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RELATIONSHIP OF SPAWNING
IN CARRIER SOCKEYE SALMON TO
INFECTIOUS HEMATOPOIETIC NECROSIS
VIRUS

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Roger R. Saft, Ph.D.

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Alaska Department of Fish & Game
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ABSTRACT

The expression of infectious hematopoietic necrosis (IHN) virus was studied in spawning adult sockeye salmon, *Oncorhynchus nerka*, at the inlet and outlet streams of Big Lake, Alaska; the experiments were conducted at Meadow and Fish Creeks. Mature fish were captured from their natural spawning grounds in August and September 1976. By testing gonadal fluid samples from individual fish on alternate days, it was determined that the virus cannot always be detected before spawning has occurred.

Similarly, the virus-carrier rate in both male and female fish was much higher after spawning than before. In the Meadow Creek experiment, 73.3% of the males and 89.3% of the females were determined to be virus carriers. After the peak of spawning, 37.5% of male fish tested had the virus, compared to only 4.2% before spawning. None of the tests on females indicated the presence of the virus prior to spawning; however, when at least half of the experimental fish had spawned, 64.8% of the females tested carried the virus.

In comparison, at Fish Creek where the fish were more sexually mature, 76.6% of the males and 98.0% of the females carried the IHN virus. Of these, 50.5% of the males and 30.5% of the females tested positive after the peak of spawning. Most virus carriers could be detected by sampling two days after spawning. Additional carrier fish were identified when negative samples were blind passaged in cell cultures.

KEY WORDS: infectious hematopoietic necrosis, sockeye salmon, *Oncorhynchus nerka*, virus carrier.

INTRODUCTION

At present, no direct means of preventing infectious hematopoietic necrosis (IHN) in fish exists, except to avoid contact of the viruses with their hosts (Amend 1976). This is done primarily by eliminating virus-positive fish populations before they are used as a hatchery egg source or moved to other locations. This is difficult to accomplish when all sources of sockeye salmon, *Oncorhynchus nerka*, in Alaska are presumed to be naturally infected with the IHN virus (Grischkowsky and Mulcahy 1982).

Consequently, detection of IHN virus is a global concern. A protocol specifying the requirements for inspection and certification of fish stocks exists (McDaniel 1979). For the detection of IHN virus carriers not having signs of the disease, it specifies that gonadal fluid samples be used.

Amend (1975) noted a higher incidence of IHN virus in female rainbow trout, *Salmo gairdneri*, several days after artificial spawning than during spawning. Unlike trout, salmon are typically killed when their eggs are removed for hatchery incubation; this means post-spawning samples are not available from those fish. This investigation addresses whether IHN virus can be detected in adult sockeye salmon before and after natural spawning occurs. For the detection of adult IHN virus carriers, it was determined that gonadal fluid samples taken after the fish had spawned were superior to samples obtained before spawning.

MATERIALS AND METHODS

Field Experiment Procedure

Experiments were conducted at Meadow Creek and Fish Creek, the respective inlet and outlet stream to Big Lake, during August and

September 1976. Adult sockeye salmon were held on natural spawning gravel in wire-mesh pen enclosures. Fish were disc tagged so that consecutive samples from individual fish could be followed. The first experiment, at Meadow Creek, used 60 fish (30 each sex), and the second experiment was conducted at Fish Creek with 108 fish (53 males and 55 females). The fish were collected by seining. For the Meadow Creek experiment, only "green" females (eggs could not be expressed by slight pressure on the abdomen) were used. The females in the Fish Creek experiment were green, "ripe" (eggs expressible), or "spawned-out" (having a flaccid abdomen, eggs absent or few in number) fish.

On the day after the fish were collected and alternate days thereafter, gonadal fluid samples were collected by stripping about 60 ml of eggs and fluid or approximately 5 ml of milt into a paper cup. Ovarian fluid was decanted off the eggs into tubes. Milt was placed directly into tubes. Fish that died between samplings were removed from the pen and discarded. Chi-square 2 X 2 analysis with a continuity correction (Chapman and Schaufele 1970) was used to test the significance of carrier rates between sexes.

