

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:

**Developing DNA Markers for the Analysis of Chum Salmon Bycatch
in Alaskan Trawl Fisheries (Phase 1)**

Summary of Work from 1 August 1 2004 to 31 December 2004.

**A.J. Gharrett
Principal Investigator
and
M. Garvin
School of Fisheries and Oceans Sciences**

19 January 2005

Overview

Chum salmon bycatch in the Gulf of Alaska and Bering Sea creates problems for the groundfish fisheries, particularly the Bering Sea trawl fisheries. Salmon returns to western Alaskan systems, which have declined in recent years, are critical to the livelihood and culture of rural Alaskans. In addition, chum salmon are the focus of a number of other issues including allocation among Alaskan users and between the U.S. and Canada. Central to bycatch questions is the origin/destination of intercepted fish. Substantial effort has been (and continues to be) devoted to genetic studies of North American chum salmon stocks, the objective of which is to resolve mixtures to their contributing components. An extensive allozyme baseline has been developed to address those questions. Unfortunately, because of the logistics required to sample tissues for allozyme analysis and the increasing costs of storing and processing the samples, most labs are ceasing their allozyme operations. In addition, for chum salmon, allozymes may not provide the fine-scale resolution needed to address some questions.

An alternative approach is the use of DNA variation; however, there are as yet no baselines for DNA markers. In this project, we are developing DNA markers, particularly single nucleotide polymorphism (SNP) markers, for application to the trawl fishery chum salmon bycatch problem. The challenge is to discover the variation, design the specific molecular tool, and verify its utility in separating stocks. In this phase of the project, we are developing markers. In the next phases, we will survey the variability in substantial portions of the geographic range of chum salmon and evaluate the success that the markers can be expected to have in resolving populations in mixtures, for example, between or within the Yukon and Kuskokwim drainages.

Accomplishments to date

1. We have the Ecotilling technique working and have preliminary data for the MHCIIb locus.
2. We adapted and further developed a technique to rapidly screen new sequences for variation as they become available. We have termed the method Decotilling. Two genes have been screened by Decotilling and PCR primers have been optimized for seven additional loci.
3. We have begun acquiring and organizing samples of chum salmon tissues and DNA. DNA has been isolated from some of the tissues. We have identified the other sources for samples of western Alaskan (ADF&G) and Asian (Russian Academy of Sciences).

Ecotilling

Ecotilling is a polymerase chain reaction (PCR)-based method for rapidly screening DNA fragments of up to 1.2kb (and most likely larger) for variation at the single nucleotide level. It was developed to screen for induced mutations in several species, mainly plants (Henikoff et al. 2003; McCallum et al. 2000; Till et al. 2004). The advantage that this technique holds over others is that only one DNA strand is cleaved, giving two sets of dye labeled fragments which can be used to more accurately identify

nucleotide differences. We have modified the technique to detect natural polymorphisms in chum salmon DNA, using 1/10 of the labeled primers that are described in the original publication. We expect to be able to reduce the dye-labeled primers even more to reduce costs. We have successfully used regular Taq polymerase, rather than the more expensive proofreading Taq cited in the literature, with no negative effects on the signal.

We are conducting preliminary screening for variants in the MCH IIB gene in samples of fish from nine populations. The literature reports 5 loci for the MCH IIB gene, which we have observed.

Decotilling

We modified a method described by Sokurenko (2001) that is a variation on Ecotilling. This method allows us to detect simple mutations and polymorphisms in large genomic regions without using a sequencer and expensive labeled primers. We term the method Decotilling (double strand cleavage Ecotilling). For this method, we altered conditions to cleave both strands of DNA rather than the single strand cleavage performed in the Ecotilling reaction. The advantage of the method is that it allows the user to look for genetic variation in numerous genes both cheaply and rapidly. Currently we can screen a 1.2kb fragment in 480 fish in one day. This uses ethidium bromide staining of agarose gels and is very cost effective.

