

Genetic Characterization of Lake Ozette Sockeye Salmon for Enhancement Recovery Strategies

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Abstract

Makah Fisheries Management (MFM), Washington Department of Fish and Wildlife (WDFW), and the Northwest Indian Fisheries Commission (NWIFC) collected genetic information on spawning aggregations of anadromous sockeye and resident kokanee in the Lake Ozette basin during 2000-2001 to help develop hatchery and recovery strategies for Lake Ozette sockeye salmon (*Oncorhynchus nerka*). Baseline genetic profiles using microsatellite DNA loci were developed by WDFW from fish collected by MFM. The data revealed large genetic differences between sockeye and kokanee populations in the Lake Ozette basin, indicating that it will be possible to use these markers to monitor potential hybridization among kokanee and sockeye salmon. Comparisons between two beach spawning aggregations, one at Olsen's Beach and one at Allen's Bay, showed that the two beach spawning aggregations were significantly different from each other in 2000. Differences also existed between cohort lineages. Allele frequencies of sockeye salmon spawning at Olsen's Beach in 1996 and 2000, which represent parents and their adult offspring, were not significantly different from each other, but sockeye salmon spawning at the Olsen's Beach in 1999 and 2000 were significantly different. Lake Ozette sockeye salmon had lower allelic diversity at microsatellite DNA loci than other *O. nerka* populations examined in Washington State, which suggested that they have had a smaller effective population size. Continued collection and analyses of archived and new Lake Ozette *O. nerka* samples from other years is necessary to provide a more complete comparison of geographical and temporal differences and to determine if genetic differences observed among beach-spawning populations are a consistent pattern.

Executive Summary

The Lake Ozette basin is located near the northwestern tip of the Olympic Peninsula in Washington State. Two distinct groups of *Oncorhynchus nerka* exist in Lake Ozette. Anadromous sockeye salmon, which are protected by the Endangered Species Act (ESA), are thought to have once spawned in tributaries and along lake beaches. Currently they are presumed to have been extirpated from tributaries, and beach spawning aggregations have declined in abundance. Earliest recorded adult returns entering Lake Ozette averaged several thousand sockeye (Kemmerich 1945) but have averaged less than 2,000 in the last 30 years. In contrast, kokanee, which spend their entire life history in freshwater, are genetically distinct, unprotected by the ESA, and are relatively abundant in several tributary spawning aggregations.

The Makah Tribe has operated a hatchery supplementation and reintroduction program for Lake Ozette sockeye salmon on a tributary to Umbrella Creek since 1982 to restore the Ozette sockeye salmon to levels that can once again provide meaningful fisheries. In response to the National Marine Fisheries Service (NMFS) decision (FR 14528, Mar. 24, 1999) to list Lake Ozette sockeye salmon as a threatened species under the ESA, the Makah Tribe, in consultation with NMFS, the U.S. Fish and Wildlife Service, and the WDFW, developed the Lake Ozette Sockeye Hatchery Genetic Management Plan (HGMP) to govern future management of its Lake Ozette sockeye salmon hatchery supplementation and reintroduction program. The HGMP combines modern hatchery management practices with a rigorous monitoring, evaluation, and research program designed to avoid, minimize, and mitigate any unintended consequences of hatchery supplementation, including undesirable genetic changes in the listed population and any adverse ecological interactions that arise from program actions.

This study provided genetic information that is essential for accomplishing these goals and other recovery activities in the basin by focusing on two objectives:

- To test the hypothesis that the two major beach spawning aggregations of sockeye salmon remaining in Lake Ozette on Olsen's Beach and Allen's Bay are components of a single, panmictic population and
- To identify genetic markers that can be used to monitor potential hybridization between sockeye salmon and kokanee resulting from reintroduction of sockeye salmon to the tributaries.

Completing these objectives is critical for addressing sections 1.1, 2.3, 3.1, and 4.5 of the *Scientific Framework for Artificial Propagation of Salmon and Steelhead* developed by the Hatchery Scientific Review Group (HSRG).

Makah Fisheries Management and WDFW biologists identified, consolidated, and collected new and archived tissue samples from different studies of sockeye salmon to create a tissue bank. From this they chose samples of three

different sockeye salmon spawning aggregations in different years (Olsen's Beach 1996, 1999, 2000; Allen's Bay 2000; Umbrella Creek 2000) and two kokanee spawning aggregations (Crooked Creek and Siwash Creek) for genetic analysis. The WDFW genetics laboratory examined these samples for genetic differences using nine microsatellite loci: *One-100*, *One-101*, *One-102*, *One-105*, *One-108*, *One-110*, *One-114*, *One-115*, and *Ots-103*.

Results of pairwise tests of population differentiation and F_{ST} values indicated that genetic differences existed both between sockeye salmon spawning at different beaches and among spawners returning in different years to the same beach. This latter difference may be more attributable to the existence of four, largely independent cohort lineages that return in different years than to allele fluctuations between generations as a result of genetic drift. Significant allele frequency differences existed between spawners from Allen's Bay 2000 and spawners from Olsen's Beach in 1996, 1999, and 2000 (Table 2). Significant differences also occurred in comparisons of sockeye salmon from Olsen's Beach among different cohort lineages (Olsen's 1996 vs. Olsen's 1999; Olsen's 1999 vs. Olsen's 2000, Table 2). We found no significant differences between parents and their adult offspring from Olsen's Beach, however (Olsen's 1996 vs. Olsen's 2000, Table 2), and F_{ST} was not significantly different from zero (Table 3). Although suggestive, these results need to be used with caution. Too few years were included in the analysis to determine whether this is a consistent pattern, especially for Allen's Bay, and the levels of differentiation among spawners from different beaches were similar to that observed between Olsen's Beach sockeye salmon and Umbrella Creek sockeye salmon, which were derived from both Allen's and Olsen's Beach spawning fish.

We concluded that microsatellite loci will provide an effective tool for monitoring potential hybridization between sockeye and kokanee in Lake Ozette. Microsatellite allele frequencies between sockeye and kokanee in Lake Ozette were very different. Jackknife classification analyses based on the multilocus genotypes documented in this study indicated that *O. nerka* could be confidently assigned to their respective sockeye salmon or kokanee life history types.

Additional work and funding are needed to expand the database for sockeye salmon to include more years so that the degree of differences between spawning aggregations can be understood. The co-managers intend to continue to collect and archive samples until this funding becomes available. Makah Fisheries Management is using these results to refine study designs to monitor hybridization between sockeye salmon and kokanee and as part of their adaptive management of artificial production.

Approach

Objective 1: Test the hypothesis that the two major beach spawning aggregations of sockeye salmon at Olsen's Beach and Allen's Bay are the same population using microsatellite DNA loci.

Task 1: Review the scientific literature for information on the biological characteristics and historical population structure of Lake Ozette basin sockeye salmon populations. Biologists from MFM collected and reviewed all the available published and unpublished information on Lake Ozette sockeye salmon that might be useful in interpreting the results of this study to make informed decisions for hatchery reform and sockeye recovery in the basin.

*Task 2: Consolidate and catalog existing genetic samples of *Oncorhynchus nerka* from the Lake Ozette basin.* Based upon Task 1, MFM and NWIFC identified a variety of investigators from universities, WDFW, and the National Marine Fisheries Service (NMFS) who have collected tissue samples from Lake Ozette sockeye salmon in Lake Ozette. Using this information, MFM and WDFW located existing archived samples from Lake Ozette sockeye salmon from return years 1994, 1995, 1996, 1998, and 1999, consolidated these samples (except for those from 1995) with collections made for this study in 2000, and developed a catalog of tissue samples for future studies. The resulting collection of preserved tissue samples is now housed at the WDFW Genetics Laboratory in Olympia, Washington.

Task 3: Collect tissue samples from sockeye salmon spawning aggregations in 2000 for genetic analysis. Makah Fisheries Management surveyed the following stream and lake areas for *O. nerka* spawners: Umbrella Creek, two unnamed tributaries to Umbrella Creek, Big River, Trout Creek and Boe Creek (tributaries to Big River), Coal Creek, Crooked Creek, Siwash Creek, South Creek, and a total of eleven lake beach areas, including the two primary anadromous sockeye salmon spawning locations. Spawn survey reaches, known kokanee and sockeye spawning locations, and the areas where the genetic samples, were collected are illustrated in Figure 1.

Sampling methods for genetic tissues varied. In return year 1999, MFM collected beach spawning sockeye for genetic tissue samples using a gillnet, with the exception of Allen's Beach, where all but one of the samples came from carcasses recovered during spawner surveys. About two thirds of the sockeye samples from Umbrella Creek in 1999 were composed of fin clips taken from live captures of spawned out adult sockeye, with the remaining third being tissue samples taken from carcasses during spawner surveys. Return year 2000 samples were collected from dead adult sockeye that had spawned or were predator mortalities, with the exception of 41 sockeye that were live-captured using a gillnet on Olsen's Beach. Pelvic fin-clip tissue samples were taken from live captures, and operculum samples were taken from carcasses. Umbrella Creek sockeye samples were collected from hatchery brood stock in 2000, all but one of these were pelvic fin clips, with a single operculum-punch sample. One kokanee operculum tissue sample was also collected from Umbrella Creek. All other tissue samples, currently stored at WDFW, were stored in 100% ethyl alcohol.

