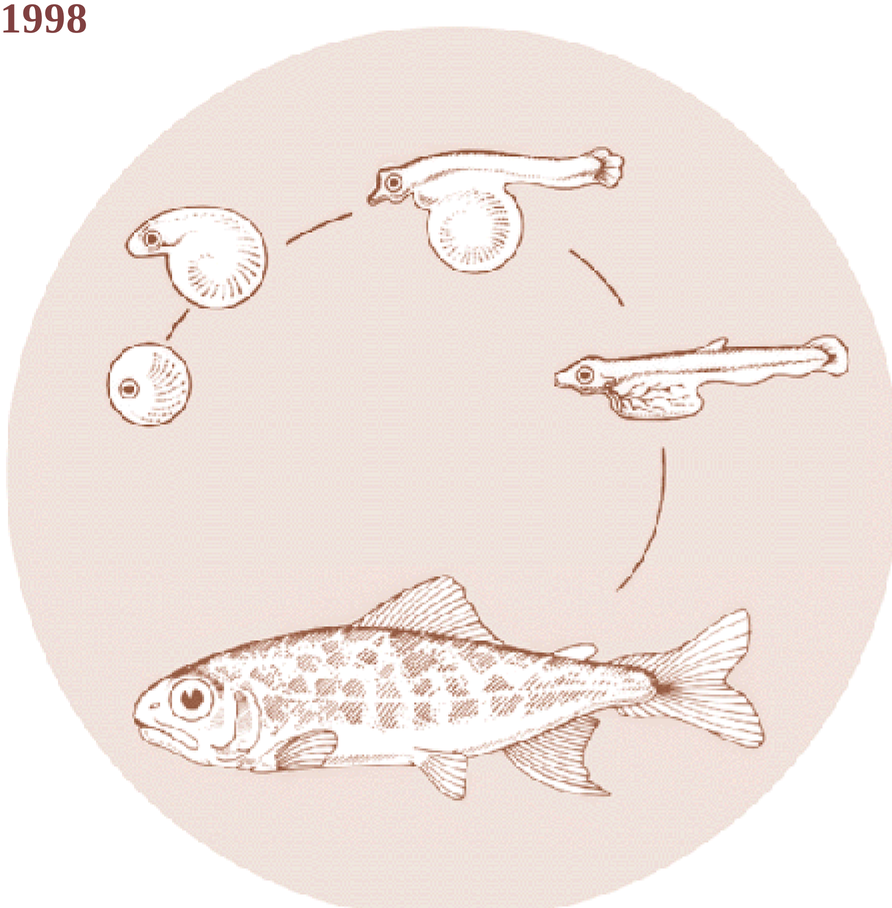
Salmonid Gamete Preservation in the Snake River Basin

Annual Report
1998



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Salmonid Gamete Preservation in the Snake River Basin

1998 Annual Report

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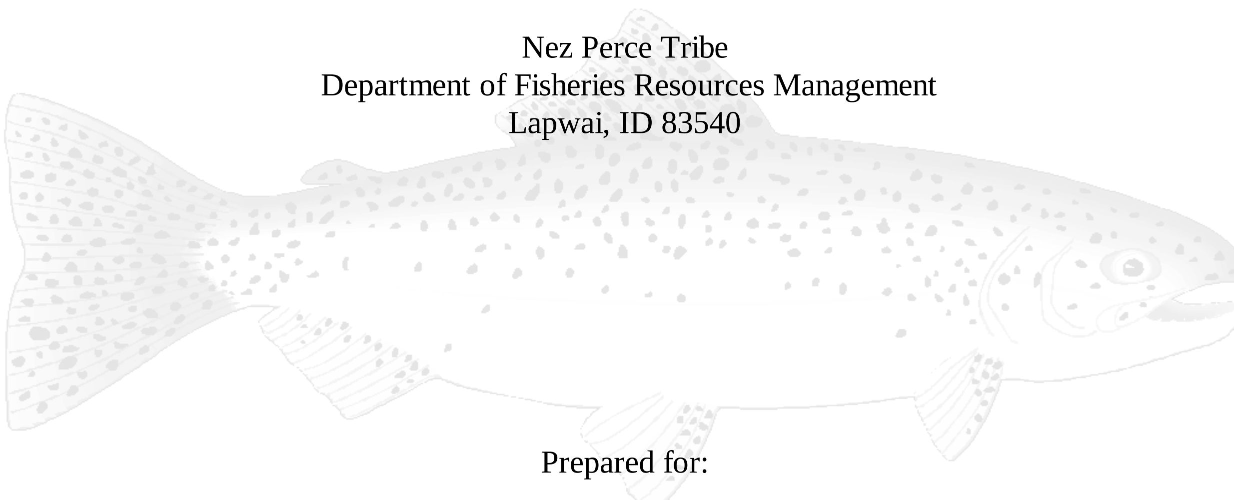

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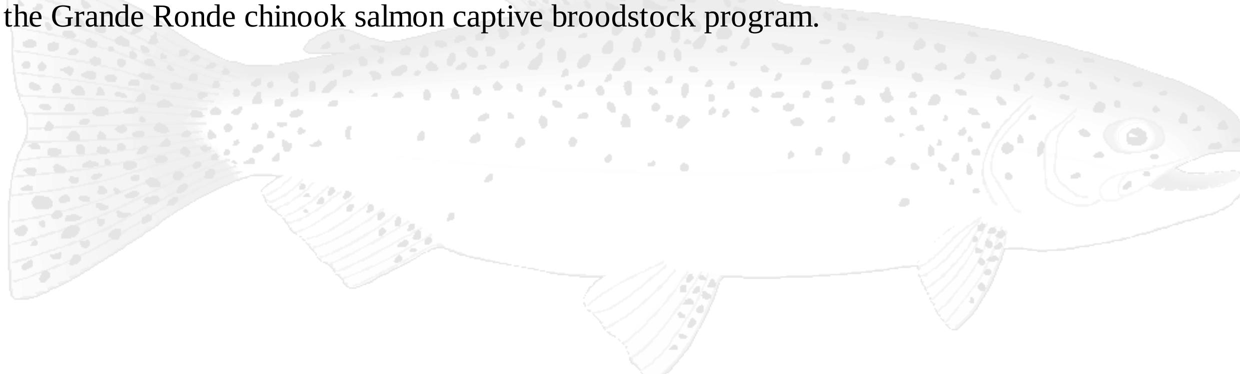
ABSTRACT

Steelhead (Oncorhynchus mykiss) and chinook salmon (Oncorhynchus tshawytscha) populations in the Northwest are decreasing. Detrimental conditions causing these decreases can be improved in some cases, but time is required. The Nez Perce Tribe (Tribe) strives to ensure availability of a representative genetic sample of the original male population by establishing and maintaining a germplasm repository. Our approach has been to sample and preserve chinook salmon genetic diversity within the major river subbasins in the Snake River basin. Gamete cryopreservation permits the creation of a genetic repository, but is not a cure for decreasing fish stock problems. The Tribe was funded in 1998 by the Bonneville Power Administration to coordinate gene banking of male gametes from Endangered Species Act (ESA) listed steelhead and spring and summer chinook salmon in the Snake River basin. In 1998, a total of 295 viable chinook salmon semen cryopreservation samples were taken from the Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River stocks), Rapid River Hatchery and Lookingglass Hatchery (Imnaha River stock). Also, twenty five male steelhead gametes from Little Sheep Creek, a tributary to the Imnaha River, were cryopreserved. A total of 561 cryopreserved samples from Snake River basin steelhead and spring and summer chinook salmon, from 1992 through 1998, are in two independent locations at the University of Idaho and Washington State University. The Tribe's cryopreservation expertise was also called upon to cryopreserve and fertilize gametes for the Grande Ronde chinook salmon captive broodstock program.



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INTRODUCTION

Snake River steelhead (Oncorhynchus mykiss) and spring and summer chinook salmon (Oncorhynchus tshawytscha) spawning aggregates have experienced significant decline in numbers over the past five decades and are now listed as threatened species under the Endangered Species Act. These declines are due to many different factors. Most are the result of human activities.