We designed and ordered primers for 13 gene sequences for chum salmon. Nine of these are functional sets that yield a single clean band by PCR. Three primer pairs failed to give good PCR results, and one has yet to be tested. So far we have detected 5 variants for the MHC II gene, and three variants for the CLOCK gene. The primary purpose of Decotilling is to answer the question, "Is there variation?" by screening pools of 5 fish each. If the answer is "yes", then Ecotilling will be used to screen individual fish.

What next?

We will continue isolating DNA from samples that we have in hand and continue to acquire samples, particularly from western Alaskan populations (from J. and L. Seeb at the ADF&G Genetics Lab). Next winter and spring we will host Dr. V. Brykov, who will bring additional Russian samples.

We will continue to develop markers from Deco- and Eco-tilling and will begin to process samples for SNP variation this winter.

We plan to conduct a demonstration of the methods the third year of the project on a sample of chum salmon caught as bycatch. It would be advisable to obtain a single sample of about 200 fish from a single location or restricted area this season to be sure that the samples were at hand when we are ready to analyze them. Samples accumulated across the entire fishery are inappropriate for the trial run of this method.

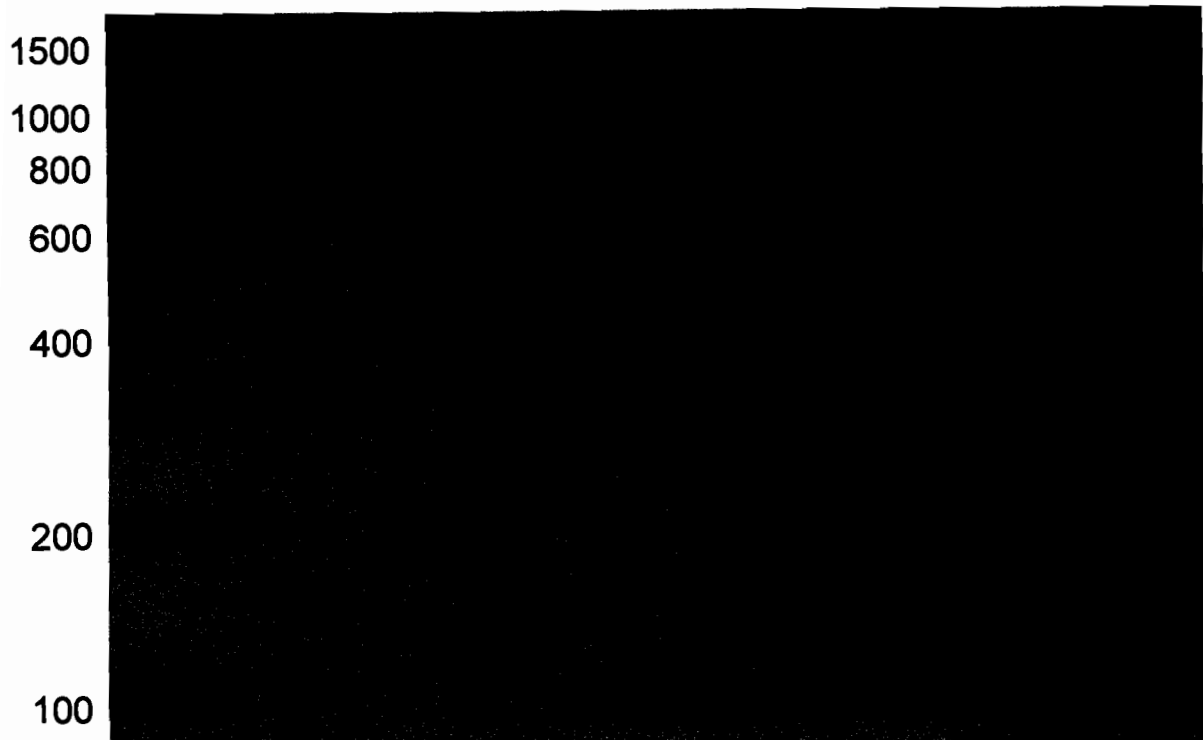


Figure 1. A Decotilling gel of MHCIIb PCR fragments from Kizhuchia River. Each lane is a pool of five fish. The arrows show the fragments produced by cleavage of heterodimer molecules at the site that has mismatched nucleotides. The numbers at the left of the gel are labels for sizes (base pairs) of the molecular marker at the right margin of the gel.

