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# CLE ELUM LAKE SOCKEYE SALMON RESTORATION FEASIBILITY STUDY, 1987 - 1988

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CLE ELUM LAKE SOCKEYE SALMON RESTORATION  
FEASIBILITY STUDY, 1987 - 1988

by

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## ABSTRACT

In 1986, a multi-year project to evaluate the biological feasibility of re-establishing anadromous sockeye salmon (Oncorhynchus nerka) runs to Cle Elum Lake in the Yakima River Basin was established between the Bonneville Power Administration (BPA) and the National Marine Fisheries Service (NMFS). This program involves the capture, spawning, and rearing of disease-free donor stock in 1987 and 1988 and assessment of juvenile outmigration and survival from Cle Elum Lake in 1989 and 1990.

Work in 1987-1988 involved collection of adult sockeye salmon from the Lake Wenatchee run and incubation and rearing of progeny as donor stock. In July 1987, 263 adults were captured at the Dryden fishway on the Wenatchee River and transferred to net-pens in Lake Wenatchee. Adults were held approximately 90 days and spawned, and the eggs were transferred to a quarantine hatchery. Pre-spawning survival was 95.1%, and all spawners were certified as being free of Infectious Hematopoietic Necrosis (IHN) and other replicating viruses. Egg viability averaged about 40%; however, eyed egg to hatch survival was over 99%.

Juveniles are being reared in quarantine, and survival to date is about 92%. The NMFS currently has over 131,000 fry (0.7 g average weight) in culture. Fry have been certified twice (at 0.12 g and 0.25 g average weight) as being free of IHN and other replicating viruses. Viral certification will continue throughout rearing.

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## INTRODUCTION

The purpose of the Cle Elum Lake Study is to assess the feasibility of restoring anadromous sockeye salmon (Oncorhynchus nerka) to the Yakima River Basin. The study focuses on Cle Elum Lake; however, the information obtained should be applicable to other irrigation storage systems within the basin. This program is a cooperative effort between the National Marine Fisheries Service (NMFS) and the Bonneville Power Administration (BPA). The initial phase of the program concentrates on providing disease-free juveniles for research, assessment of juvenile outmigration from Cle Elum Lake, and evaluation of downstream survival through the Yakima River system.

Historically the Yakima River Basin supported large runs of anadromous sockeye salmon that contributed significantly to the Columbia River harvest (Robison 1957; Mullan 1986). The development of irrigation storage reservoirs without fishways during the early 1900s eliminated these runs. However, considerable spawning and rearing habitat still exist above the four lakes on the Yakima River system that historically supported runs of anadromous sockeye salmon (Cle Elum, Kachess, Keechelus, and Bumping). Reintroducing self-sustaining anadromous sockeye salmon to these areas would significantly enhance the basin's salmonid resources.

The present program uses a modification of captive brood-stock rearing concepts developed by NMFS for restoration of threatened runs of Atlantic and Pacific salmon (Harrell et al. 1984a, 1984b, 1985). Whereas, the other NMFS brood-stock programs have centered on rebuilding depleted gene pools, no anadromous sockeye salmon presently exist in the Yakima River Basin. The Wenatchee River Basin (adjoining the Yakima Basin) has a viable anadromous sockeye salmon run with an annual spawning population of approximately 25,000 adults. Lake Wenatchee also has many geographical and limnological similarities

to the lakes of the Yakima River Basin. Therefore, returning adult sockeye salmon from the Wenatchee Basin were selected as a suitable donor stock for Cle Elum Lake.

The program began in 1987, and initial work included a literature and habitat survey, design and construction of a net-pen system for holding adults, and construction of a interim quarantine egg incubation and fry rearing facility (Slatick et al. 1988). Work during 1988 included brood-stock collection and spawning, egg incubation, and fry rearing. Outmigration studies (lake survival and downstream passage) will be conducted during the spring of 1989 and 1990, and recommendations for run restoration will be presented in 1991.

#### DESCRIPTION OF STUDY AREA

Returning adult sockeye salmon were captured at the Dryden fishway on the Wenatchee River, transported to Lake Wenatchee, held to maturity in floating net-pens, spawned, and gametes transferred to the NMFS Montlake Quarantine Hatchery in Seattle, WA (Fig. 1). All spawners were surveyed for the presence of Infectious Hemopoietic Necrosis (IHN), and all eggs were incubated in an isolated quarantine system that provided the ability to discard individual lots if a group was determined to be positive for disease. Fry are being reared and resulting smolts will be used in studies to assess outmigration potential from Cle Elum Lake and downstream survival through the Yakima River (Fig. 1). Adult collection will be repeated during 1989, and outmigration assessment is planned for spring 1989 and 1990.

