

SALMONID GAMETE PRESERVATION IN THE SNAKE RIVER BASIN

2009 Annual Report



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ABSTRACT

In 1992 the Nez Perce Tribe (NPT), in cooperation with Washington State University (WSU) and the University of Idaho (UI), established a germplasm repository in order to preserve a representative sample from the remaining salmonid diversity in the Snake River basin.

Collections focused on Chinook salmon (*Oncorhynchus tshawytscha*; Nacó'x in Nez Perce) and steelhead (*O. mykiss*; Héeyey in Nez Perce) populations that were listed as threatened species under the Endangered Species Act. Numerous species and populations in this region have already been extirpated and small population sizes in the remaining populations face threats from decreased genetic diversity resulting from genetic drift and inbreeding, reducing viability of the entire species and ecological communities. A comprehensive collection strategy was developed to maximize the preservation of within- and among-population genetic diversity from these species. In addition, the repository serves as a long-term storage facility available for other management agencies to contribute gamete samples from other populations or species. Although only male gametes can be cryopreserved at this time, the germplasm repository provides management options for future species recovery actions.

No gametes were collected in 2009. Current project objectives are to maintain the samples in repositories at WSU and UI and assist hatchery or research personnel in the use of the gametes. This report provides an overview of the samples in the repository that are available for use. A total of 2,990 Columbia River male Chinook salmon, 1,403 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.

TABLE OF CONTENTS

ABSTRACT.....	i
TABLE OF CONTENTS.....	ii
LIST OF FIGURES	iii
LIST OF TABLES	iii
ACKNOWLEDGMENTS	iv
INTRODUCTION	1
PROJECT OVERVIEW	2
Description of Chinook salmon and steelhead gamete collections.....	2
Use of Cryopreserved Gametes in 2008	5
Project Publications	6
RESULTS AND DISCUSSION.....	7
Management Recommendations.....	8
LITERATURE CITED.....	9
APPENDICES	13
Appendix A. Gamete samples collected from 1992 through 2008.....	14
Appendix B. Total number of Chinook salmon and steelhead straws preserved in the gene bank.....	17
Appendix C. Snake River Germplasm Repository Cryopreserved Semen Request Form.....	19

LIST OF FIGURES

Figure 1. Map showing the Snake River basin Chinook salmon sampling locations for 2008..... 4

Figure 2. Map showing the Snake River basin steelhead sampling locations for 2008..... 5

LIST OF TABLES

Table 1. Chinook salmon and Steelhead Major Population Group (MPGs) and populations that have germplasm preserved in the repository..... 3

Table 2. Number of hatchery- and natural-origin (HOR and NOR) Chinook salmon (3a) and steelhead (3b) preserved in the genebank for each population and the percent natural-origin by population and overall..... 6

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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. Although *in situ* actions for conserving species such as habitat protection and enhancement and harvest management are preferred, these measures frequently are not implemented until populations have reached critically low levels. Once this occurs, *ex situ* conservation strategies using artificial environments such as zoos, botanical gardens and live or cryopreserved 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, 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 were extinct, and an additional 214 salmon, steelhead, and sea-run cutthroat trout stocks were at risk of extinction. As a first step in the recovery of anadromous fish stocks, National Oceanographic and Atmospheric Administration Fisheries (NOAA) 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 (*Oncorhynchus tshawytscha*; Nacó'x in Nez Perce) and steelhead (*O. mykiss*; Héeyey in Nez Perce) in the Snake River basin. These populations warranted protection because they possess unique genetic and life history attributes of the species and thus represent distinct population segments. The recovery effort for these species has mainly focused on habitat protection and enhancement, hatchery construction, harvest controls, fish barging, and 'fish-friendly' changes in dam operation. Although these measures have been in place for decades, many populations continue to decline. Recently more intensive practices such as supplementation and captive brood programs have been implemented.

