

Report to Yukon River Panel: Project No. URE-01-14N

**Genetic stock identification of fall chum salmon in
commercial harvests, Yukon River, 2014.**

by

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and

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April 2015

Alaska Department of Fish and Game

Divisions of Commercial Fisheries



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Weights and measures (metric)		General		Mathematics, statistics	
centimeter	cm	Alaska Administrative Code	AAC	<i>all standard mathematical signs, symbols and abbreviations</i>	
deciliter	dL	all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.	alternate hypothesis	H_A
gram	g	all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.	base of natural logarithm	e
hectare	ha	at	@	catch per unit effort	CPUE
kilogram	kg	compass directions:		coefficient of variation	CV
kilometer	km	east	E	common test statistics	(F, t, χ^2 , etc.)
liter	L	north	N	confidence interval	CI
meter	m	south	S	correlation coefficient	
milliliter	mL	west	W	(multiple)	R
millimeter	mm	copyright	©	correlation coefficient (simple)	r
		corporate suffixes:		covariance	cov
Weights and measures (English)		Company	Co.	degree (angular)	$^\circ$
cubic feet per second	ft ³ /s	Corporation	Corp.	degrees of freedom	df
foot	ft	Incorporated	Inc.	expected value	E
gallon	gal	Limited	Ltd.	greater than	>
inch	in	District of Columbia	D.C.	greater than or equal to	\geq
mile	mi	et alii (and others)	et al.	harvest per unit effort	HPUE
nautical mile	nmi	et cetera (and so forth)	etc.	less than	<
ounce	oz	exempli gratia	e.g.	less than or equal to	\leq
pound	lb	(for example)		logarithm (natural)	ln
quart	qt	Federal Information Code	FIC	logarithm (base 10)	log
yard	yd	id est (that is)	i.e.	logarithm (specify base)	log ₂ , etc.
		latitude or longitude	lat. or long.	minute (angular)	'
Time and temperature		monetary symbols (U.S.)	\$, ¢	not significant	NS
day	d	months (tables and figures): first three letters	Jan, ..., Dec	null hypothesis	H_0
degrees Celsius	$^\circ\text{C}$	registered trademark	®	percent	%
degrees Fahrenheit	$^\circ\text{F}$	trademark	™	probability	P
degrees kelvin	K	United States (adjective)	U.S.	probability of a type I error (rejection of the null hypothesis when true)	α
hour	h	United States of America (noun)	USA	probability of a type II error (acceptance of the null hypothesis when false)	β
minute	min	U.S.C.	United States Code	second (angular)	"
second	s	U.S. state	use two-letter abbreviations (e.g., AK, WA)	standard deviation	SD
Physics and chemistry				standard error	SE
all atomic symbols				variance	
alternating current	AC			population sample	Var
ampere	A			sample	var
calorie	cal				
direct current	DC				
hertz	Hz				
horsepower	hp				
hydrogen ion activity (negative log of)	pH				
parts per million	ppm				
parts per thousand	ppt, ‰				
volts	V				
watts	W				

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This document should be cited as:

DeCovich, N. A. and B. M. Borba. 2015. Genetic stock identification of fall chum salmon in commercial harvests, Yukon River, 2014. Alaska Department of Fish and Game, Report to Yukon River Panel Project No. URE-01-14N, Anchorage.

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ABSTRACT

Chum salmon (*Oncorhynchus keta*) genetics samples were collected from the commercial fishery conducted in District 1 of the Lower Yukon Area. Historically the last two weeks of July include the transition of stocks from summer to fall chum salmon whereas August is dominated by fall chum salmon. Because of the entry pattern of the run (producing extreme highs and lows within two days' time) and timing of the commercial openings, more samples had to be collected (n=1,296) than would be analyzed. Each pulse fished was sampled representing pulses 1-2 (in July n=550) and 5-6 (in August n=526). Genotyping of 952 fish using 96 single nucleotide polymorphism markers has been completed at the time of this report. Initial quality control measures of genotype data reveal that all fish have genotypes for greater than 80% of markers and a genotype error rate of 0.08%, which is within the acceptable range for mixed-stock analysis.

