PACIFIC SALMON COMMISSION
TRANSBOUNDARY TECHNICAL COMMITTEE

REPORT TCTR (91)-2

TRANSBOUNDARY RIVER SOCKEYE SALMON
ENHANCEMENT ACTIVITIES, 1989 BROOD YEAR
July 1989 through October 1990

May 1991
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EXECUTIVE SUMMARY

Joint Canada/U.S. enhancement production egg-takes in the Stikine River drainage began in 1989 with the collection of 3,278,000 sockeye salmon (*Onchorhyncus nerka*) eggs from Tahltan Lake. A total of 1,110 female and 1,100 male adult sockeye salmon was removed from an escapement of 8,316 sockeye salmon, leaving 6,106 adults to spawn naturally. The eggs were transported to and incubated at the central incubation facility (CIF) at Port Snettisham, Alaska. The otoliths of the resulting embryos were mass-marked by inducing growth bands through thermal variation during egg incubation. A total of 1,041,744 marked sockeye salmon fry were planted in Tahltan Lake in the spring of 1990. In compliance with the 1989 Understanding Concerning Joint Enhancement of Transboundary River Salmon Stocks signed by the U.S. and Canada, the low return of spawners (<15,000 sockeye salmon) to Tahltan Lake in 1989 resulted in backplanting the enhanced fry into only Tahltan Lake rather into both Tahltan and Tuya Lakes.

Overall survival from egg to backplanted fry was 35%, much lower than expected. Low fertilization rates in the initial egg-takes and incubation problems at the CIF resulted in the loss of a significant number of progeny. The operational deficiencies have been corrected and preventative measures have been implemented to ensure greater egg-to-fry survival with subsequent season’s egg-takes. No disease-related problems were encountered in the 1989 joint enhancement program.

Several ancillary projects were undertaken to provide additional information relevant to joint sockeye enhancement. Disease samples taken from the 1989 Tahltan brood stock showed a major reduction in the prevalence of both infectious hematopoietic necrosis virus (IHNV) and bacterial kidney disease (BKD) compared to 1988. A disease survey of the indigenous fish species of Tuya Lake established the presence of BKD and *Mycobacteria*, but no IHNV was found.

Results from other enhancement related projects conducted in 1989 and 1990 are presented in this report, including: testing potential fry transport problems; mass marking investigations; limnological studies; a fish quality assessment from potential terminal fishing areas in the upper Taku River drainage; and brood stock holding studies at Little Tatsamenie Lake are presented in this report. Fry transport did not present any problems. The quality of fish from terminal areas was of a lower grade than sockeye salmon caught in the Taku River commercial fishery. Holding mortality of Tatsamenie brood stock was a concern, but was judged to be acceptable for an egg-take program.
INTRODUCTION

In February 1989, Canada and the United States agreed to conduct a joint fisheries enhancement project on the Stikine River. Up to 3.0 million sockeye salmon (*Onchorynchus nerka*) eggs were to be taken from Tahltan Lake in 1989 and incubated in isolation at the Port Snettisham central incubation facility (CIF). The resulting fry were to be mass-marked by thermally induced banding of the otoliths. If the 1989 sockeye salmon escapement into Tahltan Lake exceeded 15,000 adults, the fry would be distributed between Tahltan and Tuya Lakes in a manner that would maximize harvestable production. Otherwise, all fry would be planted into Tahltan Lake. Cost and harvest sharing arrangements were also documented in the Canada/U.S. agreement, as were other ancillary studies that would be used to evaluate joint enhancement projects on both the Stikine and Taku Rivers.

FISH HEALTH APPROVALS

**Canadian Approvals**

Approvals to move 1989 brood Tahltan sockeye salmon eggs to Port Snettisham hatchery and the resulting fry back to Tahltan Lake were obtained under Section 4 of the Fisheries Act, under the Federal/Provincial Importation Agreement, and from the Federal/Provincial Transplant Committee. To obtain these approvals, the Port Snettisham CIF was designated as an isolated interim rearing station and agreement to the conditions listed in Appendix A was required. A further condition of the approvals was the requirement to provide a written report to the Canadian National Registry of Fish Health following completion of the project in spring of 1990. Approvals for future activities will depend partly on the demonstrated success of this project.

