ASSESSING THE RELATIVE CONTRIBUTION OF CHINOOK SALMON GENETIC SUB-STOCKS TO THE LOWER KUSKOKWIM RIVER SUBSISTENCE FISHERY

by

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Introduction

Chinook salmon stocks throughout Western Alaska have experienced low runs in recent years (ADF&G Chinook Salmon Research Team 2013; Schindler et al. 2013). Specifically, within the Kuskokwim River, Chinook salmon abundance has declined substantially since 2007 (Liller et al. 2018; Schindler et al. 2013) with the lowest run on record occurring in 2012 (Liller et al. 2018). Beginning in 2014, unprecedented subsistence harvest restrictions were enacted, including: extended fisheries closures; gear restrictions; limited participation fisheries; Federal Special Action; and harvest permits (Tiernan et al. 2018). Resource managers have had to develop new strategies to meet escapement goals, along with other biological and socially desirable objectives including ensuring escapement is distributed proportional to abundance among various stock groups and providing equitable harvest opportunities on the limited surplus.

Early season fisheries closures have been discussed by the Alaska Department of Fish and Game (ADF&G) as the preferred strategy for achieving Chinook salmon escapement goals and addressing harvest distribution throughout the Kuskokwim River during years of low run abundance (ADF&G 2015: pages 2, 4, 7, and 10). The expectation that this strategy would be effective is predicated on the fact that over 85% of the total annual Chinook salmon exploitation occurs in the lower portion of the Kuskokwim River (Tiernan and Poetter 2015) and the timing of subsistence harvest is focused during the early portion of the annual run (Hamazaki 2008). Evidence from large-scale tagging studies indicates that Chinook salmon bound for headwater portions of the Kuskokwim River have earlier migration timing compared to fish spawning in lower river tributaries (Stuby 2007; Schaberg et al. 2010; Head et al. 2017; Smith and Liller 2017a; Smith and Liller 2017b). Therefore, early season fishing closures would allow for assessment of Chinook salmon run strength prior to providing directed harvest opportunity and potentially allow upper river sub-stocks to move through high harvest areas in the lower river before fishing begins.

While the potential for disproportionate harvest of upper river sub-stocks has been acknowledged (e.g., Templin et al. 2004, 2011; Hamazaki 2008), this aspect of the existing fishery is not currently addressed in the Kuskokwim Area Management Plan (5 AAC 07.365). The lack of regulatory guidelines in the Kuskokwim may be due in part to an incomplete understanding of sub-stock compositions within this system and the inability to detect disproportionate exploitation on certain stock components.

Until recently, the ADF&G assumed the potential for disproportionate harvest of upper river stocks was low. Radio telemetry studies conducted from 2003–2007 suggested that headwater spawning sub-stocks represented a small component (less than 10%) of the spawning escapement and had similar run timing to other stock components (Stuby 2007; Schaberg et al. 2012). More recent tagging studies conducted in 2014 and 2015 (Head et al. 2017; Smith and Liller 2017a), however, found nearly 25% of tagged fish tracking to headwater tributaries. In all years, tagging data indicate that the various geographic stock components had equal probability of being tagged and that distribution of tagged fish was similar to untagged fish (Schaberg et al. 2012). The notable differences between the earlier and more recent studies were relative run size and exploitation rate during the respective time periods. From 2002–2005 Chinook salmon runs were above average and total annual exploitation averaged 30%. In 2014 and 2015 Chinook salmon runs were below average and total annual exploitation was less than 10%. The reduction in exploitation and corresponding increase in headwaters escapement has led us to hypothesize that upper river sub-stocks have been harvested at a much higher rate, compared with others, than previously thought.

In order to obtain better estimates of sub-stock representation in Kuskokwim River Chinook salmon harvests, this project worked to enhance the existing baseline by collecting additional samples from unrepresented populations in the middle river and upper river tributaries. The existing genetic baseline for Kuskokwim River Chinook salmon included 14 populations (populations described in Templin et al. 2011 plus the Necons River), and simulation studies support the identification of upper Kuskokwim River sub-stocks in stock identification applications (Templin et al. 2011). This project then used genetic mixed stock analysis (MSA) techniques to evaluate the proportion of upper river sub-stocks captured in the 2003–2007 Kuskokwim River Chinook salmon subsistence harvests. MSA results were compared to results from upriver tagging studies to determine if upper river stock components were subjected to higher exploitation compared to lower river stock components. Results of this project provide basic information about stock specific exploitation, which is critical for sustainable fisheries management.

Objectives

1. Enhance the existing Kuskokwim River Chinook salmon genetic baseline to identify the upper Kuskokwim River sub-stock in mixed stock fisheries.
2. Determine the relative contribution of the upper Kuskokwim River sub-stock to the subsistence fishery executed in the lower portion of the Kuskokwim River.
3. Determine if the upper Kuskokwim River sub-stock has been exploited at a higher rate compared to fish returning to other areas of the Kuskokwim River.

Methods

TISSUE SAMPLING

Baseline

Baseline samples were collected from spawning aggregations of Chinook salmon throughout the Kuskokwim River drainage to compile our library of tissues (Table 1, Figure 1). Tissues were collected by ADF&G staff and collaborators, including the U.S. Fish and Wildlife Service, Kuskokwim Native Association, Native Village of Napaimute, and others. Collections were made between 1992 and 2007, and most were reported as a subset of a coastwide baseline reported in Templin et al. (2011); an additional 6 populations were collected in 2016 and 2017. When possible, the minimum target sample size for each set of spawning aggregations that might represent a population in the baseline was 95 individuals to achieve acceptable precision for estimating allele frequencies (Allendorf and Phelps 1981; Waples 1990) and to accommodate our genotyping platform. For this baseline, we selected collections (fish collected within the same year at the same location) to represent 1) demographic distribution, 2) genetic diversity, 3) geographic coverage, and 4) among-year variation of allele frequencies within locations.

Mixtures

In 2003 – 2007, scale samples were collected from the lower Kuskokwim River subsistence fishery (Table 2). Scale samples were collected from lower river subsistence fishermen who sampled their own catch (Liller et al. 2013). Each year, samples were collected from a range of gillnet mesh sizes, but large mesh gear was most common. Participating samplers represent a range of communities that collectively made up more than 50% of the total lower river subsistence harvest. Most of the samples were collected from Bethel, which is the largest community in the in the

Kuskokwim Area and where, on average, 38% of the lower river subsistence harvest occurred. Samples were collected from throughout the entire annual harvest in proportion to harvest timing.

The target sample size for each year was 196 scale samples chosen to be representative of the sub-stock composition of the harvest. Due to the low DNA quality characteristics of scale samples, we selected 380 scales per year to ensure adequate sample sizes. In order to best represent subsistence harvest over these years, whenever possible samples were selected proportional to harvest over 3 spatial strata (below Bethel, Bethel, or above Bethel), and then were distributed proportional to timing (early, middle, or late) and mesh size (small, medium, or large) within each area.

