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# Genetic Analysis of Snake River Sockeye Salmon (Oncorhynchus Nerka)

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# **Genetic Analysis of Snake River Sockeye Salmon**
## ***(Oncorhynchus nerka)***

Completion Report


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ABSTRACT

A total of 1720 *Oncorhynchus nerka* tissue samples from 40 populations were characterized using mitochondrial DNA RFLPs (Restriction Fragment Length Polymorphisms). Analysis of anadromous sockeye populations indicated the historical presence of four major maternal lineages. Thirty-five composite mitochondrial haplotypes were observed from the 40 populations of *O. nerka* sampled throughout the Pacific Northwest. Six of these composite haplotypes ranged in frequency from 7-26% overall and were commonly observed in most populations. The six haplotypes together comprised 90% of the sampled *O. nerka*. An average of 4.6 composite haplotypes were observed per population.

Genetic markers used were satisfactory in separating Redfish Lake anadromous sockeye, residual sockeye and outmigrants from the sympatric kokanee population that spawns in the Fishhook Creek tributary. Outmigrants appear to be primarily composed of progeny from resident residual sockeye, and captively-reared progeny of the captive broodstock program. Thus, residual sockeye may be considered a suitable source of genetic variation to maintain genetic diversity among captive broodstocks of anadromous sockeye. Fishhook Creek kokanee are genetically diverse and during spawning, are temporally and spatially isolated from the residual sockeye population. Eleven composite haplotypes were observed in the kokanee population. The unusually high number of haplotypes is most likely a consequence of periodic stocking of Redfish Lake with kokanee from other sources. Genetic data from Redfish Lake creel samples taken during 1996-1999 putatively indicate the incidental take of a listed resident sockeye.

# INTRODUCTION

Sockeye salmon, the anadromous form of *Oncorhynchus nerka*, generally rear in lake environments for one to three years, migrate to the ocean to mature for one to four years, and return to their natal stream or lakeshore to spawn and die (Burgner 1991). The *O. nerka* species is set apart from its congener Pacific salmonids (*O. tshawyscha, O. keta, O. gorbuscha, O. kisutch*) by exhibiting two additional freshwater life history forms, which may exist concurrently in sympatry with the primary anadromous form. The landlocked or resident form (kokanee) completes its life cycle in freshwater. Anadromous sockeye and most populations of kokanee develop bright red coloration when ready to spawn, and the males develop secondary sex characteristics (e.g. elongated jaw and humped back). Kokanee are considered wholly freshwater-adapted descendents from anadromous stocks (Ricker 1938). Recently observed genetic patterns suggest kokanee have arisen from radiating, anadromous populations independently on several occasions (Foote et al. 1989, Taylor et al. 1996). Currently, kokanee populations are numerous throughout the Pacific Northwest and exist in nursery lake environments with and without sympatric anadromous sockeye runs. Landlocked resident forms of other Pacific salmonids do exist, but only through the deliberate stocking of lakes, as in the case of pink salmon stocked in the Great Lakes (Kwain 1987). A third less common life history form is the residual, a lake-maturing nonanadromous sockeye regarded as the progeny of anadromous adults. Residual sockeye populations have a sex ratio heavily skewed toward males, do not develop strong secondary sexual characteristics and the bright red coloration associated with spawning activities, and occur sympatrically with anadromous sockeye populations (Ricker 1938). Residual lake dwelling coho have also been reported (Foerster and Ricker 1953).

Redfish Lake is the largest of the Stanley Basin lakes in the Sawtooth National Recreation Area of Central Idaho, located approximately 1,500 km from the Pacific Ocean at an elevation of 2,000 m. It is the southernmost lake supporting an anadromous sockeye run, and the only lake known to support all three life history forms of *O. nerka* (Allendorf and Waples 1996). Sockeye salmon returning to Redfish Lake travel farther and higher than any other population of sockeye in the world. These characteristics, among others, were used by Waples (1991) to support the evolutionary significance of Redfish Lake sockeye in a bid to designate these sockeye as an endangered species. Redfish Lake sockeye are the only remaining anadromous run of *O. nerka* in the Snake River Basin and thus are referred to as Snake River sockeye. Due to almost non-existent returns to Redfish Lake, this unique population of sockeye was designated as an evolutionarily significant unit (ESU), and was listed as a federally endangered species in November 1991 (57 FR 213: 1992). The small number of residual sockeye in Redfish Lake were also given protection under the Endangered Species Act (ESA) because they are considered to be descendents of listed anadromous sockeye. The sympatric kokanee population in Redfish Lake are reproductively isolated temporally and spatially from the anadromous population, and thus were excluded from the ESA listing (Waples et al. 1991).

Redfish Lake anadromous sockeye return from the ocean after two to three years, and spawn in October and November along shallow gravel beach areas of the northeast shoreline of the lake (Brannon et al. 1992). The fry emerge in the spring, rear for one to two years in limnetic areas, then migrate to the ocean in the spring as one or two year old smolts. Some fry may remain resident, forming the residual population first observed in 1992 (Waples et al. 1997). Residual sockeye spawn at approximately the same time and place as the anadromous sockeye, but are closer in size to the resident kokanee. Residual sockeye spawners measured in 1993 averaged 216 mm, while anadromous sockeye from the same year averaged 540 mm (Kline 1994). Kokanee spawners in Fishhook Creek averaged 218 mm in 1993 (Teuscher et al. 1994). The native kokanee population migrates into Fishhook Creek, the major tributary to Redfish Lake, to spawn in August and September.

Cooler inlet stream temperatures allow for earlier spawning times than the warmer shallow beach areas in the lake. Thus, the present day kokanee population is able to spawn in Fishhook Creek during August and September, while the sockeye populations that spawn in the lake areas delay until October and November when cooler lake temperatures prevail (Brannon et al. 1992). Historical accounts indicated that the sockeye population previously spawned in Fishhook Creek in August during 1887-1889 and 1893 (Evermann 1895) instead of the beach areas currently used by anadromous sockeye. The current anadromous population may not be genetically identical to the original stock, due to a 20-year interruption of the returning run by the construction of Sunbeam Dam, built in 1913 on the main Salmon River approximately 20 miles downstream from the mouth of Redfish Lake (Bjornn et al. 1968). Additionally, some out-of-basin sockeye and kokanee have been stocked to Redfish Lake, though more recent stocking is not thought to have produced any returning anadromous fish (Hall-Griswold 1990, Waples et al. 1991).

In 1901, the U.S. Fish and Fisheries Commission expanded its egg taking efforts to include “blueback” salmon (sockeye), thus beginning a long history of *O. nerka* stock transfers within the United States (Stickney 1997). For example, in 1915, the Oregon Fish Commission and the Washington Fish Commission received over 3 million eggs produced in Yes Bay, Alaska (Smith 1915). Kokanee have been propagated in captivity since the 1920’s and have been extensively distributed as a sport fish throughout the West (Stickney 1997). The Bureau of Fisheries recognized differences between anadromous sockeye and “landlocked” kokanee in 1937 (Leach 1938), but continued to stock captively raised kokanee into many areas where anadromous sockeye were historically distributed. Introduced and transferred kokanee populations sympatric with natural or wild sockeye runs may pose problems for stock identity if they significantly contribute to outmigrating cohorts.

Genetic variation has been examined in kokanee and sockeye populations in British Columbia (Wood et al. 1994), Alaska (Burger et al. 1997), the Pacific Northwest (Winans et al. 1996), and the Snake River Basin (Waples et al. 1997). This report presents genetic information from digests of several polymorphic mtDNA gene regions for 40 populations of *O. nerka* in the Pacific Northwest, including Idaho, Washington, Oregon, and British Columbia. Genetic comparisons were made of several sympatric populations of kokanee and sockeye. Natural and introduced populations of kokanee were also examined. Finally, Snake River Basin populations were examined, with special emphasis on the sympatric populations of Redfish Lake *O. nerka*, and the contributions of captive broodstock from a supplementation program operated by the Idaho Department of Fish and Game (IDFG) and the National Marine Fisheries Service (NMFS).

## MATERIALS AND METHODS

### BIOLOGICAL MATERIAL

Tissue samples of *O. nerka* were collected from Idaho, Washington, Oregon, and British Columbia. Samples of muscle, liver, blood, milt or fin tissue were stored in ethanol (70% or 95%) or lysis buffer (50 mM Tris-HCl, pH 8.0; 200 mM NaCl; 50 mM EDTA; 1% Sodium dodecyl sulfate; 0.2% Dithiothreitol), or frozen at –80°C. The majority of samples were non-lethally collected fin clips. Samples were collected over several years from multiple locations (Figure 1), covering several year classes (Table 1).

### NORTHERN IDAHO COLLECTIONS

Lake Coeur d'Alene adult kokanee were sampled from a spawning beach at the northeast end of the lake. Lake Pend O'Reille adult kokanee were sampled at the IDFG Cabinet Gorge Hatchery fish ladder.

### STANLEY BASIN COLLECTIONS

Anadromous adults returning to Redfish Lake were captured at the Redfish Lake Creek trap or the Sawtooth Hatchery trap. Spawning residual adults were captured in a Merwin trap near the shore, and spawning kokanee adults in Fishhook Creek were hand-seined or captured at a weir. Outmigrating juveniles were captured at the Redfish Lake Creek trap, and an admixture of *O. nerka* adults and juveniles were collected from creel surveys and mid-water trawl surveys.

Other Sawtooth Basin lakes sampled included Alturas, Pettit, and Stanley Lakes (Figure 2). Samples of *O. nerka* were collected from mid-water trawls of the lakes and from creel surveys. Kokanee spawners were sampled from Alturas Lake Creek and Stanley Lake Creek. Outmigrants from Alturas Lake were sampled from the Alturas Lake Creek trap.

## OTHER IDAHO COLLECTIONS

Anderson Ranch Reservoir kokanee were collected from a mid-water trawl of the lake. Deadwood Reservoir adult spawning kokanee were collected from the Deadwood River at the mouth of Wild Buck Creek. Dworshak Reservoir adult spawning kokanee were collected from Isabella Creek. Payette Lake adult spawning kokanee were collected from the North Fork of the Payette River. Warm Lake adult kokanee were collected from a spawning beach area.

## WASHINGTON COLLECTIONS

Lake Wenatchee adult anadromous sockeye were captured at the Tumwater Dam trap, held in net-pens in Lake Wenatchee until maturity, and sampled on the day they were spawned by hatchery personnel as part of their juvenile rearing supplementation program. Five other Washington *O. nerka* populations (Baker Lake, Lake Ozette, Lake Quinault, Lake Whatcom, Lake Wenatchee) were included for out-of-basin comparison to give geographic perspective to any genetic population structure observed within the Columbia Basin. Anadromous sockeye runs were sampled from Baker Lake, Lake Ozette and Lake Quinault. Kokanee were collected by mid-water trawl from Lake Whatcom near Bellingham. Adult kokanee spawners were sampled from Lake Sutherland on the Olympic Peninsula.

## OREGON COLLECTIONS

Anadromous sockeye were sampled during 1995 and 1996 at the Pelton Dam fish trap on the Deschutes River, Oregon. Samples of adult spawning kokanee from Lake Billy Chinook at Gorge Campground and juvenile kokanee from Wizard Falls Hatchery were obtained from frozen specimens stored at the National Marine Fisheries Service laboratory in Montlake, WA (Waples et al. 1997). Tissue samples of *O. nerka* were collected from Wallowa Lake gill net surveys during November 1993, and from spawning kokanee in the Wallowa River near the entrance to the lake during September 1992.

## BRITISH COLUMBIA COLLECTIONS

Samples of spawning Shuswap Lake sockeye and kokanee were collected from the Middle Shuswap River and Eagle River populations. Middle Shuswap River sockeye were sampled from Bessette Creek, whereas kokanee were sampled in a side channel downstream from Bessette Creek. Eagle River kokanee and sockeye were sampled at the confluence of Eagle River and Perry Creek. Other samples from the Fraser River drainage included anadromous sockeye from Horsefly River. Samples of kokanee and anadromous sockeye from Babine Lake spawners in Pierre Creek in the Skeena River drainage were included because of known stock transfers of *O. nerka* from this location to Redfish Lake. Kokanee samples from Pierre Creek were obtained from Dennis Rutherford of the Pacific Biological Station, Nanaimo, BC, Canada (Robison 1995). Upper Columbia River drainage samples include Okanogan River anadromous sockeye and Okanogan Lake kokanee shore spawners from Squally Point and creek spawners from Peachland Creek.  Some British Columbia populations were collected and analyzed as part of a graduate research project (Robison 1995) and are presented here to provide geographic perspective to within-Basin genetic population structure.

Table 1. Collections of *Oncorhynchus nerka* samples from 40 locations. Location numbers correspond to sampling locations diagramed in Figure 1.

| Location # | Population | N | Form | Age | Collection Date(s) |
|---|---|---|---|---|---|
| | **IDAHO** | | | | |
| **1** | Alturas Lake (Alturas Lake Creek) | **24** | kokanee | adults | Aug. 25, 1992 |
| | | | | | |
| **2** | Alturas Lake, trawl | **54** | | | |
| | 1990 | 21 | kokanee | mixed | Aug. 19, 1990 |
| | 1994 | 12 | kokanee | mixed | Sep. 7, 1994 |
| | 1995 | 21 | kokanee | mixed | Sep. 25, 1995 |
| | | | | | |
| **3** | Alturas Lake, outmigrants | **36** | | | |
| | 1991 fish trap | 6 | unknown | juveniles | May-June 1991 |
| | 1991 hatchery | 13 | unknown | juveniles | May 1991-Sep. 1993* |
| | 1992 fish trap | 8 | unknown | juveniles | Apr.-May 1992 |
| | 1992 hatchery | 9 | unknown | juveniles | May 1992-Sep. 1993* |
| | | | | | |
| **4** | Anderson Ranch Reservoir, trawl | **12** | kokanee | juveniles | Aug. 15, 1990 |
| | | | | | |
| **5** | Coeur d' Alene Lake | **22** | kokanee | adults | Nov. 20, 1990 |
| | | | | | |
| **6** | Deadwood Reservoir | **36** | | | |
| | 1990 | 16 | kokanee | adults | Sep. 8., 1990 |
| | 1992 | 20 | kokanee | adults | Sep. 17, 1992 |
| | | | | | |
| **7** | Dworshak Reservoir (Isabella Crk.) | **37** | kokanee | adults | Sep. 17, 1990 |
| | | | | | |
| **8** | Payette Lake (N. Fk. Payette R.) | **32** | | | |
| | 1990 | 16 | kokanee | adults | Sep. 7, 1990 |
| | 1992 | 16 | kokanee | adults | Sep.18, 1992 |
| | | | | | |
| **9** | Pend O'Reille Lake | **32** | kokanee | adults | Nov. 20, 1990 |
| | | | | | |
| **10** | Pettit Lake, trawl | **47** | | | |
| | 1993 | 13 | kokanee | mixed | Sep. 18, 1993 |
| | 1994 | 15 | kokanee | mixed | Sep. 8, 1994 |
| | 1995 | 19 | kokanee | mixed | Sep. 25, 1995 |
| | | | | | |
| **11** | Redfish Lake | **262** | | | |
| | 1991 | 4 | anadromous | adults | Fall return |
| | 1992 | 1 | anadromous | adult | Fall return |
| | 1993 | 8 | anadromous | adults | Fall return |
| | 1994 | 1 | anadromous | adult | Fall return |
| | 1996 | 1 | anadromous | adult | Fall return |
| | 1998 | 1 | anadromous | adult | Fall return |
| | 1999 | 7 | anadromous | adults | Fall return** |
| | 2000 | 219 | anadromous | adults | Fall return** |
| | 2001 | 20 | anadromous | adults | Fall return** |

