

Report on the occurrence of infectious hematopoietic necrosis disease in out-migrating Sockeye Salmon fry at Pinkut Creek Spawning Channel in the spring of 2021

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Report on the occurrence of infectious hematopoietic necrosis disease in out-migrating Sockeye
Salmon fry at Pinkut Creek Spawning Channel in the spring of 2021

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ABSTRACT

Garver, K.A., Hawley, L.M., Thiessen, L., Harborne, M., Berdan, C. and Lofthouse, D. 2022. Report on the occurrence of infectious hematopoietic necrosis disease in out-migrating Sockeye Salmon fry at Pinkut Creek Spawning Channel in the spring of 2021. Can. Tech. Rep. Fish. Aquat. Sci. 3478: iv + 17 p.

Infectious hematopoietic necrosis virus (IHNV) occurs naturally in wild and enhanced stocks of Sockeye Salmon, where it can result in severe infectious hematopoietic necrosis (IHN) disease. We report the occurrence of an IHN epizootic in Sockeye Salmon fry out-migrating from Pinkut Creek Spawning Channel. IHN disease was suspected during fry enumeration where upwards of 10% of early out-migrating fry exhibited external hemorrhaging. Analyses confirmed high load IHNV infections particularly at the beginning of the out-migration period. Clinically diseased fry became less apparent over the course of the out-migration period with concomitant declines in IHNV prevalence and load. Despite the decreasing progression of IHN disease over the migration period, the occurrence of the viral outbreak at Pinkut Creek Spawning Channel corresponded with a marked decrease in the egg-to-fry survival rate and in the total number of fry produced for brood year 2020. At Fulton River Spawning Channel, a nearby Babine Lake companion facility, IHNV was detected in out-migrating fry, however no signs of IHN disease or decreases in survival were noted and IHNV prevalence remained less than 10%. Whether IHNV infections lead to disease is dependent upon a myriad of environmental, virus and host factors that often interact in unforeseen ways. Herein we highlight factors potentially contributing to the IHN outbreak at Pinkut Creek Spawning Channel and make operational and research recommendations to mitigate against the occurrence of IHN disease and to better understand the triggers responsible for such outbreaks in Sockeye Salmon populations.

RÉSUMÉ

Garver, K.A., Hawley, L.M., Thiessen, L., Harborne, M., Berdan, C. and Lofthouse, D. 2022. Report on the occurrence of infectious hematopoietic necrosis disease in out-migrating Sockeye Salmon fry at Pinkut Creek Spawning Channel in the spring of 2021. Can. Tech. Rep. Fish. Aquat. Sci. 3478: iv + 17 p.

Le virus de la nécrose hématopoïétique infectieuse (VNHI) se manifeste de façon naturelle dans les stocks de saumons rouges sauvages et mis en valeur. Nous rapportons l'apparition d'une nécrose hématopoïétique infectieuse (NHI) épizootique observée dans les alevins de saumon rouge en migration sortante du chenal de ponté du ruisseau Pinkut. On a soupçonné la maladie de NHI lors du dénombrement des alevins, où plus de 10 % des alevins en migration sortante précoce présentaient une hémorragie externe. Des analyses ont confirmé les infections de VNHI à forte charge, particulièrement au début de la période de migration sortante. Les alevins cliniquement malades sont devenues moins apparentes au cours de la période de migration sortante, ce qui a entraîné une diminution concomitante de la prévalence et de la charge du VNHI. Malgré la progression décroissante de la maladie de NHI au cours de la période de migration, l'apparition de l'épidémie virale au chenal de ponté du ruisseau Pinkut a correspondu à une diminution marquée du taux de survie de l'œuf à l'alevin et du nombre total d'alevins produits pour l'année d'éclosion 2020. Au chenal de ponté de la rivière Fulton, une installation voisine du lac Babine, on a détecté le VNHI chez les alevins en migration sortante, mais aucun signe de maladie ou de diminution de la survie, et la prévalence du VNHI est demeurée inférieure à 10 %. Le fait que les infections par le VNHI devient une maladie dépend d'une myriade de facteurs environnementaux, viraux et liés à l'hôte qui interagissent souvent de manière imprévisible. Dans le présent document, nous mettons en évidence les facteurs qui ont potentiellement contribué à l'éclosion de la NHI dans le chenal de ponté du ruisseau Pinkut et nous formulons des recommandations opérationnelles et de recherche dans le but d'atténuer l'apparition de la maladie et de mieux comprendre les déclencheurs responsables de telles éclosions dans les populations de saumon rouge.

INTRODUCTION

Between 1965 and 1971, the Department of Fisheries and Oceans Canada constructed three spawning channels adjacent to Babine Lake on the Skeena River (Table 1) with the intent of increasing the number of fry in the lake thereby resulting in a corresponding increase in the number of returning adults. Since their inception, each channel is assessed annually by estimating the number of eggs deposited and the number of fry produced allowing for a determination of an egg-to-fry survival rate. In addition, yearly smolt production leaving the lake is monitored, along with the subsequent return of adult Sockeye Salmon (*Oncorhynchus nerka*) both to catch and escapement.

Table 1. Specifications of Babine Lake Spawning Channels.

Channel name	Year completed	Spawning area (m ²)	Flow (m ³ /s)	Velocity (m/s)
Fulton I	1965	11,426	2.67	0.43
Pinkut	1968	33,442	2.34	0.37
Fulton II	1971	73,154	3.60	0.52

At the onset of the 2021 fry enumeration assessments at Pinkut Creek Spawning Channel, the Operations Manager (Lindsay Thiessen) observed hemorrhaging at the base of the pelvic fins in approximately 5-10% of Sockeye Salmon fry migrating out of the channel en route to Babine lake. While fin base hemorrhages are not pathognomonic, they have been associated with fish infected with infectious hematopoietic necrosis virus (IHNV). This virus is endemic to the waters of British Columbia where it can cause infectious hematopoietic necrosis (IHN), a deadly disease in Sockeye Salmon fry. Given the severity of this disease in Sockeye Salmon, concern was raised regarding whether the out-migrating Sockeye Salmon fry in Pinkut Creek Spawning Channel were indeed suffering from IHN disease. This report details the onsite observations and laboratory tests undertaken to determine the presence of IHNV and the occurrence of IHN disease in Sockeye Salmon fry at both Pinkut Creek and Fulton River Spawning Channel complexes.