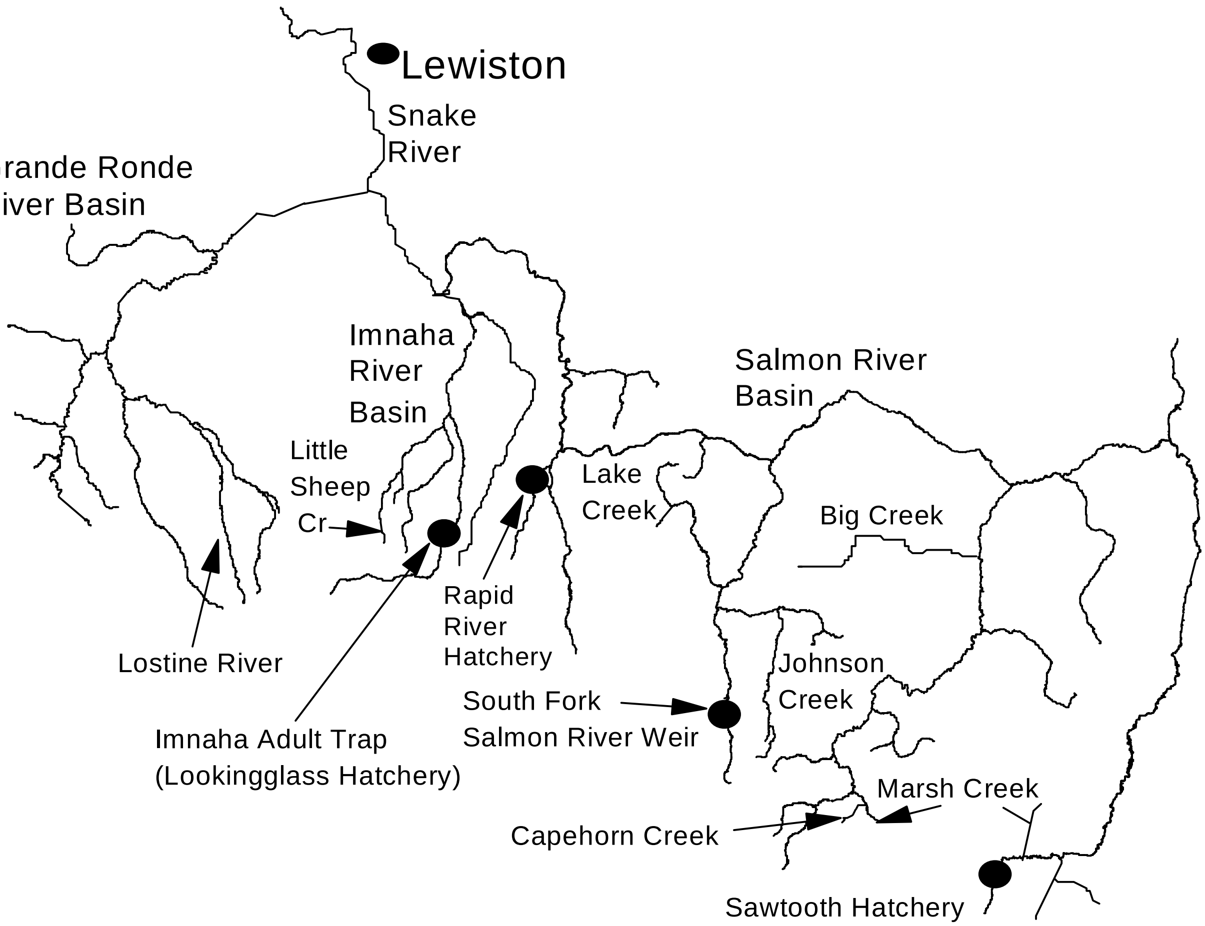
Genetic conservation through population protection and monitoring has not been successful. With the constant threat of losing genetic diversity in specific native fish stocks, the establishment of a program for the long-term storage of fish germplasm serves as insurance against population collapse and extirpation. The Tribe has ensured that a representative genetic sample of the original population exists by establishing a germplasm repository. At present, cryopreservation of semen is the best means of storing fish germplasm for extended periods of time. Cryopreserved salmonid semen will remain viable for an extended time and can be easily shipped. Ashwood-Smith (1980), Whittingham (1980), and Stoss (1983) have estimated the storage time for fish semen held in liquid nitrogen to be between 200 and 32,000 years. This storage period is more than adequate for a germplasm repository. The technology for preservation of the maternal nuclear DNA component is not available to fisheries. Successful research and development to preserve germplasm components from male and female steelhead and chinook salmon would increase future management options.

There are two important factors to be considered when developing a germplasm repository. First, this is a genetic repository and will not solve population problems of a fish stock that is at low levels of abundance and high risk of extirpation. Second, fertility of the stored semen currently is not as great as the fresh semen. The quality of the stored semen is usually a direct reflection of the quality of the sperm that was cryopreserved, and 50 to 80% motility of fresh sperm is considered good. It is desired that fertilization rates using cryopreserved semen in conventional hatchery programs would average 80% or higher. The fertilizing ability of the frozen milt has been tested against controls using fresh milt, and based on the number of eyed eggs, was approximately 60% of that achieved when using fresh milt for Atlantic salmon (Gausen 1991). There is a risk of lower fertilization rates and potential loss of eggs using cryopreserved semen. The Tribe and Washington State University conducted a small-scale fertilization trial this year using non-listed chinook salmon from Leavenworth National Fish Hatchery. The results of this study have not been finalized. Lesser fertilization rates using cryopreserved sperm may be acceptable where genetic concerns warrant them.

The Nez Perce Tribe initiated chinook salmon cryopreservation activities in 1992. The Lower Snake River Compensation Plan hatchery evaluations program, funded through the U. S. Fish and Wildlife Service has provided a valuable, though limited, amount of funding for this chinook salmon effort from 1992 through 1998. The Nez Perce Tribe was funded by Bonneville Power Administration in 1997 and 1998 to coordinate and initiate a more comprehensive gene banking effort. Male steelhead gamete cryopreservation was initiated at Little Sheep Creek in 1998. Goals of the cryopreservation project are: 1) preserve the genetic diversity of listed salmonid populations at high risk of extirpation through application of cryogenic techniques, 2) maintain gene bank locations at independent sites for the short-term, and 3) establish and maintain long-term germplasm repositories.

DESCRIPTION OF PROJECT AREA

Figure 1. Snake River basin chinook salmon and steelhead cryopreservation locations in 1998.



The Nez Perce Tribe's cryopreservation project seeks to preserve Snake River steelhead and spring and summer chinook salmon male gametes. The project area is the Snake River basin (Figure 1). In 1998, the sampling locations included: Lostine River, Big Creek, Johnson Creek, Lake Creek, Little Sheep Creek, Marsh Creek and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River spawning aggregate), Rapid River Hatchery and Lookingglass Hatchery (Imnaha River spawning aggregate). This geographically-large collection area is covered by the fisheries personnel from the McCall and Enterprise field offices as well as the Lapwai office.

METHODS

Fish handling protocol training was provided to all personnel prior to collection of adult male salmon to minimize stress on the fish. Each team member was assigned a specific duty to improve the efficiency of sample collection. The Tribe collected male kelts from the spawning grounds. The salmonid milt was collected either at the hatcheries, or by hand or dip net in the streams.

Redd counts are usually conducted on pre-determined stream reaches before handling any fish. Redd counts also determine where in each stream the collection of adults takes place. Several team members locate salmon being careful not to disturb the fish. Observations are made to visually identify male salmon. Males are identified by secondary sexual characteristics which include a kype (greatly extended, narrowed snout, turned down at tip, also an enlarged lower jaw). Females can be identified by a rounder head, thicker caudal peduncle, and a tattered, discolored caudal fin from digging the redds. No harassment of actively-spawning salmon should occur.

No one enters the water near any existing or active redds (i.e. where salmon are on the nests). A snorkeler enters the water to find solitary males, looking under cut banks, in log jams, in backwater habitats, etc. From the vantage point underwater, this person identifies fish for others to collect. It is easiest to collect the males in a constricted portion of the stream. Depending on the width of the stream, enough dip-netters position themselves downstream from the fish to adequately cover the stream. Several people get in the water upstream of the fish and herd the adult to the passive netters below. All movements should be done quietly and slowly to avoid disturbing the fish, thus making capture easier and less stressful. Any females caught are returned to the water immediately, unharmed, and the capture is recorded. Upon capture of the male, the anaesthetic bath tank is set up and filled. Captured fish are held in the stream before transfer to the anaesthetic bath tank.