*Includes hatchery mortalities

**Returns from the captive-rear program

Table 1 continued. Collections of *Oncorhynchus nerka* samples from 40 locations. Location numbers correspond to sampling locations diagramed in Figure 1.

| Location # | Population | N | Form | Age | Collection Date(s) |
|---|---|---|---|---|---|
| | **IDAHO continued** | | | | |
| **12** | Redfish Lake, outmigrants | **81** | | | |
| | 1991 | 49 | unknown | juveniles | May-June 1991 |
| | 1992 | 11 | unknown | juveniles | Apr.-May 1992 |
| | 1993 | 21 | unknown | juveniles | Apr.-June 1993 |
| **13** | Redfish Lake, Merwin trap | **22** | | | |
| | 1992 | 5 | residual | adults | Oct.-Nov. 1992 |
| | 1993 | 14 | residual | adults | Oct. 1993 |
| | 1995 | 3 | residual | adults | Oct. 1995 |
| **14** | Redfish Lake (Fishhook Ck.) | **81** | | | |
| | 1990 late spawners | 22 | kokanee | adults | Sep. 9, 1990 |
| | 1991 early spawners | 15 | kokanee | adults | Aug. 22, 1991 |
| | 1991 late spawners | 13 | kokanee | adults | Sep. 5, 1991 |
| | 1992 early spawners | 16 | kokanee | adults | Aug. 14, 1992 |
| | 1992 late spawners | 3 | kokanee | adults | Sep. 8, 1992 |
| | 1995 late spawners | 11 | kokanee | adults | Sep. 1, 1995 |
| | 1996 late spawners | 1 | kokanee | adults | Sep. 5, 1995 |
| **15** | Redfish Lake, creel survey | **145** | | | |
| | 1996 | 42 | unknown | mixed | Summer 1996 |
| | 1997 | 37 | unknown | mixed | Summer 1997 |
| | 1998 | 25 | unknown | mixed | Summer 1998 |
| | 1999 | 41 | unknown | mixed | Summer 1999 |
| **16** | Redfish Lake, trawls | **93** | | | |
| | 1990 | 8 | unknown | mixed | Aug. 20, 1990 |
| | 1994 | 9 | unknown | mixed | Sep. 6, 1994 |
| | 1995 | 8 | unknown | mixed | Sep. 26, 1995 |
| | 1996 | 45 | unknown | mixed | Sep. 10, 1996 |
| | 1997 | 23 | unknown | mixed | Sep. 2, 1997 |
| **17** | Stanley Lake (Stanley L. Creek) | **33** | | | |
| | 1992 early spawners in creek | 25 | kokanee | adults | Aug. 20, 1992 |
| | 1994 late (gill net near inlet) | 5 | kokanee | ripe adults | Oct. 1994 |
| | 1994 late spawners in creek | 3 | kokanee | adults | Nov. 1994 |
| **18** | Stanley Lake, trawls | **28** | | | |
| | 1994 | 18 | kokanee | mixed | Sep. 6, 1994 |
| | 1995 | 10 | kokanee | mixed | Sep. 27, 1995 |
| **19** | Warm Lake | **33** | | | |
| | 1990 | 16 | kokanee | adults | Oct. 26, 1990 |
| | 1992 | 17 | kokanee | adults | Sep. 12, 1992 |

Table 1 continued. Collections of *Oncorhynchus nerka* samples from 40 locations. Location numbers correspond to sampling locations diagramed in Figure 1.

| Location # | Population | N | Form | Age | Collection Date(s) |
|---|---|---|---|---|---|
| | **Washington** | | | | |
| **20** | Baker Lake | **16** | anadromous | juveniles | Spring 1993 |
| **21** | Lake Ozette | **37** | anadromous | juveniles | Jun. 1994 |
| **22** | Lake Quinault | **14** | anadromous | juveniles | Spring 1993 |
| **23** | Lake Sutherland | **20** | kokanee | adults | Nov. 1993 |
| **24** | Lake Wenatchee | **50** | | | |
| | 1990 | 32 | anadromous | adults | Sep. 25, 1990 |
| | 1996 | 18 | anadromous | adults | Sep. 25, 1996 |
| **25** | Lake Whatcom | **20** | kokanee | juveniles | Spring 1993 |
| | **Oregon** | | | | |
| **26** | Deschutes River, Pelton Dam | **17** | | | |
| | 1995 | 14 | anadromous | adults | Fall 1995 |
| | 1996 | 3 | anadromous | adults | Fall 1996 |
| **27** | Lake Billy Chinook @ Gorge Campground | **38** | kokanee | adults | Sep. 15, 1992 |
| **28** | Wallowa Lake | **31** | kokanee | mixed | Nov. 4, 1993 |
| **29** | Wallowa River | **28** | kokanee | adults | Sep. 29, 1992 |
| **30** | Wizard Falls Hatchery | **53** | kokanee | juveniles | Spring 1993 |
| | **British Columbia** | | | | |
| **31** | Babine Lake | **28** | kokanee | adults | Aug. 1988 |
| **32** | Babine Lake | **34** | anadromous | adults | Sep. 27, 1990 |
| **33** | Eagle River | **31** | kokanee | adults | Sep. 11-13, 1994 |
| **34** | Eagle River | **32** | anadromous | adults | Sep. 11-13, 1994 |
| **35** | Horsefly River | **34** | anadromous | adults | Sep. 14, 1991 |
| **36** | Okanogan Lake (Peachland Creek) | **17** | kokanee | adults | Oct. 13, 1990 |
| **37** | Okanogan Lake (Squally Pt. shore) | **20** | kokanee | adults | Oct. 26, 1990 |
| **38** | Okanogan River | **32** | anadromous | adults | Oct. 12-13, 1990 |
| **39** | Shuswap River | **30** | anadromous | adults | Oct. 7-14, 1994 |
| **40** | Shuswap River (Bessette Ck.) | **28** | kokanee | adults | Oct. 7-14, 1994 |
| | **Total** | **1720** | | | |

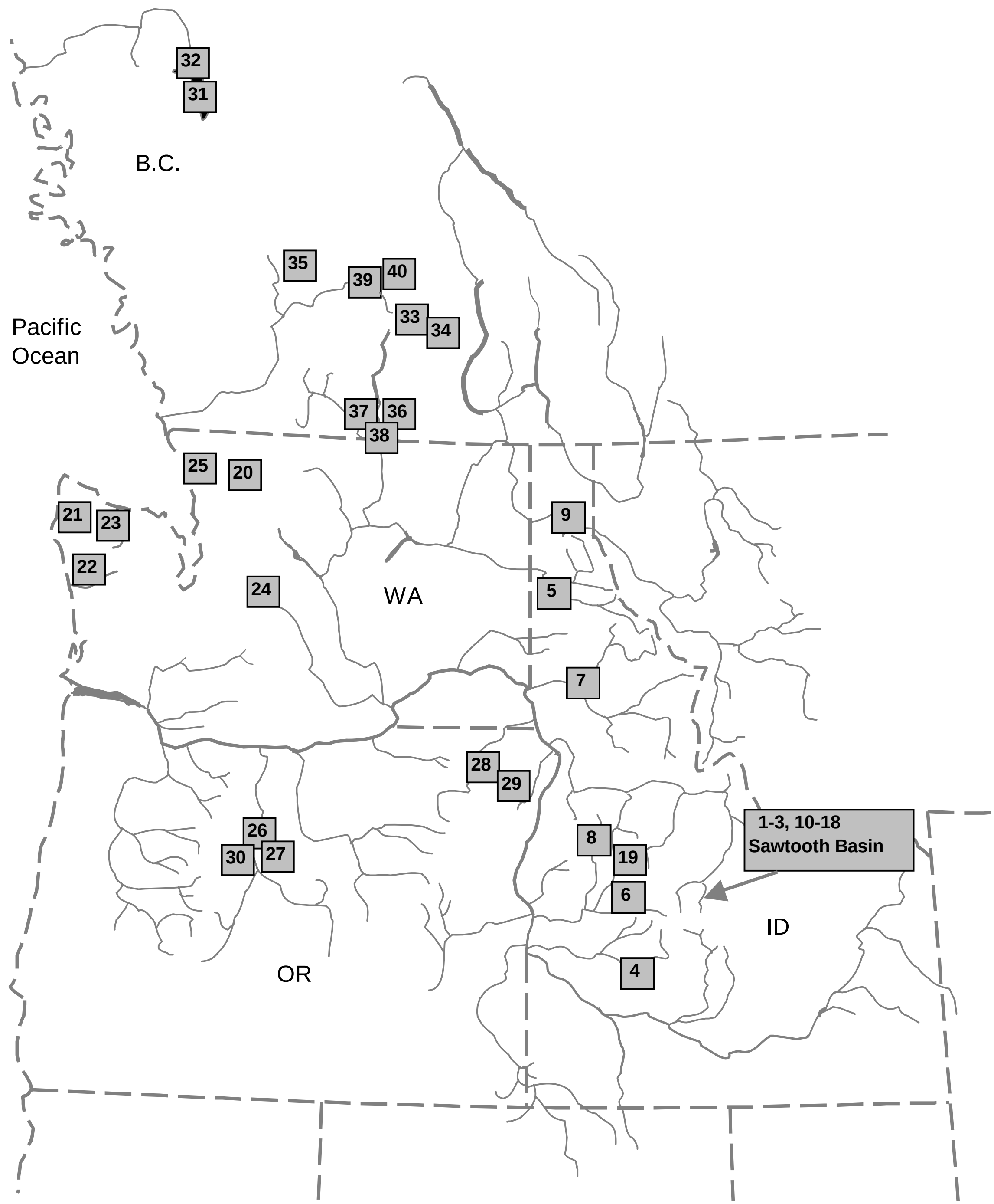


Figure 1. Sampling locations for *O. nerka* populations in British Columbia, Washington, Oregon, and Idaho. Location numbers are described in Table 1.

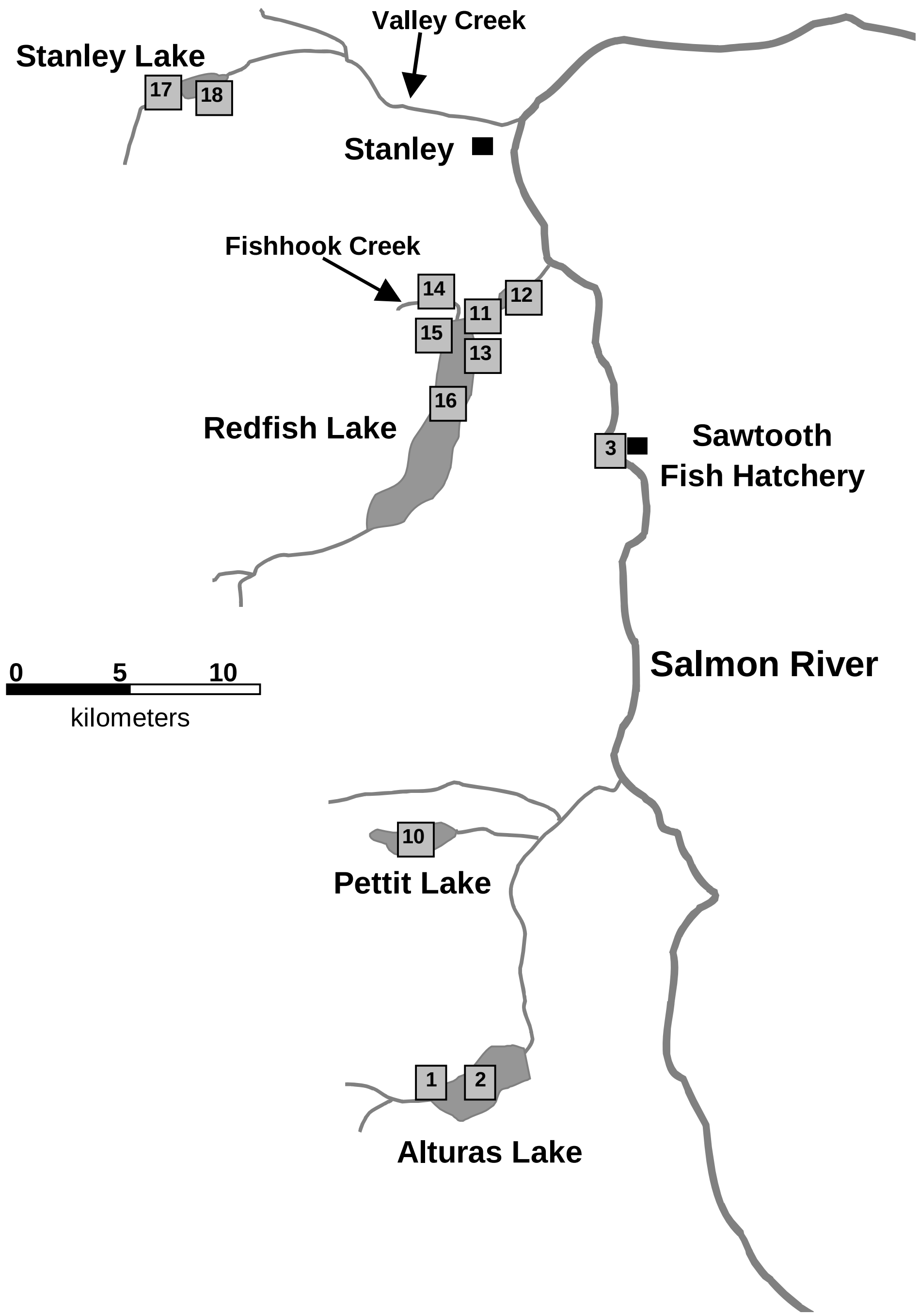


Figure 2. Detail of sockeye and kokanee sampling locations within the Sawtooth Basin. Locations numbers are described in Table 1.

## DNA EXTRACTION AND RFLP ANALYSIS

DNA was extracted from tissue samples using methods modified from Sambrook et al. (1989) and Dowling et al. (1996). The polymerase chain reaction (PCR) was used to amplify sequences from each DNA sample using nucleotide primers developed by LGL Ecological Genetics specific for the mitochondrial Cytochrome b and NADH dehydrogenase subunit 1, 2, and 5/6 gene regions (Appendix A).