MATERIALS AND METHODS

Sockeye Salmon fry enumeration

Fry enumeration extended from April 19 to June 4, 2021 at Pinkut Creek Spawning Channel and from April 26 to June 2 at Fulton River Spawning Channel. Each night, for a duration of approximately 4 hours (22:30-2:30), fry traps were deployed every half hour for 5 minutes. After five minutes, the fry are collected from the traps and either counted directly or estimated based on a volume measurement when fry were too numerous to individually count within a short timeframe. Once the fry are counted, they are immediately placed onto a water table where running water directs them to a pipe that leads to a fishway where fry out-migrate to Babine Lake via Pinkut Creek or Fulton River.

Fry sampling

As part of the spring enumeration activities, fry collections for viral analysis were first collected in late April at Fulton River Spawning Channel and during the first week of May at Pinkut Creek Spawning Channel. Fry collections occurred over the entire outmigration period. Table 2 summarizes the subset of fry included in this study.

Table 2. Summary of 2021 Sockeye Salmon fry from the Babine Lake Spawning Channel systems screened for the presence of IHNV.

Sampling Period	Sampling Date	Fulton		Pinkut	
		Channel	River	Channel	Creek
Early	April 26 th	25	25		
	May 6 th	-	-	69*	-
	May 9 th	25	25	-	-
Mid	May 18 th	25	25	-	-
	May 24 th	-	-	60	-
Late	May 28 th	25	25	-	
	May 31 st	-	-	60	60
Total		100	100	189	60

* Fry exhibiting clinical signs were targeted at this sampling event.

For Fulton River Spawning Channel, the fry collected from each location and time point were pooled into a single whirl-pak bag and immediately frozen, while at Pinkut Creek Spawning Channel, sampled fry were placed in individual 1.5mL microtubes and immediately frozen. All samples were labeled with the date and location of sampling and were shipped on dry ice for overnight delivery to the Pacific Biological Station.

Virological testing

Sockeye Salmon fry were assayed for IHNV using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and cell culture methodologies.

Fry were transferred into 2mL safe-lock tubes containing a 5mm stainless steel bead using sterile forceps. Fry arriving from Pinkut Creek Spawning Channel were transferred directly from their 1.5mL tubes whereas fry received from Fulton River Spawning Channel were thawed and placed on paper towel before being teased apart and transferred using sterile forceps. Hank's balanced salt solution (HBSS) was added to yield a 40% (w/v) homogenate and fry were homogenized using Qiagen's Tissue Lyser for 2min at 25Hz. For cell culture, the 40% homogenates were further diluted in HBSS to prepare a final 2-4% (w/v) homogenate. The homogenate was centrifuged at 2500 x g for 15 minutes and 100uL of the clarified supernatant was inoculated into duplicate wells containing monolayers of EPC cells prepared 24 hours prior to inoculation. Cells were incubated at 15°C and observed 2-3 times per week for a period of 3 weeks for the presence of cytopathic effects (CPE).

For molecular analysis, the 40% (w/v) homogenate was extracted using MagMAX™ CORE Nucleic Acid Purification kit (Applied Biosystems™) in conjunction with KingFisher™ Flex instrument. The IHNV RT-qPCR was carried out as described previously (Purcell et al., 2013). Briefly, 10µL of extracted material was used as template in cDNA generation using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™) following manufacturer's instructions. 2.5µL of cDNA product was then used as template in 25µL qPCR reactions containing 1x TaqMan™ Universal PCR master mix (Applied Biosystems™), 900nM each IHNV N 796F (5'AGA GCC AAG GCA CTG TGC G-3') and IHNV N 875R (5'-TTC TTT GCG GCT TG GTT GA-3') and 250nM IHNV N 818MGB probe (5' 6FAM-TGA GAC TGA GCG GGA CA-MGB 3'). qPCR reactions were run in duplicate on Bio-Rad CFX qPCR machine using the following thermal profile: 50°C for 2 min, 95°C 10 min followed by 40 cycles of 95°C 15 sec, 60°C 1 min. If a Ct value was registered in duplicate reactions then IHNV was conclusively "detected" while a Ct value in only one of the duplicate

reactions was considered an “inconclusive”. In instances where prevalence of IHN was calculated, a “detection” and “inconclusive” result were both considered a positive IHN finding however the portion of inconclusive results contributing to the overall prevalence was noted.

IHN genotype was determined using conventional PCR targeting the glycoprotein gene followed by nucleotide sequence analysis. Briefly 2.5µL of extracted material was used as template in 25µL Invitrogen™ SuperScript™ III One-step RT-PCR with Platinum™ Taq high fidelity reactions containing 200nM each IHN G 517+ (5’AGA GAT CCC TAC ACC AGA GAC-3’) and IHN G1209- (5’-GGT GGT GTT GTT TCC GTG CAA-3’). RT-PCR reactions were run according to the following thermal profile: 50°C for 30 min, 95°C 2 min followed by 40 cycles of 94°C 15 sec, 50°C 1 min, 68°C followed by final extension at 68°C for 5 min. PCR products were purified using ExoSAP-IT™ Express (Applied Biosystems™) then used as template in Applied Biosystems’™ BigDye™ Terminator v3.1 sequencing reactions according to manufacturer’s instructions. Sequencing reactions were purified using Qiagen DyeEx 2.0 spin columns before being run on SeqStudio™ Genetic Analyzer (Applied Biosystems™).