LABORATORY ANALYSIS

Genomic DNA were extracted using a NucleoSpin 96 Tissue Kit by Macherey-Nagel (Duren, Germany). To address our expectation of low DNA quality, which is characteristic of scale samples, we pre-amplified the DNA using standard methods prior to initiating the genotyping process. Pre-amplification has been shown to significantly improve genotyping results from archived scale samples (Smith 2010).

Samples were analyzed for 43 single nucleotide polymorphism (SNP) loci shown to differentiate between Kuskokwim River reporting groups (Templin et al. 2011; Table 3). SNP markers were genotyped using Fluidigm® 192.24 Dynamic Arrays (http://www.fluidigm.com). Each reaction was a mixture of 4µL of assay mix (1x DA Assay Loading Buffer (Fluidigm), 10x TaqMan® SNP Genotyping Assay (Applied Biosystems), and 2.5x ROX (Invitrogen)) and 5µL of sample mix (1x TaqMan® Universal Buffer (Applied Biosystems), 0.05x AmpliTaq® Gold DNA Polymerase (Applied Biosystems), 1x GT Sample Loading Reagent (Fluidigm), and 60-400ng/µL DNA) combined in a 6.75nL chamber. Thermal cycling was performed on an Eppendorf IFC Thermal Cycler as follows: an initial denaturation of 10 min at 96ºC followed by 40 cycles of 96ºC for 15 s and 60ºC for 1 min. The Dynamic Arrays were read on a BioMark™ Real-Time PCR System (Fluidigm) after amplification and scored using Fluidigm® SNP Genotyping Analysis software. Re-analysis of failed assays were performed on the Applied Biosystems Prism 7900HT Sequence Detection System. The data collected was individual genotypes for each locus. Genotype data were stored in an *Oracle* database on a network drive maintained by ADF&G Information Services.

Quality control measures were instituted to identify laboratory errors and to determine the reproducibility of genotypes. The process involved reanalysis from DNA extraction through genotyping of 8 out of every 96 fish (one row per 96 well plate; 8%) for all markers by staff not involved in the original analysis.

STATISTICAL ANALYSIS

Quality control and pooling of collections

Subsequent analyses were performed using R version 1.1.383 unless otherwise noted. Concordance between QC reproduced genotypes was evaluated, and initial filtering was conducted to ensure the quality of the data by removing SNP markers with low amounts of variation (minor allele frequency < 0.005), removing individuals with low genotyping success rate (genotyping success < 0.8), and by removing individuals that were likely duplicate samples (genotypic concordance > 0.95).

To evaluate conformance of observed genotype frequencies to Hardy-Weinberg expectations (HWE) in baseline collections, we used a Monte Carlo simulation function implemented in

Genepop v4.3 (Rousset 2008) with 10,000 burn-in steps followed by 20 batches of 5,000 iterations per batch. We combined probabilities for each collection across loci using Fisher's method (Sokal and Rohlf 1995) and removed markers that had significant departures from HWE across all collections ($P < 0.05$), or if the distribution of *p*-values across collections was indicative of nonconformance to HWE (Waples 2014).

Similarly, we examined departure from HWE for each collection across all loci.

To obtain more temporally robust estimates of allele frequencies in populations, we attempted to pool collections. Pooling of collections was only considered if sampling locations were from identical tributaries. Additionally, collections could not have significantly different allele frequencies across all loci ($p > 0.01$) as determined by Fisher's exact tests. If collections were pooled, departure from HWE of loci and collections was reassessed as stated above.

Analysis of genetic structure in baseline populations

The reporting groups *Upper Kuskokwim* and *Lower Kuskokwim* were defined by the project goals. Determination of which populations would be grouped into each reporting group considered both genetic structure of the populations sampled and geographic distance from the mouth of the Kuskokwim River. To analyze genetic structure of the sampled populations, we visualized pairwise-F_{ST} estimates among populations with neighbor-joining trees. F_{ST} estimates were computed using methods described by Weir and Cockerham (1984) and plotted with FigTree in R. We also plotted MDS plots based on Nei's genetic distance using the adegenet package in R. Finally, we computed a likelihood profile of the baseline, or the self-assignment probability for all individuals within each population based on the leave-one-out methods described by Anderson et al. (2008).

Baseline evaluation for MSA

To evaluate the ability of the baseline to accurately proportion *Upper Kuskokwim* and *Lower Kuskokwim* mixtures of Chinook salmon, we simulated mixtures using individuals of known origin from baseline collections. These simulated mixtures were constructed for use in 2 evaluation methods: 1) 100% proof tests and 2) fisheries scenario tests.

First, in what is known as 100% proof tests, half of the individuals from a single reporting group were sampled to create a mixture and analyzed against a reduced baseline created with the remaining individuals. This process was repeated to produce five replicates for each reporting group to better understand precision and accuracy of assignments given different samples. The GCL operates under the guideline that correct allocation for these single-reporting group tests should exceed 90% to be considered adequate (Seeb et al. 2000).

Second, in the fishery scenario tests, mixtures are simulated to reflect expected proportions in the fishery. Given the simplicity of having only 2 reporting groups, we evaluated many hypothetical mixture proportions some of which were not specifically expected. These tests provided an indication of the power of the baseline for MSA, without the potential issue of overestimation of power seen with 100% proof tests (Anderson et al. 2008).

Stock composition estimation of mixtures

The stock composition of fishery mixtures were estimated using the program BAYES (Pella and Masuda 2001). The Bayesian model implemented by BAYES uses a Dirichlet distribution as the prior distribution for the stock proportions. In this analysis, prior parameters for each reporting

group were defined to be equal (i.e., a "flat" prior) with the prior for a reporting group divided equally among populations within that reporting group for population prior parameters. The sum of all prior parameters was set to 1 (prior weight), which is equivalent to adding 1 fish to each mixture (Pella and Masuda 2001). We ran 5 independent Markov Chain Monte Carlo (MCMC) chains of 40,000 iterations with different starting values and discarded the first 20,000 iterations to remove the influence of the initial start values. In order to assess among-chain convergence, we examined the Gelman-Rubin shrink factors computed for all stock groups in BAYES (Gelman and Rubin 1992). If a shrink factor for any stock group in a mixture was greater than 1.2, we reanalyzed the mixture with 80,000 iterations; if a shrink factor greater than 1.2 was observed in the reanalysis, non-convergent results were averaged and noted in the tables. Estimates and 90% credibility intervals were tabulated from the combined set of the second half of the 5 chains. Credibility intervals differ from confidence intervals in that they are a direct statement of probability; i.e., a 90% credibility interval has a 90% chance of containing the true answer (Gelman et al. 2000). The credibility intervals reflect both sampling error and genetic assignment error. We repeated this procedure for each fishery mixture.