The conditions of the Canadian approvals required the following previously unanticipated activities:

i) Under condition 2 (Appendix A), a Canadian Local Fish Health Officer was required to inspect the Port Snettisham Hatchery and approve it for use as an interim rearing station.

ii) Condition 3 (Appendix A) specified that the water supply to the Port Snettisham CIF be from a fish-free water source. Long and Crater Lakes, which supply water to the hatchery, had been examined in the past and were thought to be fish-free. To confirm this, the Alaska Department of Fish and Game (ADF&G) conducted extensive surveys of both lakes, which included variable mesh gillnetting and Gee-trapping. These surveys confirmed the absence of any fish.

**United States Approvals**

Approvals were received from the Fish Pathology Section of the Fisheries Rehabilitation, Enhancement, and Development (FRED) Division of ADF&G to receive sockeye salmon eggs from Tahltan Lake, incubate them in isolation modules at the Port Snettisham CIF, and plant the resultant fry back to Tahltan or Tuya Lake.
1989 BROOD ENHANCEMENT ACTIVITIES

Tahltan Egg-Take

The egg-take was contracted to Triton Environmental Consultants Limited\(^1\). Brood stock was seined from the main spawning beach and from in front of the weir at the lake outlet. Brood stock was held for short periods in net pens until enough fish were accumulated to provide sufficient eggs to warrant a shipment. Eggs were flown to Port Snettisham using either a float-equipped Cessna-185 or a Bell-206 helicopter (one trip only). For reasons of logistics and economy, eggs and sperm were transported separately in the first five shipments and fertilized at the CIF. The eggs from groups of four females were combined with the milt from groups of four males until 1.2 million eggs were processed in this way. Fertilization rates proved to be poor using this normally accepted technique of shipping gametes separately. Therefore, the method was changed for the remaining seven egg-takes such that fertilization was conducted at Tahltan Lake and the water-hardened eggs were then shipped to the CIF. The eggs were fertilized by combining the eggs from each female with the milt from two males (one male used with the previous female and one new male) until the approximately 1.8 million remaining eggs were processed. The reasons for the poor results obtained from shipping separate gametes are not apparent. Fish culture experts from both countries who viewed the egg-take agreed that the contractors carried out a well-run operation.

Twelve egg shipments were made during the period September 3 to September 26; details are summarized below:

- Total number of females spawned: 1,110
- Total number of males spawned: 1,100
- Total number of eggs taken: 3,278,000
- Average fecundity (eggs per female): 2,952

The 1989 sockeye escapement into Tahltan Lake totaled 8,316 adults. This included the brood stock used for enhancement purposes, which represented 27% of the total escapement.

Incubation

The Port Snettisham CIF received both separate gametes and water-hardened eggs from Tahltan Lake. Based on 24-hour fertility samples, there was a significant difference in the fertilization rates between the two methodologies. Fertilization rates were estimated to be 35% and 90% for the separate-gametes and fertilized-egg methodologies, respectively. Approximately 40% of the water-hardened eggs were fertilized by the first method. The overall fertilization rate was estimated to be 71%; 2,317,000 fertilized eggs were incubated at the Port Snettisham CIF.

Upon receipt at Port Snettisham, the eggs were disinfected in an iodophor solution and placed into 10 Kitoi incubators. Water temperatures were mechanically reduced to slow the rate of egg development. Formalin treatments were administered to control the growth of fungus during incubation. Eggs were shocked at the eyed stage and the dead removed. Actual green-to-eyed egg survival was 70%.

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Tahltan sockeye eggs were noticeably smaller than those of other sockeye stocks being enhanced in Alaska and there was a concern that the Kitoi incubators would not function properly with the small egg size. A small-mesh screen was, therefore, used to prevent alevins from migrating down through the perforated plate at the bottom of the incubator and suffocating. Two Kitoi incubators were used as a control and did not contain the small-mesh screen. These modifications were made while the eyed-eggs were removed for shocking and picking.

The equipment used to separate the live and dead eggs was disinfected each time prior to picking dead eggs in a different incubator. The live eggs and recipient Kitoi incubators were also disinfected prior to reseeding. Eggs that had been removed from a specific incubator were returned to the same incubator. These stringent measures were undertaken to minimize the risk of cross-contamination and to isolate any potential outbreak of IHNV.

