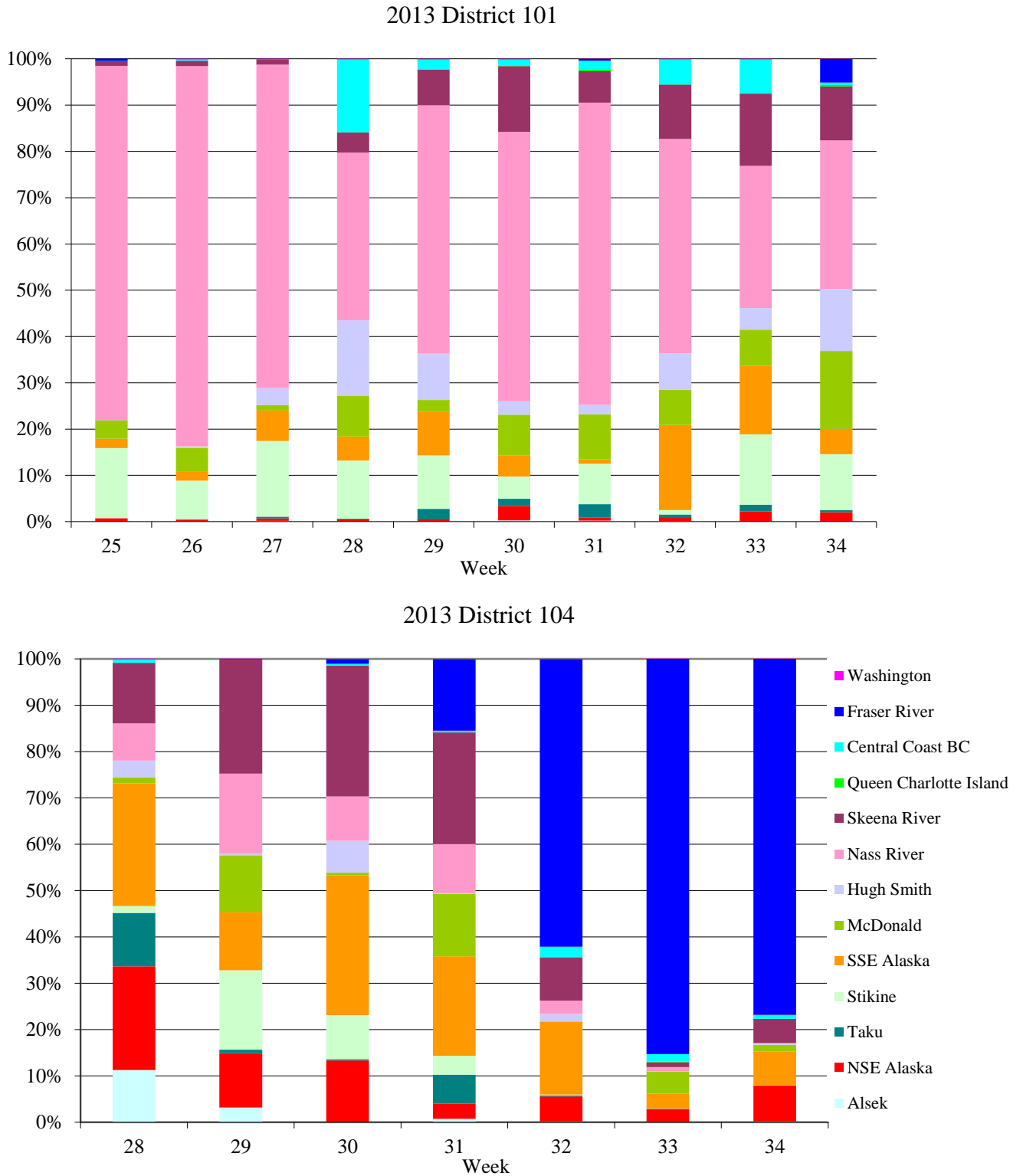


Figure 2. 2013 sockeye stock group proportions for each statistical week from the ADF&G District 101 gillnet (top panel) and 104 purse seine fisheries (lower panel).