References

- Henikoff, S., and L. Comai . 2003. Single-Nucleotide Mutations for Plant Functional Genomics. *Ann. Rev. Plant Biol.* 54:375-401.
- McCallum, C.M., L. Comai, E.A. Green, and Steven Henikoff. 2000. Targeted screening for induced mutations. *Nature Biotechnol.* 18:455-458.
- Moran, P. 2002. Current conservation genetics: building an ecological approach to the synthesis of molecular and quantitative genetic methods. *Ecol. Freshw. Fish* 11:30–55
- Sokurenko, E.V., V. Tchesnokova, A.T. Yeung, C.A. Oleykowski, E. Trintchina, K.T. Hughs, R.A. Rashid, J.M. Brint, S.L. Moseley. 2004. Detection of Simple Mutations and Polymorphisms in Large Genomic Regions. *Nuc. Acids Res.* 299:e111.
- Till, B.J., C.B., L. Comai, and S. Henikoff. 2004. Mismatch cleavage by single-strand specific nucleases. *Nuc Acids Res* 32:2632-2641.

Appendix I. Locations of samples identified to date for analysis using SNP and Ecotilling methods to quantify variation in chum salmon populations.

	Region	River System	Year	number	Form
1	N. British Columbia	Klownick Creek	1989	50	Tissue
2	N. British Columbia	Neekas Creek	1989	50	Tissue
3	W. Queen Charlotte Island	Tasu Creek	1989	40	DNA
4	E. Queen Charlotte Island	Bag Harbor	1989	50	Tissue
5	Behm Canal	Portage Creek	1986, 1988	100	Tissue
6	Behm Canal	Wilson River	1986	42	DNA
7	Behm Canal	Herman Creek	1987, 1990	100	Tissue
8	E. Prince of Wales Island	Old Tom Creek	1986, 1988	100	Tissue
9	E. Prince of Wales Island	Karta River	1983	43	DNA
10	E. Baranof Island	Kizhuchia River	1989	40	DNA
11	E. Admiralty Island	Greens Creek	1995	100	Tissue
12	Prince William Sound	Olsen	1992	100	Tissue
13	Yakutat	Alek N. Fork	2000	100	Tissue
14	Goodnews Bay	Goodnews River M. Fork	1989	50	Heart
15	Goodnews Bay	Goodnews River	1989	50	Muscle
16	Kuskokwim River	Kasigluk River	1990	73	Heart
17	Kuskokwim River	Anvik River	1989	75	Heart
18	Kuskokwim River	Kwethluk River	1989	77	Heart
19	Kuskokwin Bay	Kanektok River	1989	75	Heart
20	Port Heiden	Meshik River	1989	75	Heart
21	Nushugak Bay	Bristol Bay, Nushugak Big Creek	1988	75	Heart
22	Bristol Bay	(Naknek River) Gertrude Creek (King Salmon River)	1988, 2000	75	Heart
23	Bristol Bay		1987, 1999	60	Muscle & fin
24	Yukon River	Chulinak River	1989	100	Heart
25	Yukon River	Anvik River	1988	100	Heart
26	Yukon River	California Creek	1997	100	fin
27	Yukon River	Gisasa River	2003	294	fin
28	Yukon River	Nulato River	2003	100	fin
29	Kotzebue Sound	Kobuk River	2000	100	fin
30	W. Kamchatka	Hairasova	??	40	DNA
31	E. Kamchatka	Ossoro River	1996	40	DNA
32	Sakhalin Island	Udarnitsa River	1994	40	DNA
33	Sakhalin Island	Tym River	1995	40	DNA
34	China	Heilong River	1989	41	DNA

Appendix II. Loci being examined for suitability as SNP or Ecotilling loci.

