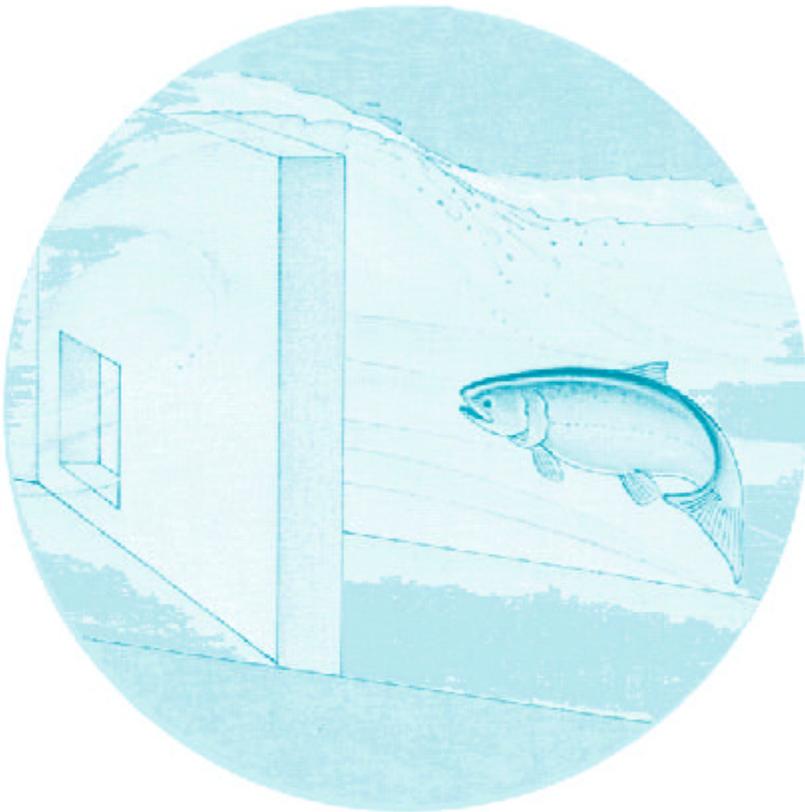


August 1991

CLE ELUM LAKE RESTORATION FEASIBILITY STUDY: FISH HUSBANDRY RESEARCH, 1989-1991

Annual Report 1991



DOE/BP-64840-3



This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views of this report are the author's and do not necessarily represent the views of BPA.

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FISH HUSBANDRY RESEARCH, 1989-1991**

Annual Report

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ABSTRACT

This report summarizes research activities for broodstock development for the Cle Elum Lake restoration feasibility study conducted by the National Marine Fisheries Service (NMFS) and the Bonneville Power Administration (**BPA**) from March 1989 to June 1991. These fish husbandry efforts involved the collection and spawning of sockeye salmon (Oncorhynchus nerka) from the Wenatchee River in 1989 and 1990 and rearing of progeny from these, and earlier, collections. During the reporting period, NMFS completed construction of a joint NMFS/BPA Stock Restoration Laboratory (at the NMFS Northwest Fisheries Science Center in Seattle, Washington) for development of fish culture techniques for restoration of depleted stocks of salmonids.

In both 1989 and 1990, 520 adult sockeye salmon were captured at the Tumwater Dam **fishway** on the Wenatchee River during late July and early August and transferred to floating net-pens in Lake Wenatchee. Fish were held to maturity in late September and early October and spawned. Green eggs and milt from spawnings at the Lake Wenatchee net-pens were transported to the Stock Restoration Laboratory for fertilization and incubation. All eggs were water hardened in iodophor after fertilization. All eggs were incubated and juveniles reared at the laboratory.

Pre-spawning survival in 1989 was about 72%; 167 male and 165 females were successfully spawned and egg viability was about 80%. In 1990, pre-spawning survival was about 87%; 167 male and 200 females were successfully spawned. However, due to water

temperature control problems, **egg viability** was only about 60%. In both years, some excess males and a few females were not spawned.

In the first 2 years of the Cle Elum Lake restoration feasibility study (1987 and **1988**), all net-pen spawners were documented as being free of infectious hematopoietic necrosis (**IHN**) virus, and progeny from all spawners were reared. However, in both 1989 and 1990, IHN was detected in about 30% of the adult fish at the net-pen complex; only juveniles from MN-negative parents were retained at the laboratory. Juveniles from positive parents were donated to the Washington Department of Fisheries (**WDF**) for outplanting in the Wenatchee River Basin. All juvenile sockeye salmon reared at the NMFS laboratory and by WDF remained free of IHN during culture, suggesting transfer of virus from parent to progeny was avoided by iodophor disinfection of eggs.

For the 1987, 1988, and 1989 broods reared at the laboratory, juvenile survival averaged about 85% to release. Survival of the 1990 brood juveniles has been 88.5%.

During the study period, juvenile sockeye salmon were released in the Yakima River Basin to assess the feasibility of anadromous salmonids recolonizing the habitat above Cle Elum Dam. These releases included almost 107,000 of the **1987-brood** juvenile sockeye salmon in fall 1988 to spring 1989; almost 90,000 of the 1988 brood in fall 1989 to spring 1990; and about 100,000 of the 1989 brood in fall 1990 to spring 1991. We currently have about 150,000 1990 brood at the laboratory; these fish will be released in the Yakima River Basin in summer 1991 to spring 1992.

A report detailing outmigration and downstream passage studies from Cle Elum Lake will be available by the end of 1991.

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INTRODUCTION

The National Marine Fisheries Service (NMFS) and the Bonneville Power Administration (BPA) are involved in a project to evaluate the feasibility of re-establishing anadromous salmon runs to Cle Elum Lake in the Yakima River Basin of Washington state. Historically, the Yakima River system supported large runs of anadromous salmonids that contributed significantly to the Columbia River harvest. Habitat destruction and overfishing drastically reduced run abundance prior to the early 1900s. Salmon runs were eliminated from upper reaches of the Yakima River Basin with development of irrigation storage reservoirs without **fishways** in the early 1900s (Robison 1957, **Mullan** 1986).

The goal of the NMFS/BPA project is to determine if it is feasible for anadromous salmonids to recolonize the habitat above Cle Elum Dam under the present format of irrigation water withdrawal from the reservoir. The primary concern is whether anadromous fish can successfully exit Cle Elum Lake and survive downstream passage through the Yakima and Columbia Rivers to the ocean.

Sockeye salmon (*Oncorhynchus nerka*) were selected since this species can best utilize the mid-water (plankton oriented) rearing habitat in Cle Elum Lake (Mongillo and Faulconer 1982). Sockeye salmon are considered by many to be difficult to culture. Disease susceptibility and low return rates led to the termination of production-scale sockeye salmon culture in Washington state in the early 1960s (**Mullan** 1986). Therefore, a first priority of the

NMFS/BPA study was developing a broodstock and juvenile culture program to provide healthy fish for studies on lake survival and downstream passage.

The broodstock development program began in 1987 (Flagg et al. 1988, 1990). The present report details fish husbandry research from March 1989 to June 1991 and reviews results from 1987 and 1988. [A report detailing outmigration and downstream passage studies from Cle Elum Lake will be available by the end of 1991.]

The sockeye salmon husbandry program uses a modification of captive broodstock rearing concepts developed by NMFS for restoration of threatened runs of Atlantic and Pacific salmon (Harrell et al. 1984a, 1984b, 1985). While other NMFS broodstock programs have centered on rebuilding depleted gene pools, no native anadromous sockeye salmon presently exist in the Yakima River Basin. Fortunately, the adjoining (Wenatchee River) basin to the north has a viable anadromous run of sockeye salmon with presumed (genetic and run-timing) similarities to the historic Yakima River Basin stock. Lake Wenatchee also has many geographical and limnological similarities to Cle Elum Lake (Mongillo and Faulconer 1982, Mullan 1986). Therefore, adult sockeye salmon returning to the Wenatchee River Basin were selected as a suitable donor stock to provide juveniles for transplanting to Cle Elum Lake.

Returning adult sockeye salmon were captured during their upstream migration in the Wenatchee River at either Dryden Dam (1987 and 1988) or Tumwater Dam (1988, 1989, and 1990), transported to Lake Wenatchee, and held to maturity in floating net-pens

(Figs. 1-2). At maturity these fish were spawned and gametes transferred to the (recently completed) **NMFS/BPA** Stock Restoration Laboratory at the **NMFS** Northwest Fisheries Science Center in Seattle, Washington (Fig. 3). All spawners were surveyed for-the presence of infectious hematopoietic necrosis (**IHN**) virus, and eggs incubated in a quarantine system. Progeny were reared at the Stock Restoration **Laboratory** for use in studies at Cle Elum Lake.