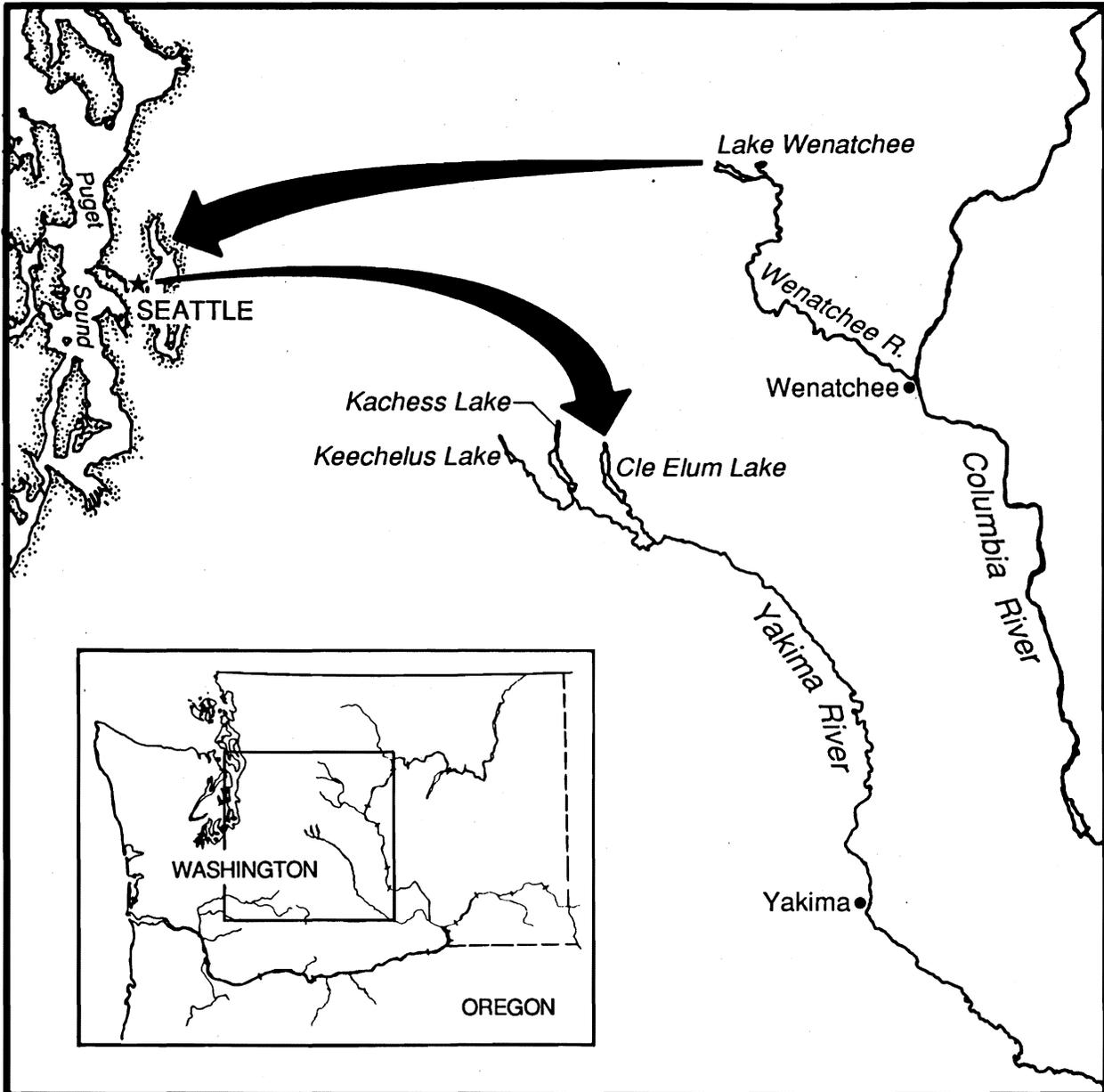


Figure 1.--Movement of salmon gametes from cage system in Lake Wenatchee to NMFS quarantine facility at the Montlake Laboratory. Arrow pointing east indicates reintroduction of disease-free juvenile sockeye salmon to Cle Elum Lake in the Yakima River Basin.

## MATERIALS AND METHODS

## Adult Collection and Holding

The net-pen system designed for holding adult sockeye salmon (Slatick et al. 1988) was installed in Lake Wenatchee (Chelan County, WA) by NMFS personnel in late June 1987. Initial siting was off-shore from U.S. Forest Service Facilities at Dirty Face Campground on the 12- to 15-m depth contour (Southwest 1/4, Section 13, Township 27 North, Range 11 East), but wind and wave conditions necessitated moving the pen nearer the north end of the lake. The pen was relocated on the 12- to 15-m depth contour between Cougar Inn and the mouth of the White River (Northwest 1/4, Section 14, Township 27 North, Range 16 East) in mid-August and remained in this location through the end of the collection and holding period in mid-October 1988. It was then removed and stored for the season. The NMFS received a Washington Department of Fisheries (WDF) Hydraulic Permit (WDF Log FNWC5TN-02, WRIA 45) and a Chelan County Planning Department determination of SEPA exemption for the 1987 siting of the two-module (4.8 by 4.8 by 7.5 m deep) pen-system.

Engineering staff from Chelan County Public Utility District instructed NMFS personnel in the use of the denil ladder and fish trapping facility at Dryden Dam on the Wenatchee River (Fig. 2), and returning adult sockeye salmon were collected during July. The fish were transported in a 6,000-liter fish transportation truck to Lake Wenatchee (Fig. 2) and transferred via a 9-m long fish hauling barge to the floating net-pens. The NMFS was issued a WDF Scientific Collection Permit (Number 87-85) and a WDF Fish Transfer Permit (Number 332-6-87) for the collection and transportation of these fish.

Maturing adults were held in the net-pen system and fed a maintenance diet of frozen krill (Euphausia pacifica) at 0.05% of body-weight/day or less.

