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Salmonid Gamete Preservation in the Snake River Basin

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SALMONID GAMETE PRESERVATION
IN THE SNAKE RIVER BASIN

2001 Annual Report

Prepared by:

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ABSTRACT

Steelhead (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus tshawytscha*) populations in the Northwest are decreasing. Genetic diversity is being lost at an alarming rate. Along with reduced population and genetic variability, the loss of biodiversity means a diminished environmental adaptability. The Nez Perce Tribe (Tribe) strives to ensure availability of genetic samples of the existing male salmonid population by establishing and maintaining a germplasm repository. The sampling strategy, initiated in 1992, has been to collect and preserve male salmon and steelhead genetic diversity across the geographic landscape by sampling within the major river subbasins in the Snake River basin, assuming a metapopulation structure existed historically. Gamete cryopreservation conserves genetic diversity in a germplasm repository, but is not a recovery action for listed fish species.

The Tribe was funded in 2001 by the Bonneville Power Administration (BPA) and the U.S. Fish and Wildlife Service Lower Snake River Compensation Plan (LSRCP) 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 2001, a total of 398 viable chinook salmon semen samples from the Lostine River, Catherine Creek, upper Grande Ronde River, Lookingglass Hatchery (Imnaha River stock), Lake Creek, the South Fork Salmon River weir, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, Pahsimeroi Hatchery, and Sawtooth Hatchery (upper Salmon River stock) were cryopreserved. Also, 295 samples of male steelhead gametes from Dworshak Hatchery, Fish Creek, Grande Ronde River, Little Sheep Creek, Pahsimeroi Hatchery and Oxbow Hatchery were also cryopreserved. The Grande Ronde chinook salmon captive broodstock program stores 680 cryopreserved samples at the University of Idaho as a long-term archive, half of the total samples. A total of 3,206 cryopreserved samples from Snake River basin steelhead and spring and summer chinook salmon, from 1992 through 2001, are stored in two independent locations at the University of Idaho (UI) and Washington State University (WSU). Two large freezer tanks are located at each university.

Recommendations for future gene banking efforts include the need for establishment of a regional genome resource bank, an emphasis on cryopreserving wild unmarked fish, continued fertility trials, and genetic analysis on all fish represented in the germplasm repository.

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INTRODUCTION

Three hundred and three freshwater fish species are present in the United States, 37% of which are at risk of extinction; 17 species have already gone extinct, mostly in this century (The Nature Conservancy 2000). At least 106 major populations of salmon and steelhead on the west coast of the United States have been extirpated, and an additional 214 salmon, steelhead, and sea-run cutthroat trout stocks are at risk of extinction (Nehlsen et al. 1991). Snake River spring and summer chinook salmon and steelhead are at high risk of extirpation with natal stream escapements of less than 200 fish, sometimes with only a few adults returning (Slaney et al. 1996). These stocks probably cannot be effectively replaced by the introduction of fish from other stocks because they are thought to be highly adapted to their spawning sites through natural genetic selection (Corley-Smith and Brandhorst 1999). These species are now listed as threatened under the ESA. In addition to the Snake River fish, a number of populations of salmonids within the Columbia River basin are at risk. With decreases in population sizes over time, the genetic diversity of these populations is declining. “The most fundamental derivative of a species is the information in its genome, currently most easily preserved in the form of DNA” (Ryder et al. 2000). Whether a population is at risk at present or not, the time to act to conserve the unique genetics of these different populations is now.

Extinction of a biological unit is irreversible because it involves the permanent loss of genetic resources capable of regenerating that unit; therefore, a program aimed at avoiding extinction must focus on conserving genetic resources (Waples 1995). 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 the preservation of genetic diversity through cryopreservation of male gametes and development of 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 long periods 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.

Although preservation of the maternal nuclear DNA component has been accomplished with some mammals (Rall and Fahy 1985, Fahning and Garcia 1992, Dobrinsky et al. 1991, Ali and Shelton 1993, Kono et al. 1988, Trounson and Mohr 1983, Hayashi et al. 1989), no similar techniques exist for fish species. Successful research and development to preserve germplasm components from female salmonids 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 localized extinction. 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% motility of fresh sperm is considered good. There is a risk of lower fertilization rates and potential loss of eggs using cryopreserved semen.

The Nez Perce Tribe initiated chinook salmon cryopreservation activities in 1992 (Armstrong and Kucera 2000). The Lower Snake River Compensation Plan hatchery evaluation program funding through the U.S. Fish and Wildlife Service has provided a valuable, though limited, amount of financial support for this effort from 1992 through 2001. Bonneville Power Administration funded the Nez Perce Tribe in 1997 to coordinate and initiate a more comprehensive gene banking effort (Faurot et al. 1998). More extensive male steelhead gamete cryopreservation was initiated in 1999 (Armstrong and Kucera 1999). Annual reports from 1997 through 2000 are on line at the BPA web site.

Goals of the cryopreservation project are: 1) preserve the genetic diversity of listed salmonid populations at high risk of extirpation through application of cryogenic techniques, 2) maintain gene bank locations at independent sites for the short-term, and 3) establish and maintain a long-term regional germplasm repository.

DESCRIPTION OF PROJECT AREA

The cryopreservation project currently seeks to preserve male spring and summer chinook salmon and steelhead gametes. The project area is the Snake River basin (Figure 1). In 2001, the sampling locations by subbasin included: Dworshak National Fish Hatchery, Fish Creek (Clearwater River subbasin); Lostine River, Catherine Creek, upper Grande Ronde River (Grande Ronde River subbasin); Lake Creek, Johnson Creek, South Fork Salmon River (SFSR), Big Creek, Capehorn Creek, Marsh Creek, Pahsimeroi Hatchery, upper Salmon River spawning aggregate at Sawtooth Hatchery (Salmon River subbasin); Imnaha River, Imnaha River spawning aggregate at Lookingglass Hatchery, Little Sheep Creek (Imnaha River subbasin); and Oxbow Hatchery (Snake River basin).

The Sweetwater Research Headquarters Office and Enterprise Field Offices cost share fisheries personnel as well as the McCall Field Office to successfully begin to cover this geographically large collection area.

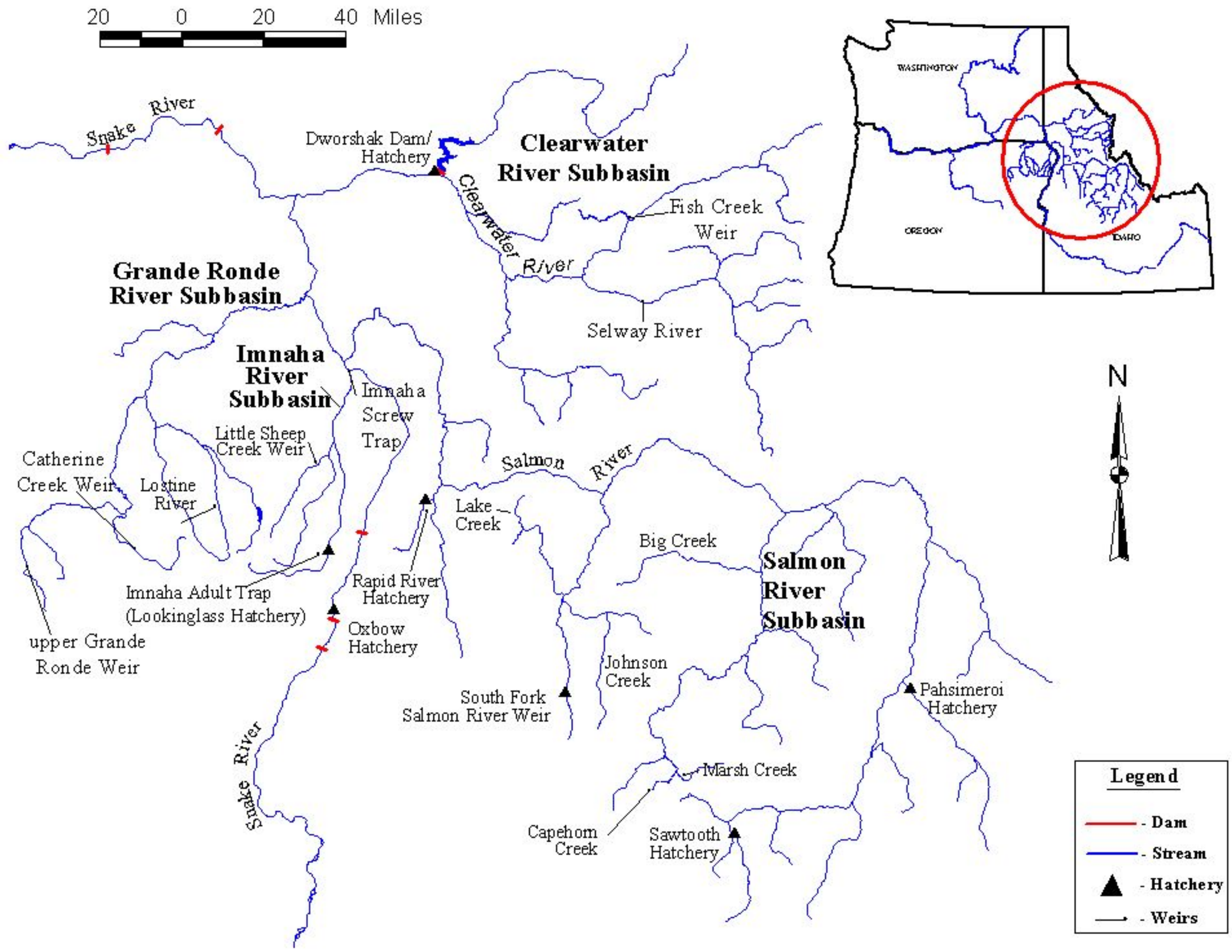

Figure 1. Snake River basin chinook salmon and steelhead cryopreservation locations in 2001.

METHODS

Fish handling protocol training was provided to all personnel prior to collection of adult male salmonids to minimize stress on the fish. Each team member was assigned a specific duty to improve the efficiency of sample collection. Semen from male kelts was collected from fish on the spawning grounds or from hatchery holding ponds. Fish were captured either by hand or dip net in the streams and sampled for sperm.

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

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 logjams, 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. Any females caught are returned to the water immediately, unharmed, and the capture is recorded.

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

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

Dip net. 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. Keep the large dip nets in the water in a line and let fish swim into the net. Net holders should be absolutely still as fish approach the nets.

Seine. Two 5' x 30-40' seine nets are set up perpendicular to the flow of water, blocking a segment of stream. The upstream net is moved slowly downstream, trapping the fish in a corral of decreasing area. 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.

