

Genetic and Phenotypic Evidence of Reproductive Isolation between Seasonal Runs of Sockeye Salmon in Bear Lake, Alaska

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Abstract.—The effective conservation of salmonids requires the recognition and preservation of populations that are diverse in genetic composition and life history. The management of sockeye salmon *Oncorhynchus nerka* in Bear Lake, Alaska, is based on the presumption that there are two, genetically isolated seasonal runs that exhibit a bimodal escapement pattern. We investigated the genetic composition and life history of the putative early and late runs in two consecutive years. Significant allele frequency differences at six microsatellite loci demonstrate restricted gene flow between the early and late runs ($F_{ST} = 0.017$). There were also significant, and presumably adaptive, differences between the runs with respect to body weight, somatic weight, ovary weight, and egg size among females after correction to equal body sizes. Further, scale pattern analysis revealed highly significant differences in the growth-at-age patterns of these runs. These results demonstrate that there are significant genetic differences between seasonal runs of sockeye salmon and provide support for the management strategy that has been employed for nearly 20 years to protect the genetic diversity of this species in Bear Lake.

Precise natal homing in Pacific salmon *Oncorhynchus* spp. has resulted in the development of discrete populations (Ricker 1972). Spatial and temporal isolation leads to a reduction in gene flow between populations, and the physical and biological characters of the different spawning environments promote adaptive divergence of the populations (MacLean and Evans 1981; Taylor 1991). This divergence is often reflected in the morphology, life history, and behavior of salmonid populations (Beacham and Murray 1987; Taylor 1991). Such differentiation has been observed among sockeye salmon *O. nerka* spawning in different lakes (Wood 1995) and those sharing freshwater systems of varying complexity and size (Ra-

leigh 1971; Gard et al. 1987; Burger et al. 1995; Quinn et al. 1995, 2001; Woody et al. 2000).

The primary aim of Pacific salmon management is to determine an escapement goal for the system or population of interest and regulate the harvest appropriately, thereby conserving the population size and genetic diversity of exploited stocks (Al-lendorf et al. 1987; Marshall et al. 1987). Identification and characterization of discrete populations within systems is key to implementing this type of management strategy. Ideally, the total annual escapement to a system would be partitioned among all spawning populations in that system; however, it is often impossible to manage harvest with this level of precision because of overlap in run timing among populations. In the case of seasonal runs (i.e., conspecific runs that differ in their time of return to the same freshwater system), harvest can be manipulated over time to conserve genetic variation in the system. A seasonal run may be composed of one or more reproductively

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isolated spawning populations, and run timing may or may not be correlated with differences in the time of spawning or emergence of the young. For example, multimodal run timing may be closely correlated with multimodal spawning or reduce to a single, unimodal peak of spawning activity.

Seasonal runs enrich the genetic variability of salmonid species and have been documented among five species of Pacific salmon (sockeye salmon: Wilmot and Burger 1985; chinook salmon *O. tshawytscha* and coho salmon *O. kisutch*: Reisenbichler and Phelps 1987; Utter et al. 1989; chum salmon *O. keta*: Phelps et al. 1994; and pink salmon *O. gorbuscha*: McGregor et al. 1998; Olsen et al. 2000) as well as steelhead *O. mykiss* (Nielsen and Fountain 1999). For example, Varnavskaya et al. (1994) found that seasonal run timing often accounted for the greatest genetic diversity among sockeye salmon populations within lake systems. Systems with seasonal runs of sockeye salmon typically have two or more lakes, with the different seasonal runs linked to the use of one or the other. For example, the two lakes in the Chignik system of Alaska support different seasonal runs of sockeye salmon (Dahlberg 1979). However, seasonal runs also exist within lakes, as in the case of Karluk Lake, Alaska (Wilmot and Burger 1985; Gard et al. 1987). Within a common nursery lake, early runs tend to spawn earlier and in colder habitats that are usually linked with upper lakes and inlet tributaries. In contrast, late runs tend to spawn later and in warmer habitats, such as lake beaches and outlet tributaries (Koenings and Burkett 1987). The differences in run timing and spawning time may be adaptations to temperature variation within lakes and among spawning habitats (Brannon 1987; Hodgson and Quinn 2002). Nevertheless, in some systems early- and late-run sockeye salmon can occupy the same spawning area sequentially (Gard et al. 1987).

The simple existence of bimodality in escapement timing into a system is not conclusive evidence of reproductive isolation between spawning or run timings within a system. For example, it has been postulated that overfishing of the middle portion of the escapement into Karluk Lake is responsible for the present bimodal distribution of the sockeye salmon in that lake (Thompson and Bevan 1954; Van Cleve and Bevan 1973). However, the bimodality of the Karluk Lake return has been observed over many decades, suggesting a natural origin for these seasonal runs (Rounsefell 1973; Koenings and Burkett 1987).

The objective of this study was to determine

whether there are two seasonal runs of sockeye salmon at Bear Lake, Alaska (Figure 1), or a single run with a protracted time of return. This study is novel in that it seeks to reveal genetic differences among putative populations based on discontinuous run timing when the degree of spatial and temporal isolation on their spawning grounds is unknown. We used two separate approaches to address the question. First, we compared the variation in allele frequencies at six microsatellite loci between early- and late-run fish to assess the degree of reproductive isolation between putative runs. Similarity between putative runs in neutral genetic markers (microsatellites) would indicate high levels of gene flow and a lack of reproductive isolation. Second, we compared early- and late-arriving sockeye salmon phenotypically using morphological and life history traits. Pacific salmon morphology and life history are composed of coevolved suites of traits that are to varying extents heritable and adaptive (Hutchings and Morris 1985; Taylor and McPhail 1985; Beacham and Murray 1987; Rogers 1987; Bromage et al. 1990; Fleming and Gross 1990; Taylor 1991; Wood 1995). Differences between putative runs in these traits would suggest adaptive differences among early- and late-run fish. Alternatively, phenotypic differences between runs could result from different states of maturity, which suggests different spawning times and also supports the presence of seasonal runs of sockeye salmon.

Study Site

Bear Lake, with a mean depth of 32 m and a maximum depth of 104 m, is an oligotrophic, glacially formed lake that is approximately 9.6 km long and less than 5 km wide (Figure 1; Honnold et al. 1996). It is approximately 14.3 km from the Bering Sea, lies at an elevation of 37.5 m above sea level, and drains into the Bering Sea at its northwestern end through its outlet tributary, the Bear River. The southeastern end has a deep glacial valley that holds Bear Creek, the largest of four and the only glacial inlet tributary of the Bear Lake system.

