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Bering Sea and Chukchi Sea Research Surveys**

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Christine M. Kondzela¹, Charles M. Guthrie III¹, Colby T. Marvin¹, Jacqueline A. Whittle¹,
Hanhvan T. Nguyen¹, Colleen Ramsower², Jeffrey R. Guyon¹

¹Auke Bay Laboratories,
Alaska Fisheries Science Center,
National Marine Fisheries Service,
National Oceanic and Atmospheric Administration,
17109 Pt. Lena Loop Road, Juneau, AK 99801, USA

²University of Arizona Genetics Core
1657 E. Helen Street, Rm 124B
Tucson, AZ 85719

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Stock Composition Analysis of Juvenile Chum and Chinook Salmon Captured on the 2012 Bering Sea and Chukchi Sea Research Surveys

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¹Auke Bay Laboratories, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 17109 Pt. Lena Loop Road, Juneau, AK 99801, USA

²University of Arizona Genetics Core
1657 E. Helen Street, Rm 124B
Tucson, AZ 85719

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Abstract

Juvenile chum (*Oncorhynchus keta*) and Chinook salmon (*O. tshawytscha*) were collected in the Bering and Chukchi seas as part of the 2012 U.S. BASIS/Arctic Ecosystem Integrated Survey (Arctic EIS) cruises. Juvenile chum salmon were more commonly encountered on the survey and 1,222 juveniles were genotyped for 11 microsatellite markers to determine their stock of origin. The most northern sample set was relatively small; juvenile chum salmon collected in the Chukchi Sea were predominantly from the Kotzebue Sound stock group. Juvenile chum salmon collected in the northern Bering Sea near Norton Sound were predominantly of Norton Sound origin. Yukon River chum salmon were present in both survey areas of the Bering Sea, but were more prevalent between lat. 60-63°N. Juvenile Chinook salmon were not encountered in the Chukchi Sea, but a small sample of 81 juveniles from the Bering Sea was genotyped for 43 single nucleotide polymorphism (SNP) markers. Most of the Chinook salmon were from the Upper Yukon, Coastal Western Alaska, and Middle Yukon stock groups. This study determined the freshwater origin of juvenile chum and Chinook salmon from the northern Bering and Chukchi seas during late-summer/fall based on genetic data and may be used to help guide future surveys of juvenile salmon abundance in western Alaska.

Introduction

Both Chinook (*Oncorhynchus tshawytscha*) and chum (*O. keta*) salmon are high priority species whose management has significant allocation, conservation, and management implications. Over the last couple of decades, declines in both chum and Chinook salmon returns in some western Alaska drainages prompted various disaster declarations by the Governor of Alaska and federal agencies (Nelson, 2011). It is unclear why salmon returns have declined recently. There is some speculation that a significant source of mortality exists during the

transition when juvenile salmon migrate out of fresh water as fry and smolts into salt water (Healey, 1982). Understanding the migration dynamics of juvenile salmon stocks and their relative abundance at sea may help determine the stresses that salmon undergo during this transition and could possibly lead to future models of estimating adult returns.

Juvenile chum and Chinook salmon were collected as part of annual U.S. BASIS cruises in the eastern Bering Sea since 2002. Juvenile chum salmon collected in 2002 and a subset in 2007 were genetically analyzed (Farley et al. 2004; Kondzela et al. 2009); samples from other years remained unanalyzed until recently (Kondzela et al., in preparation). The 2002 sample set was collected in the eastern Bering Sea between lat. 58-63°N, in an area from west of the Kuskokwim River to west of the Yukon River mouth. The 2002 sample set was genotyped for allozyme markers; mixed-stock analysis of samples from five areas showed that most of the fish were from coastal western Alaska and fall-run Yukon River stocks. The fall-run Yukon fish were found predominantly west and south of the Yukon River mouth, but not in the area west of the Kuskokwim River mouth. Contribution from northern Russian stocks was observed in the most northern and western stations. The 2007 samples from just south of the Bering Strait were predominately from northern Russian stocks, but the samples from the Chukchi Sea were from Kotzebue and Norton Sounds.

