

# Genetic Mixed-Stock Analysis and Composition of Inconnu from the Hotham Inlet and Selawik Lake Winter Subsistence Fishery

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## Genetic Mixed-Stock Analysis and Composition of Inconnu from the Hotham Inlet and Selawik Lake Winter Subsistence Fishery

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### Abstract

Information is lacking about the proportional contributions of Inconnu *Stenodus leucichthys* from the Selawik and Kobuk rivers to the winter subsistence fishery on Hotham Inlet and Selawik Lake. To address this issue, spawning Inconnu from the Selawik and Kobuk River populations were assayed for genetic variation at 20 microsatellite loci to establish a genetic baseline for mixed-stock analysis (MSA). This analysis observed significant allele frequency differences ( $P < 0.05$ ) between the Selawik and Kobuk River populations, but the magnitude of the divergence was quite low with an overall  $F_{ST}$  of 0.0002. Mixed-stock simulations revealed low estimation accuracies and high variances for the two populations. These analyses demonstrated that the genetic baseline did not provide a sufficient level of discrimination between the Selawik and Kobuk River populations for MSA.

### Introduction

There are two known Inconnu *Stenodus leucichthys* populations in northwest Alaska, one that spawns in the upper Selawik River, within the Selawik National Wildlife Refuge (Refuge), and one that spawns in the upper Kobuk River (Alt 1969; Underwood 2000; Hander et al. 2008). Inconnu from both populations live their entire life-cycle within those rivers and associated estuary systems in northwest Alaska. They overwinter in Selawik Lake, Hotham Inlet, and other associated waterways (Alt 1969, 1973; Figure 1). Tagged fish from both the Selawik and Kobuk rivers have been recaptured as far seaward as the village of Kotzebue, where the brackish water of Hotham Inlet meets the marine water of Kotzebue Sound (Taube 1996, 1997; Underwood 2000). They tolerate brackish environments but cannot survive the cold fully marine water in the winter, which approaches  $-2^{\circ}\text{C}$  under ice cover (Black 1957; U.S. Navy Hydrographic Office 1958; DeVries and Cheng 2005). Both populations are thus confined during winter to the aquatic habitat on the freshwater side of the brackish-marine divide near the village of Kotzebue.

Most Inconnu mature by 8 to 12 years of age and are capable of living for 30 years or more (Howland 1997; Brown 2000; Howland et al. 2004). Beginning in early summer, mature Inconnu begin a slow spawning migration up either the Selawik or Kobuk River, initially feeding in the lower reaches with nonspawning fish, and arriving at spawning areas in the upper reaches by late summer or fall. Nonspawning adults and immature Inconnu remain in the lower reaches of the rivers and estuaries (Alt 1969). By early September, Inconnu have completed their migration to upstream spawning areas, where they remain until late September or early October when spawning takes place. Eggs are broadcast over gravel and cobble substrate. The fertilized eggs settle into the interstitial spaces in the substrate and develop through the winter. Post-spawning Inconnu leave the area immediately, returning to the large lakes and estuaries

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for overwintering (Alt 1969; Underwood 2000). Eggs are thought to hatch in late winter or spring and larvae are carried downstream with the high waters of spring (Shestakov 1991; Bogdanov et al. 1992; Naesje et al. 1995). Tag recapture data from mark recapture studies (Taube and Wuttig 1998; Underwood 2000; Hander et al. 2008) and previous genetics analyses (Miller et al. 1998) suggest that Inconnu exhibit fidelity to their natal rivers for spawning.

Inconnu are harvested in the rivers and estuaries of northwest Alaska in all seasons of the year. Annual harvests in the region can be greater than 20,000 Inconnu, although harvest is not monitored region-wide or on a regular basis (Georgette and Loon 1990; Taube 1997; Savereide 2002; Georgette and Koster 2005; Georgette and Shiedt 2005). The winter harvest of Inconnu that takes place in Selawik Lake and Hotham Inlet is thought to be the largest regional fishery and includes fish from both populations, however, the proportional composition is not known. Population-specific harvest data would be valuable information for management of the fishery. The objectives of this project were, therefore, to (1) develop a modern genetic baseline for the two known Inconnu populations in the region that is sufficiently robust to perform a meaningful mixed-stock analysis on harvest samples; (2) estimate the proportional contributions of Selawik and Kobuk River Inconnu to spatial and temporal strata of the winter subsistence fishery; and (3) test the hypothesis that proportions of Selawik or Kobuk River Inconnu are statistically equivalent among sample areas and times (i.e., uniform distribution in overwintering habitat).

## **Study Area**

The Selawik and Kobuk rivers estuary complex is surrounded by lands consisting mainly of broad river floodplains and lake-dotted lowlands (Figure 1; USFWS 2011). The lowlands are drained primarily by the Selawik River in the central and eastern portions of the Refuge and the Kobuk River in the northwest. Most of the streams in the area are sluggish, meandering, moderately low gradient, and have numerous side sloughs. The waters of Hotham Inlet (1,120 km<sup>2</sup>; United States Census Bureau 2000) are an extension of Kotzebue Sound and the Chukchi Sea. It is brackish as a result of the considerable outflows of the Selawik and Kobuk rivers, combined with those of the Noatak River to the north. Hotham Inlet is bound on the south and west by the Baldwin Peninsula.