Key words Yukon River, chum salmon *Oncorhynchus keta*, commercial harvest, genetic stock identification, single nucleotide polymorphisms, SNP

INTRODUCTION

Fall chum salmon (*Oncorhynchus keta*) stocks of Canadian-origin have escapement objectives outlined in the Yukon River Salmon Agreement as an Annex to the Pacific Salmon Treaty while U.S. stocks including Tanana, Chandalar and Sheenjek rivers are within the purview of Alaska Department of Fish and Game's (ADF&G) escapement goal policy. Canadian-origin stocks include both the upper Porcupine River (Fishing Branch River) and the upper Yukon River mainstem. Priority for fisheries management in the State of Alaska is first to escapement, second to subsistence uses and third to other uses such as commercial harvests. However, the occurrence of fisheries for fall chum salmon along the length of the Yukon River is typically in the opposite order. Fall chum salmon spawn in the most upper reaches of the drainage (river mile-RM >800), while the largest commercial harvests (>80%) are taken in the Lower Yukon Area and the largest subsistence harvests (>90%) occur in the Upper Yukon Area which adds to the complexity of fisheries management (Figure 1). Because the majority of the commercial fishery occurs in the lower two commercial districts, collecting and analyzing genetic harvest samples would assist in prosecution of fisheries, while ensuring that sufficient numbers of chum salmon escape the fishery to achieve other objectives within the drainage. Knowledge of the stock-specific harvest and run timing of Canada-origin stocks can be applied to inseason run assessment in efforts to meet management objectives and allow for development of more accurate post-season run reconstructions which are used for assessing productivity and preseason forecasting.

This project was designed to test the potential to estimate stock composition of chum salmon harvested in the fall season commercial fisheries within District 1 of Yukon River. The commercial harvests in the Lower Yukon Area were substantial; therefore determination of stock composition in the fishery (preclude gear bias) compared to the passage upriver could potentially inform management actions to achieve escapement goals. This project uses the ADF&G genetic baseline of chum salmon populations in the Yukon River to provide regional stock composition estimates. Chum salmon will be allocated to the following stock compositions: summer, fall, U.S., Canada, and combinations thereof. Commercial fish harvests are recorded on ADF&G fish tickets which are legal documentation of transactions (receipts) of sales from fishermen to the buying stations.

OBJECTIVES

This project will analyze chum salmon genetic samples from District Y-1 commercial fishing periods during the transition from summer to fall chum salmon in mid-July when a mix of the

two runs are being harvested. In addition, samples will be collected from the two largest pulses in the remainder of the run when the largest commercial harvests occur. Attempts will be made to sample fish from both the south mouth and middle/north mouth harvests. Stock compositions will be estimated using the available chum salmon baseline of single nucleotide polymorphisms (SNPs; DeCovich et al. 2012) and allocated to the following stock compositions: Lower Summer, Upper U.S. Fall, and Canada. This is a multiyear feasibility study to account for variability in stock timing relative to the fishery in order to allow for robust statistical analysis.

METHODS

FISHERY SAMPLING

Fall chum salmon from the District Y-1 commercial fishery will be tissue sampled by ADF&G and Yukon Delta Fisheries Development Association (YDFDA) crewmembers at the local buying stations. Two methods of collecting genetic samples are most often used: 1) each tissue sample is stored in its own numbered vial allowing association with other data about the fish such as age, sex and length; and 2) tissue samples from multiple fish are pooled or mixed together in a larger container as one bulk collection, in this case based on a time frame (commercial period). In bulk collections individual tissue samples can no longer be matched with data like sex, age, or length. For all tissue sampling, axillary processes are collected and preserved in ethanol.

Samples collected from 2014 were to concentrate on areas where the majority the harvest was occurring. Targeted sampling goals included collections from each of two locations (South Mouth n=110 and Middle/North Mouth n=110) for a total of 220 per commercial period (Table 1) based on the average proportion of harvest from the two major portions of the delta (Table 2). Attempts were made to sample during commercial periods when harvest was expected to be larger than 5,000 fall chum salmon between July 16 and August 30. Harvest sampling was expected to occur during four to eight commercial periods per season based on the most recent (2011–2013) harvest pattern. Decisions about which commercial fishing periods to sample are difficult to make this low in the drainage since managers must estimate the expected catch per unit effort ahead of time. Therefore, more commercial fishing periods were sampled than will be analyzed. Post season the harvest of each commercial period relative to the abundance will be known and four commercial periods (targeted at 220 samples times 4 commercial periods; N=880) ideally will be selected for analysis. Priority for analyzing samples will be given to the commercial periods during the transition of summer to fall chum salmon during July. Second priority will be to analyze the largest commercial harvests sampled during the August fishing periods. Samples will be weighted by the total commercial harvest per sampling period and possibly also weighted by harvest at applicable sampling locations (South Mouth and Middle/North Mouth).