Results

TISSUE SAMPLING

Baseline

The baseline tissue collections analyzed in this study included 4,441 individual Chinook salmon from 24 locations representing 49 separate collections from the Kuskokwim River area (Table 1, Figure 1). In 2016 – 2017, a total of 888 genetic samples were collected from spawning populations of Chinook salmon in the Kuskokwim River drainage. These samples were collected at 6 locations, and target sample sizes of 95 fish were met at all locations. These collections were added to the existing library of baseline tissues, with collections spanning the years 1992 – 2007 and the average sample size per collection was 162. Factors that contributed to missing sample goals of 95 fish per population included: difficulties of sampling in remote locations, lack of dedicated funding to support sampling crews, challenging water conditions (e.g. brackish, swift, deep), and small spawning population size. Of the 24 locations, 18 were sampled in more than 1 year. Twenty of the sampling locations were tributaries to the Kuskokwim River, ranging from the Eek River near the river's mouth northeast to Pitka Fork in the upper Kuskokwim River drainage. In addition, 4 sample locations were in drainages that are separate from the Kuskokwim River, but which also flow into Kuskokwim Bay and are part of the Kuskokwim Management Area as defined by ADF&G: Middle Fork Goodnews, North Fork Goodnews, Arolik, and Kanektok river drainages.

Mixture

A total of 1,900 archived scales collected from the lower Kuskokwim River subsistence fishery in 2003 – 2007 were selected for stock composition analysis. For each year, 380 individuals were selected for genotyping. If a scale was missing for a selected individual, an alternative replacement was selected from the same strata.

LABORATORY ANALYSIS

For baseline collections, a total of 4,441 individuals were genotyped at all 43 SNP markers. The number of individuals genotyped per baseline collection ranged from 12 to 190 individuals. For

mixture collections, a total of 1,900 individuals were genotyped at all 43 SNP markers. The quality of mixture scale samples was high, and of 1,900 fish analyzed only 30 fish were dropped due to poor DNA quality (1.58% over all samples).

Discrepancy rates from QC analyses were low. The discrepancy rate for newly genotyped baseline collections was 0.36%, for old collections was 0.14%, and for mixture collections was 0%. The overall discrepancy rate was very low at 0.17%.

STATISTICAL ANALYSIS

Quality control and pooling of collections

SNP markers were evaluated for minor allele frequencies. Of the 43 SNPs evaluated across all collections, 5 had allele frequencies < 0.005. Furthermore, all 5 of these had minor allele frequencies less than 0.0001 and were removed from further analyses leaving 38 loci. The only mitochondrial (ploidy =1) marker was one of the 5 SNP markers removed due to potential for fixation, so all remaining markers evaluated were diploid loci.

Collections were then evaluated for genotyping success rates and for duplicate samples. Of the 4,441 samples genotyped, 54 samples were unsuccessfully genotyped at greater than 20% of the SNP markers and 18 samples were found to likely be duplicated samples. These 72 samples were removed from further analyses.

Loci and collections were tested for departures form HWE. Two loci, Ots_IL-1RA and Ots_SERPC1−209, were found to deviate from HWE and were removed from further analyses leaving 36 loci in total. No collections were found to deviate from HWE according to Fisher's summary probability over diploid loci. No SNP markers exhibited signs of linkage.

Collections were then assessed for pooling within locations. Forty seven of the 49 collections did not show significantly different allele frequencies Fisher's exact tests after being pooled within 24 distinct locations. The 3 collections that did not successfully pool (Salmon River – Pitka Fork 1995, Salmon River – Pitka Fork 2017, and Tonzona River 2017) were left un-pooled, forming 26 populations in total.

Analysis of genetic structure in baseline populations

The neighbor-joining tree based on pairwise F_{ST} values indicated that populations located above McGrath were distinct from the other populations in the dataset and tended to be more distinct from each other than were populations lower in the drainage (Figure 2). Of the populations in the upper drainage, the Blackwater and Big River populations were the most distinct. This pattern was also evident in the MDS plot, with the first and second axes representing the most genetic distance between proposed reporting groups (Figure 3). In addition, there appears to be some higher diversity in the middle drainage, notably with the Tatlawiksuk River more distinct than other populations located below McGrath.

Based on the updated population structure and management needs, populations were grouped into 2 regions. This included combining the Kuskokwim Bay populations (Middle Fork Goodnews, North Fork Goodnews, Arolik, and Kanektok rivers) with the lower and middle Kuskokwim River populations (Eek, Kwethluk, Kisaralik, Tuluksak, Aniak, Salmon – Aniak, George, Hoholitna, Kogrukluk, Stony, Necons, Cheeneetnuk, Gagaryah, Tatlawiksuk, and Takotna rivers) into a *Lower Kuskokwim* reporting group representing everything that joins the Kuskokwim River at or

downstream of McGrath; and then combining the headwaters populations (Blackwater, Big, Middle Fork Kuskokwim, Salmon – Pitka Fork, and Tonzona rivers) into a *Upper Kuskokwim* reporting group representing everything upstream of McGrath.

Baseline evaluation for MSA

These 2 reporting groups (*Lower Kuskokwim* and *Upper Kuskokwim*) were used for simulations to examine the identifiability of the defined groups in genetic stock identification (Figure 4). All 10 of the 100% proof tests (5 replicates for each of the 2 reporting groups tested) met the goal of 90% correct allocation. Average correct allocations across replicates were 97.6% for *Lower Kuskokwim* and 96.0% for *Upper Kuskokwim* reporting groups.

Similarly, reporting groups performed well in fishery scenario tests (Figure 5). Out of 30 test mixture sets, only 1 set had estimates with confidence intervals that did not include the correct allocation (replicate 3 of 10% *Lower Kuskokwim*, 90% *Upper Kuskokwim*).

Stock composition estimation of mixtures

Genetic mixed stock analysis of subsistence harvest samples to the *Lower Kuskokwim* and *Upper Kuskokwim* reporting groups showed that the lower group dominated in each year of the study (2003 – 2007; Table 4). After removing fish for unsuccessful genotypes, sample sizes for each mixture ranged from 369 – 376 fish per mixture. The contribution of *Lower Kuskokwim* ranged from 85 – 96% over the study period, while the contribution of the *Upper Kuskokwim* ranged from 4 – 15%. The average over all years was 91% *Lower Kuskokwim* and 9% *Upper Kuskokwim* fish.

Discussion

This study built upon the existing Kuskokwim River Chinook salmon baseline to better understand the genetic distinction of fish spawning in the headwaters of the drainage compared to those spawning in lower and middle portions of the drainage. In addition, the study aimed to compare results from upriver tagging studies to determine if the proportion of the upper river sub-stock in subsistence harvests has differed from the proportion of upper river spawners estimated in drainage-wide escapements in those years. Results of this project will provide context for management strategies currently under considerations. In addition, results will contribute to broader efforts aimed at determining if historical harvest practices could have led to differential reduction of the upper river sub-stock, and if so, whether long-term conservation strategies are warranted for this stock component.