Virus Assay Procedure

Samples were stored on ice and flown to the Division of Fisheries Rehabilitation, Enhancement and Development (FRED) Division fish pathology laboratory in Anchorage for preliminary processing; subsequently, samples were sent to the National Fisheries Research Center, U. S. Fish and Wildlife Service, Seattle laboratory for inoculation of cell cultures. For preliminary processing, samples were first centrifuged at 2,000 times gravity to remove solids and sperm; 2,000 units penicillin, 2,000 μg streptomycin, 1,000 μg mycostatin, and 5 mg gentamicin were then added to 2.1 ml of the clarified sample. All samples were inoculated into cells, usually within several days or a maximum of seven days after collection. This was done with 2- to 4-day-old fathead

minnow cell cultures (Gravell and Malsberger 1965) held at 15°C. Duplicate inoculations were treated with antibiotics without further dilution. Supernatants from cell cultures showing cytopathic effects (CPE) were subcultured by diluting 1:1000 and new cell cultures inoculated. If CPE was again observed, a second was subcultured. Final dilution of the samples at this point exceeded 1:1,000,000. The presence of typical IHN virus CPE at this dilution was considered presumptive evidence for the presence of IHN virus. Selected cultures exhibiting typical CPE were examined by serum neutralization tests, using antiserum specific for the IHN virus.

All test cultures were observed for 2 weeks. A blind passage was done on all negative samples by making a 1:10 dilution of supernatant and inoculating new cell cultures. Subcultures of positive samples that failed to develop CPE were also blind passaged; any sample showing CPE was then subcultured twice, as described.

RESULTS

Meadow Creek Experiment

At the beginning of this experiment, vandals killed many of the 60 fish during 7-9 August. Results from this experiment are shown in Figure 1. To avoid data bias, the destroyed fish were excluded in all considerations of the data. The detection of IHN virus in male and female gonadal fluids was highest following spawning. The cumulative virus-carrier rate in the experimental population continued to increase after spawning had peaked. IHN virus was not found in male fish on the first day, but its presence increased to 73.3% on the seventh day of the experiment. One male fish that was green on the first day remained negative throughout the study. One male, positive when first tested on 7 August, yielded negative samples 2 and 4 days later. This

