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Development of Microsatellite Genetic Markers for Kenai River Chinook Salmon

by

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Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
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

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye to fork	MEF
mideye to tail fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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ABSTRACT

Significant genetic variation exists among populations of Chinook salmon from Kenai River drainage. Recent analyses using microsatellite markers confirm the results of the earlier studies which detected differences between early- and late-run Chinook salmon based on allozyme and mitochondrial DNA markers. This report presents the results of a survey of 13 microsatellite loci (from a standardized set used by the Pacific Salmon Commission Chinook Technical Committee) in 977 individual fish representing nine Chinook salmon populations in the drainage. Average genotyping failure rate was approximately 4.5%, with the majority of failures in the Slikok Creek collection (success rate ~ 35.9%) where samples mainly came from carcass sampling. Estimates of per-locus F_{ST} ranged from 0.019 to 0.045 suggesting a level of divergence among collections that should be useful for management applications. The populations in the baseline could be separated into four groups based on geographic, behavioral, and genetic characteristics: Lower Kenai River tributaries, Kenai River mainstem, Killey River, and Quartz Creek. Simulation results indicate that contributions from these groups can be detected in fishery harvests with a high degree of precision and accuracy (mean correct allocation = 96.7%).

Keywords: Chinook salmon, *Oncorhynchus tshawytscha*, Cook Inlet, Kenai River, microsatellite.

INTRODUCTION

Kenai River supports two runs of Chinook salmon *Oncorhynchus tshawytscha* annually. Popular sport fisheries are supported by each run due to the proximity of Kenai River to major population centers, easy accessibility and large size of fish from both runs. Separate management plans have been adopted by the Alaska Board of Fisheries for each run and are based upon the ecological differences (i.e., run-timing, abundance, and spawning distribution) of each run. Those returning from mid-May through June 30 are designated as early run and are known to spawn primarily in Kenai River tributaries (Bendock and Alexandersdottir 1992; Burger et al. 1983). Tributaries which support populations of Chinook salmon include Beaver Creek, Slikok Creek, Funny River, Moose River, Killey River, Russian River, Juneau Creek, Quartz Creek, Ptarmigan Creek, and Grant Creek (Figure 1; ADF&G 1998). Benjamin Creek, tributary of the Killey River, and Crescent and Daves Creek, tributaries of Quartz Creek, also contain Chinook salmon. The average total return from 1986 to 2001 was 17,948 Chinook salmon (McKinley 2003). Early-run fish are harvested primarily by an inriver sport fishery, but also to a lesser degree by a marine sport fishery in Cook Inlet and a subsistence fishery in the estuary.

Late-run Chinook salmon return from July 1 to early August, are more numerous, and exhibit a less geographically complex spawning distribution as most are thought to spawn primarily in the mainstem Kenai River (Bendock and Alexandersdottir 1992; Burger et al. 1983; Hammarstrom et al. 1985). The entire length of the Kenai River mainstem is suitable spawning habitat for Chinook salmon. The average total return from 1986-1998 was 57,096 Chinook salmon (Hammarstrom 1993). Late-run fish are harvested primarily by an inriver sport fishery and a marine commercial set gillnet fishery, but also harvested by marine sport, commercial drift gillnet, subsistence, and personal use fisheries.

To achieve management plan objectives amid harvests by the various fisheries, timely information on stock status is required. Consequently, the Alaska Department of Fish and Game (ADF&G) monitors and manages both runs in season. The returns are monitored by a riverine sonar system located at river mile (RM) 8.5 (Miller et al. 2003). Age, sex, and length (ASL) of the inriver return is estimated from catches obtained from a drift gillnetting program near the sonar. The magnitude and ASL of the sport harvest are estimated by a creel survey (Eskelin 2007). In addition, ADF&G tallies information from personal use fishing permits (Reimer and Sigurdsson 2004) and subsistence reports from the Kenaitze Indian Tribe (Shields 2006). The

proportion of Chinook salmon harvested in the commercial set gillnet fishery on the east side of Cook Inlet (eastside setnet, ESSN) thought to be from the Kenai River is estimated postseason. In addition to supporting inseason management, research and management programs are the foundation for the long-term quantitative stock assessment of Kenai River Chinook salmon.

Despite these efforts, several issues remain to be resolved. For instance, although each run is managed as a separate breeding group, the degree of overlap in the return timing or sonar passage date by run of tributary-(early run) and mainstem-(late run) spawning Chinook salmon is not known, nor is the run composition of the sport, personal use, commercial ESSN, or subsistence harvests. The accuracy with which we assess the abundance and status of early- and late-run Kenai River Chinook salmon would be substantially improved with knowledge of these factors.

Assay genetic marker technology has been applied extensively to develop baselines for genetic stock identification (GSI) to differentiate between aggregates of stocks, stocks, and sub-stocks of Pacific salmon in various mixed stock fisheries (Seeb et al. 2007). A genetic baseline for Kenai River Chinook salmon would identify variation in population structure between and within runs and provide a means to detect, through sampling, the ecological differences that we are not able to recognize through our traditional Kenai River Chinook salmon research and management programs.

Through both mitochondrial DNA (mtDNA) and protein electrophoresis analysis Adams et al. (1994) identified genetic differences between early- and late-run spawning Chinook salmon in Kenai River drainage and concluded that Chinook salmon may segregate into genetically different early and late forms within the drainage. Since that time, advances in genetic techniques now allow for enhanced population discrimination. The focus of this project was to employ new genetic technologies to quantify genetic differences between, as well as within, early- and late-run Kenai River Chinook salmon by defining the microsatellite[1] genetic markers[2] of tributary-(early run) Chinook salmon spawning in the Killey and Funny rivers and Benjamin Creek of the Kenai River drainage and mainstem-(late run) Chinook salmon spawning in two Kenai River mainstem locations. Another aspect was to determine the feasibility of using genetic differences unique to each of these groups to detect them in run time monitoring programs and potentially in various fisheries where Kenai River Chinook salmon are harvested. Finally, the project also expands the Pacific coastwide microsatellite genetic baseline for Chinook salmon managed by the Northwest Fisheries Science Center of the National Marine Fisheries Service. Addition of these groups from Kenai River to the baseline will enhance the ability to identify more regional groups of Chinook salmon in mixtures of samples taken from various fisheries.