POPULATION STRUCTURE

In order to improve upon past applications of genetic diversity of Kuskokwim Area Chinook salmon, this study increased sample sizes and geographic coverage of the genetic baseline, particularly for headwaters tributaries. Four new populations, the Hoholitna, Blackwater, Big, and Middle Fork Kuskokwim rivers, were added. Additional samples were collected in 2016 and 2017 for the Salmon River – Pitka Fork and Tonzona rivers, increasing the number of samples representing each of these populations by an average of 138. The improved representation of the populations in the headwater tributaries led to more accurate understanding of the genetic similarity of these populations to the populations of the middle and lower Kuskokwim River. Based on what is known about Chinook spawning areas in the Kuskokwim Area (e.g. Stuby 2007), the baseline represents broad geographic coverage of most major spawning areas.

Patterns in genetic diversity seen in this study were generally similar to past analyses (e.g. Templin et al. 2004, Templin et al. 2011). Populations in the headwaters area plus the Tatlawiksuk River were the most divergent, with the largest F_{ST} values (Figures 2 and 3). Other studies showed that the Kuskokwim Bay populations, especially the Goodnews and Kanektok rivers, had greater genetic diversity. With the marker set used in this study, this relationship is most apparent for the Goodnews populations. Similar to Templin et al. 2011, our data show that the Stony, Cheeneetnuk, and Necons (a tributary of the Stony) are somewhat separated from the main group of lower and middle Kuskokwim River populations. The remaining populations are similar to each other based on the markers used in this baseline, from the Takotna River downstream to the Arolik River. This may represent a limitation of the marker set but may also represent a lack of barriers to present and/or historical gene flow between populations in this area.

BASELINE PERFORMANCE

Tests of the Kuskokwim River Chinook salmon baseline for estimating mixed stock compositions for the 2 reporting groups (*Upper Kuskokwim* and *Lower Kuskokwim*) demonstrated its effectiveness for producing precise, accurate estimates of stock composition for fisheries applications. This is similar to Templin et al. 2011, who found that a reporting group consisting of the Tatlawiksuk and Pitka Fork populations was consistently identifiable in mixtures from both Kuskokwim Bay and within the Kuskokwim River. However, Templin et al. 2011 noted that given the large amount of diversity in the upper portion of the drainage, further efforts were necessary to obtain representative samples in the area. The study described herein includes a more complete baseline, and better supports the reporting groups tested.

Reporting groups constructed based primarily on management needs and geographic location may not adequately represent the actual population structure in the Kuskokwim area. Similar to past analyses, genetic differences were more heavily weighted than geographic locations in making the determination that the Takotna River population should be included with the *Lower Kuskokwim* reporting group. At the same time, the Tatlawiksuk River population was included in the *Lower Kuskokwim* reporting group, even though it appears to be genetically distinct from many other populations in that group. Simulation tests (100% tests and fishery scenario tests) showed that these groups still performed well in mixtures.

UPPER KUSKOKWIM CONTRIBUTION TO SUBSISTENCE FISHERIES

Results from mixed stock analysis of 2003 – 2007 subsistence fishery samples showed a consistent low (4 – 15%) contribution of *Upper Kuskokwim* fish in each year. These results were compared to contributions of sub-stocks from the upper Kuskokwim River drainage as documented by telemetry studies conducted in 2003 – 2007 (Table 5, Figure 6; Schaberg et al. 2012). Our expectation, if the upper Kuskokwim River stocks have been exploited at a much higher rate than those in the lower Kuskokwim, would be to see a considerably higher proportion of fish from the Upper Kuskokwim reporting group in the subsistence mixtures in 2003 – 2007 compared to the telemetry results. In 2003 – 2007, less than 10% of tags each year migrated above McGrath (Schaberg et al. 2012). Results indicate that the subsistence harvest is only slightly enriched for the Upper Kuskokwim sub-stock (Table 5, Figure 6), and the subsistence harvest alone cannot fully explain any differences in results between tagging studies in 2002 – 2007 versus 2014 – 2016 (Schaberg et al. 2012; Head, Smith, and Liller 2017; Smith and Liller 2017). For management, while there is potential for disproportional harvest of upper Kuskokwim River stock components, there is no evidence for severe effects.

Conclusions

1. The enhanced Kuskokwim area Chinook salmon baseline can assess contributions of upper and lower stocks within the drainage.
2. The 2003 – 2007 subsistence harvest shows a consistent low contribution of *Upper Kuskokwim* fish in each year.
3. The subsistence harvest in 2003 – 2007 appears slightly enriched for the Upper Kuskokwim stock for those years compared to telemetry studies, but the subsistence harvest alone does not fully explain the relatively large proportions of Upper Kuskokwim River fish observed in recent year telemetry results.
4. Results support the potential for disproportional harvest of *Upper Kuskokwim* stocks in subsistence fisheries, but do not indicate severe effects.

Completion of Objectives

Objective 1. Enhance the existing Kuskokwim River Chinook salmon genetic baseline to identify the upper Kuskokwim River sub-stock in mixed stock fisheries.

In 2016 and 2017, baseline genetic collections for Kuskokwim River Chinook salmon occurred in the Hoholitna, Blackwater, Big, Middle Fork, Pitka Fork, and Tonzona rivers. A total of 181 samples were collected from the Hoholitna River, 151 samples were collected from the Blackwater River, 147 samples from the Big River, 135 samples from the Middle Fork River, 153 samples from the Pitka Fork, and 121 samples from Tonzona Rivers. After laboratory analysis, these samples were added to the existing baseline. Statistical analyses indicate that 2 groups (*Upper Kuskokwim* and *Lower Kuskokwim*) are evident, with the new collections clustering as expected relative to existing samples (Figure 2). These groups performed well in 100% proof tests, with an average 96.8% correct allocation to reporting group over repeated tests.

Objective 2. Determine the relative contribution of the upper Kuskokwim River stock to the subsistence fishery executed in lower portion of the Kuskokwim River.

Genetic mixed stock analysis of subsistence harvest samples to these 2 reporting groups (Upper Kuskokwim and Lower Kuskokwim) was completed from samples selected to represent harvest from each year 2003 – 2007 (Table 4). These results showed a consistently low (4 – 15%) contribution of *Upper Kuskokwim* fish to the subsistence fishery in each year.

Objective 3. Determine if the upper Kuskokwim River sub-stock has been exploited at a higher rate compared to fish returning to other areas of the Kuskokwim River.