Gene Name	Species	Reference	Gene Name	Species	Reference
clock protein (SClkF2R2)	<i>O. keta</i>	our lab	Lysozyme 2	<i>O. mykiss</i>	Moran (2000)
insulin gene for preproinsulin	<i>O. keta</i>	our lab	Metallothionine A	<i>O. mykiss</i>	Moran (2000)
MHC class II beta chain	<i>O. keta</i>	our lab	Metallothionine B	<i>O. mykiss</i>	Moran (2000)
proopiomelanocortin precursor	<i>O. keta</i>	our lab	Monomine oxidase	<i>O. mykiss</i>	Moran (2000)
Vitellogenin	<i>O. mykiss</i>	our lab	Myelin proteolipid DM20	<i>O. mykiss</i>	Moran (2000)
Band3 Protein	<i>O. mykiss</i>	Moran (2000)	myogenic factor D	<i>O. mykiss</i>	Moran (2000)
Cathepsin D	<i>O. mykiss</i>	Moran (2000)	myogenic factor D2	<i>O. mykiss</i>	Moran (2000)
C-my protooncogene	<i>O. mykiss</i>	Moran (2000)	P53 protooncogene	<i>O. mykiss</i>	Moran (2000)
Cytokeratin S, TKS	<i>O. mykiss</i>	Moran (2000)	Prolactin 2 3' region	<i>O. tschawytscha</i>	Moran (2000)
Ependymin 1	<i>O. mykiss</i>	Moran (2000)	prolactin 1	<i>O. tschawytscha</i>	Moran (2000)
Ependymin 2	<i>O. mykiss</i>	Moran (2000)	prolactin 1 3' region	<i>O. tschawytscha</i>	Moran (2000)
Estrogen Receptor	<i>O. mykiss</i>	Moran (2000)	Prolactin 2	<i>O. tschawytscha</i>	Moran (2000)
Gonadotropin	<i>O. mykiss</i>	Moran (2000)	Protamine CII	<i>O. mykiss</i>	Moran (2000)
Gonadotropin 2 beta	<i>O. tschawytscha</i>	Moran (2000)	Ran1	<i>O. mykiss</i>	Moran (2000)
Growth Hormone 2	<i>O. mykiss</i>	Moran (2000)	ras-1 proto-oncogene	<i>O. mykiss</i>	Moran (2000)
Heat shock protein 71	<i>O. mykiss</i>	Moran (2000)	Recombination act.	<i>O. mykiss</i>	Moran (2000)
Heat shock protein 90	<i>O. tschawytsch</i>	Moran (2000)	Somatolactin	<i>O. keta</i>	Moran (2000)
High Mobility GroupT2	<i>O. mykiss</i>	Moran (2000)	Somatolactin	<i>O. keta</i>	Moran (2000)
IgG H	<i>O. mykiss</i>	Moran (2000)	Stanniocalcin	<i>O. kisutch</i>	Moran (2000)
IgG M	<i>O. mykiss</i>	Moran (2000)	syr-type HMG box	<i>O. mykiss</i>	Moran (2000)
ikaros	<i>O. mykiss</i>	Moran (2000)	Transf. Growth Factor	<i>O. mykiss</i>	Moran (2000)
Inhibitor of DNA bind./diff.1	<i>O. mykiss</i>	Moran (2000)	Transferrin	<i>O. mykiss</i>	Moran (2000)
Inhibitor of DNA bind./diff.2	<i>O. mykiss</i>	Moran (2000)	Transp. Protein	<i>Salmo salar</i>	Moran (2000)
Insulin-like growth factor	<i>O. keta</i>	Moran (2000)	Vimentin	<i>O. mykiss</i>	Moran (2000)
Interleukin-8 receptor	<i>O. mykiss</i>	Moran (2000)	Vitellogenin Receptor	<i>O. mykiss</i>	Moran (2000)