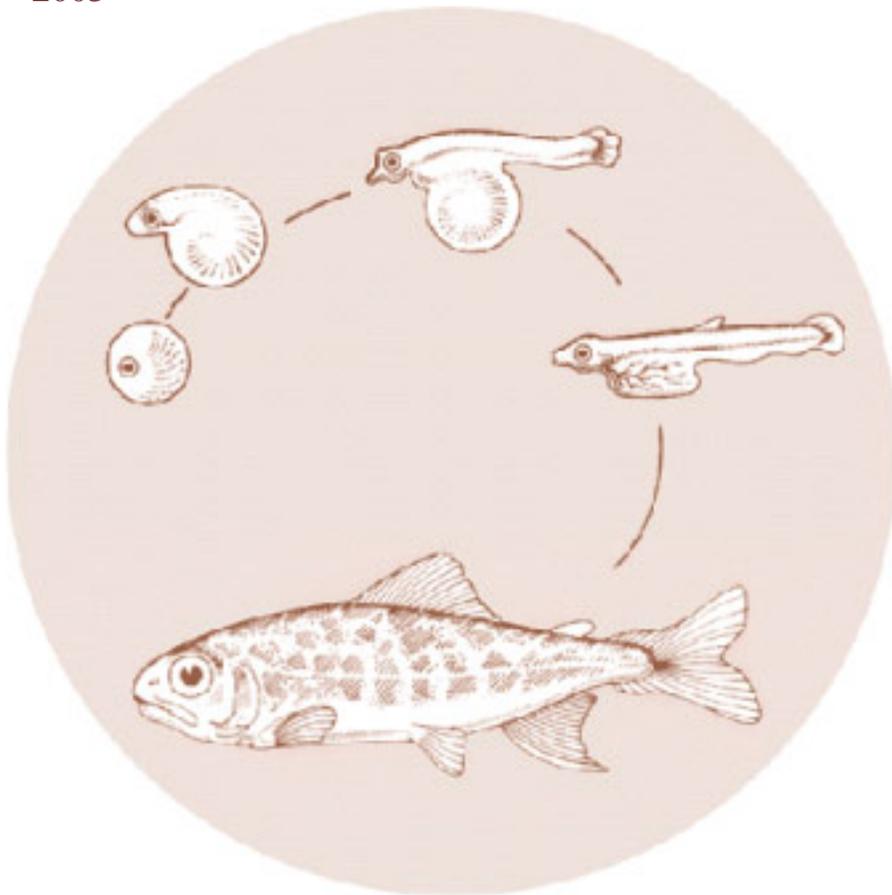


Salmonid Gamete Preservation in the Snake River Basin

Annual Report
2003



This Document should be cited as follows:

Young, William, Paul Kucera, "Salmonid Gamete Preservation in the Snake River Basin", 2003 Annual Report, Project No. 199703800, 79 electronic pages, (BPA Report DOE/BP-00004000-3)

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This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

**SALMONID GAMETE PRESERVATION
IN THE SNAKE RIVER BASIN**

2003 Annual Report



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May 2004

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Bonneville Power Administration
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P.O. Box 3621
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Project Number 97-03800
Contract Number 00004000

and

U.S. Fish and Wildlife Service
Lower Snake River Compensation Plan
1387 South Vinnell Way, Suite 343
Boise, Idaho 83709
Cooperative Agreement Number 141101J005

May 2004

ABSTRACT

In spite of an intensive management effort, chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) populations in the Northwest have not recovered and are currently listed as threatened species under the Endangered Species Act. In addition to the loss of diversity from stocks that have already gone extinct, decreased genetic diversity resulting from genetic drift and inbreeding is a major concern. Reduced population and genetic variability diminishes the environmental adaptability of individual species and entire ecological communities. The Nez Perce Tribe (NPT), in cooperation with Washington State University (WSU) and the University of Idaho (UI), established a germplasm repository in 1992 in order to preserve the remaining salmonid diversity in the region.

The germplasm repository provides long-term storage for cryopreserved gametes. Although only male gametes can be cryopreserved, conserving the male component of genetic diversity will maintain future management options for species recovery. NPT efforts have focused on preserving salmon and steelhead gametes from the major river subbasins in the Snake River basin. However, the repository is available for all management agencies to contribute gamete samples from other regions and species.

In 2003 a total of 358 viable semen samples were collected by NPT and added to the germplasm repository. This included the gametes from 268 male chinook salmon from the Lostine River, Catherine Creek, upper Grande Ronde River, Imnaha River (Lookingglass Hatchery), Lake Creek, South Fork Salmon River, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, Pahsimeroi River (Pahsimeroi Hatchery), and upper Salmon River (Sawtooth Hatchery) and the gametes from 90 male steelhead from the Little Sheep Creek, Cow Creek (Imnaha River tributary), Lightning Creek (Imnaha River tributary) and South Fork Salmon River. In addition, the Columbia River Intertribal Fish Commission contributed male gametes from 30 Wenatchee River coho salmon. To date, a total of 4,316 Columbia River male salmon and steelhead gamete samples and three Kootenai River white male sturgeon gametes are preserved in the repository. Samples are stored in independent locations at the UI and WSU.

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INTRODUCTION

The goals of genetic conservation are to reduce the possibility of extinction and ensure the recovery of a species as a functioning ecological unit of the environment. While preventative actions for conserving species such as population monitoring, habitat protection and enhancement and harvest controls are preferred, these measures frequently are not implemented until populations have reached critically low levels. Once this occurs, conservation strategies using artificial environments such as zoos, botanical gardens and live or frozen gene banks are often required (Bartley 1998). Although it is often difficult to decide when to use the more intensive actions, measures aimed at conserving the genetic diversity of a species should be implemented prior to a severe population collapse. Therefore, once a species threatened by a population collapse is identified, a combination of preventative and intensive measures should begin in order to prevent further loss of genetic diversity and preserve long term evolutionary potential.

Nehlsen et al. (1991) concluded that least 106 major populations of salmon and steelhead on the west coast of the United States are extinct, and an additional 214 salmon, steelhead, and sea-run cutthroat trout stocks are at risk of extinction. This suggests that without recovery a complete collapse of the anthropomorphic and ecological communities dependent on anadromous salmonids may occur. As a first step in the recovery of anadromous fish stocks, National Oceanographic and Atmospheric Administration Fisheries (NOAAF) listed 39 salmonid populations as threatened or endangered under the Endangered Species Act (ESA). Included in this list are all of the remaining wild populations of spring/summer and fall chinook salmon and steelhead in the Snake River basin. These populations warrant protection because they possess unique genetic and life history attributes of the species and thus represent distinct population segments.

The recovery effort for these species has mainly focused on habitat protection and enhancement, hatchery construction, harvest controls, fish barging, and 'fish-friendly' changes in dam operation. Although these measures have been in place for decades, many populations continue to decline. Recently more intensive practices such as supplementation and captive brood rearing have begun. As opposed to conventional hatcheries, these programs utilize local stocks and attempt to minimize selection during all aspects of their life history. Although it is too early to judge the success of these programs, the one thing that has been recognized is the importance of using local stocks for recovery. It is believed that natural selection created highly adapted stocks (Corley-Smith and Brandhorst 1999) and the use of these local stocks will maximize the success of the program.

The threat of a significant loss of genetic diversity in native fish stocks warrants the establishment of gene banks for the long-term storage of fish germplasm. Preserving genetic material serves as insurance against population collapse and extirpation and provides options for future management programs by providing an opportunity for rebuilding lost stocks or maintaining genetic diversity caused by population bottlenecks (Ryder et al. 2000). At present, cryopreservation of male gametes is the only means of storing fish germplasm for extended periods of time. It was estimated that the storage time for fish semen held in liquid nitrogen are between 200 and 32,000 years (Ashwood-Smith 1980; Whittingham 1980; and Stoss 1983).

Although preservation of the maternal nuclear DNA component has been accomplished with some mammals (Rall and Fahy 1985, Fahning and Garcia 1992, Dobrinsky et al. 1991, Ali and Shelton 1993, Kono et al. 1988, Trounson and Mohr 1983, Hayashi et al. 1989), it has not been accomplished with fish. Successful development of methods to preserve female gametes is an active area of research and would greatly increase the ability to recover extinct salmonid stocks.

NPT initiated chinook salmon cryopreservation activities in 1992 (Kucera and Blenden 1999) in response to the severely reduced returns of adult chinook salmon in Big Creek (a tributary of the Middle Fork Salmon River). In subsequent years, a more comprehensive gene banking effort was initiated (Faurot et al. 1998) including collections from additional chinook spawning aggregates in the Snake River basin and collections from steelhead populations in the region (Armstrong and Kucera 1999). By collecting from numerous populations of spring and summer chinook salmon and steelhead across the entire Snake River basin, we hope to preserve the greatest amount of endemic salmonid diversity. Some of this diversity is reflected by the variable size, migration and spawning timing and age structure found in different populations of these fish. For example, adult chinook salmon migrating upstream past Bonneville Dam from March through May, and June through July are categorized as spring- and summer-run fish respectively (Burner 1951). Some streams in the Snake River are considered to have only spring chinook, some mainly summer-run fish (e.g., those in the South Fork Salmon River), and some both forms (e.g., Middle Fork Salmon River and upper Salmon River). In most cases where the two forms coexist, spring-run fish spawn earlier and in the headwaters of the tributaries, whereas summer chinook spawn later and farther downstream (Matthews and Waples 1991).

Snake River basin steelhead spawning areas are well isolated from other populations and include the highest elevations for spawning (up to 2,000 meters) as well as the longest migration distance from the ocean (up to 1,500 kilometers; Busby et al. 1996). Steelhead from the Snake River basin can be categorized into two major groups known as A-run and B-run fish. The A-run group passes Bonneville Dam (Columbia River kilometer 235) before August 25 and the B-run group pass Bonneville after August 25 (CBFWA 1990, IDFG 1994). A-run steelhead are defined as predominately one ocean fish, while B-run steelhead are defined as two ocean (IDFG 1994). B-run steelhead tend to be larger, averaging 11-15 pounds (or 5-7 kilograms) with maximum size up to 35 pounds (or 16 kilograms).

This annual report details NPT germplasm preservation activities from 2003 and updates the status of the long-term repository. Goals of the cryopreservation project are: 1) preserve the genetic diversity of listed salmonid populations at high risk of extirpation through application of cryogenic techniques, 2) maintain gene bank locations at independent sites for the short-term, and 3) establish and maintain a long-term regional germplasm repository.

METHODS

Description of Spawning Aggregates

The cryopreservation project managed by NPT currently seeks to preserve male spring and summer chinook salmon and steelhead gametes in the Snake River basin (Figure 1). The large number of subbasins within this region has resulted in a genetically diverse collection of anadromous species. The following is a list of the sub-basins and locations that were sampled in 2003.

CHINOOK SALMON

Grande Ronde River Subbasin

1. Catherine Creek
2. Upper Grande Ronde River

Salmon River Subbasin

1. Lake Creek
2. Johnson Creek
3. Marsh Creek
4. Capehorn Creek
5. Big Creek
6. South Fork Salmon River Trap – McCall Fish Hatchery
7. Upper Salmon River – Sawtooth Fish Hatchery
8. Pahsimeroi River – Pahsimeroi Fish Hatchery

Imnaha River Subbasin

1. Imnaha River – Lookingglass Hatchery

STEELHEAD

Salmon River Subbasin

1. South Fork Salmon River

Imnaha River Subbasin

1. Little Sheep Creek
2. Cow Creek
3. Lightning Creek

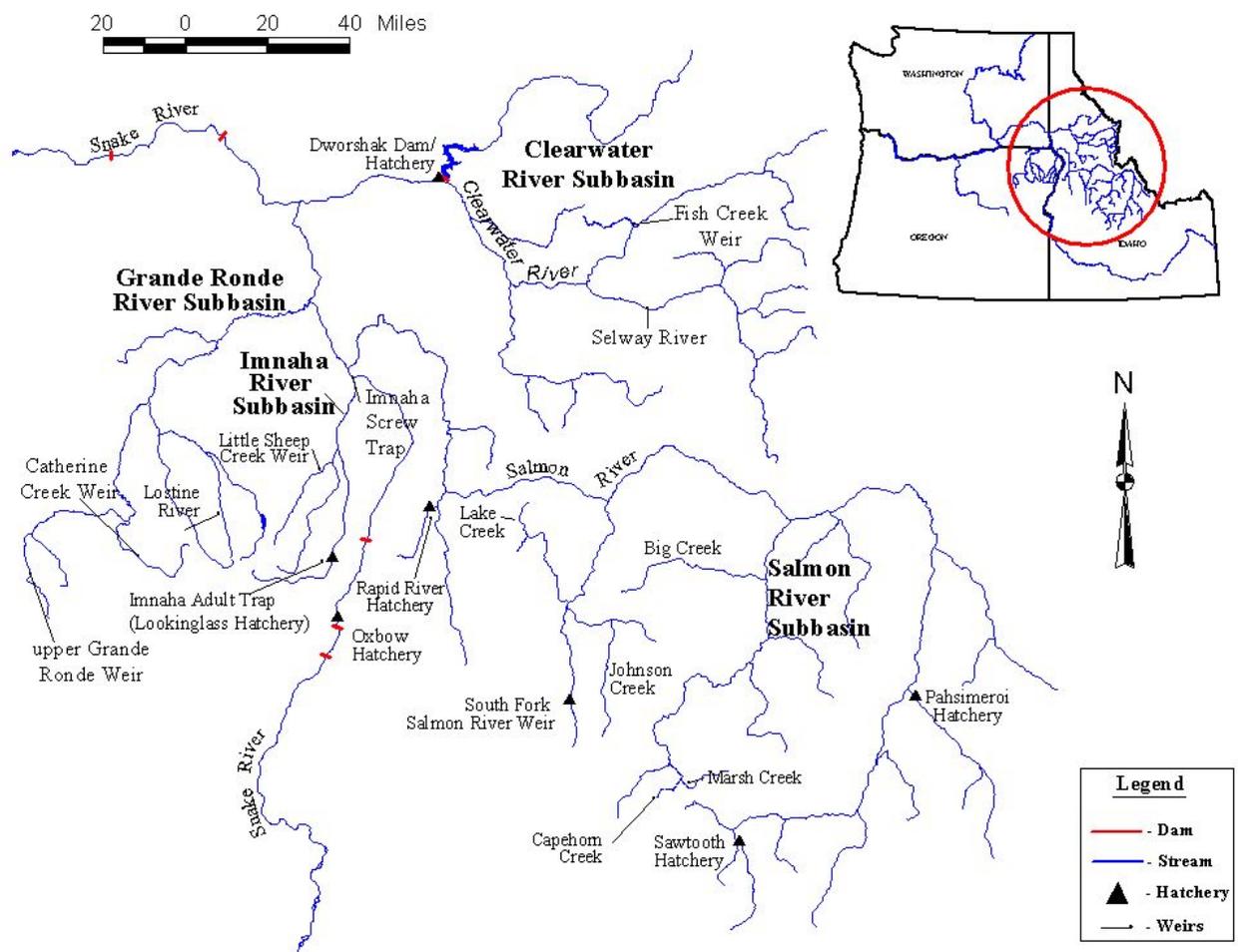


Figure 1. Map showing the Snake River basin chinook salmon and steelhead sampling locations for 2003.

Fish Collection and Handling

Chinook salmon spawning ground surveys were usually conducted on pre-determined stream reaches before handling any fish. Redd counts also determined where in each stream the collection of adult males would be most effective. Several team members located adults and visually identified male salmon, being careful not to disturb the fish. Actively spawning females and males paired with females were avoided so as not to disrupt spawning. Males were identified by secondary sexual characteristics such as a kype (greatly extended, narrowed snout, turned down at tip, also an enlarged lower jaw), large teeth, and a slim caudal peduncle that is not as worn as the female salmon. Personnel were instructed to stay away from any existing or active redds (i.e. where salmon are on the nests). A snorkeler entered the water to find solitary

males, looking under cut banks, in logjams, in backwater habitats, etc. From the vantage point underwater, this person identified fish for others to collect. Inadvertently caught females were immediately released from the net without ever being out of the water and the capture was recorded.

All adult male salmon were collected by hand or dip net in that order of preference:

Hand. Walk or swim up to the identified fish and grasp the fish at caudal peduncle, put the fish into a dip net immediately. Always keep the fish in the water, pointing upstream, until ready to place in the tank.