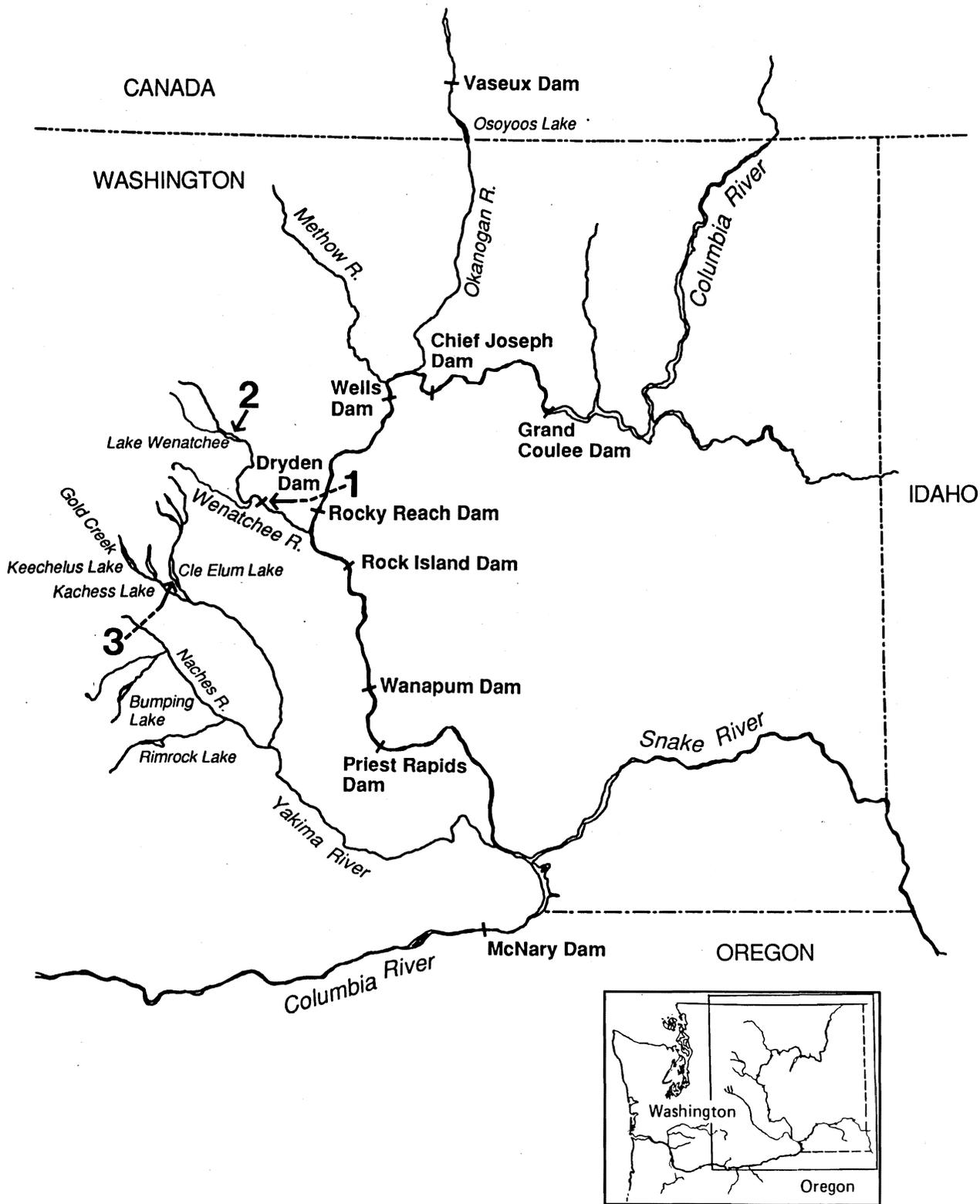


Figure 2.--Map of sockeye salmon project study area in the Mid-Columbia Basin. Arrows indicate: (1) Dryden Dam, (2) Lake Wenatchee, and (3) Cle Elum Lake.

## Spawning

The fish were sorted (visually) according to stages of maturity during mid-to-late September. To inspect potential spawners, a net-pen was raised gradually (at approximately 2.5 m/5 min) to a final depth of about 1.5 m. All fish were crowded to one side, and the pen was divided into halves. Fish were lifted by the caudal peduncle and checked for ripeness. For female fish, gentle pressure was applied anterior to the vent to determine looseness of the egg mass and, if determined to be ripe, a few eggs were expressed to visually examine egg quality. Males were checked in a similar manner to determine if milt was mature. Mature females with free-flowing eggs were loaded onto a transport barge into one section of a divided holding tank of about 25 m<sup>3</sup>, and mature males were placed in the other section of the tank. Fish not yet mature were immediately returned to the unoccupied section of the net-pen. The transport barge was then moved to the beach.

At the beach, females were killed by a blow to the head, bled by cutting the gill arches, and disinfected for 3 to 5 min in 100 ppm iodophor. Gravid females were put into individually numbered plastic bags and placed in large (approximately 0.9 m by 1.2 m by 0.6 m) totes containing crushed ice. Spawning males were anesthetized and disinfected for 3 to 5 min in 100 ppm iodophor. Sperm was then expressed carefully into individually numbered vials by gentle pressure anterior to the vent. Vials of sperm were stored on ice. After spawning, the males were killed, placed into individually numbered plastic bags, and held on ice in a large tote.

Fish and gametes were transported on ice to the NMFS Montlake Quarantine Hatchery in Seattle, WA (about a 2.5-h trip) (Fig. 1). The NMFS received a WDF permit (Number 331-6-87) for this transfer of fish and gametes. Stripping of

females and subsequent fertilization of eggs began immediately upon arrival at the quarantine hatchery. Eggs from each female were collected in separate 1-qt Zip-Loc<sup>1</sup> containers and fertilized with the sperm from one male. Fertilized eggs were water hardened in a 100 ppm iodophor solution for 10 min in the Zip-Loc containers.

#### Viral Certification

Each adult spawner was examined for the presence of replicating virus by a certified Fish Pathologist (American Fisheries Society Board) at the Battelle Marine Laboratories in Sequim, WA. In addition, 37 of the fish were also tested at virology laboratories at the University of California at Davis, CA. Samples of kidney, spleen, and ovarian fluid were tested from each spawning female, and samples of kidney, spleen, and sperm were tested from each spawning male. The testing laboratories inoculated tissue, ovarian fluid, and sperm samples onto appropriate cell lines and observed for cytopathic effects (see Appendix A for complete materials and methods). Offspring were also analyzed (by the whole body homogenization method) for replicating viruses at the Battelle Laboratories (Appendix B).

Eggs resulting from each mating were held in individual isolation incubators until results of the viral analysis were known. Fry are being reared in quarantine with no cross contamination between tanks.

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<sup>1</sup> Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

### Egg Incubation

The eggs were incubated at the NMFS Montlake Quarantine Hatchery in Seattle, WA (described in Slatick et al. 1988). Pathogen-free (dechlorinated City of Seattle) water was used for incubation. All discharge water from the quarantine incubation station was directed into a domestic sewage system and eventually through a City of Seattle sewage treatment plant (METRO).

The eggs were initially incubated in individual isolation incubators described by Novotny et al. (1985). After a 10-min period for water hardening in 100 ppm iodophor solution, the eggs were gently transferred to the incubators. Each individual incubator was numbered for identification of the female/male spawning pair. A chilled water supply using spray mist equipment provided about 0.6 liter/min of flow to each basket.

After viral certification of spawners, eyed eggs from families were combined and transferred to Heath-type incubators for hatching. Hatching incubators were supplied with about 18.9 liters/min of chilled water. Standard hatchery procedures were followed in maintaining egg lots.

### Fry Rearing

Fry rearing was conducted at the NMFS Montlake Quarantine Hatchery in Seattle, WA. Pathogen-free (dechlorinated City of Seattle) water was used for fry rearing. All discharge water from the quarantine rearing station was also directed into a domestic sewage system and eventually through a City of Seattle sewage treatment plant (METRO). At swim-up, fry were ponded into 2.4-m diameter tanks with water depths of 30.0 cm. Approximately 6,000 fish were ponded in each tank, providing an initial density of about 1.5 kg of fish/m<sup>3</sup>. Standard quarantine husbandry practices were followed in maintaining the fish.