Problems were encountered during the incubation process that caused significant mortalities in the Tahltan stock. Iron bacteria in the process water caused blockages in the small-mesh screen that was used in response to the small egg size. This channelized the water flows in the incubators and suffocated sections of the population. Sockeye alevins in the control incubators did not suffer the losses experienced by those in the incubators with the small-mesh screens. Poor water quality was also a contributing factor to the incubation losses. High siltation and low dissolved oxygen levels were caused by construction activities at the Port Snettisham hydroelectric generating plant, from which the Port Snettisham Hatchery receives its water. In combination, these factors caused a 48% mortality from fertilization to emergence. While these results are below the desired levels, the problems encountered in the pilot year are correctable and similar losses should not be experienced in future years.

Mass Marking

Marking all enhanced sockeye salmon before release is required for effective management of these stocks in a mixed-stock fishery. The efficacies of the most common and the more exotic fish-marking methods were compared using in-house previous experience and literature reviews before the technique of inducing unique patterns of bands on the otoliths of incubating sockeye embryos through varying the temperature of the incubation water was selected. This mass-marking technique was tested using brood-year 1988 eggs from the Speel Lake sockeye stock in Alaska. Three- and five-band patterns were successfully induced on otoliths of the test fish at the Port Snettisham CIF such that the two test groups of the Speel stock were discretely recognized. The examined otoliths were from embryos still in the eyed-egg stage.

After this success, the U.S. and Canadian members of the Transboundary River Enhancement Subcommittee agreed to use otolith banding for marking sockeye salmon in the joint enhancement program. In January 1990, the Tahltan Lake sockeye embryos in the CIF were mass marked by inducing a unique pattern of four otolith bands. This pattern was verified by examining otoliths extracted from eyed-egg stage embryos prior to planting the fry in Tahltan Lake. Samples for otolith-mark detection were taken from all stacks of incubators. All the enhanced fish that were examined had successfully received the mark.

Fry Transport

Since the escapement of sockeye salmon to Tahltan Lake did not exceed 15,000 fish, all of the enhanced fry were planted back into Tahltan Lake, in compliance with the 1989 Understanding Concerning Joint Enhancement of Transboundary River Salmon Stocks (Appendix B). Ideally, fry should be transported to their recipient lake when the zooplankton population in the lake has
increased to the point that it can support the additional fry recruitment. This condition normally exists several weeks after ice leaves the lake. Another important consideration is cost-effectiveness; it is more cost-effective to deliver the fry by float plane after the ice is gone. For these reasons, alevin development was controlled by water temperature manipulation during incubation. In our case, approximately 650,000 fry emerged some two weeks before the planned date of planting them in Tahltan Lake. Therefore, although the original plan was not to feed any fry, these early-emergence fry were fed at the Port Snettisham CIF for 13 to 18 days before being back planted. They gained only about 7% of their initial weight.

Before transport to Tahltan Lake, the sockeye fry were sampled for the prevalence of disease, as per conditions 9 and 10 (Appendix A) of the Canadian Fish Health approvals. This was done as a preventative measure to ensure that no fish diseases were transferred from Alaska to Canada. No diseases were detected. One lot of fry was held over for additional testing when mortalities were experienced in the incubator. The loss was attributed to environmental stresses in the incubator and not to any disease.

The targeted release date for planting the enhanced fish in Tahltan Lake was June 1, 1990. This date was chosen so that fry plants would occur subsequent to the departure of ice from the lake. A total of 1,041,744 fry were planted in Tahltan Lake between June 6 and June 25, 1990. Because transport densities were deliberately conservative during the first year, five flights were required to move the fry.

Two small test lots of about 2,000 hatchery produced fry each were held in net pens for 96 hours in Tahltan Lake and observed for any detrimental effects of the air transport. None were found. This study is discussed further later in this report.