A multi-laboratory standardized genetic baseline for the southern portion of the Chinook salmon range has been developed through a project funded by the Chinook Technical Committee (CTC) of the Pacific Salmon Committee (Moran et al. 2005; Seeb et al. 2007). The consortium chose

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[1] A *microsatellite* is a segment of DNA consisting of numerous tandem repeats of short, simple sequence motifs. An example of a microsatellite is "GAGAGAGAGAGAGAGA", where the dinucleotide motif "GA" is repeated eight times. (Source: Panzea. 2008. Molecular and functional diversity in the maize genome project, glossary. http://www.panzea.org/lit/glossary.html [Accessed April 2009]).

[2] Microsatellites are transformed into *genetic markers* by designing PCR primers specific to the hopefully unique sequence flanking the microsatellite repeat. They are particularly useful for discriminating among individuals within a population or for determining the (unknown) population of origin of an individual. (Source: Panzea. 2008. Molecular and functional diversity in the maize genome project, glossary. http://www.panzea.org/lit/glossary.html [Accessed April 2009]).

13 microsatellite loci[3] for baseline development, based on consistency in various laboratory conditions and potential information content. To date, 165 populations (approximately 22,000 individuals) have been surveyed from Southeast Alaska to California to create an initial coastwide baseline (Seeb et al. 2007). Use of this database is governed by a certification process by which laboratories interested in adding to the database must demonstrate the ability to accurately standardize results with other member laboratories.

This report presents the results of a survey of 13 CTC microsatellite loci in 14 Chinook salmon collections representing nine populations within Kenai River drainage (Table 1).

OBJECTIVES

The objective of this study is to develop a baseline of microsatellite genetic markers for Chinook salmon populations of Kenai River.

Tasks associated with this objective include:

1. Collect baseline tissue samples from populations of Chinook salmon that spawn in Killey River, Funny River, Russian River, Juneau Creek, Quartz Creek and Crescent Creek.
2. Expand the collection of baseline samples from the Kenai River mainstem and Slikok Creek to include a second mainstem population of Chinook salmon spawning downstream of Skilak Lake from RM 39.8 to RM 47.9.
3. Analyze 13 microsatellite genetic markers in all baseline samples.
4. Conduct simulation analyses to determine whether tributary- and mainstem-spawning Chinook salmon can be separated based on genetic markers.

METHODS

SAMPLE COLLECTION

Collecting tissue from Chinook salmon for genetic analysis was non-lethal; a ½ inch piece of tissue from the tip of the axillary process was removed from each fish sampled, placed in a 2mL cryovial and completely covered with a Sigma Reagent Grade 95% Alcohol (Sigma Cat. # R 8382) buffer solution until the liquid/tissue ratio was approximately 3:1. Samples were transferred and stored at room temperature until analyzed. The sample size goal for each spawning location was predicated on criterion to estimate allele frequency proportions at each locus to within five percentage points of the true values 90% of the time. This level of precision requires identification of 403 alleles[4] (Thompson 1987). Given two copies of the genetic information at each locus in each diploid individual, and assuming random mating, tissue samples from a total of approximately 200 fish at each location was needed to meet the stated precision criterion.

All Chinook salmon sampled for tissue were also sampled for age, sex, and length. Three scales were collected for aging from the left side of the body, at a point on a diagonal from the posterior

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[3] Locus (plural: *Loci*) is the particular chromosomal location of a gene, genetic marker, or other genetic feature in the genome. (Source: Panzea. 2008. Molecular and functional diversity in the maize genome project, glossary. http://www.panzea.org/lit/glossary.html [Accessed April 2009]).

[4] *Allele* is the form of a gene or genetic marker. Two different alleles of a gene or genetic marker differ because of one or more DNA sequence differences at the corresponding location (or locus) in the genome. (Source: Panzea. 2008. Molecular and functional diversity in the maize genome project, glossary. http://www.panzea.org/lit/glossary.html [Accessed April 2009]).

insertion of the dorsal fin to the anterior insertion of the anal fin, two rows above the lateral line (Welander 1940). Later, scales were pressed and age determined using procedures described by Mosher (1969). Sex was determined based on head shape, and presence of ovipositor, eggs, or milt. Length was measured from mid eye to tail fork (METF) to the nearest millimeter. After sampling Chinook salmon were released back into the water alive. Sample collection dates, logistics, and Chinook salmon capture methods varied among spawning locations. The sampling dates were based upon the time of spawning as described by Burger et al. (1983). Tributary-(early run) fish were sampled from mid to late July and mainstem- (late run) fish were sampled from mid to late August. A description of sampling activities at each location follows.

Kenai River Tributary Locations

Slikok Creek originates from the Kenai River lowlands and intersects Kenai River at approximately RM 18.5. Samples were collected from live Chinook salmon using hook-and-line gear. Spent-dead carcasses were also sampled over 6 days from July 19 to July 29, 2005.

The Killey River is the Kenai River's largest tributary and originates from Killey Glacier in the Harding Ice Field of the Kenai Mountains. It is known to be a primary spawning destination for tributary-(early run) spawning Chinook salmon (Figure 1) (Bendock and Alexandersdottir 1992; Burger et al. 1983). The confluence of the Killey and Kenai rivers is located at Kenai River RM 44.0. A base camp was established on Killey River approximately 2.0 river miles upstream of the confluence. At this location one fish wheel was positioned in a thalweg of Killey River where immigrating Chinook salmon were captured by the fish wheel baskets and deposited in a live-box affixed to the fish wheel float. Periodically, ADF&G staff removed Chinook salmon from the live-box to collect tissue and biological samples. The fish wheel was operated from June 18 to July 19, 2005 and June 15 to July 5, 2006.