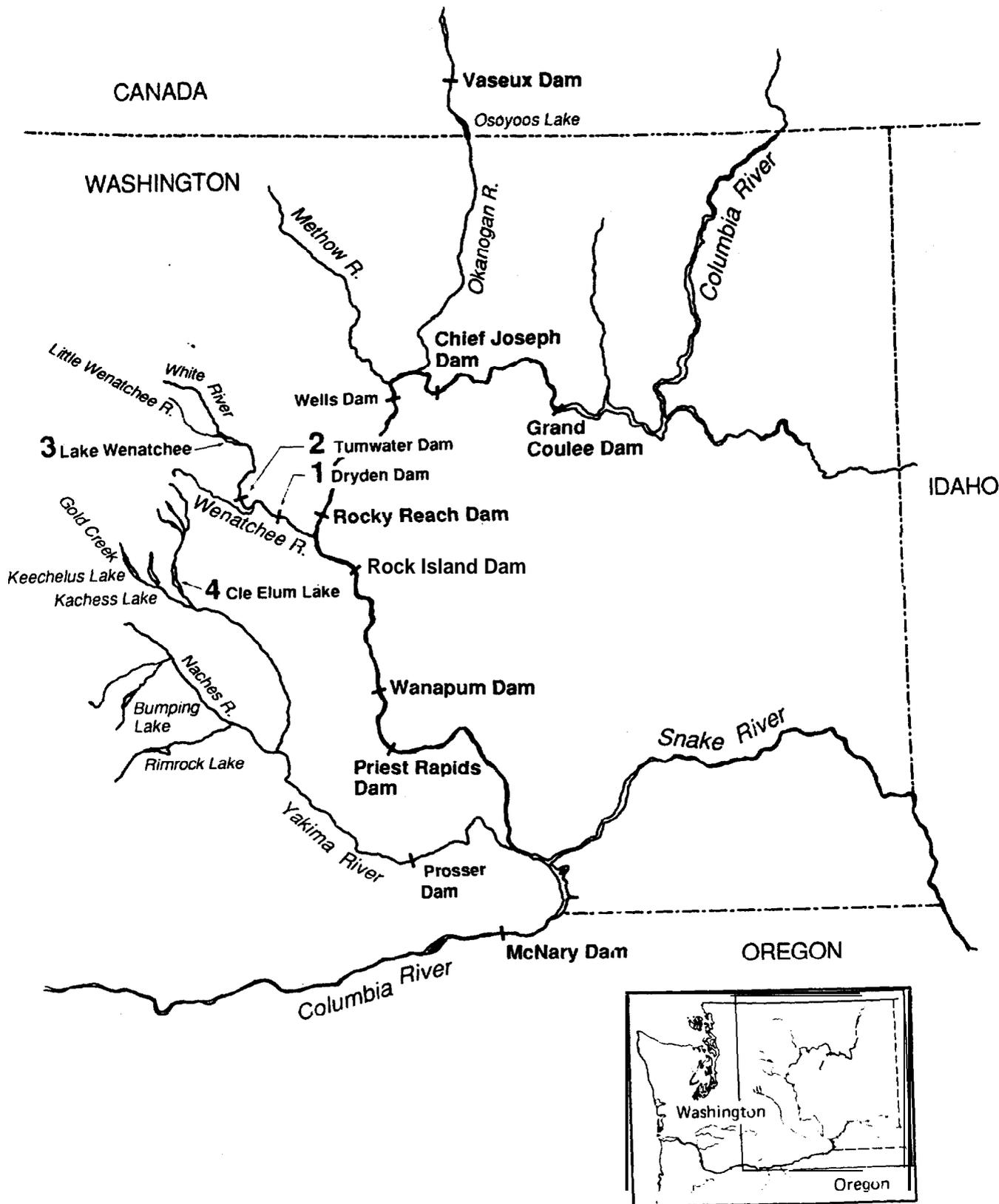


Figure 1. --Sockeye salmon study area in the Mid-Columbia River Basin, showing Cle Elum Lake, Lake Wenatchee, Tumwater Dam, and Dryden Dam.

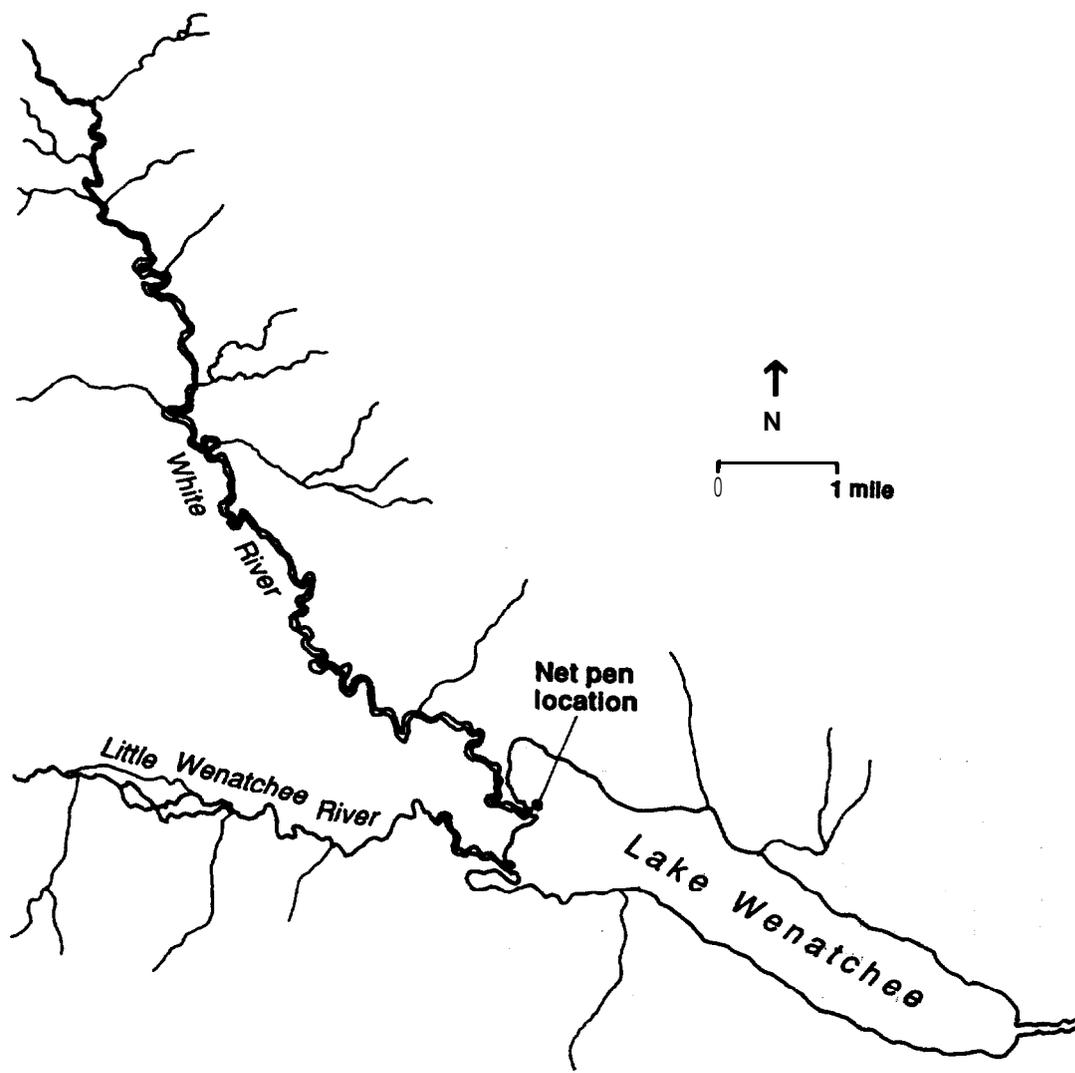


Figure 2. --Map of Lake Wenatchee and major tributaries used for spawning by sockeye salmon. NMFS net-pens for holding prespawning sockeye salmon are indicated.

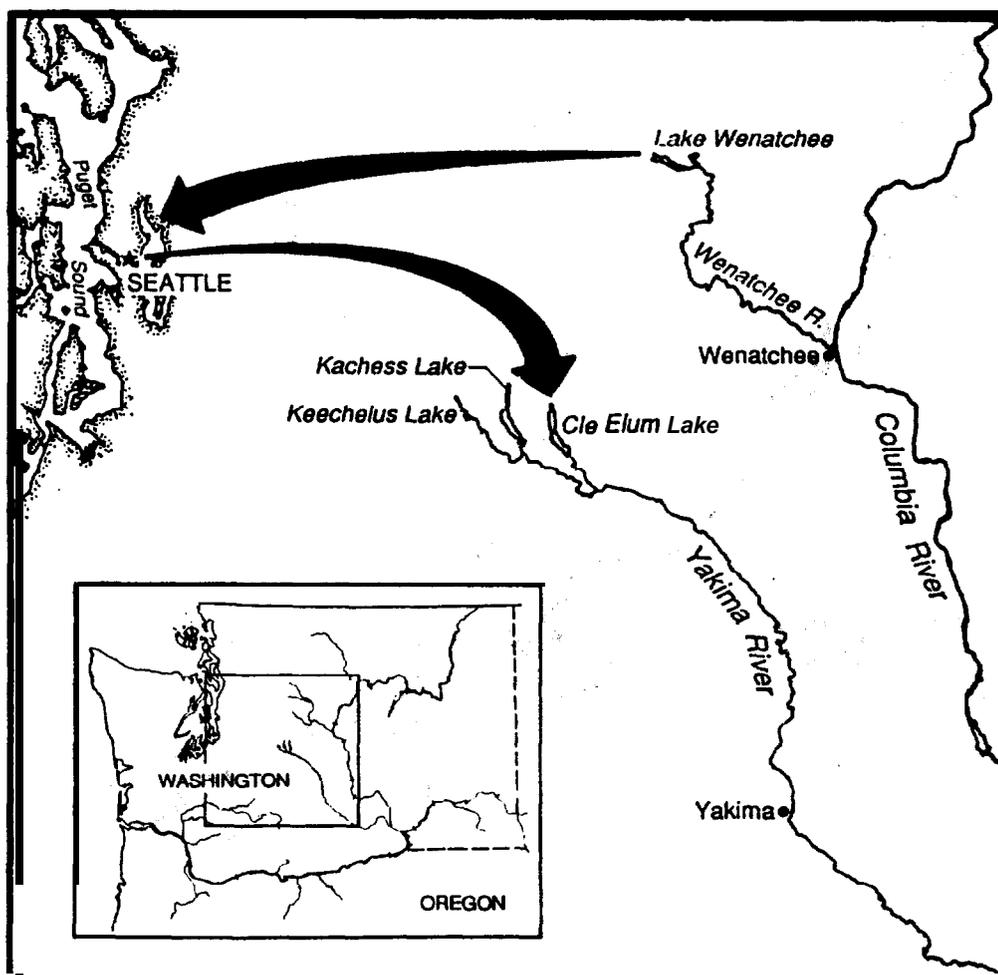


Figure 3.--Movement of salmon gametes from the net-pen system in Lake Wenatchee to the NMFS/BPA Stock Restoration Laboratory in Seattle, Washington (upper arrow). Lower arrow indicates introduction of disease-free juvenile sockeye salmon to Cle Elum Lake in the Yakima River Basin.