Both the Selawik and Kobuk rivers have extensive deltas, underlain by continuous permafrost and characterized by thaw lakes and branching channels (USFWS 2011). Jorgenson et al. (2008) identified a bulb of discontinuous permafrost that may also influence hydro-geologic landscape characteristics. The Selawik Flats begins where the river leaves a well-defined floodplain at the junction of the Kugarak River; here, it begins to flow sluggishly and form back waters and sloughs. The Kobuk River delta is larger, beginning at the mouth of the Squirrel River where channels fan outward. The Kobuk River flows into Hotham Inlet through as many as 13 separate mouths. The southern channel, the Nazuruk Channel, is the largest and enters Hotham Inlet near the west end of Selawik Lake (1,046 km<sup>2</sup>; National Atlas of the United States 2013). Another large channel is the Riley Channel; it flows from the village of Noorvik and reaches the inlet opposite Nimiuk Point. The many other channels form a well-used navigation network throughout the entire delta. The Kobuk River delta is approximately 56 km long and 40 km wide, and is the largest river delta emptying into the Chukchi Sea (USFWS 2011).

## Methods

### *Sample collection and laboratory analysis*

Tissue samples from spawning Inconnu were collected for a genetic baseline from the Selawik ( $n=382$ ) and Kobuk ( $n=389$ ) rivers in 2008 (Selawik  $n=191$ , Kobuk  $n=190$ ) and 2012 (Selawik  $n=191$ , Kobuk  $n=199$ , Figure 1). In addition, tissue samples from Inconnu were collected from the subsistence fisheries at Hotham Inlet ( $n=1,604$ ), Noorvik ( $n=113$ ), and Selawik Lake ( $n=662$ ) during the winters of 2011 and 2012 (Table 1; Figure 1). Subsistence fishery sample locations were based on work done by Savereide (2002) with the addition of locations near the village of Selawik and Noorvik (added in the late winter of 2012) (Figure 1). These general fishing locations were used on an annual basis. Sampling locations and sample timing coincided with the timing of the Inconnu subsistence fishery noted by Savereide (2002), A. Whiting (Native Village of Kotzebue (NVOK), personal communication), and F. Berry and C. Ramoth (USFWS, Selawik National Wildlife Refuge, personal communication).

Contract agreements were made with NVOK, the Native Village of Selawik (NVOS), and the Noorvik Native Community (NNC) to collect genetic samples. The Native Village of Kotzebue, NVOS, and NNC were responsible for collecting samples from subsistence harvested Inconnu and preserving them according to criteria from the USFWS Conservation Genetics Laboratory (CGL). The Native Village of Kotzebue, NVOS, and NNC sampled in locations that are geographically close to their respective community. Sample sites were documented using a global positioning system (GPS) unit and information from fishers regarding the general area(s) where sampled Inconnu were caught. Sampling procedures and GPS training were provided to collection personnel before the collection process began, including inspecting Inconnu for prior scientific project's markings with fin clips, floy tags, and radio tags.

Total genomic DNA was extracted from the tissue (~25mg) using proteinase K with the Dneasy™ DNA isolation kit (Qiagen Inc., Valencia, CA), quantified by fluorometry, and diluted to 30 ng/μl. Polymerase chain reaction (PCR) DNA amplification was used to assay genetic variation at the following microsatellite loci: *BWF2* (Patton et al. 1997); *C2-157* (Turgeon et al. 1999); *Cocl-Lav4*, *Cocl-Lav61* (Rogers et al. 2004); *Sfo-8*, *Sfo-8b* (Angers et al. 1995); *Sle001*, *Sle002*, *Sle003*, *Sle004*, *Sle005*, *Sle006*, *Sle007*, *Sle008*, *Sle009*, *Sle010*, *Sle011*, *Sle012*, *Sle013*, *Sle014* (USFWS unpublished). The PCR product was electrophoresed and visualized with the Applied Biosystems 3730 Genetic Analyzer utilizing a polymer denaturing capillary system. The sizes of bands were estimated and scored by the computer program GENEMAPPER version 4.0. Applied Biosystems GeneScan-600 LIZ size standards, 20-600 bases, were loaded in all lanes to ensure consistency of allele scores. All scores were verified manually. Alleles were scored by two independent researchers, with any discrepancies being resolved by re-running the samples in question and repeating the double scoring process until scores matched.

### *Data analysis*

Hardy-Weinberg and gametic phase equilibrium were assessed for the baseline data using the program FSTAT 2.9.3 (Goudet 1995). Genetic diversity for the loci was estimated by calculating allelic richness, observed and expected heterozygosity, and gene differentiation ( $F_{ST}$ ) with FSTAT 2.9.3 and GENEPOP 4.1 (Raymond and Rousset 1995). Estimates of effective number of alleles (Hartl and Clark 1997) were calculated in Microsoft Excel. GENEPOP 4.1 was used to conduct tests of allelic frequency homogeneity between the baseline collections.