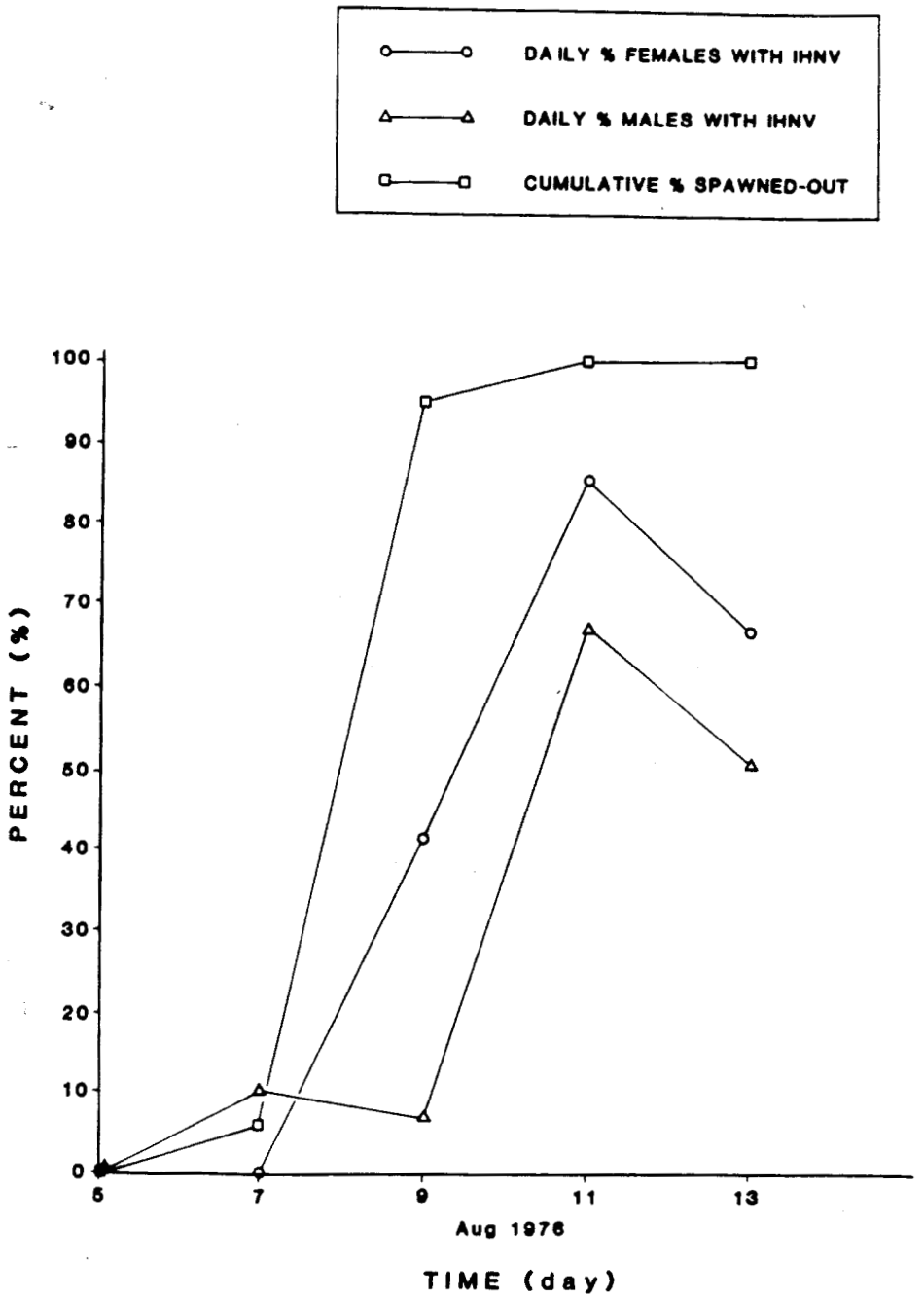

Figure 1. Relationship in sockeye salmon between infectious hematopoietic necrosis virus (IHNV) findings each day and the spawned-out condition of the females at Meadow Creek.

probably indicated a low level of virus at the limits of the sensitivity of the assay. If 8 August is taken as the normal day of spawning for the female fish in this experiment, then only one male fish was positive prior to that date; one male became positive on 9 August, and nine males became positive 2 days later, after each had yielded one negative sample.

Although only green females were placed into the pen the day before the first sample day, only 46.6% (13) of 28 female fish were still green the following day. Of these, six spawned during the experiment, and 84.6% (11) of 13 green fish eventually were determined to be positive for IHN virus. Four days usually transpired between the last categorization of "greenness" and post-spawning determinations. The results of the first test day were typically negative. The green female fish that were negative at the beginning of the experiment became positive about 48 h after they had spawned.

Of the 15 female fish that were ripe on the first sample day, 93.3% (14) were determined to be positive overall. The time of spawning was not determined for only one fish. For the 13 ripe females for which the date of spawning was determined, four were positive by the first sample day following spawning, eight by the second sample day (one negative sample intervening), and one by the third sample day (two negative samples intervening). Relative to spawning, the patterns of first appearance of the IHN virus are in Table 1.

In the Meadow Creek experiment, 73.3% (11/15) of the males and 89.3% (25/28) of the females were determined to be IHN virus carriers. The difference in carrier rates between the sexes was not significant. Almost all of the fish converted from negative to positive after the peak of spawning activity, which occurred between 7 and 9 August. Only 4.2% of the males and none of the

Table 1. Patterns of the first appearance of infectious hematopoietic necrosis (IHN) virus in sockeye salmon ovarian fluids relative to spawning at Meadow Creek and Fish Creek.

Condition of Fish at Beginning of Experiments	Isolation of IHN virus from ovarian fluid[b/]							Percent of fish exhibiting each pattern[c/]	
	Pre-spawning			Post-spawning				Meadow Creek (16)	Fish Creek (29)
	Day	1	3	5	7	9	11		
			-	-	-	+		6.2	6.9
			-	-	+			50.0	3.4
Pre-spawning			-	+				43.8	10.4
		-	+	+					6.9
			+	+					48.3
				+					13.8
Post-spawning				-	+				6.9
				-	-	-	+		3.4

a/ Virus-negative fish and fish for which the time of spawning was not determined were not included.
b/ Negative sample: -; positive sample: +.
c/ Total numbers of fish given in parentheses.

females were positive before this time. After that, the tests of 37.5% of the males and 64.8% of the females detected the IHN virus.

