

Genetic Stock Identification of Sockeye Salmon Captured in the Coastal Waters of Unalaska Island during April/May and August 1998

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Abstract: This study is part of the Auke Bay Laboratory's Ocean Carrying Capacity (OCC) which has extensively sampled salmon in the North Pacific since 1996 to obtain information on marine life history and migration patterns. Genetic stock identification techniques (protein electrophoresis) indicated that Bristol Bay stocks of immature sockeye salmon (*Oncorhynchus nerka*) made up the largest percentage in two samples taken near Unalaska Island in 1998. The substantial numbers of immature sockeye salmon captured at Cape Cheerful during May 1998 were unexpected, based on current migration models of western Alaska sockeye salmon. Immature sockeye constituted the largest percentage of our immature salmon catch captured at Cape Prominence during August 1998. This was also unexpected since immature chum salmon (*O. keta*) were the predominant catch during August 1996 and 1997 at the same location. These unexpected events may be due to changes in distribution resulting from the strong El Niño event during 1997–1998.

INTRODUCTION

Scientists from the Auke Bay Laboratory (ABL), Alaska Fisheries Science Center, National Marine Fisheries Service, conducted two surveys of salmon (*Oncorhynchus spp.*) distribution during 1998, one in the eastern Aleutian Islands and North Pacific Ocean during April and May, and one in the Gulf of Alaska during July and August (Carlson et al. 1998a, b). These surveys were part of ABL's OCC study on the dynamics of ocean residence of salmonids (National Marine Fisheries Service 1995). Two large catches of immature sockeye salmon collected north and south of Unalaska Island provided an opportunity to estimate their area of origin using a genetic stock identification technique. Sockeye salmon are a valuable food fish, and their absence in expected numbers in the 1997 and 1998 Bristol Bay fishery created substantial economic disruption (Ocean Carrying Capacity Program 1999). The ability to determine stock origins of the salmon during their early ocean life history phase may be used to forecast drops in abundance of important fisheries and protect smaller stocks from overexploitation. Knowledge of stock location in the ocean may help determine exposure to other factors that could affect abundance, e.g., interception by trawl fisheries or drift-net fisheries, or

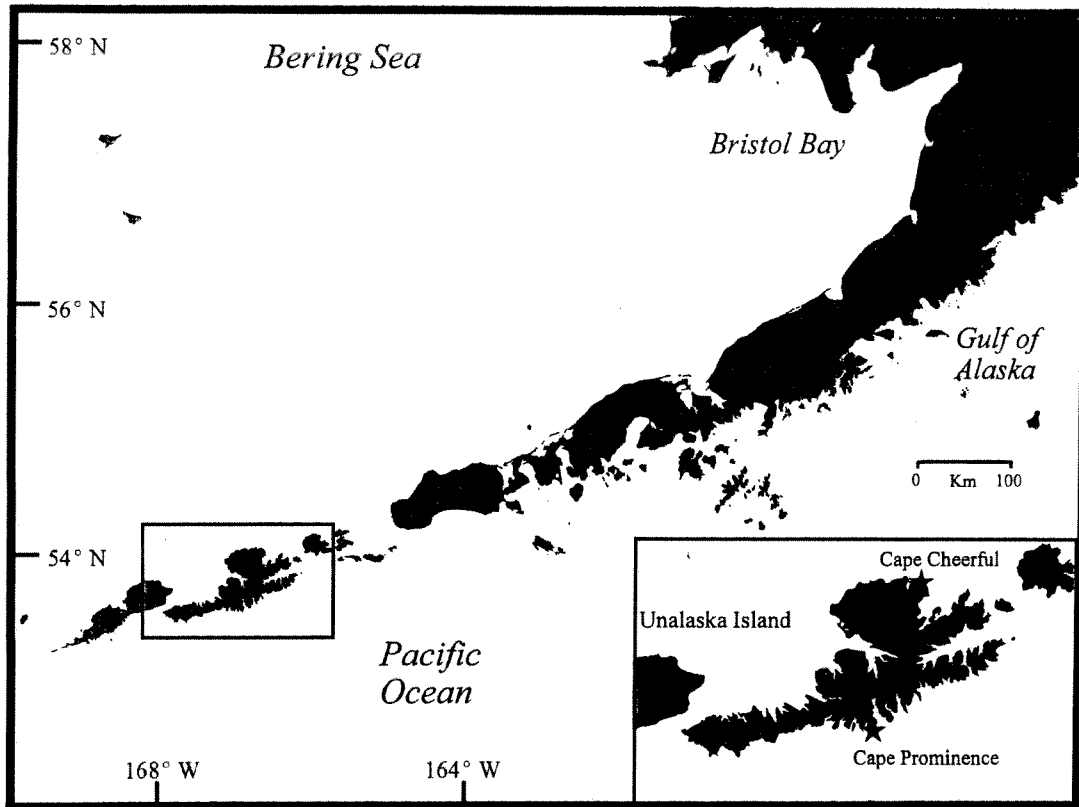
altered marine survival caused by climate changes such as El Niño.

