

Genetic Differentiation of Sockeye Salmon Subpopulations from a Geologically Young Alaskan Lake System

CARL V. BURGER¹ AND WILLIAM J. SPEARMAN²

U.S. Geological Survey, Biological Resources Division, Alaska Science Center
1011 East Tudor Road, Anchorage, Alaska 99503, USA

MATTHEW A. CRONIN

LGL Alaska Research Associates
4175 Tudor Centre Drive, Suite 202, Anchorage, Alaska 99508, USA

Abstract.—The Tustumena Lake drainage in southcentral Alaska is glacially turbid and geologically young (<2,000 years old). Previous field studies identified at least three subpopulations of sockeye salmon *Oncorhynchus nerka* at Tustumena Lake, based on the distribution and timing of spawners. The subpopulations included early-run salmon that spawned in six clearwater tributaries of the lake (mid August), lake shoreline spawners (late August), and late-run fish that spawned in the lake's outlet, the Kasilof River (late September). Our objective was to determine the degree of genetic differentiation among these subpopulations based on restriction enzyme analyses of the cytochrome *b* gene of mitochondrial DNA and analyses of four polymorphic allozyme loci. Mitochondrial DNA haplotype frequencies for outlet-spawning sockeye salmon differed significantly from those of all other subpopulations. The most common (36%) haplotype in the outlet subpopulation did not occur elsewhere, thus suggesting little or no gene flow between outlet spawners and other spatially close subpopulations at Tustumena Lake. Allele frequencies at two allozyme loci also indicated a degree of differentiation of the outlet subpopulation from the shoreline and tributary subpopulations. Allele frequencies for three tributary subpopulations were temporally stable over approximately 20 years (based on a comparison to previously published results) despite initiation of a hatchery program in two of the tributaries during the intervening period. Collectively, our results are consistent with the hypothesis that significant genetic differentiation has occurred within the Tustumena Lake drainage since deglaciation approximately 2,000 years ago.

The spawning habitats used by sockeye salmon *Oncorhynchus nerka* vary considerably and include tributaries flowing into lakes, lake shorelines, and outlet rivers from lakes (Burgner 1991). Also, some populations spawn in rivers without a nursery lake (Eiler et al. 1992). Considerable temporal variation in spawning exists among the various habitat types with earlier spawning commonly occurring in tributaries flowing into lakes than along the lake shorelines and outlets, which are used by late-run salmon (Narver 1968; Brannon 1987; Gard et al. 1987; Burgner 1991). A high level of precision in sockeye salmon homing behavior has been observed within these varying spawning habitats and times (Quinn 1985, 1993; Quinn et al. 1987).

Recent tagging studies have suggested at least three subpopulations of sockeye salmon at Tustumena Lake, a glacially turbid system in southcentral Alaska (Figure 1). Based on radio-tagging of 564 fish during a 3-year period (1989-1991), an average of 69% of the upstream migrants tagged in the lake's outlet (Kasilof River) spawned in tributaries flowing into the lake, and the remainder (31%) spawned along the lake's shoreline (Burger et al. 1995). Significant differences in the spawning time distributions between tributary (mid August) and shoreline (late August) spawners provided evidence of distinct subpopulations. The study also discovered a discrete late run (late August through mid-September) of sockeye salmon that spawned during late September in the lake's outlet river. During 1976-1995, spawning fish from two of the tributaries had been used as hatchery broodstock (described below), with offspring released into the tributaries or shoreline areas. A better understanding of the tributary, lake shoreline, and outlet subpopulations could be obtained from a genetic analysis. An earlier genetic study

¹ Present address: Abernathy Salmon Culture Technology Center, U.S. Fish and Wildlife Service, 1440 Abernathy Road, Longview, Washington 98632, USA.

² Present address: Division of Fishery Resources, U.S. Fish and Wildlife Service, 1011 East Tudor Road, Anchorage, Alaska 99503, USA.

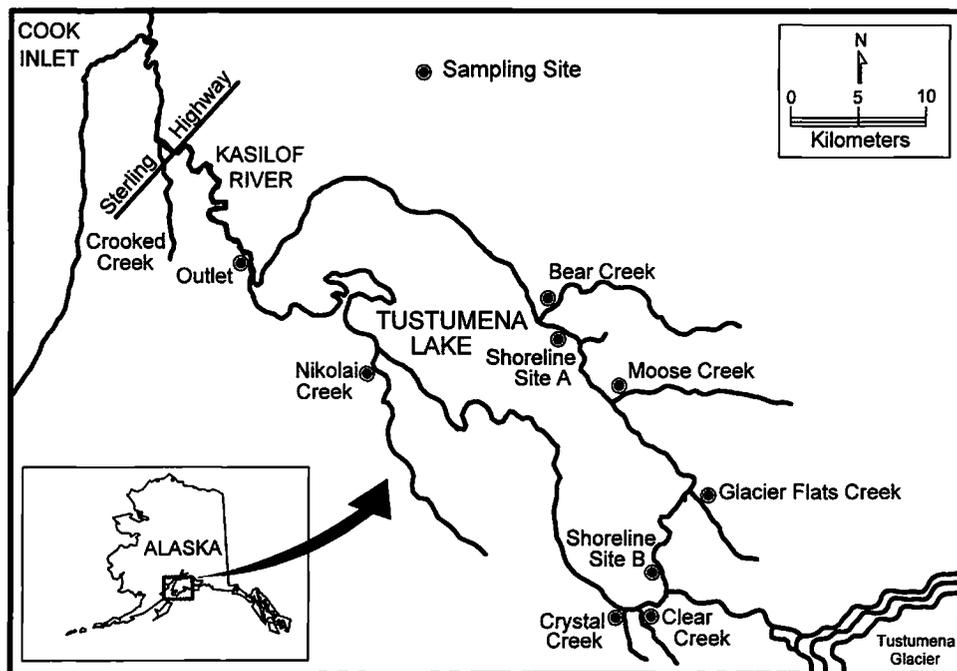


FIGURE 1.—Map of the study area showing sample locations for sockeye salmon in the Tustumena Lake drainage on the Kenai Peninsula in southcentral Alaska.

had been conducted on Tustumena tributary subpopulations (Grant et al. 1980), but the lake's shoreline and outlet were not sampled.