Finally, Table 2 summarizes data relating the rate of occurrence of negative samples from the female fish that eventually proved to be IHN virus carriers. Excluded from this table are the data from the fish that were already spawned-out at the beginning of the experiment. The IHN virus could not be detected prior to spawning; however, in 26.7% of the ripe fish and 30.8% of the green fish, positive samples were detected on the first sample day after spawning. A further 53.3% of the ripe fish provided negative samples on the first day after spawning but positive samples on the second day after spawning. One fish yielded two negative samples before providing a positive sample.

Fish Creek Experiment

This experiment was essentially identical to the one at Meadow Creek, except that more fish were used and the population was more sexually mature. Fish that lost their tags were eliminated from the experiment, reducing the total number to 97. The experiment began with seven spawned-out (13.7%), 23 ripe (58.8%), and 21 green (41.2%) females. As in the previous experiment, the detection of IHN virus in gonadal fluids increased with spawning activity (Figure 2).

Of the 51 female fish, a total of 50 were eventually determined to be positive, an IHN virus-carrier rate of 98.0%. Of these, 72.0% (36) were positive each time they were tested; this reflected the population's advanced state of the spawning cycle.

Table 2. Findings of infectious hematopoietic necrosis virus in sockeye salmon ovarian fluid relative to spawning of experimental populations at Meadow Creek and Fish Creek.[a/]

	Meadow Creek initially		Fish Creek initially	
	Ripe Fish	Green Fish	Ripe Fish	Green Fish
Positive before spawning	0% (0)[b/]	0% (0)	60.9% (14)	71.4% (15)
Positive on day spawning condition was determined	26.7% (4)	30.8% (4)	17.5% (4)	14.3% (3)
Positive two days after spawning (after one negative sample)	53.5% (8)	0% (0)	4.3% (1)	0% (0)
Positive four days after spawning (after two negative samples)	6.7% (1)	0% (0)	8.7% (2)	0% (0)
Positive six days after spawning (after three negative samples)	0% (0)	0% (0)	0% (0)	0% (0)
Time of spawning not determined	6.7% (1)	53.8% (7)	4.3% (1)	14.3% (3)
Positive	93.3% (14)	84.6% (11)	95.7% (22)	100% (21)
Negative	6.7% (1)	15.4% (2)	4.3% (1)	0% (0)
Total	100% (15)	100%(13)	100%(23)	100%(21)

a/ Fish were divided into initially green and ripe spawning conditions.

b/ Numbers in parentheses refer to fish in each category.