Genetic stock identification relies on genetic differences among stocks in relative allelic frequencies of protein-coding genes (allozymes), or many other types of genetic markers detected by electrophoresis. A sockeye salmon allozyme baseline has recently been constructed from potentially contributing stocks around the North Pacific Ocean, and was used to determine the region of origin of sockeye salmon from a seized high sea's drift net vessel, the *Ying Fa* (Wilmot et al. 1999). Our study used this baseline to identify the region of origin of immature sockeye salmon collected in two samples from the eastern Aleutian Islands in 1998.

METHODS AND MATERIALS

Immature salmon were collected during two salmon surveys of the North Pacific Ocean by the 38 m stern trawler F/V *Great Pacific*, using a midwater rope trawl (Carlson et al. 1998a, b). At less than 30 nautical miles offshore of Cape Cheerful (Fig. 1) on the Bering Sea side of Unalaska Island (approximately 54°03' N latitude and 166°40' W longitude) 440 immature sockeye salmon were collected on April 30, and May 1, 1998 (Carlson et al. 1998a).

Fig. 1. Map of southwest Alaska and Aleutian Islands. Inset shows Unalaska, ★ indicates areas of fish collection.



Three hundred immature sockeye salmon were collected within 11 nautical miles south of Cape Prominence (Fig. 1) on Unalaska Island (approximately 53°19' N latitude and 166°38' W longitude) on August 11, 1998 (Carlson et al. 1998b).

Immature sockeye salmon collected off of Cape Cheerful were frozen whole. Tissues for genetic analysis (eye, heart, muscle and liver) from immature sockeye salmon collected off of Cape Prominence were dissected on board the F/V *Great Pacific*. The whole fish and tissue samples were stored frozen at -60°C until they were shipped to ABL for further analysis.

At the ABL samples of tissue from the heart, liver, muscle, and eye were placed in individual tubes, and frozen at -80°C until analysis. Starch-gel protein electrophoretic analysis followed procedures described by Aebersold et al. (1987) and results are reported using the genetic nomenclature of the American Fisheries Society (Shaklee et al. 1990). Specific tissues and buffers used to interpret genetic variation at 73 loci for sockeye salmon followed Guthrie et al. (1994). The genotypic frequency of the locus *PGM-1** could not be determined due to null allele variation and was therefore treated as a phenotypic character. Variation at co-migrating, duplicated loci (termed isoloci, Allendorf and Thorgaard 1984) was treated as if all the variability appeared at one

locus and the other was monomorphic. This is a conservative treatment for isoloci having relatively low allelic variability (Gharrett and Thomason 1987).

