2006 LITTLE TRAPPER LAKE SOCKEYE EGG TAKE

Prepared for:

1) Pacific Salmon Commission

Transboundary Panel

2) Department of Fisheries and Oceans

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1.0 INTRODUCTION

The 2006 sockeye egg take at Little Trapper Lake was initiated as part of proposed project to establish a self-sustained anadromous sockeye population in Trapper Lake. The objectives of the proposed project are contained in previous feasibility and risk assessment reports (Mercer 2005, 2006). It was proposed that Little Trapper Lake sockeye stock would be used as the donor stock to re-colonize Trapper Lake with anadromous nerkids. Little Trapper Lake sockeye were previously used as a donor stock for a sockeye enhancement project at Trapper Lake from 1990 –1994 (PSC 1998). This project is funded by the Northern Fund of the Pacific Salmon Commission.

1.1 Description of the System

Little Trapper Lake is located in the Taku River system at the headwaters of Kowatua Creek, a tributary of the Inklin River (Figure 1). The Taku River drainage encompasses approximately 45,000 sq. km. of which 97% is in Canada. The Taku River empties into Taku Inlet in Southeast Alaska.

1.2 Little Trapper Lake Sockeye Stocks

Fisheries and Oceans Canada has contracted operation of a sockeye enumeration weir at the outlet of Little Trapper Lake from 1983 through 2006. During this period annual spawning escapements have averaged 12,860 (range 6,000 – 31,000). It is estimated that the Little Trapper sockeye stocks contributed 21% or 53,340 annually (1994-2003 average) to the total Taku River sockeye run size (TTC 2004). The Little Trapper Lake origin sockeye begin their migration up the Taku River starting mid-June continuing through to the latter part of August, with peak numbers passing through the Little Trapper Lake weir during the first week in August. Mean travel time from the Taku Inlet to Little Trapper Lake is approximately 30 days. The condition of returning sockeye at the weir site ranges from bright to sexually mature, however the majority of fish entering the lake show evidence of maturation.

Little Trapper Lake sockeye spawn in the inlet stream connecting Little Trapper and Trapper lakes, with 90% of the spawning within 1 km of the inlet. Spawning occurs from mid August through September with peak spawning typically occurring during the last week in August and first week in September.

2.0 OBJECTIVES

The objectives of the 2006 Little Trapper Lake egg take were the following:

1. To capture broodstock to obtain 1.1 million fertilized eggs or 30% of the spawning escapement, whichever is less; sufficient males for a 1:1 spawning ratio to be taken.

2. Conduct killing, spawning and fertilization procedures according to revised ADF&G 1994 Infectious Haematopoietic Necrosis Virus (IHNV) disinfection/avoidance protocol.

3. Collect 120 samples of ovarian fluid at peak spawning for IHNV detection and deliver to Alaska Department of Fish and Game (ADF&G) in Juneau.

4. Collect 120 kidney samples (posterior and anterior) for Bacterial Kidney Disease (BKD) sampling and deliver to ADF&G in Juneau.

5. Transport fertilized water-hardened eggs by float plane or helicopter to the Snettisham hatchery, Alaska; the eggs to be transported in coolers as prescribed by ADF&G protocol.

6. Maintain records detailing:
   (a) Number of males and females captured for broodstock
   (b) Number of fish retained for spawning by sex
   (c) Number of pre-spawning mortalities
   (d) Fish culture procedures
   (e) Time and method of transport
   (f) Ambient air and water temperature and egg temperature during transport.

3.0 METHODS

3.1 Mobilization

Required supplies, and materials were purchased in Vancouver and Whitehorse and shipped to Atlin. Personnel, materials, and supplies were transported to the project site via the contractors' aircraft. Egg take personnel stayed at the contractors residence at Little Trapper Lake.

3.2 Broodstock Capture, Transport, and Holding

Broodstock capture began on August 24 and continued through to September 3. The sockeye were captured in Little Trapper Lake at the mouth of the inlet stream. Capture was accomplished using a 100 m long and 8 m deep seine net set from the front of a boat.