Upon capture of the male, the anesthetic bath tank is set up and filled. Captured fish are held in the stream before transfer to the anesthetic bath tank. General anesthetics 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 male chinook. Two people set up and fill the portable tank with 35 gallons of water. Pre-measured Finquel™, tricane methanesulfonate (MS-222), is used to anesthetize the adult male salmon, with the exception of hatchery fish. Sodium bicarbonate ($NaHCO_3$) is used to buffer the acidic effect of the MS-222. It takes 1-3 minutes for the fish to be anesthetized. 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 anesthesia. Fish handling/spawning protocols of Idaho Department of Fish and Game (IDFG) are used at the Idaho hatcheries, and the adults are not anesthetized before semen samples are taken. Imnaha River chinook salmon are anesthetized at Lookingglass Hatchery. Extra care is taken with semen collection to ensure the quality of preserved samples. The sedated fish is rinsed in the fresh water of the stream before milt is collected. The abdomen of the anesthetized male chinook salmon is dried to reduce contamination of the semen samples and the milt is sampled (Figure 2).

Figure 2. Collecting chinook salmon milt from anaesthetized fish at Big Creek.

Semen samples were placed in two separately labeled Whirl Pak bags, aerated, and placed in an insulated cooler, on newspaper over wet ice. Some of the fish provide only enough semen for cryopreservation at one university. A few males are completely spawned out, so samples are not obtained. Fish biological information (fork length and mid-eye to hypural plate length, general condition, external marks) was recorded following semen collection (Figure 3). Stream water is gently poured over the salmon's head and gills to start the recovery from the MS-222 and reduce stress on the fish. Caudal fin tissue was collected and preserved in ethyl alcohol for later 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 assisted until recovered. 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.

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

Semen samples are shipped to, cryopreserved and 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 occur independently at UI and WSU within a 12-hour period. Both universities started using nitrogen vapor freezing techniques in 1997. These samples were frozen in 20 0.5 mL French straws (IMV International, Minneapolis, Minnesota) if the quantity allowed. Any excess semen was cryopreserved in larger 5.0 ml straws. We continue to enlist the assistance of Dr. Joseph Cloud, professor of Zoology in the Department of Biological Sciences at the University of Idaho, Dr. Gary Thorgaard and Paul Wheeler in the Thorgaard Lab at Washington State University, and Dr. Madison Powell, geneticist at the Fish Genetics Laboratory and Hagerman Experiment Station with the University of Idaho. These subcontractors are experts in the field of salmonid cryopreservation, reproductive physiology and/or fish genetics.

Sperm evaluation is an important component of the cryopreservation program in order to estimate the quality from the motility of the stored sperm. All semen from listed fish is stored regardless of motility. Fertility is evaluated by sperm motility (Figure 4), which is the percentage of motile sperm following the addition of a sperm activating solution (Mounib 1978). Motility correlates to post-thaw fertility.

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

There are four stages in the cooling sequence of cryopreservation of cells (Cloud and Osborne 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 extra cellular solution; 3) Cooling through the critical period - there is a net movement of water out of the cells as the temperature is constantly being reduced 4) Reduction to liquid nitrogen temperature - the frozen 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 (average 2-4 ml). A sample of 5 ml of semen is sufficient to fill 20 0.5 ml straws, due to the dilution of semen with three parts freezing solution. Depending on the motility of the thawed sperm, one 0.5 ml straw can fertilize up to 450 eggs, and a 5.0 ml straw can fertilize approximately 2,000 eggs. There is not a linear relationship of straw volume to fertilization capacity due to the heat of fusion and the surface area involved.

Two additional large liquid nitrogen tanks, one for each university, were purchased in late 1998 to serve as emergency backups and long-term repositories. Each university has two large nitrogen storage facilities (Figure 5).

Figure 5. Example of a liquid nitrogen tank where chinook salmon and steelhead germplasm is stored.

Fertilization experiments have been conducted on chinook salmon cryopreserved versus fresh sperm in order to assess our procedures. In 2001, Washington State University used eggs from a single female chinook salmon in their fertilization trial. Semen from six cryopreserved hatchery chinook males was used to fertilize the eggs. Milt from a 0.5mL straw from each of the six males tested and fresh (2 days old) semen from three males were compared (Appendix 3). University of Idaho experienced a contaminant in the incubator trays, which resulted in the death of all the embryos. No results were reported.

RESULTS

Description of Spawning Aggregates

The background of the selected spawning aggregates for cryopreservation activities have a diverse history of transfers, stocking, and straying. Chinook salmon and steelhead hatcheries in the Snake River basin that are sampled for male gamete cryopreservation have developed broodstocks in a variety of ways. It is important to understand how broodstock development, management and stocking occurred as it relates to the genetic profile of the existing stock of fish being propagated in these facilities. A genetic analysis was not conducted on any of the original hatchery or stream brood sources to the authors' knowledge. In that regard, we have compiled existing information of where the original chinook salmon and steelhead spawning aggregates brood sources were obtained.

<u>Grande Ronde River</u>

The Grande Ronde River is located in northeast Oregon and historically supported diverse and healthy populations of summer steelhead and spring chinook salmon (Carmichael et al. 1998b). Wallowa summer steelhead in the Grande Ronde subbasin is supplemented with fish from three Lower Snake River Compensation Plan (LSRCP) facilities in Oregon. The hatchery program began in 1976 with the collection of adults trapped at Ice Harbor Dam and Little Goose Dam in 1977 and 1978. It is not clear whether steelhead adults collected at these dams were destined for the Grande Ronde River subbasin. In 1979, embryos from Pahsimeroi Fish Hatchery in Idaho were incorporated into the broodstock. In addition, relatively few fish were used to develop the hatchery steelhead broodstock. To meet broodstock needs in 1991 and 1995, ODFW incorporated adults returning to Cottonwood Creek, Washington into the hatchery program. Furthermore, since the hatchery broodstock was founded it has generally been domesticated for the past 25 years.

Table 1. Broodstock history of Lookingglass Hatchery Grande Ronde spring chinook salmon (Carmichael et al. 1998b).

Brood Year	Source
1978	Rapid River
1980-1984	Carson / Willamette Hatchery
1985-1987	Carson / Lookingglass Hatchery Rapid River
1988	Rapid River
1989	Carson / Lookingglass Hatchery Rapid River
1990-1997	Rapid River / Lookingglass Hatchery
1995-2000	native Lostine, upper Grande Ronde, Catherine Creek

Since 1995, up to 50 adults from weirs on the Lostine River, upper Grande Ronde River and Catherine Creek have been collected, transported and spawned for the conventional hatchery program at Lookingglass Hatchery. Conventional supplementation means using endemic broodstock that return to a natal stream, spawning the adults in the hatchery, raising their progeny to smolt size, then returning them to their natal stream for volitional outmigration (Carmichael 1998b). Jack chinook salmon started returning to the Lostine River from the conventional program in 2000.

The Oregon Fish Commission built a dam on the Wallowa River downstream from the confluence with the Minam River in 1904, which was operational in 1905 and the permanent rock crib dam was in place in 1906 (Oregon Fish Commission 1905 and 1906). It is assumed that this structure was at least a partial, if not a permanent barrier to anadromous fish passage through the 1920's (Don Bryson NPT personal communication). The dam was for broodstock collections and spawning at a nearby hatchery. Because of freezing conditions in the hatchery in the winter, fry were released in the river at this time (Don Bryson NPT personal communication). The dam was partially destroyed in the 1920's allowing fish to pass upstream and recolonize unseeded areas (Don Bryson NPT personal communication).

Oregon Fish Commission moved approximately four to five million McKenzie River (a tributary to the Willamette River) chinook salmon eggs into the Wallowa Hatchery in 1927 and other years. These fish were released as five-inch fish into the Wallowa River (Don Bryson NPT personal communication).

The Nez Perce Tribe, ODFW and the Umatilla Tribe operate an adult weir on the upper Grande Ronde River, Catherine Creek and the Lostine River as part of the conventional chinook salmon hatchery program at Lookingglass Fish Hatchery. Several downstream migrating steelhead were captured and male kelts can be sampled from this weir.

Grande Ronde Subbasin Chinook Salmon Captive Broodstock Project

A Grande Ronde basin spring chinook salmon captive broodstock program was initiated in 1995 with the collection of juvenile salmon (500 parr) from the Lostine River, Catherine Creek and upper Grande Ronde River. Fish are reared at Lookingglass Fish Hatchery until the smolt stage and then are transferred to facilities at Bonneville Hatchery (BOH) and Manchester Marine Laboratory (MML). This allows for the evaluation of freshwater and saltwater broodstock rearing strategies. As the fish reach maturity, the MML fish are moved to BOH where they are held until they ripen and are spawned. 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 (Harbeck personal communications). Excess milt is cryopreserved from the male chinook salmon towards the end of the spawning season to have a repository of genetic material available from these captive fish. One half of each sperm sample is stored at the University of Idaho germplasm repository; as a safeguard should anything happen to the storage facility at BOH.

In previous years, portions of the Rapid River stock and Carson River (82-89) stock maintained in the Lookingglass Hatchery were released into the Upper Grande Ronde River, Wallowa River, Catherine Creek, and into Lookingglass Creek itself (Crateau 1995).

The release of Rapid River stock as well as Carson River stock has resulted in an extremely high incidence of straying in the Grande Ronde subbasin. Spawning-ground surveys revealed a considerable amount of straying. For example, in 1990, stray hatchery fish constituted an estimated 46.2% of the spawners in the Minam River, 77.8% in the Wenaha River, 40% in the Lostine River, 100% in Catherine Creek, and 50% in the Grande Ronde River. In 1992, the amount of straying was particularly high, probably because of low water levels that may have prevented access to some spawning areas. In 1993, straying was somewhat lower, but still over 45% in all populations (Carmichael 1998b).

Lostine River

The Lostine River is a tributary to the Wallowa River in the Grande Ronde subbasin in northeast Oregon. The Oregon Fish Commission built a dam on the Wallowa River downstream from the confluence with the Minam River in 1904, which was operational in 1905 and a permanent rock crib dam was in place in 1906 (Oregon Fish Commission 1906). It is assumed that this structure was at least a partial, if not a permanent barrier to Lostine River anadromous fish passage through the 1920s (Don Bryson NPT personal communication). The dam was partially destroyed in the 1920s allowing fish to pass upstream and recolonize unseeded areas (Don Bryson NPT personal communication). There have not been any deliberate outplants of chinook salmon into the Lostine River (Don Bryson NPT personal communications) prior to the recent captive and conventional hatchery program releases.

Straying of Grande Ronde chinook salmon hatchery fish from Lookingglass Hatchery occurs in the Lostine River. Based on the origin of carcasses recovered on spawning ground surveys, ODFW determined that a high proportion (20-75%) of natural spawners in the Lostine River were of Lookingglass Hatchery origin from 1986-1994 (Carmichael et al. 1998b). The broodstock history at Lookingglass Hatchery is varied and complex (Table 1).

Catherine Creek

Catherine Creek is a tributary to the Grande Ronde River southeast of La Grande, Oregon. The Nez Perce Tribe, ODFW and the Umatilla Tribe maintain a weir on Catherine Creek to trap adult steelhead and chinook salmon.

Upper Grande Ronde River

The headwaters of the Grande Ronde River are southwest of La Grande, Oregon in the Blue Mountains. The Nez Perce Tribe, ODFW and the Umatilla Tribe maintain a weir on the upper Grande Ronde River to trap adult steelhead and chinook salmon as part of the conventional chinook salmon hatchery program at Lookingglass Fish Hatchery. One downstream migrating steelhead was captured and gametes sampled were cryopreserved from this weir.