A hydroacoustic survey was conducted at Tahltan Lake in mid-July to determine sockeye fry densities. However, attempts to capture fry by trawling to determine the wild/enhanced ratio in the fry population were not successful. Beach seining revealed that substantial numbers of fry remained in the littoral zone and included both wild and enhanced fry, as determined by the presence or absence of the otolith mark, which was clearly evident on the otoliths sampled. Preliminary calculations show enhanced fry were smaller on average than wild fry (mean lengths 39.0 mm and 50.4 mm and mean weights 0.56 g and 1.3 g, respectively). The size distribution of the enhanced fry (30-53 mm and 0.18-1.46 g) was less than that of the wild fry (30-75 mm and 0.16-4.06 g) but fell within the lower portion of the wild fry range. It was estimated that approximately 54% of the fry captured in the littoral zone were of enhanced origin. If the littoral zone samples are assumed to be representative of the pelagic zone ratio of wild to enhanced fish (validity of assumption is unknown), then comparable survival of enhanced and wild fry during the first month of lake residence is indicated, based on known numbers of enhanced fry and estimated wild fry production. Surveys in August and early October showed that essentially all fry had moved offshore; attempts to capture these fry by trawl in the pelagic zone were again unsuccessful. Thus, relative survival of enhanced and wild fry could not be estimated and an evaluation of the freshwater survival of this first outplant attempt is dependent on a smolt sampling program at the Tahltan Lake in 1991.

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2 It should be noted that these measurements are for fish preserved in 95% ethanol and will require adjustment before making any comparisons with measurements taken from unpreserved fish.

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ANCILLARY STUDIES

Disease Sampling of Tahltan Brood Stock

Pathology samples were collected from adult sockeye salmon and processed during the 1989 Tahltan Lake egg-take to provide further baseline information on the prevalence of the infectious hematopoietic necrosis virus (IHNV) and bacterial kidney disease (BKD) in this stock. The 1989 sampling was also a condition of the Canadian Fish Health approvals, since sampling in 1988 indicated a high incidence of IHNV.

A total of 159 female sockeye salmon were tested for IHNV in 1989. Only three of the fish (2%) tested positive. Alaska hatchery production experience indicates that the likelihood of parent to offspring transmission greatly increases when the IHNV titer (concentration) is greater than, or equal to, $10^4$ plaque-forming units (pfu); titers of less than $10^4$ pfu are considered to be of less pathological concern. Of the three positive samples, only one had a titer in excess of $10^4$ pfu ($10^5$); the remaining two had titers of $10^2$ and $10^3$ pfu.

A total of 151 fish was tested for BKD in 1989. Seven of the fish samples (5%) were positive. Average optical density values ranged from 0.178 to 2.765 utilizing the ELISA technique of analysis.

The prevalence and degree of infection of both IHNV and BKD were much lower in the Tahltan stock in 1989 than in 1988. The number of IHNV positive fish detected may have been less in 1989 because eggs were taken soon after fish entered the spawning area; hence, there would have been less opportunity for horizontal transmission to occur from carrier fish. Also, the 1988 samples included post-spawned fish, which, depending upon the stock, can have higher prevalence and titers of IHNV. The carrier rate for the BKD agent is often influenced by stress-related environmental factors (holding condition, water temperature, etc.) and the degree of senescence in the fish sampled. The actual egg-take conditions in 1989 may have been closer to optimal. It should be noted that a few false positive tests were detected in the 1988 Indirect Fluorescent Antibody Technique tests for BKD when the samples were reanalyzed using the ELISA technique. This also could contribute to the apparently higher BKD prevalence in 1988 than in 1989. The results of the 1989 sampling support the use of the Tahltan sockeye brood stock for enhancement purposes.

Disease Sampling of Indigenous Species in Tuya and Tatsamenie Lakes

Indigenous species of fish were collected from the Tuya and Tatsamenie Lake systems by angling and netting. These fish were frozen whole within 24 hours and shipped to the Fish Disease Diagnostics Laboratory, Pacific Biological Station, Nanaimo, B.C. for analysis.

All fish were examined externally and internally for gross abnormalities. Kidney material was cultured and examined for bacterial pathogens; confirmatory staining of kidney smears was conducted as required. Kidney, gill, spleen, and pyloric caeca tissues were collected for viral assay. Tissues from up to five individual fish were pooled for the assays and the homogenates were tested with a minimal incubation period of 14 days. Internal organs from a number of the fish were examined by the parasitology staff. Results are given in detail in Appendix C and are summarized below.
Tuya Lake

Thirty-one arctic grayling (*Thymallus arcticus*), 10 Dolly Varden (*Salvelinus malma*), and 12 longnose suckers (*Catostomus catostomus*) were examined. No viral pathogens were found. *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, was found in the grayling and Dolly Varden. *Mycobacteria*, which is relatively uncommon, was detected in the arctic grayling. Parasites found included *Myxobolus* spp., a protozoan found in brain tissue (in grayling and Dolly Varden); *Diphyllobothrium* and *Eubothrium*, tapeworms (in grayling and Dolly Varden); and *Myxidium*, a myxosporidian (in Dolly Varden). No unusual parasites or abnormal levels of parasite infection were detected.