All potential captured broodstock were examined and either released in the lake or placed in a transport tub in a boat. Although selection criteria required sockeye to be free of wounds and fungus, all suitable fish in varying stages of sexual maturity were retained. The transport tub contained 200 litres of water to which was added 8 ml of "Aquacalm" (metomidate hydrochloride), resulting in a transport solution of 1 ppm. In the above concentration, "Aquacalm" has a sedative effect on the transported fish that lasts up to 24 hours after application. Use of this tranquilizer facilitated ease of handling, reduced stress to the fish, and was found in previous sockeye transport/holding projects to reduce overall pres-pawn mortality.

During transport the fish were supplied with supplementary oxygen by administering bottled oxygen at a rate of 0.5 l/min. via tygon tubing and a ceramic diffuser stone positioned on the bottom of the transport tub.

Broodstock were transported upstream to floating net pens anchored approximately 10m from the west shore and 100m from the lake inlet. Distance from the capture site to net pens was approximately 100 Approximately 20 - 25 fish were transported per trip.

Two floating net pens were assembled and used during the broodstock capture operation. One pen measured 3m x 7m x 2.2 m deep, and 1 pen measured 3m x 3m x 2.2m deep. The pens were constructed using floating frames that were made by laminating 4 cm Styrofoam between 2 pieces of 5 cm x 15 cm lumber. The upper margins of the knotless nylon net pens were fastened to the pen frames using fencing staples. Blue polyethylene tarps covered the pens and were secured with boards along the frame rim. The pens were anchored over a depth of about 10m and total pen volume was 65 m^3. Maximum broodstock holding densities were approximately 10/fish $meter^3$.

3.3 Sorting, Spawning, and Fertilization

The held broodstock were sorted by towing the net pens into shallow water near shore using a boat, removing the cover, and crowding the held fish into one end of the pen. The pen was then partitioned by sliding a pole under the net and securing it to the top of the net frame. Ripe, sorted fish were transferred to the smaller net pen in preparation for spawning the following day. The pens containing

the broodstock was re-covered and towed out into deeper water and secured to an anchored float.

A 3.5 m x 3.5 m "weatherall" tent was erected to serve as a spawning facility. Tables to hold ovadine solutions, water, coolers, and egg cups were positioned around the inside perimeter of the structure. Killing, spawning, and fertilization was conducted according to current IHNV avoidance protocol as described in the ADF&G, *Alaska Sockeye Salmon Culture Manual, 1994*. Ripe females were retrieved from the pen, killed with a club and dipped in a 200 ppm ovadine solution; the ventral area of each fish was scrubbed with a brush. The fish were then hung on a rack, and bled through a knife cut to the carotid artery. The ventral area was then dried with a paper towel. A few eggs were expelled prior to stripping the eggs into a 1.2 litre Styrofoam container. One container was used per female, with only perfect loose eggs retained. Hands and all related equipment were disinfected prior to handling and processing each fish. As well, clean paper towels were used for each fish.

After being killed, males were dipped in a 200 ppm ovadine bath, the ventral area scrubbed with a brush, and the fish hung on a rack. The belly and vent area were then wiped dry. A small quantity of milt was expressed to avoid potential contamination with iodophor. Milt from each male was expressed into 2 egg containers, each containing the eggs from 1 female. The process was repeated with a second male thus resulting in the fertilization of each female with two males. The egg container was gently swirled to mix eggs and milt, and adding approximately 200 ml of virus free water then activated the sperm. A lid was placed over the cup and the cup inverted to thoroughly mix the milt, water, and egg combination.

The eggs, milt, and water mixture was left for approximately 1 minute to ensure fertilization. The activation water was decanted and a 100 ppm ovadine solution added to fully cover the eggs. The eggs and ovadine were gently mixed and the solution then drained. Ovadine solution was again added to the container to cover the eggs with 20mm to 25mm of solution. If the presence of coagulated milt, blood, or other organic matter was observed the eggs were further rinsed a second or third time with the ovadine solution. Each container was labeled with a consecutive number and the time, and the eggs then left to water harden for at least one hour.

