

Annual Progress Report to:

**Pollock Conservation Cooperative
Research Center
School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
Fairbanks, AK 99775-7220**

for Project:

**DNA Analysis of the Origins of Chinook Salmon Bycatch
in Alaskan Trawl Fisheries**

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Overview

Chinook salmon bycatch in the Gulf of Alaska and Bering Sea, create problems for the groundfish fisheries, particularly the Bering Sea trawl fisheries. Salmon returns to western Alaskan systems have declined sharply in recent years and salmon are critically to the livelihood and culture of rural Alaskans. In addition, chinook salmon are the focus of a number of other issues ranging from Endangered Species Act concerns to allocations between the U.S. and Canada. Central to bycatch questions is the origin or destination of intercepted fish. Substantial effort has been and continues to be devoted to genetic studies of North American chinook stocks, with the objective resolving stock mixtures to their component stocks. However without data from all potential contributors --at least the predominant ones --, stock mixture analyses are not reliable. Missing from the baseline are data from Russian chinook stocks. We are collaborating with Russian geneticists to obtain genetic information for Russian chinook populations and to examine the genetic divergence between those populations and North American chinook salmon lineages that represent much of the extant chinook salmon genetic diversity. We are quantifying genetic variation using both microsatellites and mtDNA to determine if there are markers that would assist in separating Russian salmon from North American fish in groundfish bycatches. We also plan to use the data to examine the recent evolutionary history of chinook salmon.

Approach

Several activities are necessary to accomplish our goals:

- 1) Our Russian colleague, Dr. Brykov (Institute of Marine Biology, Russian Academy of Sciences), has collected some chinook salmon tissue samples from major and geographically diverse regions in Russia (the Kamchatka Peninsula) and will collect additional samples in June 2002.
- 2) We have contacted geneticists along the Pacific coast and have acquired a most of the samples we will need and have promises for the remainder. The samples come from their archives that many of the labs have maintained. The generosity of those labs makes this project possible.
- 3) A subset of samples from across the geographic range are currently being used to establish polymerase chain reaction (PCR) conditions that will efficiently amplify specific microsatellite loci chosen from the literature and from the advise of other labs using microsatellites to analyze chinook. These samples will provide us an indication of the number and size diversity of microsatellite alleles throughout the geographic range. We intend to use loci that will generate data that are compatible with as many labs as possible. We cannot complete this preliminary phase and progress with the bulk analysis of samples until Dr. Brykov arrives with his Russian samples.
- 4) After the conditions have been determined, we will try to improve the efficiency of the process as we screen selected North American populations for variation that can be compared to the variation in the Russian populations.
- 5) The same populations that were used in 3) will be used (starting in late January when Dr. Brykov arrives to work on this project with us) to thoroughly screen the mtDNA variation. Our lab scrutinizes nearly the entire mtDNA genome (97%) using restriction endonucleases to sample the variation and determine the genomic location of variation that best defines the evolutionary history. We have shown that the region harboring useful variation varies among salmon species, so this step is necessary to maximize our chances of finding the most useful stock markers.
- 6) When we have identified the mtDNA regions and restriction endonucleases that provide the most discriminating restriction sites, we will screen the populations for those sites.
- 7) Data analysis, interpretation, and reporting are a critical step, and include several phases.

Where are we?

We have obtained samples 15 rivers ranging from the Sacramento River drainage in the south through the Yukon drainage in the north (Figure 1 and Table 1). We also have agreements from Oregon Department of Fish and Wildlife to provide samples from the southern Oregon coast and from Washington Department of Fisheries to provide samples of upper Columbia River and Puget Sound samples. These collections represent most of the evolutionary lineages of chinook salmon in North America and, therefore, most of the genetic variation we expect to see. Samples provided to us by the US Fish from Russian systems were not usable, but Dr. Brykov obtained new tissues in June 2001, which were preserved in ethanol and should be excellent and he plans to sample other Russian drainages in 2002.

We have begun testing microsatellite loci on four geographically diverse North American populations. The initial purpose is to determine appropriate conditions for PCR amplification and the size range of alleles. This preliminary work will provide us with the information we need to analyze the rest of the samples. The populations we have begun with are from the American River in the Sacramento River drainage, the Hanford Reach of the Columbia River, Harding River in Southeast Alaska, and Beaver Creek in the Yukon River drainage. We will add populations from an eastern and a western Kamchatka system to the preliminary analysis when Dr. Brykov brings the samples.