Unlike the neighboring systems of the Alaska Peninsula and Bristol Bay, the escapement pattern of Bear Lake sockeye salmon is protracted and typically bimodal; fish return from early June through September (Figure 2). The limited data available from tagging studies and spawning surveys in this remote location suggest that the early- and late-returning fish spawn at different times and different places in the Bear Lake system, but the

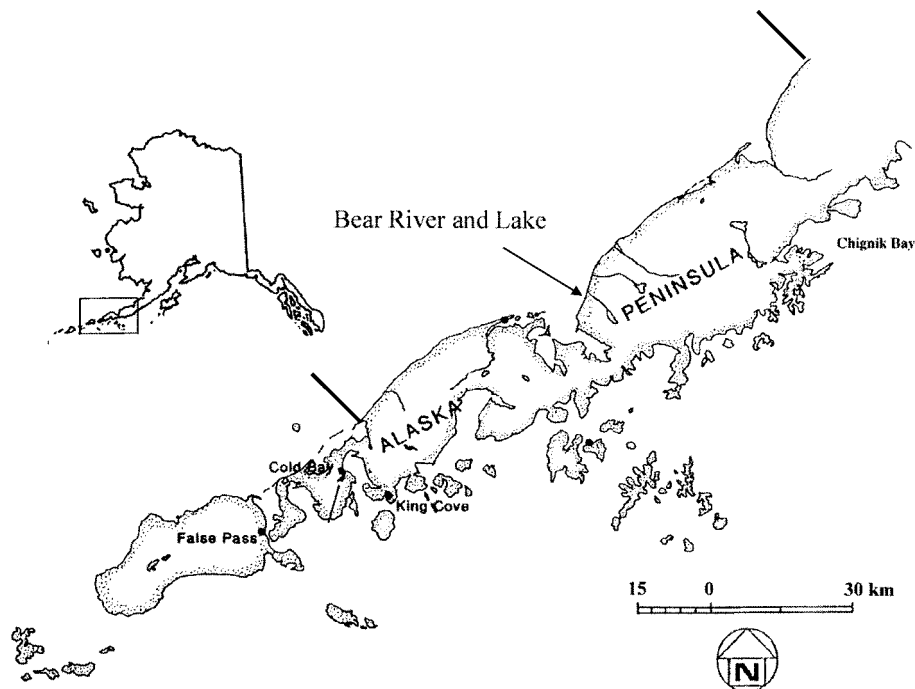


FIGURE 1.—Map of the Alaska Peninsula showing the location of the sockeye salmon fishery (between the bars) and Bear Lake (arrow). All fish sampled were collected at the confluence of Bear Lake and its outlet tributary, Bear River.

results are inconclusive (Shaul 1972). Early- and late-returning fish often differ in age composition, but not consistently so (Table 1). Similarly, the bimodal escapement pattern is not stable over years, which suggests that it is due to other factors,

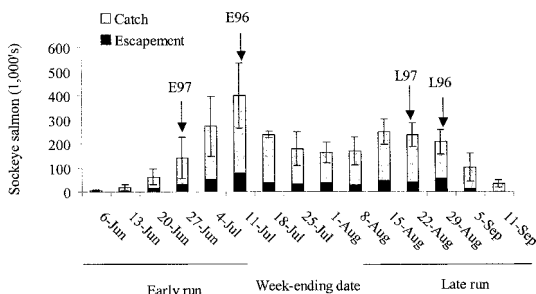


FIGURE 2.—Estimated mean run timing (as determined by catch plus escapement) of Bear Lake sockeye salmon. Catch through 11 July was estimated as the product of the North Peninsula fishery catch (C_{NP}) and the proportion of total North Peninsula escapement that returned to Bear Lake (E_B/E_{NP}). Catch after 1 August is the sum of C_{NP} and E_B , as escapement to all other North Peninsula systems ends in mid to late July. Bars represent one standard deviation, arrows the dates of phenotypic and genetic sampling. Samples are abbreviated as follows: E96 and E97, early-run fish collected in 1996 and 1997; and L96 and L97, late-run fish collected in 1996 and 1997.

such as varying fishing pressure or variation in the migration environment (Rounsefell 1973; Thomson et al. 1994; Macdonald 2000).

Bear Lake is the leading producer of sockeye salmon in the North Peninsula fishery, providing roughly one-half the annual catch of the district (ADFG 1993). Between 1962 and 1993, commercial catches of sockeye salmon in this fishery ranged from less than 200,000 to 3.8 million fish (ADFG 1993; Murphy et al. 1996). The fishery permits set netting, drift gill netting, and purse seining along the north side of the Alaska Peninsula (Figure 1). Fishing dates are established based on in-season evaluations of escapement goals in each of the area's four major systems (Murphy et al. 1996). In the absence of conclusive information to the contrary, the Alaska Department of Fish and Game manages the Bear Lake escapement as two distinct seasonal runs; this is a conservative measure designed to protect the genetic diversity of Bear Lake sockeye salmon (B. Murphy, Alaska Department of Fish and Game, personal communication).

Methods

Sample Collection

Early and late fish were defined by their historical run timing (Figure 2). All sockeye salmon

TABLE 1.—Annual and mean age composition (percent) of early-run (through 11 July) and late-run (after 1 August) Bear Lake, Alaska, sockeye salmon, 1988–1997. Age designations follow Koo (1962), the first digit indicating the number of winters spent in freshwater and the second the number of winters in the ocean; standard deviations are given in parentheses.

Year	Early-Run					
	1.2	1.3	2.1	2.2	2.3	Other
1988	0	8	1	23	68	0
1989	2	0	7	27	57	7
1990	15	2	1	37	39	6
1991	3	34	5	51	7	1
1992	4	4	6	58	27	2
1993	2	4	1	32	59	2
1994	1	7	6	27	55	4
1995	4	0	8	39	49	2
1996	5	3	12	58	21	1
1997	6	3	17	48	25	1
Mean	9.1 (4.3)	6.5 (9.8)	6.3 (5.1)	40.0 (13.1)	40.7 (20.0)	2.4 (2.3)

entering Bear Lake up until 11 July were considered early and those entering after 1 August were considered late. Fish entering the lake between 12 July and 1 August were excluded from this study, as the two proposed runs would probably overlap during this period so that individual fish could not be reliably sorted into either the early or the late sample.

Early- and late-returning adult sockeye salmon were trapped at the Bear River weir in 1996 and 1997 (Figures 1, 2). Dorsal fin clips were collected and preserved in 95% ethanol and body weight (g), length from mid-eye to fork (mm), and sex were recorded. In addition, 47 females (26 early and 21 late) and 69 females (35 early and 34 late) were collected and frozen for fecundity and egg size analyses in 1996 and 1997, respectively. Sampling of females was not random but was intended to represent the range of female body lengths present in the escapement. In addition, ADFG has collected scales from fish throughout the run annually since 1982 and kindly shared their age data and archived scale samples for growth-at-age analysis.

Genetic Comparisons

A modified rapid cell lysis procedure was used to extract DNA (Hoezel and Green 1992). Fish were screened for genetic variation at six micro-satellite loci using a multiplex described in Olsen et al. (1996) that was modified with the substitution of a single locus, *Ots103* (Nelson and Beacham 1999). Primers were purchased from GIBCO Life Technologies (Gaithersburg, Maryland). Amplifications were carried out as in Olsen et al. (1996) with an annealing temperature of 58°C. Primer concentrations ranged from 0.09 to 0.34 μ M. Allele sizes were scored with the use of an Applied Biosystems 373A sequencer (ABI 1993).

