A Microsatellite DNA Analysis of Walsh Lake kokanee

by

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Abstract

An analysis using nine microsatellite loci was used to determine the relationship between kokanee found in Walsh Lake (Cedar River Municipal Watershed) and kokanee and sockeye in the Lake Sammamish tributaries, Baker Lake, and Lake Whatcom hatchery. Measures of within-population genetic diversity revealed a low level of genetic diversity in the Walsh Lake kokanee samples. Tests of population structure and genetic distance matrices both identify samples from Walsh Lake as being unique to all other samples analyzed. The combination of low genetic diversity and unique population structure prohibits this analysis to determine any relationship or historic origin of Walsh Lake kokanee to the other samples analyzed from the archive collections at WDFW.

Introduction

Recent fieldwork by biologists in King County documented trends in kokanee spawning runs within the Lake Washington Basin indicating that some runs are declining, and that the early-run kokanee are extinct (Berge and Higgins 2003). Washington Department of Fish and Wildlife (WDFW) investigated the genetic relationships among kokanee spawning in tributaries of Lake Sammamish, Lake Whatcom Hatchery, Baker Lake, and Meadow Creek (Lake Kootenay - British Columbia; Young et al. 2004). This microsatellite DNA analysis found no evidence to suggest that kokanee from the Lake Sammamish tributaries were altered by past introductions of non-native kokanee or sockeye. These findings may provide a basis for determining how best to manage kokanee stocks remaining in the Lake Sammamish tributaries.

Surveys conducted by Seattle Public Utilities (SPU) staff confirmed historic reports of kokanee in Walsh Lake within the Cedar River Municipal Watershed (CRMW). Spawning surveys in Walsh Lake each fall, from 1997 to present, indicated that the population was quite small. Surveyors never located more

than 20 kokanee redds in any given year from 1997 to 2001. However, in 2002, an unusually large run of kokanee salmon was documented in Walsh Lake. SPU staff collected DNA samples from 87 fish during the 2002 spawning season.

I conducted a microsatellite DNA analysis on the 87 kokanee samples collected by SPU in 2002 and compared them with kokanee and sockeye samples from Lake Sammamish tributaries, Lake Whatcom Hatchery, Baker Lake, and Meadow Creek (Lake Kootenay - British Columbia). These results may help to determine if this run is native or introduced and provide a better understanding of the Walsh Lake Basin where studies are currently assessing restoration options.

Materials and Methods

A total of 87 kokanee samples were analyzed from Walsh Lake using nine microsatellite loci that had been used in the earlier WDFW analysis of kokanee and sockeye (Young et al. 2004). Tissues samples of the Walsh Lake kokanee are archived at WDFW. Eight of the nine loci were used for analysis because these loci provided enough variation to resolve differences among the populations that had been analyzed. Data from earlier collections of kokanee and sockeye were analyzed with the samples from Walsh Lake for comparison (Table 1). A map of the collection locations within the Lake Washington basin is shown in the Figure 1.

DNA was extracted using standard operating procedures following manufacturer's protocols.

Descriptions of the loci assessed in this study and PCR conditions are given in Table 2. PCR reactions were multiplexed (combinations of microsatellite loci) using an MJ Research PTC-200 thermalcycler, with a simple thermal profile consisting of: denaturation at 95^{o}C for 3 min, denaturation at 95^{o}C for 15 sec, annealing at 50^{o}C, extension at 72^{o}C for 1 min, repeat cycle (steps 2-4), final

extension at 72^{o}C for 30 minutes. PCR products for each locus were processed with an ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GeneMapper 3.0 software. Allele binning and naming were accomplished using MicrosatelliteBinner-v1h (Young, WDFW available from the author). MicrosatelliteBinner creates groups (bins) of alleles with similar mobilities (alleles with the same number of repeat units). The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

Each locus was tested for deviations from Hardy-Weinberg equilibrium (HWE) over all groups using GENEPOP 3.4 (Raymond and Rousset 1995) with 10,0000 dememorizations, 100 batches, and 2,000 iterations; all groups were also tested for deviations from HWE at each locus and over all loci. Allele frequencies were calculated using CONVERT 1.3 (Glaubitz 2003). Measures of within-population genetic diversity were calculated for each group (F_{IS}, genetic diversity, and allelic richness – the number of alleles corrected for sample size, FSTAT 2.9.3.1 (Goudet 2001); and observed and expected heterozygosity, GDA 1.1 (Lewis and Zaykin 2001)). Population structure among the groups was determined by performing pairwise genotypic population differentiation tests (GENEPOP 3.4). Statistical significance of all tests was determined using a Bonferroni corrected P-value to account for multiple, simultaneous tests (Rice 1989). Genetic distance matrices among all collections were generated using the program MSA (Microsatellite Analyzer, version 2.65, Dieringer and Schlötterer 2003) with Cavalli-Sforza and Edwards (1967) chord distance. The neighbor-joining method of Saitou and Nei (1987) and Consensus program in PHYLIP (version 3.5, Felsenstein 1989) were used to generate a dendrogram from the distance matrix calculated by MSA with 1,000 bootstrap replicates. The dendrogram generated in PHYLIP was plotted as a radial tree using TREEVIEW (version 1.6.6, Page 1996).

Results and Discussion

Good quality DNA was obtained and analyzed from the kokanee samples from Walsh Lake using an ABI-3730. Existing data for all other collections was collected on an ABI-377, and therefore needed to be aligned and converted for analysis with the samples from Walsh Lake. DNA mobility on each machine may vary; therefore data collected for the same individuals using an ABI-377 and ABI-3730 were plotted together in Microsoft Excel to determine the correction (# of base pairs) that was required to align the ABI-377 data to the data from the ABI-3730. Once the data from all collections were aligned they were binned together to ensure common allelic naming and formatted for analysis. One locus (*One-100*) was not used because the allelic variation in the data was distributed evenly across bins and could not be binned confidently.

