

SALMONID GAMETE PRESERVATION IN THE SNAKE RIVER BASIN

2006 Annual Report



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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. The 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 2006 a total of 163 viable semen samples were collected and added to the germplasm repository. This included the gametes from 141 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 22 male steelhead from the Tucannon River, Little Sheep Creek and South Fork Salmon River. To date, a total of 2,792 Columbia River male Chinook salmon, 1,390 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. Genetic analysis of a subset of Chinook salmon samples preserved in the gene bank revealed high levels of within and among population genetic diversity, validating the collection strategy. Demographic analysis of three wild populations of Chinook salmon that contributed gametes to the repository indicated that the samples were not evenly distributed, potentially decreasing the level of genetic diversity preserved in the gene bank from these populations. The significantly underrepresented Dominant Brood Year (DBY; returns from the imperiled 1995 return year) will be represented by 4 year old fish that return in 2007 will be targeted for semen samples. Gamete collection will continue in 2007 from imperiled Chinook salmon and steelhead populations of the Snake River basin, focusing on Chinook salmon from DBY 3.

This report and annual reports from 1997-2003 are available on the Internet through BPA Fish and Wildlife Publications at: <http://www.efw.bpa.gov/cgi-bin/efw/FW/publications.cgi>

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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 species abundance has declined to critically low levels or become highly fragmented. 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 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 or before they reach a level that will decrease species viability. Once genetic viability is affected, diversity has been lost and there is no way of restoring it. Thus, identifying species threatened by a loss of diversity should immediately trigger a combination of preventative and intensive measures that will 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.

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 operations. 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 been implemented. 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.

Nez Perce Tribe (NPT) initiated Chinook salmon *Ocorhynchus. 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 salmon 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 2006 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 2006.

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

5. Lake Creek
6. Johnson Creek
7. Marsh Creek
8. Capehorn Creek
9. Big Creek
10. South Fork Salmon River (SFSR - collected at the SFSR weir, McCall Fish Hatchery)
11. Upper Salmon River (collected at Sawtooth Fish Hatchery)

Imnaha River Subbasin

12. Imnaha River (collected at Lookingglass Hatchery)

STEELHEAD

Tucannon River Subbasin

13. Tucannon River (collected at Lyons Ferry Hatchery)

Salmon River Subbasin

14. South Fork Salmon River

Imnaha River Subbasin

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

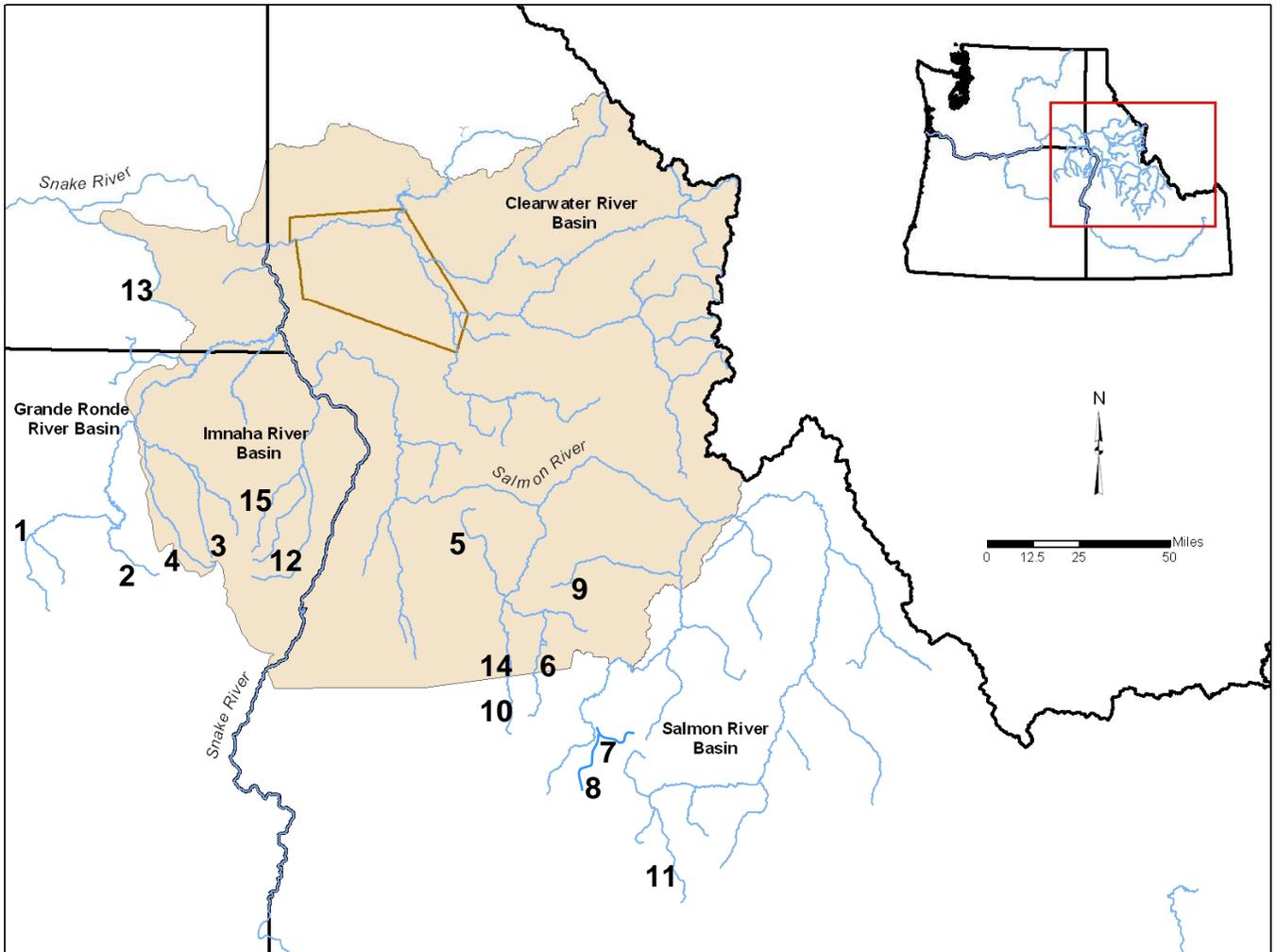


Figure 1. Map showing the Snake River basin Chinook salmon and steelhead sampling locations for 2006. Numbers correspond to the sampling locations above. The shaded area represents the territory ceded to the Nez Perce Tribe in the treaty of 1855 and the gold outline represents the present day reservation. Sampling locations included; 1) Catherine Creek, 2) upper Grande Ronde River, 3) Lostine River, 4) Minam River, 5) Lake Creek, 6) Johnson Creek, 7) Marsh Creek, 8) Capehorn Creek, 9) Big Creek, 10) South Fork Salmon River, 11) upper Salmon River, 12) Imnaha River, 13) Tucannon River, 14) South Fork Salmon River, 15) Little Sheep Creek.

Fish Collection and Handling

Spawning ground surveys for Chinook salmon 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 on 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 and filled with 135 liters of water. Fish were immobilized using anesthetic so they could be handled faster and less stressfully. The anesthesia was delivered by placing the fish in the water filled portable tank containing 90 mg/l of tricane methanesulfonate (MS-222, Finquel™) 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).

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 euthanized following production spawning. Milt was then collected using the standard protocol (see above).



Figure 2. Collecting Chinook salmon milt from anaesthetized fish.



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 (Figure 4; IMV International, Minneapolis, Minnesota). Samples were stored in large cryopreservation tanks under liquid nitrogen.