Task 4: Develop a microsatellite DNA screening protocol and genotype fish from as many relevant collections as possible based on available funding. Based on Task 1, we chose microsatellite DNA markers because earlier investigations of sockeye salmon and kokanee using allozyme electrophoresis (Winans et al. 1996; Gustafson et al. 1997) revealed relatively low levels of detectable genetic variation consisting of only a few variable loci, each having only 2-4 alleles. We believed that more variable genetic markers were needed to determine fine-scaled population interrelationships (Objective 1), to provide the necessary discriminatory power to assess possible introgression between sockeye and kokanee in the Lake Ozette basin (Objective 2, below), and to allow assessment of past and future hatchery operations. Indeed, a number of recent studies have demonstrated the power of microsatellite DNA markers to elucidate population structure and assign individuals to their most likely stock-of-origin (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).

Microsatellite DNA loci are arrays of short, repeated (mostly di-, tri-, and tetra-nucleotide) sequences occurring commonly in eukaryotic organisms (Wright and Bentzen 1994). Microsatellites generally are less than 300 base pairs in size and allelic variation is commonly due to variation in the number of times the basic repeating unit occurs (thus, microsatellite alleles are typically distinguished and named according to their sizes). Microsatellites are considered to be non-coding in that they are not known to be transcribed into RNA and, therefore, do not encode proteins. Additionally, allelic variation at most microsatellite DNA loci is assumed to be selectively neutral. Microsatellite DNA variation typically exhibits biparental, Mendelian inheritance and alleles are co-dominantly expressed, allowing an organism's genotype to be unambiguously inferred from its DNA phenotype (Ardren et al. 1999). Additionally, microsatellites evolve rapidly and often exhibit many alleles and high heterozygosities. These characteristics make microsatellites very useful markers for investigating genetic aspects of population structure.

DNA extractions and amplification

The WDFW genetics laboratory analyzed 344 sockeye salmon from five collections taken from 1996 to 2000 (Table 1). DNA was purified from all samples using commercially-available, 96-well, silica membrane-based kits (Machery-Nagel Nucleospin multi-96 tissue kits obtained from Clontech Laboratories Incorporated). Nine microsatellite DNA loci were examined using the polymerase chain reaction (PCR; see Saiki et al. 1988) and fluorescently-labeled primers. We used eight primer sequences that were obtained from sockeye salmon (Olsen et al. 2000) and one sequence (*Ots-103*) that was obtained from chinook salmon, *Oncorhynchus tshawytscha* (Small et al. 1998). Groups of three loci were multiplexed together at the PCR step to make screening more efficient and cost effective (Olsen et al. 1996; Neff et al. 2000). The specific PCR amplification protocols used were:

PCR multiplex OneA: *One-108* at 0.06 μ M; *One-110* at 0.8 μ M; and *One-100* at 0.8 μ M

PCR multiplex OneB: *One-102* at 0.06 μ M; *One-114* at 0.1 μ M; and *One-115* at 0.08 μ M

PCR multiplex OneC: *One-105* at 0.04 μ M; *Ots-103* at 0.2 μ M; and *One-101* at 0.05 μ M

All PCR amplifications used 0.2 μ M of each dNTP, 1.5 mM MgCl₂, and 0.5 unit Taq polymerase in 10 μ L reactions using the following thermal profile: an initial three minute denaturation at 92°C, followed by 38 cycles of 15 second denaturation at 92°C, 30 second annealing at 50°C, 60 second extension at 72°C; and then a final 30 minute extension at 72°C.

Genotyping

Individual genotypes, based on microsatellite DNA analysis, were identified for the nine loci with procedures established in the WDFW genetics laboratory (Shaklee and Young 2000) using a 96-lane ABI-377 automated DNA sequencer with in-lane size standards (GeneScan-500 rox; Applied Biosystems). Raw data from the DNA sequencer was processed using Genescan (v. 3.1) and Genotyper (v. 2.0; 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 that were defined based on the presumed repeat motif of each microsatellite and the observed distributions of raw Genotyper size calls for each locus. All loci screened in this study were scored as having tetranucleotide repeat motifs. DNA fragments, whose raw size estimates fell between bins, were not assigned an allelic designation and the resulting diploid genotype at the locus was zeroed so that such ambiguous data were not included in subsequent analyses. The final Excel file was used to output a genotype file that was used for statistical analysis.

Task 5: Use statistical analyses to test null hypotheses. The original design of this study (and the reason that we identified and consolidated archived samples in Task 2) was to allow a nested, hierarchical design to compare the genetic differences between sockeye salmon and kokanee (Objective 2, below), differences within sockeye salmon and kokanee attributable to different spawning locations, differences within sockeye salmon spawning at a specific location attributable to different cohort lineages, and differences within sockeye salmon spawning at a specific location attributable to different generations (Objective 1). Unfortunately, because we only received funding for one year, we could not fulfill this design. Nevertheless, for this one year study, we chose seven samples that would give us some idea of the magnitude of these differences, recognizing that the unbalanced, unreplicated design would seriously limit our inference. We chose two groups of kokanee samples (Siwash and Crooked creeks), and five groups of sockeye salmon samples (Olsen's Beach 1996, 1999, 2000; Allen's Bay 2000, and Umbrella Creek 2000). We used GENEPOP (version 3.3) of Raymond and Rousset (1995a) to calculate allele frequencies and to conduct tests of Hardy-Weinberg equilibrium, genotypic disequilibrium, and population differentiation (Raymond and Rousset 1995b). We used the computer program GDA (Lewis and Zaykin 2001) to calculate F_{ST} values and their 95% confidence intervals.

Objective 2: Identify genetic markers that could be used to monitor potential hybridization between sockeye salmon and kokanee.

Because sockeye salmon and kokanee represent different life history forms of the same species, *Onchorhynchus nerka*, it is reasonable to expect that they can successfully interbreed. Nevertheless, studies using allozyme and DNA markers have shown that sympatric populations of sockeye and kokanee from various localities in British Columbia and Washington (including Lake Ozette) are genetically distinct (Winans et al. 1996; Wood and Foote 1996). Despite such demonstrations that sympatric populations of the two forms often exhibit significant differences in allele frequencies, evidence that size-based, assortative mating occurs and promotes reproductive isolation between the smaller kokanee and larger sockeye salmon (Foote and Larkin 1988), and evidence that hybrid progeny of sockeye x kokanee matings may be less fit than either pure form (Wood and Foote 1990; Foote et al. 1992; Wood and Foote 1996), whether or not any hybridization between the two forms occurs and if so at what level it occurs remains largely unknown for any given locality (cf. Foote et al. 1989). The opportunity for such hybridization between the two forms could be especially high in situations where one or both forms are introduced into a common habitat that is outside of their historical or recent area of reproduction – as is occurring with reintroduction of sockeye salmon into Umbrella Creek and Big River in the Lake Ozette Basin. Also, if current management increases the occurrence of residualization of sockeye, their diminished size might reduce size-based impediments to interbreeding with kokanee, although there has been no evidence to indicate residualization or reduced size has occurred due to reintroduction efforts. Considering that reproductive isolation between these two forms might be diminished if hybridization and introgression occur as a result of reintroduction efforts, identifying suitable genetic markers and statistical tests that can be used to detect sockeye x kokanee hybrids was an important goal of this study.

Task 6: Collect samples of kokanee from Lake Ozette streams. MFM collected tissues from kokanee from Siwash and Crooked creeks in 2000. One kokanee sample was also collected from Umbrella Creek, where kokanee are rare. All of these samples were collected using dip-nets and rod and reel, except for a few carcasses sampled in Crooked Creek during spawner abundance surveys.

Task 7: Develop a microsatellite DNA screening protocol for genotyping kokanee. The WDFW genetics laboratory verified that the same screening protocol developed for sockeye salmon was suitable for kokanee and used this protocol to analyze a total of 100 kokanee from the two kokanee collections for microsatellite DNA variation (Table 1). Microsatellite methods are described in Task 4.

Task 8. Identify genetic markers. We used the program WHICHRUN (Banks and Eichert 2000, version 4.0 beta) to conduct jackknife analyses to investigate the feasibility of correctly assigning kokanee and sockeye salmon to their respective life history type using multilocus microsatellite DNA genotypes. All fish were initially classified as putative sockeye salmon or kokanee based on field observations, including size, external morphology of fish, and location of capture. We then used the jackknife function of WHICHRUN to remove a fish, recalculate the allele frequencies of the respective baseline population (sockeye salmon or kokanee) from which it came, and assign the fish to its most likely life history type (sockeye or kokanee), based on its multilocus genotype at the microsatellite DNA loci.

