Salmonid Gamete Preservation in the Snake River Basin

**Annual Report
2002**

This Document should be cited as follows:

Young, William, Paul Kucera, "Salmonid Gamete Preservation in the Snake River Basin ", 2002 Annual Report, Project No. 199703800, 63 electronic pages, (BPA Report DOE/BP-00004000-2)

Bonneville Power Administration
P.O. Box 3621
Portland, OR 97208

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**

2002 Annual Report

© 1995 Nez Perce Tribe

Prepared by:

William P. Young, Ph.D.
Paul A. Kucera

Nez Perce Tribe
Department of Fisheries Resources Management
P.O. Box 365
Lapwai, Idaho 83540

July 2003

Salmonid Gamete Preservation in the Snake River Basin

2002 Annual Report

Prepared by:

William P. Young, Ph.D.
Paul A. Kucera

Nez Perce Tribe
Department of Fisheries Resources Management
Lapwai, Idaho 83540

Prepared for:

U.S. Department of Energy
Bonneville Power Administration
Environment, Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97208-3621

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

July 2003

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 and the University of Idaho, 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 2002 a total of 570 viable semen samples were added to the germplasm repository. This included the gametes of 287 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 of 280 steelhead from the North Fork Clearwater River (Dworshak Hatchery), Fish Creek, Little Sheep Creek, Pahsimeroi River (Pahsimeroi Hatchery) and Snake River (Oxbow Hatchery). In addition, gametes from 60 Yakima River spring chinook and 34 Wenatchee River coho salmon were added to the repository by Washington Department of Fish and Wildlife and Columbia River Intertribal Fish Commission, respectively. To date, a total of 3,928 Columbia River salmon and steelhead gamete samples and three Kootenai River white sturgeon are preserved in the repository. Samples are stored in independent locations at the University of Idaho (UI) and Washington State University (WSU).

TABLE OF CONTENTS

ABSTRACT.....	i
TABLE OF CONTENTS.....	ii
LIST OF FIGURES	iv
LIST OF TABLES.....	v
INTRODUCTION	1
METHODS	3
Description of Spawning Aggregates	3
Fish Collection and Handling	4
Semen Handling and Cryopreservation	7
RESULTS	10
2002 Chinook Salmon Gamete Collections	10
Lostine River	10
Upper Grande Ronde.....	10
Imnaha River	12
South Fork Salmon River	12
Lake Creek	13
Johnson Creek	13
Big Creek.....	14
Capehorn Creek.....	14
Marsh Creek	14
Pahsimeroi River	15
Upper Salmon River.....	15
2002 Steelhead Gamete Collections.....	16
Snake River	16
Pahsimeroi River	16
North Fork Clearwater River.....	17
Fish Creek	17
Little Sheep Creek.....	17
Status of Germplasm Collections in the Snake River Basin.....	18
Grande Ronde River Chinook Salmon Captive Broodstock Project	18

Fertility Trials	19
Use of Cryopreserved Gametes in 2002	20
DISCUSSION	21
RECOMMENDATIONS	28
ACKNOWLEDGMENTS	29
LITERATURE CITED	30
APPENDICES	33
Appendix 1. Gamete samples collected from 1992 through 2002	34
Appendix 2. Snake River Germplasm Repository Cryopreserved Semen Request Form	37
Appendix 3. Data from chinook salmon collected in 2002.....	39
Appendix 4. Data from steelhead collected in 2002.	47

LIST OF FIGURES

Figure 1. Map showing the Snake River basin chinook salmon and steelhead sampling locations for 2002.	4
Figure 2. Collecting chinook salmon milt from anaesthetized fish at Big Creek.	6
Figure 3. Anaesthetized male chinook salmon on portable tank for measurements.	7
Figure 4. Conducting pre-freeze motility estimates on fresh chinook salmon sperm.	8
Figure 5. Example of a liquid nitrogen tank used to store chinook salmon and steelhead gametes.	9
Figure 6. Length distribution of 52 chinook salmon sampled for cryopreservation in Johnson Creek in 2002.	13
Figure 7. Length distribution of 33 chinook salmon sampled for cryopreservation in Marsh Creek in 2002.	14
Figure 8. Length distribution of 39 chinook salmon sampled for cryopreservation in the Pahsimeroi River in 2002.	15
Figure 9. Length distribution of 54 chinook salmon sampled for cryopreservation in the upper Salmon River in 2002.	16
Figure 10. Length distribution of 62 steelhead sampled from Pahsimeroi Hatchery in 2002.	17
Figure 11. Length distribution of 94 steelhead sampled in Little Sheep Creek in 2002.	18
Figure 12. Graph showing the number of gametes collected from the Imnaha River chinook salmon per effective brood year over a 5-year generation.	23
Figure 13. Graph showing the number of gametes collected from the South Fork Salmon River chinook salmon per effective brood year over a 5-year generation.	24
Figure 14. Graph showing the number of gametes collected from the North Fork of the Clearwater River per effective brood year over a 4-year generation.	25
Figure 15. Graph showing the number of gametes collected from the Pahsimeroi River (Pahsimeroi Hatchery) steelhead per effective brood year over a 4-year generation.	26
Figure 16. Graph showing the number of gametes collected from the Little Sheep Creek steelhead per effective brood year over a 4-year generation.	27
Figure 17. Graph showing the number of gametes collected from the Snake River (Oxbow Hatchery) steelhead per effective brood year over a 4-year generation.	27

LIST OF TABLES

Table 1. Locations and numbers of spring and summer chinook salmon semen samples cryopreserved in the Snake River basin in 2002.....	11
Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2002.	12
Table 3. Results of chinook salmon fertilization trial conducted at Washington State University in 2002.....	19

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.

The 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). We are attempting to preserve the endemic salmonid diversity by collecting from numerous populations of spring and summer chinook salmon and steelhead across the entire Snake River basin. 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 the NPT germplasm preservation activities from 2002 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 the 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 2002.

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

Clearwater Subbasin

1. North Fork Clearwater River - Dworshak National Fish Hatchery
2. Fish Creek

Snake River Subbasin

1. Snake River – Oxbow Hatchery

Salmon River Subbasin

1. Pahsimeroi River – Pahsimeroi Fish Hatchery

Imnaha River Subbasin

1. Little Sheep Creek

Figure 1. Map showing the Snake River basin chinook salmon and steelhead sampling locations for 2002.

Fish Collection and Handling

Chinook salmon spawning ground surveys are usually conducted on pre-determined stream reaches before handling any fish. Redd counts also determine where in each stream the collection of adult males occurs. Several team members locate the adults, being careful not to disturb the fish. Observations are made to visually identify male salmon. Males are identified by secondary sexual characteristics, which include 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. Females can be identified by a rounder head, thicker caudal peduncle, and a tattered, discolored (white) caudal fin from digging redds. No harassment of actively spawning salmon occurs.

Fish handling protocol training was provided to all personnel prior to collection of adult male salmonids to minimize stress on the fish. Each team member was assigned a specific duty to improve the efficiency of sample collection. No one entered the water near 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 are collected by hand, dip net, or seine 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. Net holders should be absolutely still as fish approach the nets.

Seine. Two 5' x 30-40' seine nets are set up perpendicular to the flow of water, blocking a segment of stream. The upstream net is moved slowly downstream, trapping the fish in a corral of decreasing area and fish can be easily collected with dip nets. In the case where multiple fish are captured, fish are quickly screened to identify those that will be sampled and the others are immediately released.

Captured fish are held in the stream while a portable tank is set up along the stream. Fish are immobilized using anesthetic so they can be handled faster and less stressfully. General anesthetics first calm the fish, then cause it successively to lose mobility, equilibrium, consciousness, and finally reflex action (Summerfelt and Smith 1990). The anesthesia is delivered by placing the fish in a portable tank filled with 35 gallons of water containing 90 mg/l of tricane methanesulfonate (MS-222, FinquelTM) anesthesia and sodium bicarbonate (NaHCO_3) to buffer the acidity of the MS-222. It takes 1-3 minutes for the fish to be anesthetized. The fish is constantly monitored while in the tank and the time to sedation is noted. The sedated fish is rinsed in the fresh water of the stream and the abdomen is dried to reduce contamination prior to collecting the milt in a plastic Whirl Pak bag (Figure 2). Fish handling/spawning protocols of Idaho Department of Fish and Game (IDFG) are used at the Idaho hatcheries, and the adults are not anesthetized before semen samples are taken. Imnaha River chinook salmon are anesthetized at Lookingglass Hatchery.

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. Scale analyses will be used to determine the brood year origin of each fish and this along with genetic analyses will provide estimates of the amount of diversity preserved from each brood year sampled. 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 was 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.

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

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 ~15 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 used to cover the ice and insulate the samples.

Semen samples were shipped to, cryopreserved and stored at both Washington State University and the University of Idaho within 12 hours of collection. Storing the samples at two facilities acts as a safeguard to protect against catastrophic loss at one facility. Because percent motility at freezing positively correlates to post-thaw fertility, sperm quality was determined (Figure 4) 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) and 5.0 ml straws. Each university first filled 20 0.5 ml straws and any leftover

semen was used to fill as many 5.0 ml straws as the volume allowed. A sample of 5 ml of semen is sufficient to fill 20 0.5 ml straws, due to the dilution of semen with three parts freezing solution. Samples were stored in large cryopreservation tanks under liquid nitrogen (Figure 5).

Figure 4. Conducting pre-freeze motility estimates on fresh chinook salmon sperm.