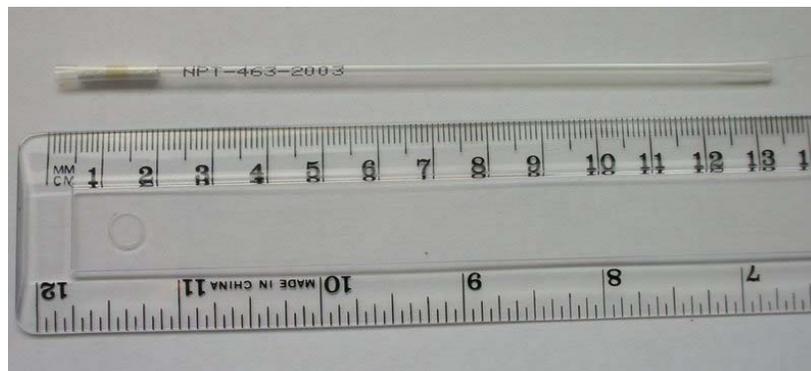


Figure 4. French Straw used for long-term storage of frozen gametes in liquid Nitrogen.

RESULTS

The Chinook salmon and steelhead spawning aggregates and hatcheries in the Snake River basin where gametes were collected in 2006 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 141 male Chinook salmon (Table 1) were collected and cryopreserved from twelve populations in 2006. Collections occurred from August 1 to September 7, 2006. Gametes were collected from 108 unmarked, natural-origin fish and 33 marked, hatchery-origin fish. Two males were recaptured from Marsh Creek and one male was captured but not sampled from Lake Creek. Five females were accidentally captured and immediately released. Motility of the sperm ranged from 0 – 90%.

Gametes from 22 male steelhead (Table 2) were collected and cryopreserved from 3 populations in 2006. Collections occurred from March 14 to May 11, 2006. 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%.

2006 Chinook Salmon Gamete Collections

Lostine River

In 2006 the gametes from 16 male Chinook salmon were cryopreserved from fish trapped at the adult weir on the Lostine River and spawned at Lookingglass Hatchery. The collection included gametes from six adipose fin clipped, hatchery-origin male and 10 unmarked, natural-origin males. Based on the length data (Appendix B), one age 3, thirteen age 4 and two age 5 fish were sampled from brood years 2003, 2002 and 2001 respectively. Collections from 1994 to 2006 have preserved a total of 170 Lostine River male gamete samples in the gene bank (Appendix A).

Minam River

In 2006 the gametes from two unmarked, natural-origin male Chinook Salmon were cryopreserved from fish captured in the Minam River. Based on length data, both were age 4, originating from brood years 2002. Collections from 2005 and 2006 have preserved a total of 6 Minam River male gamete samples in the gene bank (Appendix A).

Upper Grande Ronde

In 2006 the gametes from 13 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 10 adipose fin clipped, hatchery-origin males and 3 unmarked, natural-origin male. Based on the length data (Appendix B), all thirteen age 4 fish originated from brood year 2002. Collections from 2001 to 2006 have preserved a total of 55 Grand Ronde River male gamete samples in the gene bank (Appendix A).

Table 1. Locations and numbers of Snake River basin spring and summer Chinook salmon milt samples collected and cryopreserved in the in 2006.

Spawning Aggregate	Total Samples	Unmarked Fish ^a	Marked Fish ^b	Females Captured	Collection Dates	Sperm Motility (%)
Lostine River	16	10	6	0	8/23, 31, 9/7	10-90
Minam River	2	2	0	0	9/7	80-90
Catherine Creek	12	8	4	0	9/1, 8	20-90
Grande Ronde River	13	3	10	0	9/1, 8	50-90
Imnaha River	20	15	5	0	8/30, 9/5	10-90
South Fork Salmon River	1	0	1 ^c	0	8/25 & 27	90
Lake Creek	15	15	0	0	8/2, 7, 14	0-90
Johnson Creek	31	26	5	1	8/25, 29, 30, 9/1	50-90
Big Creek	9	9	0	1	8/1, 8 & 15	10-90
Capehorn Creek	1	1	0	0	8/16	90
Marsh Creek	15	15	0	3	8/16, 17 & 23	10-90
Upper Salmon River	13	11	2 ^c	0	8/31	0-90
Totals	141	108	33	5	8/1 – 9/7	0-90

^aNon fin-clipped fish, natural-origin

^bFin-clipped or tagged fish, hatchery-origin

^cNatural by Hatchery-origin cross supplementation fish, marked with a coded wire tag (CWT)

Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2006.

Spawning Aggregate	Total Samples	Un-marked Fish ^a	Marked Fish ^b	Females Captured	Collection Dates	Sperm Motility (%)
Tucannon River	16	16	0	0	3/14, 27	60-90
South Fork Salmon River	3	3	0	3	4/19, 20, 27, 28, 5/11	50-90
Little Sheep Creek	3	1	2	0	4/19	70-80
Totals	22	20	2	2	3/14 – 5/11	0-90

^aNon fin-clipped fish, natural origin

^bFin-clipped or tagged fish, hatchery origin

Catherine Creek

In 2006 the gametes from 12 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 four adipose fin clipped, hatchery-origin males and eight unmarked, natural-origin male. Based on the length data (Appendix B), one age 3 and eleven age 4 fish were sampled from brood years 2003 and 2002, respectively. Collections from 2001 to 2006 have preserved a total of 53 Catherine Creek male gamete samples in the gene bank (Appendix A).

South Fork Salmon River

In 2006 the gametes from one 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 male was marked (CWT), supplementation (natural by hatchery-origin cross). Based on the length data (Appendix B), the fish was age 3 originating from brood year 2003. Collections from 1996 to 2006 have preserved a total of 376 South Fork Salmon River male gamete samples in the gene bank (Appendix A). Of these, 183 were from hatchery-origin males, 85 were from supplementation males, and 106 were from unmarked, natural-origin males.

Lake Creek

In 2006 the gametes from eight unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Lake Creek. One male was recaptured and released without taking a sample. Based on the length data (Appendix B), one age 3 and seven age 4 were sampled, originating from brood years 2003 and 2002, respectively. Collections from 1996 to 2006 have preserved a total of 163 Lake Creek gamete samples in the gene bank (Appendix A).

Johnson Creek

In 2006 the gametes from 31 male Chinook salmon were cryopreserved from fish captured in Johnson Creek. The collection included gametes from 25 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, 1 male captured at the NPT Johnson Creek adult weir and immediately released upstream and 6 males netted in Johnson Creek. Duplicate gametes samples were collected from three males; all were sampled twice at the SFSR trap. Based on the length data (Appendix B), one age 3 and thirty age 4 fish were sampled, originating from brood years 2003 and 2002, respectively. Length was not determined for one fish. Collections from 1997 to 2006 have preserved a total of 374 Johnson Creek male gamete samples (Appendix A).

Big Creek

In 2006 the gametes from nine unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Big Creek. One female Chinook salmon was incidentally netted and immediately released. Based on the length data (Appendix B), all were age 4, originating from brood years 2002. Collections from 1992 to 2006 have preserved a total of 170 Big Creek male gamete samples in the gene bank (Appendix A).

Capehorn Creek

In 2006 the gametes from one unmarked, natural-origin male Chinook salmon was cryopreserved from fish captured in Capehorn Creek. Based on the length data (Appendix B), the male was age 4, originating from brood year 2002. Collections from 1997 to 2006 have preserved a total of 34 Capehorn Creek male gamete samples in the gene bank (Appendix A).

Marsh Creek

In 2006 the gametes from 15 unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Marsh Creek. Two males were recaptured and immediately released without taking an additional sample. Three females were captured and immediately released. Based on the length data (Appendix B), two age 3, eleven age 4 and two age 5 fish were sampled, originating from brood year 2003, 2002 and 2001, respectively. Collections from 1997 to 2006 have preserved a total of 113 Marsh Creek male gamete samples in the gene bank (Appendix A).