All adult male salmon sampled are collected by hand, dip net, or seine in that order of priority:

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

- <u>Dip net.</u> Stay away from active redds. Several dip netters get into position below the fish, with several people in the water upstream of the fish. The upstream people slowly herd fish towards the netters, moving slowly and quietly. Keep the large dip nets in the water in a line and let fish swim into the net. Net holders should be absolutely still as fish approach the nets.

- <u>Seine.</u> Two 5' x 30-40' seine nets are set up perpendicular to the flow of water, blocking a segment of stream. The upstream net is slowly moved downstream, trapping the fish in a corral of decreasing area. Fish are collected with dip nets. If more than one fish is captured, determine which fish are to be sampled and release the others immediately.

Figure 2. Dip netting chinook salmon in Lake Creek.

General anaesthetics first calm the fish, then cause it successively to lose mobility, equilibrium, consciousness, and finally reflex action (Summerfelt and Smith 1990). We wish to immobilize adult male salmon so they can be handled faster and less stressfully. A portable 35 gallon tank is set up along the stream to anaesthetize the male chinook. Two people are assigned to set up and fill the portable tank with seven 5-gallon buckets. Pre-measured Finquel™ tricane methanesulfonate (MS-222) is used to anaesthetize the adult male salmon, along with a sodium bicarbonate ($NaHCO_3$) buffering compound to buffer the acidic effect of the MS-222. It takes 3-5 minutes for the fish to be anaesthetized. It is important to have one person time how long the fish is in the tank, and observe the fish all the time it is undergoing anaesthesia. After the fish is released into the stream, the tank is emptied well away from the stream, so no chemicals are released into the stream proper.

Pre-measured MS-222 was also used to anaesthetize all adult salmon, along with a sodium bicarbonate buffering compound to buffer the acidic effect of the MS-222, with the exception of fish at Rapid River Hatchery, Sawtooth Hatchery and the South Fork Salmon River weir. Fish handling/spawning protocols of Idaho Department of Fish and Game (ID F&G) are used at the Idaho hatcheries, and thus the adults were not anesthetized before semen samples are taken. Imnaha River chinook salmon are anesthetized at Lookingglass Fish Hatchery. Extra care was taken with semen collection to ensure the quality of preserved samples. The abdomen of the anesthetized male chinook salmon was dried to reduce contamination of the semen samples and the milt was stripped. Some of the fish provided only enough semen for cryopreservation at one university. A few males were completely spawned out, so samples could not be obtained.

Fish biological information (fork length and mid-eye to hypural plate length, general condition, external marks) was recorded following semen collection. Caudal fin tissue was collected for genetic (DNA) analysis. Scales were taken for age assessment and scale pattern analysis. Following sampling and data collection, the anesthetized salmon were immediately returned to a slow water area and held until recovered.   When sufficient semen existed, samples were collected in two separate Whirl Pak® bags, and one bag of semen delivered to the University of Idaho and the other to Washington State University for cryopreservation. These samples were frozen in 20 0.5 ml straws if the quantity allowed. Any excess semen was cryopreserved in larger 5.0 ml straws. Semen samples were placed in two separately labeled Whirl Pak bags, aerated, and placed in an insulated cooler, on newspaper over wet ice. There was a protocol change in 1998 from filling the bags with pure oxygen to using air inside the Whirl Paks. This change is based on research conducted by the University of Idaho (Bencic et al 1999).

Samples were flown to the universities on the same day for preservation to ensure the highest

Figure 3. Collecting chinook salmon milt from anaesthetized fish at Big Creek.

quality samples possible. One Whirl Pak® bagged sample was shipped to, and the semen stored at each university as a safeguard to protect against catastrophic events that could destroy all germplasm samples if they were stored at one facility.   Cryopreservation and storage occurred independently at the University of Idaho and Washington State University within a 12-hour period. Both universities started using nitrogen vapor freezing techniques in 1997. Previous to this time, sample freezing occurred on dry ice.

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

Sperm evaluation is an important component of the cryopreservation program in order to cull poor quality sperm samples prior to freezing, and to estimate the post-thaw fertility of the stored sperm. All wild fish semen is stored regardless of motility. Fertility was evaluated by sperm motility, which is the percentage of motile sperm following the addition of a sperm activating solution (Mounib 1978). Motility correlates to post-thaw fertility.
There are four stages in the cooling sequence of cryopreservation of cells (Cloud and Osborne

Figure 4. Nez Perce Tribe Fisheries employee conducting pre-freeze motility estimates on fresh chinook salmon sperm.

1997): 1) Cooling cells to the point of ice formation - this does not appear to be a critical factor in the cryopreservation of salmonid sperm; 2) The formation of ice - the goal at this stage is to have ice form near the freezing point of the extracellular solution; 3) Cooling through the critical period - there is a net movement of water out of the cells as the temperature is constantly being reduced. The cooling rate during this phase needs to be slow enough to allow water to move out of the cells, but it must be fast enough to protect the intracellular environment from the effect of the high salt concentrations. The success of cryopreservation is dependent upon required cryoprotectants (such as dimethyl sulfoxide-DMSO) in the freezing solution. These small compounds enter the cells and protect the cells during dehydration by inhibiting ice formation. The rate at which the sperm is cooled is a critical factor in the success of the cryopreservation process, salmonid sperm optimal cooling rates are -20 to -30° C/minute (Stoss 1983), down to approximately -79° C; 4) Reduction to liquid nitrogen temperature - the frozen milt is then plunged into liquid nitrogen at -196° C.

The amount of sperm cryopreserved varied greatly by individual fish and by species. Chinook salmon produce greater volumes of milt (averaging~15 ml), whereas steelhead produce less

Figure 5. Nitrogen tank at the universities.

(average 2-4 ml) Many of the fish sampled had been actively spawning for several days and sometimes very little or no sperm was available. A sample of 5 ml of semen was sufficient to fill 20 0.5 ml straws, due to the dilution of semen with three parts freezing solution. Depending on the motility of the thawed sperm, one 0.5 ml straw can fertilize up to 450 eggs, and a 5 ml straw can fertilize approximately 2,000 eggs. There is not a direct relationship of straw volume to fertilization capacity due to the heat of fusion and the surface area involved.

RESULTS

Gametes from male chinook salmon were sampled from Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek, Capehorn Creek, the South Fork Salmon River weir, Rapid River Hatchery, Sawtooth Fish Hatchery and Lookingglass Hatchery. Sampling at hatchery facilities has been designed to collect gametes from the spectrum of the run. Male steelhead gametes were collected at an Oregon Department of Fish and Wildlife (ODF&W) weir on Little Sheep Creek , a tributary to the Imnaha River (Table 1).

Table 1. Cryopreserved samples taken from listed Snake River basin steelhead and spring and summer chinook in 1998; dates collected, unmarked (wild/natural) and marked (hatchery) fish numbers, fork length and percent sperm motility.

Spawning Aggregate	Species	Collection Dates	Unmarked Fish	Marked fish	Fork Length (mm)	Sperm Motility (%)
Lostine River	CH	Aug. 26	2	1	705-935	50-90
Little Sheep Creek	ST	April 27		25	not measured	40-90
Imnaha River	CH	Aug.14, 26 & Sept. 2, 9	30	49	520-1045	0-90
Rapid River	CH	Aug.21, 28 & Sept. 4		98	not measured	20-100
S Fork Salmon R.	CH	Aug 11,18,25	5	40	not measured	<5-100
Lake Creek	CH	Aug. 20	3		800-810	50-90
Johnson Creek	CH	Aug. 11 & Sept. 1,3	17		810-1090	10-90
Big Creek	CH	Aug. 18	1		540	80-90
Capehorn Creek	CH	Aug. 14, 19	6		635-1080	<5-50
Marsh Creek	CH	Aug. 14	2		1000-1050	20-80
Upper Salmon River	CH	Aug. 20, 27, & 31	36	5	480-1080	20-90

In 1998, a total of 295 viable chinook salmon semen cryopreservation samples were taken from six different streams in the Snake River Basin and four state hatcheries in Idaho and Oregon. Also, twenty five male steelhead gametes from Little Sheep Creek, a tributary to the Imnaha River, were cryopreserved. A minimum of 20 0.5ml straws were filled with the milt from each fish. Any excess sperm was then frozen in larger 5ml straws. Though it may look like we have a lot of straws frozen, in actuality, we do not have many fish or genetic material cryopreserved (Appendix 1). The germplasm repository inventory is shown in the Appendix 2.

