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IHN-Water Supply Study
Dworshak NFH, 1985

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Introduction

Infectious hematopoietic necrosis virus (IHNV) is the causative agent of infectious hematopoietic necrosis disease (IHN) and has caused extensive losses of steelhead fry (Salmo gairdneri) at Dworshak National Fish Hatchery (DNFH) since 1982. Losses experienced during 1982-84 were 48, 98, and 68 percent respectively. The major period of loss occurred in the hatchery nursery building where fish were less than 2 1/4 inches (250/lb.). Although not completely understood, transmission of the virus is thought to occur both vertically, through the egg, and horizontally, through the water, (Mulcahy et al 1983; Mulcahy and Pascho, 1984; Mulcahy and Pascho, 1985). The current broodstock culling program at DNFH is an attempt to control vertical transmission of the virus. Horizontal transmission is postulated from four possible sources at DNFH:

1. The reservoir water supply, where reservoir fish are carriers;
2. The river water supply, where riverine fish are carriers;
3. Virus remaining viable within the pipes, water delivery system, and rearing facilities of the hatchery;
4. Any combination of the above.

It is not known how long the IHNV can survive in a waterborne state.

The purpose of the study was to evaluate the DNFH water supply to determine the source of the horizontally-transmitted IHNV. This work was part of an IHN disease transmission project at DNFH coordinated among Dworshak Fishery Assistance Office, DNFH, Dworshak Fish Health Center, and Idaho Department of Fish and Game (IDF&G). Findings also were to be used to determine the optimum water supply source for the proposed Clearwater Hatchery to be constructed directly across the North Fork Clearwater River from DNFH.

The study was divided into two separate phases. The first phase was to document fish other than adult steelhead that carried IHNV and entailed collecting fish from Dworshak Reservoir, its tributaries, and the North Fork Clearwater River above DNFH's water intake. The second phase used steelhead fry reared at three locations in DNFH water supply to identify the geographic point where the water was carrying infectious IHNV.

Materials and Methods

Starting in September 1984, fish samples were collected from Dworshak Reservoir, its tributaries, and the North Fork Clearwater River. Special emphasis was placed on collecting spawning kokanee (Oncorhynchus nerka) from tributaries of Dworshak Reservoir (Figure 1). Periodic sampling of the above waters was continued through October 1985. Spawning kokanee from Dworshak Reservoir tributaries were collected in September and October 1984 and 1985 using a portable electrofishing unit. An 18-foot electrofishing boat was used on three separate North Fork Clearwater River fish collections. Gill nets were the primary method of sampling fish from Dworshak Reservoir, although

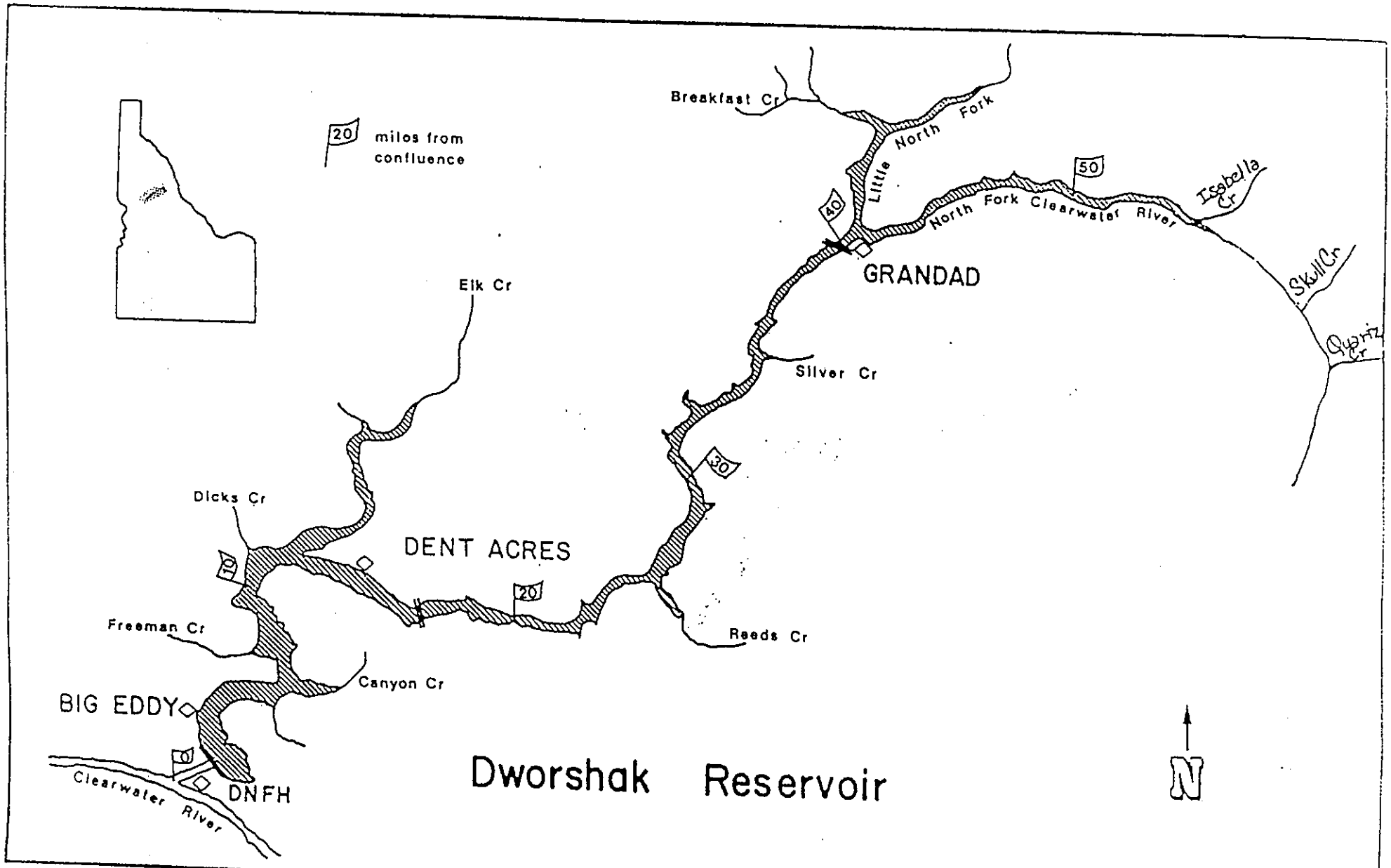

Figure 1. Dworshak Reservoir and major tributaries, North Fork Clearwater River, Idaho

some samples were taken with rod and reel and electrofishing. Analysis of kidney, spleen and ovarian fluid (in spawning kokanee) for IHNV was performed by Dworshak Fish Health Center using the epithelioma papillosum carpio (EPC) cell line according to the method of Burke and Mulcahy (1980).