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. Cryotechnology has been 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 has the potential to maintain genetic diversity and reduce extinction risk in the short term by increasing the effective population size of the population (Ballou, 1992). 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). The Nez Perce Tribe (NPT) initiated Chinook salmon cryopreservation activities in 1992 (Kucera and Blendin, 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 and steelhead populations in the Snake River basin (Armstrong and Kucera 1999).

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 gametes and embryos 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.

The Snake River Basin Germplasm Repository represents one of the largest collections of salmonid gametes in the world. Development of this repository was not meant to replace existing recovery actions, but rather compliment them and provide a buffer against future population collapse. As stated by the Convention on Biological Diversity, Article IX, *ex situ* techniques (such as gamete cryopreservation) are predominantly for the purpose of complimenting *in situ* methods. The NPT considers habitat rehabilitation, including the restoration of naturally-flowing mainstem river habitats, a priority in the recovery of anadromous fish. The crisis that prompted the initiation of this project appears to have diminished with the increased returns over the last decade. However, population growth, land development and climate change will no doubt put additional stress on the ecosystem and anadromous fish populations in the region (Lackey, 2003). Providing an alternative management option through *ex situ* techniques may be the only option available for the preservation and recovery of the existing Snake River basin anadromous fish diversity.

No collections of Chinook salmon and steelhead gametes occurred in 2009. Main activities involved long-term maintenance of the stored germplasm and assisting in the use of gametes for production or research objectives.

PROJECT OVERVIEW

Description of Chinook salmon and steelhead gamete collections

The cryopreservation project collected and preserved gametes from Snake River basin male spring/summer Chinook salmon (Table 1; Figure 1) and steelhead (Table 1; Figure 2) from 1992 – 2008, resulting in a genetically diverse collection of germplasm from these species. Collections occurred from multiple populations within all major Population groups (MPGs) within the Snake River Spring/Summer Chinook salmon and steelhead Evolutionary Significant Units (ESU). Because no collections occurred in 2009 we will not describe fish and gamete handling and storage procedures. A complete description can be found in previous annual reports (Young, 2009).

Table 1. Chinook salmon and Steelhead Major Population Group (MPGs) and populations that have germplasm preserved in the repository. Standard river kilometer site codes (Stein, et al. 2001) designated a trap location or the downstream point of a river reach where the donor fish were captured. The collection locations designated the location where gametes were collected and the origin of the targeted fish (HOR - hatchery-origin; NOR - natural-origin).

Chinook Salmon MPGs and Populations		
Population	Site Codes	Collection location
1. Grande Ronde/Imnaha River		
a. Minam River	522.271.131.016	Stream, NOR
b. Lostine River	522.271.131.042.001	Lookingglass Hatchery, HOR & NOR
c. Catherine Creek	522.271.232.032	Lookingglass Hatchery, HOR & NOR
d. Upper Grande Ronde River	522.271.307	Lookingglass Hatchery, HOR & NOR
e. Imnaha River	522.308.074	Lookingglass Hatchery, HOR & NOR
2. South Fork Salmon River MPG		
a. Lake Creek	522.303.215.059.045	Stream
b. Johnson Creek	522.303.215.060.024	Johnson Creek weir and stream, HOR and NOR
c. South Fork Salmon River	522.303.215.118	McCall Hatchery Trap, HOR and NOR
3. Middle Fork Salmon River MPG		
a. Big Creek	522.303.319.029	Stream, NOR
b. Marsh Creek	522.303.319.170	Stream, NOR
c. Capehorn Creek	522.303.319.170.010	Stream, NOR
d. Elk Creek	522.303.319.170.014	Stream, NOR
4. Upper Salmon River MPG		
a. Pahsimeroi River	522.303.489.002	Pahsimeroi Hatchery, HOR and NOR
b. upper Salmon River	522.303.617	Sawtooth Hatchery, HOR and NOR
5. Other		
a. Rapid River	522.303.140.007.006	Rapid River Hatchery, HOR
Steelhead MPGs and Populations		
Population	Site Codes	Collection location
1. Lower Snake River MPG		
a. Tucannon River	522.100	Lyons Ferry Hatchery, HOR & NOR
2. Clearwater River MPG		
a. North Fork Clearwater River	522.224.065	Dworshak National Fish Hatchery, HOR
b. Fish Creek	522.224.120.037.039	Fish Creek Trap, NOR
c. Selway River	522.224.120.037	Selway Falls fish ladder, NOR
3. Grande Ronde/Imnaha River		
a. Upper Grande Ronde River	522.271.307	Grande Ronde River Trap, NOR
b. Imnaha River	522.308.074	Lookingglass Hatchery, NOR
c. Little Sheep Creek	522.308.032.005.008	Lookingglass Hatchery, HOR & NOR
d. Lightning Creek	522.308.008	Lookingglass Hatchery, NOR
e. Cow Creek	522.308.007	Lookingglass Hatchery, NOR
4. South Fork Salmon River MPG		
a. South Fork Salmon River	522.303.215.060	Stream, NOR
b. Johnson Creek	522.303.215.060.024	Johnson Creek weir; Stream, NOR
5. Upper Salmon River MPG		
a. Pahsimeroi River	522.303.489.002	Pahsimeroi Hatchery, HOR
6. Other		
a. Snake River		Oxbow Hatchery, HOR