Dip net. Staying away from active redds, several dip netters get into position below the fish, with several people in the water upstream of the fish. The upstream people slowly herd fish towards the netters. Keep the large dip nets in the water in a line and let fish swim into the net.

Captured fish were held in the stream while a portable tank was set up along the stream. Fish were immobilized using anesthetic so they could be handled faster and less stressfully. The anesthesia was delivered by placing the fish in a portable tank filled with 135 liters of water containing 90 mg/l of tricaine methanesulfonate (MS-222, Fiquel™) anesthesia and sodium bicarbonate (NaHCO_3) to buffer the acidity of the MS-222. The fish was constantly monitored while in the tank and the time to sedation was noted. The sedated fish was rinsed in the fresh water of the stream and the abdomen dried to reduce water contamination prior to collecting the milt. Milt was collected in a plastic Whirl Pak bag by gently squeezing the abdomen (Figure 2)



Figure 2. Collecting chinook salmon milt from anaesthetized fish at Big Creek.

General biological information such as fork length, mid-eye to hypural plate length, general condition and external marks were recorded following semen collection (Figure 3). Caudal fin tissue was collected and preserved in ethyl alcohol for later genetic (DNA) analysis and scales were taken for age assessment and scale pattern analysis. Stream water was gently poured over the salmon's head and gills to start the recovery from the MS-222 and reduce stress on the fish while this information was collected. Following sampling and data collection, the anesthetized salmon were immediately returned to a slow water area and assisted until it fully recovered. After the fish is released into the stream, the tank was emptied well away from the stream to prevent the release of chemicals into the stream proper.

Spring/summer chinook salmon gametes were also collected at weirs and hatchery traps. Fish were either anesthetized by personnel working the traps or euthanased following their use in the cross matrix. Milt was then collected using the standard protocol (see above).



Figure 3. Anaesthetized male chinook salmon on portable tank for measurements.

The brood year of each sampled fish was determined initially using length data and will be modified following scale analyses if the scales provide a better estimate of age. We used the following length age relationship to determine the ages of chinook salmon: <66 cm - age 3, 66-90 cm - age 4 and >90 cm – age 5.

In 2003 we obtained an ESA section 10 permit to capture adult steelhead males by angling. The permit states that we were limited to artificial lures and barbless hooks. The preferred method involved locating male steelhead away from active redds and targeting these fish. At other times we fished deep holding water. Once hooked, fish were brought in as rapidly as possible, netted and held in the water until the anesthesia tank was set up. Sperm was taken as described for chinook salmon above. The fish were measured (fork length) and a tissue sample was taken for DNA analysis. Fish were revived by holding them in the current until they swam away. We used the following length age relationship to determine the ages of steelhead collected from the Imnaha River subbasin (Little Sheep, Cow and Lightning Creeks): <64 cm - age 3 and > 64 cm – age 4. We used the following length age relationship to determine the ages of steelhead collected from the South Fork Salmon River (B-run steelhead; data from Dworshak National Fish Hatchery): <72 cm – age 3, 72 – 93 cm – age 4 and >93 cm – age 5.

Semen Handling and Cryopreservation

The amount of semen obtained varied greatly by individual fish and by species. Chinook salmon produced greater volumes of milt (averaging > 5 ml), whereas steelhead produced less (average 2-4 ml). If greater than approximately 5 ml of semen were collected then the sample is separated into equal aliquots and poured into two separately labeled Whirl Pak bags so the sample can be sent to two independent locations for freezing. The bags are aerated using a foot pump then placed in an insulated cooler containing wet ice. Because it is critical to avoid placing the samples directly on the ice, newspaper was placed over the ice to insulate the samples.

Semen samples were shipped to, cryopreserved and stored at both WSU and the UI within 12 hours of collection. Sperm quality was determined by estimating the percentage of motile sperm following the addition of a sperm activating solution (Mounib 1978). Samples were frozen in 0.5 ml French straws (IMV International, Minneapolis, Minnesota). Samples were stored in large cryopreservation tanks under liquid nitrogen (Figure 4).

Fertilization experiments

Fertilization experiments were conducted at WSU in order to evaluate the viability of the gametes contained in the gene bank. Eggs from a single female chinook salmon were divided into equal lots and fertilized with semen from 6 cryopreserved and 3 fresh semen samples. Eggs and fresh semen were collected at Pahsimeroi Hatchery summer chinook salmon. Cryopreserved semen was obtained by thawing a single 0.5ml straw from six fish. Both the absolute and relative fertility were calculated for the cryopreserved samples. The relative fertility was calculated by dividing the average fertility from the three fresh samples by the absolute fertility from the cryopreserved samples. This calculation removed the influence of egg quality and provided an estimate of the fertilization potential of the cryopreserved samples. UI did not perform fertility trials in 2003.



Figure 4. Example of a liquid nitrogen tank used to store chinook salmon and steelhead gametes.

RESULTS

The chinook salmon and steelhead spawning aggregates and hatcheries in the Snake River basin where gametes were collected in 2003 have a diverse history of transfers, stocking, and straying. It is important to understand how the history of broodstock development, management and stocking has influenced the samples in the gene bank. A detailed description of the spawning aggregates sampled for cryopreservation can be found in Armstrong and Kucera (2001).

Gametes from 268 male chinook salmon (Table 1) were collected and cryopreserved in 2003. Collections occurred over a two-month period from August 6, 2003 to September 22, 2003. The majority of the samples, 234, were collected from unmarked, natural fish. Eleven females were accidentally captured and immediately released. Motility of the sperm ranged from 0 – 100%.

Gametes from 90 male steelhead (Table 2) were collected and cryopreserved in 2003. Collections occurred over a four-month period from Feb. 26, 2002 to June 30, 2003. Fish were collected at Little Sheep Creek adult trap, at weirs in Cow Creek and Lightning Creek and by angling in the South Fork Salmon River. Motility of the sperm ranged from 5 – 90%.

2003 Chinook Salmon Gamete Collections

Lostine River

In 2003 the gametes from 16 male chinook salmon were cryopreserved from fish trapped at the adult weir on the Lostine River and spawned at Lookingglass Hatchery. All fish were unmarked indicating that they were of natural-origin. Based on the length data (Appendix 3), two age 3, three age 4 and five age 5 fish were sampled from brood years 2000, 1999 and 1998, respectively. Length was not determined for six fish. Collections from 1994 to 2003 have preserved a total of 101 Lostine River male gamete samples in the gene bank (Appendix 1).

Upper Grande Ronde

In 2003 the gametes from 10 male chinook salmon were cryopreserved from fish trapped at the adult weir on the upper Grande Ronde River and spawned at Lookingglass Hatchery. All fish were unmarked indicating that they were natural-origin. Based on the length data (Appendix 3), seven age 4 and three age 5 fish were sampled from brood years 1999 and 1998, respectively. Collections from 2001 to 2003 have preserved a total of 27 Grand Ronde River male gamete samples in the gene bank (Appendix 1).

Catherine Creek

In 2003 the gametes from 8 male chinook salmon were cryopreserved from fish trapped at the adult weir on the Catherine Creek and spawned at Lookingglass Hatchery. All fish were unmarked indicating that they were of natural-origin. Based on the length data (Appendix 3), one age 3, three age 4 and four age 5 fish were sampled from brood years 2000, 1999 and 1998, respectively. Collections from 2001 to 2003 have preserved a total of 24 Lostine River male gamete samples in the gene bank (Appendix 1).

Table 1. Locations and numbers of spring and summer chinook salmon semen samples cryopreserved in the Snake River basin in 2003.

Spawning Aggregate	Total Samples	Unmarked Fish ^a	Marked Fish ^b	Females Captured	Collection Dates	Sperm Motility (%)
Lostine River	16	16	0	0	8/20, 27, 9/3 & 9/10	0-100
Catherine Creek	8	8	0	0	8/28 & 9/4	5-90
Grande Ronde River	10	10	0	0	8/28 & 9/4	50-100
Imnaha River	23	23	0	0	8/26, 9/2 & 9/9	10-100
S. Fork Salmon River	26	26	0	0	8/26, 29 & 9/2	0-100
Lake Creek	32	32	0	4	8/6, 11 & 8/18	0-100
Johnson Creek	54	51	3 ^c	2	8/20,22,25,26,27 & 8/29	0-100
Big Creek	31	31	0	2	8/7, 12 & 8/19	0-100
Capehorn Creek	15	15	0	2	8/15 & 8/21	5-100
Marsh Creek	16	16	0	1	8/14 & 8/21	0-100
Pahsimeroi River	15	0	15	0	9/22	30-100
Upper Salmon River	20	6	14 ^d	0	8/28	0-100
Totals	269	237	32	11	8/6 – 9/22	0-100

^aNon fin-clipped fish, natural origin

^bFin-clipped or tagged fish, hatchery origin

^cMarked with a coded wire tag (cwt; supplementation fish – hatchery x natural)

^d1 fish was adipose clipped (hatchery fish), the other 13 had a cwt (supplementation fish - hatchery x natural)

Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2003.

Spawning Aggregate	Total Samples Cryopreserved	Un-marked Fish ^a	Marked Fish ^b	Collection Dates	Sperm Motility (%)
Little Sheep Creek	70	3	67	3/25, 4/1, 15, 22, 29 & 5/6	0-90
Cow Creek	2	2	0	4/24	<10
Lightning Creek	1	1	0	5/1	<10
South Fork Salmon River	17	17	0	4/30, 5/4, 6, 7, & 16	20-90
Totals	90	23	67	3/25 – 5/16	0-90

^aNon fin-clipped fish, natural origin

^bFin-clipped or tagged fish, hatchery origin

Imnaha River

In 2003 the gametes from 23 chinook salmon were cryopreserved from fish trapped in the Imnaha River and spawned at Lookingglass Fish Hatchery. All fish were unmarked indicating that they were of natural-origin. Based on the length data (Appendix 3), eight age 3, seven age 4 and eight age 5 fish were sampled from brood years 2000, 1999 and 1998, respectively. Collections from 1994 to 2003 have preserved a total of 450 Imnaha River male gamete samples in the gene bank (Appendix 1). Of these, 209 were from marked hatchery fish and 241 were from unmarked natural fish.

South Fork Salmon River

In 2003 the gametes from 26 male chinook salmon were cryopreserved from fish trapped at the adult weir on the South Fork Salmon River (McCall Hatchery, Idaho Department of Fish and Game - IDFG). All fish were unmarked indicating that they were of natural-origin. Based on the length data (Appendix 3), three age 3, six age 4 and twelve age 5 fish were sampled from brood years 2000, 1999 and 1998, respectively. Length was not determined for 5 fish. Collections from 1996 to 2003 have preserved a total of 347 South Fork Salmon River male gamete samples in the gene bank (Appendix 1). Of these, 183 were from non-ESA-listed

hatchery fish, 81 were from ESA listed supplementation fish, and 83 were from unmarked ESA-listed natural fish.

Lake Creek

In 2003 the gametes from 32 wild males were cryopreserved from fish netted in Lake Creek. Four female chinook salmon were incidentally netted and immediately released. All fish were unmarked indicating that they were natural-origin. Four female chinook salmon were incidentally netted and immediately released. Based on the length data (Appendix 3), two age 3, sixteen age 4 and thirteen age 5 fish were sampled, originating from brood years 2000, 1999 and 1998, respectively. Length was not determined for one fish. Collections from 1996 to 2003 have preserved a total of 109 Lake Creek male gamete samples in the gene bank (Appendix 1).

Johnson Creek

In 2003 the gametes from 54 male chinook salmon were cryopreserved from fish captured in Johnson Creek and at the NPT adult weir. Nineteen fish were sampled from fish captured at the adult weir and spawned at McCall Hatchery's South Fork Salmon River facility as part of the Johnson Creek supplementation project. Of the remaining 35 fish, 16 were sampled from fish captured at the NPT Johnson Creek adult weir and 19 were netted in Johnson Creek. Two female chinook salmon were incidentally netted and immediately released. Based on the length data (Appendix 3), eight age 3, fifteen age 4 and thirty age 5 fish were sampled, originating from brood years 2000, 1999 and 1998, respectively. Length was not determined for one fish. Collections from 1997 to 2003 have preserved a total of 238 Johnson Creek male gamete samples in the gene bank (Appendix 1).

Big Creek

In 2003 the gametes from 31 male chinook salmon were cryopreserved from fish netted in Big Creek. Two adipose-clipped fish were captured but milt was not collected. All fish were unmarked indicating that they were of natural-origin. Two female chinook salmon were incidentally netted and immediately released. Based on the length data (Appendix 3), sixteen age 3, six age 4 and nine age 5 fish were sampled, originating from brood years 2000, 1999, and 1998, respectively. Collections from 1992 to 2003 have preserved a total of 134 Big Creek male gamete samples in the gene bank (Appendix 1).

Capehorn Creek

In 2003 the gametes from 15 male chinook salmon were cryopreserved from a fish netted in Capehorn Creek. All 15 fish were unmarked indicating that they were natural-origin. Two female chinook salmon were incidentally netted and immediately released. Based on the length data (Appendix 3), all fifteen fish were age 5 indicating that they originated from brood year 1998. Collections from 1997 to 2003 have preserved a total of 27 Capehorn Creek male gamete samples in the gene bank (Appendix 1).

Marsh Creek

In 2003 the gametes from 16 male chinook salmon were cryopreserved from fish netted in Marsh Creek. All 16 fish were unmarked indicating that they were natural-origin. One female chinook salmon was incidentally netted and immediately released. Based on the length data (Appendix 3), all sixteen fish were age 5 indicating that they originated from brood year 1998. Length was not determined for one fish. Collections from 1997 to 2003 have preserved a total of 87 Marsh Creek male gamete samples in the gene bank (Appendix 1).

Pahsimeroi River

In 2003 the gametes from 17 Pahsimeroi River male chinook salmon were cryopreserved from fish spawned at Pahsimeroi Hatchery. All were marked hatchery-origin fish. Based on the length data (Appendix 3), age 3, age 4 and age 5 fish were sampled, originating from brood years 2000, 1999 and 1998, respectively. Collections from 1999 to 2003 have preserved a total of 187 Pahsimeroi River male gamete samples in the gene bank (Appendix 1). Of these, 153 were from marked hatchery fish and 34 were from unmarked natural fish.

Upper Salmon River

In 2003 the gametes from 20 upper Salmon River male chinook salmon were cryopreserved from fish spawned at Sawtooth Fish Hatchery. Six, fourteen and one of the samples were obtained from unmarked natural fish, tagged supplementation fish (wild x hatchery parents) and adipose fin-clipped hatchery fish, respectively. Based on the length data (Appendix 3), two age 3, eleven age 4 and seven age 5 fish were sampled, originating from brood years 2000, 1999 and 1998, respectively. Collections from 1997 to 2003 have preserved a total of 293 upper Salmon River male gamete samples in the gene bank (Appendix 1). Of these, 55 were from marked hatchery fish, 26 were from marked supplementation fish and 214 were from unmarked natural fish.

2003 Steelhead Gamete Collections

Little Sheep Creek

In 2002 the gametes from 70 male steelhead were cryopreserved from fish spawned at the Little Sheep Creek adult weir. Of these, two were unmarked natural fish and 68 were marked hatchery fish. Based on the length data (Appendix 4), fifty-one age 3 and nineteen age 4 fish were sampled, originating from brood years 2000 and 1999, respectively. Collections from 1999 to 2003 have preserved a total of 350 Little Sheep Creek male gamete samples in the gene bank (Appendix 1). Of these, 328 were from marked hatchery fish and 22 were from unmarked natural fish (Appendix 1).

Cow Creek

In 2003 the gametes from 2 male steelhead were cryopreserved from fish captured at a NPT adult weir in Cow Creek. Both were unmarked, indicating that they were natural-origin. This is the first year collecting fish from Cow Creek

Lightning Creek

In 2003 the gametes from 1 male steelhead was cryopreserved from fish captured at a NPT adult weir in Lightning Creek. The fish was unmarked, indicating that it was of natural-origin. This is the first year collecting fish from Cow Creek

South Fork Salmon River

In 2003 the gametes from 17 unmarked male steelhead were cryopreserved from fish captured by angling in the South Fork Salmon River. The gametes from two other males were collected but not cryopreserved due to low volume and poor quality. Seven females were inadvertently captured and immediately released and two males were recaptured and immediately released. Based on the length data (Appendix 4), eight age 4 and nine age 5 were sampled. This was the first year collecting steelhead gametes from the South Fork Salmon River.

