SALMONID GAMETE PRESERVATION IN THE SNAKE RIVER BASIN

2005 Annual Report

Prepared by:

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ABSTRACT

In spite of an intensive management effort, Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) populations in the Columbia River basin have not recovered and are currently listed as threatened species under the Endangered Species Act. In addition to the loss of diversity from stocks that have already gone extinct, decreased genetic diversity resulting from genetic drift and inbreeding is a major concern. Reduced population and genetic variability diminishes the environmental adaptability of individual species and entire ecological communities. The Nez Perce Tribe (NPT), in cooperation with Washington State University (WSU) and the University of Idaho (IU), 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, this project preserves the genetic diversity of these stocks and provides management options for future species recovery actions. 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 2005 a total of 197 viable semen samples were collected and added to the germplasm repository. This included the gametes from 162 male Chinook salmon from the Lostine River, Minam River, Catherine Creek, upper Grande Ronde River, Imnaha River, Lake Creek, South Fork Salmon River, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, and upper Salmon River and, gametes from 35 male steelhead from the Tucannon River, Little Sheep Creek amd South Fork Salmon River. To date, a total of 2,651 Columbia River male Chinook salmon, 1,368 Columbia River male steelhead gamete samples, 22 Kootenai River male white sturgeon gamete samples and 9 Kootenai River male burbot gamete samples are preserved in the repository. Fertility trials performed using Chinook salmon milt collected and cryopreserved in 2005 resulted in an average fertility of 33%. Genetic analysis of Chinook salmon samples collected in 2003 using 13 microsatellite loci revealed high levels of within and among population diversity were preserved. Gamete collection will continue in 2006 from imperiled Chinook salmon and steelhead populations of the Snake River basin.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

The goals of genetic conservation are to reduce the possibility of extinction and ensure the maintenance and recovery of a species as a functioning ecological unit of the environment. While preventative actions for conserving species such as 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 (Convention on Biological Diversity).

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. 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.

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 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).

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.

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. A gene bank containing a collection of germplasm from multiple river basins preserves the greatest level of genetic diversity and enables recovery programs to use local stocks. This 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 in 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.

Cryotechnology is important in the conservation of aquatic species throughout the world (Harvey et al. 1998; Cloud and Thorgaard, 1993) and its widespread use resulted in scientific improvements enhancing its utility as a conservation tool (Cloud, 2003a; 2003b; Tiersch and Mazik, 2000; Wheeler and Thorgaard, 1991; Stoss, 1983). Using cryotechnology in a recovery program not only preserves genetic diversity for future management options, it also increases genetic diversity and reduces extinction risk in the short term by increasing the effective population size of the population (Ballou 1992). For these reasons, cryopreserved sperm has become an important part of recovery programs in the Snake River basin, especially those that fall under the Safety Net Artificial Propagation Program (SNAPP) such as the Redfish Lake Sockeye and the Grande Ronde Captive Broodstock Projects.

NPT initiated Chinook salmon (*O. tshawytscha)* 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 (*O. mykiss*) populations in the region (Armstrong and Kucera 1999). By collecting from numerous populations of spring and summer Chinook salmon and steelhead across the entire Snake River basin, we hope to preserve the greatest amount of endemic salmonid diversity.

This annual report details NPT germplasm preservation activities from 2005 and updates the status of the long-term repository.

METHODS

Description of Spawning Aggregates

The cryopreservation project managed by NPT currently seeks to preserve male spring and summer Chinook salmon and steelhead gametes in the Snake River basin (Figure 1). The large number of subbasins within this region has resulted in a genetically diverse collection of anadromous species. The following is a list of the sub-basins and locations that were sampled in 2005.

CHINOOK SALMON

Grande Ronde River Subbasin

1. Catherine Creek (collected at Lookingglass Hatchery)
2. Upper Grande Ronde River (collected at Lookingglass Hatchery)
3. Lostine River (collected at Lookingglass Hatchery)
4. Minam River

Salmon River Subbasin

1. Lake Creek
2. Johnson Creek
3. Marsh Creek
4. Capehorn Creek
5. Big Creek
6. South Fork Salmon River (SFSR - collected at the SFSR weir, McCall Fish Hatchery)
7. Upper Salmon River (collected at Sawtooth Fish Hatchery)

Imnaha River Subbasin

1. Imnaha River (collected at Lookingglass Hatchery)

STEELHEAD

Tucannon River Subbasin

1. Tucannon River (collected at Lyons Ferry Hatchery)

Salmon River Subbasin

1. South Fork Salmon River
2. Johnson Creek

Imnaha River Subbasin

1. Little Sheep Creek (collected at Little Sheep Creek weir)

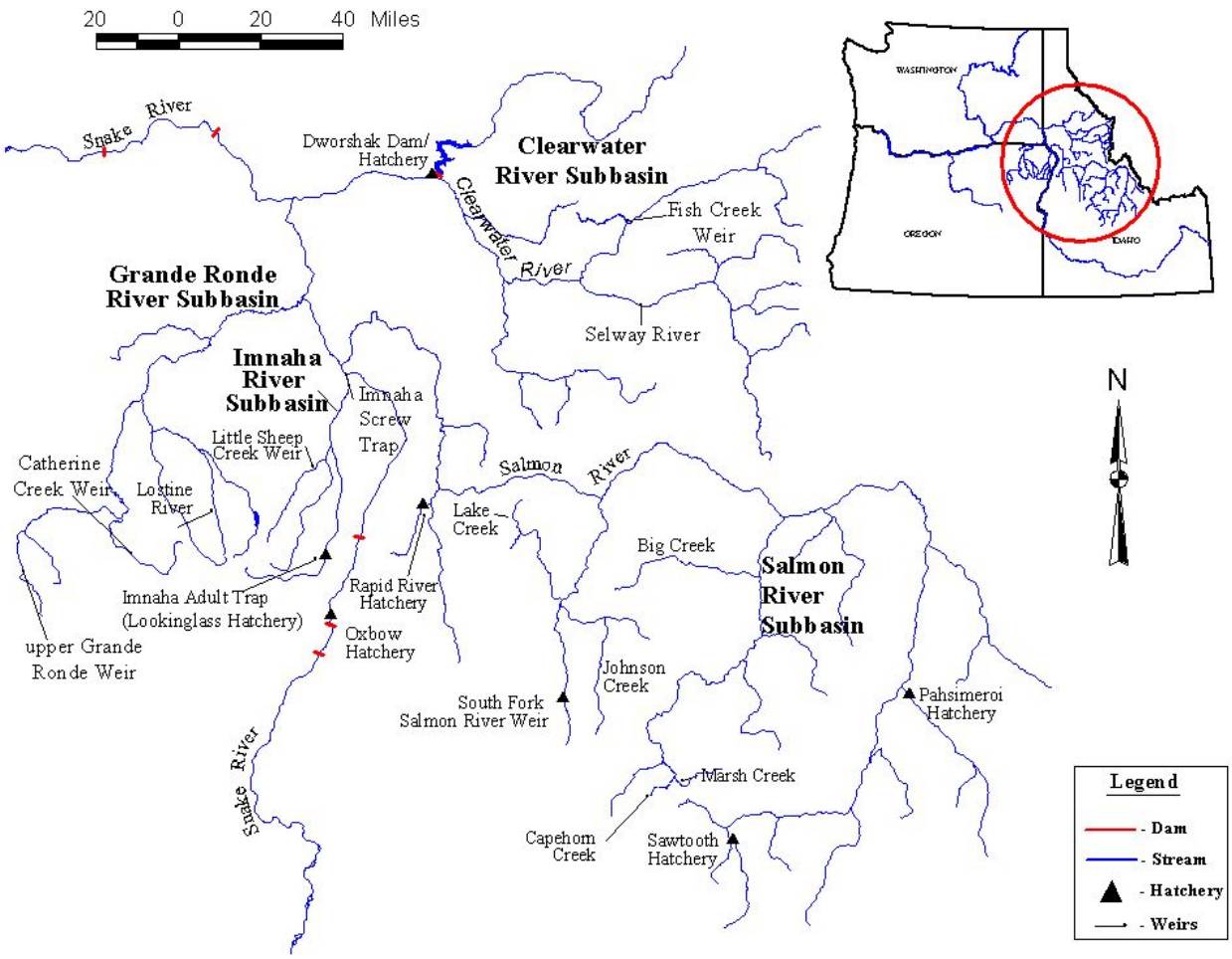

Figure 1. Map showing the Snake River basin Chinook salmon and steelhead sampling locations for 2005.

Fish Collection and Handling

Chinook salmon spawning ground surveys were determined when and where in each stream the collection of adult males would be most effective. Several team members located adults and visually identified male salmon, being careful not to disturb the fish. Actively spawning females and males paired with females were avoided so as not to disrupt spawning. Males were identified by secondary sexual characteristics such as a kype, large teeth, and a slim caudal peduncle that is not as worn as the female salmon. Personnel were instructed to stay away from any existing or active redds. 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.

All adult male salmon were collected by hand or dip net in that order of preference. Hand collections involved walking or swimming up to the identified fish and grasp the fish at caudal peduncle, putting the fish into a dip net and keeping the fish in the water, pointing upstream, until ready to place in the tank. Dip net collection involved placing several dip netters in a position downstream of the fish, being careful to avoid redds, while several upstream people slowly herd fish towards the netters. The large dip nets are held in the water in a line effectively blocking the stream until the fish swims into the net. Inadvertently caught females were immediately released from the net without ever being out of the water and the capture was recorded.

Captured fish were held in the stream while a portable tank was set up along the stream. Fish were immobilized using anesthetic so they could be handled faster and less stressfully. The anesthesia was delivered by placing the fish in a portable tank filled with 135 liters of water containing 90 mg/l of tricane methanesulfonate (MS-222, Finquel^{TM}) anesthesia and approximately 180 mg/l sodium bicarbonate ($NaHCO_3$) to buffer the acidity of the MS-222. The fish was constantly monitored while in the tank and the time to sedation was noted. The sedated fish was rinsed in the fresh water of the stream and the abdomen dried to reduce water contamination prior to collecting the milt. Milt was collected in a plastic Whirl Pak bag by gently squeezing the abdomen (Figure 2).

Figure 2. Collecting Chinook salmon milt from anaesthetized Chinook salmon.