Amplified mtDNA gene regions from a subset of samples were digested using 13 Type II restriction endonucleases to search for polymorphisms (Appendix B). The resulting mtDNA fragments were separated by electrophoresis using agarose or polyacrylamide gels. Gels were stained with ethidium bromide and restriction fragment patterns were visualized using UV light.

Fragment sizes of each restriction fragment length polymorphism (RFLP) from each gene region were estimated by comparison to a size standard, pUC-19 marker (LGL Ecological Genetics) or 100 bp marker (Invitrogen). Observed RFLPs for each combination of gene region and restriction endonuclease digest were assigned alphabetical scores (simple haplotypes). Scores for simple haplotypes were combined to form composite haplotypes for each sample (Table 2).

Table 2. Combinations of simple mitochondrial haplotypes observed among *Oncorhynchus nerka* populations of Idaho, Washington, Oregon, and British Columbia.

| **Composite Haplotype Designation** | **Simple Mitochondrial Haplotypes** (Gene Region / *Restriction Enzyme*) | | | | | |
|---|---|---|---|---|---|---|
| | Cyt. B / *Hae* III | ND 1 / *Dpn* II | ND 1 / *Hae* III | ND 1 / *Rsa* I | ND 2 / *Hae* III | ND 5-6 / *Taq* I |
| H01 | A | A | A | A | A | A |
| H02 | A | A | A | A | A | B |
| H03 | A | A | A | A | B | B |
| H04 | A | A | A | A | C | A |
| H05 | A | A | B | A | A | B |
| H06 | A | A | B | C | A | B |
| H07 | B | A | A | A | A | A |
| H08 | B | A | A | A | A | B |
| H09 | B | A | A | A | B | A |
| H10 | B | A | A | A | B | B |
| H11 | B | A | A | A | C | A |
| H12 | B | A | A | A | C | B |
| H13 | B | A | A | A | D | A |
| H14 | B | A | A | B | A | A |
| H15 | B | A | A | B | B | B |
| H16 | B | A | A | B | C | A |
| H17 | B | A | B | A | A | A |
| H18 | B | A | B | A | A | B |
| H19 | B | A | B | A | C | A |
| H20 | B | A | B | A | C | B |
| H21 | B | A | C | A | A | A |
| H22 | B | A | C | A | A | B |
| H23 | B | A | C | A | B | A |
| H24 | B | A | C | A | C | A |
| H25 | B | A | C | B | A | A |
| H26 | B | A | C | B | A | B |
| H27 | B | A | C | B | B | A |
| H28 | B | A | E | A | A | A |
| H29 | B | B | A | A | A | B |
| H30 | B | B | B | A | A | A |
| H31 | B | B | B | A | A | B |
| H32 | B | B | B | A | B | B |
| H33 | B | B | C | B | A | A |
| H34 | B | C | B | A | A | B |
| H35 | B | D | A | A | A | A |

Cyt B = cytochrome *b*, ND 1 = NADH dehydrogenase subunit 1, ND 2 = NADH dehydrogenase subunit 2, ND 5-6 = NADH dehydrogenase subunits 5 and 6.

# RESULTS

## GENERAL OBSERVATIONS

A total of 1,720 tissue samples representing 40 populations were analyzed using mitochondrial RFLPs. Sample collections representing possible admixtures of resident and anadromous life forms (e.g. trawl and creel surveys from Redfish Lake or outmigrants from Alturas Lake) are indicated in Table 1 and in following tables as being of unknown (U) origin. Restriction enzymes that yielded polymorphic banding patterns were combined from each mitochondrial gene region analyzed to form 35 composite mitochondrial RFLP haplotypes (Table 2). Additional enzymes used in this study that produced monomorphic restriction fragment patterns were not included in the composite haplotypes.

Several populations were fixed for one or more simple haplotypes (Appendix C). However, none of the populations examined were fixed for any composite haplotype. Composite haplotypes were categorized by their frequency of occurrence (Appendix D): those observed in fewer than ten samples (n=26), those observed in 10-100 samples (n=3), and those observed in more than 100 samples (n=6). The six composite haplotypes observed most often were H7, H9, H11, H18, H21, and H25 (collectively, 1,544 individuals or 89.8% of all samples). At least one of these composite haplotypes was observed in each population. Fifteen composite haplotypes were observed in and limited to single populations. For the purposes of this report, these composite haplotypes have been designated as “unique”, bearing in mind this designation is putative and subject to change upon further analysis.

Composite mtDNA haplotypes and their frequencies are summarized by sample location in Appendix E. Further separations by year class are summarized in Appendices F (Redfish Lake populations) and G (Alturas and Stanley populations, Deadwood, Payette, Warm Lake, Lake Ozette, and Lake Wenatchee).

## NORTHERN IDAHO POPULATIONS

Kokanee samples from Lake Pend O'Reille and Lake Coeur d'Alene both contained H11 as their dominant composite haplotype (frequencies 0.937 and 0.954, respectively). The H11 composite haplotype was observed at similar frequencies in Pettit Lake (Stanley Basin, ID) and Lake Whatcom, WA, and at various lesser frequencies in numerous other sampling locations. Additional minor (frequency of 5% or less of sample set) haplotypes H4 and H18 were observed in Lake Pend O'Reille; one minor haplotype, H24, was observed in Lake Coeur d'Alene. The H24 composite haplotype was observed in one individual of the sample set, and represented a unique haplotype.

## STANLEY BASIN POPULATIONS

Six populations were examined from Redfish Lake: anadromous sockeye adults, sockeye residuals, outmigrants, kokanee spawners from Fishhook Creek, and an admixture of *O. nerka* collected from creel and trawl surveys. Combined, these populations exhibited 19 composite haplotypes. Appendix E details the frequencies of composite haplotypes observed in each of the Redfish Lake populations by year of collection.

Fishhook Creek kokanee spawners collected from five brood years exhibited 11 different composite haplotypes. When separated into “early” (August) and “late” (September) spawning groups, five composite haplotypes were shared (H2, H9, H18, H25, and H31). The frequencies of H18, H25, and H31 were similar between the early and late groups (H18: 0.129 and 0.140, H25: 0.323 and 0.280, H31: 0.129 and 0.140, respectively). Of the six remaining composite haplotypes, two were observed in the early group (H21 and H33), and four in the late group (H10, H14, H15, and H29); all but H29 were minor haplotypes. The H15 haplotype was observed in one individual, and is unique to the Redfish Lake kokanee spawner population in Fishhook Creek.

Four composite haplotypes were observed in the sixteen anadromous sockeye that returned to Redfish Lake between 1991 and 1998 (H9, H23, H25, and H27). Three haplotypes (H9, H21, and H25) were observed in the 1999, 2000, and 2001 returns, which included captive-reared hatchery fish. The H23 and H27 haplotypes have not been observed since 1993. Outmigrants from Redfish Lake exhibited two major composite haplotypes, H9 and H25 (frequencies of 0.444 and 0.481, respectively), and four minor haplotypes, H7, H14, H21 and H23. Four of the haplotypes (H9, H21, H23, and H25) occur also in the anadromous sockeye returns to Redfish Lake. The H14 haplotype was also observed in Redfish Lake kokanee, and in Alturas Lake trawl samples. The H7 haplotype was also observed in Redfish Lake residuals and creel samples. Redfish Lake residual sockeye exhibited five composite haplotypes, of which four (H9, H21, H23, and H25) were shared with Redfish Lake anadromous sockeye returns and outmigrants.

Among the Redfish Lake *O. nerka* populations, four composite haplotypes, H9, H14, H21, and H25, were shared by the three putative sockeye populations with the kokanee spawners in Fishhook Creek. The frequencies of H9 were similar among the sockeye populations (0.437 for anadromous pre-1999, 0.454 for residuals, 0.444 for outmigrants), and double the kokanee frequency (0.185). Composite haplotypes H2, H10, H15, H18, H29, H31, and H33 were observed in kokanee spawners, but not in the putative sockeye populations. Similarly, composite haplotypes H7, H23 and H27 were observed in the sockeye populations, but not in the kokanee spawners.

Samples from creel and trawl surveys of Redfish Lake yielded 13 composite haplotypes. Trawl survey samples contained an admixture of adults and juveniles from the kokanee and sockeye populations, while creel survey samples contained putative kokanee caught by fishermen. Five minor haplotypes not previously seen in the putative kokanee and sockeye populations included H1, H8, H26, H30, and H32. The remaining eight haplotypes included H2, H18, H29, H31 and H33 (also observed in Fishhook Creek kokanee), H7 (also observed in residuals and outmigrants), and H9 and H25 (shared haplotypes observed in Redfish Lake kokanee and sockeye). The one fish exhibiting an H7 putative sockeye haplotype was observed in the 1996 creel survey.

Alturas Lake samples included one year of kokanee spawners from Alturas Lake Creek, two years of outmigrants and three years of admixed *O. nerka* from mid-water trawls of the lake. Four composite haplotypes were observed in all three populations, each occurring at similar frequencies among the populations: H9 (frequencies of 0.583, 0.556 and 0.463, respectively), H25 (0.208, 0.333, and 0.278), H31 (frequencies less than 0.13) and H21 (frequencies less than 0.10). Spawners and outmigrants each had one additional minor haplotype, H27 and H29, respectively. Six additional minor haplotypes were observed in trawl samples, H27 (observed also in spawners), and H10, H14, H17, H18, and H30. Appendix G details the frequencies of composite haplotypes observed in each of the Stanley Basin populations (except Redfish Lake, Appendix F) by year of collection.

Stanley Lake kokanee spawners collected from Stanley Lake Creek exhibited five composite haplotypes (H7, H9, H11, H18 and H21). When separated into “early” (September) and “late” (October/November) spawning groups, two haplotypes were observed only in the early group (H7 and H21) and three observed only in the late group (H9, H11, and H18). None of the five haplotypes was shared between the two groups. Mid-water trawls of Stanley Lake yielded only two haplotypes (H18 and H21) that were shared with the creek spawners.

Pettit Lake trawl samples yielded one major haplotype, H11, and one minor haplotype, H19. The frequency of the H11 haplotype in Pettit Lake (0.957) was similar to the frequencies seen in kokanee from Lake Pend O’Reille, Lake Coeur d’Alene, and Lake Whatcom (0.937, 0.954, and 0.850 respectively). The minor haplotype, H19, was detected in only one other population, the Wizard Falls Hatchery kokanee juveniles. Frequencies were similar for the two populations, 0.043 for Pettit Lake and 0.038 for Wizard Falls Hatchery.

## OTHER IDAHO POPULATIONS

Kokanee samples from Anderson Ranch Reservoir, Deadwood Reservoir, Dworshak Reservoir (Isabella Creek spawners), and Payette Lake (North Fork Payette River spawners) all contained H18 as their major composite haplotype (frequencies of 0.917, 0.861, 0.649, and 0.687, respectively). Additional haplotypes included H11 at

Anderson Ranch, H7 at Deadwood Reservoir, H2, H5 and H7 at Dworshak Reservoir, and H2 and H7 at Payette Lake. The H5 haplotype represented a unique haplotype that was observed in two individuals from the Dworshak sample set.

Warm Lake kokanee samples exhibited different composite haplotype distributions between the two sampling years. The H21 haplotype dominated the 1990 group with a frequency of 0.687; other haplotypes included H7, H11, and H28. The H21 haplotype was not observed in the 1992 group, being replaced in dominance by H7 and H18 (frequencies of 0.353 and 0.471, respectively); other haplotypes observed included H1 and H8.

## WASHINGTON POPULATIONS

Lake Wenatchee sockeye samples (Appendix E) exhibited two major composite haplotypes (H9 and H18) and five minor haplotypes (H2, H3, H7, H8, and H11). The H9 haplotype was observed in numerous other Columbia basin locations, but not in the out-of-basin lakes sampled in Washington. Composite haplotype H3 was observed in only one individual in the Lake Wenatchee sample set and represented a unique haplotype. Comparing samples taken in 1990 with those from 1996 (Appendix G), we observed that the two major composite haplotypes had similar frequencies (0.531 and 0.611 for H9, and 0.344 and 0.333 for H18, respectively). Four minor haplotypes (H2, H3, H7, H8) were observed in the 1990 sample set (n=32 individuals), while only one additional haplotype, H11, was observed in the 1996 sample set (n=18 individuals).

The five out-of-basin populations sampled in Washington included kokanee from Lake Sutherland and Lake Whatcom, and anadromous sockeye from Baker Lake, Lake

Ozette, and Lake Quinault. The H7 composite haplotype was observed in all five populations, comprising the major part of the sample in each of the sockeye populations (frequencies of 0.875 for Baker, 0.767 for Ozette, and 0.929 for Quinault). Additional haplotypes observed in the sockeye populations were H11 in Baker Lake, H8, H18, and H21 in Lake Ozette, and H35 in Lake Quinault. The H35 haplotype was observed in only one individual in the Lake Quinault sample set and represented a unique haplotype. Lake Ozette samples (Appendix G) were from hatchery juveniles that resulted from spawning of adults that arrived “early” or “late” in the run. Early run fish exhibited two haplotypes, H7 and H21 (frequencies 0.700 and 0.300, respectively), and late run fish exhibited three haplotypes, H7, H8 and H18 (0.823, 0.059, and 0.118). Lake Sutherland kokanee samples contained five composite haplotypes, H7, H8, H13, H17 and H18. Lake Whatcom kokanee samples also exhibited haplotypes H7 and H18, though at lower frequencies, with H11 as the observed major haplotype (frequency 0.850).

OREGON POPULATIONS

Deschutes River basin populations analyzed in this study included anadromous sockeye captured at Pelton Dam, spawning kokanee at Gorge Campground on the Metolius River, and juvenile kokanee at Wizard Falls Hatchery on the Metolius River. Three composite haplotypes were observed in all three populations, H8 (frequencies of 0.294, 0.105, and 0.151, respectively), H9 (0.176, 0.210, and 0.019), and H18 (0.411, 0.579, and 0.453). Additional haplotypes observed in the Deschutes River sockeye included H7 and H20. The H20 composite haplotype was observed in only one individual in the Deschutes sample set and represented a unique haplotype. Additional haplotypes observed in the kokanee populations included H7 and H11 in Gorge Campground spawners, and H11 (frequency 0.321) and minor haplotypes H4 and H19 in Wizard Falls Hatchery juveniles.

Wallowa Lake adult and juvenile kokanee were sampled with a gill net in 1993, and adult spawners were sampled in the Wallowa Lake River in 1992. Six composite haplotypes were observed from the gill net samples, H11 and H21 (frequencies 0.387 and 0.355, respectively), H7 and H18 (0.097 and 0.097), and minor haplotypes H4 and H8. Spawners shared three of these haplotypes at similar frequencies (H7, H8, and H21), and one haplotype, H18, at dissimilar frequencies (0.097 for gill net, 0.286 for spawners). Four additional haplotypes observed in spawners included H7, H12, H22 and H25. The H12 and H22 haplotypes were observed in one and two individuals, respectively, and both represented unique haplotypes. Combined, the Wallowa populations contain ten composite haplotypes (Appendix E).