Status of Germplasm Collections in the Snake River Basin

NPT initiated the gene bank effort in 1992 with collections of milt from Big Creek spring chinook salmon. Since that time sampling effort has increased to include chinook salmon and steelhead from most of the major river subbasins in the Snake River basin (Appendix 1). Regional support for the project was evident by the addition of cryopreserved samples collected state management agencies and Native American Tribes. These agencies utilized NPT's long-term repository to store cryopreserved gametes from other imperiled salmon populations and species in the Columbia River drainage. In 2003, the Columbia River Intertribal Fish Commission added gamete samples from 30 Wenatchee River coho to the repository. The repository also includes gamete samples from Redfish Lake sockeye (IDFG), Yakima River spring chinook salmon (Washington Department of Fish and Wildlife - WDFW), Grande Ronde River subbasin chinook salmon captive broodstock (NPT – see below) and Kootenai River white sturgeon (Armstrong and Kucera 2001).

Grande Ronde River Chinook Salmon Captive Broodstock Project

A Grande Ronde River subbasin spring chinook salmon captive broodstock program was initiated in 1995 with the collection of juvenile salmon (500 parr) from the Lostine River, Catherine Creek and upper Grande Ronde River. This program is an attempt to maximize the species reproductive potential and to preserve the population through use of acclimated smolt releases to return a threshold number of spawning chinook salmon adults to the three rivers (Mary Edwards, personal communication). Semen is cryopreserved from the male chinook

salmon in order to maintain a repository of genetic material from these captive fish. The project maintains a repository at Bonneville Hatchery. Half of the straws from each male are transported to the germplasm repository at University of Idaho as insurance against catastrophic failure at the Bonneville repository. No samples were added to the repository in 2003. The total number of samples stored in the repository from this captive broodstock project is 680. Of these, 232 were from the Lostine River, 180 were from the upper Grande Ronde River, and 268 were from Catherine Creek.

Fertility Trials

Fertility trials were conducted at WSU in order to evaluate the effectiveness of the cryopreservation protocols used in 2003. The fertility of cryopreserved semen from eight fish was compared to that from two fresh semen samples. Fresh semen and eggs were collected from two males and two female chinook salmon from Pahsimeroi Hatchery. The eggs were separated into lots of approximately 450 eggs and fertilized using the milt from a single 0.5 ml straw. Two control crosses were made using fresh milt from a Pahsimeroi Hatchery male and eggs from two Pahsimeroi Hatchery females. Average relative fertilization rate for the cryopreserved samples was 36.4% with a range from 9.6 to 71.1% (Table 3).

Table 3. Results of chinook salmon fertilization trial conducted at Washington State University in 2003.

Cross (genebank #) ¹	Fish origin	# fertile	total eggs	% fertilized	% relative fertility ²	motility
Pah 1 x NPT 162	Lake Creek	145	512	28.32	78.61	80
Pah 1 x NPT 167	Lake Creek	64	501	12.77	35.46	80
Pah 1 x NPT 172	Lake Creek	47	491	9.57	26.57	90
Pah 1 x NPT 171	Lake Creek	20	431	4.64	12.88	50
Pah 2 x NPT 174	Big Creek	45	494	9.11	11.85	90
Pah 2 x NPT 219	Big Creek	80	397	20.15	26.22	90
Pah 2 x NPT 217	Big Creek	114	401	28.43	36.99	90
Pah 2 x NPT 222	Big Creek	1	525	0.19	0.25	70
			Average	14.15	28.60	
Controls						
Pah 1 x Pah 1		116	322	0.36		
Pah 2 x Pah 2		362	471	0.77		

¹Cryopreservation straw number from WSU

²Percent fertility divided by control fertility of the cross using the same female (Pah 1 or Pah 2) Activator trial

A fertility trial was also performed in order to compare the fertility of cryopreserved sperm using two activator solutions. Gametes from three male and three female steelhead were collected at Dworshak National Fish Hatchery. Milt from each male was cryopreserved in 10 0.5ml straws, using standard protocols, and stored overnight in liquid nitrogen. Eggs were pooled and divided into lots of approximately 350 eggs. Three replicate fertilizations were performed using sperm from a single male and standard or Cossin's activator solution. Control crosses involved fresh sperm from each male that had been stored overnight in an oxygenated whirlpac bag in the refrigerator (approximately 4 C). Two replicate control crosses were made using fresh sperm from each male. Results of the experiment are presented in Table 4. The standard activator produced significantly higher fertility rates (2-factor ANOVA, $F=9.17$, $P>0.01$).

Table 4. Results of cryopreservation activator trial conducted at Washington State University in 2003.

Cross	Replicate	Standard	Cossin's
1	1	40.42	43.14
	2	64.68	39.51
	3	52.65	49.54
2	1	80.78	59.62
	2	83.41	70.35
	3	70.53	64.53
3	1	63.29	52.36
	2	60.05	55.56
	3	64.11	25.83
Control 1	1	99.11	
	2	97.10	
Control 2	1	96.72	
	2	99.19	
Control 3	1	94.98	
	2	97.37	

Use of Cryopreserved Gametes in 2003

No gametes from the repository were used in 2003.

Salmonid Genetic Analysis

An important objective of the Salmonid Gamete Preservation project is to report the genetic composition of the fish in the genebank and evaluate the effectiveness of the collection versus the extant population. Genetic diversity information from fish in the repository is used to evaluate the level genetic diversity contained in the gamete repository and serve as a baseline that can be used to monitor shifts or losses of genetic variation over time (Servheen et al. 2001).

In 2003, tissue samples were collected from the majority of chinook salmon and steelhead captured and spawned for cryopreservation. These samples will be analyzed and incorporated into a larger analysis of the within and among population spatial and temporal genetic diversity of all samples in the repository.

Steelhead Genetic Analyses

DNA samples from fish collected in 1999 through 2001 were analyzed using mitochondrial control region sequencing and 4 nuclear microsatellite loci (Appendix 4). A detailed report of the steelhead genetic analysis can be obtained from NPT (contact William Young). Mitochondrial sequence data showed two common alleles in all populations and the presence of unique alleles both among populations and year classes suggesting that a high level of mitochondrial diversity was preserved in the genebank. Although significant within and among population diversity was observed from the microsatellite analysis, small sample sizes made these results suspect. In addition, the small sample sizes likely contributed to the deviation from Hardy-Weinburg equilibrium and the lower than expected levels of heterozygosity observed in the microsatellite loci. Future analyses should include more samples from these populations in order to increase the confidence of the results.

DISCUSSION

Sustained productivity of salmonids in the Pacific Northwest is possible only if the genetic resources that are the basis of such productivity are maintained (National Research Council 1996). Much of the genetic diversity that historically existed probably has already been lost. The germplasm repository is an effort to conserve the genetic diversity that remains in existing salmon and steelhead runs and allow for future management options. Although we have attempted to sample and preserve salmonid genetic diversity within the major river subbasins in the Snake River basin, the spawning aggregates sampled represent only a small portion of the stocks in the Snake River basin. Consequently, collections should continue from these and additional populations until an adequate number of individuals have been sampled.

Since the program was initiated in 1992, NPT has been very successful cryopreserving chinook

salmon gametes from both hatchery and natural populations. In contrast, few gametes from naturally-spawned steelhead have been collected and cryopreserved. Chinook salmon spawn in late summer during periods of low water flows, making it relatively easy to spot and capture spawning adults from natural spawning grounds. Steelhead spawn in the spring during periods of high water and inclement weather making them essentially inaccessible to capture with nets or seines. Thus, a majority of the steelhead gametes came from easily accessible hatchery-origin fish. In 2003 we began collecting naturally-spawning adult male steelhead using angling. This method proved effective based on the 17 steelhead gamete samples collected from the South Fork Salmon River. We plan to increase our angling effort in 2004.

In 2003 we performed the fertility trial using cryopreserved gametes from naturally-spawning chinook salmon males. All previous fertility trials were performed using sperm collected from hatchery-spawned males. The rigorous spawning behavior displayed by naturally-spawning male chinook salmon often causes these fish to be in poor condition and produce what appears to be lower quality milt compared to that of hatchery spawned males. This may lower the potential fertility of sperm cryopreserved from naturally-spawning males and result in lowered fertility rates compared to previous trials that used hatchery spawned males. Results revealed that fertility rates from this trial were similar to previous results (Armstrong and Kucera, 1999; 2000; 2001; Young and Kucera, 2003), indicating that gametes collected from naturally-spawning males were of similar quality compared to those of hatchery-spawned males. As in previous trials there was a high level of variation in fertility rates, ranging from near zero to over 78% relative fertility. The high level of variation observed in the fertility rates was likely a function of semen quality. Scheerer and Thorgaard (1989) found that fresh and cryopreserved rainbow trout semen taken late in the spawning season (marginal-quality semen) exhibited significantly lower fertility than that taken early in the season (high-quality semen).

Previous fertility trials demonstrated that the fertility of cryopreserved sperm averages 40% compared to fresh sperm (Armstrong and Kucera, 1999; 2000; 2001; Young and Kucera, 2002). Numerous factors are believed to influence these results including sperm quality, freezing rate, extender solution, thawing rate and activator solution. In 2003 we tested Cosin's activator in an attempt to increase the fertility of cryopreserved sperm. This activator is a basic solution (pH > 8.0) containing trace concentrations of calcium that greatly increases sperm motility compared to water (Joseph Cloud, personal communication) and was compared to the standard activator used in the previous fertility trials (Armstrong and Kucera, 1999; 2000; 2001; Young and Kucera, 2002). Results demonstrated that the standard activator produced significantly higher fertilities compared to the Cossin's activator. Thus, the standard activator is preferred at this time.

Steelhead genetic analyses using mitochondria and microsatellite markers revealed relatively high levels of genetic diversity. However, an analysis of the source populations has not been performed making it impossible to determine if the samples in the genebank represent the source populations. This will need to be done in the future in order to ensure a representative sample of the genetic diversity has been collected.

Understanding the distribution of the samples obtained from an organism with a non-discrete generation time is critical for preserving the greatest level of diversity. This project set a goal of preserving gametes from at least 100 males per brood year for at least one generation from each

spawning aggregation. Equalizing the collection of milt from adults across an entire generation will theoretically result in the preservation of the greatest amount of genetic diversity. However, collecting 100 samples/year for an entire generation has not been possible given the low number of returning adults and the difficulty in capturing adult males. Generally, collections ranged from 10 – 40 samples per year per spawning aggregation. Thus it was inevitable that collections would need to continue for multiple generations in order to reach the sampling goal. For this reason we required a method that would quantify the distribution of collections that occurred over multiple generations. This method, referred to as the effective brood year (EBY) analysis, could deal with sample collections from multiple age classes over multiple years. Just as an effective population size was defined as the theoretical size of a population under ideal conditions (see Hedrick 2000 or any genetics text for an explanation of effective population size), effective brood year is the theoretical brood year an organism originated from. By analyzing the demographic makeup of the fish that contributed gametes to the collection each year, assigning them to the actual brood years that they originated, this method enabled us to estimate the overall distribution of samples in the genebank.

Generation times were calculated as the average number of years it takes for 95% of the individuals from a brood year to return. Fish were designated to actual brood years based on length/frequency data. The number of effective brood years in a generation is equal to the number of years per generation. The time it takes to collect a specified number of samples per effective brood year will vary depending on the number and age of the fish sampled each year. Fish collected as 3, 4 and 5 year olds in one year originated from 3 different brood years and thus 3 different effective brood years. The first effective brood year was arbitrarily set as the first year of collection and proceeded for the number of years in a generation. For example, let say we made two collections of 500 gamete samples, collection 1 consisted of 50 samples/year for consecutive 10 years (2 chinook salmon generations) and collection 2 consisted of 10 yearly collections of 100, 100, 0, 20, 20, 80, 80, 40, 0, 60 (2 chinook salmon generations). Assuming similar demographic composition among the years (approximately similar number of 3, 4 and 5 year old fish each brood year), the former collection would preserve more diversity compared to the latter. By evenly sampling fish over two generations, collection 1 maximized the potential diversity from the population. In contrast, collection 2 underrepresented the extant diversity of the population because certain brood years were overrepresented and others were underrepresented.

To date, none of the populations have met the goal of collecting 500 samples for chinook salmon (based on a generation time of 5 years) and 400 - 500 samples for steelhead (based on a generation time of 4 years for A-run hatchery fish and 5 years for B-run hatchery fish). However, a number of collections from non-ESA listed hatchery populations are represented by large numbers of individuals that may have an adequate number of samples to mitigate genetic diversity problems in the source populations. Young and Kucera (2002) recommended not collecting additional samples from North Fork Clearwater steelhead (Dworshak National Fish Hatchery), Pahsimeroi River steelhead (Pahsimeroi Fish Hatchery) and Snake River steelhead from Oxbow Fish Hatchery and made recommendations for future collections from Imnaha River chinook salmon, South Fork Salmon River chinook salmon and Little Sheep Creek steelhead. We will not repeat those analyses in this report, but will update the status of the 2003 collections in relation to the recommendations of Young and Kucera (2002). With the exception

of those listed above, all chinook salmon and steelhead populations listed in Appendix Table A1 and A2 do not have sufficient number of gamete samples and will require additional sample collections in 2004.

In 2003 gamete samples were collected from three populations that contain large number of samples, the Imnaha River chinook salmon, South Fork salmon River chinook salmon and Little Sheep Creek steelhead. Of these, only gamete sample collections from Little Sheep Creek were great enough in number to warrant an effective brood year analysis. The status of the 2003 collections from Imnaha River chinook salmon and South Fork salmon River chinook salmon will be discussed with respect to the recommendations of Young and Kucera (2002).

Imnaha River Chinook Salmon

Young and Kucera (2002) recommended collecting gametes from natural-origin fish in order to preserve the greatest level of diversity from this population and to concentrate collections on fish from effective brood year 1 (2003 four year old fish), as it was underrepresented in the repository. In 2003 we collected gametes from 23 natural-origin fish including 8 fish representing effective brood year 1. Thus, little was accomplished in increasing the representation of this brood year. Four-year old fish were relatively rare in 2003 across the entire Snake River basin (based on our collections) and we collected as many samples as we could from this brood year. The gene bank contains gametes from 450 Imnaha River male chinook salmon including 209 marked hatchery-origin fish and 241 wild fish.

RECOMMENDATIONS - Although a large number of samples have been collected from this population, additional collections are warranted because of the importance of this ESA-listed stock and the fact that nearly half of the samples were from hatchery-origin fish. Although hatchery-origin fish are also ESA-listed, collecting gametes from additional fish, especially of natural-origin, would preserve the greatest level of diversity from this population. EBY 1 was still underrepresented in the repository. In 2004 five-year old fish will represent this EBY and will be targeted. However, based on the return of four year olds from this brood year it is highly unlikely that we will make significant collections from 5-year old fish in 2004.

South Fork Salmon River Chinook Salmon

Young and Kucera (2002) recommended collecting gametes from natural-origin fish in order to preserve the greatest level of diversity from this population and to concentrate collections on fish from effective brood year 4 (2003 four year old fish), as it was underrepresented in the repository. In 2003 we collected gametes from 26 natural-origin fish including 6 fish representing effective brood year 4. Once again our collections were limited by a low number of 4 year-old fish returning in 2003. However, we did significantly increase the number of gametes from natural-origin fish by collecting milt directly at the trap as IDFG personnel sorted hatchery- and natural-origin fish. The gene bank now contains gametes from 347 South Fork Salmon River male chinook salmon including 183 marked hatchery-origin fish, 81 supplementation fish (hatchery-origin x natural-origin) and 83 natural-origin fish.

RECOMMENDATIONS – The 183 hatchery-origin fish are adequate as a buffer against

potential loss of diversity in the hatchery population. Additional collections are warranted for fish from effective brood year 4. In 2004 the returning 5-year old fish will represent this EBY and effort should be made to collect a large number of these fish in order to increase the representation of this EBY in the repository. As above, our collections of 5-year old fish will be limited due to the low abundance of fish from this brood year.