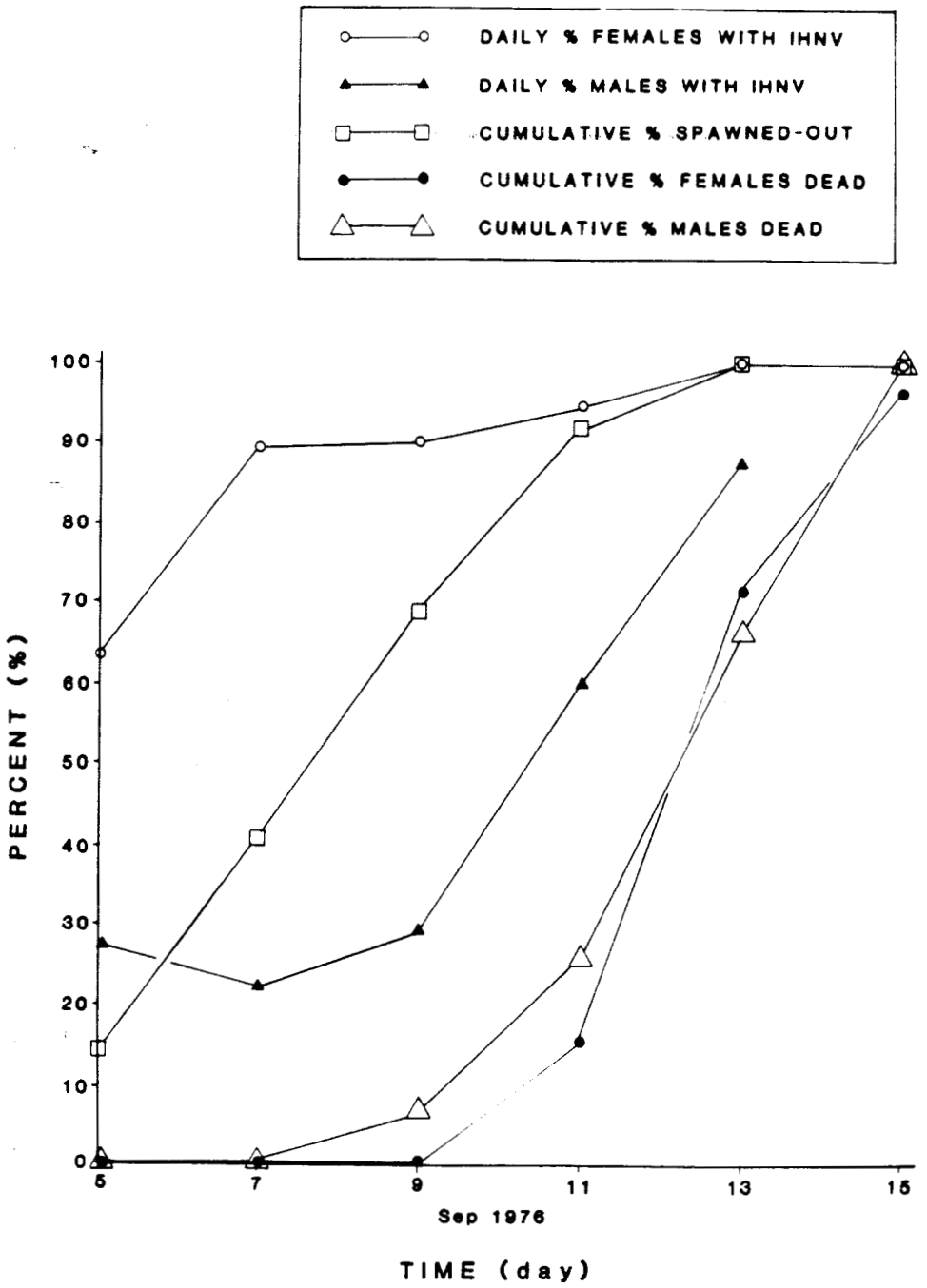

Figure 2. Relationship in sockeye salmon between infectious hematopoietic necrosis virus (IHNV) finding each day, the spawned-out condition of the females and death of both sexes at Fish Creek.

Of the seven spawned-out female fish, four were positive from the first day. One of these yielded four positive samples (over an 8-day period) before dying, two fish yielded one negative sample, and one fish yielded three negative samples before becoming positive.

All samples were positive from the 21 green female fish at the start of the experiment. Patterns of first appearance of the virus, relative to spawning, are provided in Table 1. Of the 23 ripe female fish with which the experiment began, only one fish remained negative. The time of spawning was not determined for one fish. One fish yielded one negative sample and one positive sample before spawning. Two fish yielded two negative samples after spawning, and four fish became positive on the day spawning occurred. All pre-spawning samples were positive for the remaining 14 fish (66.6%).

The data relating the pattern of occurrence of negative samples from the female fish that proved to be IHN virus carriers are summarized in Table 1. At the beginning of the experiment, spawned-out fish were excluded. Prior to spawning, 60.9% of the ripe females and 71.4% of the green females were positive. During this experiment, the peak of spawning occurred between 7 and 9 September.

As shown in Table 2, an additional 30.5% of the ripe females and 14.3% of the green females yielded positive samples on the test days after spawning. One of these fish had three negative samples before a positive sample was obtained. After this point, 50.5% of the male fish tested contained the virus, compared with 22.0% before this point.

Finally, to check the accuracy of the IHN virus prevalence data for the experimental fish, a group of 60 naturally spawned-out female fish were seined from Fish Creek. Ovarian fluid samples were taken on 15 September. All of these fish proved to be IHN

virus carriers. Since the experimental group of females was 98.0% (50/51) positive for the IHN virus, the final prevalences were comparable; however, the experimental group of males had a virus carrier rate of 76.6% (36/47). This difference between the sexes in the carrier rate was significant ($P<0.01$).