## BRITISH COLUMBIA POPULATIONS

Three populations of *O. nerka* were sampled from the Okanogan system in the upper Columbia River basin. Four composite haplotypes were observed in Okanogan Lake kokanee shore spawners, H11 and H18, and minor haplotypes H6 and H16. Both minor haplotypes were observed in only one individual each in the sample set, and both represented unique haplotypes. Okanogan Lake kokanee spawners from Peachland Creek shared major haplotypes H11 and H18 with the shore spawners, and exhibited one additional haplotype, H7. Okanogan River sockeye spawners shared two composite haplotypes with the Okanogan kokanee, H7 and H18. Additional sockeye haplotypes observed were H9 and H21.

The five Fraser River drainage populations sampled all shared three composite haplotypes, H7, H11, and H18. Additional haplotypes included minor haplotype H8 from Eagle River kokanee, and haplotype H34 from Bessette Creek sockeye. The H34 composite haplotype was observed in three individuals from the Bessette Creek sample set, and represented a unique haplotype. Frequencies of shared haplotypes were similar between sockeye and kokanee populations of Eagle River. Shuswap River sockeye and kokanee frequencies were not similar except for H18 (0.167 and 0.214, respectively).

The Babine Lake sockeye and kokanee populations (Skeena River drainage) sampled shared three composite haplotypes, H7, H11, and H21, but at different frequencies: 0.382 and 0.179 for H7 in sockeye and kokanee, respectively; 0.088 and 0.679 for H11; and 0.529 and 0.143 for H21.

# DISCUSSION

## GENETIC ANALYSIS

Previous genetic surveys have shown the genetic diversity of sockeye salmon to be extensively subdivided among major geographic regions and among different lake systems (Wood 1995, Beacham et al. 1995, Winans et al. 1996, Waples et al. 1997). This study utilized mitochondrial DNA RFLPs to analyze the genetic diversity of sockeye populations of the Pacific Northwest. Mitochondrial DNA (mtDNA) is maternally inherited in a clonal manner (without recombination), and its gene sequences referred to as haplotypes, because the mitochondrial genome is haploid. From a phylogenetic perspective, the entire mtDNA molecule represents one nonrecombining geological unit with multiple alleles (Avise 1994). Greater resolution of genetic differences can be achieved using mtDNA due to the higher evolutionary rate of the mitochondrial molecule (5-10 times higher than the nuclear). Frequencies and geographic distributions of mitochondrial DNA markers can be used to evaluate change on large spatial and temporal scales to view gene flow and population history (Sunnucks 2000). Mitochondrial haplotype frequencies can be affected by genetic drift, founder effects, gene flow, and selection. Statistical analysis can be used to estimate gene flow and population subdivision. The fragment data generated by RFLPs can be analyzed to quantify genetic differences among populations; whereas the restriction site changes among the RFLPs can be used to estimate phylogenetic distances to address issues of population structure (Palumbi and Baker, 1996). The report utilized the property of maternal inheritance to trace maternal lineages, and to identify relatedness among populations that may now be geographically separated.

## GENETIC VARIATION OF ANADROMOUS SOCKEYE POPULATIONS

Sockeye are believed to have evolved from the trout genus *Salmo* during a series of geographical isolations around the North Pacific rim as ocean elevations changed during glaciation changes early in the Pleistocene period (Burgner 1991). The adaptation of sockeye to lake environments for rearing purposes appears to require precise homing to spawning areas by time and location, leading to temporal and spatial separations that contribute to the genetic subdivision observed among populations by previous genetic studies. Ricker (1940) deemed it likely that sockeye colonized diverse regions of Japan, Kamchatka, Alaska, British Columbia, and the northwestern United States, and subsequently gave rise to nonanadromous kokanee populations. Genetic studies by Foote et al. (1989) and Taylor et al. (1996) support the divergence of kokanee from sockeye salmon that colonized watersheds within northwestern regions of North America

Using mtDNA RFLPs, this study analyzed populations of anadromous sockeye from the Pacific Northwest to evaluate evolutionary history and population structure of the Columbia River Basin sockeye. Effects of stocking on genetic diversity and population identification were also discussed.

Three extant populations of anadromous sockeye remain in the Columbia Basin: Okanogan River sockeye of Lake Osoyoos, Lake Wenatchee River sockeye of Lake Wenatchee, and Salmon River sockeye of Redfish Lake (Fulton 1970; Mullan 1986). Composite haplotypes H7 and H9 were shared by these three anadromous populations, however their haplotype distributions and frequencies were dissimilar within the Basin (Figure 4). This mosaic pattern of differentiation among populations from different lakes within a river system due to reproductive isolation is discussed in Wood (1995).

In this study, the sockeye populations of the Columbia River Basin were also compared to Puget Sound sockeye populations (Baker Lake and the Fraser Basin populations of Horsefly, Eagle River and Middle Shuswap River), Washington Coastal sockeye populations (Lake Ozette, Lake Quinault) and the Skeena River sockeye of Babine Lake. Geographic and frequency distributions of the major haplotypes observed suggested the historical presence of four major maternal lineages: H7, H9, H11, and H18. Some of the haplotype frequency distributions may be explained by the physical (geologic) history of the Pacific Northwest. Specifically, the upper Fraser River historically drained to the ocean via the upper Columbia when the lower Fraser was blocked by ice dams during deglaciation (McPhail and Lindsey 1986). Fraser drainage juvenile sockeye exiting to the ocean via the Columbia River would return the same way, possibly straying and colonizing Columbia tributaries. The H7 lineage (Figure 7) appears to have colonized the Fraser drainage first (frequencies of 0.250 – 0.567), then spread into the Okanogan during deglaciation (0.594). H7 occurs only as a minor haplotype in Lake Wenatchee and Redfish Lake. Okanogan sockeye were stocked to Lake Wenatchee during 1939-1943 (Gustafson et al. 1997), possibly accounting for the presence of the H7 haplotype. Various stocking transplants to Stanley Basin lakes in Idaho may also account for the observed low occurrence of H7 in Redfish Lake. The H9 lineage was observed only in Columbia River populations, suggesting that anadromous sockeye of this lineage entered the drainage from the mouth of the Columbia to colonize its tributaries. The H11 haplotype was observed in Fraser River populations, but not in Okanogan sockeye. Possibly the H11 lineage colonized the Fraser drainage after the lower Fraser was unblocked, and so did not connect to the Columbia River. Alternatively, H11 may have been established but then lost from the Columbia. The H18 composite haplotype was observed in the Fraser River, Okanogan River and Lake Wenatchee sockeye populations. What happened here is more difficult to interpret. Colonization of the Columbia basin from Fraser River populations during deglaciation is a possibility; alternatively, the H18 lineage may have directly colonized the Columbia via the mouth of the Columbia River. Furthermore, historical transplants of Okanogan fish to the Wenatchee system may have artificially altered the frequency of H18 in that system. A lower frequency would suggest colonization via Fraser River populations. The higher frequency observed may be the result of direct colonization via the Columbia, or the result of stocking from the Okanogan, or just very successful colonization from the Fraser populations.

Possible remnant members of the Deschutes anadromous sockeye run were sampled during 1995-1996 and found to contain the H7, H9 and H18 composite haplotypes at frequencies of 0.059, 0.176, and 0.412, respectively. The high frequency of the H18 haplotype might suggest Lake Wenatchee strays, however, similar frequencies are observed in the kokanee spawners sampled from the Metolius River Gorge Campground and Wizard Falls Hatchery.

Puget Sound sockeye populations were found to be genetically similar, sharing three composite haplotypes (two only in Baker Lake) that comprised 90-100% of the samples in each population. However, the frequency distribution of those haplotypes was dissimilar between lakes.

GENETIC VARIATION OF SYMPATRIC *O. NERKA* POPULATIONS

Five sets of sympatric *O. nerka* populations were included in this study. Redfish Lake anadromous sockeye and kokanee populations were genetically dissimilar. Of the 13 composite haplotypes observed from the two populations, eight were observed in kokanee but not in the anadromous sockeye, and two were observed in sockeye but not in the kokanee population (H23 and H27). Only three haplotypes (H9, H21 and H25) were shared both populations. This genetic dissimilarity between kokanee and anadromous sockeye supports previous genetic work on Redfish lake *O. nerka* populations by Waples et al. (1997) and Cummings et al. (1997), further justifying the exclusion of kokanee from the ESA listing. The residuals and outmigrants shared the H9, H21, H23 and H25 haplotypes with the anadromous. Only one of the eight “kokanee” haplotypes occurred in the residual and outmigrant groups (H14 in the outmigrants). Kokanee have been transplanted to Redfish Lake numerous times (Hall-Griswold 1990, Bowler 1990), perhaps explaining the presence of several composite haplotypes not shared with the sympatric anadromous sockeye population.

The formerly sympatric *O. nerka* populations of the Okanogan River/Lake system were also genetically dissimilar. Of seven observed composite haplotypes, three were observed only in kokanee, two were observed only in sockeye, and the only two haplotypes shared by both forms of *O. nerka* occurred at dissimilar frequencies. Physical barriers and transplants of *O. nerka* possibly contributed to the genetic dissimilarity observed. Okanogan River sockeye have been blocked from entering Okanogan Lake by the Vaseux Dam, 24 km above Osoyoos Lake, since 1921 (Fulton 1970), thus preventing all genetic interchange in drainages above the dam. In addition, the Grand Coulee Fish-

Maintenance Project (GCFMP) trapped all sockeye salmon at Rock Island Dam between 1939 and 1943 and transplanted them to Lake Osoyoos, Lake Wenatchee, and several fish hatcheries (Gustafson et al. 1997). Hatchery-reared descendents of sockeye captured at Rock Island and Bonneville Dams, and progeny of Lake Quinault sockeye, were also stocked into Lakes Osoyoos and Wenatchee between 1940 and 1968 (Mullan 1986).

The remaining sympatric *O. nerka* populations examined, Shuswap River sockeye and kokanee, Eagle River sockeye and kokanee, and Babine Lake sockeye and kokanee, were all genetically similar. Each sympatric group shared three haplotypes comprising 90-100% of the fish sampled in this study. On the other hand, composite haplotype frequencies for Okanogan River sockeye and Middle Shuswap River sockeye were more similar to each other than either was to haplotype frequencies for their respective sympatric kokanee population. Collectively, the H7 and H18 composite haplotypes comprised 72% and 73% of the sampled Okanogan and Shuswap sockeye, respectively, vs. 30%, 53%, and 57% of the Okanogan shore spawning kokanee, Okanogan creek spawning kokanee, and Shuswap kokanee samples, respectively. When the H11 haplotype was included, 90% - 100% of the kokanee samples were included, vs. 72% and 90% for the Okanogan and Shuswap sockeye samples, respectively. Similar results were observed by Winans et al. (1996), who hypothesized that stream capture in recent geological time (the two river systems are less than 20 km apart) may have allowed gene flow, accounting for the genetic similarity between these sockeye and kokanee populations. Robison (1995) suggested that Shuswap Lake kokanee might have been transplanted to Okanogan Lake.

Another formerly sympatric population of interest is the Deschutes River *O. nerka*. Deschutes River sockeye originally reared in Suttle Lake at the head of the Metolius River. Fish passage to Suttle Lake was subsequently blocked by installation of a dam and screen at the outlet in 1930 (Fulton 1970), with further barrier dams erected between 1925-1938 (dam at Lake Creek Lodge), in 1958 (Pelton Dam and Pelton Re-regulating Dam), and 1964 (Round Butte Dam forming Lake Billy Chinook) (Gustafson et al. 1997). However, some anadromous adults are observed each year at the Pelton Re-regulating Dam. Sockeye were sampled from the Pelton Trap and compared to Lake Billy Chinook kokanee spawners in the Metolius River (an upstream tributary of the Deschutes River). Four composite haplotypes were shared by kokanee and sockeye, comprising over 90% of the samples. Thiesfeld et al. (1999) and Oregon Dept. of Fish and Wildlife (1990) suggest that the kokanee population of Lake Billy Chinook may have originated from the original sockeye population that was native to Suttle Lake. Wild kokanee in the Deschutes are distinguished from hatchery-released kokanee by the unique color pattern of Metolius fish, a blue-black body coloration during spawning (ODFW 1995). Analysis of the Wizard Falls Hatchery haplotype distribution revealed less similarity, with the hatchery kokanee sharing only three haplotypes with the sockeye and comprising less than 65% of the fish sampled.

## GENETIC VARIATION OF KOKANEE POPULATIONS

Comparison of Stanley Basin kokanee populations revealed much diversity and very little similarity. Pettit Lake stood out as unique from the other Stanley Basin lakes by exhibiting only two composite haplotypes: a minor haplotype, H19, observed elsewhere only in Wizard Falls Hatchery juveniles; and a major haplotype, H11, occurring at frequencies similar to those seen in Northern Idaho populations, and to Lake Whatcom, WA. Hall-Griswold (1990) collected information suggesting that Pettit had been stocked with Anderson Ranch kokanee, from Lake Pend O'Reille, which in turn, had originated in Flathead Lake. Waples (1995) traced the repercussions of the early part of this stocking history. In 1916, kokanee (believed to be from Lake Whatcom) were mistakenly transplanted from an Oregon hatchery into Flathead Lake, MT. The fish thrived and spread via the Clark Fork River to Lake Pend O'Reille, ID, which subsequently served as a source of broodstock for transplants to Coeur d'Alene (ID) and elsewhere. Lakes Whatcom, Pettit, Coeur d'Alene, and Pend O'Reille fish all share composite haplotype H11, at frequencies of 0.85 to 0.96, which would back up the veracity of the rather incomplete stocking records, previously supplemented only by personal communications. Genetic work by Winans et al. (1996) also showed the strong genetic similarity between Lakes Whatcom, Coeur d'Alene, and Pend O'Reille, which are all late-spawning stocks. Anderson Ranch *O. nerka* sampled in 1990 contained the H11 haplotype, but at a frequency of only 0.083; its major haplotype was H18 as 0.917. During 1983-1985, Anderson Ranch was stocked with over 445,000 early-spawning fish (IDFG stocking records http://www2.state.id.us/fishgame/fish/fishstocking/index.htm) that have apparently supplanted the Whatcom related haplotype.

Comparison of other Snake River kokanee populations sampled revealed a high degree of genetic similarity between Anderson Ranch Reservoir, Deadwood Reservoir, Dworshak Reservoir, and Payette Lake. Their similarity reflects the transfers of kokanee that occurred between these lake systems (Waples et al. 1997, Winans et al. 1996).