RESULTS

Field observations

Pinkut Creek Spawning Channel

The occurrence of external hemorrhaging was most commonly observed among the early out-migrants of Sockeye Salmon fry at Pinkut Creek Spawning Channel (Figure 1). During the first two weeks of outmigration (Weeks of April 19 and 26) hemorrhaging was visible on the surface of approximately 5-10% of fry enumerated, however these observations greatly diminished over the out-migration period such that less than 1% of fry caught had visible signs of disease during subsequent enumerations.

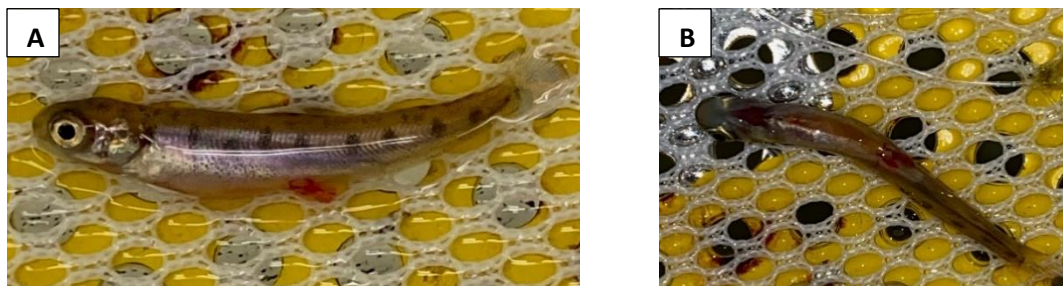


Figure 1. Hemorrhaging observed at (A) the base of the pelvic fin and (B) dorsal fin area of out-migrating Sockeye Salmon fry from Pinkut Creek Spawning Channel.

Fulton River Spawning Channel

No notable signs of disease were observed in out-migrating fry during the enumeration activities at Fulton River Spawning Channel.

Virological testing

Molecular detection

At Pinkut Creek Spawning Channel a total of 249 fry were tested for IHN with 189 fry originating from the spawning channel and 60 fry originating from the creek. The overall prevalence of IHN in the out-

migrating fry from this system was 33% with IHNV infection levels ranging from extremely high (Ct value 13.37) to very low (Ct 38.78) which often resulted in an inconclusive detection due to the levels being near the limit of detection of the IHNV test (Figure 2). The frequency and intensity of IHNV infections was highest early in the out-migration period, although it is worthwhile noting that the early collections were biased towards fry with signs of disease which likely influenced the prevalence. Nevertheless, as the higher IHNV detection frequency within the early out-migrants coincided with the higher frequency of clinical signs of disease, the progression of IHNV infections as determined through the sampling was likely a good reflection of the outbreak in the channel. The prevalence of IHNV during this early out-migration period was 81% (56 out of 69 fry) with mean viral loads nearing a Ct value of 20 (Figure 2, Table 3). However, IHNV detections in subsequent samples collected over the course of the out-migration progressively declined with only 10% (6/60) of fry sampled at the end of the out-migration period containing levels of virus detectable by the RT-qPCR assay (Figure 2, Table 3). Similarly, at this later stage of the out-migration period, Sockeye fry originating from Pinkut Creek showed minimal infection with IHNV (Table 3).

At Fulton River Spawning Channel, a total of 200 fry were tested for IHNV with 100 originated from Channel II and 100 originating from the river. The fry that were screened for IHNV were collected during the beginning (April 26th, May 6th), middle (May 18th), and end (May 28th) of the out-migration period (n=25 per date). Overall prevalence of IHNV in the channel for the out-migration period was 8% with all infections being very low intensity and Ct values ranging from 36.61 to 37.65 (Figure 2). Similarly, IHNV was of low prevalence (3%) and low intensity in the river samples, however one fry sampled on April 26th registered an extremely high load infection (Ct 14.67) suggesting that at least one fish likely had IHN disease. IHNV was detected in all other samples from this time point, however the level of virus detected in the other specimens was much lower (Ct values > 29). Given that all fry sampled at this time point were placed together in a single bag, the potential for cross contamination of all samples from the one highly infected fry cannot be ruled out. Consequently, the true prevalence for this sample time point is unknown and therefore only the highly infected fry is represented by a triangle in Figure 2.