General biological information such as fork length, mid-eye to hypural plate length, general condition and external marks were recorded following semen collection (Figure 3). Caudal fin tissue was collected and preserved in ethyl alcohol for later genetic (DNA) analysis and scales were taken for age assessment and scale pattern analysis. Stream water was gently poured over

the salmon's head and gills to start the recovery from the MS-222 and reduce stress on the fish while this information was collected. Following sampling and data collection, the anesthetized salmon were immediately returned to a slow water area and assisted until it fully recovered. After the fish is released into the stream, the tank was emptied well away from the stream to prevent the release of chemicals into the stream proper.

Spring/summer Chinook salmon gametes were also collected at weirs and hatchery traps. Fish were either anesthetized by personnel working the traps or euthanatized following production spawning. Milt was then collected using the standard protocol (see above).

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

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

In 2003 we obtained ESA section 10 permit approval to capture adult steelhead males by angling (Permit # 1134). The permit states that we were limited to artificial lures and barbless hooks. The preferred method involved locating male steelhead away from active redds and targeting these fish. At other times we fished deep holding water. Once hooked, fish were brought in as rapidly as possible, netted and held in the water until the anesthesia tank was set up. Sperm was taken as described for Chinook salmon above. The fish were measured (fork length) and a tissue sample was taken for DNA analysis. Fish were revived by holding them in the current until they swam away. We used the following length age relationship to determine the ages of steelhead collected from the Imnaha River subbasin (Little Sheep, Cow and

Lightning Creeks): <64 cm - age 3 and > 64 cm – age 4. We used the following length age relationship to determine the ages of steelhead collected from the South Fork Salmon River (B-run steelhead; data from Dworshak National Fish Hatchery): <72 cm – age 3, 72 – 93 cm – age 4 and >93 cm – age 5.

Semen Handling and Cryopreservation

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

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

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

RESULTS

The Chinook salmon and steelhead spawning aggregates and hatcheries in the Snake River basin where gametes were collected in 2005 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 162 male Chinook salmon (Table 1) were collected and cryopreserved from eleven populations in 2005. Collections occurred over a two-month period from August 2, 2005 to September 9, 2005. Gametes were collected from 125 unmarked, natural-origin fish and 37 marked, hatchery-origin fish. Seven males were recaptured and 3 adipose fin clipped males were captured in natural-origin streams (1 each in Lake, Big and Capehorn Creeks). Five females were accidentally captured and immediately released. Motility of the sperm ranged from 0 – 90%.

Gametes from 35 male steelhead (Table 2) were collected and cryopreserved from three populations in 2005. Collections occurred over a two-month period from March 8 to May 12, 2005. Gametes were collected at Little Sheep Creek adult trap, Lyons Ferry Hatchery (Tucannon River steelhead) and by angling in the South Fork Salmon River. Motility of the sperm ranged from 0 – 90%.

2005 Chinook Salmon Gamete Collections

Lostine River

In 2005 the gametes from 14 male Chinook salmon were cryopreserved from fish trapped at the adult weir on the Lostine River and spawned at Lookingglass Hatchery. The collection included gametes from 1 adipose fin clipped, hatchery-origin male and 13 unmarked, natural-origin males. Based on the length data (Appendix B), eleven age 4 and three age 5 fish were sampled from brood years 2001 and 2000 respectively. Collections from 1994 to 2005 have preserved a total of 154 Lostine River male gamete samples in the gene bank (Appendix A).

Minam River

In 2005 the gametes from four unmarked, natural-origin male Chinook Salmon were cryopreserved from fish captured in the Minam River. Based on length data, two age 4 and two age 5 fish were sampled, originating from brood years 2001 and 2000, respectively. This was the first year that gametes were collected from males in the Minam River.

Table 1. Locations and numbers of spring and summer Chinook salmon milt samples cryopreserved in the Snake River basin in 2005.

Spawning Aggregate	Total Samples	Unmarked Fish[a]	Marked Fish[b]	Females Captured	Collection Dates	Sperm Motility (%)
Lostine River	14	13	1	0	8/23, 31, 9/7	50-90
Minam River	4	4	0	0	9/7	50-80
Catherine Creek	10	1	9	0	9/1, 8	70-90
Grande Ronde River	7	1	6	0	9/1, 8	70-90
Imnaha River	12	11	1	0	8/30, 9/5	50-90
S. Fork Salmon River	11	9	2[c]	0	8/25 & 27	0-90
Lake Creek	20	20	0	1	8/3, 10, 15, 22	0-90
Johnson Creek	48	41	7[c]	0	8/17,19, 23,24, 26, 27, 31	50-90
Big Creek	6	6	0	2	8/2, 11 & 16	60-90
Capehorn Creek	6	6	0	0	8/12, 18	50-90
Marsh Creek	6	6	0	2	8/12 & 18	60-90
Upper Salmon River	18	7	11	0	9/1 & 9	50-90
Totals	162	125	37	5	8/2 – 9/9	0-90

[a]Non fin-clipped fish, natural-origin
[b]Fin-clipped or tagged fish, hatchery-origin
[c]Natural by Hatchery-origin cross supplementation fish, marked with a coded wire tag (CWT) and/or visual implant elastomer (VIE) tag

Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2005.

Spawning Aggregate	Total Samples	Un-marked Fish[a]	Marked Fish[b]	Females Captured	Collection Dates	Sperm Motility (%)
Little Sheep Creek	11	1	10	0	4/19	0-90
Tucannon River	22	22	0	0	3/8	0-90
South Fork Salmon River	2	2	0	3	4/8, 16, 20, 21, 26, 5/3, 5 12	50-90
Totals	35	25	10	3	3/8 – 5/12	0-90

[a]Non fin-clipped fish, natural origin
[b]Fin-clipped or tagged fish, hatchery origin

Upper Grande Ronde

In 2005 the gametes from seven male Chinook salmon were cryopreserved from fish trapped at the adult weir on the upper Grande Ronde River and spawned at Lookingglass Hatchery. The collection included gametes from seven adipose fin clipped, hatchery-origin males and one unmarked, natural-origin male. Based on the length data (Appendix B), one age 3 and six age 4 fish were sampled from brood years 2002 and 2001, respectively. Collections from 2001 to 2005 have preserved a total of 42 Grand Ronde River male gamete samples in the gene bank (Appendix A).

Catherine Creek

In 2005 the gametes from ten male Chinook salmon were cryopreserved from fish trapped at the adult weir on the Catherine Creek and spawned at Lookingglass Hatchery. The collection included gametes from nine adipose fin clipped, hatchery-origin males and one unmarked, natural-origin male. Based on the length data (Appendix B), one age 3 and nine age 4 fish were sampled from brood years 2002 and 2001, respectively. Collections from 2001 to 2005 have preserved a total of 41 Catherine Creek male gamete samples in the gene bank (Appendix A).

Imnaha River

In 2005 the gametes from twelve Chinook salmon were cryopreserved from fish trapped in the Imnaha River and spawned at Lookingglass Fish Hatchery. The collection included gametes from one adipose fin clipped, hatchery-origin male and 11 unmarked, natural-origin males. Based on the length data (Appendix B), 11 age 4 and one age 5 fish were sampled from brood years 2001 and 2000, respectively. Length was not determined for one fish. Collections from 1994 to 2005 have preserved a total of 487 Imnaha River male gamete samples in the gene bank (Appendix A). Of these, 210 were from marked hatchery-origin males and 253 were from unmarked natural-origin males.

South Fork Salmon River

In 2005 the gametes from eleven male Chinook salmon were cryopreserved from fish trapped at the adult weir on the South Fork Salmon River (McCall Hatchery, Idaho Department of Fish and Game - IDFG). The collection included gametes from two marked (CWT), supplementation males (natural by hatchery-origin cross) and 9 unmarked, natural-origin males. Based on the length data (Appendix B), three age 3 and eight age 4 fish were sampled from brood years 2002 and 2001, respectively. Collections from 1996 to 2005 have preserved a total of 375 South Fork Salmon River male gamete samples in the gene bank (Appendix A). Of these, 183 were from non-ESA-listed hatchery-origin males, 84 were from ESA listed, supplementation males, and 106 were from unmarked, ESA-listed natural-origin males.

Lake Creek

In 2005 the gametes from 20 unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Lake Creek. One female Chinook salmon was incidentally netted and immediately released. One adipose-clipped fish was captured and released (milt was not collected) and one male was recaptured and released without taking an additional sample. Based on the length data (Appendix B), one age 3, 15 age 4 and four age 5 fish were sampled, originating from brood years 2002, 2001 and 2000, respectively. Collections from 1996 to 2005 have preserved a total of 155 Lake Creek male gamete samples in the gene bank (Appendix A).

Johnson Creek

In 2005 the gametes from 48 male Chinook salmon were cryopreserved from fish captured in Johnson Creek. The collection included gametes from 31 males captured at the Johnson Creek adult weir and spawned at McCall Hatchery's South Fork Salmon River facility as part of the Johnson Creek supplementation project, 5 males captured at the NPT Johnson Creek adult weir and immediately released upstream and 15 males netted in Johnson Creek. Duplicate gametes samples were collected from three males; one male was captured at the weir and later netted in the creek, one male was sampled at the SFSR trap and later netted in the creek and one male was sampled twice at the SFSR trap. Based on the length data (Appendix B), four age 3, 42 age 4 and one age 5 fish were sampled, originating from brood years 2002, 2001 and 2000, respectively. Length was not determined for one fish. Collections from 1997 to 2005 have preserved a total of 343 Johnson Creek male gamete samples (Appendix A).

Big Creek

In 2005 the gametes from six unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Big Creek. One adipose-clipped male was captured (milt was not collected) and two males were recaptured and released without taking an additional sample. Milt from one male was transported to UI but was not frozen due to zero sperm motility. Two female Chinook salmon were incidentally netted and immediately released. Based on the length data (Appendix B), one age 3, four age 4 and one age 5 fish were sampled, originating from brood years 2002, 2001 and 2000, respectively. Collections from 1992 to 2005 have preserved a total of 161 Big Creek male gamete samples in the gene bank (Appendix A).

Capehorn Creek

In 2005 the gametes from six unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Capehorn Creek. One adipose-clipped fish was captured (milt was not collected) and one male was recaptured and released without taking an additional sample (it was also adipose clipped and possessed markings that indicated that it was a recapture of the same adipose clipped male). Based on the length data (Appendix B), five age 4 and one age 5 fish were sampled, originating from brood year 2001 and 2000, respectively. Collections from 1997 to 2005 have preserved a total of 33 Capehorn Creek male gamete samples in the gene bank (Appendix A).

