NPAFC
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Jacqueline A. Whittle, Christine M. Kondzela, and Jeffrey R. Guyon

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Alaska Fisheries Science Center, Auke Bay Laboratories
17109 Pt. Lena Loop Road, Juneau, AK 99801, USA

Submitted to the

NORTH PACIFIC ANADROMOUS FISH COMMISSION

by

United States of America

April 2017

THIS PAPER MAY BE CITED IN THE FOLLOWING MANNER:
Whittle, J.A., C.M. Kondzela, and J.R. Guyon. 2017. Comparative analysis of microsatellite markers on coastal western Alaska chum salmon stocks. NPAFC Doc. 1703. 12 pp. National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service (NMFS), Alaska Fisheries Science Center, Auke Bay Laboratories (Available at http://www.npafc.org).

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Auke Bay Laboratories, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 17109 Pt. Lena Loop Road, Juneau, AK 99801, USA

Keywords: western Alaska, chum salmon, microsatellite markers, genetic stock identification

Abstract

Chum salmon (O*ncorhynchus keta*) populations from five reporting groups in coastal Western Alaska were genotyped for 11 new microsatellite markers and 25 existing microsatellite markers to determine if discrimination among these reporting groups was possible, and if a larger number of markers could improve stock discrimination. G-test and F_{ST} values for the two panels were similar, with highly significant differences between the majority of population pairs. Stock aggregation results from principal component analyses and phylogenetic trees were nearly identical for the two panels. Baseline 100% simulation analyses indicated that the addition of the 11 new genetic markers slightly increased the ability to distinguish reporting groups in mixed-stock applications.

Introduction

Stock-specific identification of chum salmon (O*ncorhynchus keta*) intercepted or harvested in federally and state managed fisheries is of great interest to user groups and managers. Currently it is difficult to discriminate among chum salmon stocks in coastal western Alaska. Efforts to improve discrimination of these stocks has been ongoing for decades (e.g., Beacham et al. 2009a, Seeb et al. 2011, Seeb and Crane 1999), and finding genetic markers capable of distinguishing these stocks could aid in fisheries management and in understanding the effects of fisheries on chum salmon stocks in Western Alaska.

Recently, a collaborative chum salmon baseline development project (hereafter abbreviated as CIAP-WASC), funded by the State of Alaska, Coastal Impact Assistance Program (CIAP), was completed by the University of Alaska, Alaska Department of Fish and Game, National Marine Fisheries Service, and Western Alaska Salmon Coalition (WASC). With the objective to provide information that could assist fisheries managers to more accurately identify genetic stocks and to determine the origin of chum salmon catch and bycatch from Alaska fisheries, this collaboration increased the number of microsatellite markers available from 13 to 25 for genetic analyses of chum salmon. During the CIAP-WASC project, a new set of chum

salmon microsatellite markers became available (Tsukagoshi et al. 2015). Here, we evaluate two panels of markers; the first panel is the 25 microsatellite markers used in the CIAP-WASC project and the second panel is 11 of the recently published microsatellite markers (Tsukagoshi et al. 2015). Our report evaluates the effects of different numbers of markers on discriminatory power in stock identification and mixed stock analyses of western Alaska chum salmon populations.

Materials and Methods

Sample Collection and Microsatellite Genotyping

Representative samples from two populations each from four regions in coastal Western Alaska (Norton Sound, Lower Yukon, Kuskokwim, and Bristol Bay) and one control region (Middle Yukon) (Fig. 1, Table 1) were used to compare these markers.