Table 6. Stock composition of weekly mixtures of sockeye salmon the 2013 District 101 commercial gillnet fishery.

	Week 25			Week 26			Week 27			Week 28		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	0.1	0.24	(0.0,0.7)	0.1	0.21	(0.0,0.6)	0.2	0.62	(0.0,2.1)	0.1	0.30	(0.0,0.7)
NSE Alaska	0.7	0.97	(0.0,3.3)	0.3	0.58	(0.0,2.0)	0.6	1.01	(0.0,3.6)	0.5	0.94	(0.0,3.3)
Taku	pooled with Stikine			0.2	0.56	(0.0,1.4)	0.4	0.61	(0.0,2.1)	0.1	0.31	(0.0,1.0)
Stikine	15.1	2.67	(10.2,20.6)	8.3	2.24	(4.3,13.1)	16.4	2.97	(10.8,22.4)	12.6	3.45	(6.2,19.7)
SSE Alaska	2.0	1.45	(0.1,5.5)	1.9	1.15	(0.3,4.7)	6.6	2.47	(2.3,11.8)	5.2	2.32	(1.6,10.6)
McDonald	4.0	1.79	(0.9,7.9)	5.2	2.01	(0.0,9.1)	1.2	1.78	(0.0,6.0)	8.7	3.19	(3.1,15.5)
Hugh Smith	0.0	0.11	(0.0,0.2)	0.4	0.93	(0.0,3.5)	3.6	1.97	(0.0,7.6)	16.4	3.30	(10.2,23.2)
Nass River	76.5	2.84	(70.7,81.9)	82.2	2.61	(76.8,87.0)	69.9	2.95	(64.0,75.5)	36.2	3.18	(30.1,42.6)
Skeena River	1.1	0.77	(0.0,3.0)	1.2	0.98	(0.0,3.6)	1.1	0.97	(0.0,3.3)	4.4	1.57	(1.8,7.9)
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	0.05	(0.0,0.0)
Central Coast BC	0.0	0.08	(0.0,0.1)	0.3	0.77	(0.0,2.8)	0.0	0.22	(0.0,0.4)	15.8	3.01	(10.3,22.1)
Fraser River	0.5	0.46	(0.0,1.7)	0.1	0.15	(0.0,0.5)	0.0	0.14	(0.0,0.4)	0.1	0.21	(0.0,0.7)
Washington	0.0	0.00	(0.0,0.0)	0.0	0.10	(0.0,0.2)	0.1	0.36	(0.0,1.3)	0.0	0.14	(0.0,0.3)

	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	0.0	0.18	(0.0,0.4)	0.3	0.73	(0.0,2.7)	0.2	0.53	(0.0,1.9)	0.1	0.41	(0.0,1.4)
NSE Alaska	0.4	0.73	(0.0,2.6)	3.2	2.08	(0.0,7.7)	0.6	1.30	(0.0,4.7)	0.9	0.95	(0.0,3.4)
Taku	2.3	4.01	(0.0,13.8)	1.5	2.39	(0.0,8.6)	2.9	2.72	(0.0,11.4)	0.6	1.33	(0.0,4.7)
Stikine	11.5	4.25	(1.0,18.9)	4.8	3.17	(0.0,11.0)	8.8	3.68	(0.0,15.3)	0.9	1.93	(0.0,6.8)
SSE Alaska	9.4	2.51	(4.9,14.7)	4.5	2.30	(0.4,9.4)	1.0	1.03	(0.0,3.6)	18.4	3.49	(11.6,25.4)
McDonald	2.6	2.80	(0.0,9.0)	8.9	2.95	(3.8,15.1)	9.8	3.43	(3.4,16.4)	7.7	3.88	(0.0,15.8)
Hugh Smith	10.1	2.79	(5.0,15.8)	2.9	2.65	(0.0,8.5)	2.1	2.38	(0.0,7.4)	7.8	3.26	(1.2,14.6)
Nass River	53.7	3.58	(46.6,60.6)	58.2	3.56	(51.1,65.0)	65.3	3.18	(58.9,71.4)	46.3	3.39	(39.7,53.0)
Skeena River	7.7	2.16	(4.0,12.4)	14.2	2.78	(9.3,20.1)	6.8	1.89	(3.6,10.9)	11.8	2.53	(7.2,17.1)
Queen Charlotte I.	0.0	0.07	(0.0,0.1)	0.0	0.08	(0.0,0.1)	0.3	0.39	(0.0,1.4)	0.0	0.06	(0.0,0.0)
Central Coast BC	2.2	1.83	(0.0,6.2)	1.4	1.45	(0.0,4.9)	2.0	1.61	(0.0,5.7)	5.4	2.11	(1.9,10.1)
Fraser River	0.1	0.17	(0.0,0.5)	0.1	0.18	(0.0,0.5)	0.4	0.46	(0.0,1.6)	0.1	0.20	(0.0,0.6)
Washington	0.1	0.27	(0.0,0.9)	0.1	0.25	(0.0,0.9)	0.0	0.06	(0.0,0.1)	0.0	0.15	(0.0,0.3)