After water hardening the ovadine solution was poured off and the water hardened eggs poured into a cooler lined with a plastic bag. The eggs from 15 - 18 females were placed into each cooler. Approximately 4 to 5 litres of virus free water were added to each bag covering the eggs to a depth of 40mm to 50mm. The bags were twisted closed and sealed with a tight fitting elastic band. If required, crushed ice was placed under and around the bags in each cooler. Labels were placed in each cooler detailing time of pooling and number of females. The coolers were stored in the shade until transport.

Average fecundities for each egg take were determined on site. This was accomplished by weighing the

pooled eggs from 10 females, extracting a small sub sample (150 - 200 eggs), weighing and counting the eggs in the sub sample, and expanding this number for the pooled weight of the eggs from all 10 females. A 10 kg spring scale was used to weigh the pooled eggs, and a 300 g. electronic balance was used for weighing the sub-sample. The fecundities of 3 or 4 lots of 10 females from each egg take were determined to estimate an average fecundity.

Virus free water for milt activation and pooling of eggs, as well as ice for cooling was supplied by Snettisham Hatchery. Water for the ovadine solutions used in the water hardening process was obtained from the Trapper Lake inlet stream.

3.4 Egg Transport

Three egg take flights were conducted using the contractors Maule aircraft. All egg take flights were conducted on the same day as the egg take with all fertilized eggs being delivered on the same day they were removed from the fish.

3.5 Disease Sampling

One hundred kidney samples were taken for BKD analysis over 2 egg takes (Table 1). Anterior and posterior kidney tissue samples were collected from each sampled fish using a sterilized scalpel, and placed in individual whirlpak bags. Sampling instruments were disinfected with a 300 ppm ovadine solution and wiped dry between each fish. A 50/50 male to female ratio was sampled.

Ovarian fluid samples were obtained during egg takes two and three. These were obtained using a 7 ml. disposable pipette inserted into the egg container and withdrawing ovarian fluid that had settled to the base of the container. The ovarian fluid was then transferred to a labeled centrifuge tube. A total of 113 ovarian fluid samples were collected (Table 1).

All IHNV and BKD samples were stored before and during transport in a small cooler containing ice, and were delivered to Snettisham hatchery along with the egg shipments. From Snettisham the samples were forwarded to the ADF&G Fish Pathology Lab in Juneau.

4.0 Demobilization

All materials and equipment remain at Little Trapper Lake. The net pen frames, spawning shed, and vexar pens were dismantled and returned to the contractors residence. Egg transport coolers, related

equipment, net pens, and miscellaneous gear were dried and stored in the contractors cabin at the camp site.

4.0 RESULTS

4.1 Little Trapper Lake Weir Operation

Compilation and interpretation of the 2005 Little Trapper Lake sockeye enumeration weir data will be performed by DFO Whitehorse. The following is a brief summary of the 2006 weir operation results.

Summary results of the 2006 Little Trapper Lake weir operation are listed in Table 2. A total of 25,139 sockeye were counted through the weir. Available spawning escapement was 24,431 after 708 fish were removed for broodstock. The run timing and pattern was somewhat later than that of past years. A representative sample of 750 sockeye was sampled for sex, scales, and length during the course of the weir operation. A weighted preliminary sex ratio obtained from sampling results and weighted by week indicated that 40% of the run were females (10,056) and 60% males (Table 1). The taking of 396 females for broodstock would leave approximately 9,650 females for natural spawning. An additional 60 females and 11males were released un-spawned on September 15 at the end of the project. The released fish were not sexually mature and were considered to be in relatively good condition

4.2 Broodstock Capture and Holding

A total of 708 sockeye (310 males and 398 females) were captured and placed in net pens. This represented 2.8% of the total escapement into Little Trapper Lake. However, the weir sampling weighted by week indicated that 10,556 females passed through the weir. Therefore, the 398 held females comprised approximately 3.9% of the total estimated number of female sockeye entering the system in 2006.