The test-fish crew operating out of the Middle/North Mouth camp (Figure 2) assisted with collecting samples directly from that portion of the delta. During commercial openings at least one of their two daily drifts were cancelled (usually the evening drift) therefore instead of sampling the test fish catch they were assigned to sample the commercial harvest. One option for collecting tissue samples from fishermen's catch was to sample while they are waiting to offload on a nearby tender. Another option involved actually sampling from the fishermen's boat as they fish (based on good rapport with the fishermen). These options best assured that the samples were from the area of interest. Sampling from Middle/North mouth camp allowed for samples

from specific areas of interest (Statistical Areas 334-15, 334-16 and Kwikpak Pass portion of 334-17; Figure 2). The remote crew had access to potentially three different fishing areas including Middle Mouth proper (334-15), North Mouth proper (334-16) and the area upstream where the two mouths converge (334-17). If samples were collected from more than one of the areas they were stored in separate bulk bottles. The last and most difficult option was to track down individual tender arrival times from the areas of interest and bulk sample when it arrived at the buyer's station in Emmonak.

The South Mouth (Statistical Areas 334-12, 334-13 and 334-14; Figure 2) samples were collected in bulk at the buyer's station in Emmonak as the fish were delivered to the dock along with typical ASL sampling efforts. Commercial catch ASL and genetic sampling normally occurred on some of the first deliveries, therefore Statistical Areas 334-13 and 334-14 were most likely represented based on proximity to the buying station. Samples from Statistical Areas 334-17 and 334-18 were excluded or collected separately from the lower areas since they would potentially be a mix of South and Middle/North mouth harvests. The harvest and sampling regimes were evaluated post season based on groupings of statistical areas covering each distributary.

GENETIC ANALYSIS

Genomic DNA was extracted using a DNeasy® 96 Tissue Kit by QIAGEN® (Valencia, CA). Samples were analyzed using Fluidigm® 96.96 Dynamic Arrays (<http://www.fluidigm.com>). The Fluidigm® 96.96 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame. On one side of the frame, there are 96 inlets to accept DNA extracts from individual fish and on the other are 96 inlets to accept the assay cocktails for each SNP marker. Once in the wells, the components are pressurized into the chip using the IFC Controller HX (Fluidigm®). The 96 samples and 96 assays are then systematically combined into 9,216 parallel reactions. Each reaction is a mixture of 4 microliters (µl) of assay mix (1x DA Assay Loading Buffer [Fluidigm®], 10x TaqMan® SNP Genotyping Assay [Applied Biosystems], and 2.5x ROX [Invitrogen]), and 5 µl 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/ul DNA) combined in a 6.7 nanoliter (nL) chamber. Thermal cycling was performed on an Eppendorf IFC Thermal Cycler as follows: an initial "hot mix" for 30 minutes at 70°C, then denaturation for 10 minutes at 96°C followed by 40 cycles of 96°C for 15 seconds and 60°C for 1 minute. The Dynamic Arrays was read on a BioMark™ Real-Time PCR System (Fluidigm®) after amplification and scored using Fluidigm® SNP Genotyping Analysis software.

Quality control (QC) methods consisted of re-extracting DNA from 8% of project fish and re-genotyping them for the original SNPs in a separate process. Discrepancy between genotypes between the original and QC genotypes measure error rates and are capable of identifying extraction, assay plate, and genotyping errors. After laboratory errors are corrected, error rates in the original genotyping were estimated as half the discrepancy rate by assuming that the discrepancies among analyses were due equally to errors during the original genotyping and to errors during QC, and by assuming that at least one of these assays produced the correct genotype.