Dworshak National Fish Hatchery (North Fork Clearwater River)

Dworshak National Fish Hatchery is located at the confluence of the North Fork and the main stem of the Clearwater River near Ahsahka, Idaho. The U.S. Army Corps of Engineers, as mitigation for Dworshak Dam, which blocks anadromous fish passage to the North Fork

Clearwater River, built Dworshak National Fish Hatchery (DNFH) in 1969. The steelhead broodstock was started from native B-run steelhead returning to the North Fork to spawn (Howell et al. 1985b, IDFG et al. 1990, Rhine 1998). Based on allele frequencies, Milner (1997) reported that North Fork Clearwater steelhead were distinguishable from all other Columbia basin steelhead by the high frequency of the allele for peptidase locus. Spawning stock is comprised of three age classes; I-, II-, and III- ocean fish. This nomenclature refers to the number of complete years fish have spent in saltwater. Fish are actually two years older than this system indicates, as they are reared for one year in the hatchery and spend another year migrating to and from the ocean (Burge et al. 2000). The DNFH is operated during the fall to insure inclusion of sufficient early arriving steelhead (~500 adults) into the hatchery gene pool. The trap is then reopened from February through April to capture broodstock from the mid and late portions of the run (Burge et al. 2000). The 1999-2000 return marked the 28th year that artificially spawned North Fork Clearwater River steelhead have returned to DNFH (Burge et al. 2000). At present, the Dworshak NFH population is considered to be part of the Snake River ESU. As such, Dworshak Hatchery steelhead fish are ESA-listed as threatened. The Biological Review Team concluded that the Snake River Basin steelhead ESU is not presently in danger of extinction, but it is likely to become endangered in the foreseeable future (Busby et al. 1996).

Selway River

The Selway and Lochsa rivers converge to form the Middle Fork Clearwater River in central Idaho. The Selway River is designated as a wild and scenic river. There is no history of any steelhead ever being stocked in the Selway River. Lewiston Dam was built at the mouth of the mainstem of the Clearwater River in 1927. This structure prevented passage of spring, summer and fall chinook salmon from at least 1927 to 1940, although steelhead was evidently able to pass (Milner 1997). Steelhead, which managed to hang on during the dam building era, is no longer abundant or distributed as widely.

Fish Creek

Fish Creek is a tributary to the Lochsa River in the upper Clearwater River subbasin in northeast Idaho. Fish Creek had 80,000 Dworshak Hatchery B-run steelhead fry stocked into it in 1979 and 20,000 in 1980 and nothing since. There have been numerous out plants in the Lochsa River system (tributaries and mainstem) in the 1970’s and early 1980’s, but the last record of Dworshak stocking in the Lochsa drainage is in 1982.

Rapid River Hatchery (Rapid River)

Rapid River is a tributary to the Little Salmon River south of Riggins in central Idaho. The Little Salmon River joins the Salmon River approximately 5 miles downstream from the Rapid River confluence. Rapid River Hatchery was constructed in 1964 through the Idaho Power Company (IPC) as part of the mitigation for spring chinook salmon losses due to the construction and operation of Brownlee Dam, Oxbow Dam, and Hells Canyon Dam on the Snake River (IDFG et al. 1990, Howell et al. 1985a). Broodstock was obtained from adult collection at Hells Canyon Dam from 1964 through 1968. These fish originated from the mid-Snake River tributaries such as Eagle Creek, Powder River and the Weiser River (Howell et al. 1985a, IDFG et al. 1990). As such, this hatchery brood source represents the genetic diversity contained from these chinook salmon subpopulations. In addition, the hatchery broodstock probably contains natural spring and summer chinook salmon from Rapid River.

Lake Creek

Lake Creek and Summit Creek converge to form the Secesh River in central Idaho near Burgdorf. The Secesh River summer chinook run is a wild native run (Kiefer et al. 1996). Welsh and Corley (1968) reported that 112 adult summer chinook were transplanted into Summit Creek in 1966. The fish were hauled from the adult trap below Hells Canyon Dam into an area of Summit Creek blocked by a barrier, which was subsequently removed. Frequent observations of the transplanted fish revealed only 10 redds, there was no further information regarding evidence of spawning success or any resulting population. One hundred and eighty nine redds were counted in parts of the spawning areas of the Secesh River drainage that year (Kiefer et al. 1996).

Straying of hatchery fish (marked), presumably from McCall Fish Hatchery (MFH) South Fork Salmon River (SFSR) program has been noted in Lake Creek and the Secesh River. This straying is an infrequent occurrence on the order of 4 % from 1992-2001 for Lake Creek (ISS unpublished data 2002).

Johnson Creek

Johnson Creek is a tributary of the East Fork South Fork Salmon River in central Idaho near the town of Yellow Pine. The Johnson Creek summer chinook salmon population has received supplementation outplants of SFSR summer chinook salmon reared at MFH in 1984 through 1988 (Table 2). These outplants primarily occurred as fingerling releases and were distributed from the headwater reaches to the Ice Hole in Johnson Creek. However, no evaluations of these releases were conducted. It is not known how these supplementation efforts have affected the Johnson Creek summer chinook salmon population in terms of stock mixing, population interactions, spawning distribution, or genetic effects. Minimal straying has occurred into Johnson Creek, on the order of one fish with a missing adipose, probably from the MFH SFSR program, per year.

The Nez Perce Tribe implemented the Johnson Creek artificial propagation enhancement (JCAPE) program to supplement summer chinook in Johnson Creek to prevent extirpation of the spawning aggregate, preserve genetic, ecological, and behavioral attributes of these fish, and to build a naturally-self sustaining population.   Monitoring and evaluation of this project began in 1998. Native Johnson Creek chinook salmon are trapped and spawned and their progeny reared at the McCall Fish Hatchery, and released into Johnson Creek as a yearling smolt. The JCAPE project released 78,950 summer chinook fingerlings into Johnson Creek on March 27, 2001.

Two hundred pairs of chinook salmon of marked (double opercle and adipose fin clipped hatchery fish from MFH were outplanted in Meadow Creek and the East Fork South Fork Salmon River above Meadow Creek. These two locations are approximately ten miles upstream of the mouth of Johnson Creek. Several of these fish were detected in Johnson Creek in 2001. Estimated adult hatchery reared salmon composition in Johnson Creek has ranged from 0 to 12.5% from 1992 to 2000 (Kucera unpublished data).

Table 2. South Fork Salmon River summer chinook salmon releases in Johnson Creek. (NPT and PRRG 2001).

SFSR Brood Year	Release Date	Fish Released	Fish Size
BY 1984	8/02/85	50,000	Fry
BY 1985	5/09/86	177,606	Fry
BY 1986	5/05/87	90,000	Fry
BY 1986	6/12/87	28,400	Fry
BY 1987	5/09/88	194,600	Fry
BY 1987	5/31/88	259,200	Fry
BY 1988	8/8-10/89	290,000	Fingerling
Total Fish Released	1985-1989	1,290,306	Fry/Fingerling

McCall Fish Hatchery (South Fork Salmon River)

McCall Hatchery is located in McCall, Idaho on the North Fork Payette River. McCall Fish Hatchery was constructed in 1980 through the LSRCP program to supplement summer chinook salmon in the SFSR. The South Fork Salmon River is in central Idaho and is a tributary to the main Salmon River. The LSRCP hatchery program provides mitigation for salmon and steelhead losses incurred by the construction and operation of the four lower Snake River hydroelectric dams. The broodstock was initially collected at Little Goose Dam in 1978, at Lower Granite Dam in 1979, and at Lower Granite Dam and the SFSR in 1980 (Howell et al. 1985a, Kucera 1987, IDFG et al. 1990). After 1980, adult returns to the SFSR were used for broodstock purposes. As such, the broodstock is a thought to be a mixture mainly of Snake River summer chinook, but may also contain genetic diversity from spring chinook salmon subpopulations.

Big Creek

Big Creek is a tributary to the Middle Fork Salmon River in the Frank Church Wilderness of No Return in central Idaho. Big Creek chinook salmon are wild fish, which have never been supplemented. Two adipose-clipped male chinook were identified in the upper spawning index area in 2001.

Capehorn Creek

Capehorn Creek is a tributary of Marsh Creek at the headwaters of the Middle Fork Salmon River in central Idaho. In 1975, 22,000 spring chinook salmon fry from Rapid River Hatchery were outplanted in Capehorn Creek (Matthews and Waples 1991).

Marsh Creek

Marsh Creek joins Bear Valley Creek to form the Middle Fork Salmon River. Most of this drainage is in the Frank Church River of No Return Wilderness except for the upper headwaters areas. IDFG has operated an adult weir on Marsh Creek upstream of Capehorn Creek. A screw trap is in place upstream from the weir location to enumerate and PIT tag outmigrating juvenile salmonids.

Pahsimeroi Hatchery Chinook Program (Pahsimeroi River)

The Pahsimeroi Hatchery is located one mile upstream of the mouth of the Pahsimeroi River in east central Idaho near the town of Ellis. The Idaho Power Company (IPC) as part of the mitigation constructed Pahsimeroi Hatchery in the mid 1960's for chinook salmon and steelhead losses due to the construction and operation of Brownlee Dam, Oxbow Dam, and Hells Canyon Dam on the Snake River (IDFG et al. 1990). Broodstock for the Pahsimeroi Hatchery chinook program was developed from native summer chinook in the Pahsimeroi River in 1968 (Howell et al. 1985a). When the hatchery was expanded in 1980, a spring chinook salmon program was started and the summer chinook program continued. In 1982-1985, over 700 females from the Hayden Creek hatchery in the Lemhi River subbasin were spawned and added to the Pahsimeroi broodstock. In 1987, the hatchery program converted to a summer chinook salmon program and eggs were obtained from the South Fork Salmon River (McCall Hatchery). Transplants from Rapid River spring chinook occurred in 1982, 1987-1989. Skamania hatchery embryos were also transferred to Pahsimeroi Hatchery and the smolts were released into the East Fork Salmon River (Brannon et al. 2000). The genetic diversity contained within the current stock includes at least spring and summer chinook from the Pahsimeroi River, Rapid River (see Rapid River above), Lemhi River and SFSR, and Skamania Hatchery chinook salmon (see MFH above). It is unclear what other program modifications may have occurred. The broodstock makeup has been stable since 1990, meaning only the fish returning to the hatchery have been used for spawning purposes.

Chinook salmon spawned at Pahsimeroi Hatchery are reared at the Sawtooth Hatchery for the first year, and then transferred as 4-5" juveniles to Pahsimeroi Hatchery's upper facility (well water) in September. These fish are released in April directly into the Pahsimeroi River at the weir approximately 1 mile upstream from the confluence with the Salmon River (Garlie personal communication).

As far as pathogens that the Pahsimeroi Hatchery chinook are exposed to, the major concern is bacterial kidney disease (BKD). The fish are treated with erytromycin upon entry to the hatchery.

All the marked and unmarked salmon (brood year 96-98) returning to Pahsimeroi this year is ESA listed (Garlie personal communication).