Chinook salmon are the least abundant of the Pacific salmon species in Alaska (Healey 1991). Not surprisingly, the number of juvenile Chinook salmon collected from the 2012 eastern Bering Sea survey was smaller than that of chum salmon. Genetic stock composition estimates for the 2002-2006 juvenile Chinook salmon samples from the eastern Bering Sea were completed by the Alaska Department of Fish and Game (ADF&G; Murphy et al. 2009), and the 2009-2011 samples have recently been analyzed by the National Marine Fisheries Service (Murphy et al., in preparation). Juvenile Chinook salmon from those two multi-year datasets were primarily from Coastal Western Alaska, Middle Yukon, and Upper Yukon stock groups.

Our study reports the sample locations and genetic stock composition estimates for juvenile Chinook and chum salmon collected from the Bering Sea and Chukchi Sea on the 2012 U.S. BASIS/Arctic Ecosystem Integrated Survey (Arctic EIS) research cruises. These results will help elucidate the migrations of juvenile salmon in western Alaska as they emigrate from freshwater to marine environments, and are expected to complement the mixed-stock analyses of the juvenile salmon samples collected from the eastern Bering Sea and Chukchi Sea in other years.

Materials and Methods

Sample Collection and DNA Extraction

Juvenile salmon samples were collected in the Bering Sea and Chukchi Sea as part of the 2012 U.S. BASIS/Arctic EIS surveys, following the methods described in Farley et al. (2005).

DNA was extracted from the tails or opercles of the juvenile salmon with a DNeasy® Blood and Tissue Kit (Qiagen, Inc., Germantown, Maryland)¹ or Corbett reagents (Corbett Robotics Pty. Ltd., Australia), and processed with a X-Tractor Gene™ CAS-1820 robot as described by the manufacturer (Corbett Robotics). Extracted DNA was stored in 96-well DNA plates at -20°C.

Genetic Baselines

Allele frequencies of the 381-population Pacific Rim chum salmon microsatellite baseline (Beacham et al. 2009) were downloaded from the Fisheries and Oceans Canada (DFO) Molecular Genetics web page (http://www-sci.pac.dfo-mpo.gc.ca/mgl/data_e.htm). Baseline files were created with Excel (Microsoft, Inc.) for 11 of the 14 markers that we routinely use in our laboratory for mixed-stock analyses (McCraney et al. 2012). The species-wide Chinook salmon baseline provided by the ADF&G (Templin et al. 2011) contains a set of 43 single nucleotide polymorphism (SNP) markers all of which were used in the stock composition analyses of our report. The SNP baseline contains genetic information for 172 populations of Chinook salmon grouped into 11 geographic regions.

Genotyping – Chum Salmon

The juvenile chum salmon samples were assayed for 11 microsatellite loci (Beacham et al. 2009)–*Oki100*, *Omm1070*, *Omy1011*, *One101*, *One102*, *One104*, *One114*, *Ots103*, *Ots3*, *Ots68*, and *Ssa419*–with a Qiagen® Multiplex PCR Kit following the manufacturer’s protocols. Thermal cycling for the amplification of DNA fragments with the polymerase chain reaction (PCR) was performed on a dual 384-well GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, California). Samples from the PCR reactions were diluted into 96-well plates for analysis by a 16-capillary, 36 cm array on the ABI 3130xl Genetic Analyzer (Applied Biosystems).

Genotypes were double-scored with GeneMapper® software, Version 4.0 (Applied Biosystems) and exported to Excel spreadsheets for further analysis. Of the 1,412 samples analyzed, 1,222 were genotyped for 8 or more of the markers (average 10.8 markers). The remaining 188 samples were deleted either due to a lower number of successfully genotyped markers or an excess of homozygosity; the data from one sample from each of two pairs of duplicates were deleted. Most of the loss of genotype data was associated with samples collected early in the survey. Quality control of sample handling and genotyping was examined by plating DNA from 12.5% of the successfully genotyped samples that were then re-processed for genotyping as described above. Overall, the genotyping error rate was <1%.

Microsatellite allele designations were converted to match those in the DFO chum salmon microsatellite baseline (Beacham et al. 2009) from a conversion table that was developed

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

by genotyping samples shared between the laboratories. Converted genotypes were then formatted into mixture files that were compatible with BAYES software.