A detailed description of the freezing process can be found in Cloud and Osborne (1997). We will briefly describe the important aspects of the process here. There are four stages in the cooling sequence of cryopreservation of cells: 1) Cooling cells to the point of ice formation - this does not appear to be a critical factor in the cryopreservation of salmonid sperm; 2) The formation of ice - the goal at this stage is to have ice form near the freezing point of the extra cellular solution; 3) Cooling through the critical period - there is a net movement of water out of the cells as the temperature is constantly being reduced 4) Reduction to liquid nitrogen temperature - the frozen sperm is then plunged into liquid nitrogen at -196°C .

We continue to enlist the assistance of Dr. Joseph Cloud, professor of Zoology in the Department of Biological Sciences at the University of Idaho, Dr. Gary Thorgaard and Paul Wheeler in the Thorgaard Lab at Washington State University, and Dr. Madison Powell, geneticist at the Fish Genetics Laboratory and Hagerman Experiment Station with the University of Idaho. These subcontractors are experts in the field of salmonid cryopreservation, reproductive physiology and/or fish genetics.

Figure 5. Example of a liquid nitrogen tank used to store chinook salmon and steelhead gametes.

Fertilization experiments were conducted at Washington State University 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 Lyons Ferry Hatchery from Umatilla River fall chinook salmon that strayed into the Snake River. Cryopreserved semen was obtained by thawing a single 0.5ml straw from six non-ESA listed chinook salmon. 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 removes the influence of egg quality and provides an estimate of the fertilization potential of the cryopreserved samples. University of Idaho did not perform fertility trials in 2002.

RESULTS

The chinook salmon and steelhead spawning aggregates and hatcheries in the Snake River basin where gametes were collected in 2002 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 286 male chinook salmon (Table 1) were collected and cryopreserved in 2002. Collections occurred over a two-month period from August 12, 2002 to October 1, 2002. The majority of the samples, 225, were collected from unmarked, natural fish. Six females were accidentally captured and immediately released. Motility of the sperm ranged from 5 – 90%.

Gametes from 284 male steelhead (Table 2) were collected and cryopreserved in 2002. Collections occurred over a four-month period from Feb. 26, 2002 to June 30, 2002. All but the three samples from Fish Creek were collected at hatchery facilities with a majority of the samples collected from marked hatchery fish (278/281). Motility of the sperm ranged from 5 – 90%.

2002 Chinook Salmon Gamete Collections

Lostine River

In 2002 the gametes from 19 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), one age 3, six age 4 and five age 5 fish were sampled from brood years 1999, 1998 and 1997, respectively. Length was not determined for one fish. Currently, gametes from 85 Lostine River males sampled from 1994 to 2002 are preserved in the gene bank (Appendix 1).

Upper Grande Ronde

In 2002 the gametes from eight 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), six age 4 and two age 5 fish were sampled from brood years 1998 and 1997, respectively. Currently, gametes from 17 Grand Ronde River males sampled from 2001 and 2002 are preserved 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 2002.

Spawning Aggregate	Total Samples	Unmarked Fish ^a	Marked Fish ^b	Females Captured ^c	Collection Dates	Sperm Motility (%)
Lostine River	19	19	0	0	8/28, 9/4 & 9/11	20-90
Catherine Creek	5	5	0	0	8/30 & 9/11	10-90
Grande Ronde River	8	8	0	0	8/30 & 9/11	10-90
Imnaha River	7	7	0	0	8/28 & 9/11	10-90
S. Fork Salmon River	23	0	23 ^d	0	8/27 & 9/3	5-90
Lake Creek	18	15	3	2	8/12 & 8/19	20-90
Johnson Creek	58	58	0	2	8/21,23,27,28,30 & 9/3,4	10-90
Big Creek	21	20	1	0	8/13 & 8/20	50-90
Capehorn Creek	1	1	0	0	8/16	60
Marsh Creek	34	34	0	2	8/15,16 & 8/22	20-90
Pahsimeroi River	39	5	34 ^e	0	9/24 & 10/1	10-90
Upper Salmon River	53	53	0	0	8/22,29 & 9/5	10-90
Totals	286	225	61	6	8/12 – 10/1	5-90

^aNon fin-clipped fish

^bFin-clipped or tagged fish, hatchery origin

^cFemales were immediately released

^dNot ESA listed

^e33/34 of these fish were ESA listed

Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2002.

Spawning Aggregate	Total Samples Cryopreserved	Un-marked Fish ^a	Marked Fish ^b	Collection Dates	Sperm Motility (%)
Little Sheep Creek	93	3	90	4/2,9,23,30 & 5/14	50-90
Snake River Oxbow Hatchery	58	0	58 ^c	4/15,18,22	50-90
North Fork Clearwater River	63	0	63 ^c	2/26 3/19,26 & 4/9,30	50-90
Fish Creek	3	3	0	6/25 & 6/30	<10
Pahsimeroi River	63	0	63 ^c	4/19,24 & 5/1	20-90
Totals	280	6	274	2/26 – 6/30	10-90

^aNon fin-clipped fish, natural origin

^bFin-clipped or tagged fish, hatchery origin

^cNot ESA listed

Imnaha River

In 2002 the gametes from seven 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), one age 3, four age 4 and two age 5 fish were sampled from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 427 Imnaha River males sampled from 1996 through 2002 are preserved in the gene bank (Appendix 1). Of these, 209 were from marked hatchery fish and 218 were from unmarked natural fish.

South Fork Salmon River

In 2002 the gametes from 23 male chinook salmon were cryopreserved from fish trapped at the adult weir on the South Fork Salmon River (McCall Hatchery, IDFG). All fish were adipose fin-clipped indicating that they were of hatchery-origin. Lengths were not obtained on these fish. Currently, gametes from 323 South Fork Salmon River males sampled from 1996 through 2002 are preserved in the gene bank (Appendix 1). Of these, 183 were from non-ESA-listed marked

hatchery fish, 55 were from marked ESA listed supplementation fish, and 83 were from unmarked ESA-listed natural fish.

Lake Creek

In 2002 the gametes from 18 wild males were cryopreserved from fish netted in Lake Creek. Two female chinook salmon were incidentally netted and immediately released. All fish were unmarked indicating that they were natural-origin. Based on the length data (Appendix 3), one age 3, thirteen age 4 and four age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 77 males sampled from 1996 through 2002 are preserved in the gene bank (Appendix 1).

Johnson Creek

In 2002 the gametes from 58 male chinook salmon were cryopreserved from fish captured in Johnson Creek and at the NPT adult weir. Thirty-one 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 27 fish, 12 were sampled from fish captured at the NPT Johnson Creek adult weir and 15 were netted in Johnson Creek. Two female chinook salmon were incidentally netted and immediately released. Based on the length frequency distribution (Figure 6), two age 3, forty-one age 4 and eight age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Length was not determined for two fish. Currently, gametes from 184 males sampled from 1996 through 2002 are preserved in the gene bank (Appendix 1).

Figure 6. Length distribution of 52 chinook salmon sampled for cryopreservation in Johnson Creek in 2002.

Big Creek

In 2002 the gametes from 21 male chinook salmon were cryopreserved from fish netted in Big Creek. One adipose-clipped fish was captured and semen was collected. The remaining 20 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), one age 3, nineteen age 4 and one age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 102 males sampled from 1992 through 2002 are preserved in the gene bank (Appendix 1).

Capehorn Creek

In 2002 the gametes from one wild male chinook salmon were cryopreserved from a fish netted in Capehorn Creek. Currently, gametes from 12 males sampled from 1997 through 2002 are preserved in the gene bank (Appendix 1).

Marsh Creek

In 2002 the gametes from 34 male chinook salmon were cryopreserved from fish netted in Marsh Creek. All 34 fish were unmarked indicating that they were natural-origin. Six fish originally captured and spawned on August 16 were recaptured August 22. One fresh-looking adipose fin clipped fish was captured on August 22 but did not produce milt suggesting that it was not ripe. Based on the length frequency distribution (Figure 7), zero age 3, twenty-two age 4 and eleven age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Length was not determined for one fish. Currently, gametes from 72 males sampled from 1997 through 2002 are preserved in the gene bank (Appendix 1).

Figure 7. Length distribution of 33 chinook salmon sampled for cryopreservation in Marsh Creek in 2002.

Pahsimeroi River

In 2002 the gametes from 39 Pahsimeroi River male chinook salmon were cryopreserved from fish spawned at Pahsimeroi Hatchery. Of these, 34 were marked hatchery-origin fish and 5 were unmarked natural fish. Based on the length frequency distribution (Figure 8), five age 3, twenty age 4 and fourteen age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 170 males sampled from 1997 through 2002 are preserved in the gene bank (Appendix 1). Of these, 136 were from marked hatchery fish and 34 were from unmarked natural fish.

Figure 8. Length distribution of 39 chinook salmon sampled for cryopreservation in the Pahsimeroi River in 2002.

Upper Salmon River

In 2002 the gametes from 54 upper Salmon River male chinook salmon were cryopreserved from fish spawned at the Sawtooth Fish Hatchery. Thirty-two of the samples were obtained from unmarked natural fish and 12 of the samples were obtained from supplementation fish (wild x hatchery parents). Based on the length frequency distribution (Figure 9), two age 3, thirty age 4 and seventeen age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 274 males sampled from 1997 through 2002 are preserved in the gene bank (Appendix 1). Of these, 54 were from marked hatchery fish, 12 were from marked supplementation fish and 208 were from unmarked natural fish.