Pahsimeroi Hatchery Steelhead Program (Pahsimeroi River)

Idaho's hatchery steelhead program began in 1965 after the Federal Power Commission ordered IPC to transplant Snake River steelhead trapped at Hells Canyon Dam to the Salmon River for mitigation due to the Hells Canyon dam complex construction on the Snake River. This Snake

River stock has been the basis for all hatchery A-strain programs in the Salmon River (Ball 1998). B-run steelhead from Dworshak National Fish Hatchery was released in the Pahsimeroi River in 1973 - 1974 (Ball 1998, IDFG et al. 1990). Skamania (Washington state) Hatchery embryos were also transferred to Pahsimeroi Hatchery and smolts grown in the Pahsimeroi Hatchery were released into the East Fork of the Salmon River (Brannon et al. draft 2001).

Fertilized eggs from the Pahsimeroi hatchery are immediately flown to the Oxbow Hatchery for early rearing then Niagara Springs until they are directly released into the Pahsimeroi River at the weir. This off-site rearing is due to the presence of Myxosoma cerebralis spores, which enter very young fishes and infect skeletal tissues prior to ossification of the bones and are thus trapped within the bone as the fish ages (Post 1987). Whirling disease is a chronic debilitating, highly infectious disease of salmonids and is a problem for the steelhead at Pahsimeroi Hatchery

Other diseases that have occurred in the Pahsimeroi Hatchery steelhead historically are coldwater disease or fin rot, furunculosis, bacteria gill disease, and infectious pancreatic necrosis (IPN). There have been no outbreak lately because of the lower densities in which the juveniles are reared (Garlie personal communication).

All wild/natural (unmarked) steelhead are ESA-listed, but the Pahsimeroi hatchery fish are not ESA-listed. All IDFG hatchery fish in Idaho are adipose-clipped (Garlie personal communication).

Sawtooth Hatchery (Upper Salmon River)

The Sawtooth Hatchery is located on the upper Salmon River south of Stanley, Idaho in Sawtooth Mountains. Sawtooth Hatchery was constructed and became operational in 1985 (Hassemer 1998). It is an LSRCP facility designed to compensate spring chinook salmon in the upper Salmon River. These spring chinook must migrate 900 plus miles from the ocean and spawn and rear at an elevation over 6,000 feet. Broodstock development occurred from adult collections on the East Fork Salmon River and the upper Salmon River (Hassemer 1998, IDFG et al. 1990). During the early and late 1970's Rapid River hatchery chinook salmon were released into the East Fork Salmon River and upper Salmon River in response to severe declines in adult escapement (Howell et al. 1985a, Kucera 1987, Hassemer 1998). A total of 985,400 Rapid River hatchery smolts were released in 1978 and 1,012,300 smolts were released in 1979 Howell et al. 1985a). Another report states 914,000 Rapid River smolts were released in 1979 at the present site of Sawtooth Hatchery, and at least 500 returned as adults in 1981 (Yundt personal communication, Waples et al. 1991). Adult broodstock collection has occurred in the upper Salmon River since 1981 (Howell et al. 1985a). The current hatchery broodstock may reflect the genetic diversity contained in both the upper Salmon River chinook and the Rapid River hatchery chinook.

Imnaha River

Historically, "the Imnaha River subbasin of northeast Oregon supported a vital run of steelhead. Correlated with the completion of the dams on the lower Snake River, however, run sizes began to diminish" (Whitesel et al. 1998). Neither the number nor the characteristics of natural steelhead in the Imnaha River basin have appeared to respond to the supplementation program (mentioned below) (Whitesel et al. 1998).

Lookingglass Hatchery (Imnaha River)

Lookingglass Hatchery is an LSRCP facility in the Grande Ronde subbasin in northeast Oregon near Elgin, constructed in 1982 with adult chinook salmon trapping and smolt acclimation facilities on the Imnaha River, in the Imnaha subbasin. The LSRCP hatchery program provides mitigation for salmon and steelhead losses incurred by the construction and operation of the four lower Snake River dams. Native Imnaha River chinook salmon was collected in 1982 for the supplementation program (Howell et al. 1985a, Carmichael et al. 1998a) and only this stock has been used.

Little Sheep Creek

Little Sheep Creek is a tributary to the Imnaha River in northeast Oregon. The summer steelhead LSRCP supplementation program in the Imnaha River began with the trapping of native steelhead adults in Little Sheep Creek in 1982 (Howell et al. 1985b). In 1983, hatchery-reared smolts were released into Little Sheep Creek and the Imnaha River and adults began to return to the basin in 1985 (Whitesel et al. 1998). The collection, holding and spawning of natural and hatchery adults occurs at a permanent facility on Little Sheep Creek. The embryos are transferred to Wallowa Hatchery for incubation to eyed state. At eye up, the fish are taken to Irrigon Hatchery for rearing to smolts. Then, the smolts are acclimated and released at the Little Sheep Creek facility. Hatchery-produced and naturally produced steelhead from the 1989 and 1990 broods were genetically indistinguishable based on allozyme analyses conducted by Dr. Robin Waples (Whitesel et al. 1998).

Oxbow Fish Hatchery (Snake River)

The Oxbow Hatchery is located downstream from Oxbow Dam on the Snake River in west central Idaho. The Idaho Power Company (IPC) as part of the mitigation constructed Oxbow Hatchery in the 1962 for chinook salmon and steelhead losses due to the construction and operation of Brownlee Dam, Oxbow Dam, and Hells Canyon Dam on the Snake River. Broodstock for the Oxbow Hatchery steelhead program was developed from native steelhead in the Snake River in 1968 (Hills IDFG personal communication). No major disease outbreak in the steelhead broodstock has occurred in the last 4 years since 1999 (Hills IDFG personal communication).

2001 Cryopreservation Sample Collections

Gametes from 398 male chinook salmon were sampled from Lostine River, Catherine Creek, upper Grande Ronde River, Lookingglass Hatchery, Lake Creek, the SFSR weir, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, Pahsimeroi Hatchery, and Sawtooth Fish Hatchery (Table 3). Sampling at hatchery facilities has been designed to collect gametes from the spectrum of the run.

Male steelhead gametes were collected at the upper Grande Ronde River, at the ODFW weir on Little Sheep Creek, a tributary to the Imnaha River, Dworshak Hatchery, Fish Creek, Pahsimeroi Hatchery, and at Oxbow Hatchery (Table 4). A total of 295 steelhead gametes were colleted and cryopreserved in 2001.

Table 3. Snake River basin spring and summer chinook salmon spawning aggregates sampled for semen collection in 2001. Includes total samples cryopreserved, unmarked (wild/natural) and marked (hatchery) fish numbers, dates collected, and the range of percent sperm motility.

Spawning Aggregate	Total Samples Cryopreserved	Un-marked Fish	Marked Fish	Collection Dates	Sperm Motility (percent)
Lostine River	33	33	0	8/30 & 9/5, 11	20-90
Catherine Creek	11	11	0	8/30 & 9/5, 11	50-90
Grande Ronde River	9	9	0	8/30 & 9/5, 11	5-90
S. Fork Salmon River McCall Hatchery	44	25	19*	9/4, 7, 13	5-90
Lake Creek	28	27	1	8/13, 17, 22	0-90
Johnson Creek	62	62	0	8/15, 21,22,24, & 9/4, 6	0-90
Big Creek	50	49	1	8/10, 14, 21	0-90
Capehorn Creek	2	2	0	8/24	60-80
Marsh Creek	24	24	0	8/16, 23, 24	5-90
Pahsimeroi River	50	15	35	9/23 & 10/2	5-90
Upper Salmon River	48	48	0	8/27 & 9/10	20-90
Imnaha River Lookingglass Hatchery	37	37	0	8/28 & 9/7, 11	20-90
Totals and Range	398	342	56	8/10-10/2	0-90

* not ESA listed

Table 4. Snake River basin steelhead spawning aggregates sampled for semen collection in 2001. Includes total samples cryopreserved, unmarked (wild/natural) and marked (hatchery) fish numbers, dates collected, and the range of percent sperm motility.

Spawning Aggregate	Total Samples Cryopreserved	Unmark Fish	Marked Fish	Collection Dates	% Sperm Motility
Grande Ronde River	1	1	0	5/23	50
Snake River, Oxbow	73	0	73	4/9, 16, 23 & 5/3	50-90
NFk Clearwater River Dworshak Hatchery	81	0	81	2/20, 3/13, 20, & 4/3, 17	50-90
Fish Creek	1	1	0	6/11	80
Johnson Creek	1	1	0	6/7	70
Pahsimeroi River*	60	0	60	4/6, 25	20-90
Little Sheep Creek	78	7	71	4/3,10,17,24,5/1,8,15	50-90
Totals and Range	295	17	277	2/20-6/11	20-90

Lostine River – chinook salmon

The Lostine River flows into the Wallowa River, which empties into the Grande Ronde River. A total of 33 male chinook salmon, mostly 4-year olds (Figure 6), were sampled in August and September, 2001. These fish were spawned at Lookingglass Hatchery, originally collected at the adult weir on the Lostine River. Cryopreservation samples were taken with excess milt collected from the conventional hatchery program. This was the eighth consecutive year of cryopreservation sampling in the Lostine River. A total of 66 cryopreserved semen samples taken from 1994 to 2001 are now in storage at the universities.

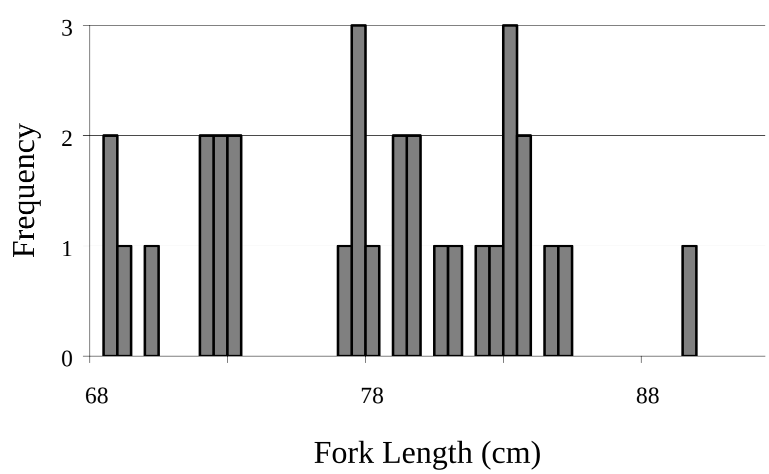

Figure 6. Length frequency distribution of chinook salmon sampled for cryopreservation in the Lostine River in 2001.

Grande Ronde River - steelhead

One steelhead kelt was sampled at a weir in the upper Grande Ronde River administered by the Confederated Tribes of the Umatilla Indian Reservation. This sample was collected by Umatilla staff at the weir and frozen the next day at the University of Idaho, with a sperm motility of 50%.

Dworshak National Fish Hatchery (North Fork Clearwater River) – steelhead

Semen was cryopreserved from 81-hatchery steelhead that were held for spawning at the Dworshak National Fish Hatchery in Ahsahka, Idaho on the Clearwater River. All of these fish were hatchery origin steelhead, which means they are not ESA listed fish. The spectrum of the run was sampled. This was the third year of steelhead cryopreservation sampling at this hatchery. There are a total of 232 steelhead samples in storage from Dworshak now.

