

AN INVESTIGATION OF *ICHTHYOPHONUS* INFECTION RELATED IN-RIVER
MORTALITY IN YUKON RIVER CHINOOK SALMON FROM MULTIPLE KNOWLEDGE
SYSTEMS: INDIGENOUS, LOCAL, AND WESTERN SCIENCE

by

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Abstract

The recent historic low salmon returns in the Yukon River contribute to an ongoing humanitarian crisis for subsistence users and Indigenous Peoples with cultural and well-being ties to salmon. After over a decade of relative calm, the marine parasite *Ichthyophonus* has undergone a resurgence and is once again infecting Yukon River Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo, hereafter referred to as king salmon) at high rates, with currently unknown consequences for survival of migrating adults. This thesis pairs multiple knowledge systems to assess the prevalence, severity, and impact of *Ichthyophonus* on adult Yukon River king salmon during their upriver spawning migration. Organized as two chapters, I conducted work that suggests highly infected king salmon may experience mortality before reaching spawning grounds due to *Ichthyophonus* and its interactions with other stressors. Infection severity significantly declined as fish migrated upriver, consistent with selective mortality of severely diseased fish. However, infection prevalence remained largely unchanged during migration, which we attribute to detection limits of testing coupled with infection progression. Through dialogues at community gatherings facilitated in two fishing-dependent Yukon River communities, I sought to co-interpret research findings alongside the Tribal Council and community members. Participants highlighted *Ichthyophonus* as only one of a myriad of co-occurring challenges faced by Yukon River king salmon and consistently voiced concerns that a focus on disease would be used to assign blame for salmon declines that they felt were more attributable to factors such as marine bycatch, and overfishing. Overall, the approach and findings contribute to an elevation of, and stronger inclusion of, Indigenous Knowledge, Tribal governance, local knowledge, and ecological stewardship within western fisheries science and management. While this thesis provides evidence that *Ichthyophonus* may indeed contribute to king salmon mortality in-river, it should not be used to diminish the voices of community dialogue participants who emphasized the importance of considering the impacts of industrial fisheries, broader environmental factors, as well as historical and cross-jurisdictional management practices when explaining the low salmon abundance and population declines. I dream this thesis will contribute to mending a proverbial collective fishing net to steward fisheries by uplifting Tribal sovereignty and governance, intentionally weaving together many ways of knowing to guide us toward more equitable, respectful, and restorative salmon management for future generations.

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Chapter 1: General Introduction

1.1 Positionality statement

Waqaa wiinga Nukalpiaq cali-llu Qemeryarpak. Hello, my name is Keith D. Herron. I am a Yup'ik biologist and scholar. My family comes from Bethel, Alaska; however, I grew up in the communities of Wrangell, Seward and Kenai, Alaska. I am an Orutsararmiut Native Council Tribal Member and Calista shareholder by descent. I completed my Bachelor of Science degree in Biological Sciences in December of 2020 at the University of Alaska Anchorage supported by the 'Alaska Native' Science and Engineering Program (ANSEP). During my undergraduate journey, ANSEP helped provide the opportunity for several internships related to working with natural and cultural resources in Alaska with the United States Fish and Wildlife Service (USFWS) and National Park Service. I continued my university education in August of 2021 as a graduate student pursuing a Master of Science degree in Fisheries at the University of Alaska Fairbanks (UAF) supported by the Tamamta program (NSF #2022190). Prior to starting graduate school, I began working for the USFWS in March of 2021 as a biologist, assistant subsistence fishery manager, and tribal liaison for fisheries on the Yukon River, providing the opportunity to merge my graduate school thesis and work-related research. With this opportunity, I embraced the journey of researching Yukon salmon health with relational accountability to the fish, the communities and Indigenous peoples of the Yukon River who continue to practice traditional stewardship to care for our salmon relatives.

1.2 Institutional permits and consultations

The collection of biological samples discussed as part of this thesis was approved by the University of Alaska Fairbanks IACUC, as well as collected under State of Alaska Department of Fish and Game (ADF&G) Aquatic Resource Permits. Consultations with the Tanana Tribal Council and Gwichyaa Zhee Tribal Government were conducted prior to the research. Both Tribal governments gave their support for the project. The University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) approval number is 2031286-2 (see 1.7 Appendix 1). The ADF&G Aquatic Resource Permit numbers are SF2022-145 (see 1.8 Appendix 2) and SF2023-041 (see 1.9 Appendix 3). The University of Fairbanks Institutional Review Board (IRB) approval number is 1146180-2. This research protocol was used for hosting community dialogues about this study.

1.3 Project background

In addition to addressing the impacts of *Ichthyophonus spp.* infection on Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo, hereafter referred to as king salmon) during the homeward spawning migration from a western science perspective, this graduate thesis uplifts Indigenous Knowledge (IK), Tribal governance, and Tribal sovereignty, as well as local knowledge (LK). This research takes place in the Yukon River drainage, which is the ancestral homelands to Indigenous Peoples, who have stewarded and developed cultural and spiritual relationships to salmon for millennia (Black, 2017; Carothers et al., 2021). Salmon species have also become vital to settlers in the Yukon River watershed (Brown & Godduhn, 2015). Throughout the research process, I aimed to co-interpret my graduate research outcomes utilizing methodologies which respect and integrate Indigenous ways of knowing, as well as building equitable partnerships between communities and myself as a researcher. My intent was to integrate multiple knowledge systems together, not only to contribute to scientific understanding of the impact the parasitic pathogen *Ichthyophonus spp.* (hereafter referred to as *Ichthyophonus*) has on Yukon River king salmon during upriver migration, but also strengthen community engagement, foster trust, and empower the communities. In the research process, I worked alongside communities with the help of community members to share their knowledge and be a part of the research process that helps gather information to inform management of fisheries (Castleden et al., 2012; Wallerstein & Duran, 2010; Wilson et al., 2018).

Through a western science lens, this thesis explores a disease of interest in Yukon River fish – ichthyophoniasis. I, as part of a collaborative team, explored evidence that suggested *Ichthyophonus* could be a significant source of in-river mortality during spawning migration for Yukon River king salmon. From an IK lens, this research explored the impact of *Ichthyophonus* on fish health more generally in collaboration with two Yukon River Tribal Governments – Rampart Village Council and Gwichyaa Zhee Tribal Government, and by directly working alongside Indigenous and non-Indigenous fishermen from the communities of Tanana and Fort Yukon. Additionally, we explored LK by highlighting a local subsistence fisherman who has monitored *Ichthyophonus* infection within his harvest for two decades.

Ichthyophonus is a parasitic pathogen of fish (McVicar, 1999). Individuals infected with *Ichthyophonus* remain so for the duration of their lives, though the effects of the pathogen on each host can vary widely, with noted variation in species and stock susceptibility (Hershberger,

2012). Subclinical infections can also occur, meaning the pathogen can be present at low infectious levels considered to have negligible impacts on overall salmon health. These low-level infections may be missed by visually seeing common signs of disease related to *Ichthyophonus* infection within host tissue (Zuray et al., 2012; Kocan et al., 2003, 2004). However, disease severity in king salmon is known to increase with stress and deteriorating body condition that occurs during migration to their natal spawning grounds (Kocan & Hershberger, 2006), and once the disease becomes more severe, visual detection of common signs of disease related to *Ichthyophonus* infection is considered reliable to assess infection prevalence (Zuray et al., 2012).

People from the Yukon River are able to both detect *Ichthyophonus* visually in king salmon and have been reporting disease trends across the river for many years. Infected fish may show visual signs of disease such as lesions in the form of white spots on the heart and elsewhere in the organs and meat. Subsistence harvesters also report that the fillets from infected fish can also have an unusual “sweet” or “tangy” smell and do not dry well, which can impact common fish preservation practices. Such characteristics can lead to a considerable loss of subsistence harvest, as well as compensatory harvest of additional fish to replace the inedible fish (Kocan et al., 2003, 2004; Kahler et al., 2011). *Ichthyophonus* primarily infects cardiac muscle and spreads to skeletal muscle in more severe cases, reducing swimming performance and stamina, which may lead to mortality of king salmon during upriver spawning migration (Kocan et al., 2003, 2004; Kocan & Hershberger, 2006). A host inflammatory response against the parasite contributes to the grossly visible white spots seen in clinical disease. Microscopically, these spots are composed of a granulomatous host response, where immune cell types surround the multinucleated spherical parasitic ‘schizonts’, the resting stage of the parasite. These schizonts can appear in all organs of the fish, such as the spleen, liver, kidney, skeletal muscle, and, most prominently, the heart (Kocan et al., 2003, 2004; Kahler et al., 2007). Of course, not all king salmon are infected with *Ichthyophonus*, and not all infected fish will progress to clinical disease, defined as having laboratory confirmation of infection as well as the paired visual signs of disease (Zuray et al., 2012). Subclinical infections can be without host response; however, presence of the parasites in host tissue can still allow for laboratory confirmation of infection.

The Indigenous stewardship and caretaking of salmon that has characterized salmon management along the Yukon River since time immemorial, is now complicated by additional state, national, and international management. The Yukon River salmon harvest regulations are

determined by estimates of how many individual fish enter the river near the mouth at the Pilot Station Sonar site operated by the Alaska Department of Fish and Game (ADF&G) and the number of salmon by species predicted to reach their spawning grounds. In 2019, a major discrepancy was noticed by fishery management agencies between sonar sites run by ADF&G, where the number of Canadian-origin king salmon within the Yukon River were estimated at a lower-river assessment site at Pilot Station Sonar (km 195) and at an upper-river site at Eagle Sonar (km 1950). The estimated count from the lower-river site provides fishery management agencies an estimated abundance of each salmon species entering the Yukon River. Management agencies then use these abundance estimates to determine fishing opportunity based on estimated harvest surplus above estimated stock escapement.

From 2019 to 2023, considerably fewer Canadian-origin king salmon passed the Eagle sonar up-river site compared to expected numbers based on the Pilot Station Sonar lower-river site, suggestive of mortality of migrant kings after passing the sonar (JTC, 2025). Beginning in 2019, large discrepancies between estimates of Canadian-origin king salmon at the lower-river assessment site at Pilot Station Sonar and upper-river site at Eagle Sonar occurred consistently each year. Understanding these discrepancies is a top priority for fishery management agencies, since these discrepancies hamper the ability of management bodies to use lower-river assessments to equitably manage Yukon River king salmon within the Yukon River drainage, to determine if harvestable surplus is available, as well as achieve escapement goals set by the Pacific Salmon Treaty (JTC, 2025). Some inconsistencies from in-river sonar estimates are expected, since individual species abundances are estimated by apportioning the total number of sonar hits among individual species (and among areas of origin where applicable) based on the observed proportions harvested (and genetically assigned to areas of origin) in a test fishery (Lozori & Dreese, 2019). Based on trend estimates starting in 2005 through 2018, no major trends of overestimating or underestimating the Canadian-origin king salmon escapement had occurred across multiple seasons between the sonar sites prior to 2019. However, the predicted number of Canadian-origin king salmon at Pilot Station Sonar overestimated the actual Canadian escapement by a significant margin for five consecutive years, 2019-2023 (Figure 1.1). During this time, the actual Eagle Sonar count plus estimated U.S. harvest of Canadian-origin king salmon was 33-52% smaller than expected based on the point estimates (F. West, ADF&G, personal communication, March 26, 2025).

If en route mortality between the lower- and upper-river sonar sites is a source for the discrepancies in population estimates, it is important to identify the sources of mortality and the degree of impact to accurately quantify the number of homeward migrating salmon. One potential source of mortality during upriver migration could be due to *Ichthyophonus*, known to be contracted by king salmon during their marine life stage (McVicar, 1999). This parasitic pathogen has been historically monitored in the middle Yukon River at the Rapids in between the communities of Tanana and Rampart by Stanley Zuray, a local subsistence fisherman, in most years during the last two decades (2004-2013, 2018 and 2020-2021), with Mr. Zuray reporting high levels of *Ichthyophonus* prevalence in 2020 (Figure 3.1). At this time, Mr. Zuray advocated that fishery management agencies take the reemergence of *Ichthyophonus* seriously and consider the impacts on the king salmon spawning population (Zuray, 2020). During this time, the Eagle Sonar upper-river estimate for the number of Canadian-origin king salmon that made it to the U.S./Canada border were much lower than expected based on the Pilot Station Sonar lower-river estimate for the number of Canadian-origin king salmon which entered the Yukon River. The estimated disparity and, at the same time, high *Ichthyophonus* prevalence occurred over four consecutive years (2020-2023), suggesting apparent mortality during upriver migration could be linked to *Ichthyophonus* infection.

To accurately assess en route mortality of salmon moving forward, it is essential to consider all the potential sources of mortality and their interactions. In 2019, fishery management agencies provided liberal subsistence salmon fishing opportunity on king salmon based on the lower river estimate which showed escapement goals would be exceeded. This high lower river estimate could have misled fishery management agencies to believe the Canadian-origin king salmon run was more numerous than expected or unknown en route mortality could have occurred between the sonar projects. Stanley Zuray did not report any concerning amount of *Ichthyophonus* within his subsistence harvest at the Rapids in 2019; however, he did not quantify the percent of *Ichthyophonus* within his harvest as in previous years. The leading hypothesis to explain the difference between estimates in 2019 and other years was that king salmon may have experienced extreme heat stress, another potential factor that could cause en route mortality of Yukon River salmon independent of *Ichthyophonus* infection (Westley, 2020; von Biela et al., 2020, 2022). Alternatively, there is no evidence to exclude the possibility there was a high prevalence of *Ichthyophonus* in 2019 and environmental conditions such as high-

water temperature could have caused the *Ichthyophonus* disease within infected king salmon to progress to a lethal level much sooner than in years with lower or normal water temperatures (Kocan et al., 2009). Meaning the point at which large-scale en route mortality occurred shifted downstream due to elevated in-river temperatures and the majority of clinically diseased fish may have perished before monitoring occurred in the middle Yukon River at the Rapids. And of course, these effects are not mutually exclusive and can be occurring concomitantly.

Annual monitoring and working in collaboration with Tribal Governments and local communities for the potential sources that can cause en route mortality of salmon can help provide a more complete assessment of the fitness of migrating Yukon River king salmon. Historical monitoring for *Ichthyophonus* at the Rapids led by Stanley Zuray shows how *Ichthyophonus* prevalence varies annually within harvest at his fish camp from 2004-2013, 2018, and 2020-2023 (Figure 3.1). This continued monitoring across multiple years began to suggest that a high prevalence of *Ichthyophonus* coincided with years where there was a large discrepancy between the Pilot Station and Eagle Sonar estimates of Canadian-origin king salmon. In 2020, Mr. Zuray opportunistically sent 36 samples to independent researcher Dr. Richard Kocan, who has a lifetime of experience studying *Ichthyophonus*, to confirm the presence of *Ichthyophonus* by histopathology laboratory procedures. Of the 36 samples, 16 were confirmed positive by histopathology (44.4%), and 17 (47.2%) exhibited visual signs of infection. This information prompted fishery management agencies to consider *Ichthyophonus* as a significant source of en route mortality within Yukon River king salmon, something previously hypothesized as well (Kocan et al., 2003, 2004).

In 2021, ADF&G sampled 200 king salmon for *Ichthyophonus* using the diagnostic tool explant tissue culture, with findings which indicated 44% of randomly sampled fish were infected with *Ichthyophonus* at Pilot Station Sonar. The U.S Fish and Wildlife Service (USFWS) in partnership with Stanley Zuray at the Rapids sampled 62 king salmon from incidental harvest in a 10.16 cm (4 inch) mesh set gillnet. Of the 62 samples, 24 were confirmed positive by histopathology (38.7%). A total of 56 samples of the 62 samples were examined for the presence or absence of visual lesions, and 17 out of these 56 (30.4%) had visual signs of infection consistent in appearance with *Ichthyophonus* infection. The 2021 Rapids samples were largely dominated by males; no sex was identified on 11 (17.7%) fish sampled, 8 (12.9%) were identified as female, and 43 (69.4%) were identified as male. This high prevalence of

Ichthyophonus infection again coincided with a higher Pilot Station Sonar estimate of Canadian-origin king salmon compared to the actual passage of Canadian-origin king salmon at Eagle Sonar. The prevalence of *Ichthyophonus* infection in 2020 and 2021 was well above the threshold of 25% set by the Yukon River Panel Joint Technical Committee (JTC) to monitor and research the impacts of *Ichthyophonus* within the Yukon River drainage (JTC, 2022). However, the only continuous historical monitoring and reporting was done by Stanley Zuray at his Rapids Research Center by monitoring his subsistence harvest for any type of baseline assessment of the disease overtime, revealing an urgent need for a coordinated cross-watershed effort to quantify the occurrence and potential impact of *Ichthyophonus* infection.