Effects of Blind Passage

A total of 143 samples were processed for the Meadow Creek experiment; two (1.3%) of these were initially negative on cell cultures but became positive upon blind passage. All virus-positive samples from this experiment remained positive on subculture. There were no mixed reactions (i.e., one positive and one negative) in the duplicate cell cultures used for the examination of each sample.

A total of 357 samples were processed for the Fish Creek experiment. Of these, two (0.5%) initially negative samples became positive on blind passage, nine (2.5%) initially positive samples reverted to negative after subculturing (indicating sample toxicity), and 24 samples (6.7%) gave mixed positive and negative reactions on the replicate cell cultures used. The latter cultures maintained their mixed status after blind passage or subculture.

DISCUSSION

The virus that causes IHN is present in virtually every Alaskan population of sockeye salmon examined (Grischkowsky and Amend 1976). Results from the Meadow Creek and Fish Creek experiments show that the virus is not always detectable in gonadal fluids. Samples taken before spawning indicated a lower virus-carrier rate than ones taken after spawning; this agrees with Amend (1975) for rainbow trout and Mulcahy et al. (1983) for

sockeye salmon. Had the fish used in the Meadow Creek experiment been sampled before spawning for a broodstock certification, IHN virus would have been extremely difficult to detect and the population probably judged virus-free or having a low virus-carrier rate.

Although the test results for some fish were positive before spawning, the appearance of virus in gonadal fluids continued to increase after spawning until all carriers expressed virus in their gonadal fluids. The incidence of IHN virus increased as the fish progressed from ripening through spawning and death. However, as carcasses were removed every 48 h, their deterioration was not likely a factor in this increase. The release of virus from spawning and the subsequent gill uptake potentially could have occurred (Mulcahy et al. 1983); however, no quantitative virus data was collected.

At Fish Creek, the female carrier rates of the control fish and experimental fish were both at or near 100%. Although a few fish had two or even three negative post-spawning samples (four to six days), most virus carriers could be detected two days after spawning. Consequently, when the greatest sensitivity and accuracy in detection of IHN virus adult salmon is required, naturally spawned-out fish should be used as the sample population. Amend (1975) found that IHN virus could not be isolated from immature carrier rainbow trout held in a laboratory, but when spawning occurred, the virus could be detected.

The IHN virus is highly detectable at the terminal extreme of the host's life cycle. During spawning activity, it is probably transmitted to a new generation.

Amend (1975) found no significant difference in carrier rates between sexes of rainbow trout. However, with the sockeye salmon of both experiments, the male carrier rate of IHN virus was

numerically lower than that of the females, but only significantly so at Fish Creek. Earlier, Grischowsky and Amend (1976) found the IHN virus in milt from only four of eight sockeye salmon sampling sites, compared with all 16 sites where ovarian fluids had been positively tested. When they found the virus, it always had a low prevalence in males. Since the virus-carrier rate of the males used in the Meadow Creek and Fish Creek experiments exceeded 70%, males should also be considered a major source of IHN virus contamination.

For critical work, blind passage of negative samples will reveal the presence of additional virus carriers. Since the population of salmon from which we obtained our experimental groups of fish had virus-carrier rates that were very high, the impact of two or three samples having been incorrectly diagnosed is slight. When there are few samples or a very low carrier rate, blind passage can aid in detecting the presence of all carriers.

The incidence and titer levels of the virus were lower in milt than in internal organs of the same fish (Mulcahy et al. 1982). Mulcahy and Pascho (1984) showed that IHN virus was rapidly adsorbed by the sperm of chinook salmon, *Oncorhynchus tshawytscha*, and steelhead trout, *S. gairdneri*. This may be an explanation for the lower virus prevalence in some milt sampled. Ovarian fluids of post-spawning sockeye salmon are the samples of choice for the detection of IHN virus. Hatchery spawning operations that are dependent upon low IHN virus-carrier rates should utilize sockeye salmon in their most advanced spawning condition to determine the real incidence within that sex. Male sockeye salmon should also be tested for the virus.

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