A sockeye salmon allozyme baseline was recently constructed (Wilmot et al. 1999) containing data for 165 populations: seven populations from Russia (ABL, unpublished data), 30 from western Alaska (Varnavskaya et al. 1994; Everett and Wilmot, ABL unpublished data; W. Templin, Alaska Department of Fish and Game [ADF&G], 333 Raspberry Rd., Anchorage, Alaska 99518, U.S.A., personal communication), 52 from southcentral Alaska (W. Templin, personal communication; Seeb et al. in press) 37 from southeastern Alaska (Guthrie et al. 1994; ABL unpublished data) 34 from British Columbia (Wood et al. 1994; C. Wood, Pacific Biological Station, Nanaimo BC, Canada V9R5K6, personal communication), and five from Washington (Winans et al. 1996). For this study we modified the sockeye allozyme baseline to include 150 populations to form nine regional groups: Western Alaska, Bristol Bay, Alaska Peninsula, Kodiak, Southcentral Alaska, Eastern Gulf of Alaska, Southeastern Alaska, British Columbia, and Fraser River (Table 1). Washington and Russian populations were omitted since they were not likely to be potential contributors due to geographic distance and were missing data for four loci. In doing so, the number of available loci in-

Table 1. Sockeye salmon populations sampled for genetic analysis, and source of the data.

Site	Source	Site	Source*
Western Alaska			
Stoney River	1	Talkeetna River Slough	4
Kanektok River	1	Stephan Lake	4
Kagati River	1	Susitna River Slough	4
Goodnews River	1,3	Red Shirt Lake	4
Bristol Bay			
Togiak River	1	Birch Creek	4
Igushik River	1	Byers Lake	4
Wood River	1	Larson Lake	4
Nushagak River	1	Chelatna Lake	4
Battle Creek	1	WF Yetna River	4
Kulik Creek	1	Hewitt/Whiskey Lake	4
Belinda Creek	1	Shell Lake	4
Copper River	1,2	Trinity/Movie Lake	4
Gibraltar Creek	1,2	Judd Lake	4
Iliamna River	1,2	Sixmile Creek	4
Lower Talarik Creek	1	Jim Creek	4
Fuel Dump Island	2	Fish Creek	4
Knutson Creek	2	Cottonwood Creek	4
Woody Island	2	Nancy Lake	4
Lake Clark	1	Swanson River	4
Margot Creek	1	Bishop Creek	4
Upatree Creek	1	Daniels Lake	4
Bear Creek	1	Russian River above/Early	4
Bible Creek	1	Russian River Above/Late	4
Featherly Creek	1	Russian River below	4
Franks Creek	1	Kenai River	4
Kejulik River	1	Skilak Lake Outlet	4
Ruth River	1	Quartz Creek	4
Ugashik River	1	Ptarmigan Creek	4
Alaska Peninsula			
Bear River	1	Hidden Creek	4
Sapsuk Lake	1	Tem Lake	4
Alec River	3	Moose Creek	4
Chignik Lake	3	Johnson/Railroad Creek	4
Kodiak			
Saltery Creek	3	Kasilof River	4
Malina Lake	3	Coal Creek	4
Frazer Lake	5	Chilligan River	4
Little Kitoi	3	Packers Lake	4
Afognak Lake	3	Crescent Lake	4
Southcentral Alaska			
Mama/Papa Bear Lake	4	McArthur River	4
Bering Lake	3	Wolverine Creek	4
Eastern Gulf of Alaska			
Situk Lake	5,6	Delight Lake	3
Old Situk River	5	Eshamy Lake	3
East Alsek River	5,6	Coghill Lake	3
Klukshu Lake	7	Eyak Lake	3
Southeastern Alaska			
Sitkoh Lake	6	British Columbia	
Eva Lake	5	Lower Taku River	6
Kook Lake	5	Upper Taku River	6
Ford Arm Lake	5	Little Trapper	5,6
Redoubt Lake Beach	5	Little Tatsamenie	5,6,7
Redoubt Lake Outlet	5	Tahltan Lake	5,7
Redfish Lake	5	Bowser Lake	7,8
		Bonney Lake	7,8
		Damdochax Lake	7,8
		Alastair Lake	7,8
		Williams Creek	7,8
		McDonnell Lake	7,8
		Bear Lake	7,8
		Sustat River	7

continue...

Table 1. continued.

