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CLE ELUM LAKE SOCKEYE SALMON RESTORATION FEASIBILITY STUDY

Annual Report 1988

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CLE ELUM LAKE SOCKEYE SALMON RESTORATION FEASIBILITY STUDY, 1988-1989

Annual Report

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ABSTRACT

This report summarizes research activities conducted by the National Marine Fisheries Service (NMFS) from July 1988 through March 1989 relating to the Cle Elum Lake sockeye salmon restoration feasibility study. During this period, efforts focused on collection and spawning of adult sockeye salmon (Oncorhynchus nerka nerka) from the Wenatchee River, incubation of eggs from the 1988-brood, and the rearing of juveniles from the 1987-brood.

In late July and early August 1988, 520 adult sockeye salmon were captured at fishways on the Wenatchee River and transferred to net-pens in Lake Wenatchee. Fish were held to maturity in late September and early October, spawned, and eggs incubated at a quarantine hatchery in Seattle, WA. Pre-spawning survival was over 85.0% and egg viability averaged about 80%. Some excess males were not spawned.

The 336 sockeye salmon successfully spawned from the net-pens at Lake Wenatchee were surveyed for the presence of infectious hematopoietic necrosis (IHN) and other replicating viruses. In addition, 13 and 5 sockeye salmon spawners were surveyed from spawning grounds on the White and Little Wenatchee Rivers, respectively, from within the Lake Wenatchee system. All spawners from the net-pen system were free of IHN, while 89% from the spawning grounds were positive for the virus.

Survival of 1987-brood juveniles in culture at the Montlake Quarantine Hatchery has been good, with over 80% survival from eyed eggs to smolt. About 25,000 freeze-branded and coded-wire-tagged (CWT) fish were released into Cle Elum Lake in mid-November 1988 (at 6- to 8-g average weight) to assess overwinter survival and outmigration potential. We are presently holding over 93,000 (10- to 15-g average weight) 1987-brood Lake Wenatchee sockeye salmon juveniles for release in Cle Elum

Lake or the Cle Elum and Yakima Rivers in spring 1989. These fish have been certified three times as being free of IHN.

Survival of the 1988-brood to March 1989 has been good, with about 93% survival from eyed-egg to fry. The NMFS presently has over 350,000 1988-brood Lake Wenatchee sockeye salmon fry in culture; these fish will be used in outmigration studies in the Yakima River Basin in 1990. The NMFS is presently enlarging fish rearing capacity at the Montlake quarantine hatchery to accommodate these fish.

CONTENTS

INTRODUCTION

In 1986, a project to evaluate the feasibility of re-establishing anadromous sockeye salmon (Oncorhynchus nerka nerka) runs to Cle Elum Lake in the Yakima River Basin of Washington State was established between the Bonneville Power Administration (BPA) and the National Marine Fisheries Service (NMFS). Historically, the Yakima River Basin supported large runs of anadromous sockeye salmon. However, these runs were eliminated with the development of irrigation storage reservoirs without fishways during the early 1900s (Robison 1957; Mullan 1986). A major focus of the initial phase of the program (1987-1988) was acquiring a suitable donor stock to provide healthy juveniles for studies on lake survival and downstream passage.

During the first year of the program (1987-1988), NMFS successfully obtained (1987) donor stock sockeye salmon that were certified free of infectious hematopoietic necrosis (IHN) and began rearing the progeny (Flagg et al. 1988). The current report summarizes research activities from July 1988 to March 1989 relating to the Cle Elum Lake sockeye salmon restoration feasibility study. During this period, efforts focused on collection and spawning of (1988 donor stock) adult sockeye salmon from the Wenatchee River, incubation of eggs, and the rearing of juveniles from both the 1987- and 1988-broodstock. Outmigration studies (lake survival and downstream passage) will be conducted during the spring of 1989 and 1990, and recommendations for run restoration presented in 1991.

In 1988, returning adult sockeye salmon were captured at either the Dryden or Tumwater fishways on the Wenatchee River, transported to Lake Wenatchee, and held to maturity in floating net-pens. At maturity these fish were spawned and gametes transferred to the NMFS Montlake quarantine hatchery in Seattle, Washington (Fig. 1). All spawners were surveyed for the presence of IHN, and eggs incubated in an isolated

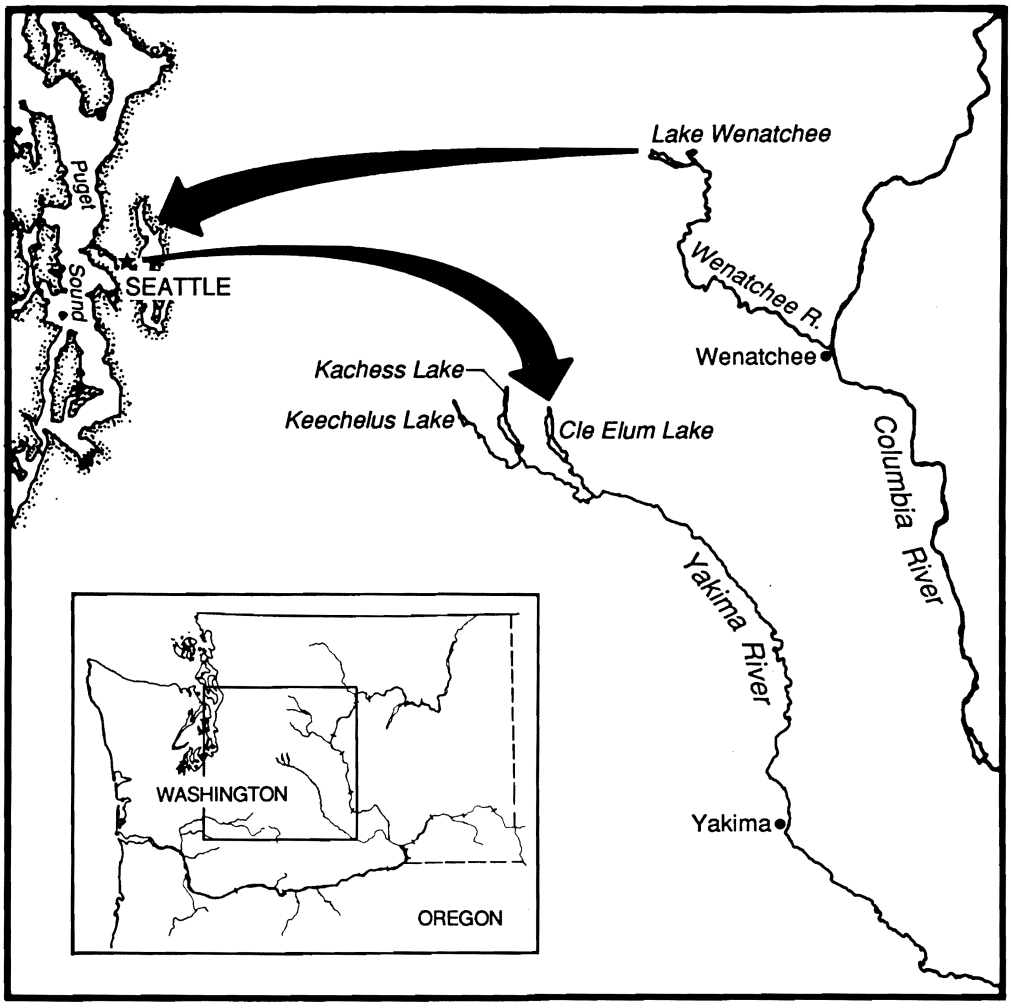

Figure 1.--Movement of salmon gametes from the cage system in Lake Wenatchee to the NMFS Montlake quarantine hatchery in Seattle, Washington. Lower arrow indicates introduction of disease-free juvenile sockeye salmon to Cle Elum Lake in the Yakima River Basin.

quarantine system (Novotny et al. 1985). In addition, sockeye salmon spawners from Lake Wenatchee system spawning grounds on the White and Little Wenatchee Rivers, and 45 kokanee (O. n. kennerlyi) from the recipient watershed (Gold Creek in the Yakima River Drainage) (Fig. 2), were surveyed for IHN.

Juveniles from 1987 spawnings (described in Flagg et al. 1988) 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. 2) during 1989. Progeny from the 1988 spawning will be used in outmigration studies in 1990. Adult collection will be repeated during 1989 and 1990.

MATERIALS AND METHODS

Adult Collection and Holding

A set of four floating modular net-pens was installed by NMFS personnel in Lake Wenatchee, Chelan County, Washington in late June 1988. The 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. 3). 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. The pens remained in the lake until mid-October 1988 when they were removed and stored for the season.