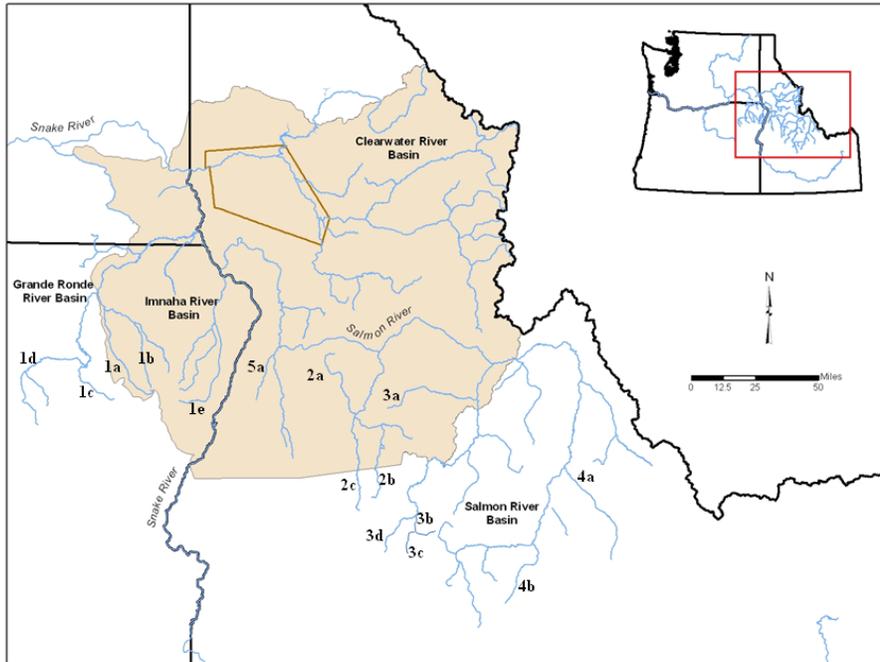


Figure 1. Map showing the Snake River basin Chinook salmon sampling locations for 2008. The outlined area within the shaded region represents the current Nez Perce Tribe reservation boundary. The shaded area represents the Indian Claims Commission (ICC) area. Sample locations included; 1a) Minam River; 1b) Lostine River; 1c) Catherine Creek; 1d) Grande Ronde River; 1e) Imnaha River; 2a) Lake Creek; 2b) Johnson Creek; 2c) South Fork Salmon River; 3a) Big Creek; 3b) Marsh Creek; 3c) Capehorn Creek; 3d) Elk Creek; 4a) Pahsimeroi River; 4b) upper Salmon River; 5a) Rapid River.