Genotyping – Chinook salmon

The juvenile Chinook salmon samples were genotyped for the 43 SNP DNA markers represented in the Chinook salmon baseline with a “Matrix-assisted laser desorption/ionization - time of flight” (MALDI-TOF) method performed by using a Sequenom MassARRAY iPLEX platform (Gabriel et al. 2009). In addition to internal MALDI-TOF chip controls, ten previously genotyped samples were included on each chip during the analyses and resulting genotypes were compared to those from ADF&G, which used TaqMan® chemistries (Applied Biosystems). Concordance rates of 99.9% between the two chemistries confirmed the compatibility of both genotyping methods. Of the 90 samples analyzed, 81 samples were successfully genotyped for 35 or more of the 43 SNP markers (average of 41 SNPs).

Stock Composition Analysis

Stock composition estimates were determined with a Bayesian (BAYES; Pella and Masuda 2001) approach by comparing mixture genotypes with allele frequencies from reference baseline populations. For each BAYES analysis, Monte Carlo chains starting at disparate values of stock proportions for each region 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 was used for all baseline populations. The stock composition analyses were completed for a chain length of 10,000 with the first 5,000 discarded as burn-in and convergence of the chains to posterior distributions of stock proportions was determined with Gelman and Rubin (1992) shrink factors.

Baseline evaluation

The chum salmon baseline data were examined to determine major regional stock groupings of populations that would then be used for mixed-stock analyses of the chum salmon samples. Larger regional stock groupings can increase estimation accuracy and provide a means to compare similar studies. Population genetic structure was examined in two ways. First, structure was examined in the software NT-SYS (Applied Biostatistics, Inc.) with a principal coordinate analyses of chord distances (Cavelli-Sforza and Edwards, 1967) that were calculated from the allele frequencies of the baseline populations. Second, baseline simulation analyses were performed with SPAM software (Version 3.7; ADF&G, 2003) to evaluate the effectiveness of the baseline to allocate stocks to the correct regions. Hypothetical mixtures of 400 fish from single stock groupings were compared with the baseline to determine the percentage that reallocated back to the correct stock group. Simulations were done with baseline population resampling, a conservative method that incorporates the sensitivity of the baseline to small changes in allele frequency differences.

Results

Sample collection and distribution

Between August 6 and September 26, 2012, stations along the eastern Bering Sea and Chukchi Sea shelf from longitudinal meridians 159-168°W and from lat. 60-69°N were sampled for juvenile chum and Chinook salmon. The sampling locations for the successfully genotyped juvenile chum and Chinook salmon are shown in Figures 1A and 1B, respectively.

Chum salmon - stock composition

The number of successfully genotyped juvenile chum salmon samples from each of the three areas depicted in Figure 1A is shown in Table 1. Stock composition estimates were made for the total 1,222 sample set and for each sample set from the three areas. When six large regional baseline stock groupings were used in the stock composition analyses, most of the juvenile chum salmon samples were estimated to be from the Coastal Western Alaska stock group. A smaller proportion from the Upper/Middle Yukon stock group was present in the Bering Sea, but absent in the Chukchi Sea (Figure 2A).

To determine the relative contribution of more local stocks, the coastwide baseline was refined to 58 western Alaska/Arctic populations—from northern Bristol Bay to the Peel River in the Arctic—in five temporal-spatial stock groupings. The middle Yukon, including the Koyukuk and Tanana rivers, has both a summer and fall run of chum salmon. Some of the middle Yukon River populations were included with the Yukon Fall (upper river) stock group based on principle coordinate analysis of the baseline (not shown). The simulation results identify the difficulty of separating the coastal western Alaska chum salmon populations (Table 2); however, a stock composition analysis can provide at least a relative measure of contribution. Stock composition estimates made with this smaller baseline showed that samples collected between lat. 60-63°N were predominantly of Yukon River origin (Summer and Fall stock groups). About half the fish collected between lat. 64-66°N were from the Yukon River and most of the remainder from the Norton Sound stock group, whereas 94% of the Chukchi Sea collection was from the Kotzebue Sound stock group (Table 2B).