MATERIALS AND METHODS

Adult Fish Collection and Holding

In mid July 1988, NMFS in cooperation with Chelan County Public Utility District, installed a temporary fish trap in the **fishway** at Tumwater Dam on the Wenatchee River (Flagg et al. 1990) (Fig. 1). This trap consisted of approximately 15 m of (NMFS owned) Denil steep-pass fish ladder (as described in Rajaratnam and Katopodis 1984) coupled to a fish capture tank. In 1990, this facility was modified to provide for bypass of non-target fish (e.g., chinook salmon and steelhead) directly to the river via a flip-gate and exit flume.

All adult sockeye salmon captured at Tumwater Dam were transported to Lake Wenatchee (Figs. 1-2) in a **6,000-liter** fish transportation truck and transferred via a 9-m long fish-hauling barge to the floating net-pens. Each year, NMFS was issued a WDF Scientific Collection Permit (Numbers 89-73 and 90-93) and a WDF Fish Transfer Permit (Numbers 631-4-89 and 940-6-90) for the collection and transportation of these fish.

In both 1989 and 1990, a set of four floating modular net-pens were installed in Lake Wenatchee in mid-July in a manner similar to that in 1987 and 1988 (Flagg et al. 1988, 1990). The net-pens were located near the upper **end** of the lake in 12- to 15-m depth and were within 500 m of the mouth of the White River (Fig. 2). Each 4.8-m square module consisted of wood and steel walkways supported by Styrofoam floats and contained one 7.5-m deep net with 3.8-cm

stretch mesh. In 1989, these net-pens were sited side-by-side in a 2- by 2-pen matrix. In 1990, a central work platform was added to the float complex, providing about 4 m separation between each pen. The net-pens remained in the lake until mid-October each year, when they were removed and stored for the season.

The net-pen modules were sited under permits issued for the pen system [Washington Department of Fisheries (WDF) Hydraulic Permits (Numbers 00-37408-01, 02, and 03); Chelan County Shoreline Management Permit (Number 1586); Washington Department of Ecology Substantial Development Permit (Number 590-14-7804); and Washington Department of Natural Resources Right of Entry Permit (Number 20-012737)].

All fish were held in the net-pen system to maturity. In 1989, fish in three of the four pens were fed a maintenance diet of frozen krill (Euphausia pacifica) at about 0.5% of body-weight/day, or less, while fish in the fourth pen were not fed during holding. In 1990, fish in two of the four net-pens received the krill ration.

Spawning

In 1989 and 1990, adult sockeye salmon held in the net-pens were sorted by appearance according to stages of maturity during mid-to-late September in a manner similar to previous years (Flagg et al. 1988, 1990). To inspect potential spawners, each net-pen was raised gradually (approximately 2.5 m/5 minutes) 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 then 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 ripe, a few eggs were expressed to visually examine egg quality. Males were checked for milt in a similar manner.

Mature females with free-flowing eggs were loaded onto a transport barge into one section of a divided holding tank of about 25 m³, 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 and the fish were spawned in a portable spawning trailer (Flagg et al. 1990). All discharge water from the trailer was routed through a central drain to a settling basin and periodically disinfected with a solution of 100 ppm iodophor.

At the trailer, fish were killed by a blow to the head, placed in a V-board, and bled by cutting the gill arches. After bleeding, carcasses were disinfected in 100 ppm iodophor. Female carcasses were opened by surgical incision of the abdomen and eggs collected into individually numbered plastic bags. Milt from males was expressed into individually numbered vials by gentle pressure anterior to the vent.

All eggs and milt were transported to the Stock Restoration Laboratory in Seattle, Washington (Fig. 3) (about a 2.5-hour trip) in insulated coolers containing ice. Some adults were transported live to the laboratory and later spawned. Each year, **NMFS** received a **WDF** Fish Transfer Permit (**Numbers 630-4-89 and 939-6-90**) for this transfer of fish and gametes.

Fertilization of eggs began immediately upon arrival at the laboratory. Ovarian fluid was rinsed from each (individual female) egg lot. Each egg lot was then placed in a 1-liter plastic container and (dry method) fertilized with the sperm from one male. Fertilized eggs were water hardened for an initial 3 to 5 minutes and then disinfected in 100 ppm iodophor solution for an additional 10 minutes.

Viral Certification

Each adult male and female spawner was examined for the presence of IHN and other replicating viruses by a certified Fish Pathologist (American Fisheries Society Board). In 1989, the viral survey was conducted by the Battelle Marine Laboratory in Sequim, Washington. In 1990, the analysis was conducted by the WDF virology laboratory in Olympia, Washington.

In both 1989 and 1990, individual ovarian and milt reproductive fluid samples from spawners were tested. [Investigators generally agree that IHN virus must be present in the reproductive fluids for vertical transmission to progeny.] However, for confirmation, kidney and spleen tissues were also sampled in some cases. The testing laboratory inoculated samples onto appropriate cell lines and observed for cytopathic effects (see Appendixes A and B for materials and methods). Fry and juvenile sockeye salmon were **also** analyzed for replicating viruses by the laboratories.

Facilities and Fish Rearing

During the reporting period, NMFS completed construction and remodeling of a fish hatchery at the Northwest Fisheries Science Center in Seattle, Washington. This Stock Restoration Laboratory was dedicated for operation in June 1990 and provides the NMFS/BPA project with a centralized facility in which to rear progeny from donor stocks under controlled conditions. The laboratory conforms to state and federal isolation and quarantine standards. Over 280 isolation incubators (Novotny et al. 1985) are available to hold eggs from individual paired matings. In addition, there are 23 1.2-m and 32 1.8-m circular juvenile fish rearing tanks. Fish rearing space within the laboratory is segmented into isolation areas to maintain quarantine standards.

The facility is supplied with pathogen-free water processed through a series of dechlorinators and chillers to ensure quality. Water quantity, quality, and fish rearing parameters are monitored by a computerized system. The laboratory accommodates up to 250,000 juvenile salmon and has the capability to continually provide healthy fish for rebuilding depleted runs.

In 1989 and 1990, eggs were incubated and fish reared at the laboratory even though it was under construction during much of this time. In both years, after water hardening and disinfection, the eggs were transferred to isolation incubators numbered for identification of the female/male spawning pair. After hatching, fish were reared using standard fish culture methods.

RESULTS

During the reporting period, juvenile sockeye salmon from 1987- and 1988-brood Lake Wenatchee donor sockeye salmon egg collections (Flagg et al. 1988, 1990) were reared at the Stock Restoration Laboratory. In addition, during this period, 1989- and 1990-brood eggs were collected, incubated, and progeny reared at the laboratory. An objective of the program was to provide natural size smolts for outmigration studies. Therefore, egg incubation and fish rearing temperature and feed ration were adjusted to obtain target size for release (6 to 15 g and 80 to 130 mm depending on release date).

Generally, water temperature during egg incubation was maintained between 4 and 12°C and fry-to-juvenile rearing temperature was maintained between 5 and 17°C through a chiller system. Fish were fed a commercial ration (either Biodiet or Moore Clarke Semi-moist¹) at 5% of body-weight/day for the first 30 days and 3% of body-weight/day, or less, thereafter. [This food level is about mid-way between the optimum and maximum ration for juvenile sockeye salmon defined by Brett et al. (1969). This combination of temperature and ration was intended to provide a growth profile close to natural while maintaining fish health and quality.]

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

1987 Brood

During 1987, 263 certified IHN-free adult sockeye salmon were collected from the Wenatchee River system. Adult survival to spawning was about 95%. Egg viability was about 40%; subsequent tests indicated this low viability was due to direct fertilization in iodophor disinfectant, an effective spermicide. This process was modified to allow for fertilization prior to disinfection, and in later years viability improved. Survival to hatch was almost 99%; survival to fry was about 92% (Flagg et al. 1988, 1990). The **1987-brood** juvenile population was certified (by Battelle) in March, April, September 1988, and March 1989 as IHN-free.

Cumulative mortality of the 1987 brood from swim-up to release was about 11% (Fig. 4). Growth (length and weight) of these fish was maintained within target criteria during culture (Figs. 5-6).

Almost 107,000 **1987-brood** juvenile sockeye salmon were released in the Yakima River Basin. About 25,000 of these fish were released into Cle Elum Lake in fall 1988 (Flagg et al. 1990). The remaining about 82,000 fish were released in spring 1989. All of the fish were freeze branded and coded-wire tagged. In addition, over 3,000 of the fish were PIT tagged.