Lostine River

The Lostine River is a tributary to the Wallowa River which empties into the Grande Ronde River. Four male chinook salmon were sampled on August 26; three samples were collected, the fourth male produced no semen. One of the three samples (Table 1) collected in 1998, sample number NPT98-002LR had a poor adipose fin clip, indicating it probably was of hatchery origin (Blenden personal communication). Field efforts were hampered by the refusal of a landowner in the prime spawning area to allow project personnel access to his property. This was the fifth consecutive year of cryopreservation sampling in the Lostine River. A total of 13 cryopreserved semen samples taken from 1994 to 1998 are now in the germplasm repository.

Little Sheep Creek (steelhead)

Steelhead semen was collected at the adult weir on Little Sheep Creek which is operated by the Oregon Department of Fish and Wildlife. Low volume of semen was collected from these recycled steelhead. Because of the small quantity, Washington State University froze the first 12 samples and the University of Idaho cryopreserved the second 13 samples. No lengths, scales nor fin punches were taken.

Lookingglass Fish Hatchery (Imnaha River stock)

Semen was cryopreserved from 79 Imnaha River chinook salmon that were held for spawning at the ODF&W Lookingglass Fish Hatchery. A total of 30 natural and 49 hatchery fish were cryopreserved. Two of these 49 hatchery fish were ventral fin-clipped, which means they are ESA non-listed fish (hatchery crossed with hatchery fish). The chinook males ranged in fork length from 520 to 1,045 mm. A study of the length frequencies is shown in Figure 6 using an n = 71, mean = 761.4, and a standard deviation = 177.8. This was the third year of cryopreservation sampling with a total of 152 cryopreserved semen samples taken from 1996 to 1998.

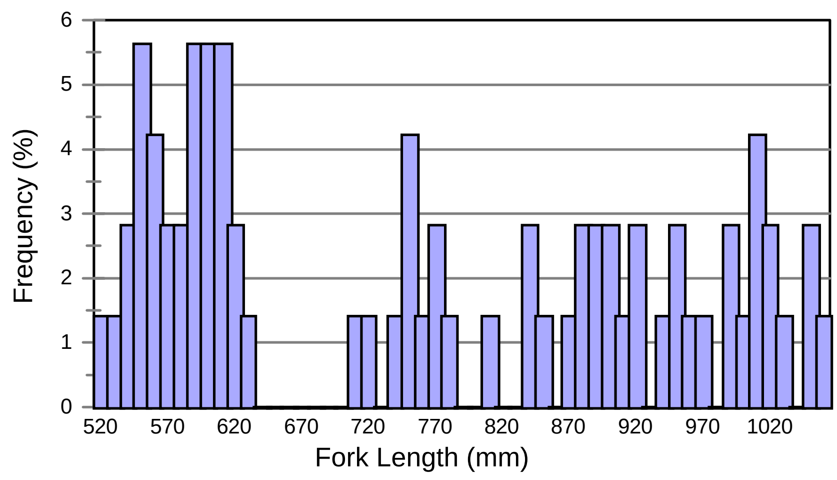


Figure 6. 1998 Imnaha River chinook salmon length frequency.

Rapid River Hatchery

Rapid River hatchery chinook salmon semen was collected at the hatchery starting in 1998. Milt from 98 samples chinook salmon considered excess to the production needs was collected and cryopreserved. These fish are not ESA-listed fish.

South Fork Salmon River (adult weir)

Forty-five fish were sampled over a three-week period of three days at the South Fork Salmon River weir with IDFG hatchery personnel. The breakdown of the origin of these fish is as follows: 32 were adipose fin-clipped (hatchery origin), 5 were natural fish, 7 had a right ventral fin-clip (5-year old) and 1 had a left ventral fin-clip (4-year old). Ventral fin-clipped fish are supplementation fish meaning they are the progeny of a wild and a hatchery parent. This was the third year of cryopreservation sampling at the South Fork Salmon River weir. A total of 109 cryopreserved semen samples taken from 1996 to 1998 are now in storage.

Lake Creek

Three wild fish were sampled in Lake Creek on August 20. In 1998, a total of 44 redds were counted during four passes. Most of the cryopreservation sampling took place in the reach from Willow Creek to Corduroy Creek, where 5 new redds, and 2 carcasses were counted on 8/19. This was the third year of cryopreservation sampling in Lake Creek, a tributary of the Secesh River in the South Fork Salmon River watershed. A total of 10 cryopreserved semen samples taken in 1996, 1997 and 1998 are now in storage.

Johnson Creek

Sixteen wild salmon semen samples were cryopreserved from Johnson Creek in 1998. The Nez Perce Tribe initiated a supplementation program in 1998 on this stream, with a adult weir in place. Six fish were sampled at the South Fork Salmon River weir, where Johnson Creek adults were held and spawned for artificial propagation. Fifteen fish were captured in the stream on September 1 and 3, though semen samples from only ten fish were cryopreserved. Their sizes ranged from 810 to 1,090 mm in fork length. Ninety percent (62 redds were counted by 9/1/98 of the total 69 redds counted on 9/18/98) of the redds constructed in the collection area were completed when cryopreservation collection took place. Johnson Creek is a tributary of the East Fork South Fork Salmon River. This was the second year of cryopreservation sampling in Johnson Creek, for a total of 24 semen samples cryopreserved.

Big Creek

Big Creek, a tributary to the Middle Fork Salmon River, has been sampled for seven years. Only one jack chinook salmon was sample d in Big Creek in 1998. Big Creek experienced three consecutive years (1994-1996) of cohort collapse when samples could not be obtained. A total of 24 cryopreserved semen samples taken in 1992, 1993, 1997 and 1998 are now in storage. Half a dozen females were identified on August 18, 1998. A total of 13 redds were found in the index area and 16 in Smith and Jacob's Ladder Creeks this season.

Capehorn Creek

This was the second year of cryopreservation sampling in Capehorn Creek, a headwater tributary to the Middle Fork Salmon River, and six fish were sampled here in 1998. A total of 39 redds were counted in Capehorn Creek on August 12 and 13, 1998. The Nez Perce Tribe's cryopreservation crew sampled this stream the day after Idaho Department of Fish and Game had their redd count training, after learning the places where active spawning was occurring. There are eight semen samples from the two years of cryopreservation sampling in Capehorn Creek in the gene bank.

Marsh Creek

This was the second year of cryopreservation sampling in Marsh Creek, a headwater stream of the Middle Fork Salmon River. In 1998, two fish were sampled. The Nez Perce Tribe's cryopreservation crew sampled this stream after Idaho Department of Fish and Game conducted their redd count training here and numbers of fish/carcasses, and percent spawning had been estimated. Eleven redds were counted in Marsh Creek in 1998. The sperm of six fish has been cryopreserved in the past two years.

Sawtooth Fish Hatchery (upper Salmon River and East Fork Salmon River stock)

Forty one fish held in the Sawtooth Fish Hatchery from the upper Salmon River were sampled in 1998. Male chinook collected ranged in fork length from 480 to 1080 mm. A study of the length frequencies is shown in Figure 7 using an n = 41, mean = 875.8, and a standard deviation = 156.0. Only 12% of the chinook sampled in 1998 at the Sawtooth Hatchery were of hatchery origin, the rest were natural, whereas 75% of the fish sampled in 1997 were from the hatchery. There are 92 samples from the upper Salmon River spawning aggregate(1997-98) represented in the repository.
The Grande Ronde chinook salmon captive broodstock program started spawning fish from the Lostine River, Catherine Creek and the upper Grande Ronde River this year at Bonneville Fish Hatchery in Oregon. These fish were collected as parr from the stream and captively-reared.

