

**A Microsatellite DNA Based Characterization of
Lake Washington/Lake Sammamish
Kokanee and Sockeye Salmon,
with Notes on Distribution, Timing, and Morphology**

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Summary

Microsatellite DNA data for 9 loci in 774 fish were used to characterize collections of kokanee and sockeye salmon (*Oncorhynchus nerka*) from the Lake Washington/Lake Sammamish watershed. These data were analyzed to elucidate the genetic interrelationships among the populations represented by these collections and to evaluate possible effects of known or hypothetical historical kokanee introductions. High levels of genetic variation were seen at all loci (5-49 alleles per locus) and genotypic proportions were in Hardy-Weinberg equilibrium in the vast majority of cases. Explicit tests of population subdivision revealed that all collections were significantly different from one another except for the comparison of the combined Bear, Little Bear, and North creeks kokanee collection vs. the combined Bear and North creeks sockeye collection. F_{ST} values indicated population subdivision among many of the collections. Multidimensional scaling plots using the F_{ST} data revealed that the early-run (August/September) Issaquah Creek kokanee and the late-run (November/December) kokanee in the three Lake Sammamish tributaries (Lewis, Ebright, and Laughing Jacobs creeks) were distinct from other *O. nerka* in the watershed and that they were quite different from both the Lake Whatcom (north Puget Sound) and Meadow Creek (upper Columbia River) kokanee stocks. We concluded that there was no compelling evidence that either of these two groups of kokanee (early-run Issaquah Creek or late-run Lake Sammamish tributaries) has resulted from, or been substantially altered by, past introductions of non-native kokanee or sockeye.

Introduction

The Lake Washington/Lake Sammamish basin is one of five watersheds in Washington that historically supported native kokanee (*Oncorhynchus nerka*) populations (reviewed in Pfeifer, 1995, and Hendry, 1996). Additionally, it is one of about seven watersheds that supports native populations of sockeye salmon, the anadromous form of (*O. nerka*) (Shaklee et al., 1996). Despite being considered the same species, sockeye (anadromous) and kokanee (non-anadromous) are biologically and genetically distinct (Foote et al., 1989, Wood and Foote, 1990; Winans et al., 1993).

Historically, the Bear Creek system supported September/October-run kokanee populations estimated to number in the tens of thousands based on annual egg take records of the Washington Department of Game (WDG). Prior to 1982, the August/September-run of kokanee spawning in Issaquah Creek numbered between 400 and 1,000 (or more) fish annually (Pfeifer, 1995 and Berggren, 1974 cited in Pfeifer). Other tributaries to Lake Sammamish have supported November/December-runs of kokanee at least since the 1930's. However, little is known about these fish and they have often been assumed (e.g., Fletcher, 1973) to have originated from the substantial fry plants of Lake Whatcom kokanee prior to 1978 (Hendry, 1995; Pfeifer, 1995; Ostergaard, 1996).

Few historical records exist regarding the past status of anadromous sockeye in Bear Creek or Issaquah Creek. Large numbers of Baker River (Skagit River) and Cultus River (British

Columbia) stocks were introduced in the first half of the 20th century suggesting numbers of native sockeye were relatively low (Hendry, 1996). However, several investigations of genetic relationships among Lake Washington/Sammamish sockeye have revealed the distinctness of Bear Creek sockeye with respect to Baker River and Cultus stocks and pointed to a strong native component in these fish (Seeb and Wishard, 1977; Hendry, 1996; Gustafson et al., 1997).

In recent years, several of these populations have experienced declining abundances or relatively low abundance over time (Issaquah kokanee Pfeifer, 1995, Berggren, 1974 cited in Pfeifer; Lake Washington/Sammamish tributaries and Lake Washington beach spawning sockeye Washington Department of Fish and Wildlife and Western Washington Treaty Tribes, 1994; late-run Lake Sammamish tributary kokanee Ostergaard, 1996 & 1998). While the causes for these declines have not been rigorously established, they likely include myriad effects due to urbanization in the region; loss/degradation of habitat; harvest; introduction and proliferation of non-native fish species; and some hatchery management practices aimed at eliminating kokanee as a means of controlling IHN disease.

Particular concern has been focused on the early-run kokanee population in Issaquah Creek for three reasons. First, the abundance of this population has declined to extremely low numbers in recent years. Second, this population has been described as exhibiting "early entry run timing" with spawning occurring primarily in mid August. This period of spawning contrasts with that of other kokanee and sockeye populations in the basin, which is late September through early January (Pfeifer, 1995; Ostergaard, 1998). Third, the genetic distinctiveness of the early-run Issaquah Creek kokanee population has been previously recognized based on both allozyme electrophoretic data (Seeb and Wishard, 1977; Hendry, 1995; Hendry et al., 1996) and, more recently, microsatellite DNA data (Bentzen and Spies, 2000). In contrast, little attention has been paid to the late run kokanee of the Sammamish Basin, primarily because managers assumed that they originated from Lake Whatcom stock fish planted in the basin from 1940 to 1978. However, no genetic evidence currently exists to support or refute this assumption.

The present study was undertaken to genetically characterize the kokanee and sockeye populations in the Lake Washington/Lake Sammamish basin, to assess their interrelationships, and to investigate whether or not some or all of the currently extant populations were derived from plants of hatchery fish from other areas. Microsatellite DNA markers (Wright and Bentzen, 1994) were chosen because earlier work done on sockeye and kokanee using allozyme electrophoresis (e.g., Wood et al., 1994; Winans et al., 1996) revealed relatively low levels of detectable genetic variation consisting of relatively few variable loci and we believed that more variable genetic markers were needed in this study. Indeed, a number of recent investigations have demonstrated the power of microsatellite DNA markers to elucidate population structure (Small et al., 1998, Banks et al., 1999, 2000, Beacham et al., 1999a & b, Olsen et al., 2000a, Shaklee and Young, 2000, Young and Shaklee, 2000 and 2001).

Materials and Methods

Spawner surveys and sample collection

Washington Department of Fish and Wildlife (WDFW) and King County Department of Natural Resources (KCDNR) personnel conducted weekly spawner escapement surveys on index reaches of Issaquah Creek (RM [river mile] 1.2 to 4.5) and East Fork Issaquah Creek (RM 0 to 3.5) between 1 August and 10 September for early-run kokanee. From 18 September to 30 October, WDFW and KCDNR personnel surveyed index reaches of Bear Creek (RM 0.2 to 0.8 and RM 3.5 to 5.5), Little Bear Creek (RM 2.0 to 3.5), and North Creek (RM 1.0 to 2.5). From 30 October to 7 January, WDFW and KCDNR personnel surveyed index reaches of Lewis Creek (RM 0 to 0.5), Ebright Creek (RM 0 to 0.2), and Laughing Jacobs Creek (RM 0 to 1.0). Index reaches were designated to contain known spawning habitat and subsequent aggregations of kokanee and sockeye. During each survey, counts of live fish and of carcasses were made. Conservative escapement estimates were based on live fish counts (due to variability of carcass persistence in the streams and poor confidence in estimating proportions of unobserved fish). This process assumed an average stream life of 12 days. During the surveys, and on other occasions, fin tissue samples for subsequent DNA analysis were collected from both carcasses and live fish, generally in proportion to the estimated numbers of fish in the stream.

DNA samples and extractions

We analyzed a total of 774 *O. nerka* from 13 collections in this study (see table below). The map of the Lake Washington/Lake Sammamish basin in Figure 1 shows the locations of the streams sampled in the region. Samples from three locations outside of this region were also included in the study to provide additional perspective on the results. One was a collection of kokanee from

collection code	Collection name (WRIA ¹ number)	species/form	N
00HA	Ebright Creek (08.0149)	kokanee	100
00DX	Lewis Creek (08.0162)	kokanee	100
00DY	Laughing Jacobs Creek (08.0166)	kokanee	55
00LE	Lake Whatcom WDFW Hatchery	kokanee	100
00DW	Bear Creek ² (08.0105)	kokanee	48
00HB	Little Bear Creek ² (08.0080)	kokanee	25
00HC	North Creek ² (08.0070)	kokanee	9
93WA	Issaquah Creek (08.0178)	early kokanee	13
00RP	North Creek ³	sockeye	11
00MY	Bear ³ Creek	sockeye	52
00MX	Issaquah Creek	sockeye	61
00LF	Baker Lake	sockeye	100
99OE	Meadow Creek (B.C.) Hatchery	kokanee	100

¹ WRIA = Water Resource Inventory Area

² these three collections were combined for most statistical analyses

² these two collections were combined for most statistical analyses

Lake Whatcom, which is located just east of the town of Bellingham in northern Puget Sound. This stock has been propagated at a WDFW hatchery for decades and has been introduced into many locations throughout Washington, including the Lake Washington/Lake Sammamish basin. Another was a collection of kokanee from Meadow Creek, and tributary to the North Arm of Lake Kootenai in British Columbia, from the upper Columbia River basin. The third was a collection of sockeye from Baker Lake in the Skagit River drainage in north Puget Sound. One of the 13 collections (Issaquah Creek early-run kokanee) was obtained in 1993, one was taken in 1999 (Meadow Creek kokanee), and the remaining eleven collections were obtained in 2000. The collection of Meadow Creek kokanee consisted of juveniles sampled at the Spokane Hatchery. All other collections were of adult fish. Small fin clip or opercle punches from adults or whole juveniles were preserved in 100% ethanol until DNA was extracted.

DNA extractions for three sets of samples (samples 00HA 1-100, 00DX 1-92, and 99OE 1-100) were done using an ammonium acetate precipitation purification following proteinase K digestion of the tissue samples. DNA extractions for all other samples were done using commercially available, 96-well silica membrane based kits (i.e., Machery-Nagel Nucleospin multi-96 tissue kits or Qiagen DNeasy 96 tissue kits).