1988-Brood

During 1988, 520 adult sockeye salmon were collected from the Wenatchee River system. Adult survival to spawning was about 85%; all spawners were certified IHN-free. Egg viability was about 80%; survival to hatch was almost 99%; survival to fry was about 93%

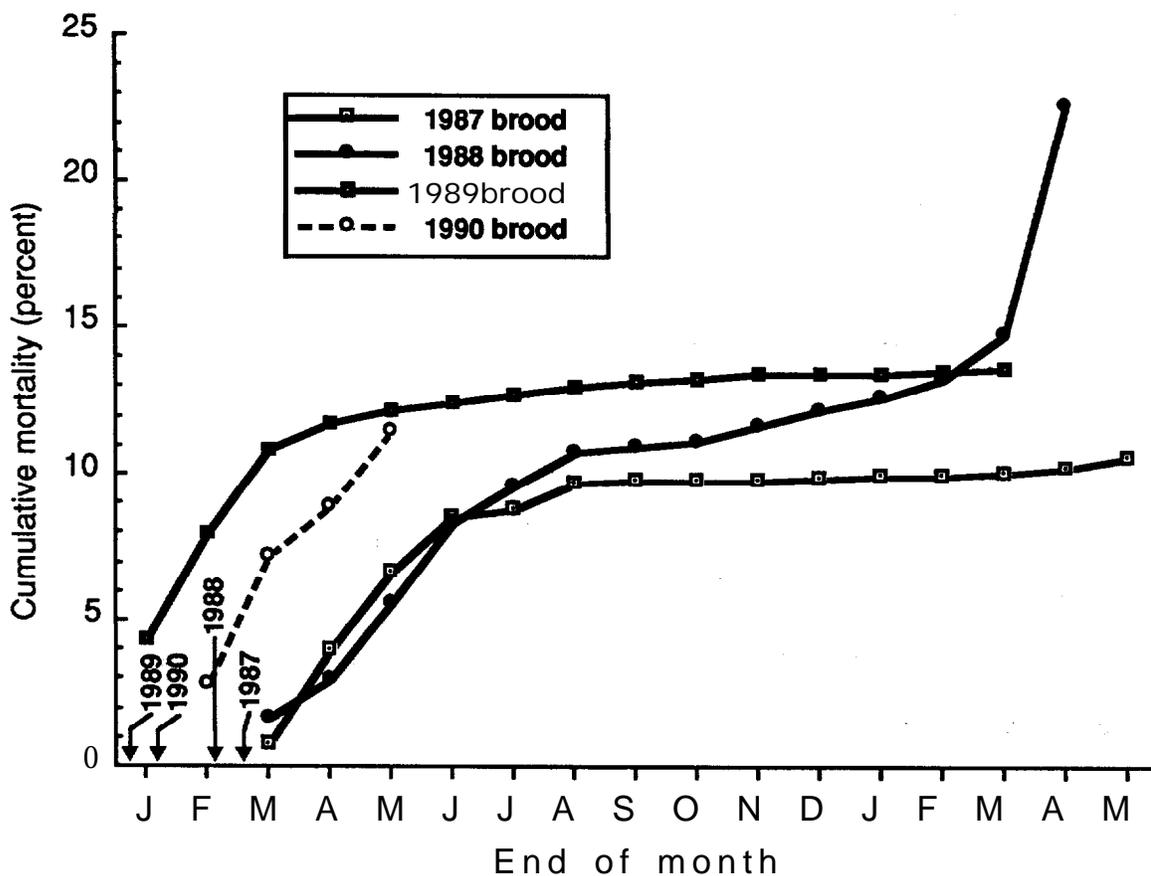


Figure 4. --Cumulative mortality for the 1987- to 1990-brood juvenile sockeye salmon reared at the NMFS/BPA Stock Restoration Laboratory. Arrows indicate start of rearing dates. The majority of 1987-, 1988-, and 1989-brood juveniles were released in the Yakima River Basin from November to March as yearling fish.

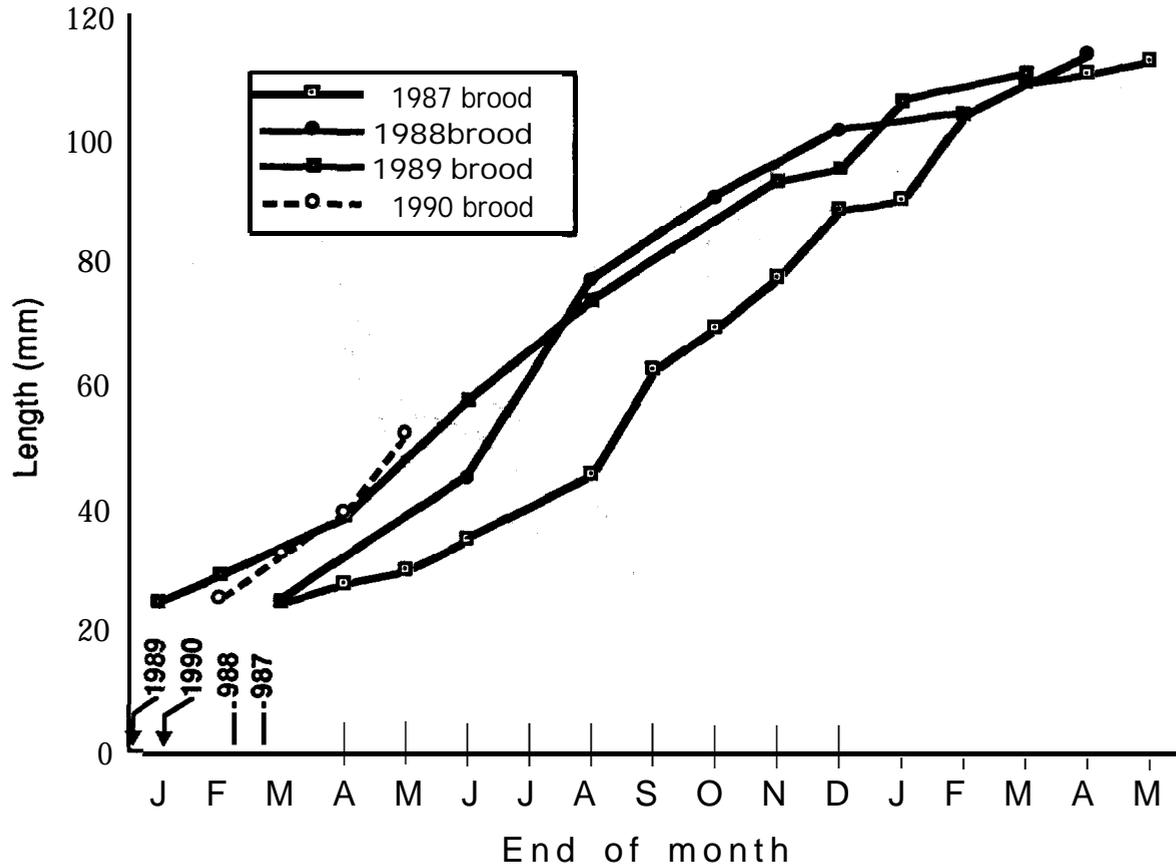


Figure 5. --Growth (length) for the 1987- to 1990-brood juvenile sockeye salmon reared at the NMFS/BPA Stock Restoration Laboratory. Arrows indicate start of rearing dates. The majority of 1987-, 1988-, and 1989-brood juveniles were released in the Yakima River Basin from November to March as yearling fish.

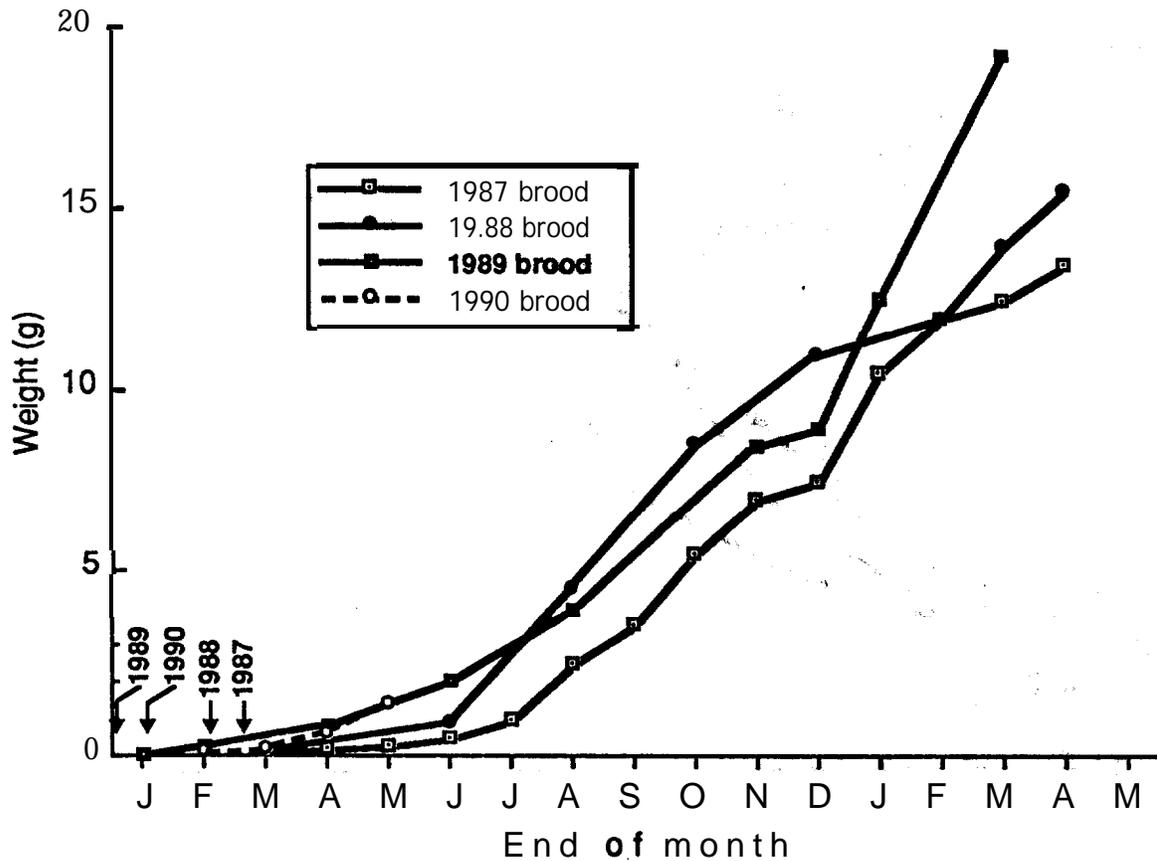


Figure 6. --Growth (weight) for the 1987- to 1990-brood juvenile sockeye salmon reared at the NMFS/BPA Stock Restoration Laboratory. Arrows indicate start of rearing dates. The majority of 1987-, 1988-, and 1989-brood juveniles were released in the Yakima River Basin from November to March as yearling fish.