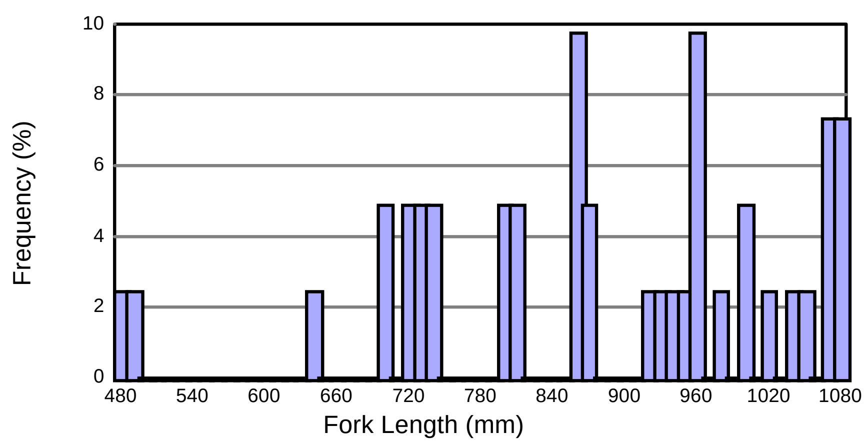


Figure 7. 1998 Salmon River chinook salmon length frequency.

Three different rearing strategies were used: freshwater normal-feeding, freshwater accelerated-feeding and saltwater. The Nez Perce Tribe Fisheries staff was requested by ODFW assist in thawing cryopreserved sperm to fertilize eggs as directed by the spawning matrix. A total of 319 fish (120 females) were spawned which resulted in an estimated 216,000 embryos (Harbeck personal communication). Males that were not used in the spawning were cryopreserved (101 samples were frozen).

Table 2. Cryopreserved samples from Snake River spring and summer chinook and steelhead.

Fertilization experiments were conducted at Washington State University using ESA non-listed

Spawning Aggregate	**Number of Samples Cryopreserved**							**Number Samples /stream**
	1998	**1997**	**1996**	**1995**	**1994**	**1993**	**1992**	
Lostine River	3	2	3	1	4			13
Little Sheep Cr. (steelhead)	25							25
Imnaha River	79	40	33					152
Rapid River	98							98
South Fork Salmon River	45	45	19					109
Lake Creek	3	4	3					10
Johnson Creek	17	7						24
Big Creek	1	6	0	0	0	10	7	24
Capehorn Creek	6	2						8
Marsh Creek	2	4						6
Upper Salmon River	41	51						92
Total number of Samples	320	161	58	1	4	10	7	561

chinook salmon gametes acquired from Leavenworth National Fish Hatchery on August 26, 1998. These small-scale trials are needed to compare fresh and frozen/thawed semen fertilization of eggs. Preliminary results from these studies show an overall low fertilization rate. Fertilization at Leavenworth on the same day our experiment was conducted, showed a 97.6% survival rate from fresh sperm fertilizing fresh eggs (Davis personal communication). Approximately 10% of the eyed eggs have been kept for raising the fish to parr-size for meristic counts of bilateral asymmetry. No results have been finalized yet, but will be reported in the 1999 annual report.

DISCUSSION

Semen samples from 320 wild/natural and hatchery steelhead and spring and summer chinook salmon were collected for cryopreservation in 1998. More semen samples for cryopreservation were collected this year than from the combined efforts of 1992 through 1997 (266). The five hatchery sampling locations provided 288 of the 320 samples for the cryopreservation program in 1998. Of the six stream sites sampled, only Johnson Creek produced more than a minimal sample number, with 17 samples. Adult salmon spawner escapement in 1998 was not strong and a large sample size was not obtained; this could also be attributed to limited time at each stream and the constraint of collecting only spawned-out male fish.

Sustained productivity of salmonids in the Pacific Northwest is possible only if the genetic resources that are the basis of such productivity are maintained (National Research Council 1996). Much of the genetic diversity that historically existed probably has already been lost. The Tribe's germplasm repository is an effort to conserve male genetic diversity that remains in existing salmon runs and steelhead runs for future management options. The spawning aggregates sampled represent only a small portion of the stocks in the Snake River basin. The Nez Perce Tribe has attempted to sample and preserve chinook salmon genetic diversity within the major river subbasins in the Snake River basin. An adequate number of individuals should be sampled from each genetically unique conservation unit to ensure conserving the genetic diversity contained in the runs of chinook salmon and steelhead.

Sampling of male chinook salmon was restricted to the later part of the spawning period to avoid harassing non-spawned-out fish. By limiting the sampling period, the genetic diversity contained in early spawning fish may not be saved in the germ plasm repository. Although it would be very time-consuming and labor-intensive to observe and sample early spawning fish while avoiding harassment of fish that were not yet ready for spawning, it should be investigated.

The urgency to create a germ plasm repository becomes apparent when reviewing the status of the runs (Kucera 1998) and the number of samples being preserved. The number of samples collected in 1998 was minimal. In previous years, such as 1994-1995, fish were not found in some streams for sampling.

All of the 1997 cryopreserved samples stored at WSU were accidentally thawed due to a human error (the lids were left off the freezers). Some of the DNA in the semen has been salvaged for genetic analysis. The duplicate set of those 1997 samples is safely at the University of Idaho. However, cryopreserved semen from 28 chinook samples from 1997 were lost since they did not have a duplicate set at the University of Idaho. A liquid nitrogen tank was installed at Washington State University in 1998 to accommodate more cryopreserved samples. Also, two additional large tanks were purchased for both universities to serve as emergency backups and long-term repositories. Each university now has two large nitrogen storage facilities which are expected to be fully operational in 1999. We plan to move half of those 1997 samples at the University of Idaho to the freezer at Washington State University when both nitrogen tanks are in operation.

Fertilization rates using cryopreserved semen have been highly variable. The Washington Department of Fish and Wildlife observed mean fertilization rates of fall chinook eggs using cryopreseved semen of 50-72% with individual ranges from 6.1-91.5% (Mendel memo 1996). The Nez Perce Tribe found an average fertilization rate of 47.3% (range 19.4-70.4%) using cryopreserved semen from Dworshak Hatchery steelhead. The Tribe also found a mean fertilization rate of 20% (range 0-42.9%) using Imnaha River chinook semen that ODF&W had cryopreserved. The major theme in cryopreservation of milt seems to be much variation between males and low expectation of wholesale success of fertilization. The most important problem in cryopreserving male gametes and the quality of semen probably resides in the physiology of the maturation of spermatozoa within the seminal fluid generated by the sperm ducts (Purdom 1993).

RECOMMENDATIONS

The genetic diversity within existing spawning aggregates is not replace able and should be conserved to protect present and future opportunities, including the evolutionary process in salmon (National Research Council 1996). The recommendation for the gamete preservation project is to cryopreserve gametes from as many genetically diverse conservation units as possible. Coordination with other state and federal agencies can help to identify any unique spawning aggregates. Further genetic analysis may help separate different salmon and steelhead subpopulations.

The DNA from the caudal fin punches taken from most of the fish that the Nez Perce Tribe has cryopreserved the semen from may help to begin to define and separate subpopulations within the Snake River basin. This analysis will indicate if the different spawning aggregates sampled are genetically different enough to be considered subpopulations. This is important because the goal is to preserve genetic diversity among the salmonid subpopulations in the Snake River basin. The relative frequency of polymorphism (number of loci where variation occurs) would help determine the amount of genetic diversity in the population. The Fish Genetics Laboratory at the University of Idaho currently has 264 tissue samples, and genetic analysis is underway and results will be reported next year.

It is anticipated that hatchery programs will be needing to use cryopreserved semen in low return years forecasted for 1999-2000. Similarly, the chinook salmon captive brood program in the Grande Ronde River has requested use of cryopreserved semen as well. At least two types of spawning protocols have been employed in broodstock management: random spawning matrices and those based on genetic selection (dissimilarity matrices). Broodstock management spawning matrices developed using either strategy, within the Snake River basin, must balance genetic diversity and survival expectations relative to current structure of the metapopulation. Further discussion with geneticists is highly recommended before any cryopreserved semen is thawed and used in hatchery production programs.

A genetic matrix will be completed from cryopreserved semen in the germplasm repository to determine lineage. A dissimilarity matrix using DNA analysis will be developed to identify directly related individuals. This matrix would then be available if geneticists determine that a genetic selection mating protocol was preferred.