For the test fish rearing phase of the study, three sites were prepared; on Dworshak Reservoir (Dam), in the hatchery pump house (PH) and in the wet lab at the hatchery (Lab). Four circular tanks equipped with degassers and screen lids were placed at each site. The Dam site, located at Dworshak Reservoir on a floating barge behind the dam, was supplied by water from Dworshak Reservoir. Water was pumped from various depths to maintain desired temperatures. The PH site received water from the hatchery's fire maintenance water supply which is pumped from the North Fork Clearwater River and passes through the first aeration chamber. The Lab site, located on the hatchery, was supplied by water from the hatchery's raw water system which also services the nursery and ponds.

Swim-up fry used for the study were progeny from IHNV-negative steelhead adults spawned at DNFH during takes 12 and 13, on April 16 and 23 respectively. Eggs were held in raw water at DNFH until IHNV status was determined for the adults. Eyed eggs were then moved to Kooskia NFH which has no history of the virus in young steelhead. Eggs were randomly mixed, put in Heath incubator trays, and kept at Kooskia NFH until they developed to swim-up fry. The first load of 135,000 swim-up fry from take 13, moved on June 4, suffered high mortality during transport from Kooskia NFH back to the study sites. Survivors (n=6336) were placed in tank 1 in the Lab. Tanks 1, 2, and 4 at the PH and Dam, and tanks 2 and 4 in the Lab, received the remaining fry from take 13 (n = 20,454 fish/tank; range = 18,761 - 22,777 fish/tank) which were 2200 fish/lb. at the time of transport. Tank 3 at each site was loaded with an average of 21,087 fish/tank (range = 19,390 - 22,256 fish/tank) from take 12. These fish were slightly larger (1800 fish/lb.) than those from take 13. Mortalities, due to transport, and dead eggs were not picked before loading each tank; therefore, dead fish from the first two days after transport were not included in analysis and graphing of mortalities.

Tanks were cleaned once daily. Mortalities were picked and counted daily. To limit tank to tank contamination, each tank was assigned its own cleaning brush and mort net. These were disinfected daily in separate buckets containing a benzalkonium chloride solution. Disposable gloves, changed between tanks, were worn when handling fish and equipment.

Fish were fed 10 times each day at 45-minute intervals. Separate feed buckets were maintained for each site, and disposable gloves were worn when handling feed. OMP starter mash was fed from the start of the study until fry reached 700/lb. Fry were fed OMP 1/32-inch pellets while they were 700-450/lb.; 3/64-inch pellets were fed the remainder

of the study. The tank study concluded when fry attained a size of approximately 250/lb.

Pound counts began 2 weeks after arrival of fry at the test sites and continued once each week. From each tank, three subsamples were weighed and counted. A mean number of fish per pound and mean lengths were calculated from these data. Each site was assigned a set of sampling nets which were disinfected in benzalkonium chloride solution between tanks.

Temperature, dissolved oxygen, nitrogen saturation, and flows were monitored at each site; flow and density indices were calculated for each tank. We attempted to keep similar water temperatures at all sites. Water temperatures were taken twice each day, and minimum/maximum thermometers were read daily. Dissolved oxygen (DO) concentrations were measured weekly from each tank using the Winkler method or, less frequently, a DO meter. Contamination between tanks was avoided by use of disposable cups to obtain water from tanks for DO sampling. A Weiss saturometer was used weekly to measure nitrogen saturation of incoming water at each site. Water flows of 10 gallons per minute (gpm) per tank at the start of the study were increased gradually to 20 gpm at the PH and Lab sites. Maximum flows at the Dam did not exceed 13 gpm per tank due to piping limitations.

Dead or moribund fry from each tank were periodically collected in sterile polyethylene beakers to test for IHNV. Whole fish or viscera in pools of up to five fish were homogenized 1:10 (w/v) in Hanks balanced salt solution and centrifuged at 4°C for 10 minutes at about 1800 X G. Serial tenfold dilutions of supernate were made, and 0.2 ml of each dilution inoculated in duplicate on 75% to 100% confluent cultures of the epithelioma papillosum carpio (EPC) cell line. Inoculated cultures were held for 1 hour, overlayed with 0.75% methylcellulose and incubated for 8 days at 15°C. Cultures were examined daily and fixed and stained on day eight. Samples yielding plaques characteristic of IHNV infection (Burke and Mulcahy, 1980) were considered positive for presence of the virus.

Results

During the fall of 1984 and spring, summer, and fall of 1985, 2167 fish were collected from Dworshak Reservoir, its tributaries and the North Fork Clearwater River. Twelve species were captured with kokanee being the most common (Table 1). Of all the species sampled and analyzed for IHNV, only thirteen specimens of kokanee were found to have IHNV; three in 1984 and ten in 1985. These thirteen kokanee were only lightly infected and all came from tributaries of Dworshak Reservoir (Table 2). These IHN positive fish also came from the latter portion of the kokanee spawning in these tributaries, and the fish were physically quite deteriorated. Four of the positive samples in 1985 came from resampling the last fourteen ovarian fluid after nine additional

Table 1. Number of fish by species and capture location assayed for IHNV, 1984 and 1985.