Little Tatsamenie Lake

Nineteen lake trout (*Salvelinus namaycush*) and 10 Dolly Varden were examined. No significant viral or bacterial agents were detected. Parasites found included *Diplostomum* sp. and *Tetracotyle* sp., trematode worms (in lake trout and Dolly Varden); *Myxobolus articus* (in lake trout and Dolly Varden); and *Eubothrium salvelini* (Dolly Varden). These parasites are all common in wild fish.

**Fry Transport Studies**

Studies were done to examine the possibility of mortality resulting from aerial transport of fry. Specifically, there was a concern that gas bubbles might form in the blood as a result of changes in elevation, similar to the "bends" which SCUBA divers can experience. Both Canada and the U.S. conducted tests in 1989 to simulate the conditions that would occur during aerial transport of fry from Port Snettisham to Tahltan Lake. These studies were followed with a joint holding study at Tahltan Lake in 1990 designed to provide immediate information on any induced transport mortalities.

**Canadian Studies**

A test was conducted on April 20, 1989, using newly emergent sockeye fry from Weaver Creek spawning channel on the Fraser River system. One hundred fry were placed in each of two 5-gallon plastic buckets fitted with battery-operated aerators. The buckets of fry were transported by truck from near sea level to a nearby ski mountain, an elevation change of 970 meters (3180 feet) in 25 minutes, for a mean rate of climb of 38.8 m/min (127 feet/min). They were held at this altitude for 30 minutes, then driven back to Weaver Creek. The buckets were monitored for changes in temperature and total dissolved gas pressure. An aneroid barometer was used to determine changes in elevation. Upon return to Weaver Creek, the fry were held in the buckets for one hour, then placed in holding pens located in the creek.

Except for some immediate mechanical-related mortality in one bucket, all fish appeared healthy after four days of holding. Temperatures during the trial remained quite constant (11.7 - 11.9°C). The net change in dissolved gas pressure, adjusted for altitude change, was 25 mm Hg. Maximal calculated total gas pressure was 103.8%.
Alaskan Studies

In the 1989 test conducted by ADF&G, 1988 brood Speel Lake sockeye fry were loaded into an airplane and their behavior was monitored during a flight simulating the conditions of a trip to Tahltan Lake. No immediate mortalities occurred. There were no indications that serious problems would be encountered during transport to Tahltan Lake.

Joint Studies

In 1990, two net pens were installed in Tahltan Lake to observe samples of hatchery produced fry after transport from Port Snettisham. Approximately 2,000 fry per group were held and examined. No mortality or unusual behavior was observed after holding the fry for 96 hours. In conclusion, if proper aerial transport protocol is followed, no fry mortality should occur. The protocol used was to load fry into water slightly below saturation of dissolved gases, to monitor the dissolved gases and add oxygen only when its concentration dropped to a critical level, and to maintain aircraft ascent to less than 100 feet per minute.

Fish Quality Assessment

Samples of adult sockeye salmon for quality testing were collected at the Little Tatsamenie and Little Trapper Lake weirs in 1989. The fish were bled and then flown directly to Whitehorse, Yukon. Thirty fish were sampled from Little Trapper on August 16. Fifteen fish were sent to each of two Whitehorse fish processing firms and subsamples were sent to the CDFO Field Inspection Laboratory in Richmond, B.C. for more detailed analysis. Little Tatsamenie sockeye salmon were sampled twice. The first sample was taken on August 16 and consisted of 30 fish, 15 of which were sent to each of the two Whitehorse fish processing firms. A second sample of three fish was taken on September 2 and sent to the CDFO Field Inspection Laboratory in Richmond, B.C.

Changes in the appearance of the flesh was observed throughout the Little Trapper run. Later migrating fish appeared to be in better condition than earlier migrants. This was likely a result of delayed migration of early-run fish because of abnormally high water conditions. Approximately 90% of the run had passed through the Little Trapper weir at the time the sample was taken there. On the Little Tatsamenie sample dates of August 16 and September 2, approximately 17% and 62% of the run, respectively, had passed through the weir. No obvious temporal differences were observed in the flesh appearance from Little Tatsamenie fish.