DNA amplification

We amplified the nine microsatellite DNA loci of interest via the polymerase chain reaction (PCR; see Saiki et al., 1988) using fluorescently labeled primers. All of the loci screened are reported to be tetranucleotide repeats, eight of them were isolated from sockeye salmon (Olsen et al., 2000b) and one (*Ots-103*) was isolated from chinook salmon, *Oncorhynchus tshawytscha* (Small et al., 1998). The specific PCR amplification protocols we used were:

PCR multiplex OneA: One-108 at 0.06 μ M; One-110 at 0.1 μ M; and One-100 at 0.4 μ M
 PCR multiplex OneB: One-102 at 0.075 μ M; One-114 at 0.1 μ M; and One-115 at 0.06 μ M
 PCR multiplex OneC: One-105 at 0.04 μ M; Ots-103 at 0.2 μ M; and One-101 at 0.06 μ M

The thermal profile for all three multiplex PCRs (Olsen et al., 1996) was the same: an initial 3 min denaturation at 92°C, followed by 38 cycles of 15 sec denaturation at 92°C, 30 sec annealing at 50°C, and 60 sec extension at 72°C; and then a final 30 min extension at 72°C.

Microsatellite DNA data collection

Microsatellite DNA analysis was conducted for the nine loci identified above using procedures established in our lab (Shaklee and Young, 2000). We collected microsatellite data using a 96-lane ABI-377 automated DNA sequencer utilizing in-lane size standards (GeneScan-500 rox; Applied Biosystems). Raw data from the DNA sequencer was processed using Genescan (v. 3.0)

and Genotyper (v. 2.5) (Applied Biosystems). The microsatellite DNA patterns of all samples were independently scored by two biologists and all scoring discrepancies were reviewed and resolved. The output tables from Genotyper were imported into MS Excel, where allele calling was accomplished using size bins defined based on the presumed repeat motif of each microsatellite and the observed distributions of raw Genotyper size calls for each locus. DNA fragments whose raw size estimates fell between bins were zeroed and not included in subsequent analyses. Despite being described as having a tetranucleotide repeat motif, we scored variation at *One-110* as if this locus had a dinucleotide repeat motif because of the large number of raw size estimates that seemed to fit this model. The final Excel file was used to output a genotype file that was used for statistical analysis.

Statistical analyses of DNA data

We used the program GENEPOP (version 3.3) of Raymond and Rousset (1995a) to calculate allele frequencies and to conduct tests of Hardy-Weinberg equilibrium, linkage disequilibrium, and population differentiation.

Results and Discussion

Summary of biological observations

Weekly spawner escapement surveys conducted between August and January of 2001 revealed three major groups of kokanee and two major groups of sockeye spawning in the Lake Washington/Lake Sammamish Basin based on distribution and timing (Figure 2).

Surveys for early-run kokanee were conducted in Issaquah Creek and many of its tributaries (i.e., mainstem Issaquah, East Fork Issaquah [WRIA 08.0183], McDonald [WRIA 08.0212], Fifteenmile [WRIA 08.0207], Carey [WRIA 08.0218], and Holder [headwaters of Issaquah Creek above confluence with Carey Creek] creeks, and in Tibbits Creek [WRIA 08.0169]) another south Lake Sammamish tributary. Two presumed early-run kokanee were observed in the Issaquah Creek drainage during the third and fourth weeks of August, one of which was responsible for the construction of a redd immediately downstream of the Issaquah Creek Hatchery. Surveys of two other Lake Sammamish tributaries (Laughing Jacobs and Vasa [WRIA 08.0156] creeks) and of several Sammamish River tributaries (Swamp [WRIA 08.0059], North, Bear, Cottage Lake [WRIA 08.0122], and Little Bear creeks) were also conducted between 25 July and 31 August to look for early-run kokanee. Only one additional kokanee was observed in these early surveys (in Little Bear Creek on 31 August).

A second group of kokanee entered the north tributaries of the Sammamish River from early September through late October. These fish were dull olive to brown in color and ranged from 250 to 360 mm in fork length (FL). Of the estimated escapement of 170 kokanee in these creeks, most were males. The kokanee runs in Bear, Little Bear and North creeks occurred at virtually the same time as the run of over 35,000 sockeye that spawned in the same reaches of these three tributaries (Figure 2).

A third group of kokanee entered east and south Lake Sammamish tributaries from October through early January (Figure 2). These fish were morphologically distinct from the kokanee mentioned above with heavy spotting along their entire dorsal surface and both lobes of their caudal fins and with varying degrees of red coloration laterally. They ranged from 340 to 520 mm (FL) with an estimated total escapement of 620 fish. Only three adult sockeye were observed in the tributaries used by these kokanee. Kokanee timing in these Lake Sammamish tributaries was later than that of the second major group of sockeye in the basin, the East Fork Issaquah sockeye. This sockeye run peaked October 26th with a total estimated escapement of 10,700 fish. Kokanee were not observed in East Fork Issaquah Creek during this time.

DNA

Preliminary statistical tests of all individual collections indicated that the three kokanee collections from North Creek, Bear Creek, and Little Bear Creek (= "north tribs") were very similar (data not shown) and, because we had only small samples from each, we combined these three collections for subsequent analysis. For similar reasons (data not shown), we combined the North Creek and Bear Creek sockeye collections for subsequent statistical analyses.

Allele frequencies, allele codes and estimated allele sizes in base pairs (bp), and the numbers of alleles successfully scored at each locus for each of the 10 collections used in most analyses are reported in Appendix 1.

Tests of Hardy-Weinberg equilibrium at each locus in each of the 10 collections (total of 90 tests) revealed significant or nearly significant departures at five cases: *One-100* in the Bear/North sockeye combined collection, *One-101*, *Ots-103*, and *One-114* in the Lewis collection, and *Ots-103* in the Laughing Jacobs collection. In addition to being out of Hardy-Weinberg equilibrium in the combined Bear/North sample, in several collections *One-100* had a relatively large number of microsatellite raw size calls that fell outside of the bins used for allele calling. For this reason, we conducted some statistical analyses using the full set of nine loci and others using the eight loci remaining after omitting *One-100*.

Because of the observed deviations from Hardy-Weinberg equilibrium at several loci, we conducted explicit tests of population subdivision among pairs of populations using both genotypic (no assumption of Hardy-Weinberg equilibrium) and genic (allelic) data to assess their interrelationships (Raymond and Rousset, 1995b). The results of these tests using genotypic data for all nine loci are shown in Table 1 while those using genotypic data for eight loci are shown in Table 2. The results of tests using allelic data (genic differentiation test) are summarized in Table 3. We believe these statistical results provide compelling evidence for significant genetic divergence among the populations tested, except for the pairwise test of Bear/North sockeye vs. north Tribs (Bear, Little Bear, & North) kokanee, which was not statistically significant. Genetic similarity, spatial and temporal overlap, and morphological characteristics support the assertion that adfluvial *O. nerka* entering the north tributaries may actually be residualized sockeye.

We investigated the reliability of these results by resampling the genotypes observed in one of the

collections (Lake Whatcom kokanee) to generate 15 replicate samples and then running the genic differentiation test on the resulting files. The results of this analysis are summarized in Table 4. Although eight of the 105 pairwise tests were significant at the $P = 0.5$ level, approximately five would have been expected for this number of tests, and none were statistically significant if an adjusted α -level of 0.00048 is used. Thus, we concluded that these resamples were basically not significantly different from one another. Given this outcome, we believe that the large proportion of significant outcomes shown in tables 1-3 are a reliable indication of relationships and not some sort of statistical artifact.

We also calculated Wright's fixation index (F_{ST}) for all pairwise comparisons (using nine and eight loci) and these results are shown in Table 5. The F_{ST} values provide evidence for the distinctiveness of both the Issaquah Creek early-run kokanee (even though only 13 samples were analyzed and one of these yielded no data) and, collectively, of the kokanee in Ebright, Lewis, and Laughing Jacobs creeks.

Finally, we conducted multidimensional scaling analysis (with a minimum spanning tree connecting each collection with its most similar neighbor) using the F_{ST} values. The results of this analysis are shown in Figure 3 using all nine loci and in Figure 4 using only eight loci (*One-100* omitted). These figures emphasize the distinctiveness of the early run Issaquah Creek kokanee, and of the kokanee in Ebright, Lewis, and Laughing Jacobs creeks. They also show that each of these populations is quite distinct from the Lake Whatcom Hatchery kokanee stock, from the Meadow Creek stock from the upper Columbia River basin, and from the Baker Lake sockeye stock.

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Table 1. Lake Washington/Lake Sammamish and other *O. nerka* collections tested for population differentiation using the method of Raymond and Rousset, 1995b). Genotypic data for 9 loci were used in these tests; k = kokanee; s = sockeye

P-value for each population pair across all 9 microsatellite DNA loci (Fisher's method)