Marsh Creek

In 2005 the gametes from six unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Marsh Creek. One adipose fin clipped Chinook salmon was observed by a snorkeler, but not captured. One male was recaptured and immediately released without taking an additional sample. Two females were captured and immediately released. Based on the length data (Appendix B), one age 3 and five age 4 fish were sampled indicating that they originated from brood year 2002 and 2001, respectively. Collections from 1997 to 2005 have preserved a total of 98 Marsh Creek male gamete samples in the gene bank (Appendix A).

Upper Salmon River

In 2005 the gametes from 18 upper Salmon River male Chinook salmon were cryopreserved from fish spawned at Sawtooth Fish Hatchery. The collection included gametes from 7 unmarked, natural-origin males and 11 adipose fin clipped, hatchery-origin males. Based on the length data (Appendix B), one age 1, fifteen age 4 and two age 5 fish were sampled, originating from brood year 2002, 2001 and 2000, respectively. Collections from 1997 to 2005 have preserved a total of 336 upper Salmon River male gamete samples in the gene bank (Appendix A). Of these, 78 were from marked hatchery fish, 26 were from marked supplementation fish and 234 were from unmarked natural fish.

2005 Steelhead Gamete Collections

Tucannon River

In 2005 the gametes from 22 natural-origin male steelhead were cryopreserved from fish spawned at Lyons Ferry Hatchery with assistance of the Washington Department of Fish and Wildlife (WDFW). Based on the length data (Appendix C), six age 3 and two age 4 fish were sampled, originating from brood years 2002 and 2001, respectively. Lengths of 14 fish were unknown. This was the first year of collection from Tucannon River steelhead.

Little Sheep Creek

In 2005 the gametes from 11 male steelhead were cryopreserved from fish spawned at the Little Sheep Creek adult weir. The collection included gametes from 10 adipose fin clipped hatchery-origin males and one unmarked, natural-origin male. Based on the length data (Appendix C), ten age 3 and one age 4 fish were sampled, originating from brood years 2002 and 2001, respectively. Collections from 1999 to 2005 have preserved a total of 461 Little Sheep Creek male gamete samples in the gene bank (Appendix A). Of these, 435 were from marked hatchery-origin fish and 26 were from unmarked natural-origin fish (Appendix A).

South Fork Salmon River

In 2005 the gametes from 2 unmarked, natural-origin male steelhead were cryopreserved from fish captured by angling in the South Fork Salmon River. Three females were inadvertently captured and immediately released. Based on the length data (Appendix C), both fish were age 4. Collections from 2003 to 2005 have preserved a total of 46 natural-origin SFSR male gamete samples in the gene bank (Appendix A).

Johnson Creek

In 2005 no Johnson Creek steelhead were captured by angling in Johnson Creek. Collections from 1999 to 2005 have preserved a total of four Johnson Creek male gamete samples in the gene bank (Appendix A).

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 expanded to include Chinook salmon and steelhead from most of the major river subbasins in the Snake River basin (Appendix A). Regional support for the project was evident by the addition of cryopreserved samples collected by state management agencies and Native American Tribes. These agencies utilized NPT's

long-term repository to store cryopreserved gametes from other imperiled salmon populations and species in the Columbia River drainage. The repository also includes gamete samples from Redfish Lake sockeye (IDFG), Yakima River spring Chinook salmon (Washington Department of Fish and Wildlife - WDFW), Grande Ronde River subbasin Chinook salmon captive broodstock programs (NPT, ODFW, Confederated Tribes of the Umatilla Indian Reservation), Clearwater River coho salmon (Columbia River Intertribal Fish Comission), Kootenai River white sturgeon (Kootenai Tribe) and Kootenai River Burbot (Kootenai Tribe).

Idaho Fish and Game Department (IDFG) transferred cryopreserved Pahsimeroi River gamete samples from the IDFG Eagle Lab to the storage tanks at WSU. These samples were collected in 1999 and 2000 and had been stored at the Eagle, ID Lab. Transferring them will better maintain security of these samples.

Grande Ronde River Chinook Salmon Captive Broodstock Project

A Grande Ronde River subbasin spring Chinook salmon captive broodstock program, co-managed by Oregon Deparment of Fish and Wildlife, Confederated Tribes of the Umatilla Indian Reservation and NPT, was initiated in 1995 with the collection of juvenile salmon 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 (Kline et al. 2003). Semen was 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 2005. 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 and UI to evaluate the fertility of cryopreserved Chinook salmon sperm collected in 2005. Eggs from a single female and fresh sperm from a single male Snake River Fall Chinook salmon stray (coded wire tag confirmed Umatilla River-origin) were obtained from the Nez Perce Tribal Hatchery. Cryopreserved sperm from fish captured in 2005 were used to fertilize lots of approximately 100 eggs. A single 0.5 ml straw was thawed by immersing it in a 10° C water bath for 10 seconds, removing it to scrape the ice that forms on the straw, and immersing it for another 20 seconds in the water bath. The straw was immediately cut open and the partially frozen milt was poured on the eggs. A sperm activator solution was added and the eggs and sperm were mixed by gently swirling the container. After one minute the activator was poured off and the eggs were rinsed twice with 10° C water. Four additional egg lots were fertilized using an equivalent volume of fresh sperm as a control. The rinsed eggs were then poured into small compartments in a Heath incubation tray. At 10 days post fertilization (streak stage) the eggs were cleared using Stockard's solution and

the successfully fertilized eggs were quantified.

Results of the fertility trials are presented in Table 3. Absolute fertilization rate was determined by dividing the number of fertilized eggs by the total number of eggs. Relative fertilization rate was determined by dividing the absolute fertilization rate by the average control fertilization rate. This transformation reduces the affect of egg quality, allows the results from both labs to be combined and is a better measure of potential sperm quality. Relative fertilities ranged from zero to 89%. Average relative fertility from the WSU trial was 34.78 (S.E = 11.08) and the average relative fertility from the UI trial was 32.67 (S.E. = 10.20).

Straws with a range of prefreeze motility percentages were used in the trial in order to determine the relationship between prefreeze motility and fertilization rate. Prefreeze motility rates ranged from 20% to 90%. Regression analysis of relative fertility (arcsine transformed) and prefreeze motility resulted in a significant slope ($P<0.011$) and an $R^2 = .407$. The relationship was presented in Figure 5. These results suggest that prefreeze sperm motility was marginally effective at predicting relative post thaw fertility. An analysis of all fertility trial data (1999-2005) did not confirm this relationship (data presented in Project Annual Reports 1999 - 2003). Results over 66 crosses produced a significant slope ($P<0.019$) but an $R^2 =0.0827$, indicating a slight but biologically insignificant relationship between prefreeze motility and relative fertility (Figure 6).

Table 3. 2005 results of the fertility trials conducted at WSU and UI including sperm source, prefreeze motility, total eggs fertilized, successfully fertilized eggs and fertilization rate.

Sperm source	Prefreeze motility (%)	Total eggs	Fertilized eggs	Absolute fertilization rate	Relative fertilization rate
Control WSU #1	90	94	92	97.87	n/a
Control WSU #2	90	95	92	96.84	n/a
NPT-137-05	90	107	28	26.17	26.87
NPT-114-05	90	112	62	55.36	56.83
NPT-62-05	50	90	1	1.11	1.14
NPT-77-05	60	116	31	26.72	27.44
NPT-135-05	70	115	69	60.00	61.60
Control UI #1	80	107	92	85.98	n/a
Control UI #2	90	112	104	92.86	n/a
NPT-015-2005	60	98	2	2.04	2.28
NPT-185-2005	60	95	23	24.21	27.08
NPT-198-2005	80	110	71	64.55	72.20
NPT-082-2005	90	76	15	19.74	22.08
NPT-181-2005	60	98	3	3.06	3.42
NPT-109-2005	80	97	38	39.18	43.82
NPT-165-2005	80	116	93	80.17	89.68
NPT-018-2005	20	108	0	0.00	0.00
NPT-147-2005	80	114	61	53.51	59.85
NPT-070-2005	50	107	6	5.61	6.27

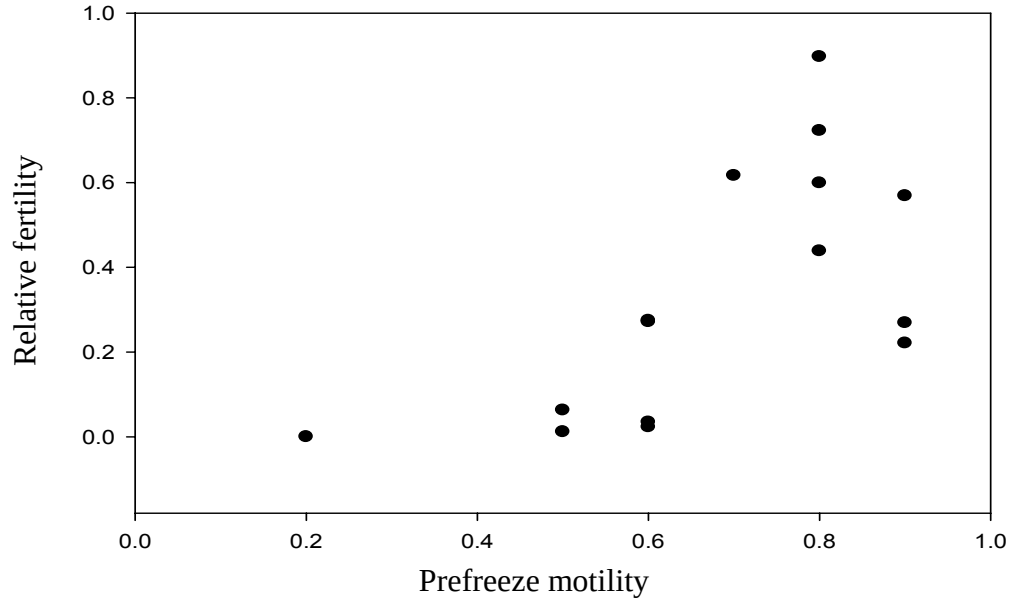

Figure 5. Scatter plot showing the relationship between prefreeze sperm motility and fertilization rate for the 2005 fertility trial.