The reports received from the two Whitehorse processing firms indicated that the market value of the fish was significantly lower than that of fish harvested on the lower Taku River. Most fish collected were severely blushed but had firm flesh. The meat color ranged from red marbled with white to a complete grey white color. Because of the blushing, one processor suggested that, if marketed as a fresh or fresh-frozen product, the skin would have to be removed.

Samples sent to the Field Inspection Laboratory were analyzed for moisture and fat content. Little Tatsamenie samples had moisture contents ranging from 78.6% to 79.5% and fat content ranging from 0.6% to 0.8%. Moisture and fat content values for the Little Trapper Lake samples ranged from 78% to 79% and 1.0% to 1.6%, respectively. For reference, the fat content of ocean-caught sockeye salmon usually ranges from 6% to 16%.

Taste tests were conducted by both CDFO Field Inspection Laboratory staff and the staff of the processing plants. These tests indicated a hint of a musty flavor. This is common to fish caught in fresh water in an advanced state of maturity.
Roe recovery was approximately 3.5% of the whole weight of the females. The roe was of good quality. Approximately 75% was graded as number 1 quality while 25% was graded as lower number 2 quality.

In summary, fish quality ranged from low domestic grade to utility grade and the fish were judged to be most suitable for smoking or canning; it is unlikely they could be marketed as a fresh or fresh-frozen product.

**Limnological Sampling**

Canada conducted a small amount of limnological sampling in 1989 to expand baseline data on various lakes involved in the transboundary enhancement projects. This work included:

a) Collection of plankton samples from Trapper, Little Trapper, Tatsamenie, Tahlton, and Tuya Lakes at four times throughout the ice-free period. These samples will provide information on seasonal variability in food supply and carrying capacity (sampling has just been concluded and samples still have to be analyzed).

b) Chemical and physical observations of Tahlton Lake water quality were conducted on September 12. These included Secchi depth (water clarity), temperature profiles, and measurement of nutrient levels (total phosphorous and total dissolved solids). Results were very similar to those observed in 1987 and 1988, with extremely clear water and a deep thermocline. Nutrient levels remained fairly high and the lake still appears capable of supporting substantially higher numbers of juvenile sockeye salmon than those that have resulted from natural spawning in recent years.

**Brood Stock Holding Studies at Little Tatsamenie Lake**

A small sample of adult sockeye salmon was captured at the Little Tatsamenie Lake weir on August 25, 1989. Based on the 1989 weir count, 23% of the sockeye run had migrated through the weir prior to August 25. Fish from the sample were held in net pens located near the weir site in the outlet stream to determine mortality rates and the holding period required for maturation. A total of 16 male and 14 female sockeye salmon was collected. Male and female sockeye were held in separate net pens positioned side by side in the stream. The captive fish were examined externally daily and released when they reached spawning condition. Male sockeye matured earlier than the females. All males were released on or before September 10, while the last group of females did not reach spawning condition until September 26. Holding mortality for female sockeye was 1/14 or 7%; mortality in males was higher, 4/16 or 25%. Daily mean water temperatures during this period were between 13°C and 14.5°C the first week, held steady at 13°C the second week, and dropped steadily to 10°C by September 26. Temperatures above 12°C may contribute to increased mortalities.

The results of this study indicate that female brood stock collected in late August and held in net pens in the river may have to be held for up to 33 days before they reach spawning condition. Male sockeye appear to reach spawning condition earlier and may have to be held for approximately 17 days. The potential of holding fish in the lake has not been examined. It is possible lake temperatures may be more suitable than those of the outlet stream.
ADF&G is in the process of establishing a permanent CIF at Port Snettisham. This facility is the critical egg incubation and fry delivery system needed for joint transboundary river sockeye salmon enhancement projects. An interim CIF at Port Snettisham is in use until the permanent facility is completed.