Species	Dworshak Reservoir Tributaries	Dworshak Reservoir	North Fork Clearwater River (Below Dam)	Number Sampled 1984	Number Sampled 1985	Total Fish Sampled
Kokanee (*Oncorhynchus nerka*)	1528*	197	125	1020	830	1850
Rainbow trout (*Salmo gairdneri*)	0	61	74	65	70	135
Chinook salmon (*Oncorhynchus tshawytasha*)	0	0	26	1	25	26
Whitefish (*Prosopium williamsoni*)	0	4	34	16	22	38
Bull trout (*Salvelinus malma*)	0	3	0	3	0	3
Squawfish (*Ptychocheilus oregonensis*)	0	3	26	25	4	29
Suckers (*Catostomus sp.*)	0	10	57	41	26	67
Smallmouth bass (*Micropterus dolomieui*)	0	5	1	1	5	6
Black Crappie (*Pomoxis nigromaculatus*)	0	2	0	2	0	2
Redside shiner (*Richardsonius balteatus*)	0	8	0	8	0	8
Peamouth (*Mylocheilus caurinus*)	0	0	2	2	0	2
Sculpin (*Cottus sp.*)	0	0	1	0	1	1
Totals	1528	293	346	1184	983	2167

*857 spawning kokanee were sampled in the fall 1984 and 671 spawning kokanee were sampled in the fall 1985. Of these 3 fish were found positive for IHN in 1984 and 10 were found positive in 1985.

Table 2. Location and time of collection of IHN positive fish kokanee salmon, (*Oncorhynchus nerka*), North Fork Clearwater River and tributaries, 1984-85.

Location	Date Positive IHN Fish Collected	Number of IHN Positive Fish	Sex of Positive Fish
Breakfast Creek	10/16/84	2	F
Skull Creek	10/18/84	1	F
Breakfast Creek	9/20/85-9/26/85	4	F
Skull Creek	9/27/85	1	F
Isabella Creek	9/20/85	1	M
	10/16/85	4*	F

*These four positive females were from resampling ovarian fluid that was incubated an additional 9 days.

days incubation. These were the only samples treated this way and so it is not possible to determine if previous samples would have yielded similar results.

Only a few kokanee could be found in Elk Creek and very few spawning kokanee were taken later than mid-October. We believe that the late-spawning kokanee originally planted in Elk Creek have, for the most part, disappeared.

Test tanks at all three sites functioned well throughout the second phase of the study period. Test tank studies started on June 4 and terminated on July 25-28.

As the test fish in the tanks grew and required more space and oxygen, some were removed from each tank on July 10 and 11. The number of fish removed from each tank varied and depended on the amount needed to restore the flow index to 1.0.

No parasites, bacterial or viral pathogens were detected in fish reared at the Dam; however, a few fish were observed with an internal fungal infection. Early in the study, these fish grew slower than fish at the other two sites (Tables 3, 4, and 5).

Mean mortality at the Dam was highest in tank 1 and lowest in tank 3 (Table 5). Mortalities in all Dam tanks remained relatively low after fish reached about 1400/lb. (Table 3; Figure 2).

IHNV was never detected from fish at the PH site; however, a fungal infection, causing hemorrhaging near the vent and lower intestine, was found in fish from tank 4 on June 14. Swollen, red vents were noticed also on morts picked from other tanks at each site. The infection, occasionally seen in first feeding fry, lasted only a short time and was seen in all tanks as a rapid rise and fall in mortality occurring around June 14 (Figures 2, 3, and 4).

Mortalities in tanks 1 and 4 of the PH increased again about July 6 (Figure 3). IHNV was suspected, but was not confirmed. Several dead or moribund fry had enlarged spleens, pale livers, and no food in the gut. *Pseudomonas spp.* and an enteric bacteria (not *Yersinia ruckeri* which causes Enteric Red Mouth) were isolated from kidney tissue. No treatment was administered and mortality remained elevated in these tanks throughout the remainder of the study. The fluke *Sanguinicola spp.* was also found in the gills in low levels.

Tanks 1 and 4 at the PH site had considerably higher mean mortalities than did tanks 2 and 3; and tank 1 showed the widest variation (Table 6).

IHNV was confirmed at the Lab site. Fry in the Lab remained free of infection until July 3 when IHNV was isolated from moribund fish in tank 2. Mortality in this tank increased steadily from July 9 to the

Table 3. Weekly sampling information for Dam site, 1985.

Tank # Date sampled	Size * (fish/lb)	Mean total length (mm)	Density ** index	Flow ** index	Mean temp (°F)	During sample week D.O. (ppm)	During sample week Nitrogen saturation (%)
#1							
Start-6/4	2200	27.0	.07 (.07)	0.96 (0.89)	49.0	--	--
6/19	1408	31.3	.09 (.09)	1.00 (0.92)	50.5	10.1	100.5
6/26	1056	34.4	.11 (.10)	1.20 (1.11)	56.0	9.3	102.6
7/3	730	39.0	.14 (.13)	1.53 (1.41)	54.5	8.5	101.2
7/9	529	45.9	.17 (.15)	1.79 (1.65)	57.0	7.4	105.1
7/16***	382	48.3	.12 (.10)	1.31 (1.12)	57.0	8.2	101.9
7/23	257	55.2	.16 (.14)	1.70 (1.46)	54.5	8.0	105.2
7/25	237	56.7	.17 (.14)	2.16 (1.85)	54.0	8.0	105.2
#2							
Start-6/4	2200	27.0	.11 (.09)	0.96 (0.84)	49.0	--	---
6/19	1408	31.3	.14 (.12)	1.09 (0.95)	50.5	10.3	100.5
6/26	1056	34.4	.17 (.14)	1.32 (1.13)	56.0	9.5	102.6
7/3	730	39.0	.21 (.18)	1.72 (1.48)	54.5	8.7	101.2
7/9	514	43.8	.27 (.23)	2.16 (1.86)	57.0	8.2	105.1
7/16***	356	49.5	.16 (.11)	1.27 (0.86)	57.0	8.8	101.9
7/23	262	54.8	.19 (.13)	1.55 (1.08)	54.5	8.6	105.2
7/25	237	56.7	.20 (.14)	1.40 (0.98)	54.0	8.6	105.2
#3							
Start-6/4	1800	28.8	.09 (.08)	1.20 (1.09)	51.0	--	---
6/19	1067	34.3	.13 (.11)	1.27 (1.15)	50.5	10.0	100.5
6/26	801	37.8	.15 (.14)	1.53 (1.38)	56.0	9.1	102.6
7/3	627	41.0	.18 (.16)	1.79 (1.61)	54.5	8.0	101.2
7/9	431	46.2	.23 (.21)	2.30 (2.08)	57.0	8.7	105.1
7/16***	351	49.7	.11 (.09)	1.12 (0.88)	57.0	8.3	101.9
7/23	249	55.7	.14 (.11)	1.41 (1.10)	54.5	8.5	105.2
7/25	234	56.9	.15 (.12)	1.74 (1.36)	54.0	8.5	105.2
#4							
Start-6/4	2200	27.0	.07 (.07)	0.96 (0.89)	49.0	--	---
6/19	1408	31.3	.09 (.09)	1.02 (0.94)	50.5	10.2	100.5
6/26	1056	34.4	.11 (.10)	1.23 (1.13)	56.0	9.4	102.6
7/3	730	39.0	.14 (.13)	1.56 (1.43)	54.5	7.9	101.2
7/9	480	45.4	.19 (.17)	2.03 (1.86)	57.0	8.1	105.1
7/16***	348	49.9	.12 (.10)	1.25 (1.04)	57.0	8.1	101.9
7/23	244	56.1	.15 (.12)	1.58 (1.31)	54.5	8.4	105.2
7/25	227	57.5	.15 (.13)	1.53 (1.27)	54.0	8.4	105.2