Population pair	Chi2	df	P-value
Lewis-k vs Ebright-k	Infinity	18	Highly sign.
Laughing Jacobs-k vs Ebright-k	88.021	18	0.00000
Laughing Jacobs-k vs Lewis-k	71.834	18	0.00000
north Tribs-k vs Ebright-k	Infinity	18	Highly sign.
north Tribs-k vs Lewis-k	Infinity	18	Highly sign.
north Tribs-k vs Laughing Jacobs-k	Infinity	18	Highly sign.
Issaquah-k vs Ebright-k	Infinity	18	Highly sign.
Issaquah-k vs Lewis-k	Infinity	18	Highly sign.
Issaquah-k vs Laughing Jacobs-k	Infinity	18	Highly sign.
Issaquah-k vs north Tribs-k	Infinity	18	Highly sign.
L Whatcom-k vs Ebright-k	Infinity	18	Highly sign.
L Whatcom-k vs Lewis-k	Infinity	18	Highly sign.
L Whatcom-k vs Laughing Jacobs-k	Infinity	18	Highly sign.
L Whatcom-k vs north Tribs-k	Infinity	18	Highly sign.
L Whatcom-k vs Issaquah-k	Infinity	18	Highly sign.
Issaquah-s vs Ebright-k	Infinity	18	Highly sign.
Issaquah-s vs Lewis-k	Infinity	18	Highly sign.
Issaquah-s vs Laughing Jacobs-k	Infinity	18	Highly sign.
Issaquah-s vs north Tribs-k	95.399	18	0.00000
Issaquah-s vs Issaquah-k	Infinity	18	Highly sign.
Issaquah-s vs L Whatcom-k	Infinity	18	Highly sign.
Bear & North-s vs Ebright-k	Infinity	18	Highly sign.
Bear & North-s vs Lewis-k	Infinity	18	Highly sign.
Bear & North-s vs Laughing Jacobs-k	Infinity	18	Highly sign.
Bear & North-s vs north Tribs-k	28.149	18	0.05983
Bear & North-s vs Issaquah-k	Infinity	18	Highly sign.
Bear & North-s vs L Whatcom-k	Infinity	18	Highly sign.
Bear & North-s vs Issaquah-s	84.913	18	0.00000
Baker L-s vs Ebright-k	Infinity	18	Highly sign.
Baker L-s vs Lewis-k	Infinity	18	Highly sign.
Baker L-s vs Laughing Jacobs-k	Infinity	18	Highly sign.
Baker L-s vs north Tribs-k	Infinity	18	Highly sign.
Baker L-s vs Issaquah-k	Infinity	18	Highly sign.
Baker L-s vs L Whatcom-k	Infinity	18	Highly sign.
Baker L-s vs Issaquah-s	Infinity	18	Highly sign.
Baker L-s vs Bear & North-s	Infinity	18	Highly sign.
Meadow, BC-k vs Ebright-k	Infinity	18	Highly sign.
Meadow, BC-k vs Lewis-k	Infinity	18	Highly sign.
Meadow, BC-k vs Laughing Jacobs-k	Infinity	18	Highly sign.
Meadow, BC-k vs north Tribs-k	Infinity	18	Highly sign.
Meadow, BC-k vs Issaquah-k	Infinity	18	Highly sign.
Meadow, BC-k vs L Whatcom-k	Infinity	18	Highly sign.
Meadow, BC-k vs Issaquah-s	Infinity	18	Highly sign.
Meadow, BC-k vs Bear & North-s	Infinity	18	Highly sign.
Meadow, BC-k vs Baker L-s	Infinity	18	Highly sign.

Genepop (Version 3.3): Genotypic differentiation for each population pair

File:nfinal9 (L WA-Samm resolved scores-w-LkRoos bins 7 May 01)

Number of populations detected: 10 Number of loci detected: 9

note: geographically proximate collections (of similar life-history type) with small N were combined before testing

Markov chain parameters Dememorization: 1000 Batches: 1000 Iterations per batch: 1000

Normal ending

Table 2. Lake Washington/Lake Sammamish and other *O. nerka* collections tested for population differentiation using the method of Raymond and Rousset, 1995b). Genotypic data for 8 loci (One-100 omitted) were used in these tests; k = kokanee; s = sockeye

P-value for each population pair across 8 microsatellite DNA loci (Fisher's method)

Population pair	Chi2	df	P-value
Lewis-k vs Ebright-k	Infinity	16	Highly sign.
Laughing Jacobs-k vs Ebright-k	76.261	16	0.00000
Laughing Jacobs-k vs Lewis-k	71.607	16	0.00000
north Tribs-k vs Ebright-k	Infinity	16	Highly sign.
north Tribs-k vs Lewis-k	Infinity	16	Highly sign.
north Tribs-k vs Laughing Jacobs-k	Infinity	16	Highly sign.
Issaquah-k vs Ebright-k	Infinity	16	Highly sign.
Issaquah-k vs Lewis-k	Infinity	16	Highly sign.
Issaquah-k vs Laughing Jacobs-k	Infinity	16	Highly sign.
Issaquah-k vs north Tribs-k	Infinity	16	Highly sign.
L Whatcom-k vs Ebright-k	Infinity	16	Highly sign.
L Whatcom-k vs Lewis-k	Infinity	16	Highly sign.
L Whatcom-k vs Laughing Jacobs-k	Infinity	16	Highly sign.
L Whatcom-k vs north Tribs-k	Infinity	16	Highly sign.
L Whatcom-k vs Issaquah-k	Infinity	16	Highly sign.
Issaquah-s vs Ebright-k	Infinity	16	Highly sign.
Issaquah-s vs Lewis-k	Infinity	16	Highly sign.
Issaquah-s vs Laughing Jacobs-k	Infinity	16	Highly sign.
Issaquah-s vs north Tribs-k	75.974	16	0.00000
Issaquah-s vs Issaquah-k	Infinity	16	Highly sign.
Issaquah-s vs L Whatcom-k	Infinity	16	Highly sign.
Bear & North-s vs Ebright-k	Infinity	16	Highly sign.
Bear & North-s vs Lewis-k	Infinity	16	Highly sign.
Bear & North-s vs Laughing Jacobs-k	Infinity	16	Highly sign.
Bear & North-s vs north Tribs-k	25.931	16	0.05501
Bear & North-s vs Issaquah-k	Infinity	16	Highly sign.
Bear & North-s vs L Whatcom-k	Infinity	16	Highly sign.
Bear & North-s vs Issaquah-s	Infinity	16	Highly sign.
Baker L-s vs Ebright-k	Infinity	16	Highly sign.
Baker L-s vs Lewis-k	Infinity	16	Highly sign.
Baker L-s vs Laughing Jacobs-k	Infinity	16	Highly sign.
Baker L-s vs north Tribs-k	Infinity	16	Highly sign.
Baker L-s vs Issaquah-k	Infinity	16	Highly sign.
Baker L-s vs L Whatcom-k	Infinity	16	Highly sign.
Baker L-s vs Issaquah-s	Infinity	16	Highly sign.
Baker L-s vs Bear & North-s	Infinity	16	Highly sign.
Meadow, BC-k vs Ebright-k	Infinity	16	Highly sign.
Meadow, BC-k vs Lewis-k	Infinity	16	Highly sign.
Meadow, BC-k vs Laughing Jacobs-k	Infinity	16	Highly sign.
Meadow, BC-k vs north Tribs-k	Infinity	16	Highly sign.
Meadow, BC-k vs Issaquah-k	Infinity	16	Highly sign.
Meadow, BC-k vs L Whatcom-k	Infinity	16	Highly sign.
Meadow, BC-k vs Issaquah-s	Infinity	16	Highly sign.
Meadow, BC-k vs Bear & North-s	Infinity	16	Highly sign.
Meadow, BC-k vs Baker L-s	Infinity	16	Highly sign.

Genepop (Version 3.3): Genotypic differentiation for each population pair

File:nfinal8 (L WA-Samm 8 loci resolved scores-w-LkRoos bins 7 May 01)

Number of populations detected: 10

Number of loci detected: 8

note: geographically proximate collections (of similar life-history type) with small N were combined before testing

Markov chain parameters - Dememorization: 1000

Batches: 1000

Iterations per batch: 1000

Normal ending

Table 3. Lake Washington/Lake Sammamish and other *O. nerka* collections tested for population differentiation using the method of Raymond and Rousset, 1995b). Genic (allelic) data for 8 loci (*One-100* omitted) were used in these tests; k = kokanee; s = sockeye

P-value for each population pair across 8 microsatellite DNA loci (Fisher's method)

Population pair	Chi2	df	P-value
Ebright-k & Lewis-k	Infinity	16	Highly sign.
Ebright-k & Laughing Jacobs-k	77.950	16	0.00000
Ebright-k & north Tribs-k	Infinity	16	Highly sign.
Ebright-k & Issaquah-k	Infinity	16	Highly sign.
Ebright-k & L Whatcom-k	Infinity	16	Highly sign.
Ebright-k & Issaquah-s	Infinity	16	Highly sign.
Ebright-k & Bear & North-s	Infinity	16	Highly sign.
Ebright-k vs Baker L-s	Infinity	16	Highly sign.
Ebright-k vs Meadow, BC-k	Infinity	16	Highly sign.
Lewis-k vs Laughing Jacobs-k	74.104	16	0.00000
Lewis-k vs north Tribs-k	Infinity	16	Highly sign.
Lewis-k vs Issaquah-k	Infinity	16	Highly sign.
Lewis-k vs L Whatcom-k	Infinity	16	Highly sign.
Lewis-k vs Issaquah-s	Infinity	16	Highly sign.
Lewis-k vs Bear & North-s	Infinity	16	Highly sign.
Lewis-k vs Baker L-s	Infinity	16	Highly sign.
Lewis-k vs Meadow, BC-k	Infinity	16	Highly sign.
Laughing Jacobs-k vs north Tribs-k	Infinity	16	Highly sign.
Laughing Jacobs-k vs Issaquah-k	Infinity	16	Highly sign.
Laughing Jacobs-k vs L Whatcom-k	Infinity	16	Highly sign.
Laughing Jacobs-k vs Issaquah-s	Infinity	16	Highly sign.
Laughing Jacobs-k vs Bear & North-s	Infinity	16	Highly sign.
Laughing Jacobs-k vs Baker L-s	Infinity	16	Highly sign.
Laughing Jacobs-k vs Meadow, BC-k	Infinity	16	Highly sign.
north Tribs-k vs Issaquah-k	Infinity	16	Highly sign.
north Tribs-k vs L Whatcom-k	Infinity	16	Highly sign.
north Tribs-k vs Issaquah-s	78.296	16	0.00000
north Tribs-k vs Bear & North-s	27.613	16	0.03515
north Tribs-k vs Baker L-s	Infinity	16	Highly sign.
north Tribs-k vs Meadow, BC-k	Infinity	16	Highly sign.
Issaquah-k vs L Whatcom-k	Infinity	16	Highly sign.
Issaquah-k vs Issaquah-s	Infinity	16	Highly sign.
Issaquah-k vs Bear & North-s	Infinity	16	Highly sign.
Issaquah-k vs Baker L-s	Infinity	16	Highly sign.
Issaquah-k vs Meadow, BC-k	Infinity	16	Highly sign.
L Whatcom-k vs Issaquah-s	Infinity	16	Highly sign.
L Whatcom-k vs Bear & North-s	Infinity	16	Highly sign.
L Whatcom-k vs Baker L-s	Infinity	16	Highly sign.
L Whatcom-k vs Meadow, BC-k	Infinity	16	Highly sign.
Issaquah-s vs Bear & North-s	78.424	16	0.00000
Issaquah-s vs Baker L-s	Infinity	16	Highly sign.
Issaquah-s vs Meadow, BC-k	Infinity	16	Highly sign.
Bear & North-s vs Baker L-s	Infinity	16	Highly sign.
Bear & North-s vs Meadow, BC-k	Infinity	16	Highly sign.
Baker L-s vs Meadow, BC-k	Infinity	16	Highly sign.