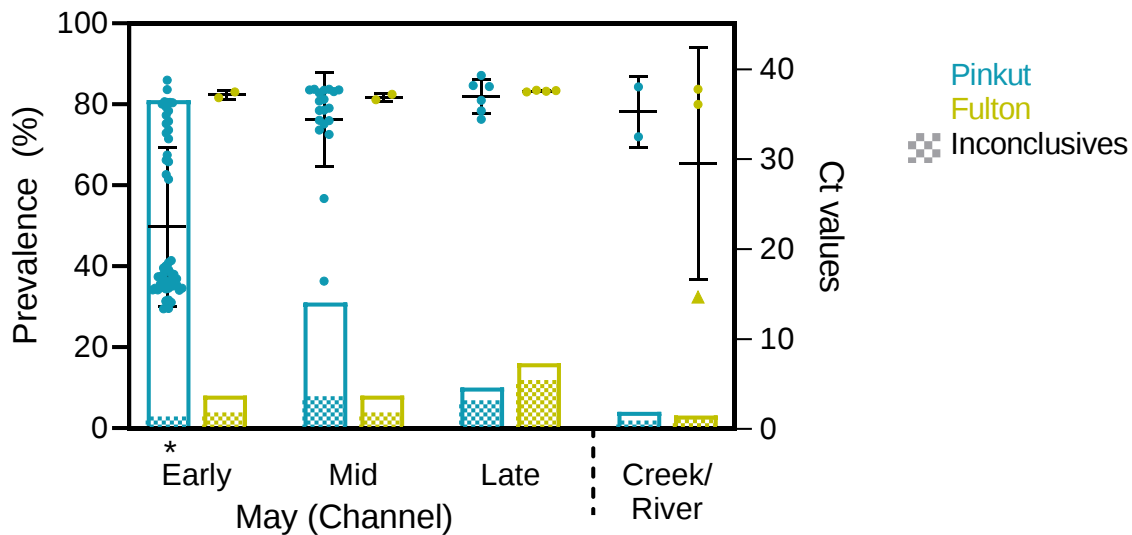


Figure 2. RT-qPCR detection of IHNV in out-migrating Sockeye Salmon fry at Babine Lake Spawning Channels. Individual (dot) and mean (line) IHNV loads and infection prevalence (bars) measured during the early, mid and late out-migration period. Overall prevalence of IHNV infection per sampling time calculated based upon number of detections and inconclusive results with the portion of inconclusive results denoted by checkerboard fill. Asterisk denotes sampling time point whereby fry with clinical signs were targeted.

Table 3. IHNV RT-qPCR results and IHNV prevalence in 2021 spring out-migrating Sockeye fry sampled from the channel and creek at Pinkut Creek Spawning Channel.

RT-qPCR Result	Sampling period in Pinkut Channel				Pinkut Creek
	Early	Mid	Late	Overall	
Detected	54 (78.3%)	14 (68.3%)	2 (3.3 %)	70 (37.0%)	1 (1.7%)
Inconclusive	2 (2.9%)	5 (8.3%)	4 (6.7%)	11 (5.8%)	1 (1.7%)
Not Detected	13 (18.8%)	41 (68.3%)	54 (90.0%)	108 (57.1%)	58 (96.7%)
Total	69	60	60	189	60

Virus genotyping

A subset of the IHNV detections from both Pinkut Creek and Fulton River Spawning Channels were confirmed by IHNV specific conventional PCR and nucleotide sequencing to determine the IHNV genotype. The IHNV detected at Pinkut Creek and Fulton River Spawning Channels were found to be identical to each other, based upon a 303 base fragment of the IHNV glycoprotein gene, and grouped into the endemic U genotype. Particularly, this exact IHNV sequence has been identified previously from numerous locations throughout British Columbia including from spawning adult Sockeye Salmon collected in 2015 and 2016 from Pinkut Creek and Fulton River Spawning Channels.

Cell culture

As RT-qPCR detects viral RNA and does not verify replicating virus, cell culture testing was performed on a portion of the fry collected from the Babine Lake Spawning Channels to confirm the presence of infectious (replicating) virus. From a total of 123 fry tested, 46 samples produced cytopathic effect (CPE) in cell culture revealing the presence of replicating virus. In evaluating the agreement of results between cell culture and RT-qPCR among the 123 samples, the RT-qPCR was determined to be the more sensitive test often detecting IHNV when cell culture was deemed negative (Figure 3). Typically IHNV detections of low viral load ($Ct > 30$) escaped detection by cell culture while the highly infected samples ($Ct < 20$) resulted in CPE of the cell culture (Figure 3). These differences in test sensitivity are important to consider when applying the test. In instances where high viral loads are anticipated such as when clinical signs of disease are observed, cell culture testing would be suitable. However in scenarios where the infection status of the population is unknown, the more sensitive RT-qPCR assay should be employed to reduce the potential for a false negative. For instance, of the eleven fry from the Fulton sample set that proved positive by RT-qPCR, only one proved positive by cell culture highlighting the importance of selecting the appropriate test method.

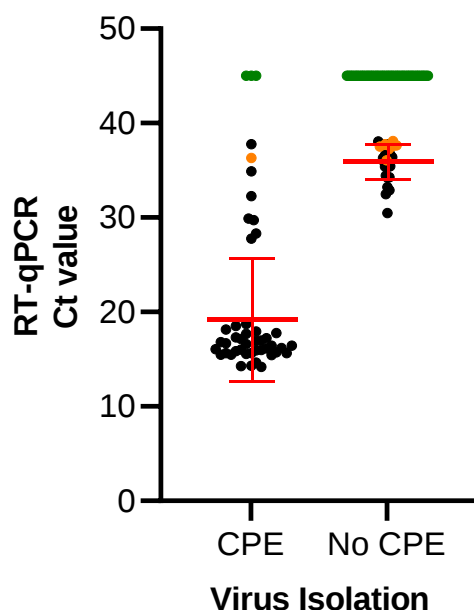


Figure 3. IHNV screening results of Sockeye Salmon fry ($n=123$) tested via cell culture and RT-qPCR. Individual RT-qPCR results (dots) identifying fry as either negative (green), inconclusive (orange) or detected (black) for IHNV with mean ($\pm$ standard deviation) loads shown in red brackets.

DISCUSSION

The IHN virus occurs in wild and cultured salmon and trout populations over a geographic range spanning from Alaska to California and in-land to Idaho and northern Montana. In British Columbia, the virus is predominately detected in Sockeye Salmon at the fry and spawning adult life stages. In some instances, IHNV infection of Sockeye fry has resulted in severe disease and mortality most often during

the egg to fry stage. In particular, the occurrence of such IHN disease outbreaks in British Columbia has been documented for two different stocks of Sockeye Salmon. The first epizootic occurred in the wild stock of Chilko Lake, BC in the spring of 1973 and resulted in an estimated reduction of 23.7 million fry migrating into the lake. This marked the lowest egg-to-fry survival ever recorded over the period from 1949 to 1973 (Williams and Amend, 1976). Similarly, an IHN outbreak at Weaver Creek Spawning Channel in 1987 caused devastating losses in Sockeye Salmon fry with nearly 50% mortality occurring among the 16.8 million out-migrating fry (Traxler and Rankin, 1989).