Figure 9. Length distribution of 54 chinook salmon sampled for cryopreservation in the upper Salmon River in 2002.

2002 Steelhead Gamete Collections

Snake River

The gametes from 58 Snake River male steelhead were cryopreserved from fish spawned at Oxbow Hatchery. All were marked, hatchery-origin fish. Length was not determined for these fish. Currently, gametes from 305 hatchery-origin males collected from 1997 through 2002 from this population are preserved in the gene bank (Appendix 1).

Pahsimeroi River

The gametes from 63 Pahsimeroi River male steelhead were cryopreserved from fish spawned at Pahsimeroi Hatchery. All were marked, hatchery-origin fish. Based on the length frequency distribution (Figure 10), fifty-five age 3 and seven age 4 fish were sampled, originating from brood years 1999 and 1998, respectively. Length was not determined for one fish. Currently, gametes from 210 hatchery-origin males collected from 1999 through 2002 from this population are preserved in the gene bank (Appendix 1).

Figure 10. Length distribution of 62 steelhead sampled from Pahsimeroi Hatchery in 2002.

North Fork Clearwater River

In 2002 the gametes from 63 North Fork Clearwater River male steelhead were cryopreserved from fish spawned at Dworshak National Fish Hatchery. Length was determined for 22 fish. Based on the length data (Appendix 3), two age 3, twelve age 4 and eight age 5 fish were sampled, originating from brood years 1999, 1998 and 1997, respectively. Currently, gametes from 295 hatchery-origin males collected from 1999 through 2002 from this population are preserved in the gene bank (Appendix 1).

Fish Creek

In 2002 the gametes from three male steelhead were cryopreserved from fish captured at the IDFG weir in Fish Creek. These samples were obtained from downstream migrating wild kelts captured in June. Currently, gametes from 15 wild males sampled from 1993 through 2002 from this subpopulation are preserved in the gene bank (Appendix 1).

Little Sheep Creek

In 2002 the gametes from 93 male steelhead were cryopreserved from fish spawned at the Little Sheep Creek adult weir. Of these, three were unmarked natural fish and 92 were marked hatchery fish. Based on the length frequency distribution (Figure 11), seventy-two age 3 and twenty age 4 fish were sampled, originating from brood years 1999 and 1998, respectively. Length was not determined on one fish. Currently, gametes from 280 males sampled from 1999 through 2002 are preserved in the gene bank. Of these, 260 were from marked hatchery fish and 20 were from unmarked natural fish (Appendix 1).

Figure 11. Length distribution of 94 steelhead sampled in Little Sheep Creek in 2002.

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). In addition, state management agencies have utilized the long-term repository to store cryopreserved gametes from other imperiled salmon populations and species in the Columbia River drainage. In 2002, Washington Department of Fish and Wildlife (WDFW) added gamete samples from 60 Yakima River spring chinook and Columbia River Intertribal Fish Commission added gamete samples from 34 Wenatchee River coho to the repository. The repository also includes gamete samples from Redfish Lake sockeye (IDFG), Yakima River spring chinook salmon (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 2002. 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 2002. The fertility of cryopreserved semen from six nonESA-listed hatchery fish were compared to that from three fresh semen samples. Fresh semen and eggs were collected from three males and one female Umatilla River fall chinook that strayed to Lyons Ferry Hatchery on the Snake River. The eggs were partitioned and fertilized with the six frozen and three fresh semen samples. 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 2002.

Male	Pre-freeze Motility	Eyed Eggs /Total	Percent Fertilized	Relative Percent Fertilized ²
NPT-415-02 ¹	90	130/224	58.0	70.6
NPT-423-02 ¹	80	139/238	58.4	71.1
NPT-582-02 ¹	80	49/306	16.0	19.5
NPT-588-02 ¹	90	23/246	9.3	11.3
NPT-599-02 ¹	90	18/229	7.9	9.6
Average			29.9	36.4
C1 (fresh)		160/206	77.7	
C2 (fresh)		186/208	88.5	
C3 (fresh)		219/272	80.5	
Average			82.2	

¹Cryopreservation straw number from WSU

²Percent fertility divided by mean fresh fertility (82.2%)

Use of Cryopreserved Gametes in 2002

In 2002 Oregon Department of Fish and Wildlife (ODFW) Oxbow Hatchery requested cryopreserved Imnaha River chinook salmon gametes for use in their broodstock spawning matrix. The request was made by the agency in order to meet the goal of using a preset percentage of wild fish in a supplementation program. Following a risk/benefit analysis, the Snake River Germplasm Committee (SRGC) convened for the first time to evaluate the cryopreserved semen request. The SRGC determined that gametes from individuals with greater than five 5.0 ml straws in the gene bank could be used without jeopardizing the goals of the long-term repository. Accordingly, one 5.0 ml straw from 15 natural chinook salmon was removed from the repository tanks and transported to Oxbow Hatchery in a nitrogen dry shipper. The SRGC forwarded a memo to ODFW with suggested recommendations and conditions for use of the cryopreserved semen. Included in this memo was the disclaimer that fertilization rates using the frozen sperm would be significantly lower than that expected from fresh sperm. The memo further requested that the fertilized eggs would be held separately in order to evaluate the fertilization rate of each cross.

NPT staff assisted with the fertilizations using the cryopreserved gametes at Oxbow Hatchery based on experience with the use of cryopreserved semen from the Grande Ronde River chinook salmon Captive Broodstock Project. WSU provided the protocol for performing fertilizations using 5.0 ml straws and trained staff prior to conducting the fertilizations using the ESA-listed Imnaha River Chinook salmon gametes.

Numerous problems were encountered while performing the fertilizations using the protocols provided from the expert advisors at WSU. Most importantly, 4/5 of the 5.0 ml straws obtained from the cryopreservation tanks at WSU and 4/10 of the straws obtained from the cryopreservation tanks at U of I shattered as they were being thawed. This resulted in a complete loss of a portion of the straws. In addition, other straws that were used did not appear to thaw correctly. Protocols indicate that the semen should be slushy when it is put on the eggs. In contrast, many of the semen samples contained large chunks of ice. Given the concern, staff delayed spawning and contacted WSU for advice. WSU personnel indicated that the sperm should maintain viability as long as the semen was still frozen when it makes contact with the eggs and suggested increasing the temperature of the water bath in order to change the thaw rate. Of greater concern was the safety hazard posed by the exploding straws. The 5.0 ml straws were sealed using steel or glass beads. When the straws shattered the beads shot out at dangerously high velocities. Although beads are commonly used for sealing large cryopreservation straws (Joe Cloud, University of Idaho, personal communication), the danger posed by exploding straws was not anticipated. Thus, water baths did not have lids to contain the hazard. Serious injury could have resulted if a bead struck personnel conducting the fertilizations.

Despite the SRGC request that the eggs fertilized using the cryopreserved sperm be held separate to monitor fertilization rates, the eggs used in this trial were combined with eggs fertilized with fresh sperm so their fertilization rate could not be determined. It was suggested that ODFW upper-level managers never communicated this requirement to the on-site hatchery personnel.

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 verses the extant population. Genetic information will be used in conjunction with the biological data to assist in the use of the cryopreserved germplasm in management applications such as captive broodstock or supplementation programs. Genetic diversity information from fish in the repository can also serve as a baseline that can be used to monitor shifts or losses of genetic variation over time (Servheen et al. 2001). Further genetic analysis will help determine genetic structure of chinook salmon (Brannon et al. 2002) and steelhead populations and allow for the evaluation of the genetic diversity contained in the gamete repository.

In 2002, tissue samples were collected from all wild chinook salmon captured and spawned for cryopreservation. In addition, tissue samples were collected from all hatchery chinook salmon and steelhead spawned for cryopreservation except a portion of the steelhead from Dworshak National Fish Hatchery. Genetic analyses from the tissue samples collected from 1992 through 2002 have not been completed. As this is an on going task, we expect the analyses of samples collected from 1992 through 2001 to be completed in 2003.

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 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, the NPT has been very successful cryopreserving wild chinook salmon gametes. In contrast, few wild steelhead gametes 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. Steelhead spawn in the spring during periods of high water and inclement weather. Thus, a majority of the steelhead gametes came from easily accessible hatchery-origin fish. Future efforts should concentrate on sampling wild/natural steelhead.

Fertility trials indicated that cryopreservation techniques are effective at maintaining the fertility of frozen sperm. 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). The three samples that gave the lowest fertility rates in our trial (Table 3) were all collected on the last day of spawning

at Pahsimeroi Hatchery, suggesting that collection time could be a factor in fertility. Additional research will be required to confirm these results.

Clearly the use of 5.0 ml straws using the technology currently employed is not an option. Not only is it unlikely to produce reliable fertilizations, but it also poses a serious danger to personnel involved. Following this incident, NPT has placed a moratorium on freezing gametes in 5.0 ml straws. Obviously alternate technologies for freezing large volumes of semen are needed. In addition, functional thawing protocols need to be developed for the thousands of 5.0 ml straws currently in the repository. University experts will be contacted and requested to provide recommendations and useful data on the use of 5.0 ml straws.

This project set a goal of cryopreserving gametes from at least 100 males per brood year for at least one generation from each spawning aggregation. To date, none of the collections have met that objective. 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. Examples include North Fork Clearwater steelhead from Dworshak National Fish Hatchery, Pahsimeroi River steelhead from Pahsimeroi Fish Hatchery and Snake River steelhead from Oxbow Fish Hatchery. Other collections such as Imnaha River chinook salmon, South Fork River chinook salmon and Little Sheep Creek steelhead have large numbers of stored gametes but may warrant additional collections because a large proportion of the males were of hatchery-origin and the addition of wild males would greatly enhance the diversity of the collections. In addition, collection and cryopreservation of milt in large volumes, such as 5.0 ml straws, would enable more flexibility for standard hatchery production.