Assessment of genetic variability within and among subpopulations is an essential component of fishery management and is often useful in identifying distinct spawning groups (Allendorf et al. 1987). Biologists cannot rely on meristic and ecological differences among subpopulations to differentiate breeding groups; thus, genetic methods have become increasingly useful to discriminate stocks of fish (Wilmot and Burger 1985; Wilson et al. 1987). Because of the ability to analyze many genetically encoded loci and large numbers of fish, allozyme electrophoresis has been the most widely used technique to describe the population genetic structure of salmonid fishes (Allendorf and Phelps 1981; Gharrett et al. 1987; Utter et al. 1989). Recently, analysis of mitochondrial DNA (mtDNA; Cronin et al. 1993; Nielsen et al. 1994; Park and Moran 1995) variation using the polymerase chain reaction (PCR) has also been used to assess population genetic structure. Mitochondrial DNA is maternally inherited without recombination and, on average, evolves faster than nuclear genes (Lansman et al. 1981; Birky et al. 1983; Wilson et al. 1985). This difference may result in greater

spatial or temporal differentiation of mtDNA haplotype frequencies than for the nuclear genome (Birky et al. 1983). Analysis of mtDNA and allozymes together may improve the ability to discriminate runs of salmon that spawn under different ecological conditions or in different drainages (Adams et al. 1994).

Several researchers have used either mtDNA or allozyme analysis to describe the population structure and degree of differentiation of sockeye salmon subpopulations. On a broad scale encompassing the range of the species, molecular genetic techniques have been used to address prevalent theories on the origins and history of sockeye salmon colonization (Bickham et al. 1995; Wood 1995). On a smaller scale, Wilmot and Burger (1985) used allozyme analysis to show that early- and late-run sockeye salmon spawning in each of the Karluk and Russian river drainages (Alaska) were genetically differentiated. Varnavskaya et al. (1994) found the greatest genetic differentiation among sockeye salmon subpopulations that exhibited temporal and spatial segregation during spawning. Based on estimates of gene flow among lakes (Quinn et al. 1987) and hierarchical analyses of gene diversity within drainages (Wood 1995), nursery lakes have been suggested as appropriate

units for defining distinct sockeye salmon stocks (Wood et al. 1994). However, on a smaller geographic scale, appropriate units also may be individual spawning areas (for example, tributaries and lake outlets; Varnavskaya et al. 1994).

The objective of our study was to determine the degree of allozyme and mtDNA differentiation among sockeye salmon that spawn in the tributaries, shoreline areas, and outlet river of Tustumena Lake. Specifically, we wanted to (1) determine whether the ecological differences (temporal and spatial) observed between the subpopulations in the previous study (Burger et al. 1995) were accompanied by genetic differentiation; (2) assess whether any genetic evidence linked the present shoreline spawners at Tustumena Lake to an origin associated with the inception of hatchery enhancement (1976) in the drainage (see below); and (3) assess whether any temporal differences existed between our allozyme allele frequencies and those of other investigators (Grant et al. 1980; Seeb et al., in press) who had conducted genetic research on Tustumena sockeye salmon.

Study Site

Tustumena Lake (60°10'N, 150°55'W) is one of the largest producers of sockeye salmon on Alaska's Kenai Peninsula (Figure 1). Draining more than 1,375 km², this oligotrophic system is host to 140,000 to more than 500,000 sockeye salmon that may return to spawn in a given year (Kyle 1992). Although several clear-water tributaries drain into the lake, the lake and its outlet (Kasilof River) are glacially turbid (52 nephelometric turbidity units) and, although the lake is as deep as 320 m, its euphotic zone is limited to a depth of 1.1 m (Koenings and Burkett 1987; Lloyd et al. 1987). The turbidity results from the intrusion of meltwater from nearby Tustumena Glacier (Figure 1), presently located 7 km from the lake's southwest shoreline. The glacier, however, apparently protruded into the lake as recently as 2,000 years ago (Karlstrom 1964). Although sockeye salmon do not presently inhabit the unnamed river that drains the glacier, they spawn extensively in Bear, Glacier Flats, and Nikolai creeks and to a lesser degree in Moose, Crystal, and Clear creeks, from early to mid August (Figure 1; Burger et al. 1995). Sockeye salmon spawn in shoreline areas of Tustumena Lake during late August and in the lake's outlet from mid to late September (Burger et al. 1995).

Burger et al. (1995) noted that 20–40% of returning adult sockeye salmon counted by sonar in

the lake's outlet could not be accounted for during subsequent spawning ground surveys in the lake's clear tributaries. This discrepancy existed for several years (Kyle 1992) and suggested either substantial annual counting errors or that a large number of sockeye salmon were spawning in the lake itself. The latter possibility was confirmed through radio-tagging studies by Burger et al. (1995).

Hatchery-incubated sockeye salmon were released into Tustumena Lake beginning in 1976 (Kyle 1992) after a genetic analysis of tributary spawners sampled during summers of 1975 and 1976 had been made (Grant et al. 1980). Several hundred adult sockeye salmon spawners were captured from Glacier Flats Creek in most years and from Bear Creek in all years from 1976 to 1995. Eggs were artificially fertilized and incubated in a hatchery at nearby Crooked Creek (Figure 1). The resulting fry were released into Bear and Glacier Flats creeks from 1976 to 1985 but directly into the lake near the mouths of those two tributaries from 1986 onward. The lake release strategy possibly could have led to juvenile imprinting and subsequent colonization along the lake's shoreline by hatchery sockeye salmon. However, discrepancies between the sonar counts and stream surveys existed since the first use of sonar in the outlet in 1974, before the inception (1976) of the hatchery releases. These data suggested that shoreline spawners had been present before the hatchery program at Tustumena Lake began.