Genepop (Version 3.3), Genic differentiation for each population pair

File:nfinal8 (L WA-Samm resolved scores-w-LkRoos bins 7 May 01)

Number of populations detected: 10 Number of loci detected: 8

note: geographically proximate collections (of similar life-history type) with small N were combined before testing

Markov chain parameters - Dememorisation: 1000 Batches: 1000 Iterations per batch: 1000

Normal ending

Table 4. Results of pairwise genic differentiation tests of 15 resamplings of the Lake Whatcom Hatchery kokanee stock (n= 100 per resampling). [highlighted outcomes significant at $P < 0.05$ before correction for multiple testing; total of 105 pairwise tests; adjusted $\alpha = 0.05/105 = 0.00048$]

P-value for each population pair across all loci(Fisher's method)

Population pair	Chi2	df	P-value	Population pair	Chi2	df	P-value
11-100 & 12-100	16.845	18	0.53376	21-100 & 31-100	16.494	18	0.55813
11-100 & 13-100	26.990	18	0.07919	21-100 & 32-100	20.473	18	0.30681
11-100 & 14-100	15.770	18	0.60860	21-100 & 33-100	17.509	18	0.48841
11-100 & 21-100	35.993	18	<u>0.00707</u>	21-100 & 41-100	36.731	18	<u>0.00568</u>
11-100 & 22-100	22.948	18	0.19261	21-100 & 42-100	30.328	18	<u>0.03437</u>
11-100 & 23-100	21.430	18	0.25825	21-100 & 43-100	19.414	18	0.36675
11-100 & 24-100	19.270	18	0.37538	21-100 & 44-100	37.044	18	<u>0.00517</u>
11-100 & 31-100	22.268	18	0.22031	22-100 & 23-100	19.408	18	0.36709
11-100 & 32-100	18.294	18	0.43648	22-100 & 24-100	14.385	18	0.70366
11-100 & 33-100	24.094	18	0.15197	22-100 & 31-100	19.222	18	0.37829
11-100 & 41-100	22.521	18	0.20967	22-100 & 32-100	20.947	18	0.28210
11-100 & 42-100	26.632	18	0.08617	22-100 & 33-100	23.018	18	0.18989
11-100 & 43-100	16.426	18	0.56287	22-100 & 41-100	17.882	18	0.46345
11-100 & 44-100	24.462	18	0.14048	22-100 & 42-100	14.035	18	0.72679
12-100 & 13-100	15.298	18	0.64145	22-100 & 43-100	18.997	18	0.39203
12-100 & 14-100	21.753	18	0.24313	22-100 & 44-100	16.778	18	0.53838
12-100 & 21-100	38.726	18	<u>0.00310</u>	23-100 & 24-100	13.970	18	0.73107
12-100 & 22-100	16.610	18	0.55007	23-100 & 31-100	13.908	18	0.73504
12-100 & 23-100	22.440	18	0.21303	23-100 & 32-100	16.631	18	0.54856
12-100 & 24-100	22.248	18	0.22115	23-100 & 33-100	14.689	18	0.68318
12-100 & 31-100	18.714	18	0.40964	23-100 & 41-100	11.339	18	0.87940
12-100 & 32-100	16.798	18	0.53706	23-100 & 42-100	22.946	18	0.19268
12-100 & 33-100	23.234	18	0.18177	23-100 & 43-100	15.825	18	0.60479
12-100 & 41-100	19.780	18	0.34531	23-100 & 44-100	18.741	18	0.40794
12-100 & 42-100	25.449	18	0.11304	24-100 & 31-100	19.682	18	0.35100
12-100 & 43-100	22.620	18	0.20561	24-100 & 32-100	12.948	18	0.79464
12-100 & 44-100	12.906	18	0.79712	24-100 & 33-100	12.918	18	0.79644
13-100 & 14-100	15.826	18	0.60468	24-100 & 41-100	20.009	18	0.33230
13-100 & 21-100	24.087	18	0.15220	24-100 & 42-100	29.764	18	<u>0.03981</u>
13-100 & 22-100	14.362	18	0.70518	24-100 & 43-100	15.329	18	0.63929
13-100 & 23-100	19.702	18	0.34982	24-100 & 44-100	19.427	18	0.36595
13-100 & 24-100	13.043	18	0.78904	31-100 & 32-100	14.503	18	0.69580
13-100 & 31-100	23.741	18	0.16369	31-100 & 33-100	18.257	18	0.43887
13-100 & 32-100	18.443	18	0.42683	31-100 & 41-100	17.239	18	0.50673
13-100 & 33-100	20.993	18	0.27977	31-100 & 42-100	23.493	18	0.17234
13-100 & 41-100	23.616	18	0.16800	31-100 & 43-100	15.751	18	0.60995
13-100 & 42-100	23.609	18	0.16827	31-100 & 44-100	26.645	18	0.08590
13-100 & 43-100	17.507	18	0.48853	32-100 & 33-100	19.490	18	0.36223
13-100 & 44-100	20.647	18	0.29761	32-100 & 41-100	21.417	18	0.25891
14-100 & 21-100	24.706	18	0.13322	32-100 & 42-100	16.069	18	0.58771
14-100 & 22-100	21.620	18	0.24930	32-100 & 43-100	18.778	18	0.40561
14-100 & 23-100	15.200	18	0.64817	32-100 & 44-100	16.143	18	0.58253
14-100 & 24-100	11.679	18	0.86338	33-100 & 41-100	23.994	18	0.15524
14-100 & 31-100	30.168	18	<u>0.03585</u>	33-100 & 42-100	24.568	18	0.13730
14-100 & 32-100	18.393	18	0.43005	33-100 & 43-100	15.226	18	0.64642
14-100 & 33-100	16.872	18	0.53191	33-100 & 44-100	19.308	18	0.37308
14-100 & 41-100	12.934	18	0.79547	41-100 & 42-100	12.347	18	0.82884
14-100 & 42-100	11.869	18	0.85393	41-100 & 43-100	25.290	18	0.11713
14-100 & 43-100	6.758	18	0.99201	41-100 & 44-100	19.446	18	0.36486
14-100 & 44-100	12.015	18	0.84649	42-100 & 43-100	27.148	18	0.07626
21-100 & 22-100	30.733	18	<u>0.03090</u>	42-100 & 44-100	17.438	18	0.49323
21-100 & 23-100	20.951	18	0.28188	43-100 & 44-100	27.277	18	0.07394
21-100 & 24-100	26.343	18	0.09218				

Normal ending

Genepop (Version 3.3), Genic differentiation for each population pair

File:res100 (15 independent samples [n=100] drawn from Lake Whatcom collection 00LE)

Number of populations detected: 15

Number of loci detected: 9

Markov chain parameters Dememorisation: 1000 Batches: 600 Iterations per batch: 1000

Table 5. Wright's F_{ST} values for all pairwise comparisons of Lake Washington/Lake Sammamish and other *O. nerka*.

Estimated pairwise F_{ST} values using all 9 microsatellite DNA loci:

pop	Ebrig-k	Lewis-k	Laugh-k	nTrib-k	Issaq-k	Lwhat-k	Issaq-s	BearN-s	Baker-s
Lewis-k	0.0095								
Laugh-k	0.0148	0.0125							
nTrib-k	0.0466	0.0481	0.0634						
Issaq-k	0.0849	0.0688	0.0969	0.0584					
LWhat-k	0.0607	0.0644	0.0734	0.0248	0.0701				
Issaq-s	0.0428	0.0429	0.0620	0.0090	0.0577	0.0216			
BearN-s	0.0511	0.0463	0.0634	0.0030	0.0576	0.0211	0.0071		
Baker-s	0.0749	0.0824	0.0961	0.0309	0.1092	0.0479	0.0373	0.0380	
Meado-k	0.0549	0.0471	0.0569	0.0292	0.0734	0.0353	0.0334	0.0287	0.0621

The file nfinal.mig contains the matrix ready for further analysis Normal ending

Estimated pairwise F_{ST} values using 8 microsatellite DNA loci (One-100 omitted):

pop	Ebrig-k	Lewis-k	Laugh-k	nTrib-k	Issaq-k	Lwhat-k	Issaq-s	BearN-s	Baker-s
Lewis-k	0.0101								
Laugh-k	0.0147	0.0136							
nTrib-k	0.0460	0.0491	0.0648						
Issaq-k	0.0846	0.0699	0.0999	0.0655					
LWhat-k	0.0620	0.0669	0.0757	0.0241	0.0759				
Issaq-s	0.0429	0.0440	0.0640	0.0074	0.0602	0.0217			
BearN-s	0.0514	0.0483	0.0661	0.0024	0.0640	0.0214	0.0060		
Baker-s	0.0767	0.0857	0.0994	0.0327	0.1197	0.0502	0.0386	0.0397	
Meado-k	0.0584	0.0498	0.0595	0.0293	0.0775	0.0366	0.0352	0.0296	0.0659

The file nfinal8.mig contains the matrix ready for further analysis Normal ending

Codes for pop names:

Ebrig-k	Ebright Creek kokanee
Lewis-k	Lewis Creek kokanee
Laugh-k	Laughing Jacobs Creek kokanee
nTrib-k	North, Bear, & Little Bear Creeks kokanee
Issaq-k	Issaquah Creek kokanee
Lwhat-k	Lake Whatcom Hatchery kokanee
Issaq-s	Issaquah Creek sockeye
BearN-s	Bear & North creeks sockeye
Baker-s	Baker Lake sockeye
Meado-k	Meadow Creek (Kootnei Lake, B.C.) kokanee