Electrophoresis time was approximately 10 h per gel. Alleles were scored and results were tabulated by Genotyper software (ABI 1996). A control sample was included in each gel to confirm that genotyping was consistent across gels.

Tests for departures from Hardy–Weinberg proportions (Guo and Thompson 1992) and the heterogeneity of allele frequencies were performed in GENEPOP (Raymond and Rousset 1995). Hierarchical fixation indexes (F_{ST} statistics), which measure the degree of genetic divergence, were computed in FSTAT (Goudet 1995) according to Weir and Cockerham (1984). Principal components analysis was performed with the covariance matrix of allele frequencies in MINITAB (Ryan et al. 1985) after omitting the largest allele at each locus to allow for the nonindependence of allele frequencies within a locus. Sequential Bonferroni adjustments were made for all multiple comparisons (Rice 1989).

Phenotypic Comparisons

Ovary weight, fecundity, and egg size.—All of the fish sampled were silver in color, with bright red–orange flesh (Ramstad 1998) indicating that none had started the final stage of maturation when ovary and egg size change rapidly and carotenoids are mobilized and transported from the flesh to the skin (Foote et al. 1994; Craig and Foote 2001). Ovaries were removed and weighed, and three subsamples (approximately one-quarter of the total ovary weight) were collected from one ovary. Eggs were loosened from their skeins by exposure to modified Gilson's fluid for approximately 60 d (Gunderson 1993). Eggs were counted and the mean egg weight for each fish determined by weighing three sets of five randomly chosen eggs. Total fecundity was estimated by multiplying the

TABLE 1.—Extended.

Late-Run					
1.2	1.3	2.1	2.2	2.3	Other
1	1	13	55	30	0
2	0	8	79	11	1
4	1	0	85	7	2
11	2	10	68	7	2
0	0	15	71	10	3
1	1	19	44	32	3
0	2	1	83	9	5
1	1	15	66	18	0
1	1	16	47	21	14
1	0	21	67	10	2
2.2 (3.3)	0.8 (0.6)	11.8 (7.1)	66.5 (14.3)	15.5 (9.2)	3.3 (4.2)

total gonad weight by the ratio of the number of eggs in the subsample to the weight of the subsample. Somatic weight was estimated by subtracting the ovary weight from the total body weight.

Growth at age.—Total body growth is correlated with scale radius and annuli width, the latter being determined by the number of circuli in the annulus and the distances between them (Bilton and Robins 1971; Fisher and Percy 1990). Therefore, scales of early- and late-returning fish were measured to assess differences in growth at age. Since 1988, the early and late escapements have on average consisted of 40% and 67% age-2.2 fish (Table 1). This single, shared age (designations according to Koo 1962, i.e., two winters in freshwater followed by two winters at sea) was selected for scale pattern analysis to control for age effects. Approximately 150 scales (50 early [return dates of 8 June to 11 July] and 100 late [2–30 August]; equal sex ratio per year and run timing) were measured from fish returning in each of 12 years (1981, 1985–1986, and 1988–1996).

The freshwater and marine growth zones of the scales were measured with an optical pattern recognition system (OPRS Model 512; BioSonics, Inc., Seattle). Freshwater measurements were made at 113 \times magnification and include the distances from the focus of the scale to the outer edge of the first annulus (FW1), the second annulus (FW2), and the plus zone (PLUS). The distance across the second-year zone (FW2) and the amount of plus growth (PLUS) were estimated as the difference between measures. The number of circuli was recorded and the average distance between circuli calculated in each freshwater zone. Measurements of marine growth were made at 56 \times magnification and include the distance from the

focus of the scale to the outer edge of the first marine annulus (SW1), the second marine annulus (SW2), and the edge of the scale (EDGE). The total distance across the first (SW1) and second marine growth zones (SW2) and the amount of edge growth (EDGE) were calculated by taking the difference between measures.

The same reader measured all scales. We controlled for possible temporal differences in the reader's level of experience in two ways. First, measurements of early and late fish were alternated within years. Second, the reader remeasured scales ($n = 172$) from years representing all levels of her experience (1981, 1985, 1990, and 1996) and the initial and repeated measures were compared.

Data analysis.—The log_e transformed body, somatic, and ovary weights and fecundity and the untransformed egg weight of early and late fish of similar maturities were compared by analysis of covariance (ANCOVA). The logarithm of length was used as the covariate and removed the effects of both body size and age (Ramstad 1998). Traits were adjusted to a common length within run timings based on a pooled slope estimate from SYSTAT (1992). When interaction effects between run timing and length were absent (i.e., the slopes were equal), the interaction term was dropped from the model and the adjusted values (least-squares estimates of the group means) are presented; the differences were also tested for significance. When significant interaction effects between run timing and length were present, separate linear regression equations for each run timing are presented. Not all collected fish were included in every analysis because several samples were destroyed before processing was completed. In addition, analysis of the residuals indicated that one fish from the 1996

TABLE 2.—Expected heterozygosity (H_e) and the number of alleles (A) of Bear Lake sockeye salmon per year, run timing, and microsatellite locus. Samples consist of 50 fish (equal sex ratio) per run timing and year and 100 fish per run timing pooled over years. The total number of alleles per locus is given in parentheses.

Locus	H_e and A	1996		1997		Years pooled		Mean per locus
		Early	Late	Early	Late	Early	Late	
<i>Oneμ1</i>	H_e	0.26	0.30	0.19	0.17	0.22	0.23	0.23
	A (5)	5	4	4	3	5	4	4.0
<i>Oneμ11</i>	H_e	0.65	0.60	0.62	0.60	0.64	0.60	0.62
	A (4)	4	3	3	3	4	3	3.3
<i>Oneμ2</i>	H_e	0.72	0.77	0.83	0.77	0.78	0.77	0.77
	A (11)	7	11	9	8	9	11	8.8
<i>Oneμ8</i>	H_e	0.81	0.86	0.82	0.83	0.81	0.84	0.83
	A (14)	11	13	9	9	13	13	10.5
<i>Ots103</i>	H_e	0.91	0.87	0.90	0.89	0.91	0.88	0.89
	A (17)	15	15	15	13	17	15	14.5
<i>Ssa85</i>	H_e	0.84	0.83	0.79	0.84	0.82	0.84	0.83
	A (15)	13	12	10	12	13	13	11.8
Mean	H_e	0.70	0.70	0.69	0.68	0.70	0.69	0.69
	A	9.2	9.7	8.3	8.0	10.2	9.8	8.8

fecundity sample was an outlier. Thus, sample sizes are not constant across comparisons.

Two-way analysis of variance (ANOVA) tested for significant effects of year and run timing on growth within life history phases (FW1, FW2, PLUS, SW1, SW2, and EDGE) and cumulative growth across life history phases between the early- and late-returning Bear Lake sockeye salmon. The number of and average distance between circuli in the freshwater scale zones were similarly compared among run timings by means of two-way ANOVA. Scales displaying a reabsorption of more than three circuli were omitted from the EDGE and total growth analyses, and outliers were identified by analysis of residuals within each comparison and removed. Thus, sample sizes vary slightly and adjusted means are not strictly additive among comparisons.