The following is a description of the subpopulations where a large number of gamete samples have been cryopreserved and recommendations for future collections. Generation times were calculated as the average number of years it takes for 95% of the individuals from a brood year to return. This was calculated using fork length or tagging data and is specific to each subpopulation. Because length was not determined for every fish sampled, an estimate of the number of fish from each brood year was determined based on the run composition for that particular year.

Fish were designated to specific brood years based on length/frequency data. We use the concept of effective brood years to represent the collections from multiple brood years over two or more generations. 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. All fish that were collected over multiple generations are placed in a single effective brood year, depending on the year of collection and age. For example, under this concept four year old fish collected in years one and five from a population with a four-year generation are grouped in the same effective brood year. Similarly, four-year old fish from year one and three year old fish from year 4 are grouped into the same effective brood year.

Imnaha River Chinook Salmon

The gene bank contains gametes from 427 Imnaha River male chinook salmon including 209 marked hatchery-origin fish and 218 wild fish. ODFW hatchery managers designate age groups by the following lengths: <66 cm, age 3; 66 – 90 cm, age 4; and >90 cm, age 5. Using these lengths along with the run composition for each year, the brood years represented in the gene bank was calculated (Figure 12).

Figure 12. Graph showing the number of gametes collected from the Imnaha River chinook salmon per effective brood year over a 5-year generation.

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 wild-origin, would preserve the greatest level of diversity from this population. In addition, effective brood year 1 is underrepresented in the repository. In 2003 four-year old fish will represent this brood year and should be targeted.

South Fork Salmon River Chinook Salmon

The gene bank contains gametes from 323 South Fork Salmon River male chinook salmon including 185 marked hatchery-origin fish, 55 supplementation fish and 83 wild fish. IDFG hatchery managers designate age groups by the following lengths: <66 cm – age 3, 66 – 90 cm – age 4 and >90 cm – age 5. Using these lengths along with the run composition for each year, the brood years represented in the gene bank was calculated (Figure 13).

Figure 13. Graph showing the number of gametes collected from the South Fork Salmon River chinook salmon per effective brood year over a 5-year generation.

RECOMMENDATIONS – The 185 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 2003 the four-year old fish that return will represent this brood year and effort should be made to collect a large number of these fish in order to increase the representation of this brood year in the repository. Future collections should concentrate effort on increasing the number of natural fish from this population. Natural fish used in the SFSR supplementation program are spawned late in the day, to close to or after the time that the plane has departed for the Universities. Cryopreserving gametes on site or transporting them the following morning would increase the number of natural fish in the collections.

North Fork Clearwater River Steelhead

The gene bank contains gametes from 295 North Fork Clearwater River male steelhead. USFWS hatchery managers designate age groups by the following lengths: <72 cm – age 3, 72 – 93 cm – age 4 and >93 cm – age 5. The generation time of this population is four years since on average 95.4 % of fish return as 3 or 4 year olds (Burge et al. 2000). 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 14).

Figure 14. Graph showing the number of gametes collected from the North Fork of the Clearwater River per effective brood year over a 4-year generation.

RECOMMENDATIONS - Additional collections are not warranted because a large number of samples will provide a buffer against genetic diversity loss in this non ESA-listed hatchery population. Previous collections were predominantly frozen in 0.5 ml straws. Collecting and freezing gametes in 5.0 ml straws should be considered, as it would provide the large volumes required for conventional hatchery situations.

Pahsimeroi River Steelhead

The gene bank contains gametes from 210 Pahsimeroi River male hatchery-origin steelhead. IDFG hatchery managers designate age classes by the following lengths: <66 cm – age 3 and >66 cm – age 4 and the generation time for this population is four years since nearly all fish return as 3 and 4 year olds (Garlie and Engemann, 2000). 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 15).

RECOMMENDATIONS - Additional collections are not warranted because a large number of samples will provide a buffer against genetic diversity loss in this non ESA-listed hatchery population. Collecting natural Pahsimeroi River steelhead as they are passed above the adult weir is recommended.

Figure 15. Graph showing the number of gametes collected from the Pahsimeroi River (Pahsimeroi Hatchery) steelhead per effective brood year over a 4-year generation.

Little Sheep Creek Steelhead

The gene bank contains gametes from 280 Little Sheep Creek male steelhead including 260 marked hatchery-origin fish and 20 natural fish. 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 Flesher, ODFW, personal communication). The generation time of the wild fish is 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 16).

RECOMMENDATIONS - Additional collections are warranted because this hatchery population is ESA-listed and a low number of natural-origin fish have been sampled. Future collections should continue until at least 100 fish from each brood year are in the repository. In addition, increasing the collection of natural-origin fish will maximize the diversity of the collection from this drainage.

Snake River Steelhead (Oxbow Hatchery)

The gene bank contains gametes from 307 Snake River male hatchery-origin steelhead. IDFG hatchery managers designate age groups by the following lengths: <66 cm – age 3 and >66 cm – age 4 and generation time for this population is 4 years since nearly all fish return as 3 and 4 year olds (Hills 2001). 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 17).

Figure 16. Graph showing the number of gametes collected from the Little Sheep Creek steelhead per effective brood year over a 4-year generation.

RECOMMENDATIONS - Additional collections are not warranted because a large number of samples will provide a buffer against genetic diversity loss in this non ESA-listed hatchery population.

Figure 17. Graph showing the number of gametes collected from the Snake River (Oxbow Hatchery) steelhead per effective brood year over a 4-year generation.

One request for cryopreserved gametes was made in 2002. In the future, 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. Focus steelhead collections on wild populations throughout the Snake River basin.
3. Continually evaluate the repository collection and modify in order to maximize diversity.
4. Continue tissue sample collections from all of the fish that are sampled in order to perform critical genetic analyses.
5. Complete the genetic analyses on samples collected to date to examine the diversity within the repository and to decide if continued collections are warranted.
6. Research techniques to optimize 5.0 ml straw freezing and thawing protocols that will improve fertilization rates.
7. Continue fertility trials on cryopreserved gametes in order to evaluate the freezing techniques.
8. Establish a Regional Germplasm Repository for gene conservation of imperiled fish and wildlife species.

ACKNOWLEDGMENTS

The Nez Perce Tribe would like to thank: Dr. Joe Cloud and Wendy Lawrence at the University of Idaho, Dr. Gary Thorgaard, Paul Wheeler, Steve Patton, Rob Drew, and Brad Cunningham at Washington State University for cryopreservation assistance, the storage facilities, and recommendations to make this a better program. The U.S. Fish and Wildlife Service Lower Snake River Compensation Plan program provided 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, Santee Penny, Levi Pinkham, 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 cooperation and patience during the hectic chinook salmon collecting season.

We greatly appreciate the cooperation and assistance of: Gene McPherson at the Idaho Department Fish and Game McCall Fish Hatchery, Greg Davis at Oregon Department of Fish and Wildlife Wallowa Hatchery, Bob Lund at the Oregon Department of Fish and Wildlife Lookingglass Hatchery, Todd Garlie and Doug Engelman at Pahsimeroi Hatchery, Kent Hill at Oxbow Hatchery, Bob Semple at Dworshak National Fish Hatchery, Idaho Power for the use of its Oxbow airstrip, and Brent Snider at the Sawtooth Fish Hatchery. We are grateful to Dr. Rolf Inglemann for helping transport steelhead samples from Dworshak to the University of Idaho. 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.

LITERATURE CITED

- Ali, J. and J. N. Shelton. 1993. Successful vitrification of day-6 sheep embryos. *Journal of Reproduction and Fertility*. 99: 65-70.
- Armstrong, R. and P. A. Kucera. 1999. Salmonid Gamete Preservation in the Snake River Basin. 1998 Annual Report. Prepared for Bonneville Power Administration. Nez Perce Tribe Department of Fisheries Resources Management. Lapwai, Idaho.
- Armstrong, R. D. and P. A. Kucera. 2000. Salmonid Gamete Preservation in the Snake River Basin. 1999 Annual Report. Prepared for Bonneville Power Administration. Nez Perce Tribe Department of Fisheries Resources Management. Lapwai, Idaho.
- Armstrong, R. D. and P. A. Kucera. 2001. Salmonid Gamete Preservation in the Snake River Basin. 2000 Annual Report. Prepared for Bonneville Power Administration. Nez Perce Tribe Department of Fisheries Resources Management. Lapwai, Idaho.
- Ashwood-Smith, M. J. 1980. Low temperature preservation of cells, tissues and organs. Pages 19-44 *in* M. J. Ashwood-Smith and J. Farrant, editors. *Low Temperature Preservation in Medicine and Biology*. Pitman Medical Ltd., Tunbridge Wells, Kent, UK.
- Bartley, D. 1998. *Ex Situ* conservation, gene banks, and responsible fisheries. *Action Before Extinction*. B. Harvey, C. Ross, D Greer and J. Carolsfeld, eds. World Fisheries Trust, Victoria, B.C., Canada.
- Brannon, E., M. Powell, T. Quinn, and A. Talbot. 2002. Population structure of Columbia River chinook salmon and steelhead trout. University of Idaho, Center for Salmonid and Freshwater Species at Risk. Moscow, ID.
- Burge, H.L., M. Faler, R.B. Roseberg, and R.N. Jones. 2000. Adult steelhead returns to Dworshak NFH in 1999-00 and prognosis for 2000-01. Appendix B. U.S. Fish and Wildlife Service. Ahsahka, Idaho.
- Burner, C.J. 1951. Characteristics of spawning nests of Columbia River salmon. *Fish. Bull.* 1:1-50.
- Busby, P.J., T.C. Wainwright, G.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz, and I.V. Lagomarsino. 1996. Status of West Coast Steelhead from Washington, Idaho, Oregon, and California. NOAA Technical Memo. NMFS-NWFSA-27. Department of Commerce National Marine Fisheries Service. Seattle, Washington.
- Cloud, J. G., and C. Osborne. 1997. Cryopreservation of Salmonid Sperm. University of Idaho, Moscow, Idaho.
- Columbia Basin Fish and Wildlife Authority (CBFWA). 1990. Integrated system plan for salmon and steelhead production in the Columbia River Basin. Columbia Basin System Planning,