Site	Source	Site	Source*
Benzeman Lake	5	Johnson Creek	7
Chilkoot Lake	6	Nanika River	7,8
Chilkat Lake	6	Fulton River Channel	7
Lace River	5	Pierre Creek	7,8,9
Windfall Lake	6	Pinkut River Channel	7
Auke Lake	5,6	Morrison River	7,8
Steep Creek	1,5	Kimsquit Lake	7,8
Yehring Creek	6	Tenas Lake	7,8
Speel Lake	5,6	Owikeno Lake	7,8
Crescent Lake-SEAK	5	Fraser River	
Thoms Lake	5,6	Weaver Channel	7
Naha River	5,6	Birkenhead River	7
Hugh Smith Lake	5,6	Gates Channel	7
McDonald Lake	5,6	Chilko Lake	7
Kutlaku Lake	5,6	Stellako River	7
Alecks Lake	5,6	Nadina Channel	7
Red Bay Lake	5,6	Gluskie Creek	7
Salmon Bay Lake	5,6	Dust Creek	7
Karta River	5,6	Shale Creek	7
Luck Lake	6	Narrows Creek	7
Kegan Lake	6	Middle River	7
Klakas Lake	6	Adams River	7,8
		Shuswap River	7,9

*Sources:

- 1 W.J. Spearman, USFWS, Anchorage, AK (personal communication)
- 2 Varnavskaya et al. (1994)
- 3 W. Templin, ADF&G, Anchorage, AK (personal communication)
- 4 Seeb et al. (In Press)
- 5 C.M. Guthrie, ABL, Juneau, AK, (unpublished data)
- 6 Guthrie et al. (1994)
- 7 C. Wood et al. (1994)
- 8 C. Wood, CDFO, Nanaimo, BC (personal communication)
- 9 Winans et al. (1996)

creased from 18 (10 loci/4 isoloci) to 22 (14 loci/4 isoloci). The loci used were: *sAAT-1,2**, *mAAT-1**, *sAH-1**, *ALAT**, *GPIB-1,2**, *sIDHP-1**, *sIDHP-2**, *LDHB-1**, *LDHB-2**, *MPI**, *sMDHA-1,2**, *sMDHB-1,2**, *sMEP-1**, *mMEP-1**, *PEPLT**, *PGM-1**, *PGM-2**, and *sSOD-1**. Conditional maximum likelihood estimates (MLE) of stock composition collections were calculated for the two sockeye collections using the Statistics Program for Analyzing Mixtures developed by ADF&G (SPAM ver. 3.2, ADF&G 1997) based on the GIRLS (Masuda et al. 1991) and CONJA-S (Pella et al. 1996) algorithms. Standard errors of stock composition estimates were determined by 500 bootstrap resamplings of baseline and mixture samples (Efron and Tibshirani 1986). The reliability of stock composition estimates was evaluated by conducting simulation studies of the baseline using SPAM (ver. 3.2). Simulated baseline samples of sizes equal to actual baseline samples were generated by 500 bootstrap resamplings. Each simulated mixture ($n = 300$) was composed of 100% of the region under study, with each regional population contributing equally to the mixture. Genotypes of individuals in these hypothetical mixtures of known composition were generated from baseline

allele frequencies assuming independence of loci and Hardy-Weinberg equilibrium. The allocate sum procedure was followed, whereby estimated contributions of the individual populations were first calculated, and then summed into regional groups.

RESULTS

Ninety-eight percent of all immature salmon captured at Cape Prominence in August of 1998 were sockeye salmon (Carlson et al. 1998b). This was unexpected since immature chum salmon (*O. keta*) constituted the largest percentage of our immature salmon catch at the same location during August in 1996 (77%) and 1997 (66%) (Carlson et al. 1996, 1997).

Seventeen (11 loci/3 isoloci) of the loci used in the baseline were variable in the mixtures: *mAAT-1**, *sAH-1**, *ALAT**, *GPIB-1,2**, *LDHB-1**, *LDHB-2**, *sMDHA-1,2**, *sMDHB-1,2**, *sMEP-1**, *mMEP-1**, *PEPLT**, *PGM-1**, *PGM-2**, and *sSOD-1**. The mixture samples were variable at 22 additional loci (16 loci/3 isoloci) that were missing from the following baseline regions: Bristol Bay (*G3PDH-1,2**, *G3PDH-4**, *mIDHP-1**, *LDH-A2**, *PEPC**, *MAH-4**

and *PGDH**); British Columbia (*GPIA**, *PEPD-1**, *TPI-1,2**, *TPI-3**, and *TPI-4**); Bristol Bay and British Columbia (*MAH-1,2*, *MAN**, *CKA2**, *CKC2**, *FDHG**, *FH**, and *GAPDH-2**). *PGK-2** was also variable, but baseline data were only available from Southeast Alaska.