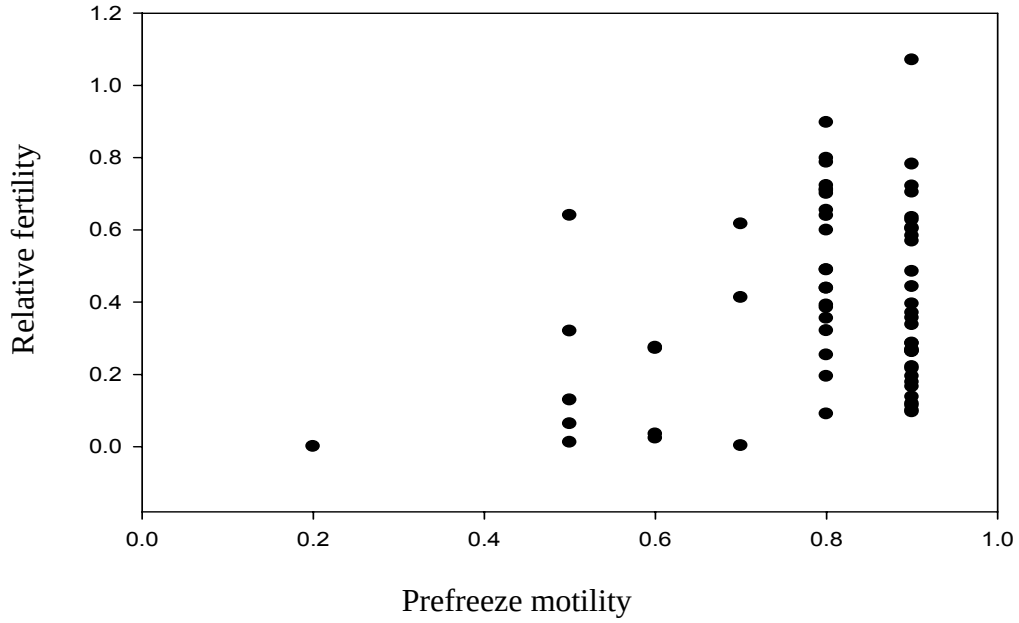

Figure 6. Scatter plot showing the relationship between prefreeze sperm motility and fertilization rate for all fertility trials from 1999 though 2005.

Use of Cryopreserved Gametes in 2005

No gametes from the repository were requested or used in 2005.

Salmonid Genetic Analysis

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

In 2005, tissue samples were collected from the majority of Chinook salmon and steelhead captured and spawned for cryopreservation. These samples will be analyzed and incorporated into a larger analysis of the within and among population spatial and temporal genetic diversity of all samples in the repository.

Genetic analysis results from Chinook salmon samples collected in 2003 were completed in 2005. Genetic analysis results from 7 populations analyzed using 13 microsatellite loci were presented in Table 4. All populations demonstrated relatively high levels of gene and allelic diversity. Exact test for deviation from Hardy Weinberg (H-W) equilibrium revealed that all populations except Big Creek were in H-W equilibrium.

Table 4. Populations of Chinook salmon and results of genetic analyses including the number of samples (N), gene diversity, allelic diversity, allelic richness and exact test probabilities for deviation from H-W equilibrium.

Population	N	Gene diversity (H_e)	Allelic diversity (alleles/locus)	Allelic richness/locus	Hardy-Weinberg Exact Tests
Lake Creek	31	0.7458	10.4	5.8	P>0.9043
SFSR	29	0.7807	12.9	6.6	P>0.9373
Big Creek	31	0.7441	10.9	5.7	P>0.0162*
Marsh Creek	16	0.7359	8.7	5.8	P>0.2061
Capehorn Creek	14	0.7458	8.0	5.8	P>0.4233
Salmon River	19	0.7530	9.9	6.2	P>0.4225
Pahsimeroi R	15	0.7111	7.1	5.6	P>0.3618

* Significant deviation from H-W equilibrium.

Significant among population genetic differentiation was revealed (Fst = 0.0323; Fisher's Exact Test, P<0.00001). An UPGMA (Unweighted Pair Group Method with Arithmatic Mean) phylogenetic tree produced using a Nei's (1972) genetic distance matrix revealed the relationship

of the populations (Figure 7). Bootstrap analysis was performed to assess the reliability of the branch nodes. Overall support for the tree was low, however moderately supported groups (>50%) include the Lake Creek and SFSR group, the Lake Creek, SFSR and upper Salmon River group and the Middle Fork Salmon River group (Marsh, Capehorn and Big Creeks). Low sample sizes (< 20 samples) for many of the populations likely limited overall robustness of the tree.

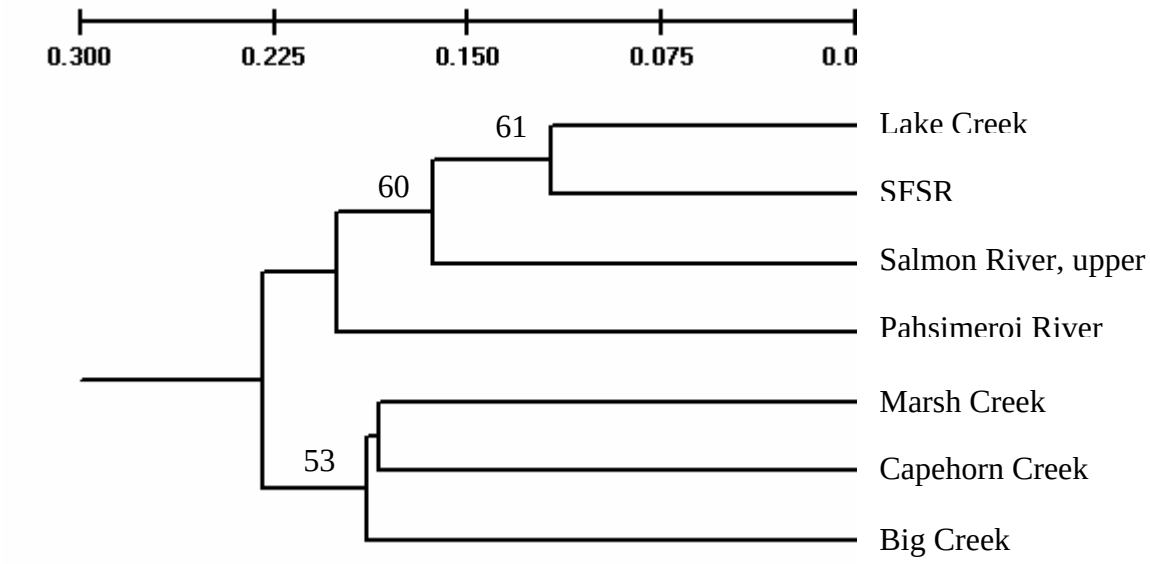

Figure 7. UPGMA cluster using Nei's (1972) original distance on Salmon River Chinook salmon populations where gametes were collected for cryopreservation in 2003. Bootstrap values (shown in %) greater than 50% were shown.

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). Because a significant portion of the genetic diversity that historically existed in the Snake River basin has already been lost, the germplasm repository is an effort to conserve the genetic diversity that remains in extant salmon and steelhead populations. In reality, the genetic diversity preserved by this project may still only represent a small portion of the total genetic diversity in the Snake River basin. Consequently, collections should continue until we can confirm that an adequate representation of the current diversity has been preserved.

Since the program was initiated in 1992, NPT has been very successful cryopreserving Chinook salmon gametes from both hatchery and natural populations. In contrast, few gametes from naturally-spawned steelhead have been collected and cryopreserved. Chinook salmon spawn in late summer during periods of low water flows, making it relatively easy to spot and capture spawning adults from natural spawning grounds. Steelhead spawn in the spring during

periods of high water and inclement weather making them essentially inaccessible to capture with nets or seines. Thus, a majority of the steelhead gametes came from easily accessible hatchery-origin fish. In 2003 and 2004 we successfully collected naturally-spawning adult male steelhead using angling. In 2005 we only collected gamete samples from two SFSR steelhead using this method. Steelhead spawner abundance was significantly lower in 2005 compared to the previous two years. Male steelhead were often observed paired with a female on a redd making it impossible to attempt to capture the male without disturbing the spawning female. In previous years we observed large numbers of males cruising the spawning areas and could effectively target them without disrupting actively spawning females. It appears that male behavior was influenced by spawner abundance, with less aggression and competition for females in 2005.

Fertility trials conducted in 2005 demonstrated that the collection, handling and freezing of gametes was similar to previous years. This year we purposely used straws that contained milt with a wide range prefreeze motilities (20-90%) in order to better evaluate the sample collection. In previous trials nearly all of the straws used for fertility trials contained milt with high prefreeze motility (80-90%). Fertilization using samples with low pre-freeze motility (<60%) revealed a significant decrease in relative fertility, indicating lower expected fertility. The inclusion of these samples likely influenced the regression analysis; analyzing a wide range of prefreeze motilities produced an $R^2 = 0.407$. This suggests that prefreeze motility was a reasonable indicator of relative fertililty. However, when a regression analysis was performed using the fertility trial data from 1999 through 2005 (66 fertilizations) the relationship was not supported ($R^2 = 0.0827$). The data used in the combined year regression analysis had few prefreeze motilities below 70% and a very large range of relative fertilities for a given prefreeze motility (Figure 6). For example, relative fertilities from crosses conducted using frozen milt with a prefreeze motility of 90% (N=32) ranged from 9.5% to 107%. This low precision reduced support for the relationship and resulted in poor predictive ability of prefreeze motility when assessing relative fertility. Results from the 2005 trial did suggest that samples with low prefreeze motility will on average produce lower fertility rates.

Tissue samples were collected from nearly all fish sampled in 2005. The Chinook salmon samples were sent to the Hagerman Aquaculture Research Institution where they were added to the collection from previous year. The samples collected in 2005 will be genotyped and analyzed in 2006.

In 2005 the Hagerman Aquaculture Research Institution began using a region-wide standardized set of 13 microsatellite loci that provide tremendous statistical power of analysis. DNA analysis results for cryopreserved samples collected in 2003 were completed and used to characterize the genetic diversity of the samples from each population preserved in the gene bank. Results revealed a high level of gene and allelic diversity was preserved in the gene bank samples. Although the diversity of the source populations is not know at this time, the diversity levels were of similar magnitude to other Chinook salmon populations in the region (Shawn Narum, Hagerman Aquaculture Research Institution, personal communication). The availability of the standardized microsatellite loci will enable us to use genetic data collected from other projects to directly compare the cryopreserved samples to the source populations and accurately evaluate the level of diversity preserved.