- 449 p. and 40 subbasin volumes. (Available from Northwest Power Planning Council, 851 S.W. Sixth, Suite 1100, Portland, OR 97204-1348.)
- Corley-Smith, G.E. and B.P. Brandhorst. 1999. Preservation of endangered species and populations: A role of genome banking, somatic cell cloning, and androgenesis? *Molecular Reproduction and Development*. 53:363-367.
- Dobrinsky, J.R., F.F. Hess, R.T. Duby, J. M Robl. 1991. Cryopreservation of Bovine Embryos by vitrification. *Theriogenology*. Vol. 35 No.1.
- Fahning, M.L. and M.A. Garcia. 1992. Status of cryopreservation of embryos from domestic animals. *Cryobiology* 29:1-18.
- Faurot, D., R. Armstrong, P. A. Kucera, and M. L. Blenden. 1998. Cryopreservation of adult male spring and summer chinook salmon gametes in the Snake River Basin. 1997 Annual Report. Prepared for Bonneville Power Administration. Nez Perce Tribe Department of Fisheries Resources Management. Lapwai, Idaho.
- Garlie, T. and Engemann, D. 2000. Pahsimeroi Hatchery Summer Steelhead Run Report Brood Year 2000. Pahsimeroi Hatchery, Ellis ID.
- Hayashi, S., K. Kobayashi, J. Mizuno, K. Saitoh, S. Hirano. 1989. Birth of piglets from frozen embryos. *The Veterinary Record*. July 8, 1989.
- Hills, K. 2001. Oxbow Fish Hatchery 1999 Steelhead Brood Year Report and 1998 Spring Chinook Brood Year Report. IDFG 00-55. Idaho Fish and Game and Idaho Power.
- Idaho Department of Fish and Game (IDFG). 1994. Documents submitted to the ESA Administrative Record for west coast steelhead by Eric Leitzinger, 18 October 1994. (Available from Environmental and Technical Services Division, National Marine Fisheries Service, 525 N.E. Oregon Street, Suite 500, Portland, OR 97232.)
- Kono, T., O. Suzuki, and Y. Tsunoda. 1988. Cryopreservation of rat blastocysts by vitrification. *Cryobiology*. 25: 170-173
- Kucera, P.A. and M.L. Blenden. 1999. Lower Snake River Compensation Plan Hatchery Evaluation Studies Annual Project Report. Prepared for the U.S. Fish and Wildlife Service LSRCP Program. Nez Perce Tribe Department of Fisheries Resources Management. Lapwai, Idaho.
- Matthews, G.M., and R.S. Waples. 1991. Status review for Snake River Spring and Summer Chinook Salmon. National Marine Fisheries Service, Seattle, Washington.
- Mounib, M. S. 1978. Cryogenic preservation of fish and mammalian spermatozoa. *Journal of Reproductive Fertility* 53:13-18.

National Research Council. 1996. Pages 145-163 *in* Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington, DC.

Nehlsen, W., J. E. Williams and J. A. Lichantowich. 1991. Pacific Salmon at Crossroads: Stocks at Risk from California, Oregon, Idaho and Washington. *Fisheries* 16(2): 4-20.

Rall, W.F. and G.M. Fahy. 1985. Ice-free cryopreservation of mouse embryos at -196°C by vitrification. *Nature*. Vol. 313. P.573-575.

Ryder, O.A., A. McLaren, S. Brenner, Y.Zhang, K. Benirschka. 2000. DNA Banks for Endangered Animal Species. *Science*. Volume 288. Page 275.

Servheen G. and 16 coauthors. 2001. Salmon Subbasin Summary, Northwest Power Planning Council. <http://www.cbfwa.org/files/province/mtnsnake/subsum.html>.

Scheerer, P.D. and G.H. Thorgaard. 1989. Improved fertilization by cryopreserved rainbow trout semen treated with theophylline. *Prog. Fish. Cult.* 51:179-182.

Stoss, J. 1983. Fish gamete preservation and spermatozoan physiology. Pages 305-350 *in* W. S. Hoar, D. J. Randell, and E. M. Donaldson editors. *Fish Physiology*. Vol. 9, part B, Academic Press, New York.

Summerfelt, R.C. and L.S. Smith. 1990. Anesthesia, Surgery, and Related Techniques. Page 213. *in* C.B. Schreck and P. B. Moyle editors. *Methods for Fish Biology*. American Fisheries Society, Bethesda, Maryland.

Trounson, A. and L. Mohr. 1983. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature*. Vol. 305. P. 707-709.

Whittingham, D.G. 1980. Principles of embryo preservation. Pages. 65-83 *in* M. J. Ashwood-Smith and J. Farrant editors. *Low Temperature Preservation in Medicine and Biology*. Pitman Medical Ltd., Tunbridge Wells, Kent, England.

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 2002.

Spawning Aggregate	Year											Totals
	2002	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992	
Lostine River	19	33	18	2	3	2	3	1	4			85
Upper Grande Ronde River	8	9										17
Catherine Creek	5	11										16
Rapid River			51	68	98							217
South Fork Salmon River	23	44	54	93	45	45	19					323
Lake Creek	18	28	15	6	3	4	3					77
Johnson Creek	58	62	35	5	17	7						184
Big Creek	21	50	7	0	1	6	0	0	0	10	7	102
Capehorn Creek	1	2	1	0	6	2						12
Marsh Creek	34	24	7	0	2	4						71
Pahsimeroi River	39	50	50	31								170
Upper Salmon River	54	48	40	40	41	51						274
Imnaha River	7	37	71	95	79	41	33	42	22			427
Totals	287	398	349	340	295	162	58	43	26	10	7	1975

Table A2. Snake River basin steelhead samples cryopreserved from 1993 through 2002.

Spawning Aggregate	Year								Totals
	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
Johnson Creek		1		2					3
Pahsimeroi River	63	60	40	47					210
Imnaha River			2						2
Little Sheep Creek	93	78	52	25	25	5			278
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 2002.

Table A3. Collection date, fork lengths, percent motilities and number of straws from chinook salmon collected in 2002.

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Big Creek	08/13/02	76	80	20	7	80	20	6
Big Creek	08/13/02	82.4	70	20		80	10	0
Big Creek	08/13/02	73	70	20				
Big Creek	08/13/02	73	70	20	4	80	20	4
Big Creek	08/13/02	78.5	80	20	3	50	20	0
Big Creek	08/13/02	75.5			6	50	20	0
Big Creek	08/13/02	75	80	20		50	20	0
Big Creek	08/13/02	75.5	90	20	4	80	10	0
Big Creek	08/13/02	82.5	80	20				
Big Creek	08/13/02	73.5	70	20	2	60	20	0
Big Creek	08/13/02	74.5	80	20	4	90	20	4
Big Creek	08/13/02	64	80	20		80	20	0
Big Creek	08/13/02	75.5	70	10				
Big Creek	08/13/02	76.5	80	20	2	80	6	0
Big Creek	08/20/02	74.5	60	20	2			
Big Creek	08/20/02	93.5	70	20	4			
Big Creek	08/20/02	73.5	80	20	5			
Big Creek	08/20/02	83.5	50	20	1			
Big Creek	08/20/02	77	80	20	5			
Big Creek	08/20/02	70.5	80	20	2			
Big Creek	08/20/02	75.5	90	20	1			
Cape Horn Creek	08/16/02	77.5	70	20	1	50	20	0
Catherine Creek	08/30/02	73.4				70	20	5
Catherine Creek	08/30/02	75.5	90	20	5			
Catherine Creek	08/30/02	68.2				70	20	5
Catherine Creek	09/11/02	65.4				10	20	0
Catherine Creek	09/11/02	58.3	90	30	5			
Grande Ronde River	08/30/02	77				90	20	0
Grande Ronde River	08/30/02	70	90	20	3			
Grande Ronde River	08/30/02	71.1				60	20	5
Grande Ronde River	08/30/02	79.5	70	20	5	20	20	0
Grande Ronde River	08/30/02	76	80	20	5			
Grande Ronde River	08/30/02	90.6	70	20	5	10	20	0
Grande Ronde River	09/11/02	73.5				30	20	
Grande Ronde River	09/11/02	97.7	70		3			
Imnaha River	08/28/02	72.5				80	20	1
Imnaha River	08/28/02	82.7	80	20	4			
Imnaha River	08/28/02	80				80	20	5