A total of 860 kokanee/sockeye samples from 12 locations were compared and samples with genotypes for five or more of the eight loci were included (752 samples) in analyses (Table 1). Allele frequencies for all collections and loci are shown in Appendix 1. There were large differences in the allele frequencies among different collections that were analyzed. Allele frequency differences among populations can occur for multiple reasons. The presence of common alleles among collections may imply that samples were from the same historic population. The absence of common alleles from collections may result from isolation and resulting differences in genetic history among populations. Founder effects, population bottlenecks, and genetic drift will also result in the loss of alleles, which is a function of effective population size. All of these factors will affect the frequency of alleles within a population or collection, and may also limit our ability to establish the historical relationships among the collections. The allele frequencies of the Walsh Lake kokanee for example are very different from the other collections. This difference may result from (1) a historical difference between fish from this locality, and fish from other localities, or (2) common

history masked by genetic drift. We are unable to determine the historic origin of the Walsh Lake kokanee based on the samples and loci used in this analysis.

Kokanee from Lake Whatcom Hatchery and Meadow Creek have a relatively high frequency of an alternate two-based allele at *One-110* as seen by Young et al. (2004). *One-100* has a tetranucleotide repeat, but an alteration or mutation has created dinucleotide repeats resulting in the alternate two based alleles. This alternate allele is seen at low frequencies in the Bear Creek, Little Bear Creek, and Issaquah Creek samples. The presence of the two-based alternate alleles in the Bear Creek basin and Issaquah Creek may indicate an historic relationship to Lake Whatcom Hatchery. Stocking records of kokanee and sockeye from non-basin sources show that Lake Whatcom kokanee were planted into both Issaquah and Bear Creek (Kolb 1971 and Gustafson et al. 1997). The presence of the two-based alternate allele in those populations suggests a possible remnant of genes from the Lake Whatcom Hatchery. The absence of the two-based alternate allele in other populations, including Walsh Lake, may be a result of genetic drift, and not a function of historical relationships.

Observed heterozygosity ranged from 0.6000 (*One-105*) to 0.9033 (*One-101*) among the eight loci that were scored (Table 2), and observed heterozygosity for collections ranged from 0.6923 (Walsh Lake) to 0.9647 (Lake Whatcom Hatchery; Table 3). The number of alleles observed ranged from 7 (One-105) to 47 (*One-101*) and the observed allele size range at each locus is shown in Table 2.

Tests for conformance to Hardy-Weinberg expectations showed no significant deviations for any of the eight loci when tested among all collections, therefore all loci were included in the analyses (Table 2). Within collections there were only three loci that had any significant deviation from Hardy-Weinberg expectations after Bonferroni correction was applied (0.05/96 = 0.0005): *One-114* in Walsh Lake, *One-115* in Meadow Creek, and *One-101* in Lewis Creek. These few

significant differences do not indicate any problem with the samples or loci so they were included in all analyses.

Tests for genotypic differentiation among all collections revealed significant differences with the exception of three collections: kokanee from Bear Creek, kokanee from Little Bear Creek, and sockeye from Bear Creek (Table 4). Young et al. (2004) identified this same pattern between the kokanee and sockeye collections in the Bear Creek and Little Bear Creek drainages and they concluded that all three collections were sockeye (the kokanee had anadromous mothers based on analyses of Sr:Ca ratio in otolith primordia). This report does not address the conclusion of Young et al. (2004), but specifically identifies the Walsh Lake collection as being highly significantly different from all other collections analyzed.

Tests for allelic richness and gene diversity revealed the lowest allelic richness (4.94) and diversity (0.67) in the Walsh Lake kokanee collection (Table 5). The average of allelic richness and gene diversity for the other seven collections of kokanee was 10.27 and 0.86 respectively. Average allelic richness and gene diversity of the four collections of sockeye was 8.89 and 0.83 respectively. These results suggest that the kokanee in Walsh Lake may have a reduced effective population size (undergone a bottleneck or was founded by a few individuals), and therefore have lost genetic variation due to genetic drift or founder effect.

The consensus neighbor-joining tree generated using Cavalli-Sforza and Edwards (1967) distances revealed only one group that clustered together with bootstrap support that was over 80% (Figure 2). This group included the three collections from the Lake Sammamish tributaries (Lewis Creek, Laughing Jacobs Creek, and Ebright Creek) with bootstrap support of 99%. The distribution of the collections in the tree is generally consistent with Young et al. (2004), however

this distribution does not provide any evidence of genetic structure with the exception of the three Lake Sammamish tributaries.

Conclusions

The neighbor-joining and genotypic differentiation analyses identified all collections as unique with the exception of the Lake Sammamish tributaries (neighbor-joining tree) and the collections from the Bear and Little Bear Creek watershed (tests of population differentiation). The lack of genetic diversity, the low allelic richness values, and the observed heterozygosity detected in the Walsh Lake samples suggests these fish have undergone a reduction in population size or the population was founded by a few individuals and therefore a reduced level of genetic variability was observed. This lack of genetic variability within the Walsh Lake samples limits our ability to determine ancestry or the historic origin of Walsh Lake kokanee.

The analysis of *O. nerka* by Spies (2002) concluded that there was a close genetic relationship between *O.nerka* from the Cedar River basin (including fish from Walsh Lake) to sockeye from Baker Lake. This relationship was based on the results of two neighbor-joining trees using both Cavalli-Sforza and Edwards (1967) and Nei (1972) genetic distance. Spies (2002) cited over 60% bootstrap support for that cluster and states that the genetic distances values between those locations also supports the grouping. In contrast, the results of the assignment tests by Spies (2002) does not assign any of the Cedar River (including Walsh Lake) samples to Baker Lake suggesting the genetic relationship between the two populations is not that close.

This current analysis does not support Spies' conclusion that Walsh Lake is from a Baker Lake origin. There is no bootstrap support for a cluster of Walsh Lake kokanee and Baker Lake sockeye in this neighbor-joining tree. There are however, differences between the two analyses (number of loci and samples

analyzed). Spies (2002) analysis included 17 individuals sampled over four years (1997 – 2000) from Walsh Lake while 87 samples were used from one sampling year (2002) in this analysis. The analysis by Spies (2002) also used fourteen loci while only eight loci was used in this analysis (four loci in common).