Results

Genetic Comparisons

All six loci were polymorphic (Table 2). The mean number of alleles over all samples (1996 early, 1997 early, 1996 late, and 1997 late) was 8.8, ranging from 3.3 (*Oneμ11*) to 14.5 (*Ots103*). The mean expected heterozygosity over all samples was 0.69. There was no evidence of deviation from Hardy–Weinberg proportions at any locus for any sample after sequential Bonferroni correction for multiple tests. However, prior to correction, the 1996 early sample was significantly different from Hardy–Weinberg proportions at a single locus (*Ssa85*; $P = 0.025$) and marginally so at another (*Ots103*; $P = 0.079$). Without Bonferroni correction, the 1996 late sample was also signifi-

cantly different at a single locus (*Ssa85*; $P = 0.023$). In each of these cases there was a deficit of heterozygotes, suggesting a possible Wahlund effect and therefore multiple spawning populations with the runs. All other loci in every sample were well within Hardy–Weinberg proportions ($P = 0.120$ – 1.0).

There were no significant differences in allele frequencies (Appendix 1) between years ($P > 0.05$ after Bonferroni correction) at any locus or over all loci for either the early- or late-run fish. Without Bonferroni correction, early-run fish sampled in different years differed significantly in allele frequencies at one locus (*Oneμ2*; $P = 0.013$). Inter-annual estimates of F_{ST} were not significant (early-run fish: $F_{ST} = 0.004$, $P = 0.091$; late-run fish: $F_{ST} = -0.04$, $P = 0.952$).

Allele frequencies differed significantly between early- and late-run fish within years and when years were pooled. In 1996, allele frequencies differed significantly at four of the six loci (*Oneμ1*, *Oneμ2*, *Oneμ11*, and *Ots103*; $P < 0.05$ after Bonferroni correction) and over all loci ($P < 0.001$). Without Bonferroni correction, early- and late-run fish collected in 1996 differed in allele frequencies at an additional locus (*Oneμ8*; $P = 0.025$). The estimated F_{ST} between early- and late-run fish in 1996 was 0.015 ($P = 0.001$). In 1997, allele frequencies differed significantly between early- and late-run fish at one locus (*Oneμ11*; $P < 0.05$ after Bonferroni correction) and over all loci ($P < 0.001$). Another two loci were marginally different in allele frequencies prior to Bonferroni correction (*Oneμ1*; $P = 0.078$ and *Oneμ11*; $P = 0.063$). The estimated F_{ST} between early- and late-

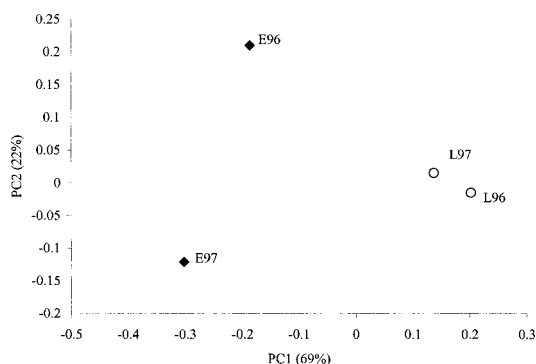


FIGURE 3.—First and second principal components of the allele frequencies at six microsatellite loci for Bear Lake sockeye salmon that returned early and late in 1996 and 1997. The percentages in parentheses indicate the percent of variation explained by each principal component.

run fish in 1997 was 0.019, and this was significantly greater than zero ($P = 0.001$). There were significant differences in allele frequencies between early- and late-run fish (years pooled) at three loci (*Oneμ1*, *Oneμ11*, and *Ots103*; $P < 0.05$ after Bonferroni correction) and over all loci ($P < 0.001$). Without Bonferroni correction, early- and late-run fish differed significantly in allele frequencies at an additional locus (*Oneμ2*; $P = 0.017$) and marginally at another (*Oneμ8*; $P = 0.057$). The estimated F_{ST} between early- and late-run fish

with years pooled was 0.017, and this was significantly greater than zero ($P = 0.001$).

Principal components analysis reduced allele frequencies to two axes that explain 91% of the total genetic variation among sampled fish (Figure 3). The first principal component explains 69% of the variation and differentiates between early- and late-run fish. The second component explains 22% of the variation, differentiates between early-run fish sampled in different years, and suggests greater interannual variation in allele frequencies among early- than among late-run fish.

Phenotypic Comparisons

Ovary weight, fecundity, and egg size.—Early females weighed more per unit body length than late females in each year and in both years combined (Table 3). Adjusted to a common length, the mean body weight of the early fish (years pooled) was approximately 7% (137 g) greater than that of the late fish. Interannual variation was insignificant between early samples ($P = 0.35$) and significant ($P = 0.04$) between late samples (Table 4). Early fish also had significantly greater somatic weights per unit body length than late fish in 1996, 1997, and both years pooled (Table 3). The adjusted mean somatic weight of early fish (years pooled) was approximately 6% (112 g) greater than that of late fish. Interannual variation was insignificant between early samples ($P = 0.90$) and marginally

TABLE 3.—Differences in the body weight, somatic weight, and fecundity of early- and late-run Bear Lake sockeye salmon. Means are adjusted to a common body length within each comparison, and 95% confidence intervals are shown in parentheses. Significance ($P < 0.05^*$, $P < 0.01^{**}$) was tested by two-way analysis of variance with run timing and \log_e transformed body length as factors; interaction between factors was insignificant and was excluded from the model in all comparisons. Sample sizes are not consistent among comparisons owing to the removal of outliers identified within comparisons and the destruction of several samples before processing was completed.

Variable	1996	1997	Years pooled
Body weight (g)			
Early run	2,149.5 (2,094.4–2,206.1)	2,136.7 (2,077.7–2,197.3)	2,113.3 (2,071.4–2,156.0)
Late run	1,958.6 (1,904.6–2,014.2)	1,917.9 (1,861.2–1,976.3)	1,976.3 (1,937.2–2,016.3)
N^a	24, 21	33, 32	46, 53
F	25.0**	22.6**	20.0**
Somatic weight (g)			
Early run	1,992.2 (1,956.7–2,028.4)	1,998.2 (1,939.1–2,059.1)	1,986.2 (1,950.8–2,022.3)
Late run	1,927.5 (1,885.6–1,970.4)	1,822.6 (1,768.7–1,878.1)	1,874.3 (1,837.2–1,912.2)
N^a	26, 21	33, 32	59, 53
F	5.4*	15.5**	17.8**
Fecundity			
Early run	3,408.4 (3,171.6–3,662.9)	2,919.0 (2,716.2–3,136.9)	3,026.0 (2,861.2–3,200.3)
Late run	3,281.3 (3,108.8–3,463.4)	2,864.1 (2,665.1–3,077.9)	3,032.1 (2,884.2–3,187.5)
N^a	12, 21	33, 33	45, 54
F	0.7	0.1	0.0

^a Early and late runs, respectively.