In regards to the occurrence of IHN disease in the spring of 2021 at Pinkut Creek Spawning Channel, the level of mortality incurred in the out-migrating Sockeye Salmon fry remains uncertain. The occurrence of the viral outbreak corresponded with a marked decrease in the egg-to-fry survival rate and in the total number of fry produced from the project for the 2020 brood year. Total fry production (channel + creek) for the Pinkut project for the 2020 brood year is estimated at 11.2M, while annual fry production has averaged 53.5 M over the past four decades (Figure 4).

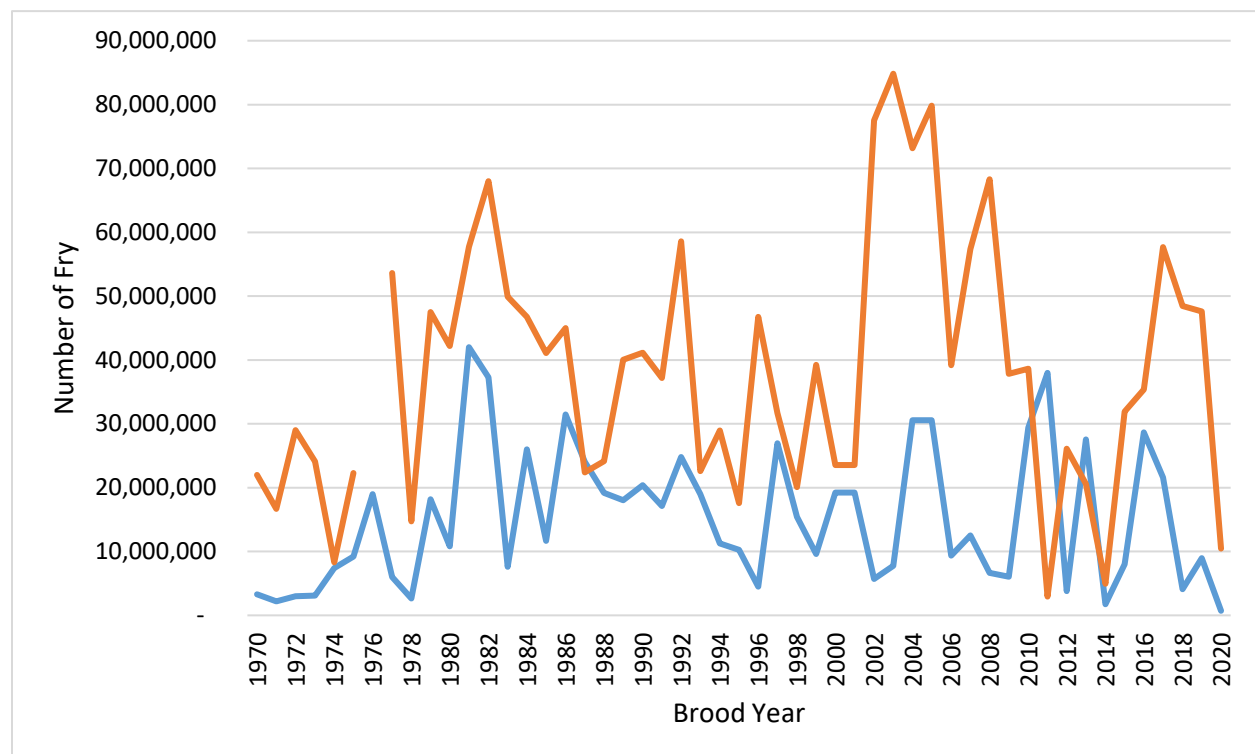


Figure 4. Annual number of out-migrating Sockeye Salmon fry originating from Pinkut Creek (blue) or Pinkut Creek Spawning Channel (orange).

While dead and dying fry were not observed accumulating along the channel's banks and/or back eddies as was observed during the IHN outbreak at Weaver Creek Spawning Channel, mortalities among symptomatic fry that were held for short periods during enumeration suggests that Sockeye Salmon likely died of IHN soon after leaving Pinkut Creek Spawning Channel.

The detection of high levels of IHN in association with the presence of clinical signs of disease suggests the occurrence of an IHN epizootic in out-migrating Sockeye Salmon fry in Pinkut Creek Spawning Channel (Figure 2). The coinciding reduction of IHN levels, prevalence, and diminished presence of clinical signs of disease reveals a decreasing progression of IHN disease over the migration period at

Pinkut Creek Spawning Channel. Although the reason for the declining severity of the disease at Pinkut Creek Spawning Channel is not known, it is worthwhile noting that a similar decline in IHN disease progression was observed at Weaver Creek Spawning Channel during the 1987 outbreak. In both instances, prevalence of IHNV and associated disease signs were highest during the early part of the out-migration period. Interestingly, although one fry from Fulton River sampled at the beginning of the out-migration contained high viral loads typical of fish with IHN disease, the prevalence of IHNV remained low over the course of the migration with no discernable clinical signs of disease or notable mortality observed. Furthermore, despite the presence of IHNV in the river and channel (Figure 2), no corresponding decrease in survivals were noted, in fact egg-to-fry survivals in the River were unusually high (27.6%) suggesting that an epizootic did not ensue within Fulton river or the spawning channel. Undoubtedly the progression of IHN disease is multifactorial and involves complex interactions between the virus, host, and environment nevertheless, the lack of an IHN outbreak in the Fulton fry population despite the presence of virus suggests the possibility of implementing effective disease mitigation measures in Sockeye Salmon spawning channels.

In the case of the 1987 IHN epizootic at Weaver Creek Spawning Channel, no notable adverse environmental conditions, such as water flow, oxygen level, temperature, lack of ice formation, or abundance of algal growth were noted during the 1986 brood year (Traxler and Rankin, 1989). Conversely, in the year leading up to the IHN epizootic at Pinkut Creek Spawning Channel, one environmental anomaly stands out. In the summer of 2020, precipitation levels were exceptional for the Babine area. The weather station at Fulton River Spawning Channel had measurable precipitation on 27 of 31 days in August prohibiting the usual drying of the spawning channel gravel. Additionally, at the Pinkut Creek facility, the cleaning regiment for the spawning channel gravel which typically took channel personnel approximately 14 days (12 hours/day) was performed by a contractor over a total of 4.5 days (8 hours/day).