Warm Lake samples taken in 1990 showed a composite haplotype frequency distribution different from the other Snake River populations. Winans et al. (1996) described the Warm Lake kokanee population as a late-spawning population that utilized shore areas, and based on genetic data, has been isolated from other populations for a considerable time. In 1990, Warm Lake was stocked with 49,900 early-spawning kokanee from MacKay Hatchery (IDFG stocking records), which according to Waples et al. (1997) are Deadwood Reservoir stock. Subsequent sampling of Warm Lake kokanee in 1992 revealed an altered haplotype frequency distribution, which including an increase in the frequency of composite haplotypes H7 and H18, the only two haplotypes observed in our Deadwood Reservoir samples. Additional sampling of Warm Lake would reveal if the genetic changes from the 1990 stocking have dissipated over time.

Two Snake River kokanee populations occur in Wallowa Lake, where sockeye and kokanee existed sympatrically until a dam built in 1916 at the outlet of Wallowa Lake blocked the corridor for the Wallowa Lake sockeye (ODFW 1995). One population spawns in Wallowa River, and the other in the lake. While the two populations share four of ten observed composite haplotypes, their haplotype frequency distributions are otherwise dissimilar. Waples et al. (1997) suggested that the lake spawners were genetically similar to the northern Idaho populations. Two composite haplotypes shared by Lake Pend O'Reille and Wizard Falls Hatchery kokanee were observed in the lake spawners; a third shared haplotype was observed in both Wallowa spawner groups. Both Wallowa kokanee populations shared two haplotypes with the Snake River kokanee populations of Anderson Ranch Reservoir, Deadwood Reservoir, Dworshak Reservoir, and Payette Lake, though at lower frequencies. Further sampling of spawners from

Wallowa Lake and River would give a clearer picture of the genetic structure of the two populations.

Lake Sutherland kokanee occur outside the Columbia River drainage, but 70% of sampled fish contained two of the composite haplotypes (H7, H18) observed in the Snake River populations of Anderson, Deadwood, Dworshak, and Payette (comprising 89%-92% of samples). Haplotypes H7 and H18 were observed in most of the study populations, and represented 12.2% and 16.2% of the samples, respectively.

Six of the 35 composite haplotypes observed in this study ranged in frequency from 7-26% overall and are commonly observed in most populations. The six haplotypes together comprised 90% of the sampled *O. nerka*.

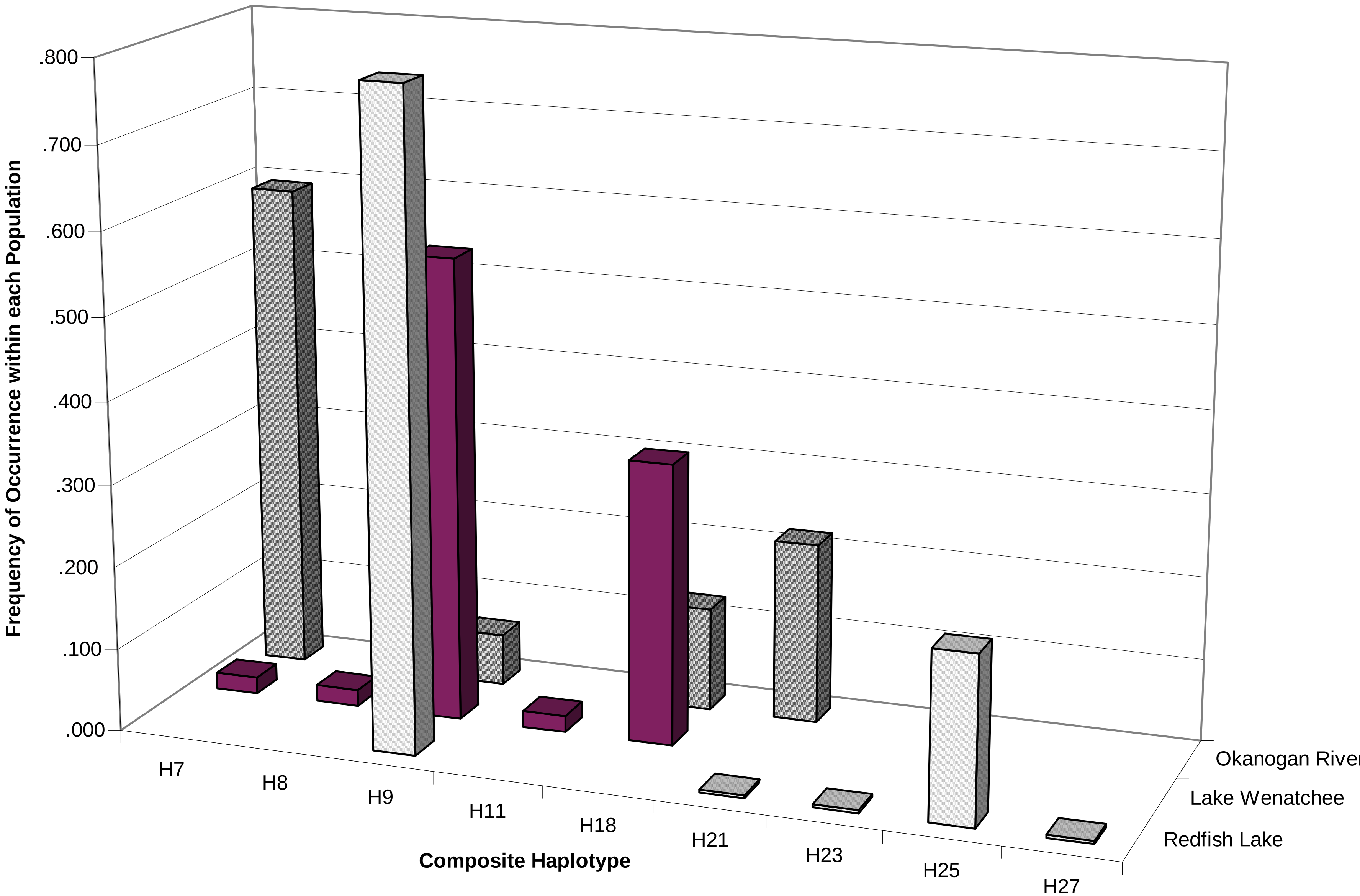


Figure 3. Composite haplotype frequency distribution for anadromous sockeye populations of the Columbia basin.

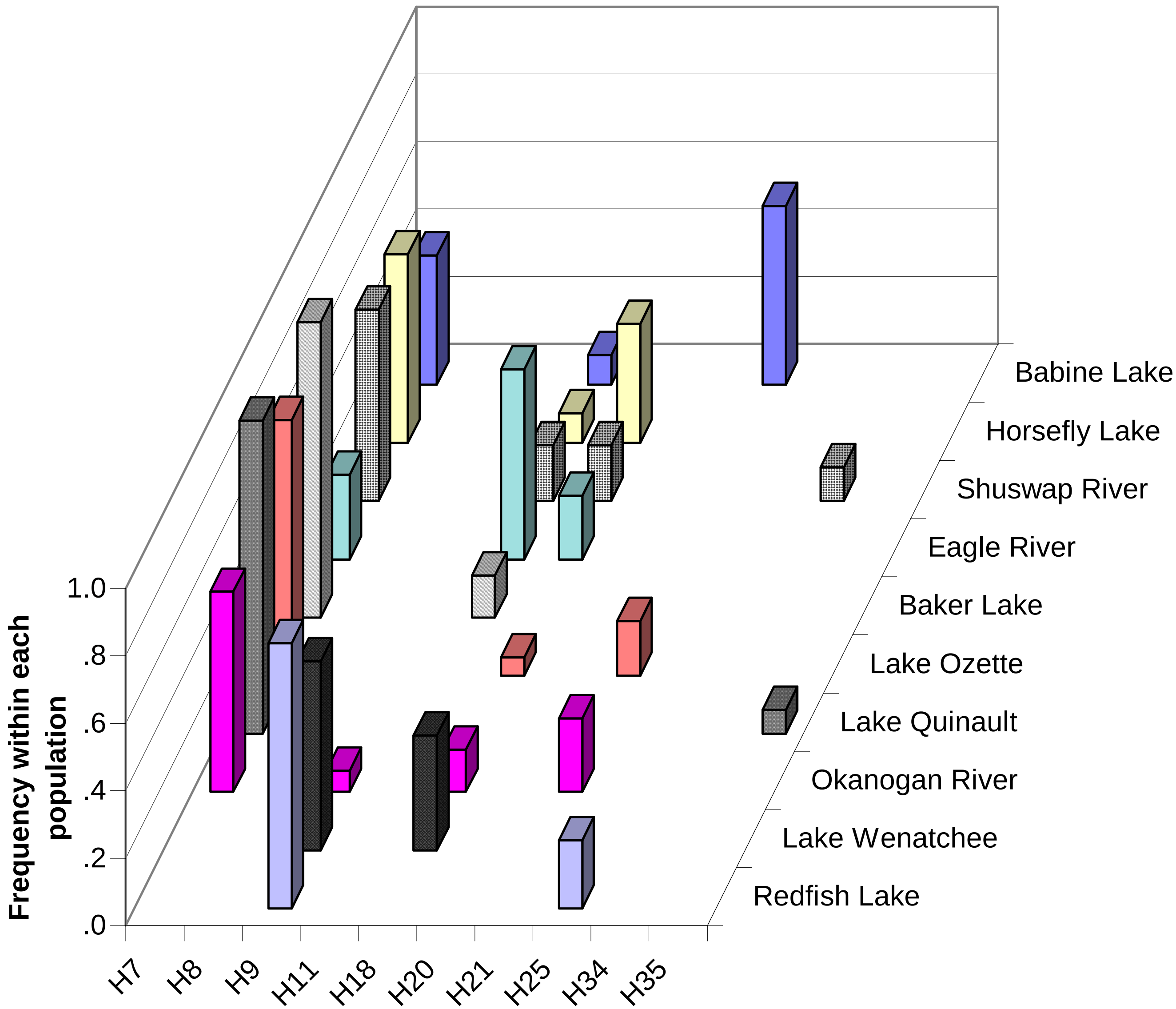


Figure 4. Composite haplotype frequency distribution for anadromous sockeye populations of the Pacific Northwest.

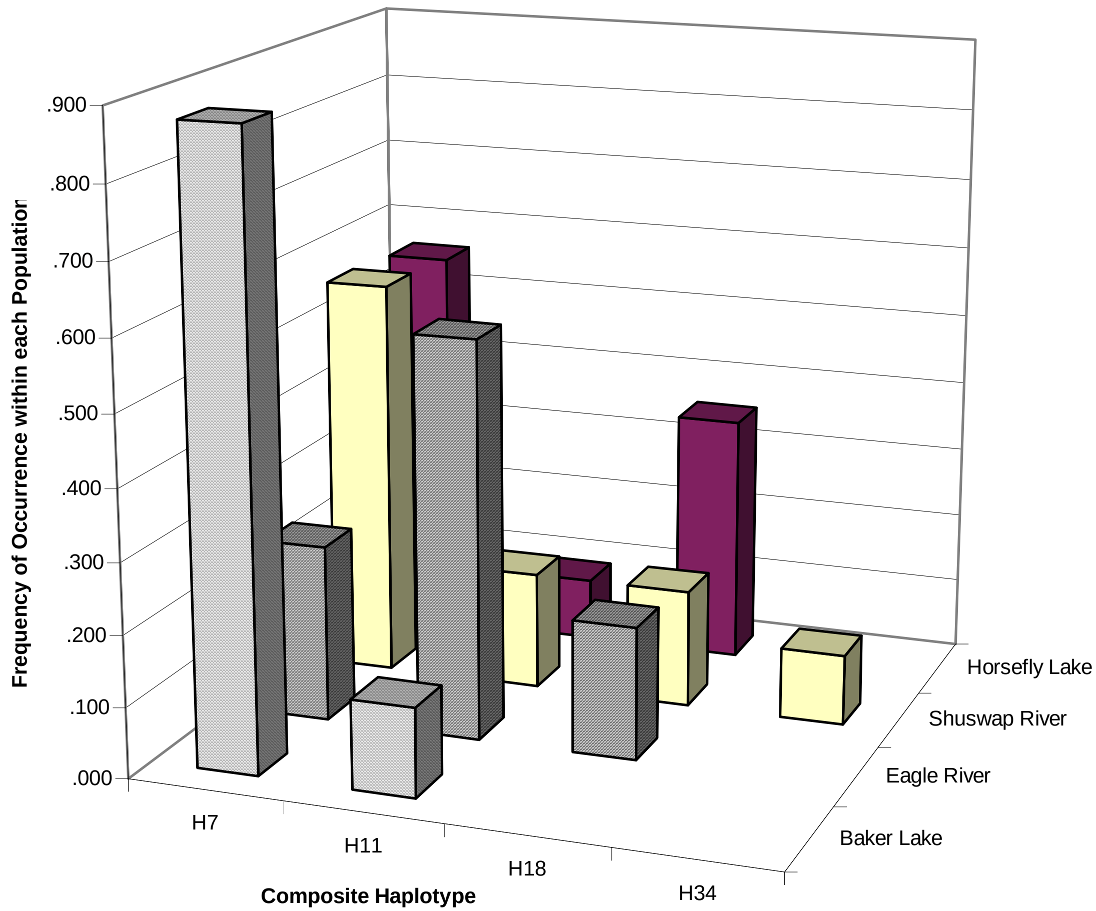


Figure 5. Composite haplotype frequency distribution for anadromous sockeye populations of the Puget Sound area.