To evaluate the extent to which ichthyophoniasis causes en route mortality in Yukon River king salmon, this research project partners with Tribal Governments, the State of Alaska, and the US Federal Government, alongside Indigenous and local fishermen to collect samples to characterize the prevalence and severity of *Ichthyophonus* in Yukon River king salmon at the Rapids and Fort Yukon (km 1612), as well as co-interpret research outcomes utilizing multiple perspectives. Concurrently, ADF&G utilized their established Pilot Station and Eagle Sonar sites to collect samples allowing for comparison across four sites within the Alaska portion of the Yukon River. This project aimed to accurately characterize the prevalence and severity of *Ichthyophonus* within Yukon River king salmon at four locations: Pilot Station, the Rapids, Fort Yukon, and Eagle Sonar (Figure 2.2). An important factor to recognize is that king salmon entering the Yukon River spawn at a variety of destinations, including major tributaries such as Koyukuk, Tanana, and others (Brown et al., 2017). However, Canadian-origin king salmon make up 42% of the entire Yukon drainage total king salmon stock annually (JTC, 2025). These fish migrate through the entire U.S. portion of the Yukon River and continue into Canada before spawning further upstream. These various stocks can be identified through genetic stock identification, and the Canadian-origin stock is of particular interest to this study given the long migration distance, which creates the unfortunate scenario for large-scale en route mortality due to ichthyophoniasis. The likelihood for this scenario is based on known length of time for Canadian-origin king salmon migration surpassing the known length of time for *Ichthyophonus* infection to mature to lethal disease levels (Kocan & Hershberger, 2006; Kocan et al., 2009), suggesting en route mortality due to *Ichthyophonus* is most likely to occur in upper reaches of the Yukon River (Kocan et al., 2004). Indigenous and local knowledge in the upper Yukon River

has suggested low clinical *Ichthyophonus* prevalence in subsistence harvest, relative to local reports of high clinical *Ichthyophonus* prevalence in the Rapids subsistence harvest, which strongly implicates mortality between the middle- and upper-river sites.

The overall objectives for this thesis aimed to be solution oriented by: (1) conducting a research study to investigate the impacts of *Ichthyophonus* on Yukon River king salmon during upriver spawning migration, (2) co-interpreting research findings with Tribal Government councils and community members at early stages of the process to explore the health of Yukon River king salmon based on multiple knowledge systems (Indigenous, local, and western), and (3) co-develop research recommendations moving forward for a more complete assessment for the health of king salmon in the Yukon River. The overall information gained through this graduate research process should ultimately be helpful to better inform equitable fishery management decisions focused on restoring salmon runs and health.

1.4 Authorship and funding

This thesis research is a compilation of two chapters intended as stand-alone peer-reviewed publications, not including the general introduction (Chapter 1) and general conclusion (Chapter 4). Chapter 2 and 3, where I serve as the lead author, has several co-authors who collaborated on this writing, including Dr. Peter Westley, Dr. Jessica Black, Dr. Morag Clinton, Dr. Courtney Carothers, and Dr. Scott T. Walter. All funding for the research itself was provided by the United States Fish and Wildlife Service, and the Alaska Department of Fish and Game, including Congressionally Directed Stewardship Funds secured by Senator Murkowski and the Alaska Sustainable Salmon Fund. The Wakefield Chair of Fisheries and Ocean Sciences provided funding support on unexpected lab assistance. Stipend and educational costs were supported by the NSF Tamamta Program (#2022190). Travel funding for community visits was provided by the USFWS for myself and Scott T. Walter. Travel funding for other co-authors was provided by Tamamta and the Wakefield Chair of Fisheries and Ocean Sciences, and items such as participatory meeting gifts for community meeting attendees were sponsored by the Tamamta Program.

A complete list of all co-authors and their affiliations are as follows: Keith D. Herron, UAF, USFWS; Peter Westley, UAF; Jessica Black, UAF; Morag Clinton, Sitka Sound Science Center, UAF; Courtney Carothers, UAF, and Scott T. Walter, USFWS.

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1.6 Figures

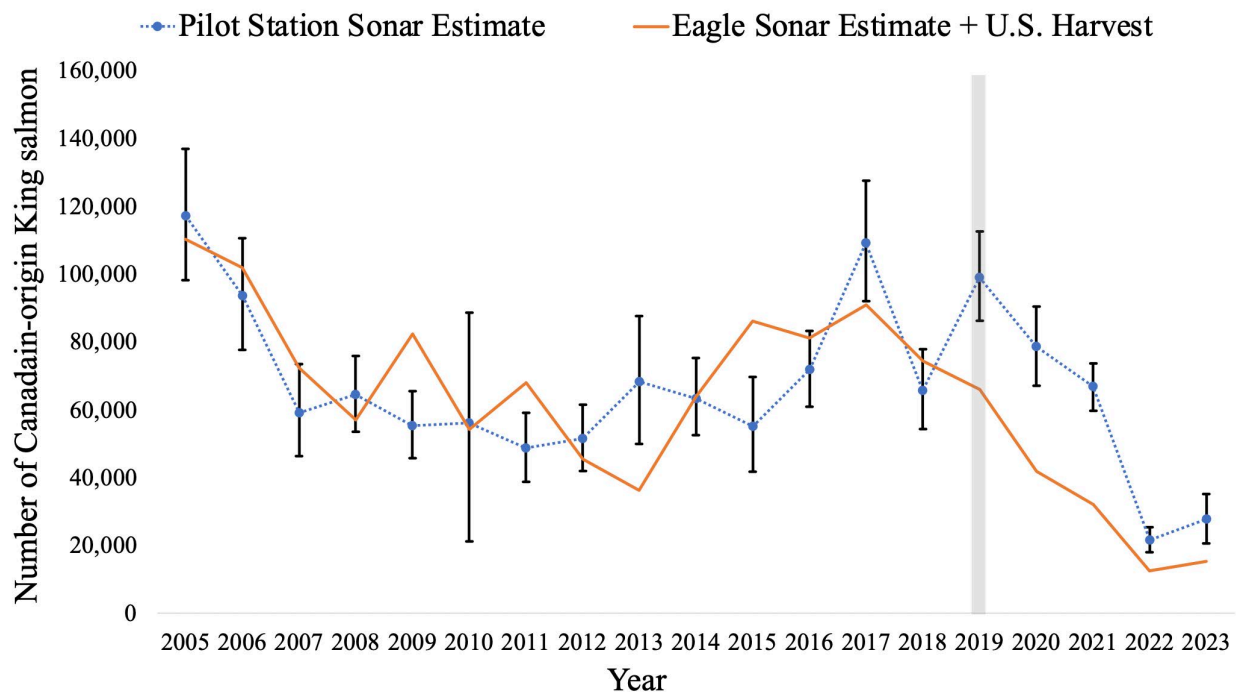


Figure 1.1. Comparison between the number of Canadian-origin king salmon estimated at Pilot Station Sonar and the number estimated at Eagle Sonar plus the estimated U.S. harvest of the Canadian-origin stock between Pilot Station and Eagle Sonar, 2005-2023 (F. West, ADF&G, personal communication, March 26, 2025). Vertical line shows 2019 when large discrepancies between Pilot and Eagle became apparent, suggesting in-river mortality of migrating adult king salmon between Pilot and Eagle.

1.7 Appendix 1. IACUC approval letter



(907) 474-7800
(907) 474-5993 fax
uaf-iacuc@alaska.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

June 2, 2023

To: Peter Westley
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [2031286-2] An investigation of Ichthyophonus sp. in Yukon River Chinook salmon
(Kiagtaq, Taryaqvak, Ggaal, Gath, Luk Choo, Oncorhynchus tshawytscha) from multiple
perspectives (western, local, and Indigenous)

The IACUC reviewed and approved the New Project referenced above by Designated Member Review.

Received:	May 12, 2023
Approval Date:	May 26, 2023
Initial Approval Date:	May 26, 2023
Expiration Date:	May 26, 2024

This action is included on the June 8, 2023 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

1.8 Appendix 2: 2022 ADF&G aquatic resource permit



**STATE OF ALASKA
DEPARTMENT OF FISH AND GAME**
PO Box 115526
JUNEAU, ALASKA 99811-5526

Permit No. **SF2022-145**

Expires: **12/31/2022**

AQUATIC RESOURCE PERMIT
(For Scientific/Collection Purposes)

This permit authorizes:

Fred West

(whose signature is required on page 2 for permit validation)

Of

Alaska Department of Fish and Game, Division of Commercial Fisheries
333 Raspberry Road, Anchorage, AK 99518
(907) 267-2237 fred.west@alaska.gov

to conduct the following activities from **May 15, 2022** to **December 31, 2022** in accordance with AS 16.05.930, AS 16.05.340(b), and 5 AAC 41.

Purpose: To authorize participation of non-departmental personnel in a collaborative Yukon River Chinook salmon *Ichthyophonus* disease study, coordinated by ADF&G-DCF staff, as outlined in ADF&G Cooperative Agreement COOP #22-064.

Location: Yukon River (AWC #334-10-11000 through #334-45-11000) drainage, including (but not limited to) sampling operations at Pilot Station, Rampart Rapids, Fort Yukon, and Eagle.

Species: Chinook salmon

Method of Capture: Personnel may use department approved methods for capturing of fish.

Final Disposition: Personnel may use department approved methods for sampling fish. Numbers of fish sampled should be in line with the basic objectives of agency programs.
Pathogen tissues collected under this permit will be sent to the ADF&G Fish Pathology Laboratory in Anchorage or the University of Washington (Dr. Kocan's Lab) for processing.
Genetic tissues collected under this permit will be sent to and archived at the ADF&G Gene Conservation Lab.
Mortalities that occur under this permit (including unintentional and non-target species) may be donated to local communities and included in ADF&G-DCF subsistence harvest estimates.

COLLECTION REPORT DUE January 31, 2023: see **Stipulation #2** for more information. Data from such reports are considered public information. Reports must be submitted by email (dfg.dsf.permitcoordinator@alaska.gov) or by mail to: Alaska Department of Fish and Game, Division of Sport Fish-HQ, PO Box 115526, Juneau, AK 99811-5526, attention: Permit Coordinator. A report is required whether or not collecting activities were undertaken.

GENERAL CONDITIONS, EXCEPTIONS, AND RESTRICTIONS

1. This permit must be carried by person(s) specified during approved activities who shall show it on request to persons authorized to enforce Alaska's fish and game laws. This permit is nontransferable and will be revoked or renewal denied by the Commissioner of Fish and Game if the permittee violates any of its conditions, exceptions, or restrictions. No redelegation of authority may be allowed under this permit unless specifically noted.
2. No specimens taken under authority hereof may be sold, bartered, or consumed. All specimens must be deposited in a public museum or a public scientific or educational institution unless otherwise stated herein. Subpermittees shall not retain possession of live animals or other specimens.
3. The permittee shall keep records of all activities conducted under authority of this permit, available for inspection at all reasonable hours upon request of any authorized state enforcement officer.
4. Permits will not be renewed until detailed reports, as specified in the Stipulations section, have been received by the department.
5. **UNLESS SPECIFICALLY STATED HEREIN**, this permit does not authorize the exportation of specimens or the taking of specimens outside of existing regulations.

Permit Coordinator
Division of Sport Fish

R.T. Taulhe for

Director
Division of Sport Fish

5-9-2022

Date

1.9 Appendix 3: 2023 ADF&G aquatic resource permit



STATE OF ALASKA
DEPARTMENT OF FISH AND GAME
PO Box 115526
JUNEAU, ALASKA 99811-5526

Permit No. **SF2023-041**

Expires: **12/31/2023**

AQUATIC RESOURCE PERMIT
(For Scientific/Collection Purposes)

This permit authorizes:

Fred West

(whose signature is required on page 2 for permit validation)

Of

Alaska Department of Fish and Game, Division of Commercial Fisheries
333 Raspberry Road, Anchorage, AK 99518
(907) 267-2237 fred.west@alaska.gov

to conduct the following activities from **May 15, 2023** to **December 31, 2023** in accordance with AS 16.05.930, AS 16.05.340(b), and 5 AAC 41.

Purpose: To authorize participation of non-departmental personnel in a collaborative Yukon River Chinook salmon *Ichthyophonus* disease study, coordinated by ADF&G-DCF staff, as outlined in ADF&G Cooperative Agreement COOP #22-064.

Location: Yukon River (AWC #334-10-11000 through #334-45-11000) drainage, including (but not limited to) sampling operations at Pilot Station, Rampart Rapids, Fort Yukon, and Eagle.

Species: Chinook salmon

Method of Capture: Personnel may use department approved methods for capturing fish.

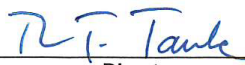
Final Disposition: Personnel may use department approved methods for sampling fish. Numbers of fish sampled should be in line with the basic objectives of agency programs.
Pathogen tissues collected under this permit will be sent to the ADF&G Fish Pathology Laboratory in Anchorage or the University of Washington (Dr. Kocan's Lab) for processing.
Genetic tissues collected under this permit will be sent to and archived at the ADF&G Gene Conservation Lab.
Mortalities that occur under this permit (including unintentional and non-target species) may be donated to local communities and included in ADF&G-DCF subsistence harvest estimates.

COLLECTION REPORT DUE January 31, 2024; see **Stipulation #2** for more information. Data from such reports are considered public information. Reports must be submitted by email (dfg.dsf.permitcoordinator@alaska.gov) or by mail to: Alaska Department of Fish and Game, Division of Sport Fish-HQ, PO Box 115526, Juneau, AK 99811-5526, attention: Permit Coordinator. A report is required whether or not collecting activities were undertaken.

GENERAL CONDITIONS, EXCEPTIONS, AND RESTRICTIONS

1. This permit must be carried by person(s) specified during approved activities who shall show it on request to persons authorized to enforce Alaska's fish and game laws. This permit is nontransferable and will be revoked or renewal denied by the Commissioner of Fish and Game if the permittee violates any of its conditions, exceptions, or restrictions. No redelegation of authority may be allowed under this permit unless specifically noted.
2. No specimens taken under authority hereof may be sold, bartered, or consumed. All specimens must be deposited in a public museum or a public scientific or educational institution unless otherwise stated herein. Subpermittees shall not retain possession of live animals or other specimens.
3. The permittee shall keep records of all activities conducted under authority of this permit, available for inspection at all reasonable hours upon request of any authorized state enforcement officer.
4. Permits will not be renewed until detailed reports, as specified in the Stipulations section, have been received by the department.
5. **UNLESS SPECIFICALLY STATED HEREIN**, this permit does not authorize the exportation of specimens or the taking of specimens outside of existing regulations.

Permit Coordinator
Division of Sport Fish



Director
Division of Sport Fish

2-2-2023

Date

Chapter 2: Associations between ichthyophoniasis disease progression and en route mortality of upstream migrating adult Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo) in the Yukon River, Alaska

2.1 Abstract

Ichthyophoniasis, caused by the parasitic pathogen *Ichthyophonus* spp., is a potential factor contributing to en route mortality during spawning migrations in Yukon River Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo), yet the extent to which it may be impacting salmon fitness is unknown. Here we investigate the prevalence and severity of *Ichthyophonus* infection in upstream migrating adult king salmon in the longest freshwater salmon migration on Earth (3,200 river kilometers [rkm]). We sampled 878 individuals at four sites across 1000 rkm along the Yukon River mainstem in Alaska during the 2022 and 2023 migration seasons and conducted histopathological analyses to assess disease progression. Consistent with the hypothesis of en route mortality of the most severely infected and diseased individuals, we observed that infection severity significantly declined as fish migrated upriver. In contrast, the prevalence of infected individuals remained largely unchanged throughout the migration, which we attribute to the zero-sum balance of losing sick individuals with the gaining of individuals that were below testing thresholds lower in the river due to disease progression. Statistical modeling estimated infection severity declined by 98% from lower- to upper-river sites in 2022 and by 82% in 2023, consistent with the hypothesis of selective mortality of severely diseased fish as they migrated upstream. These findings strongly suggest *Ichthyophonus* as a clear and present factor linked to en route mortality of Yukon River king salmon. Future research is needed to develop and integrate non-lethal diagnostic methods and to pair with telemetry to measure en route mortality while considering cumulative impacts of other known stressors. Understanding the impacts of *Ichthyophonus* on migration success is essential and urgently needed for effective fishery management and plans for salmon population recovery from a severely depleted state.