Tests for deviation from H-W equilibrium can detect the presence of non-random mating, selection or migration in a population (Hedrick, 2000; Frankham et al. 2002). Exact tests for deviation from H-W equilibrium revealed that the samples from Big Creek were not in H-W

equilibrium. Demographic analysis of the Big Creek samples from 2003 revealed 52%, 19% and 29% of the samples were from age 3, age 4 and age 5 year old fish, respectively. Normally, a large majority of Columbia River stream-type Chinook salmon spawn at age 4 (Groot and Margolis, 1991). The 2003 Big Creek samples were predominately age 3 and age 5 males, and sampling effects associated with a lack of age 4 fish may have accounted for the deviation from H-W expectations. Interestingly, the other populations sampled in 2003 were disproportionably age 5 fish, with few age 3 or age 4 males. Why these did not show a similar departure from H-W expectations was interesting and may indicate that the Big Creek population was significantly affected by the population bottleneck in the mid 1990's compared to the other Snake River populations. The age 4 fish from Big Creek returning in 2003 were from the Effective Brood Year that was highly underrepresented in all populations in the Snake River basin (see below), and this brood year can be linked back to the year of lowest abundance (1995) in the Snake River basin.

Significant among population diversity was revealed using Fisher's Exact tests for population differentiation and the UPGMA dendogram based on a Nei's genetic distance matrix. These results indicate a high level of among population diversity exists within the Salmon River subbasin and provide support for the collection strategy (Young et al, in prep). Low bootstrap values for the UPGMA tree likely resulted from small sample sizes for many of the populations (<20 samples). In spite of this, the relationships revealed by the tree largely followed expected distributions based on spatial and geographic organization within the Salmon River basin.

Understanding the distribution of the samples obtained from an organism with a non-discrete generation time is critical for preserving the greatest level of diversity. This project set a goal of preserving gametes from at least 100 males per brood year for at least one generation from each spawning aggregation (Young et al, in prep). Equalizing the collection of milt from adults across an entire generation will preserve of the greatest amount of genetic diversity. However, collecting 100 samples/year for an entire generation has not been possible given the low number of returning adults and the difficulty in capturing adult males. Generally, collections ranged from 10 – 40 samples per year per spawning aggregation. Thus, it was inevitable that collections would need to continue for multiple generations in order to reach the sampling goal. For this reason we developed a method that would quantify the distribution of collections that occurred over multiple generations. This method, referred to as the Effective Brood Year (EBY) analysis, could deal with sample collections from multiple age classes over multiple years. An EBY is defined as the theoretical brood year an individual originated from over multiple generations. Analyzing the demographic makeup of the fish that contributed gametes to the collection each year enabled assignment to an actual brood year, and enables an estimation of the brood year that produced these fish. Although this method looses precision more than two generations back, it still accurately estimates the overall distribution of samples in the genebank over short time periods (2 generations).

Generation times were calculated as the average number of years it takes for 95% of the individuals from a brood year to return. Fish were designated to actual brood years based on length/frequency data. The number of effective brood years in a generation is equal to the number of years per generation. The time it takes to collect a specified number of samples per effective brood year will vary depending on the number and age of the fish sampled each year. Fish collected as 3, 4 and 5 year olds in one year originated from 3 different brood years and thus 3 different effective brood years. The first effective brood year was arbitrarily set as the first year of collection and proceeded for the number of years in a generation. For example, let say

we made two collections of 500 gamete samples, collection 1 consisted of 50 samples/year for 10 consecutive years (2 Chinook salmon generations) and collection 2 consisted of 10 yearly collections of 100, 100, 0, 20, 20, 80, 80, 40, 0, 60 (2 Chinook salmon generations). Assuming similar demographic composition among the years (approximately similar number of 3, 4 and 5 year old fish each brood year), the former collection would preserve more diversity compared to the latter. By evenly sampling fish over two generations, collection 1 maximized the potential diversity from the population. In contrast, collection 2 underrepresented the extant diversity of the population because certain brood years were overrepresented and others were underrepresented.

The Little Sheep Creek steelhead gamete collection has reached the goal of collecting at least 100 individuals/year for an entire generation. In addition, a number of Chinook salmon and steelhead collections are represented by large numbers of individuals that may have an adequate number of samples to mitigate genetic diversity problems in the source populations. Young and Kucera (2003) recommended not collecting additional samples from North Fork Clearwater steelhead (Dworshak National Fish Hatchery), Pahsimeroi River steelhead (Pahsimeroi Fish Hatchery) and Snake River steelhead from Oxbow Fish Hatchery and made recommendations for future collections from Imnaha River Chinook salmon, South Fork Salmon River Chinook salmon and Little Sheep Creek steelhead. We will not repeat those analyses in this report, but will update the status of the 2005 collections in relation to the recommendations of Young and Kucera (2003). In addition, we present the EBY analysis from the Johnson Creek population. With the exception of those listed above, all Chinook salmon and steelhead populations listed in Appendix Table A1 and A2 do not have sufficient number of gamete samples and will require additional sample collections in 2006.

Imnaha River Chinook Salmon

Young and Kucera (2003) recommended collecting gametes from natural-origin fish in order to preserve the greatest level of diversity from this population and to concentrate collections on fish from the underrepresented EBY (EBY 1). In 2005 we collected gametes from 12 natural-origin fish, but no fish representing EBY 1 were collected. Fish from this EBY remain underrepresented in the repository (Figure 5) and will not be available again for collection until 2007. In fact, fish from this EBY were relatively rare across the entire Snake River basin (based on our collections). The gene bank contains gametes from 487 Imnaha River male Chinook salmon including 222 marked hatchery-origin fish and 265 wild fish.

RECOMMENDATIONS - Although a large number of samples have been collected from this population, additional collections, focusing on wild-origin fish, are warranted because of the importance of this ESA-listed population and the fact that nearly half of the samples were from hatchery-origin fish. Focusing our collection on natural-origin fish will preserve the greatest level of diversity from this population.

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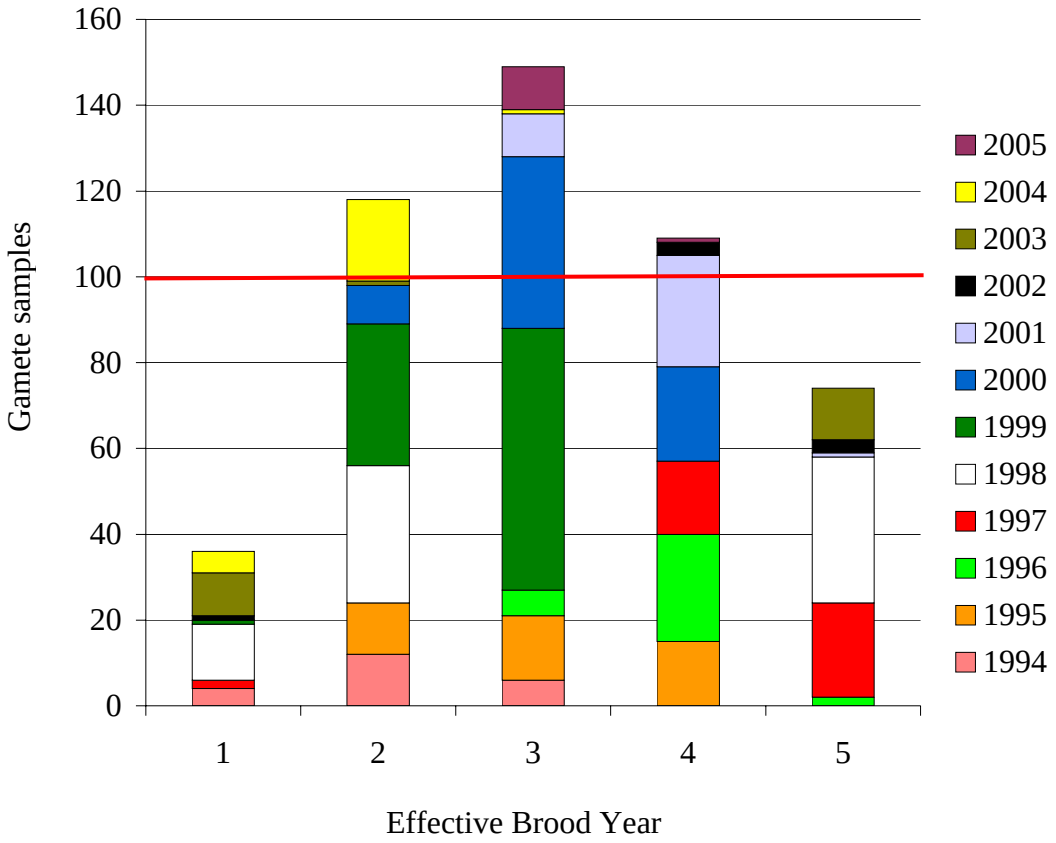

Figure 8. Graph showing the number of gametes collected from Imnaha River Chinook salmon per EBY over a 5-year generation. The red line represents the targeted number of samples for each EBY.

South Fork Salmon River Chinook Salmon

Young and Kucera (2003) recommended collecting gametes from natural-origin fish in order to preserve the greatest level of diversity from this population and to concentrate collections on fish from the underrepresented EBY (EBY 4). In 2005 we collected gametes from 11 natural-origin fish however, no fish representing EBY 4 were obtained. Fish from this EBY remain underrepresented in the repository (Figure 6) and will not be available again for collection until 2007. Similar to the previous two years, we significantly increase the number of gametes from natural-origin fish by collecting milt directly at the trap as IDFG personnel sorted hatchery- and natural-origin fish. The gene bank now contains gametes from 375 South Fork Salmon River male Chinook salmon including 185 marked hatchery-origin fish, 83 supplementation fish (hatchery-origin x natural-origin) and 107 natural-origin fish.

RECOMMENDATIONS – The 185 hatchery-origin fish are adequate as a buffer against potential loss of diversity in the hatchery population. Increasing the collection of wild-origin fish will be a priority as it maximizes the diversity of the collection.