Chinook salmon - stock composition

After SNP genotyping, the 81 juvenile Chinook salmon samples were analyzed as a single dataset due to the small number of samples available (Table 1). Based on the coastwide Chinook salmon baseline aggregated into 11 large regions, the juvenile Chinook samples allocated primarily to the Coastal Western Alaska, Middle Yukon, and Upper Yukon stock groups (Figure 3A). The genetic variance for those estimates was relatively large, a result of the small sample set. To minimize potential cumulative effects from the misassignment of low stock estimates, the baseline was refined to the five most proximal Bering Sea stock groups and the mixed stock analysis was repeated. With a localized baseline, all samples allocated to the Coastal

Western Alaska and Yukon stock groups in proportions nearly identical to those estimated with the full baseline (Figure 3B).

Discussion

Juvenile chum and Chinook salmon samples were collected in late-summer/fall 2012 from U.S. BASIS/Arctic EIS research surveys in the Bering Sea and Chukchi Sea. Genetic stock composition analyses show that juvenile salmon from multiple stock groups in western Alaska had migrated into the Bering and Chukchi seas at the time of the surveys. Due to the large spatial pooling of samples used in these analyses, the extent of population mixing on the continental shelf during the first summer at sea is not known. The stock estimates support an essentially westward (offshore) and southern migration of juvenile Chinook and chum salmon from the Yukon River.

The stock composition estimates for chum salmon show that the Yukon River stocks were most common between 60-63°N, an area located just south of the river mouth. Given the genetic similarity between lower Yukon and Kuskokwim stocks, it is possible that some Kuskokwim fish could have misallocated to the Summer Yukon stock group (Table 2). However, the relatively minor contribution of the Kuskokwim/Northeastern Bristol Bay stock group to the 60-63°N collection suggests that these southern Bering Sea stocks were not large contributors. The 2012 juvenile chum salmon samples from the Chukchi Sea were caught nearshore in mid-August above Cape Lisburne, where sea surface temperatures in August-September were relatively warm (Lisa Eisner, unpublished data; https://web.sfos.uaf.edu/wordpress/arcticeis/?page_id=209, accessed 3/26/14). Results from our study corroborate those of a previous analysis in which Kotzebue stocks dominated the juvenile chum salmon samples collected in early September 2007 from nearly the same location in the Chukchi Sea (Kondzela et al. 2009). We speculate that survival of juvenile chum salmon in the Chukchi Sea requires migration southward before sea ice formation to overwinter in the southern Bering Sea. In 2012, chum salmon from the Kotzebue Sound region were not present in samples collected in the eastern Bering Sea, off the Yukon River more than a month after they were detected in the Chukchi Sea. Kotzebue region chum salmon are genetically distinct and the 2011 parent abundance was high (Menard et al. 2012), so if the juveniles migrated south into the Bering Sea, perhaps they moved farther offshore than the area surveyed, or migrated south later in the season. The possibility of a later migration time is supported by the record low sea ice cover minimum in the Arctic Ocean in September 2012 (Perovich et al. 2013).

Stock composition estimates for the 2012 juvenile Chinook salmon were similar to those from previous years (Murphy et al. 2009) with the Upper Yukon stock group having the highest contribution, followed closely by the Coastal Western Alaska and Middle Yukon stock groups. The Coastal Western Alaska stock group includes many populations south of the Yukon River

(Templin et al. 2011). Because juvenile Chinook from the Yukon River are thought to migrate offshore in a southwesterly direction (Farley et al. 2005), the Coastal Western Alaska portion of Chinook captured in the 2012 survey is likely to be from the lower Yukon River.

The genetic data generated from our study will support ongoing investigations of Yukon River juvenile salmon migration, abundance, and the inter-annual variation of proportions of summer and fall-run chum salmon. The samples from 60-63°N contained the largest number of Yukon River chum and Chinook salmon, and given the wide interest in the salmon resources of the Yukon River drainage, supports future survey effort in that area of the eastern Bering Sea.

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The findings and conclusions are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service.

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Table 1. Number of genotyped juvenile chum and Chinook salmon collected between August 6 and September 26, 2012 from the Bering Sea and Chukchi Sea research surveys.