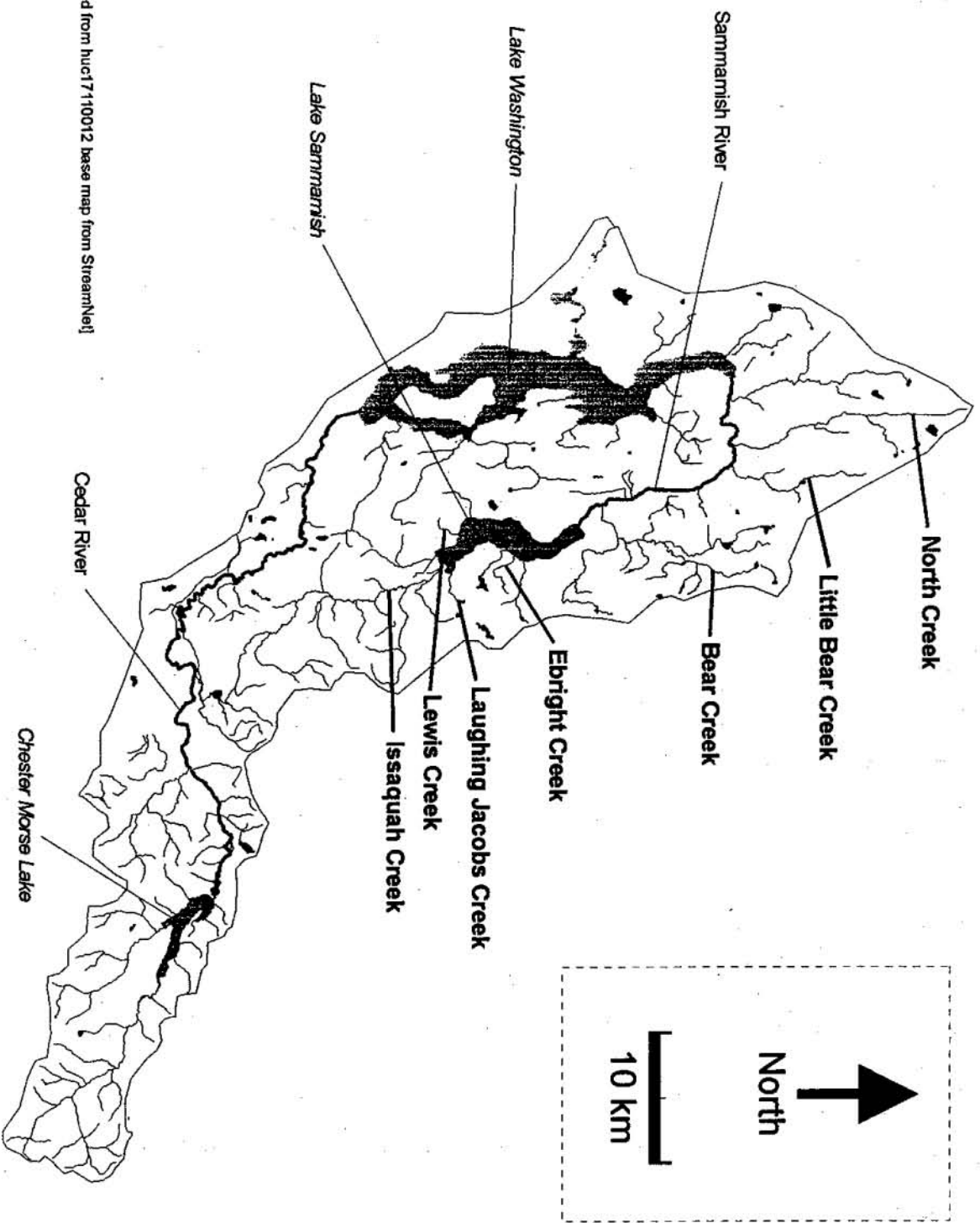
Genepop (Version 3.3): Pairwise IIS for population pairs

(F_{ST} is estimated as in Weir and Cockerham 1984)

File:nfinal9 (L WA-Samm resolved scores-w-LkRoos bins 7 May 01)

Number of samples detected: 10 Number of loci detected: 9

Figure 1. Map of the Lake Washington/Lake Sammamish watershed.



[map created from huc17110012 base map from StreamNet]

Figure 2. Run timing and relative numbers of five major groups of *O. nerka* in the Sammamish Basin based on 2001 surveys.

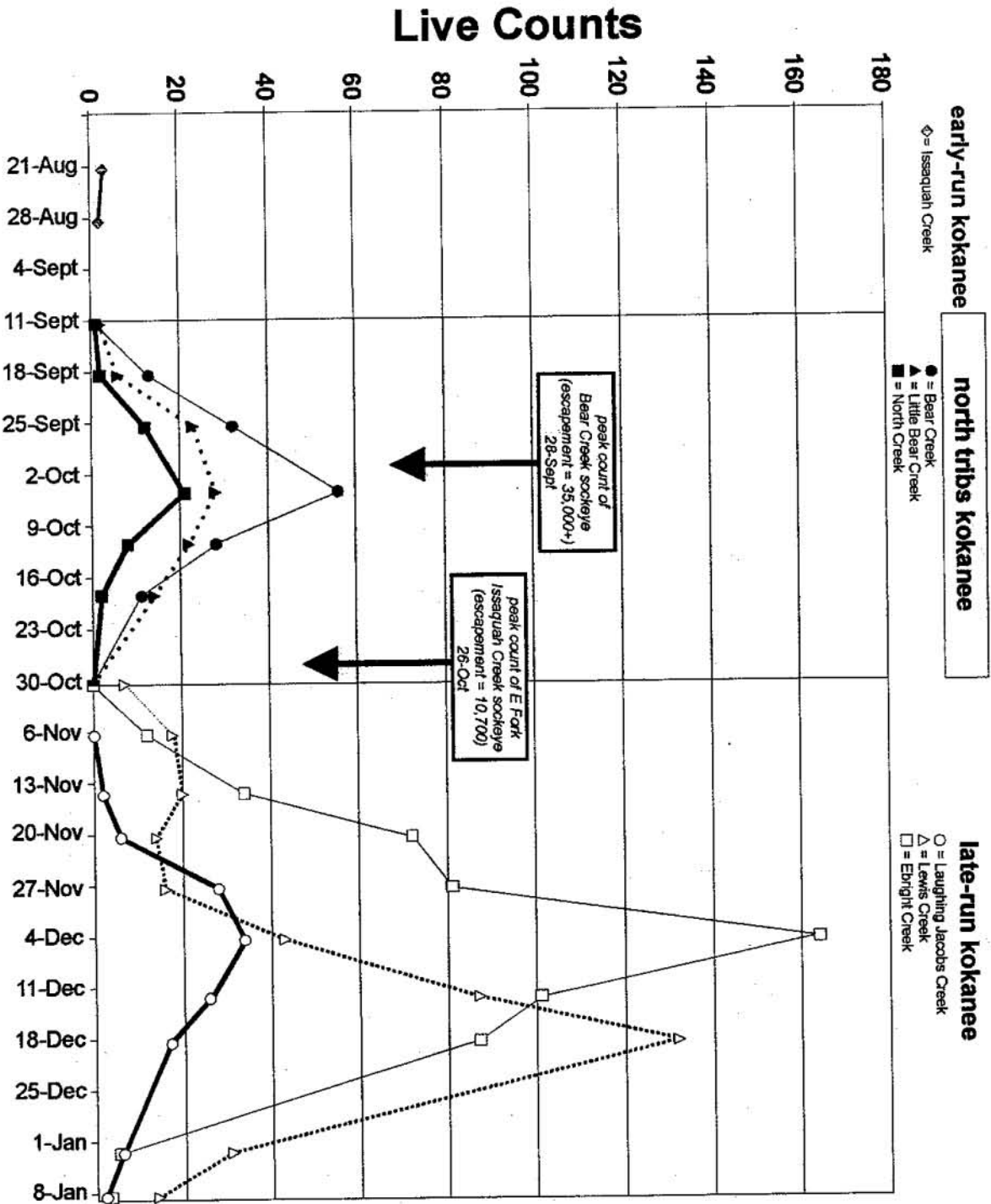
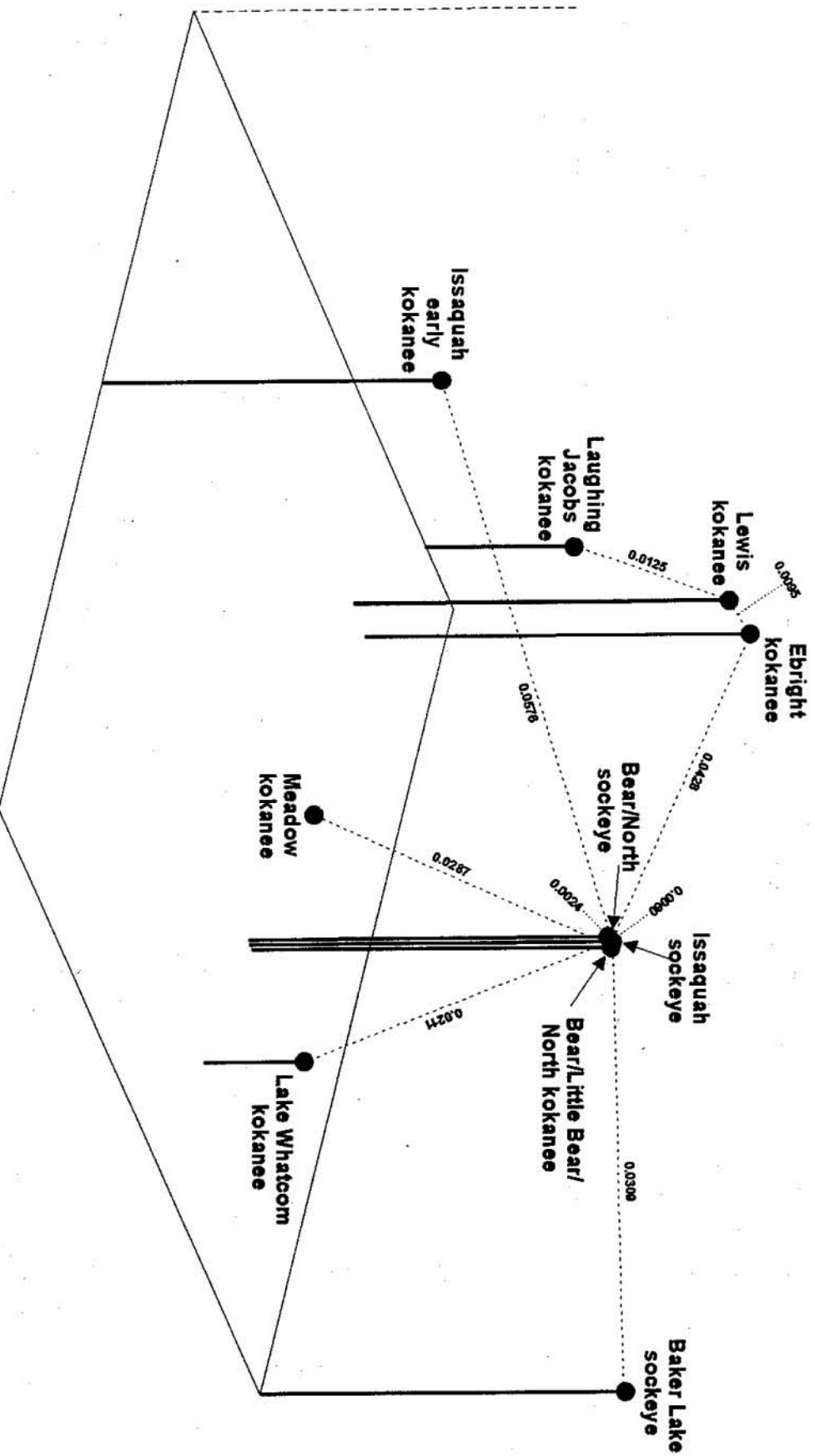