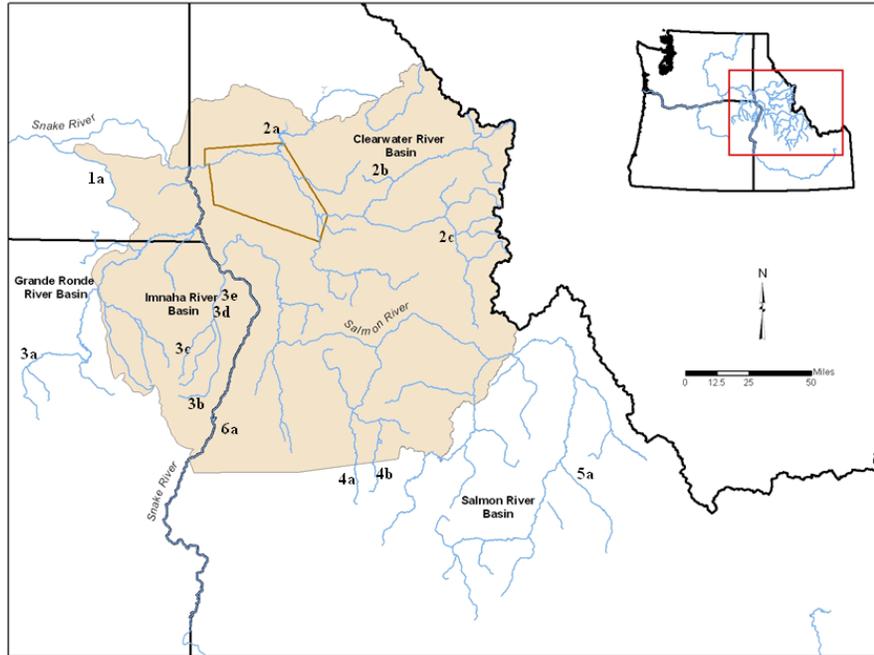


Figure 2. Map showing the Snake River basin steelhead sampling locations for 2008. The outlined area within the shaded region represents the current Nez Perce Tribe reservation boundary. The shaded area represents the Indian Claims Commission (ICC) area. Sample locations included; 1a) Tucannon River; 2a) North Fork Clearwater River; 2b) Selway River; 2c) Fish Creek; 3a) Grande Ronde River; 3b) Imnaha River; 3c) Little Sheep Creek; 3d) Lightning Creek; 3e) Cow Creek; 4a) South Fork Salmon River; 4b) Johnson Creek; 5a) Pahsimeroi River; 6a) Snake River.

Composition of hatchery- and natural-origin Chinook salmon and steelhead are presented in Table 2a and Table 2b, respectively. Greater numbers of natural-origin Chinook salmon were preserved, with adequate numbers of hatchery-origin individuals to capture the diversity represented in these populations (Imnaha River, Pahsimeroi River, Rapid River and SFSR). In contrast, few natural-origin steelhead were collected.

Use of Cryopreserved Gametes in 2008

There were no requests for germplasm from the gene bank in 2008.

Table 2. Number of hatchery- and natural-origin (HOR and NOR) Chinook salmon (3a) and steelhead (3b) preserved in the genebank for each population and the percent natural-origin by population and overall.