TABLE 4.—Interannual differences in the body weight, somatic weight, and fecundity of early- and late-run Bear Lake sockeye salmon. See the caption to Table 3 for additional details.

Variable	Early run	Late run
Body weight (g)		
1996	2,406.7 (2,330.9–2,484.9)	1,791.8 (1,735.4–1,850.1)
1997	2,450.4 (2,401.9–2,499.9)	1,716.4 (1,675.7–1,758.1)
<i>N</i> ^a	13, 33	21, 32
<i>F</i>	0.9	4.4*
Somatic weight (g)		
1996	2,215.0 (2,162.5–2,268.8)	1,702.8 (1,649.1–1,758.1)
1997	2,219.4 (2,175.5–2,264.3)	1,629.5 (1,587.6–1,672.4)
<i>N</i> ^a	26, 33	21, 32
<i>F</i>	0.0	4.2
Fecundity		
1996	3,729.4 (3,367.8–4,129.9)	2,975.0 (2,768.3–3,197.1)
1997	3,235.7 (3,047.3–3,435.8)	2,657.1 (2,512.4–2,810.2)
<i>N</i> ^a	12, 33	21, 33
<i>F</i>	5.5*	5.8*

^a 1996 and 1997, respectively.

significant ($P = 0.05$) between late samples (Table 4). While there was significant interannual variation ($P = 0.02$) in fecundity within early- and late-run fish, there were no differences between runs in either year ($P = 0.42$ in 1996, 0.74 in 1997) or years pooled ($P = 0.97$; Tables 3, 4). Differences in the outputs of various methods to adjust fish to a common length are minimal (e.g., Foote et al. 1999) and do not account for the differences presented.

Ovary size increased with female body length in both early- and late-run fish (years pooled), with a significant difference in elevation ($F_{1,108} = 65.176$, $P < 0.001$) but no difference in slope ($F_{1,107} = 0.651$, $P = 0.421$) between the lines (Figure 4). The regression equations describing this

relationship for the early- and late-run fish (years pooled) are as follows: (1) $\log_e(\text{ovary weight}) = -15.376 + 3.250 \cdot \log_e(\text{length})$ and (2) $\log_e(\text{ovary weight}) = -13.1027 + 2.828 \cdot \log_e(\text{length})$. Adjusted mean ovary weight (years pooled) was 142.2 g (95% confidence interval, 133.0–155.4 g) and 90.2 g (83.9–98.8 g) for early- and late-run fish. Ovary weight was significantly greater among early-run fish in both 1996 ($F_{1,43} = 4.899$, $P = 0.032$) and 1997 ($F_{1,62} = 34.464$, $P < 0.001$). There was also significant interannual variation in ovary weight for both early-run ($F_{1,55} = 8.885$, $P = 0.004$) and late-run ($F_{1,49} = 11.816$, $P = 0.001$) fish.

Egg size increased with female body length in early-run fish but not in late-run fish (Figure 5), as

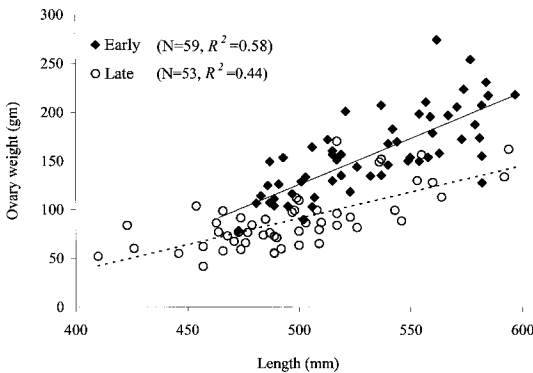


FIGURE 4.—Regressions of ovary weight on fish body length for early- and late-run Bear Lake sockeye salmon, with data pooled over years. Although all statistics were computed with \log_e transformed values, the untransformed data are presented here for ease of interpretation.

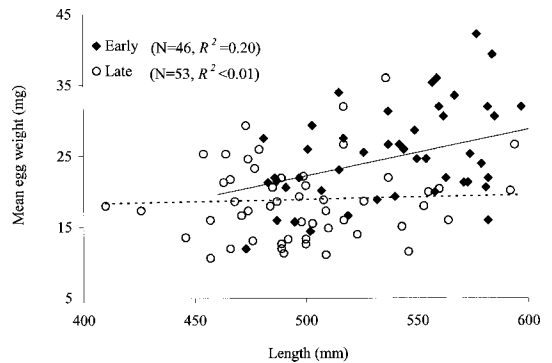


FIGURE 5.—Regressions of egg weight on \log_e transformed body length for early- and late-run Bear Lake sockeye salmon, with data pooled over years. Untransformed body length data are presented here for ease of interpretation.

TABLE 5.—Growth at age of early- and late-run Bear Lake sockeye salmon. Data are distances across freshwater and marine zones in micrometers, with standard errors in parentheses. Zones are defined in terms of the boundaries of the first freshwater annulus (FW1), the second freshwater annulus (FW2), the plus-growth area (PLUS), the first marine annulus (SW1), the second marine annulus (SW2), and the edge growth area (EDGE). The significance of run timing effects was tested by two-way ANOVA; $P < 0.05^*$, $P < 0.01^{**}$. Sample sizes vary and adjusted means are not strictly additive among comparisons owing to the removal of outliers and re-absorbed scales within comparisons.

Scale measure	<i>N</i> (early, late)	Early	Late	<i>F</i>
Freshwater growth				
FW1				
Total distance	630, 1,196	282.7 (1.8)	206.7 (1.3)	1,223.7**
Mean intercirculi distance	630, 1,194	30.9 (0.2)	31.2 (0.1)	2.0
Mean number of circuli	630, 1,196	9.2 (0.1)	6.7 (0.1)	1,364.3**
FW2				
Total distance	630, 1,196	249.9 (1.7)	262.8 (1.2)	38.8**
Mean intercirculi distance	628, 1,195	23.2 (0.1)	23.9 (0.1)	30.2**
Mean number of circuli	630, 1,196	10.7 (0.1)	11.0 (0.1)	9.8**
PLUS				
Total distance	629, 1,196	52.1 (1.6)	51.2 (1.2)	0.2
Mean intercirculi distance	629, 1,196	15.2 (0.5)	13.6 (0.4)	6.8**
Mean number of circuli	630, 1,196	1.9 (0.1)	1.8 (0.1)	4.4*
Marine growth				
SW1	627, 1,197	965.0 (5.4)	951.0 (3.9)	4.5*
SW2	627, 1,196	710.9 (4.3)	756.7 (3.1)	76.8**
EDGE	628, 1,197	232.7 (5.7)	321.0 (4.1)	157.8**

evidenced by a significant interaction between fish length and run timing in determining egg size ($F_{1,95} = 6.437$, $P = 0.013$). Early fish had greater egg size per unit body length than late fish when years were pooled ($F_{1,95} = 6.210$, $P = 0.014$). The regression equations describing this relationship for early- and late-run fish (years pooled) are as follows: (1) egg weight = $-0.2591 + 0.0452 \cdot \log_e(\text{length})$ and (2) egg weight = $0.0026 + 0.0026 \cdot \log_e(\text{length})$. The greater egg size among early than late fish was significant in 1996 ($F_{1,30} = 10.001$, $P = 0.004$) but not in 1997 ($F_{1,61} = 1.172$, $P = 0.283$), though the same pattern was evident. Interannual variability was not significant for early fish ($F_{1,43} = 1.387$, $P = 0.245$) but was significant among late fish ($F_{1,50} = 6.345$, $P = 0.015$).