Results from genetic mixed stock analysis were compared to telemetry studies conducted in respective years (Table 5, Figure 6; Schaberg et al. 2012). Results indicated that the subsistence harvest is only slightly enriched for the Upper Kuskokwim sock, and the subsistence harvest alone cannot fully explain any differences in results between tagging studies in 2002 – 2007 versus 2014 – 2016 (Table 5, Figure 6; Schaberg et al. 2012; Head et al. 2017; Smith and Liller 2017). While the upper Kuskokwim River sub-stock may be exploited at slightly higher rates, there is no evidence for severe effects.

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Table 1. Tissue collections used to describe the genetic structure of Chinook salmon from the Kuskokwim area, including reporting group, tissue collection location, collection (Col) and population (Pop) numbers, the years collected, and the numbers of individuals included in baseline analyses. Numbers of individuals include the number of samples initially genotyped for the set of 43 SNPs (Initial), removed for missing loci (Miss), removed for duplicate genotypes (Dup), and the number of individuals incorporated into the baseline (Final). Population numbers correspond to Figure 1.

					No. of Individuals			
Reporting Group	Location	Col	Pop	Year	Initial	Miss	Dup	Final
Lower Kuskokwim	Middle Fork Goodnews River	1	1	1992	95	19	0	76
		2	1	1993	40	0	0	40
		3	1	2005	160	0	0	160
	North Fork Goodnews River	4	2	2006	170	1	2	167
	Arolik River	5	3	2005	149	0	1	148
	Kanektok River	6	4	1992	34	1	0	33
		7	4	1993	46	0	0	46
		8	4	2005	165	0	1	164
	Eek River	9	5	2002	96	3	0	93
		10	5	2005	77	0	0	77
	Kwethluk River	11	6	2001	96	0	2	94
		12	6	2007	95	0	0	95
	Kisaralik River	13	7	2001	96	2	0	94
		14	7	2005	95	0	0	95
	Tuluksak River	15	8	1993	50	0	0	50
		16	8	1994	96	0	1	95
		17	8	2005	50	0	0	50
		18	8	2007	95	0	0	95
	Aniak River	19	9	2005	84	5	0	79
	Salmon River - Aniak	20	10	2002	96	2	0	94
		21	10	2006	156	0	0	156
	George River	22	11	2002	96	1	0	95
		23	11	2005	95	0	0	95
	Hoholitna River	24	12	2016	180	0	0	180
	Kogrukluk River	25	13	1992	50	0	1	49
		26	13	1993	50	0	0	50
		27	13	2005	50	0	0	50
		28	13	2007	44	0	0	44
	Stony River	29	14	1994	96	1	1	94
	Necons River	30	15	2006	23	0	0	23
		31	15	2007	178	2	1	175
	Cheeneetnuk River	32	16	2002	96	3	0	93
		33	16	2006	21	0	0	21
	Gagaryah River	34	17	2006	190	0	0	190
	Tatlawiksuk River	35	18	2002	96	2	0	94
		36	18	2005	95	0	0	95
	Takotna River	37	19	2005	80	0	0	80
		38	19	2007	95	0	0	95
Upper Kuskokwim	Blackwater River	39	20	2016	90	1	0	89
		40	20	2017	61	3	0	58
	Big River	41	21	2016	54	0	0	54
		42	21	2017	95	3	0	92
	Middle Fork Kuskowkim River	43	22	2016	12	0	1	11
		44	22	2017	122	2	0	120

-continued-

Table 1. (continued)

Reporting Group	Location	Col	Pop	Year	No. of Individuals			
					Initial	Miss	Dup	Final
Upper Kuskokwim (cont.)	Salmon River - Pitka Fork	45	23	1995	96	0	0	96
		46	24	2017	155	0	0	155
	Tonzona River	47	25	2006	33	1	0	32
		48	25	2007	26	0	7	19
		49	26	2017	121	2	0	119

Table 2. Summary of scale samples available for estimating harvest composition.

Year	Sample Size	Number of Samplers [a]	Number of Communities [b]	Percent Harvest [c]	Sample Days [d]	Timing Index [e]
2003	1,974	32	5	59%	40	1
2004	2,290	21	4	59%	29	4
2005	2,799	30	5	57%	30	1
2006	1,917	23	4	53%	27	0
2007	2,610	32	3	51%	37	4

[a] Number of samplers that collected scale samples.
[b] Number of communities that were represented by participating samplers.
[c] Percent of the total lower river subsistence harvest represented by sampled communities.
[d] Number of sample days on which sampling occurred.
[e] The number of days between the median sample timing and median harvest timing.

Table 3. Sources for single nucleotide polymorphisms surveyed in collections of Chinook salmon from Kuskowkim River and rational for removing specific loci from analysis.

Assay Name	Source [1]	Removal rational [2]
Ots_GTH2B-550	a	-
Ots_NOD1	a	-
Ots_E2-275	b	-
Ots_arf-188	b	MAF
Ots_AsnRS-60	b	-
Ots_C3N3	e	MAF
Ots_ETIF1A	c	-
Ots_FARSLA-220	d	-
Ots_FGF6A	a	-
Ots_FGF6B	a	-
Ots_GH2	e	-
Ots_GPDH-338	b	-
Ots_GPH-318	d	-
Ots_GST-207	d	-
Ots_GST-375	d	MAF
Ots_HGFA-446	b	MAF
Ots_hnRNPL-533	d	-
Ots_HSP90B-100	d	-
Ots_HSP90B-385	d	-
Ots_IGF-I.1-76	b	-
Ots_Ikaros-250	b	-
Ots_il-1racp-166	b	HWE
Ots_LEI-292	d	-
Ots_MHC1	e	-
Ots_MHC2	e	-
Ots_ZNF330-181	b	-
Ots_LWSop-638	b	-
Ots_SWS1op-182	b	-
Ots_P450	e	-
Ots_Prl2	e	-
Ots_ins-115	b	-

-continued-

Table 3. (continued)

Assay Name	Source [1]	Removal rational [2]
Ots_RFC2-558	b	MAF
Ots_SClkF2R2-135	b	-
Ots_SERPC1-209	d	HWE
Ots_SL	e	-
Ots_TAPBP	c	-
Ots_Tnsf	e	-
Ots_u202-161	b	-
Ots_u211-85	b	-
Ots_U212-158	b	-
Ots_u4-92	b	-
Ots_u6-75	b	-
Ots_Zp3b-215	b	-
Ots_RAG3	a	-
Ots_S71	a	-

[1] Marker sources: a) Northwest Fisheries Science Center-NOAA (Unpublished); b) Smith et al. 2005a; c) Washington State University Vancouver (Unpublished); d) Smith et al. 2007; e) Smith et al. 2005b.

[2] Reasons for not including loci in analysis: Minor allele frequency at locus less than 0.005 (MAF); Locus out of Hardy-Weinberg equilibrium (HWE).

Table 4. Sample size, estimated stock composition (mean), standard deviation (SD), median, upper and lower bounds of the 90% credibility intervals, for Kuskokwim River Chinook salmon subsistence fishery samples collected 2003 – 2007.