Genetic stock analysis will begin after 3 years of harvest samples are available. At that time, genotypes will be retrieved from LOKI and imported into *R* (R Development Core Team 2010).

All subsequent analyses will be performed in R unless otherwise noted. Prior to MSA, two statistical QC analyses will be conducted to ensure that only quality genotypic data are included in the estimation of stock compositions. First, individuals missing substantial genotypic data will be removed from further analyses. Individuals missing genotypes for 20% or more of loci will be excluded, because these individuals likely have poor-quality DNA. The inclusion of individuals with poor-quality DNA can introduce genotyping errors and reduce accuracy and precision of MSA. Second, individuals with duplicate genotypes will be identified and removed from further analyses. Duplicate genotypes can occur as a result of sampling or extracting the same individual twice, and will be defined as pairs of individuals sharing the same genotype in 95% of markers screened. The individual with the most missing data from each duplicate pair will be removed from further analyses.

RESULTS AND DISCUSSION

Fall season fishery management began in lower Yukon River District Y-1 on July 16, 2014. The very first commercial period on July 17 appeared to be on a large group of chum salmon that met the criteria and therefore samples were collected. There were twelve commercial periods of which six were sampled. The run consisted of six pulses two in July, one August 1, and three in the latter half of August (Table 3 and Figure 3). Five of the commercial sampling periods landed on pulses of chum salmon and one missed (collection 3) and ended up between pulses 4 and 5. Collection 3 was not analyzed because the harvest was extremely low (approximately 1,000 chum salmon) and collection 6 was not analyzed because it occurred on the same pulse of fish as collection 5 with an equivalent harvest.

The number of samples collected in the field per bulk bottle were slightly different (± 3) than counted in the lab. Overall there were 952 fish (Table 3) genotyped for 96 markers (Table 4) in this study for a total of 91,392 genotypes. Of these, 80 fish were re-analyzed (beginning with the DNA extraction step). Of the 7,680 genotypes in the QC analysis, 13 genotypes, or 0.17%, deviated from the original. Discrepancies did not appear to be related to a laboratory error. Assuming that the discrepancies among analyses were due equally to errors during the original genotyping and to errors during QC, the project error rate is estimated to be 0.08%. No fish were missing genotypes for greater than 20% of markers.

Stock composition will be estimated in a hierarchical manner with harvest apportioned to the following groups in each level: a) summer and fall, b) U.S. and Canada, and c) Lower Summer, U.S. Fall, and Canada. This will be completed for the 2014 sample in June of 2015. At the conclusion of four years of analyses (spring 2016), a comprehensive report detailing the results for 2012 through 2015 will be completed.

ACKNOWLEDGEMENTS

This investigation was financed by the Yukon River Restoration and Enhancement Fund and the Department of Fish and Game under Project URE-01-14N. The authors would like to thank all the individuals that collected samples out of Emmonk including: Yukon Delta Fisheries Development Association employees Evan Charles and Henry Uisok alongside Alaska Department of Fish and Game employees Keegan Birchfield, Amy Bower, Jeff Estensen, Tara Harrington, Joel Hunt, and Mick Leach.

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TABLES AND FIGURES

Table 1.-Genetic sampling goals for chum salmon in the fall season commercial fishery in District Y-1 of the Yukon River, 2014.

Pulse Number	Date Range	South Mouth	Middle/North Mouths	Total per Stratum
1	16-Jul	110	110	220
2	thru	110	110	220
3		110	110	220
4	30-Aug	110	110	220

Table 2.-Average (2005–2013) commercial harvest proportions by statistical area as they relate to the individual river mouths targeted for genetic sampling.

Mouth Name	Statistical Area	Sampling Location	Average Harvest Proportion
Black River	334-11	Do Not Sample	0.00
	334-19	Do Not Sample	0.00
South	334-12	Emmonak Plant	0.17
	334-13	Emmonak Plant	0.07
	334-14	Emmonak Plant	0.11
	South 334-17	Do Not Sample	0.14
Middle	334-15	On site Tender	0.13
North	334-16	On site Tender	0.14
Middle/North	North 334-17	On site Tender	0.14
Mixed Mouths	334-18	Do Not Sample	0.09

Table 3.–Genetic sampling goals for chum salmon in the fall season commercial fishery in District Y-1 of the Yukon River, 2014.