Broodstock capture occurred over 10 days from August 24 through September 3. The fish were held over a period of approximately 15-20 days, ranging from August 23 to September 15. The above average escapement into Little Trapper Lake in 2006 should have made broodstock capture relatively easy. Knowing the escapement was large it was decided to refrain from broodstock capture until the latter part of August. This tactic was employed to reduce the broodstock maturation holding time. However a significant flood event[1] occurred in the system just before broodstock capture was scheduled to occur. Typically large numbers of sockeye hold at the inlet stream mouth prior to entering the

spawning stream. Because of the very high flow and volume of the inlet stream fewer fish than expected were immediately available for capture. In addition large numbers of spawned out and partially spawned sockeye had been flushed out of the inlet stream. As a result, broodstock capture took longer than anticipated.

Female and male pre-spawn mortality was 2(0.5%) and 4(1.0%) of held fish, respectively (Table 1). Both the female and male broodstock pre-spawn mortality rate was considered low relatively to other similar projects where broodstock are penned to wait for sexual maturation.

4.3 Spawning and Fertilization

The spawning and egg transport summary is listed in Table 1. A total of 336 females and 295 males were
spawned over 3 egg takes. The average measured fecundity was 3,400. The mean calculated fecundity was used to determine the number of eggs shipped on that date. Using this method, it was estimated that 1,142,000 green eggs were delivered to Snettisham Hatchery. Subsequent to shocking and picking the measured mean fecundity determined by Snettisham Hatchery was 3,342 (Appendix 2).

Fertilization rates (2-4 cell survival as determined by Snettisham Hatchery) ranged from 96% to 100%, with a non-weighted average over the three egg takes of 93.0%. At the time of this report preparation the survival to 100 CTU's ranged from 87% to 96% with a non-weighted average of 92%. This fertilization rate is similar to that obtained from other Transboundary Egg take projects. After shocking and picking the measured survival rate was 89.7% resulting in approximately 995,000 fertilized eggs currently incubating at Snettisham Hatchery (Appendix 2). .

4.4 Egg Transport

All egg transport flights were conducted using fixed wing aircraft. Efforts were made to deliver the fertilized eggs to Snettisham on the same day the fish were spawned and this was accomplished for all egg takes.

4.5 Disease and Otolith sampling

The 2006 IHNV and BKD, and otolith sampling regime are presented in Table 1. One hundred kidney (BKD detection) samples and 113 ovarian fluid (IHNV) samples were collected and sent to Snettisham to be forwarded to the Fish Pathology Lab in Juneau. The results of the 2006 Little Trapper Lake

[1] The flood event that occurred, although not unprecedented, was atypical of prevailing water conditions normally

sockeye pathology screenings are presented in Appendix 1.

A total of 0 of the 100 BKD samples tested positive for *Renibacterium solarium*. Typically the Little Trapper Lake sockeye stock has a relatively low prevalence of BKD, ranging from 0.7% to 13.2%. (PSC 1998). The prevalence of the IHN virus detected in the 2006 Little Trapper Lake broodstock was 57/112 (50.9%) of the samples containing > 10^4 PFU's. This proportion of IHNV positive fish in the Little Trapper Lake stocks is considered to be in the mid-range compared to past pathology screenings. The virus prevalence has ranged from 13% to 97% when tested during the period 1990 through 1994 (PSC 1998).

5.0 Discussion

The objectives of the 2006 egg take at Little Trapper Lake were achieved. Due to flood events, broodstock capture took longer than anticipated. However, the egg take schedule was not delayed and maturation of the broodstock occurred as anticipated. Holding mortality was negligible. Scheduling of egg takes to coincide with weather conducive to flying to the hatchery resulted in the egg deliveries occurring on the same day the eggs were taken. The fertilization rates of the delivered eggs were somewhat below expected but the percentage of live seeded eggs is well within the expected norm. If required, it is anticipated the 2006 egg take project goals could be repeated in the future.

encountered at that time of year.

Table 1. Little Trapper Lake 2006 egg take summary.