## RESULTS AND DISCUSSION

## Adult Collection and Holding

During the last 2 weeks of July, 263 adult sockeye salmon were trapped at Dryden Dam on the Wenatchee River (Table 1). Fish were transported by tank truck to net-pens anchored in the north end of Lake Wenatchee and held (at about 1.5 kg/m<sup>3</sup>) until spawning during late September and early October. Total mortality during the approximately 90-day pre-spawning holding period was 13 fish (4.9%).

During the 1987 holding period, the fish fed actively on a maintenance diet of frozen krill. When the fish were captured at Dryden Dam many had open wounds and abrasions. However, by the time of spawning most external wounds had healed and fish were vigorous. We feel that feeding contributed to this success, as krill contains relatively high levels of Vitamin A, an essential factor in epithelial regeneration in fish (Halver 1972).

## Spawning

Gametes from 232 adult sockeye salmon (137 females and 95 males) were collected between 24 September and 13 October 1987 (Table 2). All females that survived to maturity were spawned; however, 18 surviving males were judged to have poor sperm quality and were not spawned. Spawnings were conducted using individual male:female pairs; however, because of unequal numbers, some males were used for multiple spawnings (Table 3). The fork length of male spawners averaged 544 mm whereas females averaged 519 mm (Table 2).

Females had an average fecundity of 2,644 eggs. However, fertilization success was lower than expected (averaging about 40%) (Tables 2 and 3). There

Table 1.--Inventory of adult Lake Wenatchee stock sockeye salmon captured at the Dryden Dam fish facility on the Wenatchee River in 1987.

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Date	Daily total	Cumulative total
14 July 87	19	19
15 July 87	11	30
16 July 87	6	36
17 July 87	22	58
19 July 87	5	63
21 July 87	4	67
22 July 87	17	84
23 July 87	29	113
24 July 87	37	150
25 July 87	18	168
27 July 87	17	185
29 July 87	31	216
30 July 87	29	245
31 July 87	18	263

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Table 2.--Spawning dates, number of females and males spawned, and average length, fecundity, and egg viability of Lake Wenatchee sockeye salmon spawned from NMFS net-pens in 1987.

Date	Number spawned			Length		Average	
	Female	Male	Total	Female (mm)	Male (mm)	Fecundity (No.)	Egg viability (%)
24 Sept	13	12	25	528	532	2,888	49.1
01 Oct	64	56	120	517	546	2,597	33.2
06 Oct	25	12	37	514	561	2,738	47.7
08 Oct	22	9	31	522	551	2,546	48.5
13 Oct	13	6	19	529	542	2,615	32.8
TOTAL	137	95	232	—	—	—	—
AVERAGE <sup>a</sup>				519	544	2,644	39.8

<sup>a</sup> Combined average of all female (n = 137) and male (n = 95) spawners.

Table 3.--Spawner identification numbers (full-sib families), fecundity, and egg survival for Lake Wenatchee stock sockeye salmon spawned from NMFS net-pens in 1987.

Date spawned	Mating pair		Total eggs (No.)	Live eggs	
	Female	Male		Eyed (No.)	Viability (%)
24 Sept 87	1	4	2,950	1,064	36.1
	2	2	2,363	904	38.3
	3	3	1,924	0	0.0
	4	1	2,638	66	2.5
	5	5	2,650	150	4.5
	6	6	2,989	00	0.0
	7	7	2,556	2,238	87.5
	8	8	2,400	2,019	84.1
	9	9	3,685	2,730	74.1
	10	9	2,717	1,625	59.8
	11	12	3,832	3,487	91.0
	12	13	3,973	3,556	89.5
	13	14	2,873	2,066	71.9
	01 Oct/87	14	101	2,358	240
15		102	2,599	920	35.4
16		103	1,792	1,281	71.5
17		104	2,735	00	0.0
18		105	2,321	28	1.2
19		106	2,363	1,673	70.8
20		107	2,227	1,912	85.9
21		108	3,191	118	3.7
22		109	3,732	104	2.8
23		110	2,433	1,543	63.4
24		111	2,843	2,684	94.4
25		112	2,851	67	2.4
26		113	1,368	00	0.0
27		114	3,052	15	0.5
28		115	2,639	2,504	94.9
29		116	2,747	107	3.9
30		117	2,625	2,443	93.1
31		118	3,768	177	4.7
32		119	2,807	2,251	80.2
33		120	2,194	1,614	73.6
34		121	2,920	2,541	87.0
35		122	2,439	00	0.0
36		123	1,817	41	2.3
37		124	2,766	21	0.8
38		127	2,780	3	0.1
39		125	2,588	33	1.3
40	130	4,612	765	16.6	

Table 3.--Continued.

Date spawned	Mating pair		Total eggs (No.)	Live eggs	
	Female	Male		Eyed (No.)	Viability (%)
	41	126	1,981	1,648	83.2
	42	128	3,139	61	1.9
	43	129	2,516	2,250	89.4
	44	131	2,107	704	33.4
	45	132	2,776	2,480	89.3
	46	133	3,113	53	1.7
	47	134	2,373	2,187	92.2
	48	135	2,333	159	6.8
	49	136	2,072	225	10.9
	50	137	2,602	117	4.5
	51	138	2,780	1,311	47.2
	52	139	2,694	59	2.2
	53	140	3,045	2,407	79.0
	54	141	2,055	1,527	74.3
	55	142	2,338	406	17.4
	56	143	3,006	2,283	75.9
	57	144	1,997	1,549	77.6
	58	145	2,225	2,173	97.7
	59	146	3,232	2,619	81.0
	60	147	3,508	3,020	86.1
	61	148	2,800	1	0.0
	62	149	2,514	0	0.0
	63	150	2,487	1	0.0
	64	151	2,741	0	0.0
	65	152	1,786	0	0.0
	66	153	2,869	201	7.0
	67	154	2,106	265	12.6
	68	155	2,202	32	1.5
	69	156	2,242	30	1.3
	70	143	1,705	307	18.0*
	71	144	2,845	1,274	44.8*
	72	145	2,488	9	0.4*
	73	146	2,790	895	32.1*
	74	147	2,218	51	2.3*
	75	148	2,404	73	3.0*
	76	149	2,748	174	6.3*
	77	150	2,826	1,291	45.7*
06 Oct 87	78	211	3,233	257	7.9
	79	212	2,276	2,196	96.5
	80	205	2,202	4	0.2

Table 3.--Continued.