It may be possible to address the question of historic origin more adequately if other genetic markers (e.g. mtDNA sequencing) are used. Mitochondrial DNA sequences would have a slower mutation rate that would provide a means of evaluating the different stocks before they became significantly differentiated.

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Literature Cited

Berge, H.B. and K. Higgins. 2003. The current status of kokanee in the greater Lake Washington Watershed. King County Department of Natural Resources and Parks, Water and Land Resources Division. Seattle, Washington. 50pp.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Dieringer, D., and C. Schlötterer. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3:167-169.

Glaubitz, J.C. 2003. CONVERT (version 1.2): A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. http://www.agriculture.purdue.edu/fnr/html/faculty/Rhodes/Students%20and%20Staff/glaubitz/software.htm.

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (version 3.2). Cladistics 5:164-166.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from Goudet (1995). Available from http://www.unilch/izea/softwares/fstat.html.

Gustafson, R.G., T.C. Wainwright, L.T. Parker, and R.S. Waples. 1997. Status review of sockeye salmon from Washington and Oregon. U.S. Department of Commerce, NOAA, NMFS, Seattle, WA, 282 pp.

Kolb, R. 1971. A review of Lake Washington sockeye (*oncorhynchus nerka*) age and racial characteristics as determined by scale analysis. State of Washington, Department of Fisheries, Management and Research Division, Seattle, WA 8 pp.

Lewis, P. O. and D. Zaykin. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12:357-358.

Raymond, M. and F. Rousset. 1995. GENEPOP (Version 3.3): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Spies, I.B. 2002. The origin of sockeye salmon (*Oncorhynchus nerka*) in the Lake Washington Watershed, Washington State: A reappraisal based on microsatellite data. Masters thesis submitted to the University of Washington graduate school.

Young, S.F., M.R. Downen, and J.B. Shaklee. 2004. Microsatellite DNA data indicate distinct native populations of kokanee, *Oncorhynchus nerka*, persist in the Lake Sammamish Basin, Washington. Environmental Biology of Fishes 69:63-79.

Table 1. Collection code and location of kokanee and sockeye and the number of samples analyzed with the Webster Creek kokanee.

Collection code	Collection location	Species	Total # collected	# samples used
96ET	Lake Ozette	sockeye	30	25
99OE	Meadow Creek	kokanee	100	94
00DW	Bear Creek	kokanee	48	41
00DX	Lewis Creek	kokanee	100	72
00DY	Laughing Jacobs Creek	kokanee	55	53
00HA	Ebright Creek	kokanee	100	74
00HB	Little Bear Creek	kokanee	27	16
00LE	Whatcom hatchery	kokanee	100	96
00LF	Baker Lake	sockeye	100	92
00MX	Issaquah Creek	sockeye	61	60
00MY	Bear Creek	sockeye	52	42
05MX*	Webster Creek	kokanee	87	87

* - the Webster Creek collection was made in 2002, but issued a code for 2005. All data files identify the collection with the 05MX code.

Table 2. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci. Also included are the P-values for deviations from Hardy-Weinberg equilibrium (HWE) and the observed and expected heterozygosity (H_e and H_o) for each locus and collection.

						Heterozygosity	
Multiplex	Locus	Annealing temp ^oC	# Alleles/ Locus	Allele Size Range (bp)	HWE - pvalue	Observed	Expected
One-A	*One-100**	50	46	245 - 484	x	x	x
	*One-108**	50	25	179 - 273	0.3257	0.8458	0.9169
	*One110**	50	33	209 - 297	0.2862	0.8668	0.9266
One-B	*One-102**	50	17	196 - 260	0.6049	0.8273	0.8696
	*One-114**	50	25	201 - 297	0.1451	0.8788	0.9232
	*One-115**	50	16	176 - 237	0.6675	0.8450	0.9054
One-C	*One-101**	50	47	171 - 355	0.4980	0.9033	0.9472
	*One-105**	50	7	126 - 155	0.1164	0.6000	0.7191
	*Ots-103**	50	24	131 - 231	0.1160	0.8762	0.9241

*One-100** was dropped from statistical analyses.

Table 3. Expected and Observed Heterozygosity, Hardy-Weinberg p-value, and F_{IS} for each collection location.

	Heterozygosity		HWE	
	Observed	Expected	p-value	F_{IS}
Bear Cr. kokanee	0.8919	0.9315	0.4184	-0.008
Lewis Cr. kokanee	0.8429	0.8081	0.1796	0.026
Laughing Jacobs Cr. kokanee	0.8431	0.8672	0.4211	0.017
Ebright Cr. kokanee	0.8286	0.8339	0.6906	-0.016
L Bear Cr. kokanee	0.7857	0.9127	0.8895	-0.031
Whatcom Hatchery kokanee	0.9647	0.9350	0.0421	0.023
Baker L. sockeye	0.7722	0.8170	0.2004	0.004
Issaquah Cr. sockeye	0.8269	0.9029	0.0327	0.025
Bear Cr. sockeye	0.8919	0.9100	0.3559	0.013
Webster Cr. kokanee	0.6923	0.6484	0.8860	-0.029
L. Ozette sockeye	0.8636	0.8626	0.4546	0.016
Meadow Cr. kokanee	0.9302	0.9133	0.7097	0.001

Table 4. Genotypic differentiation of kokanee and sockeye collections analyzed. Pairwise comparisons that were not-significantly different are highlighted in black with white type. Pairwise comparisons were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989; 78 comparisons; alpha = 0.05/78 = 0.0006).

	Bear Cr. kokanee	**Lewis Cr.** kokanee	**Laughing Jacobs Cr.** kokanee	**Ebright Cr.** kokanee	**L Bear Cr.** kokanee	**Whatcom Hatchery** kokanee	**Baker L.** sockeye	**Issaquah Cr.** sockeye	**Bear Cr.** sockeye	**Webster Cr.** kokanee	**L. Ozette** sockeye	**Meadow Cr.** kokanee
Bear Cr. kokanee	----											
Lewis Cr. kokanee	0.0000	----										
Laughing Jacobs Cr. kokanee	0.0000	0.0000	----									
Ebright Cr. kokanee	0.0000	0.0000	0.0000	----								
L Bear Cr. kokanee	**0.5333**	0.0000	0.0000	0.0000	----							
Whatcom Hatchery kokanee	0.0000	0.0000	0.0000	0.0000	0.0000	----						
Baker L. sockeye	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	----					
Issaquah Cr. sockeye	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	----				
Bear Cr. sockeye	**0.0733**	0.0000	0.0000	0.0000	**0.0038**	0.0000	0.0000	0.0000	----			
Webster Cr. kokanee	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	----		
L. Ozette sockeye	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	----	
Meadow Cr. kokanee	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	----

Table 5. Allelic richness and gene diversity per locus and population of kokanee and sockeye in the Lake Sammamish and Cedar River basin with several outlying collections for comparison.