Upper Salmon River

In 2006 the gametes from 13 upper Salmon River male Chinook salmon were cryopreserved from fish spawned at Sawtooth Fish Hatchery. The collection included gametes from 11 unmarked, natural-origin males and 2 CWT, supplementation hatchery-origin males. Based on the length data (Appendix B), one age 3 and twelve age 4 fish were sampled, originating from brood year 2003, and 2002 respectively. Length was not determined for one fish. Collections from 1997 to 2000 have preserved a total of 349 upper Salmon River male

gamete samples in the gene bank (Appendix A). Of these, 78 were from marked hatchery fish, 28 were from marked supplementation fish and 244 were from unmarked natural fish.

Imnaha River

In 2006 the gametes from 20 Chinook salmon were cryopreserved from fish trapped in the Imnaha River and spawned at Lookingglass Fish Hatchery. The collection included gametes from 5 adipose fin clipped, hatchery-origin male and 15 unmarked, natural-origin males. Based on the length data (Appendix B), two age 3, sixteen age 4 and two age 5 fish were sampled from brood years 2003, 2002 and 2001, respectively. Collections from 1994 to 2006 have preserved a total of 507 Imnaha River male gamete samples in the gene bank (Appendix A). Of these, 215 were from marked hatchery-origin males and 268 were from unmarked natural-origin males.

2006 Steelhead Gamete Collections

Tucannon River

In 2006 the gametes from 16 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), 7 one-ocean and 9 two-ocean fish were sampled. Collections from 2005 to 2006 have preserved a total of 38 Tucannon River male gamete samples in the gene bank (Appendix A).

South Fork Salmon River

In 2006 the gametes from 3 unmarked, natural-origin male steelhead were cryopreserved from fish captured by angling in the South Fork Salmon River. One adipose fin-clipped, hatchery-origin male was captured and released and one unmarked wild-origin male was captured but not sampled (did not produce milt). Two females were inadvertently captured and immediately released. Based on the length data (Appendix C), one fish was two ocean and two were one ocean fish. Collections from 2003 to 2006 have preserved a total of 49 natural-origin SFSR male gamete samples in the gene bank (Appendix A).

Johnson Creek

In 2006 no Johnson Creek steelhead were captured by angling in Johnson Creek. Collections from 1999 to 2006 have preserved a total of four Johnson Creek male gamete samples in the gene bank (Appendix A).

Little Sheep Creek

In 2006 the gametes from 3 male steelhead were cryopreserved from fish spawned at the Little Sheep Creek adult weir. The collection included gametes from two adipose fin clipped hatchery-origin males and one unmarked, natural-origin male. Based on the length data (Appendix C), two age 3 and one age 4 fish were sampled, originating from brood years 2003 and 2002, respectively. Collections from 1999 to 2006 have preserved a total of 464 Little Sheep

Creek male gamete samples in the gene bank (Appendix A). Of these, 437 were from marked hatchery-origin fish and 27 were from unmarked natural-origin fish (Appendix A).

Cow and Lightning Creeks

In 2006 we attempted to collect gametes from male steelhead captured in the abundance monitoring weirs in Cow and Lightning creeks in the Imnaha River subbasin. Personnel were available if any ripe males were captured, but we did not collect any gametes. We will attempt to collect from these creeks again in 2007. Collections from 1999 to 2006 have preserved a total of 2 Cow Creek and 1 Lightning 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 Yakima River spring Chinook salmon (WDFW), Grande Ronde River subbasin Chinook salmon captive broodstock programs (NPT, ODFW, Confederated Tribes of the Umatilla Indian Reservation - CTUIR), Clearwater River coho salmon (Columbia River Intertribal Fish Commission - CRITFC), Kootenai River white sturgeon (Kootenai Tribe) and Kootenai River Burbot (Kootenai Tribe).

Grande Ronde River Chinook Salmon Captive Broodstock Project

A Grande Ronde River subbasin spring Chinook salmon captive broodstock program, co-managed by ODFW, CTUIR 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 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 semen 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 2006. 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 in 2006 consisted of a large experiment conducted at Bonneville Fish Hatchery that investigated the relationship between cryopreserved sperm volume, egg number,

and fertility. Previous recommendations suggest a single 0.5 ml straw was adequate to fertilize up to 500 eggs (Joe Cloud, personnel information) however, the adequacy of this method is untested. We examined fertility of fall Chinook salmon using 1, 2, 4 and 8; 0.5 ml straws used to fertilize batches of 250, 500 and 1000 eggs.

Methods

We collected sperm from eight fall Chinook salmon males and pooled eggs from 14 females. Pre-freeze motility estimates for all males used in the experiments were established by placing a small amount of activator solution (about 200 μL) and a small amount of live sperm (about 10 μL) on a glass slide and viewing cells under a stereo microscope. Estimates were based on the percent of the field with actively moving sperm cells. All males had > 95% pre-freeze motility estimates. Sperm from each male was cryopreserved using the standard protocol (see above). Eggs were divided into groups of 250, 500 and 1,000 eggs and fertilized with 1, 2, 4 and 8 straws for each group of eggs. Straws were thawed in a water bath for approximately 10 seconds at 11.67° C, quickly dried with a paper towel, then both ends cut to release the thawed sperm over the eggs. A sperm activator solution consisting 0.9% NaCl, 0.01M Tris (HCl), 0.02M glycine, pH 9 was added to egg and sperm groups for 5 minutes to facilitate fertilization. After fertilization eggs were rinsed, poured into screen boxes to isolate them and incubated in 11.67° C water. After incubating for approximately 14 hours embryo development was arrested using Carnoy's solution (glacial acetic acid solution) at the 4-cell stage. A sub sample of 50 eggs per group was examined for the presence of cell division to estimate percent fertilization.

We used analysis of variance (ANOVA) to analyze the data using transformed fertility data (arc sine square root transformation). We used multivariate ANOVA (MANOVA) to evaluate the relationship between fertility and males, eggs, and straws and their two-way interactions, and used ANOVA to evaluate the relationship between estimated thawed sperm motility and fertilization rate. In addition we used regression to examine the change in fertilization rate with a changing number of straws for all egg lots and for each individual egg lot size. We considered all tests to be significant at $\alpha = 0.05$.

Results

Fertility increased with the number of straws used (Figure 5) and decreased as the number of eggs increased (Figure 6). There was no significant difference between 8 vs. 4 straws or 1 vs. 2 straws, however 8 or 4 straws was better than 1 or 2 straws (Figure 5) and fertility for 250 eggs was better than 500 eggs, which was better than 1,000 eggs (Figure 6). Fertility increased with the number of straws that you used ($P < 0.0001$; Figure 7) at a rate of 2.4% per extra straw ($r^2 = 0.2954$). This was also true within egg batch sizes. For 250 eggs, fertility increased at a rate of 3.0% per straw ($P = 0.0006$; $r^2 = 0.3256$). For 500 eggs, fertility increased at a rate of 2.4% per straw ($P < 0.0001$; $r^2 = 0.4438$). For 1,000 eggs, fertility increased at a rate of 1.8% per straw ($P = 0.0001$; $r^2 = 0.3907$; Figure 7). Fertilization rate was significantly related to the individual male ($P < 0.0001$), but not the estimated motility of the thawed sperm ($P = 0.1128$). There were no interactions among the variables ($P > 0.6770$).

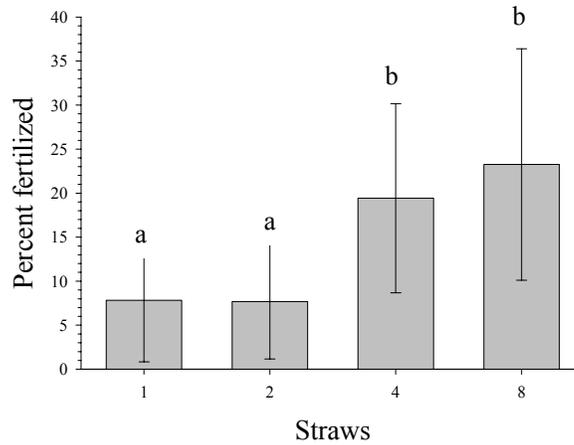


Figure 5. Mean fertility rate (+1 SD) for 1, 2, 4, or 8 straws over the combined data from 250, 500 and 1000 egg lots. Different letters represent significant differences among the egg lot numbers.