Benjamin Creek originates from Twin Lakes in the Kenai Mountains (Figure 1). It is a second order Kenai River tributary that enters Killey River approximately 31 river miles upstream of the Killey-Kenai river confluence. Benjamin Creek is remote and accessible only via aircraft during the summer months. A helicopter was used to drop a 3-person crew at Benjamin Creek just upstream of its confluence with Killey River where a temporary field camp was established. Chinook salmon were captured using hook-and-line gear and a 5 fathom long 4.75 in (stretched mesh) gillnet. Samples were collected from live fish at Benjamin Creek from August 2 to August 5, 2005 and from July 22 to July 25, 2006.

The Funny River originates from the Kenai Mountains and joins Kenai River at approximately RM 30 (Figure 1). The Upper Funny River is remote and accessible only via aircraft in summer. Between July 25 and July 26, 2005, a 4-person crew, that had been dropped off via helicopter to access the upper section of Funny River, traveled downstream in inflatable rafts to the confluence with Kenai River capturing and sampling Chinook salmon with hook-and-line gear and a 5 fathom long 4.75 in (stretched mesh) gillnet along the way. In 2006 a weir operated cooperatively with the United States Fish and Wildlife Service Kenai Fisheries Assistance Office was installed from May 16 to October 2 approximately 1.5 miles upstream of the Funny–Kenai River confluence. During 2 days each week between June 16 and July 27 Chinook salmon that entered the trap to pass through the weir were captured and sampled.

The Russian River intersects Kenai River at about RM 74. The Russian River originates from an interconnected lake system comprised of Upper and Lower Russian Lakes. A falls is located between the lower lake and the Kenai River confluence. A weir located at the outlet of the lower

lake is used to assess sockeye salmon *Oncorhynchus nerka* escapement. Chinook salmon that migrate through the falls to spawn in the Russian River drainage were sampled at the weir and released back into the water alive during August of 2005.

Juneau Creek originates from the Kenai Mountains and joins Kenai River at about RM 81. Juneau Creek contains a barrier falls approximately 3.5 miles upstream of its confluence with Kenai River. Juneau Creek is accessible via a Chugach National Forest trail system and boat from the Kenai River. Chinook salmon were captured using hook-and-line gear from July 29 to August 5, 2005.

The Quartz Creek drainage is comprised of several small tributaries including Crescent, Dave's and Devils creeks. These creeks begin as mountain drainages except for Crescent Creek which originates from Crescent Lake. Quartz Creek flows into the eastside of the North Arm of Kenai Lake. Samples were collected from live Chinook salmon using hook-and-line gear from Quartz Creek from July 28 to August 15 and at Crescent Creek from July 25 to August 15, 2006.

Kenai River Mainstem Locations

Mainstem Site 1 extends from RM 21.8 upstream to RM 36.0 and was sampled from August 12 to August 20, 2003 and from August 13 to August 25, 2004. Mainstem Site 2 extends from RM 39.8 upstream to RM 47.9 and was sampled from August 18 to September 7, 2006 (Figure 1).

Chinook salmon were captured by deploying drift gillnets from a riverboat. Crews drifted a gillnet through areas likely holding Chinook salmon. Drifts were terminated when either: (1) the crew believed at least one Chinook salmon was in the net, (2) the net was drifting off course, (3) the net became snagged on the bottom or was not fishing properly, or (4) the end of the targeted area had been reached. Chinook salmon captured were untangled from the net and placed in a portable restraint cradle (Larson 1995) to be sampled as described above. Specifications of nets used and operational procedures varied between sites. Chinook salmon sampled from Site 1 were captured incidental to a drift gillnetting program designed to capture adult coho salmon *Oncorhynchus kisutch* and were not sampled for age, sex, and length information (Massengill 2007; Massengill and Carlon 2007). In this program a 4.75 in (stretched mesh) multi-strand monofilament gillnet 29 meshes deep and 5 fathoms long was used. At Site 2, Chinook salmon were the targeted species and one gillnet, selected from a variety of different sized gillnets, was used that best suited the water conditions for each drift. Gillnets included the size specified above as well as 5.0 and 7.5 in (stretched mesh) multi-strand monofilament gillnet of various mesh depths (30-80 mesh deep) and lengths (5-10 fathoms).

Laboratory Analysis

Sample selection

The laboratory time and funding available were not sufficient to analyze all samples collected as part of this project. The following criteria were used to determine which samples would be analyzed: (1) represent each of the spawning locations sampled, (2) represent multiple brood years when possible, and (3) analyze up to 95 individuals from each collection and location.

Microsatellite assay

DNA was extracted from tissues using DNeasy 96 Tissue Kits (QIAGEN). Polymerase chain reaction (PCR) was carried out in 384-well reaction plates in 10µl reaction volumes (10mM Tris-HCl, 50mM KCl, 0.2mM each dNTP, 0.50 units *Taq* DNA polymerase (Promega) using Dual

384-Well GeneAmp Thermal Cyclers (Applied Biosystems). Primer concentrations, MgCl concentration and the corresponding annealing temperature for each locus are available upon request. PCR fragment analysis was done on an Applied Biosystems 3730 capillary DNA sequencer. 0.5µl PCR product was loaded into a 96-well reaction plate along with 0.4µl of GS500LIZ internal lane size standard and 9.0µl of Hi-Di (Applied Biosystems). PCR bands were visualized and separated into bin sets using AB GeneMapper software v4.0.