* Only 2 of 4 tanks were sampled 6/19, 6/26, and 7/3. It was assumed that tanks 1, 2, and 4 were the same during this period since they received eggs from the same take.

** Parentheses enclose adjusted indices using revised running totals calculated after total fish biomass in each tank was weighed at the completion of the study.

*** Tanks were split out on 7/10 to maintain desired density indices of .20 and flow indices of 1.0.

Table 4. Weekly sampling information for the Pump House (PH) site, 1985.

Tank # Date sampled	Size * (fish/lb)	Mean total length (mm)	Density ** index	Flow ** index	Mean temp (°F)	During sample week D.O. (ppm)	During sample week Nitrogen saturation (%)
#1							
Start-6/4	2200	27.0	.08 (.08)	0.84 (0.84)	53.0	--	---
6/19	1181	33.2	.11 (.11)	0.90 (0.90)	54.5	10.0	101.4
6/26	870	36.7	.13 (.13)	1.09 (1.10)	55.5	8.9	100.6
7/3	565	42.4	.17 (.17)	0.87 (0.88)	56.5	9.2	100.3
7/9	464	44.5	.20 (.20)	1.01 (1.01)	57.0	8.5	102.8
7/16***	323	51.1	.25 (.25)	1.24 (1.24)	56.5	8.5	102.3
7/23	265	54.6	.28 (.28)	1.40 (1.41)	54.0	8.5	104.4
7/28	238	56.6	.29 (.30)	2.00 (2.01)	57.5	8.5	104.4
#2							
Start-6/4	2200	27.0	.07 (.06)	0.96 (0.88)	53.0	--	---
6/19	1181	33.2	.10 (.09)	1.19 (1.08)	54.5	9.4	101.4
6/26	870	36.7	.12 (.11)	1.46 (1.33)	55.5	8.9	100.6
7/3	565	42.4	.16 (.15)	1.16 (1.06)	56.5	8.9	100.3
7/9	431	44.1	.20 (.19)	1.46 (1.33)	57.0	8.1	102.8
7/16***	345	50.0	.15 (.13)	1.10 (0.95)	56.5	8.6	102.3
7/23	267	54.5	.18 (.16)	1.30 (1.13)	54.0	8.1	104.4
7/28	235	56.8	.20 (.17)	1.89 (1.64)	57.5	8.1	104.4
#3							
Start-6/4	1800	28.8	.08 (.07)	1.21 (1.06)	52.5	--	---
6/19	1091	34.1	.11 (.10)	1.37 (1.20)	54.5	9.6	101.4
6/26	791	37.9	.14 (.12)	1.70 (1.49)	55.5	8.8	100.6
7/3	575	42.2	.18 (.15)	1.26 (1.10)	56.5	8.6	100.3
7/9	412	48.0	.21 (.19)	1.54 (1.35)	57.0	7.8	102.8
7/16***	322	51.2	.17 (.13)	1.20 (0.96)	56.5	8.4	102.3
7/23	257	55.2	.19 (.16)	1.39 (1.12)	54.0	8.3	104.4
7/28	221	58.0	.21 (.17)	2.05 (1.64)	57.5	8.3	104.4
#4							
Start-6/4	2200	27.0	.07 (.07)	0.96 (0.97)	53.0	--	---
6/19	1181	33.2	.10 (.10)	1.16 (1.18)	54.5	10.0	101.4
6/26	870	36.7	.12 (.12)	1.43 (1.44)	55.5	9.2	100.6
7/3	565	42.4	.16 (.16)	1.14 (1.15)	56.5	8.6	100.3
7/9	469	44.2	.18 (.18)	1.30 (1.32)	57.0	8.0	102.8
7/16***	397	47.7	.15 (.15)	1.06 (1.08)	56.5	8.5	102.3
7/23	273	54.1	.18 (.19)	1.32 (1.35)	54.0	8.4	104.4
7/28	268	54.4	.18 (.19)	1.76 (1.80)	57.5	8.4	104.4

* Only 2 of 4 tanks were sampled 6/19, 6/26, and 7/3. It was assumed that tanks 1, 2, and 4 were the same during this period since they received eggs from the same take.

** Parentheses enclose adjusted indices using revised running totals calculated after total fish biomass in each tank was weighed at the completion of the study.

*** Tanks were split out on 7/11 to maintain desired density indices of .20 and flow indices of 1.0.

Table 5. Weekly sampling information for the Lab site, 1985.