Little Sheep Creek Steelhead

Young and Kucera (2002) recommended collecting gametes from hatchery- and natural-origin fish in order to preserve the greatest level of diversity from this population. In 2003 we collected gametes from 70 Little Sheep Creek male steelhead including 53 from effective brood year 3 and 19 from effective brood year 4. Sixty-eight were marked hatchery-origin fish and two were unmarked natural-origin fish. The gene bank contains gametes from 350 Little Sheep Creek male steelhead including 328 marked hatchery-origin fish and 22 natural-origin fish. Oregon Department of Fish and Wildlife (ODFW) hatchery managers designate age groups by the following lengths: <64 cm – age 3 and >64 cm – age 4 and the generation time of the hatchery population is 4 years since nearly all fish return as 3 and 4 year olds (Mike Fleisher, ODFW, personal communication). The generation time of the natural-origin fish was unknown. Using these lengths along with the run composition for each year, the number of fish from each brood year represented in the gene bank was calculated (Figure 5).

RECOMMENDATIONS - Additional collections are warranted because this hatchery population is ESA-listed and a low number of natural-origin fish have been sampled. Based on the collections, we estimate that the number of gametes from EBY 3 is adequate to meet future requirements. Future collections should continue from fish representing the other 3 EBYs until at least 100 fish from each brood year are in the repository. Increasing the collection of natural-origin fish from all brood years will maximize the diversity of the collection from this drainage.

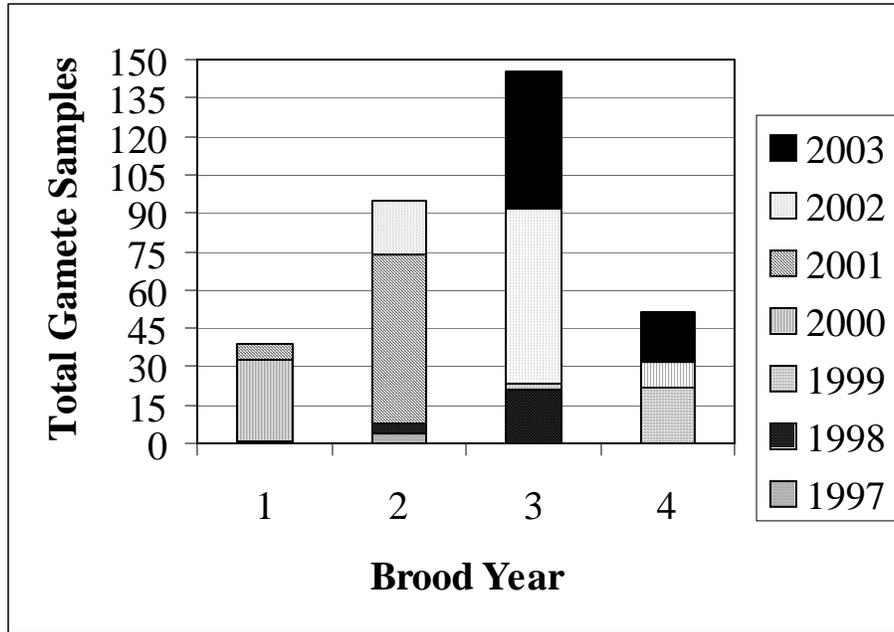


Figure 5. Graph showing the number of gametes collected from the Little Sheep Creek steelhead per effective brood year over a 4-year generation.

Although no requests for cryopreserved gametes were made in 2003, we believe that more requests will be made to use cryopreserved semen in hatchery production programs and in research. We recommend and support only the ethical use of cryopreserved genetic material from the germplasm repository. The judicious use of this vital genetic resource is imperative. To that end, we will provide criteria for accessing and using cryopreserved semen samples from the germplasm repository that will assist in rational use and inventory management. A form has been developed to request cryopreserved semen from the germplasm repository and is available for use (Appendix 2). The semen request form's main function is for inventory management of the 0.5ml straws and 5.0 ml straws. Semen requests are reviewed by the Snake River Germplasm Repository Committee to ensure rational use. A database of the germplasm inventory has been established and is available for use.

RECOMMENDATIONS

1. Continue collecting gametes from chinook salmon populations throughout the Snake River basin.
2. Utilize angling as a method of collecting gametes from steelhead populations throughout the Snake River basin.
3. Complete a genetic analysis of the chinook salmon contained in the genebank and compare it to the source populations.
4. Continue tissue sample collections from all of the fish that are sampled in order to perform critical genetic analyses.

5. Research techniques to optimize 5.0 ml straw freezing and thawing protocols that will improve fertilization rates.
6. Continue fertility trials on cryopreserved gametes in order to evaluate the freezing techniques.
7. Establish a Regional Germplasm Repository for gene conservation of imperiled fish and wildlife species.

ACKNOWLEDGMENTS

We would like to thank Dr. Joe Cloud and Wendy Lawrence from the University of Idaho and Dr. Gary Thorgaard, Paul Wheeler, Steve Patton and Darin Smith from Washington State University for cryopreservation assistance, maintaining the storage facilities, and recommendations that made this a better program. We also thank the U.S. Fish and Wildlife Service Lower Snake River Compensation Plan program for providing cost-share funds for cryopreservation activities.

We also thank the hard work and cooperation of our Nez Perce Tribe field crews: Mary Edwards, Raphael Johnnie, John Gebhards, Mike Busby, Cameron Albee, Dave Faurot, Ryan Jain, Mitch Daniel, Mike Blenden, Sarah Aavedel, Jay Hesse, Doug Nelson, Jason Vogel, Rob Hill, Carl East and Jim Harbeck. A special thanks to Robyn Armstrong, the former project leader, for her continuing advice and assistance.

We greatly appreciate the cooperation and assistance of: Gene McPherson from the Idaho Department Fish and Game McCall Fish Hatchery, Greg Davis from Oregon Department of Fish and Wildlife Wallowa Hatchery, Bob Lund from the Oregon Department of Fish and Wildlife Lookingglass Hatchery, Todd Garlie and Doug Engelman from Pahsimeroi Hatchery, Bob Simple from Dworshak National Fish Hatchery, and Brent Snider from the Sawtooth Fish Hatchery. We thank Bob Simple and Thomas Trock from Dworshak National Fish Hatchery for providing steelhead gametes used to examine the effects of different activators and Todd Garlie and Doug Engelman from Pahsimeroi Hatchery for providing chinook gametes used in the fertility trial. Also, thanks again to Interstate Aviation for being so flexible about our schedules and flying the chinook and steelhead gametes.

The Nez Perce Tribe is appreciated for administrative support of this project.

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This report and annual reports from 1997-2001 are available on the Internet through BPA Fish and Wildlife Publications at:

<http://www.efw.bpa.gov/cgi-bin/efw/FW/publications.cgi>

APPENDICIES

Appendix 1. Gamete samples collected from 1992 through 2002

Table A1. Snake River basin chinook salmon samples cryopreserved from 1992 through 2003.

Spawning Aggregate	Year												Totals
	2003	2002	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992	
Lostine River	16	19	33	18	2	3	2	3	1	4			101
Upper Grande Ronde River	10	8	9										27
Catherine Creek	8	5	11										24
Rapid River				51	68	98							217
South Fork Salmon River	26	23	44	54	93	45	45	19					347
Lake Creek	32	18	28	15	6	3	4	3					109
Johnson Creek	54	58	62	35	5	17	7						238
Big Creek	31	21	50	7	0	1	6	0	0	0	10	7	134
Capehorn Creek	15	1	2	1	0	6	2						27
Marsh Creek	16	34	24	7	0	2	4						87
Pahsimeroi River	17	39	50	50	31								187
Upper Salmon River	20	54	48	40	40	41	51						293
Imnaha River	23	7	37	71	95	79	41	33	42	22			450
Totals	268	286	398	349	340	295	162	58	43	26	10	7	2242

Table A2. Snake River basin steelhead samples cryopreserved from 1993 through 2003.

Spawning Aggregate	Year									Totals
	2003	2002	2001	2000	1999	1998	1997	1994	1993	
North Fork Clearwater River		63	81	89	62					295
Selway River								5*		5
Fish Creek		3	1	1					10*	15
Grande Ronde River			1	1						2
South Fork Salmon River	17									17
Johnson Creek			1		2					3
Pahsimeroi River		63	60	40	47					210
Imnaha River				2						2
Little Sheep Creek	70	95	78	52	25	25	5			350
Cow Creek	2									2
Lightning Creek	1									1
Snake River		58	73	98	76					305
Totals		280	295	281	214	25	5	5	10	1115

* Samples collected by the USGS/ National Biological Survey.

Appendix 2. Snake River Germplasm Repository Cryopreserved Semen Request Form

Snake River Germplasm Repository Committee
P.O. Box 1942, 125 South Mission St
McCall, ID 83638
Phone: (208) 634-5290
Fax: (208) 634-4097

Snake River Germplasm Repository Cryopreserved Semen Request Form

Name: _____ Affiliation: _____
Phone number: (_____) _____ Address: _____
Date of request: _____ Date need by: _____
Species/stock requested: _____ Hatchery or wild/natural: _____
Number of straws needed: _____ 0.5ml, _____ 5.0ml
Reason for request (clearly demonstrate need or type of hatchery program): _____

Fertilization experience using cryopreserved semen: _____

Name, address, and phone number of person samples should be delivered to: _____

Please use additional papers as necessary.

The salmon managers of the Snake River Basin are concerned with how cryopreserved samples are being used and retain the right to refuse samples for inappropriate use of the threatened salmonid species gametes. The Nez Perce Tribe can arrange to deliver and assist in the fertilization of eggs. Please call William Young at the McCall Field Office (address above) to coordinate transfer. The Nez Perce Tribe also may request data on the performance of the semen (percent of eggs fertilized, post-thaw sperm motility, etc.).

Signature: _____ Date: _____

Appendix 3. Data from chinook salmon collected in 2003.

Table A3. Collection date, fork lengths, percent motilities and number of straws from chinook salmon collected in 2003.

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Big Creek	8/7/03	56	50	20	10	20
Big Creek	8/7/03	90.5	90	20	80	20
Big Creek	8/7/03	104.5	2	20	20	20
Big Creek	8/7/03	85	0		0	10
Big Creek	8/7/03	110	0		0	20
Big Creek	8/7/03	55	50	20	20	10
Big Creek	8/7/03	107	2	20	20	20
Big Creek	8/7/03	105			50	20
Big Creek	8/7/03	107	90	20	50	20
Big Creek	8/7/03	62	70	10		
Big Creek	8/12/03	69	90	20		
Big Creek	8/12/03	71.5			100	13
Big Creek	8/12/03	71	50	20	90	20
Big Creek	8/12/03	76.5	5	20	10	20
Big Creek	8/12/03	91	80	20		
Big Creek	8/12/03	52.5	60	20	80	11
Big Creek	8/12/03	49.5	70	20	80	10
Big Creek	8/12/03	107	90	20	5	20
Big Creek	8/12/03	50.5	90	20	90	11
Big Creek	8/12/03	108	20	18	100	10
Big Creek	8/12/03	49.5	60	20	100	16
Big Creek	8/19/03	56	90	20	80	12
Big Creek	8/19/03	59	50	20	90	19
Big Creek	8/19/03	57	90	20	100	20
Big Creek	8/19/03	49.5			100	20
Big Creek	8/19/03	55	90	20		
Big Creek	8/19/03	58.5	70	20	0	20
Big Creek	8/19/03	74.5	50	20	90	20
Big Creek	8/19/03	59			80	9
Big Creek	8/19/03	55	50	20		
Big Creek	8/19/03	56			90	20
Capehorn Creek	8/15/03	101.5	60	20	90	19
Capehorn Creek	8/15/03	106	90	20	50	20
Capehorn Creek	8/15/03	100.5	60	20	90	20
Capehorn Creek	8/15/03	106.5	90	20	100	20
Capehorn Creek	8/15/03	103.5	10	20	100	20
Capehorn Creek	8/15/03	110			90	20
Capehorn Creek	8/15/03	100	5	20	100	20

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Capehorn Creek	8/15/03	104.5	90	20	100	2
Capehorn Creek	8/15/03	105	90	20		
Capehorn Creek	8/15/03	103	10	20	5	20
Capehorn Creek	8/15/03	105.5			90	12
Capehorn Creek	8/21/03	108	0	0	100	20
Capehorn Creek	8/21/03	111.5	10	20		
Capehorn Creek	8/21/03	114	70	20	100	20
Catherine Creek	8/28/03	89.5			50	16
Catherine Creek	8/28/03	71.5	70	20		
Catherine Creek	8/28/03	93.5			90	20
Catherine Creek	8/28/03	90	70	20		
Catherine Creek	9/4/03	92.5	10	20	5	20
Catherine Creek	9/4/03	92.5	90	20	5	20
Catherine Creek	9/4/03	80	90	20	5	20
Catherine Creek	9/4/03	64.5	20	20	90	20
Grande Ronde R.	8/28/03	76.5	80	20	100	20
Grande Ronde R.	8/28/03	91.5			80	17
Grande Ronde R.	8/28/03	870	70	20		
Grande Ronde R.	8/28/03	88			100	16
Grande Ronde R.	9/4/03	89.5			90	20
Grande Ronde R.	9/4/03	95.5	80	20		
Grande Ronde R.	9/4/03	83	70	20	50	20
Grande Ronde R.	9/4/03	87			90	20
Grande Ronde R.	9/4/03	91.5	70	20		
Grande Ronde R.	9/4/03	68	70	20	80	20
Imnaha River	8/26/03	73	90	20		
Imnaha River	8/26/03	83			90	20
Imnaha River	8/26/03	78.5	60	20		
Imnaha River	8/26/03	74.5			90	20
Imnaha River	8/26/03	89.5	90	20		
Imnaha River	8/26/03	83.5	70	20	100	20
Imnaha River	8/26/03	43	80	20	100	20
Imnaha River	8/26/03	99	70	20	100	20
Imnaha River	8/26/03	106.5	40	20	100	20
Imnaha River	8/26/03	75	90	19	100	20
Imnaha River	9/2/03	100.5	90	20	50	20
Imnaha River	9/2/03	94.5	90	20	50	20
Imnaha River	9/2/03	68.5	80	20	90	20
Imnaha River	9/2/03	110.5			100	20
Imnaha River	9/2/03		80	20	100	20
Imnaha River	9/2/03		90	20	90	20
Imnaha River	9/2/03		0	0	10	20

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Imnaha River	9/2/03	99.5	90	20	90	20
Imnaha River	9/9/03		90	20	90	20
Imnaha River	9/9/03		70	20		
Imnaha River	9/9/03		90	20	90	20
Imnaha River	9/9/03		80	20	100	20
Johnson Creek	8/20/03	69			100	20
Johnson Creek	8/20/03	101	90	20		
Johnson Creek	8/20/03	81			90	19
Johnson Creek	8/20/03	106	80	20		
Johnson Creek	8/20/03	100	60	20	100	14
Johnson Creek	8/20/03	81			100	20
Johnson Creek	8/20/03	104	5	20		
Johnson Creek	8/20/03	107	40	20	70	20
Johnson Creek	8/20/03	95	90	20	90	15
Johnson Creek	8/20/03	100	50	20	100	20
Johnson Creek	8/20/03	55			90	13
Johnson Creek	8/22/03	96	90	20	100	20
Johnson Creek	8/22/03	88	70	20	10	20
Johnson Creek	8/22/03	95	70	20	100	20
Johnson Creek	8/22/03	75	80	20	100	20
Johnson Creek	8/22/03	93	90	10	100	18
Johnson Creek	8/22/03	87	90	20	100	20
Johnson Creek	8/22/03	95	50	20	80	20
Johnson Creek	8/22/03	78	90	20	100	10
Johnson Creek	8/22/03	77	80	20	10	12
Johnson Creek	8/22/03	85	90	20	50	20
Johnson Creek	8/22/03	81	80	20	90	20
Johnson Creek	8/22/03	77	10	20	50	15
Johnson Creek	8/22/03	52	70	20	80	20
Johnson Creek	8/22/03	70	90	20		
Johnson Creek	8/22/03	101			100	13
Johnson Creek	8/22/03	93	90	20		
Johnson Creek	8/25/03	99			100	20
Johnson Creek	8/25/03	89	10	20		
Johnson Creek	8/25/03	91	70	20	100	20
Johnson Creek	8/25/03	54			100	5
Johnson Creek	8/25/03	106	10	20		
Johnson Creek	8/25/03	115	10	10	90	20
Johnson Creek	8/25/03	99	40	20	100	10
Johnson Creek	8/25/03	106	10	20	50	20
Johnson Creek	8/25/03	53			75	10
Johnson Creek	8/25/03	92	40	20	0	10