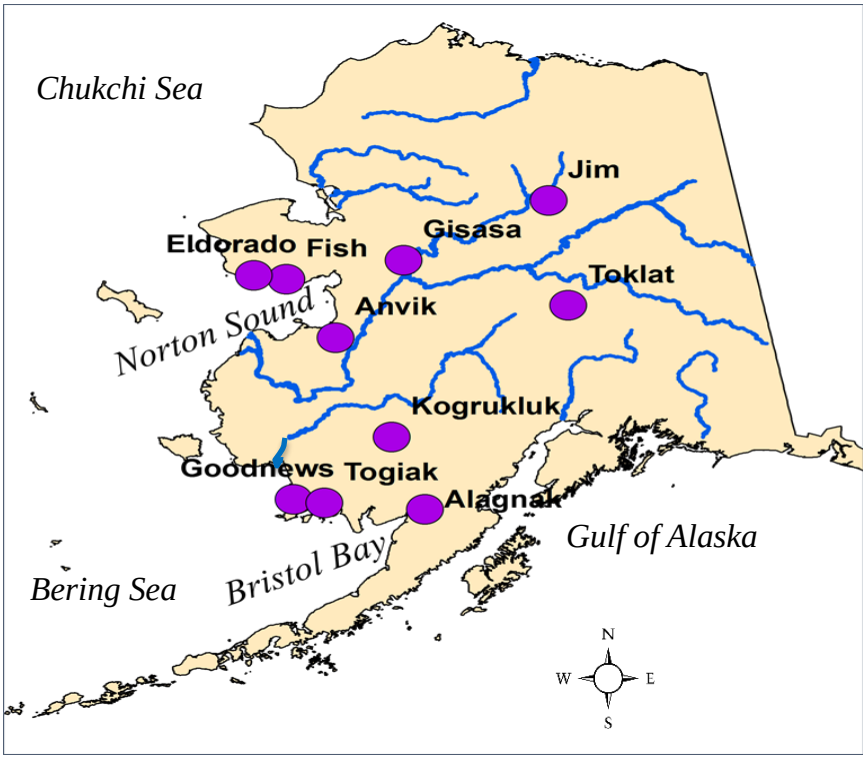


Figure 1. -- Sampling locations of 10 populations of chum salmon genotyped for 36 microsatellite markers.

Table 1. -- Reporting groups and sample sizes of 10 populations of chum salmon genotyped for 36 microsatellite markers.

5-region reporting group	Population	Collection year	Sample size
Norton Sound	Eldorado River	2005	95
	Fish River	2004	95
Middle Yukon	Jim River	2002	95
	Toklat River	1993	95
Lower Yukon	Anvik River	1992	95
	Gisasa River	2004	95
Kuskokwim	Goodnews River	2007	95
	Kogrukluk River	2007	95
Bristol Bay	Alagnak River	2010	95
	Togiak River	1994	95

Genomic DNA was obtained from the Alaska Department of Fish and Game (ADFG, Gene Conservation Laboratory, Anchorage, Alaska). Ninety-five samples from each population (Table 1) were genotyped for the following 36 microsatellite markers: *Oke11, Oke4* (Buchholz et al. 2001); *Oki2, Oki18, Oki23* (Smith et al. 1998); *Oki100* (Beacham et al. 2009a); *Omm1070* (Rexroad et al. 2001); *Omy1011* (Spies et al. 2005); *One101*, *One102*, *One104*, *One106, One108, One109, One111, One114* (Olsen et al. 2000), *Oneu18* (Scribner et al. 1996), *Ots103* (Beacham et al. 1999); *Ots3* (Greig et al. 1999); *Otsg253, Otsg311, Otsg68* (Williamson et al. 2002); *Ots7* (Banks et al. 1999); *Ssa407, Ssa419* (Cairney et al. 2000); and *Chums2, Chums13, Chums116, Chums117, Chums120, Chums122, Chums127, Chums134, Chums136, Chums137, Chums139* (Tsukagoshi et al. 2015).

Thermal cycling for the amplification of DNA fragments with polymerase chain reaction (PCR) was performed on a dual 384-well GeneAmp PCR System 9700 (Applied Biosystems, Inc.). Samples from the PCR reactions were diluted into 96-well plates for analysis by a 48-capillary, 36 cm array on the ABI 3730*xl* Genetic Analyzer (Applied Biosystems, Inc.). Genotypes were double-scored with GeneMapper 5.0 software (Applied Biosystems, Inc.) and exported to Excel (Microsoft, Inc.) spreadsheets.