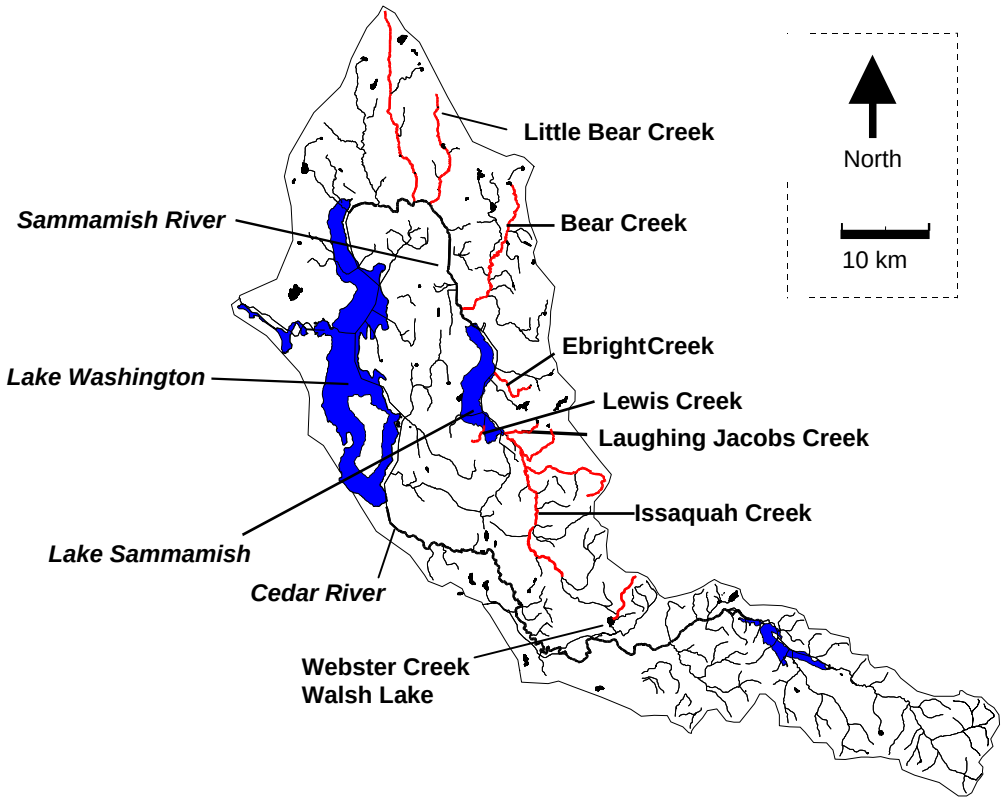
Allelic Richness

	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Walsh Lake kokanee	L. Ozette sockeye	Meadow Cr. kokanee
One-108	12.74	7.78	9.07	9.45	10.71	13.20	7.48	11.12	11.44	4.77	8.49	10.91
One-110	11.49	10.35	9.13	10.34	9.32	15.29	8.47	11.80	11.35	5.05	6.10	12.07
One-102	10.03	7.64	6.50	6.35	11.00	10.23	6.05	7.94	8.34	3.29	5.10	10.96
One-114	13.79	9.72	9.93	9.42	12.12	12.45	10.04	12.48	12.86	6.33	8.99	12.41
One-115	11.62	8.81	8.75	8.91	10.19	10.22	7.60	10.85	10.80	4.47	4.27	9.98
One-101	16.68	11.24	12.61	11.91	15.41	18.06	10.56	13.59	13.64	6.19	9.74	17.22
One-105	5.01	3.82	3.59	3.50	5.59	4.11	4.28	4.20	4.73	2.77	2.91	4.01
Ots-103	10.70	10.22	9.66	9.65	12.78	12.21	7.52	11.48	12.53	6.69	7.62	14.14
Average	11.51	8.70	8.65	8.69	10.89	11.97	7.75	10.43	10.71	4.94	6.65	11.46

Gene Diversity

	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Walsh Lake kokanee	L. Ozette sockeye	Meadow Cr. kokanee
One-108	0.93	0.81	0.87	0.83	0.92	0.94	0.82	0.90	0.91	0.65	0.86	0.91
One-110	0.89	0.89	0.85	0.88	0.82	0.95	0.86	0.91	0.91	0.75	0.76	0.91
One-102	0.88	0.84	0.74	0.78	0.93	0.90	0.82	0.83	0.86	0.67	0.73	0.91
One-114	0.94	0.88	0.87	0.87	0.93	0.93	0.88	0.92	0.93	0.79	0.89	0.92
One-115	0.92	0.85	0.78	0.84	0.89	0.88	0.85	0.91	0.91	0.67	0.58	0.89
One-101	0.96	0.90	0.90	0.91	0.95	0.97	0.90	0.93	0.92	0.75	0.81	0.96
One-105	0.72	0.63	0.66	0.68	0.64	0.71	0.67	0.73	0.70	0.32	0.41	0.58
Ots-103	0.91	0.90	0.86	0.86	0.93	0.93	0.82	0.92	0.93	0.80	0.84	0.94
Average	0.89	0.84	0.81	0.83	0.88	0.90	0.83	0.88	0.88	0.67	0.74	0.88