(Flagg et al. 1990). Unfortunately, on 17 June 1989 (a Saturday), an accident at the NMFS Montlake Hatchery resulted in the death of approximately 250,000 of the 375,000 **1988-brood** sockeye salmon fry at the Stock Restoration Laboratory. [The laboratory was under construction; a backhoe operator ruptured a water supply line, causing low flows and oxygen depletion].

Approximately 125,000 **1988-brood** fish survived the accident. Cumulative mortality of these fish from June 1989 to release was about 15%. Overall, swim-up to release mortality (adjusted for fish killed in the accident) was about 23% (Fig. 4). The **1988-brood** juvenile population was certified (by Battelle) in March, May, and September 1989, and (by WDF) in March 1990 as IHN-free. Growth (length and weight) of the 1988 brood was maintained within target criteria during culture (Figs. 5-6).

Almost 90,000 **1988-brood** juvenile sockeye salmon were released in the Yakima River Basin. About 25,000 of these fish were released into Cle Elum Lake in November 1989. The remaining about 64,000 fish were released in spring 1990. All of the fish were freeze branded and coded-wire tagged. In addition, over 8,000 of the fish were PIT tagged.

1989 Brood

During late July through early August 1989, 520 adult sockeye salmon were captured at the Tumwater Dam **fishway** on the Wenatchee **River** and transferred to the net-pens in Lake Wenatchee (Table 1). **Fish were held** for 60 to 75 days at a density of about 1.5 kg/m³

Table 1.--Inventory of salmonids captured at the Tumwater Dam fish facility on the Wenatchee River, 1989.

Date (July)	Time of trap operation (h)	Number of fish trapped/day			
		Sockeye		Chinook'	Steelhead'
		NMFS'	WDF ^b		
18	0430 to 1200	16	0	0	0
	1930 to 2100	34	0	0	0
19	0500 to 1130	126	80	16	0
20	0530 to 0800	123	0	11	0
	0930 to 1100	2	75	2	0
21	0530 to 0800	80	45	7	0
24	0530 to 0950	60	31	15	1
25	0510 to 0745	55	52	6	0
26	0510 to 0815	<u>24</u>	<u>16</u>	<u>4</u>	<u>0</u>
TOTAL		520	299	61	1

^a Fish transported to Lake Wenatchee and held in four NMFS net-pens at 130 fish/pen.

^b Fish collected by WDF for brood stock.

^c Fish trapped and returned to river immediately upstream of dam.

(130 fish/pen) to maturity in late September to early October and spawned (Table 2). The net-pens were checked daily and total mortality during the (pre-spawning) holding period was 146 fish. Pre-spawning survival in 1989 was 72%; 167 males and 165 females were successfully spawned (Tables 2-3). All females that survived to maturity were spawned; however, 39 excess males were not spawned.

There was no apparent relationship between feeding of fish during prespawning holding in the pens and 1) survival to spawning or 2) egg viability (Table 3). The fork length of male spawners in 1989 averaged 525 mm, while females averaged 490 mm. Fecundity averaged 2,725 eggs per female (Table 2).

In 1989, tests showed that approximately 30% of the spawning fish had reproductive fluids that were positive for IHN--about 54% for females and less than 6% for males (Table 3 and Appendix A). This resulted in 67 paired matings certified as (reproductive fluid) IHN-negative.

1989-brood eggs were water-hardened, disinfected in 100 ppm iodophor, and incubated in isolation. Egg viability was about 77% (Table 2). In December 1989, NMFS donated about 250,000 eggs from groups where the reproductive fluids were IHN-positive to WDF for enhancement projects in the Wenatchee River Basin. NMFS retained about 125,000 **1989-brood** eggs from IHN-negative parents.

Initiation of egg incubation ranged from 25 September (first spawning) to 5 October 1989 (final spawning). Hatching began on 22 November and ended on 15 December 1989. During incubation in 1989, temperature ranged from 12.4 (on 25 September) to 7.0°C (on

Table 2.--Spawning dates, number of females and **males**, and average fecundity and egg viability of Lake Wenatchee sockeye salmon spawned from net-pens, 1989.

Date	Number spawned		Average	
	Female	Male	Fecundity (number of eggs)	Egg viability (%)
25 Sep	45	45	2,721	79.2
26 Sep	68	69	2,607	76.9
27 Sep				
28 Sep	20	20	2,490	85.5
2 Oct	12	12	2,329	61.6
5 Oct	<u>4</u>	<u>5</u>	<u>3,035</u>	<u>60.3</u>
TOTAL	165	167		
AVERAGE ^a			2,725	77.1

^a Combined average of all female spawners (n = 165).

Table 3. --Survival, egg viability, and IHN viral incidence in spawning sockeye held in net-pens in Lake Wenatchee, 1989.

	Pen	
	A ^a	B ^b
Survival (%)		
- Males	78.1	78.5
- Females	63.6	66.2
- Total	70.8	72.3
Average Egg Viability (%)	83.0	71.0
<u>IHN positive (%)</u>		
-reproductive fluids		
- Males	5.1	2.4
- Females	30.0	32.6
-Kidney/spleen		
- Males	63.4	82.5
- Females	75.0	87.8
	C ^b	D ^b
Survival (%)		
- Males	78.6	83.6
- Females	65.0	62.3
- Total	72.3	72.3
Average Egg Viability (%)	76.0	78.0
<u>IHN positive (%)</u>		
-reproductive fluids		
- Males	5.0	9.3
- Females	58.9	93.0
-Kidney/spleen		
- Males	88.9	79.1
- Females	100.0	100.0

^a Not fed during holding.

^b Fed frozen krill (*Euphausia pacifica*) at about 0.5% of body-weight/day, or less, during prespawning holding.

15 December); during the alevin-to-swim-up stage temperatures ranged from 8.9 (on 11 December 1989) to **4.5°C** (in February 1990). **Egg** fertilization to hatching required about 700 (**°C**) incubation temperature units, whereas hatch to swim-up required approximately 420 temperature units.

In early February 1990, about 125,000 **1989-brood** swim-up fry were moved from the incubators to 1.2-m diameter tanks at an initial fish density of about 6.0 **kg/m³**. Water depth in the tanks was set at 10 to 15 cm with an inflow of about 4 to 6 liters/minute. These densities and flows were maintained through March 1990; water temperature ranged from 6.5 to **8.0°C**.

Beginning in July 1990, **1989-brood** juveniles were transferred to 1.8-m diameter tanks. Water depth in these tanks was set at 43 to 46 cm with an inflow of 16.3 to 33.3 liters/minute. Fish were held in these tanks throughout the remainder of the reporting period. Fish density in the tanks ranged from about 3.7 to about 47.6 **kg/m³**; water temperature ranged from **4.5 to 20.1°C**.

The **1989-brood** juvenile sockeye salmon were certified as **IHN-**free by WDF in March, April, and September 1990. Growth (length and weight) of the 1989 brood was maintained within target criteria during culture (Figs. 5-6). Cumulative mortality of the **1989-brood** fish through the entire culture cycle was under 14% (Fig. 4).

Over 100,000 **1989-brood** juvenile sockeye salmon were released in the Yakima River Basin. About 75,000 of these fish were released into Cle Elum Lake from November 1990 to January 1991. The remaining about 25,000 fish were released in spring 1991. All of

the fish were freeze branded and coded-wire tagged. In addition, over 9,000 of the fish were PIT tagged.

1990 Brood

During late July through early August 1990, 520 adult sockeye salmon were captured at the Tumwater Dam **fishway** on the Wenatchee River and transferred to the net-pens in Lake Wenatchee (Table 4). Fish were held for 60 to 75 days at a density of about 1.5 **kg/m³** (130 fish/pen) to spawning in late September to early October. The net-pens were checked daily and total mortality during the (**pre-spawning**) holding period was 66 fish. Adult survival from capture' to (initiation of) spawning was 87.3%; 167 males and 200 females were successfully spawned (Tables 5-6). Some females and males were not spawned.

There was no apparent relationship between feeding of prespawning adult fish in the pens in 1990 and either survival from adult capture to spawning or egg viability (Table 6). The fork length of male spawners in 1990 averaged 509 mm, while females averaged 487 mm. Fecundity averaged 2,225 eggs per female (Table 5).

In 1990, tests showed that approximately 21% of the spawning fish had reproductive fluids that were positive for IHN-- about 33.5% for females and 6.7% for males (Table 6 and Appendix B). This resulted in 112 paired matings certified as (reproductive fluid) IHN-negative and 88 as IHN-positive. This is similar to results of spawning in 1989.

Table 4.--Inventory of salmonids captured at the Tumwater Dam fish facility on the Wenatchee River, 1990.