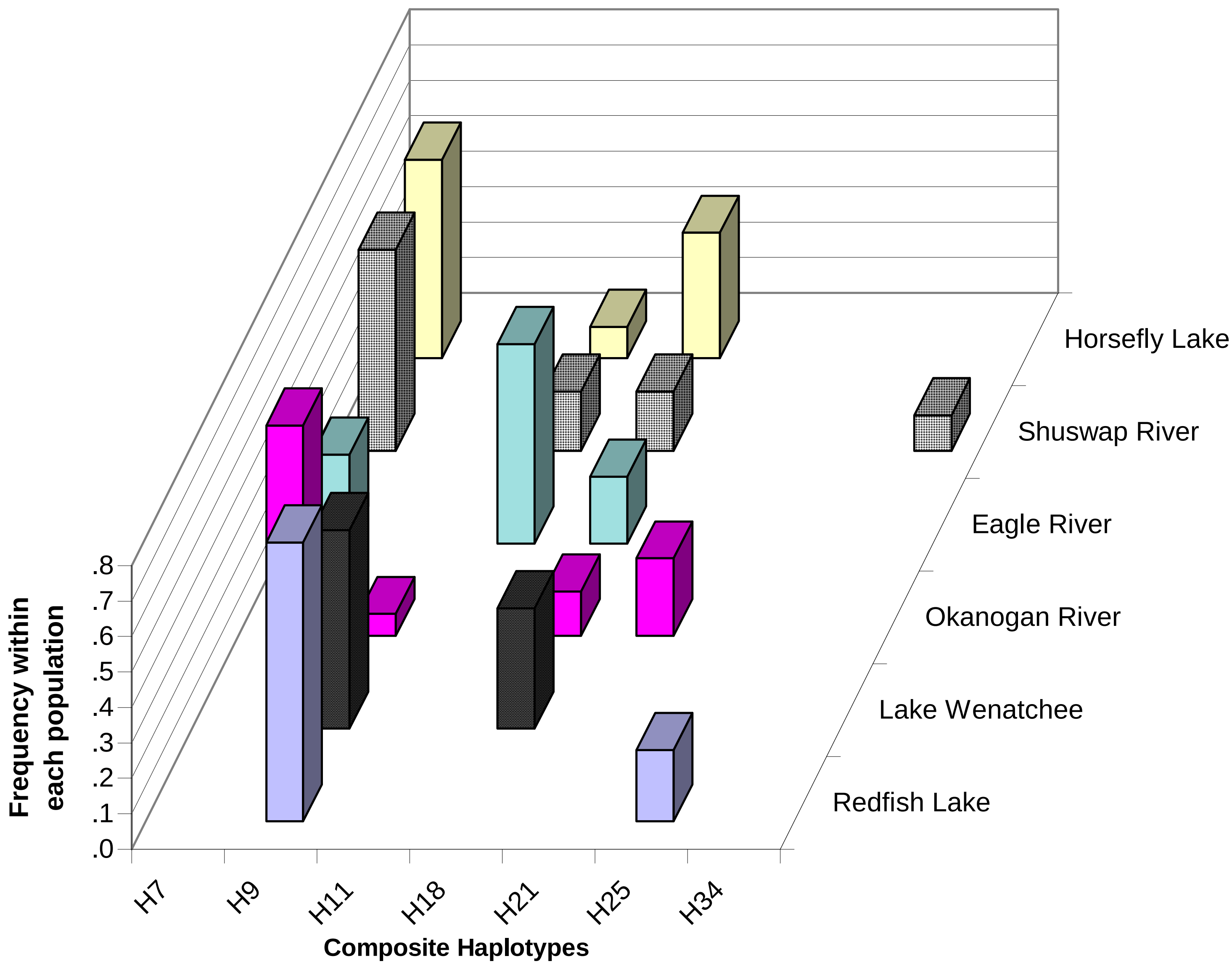


Figure 6. Composite haplotype frequencies (minor haplotypes excluded) of anadromous sockeye populations of the Fraser and Columbia basins.

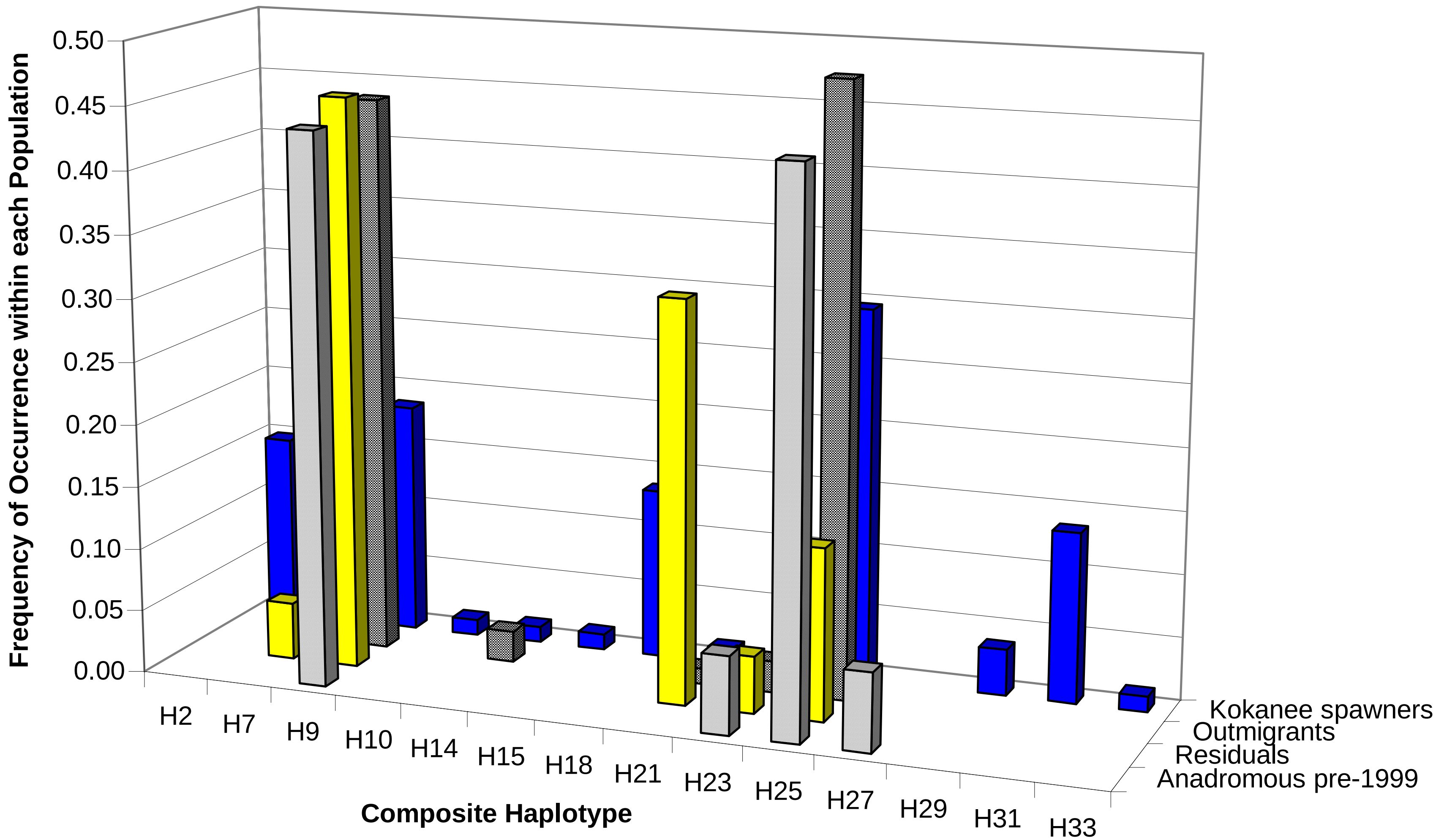


Figure 7. Composite haplotype frequencies of sympatric *O. nerka* populations of Redfish Lake, ID.

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Appendix A. Primer sequences used to PCR amplify specific mitochondrial gene regions in *Oncorhynchus nerka*.

Cytochrome *b*

| | |
|---|---|
| #765 | $^{5'}$GAA AAA CCA YCG TTG TWA TTC AAC T$^{3'}$ |
| #766 | $^{5'}$GTT TAA TTA GAA TYT YAG CTT TGG G$^{3'}$ |

NADH dehydrogenase subunit 1

| | |
|---|---|
| #381 | $^{5'}$ACC CCG CCT GTT TAC CAA AAA CAT$^{3'}$ |
| #563B | $^{5'}$GGT TCA TTA GTG AGG GAA GG$^{3'}$ |

NADH dehydrogenase subunit 2

| | |
|---|---|
| #461 | $^{5'}$GGC TCA GGC ACC AAA TAC TAA$^{3'}$ |
| #562 | $^{5'}$TAA GCT ATC GGG CCC ATA CC$^{3'}$ |

NADH dehydrogenase subunit 5/6

| | |
|---|---|
| #763RAI | $^{5'}$AAT AGC TCA TCC ATT GGT CTT AGG$^{3'}$ |
| #764RAI | $^{5'}$TAA CAA CGG TGG TTT TTC AAG TCA$^{3'}$ |

Y = C, T

W = A, T

Appendix B. Restriction endonucleases used to digest PCR amplified mitochondrial gene regions in this study. Number of polymorphic patterns observed indicated by (#).

| **Gene Region** | **Enzyme** | **Restriction Fragment Pattern** |
|---|---|---|
| Cyt. B | *Hae III* | Polymorphic (2) |
| Cyt. B | *Bfa I* | Polymorphic* |
| ND 1 | *Dde I* | Monomorphic |
| ND 1 | *Dpn II* | Polymorphic (4) |
| ND 1 | *Hae III* | Polymorphic (5) |
| ND 1 | *Hha I* | Monomorphic |
| ND 1 | *Hinf I* | Monomorphic |
| ND 1 | *Hpa I* | Monomorphic |
| ND 1 | *Nla III* | Monomorphic |
| ND 1 | *Rsa I* | Polymorphic (4) |
| ND 1 | *Taq I* | Monomorphic |
| ND 2 | *Hae III* | Polymorphic (4) |
| ND 5/6 | *Taq I* | Polymorphic (2) |

**Bfa I* was polymorphic but was dropped from further analyses because of unreliable enzyme digestion.

Appendix C. Summary of simple haplotypes by gene region and restriction endonuclease for each population.

| | | Simple Haplotypes by Gene region and *Restriction endonuclease* | | | | | |
|---|---|---|---|---|---|---|---|
| | | CYT B | ND 1 | ND 1 | ND 1 | ND 2 | ND 5/6 |
| **POPULATION** | **FORM** | *Hae III* | *Dpn II* | *Hae III* | *Rsa I* | *Hae III* | *Taq I* |
| **IDAHO** | | | | | | | |
| **Northern Idaho** | | | | | | | |
| Lake Pend O'Reille | K | A,B | A | A,B | A | A,C | A,B |
| Lake Coeur d' Alene | K | B | A | A,C | A | C | A |
| **Stanley Basin** | | | | | | | |
| Alturas Lake trawl | U | B | A,B | A,B,C | A,B | A,B | A,B |
| Alturas Lake spawners in Alturas L. Creek | K | B | A,B | A,B,C | A,B | A,B | A,B |
| Alturas Lake outmigrants | U | B | A,B | A,B,C | A,B | A,B | A,B |
| Pettit Lake trawl | K | B | A | A,B | A | C | A |
| Redfish Lake anadromous adults | S | B | A | A,C | A,B | A,B | A |
| Redfish Lake residuals | S | B | A | A,C | A,B | A,B | A |
| Redifish Lake outmigrants | U | B | A | A,C | A,B | A,B | A |
| Redfish Lake spawners in Fishhook Creek | K | A,B | A,B | A,B,C | A,B | A,B | A,B |
| Redfish Lake creel | U | A,B | A,B | A,B,C | A,B | A,B | A,B |
| Redfish Lake trawl | U | A,B | A,B | A,B,C | A,B | A,B | A,B |
| Stanley Lake spawners in Stanley Lake Creek | K | B | A | A,B,C | A | A,B,C | A,B |
| Stanley Lake trawl | U | B | A | B,C | A | A | A,B |
| **Other Idaho** | | | | | | | |
| Anderson Ranch Reservoir | K | B | A | A,B | A | A,C | A,B |
| Deadwood Reservoir | K | B | A | A,B | A | A | A,B |
| Dworshak Reservoir spawners in Isabella Creek | K | A,B | A | A,B | A | A | A,B |
| Payette Lake spawners in N. Fk Payette River | K | A,B | A | A,B | A | A | A,B |
| Warm Lake 1990 | K | B | A | A,C,E | A | A,C | A |
| Warm Lake 1992 post-stocking | K | A,B | A | A,B | A | A | A,B |

Appendix C continued. Summary of simple haplotypes by gene region and restriction endonuclease for each population.

| POPULATION | FORM | Simple Haplotypes by Gene region and *Restriction endonuclease* | | | | | |
|---|---|---|---|---|---|---|---|
| | | CYT B *Hae III* | ND 1 *Dpn II* | ND 1 *Hae III* | ND 1 *Rsa I* | ND 2 *Hae III* | ND 5/6 *Taq I* |
| **WASHINGTON** | | | | | | | |
| Baker Lake | S | **B** | **A** | **A** | **A** | A,C | **A** |
| Lake Ozette, early and late combined | S | **B** | **A** | A,B,C | **A** | **A** | A,B |
| Ozette early run | S | **B** | **A** | A,C | **A** | **A** | **A** |
| Ozette late run | S | **B** | **A** | A,B | **A** | **A** | A,B |
| Lake Quinault | S | **B** | A,D | **A** | **A** | **A** | **A** |
| Lake Sutherland | K | **B** | **A** | A,B | **A** | A,D | A,B |
| Lake Wenatchee, 1990 and 1996 combined | S | A,B | **A** | A,B | **A** | A,B,C | A,B |
| 1990 | S | A,B | **A** | A,B | **A** | A,B | A,B |
| 1996 | S | **B** | **A** | A,B | **A** | A,B,C | A,B |
| Lake Whatcom | K | **B** | **A** | A,B | **A** | A,C | A,B |
| **OREGON** | | | | | | | |
| Deschutes River @ Pelton Dam fish trap | S | **B** | **A** | A,B | **A** | A,B,C | A,B |
| L. Billy Chinook spawners @ Gorge Campground | K | **B** | **A** | A,B | **A** | A,B,C | A,B |
| Wizard Falls Hatchery juveniles | K | A,B | **A** | A,B | **A** | A,B,C | A,B |
| Wallowa Lake gillnet | K | A,B | **A** | A,B,C | **A** | A,C | A,B |
| Wallowa Lake spawners in Wallowa River | K | **B** | **A** | A,B,C | A,B | A,C | A,B |
| **BRITISH COLUMBIA** | | | | | | | |
| **Upper Columbia River Drainage** | | | | | | | |
| Okanogan Lake shorespawners @ Squally Point | K | A,B | **A** | A,B | A,B,C | A,C | A,B |
| Okanogan Lake spawners in Peachland Creek | K | **B** | **A** | A,B | **A** | A,C | A,B |
| Okanogan River anadromous adults | S | **B** | **A** | A,B,C | **A** | A,B | A,B |
| **Fraser River Drainage** | | | | | | | |
| Horsefly River anadromous adults | S | **B** | **A** | A,B | **A** | A,C | A,B |
| Shuswap Lake spawners in: | | | | | | | |
| Eagle River @ mouth of Perry Creek | K | **B** | **A** | A,B | **A** | A,C | A,B |
| Eagle River @ mouth of Perry Creek | S | **B** | **A** | A,B | **A** | A,C | A,B |
| M. Shuswap R. side channel below Bessette Crk | K | **B** | **A** | A,B | **A** | A,C | A,B |
| M. Shuswap R. in Bessette Creek | S | **B** | A,C | A,B | **A** | A,C | A,B |
| **Skeena River Drainage** | | | | | | | |
| Babine Lake spawners in Pierre Creek | K | **B** | **A** | A,C | **A** | A,C | **A** |
| Babine Lake spawners in Pierre Creek | S | **B** | **A** | A,C | **A** | A,C | **A** |

Appendix D. Composite haplotypes and their simple haplotype digest scores sorted by occurrence (number of samples).