Figure 1. Map of collection sites in the Lake Washington basin



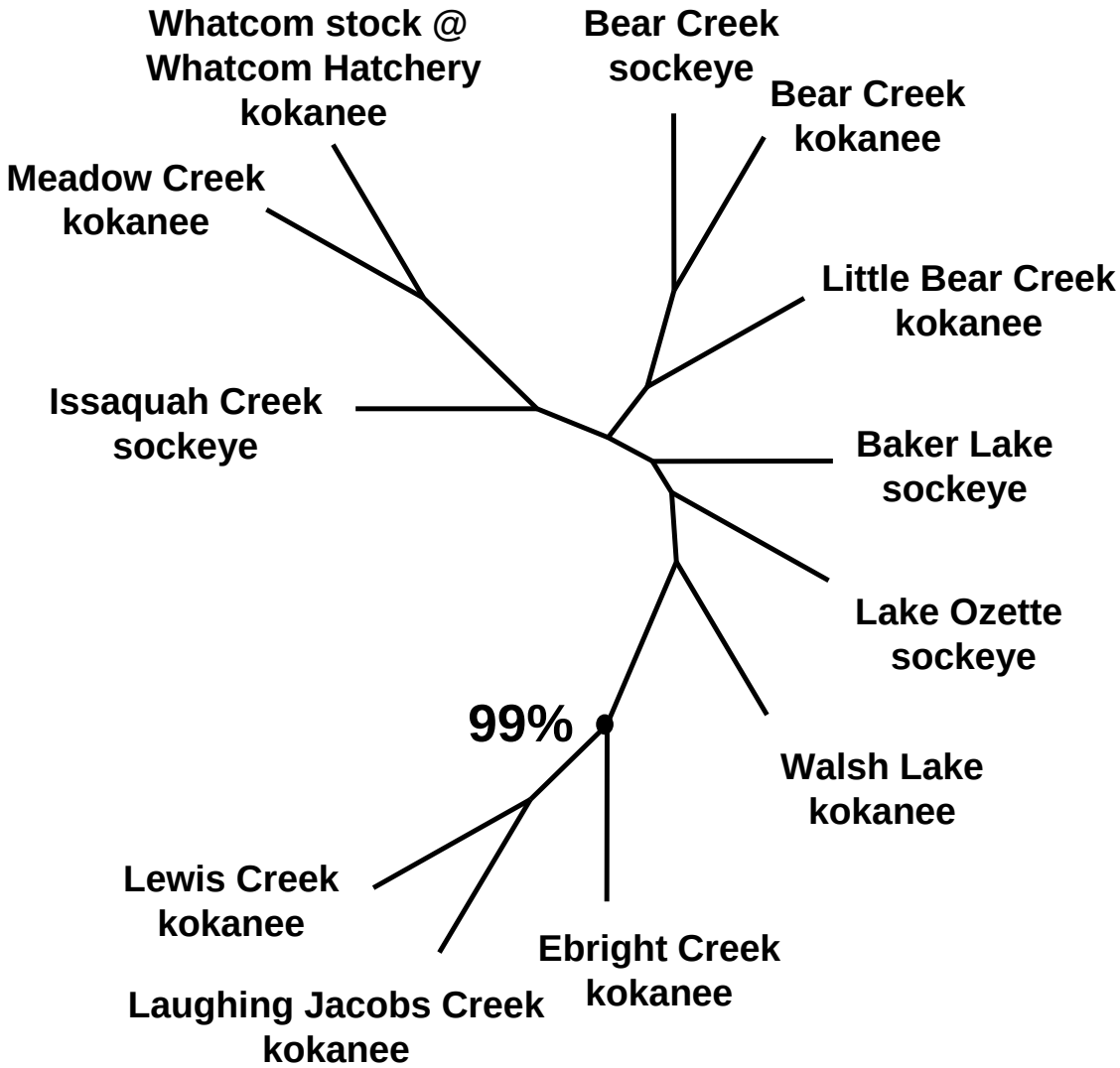


Figure 2. Relationship of 12 kokanee and sockeye populations from tributaries of Cedar River (Walsh Lake), Lake Sammamish, Lake Whatcom Hatchery, Baker Lake, and Meadow Creek (Lake Kootenay - British Columbia) based on the genetic distance matrix using Cavalli-Sforza and Edwards (1967) chord distance. The cluster of the three tributaries from the Lake Sammamish basin are the only ones to have bootstrap support (99%).

Appendix 1. Allele frequencies

One-108	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	37	70	51	70	14	85	79	52	37	78	22	86
179	---	---	---	---	---	0.006	---	---	---	---	---	---
183	0.041	---	---	---	0.036	0.012	---	---	0.068	---	---	---
187	0.014	---	---	---	0.036	0.077	---	0.010	0.014	---	---	---
191	0.014	---	---	---	---	0.082	0.044	0.058	0.027	---	0.023	0.087
195	0.068	---	0.010	0.014	0.071	0.094	0.304	0.019	0.068	---	0.159	0.058
199	0.081	---	---	---	0.143	0.024	0.241	0.067	---	0.026	---	0.087
203	0.068	0.057	---	0.036	0.071	0.047	0.070	0.048	0.068	---	0.205	0.105
207	0.095	0.014	0.029	0.007	---	0.088	0.082	0.058	0.081	0.180	0.227	0.140
209	0.041	---	---	---	---	---	---	---	---	---	---	---
212	0.149	0.121	0.177	0.071	0.179	0.118	0.025	0.077	0.162	0.013	0.023	0.111
215	0.108	0.036	0.069	0.064	0.107	0.077	0.019	0.183	0.176	0.532	0.159	0.111
218	---	---	0.078	0.036	---	---	---	---	---	---	---	---
220	0.081	---	0.029	0.036	0.143	0.077	0.076	0.164	0.054	0.006	0.023	0.081
224	0.054	0.150	0.118	0.186	---	0.024	---	0.058	0.122	0.192	---	0.064
228	0.014	0.064	0.020	0.057	0.036	0.035	---	0.048	0.027	---	---	0.081
232	0.068	0.364	0.245	0.336	0.143	0.047	0.133	0.135	0.054	0.051	0.068	0.023
236	0.054	0.021	0.029	0.036	0.036	0.053	---	0.039	0.027	---	---	0.017
240	0.041	0.043	0.059	0.071	---	0.065	0.006	0.010	0.041	---	---	0.006
244	---	---	---	0.007	---	0.024	---	0.019	0.014	---	---	0.017
248	---	---	---	---	---	0.029	---	0.010	---	---	---	0.006
252	0.014	---	---	---	---	0.006	---	---	---	---	---	---
256	---	---	---	---	---	0.018	---	---	---	---	---	---
260	---	0.129	0.137	0.043	---	---	---	---	---	---	0.046	---
264	---	---	---	---	---	---	---	---	---	---	0.068	---
273	---	---	---	---	---	---	---	---	---	---	---	0.006

Appendix 1 continued.