Fish Creek – steelhead

One natural steelhead kelt was captured moving downstream at the Idaho Department of Fish and Game (IDFG) weir and artificially spawned to collected sperm on June 11, 2001. The sperm motility rated 80%.

South Fork Salmon River weir – chinook salmon

Forty-four fish were sampled over a period of two weeks in September from the South Fork Salmon River (SFSR) and at McCall Hatchery weir with IDFG hatchery personnel. Fourteen of the fish were sampled in the SFSR were immediately upstream of the hatchery weir. Four of those fourteen (29%) were adipose clipped fish, meaning some hatchery fish were above the weir. The breakdown of the origin of all of the sampled fish is as follows: 19 were adipose fin-clipped (hatchery reared), 15 were supplementation fish (marked with a left ventral fin clop), 25 were natural fish. This was the sixth year of cryopreservation sampling at the SFSR weir, with 300 cryopreserved semen samples taken from 1996 to 2001 now in storage.

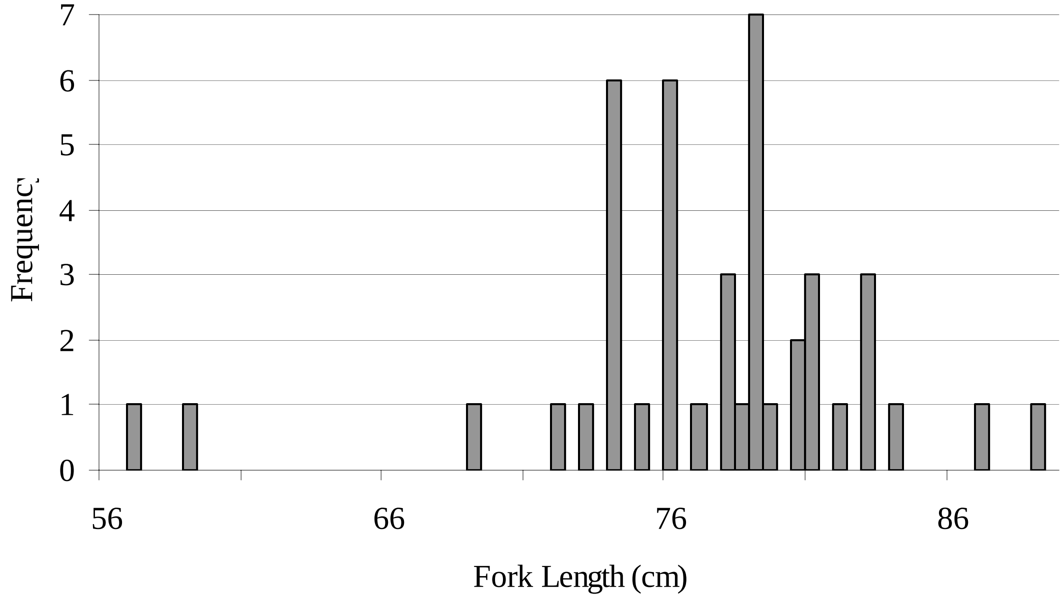

Figure 7. Length frequency distribution of chinook salmon sampled for cryopreservation in the South Fork Salmon River in 2001.

Lake Creek– chinook salmon

Twenty-eight wild adult male fish were sampled in Lake Creek on August 13th, 17th and 22nd. Three female chinook salmon were netted and immediately released upon identification. Four year old fish comprised the majority of fish sampled (Figure 8). Sperm motility for the Lake Creek samples ranged from 0-90%. There were 27 unmarked fish and one marked fish sampled. This was the sixth year of cryopreservation sampling in Lake Creek, a tributary of the Secesh River in the South Fork Salmon River watershed. A total of 59 cryopreserved semen samples taken in 1996 through 2001 are now in storage.

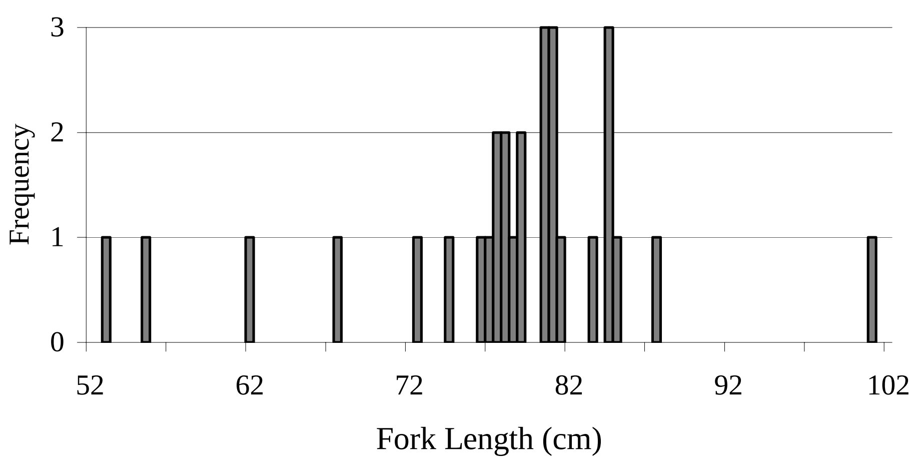

Figure 8. Length frequency distribution of chinook salmon sampled for cryopreservation in Lake Creek in 2001.

Johnson Creek – chinook salmon

Sixty two natural chinook salmon semen samples was cryopreserved from Johnson Creek in 2001. Seven fish were netted in Johnson Creek and spawned artificially. The Nez Perce Tribe initiated a supplementation program on Johnson Creek and the adult weir was in place in 2001. Of the remaining 55 chinook, 47 gametes samples were collected at the weir in mid August on Johnson Creek and 8 were sampled for cryopreservation purposes after broodstock fertilizations occurred at the SFSR trap in September. The spectrum of the run was sampled. A high proportion of the adults sampled were age IV fish (Figure 9). Johnson Creek is a tributary of the East Fork South Fork Salmon River. This was the fifth year of chinook cryopreservation sampling in Johnson Creek, for a total of 126 semen samples cryopreserved.

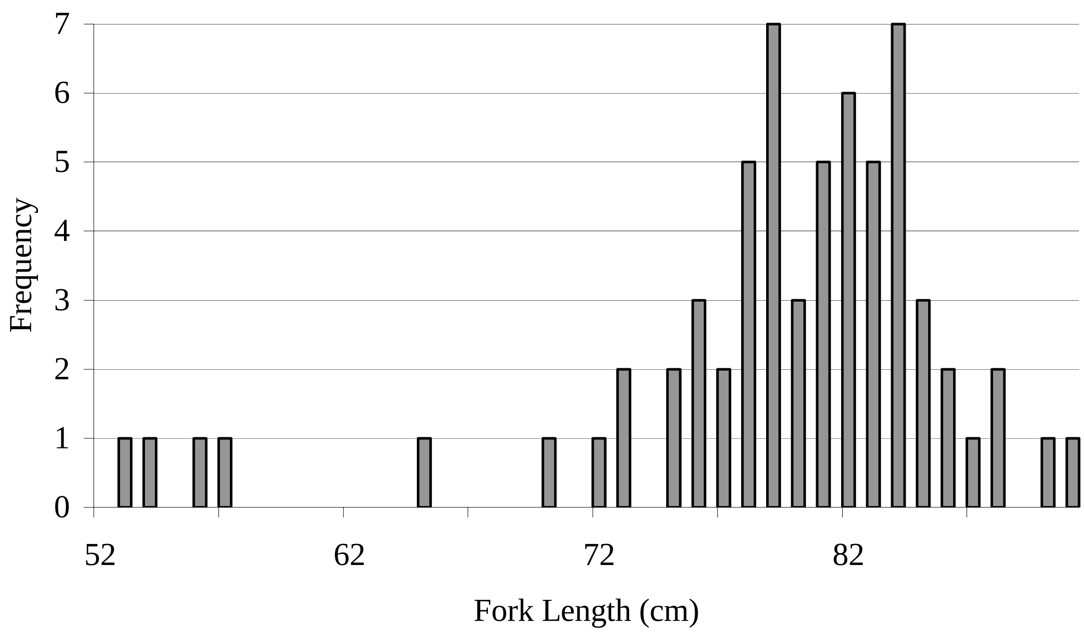

Figure 9. Length frequency distribution of chinook salmon sampled for cryopreservation in Johnson Creek in 2001.

Big Creek – chinook salmon

The Tribe has been attempting to collect semen from chinook salmon in Big Creek, a tributary to the Middle Fork Salmon River, for nine years. Big Creek experienced three consecutive years (1994-1996) of cohort collapse when samples were not obtained. No salmon were netted in 1999 in the stream either. In 2001, 50 male chinook were sampled for cryopreservation purposes. This represents nearly twice the number of samples collected in the previous nine years. The vast majority of adults collected were four-year-old fish, resulting from brood year 1997 production (Figure 10). One male was sampled on two different days. An adipose-clipped male was sampled with no coded wire tag found in the fish. Five females were netted incidental to adult male collections. A total of 81 cryopreserved semen samples taken from 1992 to 2001 are now in storage.

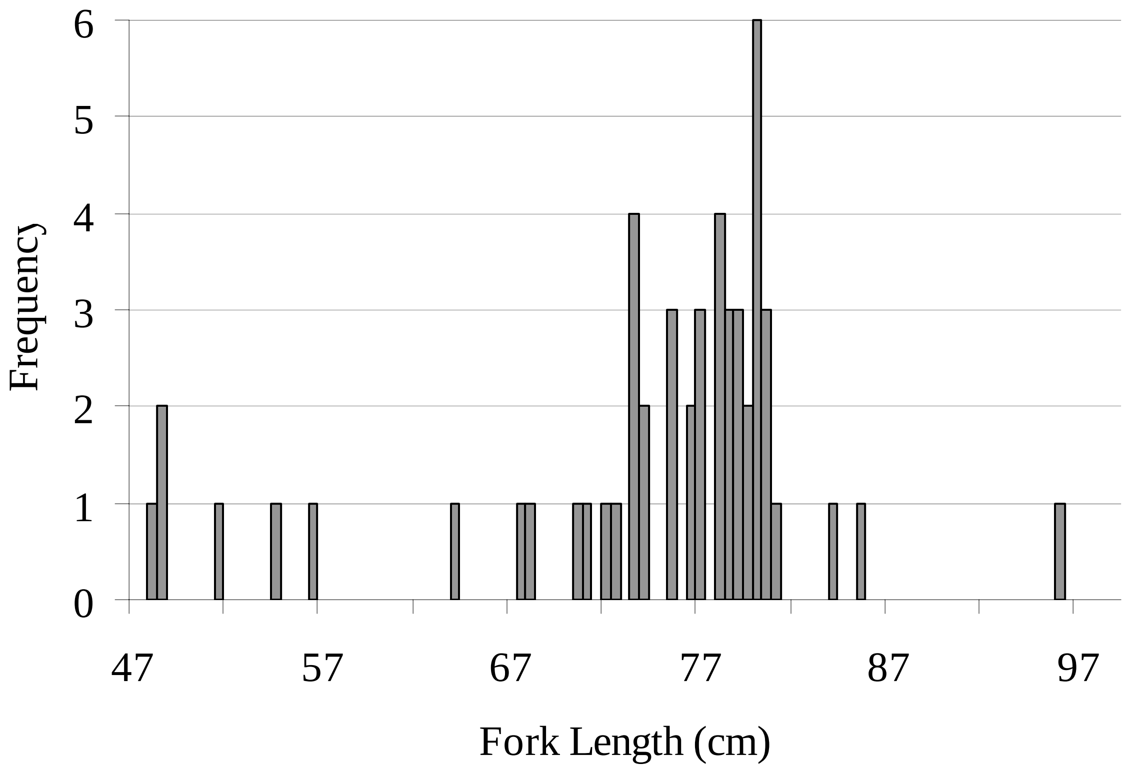

Figure 10. Length frequency distribution of chinook salmon sampled for cryopreservation in Big Creek in 2001.