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Imnaha River	08/28/02	88.5	90	20	3			
Imnaha River	09/11/02	95.3	80	30	5			
Imnaha River	09/11/02	81.5				10	20	
Imnaha River	09/11/02	58	80	30	3			
Johnson Creek	08/23/02		90	20	6			
Johnson Creek	08/23/02	83	90	20	4			
Johnson Creek	08/23/02	82	80	20	7			
Johnson Creek	08/23/02	74	90	20	7			
Johnson Creek	08/23/02	77	90	20	6			
Johnson Creek	08/23/02	73	80	20	7			
Johnson Creek	08/23/02	85	90	20	7			
Johnson Creek	08/23/02	79	90	20				
Johnson Creek	08/23/02	85	80	20	1			
Johnson Creek	08/23/02	83						
Johnson Creek	08/23/02	81	80	20				
Johnson Creek	08/23/02		90	20	3			
Johnson Creek	08/23/02	81	80	20				
Johnson Creek	08/23/02	76	80	20				
Johnson Creek	08/27/02	87				70	20	3
Johnson Creek	08/27/02	87				80	20	5
Johnson Creek	08/27/02	58				90	16	
Johnson Creek	08/27/02	78				70	20	2
Johnson Creek	08/28/02	88	0			70	20	5
Johnson Creek	08/28/02	88				40	20	
Johnson Creek	08/28/02	83	80	20				
Johnson Creek	08/28/02	84	80	20	5	30	20	
Johnson Creek	08/28/02	80	80	20	3	30	20	
Johnson Creek	08/28/02	90	0			80	20	5
Johnson Creek	08/28/02	83				80	20	5
Johnson Creek	08/28/02	80	90	20	1	70	20	1
Johnson Creek	08/28/02	79	80	10				
Johnson Creek	08/28/02	79	80	20	2	50	20	4
Johnson Creek	08/28/02	91				80	20	3
Johnson Creek	08/28/02	80	70	20	5			
Johnson Creek	08/28/02	80				80	20	2
Johnson Creek	08/28/02	79	80	20	5	60	19	5
Johnson Creek	08/28/02	67	90	20	2			
Johnson Creek	08/28/02	45				80	10	
Johnson Creek	08/28/02	81	10			80	20	5
Johnson Creek	08/30/02		80	20				
Johnson Creek	08/30/02	83	20			10	20	
Johnson Creek	08/30/02	87				10	20	

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Johnson Creek	08/30/02		80	20	4	50	20	3
Johnson Creek	08/30/02	83				80	20	5
Johnson Creek	08/30/02	85	80	20	3	50	20	
Johnson Creek	08/30/02	73	90	20	5	20	20	
Johnson Creek	08/30/02	92	90	20	5			
Johnson Creek	08/30/02	87	80	20	3	20	20	
Johnson Creek	08/30/02	76	90	20	5	80	20	5
Johnson Creek	08/30/02	88	70	20	5	80	20	5
Johnson Creek	08/30/02		50	20	4	10	20	
Johnson Creek	09/03/02	92	80	20	4	20	20	
Johnson Creek	09/03/02	79	90	20	3			
Johnson Creek	09/03/02	86				40	20	
Johnson Creek	09/03/02	87	80	20	5			
Johnson Creek	09/03/02	81	80	20	5	70	20	5
Johnson Creek	09/04/02	92	60	20	5			
Johnson Creek	09/04/02	77				20	20	
Johnson Creek	09/04/02	83	80	20	5			
Johnson Creek	09/04/02					70	8	
Johnson Creek	09/04/02	75	90	20	3	70		
Johnson Creek	09/04/02	76	80	20	5			
Lake Creek	08/12/02	55	30	10				
Lake Creek	08/12/02	87.8	80	20	5	50	20	0
Lake Creek	08/12/02	80.5	80	20	3	50	20	0
Lake Creek	08/12/02	91.5	90	20	4	20	20	
Lake Creek	08/12/02	78	70	20	4	70	20	5
Lake Creek	08/12/02	75.2	80	20				
Lake Creek	08/12/02	72.1	90	20	2	80	20	1
Lake Creek	08/12/02	99				20	20	0
Lake Creek	08/12/02	80				80	10	0
Lake Creek	08/12/02	82.5	90	20	1	80	19	0
Lake Creek	08/19/02	74	80	20	7			
Lake Creek	08/19/02	69	80	20	7			
Lake Creek	08/19/02	80	90	20	7			
Lake Creek	08/19/02	74	70	20				
Lake Creek	08/19/02	83	80	10				
Lake Creek	08/19/02	77	70	20	1			
Lake Creek	08/19/02	95	80	20	6			
Lake Creek	08/19/02	78	90	20	7			
Lostine River	08/28/02	74.4				50	20	1
Lostine River	08/28/02	97.5	90	20	7			
Lostine River	08/28/02	94.5				80	20	5
Lostine River	09/04/02	71.5				80	20	2

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Lostine River	09/04/02		80	20	5			
Lostine River	09/04/02	82.9				50	20	0
Lostine River	09/04/02	84	80	20	5	40	20	0
Lostine River	09/04/02	99	90	20	5	50	20	2
Lostine River	09/04/02	79	70	20	5			
Lostine River	09/04/02	66.6				80	20	3
Lostine River	09/04/02	74	90	20	5	20	20	3
Lostine River	09/04/02	76.5	80	20	5			
Lostine River	09/04/02	88.7				80	20	1
Lostine River	09/04/02	78.9	80	20	5			
Lostine River	09/04/02	76.2				80	20	2
Lostine River	09/11/02	78.5				50	20	
Lostine River	09/11/02	102	90	30	5			
Lostine River	09/11/02	55.3				40	20	
Lostine River	09/11/02	76	80	30	5			
Marsh Creek	08/15/02	87	0			60	16	0
Marsh Creek	08/15/02	92	70	20	5	40	20	0
Marsh Creek	08/15/02	76.5	40	10				
Marsh Creek	08/15/02	76.5	0			80	20	5
Marsh Creek	08/15/02	99	40	20	5	70	20	0
Marsh Creek	08/15/02	86	80	20	2	20	20	0
Marsh Creek	08/15/02	91.5	70	20		80	20	3
Marsh Creek	08/15/02	78.5	70	20		90	20	5
Marsh Creek	08/15/02	76				70	20	3
Marsh Creek	08/15/02	95	70	20	2	90	20	4
Marsh Creek	08/15/02	71	80	20	2			
Marsh Creek	08/15/02	85	80	20	2	60	20	0
Marsh Creek	08/15/02	85	80	20		80	20	4
Marsh Creek	08/15/02	88.5	70	20	2	90	20	4
Marsh Creek	08/15/02	80.5	80	20	1	70	20	2
Marsh Creek	08/16/02	77	70	20	3			
Marsh Creek	08/16/02	86.5	70	20				
Marsh Creek	08/16/02	69.5	80	10		50	19	0
Marsh Creek	08/16/02	82.5	90	10		90	10	0
Marsh Creek	08/16/02	71	80	20	2	30	20	0
Marsh Creek	08/16/02	71	90	20	2	90	20	0
Marsh Creek	08/16/02	110.5	0			50	20	0
Marsh Creek	08/16/02	81				50	5	0
Marsh Creek	08/22/02	92.5	80	20	6			
Marsh Creek	08/22/02	97	90	20	7			
Marsh Creek	08/22/02	93	80	20	4			
Marsh Creek	08/22/02	94	70	20	7			

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Marsh Creek	08/22/02	85.5	70	20	7			
Marsh Creek	08/22/02	71	90	20	1			
Marsh Creek	08/22/02	105	80	20	7			
Marsh Creek	08/22/02	69.5	60	20	2			
Marsh Creek	08/22/02	78.5	80	20	7			
Marsh Creek	08/22/02	83	90	20	7			
Pahsimeroi River	09/24/02	65	90	20				
Pahsimeroi River	09/24/02	92	70	20		50	20	
Pahsimeroi River	09/24/02	66	90	20		60	20	
Pahsimeroi River	09/24/02	78.5	80	20		30	20	
Pahsimeroi River	09/24/02	96	90	20		70	20	
Pahsimeroi River	09/24/02	85	80	20		70	19	
Pahsimeroi River	09/24/02	84	70	20		70	20	
Pahsimeroi River	09/24/02	106	30	20		80	20	
Pahsimeroi River	09/24/02	84	90	20		70	20	
Pahsimeroi River	09/24/02	86	80	20		30	20	
Pahsimeroi River	09/24/02	92	10			10	20	
Pahsimeroi River	09/24/02	83	70	20		30	20	
Pahsimeroi River	09/24/02	79.5	40			50	19	
Pahsimeroi River	09/24/02	86.5	90	20		30	20	
Pahsimeroi River	09/24/02	100	70	20		30	20	
Pahsimeroi River	09/24/02	82.5	90	20		80	20	
Pahsimeroi River	09/24/02	102	90	20		10	20	
Pahsimeroi River	09/24/02	64	70	20		80	20	
Pahsimeroi River	09/24/02	51.5	40			80	20	
Pahsimeroi River	09/24/02	62.5	90	20		50	20	
Pahsimeroi River	10/01/02	90	80	20		50	20	
Pahsimeroi River	10/01/02	79	80	20		80	20	
Pahsimeroi River	10/01/02	97	80	20		70	20	
Pahsimeroi River	10/01/02	83	20			50	20	
Pahsimeroi River	10/01/02	96	90	20		20	20	
Pahsimeroi River	10/01/02	100	40			60	20	
Pahsimeroi River	10/01/02	95	90	20		60	20	
Pahsimeroi River	10/01/02	82	90	20		50	20	
Pahsimeroi River	10/01/02	87	90	20		50	20	
Pahsimeroi River	10/01/02	86	80	20		70	20	
Pahsimeroi River	10/01/02	86	90	20		80	20	
Pahsimeroi River	10/01/02	84	90	20		40	20	
Pahsimeroi River	10/01/02	83	90	20		20	20	
Pahsimeroi River	10/01/02	93	90	20		20	20	
Pahsimeroi River	10/01/02	83	40			50	20	
Pahsimeroi River	10/01/02	87	90	20		70	20	