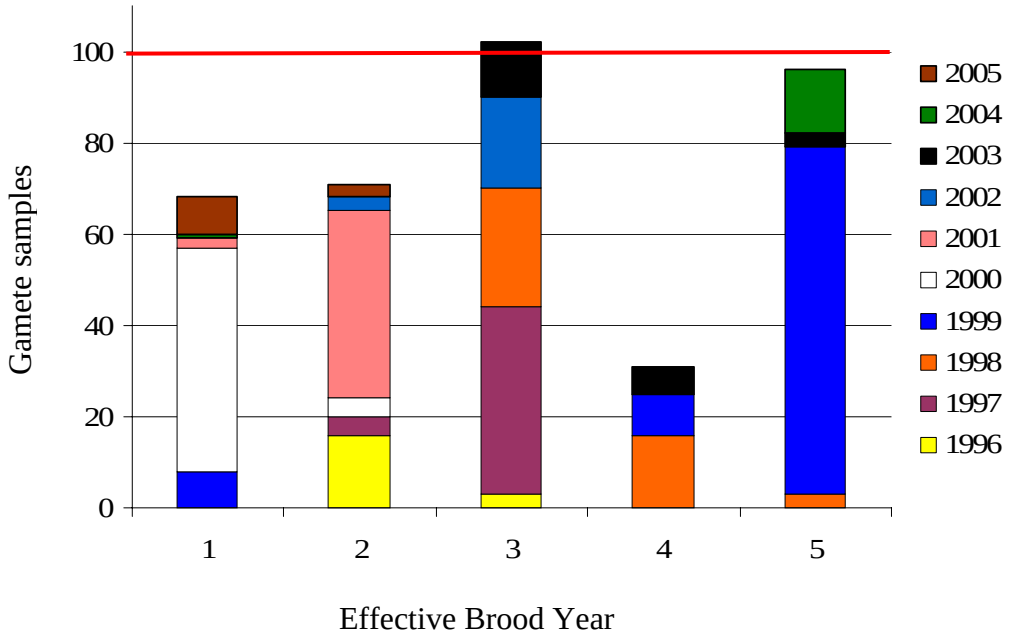

Figure 9. Graph showing the number of gametes collected from South Fork Salmon River Chinook salmon per EBY over a 5-year generation. The red line represents the targeted number of samples for each EBY.

Johnson Creek Chinook Salmon (East Fork South Fork Salmon River Population)

This is the first year that the effective brood year analysis was done for Johnson Creek collection. One EBY (EBY 2) was significantly underrepresented in this population, similar to that observed in the other populations (Figure 10). In 2005 we collected gametes from 48 natural-origin fish however, no fish representing effective brood year 2 were obtained. Fish from effective brood year 4 will not be available again for collection until 2007. The gene bank now contains gametes from 343 Johnson Creek male Chinook salmon including 306 natural-origin fish, 25 supplementation fish (hatchery-origin x natural-origin) and12 of unknown origin.

RECOMMENDATIONS – The Johnson Creek Artificial Propagation and Evaluation Project traps adult Chinook salmon in Johnson Creek and spawns natural-origin fish as part of a conservation hatchery program. This enables the collection of a relative large number of natural-origin males from this population and their cooperation is largely responsible for the size of the collection. Collection should continue until the collection goals are met and JCAPE personnel feel they have enough gametes banked to provide a secure source of germplasm for the continued propagation activities.

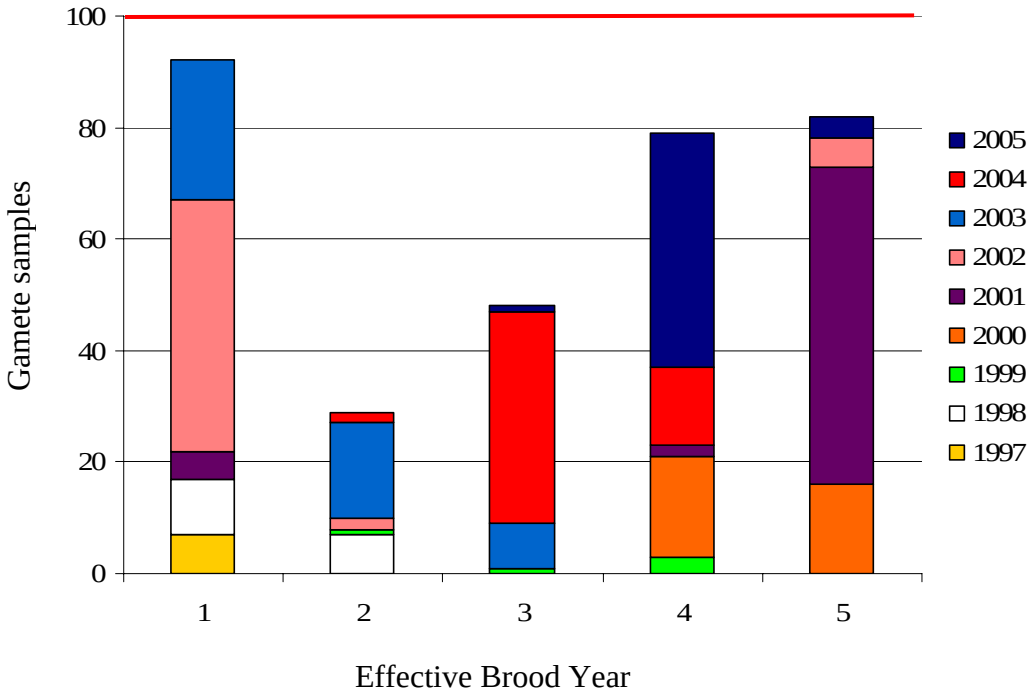

Figure 10. Graph showing the number of gametes collected from Johnson Creek Chinook salmon per EBY over a 5-year generation. The red line represents the targeted number of samples for each EBY.

Little Sheep Creek Steelhead

Young and Kucera (2003) recommended collecting gametes from hatchery- and natural-origin fish in order to preserve the greatest level of diversity from this population. In 2005 we collected gametes from 11 Little Sheep Creek male steelhead including ten marked hatchery-origin fish and one unmarked natural-origin fish. The gene bank contains gametes from 461 Little Sheep Creek male steelhead including 435 marked hatchery-origin fish and 26 natural-origin fish. Oregon Department of Fish and Wildlife (ODFW) hatchery managers designate age groups by the following lengths: <64 cm – age 3 and >64 cm – age 4 and the generation time of the hatchery population is 4 years since nearly all fish return as 3 and 4 year olds (Mike Flesher, ODFW, personal communication). This does not accurately represent the age of wild fish since they often remain in fresh water for more than 2 years and therefore generation time of the natural-origin fish was unknown. Of the 11 fish sampled, three were from EBY 4 and eight were from EBY 1. Using these lengths along with the run composition for each year, the number of fish from each EBY represented in the gene bank was calculated (Figure 7).

RECOMMENDATIONS – The Little Sheep Creek steelhead collection of 461 samples meets the goal of collection 100 fish per brood year for a complete generation. In 2004 we recommended a limited collection targeting natural-origin males and freezing milt in 5.0 ml straws. Unfortunately we were only able to collect milt from one natural-origin male, but did freeze

gametes in 5.0 ml straws. We will continue collecting natural-origin fish in 2006.

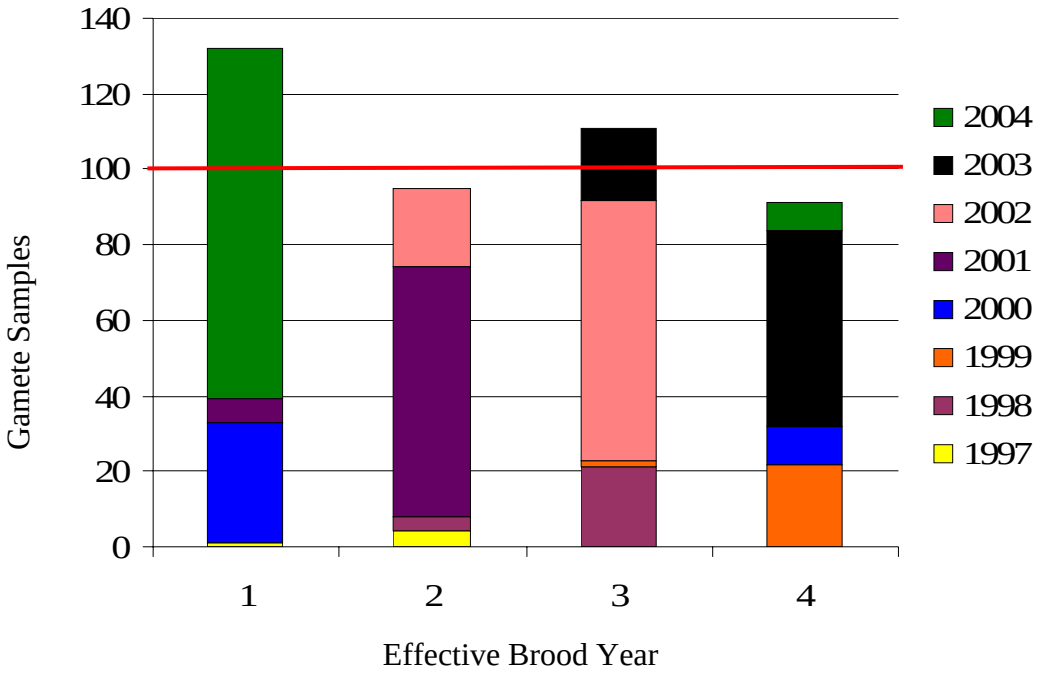

Figure 11. Graph showing the number of gametes collected from the Little Sheep Creek steelhead per Effective Brood Year over a 4-year generation. The red line represents the targeted number of samples for each EBY.

EBY analysis on the Chinook salmon collections revealed an interesting pattern. In all cases one EBY was significantly underrepresented in the each collection. Although this underrepresented EBY was numbered differently, depending on the year the collections began, all could be traced back to the 1995 brood year. Abundance of adults that returned in 1995 was the lowest ever recorded for the Snake River basin populations, resulting in severe demographic bottlenecks. EBY analysis revealed that the number of adults that returned from this brood year was significantly reduced in 1998, 1999 and 2000 as age 3, age 4 and age 5 adults, respectively. Because Snake River spring Chinook predominantly spawn as age 4 adults, the low returns in 1995 had the greatest impact on 1999 adult returns. The combination of low adult abundance and/or environmental conditions in 1999 apparently decreased production and significantly reduced the abundance of returning adults from this brood year. Consequently, returns were low for 2002, 2003 and 2004 as age 3, age 4 and age 5 adults, respectively. Although these affects will decrease over time, the fact that they persisted for multiple generations suggest that recovery could be slow following a severe bottleneck.

Although no requests for cryopreserved gametes were made in 2005, 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 D). The semen request form's main function is for inventory management of the 0.5ml straws and 5.0 ml straws. The Snake River Germplasm Repository Committee, consisting of Tribal and University personnel, meets following a request for germplasm and decides how best to honor the request. The main decision factors are availability, scientific merit and ESA compliance.