Data collection

Genetic data were collected as individual multi-locus genotypes[5] for the 13 microsatellite loci currently included in the Chinook Technical Committee (CTC) standardized database (Table 2). According to the convention implemented by the CTC, at each locus, a standardized allele is one that has a recognized holotype[6] specimen from which the standardized allele can be reproduced using commonly applied fragment analysis techniques. The determination of whether or not an allele is standardized is made by the curator of the locus from which the allele is derived. The curator of a locus is responsible for distributing a document which lists each allele for a particular locus and a corresponding holotype specimen from which a unique allele can be reproduced. By the process of sizing the alleles from the holotype specimens, any individual laboratory should be able to convert allele sizes obtained in the laboratory to standardized allele names.

Genotype data were stored as GeneMapper (*.fsa) files on a network drive that was backed up nightly. Long-term storage of the data was ensured by entering it in the Gene Conservation Laboratory's Oracle database, LOKI.

Quality control methods

Several measures were implemented to insure the quality of data produced.

1. Each sample that arrived in our laboratory was assigned a unique accession identifier code. At the time DNA was extracted or analyzed from each sample, a sample sheet was created that linked each individual sample's code to a specific well number in a uniquely numbered 96-well plate. This sample sheet then followed the sample through all phases of a project, minimizing the risk of misidentification of samples through human-induced errors.
2. Genotypes were assigned to individuals using a double-scoring system. Two observers independently produced allele scores for an entire project before the two data sets were compared. Discrepancies between the two sets of scores were then resolved with one of three possible outcomes: (1) one score was accepted and the other rejected, (2) both scores were rejected and the score was blanked, or (3) the sample was rerun.
3. Approximately 8% of the individuals, eight samples from each 96-well DNA extraction plate, were reanalyzed for all loci. Discrepancies here indicate an error in the process and may require reanalysis of the entire set where the error is located. This process insured that the data are reproducible and any errors created from the processing of individual plates were corrected.

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[5] *Genotype* is the genetic makeup of an organism or group of organisms as determined by the combination of alleles located on homologous chromosomes that determines a specific characteristic or trait (Source: The American Heritage dictionary of the English Language.–4th ed.).

[6] *Holotype* is the single specimen or illustration designated as the type for naming a species or subspecies or used as the basis for naming a species or subspecies when no type has been selected (Source: The American Heritage dictionary of the English Language–4th ed.).

4. The final data were checked for duplicated multi-locus genotypes for indication of errors caused prior to extraction of the DNA. When duplicate genotypes were found, the genotype was attributed to the first individual and subsequent individuals with the same genotype were removed from the analysis to insure that any given individual does not appear more than once in the baseline.

Statistical analysis

Individual genotype data were summarized as allele frequencies for each microsatellite locus in each collection. When multiple collections were available from the same population, these collections were combined to represent the population. A minimum sample size of 50 individuals was used for inclusion of a population in the population structure analysis. Because Chinook salmon are diploid organisms, this is a minimum of 100 samples from the gene pool for determining allele frequencies at each locus. Collections with smaller sample sizes were pooled with collections from the same tributary if the log likelihood ratio statistic (Weir 1990) detected no significant difference in allele frequency estimates between the collections.

Estimates of the population frequency of individual alleles for each locus were calculated from the observed frequency of the allele in the representative sample. The numbers of alleles at each locus were calculated for each population. Observed and expected heterozygosity was calculated using *FSTAT* (Goudet 1995), and conformation of genotype frequencies to Hardy-Weinberg equilibrium (HWE) expected ratios was assessed using the exact test in *GENEPOP* (Raymond and Rousset 1995). The significance of departures from HWE for each locus in each population was determined using α=0.05 adjusted for the number of loci (n=13) assayed in each population using the Bonferroni adjusted significance levels ($\acute{\alpha} = \alpha/n = 0.0038$).

Two measures of population subdivision were calculated from allele frequency differences: Cavalli-Sforza and Edwards (CSE)' chord distances (Cavalli-Sforza and Edwards 1967) and F_{ST} (Weir and Cockerham 1984). *FSTAT* was used to calculate F_{ST} values. Population structure was visualized as a tree based on unweighted pair-group method with arithmetic mean (UPGMA, Sneath and Sokal 1973) using *PHYLIP* version 3.6, (Felsenstein 2004) to view genetic similarities between populations reflected in the interpopulation chord distances.

Simulation analyses

Simulations were conducted to evaluate the accuracy and precision of the genetic baseline to provide compositional estimates of mixtures of Chinook salmon taken from within the Kenai River. These simulations were used to help assess whether the baseline of allele frequencies at the 13 microsatellites would provide sufficient information to identify individual stocks or groups of stocks (reporting groups) in mixtures. Reporting groups for genetic stock identification of Kenai River Chinook salmon were defined based on a combination of genetic similarity, geographic features, and management applications.

Once reporting groups were defined, simulations were performed using the Statistical Package for Analyzing Mixtures (SPAM version 3.7, Debevec et al. 2000). Baseline and mixture genotypes were randomly generated from the baseline allele frequencies assuming Hardy-Weinberg equilibrium. Each simulated mixture (N = 400) was composed 100% of the stock or reporting group under study. When a reporting group mixture was simulated, all stocks in the reporting group contributed equally to the mixture. Average estimates of mixture proportions and 90% confidence intervals were derived from 1,000 simulations. Reporting groups with

mean correct estimates of 90% or better are considered highly identifiable in fishery applications. Reporting groups with mean correct estimates lower than 90% can still be considered identifiable in mixtures, but sources of misallocation should be considered when interpreting the results.