Commercial Period / Collection Number	Start Time	Start Date/ Sample Date	Hours Fished	Harvest Amount	Pulse Number	Number of Samples		
						South Mouth	Middle & North Mouths	Total per Strata
1/1	4:00 PM	17-Jul	6	12,524	1	112*	15*	127
2/2	6:00 PM	21-Jul	6	8,520	2	228*	187*	415
3/0	4:00 PM	25-Jul	6	3,746		NA	NA	
4/0	4:00 PM	28-Jul	6	1,694		NA	NA	
5/3	4:00 PM	15-Aug	6	1,048	4	220		220
6/4	5:00 PM	19-Aug	6	10,011	5	168*	27*	195
7/5	12:00 PM	25-Aug	9	3,994	6	215*		215
8/6	5:00 PM	26-Aug	4	4,342	6	NA	110	110
9/0	12:00 PM	28-Aug	9	3,564		NA	NA	
10/0	12:00 PM	1-Sep	9	1,529		NA	NA	
11/0	9:00 AM	3-Sep	9	478		NA	NA	
12/0	11:00 AM	5-Sep	9	373		NA	NA	
Total			85	51,823		943	339	1,282
Total Analyzed						723	229	952

Note: Sample sizes by mouth noted with an asterisk (*) were included for genotyping in this analysis.

Table 4.–Assay name and source of the 96 SNP markers used in this study.

Assay	Source	Assay	Source	Assay	Source
<i>Oke_ACOT-100</i>	A	<i>Oke_LAMP2-186</i>	A	<i>Oke_U1022-139</i>	A
<i>Oke_AhR1-78</i>	B	<i>Oke_mgll-49</i>	A	<i>Oke_U1023-147</i>	A
<i>Oke_arf-319</i>	C	<i>Oke_MLRN-63</i>	A	<i>Oke_U1024-113</i>	A
<i>Oke_ATP5L-105</i>	A	<i>Oke_Moesin-160</i>	C	<i>Oke_U1025-135</i>	A
<i>Oke_azin1-90</i>	A	<i>Oke_nc2b-148</i>	A	<i>Oke_u200-385</i>	C
<i>Oke_brd2-118</i>	A	<i>Oke_ND3-69</i>	A	<i>Oke_U2006-109</i>	A
<i>Oke_brp16-65</i>	A	<i>Oke_NUPR1-70</i>	A	<i>Oke_U2007-190</i>	A
<i>Oke_CATB-60</i>	A	<i>Oke_pgap-111</i>	A	<i>Oke_U2011-107</i>	A
<i>Oke_ccd16-77</i>	A	<i>Oke_pgap-92</i>	A	<i>Oke_U2015-151</i>	A
<i>Oke_CD81-108</i>	A	<i>Oke_PPA2-635</i>	B	<i>Oke_U2025-86</i>	A
<i>Oke_CD81-173</i>	A	<i>Oke_psm9-57</i>	A	<i>Oke_U2029-79</i>	A
<i>Oke_CKS1-94</i>	A	<i>Oke_rab5a-117</i>	A	<i>Oke_U2031-37</i>	A
<i>Oke_CKS-389</i>	D	<i>Oke_ras1-249</i>	B	<i>Oke_U2032-74</i>	A
<i>Oke_Cr30</i>	A	<i>Oke_RFC2-618</i>	C	<i>Oke_U2034-55</i>	A
<i>Oke_Cr386</i>	A	<i>Oke_RH1op-245</i>	C	<i>Oke_U2035-54</i>	A
<i>Oke_ctgf-105</i>	B	<i>Oke_RS27-81</i>	A	<i>Oke_U2037-76</i>	A
<i>Oke_DCXR-87</i>	A	<i>Oke_RSPRY1-106</i>	A	<i>Oke_U2041-84</i>	A
<i>Oke_e2ig5-50</i>	A	<i>Oke_serpin-140</i>	C	<i>Oke_U2043-51</i>	A
<i>Oke_eif4g1-43</i>	A	<i>Oke_slc1a3a-86</i>	A	<i>Oke_U2048-91</i>	A
<i>Oke_f5-71</i>	A	<i>Oke_sylc-90</i>	A	<i>Oke_U2050-101</i>	A
<i>Oke_FANK1-166</i>	A	<i>Oke_TCP1-78</i>	B	<i>Oke_U2053-60</i>	A
<i>Oke_FBXL5-61</i>	A	<i>Oke_Tf-278</i>	B	<i>Oke_U2054-58</i>	A
<i>Oke_gdh1-191</i>	A	<i>Oke_thic-84</i>	A	<i>Oke_U2056-90</i>	A
<i>Oke_gdh1-62</i>	A	<i>Oke_U1002-262</i>	A	<i>Oke_U2057-80</i>	A
<i>Oke_GHII-3129</i>	B	<i>Oke_U1008-83</i>	A	<i>Oke_U212-87</i>	C
<i>Oke_glr1-78</i>	A	<i>Oke_U1010-251</i>	A	<i>Oke_u217-172</i>	C
<i>Oke_GPDH-191</i>	C	<i>Oke_U1012-241</i>	A	<i>Oke_U302-195</i>	B
<i>Oke_GPH-105</i>	B	<i>Oke_U1015-255</i>	A	<i>Oke_U502-241</i>	B
<i>Oke_HP-182</i>	B	<i>Oke_U1016-154</i>	A	<i>Oke_U504-228</i>	B
<i>Oke_il-1racp-67</i>	C	<i>Oke_U1017-52</i>	A	<i>Oke_U506-110</i>	B
<i>Oke_IL8r2-406</i>	A	<i>Oke_U1018-50</i>	A	<i>Oke_U507-286</i>	B
<i>Oke_KPNA2-87</i>	B	<i>Oke_U1021-102</i>	A	<i>Oke_U509-219</i>	B