	Week 33			Week 34		
	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	0.1	0.28	(0.0,0.8)	0.1	0.29	(0.0,0.6)
NSE Alaska	2.2	1.70	(0.0,6.3)	2.0	1.59	(0.1,6.0)
Taku	1.5	2.88	(0.0,9.9)	0.4	1.99	(0.0,6.8)
Stikine	15.2	4.64	(6.6,24.7)	12.1	5.64	(0.0,22.5)
SSE Alaska	14.9	4.03	(7.8,23.6)	5.5	4.64	(0.0,16.7)
McDonald	7.8	3.97	(1.2,16.5)	16.8	6.22	(6.0,30.1)
Hugh Smith	4.7	3.62	(0.0,12.3)	13.4	6.75	(0.0,26.5)
Nass River	30.8	3.57	(24.0,38.0)	32.1	4.02	(24.5,40.2)
Skeena River	15.6	2.99	(10.2,21.9)	11.6	3.13	(6.2,18.4)
Queen Charlotte I.	0.0	0.13	(0.0,0.2)	0.4	0.74	(0.0,2.6)
Central Coast BC	7.4	3.23	(1.5,14.3)	0.5	1.67	(0.0,6.3)
Fraser River	0.1	0.20	(0.0,0.6)	5.1	1.98	(1.9,9.6)
Washington	0.0	0.11	(0.0,0.2)	0.0	0.16	(0.0,0.2)

Table 7. Stock composition of weekly mixtures of sockeye salmon in the 2013 District 104 commercial purse seine fishery.

	Week 28			Week 29			Week 30			Week 31		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	11.3	2.99	(6.0,17.6)	3.2	2.39	(0.0,9.1)	0.1	0.36	(0.0,1.1)	0.7	1.39	(0.0,4.9)
NSE Alaska	22.4	4.73	(13.5,32.0)	11.7	6.56	(1.3,25.5)	13.3	5.75	(3.0,24.8)	3.3	1.62	(0.9,7.1)
Taku	11.5	4.79	(0.8,20.6)	0.7	2.50	(0.0,9.5)	0.2	0.99	(0.0,2.8)	6.3	5.78	(0.0,17.6)
Stikine	1.5	3.14	(0.0,13.0)	17.2	8.66	(0.0,33.3)	9.5	8.29	(0.0,26.1)	4.0	5.61	(0.0,17.3)
SSE Alaska	26.4	4.75	(17.7,36.3)	12.5	4.72	(4.7,23.1)	30.2	6.10	(18.9,42.7)	21.5	4.42	(13.1,30.4)
McDonald	1.3	3.12	(0.0,11.1)	12.3	4.33	(4.6,21.5)	0.6	1.96	(0.0,7.5)	13.5	3.92	(6.5,21.7)
Hugh Smith	3.6	3.03	(0.0,10.1)	0.3	1.21	(0.0,4.3)	6.9	3.66	(0.0,14.2)	0.1	0.45	(0.0,1.3)
Nass River	8.1	2.53	(3.9,13.7)	17.2	4.34	(8.8,26.0)	9.6	2.88	(4.6,15.8)	10.7	2.49	(6.3,16.0)
Skeena River	13.1	2.89	(7.9,19.2)	24.6	4.58	(16.5,34.5)	28.2	4.03	(20.6,36.4)	24.1	3.39	(17.8,31.0)
Queen Charlotte I.	0.0	0.07	(0.0,0.1)	0.0	0.11	(0.0,0.1)	0.1	0.26	(0.0,0.8)	0.0	0.06	(0.0,0.0)
Central Coast BC	0.8	1.48	(0.0,5.1)	0.0	0.33	(0.0,0.4)	0.4	1.12	(0.0,4.2)	0.4	1.41	(0.0,5.3)
Fraser River	0.1	0.21	(0.0,0.7)	0.1	0.30	(0.0,0.9)	1.0	0.87	(0.0,3.2)	15.4	2.82	(10.3,21.3)
Washington	0.0	0.12	(0.0,0.2)	0.0	0.15	(0.0,0.3)	0.1	0.36	(0.0,1.2)	0.1	0.35	(0.0,1.2)

