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INFECTIOUS HEMATOPOIETIC NECROSIS
IN ALASKAN CHUM SALMON

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ABSTRACT

Prior to 1985, two cases of infectious hematopoietic necrosis (IHN) had been detected in Alaskan chum salmon, *Oncorhynchus keta*, alevins and fry. The first occurred in 1982 at Kitoi Bay Hatchery; the second, in 1984 at Russell Creek Hatchery. These outbreaks were typical of IHN in their severity and in the gross and histopathological features observed. The causative virus (IHNV) was cultured and identified by serum neutralization. Subsequent to the Kitoi Bay episode, chum salmon fingerlings were challenged in the laboratory with IHNV isolates from the Kitoi Bay Hatchery epizootic and from the ovarian fluid of kokanee, *Oncorhynchus nerka*, adults of Big Kitoi Lake. Although the challenged chum salmon were older than the fish involved in the hatchery episodes, they were susceptible to the virus and incurred significant mortalities.

This report documents the experimental and natural infection of chum salmon with IHNV under hatchery conditions. Both hatchery cases were most likely instances of horizontal waterborne transmission of IHNV from sockeye salmon or kokanee adults in the water supplies. The established susceptibility of chum salmon to IHNV should be addressed when planning for chum and sockeye salmon propagation, enhancement, and stocking.

KEY WORDS: infectious hematopoietic necrosis, chum salmon, *Oncorhynchus keta*, viral epizootics.

INTRODUCTION

Infectious hematopoietic necrosis (IHN), previously a cause of numerous epizootics in Alaskan sockeye salmon, has recently caused mortalities in chum salmon at two locations in Alaska. During the 1982 epizootic at Kitoi Bay Hatchery, 900 of 1850 Sturgeon River chum salmon alevins and fry died. In 1984 an

epizootic occurring at Russell Creek Hatchery killed 400,000 of 12.3 million chum salmon alevins. Although IHN has been reported in chum salmon fry in Japan (Sano et al. 1977), this epizootic was the first North American finding in this species.

IHN virus (IHNV) is the only virus known to cause disease in the salmonid populations in Alaska. From 1973 through 1984, IHN mortalities in sockeye salmon alevins have occurred at many sites throughout Alaska (Figure 1). The distribution of IHNV in carrier adult sockeye salmon is widespread throughout Alaska. Grischkowsky and Amend (1976) previously reported its presence in 16 sockeye salmon populations. Additionally, IHNV has been detected in every Alaskan sockeye salmon population examined by our laboratory (63); each population sample consisted of a minimum of 194 fish. This sample size allowed detection of about a 2% prevalence, at a 95% confidence interval (Ossiander and Wedemeyer 1973). Consequently, these results indicate that IHNV is ubiquitous in Alaska. The contamination of the hatchery water supplies by kokanee or sockeye salmon carrying IHNV is considered to be the probable cause of the subsequent chum salmon epizootics reported here.

There have been reports of five other viruses affecting chum salmon inside and outside of Alaska: chum salmon virus (Winton et al. 1981), erythrocytic necrosis virus (Evelyn and Traxler 1978; Rohovec and Amandi 1981), *Oncorhynchus masou* virus (Kimura et al. 1981), yamane tumor virus (Sano et al. 1983), and infectious pancreatic necrosis virus (Hah et al. 1984). The mortalities at Kitoi Bay and Russell Creek Hatcheries did not appear to have any of the above viral origins. These two cases of mortalities in chum salmon were determined to be attributable to IHN by using the following techniques: (1) direct observation of gross abnormalities, (2) histopathological studies, (3) tissue culture assays, and (4) serum neutralization. Using the Kitoi Bay viral isolate to challenge Sturgeon River chum salmon fingerlings,