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Johnson Creek	8/25/03	58	10	20		
Johnson Creek	8/25/03	89	30	20	90	20
Johnson Creek	8/25/03	93			100	20
Johnson Creek	8/25/03	97	10	20	100	20
Johnson Creek	8/25/03	75	40	20	90	12
Johnson Creek	8/26/03	79	60	10		
Johnson Creek	8/27/03	109			90	20
Johnson Creek	8/27/03	59	90	20	100	12
Johnson Creek	8/27/03	91	10	20	10	12
Johnson Creek	8/27/03	91	60	20	90	9
Johnson Creek	8/27/03	52	20	18		
Johnson Creek	8/27/03	94			90	14
Johnson Creek	8/27/03	n	50	20		
Johnson Creek	8/27/03	80	20	20	90	14
Johnson Creek	8/27/03	51	90	19	90	12
Johnson Creek	8/29/03	97	50	20		
Johnson Creek	8/29/03	103	70	20		
Lake Creek	8/6/03				50	20
Lake Creek	8/6/03	86	80	20	70	20
Lake Creek	8/6/03	78	90	20	5	10
Lake Creek	8/6/03	82.5	90	20	90	17
Lake Creek	8/6/03	87	60	20	5	20
Lake Creek	8/6/03	82.5	90	20	90	10
Lake Creek	8/6/03	106	80	20	30	20
Lake Creek	8/6/03	77	80	20	5	20
Lake Creek	8/6/03	81	60	20	0	14
Lake Creek	8/6/03	82.5	70	20	0	20
Lake Creek	8/6/03	100	0	0	5	16
Lake Creek	8/11/03	102	1-2		80	20
Lake Creek	8/11/03	100	80	20	90	20
Lake Creek	8/11/03	102	50	20		
Lake Creek	8/11/03	98	5	20	80	15
Lake Creek	8/11/03	95	90	20	90	20
Lake Creek	8/11/03	97			80	20
Lake Creek	8/11/03	103	80	20	30	20
Lake Creek	8/11/03	82	80	20	80	20
Lake Creek	8/11/03	85	90	20	80	14
Lake Creek	8/11/03	88	80	20	50	20
Lake Creek	8/11/03	104	50	20	100	20
Lake Creek	8/11/03	78	90	20	90	20
Lake Creek	8/11/03	75	40	20	100	3
Lake Creek	8/18/03	84.5	40	20	100	19

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Lake Creek	8/18/03	88	60	20	80	16
Lake Creek	8/18/03	88	80	20	80	20
Lake Creek	8/18/03	58	80	20		
Lake Creek	8/18/03	105	80	20	100	20
Lake Creek	8/18/03	97	90	20	0	20
Lake Creek	8/18/03	99	60	20	90	20
Lake Creek	8/18/03	60			90	20
Lostine River	8/20/03	94	90	20	70	20
Lostine River	8/20/03	106			90	20
Lostine River	8/27/03	102			80	20
Lostine River	8/27/03	97	90	20		
Lostine River	8/27/03	85.5	20	20	90	20
Lostine River	8/27/03	55			100	18
Lostine River	9/3/03	83.5	90	20		
Lostine River	9/3/03	105	20	20	90	20
Lostine River	9/3/03		90	20	80	20
Lostine River	9/3/03		80	20	50	20
Lostine River	9/3/03	59			80	20
Lostine River	9/3/03		70	20	90	20
Lostine River	9/10/03	77.5			80	17
Lostine River	9/10/03		50	20	90	20
Lostine River	9/10/03		60	20	100	20
Lostine River	9/10/03		90	20	100	20
Marsh Creek	8/14/03	102	20	20	30	20
Marsh Creek	8/14/03	102.5	0	0	90	20
Marsh Creek	8/14/03	108.5	50	20	100	20
Marsh Creek	8/14/03	93	90	20	5	20
Marsh Creek	8/14/03	108	60	20	0	20
Marsh Creek	8/14/03	105	10	10	0	12
Marsh Creek	8/14/03	111	0	0	5	20
Marsh Creek	8/14/03	112.5	80	20	100	20
Marsh Creek	8/14/03	107			100	20
Marsh Creek	8/14/03	108	70	20		
Marsh Creek	8/14/03	102.5	90	20	100	20
Marsh Creek	8/14/03	105.5	0	0	90	7
Marsh Creek	8/21/03	105	90	20	100	16
Marsh Creek	8/21/03	107.5	5	20	100	20
Marsh Creek	8/21/03	94	90	20	90	20
Marsh Creek	8/21/03	106	10	20	100	20
Pahsimeroi Hatchery	9/22/03	-	0		80	20
Pahsimeroi Hatchery	9/22/03	-	50	20	80	18
Pahsimeroi Hatchery	9/22/03	-	0		30	20

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
Pahsimeroi Hatchery	9/22/03	640			90	20
Pahsimeroi Hatchery	9/22/03	-	70	20		
Pahsimeroi Hatchery	9/22/03	-	90	20	20	20
Pahsimeroi Hatchery	9/22/03	-	90	20	50	20
Pahsimeroi Hatchery	9/22/03	-	70	20	90	20
Pahsimeroi Hatchery	9/22/03	-	40	20	90	20
Pahsimeroi Hatchery	9/22/03	-			50	14
Pahsimeroi Hatchery	9/22/03	815			100	20
Pahsimeroi Hatchery	9/22/03	-			100	20
Pahsimeroi Hatchery	9/22/03	-			70	20
Pahsimeroi Hatchery	9/22/03	-			90	20
Pahsimeroi Hatchery	9/22/03	570			90	10
SFSR	8/26/03				90	20
SFSR	8/26/03	102	30	20	10	18
SFSR	8/26/03	92			80	17
SFSR	8/26/03	102	60	20	20	12
SFSR	8/26/03		60	10		
SFSR	8/26/03	98	90	20	100	19
SFSR	8/26/03	102	20	20	20	20
SFSR	8/26/03	100			100	20
SFSR	8/29/03	99	40	20	90	20
SFSR	8/29/03	73	80	20	90	13
SFSR	8/29/03	78	90	20	100	11
SFSR	8/29/03		20	20	100	15
SFSR	8/29/03				5	20
SFSR	8/29/03	105	90	20	100	7
SFSR	8/29/03	84			90	13
SFSR	8/29/03	91	0	0	0	7
SFSR	9/2/03	77	90	20		
SFSR					100	17
SFSR	9/2/03	91	70	20	50	20
SFSR	9/2/03	99	20	20	100	20
SFSR	9/2/03	101	0		5	20
SFSR	9/2/03	49	70	20	90	20
SFSR	9/2/03	57	40	20	100	20
SFSR	9/2/03	57	90	20	100	14
SFSR	9/2/03	80	20	20	90	11
SFSR	9/2/03	68			100	20
upper SR, Sawtooth	8/28/03	107	20	20	90	18
upper SR, Sawtooth	8/28/03	100	50	20	80	20
upper SR, Sawtooth	8/28/03	75			5	11
upper SR, Sawtooth	8/28/03	53	80	10		

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI
upper SR, Sawtooth	8/28/03	58			5	13
upper SR, Sawtooth	8/28/03	79	0	0	5	14
upper SR, Sawtooth	8/28/03	87	0	0	0	20
upper SR, Sawtooth	8/28/03	95	80	20	80	12
upper SR, Sawtooth	8/28/03	77	90	20	50	13
upper SR, Sawtooth	8/28/03	85	90	20		
upper SR, Sawtooth	8/28/03	99	90	0	100	8
upper SR, Sawtooth	8/28/03	87	90	20	90	20
upper SR, Sawtooth	8/28/03	93	50	20	70	12
upper SR, Sawtooth	8/28/03	80			90	16
upper SR, Sawtooth	8/28/03	81			100	13
upper SR, Sawtooth	8/28/03	99	90	20		
upper SR, Sawtooth	8/28/03	98	90	20	100	17
upper SR, Sawtooth	8/28/03	72	50	20	90	20
upper SR, Sawtooth	8/28/03	88	90	20	90	20
upper SR, Sawtooth	8/28/03	77	20	10		
Totals				3814		3929

Appendix 4. Data from steelhead collected in 2003.

Table A4. Collection date, fork lengths, percent motilities and number of straws from steelhead collected in 2003.

Collection Site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml straws at WSU	UI Motility (%)	Number of 0.5 ml straws at UI
Cow Creek	4/24/03	610	5	20		
Cow Creek	4/24/03	590	30	20		
Lightning Creek	5/1/03	570			0	30
Little Sheep Creek	3/25/03	760	80	20		
Little Sheep Creek	3/25/03	583	70	20		
Little Sheep Creek	3/25/03	538	90	20		
Little Sheep Creek	3/25/03	593	90	20		
Little Sheep Creek	3/25/03	763	80	20		
Little Sheep Creek	3/25/03	778	90	20		
Little Sheep Creek	3/25/03	585	80	20		
Little Sheep Creek	3/25/03	606	70	20		
Little Sheep Creek	3/25/03	603	90	20		
Little Sheep Creek	4/1/03	710			80	18
Little Sheep Creek	4/1/03	603			90	15
Little Sheep Creek	4/1/03	737			70	20
Little Sheep Creek	4/1/03	609			80	20
Little Sheep Creek	4/1/03	596			80	16
Little Sheep Creek	4/1/03	715			70	17
Little Sheep Creek	4/1/03	535			80	16
Little Sheep Creek	4/1/03	735			80	18
Little Sheep Creek	4/1/03	649			80	20
Little Sheep Creek	4/15/03	565	80	20		
Little Sheep Creek	4/15/03	608	90	20		
Little Sheep Creek	4/15/03	565	70	20		
Little Sheep Creek	4/15/03	640	90	20		
Little Sheep Creek	4/15/03	625	80	20		
Little Sheep Creek	4/15/03	605	90	20		
Little Sheep Creek	4/15/03	575	70	20		
Little Sheep Creek	4/15/03	556	70	20		
Little Sheep Creek	4/15/03	605	90	20		
Little Sheep Creek	4/15/03	780	30	0		
Little Sheep Creek	4/15/03	570	90	20		
Little Sheep Creek	4/15/03	620	70	20		
Little Sheep Creek	4/15/03	615	80	20		
Little Sheep Creek	4/15/03	530	50	20		
Little Sheep Creek	4/15/03	675	70	20		

Collection Site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml straws at WSU	UI Motility (%)	Number of 0.5 ml straws at UI
Little Sheep Creek	4/15/03	600	50	20		
Little Sheep Creek	4/22/03	585			80	17
Little Sheep Creek	4/22/03	620			70	10
Little Sheep Creek	4/22/03	720			80	17
Little Sheep Creek	4/22/03	595			70	18
Little Sheep Creek	4/22/03	622			80	36
Little Sheep Creek	4/22/03	542			80	20
Little Sheep Creek	4/22/03	629			80	20
Little Sheep Creek	4/22/03	643			80	17
Little Sheep Creek	4/22/03	619			60	40
Little Sheep Creek	4/22/03	658			80	19
Little Sheep Creek	4/22/03	545			80	29
Little Sheep Creek	4/22/03	755			80	30
Little Sheep Creek	4/22/03	590			80	20
Little Sheep Creek	4/22/03	668			60	16
Little Sheep Creek	4/22/03	595			70	16
Little Sheep Creek	4/22/03	580			80	36
Little Sheep Creek	4/22/03	613			20	20
Little Sheep Creek	4/22/03	569			60	16
Little Sheep Creek	4/22/03	570			60	15
Little Sheep Creek	4/22/03	677			80	40
Little Sheep Creek	4/22/03	650			70	19
Little Sheep Creek	4/29/03	580				
Little Sheep Creek	4/29/03	658				
Little Sheep Creek	4/29/03	600				
Little Sheep Creek	4/29/03	551				
Little Sheep Creek	4/29/03	625				
Little Sheep Creek	4/29/03	629	90	20		
Little Sheep Creek	4/29/03	549	90	20		
Little Sheep Creek	4/29/03	593	90	20		
Little Sheep Creek	5/6/03	570			70	20
Little Sheep Creek	5/6/03	631			60	20
Little Sheep Creek	5/6/03	597			70	20
Little Sheep Creek	5/6/03	614			70	20
Little Sheep Creek	5/6/03	603			50	20
Little Sheep Creek	5/6/03	600			60	10
Little Sheep Creek	5/6/03	709			70	20
Little Sheep Creek	5/6/03	598			80	20

Collection Site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml straws at WSU	UI Motility (%)	Number of 0.5 ml straws at UI
South Fork Salmon River	4/30/03	860	60	20		
South Fork Salmon River	4/30/03	900	70	20		
South Fork Salmon River	4/30/03	900	90	40		
South Fork Salmon River	4/30/03	950	60	20		
South Fork Salmon River	4/30/03	950	10	20		
South Fork Salmon River	4/30/03	980	90	0		
South Fork Salmon River	5/4/03	900	?	20		
South Fork Salmon River	5/4/03	990	?	20		
South Fork Salmon River	5/4/03	870	?	20		
South Fork Salmon River	5/6/03	770	80	20		
South Fork Salmon River	5/6/03	950	10	20		
South Fork Salmon River	5/6/03	790	50	20		
South Fork Salmon River	5/6/03	880	90	18		
South Fork Salmon River	5/6/03	900	70	20		
South Fork Salmon River	5/6/03	880	90	20		
South Fork Salmon River	5/7/03	750	90	20		
South Fork Salmon River	5/7/03	690	50	20		
TOTALS				918		811

Appendix 5. Steelhead Genetics Report

GENETIC DIVERSITY IN *Oncorhynchus mykiss* FROM THE SNAKE RIVER BASIN AND EASTERN OREGON

A report to the Nez Perce Tribe January 2003

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INTRODUCTION AND OVERVIEW

The following report discusses the genetic diversity of steelhead trout (*Oncorhynchus mykiss*) collected by the Nez Perce Tribe for cryopreservation of male gametes in the years 1999, 2000, and 2001. We have analyzed both mitochondrial DNA D-loop sequence and four microsatellite loci to discern genetic diversity and the relationship among populations. The variable sites chosen for this analysis coincide with those utilized in other population level analyses for *Oncorhynchus mykiss*, particularly for those aimed at differentiating steelhead and rainbow trout in California, Oregon, Washington, and Alaska (Nielsen et al., 1994a, 1994b, 1997; Wenburg et al., 1997; Nielsen and Fountain, 1999; Nielsen, 1999). These variable sites are putatively neutral, have higher rates of mutation than expressed genes, and thus can provide fine-level differentiation between closely related populations of organisms (Nielsen, 1999 and references therein).

The *O. mykiss* sampled were captured at hatchery weirs in 1999, 2000, and 2001, and included fish from Dworshak National Fish Hatchery, Oxbow Hatchery, Pahsimeroi Hatchery, Little Sheep Creek in northeastern Oregon, Imnaha River in Oregon, Grande Ronde River, Fish Creek, and the Selway River (Table 1). Dworshak hatchery was established to mitigate losses of North Fork Clearwater River drainage steelhead following installation of Dworshak dam. Oxbow and Pahsimeroi are funded by the Idaho Power Company and operated by the Idaho Department of Fish and Game to mitigate for salmon and steelhead losses due to the building of the Hells Canyon Complex of dams. Each of the hatcheries has its own unique history regarding stock establishment for hatchery populations. With this in mind, we have attempted to discuss the putative relationships of these hatchery stocks, as well as the relationship of natural Pahsimeroi River steelhead with all other populations.

Table 1. Population information for genetic study of *O. mykiss* for the Nez Perce Tribe (1999-

2001).