Tank # Date sampled	Size * (fish/lb)	Mean total length (mm)	Density ** index	Flow ** index	Mean temp (°F)	During sample week D.O. (ppm)	Nitrogen saturation (%)
#1 ****							
Start-6/4	2200	27.0	(.02)	(0.27)	53.0	--	---
6/19	967	35.5	(.03)	(0.34)	52.5	10.1	101.6
6/26	820	37.5	(.03)	(0.38)	56.0	10.0	99.5
7/3	655	40.4	(.04)	(0.28)	56.5	9.4	100.2
7/9	366	50.1	(.06)	(0.41)	57.5	8.6	101.5
7/16***	257	55.2	(.07)	(0.52)	56.5	9.7	99.5
7/23	204	59.6	(.09)	(0.61)	54.0	9.0	103.5
7/28	188	61.2	(.09)	(0.65)	58.0	9.0	103.5
#2							
Start-6/4	2200	27.0	.07 (.06)	0.96 (0.89)	53.0	--	---
6/19	967	35.5	.11 (.10)	1.24 (1.13)	52.5	10.3	101.6
6/26	820	37.5	.12 (.11)	1.46 (1.26)	56.0	9.3	99.5
7/3	655	40.4	.14 (.13)	1.03 (0.95)	56.5	8.9	100.2
7/9	476	44.6	.18 (.16)	1.28 (1.18)	57.5	8.5	101.5
7/16***	383	48.3	.14 (.14)	1.02 (1.01)	56.5	9.1	99.5
7/23	302	52.3	.16 (.16)	1.16 (1.15)	54.0	8.8	103.5
7/28	261	54.9	.17 (.17)	1.25 (1.23)	58.0	8.8	103.5
#3							
Start-6/6	1800	28.8	.12 (.10)	1.20 (0.95)	53.0	--	---
6/19	1007	35.0	.17 (.13)	1.35 (1.06)	52.5	10.0	101.6
6/26	730	39.0	.21 (.16)	1.66 (1.31)	56.0	9.0	99.5
7/3	590	41.8	.24 (.19)	1.23 (0.97)	56.5	8.7	100.2
7/9	429	45.0	.31 (.24)	1.58 (1.24)	57.5	8.1	101.5
7/16***	325	51.0	.23 (.15)	1.16 (0.77)	56.5	9.0	99.5
7/23	247	55.9	.27 (.18)	1.39 (0.92)	54.0	9.1	103.5
7/28	223	57.8	.29 (.19)	1.65 (1.10)	58.0	9.1	103.5
#4							
Start-6/4	2200	27.0	.07 (.06)	0.96 (0.80)	53.0	--	---
6/19	967	35.5	.11 (.09)	1.25 (1.03)	52.5	10.1	101.6
6/26	820	37.5	.13 (.10)	1.39 (1.15)	56.0	9.5	99.5
7/3	655	40.4	.14 (.11)	1.03 (0.86)	56.5	8.8	100.2
7/9	483	43.2	.18 (.15)	1.32 (1.09)	57.5	7.8	101.5
7/16***	358	49.4	.16 (.13)	1.18 (0.91)	56.5	9.3	99.5
7/23	292	52.9	.19 (.14)	1.34 (1.03)	54.0	9.2	103.5
7/28	253	55.4	.20 (.16)	1.47 (1.13)	58.0	9.2	103.5

* Only 2 of 4 tanks were sampled 6/19, 6/26, and 7/3. It was assumed that tanks 1, 2, and 4 were the same during this period since they received eggs from the same take.

** Parentheses enclose adjusted indices using revised running totals calculated after total fish biomass in each tank was weighed at the completion of the study.

*** Tanks were split out on 7/11 to maintain desired density indices of .20 and flow indices of 1.0.

**** Tank #1 contained survivors from several trays transported under poor conditions. Total pounds and numbers of steelhead put into the tank originally are unknown.

Table 6. Daily mortality means and standard deviations of tanks at all sites, 1985.

Tank And Location	Mean Mortality*	Mean Mortality Percentage	SD.
DAM			
Tank 1	36	.20	53.9144
Tank 2	25	.17	30.5373
Tank 3	21	.11	32.5634
Tank 4	31	.17	38.7978
		$\overline{X}$ = .17	
PUMP HOUSE			
Tank 1	41	.36	83.8867
Tank 2	12	.07	13.1016
Tank 3	12	.07	18.4314
Tank 4	45	.24	31.9728
		$\overline{X}$ = .20	
LAB			
Tank 1	8	.15	14.746
Tank 2	48	.30	50.7674
Tank 3	10	.08	10.0439
Tank 4	18	.12	32.3859
		$\overline{X}$ = .17	

* Rounded to the nearest fish.