Methods

Skeletal muscle and caudal fin tissues were collected from sockeye salmon at known spawning areas in the Tustumena Lake drainage from early August through early September of 1992. Samples were obtained from actively spawning, partially spawned, or recently spent salmon in tributaries, shoreline areas, and the lake outlet. The tissues were frozen at -70°C until analyzed, except for caudal fin samples, which were air-dried at ambient temperature in the field and stored at -20°C in the laboratory. Early-run sockeye salmon were sampled with dip nets in Bear, Moose, Glacier Flats, Clear, Crystal, and Nikolai creeks about 1–2 km upstream of the lake from early to mid August. Shoreline spawners were captured with a gill net during late August at two major spawning areas in the lake (Burger et al. 1995; Figure 1). Late-run salmon were sampled via gill net in the upper Kasilof River (about 3 km below the outlet from Tustumena Lake) during early September. The peak spawning times of Tustumena drainage sock-

eye salmon had been determined in previous studies (Burger et al. 1995) and were confirmed during this sampling.

Mitochondrial DNA analyses were performed on caudal fin samples from the six Tustumena Lake tributaries, the two shoreline sites (A and B), and the lake outlet spawning area. We also analyzed populations (sampled during 1992) from Hidden Lake and Ptarmigan Creek in the nearby Kenai River system, from Bear River in the Bristol Bay region of western Alaska, and from the Kamchatka River, Russia.

Allozyme analyses were performed on skeletal muscle samples from a subset of the Tustumena drainage sockeye salmon analyzed for mtDNA that included the three tributaries most heavily used by sockeye salmon at Tustumena Lake (Bear, Glacier Flats, and Nikolai creeks), the two shoreline spawning sites (A and B), and the lake outlet (Figure 1).

MtDNA analysis.—Genomic DNA extraction was begun by incubating caudal fin samples in 500 μ L of salt-tris-EDTA (STE) buffer (0.1 M NaCl, 10 mM tris-HCl, 1 mM EDTA, pH 8.0), 50 μ L of 20% SDS (sodium dodecyl sulfate), and 10 μ L of proteinase-K (10 mg/mL) at 65°C for at least 30 min. Ammonium acetate (250 μ L of a 4°C, 7.5 M stock) was added, then the samples were incubated on ice for 1 h and centrifuged at 9,000 \times gravity (*g*) for 5–10 min. The supernatant was transferred to new tubes. Nucleic acids were precipitated with 1 mL of 95% ethanol and centrifuged at 9,000 \times *g* for 5–10 min. The 95% ethanol was decanted and the nucleic acid pellet was washed with 70% ethanol. Nucleic acid pellets were air-dried and resuspended in tris-EDTA (TE) buffer (10 mM tris, 1 mM EDTA, pH 7.6).

The mtDNA cytochrome *b* gene was amplified with PCR by using the following oligonucleotide primers:

LGL 765, 5'-GAAAAACCA(CT)
CGTTGT(TA)ATTCAACT-3'

and

LGL 287, 5'-GAGCTACTAGGGCAGGCTCA-3' (Bickham et al. 1995). The oligonucleotide base sequences are adenine (A), cytosine (C), guanine (G), and thymine (T).

Each PCR reaction was composed of 0.1–0.5 μ g genomic DNA; 5 μ L 10 \times buffer (0.1 M tris-HCl, pH 8.5; 0.025 M MgCl₂; 0.5 M KCl; 1 μ g/ μ L bovine serum albumin); 5 μ L deoxynucleotide triphosphate (dNTP) mix (2 mM each of dATP, dTTP,

dCTP, and dGTP, in 10 mM tris-HCl, pH 7.9); 5 μ L of a 2 μ M solution of each of the two primers; 1.25–2.50 units of *Taq* polymerase; and deionized water for a final volume of 50 μ L. The amplification reaction consisted of a one-cycle denaturation of 95°C for 4 min followed by 32 cycles of 95°C for 45 s, 50°C for 30 s, and 70°C for 2.5 min.

The PCR products were digested with restriction enzymes *Bfa* I, *Bsa* I, and *Bst* E II, run on 2% agarose gels with tris-boric acid-EDTA (TBE) and tris acetate-EDTA (TAE) buffers (Sambrook et al. 1989), stained with ethidium bromide, and photographed under ultraviolet light (wavelength = 312 nm). The sizes of restriction fragments were estimated by comparison with a standard (lambda phage DNA digested with *Hind* III or *Bst* E II). Restriction fragment patterns were visually identified from the gels and photos. Haplotypes were defined by the composite restriction fragment patterns for *Bst* E II and *Bfa* I (Bickham et al. 1995; Lansman et al. 1981).

Allozyme analysis.—Protein electrophoresis followed the techniques described by Aebersold et al. (1987) and Gall et al. (1989). Gene nomenclature and enzyme numbers (EC) follow the recommendations of the International Union of Biochemistry and Molecular Biology, Nomenclature Committee (IUBMBNC 1992) and Shaklee et al. (1990). Seven protein-coding loci were screened in skeletal muscle: *sAAT-1,2** for aspartate amino transferase (EC 2.6.1.1), *ALAT** for alanine amino transferase (EC 2.6.1.2), *ESTD** for esterase D (EC 3.1.*.*), *GPI-B1,2** for glucose-6-phosphate dehydrogenase (EC 5.3.1.9), *LDH-B2** for lactate dehydrogenase (EC 1.1.1.27), *sMDH-B1,2** for malate dehydrogenase (EC 1.1.1.37), and *PGM-2** for phosphoglucomutase (EC 5.4.2.2). Goodness-of-fit tests (Richardson et al. 1986) indicated that the observed allozyme variation at the duplicated locus *GPI-B1,2** could be described by a single locus model, so the variation was assigned to *GPI-B1** for analyses.