2.2 Introduction

The lives of Pacific salmon and the people of the Yukon River have been entwined for at least 11,500 years (Halffman et al., 2015). Beyond this archaeological evidence from a western

science perspective, we know that since time immemorial the Indigenous peoples of the Yukon River have lived in reciprocal relationship with salmon, rooted in cultural identity, shaping deep spiritual, social, physical, emotional, and well-being ties to salmon (Carothers et al., 2021). A violent history of colonialism uprooted traditional salmon governance; under western fisheries management, the salmon fishery of the Yukon River has been in a state of chronic duress. Since 1981 when accurate stock and age estimates began being systematically collected, the Yukon River Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo) population has steadily declined and recently collapsed to historically low returns, prompting complete Chinook salmon fishery closures since 2021 (Figure 2.1, JTC, 2025). Chinook salmon (hereafter referred to as king salmon to honor the name used along the river), have original Indigenous names including Taryaqvak in Yup'ik, Ggaal in Denaakk'e/Koyukon Athabascan, Gath in Benhti Kenaga/Lower Tanana, and Łuk Choo in Gwich'in (SASAP, 2025b). Fishery closures cause severe hardship to Yukon subsistence users not only due to the loss of food security, but loss of traditional and spiritual practices central to Indigenous people's well-being and health (Donkersloot et al., 2020; Moncrieff, 2017; Peltola, Woods, and Bahnke, 2021). Salmon are a substantial part of Yukon subsistence diets in the region and account for around 40% of the wild resources harvested by Yukon subsistence communities when salmon harvest occurs (SASAP, 2025a). The Yukon River salmon have also overtime become important to settlers of the area. Salmon of many species have also provided economic value to people living along the river in the form of commercial fisheries throughout the drainage (Brown & Godduhn, 2015). Given the supreme importance of salmon to the people of the Yukon River, substantial efforts have gone into understanding the root causes of these declines.

In no particular order, there are many suspected factors associated with the decline of Yukon king salmon including, but not limited to, marine bycatch (Ianelli & Stram, 2015), stock overharvest in a mixed-stock fishery (Connors et al., 2022), size selective overharvest and associated declines in size and age of spawners (Ohlberger et al., 2023; Oke et al., 2020), marine environmental changes impacting prey quality (Farley et al., 2024), competition in the marine environment with hatchery fish and other competitors (Cunningham et al., 2018; Feddern et al., 2024), the rise of marine mammal and shark predators (Ohlberger et al., 2019; Seitz et al., 2019), heat stress during upriver spawning migration (von Biela et al., 2020, 2022), and overarching climate change (Schoen et al., 2017; Crozier et al., 2021). To be clear, evidence does not support

subsistence harvest as causing the decline of Yukon king salmon as the estimated subsistence harvest over time remained relatively consistent prior to restrictions and necessary closures from other causes.

The Yukon River drainage king salmon amount necessary for subsistence based on historical subsistence harvest is 45,500 - 66,704 fish (Padilla & Hamazaki, 2024). From 1981 through 1997, 12% of the yearly runs on average were harvested by subsistence users (JTC, 2025). Before salmon runs were systematically tracked, the historic Yukon River salmon runs supported traditional and customary use (subsistence) for countless generations continuously. However, as the population declined due to other causes, the exploitation rate of the fishery increases with any form of harvest. In the 1980s, the Yukon River saw an increase of commercially harvested king salmon, and on average nearly 30% of the yearly runs were taken commercially from 1981 through 1997 (JTC, 2025). In 1998, the abundance of Yukon River king salmon saw its first major decline with another major decline in 2007, followed by historically low returns since 2021 (Figure 2.1). As the Yukon king salmon fishery declined, the commercial fishery was first restricted and then ultimately closed with no legal sale during the king salmon season since 2010 (JTC, 2025). Similarly, subsistence fishery restrictions began in the early 2000's and the harvest has varied annually depending on the run size and harvest restrictions. Complete fishery closures started in 2021 due to historically low king salmon returns. The amount necessary for subsistence for Yukon king salmon has only been reached once between 2008 and 2024 (JTC, 2025). For many residents of the Yukon River, commercial fishing provided the means to sustain customary and traditional ways of life, as they would use the money received from commercial fishing to purchase gear to support subsistence harvest (Moncrieff, 2017).

While changes in freshwater conditions are associated with declines in salmon abundance, productivity (Howard & von Biela, 2023) and juvenile survival (Miller & Weiss, 2023), changes in marine conditions are also connected to salmon survival prior to spawning (Cunningham et al., 2018; Feddern et al., 2024). King salmon spend most of their life at sea where they gain more than 99% of their final weight (Quinn, 2018). It is here, and most likely through their diet, where king salmon of the Yukon River are exposed to another potential driver limiting their recovery: the disease-causing pathogen *Ichthyophonus* (Kocan et al., 2009). *Ichthyophonus* is a parasitic pathogen (McVicar, 1999) that can infect salmon and cause disease.

Fish with established *Ichthyophonus* infection remain so for the duration of their lives, though the effects of the pathogen on individuals can vary widely (Hershberger, 2012). The drivers of this variation in clinical consequences, and the exact source of *Ichthyophonus* for Yukon River king salmon, is yet to be fully explored. It is thought transmission of *Ichthyophonus* occurs in the ocean through the ingestion of infected prey (Jones & Dawe, 2002; Kocan et al., 2004), or through a waterborne free-floating stage of the pathogen (Kocan, 2018). However, infection severity in king salmon is thought to increase with stress and deteriorating body condition that occurs during migration to their spawning grounds (Kocan & Hershberger, 2006; Kocan et al., 2009). Despite marine infection, the clinical impacts of *Ichthyophonus* appear to be most severe for king salmon during their spawning migrations.

Ichthyophonus primarily infects cardiac muscle and in more severe cases can be observed in other tissue types, such as skeletal muscle, reducing swimming performance and stamina of infected salmonids (Kocan et al., 2003, 2004, 2006, 2011). A strong inflammatory response against the parasite often results in grossly visible lesions, which microscopically appear as multinucleated spherical structures, the resting stage of the parasite commonly referred to as schizonts. The inflammation caused by the schizonts can appear in all organs of the fish, such as the spleen, liver, kidney, skeletal muscle (fillets), and more commonly, the heart (Kocan et al., 2003, 2004; Kahler et al., 2007). It was through the gross visual signs of infection that Yukon River fishermen began to sound the alarm regarding the health of returning king salmon (Zuray et al., 2012; Zuray, 2020).

Although *Ichthyophonus* has likely been present at some level throughout history, the disease has been documented in the Yukon River sporadically for nearly 40 years. In 1986, Bill Fliris, a fisherman from the community of Tanana (rkm 1118), recalled one of his harvested king salmon did not dry properly and had lesions (Zuray, 2020; S. Zuray, personal communication, July 26, 2022), and at the time did not recognize the incident to be related to *Ichthyophonus*. The following year in 1987, he reported several more harvested king salmon had visual signs of infection, and his report aligns with other confirmed *Ichthyophonus* cases within the Yukon River. In 1988, an Alaska state pathologist first confirmed *Ichthyophonus* within a Yukon River king salmon from the Koyukuk drainage, a tributary of the Yukon River (Anchorage Fish Pathology Laboratory disease history database, June 1988; Kahler et al., 2011). Fishers from the Yukon River have been able to both detect suspected *Ichthyophonus* in king salmon and see

disease trends across years and locations. *Ichthyophonus* has been monitored at the middle Yukon River Rapids site (rkm 1175) by Stanley Zuray, a naturalist and subsistence fisherman, in most years during the last two decades (2004-2013, 2018 and 2020-2021). He documented the number of king salmon that exhibited visual signs of infection, such as white lesions in the form of spots on king hearts, typical of *Ichthyophonus* infection (Zuray et al., 2012; Zuray, 2020). Kocan et al. (2004) investigated *Ichthyophonus* in Yukon River king salmon between 1999 to 2003 and found slightly above 30% of kings were infected with *Ichthyophonus* over that 5-year span. After 2003, the rate of infection prevalence steadily decreased, shown by the monitoring efforts led by Stanley Zuray. Based on Mr. Zuray visualizing signs of disease typical of *Ichthyophonus* infection (Zuray et al., 2012), the prevalence at the Rapids (rkm 1175) was around 20% in 2004, and dropped to as low as nearly 2% in 2018 (S. Zuray, personal communication, October 14, 2021, Chapter 3). After multiple years of relatively low *Ichthyophonus* prevalence in Yukon king salmon, infection resurged to nearly 45% of returning king salmon sampled at Rapids in 2020 (Zuray, 2020) and 30% in 2021 from incidental harvest in a 10.16 cm (4 inch) mesh set gillnet used to target non-salmon (S. Zuray, personal communication, October 14, 2021, Chapter 3). Around this same time, fishery management agencies noticed the estimate for king salmon at the Eagle Sonar in the upper river near the US/Canada border was much lower than what was expected to arrive based on Pilot Station Sonar counts, while accounting for harvest. The discrepancy in the numbers of fish observed to arrive at the border is consistent with predictions of en route mortality of upstream migrating individuals. Over the course of five consecutive years (2019-2023), the number of Canadian-origin king salmon accounted for postseason, which includes the Eagle Sonar estimate plus the estimated U.S. harvest on the Canadian-origin stock, was on average 44% fewer than what was estimated at the Pilot Station Sonar, ranging from 33% in 2019 to 52% in 2021. The postseason estimates in 2020, 2022, and 2023 were 47%, 42%, and 45% lower than expected respectively (F. West, ADF&G, personal communication, March 26, 2025). This suggests a significant level of en route mortality occurred annually during this span.

Here we explore the potential cause of en route mortality for king salmon due to ichthyophoniasis, a disease caused by *Ichthyophonus* infection. To the extent that the pathogen and other stressors interact during the arduous upstream migration, we hypothesize that infection prevalence and severity of infection will increase from the lower- to middle-river as the disease

progresses in infected fish, then infection rate and severity will decrease from the middle- to upper-river, consistent with the selective death of severely diseased individuals. The infection decrease between the middle- and upper-river would suggest en route mortality occurred due to *Ichthyophonus*, as the sickest fish are no longer in the population by the time they reach the upper-river. Our specific objectives were to 1) quantify *Ichthyophonus* infection prevalence (defined as confirmed presence or apparent absence of parasitic schizonts in heart tissue) based on histopathology at four sampling sites in the Yukon River drainage to assess how the infection progresses while controlling for age, sex, length, genetic stock groupings, capture method, water temperature, and day of year, and 2) quantify *Ichthyophonus* infection severity (defined as number of schizonts in heart tissue) in a similar manner to evaluate the evidence to suggest a linkage between *Ichthyophonus* infection and mortality of Yukon River king salmon during spawning migration.

2.3 Methods

2.3.1 Study area

The Yukon River drainage, covering an area of 854,000 km², is the fourth largest river basin in North America (Brabets et al., 2000). Spanning over 3,200 rkm, the mainstem of the river flows from its headwaters in Canada across Alaska to the Bering Sea. The most upstream stocks of king salmon undertake the longest freshwater migration among salmon species to reach their spawning grounds in Canada, traveling the entire Yukon River mainstem (Brown et al., 2017). The ecosystem of the Yukon River provides fishery resources that sustain customary and traditional ways of life to over 60 communities in both Alaska and Canada.

2.3.2 Fish sampling

A total of 878 king salmon were sampled at four locations on the Yukon River mainstem (Figure 2.2) within the Alaska portion of the drainage from June to August (2022-2023; Table 2.1). Sampling sites were Pilot Station Sonar (rkm 195), Rapids (rkm 1175), Fort Yukon (rkm 1612), and Eagle Sonar (rkm 1950). Given site-specific characteristics and variable logistics, fish capture techniques differed across the four sampling sites. At Pilot Station and Eagle Sonar, fish were captured using multiple drift gillnets ranging from 6.4 cm (2.5 inch) to 21.6 cm (8.5 inch) in mesh size as part of test fishery projects operated by the Alaska Department of Fish and Game

(ADF&G) to collect salmon migration data. The United States Fish and Wildlife Service (USFWS) operated the Rapids and Fort Yukon sites where Indigenous and/or local subsistence fishermen were contracted to capture fish using fish wheels. Fish wheels are large water-powered, and shore-based gear that involves a raft, revolving baskets and paddles to help rotate the wheel situated out in the river held in place with support beams to the riverbank. A lead, in the form of a wooden fence, is placed between the wheel and the shore to direct fish toward the rotating baskets. The fish wheel fishing depth varies depending on the area the wheel was constructed to rotate in the river, often the basket can be lowered and raised to account for fishing depth. The basket is situated to rotate near the bottom of the riverbed. The basket depth used in this study ranged from 2.44m (8 ft) at Fort Yukon to 4.42m (14.5 ft) at Rapids (Figure 2.3).

Effort was made to sample throughout the typical timing of the king salmon run, while considering in-season salmon run timing and estimated swim speeds to spread out the number of fish sampled based on run strength (Eiler et al., 2014). However, the total number of fish sampled each day was often limited due to the number of fish present in low run abundance years. Additionally, the upper river sampling site changed from Eagle Sonar in 2022 to Fort Yukon in 2023; due to community concerns about lethally sampling depleted runs, and the sampling size varied at each location (Table 2.1).

2.3.3 Samples collected and measurements

For each king salmon, we recorded the date and time of capture and capture method (fish wheel or drift gillnet mesh size). Individuals were euthanized as quickly as possible upon capture with blunt force trauma to the head as per permitting requirements (UAF IACUC #2031286-2). Fish were necropsied within three hours of capture to ensure the level and stage of *Ichthyophonus* is representative of the actual infection near the time of capture as the parasite begins to germinate hours after death of the host (Storesund et al., 2022). We collected heart tissue from each king salmon for infection diagnosis using histopathology. Each sample consisted of approximately 1 cm³ of cardiac tissue taken from the apex of the heart. Heart tissue was preserved in 10% neutral buffered formalin. Sex was determined by internal inspection of sperm or egg sacks (Brown et al., 2017) and fish length was measured from mid-eye to fork of tail. Scales were collected for laboratory age determination (Eaton, 2015) and a pelvic fin clip

was collected for genetic stock identification through laboratory procedures (Lee et al., 2021) conducted by the Alaska Department of Fish and Game age and genetics laboratories. The demographics of the total sample size can be found in Table 2.1.

2.4 Laboratory analyses

Historically, polymerase chain reaction (PCR; Whipps et al., 2006; Hamazaki et al., 2013), histopathology, and explant tissue culture (Hamazaki et al., 2013; Kocan et al., 2003, 2004; Kocan & Hershberger, 2006; Whipps et al., 2006; Zuray et al., 2012) have been used to diagnose *Ichthyophonus* infection. In research that used all three methods for Yukon king salmon, mean percent positive rates of infection were largely congruous across all three methods to measure infection prevalence (Whipps et al., 2006). Given its utility to describe both infection prevalence and severity, we determined to evaluate *Ichthyophonus* infection via histopathology, which remains an accepted standard for pathogen observation and presumptive diagnosis (Hershberger, 2012). Previous research using single sections of heart tissue has noted histopathology can miss low level infections, yielding false negatives (White et al., 2013). Here we increased the number of sections observed to increase the sensitivity of the diagnostic test to measure both the prevalence and severity of *Ichthyophonus* infection.

2.4.1 Histopathology

Heart samples from king salmon collected at Pilot Station (2022-2023) and Eagle Sonar (2022) were evaluated for *Ichthyophonus* at the ADF&G Pathology Laboratory. Samples from Rapids (2022-2023) and Fort Yukon (2023) were analyzed at the Animal Disease Diagnostic Laboratory at Washington State University, under the direction of an independent researcher with extensive experience, Dr. Richard Kocan (2022), and at the US Geological Survey's Western Fisheries Research Center (2023).

For laboratory preparation, heart tissue samples were trimmed and stored in histology cassettes placed in 70% ethanol. These samples were processed according to a standard histological approach including dehydration and clearing followed by infiltration and embedding in paraffin wax. Tissue plugs within wax blocks were cut into five 5 µm thick sections with 100 µm between each section to avoid sections that contained portions of the same schizont, thus eliminating the risk of double counting schizonts in serial slices. However, 50 heart samples at

Rapids in 2022 only had 4 sections due to a preparation error, which was accounted for when calculating the heart tissue volume used to model the infection severity. The five (or four) sections per fish were mounted on microscope slides and stained with Periodic-acid Schiff (PAS) reagent. Finally, the number of *Ichthyophonus* schizonts in all mounted sections were counted using brightfield microscopy.

Two separate adjustments were made to accurately model infection severity. First, to account for potential observer differences in *Ichthyophonus* schizont counts across three laboratories (referred to here as ADF&G, USGS, and Kocan), a random subset representing 20% of the 2023 Fort Yukon and Rapids samples ($n = 54$) was blindly evaluated by each of the three laboratories. Paired t-tests revealed significant differences between USGS and Kocan counts ($p = 0.014$), as well as between USGS and ADF&G counts ($p = 0.004$), with ADF&G and Kocan counting a higher number of schizonts on average. This was likely due to counting that included more ambiguous lesions associated with *Ichthyophonus* presence. For logistical and practical purposes, as well as to further minimize the risk of double counting of single schizont lesions and associated host response, the Kocan and ADF&G schizont counts were adjusted to be comparable to USGS schizont counts based on linear regression.