Capehorn Creek – chinook salmon

Two male salmon were sampled at Capehorn Creek on August 24, 2001. Few live adult males were observed during sample collection. Eleven samples are in storage from this headwater stream in the Middle Fork Salmon River drainage.

Marsh Creek – chinook salmon

Preliminary chinook salmon redd count data showed 185 redds in the Marsh Creek drainage in 2001. A total of 24 semen samples were collected in Marsh Creek in 2001. The salmon sampled were all 4 and 5 year old males (Figure 11). There are 37 samples from 1997 on cryopreserved in the gene bank.

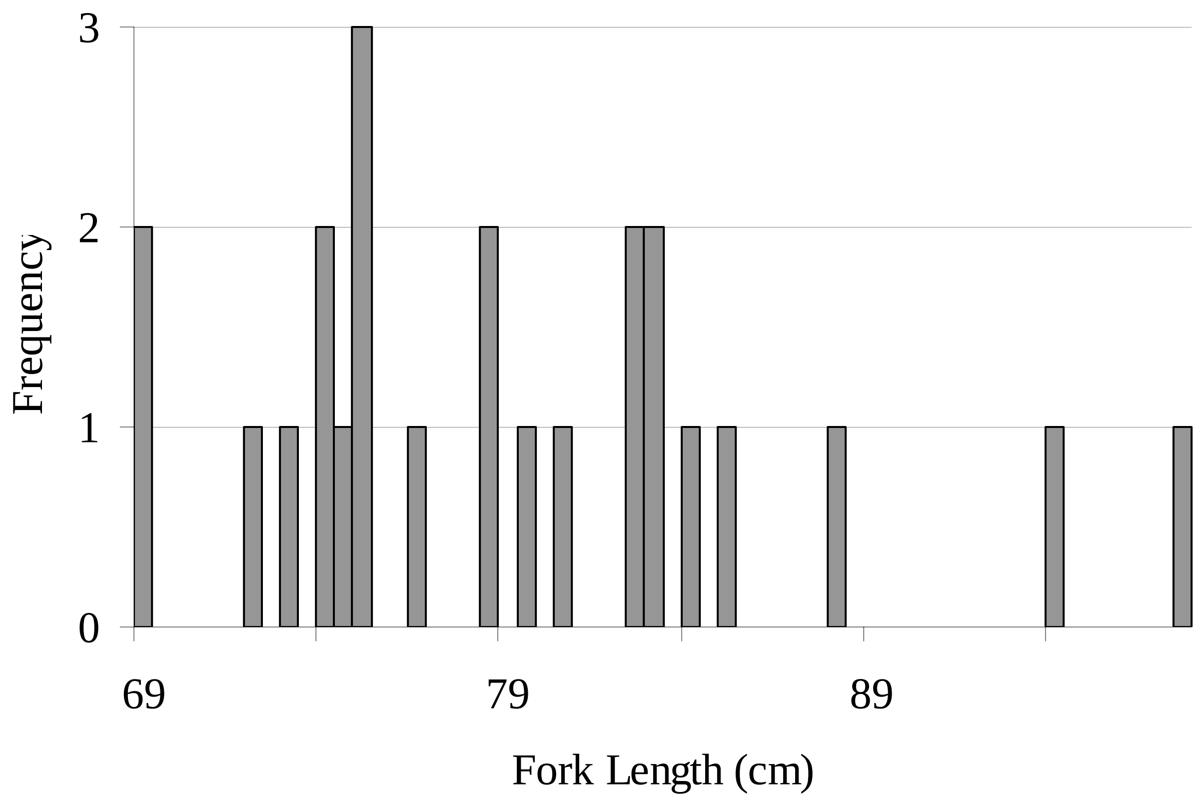

Figure 11. Length frequency distribution of chinook salmon sampled for cryopreservation in Marsh Creek in 2001.

Pahsimeroi Hatchery (Pahsimeroi River) – chinook salmon and steelhead

Summer chinook salmon and A-run steelhead semen was collected at the Pahsimeroi Hatchery starting in 1999. This year 50 chinook milt samples (from 35 marked and 15 unmarked salmon) considered excess to the production needs, was cryopreserved. All the marked and unmarked salmon (BY96, BY97 and BY98) returning to Pahsimeroi this year were ESA-listed. Pahsimeroi Hatchery steelhead are adipose fin- clipped and are not ESA-listed. Steelhead (60 milt samples) were sampled and frozen. A total of 131 chinook and 147 steelhead gametes are now present in the germplasm repository.

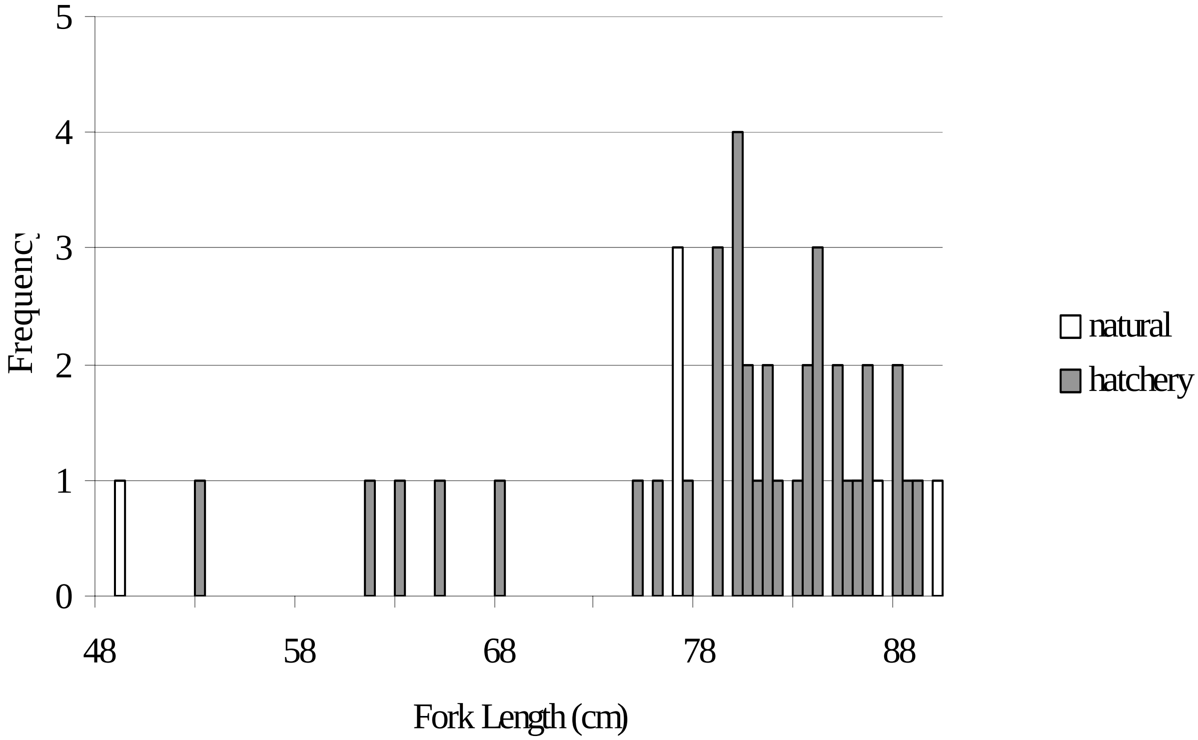

Figure 12. Length frequency distribution of chinook salmon sampled for cryopreservation in the Pahsimeroi River in 2001.

Sawtooth Fish Hatchery (upper Salmon River) – chinook salmon

Forty-eight fish held in the Sawtooth Fish Hatchery from the upper Salmon River were sampled in 2001. All of the 44 chinook sampled in 2001 were unmarked, presumed natural. A total of 220 samples from the Sawtooth Hatchery have been frozen.

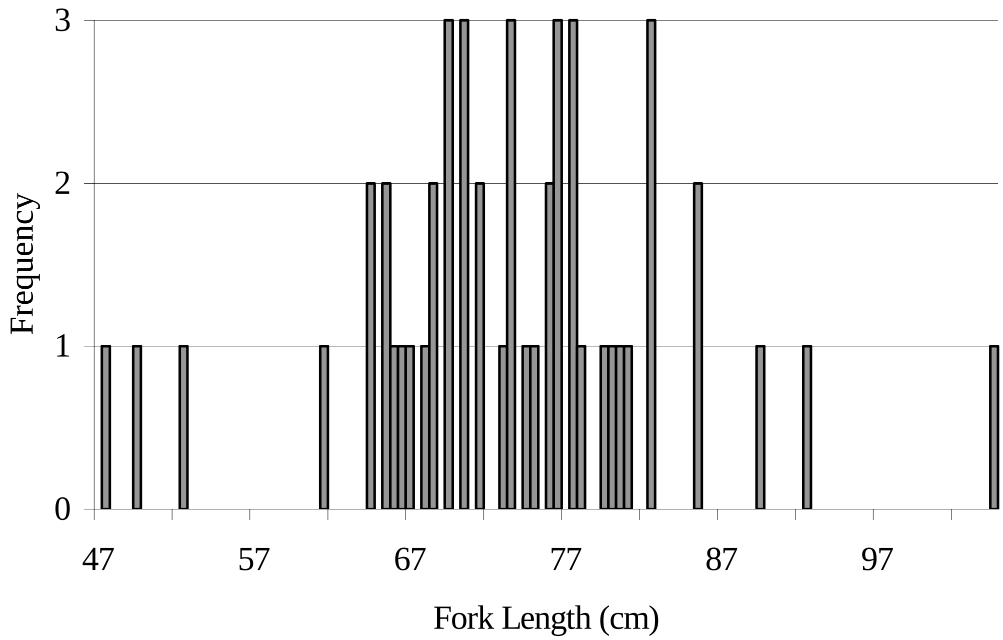

Figure 13. Length frequency distribution of chinook salmon sampled for cryopreservation in the upper Salmon River in 2001.