We chose eight microsatellite loci to evaluate from several possible loci that have been used successfully for chinook salmon. The loci we chose are ones used either by several different laboratories or used routinely by the University of California Davis lab in their ongoing analyses of mixed fisheries (Table 2). Very preliminary results for two microsatellite loci (10 individuals from each of American River, Hanford Reach, Harding River, and Beaver Creek) show interesting differences in allele composition among them:

<i>Oneμ 13</i>		Allele s										
Population	n	141	147	149	151	153	163	165	167	169	171	173
American River	10		0.05	0.45	0.10	0.20	0.05			0.05	0.10	
Hanford Reach	10	0.05		0.25	0.45				0.05	0.15	0.05	
Harding River	10	0.05		0.10	0.15	0.05	0.20	0.15	0.20	0.10		
Beaver Creek	5				0.30	0.50					0.10	0.10

<i>Ots 3</i>		Allele s									
Population	n	80	82	86	88	90	92	94	96	98	
American River	10	0.10			0.15		0.20	0.30	0.05	0.20	
Hanford Reach	10		0.05	0.05		0.25	0.40	0.10	0.10	0.05	
Harding River	9		0.22		0.06	0.22	0.22	0.17		0.11	
Beaver Creek	9		0.17			0.39	0.44				

We are also extracting DNA from small numbers of individuals for each population and amplifying mtDNA fragments to assure the quality of the tissue samples. We will begin the preliminary mtDNA analysis in late January 2002 when Dr. Brykov arrives.