Findings

Task 1: Literature Review

Understanding population structure is important for assessing the overall trends in abundance in Lake Ozette sockeye salmon. The overall abundance of naturally-produced Lake Ozette sockeye salmon has probably declined substantially from historical levels, although precise estimates of historical abundance were poorly documented. In the earliest quantitative estimate of abundance, Kemmerich (1945) estimated that the number of sockeye entering the lake in 1924-1926 ranged from three to six thousand fish, which is approximately twice the current run size, even without considering that the historical estimates were presumably conducted upstream from fisheries in or near to the Ozette River. In the 1997 sockeye salmon status review, NMFS reported that recent escapements averaged below 1,000 adults per year and in low abundance years dropped to only a few hundred fish. Their findings suggested that low abundance trends created a degree of risk for loss of population genetic and demographic variability that left little room for further declines before abundances reached critically low levels (Gustafson et al. 1997). Recent abundance trends and reexamination of methods for estimating abundances indicate that sockeye abundance may be relatively stable or increasing in recent years, presumably with the help of the hatchery supplementation and reintroduction program to the tributaries. Based on recent adult counts monitored by underwater video camera, the run size has ranged from a low of 1,133 to a high of 4,400 adult sockeye entering Lake Ozette (return years 1997 – 2000), and has averaged more than 2,250 adults (MFM unpublished data; MFM 2000).

Mature adults return to freshwater during an extended period from spring through late-summer. Historically, most Ozette sockeye salmon spawned on lake beaches, or in tributaries (Jacobs et al. 1996) where they are being reintroduced after they were presumably extirpated earlier in this century. The two remaining principle shoreline spawning areas for Lake Ozette sockeye salmon are on Olsen's Beach, located on the lake's eastern shore north of Siwash Creek, and on Allen's Bay, on the lake's southwestern shore (MFM 2000, Jacobs et al. 1996). To determine whether the relative changes in abundance at Olsen's Beach and Allen's Bay reflect fluctuations in a single population, Objective 1 of this study examined these aggregations for genetic differences that might indicate that they are subpopulations. Genetic differences among different spawning aggregations also have significant implications for how and where brood stock are selected, what methods are used, and where their progeny should be released, if artificial production is used to reintroduce and supplement beach spawning.

Because both sockeye salmon and kokanee from other localities have been transplanted from hatcheries into the Lake Ozette basin, genetic (and other) characterizations of the extant populations in Lake Ozette are not entirely straightforward. According to Gustafson et al., (1997; pp. 65-66), sockeye introductions included over 450,000 fingerlings in 1937 (Baker Lake stock and probably Fraser River and Quinault stocks as well) and 120,000 fry from Quinault Lake in 1983 while over 100,000 kokanee fry were introduced from the Lake Crescent trout hatchery (in 1940) and a second introduction (from an unknown source) occurred in 1958. Although no significant returns from these plants are documented, it is not known whether any fish from these introductions survived and later spawned in the Lake Ozette basin.

Previous analyses indicated that Ozette sockeye were genetically distinct from other sockeye populations in Puget

Sound (Winans et al. 1996), and from other Washington coastal populations (Gustafson et al. 1997). Dlugokenski et al. (1981) suggested that sockeye salmon spawning at Allen's Bay and Olsen's Beach might also be different subpopulations based on spawn timing. Hershberger et al. (1982) surveyed genetic variation at 37 allozyme loci (only two of which were polymorphic) in sockeye salmon from Lake Ozette and based on the variation at *PGM-1**, suggested that two groups (or populations) of sockeye salmon might be present in Lake Ozette, separated by a difference in run-timing. Gustafson et al. (1997) found differences in allozyme frequencies among the Allen's and Olsen's beach-spawning aggregations, but the differences between years at the same spawning location were as great as the differences between beaches. This suggested that the observed differences among spawning beaches could reflect chance temporal changes in allele frequencies caused by genetic drift rather than due to geographical isolation.

Most sockeye salmon spawning in Lake Ozette begins by late-October to early-November and is completed by late-December, but Dlugokenski et al. (1981) reported relatively large numbers of spawners on Allen's Beach in January of 1979, with significant numbers spawning into February of 1979. In recent years (1998-2000), however, peak spawning on Allen's Bay beach, like Olsen's Beach, has occurred prior to January. In 1999 and 2000, MFM surveyed for a later beach spawning component in Lake Ozette. In return year 1999, only a few redds and one adult sockeye (February 4, 2000) were observed on lake beaches in surveys conducted from early-January through February 15, 2000 (MFM 2000), but the observations may have been confounded by a flood of a magnitude with a 50 to 100+ year expected recurrence interval that occurred on December 15, 1999. This flood reduced visibility to less than one meter in the lake for over a month, and resulted in water depths more than a meter deeper than average on the spawning beaches, hampering survey efforts significantly. In return year 2000, spawning was observed through January 22, 2001, on Allen's Bay beach, and through January 31, 2001, on Olsen's Beach. On January 11, 2001, 92 sockeye and 26 redd complexes were observed at Olsen's Beach and Allen's Bay beach, combined.

Review of the age structure of Lake Ozette sockeye salmon suggested that the population consisted of four different lineages. Mature adults return to Lake Ozette almost exclusively as four-year-olds, which means that each year, over four years, a different lineage of adults returns to spawn. Recent scale analyses conducted by WDFW on 1998 brood stock revealed that 71/71 (100%) were age 4₂. Scale analysis conducted in conjunction with a 1994 genetics study identified 80 of 81 samples as four-year-olds, and one as a 5₂. A small number of putative three-year-olds (age estimated from observed fish size and/or fin clip status) have been observed passing by the weir in the Ozette River and on the spawning grounds (MFM 2000). Dlugokenski et al. (1981) identified only a few three-year-old fish in surveys between 1977 and 1979. In adult tagging studies conducted in 2001, several fin-clipped adult sockeye salmon were captured as they returned to the lake in June. These fish presumably originated from brood year 1996 or 1998 hatchery releases when released fingerlings were fin-clipped, because no sockeye salmon were clipped from brood year 1997. This suggested that the larger clipped adults were likely age 5₂ or 5₃ and that smaller returns were likely age 3 (MFM unpublished data). This may also indicate that hatchery practices are altering the age-of-return for some of the adults or that small numbers of returning adults comprised of these other age classes were not previously observed arising from natural lake production.

Lake Ozette sockeye salmon spend over a year in freshwater before migrating to the ocean. Fry rear in the lake for about one year before undergoing smoltification and migrate to sea at an unusually large size, which has been attributed to a highly productive lake environment (MFM 2000; Jacobs et al. 1996). Dlugokenski et al. (1981) reported that they were the third largest sockeye salmon smolts reported in the literature. Burgner (1987) found that Lake Ozette sockeye smolts weighed more than twice the average weight of sockeye smolts measured in 34 different lake systems.

Kokanee

Although kokanee—a resident form of *Oncorhynchus nerka* that is native to Lake Ozette and that spends its complete life history in freshwater—have evolved independently from other populations of kokanee and from anadromous sockeye salmon (Gustafson et al. 1997), ecologically the juveniles are similar. Juveniles of both life history forms presumably occupy the same habitat as post-emergent fry and while rearing in the pelagic zone of Lake Ozette until the sockeye salmon undergo smoltification. Hydroacoustic and net surveys estimated that of the total offshore fish abundance estimate (439,000), approximately 395,000 (90%) were kokanee and sockeye (Beauchamp et al. 1995). Adult kokanee spawners are present in the lake and in tributaries from late-October to January annually. The spawning population size of kokanee has not been well studied in the Lake Ozette basin, but Beauchamp et al. (1995) estimated that approximately 7,500 kokanee may spawn in the tributaries annually.

A potential risk of reintroducing sockeye salmon into the tributaries is that they might hybridize with kokanee, leading to reduced fitness in the progeny. In some lake systems with kokanee and sockeye salmon, 20% of the fertilization of sockeye salmon eggs has been attributed to male kokanee (Wood and Foote 1996). Previous genetic analyses, however, suggested that little or no genetic exchange has occurred in Lake Ozette (Gustafson et al. 1997). Numerous observations of small numbers of kokanee-sized *O. nerka* have been observed on spawning beaches in Lake Ozette and in Umbrella Creek spawning with sockeye salmon (MFM unpublished data). In 1998, 13 kokanee-sized *O. nerka* were observed contemporaneously with 96 sockeye, during peak spawner counts in Umbrella Creek. Only three of an estimated escapement of 400 adults in 1999 and less than 10 of an estimated escapement of 2,500 (MFM preliminary estimate) adults in 2000 were kokanee-sized *O. nerka* in Umbrella Creek. In 1999, none of the kokanee-sized fish were observed spawning with sockeye, while in 1998 and 2000, sockeye and kokanee-sized *O. nerka* were seen spawning together. We do not know if the kokanee-sized fish observed in sockeye spawning areas in Umbrella Creek in 1998 and 2000 were kokanee, residualized sockeye, or small anadromous sockeye salmon. Adult kokanee in spawning coloration were observed (Pat Gearin, National Marine Fisheries Service Marine Mammal Laboratory, personal communication) and one spawned out kokanee was recovered (MFM unpublished data 2000) near spawning beaches. Adult kokanee in spawning coloration have previously been captured on spawning beaches during brood stock collections (Bill Mahone, personal communication, 1998).

