

**Status Report for Genetic Stock Identification Studies
of Pacific Rim Sockeye Salmon**

by

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ABSTRACT

Sockeye salmon populations from the Pacific Rim form mixed-stock aggregations during their ocean residency. Identification of sockeye salmon harvested from these mixtures has been an ongoing challenge for management agencies. Classification methods based on environmentally mediated traits such as scale pattern analysis or parasite load are only marginally useful. Substantial allozyme baselines have been constructed and genetic stock identification methods based on these baselines have been used successfully. We report on the progress of integrating these baselines. The current allozyme baseline will eventually include allozyme data for as many as 51 loci from over 40,380 individuals from 280 sites ranging across the Pacific Rim from Washington State to Russia.

INTRODUCTION

Sockeye salmon (*Oncorhynchus nerka*) from North America and Asia migrate into the North Pacific Ocean generally spending two to three years in the ocean before returning to their natal streams to spawn (Burgner 1991). The salmon form aggregations composed of numerous stocks during their ocean residency and freshwater migrations. Identification of stock compositions in mixtures of sockeye salmon caught in international waters, in the U.S. Exclusive Economic Zone, and in the major river systems leading to spawning tributaries, has been an ongoing challenge for fisheries biologists and management agencies throughout the Pacific Rim.

In Alaska, migrating sockeye salmon are harvested in fisheries including Bristol Bay, Alaska Peninsula, Kodiak Island, Cook Inlet, and Southeast Alaska. In addition, sockeye salmon have been harvested by foreign fishing vessels fishing illegally in international waters of the North Pacific (Wilmot et al. 1999, 2000). Identifying stock composition of fish within these legal and illegal fisheries is important to manage the fisheries, assess the effects of the fisheries on conservation, and to provide forensic information to law enforcement agencies.

Identifying stock compositions of mixed fisheries of sockeye salmon has been attempted with age and parasite composition analyses and genetic analyses. Age composition analyses have limited resolving power because of a lack of differences among stocks (Wilmot et al. 1999). Parasite composition data are limited by lack of differences especially within regions and by potential distribution changes in parasites since some studies were done 40 years ago (Wilmot et al. 1999). Although genetic data consisting of microsatellite, mtDNA, and minisatellite markers have been used to characterize the population genetic structure of sockeye salmon (Beacham et al. 1995; Seeb et al. 1998; Allendorf and Seeb 2000), only allozyme marker data exist from all areas of the Pacific Rim (Guthrie et al. 1994; Varnavskaya et al. 1994; Wood et al. 1994; Winans et al. 1996; Seeb et al. 2000). Allozymes have been used to successfully identify components of mixtures using genetic stock identification (GSI) methods in Cook Inlet, Alaska (Grant et al. 1980; Seeb et al. 2000). A limited set of allozyme loci has also been successfully used in GSI to identify the origin of sockeye salmon originating from throughout the Pacific Rim (Wilmot et al. 1999, 2000; Guthrie et al. 2000). Standardization of additional allozyme alleles and subsequent statistical analysis to determine reporting groups are needed to increase the power and assess the accuracy of the allozyme data to identify stock compositions of mixed fisheries throughout the Pacific Rim.

Developing a comprehensive for allozyme database from the North Pacific requires cooperation among all the laboratories collecting data on sockeye salmon. This database is in the process of being standardized among laboratories and will be maintained by National Marine Fisheries Service (NMFS; Auke Bay Lab (ABL), Juneau. We anticipate that it will eventually include allozyme data for as many as 51 loci from over 40,380 individuals from 280 sites ranging across the Pacific Rim from Washington State to Russia (Table 1). Data have been collected by the ABL, the NMFS Northwest Fisheries Science Center (NWFSC), Seattle, the Alaska Department of Fish and Game (ADF&G; Gene Conservation Lab (GCL), Anchorage); United

States Fish and Wildlife Service (USFWS; Alaska Fish and Wildlife Research Center (AFWRC), Anchorage); the Canadian Department of Fisheries and Oceans (CDFO; Pacific Biological Station (PBS), Nanaimo); and with assistance from the Pacific Research Institute of Fisheries and Oceanography in Kamchatka, Russia (Table 1). Additional data collection is ongoing by the GCL.