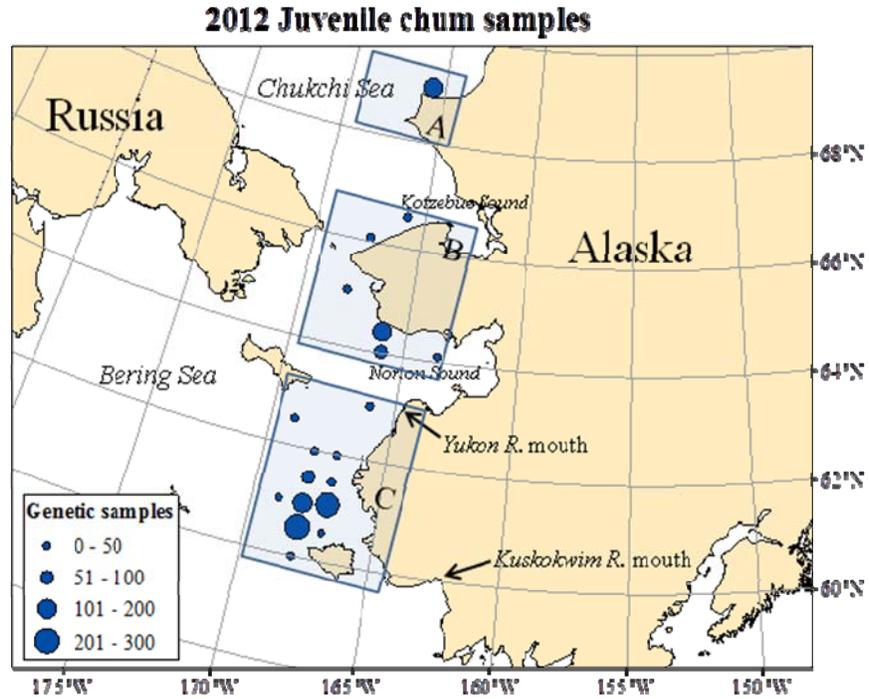
| Location | Date of chum collections | Number of chum samples | Number of Chinook samples |
|-----------------|---------------------------------|-------------------------------|----------------------------------|
| 60 to 63°N | Sep 18-25 | 880 | 64 |
| 64 to 66°N | Aug 8, Sep 12-17 | 228 | 17 |
| 69°N | Aug 8 | 114 | 0 |
| Total | | 1,222 | 81 |

Table 2. Chum salmon baseline simulation analysis – 100% simulations for 5 western Alaska regions with 11 microsatellite loci. NEBB = northeastern Bristol Bay.

| Region | Yukon Summer | Yukon Fall | Norton Sound | Kuskokwim-NEBB | Kotzebue |
|----------------|---------------------|-------------------|---------------------|-----------------------|-----------------|
| Yukon Summer | 0.799 | 0.047 | 0.070 | 0.217 | 0.019 |
| Yukon Fall | 0.022 | 0.935 | 0.006 | 0.007 | 0.005 |
| Norton Sound | 0.129 | 0.009 | 0.883 | 0.239 | 0.273 |
| Kuskokwim-NEBB | 0.040 | 0.004 | 0.023 | 0.522 | 0.009 |
| Kotzebue Sound | 0.005 | 0.001 | 0.014 | 0.003 | 0.666 |

Figure 1. Sampling locations from the 2012 Bering Sea and Chukchi Sea research surveys: juvenile chum salmon in Panel A, and juvenile Chinook salmon in Panel B. Relative sample sizes are indicated by the size of the blue dots. Areas A, B, and C in Panel A at lat. 69°N, 64-66°N, and 60-63°N, respectively, encompass the three chum salmon sample sets used for stock composition analyses.

A.



B.