Task 2, 3, 6: Consolidation of Existing Tissue Samples.

Makah Fisheries Management and WDFW compiled and consolidated a detailed inventory of tissue samples of *O. nerka* spawning aggregations from this and previous studies in the Lake Ozette basin for use in this study and possible future investigations. The inventory now contains six years of samples from Olsen's Beach (573 samples), five years of Allen's Bay samples (244 samples), two years of samples from Umbrella Creek adults (159 samples), one year of samples from Umbrella Creek fry (105 natural-origin and 46 hatchery-origin samples), and one year of kokanee samples (100 from Siwash Creek and 142 from Crooked Creek).

Task 4, 7: Develop a microsatellite DNA screening protocol and genotype samples.

All nine loci were successfully examined and were polymorphic in all samples. Numbers of alleles per locus ranged from a low of three at *One-105* in the Olsen's Beach 1996 and 2000 sockeye collections to 33 at *One-101* in the Crooked Creek kokanee collection (Appendix 1). Tests of observed genotypic proportions did not identify any significant deviations (after correction for multiple testing) from Hardy-Weinberg equilibrium expectations, which indicated undetected null alleles were not present at substantial frequencies at any of the nine loci and that the collections could be treated as randomly-mating populations.

Pairwise tests of genotypic disequilibrium indicated that each of these loci could be treated as unlinked. Results of the pairwise tests for the nine loci yielded only four statistically significant deviations (*One-108* x *One-110*, *One-108* x *One-101*, *One-110* x *One-101*, and *One-115* x *Ots-103*). Three of these pairs were significant in only one collection: *One-108* x *One-110* in Olsen's Beach in 1999, *One-108* x *One-101* in Allen's Bay; and *One-115* x *Ots-103* in Siwash. The *One-110* x *One-101* comparison was not significant in any individual collection. Two other comparisons, which were not significant over all collections were each significant in one collection: *One-115* x *One-105* in Olsen's Beach 2000 and *One-115* x *One-101* in Umbrella Creek. These results are consistent with those of Young et al., (2001) and Young and Shaklee (2001), who found no evidence of close linkage among any of these loci in other sockeye salmon or kokanee populations in the Lake Sammamish basin or in kokanee populations from Lake Roosevelt or Lake Whatcom in Washington state, or from Meadow Creek in British Columbia.

At eight of the nine loci screened, the Lake Ozette kokanee samples had significantly more alleles than the sockeye salmon (Wilcoxon's signed rank test; $P < 0.01$), even though fewer kokanee were screened (total $N = 100$) than sockeye (total $N = 344$). We also compared the numbers of alleles per locus observed in this study for Lake Ozette sockeye salmon and kokanee with the numbers found in 11 other *O. nerka* populations (6 sockeye salmon and 4 kokanee populations from various locations in Washington state and one kokanee population from Meadow Creek in British Columbia), as reported by Young et al. (2001) and Young and Shaklee (2001); see Appendix 2. Comparisons were not meaningful at *One 110** because the data for this locus in the other collections were not comparable to those in this study. Nevertheless, at the remaining eight loci (*One-100**, *One-101**, *One-102**, *One-105**, *One-108**, *One-114**, *One-115**, and *Ots-103**), the mean numbers of alleles observed in the Lake Ozette sockeye salmon and kokanee collections were significantly smaller than the mean numbers observed in the other *O.*

nerka collections (Wilcoxon's signed rank test; $P < 0.005$ for sockeye salmon and $P < 0.01$ for kokanee; Appendix 2). Lake Ozette sockeye salmon had the fewest alleles at all loci. At *One-101**, Lake Ozette kokanee had more alleles (average of 30 alleles per collection) than the other three *O. nerka* populations compared (average of 24.3 alleles per collection). This suggested that Lake Ozette sockeye salmon have experienced a smaller effective population size, either as a result of overall reduced abundance, finer population structure, or a recent genetic bottleneck. Similar reduction in genes related to fitness can increase the risk of population extinction from fixation of deleterious alleles lower productivity from loss of favorable alleles and inbreeding depression.

Task 5: Test null hypotheses that Allen's Bay and Olsen's Beach are a single, panmictic population.

Results of pairwise tests of population differentiation and F_{ST} values indicated that statistically significant genetic differences existed between sockeye salmon spawning at different beaches and among spawners returning in different years to the same beach. This latter difference may be more attributable to the existence of four, largely independent cohort lineages that return in different years than to allele fluctuations between generations as a result of genetic drift. Significant allele frequency differences existed between spawners from Allen's Bay 2000 and spawners from Olsen's Beach in 1996, 1999, and 2000 (Table 2). Significant differences also occurred in comparisons of sockeye salmon from Olsen's Beach among different cohort lineages (Olsen's Beach 1996 vs. 1999; Olsen's Beach 1999 vs. 2000, Table 2). No significant differences occurred between parents and their adult offspring, however (Olsen's Beach 1996 vs. 2000, Table 2) and F_{ST} was not significantly different from zero (Table 3).

Although the exact genealogy of the sockeye salmon recently colonizing Umbrella Creek is unknown, they most likely originated from Umbrella Creek hatchery offspring of sockeye salmon taken largely from Olsen's Beach although significant numbers of spawners from Allen's Beach also contributed to past broods. The genetic data show a strong similarity to sockeye salmon from the Olsen's Beach aggregation. No significant differences were detected between Umbrella Creek 2000 and Olsen's Beach 1996 sockeye salmon (Table 2). However, small but statistically significant differences were detected between sockeye salmon from Umbrella Creek 2000 and Olsen's Beach in 1999 and 2000. These differences may be due to genetic differences between cohort lineages, different combinations of spawners taken from both Allen's and Olsen's Beaches in the parent brood years 1995 and 1996 as brood stock, or genetic drift in the natural-spawning river population. F_{ST} values between Umbrella Creek and Olsen's Beach and Allen's Bay (0.006 and 0.012, respectively) were similar to F_{ST} values detected between Olsen's Beach and Allen's Bay in different years (Table 3).

Although suggestive, these results should be interpreted with caution. These results and earlier studies (Winans et al. 1996) indicate the different subpopulations may exist in Lake Ozette. Too few years were included in the analyses, however, to determine whether the patterns we observed here are consistent over time, especially for Allen's Bay where only a single collection of fish from one brood year (2000) were analyzed. Likewise, although exact tests of population differentiation showed significant differences between Olsen's Beach and Allen's Bay (Table 2), F_{ST} was not significantly different from zero for sockeye salmon returning to Olsen's Beach and Allen's Bay in 2000 (Table 2). In addition, F_{ST} values between beaches and years were similar to those observed between Olsen's Beach and Umbrella Creek sockeye salmon.

It is important to consider that returns to Umbrella Creek in all brood years were derived from differing combinations of adult sockeye captured from both Olsen's and Allen's Beaches. Also, a varying component of past releases onto the spawning beaches were comprised of fish from other beaches (Allen's Beach-origin fish released onto Olsen's and vice versa). However, on the one year where it was possible to monitor marked adult returns resulting from lake releases (the 1998 return from 1994 releases onto Allen's and Olsen's Beaches), no returns were recovered from Allen's Beach, less than 5% were recovered from Olsen's Beach, and more than 95% returned to Umbrella Creek.

Task 8: Identify genetic markers to monitor potential hybridization between sockeye salmon and kokanee.

Microsatellite allele frequencies between sockeye salmon and kokanee in Lake Ozette were very different. Significant differences in genotype and allele frequencies between kokanee and sockeye salmon occurred at nearly all nine loci (Table 2). Pairwise F_{ST} between kokanee and sockeye salmon ranged from 0.099 to 0.110, compared to values between sockeye salmon aggregations that ranged from 0.005 to 0.008 (Table 3). Visual inspection indicated that different allele frequencies occurred at *One-100**335 and *343; *One-101**217, *221, *225, and *309; *One-102**236 and *240; *One-105**137; *One-108**202, and *214; *One-110**240 and *256; *190, *One-114**270 and *286;

*One-115*198* and **206*; and *Ots-103*200* (Appendix 1). We observed several alleles that occurred in kokanee at high frequencies but were absent or rare (<0.015) in sockeye salmon aggregations. These included: *One-101*297*, *One-102*248*, *One-105*133*, *One-108*186*, *One-110*264*, **272*, **276*, and **280*, *One-114*250*, and *One-115*186*. Sockeye salmon also had alleles that were absent in the kokanee: *Ots-103*156*, *One-100*311*, *One-108*262*, *One-110*236* and **252*, and *One-114*290*.

Tests of genetic differentiation between the two kokanee collections provided no evidence that they represented different subpopulations. No differences in allele or genotype frequencies occurred between kokanee collected from Siwash and Crooked Creeks in 2000, based on Raymond and Rousset's (1995b) tests for population differentiation, and F_{ST} was zero.