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Pahsimeroi River	10/01/02	88	80	20		50	20	
Pahsimeroi River	10/01/02	84	90	20		40	20	
Pahsimeroi River	10/01/02	90	90	20		50	20	
Upper Salmon River	08/22/02	83.7	80	20	3			
Upper Salmon River	08/22/02	88.5	80	10				
Upper Salmon River	08/22/02	95.5	90	20	6			
Upper Salmon River	08/22/02	71.5	90	20	1			
Upper Salmon River	08/22/02	102	80	20	7			
Upper Salmon River	08/22/02	100	80	20	3			
Upper Salmon River	08/22/02	100	80	20	1			
Upper Salmon River	08/22/02	80	90	20				
Upper Salmon River	08/22/02	74	80	10				
Upper Salmon River	08/22/02	74.5	80	20				
Upper Salmon River	08/22/02	74	80	20	7			
Upper Salmon River	08/22/02	72	70	20	7			
Upper Salmon River	08/22/02	84	90	20	2			
Upper Salmon River	08/22/02	91	80	20	7			
Upper Salmon River	08/22/02	68	80	20	7			
Upper Salmon River	08/29/02	83	90	20	2	80	20	4
Upper Salmon River	08/29/02	82	10			70	20	
Upper Salmon River	08/29/02	66	50	20				
Upper Salmon River	08/29/02	103	70	20	5	70	20	
Upper Salmon River	08/29/02	108	70	20	5	80	20	
Upper Salmon River	08/29/02	78	90	20	5	80	20	
Upper Salmon River	08/29/02	111	80	20	5	80	20	4
Upper Salmon River	08/29/02	74	90	20	2	80	20	5
Upper Salmon River	08/29/02	59				90	20	5
Upper Salmon River	08/29/02	59	90	20	3	90	20	1
Upper Salmon River	08/29/02	92	10			80	20	5
Upper Salmon River	08/29/02	81	80	20	5	70	20	5
Upper Salmon River	08/29/02	80	90	20	5	70	20	5
Upper Salmon River	08/29/02	105	90	20	5	80	20	5
Upper Salmon River	08/29/02	110	70	20	5	80	20	5
Upper Salmon River	08/29/02	91	80	20	5	80	20	5
Upper Salmon River	08/29/02	76	80	20	4	80	20	5
Upper Salmon River	08/29/02	74	80	20	5	80	20	4
Upper Salmon River	08/29/02	84	70	20	5	60	20	5
Upper Salmon River	09/05/02	102	90	20	5			
Upper Salmon River	09/05/02	110	90	20				
Upper Salmon River	09/05/02	81	80	20	5			
Upper Salmon River	09/05/02	68	80	20	4			
Upper Salmon River	09/05/02	105	80	20	4			

Collection site	Collection Date	Fork Length (cm)	WSU Motility (%)	Number of 0.5 ml Straws at WSU	Number of 5.0 ml Straws at WSU	UI Motility (%)	Number of 0.5 ml Straws at UI	Number of 5.0 ml Straws at UI
Upper Salmon River	09/05/02	72	90	20	4			
Upper Salmon River	09/05/02	79	90	20	5			
Upper Salmon River	09/05/02	110	90	20	3			
Upper Salmon River	09/05/02	82	80	20	5			
Upper Salmon River	09/05/02	81	90	20	5			
Upper Salmon River	09/05/02	75	90	20	5			
Upper Salmon River	09/05/02	86	90	20	5			
Upper Salmon River	09/05/02	79	90	20	5			
Upper Salmon River	09/05/02	78	80	20	5			
Upper Salmon River	09/05/02	70	90	20	4			
Upper Salmon River	09/05/02	82	90	20	5			
Upper Salmon River	09/05/02	73	90	20	5			
Upper Salmon River	09/05/02	75	80	20				
Upper Salmon River	09/05/02	74	70	20				
Upper Salmon River	09/05/02	77	90	20				
South Fork Salmon R	08/27/02	83	90	20	5			
South Fork Salmon R	08/27/02					80	20	3
South Fork Salmon R	08/27/02			20	5			
South Fork Salmon R	08/27/02					80	20	4
South Fork Salmon R	08/27/02		40			80	20	5
South Fork Salmon R	08/27/02		70	20	3	80	20	
South Fork Salmon R	08/27/02		90	20	5	80	20	5
South Fork Salmon R	08/27/02		90	20	5	80	20	5
South Fork Salmon R	08/27/02		80	20	5	80	20	3
South Fork Salmon R	08/27/02		90	20	5	70	20	3
South Fork Salmon R	08/27/02		20			50	20	5
South Fork Salmon R	08/27/02		40			60	20	5
South Fork Salmon R	08/27/02		80	20	4	60	20	4
South Fork Salmon R	08/27/02		70	20	5	80	20	3
South Fork Salmon R	08/27/02		80	20	5	80	20	5
South Fork Salmon R	09/03/02		70	20	5	80	20	3
South Fork Salmon R	09/03/02					5	19	
South Fork Salmon R	09/03/02		50	20				
South Fork Salmon R	09/03/02		80	20	5	90	20	5
South Fork Salmon R	09/03/02		80	20	5	40	20	
South Fork Salmon R	09/03/02		80	20	5	40	20	
South Fork Salmon R	09/03/02		90	20				
South Fork Salmon R	09/03/02		10			70	20	5
Totals				4440	686		3275	284

Appendix 4. Data from steelhead collected in 2002.

Table A4. Collection date, fork lengths, percent motilities and number of straws from steelhead collected in 2002.