| Composite haplotype | Simple hapotype digest scores | Number of samples | Frequency of haplotype overall | Number of populations containing haplotype |
|---|---|---|---|---|
| *Composite haplotypes occurring in fewer than 10 samples* | | | | |
| H03 | AAAABB | 1 | 0.0006 | 1 |
| H06 | AABCAB | 1 | 0.0006 | 1 |
| H12 | BAAACB | 1 | 0.0006 | 1 |
| H15 | BAABBB | 1 | 0.0006 | 1 |
| H16 | BAABCA | 1 | 0.0006 | 1 |
| H20 | BABACB | 1 | 0.0006 | 1 |
| H24 | BACACA | 1 | 0.0006 | 1 |
| H35 | BDAAAA | 1 | 0.0006 | 1 |
| H01 | AAAAAA | 2 | 0.0012 | 2 |
| H05 | AABAAB | 2 | 0.0012 | 1 |
| H10 | BAAABB | 2 | 0.0012 | 2 |
| H13 | BAAADA | 2 | 0.0012 | 1 |
| H22 | BACAAB | 2 | 0.0012 | 1 |
| H26 | BACBAB | 2 | 0.0012 | 1 |
| H28 | BAEAAA | 2 | 0.0012 | 1 |
| H30 | BBBAAA | 2 | 0.0012 | 2 |
| H32 | BBBABB | 2 | 0.0012 | 1 |
| H33 | BBCBAA | 2 | 0.0012 | 2 |
| H04 | AAAACA | 3 | 0.0017 | 3 |
| H27 | BACBBA | 3 | 0.0017 | 3 |
| H34 | BCBAAB | 3 | 0.0017 | 1 |
| H14 | BAABAA | 4 | 0.0023 | 3 |
| H19 | BABACA | 4 | 0.0023 | 2 |
| H23 | BACABA | 4 | 0.0023 | 3 |
| H29 | BBAAAB | 7 | 0.0041 | 3 |
| H17 | BABAAA | 9 | 0.0052 | 3 |
| *Composite haplotypes occurring in 10-100 samples* | | | | |
| H02 | AAAAAB | 25 | 0.0145 | 6 |
| H08 | BAAAAB | 29 | 0.0169 | 12 |
| H31 | BBBAAB | 57 | 0.0331 | 6 |
| *Composite haplotypes occurring in more than 100 samples* | | | | |
| H21 | BACAAA | 124 | 0.0721 | 16 |
| H07 | BAAAAA | 206 | 0.1198 | 27 |
| H25 | BACBAA | 237 | 0.1378 | 10 |
| H11 | BAAACA | 251 | 0.1459 | 21 |
| H18 | BABAAB | 278 | 0.1616 | 29 |
| H09 | BAAABA | 448 | 0.2605 | 15 |
| | | N=1720 | | |

Appendix E. Composite mtDNA haplotypes H1-H35 (Table 2) and their frequencies observed among 1720 *O. nerka* from 40 sample locations in the Pacific Northwest. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Population | Form | N | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 | H13 | H14 | H15 | H16 | H17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **IDAHO** | | | | | | | | | | | | | | | | | | | |
| **Northern Idaho** | | | | | | | | | | | | | | | | | | | |
| Lake Pend O'Reille | K | **32** | - | - | - | .0313 | - | - | - | - | - | - | .9375 | - | - | - | - | - | - |
| Lake Coeur d' Alene | K | **22** | - | - | - | - | - | - | - | - | - | - | .9545 | - | - | - | - | - | - |
| **Stanley Basin** | | | | | | | | | | | | | | | | | | | |
| Alturas Lake trawl | U | **54** | - | - | - | - | - | - | - | - | .4630 | .0185 | - | - | - | .0185 | - | - | .0370 |
| Alturas Lake spawners in Alturas L. Creek | K | **24** | - | - | - | - | - | - | - | - | .5833 | - | - | - | - | - | - | - | - |
| Alturas Lake outmigrants | U | **36** | - | - | - | - | - | - | - | - | .5556 | - | - | - | - | - | - | - | - |
| Pettit Lake trawl | K | **47** | - | - | - | - | - | - | - | - | - | - | .9574 | - | - | - | - | - | - |
| Redfish Lake anadromous adults | S | **262** | - | - | - | - | - | - | - | - | .7863 | - | - | - | - | - | - | - | - |
| Redfish Lake residuals | S | **22** | - | - | - | - | - | - | .0455 | - | .4545 | - | - | - | - | - | - | - | - |
| Redifish Lake outmigrants | U | **81** | - | - | - | - | - | - | .0123 | - | .4444 | - | - | - | - | .0247 | - | - | - |
| Redfish Lake spawners in Fishhook Creek | K | **81** | - | .1481 | - | - | - | - | - | - | .1852 | .0123 | - | - | - | .0123 | .0123 | - | - |
| Redfish Lake creel | U | **145** | - | .0345 | - | - | - | - | .0069 | .0138 | .3172 | - | - | - | - | - | - | - | - |
| Redfish Lake trawl | U | **93** | .0108 | .0215 | - | - | - | - | - | .0108 | .3548 | - | - | - | - | - | - | - | - |
| Stanley Lake spawners in Stanley Lake Creek | K | **33** | - | - | - | - | - | - | .2121 | - | .0303 | - | .1212 | - | - | - | - | - | - |
| Stanley Lake trawl | U | **28** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Other Idaho** | | | | | | | | | | | | | | | | | | | |
| Anderson Ranch Reservoir | K | **12** | - | - | - | - | - | - | - | - | - | - | .0833 | - | - | - | - | - | - |
| Deadwood Reservoir | K | **36** | - | - | - | - | - | - | .1389 | - | - | - | - | - | - | - | - | - | - |
| Dworshak Reservoir spawners in Isabella Cre | K | **37** | - | .0541 | - | - | .0541 | - | .2432 | - | - | - | - | - | - | - | - | - | - |
| Payette Lake spawners in N. Fk Payette Rive | K | **32** | - | .0938 | - | - | - | - | .2188 | - | - | - | - | - | - | - | - | - | - |
| Warm Lake | K | **33** | .0303 | - | - | - | - | - | .2121 | .0606 | - | - | .0606 | - | - | - | - | - | - |
| **WASHINGTON** | | | | | | | | | | | | | | | | | | | |
| Baker Lake | S | **16** | - | - | - | - | - | - | .8750 | - | - | - | .1250 | - | - | - | - | - | - |
| Lake Ozette | S | **37** | - | - | - | - | - | - | .7568 | .0270 | - | - | - | - | - | - | - | - | - |
| Lake Quinault | S | **14** | - | - | - | - | - | - | .9286 | - | - | - | - | - | - | - | - | - | - |
| Lake Sutherland | K | **20** | - | - | - | - | - | - | .2500 | .0500 | - | - | - | - | .1000 | - | - | - | .1500 |
| Lake Wenatchee | S | **50** | - | .0200 | .0200 | - | - | - | .0200 | .0200 | .5600 | - | .0200 | - | - | - | - | - | - |
| Lake Whatcom | K | **20** | - | - | - | - | - | - | .1000 | - | - | - | .8500 | - | - | - | - | - | - |
| **OREGON** | | | | | | | | | | | | | | | | | | | |
| Deschutes River @ Pelton Dam fish trap | S | **17** | - | - | - | - | - | - | .0588 | .2941 | .1765 | - | - | - | - | - | - | - | - |
| L. Billy Chinook spawners @ Gorge Campgr | K | **38** | - | - | - | - | - | - | .0263 | .1053 | .2105 | - | .0789 | - | - | - | - | - | - |
| Wizard Falls Hatchery juveniles | K | **53** | - | - | - | .0189 | - | - | - | .1509 | .0189 | - | .3208 | - | - | - | - | - | - |
| Wallowa Lake gillnet | K | **31** | - | - | - | .0323 | - | - | .0968 | .0323 | - | - | .3871 | - | | - | - | - | - |
| Wallowa Lake spawners in Wallowa River | K | **28** | - | - | - | - | - | - | .1071 | .0714 | - | - | - | .0357 | | - | - | - | .1429 |

Appendix E continued. Composite mtDNA haplotypes H1-H35 (Table 2) and their frequencies observed among 1720 *O. nerka* from 40 populations in the Pacific Northwest. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Population | Form | H18 | H19 | H20 | H21 | H22 | H23 | H24 | H25 | H26 | H27 | H28 | H29 | H30 | H31 | H32 | H33 | H34 | H35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **IDAHO** | | | | | | | | | | | | | | | | | | | |
| **Northern Idaho** | | | | | | | | | | | | | | | | | | | |
| Lake Pend O'Reille | K | .0313 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lake Coeur d' Alene | K | - | - | - | - | - | - | .0455 | - | - | - | - | - | - | - | - | - | - | - |
| **Stanley Basin** | | | | | | | | | | | | | | | | | | | |
| Alturas Lake trawl | U | .0370 | - | - | .0926 | - | - | - | .2778 | - | .0185 | - | - | .0185 | .0185 | - | - | - | - |
| Alturas Lake spawners in Alturas L. Creek | K | - | - | - | .0417 | - | - | - | .2083 | - | .0417 | - | - | - | .1250 | - | - | - | - |
| Alturas Lake outmigrants | U | - | - | - | .0278 | - | - | - | .3333 | - | - | - | .0278 | - | .0556 | - | - | - | - |
| Pettit Lake trawl | K | - | .0426 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Redfish Lake anadromous adults | S | - | - | - | .0038 | - | .0038 | - | .2023 | - | .0038 | - | - | - | - | - | - | - | - |
| Redfish Lake residuals | S | - | - | - | .3182 | - | .0455 | - | .1364 | - | - | - | - | - | - | - | - | - | - |
| Redifish Lake outmigrants | U | - | - | - | .0123 | - | .0247 | - | .4815 | - | - | - | - | - | - | - | - | - | - |
| Redfish Lake spawners in Fishhook Creek | K | .1358 | - | - | .0123 | - | - | - | .2963 | - | - | - | .0370 | - | .1358 | - | .0123 | - | - |
| Redfish Lake creel | U | .0483 | - | - | - | - | - | - | .3724 | - | - | - | .0207 | - | .1793 | - | .0069 | - | - |
| Redfish Lake trawl | U | .0645 | - | - | - | - | - | - | .3333 | .0215 | - | - | - | .0108 | .1505 | .0215 | - | - | - |
| Stanley Lake spawners in Stanley Lake Creek | K | .0909 | - | - | .5455 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Stanley Lake trawl | U | .1071 | - | - | .8929 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Other Idaho** | | | | | | | | | | | | | | | | | | | |
| Anderson Ranch Reservoir | K | .9167 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Deadwood Reservoir | K | .8611 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Dworshak Reservoir spawners in Isabella Cre | K | .6486 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Payette Lake spawners in N. Fk Payette Rivei | K | .6875 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Warm Lake | K | .2424 | - | - | .3333 | - | - | - | - | - | - | .0606 | - | - | - | - | - | - | - |
| **WASHINGTON** | | | | | | | | | | | | | | | | | | | |
| Baker Lake | S | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lake Ozette | S | .0541 | - | - | .1622 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lake Quinault | S | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | .0714 |
| Lake Sutherland | K | .4500 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lake Wenatchee | S | .3400 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lake Whatcom | K | .0500 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **OREGON** | | | | | | | | | | | | | | | | | | | |
| Deschutes River @ Pelton Dam fish trap | S | .4118 | - | .0588 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| L. Billy Chinook spawners @ Gorge Campgr | K | .5789 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Wizard Falls Hatchery juveniles | K | .4528 | .0377 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Wallowa Lake gillnet | K | .0968 | - | - | .3548 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Wallowa Lake spawners in Wallowa River | K | .2857 | - | - | .2500 | .0714 | - | - | .0357 | - | - | - | - | - | - | - | - | - | - |

Appendix E continued. Composite mtDNA haplotypes H1-H35 (Table 2) and their frequencies observed among 1720 *O. nerka* from 40 populations in the Pacific Northwest. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Population | Form | N | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 | H13 | H14 | H15 | H16 | H17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BRITISH COLUMBIA** | | | | | | | | | | | | | | | | | | | |
| **Upper Columbia River Drainage** | | | | | | | | | | | | | | | | | | | |
| Okanogan Lake shorespawners @ Squally Poi | K | **20** | - | - | - | - | - | .0500 | - | - | - | - | .6000 | - | - | - | - | .0500 | - |
| Okanogan Lake spawners in Peachland Creek | K | **17** | - | - | - | - | - | - | .0588 | - | - | - | .4706 | - | - | - | - | - | - |
| Okanogan River anadromous adults | S | **32** | - | - | - | - | - | - | .5938 | - | .0625 | - | - | - | - | - | - | - | - |
| **Fraser River Drainage** | | | | | | | | | | | | | | | | | | | |
| Horsefly River anadromous adults | S | **34** | - | - | - | - | - | - | .5588 | - | - | - | .0882 | - | - | - | - | - | - |
| Shuswap Lake spawners in: | | | | | | | | | | | | | | | | | | | |
| Eagle River @ mouth of Perry Creek | K | **31** | - | - | - | - | - | - | .1613 | .0323 | - | - | .5161 | - | - | - | - | - | - |
| Eagle River @ mouth of Perry Creek | S | **32** | - | - | - | - | - | - | .2500 | - | - | - | .5625 | - | - | - | - | - | - |
| M. Shuswap R. side channel | K | **28** | - | - | - | - | - | - | .3571 | - | - | - | .4286 | - | - | - | - | - | - |
| M. Shuswap R. in Bessette Creek | S | **30** | - | - | - | - | - | - | .5667 | - | - | - | .1667 | - | - | - | - | - | - |
| **Skeena River Drainage** | | | | | | | | | | | | | | | | | | | |
| Babine Lake spawners in Pierre Creek | K | **28** | - | - | - | - | - | - | .1786 | - | - | - | .6786 | - | - | - | - | - | - |
| Babine Lake spawners in Pierre Creek | S | **34** | - | - | - | - | - | - | .3824 | - | - | - | .0882 | - | - | - | - | - | - |
| **Total** | | **1720** | .0012 | .0145 | .0006 | .0017 | .0012 | .0006 | .1198 | .0169 | .2605 | .0012 | .1459 | .0006 | .0012 | .0023 | .0006 | .0006 | .0052 |