Date spawned	Mating pair		Total eggs (No.)	Live eggs	
	Female	Male		Eyed (No.)	Viability (%)
	81	206	2,525	167	6.6
	82	207	2,236	1,399	62.6
	83	208	3,408	2,298	67.4
	84	209	2,479	2,044	82.4
	85	201	3,952	1,855	46.9
	86	210	969	742	76.6
	87	202	3,419	3,272	95.7
	88	203	2,797	152	5.4
	89	211	3,112	5	0.2
	90	212	3,102	650	21.0
	91	201	2,032	761	37.5
	92	201	3,830	3,426	89.5
	93	202	3,158	3,098	98.1
	94	203	2,623	27	1.0
	95	204	2,634	2,552	96.9
	96	205	2,124	596	28.1
	97	206	2,297	2	0.1
	98	207	2,809	2,499	89.0
	99	208	2,896	2,715	93.8
	100	202	3,169	2,275	71.8
	101	203	3,429	328	9.6
	102	204	1,757	127	7.2
08 Oct 87	103	301	2,579	2,120	82.2
	104	302	1,812	1,351	74.6
	105	303	2,206	242	11.0
	106	304	2,991	1,042	34.8
	107	305	2,427	2,037	83.9
	108	306	2,205	1,324	60.0
	109	307	2,938	1,729	58.8
	110	308	2,741	2,208	80.6
	111	309	2,604	692	26.6
	112	301	2,488	433	17.4
	113	302	2,980	2,241	75.2
	114	303	1,085	493	45.4
	115	304	3,149	1,724	45.7
	116	305	2,311	435	18.8
	117	306	2,611	99	3.8
	118	307	2,927	2,092	71.4
	119	308	2,409	1,567	65.0
	120	309	2,708	1	0.0

Table 3.--Continued.

Date spawned	Mating pair		Total eggs (No.)	Live eggs	
	Female	Male		Eyed (No.)	Viability (%)
	121	301	2,785	1,583	56.8
	122	302	2,818	817	29.0
	123	303	2,458	1,405	57.2
	124	304	2,784	1,671	60.0
13 Oct 87	125	401	1,921	1,567	81.6
	126	402	2,812	2,175	77.3
	127	403	3,138	46	1.5
	128	404	3,121	462	14.8
	129	405	3,233	2,242	69.3
	130	406	2,832	1,949	68.8
	131	401	2,590	659	25.4
	132	402	2,230	767	34.4
	133	403	1,793	3	0.2
	134	404	2,131	860	40.4
	135	405	2,605	0	0.0
	136	406	2,447	250	10.2
	137	401	3,150	98	3.1
Total			362,267	146,663	
Average			2,644	1,070	39.8
Eggs used in experiment				142,589**	

\* Indicates egg lots (females Number 70 to 77) removed from experiment due to loss of viral samples during processing.

\*\* Reflects removal of a total of 4,074 eggs from females Number 70 to 77.

is no apparent correlation between fecundity, egg viability, spawner size, or date of spawning (Table 2). It is believed that extensive handling and disinfection procedures (implemented in an effort to lessen IHN transfer risk) contributed to the low fertilization rate. Of particular concern was the addition of iodophor disinfectant (a known spermicide) during fertilization. In the future, fertilization protocol will include draining ovarian fluid from eggs prior to addition of sperm, allowing the sperm time to penetrate the egg before addition of iodophor solution. This revised method allows adequate time for fertilization while maintaining disinfection standards (Groberg<sup>2</sup>). It is believed these procedures will improve egg fertilization rates in the future.

#### Viral Certification

Viral certification was done by the Battelle Marine Research Laboratory on 129 female and 95 male spawners and 2 non-spawning males [detection of viral infection in either parent would have necessitated destroying all eggs from that particular mating]. Battelle Laboratories reported no indication of replicating virus in any of the adult sockeye salmon tested (100% viral free -- Appendix A). However, samples from 8 female spawners (females Number 70 to 77) were lost during processing and, although there was no reason to suspect IHN infection, the eggs from these lots were destroyed (Appendix A and Table 3). Thirty-seven samples (females Number 78 to 102 and males Number 201 to 212) were also cross checked at the University of California Virology Laboratory and independently determined to be free of replicating viruses (Appendix A).

A major concern in this project has been the potential for IHN infection. This viral disease can cause major losses of eggs and juveniles in salmon and

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<sup>2</sup> W. Groberg, Oregon Department of Fish and Wildlife, PO Box 59, Portland, Oregon 97207, pers. commun. December 1987.

steelhead hatcheries and is particularly prevalent in sockeye salmon on spawning grounds. Infection rates of over 90% have been reported for sockeye salmon on their spawning grounds in the Little Wenatchee and White Rivers. A concern is that IHN could, potentially, infect other salmonid species when offspring from the Wenatchee River sockeye salmon are used in outmigration studies in the Yakima Basin. However, preliminary information indicates that IHN may be primarily contracted and/or spread by adults after they reach the spawning grounds.

The Washington Department of Fisheries (WDF) conducted pilot studies on three separate occasions (1983, 1984, and 1985) using 20 to 120 sockeye salmon captured at the Hiram M. Chittenden Locks on the Lake Washington Ship Canal. These fish return to the Cedar River and normally have a high (90%+) incidence of IHN on the spawning ground. In all 3 years of the WDF study, the sockeye salmon adults were held to maturity away from the spawning grounds and were determined to be IHN free at spawning (Amos et al. 1983; Hopper et al. 1984; LeVander et al. 1985). A possible carrier for IHN has recently been identified in the salmon leech (Pisicola salmositica) in the Cedar River and may serve as a reservoir to infect returning fish (Winton<sup>3</sup>).

Host vector transmittal may, potentially, be avoided by holding maturing adults away from the spawning ground, thereby limiting exposure to IHN virus. The results of our experiments in the Lake Wenatchee system are similar to those of WDF's in the Lake Washington system. In both cases, IHN was not detected in sockeye salmon spawners held away from the rivers used for spawning (the Wenatchee fish were held in net-pens within 1,000 m of the mouth of the White

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<sup>3</sup> J. Winton, U.S. Fish and Wildlife Service, National Fisheries Research Center, Sand Point Naval Support Activity, Building 204, Seattle, WA 98115, pers. commun. February 1988.