PROGRESS TO DATE

Baseline Collections:

To date, the majority of collections have been analyzed (Table 1) using 51 loci (Table 2). Fifty-two additional sockeye salmon collections from Western Alaska and the Alaska Peninsula will be analyzed by the GCL (Table 1) by the fall of 2002.

Allele Standardization Within and Among Laboratories:

Alleles have been standardized within each participating laboratory, and standardization among laboratories is in progress. The ABL has completed standardization of allozyme alleles between itself and the AFWRC and PBS laboratories. The GCL and ABL have initiated standardization among remaining laboratories, and progress for each allele is presented in Table 3. First priority is aimed at alleles appearing at a frequency at or above 0.05 within any single collection. Alleles will be pooled for statistical analyses under two conditions: 1) if mobility differences are not replicable within or among laboratories or 2) if differences in mobility are minor and differentiation among alleles would be too complex. NWFSC has submitted samples to GCL for some alleles (Table 3), and laboratory analysis of these samples is pending.

Statistical Analyses:

After allozyme alleles have been standardized across laboratories, reporting groups will be delineated and simulations conducted to assess the accuracy and precision of the stock composition estimates following the methods of Teel et al. (1999).

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Table 1. Collection sites, data sources, and sample sizes for collected for allozyme tissue collections of anadromous sockeye salmon from Pacific Rim. Sites ordered alphabetically within regions or drainages which are ordered from West to East. Sources followed by an asterisk (*) indicate that the tissue collection has been made, but allozyme data collection is pending.

Site	Source	N	Site	Source	N
Japan					
Abira River	12	80			
Russia					
Belia River	5	90	Kamchatka River	5	80
Bistraya River	3	56	Kamchatka River Early	3	80
Bolshaya River	3	28	Kamchatka River Late	3	119
Dvn-Yurta River	5	91	Kitilgina River Early	3	28
Etamink Bay	12	50	Kronoka Lake	12	100
Hairusova River	5	150	Kuril Lake	5	249
Hapiza River Early	3	100	North River	12	53
Hapiza River Late	3	81	Yelovka River	5	110
Irurup Island	12	60			
Norton Sound					
Salmon Lake	3*	100			
Kuskokwim Delta					
Goodnews Weir	1,3	174	Kagati River	1	50
Stoney River	1	75	Kanektok River	1	50
Togiak River					
Gechiak Lake	3*	100	Togiak River	1	50
Togiak Lake	3*	100			
Wood River					
Agulowok River	3*	100	Lake Kulik	3*	102
Agulupak River	3*	100	Lynx Creek	3*	100
Bear Creek	3*	150	Pick Creek	3*	100
Happy Creek	3*	107	Wood River	1	50

Table 1. continued.

Site	Source	N	Site	Source	N
Nushagak River					
Allen River	3*	100	Mulchatna River B	3*	65
Allen River Beach	3*	100	Nushagak River	1	50
Igushik River	1	50	Nuyakuk Lake (North Shore)	3	100
King Salmon River	3*	100	Nuyakuk Lake (South Shore)	3*	100
Klutapuk Creek	3*	100	Nuyakuk River	3*	100
Kogrukluk River	3*	100	Stuyahok River	3*	100
Koktuli River	3	100	Tikchik River	3*	100
Lake Chauekuktuli (north shore)	3*	100	Upper Nushagak River	3*	100
Mulchatna River A	3*	100			
Kvichak River					
Battle Creek	1,3*	150	Knutson Bay	3*	100
Bear Creek	3*	100	Knutson Creek	1	50
Belinda Creek	1	50	Kulik Creek	1,3*	151
Chinkelyes Creek	3	98	Lower Talarik Creek	1,3	220
Copper River	1,2	101	Moraine Creek	3*	100
Dennis Creek	3*	100	Nick N Creek	3*	100
Dream Creek	3*	103	Newhalen River	1	50
Finger Beach	3	100	Southeast Creek	3*	100
Fuel Dump Island Beach	2,3	148	Tazimina River	3*	150
Gibraltar Creek	1,2	109	Tommy Creek	3*	100
Gibraltar River	3	100	Triangle Island	3*	100
Iliamna River	1,2	109	Woody Island	2,3*	151
Kijik Lake	3	151			
Naknek River					
American River	3*	100	Margot Creek	1,3*	150
Headwater Creek	3*	100	Upatree Creek	1,3	150
Idavain Creek	3	100			
Egegik River					
Bear Creek	1	50	Featherly Creek	1,3*	150
Becharof Creek	3	100	Franks Creek	1	50
Bible Creek	1	50	Kejulik River	1,3*	150
Cabin Creek	3	100	Ruth River	1,3*	150
Cleo Creek	3*	100			

Table 1. continued.