The NMFS received Washington Department of Fisheries (WDF) Hydraulic Permits (Numbers 00-37408-01, 02, and 03); a Chelan County Shoreline Management Permit (Number 1586); a Washington State Department of Ecology (DOE) Substantial Development Permit (Number 590-14-7804); and a Washington State Department of Natural Resources (DNR) Right of Entry Permit (Number 20-012737) for siting the pen system.

Fish trapping efforts at Dryden Dam (Fig. 2) commenced the first week in July 1988. However, high-water conditions allowed most of the sockeye salmon to avoid the

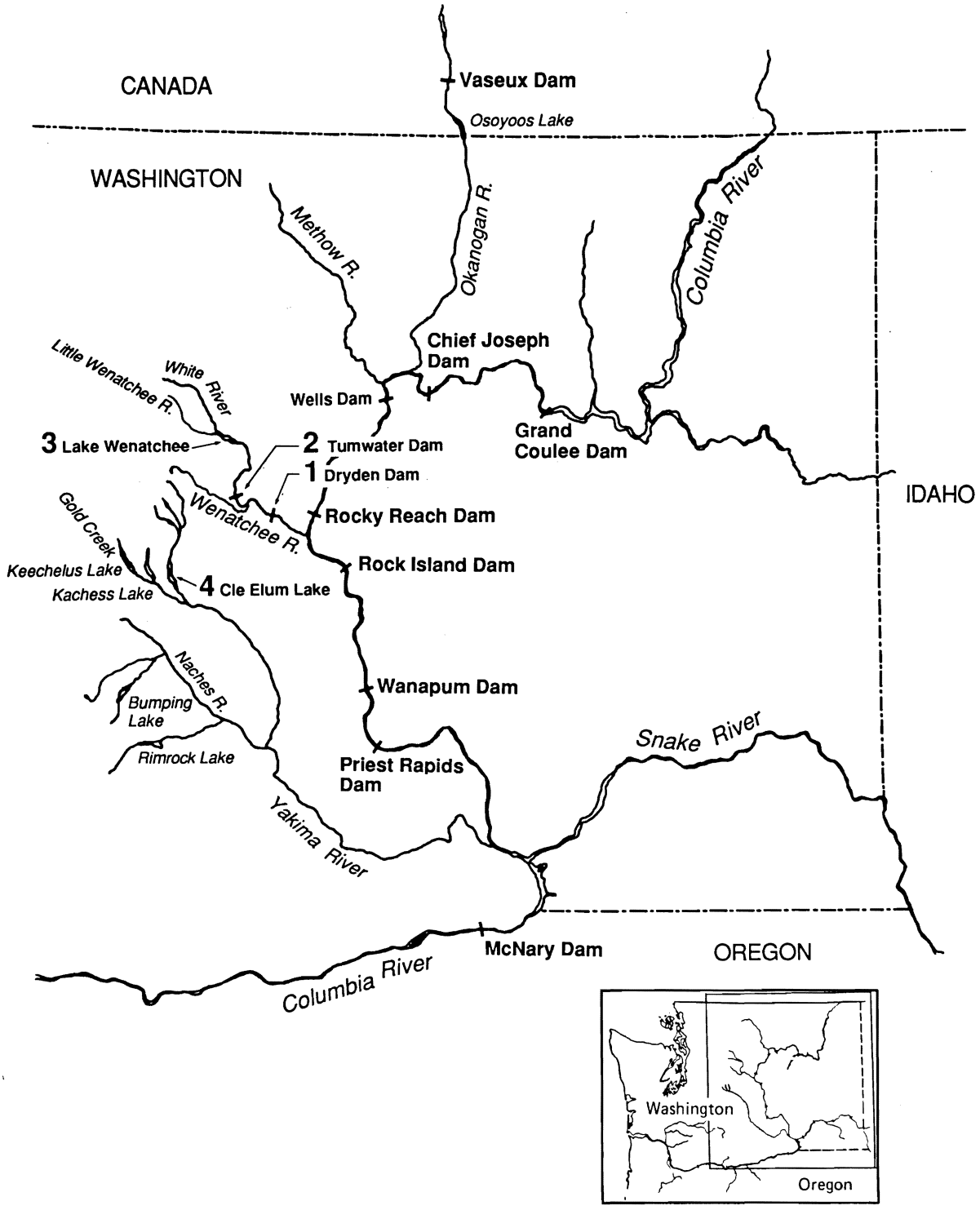

Figure 2.--Map of sockeye salmon project study area in the Mid-Columbia Basin. Arrows indicate: (1) Dryden Dam, (2) Tumwater Dam, (3) Lake Wenatchee, and (4) Cle Elum Lake.

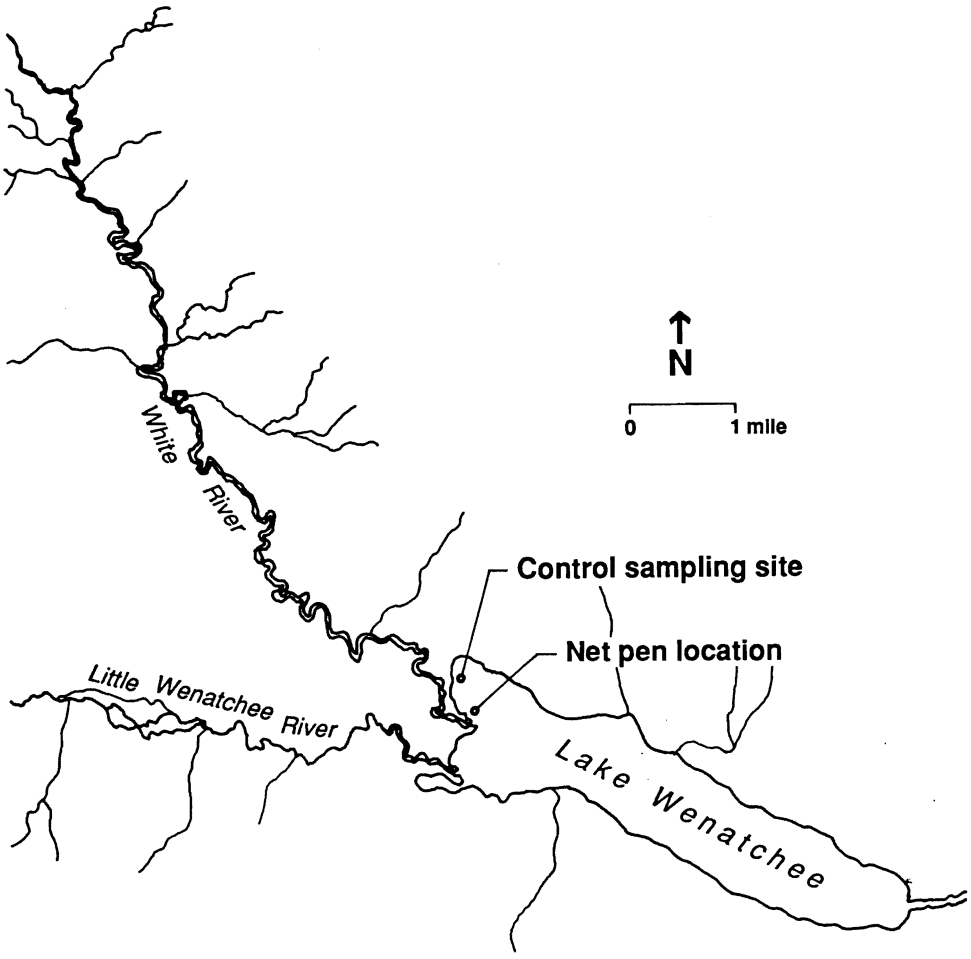

Figure 3.--Map of Lake Wenatchee and major tributaries.

fishway and trap and to continue upriver. Therefore, adult collection efforts were shifted upstream to Tumwater Dam. The NMFS obtained permission from Chelan County PUD and WDF to construct a temporary trap in the fishway at Tumwater Dam (Fig. 2), and trapping operations at that site began on 29 July 1988.

All adult fish were transported in a 6,000-liter fish transportation truck to Lake Wenatchee (Figs. 2 and 3) 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 88-45) and a WDF Fish Transfer Permit (Number 429-3-88) for the collection and transportation of these fish.

All fish were held in the net-pen system and fed a maintenance diet of frozen krill (Euphausia pacifica) at 0.5% of body-weight/day, or less, to maturity. The effects of holding and feeding these fish on water quality and the benthos was monitored throughout the holding period (Appendix A).