Population	Collection Site	Year-classes	Age	Historical makeup	Population information
Clearwater (CW) Idaho	Dworshak Nat. Fish Hatchery	1999 2000 2001	Returning adults	North Fork Clearwater River	B-run hatchery fish
Oxbow, Snake River (OX) Idaho	Hell's Canyon Dam	1999 2000 2001	Returning adults	Snake River	Early returning A- and B-run, some wild fish
Pahsimeroi (PR) Idaho	Pahsimeroi Hatchery and river weir	1999 2000 2001	Returning adults and wild kelts	Snake River	A-run, hatchery not listed, ESA-listed wilds released to spawn
Johnson Creek (JC) Idaho	Johnson Creek screw trap (NPT)	1999	Juvenile	South Fork Salmon River Basin	Resident rainbows or steelhead?
Little Sheep Creek (LSC) Oregon	Wallowa Hatchery	1999 2000 2001	Returning adults	Little Sheep Creek, Imnaha River	A-run, ESA-listed Very few wild fish
Imnaha River (IMR) Oregon	Imnaha River screw trap (NPT) lower river	2000	Adults	Supplemented under LCCRP with Little Sheep Creek (Wallowa Hatchery) summer steelhead	Summer steelhead
Fish Creek (FSH) Idaho	Fish Creek, Idaho	1993 2000	Spawning adults (1993) Wild kelt (2000)	B-run Dworshak steelhead stocked in 1979, 1980	Tributary to Lochsa River (outplants through 1982 by Dworshak)
Selway River (SEL) Idaho	Selway River, Idaho	1994	Spawning adults	Natural stocks	Wild and Scenic River, protected

MATERIALS AND METHODS

Tissue collection and DNA extraction

Steelhead trout (*Oncorhynchus mykiss*) tissue samples were collected in Idaho and Oregon at collection weirs and traps during spring spawning by the Nez Perce Tribe. Fin clips or opercular punches were collected at hatcheries located on the Snake River, Pahsimeroi River, North Fork Clearwater River, Little Sheep Creek (Oregon), Johnson Creek, Imnaha River, Fish Creek, and Grande Ronde River in 1999 and 2000. Additional Fish Creek and Selway River tissues were collected in 1993 and 1994, respectively. A summary of the origin and makeup of these populations is provided in Table 1. Most fish for this 2001 genetic analysis were of hatchery origin and collected from February to May at four locations: Dworshak National Fish Hatchery, Oxbow Hatchery, Little Sheep Creek, and Pahsimeroi Hatchery. Tissues from fish were collected using either opercular punches or caudal fin clips. Tissue was immediately

placed in 6 ml tubes containing 80% ethanol for storage and shipment. Total genomic DNA was extracted from 1 mm² tissue samples using the Puregene[®] Isolation Kit D-5000A and solid tissue protocol (Gentra Systems). Extracted DNA was then quantified using the Hoefer DNA fluorometer Model TKO 100 using Hoechst dye. Samples were diluted in Tris-EDTA (TE) to 50ng/μl prior to PCR amplification.

Mitochondrial Sequencing

PCR amplification of the highly variable 3' end of the mitochondrial control region was carried out using primers known to amplify this region in salmonids (Nielsen et. al., 1994, and references therein). Two primers were used, S-phe (5'-GCTTTAGTTAAGCTACG-3') and P2 (5'-TGTTAAACCCCTAAACCAG-3'), for synthesis of the 193 bp amplified product which includes the 3' end of the control region along with 5 bp of the adjacent phenylalanine tRNA gene. Double stranded PCR amplifications were carried out in 40 μl reactions containing 8.0 μl 5X Buffer C (300mM Tris-HCL, 75mM (NH₄)₂SO₄, 12.5mM MgCl₂), 3.2 μl 10mM dNTP's (2.5mM dATP, 2.5mM dCTP, 2.5mM dGTP, 2.5mM dTTP), 0.8 μl DMSO, 6.0 μl of each primer (4 pM/μl), 13.3 μl ddH₂O, 0.2 μl *Taq* DNA polymerase (5 U/μl, GibcoBRL). Amplifications were performed in an AMPLITRONII (Thermolyne) for 40 cycles of denaturation at 95°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 2 min 30 s with a 4°C chill upon completion.

Amplified double stranded products (5 μl) were then electrophoresed on 1% agarose gels. Gels were stained with an ethidium bromide solution and visualization of DNA was performed using UV trans-illumination. Successfully amplified products were purified using the GeneClean III (Bio 101, Inc.) for use in the second PCR reactions to produce single stranded DNA for sequencing. Single stranded DNA amplification was carried out using fluorescent dyeterminator biochemistry. The cycle sequencing reaction mixture contained 2.0 μl terminator dye premix (Perkin Elmer/ABI), 1.0 μl DMSO, 0.5 μl primer (4 pM/μl), 3.5 μl ddH₂O, 2.0 μl 2.5X Sequence Buffer (5 mM MgCl₂, 200mM Tris-HCl), and 1.0 μl purified DNA for a total volume of 10.0 μl. Sequencing reactions were run for 25 cycles of denaturation at 96°C for 30 s, annealing at 50°C for 15 s, and extension at 60°C for 4 min. with a 4°C chill upon completion. After cycle sequencing, excess unincorporated dye terminators were removed by running samples through 400 μl of Sephadex G-50 in Centri-Sep spin columns (Princeton Separations, Inc.) and vacuum dried. Dried samples were resuspended in 1.0 μl of loading buffer (five parts deionized formamide to *One* part 30 mg/ml Blue Dextran). DNA sequencing was performed on an ABI 377 automated sequencer using 6% acrylamide gel. DNA sequences were assembled and analyzed using the Sequencher[™] 3.1 computer program (Gene Codes Corporation).

Microsatellites

Four microsatellite loci were chosen based upon their polymorphisms and use in previous studies of other *O. mykiss* populations, including the 1999 and 2000 NPT

steelhead genetics reports. *Omy77*, *One2*, *One6*, and *One8* were used and have been named previously according to the species in which they were isolated; *Omy77* was isolated in *O. mykiss* and the *One* microsatellites were isolated in *Oncorhynchus nerka*. Microsatellite loci were amplified from fluorescent labeled forward and unlabeled reverse primers developed previously for *Omy77* (Morris et al., 1996) and *One2*, *One6*, and *One8* (Scribner et al., 1996). *Omy77* and *One2* were amplified in single reactions, while *One6* and *One8* were amplified in the same polymerase chain reaction (PCR) duplex reaction. PCR was performed in a total volume of 20 μ L containing 2.5 mM MgCl₂ for *Omy77* and *One6,8* duplex or 1.5 mM MgCl₂ for *One2*, 1X PCR buffer without MgCl₂ (Gibco BRL), 250 μ M each of dATP, dCTP, dGTP, dTTP, 0.25 μ L of Taq DNA polymerase (5 units/ μ L), and 100 ng of sample DNA. Primer concentrations were 0.125 μ M for *One2*, 0.05 μ M for *One6* and 0.1 μ M for *One8* in duplex, and 0.075 μ M for *Omy77*. The PCR profile for *Omy77* amplifications was 95°C for 3 minutes (pre-dwell), 35 cycles of 1 minute at 95°C (denature), 1 minute at annealing temperature (described below), 2 minutes at 72°C (extend), followed by 5 minutes at 72°C (post-dwell). The annealing temperature was 50°C for *Omy77*. *One2* and the *One6*, *One8* duplex were run in 'touchdown' PCR conditions with the same pre-dwell, denature, extension, and post-dwell parameters, but with the 1 minute annealing steps as follows: 2 cycles each at 62°C, 60°C, and 58°C followed by 30 cycles at 55°C anneal.

Microsatellite alleles for each sample and locus were separated by 5% denaturing polyacrylamide gel electrophoresis on the ABI 377 Sequencing system (Perkin Elmer). Prior to gel electrophoresis, samples were diluted in deionized formamide, blue dextran dye, and Genescan ROX-500 and denatured for 2 minutes at 95°C. Sizing of microsatellite alleles was determined with Genescan ROX-500 size standard run within each sample and analyzed with Genescan and Genotyper software (Perkin Elmer). Allele sizes in base pairs include the total size of the PCR product. Allele frequencies and observed versus expected heterozygosities (H_o and H_e , respectively) for each population were calculated using the Genepop program v3.1c (Raymond and Rousset, 1996). Using Genepop, alleles were randomized across populations to assess deviation from expected heterozygosity under Hardy-Weinberg equilibrium. The proportions of randomizations giving larger H_e than H_o was calculated and is reported as significance levels for testing whether observed and expected heterozygosities are significantly different. F_{ST} estimates were performed as per Weir and Cockerham (1984) to calculate intra-class correlation using allele frequency; F_{ST} estimates were subsequently used for pairwise genetic estimates in the Genepop program.

RESULTS AND DISCUSSION

Mitochondrial Haplotypes

Analysis of the 193 base pair sequence identified eight variable sites in the populations studied. In 1999, five mitochondrial haplotypes were identified. Two haplotypes, ST19 and ST21, were unique to the populations studied. ST19 was found in populations from Little Sheep Creek, Oxbow Hatchery, and wild Pahsimeroi River samples. In 1999, a single individual from the Oxbow Hatchery had haplotype ST21. In 2000, three additional mitochondrial haplotypes not observed in 1999 were found; ST21 observed in 1999 was not present in any 2000 or 2001 samples. Haplotypes ST23 and ST24 were present only in 2000. In 2001, one individual from Dworshak National Fish Hatchery was identified with the ST25 haplotype (Table 2). The most common haplotype for the years 1999, 2000, and 2001 was ST1, occurring at a 60.9% frequency; ST2 occurred all three years at a 6.8% frequency; ST9 was the second most common haplotype for all three years, occurring at a frequency of 18.2%; ST19 was present all three years at a 9.9% frequency. ST21, present only in 1999, occurred at 0.52% frequency; ST22, present in 2000 and 2001, occurred at 1.04% frequency; ST23, present only in 2000, occurred at 1.04% frequency; ST24, also present only in 2000, occurred at 1.04% frequency; and ST25, present only in 2001, occurred at 0.52% frequency. Maximum sequence divergence between haplotypes was 2.1% with a mean distance of 1.0%.

Table 2. Mitochondrial haplotype variability for *Oncorhynchus mykiss* collected from the Snake and Salmon River drainages. *Numbers correspond to Digby et al., 1992. Variable nucleotide sites are indicated in red. ST19, ST21, ST22, ST23, ST24, and ST25 are mitochondrial haplotypes unique to study (shaded).

	Variable Sites							
haplotype	25	60	101	136	148	172	186	244
ST1	A	T	A	G	G	T	G	G
ST2	A	C	A	G	G	T	G	G
ST9	A	T	A	G	G	T	A	G
ST19	A	T	A	G	G	C	G	G
ST21	A	T	A	G	T	T	A	G
ST22	A	T	A	A	G	T	G	G
ST23	A	T	A	G	G	T	G	A
ST24	G	C	A	G	G	T	G	G
ST25	A	T	G	G	G	T	G	G

Table 3. Pairwise distance matrix for 9 steelhead mtDNA haplotypes.

mtDNA* haplotype	1	2	9	19	21	22	23	24	25
1	-	0.5	0.5	0.5	1.0	0.5	0.5	1.0	0.5
2	1	-	1.0	1.0	1.6	1.0	1.0	0.5	1.0
9	1	2	-	1.0	0.5	1.0	1.0	1.6	1.0
19	1	2	2	-	1.6	1.0	1.0	1.6	1.0
21	2	3	1	3	-	1.6	1.6	2.1	1.6
22	1	2	2	2	3	-	1.0	1.6	1.6
23	1	2	2	2	3	2	-	1.6	1.0
24	2	1	3	3	4	3	3	-	1.6
25	1	2	2	2	3	2	2	3	-

*percent sequence divergence above diagonal and number of differences below diagonal

Haplotype variation was present between populations and among year-classes within single populations (Fig 1). Within the 1999 studied populations, mitochondrial haplotype diversity was greatest in the Oxbow and Wallowa Hatcheries, with each having four mitochondrial haplotypes. Although only three haplotypes were observed in the Oxbow populations during the 2000 study, one haplotype, ST22, was new. An additional haplotype, ST24, was found in the Little Sheep Creek population during the 2000 study along with the previous four haplotypes. High mitochondrial haplotype diversity at these locations indicate a good representation of the source populations, provided no additional supplementation from outside sources has occurred. The inadvertent advancement of run timing at Oxbow Hatchery and subsequent attempts to return the run to more natural timing may have altered the mitochondrial haplotype frequency distribution (Armstrong, personal communication). Unfortunately this cannot be determined since return rates of native fish to Hells Canyon Dam, the collection source for the hatchery, are extremely low. Comparisons between wild and hatchery fish from Little Sheep Creek may be needed to ensure the status of this ESA listed run. In 2001, three haplotypes were identified in the Little Sheep Creek population. Both ST1 and ST19 haplotypes were present in hatchery and wild populations; however, ST2 was present only within the hatchery sample and not in wild fish.

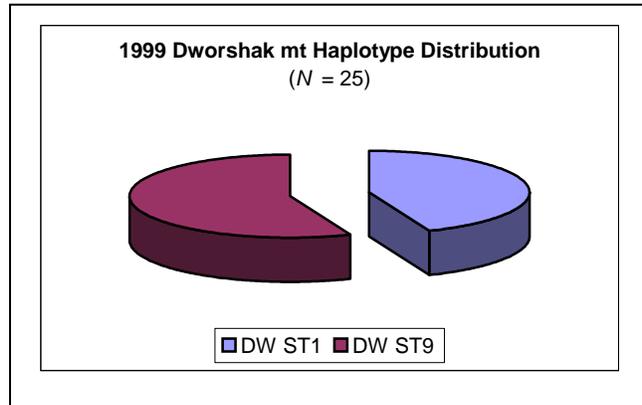
Samples from Dworshak Hatchery contained only two mitochondrial haplotypes, ST1 and ST9, in 1999, but additional haplotypes unique to Dworshak were observed in 2000 (ST23) and 2001 (ST25). The additions of ST 23 in 2000 and ST 25 in 2001 may indicate year class differences in mitochondrial haplotypes, but due to low sample size this cannot be assured. The Dworshak population's distinctive mitochondrial frequency distribution in 1999 and 2000 is consistent with allozyme frequencies unique to this population (Williams, 1994).

Individuals from Pahsimeroi Hatchery exhibited only two haplotypes in 2000, ST1 and ST9; in 2001, two Pahsimeroi individuals were found to have ST19 haplotypes. Haplotype information for hatchery fish is indicative of the stock collection from the Snake River; the absence of ST2 could again indicate the presence of year class

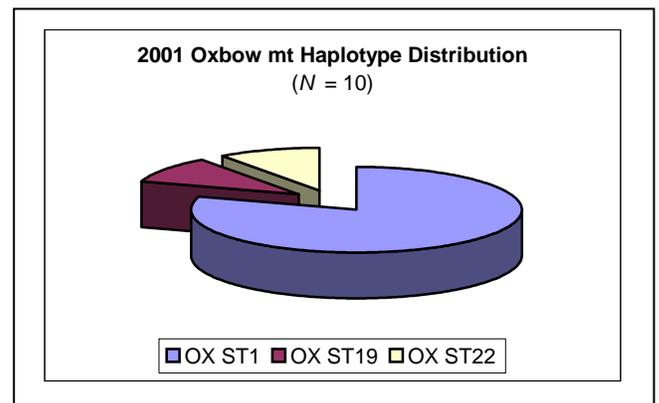
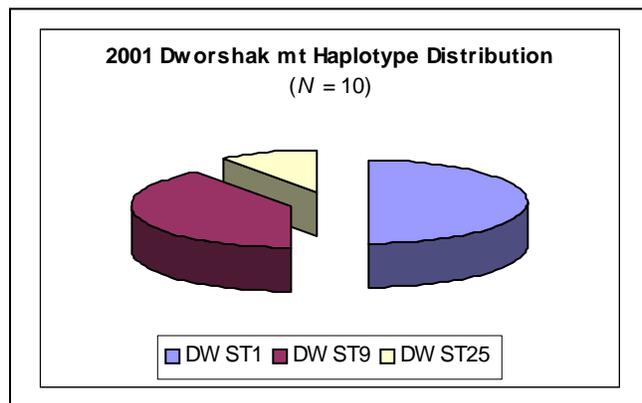
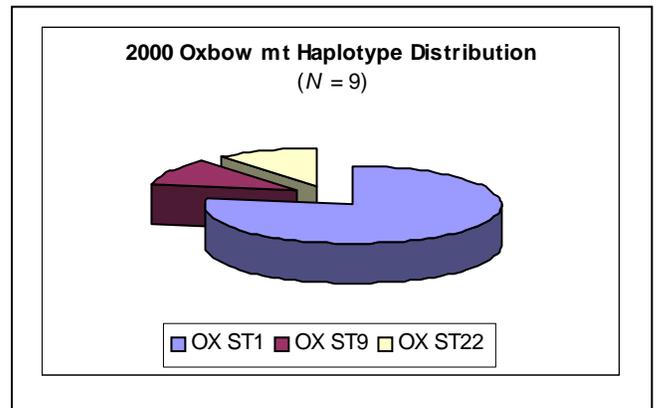
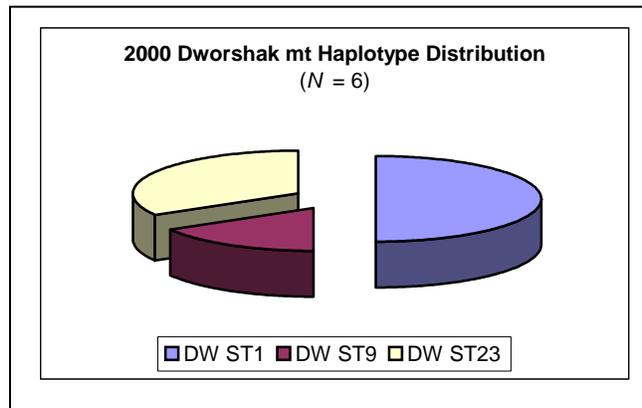
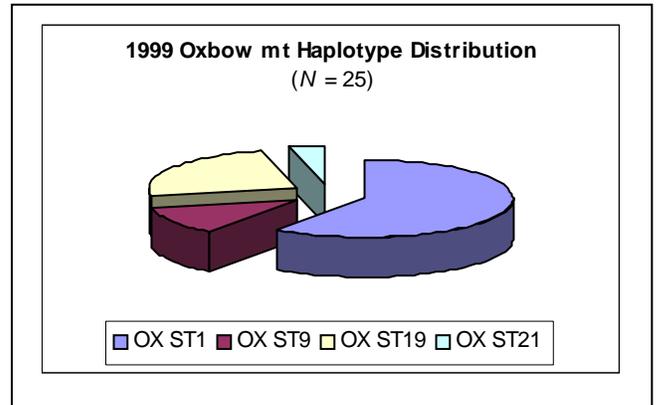
differences.