Data analysis.—Allozyme genotype frequencies were evaluated for Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test (Richardson et al. 1986). Hierarchical tests of homogeneity using the log-likelihood ratio statistic (*G*; Sokal and Rohlf 1981) assessed differentiation of frequencies of allozyme alleles and mtDNA haplotypes among sampling locations. Frequencies were considered significantly different at $P < 0.05$. Genetic relationships among sampling sites also were assessed with neighbor-joining (NJ; Saitou and Nei 1987)

TABLE 1.—Mitochondrial DNA cytochrome *b* fragment patterns (haplotypes A–C) of sockeye salmon from the Tustumena Lake drainage, Alaska, for restriction enzymes *Bfa* I and *BstE* II. Data are numbers of base pairs in fragments.

Fragment pattern		
A	B	C
<i>Bfa</i> I		
747	658	
222	222	572
93	93	174
67	84	93
	67	67
<i>BstE</i> II		
1,267	840	
	373	

cluster analysis of matrices of pairwise Cavalli-Sforza and Edwards (1967) chord genetic distances. Probabilities for the nodes of the resulting majority-rule consensus dendrograms were derived from bootstrap analyses (500 replicates) by using PHYLIP 3.5c software (Felsenstein 1994). The Kamchatka River, Russia, was used as an outgroup for the NJ analysis by using mtDNA data from this study and allozyme data of Varnavskaya et al. (1994).

To evaluate the temporal stability of allozyme variation, allele frequencies for *ALAT**, *GPI-B1**, *LDH-B2**, and *PGM-2** from our study were compared with those reported in two other studies of sockeye salmon from Bear, Glacier Flats, and Nikolai creeks. Grant et al. (1980) obtained data from samples collected during two field seasons (1975 and 1976), and Seeb et al. (in press) provided data for 3 years (1992–1994). For each creek and each locus, we compared allele frequencies (*G*-tests of homogeneity; Sokal and Rohlf 1981) from the resulting three data sets, with data from multiple years for each creek and locus being pooled within each of the 1975–1976 and 1992–1994 data sets.

Results

Mitochondrial DNA

The mtDNA cytochrome *b* PCR product was 1.2 kilobases (kb) in length, as reported by Bickham et al. (1995). Polymorphic restriction fragment patterns were observed for all three restriction enzymes. However, only *Bfa* I and *BstE* II data were used for analysis (Table 1), as the *Bsa*I patterns

TABLE 2.—Mitochondrial DNA cytochrome *b* haplotype frequencies of sockeye salmon from nine sites in the Tustumena Lake drainage and four distant locations; *N* = number of fish sampled.

Location and population	<i>N</i>	Frequency for haplotype:			
		AA	BA	AB	AC
Tustumena Lake drainage					
Tributaries					
Bear Creek	48	0.83	0.17		
Moose Creek	50	0.90	0.10		
Glacier Flats Creek	50	0.94	0.06		
Clear Creek	55	0.89	0.11		
Crystal Creek	48	0.85	0.15		
Nikolai Creek	60	0.83	0.17		
Shoreline					
Site A	58	0.81	0.19		
Site B	49	0.90	0.10		
Outlet					
Kasilof River	47	0.32	0.28	0.04	0.36
Other drainages					
Kenai River					
Ptarmigan Creek	15	0.60	0.27	0.13	
Hidden Lake	15	0.60	0.07	0.33	
Bristol Bay					
Bear River	15	0.33	0.67		
Russia					
Kamchatka River	14	0.07	0.93		

varied consistently with those for *Bfa* I and did not enhance resolution of genetic differentiation. Restriction enzyme *Bfa* I recognized the nucleotide sequence CTAG and *BstE* II recognized GGTNACC. Digestion of the cytochrome *b* mtDNA segment with *BstE* II resulted in a single 1.2-kb fragment (no *BstE* II restriction site) or two fragments approximately 0.840 and 0.373 kb (one *BstE* II restriction site). Digestion with *Bfa* I resulted in three different fragment patterns with four (pattern A) or five (patterns B and C) fragments (Table 1). Haplotypes were identified by the composite fragment pattern for *BstE* II and *Bfa* I. The first letter of the haplotype designation refers to the fragment pattern for *BstE* II and the second letter for *Bfa* I (e.g., a fish with fragment pattern A for *BstE* II and fragment pattern B for *Bfa* I has haplotype AB). Four haplotypes were identified: AA, BA, AB, AC (Table 2).

Sequence analysis (Bickham et al. 1995) of portions of the mtDNA cytochrome *b* gene showed the location of the single *BstE* II site and two of the *Bfa* I sites, which define all of our haplotypes except haplotype AC. Haplotype AC was not identified by Bickham et al. (1995) and resulted from a new *Bfa* I site created by a G to A transition at position 15436 (J. C. Patton, LGL Ecological Genetics, Inc., unpublished data). This haplotype

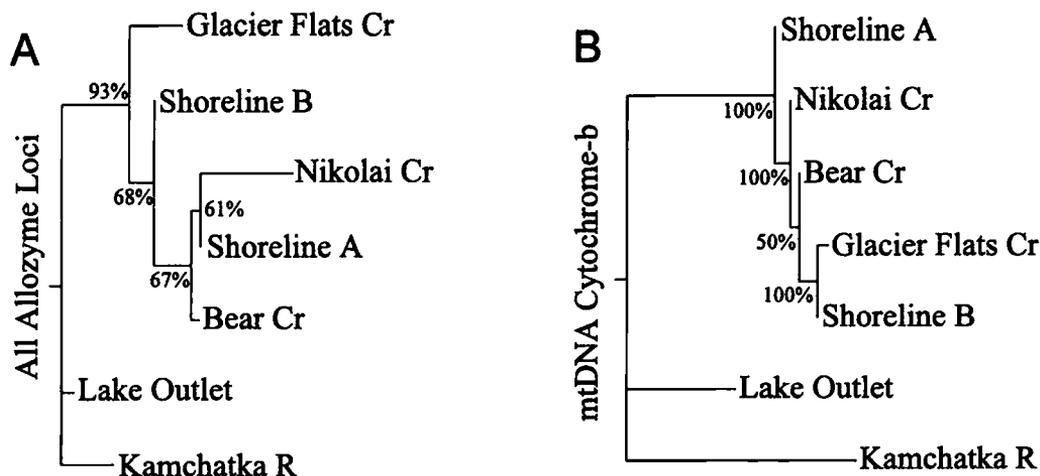


FIGURE 2.—Neighbor-joining dendrograms for (A) combined allozyme loci and (B) mitochondrial DNA cytochrome *b*, showing relationships among six populations of sockeye salmon from the Tustumena Lake drainage, Alaska, and the Kamchatka River (used as an outgroup). Percentages show results of consensus analysis based on 500 bootstraps. See Appendix for pairwise distances.