Date (July)	Time of trap operation (h)	Number of fish trapped/day				
		Sockeye			Chinook"	Steelhead"
		NMFS ^a	WDF ^b	Other"		
24	0530 to 0700	98	0	57	9	0
	1210 to 1340	164	0	100	7	0
	1610 to 1750	0	0	25	2	0
25	0500 to 1645	128	326	397	19	0
26	0720 to 0939	<u>132</u>	<u>7</u>	<u>85</u>	1	0
TOTAL		522	333	664	38	0

^a Fish transported to **net-pens** in Lake Wenatchee and held in four NMFS net-pens at 130 fish/pen.

^b Fish collected by WDF for brood stock.

^c Fish trapped **and** returned to river.

Table 5.--Spawning dates, number of females and **males**, and average fecundity and egg viability of Lake Wenatchee sockeye salmon spawned from NMFS net-pens, 1990.

Date	Number spawned		Average	
	Female	Male	Fecundity (number of eggs)	Egg viability (%)
18 Sep	13	13	2,325	85.7
24 Sep	24	7	2,710	83.6
25 Sep	32	32	2,267	80.5
26 Sep	35	35	2,381	54.7
2 Oct	42	36	1,998	48.9
3 Oct	21	21	2,112	31.8
10 Oct	<u>33</u>	<u>23</u>	<u>1,988</u>	<u>57.6</u>
TOTAL	200	167		
AVERAGE ^a			2,225	61.4

^a Combined average of all female spawners (n = 200).

Table 6. --Survival, egg viability, and IHN viral incidence in spawning sockeye held in net-pens in Lake Wenatchee, 1990.

	Pen	
	A ^a	B ^a
Survival ^b (%)		
- Males	98.4	98.4
- Females	98.5	98.6
- Total	98.5	98.5
Average Egg Viability (%)	50.5 ^c	
<u>IHN positive (%)</u>		
-reproductive fluids		
- Males	2.8 ^c	
- Females	21.2 ^c	
	C ^d	D ^d
Survival ^b (%)		
- Males	94.8	98.5
- Females	98.6	95.2
- Total	96.9	96.9
Average Egg Viability (%)	61.0 ^c	
<u>IHN positive (%)</u>		
-reproductive fluids		
- Males	11.1 ^c	
- Females	65.4 ^c	

^a Not fed during holding.

^b Survival to first sorting for spawning. An additional 25 females died from pens A and B, and 30 females from pens C and D, prior to spawning. In addition, some males were not spawned.

^c Average of pens A and B.

^d Fed frozen krill (Euphausia pacifica) at about 0.5% of body-weight/day, or less, during prespawning holding.

^e Average of pens C and D.

1990-brood eggs from NMFS spawners were water-hardened, disinfected in 100 ppm iodophor ,and incubated in isolation. In January 1991, NMFS donated about 103,000 alevins from groups where the reproductive fluids were IHN-positive to WDF for enhancement projects in the Wenatchee River Basin. NMFS retained about 150,000 1990 brood from IHN-negative parents.

Egg viability for the 1990 brood was about 60% (Table 5). We believe eyed-egg survival was compromised by a power outage and chiller failure in early October that resulted in a 5°C temperature spike (to about 17°C) for about 12 hours.

Initiation of egg incubation for the 1990 brood ranged from 18 September (first spawning) to 10 October 1990 (final spawning) (Table 5). Hatching began on 25 November 1990 and ended on 10 January 1991. During incubation, temperature ranged from 13.9 (on 18 September) to 6.2°C (on 21 December) whereas alevin incubation temperatures ranged from 8.3 (on 7 December) to 6.2°C (on 21 December). Egg fertilization to hatching required an average of 678 (°C) incubation temperature units, whereas hatch to swim-up averaged 426 temperature units.

In late January to early February 1991, about 150,000 1990 brood swim-up fry were move from the incubators to 1.2-m diameter tanks at an initial fish density of about 6.0 kg/m³. Water depth in the tanks was set at 10 to 15 cm with an inflow of about 4 to 6 liters/minute. These densities and flows were maintained through the remainder of the reporting period.

Cumulative mortality of the **1990-brood** fish through the first 4 months of rearing was 11.5% (Fig. 4). Initial growth (length and weight) of the 1990 brood was similar to the other (1987-1989) broods (Figs. **5-6**). The **1990-brood** juvenile sockeye salmon were certified as IHN-free by WDF in February and June 1991. These fish will be held at the Stock Restoration Laboratory and periodically surveyed for IHN throughout the rearing period.

The 1990 brood will be released in the Yakima River Basin in summer 1991 and spring 1992.

DISCUSSION

A **major** component of the Cle Elum Lake Restoration Feasibility Study has been the development of a suitable sockeye salmon donor stock for transplanting to the Yakima River Basin. At the onset of the program in 1987; sockeye salmon were considered by many investigators to be difficult to culture. High prespawning mortality, low egg viability, inability to mature in captivity, and low fry-to-smolt survival were viewed as potential **limits to success** (Mullan 1986, ADFG 1988, Amos et al. 1989, Burkett 1989, Meyers et al. 1990).

Fortunately, our experience has been much more positive than most previous work with sockeye salmon. During the **last 4 years (1987-1990)**, NMFS routinely held from 240 to 520 wild adult sockeye salmon captured from the Wenatchee River in net-pens in Lake Wenatchee for 60 to 90 days prior to spawning. Prespawning survival ranged from about 72 to 95% (4-year mean = 85%). This is much

better than the approximately 25% average adult capture-to-spawning survival described by **Mullan** (1986) for Columbia River sockeye salmon held in raceways and ponds. Fish in our studies uniformly completed maturation with no evidence of delays or decreases in reproductive readiness.

A first key to development of a donor stock for Cle Elum Lake was the adaptation of seawater net-pen culture techniques [developed at the NMFS Marine Experimental Station near Manchester, Washington for restoration of threatened runs of Atlantic and Pacific salmon (**Harrell et al. 1984a, 1984b, 1985**)] to the freshwater environment at Lake Wenatchee. The major modification, other than siting in fresh water, was providing 7.5-m deep pens to allow the fish access to depth during holding. The majority of the **time**, fish remained near the pen bottoms; however, they were free to range to preferred depths. We feel that low-density captive holding of prespawning adults in lacustrine net-pens provides a much more natural (and, therefore, beneficial) environment than (commonly used) hatchery raceways and ponds.

Feeding is not a common husbandry strategy when holding prespawning salmonids. However, unpublished research at the NMFS Manchester and Montlake laboratories indicate that many species of salmon will feed to maturity in captivity. Our studies at Lake Wenatchee indicate that captured wild sockeye salmon will accept natural feeds (e.g., krill) during maturation. The adult fish routinely accepted up to 0.5% body weight/day from July to

September--preferentially decreasing their ration amount to near zero at final maturation in late September and early October.

During 1989 and 1990, there was no apparent relationship between fish health, survival, and egg quality between the fed and unfed groups (Tables 3 and 6). However, other NMFS research (unpublished) suggests that feeding of salmon until maturity in captivity may benefit survival and reproductive success. Although we have conflicting data in the present study as to the biological effectiveness of feeding during prespawning holding, the usefulness of such feeding should not be dismissed. The preference for feeding during maturation suggests that nutrition may be a worthwhile component in maximizing reproductive success of cultured fish. At a minimum, feeding during prespawning holding **enticed** fish from depth in the pen and allowed culturists to visually assess conditions of the adult fish without handling.

Yearly incidence of IHN virus in naturally spawning Lake Wenatchee sockeye salmon was seen as a possible barrier to transplanting juveniles to the Yakima River Basin. Although the Yakima River Basin is considered an IHN-positive watershed, policy and reason dictate that only IHN-negative fish be transferred into the basin.

Traditionally, IHN viral disease has been viewed as the most formidable obstacle to successful culture of sockeye salmon (Wolf 1988, Meyers et al. 1990). IHN is an acute, systemic, and often virulent rhabdovirus with an affinity for renal blood-forming tissues (Wolf 1988). The virus has been isolated from all juvenile

and maturing adult life-stages for **sockeye salmon** of both hatchery and wild origin (Mulcahy et al. 1983, Traxler 1986, Wolf 1988). However, the (pre-immune competent) swim-up-to-fingerling stage hatchery fish are most at risk for IHN disease outbreaks. IHN was a major factor in termination of most sockeye salmon culture in Washington state in the 1960s (**Mullan** 1986).

All spawners from the **NMFS** net-pens in Lake Wenatchee were surveyed for the presence of IHN each year. Even though the **net-** pens at Lake Wenatchee were sited within 500 m of the mouth of a major spawning stream (the White River), IHN incidence in **NMFS pen-** held fish bore little resemblance to levels in natural (instream) spawners. NMFS surveys of sockeye salmon on spawning grounds in the Lake Wenatchee system indicated an IHN prevalence of 89% in 1988 and only 8% in 1989. IHN prevalence on the spawning grounds in the Lake Wenatchee system was not documented 1987 **and 1990; however, it** often approaches 90% (S. Roberts'). In 1987 and 1988, IHN was not detected in any sockeye salmon spawners from the net-pens; however, in 1989 and 1990, 30 and **21%**, respectively, of the net-pen spawners tested positive for IHN virus.

Several investigators have suggested that IHN may be primarily contracted and spread through horizontal transmission from a low number of heavily infected carriers (Wolf 1988, Amos et al. 1989, Meyers et **al.** 1990). Our results support this hypothesis. It is probable that no IHN-carrier fish were captured in 1987 and 1988.

² Steve Roberts, Washington State Department of Wildlife, 1421 Anne Avenue, Wenatchee, WA 98801. Pers. **commun.**, December 1988.