Second, to account for the variation in tissue per sample, the volume of heart tissue sampled was measured in all the *Ichthyophonus* positive samples. To calculate the heart sample volume, we used the formula: $V = A \times h$, where: V = total volume of the appraised area (mm^3); A = area of the serial sections (mm^2); and h = depth of 1 serial section (0.005 mm) $\times$ number of sections in sample. For all samples in which *Ichthyophonus* was not detected, the number of schizonts was set to zero. The area of each serial section was measured by microscopy with a calibrated polygon of interest tool.

2.4.2 Genetic stock groupings

Genomic DNA was extracted from collected pelvic fin clip samples and genotyped by the ADF&G genetic laboratory to assign a stock grouping (Table 2.1). The genetic stock groups assigned to each sampled fish corresponded to one of 5 regional categories. This includes the category Lower US representing tributaries in the lower Yukon River within Alaska, such as the Andreafsky River which is below our lower river sampling site, the Anvik River, the Gisasa River in the lower Koyukuk River and the Tozitna River below the community of Tanana. The

Koyukuk category represents the upper Koyukuk River tributaries, such as the South Fork Koyukuk and Henshaw Creek. The Tanana category represents Tanana River tributaries, such as the Kantishna, Chena and Salcha rivers. The Upper US category represents tributaries in the upper Yukon River within Alaska, such as the Teedriinjik and Sheenjek rivers. The Canada category represents both the tributaries off the mainstem Yukon River and Porcupine River in Canada. Each reporting group is made up of the genetic baseline information from several major king salmon populations within the area (Lee et al., 2021).

2.5 Statistical analyses

2.5.1 Covariate selection

To quantify the patterns of *Ichthyophonus* infection across the Yukon River king salmon migration, we assessed how the infection prevalence and severity changes across sampling sites while accounting for several important covariates. Initially, we identified potential covariates that may impact the *Ichthyophonus* infection rate. Covariates such as capture method, genetic stock groupings, and maximum daily water temperature were considered but were excluded because they are confounded with our main covariate of interest, sampling site. Additionally, we considered the total freshwater and saltwater age of the fish in the prevalence model selection process. However, after applying backward selection in the prevalence model, age was ultimately excluded from our analysis. The remaining covariates considered were the sampling date (expressed as day of year), sex, and body length (measured from the middle of the eye to the fork of the tail in millimeters). These covariates were checked for collinearity, and no significant associations were found. Since the chosen covariates of interest had complete datasets, meaning there was no missing data present from fish that were not ageable or not assigned to a genetic stock grouping, we were able to include all the sampled fish in our analysis.

2.5.2 Model selection

To assess the effect of covariates on *Ichthyophonus* infection prevalence and severity, we used several generalized linear models (Zuur et al., 2009a, 2009b; Zeileis et al., 2008). We used backward model selection to identify the most relevant covariates based on Akaike Information Criterion (AIC). Starting with a full model that included all the potential covariates as main effects, we sequentially removed variables using backward selection until only the most

significant covariates remained in the model. Sampling site was retained throughout the backward selection process given our hypotheses to compare *Ichthyophonus* infection prevalence and severity among sampling sites. No interaction terms were included in the model selection, as we did not have prior hypotheses regarding interactions between covariates.

2.5.3 Prevalence model formulation

To evaluate how *Ichthyophonus* infection prevalence varies across sampling sites each year, we applied a logistic regression model. Logistic regression is used to model binary outcomes, where the response variable has a value of 1 indicating *Ichthyophonus* infection and 0 indicating no infection. The model used the logit link function to estimate the log-odds of infection based on various explanatory covariates, such as sampling site, day of year, body length, sex, and age of the fish in the full model. The model estimates the probability (i.e., prevalence) of infection. The full model was applied separately for each sampling year as follows:

$$\text{logit}(\pi_i) = \ln \left(\frac{\pi_i}{1 - \pi_i} \right) = \beta_0 + \beta_l x_{li} + \dots + \beta_p x_{pi}$$

Where, π_i represents the probability of infection for fish i , β_0 represents the intercept, β_p represents the coefficients for each covariate x_{pi} , and p represents the total number of covariates in the model.

2.5.4 Severity model formulation

To assess the severity of *Ichthyophonus* infection, we used a zero-inflated negative binomial (ZINB) regression model. This model was chosen due to the presence of a high number of zeros in the data indicating no *Ichthyophonus* infection. The zero-inflated negative binomial regression accounts for both the excess of zeros and potential overdispersion in the count data. The dispersion parameter ($\log(\theta)$) was highly significant in both years, confirming the presence of overdispersion and supports the use of this model type in our analysis (Table 2.3). The model was fit to evaluate the relationship between explanatory covariates and the severity of *Ichthyophonus* infection, with severity defined as the count of *Ichthyophonus* schizonts as the response variable. The full model included covariates such as sampling site, day of year, body length, and sex, along with a control variable for tissue volume, assuming linear relationships on the log-scale. The ZINB model consists of two parts: the negative binomial component for the

count of schizonts and the zero-inflated component to handle the excess zeros. The model was applied separately to each sampling year.

The negative binomial part is expressed as:

$$\ln(\mu_i) = \beta_0 + \beta_1 x_{1i} + \dots + \beta_p x_{pi} + \ln(\text{offset}_i)$$

Where μ_i represents the predicted count of *Ichthyophonus* schizonts for infected fish i , β_0 represents the intercept term, $\beta_0 - \beta_p$ represent the coefficients for covariates $x_{0j} - x_{pj}$, p represents the total number of covariates and $\ln(\text{offset}_i)$ represents a fixed offset term for each fish i to account for sample volume.

The zero-inflation component is expressed as:

$$\text{logit}(\pi_i) = \ln\left(\frac{\pi_i}{1-\pi_i}\right) = \gamma_0 + \gamma_1 x_{1i} + \dots + \gamma_p x_{pi} + \ln(\text{offset}_i)$$

Where π_i represents the probability of no infection for fish i , γ_0 represents the intercept term, γ_p represents the explanatory covariates, p represents the total number of covariates and $\ln(\text{offset}_i)$ represents a fixed offset term for each fish i .

2.5.5 Hypothesis testing

Based on the model results, we then used estimated marginal means to calculate adjusted means for *Ichthyophonus* prevalence and severity from each sampling site to test our hypotheses. We used a Bonferroni correction for multiple pairwise comparisons among sampling sites in each year.

2.6 Results

2.6.1 *Ichthyophonus* infection prevalence

Overall, in both sampling years, the *Ichthyophonus* infection prevalence did not change during upriver spawning migration. Although the change was not significantly different among sampling sites, a slight negative trend in infection prevalence is observed during upriver migration (Figure 2.4). The full model for each sampling year was set up with the same covariates to assess the relationship between factors that could impact the likelihood of *Ichthyophonus* infection in king salmon, including sampling site, day of year, body length, sex, and age of the fish. The best fit model differed between sampling years. In 2022, sampling site, day of year, and sex were included in the final model (Table 2.2). In 2023, the only covariate included in the final model was sampling site. The 2022 model identified sex ($p = 0.002$) as the

only significant predictor for *Ichthyophonus* prevalence. Males were 54% ($e^{-0.612}$) less likely to be infected with *Ichthyophonus* compared with females in the 2022 sampling season. All the other covariates considered, including sampling site in the 2023 model did not show significant effects on predicting *Ichthyophonus* prevalence ($p > 0.05$).

2.6.2 *Ichthyophonus* infection severity

Overall, in both sampling years, the *Ichthyophonus* infection severity in infected fish significantly decreased during upriver migration (Figure 2.5). The best fit model from both years included the tissue volume control variable, sampling site, day of year and body length; however, sex was only included in the 2022 model.

Based on the results from the 2022 negative binomial component (Table 2.3), the explanatory variables sampling site, day of year, body length, and sex were all significant predictors of *Ichthyophonus* infection severity in infected fish ($p < 0.05$ for all). Additionally, infection severity in infected fish increased as the season progressed, with day of year showing a positive effect (estimate = 0.082, $p < 0.001$), indicating that the severity of infection tended to increase as the year advanced. The infection severity in infected fish also increased with body length showing a slight increase in infection severity for larger fish (estimate = 0.004, $p = 0.04$). Infected males exhibited significantly higher infection severity compared to infected females (estimate = 0.872, $p = 0.005$). The zero-inflation component takes a deeper look at the factors associated with the probability of not being infected with *Ichthyophonus*, and sex was the only significant predictor of having zero infection severity. Males exhibited a significantly higher likelihood of having zero infection severity compared to females (estimate = 0.693, $p = 0.013$). The other variables, including sampling site, day of year, and body length, did not show significant effects on the likelihood of having zero infection severity ($p > 0.05$ for all).

Based on the results from the 2023 negative binomial model (Table 2.3), sampling site, day of year, and body length were significant predictors of *Ichthyophonus* infection severity in infected fish ($p < 0.05$ for all). Additionally, infection severity in infected fish increased as the season progressed, with day of year showing a positive effect (estimate = 0.047, $p = 0.003$), indicating that the severity of infection increased as the year advanced. The infection severity in infected fish also increased with body length showing a slight increase in infection severity for larger fish (estimate = 0.005, $p < 0.001$). The zero-inflation component showed none of the

variables had significant effects on the likelihood of having zero infection severity ($p > 0.05$ for all).

2.7 Hypothesis testing

2.7.1 *Ichthyophonus* infection prevalence

Pairwise comparisons between the sampling sites in 2022, while controlling for the effects of other influential variables, suggest that the infection prevalence at Pilot Station Sonar was not different compared to Rapids ($p = 0.720$), Pilot Station Sonar was not different compared to Eagle Sonar ($p = 1$), and Rapids was not different compared to Eagle Sonar ($p = 1$). These findings suggest the infection prevalence does not differ significantly among sampling sites. Results were similar in 2023 with no significant differences observed for infection prevalence among sampling sites (Figure 2.4). The *Ichthyophonus* infection prevalence (= probability of infection) at each sampling site is shown in Table 2.4, along with the 95% confidence interval for the predicted probability of infection. Overall, the annual *Ichthyophonus* infection prevalence including all the sampling sites was 34.7% (157/452) in 2022, and 38.7% (165/426) in 2023.

2.7.2 *Ichthyophonus* infection severity

Pairwise comparisons between the sampling sites in 2022, while controlling for the effects of other influential variables, suggest the infection severity at Pilot Station Sonar was significantly higher than at Rapids ($p = 0.011$), Pilot Station Sonar was significantly higher than Eagle Sonar ($p = 0.001$), and Rapids was significantly higher than Eagle Sonar ($p = 0.001$). These findings suggest that king salmon at Pilot Station Sonar had the highest infection severity and infection severity in infected fish significantly decreased by the time king salmon reached both Rapids and Eagle Sonar (Figure 2.5).

Using the same approach for the 2023 sampling season, the pairwise comparisons suggest the infection severity at Pilot Station Sonar was not significantly different than at the Rapids ($p = 0.064$), Pilot Station Sonar was significantly higher than Fort Yukon ($p = 0.015$), and Rapids was not significantly different from Fort Yukon ($p = 0.277$). These findings suggest that king salmon at Pilot Station Sonar had the highest infection severity and infection severity in infected fish significantly decreased by the time king salmon reached Fort Yukon (Figure 2.5). The predicted

Ichthyophonus infection severity at each sampling site by year is shown in Table 2.5, along with the 95% confidence interval.

2.8 Discussion

In this study, we explored the association between ichthyophoniasis disease progression and en route mortality of adult king salmon during spawning migration in the Yukon River. Results of this work yielded the following insights. First, counter to our predictions, we observed that the *Ichthyophonus* infection prevalence did not change across sampling sites. Second, in contrast to prevalence, *Ichthyophonus* infection severity in infected fish significantly differed among sampling sites in a manner consistent with patterns predicted should en route mortality of diseased individuals be occurring. Third, we detected the influence of migration timing (i.e., day of year), sex, and body length when predicting *Ichthyophonus* infection. A key message of our work is to include a measure of infection severity in future research that aims to quantify *Ichthyophonus* related en route mortality. Quantifying infection prevalence is valuable information for disease surveillance and to be aware of the potential harm the disease may have on migrating salmon and its annual variation; however, it is unlikely to be informative for quantifying mortality as infection rates did not progress as predicted during upstream migration and when comparing among sampling sites. This lack of variation in prevalence is likely due to the lower end sensitivity of the diagnostic test. Since the prevalence estimate remained consistent across our sampling sites, we provide evidence that future monitoring efforts can obtain an accurate estimate for *Ichthyophonus* prevalence anywhere in the Alaska portion of the Yukon River mainstem. Taken as a whole, our results strongly suggest a linkage between *Ichthyophonus* and adult upstream en route mortality of king salmon in the Yukon River.

2.8.1 *Ichthyophonus* infection prevalence

Counter to predictions, we detected no significant difference in *Ichthyophonus* infection prevalence among sampling sites, which was not consistent with predictions of upstream en route mortality. The lack of any difference between infection prevalence among sampling sites could have arisen from multiple non-mutually exclusive reasons. One explanation could be that the sensitivity of the histopathology diagnostic test provides the illusion of fixed rates of infection as diseased individuals drop out and disease progression occurs sufficiently in others such that the

infection became detectable. Said another way, some king salmon may have become detectable by the diagnostic test at the upriver sampling locations, as the infection progresses to levels within the sensitivity of the test. This would provide the perception that the infection prevalence is staying consistent throughout the drainage, even if some en route mortality occurred. Additionally, infection prevalence does not provide any information regarding the infectious burden or overall impact on the host. This interpretation of how the *Ichthyophonus* infection is progressing in king salmon during migration is based on evidence suggesting they are not able to naturally clear the infection once established in the host (Hershberger, 2012), and the infection continues to progress during upriver migration (Kocan et al., 2004; Kocan & Hershberger, 2006).

Although we did not see differences in infection prevalence among sampling sites, we did detect a difference between the sexes. When accounting for sex, female king salmon were 54% more likely to be infected compared with males during the 2022 sampling season. This aligns with reports of female salmon being more susceptible to infection than males (Kocan et al., 2003, 2004; Kocan & Hershberger, 2006; Hinch et al., 2021; von Biela et al., 2022). Previous research suggests that females may be more impacted by *Ichthyophonus* infection, therefore, we focus on why females may be more impacted. During the long spawning migration journey, salmon rely on their energy stores, as they are not feeding during this period of their lifespan (Brett, 1995). The energetic requirements of female salmon during migration are greater compared to male salmon as they require a larger portion of their stored energy to develop eggs (Crossin et al., 2004). Additionally, a study on female sockeye salmon showed the cardiac capacity of female salmon is lower compared to male salmon and can cause oxygen deficiency adding to their susceptibility of mortality (Hinch et al., 2021).

Previous research has suggested the prevalence of *Ichthyophonus* increases later in migration timing (Zuray et al., 2012). However, in that research they looked at the number of weeks into the salmon migration. We used the continuous numeric value for migration timing, represented as the day of year, and did not see the same trend with respect to infection prevalence in the 2022 data. In 2023, the only variable assessed in the analysis was the sampling site since we forced the sampling site to be included in the model selection process to compare how the infection prevalence changes across the sampling sites. None of the variables considered were influential for predicting the outcome for infection prevalence in the 2023 season.

2.8.2 *Ichthyophonus* infection severity

In contrast to prevalence, the *Ichthyophonus* infection severity in infected fish significantly decreased as fish migrated upriver. Assuming Yukon king salmon do not recover from *Ichthyophonus* infection once established (Hershberger, 2012), we interpret the decrease of infection severity as being representative of severely infected king salmon dying from ichthyophoniasis, while the low or non-infected fish remain, accounting for the perceived decrease of infection severity during upriver migration. Based on our initial hypothesis, we did not expect the *Ichthyophonus* infection severity in infected fish to be most severe in the lower river at Pilot Station Sonar. The pathology results show the fish at Pilot Station Sonar had high infectious burden; however, the disease had not progressed to clinical levels in the host and, thus, is not apparent to harvesters. The high infection burden in the lower river is contradictory to results from Kocan and Hershberger (2006), who showed an increase of infection severity using categorized grades based on the number of schizonts present in heart tissue and compared king salmon sampled in the lower Yukon River at Emmonak (rkm 39) to middle Yukon River near Tanana (rkm 1110). The different methodologies used to measure infection severity could have contributed to this discrepancy in results. Zuray et al. (2012) described clinical disease is achieved when visual signs of infection are paired with the positive infection confirmation from laboratory diagnostic tests. By measuring the inflammation surrounding the schizonts within the tissue at each sampling site, one may be able to describe why the infections at Pilot Station Sonar are not yet visible via white lesions on the surface of the heart tissue. The schizonts in the samples from the middle- and upper-river sampling sites, where visual lesions are more common, likely have more tissue damage surrounding the schizonts which is causing inflammation that is visual via macroscopic lesions. This may also prove beneficial to understand how the infection is impacting proper physiological function of the heart at the host level, moving beyond estimated infection prevalence or predicted severity at the population level.