Fig. 1 Mitochondrial haplotype distribution by population and year class. (a) Dworshak. (b) Oxbow. (c) Pahsimeroi. (d) Little Sheep.

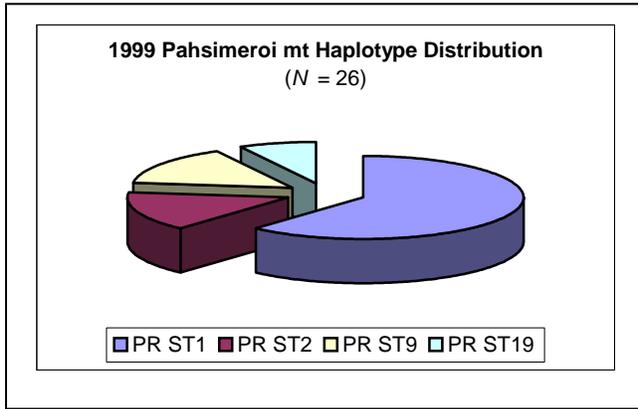
(a)



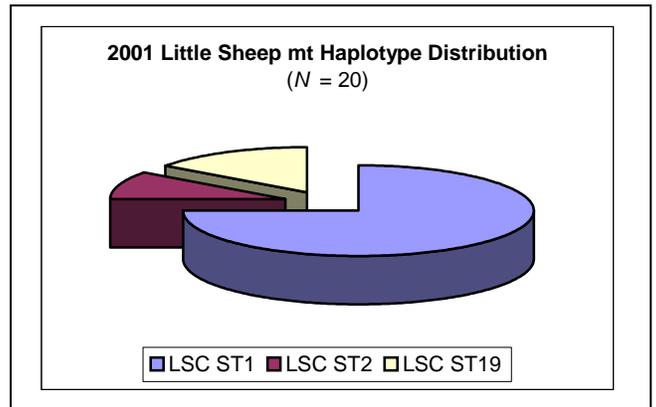
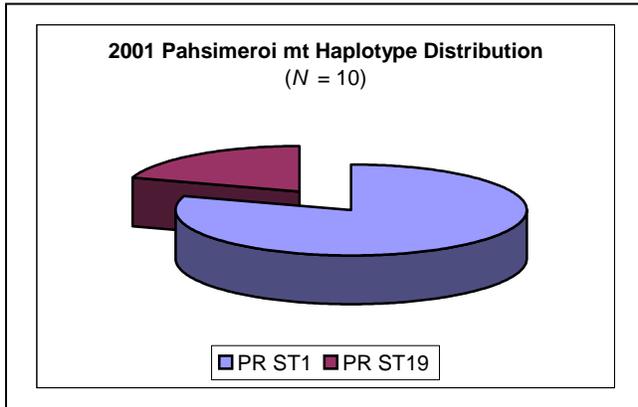
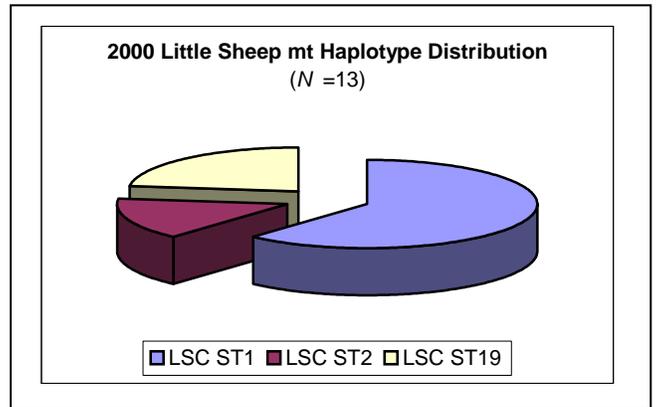
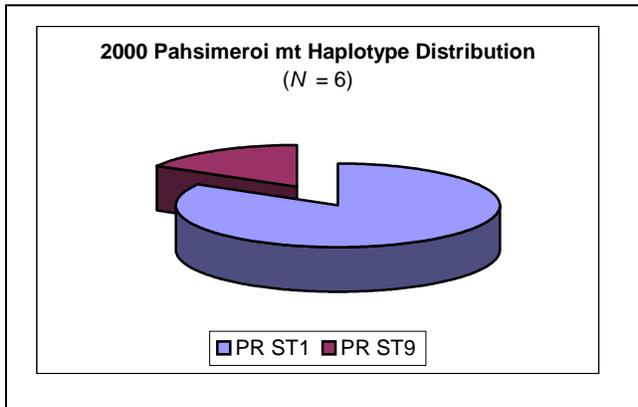
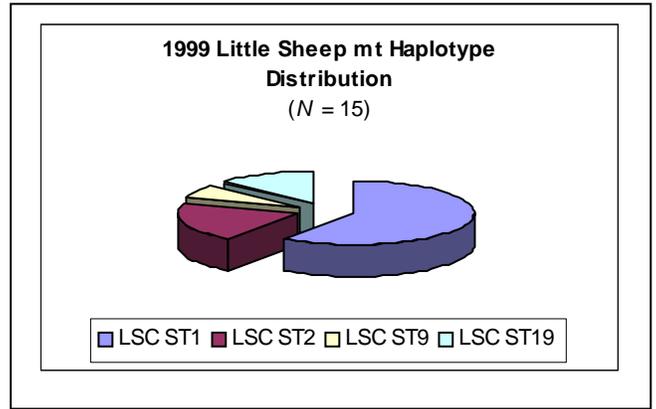
(b)



(c)



(d)



Microsatellites

Hardy-Weinberg test- Tests for Hardy-Weinberg equilibrium were performed to confirm the microsatellite loci chosen for this study were neutral markers. Of the four microsatellite loci examined, p-values for each locus varied among populations (Table 4). For *Omy77*, p-values ranged from 0.0362 for Little Sheep to 0.4168 for Oxbow. *One2* p-values ranged from less than .05 for both wild Little Sheep and Pahsimeroi populations to 0.2038 for the Oxbow population. *One6* p-values ranged from less than .05 for both the hatchery Little Sheep and Oxbow populations to 0.2487 for Dworshak. *One8* p-values ranged from less than .05 for both hatchery and wild Little Sheep individuals and Dworshak to 0.6205 for the Oxbow population.

Among all populations, *Omy77* had a p-value of 0.1690; *One2* had a p-value of 0.1446; *One6*'s p-value was 0.0791 for the pooled populations; and *One8*'s p-value was 0.1561. While certain loci within populations had p-values less than 0.05, pooled population p-values were all greater than 0.05, indicating the four loci examined are inherited in a Mendelian fashion and lie within H-W equilibrium. In other studies, *Omy77*, *One2*, *One6*, and *One8* have been shown to be neutral markers (Nielsen 1999); therefore our assumption is merited.

Table 4: Tests for Hardy-Weinberg equilibrium for each locus at each sample population: Oxbow, Dworshak, Little Sheep, and Pahsimeroi. Lower table contains pooled populations as listed (p-values < 0.05 indicate rejection of the null hypothesis and locus is not in H-W equilibrium).

Population	<i>Omy77</i>	<i>One2</i>	<i>One6</i>	<i>One8</i>
<i>Oxbow</i>	0.4168	0.2038	0.0000	0.6205
Dworshak	0.3021	0.0700	0.2487	0.0460
Little Sheep	0.0362	0.4406	0.0006	0.0006
Little Sheep (wild)	0.0387	0.0081	0.0982	0.0224
Pahsimeroi	0.0899	0.0003	0.0480	0.0909

	<i>Omy77</i>	<i>One2</i>	<i>One6</i>	<i>One8</i>
Pooled Populations	0.1690	0.1446	0.0791	0.1561

Population variation- Allelic frequencies varied across loci (Table 5). Sixteen different alleles were scored for *Omy77*. The 114, 116, and 120 bp alleles were present in all four populations. For the Oxbow population, eight alleles were identified with 114 and 116 bp alleles the most common, occurring at frequencies of .250 and .350 respectively. In both 1999 and 200, a 122 bp allele was most common at the *Omy77* locus in Oxbow individuals. In the 2001 sample, a 106 bp allele was unique to the Oxbow population. In the Dworshak population, eight alleles were identified at *Omy77* with a 114 bp allele occurring at the highest frequency of .650. In 2000, 116, 118, and 124 bp alleles were most common; in

1999, the 132 and 125 bp alleles occurred most frequently. No private alleles were present in the Dworshak population in 2001. Nine alleles were identified in the hatchery reared Little Sheep population at *Omy77* with a 114 bp allele most common occurring at a frequency of .250. In 2000, a 122 bp allele was most common while a 118 bp allele was most common in 1999. For 2001, 92 and 98 bp alleles were unique to Little Sheep hatchery-reared individuals. In wild Little Sheep individuals, seven alleles were identified with a 118 bp allele occurring at the highest frequency of .333. Two alleles, 106 bp and 118 bp, were present in the wild population but not in hatchery-reared individuals. No private alleles occurred in wild individuals sampled. Ten alleles were scored for the Pahsimeroi population at *Omy77*, with 112 and 114 bp alleles being most common in 2001 occurring at a frequency of .222; in both 1999 and 2000, a 122 bp allele was most common. Alleles 102 bp and 112 bp were unique to Pahsimeroi.

At *One2*, twenty alleles were identified across all populations. For the Oxbow population, five alleles were identified in 2001 with a 232 bp allele being most common; in 2000, 236 and 256 bp alleles were most common, while a 254 bp allele occurred most frequently in 1999. Alleles of 230, 234, 236, and 252 bp were unique to Oxbow for 2001. Four alleles were identified at the *One2* locus for the Dworshak population in 2001, with the most common alleles being 224 and 232 bp occurring at a frequency of .333; in 2000 a 238 bp allele was most common; in 1999, a 250 bp allele was most common at the *One2* locus. The 210 bp allele was unique to Dworshak individuals. Six alleles were scored at *One2* in the Little Sheep hatchery-reared population with 254 and 260 bp alleles being most common; in 2000, 228, 230, and 244 bp alleles were most common; in 1999, a 254 bp allele occurred at the highest frequency at the *One2* locus for the Little Sheep population. Alleles unique to hatchery-reared Little Sheep individuals in 2001 include: 260, 268, 270, 272, and 284 bp. Wild Little Sheep individuals had five alleles present at *One2* with 244, 258, and 282 bp alleles occurring at a frequency of .250; the other two alleles, 232 and 246 bp, occurred at a frequency of .125. A 244 bp and 282 bp allele were unique to wild Little Sheep individuals. Pahsimeroi individuals had five alleles identified at the *One2* locus in 2001, with a 228 bp allele being most common occurring at a frequency of .333. In 2000, a 232 bp allele occurred at the highest frequency, while in 1999, a 256 bp allele was most common. The remaining four alleles identified in 2001, 226, 246, 256, and 258 bp, occurred at a frequency of .167 with the 256 bp allele unique to Pahsimeroi individuals.

Eighteen alleles were identified at the *One6* locus. Oxbow featured eight alleles at *One6* with a 250 bp allele most common at a frequency of .333. In 2000, a 264 bp allele occurred most frequently; in 1999, a 250 bp allele was most common. A 230 bp allele was unique to Oxbow individuals at the *One6* locus in 2001. In the Dworshak population, eight alleles were also identified at the *One6* locus, with a 250 bp allele occurring at the highest frequency of .350, while a 266 bp allele was unique to Dworshak in 2001. In 1999 and 2000, a 254 bp allele was most common at *One6* for Dworshak individuals. At the *One6* locus in hatchery-reared Little

Sheep individuals, nine alleles were identified with 236 and 250 bp alleles occurring at the highest frequency of .250. Alleles of 232 bp and 272 bp were unique to the hatchery-reared Little Sheep population. In 2000, a 250 bp allele was most common at *One6*, while a 264 bp allele was most common in 1999. Six alleles were scored in wild Little Sheep individuals at the *One6* locus in 2001 with a 250 bp allele being most common occurring at frequency of .500. No private alleles were found in the wild Little Sheep population. Pahsimeroi individuals had seven alleles present at *One6*. In 2001, a 254 bp allele was most common at a frequency of .357, while the 258 bp allele was unique to Pahsimeroi. In 2000, a 254 bp allele was most common while in 1999 a 250 bp allele occurred most frequently at *One6* for Pahsimeroi individuals.

Thirteen alleles were identified at the *One8* locus across all four populations. In the Oxbow population, seven alleles were present in 2001 with a 146 bp allele occurring at the highest frequency of .250. 152, 154, 156, and 164 bp alleles were present only within the Oxbow population for 2001. In 2000, 158 and 166 bp alleles were most common; in 1999, a 150 bp allele occurred at the highest frequency. In the Dworshak population, eight alleles were identified at the *One8* locus, with a 162 bp allele being most common at a frequency of .300. In 2000, a 150 bp allele occurred most frequently while a 166 bp allele was most common in 1999. In 2001, a 170 bp allele was unique to Dworshak. Five alleles were present at *One8* for hatchery-reared Little Sheep individuals, with a 150 bp allele occurring at the highest frequency of .409. Alleles of 158 bp and 166 bp were most common at *One8* in 1999 and 2000 for Little Sheep individuals. No private alleles were scored for hatchery-reared Little Sheep individuals at *One8* in 2001. In the wild Little Sheep individuals, five alleles were scored with a 150 bp allele being most common at a frequency of .400. No private alleles were present in wild Little Sheep individuals. In the Pahsimeroi population, four alleles at the *One8* locus were identified with 150 and 158 bp alleles occurring most frequently at .333. No private alleles were found in the Pahsimeroi population at the *One8* locus. In 1999, a 160 bp allele was most common while in 2000, 158 and 162 bp alleles occurred at the highest frequency for the *One8* locus.

Table 5: Allele frequencies at *Omy77*, *One2*, *One6*, and *One8* for steelhead sampling groups: Oxbow, Dworshak, Little Sheep, and Pahsimeroi.

Omy77

Allele	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)	Pahsimeroi
92			.042		
98			.042		
102					.111
106	.050				
108		.050		.083	.056
112					.222
114	.250	.650	.250	.083	.222
116	.350	.050	.125	.167	.111
118		.050		.333	
120	.050	.050	.125	.167	.056
122	.050		.1167	.083	.056
124		.050	.042		.056
126	.100	.050			.056
128	.100	.050			
130	.050		.125		.056
132			.083	.083	

One2

Allele	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)	Pahsimeroi
210		.167			
224		.333			
226		.167			.167
228					.333
230	.167				
232	.333	.333		.125	
234	.167				
236	.167				
244				.250	
246				.125	.167
252	.167				
254			.333		
256					.167
258				.250	.167
260			.250		
268			.083		
270			.083		
272			.167		
282				.250	
284			.083		

One6

Allele	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)	Pahsimeroi
228	.056				.143
230	.111				
232			.050		
234			.050	.143	
236	.111	.100	.250		.071
238		.100		.071	
240	.056	.050			
242	.111	.150			
246			.100	.071	
248	.111		.050	.143	
250	.333		.250	.500	
252		.100	.050	.071	
254	.111	.350			.357
256		.050			.071
258					.071
262			.050		.143
264					.143
266		.100			
272			.100		

One8

Allele	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)	Pahsimeroi
146	.250		.136	.200	
150		.150	.409	.400	.333
152	.083				
154	.083				
156	.167				
158	.167	.150	.318	.100	.333
160		.150		.200	.250
162		.300			
164	.083				
166	.167	.050		.100	
168		.050	.045		
170		.100			.083
172		.050	.091		

Levels of Heterozygosities- Observed levels of heterozygosities were lower than expected levels of heterozygosities for all loci (Table 6). For the Oxbow population, *Omy77* had an observed heterozygosity (HO) frequency of 7 compared to an expected heterozygosity (HE) of 8.263; *One2* had an HO of value of 2 compare to an HE value of 2.800; *One6* had an HO value of 4 compared to an

HE of 7.824; locus *One8* had an HO value of 5 compared to an HE value of 5.455.