In 1989 and 1990, one to several of the captured fish may have been carriers that served as a focus for spread of IHN in net-pen-held fish.

Capture, or absence, of infected carrier fish best explains the marked dissimilarities we noted between IHN occurrence in natural and captive spawners. However, infection from an alternate host vector cannot be ruled out.

During past decades, stringent and sometimes severe measures have been undertaken to "control" IHN. Culture of susceptible species (particularly sockeye salmon) was terminated at many hatcheries (Mullan 1986, Wolf 1988). At hatcheries where culture of IHN-susceptible species (e.g., chinook salmon) continued, policy and practice included broodstock culling, destruction of eggs from IHN-positive parents, and total destruction of juvenile populations when IHN **was** detected (Wolf 1988, Amos et al. 1989). These efforts were undertaken in hopes of disrupting transmission of the IHN virus. Unfortunately, these "destroy and disinfect" policies often had greater negative impact to the host (fish) population than to the disease. In many cases, yearly viral prevalence in returning broodstock was unchecked while wholesale destruction of eggs left enhancement goals unfulfilled.

During the **1980s**, pathologists became increasingly aware that most, if not all, parent-to-progeny transmission of IHN virus occurs through egg-associated binding of virus to the outside of the egg membrane rather than by true vertical (within egg). routes (Wolf 1988, Yoshimizu et al. 1989). This new understanding of the **virus-**

host relationship provided the possibility that surface disinfection of fertilized gametes with virucidal agents (e.g., iodophor) could be used to minimize IHN occurrence at hatcheries.

From the onset of our program (in 1987), **NMFS** believed surface disinfection of eggs in iodophor (100 ppm/10 minutes), coupled with standard quarantine practices (including isolated egg incubation and fish rearing in a pathogen-free water), was a key to successful hatchery culture of sockeye salmon. We developed the **NMFS/BPA** Stock Restoration Laboratory as a model quarantine and isolation fish hatchery to refine methods for production of healthy, IHN-free, juvenile sockeye salmon. In addition, we initiated individual certification of spawners, and serial certification of juveniles, to ensure that only IHN-negative sockeye salmon juveniles were outplanted in the Yakima River Basin.

NMFS has reared or is rearing progeny from all 1987-1990 **egg-**takes from Lake Wenatchee sockeye salmon. In 1987 and 1988, all spawners were negative for IHN and fish from all groups were reared. However, in 1989 and 1990, we only retained juveniles from **IHN-**negative parents; juveniles from positive parents were donated to WDF for enhancement in the Wenatchee River Basin. For the 1987, 1988, and 1989 broods reared at the laboratory to release (the 1990 brood is still in culture), egg-to-smolt survival ranged from about 75 to 90% (3-year mean = 84%). Survival of the 1990 brood from ponding has been 88.5%. This is much better than the variable (and often low) hatchery survival described by **Mullan** (1986) for juvenile Columbia River sockeye salmon.

Importantly, **all juvenile** sockeye salmon reared at the NMFS/BPA Stock Restoration Laboratory have remained free of IHN during culture. [In addition, 1989 and 1990 broods from IHN-positive parents donated to WDF remained free of IHN (to release and to date, respectively) even though they were reared in the IHN-positive environment of Lake Wenatchee (K. Hopper?).] We feel the treatment of eggs in iodophor was instrumental to production of healthy juveniles. However, successful culture of sockeye salmon requires that stringent quarantine standards be maintained during all phases. In addition, wherever possible, pathogen-free water should be used for egg incubation and fish rearing.

It is encouraging to note that acceptance of disinfection and isolation procedures is augmenting enhancement of many **IHN-**susceptible stocks of salmon in the Pacific Northwest and Alaska. Meyers et al. (1990) detail procedures employed by the Alaska Department of Fish and Game since the mid-1980s to develop a successful sockeye salmon culture program. Federal and state hatcheries in Washington state are now rearing IHN-positive stocks of chinook salmon (*O. tshawytscha*) and sockeye salmon at some facilities (K. Hopper³, R. Brunson⁴). These programs rely on combinations of adult certification, iodophor disinfection of eggs,

³ Kathleen A. Hopper, Washington State Department of Fisheries, 115 General Administration Building, Olympia, WA 98504. Pers. commun., January 1991.

⁴ Ray Brunson, U.S. Fish and Wildlife Service, Fisheries Assistance Office, Olympia, WA 98504. Pers. **commun.**, November 1990.

and isolated egg incubation and fish rearing to produce healthy juveniles from IHN-susceptible stocks of salmon.

The husbandry techniques utilized by NMFS have provided a stable egg supply to produce juvenile sockeye salmon for the Cle Elum Lake Restoration Feasibility Study. From 1988 to 1991, juvenile sockeye salmon were released in the Yakima River Basin to study the feasibility of anadromous salmonids recolonizing the habitat above Cle Elum Dam. These releases included almost 107,000 of the **1987-brood** juvenile sockeye salmon from fall 1988 to spring 1989; almost 90,000 of the 1988 brood from fall 1989 to spring 1990; and about 100,000 of the 1989 brood from fall 1990 to spring 1991. We currently have about 150,000 1990 brood at the laboratory; these fish will be released in the Yakima River Basin in summer 1991 and spring 1992.

These juvenile fish releases were made to evaluate fish outmigration from Cle Elum Lake, fish trapping and bypass systems at Cle Elum Dam, downstream fish migration rates through the Yakima River system, and recovery rates at fish collection facilities (e.g., Prosser and **McNary** Dams). The first adult sockeye salmon from releases of **1987-brood** juveniles in the Yakima River Basin are expected to return to the Yakima River in July 1991. A report detailing outmigration and downstream passage studies from Cle Elum Lake and adult contribution will be available by the end of 1991.

ACKNOWLEDGMENTS

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APPENDIX A

Viral Certification

1989

**ANALYSIS OF LAKE WENATCHEE SOCKEYE SALMON
FOR INFECTIOUS HEMATOPOIETIC NECROSIS
VIRUS (IHN) AND INFECTIOUS PANCREATIC
NECROSIS VIRUS (IPN) - 1989**

R. A. Elston

**Battelle/Marine Sciences Laboratory
Sequim, Washington**

June 1990

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**Pacific Northwest Laboratory
Richland, Washington 99352**

Introduction

The National Marine Fisheries Service (NMFS) is involved in a sockeye salmon restoration program for the Yakima River system. In 1967, 1966 and 1969, donor stock returning sockeye salmon were captured in the Wenatchee River in late July and early August, transported upstream about 26 miles to Lake Wenatchee, and held in captivity in net-pens to maturity in September and October. Eggs are incubated and fry reared in quarantine and, if determined to be free of important fish viruses, resultant juveniles will be used for stock restoration purposes.

In all three years, spawning sockeye salmon were examined by the Battelle Marine Sciences Laboratory for the presence of infectious hematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN) viruses. Sockeye salmon are particularly susceptible to IHN virus which often causes mortalities of young fish. It has generally been believed that many returning adult fish are infected with IHN virus and that the offspring may become infected by intraovarian transmission of the virus. Support for this belief has been based, in part, on a high frequency of heavily infected spawning fish on spawning grounds or in hatcheries in areas where the disease is enzootic.

In 1966 and 1969, we examined 226 and 334 respectively, Lake Wenatchee stock sockeye salmon moved to Lake Wenatchee and found that none were infected with IPN or IHN virus. In this report we provide the results of virological examinations of 334 Lake Wenatchee stock sockeye salmon spawned from net-pens in 1969 as well as 46 sockeye salmon which migrated upriver through Lake Wenatchee and were captured on spawning grounds.

Materials and Methods

Adult returning sockeye salmon (*Oncorhynchus nerka*) were captured by National Marine Fisheries Service personnel during July and August, 1969 at the Tumwater dam on the Wenatchee River (about 20 miles downstream from Lake Wenatchee). After capture, the fish were transported to Lake Wenatchee where they were held in net-pens until maturity. The net-pen site was located about 100 yards from the mouth of the White River. Fish were spawned and samples collected for virological examination (n=334) at the Lake Wenatchee site in September and early October 1969. In addition, 24 adult fish were captured from spawning grounds on the Little Wenatchee River, and 24 adult fish captured from spawning grounds on the White River in late September and early October, respectively. Both of these capture sites were between one-half and three miles upstream from the mouth of these waterways on Lake Wenatchee. Reproductive fluids from all fish were individually collected, diluted in phosphate buffered saline (PBS) containing polyethylene glycol (PEG) and an 6-fold normal concentration of antibiotics; spleen-kidney tissues from file-fish pools were placed in an identical buffer-antibiotic-PEG solution. All materials were transported on ice to the Battelle Marine Sciences Laboratory and processed for inoculation onto tissue cultures within 72 hours of collection. The general methods described by Amos (1985) were used in the examinations. The following specific techniques were

employed. Reproductive fluids and homogenized kidney-spleen extract were inoculated onto two cell lines (EPC and CHSE-214) at two dilutions each (1/2 and 1/10 for reproductive fluids and 1/10 and 1/80 for kidney-spleen tissue pools) and incubated at 15-18°C. All samples were subcultured, either following the appearance of presumptive cytopathic effect or fourteen days following inoculation onto the cultures. Selected individual tissue cultures which displayed cytopathic effect on primary and secondary culture were tested for the presence of IHN virus using a serum neutralization procedure. Anti-IHN virus antiserum prepared against virus isolated against Cedar River, Washington IHN virus isolated from sockeye salmon, (supplied by J. Winton, National Fisheries Research Center, U.S. Dept. Interior, Seattle, WA.) was used in the neutralization procedure.