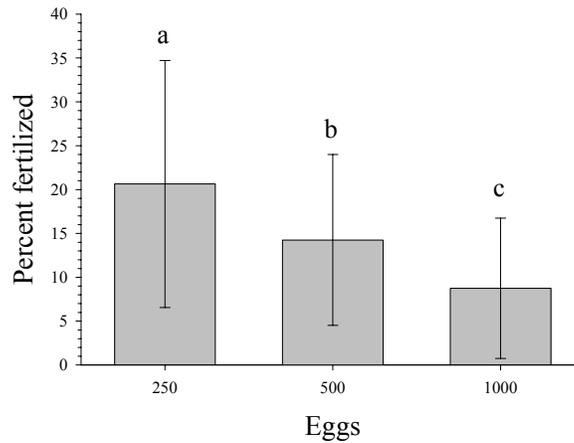


Figure 6. Mean fertility rate (+1 SD) for 250, 500, or 1,000 eggs over the combined data from 1, 2, 4, and 8 straw numbers. Different letters represent significant differences among the egg lot numbers.

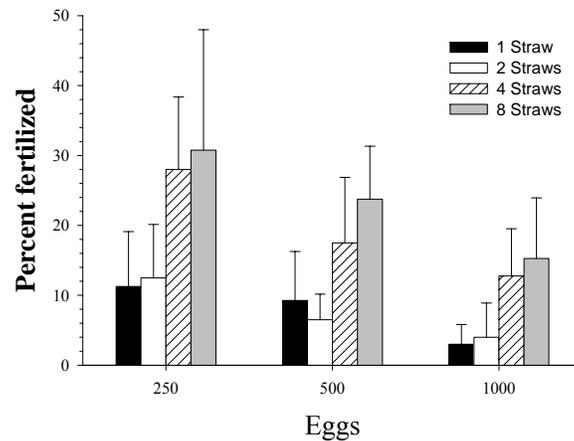


Figure 7. Mean fertility rate (\pm 1 SD) using 1, 2, 4, or 8 straws for 250, 500, or 1,000 eggs.

2006 Use of Cryopreserved Gametes

No gametes from the repository were requested or used in 2006.

Chinook Salmon 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 2006, 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. Presented below are results from the genetic analysis of samples collected from 2001 through 2005.

Methods

Chinook salmon tissue samples were taken with a hole punch from the caudal fin of fish that contributed gametes to the genebank. Tissue was stored either in 100% ethanol or lysis buffer (0.5 M EDTA, pH 8.0, 2 M Tris, pH 7.5, 5 M NaCl, 20% SDS). Extraction of DNA and microsatellite analysis followed the protocols in Narum et al. (2004). The DNA was amplified via polymerase chain reaction (PCR) using 13 microsatellite loci including; Ots211, Ots212, OMM1080, Ogo4, Ogo2, Ots201, OtsG474, Ots208b, Ots3M, Oki100, Ssa408, Ots9 and Ots213. A total of 627 samples from 11 populations were analyzed. (Table 4). Preliminary results revealed no significant difference between Marsh Creek and Capehorn Creek samples, so they were combined for all subsequent analyses.

Results

Results of general genetic diversity analyses are presented in Table 3. All populations demonstrated relatively high levels of gene and allelic richness. Exact test for deviation from Hardy Weinberg (H-W) equilibrium revealed that no populations deviated from H-W equilibrium when Bonferroni corrections for multiple comparisons were performed.

Fisher's exact test revealed significant population differentiation among the 10 major population groups ($F_{st} = 0.0323$; Fisher's Exact Test, $P < 0.00001$; Raymond and Rousset 1995). An UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram produced using a Nei's (1972) original genetic distance matrix revealed the relationship of the populations (Figure 8). Bootstrap analysis was performed to assess the reliability of the branch nodes. Overall bootstrap support for the tree was low.

Table 3. Populations of Chinook salmon and results of genetic analyses including the number of samples (N), collection years, gene diversity, allelic richness and exact test probabilities for deviation from H-W equilibrium.

Population	N	Gene Diversity (H _e)	Allelic Richness	Hardy-Weinberg Exact Tests (S.E)
Lostine River	83	0.7612	9.19	P=0.366 (0.032)
Catherine Creek	21	0.7633	9.36	P=0.649 (0.029)
Upper Grande Ronde	25	0.7848	10.23	P=0.655 (0.030)
Imnaha River	59	0.7853	10.23	P=0.871 (0.021)
Lake Creek	119	0.7635	8.93	P=0.806 (0.023)
South Fork Salmon R.	53	0.7950	10.37	P=0.029 (0.010)
Big Creek	83	0.7657	9.46	P=0.627 (0.036)
Marsh/Capehorn Creek	87	0.7847	9.72	P=0.487 (0.034)
Upper Salmon River	66	0.7798	10.01	P=0.756 (0.027)
Pahsimeroi R	31	0.7685	8.66	P=0.467 (0.027)
Overall	627	0.7752	10.46	P=0.634 (0.035)

The major population groups with the largest sample sizes were separated into subpopulations based on collection year and analyzed for population differentiation. These populations included Big Creek, Marsh Creek, Lake Creek, SFSR, Pahsimeroi River, upper Salmon River, Lostine River and Imnaha River. Samples sizes were small for many subpopulations (<20), so a comprehensive analysis was not possible and results should be taken with caution. Hierarchical analysis of subpopulations within the major population groups revealed significant differentiation among subpopulations within major population groups (Fisher's Exact Test, $P < 0.001$; Figure 9). An UPGMA dendrogram produced using a Nei's (1972) original genetic distance matrix revealed the relationship of the subpopulations within populations (Figure 9). Generally, branch support for the relationships among subpopulations within populations was relatively high and support for relationships among population groups was low, similar to the major population groups analysis above (Figure 8), and represented regional geographic differences.

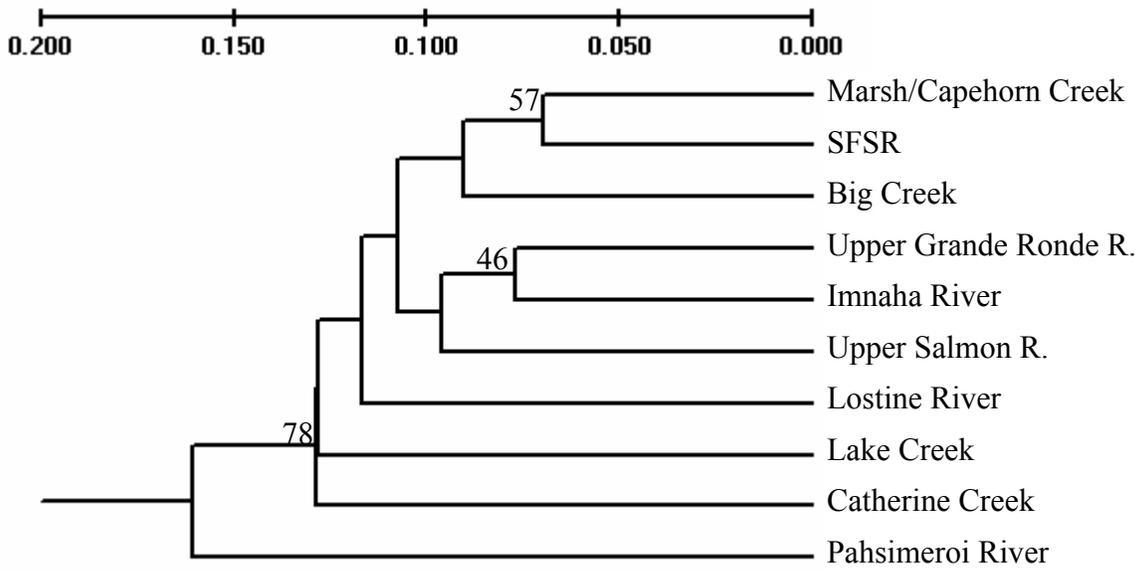


Figure 8. UPGMA dendrogram produced using Nei's (1997) original distance on Snake River basin Chinook salmon populations where gametes were collected for cryopreservation. Bootstrap values (shown in %) greater than 45% were shown.