Spawning

During mid-to-late September the fish were sorted by appearance according to stages of maturity in the same manner as in 1987 (Flagg et al. 1988). To inspect potential spawners, a net-pen was raised gradually (at 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 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 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 m^3, 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.

During 1988, spawning of fish at Lake Wenatchee was conducted in a (2.4- by 9.8-m) fish-marking trailer modified by NMFS as a portable quarantine fish spawning station. Interior modifications to the trailer included a water table with a screen and multiple V-board arrangement for individual holding of up to 17 fish at a time. This table was supplied with a lake water supplied spray-mist system for cooling and flushing of carcasses and with tanks for disinfection. 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. In most cases, eggs and milt were removed from the fish prior to transport to the NMFS Montlake quarantine hatchery. Female carcasses were opened by a cut from the vent to 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 carcasses (from spawned and non-spawned fish) were placed into individually numbered plastic bags. All eggs, milt, and carcasses were transported to the NMFS Montlake quarantine hatchery (about a 2.5-hour trip) in large (approximately 0.9- by 1.2- by 0.6-m) totes containing crushed ice. The NMFS received a WDF Fish Transfer Permit (Number 428-3-88) for this transfer of fish and gametes. Fertilization of eggs began immediately upon arrival at the hatchery. Eggs from individual females were placed in 1-qt plastic containers and fertilized with the sperm from one male. In most cases, fertilized eggs were water hardened for an initial 3 to 5 minutes and then disinfected in 100 ppm iodophor solution for an additional 10 min. However, in an effort to determine causes of low fertility in 1987, eggs from ten females were fertilized and water-hardened in 100 ppm iodophor in a method similar to that used in 1987 (Flagg et al. 1988).

Viral Certification

As in 1987, each adult spawner was examined for the presence of replicating virus by a certified Fish Pathologist (American Fisheries Society Board) at the Battelle Marine Laboratory in Sequim, Washington. Kidney, spleen, and reproductive fluid samples were removed for viral analysis of net-pen spawners after transport to the NMFS quarantine hatchery. In 1988, 13 naturally spawning sockeye salmon from the White River and 5 from the Little Wenatchee River were also surveyed for IHN (Fig. 3). In addition, 45 spawning kokanee were examined from the recipient watershed (Gold Creek in the Yakima River Drainage). Samples from natural spawners were collected on the spawning grounds.

The testing laboratory inoculated tissue, ovarian fluid, and sperm samples onto appropriate cell lines and observed for cytopathic effects (see Appendix B for materials and methods). Eggs from each mating were held in individual isolation incubators until results of the viral analysis were known. Offspring were also analyzed (by the whole body homogenization method) for replicating viruses (Appendix C).

Egg Incubation and Juvenile Rearing

In 1988, the 1988-brood eggs were incubated at the NMFS Montlake quarantine hatchery in a manner similar to that in 1987 (Flagg et al. 1988). Pathogen-free, dechlorinated City of Seattle water was used for incubation, and all discharge water was treated to eliminate pathogens. After water hardening, the eggs were transferred to individual isolation incubators numbered for identification of the female/male spawning pair (Novotny et al. 1985). After eyeing, eggs from families certified as being IHN-free were combined and transferred to Heath-type incubators for hatching. Standard hatchery procedures were followed during egg incubation.

Rearing of 1987- and 1988-brood juveniles was also conducted at the NMFS Montlake quarantine hatchery. Juveniles were reared in 1.2- and 2.4-m diameter tanks

using methods to minimize potential cross contamination. Standard husbandry practices were followed in maintaining the fish.

Outmigration Releases

In early November 1988, about 25,000 (6- to 10-g average weight) 1987-brood juvenile sockeye salmon were freeze-branded and coded-wire-tagged from the approximately 110,000-fish population at the NMFS Montlake quarantine hatchery. A 60-fish subsample of these were stressed (by dewatering for about 1 minute), sacrificed after 48-hour holding, and sent to the Battelle Marine Laboratory for viral analysis.

On 16 and 17 November 1988, two replicates of approximately 12,500 fish each were transported from the hatchery to Cle Elum Lake and released in the lake at a site between the dam and the U.S. Forest Service Wish-Poosh Campground (Fig. 4). Replicate releases were freeze-branded with right anterior (RA) Y in position 1 and 2 and coded-wire-tagged with codes 23-22-53 and 23-22-54, respectively.

RESULTS AND DISCUSSION

Adult Collection and Holding

Between the last 2 weeks of July and the first week of August 1988, 3 and 517 adult sockeye salmon were trapped at Dryden Dam and Tumwater Dam, respectively, on the Wenatchee River (Table 1). The Dryden Dam fish facility was used exclusively in 1987 (Flagg et al. 1988) during a period when river flows rarely exceeded 1,300 cfs. However, 1987 was a low-water year in the Wenatchee River Basin. In 1988, the river flows during the trapping period were usually above 1,500 cfs. These, more typical, water flows allowed most of the sockeye salmon to avoid the fishway and trap and continue upriver by jumping the low-head irrigation dam at Dryden. Therefore, adult collection efforts were shifted upstream to Tumwater Dam during the last week of July 1988 (Table 1).

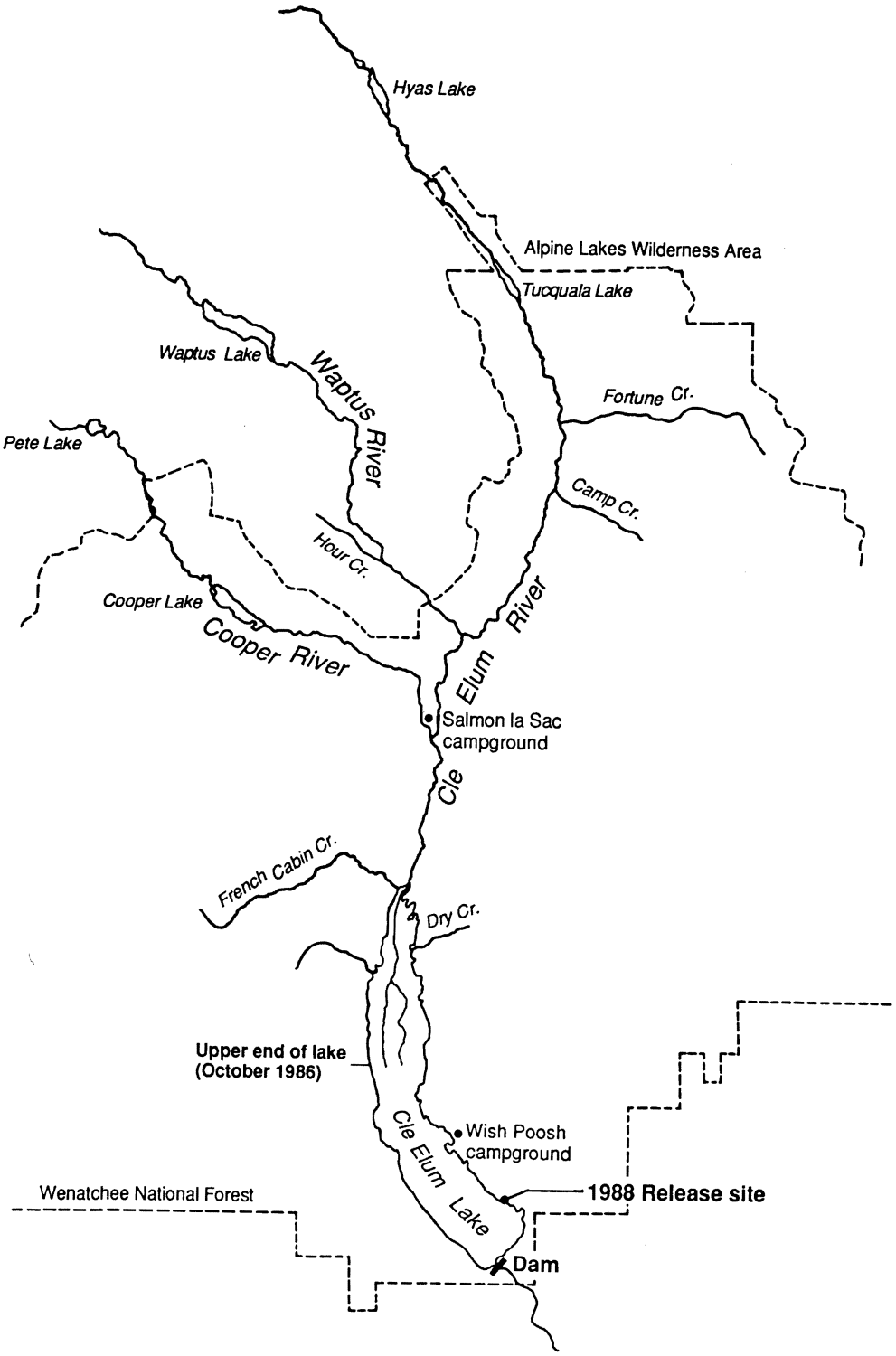

Figure 4.--Map of Cle Elum Lake and major tributaries.