Little Sheep Creek - steelhead

Steelhead semen was collected from 78 fish at the adult weir on Little Sheep Creek, which is operated by ODFW. Seventy-one of these fish had an adipose or an adipose plus left ventral fin clip, and gametes were collected from 7 unmarked fish. The Tribe has samples from Little Sheep steelhead every year since 1997 for a total of 185 cryopreserved samples.

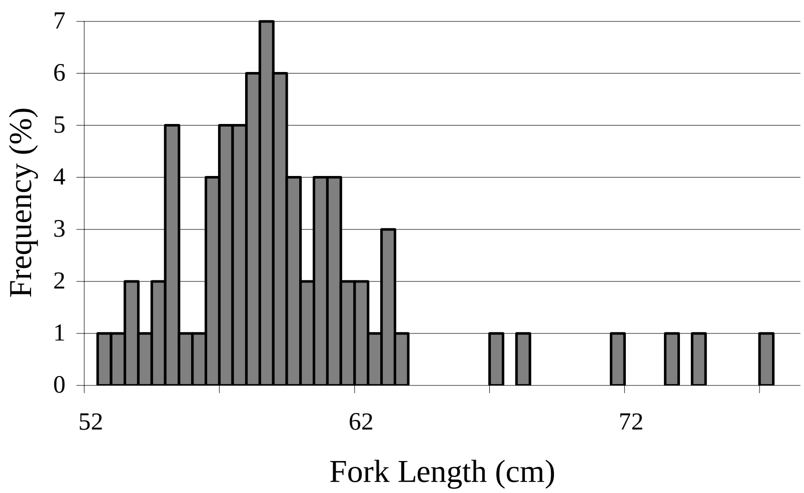

Figure 14. Length frequency distribution of steelhead sampled in Little Sheep Creek in 2001.

Lookingglass Fish Hatchery (Imnaha River) – chinook salmon

Sperm was cryopreserved from 37 natural Imnaha River chinook from Lookingglass Fish Hatchery. All of these fish were unmarked natural chinook salmon and are ESA listed fish. Four year old adults represented the majority of fish sampled (Figure 15). These samples were collected in coordination with the ODFW during hatchery spawning operations. A total of 420 cryopreserved semen samples taken from 1994 - 2001 are now in storage.

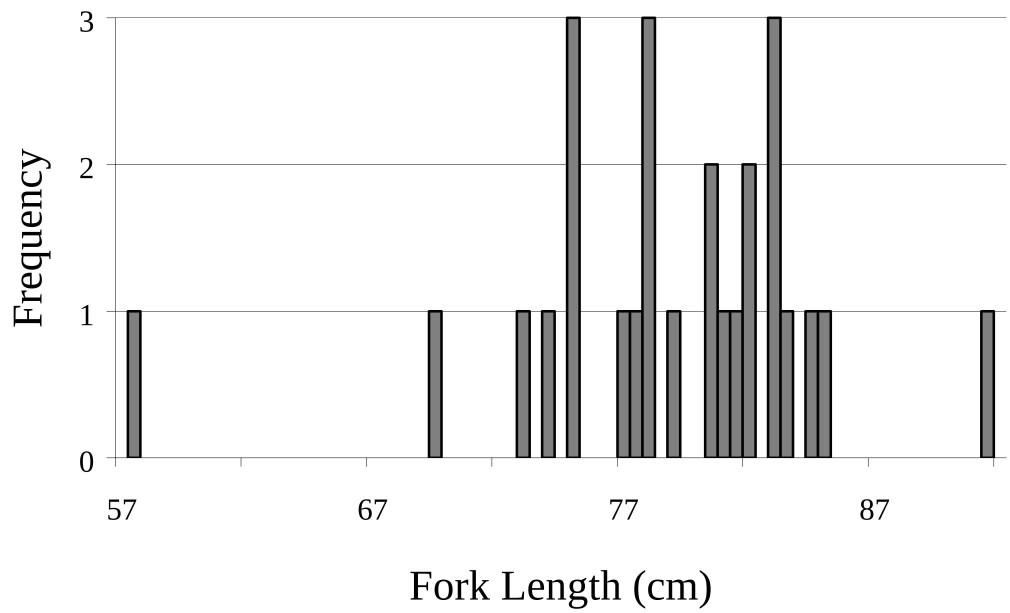

Figure 15. Length frequency distribution of chinook sampled for cryopreservation in the Imnaha River in 2001.

Oxbow Hatchery (Snake River) - steelhead

Snake River steelhead milt was sampled at Oxbow Hatchery for the first time in 1999. In 2001, hatchery steelhead milt (73 samples) considered excess to the production needs was collected and cryopreserved over the spectrum of the spawning season from early March through April. These fish are trapped below Hells Canyon dam in the fall and again starting in February. Wild, unmarked fish are released to spawn.

Fertility Trials

The 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% motility of fresh sperm is considered good. There is a risk of lower fertilization rates and potential loss of eggs using cryopreserved semen. Fertility trials are conducted every year to test the viability of the cryopreserved chinook salmon sperm. Lower fertilization rates using cryopreserved sperm may be acceptable where genetic concerns warrant them.

Fertilization experiments were conducted again at WSU 2001 using chinook salmon gametes. WSU acquired the eggs from one female chinook salmon at Quinault Hatchery in Washington on November 19. The milt from 6 males in the germplasm repository frozen in August 2001 fertilized those eggs on the next day.

Average fertilization rate for the WSU experiment was 17.4% with a range from 5.9 to 41.3%.

Salmonid Genetic Analysis

One objective of the Salmonid Gamete Preservation project is to have each individual fish, which is cryopreserved, in the germplasm repository genetically analyzed. This genetic information will be used in conjunction with the biological data to characterize each unique individual and aid in the application and use of the cryopreserved germplasm. The establishment of genetic baselines for salmon and steelhead is a key element for identifying stock or management units within populations and conserving existing genetic resources. Also, baselines provide standards against which shifts or losses of genetic resources through various management practices (e.g. supplementation or hatchery practices) can be monitored (Salmon Subbasin Summary 2001).

The University of Idaho Center for Salmonid and Freshwater Species at Risk and the Columbia River Inter-Tribal Fish Commission (CRITFC) are currently genetically analyzing the chinook salmon collected in 1992-1997 and 2000-2001. These results will be reported in 2002 and will be added to previous genetic reports from chinook sampled in 1998-1999 with new markers and analyses are newly developed. This final cumulative report will incorporate all the chinook salmon currently in the germplasm repository. In order to analyze some of the individuals for whom no tissue samples were collected, it was necessary to thaw a single 0.5mL straw and analyze the DNA in the sperm.

Washington State University is analyzing a subset of the steelhead sampled and cryopreserved in 2001. A report of results will be finished in May of 2002 and added to the past 2 years of analysis.

It is also important to remember that both of the above genetic analyses were conducted on putatively neutral markers that are not subject to natural selection, as compared to adaptive markers. It is difficult to analyze adaptive markers, so this is rarely done. Some adaptive traits are: distance migrated, size, run timing, smolt emergence, and habitat characteristics (such as water temperature, velocity, water quality) chosen by the fish.

DISCUSSION

Semen samples from 693 wild/natural and hatchery steelhead and spring and summer chinook salmon were collected for cryopreservation in 2001. Also, 610 samples from the Grande Ronde chinook salmon captive broodstock are in the germplasm repository. A total of 3,206 samples from chinook salmon, steelhead, Snake River sockeye and Kootenai River white sturgeon are stored in the repository. Chinook salmon spawning aggregates to sample were chosen based upon geographic distribution. More semen samples for cryopreservation were collected this year than any other year (see Appendix 1).

A total of 398 spring and summer chinook were sampled with 87% of those fish being of natural origin. Adult chinook salmon migrating upstream past Bonneville Dam from March through May, and June through July are categorized as spring- and summer-run fish respectively (Burner 1951). Some streams in the Snake River are considered to have only spring chinook, some mainly summer-run fish (e.g., those in the SFSR), and some both forms (e.g., Middle Fork Salmon River and upper Salmon River). In most cases where the two forms coexist, spring-run fish spawn earlier and in the headwaters of the tributaries, whereas summer chinook spawn later and farther downstream (Matthews and Waples 1991). We are sampling chinook in the major river subbasins in the Snake River drainage, thereby preserving gametes from both runs.

Sampling of male chinook salmon in the streams covered the entire spawning period for the first time in 2001. Care was taken to observe the males in the streams so as not to disturb actively spawning males and not negatively affect reproductive success. This year the genetic diversity contained in the entire spectrum of the run was preserved in the germplasm repository. Our sampling strategy involved collecting gametes from males in high density spawning areas every week for 3 weeks. Redd counts usually preceded the cryopreservation crew's field collections to determine the status of spawning activities. Because salmonids produce excess germplasm and regenerate milt readily, it makes sense to sample male steelhead and chinook salmon before and after they spawn.

Snake River Basin steelhead spawning areas are well isolated from other populations and include the highest elevations for spawning (up to 2,000 meters) as well as the longest migration distance from the ocean (up to 1,500 kilometers) (Busby et al. 1996). Collectively, the environmental factors of the Snake River Basin result in a river that is warmer and more turbid, with higher pH and alkalinity, than most others in the species' range (Busby et al. 1996). The majority of natural stocks for which there is data has been declining. Parr densities in natural production areas have been substantially below estimated capacity in recent years (Busby et al. 1996). Downward trends and low parr densities indicate a particularly severe problem for B-run steelhead, the loss of which would substantially reduce life history diversity within the Snake River ESU (Busby et al. 1996).

A total of 295 steelhead male gametes were cryopreserved (277 were hatchery fish, 17 were unmarked wild/natural fish). Both of the major extant groups (A and B-run) of steelhead in the Snake River basin were sampled. It has been challenging to sample natural steelhead. There are few collection facilities, high water, inclement weather for flying samples, and difficulties in identifying spawning aggregates combine to hinder stream collections. Thus, mainly Snake River hatchery A-run and Dworshak hatchery B-run fish have been preserved. The A-group passes Bonneville Dam (Columbia River kilometer 235) before August 25 and the B-group pass Bonneville after August 25 (CBFWA 1990, IDFG 1994), but both reach the lower Snake River dams at about the same time. A-run steelhead are defined as predominately one ocean fish, while B-run steelhead are defined as two ocean (IDFG 1994). A-run steelhead are believed to occur throughout the steelhead-bearing streams of the Snake River basin (Busby et al. 1996). B-run steelhead are thought to be produced only in the Clearwater River, Middle Fork Salmon River and South Fork Salmon River (IDFG 1994). Steelhead of the B-group are larger, averaging 11-15 pounds (or 5-7 kilograms) with maximum size up to 35 pounds (or 16 kilograms).

The data that are available from scales show a high degree of variability in age structure, from 4-year-old spawners in the Clearwater River (Whitt 1954) to 7-year-old spawners in the South Fork Salmon River (BPA 1992).

Sampling of wild/natural steelhead is restricted to downstream migrating kelts at Fish Creek, Grande Ronde, Catherine Creek and Imnaha River and tributary weirs and the Lake Creek, Secesh River and Johnson Creek juvenile outmigrant screw traps.