RESULTS

LABORATORY ANALYSIS

A total of 1,444 Chinook salmon from 14 collections representing nine populations were available for analysis (Table 1). From these, genotypes were assayed from 977 individuals based on the selection criteria. Criteria 1 and 2 were met by analyzing representative samples from each location and brood year available. Criterion 3 was generally met, but in some situations more or fewer individuals were run due to collection method, sample quality, or laboratory scheduling. The overall failure rate for successfully assaying genotypes at the 13 CTC microsatellites was 4.5%. Most failures occurred in the samples from Slikok Creek (success rate approximately 35.9%) and were due to poor tissue quality. The quality control checks employed demonstrated an error rate of 0%. The quality control checks also revealed pairs of individuals in some collections that had identical multi-locus genotypes. The following populations had individuals with duplicate genotypes: Benjamin Creek (1 pair), Quartz Creek (2 pairs), and Juneau Creek (2 pairs). In most cases, duplicates appear to have been the result of sampling the same fish in neighboring vials.

Age, sex, and length data summaries for all Chinook salmon that were sampled are presented in Appendices A1 to A12.

STATISTICAL ANALYSIS

An average of 47% of the alleles (range: from 21 to 65%) observed by the CTC in the survey of southern Chinook salmon populations were observed in the nine populations in Kenai River (Table 2). Survey of the Kenai River populations revealed three alleles not included in the list of standardized CTC allele designations. These alleles were pooled with standardized alleles following procedures established by the CTC. The new allele *Ssa408**225 (ADF&G designation) was only observed in salmon from Funny River, Slikok Creek, Killey River, and Benjamin Creek. In addition, the *Ssa408**221 was only found in the collections from Funny and Killey rivers and Benjamin Creek.

Four populations had collection sample sizes too small for inclusion in the population structure analysis: Crescent Creek, Quartz Creek, Juneau Creek, and Russian River. Crescent Creek is a tributary to Quartz Creek so these collections were combined to represent Quartz Creek (Table 3; G = 279.63, DF = 239, P = 0.036). The Juneau Creek and Russian River populations were not included in further analysis.

After correcting for multiple tests, significant departures from HWE were found at *Ssa408* in six of the seven populations; Kenai Mainstem – Site 1 was the exception (P = 0.45). This locus demonstrated a consistent excess of homozygote[7] genotypes in all populations and was dropped from further analysis. Using the remaining 12 loci, significant departures from HWE (adjusting

[7] *Homozygote* is an organism that has the same alleles at a particular gene locus on homologous chromosomes (Source: The American Heritage dictionary of the English Language.–4th ed.).

for the number of tests) were found in only one population: Slikok Creek at *Oki100* and *Ots208b*. Each significant departure from HWE was due to homozygote excess.

Genetic differences between populations were measured using CSE distances calculated from allele frequencies at the 13 CTC microsatellites. Visualizing these interpopulation distances with a UPGMA tree showed four major clusters of populations which appear to be structured largely by tributaries (Figure 2). Each of the major branches on the tree corresponds to a subdrainage within the greater Kenai River drainage (considering the mainstem spawning locations to be a subset of the whole).

SIMULATION ANALYSES

Reporting groups for mixed stock analysis of Chinook salmon in Kenai River were defined based on a combination of geographic features, management applications, and genetic structure revealed in this analysis: (1) Lower Kenai – Slikok Creek and Funny River, (2) Kenai River Mainstem – Site 1 (rkm 35-58) and Site 2 (rkm 64-77), (3) Killey River – Killey River and Benjamin Creek, and (4) Quartz Creek – Quartz Creek and Crescent Creek. Simulation studies based on this structure indicate that these reporting groups are highly identifiable in mixtures (Table 4). When simulated mixtures composed entirely from a single reporting group were treated as mixtures of unknown origin more than 94% of the mixture was correctly identified to region-of-origin. Additional simulations conducted with simulated mixtures composed entirely from a single population provided mean correct allocations to population that were all greater than 80% (Table 5). In each of these cases, the largest portion of the misallocation was attributed to the other population in the population group.

DISCUSSION

The objective of this project was to develop a baseline of standardized microsatellite markers for the Chinook salmon populations of Kenai River. To meet this objective, it was necessary to collect or expand the existing collections for Chinook salmon that spawn in Kenai River Mainstem, Slikok Creek, Killey River, Funny River, Russian River, Juneau Creek, and Quartz Creek. Tissue samples were collected from 10 locations including two spawning areas in the Kenai River Mainstem and Benjamin Creek, a tributary to Killey River. In general, the success rates for determining genotypes in each population were very high and meet the requirements for inclusion in the baseline, with the exception of Slikok Creek. The tissue samples from Slikok Creek were collected from fish carcasses and were generally in poor condition. However, inclusion of the population in the baseline is probably still warranted, but additional individuals from this population should be added in the near future.

Population structure of Kenai River Chinook salmon is similar to that described previously by Adams et al. (1994) using allozymes and mitochondrial DNA markers; there are genetic differences between the Kenai River Mainstem spawners (late run) and tributary spawners (early run). The additional new tributary populations surveyed here demonstrate further genetic diversity within the early run. These populations appear to be grouped by geographic proximity. The results of this project allow a more comprehensive representation of Chinook salmon populations of Kenai River.

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TABLES

Table 1.–Collections of Kenai River drainage Chinook salmon tissue samples used in the survey of 13 standardized microsatellites, 2003–2006.

Collection number	Population	Sampling locations (tributary of)	River mile(s) [a]	Year	Number of Chiook salmon tissue samples Collected [b]	Analyzed
1	1	Quartz Creek (Kenai Lake)	NA	2006	34	34
2		Crescent Creek (Quartz Creek)	NA	2006	165	44
3	2	Juneau Creek	81.0	2005	32	32
4	3	Russian River	74.0	2005	24	24
5	4	Killey River - weir	44.0	2005	68	68
6				2006	198	95
7	5	Benjamin Creek (Killey River)	NA	2005	56	56
8				2006	150	95
9	6	Kenai River, Mainstem–Site 1	21.8 to 36.0	2003	101	80
10				2004	96	39
11	7	Kenai River, Mainstem–Site 2	39.8 to 47.9	2006	200	183
12	8	Funny River	30.0	2005	37	37
13				2006	183	95
14	9	Slikok Creek	18.5	2005	100	95
				Total	1,444	977

Note: NA = not applicable.