Table 1.--Inventory of salmon captured at Dryden Dam or Tumwater Dam fish facilities on the Wenatchee River, 1988.

Date	Time of trap operation (h)	Number of fish trapped/day		
		Sockeye	Chinook[a]	Steelhead[b]
Dryden Dam:				
22 Jul 88	0500 to 1800	3[c]	0	0
Tumwater Dam:				
29 Jul 88	0500 to 1800	114[c]	7 (1 jack)	2
30 Jul 88	0528 to 0830 0900 to 1600	165[c]	22	4
31 Jul 88	0615 to 1150	177[c]	12	3
1 Aug 88	0815 to 1232 1420 to 1820	47[c]	26 (4 jacks)	0
2 Aug 88	0610 to 1800	14[c] 89[d]	41 (4 jacks)	0
3 Aug 88	0600 to 1145	84[d]	4	0

[a] Chinook salmon returned to river, n = 112 total.
[b] Steelhead returned to river, n = 9 total.
[c] Transported to net-pens in Lake Wenatchee, n = 520 total.
[d] Scale-sampled by Columbia River Inter-Tribal Fish Commission and returned to river, n = 173 total.

During trapping at Tumwater Dam, incidental numbers of chinook salmon (O. tshawytscha) and steelhead (O. mykiss) were also collected and released to the river (Table 1). In addition, 173 sockeye salmon adults were collected at Tumwater Dam in cooperation with the Columbia River Inter-Tribal Fish Commission (CRITFC), sampled for scale analysis, and returned to the river. Profiling by CRITFC indicated that, in 1988, the sockeye salmon run returning to Lake Wenatchee was made up of a nearly even distribution of three predominate age-classes (1 freshwater:2 ocean; 2 freshwater:2 ocean; and 1 freshwater:3 ocean)(Swartzburg[1]).

A total of 520 sockeye salmon were collected for donor-stock spawning, transported by tank truck to net-pens anchored in the north end of Lake Wenatchee, and held (at about 1.5 kg/m^3) until spawning during late September and early October. The net-pens were checked daily and total documented mortality during the (pre-spawning) holding period was 73 fish (14%). However, during the spawning period, a hole in a net-pen was noted. We believe an additional 62 fish escaped from the net-pens during the holding period. The prespawning survival of sockeye salmon held in net-pens in Lake Wenatchee was over 90% in 1987 (Flagg et al. 1988) and over 85% in 1988. These prespawning survival rates are much higher than reported for prespawning sockeye salmon held in hatchery raceways and ponds (Mullan 1986, Amos et al. in press).

Feeding is not a common (hatchery) strategy when holding prespawning salmonids. However, the NMFS has conducted other captive broodstock programs where Atlantic and Pacific salmon were reared to maturity in seawater net-pens (Harrell et al. 1984a, 1984b, 1985). These studies suggested that maturing fish will accept a ration level of about 0.5% body weight/day; we used this guide for establishing the feed rates in the present study. In both the 1987 and 1988 studies, the fish held in the net-pens fed

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[1] Matthew Swartsburg, CRITFC, 975 S.E. Sandy Blvd., Suite 202, Portland, OR 97214, pers. commun. October 1988.

actively on the maintenance diet of krill. It is probable that both the net-pen environment (providing access to lake water to a depth of about 7.5 m) and supplemental feeding contributed to the high survival observed in these studies.

During 1988, NMFS evaluated the environmental effects of holding adult sockeye salmon in the net-pens. The results indicated that holding fish in the net-pens had no adverse effect on water quality or the benthos (Appendix A).

Spawning

Gametes were collected from 336 adult sockeye salmon (175 females and 161 males) between 21 September and 6 October 1988 (Table 2). All females that survived to maturity were spawned; however, 49 surviving males 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 557 mm, while females averaged 492 mm with an average fecundity of 2,688 eggs and egg viability of 79.0% (Table 2 and 3).

In 1988, egg viability was much better than the 40% average documented in 1987 (Flagg et al. 1988). The low viability in 1987 was attributed to the addition of iodophor disinfectant during fertilization. Therefore, in 1988, fertilization protocol was modified to include draining ovarian fluid from eggs prior to addition of sperm and allowing a fertilization period (in water) before addition of iodophor solution. Tests conducted in 1988 substantiate this modification in protocol (from 1987 methods) and indicated that eggs directly fertilized in iodophor had a much lower viability (about 18% average) than eggs fertilized in water prior to disinfection in iodophor (over 90% average viability). In the future, NMFS will use a protocol allowing 3 to 5 minutes of fertilization and water hardening prior to disinfection in 100 ppm iodophor for 10 minutes. We believe this procedure allows adequate time for fertilization while maintaining disinfection standards.

Table 2.--Spawning dates; number of females and males; and average length, fecundity, and egg viability of Lake Wenatchee sockeye salmon spawned from NMFS net-pens, 1988.

Date	Number spawned		Average			
			Length		Fecundity	Egg
	Female	Male	Female (mm)	Male (mm)	(number of eggs)	viability (%)
21 Sep 88	4	1	466	532	2,304	96.1
22 Sep 88	2	1	517	546	3,166	93.8
26 Sep 88	16	16	514	561	2,164	75.4
27 Sep 88	39	34	522	551	2,880	84.6
28 Sep 88	52	52	529	542	2,922	76.9
29 Sep 88	47	42			2,578	79.1
3 Oct 88	14	14			2,318	73.8
6 Oct 88	1	1			2,459	0.0
TOTAL	175	161				
AVERAGE[a]			519	544	2,688	79.0

a Combined average of all female (n = 175) and male (n = 161) spawners.

Table 3.--Spawner identification numbers, fecundity, and egg survival for Lake Wenatchee sockeye salmon spawned from NMFS net-pens, 1988.

Date spawned	Mating pair ID number		Total eggs	Live eggs	
	Female	Male		Eyed (no.)	Viability (%)
21 Sep 88	001	001	3,290	3,113	94.6
	002	001	1,901	1,812	95.3
	003	001	2,087	1,987	95.2
	004	001	1,939	1,925	99.3
22 Sep 88	005	002	2,757	2,544	92.3
	006	002	3,575	3,407	95.3
26 Sep 88	101	101	1,154	960	83.2
	102	102	3,379	2,865	84.8
	103	103	1,456	877	60.2
	104	104	3,387	1,944	57.4
	105	105	1,987	1,710	86.1
	106	106	2,277	138	6.1
	107	107	701	671	95.7
	108	108	2,195	2,049	93.3
	109	109	2,581	201	7.8
	110	110	2,853	2,686	94.1
	111	111	1,216	1,097	90.2
	112	112	1,559	1,520	97.5
	113	113	2,248	2,150	95.6
	114	114	3,042	2,876	94.2
	115	115	2,182	1,539	70.5
	116	116	2,414	2,183	90.4
27 Sep 88	201	201	2,571	0	0.0
	202	202	2,078	1,889	90.9
	203	203	3,898	2,593	66.5
	204	204	3,381	3,102	91.7
	205	205	2,187	1,906	87.2
	206	206	3,784	2,992	79.1
	207	207	2,757	2,330	84.5
	208	208	3,730	3,551	95.2
	209	209	2,822	2,522	89.4
	210	210	3,733	3,176	85.1
	211	211	2,371	2,309	97.4
	212	212	2,645	2,320	87.7*
	213	213	2,309	2,272	98.4

Table 3.--Continued.