One-110	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	38	70	50	70	16	83	75	54	37	83	21	86
209	---	0.021	0.020	0.121	---	---	---	---	---	---	---	---
221	0.013	---	---	---	---	---	---	---	0.014	---	---	---
225	0.013	---	---	---	---	---	0.073	0.019	0.054	0.018	---	---
229	0.013	---	---	0.093	0.031	0.006	---	0.009	0.041	---	---	---
233	0.040	0.071	0.040	0.064	0.031	---	---	0.009	0.014	0.181	0.024	0.017
235	0.013	---	---	---	---	0.006	---	---	---	---	---	---
237	0.013	0.229	0.290	0.257	0.063	0.030	0.047	0.093	0.081	---	0.048	0.052
241	0.237	0.129	0.090	0.064	0.125	0.072	0.147	0.120	0.122	0.386	0.214	0.169
245	0.079	0.043	0.060	0.021	0.156	0.072	0.253	0.065	0.135	0.024	---	0.145
248	---	---	---	---	---	0.048	---	---	---	---	---	0.006
250	0.040	0.129	0.200	0.129	0.031	0.060	0.040	0.093	0.068	---	---	0.140
251	0.026	---	---	---	---	0.018	---	---	0.014	---	---	0.029
253	0.118	0.036	0.010	0.029	0.063	0.024	0.040	0.083	0.081	0.006	0.143	0.064
255	---	---	---	---	---	0.030	---	---	---	---	---	0.006
257	0.026	0.029	---	0.007	---	0.018	0.007	0.046	0.027	---	0.405	0.058
259	0.013	0.014	---	---	---	0.042	---	---	---	---	---	0.006
261	0.197	0.086	0.040	0.064	0.375	0.024	0.060	0.194	0.176	---	0.143	0.052
263	---	---	---	---	---	0.108	---	0.009	---	---	---	0.006
265	0.013	0.007	0.020	0.014	0.063	0.024	0.013	0.028	0.041	---	---	0.076
267	---	---	---	---	---	0.042	---	---	---	---	---	0.012
269	0.053	0.007	0.010	0.007	---	0.054	0.153	0.065	0.108	0.163	0.024	0.047
271	---	---	---	---	---	0.090	---	---	---	---	---	---
273	0.040	0.036	0.090	0.021	---	0.006	0.160	0.056	---	---	---	0.035
275	---	---	---	---	0.063	0.054	---	---	---	---	---	0.035
277	---	0.079	0.030	0.007	---	0.036	---	0.046	0.014	0.223	---	0.006
279	---	---	---	---	---	0.024	---	---	---	---	---	0.006
281	0.040	---	---	0.036	---	0.048	---	0.037	0.014	---	---	0.006
283	---	---	---	---	---	0.012	---	---	---	---	---	0.017
285	0.013	---	---	---	---	0.036	---	0.028	---	---	---	---
289	---	0.086	0.100	0.064	---	0.006	---	---	---	---	---	0.006
292	---	---	---	---	---	---	---	---	---	---	---	0.006
293	---	---	---	---	---	0.006	---	---	---	---	---	---
297	---	---	---	---	---	---	0.007	---	---	---	---	---

Appendix 1 continued.

One-102	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	38	57	52	58	13	93	89	56	39	84	21	89
196	---	---	---	---	---	---	---	---	---	---	---	0.023
200	0.053	---	---	---	0.039	---	0.107	0.036	---	0.012	---	0.006
204	---	---	---	---	---	---	---	---	---	---	---	0.146
208	0.040	---	---	---	0.115	0.075	---	---	0.013	---	---	0.017
212	---	---	---	---	0.039	0.038	---	---	0.013	---	---	---
216	---	0.026	---	0.009	---	0.027	---	---	---	---	---	0.011
220	0.026	0.088	0.039	0.052	0.039	0.032	0.006	0.018	---	---	0.048	0.062
225	0.105	0.026	0.058	0.035	0.115	0.145	---	0.009	0.077	---	---	0.140
229	0.026	0.184	0.433	0.190	0.077	0.065	---	0.027	0.115	---	0.024	0.124
233	0.118	0.070	0.058	0.078	0.115	0.108	---	0.107	0.167	---	0.357	0.073
237	0.250	0.175	0.087	0.233	0.115	0.134	0.135	0.259	0.231	0.405	0.214	0.124
241	0.105	0.281	0.260	0.353	0.115	0.113	0.242	0.250	0.167	0.327	0.333	0.067
245	0.118	0.114	0.048	0.052	---	0.161	0.253	0.143	0.141	0.256	0.024	0.073
249	0.092	0.026	0.019	---	0.154	0.043	0.135	0.080	0.026	---	---	0.062
253	0.013	0.009	---	---	0.077	0.038	---	0.018	0.026	---	---	0.028
257	0.026	---	---	---	---	0.022	0.124	0.054	0.013	---	---	0.028
260	0.026	---	---	---	---	---	---	---	0.013	---	---	0.017

Appendix 1 continued.