2a.				2b.			
Location	HOR	NOR	% NOR	Location	HOR	NOR	% NOR
Big Creek	4	183	97.9	Cow Creek	0	4	100
Capehorn Creek	0	42	100	NF Clearwater River	295	0	0
Catherine Creek	17	59	77.6	Fish Creek	0	15	100
Elk Creek	0	1	100	Grande Ronde River	0	2	100
Grande Ronde River	7	52	88.1	Imnaha River	0	2	100
Imnaha River	212	307	59.2	Johnson Creek	0	4	100
Johnson Creek	35	378	91.5	Lightning Creek	0	3	100
Lake Creek	5	198	97.5	Little Sheep Creek	433	31	6.7
Lostine River	33	143	81.3	Pahsimeroi River	207	0	0
Marsh Creek	0	142	100	Selway River	1	4	80
Minam River	0	6	100	SFSR	0	50	100
Pahsimeroi River	126	79	38.5	Snake River	302	4	1.3
Rapid River	216	0	0	Tucannon River	0	51	100
SFSR	199	175	46.8	Totals (Ave. % NOR)	1238	170	(12.1)
Salmon River, upper	75	277	78.7				
Totals (Ave. % NOR)	929	2042	(68.7)				

Project Publications

One peer-reviewed manuscript was published in 2009 (see abstract below; Young et al. 2009). Previous publications included Annual Reports from 1999 through 2009 (Armstrong and Kucera, 1999; Armstrong and Kucera, 2000; Armstrong and Kucera, 2001; Young and Kucera, 2002; Young, 2003; Young, 2004; Young, 2005; Young, 2006; Young, 2007; Young, 2008; Young, 2009) and a peer-reviewed manuscript in 2000 (Cloud et al. 2000).

*No increase in developmental deformities or fluctuating asymmetry in rainbow trout (*Oncorhynchus mykiss*) produced with cryopreserved sperm.*

William P. Young, Kathryn Frenyea, Paul A. Wheeler, Gary H. Thorgaard

Although cryopreservation is a widely accepted tool in animal breeding and human reproduction, questions have arisen regarding the health and viability of fish sired by cryopreserved sperm. We examined rainbow trout families produced using fresh and cryopreserved milt to determine if sperm cryopreservation negatively influenced early development. Fresh and cryopreserved milt from 3 males were used to fertilize eggs from 6 females in a 3×6 factorial design. Survival to eye, survival to fry, proportion of fry deformities and developmental stability were compared for fresh vs. cryopreserved milt. Results revealed a significant reduction in survival to eye ($P = 0.001$) and survival to fry ($P = 0.001$) for families sired using cryopreserved milt. Survival during the interval from eye to fry did not differ among the groups ($P = 0.127$), indicating that survival differences occurred prior to the eye stage. These results could be explained by reduced fertilization success of cryopreserved sperm. We examined developmental differences by analyzing the proportion of haploid embryos at the eyed stage, the proportion of fry deformities at hatch and fluctuating asymmetry (FA) of pectoral fin rays. Results revealed no significant difference between the groups. Our observations that surviving fry produced using cryopreserved sperm showed no differences in early development suggest that sperm cryopreservation is a viable option for use in breeding programs and in conservation and recovery of imperiled salmonid populations.

RESULTS AND DISCUSSION

This program began gamete collection and preservation in 1992, with large scale collections occurring from 1997 through 2008. The goal of the collection strategy was to preserve gametes from at least 500 individuals from a minimum of 2 subpopulations from each MPG in the Snake River basin. Although the numerical goal of 500 individuals per population was only met for one population, Imnaha River Chinook salmon, gamete collections from numerous populations of Chinook salmon across the Snake River basin were relatively large, and likely adequate, for moderate recovery actions (Appendix A, Table A1). In contrast, gamete collections from steelhead have had limited success. Although steelhead gametes were preserved from a large number of geographically distinct populations, most collections numbered fewer than 15 individuals (Appendix A, Table A2), which is not nearly adequate for genetic diversity preservation or population recovery. The only steelhead populations with adequate collections were from a few large hatchery programs that were not at high risk of extirpation. Consequently, limited genetic diversity has been preserved from Snake River basin steelhead. Steelhead populations should be closely monitored and, if warranted, alternative methods of genetic diversity preservation should be explored.