Site	Source	N	Site	Source	N
Ugashik River					
Black Creek	1	50	Ugashik Narrows	1,3	150
Deer Creek	1,3*	150	Ugashik Creek	1,3*	149
Outlet Stream	1,3	150			
Northern Alaska Peninsula					
Bear River Late	3	100	Nelson River	3*	100
Bear River Early	1,3	175	Ocean River	3*	100
Hoodoo Lake (Sapsuk Lake)	1,3	150	Sandy Lake	3	100
Landlocked Creek	3*	72	Willie Creek	3*	81
Aleutian Islands					
Summer Bay Lake	3	100			
Southern Alaska Peninsula					
Alec River	3	100	Chignik River	3	100
Big Springs	3	100	Clark River October	3	100
Black Lake	3	100	Clark River September	3	100
Boulevard Creek	3	100	Fan Creek	3	100
Broad Creek	3	100	Hatchery Beach October	3	100
Chiaktuak Creek August	3	100	Hatchery Beach September	3	100
Chiaktuak Creek October	3	50	Orzinski Creek	3	100
Chiaktuak Creek September	3	94	West Fork	3	100
Kodiak/Afognak Islands					
Afognak Lake	3	80	Saltery Lake	3	200
Ayakulik River weir	3	100	Thumb River (lower) Late	3	100
Little Kitoi Lake	3	100	Thumb River (upper) Early	3	100
Little River Lake	3	100	Uganik Lake	3	100
Malina Creek	3	80	Upper Station (lower) Late	3	100
O'Malley River	3	100	Upper Station (upper) Early	3	200
Paul's Lake	3	70	Frazer Lake	11	223
Portage Lake	3	100			
Western Cook Inlet					
Beluga River	4	100	McArthur River	4	100
Chilligan River	4	150	Packers Lake	4	200
Coal Creek	4	200	Wolverine Creek	4	100
Crescent Lake	4	250			

Table 1. continued.

Site	Source	N	Site	Source	N
Susitna River					
Birch Creek	4	100	Red Shirt Lake	4	34
Byers Lake	4	100	Shell Lake	4	200
Chelatna Lake	4	200	Stephan Lake	4	150
Hewitt and Whiskey lakes	4	100	Susitna River sloughs	4	156
Judd Lake	4	200	Talkeetna River sloughs	4	79
Larson Creek	4	200	Trinity and Movie lakes	4	200
Mama and Papa Bear lakes	4	50	West Fork Yentna River	4	200
Knik Arm					
Big Lake	4	95	Jim Creek	4	100
Cottonwood Creek	4	100	Nancy Lake	4	100
Fish Creek	4	300	Sixmile Creek.	4	100
North East Cook Inlet					
Bishop Creek	4	100	Swanson River	4	100
Daniels Lake	4	200			
Kenai River					
Bear Creek	3*	100	Ptarmigan Creek	4	200
Btwn Kenai/Skilak lakes site 1	4	100	Quartz Creek	4	200
Btwn Kenai/Skilak lakes site 2	4	100	Railroad Creek	4	100
Btwn Kenai/Skilak lakes site 3	4	150	Russian River Weir Early	4	100
Btwn Kenai/Skilak lakes site 4	4	50	Russian River Weir Late	4	200
Btwn Kenai/Skilak lakes site 5	4	100	Russian River Below Falls	4	100
Btwn Kenai/Siklak lakes site 6	4	299	Russian Lake Upper, Shore	3*	100
Goat Creek	3*	100	Russian Lake Upper, Outlet	3*	100
Hidden Creek	4	200	Skilak Lake Outlet	4	800
Johnson Creek	4	88	Tern Lake	4	150
Moose Creek	4	200			
Kasilof River					
Bear Creek	4	200	Seepage Creek	4	100
Glacier Flats Creek	4	300	Tustumena Lake site 1	4	50
Moose Creek	4	200	Tustumena Lake site 2	4	50
Nikolai Creek	4	200	Tustumena Lake site 3	4	27

Table 1. continued.