Based on the multilocus genotypes documented in this study, we concluded that microsatellite loci will provide an effective tool for monitoring potential hybridization between sockeye and kokanee in the Lake Ozette basin (Figure 2). Nearly all of the fish identified in the field as sockeye were grouped with an approximately normal distribution of positive likelihood ratio scores (= probability of sockeye / probability of kokanee) and nearly all of those identified in the field as kokanee formed a group with negative likelihood ratio scores. Only three of the 419 fish were assigned to a genetic type ("kokanee") that differed from how they were classified by MFM samplers ("sockeye"; Figure 2a). One fish that was included in the Allen's Bay sockeye salmon collection had a 9-locus genotype that was approximately 10^9 times more likely to be that of a kokanee than a sockeye salmon. That individual was actually identified in the field as a kokanee but was sampled among sockeye spawners and was assigned a collection identifier intended for Allen's Bay sockeye salmon. Although a note from the field samplers indicated the individual was a kokanee, the proper identification was overlooked by WDFW genetics laboratory and MFM sampling staffs until after the genetic analyses had been completed. This confusion fortuitously resulted in what can be considered to have been a successful double blind test of the genetic method to identify an unknown sample based upon its genetic profile.

Upon examination of the other two fish that were identified as sockeye salmon at the time of capture, but classified as kokanee based on their microsatellite DNA genotypes, we found that each of them had only been successfully scored at one of the nine loci screened. We then went back to the data set and removed all fish that had been scored at fewer than five of the nine loci screened and plotted their likelihood ratio scores (Figure 2b). When only fish with scores at five or more loci were considered, many of the individuals with the most ambiguous assignments were eliminated (compare boxed area of plots in Figure 2a and 2b).

The apparent similarity of Umbrella Creek sockeye salmon and kokanee at *One-101*221* and *One-100*331* and **351* could indicate potential hybridization, but it is unlikely that this was the plausible explanation. Although we have not applied direct statistical tests to identify potential hybrids, we believe that inspection of the allele frequency data in Appendix 1 provides strong evidence that hybridization between sockeye salmon and kokanee has not occurred in Umbrella Creek to any measurable degree. There are a large number of pronounced allele frequency differences between the Umbrella Creek Hatchery sockeye salmon collection and the two kokanee collections analyzed from the Lake Ozette basin (Siwash and Crooked creeks). These differences included: alleles found at relatively high frequencies in Umbrella Creek sockeye but absent from both kokanee collections (*Ots-103*156*, *One-114*290*, *One-110*236* & **252*, and *One-100*311*), alleles found at relatively high frequencies in the two kokanee collections but absent in the Umbrella Creek Hatchery sockeye collection (*One-108*186*; *One-114*250*; *One-110*264*, **272*, **276*, **280*, & **284*; *One-100*343*; *One-101*297*; *One-102*248*; and *Ots-103*184*), and alleles showing large frequency differences between the Umbrella Creek sockeye collection and the kokanee collections (*One-114*286*, *One-110*240* & *256*; *One-100*335*; *One-101*217*, & **225*; *One-102*240*; *One-115*186*, **190*, **198*, & **206*; *Ots-103*176*; and *One-105*133* & **137*). Given these frequency differences between the Umbrella Creek sockeye and kokanee, we believe that the similarities noted above for three alleles (*One-101*221* and *One-100*331* & **351*) likely reflect similarity due to random drift and are not indicative of interbreeding between sockeye and kokanee.

Need for Additional Work

The need for additional work focuses on three areas:

- Monitoring hybridization of kokanee and sockeye salmon
- Developing a database that will allow us to confidently describe the population structure of sockeye salmon

- Investigating the genetic changes that may occur as beach-spawning sockeye salmon recolonize tributaries

We are confident that we have identified genetic markers for monitoring hybridization between kokanee and sockeye salmon. We hope to secure funding to study potential hybridization using hybrid analyses (Campton 1987). In addition, it would be useful to expand the data for kokanee to different year-classes, which will allow us to estimate within-kokanee diversity, increase our ability to distinguish sockeye salmon and kokanee, and to identify hybrids. Demographic studies to estimate straying rates of sockeye salmon as they are reintroduced to the tributaries will continue to be invaluable for interpreting future genetic comparisons among *O. nerka* spawning aggregations. The Lake Ozette sockeye HGMP monitoring program encompasses these needed assessments, and additional funding to characterize the genetic composition of different *O. nerka* spawning aggregations will allow co-managers to conserve the genetic composition of this ESU while recovery actions are undertaken.

We will be unable to conclude whether the patterns we have seen in this study among beach-spawning sockeye salmon are representative of the true population structure until we have funding to build and analyze a database with more year-classes and potentially more geographical samples. The original design of this study—which was to use a nested design to detect differences among sockeye attributable to different spawning locations, differences within sockeye salmon spawning at a specific location attributable to different cohort lineages, and differences within sockeye salmon spawning at a specific location attributable to different generations (Objective 1)—remains the best design for identifying *O. nerka* spawning aggregation substructure in Lake Ozette. Until that is possible, the co-managers will continue to collect and archive samples from the known spawning beaches.

One of the most exciting areas for addition research is to study the adaptation by beach-spawning sockeye to tributary-spawning in Umbrella Creek and Big River as they are reintroduced. Little is known about the genetic challenges of reintroducing fish to areas where they have been extirpated or the potential role of hatcheries in contributing to such supplementation. Efforts will continue to be aimed at determining how different hatchery practices enhance or delay this adaptation.

Evaluation

The objectives of our study were to:

- Test the hypothesis that the two major beach-spawning aggregations of sockeye salmon in Lake Ozette, one on Olsen’s Beach and the other on Allen’s Bay, are the same population using microsatellite DNA loci.
- Identify genetic markers that could be used to monitor potential hybridization between sockeye salmon and kokanee.
- Communicate results.
- Implement hatchery reform through adaptive management.

Completing these objectives is critical for addressing sections 1.1, 2.3, 3.1, and 4.5 of the *Scientific Framework for Artificial Propagation of Salmon and Steelhead* developed by the Hatchery Scientific Review Group (HSRG).

We tested the hypothesis that sockeye salmon from Olsen’s Beach and Allen’s Bay were the same population and concluded that genetic differences existed both between sockeye salmon spawning at different beaches and among spawners returning in different years to the same beach. Although this suggested that the two spawning beaches may be different subpopulations, we could not be confident about this conclusion because fish spawning in different years were also different and we had insufficient funding to resolve and compare temporal and geographical variation adequately. Funding to analyze existing archived samples and additional samples from future year that continue to be collected will help to resolve this issue.

We successfully identified nine microsatellite loci that can be used to monitor potential hybridization between sockeye salmon and kokanee.

This report represents the first effort to communicate these results, except for the presentation to the HSRG at their annual review. These data will be shared with other scientists and managers, such the National Marine Fisheries Service and the Puget Sound Technical Recovery Team, which has the responsibility for identifying populations

within evolutionarily significant units (ESU's) listed under the Endangered Species Act and for developing federal recovery goals for those populations. This report will also be available at the NWIFC website. We hope to secure additional funding to complete the original (more extensive) objectives of this study before submitting our results for publication in the peer-reviewed literature.

Makah Fisheries Management staff are committed to implementing hatchery reform through adaptive management and have described in detail their current and near-future supplementation, monitoring, and adaptive management actions for this ESU within the Lake Ozette Sockeye Hatchery and Genetic Management Plan (HGMP). This study provided an initial genetic baseline for all *O. nerka* spawning aggregations in the Ozette basin, including sockeye salmon that have recently recolonized Umbrella Creek. The genetic data from this study will also be used to design regular sampling and analysis for potential hybridization of kokanee and sockeye salmon, which will be incorporated into new versions of the HGMP. In addition, samples from lake-spawning beaches will continue to be collected for future analysis to help resolve spawning aggregation substructure of sockeye salmon in Lake Ozette. This information will be necessary for guiding brood stock collection, release, and monitoring, if it becomes necessary to use artificial production to reintroduce or supplement beach-spawning aggregations.

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Table 1. Details of the seven collections analyzed in the present study.

Sampling Location	Code	Sampling Period	River Mile		Number of Samples		% Adipose Clipped	Spawning Status (%)			Sex (%)	
			Upper	Lower	Collected	Analyzed		Green	Ripe	Spent	Male	Female
Olsen's Beach	96ET	24 Nov - 18 Dec 1996	N/A	N/A	100	75	-	-	-	-	46	54
Olsen's Beach	99PX	11 Nov - 23 Dec 1999	N/A	N/A	76	50	0%	2	43	55	64	36
Olsen's Beach	00MK	8 Nov - 4 Jan 2001	N/A	N/A	100	92	0%	36	4	60	47	53
Allen's Bay	00ML	21 Nov 2000 - 31 Jan 2001	N/A	N/A	77	77	0%	?	?	56	53	47
Umbrella Creek	00MM	26 Oct 2000 - 14 Jan 2001	4.78	2.0	109	75	31%	4	94	2	55	45
Crooked Creek	00MR	9 - 21 Nov 2000	4.8	0.5	100	50	0%	1	87	12	54	46
Siwash Creek	00MS	10 - 14 Nov 2000	1.59	1.33	100	50	0%	-	-	-	57	43

Table 2. Unadjusted P values for pairwise tests for population differentiation at nine loci for seven collections of *O. nerka* in the Lake Ozette basin. Tests of allele frequency (genic) differences are above the diagonal and tests of genotypic differences are below the diagonal. Adjusted critical level for multiple comparisons is 0.0014 ($\alpha = 0.05/36$ comparisons). Numbers of loci that were individually significantly different in the comparisons are denoted by parentheses. Comparisons that were not significantly different are shaded.