Sources: A) International Program for Salmon Ecological Genetics at the University of Washington; B) Elfstrom et al. 2007; C) Smith et al. 2005a; and D) Smith et al. 2005b.

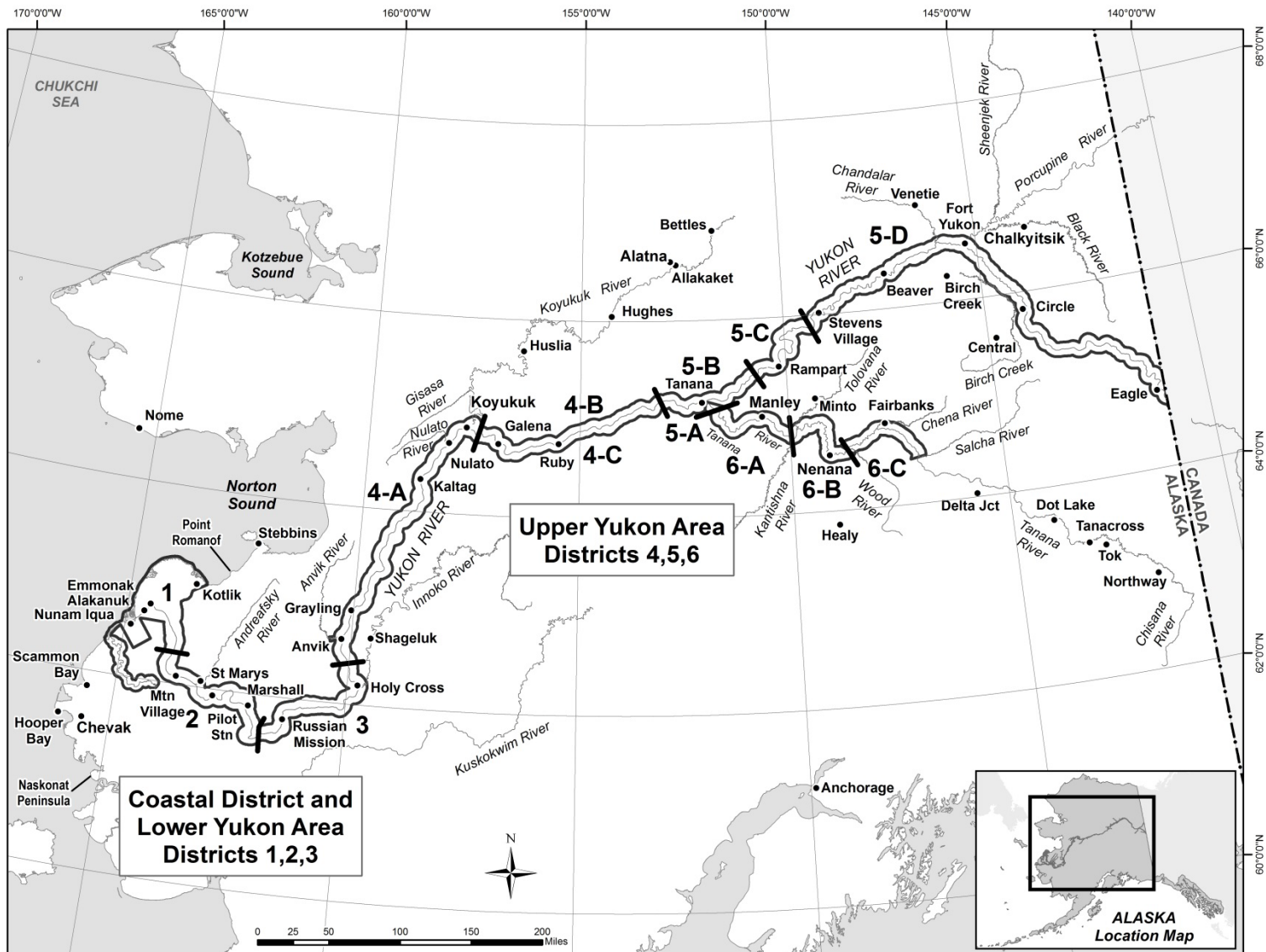


Figure 1.—Location of the fishing districts used for management of salmon fisheries in the United States portion of the Yukon River drainage.