Date spawned	Mating pair ID number		Total eggs	Live eggs	
	Female	Male		Eyed (no.)	Viability (%)
	214	214	1,965	1,790	91.1
	215	215	2,685	2,011	74.9
	216	216	2,219	2,001	90.2
	217	217	3,818	3,587	93.9
	218	218	2,489	2,326	93.5
	219	219	1,808	1,772	98.0
	220	220	3,801	3,630	95.5
	221	221	1,883	1,673	88.8
	222	222	2,557	2,445	95.6
	223	223	3,573	3,374	94.4
	224	224	2,767	2,713	98.0
	225	225	2,907	823	28.3
	226	226	3,062	2,934	95.8
	227	227	3,242	2,989	92.2
	228	228	2,692	2,514	93.4
	229	229	2,048	2,025	98.9
	230	230	3,830	2,153	56.2
	231	231	2,759	2,632	95.4
	232	231	2,507	75	3.0
	007	003	2,152	2,117	98.4
	008	004	3,171	3,106	98.0
	009	005	3,558	3,495	98.2
	010	003	3,767	3,373	89.5
	011	004	3,336	3,136	94.0
	012	005	2,045	1,992	97.4
	013	005	3,437	3,326	96.8
28 Sep 88	301	301	3,008	2,824	94.0
	302	302	1,734	69	4.0
	303	303	2,768	426	15.4
	304	304	3,639	3,306	90.8
	305	305	4,010	2,207	55.0
	306	306	3,247	3,134	96.5
	307	307	2,939	2,219	75.5
	308	308	1,688	1,292	76.5
	309	309	2,705	2,640	97.6
	310	310	2,810	2,651	94.3
	311	311	4,271	3,405	79.7
	312	312	3,146	1,278	40.6
	313	313	2,310	2,176	94.2
	314	314	2,992	2,499	83.5

Table 3.--Continued.

Date spawned	Mating pair ID number		Total eggs	Live eggs	
	Female	Male		Eyed (no.)	Viability (%)
	315	310	2,714	2,624	96.7
	316	316	2,636	2,436	92.4
	317	317	3,005	2,877	95.7
	318	318	1,802	1,321	73.3
	319	319	3,660	3,283	89.7
	320	320	2,193	2,123	96.8
	321	321	3,723	3,358	90.2
	322	322	3,076	1,712	55.7
	323	323	2,137	1,989	93.1
	324	324	2,914	2,771	95.1
	325	325	3,593	3,273	91.1
	326	326	3,369	2,743	81.4
	327	327	1,794	1,724	96.1
	328	328	3,044	791	26.0
	329	329	2,350	1,193	50.8
	330	330	3,208	2,381	74.2
	331	331	3,330	3,021	90.7
	332	332	3,000	2,711	90.4
	333	333	2,930	2,717	92.7
	334	334	2,748	2,565	93.3
	335	335	3,459	3,306	95.6
	336	336	1,819	1,778	97.7
	337	337	3,189	2,814	88.2
	338	338	2,382	2,338	98.2
	339	339	2,459	2,294	93.3
	340	340	3,920	2,929	74.7
	341	341	3,402	3,189	93.7
	342	342	2,788	2,547	91.4
	343	343	3,603	3,216	86.3
	344	344	3,642	3,061	84.0
	345	345	1,949	282	14.5
	346	346	2,314	91	3.9
	347	347	3,536	2,560	72.4
	348	348	2,191	1,471	67.1
	349	349	3,061	386	12.6
	350	350	4,031	3,173	78.7
	351	351	2,732	2,659	97.3
	352	352	2,956	2,509	84.9
29 Sep 88	401	401	2,584	1,866	72.2
	402	402	3,283	2,085	63.5

Table 3.--Continued.

Date spawned	Mating pair ID number		Total eggs	Live eggs	
	Female	Male		Eyed (no.)	Viability (%)
	403	403	3,306	1,800	54.4
	404	404	3,049	1,762	57.8
	405	405	510	143	26.3
	406	406	2,060	1,285	62.4
	407	407	2,459	2,416	98.3
	408	408	3,100	2,073	66.9
	409	409	2,451	2,371	96.7
	410	410	3,036	2,864	94.3
	411	411	2,891	1,192	41.2
	412	412	2,389	704	29.5
	413	413	2,309	2,203	95.4
	414	414	2,456	2,324	94.6
	415	415	2,512	766	30.5
	416	416	2,511	2,243	89.3
	417	417	2,372	2,170	91.5
	418	418	2,522	2,049	81.2
	419	419	2,733	2,711	99.2
	420	420	2,221	2,169	97.7
	421	421	2,368	1,103	46.6
	422	422	2,501	1,998	79.9
	423	423	2,744	2,505	91.3
	424	424	2,355	2,106	89.4
	425	425	2,110	2,047	97.0
	426	426	2,557	1,608	62.9
	427	427	2,589	2,364	91.3
	428	428	4,581	3,888	84.9
	429	429	1,471	1,418	96.4
	430	430	2,489	2,148	86.3
	431	431	2,766	1,130	40.9
	432	432	2,408	2,382	98.9
	433	433	2,567	2,316	90.2
	434	434	2,049	1,993	97.3
	435	435	2,736	2,684	98.1
	436	436	2,634	2,503	95.0
	437	437	2,973	2,592	87.2
	438	438	3,098	268	8.7
	380	380	3,462	3,332	96.2
	381	380	2,794	2,646	94.7
	382	380	1,770	1,706	96.4
	383	381	3,294	3,191	96.9
	384	381	3,059	2,988	97.7

Table 3.--Continued.

Date spawned	Mating pair ID number		Total eggs	Live eggs	
	Female	Male		Eyed (no.)	Viability (%)
	385	382	2,845	2,744	96.4
	386	382	2,031	1,917	94.4
	387	383	2,119	2,085	98.4
	388	383	2,063	1,275	61.8
3 Oct 88	501	501	2,624	1,763	67.2
	502	502	49	5	10.2
	503	503	2,953	2,032	68.8
	504	504	2,678	1,624	60.6
	505	504	2,168	1,031	47.6
	506	505	2,762	577	20.9
	507	506	2,212	2,178	98.5
	508	507	3,210	2,968	92.5
	509	508	2,348	2,231	95.0
	510	509	3,233	3,182	98.4
	511	510	3,284	2,895	88.2
	512	511	1,045	929	88.9
	513	512	2,314	2,300	99.4
	514	513	1,583	1,541	97.3
6 Oct 88	601	601	2,459	0	0.0
Total			472,570	374,959	
Average			2,685	2,142	79.0
Eggs used in experiment				372,639**	

* Indicates egg lot (female Number 212) removed from experiment due to loss of viral samples during processing.

** Reflects removal of a total of 2,320 eggs (from female Number 212) from the study.

Viral Certification

High rates of IHN infection have been reported for sockeye salmon spawning in the Little Wenatchee and White Rivers (Roberts[2]). In 1988, we documented an 89% incidence of IHN infection in Lake Wenatchee sockeye salmon surveyed on the spawning grounds (Appendix B). This viral disease can cause major losses of eggs and juveniles in salmonids in fish hatcheries and is usually prevalent in sockeye salmon. A concern has been that IHN could infect other salmonid species when offspring from the Wenatchee River sockeye salmon are used in outmigration studies. To address this concern, all spawners from the NMFS net-pens in Lake Wenatchee were surveyed for the presence of IHN.

In 1987, all spawners from the NMFS net-pens were certified as 100% IHN-free (Flagg et al. 1988). In 1988, a total of 336 (175 female and 161 male) sockeye salmon spawners from the NMFS net-pens in Lake Wenatchee were surveyed (Appendix B). Detection of viral infection in either parent would have necessitated destroying all eggs from that particular mating. As in 1987, Battelle Laboratories reported no indication of replicating virus in any of the sockeye salmon spawned from NMFS net-pens in Lake Wenatchee. However, samples from one fish (female Number 212) were contaminated with bacteria (preventing viral analysis) and, although there was no reason to suspect IHN infection, the eggs from this lot were destroyed (Appendix B and Table 3).

In both 1987 and 1988, IHN was not detected in sockeye salmon spawners held away from the rivers used for spawning, even though the net-pens were within 500 m of the mouth of the White River and within the river's outflow plume. Based on the absence of IHN infection in these fish, NMFS and Battelle pathologists suggest that IHN may be primarily contracted or spread by adults after they reach the spawning grounds (Appendix B). In addition, 1987-brood Lake Wenatchee juvenile sockeye

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[2] Steve Roberts, Washington Department of Wildlife, 1421 Anne Avenue, Wenatchee, WA 98801, pers. commun. December 1988.

salmon under culture by NMFS have been periodically checked and determined to be free of replicating viruses (Flagg et al. 1988; Appendix C). This, apparently, substantiates the lack of vertical transmission of virus from net-pen fish. IHN infections may be avoided by holding maturing adults away from the spawning ground, thereby limiting exposure to IHN virus.