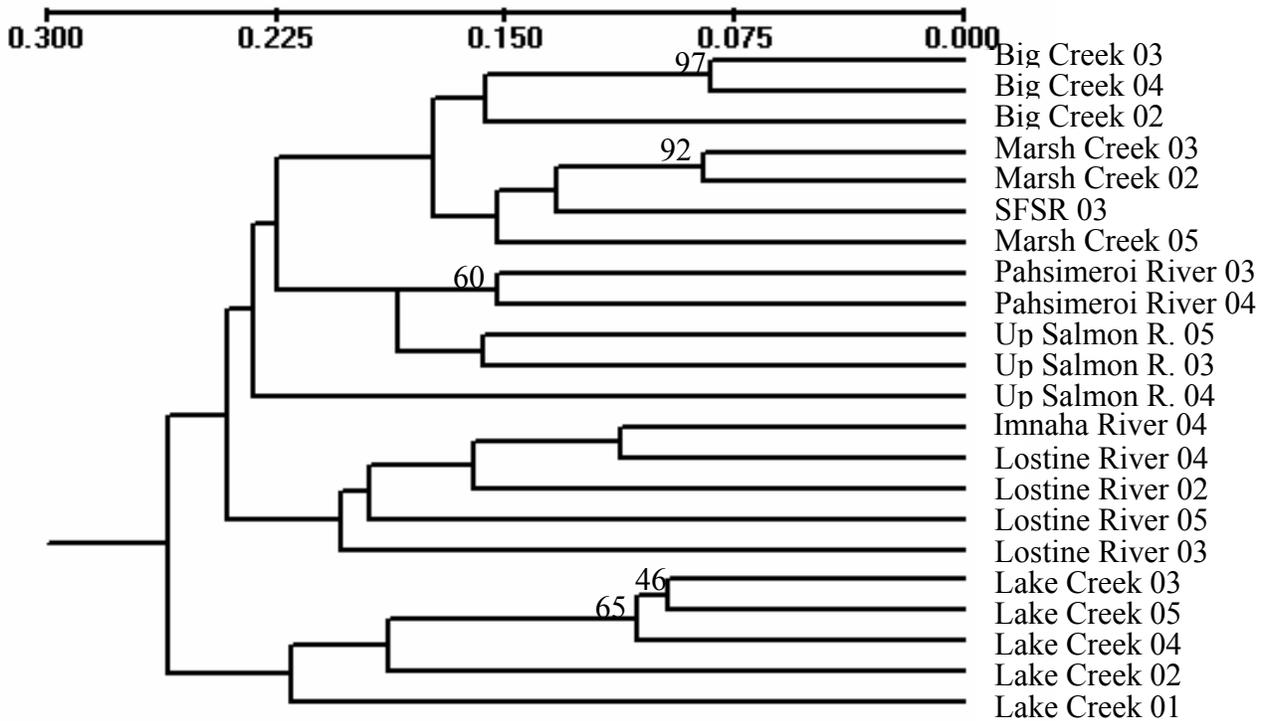


Figure 9. UPGMA dendrogram produced using Nei's (1972) original distance on Snake River basin Chinook salmon subpopulations where gametes were collected for cryopreservation. Bootstrap values (shown in %) greater than 45% were shown.

Chinook Salmon Brood Year Analysis

Dominant Brood Year Analysis

Understanding the distribution of the samples obtained from an organism with a non-discrete generation time is critical for preserving the greatest level of genetic diversity. This project set a goal of preserving gametes from at least 500 males from each population sample (Young et al, in prep). Equally weighting the collection of milt from adults across the entire sampling period will preserve the greatest amount of genetic diversity. Given a stable population size and age structure, this is best accomplished by collecting 100 samples per year for an entire generation. However, this has not been possible given the high variation in population size and the difficulty in capturing adult males. Generally, collections ranged from 0 – 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 Dominant Brood Year (DBY) analysis, could deal with sample collections from multiple age classes over multiple years and several generations.

A DBY is defined as the brood year that most influenced an individual calculated over multiple generations. The DBY is determined by calculating the brood year of an individual then using age structure of the population to estimate the most probable brood year of its grand parents and great grandparents. This essentially determines the previous brood years that made the greatest genetic contribution to an individual. Although we expect the influence to decrease more than two generations back, it still estimates the overall distribution of samples in the gene bank over short time periods (2-3 generations).

For example, comparing two collections of 400 gamete samples makes it possible to estimate the genetic contribution of each brood year in the collection. Collection 1 consisted of 50 samples/year for 2 Chinook salmon generations (8 years) and collection 2 consisted of the following sample collections: year 1 = 80; year 2 = 10; year 3 = 60; year 4 = 50; year 5 = 90; year 6 = 10; year 7 = 30 and; year 8 = 70 samples. DBY 1 are collections from years 1 and 5, DBY 2 are collections from years 2 and 6, DBY 3 are collections from years 3 and 7 and DBY 4 are collections from years 4 and 8. Assuming similar demographic composition among the years (similar number of 3, 4 and 5 year old fish each brood year), the former collection would theoretically preserve more diversity compared to the latter by evenly sampling fish over two generations. In contrast, collection 2 did not capture as much of the genetic diversity because DBY 1 was overrepresented (170 samples) and DBY 2 was underrepresented (20 samples).

In this report, we conducted DBY analysis on three natural-origin populations of Chinook salmon, Big Creek, Lake Creek and Marsh/Capehorn Creeks. Previous annual reports (Young, 2006; Young, 2005) provided DBY analysis for large collections of hatchery-origin Chinook salmon (This analysis was named Effective Brood Year (EBY) analysis in previous reports). These analyses will not be repeated in the report since the 2006 collections from those populations were relatively small. However, we will continue to collect based on our recommendations from Young (2006).

Methods

Spring/summer Chinook salmon from the Snake River basin have a generation time of approximately 4 years (Groot and Margolis, 1991). Males return to spawn after spending 1, 2 or 3 years in the ocean and females return to spawn after spending 2 or 3 years in the ocean. Initially the demographic makeup of fish that contributed gametes to the collection each year were assigned to an actual brood year using length frequency data provided by hatcheries (see methods section of this document, page 7). Length frequency determined that 68.7 % of natural-origin male salmon collected by this project returned as age 4 fish after spending 2 years in the ocean (Figure 10). The number of DBYs was equal to four (the number of years per generation). Fish collected as 3, 4 and 5 year olds in one year originated from 3 different brood years and thus 3 different DBYs. The first DBY was arbitrarily determined by subtracting 4 (the generation time) from the first year of collection and proceeded for the number of years in a generation. For this analysis 1996 was the first year of collections, so the DBY 1 corresponded to brood year 1991, DBY 2 corresponded to brood year 1992, DBY 3 corresponded to brood year 1993, DBY 4 corresponded to brood year 1994. For a 4 year generation the progression would start again with DBY 1 for brood year 1995, DBY 2 for brood year 1996 and so on. Summing the total number of fish from each DBY provided an estimate of the number and relative yearly distribution of fish preserved in the gene bank.