(and hence this nucleotide substitution) occurs only in the lake outlet subpopulation (Table 2). For our samples, there may be additional DNA sequence variation, which was undetected in our analysis with only three restriction enzymes, but our method allowed for a relatively rapid analysis and a minimum estimate of variation (Cronin et al. 1993; Adams et al. 1994).

The mtDNA haplotype frequencies were generally similar among the tributary and shoreline subpopulations of the Tustumena Lake system, whereas the outlet subpopulation was differentiated from the shoreline and tributary subpopulations (Tables 2, 3; Figures 2, 3). This distinction was due primarily to the unique occurrence of the AC haplotype in relatively high frequency (36%) in the outlet subpopulation. Haplotype AB was also absent from the shoreline and tributary subpopulations and occurred in 4% of the outlet subpopulation. In addition, haplotype AA occurred in lower frequency and haplotype BA occurred in higher frequency in the outlet subpopulation than in the shoreline and tributary subpopulations.

Hierarchical tests of homogeneity (Table 3) showed no significant differences in mtDNA haplotype frequencies among the Tustumena Lake tributaries, between the two shoreline spawning areas, or among the tributaries and shoreline areas. However, a highly significant ($P < 0.001$) difference was observed among the outlet, tributary, and shoreline spawners (Table 3). This result indicates considerable genetic differentiation of the outlet

spawners from all other Tustumena Lake sockeye salmon, as indicated in the NJ dendrogram for mtDNA (Figure 2B). The 100% bootstrap value for the node separating the outlet from the tributaries and shoreline in the NJ dendrogram gives strong support to this result.

We compared the mtDNA haplotype frequencies of the Tustumena sockeye salmon subpopulations with others from a wide geographic distribution including the Kenai River and Bristol Bay in Alaska and the Kamchatka River in Russia. As indicated in the NJ dendrogram (Figure 3), differentiation among these groups was greater than within the Tustumena system (Tables 2, 3). Significant differentiation of mtDNA haplotype frequencies was observed between the Kenai River and the Bristol Bay and Russian samples and among all sample locations (Table 3). The mtDNA haplotype AB, which was rare in the lake outlet subpopulation (Kasilof River) and absent in the shoreline and tributary subpopulations, was fairly common in the two Kenai River subpopulations (13% and 33%; Table 2). This distribution was reflected in the two Kenai River subpopulations (Ptarmigan Creek and Hidden Lake) clustering together in the NJ analysis (Figure 3). The Bristol Bay (Bear River) and Russian (Kamchatka River) subpopulations clustered together and had relatively high frequencies of haplotype BA (0.67 and 0.93; Table 2) compared with the Kenai and Tustumena subpopulations (range, 0.06–0.28).

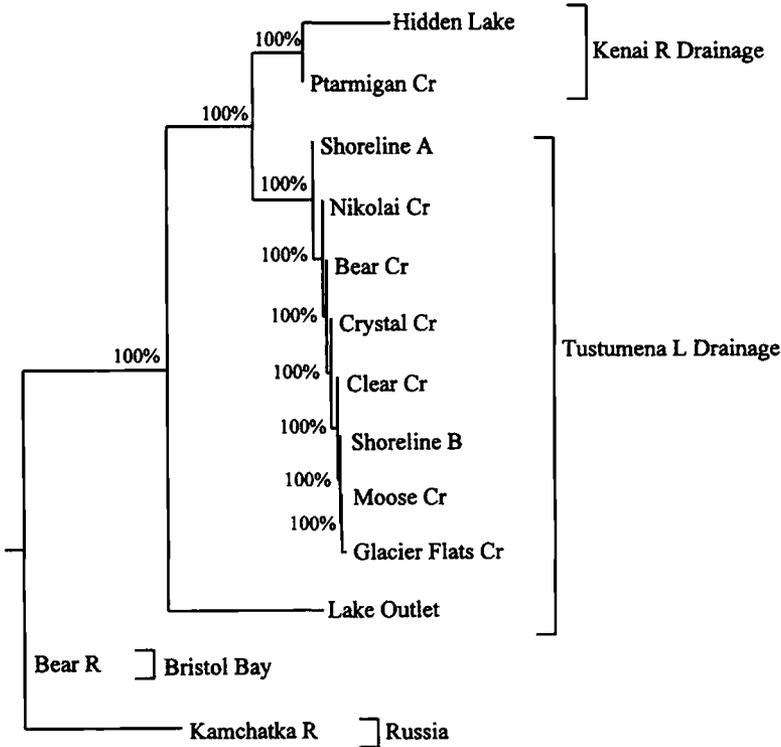


FIGURE 3.—Neighbor-joining dendrogram for mitochondrial DNA cytochrome *b* showing relationships among sockeye salmon from the Tustumena Lake drainage, Alaska, and other locations. (Kamchatka River sockeye salmon were used as an outgroup.) Percentages show results of consensus analysis based on 500 bootstraps. See Appendix for pairwise distances.

Allozymes

Mean heterozygosities, H (SE), for the seven allozyme loci were: Bear Creek, $H = 0.191$ (0.088); Glacier Flats Creek, $H = 0.194$ (0.090);

TABLE 3.—Hierarchical tests of homogeneity (G ; Sokal and Rohlf 1981) for mitochondrial DNA cytochrome *b* haplotypes of sockeye salmon from the Tustumena Lake drainage, Alaska, and other locations. An asterisk (*) indicates a statistically significant value.