Egg Incubation and Juvenile Rearing

Survival of the 1987-brood juveniles has been over 75% from ponding in early 1988 (Flagg et al. 1988) to March 1989. We have over 80,000 of these 10- to 15-g fish available for outmigration studies in the Yakima River Basin in spring 1989.

Incubation of the 1988-brood was initiated in late September and early October 1988, and all fish hatched during January and February 1989. Eyed egg to hatch survival generally averaged over 99%. A total of about 362,500 1988-brood swim-up fry survived to ponding. Ponding of swim-up fry began on 3 March 1989 and ended on 12 March 1989.

Survival to March 1989 has been good, with about 93% survival from eyed egg to fry. Most mortalities were associated with genetic anomalies or normal attrition. The NMFS presently has over 350,000 1988-brood Lake Wenatchee sockeye salmon fry in culture; these fish will be used in outmigration studies in the Yakima River Basin in 1990. Quarantine standards are being maintained in the culture facility, and IHN certification will be conducted through smoltification. The NMFS is presently enlarging fish rearing capacity at the Montlake quarantine hatchery to accommodate both 1987- and 1988-brood sockeye salmon juveniles and progeny anticipated from the upcoming 1989 egg-take.

Outmigration Releases

In fall 1988, about 25,000 1987-brood juvenile sockeye salmon were released into Cle Elum Lake to assess overwinter survival and outmigration potential. Immediately

after transfer to the lake, the fish appeared vigorous and active and we presume survival will be good. Outmigration success for these groups will be evaluated in 1989 by springtime recaptures at the Prosser fish collection facility on the lower Yakima River. The NMFS is freeze branding over 80,000 additional 1987-brood juveniles to be used in outmigration releases during spring 1989. In addition, we have PIT-tagged almost 3,000 fish and are installing a PIT-tag detection system at Prosser.

SUMMARY

In 1987 and 1988, 263 and 520 adult sockeye salmon, respectively, were taken from the Wenatchee River and held to maturity in net-pens in Lake Wenatchee. All spawners were surveyed for the presence of IHN. In both years, 100% of the spawners from the net-pens were IHN-free, while natural river spawners had an infection rate of nearly 90%. The 1987-brood juveniles were periodically surveyed for viruses and remained negative; 1988-brood juveniles will also be surveyed during culture. It appears that adult sockeye salmon held to maturity away from the spawning ground do not contract, express, or transmit IHN. This technique may be useful where IHN-free progeny are desired. Captive holding of IHN-susceptible adults away from the spawning grounds may become a valuable management technique.

The NMFS is continuing to develop brood-stock holding and culture techniques that offer potential in the management of IHN problems. We are planning to collect 520 sockeye salmon adults in summer 1989. All of these fish will be held to maturity off the spawning grounds in net-pens in Lake Wenatchee. All spawners will be checked for IHN; supplemental data should be available in fall 1989 to confirm whether an IHN-free status can be repeated.

The NMFS began outmigration studies in Cle Elum Lake using donor juveniles from the Wenatchee stock. Lake survival and downstream passage studies will be

conducted during the spring of 1989 and 1990, and recommendations for run restoration presented in 1991.

ACKNOWLEDGMENTS

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APPENDIX A

Environmental Effects of Net-Pens

Appendix A--Environmental effects of net-pens.

INTRODUCTION

The Shoreline Management Permit for the temporary National Marine Fisheries Service (NMFS) net-pen facility at Lake Wenatchee required monitoring of environmental effects in 1988. This report addresses effects on water quality and the benthos from holding maturing sockeye salmon in net-pens in Lake Wenatchee during this period.

MATERIALS AND METHODS

Adult Collection and Holding

A net-pen system designed for holding adult sockeye salmon was installed in Lake Wenatchee (Chelan County, Washington) by NMFS personnel in late June 1988. Four (4.8- by 4.8- by 7.5-m deep) net-pen modules were installed in 1988. These pens were located on the 12- to 15-m depth contour near the mouth of the White River (Northwest 1/4, Section 14, Township 27 North, Range 16 East) and were in place from early July to mid-October 1988. The pens were then removed and stored for the season.

NMFS received Washington Department of Fisheries (WDF) Hydraulic Permits (Numbers 00-37408-01, 02, and 03); a Chelan County Shoreline Management Permit (Number 1586); a Washington State Department of Ecology (DOE) Substantial Development Permit (Number 590-14-7804); and a Washington State Department of Natural Resources (DNR) Right of Entry Permit (Number 20-012737) for siting the pen system.

Fish trapping at Dryden Dam began the first week in July 1988. However, high-water conditions allowed most of the sockeye salmon to avoid the fishway and trap, and to continue upriver. Therefore, adult collection efforts were shifted upstream to

Tumwater Dam. NMFS obtained permission from Chelan County PUD and WDF to construct a temporary trap in the fishway at Tumwater Dam; trapping operations began at that site on 29 July 1988.

Fish were transported in a 6,000-liter fish transportation truck to Lake Wenatchee and transferred via a 9-m fish hauling barge to the floating net-pens. NMFS was issued a WDF Scientific Collection Permit (Number 88-45) and a WDF Fish Transfer Permit (Number 429-3-88) for the collection and transportation of these fish.

Fish were fed a maintenance diet of frozen krill (Euphausia pacifica) at about 0.5% of body-weight/day and held to maturity in the net-pen system.

Environmental Monitoring at Pen Site

We monitored the effects of uneaten feed and fecal wastes on the benthos by sampling sediments at the pen site and at a control site located about 60 m offshore of the Cougar Inn. Sediment cores were collected by divers from a depth of 12 to 15 m during the period the pens were in the water. Sampling periods were on 21-22 June and 13-14 October. Both pen and control sampling sites covered about a 15-m^2 area of lake bottom; samples were taken randomly from each quadrant using an 18.0-cm^2 core sampler. All benthic samples were fixed in 4% buffered formaldehyde. Four core samples from each sampling site were selected at random. These core samples were screened on 1.0-mm sieves and all macrobenthic organisms were sorted to major taxa and enumerated using standard methods (Pennak 1978). Data from each core sample were adjusted to represent a 1.0-m^2 area of lake bottom.

Water temperatures and dissolved oxygen levels were monitored at the pen and control sites and were recorded to the nearest 0.1°C and 0.1 ppm, respectively. Light absorption in the water column was observed to the nearest 0.1 m using a secchi disk.

RESULTS AND DISCUSSION

Adult Collection and Holding

Between the last 2 weeks of July and the first week of August 1988, three adult sockeye salmon were trapped at Dryden Dam and 517 at Tumwater Dam on the Wenatchee River. Fish were transported by tank truck to the net-pens and held at a density of about 1.5 kg/m^3 until spawned in late September and early October. Individual weights of the fish ranged from about 1.5 kg to 3.0 kg and total biomass in the pens was about 1,000 kg. During this study, and a previous study in 1987 (Flagg et al. 1988), the fish fed actively on a maintenance diet of frozen krill.

Environmental Monitoring at Pen Site

Water quality and abundance of benthic organisms were monitored and compared between the net-pen and control site during the period fish were held in the pens. During the early part of the monitoring period, water quality measurements were not taken at the control site; however, enough data are available to make useful comparisons. Throughout the monitoring period, water temperature and dissolved oxygen and light absorption were similar at the two sites (Appendix Table 1). Dissolved oxygen levels usually remained near saturation at both sites and never dropped below 95%. These comparisons indicate that lake water quality was not affected by the holding of fish in pens.

The potential effects on the benthos from organic wastes from uneaten krill and fish feces from the penned sockeye salmon were documented by comparing bottom samples taken at the pen and control sites. There was no apparent difference in the average number of invertebrates in the benthic samples from the pen and control sites at either the beginning or end of the fish-holding period (Appendix Table 2). In addition, at the end of the holding period the divers indicated that there was no visual accumulation of organic debris below the pens.

Species abundance in the benthic samples (Table 2) was primarily limited to aquatic oligochaetes (Tubificidae)--the dominate forms in most lakes at depths exceeding 1 m (Pennak 1978). The 2,000 to 2,500 individuals per m^2 observed in the present study are considerably less than the 8,000 or more per m^2 often observed in enriched lakes (Pennak 1978). The evidence indicates that holding and feeding of maturing sockeye salmon at the pen site in Lake Wenatchee did not have an adverse affect on the benthos.

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Appendix Table 1.--Water temperature, dissolved oxygen levels, and water clarity at the net-pen and control site in Lake Wenatchee during the 1988 sockeye salmon holding period.