Data Analyses

Population structure and genetic differentiation were examined with Hardy-Weinberg equilibrium tests, G-tests, F_{ST} values, principal component analyses, phylogenetic trees, and ONCOR simulations. Deviation from Hardy-Weinberg proportions (initial $P < 0.05$) for each of the 36 microsatellite markers for each population was tested in GENEPOP 4.2.1 (Rousset 2008) with Markov chain parameters of 10,000 dememorization, 20 batches, and 5,000 iterations. Allele frequency exact tests for population differentiation between all pairs of populations were performed in GENEPOP 4.2.1 with Markov chain parameters of 10,000 dememorization, 100

batches, and 5,000 iterations. Pairwise F_{ST} tests of differentiation were performed in FSTAT 2.9.3.2 (Goudet 2001). Principal component analysis based on arcsine-square root transformed allele frequencies (with low frequency alleles of <0.05 for all 10 populations removed) was performed in Minitab 17.3.1 (Minitab, Inc. 2010). Phylogenetic trees were examined in PHYLIP 3.63 (Felsenstein 2005) with Nei's genetic distance and neighbor-joining trees, and tree files were visualized with Mega 7.0.21 (Kumar et al. 2015). Genetic stock assignment was evaluated with 100% simulations in ONCOR (Kalinowski et al. 2008) with simulated mixtures of 200 fish conducted with 100 simulations for both sets of 25 (CIAP-WASC) and 36 (CIAP-WASC and 11 new) markers.

Results

Of the 950 samples genotyped in the 11 microsatellite marker panel, 92% were successfully genotyped for eight or more loci. Samples not meeting this genotyping criteria were removed from further analyses. All genotypes from the final sample set for the 25 microsatellite panel from the CIAP-WASC project were used.

Of the tests to evaluate Hardy-Weinberg proportions for the 36 microsatellite markers, three markers from the 25 microsatellite marker panel were out of equilibrium: *Oki18* departed from equilibrium in five populations, *One106* in seven populations, and *One108* in nine populations. These three markers were removed from further analyses, with the exception of the 100% simulation analyses. The remaining 33 markers (22 markers from the original 25 CIAP-WASC marker panel and all markers from the 11 new marker panel) had no significant departure from Hardy-Weinberg equilibrium in any of the 10 populations. For all but three pairwise population comparisons (two G-tests and one F_{ST} test) the G-tests and F_{ST} values had similar results for the 22 and 33 marker panels, with highly significant differences between nearly all population pairs (Table 2).

Principle component analysis was performed for the 11 new markers, the 22 CIAP-WASC markers, and all 33 markers. Results of the three marker panels were very similar, suggesting that all markers are measuring the same divergence among populations (Fig. 2). In the 22 and 33 marker panels, Norton Sound (Fish and Eldorado Rivers) and Middle Yukon (Jim and Toklat Rivers) diverged from other populations, and the two populations in the Kuskokwim reporting group clustered more closely with other populations: the Goodnews River with Bristol Bay and the Kogrukluk River with the Lower Yukon (Anvik and Gisasa Rivers). The main difference between the three marker panels was the position of Alagnak River, which grouped more closely with Kogrukluk, Anvik, and Gisasa Rivers in the 11 marker panel and with Goodnews and Togiak Rivers in the 22 and 33 marker panel. Neighbor-joining tree results for the 22 and 33 microsatellite marker panels were similar to each other, mirroring the principle component analyses results for the two panels (Fig. 3).

Table 2. -- Pairwise G-tests and F_{ST} values of 10 populations for 22 microsatellite markers (top table) and 33 microsatellite markers (bottom table). P-values from G-tests of allele frequencies are above the diagonal. Significance after Bonferroni correction (initial P < 0.05) for multiple testing are indicated in bold. HS = highly significant. F_{ST} values are below the diagonal; values significantly > 0 are indicated in bold.