[a] Single river mile measured at the tributary's confluence with Kenai River. A range of river miles denote a reach of Kenai River where samples were collected.

[b] One 1/2 inch piece of tissue from the tip of the axillary process was removed from each fish.

Table 2.-The 13 microsatellite loci detected in the Kenai River Chinook salmon analysis with the observed heterozygosity, expected heterozygosity, and F_{ST} for each.

Microsatellites			Heterozygosity			Alleles		
						Number in each dataset		Range of sizes in the combined CTC/Kenai datasets (base pairs) [a]
Number	Locus name	Citation	Observed (H_o)	Expected (H_e)	F_{ST}	CTC [a]	Kenai	
1	*Ogo2*	Olsen et al. 1998	0.722	0.697	0.024	27	10	200-258
2	*Ogo4*	Olsen et al. 1998	0.711	0.715	0.045	20	13	130-170
3	*Oki100*	DFO unpublished [b]	0.910	0.929	0.021	53	25	160-365
4	*Omm1080*	Rexroad et al. 2001	0.954	0.950	0.025	74	44	160-460
5	*Ots201b*	Greig et al. 2003	0.881	0.879	0.043	52	24	130-345
6	*Ots208b*	Greig et al. 2003	0.925	0.941	0.019	58	32	140-380
7	*Ots211*	Greig et al. 2003	0.888	0.895	0.038	47	23	195-350
8	*Ots212*	Greig et al. 2003	0.791	0.844	0.032	37	17	120-265
9	*Ots213*	Greig et al. 2003	0.934	0.914	0.025	55	27	175-410
10	*Ots3M*	Banks et al. 1999	0.453	0.485	0.041	19	7	120-170
11	*Ots9*	Banks et al. 1999	0.506	0.496	0.026	10	4	97-115
12	*OtsG474*	Williamson et al. 2002	0.275	0.280	0.024	19	4	140-220
13	*Ssa408*	Cairney et al. 2000	0.716	0.875	0.034	39	22	180-320

Note: For comparison, the number of alleles in the Chinook Technical Committee (CTC) and the Kenai River datasets as well as the range of allele sizes in the combined datasets are included.

[a] Based on the CTC baseline updated February 2006.

[b] Personal communication, K. Miller, Department of Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada.

Table 3.-Sample sizes by locus for 13 microsatellite loci assayed in Chinook salmon from populations in the Kenai River and mean sample size by population.

Microsatellite locus name	Kenai River Chinook salmon population sample sizes						
	Slikok Creek	Kenai River Mainstem Site 1	Funny River	Kenai River Mainstem Site 2	Killey River	Benjamin Creek	Quartz Creek
Ogo2	56	119	131	179	160	150	76
Ogo4	58	119	131	179	160	149	76
Oki100	63	117	131	180	159	150	75
Omm1080	65	115	130	181	159	149	75
Ots201b	66	118	131	180	160	150	76
Ots208b	56	118	130	178	159	150	75
Ots211	61	118	131	181	160	150	76
Ots212	55	119	131	180	160	150	76
Ots213	63	119	130	181	160	150	76
Ots3M	65	119	131	181	160	150	76
Ots9	63	119	131	181	160	150	76
OtsG474	62	119	131	179	160	150	76
Ssa408	57	118	124	176	137	121	74
Mean	60.8	118.2	130.2	179.7	158.0	147.6	75.6

Table 4.-Mean reporting group allocations of simulated mixtures of Kenai River Chinook salmon from the baseline of 13 microsatellites.

Reporting group	Mean	90% CI
Quartz Creek	0.944	(0.917 - 0.969)
Lower Kenai River	0.989	(0.975 - 1.000)
Kenai River Mainstem	0.968	(0.942 - 0.989)
Killey River	0.955	(0.926 - 0.980)

Note: Each set of mixtures (N = 400) was created from a single reporting region based on allelic frequencies for that region. The results reported are the mean and bounds of the middle 90% (CI) of correct allocations from 1,000 bootstrap iterations.

Table 5.-Mean allocations of simulated mixtures composed entirely of individuals from each population of Kenai River Chinook salmon.

	Mixture composition						
Estimated contribution	Quartz Creek	Kenai River Mainstem Site 1	Kenai River Mainstem Site 2	Benjamin Creek	Killey River	Funny River	Slikok Creek
Quartz Creek	**0.944**	0.002	0.003	0.001	0.001	0.002	0.004
Kenai River Mainstem Site 1	0.017	**0.815**	0.110	0.002	0.002	0.006	0.007
Kenai River Mainstem Site 2	0.022	0.172	**0.881**	0.001	0.002	0.004	0.009
Benjamin Creek	0.001	0.001	0.001	**0.878**	0.076	0.012	0.005
Killey River	0.003	0.002	0.001	0.098	**0.885**	0.030	0.011
Funny River	0.008	0.006	0.003	0.019	0.031	**0.907**	0.162
Slikok Creek	0.005	0.002	0.002	0.002	0.004	0.039	**0.802**

Note: Entries along the diagonal in bold type indicate the mean correct proportional allocation (from 1,000 bootstraps iterations) to the population comprising the mixture. Columns sum to 1.0.