Figure 3. Multidimensional scaling plot of F_{ST} values (using 9 microsatellite DNA loci) for Lake Washington/Lake Sammamish *O. nerka* [stress2 = 0.01716 indicates an excellent fit]. The minimum spanning tree (dashed lines) connects each population with the population most similar to it.



Appendix 1 (cont.). Allele frequencies in 10 collections of *Oncorhynchus nerka* from the Lake Washington & Lake Sammamish basins and from three other locations.
(Na = numbers of alleles successfully scored)

One-116										
code	size (bp)	Elbright-k	Lewis-k	Laugh-k	nTrib-k	Issac-k	LWWhet-k	Issac-s	Beard-s	Baker-s
1	174	0	0	0	0.008	0	0.010	0	0.018	0
2	178	0	0.008	0	0.024	0	0.036	0.061	0.018	0.031
3	182	0.036	0.016	0.019	0.008	0	0.036	0.079	0	0.026
4	186	0.022	0.016	0.028	0.056	0	0.046	0.026	0.114	0
5	190	0	0.039	0.009	0.040	0	0.057	0.044	0.123	0.005
6	184	0.036	0.148	0.132	0.137	0.150	0.062	0.132	0.123	0.119
7	198	0.203	0.117	0.019	0.202	0.050	0.124	0.158	0.149	0.155
8	202	0.058	0.016	0.028	0.040	0.150	0.206	0.088	0.098	0.115
9	206	0.058	0.086	0.075	0.097	0	0.222	0.070	0.132	0.124
10	210	0.022	0	0.019	0.065	0.350	0.072	0.096	0.061	0.082
11	214	0.065	0.102	0.047	0.137	0	0.021	0.132	0.114	0.253
12	218	0.014	0.065	0.075	0.065	0.150	0.031	0.053	0.035	0.134
13	222	0.304	0.287	0.434	0.040	0.050	0.041	0.018	0.026	0.005
14	226	0.116	0.086	0.085	0.056	0.100	0.031	0.035	0.009	0.016
15	230	0.065	0.016	0.028	0.008	0	0.005	0	0.018	0
16	234	0	0	0	0.016	0	0	0.009	0	0.005
17	254	0	0	0	0	0	0	0	0	0.005
Na	138	128	106	124	20	194	114	114	184	192

One-102										
code	size (bp)	Elbright-k	Lewis-k	Laugh-k	nTrib-k	Issac-k	LWWhet-k	Issac-s	Beard-s	Baker-s
1	196	0	0	0	0	0	0	0	0	0.029
2	200	0	0	0	0.049	0	0	0.036	0	0.006
3	204	0	0	0	0	0	0.074	0	0.010	0.144
4	208	0	0	0	0.059	0	0.043	0	0.010	0.017
5	212	0	0	0	0.010	0	0.043	0	0.010	0
6	216	0.010	0.029	0	0	0	0.032	0.018	0	0.011
7	220	0.059	0.096	0.038	0.028	0	0.032	0	0.080	0.006
8	224	0.040	0.029	0.058	0.108	0.050	0.144	0.009	0.080	0.144
9	228	0.218	0.212	0.433	0.039	0	0.064	0.027	0.090	0.115
10	232	0.089	0.077	0.058	0.118	0.350	0.106	0.100	0.130	0.075
11	236	0.129	0.106	0.087	0.216	0.450	0.133	0.264	0.220	0.109
12	240	0.396	0.279	0.260	0.108	0	0.112	0.245	0.150	0.120
13	244	0.059	0.135	0.048	0.088	0	0.160	0.145	0.180	0.246
14	248	0	0.029	0.019	0.108	0	0.048	0.082	0.090	0.063
15	252	0	0.010	0	0.029	0.150	0.037	0.016	0.020	0.029
16	256	0	0	0	0.020	0	0.021	0.055	0.010	0.023
17	260	0	0	0	0.020	0	0	0	0.010	0.006
18	272	0	0	0	0	0	0	0	0	0.006
Na	101	104	104	102	20	188	110	100	175	174

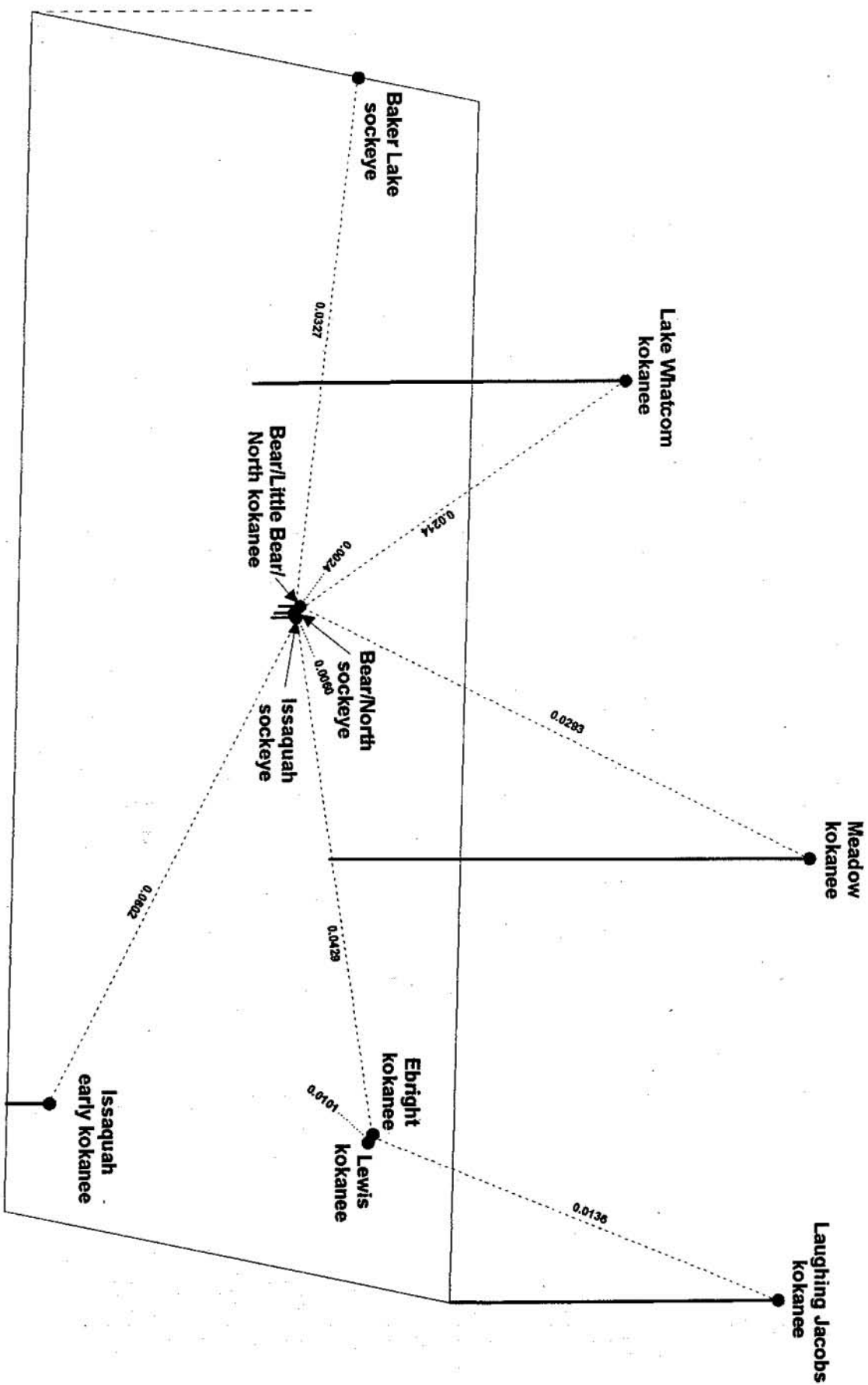
One-114										
code	size (bp)	Elbright-k	Lewis-k	Laugh-k	nTrib-k	Issac-k	LWWhet-k	Issac-s	Beard-s	Baker-s
1	202	0	0	0	0	0	0.005	0	0	0
2	206	0.051	0.021	0.046	0.008	0	0.005	0.009	0	0
3	210	0	0	0	0	0	0.010	0	0.009	0
4	214	0	0.021	0.019	0.032	0	0.010	0.036	0.037	0
5	218	0.026	0.056	0.065	0.016	0.300	0.026	0.036	0.009	0.005
6	222	0.171	0.125	0.130	0.032	0.050	0.005	0.018	0.019	0.026
7	226	0	0	0	0.079	0.050	0	0.045	0.065	0.010
8	230	0.032	0.056	0.028	0.016	0	0.021	0.045	0.037	0.005
9	234	0.006	0	0.040	0	0	0.072	0.036	0.037	0.047
10	238	0.058	0.076	0.083	0.008	0	0.072	0.080	0.083	0.052
11	242	0.019	0.028	0.019	0.079	0.150	0.088	0.080	0.120	0.047
12	246	0.103	0.118	0.028	0.095	0.100	0.093	0.098	0.141	0.120
13	250	0.032	0.048	0.009	0.048	0.100	0.139	0.036	0.019	0.130
14	254	0.205	0.243	0.157	0.063	0.050	0.057	0.036	0.028	0.078
15	258	0.090	0.028	0.111	0.071	0.100	0.093	0.045	0.042	0.078
16	262	0.167	0.167	0.259	0.079	0.050	0.072	0.116	0.093	0.042
17	266	0.026	0	0.009	0.127	0.050	0.103	0.188	0.193	0.057
18	270	0.006	0.007	0.019	0.056	0	0.052	0.071	0.056	0.021
19	274	0	0	0.009	0.087	0	0.015	0.036	0.037	0.016
20	278	0.006	0	0	0.016	0	0.062	0.054	0.028	0.073
21	282	0.006	0.007	0.009	0.016	0	0.026	0	0.065	0.028
22	286	0	0	0	0.008	0	0.010	0	0.009	0.042
23	290	0	0	0	0.016	0	0.005	0	0	0.026
24	294	0	0	0	0	0	0	0.036	0	0.010
25	298	0	0	0	0	0	0	0	0	0.005
26	314	0	0	0	0	0	0	0	0	0.005
Na	156	144	108	125	20	194	112	108	192	192