	Pen Site					Control Site *				
		Temperature		Dissolved oxygen			Temperature		Dissolved oxygen	
Date	Turbidity (m)	Bottom (°C)	Surface (°C)	Bottom (ppm)	Surface (ppm)	Turbidity (m)	Bottom (°C)	Surface (°C)	Bottom (ppm)	Surface (ppm)
05 Aug 88	5.2	14.1	14.7	9.5	9.4	---	----	----	---	---
06 Aug 88	5.1	11.8	12.2	10.6	10.0	---	----	----	---	---
07 Aug 88	5.0	13.5	15.5	9.6	9.8	---	----	----	---	---
08 Aug 88	4.7	13.9	15.8	9.7	9.3	---	----	----	---	---
09 Aug 88	4.8	13.2	15.1	9.8	9.4	---	----	----	---	---
10 Aug 88	5.2	14.1	15.1	10.2	9.6	---	----	----	---	---
11 Aug 88	5.8	14.0	14.1	10.0	9.9	---	----	----	---	---
12 Aug 88	5.0	14.5	17.7	9.7	9.3	---	----	----	---	---
13 Aug 88	5.1	12.0	13.5	9.8	9.6	---	----	----	---	---
14 Aug 88	5.2	12.7	14.0	9.8	9.6	---	----	----	---	---
15 Aug 88	5.6	13.8	14.1	9.9	9.9	---	12.0	12.5	---	---
16 Aug 88	5.7	13.8	14.0	9.8	9.9	---	14.0	14.2	---	---
17 Aug 88	4.9	14.2	15.2	9.6	9.7	---	15.2	15.8	---	---
18 Aug 88	5.8	14.0	15.1	9.7	9.7	---	14.2	15.8	---	---
19 Aug 88	5.8	14.0	15.3	10.0	9.5	---	15.1	15.5	---	---
20 Aug 88	5.2	14.1	15.1	9.9	9.4	---	15.1	15.8	---	---
21 Aug 88	6.2	14.0	15.0	9.9	9.8	---	16.0	16.0	---	---
22 Aug 88	6.1	14.2	15.5	9.6	9.4	---	14.8	15.1	---	---
23 Aug 88	6.6	14.8	15.8	---	---	---	15.0	15.2	---	---
24 Aug 88	6.9	15.2	16.5	9.4	8.9	---	15.2	16.2	---	---
25 Aug 88	6.2	14.0	14.9	9.6	9.3	---	14.1	14.5	---	---
26 Aug 88	6.8	14.2	14.8	9.8	9.4	---	14.3	14.9	---	---
27 Aug 88	6.9	15.5	16.8	9.4	9.0	---	16.0	17.0	---	---
28 Aug 88	6.2	16.0	17.5	9.2	8.9	---	16.8	17.5	---	---
29 Aug 88	5.2	15.0	16.0	9.4	9.2	---	14.9	15.9	---	---
30 Aug 88	6.8	14.1	14.6	9.5	9.3	---	14.9	15.8	---	---
31 Aug 88	7.0	15.0	17.5	9.3	9.0	---	16.5	17.0	---	---
01 Sep 88	7.1	15.0	17.5	9.5	9.1	6.4	16.5	17.0	9.3	9.1
02 Sep 88	6.1	16.0	18.5	9.3	9.1	6.1	16.0	18.5	9.1	8.9
03 Sep 88	6.0	15.5	17.5	9.2	8.9	5.8	15.5	17.0	9.4	9.0
04 Sep 88	6.2	15.5	18.5	9.2	8.9	6.0	15.5	18.0	9.3	9.1
05 Sep 88	6.0	16.0	18.5	9.3	8.8	6.0	16.0	18.0	9.3	9.0
06 Sep 88	5.2	16.0	17.5	9.4	9.2	5.2	15.9	17.6	9.4	9.2
07 Sep 88	5.2	15.3	15.8	9.4	9.2	5.0	15.5	16.3	---	---
08 Sep 88	6.2	15.1	15.2	9.6	9.3	7.1	15.3	15.9	9.4	9.2
09 Sep 88	5.6	15.0	15.2	9.3	9.2	7.0	14.5	15.0	9.4	9.5
10 Sep 88	5.9	14.0	15.0	9.5	9.5	6.1	14.5	14.8	9.2	9.1
11 Sep 88	7.2	14.0	14.5	9.5	9.5	6.4	14.0	14.0	9.5	9.5
12 Sep 88	5.8	15.0	16.5	9.6	9.5	6.7	15.0	16.0	9.5	9.4
13 Sep 88	6.8	15.5	16.0	9.5	9.5	6.9	15.0	15.5	9.5	9.6
14 Sep 88	7.4	15.0	16.0	9.4	9.4	6.0	15.0	16.0	9.6	9.4
15 Sep 88	7.2	15.0	15.8	9.6	9.5	6.0	15.0	15.2	9.7	9.5
16 Sep 88	7.0	13.9	14.0	9.8	9.6	6.0	14.1	14.4	9.7	9.6
17 Sep 88	7.0	13.8	14.0	---	---	6.0	14.0	14.1	---	---
18 Sep 88	6.8	13.5	13.8	9.6	9.6	6.8	13.5	13.8	9.6	9.6
19 Sep 88	6.6	14.0	14.5	9.6	9.6	6.5	14.0	14.5	9.6	9.5
20 Sep 88	8.0	14.0	15.0	9.6	9.5	8.2	14.0	15.0	9.6	9.5
21 Sep 88	7.0	14.0	14.5	9.5	9.5	7.1	14.0	14.5	9.5	9.5
22 Sep 88	7.5	13.5	13.5	9.6	9.6	5.9	13.5	13.5	9.6	9.6
23 Sep 88	6.3	13.5	13.5	9.7	9.7	6.0	13.5	13.5	9.6	9.6
24 Sep 88	6.8	13.0	13.0	9.7	9.8	6.5	13.0	13.0	9.7	9.7

Appendix Table 1.--Continued.

25 Sep 88	6.4	13.0	13.5	9.8	9.8	6.2	13.0	13.5	9.8	9.8
26 Sep 88	6.5	13.5	13.5	9.8	9.6	7.2	13.5	13.5	9.8	9.8
27 Sep 88	6.8	13.0	13.5	9.8	9.7	7.0	13.0	13.5	9.7	9.8
28 Sep 88	7.0	13.0	14.0	9.7	9.7	7.1	13.5	14.0	9.7	9.7
29 Sep 88	7.5	13.5	14.0	9.6	9.4	7.2	13.2	14.0	9.6	9.4
30 Sep 88	6.8	13.0	14.0	9.8	9.8	7.1	13.5	13.5	9.8	9.8
01 Oct 88	7.4	13.5	14.0	9.7	9.8	7.6	13.5	14.0	9.8	9.8
02 Oct 88	7.2	13.5	14.5	9.8	9.7	6.8	13.5	14.5	9.7	9.8

* Dashes indicate no data available.

Appendix Table 2.--Benthic data from net-pen and control site at Lake Wenatchee, 1988.

Date/ sample type	Number of organisms			
	Oligochaetes[1]		Pelecypods[2]	
	Observed[3]	Expanded[4]	Observed[3]	Expanded[4]
21 June				
pen-site 1	4	887	0	0
pen-site 2	11	2,412	0	0
pen-site 3	9	1,973	0	0
pen-site 4	11	2,412	0	0
Average		1,921		0
22 June				
control-site 1	4	887	0	0
control-site 2	21	4,605	0	0
control-site 3	10	2,193	0	0
control-site 4	3	657	0	0
Average		2,085		0
13 October				
pen-site 1	18	3,947	0	0
pen-site 2	19	4,166	0	0
pen-site 3	8	1,754	3	658
pen-site 4	2	438	0	0
Average		2,576		165
14 October				
control-site 1	9	1,973	2	438
control-site 2	13	2,850	0	0
control-site 3	11	2,412	0	0
control-site 4	12	2,631	0	0
Average		2,467		110

[1] Primarily 5- to 15-mm long by 1- to 3-mm diameter Tubificidae.
[2] Immature 2- to 4-mm diameter bivalves.
[3] Number observed in 18.0-cm diameter core sampler.
[4] Expanded to represent number per m^2.

APPENDIX B

Viral Certification

of

Spawners

ANALYSIS OF LAKE WENATCHEE SOCKEYE SALMON FOR INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHN) AND INFECTIOUS PANCREATIC NECROSIS VIRUS (IPN) - 1988

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Sequim, Washington

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Richland, Washington 99352

INTRODUCTION

The National Marine Fisheries Service (NMFS) is involved in a sockeye salmon restoration program for the Yakima River system. In 1987 and 1988, donor stock returning sockeye salmon were captured in the Wenatchee River in late July and early August, transported upstream about 20 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 both 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. 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 carrier fish on spawning grounds or in hatcheries in areas where the disease is enzootic.