RECOMMENDATIONS

1. Continue collecting gametes from Chinook salmon populations throughout the Snake River basin.
2. Utilize angling as a method of collecting gametes from steelhead populations throughout the Snake River basin.
3. Complete a genetic analysis of the Chinook salmon contained in the genebank and compare it to the source populations.
4. Continue tissue sample collections from all of the fish that are sampled in order to perform critical genetic analyses.
5. Research techniques to optimize 5.0 ml straw freezing and thawing protocols that will improve fertilization rates.
6. Continue fertility trials on cryopreserved gametes in order to evaluate the freezing techniques.
7. Work to establish a Regional Germplasm Repository for gene conservation of imperiled fish and wildlife species.
8. Female cryotechnology research
9. Improve steelhead collection techniques/options.

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

APPENDICIES

Appendix A. Gamete samples collected from 1992 through 2005

Table A1. Snake River basin Chinook salmon samples cryopreserved from 1992 through 2005.

Spawning Aggregate	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992	Totals
Lostine River	14	39	16	19	33	18	2	3	2	3	1	4			154
Minam River	4														4
Upper Grande Ronde River	7	8	10	8	9										42
Catherine Creek	10	7	8	5	11										41
Rapid River						51	68	98							217
South Fork Salmon River	11	15	26	23	44	54	93	45	45	19					375
Lake Creek	20	26	32	18	28	15	6	3	4	3					155
Johnson Creek	48	60	51	58	62	35	5	17	7						343
Big Creek	6	22	31	21	50	7	0	1	6	0	0	0	10	7	161
Capehorn Creek	6	0	15	1	2	1	0	6	2						33
Marsh Creek	6	5	16	34	24	7	0	2	4						98
Pahsimeroi River		20	15	39	50	50	31								205
Upper Salmon River	18	25	20	54	48	40	40	41	51						336
Imnaha River	12	25	23	7	37	71	95	79	41	33	42	22			487
Totals	162	252	263	286	398	349	340	295	162	58	43	26	10	7	2,651

Table A2. Snake River basin steelhead samples cryopreserved from 1993 through 2005.

Spawning Aggregate	2005	2004	2003	2002	2001	2000	1999	1998	1997	1994	1993	Totals
Tucannon River	22											22
North Fork Clearwater River				64	81	89	62					296
Selway River										5*		5
Fish Creek				3	1	1					10*	15
Grande Ronde River					1	1						2
South Fork Salmon River	2	24	17									43
Johnson Creek		1			1		2					4
Pahsimeroi River				63	60	40	47					210
Imnaha River						2						2
Little Sheep Creek	11	100	70	95	78	52	25	25	5			461
Cow Creek			2									2
Lightning Creek			1									1
Snake River				58	73	98	76					307
Totals	35	125	90	280	295	281	214	25	5	5	10	1,368

* Samples collected by the USGS/ National Biological Survey.

Appendix B. Data from Chinook salmon collected in 2005.

Table A3. Collection date, fork lengths, percent motilities and number of straws from Chinook salmon collected in 2005.

Location	Date	Fork length (cm)	Genebank #	WSU motility (%)	WSU # of 0.5 ml straws	UI motility (%)	UI # of 0.5 ml straws
Big Creek	8/2/2005	74	NPT-003-2005			70	20
Big Creek	8/2/2005	102	NPT-004-2005			90	20
Big Creek	8/11/2005	73	NPT-015-2005			60	20
Big Creek	8/11/2005	72	NPT-067-2005	10	20	60	15
Big Creek	8/16/2005	77	NPT-068-2005	50	20	60	20
Big Creek	8/16/2005	71	NPT-069-2005	90	20	80	20
Lake Creek	8/3/2005	74	NPT-005-2005			90	20
Lake Creek	8/3/2005	77	NPT-006-2005			0	10
Lake Creek	8/3/2005	83	NPT-007-2005			80	20
Lake Creek	8/3/2005	73	NPT-008-2005			0	20
Lake Creek	8/10/2005	79	NPT-009-2005			70	20
Lake Creek	8/10/2005	76	NPT-010-2005			70	20
Lake Creek	8/10/2005	79	NPT-011-2005			80	20
Lake Creek	8/10/2005	71	NPT-012-2005			70	10
Lake Creek	8/10/2005	83	NPT-013-2005			10	20
Lake Creek	8/10/2005	103	NPT-014-2005			90	20
Lake Creek	8/15/2005	72	NPT-061-2005	90	20	80	20
Lake Creek	8/15/2005	66	NPT-062-2005	50	20		
Lake Creek	8/15/2005	101	NPT-063-2005	70	20	70	20
Lake Creek	8/15/2005	93	NPT-064-2005	0	0	60	20
Lake Creek	8/15/2005	79	NPT-065-2005			90	20
Lake Creek	8/15/2005	77	NPT-066-2005	30	20		
Lake Creek	8/22/2005	76	NPT-089-2005			70	20
Lake Creek	8/22/2005	83	NPT-090-2005			80	15
Lake Creek	8/22/2005	81	NPT-091-2005			20	20
Lake Creek	8/22/2005	72	NPT-092-2005	80	20		
Marsh Creek	8/12/2005	85	NPT-016-2005			80	20
Marsh Creek	8/12/2005	76	NPT-017-2005			80	20
Marsh Creek	8/12/2005	82	NPT-018-2005			20	20
Marsh Creek	8/18/2005	77	NPT-073-2005	90	20	90	15
Marsh Creek	8/18/2005	79	NPT-074-2005	60	20	80	20
Marsh Creek	8/18/2005	65	NPT-075-2005	50	20	90	15
Capehorn Creek	8/12/2005	79	NPT-019-2005			90	20
Capehorn Creek	8/12/2005	71	NPT-020-2005			70	20
Capehorn Creek	8/12/2005	69	NPT-021-2005			50	20
Capehorn Creek	8/18/2005	70	NPT-076-2005	70	20	90	20
Capehorn Creek	8/18/2005	86.5	NPT-077-2005	60	20	60	20
Capehorn Creek	8/18/2005	96	NPT-078-2005	-	20	90	20
Johnson Creek	8/17/2005	72	NPT-070-2005	70	20	50	20
Johnson Creek	8/17/2005	77	NPT-071-2005	70	20	90	15
Johnson Creek	8/17/2005	81	NPT-072-2005	50	20	90	20
Johnson Creek	8/19/2005	76	NPT-079-2005	80	20	70	20
Johnson Creek	8/19/2005	78	NPT-080-2005	90	20	80	20
Johnson Creek	8/19/2005	77	NPT-081-2005	90	20	80	20
Johnson Creek	8/19/2005	81	NPT-082-2005	60	20	90	20
Johnson Creek	8/19/2005	80	NPT-083-2005	40	20	90	20
Johnson Creek	8/19/2005	82	NPT-084-2005	90	20	80	20
Johnson Creek	8/19/2005	78	NPT-085-2005	90	20		
Johnson Creek	8/19/2005	76	NPT-086-2005			80	15
Johnson Creek	8/19/2005	77	NPT-087-2005	90	10		
Johnson Creek	8/19/2005	70	NPT-088-2005			90	20
Johnson Creek	8/23/2005	81	NPT-093-2005			70	15
Johnson Creek	8/23/2005	78	NPT-094-2005	90	20	90	15
Johnson Creek	8/23/2005	86	NPT-095-2005	90	20		
Johnson Creek	8/23/2005	75	NPT-096-2005	70	20	90	15
Johnson Creek	8/23/2005	76	NPT-097-2005			90	10
Johnson Creek	8/23/2005	77	NPT-098-2005	90	20	90	15
Johnson Creek	8/23/2005	71	NPT-099-2005	90	20	90	15

Location	Date	Fork length	Genebank #	WSU motility	WSU # of 0.5 ml straws	UI motility	UI # of 0.5 ml straws
Johnson Creek	8/23/2005	78	NPT-100-2005	80	20	80	15
Johnson Creek	8/24/2005	90	NPT-105-2005	50	20	50	15
Johnson Creek	8/24/2005	83	NPT-106-2005	90	20	90	10
Johnson Creek	8/24/2005	53	NPT-107-2005	90	10	90	10
Johnson Creek	8/24/2005	78	NPT-108-2005	90	20	90	10
Johnson Creek	8/24/2005	80	NPT-109-2005	20	10	80	20
Johnson Creek	8/24/2005	74	NPT-110-2005	90	10	70	10
Johnson Creek	8/24/2005	76	NPT-111-2005	50	20		
Johnson Creek	8/24/2005	77	NPT-112-2005	90	20	90	20
Johnson Creek	8/24/2005	-	NPT-113-2005			80	20
Johnson Creek	8/26/2005	76	NPT-121-2005			90	20
Johnson Creek	8/26/2005	83	NPT-122-2005	90	20		
Johnson Creek	8/26/2005	73	NPT-123-2005			90	20
Johnson Creek	8/26/2005	80	NPT-124-2005	90	20		
Johnson Creek	8/26/2005	81	NPT-125-2005			90	20
Johnson Creek	8/26/2005	75	NPT-126-2005	90	20		
Johnson Creek	8/26/2005	72	NPT-127-2005	90	20	90	20
Johnson Creek	8/26/2005	79	NPT-128-2005	90	20	80	10
Johnson Creek	8/27/2005	85	NPT-134-2005	90	20	80	20
Johnson Creek	8/27/2005	71	NPT-135-2005	70	20	80	20
Johnson Creek	8/27/2005	74	NPT-136-2005			90	20
Johnson Creek	8/27/2005	50	NPT-137-2005	80	20		
Johnson Creek	8/27/2005	71	NPT-138-2005			80	10
Johnson Creek	8/31/2005	50	NPT-139-2005	10	20		
Johnson Creek	8/31/2005	79	NPT-147-2005	90	20	80	20
Johnson Creek	8/31/2005	76	NPT-148-2005	-	20	90	20
Johnson Creek	8/31/2005	75	NPT-149-2005	90	20	90	20
Johnson Creek	8/31/2005	51	NPT-150-2005	90	20	90	20
Johnson Creek	8/31/2005	73	NPT-151-2005	70	20	90	15
Johnson Creek	8/31/2005	71	NPT-152-2005	90	20	90	10
Johnson Creek	8/31/2005	71	NPT-153-2005	50	20	80	20
SFSR trap	8/25/2005	70	NPT-114-2005	90	20	80	20
SFSR trap	8/25/2005	76	NPT-115-2005	70	10	80	10
SFSR trap	8/25/2005	85	NPT-116-2005	90	20	90	20
SFSR trap	8/25/2005	54	NPT-117-2005	90	20	80	15
SFSR trap	8/25/2005	84	NPT-118-2005	70	20	90	20
SFSR trap	8/25/2005	74	NPT-119-2005	90	20		
SFSR trap	8/25/2005	70	NPT-120-2005	90	20	80	10
SFSR trap	8/27/2005	63	NPT-129-2005	90	20		
SFSR trap	8/27/2005	71	NPT-130-2005			0	20
SFSR trap	8/27/2005	67	NPT-131-2005	90	20		
SFSR trap	8/27/2005	66	NPT-132-2005			80	20
Sawtooth FH	9/1/2005	≈70	NPT-159-2005			90	20
Sawtooth FH	9/1/2005	>100	NPT-160-2005	50	20		
Sawtooth FH	9/1/2005	≈80	NPT-161-2005			90	20
Sawtooth FH	9/1/2005	≈70	NPT-163-2005			50	20
Sawtooth FH	9/1/2005	80	NPT-164-2005	90	20		
Sawtooth FH	9/1/2005	68	NPT-165-2005			80	20
Sawtooth FH	9/1/2005	76	NPT-166-2005	90	20		
Sawtooth FH	9/1/2005	67	NPT-167-2005			80	20
Sawtooth FH	9/1/2005	68	NPT-168-2005	60	20	80	10
Sawtooth FH	9/9/2005	62	NPT-200-2005	70	20		
Sawtooth FH	9/9/2005	>95	NPT-201-2005	90	20		
Sawtooth FH	9/9/2005	≈70	NPT-202-2005	90	20	90	20
Sawtooth FH	9/9/2005	73	NPT-203-2005	90	20	90	20
Sawtooth FH	9/9/2005	72	NPT-204-2005	90	20	90	20
Sawtooth FH	9/9/2005	72	NPT-205-2005	90	20		
Sawtooth FH	9/9/2005	66	NPT-206-2005	90	20	90	20
Sawtooth FH	9/9/2005	79	NPT-207-2005	90	20		20
Sawtooth FH	9/9/2005	70	NPT-208-2005	90	40	90	
Lostine River	8/23/2005	76.5	NPT-101-2005			80	20
Lostine River	8/23/2005	77	NPT-102-2005	90	20		
Lostine River	8/23/2005	91	NPT-103-2005	60	20	90	10