FIGURES

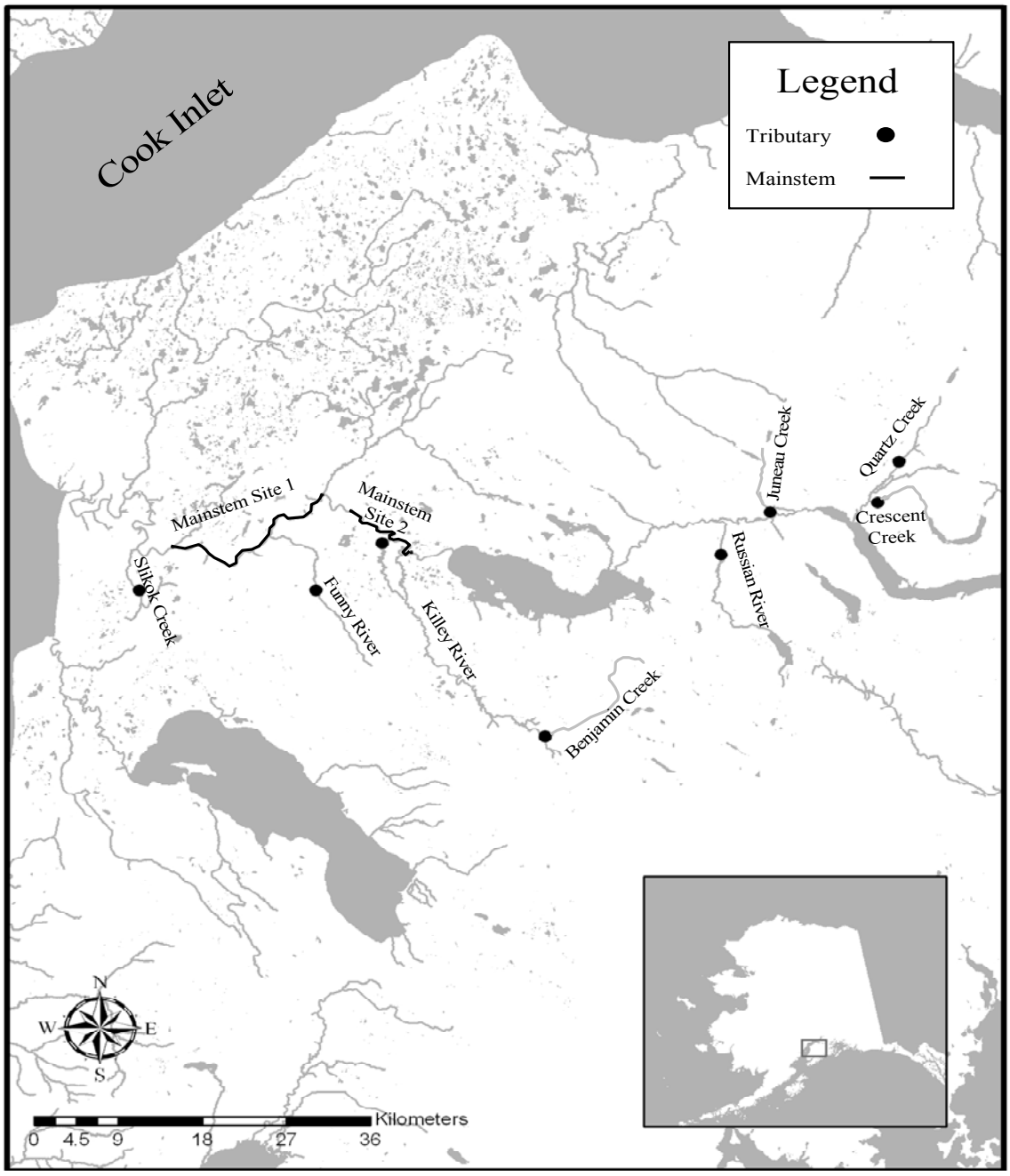

Figure 1.-Map of Chinook salmon collection locations in Kenai River drainage.

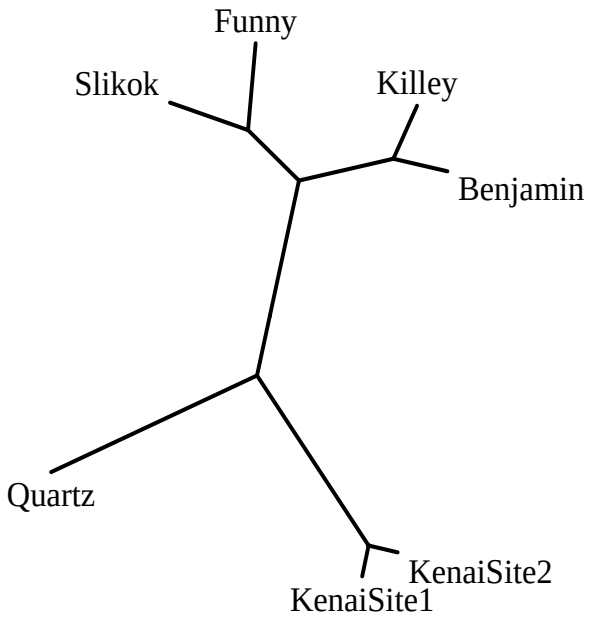

Figure 2.-Unweighted paired group-mean clustering tree based on genetic distances (Cavalli-Sforza and Edwards chord distances) between pairs of Chinook salmon populations in Kenai River drainage.