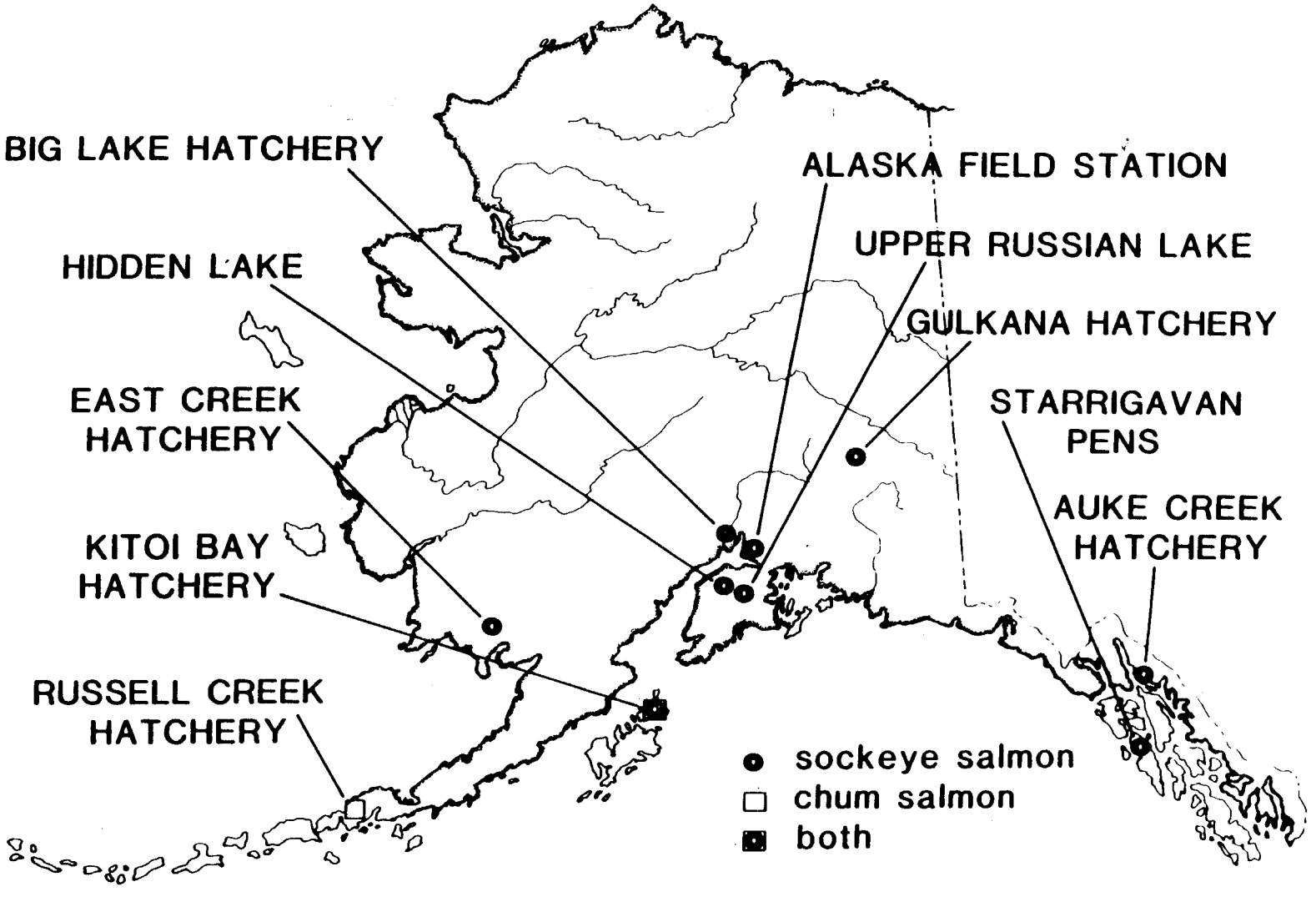

Figure 1. Sites of chum and sockeye salmon infectious hematopoietic necrosis mortalities in Alaska from 1973 to 1984. All were facilities operated by FRED except the U.S. Fish and Wildlife Service, Alaska Field Station.

Koch's postulates were fulfilled for IHN by lab challenge; and the experimental disease was compared to that observed in field cases.

MATERIALS AND METHODS

Traditional Tissue Culture Assay

A nonquantitative tissue culture assay, termed traditional assay (Fried 1984), was used to detect the presence of virus. Fish tissues were collected and held on ice or at 4°C for a maximum of 48 h until processed; diluted 1:9 in Eagle's minimum essential medium containing Earle's salts, gentamicin, fungizone, and 10% tryptose phosphat; and buffered to pH 7.2 with tris (hydroxymethyl) amino methanehydrochloride and sodium bicarbonate (MEM-0-TRIS) (Burke and Mulcahy 1980; Fried 1984). The samples were then homogenized and centrifuged at 2,000 times gravity for 20 min; the supernatant was incubated for 2 to 4 h at room temperature in gentamicin and mycostatin. Ninety-six or 24-well polystyrene tissue culture plates were used for growing confluent-cell monolayers of *epithelioma papulosum cyprini* (EPC) cells in MEM-TRIS supplemented with 10% fetal-bovine serum and 1-glutamine (MEM-10-TRIS). The cells were inoculated with serial dilutions of the samples and then incubated at 15°C for 12-15 days. Cultures were checked for toxicity prior to the third day and for cytopathic effect (CPE) from the third through the 15th day. Initial positives were subcultured to at least 10^{-6} final dilution. The identities of selected viral isolates were confirmed by serum neutralization (Fried 1984), using rabbit IHNV antiserum from the National Fisheries Research Center, U.S. Fish and Wildlife Service.