Whether such operational alterations at Pinkut Creek Spawning Channel influenced or contributed to the 2021 IHN epizootic remains unknown. Nevertheless, it's worthwhile noting that with previous accounts of IHN disease in Sockeye spawning channels, the condition of the intra-gravel environment was purported as an important consideration in the context of the IHNV outbreak. In particular, it was postulated that an IHNV outbreak during the out-migration of Sockeye Salmon fry from Fulton River Spawning Channel in 1984, was precipitated by high algal counts which occurred as a consequence of sub-optimal gravel cleaning (Appendix 1). Migrating fry were observed entangled in thick strands of *Ulothrix* and it was hypothesized that the presence of *Gomphonema* mats on the gravel reduced water exchange among eggs and alevins and further trapped swim-up fry. It's noteworthy that during the 2021 spring out-migration period at Pinkut Creek a higher abundance of algal mats were observed covering surfaces throughout the channels however, dead or dying fry were not observed entangled within the algae (Lindsay Thiessen, pers. comm.). Furthermore, it remains unknown as to whether intra-gravel water exchange in Pinkut Creek Spawning Channel was compromised as a result of the abundance of algae over the gravel substrate.

As noted previously, IHNV is endemic to western North American and infections of North Pacific Sockeye Salmon stocks occur naturally however, whether disease ensues in the infected hosts is dependent upon interactions of the host, virus, and environment. Host densities, water quality, and stress have been shown to be important factors in the susceptibility of fish to disease (Wedemeyer 1970, Wedemeyer and Ross 1973, Wood 1974). Knowledge concerning virus prevalence in Sockeye stocks and the factors contributing to outbreaks are necessary to mitigate IHN disease. In wild populations, the potential to

alter the trajectory of IHN disease is often limited due to an inability to modify the natural environmental conditions however, in spawning channel populations where conditions such as water flow, gravel size, and temperature can be optimized, there is the potential to lessen the occurrence and/or severity of IHN outbreaks. Based on previous IHN disease events in Sockeye Salmon spawning channel populations, it's postulated that the prevalence/intensity of IHNV in returning adults, the number of spawning adults loaded into the channels, and the spawning ground conditions (gravel, flows, water temperatures, algal presence) are all factors that contribute to an outbreak of IHN in a spawning channel setting. Consequently operational activities adhering to optimal adult loading densities, the flushing of adult carcasses, and ensuring adequate gravel cleaning should collectively mitigate the risk of IHN disease in Sockeye Salmon spawning channel populations. Further it is recommended that additional research be undertaken to assess the impacts of IHNV in the Babine Lake Spawning Channel systems and to better understand the factors driving the occurrences of IHN disease in these Sockeye Salmon populations.

REFERENCES

- Purcell, M.K., Thompson, R.L., Garver, K.A., Hawley, L.M., Batts, W.N., Sprague, L., Sampson, C., Winton, J.R. (2013) Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV). *Dis. Aquat. Organ.* 106:103-115 DOI: [10.3354/dao02644](https://doi.org/10.3354/dao02644).
- Traxler, G. S. and Rankin, J. B. 1989. An infectious hematopoietic necrosis epizootic in sockeye salmon *Oncorhynchus nerka* in Weaver Creek spawning channel, Fraser River system, B.C., Canada. *Dis. Aquat. Org.* 6: 221-226. DOI: [10.3354/dao006221](https://doi.org/10.3354/dao006221)
- Wedemeyer, G. A. (1970). The role of stress in the disease resistance of fishes. *In*: Symposium on diseases of fishes and shellfishes. *Am. Fish Soc. Snieszko, S. F., (Ed.) Spec. Publ.* 5:30-35
- Wedemeyer, G. A. and Ross, A. J. (1973). Nutritional factors in the biochemical pathology of corynebacterial kidney disease in the coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Bd Can.* 30: 296-298. DOI: [10.1139/f73-052](https://doi.org/10.1139/f73-052)
- Williams, I.V. and Amend, D.F. 1976. A natural epizootic of infectious hematopoietic necrosis in fry of sockeye salmon (*Oncorhynchus nerka*) at Chilko Lake, British Columbia. *J. Fish. Res. Bd. Can.* 33: 1564-1567. DOI: [10.1139/f76-196](https://doi.org/10.1139/f76-196)
- Wood, J.W. (1974) Diseases of Pacific salmon: their prevention and treatment. 2nd ed. Olympia, Washington: State of Washington, Department of Fisheries, Hatchery Division.

APPENDIX 1

Government of Canada / Gouvernement du Canada

File: *Research programs / 1000*

MEMORANDUM / NOTE DE SERVICE

TO: Cam West
A/Head, North Coast Unit
Enhancement Operations

FROM: Sam Bowman
Support Biologist
Enhancement Operations

SECURITY - CLASSIFICATION - DE SECURITE

OUR FILE/NOTRE REFERENCE

YOUR FILE/NOTRE REFERENCE

DATE: June 17, 1985

SUBJECT: BABINE SOCKEYE SPAWNING CHANNELS - ALGAE & IHN PROBLEMS
OBJET:

		Egg-to-Fry Survival Rates (%)			
Location		1982	1983	1984	1985
Fulton Channel 2	(1970-81) 45	27	27	7	61
Fulton Channel 1	(1967-81) 47	52	38	20	39
Pinkut Channel	(1969-81) 39	83	69	51	64

Algal Outbreaks:

- after 1968, when the Fulton River dam was constructed, green algal mats started to appear in the river (A. Lill, pers. comm.)
- at the top end of Channel 2, there has always been green algae growth (*Ulothrix* sp.; C. Harrison, pers. comm.)
- during February to June 1982, a serious algal outbreak of *Gomphonema* occurred at the peak of fry migration, establishing itself in the upper legs of Channel 2 and gradually covering the entire channel in one to two weeks time
- during February to June 1983, another outbreak of *Gomphonema* occurred in Channel 2, earlier and more rapidly than in 1982. *Gomphonema* was also observed in Channel 1 for the first time, although to a much lesser extent
- during February to June 1984, *Gomphonema* blooms occurred in both Channels 1 and 2 at Fulton, and also at Pinkut Channel for the first time; a *Ulothrix* bloom occurred at Channel 2 in conjunction with the *Gomphonema*, compounding the problem

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- the Gomphonema which appeared at Pinkut Channel during the 1984 fall program was physically removed in December after which it ceased to be a problem
- during February to June 1985, Gomphonema growth occurred later in Channel 2 than past years and did not appear to be a serious problem until early May, while algal growth in Channel 1 was minimal.

Potential Causes:

- 1) Reservoir level
 - 1982 - unusually low winter and spring levels
 - 1983 - " low " "
 - 1984 - normal spring levels
 - 1985 - higher than normal levels
- 2) Sunshine hours
 - 1982 - March/April 32% greater than 1973-81 mean
 - 1983 - " 33% greater " " "
 - 1984 - " 6% greater " " "
 - 1985 - " 13% greater " " "
- 3) Total gas pressure
 - no correlation found (K. Wilson, 1984).
- 4) Fertilizer input
 - from applications on the conifers planted on the berms
 - no correlation found (K. Wilson, 1984).
- 5) Nutrient levels
 - from decomposition of dead eggs and alevins, as well as, spawned-out adults
 - water quality analyses were conducted from November 1983 - March 1984 and from January - May 1985
 - Ortho-phosphate levels rarely exceeded the maximum recommended concentration of 10 ug/L dissolved P (M. Bothwell, pers. comm.)
 - nitrite, nitrate and ammonia levels were all within recommended criteria.
- 6) Invertebrate density
 - the importance of benthic invertebrate grazing rates on the growth of periphytic algae was studied
 - Experiment #1 was conducted from November 1983 - February 1984; Experiment #2 from March - May 1984
 - during the annual tunnel inspection at the Fulton facility, when Channel #2 is dried for gravel cleaning operations (in the summer after fry migration is complete), it was suggested that the intragravel invertebrate community was being disrupted reducing the natural biological control on periphyton growth

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- the results of Experiments #1 and #2 showed large differences in the number of invertebrate grazers between Channels 1 and 2 at Fulton River and smaller differences between legs within each channel.

Experiment #1		Channel 1 (#/m ²)		Channel 2 (#/m ²)
(Nov.83-Feb.84)	Leg 1	213,000	Leg 1	8,000
	3	193,000	4	46,000
	7	147,000	9	81,000
			15	71,000
		$\bar{x} = 84,000$		$\bar{x} = 51,000$

Experiment #2		Channel 1 (#/m ²)		Channel 2 (#/m ²)	Upper R. (#/m ²)	Lower R. (#/m ²)
(Mar.-May 84)	Leg 1	59,000	Leg 1	2,000		
	7	24,000	4	2,000		
			15	18,000		
		$\bar{x} = 41,000$		$\bar{x} = 7,000$	26,000	53,000

- these experiments were repeated during 1984/85 and results for the corresponding time periods were as follows:

Nov.-Feb.85	<u>Channel 1</u> (% of Exp.1 Levels)	<u>Channel 2</u> (% of Exp.1 Levels)	
Leg 1	35%	Leg 1	25%
3	30%	4	13%
7	27%	9	16%
		15	<u>45%</u>
	<u> </u>		
$\bar{x}$	= 31%	$\bar{x}$	= 26%

Mar.-Apr.85	Channel 1 (% of Exp.1)	Channel 2 (% of Exp.1)	Upper R. (%)	Lower R. (%)
Leg 1	20%	Leg 1	17%	
Leg 7	88%	Leg 4	32%	
		15	114%	
	$\bar{x} = 41\%$	$\bar{x} = 101\%$	$\bar{x} = 30\%$	$\bar{x} = 12\%$

- keeping Channel 2 wet during the summer of 1984 did not seem to have any affect on invertebrate population levels in winter; however, the number of invertebrates stabilized in Channel 2 during Mar./Apr.1985 at levels similar to those found during spring 1984 (Experiment #2), despite the fact that invertebrate levels in Channel 1 and the upper and lower river sites were markedly down (12-41%) from levels measured during Experiment #2.

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7) Periphyton (algal) growth

- the accumulation of algae (chlorophyll) on artificial (open-celled styrofoam) substrates was measured as part of Experiments # 1 and 2. In addition, alkaline phosphatase activity was measured to determine if phosphate (P) is the nutrient limiting algal growth in Channels 1 and 2. Preliminary conclusions were as follows:

- both channels showed signs of P-limitation
- Channel 2 appeared to be more nutrient limited than Channel 1 or Pinkut channel
- Leg 1 of Ch. 2 was the most nutrient limited (Exp.2)
- chlorophyll accumulation in Channel 2 was much lower ($\frac{1}{4}$) than the rate measured in Channel 1, despite visual estimates to the contrary
- chlorophyll accumulation in Pinkut channel was intermediate between Fulton Channels 1 and 2
- in Pinkut channel, chlorophyll levels increased in a downstream direction
- in Channel 2, chlorophyll levels decreased slightly in a downstream direction
- a relationship between invertebrate densities and periphyton growth rates was not apparent.

N.B. The effect of maintaining water flow in Channel 2 during cleaning operations in summer 1984 appeared to facilitate increased algal growth coincident with increased invertebrate numbers in the channel, negating the effect of a large invertebrate grazing population.