Ots-103										
code	size (bp)	Elbright-k	Lewis-k	Laugh-k	nTrib-k	Issac-k	LWWhet-k	Issac-s	Beard-s	Baker-s
1	132	0	0	0	0	0	0	0	0	0.005
2	140	0	0	0	0	0	0	0	0	0.005
3	144	0	0	0	0	0	0.015	0	0	0.031
4	148	0	0.006	0	0	0	0.010	0	0	0.016
5	152	0	0	0	0	0	0.046	0.008	0	0.057
6	156	0	0	0.009	0.007	0.083	0.051	0.042	0.018	0
7	160	0.027	0.017	0.019	0.065	0	0.087	0.017	0.046	0.005
8	164	0.112	0.092	0.139	0.058	0	0.077	0.118	0.083	0.078
9	168	0.032	0.040	0.056	0.043	0	0.077	0.118	0.083	0.078
10	172	0.069	0.081	0.009	0.022	0.042	0.122	0.126	0.018	0.010
11	176	0.085	0.029	0.074	0.123	0.042	0.046	0.134	0.165	0.078
12	180	0.271	0.191	0.287	0.087	0	0.036	0.034	0.037	0.217
13	184	0.048	0.088	0.028	0.130	0.250	0.107	0.067	0.063	0.045
14	188	0.059	0.150	0.072	0.072	0.375	0.107	0.067	0.063	0.042
15	192	0.112	0.092	0.056	0.087	0.083	0.082	0.050	0.073	0.182
16	196	0	0	0.019	0.087	0	0.082	0.034	0.138	0.115
17	200	0.011	0.012	0.019	0.087	0	0.036	0.008	0.055	0.010
18	204	0.027	0.046	0.093	0.022	0.042	0	0.008	0.046	0.005
19	208	0.128	0.110	0.065	0.072	0	0	0.067	0.028	0
20	212	0.021	0.035	0	0.029	0	0	0.008	0.028	0.021
21	216	0	0	0	0.007	0	0.005	0	0	0.005
22	220	0	0	0	0	0	0	0	0	0.010
23	224	0	0	0	0	0	0	0	0	0.005
24	232	0	0	0	0	0	0	0	0	0.005
Na	188	173	108	138	24	196	119	109	198	192

Appendix 1. Allele frequencies in 10 collections of *Oncorhynchus nerka* from the Lake Washington & Lake Sammamish basins and from three other locations.
(Na = numbers of alleles successfully scored)

One-100														One-101														
code	size (bp)	Enright-k	Lewis-k	Laugh-k	nTrib-k	Isaac-k	LWhit-k	Isaac-s	BearN-s	Baker-s	Mead-k			code	size (bp)	Enright-k	Lewis-k	Laugh-k	nTrib-k	Isaac-k	LWhit-k	Isaac-s	BearN-s	Baker-s	Mead-k			
1	247	0	0	0	0	0	0	0	0	0	0.019			1	169	0	0	0	0	0	0	0	0	0	0.005			
2	251	0.010	0.033	0	0	0	0	0.038	0	0	0			2	173	0	0	0	0	0	0.010	0	0	0	0	0.005		
3	255	0	0	0	0	0	0	0	0	0.050	0			3	177	0	0	0.010	0	0	0.021	0	0.011	0	0	0	0	
4	259	0	0.011	0	0	0	0	0	0	0.019	0			4	181	0	0	0	0	0	0	0	0	0.013	0	0.011	0	
5	263	0	0	0	0	0	0	0	0	0	0.006			5	185	0.019	0	0	0.043	0	0.021	0.016	0	0	0.027	0	0.027	
6	267	0	0	0	0	0	0	0	0	0	0.013			6	189	0	0	0	0.034	0	0.037	0	0.022	0.013	0	0	0	
7	271	0	0	0	0	0	0	0	0	0	0.013			7	193	0	0	0	0	0.174	0.063	0	0	0	0	0	0	
8	287	0	0	0	0	0	0	0.026	0	0	0			8	197	0	0	0.010	0.009	0	0.016	0.008	0	0	0.027	0	0.027	
9	295	0	0	0.015	0	0	0.006	0.038	0	0	0			9	201	0.013	0	0.030	0.026	0	0.047	0.016	0.032	0	0.032	0	0.032	
10	299	0	0	0.015	0.010	0	0.006	0	0.011	0.013	0.013			10	205	0.006	0.013	0.013	0.020	0.034	0.058	0.008	0	0	0.005	0	0.005	
11	303	0	0	0.015	0	0	0.006	0.026	0.011	0.013	0.006			11	209	0.063	0.092	0.090	0	0.130	0.021	0.025	0.032	0	0	0	0	
12	307	0	0	0	0	0.042	0.026	0	0.065	0.076	0.006			12	213	0.063	0.039	0.080	0.034	0	0.037	0.008	0.022	0.038	0.005	0	0.005	
13	311	0	0.011	0	0.010	0	0.051	0	0	0.013	0.013			13	217	0	0	0	0.008	0	0.047	0.016	0.011	0	0.005	0	0.005	
14	315	0	0.011	0	0	0	0.038	0	0.065	0	0.050			14	221	0	0	0	0.009	0	0.037	0.008	0	0.013	0	0	0	
15	319	0.048	0.098	0.197	0	0	0.032	0.077	0.032	0	0.013			15	225	0.006	0	0	0.009	0	0.052	0.016	0.022	0	0.005	0	0.005	
16	323	0	0	0	0.010	0.083	0.077	0.103	0.022	0.013	0.025			16	229	0	0	0	0.026	0	0.021	0	0	0.006	0	0.021	0	
17	327	0.029	0.033	0	0.030	0.083	0.071	0.026	0.043	0.013	0.006			17	233	0	0	0	0	0.043	0.026	0	0	0	0	0	0	
18	331	0	0	0	0.061	0.083	0.154	0.013	0.043	0.096	0.013			18	237	0	0	0	0	0	0.010	0	0	0	0	0	0	
19	335	0.010	0	0.015	0.081	0.125	0.141	0.077	0.086	0.102	0.044			19	241	0	0	0	0.017	0	0.005	0	0.011	0	0	0	0	
20	339	0.048	0.098	0.061	0.091	0.167	0.096	0.013	0.183	0.121	0.063			20	245	0	0	0	0.009	0	0.010	0	0	0.006	0	0	0	
21	343	0.124	0.087	0.121	0.152	0.042	0.058	0.064	0.118	0.044	0.044			21	249	0	0	0	0.009	0	0.005	0.008	0.022	0	0.005	0	0.005	
22	347	0	0.022	0.030	0.212	0.292	0.019	0.064	0.097	0.057	0.038			22	253	0	0	0	0.009	0	0.005	0.008	0	0	0.011	0	0.011	
23	351	0.076	0.120	0.121	0.091	0.083	0.019	0.064	0.097	0.057	0.038			23	257	0	0	0	0	0	0.005	0	0	0	0.005	0	0.005	
24	355	0.019	0.033	0.015	0.061	0	0.013	0.064	0.054	0.032	0.019			24	261	0	0	0	0	0	0.011	0	0	0	0.005	0	0.011	
25	359	0.181	0.120	0.121	0.020	0	0.058	0.026	0.011	0.006	0.044			25	265	0	0	0	0	0	0.005	0	0	0	0.032	0	0.032	
26	363	0.086	0.109	0.091	0.010	0	0.019	0.013	0.022	0.013	0.081			26	269	0	0.013	0.020	0	0	0.031	0	0	0	0.032	0	0.032	
27	367	0.019	0.011	0.045	0.020	0	0.032	0.026	0.011	0	0.081			27	273	0	0	0	0	0	0.031	0	0	0	0.032	0	0.032	
28	371	0.105	0.065	0.030	0.010	0	0	0.013	0.022	0.013	0.081			28	277	0.006	0.013	0	0.009	0	0.026	0.033	0.022	0	0.027	0	0.027	
29	375	0.096	0.011	0.015	0.020	0	0	0.051	0.011	0	0.063			29	281	0.050	0.007	0.040	0.009	0.087	0.026	0.082	0.011	0	0.043	0	0.043	
30	379	0.019	0.011	0.061	0	0	0	0.051	0	0	0.019			30	285	0.013	0.089	0	0.017	0	0.016	0.033	0.054	0.057	0.037	0	0.037	
31	383	0.029	0.011	0.015	0.030	0	0	0.051	0.032	0.025	0.025			31	289	0.019	0.020	0	0.009	0	0.026	0.043	0.054	0.108	0.053	0	0.053	
32	387	0.010	0	0	0	0	0.006	0.013	0.011	0	0.038			32	293	0	0.007	0.020	0.052	0	0.047	0.057	0.054	0.108	0.053	0	0.053	
33	391	0.067	0.054	0.015	0.010	0	0	0.013	0	0.025	0.025			33	297	0.013	0	0	0.095	0	0.016	0.047	0.049	0.129	0.108	0.027	0	0.027
34	395	0	0	0	0	0	0	0	0	0	0.019			34	301	0.188	0.178	0.250	0.103	0.087	0.047	0.047	0.054	0.133	0.074	0	0.074	
35	399	0	0.022	0	0	0	0	0	0.011	0.064	0			35	305	0.044	0.158	0.040	0.052	0.087	0.052	0.098	0.108	0.063	0.080	0	0.080	
36	403	0.019	0.033	0	0	0	0	0	0.032	0.013	0.019			36	309	0.094	0.105	0.070	0.043	0.043	0.047	0.098	0.043	0.057	0.037	0	0.037	
37	415	0	0	0	0	0	0	0	0	0.006	0.006			37	313	0	0	0	0.095	0	0.010	0.049	0.108	0.027	0	0.027		
38	419	0	0	0	0	0	0	0	0	0.019	0.019			38	317	0.075	0.046	0.040	0.009	0.217	0.010	0.016	0.111	0	0.032	0	0.032	
39	427	0	0	0	0	0	0	0	0.025	0	0			39	321	0.056	0.020	0.020	0.060	0	0.037	0.016	0.065	0.190	0.032	0	0.032	
40	431	0	0	0	0.030	0	0	0.013	0.011	0.064	0			40	325	0.006	0	0.040	0.052	0.087	0.031	0	0.097	0.139	0.032	0	0.032	
41	435	0	0	0	0.020	0	0	0	0.032	0.083	0.013			41	329	0.156	0.053	0.120	0	0	0.021	0.025	0.011	0.019	0.005	0	0.005	
42	439	0.010	0	0	0	0	0	0	0.013	0.013	0.013			42	333	0.038	0.039	0.040	0.026	0	0.005	0.016	0.054	0	0.021	0	0.021	
43	443	0	0	0	0.020	0	0	0.026	0	0.096	0.006			43	337	0.019	0.059	0.030	0.026	0	0	0.049	0	0	0.021	0	0.021	
44	447	0	0	0	0	0	0	0.051	0.022	0.051	0			44	341	0.006	0.020	0.010	0.026	0	0	0	0.011	0	0.005	0	0.005	
45	451	0	0	0	0	0	0	0	0	0.006	0.006			45	345	0	0.013	0.020	0	0	0	0	0	0	0.005	0	0.005	
46	463	0	0	0	0	0	0	0	0.022	0	0.006			46	349	0	0.007	0	0	0.043	0.005	0	0	0.013	0	0.013		
47	467	0	0	0	0	0	0	0	0	0	0.006			47	353	0.050	0	0	0	0.087	0	0	0	0	0.016	0	0.016	
48	475	0	0	0	0	0	0	0	0	0	0.013			Na	160		152	100	116	23	191	122	93	158	188			
49	483	0	0	0	0	0	0	0	0	0	0.013																	