Egg Take No.	1	2	3
Broodstock Sort Date	Sept. 8	Sept.13	Sept. 15
Egg Take Date	Sept. 9	Sept. 14	Sept. 16
Date Eggs Delivered	Sept. 9	Sept. 14	Sept. 16
# Females Spawned	106	117	113
Estimated Fecundity	3,400	3,400	3,400
# Eggs Delivered	360,400	397,800	384,200
Cum. # Eggs Delivered	360,400	758,200	1,142,400
Fertilization Rate	96.0%	94.0%	95.0%
# Viable Eggs Delivered	345,984	373,932	364,990
Cum # Viable Eggs	345,984	719,916	1,084,906
Cum. Male Prespawn Mort.	2	3	4
Cum. Female Prespawn Mort.	1	1	2
# BKD Samples	0	50	50
# IHNV Samples	0	97	16
Water Temp.	9.0	9.0	8.0
Air Temp. (min)	7	8	5
Transport Method	Maule	Maule	Maule

No. Females Spawned	336	No. Males Held	310
No. Males Spawned	295	No. Females Held	398
Average fecundity	3,342	No. Females Released Unspawned	60
# Eggs To Hatchery	1,142,400	No. Males Released Unspawned	11
# Fertilized Eggs to Hatchery	1,073,856	Male Prespawn Mortality	1.3%
(Based on 2-4 cell % surv.		Female Prespawn Mortality	0.5%
estimates by Snettisham)		Avg. Fertilization Rate	95%

Total weir count	25,139
Total female escapement at weir (weighted sampling)	10,056
Number females removed for broodstock	398

Table 2. Little Trapper Lake 2006 weir count.

Date	Daily Count	Cum Count	Sampled Daily	Sampled Cum	Daily Tags Recovered	Cum Tags Recovered	Daily Observed	Cum Observed	Cum Tags
27-Jul	0	0	0	0	0	0	0	0	
28-Jul	0	0	0	0	0	0	0	0	0
29-Jul	0	0	0	0	0	0	0	0	0
30-Jul	0	0	0	0	0	0	0	0	0
31-Jul	0	0	0	0	0	0	0	0	0
1-Aug	154	154	0	0	2	2	0	0	2
2-Aug	446	600	20	20	1	3	2	2	5
3-Aug	268	868	20	40	3	6	1	3	9
4-Aug	1077	1945	70	110	14	20	2	5	25
5-Aug	824	2769	70	180	11	31	3	8	39
6-Aug	1256	4025	50	230	17	48	3	11	59
7-Aug	1536	5561	70	300	19	67	3	14	81
8-Aug	2509	8070	70	370	28	95	9	23	118
9-Aug	2707	10777	70	440	34	129	13	36	165
10-Aug	2015	12792	60	500	36	165	12	48	213
11-Aug	1261	14053	30	530	22	187	9	57	244
12-Aug	2104	16157	40	570	31	218	24	81	299
13-Aug	1564	17721	20	590	27	245	14	95	340
14-Aug	775	18496	10	600	14	259	8	103	362
15-Aug	739	19235	20	620	15	274	10	113	387
16-Aug	157	19392	20	640	7	281	2	115	396
17-Aug	400	19792	20	660	13	294	6	121	415
18-Aug	1407	21199	0	660	30	324	14	135	459
19-Aug	573	21772	30	690	16	340	7	142	482
20-Aug	247	22019	0	690	7	347	1	143	490
21-Aug	418	22437	20	710	8	355	2	145	500
22-Aug	318	22755	0	710	13	368	1	146	514
23-Aug	303	23058	0	710	7	375	3	149	524
24-Aug	175	23233	10	720	4	379	1	150	529
25-Aug	56	23289	10	730	7	386	0	150	536
26-Aug	415	23704	10	740	2	388	7	157	545
27-Aug	279	23983	10	750	4	392	4	161	553
28-Aug	101	24084	0	750	2	394	0	161	555
29-Aug	438	24522	0	750	7	401	5	166	567
30-Aug	392	24914	0	750	10	411	3	169	580
31-Aug	195	25109	0	750	3	414	0	169	583
1-Sep	30	25139	0	750	0	414	0	169	583
2-Sep		25139		750		414		169	583