River). The NMFS will be spawning 520 Lake Wenatchee stock anadromous sockeye salmon in 1988, and all of these fish will be held off the spawning grounds in net-pens in Lake Wenatchee to maturity. All spawners will be checked for IHN, and we should have supplemental data available in fall 1988 to confirm whether an IHN free status can be maintained on a repeatable basis. It is possible that the captive holding of IHN susceptible adults away from the spawning grounds may become a valuable management technique.

#### Egg Incubation

Initiation of egg incubation ranged from 24 September (first spawning) to 13 October 1987 (final spawning) (Table 3). Hatching began on 10 December 1987 and ended on 18 January 1988. In an effort to coincide with natural hatch timing, chilled water was used throughout incubation to provide the longest possible period between fertilization and ponding of fry. During incubation, the temperature ranged from 11.0° (September) to 6.5°C (January) whereas during the alevin-to-swim-up stage temperatures ranged from 6.5° (January) to 4.5°C (March). Egg fertilization to hatching required an average of approximately 750 (°C) incubation temperature units (range 697 to 773) whereas hatch to swim-up averaged about 420 temperature units (range 393 to 498). Although, as indicated previously, egg viability averaged only about 40%, eyed egg to hatch survival generally averaged over 99%. This suggests that the low egg viability was an acute (fertilization) problem rather than a chronic problem associated with egg quality.

#### Fry Rearing

A total of about 143,000 swim-up fry survived to ponding (96.8% survival from eyed egg). Ponding of swim-up fry began on 29 February and ended on

19 April 1988. However, the majority of the fish (120,029) were ponded between 17 March and 6 April 1988.

Survival to July was good, with about 92% survival from eyed egg to fry (Fig. 3). Most mortalities were associated with genetic anomalies or normal attrition. The NMFS presently has over 131,000 Lake Wenatchee stock sockeye salmon fry in culture. Mortality is minimal at this time, and survival is expected to remain high throughout the culture period.

One objective of the sockeye salmon rearing program is to provide 10- to 15-g smolts for outmigration studies in spring 1989, and ration and rearing temperature regimes are being adjusted to obtain this target size. Water temperature during fry rearing will be maintained between 8° and 15°C through a chiller system, and fry are fed a commercial ration (either Biodiet or Moore Clarke Semi-moist). Initial feed ration was set at 5% of body-weight/day for the first 30 days and 3% of body-weight/day thereafter. This feed level is about mid-way between the optimum and maximum ration for juvenile sockeye salmon defined by Brett (1969). This combination of temperature and ration should provide a growth profile close to natural while maintaining fish health and quality. To date (July 1988), the average weight of the sockeye salmon fry in culture is 0.7 g (Fig. 4).

As of July 1988, the Lake Wenatchee stock sockeye salmon fry under culture by NMFS were checked twice (60 0.12-g average fry on 9 March 1988 and 60 0.25-g average fry on 22 April 1988) and were determined to be free of replicating viruses. Quarantine standards are being maintained in the culture facility, and IHN certification of juveniles will continue through smoltification. The NMFS is presently enlarging fish rearing capacity at the

# 1987 BROOD SOCKEYE SALMON

## SURVIVAL FROM EYED EGGS

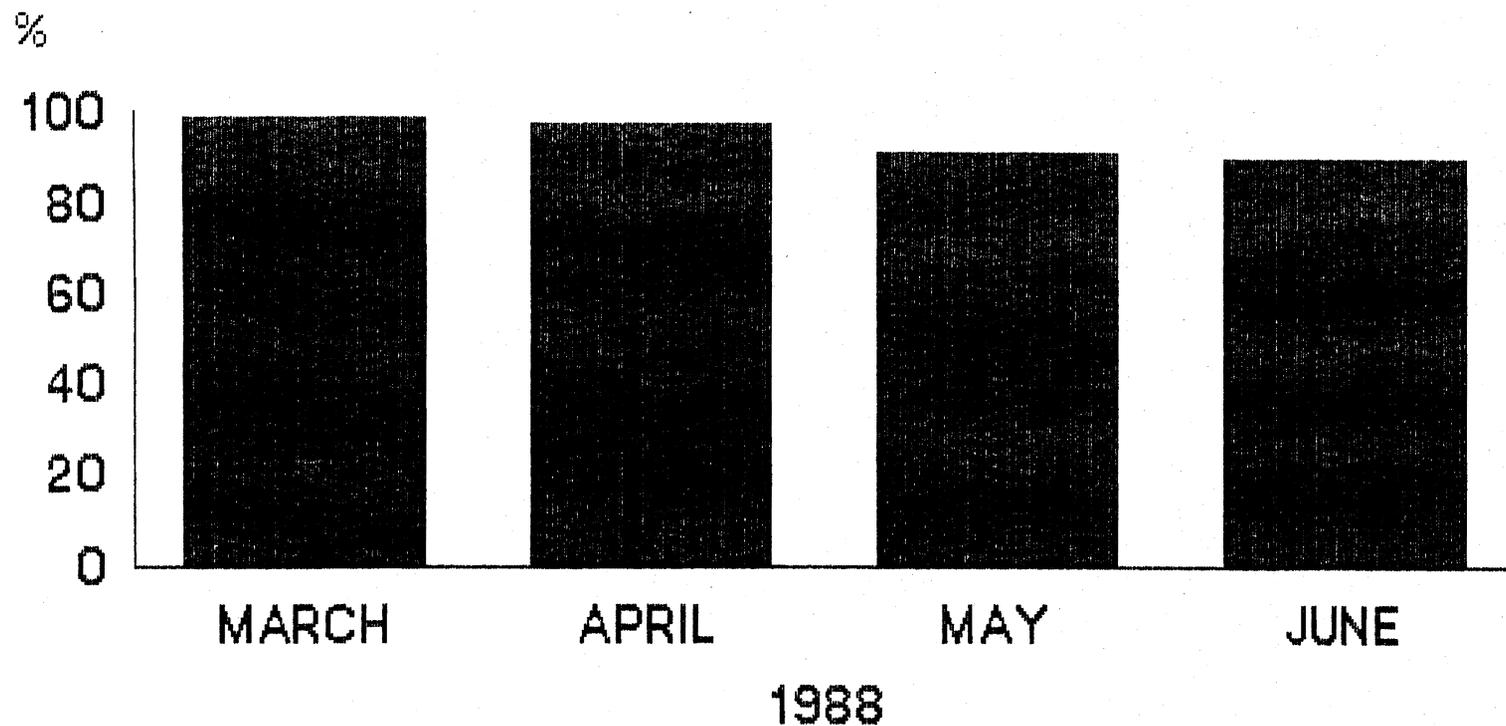


Figure 3.--Survival from eyed egg for the 1987 brood Lake Wenatchee stock sockeye salmon reared at the NMFS Montlake Quarantine Hatchery in 1988.