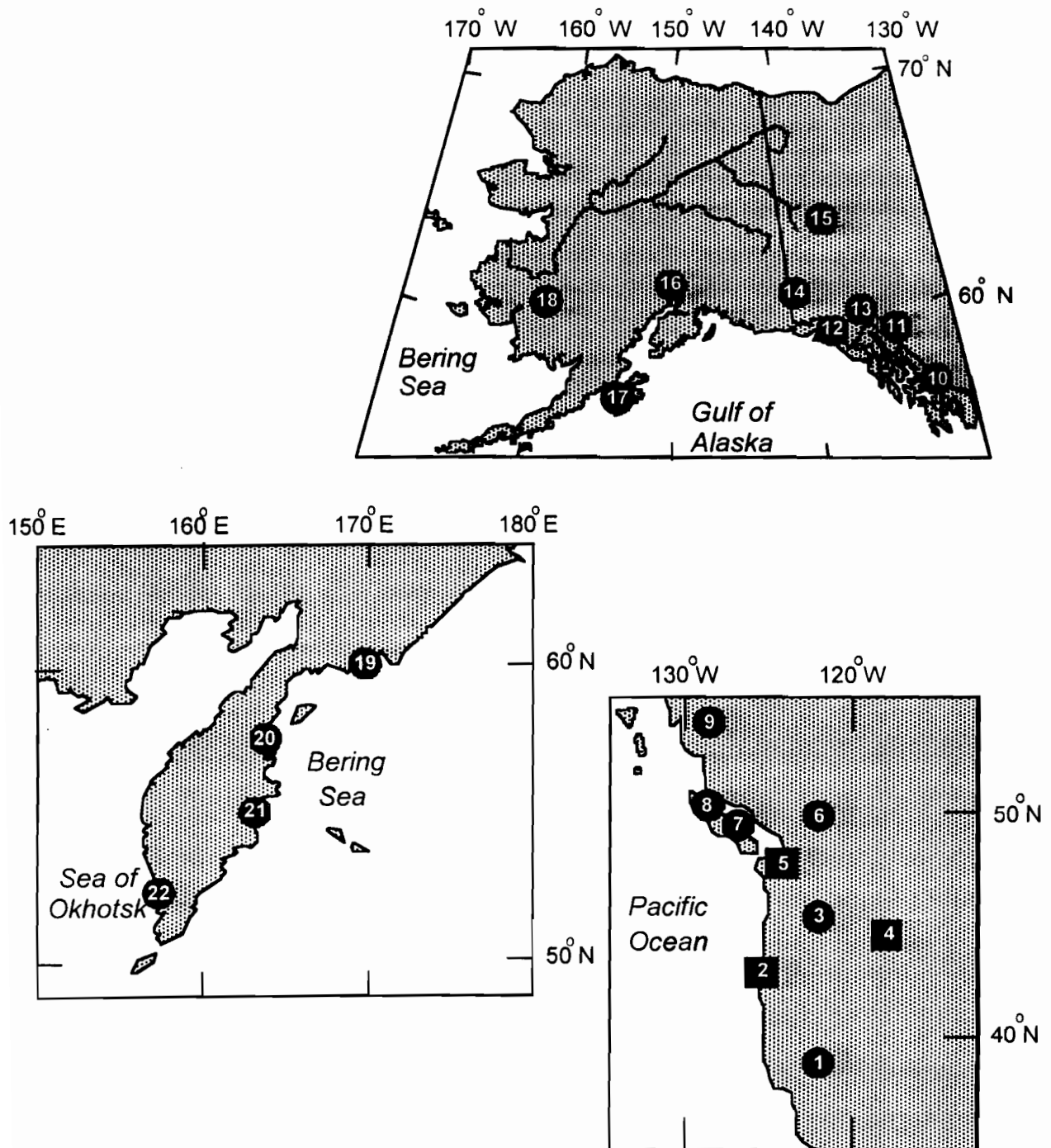


Figure 1. Chinook salmon populations sampled for microsatellite and mtDNA analysis. Circles are collections we have acquired and squares represent collections we expect to acquire.

Table 1. Chinook salmon samples available for analysis: geographic origin, sample size, DNA quality (if tested), tissue and storage, and the agency providing the samples.

Map no.	River	Region	Sample size	Life stage	DNA Quality	Sample type and storage	Providing agency
1	American River	Sacramento drainage	40	adult	Good	heart in EtOH	NWFSC
3	Hanford Reach	Columbia drainage	50	adult	Good	heart in EDTA/DMSO	WDFW
6	North Thompson River	Frazer Drainage	47	adult	untested	DNA	DFO
7	Big Quclicum River	Vancouver Island	30	adult	untested	DNA	U. Toronto
8	Nimpkish River	Vancouver Island	30	adult	untested	DNA	U. Toronto
9	Hirsch Creek	Skeena Drainage	47	adult	untested	DNA	DFO
10	Harding River	Back Behm Canal	65	adult	Good	heart in EtOH	ABL
11	Nakina River	Taku drainage	94	adult	untested	heart in EtOH	ABL
12	Klukshu River	Alsek Drainage	45	adult	untested	heart in EtOH	ABL
13	Takhini River	Yukon Drainage	26	adult	untested	heart in EtOH	USFWS
14	Beaver Creek	Yukon Drainage	100	adult	Good	heart in EtOH	USFWS
15	McQuesten River	Yukon Drainage	50	juvenile	untested	heart in EtOH	USFWS
16	Susitna River	Cook Inlet	95	adult	untested	DNA	USFWS
17	Karluk River	Kodiak Island	50	adult	untested	DNA	USFWS
17	Karluk River	Kodiak Island	126	adult	untested	heart in EtOH	USFWS
18	Kwethluk River	Kuskokwim River	50	juvenile	untested	heart in EtOH	USFWS
19	Pahkacha River	E. Kamchatka Peninsula	14	adult	untested	heart in EtOH	IMB
20	Hailulia River	W. Kamchatka Peninsula	40	adult	untested	heart in EtOH	IMB
21	Kamchatka River	E. Kamchatka Peninsula	57	adult	untested	heart in EtOH	IMB
22	Bolshaya River	W. Kamchatka Peninsula	50	adult	untested	heart in EtOH	IMB
2	not yet received	Oregon coast	est 50	adult		heart in EtOH	ODFW
4	not yet received	Upper Columbia	est 50	adult		heart in EtOH	WDFW
5	not yet received	Puget Sound	est 50	adult		heart in EtOH	WDFW

Table 2. Microsatellite loci used by laboratories conducting chinook salmon genetics work, used routinely (X) or recommended (x), which we are testing.

Microsatellite locus	UCDavis	SeaStar	ADFG/USFWS	University of Toronto
<i>Onc 13^a</i>	X	X	X	
<i>Ots 1^d</i>		X	X	X
<i>Ots 2^d</i>	X	X	X	
<i>Ots 3^d</i>	X	X		X
<i>Ots 9^d</i>	X	X		
<i>Ots 10^d</i>	X	X		
<i>Ots 104^c</i>	X	X		
<i>Ots 107^c</i>	X	X	X	X

- Chinook salmon returns have declined, jeopardizing marine fisheries through closures of CTSAs - need to identify region.

- mixture of Asian N.A. fish in BS and BDA

① environmental markers - scale patterns

② Tagging - otoliths, coded wire tags

③ Genetic:

allozymes - excellent N.A. baseline - tentatively to be expanded to include Asian.
DNA-based - no broad / standard baseline

- Little Asian baseline, Russians know little

- Mitochondrial DNA evolves faster than most nuclear genes
no recombination

- Sample June 2001 & June 2002

- Acquire reference N.A. samples from other labs

- Looking for genetic markers that individually or in aggregate delineate between Asian & stocks.

Long

- will never be able to pull a Chinook salmon off a vessel and tell where it came from. / can look at a sample and determine ^{from the markers} probability of origin.

- Scale pattern analysis highly overrated