Design of the permanent CIF is near completion. A competitive bid process will be initiated in December 1990 for the construction contract. Construction is scheduled to be complete by the end of 1991. The new CIF should be operational for the 1992 brood. Delays in the construction of this facility have been caused by complications in obtaining permits for construction and in obtaining approvals to administer the construction process.
APPENDICES
Appendix A  Conditions for Canadian Fish Health Transport Approvals

1) The sockeye eggs must be incubated and hatched, and the resulting fry reared in strict isolation from all other fish or eggs on the Port Snnettisham Hatchery site.

2) The hatchery must be inspected and approved by a Canadian Local Fish Health Officer (LFHO) from the Pacific Region. This inspection and approval is required for each year the hatchery is operated as an interim rearing station for Canadian fish stocks.

3) The water supply to the isolation site must be from a fish-free water source.

4) The isolation site must be completely disinfected before any eggs from B.C. arrive on site.

5) The isolation site must be walled and self-contained with a single entrance.

6) No persons other than those authorized by the Port Snnettisham Hatchery manager will be permitted access to the isolation area. All access will be under conditions approved by the Canadian LFHO.

7) All eggs collected in B.C. must be surface disinfected immediately after fertilization. Care must be taken to avoid recontamination of the disinfected eggs by water which is not from a fish-free source.

8) Following hatch of the B.C. eggs, all mortalities in the resulting fry will be carefully recorded and made available to the Canadian LFHO.

9) All shipments of fry returned to B.C. will be tested for IHNV at a 95% probability of detecting a 2% disease incidence, according to protocols outlined in the manual of compliance to the Canadian Fish Health Protection Regulations (CFHPR).

10) No fry will be shipped to B.C. which are undergoing an active outbreak of any of the disease agents listed in Schedule II of the CFHPR.

11) All sex products collected and used in this project must be from wild stock that have been tested for IHNV and BKD at the 95% probability of detecting a 2% disease incidence using the protocols outlined in the manual of compliance to the CFHPR.

12) Sex products will not be taken from any fish showing gross signs (external or internal) of BKD.
In order to implement the Understanding signed February, 1989, where it deals with joint enhancement of the Taku River, the Parties agree:

I. Project Selection:

In 1990, up to 2.5 million sockeye salmon eggs will be taken from each of Tatsamenie and Little Trapper lake stocks. Prior to the 1991 eggtakes, project selection will be reevaluated taking into account experience gained during the initial year of enhancement and further appraisal of alternative projects. Eggs will be incubated at the Port Snettisham central incubation facility. Fry will be backplanted into the agreed lake system.

Eggtakes will continue at a minimum of 5 million eggs per year through 1994. By 1995, the Parties agree to embark upon an expanded enhancement program to maximize sockeye salmon production from Tatsamenie Lake, based upon estimated juvenile rearing capacity, or to develop alternative projects of similar potential, subject to:

a) successful renegotiation by the two Parties of the harvest share of wild and enhanced fish, the new harvest shares to start up when returns from the increased level of production are realized;

b) evidence that enhancement of Taku River sockeye to date has been successful; and

c) expectation of a favorable benefit:cost ratio for both Parties.

II. Harvest Sharing:

i) To avoid impacts on co-migrating stocks and species, exploitation rates applied to Taku River sockeye in existing mixed stock fisheries in Canada and the United States, shall be at levels compatible with the maintenance of wild stocks.

ii) The Parties agree to manage the returns of Taku River sockeye to ensure that each country obtains catches in their existing fisheries equivalent to each country's share of wild sockeye and a 50% share of enhanced sockeye.

iii) In November 1999, the Parties shall review the harvest sharing arrangement for both wild and enhanced fish returning to the Canadian section of the Taku River.
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III. Cost Sharing:

The country on whose soil the work is conducted shall bear the costs of programs associated with producing enhanced fish. The costs of producing these enhanced fish shall be shared as follows:

i) To be paid by Canada:
   a) Egg take;
   b) Egg transport;
   c) Smolt sampling;
   d) Sampling and numerical analysis necessary to determine the contribution of enhanced Taku River sockeye stocks to Canadian fisheries;
   e) Limnology sampling and hydroacoustics; and
   f) Investigations to determine the feasibility of using sockeye from terminal areas, surplus to brood stock and spawning requirements in enhanced systems, for cost recovery purposes.

ii) To be paid by the United States:
   a) Construction and operation of that portion of the Port Snettisham central incubation facility that is dedicated to enhancement projects on the transboundary rivers;
   b) Transport of fry to the enhancement site;
   c) Sampling and analysis necessary to determine the contribution of the enhanced transboundary river sockeye salmon to the United States fisheries; and
   d) Processing of sockeye otolith samples collected in the Taku River.

iii) Projects to be funded jointly:
   a) Disease sampling and analysis; and
   b) Identification and evaluation of alternative sockeye salmon enhancement opportunities in the Taku River.
Appendix C  Details of Disease and Parasite Sampling of Indigenous Fish in Tuya and Tatsamenie Lakes

**TUYA LAKE**

Arctic Grayling (*Thymallus arcticus*): 31 fish were collected from Tuya Lake.