22 markers	Alagnak	Anvik	Eldorado	Fish	Gisasa	Goodnews	Jim	Kogrukluk	Togiak	Toklat
Alagnak		**0**	**0**	**0**	**0**	**0.0001**	**HS**	0.0127	**0**	**HS**
Anvik	**0.0039**		**0**	**0**	**0**	**0**	**HS**	0.0081	**HS**	**HS**
Eldorado	**0.0041**	**0.0021**		0.0111	**0**	**0**	**HS**	0.0133	**HS**	**HS**
Fish	**0.0054**	**0.0027**	0.0004		**0**	**0**	**HS**	0.0098	**HS**	**HS**
Gisasa	**0.0056**	0.0014	**0.0024**	**0.0021**		**HS**	**HS**	**0.0006**	**HS**	**HS**
Goodnews	0.0019	**0.0038**	**0.0039**	**0.0047**	**0.0062**		**HS**	**0.0000**	**0**	**HS**
Jim	**0.0175**	**0.0121**	**0.0148**	**0.0143**	**0.0108**	**0.0191**		**HS**	**HS**	**0**
Kogrukluk	0.0014	0.0008	0.0010	0.0011	0.0022	**0.0024**	**0.0141**		**0**	**HS**
Togiak	**0.0037**	**0.0083**	**0.0082**	**0.0090**	**0.0099**	**0.0024**	**0.0251**	**0.0060**		**HS**
Toklat	**0.0229**	**0.0179**	**0.0198**	**0.0187**	**0.0147**	**0.0229**	**0.0051**	**0.0180**	**0.0295**	

33 markers	Alagnak	Anvik	Eldorado	Fish	Gisasa	Goodnews	Jim	Kogrukluk	Togiak	Toklat
Alagnak		**0**	**HS**	**HS**	**0**	**0**	**HS**	**0.0007**	**0**	**HS**
Anvik	**0.0036**		**0**	**0**	**0.0001**	**0**	**HS**	0.0253	**HS**	**HS**
Eldorado	**0.0041**	**0.0022**		0.0158	**0**	**HS**	**HS**	0.0145	**HS**	**HS**
Fish	**0.0045**	**0.0022**	0.0004		**0**	**HS**	**HS**	0.0014	**HS**	**HS**
Gisasa	**0.0057**	0.0010	**0.0027**	**0.0021**		**HS**	**HS**	0.0013	**HS**	**HS**
Goodnews	**0.0025**	**0.0031**	**0.0038**	**0.0037**	**0.0046**		**HS**	**0**	**0**	**HS**
Jim	**0.0151**	**0.0102**	**0.0132**	**0.0123**	**0.0089**	**0.0148**		**HS**	**HS**	**HS**
Kogrukluk	0.0020	0.0002	0.0012	0.0013	0.0020	**0.0020**	**0.0112**		**HS**	**HS**
Togiak	**0.0041**	**0.0075**	**0.0072**	**0.0080**	**0.0090**	**0.0026**	**0.0214**	**0.0065**		**HS**
Toklat	**0.0198**	**0.0149**	**0.0166**	**0.0159**	**0.0124**	**0.0180**	**0.0047**	**0.0151**	**0.0248**	