Results

DBY analysis on combined data from Chinook salmon collections from Lake Creek, Big Creek, and Marsh Creek (including Capehorn Creek) indicated that DBY 3 was significantly underrepresented in the collection (Figure 10). This DBY corresponded to brood year 1995, which returned the lowest number of spring/summer Chinook salmon to the Snake River basin (over Lower Granite Dam) on record. Thus, this analysis revealed that the effects of that return year persisted for at least 2 generations and may represent a significant loss of genetic diversity for the species across a wide geographic range. Because Snake River spring Chinook predominantly spawn as age 4 adults, the low returns in 1995 had the greatest impact on 1999 adult returns, then again in 2003 (Figure 10). Although these effects will likely decrease over time, the fact that they persisted for multiple generations suggest that recovery could be slow following a severe bottleneck. Adult age-4 Chinook salmon returning in 2007 represent DBY3 and will be targeted for collection. Unfortunately, run predictions for 2007 suggest another year of low returns, resulting from poor environmental conditions and potential demographic effects demonstrated by this analysis.

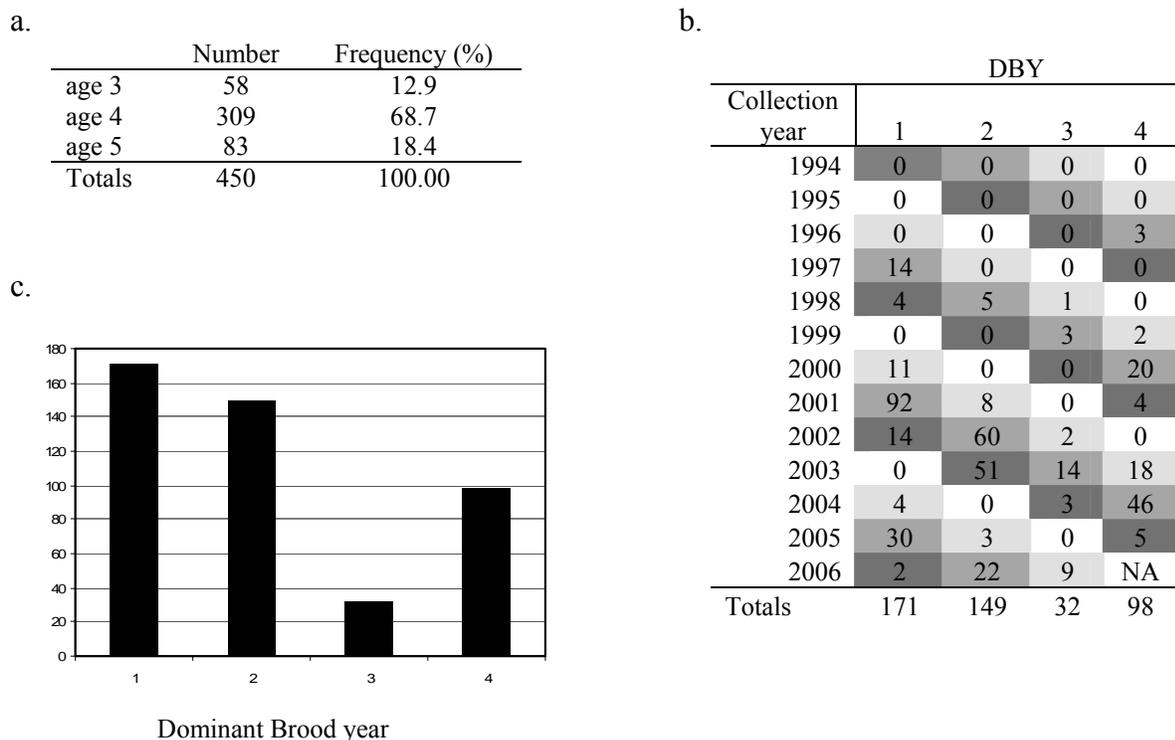


Figure 10. Dominant Brood Year (DBY) analysis of combined data from three natural-origin Chinook salmon populations, Lake Creek, Big Creek and Marsh Creek, from the Snake River basin. a. Number and frequency of adult males from each age collected from the three populations from 1996 through 2006. b. Table showing the collection year and corresponding DBY for combined data from 1994 through 2006. Shades of gray represent fish age, dark gray – age-5; medium gray – age 4 and; light gray – age-3. c. Bar graph representing the combined collections and DBY designation from 1994 through 2006.

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. Since 2003 we successfully collected naturally-spawning adult male steelhead using angling. In 2006 we collected gamete samples from three SFSR steelhead using this method. Steelhead spawner abundance was significantly lower in 2006, similar to that observed in 2005 (Young, 2006), and significantly lower than 2003 and 2004. 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 2006.

Results from the large fertility trial conducted in 2006 not only revealed important ways to maximize fertility of cryopreserved sperm, it also identified factors that need to be addressed in future research. Although fertilities were generally very low, significant differences in fertility related to sperm volume and egg number were observed. We concluded that increased fertilization rates can be obtained by increasing the number of straws (sperm volume) and suggest using at least 4 straws to fertilize 250 eggs. The low fertility was likely affected by the low post-freeze sperm motility, ranging from 0 to 20% (data not shown). The motility decrease observed following the freezing process suggested significant sperm damage occurred during the freezing procedure. Future research will explore variables that may have contributed to the sperm damage and low fertilization rates such as the relationship between water temperature and thawing time, use of different cryoprotectants in the freezing process and freezing sperm in pellet form versus straws.

Genetic population analysis of 627 Chinook salmon samples from 10 populations revealed that significant within- and among-population genetic diversity has been preserved in the genebank. Genetic diversity preservation is the overriding goal of this project and empirical confirmation of the amount and underlying structure justifies the collection strategy that was begun in 1992.

Demographic analysis of the three wild populations of Chinook salmon that contributed gametes to the repository indicated that the samples were not evenly distributed, potentially decreasing the level of genetic diversity preserved in the gene bank from these populations. The significantly underrepresented DBY 3 will be represented by 4 year old fish that return in 2007. We will target collections from age 4 fish in 2007.

No requests for cryopreserved gametes were made in 2006. We support and promote the use of gametes from the repository, creating a living gene bank that will aid the recovery of Snake River salmonids. 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 Endangered Species Act (ESA) compliance.

RECOMMENDATIONS

1. Continue collecting gametes from Chinook salmon populations throughout the Snake River basin until the goal of at least 500 samples are preserved from 2 populations per major population group (MPG).
2. Focus collections on natural populations without hatchery programs and age 4 adults, representing DBY 3, which are underrepresented in the gene bank for all populations.
3. Utilize angling as a method of collecting gametes from steelhead populations throughout the Snake River basin.
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 fertility using cryopreserved sperm.
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. Explore improved steelhead collection techniques/options.

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APPENDICIES

Appendix A. Gamete samples collected from 1992 through 2006

Table A1. Snake River basin Chinook salmon samples cryopreserved from 1992 through 2006.

Spawning Aggregate	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995	Pre- 1994	Totals
Lostine River	16	14	39	16	19	33	18	2	3	2	3	1	4	170
Minam River	2	4												6
Upper Grande Ronde River	13	7	8	10	8	9								55
Catherine Creek	12	10	7	8	5	11								53
Rapid River							51	68	98					217
South Fork Salmon River	1	11	15	26	23	44	54	93	45	45	19			376
Lake Creek	8	20	26	32	18	28	15	6	3	4	3			163
Johnson Creek	31	48	60	51	58	62	35	5	17	7				374
Big Creek	9	6	22	31	21	50	7	0	1	6	0	0	17	170
Capehorn Creek	1	6	0	15	1	2	1	0	6	2				34
Marsh Creek	15	6	5	16	34	24	7	0	2	4				113
Pahsimeroi River			20	15	39	50	50	31						205
Upper Salmon River	13	18	25	20	54	48	40	40	41	51				349
Imnaha River	20	12	25	23	7	37	71	95	79	41	33	42	22	507
Totals	141	162	252	263	286	398	349	340	295	162	58	43	26	2,792

Table A2. Snake River basin steelhead samples cryopreserved from 1993 through 2006.