The correct allocation to region of origin of fish in the simulated mixture samples that contained fish from a single region (100% simulations) ranged from a low of 47.2% for Western Alaska to a high of 94.1% for the Fraser River (Table 2A). Results for the other regions were: Bristol Bay 72.0%, Alaska Peninsula 81.3%, Kodiak 74.1%, Southcentral Alaska 66.4%, Eastern Gulf of Alaska 61.8%, Southeast Alaska 65.7% and non-Fraser River British Columbia 71.8%.

Bristol Bay was the largest component of the estimates of origin by region for the immature sockeye salmon sampled near Cape Cheerful and Cape Prominence at 49.6% and 38.6%, respectively (Table 2B). Cape Cheerful also had statistically significant numbers of fish from the Alaska Peninsula (10.8%), southcentral Alaska (17.7%), and non-Fraser River British Columbia (1.7%). Cape Prominence had statistically significant numbers from Kodiak (10.9%), southcentral Alaska (18.7%), southeast Alaska

(14.0%), and British Columbia non-Fraser River (9.6%).

DISCUSSION

The largest contributing region to both collections of immature sockeye salmon was Bristol Bay (Table 2). This is not unexpected given Unalaska Island's proximity to Bristol Bay. The collection from Cape Prominence shows a greater contribution from southcentral and southeastern populations, perhaps because this location is closer to more southern populations than those collected from Cape Cheerful.

Simulation studies were conducted with the sockeye salmon baseline to evaluate the reliability of stock composition estimates. Western Alaska had the poorest reliability, while Fraser River had the highest (Table 2). Misallocation of the mixture samples could be caused by the absence of important populations from the baseline (i.e., Karluk and Copper rivers) or by the absence of highly polymorphic loci from the baseline (i.e., *PEPC**, *MAH1*, *2**). The majority of misallocation for the 100% simulation of western Alaska went to nearby regions; Bristol Bay (25.5%), and Alaska Peninsula (8.3%), and a surprising amount to southeast Alaska (10.1%). Reliability may be enhanced by using all 62 variable loci found

Table 2. (A) Mean estimated proportion of sockeye salmon by region for 500 bootstrap simulations where each region comprises 100% of the mixture (*n* = 300). Boldface numbers are the correct allocation and should equal one. (B) Regional origin estimates of sockeye salmon from Cape Cheerful and Cape Prominence. Standard deviations are in parentheses.

Estimated Region	(A) 100% Simulations					
	Western Alaska	Bristol Bay	Alaska Peninsula Peninsula	Kodiak	Southcentral Alaska	Eastern Gulf of Alaska
Western Alaska	0.4730 (0.1362)	0.0371 (0.0690)	0.0102 (0.0239)	0.0029 (0.0100)	0.0284 (0.0354)	0.0114 (0.0255)
Bristol Bay	0.2554 (0.1280)	0.7197 (0.1172)	0.0514 (0.0604)	0.0402 (0.0573)	0.1523 (0.1021)	0.0847 (0.0669)
Alaska Peninsula	0.0833 (0.0688)	0.0293 (0.0389)	0.8128 (0.0856)	0.0054 (0.0138)	0.0258 (0.0316)	0.0070 (0.0147)
Kodiak	0.0133 (0.0265)	0.0243 (0.0368)	0.0066 (0.0205)	0.7412 (0.1200)	0.0277 (0.0407)	0.0024 (0.0101)
Southcentral Alaska	0.1010 (0.0725)	0.0890 (0.0620)	0.0638 (0.0520)	0.1866 (0.1072)	0.6636 (0.1116)	0.1144 (0.0791)
Eastern Gulf of Alaska	0.0043 (0.0116)	0.0088 (0.0184)	0.0017 (0.0059)	0.0010 (0.0038)	0.0113 (0.0208)	0.6184 (0.1068)
Southeast Alaska	0.0398 (0.0395)	0.0411 (0.0456)	0.0344 (0.0406)	0.0123 (0.0196)	0.0450 (0.0366)	0.0825 (0.0540)
British Columbia Non-Fraser	0.0269 (0.0327)	0.0464 (0.0385)	0.0173 (0.0212)	0.0098 (0.0163)	0.0281 (0.0268)	0.0426 (0.0368)
British Columbia Fraser	0.0029 (0.0063)	0.0045 (0.0085)	0.0018 (0.0052)	0.0006 (0.0021)	0.0178 (0.0150)	0.0365 (0.0374)