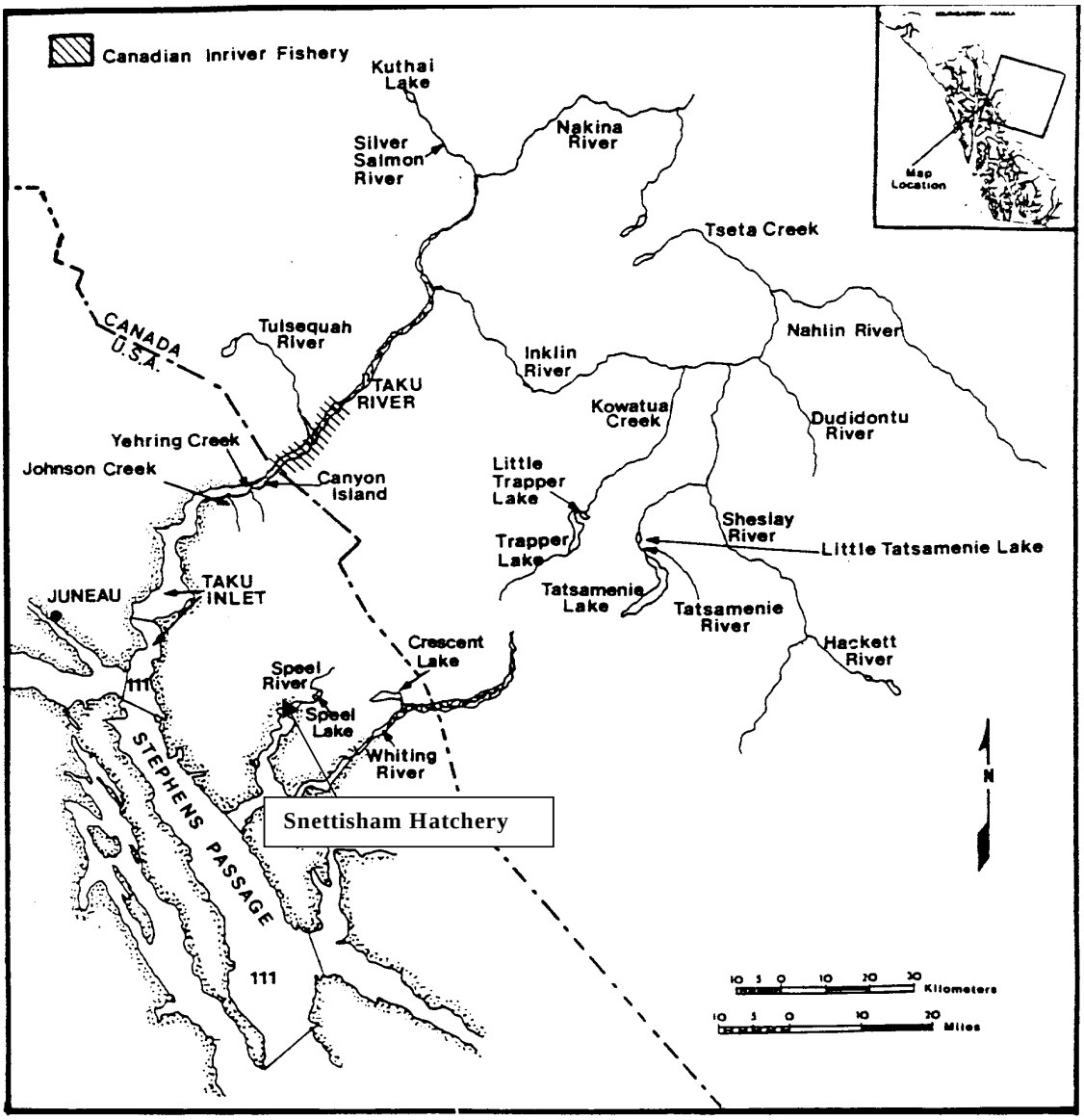

Figure 1. Location of Little Trapper Lake in Taku River watershed.

Appendix 2.