Effective population size ( $N_e$ ) was estimated for the baseline collections using the linkage disequilibrium method implemented in  $N_E$ ESTIMATOR 2.01 (Do et al. 2014).

Mixed-stock analysis of simulated and known-origin mixtures was conducted to determine if there was sufficient genetic divergence between the baseline collections to apportion fishery mixtures. The program ONCOR (Kalinowski et al. 2007) was used to conduct simulations with mixture proportions varying from 0% to 100% at 25% increments for each baseline collection. Size of simulated mixtures was set to 100 fish, and 1,000 simulations were performed for each mixture scenario. The stock compositions of the simulated mixtures were estimated by ONCOR using conditional maximum likelihood (CML).

For the known-origin mixture analysis, samples were randomly removed from the baseline collections and used as a mixture independent of the baseline. The known-origin mixtures were assembled such that the proportions ranged from 0% to 100% at 25% increments for each collection. This process was repeated three times. The sample size for each known-origin mixture was 100. The stock compositions of the mixtures were estimated by Bayesian and CML mixture modeling using the programs cBAYES (Neaves et al. 2005) and ONCOR. Variances of the Bayesian estimates were estimated from nine Markov chain–Monte Carlo simulations, which were iteratively sampled 60,000 times each. Variances for the CML estimates were estimated by bootstrapping the baseline and mixture 1,000 times.

The Inconnu samples collected from the Hotham Inlet, Noorvik, and Selawik Lake subsistence fisheries were analyzed for stock composition using the baseline data and Bayesian and CML mixture modeling as described above.

## Results

All loci were in Hardy-Weinberg and gametic phase equilibrium. Estimates of genetic diversity for the loci ranged from 3–36 for number of alleles, 3.0–34.2 for allelic richness, 1.1–17.0 for effective number of alleles, 0.0986–0.9519 for observed heterozygosity, 0.0992–0.9409 for expected heterozygosity, and 0.0000–0.0028 for  $F_{ST}$  (Table 2). There were significant allele frequency differences ( $P < 0.05$ ) between the Selawik and Kobuk River collections, but the magnitude of the divergence was quite low with an overall  $F_{ST}$  of 0.0002. The  $N_e$  estimates were large: 3,325 (1,757–22,175) for the Selawik River population and 10,741 (95% confidence interval, 2,857– $\infty$ ) for the Kobuk River population.

Mixed-stock simulations revealed low estimation accuracies and high variances for the Selawik and Kobuk collections (Table 3, Figure 2). Each collection had an accuracy of only 67% when comprising the entire mixture. Moreover, the estimates for the two collections over the range of mixture scenarios were comparable, indicating a lack of distinctiveness in the data. The known-mixture analysis further indicated that the stock composition estimates lacked accuracy and precision (Table 4, Figure 3). Over the three series of mixture scenarios, the estimates diverged widely from the expected values. The most egregious error occurred in the first mixture series when the expected value for Kobuk was 100% but the estimate was only 23.6%.

The stock composition estimates for the subsistence fisheries lacked precision (Table 5, Table 6, Figure 4). The large standard deviations and overlapping intervals preclude using these data for a quantitative assessment of fishery harvest of Inconnu from the Selawik and Kobuk rivers. Furthermore, the level of imprecision and absence of a geographic pattern prevents even a qualitative interpretation of whether the mixtures are more one collection than the other.

## Discussion

Inconnu from the Selawik and Kobuk rivers exhibit a low degree of genetic divergence ( $F_{ST}=0.0002$ ) that is marginally significant. The genetic similarity is surprising given that Miller et al. (1998) observed significant genetic divergence at two loci between Inconnu from these rivers. We attempted to replicate those results but attained different scores for a number of samples, which rendered insignificant results. Improvements in laboratory technology since the time of the Miller et al. (1998) study likely explain the difference in scores. We used a Caliper Life Sciences LabChip whereas Miller et al. (1998) used agarose gels to electrophorese DNA fragments. A LabChip offers greater sensitivity and resolution of DNA fragments compared to agarose gels (Vitale 2000).

The genetic similarity is also unexpected considering the apparent separation of Inconnu from the two rivers based on tagging data. Tagged fish from both rivers have been located in the Hotham Inlet and Selawik Lake, but fish have not been observed straying between the rivers (Taube and Wuttig 1998; Underwood 2000). Undetected gene flow or the combination of large  $N_e$  and limited time of separation could explain the genetic similarity. Genetic drift between populations decreases as  $N_e$  increases. Inconnu from the Selawik and Kobuk rivers have large  $N_e$  estimates and past glaciations covered this area (Fernald 1964; Lindsey and McPhail 1986), so this combination is a distinct possibility. A similar situation occurs for Chum Salmon *Oncorhynchus keta* from western Alaska. Despite large geographic distances among populations, western Alaska Chum Salmon show little divergence due to historical connections (Garvin et al. 2013).