Site	Source	N	Site	Source	N
South Kenai Peninsula					
Delight River	3	71	English Bay	3	300
Western Prince William Sound					
Coghill Lake	3	300	Main Bay Hatchery Eshamy	3	250
Eshamy Lake	3	100	Main Bay Hatchery Eyak	3	150
Main Bay Hatchery Coghill	3	400			
Eastern Prince William Sound					
Bering Lake	3	50	Eyak Lake	3	180
Cordova	3	31	McKinley Lake	3	100
Erb Creek	3	100	Miners Lake	3	100
Copper River					
Gulkana Hatchery Broodstock	3	16			
Southeastern Alaska					
Alecks Lake	5,6	154	Luck Lake	6	213
Auke Lake	5,6	216	McDonald Lake	5,6	407
Benzeman Lake	5	187	Naha River	5,6	384
Chilkat Lake	6	245	Old Situk River	5	106
Chilkoot Lake	6	137	Red Bay Lake	5,6	173
Crescent Lake	5,6	193	Redfish Lake	5	100
East Alsek River	5,6	178	Redoubt Lake Beaches	5	92
Eva Lake	5	100	Redoubt Lake Outlet	5	116
Ford Arm Lake	5	100	Salmon Bay Lake	5,6	258
Hugh Smith Lake	5,6	301	Sitkoh Lake	6	197
Karta River	5,6	302	Situk Lake	5,6	198
Kegan Lake	6	287	Speel Lake	5,6	200
Klakas Lake	6	159	Steep Creek	5,6	131
Kook Lake	5	100	Tahltan Lake	5,7	500
Kutlaku Lake	5,6	100	Thoms Lake	5,6	235
Lace River	5	59	Upper Taku River	6	421
Little Tatsamenie Lake	5,6,7	362	Windfall Lake	6	73
Little Trapper	5,6	308	Yehring Creek	6	217
Lower Taku River	6	80			

Table 1. continued.

Site	Source	N	Site	Source	N
British Columbia					
Alastair Lake	7,8	350	Morrison River	7,8	100
Bear Lake	7,8	152	Nanika River	7,8	126
Bonney Lake	7,8	280	Owikeno Lake	7,8	300
Bowser Lake	7,8	262	Pierre Creek	7,8,9	433
Damdochax Lake	7,8	299	Pinkut River Channel	7	1049
Fulton River Channel	7	1192	Sustat River	7	93
Johnson Creek	7	60	Swan Lake	7,8	100
Kimsquit Lake	7,8	100	Tenas Lake	7,8	100
Klukshu Lake	7	76	Williams Creek	7,8	279
McDonnell Lake	7,8	162			
Frasier River					
Adams River	7,8	100	Middle River	7	90
Birkenhead River	7	250	Nadina Channel	7	98
Chilko Lake	7	99	Narrows Creek	7	368
Dust Creek	7	101	Shale Creek	7	130
Gates Channel	7	99	Shuswap River	7,9	342
Gluskie Creek	7	42	Stellako River	7	100
Horsefly River	7,8	100	Weaver Channel	7	100
Long Lake	7	284			
Washington					
Baker Lake	9	80	Okanagan River	7,8,9	133
Cedar River	9	100	Ozette Lake	9	34
Lake Washington	9	100	Quinault River	10	93

Sources:

- 1 - W. J. Spearman, USFWS, Anchorage, AK (personal communication)
- 2 - Varnavskaya et al. (1994)
- 3 - Gene Conservation Lab, ADF&G, Anchorage, AK (unpublished data)
- 4 - Seeb et al. (2000)
- 5 - C. M. Guthrie, NMFS, Juneau, AK (unpublished data)
- 6 - Guthrie et al. (1994)
- 7 - Wood et al. (1994)
- 8 - C. Wood, CDFO, Nanaimo, BC (personal communication)
- 9 - Winans et al. (1996)
- 10 - G. A. Winans, NMFS, Seattle, WA (unpublished data)
- 11 - F. Bowers, ADF&G, Dutch Harbor, AK (personal communication)
- 12 - Winans and Urawa (2000)