Criteria for accessing and using cryopreserved semen samples from the germplasm repository are being developed by the Nez Perce Tribe. A central database has been established for inventory purposes.

In the future, we believe that more and more requests will be made for use of cryopreserved semen in hatchery production programs and in research. We recommend and support only the ethical use of cryopreserved genetic material that has been preserved in the germplasm repository.

The Nez Perce Tribe initiated cryopreserving steelhead semen from adults returning in 1998 to Little Sheep Creek, a tributary of the Imnaha River. The Tribe plans to expand steelhead cryopreservation in 1999 include: Dworshak National Fish Hatchery (North Fork Clearwater River); Oxbow Hatchery which spawns Snake River steelhead trapped below Hell's Canyon Dam; Pahsimeroi Hatchery; and Fish Creek, a tributary to the Lochsa River.

It is expected with the listing of bull trout as a threatened species, and the petition to list westslope cutthroat trout that more requests for cryopreserving male gametes from these species will occur. It is wise to move proactively to cryopreserve genetic diversity while the spawning aggregates of these species are relatively healthy instead of reacting to threatened populations levels.

SUMMARY AND CONCLUSION

The cryopreservation project currently has a total of 536 chinook salmon and 25 steelhead semen samples in the germplasm repository at the University of Idaho and Washington State University. A total of 294 viable chinook salmon semen cryopreservation samples were taken in 1998 from the Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek, Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River stock), Rapid River Hatchery, and Lookingglass Hatchery (Imnaha River stock). The first steelhead semen was collected and 25 samples cryopreserved from Little Sheep Creek in Oregon. A germplasm repository for storage of these samples is in place with backup freezer tanks available at each university. The backup freezer tanks act as a safeguard mechanism in case of tank failure within each university, as well as long-term archive storage that is not disturbed.

Semen collection from within a spawning aggregate should continue until sufficient genetic diversity from as many salmonid subpopulations as possible is represented in the germplasm repository. It is estimated that 200 individual samples are needed to establish a breeding program (Cloud personal communication). Fish in some streams such as Big Creek are low in abundance and may require a longer sampling period. Sampling of each spawning aggregate should continue until sufficient genetic material has been acquired. The goal of the gene bank is to have at least 100 samples per year from each location, covering at least five collection years.

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APPENDIX

Appendix 1. Total number of cryopreserved samples taken from Snake River basin spring and summer chinook and steelhead in 1998 and the total number of 0.5 ml and 5.0 ml straws in storage from 1992-1998.

Spawning Aggregate	Total number of 0.5 ml straws, 1998	Total number of 5.0 ml straws,1998	Total number of straws,1992-1998
Lostine River	120	20	669
Little Sheep Creek	255	13	268
Imnaha River	2,225	138	4,344
Rapid River	2,335	54	2,389
South Fork Salmon River	1,565	83	3,087
Lake Creek	100	11	298
Johnson Creek	640	66	852
Big Creek	30	0	787
Capehorn Creek	210	6	263
Marsh Creek	80	5	179
Upper Salmon River	1,500	149	2,806
Totals	9,060	545	15,943

Appendix 2. Adult male chinook salmon and steelhead semen collected from Lostine River, Little Sheep Creek, Lake Creek, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, South Fork of the Salmon River weir, Sawtooth Hatchery (upper Salmon River stock), Rapid River Hatchery and Lookingglass Hatchery (Imnaha River stock); sample identification number, sperm motility, and number and totals of 0.5 ml and 5.0 ml straws cryopreserved at the University of Idaho and Washington State University from 1992-1998.

CHINOOK SALMON AND STEELHEAD CRYOPRESERVATION DATA - 1998

(total number of straws from 1992-1998 for each location is shaded)

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
8/18/98	BIG CREEK	NPT 98-001BC	53	90	20		80	10		
					254	47		472	14	787
8/14/98	CAPE HORN CREEK	NPT 98-001 CH	29	40	20		20	20	2	
8/14/98	CAPE HORN CREEK	NPT 98-002 CH	30	10	20		10	10		
8/14/98	CAPE HORN CREEK	NPT 98-003CH	31	5	20		50	20	3.5	
8/14/98	CAPE HORN CREEK	NPT 98-004 CH	32	10	20		30	20		
8/14/98	CAPE HORN CREEK	NPT 98-005 CH	33	20	20		20	20		
8/14/98	CAPE HORN CREEK	NPT 98-006 CH	57				<5	20		
					100	0		150	13	263
8/14/98	IMNAHA RIVER(LookglassH)	NPT 98-001 IM	25	5	20		80	20	1	
8/14/98	IMNAHA RIVER(LookglassH)	NPT 98-004 IM	26	60	20	4	15	20	5	
8/19/98	IMNAHA RIVER(LookglassH)	NPT 98-005 IM	54	10	20		0	10		
8/19/98	IMNAHA RIVER(LookglassH)	NPT 98-006 IM	55	50	20					
8/19/98	IMNAHA RIVER(LookglassH)	NPT 98-007 IM	56				50	8		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-008 IM	125	90	20	4	50	20		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-009 IM	126	80	20	4	60	20	4	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-010 IM	127	90	20	4	5			
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-011 IM	128	70	20		80	20	2	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-012 IM	129				90	10		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-013 IM	130	90	20	4	80	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-014 IM	131	80	20	4	50	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-015 IM	132	90	20	4	90	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-016 IM	133	80	6					
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-017 IM	134	90	20	4	80	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-018 IM	135	90	20	4	90	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-019 IM	136	50	20	4	70	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-020 IM	137				90	20	2	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-021 IM	138	80	20		90	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-022 IM	139	90	20					
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-023 IM	140				80	10		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-024 IM	141	90	20	4	80	20	5	
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-025 IM	142				90	10		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-026 IM	143	90	19					
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-029 IM	145	90	20	2				
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-030 IM	146	90	20		90	10		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-031 IM	147	90	20	4	80	20		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-032IM	148	90	20		80	20		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-033IM	149				80	20		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-034IM	150	90	10					
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-035IM	151				90	10		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-037IM	152	60	10		90	20		
8/26/98	IMNAHA RIVER(LookglassH)	NPT 98-039IM	153	80	20					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-040IM	225	80	20	4	70	20		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-041IM	226	50	20	5	50	20		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-042IM	227	80	20	5	20			
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-043IM	228	80	20	5	80	20	5	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-044IM					80	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-045IM	230	90	20					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-046IM	231	80	20	5	80	20	5	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-047IM	232				90	20		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-048IM	233	50	20					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-049IM					80	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-050IM	235	90	10					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-051IM					80	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-052IM	237	90	10					