Sustained productivity of salmonids in the Pacific Northwest is possible only if the genetic resources that are the basis of such productivity are maintained (National Research Council 1996). Much of the genetic diversity that historically existed probably has already been lost. The germplasm repository is an effort to conserve the genetic diversity that remains in existing salmon 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. We have attempted to sample and preserve salmonid 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.

The urgency to create a germplasm repository becomes apparent when reviewing the past status of the runs (Kucera 1998) and the number of samples being preserved.

The use of cryopreserved sperm to fertilize eggs in a hatchery setting enables hatchery managers to produce threatened salmonids via captive broodstock programs or to enhance genetic diversity in conventional hatchery programs. Captive breeding programs require consideration of small population vulnerabilities to preserve high levels of genetic diversity (Ryder et al. 2000). It is estimated that 200 unrelated individual samples are needed to establish a breeding program (Cloud - personal communication).

The "ownership" of the genetic resources and responsibility for the timing and circumstances of attempts at restoration is a complicated issue to be resolved. Many of the controversial practical and ethical issues related to captive breeding populations and their reintroduction into restored and changing natural habitats apply to restoration via genome banking (Corley-Smith and Brandhorst 1999). In the future, we believe that more requests will be made to use cryopreserved semen in hatchery production programs and in research. We recommend and support only the ethical use of cryopreserved genetic material from the germplasm repository. The judicious use of this vital genetic resource is imperative. To that end, we will provide criteria for accessing and using cryopreserved semen samples from the germplasm repository that will assist in rational use and inventory management. A form has been developed to request cryopreserved semen from the germplasm repository and is available for use (Appendix 2). The semen request form's main function is for inventory management of the 0.5mL straws and 5.0 mL straws. Semen requests are further reviewed by the Snake River Germplasm Repository Committee to further ensure rational use. A database of the germplasm inventory has been established and is available for use.

Other Aquatic Gene Banking Efforts in the Columbia River Basin

Idaho Department of Fish and Game have been cryopreserving Redfish Lake sockeye salmon as part of the captive sockeye program. They also freeze male gametes from chinook salmon from the Lemhi River, Yankee Fork and the East Fork Salmon River from the captive rearing project. Three Kootenai River white sturgeon male gametes are also stored in the UI tanks. The Tribe's captive broodstock evaluation project cryopreserves male gametes from the captive Lostine River, Grande Ronde and Catherine Creek chinook salmon broodstocks at Bonneville Hatchery. Washington Department of Fish and Wildlife freezes Snake River fall chinook and spring chinook from the Tucannon River. The Washington Department of Fish and Wildlife also cryopreserved some Yakima River spring chinook at the Yakima Nation Cle Elum Hatchery as part of an experiment. The Tribe is storing those gametes for that program.

RECOMMENDATIONS

The genetic diversity within existing chinook salmon and steelhead spawning aggregates is not replaceable and should be conserved to protect present and future options (National Research Council 1996). The recommendation of the gamete preservation project is to cryopreserve gametes from as many genetically diverse conservation units as possible. Coordination with other Tribal, state and federal agencies will continue to help identify unique spawning aggregates. Further genetic analysis may help determine population structure of chinook salmon (Brannon et al. draft 2000) and steelhead subpopulations.

Snake River chinook salmon and steelhead are in a downward spiral toward extinction. This is the case with the wild and hatchery fish. The gene bank has an inventory of both wild and hatchery salmonids, though many more hatchery fish, due to the ease of collections at a known time and location. The collections in 2001 emphasized semen collection from wild/natural fish.

The cryopreservation project would benefit from input from Tribal, state, and federal agencies to identify steelhead conservation units for gamete cryopreservation.

The technology to freeze female genetic material is still elusive to fisheries science. Fish eggs or embryos cannot currently be successfully cryopreserved and since the mitochondrial DNA is maternally inherited, the mitochondrial genome of fish populations are presently not being preserved in these programs. One method of obtaining eggs from stored materials would be to cryopreserved embryonic cells, which could later be transplanted into female recipient embryos (Nilsson and Cloud 1993). The University of Idaho's Dr. Joseph Cloud is developing the methodology to freeze ovaries from sexually immature female salmonids and to transplant these ovaries into compatible recipients. This research has started with the premise that male reproductive components are easier to cryopreserved than female's. They have successfully transplanted testis from isogenic rainbow trout to donor male trout (Nagler et al. 2001). This is significant research closer to freezing female genetic material.

Fertility trials are conducted after the chinook spawning season as a standard practice. The trials involve fertilizing eggs with cryopreserved versus fresh sperm.

The goal of the gene bank is to preserve genetic diversity. Diversity can be quantified with analysis of the DNA from sampled fish. We recommend that the genetic analysis and report of results keep current with the cryopreservation of the gametes to make project evaluation possible. Reports of genetic analysis results of all of the chinook salmon sampled and cryopreserved should be completed in 2002.

It is expected with the ESA listing of bull trout as a threatened species, that more requests for cryopreserving male gametes from this 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 population levels. We recommend cryopreserving male gametes from bull trout in 2002.

Currently a limited and relatively uncoordinated effort exists to preserve tissues, gametes and frozen viable cells (Ryder et al. 2000). Sagacious managers of gene banks should coordinate at a national and worldwide level. Using the experience gained in the development of the Snake River Germplasm Repository, the Nez Perce Tribe has proposed to immediately establish a Regional Salmonid Germplasm Repository and cryopreserve ESA-listed chinook salmon, steelhead, bull trout and other rare salmonids in the Columbia River basin. This facility will have state-of-the-art technology, laboratory, instruments and the capability of evaluating, cryopreserving, storing fish sperm and maintaining the inventory of samples from a large number of populations in an efficient and secure manner. The development of a comprehensive fish germplasm repository for populations at risk can provide a tangible and quantitative solution to potential loss of biodiversity.

SUMMARY

The cryopreservation project currently has a total of 2,368 chinook salmon and 835 steelhead semen samples in the germplasm repositories at the University of Idaho and Washington State University. We collected 1,758 of the chinook gametes and we are storing 610 samples for the Grande Ronde chinook salmon captive broodstock program. Each university has two freezer tanks, with a third tank coming on-line in 2002, which act as a safeguard mechanism in case of primary tank failure. They also serve as long-term archival storage, which is not disturbed.

Semen collection should continue until sufficient genetic diversity from as many salmonid subpopulations as possible is represented in the germplasm repository. Fish in some streams such as Big Creek have been low in abundance and may require a longer sampling period. Genetic analysis needs to continue annually as semen samples are collected for cryopreservation. Assessment of genetic resources can serve a valuable planning purpose in the overall conservation effort, as well as in determination and management of the well being of species and the richness of ecosystems (Ryder et al. 2000).

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APPENDICES

APPENDIX 1. Tables of Samples Collected.

Table 1. Snake River Basin Chinook Salmon Cryopreserved from 1992-2001.

Spawning Aggregate	Number of Samples Cryopreserved, by year										Total # Sample /stream
	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992	
Lostine River	33	18	2	3	2	**3**	**1**	**4**			**66**
Lostine River (captive brood)	232 reported as cohort class not by spawning years										**232**
Upper Grande Ronde River	9										**9**
Upper Grande Ronde River (captive brood)	180 reported as cohort class not by spawning years										**180**
Catherine Creek	11										**11**
Catherine Creek (captive brood)	268 reported as cohort class not by spawning years										**268**
Rapid River		51	68	98							**217**
South Fork Salmon River	44	54	93	45	45	19					**300**
Lake Creek	28	15	6	3	4	3					**59**
Johnson Creek	62	35	5	17	7						**126**
Big Creek	50	7	0	1	6	0	0	0	10	7	**81**
Capehorn Creek	2	1	0	6	2						**11**
Marsh Creek	24	7	0	2	4						**37**
Pahsimeroi River	50	50	31								**131**
Up Salmon River	48	40	40	41	51						**220**
Imnaha River	37	71	95	79	41	33	42	22			**420**
Total # of Samples/year	**398**	**349**	**340**	**295**	**162**	**58**	**43**	**26**	**10**	**7**	**2368**

Shaded fields are samples, which have already been genetically analyzed.

Table 2. Snake River Basin Steelhead Cryopreserved in 1993-2001.

Spawning Aggregate	Number of samples per year							Total # samples per stream
	2001	2000	1999	1998	1997	1994	1993	
North Fork Clearwater River Dworshak Nat. Hatchery	81	89	62					**232**
Selway River						5*		**5**
Fish Creek	1	1					10*	**12**
Grande Ronde River	1	1						**2**
Johnson Creek	1		2					**3**
Pahsimeroi River Pahsimeroi Hatchery	60	40	47					**147**
Imnaha River		2						**2**
Little Sheep Creek	78	52	25	25	5			**185**
Snake River Oxbow Hatchery	73	98	76					**247**
Total # sample per year	**295**	**281**	**214**	**25**	**5**	**5**	**10**	**835**

* Samples collected by the USGS/ National Biological Survey.

APPENDIX 2. Snake River Germplasm Repository Cryopreserved Semen Request Form

Snake River Germplasm Repository Committee
P.O. Box 1942, 125 South Mission St
McCall, ID 83638
Phone: (208) 634-5290
Fax: (208) 634-4097

Snake River Germplasm Repository Cryopreserved Semen Request Form

Name: ____Affiliation: _____________________
Phone number: (______)__________________________Address: _______________________
Date of request: _________________________________Date need by: ___________________
Species/stock requested: __________________________Hatchery or wild/natural: _________
Number of straws needed: _______0.5ml, _______5.0ml
Reason for request (clearly demonstrate need or type of hatchery program): _______________
____
____
____
____
____
____
Fertilization experience using cryopreserved semen: __________________________________
____
____
____
Name, address, and phone number of person samples should be delivered to: ______________
____
____

Please use additional papers as necessary.

The salmon managers of the Snake River Basin are concerned with how cryopreserved samples are being used and retain the right to refuse samples for inappropriate use of the threatened salmonid species gametes. The Nez Perce Tribe can arrange to deliver and assist in the fertilization of eggs. Please call Robyn Armstrong at the McCall Field Office (address above) to coordinate transfer. The Nez Perce Tribe also may request data on the performance of the semen (percent of eggs fertilized, post-thaw sperm motility, etc.).

Signature: ____Date: ________________

APPENDIX 3. Washington State University Fertilization Trial – 2001

2001 Chinook Fertility Trial at Washington State University

Performed with eggs (one female sampled) and sperm (3 males sampled) from Quinault Tribal Hatchery collected 11-19-01. Fertilization experiment performed on 11-21-01.

Male used WSU straw #	Pre-freeze motility	Fertilization/total	%	Relative %*
NPT-612-01	90	13/144	9.0	13.7
NPT-613-01	90	52/126	41.3	62.7
NPT-618-01	-	16/141	11.3	17.1
NPT-692-01	80	32/126	25.4	38.5
NPT-694-01	90	14/120	11.7	17.8
NPT-697-01	80	6/102	5.9	9.0
			Average 17.4	Average 26.5

* % divided by mean fresh fertility (65.9%)

Q1 (fresh)	65/123	52.8
Q2 (fresh)	66/94	70.2
Q3 (fresh)	83/111	74.8
	Average	65.9