The level of divergence observed between Inconnu from the Selawik and Kobuk rivers is not adequate for mixed-stock analysis. The mixed-stock simulations gave the first indication that stock composition estimation would be problematic. The evaluation process continued with analyses of the subsistence fishery samples to ascertain if the results made biogeographical sense. The mixtures were collected across an area where biogeographical patterns of mixture composition may be evident, which would be supportive of the potential for the estimates to be used for a qualitative assessment of the fishery composition (i.e., more one stock than the other). However, the high variances of the estimates obscured any potential association between stock composition and geographic location. The known-origin mixture analyses confirmed that the genetic baseline does not provide discrimination between Inconnu from the Selawik and Kobuk rivers.

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**Table 1. Inconnu genetic samples collected from the subsistence fishery in Hotham Inlet, Selawik Lake, and Noorvik during the winters of 2011 and 2012.**

Sample Area	Winter 2011–2012		Winter 2012–2013		Total
	Late	Early	Late	Early	
Hotham Inlet East	203	199	198	200	800
Hotham Inlet West	201	201	202	200	804
Selawik Lake	203	64	199	196	662
Noorvik	---a	---	---	113	113
Total	607	464	599	709	2,379

<sup>a</sup> dashed line indicates that no samples were obtained during the sample period

**Table 2. For each locus across baseline collections: number of alleles, allelic richness ( $A_R$ ), effective number of alleles ( $A_E$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and measure of genetic divergence ( $F_{ST}$ ).**

Locus	Alleles	$A_R$	$A_E$	$H_O$	$H_E$	$F_{ST}$
<i>BWF2</i>	4	3.7	2.0	0.5228	0.4982	0.0000
<i>C2-157</i>	7	6.0	2.2	0.5618	0.5499	0.0011
<i>Cocl-Lav4</i>	4	3.7	1.1	0.1117	0.1113	0.0028
<i>Cocl-Lav61</i>	4	3.5	1.8	0.4429	0.4385	0.0000
<i>Sfo-8</i>	4	3.4	1.1	0.0533	0.0520	0.0020
<i>Sfo-8b</i>	19	16.7	6.1	0.8252	0.8364	0.0000
<i>Sle001</i>	3	2.5	1.8	0.4479	0.4531	0.0019
<i>Sle002</i>	9	8.7	2.9	0.6680	0.6577	0.0000
<i>Sle003</i>	3	3.0	1.1	0.0986	0.0992	0.0000
<i>Sle004</i>	23	21.3	11.2	0.9035	0.9102	0.0013
<i>Sle005</i>	18	17.1	10.7	0.8987	0.9062	0.0006
<i>Sle006</i>	11	10.9	4.4	0.7652	0.7723	0.0000
<i>Sle007</i>	11	9.6	1.9	0.4462	0.4662	0.0000
<i>Sle008</i>	20	19.2	3.9	0.7468	0.7423	0.0000
<i>Sle009</i>	6	5.4	1.4	0.3104	0.3089	0.0004
<i>Sle010</i>	17	16.1	6.8	0.8312	0.8523	0.0004
<i>Sle011</i>	18	17.8	4.7	0.7403	0.7862	0.0000
<i>Sle012</i>	35	31.0	14.5	0.9157	0.9309	0.0001
<i>Sle013</i>	12	10.9	4.0	0.7393	0.7491	0.0008
<i>Sle014</i>	36	34.2	17.0	0.9519	0.9409	0.0008

**Table 3. Mixed-stock analysis of simulated mixtures using ONCOR. Simulations were conducted with proportions varying from 0% to 100% for each collection at 25% increments.**

	Expected	Estimate	SD	95 CI	
Kobuk	100.0	67.0	9.5	48.9	85.7
Selawik	0.0	33.0	9.5	14.3	51.1
Kobuk	75.0	58.7	9.2	39.8	76.5
Selawik	25.0	41.4	9.2	23.6	60.2
Kobuk	50.0	49.2	9.5	30.2	68.3
Selawik	50.0	50.8	9.5	31.7	69.8
Kobuk	25.0	41.3	9.3	22.5	59.8
Selawik	75.0	58.7	9.3	40.2	77.5
Kobuk	0.0	32.7	9.5	13.4	51.5
Selawik	100.0	67.3	9.5	48.5	86.6