In 1987, we examined 226 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 336 Lake Wenatchee stock sockeye salmon spawned from net-pens in 1988 as well as 18 sockeye salmon which migrated upriver through Lake Wenatchee and were captured on spawning grounds. Kokanee salmon from the Yakima watershed were also examined.

MATERIALS AND METHODS

Adult returning sockeye salmon (Oncorhynchus nerka) were captured by National Marine Fisheries Service personnel between 31 July and 5 August, 1988, 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 300 yards from the mouth of the White River. Fish were spawned and samples collected for virological examination (n=336) at the Lake Wenatchee site from 20 September to 6 October 1988. In addition, five adult fish were captured from spawning grounds on the Little Wenatchee River, and 13 adult fish captured from spawning grounds on the White River on 29 September and 5 October, respectively. Both of these capture sites were between one-half and three miles upstream from the mouth of these waterways on Lake Wenatchee. On each of two dates, 3 October and 12 October 1988, 22 kokanee, (O. nerka kennerlyi) were captured in Gold Creek in the Yakima River system.

Reproductive fluids and pooled kidney-spleen tissues from all fish, except the Gold Creek samples, were individually collected and diluted or placed in phosphate buffered saline (PBS) containing an 8-fold normal concentration of antibiotics and transported to the Battelle Marine Sciences Laboratory. All samples were 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. All fish samples were processed individually except the Gold Creek sockeye samples which were processed as pooled 4 to 5 fish tissue samples. 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. Cultures were either observed for 28 days for cytopathic effect or subcultured (blind passed) prior to 28 days and subsequently held and observed for 28 days. Of the 336 Lake Wenatchee sockeye samples, 209 were subcultured, all of the CPE positive White River and Little Wenatchee River sockeye samples were subcultured and 3 of 10 tissue pools of the Gold Creek kokanee samples were subcultured. Selected individual tissue cultures which displayed cytopathic effect on primary and secondary cultures 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

No IHN or other virus was detected in 335 tissue samples from fish spawned from net-pens in Lake Wenatchee (Table 1). In one sample (fish number 212, collected September 27) the tissue cultures were contaminated with bacteria and the cultures were discarded. The NMFS was notified of this result and advised to destroy the eggs resulting from this female since it was not possible to obtain definitive results regarding the presence of the virus.

Sixteen of 18 fish (89%) collected from the spawning grounds on the White River and Little Wenatchee River, both upstream from the Lake Wenatchee net-pen site, were positive for the virus. These tissue cultures displayed cytopathic effect (CPE) typical of that produced by IHN virus. The concentration of virus in the tissues was not determined by titration but CPE occurred within 2 days of inoculation of the tissues cultures and completely destroyed most of the culture monolayers within 4 days. Following subculture, two samples of tissue culture fluid from fish from each river were verified to contain IHN virus by the serum neutralization method. None of the 10 pools of fish tissues from the Gold Creek fish samples were positive for virus.

Table 1. Sockeye Salmon and Kokanee Examined for IHN and IPN Viruses on EPC and CHSE-214 Cell Lines

Date Site Collected	Number of Fish		Number of Fish Positive for Virus	IHN Serum Neutralization Positive/Tested
	Female	Male		
9/20/88 to 10/88	174		0/174	NA[1]
Lake Wenatchee net-pen		161	0/161	NA
9/29/88	4		4/4	2/2
Little Wenatchee River		1	1/1	
10/5/88	13		11/13	2/2
White River		0	NA	
10/3/88	5 pools		0/5 pools	NA
Gold Creek				
10/12/88	5 pools		0/5 pools	NA
Good Creek				

[1]NA = not applicable.

DISCUSSION

In 1988, all of the sockeye salmon spawned from net-pens in Lake Wenatchee were negative for IHN and IPN viruses. These results are consistent with 1987 results in which all 226 sockeye salmon spawned from net-pens in Lake Wenatchee were negative for the fish viruses (Flagg et al. 1988). Importantly, the current study demonstrated that fish from the same population which reach spawning grounds naturally are infected with IHN virus at a high prevalence (89%). Although the virus concentration was not determined by titration in the White River and Little Wenatchee River samples, the rapid onset of CPE and rapid destruction of the tissue culture monolayers indicate a relatively high concentration of virus present in the samples. Interestingly, the sites from which positive fish were sampled were one to three miles from the net-pen site.

Results of the Gold Creek samples from the Yakima River system suggest that at least this watershed in the recipient Yakima drainage is IHN virus-free. However, the IHN virus status of the Yakima River system should be confirmed by further samples from a broader distribution of watersheds within the system.

Several hypotheses can be advanced to explain the lack of detectable IHN virus in the net-pen fish in contrast to the typical observation of high levels of infection intensity and prevalence on sockeye salmon spawning grounds:

1) Sockeye salmon may not be latently infected throughout their life span. The IHN infection may result from exposure to the virus once the fish reach the spawning grounds. This hypothesis implies the presence of an alternative host for the virus which produces and sheds sufficient amounts of virus to infect a high proportion of the population at the time the fish return to the spawning ground.

2) The fish may be selectively infected with IHN at a very low rate prior to the time they reach the spawning grounds. Under this hypothesis the carrier rate could have been so low in the currently reported study that more than 335 fish would have to have been sampled in order to detect the virus. However, based on the assumption of random sampling and a binomial distribution (with 335 fish sampled out of an assumed population of 8,000 fish) the probability that the population is greater than 99% disease free is at least 97%. Thus, we have established with 97% confidence that the population is at least 99% disease free. If there were actually a very low carrier rate, this hypothesis would require that the carriers be infected at a high enough intensity so that they shed sufficient virus in order to infect the other fish on the spawning ground.

3) A large proportion of sockeye may be latently infected with IHN prior to reaching the spawning ground. Under this hypothesis, the stress of returning to the spawning ground would likely trigger the replication of virus to the relatively high titer seen in fish sampled from the spawning ground. This hypothesis implies that the salmon in the net-pens are not subjected to the same level of stress or whatever factors trigger the expression of viral replication and remain latently infected. Consequently, the tissue culture assays commonly employed to detect infected fish, as used in this study, are not sufficiently sensitive or appropriate to detect the virus in these fish.

A more definitive study will be required to validate any of the above hypotheses. However, the first hypothesis seems attractive at this point, given the lack of virus detected in 335 fish, the rapid infection of a high proportion of individuals on the spawning grounds and the apparent lack of virus in the brood.

This method of holding maturing fish in net-pens appears to be an important resource management tool for sockeye salmon. Previously, it has been believed that a high proportion of adult sockeye are infected with IHN virus and that the virus is transmitted to the fry. These NMFS studies have demonstrated that virus-free adult fish and reproductive products can be

readily obtained and offspring safely reared. Thus, it is now possible to obtain sockeye salmon brood fish and utilize their offspring to restore depleted runs without fear of spreading IHN virus.

REFERENCES

Amos, K. H. (ed.) 1985. Procedures for the Detection and Identification of Certain Fish Pathogens. 3rd ed. Fish Health Section, American Fisheries Society, Corvallis, Oregon.

Flagg, T. A., J. L. Mighell, E. S. Slatick, and L. W. Harrell. 1988. Cle Elum Sockeye Program: A Study to Assess the Feasibility of Restoring Anadromous Sockeye Salmon runs to Irrigation Reservoirs within the Yakima River Basin. Annual Report of Research to BPA. Coastal Zone and Estuarine Studies Division, Northwest and Alaska Fisheries Center, NMFS NOAA. (In press).

APPENDIX C

Viral Certification

of

Fry

Battelle
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October 18, 1988

Dr. Lee Harrell
National Marine Fisheries Service
Manchester Experiment Station
P.O. Box 38
Manchester, WA 98353

Dear Dr. Harrell:

I am writing in reference to testing we conducted for you to determine the presence of fish viruses in samples you submitted to the laboratory. On September 20, 1988, you submitted 12 pools of 5 sockeye salmon fry each from the Montlake facility coded MTLK 1-12. We tested these fish for the presence of known fish viruses utilizing standard methods (American Fisheries Society, Fish Health Section, Bluebook, K. Amos, ed., 3rd edition).

Using these methods we did not detect any evidence of known fish viruses.

Sincerely,

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