Table 2. Stain protocols used by ADF&G to resolve enzyme coding loci in Alaska sockeye salmon samples. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	<i>sAAT-1,2*</i>	H	ACE 7.2
		<i>sAAT-3*</i>	E	TBCL
		<i>mAAT-1*</i>	H	ACE 7.2
		<i>mAAT-2*</i>	L	ACE 7.0
Aconitate hydratase	4.2.1.3	<i>mAH-1,2*</i>	H	ACE 7.2
		<i>mAH-4*</i>	H	ACE 7.2
		<i>sAH*</i>	L	ACE 7.0
Alanine aminotransferase	2.6.1.2	<i>ALAT*</i>	M	KG
Creatine kinase	2.7.3.2	<i>CK-A2*</i>	M	TBCLE
		<i>CK-B*</i>	E	ACE 7.0
		<i>CK-C1*</i>	E	ACE 7.0
		<i>CK-C2*</i>	E	ACE 7.0
Formalin dehydrogenase	1.2.1.1	<i>FDHG*</i>	L	TBE
Glyceraldehyde-3-phosphate	1.2.1.12	<i>GAPDH-2*</i>	H	ACE 7.0
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<i>G3PDH-1,2*</i>	M	ACE 7.0
		<i>G3PDH-3*</i>	H	ACE 7.0
		<i>G3PDH-4*</i>	H	ACE 7.0
Glucose-6-phosphate isomerase	5.3.19	<i>GPI-B1,2*</i>	M	TBCLE
		<i>GPI-A*</i>	M	TBCLE
L-Idditol dehydrogenase	1.1.1.14	<i>IDDH-1*</i>	L	TBCL
		<i>IDDH-2*</i>	L	TBCL

Table 2. continued.

Enzyme or Protein	Enzyme Number	Locus	Tissue	Buffer
Isocitrate dehydrogenase (NADP+)	1.1.1.42	<i>mIDHP-1*</i>	H	ACE 7.0
		<i>mIDHP-2*</i>	H	ACE 7.0
		<i>sIDHP-1*</i>	L	ACE 7.0
		<i>SIDHP-2*</i>	L	ACE 7.0
L-Lactate dehydrogenase	1.1.1.27	<i>LDHA-2*</i>	M	ACE 7.0
		<i>LDHB-1*</i>	M	TBCLE
		<i>LDHB-2*</i>	L	TBE
Malate dehydrogenase	1.1.1.37	<i>sMDH-A1,2*</i>	H	ACN 7.0
		<i>sMDH-B1,2*</i>	H	ACN 7.0
Malic enzyme (NADP+)	1.1.1.40	<i>mMEP-1*</i>	M	ACN 7.0
Mannose-6-phosphate isomerase	5.3.1.8	<i>MPI*</i>	L	TBE
Dipeptidase	3.4.13.18	<i>PEPA*</i>	M	TBCL
Tripeptide aminopeptidase	3.4.11.4	<i>PEPB-1*</i>	H	TBE
Peptidase-C	3.4.-.-	<i>PEPC*</i>	E	KG
Proline dipeptidase	3.4.13.9	<i>PEPD-2*</i>	H	TBE
Peptidase-LT	3.4.-.-	<i>PEPLT*</i>	M	TBCLE
Phosphogluconate dehydrogenase	1.1.1.44	<i>PGDH*</i>	L	ACE 7.0
Phosphoglucomutase	5.4.2.2	<i>PGM-1*</i>	H	ACE 7.2
		<i>PGM-2*</i>	M	TBCLE
Superoxide dismutase	1.15.1.1	<i>sSOD-1*</i>	L	TBE
Triose-phosphate isomerase	5.3.1.1	<i>TPI-1,2*</i>	E	KG
		<i>TPI-3*</i>	E	KG
		<i>TPI-4*</i>	E	KG

Table 3. Status of allozyme loci and variant alleles under standardization for a Pacific Rim sockeye salmon database. Loci previously used in mixed-stock analyses of seized vessels (Wilmot et al. 1999, 2000) and loci where incomplete data exist for British Columbia (B.C.) and Washington (WA) are indicated. Status notation: 1 – allele standardized between the GCL and the ABL; 2 – Allele did not meet minimum allele frequency criteria and was not standardized; 3 – Further investigation needed to standardize between GCL and ABL. Standardization between GCL and ABL and NWFSC is pending further investigation. Alleles for which samples have been sent from NWFSC to GCL are indicated with a carrot (^) after the allele in the status column.