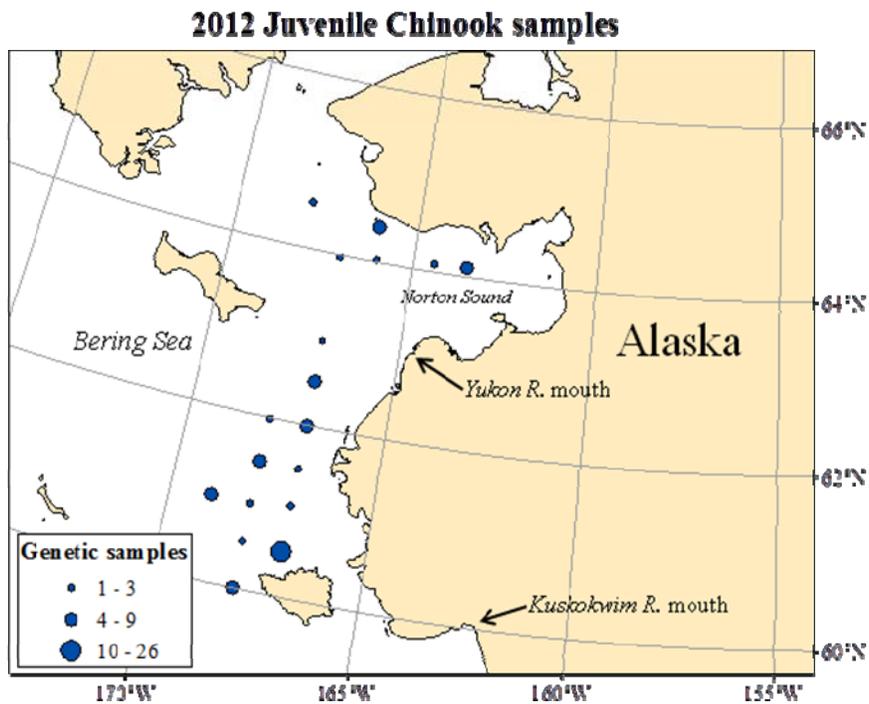
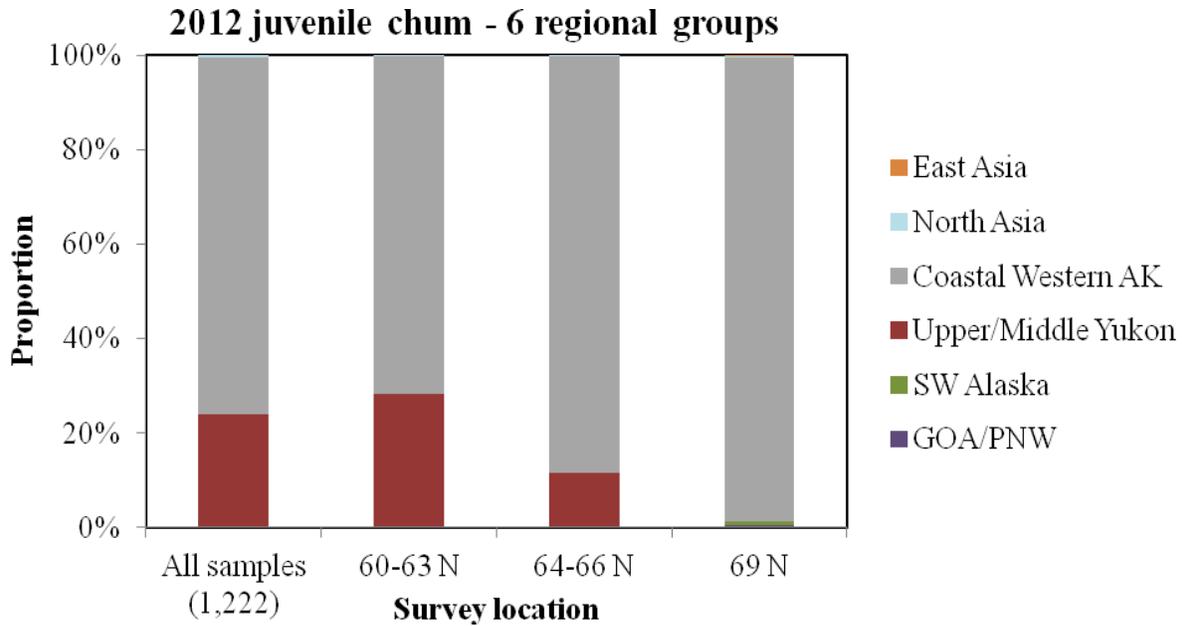


Figure 2. BAYES stock composition estimates for juvenile chum salmon samples from the 2012 Bering Sea and Chukchi Sea research surveys. Estimates from analyses that used a coastwide baseline (panel A), and a more localized western Alaska baseline (panel B). GOA = Gulf of Alaska, PNW = Pacific Northwest, NEBB = northeastern Bristol Bay

A.



B.