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-053IM	238	80	20	5	80	20	1	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-054IM	239	90	20		90	20	1	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-055IM	240	90	20	5	70	20	2	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-057IM	242	90	20		50	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-058IM	243	70	20	5	70	20	3	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-059IM	244	90	20	5	50	20	1	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-060IM					80	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-061IM	246	90	20					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-062IM	247	90	20		90	10		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-063IM	248	80	20	3	40	20	2	
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-064IM	249	90	20	3	50	20		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-065IM					60	9		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-066IM	251	90	20		70	19		
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-067IM	252	90	10					
9/2/98	IMNAHA RIVER(LookglassH)	NPT 98-068IM					90	19		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-069IM	294	80	10		60	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-070IM	295	80	20	2	70	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-071IM	296	80	20	2	80	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-072IM	297	80	20		90	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-073IM	298	90	20	2	40	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-074IM	299	90	10					
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-075IM	300	90	20	2	50	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-076IM	301				70	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-077IM	302	50	20					
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-078IM	303	80	20	4	80	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-079IM	304	80	20	2	50	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-080IM	305				50	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-081IM	306	70	20	5	20	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-082IM	307	80	20	4	80	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-083IM	308	80	15					
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-084IM	309	80	20	4	20	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-085IM	310	40	20	5	50	20		
9/9/98	IMNAHA RIVER(LookglassH)	NPT 98-086IM	311	80	20	2				
					1770	134		2302	138	4344
8/11/98	JOHNSON CREEK	NPT 98-001JC	13	70	39					
8/11/98	JOHNSON CREEK	NPT 98-002JC	14	90	40					
8/11/98	JOHNSON CREEK	NPT 98-003JC	16	60	40					
8/11/98	JOHNSON CREEK	NPT 98-004JC	18	80	40					
8/11/98	JOHNSON CREEK	NPT 98-005JC	19	10	40					
8/11/98	JOHNSON CREEK	NPT 98-006JC	21	60	40					
9/1/98	JOHNSON CREEK	NPT 98-007JC	222		0		50	20		
9/1/98	JOHNSON CREEK	NPT 98-008JC	223	80	20	5	90	20	3	
9/1/98	JOHNSON CREEK	NPT 98-009JC	224	70	20	5	50	20	5	
9/3/98	JOHNSON CREEK	NPT 98-010JC	255	90	20	3	90	20	5	
9/3/98	JOHNSON CREEK	NPT 98-011JC	256	60	20	3	50	20		
9/3/98	JOHNSON CREEK	NPT 98-012JC	257	80	20	4	90	20	5	
9/3/98	JOHNSON CREEK	NPT 98-013JC	258	80	20	5	90	20	5	
9/3/98	JOHNSON CREEK	NPT 98-014JC	259	80	20	2	0			
9/3/98	JOHNSON CREEK	NPT 98-015JC	260	90	20	2	80	20	4	
9/3/98	JOHNSON CREEK	NPT 98-016JC	261	90	20	4	90	20	2	
9/3/98	JOHNSON CREEK	NPT 98-017JC	262	70	20	2	90	21	2	
					439	35		331	47	852
8/20/98	LAKE CREEK	NPT 98-001 LC	62	90	20		70	20	2	
8/20/98	LAKE CREEK	NPT 98-002 LC	63	80	20	2	50	20	5	
8/20/98	LAKE CREEK	NPT 98-003 LC	64				90	20	2	
					89	2		195	12	298
4/27/98	LITTLE SHEEP CREEK	NPT 98-001 LSC	1	90	11					
4/27/98	LITTLE SHEEP CREEK	NPT 98-002 LSC	2	90	15					
4/27/98	LITTLE SHEEP CREEK	NPT 98-003 LSC	3	90	15					
4/27/98	LITTLE SHEEP CREEK	NPT 98-004 LSC	4	90	20					
4/27/98	LITTLE SHEEP CREEK	NPT 98-005 LSC	5	90	15					
4/27/98	LITTLE SHEEP CREEK	NPT 98-006 LSC	6	90	13					
4/27/98	LITTLE SHEEP CREEK	NPT 98-007 LSC	7	80	14					
4/27/98	LITTLE SHEEP CREEK	NPT 98-008 LSC	8	80	5					
4/27/98	LITTLE SHEEP CREEK	NPT 98-009 LSC	9	80	9					
4/27/98	LITTLE SHEEP CREEK	NPT 98-0010 LSC	10	80	15					
4/27/98	LITTLE SHEEP CREEK	NPT 98-0011 LSC	11	80	10					
4/27/98	LITTLE SHEEP CREEK	NPT 98-0012 LSC	12	80	13					
4/27/98	LITTLE SHEEP CREEK	NPT 98-0013 LSC	13				50	5	4	

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
4/27/98	LITTLE SHEEP CREEK	NPT 98-0014 LSC	14				70	8	2	
4/27/98	LITTLE SHEEP CREEK	NPT 98-0015 LSC	15				70	10		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0016 LSC	16				50	10		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0017 LSC	17				50	10		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0018 LSC	18				80	10		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0019 LSC	19				80		4	
4/27/98	LITTLE SHEEP CREEK	NPT 98-0020 LSC	20				80	9	1	
4/27/98	LITTLE SHEEP CREEK	NPT 98-0021 LSC	21				70	5		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0022 LSC	22				70	20		
4/27/98	LITTLE SHEEP CREEK	NPT 98-0023 LSC	23				70	4	1	
4/27/98	LITTLE SHEEP CREEK	NPT 98-0024 LSC	24				70	2	1	
4/27/98	LITTLE SHEEP CREEK	NPT 98-0025 LSC	25				40	7	0	
					155			100	13	268
8/26/98	LOSTINE RIVER	NPT 98 001 LR	122	90	20	4	80	20	5	
8/26/98	LOSTINE RIVER	NPT 98 002 LR	123	80	20	4	80	20	5	
8/26/98	LOSTINE RIVER	NPT98-003 LR	124	90	20	2	50	20	4	
					243	24		378	24	669
8/14/98	MARSH CREEK	NPT98-001MC	27	40	20		80	20	5	
8/14/98	MARSH CREEK	NPT98-002 MC	28	50	20		20		20	
					40	0		120	19	179
8/21/98	RAPID RIVER (hatchery)	NPT98-001 RR	65				90	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-002 RR	66				90	20	5	
8/21/98	RAPID RIVER (hatchery)	NPT98-003 RR	67				90	3		
8/21/98	RAPID RIVER (hatchery)	NPT98-005 RR	68				80	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-006 RR	69				90	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-007 RR	70				90	20		
8/21/98	RAPID RIVER (hatchery)	NPT98-008 RR	71				90	20	1	
8/21/98	RAPID RIVER (hatchery)	NPT98-010 RR	72				90	14		
8/21/98	RAPID RIVER (hatchery)	NPT98-011 RR	73				90	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-012 RR	74				80	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-013 RR	75				50	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-014 RR	76				100	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-015 RR	77				80	20		
8/21/98	RAPID RIVER (hatchery)	NPT98-016 RR	78				80	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-018 RR	79				70	40	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-019 RR	80				100	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-020 RR	81				100	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-021 RR	82				90	20	1	
8/21/98	RAPID RIVER (hatchery)	NPT98-022 RR	83				90	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-023 RR	84				100	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-024 RR	85				100	20		
8/21/98	RAPID RIVER (hatchery)	NPT98-025 RR	86				100	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-026 RR	87				100	14		
8/21/98	RAPID RIVER (hatchery)	NPT98-027 RR	88				90	20		
8/21/98	RAPID RIVER (hatchery)	NPT98-028 RR	89				90	7		
8/21/98	RAPID RIVER (hatchery)	NPT98-029 RR	90				90	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-030 RR	91				90	7		
8/21/98	RAPID RIVER (hatchery)	NPT98-031 RR	92				100	20	4	
8/21/98	RAPID RIVER (hatchery)	NPT98-032 RR	93				90	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-033 RR	94				70	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-034 RR	95				50	5		
8/21/98	RAPID RIVER (hatchery)	NPT98-035 RR	96				80	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-036 RR	97				100	7		
8/21/98	RAPID RIVER (hatchery)	NPT98-037 RR	98				70	20	2	
8/21/98	RAPID RIVER (hatchery)	NPT98-038 RR	99				80	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-039 RR	100				100	10		
8/21/98	RAPID RIVER (hatchery)	NPT98-040 RR	101				100	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-041 RR	172	80	10					
8/28/98	RAPID RIVER (hatchery)	NPT98-042 RR	173	70	20		80	8		
8/28/98	RAPID RIVER (hatchery)	NPT98-043 RR	174	80	20		90	14		
8/28/98	RAPID RIVER (hatchery)	NPT98-044 RR	175	90	20		90	13		
8/28/98	RAPID RIVER (hatchery)	NPT98-045 RR	176				90	4		
8/28/98	RAPID RIVER (hatchery)	NPT98-046 RR	177WSU	90	10					
8/28/98	RAPID RIVER (hatchery)	NPT98-047 RR	177UI, 178WSU	70	10		80	15		
8/28/98	RAPID RIVER (hatchery)	NPT98-048 RR	178				90	4		
8/28/98	RAPID RIVER (hatchery)	NPT98-049 RR	180	90	10					
8/28/98	RAPID RIVER (hatchery)	NPT98-050 RR	181	90	20		90	7		
8/28/98	RAPID RIVER (hatchery)	NPT98-051 RR	182	90	20		90	16		