**Table 4. Known-origin mixture analysis using cBAYES and ONCOR.**

Mix 1	Expected	cBAYES				ONCOR		
		Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	100.0	23.6	17.5	0.3	60.7	38.6	20.7	59.0
Selawik	0.0	76.4	17.5	39.2	99.7	61.4	41.0	79.3
Kobuk	75.0	34.9	21.5	0.8	76.5	51.1	30.8	68.0
Selawik	25.0	65.1	21.5	23.5	99.2	48.9	32.0	69.2
Kobuk	50.0	64.9	31.1	1.4	99.9	59.0	38.9	75.5
Selawik	50.0	35.1	31.1	0.1	98.6	41.0	24.5	61.1
Kobuk	25.0	77.7	16.5	42.0	99.9	66.0	42.1	80.1
Selawik	75.0	22.3	16.5	0.1	58.0	34.1	19.9	57.9
Kobuk	0.0	25.0	17.7	0.3	63.3	38.8	24.7	62.8
Selawik	100.0	75.0	17.7	36.7	99.6	61.2	37.2	75.3
Mix 2								
	Expected	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	100.0	56.5	25.1	6.9	99.5	52.0	29.1	68.1
Selawik	0.0	43.5	25.1	0.5	93.1	48.0	31.9	70.9
Kobuk	75.0	59.9	23.8	8.4	99.4	52.9	32.6	69.1
Selawik	25.0	40.1	23.8	0.6	91.6	47.1	30.9	67.4

Table 4. continued.

	cBAYES					ONCOR		
	Expected	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	50.0	36.1	17.8	5.5	73.0	43.8	27.6	65.9
Selawik	50.0	63.9	17.8	27.0	94.5	56.2	34.1	72.4
Kobuk	25.0	46.7	20.2	2.9	81.1	50.1	31.6	71.9
Selawik	75.0	53.3	20.2	18.9	97.1	49.9	28.1	68.4
Kobuk	0.0	20.0	17.6	0.1	58.5	38.8	28.4	66.4
Selawik	100.0	80.0	17.6	41.5	99.9	61.2	33.6	71.6
Mix 3								
	Expected	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	100.0	63.8	20.8	20.3	99.7	54.9	29.9	71.7
Selawik	0.0	36.2	20.8	0.3	79.6	45.1	28.3	70.1
Kobuk	75.0	67.8	19.6	32.4	99.7	62.6	37.0	75.9
Selawik	25.0	32.2	19.6	0.3	67.6	37.5	24.1	63.0
Kobuk	50.0	46.1	23.9	3.0	94.0	43.8	26.8	66.9
Selawik	50.0	53.9	23.9	5.9	97.0	56.2	33.1	73.2
Kobuk	25.0	28.9	19.4	0.4	69.1	40.6	25.1	69.3
Selawik	75.0	71.1	19.4	30.9	99.6	59.4	30.7	74.9
Kobuk	0.0	23.2	15.9	0.7	61.5	37.8	23.9	62.9
Selawik	100.0	76.8	15.9	38.5	99.3	62.2	37.1	76.1

**Table 5. Stock composition estimates for winter 2011–2012 subsistence fisheries. HIE=Hotham Inlet east, HIW=Hotham Inlet west, SL=Selawik Lake.**

2011–2012	cBAYES				ONCOR		
HIE fall	<i>n</i> =195						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	79.5	15.7	45.2	99.8	58.3	38.4	72.0
Selawik	20.5	15.7	0.1	54.8	41.7	28.0	61.6
HIE Spring	<i>n</i> =201						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	85.6	16.6	48.8	99.9	61.3	43.0	74.6
Selawik	14.4	16.6	0.0	51.1	38.7	25.4	57.0
HIW fall	<i>n</i> =186						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	63.3	28.6	0.7	97.7	55.8	39.6	71.9
Selawik	36.7	28.6	2.3	99.3	44.2	28.1	60.4
HIW Spring	<i>n</i> =195						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	84.2	15.2	52.0	99.9	63.7	43.0	74.8
Selawik	15.8	15.2	0.1	48.0	36.3	25.2	57.0
SL fall	<i>n</i> =60						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	37.8	26.4	0.3	88.7	55.8	26.3	79.9
Selawik	62.2	26.4	11.3	99.7	44.2	20.1	73.7
SL spring	<i>n</i> =198						
	Estimate	SD	95 CI		Estimate	95 CI	
Kobuk	42.2	21.5	0.2	74.4	54.8	35.7	67.4
Selawik	57.8	21.5	25.6	99.8	45.2	32.6	64.3

**Table 6. Stock composition estimates for winter 2012–2013 subsistence fisheries. HIE=Hotham Inlet east, HIW=Hotham Inlet west, SL=Selawik Lake, NOR=Noorvik.**

2012–2013	cBAYES			ONCOR		
HIE fall	<i>n</i> =193					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	92.5	7.9	70.8 100.0	70.0	44.6	77.9
Selawik	7.5	7.9	0.0 29.2	30.0	22.1	53.4
HIE Spring	<i>n</i> =156					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	63.9	17.5	30.8 96.6	57.2	37.7	70.4
Selawik	36.1	17.5	3.4 69.2	42.8	29.6	62.3
HIW fall	<i>n</i> =186					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	90.0	10.9	60.3 100.0	59.8	40.7	74.7
Selawik	10.0	10.9	0.0 39.7	40.2	25.3	59.3
HIW Spring	<i>n</i> =141					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	59.7	16.6	27.4 91.6	55.4	33.3	71.7
Selawik	40.3	16.6	8.4 72.5	44.6	28.3	66.7
SL fall	<i>n</i> =196					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	79.0	14.2	49.7 99.9	58.3	39.8	70.7
Selawik	21.0	14.2	0.1 50.3	41.7	29.3	60.2
SL spring	<i>n</i> =176					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	71.3	15.4	41.8 97.0	61.3	40.2	72.7
Selawik	28.7	15.4	3.0 58.2	38.7	27.3	59.8
NOR fall	<i>n</i> =57					
	Estimate	SD	95 CI	Estimate	95 CI	
Kobuk	82.1	17.1	39.4 99.9	73.4	34.2	93.0
Selawik	17.9	17.1	0.0 60.5	26.7	7.0	65.8