Problems associated with Algal Blooms:

1) IHN

- was first reported in the Fulton system in 1977 among the river, Channel 1 and 2 sockeye fry (G. Hoskins, pers.comm.). Diagnostic tests conducted on fry from the 1983 migration in Channel 2 showed some incidence of the virus
- external signs of IHN were found throughout the 1984 fry migration at the Fulton channels; in Channel 2 approx. 5-10% of the fry at the start of the migration, 1-2% in the middle, and 5% at the end showed positive IHN signs
- during the Channel 2 migration in 1984, indications were that the fry emerged 4-6 weeks earlier than usual. Downstream trapping results showed that 60-80% of the early migrants were alevins with only a very limited degree of yolk absorption
- high trap losses and susceptibility of the fry to handling stress indicated an acute outbreak of IHN during 1984. Furthermore, it was expected that heavily infected sockeye alevins and fry would in all likelihood die, some within the gravel (G. Hoskins, pers.comm.)

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- it was postulated that the early migration and low egg-to-fry survival experienced during 1984 may have been caused by the IHN infection and it was assumed that the IHN was precipitated by a deteriorating environment, i.e. the algal mat and poor intra-gravel conditions (G. Hoskins, pers. comm.)
- no IHN viral outbreaks have yet been reported at Pinkut Creek
- diagnostic testing for IHN for 1984 brood Fulton and Pinkut sockeye indicated high viral titers in adults collected (particularly for later run fish in Fulton River and Channel 2), but 0% incidence of IHN for eggs and sac fry collected during hydraulic sampling in November 1984 and February 1985.

2) Sub-optimal intragravel environment

- eggs and alevins may have been stressed within the substrate due to poor water exchange caused by the presence of Gomphonema mats (in 1982, 1983 and 1984) which may have resulted in low dissolved O₂ and high levels of metabolic wastes, especially at night when the algae respire
- the decaying eggs and alevins would have contributed an increasing level of organics to the channels which may have fueled the growth of the algae
- migrating fry during 1984 were observed entangled in the thick strands of Ulothrix in Channel 2 and it was further postulated that swim-up fry were becoming trapped in the gravel by the heavy mats of Gomphonema overlaying the substrate
- dissolved O₂ levels measured from March - May 1985 over 24-hr. periods showed adequate concentrations (12-13 p.p.m.) in the intragravel environment prior to and during the spring outmigration of sockeye fry.

Operational Solutions for Algal Blooms:

- an immediate solution to the problem of the Gomphonema mats which existed as impermeable barriers to swim-up fry during the 1983 and 1984 fry outmigrations was to operate an 8-wheeled all terrain vehicle (Argo) in the channels at Fulton and Pinkut to physically remove the algal mats
- gravel cleaning methods or "scarification" of the gravel was conducted differently during the summer of 1984 using two "hydraulic rakes" dragged side-by-side as opposed to a single machine which did not cover the entire width of the channel legs
- at Fulton, during winter and spring 1984/85, the channel and river discharges were manipulated up and down on a regular basis to reduce algal growth in the hope that fluctuating water level would help prevent algal colonization and the periodic higher flows would flush out the algae that had accumulated during the periods of normal channel flow (J. Smith/S. Barnetson, pers. comm.).

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- at Pinkut, where the channel is subject to icing, frazzle ice was allowed to form during cold snaps in winter 1984/85, warmer water would then be pumped into the channel from Babine Lake while concurrently increasing the discharge in the channel. The frazzle ice would in this way be flushed out of the channel, peeling the mats of algae off the gravel in the process
- further cleaning efforts were made at Fulton Channel 2 where the diffuser grates at the end of each leg were cleaned of Gomphonema and Ulothrix growth to prevent migrating sockeye fry from becoming entangled during the 1985 spring outmigration.

Recommended Strategies for Channel Operations:

- during the algae/IHN crisis of 1982-84, it was suggested that complete removal and cleaning of the gravel in Channel 2 was the best solution to counteract the low egg-to-fry survival rates being experienced at the time. This would be an extremely expensive option if undertaken
- during channel cleaning operations in 1985, the plan for Fulton and Pinkut channels is to conduct scarification of the gravel using the side-by-side technique as in 1984
- in addition, at Fulton Channel 2, the upper 2-4 legs will receive two or more passes with the scarifier and water quality parameters (OPO_4 , NO_2 , NO_3 , NH_3 , filterable and non-filterable residues) will be monitored downstream of these intensively cleaned legs to determine the effectiveness of this strategy
- the diffuser screens at Channel 2 will be thoroughly cleaned with a fire hose to remove organic debris which has accumulated since 1969
- the concrete berms which may act as a "seed" area for algal growth will also be cleaned with fire hoses
- during future fall operations, every attempt should be made to remove sockeye carcasses from the spawning channels
- whenever reservoir levels permit, channel discharges should be manipulated during the winter to help prevent algal colonization
- the factor over which we have the most control is the density at which we load sockeye adults into the Fulton and Pinkut spawning channels. From the following table it is apparent that higher loading densities in Channel 2 from 1981-83 did not result in corresponding higher fry production during 1982-84:

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Fulton Channel 2

<u>Brood Year</u>	<u>Total Spawning Adults</u>	<u>Eggs Deposited (x 10⁶)</u>	<u>Fry Production (x 10⁶)</u>
1969-80	92,000	162.6	71.3
1981	145,000	198.1	53.3
1982	116,000	201.6	54.0
1983	165,000	191.8	14.0
1984	110,000	163.2	99.9

- it is thus recommended that Channel 2 at Fulton River be loaded at a maximum acceptable level of 110,000 sockeye adults in order to realize maximum fry production
- it is also recommended that the investigation into the impact of IHN in the Babine system as suggested by Garth Traxler of PBS be continued.

SB

Sam Bowman

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