Beyond the effect of sampling site, there were several factors influential in predicting the estimate for infection severity in infected king salmon, including migration timing, sex, and body length. The predicted infection severity increased as the migration season progressed. This result could represent that infected fish have reduced swimming stamina and speed (Kocan et al., 2003, 2004); additionally, body size affects swimming speeds (Kraskura et al., 2024). This makes the interpretation difficult as the effects are confounded. In 2022, males had higher infection severity

than females. Sampled females entered the river with lower infection and infection severity decreased during upriver migration. Males entered the river with higher infection and infection severity decreased during upriver migration, consistently maintaining slightly higher infection severity at each location compared to females. Overall, the infection severity continued to decrease during upriver migration for both sexes, consistent with enroute mortality. Although, in 2022, males seemed to have slightly higher infection severity compared to females when infected, males also were less likely to be infected, which aligns with females having higher infection prevalence that year. Fish with longer body length showed a slight increase in infection severity in both sampling years. Zuray et al. (2012) noted an increase of *Ichthyophonus* infection in larger Yukon king salmon. Both sex and body size have been shown to be related to infection susceptibility (Hinch et al., 2021; Zuray et al., 2012).

2.8.3 Caveats

There are several limitations from this analysis based only on two years of data. We recognize the previous research that suggests accounting for the interannual variation in infection prevalence over multiple years (Kocan et al., 2003, 2004; Zuray et al., 2012). This research information comes at a time with relatively high *Ichthyophonus* prevalence similar to the infection prevalence in the early 2000s (Kocan et al., 2003, 2004). Additionally, we recognize future monitoring is important to understand the impact of *Ichthyophonus* infections on the annual return of adult spawner salmon to maintain the opportunity to compare the impact of infection interannually and across larger time scales. However, non-lethal tools for monitoring are urgently needed to reduce the number of lethally sampled fish. In our analysis, we excluded several covariates that were confounded with sampling sites given the hypotheses we were interested in testing. We excluded capture method, genetic stock groupings, maximum daily water temperature, and total age of fish in our analysis. Several of these factors could impact the initial *Ichthyophonus* infection rates; however, they were not formally included in our analysis for interpretation because their relative influence could not be resolved by the available data.

Although we did not formally analyze the likelihood of *Ichthyophonus* infection across the various genetic stock groupings, we did observe *Ichthyophonus* infection in all the genetic groupings. However, we had a very small sample size from the upper Koyukuk genetic stock grouping, and we did not sample fish from a major producing stock in the lower Yukon River,

the Andreafsky River, given that our downriver sampling site of Pilot Station Sonar is above that location. It appears migration distance is likely a key variable to consider for the likelihood of en route mortality due to *Ichthyophonus*, given the time and energetic capacity required for long spawning migrations (Kocan & Hershberger, 2006; Kocan et al., 2009). However, genetics may be an important factor in progression of disease, based on variable immunocompetency and susceptibility to infection across different stocks (Hershberger et al., 2010).

We also excluded water temperature from our analysis as it was confounded with sampling sites given that daily water temperatures were higher at the more upriver sampling locations. In future research, water temperature should be considered given the other consequences of water temperature and thermal stress on fish (von Biela et al., 2020, 2022), the interaction between temperature and disease progression (Kocan et al., 2009) and the interaction between multiple stressors inhibiting king salmon at the same time during their upriver spawning migration (Hinch et al., 2021; Westley, 2020). This could be achieved by tagging fish during upriver migration paired with non-lethal diagnostics.

Another factor we did not include in our final analysis was the total age of each salmon sampled which was calculated from the freshwater and saltwater age of the fish, as it was removed during the backward model selection process for infection prevalence and was excluded from the infection severity model selection process. Previous research has shown prevalence of *Ichthyophonus* increases with body length (Zuray et al., 2012). Other research shows age and body length are often related (Ohlberger et al., 2023). Given this information we thought the fish that spent more time out in the ocean may have a higher chance of being infected. We thus opted to include and interpret body length rather than age in our analysis given that some of our age categories had a small sample size.

2.8.4 Management implications

To accurately assess en route mortality of salmon moving forward, it is essential to consider all the potential sources of mortality and their interactions to best inform management decisions, estimates of spawning migration success, and stock estimates. Likely, disease alone is not causing large-scale en route mortality, but the combination of multiple stressors may be too much to overcome during migration (Hinch et al., 2021; Westley, 2020). Therefore, this study has a very narrow scope by only evaluating the impacts of *Ichthyophonus* on adult salmon during

upriver spawning migration without directly assessing the impacts of other stressors. We acknowledge the many other potential impacts on salmon during their juvenile and marine life stages. The impact of disease is important to consider when discussing all the issues that can impact Yukon River king salmon throughout their lifespan. It is important to move beyond tracking infection prevalence during migration and look at how the infection severity is progressing in the fish at both the population and the individual host level. The current downside is that there are no non-lethal methods confirmed to accurately diagnose *Ichthyophonus* infection. Therefore, non-lethally sampling fish for *Ichthyophonus* and tagging them to track the migration is not possible at this time but would be very useful in the future to quantify mortality while considering cumulative impacts of other known stressors.

Given the salmon and humanitarian crisis described in the introduction, the study team, subsistence fisherman, and Tribal governments entered this study that required lethal sampling of salmon with careful consideration and procedures to distribute study fish to the communities near each sampling site (see Chapter 3). This study is one of the first to quantify *Ichthyophonus* infection severity across the Yukon River king salmon migration route and compare between multiple locations. Like the previous *Ichthyophonus* studies in the Yukon River that looked at *Ichthyophonus* infection prevalence, the results show limitations to suggest en route mortality solely based on infection prevalence; however, the archive of collected samples present the opportunity for future investigation and interpretation. The infection severity results show the disease impacts each host differently across various stages of upriver migration and is suggestive of the potential linkage between en route mortality and *Ichthyophonus* infection.

2.8.5 Beyond the fish

Ichthyophonus has been detected in the Yukon River sporadically for nearly 40 years. The hypothesis at the forefront of this work that suggests *Ichthyophonus* could be causing en route mortality of king salmon was largely based on knowledge from some Indigenous and local fishermen, which pointed to the upper reaches of the Yukon River as a hotspot for *Ichthyophonus* related mortality. Beyond the potential impacts of *Ichthyophonus*, other Indigenous and local fishermen raised a myriad of other causes contributing to the continued low Yukon River salmon abundance. Continuing to uplift Indigenous and local knowledge systems alongside western science in future research is essential for better understanding the stressors and negative impacts

on salmon species, as well as for stewarding, managing, and conserving the relationship between salmon, people, and place.

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2.10 Figures and tables

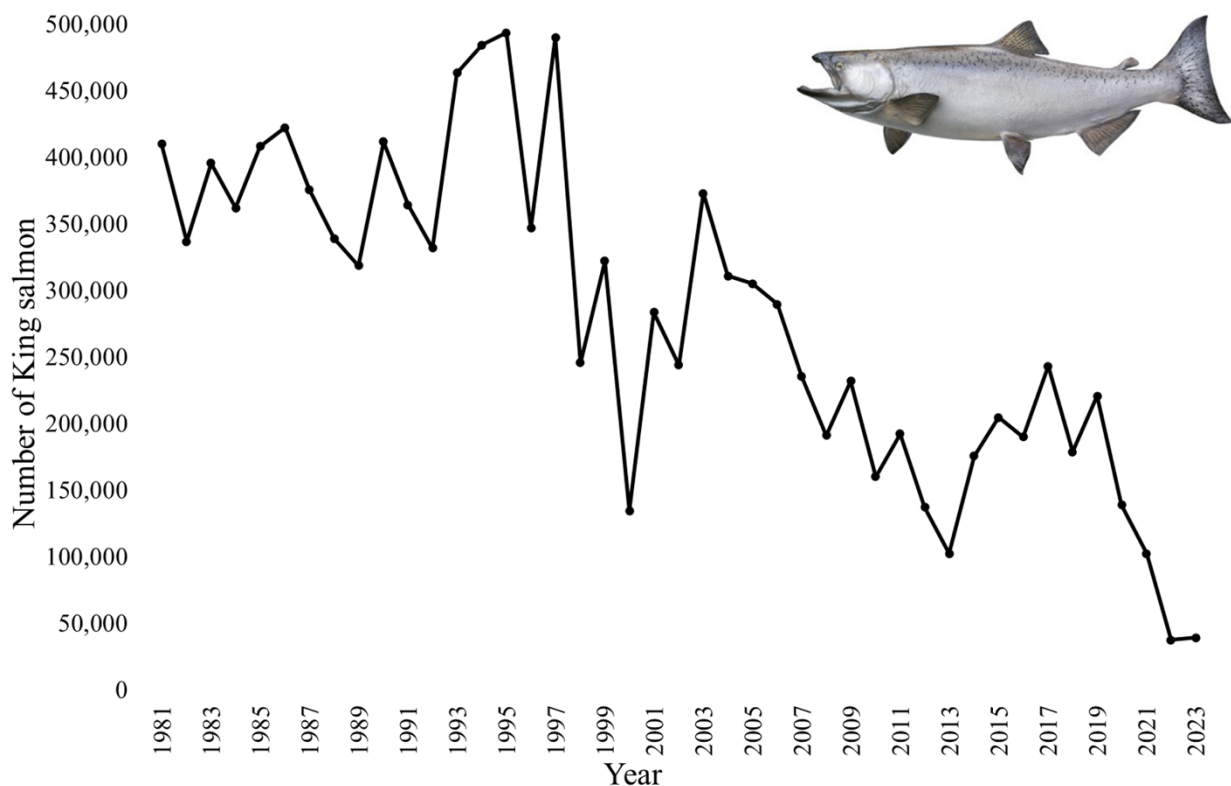


Figure 2.1. Yukon River king salmon drainage wide total run size estimates from 1981 to 2023. The total run estimates are based on historical data from assessment projects that estimate mainstem passage, harvests, tributary escapements, stock-proportions, and age compositions integrated into a state-space run reconstruction model (JTC, 2025).



Figure 2.2. The four sampling sites on the Yukon River mainstem are bolded and marked by stars and include Pilot Station Sonar (rkm 195), Rapids (rkm 1175), Fort Yukon (rkm 1612), and Eagle Sonar (rkm 1950). The two communities nearest to the Rapids, Tanana (rkm 1118) and Rampart (rkm 1228), are noted with circles. The river kilometer (rkm) metric represents the distance from the mouth of the river to each specific river location.



Figure 2.3. Fish wheel with baskets lifted out of the water located at the Rapids sampling site.

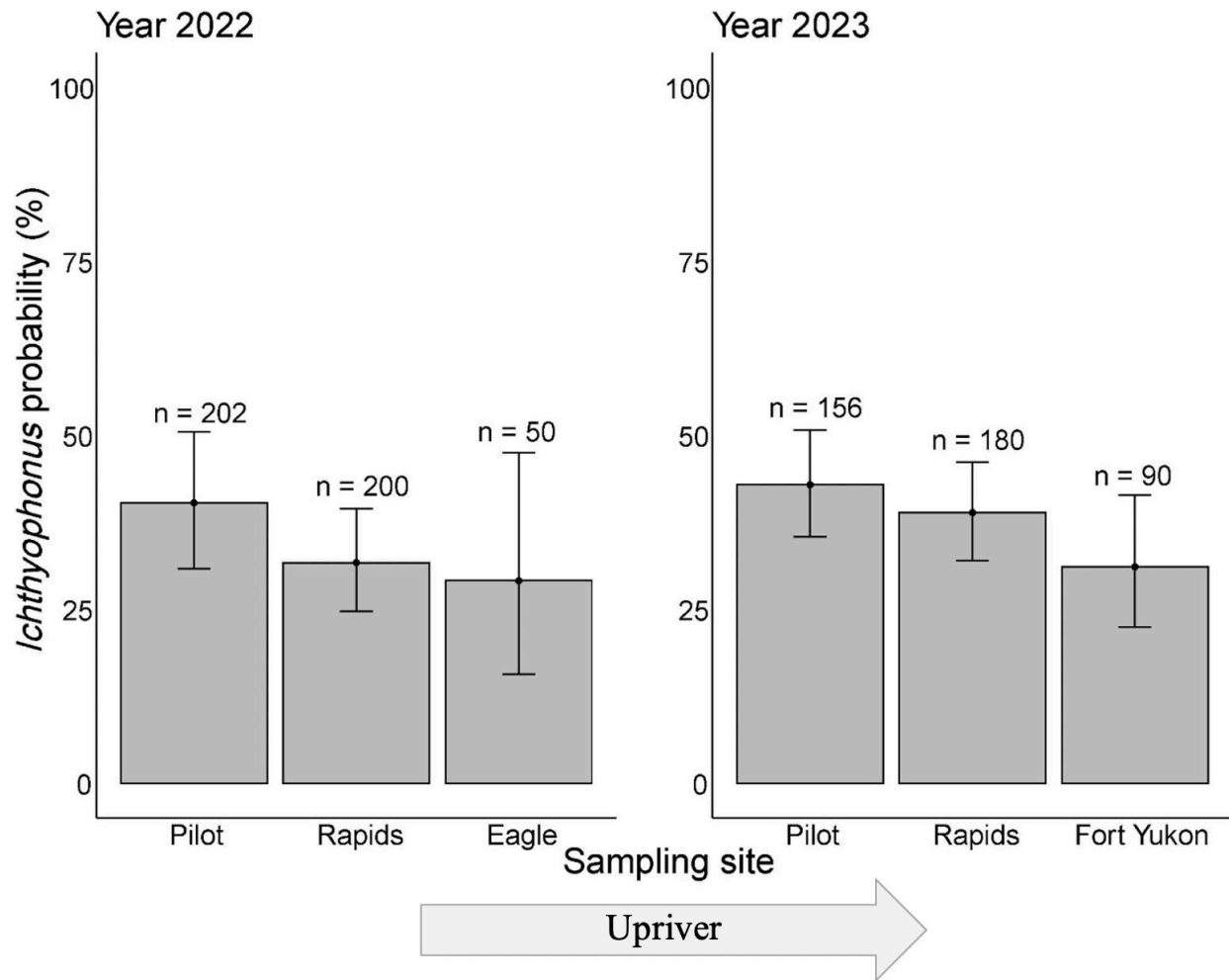


Figure 2.4. *Ichthyophonus* infection probability (i.e., prevalence) based on the best fit model for each year separately. The graph displays the estimated marginal means for infection probability across each sampling site during the 2022 and 2023 season, along with the 95% confidence interval for each estimate. Note the different sampling sites in 2022 and 2023, and specified sample size (n). Sites are arranged from downriver to upriver, left to right.