Plaque-Quantitative Assay

Virus titers were determined using the plaque-quantitative assay. The samples were initially held and processed as

previously described, except no incubation in antibiotics was used prior to inoculation of the cell layer. Inoculation, incubation, and staining followed procedures previously reported (Burke and Mulcahy 1980; Burke and Grischkowsky 1984). Plaques were microscopically examined, and the CPE was found to be characteristic of that caused by IHNV.

Histopathological Methods

Standard histological methods were used to examine the disease in the hatchery episodes and to investigate associated histopathology within the lab challenge experiment. Bouin's fluid was used as a preservative and decalcifying agent. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin or Gram stain (Brown and Hopps 1973).

Lab Challenge

A challenge of the two chum salmon stocks (Sturgeon River and Russell Creek) was performed in the laboratory during July and August 1983. These stocks were involved in the hatchery epizootics and tested against two Kitoi IHNV isolates. The mean total lengths and weights (N=30) were 57.2 mm (SD=5.2) and about 2 g 43.9 mm (SD=4.4) and approximately 1 g for Sturgeon River and Russell Creek chum salmon, respectively. These fish were larger than those usually affected by IHN under hatchery circumstances.

The Sturgeon River salmon fingerlings were divided into six groups of 20 each. The fish in four groups were challenged with IHNV isolated either from the Big Kitoi Lake kokanee ovarian fluid or the Kitoi Bay Hatchery chum salmon undergoing the epizootic. Two equivalent groups of 20 fingerlings each were used as unchallenged controls and interspersed between IHNV treatment tanks. The Russell Creek chum salmon were similarly tested using eight tanks. Duplicate tanks containing 20 fish each were tested for each virus, and four tanks of 20 fish each became the controls.

The challenge consisted of immersing the fish for 1 h in 10^4 to 10^5 plaque-forming units (pfu) of virus per milliliter of static, chilled, and aerated well water. The challenged fish were held in well water at 10°C ($\pm$ 1°C) in 19-liter flow-through fiberglass tanks. The fish were examined daily, and dead fish were removed for plaque assay. Fish used for histopathological sampling were removed when moribund; although in the tabulation of mortality, they were included as dead. Additionally, at the end of the experiment, three live fish were sacrificed from each of the six control tanks and assayed for virus. The test results for 25 days were tabulated and analyzed using chi-square 2 X 2 evaluations (Chapman and Schaufele 1970).

RESULTS

Clinical Signs

Behavior of the infected chum salmon during the two hatchery epizootics and the challenge experiment closely resembled that observed in previous Alaskan outbreaks of IHN in sockeye salmon alevins. Fish were anorexic, listless at the water's surface, and often prostrate on the tank bottom; they had erratic movement when stimulated and would swim in a tail-down aspect with rapid irrigation by gill opercula.

Gross external characteristics of diseased alevins, fry, and fingerlings included (1) darkened dorsal surface or tails; (2) trailing fecal casts; (3) cephalic depressions or swellings; (4) exophthalmos; (5) hemorrhaging from the vent and along the lateral or ventral surfaces, base of the fins, the buccal area, the cornea, opercula and yolk sac; (6) scoliosis; (7) lordosis; and (8) coagulated yolk (Figure 2). Internally, the following characteristics were observed: (1) abundant pale-to-red-tinged ascitic fluids and (2) edema of the liver.

Figure 2. Chum salmon fry from the Kitoi Bay Hatchery infectious hematopoietic necrosis epizootic showing cephalic depression (arrow) and scoliosis. Bar is 10 mm.

Microscopic Pathology

Histopathologic features of chum salmon fingerlings from the lab challenge and from both hatchery epizootics appeared analogous. Although the effect of IHN in chum salmon was similar to that in sockeye salmon, it was less severe. The most pronounced lesion occurred in the kidney. Varying degrees of kidney necrosis were evident in infected fish; these were emphasized by the reduction of hematopoietic tissue. Less severe necrosis was characterized by karyorrhexis and pyknosis of the interstitial hematopoietic cells and the appearance of small voids in the tissue. More severe lesions included local concentrations of nuclear debris with larger parenchymal voids and karyorrhexis of renal tubular epithelium.

The end-stage kidney showed massive voids in hematopoietic tissue (Figures 3, 4, and 5) containing isolated renal tubules. Other frequent lesions included (1) vacuolar degeneration, karyorrhexis, and pyknosis of cells within splenic red pulp (Figure 6); (2) liver edema; and (3) cellular pyknosis, karyorrhexis, and presence of granular debris within the mucosal epithelium of the pyloric caecum and pancreatic-acinar epithelium. Occasional lesions occurred in the liver, including hepatocyte vacuolization, karyorrhexis, and hyperchromatic staining.