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
8/28/98	RAPID RIVER (hatchery)	NPT98-052 RR	183	80	10		80	5		
8/28/98	RAPID RIVER (hatchery)	NPT98-053 RR	184WSU	80	10					
8/28/98	RAPID RIVER (hatchery)	NPT98-054 RR	184UI, 185WSU	90	10		90	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-055 RR	186	90	20		80	20		
8/28/98	RAPID RIVER (hatchery)	NPT98-056 RR	187	60	10		90	20		
8/28/98	RAPID RIVER (hatchery)	NPT98-057 RR	188	80	10		70	20		
8/28/98	RAPID RIVER (hatchery)	NPT98-058 RR	189	90	10		90	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-059 RR	190	90	20		70	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-060 RR	191	90	10		70	9		
8/28/98	RAPID RIVER (hatchery)	NPT98-061 RR	192	80	20		90	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-062 RR	193				80	12		
8/28/98	RAPID RIVER (hatchery)	NPT98-063 RR	194	90	10		90	19		
8/28/98	RAPID RIVER (hatchery)	NPT98-064 RR	195	90	20		90	20	3	
8/28/98	RAPID RIVER (hatchery)	NPT98-065 RR	196	90	20		80	16		
8/28/98	RAPID RIVER (hatchery)	NPT98-066 RR	197	90	20		90	14		
8/28/98	RAPID RIVER (hatchery)	NPT98-067 RR	198	90	20		80	20	1	
8/28/98	RAPID RIVER (hatchery)	NPT98-068 RR	199	90	20		70	20	1	
8/28/98	RAPID RIVER (hatchery)	NPT98-069 RR	200	90	10		80	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-070 RR	201UI	60	20		90	10		
8/28/98	RAPID RIVER (hatchery)	NPT98-071 RR	202UI	40	10		90	10		
9/4/98	RAPID RIVER (hatchery)	NPT98-072 RR	263	50	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-073 RR	264				20	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-074 RR	265	80	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-075 RR	266	80	20		90	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-076 RR	267	50	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-077 RR	268	90	20		90	20	5	
9/4/98	RAPID RIVER (hatchery)	NPT98-078 RR	269	80	20		90	20	2	
9/4/98	RAPID RIVER (hatchery)	NPT98-079 RR	270	80	20		90	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-080 RR	271	90	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-081 RR	272	80	20		50	16		
9/4/98	RAPID RIVER (hatchery)	NPT98-082 RR	273	80	20		90	20	2	
9/4/98	RAPID RIVER (hatchery)	NPT98-083 RR	274	90	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-085 RR	276	90	20		90	20	1	
9/4/98	RAPID RIVER (hatchery)	NPT98-086 RR	277	80	20		70	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-087 RR	278	90	20		90	20	4	
9/4/98	RAPID RIVER (hatchery)	NPT98-088 RR	279	70	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-089 RR	280	80	20		90	20	5	
9/4/98	RAPID RIVER (hatchery)	NPT98-090 RR	281	90	20		50	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-091 RR	282				30	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-092 RR	283	80	20		70	20	4	
9/4/98	RAPID RIVER (hatchery)	NPT98-093 RR	284	90	20		30	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-094 RR	285	70	20					
9/4/98	RAPID RIVER (hatchery)	NPT98-095 RR	286	90	20		90	20	1	
9/4/98	RAPID RIVER (hatchery)	NPT98-096 RR	287				70	16		
9/4/98	RAPID RIVER (hatchery)	NPT98-097 RR	288	90	20					
9/4/98	RAPID RIVER (hatchery)	NPT98-098 RR	289	90	20		70	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-099 RR	290	90	20		70	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-100 RR	291	90	20		90	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-101 RR	292	90	20		30	20		
9/4/98	RAPID RIVER (hatchery)	NPT98-102 RR	293				90	20		
					940			1395	54	2389
8/20/98	SALMON RIVER (Sawtooth)	NPT 98-001 SR	58	80	20	2	50	20	4	
8/20/98	SALMON RIVER (Sawtooth)	NPT 98-002 SR	59	80	20	2	70	20	5	
8/20/98	SALMON RIVER (Sawtooth)	NPT 98-003 SR	64,60	80	20	5	20	20		
8/20/98	SALMON RIVER (Sawtooth)	NPT 98-004 SR	61	80	20	5	50	20	3	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-005 SR	154	80	20	2	90	20	5	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-006 SR	155	90	20	2	90	20	1	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-007 SR	156	90	20	2	90	20	2	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-008 SR	157	90	20		95	20	2	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-009 SR	158	90	5		90	20		
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-010 SR	159	80	20		50	20		
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-011 SR	160	70	20		50	20		
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-012 SR	161	90	20		80	20	5	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-013 SR	162	90	20		70	20	5	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-014 SR	163	80	20		90	20	3	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-015 SR	164	90	20		80	20		
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-016 SR	165	90	20	2	70	20	5	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-017 SR	166	90	20		90	20		

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-018 SR	168UI, 167WSU	80	20	2	80	20	3	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-019 SR	167WSU	90	20					
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-020 SR	169	90	20		60	20	5	
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-021 SR	170	90	20		70	10		
8/27/98	SALMON RIVER (Sawtooth)	NPT 98-022 SR	171	90	20		80	20	2	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-023 SR	203				90	20	2	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-024 SR	204	90	20		80	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-025 SR	205	80	20		50	10		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-026 SR	206	70	20		70	20	5	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-027 SR	207	90	20	4	50	10		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-028 SR					50	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-029 SR	209	80	20	5	80	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-030 SR	210	90	20	2	80	20	3	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-031 SR	211	90	5					
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-032 SR	212	90	20		80	20	3	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-033 SR	213	70	20		90	20	2	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-034 SR	214	80	20	4	90	20	5	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-035 SR	215	80	20	4	50	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-036 SR	216	70	20	5	30	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-037 SR	217	80	20		90	20	5	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-038 SR	218	90	20		90	20	5	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-039 SR	219	90	20	2	80	20	5	
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-040 SR	220	90	20	2	50	20		
8/31/98	SALMON RIVER (Sawtooth)	NPT 98-041 SR	221	90	20	2	80	20	5	
					750	59		1760	237	2806
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-002SF	15	90	40					
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-004SF	17	90	40					
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-005SF	22	90	10					
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-007SF	23	50	40					
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-008SF	20	90	40					
8/11/98	SOUTH FORK SALMON (weir)	NPT 98-009SF	24	20	40					
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-012SF	34	80	20					
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-014SF	35				70	14		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-015SF	36	90	20					
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-016SF	37	80	20		80	15		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-017SF	38	90	20	2	80	20	4	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-018SF	39	90	20	2	50	20	2	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-019SF	40	90	20		80	20	5	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-020SF	41	80	20		80	14		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-021SF	42	70	20	2	30	20	1	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-022SF	43				80	10		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-023SF	44	90	20		50	20	4	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-024SF	45	80	20		50	10		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-025SF	46	40	20		<5	20		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-026SF	47	90	20		10	15		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-027SF	48	90	20					
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-028SF	49				80	10		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-029SF	50	50	20		50	20	3	
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-030SF	51	80	20		70	10		
8/18/98	SOUTH FORK SALMON (weir)	NPT 98-031SF	52	80	15		80	10		
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-032SF	102	80	20		90	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-033SF	103	80	20		90	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-034SF	104	90	20		90	20	2	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-035SF	105	90	20		30	20		
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-036SF	106	90	20		90	12		
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-037SF	107	90	20		90	20	3	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-039SF	108	90	20		90	20	2	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-040SF	109	80	20		90	20	2	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-041SF	110	70	20		90	20	2	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-042SF	111	90	20		80	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-043SF	112	70	20		80	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-044SF	113	80	20		80	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-045SF	114	80	20		100	20		
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-046SF	115	90	20		90	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-047SF	116	80	20		90	20	3	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-048SF	117	90	20		90	20	1	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-049SF	118	80	20		90	20		
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-050SF	119	90	20		90	20	5	

DATE	STREAM	STREAM ID. #	STRAW #	WSU % SPERM MOTILITY	WSU STRAW 0.5 ML	WSU STRAW 4.0 ML	UNIV-ID % SPERM MOTILITY	UNIV-ID STRAW 0.5 ML	UNIV-ID STRAW 4.0 ML	TOTAL # STRAWS
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-051SF	120	80	20		90	20	5	
8/25/98	SOUTH FORK SALMON (weir)	NPT 98-052SF	121	80	20		90	20	3	
					1200	6		1744	137	3087
Total number of cryopreserved semen straws in universities repositories as of 12/28/98 ---										15943