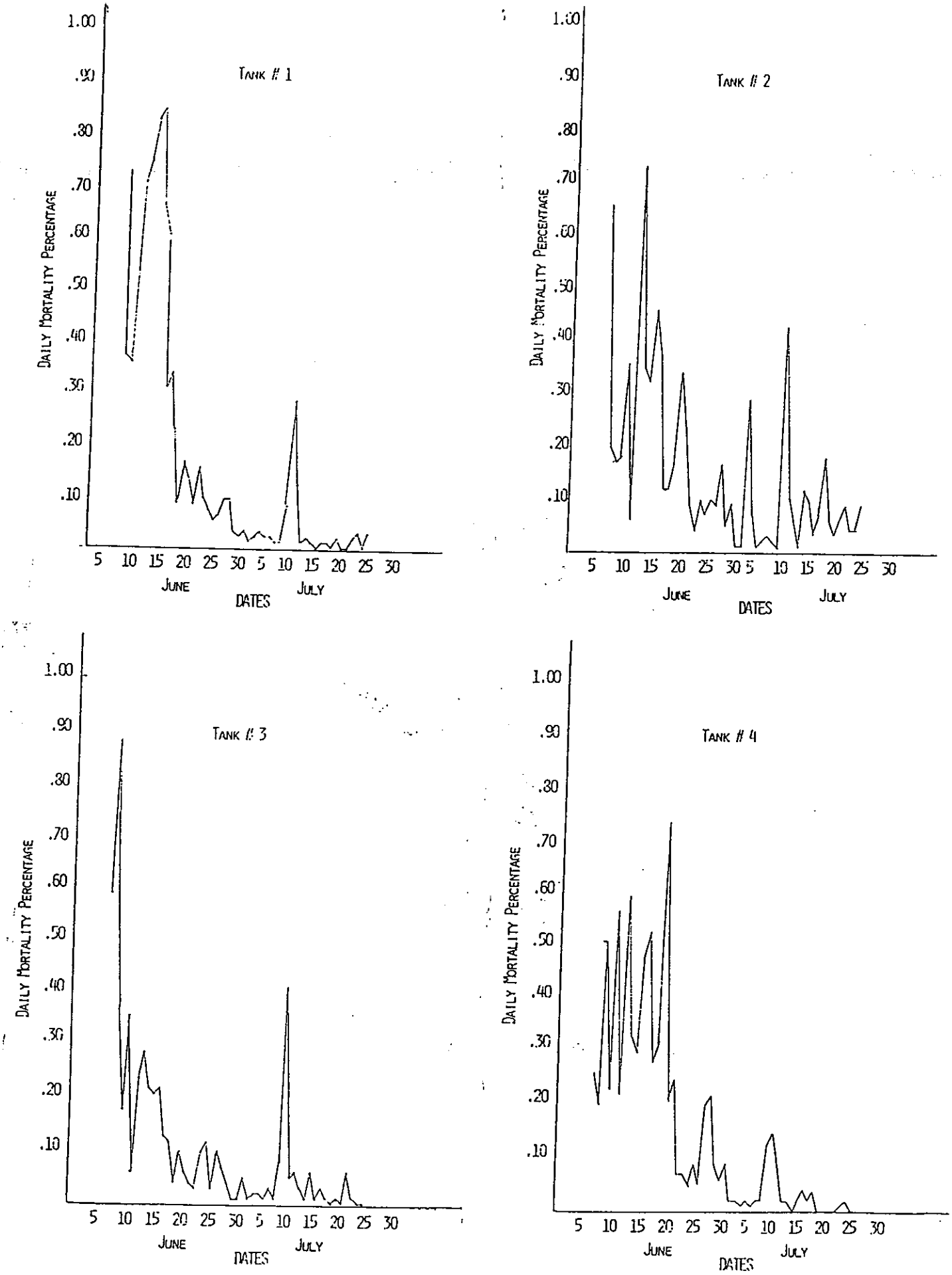

Figure 2. Percentage of steelhead mortalities per day at the Dam site, 1985.

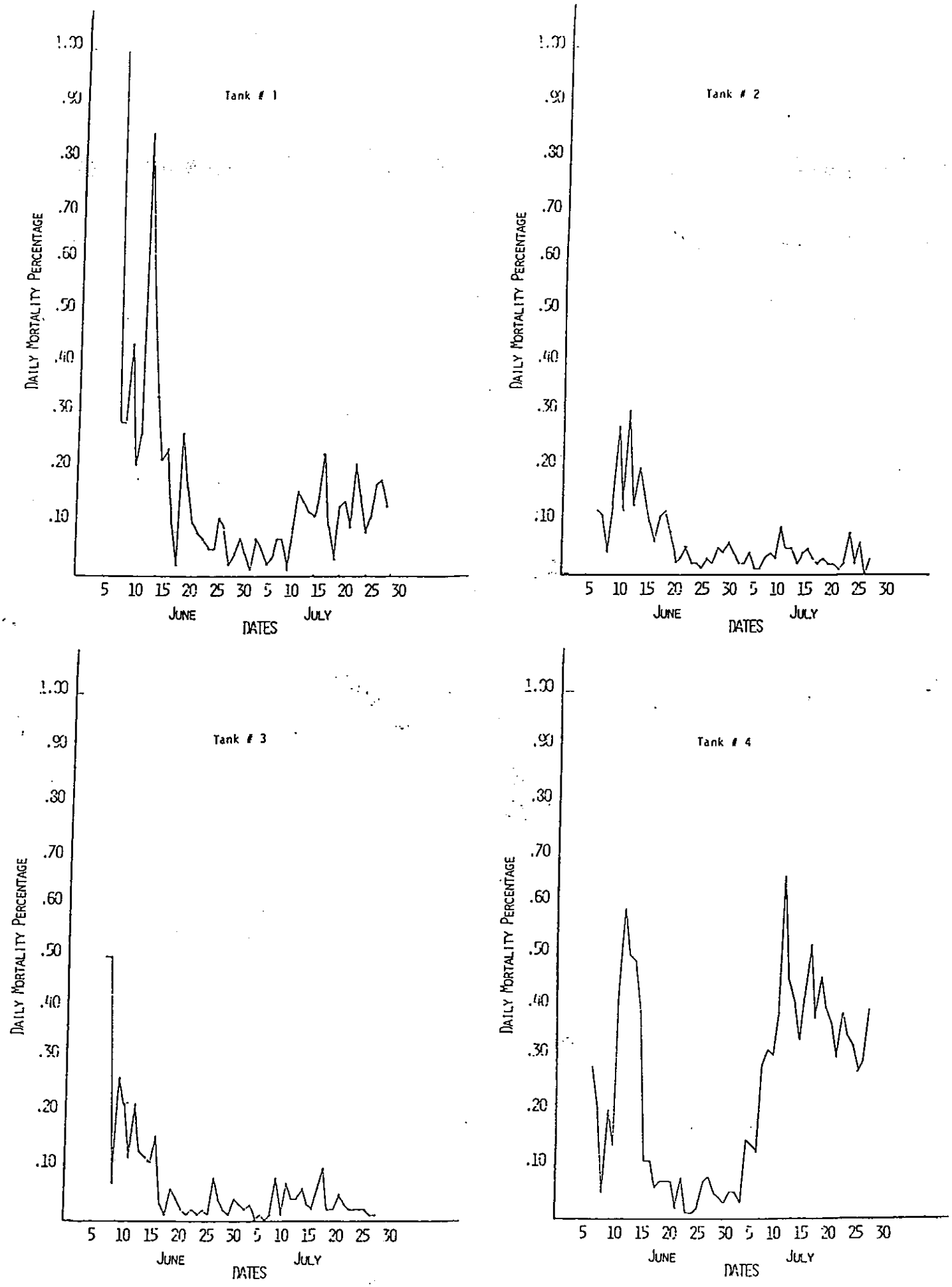

Figure 3. Percentage of steelhead mortalities per day at the Pump House (PH) site, 1985.