Appendix E continued. Composite mtDNA haplotypes H1-H35 (Table 2) and their frequencies observed among 1720 *O. nerka* from 40 populations in the Pacific Northwest. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Population | Form | H17 | H18 | H19 | H20 | H21 | H22 | H23 | H24 | H25 | H26 | H27 | H28 | H29 | H30 | H31 | H32 | H33 | H34 | H35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BRITISH COLUMBIA** | | | | | | | | | | | | | | | | | | | | |
| **Upper Columbia River Drainage** | | | | | | | | | | | | | | | | | | | | |
| Okanogan Lake shorespawners @ Squally Poi | K | - | .3000 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Okanogan Lake spawners in Peachland Creek | K | - | .4706 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Okanogan River anadromous adults | S | - | .1250 | - | - | .2188 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Fraser River Drainage** | | | | | | | | | | | | | | | | | | | | |
| Horsefly River anadromous adults | S | - | .3529 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Shuswap Lake spawners in: | | | | | | | | | | | | | | | | | | | | |
| Eagle River @ mouth of Perry Creek | K | - | .2903 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Eagle River @ mouth of Perry Creek | S | - | .1875 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M. Shuswap R. side channel | K | - | .2143 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M. Shuswap R. in Bessette Creek | S | - | .1667 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | .1000 | - |
| **Skeena River Drainage** | | | | | | | | | | | | | | | | | | | | |
| Babine Lake spawners in Pierre Creek | K | - | - | - | - | .1429 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Babine Lake spawners in Pierre Creek | S | - | - | - | - | .5294 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Total** | | .0052 | .1616 | .0023 | .0006 | .0721 | .0012 | .0023 | .0006 | .1378 | .0012 | .0017 | .0012 | .0041 | .0012 | .0331 | .0012 | .0012 | .0017 | .0006 |

Appendix F. Composite mtDNA haplotypes (H#, refer to Table 2) and their frequencies observed among 684 *O. nerka* from six Redfish Lake sample populations, separated by collection date. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Form | Population | N | H1 | H2 | H7 | H8 | H9 | H10 | H14 | H15 | H18 | H21 | H23 | H25 | H26 | H27 | H29 | H30 | H31 | H32 | H33 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Idaho - Redfish Lake** | | | | | | | | | | | | | | | | | | | | | |
| **S** | **Anadromous** | **262** | - | - | - | - | .7863 | - | - | - | - | .0038 | .0038 | .2023 | - | .0038 | - | - | - | - | - |
| | 1991 | 4 | - | - | - | - | .2500 | - | - | - | - | - | - | .7500 | - | - | - | - | - | - | - |
| | 1992 | 1 | - | - | - | - | 1.0000 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 1993 | 8 | - | - | - | - | .5000 | - | - | - | - | - | .1250 | .2500 | - | .1250 | - | - | - | - | - |
| | 1994 | 1 | - | - | - | - | - | - | - | - | - | - | - | 1.0000 | - | - | - | - | - | - | - |
| | 1996 | 1 | - | - | - | - | - | - | - | - | - | - | - | 1.0000 | - | - | - | - | - | - | - |
| | 1998 | 1 | - | - | - | - | 1.0000 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 1999* | 7 | - | - | - | - | .8571 | - | - | - | - | - | - | .1429 | - | - | - | - | - | - | - |
| | 2000* | 219 | - | - | - | - | .8356 | - | - | - | - | .0046 | - | .1598 | - | - | - | - | - | - | - |
| | 2001* | 20 | - | - | - | - | .5000 | - | - | - | - | - | - | .5000 | - | - | - | - | - | - | - |
| | 1991-1998 | 16 | - | - | - | - | 0.4375 | - | - | - | - | - | .0625 | .4375 | - | .0625 | - | - | - | - | - |
| | 1999-2001* | 246 | - | - | - | - | 0.8089 | - | - | - | - | .0041 | - | .1870 | - | - | - | - | - | - | - |
| **S** | **Residuals** | **22** | - | - | .0455 | - | .4545 | - | - | - | - | .3182 | .0455 | .1364 | - | - | - | - | - | - | - |
| | 1992 | 5 | - | - | - | - | .6000 | - | - | - | - | - | .2000 | .2000 | - | - | - | - | - | - | - |
| | 1993 | 14 | - | - | .0714 | - | .3571 | - | - | - | - | .5000 | - | .0714 | - | - | - | - | - | - | - |
| | 1995 | 3 | - | - | - | - | .6667 | - | - | - | - | - | - | .3333 | - | - | - | - | - | - | - |
| **S** | **Outmigrants** | **81** | - | - | .0123 | - | .4444 | - | .0247 | - | - | .0123 | .0247 | .4815 | - | - | - | - | - | - | - |
| | 1991 | 49 | - | - | .0204 | - | .4286 | - | .0408 | - | - | .0204 | .0408 | .4490 | - | - | - | - | - | - | - |
| | 1992 | 11 | - | - | - | - | .6364 | - | - | - | - | - | - | .3636 | - | - | - | - | - | - | - |
| | 1993 | 21 | - | - | - | - | .3810 | - | - | - | - | - | - | .6190 | - | - | - | - | - | - | - |
| **K** | **Fishhook Creek** | **81** | - | .1481 | - | - | .1852 | .0123 | .0123 | .0123 | .1358 | .0123 | - | .2963 | - | - | .0370 | - | .1358 | - | .0123 |
| | Sep. 9, 1990 | 22 | - | .2727 | - | - | .1364 | .0455 | .0455 | .0455 | .1364 | - | - | .0909 | - | - | - | - | .2273 | - | - |
| | Aug. 22, 1991 | 15 | - | .0667 | - | - | .2000 | - | - | - | .2000 | - | - | .3333 | - | - | - | - | .1333 | - | .0667 |
| | Sep. 5, 1991 | 13 | - | .1538 | - | - | .1538 | - | - | - | .1538 | - | - | .4615 | - | - | - | - | .0769 | - | - |
| | Aug. 14, 1992 | 16 | - | .0625 | - | - | .3750 | - | - | - | .0625 | .0625 | - | .3125 | - | - | - | - | .1250 | - | - |
| | Sep. 8, 1992 | 3 | - | - | - | - | - | - | - | - | .6667 | - | - | .3333 | - | - | - | - | - | - | - |
| | Sep. 1, 1995 | 11 | - | .1818 | - | - | .0909 | - | - | - | - | - | - | .4545 | - | - | .2727 | - | - | - | - |
| | Sep. 5, 1996 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.0000 | - | - |
| | Early: Aug 14-22 | 31 | - | .0645 | - | - | .2903 | - | - | - | .1290 | .0323 | - | .3226 | - | - | - | - | .1290 | - | .0323 |
| | Late: Sep 1-9 | 50 | - | .2000 | - | - | .1200 | .0200 | .0200 | .0200 | .1400 | - | - | .2800 | - | - | .0600 | - | .1400 | - | - |
| **U** | **Creel survey** | **145** | - | .0345 | .0069 | .0138 | .3172 | - | - | - | .0483 | - | - | .3724 | - | - | .0207 | - | .1793 | - | .0069 |
| | Summer 1996** | 42 | - | .0714 | .0238 | - | .2857 | - | - | - | .0238 | - | - | .3333 | - | - | .0238 | - | .2143 | - | .0238 |
| | Summer 1997** | 37 | - | - | - | - | .2432 | - | - | - | .0811 | - | - | .4865 | - | - | .0270 | - | .1622 | - | - |
| | Summer 1998** | 25 | - | .0400 | - | - | .3600 | - | - | - | .0800 | - | - | .4000 | - | - | - | - | .1200 | - | - |
| | Summer 1999** | 41 | - | .0244 | - | .0488 | .3902 | - | - | - | .0244 | - | - | .2927 | - | - | .0244 | - | .1951 | - | - |

Appendix F continued.  Composite mtDNA haplotypes (H#, refer to Table 2) and their frequencies observed among 684 *O. nerka* from six Redfish Lake sample populations, separated by collection date.  Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Form | Population | N | H1 | H2 | H7 | H8 | H9 | H10 | H14 | H15 | H18 | H21 | H23 | H25 | H26 | H27 | H29 | H30 | H31 | H32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **U** | **Trawl survey** | **93** | .0108 | .0215 | - | .0108 | .3548 | - | - | - | .0645 | - | - | .3333 | .0215 | - | - | .0108 | .1505 | .0215 |
| | Aug. 20, 1990 | 8 | - | - | - | - | - | - | - | - | .1250 | - | - | .5000 | - | - | - | - | .3750 | - |
| | Sep. 6, 1994 | 9 | - | - | - | .1111 | .3333 | - | - | - | - | - | - | .5556 | - | - | - | - | - | - |
| | Sep. 26, 1995 | 8 | - | - | - | - | .5000 | - | - | - | - | - | - | .5000 | - | - | - | - | - | - |
| | Sep. 10, 1996 | 45 | .0222 | - | - | - | .3778 | - | - | - | .0667 | - | - | .2222 | .0444 | - | - | .0222 | .2000 | .0444 |
| | Sep. 2, 1997 | 23 | - | .0870 | - | - | .3913 | - | - | - | .0870 | - | - | .3478 | - | - | - | - | .0870 | - |
| | Total | **684** | .0015 | .0278 | .0044 | .0044 | .5058 | .0015 | .0044 | .0015 | .0351 | .0146 | .0058 | .2982 | .0029 | .0015 | .0088 | .0015 | .0746 | .0029 |

*Returns from the captive program

**Includes stocking from the captive program

Appendix G. Composite mtDNA haplotypes (H#, refer to Table 2) and their frequencies observed among *O. nerka* from Idaho and Washington sample populations, separated by collection date. Form types are resident kokanee (K), anadromous sockeye or residual (S), and unknown (U).

| Form | Population | N | H1 | H2 | H3 | H7 | H8 | H9 | H10 | H11 | H14 | H17 | H18 | H19 | H21 | H25 | H27 | H28 | H29 | H30 | H31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Idaho - Stanley Basin** | | | | | | | | | | | | | | | | | | | | | |
| U | Alturas Lake trawl | **54** | - | - | - | - | - | .4630 | .0185 | - | .0185 | .0370 | .0370 | - | .0926 | .2778 | .0185 | - | - | .0185 | .0185 |
| | Aug. 19, 1990 | 21 | - | - | - | - | - | .4286 | .0476 | - | - | .0952 | - | - | .0952 | .2381 | .0476 | - | - | - | .0476 |
| | Sep. 7, 1994 | 12 | - | - | - | - | - | .5833 | - | - | - | - | .1667 | - | .1667 | .0833 | - | - | - | - | - |
| | Sep. 25, 1995 | 21 | - | - | - | - | - | .4286 | - | - | .0476 | - | - | - | .0476 | .4286 | - | - | - | .0476 | - |
| U | Alturas Lake Outmigrants | **36** | - | - | - | - | - | .5556 | - | - | - | - | - | - | .0278 | .3333 | - | - | .0278 | - | .0556 |
| | 1991 | 19 | - | - | - | - | - | .5263 | - | - | - | - | - | - | .0526 | .3684 | - | - | .0526 | - | - |
| | 1992 | 17 | - | - | - | - | - | .5882 | - | - | - | - | - | - | - | .2941 | - | - | - | - | .1176 |
| K | Pettit Lake trawl | **47** | - | - | - | - | - | - | - | .9574 | - | - | - | .0426 | - | - | - | - | - | - | - |
| | Sep. 18, 1993 | 13 | - | - | - | - | - | - | - | .8462 | - | - | - | .1538 | - | - | - | - | - | - | - |
| | Sep. 8, 1994 | 15 | - | - | - | - | - | - | - | 1.0000 | - | - | - | - | - | - | - | - | - | - | - |
| | Sep. 25, 1995 | 19 | - | - | - | - | - | - | - | 1.0000 | - | - | - | - | - | - | - | - | - | - | - |
| K | Stanley L. (Crk. spawners) | **33** | - | - | - | .2121 | - | .0303 | - | .1212 | - | - | .0909 | - | .5455 | - | - | - | - | - | - |
| | "early" Aug. 20, 1992 | 25 | - | - | - | .2800 | - | - | - | - | - | - | - | - | .7200 | - | - | - | - | - | - |
| | "late" Oct. 1994 | 5 | - | - | - | - | - | .2000 | - | .6000 | - | - | .2000 | - | - | - | - | - | - | - | - |
| | "late" Nov. 1994 | 3 | - | - | - | - | - | - | - | .3333 | - | - | .6667 | - | - | - | - | - | - | - | - |
| K | Stanley Lake trawl | **28** | - | - | - | - | - | - | - | - | - | - | .1071 | - | .8929 | - | - | - | - | - | - |
| | Sep. 6, 1994 | 18 | - | - | - | - | - | - | - | - | - | - | - | - | 1.0000 | - | - | - | - | - | - |
| | Sep. 27, 1995 | 10 | - | - | - | - | - | - | - | - | - | - | .3000 | - | .7000 | - | - | - | - | - | - |
| **Idaho - Other** | | | | | | | | | | | | | | | | | | | | | |
| K | Deadwood | **36** | - | - | - | .1389 | - | - | - | - | - | - | .8611 | - | - | - | - | - | - | - | - |
| | Sep. 8., 1990 | 16 | - | - | - | .1250 | - | - | - | - | - | - | .8750 | - | - | - | - | - | - | - | - |
| | Sep. 17, 1992 | 20 | - | - | - | .1500 | - | - | - | - | - | - | .8500 | - | - | - | - | - | - | - | - |
| K | Payette (River spawners) | **32** | - | .0938 | - | .2188 | - | - | - | - | - | - | .6875 | - | - | - | - | - | - | - | - |
| | Sep. 7, 1990 | 16 | - | .0625 | - | .1875 | - | - | - | - | - | - | .7500 | - | - | - | - | - | - | - | - |
| | Sep.18, 1992 | 16 | - | .1250 | - | .2500 | - | - | - | - | - | - | .6250 | - | - | - | - | - | - | - | - |
| K | Warm Lake | **33** | .0303 | - | - | .2121 | .0606 | - | - | .0606 | - | - | .2424 | - | .3333 | - | - | .0606 | - | - | - |
| | Oct. 26, 1990 | 16 | - | - | - | .0625 | - | - | - | .1250 | - | - | - | - | .6875 | - | - | .1250 | - | - | - |
| | post-stocking Sep. 12, 1992 | 17 | .0588 | - | - | .3529 | .1176 | - | - | - | - | - | .4706 | - | - | - | - | - | - | - | - |
| **Washington** | | | | | | | | | | | | | | | | | | | | | |
| S | Lake Ozette | **37** | - | - | - | .7568 | .0270 | - | - | - | - | - | .0541 | - | .1622 | - | - | - | - | - | - |
| | early | 20 | - | - | - | .7000 | - | - | - | - | - | - | - | - | .3000 | - | - | - | - | - | - |
| | late | 17 | - | - | - | .8235 | .0588 | - | - | - | - | - | .1176 | - | - | - | - | - | - | - | - |
| S | Lake Wenatchee | **50** | - | .0200 | .0200 | .0200 | .0200 | .5600 | - | .0200 | - | - | .3400 | - | - | - | - | - | - | - | - |
| | Fall 1990 | 32 | - | .0313 | .0313 | .0313 | .0313 | .5313 | - | - | - | - | .3438 | - | - | - | - | - | - | - | - |
| | Sep. 25, 1996 | 18 | - | - | - | - | - | .6111 | - | .0556 | - | - | .3333 | - | - | - | - | - | - | - | - |