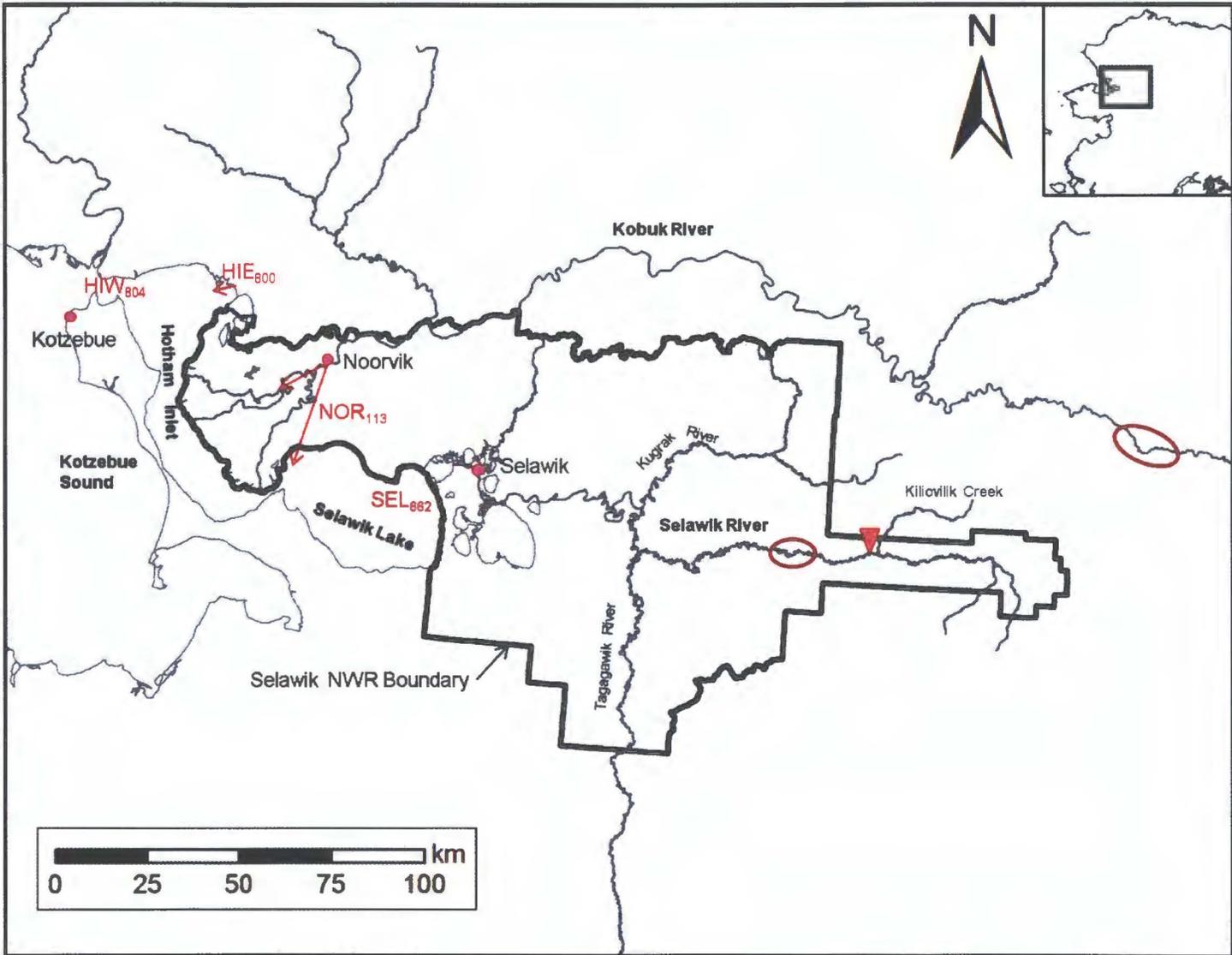
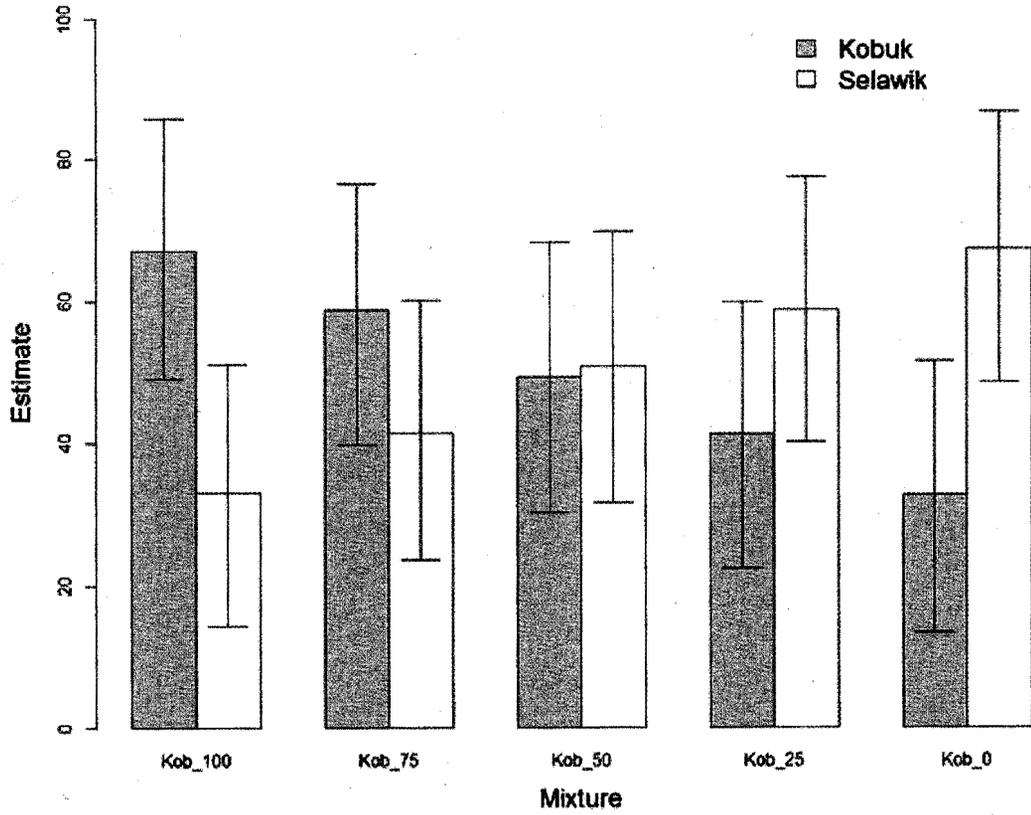