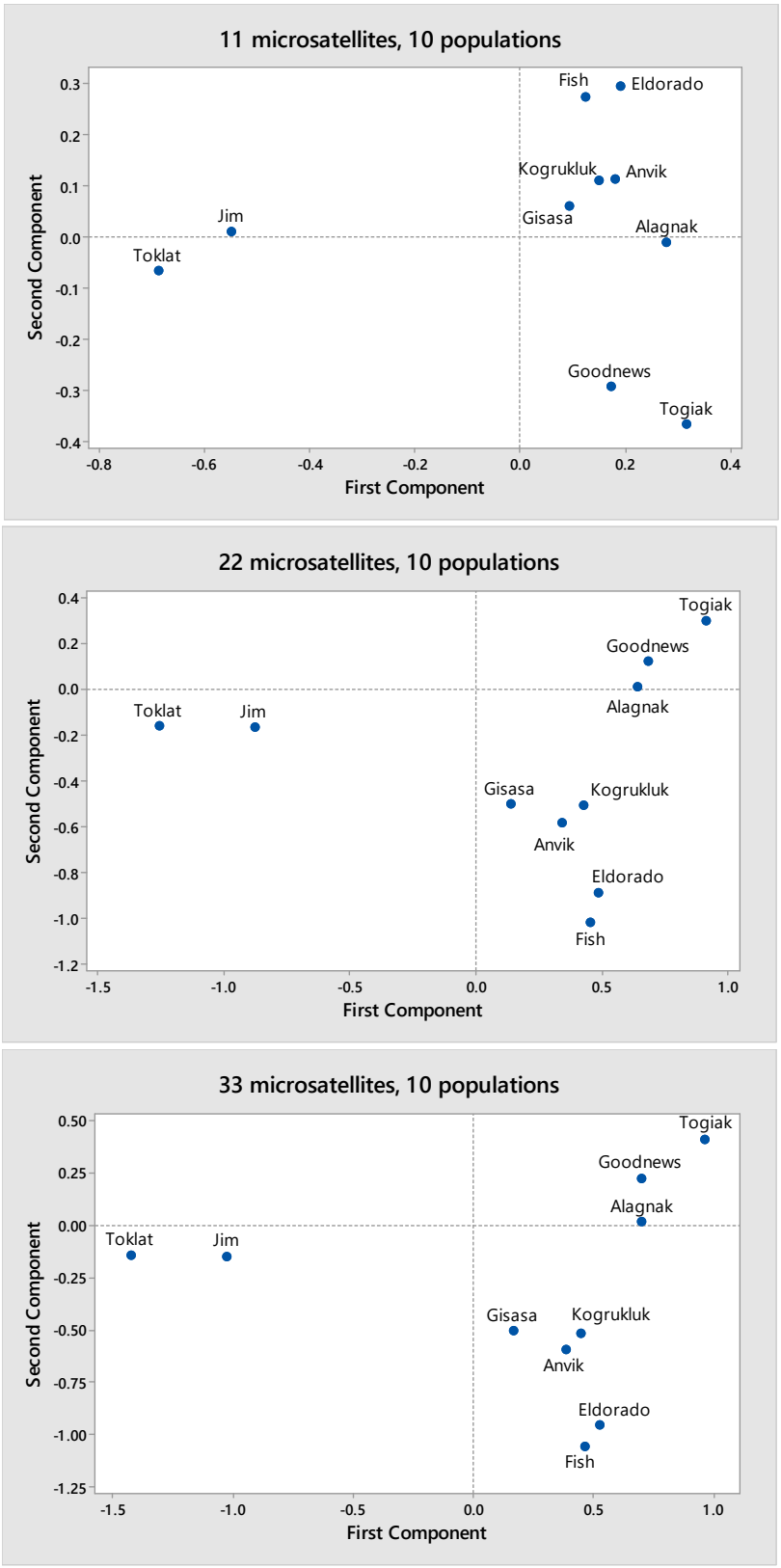
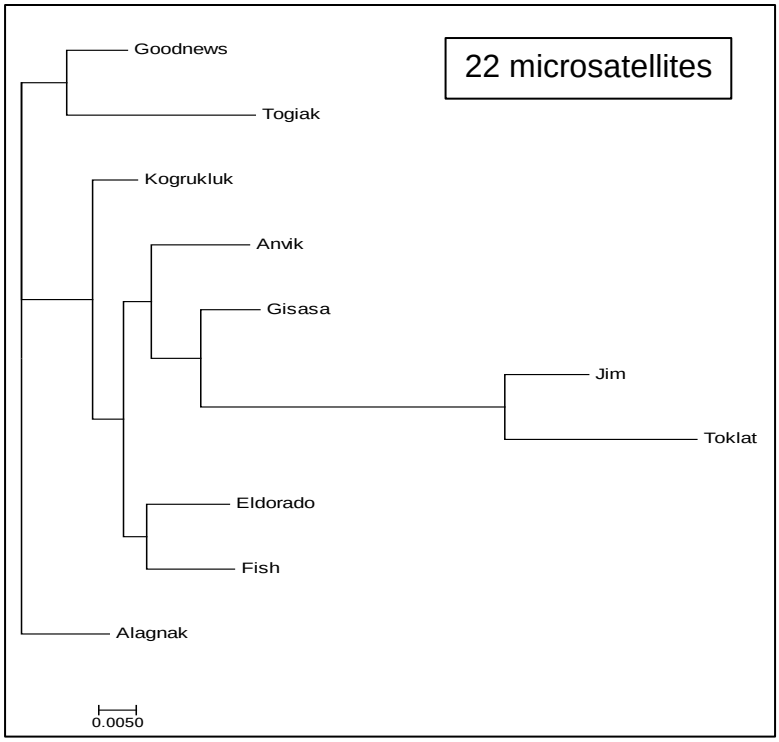