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
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02	93	90	10		
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02		80	20		
North Fork Clearwater	2/26/02		80	20		
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02		90	18		
North Fork Clearwater	2/26/02		90	20		
North Fork Clearwater	2/26/02		70	20		
North Fork Clearwater	2/26/02		80	20		
North Fork Clearwater	2/26/02		70	20		
North Fork Clearwater	2/26/02		70	20		
North Fork Clearwater	2/26/02		60	20		
North Fork Clearwater	3/19/02		80	20		
North Fork Clearwater	3/19/02		70	20		
North Fork Clearwater	3/19/02		80	20		
North Fork Clearwater	3/19/02		80	20		
North Fork Clearwater	3/19/02		90	20		
North Fork Clearwater	3/19/02		90	20		
North Fork Clearwater	3/19/02		80	20		
North Fork Clearwater	3/19/02		80	20		
North Fork Clearwater	3/19/02		90	20		
North Fork Clearwater	3/19/02		90	20		
North Fork Clearwater	3/19/02		70	20		
North Fork Clearwater	3/19/02		90	20		
North Fork Clearwater	3/26/02	75			70	20
North Fork Clearwater	3/26/02	93			80	20
North Fork Clearwater	3/26/02				80	20
North Fork Clearwater	3/26/02				80	20
North Fork Clearwater	3/26/02	85			80	11
North Fork Clearwater	3/26/02	89			70	20
North Fork Clearwater	3/26/02	95			70	20
North Fork Clearwater	3/26/02				80	20
North Fork Clearwater	3/26/02				70	20
North Fork Clearwater	3/26/02	89			70	20
North Fork Clearwater	3/26/02				70	20
North Fork Clearwater	3/26/02	88			70	20
North Fork Clearwater	3/26/02	94			60	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
North Fork Clearwater	3/26/02	92			70	20
North Fork Clearwater	3/26/02	70			70	20
North Fork Clearwater	3/26/02	99			80	20
North Fork Clearwater	3/26/02	92			70	20
North Fork Clearwater	4/9/02				80	20
North Fork Clearwater	4/9/02				50	20
North Fork Clearwater	4/9/02				80	15
North Fork Clearwater	4/9/02	102			80	20
North Fork Clearwater	4/9/02	90			70	20
North Fork Clearwater	4/9/02				80	20
North Fork Clearwater	4/9/02				80	20
North Fork Clearwater	4/9/02	92			80	20
North Fork Clearwater	4/9/02				90	20
North Fork Clearwater	4/9/02	89			50	10
North Fork Clearwater	4/9/02	95			90	20
North Fork Clearwater	4/9/02	69			90	20
North Fork Clearwater	4/9/02	93			80	20
North Fork Clearwater	4/9/02	88			90	20
North Fork Clearwater	4/30/02				50	20
North Fork Clearwater	4/30/02				20	14
North Fork Clearwater	4/30/02				50	15
North Fork Clearwater	4/30/02	74			70	10
North Fork Clearwater	4/30/02				70	12
Fish Creek	6/25/02	67	<1	20		
Fish Creek	6/30/02	68	<1	20		
Fish Creek	6/30/02	85	<1	20		
Little Sheep Creek	4/2/02	61			70	17
Little Sheep Creek	4/2/02	56			70	17
Little Sheep Creek	4/2/02	64			70	15
Little Sheep Creek	4/2/02	71			70	15
Little Sheep Creek	4/2/02	58			50	15
Little Sheep Creek	4/2/02	57			70	19
Little Sheep Creek	4/2/02	70			50	20
Little Sheep Creek	4/2/02	59			70	20
Little Sheep Creek	4/2/02				70	18
Little Sheep Creek	4/2/02	72			70	20
Little Sheep Creek	4/2/02	71			50	20
Little Sheep Creek	4/9/02	59	90	20		
Little Sheep Creek	4/9/02	66	90	20		
Little Sheep Creek	4/9/02	64	80	20		
Little Sheep Creek	4/9/02	61	80	20		
Little Sheep Creek	4/9/02	59	90	20		
Little Sheep Creek	4/9/02	85	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
Little Sheep Creek	4/9/02	75	80	20		
Little Sheep Creek	4/9/02	54	90	20		
Little Sheep Creek	4/9/02	62	90	20		
Little Sheep Creek	4/9/02	62	90	20		
Little Sheep Creek	4/9/02	63	80	20		
Little Sheep Creek	4/9/02	65	90	20		
Little Sheep Creek	4/9/02	61	80	20		
Little Sheep Creek	4/9/02	70	90	20		
Little Sheep Creek	4/9/02	73	70	20		
Little Sheep Creek	4/9/02	57	70	20		
Little Sheep Creek	4/9/02	57	80	20		
Little Sheep Creek	4/9/02	56	90	20		
Little Sheep Creek	4/9/02	62	90	20		
Little Sheep Creek	4/9/02	61	70	20		
Little Sheep Creek	4/23/02	56	90	20		
Little Sheep Creek	4/23/02	71	90	20		
Little Sheep Creek	4/23/02	70	80	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	65	90	20		
Little Sheep Creek	4/23/02	67	90	20		
Little Sheep Creek	4/23/02	52	90	20		
Little Sheep Creek	4/23/02	62	80	20		
Little Sheep Creek	4/23/02	61	80	20		
Little Sheep Creek	4/23/02	60	80	20		
Little Sheep Creek	4/23/02	61	80	20		
Little Sheep Creek	4/23/02	60	90	20		
Little Sheep Creek	4/23/02	60	70	20		
Little Sheep Creek	4/23/02	61	80	20		
Little Sheep Creek	4/23/02	61	80	20		
Little Sheep Creek	4/23/02	64	70	20		
Little Sheep Creek	4/23/02	65	70	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	58	90	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	56	90	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	61	90	20		
Little Sheep Creek	4/23/02	60	90	20		
Little Sheep Creek	4/23/02	55	90	20		
Little Sheep Creek	4/23/02	69	90	20		
Little Sheep Creek	4/23/02	61	90	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	4/23/02	59	90	20		
Little Sheep Creek	4/23/02	60	70	20		
Little Sheep Creek	4/23/02	60	90	20		
Little Sheep Creek	4/23/02	60	90	20		
Little Sheep Creek	4/23/02	54	80	20		
Little Sheep Creek	4/23/02	75	90	20		
Little Sheep Creek	4/30/02	59	90	20		
Little Sheep Creek	4/30/02	69	90	20		
Little Sheep Creek	4/30/02	61	90	20		
Little Sheep Creek	4/30/02	61	90	20		
Little Sheep Creek	4/30/02	59	70	20		
Little Sheep Creek	4/30/02	60	70	20		
Little Sheep Creek	4/30/02	59	90	20		
Little Sheep Creek	4/30/02	62	90	20		
Little Sheep Creek	4/30/02	58	90	20		
Little Sheep Creek	4/30/02	57	80	20		
Little Sheep Creek	4/30/02	67	80	20		
Little Sheep Creek	4/30/02	59	80	20		
Little Sheep Creek	4/30/02	58	70	20		
Little Sheep Creek	4/30/02	63	80	20		
Little Sheep Creek	4/30/02	60	70	20		
Little Sheep Creek	4/30/02	58	90	20		
Little Sheep Creek	4/30/02	73	80	20		
Little Sheep Creek	4/30/02	73	80	20		
Little Sheep Creek	4/30/02	60	70	20		
Little Sheep Creek	4/30/02	55	90	20		
Little Sheep Creek	5/14/02	62	80	20		
Little Sheep Creek	5/14/02	60	90	20		
Little Sheep Creek	5/14/02	57	90	20		
Little Sheep Creek	5/14/02	59	90	10		
Little Sheep Creek	5/14/02	59	80	20		
Little Sheep Creek	5/14/02	59	80	20		
Little Sheep Creek	5/14/02	60	90	20		
Pahsimeroi River	4/19/02	57		20		
Pahsimeroi River	4/19/02	60	80	20		
Pahsimeroi River	4/19/02	59	90	20		
Pahsimeroi River	4/19/02	61	70	20		
Pahsimeroi River	4/19/02	63	80	20		
Pahsimeroi River	4/19/02	63	80	10		
Pahsimeroi River	4/19/02	58	80	20		
Pahsimeroi River	4/19/02	58	80	20		
Pahsimeroi River	4/19/02	64	80	20		
Pahsimeroi River	4/19/02	61	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
Pahsimeroi River	4/19/02	68	90	20		
Pahsimeroi River	4/19/02	61	80	20		
Pahsimeroi River	4/19/02	60	80	20		
Pahsimeroi River	4/19/02	72	80	20		
Pahsimeroi River	4/19/02	77	70	20		
Pahsimeroi River	4/19/02	55	90	20		
Pahsimeroi River	4/19/02	66	90	20		
Pahsimeroi River	4/19/02	61	90	20		
Pahsimeroi River	4/19/02	74	90	0		
Pahsimeroi River	4/24/02	66	60	20		
Pahsimeroi River	4/24/02	58	70	20		
Pahsimeroi River	4/24/02		80	20		
Pahsimeroi River	4/24/02	62	50	20		
Pahsimeroi River	4/24/02	63	80	20		
Pahsimeroi River	4/24/02	66	70	20		
Pahsimeroi River	4/24/02	67	50	20		
Pahsimeroi River	4/24/02	63			70	20
Pahsimeroi River	4/24/02	63			70	20
Pahsimeroi River	4/24/02	58			50	20
Pahsimeroi River	4/24/02	65			80	20
Pahsimeroi River	4/24/02	64			30	20
Pahsimeroi River	4/24/02	69			70	20
Pahsimeroi River	4/24/02	61			50	20
Pahsimeroi River	4/24/02	62			50	19
Pahsimeroi River	4/24/02	61			50	20
Pahsimeroi River	4/24/02	62			70	20
Pahsimeroi River	4/24/02	57			30	20
Pahsimeroi River	4/24/02	61			80	20
Pahsimeroi River	4/24/02	62			70	20
Pahsimeroi River	4/24/02	56			50	20
Pahsimeroi River	5/1/02	63			70	20
Pahsimeroi River	5/1/02	59			50	20
Pahsimeroi River	5/1/02	62			20	20
Pahsimeroi River	5/1/02	58			70	20
Pahsimeroi River	5/1/02	64			70	20
Pahsimeroi River	5/1/02	56			50	20
Pahsimeroi River	5/1/02	58			70	20
Pahsimeroi River	5/1/02	67			50	20
Pahsimeroi River	5/1/02	62			50	20
Pahsimeroi River	5/1/02	63			50	20
Pahsimeroi River	5/1/02	63			70	12
Pahsimeroi River	5/1/02	60			50	20
Pahsimeroi River	5/1/02	58			50	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 River	5/1/02	60			50	20
Pahsimeroi River	5/1/02	62			70	15
Pahsimeroi River	5/1/02	56			50	20
Pahsimeroi River	5/1/02	62			50	20
Pahsimeroi River	5/1/02	70			50	16
Pahsimeroi River	5/1/02	58			50	20
Pahsimeroi River	5/1/02	62			70	20
Pahsimeroi River	5/1/02	68			20	20
Pahsimeroi River	5/1/02	59			50	20
Pahsimeroi River	5/1/02	67			50	20
Snake River	4/15/02				80	20
Snake River	4/15/02				70	20
Snake River	4/15/02				70	20
Snake River	4/15/02				60	20
Snake River	4/15/02				80	20
Snake River	4/15/02				70	20
Snake River	4/15/02				70	20
Snake River	4/15/02				70	20
Snake River	4/15/02				80	20
Snake River	4/15/02				90	20
Snake River	4/15/02				80	20
Snake River	4/15/02				80	20
Snake River	4/15/02				70	20
Snake River	4/15/02				60	20
Snake River	4/15/02				80	20
Snake River	4/15/02				70	20
Snake River	4/15/02				80	20
Snake River	4/15/02				80	20
Snake River	4/15/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				80	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				70	20
Snake River	4/18/02				50	20
Snake River	4/18/02				80	20
Snake River	4/18/02				50	20
Snake River	4/18/02				50	20
Snake River	4/18/02				50	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
Snake River	4/18/02				50	20
Snake River	4/18/02				60	20
Snake River	4/18/02				50	20
Snake River	4/18/02				50	20
Snake River	4/18/02				50	20
Snake River	4/18/02				50	20
Snake River	4/22/02				70	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02				80	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	20
Snake River	4/22/02					10
Snake River	4/22/02				80	20
Snake River	4/22/02				90	20
Snake River	4/22/02				70	20
Snake River	4/22/02				80	20
Snake River	4/22/02				90	20
Snake River	4/22/02				90	10
Snake River	4/22/02				70	20
Snake River	4/22/02				90	20
Snake River	4/22/02				70	20
Totals				2708		2725