In addition, other microscopic pathology included (1) distal epithelial pyknosis and hyperplasia of gill lamellae; (2) karyorrhexis in erythrocytes; and (3) hemorrhage, edema, and heavy melanization of the peritoneum. Viral infection also produced lesions in the gut consisting of necrotic mucus and rounded mucosal epithelial cells, multinucleation of lymphatic cells in the lamina propria, hypersecretion of mucus, and edema of villi. During the peak of the mortality, 89% of the fry samples at Kitoi Bay Hatchery were found to have histopathology typical of IHN.

Figure 3. Kidney of a chum salmon fry from the Kitoi Bay Hatchery infectious hematopoietic necrosis epizootic exemplifying edema (arrow), voids in hematopoietic tissue, pyknosis, hemorrhage, and cellular debris. Hematoxylin and eosin stains. Bar is 50 µm.

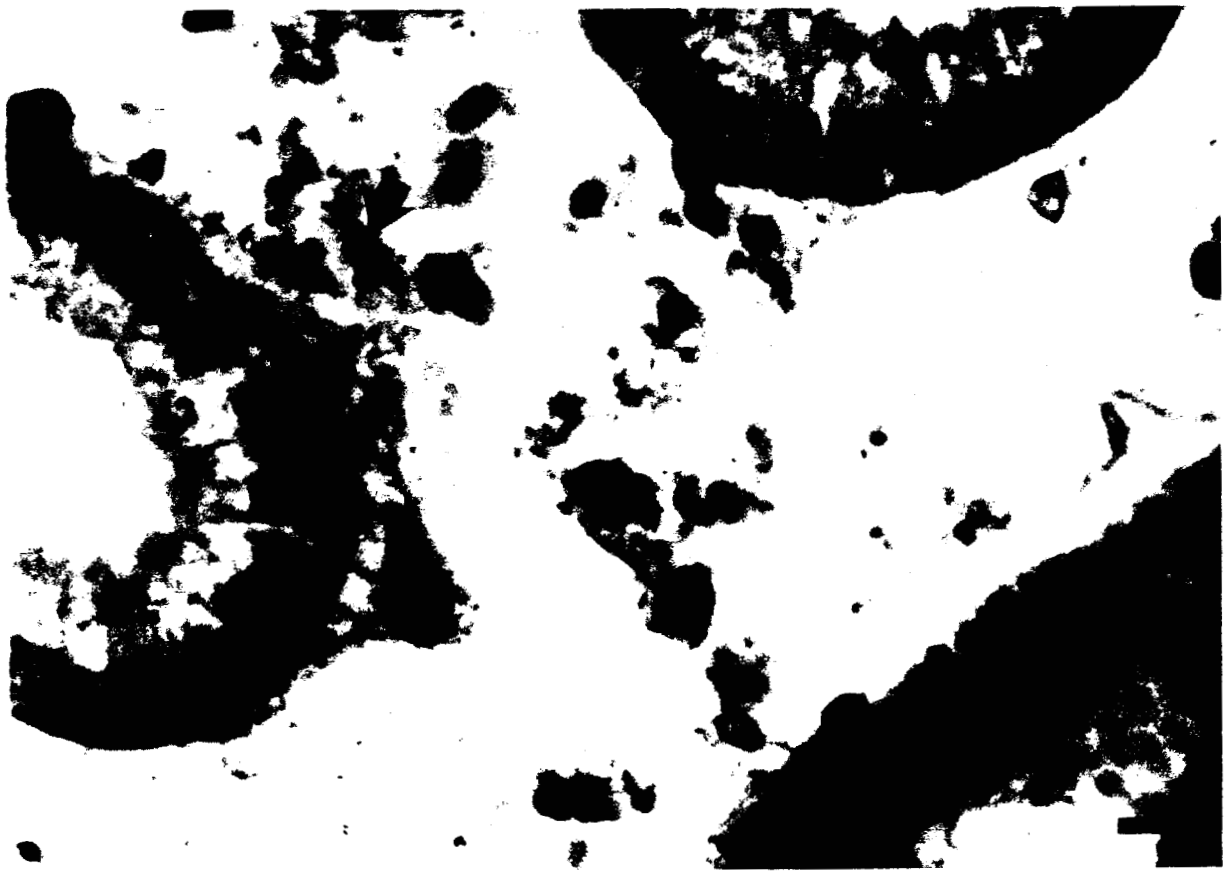

Figure 4. Kidney of a chum salmon fry from the Kitoi Bay Hatchery infectious hematopoietic necrosis epizootic illustrating renal tubules in voids lacking hematopoietic tissue, hematopoietic cell debris (arrow), pyknosis, and karyorrhectic nuclei of renal tubular epithelium. Hematoxylin and eosin stains. Bar is 100 µm.