Figure. 2. -- Principle component analyses of 10 western Alaska chum salmon populations (Table 1) based on 11, 22, and 33 microsatellite markers.



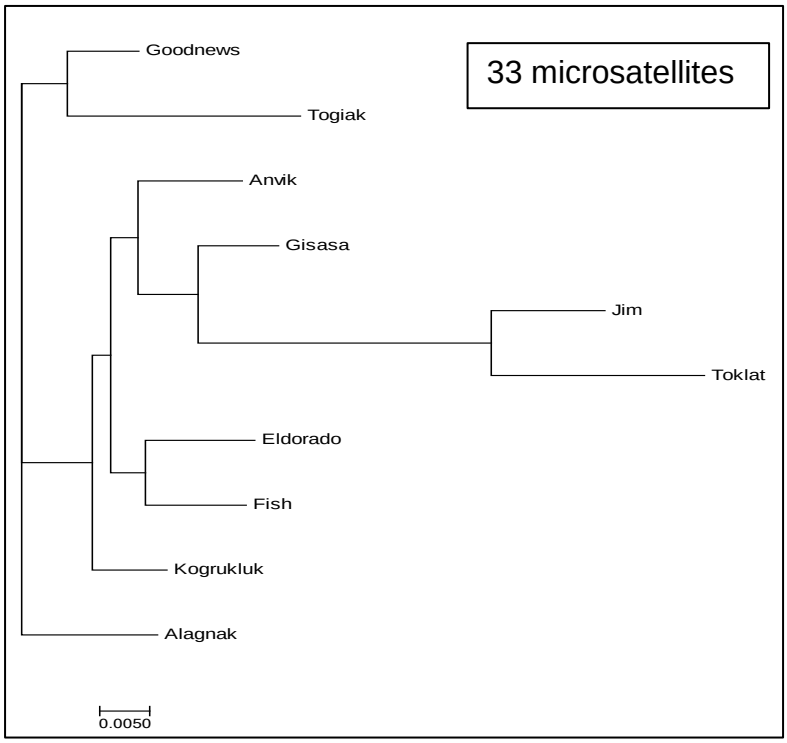


Figure 3. – Neighbor-joining trees of 10 western Alaska chum salmon populations (Table 1) based on Nei's distance of 22 and 33 microsatellite markers.

The principal component analyses and neighbor-joining trees supported aggregation of the 10 populations into four reporting groups that include: Middle Yukon (Jim and Toklat Rivers), Western Alaska-not coastal (Gisasa, Anvik, and Kogrukluk Rivers), Norton Sound (Eldorado and Fish Rivers), and Coastal Bristol Bay (Alagnak, Goodnews, and Togiak Rivers). Simulation analyses of the populations into these four reporting groups for the two marker panels (25 and 36 markers) were compared (Table 3). The 36-marker panel performed incrementally better in the 100% simulation analyses, suggesting that the additional markers provided information helpful for stock discrimination. The best reallocation was for the Middle Yukon reporting group (93%), followed by Coastal Bristol Bay (75%), Western Alaska-not coastal (67%), and Norton Sound (66%) reporting groups.

Table 3. -- Genetic stock composition 100% simulation analysis conducted in the program ONCOR, based on a simulated mixture of 200 fish and 100 simulations. The mean proportion of correct allocations back to the population of origin and reporting group are under "Population" and "Report Group", respectively. The reporting group to which the population was assigned is under "Reporting group". Results are shown for the 25 marker panel (top) and the 36 marker panel (bottom).