Spawning Aggregate	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1994	1993	Totals
Tucannon River	16	22											38
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	3	2	24	17									46
Johnson Creek			1			1		2					4
Pahsimeroi River					63	60	40	47					210
Imnaha River							2						2
Little Sheep Creek	3	11	100	70	95	78	52	25	25	5			464
Cow Creek				2									2
Lightning Creek				1									1
Snake River					58	73	98	76					307
Totals	22	35	125	90	280	295	281	214	25	5	5	10	1,390

* Samples collected by the USGS/ National Biological Survey.

Appendix B. Data from Chinook salmon collected in 2006.

Table A3. Collection date, fork lengths, percent motilities and number of straws from Chinook salmon collected in 2006.

Location	Date	Fork length (cm)	Genebank #	WSU motility (%)	WSU # of 0.5 ml straws	Genebank # (if different at UI)	UI motility (%)	UI # of 0.5 ml straws
Big Creek	8/1/2006	65	NPT-001-2006	80	20		50	20
Big Creek	8/1/2006	69	NPT-002-2006	UI			80	20
Big Creek	8/8/2006	70	NPT-007-2006	UI			80	30
Big Creek	8/15/2006	83	NPT-012-2006	10	20		80	15
Big Creek	8/15/2006	70	NPT-013-2006	90	20		80	10
Big Creek	8/15/2006	73	NPT-014-2006	90	20		80	10
Big Creek	8/15/2006	69	NPT-015-2006	UI			90	20
Big Creek	8/15/2006	72	NPT-015-2006	80	20	NPT-016-2006	60	10
Big Creek	8/15/2006	62	NPT-017-2006	90	20		90	20
Lake Creek	8/7/2006	55	NPT-003-2006	70	20		80	10
Lake Creek	8/7/2006	69	NPT-004-2006	70	20		80	20
Lake Creek	8/7/2006	74	NPT-005-2006	0	0		0	20
Lake Creek	8/7/2006	80	NPT-006-2006	90	20		90	20
Lake Creek	8/14/2006	76	NPT-008-2006	90	20		WSU	
Lake Creek	8/14/2006	77.5	NPT-009-2006	90	20		WSU	
Lake Creek	8/14/2006	64.5	NPT-010-2006	80	20		WSU	
Lake Creek	8/14/2006	78	NPT-011-2006	90	20		WSU	
Marsh Creek	8/16/2006	75	NPT-19-2006	UI			50	10
Marsh Creek	8/16/2006	96.5	NPT-20-2006	80	20		90	20
Marsh Creek	8/16/2006	77	NPT-21-2006	90	20		80	20
Marsh Creek	8/17/2006	67	NPT-22-2006	80	20		80	10
Marsh Creek	8/17/2006	62.5	NPT-23-2006	80	20		80	15
Marsh Creek	8/17/2006	107	NPT-24-2006	70	20		WSU	
Marsh Creek	8/23/2006	59	NPT-025-2006	90	20		90	15
Marsh Creek	8/23/2006	68.5	NPT-026-2006	80	20		90	20
Marsh Creek	8/23/2006	68.5	NPT-027-2006	80	20		80	20
Marsh Creek	8/23/2006	59	NPT-028-2006	10	20		80	15
Marsh Creek	8/23/2006	69	NPT-029-2006	90	20		90	20
Marsh Creek	8/23/2006	77.5	NPT-030-2006	0	0		70	20
Marsh Creek	8/23/2006	71.5	NPT-031-2006	80	20		70	10
Marsh Creek	8/23/2006	66	NPT-032-2006	80	20		80	20
Marsh Creek	8/23/2006	69	NPT-033-2006	10	20		WSU	
Capehorn Creek	8/16/2006	65	NPT-018-06	90	20		WSU	
SFSR	9/1/2006	58	NPT-099-2006	90	20		WSU	
Johnson Creek	8/25/2006	87	NPT-034-2006	80	20		90	20
Johnson Creek	8/25/2006	84	NPT-035-2006	80	20		70	20
Johnson Creek	8/25/2006	80	NPT-036-2006	70	20		90	20
Johnson Creek	8/25/2006	73	NPT-037-2006	70	20		90	20
Johnson Creek	8/25/2006	73	NPT-038-2006	80	20		90	20
Johnson Creek	8/25/2006	73	NPT-039-2006	80	20		90	20
Johnson Creek	8/25/2006	79	NPT-040-2006	60	20		90	20
Johnson Creek	8/25/2006	75	NPT-041-2006	50	20		90	20
Johnson Creek	8/25/2006	69	NPT-042-2006	80	20		80	20
Johnson Creek	8/25/2006	-	NPT-043-2006	80	20		90	20
Johnson Creek	8/25/2006	81	NPT-044-2006	80	20		90	20
Johnson Creek	8/25/2006	70	NPT-045-2006	50	20		90	20
Johnson Creek	8/25/2006	76	NPT-046-2006	80	17		80	20
Johnson Creek	8/29/2006	84	NPT-047-2006	UI			90	20
Johnson Creek	8/29/2006	76	NPT-048-2006	80	20		WSU	
Johnson Creek	8/29/2006	76	NPT-049-2006	UI			90	10
Johnson Creek	8/29/2006	72	NPT-050-2006	80	20		90	20
Johnson Creek	8/29/2006	78	NPT-051-2006	50	20		WSU	
Johnson Creek	8/29/2006	72	NPT-052-2006	90	20		90	20
Johnson Creek	8/29/2006	73	NPT-053-2006	UI			80	20
Johnson Creek	8/29/2006	78	NPT-054-2006	60	20		WSU	
Johnson Creek	8/29/2006	79	NPT-055-2006	80	20		WSU	
Johnson Creek	8/29/2006	74	NPT-056-2006	60	20		90	20
Johnson Creek	8/29/2006	79	NPT-057-2006	70	20		90	20