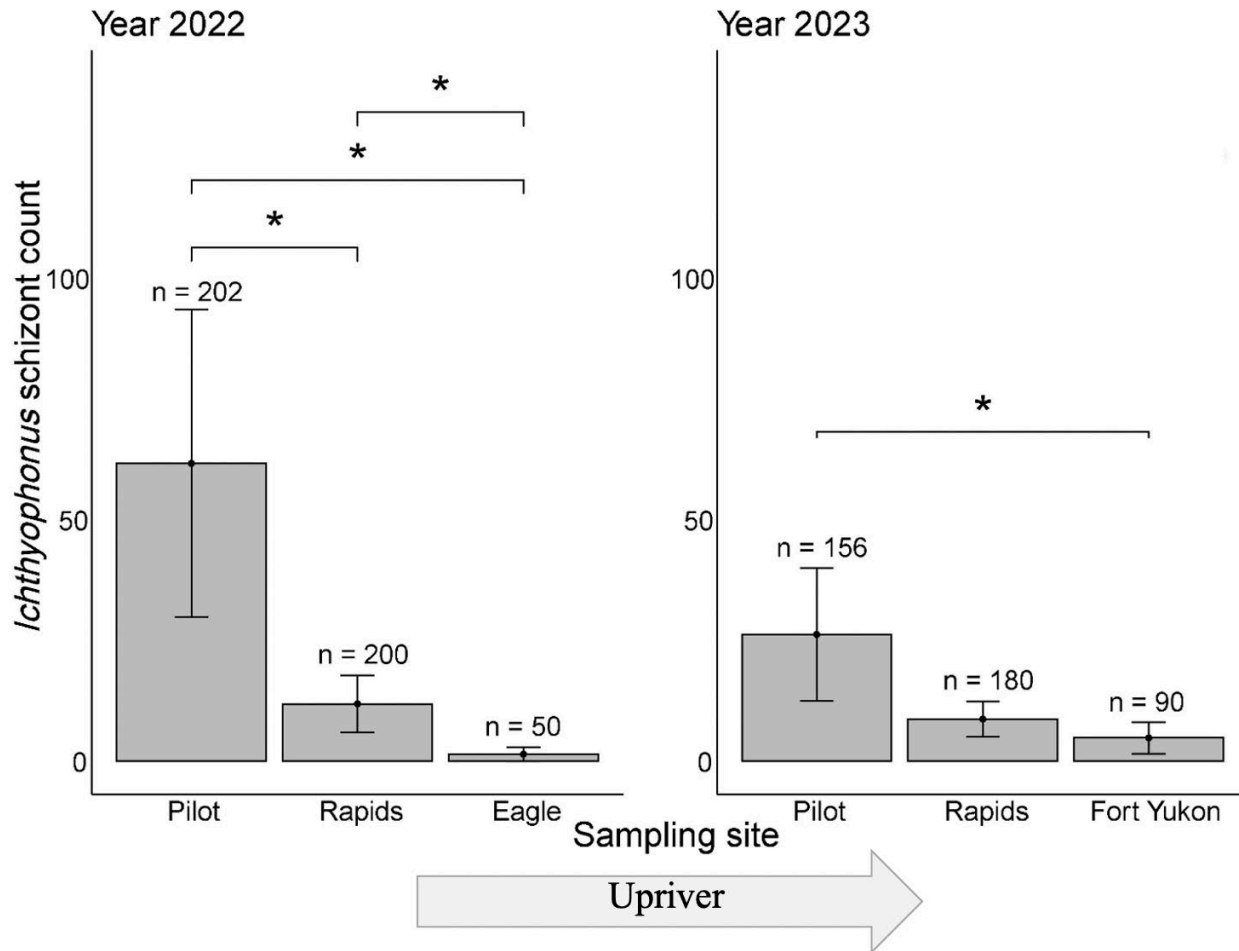


Figure 2.5. *Ichthyophonus* predicted mean schizont count (i.e., infection severity in infected fish) based on the best fit model for each year separately. The graph displays the estimated marginal means for the predicted mean schizont count across each sampling site during the 2022 and 2023 season, along with the 95% confidence interval for each estimate. Significant differences are indicated by the asterisks. Note the different sampling sites in 2022 and 2023, and specified sample size (n). Sites are arranged from downriver to upriver, left to right.

Table 2.1. Total Yukon River king salmon sampled during the 2022 and 2023 migration seasons, including the sample size (n) at each site, average body length in millimeters, male-to-female sex ratio, total age range, and assigned genetic reporting groups.

Year	Sampling site	tkm ^a	Sampling dates	Avg. body length and overall range (mm)												Kings assigned to stock reporting groups ^b												n															
				Male				Female				Overall				Male total age range ^c				Female total age range ^c				Canada					UpperUS				Tanana				Koyukuk				LowerUS		
2022	Pilot Station Sonar ^b	195	Jun. 10 - Jul. 15	660	776	713	380 - 914	3	4	5	6	7	8	NA ^e	5	6	7	NA ^e	89	6	29	2	65	11	110	92	202																
	Rapids ^d	1,175	Jul. 1 - Jul. 28	656	788	713	283 - 930	3	23	53	20	1	1	12	8	55	9	15	169	27	1	0	0	3	113	87	200																
	Eagle Sonar ^b	1,950	Jul. 14 - Aug. 10	737	808	770	582 - 910	0	1	15	8	1	0	2	3	17	1	2	50	0	0	0	0	0	0	27	23	50															
Annual totals				666	785	719	283 - 930	3	43	124	45	5	1	29	23	134	17	28	308	33	30	2	65	14	250	202	452																
Annual percentages ^f (%)								1.2	17.2	49.6	18.0	2.0	0.4	11.6	11.4	66.3	8.4	13.9	68.1	7.3	6.6	0.4	14.4	3.1	55.3	44.7																	
2023	Pilot Station Sonar ^b	195	Jun. 10 - Jul. 17	682	782	717	380 - 910	1	8	63	14	1	0	14	16	28	3	8	67	7	35	2	41	4	101	55	156																
	Rapids ^d	1,175	Jul. 2 - Jul. 27	671	777	700	465 - 924	1	18	75	17	2	0	17	10	33	1	6	147	28	0	0	5	0	130	50	180																
	Fort Yukon ^f	1,612	Jul. 10 - Aug. 5	693	782	714	538 - 902	0	3	49	10	1	0	6	7	11	1	2	87	1	0	0	0	2	69	21	90																
Annual totals				679	780	709	380 - 924	2	29	187	41	4	0	37	33	72	5	16	301	36	35	2	46	6	300	126	426																
Annual percentages ^f (%)								0.7	9.7	62.3	13.7	1.3	0.0	12.3	26.2	57.1	4.0	12.7	70.65	8.45	8.2	0.5	10.8	1.4	70.4	29.6																	
Overall totals				673	783	714	283 - 930	5	72	311	86	9	1	66	56	206	22	44	609	69	65	4	111	20	550	328	878																
Overall percentages ^f (%)								0.9	13.1	56.5	15.6	1.6	0.2	12.0	17.1	62.8	6.7	13.4	69.4	7.85	7.4	0.45	12.6	2.3	62.6	37.4																	
^a River kilometer (tkm) represents the distance from the mouth of the river																																											
^b Sampling data obtained from the Alaska Department of Fish and Game																																											
^c Number of sampled Kings not ageable due to scale regeneration or damage																																											
^d Number of sampled Kings not assigned to a reporting group due to failed genotyping																																											
^e Sampling data obtained from the United States Fish and Wildlife Service																																											
^f Total age percentages were calculated by total number of males or females sampled separately annually and overall. Sex ratio and reporting group percentages were calculated by total number of annual and overall samples.																																											

¹ River kilometer (tkm) represents the distance from the mouth of the river

² Sampling data obtained from the Alaska Department of Fish and Game

³ Number of sampled Kings not ageable due to scale regeneration or damage

⁴ Number of sampled Kings not assigned to a reporting group due to failed genotyping

⁵ Sampling data obtained from the United States Fish and Wildlife Service

⁶ Total age percentages were calculated by total number of males or females sampled separately annually and overall. Sex ratio and reporting group percentages were calculated by total number of annual and overall samples.

Table 2.2. The best fit logistic regression model that includes our main factor of interest, sampling site, analyzed separately for each sampling year for *Ichthyophonus* infection prevalence.

Year	Variable	Estimate	SE	z value	p value
2022	Intercept	-4.208	2.575	-1.634	0.102
	Rapids	-0.376	0.320	-1.175	0.240
	Eagle	-0.496	0.538	-0.922	0.357
	DOY	0.022	0.014	1.511	0.131
	Sex (Male)	-0.612	0.201	-3.044	0.002
2023	Intercept	-0.284	0.162	-1.756	0.079
	Rapids	-0.168	0.223	-0.755	0.450
	Fort Yukon	-0.511	0.279	-1.830	0.067

Table 2.3. The best fit zero-inflated negative binomial regression model analyzed separately for each sampling year for *Ichthyophonus* infection severity. This includes the count model coefficients (i.e., infected fish) and zero-inflation model coefficients (i.e., probability of no infection).

Year	Variable	Estimate	SE	z value	p value
2022 Count Model Coefficients					
	Intercept	-14.972	3.337	-4.487	< 0.001
	Rapids	-1.288	0.373	-3.454	< 0.001
	Eagle	-3.663	0.639	-5.732	< 0.001
	DOY	0.082	0.015	5.317	< 0.001
	Length	0.004	0.002	1.970	0.049
	Sex (Male)	0.872	0.312	2.796	0.005
	Log(θ)	-0.907	0.254	-3.565	< 0.001
Zero-Inflation Model Coefficients					
	Intercept	1.470	3.332	0.441	0.659
	Rapids	0.608	0.364	1.668	0.095
	Eagle	0.236	0.638	0.370	0.711
	DOY	-0.013	0.016	-0.770	0.441
	Length	-0.001	0.002	-0.411	0.681
	Sex (Male)	0.693	0.278	2.495	0.013
2023 Count Model Coefficients					
	Intercept	-10.599	3.064	-3.459	< 0.001
	Rapids	-0.972	0.370	-2.624	0.009
	Fort Yukon	-1.465	0.497	-2.948	0.003
	DOY	0.047	0.016	2.926	0.003
	Length	0.005	0.001	4.486	< 0.001
	Log(θ)	-1.313	0.267	-4.910	< 0.001
Zero-Inflation Model Coefficients					
	Intercept	-6.905	3.830	-1.803	0.071
	Rapids	0.340	0.447	0.762	0.446
	Fort Yukon	0.571	0.547	1.044	0.297
	DOY	0.021	0.019	1.108	0.268
	Length	0.001	0.002	0.665	0.506

Table 2.4. The 2022 and 2023 *Ichthyophonus* infection probability (i.e., prevalence) based on logistic regression model and 95% Confidence Intervals (CI) for each sampling site.

Year	Sampling site	Probability (%)	95% CI
2022	Pilot Station Sonar	40.3	(30.8, 50.5)
	Rapids	31.7	(24.7, 39.5)
	Eagle Sonar	29.1	(15.7, 47.5)
2023	Pilot Station Sonar	42.9	(35.4, 50.8)
	Rapids	38.9	(32.0, 46.2)
	Fort Yukon	31.1	(22.4, 41.4)

Table 2.5. The 2022 and 2023 *Ichthyophonus* infection severity (i.e., predicted mean schizont count in infected fish) computed from the zero-inflated negative binomial model and 95% confidence intervals (CI) for each sampling site.

Year	Sampling site	Predicted schizont count	95% CI
2022	Pilot Station Sonar	61.6	(29.8, 93.4)
	Rapids	11.8	(5.9, 17.7)
	Eagle Sonar	1.4	(-0.1, 2.9)
2023	Pilot Station Sonar	26.1	(12.3, 39.9)
	Rapids	8.6	(5.0, 12.3)
	Fort Yukon	4.7	(1.5, 8.0)

Chapter 3: Heartbeat of the Yukon: Combining Indigenous, local, and western knowledge systems to monitor the impacts of *Ichthyophonus* on Yukon River Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo)

3.1 Abstract

Indigenous, local, and western knowledge from the Yukon River has raised concerns about the impact of *Ichthyophonus spp.*, a parasitic pathogen, on fish health and survival during upriver spawning migration. This study pairs multiple knowledge systems to assess *Ichthyophonus* prevalence, severity, and its impact on Yukon River Chinook salmon (*Oncorhynchus tshawytscha*; king; Taryaqvak; Ggaal; Gath; Łuk choo). In collaboration with Indigenous and local subsistence fishermen from fishing-dependent communities, we developed a standardized technique for visually identifying *Ichthyophonus* infection in the field based on viewing heart lesions on king salmon hearts at multiple Yukon River sampling sites using laboratory diagnostics to confirm infection. Sampling was conducted along the Yukon River in 2022 to 2023, revealing that visual detection accuracy increased at upriver locations where the infection had progressed in Yukon king salmon to exhibit visual signs of infection. Statistical model results suggest that highly infected king salmon might experience mortality before reaching spawning grounds due to *Ichthyophonus* infection, with the potential for other stressors to influence infection prevalence and severity. To harness the wisdom of Indigenous knowledge holders, we hosted community workshops in collaboration with Tribal Governments to co-interpret research findings. We emphasize that while *Ichthyophonus* may contribute to king salmon mortality during upriver migration, broader fisheries, environmental, and management factors must also be considered. Participants stressed the importance of not elevating disease above other factors contributing to the continued low salmon abundance and collapse, as it is only one issue Yukon king salmon face throughout their lives. Workshop participants also highlighted the importance of Tribal-led, -informed, and -designed research, as well as community-based monitoring that integrates Indigenous Knowledge (IK). Our findings contribute to ongoing efforts to better respect, elevate, and collectively benefit from IK, Tribal governance, and ecological stewardship within fisheries science and management.

3.2 Introduction

Dramatic ecosystem changes are increasingly altering traditional ways of life in rural Alaska communities, having particularly profound effects on Indigenous communities who have long stewarded their environments (Carothers et al., 2021). Indigenous Peoples worldwide continue to steward their surrounding unceded traditional lands, waters, animals, and plants, despite the national and state governments who may challenge jurisdictional authority to do so. This continued stewardship generates deep spiritual and cultural connections to the ecosystems of which people are an integral part (Black, 2017; Carothers et al., 2021; Harris and Wasilewski, 2004; Justin & Black, 2019). The unwell state or feeling of uncertainty regarding the health and well-being of ecosystems in the Arctic and Subarctic places immense strain on Indigenous communities and cultures. It is important to recognize that this strain is not limited to food security and sovereignty, but also exemplifies the loss of cultural practices central to individual and community wellbeing or health (Greenwood & de Leeuw, 2007; Carothers et al., 2021). Abrupt and dramatic declines in the abundance and availability of customary and traditionally harvested species, such as Chinook salmon (*Oncorhynchus tshawytscha*) has contributed to disconnection of people from the lands, waters, and place through regulations restricting harvest and access to the ecosystem essential to Indigenous peoples' ways of life (Black & Stevens, 2019).

The concern Indigenous Peoples' have for their wellbeing and health extends to their concern for the health and long-term survival of the plants, animals, fish, and waters they have always stewarded and continue to care for (Justin & Black, 2019). For example, in Alaska, the extreme importance of salmon and other subsistence resources is evident in public testimonies and advocacy regarding the challenges communities face and erasure of Indigenous ways of life in many western spaces. Tribal advocates fight to influence more regulations or action that better protect their ways of life and equity within western management processes, such as the State of Alaska Board of Fisheries, the Federal Subsistence Board, and the North Pacific Fishery Management Council. In the Yukon River drainage, for instance, Chinook and chum (*Oncorhynchus keta*) salmon populations have declined significantly, leading to disastrously low Chinook, summer chum, and fall chum salmon returns that prompted complete fishery closures in 2021 and 2022. Only limited opportunities to harvest summer chum were available in 2023 and 2024, with closures of Chinook and fall chum fisheries (JTC, 2025). The upper Yukon River

has had heavy restrictions or complete salmon closures since 2020. Low runs and subsequent closures have had devastating impacts on the communities that rely heavily on these important cultural keystone species for their physical, social, cultural, and spiritual health (Garibaldi & Turner, 2004). In prior times when Chinook salmon runs were low, the Yukon communities and people could rely on chum salmon (Ransbury et al., 2022), but both Chinook and chum salmon in recent years have had low returns resulting in heavy restrictions or no salmon fishing opportunity, which increases the devastating impacts on Yukon communities. Prior to these significant changes, salmon made up approximately 40% of the wild harvest for those living along the Yukon River (SASAP, 2025a).

Salmon are the lifeblood of Indigenous and rural people in Yukon River communities, and the relationship between salmon and people runs deep. Chinook salmon (hereafter referred to as “king salmon” to honor the name used along the river) have original Indigenous names in the Yukon River, including Taryaqvak in Yup’ik; Ggaal in Denaakk’e/Koyukon Athabaskan; Gath in Benhti Kenaga/Lower Tanana; and Łuk Choo in Gwich’in (SASAP, 2025b). The Yukon River drainage is the ancestral homeland of many Indigenous Peoples who have long stewarded and developed rich cultural and spiritual relationships with salmon (Black, 2017; Carothers et al., 2021). The Yukon River Drainage encompasses 55 Federally Recognized Tribal Governments in Alaska, with inherent rights to Tribal sovereignty, self-governance, and self-determination (U.S. Constitution art. I, § 8, cl. 3).

Given the ongoing decline in the abundance and body size of salmon, as well as their overall health, coupled with their extreme cultural, societal, and ecosystem importance necessitates future monitoring to equitably manage culturally important species, such as salmon. To best assess the health of fish in the Yukon River drainage well into the future, more Tribally informed, designed, and led research is critical to ensure that the research does not harm the communities and cultures involved, but remains relevant and culturally grounded, and includes the best possible information (Herkshan et al., 2024). Thus, research must respect and uplift Indigenous Knowledge (IK) systems and values within the current framework for fisheries management in Alaska, benefiting federal and state agencies, Tribal Governments, resource users, and, most importantly, the fish.