Test	G	df	P
Among tributaries	4.52	5	0.477
Between shoreline sites	1.65	1	0.199
Among tributaries and shoreline sites	6.57	7	0.475
Among tributaries, shoreline sites, and outlet	118.53	24	0.001*
Between Kenai River sites	3.26	2	0.196
Among outlet and Kenai River sites	29.94	6	0.001*
Between Bristol Bay and Russian sites	3.27	1	0.071
Among Kenai River, Bristol Bay, and Russian sites	35.031	6	0.001*
Among all groups	215.10	36	0.001*

Nikolai Creek, $H = 0.229$ (0.092); shoreline site A, $H = 0.162$ (0.079); shoreline site B, $H = 0.157$ (0.075); and lake outlet spawners, $H = 0.191$ (0.088). Four of the seven allozyme loci were polymorphic: *ALAT**, *GPI-B1**, *LDH-B2**, and *PGM-2**. The observed relative mobilities of homomeric allozymes corresponded with those reported by Guthrie et al. (1994). Three alleles were detected for *ALAT** and two alleles were detected for each of the other three polymorphic loci. All nine alleles were present in each of the six subpopulations analyzed (Table 4).

Allozyme allele frequencies were generally similar among the six sampling locations (Table 4). Tests of homogeneity of the allele frequencies showed significant differences among the three tributaries for *GPI-B1** and between the two shoreline spawning areas for *LDH-B2** (Table 5). There were nonsignificant but nonoverlapping differences between the tributary and shoreline spawners (*LDH-B2** and *PGM-2**) and among tributary, shoreline, and outlet spawners (*LDH-B2** and *GPI-B1**). Nonoverlapping allele frequencies between

TABLE 4.—Allele frequencies for four polymorphic allozyme loci and up to six populations of sockeye salmon from the Tustumena Lake drainage, Alaska. Sample sizes (*N*) apply to all loci examined for a given population.

Location and population	<i>N</i>	<i>ALAT*</i>			<i>GPI-BI*</i>		<i>LDH-B2*</i>		<i>PGM-2*</i>	
		*100	*95	*91	*100	*132	*100	*115	*-100	*-93
Present Study										
Tributaries										
Bear Creek	47	0.564	0.096	0.340	0.968	0.032	0.840	0.160	0.574	0.426
Glacier Flats Creek	50	0.490	0.210	0.300	0.980	0.020	0.810	0.190	0.610	0.390
Nikolai Creek	57–58	0.561	0.123	0.316	0.897	0.103	0.836	0.164	0.647	0.353
Shoreline										
Site A	50	0.540	0.110	0.350	0.960	0.040	0.850	0.150	0.760	0.240
Site B	49	0.490	0.143	0.367	0.959	0.041	0.939	0.061	0.653	0.347
Outlet										
Kasilof River	37–44	0.386	0.148	0.466	0.977	0.023	0.795	0.205	0.640	0.360
Previous Studies										
Tributaries										
Bear Creek ^a	200	0.575	0.102	0.323	1.000	0.000	0.887	0.113	0.640	0.360
Bear Creek ^b	199–200	0.548	0.111	0.342	0.942	0.058	0.870	0.130	0.668	0.333
Glacier Flats Creek ^a	200	0.613	0.115	0.272	1.000	0.000	0.917	0.083	0.656	0.344
Glacier Flats Creek ^b	298–300	0.549	0.143	0.309	0.972	0.028	0.873	0.127	0.678	0.322
Nikolai Creek ^a	159	0.597	0.123	0.280	0.953	0.047	0.887	0.113	0.670	0.330
Nikolai Creek ^b	199–200	0.558	0.136	0.307	0.938	0.062	0.883	0.118	0.688	0.313

^a As reported by Grant et al. (1980).

^b As reported by Seeb et al. (in press).

outlet and combined tributary and shoreline samples occurred for *ALAT** and *PGM-2**. This difference, which was significant for *ALAT**, was reflected in the distinct clustering of the outlet fish (Figure 2A).

Comparison of our results with two other allozyme data sets from Tustumena Lake sockeye salmon included four loci and three sampling locations that were common to all three studies (Table 4). Allele frequencies were generally similar for each study, but we compared frequencies for each location among years. Of 12 tests of homogeneity, three were significant including *GPI-BI** in Bear Creek ($G = 32.595$, $df = 2$, $P = 0.001$), *GPI-BI** in Glacier Flats Creek ($G = 17.655$, $df = 2$, $P = 0.001$), and *LDH-B2** in Glacier Flats Creek ($G = 10.045$, $df = 2$, $P = 0.007$).

Discussion

Our results suggest that a genetically distinct subpopulation of sockeye salmon spawns in the outlet (Kasilof River) of Tustumena Lake. The high frequency (36%) of the mtDNA haplotype AC in the outlet-spawning subpopulation, and its absence in any of the other subpopulations at Tustumena Lake, indicates a considerable restriction of gene flow. The distinct *ALAT** distribution reflected in the NJ analysis of allozymes also suggests differentiation of the outlet spawners from fish spawning in tributaries and along the shoreline (Figure 2A). These findings support a previous study (Burger et al. 1995) that documented a separate subpopulation of outlet spawners based on temporal and spatial differences during spawning.

TABLE 5.—Hierarchical tests of homogeneity (*G*) for four allozyme loci and six subpopulations of sockeye salmon from the Tustumena Lake drainage, Alaska. An asterisk next to the *P* value indicates a statistically significant difference.

Test	<i>ALAT*</i>			<i>GPI-BI*</i>			<i>LDH-B2*</i>			<i>PGM-2*</i>		
	<i>G</i>	<i>df</i>	<i>P</i>	<i>G</i>	<i>df</i>	<i>P</i>	<i>G</i>	<i>df</i>	<i>P</i>	<i>G</i>	<i>df</i>	<i>P</i>
Among tributaries	5.67	4	0.226	8.42	2	0.015*	0.38	2	0.827	1.14	2	0.565
Between shoreline sites	0.71	2	0.702	<0.01	1	0.977	4.24	1	0.039*	2.74	1	0.098
Among tributaries and shoreline sites	7.33	8	0.501	8.98	4	0.062	8.86	4	0.065	8.65	4	0.071
Between outlet and tributaries plus shoreline sites	6.79	2	0.034*	1.44	1	0.231	1.87	1	0.172	0.02	1	0.896
Among tributaries, shoreline sites, and outlet	14.07	10	0.170	10.41	5	0.064	10.73	5	0.057	8.68	5	0.123

It also is similar to a study of Kasilof River chinook salmon *O. tshawytscha* that showed significant mtDNA differentiation of early- and late-run spawners (Adams et al. 1994). The significant mtDNA differentiation of early- and late-run salmon of two species in the same drainage may indicate similar colonization histories or selection pressures.