Growth at age.—A highly significant ($P < 0.001$) and positive correlation between fish length and total scale radius was confirmed by regression analysis for both early- and late-run fish. No significant differences were found between initial and repeated scale measures (two-way ANOVA; $P > 0.05$), so that scale measurements did not vary with the reader's experience level. Two-way ANOVA of growth measures revealed significant ($P < 0.05$) year and interaction effects in all measures except for the interaction of year and run timing on the mean intercirculi distance of the FW2 zone ($P = 0.722$). Nevertheless, run timing effects were high-

ly significant in 10 of 12 scale characteristics, and the pattern between early and late runs was highly consistent over years. To aid in the presentation of pertinent results, we report tests of significance for run timing effects (Table 5).

In each of the 12 years sampled, early fish displayed greater freshwater growth (as determined by total distance and the mean number of circuli) in their first year (FW1) than late fish (Table 5). There was no significant difference in intercirculi distance. In contrast, late fish exhibited higher growth in the second year in freshwater (FW2) in all measures. For overall growth, this pattern was present in 10 of the 12 years sampled; it was present in all years for intercirculi distance and in 8 years for the number of circuli in the FW2 zone. Total spring growth prior to outmigration as smolts (PLUS) did not differ significantly between early and late fish, though early fish had significantly greater mean intercirculi distances and circuli numbers (Table 5).

Early fish grew more overall in their first year at sea (SW1) than late fish (Table 5), a pattern that was clear in 9 of the 12 years sampled. In contrast, late fish experienced greater growth in the second year at sea (SW2) than early fish in 10 of the 12 years sampled and had greater growth in the spring and summer prior to their return to Bear Lake for spawning (EDGE) in all 12 years.

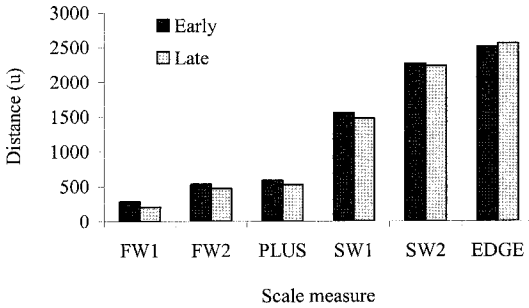


FIGURE 6.—Additive growth of early- and late-run Bear Lake sockeye salmon. Columns indicate the mean distances (μ = μm) from the focus of the scale to the outer edge of the first annulus (FW1), second annulus (FW2), plus zone (PLUS), first marine annulus (SW1), second marine annulus (SW2), and scale edge (EDGE). Error bars are not included, as they would be too small to depict. Significant ($P < 0.001$) differences in run timing were found for each measure.

The greater size of the early fish persisted through the second year in freshwater (the distance was $532.6 \pm 2.1 \mu\text{m}$ [mean \pm SE] versus $469.5 \pm 1.5 \mu\text{m}$; $F_{1,1802} = 572.703$, $P < 0.001$;) and out-migration ($584.5 \pm 2.5 \mu\text{m}$ versus $520.7 \pm 1.8 \mu\text{m}$; $F_{1,1801} = 446.249$, $P < 0.001$), though late fish grew more than early fish in the second year in Bear Lake (Figure 6). Early fish were larger on average than late fish at the end of their first ($1,550.8 \pm 4.6 \mu\text{m}$ versus $1,473.4 \pm 3.3 \mu\text{m}$; $F_{1,1800} = 183.255$, $P < 0.001$) and second ($2,261.7 \pm 6.1 \mu\text{m}$ versus $2,230.1 \pm 4.4 \mu\text{m}$; $F_{1,1800} = 17.891$, $P < 0.001$) years of ocean life, regardless of the greater growth of the late fish in their second year at sea. Total additive growth, measured as the distance from the focus to the outer edge of the scale, was slightly greater for late fish than for early fish ($2,551.1 \pm 5.2 \mu\text{m}$ versus $2,503.7 \pm 7.3 \mu\text{m}$), largely as a result of the much greater edge growth exhibited by the late fish (Table 5). The body size similarity between run timings indicated by scale growth is supported by the actual measurements of returning adults at the weir ($474.6 \pm 1.3 \text{ mm}$ for early fish versus $477.8 \pm 0.9 \text{ mm}$ for late fish; $F_{1,1803} = 4.311$, $P = 0.038$). Thus, the phenotypic data overall suggest that early-run fish are shorter but heavier for their length than late-run fish.

Discussion

The goal of fisheries management is to conserve the population size and genetic diversity of exploited stocks (Allendorf et al. 1987; Marshall et al. 1987). For salmon, this can be complicated by

overlapping run times (which result in mixed-stock fisheries) and by the lack of knowledge of the genetic structures of exploited populations. Such complications call for a conservative approach to harvest. Though information on genetic population structure is lacking, the Alaska Department of Fish and Game assumes that the Bear Lake sockeye salmon fishery consists of two reproductively isolated runs. This strategy is based on the bimodal pattern of escapement timing and age composition for Bear Lake sockeye salmon. Our genetic, phenotypic, and life history analyses show that significant population structuring exists in Bear Lake and that this diversity is partitioned temporally based on when migrating adults return to the lake. These new data support the current fishery management strategy and suggest that further evaluation of the population structure of Bear Lake sockeye salmon is warranted.

Genetic Evidence of Reproductively Isolated Runs

Significant differences in allele frequencies suggest that there is limited gene flow between early- and late-run fish. Estimates of F_{ST} indicate a small but significant amount of genetic differentiation (Wright 1978) between the early- and late-run fish in each year sampled and when the years were pooled. The differentiation between runs of Bear Lake sockeye salmon is similar in magnitude to the divergence between seasonal runs in other systems and salmonid species. Varnavskaya et al. (1994) used the variation at allozyme loci to calculate an F_{ST} of 0.014 between early- and late-run sockeye salmon in Karluk Lake, Alaska. Early and late runs of pink salmon in the Dungeness River of Washington are divergent at microsatellite loci ($F_{ST} = 0.020$; Olsen et al. 2000). Fall and summer chinook salmon in the San Joaquin system of California have an F_{ST} of 0.016 (allozymes; Bartley et al. 1992). Summer and winter runs of steelhead of the Middle Fork Eel River of California have an F_{ST} of 0.010 (microsatellites; Nielsen and Fountain 1999). Based on relative genetic diversity indices, 0.9% of the total genetic diversity found among chinook salmon in Washington, Oregon, and California (Utter et al. 1989) and 0.8% of the diversity among chum salmon in the Pacific Northwest (Phelps et al. 1994) is attributable to differences between seasonal runs. This divergence is much greater than that found between early and late components of single populations of sockeye salmon (mean $F_{ST} = 0.005$; Woody et al. 2000).