	Sockeye Salmon					Kokanee	
	Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
Olsen's Beach 1996	-	<0.00000 (1)	=0.12366 (0)	=0.00101 (1)	=0.24505 (0)	<0.00000 (9)	<0.00000 (9)
Olsen's Beach 1999	<0.00000 (3)	-	<0.00000 (1)	<0.00000 (2)	<0.00000 (3)	<0.00000 (9)	<0.00000 (9)
Olsen's Beach 2000	=0.16974 (0)	<0.00000 (3)	-	=0.00004 (1)	=0.00004 (1)	<0.00000 (9)	<0.00000 (9)
Allen's Bay 2000	=0.00483 (2)	<0.00000 (3)	=0.00006 (1)	-	<0.00000 (1)	<0.00000 (9)	<0.00000 (9)
Umbrella Creek 2000	=0.32761 (2)	<0.00000 (3)	=0.00009 (2)	=0.00029 (1)	-	<0.00000 (9)	<0.00000 (9)
Crooked Creek 2000	<0.00000 (3)	<0.00000 (9)	<0.00000 (9)	<0.00000 (9)	<0.00000 (9)	-	=0.55769 (0)
Siwash Creek 2000	<0.00000 (9)	<0.00000 (9)	<0.00000 (9)	<0.00000 (9)	<0.00000 (9)	=0.59351 (0)	-

Table 3 Nine-locus F_{ST} values and 95% confidence intervals (in parentheses) for *O. nerka* collections from the Lake Ozette basin.

	Sockeye Salmon				Kokanee	
	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
Olsen's Beach 1996	0.023 (0.040, 0.008)	0.003 (0.011, -0.002)	0.013 (0.030, 0.001)	0.001 (0.006, -0.004)	0.108 (0.156, 0.066)	0.104 (0.150, 0.063)
Olsen's Beach 1999	--	0.007 (0.011, 0.003)	0.017 (0.026, 0.009)	0.020 (0.027, 0.013)	0.115 (0.148, 0.082)	0.109 (0.140, 0.077)
Olsen's Beach 2000		--	0.007 (0.017, 0.000)	0.006 (0.120, 0.001)	0.110 (0.151, 0.072)	0.103 (0.141, 0.066)
Allen's Bay 2000			--	0.012 (0.032, 0.001)	0.108 (0.147, 0.072)	0.100 (0.014, 0.065)
Umbrella Creek 2000				--	0.111 (0.153, 0.072)	0.105 (0.144, 0.068)
Crooked Creek 2000					--	-0.001 (0.002, -0.004)

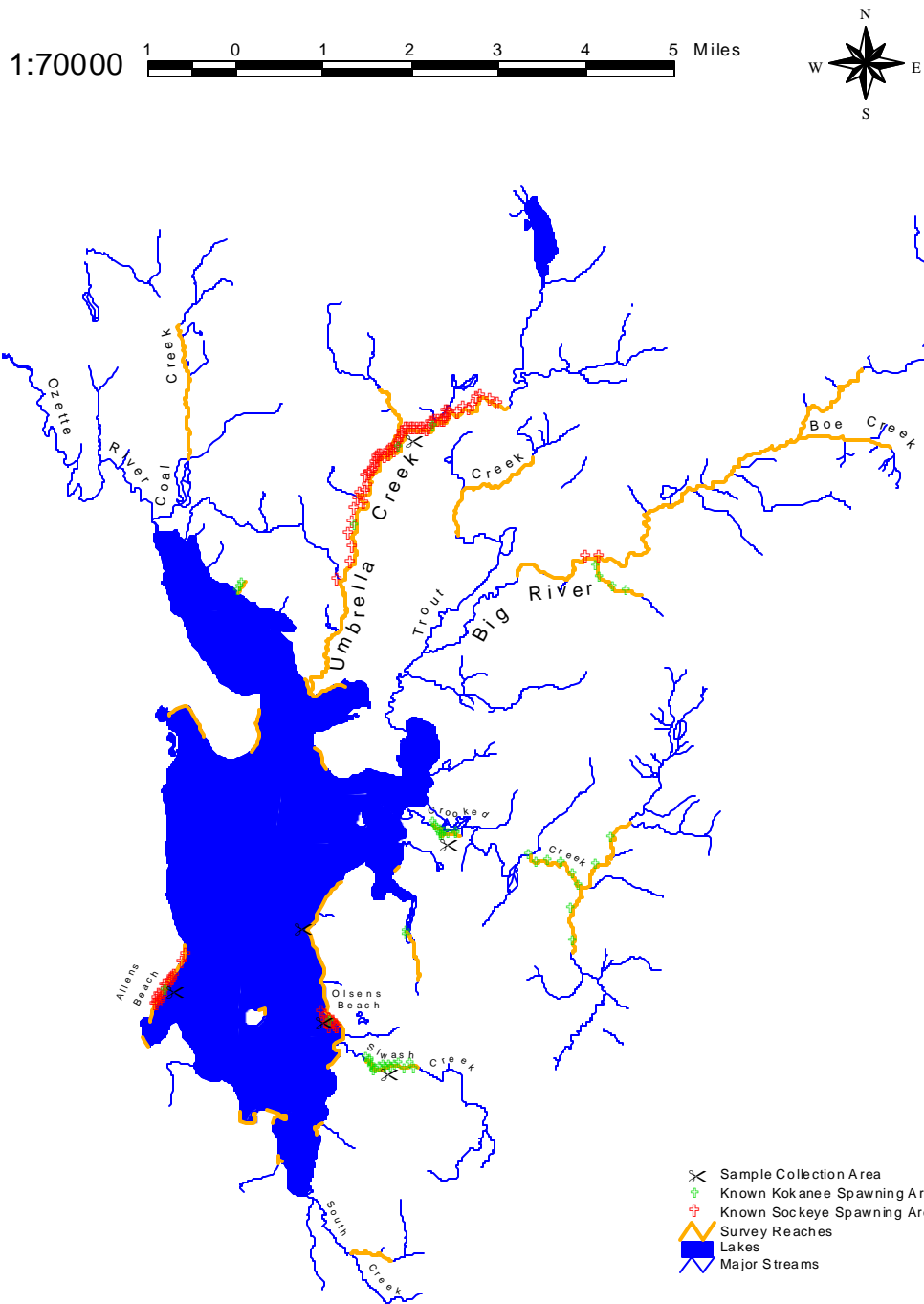


Figure 1. Ozette basin survey reaches, known spawning areas, and genetic sample collection sites.
(Oncorhynchus nerka)