25 Markers	**Population**		**Report group**		
Population	**Mean**	**S.D.**	**Mean**	**S.D.**	**Reporting Group**
Alagnak	0.42	0.05	0.65	0.04	BB-Coastal
Goodnews	0.45	0.04	0.71	0.04	BB-Coastal
Togiak	0.62	0.04	0.84	0.03	BB-Coastal
Jim	0.74	0.04	0.83	0.03	MiddleYukon
Toklat	0.82	0.03	0.96	0.01	MiddleYukon
Eldorado	0.55	0.05	0.70	0.04	NortonSound
Fish	0.44	0.05	0.59	0.05	NortonSound
Anvik	0.48	0.04	0.71	0.04	WestAK-not coastal
Gisasa	0.42	0.03	0.70	0.04	WestAK-not coastal
Kogrukluk	0.33	0.04	0.54	0.04	WestAK-not coastal

36 Markers	**Population**		**Report group**		
Population	**Mean**	**S.D.**	**Mean**	**S.D.**	**Reporting Group**
Alagnak	0.47	0.04	0.68	0.04	BB-Coastal
Goodnews	0.48	0.04	0.73	0.04	BB-Coastal
Togiak	0.63	0.04	0.85	0.03	BB-Coastal
Jim	0.80	0.04	0.88	0.03	MiddleYukon
Toklat	0.85	0.03	0.97	0.02	MiddleYukon
Eldorado	0.54	0.04	0.69	0.04	NortonSound
Fish	0.44	0.04	0.62	0.04	NortonSound
Anvik	0.48	0.04	0.76	0.04	WestAK-not coastal
Gisasa	0.41	0.04	0.71	0.04	WestAK-not coastal
Kogrukluk	0.31	0.04	0.55	0.05	WestAK-not coastal

Discussion

Western Alaska is a large geographic area with diverse habitat for chum salmon, but previous analyses have found limited stock differentiation in the coastal summer-run populations in this area (Beacham et al. 2009b, Habicht et al. 2012, Olsen et al. 2011, Seeb et al. 2011, and Seeb et al. 2012). Lack of divergence can result from a number of reasons, including interbreeding between populations and similar selective pressures. For example, recent physical connections between the Yukon River and Kuskokwim River have been postulated as a reason for the lack of differentiation of chum salmon in the lower portions of those rivers (Garvin et al. 2013). We investigated the ability of 11 new microsatellite markers to support stock separation in western Alaska chum salmon, to determine if increasing the number of markers could aid in discriminating among chum salmon stocks in coastal western Alaska, and to determine to what extent mixed stock analyses is likely to be feasible with additional markers.

Genetic diversity tests (G-tests and F_{ST}) indicated significant differences in nearly all pairwise population comparisons for both the 22 and 33 microsatellite marker panels. Phylogenetic trees had similar structure for the two microsatellite marker panels. Principal component analyses, performed for 11, 22, and 33 microsatellite markers, displayed similar results, with the exception of one population (Alagnak), which grouped with a different cluster in the 11 marker panel than in the 22 and 33 marker panels. Although our study had a limited number of populations from a large geographic area, the ONCOR simulations showed that stock discrimination and reporting group accuracy increased overall with the larger panel of markers. The improvement in stock discrimination presented here should encourage studies with additional markers, populations, and increased sample sizes to determine whether geographically finer-scale stock identification in coastal western Alaska populations can be further improved upon. With recent changes in DNA sequencing technology (e.g., Campbell et al. 2015), better separation of chum salmon stocks or reporting groups of stocks in western Alaska and other areas may be possible.

Acknowledgements

Genotyping for our study was funded by the National Marine Fisheries Service (NMFS) through the CIAP-WASC project. We thank William Templin of the Alaska Department of Fish and Game and Megan McPhee of the University of Alaska, Fairbanks for collaborative support, and Sharon Wildes of Auke Bay Laboratories for statistical software support.

The findings and conclusions are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service.

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