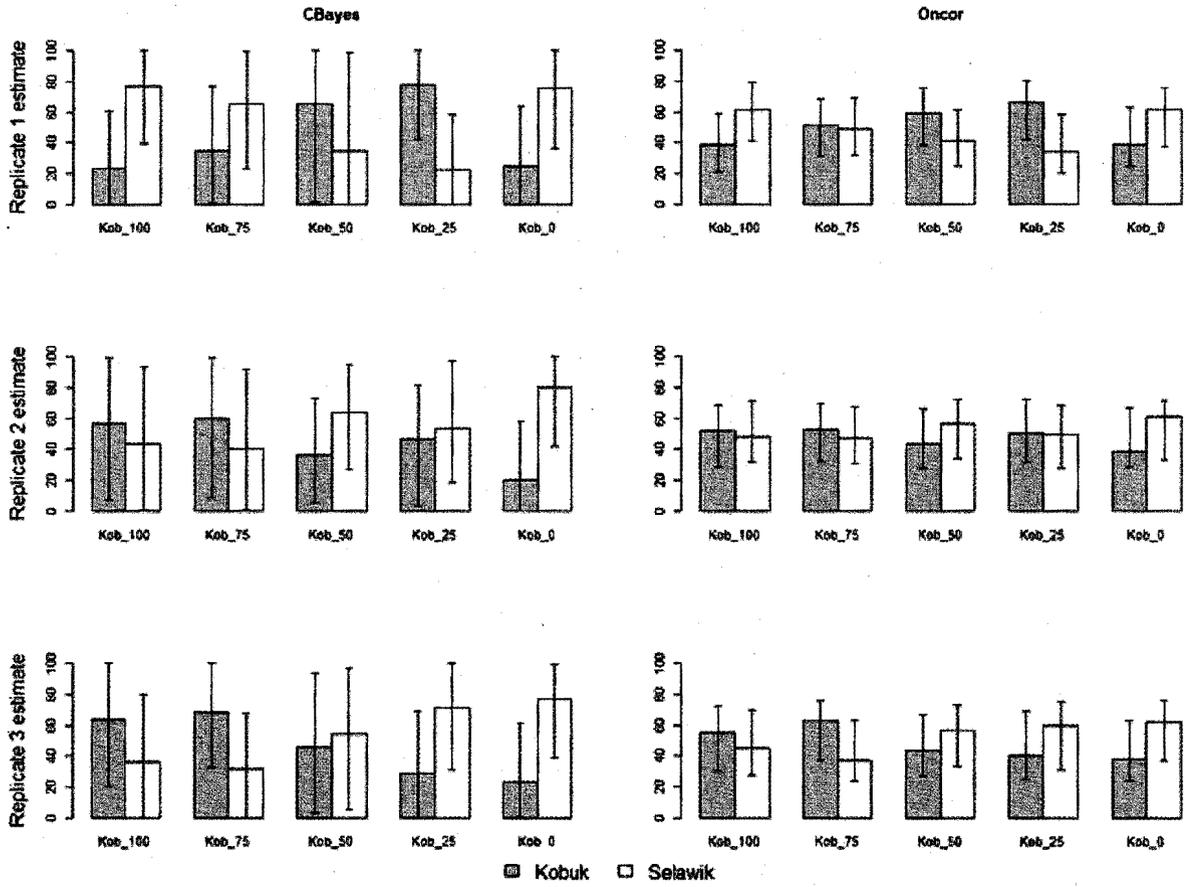