Only when compared to historic levels of Snake River basin anadromous fish diversity can the true success of the project be evaluated. Assessing the amount of diversity preserved compared to contemporary levels revealed that the project captured a significant portion of the extant Chinook salmon diversity in the Snake River basin, but not that of steelhead. From a historical perspective a relatively limited amount of genetic diversity was preserved by the project because a large proportion had been lost prior to the initiation of the project (Young, 2009). This did not mean that the project was a failure, in contrast, the effort produced one the largest and most diverse collections of anadromous fish germplasm in the world.

Maximizing demographic diversity of fish that contributed gametes to the gene bank was also a critical goal of the project, including collections from fish of multiple origins (HOR and NOR) and ages. Collecting gametes from HOR Chinook salmon and steelhead was relatively easy and large numbers of samples were preserved from a few large hatchery programs. Greater difficulties were encountered in our attempts at capturing NOR fish, especially steelhead. Although we developed methods that allowed for the collection of gametes from relatively large numbers of NOR Chinook salmon, these methods were not successful in the collection of NOR steelhead. Thus, no predominantly NOR steelhead population has adequate material preserved in the gene bank. Age structure comparisons of Chinook salmon that contributed gametes to the gene bank with that of other NOR and HOR populations revealed similar compositions, indicating that the gamete sampling effort collected a representative sample of males from the spawning population (Young, 2009). For Chinook salmon, HOR collections exhibited a greater percentage of age 3 fish and a lower percentage of age 5 fish compared to NOR populations, similar to that observed in hatcheries and natural populations in the region. Age structure analysis of steelhead populations was not possible due to lack of samples.

Gametes previously collected were sealed in 0.5 ml and 5.0 ml straws and stored submersed in liquid nitrogen. The repository contains gametes from 2,990 unique Chinook salmon (Appendix A) preserved in 85,087 one-half ml straws and 5,529 five ml straws and gametes from 1,403 unique steelhead (Appendix A) preserved in 26,021 one-half ml straws and 279.5 five ml straws (Appendix B). This represented an average of 28.6 and 18.5 one-half ml straws per individual Chinook salmon and steelhead, respectively. Previous research has

determined that a single one-half ml straw can successfully fertilize approximately 400 eggs (unpublished results). Approximately half of the material from each individual was set aside and will comprise a long-term repository, accessible only for significant recovery actions were required. The remaining material is made available for broodstock management or research at regional hatcheries in the Snake River Basin. We also began coordination with the United States Department of Agriculture, National Animal Germplasm Program to transfer approximately half of the samples to their facility in Fort Collins, CO for long-term, secure storage. The transfer will begin in 2010.

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 (Appendix D). The Snake River Germplasm Repository Committee, consisting of NPT, NOAA Fisheries 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.

Management Recommendations

- Maintain WSU and UI repositories.
- Transfer subset of samples to the United States Department of Agriculture, National Animal Germplasm Program for long-term storage.
- Continue coordinating the Snake River Germplasm Repository Committee oversight of the germplasm request and use process.
- Explore opportunities to expand steelhead sample collections in the Snake River basin, especially from NOR populations.
- Continue opportunistic Chinook salmon sample collections.
- Continue to explore alternative ex situ methods, especially related to the preservation of female germplasm.
- Include cryopreservation collections and use guidance in ESA recovery plans.
- Encourage/promote sample collections for populations not currently at risk.

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This report and annual reports from 1997-2007 are available on the Internet through BPA Fish and Wildlife Publications at:

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

APPENDICIES

Appendix A. Gamete samples collected from 1992 through 2008

Table A1. Annual Snake River basin Chinook salmon samples cryopreserved from 1992 through 2008.