Locus	Previously used	Incomplete B.C. / WA	Allele	Status
<i>mAAT-1*</i>	X		-83	1
<i>mAAT-2*</i>			-73	1
			-129	pooled w/*100
			-191	2
<i>sAAT-1,2*</i>	X		77	1
			122	1
<i>sAAT-3*</i>			117	3
<i>mAH-1,2*</i>		X	75	3
			133	1
			65	3
			111	3
<i>mAH-4*</i>			81	1
			114	1
			84	pooled w/*81
<i>sAH*</i>			117	1
			83	1
			75	pooled w/*83
			65	3
			125	1

Table 3. continued

Locus	Previously used	Incomplete B.C. / WA	Allele	Status
<i>ALAT*</i>	X		91	1
			108	1
			86	2
			95	1
<i>CK-A2*</i>		X	125	2
			86	2
<i>CK-B*</i>			102	2
<i>CK-C1*</i>			78	2
			135	2 [^]
<i>CK-C2*</i>		X	90	2
<i>FDH*</i>		X	108	pooled w/*100
			128	2
			54	2
<i>G3PDH-1,2*</i>			-150	1 [^]
			-175	pooled w/*150
			0	1
			-213	1
<i>G3PDH-3*</i>				
<i>G3PDH-4*</i>			108	1
			88	pooled w/*100
			76	1
<i>GAPDH-2*</i>		X	50	3
			208	3
<i>GPI-B1,2*</i>	X		132	1
			143	1
			-100	1

Table 3. continued

Locus	Previously used	Incomplete B.C. / WA	Allele	Status
<i>GPI-A*</i>		X	94	pooled w/*86
			107	1
			86	1
<i>mIDHP-1*</i>			33	1
			77	pooled w/*33^
<i>mIDHP-2*</i>				
<i>sIDHP-1*</i>	X		72	1
			84	1^
			61	1
			118	1
			94	pooled w/*100
<i>sIDHP-2*</i>	X		125	1
			92	2
			75	3
			115	pooled w/*100
			143	1
<i>LDH-A2*</i>			150	3^
<i>LDH-B1*</i>	X		123	3
			80	3
<i>LDH-B2*</i>	X		110	1
			85	2
			105	2
<i>PEPA*</i>			106	1^
			92	2
			88	3
<i>PEPB-1*</i>			130	1
			163	pooled w/*130

Table 3. continued

Locus	Previously used	Incomplete B.C. / WA	Allele	Status
<i>PEPC</i> *			105	1
			97	2
<i>PEPD-2</i> *		X	113	1
			94	2
			107	pooled w/*113
<i>PEPLT</i> *			88	1 [^]
			114	3
<i>sMDH-A1,2</i> *	X		64	1
			46	1
			147	1
<i>sMDH-B1,2</i> *	X		65	1
			120	3 [^]
			60	1
			116	3
			131	3
			105	2
			123	3
			128	3
<i>mMEP-1</i> *			125	1
			80	pooled w/*100
			58	1
<i>MPI</i> *	X		105	1
			91	1
			107	pooled w/*105
			96	1
<i>PGDH</i> *			90	1
			86	pooled w/*90
			107	1

Table 3. continued

Locus	Previously used	Incomplete B.C. / WA	Allele	Status
<i>PGM-1*</i>	X		null	1
			-180	1
			-80	1
<i>PGM-2*</i>	X		136	1
			186	1
			57	1
<i>sSOD-1*</i>	X		160	2
			48	1
			75	1
<i>TPI-1,2*</i>		X	54	3
			-173	3
			-82	3
<i>TPI-3*</i>		X	110	2
			98	3
<i>TPI-4*</i>		X	106	3
			97	3
			105	pooled w/*106^
			107	pooled w/*106