Johnson Creek	8/30/2006	69	NPT-066-2006	70	20		WSU	
Johnson Creek	8/30/2006	79	NPT-067-2006	90	20		90	20
Johnson Creek	8/30/2006	60	NPT-068-2006	90	20		90	20
Johnson Creek	8/30/2006	77	NPT-069-2006	90	20		80	20
Johnson Creek	8/30/2006	68	NPT-070-2006	60	20		80	15
Johnson Creek	8/30/2006	72	NPT-071-2006	70	20		90	20
Johnson Creek	8/30/2006	71	NPT-072-2006	60	20		90	20
Johnson Creek	9/1/2006	76	NPT-100-2006	90	20		WSU	
Johnson Creek	9/1/2006	79	NPT-101-2006	90	20		WSU	
Johnson Creek	9/1/2006	78	NPT-102-2006	90	20		WSU	
Johnson Creek	9/1/2006	73	NPT-103-2006	90	20		WSU	
Upper Salmon River	8/31/2006	-	NPT-077-2006	UI			0	20
Upper Salmon River	8/31/2006	63	NPT-078-2006	90	20		WSU	
Upper Salmon River	8/31/2006	77	NPT-079-2006	UI			70	20
Upper Salmon River	8/31/2006	70	NPT-080-2006	90	20		WSU	
Upper Salmon River	8/31/2006	73	NPT-081-2006	UI			70	20
Upper Salmon River	8/31/2006	54	NPT-082-2006	90	20		WSU	
Upper Salmon River	8/31/2006	70.5	NPT-083-2006	UI			80	20
Upper Salmon River	8/31/2006	64	NPT-084-2006	20	20		WSU	
Upper Salmon River	8/31/2006	84	NPT-085-2006	UI			80	20
Upper Salmon River	8/31/2006	77	NPT-086-2006	80	20		WSU	
Upper Salmon River	8/31/2006	72	NPT-087-2006	UI			70	20
Upper Salmon River	8/31/2006	77	NPT-088-2006	70	20		WSU	
Upper Salmon River	8/31/2006	75	NPT-089-2006	UI			90	20
Lostine River	8/30/2006	105.5	NPT-73-2006	20	0		40	20
Lostine River	8/30/2006	87	NPT-74-2006	10	20		40	20
Lostine River	8/30/2006	73	NPT-75-2006	80	20		70	20
Lostine River	8/30/2006	79	NPT-76-2006	UI			90	10
Lostine River	9/6/2006	79	NPT-116-2006	UI			70	40
Lostine River	9/6/2006	73	NPT-117-2006	UI			90	40
Lostine River	9/6/2006	83	NPT-118-2006	UI			90	40
Lostine River	9/6/2006	81	NPT-119-2006	UI			90	40
Lostine River	9/6/2006	83	NPT-120-2006	UI			90	4
Lostine River	9/6/2006	96	NPT-121-2006	UI			70	40
Lostine River	9/6/2006	73	NPT-122-2006	UI			90	13
Lostine River	9/6/2006	82	NPT-123-2006	UI			90	10
Lostine River	9/6/2006	70	NPT-124-2006	UI			90	32
Lostine River	9/6/2006	77	NPT-125-2006	UI			90	38
Lostine River	9/6/2006	78.5	NPT-126-2006	UI			90	40
Lostine River	9/6/2006	66	NPT-127-2006	UI			80	37
Catherine Creek	8/31/2006	86	NPT-090-2006	90	20		90	20
Catherine Creek	8/31/2006	76	NPT-091-2006	90	20		90	20
Catherine Creek	9/14/2006	71.5	NPT-137-2006	50	20		80	20
Catherine Creek	9/14/2006	81	NPT-138-2006	20	20		80	20
Catherine Creek	9/14/2006	75	NPT-139-2006	50	20		80	20
Catherine Creek	9/14/2006	82	NPT-140-2006	70	20		80	20
Catherine Creek	9/14/2006	73	NPT-141-2006	80	20		80	19
Catherine Creek	9/14/2006	75	NPT-142-2006	70	20		90	20
Catherine Creek	9/14/2006	72	NPT-143-2006	70	20		90	20
Catherine Creek	9/14/2006	62.5	NPT-144-2006	UI			80	20
Catherine Creek	9/14/2006	80.5	NPT-145-2006	90	20		90	20
Catherine Creek	9/14/2006	73	NPT-146-2006	60	20		90	20
Grande Ronde River	8/31/2006	75	NPT-092-2006	90	20		90	20
Grande Ronde River	8/31/2006	76	NPT-093-2006	90	0		90	10
Grande Ronde River	8/31/2006	76	NPT-094-2006	90	20		90	20
Grande Ronde River	8/31/2006	80	NPT-095-2006	90	20		90	10
Grande Ronde River	8/31/2006	74	NPT-096-2006	80	20		90	20
Grande Ronde River	8/31/2006	68.5	NPT-097-2006	90	20		WSU	
Grande Ronde River	8/31/2006	75	NPT-098-2006	80	20		90	20
Grande Ronde River	9/14/2006	72	NPT-130-2006	90	20		70	20
Grande Ronde River	9/14/2006	86	NPT-131-2006	60	20		80	20
Grande Ronde River	9/14/2006	73	NPT-132-2006	70	20		80	15
Grande Ronde River	9/14/2006	76	NPT-133-2006	50	10		70	20
Grande Ronde River	9/14/2006	80.5	NPT-134-2006	80	20		90	10
Grande Ronde River	9/14/2006	71	NPT-135-2006	80	10		80	10
Imnaha River	8/29/2006	73	NPT-058-2006	10	10		>20	10

Imnaha River	8/29/2006	76.5	NPT-059-2006	90	20		90	20
Imnaha River	8/29/2006	75	NPT-060-2006	50	20	NPT-061-2006	90	20
Imnaha River	8/29/2006	76	NPT-060-2006	UI			90	15
Imnaha River	8/29/2006	76.5	NPT-062-2006	20	20		80	10
Imnaha River	8/29/2006	73.5	NPT-063-2006	60	20		90	15
Imnaha River	8/29/2006	75	NPT-064-2006	80	20		80	10
Imnaha River	8/29/2006	69	NPT-065-2006	50	20		90	10
Imnaha River	9/5/2006	71	NPT-104-2006	80	20		90	10
Imnaha River	9/5/2006	68.5	NPT-105-2006	80	20		90	20
Imnaha River	9/5/2006	93	NPT-106-2006	20	20		90	20
Imnaha River	9/5/2006	68	NPT-107-2006	70	20		90	20
Imnaha River	9/5/2006	72	NPT-108-2006	75	20		90	20
Imnaha River	9/5/2006	66	NPT-109-2006	80	20		90	20
Imnaha River	9/5/2006	90	NPT-110-2006	70	20		90	20
Imnaha River	9/5/2006	81	NPT-111-2006	90	20		90	20
Imnaha River	9/5/2006	66	NPT-112-2006	40	20		90	15
Imnaha River	9/5/2006	72	NPT-113-2006	70	20		90	20
Imnaha River	9/5/2006	70	NPT114-2006	70	20		90	15
Imnaha River	9/5/2006	69	NPT-115-2006	70	10		80	10
Minam River	9/6/2006	71	NPT-128-2006	UI			80	20
Minam River	9/7/2006	69	NPT-128-06	90	20	NPT-129-2006	80	10

Appendix C. Data from steelhead collected in 2006.

Table A4. Collection date, fork lengths, percent motilities and number of straws from steelhead collected in 2006.

Location	Date	Fork Length	Age (by scale)	WSU Gene Bank #	UI Gene Bank #	Motility	# 0.5 ml straws
Little Sheep Creek	4/18/2006	710		NPT-224-2005		80	10
Little Sheep Creek	4/18/2006	615		NPT-225-2005		80	10
Little Sheep Creek	4/18/2006	508		NPT-226-2005		70	10
Tucannon River	3/14/2006	56	1.1	NPT-209-2005	NPT-200-2005	70	39
Tucannon River	3/14/2006	61	2.1	NPT-210-2005	NPT-201-2005	70	40
Tucannon River	3/14/2006	76	2.2	NPT-119-2005	NPT-202-2005	70	40
Tucannon River	3/14/2006	78	2.2	NPT-212-2005	NPT-203-2005	80	40
Tucannon River	3/14/2006	78	2.2	NPT-213-2005	NPT-204-2005	60	30
Tucannon River	3/14/2006	57	2.1	NPT-214-2005		-	20
Tucannon River	3/14/2006	63.5	2.1	NPT-206-2005		70	10
Tucannon River	3/14/2006	65	2.2	NPT-216-2005		-	20
Tucannon River	3/14/2006	58.5	2.1	NPT-208-2005		70	10
Tucannon River	3/14/2006	61	2.1	NPT-218-2005		-	20
Tucannon River	3/14/2006	73	2.2	NPT-210-2005		70	20
Tucannon River	3/27/2006	62	2.1	NPT-219-2005		-	20
Tucannon River	3/27/2006	77	R.2	NPT-220-2005		70	20
Tucannon River	3/27/2006	76	1.2	NPT-221-2005		-	20
Tucannon River	3/27/2006	70	1.2	NPT-222-2005		80	10
Tucannon River	3/27/2006	63.5	2.2	NPT-223-2005		90	20
SFSR	4/27/2006	85		NPT-463-2003		-	20
SFSR	4/27/2006	69		NPT-464-2003		-	20
SFSR	5/11/2006	77		NPT-465-2003		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).