Year	Sample Size	Reporting Group	Mean	SD	Median	90% CI	
						5%	95%
2003	369	Lower	0.85	0.05	0.85	0.77	0.93
		Upper	0.15	0.05	0.15	0.07	0.23
2004	375	Lower	0.87	0.05	0.88	0.79	0.95
		Upper	0.13	0.05	0.12	0.05	0.21
2005	370	Lower	0.95	0.04	0.96	0.88	1.00
		Upper	0.05	0.04	0.04	0.00	0.12
2006	376	Lower	0.91	0.04	0.91	0.84	0.97
		Upper	0.09	0.04	0.09	0.03	0.16
2007	373	Lower	0.96	0.03	0.96	0.89	1.00
		Upper	0.04	0.03	0.04	0.00	0.11

Table 5. Telemetry results from 2003 – 2007, 2014 – 2016; summarized from Schaberg *et al* (2012), Head, Smith, and Liller (2017), Smith and Liller (2017a), and Smith and Liller (2017b).

Year[1]	Sample Size	Fate[2]	Number of Tags Assigned	Proportion
2003[a]	460	Lower	428	0.93
		Upper	32	0.07
2004[a]	318	Lower	311	0.98
		Upper	7	0.02
2005[a]	410	Lower	393	0.96
		Upper	17	0.04
2006[a]	463	Lower	441	0.95
		Upper	22	0.05
2007[a]	327	Lower	315	0.96
		Upper	12	0.04
2014[b]	329	Lower	259	0.79
		Upper	70	0.21
2015[c]	486	Lower	367	0.76
		Upper	119	0.24
2016[d]	398	Lower	350	0.88
		Upper	48	0.12

[1] Sources for each year: a) Schaberg *et al* (2012); b) Head, Smith, and Liller (2017); c) Smith and Liller (2017a); and d) Smith and Liller (2017b).

[2] Fate assigned based on reporting groups, where "Upper" corresponds to "Above McGrath" in Schaberg *et al* (2012), "Subarea 8" in Head, Smith, and Liller (2017), "Subarea 10" in Smith and Liller (2017a), and "Subarea 9" in Smith and Liller (2017b).

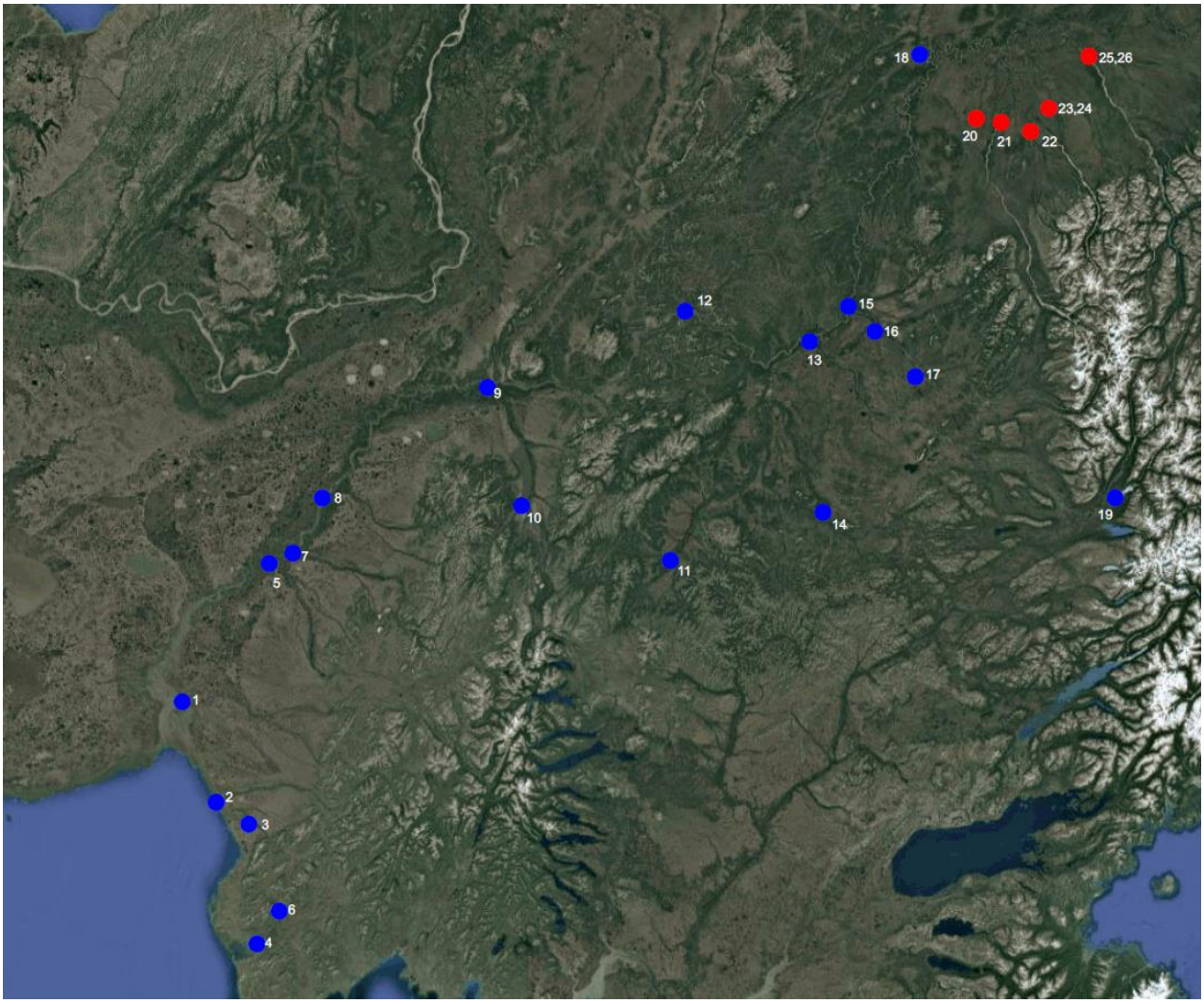

Figure 1. Location of the 26 populations represented in the Kuskokwim Chinook salmon baseline. Note, numbers correspond to population numbers in Table 1, and colors correspond to reporting groups: red = *Upper Kuskokwim*, blue = *Lower Kuskokwim*.

Figure 2. Consensus neighbor-joining tree based upon pairwise FST between 26 populations of Chinook salmon included in the Kuskokwim River baseline. Note, colors correspond to reporting groups: red = *Upper Kuskokwim*, blue = *Lower Kuskokwim*.