One-114	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	41	66	53	70	16	94	92	56	41	84	21	92
201	---	---	---	---	---	0.005	---	---	---	---	---	---
205	---	0.023	0.047	0.050	0.031	0.005	---	0.009	---	---	---	---
209	---	---	---	---	---	---	---	0.009	---	---	---	---
213	0.049	0.023	0.019	---	---	0.011	---	---	0.012	---	---	---
217	0.024	0.061	0.066	0.021	---	0.027	0.005	0.036	0.037	0.054	---	0.005
221	0.037	0.121	0.132	0.157	0.031	0.005	---	0.018	0.012	0.250	---	0.011
226	0.037	---	---	---	0.094	---	0.027	---	0.012	---	---	0.033
230	0.024	0.053	0.028	0.029	---	0.021	0.011	0.045	0.073	---	---	---
234	0.049	---	---	---	0.031	0.043	0.005	0.036	0.049	---	---	0.044
238	0.012	0.083	0.085	0.057	---	0.069	0.065	0.018	0.012	---	---	0.054
242	0.073	0.030	0.019	0.021	0.125	0.090	0.228	0.080	0.073	0.006	0.191	0.044
246	0.085	0.114	0.028	0.100	0.125	0.090	0.087	0.098	0.122	0.036	0.048	0.141
250	0.061	0.053	0.009	0.036	---	0.138	0.141	0.071	0.134	---	---	0.125
254	0.073	0.227	0.151	0.229	0.063	0.053	0.147	0.036	0.012	0.196	---	0.136
258	0.085	0.023	0.104	0.086	0.063	0.096	0.065	0.045	0.024	---	0.143	0.076
261	0.085	0.174	0.264	0.179	0.063	0.069	0.044	0.116	0.073	0.316	0.119	0.082
265	0.134	---	0.009	0.021	0.156	0.106	0.054	0.188	0.146	0.066	0.143	0.060
269	0.049	0.008	0.019	---	0.063	0.053	---	0.071	0.061	---	0.167	0.022
273	0.073	---	0.009	---	0.125	0.016	0.016	0.036	0.037	---	0.024	0.027
277	0.012	---	---	0.007	---	0.064	0.076	0.054	0.024	0.077	0.024	0.049
281	0.012	0.008	0.009	0.007	0.031	0.021	0.022	---	0.061	---	---	0.011
285	0.012	---	---	---	---	0.011	0.005	---	0.012	---	0.071	0.038
289	---	---	---	---	---	0.005	---	---	---	---	0.071	0.027
293	0.012	---	---	---	---	---	---	---	---	---	---	0.011
297	---	---	---	---	---	---	---	0.036	0.012	---	---	0.005

Appendix 1 continued.

One-115	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	41	60	53	67	16	94	92	57	42	80	22	92
176	0.012	---	---	---	---	0.011	---	---	0.024	---	---	0.016
180	0.024	0.008	---	---	0.031	0.037	---	0.061	0.024	---	---	0.027
184	0.012	0.008	0.019	0.037	---	0.037	0.027	0.079	---	---	0.023	0.054
188	0.024	0.017	0.028	0.022	0.125	0.048	---	0.026	0.119	---	---	0.082
192	0.061	0.033	0.009	---	---	0.059	0.005	0.044	0.107	---	---	0.103
196	0.122	0.150	0.132	0.037	0.156	0.064	0.120	0.132	0.119	0.163	---	0.174
200	0.159	0.125	0.019	0.202	0.250	0.122	0.158	0.158	0.179	0.019	0.568	0.163
204	0.049	0.017	0.028	0.060	0.031	0.202	0.103	0.088	0.048	---	0.046	0.114
208	0.110	0.083	0.076	0.045	0.063	0.223	0.125	0.070	0.095	0.013	0.318	0.114
212	0.073	---	0.019	0.022	0.063	0.069	0.082	0.097	0.071	0.475	0.046	0.071
216	0.134	0.108	0.047	0.067	0.125	0.021	0.250	0.132	0.119	0.281	---	0.044
220	0.061	0.058	0.076	0.015	0.094	0.032	0.125	0.053	0.024	0.050	---	0.011
225	0.049	0.300	0.434	0.306	0.031	0.043	---	0.018	0.024	---	---	0.005
229	0.073	0.075	0.085	0.119	0.031	0.027	0.005	0.035	0.012	---	---	0.016
233	0.012	0.017	0.028	0.067	---	0.005	---	---	0.012	---	---	---
237	0.024	---	---	---	---	---	---	0.009	0.024	---	---	0.005

Appendix 1 continued.

One-101	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	39	68	49	69	14	94	77	60	37	80	23	93
171	---	---	---	---	---	---	---	---	---	---	---	0.005
175	---	---	---	---	---	0.011	---	---	---	---	---	---
179	---	---	0.010	---	---	0.021	---	---	---	---	---	---
183	---	---	---	---	---	---	0.013	---	---	---	---	0.011
187	0.026	---	---	0.022	0.107	0.021	---	0.017	---	---	---	0.027
191	0.039	---	---	---	0.036	0.032	0.013	---	0.014	---	---	---
195	---	---	---	---	---	0.064	---	---	---	---	0.022	---
199	0.013	---	0.010	---	---	0.021	---	0.008	---	---	0.022	0.027
203	0.013	---	0.031	0.015	---	0.048	---	0.017	0.027	---	0.022	0.032
207	0.051	0.007	0.020	0.007	---	0.059	---	0.008	---	---	0.022	0.005
211	---	0.096	0.092	0.073	---	0.021	---	0.017	0.041	---	---	---
215	0.039	0.037	0.071	0.058	0.036	0.032	0.033	0.008	0.014	0.025	0.065	0.005
219	0.013	---	---	---	---	0.048	---	0.017	---	---	0.370	0.005
223	0.013	---	---	---	---	0.032	0.013	0.008	---	---	0.087	---
228	0.013	---	---	0.007	---	0.053	---	0.017	0.014	---	0.217	0.005
232	0.013	---	---	---	0.071	0.021	0.007	---	---	---	0.022	---
236	0.039	---	---	---	0.071	0.027	---	---	---	---	---	0.022
240	---	---	---	---	---	0.011	---	---	---	---	---	---
244	---	---	---	---	---	0.005	---	---	---	---	---	0.011
248	0.013	---	---	---	0.036	0.011	0.007	---	---	---	---	---
252	0.013	---	---	---	---	0.005	---	---	0.014	---	---	0.011
256	0.013	---	---	---	---	0.005	---	0.008	---	---	---	0.005
259	---	---	---	---	---	---	---	---	---	---	---	0.005
263	---	---	---	---	---	0.005	---	---	---	---	---	0.011
267	---	---	---	---	---	0.005	---	---	0.014	---	---	0.032
271	0.013	0.007	0.020	---	---	0.011	---	---	0.014	---	---	0.027
275	---	---	---	---	---	0.032	---	---	---	---	---	0.027
279	---	0.015	---	0.007	---	---	---	0.033	0.027	---	0.044	0.070
283	0.013	0.007	0.041	0.044	---	0.027	---	0.083	0.014	0.006	---	0.038
287	0.026	0.088	---	0.007	---	0.016	0.058	---	0.054	0.013	---	0.065
291	---	0.015	---	0.015	0.036	0.027	0.007	0.033	0.014	0.019	---	0.038
295	0.064	0.007	0.020	---	0.036	0.048	0.020	0.100	---	0.300	---	0.043
299	0.103	---	---	0.015	0.071	0.016	0.110	0.058	0.068	0.019	---	0.048

Appendix 1 continued.