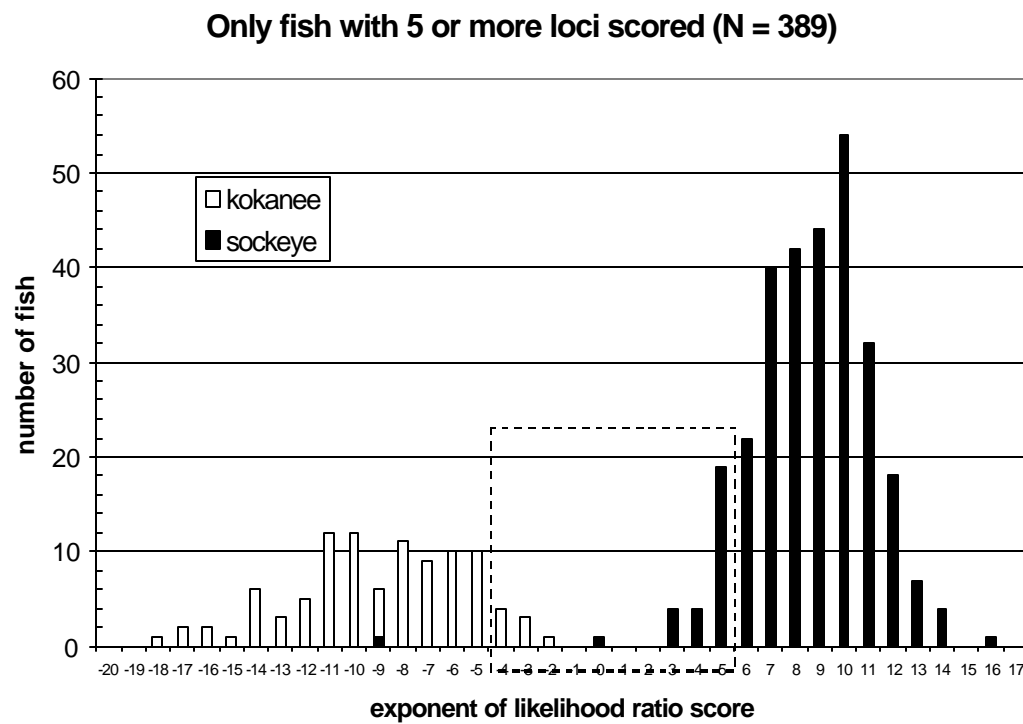
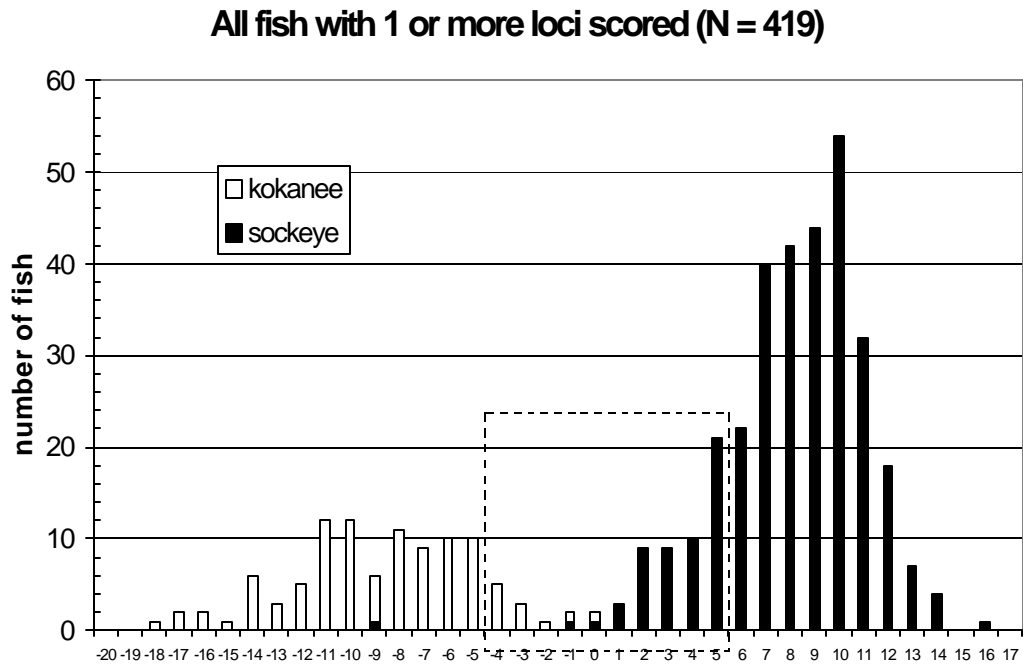


Figure 2. Distribution of individual assignments of sockeye salmon and kokanee based on microsatellite DNA..

Appendix 1. Allele frequencies at nine microsatellite DNA loci in 7 collections of *Oncorhynchus nerka* from the Lake Ozette basin. ('-' = allele not observed in collection; a = number of different alleles observed; n = number of individuals successfully scored; N = number of individuals analyzed)

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-100*</i>	299	-	-	-	-	-	0.020	-
	303	0.029	-	0.007	0.053	0.023	-	-
	307	-	-	0.014	0.013	-	-	-
	311	0.100	0.202	0.115	0.342	0.091	-	-
	315	0.057	0.011	0.014	-	-	0.080	0.060
	319	0.029	0.106	0.101	0.066	0.023	0.060	0.020
	323	0.071	0.021	0.034	0.013	-	0.040	0.040
	327	-	-	-	-	-	0.040	-
	331	-	-	-	-	0.136	0.060	0.100
	335	0.514	0.468	0.419	0.276	0.477	0.060	0.100
	339	0.129	0.074	0.169	0.171	0.023	0.100	0.120
	343	-	0.053	0.007	0.026	-	0.240	0.200
	347	-	-	-	0.013	-	0.020	0.080
	351	0.014	0.011	0.034	-	0.114	0.120	0.120
	355	0.029	-	0.041	0.026	0.068	0.020	0.020
	359	0.029	0.053	0.047	-	0.045	0.060	0.020
	363	-	-	-	-	-	0.020	0.100
	379	-	-	-	-	-	0.040	-
	387	-	-	-	-	-	-	0.020
	415	-	-	-	-	-	0.020	-
	a	10	9	12	10	9	16	13
	n	35	47	74	38	22	25	25
	N	75	50	92	76	75	50	50

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-101</i> *	177	-	-	-	-	-	0.010	-
	181	-	-	-	-	-	0.010	-
	185	-	-	-	-	-	0.031	0.010
	189	-	-	-	-	0.013	0.041	-
	193	0.032	-	-	-	0.007	0.021	0.010
	197	0.032	-	0.023	0.054	0.040	0.031	0.010
	201	0.032	0.054	0.023	0.022	0.040	-	0.010
	205	0.016	0.033	0.015	0.011	0.013	-	0.021
	209	0.016	0.043	0.045	0.054	0.027	0.041	0.063
	213	0.065	-	0.098	-	0.033	0.010	0.010
	217	0.306	0.554	0.462	0.467	0.420	0.031	0.031
	221	0.097	0.087	0.098	0.120	0.027	0.010	-
	225	0.226	0.065	0.114	0.163	0.240	0.021	0.031
	229	0.065	0.022	0.038	0.043	0.027	0.010	0.010
	233	0.016	0.087	-	0.022	0.020	0.021	-
	237	-	-	-	-	-	0.010	-
	245	-	-	-	-	-	0.010	-
	253	-	-	-	-	-	0.010	0.010
	265	-	-	-	-	-	0.010	0.010
	269	-	0.011	-	-	-	-	-
	273	-	-	-	-	-	-	0.010
	277	0.032	-	0.008	-	0.027	-	0.010
	281	-	-	-	-	-	0.021	-
	285	-	-	-	-	-	0.010	0.010
	289	-	-	0.008	-	-	0.041	0.042
	293	-	-	-	-	-	0.021	0.073
	297	-	-	-	-	-	0.062	0.104
	301	-	-	0.053	-	0.013	0.134	0.094
	305	0.032	0.033	0.008	0.043	0.033	0.041	0.104
	309	0.032	-	0.008	-	0.020	0.113	0.115

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-101*</i>	313	-	0.011	-	-	-	0.052	0.052
	317	-	-	-	-	-	0.041	0.042
	321	-	-	-	-	-	0.052	0.042
	325	-	-	-	-	-	0.010	0.010
	329	-	-	-	-	-	0.021	0.042
	333	-	-	-	-	-	0.010	0.021
	337	-	-	-	-	-	0.021	-
	341	-	-	-	-	-	0.021	-
	a	14	11	14	10	16	33	27
	n	31	46	66	46	75	49	48
N	75	50	92	76	75	50	50	
<i>One-102*</i>	208	-	-	0.037	0.029	0.020	0.031	0.020
	212	-	-	-	-	-	0.073	0.031
	216	-	-	-	-	-	-	0.031
	220	0.071	0.063	0.062	0.049	0.074	0.156	0.143
	224	-	0.010	0.006	-	-	0.073	0.041
	228	0.029	0.021	0.025	0.020	0.034	0.104	0.061
	232	0.329	0.271	0.333	0.363	0.270	0.135	0.265
	236	0.229	0.406	0.296	0.343	0.311	0.198	0.184
	240	0.300	0.115	0.222	0.137	0.270	0.125	0.112
	244	0.043	0.115	0.019	0.059	0.020	0.021	0.031
	248	-	-	-	-	-	0.063	0.061
	252	-	-	-	-	-	-	0.020
	256	-	-	-	-	-	0.021	-
	a	6	7	8	7	7	11	12
	n	35	48	81	51	74	48	49
	N	75	50	92	76	75	50	50

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-105*</i>	125	-	-	-	-	-	0.010	-
	129	0.141	0.362	0.287	0.260	0.226	0.133	0.191
	133	-	0.011	-	-	0.007	0.449	0.404
	137	0.808	0.574	0.667	0.730	0.726	0.357	0.330
	141	0.051	0.053	0.047	0.010	0.041	0.051	0.074
	a	3	4	3	3	4	5	4
	n	39	47	75	50	73	49	47
	N	75	50	92	76	75	50	50
<i>One-108*</i>	182	-	0.032	-	-	-	0.054	0.094
	186	-	-	-	-	-	0.122	0.125
	190	0.014	-	0.014	-	0.015	0.081	0.115
	194	0.162	0.096	0.169	0.100	0.144	0.216	0.125
	198	0.041	0.053	0.081	0.037	0.038	0.135	0.188
	202	0.270	0.309	0.324	0.263	0.242	0.095	0.146
	206	0.176	0.128	0.176	0.175	0.212	0.149	0.115
	210	0.041	-	0.007	0.037	0.015	0.068	0.010
	214	0.122	0.234	0.115	0.113	0.076	0.014	0.010
	218	0.027	-	-	0.013	0.068	0.027	0.021
	222	-	-	-	-	-	0.027	0.021
	226	-	0.011	0.007	0.013	-	-	-
	230	0.068	0.032	0.020	0.138	0.068	-	0.010
	234	-	-	-	-	-	-	0.010
	238	-	-	0.007	-	-	0.014	0.010
	246	-	-	-	-	0.008	-	-
	258	0.041	0.021	0.041	0.063	0.061	-	-
	262	0.041	0.085	0.041	0.050	0.038	-	-
	270	-	-	-	-	0.015	-	-
	a	11	10	12	11	13	12	14
n	37	47	74	40	66	37	48	
N	75	50	92	76	75	50	50	