Figure 4. Multidimensional scaling plot of F_{ST} values (using 8 microsatellite DNA loci) for Lake Washington/Lake Sammamish *O. nerka* [stress2 = 0.01785 indicates an excellent fit]. The minimum spanning tree (dashed lines) connects each population with the population most similar to it.



Appendix I (cont.). Allele frequencies in 10 collections of *Oncorhynchus nerka* from the Lake Washington & Lake Sammamish basins and from three other locations.

(Na = numbers of alleles successfully scored)

One-108											
code	size (bp)	Ebright-k	Lewis-k	Laugh-k	nTrib-k	Issaq-k	LWhat-k	Issaq-s	BeartN-s	Baker-s	Mead-k
1	178	0	0	0	0	0	0.006	0	0	0	0
2	182	0	0	0	0.038	0.042	0.012	0	0.052	0	0
3	186	0	0	0	0.019	0	0.076	0.009	0.031	0	0
4	190	0	0	0	0.009	0	0.082	0.057	0.031	0.044	0.087
5	194	0.014	0	0.011	0.066	0	0.094	0.019	0.073	0.306	0.058
6	198	0	0	0	0.123	0.083	0.024	0.066	0.010	0.237	0.087
7	202	0.035	0.056	0	0.066	0	0.047	0.047	0.063	0.069	0.105
8	206	0.007	0.014	0.032	0.066	0.125	0.088	0.057	0.083	0.081	0.140
9	210	0.077	0.127	0.191	0.160	0	0.118	0.085	0.177	0.025	0.110
10	214	0.070	0.035	0.074	0.123	0	0.076	0.179	0.156	0.019	0.110
11	218	0.035	0	0.032	0.094	0	0.076	0.170	0.073	0.081	0.081
12	222	0.182	0.134	0.128	0.038	0	0.024	0.057	0.115	0	0.064
13	226	0.056	0.063	0.021	0.019	0.167	0.035	0.047	0.021	0	0.081
14	230	0.343	0.373	0.266	0.094	0.250	0.047	0.132	0.042	0.131	0.023
15	234	0.042	0.021	0.032	0.047	0.292	0.053	0.038	0.021	0	0.017
16	238	0.077	0.049	0.064	0.028	0.042	0.065	0.009	0.031	0.006	0.006
17	242	0.007	0	0	0	0	0.024	0.019	0.021	0	0.017
18	246	0	0	0	0	0	0.029	0.009	0	0	0.006
19	250	0	0	0	0.009	0	0.006	0	0	0	0
20	254	0	0	0	0	0	0.018	0	0	0	0
21	258	0.056	0.127	0.149	0	0	0	0	0	0	0
22	270	0	0	0	0	0	0	0	0	0	0.006
Na	143	142	94	106	24	170	106	96	160	172	

One-110											
code	size (bp)	Ebright-k	Lewis-k	Laugh-k	nTrib-k	Issaq-k	LWhat-k	Issaq-s	BeartN-s	Baker-s	Mead-k
1	222	0	0	0	0.010	0	0	0	0.010	0	0
2	226	0	0	0	0.010	0	0	0	0.020	0.040	0.075
3	230	0.117	0	0	0.019	0	0.007	0.010	0.030	0	0
4	234	0.083	0.088	0.046	0.038	0.208	0	0.010	0.020	0	0.015
5	236	0	0	0	0.010	0	0.007	0	0	0	0
6	238	0.325	0.275	0.333	0.029	0.250	0.033	0.100	0.081	0.027	0.067
7	242	0.100	0.127	0.103	0.221	0.042	0.080	0.130	0.131	0.150	0.209
8	246	0.025	0.049	0.080	0.115	0	0.080	0.070	0.121	0.259	0.157
9	248	0	0	0	0	0	0.053	0	0	0	0.007
10	250	0.150	0.157	0.207	0.038	0.083	0.073	0.100	0.061	0.041	0.164
11	252	0	0	0	0.019	0.083	0.020	0	0.040	0	0.022
12	254	0.025	0.020	0.011	0.115	0.125	0.027	0.070	0.071	0.041	0.052
13	256	0	0	0	0	0	0.020	0	0	0	0.007
14	258	0.008	0.029	0	0.019	0	0.020	0.030	0.020	0.007	0.067
15	260	0	0.010	0	0.010	0	0.020	0	0	0	0.007
16	262	0.033	0.078	0.011	0.221	0	0.027	0.180	0.192	0.054	0.060
17	264	0	0	0	0	0	0.100	0.010	0	0	0.007
18	266	0.008	0.010	0.023	0.010	0.208	0.027	0.020	0.040	0.014	0.067
19	268	0	0	0	0	0	0.047	0	0	0	0.015
20	270	0.008	0	0.011	0.038	0	0.040	0.070	0.091	0.163	0.030
21	272	0	0	0	0	0	0.080	0	0	0	0
22	274	0.025	0.039	0.080	0.029	0	0.007	0.060	0	0.163	0.030
23	276	0	0	0	0	0	0.053	0	0	0	0
24	278	0.017	0.039	0.034	0.010	0	0.033	0.050	0.020	0	0
25	280	0	0	0	0	0	0.027	0	0	0	0
26	282	0.033	0	0	0.029	0	0.053	0.040	0.030	0	0.007
27	284	0	0	0	0	0	0.013	0	0	0	0.007
28	286	0	0	0	0.010	0	0.040	0.030	0	0	0
29	290	0.042	0.078	0.057	0	0	0.007	0	0	0	0
30	294	0	0	0	0	0	0.007	0	0	0	0
31	296	0	0	0	0	0	0	0	0	0.007	0
Na	120	102	87	104	24	150	100	99	147	134	

One-105											
code	size (bp)	Ebright-k	Lewis-k	Laugh-k	nTrib-k	Issaq-k	LWhat-k	Issaq-s	BeartN-s	Baker-s	Mead-k
1	125	0.007	0.007	0	0.032	0	0	0.008	0.049	0.013	0
2	129	0.362	0.493	0.349	0.274	0.636	0.120	0.342	0.390	0.199	0.258
3	133	0.296	0.342	0.443	0.194	0.182	0.177	0.150	0.122	0.167	0.595
4	137	0.316	0.125	0.179	0.419	0.136	0.344	0.342	0.354	0.500	0.089
5	141	0.020	0.033	0.028	0.048	0.045	0.354	0.158	0.085	0.122	0.042
6	145	0	0	0	0	0	0.005	0	0	0	0.016
7	153	0	0	0	0.032	0	0	0	0	0	0
Na	152	152	106	124	22	192	120	82	156	190	