# 1987 BROOD SOCKEYE SALMON GROWTH

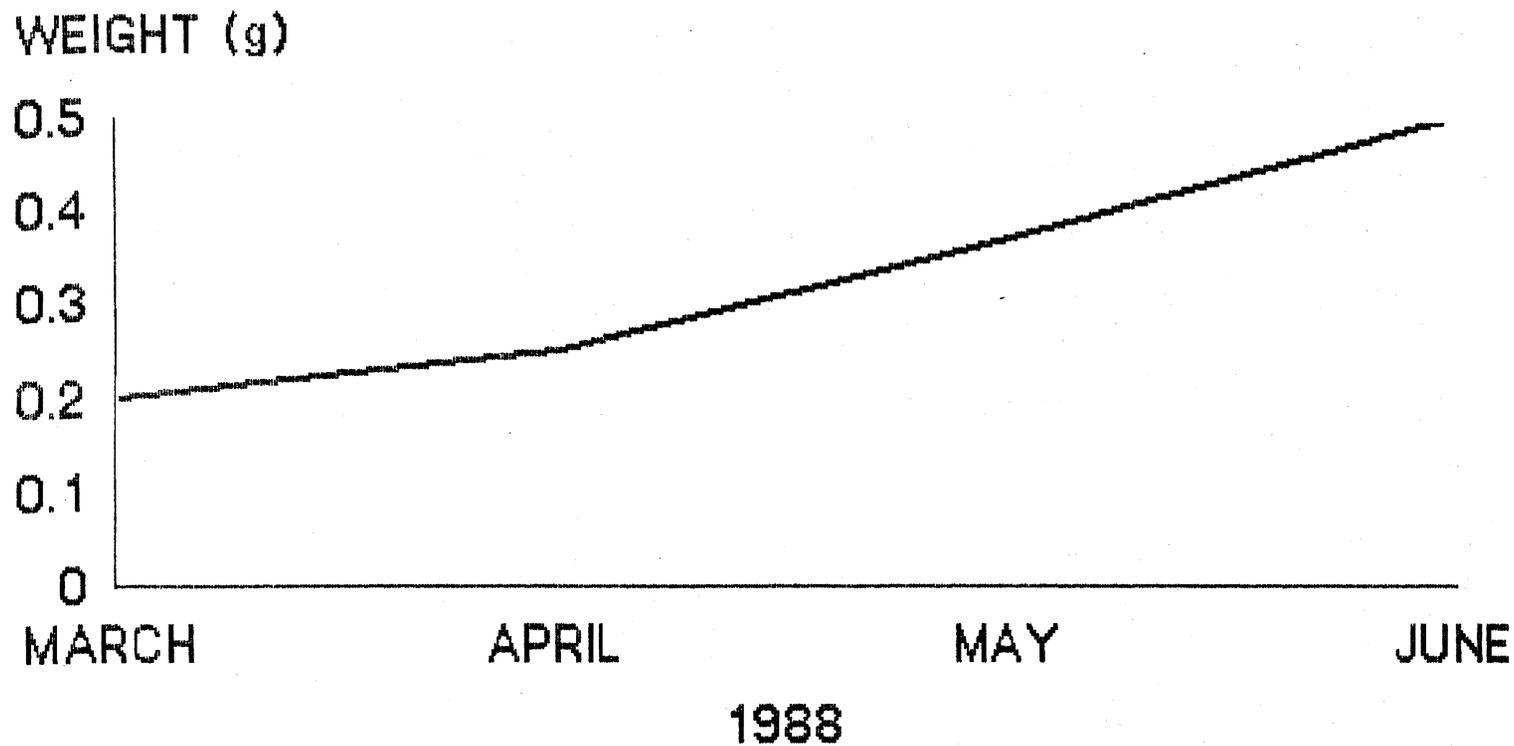


Figure 4.--Growth from swim-up for the 1987 brood Lake Wenatchee stock sockeye salmon reared at the NMFS Montlake Quarantine Hatchery in 1988.

Montlake Quarantine Hatchery to accommodate both the 1987 sockeye salmon juveniles and progeny anticipated from the upcoming 1988 egg take.

#### SUMMARY AND CONCLUSIONS

All project goals were accomplished to date. Donor stock adults from Lake Wenatchee were successfully captured and spawned. Quarantine incubation and rearing facilities were constructed (and are being expanded) and fry survival is high. The NMFS currently has about 131,000 disease-free sockeye salmon fry in culture to provide for outmigration studies in Cle Elum Lake in 1989. In addition, NMFS is planning on collecting 520 sockeye salmon adults in summer 1988 to provide juveniles for outmigration studies in 1990. The NMFS is continuing to develop brood-stock holding and culture techniques that offer potential as management tools around IHN problems.

ACKNOWLEDGMENTS

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We acknowledge, with thanks, the contributions of Anthony Novotny and Carl Sims to this project.

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APPENDIX A

Viral Certification  
of  
Spawners

ANALYSIS OF CLE ELUM SOCKEYE SALMON  
FOR INFECTIOUS HEMATOPOIETIC NECROSIS  
VIRUS (IHN) AND INFECTIOUS PANCREATIC  
NECROSIS VIRUS (IPN)

M. L. Kent  
R. A. Elston

Battelle/Marine Research Laboratory  
Sequim, Washington

February 1988

Prepared for U.S. National Marine  
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under a Related Services Agreement  
with the U.S. Department of Energy  
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory  
Richland, Washington 99352

## Introduction

Spawning sockeye salmon were examined for the presence of IHN virus as part of the National Marine Fisheries Service sockeye salmon enhancement project in Washington State. Infectious hematopoietic necrosis causes acute viremia, resulting in severe necrosis of the kidney and spleen and massive mortalities in fry or fingerling salmon. It is suspected that the virus is transmitted vertically from the parents in reproductive fluid or directly within the egg (Mulcahy and Pascho, 1985). Although not proven by definitive studies, it is widely believed that most, if not all, of adult sockeye salmon are infected with IHN virus at the time of spawning.