Genetic differentiation among sockeye salmon within drainages has been detected with allozyme analysis (Wilmot and Burger 1985; Varnavskaya et al. 1994; Wood et al. 1994), but comparatively fewer studies have been conducted with mtDNA analysis (Bickham et al. 1995). Our results show substantial differentiation of mtDNA haplotype frequencies between subpopulations (outlet spawners versus shoreline and tributary spawners) that reproduce in temporally and spatially close spawning habitats. Sockeye salmon that reproduce in the lake's outlet have a spawning time that differs from shoreline and tributary spawners by only about 4–6 weeks, and the spawning areas of the Tustumena subpopulations we analyzed are, on average, less than 30 km apart. The unique haplotype we report for the outlet-spawning fish was found in no other subpopulation of sockeye salmon we analyzed, despite a wide geographic sampling. The greater differentiation of the outlet subpopulation revealed by mtDNA versus allozymes may be a consequence of a faster rate of evolution of the mitochondrial than the nuclear genome, despite observations that the cytochrome *b* gene is among the most conserved and slowest evolving in the mtDNA molecule (Brown 1985).

The marginal degree of allozyme differentiation between tributary and shoreline spawners ($P \leq 0.071$ for three of four loci; Table 5) was not nearly as great as that exhibited by the outlet spawners. However, because one of four allozyme loci showed statistically significant differences among the tributaries, gene flow may also be restricted among these streams. Further study with a wider range of loci or more sensitive genetic markers, such as microsatellites or minisatellites (Beacham et al. 1995; Park and Moran 1995), may better resolve the relationships among sockeye salmon runs in Tustumena Lake tributaries.

Because hatchery supplementation can affect wild salmonid populations (Utter et al. 1993; Holland-Bartels et al. 1994), we wanted to assess whether there was genetic evidence for an origin of the shoreline spawners in Tustumena Lake from hatchery practices that released fry in two of the lake's tributaries (1976–1985) and directly into the

lake (1986–present). There was little differentiation between the two shoreline subpopulations and the tributaries (Bear and Glacier Flats creeks) used as hatchery donors from 1976 to present (Figure 2). Also, comparison of our data with those provided by other investigators suggested little temporal change in allele frequencies during about 20 years among Tustumena sockeye salmon subjected to hatchery supplementation (Table 4). However, sonar counts and tributary surveys (Kyle 1992) suggested that shoreline spawners might have been present in the lake when hatchery enhancement commenced in the mid-1970s. Sonar had counted more fish entering the lake than could be accounted for in the tributary surveys alone. In concert, results were insufficient to determine whether hatchery egg-take and release programs, initiated at Bear and Glacier Flats creeks, contributed to colonization of shoreline habitat by sockeye salmon at Tustumena Lake. Perhaps because sockeye salmon indigenous to Tustumena Lake (those from Bear and Glacier Flats creeks) were used by the hatchery program (in accordance with a State of Alaska fish genetics policy), there were few discernible genetic effects of the hatchery releases on other spawners in the drainage. Nevertheless, future hatchery supplementation plans in the drainage should consider that multiple subpopulations may exist that could be affected by new hatchery release strategies. Our results also suggest that previously established genetic baselines can remain temporally stable, enhancing the value of the baselines for application in genetic stock identification programs. The observed level of variation among year-classes lies within a range yielding reliable estimates of mixed-stock composition when applied to fisheries of other Pacific salmon species (Waples and Teel 1990; Brodziak et al. 1992).

Outlet Spawner Colonization and Evolution

The genetic differentiation of the outlet-spawning sockeye salmon from those spawning in tributary and shoreline areas of Tustumena Lake might have resulted from separate colonization and founder effects or from common colonization followed by restriction of gene flow and genetic drift. Gene flow from the outlet to the lake is apparently restricted, particularly because the mtDNA haplotype that was abundant in fish in the outlet was absent in sockeye salmon from either the lake or its tributaries. Because mtDNA is maternally inherited, this is specifically restriction of female-mediated gene flow. Male-mediated gene flow may be occurring. Also, the possibility of gene flow

from the lake to the outlet cannot be excluded. Although the presence of the unique AC haplotype in the outlet spawners seems to also suggest a separate colonization event, this haplotype might have been present in all subpopulations, eventually disappearing from the tributary and shoreline spawners through genetic drift or natural selection. However, our inability to detect haplotype AC in other geographically distant subpopulations indicates its overall rarity. This haplotype is defined by a single nucleotide substitution that might have occurred after colonization in the outlet subpopulation.

As glaciers recede, cold, turbid meltwater is discharged into downstream areas (such as lakes and main-stem rivers) as the drainage's lateral tributaries slowly begin to clear and warm. When no longer influenced by the glacier, the tributaries transport relatively clear surface runoff that is likely to be warmer than meltwater from the receding glacier. Also, lateral tributaries are comparatively smaller and shallower in surface area than are lakes and outlet rivers—factors that may contribute to the rate of tributary warming after glacial recession and to colonization by spawning sockeye salmon. Colonization of the lake and river outlet (commonly formed by a retreating glacier) would probably occur much later, depending on the rate of recession and the degree of warming that occurred. Consistent with this line of reasoning, cold water temperatures ($<1^{\circ}\text{C}$) persist in the 7-km unnamed river draining Tustumena Glacier during summer (Burger et al. 1995). This drainage has not yet been colonized by sockeye salmon (the extremely cold water temperature is a likely factor), and it was never used by any of the radio-tagged fish in a previous study (Burger et al. 1995). Such observations support an argument that use of outlet and shoreline spawning habitat probably occurred later than colonization of the lake's lateral tributaries because the shoreline and outlet were among the last habitats to warm as the glacier retreated from the southwest portion of Tustumena Lake.