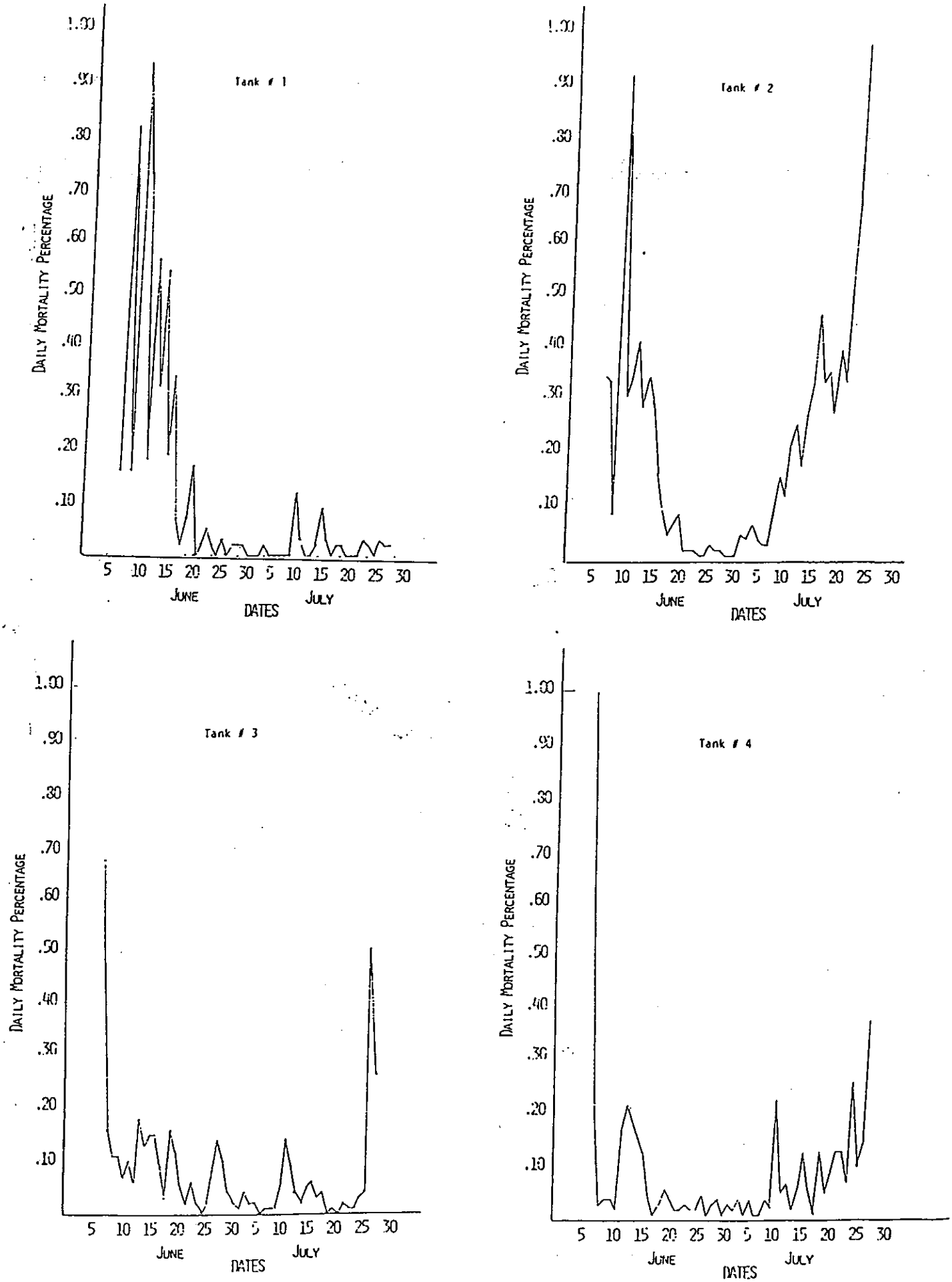

Figure 4. Percentage of steelhead mortalities per day at the Lab site, 1985.

completion of the study, and displayed the typical mortality curve associated with a chronic IHN loss (Figure 4). IHNV was reisolated at the Lab in tank 2 fry by later analyses. The virus was found in one mort from tank 3 collected on July 15. It was never reisolated from fish in this tank. Mean mortality was high in tank 2 and remained exceptionally low in tank 3 (Table 6). Test fish from the separate sites were put in rearing ponds at DNFH for further rearing and evaluation on July 25-28. On October 1, 1985, the fish were about 50/lb. and all lots had experienced IHN mortality of 14 to 17% since ponding July 26-29. This percentage loss is typical of the chronic IHN mortality experienced in the outdoor rearing ponds at DNFH (Joe Lientz, personal communication, 1985). Thus, all off-site test lots contracted IHN and displayed IHN disease losses following ponding.

Discussion

From these study results and DNFH Ozone Study results (Pratschner et al, 1985), we believe that the source of horizontally transmitted IHNV at DNFH is the river water supply and/or the hatchery facilities. However, results are not totally definitive, and further work is necessary to confirm the exact source of horizontal transmission at the hatchery.

In this study, we could not show that Dworshak Reservoir was a source of IHNV infection for steelhead. Our failure to detect any infection in steelhead we reared at the Dam may have been a function of chance or timing. We have been able to isolate IHN virus in spawning kokanee from the reservoir tributaries during the fall of 1984 and 1985. However, we found the spawning kokanee to have a very low incidence of IHNV with only 3 out of 857 kokanee in 1984 and 10 of 671 in 1985. Because there are four recognized strains of IHNV, we have made arrangements to have the kokanee IHNV analyzed to strain. Importance of this information will depend on whether the kokanee IHNV strain is the same as that infecting DNFH steelhead. The failure to detect IHNV in nonspawning salmonids from any of our sampling locations is not surprising since it has been reported that the virus can only be detected in spawning adults salmonids or in fish dying from IHN disease (Mulcahy, et al 1982). Likewise, IHNV has never been reported to occur in nonsalmonid species (Post,1983) so our failure to detect this virus in the nonsalmonids we captured was not surprising. Before rejecting the reservoir as a virus source, the study should be repeated when kokanee are spawning. If fry at the Dam site remain virus free during this critical time, reservoir water might be discounted as an IHNV source.

IHNV shed from spawning kokanee could not immediately infect steelhead fry being reared in Dworshak NFH because of timing of the steelhead production cycle. Kokanee spawn in the fall and steelhead being held at Dworshak NFH in the fall, are normally larger than 50/lb, a size

where they are much less susceptible to IHNV. The size that steelhead are most susceptible to IHNV is in the fry stage, smaller than 250/lb., while they are being held in the nursery from May through July.

The fact that IHNV was detected in fish migrating from the reservoir to spawn indicated that a strain of the virus is present in the reservoir system. Therefore, this water should be considered potentially infectious for IHN disease, regardless of experimental fish rearing results. Dilution by the tremendous volume of water present in the reservoir may make this water source the least infectious of the three sources tested and probably the most desirable for the proposed Clearwater Hatchery.