External Examination: Two fish had hemorrhagic lesions near the tail fin.

Internal Examination: Postmortem change did not allow proper evaluation.

Bacteria: *Mycobacteria*, a relatively uncommon bacteria, were found in Ziehl-Nielson stain smears of the external lesions. *Mycobacteria* were also found in kidney smears of 7 of the 31 fish examined.

*Renibacterium salmoninarum*, the causative agent of Bacterial Kidney Disease, were found in kidney smears of 19 of the 31 fish.

Virus: No cytopathic effects were observed in tissue cultures.

Parasites: Ten fish were examined for parasites. No abnormal parasites or unusual levels of parasite infection were detected. *Myxobolus spp.*, a protozoan, was found in the brain tissue of all 10 fish. This is a commonly occurring parasite which is not known to affect the health of the fish and does not affect its food quality. *Diphyllobothrium* (tapeworm) was found in the viscera of all 10 fish.

Comment: All fish analyzed were in post-spawning condition.

Dolly Varden (*Salvelinus malma*): 10 fish were collected from Tuya Lake.

External Examination: No abnormalities were observed.

Internal Examination: Postmortem change did not allow proper evaluation.

Bacteria: Low numbers of *Renibacterium salmoninarum* were found in the kidneys of 2 fish.

Virus: No cytopathic effects were observed in the tissue cultures.

Parasites: Four fish were examined for parasites. No unusual parasites or abnormal levels of parasite infection were detected. *Myxobolus spp.* was found in brain tissue of 2 fish. Two types of tapeworm, *Diphyllobothrium* (2 fish) and *Eubothrium salvelini* (2 fish) were found on the viscera and in the intestine, respectively. *Myxidium* (a Myxosporidian) was found in the urinary bladder of 2 fish.

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TUYA LAKE (Cont.)

Dolly Varden (cont.)

Comment: Two fish were pre-spawners. The remainder were likely post-spawners; however this could not be confirmed because of post-mortem changes.

Longnose Suckers (*Catostomus catostomus*): 12 fish were collected from Tuya Lake.

External Examination: No abnormalities observed.

Internal Examination: No abnormalities observed.

Bacteria: No bacterial pathogens were found.

Virus: No viral assay was done.

Parasites: Four fish were examined; no parasites were found.

LITTLE TATSAMENIE LAKE

Lake Trout (*Salvelinus namaycush*): 19 fish were collected from Little Tatsamenie Lake in a spawning area located near the outlet.

External Examination: No abnormalities were observed.

Internal Examination: No abnormalities were observed.

Bacteria: No significant bacteria were found.

Virus: No cytopathic effects were observed in tissue culture.

Parasites: All 19 fish were examined. All had heavy infections of *Diplostomum sp.* metacercaria in the eyes and *Tetracotyle sp.* metacercaria on the pericardium of the heart (both are Trematode worms). *Myxobolus articus* was found in the brain tissue of 7 fish. These parasites are common in wild fish.

Comments: All fish were in spawning or post-spawning condition.

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LITTLE TATSAMENIE LAKE (Cont.)

Dolly Varden (Salvelinus malma): 10 fish were collected from Little Tatsamenie Lake.

External Examination: No abnormalities observed.

Internal Examination: No abnormalities observed.

Bacteria: No significant bacteria were found.

Virus: No cytopathic effects were observed in tissue culture.

Parasites: Eight fish were examined. *Eubothrium salvelini* was found in the ceca-intestine of 7. *Tetracotyle sp.* metacercaria were found in the heart of one fish and *Diplostomum sp.* in the eyes of another. These parasites are common in wild fish.

Comment: One specimen was in post-spawning condition; state of maturity of the remainder was not determined.