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Western Alaska Salmon Stock Identification Program Technical Document 9: Chum Salmon SNP Discovery, First Method

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

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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)

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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Note: This document serves as a record of communication between the Alaska Department of Fish and Game Commercial Fisheries Division and the Western Alaska Salmon Stock Identification Program Technical Committee. As such, these documents serve diverse ad hoc information purposes and may contain basic, uninterpreted data. The contents of this document have not been subjected to review and should not be cited or distributed without the permission of the authors or the Commercial Fisheries Division.

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ABSTRACT

Uncertainty about the magnitude, frequency, location, and timing of the nonlocal harvest of sockeye and chum salmon in Western Alaska fisheries was the impetus for the Western Alaska Salmon Stock Identification Project (WASSIP). The project was designed to use genetic data in mixed stock analysis (MSA) to reduce this uncertainty. A baseline of allele frequencies is required for use in MSA to estimate the stock of origin of harvested fish. The single nucleotide polymorphism (SNP) baseline for chum salmon *Oncorhynchus keta* to be used in MSA for WASSIP is in a state of perpetual improvement. To meet the standards set by the Advisory Panel an emphasis was placed on selecting more markers to increase resolution among the four regional areas of coastal western Alaska. The Alaska Department of Fish and Game began the process of discovering additional SNP markers for chum salmon through a contract with International Program for Salmon Ecological Genetics (IPSEG; http://www.fish.washington.edu/research/ipseg/research.html) at the University of Washington. This process was published in an article in the journal *Molecular Ecology Resources* and this technical document highlights the importance of the article to WASSIP, gives a brief overview, and directs the Technical Committee and the Advisory Panel and any other interested readers to the published article.

Key words: Western Alaska Salmon Stock Identification Project, WASSIP, chum salmon, *Oncorhynchus keta*, mixed stock analysis, genetic baseline, single nucleotide polymorphism, SNP

INTRODUCTION

Early in the development process for the Western Alaska Salmon Stock Identification Project (WASSIP) it was clear that the resolution possible to distinguish among regional areas for chum salmon spawning in coastal western Alaska regional areas (Norton Sound, lower Yukon River, also called Yukon Coastal, Kuskokwim River, and Bristol Bay) was not going to be sufficient to meet the standards set by the Advisory Panel with available genetic markers, including the recently developed SNP markers (see Jasper et al. 2012 and Seeb et al. 2011 for the current panel of 53 SNPs). These four regional areas define important units for management, yet when treated as separate reporting groups the four areas could not be distinguished sufficiently using the 53-marker set. The Alaska Department of Fish and Game (ADF&G) Gene Conservation Laboratory began the process of discovering additional SNP markers for chum salmon through a contract with International Program for Salmon Ecological Genetics (IPSEG; http://www.fish.washington.edu/research/ipseg/research.html) at the University of Washington. These efforts were based on cDNA sequences from two chum salmon sampled from the Susitna (Cook Inlet) and Delta (Yukon River) rivers. This process has been published in *Molecular Ecology Resources* (Seeb et al. 2011) which was submitted to the WASSIP Technical Committee for review and comment as Technical Document 9. This process added 37 validated SNPs to those already available for use in chum salmon for WASSIP. The SNPs discovered through this and other efforts will be assayed in 30 populations and a subset of the best 96 SNPs will be used for mixed stock analysis in WASSIP.

METHODS

The methods followed by Seeb et al. (2011) define a new "SNP discovery validation pipeline" for use in nonmodel organisms like chum salmon. Samples were collected from two male chum salmon, their RNA extracted, and normalized cDNA libraries constructed for each fish using 454 FLX pyrosequencing. Sequence assembly employed CLC Genomic Workbench version 4.0 and successful initial candidate SNPs were selected. Potential SNP candidates then underwent a four-step validation process: (1) SNP selection and primer testing; (2) High-resolution melt curve analysis; (3) Sanger sequencing, and (4) High-throughput genotyping and population analysis. For more on the methods visit the complete online version of the article in the journal *Molecular*

Ecology Resources (copyright Wiley-Blackwell Publishers: DOI: 10.1111/j.1755-0998.2010.02936.x).

RESULTS

This process added 37 validated SNPs in addition to the 53 SNPs already available for use in chum salmon for WASSIP. To view results and figures, visit the complete online version of the article in the journal *Molecular Ecology Resources* (copyright Wiley-Blackwell Publishers: DOI: 10.1111/j.1755-0998.2010.02936.x).

DISCUSSION

The SNPs discovered through Seeb et al. (2011) and other efforts will be assayed in 30 populations at the Gene Conservation Laboratory and a subset of the best 96 SNPs will be used for mixed stock analysis in WASSIP. For an outline of the process that GCL intends to use see DeCovich et al. 2012. The Gene Conservation Laboratory will select the set of 96 SNPs that maximizes the likelihood of providing the resolution necessary to meet the objectives of WASSIP.

ACKNOWLEDGMENTS

The Technical Document series served as a record of communication between the Alaska Department of Fish and Game Commercial Fisheries Division and the Western Alaska Salmon Stock Identification Program (WASSIP) Technical Committee during the implementation of the program. The authors would like to thank the WASSIP Technical Committee and Advisory Panel for their constructive input on each of the documents throughout the project. The authors would also like to thank Erica Chenoweth who coordinated and prepared the Technical Document series for publication and Publication Specialists Amy Carroll and Joanne MacClellan for implementing the series into Regional Information Reports.

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QUESTIONS FOR THE TECHNICAL COMMITTEE

This document is provided for informational purposes and we have no specific questions. However, any comment or review that you might have would be appreciated.