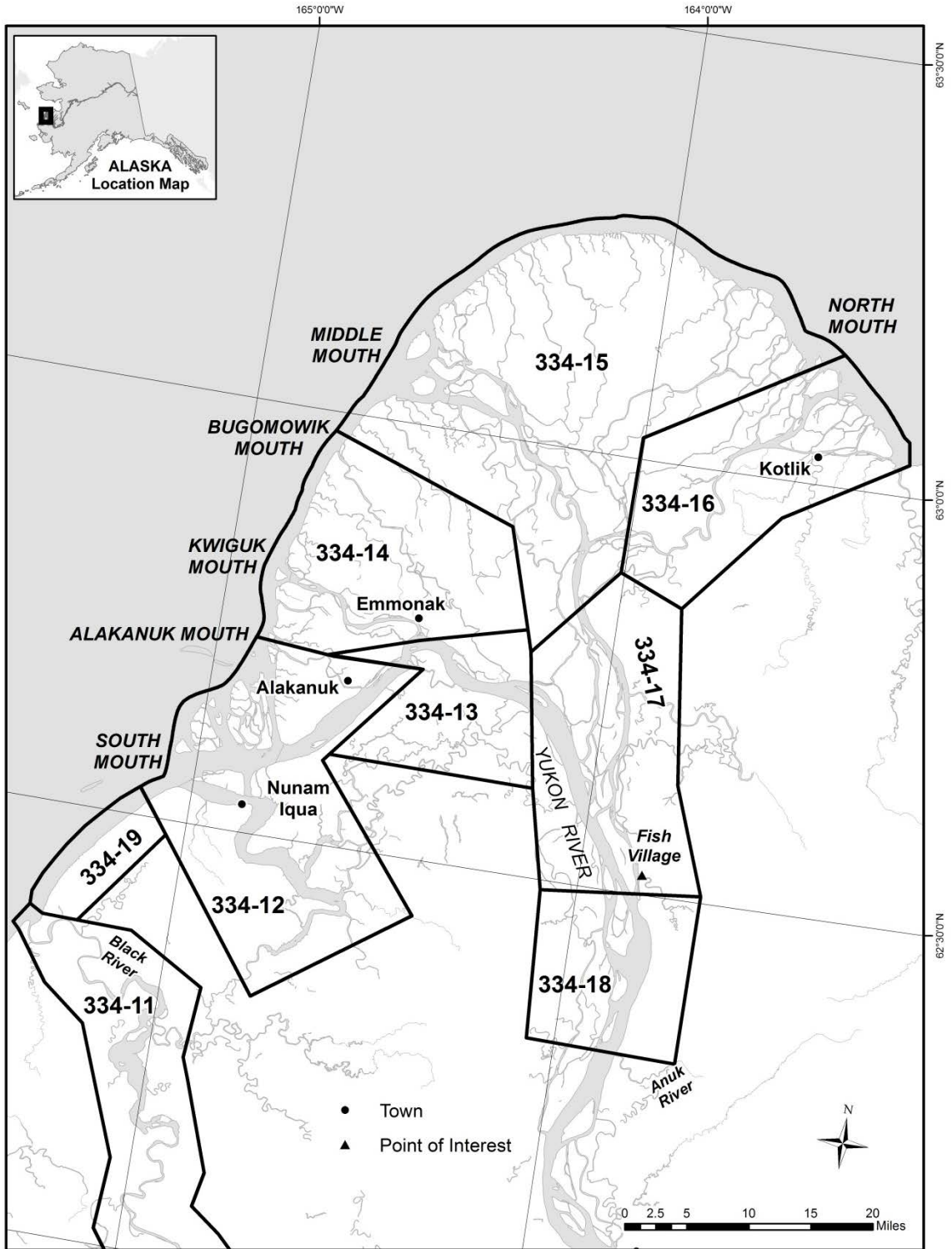


Figure 2.—District Y-1 of the Lower Yukon Area showing commercial statistical areas, Yukon River.

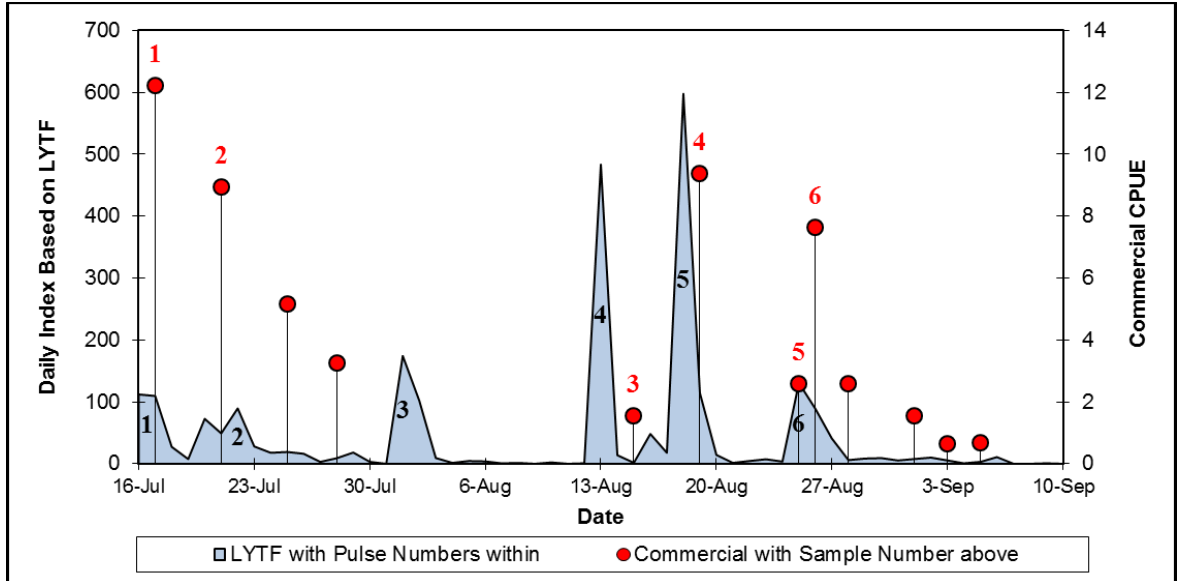


Figure 3.—Fall season passage of chum salmon based on Lower Yukon Test Fish (LYTF) catch per unit effort (CPUE) with pins denoting the CPUE of the commercial periods by date, labels within denote pulse number and labels above pins represent the periods that were sampled for genetics, Yukon River, 2014.