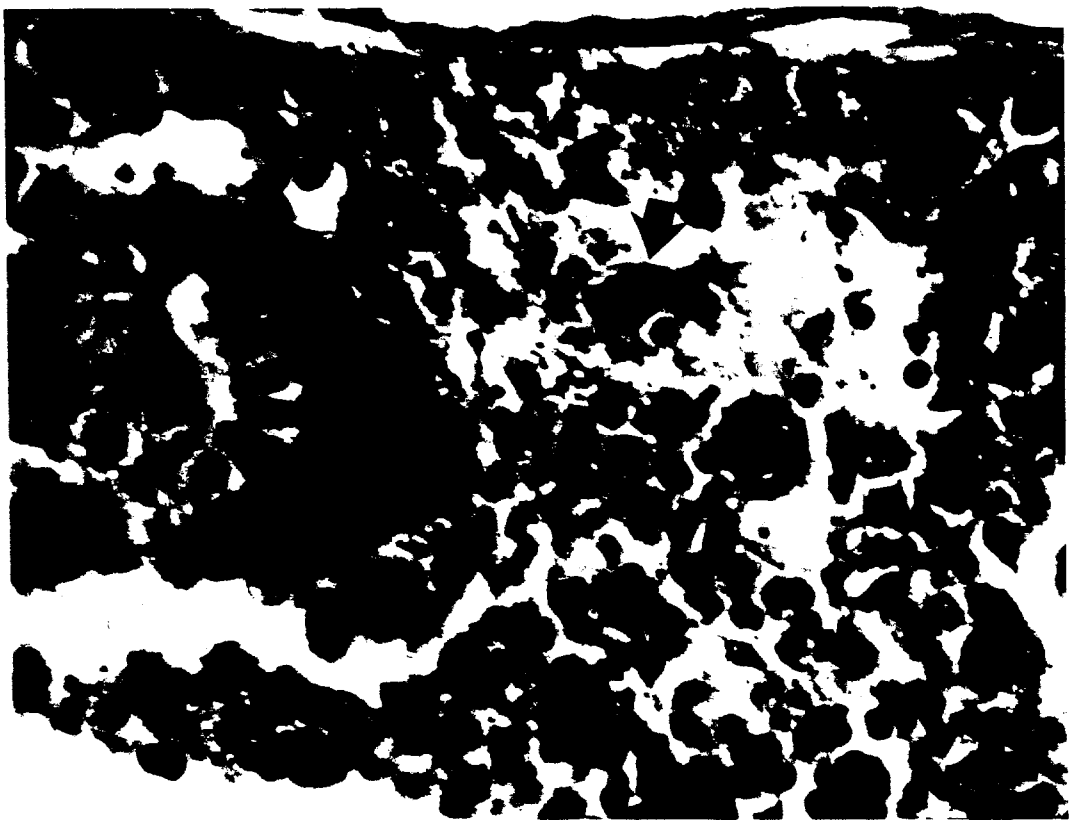

Figure 5. Kidney of a chum salmon fingerling with experimental infectious hematopoietic necrosis showing pyknosis (arrow), karyorrhexis, and cellular debris. Hematoxylin and eosin stains. Bar is 20 µm.

Figure 6. Spleen of a chum salmon alevin from the Russell Creek Hatchery infectious hematopoietic necrosis epizootic showing generalized necrosis, karyorrhexis (arrow), and pyknosis of cells. Hematoxylin and eosin stains. Bar is 20 µm.

Epizootiology

Sturgeon River chum salmon stock was first introduced to Kitoi Bay Hatchery in 1979, but a mortality in that species from IHNV did not occur until 1982. A water manifold failure in December 1981 resulted in the death of close to 90% of the original 465,000 alevins and eggs. Survivors in one incubator were removed and placed in other incubators. Emerging fry began dying during early March 1982. During the first week, the mortality rate was as high as 14%/day, and it reached 36%/day during the next week. The water temperature was approximately 2°C. Subsequently, emerging fish were placed in a seawater pen, but mortalities continued. An estimated total of 1,850 chum salmon alevins emerged; of these, 49% died from IHN prior to destruction. During the five weeks of the epizootic, mean virus titers of moribund alevins and fry were consistently greater than 10^8 pfu/g. The survivors were destroyed, and the hatchery was sanitized.

Since this was the first known IHN epizootic in Alaskan chum salmon and the virus had not yet been isolated from adults, the question arose as to the source of the virus. There has been a history of IHNV involvement at Kitoi Bay Hatchery, including four IHN epizootics in sockeye salmon prior to 1981; sockeye salmon fry or smolts had been introduced into its hatchery water supply (Big Kitoi Lake and Kitoi Creek) during 5 different years from 1965 to 1973. During the chum salmon epizootic, sockeye salmon were not being reared at the hatchery. However, to assess the probability of waterborne contamination, 65 spawning kokanee were sampled at Big Kitoi Lake during October 1982. A 97% incidence of IHNV and titers of up to 10^9 pfu/ml in ovarian fluid were found. The adult chum salmon brood stock is unlikely to have been the source of virus, as no virus was detected in 198 ovarian fluid samples collected from fully mature Sturgeon River chum salmon in subsequent years.