APPENDIX A. AGE, SEX, AND LENGTH OF CHINOOK SALMON SAMPLED IN KENAI RIVER DRAINAGE, 2005-2006

Appendix A1.-Length at age by sex of Chinook salmon sampled for genetic tissue, Quartz Creek, July 28 to August 15, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			4	1		7
Mean length			844	985		875
SE Mean length			5			20
Males						
Number sampled		9	10	1		27
Mean length		632	832	920		760
SE Mean length		12	12			22
Both sexes combined						
Number sampled		9	14	2		34
Mean length		632	835	953		784
SE Mean length		12	9	33		19

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A2.-Length at age of Chinook salmon sampled for genetic tissue, Crescent Creek, July 25 to August 15, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			29	11		57
Mean length			842	957		876
SE Mean length			9	12		9
Males						
Number sampled		17	32	9		109
Mean length		637	808	963		763
SE Mean length		14	9	29		12
Both sexes combined						
Number sampled		17	61	20		166
Mean length		637	824	960		819
SE Mean length		14	7	14		12

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A3.-Length at age of Chinook salmon sampled for genetic tissue, Juneau Creek, July 29 to August 5, 2005.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			1	2	1	5
Mean length			860	935	1,010	942
SE Mean length				25		26
Males						
Number sampled	1	6	4	2	1	26
Mean length	420	673	748	960	1,020	773
SE Mean length		5	14	20		29
Both sexes combined						
Number sampled	1	6	5	4	2	31
Mean length	420	673	770	948	1,015	800
SE Mean length		5	25	15	5	27

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A4.-Length at age of Chinook salmon sampled for genetic tissue, Russian River, August 11 to August 26, 2005.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			2	7		11
Mean length			848	1,019		980
SE Mean length			18	18		24
Males						
Number sampled			4	3		13
Mean length			891	962		913
SE Mean length			7	21		16
Both sexes combined						
Number sampled			6	10		24
Mean length			877	1,002		943
SE Mean length			11	16		16

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A5.-Length at age of Chinook salmon captured in the fish wheel, Killey River, June 18 to July 19, 2005.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			6	7		19
Mean length			812	994		870
SE Mean length			30	15		32
Males						
Number sampled	8	13	7	5	1	49
Mean length	358	591	827	1,046	1,070	639
SE Mean length	13	18	41	8		35
Both sexes combined						
Number sampled	8	13	13	12	1	68
Mean length	358	591	820	1,016	1,070	704
SE Mean length	13	18	25	12		29

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A6.-Length at age by sex of Chinook salmon sampled for genetic tissue, Killey River, June 15 to July 5, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled		2	13	16		39
Mean length		518	849	935		864
SE Mean length		23	12	16		20
Males						
Number sampled	9	36	27	43	2	156
Mean length	464	598	817	993	1,010	790
SE Mean length	37	8	17	11	30	17
Both sexes combined						
Number sampled	9	38	40	59	2	195
Mean length	464	594	828	977	1,010	805
SE Mean length	37	9	12	10	30	14

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A7.-Length at age of Chinook salmon sampled for genetic tissue, Benjamin Creek, August 2 to August 5, 2005.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			6	3	2	21
Mean length			845	931	1,015	913
SE Mean length			12	14		21
Males						
Number sampled		2	4	4	1	35
Mean length		625	783	953	1,045	814
SE Mean length			22	46		29
Both sexes combined						
Number sampled		2	10	7	3	56
Mean length		625	820	943	1,025	851
SE Mean length			15	26	10	21

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A8.-Length at age by sex of Chinook salmon sampled for genetic tissue, Benjamin Creek, July 22 to July 25, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			9	18		44
Mean length			983	958		941
SE Mean length			12	7		8
Males						
Number sampled	6	27	12	24	5	106
Mean length	389	609	826	1,037	1,029	807
SE Mean length	36	8	29	11	12	23
Both sexes combined						
Number sampled	6	27	21	42	5	150
Mean length	389	609	854	1,003	1,029	845
SE Mean length	36	8	18	9	12	18

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A9.-Length at age by sex of Chinook salmon sampled for genetic tissue, Kenai River Mainstem–Site 2, August 18 to September 7, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled			11	29	2	56
Mean length			896	1,004	1,070	995
SE Mean length			19	9	25	10
Males						
Number sampled	2	37	19	32	4	144
Mean length	483	630	827	1,050	1,114	864
SE Mean length	53	7	20	10	29	18
Both sexes combined						
Number sampled	2	37	30	61	6	200
Mean length	483	630	852	1,028	1,099	901
SE Mean length	53	7	15	7	21	14

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A10.-Length at age of Chinook salmon sampled for genetic tissue, Funny River, July 25 to July 26, 2005.

	Ocean Age				
	1	2	3	4	Total[a]
Females					
Number sampled			7	3	12
Mean length			814	880	837
SE Mean length			11	10	11
Males					
Number sampled	1	8	6	2	25
Mean length	400	591	813	870	704
SE Mean length		14	12	10	26
Both sexes combined					
Number sampled	1	8	13	5	37
Mean length	400	591	814	876	747
SE Mean length		14	8	7	20

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A11.-Length at age by sex of Chinook salmon sampled for genetic tissue, Funny River, June 16 to July 27, 2006.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled		2	14	13		39
Mean length		565	784	915		832
SE Mean length		20	9	13		16
Males						
Number sampled	9	53	35	10		144
Mean length	424	615	786	953		693
SE Mean length	25	6	11	25		13
Both sexes combined						
Number sampled	9	55	49	23		183
Mean length	424	613	786	932		723
SE Mean length	25	6	8	13		12

[a] Total number sampled does not sum across rows due to regenerated/illegible scales.

Appendix A12.-Length at age of Chinook salmon sampled for genetic tissue, Slikok Creek, July 21 to July 29, 2005.

	Ocean Age					
	1	2	3	4	5	Total[a]
Females						
Number sampled		1	28	1		39
Mean length		560	809	970		806
SE Mean length			10			11
Males						
Number sampled	2	14	29	3		60
Mean length	440	630	810	957		750
SE Mean length	10	18	9	27		16
Both sexes combined						
Number sampled	2	15	57	4		99
Mean length	440	625	809	960		773
SE Mean length	10	17	7	20		11

[a] Total number sampled does not sum across rows due to regenerated/illegible scales; one fish not sampled for sex or length.