3.2 Indigenous research frameworks

Projects that aim to engage Indigenous communities and knowledge systems should not extract IK but instead aim to co-produce and appropriately recognize IK holders throughout the entire research process and beyond (Reid et al., 2021). This requires researchers to develop and build relationships within each community, which should be continuous and reciprocal (Jumper-Reeves et al., 2014). Therefore, we discuss how to conduct Tribal-led research following the values presented in Harris and Wasilewski (2004), – relationship, responsibility, reciprocity, and redistribution – which can serve as a model for other projects seeking to work in respectful collaboration with Indigenous communities. Reid et al. (2021) also provides an Indigenous framework, known as “Two-Eyed Seeing”, to pair western and IK systems to better understand and govern fisheries. This framework allows the researcher to push against the common narrative that IK systems are only meaningful when they align with, or are affirmed by, western knowledge. The desired use of this framework is to appropriately recognize IK as their own distinct and valid knowledge systems developed through direct relation and stewardship from Indigenous Peoples and their surroundings for millennia. This approach aims to pair the best of western and IK systems to equitably, and more thoroughly, answer research questions and inform research outcomes by centering research methodology and processes focused on maintaining relationships and respect for all living and non-living beings.

Inspired and guided by the framework of “Two-Eyed Seeing”, we sought to engage communities authentically and intentionally through participation during the sample collection process and through community workshops to co-interpret the results and outcomes at an early stage of development. Here we use the term “co-interpret” to refer to the collaborative process where the research team and community members work together to think about research results to uplift multiple knowledge systems for more inclusive and transparent research outcomes (Cashman et al., 2008). This involved holding dialogues where all knowledge and experiences were welcomed to contextualize the research outcomes by creating spaces to listen to the voices of community members and critically thinking through their feedback when results were interpreted.

The topic of objectivity is raised in literature regarding both western and IK systems. Western knowledge is largely described as quantitative, literate, and objective, while IK can be presented as qualitative, anecdotal, and subjective (Reid et al., 2021). The ontological, what

researchers can acquire knowledge about, and epistemological, how knowledge is created, realities are different between these knowledge systems (Moon & Blackman, 2014). Often in western science epistemology, observers can be separate from phenomena being observed, whereas in many Indigenous epistemologies observers are interconnected and inseparable from the ecosystem. Going further, western science is based on understanding that human beings are above the natural world, and can make predictions, manage, or control natural outcomes, whereas many Indigenous ontologies see salmon, for example, with agency and self-determination (Wilson, 2008). Recognizing these false dichotomies and perceived hierarchy, researchers can help change the common phrases that describe the two systems of knowledge. Dynamic inclusivity requires all knowledge systems and diverse perspectives to be valued in the research process to generate more inclusive outcomes based on multiple knowledge systems (Harris & Wasilewski, 2004).

Here we provide a few definitions for IK:

[IK] is holistic, garnered from experience within one's own Indigenous context and in relationship to all living beings, centered on spirituality and Indigenous values of respect, relationships, and reciprocity, among others. This knowledge is acquired from direct experience "out on the land", hunting, fishing, and gathering, thus it is adaptive. This knowledge is also accumulated over thousands of years of living in connection with one's Creator, thus it is cumulative, passed down generation to generation, each generation building upon the previous generation's knowledge. It is knowledge acquired in dreams, prayers, memories, and everyday experiences. It is knowledge that demonstrates how each person, the community, the land, water, and animals can and should live in continual balance (Black, 2017).

The Inuit Circumpolar Council (ICC) defines IK as:

A systematic way of thinking applied to phenomena across biological, physical, cultural, and spiritual systems. It includes insights based on evidence acquired through direct and long-term experiences and extensive and multi-generational observations, lessons and skills. It has developed over millennia and is still developing in a living process, including knowledge acquired today and in the future, and it is passed on from generation to generation...Under this definition, IK goes beyond observations and ecological

knowledge, offering a unique ‘way of knowing’ to identify and apply to research needs which will ultimately inform decision makers (Johnson, 2016).

IK is inseparable from the knowledge holders or keepers and the environment in which it is embedded (McGregor, 2004). Place is crucial when discussing IK, as it is always contextualized by the historical, current, and changing environments (McGregor, 2004). Therefore, this thesis research attempted to create safe spaces “within place” (each community) to discuss recognition of IK systems in western research to create more equitable fisheries research, management, and decision-making processes by centering inclusive and intentional research that solicits, respects, and uplifts IK systems (Reid et al., 2021). The importance of soliciting IK in place allows the knowledge holder to share their lived experience in context to the environment that has shaped that knowledge and in the physical safety of their own home and context. When researchers are immersed in the place of knowledge sharing, they are better able to understand lived experiences and contextualize the knowledge, making it more meaningful (Wilson, 2008). This approach supports a more equitable research process by ensuring that IK holders are heard and understood and can help influence more inclusive management spaces by pairing the best of both Indigenous and western knowledge systems (Reid et al., 2021). Learning from IK holders in place ensures that [western] scientists feel and hear what is being shared in a deeper way. Conducting the research in this way challenges the status quo of fisheries monitoring from a solely western science perspective, and instead fosters true cooperation with sovereign Tribal Governments (NSF 24-1). Additionally, it is important to distinguish the difference between local knowledge (LK) and IK systems, as they are often referred to together, but are not the same. LK usually refers to knowledge generated by interacting with the local environment, providing knowledge context to place, which is usually held by the resource and land users independent of deep cultural ties to the region (Johnson, 2016).

3.3 Objectives

The objectives of this chapter were to 1) co-interpret *Ichthyophonus* investigation results through dialogue workshops with individuals from two Yukon River communities, and to 2) develop a standardized technique based on LK for collecting *Ichthyophonus* infection data from visual heart lesions caused by *Ichthyophonus* infection in king salmon, utilizing western laboratory diagnostics for disease confirmation. These objectives were chosen to build a

foundation of respect for the Koyukon and Gwich'in communities, their lands, and values to ensure that community priorities are included in the project outcomes, as a graduate student supported by the Tamamta program (NSF #2022190) and an employee for the United States Fish and Wildlife Service.

3.4 Methods

3.4.1 Co-interpreting the impact of *Ichthyophonus* on king salmon with Indigenous Knowledge holders

To ensure our project objectives were achieved, we started to critically think through how the graduate thesis outcomes were developed, shifting research processes that are purely transactional to pushing for research to be Tribal-led or co-developed alongside Tribal Nations from the inception of research ideas or questions. Methods included hosting dialogue workshops with two Yukon River Tribal Governments and the communities they represent and working directly with subsistence fishermen with immense knowledge of the study area throughout the research process. The goals were to build shared understandings of the underlying issues facing Yukon River salmon and foundations capable of co-interpreting research results and proposing fish health monitoring programs where IK regarding fish health is solicited, respected, and uplifted. Such a program is not limited to studying the impacts of *Ichthyophonus* on salmon health but aims to address and understand overall fish health from the perspective of IK systems. In this thesis research, we focused on the context of the impacts of *Ichthyophonus* on king salmon in the Yukon River during upriver migration. IK related to this topic of interest could include, but is not limited to, a historical context of disease cycles, associated climate or weather patterns linked to unhealthy or healthy fish, overall fish health impacts on harvest quality, less visible detection of disease in tissue upriver compared to lower river locations, potential teachings to inform solution-based approaches for successful monitoring efforts, alternative research methods or project outcomes, and cultural teachings or values associated with respectfully harvesting salmon and other fish and wildlife species that sustain Alaska Native Peoples.

In collaboration with the Tribal Governments of Rampart (Rampart Village Council) and Fort Yukon (Gwichyaa Zhee Gwich'in Tribal Government), we conducted community dialogue workshops in 2024 to present our findings from the 2022 and 2023 sampling efforts. Based on

the findings in Chapter 2, *Ichthyophonus* prevalence remained stable across the sampling sites, and infection severity (classified using histology) significantly declined as fish migrate upriver during spawning migration based on histopathology. Lack of detection upriver suggests that highly infected individuals experience mortality during migration before reaching spawning grounds due to *Ichthyophonus* and the extent the pathogen interacts with other stressors. We shared this information with the Tribal Governments and two communities located on the Yukon River mainstem, Rampart and Fort Yukon, Alaska. We invited the Tribal Council from each community by collaborating with the Tribal Government administrators, sharing the meeting on local radio stations, and personal communications. Additionally, we shared in the meeting announcements that food and participatory door prizes would be shared with the participants. These workshops provided a space for community members and Tribal representatives to discuss and co-interpret the impacts of *Ichthyophonus* on king salmon during upriver migration, share their perspectives and concern about king salmon overall health, as well as discuss the broader issues facing Yukon salmon management and potential future research directions from their perspective. To share details about the research process and the results we were attempting to co-interpret, we shared preliminary results from Chapter 2 by summarizing key findings in handout form. We walked the meeting participants through the summarized results by presenting the handout and asking general questions about how they interpret the results being shared. We encouraged dialogue throughout the presentation surrounding the project results and general impacts to salmon. Our research team documented each community dialogue by taking written notes throughout the discussions to capture generalized sentiments shared to co-interpret project results. The dialogue notes were reflected upon during interpretation of results and writing of this thesis. The quotes captured and shared in this thesis writing were not attributed to specific participants but taken in a manner to capture key themes.

3.4.2 Local and western knowledge

3.4.2.1 Study area

The Yukon River drainage, covering an area of 854,000 km², is the fourth largest river basin in North America (Brabets et al., 2000). Spanning over 3,200 river kilometers, the mainstem of the river flows from its headwaters in northern British Columbia, Canada all the way through Alaska to the Bering Sea. The ecosystem of the Yukon River provides fishery

resources for customary and traditional use to over 60 communities in both Alaska and Canada. It has been documented and shared that many factors contribute to the salmon declines (Cunningham et al., 2018; Feddern et al., 2024; Howard & von Biela 2023; Miller & Weiss, 2023; Schoen et al., 2017). In this chapter, we explore the resurgence of the parasitic pathogen *Ichthyophonus* as a significant factor adding stress on Yukon River king salmon and limiting recovery. Since 2004, an estimate of visual *Ichthyophonus* disease prevalence has been reported annually by Stanley Zuray at his family fish camp (known as the Rapids Research Center) in between Tanana and Rampart, Alaska (Zuray et al., 2012; S. Zuray, personal communication, October 14, 2021; Figure 3.1). This monitoring was led by Mr. Zuray, who is a non-Indigenous Alaskan resident of Tanana, Alaska, and has lived in the area for around 50 years. Similar observations of *Ichthyophonus* infection have been made throughout the Yukon River by many Indigenous and non-Indigenous Yukon River fishers over time and helped support the hypothesis that *Ichthyophonus* could be one possible cause of mortality of king salmon during upriver spawning migration. This knowledge has been shared in Yukon fishery meetings, post-season harvest surveys, and by organizations such as the Yukon River Drainage Fisheries Association, that help uplift the voices of the Yukon people within salmon management and research (Zuray, 2020).

3.4.2.2 Field data collection

A total of 878 king salmon were sampled at four locations (Chapter 2) on the Yukon River mainstem within the Alaska portion of the drainage from June to August (2022-2023) including: Pilot Station Sonar, Rapids, Fort Yukon (2023), and Eagle Sonar (2022). At Pilot Station and Eagle Sonar, fish were captured as part of test fishery projects operated by the Alaska Department of Fish and Game (ADF&G). The United States Fish and Wildlife Service (USFWS) operated the Rapids and Fort Yukon sites where Indigenous and/or local subsistence fishermen were hired to help assist and be a part of the project. The partnership with the subsistence fishermen was vital to the project success as we focused on uplifting Indigenous and local expertise. We collaborated with subsistence fishermen to provide their expertise, knowledge, and fishing gear to harvest the sampled fish. The time spent out on the river discussing issues that salmon face helped shape the co-interpretation of the project results, as well as successfully completing the work. This partnership with the fishermen was central to the

Indigenous research methods used in our research process by building reciprocal relationships between the research team and fishermen (Harris & Wasilewski, 2004) and cultivating understanding of the research based on multiple perspectives (Reid et al., 2021). The total number of fish sampled each day was often limited due to the number of fish present in low run abundance years. Additionally, the upper river sampling site changed from Eagle in 2022 to Fort Yukon in 2023, due to community concerns about lethally sampling depleted runs. After sample collection fish were provided to the communities near each sampling location for subsistence use. The Tribal Governments in Tanana, Rampart, and Fort Yukon provided the sampled fish to elders and community members based on their own process.

3.4.2.3 Samples collected

Following capture, fish were euthanized as quickly as possible upon capture with blunt force trauma to the head as per an Institutional Animal Care and Use Committee protocol (UAF IACUC #2031286-2). Fish were necropsied no longer than three hours after capture to ensure the level and stage of *Ichthyophonus* was representative of the actual infection near the time of capture as the parasite begins to germinate hours after death of the host (Storesund et al., 2022). We avoided cross-contamination of samples by using disposable sampling utensils, keeping workstation sanitized between each sampled fish by using Clorox wipes to clean the sampling table, and laying down new parchment paper on the table after each sampled fish. Additionally, we changed gloves when switching from external to internal samples on each sampled fish. We collected heart tissue from each king salmon for histopathology and quantitative polymerase chain reaction (qPCR) for diagnosis of *Ichthyophonus* infection as part of the collaborative project between ADF&G and USFWS. Each histopathology sample consisted of approximately 1 cm³ of tissue taken from the apex of the heart. Each qPCR sample consisted of approximately 1 cm³ from the remaining heart tissue then cut into 4 smaller square sections. Heart tissue was stored in 10% formalin for histological diagnosis and in buffer solution (Camacho-Sanchez et al., 2013) for qPCR diagnosis. Before heart tissue was processed, we visually inspected the heart surface for lesions typical of *Ichthyophonus* infection in the field. By documenting the lesions on king salmon hearts, we were able to calculate visual presence and severity of *Ichthyophonus* infection. Hearts were washed using fresh water to remove any blood before inspecting for lesions.

By utilizing the methods developed by Mr. Zuray (Zuray et al., 2012), we outline a standardized method for assessing lesions typical of *Ichthyophonus* infection, specifically focusing on the visible lesions (i.e., white spots; Figure 3.2) on king salmon hearts at several sampling sites along the Yukon River. The visual identification technique was taught to me by Mr. Zuray in the field at his fish camp. We collected this information at several sampling sites to support training more subsistence harvesters who wish to monitor for *Ichthyophonus* infection in harvested salmon in other regions of the river in the future. The visual detection of *Ichthyophonus* infection, based on the presence of lesions, has proven to be accurate when compared to explant tissue culture results near the Rapids in the Yukon River (Zuray et al., 2012); however, this field and visual assessment technique has not previously been utilized in other areas of the Yukon drainage. This protocol is robust for identifying clinical disease but may miss less severe or subclinical infections. Clinical disease is defined as a positive laboratory diagnosis accompanied by visible lesions on the surface of the heart caused by infection, while subclinical infection is confirmed through laboratory diagnostic tests, but the host showed no external signs of infection (Kocan et al., 2004).

By documenting the number of visual lesions on the heart typical of *Ichthyophonus* infection, we estimated both the presence and severity of *Ichthyophonus* infection. Visual infection prevalence can be determined by the proportion of fish exhibiting three or more visible lesions, while severity was measured by the estimated number of visible lesions on the heart surface. These visual infection assessments were paired with laboratory diagnostic tests, including histopathology (Kocan et al., 2011) and quantitative polymerase chain reaction (qPCR; White et al., 2013) to assess the accuracy of the visual lesion detection method for identifying presence of *Ichthyophonus* infection at various sampling sites in the Yukon River drainage.

Although previous work has used one tissue section for diagnosis of *Ichthyophonus* infection via histopathology, we increased the number of tissue sections from each heart collected to increase the sensitivity of the diagnostic test. The increased number of sections observed helps account for the random distribution of parasites within tissue, as other studies using a single section have noted histopathology misses low level infections, yielding false negatives (White et al., 2013). Both the histopathology and qPCR diagnostic tests provide a presence and absence measure for *Ichthyophonus* infection. Histopathology is also an established approach to quantifying severity, in which *Ichthyophonus* infection severity is estimated based

on the number of parasites/mm³ of tissue. Although not a widely recognized approach to assessment of infectious severity, qPCR can also be utilized to estimate infectious burden through examining the number of parasite gene copies/mg of tissue.

3.5 Laboratory diagnostics

Various methods for assessing *Ichthyophonus* have been explored in Yukon king salmon, including polymerase chain reaction (PCR; Whipps et al., 2006; Hamazaki et al., 2013), histopathology, and explant tissue culture (Hamazaki et al., 2013; Kocan et al., 2004; Kocan & Hershberger, 2006; Whipps et al., 2006; Zuray et al., 2012). In research that used all three methods for Yukon king salmon, mean percent positive rates of infection were largely congruous across all three methods to measure infection prevalence (Whipps et al., 2006). Here we used visual detection, histopathology, and quantitative polymerase chain reaction (qPCR) for assessing *Ichthyophonus* infection.