Water temperature seems to be an important determinant in salmonid spawning time, which in some populations can be altered by thermal manipulation (Morrison and Smith 1986). The warmer temperatures observed in the spawning areas used by some late-run sockeye salmon (Brannon 1987) and the moderating effects of lakes on outlet rivers (Carmack et al. 1979) might have led to selection for later spawning times in outlet-spawning salmon. This temporal difference may be a factor in the differentiation of the outlet spawners

and other subpopulations at Tustumena Lake. After many years of deglaciation, a lake's outlet river eventually becomes considerably warmer than its lateral tributaries. In Kamloops Lake, British Columbia, for example, a 2-year study recorded a peak summer temperature of 15°C in the lake's inlet, whereas water temperature peaked at about 17°C in the lake's outlet (Carmack et al. 1979). At Tustumena Lake, summer temperatures in the tributaries peak at 11°C (late July), whereas those in the lake's outlet peak in early September at up to 13.5°C (C. Burger, unpublished data). Thus, the late-run outlet spawners reproduce in an area directly influenced (warmed) by a large lake. Late-run sockeye salmon in Alaska's Russian and Karluk rivers also spawn in lake outlets (water temperature peaked at 14°C in a Karluk Lake tributary and at 17°C in the outlet; C. Burger, unpublished data), and these fish are genetically differentiated, based on allozyme analyses (Wilmot and Burger 1985). Late-run chinook salmon in the Kenai (Burger et al. 1985) and Kasilof rivers (Faurot and Jones 1990) spawn later than other subpopulations in southcentral Alaska, and both spawn in lake outlet rivers where warming influences are greatest. A recent study on Kenai and Kasilof river chinook salmon by Adams et al. (1994) demonstrated significant genetic differences between early- and late-run forms in each river. As in our study, those researchers found that the most common mtDNA haplotype among late-run chinook salmon (59% of fish) occurred in only 9% of early-run fish. Thus, late runs of two species (chinook salmon and sockeye salmon) each spawn in the upper Kasilof River just downstream from the warming influence of Tustumena Lake, and each is genetically (mtDNA) differentiated from its early-run cohort subpopulations.

Our results suggest that there is a genetic basis to the ecological differences observed in the spawning times and areas of early (tributary) and late (outlet) forms of sockeye salmon. Like Wood (1995), who determined a very low effective straying rate ($<1\%$) between ecologically different forms of sockeye salmon, our results imply little straying by outlet spawners at Tustumena Lake despite the fact that outlet-spawning sockeye salmon reproduce within 5–40 km of tributary and shoreline spawners. This difference suggests that locally adapted, somewhat isolated subpopulations of sockeye salmon can occur within very small temporal and spatial scales. Sockeye salmon have been known to colonize new habitat quickly after glacial recessions (Milner and Bailey 1989). Our

results suggest that genetic differentiation also can occur within a short (<2,000 years) geologic time frame.

Acknowledgments

We thank Leslie Holland-Bartels, Eric Knudsen, and Kim Scribner (U.S. Geological Survey, Biological Resources Division, BRD) and Jim and Lisa Seeb (Alaska Department of Fish and Game) for reviewing earlier drafts of this manuscript. Anthony Gharrett (University of Alaska, Fairbanks, School of Fisheries and Ocean Sciences) and Fred Utter (University of Washington) provided review and recommendations that greatly improved the quality of this paper. Reg Reisenbichler (BRD) provided helpful insight during discussions of postglacial salmon colonization. Jim Finn (BRD) oversaw sample collection for this study, assisted by Ken Cash, Dean Cramer, Kurt Hensel, and Dan Nelson. Natalya Varnavskaya (Kamchatka Research Institute of Fisheries and Oceanography) provided samples from the Kamchatka River. Charles Guthrie and Richard Wilmot (National Marine Fisheries Service, Auke Bay Laboratory) kindly provided laboratory protocol and recommendations for protein analyses. John Patton and Bobbi Pierson (LGL Ecological Genetics, Inc.) assisted with mtDNA and data analyses. Blair Flannery and Steve Miller (U.S. Fish and Wildlife Service, USFWS) processed tissue samples. We thank Jim and Lisa Seeb for allowing us to use their allozyme data on Tustumena sockeye salmon. We also thank the Kenai Fishery Resource Office and the Kenai National Wildlife Refuge (USFWS) for assistance with field logistics.

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Received October 25, 1995

Accepted May 20, 1997

Appendix: Pairwise Distances

TABLE A.1.—Matrices of pairwise genetic chord distances (Cavalli-Sforza and Edwards 1967) for four combined allozyme loci (below the diagonal), and mitochondrial DNA cytochrome *b* haplotypes (above the diagonal) showing relationships among six populations of sockeye salmon from the Tustumena Lake drainage, Alaska, and the Kamchatka River, Russia (used as an outgroup).

Population	Bear Creek	Glacier Flats Creek	Nikolai Creek	Shoreline A	Shoreline B	Lake outlet	Kamchatka River
Bear Creek	—	0.0210	0.0000	0.0005	0.0071	0.3553	0.4818
Glacier Flats Creek	0.0182	—	0.0210	0.0275	0.0037	0.4292	0.6764
Nikolai Creek	0.0158	0.0322	—	0.0005	0.0071	0.3553	0.4818
Shoreline A	0.0008	0.0151	0.0114	—	0.0111	0.3470	0.4554
Shoreline B	0.0056	0.0091	0.0134	0.0024	—	0.3947	0.5921
Lake outlet	0.0221	0.0199	0.0416	0.0176	0.0099	—	0.4534
Kamchatka River	0.0195	0.0257	0.0572	0.0201	0.0182	0.0098	—