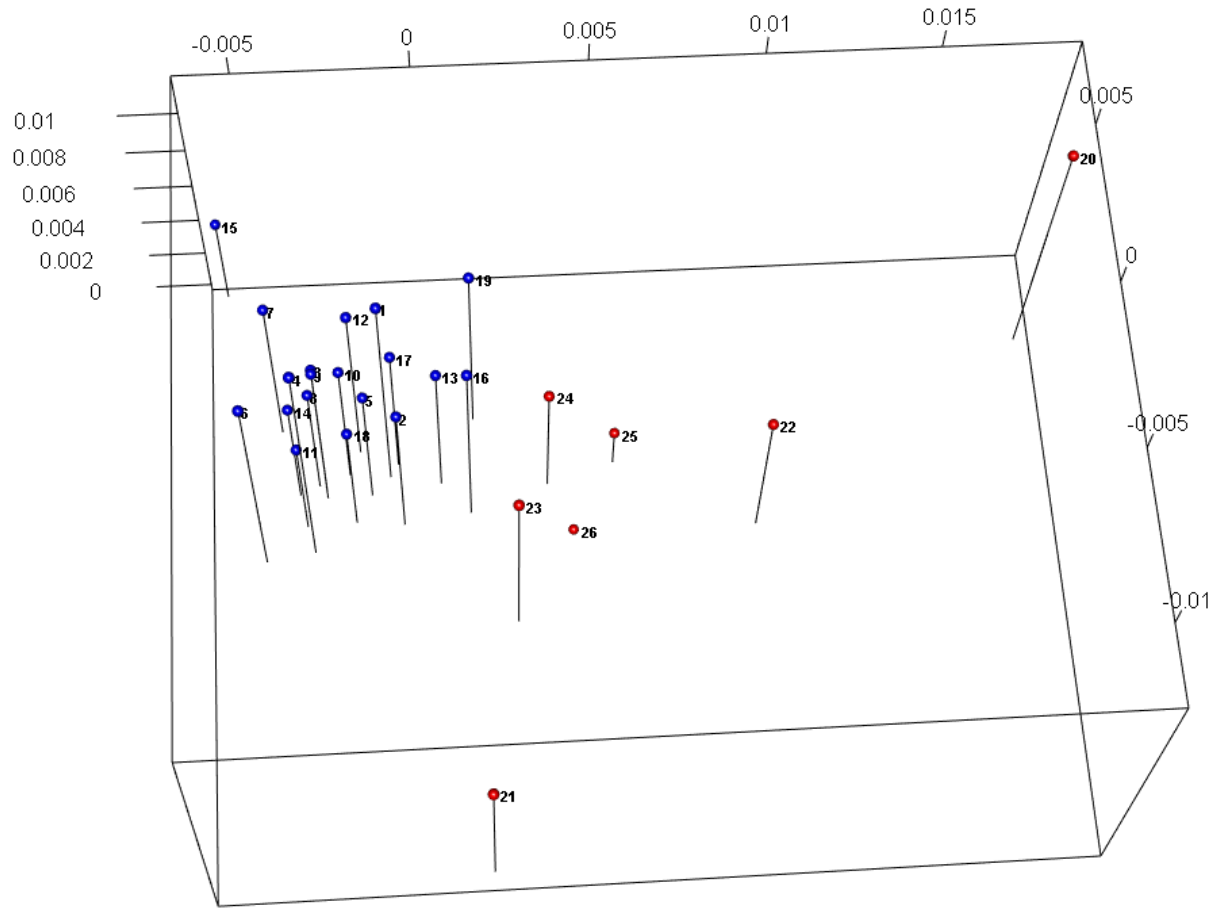

Figure 3. Multidimensional scaling plots based on Nei's genetic distances between 26 populations of Chinook salmon included in the Kuskokwim area baseline. Note, numbers correspond to population numbers in Table 1, and colors correspond to reporting groups: red = *Upper Kuskokwim*, blue = *Lower Kuskokwim*.

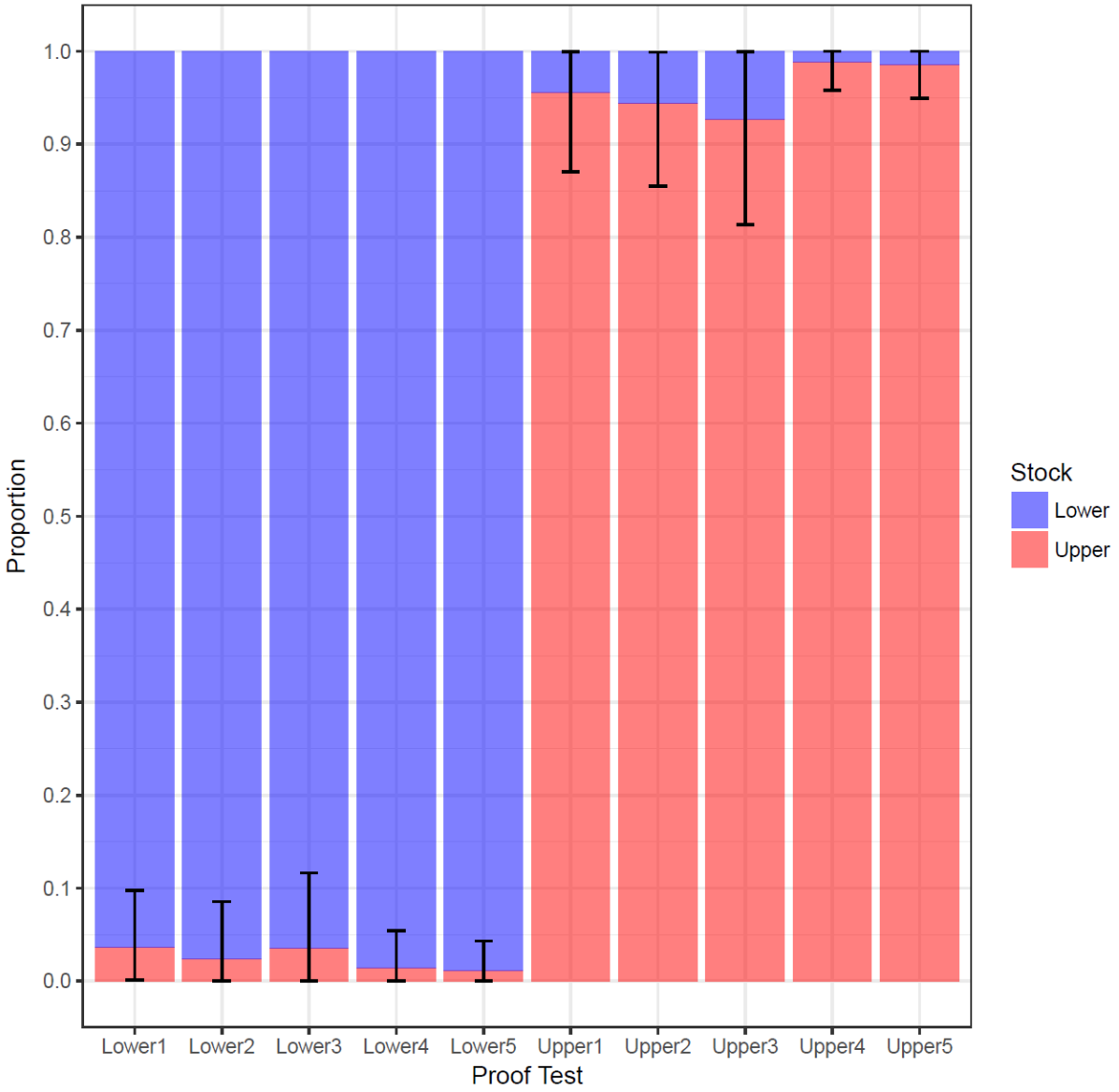

Figure 4. Results of repeated proof testing for 2 reporting groups in the Kuskokwim area Chinook salmon baseline including 90% credibility intervals. Details on reporting groups can be found in Table 1.