Estimated Region	(A) 100% Simulations			(B) Origin Estimates	
	Southeast Alaska	British Columbia Non-Fraser	British Columbia Fraser	Cape Cheerful	Cape Prominence
Western Alaska	0.0132 (0.0281)	0.0088 (0.0263)	0.0004 (0.0022)	0.0204 (0.0328)	0.0035 (0.0725)
Bristol Bay	0.0889 (0.0740)	0.1397 (0.0971)	0.0030 (0.0068)	0.4958 (0.1529)	0.3860 (0.1900)
Alaska Peninsula	0.0785 (0.0708)	0.0116 (0.0230)	0.0008 (0.0029)	0.1057 (0.0983)	0.0703 (0.0923)
Kodiak	0.0056 (0.0134)	0.0047 (0.0136)	0.0002 (0.0012)	0.0625 (0.1030)	0.1088 (0.0539)
Southcentral Alaska	0.0833 (0.0602)	0.0539 (0.0472)	0.0191 (0.0197)	0.1771 (0.1655)	0.1871 (0.1274)
Eastern Gulf of Alaska	0.0111 (0.0187)	0.0038 (0.0113)	0.0023 (0.0074)	0.0146 (0.0343)	0.0099 (0.0422)
Southeast Alaska	0.6569 (0.0980)	0.0422 (0.0401)	0.0244 (0.0228)	0.0292 (0.0297)	0.1398 (0.0684)
British Columbia Non-Fraser	0.0450 (0.0402)	0.7182 (0.1025)	0.0092 (0.0153)	0.0733 (0.0526)	0.0760 (0.0748)
British Columbia Fraser	0.0175 (0.0260)	0.0172 (0.0169)	0.9407 (0.0341)	0.0167 (0.0162)	0.0086 (0.0122)

in sockeye salmon that are not currently available for all the baseline populations (Pella and Milner 1987). It was shown in Utter et al. (1992) that additional loci in the chinook salmon (*O. tshawytscha*) baseline allowed previously indistinguishable populations to be genetically separated. Simulation studies for the 36 loci (30 loci, 3 isoloci) 254 population chinook salmon baseline (Teel et al. 1999) showed a high reliability of discrimination for 29 regions (26 > 90%; 3 > 86%).

Genetic differentiation in sockeye salmon populations can resemble a mosaic, rather than a strong regional structure (Wood et al. 1994). For example, populations within a river system can be more easily identified than those among river systems, e.g., Kamchatka River in Russia, and the upper Taku River in British Columbia have high frequencies of the *LDHB2*115* allele while populations just east and west of the Taku River have a low frequency (Guthrie et al. 1994; Varnavskaya et al. 1994). Complicating matters are the two life histories of sockeye salmon; the "lake-type" and the "river/sea-type" (Gustafson and Winans 1999). A preliminary allozyme study (Gustafson and Winans 1999) has shown that the "river/sea-type" is a genetically cohesive group throughout a 2000 km geographic region relative to the mosaic genetic structure of the "lake-type" sockeye. Preliminary results on ages based on an analysis on preferred scales of immature sockeye salmon taken at both locations showed that the majority the salmon were ages 1.1 (~2/3) and 2.1 (~1/3) indicating these fish were "lake-type". Perhaps incorporating the life history information with the genetic data could improve the mixed fishery analysis; baseline populations with primarily "river/sea-type" age structure could be omitted from the analysis.

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