Principal components analysis suggests that there is greater interannual variation in allele fre-

quencies among early-run fish than among late-run fish (Figure 3). This finding is supported by the differences in allele frequencies at a single locus (*Oneμ2*; $P = 0.013$, but the result was not significant after Bonferroni correction) and may reflect limited gene flow between return times within the early run. Run timing is highly heritable (Gharrett and Smoker 1993; Smoker et al. 1998; Quinn et al. 2000), and our sampling dates in 1996 and 1997 varied by 13 d for the early fish and only 4 d for the late fish (Figure 2). Leary et al. (1989) and Fishback et al. (2000) found significant differences in allozyme allele frequencies among rainbow trout spawned on different days throughout a single spawning season. Thus, interannual allele frequency differences between the early samples could reflect within-population temporal differences in allele frequencies.

Alternatively, the interannual variation among early-run fish may be due to spatially or temporally isolated spawning populations that are unequally represented in the two sample years. It is unknown how early and late run timing correlates with spawning time or location in the Bear Lake system. However, it is known from personal observations and previous spawning surveys (Shaul 1972) that there are multiple spawning locations in Bear Lake, which suggests the presence of multiple, spatially distinct spawning populations within either the early run, the late run, or both. Further, a deficit of heterozygotes at two of six loci (neither significant after Bonferroni correction) suggests the presence of multiple spawning populations within the early run. Therefore, the interannual variation may reflect differences in allele frequencies between different spawning populations within the early run. Even so, interannual variation in allele frequencies at neutral molecular markers is typically very low (<1%) within sockeye salmon populations (Wood 1995; Wood and Foote 1996) and was not significant (after correction for multiple comparisons) within the early and late runs of Bear Lake sockeye salmon.

Phenotypic Evidence of Reproductively Isolated Runs

Ovary weight, fecundity, and egg size.—Early fish have greater ovary and egg weight relative to body size than late fish. Because early and late fish have similar fecundities, the increased ovary weight of the early fish is due primarily to increased egg size and not egg number. These results are corroborated by observations of local canneries that value early- over late-run fish for the high

quality of their roe (i.e., larger egg and ovary sizes; Gary Johnson, Peter Pan Seafoods, Inc., personal communication). While there was significant interannual variation within run timings in all of the traits measured (after correction for body size), it was much smaller than the interseasonal variation. Greater phenotypic differences within years are not to be expected on the basis of environmental effects alone because fish of common spawning and brood years will probably experience more similar environments than fish spawning in different years and derived from different brood years. For this reason, interseasonal phenotypic variation in ovary weight and other measures probably reflects genetic structuring in addition to environmental effects.

Had the study fish been from the same spawning population and their run timing been correlated with their spawning time, we would have expected them to have roughly similar egg weights (Hendry et al. 1999), but they did not (Figure 5). Indeed, not only were the adjusted egg weights different between early and late fish, the relationship with body size also differed. Similarly, had the early and late samples been drawn from a single panmictic run with a synchronized spawning time, we would have expected the early fish to have smaller ovaries and egg sizes than the late fish because the late fish were sampled 6–8 weeks after the early fish and ovary weight and egg size increase dramatically in the weeks preceding maturity (Foote et al. 1994; Hendry et al. 2000). However, we observed the opposite pattern. Finally, if run timing is correlated with spawning time, we would expect the early-run fish to return at a more advanced state of sexual maturity, and thus with larger ovaries and eggs, than the late-run fish. Our general measures of maturity (body color, flesh color, and all eggs being in an unovulated state) indicate no clear difference in the maturity of early- and late-returning fish. However, differences in egg size could still be due to differences in the state of maturity because ovary mass in sockeye salmon begins to increase slowly approximately 3 months prior to spawning (Hendry et al. 2000). Differences in egg weight between populations at different states of maturity are sometimes maintained through full maturity (Craig 1985; C. J. Foote and Wood, Canada Department of Fisheries and Oceans, unpublished data). Thus, the data suggest that early-run fish spawn earlier and have larger eggs at spawning than late-run fish.

Similar patterns between the seasonal runs of other salmonids have been reported. The early-

spawning sockeye salmon of Morris Creek (Fraser River) have larger eggs than the late-spawning Cultus Lake population of the same system (Robertson 1922). Also, the summer forms of chum and Atlantic salmon *Salmo salar* have greater ovary-body weight ratios than their autumn counterparts (Berg 1959). Finally, Tallman and Healey (1991) showed that fall run chum salmon in a small creek had significantly larger eggs than winter run fish. Thus, our findings are consistent with the hypothesis that Bear Lake sockeye salmon are differentiated into seasonally distinct and reproductively isolated runs.

Growth at age.—Scale pattern analysis suggests that generalized growth patterns are the same for early and late fish. Both have high growth rates through their second winter in freshwater and reduced growth in the spring of their seaward migration. At sea, both early and late fish grow considerably through their second winter and relatively little as they mature in the spring of their spawning migration. In general, the late fish exhibit less total additive growth than the early fish until the end of their second winter at sea, although the total difference at this point, while significant, is slight. The difference between the early and late fish in the amount of edge growth is not surprising given that the late fish return to Bear Lake in late summer and early fall while the early fish return to Bear Lake in early summer. Because there is no lagoon for holding, the fish enter the Bear River directly from the Bering Sea, so that the delayed return of the late fish translates into an additional summer growing season.

The difference in the growth-at-age pattern between early- and late-run fish appears to stem from differences that are accrued in the first year of life. The greater growth of early fish may be due to a larger size at emergence. A larger size at emergence would be expected given that early fish have larger eggs than late fish (Thorpe et al. 1984; Hutchings 1991). Such a size difference could confer an advantage on the early fish in competing for food and result in greater growth in the first year.

The greater growth of the early fish in their first freshwater year may also be due to an earlier emergence time. If early- and late-run fish have similar growth rates, the difference in the overall number of circuli (and therefore growth) would be due to the absolute amount of time spent feeding in the lake. Since these fish share a common nursery lake, the first winter check would be similar in time; thus, any additional time spent feeding in the lake in the first year would be due to an earlier emer-

gence time. This pattern has been observed between spring and fall run sockeye salmon in Karluk Lake, Alaska, where emergence occurs 8–10 weeks earlier for spring than for fall run fish (Koenings and Burkett 1987). Also, early-run pink salmon in Auke Creek, Alaska, emigrate from freshwater before late-run fish (Gharrett and Smoker 1993).