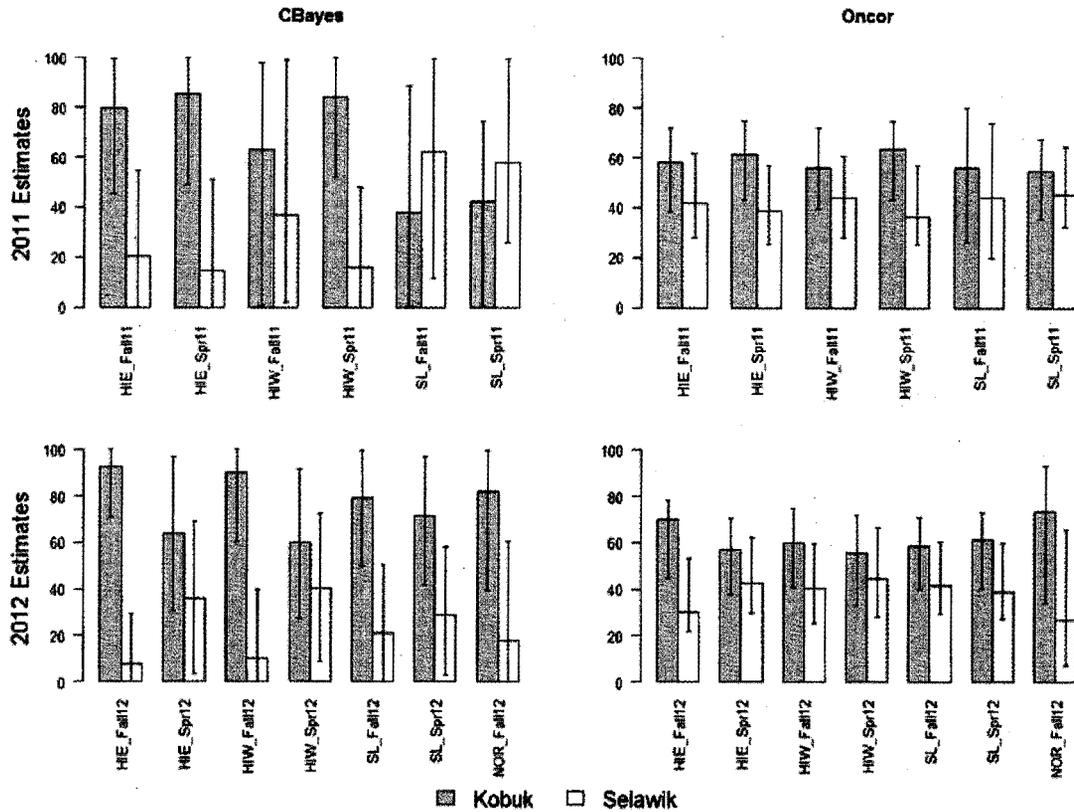
Figure 1. Hotham Inlet West and East (HIW and HIE), Noorvik (NOR), and Selawik (SEL) general sampling areas. Combined numbers of Inconnu genetic samples collected during winters 2011–2012 and 2012–2013 are in the red subscript. Red ellipses are the Selawik and Kobuk River Inconnu spawning areas and the red triangle is the location of the permafrost thaw slump.



**Figure 2. Histogram showing mixed-stock analyses of simulated mixtures using ONCOR. Simulations were conducted with proportions varying from 100% Kobuk (0% Selawik) to 0% Kobuk (100% Selawik) at 25% increments. Bars indicate the 95% confidence intervals for each estimate.**



**Figure 3. Histogram showing replicate mixed-stock analyses of known mixtures using cBayes and Oncor. The mixtures was created by drawing 100 individuals at random from the baseline with proportions varying from 100% Kobuk (0% Selawik) to 0% Kobuk (100% Selawik) at 25% increments. Bars indicate the 95% confidence intervals for each estimate.**



**Figure 4. Histogram showing mixed-stock analyses for fall and spring fishery collections from 2011 and 2012. Stock composition estimates were made using cBayes and Oncor. Locations are Hotham Inlet east (HIE), Hotham Inlet west (HIW, Selawik Lake (SL) and Noorvik (NOR). Bars indicate the 95% confidence intervals for each estimate. Stock composition estimates for 2012 subsistence fisheries. HIE=Hotham Inlet east, HIW=Hotham Inlet west, SL=Selawik Lake, NOR=Noorvik.**

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