This belief has been based primarily on examinations of sockeye from their natural spawning grounds. In the present study, potential sockeye brood stock were captured before they reached their spawning grounds and maintained in freshwater net pens at Lake Wenatchee, Washington until spawning. This was done to enhance the survival of these fish and to determine if this procedure would reduce the prevalence of the virus in spawning fish.

## Materials and Methods

Kidney and spleen tissues, and reproductive fluids were collected from brood stock sockeye salmon by National Marine Fisheries Service personnel from 24 September through 14 October 1987. Samples were collected shortly after spawning, placed in tissue culture medium containing 8X penicillin and streptomycin (800  $\mu\text{g/ml}$ ) and delivered to the Battelle Marine Laboratory within 24 h. The milt, ovarian fluid, kidney, and spleen of spawning males (n= 97) and females (n= 129) were assayed for the presence of IHN and IPN virus on EPC and CHSE-214 cell lines using standard techniques as described by Amos (1985). All fish were assayed individually. Tissues were homogenized and incubated at 4°C in tissue culture medium with 8X penicillin/streptomycin solution for 1-3 days prior to culture inoculation. After centrifugation, the supernatant was inoculated onto cells in 24 well plates at the following concentrations: 1:5 and 1:20 for reproductive fluid and 1:20 and 1:50 for spleen and kidney tissues. Cultures were incubated at 15°C, and suspect cultures were blind passed after 14 days.

For confirmatory diagnosis, 37 of these fish were also assayed at the fish pathology laboratory, University of California, Davis using similar methods. Positive controls were also employed in the study; both cell lines were tested for susceptibility to IHN and IPN viruses as described by Amos (1985) .

### Results

A total of 226 sockeye brood stock were assayed at the Battelle Marine Research Laboratory, and 37 of these fish were also assayed by the fish pathology laboratory at U.C. Davis (Table 1). No evidence suggested virus infections; no cytopathic effects indicative of IHN or IPN viruses were observed from any of the individual samples at any dilution on either the CHSE-214 or EPC cell lines. In the positive controls, both cell lines exhibited CPE when exposed to IHN or IPN virus. An additional eight females (numbers 70-77) were spawned on 10/1/87, but tissues from these fish were accidentally discarded and, therefore, not assayed for virus.

### Discussion

The lack of detectable virus in the brood stock indicates that these fish were free of IHN and IPN viruses at the time of spawning. Therefore, the progeny of these fish will likely be free of IHN virus if maintained in a virus-free environment.

These results demonstrate, contrary to commonly held beliefs, that not all returning sockeye salmon are infected with IHN virus. It is not known if salmon are carriers of IHN throughout their life, or if they become reinfected upon reaching their respective spawning grounds. Why the sockeye salmon in the present study were virus-free was not determined, but it appears capture of the fish prior to reaching the spawning ground prevented infection, or at least expression of the virus.

Table 1. Cle Elum sockeye salmon (*Oncorhynchus nerka*) examined for IHN and IPN viruses. No fish tested positive for IHN or IPN viruses as determined by lack of cytopathologic effects on CHSE-214 and EPC cell cultures.

<u>DATE COLLECTED</u>	<u>FISH ASSAYED</u> (sex, fish number)	<u>NUMBER OF FISH</u>	
		<u>female</u>	<u>male</u>
9/24/87	females: 1-13 males: 1-14	13	14
10/1/87	females: 14-69 males: 101-156	56	56
10/6/87	females: 78-102* males: 201-212*	25	12
10/8/87	females: 103-124 males: 301-309	22	9
10/13/87	females: 125-137 males: 401-406	13	6
	Total by sex	<u>129</u>	<u>97</u>
	Total	226	

\* fish also sent to U.C. Davis for virus analyses

### References

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APPENDIX B

Viral Certification  
of  
Fry



Pacific Northwest Division  
Marine Research Laboratory  
439 West Sequim Bay Road  
Sequim, Washington 98382  
(206) 683-4151

June 10, 1988

Dr. L. H. Harrell  
National Marine Fisheries Service  
Manchester Experiment Station  
P.O. Box 38  
Manchester, Washington 98353

Dear Dr. Harrell:

I am writing in reference to sockeye salmon fry which we have analyzed for you for the presence of known fish viruses. These fry were offspring of brood stock which you obtained from the Yakima drainage and have held in quarantine in the Northwest and Alaska Fisheries Center in Seattle. As you know, we conducted virological examination of eggs and tissues from these brood last fall and did not find evidence of fish viruses.

On March 9, 1988 (our reference Number CMDC-88-13) and again on April 22, 1988 (our reference number S88-2), we received 60 fry sampled from the above referenced fish from your quarantine facility. The tissues from these fish were processed and evaluated on tissue cultures in accordance with the American Fisheries Society Fish Health Bluebook (Amos, K. H., ed., 1985, Procedures for the Detection and Identification of Certain Fish Pathogens, 3rd Edition). Using these methods, we did not detect any evidence of fish viruses known to be pathogenic to salmonids.

Sincerely,

A handwritten signature in black ink, appearing to read "R. Elston", written over the word "Sincerely,".

Ralph Elston  
Senior Research Scientist  
Fish Pathologist #5  
Fish Health Section,  
American Fisheries Society

at

cc: M. Landolt

APPENDIX C

Budget Information

Budget Information  
(July 1987 to July 1988)

A. Summary of Expenditures

- Personnel Services and Benefits	\$264,821.36
- Travel and Transportation of Persons	11,869.87
- Transportation of Things	5,591.00
- Rents, Communications, & Utilities	2,018.61
- Printing and Reproduction	281.53
- Contract and Other Services	20,474.02
- Supplies and Materials	26,521.17
- Equipment	0.00
- Grants	0.00
- Support Cost (Including DOC ovhd.)	1,709.83

TOTAL	\$300,466.22
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B. Major items purchased:

- 11 ft Boston Whaler, 15 hp Johnson motor, and Easyloader trailer.
- Hach Chlorine analyzer.

The following sensitive items were purchased:

- Panasonic Video Camera/recorder and monitor.
- Konica 35 mm slide camera.