	Week 32			Week 33			Week 34		
	Mean	SD	95% PI	Mean	SD	95% PI	Mean	SD	95% PI
Alsek	0.2	0.70	(0.0,2.5)	0.1	0.40	(0.0,1.4)	0.2	0.57	(0.0,1.9)
NSE Alaska	5.4	3.63	(0.1,12.8)	2.8	2.30	(0.0,7.5)	7.8	3.50	(1.0,15.1)
Taku	0.2	0.88	(0.0,2.0)	0.1	0.21	(0.0,0.5)	0.1	0.38	(0.0,0.8)
Stikine	0.2	0.89	(0.0,2.4)	0.1	0.26	(0.0,0.6)	0.1	0.25	(0.0,0.6)
SSE Alaska	15.7	4.06	(8.2,24.1)	3.2	1.70	(0.6,7.1)	7.2	3.69	(1.1,15.0)
McDonald	0.1	0.60	(0.0,1.4)	4.8	2.11	(0.8,9.2)	1.5	2.38	(0.0,7.9)
Hugh Smith	1.7	2.33	(0.0,7.6)	0.0	0.26	(0.0,0.5)	0.3	1.02	(0.0,3.8)
Nass River	2.9	1.38	(0.8,6.1)	0.9	0.68	(0.1,2.6)	0.1	0.26	(0.0,0.7)
Skeena River	9.4	2.50	(5.0,14.8)	1.1	0.85	(0.1,3.2)	5.3	2.01	(2.0,9.8)
Queen Charlotte I.	0.0	0.09	(0.0,0.1)	0.1	0.27	(0.0,0.9)	0.0	0.11	(0.0,0.1)
Central Coast BC	2.3	2.44	(0.0,7.9)	1.6	1.29	(0.0,4.5)	0.8	1.43	(0.0,4.9)
Fraser River	62.1	4.08	(54.0,69.9)	85.3	2.36	(80.4,89.6)	76.8	3.81	(68.9,83.9)
Washington	0.1	0.27	(0.0,0.6)	0.0	0.08	(0.0,0.1)	0.0	0.20	(0.0,0.4)

Table 8. Estimated numbers of sockeye salmon caught in the 2013 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>	<i>District 101 Gillnet</i>		<i>District 104 Seine</i>	
		<i>Prior to 31</i>	<i>Total</i>	<i>Prior to 31</i>	<i>Total</i>
1	Alsek	43	58	688	853
2	NSE Alaska	380	450	2,159	4,939
3	Taku	296	467	627	1,399
4	Stikine	5,302	5,925	1,083	1,591
5	SSE Alaska	2,051	2,535	3,184	9,740
6	McDonald	2,252	3,013	495	3,421
7	Hugh Smith	2,039	2,371	521	839
8	Nass River	30,921	35,407	1,426	3,338
9	Skeena River	1,886	2,591	2,799	7,888
10	Queen Charlotte I.	3	19	4	35
11	Central Coast BC	1,135	1,379	57	965
12	Fraser River	60	107	52	41,791
13	Washington	21	22	6	30
<i>Totals</i>		46,390	54,342	13,101	76,831