ACCESSION NO: 07-0517

ALASKA DEPARTMENT OF FISH AND GAME
JUNEAU FISH PATHOLOGY LABORATORY, CF DIVISION
3333 Old Glacier Highway - PO Box 25526, Juneau, AK 99802-5526
Phone: (907) 465-3577

REPORT OF LABORATORY EXAMINATION

LOT (YEAR, STOCK, SPECIES): Little Trapper Lake sockeye salmon *Oncorhynchus nerka*

FACILITY: Snettisham Hatchery

CONTACT PERSON/ADDRESS: Rick Focht, DIPAC, 2697 Channel Drive, Juneau, AK 99801

SAMPLE DATE: 9/13, 9/16/06 **DATE SAMPLE RECEIVED:** 9/15, 9/23/06

SPECIMEN TYPE: Kidney tissues (K)/ovarian fluids (OF) **LIFE STAGE:** Adult **STATE:** Refrigerated/Frozen

NUMBER IN SAMPLE: 50 (K), 112 (OF) **WILD:** Mixed wild and transplants

HISTORY/SIGNS: Prevalence of IHNV and Rs bacteria detected in this sockeye salmon stock

REASON FOR SUBMISSION: TBR agreement to examine for IHNV and the Rs antigen by ELISA

FINAL REPORT DATE: 10/16/06

CLINICAL FINDINGS:

ELISA: 0/50 kidney tissues positive for *Renibacterium salmoninarum* (Rs). Mean optical density values ≥ 0.060 were considered positive for the Rs antigen.

VIROLOGY: 57/112 (50.9%) positive for IHNV. Ovarian fluids processed by plaque assay on EPC cells at 15°C for 7 days. The minimum level of detection was 10 PFU/ml of sample. The last 16 samples were received frozen

TITER DISTRIBUTION

Titer	Neg	101	102	103	104	105	106	>107
# of fish	55	8	15	5	6	4	6	13
% of fish	49.1	7.1	3.4	4.5	5.4	3.6	5.4	11.6

Titers ^{3} 104 = 29/57 (50.9%) of positive fish

COMMENTS/RECOMMENDATIONS:

None of the kidney tissues submitted were positive for Rs antigen.

Half of the ovarian fluid samples were positive for presumptive IHNV with over 50% of virus-positive fish having high titers indicating a moderate risk for vertical transmission of IHNV. The last 16 ovarian fluids collected on 9/16 were received frozen which may have compromised the sensitivity of virus isolation.

When last examined from 1990 through 1994 adult prevalences of IHNV in this sockeye salmon stock ranged from 13% to 97% while Rs prevalences ranged from 0.7% to 13%.

FISH HEALTH INVESTIGATOR(s): T.R. Meyers

TECHNICAL ASSISTANCE: I. Conte, R. Young

Appendix 2. Summary of Little Trapper Lake egg receipts at Snettisham Hatchery, 2006.

EGG PICKING SUMMARY

MODULE IM-6

Hatch Code **6** / RBr code **1:1.6** Prehatch Image **I I I I I I**

EGGTAKE DATE	DATE RECIEVED	EGG LOT NUMBER	INCUBATOR NUMBER	SHOCK DATE	SHOCK CTU'S	PICK DATE	KG LIVE	EGGS/ KG	TOTAL LIVE	KG DEAD	EGGS / KG	TOTAL DEAD	TOT. GRN EGGS	% SURV.	# OF FEM.
09/09/06	09/09/06	TRAP 01	2a	10/23/06	314.2	10/24/06	20.284	7,080	143,611	3.443	7,480	25,754	169,364	84.8	52
09/09/06	09/09/06	TRAP 01	2b	10/23/06	314.2	10/24/06	22.092	7,330	161,934	2.557	7,730	19,766	181,700	89.1	52
09/13/06	09/13/06	TRAP 02	3a	10/25/06	300.1	10/26/06	25.261	7,210	182,132	2.537	7,440	18,875	201,007	90.6	61
09/13/06	09/13/06	TRAP 02	3b	10/25/06	300.1	10/26/06	22.266	7,350	163,655	2.526	7,580	19,147	182,802	89.5	54
09/16/06	09/16/06	TRAP 03	4a	10/30/06	315.2	10/31/06	24.697	7,180	177,324	1.841	7,280	13,402	190,727	93.0	59
09/16/06	09/16/06	TRAP 03	4b	10/30/06	315.2	10/31/06	23.626	7,030	166,091	2.388	7,410	17,695	183,786	90.4	54
Totals & Averages					**309.8**		**138.226**	**7,197**	**994,747**	**15.292**	**7,487**	**88,886**	**1,109,386**	**89.7**	**332**