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-110*</i>	232	0.014	-	-	0.012	0.008	-	-
	236	0.054	0.064	0.075	0.073	0.054	-	-
	240	0.203	0.287	0.274	0.220	0.238	-	0.014
	244	-	0.043	0.014	0.012	0.015	-	0.014
	248	-	-	0.014	-	0.008	-	0.028
	252	0.149	0.255	0.130	0.085	0.138	-	-
	256	0.432	0.287	0.342	0.354	0.415	0.048	0.028
	260	0.135	0.043	0.151	0.232	0.115	0.032	0.083
	264	-	0.011	-	-	-	0.113	0.097
	268	0.014	0.011	-	0.012	0.008	0.081	0.069
	272	-	-	-	-	-	0.161	0.111
	276	-	-	-	-	-	0.210	0.125
	280	-	-	-	-	-	0.177	0.181
	284	-	-	-	-	-	0.048	0.125
	288	-	-	-	-	-	0.032	0.083
	292	-	-	-	-	-	0.032	0.014
	296	-	-	-	-	-	0.016	-
	300	-	-	-	-	-	0.032	0.014
	308	-	-	-	-	-	-	0.014
	316	-	-	-	-	-	0.016	-
a		7	8	7	8	9	13	15
n		37	47	73	41	65	31	36
N		75	50	92	76	75	50	50

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-114*</i>	214	-	-	-	-	-	0.010	-
	218	-	-	-	-	-	0.021	0.051
	222	-	-	-	-	-	0.010	0.031
	226	-	-	-	-	-	0.031	0.010
	230	-	0.031	0.006	0.019	0.014	0.021	0.061
	234	-	0.010	-	-	-	0.052	0.031
	238	0.015	-	0.006	0.009	0.027	0.073	0.092
	242	0.121	0.125	0.167	0.132	0.103	0.115	0.092
	246	0.030	0.010	0.031	0.028	0.041	0.125	0.051
	250	-	-	-	-	-	0.115	0.102
	254	-	-	0.025	-	0.034	0.052	0.071
	258	0.167	0.083	0.136	0.142	0.137	0.052	0.112
	262	0.121	0.063	0.086	0.151	0.151	0.104	0.071
	266	0.121	0.146	0.093	0.057	0.055	0.083	0.010
	270	0.136	0.156	0.154	0.113	0.137	0.031	0.031
	274	0.045	0.031	0.012	0.075	0.027	0.052	0.092
	278	0.045	0.063	0.019	0.019	0.007	0.021	0.041
	282	-	-	-	-	-	0.010	0.031
	286	0.121	0.188	0.136	0.142	0.199	0.021	0.020
	290	0.076	0.094	0.111	0.104	0.068	-	-
	294	-	-	0.019	0.009	-	-	-
	a	11	12	14	13	13	19	18
	n	33	48	81	53	73	48	49
	N	75	50	92	76	75	50	50

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>One-115*</i>	174	-	-	-	-	-	0.010	0.010
	178	-	0.032	-	-	-	0.063	0.122
	182	0.014	0.032	-	-	-	0.052	0.031
	186	-	-	0.012	-	0.007	0.146	0.102
	190	-	0.021	-	-	0.020	0.146	0.194
	194	0.041	0.032	0.012	0.029	0.020	0.104	0.041
	198	0.541	0.521	0.542	0.382	0.480	0.125	0.112
	202	0.054	-	0.030	0.029	0.034	0.094	0.153
	206	0.284	0.309	0.367	0.480	0.378	0.052	0.122
	210	0.068	0.043	0.036	0.078	0.061	0.115	0.051
	214	-	0.011	-	-	-	0.052	0.051
	218	-	-	-	-	-	0.031	-
	222	-	-	-	-	-	0.010	0.010
	a	6	8	6	5	7	13	12
	n	37	47	83	51	74	48	49
	N	75	50	92	76	75	50	50

Appendix 1. Continued.

Locus	Allele (base pairs)	Sockeye Salmon					Kokanee	
		Olsen's Beach 1996	Olsen's Beach 1999	Olsen's Beach 2000	Allen's Bay 2000	Umbrella Creek 2000	Crooked Creek 2000	Siwash Creek 2000
<i>Ots-103*</i>	156	0.298	0.229	0.214	0.263	0.311	-	-
	160	-	-	-	-	-	-	0.023
	164	-	-	-	-	-	0.021	-
	168	0.024	0.073	0.032	0.035	0.034	0.094	0.102
	172	0.048	0.052	0.039	-	0.034	0.073	0.102
	176	0.024	0.052	0.039	0.018	0.027	0.156	0.250
	180	0.214	0.115	0.195	0.237	0.291	0.135	0.170
	184	-	-	0.019	0.009	-	0.125	0.068
	188	-	-	0.006	0.018	-	0.063	0.057
	192	-	-	-	-	-	0.073	0.023
	196	0.119	0.198	0.169	0.175	0.142	0.042	0.011
	200	0.131	0.250	0.195	0.202	0.101	0.021	0.045
	204	0.012	0.010	0.006	0.009	0.020	0.052	0.034
	208	-	-	-	0.009	-	0.063	0.045
	212	0.131	0.021	0.084	0.026	0.041	-	0.011
	216	-	-	-	-	-	0.031	0.045
	220	-	-	-	-	-	0.042	-
	228	-	-	-	-	-	0.010	0.011
	a	9	9	11	11	9	15	15
	n	42	48	77	57	74	48	44
N	75	50	92	76	75	50	50	

Appendix 2. Numbers of alleles observed at each locus in eleven collections of *O. nerka* in other Washington populations versus Lake Ozette. S = anadromous life history form (sockeye salmon); K = resident life history form (kokanee); U = life history form undetermined.

Locus	Numbers of Different Alleles												Lake Ozette			
	Ebright Creek (K)	Lewis Creek (K)	Laughing Jacobs Creek (K)	N Lake Washington tributaries (U)	Lake Whatcom Hatchery (K)	Issaquah Creek (S)	Bear & North creeks (S)	Baker Lake (S)	Meadow Creek (K)	Nespelem River (K)	Lake Roosevelt (K)	Mean	Low	High	(S)	(K)
<i>One-100*</i>	19	21	18	21	20	26	21	21	41	24	21	23.0	18	41	10.0	14.5
<i>One-101*</i>	22	20	20	31	39	24	27	18	38	16	12	24.3	12	39	13.0	30.0
<i>One-102*</i>	8	10	8	14	13	11	12	7	17	15	13	11.6	7	17	7.0	11.5
<i>One-105*</i>	5	5	4	6	5	5	5	5	5	6	5	5.1	4	6	3.4	4.5
<i>One-108*</i>	13	10	11	16	20	16	16	10	16	16	12	14.2	10	20	11.4	13.0
<i>One-114*</i>	16	14	16	22	21	18	21	16	22	20	18	18.5	14	22	12.6	18.5
<i>One-115*</i>	12	13	13	16	15	14	15	10	16	14	11	13.5	10	16	6.4	12.5
<i>Ots-103*</i>	13	14	14	16	17	17	16	13	24	21	18	16.6	13	24	9.8	15.0

Appendix 3. Summary of available Lake Ozette basin *O. nerka* tissue collections for DNA analysis by collection year. (N = number of fish in sample, NMFS = National Mzrine Fisheries Service, Northwest Fisheries Science Center; UI = Matt Powell at the University of Idaho). All fish were adults unless otherwise noted. Sources of fish not currently archived at WDFW are noted.

Life History Form & Location	1994		1995			1996		1998			1999		2000	
	Code	N	Code	N	Source	Code	N	Code	N	Source	Code	N	Code	N
Sockeye														
"Lake Ozette" (exact location(s) unknown)	94LG	4												
Olsen's Beach	94LG	16	---	50	NMFS	96ET	100	---	138	UI	99PX	76	00MK	100
Olsen's Beach; (used as broodstock)	94LG	28									99PY	29		
Allen's Bay	94LG	32	---	33	NMFS	96ES	101	---	27	UI	99PZ	12	00ML	71
Big River								---	3	UI	---	2		
Umbrella Creek								---	1	UI	99QA	58	00MM	101
											---	4		
Umbrella Creek (natural fry; 20-30mm)											99QB	105		
hatcheryfry; 55-80mm)											99QC	46		
Crooked Creek			---	?	NMFS?									
Kokanee														
Umbrella Creek													00MN	1
Siwash Creek													00MS	100
Crooked Creek			---	42	NMFS								00MR	100