Location	Date	Fork length	Genebank #	WSU motility	WSU # of 0.5 ml straws	UI motility	UI # of 0.5 ml straws
Lostine River	8/23/2005	75.5	NPT-104-2005	70	20		
Lostine River	8/31/2005	93.5	NPT-154-2005			90	20
Lostine River	8/31/2005	73	NPT-155-2005	5	20		
Lostine River	8/31/2005	91	NPT-156-2005			80	20
Lostine River	8/31/2005	74	NPT-157-2005	5	20		
Lostine River	8/31/2005	85	NPT-158-2005			90	20
Lostine River	8/31/2005	82	NPT-159-2005	90	20		
Lostine River	9/7/2005	79.5	NPT-188-2005			80	20
Lostine River	9/7/2005	77	NPT-189-2005			90	20
Lostine River	9/7/2005	72	NPT-190-2005			80	20
Lostine River	9/7/2005	76	NPT-191-2005			90	20
Grande Ronde River	9/1/2005	86.5	NPT-169-2005	90	20	70	20
Grande Ronde River	9/1/2005	82.5	NPT-170-2005	60	20		
Grande Ronde River	9/1/2005	72	NPT-171-2005	70	20	90	20
Grande Ronde River	9/1/2005	81.5	NPT-172-2005	50	20	80	20
Grande Ronde River	9/1/2005	68.5	NPT-173-2005	90	20	80	20
Grande Ronde River	9/8/2005	54	NPT-192-2005	90	20	80	20
Grande Ronde River	9/8/2005	67.5	NPT-193-2005			90	20
Catherine Creek	9/1/2005	75.5	NPT-174-2005			80	10
Catherine Creek	9/1/2005	70	NPT-175-2005	90	20		
Catherine Creek	9/1/2005	72	NPT-176-2005			80	10
Catherine Creek	9/1/2005	74	NPT-177-2005	90	20		
Catherine Creek	9/8/2005	71	NPT-194-2005	70	20	80	20
Catherine Creek	9/8/2005	70	NPT-195-2005	90	20		
Catherine Creek	9/8/2005	75	NPT-196-2005			70	10
Catherine Creek	9/8/2005	75	NPT-197-2005	70	20	90	20
Catherine Creek	9/8/2005	75	NPT-198-2005	60	20	80	20
Catherine Creek	9/8/2005	Unk	NPT-199-2005	60	20	80	10
Minam River	9/7/2005	81	NPT-184-2005	10	20	80	20
Minam River	9/7/2005	101	NPT-185-2005	5	20	60	20
Minam River	9/7/2005	80	NPT-186-2005	20	20	50	10
Minam River	9/7/2005	101	NPT-187-2005	20	20		
Imnaha River	8/30/2005	70	NPT-139-2005	90	20		
Imnaha River	8/30/2005	80	NPT-140-2005	70	20	90	20
Imnaha River	8/30/2005	71	NPT-141-2005	90	20	80	20
Imnaha River	8/30/2005	82.5	NPT-142-2005	90	20	80	20
Imnaha River	8/30/2005	71	NPT-143-2005			50	15
Imnaha River	8/30/2005	73	NPT-145-2005			70	10
Imnaha River	9/5/2005	73	NPT-178-2005	90	20	80	20
Imnaha River	9/5/2005	82	NPT-179-2005	10	20	60	10
Imnaha River	9/5/2005	Unk	NPT-180-2005	50	20	50	10
Imnaha River	9/5/2005	79	NPT-181-2005	5	20	60	20
Imnaha River	9/5/2005	78	NPT-182-2005			90	10
Imnaha River	9/5/2005	116	NPT-183-2005	50	20	80	20

Appendix C. Data from steelhead collected in 2005.

Table A4. Collection date, fork lengths, percent motilities and number of straws from steelhead collected in 2005.

Location	Date	Fork Length	Fin Clip	Gene Bank #	Motility	# 0.5 ml straws
Little Sheep Creek	4/19/2005	72	n	NPT-055-05	70	20
Little Sheep Creek	4/19/2005	59	y	NPT-056-05	70	20
Little Sheep Creek	4/19/2005	59	y	NPT-057-05	80	20
Little Sheep Creek	4/19/2005	61	y	NPT-058-05	80	20
Little Sheep Creek	4/19/2005	59	y	NPT-059-05	90	20
Little Sheep Creek	4/19/2005	57.5	y	NPT-060-05	80	20
Little Sheep Creek	4/19/2005	58	y	NPT-061-05	70	2 (5.0 ml)
Little Sheep Creek	4/19/2005	60	y	NPT-062-05	90	2 (5.0 ml)
Little Sheep Creek	4/19/2005	60	y	NPT-063-05	80	2 (5.0 ml)
Little Sheep Creek	4/19/2005	58	y	NPT-064-05	90	2 (5.0 ml)
Little Sheep Creek	4/19/2005	58	y	NPT-065-05	90	2 (5.0 ml)
Tucannon River	3/8/2005	55	n	NPT-031-05	60	19
Tucannon River	3/9/2005	55	n	NPT-032-05	70	10
Tucannon River	3/10/2005	55	n	NPT-033-05	80	20
Tucannon River	3/11/2005	78.5	n	NPT-034-05	80	20
Tucannon River	3/12/2005	59.5	n	NPT-035-05	70	20
Tucannon River	3/13/2005	59.5	n	NPT-036-05	70	20
Tucannon River	3/14/2005	59	n	NPT-037-05	80	20
Tucannon River	3/15/2005	74.5	n	NPT-038-05	70	20
Tucannon River	3/16/2005	66	n	NPT-039-05	90	20
Tucannon River	3/17/2005	71	n	NPT-041-05	90	10
Tucannon River	3/18/2005	57.5	n	NPT-042-05	80	20
Tucannon River	3/19/2005	73.5	n	NPT-043-05	80	20
Tucannon River	3/20/2005	58.5	n	NPT-044-05	80	20
Tucannon River	3/21/2005	56	n	NPT-045-05	70	20
Tucannon River	3/22/2005	73	n	NPT-046-05	90	20
Tucannon River	3/23/2005	64.5	n	NPT-048-05	80	10
Tucannon River	3/24/2005	65.5	n	NPT-049-05	80	20
Tucannon River	3/25/2005	61.5	n	NPT-050-05	90	20
Tucannon River	3/26/2005	73	n	NPT-051-05	0	20
Tucannon River	3/27/2005	83.5	n	NPT-052-05	90	20
Tucannon River	3/28/2005	75	n	NPT-053-05	70	20
Tucannon River	3/29/2005	64.5	n	NPT-054-05	80	20
SFSR	4/20/2005	82	n	NPT-17-05	90	20
SFSR	5/3/2005	85	n	NPT-18-05	50	20

Appendix D. Snake River Germplasm Repository Cryopreserved Semen Request Form

NEZ PERCE TRIBE
Department of Fisheries Resources Management
Administration • Enforcement • Harvest • Production • Research • Resident Fish • Watershed

MCCALL FIELD OFFICE
125 S. Mission St. • McCall, ID 83638
Phone: (208) 634-5290 • Fax: (208) 634-4097

Cryopreserved Semen Request Form

Name:

Affiliation:

Phone number:

Email address:

Date needed by:

Species/stock requested: _____Hatchery or wild/natural: _____

Number of straws needed: _____0.5ml, _____5.0ml

Reason for request (clearly demonstrate need):

_____
_____
_____
_____
_____
_____

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

_____
_____

Please provide additional information as necessary (Annual Operating Plan, Management Plan, etc.). You will be contacted by phone or email to discuss the request and coordinate the transfer. The Nez Perce Tribe will assist in the fertilization of eggs and expects adequate monitoring of the results (percent of eggs fertilized, post-thaw sperm motility, etc.).

Signature: _____Date: _____

Contact William Young at the above address (or by email: billy@nezperce.org) if you would like additional information about the gene bank or the request process. Management agencies in the Columbia River Basin are concerned with the inappropriate use of cryopreserved gametes and retain the right to refuse unjustifiable requests. See the Listed Stock Gamete Preservation Annual Reports or the management plan for additional information (www.nezperce.org/%7Edfrm/research/gametes.html).