One-101 cont.	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	39	68	49	69	14	94	77	60	37	80	23	93
303	0.090	0.184	0.245	0.181	0.071	0.048	0.136	0.150	0.041	0.344	0.022	0.075
307	0.051	0.177	0.041	0.051	0.071	0.053	0.058	0.100	0.108	---	0.044	0.081
311	0.064	0.118	0.071	0.101	---	0.048	0.058	0.092	0.041	0.025	0.044	0.032
315	0.077	---	---	---	0.179	0.011	0.110	0.050	0.135	---	---	0.027
319	0.013	0.044	0.041	0.080	---	0.011	---	0.017	0.014	---	---	0.043
323	0.051	0.022	0.020	0.058	0.036	0.037	0.195	0.067	0.054	0.200	---	0.032
326	0.051	---	0.041	---	0.071	0.027	0.130	---	0.189	---	---	0.032
331	---	0.052	0.122	0.167	---	0.021	0.020	0.025	0.014	0.006	---	0.043
335	0.039	0.037	0.041	0.022	---	0.005	---	0.017	0.068	---	---	0.005
339	0.013	0.052	0.031	0.015	0.036	---	---	0.050	---	0.044	---	0.022
343	0.026	0.022	0.010	0.007	0.036	---	---	---	0.014	---	---	0.005
347	---	0.007	0.020	---	---	---	---	---	---	---	---	0.005
351	---	0.007	---	---	---	0.005	0.013	---	---	---	---	0.016
355	---	---	---	0.051	---	---	---	---	---	---	---	0.005

One-105	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	38	69	51	73	16	94	77	60	30	85	24	93
126	0.026	0.007	---	0.007	0.031	---	0.013	0.008	0.050	---	---	---
130	0.276	0.500	0.333	0.356	0.219	0.122	0.195	0.342	0.450	0.053	0.188	0.263
134	0.224	0.333	0.451	0.308	0.094	0.170	0.169	0.150	0.150	0.135	---	0.586
138	0.395	0.123	0.186	0.315	0.563	0.346	0.500	0.342	0.283	0.812	0.750	0.091
142	0.040	0.036	0.029	0.014	0.063	0.356	0.123	0.158	0.067	---	0.063	0.043
147	---	---	---	---	---	0.005	---	---	---	---	---	0.016
155	0.040	---	---	---	0.031	---	---	---	---	---	---	---

Appendix 1 continued.

Ots-103	Bear Cr. kokanee	Lewis Cr. kokanee	Laughing Jacobs Cr. kokanee	Ebright Cr. kokanee	L Bear Cr. kokanee	Whatcom Hatchery kokanee	Baker L. sockeye	Issaquah Cr. sockeye	Bear Cr. sockeye	Webster Cr. kokanee	L. Ozette sockeye	Meadow Cr. kokanee
	41	71	52	74	16	96	91	59	40	85	24	94
131	---	---	---	---	---	---	---	---	---	---	---	0.005
140	---	---	---	---	---	---	---	---	---	---	---	0.005
144	---	---	---	---	---	0.016	---	---	---	---	---	0.032
148	---	---	---	---	---	0.010	0.006	---	---	---	---	0.016
152	---	---	---	---	---	0.047	---	0.009	---	---	---	0.016
156	---	---	0.010	---	0.031	0.052	---	0.042	0.025	---	0.271	0.059
160	0.073	0.021	0.019	0.027	---	0.083	0.006	0.017	0.038	---	---	0.053
163	0.061	0.113	0.058	0.108	0.063	0.099	0.017	0.119	0.088	0.077	---	0.064
167	0.049	0.042	0.125	0.034	0.031	0.089	0.077	0.119	0.088	0.012	0.042	0.032
171	0.012	0.092	0.010	0.061	0.031	0.125	0.011	0.136	0.013	---	0.021	0.059
175	0.159	0.035	0.077	0.074	---	0.037	0.297	0.136	0.100	---	0.042	0.075
179	0.085	0.162	0.298	0.297	0.156	0.037	0.214	0.034	0.038	---	0.208	0.075
183	0.159	0.106	0.029	0.047	0.094	0.063	0.050	0.085	0.050	0.259	---	0.090
187	0.061	0.127	0.135	0.054	0.094	0.109	0.033	0.068	0.075	0.312	---	0.043
190	0.073	0.099	0.048	0.115	0.156	0.083	0.187	0.051	0.088	0.077	---	0.048
194	0.098	---	0.019	---	0.063	0.042	0.088	0.068	0.100	0.147	0.104	0.117
198	0.085	0.007	0.019	0.014	0.125	0.068	---	0.034	0.113	0.065	0.208	0.080
202	0.012	0.049	0.096	0.020	0.031	0.037	0.011	0.009	0.075	---	0.021	0.021
206	0.073	0.106	0.058	0.122	0.063	---	0.006	0.009	0.063	0.053	---	0.027
210	---	0.042	---	0.027	0.031	---	---	0.059	0.038	---	0.083	0.043
214	---	---	---	---	0.031	0.005	---	0.009	0.013	---	---	0.021
218	---	---	---	---	---	---	---	---	---	---	---	0.005
223	---	---	---	---	---	---	---	---	---	---	---	0.011
231	---	---	---	---	---	---	---	---	---	---	---	0.005