The second chum salmon IHN epizootic occurred in April and May 1984 at the Russell Creek Hatchery. Severe mortality (50% to 70%) occurred in two incubators; approximately 400,000 alevins died. In those affected incubators, premature emergence predominated, and the mean virus titers of the chum salmon alevins exceeded 10^7 pfu/g. Fish from the remaining 32 incubators emerged normally and appeared healthy. However, when sampled by traditional assay, virus was detected in the fish from 12 of these incubators. All incubators were on the same water supply at 4° to 5°C. Periods of water recirculation had occurred prior to the outbreak, and fry were being collected in common raceways. After the extent of the viral infection had been determined, the surviving 11.9 million fish were destroyed to prevent possible production of a carrier population and the hatchery was sanitized.

The IHN epizootic at Russell Creek Hatchery, unlike that at Kitoi Bay Hatchery, was not preceded by a long history of IHN involvement. However, each year several hundred sockeye salmon and thousands of chum salmon adults pass above the hatchery to spawn in water that supplies the hatchery. No virus was found in the ovarian fluid from 134 fully mature chum salmon. Because of difficulties involved in capturing fully mature sockeye salmon upstream from the hatchery, only 12 unripe fish have been tested for the virus, and all results were negative.

Lab Challenge

Challenges of Sturgeon River and Russell Creek chum salmon with either the chum salmon or the kokanee virus isolates caused significant mortalities (Figure 7) when compared to control fish ($P<0.05$). Highly significant mortality ($P<0.001$) for the two virus isolates occurred by the seventh and eighth days after challenge in the Sturgeon River chum salmon and by the eighth and 14th days for the Russell Creek chum salmon. The Sturgeon River chum salmon cumulative mortality reached 100% when