In addition to earlier emergence and greater size at emergence, segregation within Bear Lake itself could explain the greater growth of the early fish in their first year of life. However, this is unlikely given the small size, low species diversity, and homogeneity of juvenile rearing habitat in Bear Lake. Regardless of the mechanisms creating these different scale patterns, early and late Bear Lake sockeye salmon exhibit clear and consistent differences in their growth-at-age patterns in their residence both in freshwater and in the marine environment. This suggests life history differentiation between run timings in time at emergence, size at emergence, or size at outmigration and thus that there is reproductive isolation between the early- and late-arriving sockeye salmon of Bear Lake.

Seasonal Runs

The sockeye salmon of Bear Lake comprise two seasonal runs that are differentiated in population genetic structure and life history. There are significant genetic and phenotypic differences between the runs that persist across years and are much greater than the interannual variation in these traits. These data suggest that the early and late runs are subject to different selection regimes, are reproductively isolated, and have evolved different suites of life history characteristics. Differences in run timing contribute significantly to this isolation, though it is likely that different spawning areas within Bear Lake itself also contribute to the isolation and coevolution of early- and late-run sockeye salmon.

The factors that promote or allow for seasonal runs in some systems but not others are unknown. Suitable spawning areas with different temperature regimes may be important in maintaining this adaptive divergence. Brannon (1987) suggested that differences in temperature select for run timing by necessitating that fish that spawn in areas with colder rearing temperatures deposit their eggs earlier in the spawning season to allow for the development and optimum emergence timing of their young. In addition, high lake temperatures may serve as a thermal stimulus that encourages the separation of seasonal spawning populations

(Hodgson and Quinn 2002). Koenings and Burkett (1987) have observed that the maximum water temperature in Karluk Lake corresponds to minimum spawning activity followed by the beginning of spawning by the late run.

The factors that promote seasonal runs of sockeye salmon in Bear Lake have yet to be determined. In contrast to the juvenile rearing habitat, the spawning habitat in the Bear Lake system is highly heterogeneous and includes lake beaches, an outlet tributary that is influenced by warm lake temperatures, and inlet tributaries that are fed by glacial meltwater, springs, and runoff. This habitat heterogeneity may provide a variety of selection regimes that promote different run timings among Bear Lake sockeye salmon. For example, the early-run sockeye salmon of Karluk Lake spawn primarily in the inlet tributaries, while the late-run fish spawn primarily on lake beaches (Gard et al. 1987). Observation of different spawning grounds based on return time would ultimately confirm inferences made from these data. However, such data alone would not explain why sockeye salmon return to Bear Lake at different times rather than synchronizing their return and simply spawning at different times. The latter is the case, for example, in the sockeye salmon runs of nearby Bristol Bay: the majority of the returning fish move through the fishery within a 10-d period, but spawning varies by months among the different populations. Given the significant marine growth of the late-returning fish during the summer prior to spawning, it seems likely that it is more profitable in terms of growth and fecundity for those fish that spawn late to return late.

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Appendix: Microsatellite Allele Frequencies

TABLE A.1.—Microsatellite allele frequencies of Bear Lake sockeye salmon by locus, run timing, and year; sample sizes are given in parentheses in the column headings.

Locus and allele	1996		1997		Years pooled	
	Early (50)	Late (50)	Early (50)	Late (50)	Early (100)	Late (100)
<i>Onc1</i>						
110	0.030	0.010			0.015	0.005
112	0.060	0.030	0.010	0.010	0.035	0.020
114	0.860	0.830	0.900	0.910	0.880	0.870
116	0.020	0.130	0.040	0.080	0.030	0.105
118	0.030		0.050		0.040	
<i>Onc11</i>						
139	0.010				0.005	
145	0.420	0.540	0.340	0.540	0.380	0.540
149	0.380	0.170	0.490	0.180	0.435	0.175
155	0.190	0.290	0.170	0.280	0.180	0.285
<i>Onc2</i>						
263		0.010	0.010		0.005	0.005
265	0.060	0.040	0.060	0.020	0.060	0.030
267		0.010				0.005
269	0.130	0.070	0.070	0.110	0.100	0.090
271	0.480	0.430	0.300	0.420	0.390	0.425
273	0.120	0.030	0.130	0.090	0.125	0.060
275		0.080	0.050	0.120	0.025	0.100
277	0.080	0.110	0.110	0.110	0.095	0.110
279	0.120	0.170	0.210	0.110	0.165	0.140
281	0.010	0.040	0.060	0.020	0.035	0.030
283		0.010				0.005
<i>Onc8</i>						
188	0.01	0.020		0.010	0.005	0.015
192		0.010				0.005
194	0.020	0.010	0.030	0.010	0.025	0.010
196		0.010	0.010		0.005	0.005
198	0.290	0.190	0.210	0.180	0.250	0.185
202	0.050	0.090	0.080	0.090	0.065	0.090
204	0.280	0.150	0.320	0.240	0.300	0.195
206	0.160	0.220	0.140	0.250	0.150	0.235
208	0.060	0.170	0.100	0.100	0.080	0.135
212		0.040	0.040	0.020	0.020	0.030
214	0.080	0.070	0.070	0.100	0.075	0.085
216	0.010	0.010			0.005	0.005
218	0.020				0.010	
232	0.020	0.010			0.010	0.005
<i>Ots103</i>						
273	0.010				0.005	
277	0.070	0.030	0.040	0.080	0.055	0.055
285	0.030	0.030	0.030	0.010	0.030	0.020
289	0.090	0.020	0.030	0.020	0.060	0.020
293	0.030	0.110	0.020	0.080	0.025	0.095
297	0.030	0.050	0.070	0.050	0.050	0.050
301	0.080	0.020	0.140	0.050	0.110	0.035
305	0.130	0.040	0.100	0.050	0.115	0.045
309	0.160	0.270	0.180	0.240	0.170	0.255
313	0.010	0.050	0.030	0.060	0.020	0.055
317	0.040	0.020			0.020	0.010
321	0.110	0.050	0.070	0.080	0.090	0.065

TABLE A.1.—Continued.

Locus and allele	1996		1997		Years pooled	
	Early (50)	Late (50)	Early (50)	Late (50)	Early (100)	Late (100)
325	0.120	0.180	0.130	0.140	0.125	0.160
329	0.080	0.090	0.100	0.120	0.090	0.105
333			0.010		0.005	
337	0.010	0.020	0.010	0.020	0.010	0.020
341		0.020	0.040		0.020	0.010
<i>Ssa85</i>						
129	0.120	0.200	0.210	0.220	0.165	0.210
131	0.030	0.040	0.020	0.020	0.025	0.030
133	0.050	0.070	0.050	0.080	0.050	0.075
135	0.040	0.060	0.040	0.050	0.040	0.055
137	0.180	0.100	0.130	0.120	0.155	0.110
139	0.040	0.040		0.010	0.020	0.025
141	0.080	0.040	0.030	0.050	0.055	0.045
143	0.310	0.320	0.360	0.280	0.335	0.300
145	0.090	0.090	0.140	0.120	0.115	0.105
149		0.010				0.005
165	0.030	0.010	0.010	0.020	0.020	0.015
173	0.010				0.005	
175	0.010		0.010	0.010	0.010	0.005
177	0.010				0.005	
181		0.020		0.020		0.020