Our study could not directly demonstrate that the infectious virus was entering the hatchery via the North Fork Clearwater River. IHNV was never confirmed in any PH fry even though these fish were stressed by fungal, bacterial, and parasitic infections. It may be that this study was initiated too late for optimal transmission of the virus since few adult steelhead remained in the North Fork Clearwater River when test fish were placed in PH tanks. It has been the experience at DNFH that the most severe IHN mortalities occur in steelhead fry started in the nursery building when large numbers of adult steelhead are present in the river and presumably shedding virus into the hatchery water supply (Joe Lientz, personal communication, 1985). Rearing of steelhead fry at the PH when adult steelhead are present in greater numbers in the North Fork Clearwater River may yield different results.

Adult steelhead spawned each week at Dworshak NFH during 1985 had IHNV incidences ranging from 0 to 75% with a mean for all fish spawned of 13.6% (Greg Pratschner, personal communication). Fish spawning in the North Fork Clearwater or caught by anglers would presumably have similar incidences at the virus.

A source of IHNV may lie between the PH and the Lab site since;

1) IHNV was never isolated from fish in the PH.
2) The PH site receives water passed only through the hatchery's first aeration chamber, and
3) IHNV was isolated from fish reared in the Lab, which receives water passed through the raw water system, including the first aeration chamber and the piping system to the nursery building.

It may be that the hatchery raw water system is a source of IHNV at DNFH. However, the virus may have entered the raw water system supplying the Lab tanks prior to the study. Most of the adult steelhead spawning in the North Fork Clearwater River above the hatchery intake had already spawned by June 4, the starting date of our rearing study. Prior to this time, Dworshak NFH was drawing water from the river into their raw water supply, which could also draw in virus presumably shed by spawning adult steelhead. Evidence to

support this theory is shown by the fact that steelhead fry reared in Dworshak's nursery building prior to initiation of our study suffered high mortalities from IHN even though the complete water supply system and tanks were disinfected prior to use (Pratschner et al, 1985).

The source of virus in the Lab may also have been due to inadvertant contamination by study personnel through the use of contaminated equipment. It is possible the wet lab floor harbored IHNV brought in by hatchery or study personnel from DNFH's nursery, which had been experiencing IHN mortalities since mid-May. Virus may then have been introduced to Lab tanks by the screen covers, which were set on edge on the wet floor while tanks were being cleaned. It is difficult to explain why virus was isolated from only two tanks if this were the case, however, since screens from all tanks in the Lab were handled this way.

Another possible source of virus in the Lab is from fry used for the study. Eggs from which the fry were hatched were supposedly from IHNV-negative parents. Due to time and space limitations, viral assays to determine IHNV status of parent steelhead were completed after 8 days incubation. Parent fish identified as negative at that time may indeed have been positive, however, since on occasion IHNV does not form identifiable plaques within 8 days and further incubation on blind passages may reveal plaques (McDaniel 1979). This was the case in several samples from steelhead spawned for DNFH production rearing this year and in our testing of spawning kokanee from tributaries of Dworshak Reservoir this year. Examination of the assays after 8 days revealed no evidence of viral plaquing, but after later examination plaques were evident (Colleen Poe, personal communication, 1985). This phenomenon could have occurred in samples taken from the fish spawned for this study. If this was the source of IHNV, it is difficult to explain why only two Lab tanks had the virus isolated from them since eggs were mixed and used randomly to supply fish for tanks at each location.

The IHN infection observed in our Lab test tanks differed from that observed in DNFH nursery-ozone test during 1985. Generally, the Lab reared fry experienced initial IHN mortalities at a larger size than fry in DNFH nursery. Also, total numbers of fry lost to IHN in infected tanks was much less in our Lab tests. This observation, however, may be a direct result of the fact that Lab test fish contracted the infection at a larger size and thus were not as susceptible as the smaller sized fish. Because the classic mortality rate of IHN developed in only one Lab tank and because the other positive IHN Lab tank (which was identified from only one fish), could not be reconfirmed; we consider this as only 25% infection rate from IHN in the Lab tests. DNFH on the other hand experienced 11 of 14 (78%) tanks on non-ozonated water contained fish dying of IHNV. Mortality in nearly all the raw water tanks in the DNFH nursery demonstrated the typical classical explosive mortality rate associated with IHN and fish in all but one of these tanks were eventually

destroyed (Pratschner et al, 1985). Our Lab test tanks could have experienced less total IHN mortality because of: 1) size difference at first loss as mentioned above, 2) difference in the way the eggs were handled (our test fish were hatched at Kooskia NFH where IHN has never been recorded in small steelhead while DNFH test fish were hatched at DNFH), or 3) outside factors such as differences in handling and care, differences in tank configuration, differences in specific locations, and length of experiment.

Of all the above listed differences, we believe that hatching eggs at Kooskia probably benefited the survival of our fry the most. Past experiences at DNFH where eggs were hatched at Kooskia NFH and returned to Dworshak NFH at 250/lb. and even some at 600/lb. have demonstrated higher survival relative to IHN (Pratschner, personal communication, 1985).

Recommendations

Uncertainties arising from the study cloud interpretation of results. We suggest that the key portions of the study be repeated to alleviate doubts. A better approach would be to use rainbow trout or steelhead swim-up fry from a hatchery without a history of IHNV. This would negate any possibility of receiving fry that are progeny of adults with low titers of IHNV that might go undetected during culling. Ideally, the PH site should receive water directly from the North Fork Clearwater River so that the hatchery's water supply system could be completely isolated. The entire study should be conducted when large numbers of adult steelhead are in the North Fork Clearwater River, so this suspected source could be evaluated further. It would also be worthwhile to conduct the study when kokanee in the reservoir are spawning. Thus, this water source could be further evaluated. However, since DNFH does not have steelhead fry (less than 250/lb.) on station in the fall when kokanee normally spawn, this possible source of infection may be nonviable as a source of contamination for the hatchery's water supply.

The combination of the ozone studies at DNFH and these off-site rearing tests yielded no absolute conclusions. They did, however, give some indication that:

1. Water from the reservoir and, to a lesser extent, water which had not passed through the DNFH supply system provides a more satisfactory rearing medium than DNFH's current nursery supply.

2. Horizontal transmission of IHNV is more important than vertical transmission at DNFH when broodstock culling is used.

3. Ozone disinfection of water can reduce the extent of early rearing mortality due to IHNV in the existing water supply system at DNFH.

Based on these indications, we recommend that the water supply for the Clearwater Hatchery be drawn from Dworshak reservoir. We further recommend that the reservoir source be provided for DNFH if possible.

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