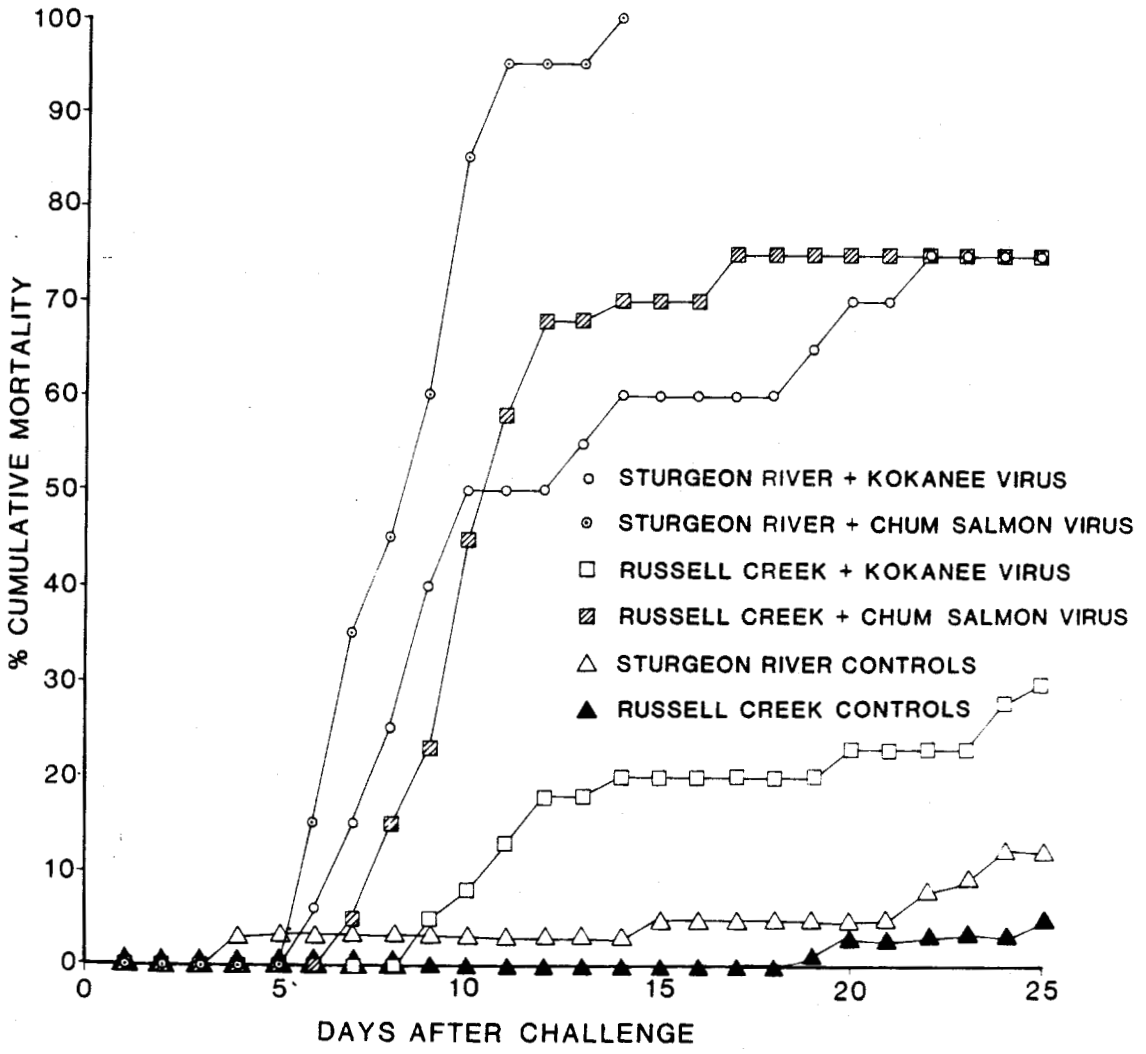

Figure 7. Mortality in Sturgeon River and Russell Creek chum salmon fingerlings challenged with Big Kitoi Lake kokanee virus or Kitoi Bay Hatchery chum salmon virus compared to fish not challenged with virus.

challenged with the Kitoi Bay Hatchery chum salmon virus isolate and stabilized at 75% after 22 days when challenged with the kokanee virus isolate; while deaths abated in the Russell Creek chum salmon essentially by the 18th day at 75% and 20% for the two virus isolates, respectively. The control-tank fish showed no signs of IHN and no virus was isolated from them. These included 11 Sturgeon River (five dead and six sacrificed) and 15 Russell Creek fish (three dead and 12 sacrificed). Cumulative mortality in control fish was 0% to 15%. These values were significantly different ($P<0.001$), compared to the 30% to 100% mortality for challenged fish.

Both virus isolates caused clinical signs similar to those observed in the epizootics. Virus was isolated from almost all challenged fish examined. The IHNV was detected in 85% (23 of 27) of the Sturgeon River chum salmon and 98% (41 of 42) of the Russell Creek chum salmon. In all, virus was found in 93% (64/69) of the challenged fish that died and in none of the 26 control fish assayed. Mean IHNV titers of these whole fish containing virus were about 10^7 to 10^8 pfu/g (Table 1).

DISCUSSION

This is the first report of findings of IHN and virally induced mortality in chum salmon in North America. No other agents were detected that could explain these mortalities. Clinical signs of IHN in our chum salmon were similar to those described for sockeye salmon (Amend et al. 1969), except for the occasional presence of previously unreported cephalic depressions. Internally, edema was much more obvious in the chum salmon than formerly found in Alaskan sockeye salmon alevins, fry, and fingerlings.

Table 1. Infectious hematopoietic necrosis virus (IHNV) titers (plaque-forming units/gram whole fish tissue) in mortalities of two chum salmon stocks challenged with two virus isolates.

IHNV isolate	STURGEON RIVER STOCK Mean (range)	*N*	IHNV not detected*	RUSSELL CREEK STOCK Mean (range)	*N*	IHNV not detected*
Big Kitoi Lake kokanee	6.4×10^7 (1.2×10^5-2.2×10^8)	13	4	9.2×10^7 (6.0×10^4-6.7×10^8)	9	1
Kitoi Bay Hatchery chum salmon	4.6×10^7 (7.8×10^5-1.9×10^8)	14	0	2.2×10^7 (1.0×10^4-7.5×10^7)	33	0

*These fish were not included in determination of mean titers.

In general, the histopathology of IHN in chum salmon was more subtle than for sockeye salmon alevins and fingerlings (Yasutake and Amend 1972; Yasutake 1970, 1975) and similar to that described for sockeye salmon smolts and yearlings (Burke and Grischkowsky 1984; Yasutake 1978). Foci of necrosis within the hematopoietic tissue of the kidney were more often seen than the presence of massive voids that are typical with sockeye salmon. Additionally present in advanced cases were renal tubule necrosis and splenic abnormalities; however, no hepatic focal degeneration was evident, as previously reported in sockeye salmon and rainbow trout (Yasutake 1970). Many abnormalities of the gut were noted, as previously described for sockeye salmon by Yasutake and Amend (1972): the collection of granular debris, lymphatic cell necrosis, and hypersecretion of mucus; the latter abnormality results in the trailing fecal casts first reported by Amend et al. (1969).