Results

Results presented in Table 1 indicate that some fish from all pens tested positive for virus (confirmed by selected serum neutralization tests to be IHN virus). The proportion of males positive by examination of individual reproductive fluids ranged from 2.4% in Pen B to 9.3% in Pen D. However, the five-fish pooled male samples of spleen-kidney tissues indicated maximum ranges of infection of 63.4% in Pen A to 88.9% in Pen C. The results of the pooled spleen-kidney samples are most probably an overestimate of the true infection prevalences (due to pooling) but the markedly higher prevalences than obtained by examining reproductive fluids indicate that spleen-kidney tissues are more likely to detect virus in male fish. The proportion of females positive by examination of individual reproductive fluids ranged from 30% in Pen A to 93% in Pen D.

None of the 24 fish from the White River were positive for virus while 16.7% (4/24) of the Little Wenatchee River fish were positive for IHN virus.

Table 1. Number (%) Sockeye salmon positive for IHN virus.

	<u>Sample Source</u>			
	<u>Pen A</u>	<u>Pen B</u>	<u>Pen C</u>	<u>Pen D</u>
Males, n=	41	41	40	46
Repro. fl.	2/39 (5.1)	1/41 (2.4)	2/40 (5.0)	4/43 (9.3)
Spl/Kid	26/41 (63.4)	33/40 (82.5)	32/36 (88.9)	34/43 (79.1)
Females, n =	40	44	39	43
Repro. fl.	12/40 (30.0)	14/43 (32.6)	23/39 (59.0)	40/43 (93)
Spl/kid	30/40 (75)	36/41 (87.8)	39/39 (100)	40/40 (100)
All fish, n=	81	85	79	69
Repro. fl.	13/79 (17.7)	15/84 (17.9)	25/79 (31.6)	45/86 (52.3)
Spl/kid	56/81 (69.1)	69/81 (85.2)	71/75 (94.7)	74/83 (89.2)

Discussion

In 1987 and 1988 all of the sockeye salmon spawned from net-pens in Lake Wenatchee were negative for IHN and IPN viruses (Flagg et al 1988,1990). In 1988, even though the net-pen fish were all negative for the virus, 89% of the fish (n= 18) from the spawning grounds were positive for IHN virus. This contrasts with the 1989 results in which the four net pens had prevalences (based on evaluation of individual reproductive fluids) from 17.7% to 52.3% and only 8.4% of the fish from the spawning ground were virus positive.

The reason that virus positive fish were found in the net-pens in 1959 but not in 1987 or 1988 is not known. It is possible that a very low proportion of carrier fish exists and that one or more of these was transferred to the pens in 1939 but not in 1987 or 1988. Alternatively, it is possible that the pens were contaminated with the virus by another source but there is no direct evidence for any such source. Examination of the results for the female fish shows a wide range of fish positive for the virus, depending on which pen they resided in. Pen A had only 30% positive while Pen D had 93% positive, suggesting that the infection may have started in or near Pen D and spread to the other pens.

Further studies in subsequent years should be conducted to determine the long term annual occurrence of IHN in these fish.

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APPENDIX B
Viral Certification
1990

SEPH R. BLUM
Director



STATE OF WASHINGTON
DEPARTMENT OF FISHERIES

115 General Administration Building • Olympia, Washington 98504 • (206) 753-6600 • (SCAN) 234-6600

November 8, 1990

Tom Flagg
NMFS
Manchester Field Station
P.O. Box 38
Manchester, WA 98353

Dear Tom,

Attached are the results for the Lake Wenatchee sockeye samples that we processed for you. Ovarian fluids (OF) and milt samples were tested on EPC cell line by plaque assay held at 15°C for 14 days. OF were plated at three log 10 dilutions and milts were spun and the supernatants plated at one dilution. The OF from the first three eggtakes and twelve pools of kidney/spleen tissues (five fish/pool) were plated on CHSE,,, cell line to fulfill certification requirements for viruses other than IHN. Seven of 12 kidney/spleen pools were positive for IHN. The minimum levels of detection were 10 pfu/ml for OF and milt and 200 pfu/ml for kidney/spleen pools.

If you have any questions please give me a call.

Sincerely,

Joan Thomas
Joan Thomas

1990 Lake Wenetchee sockeye salmon

Spawn Date	Fish No.	IHNV pfu/ml		
		Ovarian EPC	-Fluids CHSE	Milt EPC
9/8	1	1×10^3		
	2	$>10^5$	+	
	3	10		
	4	4×10^4	+	
	5			
	6	10		
	7			
	8			
	9			
	10			
	11			
	12	4×10^4	+	
	13			
9/24 at Montlake	1	5×10^1	+	
	2	20		
	3	20	-	
	4			
	5	20		
	6	-		
	7	5×10^3	+	
	8			no sample
	9			no sample
	10			no sample
	11			no sample
	12			no sample
	13			no sample
	14			no sample
	15			no sample
	16			no sample
	17	$>10^5$	+	no sample
	18	$>10^5$	+	no sample
	19			no sample
	20			no sample
	21	20		no sample
	22			no sample
	23			no sample
	24			no sample
9/25	14	-		
	15	$>10^5$	+	
	16	2×10^2		
	17	-	-	
	18	2×10^3	+	
	19	$>10^5$	+	
	20			-
	21	-	-	
	22	7×10^3	-	
	23	$>10^5$	+	
	24	$>10^5$	+	

Spawn Date	Fish No.	Ovarian EPC	Fluids CHSE	Milt EPC
	25	-	-	
	26	-	-	-
	27	-	-	-
	28	2×10^2	+	-
	29	-	-	-
	30	2×10^4	-	-
	31	-	-	-
	32	$> 10^5$	+	-
	33	-	-	-
	34	-	-	-
	35	10	+	-
	36	-	-	-
	37	-	-	-
	38	-	-	-
	3	-	-	-
	40	1×10^3	-	-
	41	$> 10^5$	+	-
	42	-	-	10
	43	-	-	-
	44	-	-	-
	45	10	-	-
	46	no sample	-	-
	47	no sample	-	-
9/26	46	-	-	no sample
	47	-	-	. no sample
	48	-	-	-
	49	-	-	-
	50	-	-	-
	51	-	-	-
	52	-	-	-
	53	-	-	-
	54	-	-	-
	55	-	-	-
	56	-	-	-
	57	-	-	-
	58	-	-	-
	59	-	-	-
	60	- (tox @ 10^0)	-	-
	61	-	-	-
	62	-	-	-
	63	- (tox @ 10^0)	-	-
	64	-	-	-
	65	10	-	-
	66	-	-	-
	67	-	-	-
	68	-	-	-
	69	-	-	-
	70	-	-	-
	71	-	-	-
	72	-	-	-
	73	-	-	-

Spawn Date	Fish No.	'Ovarian Fluids EPC	CHSE	Milt EPC
	74			
	75			
	76			
	77			
	78			
	79			
	80			
	81	no sample		
	82	no sample		
	83	no sample		
10/2	81	- (tox @ 10")		
	82	-		-
	83	2x10 ⁴		-
	84	>10 ⁵		-
	85	10		-
	86	- (tox @ 10 ⁰)		90
	87	-		-
	88	2x10 ²		-
	89	-		-
	90	- (tox @ 10 ⁰)		-
	91	>10 ⁵		-
	92	7x10 ²		-
	93	- (tox @ 10 ⁰)		-
	94	6x10 ⁴		20
	95	4x10 ²		-
	96	7x10 ²		-
	97	2x10 ²		-
	98	10		-
	99	-		90
	100	2x10 ²		-
	101	- (tox @ 10 ⁰)		-
	102	-		10
	103	3x10 ⁴		-
	104	25		no sample
	105	4x10 ²		-
	106	3x10 ²		two samples #1 - & #2 lo
	107	1x10 ⁵		-
	108	-		-
	109	4x10 ²		
	110	40		
	111	1x10 ²		
	112	>10 ⁵		
	113	1x10 ²		
	114	>10 ⁵		
	115	>10 ⁵		
	116	-		
	117	3x10 ²		no sample
	118	2x10 ²		no sample
	119	3x10 ²		no sample
	120	>10 ⁵		no sample
	121	20		no sample

Spawn Date	Fish No.	Ovarian EPC	Fluids CHSE	Milt EPC
	122	$>10^5$		no sample
10/3	123	-		
	124	- (tox @ 10^0)		
	125	- (tox @ 10^0)		
	126			
	127			
	128			
	129	- (tox @ 10^0)		20
	130			
	131			
	132			
	133			
	134			
	135			
	136			
	137			
	138	-		
	139	1×10^3		
	140	- (tox @ 10^0)		
	141			
	142			no sample
	143			no sample
	144	no sample		no sample
10/10	145	10		2×10^2
	146	-		10
	147	1×10^4		10
	148	-		-
	149	-		$>10^3$
	150	-		-
	151	-		-
	152	- (tox @ 10^0)		no sample
	153	-		-
	154	10		-
	155	- (tox @ 10^0)		-
	156	-		-
	157	- (tox @ 10^0)		-
	158	-		-
	159	-		-
	160	-		-
	161	- (tox @ 10^0)		-
	162	20		-
	163	-		-
	164	-		-
	165	-		-
	166	-		-
	167	-		-
	168	3×10^4		no sample
	169	-		no sample
	170	-		no sample
	171	-		no sample
	172	10		no sample

Spawn Date	Fish No.	Ovarian EPC	Fluids CHSE	Milt EPC
	173			no sample
	174			no sample
	175	-		no sample
	176	2×10^4		no sample
	177	- (tox @ 10^0)		no sample

TOTALS: OF - 67/200 positive for IHNV
Milt - 11/164 positive for IHNV
Pairs - 74/200 positive for IHNV assuming that the same number OF and milt were paired together.