Spawning Aggregate	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	Pre- 1995	Totals
Lostine River	3	4	16	14	39	16	19	33	18	2	3	2	3	5	177
Minam River			2	4											6
Grande Ronde River	0	4	13	7	8	10	8	9							59
Catherine Creek	12	13	12	10	7	8	5	11							78
Rapid River									51	68	98				217
SFSR			1	11	15	26	23	44	53	93	45	45	19		375
Lake Creek	35	5	8	20	26	32	18	29	15	6	3	4	3		204
Johnson Creek	22	17	31	48	60	54	57	64	35	5	17	7			417
Big Creek	18	2	9	6	22	31	20	51	7	0	1	6	0	16	189
Capehorn Creek	5	2	1	6	0	15	2	2	1	0	6	2			42
Marsh Creek	17	13	15	6	5	16	33	24	7	0	2	4			142
Elk Creek		1													1
Pahsimeroi River					20	15	39	52	49	31					206
Upper Salmon River		5	13	18	25	20	54	49	40	40	41	51			356
Imnaha River	11		20	12	25	29	7	37	71	94	79	40	33	63	521
Totals	123	66	141	162	252	272	285	405	347	339	295	161	58	84	2990

Table A2. Annual Snake River basin steelhead samples cryopreserved from 1993 through 2008.

Spawning Aggregate	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1994	1993	Totals
Tucannon River	0	11	16	24											51
North Fork Clearwater River							63	81	89	62					295
Selway River													5*		5
Fish Creek							3	1	1					10*	15
Grande Ronde River								1	1						2
South Fork Salmon River	0	1	3	2	24	16									46
Johnson Creek					1			1		2					4
Pahsimeroi River							63	57	40	47					207
Imnaha River									2						2
Little Sheep Creek	0		3	11	101	71	93	77	52	25	25	5			463
Cow Creek		2				2									4
Lightning Creek		2				1									3
Snake River							58	74	98	76					306
Totals	0	16	22	37	126	90	280	292	283	212	25	5	5	10	1,403

*Samples collected by the USGS/ National Biological Survey.

Appendix B. Total number of Chinook salmon and steelhead straws preserved in the gene bank

Table B1. Snake River basin Chinook salmon 0.5 ml and 5.0 ml straws preserved in the genebank from 1992 through 2008.

Location	Unique individual samples	0.5 ml straws	5.0 ml straws	Average # of 0.5 ml straws/ individual
Big Creek	190	5877	240	31.4
Capehorn Creek	41	1263	31	30.1
Catherine Creek	78	1911	45	25.1
Elk Creek	1	20	0	20.0
Grande Ronde River	59	1582	53	26.8
Imnaha River	518	12576	727	24.2
Johnson Creek	413	12669	796	30.7
Lake Creek	203	6151	205	30.3
Lostine River	177	5159	275	29.3
Marsh Creek	143	4271	303	30.1
Minam River	6	180	0	30.0
Pahsimeroi	205	6822	394	33.3
Rapid River	217	5148	191	23.8
SFSR (McCall Hatchery)	376	11130	1201	29.8
upper Salmon (Sawtooth Hatchery)	354	10328	1068	29.3
Totals	2981	85087	5529	28.6

Table B2. Snake River basin steelhead 0.5 ml and 5.0 ml straws preserved in the genebank from 1993 through 2007.

Location	Unique individual samples	0.5 ml straws	5.0 ml straws	Average # of 0.5 ml straws/ individual
Cow Creek	4	77	0	19.3
Dworshak NFH	295	5612	14	19.0
Fish Creek	15	122	111	8.1
Grande Ronde River	2	37	0	18.5
Imnaha River	2	0	0	0.0
Johnson Creek	4	80	8	20.0
Lightning Creek	3	67	0	22.3
Little Sheep Creek	464	8492	2	18.3
Pahsimeroi River	207	4077	53	19.7
Selway River	5	0	32.5	0.0
SFSR	50	1003	0	20.1
Snake River	306	5890	59	19.2
Tucannon River	51	992	0	19.5
Totals	1408	26449	279.5	18.8

Apenndix C. 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).