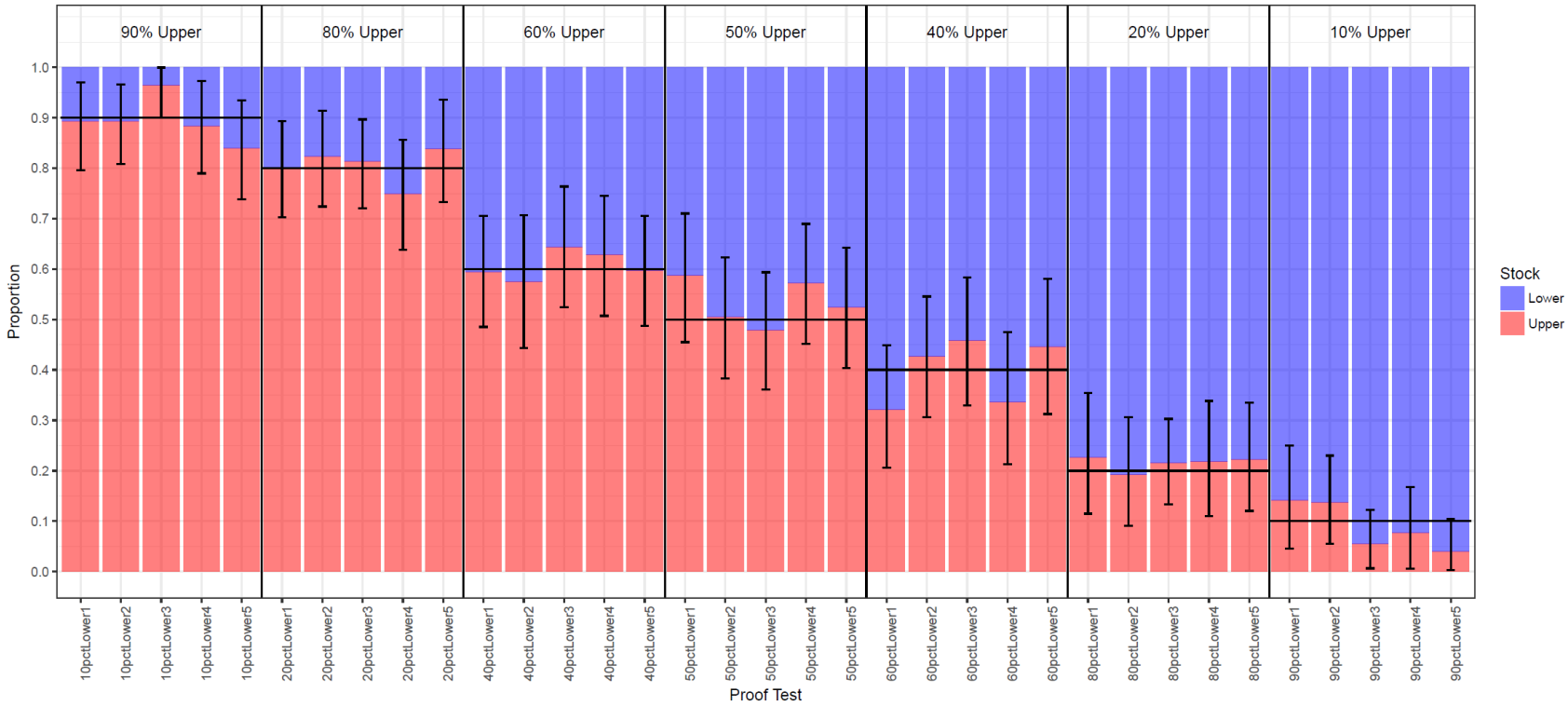

Figure 5. Results of fishery scenario testing for 2 reporting groups in the Kuskokwim area Chinook salmon baseline including 90% credibility intervals. Details on reporting groups can be found in Table 1.

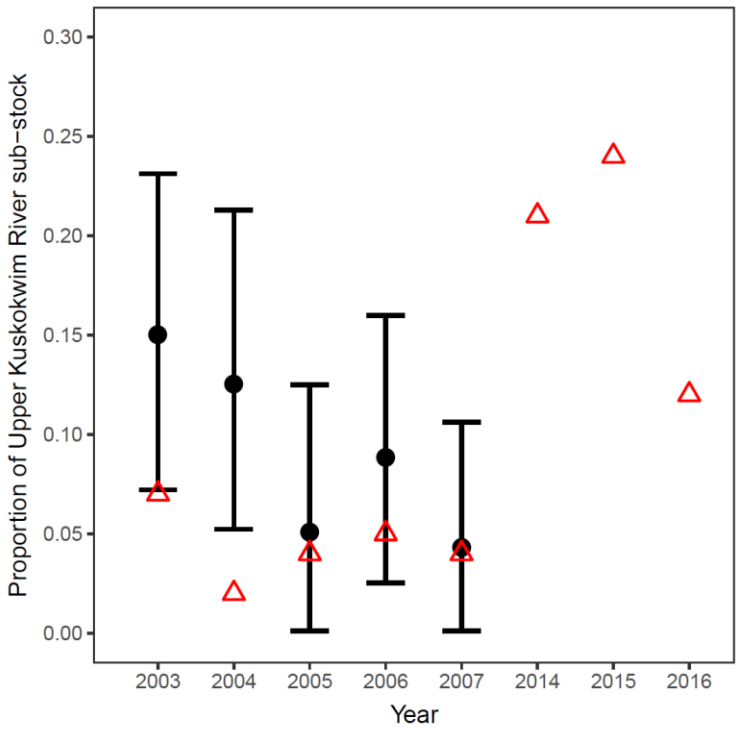

Figure 6. Comparison of Upper Kuskokwim River Chinook salmon sub-stock proportions estimated in subsistence harvests in 2003-2007 (black circles with 90% credibility intervals) as compared to drainage-wide estimates compiled from telemetry studies in 2003-2007 and 2014-2015 (red triangles, see Table 5).