Little is known about IHNV subtyping. Mean plaque diameters may be useful in identifying strains of IHNV, because plaque diameters of isolates tend to increase as the latitude of the geographic source of the isolate increases (Mulcahy et al. 1984; Leong et al. 1981). We tested the IHNV plaque diameters (Appendix Table 1), following the procedures of the plaque-quantitative assay, and found the chum salmon virus from Kitoi Bay Hatchery had a mean diameter of 450 µm (SD=42). In comparison, by using one-way analysis of variance (Guenther 1964), four Alaskan sockeye salmon isolates from a different area had a larger mean value of 541 µm (SD=50: $P<0.001$). However, additional studies need to be completed before we can make conclusions regarding subtyping.

Horizontal waterborne transmission of IHN has been well documented (Mulcahy et al. 1983; Wingfield and Chan 1970) and seems to have been the major factor in these chum salmon epizootics at Kitoi Bay and Russell Creek Hatcheries. Viral titers of the magnitude found in Big Kitoi Lake kokanee could

certainly have resulted in substantial downstream flow of virus particles during spawning. At Russell Creek Hatchery, the sockeye salmon in the water supply above the hatchery are presumed to be a source of viral contamination and ultimately linked with the chum salmon epizootic, but the sockeye salmon have not been adequately tested for IHNV. Because no IHNV-free Alaskan populations of sockeye salmon have been found, it is unlikely that Russell Creek's sockeye salmon population would be free of the agent. That population should be tested prior to continued operation of the hatchery facility with chum salmon. Because no virus was found in either of the spawning chum salmon populations, there is no evidence to implicate vertical transmission from parents to progeny.

On the other hand, at Russell Creek Hatchery, there is also the possibility that contamination of the eggs was directly caused by the clothing of field personnel. These personnel had handled sockeye salmon adults at the weir and subsequently had recreationally fished in the upper watershed areas without sanitizing their field clothing.

Since the epizootic, changes have been made to the facility and operating procedures at Kitoi Bay and Russell Creek Hatcheries. These changes have been designed to prevent or limit a recurrence of IHN. Wooden incubators in use at the Kitoi Bay Hatchery have since been replaced with aluminum ones, which are more easily disinfected, and an ultraviolet sterilizer has been placed in the influent water. To reduce the virus source, many prespawning and spawning kokanee have been removed from Big Kitoi Lake and Kitoi Creek. Since 1982 over 12,200 kokanee, representing an unknown percentage of the population, have been eliminated in an attempt to eradicate them.

At Russell Creek Hatchery, changes include (1) physical isolation of egg-take tasks, (2) increased use of disinfectant, and (3) elimination of use of the same external clothing for fish

culture and sport fishing activities. Instead of direct use of water from the stream as the major water source, the hatchery now uses a shallow well, and water is no longer being recirculated throughout that hatchery. Since these changes, neither hatchery has had a recurrence of IHN, despite continued propagation of chum salmon.

Further, stress caused by water reuse and handling predisposes older fish to IHN (Warren 1983). Both Alaskan chum salmon epizootics were preceded by stressors. At Kitoi Bay, it was water flow interruption; at Russell Creek, it was exposure of developing eggs and alevins to silted water, the ciliated parasite *Trichodina*, and formalin treatments. These stressors may have played some role in precipitating an outbreak when virus was present in contaminated water.

Indeed, the potential for more IHN epizootics in chum salmon emphasizes the need for preventive measures. Chum salmon should not be incubated or reared in surface water containing sockeye salmon or kokanee, unless adequate depuration of the water is first accomplished or these species are removed from the water supply. Chum salmon brood stocks that are potentially in contact with IHNV should be sufficiently screened for the virus to allow for a 2% prevalence detection. The susceptibility of chum salmon to IHNV should be considered when making decisions regarding chum and sockeye salmon propagation, enhancement, and stocking.

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APPENDIX

Appendix Table 1. Plaque diameter comparisons for infectious hematopoietic necrosis virus (IHNV) isolates from four sockeye salmon from the Gulkana River near Paxson and one Kitoi Bay chum salmon isolate. Mean diameters were established from 20 virus plaques from each of eight replicate 60 mm polystyrene plates. Monolayers within the plates were *epithelioma papulosum cyprini* cells.

Replicates	IHNV Plaque diameters (µm) 1/ 3/				
	sockeye salmon 2/				chum salmon
1	523	534	540	560	460
2	514	560	569	549	502
3	526	509	606	629	431
4	486	518	626	540	386
5	537	560	583	537	506
6	503	566	623	609	434
7	503	520	560	489	414
8	483	540	540	377	466
$\bar{X}$	509	538	581	536	450

1/ Most of the variance was associated with the plaque diameters of the sockeye salmon isolates compared with the chum salmon isolates ($P<0.001$ using one way analysis of variance).

2/ A lesser degree of variance was associated with plaque diameters of the sockeye salmon isolates ($P<0.005$).

3/ The cytopathic effect produced by all of the virus isolates was indistinguishable.