Within the Dworshak population, *Omy77* had an HO value of 5 compared to an HE value of 5.895; *One2* had an HO of value of 1 compare to an HE value of 2.600; *One6* had an HO value of 7 compared to an HE of 8.526; locus *One8* had an HO value of 6 compared to an HE value of 8.684.

In hatchery-reared Little Sheep individuals, locus *Omy77* had an HO value of 9 compared to an HE value of 10.652; *One2* had an HO of value of 5 compared to an HE value of 5.091; *One6* had an HO value of 5 compared to an HE of 8.842; locus *One8* had an HO value of 4 compared to an HE value of 8.095. Wild Little Sheep individuals had an HO value of 3 compared to an HE value of 5.273 for locus *Omy77*; *One2* had an HO of value of 1 compare to an HE value of 3.571; *One6* had an HO value of 4 compared to an HE of 5.231; locus *One8* had an HO value of 2 compared to an HE value of 4.111.

Pahsimeroi individuals, *Omy77* had an HO value of 7 compared to an HE value of 8.177; *One2* had an HO of value of 0 compare to an HE value of 5.091; *One6* had an HO value of 5 compared to an HE of 6.000; locus *One8* had an HO value of 3 compared to an HE value of 4.636.

Table 6: Sample by population and locus, range of alleles, number of alleles, expected and observed heterozygosity, percent private alleles, and number of individuals scored.

Oxbow

Locus	R	A	HE	HO	%PA	N
<i>Omy77</i>	106-130	7	8.2632	7	0.063	10
<i>One2</i>	230-252	5	2.8000	2	0.200	3
<i>One6</i>	228-254	8	7.8235	4	0.056	9
<i>One8</i>	146-166	7	5.4545	5	0.308	6

Dworshak

Locus	R	A	HE	HO	%PA	N
<i>Omy77</i>	108-128	8	5.8947	5	0.000	10
<i>One2</i>	210-232	4	2.6000	1	0.100	3
<i>One6</i>	236-266	8	8.5263	7	0.056	10
<i>One8</i>	150-172	8	8.6842	6	0.154	10

Little Sheep

Locus	R	A	HE	HO	%PA	N
<i>Omy77</i>	92-132	9	10.6522	9	0.286	12
<i>One2</i>	254-284	6	5.0909	5	1.000	6
<i>One6</i>	232-272	9	8.8421	5	0.222	10
<i>One8</i>	146-172	5	8.0952	4	0.000	11

Little Sheep (wild)

Locus	R	A	HE	HO	%PA	N
<i>Omy77</i>	108-132	7	5.2727	3	0.000	6
<i>One2</i>	232-282	5	3.5714	1	0.400	4
<i>One6</i>	234-252	6	5.2308	4	0.000	7
<i>One8</i>	146-166	5	4.1111	2	0.000	5

Pahsimeroi

Locus	R	A	HE	HO	%PA	N
<i>Omy77</i>	102-130	10	8.1765	7	0.125	9
<i>One2</i>	226-258	5	5.0909	0	0.100	6
<i>One6</i>	228-264	7	6.0000	5	0.056	7
<i>One8</i>	150-170	4	4.6364	3	0.000	6

Range of allele sizes (R), number of alleles observed (A), expected (HE) and observed (HO) heterozygosities, percent private alleles (%PA), and individuals scored (N), are given for each locus in each population.

F-statistics- F_{ST} represents a measure of the Wahlund principle, where reduction in heterozygosity results due to population subdivision (Wahlund, 1928); therefore F_{ST} should effectively serve as a measure of heterozygosity deficit relative to expectations under Hardy-Weinberg equilibrium (Hartl and Clark, 1997). Estimates of F_{ST} were performed as per Weir and Cockerham 1984 (Table 7). *Omy77* had an F_{ST} value of 0.0578; *One2* had an F_{ST} value of 0.0391; *One6* had an F_{ST} value of 0.0620; *One8* had an F_{ST} value of 0.0339.

Ideally, F_{ST} estimates should correct for the effects of a limited number of subpopulations containing a small number of individuals; however corrections for limited sample sizes tend to be complex and give rise to additional issues. F_{ST} values reported here are based on the assumption that limited sample sizes collected are an accurate reflection of the genetic diversity found throughout subpopulations analyzed. Allele frequencies among subpopulations can differ due to random processes (genetic drift) as well as by natural selection and migration among subpopulations; difficulties in assigning the cause of variation among subpopulations do not invalidate the usefulness of F_{ST} as a measure of genetic differentiation (Hartl and Clark, 1997).

Table 7: Intra class correlation using allele frequency (F-statistics are estimated (F_{WC}) as in Weir and Cockerham 1984). Lower table summarizes F-statistics across populations.

Omy77:

Genotypes:	2 12 14 6 8 16 8 18 14 20 14 14 14 16 18 14 20 14 16 20 24 18 20
22 92	
Pop.	12 12 14 16 16 16 18 18 20 20 22 24 26 26 26 28 28 30 30 30 30 32
32 32 98 All	

Oxbow	0 0 1 1 0 2 0 0 0 0 1 0 1 1 0 1 1 0 1 0 0 0
0 0 0 10	
Dworshak	0 0 5 0 1 0 0 0 1 0 0 1 0 0 1 1 0 0 0 0 0 0
0 0 0 10	
Little Sheep	0 0 1 0 0 1 0 0 0 1 3 0 0 0 0 0 0 1 1 0 1 0
1 1 1 12	
Little Sheep (wild)	0 0 0 0 0 1 1 1 0 1 1 0 0 0 0 0 0 0 0 0 0 1
0 0 0 6	
Pahsimeroi	2 1 1 0 1 0 0 0 0 0 1 1 0 1 0 0 0 0 0 1 0 0
0 0 0 9	
All:	2 1 8 1 2 4 1 1 1 2 6 2 1 2 1 2 1 1 2 1 1 1 1
1 1 47	

$F_{ST} = 0.057787$

One2:

Genotypes:	24 10 26 28 32 30 44 32 46 34 56 58 54 54 60
72 82 54	
Pop.	24 26 26 28 32 36 44 46 46 52 56 58 60 68 70 72
82 84 All	

Oxbow	0 0 0 0 1 1 0 0 0 1 0 0 0 0 0 0 0 0
3	
Dworshak	1 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
3	
Little Sheep	0 0 0 0 0 0 0 0 0 0 0 0 2 1 1 1 0 1
6	

Little Sheep (wild)	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0
4																		
Pahsimeroi	0	0	1	2	0	0	0	0	1	0	1	1	0	0	0	0	0	0
6																		
All:	1	1	1	2	2	1	1	1	1	1	1	2	2	1	1	1	1	1
22																		

$F_{ST} = 0.039122$

One6:

Genotypes:
 30 32 34 28 36 36 36 28 40 42 38 46 36 48 50 50 38 42 52 54 28
 54 54 62 66 72
Pop: 30 34 34 36 36 38 40 42 42 42 46 46 50 50 50 52 54 54 54 54 56
 56 58 64 66 72 All

Oxbow 1 0 0 0 1 0 0 1 1 0 0 0 0 2 2 0 0 0 0 1 0
 0 0 0 0 0 9
Dworshak 0 0 0 0 0 1 1 0 0 1 0 0 0 0 0 0 1 1 2 1 0
 1 0 0 1 0 10
Little Sheep 0 1 0 0 2 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 0
 0 0 1 0 1 10
Little Sheep (wild) 0 0 1 0 0 0 0 0 0 0 1 0 0 2 2 1 0 0 0 0 0
 0 0 0 0 0 7
Pahsimeroi 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 1
 0 1 2 0 0 7

 All: 1 1 1 1 3 1 1 1 1 1 1 1 1 5 5 2 1 1 2 4 1 1
 1 3 1 1 43

$F_{ST} = 0.062045$

One8:

Genotypes:
 46 50 54 46 50 51 56 58 58 60 62 46 58 64 50 58 60
 62 62 72
Pop: 46 50 56 58 58 58 58 58 60 60 62 66 66 66 68 70 70
 70 72 72 All

Oxbow 1 0 1 0 0 1 1 0 0 0 0 1 0 1 0 0 0 0
 0 0 6
Dworshak 0 1 0 0 0 0 0 0 1 1 2 0 1 0 1 1 0 1
 1 0 10
Little Sheep 0 4 0 3 0 0 0 2 0 0 0 0 0 0 1 0 0 0
 0 1 11
Little Sheep (wild) 0 2 0 1 0 0 0 0 0 1 0 1 0 0 0 0 0 0
 0 0 5
Pahsimeroi 0 1 0 0 2 0 0 1 0 1 0 0 0 0 0 0 1 0
 0 0 6

 All: 1 8 1 4 2 1 1 3 1 3 2 2 1 1 2 1 1 1 1 1 1
 38

$F_{ST} = 0.033924$

F-statistics across all populations:

Locus	F_{ST}
-----	-----
<i>Omy77</i>	0.0578
<i>One2</i>	0.0391
<i>One6</i>	0.0620
<i>One8</i>	0.0339
All:	0.0691

Pairwise genetic distance- Pairwise genetic distance was estimated using the Weir and Cockerham (1984) method to calculate F_{ST} ; pairwise F_{ST} scores (reduction in heterozygosity due to population subdivision) are listed in Table 8. *Omy77* pairwise F_{ST} values ranged from -0.0001 for the hatchery-reared Little Sheep/Pahsimeroi populations to 0.1159 for Oxbow/Dworshak populations; *One2* pairwise F_{ST} values ranged from -0.1250 for Oxbow/Dworshak populations to 0.0986 for the Oxbow/hatchery-reared Little Sheep populations; *One6* pairwise F_{ST} values ranged from -0.195 for the Oxbow/hatchery-reared Little Sheep populations to 0.1845 for the wild Little Sheep/Pahsimeroi populations; *One8* pairwise F_{ST} values ranged from -0.0416 from the hatchery-reared Little Sheep/wild Little Sheep populations to 0.0912 for the Oxbow/Pahsimeroi populations. Pairwise F_{ST} values for all loci ranged from -0.0008 for the Oxbow/wild Little Sheep populations to 0.0930 for the Dworshak/hatchery-reared Little Sheep population.

According to Wright (1978), F_{ST} values less than $.05$ may be considered as indicating little genetic differentiation between populations; F_{ST} values from $.05$ to $.15$ may be considered to indicate moderate genetic differentiation; and F_{ST} values greater than $.15$ may be considered to indicate great genetic differentiation between populations. Pairwise F_{ST} values greater than $.15$ were calculated for *Omy77* and *One6* in the wild Little Sheep/Dworshak populations, and at *One6* in the Pahsimeroi/wild Little Sheep populations. Pairwise F_{ST} values from $.05$ to $.15$ were calculated at *Omy77* Dworshak/Oxbow populations and the hatchery-reared Little Sheep/Dworshak populations. Pairwise F_{ST} values from $.05$ to $.15$ were calculated at *One2* for the hatchery-reared Little Sheep/Oxbow, hatchery-reared Little Sheep/Dworshak, wild Little Sheep/hatchery-reared Little Sheep, and Pahsimeroi/hatchery-reared Little Sheep populations. At *One6*, pairwise F_{ST} values from $.05$ to $.15$ were found in Dworshak/Oxbow, Pahsimeroi/Oxbow, and Pahsimeroi/hatchery-reared Little Sheep populations. Pairwise F_{ST} values from $.05$ to $.15$ at *One8* were calculated for the Dworshak/ Oxbow, hatchery-reared Little Sheep/Oxbow, hatchery-reared Little Sheep/Dworshak, and Pahsimeroi/Oxbow populations. All other pairwise F_{ST} values were less than $.05$. Pairwise F_{ST} values for all loci were less than $.05$ for all population pairs except wild Little Sheep/Dworshak and hatchery-reared Little Sheep/ Dworshak populations.

Table 8: Pairwise IIS for population pairs (F_{ST} estimated as in Weir and Cockerham 1984).

Estimates for each locus:

Omy77:

population	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)
Dworshak	0.1159			
Little Sheep	0.0102	0.0924		
Little Sheep (wild)	0.0443	0.1880	0.0195	
Pahsimeroi	0.0190	0.1016	- 0.0001	0.0337

One2:

population	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)
Dworshak	-0.1250			
Little Sheep	0.0986	0.1138		
Little Sheep (wild)	-0.0546	-0.0457	0.0962	
Pahsimeroi	0.0081	- 0.0041	0.1083	-0.0343

One6:

population	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)
Dworshak	0.0570			
Little Sheep	-0.0195	0.0893		
Little Sheep (wild)	-0.0179	0.1744	0.0141	
Pahsimeroi	0.0627	-0.0009	0.0797	0.1845

One8:

population	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)
Dworshak	0.0663			
Little Sheep	0.0838	0.0740		
Little Sheep (wild)	0.0262	0.0176	-0.0416	
Pahsimeroi	0.0912	0.0131	-0.0275	-0.0664

Estimates for all loci:

population	Oxbow	Dworshak	Little Sheep	Little Sheep (wild)
Dworshak	0.0277			
Little Sheep	0.0449	0.0930		
Little Sheep (wild)	-0.0008	0.0833	0.0267	

Pahsimeroi 0.0453 0.0259 0.0455 0.0363

Microsatellite loci reliability- As per conversations with the Nez Perce Tribe, and among groups interested in Snake and Salmon River steelhead genetics, there is a need to prioritize sampling and standardize loci for genetic analyses. To this end, we feel the microsatellite loci chosen for this study have been useful, but shall comment on the reliability of each. *Omy77* and *One6* have been the most reliably amplified and scored. *One8* is reliably amplified, but can be difficult to score due to complex patterns of stuttering. Finally, we do not recommend the use of *One2* in future studies due to extreme stuttering and susceptibility to erroneous sizing of alleles for this locus. As reported in previous years, we believe the loci chosen for these analyses accurately depict the relationship among populations, and for the objectives of this project, do not believe that the addition of more loci would significantly alter the relationships observed.

Conclusions and Recommendations

Mitochondrial DNA haplotypes and microsatellite allelic distribution appear to have a surprising level of diversity between populations and among year-classes within single populations. Both mitochondrial DNA haplotypes and microsatellite allele frequencies varied between 1999, 2000, and 2001, indicating there are differences in the genetic diversity of these populations between the three years. With these differences, it is difficult to make conclusions about the overall relationships among some stocks.

In future analyses, we would recommend the continued use of *Omy77*, *One6*, and *One8* for microsatellite scoring, with possible choice of new loci that may be more reliably scored. Continued sampling and similar genetic analyses will aid delineating the relationships among populations over time. Small sample sizes make it difficult to infer with certainty relationships between hatchery-reared and 'wild' fish. In this 2001 study, it appears hatchery-reared Little Sheep individuals accurately reflect the genetic composition of wild Little Sheep fish. F_{ST} values at *Omy77*, *One6*, and *One8* were all less than .05 for pairwise genetic distance estimates. *One2*'s pairwise genetic distance estimate was greater than .05 ($F_{ST} = .0962$), but this may be due to erroneous scoring and exceedingly small sample size for the *One2* locus. This data suggests little genetic variation is present between 'wild' and hatchery-reared Little Sheep fish.

Although we realize the limitations in obtaining large sample sizes, especially for 'wild' fish, we feel future efforts for genetic analyses of these populations could benefit from larger sample sizes within the same year classes for better dissection of population relationships and substructure (minimum 10 individuals); and a priority should be placed on assessing genetic relationships between 'wild' and hatchery-reared fish collected at the same locations.

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