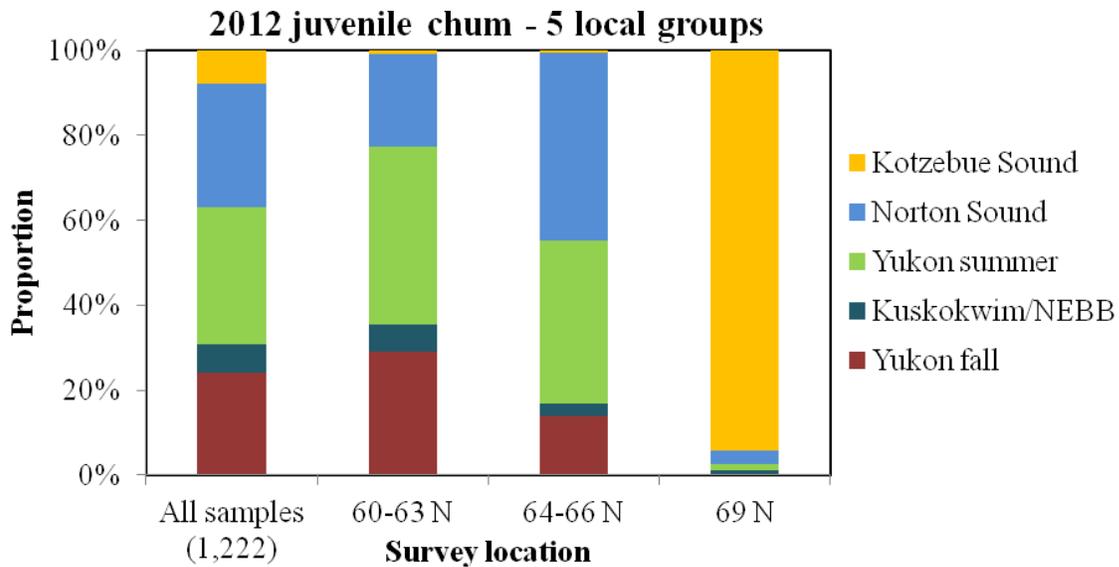
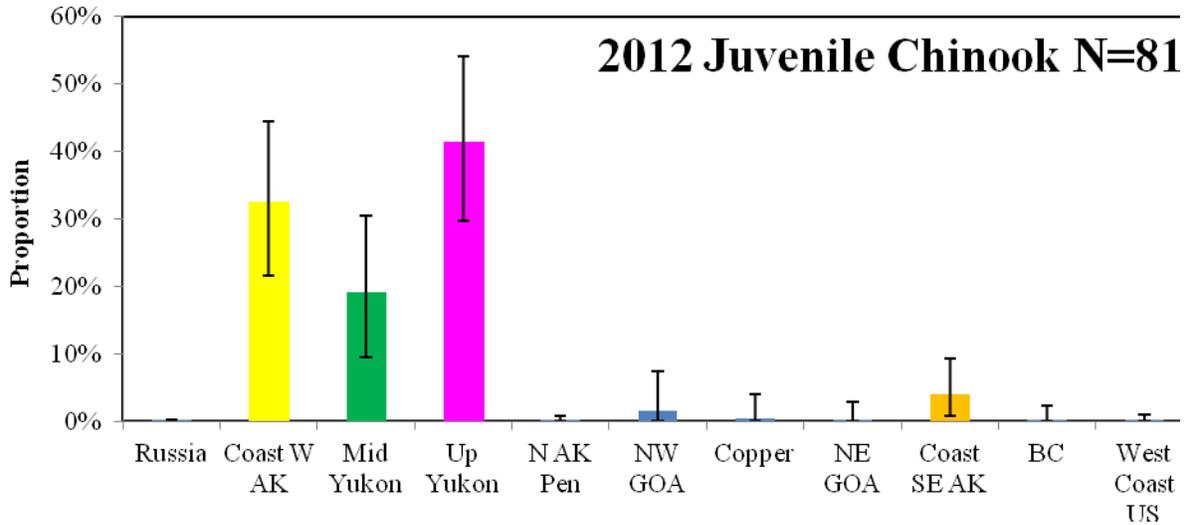


Figure 3. BAYES stock composition estimates (\pm credible intervals) for juvenile Chinook salmon from the 2012 Bering Sea research survey. Estimates from analyses that used a coastwide baseline (panel A), and a more localized western Alaska baseline (panel B).

A.



B.