3.5.1 Visual detection (field-based assessment)

Visual detection of *Ichthyophonus* infection in the field by inspecting king salmon hearts for visible lesions was 97.8% accurate when compared to explant tissue culture confirmation (Zuray et al., 2012). Visual identification of white lesions on internal organs can provide real time estimation of infection prevalence and severity by sampling harvest. Increased numbers of visual lesions correlate with an increase of infection within the host (Zuray et al., 2012). Infection prevalence in harvest can be calculated by using the following equation: Prevalence (%) = 100 * [{number of fish with 3+ visible lesions}/ total fish sampled]. The infection severity can be estimated for each fish sampled by counting the number of visual lesions present on each side of the heart. Heart lesion identification followed methods developed and taught by Stanley Zuray (Figure 3.2).

3.5.2 Histopathology (laboratory diagnostic)

Heart samples from king salmon collected at Pilot Station (2022-2023) and Eagle Sonar (2022) were evaluated for *Ichthyophonus* at the ADF&G Pathology Laboratory. Samples from Rapids (2022-2023) and Fort Yukon (2023) were analyzed at the Animal Disease Diagnostic Laboratory at Washington State University, under the direction of independent researcher with

extensive experience, Dr. Richard Kocan (2022) and at the US Geological Survey's Western Fisheries Research Center (2023).

For laboratory preparation, heart tissue samples were trimmed and stored in histology cassettes placed in 70% ethanol. These samples were processed according to a standard histological approach including dehydration and clearing followed by infiltration and embedding in paraffin wax. Tissue plugs within wax blocks were cut into five 5 μm thick sections with 100 μm between each section to avoid sections that contained portions of the same schizont (parasite within tissue), thus eliminating the risk of double counting schizonts in serial slices. However, 50 heart samples from Rapids in 2022 only had 4 sections due to a preparation error, which was accounted for when calculating the heart tissue volume used to calculate the infection severity. The five (or four) sections per fish were mounted on microscope slides and stained with Periodic-acid Schiff (PAS) reagent. Finally, the number of *Ichthyophonus* schizonts in all mounted sections were counted using brightfield microscopy.

Similar to direct observation of visual lesions on organs in the field, histopathology allows for the direct observation of the parasitic pathogen in host tissue, thus reducing the chance for a misdiagnosis or false positive of infection. The stain used can eliminate organisms that resemble *Ichthyophonus* but can only infer the identity of *Ichthyophonus*; thus, investigator familiarity with parasite morphology is relied on for identification until there is positive laboratory confirmation of infection (i.e., qPCR). A drawback to the use of standard single-plane histopathology as a survey tool or for determining infection prevalence, is that only a small portion of host tissue is examined, ~ 5 μm thick slice of tissue. Because *Ichthyophonus* parasites vary in size and can be widely dispersed throughout the host tissue, viewing only a single section of tissue increases the probability of missing early and low-level infections; this results in underestimating infection prevalence (White et al., 2013). This problem was addressed by increasing the number of serial sections, thus increasing the probability of detecting the organism in low-level infections. Another technical problem is overestimating the number of parasites by counting multiple cuts through the same parasite, which can occur when the cells exceed 100-150 μm . This was remedied by increasing the spacing between sections.

Beyond assessing infection prevalence, we aimed to evaluate infection severity. Two separate adjustments were made to accurately calculate infection severity. First, to account for potential observer differences in *Ichthyophonus* schizont counts across three laboratories

(referred to here as ADF&G, USGS, and Kocan), a random subset representing 20% of the 2023 Fort Yukon and Rapids samples ($n = 54$) was blindly evaluated by each of the three laboratories. Paired t-tests revealed significant differences between USGS and Kocan counts ($p = 0.014$), as well as between USGS and ADF&G counts ($p = 0.004$), with ADF&G and Kocan counting a higher number of schizonts on average. This was likely due to counting that included more ambiguous lesions associated with *Ichthyophonus* presence. For logistical and practical purposes, as well as to further minimize the risk of double counting of single schizont lesions and associated host response, the Kocan and ADF&G schizont counts were adjusted to be comparable to USGS schizont counts using linear regression.

Second, to account for the variation in tissue per sample, the volume of heart tissue sampled was measured in all the *Ichthyophonus* positive samples. To calculate the heart sample volume, we used the formula: $V = A \times h$, where: V = total volume of the appraised area (mm^3); A = area of the serial sections (mm^2); and h = depth of 1 serial section (0.005 mm) $\times$ number of sections in sample. For all samples in which *Ichthyophonus* was not detected, the number of schizonts was set to zero. The area of each serial section was measured by microscopy with a calibrated polygon of interest tool.

The total adjusted schizont count was divided by the measured tissue volume to estimate the number of schizonts (i.e., parasites) per mm^3 of tissue.

3.5.3 Quantitative polymerase chain reaction (laboratory assessment)

Polymerase chain reaction can be used to confirm *Ichthyophonus* presence in tissue and detect viable and non-viable parasites that may have died or lost viability between sample collection and lab analysis (Lowe et al., 2018), as often occurs when transporting material from remote field camps in Alaska. This method can also be used for high through-put screening of large sample sizes. Quantitative polymerase chain reaction (qPCR) can also be used to estimate infection severity, however there are some important limitations to this approach. Infection severity estimated by qPCR uses the number of target gene copies per mg of tissue as a proxy for parasite infection level, which is susceptible to contamination and the frequent inherent biases introduced by molecular amplification (Aird et al., 2011; Polz & Cavanaugh, 1998). However, the qPCR for *Ichthyophonus* (White et al., 2013) has been published and validated for parasite detection and qPCR has been shown to be correlated with and even more sensitive than

histopathology (without serial sections) and conventional PCR (White et al., 2013) for *Ichthyophonus* detection.

In qPCR, only a small amount of tissue (10-50mg) is necessary for testing, which may fail to detect infections due to the uneven distribution of the parasite in large hosts and the variable size of parasites (Kocan et al., 2011). This was addressed by homogenizing the entire 1cm³ of heart tissue then sub-sampling approximately 20mg to be tested. This increases sensitivity as the subsample given that it was taken from a much larger piece of homogenized tissue. Since qPCR cannot differentiate between viable and non-viable parasites, paired histopathology confirms viable parasites were present, however we did not account for variation in parasite size, or the biases introduced by batch processing of samples for targeted amplification. These remain an inherent challenge with qPCR and are discussed below.

3.6 Statistical analyses

In this study, we compared the visual identification of lesions typical of *Ichthyophonus* infection to histopathology and qPCR results to assess its efficacy in confirming *Ichthyophonus* infection at multiple locations along the Yukon River, as discussed in results. To assess how the visual identification of lesions compared to the confirmation diagnostic of histopathology, confirming the presence of the parasite in tissue using microscopy, and qPCR, the presence of parasite DNA within the tissue, we analyzed two different aspects, the visual lesions presence and severity typical of *Ichthyophonus* infection. For visual lesion presence, we compared the agreement between presence of visual lesions typical of *Ichthyophonus* infection and the laboratory confirmation of infection from the histopathology and qPCR diagnostic tests. For visual infection severity, we used a linear regression to compare the estimated visual lesion count in the field to both estimates for *Ichthyophonus* severity obtained from the diagnostic tests.

3.7 Results and discussion

In this chapter, we worked alongside subsistence fishermen during sample collection and sought to co-interpret the association between *Ichthyophonus* disease progression and in-river mortality of adult king salmon during spawning migration in the Yukon River during community meetings. Results of this work yielded the following insights. First, there were concerns by community participants about impacts of disease on king salmon migration, success and health,

but it was clear that individuals were worried disease would be used to assign blame above other factors that have contributed to the salmon population declines. Community meeting participants stressed the importance for disease not to become a scapegoat, dismissing the impacts of marine bycatch and all the other factors that impact salmon throughout their lifespan (Lamborn et al., 2025). Second, community participants highlighted the importance of future community-based and Tribal-led monitoring and research that includes IK and youth. Third, we highlight results based on methods following the lead of local-led monitoring to track *Ichthyophonus* prevalence and severity across several sampling sites. Overall, we describe the importance of incorporating multiple perspectives in understanding the health of salmon populations and fostering respectful and meaningful collaborations with Indigenous communities during the research process.

3.7.1 Co-interpreting the impact of *Ichthyophonus* on king salmon

A major theme from the community meetings held in collaboration with Rampart and Fort Yukon Tribal Governments was understanding the breadth of root causes that led to the decline of king salmon. We heard participants passionately discuss mismanagement of Yukon River salmon fisheries, historical overharvest in commercial fisheries within the Yukon River, declining salmon size, marine bycatch within industrial fisheries, marine and in-river environmental changes, the rise of marine mammal and shark predators including in-river predators such as sheefish and pike, climate change, warming water temperatures in-river, manmade contamination issues due to mining, as well as impacts of disease.

Several key questions surrounding the impacts of *Ichthyophonus* were discussed throughout the presentation that shared project results from *Ichthyophonus* monitoring in 2022 and 2023 to co-interpret results. We discussed what fishers have observed over time, such as seeing white heart lesions (Figure 3.2). Participants noted they have seen spots in the organs before, but they spend more time looking at the meat (fillets) during processing of king salmon and less time looking at salmon hearts. Other participants noted seeing visual signs of disease all the time, such as white spots (i.e., lesions typical of *Ichthyophonus* infection) on the heart, soft mushy hearts, and salmon that smell bad and typically the smell reported is an unusual “sweet” or “tangy” smell. Some participants noted the salmon meat is softer now compared to historically, and some noted visual signs of disease in the meat. Other participants noted salmon

are much smaller now, and have a “lower oil content”, and do not taste as good as they used to, when we asked about the other health issues participants have observed in salmon.

Another topic we discussed was related to the decrease of *Ichthyophonus* infection severity during upriver migration and if this is suggestive of *Ichthyophonus* related mortality (Chapter 2). Some participants suggested this trend could mean highly infected fish are dying in-river. However, others noted, “If this (*Ichthyophonus*) is the reason fish are dying between Pilot [Station Sonar] and Fort Yukon, why wouldn’t we (Yukon River residents) see kings washed up on the banks in the river? We (community of Fort Yukon) don’t see that...dog (chum) salmon wash up on gravel bars when they die, why don’t we see dead kings on the [river] banks?” Similar thoughts about not seeing dead kings on riverbanks, in agreement, were brought up during the Rampart meeting; however, participants in Rampart also suggested, “the large kings will sink and not be seen.” Other participants noted, “Salmon could instead be traveling to tributaries they’ve never traveled before. People that fish keep bringing it up that salmon are going down different waterways and disappearing.” Participants discussed fish movement patterns in the river could be changing due to warming water in the mainstem, and fish are seeking colder water.

The next major question discussed was, “When the communities on the Yukon River are fishing again, how can researchers work better with communities to monitor fish health or increase the amount of community-based and tribal-led research?” Some participants said, “The fishermen are the ones who should do the health assessment, and to let them know who to contact when they notice abnormalities in the salmon.” Another person suggested that the question is missing something, and we should instead talk about:

Commercial bycatch out in the ocean, and how to sample periodically from wild salmon in the ocean to see if those fish are sick. Additionally, they should be collecting fish and freezing them to allow stored fish from history to be looked at.... The historical data can be looked at again in the future when something happens, and if something was missed...with warmer water harmful organisms are flourishing.

Another participant noted:

Our community is overwhelmed. Problems are quadrupling, and people aren’t talking to each other. How are we (the community) communicating with each other? We (the community) need to start doing what they’re saying (community-based monitoring)

instead of other entities telling us how they think things are going.... We (the community) should have our own say and our own way of monitoring things, and we (the community) should be the ones doing this (sampling) ourselves but are grateful they (the research team) are here to tell us what they did. However, it would be better for our community to do that work.

Other participants voiced the copyright and ownership of information being discussed when discussing historical observations about the impacts to salmon from the perspective of the people on the river. Participants expressed concerns surrounding the contributions of community members' time and effort to the studies, surveys, and final reports of other research that has never come back to the Yukon River. Similarly, participants highlighted that researchers must be doing more of these meetings to engage with the communities. They said, "We (the community) don't see researchers and biologists, they don't visit.... As fishery biologists and employees of organizations that help manage land and water resources and conduct research, they should visit, and that the community has already sacrificed too much." Other participants said, "Researchers need to work together with Yukon Tribes and come up with a plan so there is accountability for the lack of fish and we the people can help to ensure fish are present for generations to come in the water for subsistence." Overall, participants supported more Tribal-led, -informed, and -designed research that includes people and knowledge from the Yukon River, as well as more research that involves youth.

Through this research we also discussed the major question, "Do the participants think king salmon could be dying in-river due to *Ichthyophonus* infection, keeping in mind all the other issues salmon face, and how the shared information should influence fisheries management?" Overall participants said *Ichthyophonus* is an issue we should be concerned about; however, a few participants highlighted disease as an issue increased by the warming environment and the combination of other stressors related to upriver migration. A few participants reflected on the issues of dealing with *Ichthyophonus* related mortality in management would only create a more conservative management approach and may restrict subsistence fishing more in the future. Similar themes were highlighted by multiple participants that the in-river communities are already negatively impacted by the effects of marine bycatch and the behavior of the industrial trawlers, and accounting for *Ichthyophonus* related mortality in-river would only be another issue that limits in-river subsistence users' ability to fish. During

the community workshops, it was evident that the participants were grateful to be a part of the dialogue discussing project results. They were understandably frustrated with the state of Yukon River fisheries management and the lack of Yukon salmon. The participants voiced sentiments of concern, such as the in-river people have already sacrificed everything and are forgoing harvest to conserve salmon, yet the major issue facing Yukon River salmon has not been addressed in the marine environment with the bycatch of salmon from ocean fisheries intercepting Yukon River salmon. There was frustration that yet another project, focused on what is happening to the king salmon in-river, but nothing is being done in the marine environment to distribute the equity of conservation across all the user groups in the salmon life cycle instead of conservation being placed solely on the in-river subsistence people, as they have “already sacrificed everything.”

3.7.2 Visual *Ichthyophonus* detection (field-based assessment)

To help uplift and support the discussions brought up by community meeting participants for more community-based and Tribal-led research that includes people and knowledge from the Yukon River, we highlight our results that followed methods developed by Stanley Zuray to monitor *Ichthyophonus* infection within his subsistence harvest. We compare the agreement between visual detection of heart lesions and laboratory confirmation of *Ichthyophonus* infection by location of harvest (Table 3.1 and 3.2). In Table 3.1, visual lesions are defined by the presence (Yes) and absence (No) of lesions typical of *Ichthyophonus* infection. The king salmon that were considered positive for *Ichthyophonus* infection was determined by the presence or absence of *Ichthyophonus* infection from either histological or qPCR infection confirmation. The n column represents the number of occurrences for each combination of Yes and No for each category of infection status based on visual identification and laboratory methods. The column that shows the number of lesions present on the heart only shows the number of lesions on hearts noted to have visual lesions typical of *Ichthyophonus* infection, but did not have a paired diagnosis confirming *Ichthyophonus* presence. Table 3.2 shows the percentage agreement and disagreement between visual lesions and the confirmation of *Ichthyophonus* infection across each Yukon River sampling site. If visual lesions typical of *Ichthyophonus* infection were present on the heart surface at any of the sampling sites within the Yukon River drainage, then it was related to *Ichthyophonus* infection 96.2% (229/238) of the time.

The detection accuracy of *Ichthyophonus* infection by visual lesions in king salmon is significantly influenced by the location of sampling, as the infection progresses to clinical disease in fish as they migrate upriver (Kocan et al., 2004; Kocan & Hershberger, 2006). At Pilot Station Sonar, located in the lower river, a high presence of infection is observed based on confirming *Ichthyophonus* infection by laboratory diagnostic techniques, but this does not translate into visually seeing signs of infection on the heart of king salmon and clinical disease. This discrepancy between the visual signs of infection and the accuracy of diagnosing *Ichthyophonus* infection in king salmon points to challenges in detecting the disease accurately based on what people can visually see in their harvest dependent on harvest location.

In the lower river at Pilot Station Sonar, visual heart lesions were only 25% accurate at diagnosing *Ichthyophonus* infection status. However, as fish migrate upriver to the other sampling sites, such as Rapids and Fort Yukon, the accuracy of detecting *Ichthyophonus* infection based on visual lesions improves dramatically. At these locations, the overall accuracy of diagnosing *Ichthyophonus* infection based on visual lesions in king salmon rises to 91.4% at the Rapids and 93.9% at Fort Yukon. These results suggest that the historical monitoring efforts led by Stanley Zuray at his Rapids Research Center are an accurate reflection of *Ichthyophonus* prevalence in the middle Yukon River over the past two decades (Figure 3.1). Historically, Stan Zuray only counted the king salmon with 3+ lesions present on the heart to be related to *Ichthyophonus* infection, in part to avoid human error in observation; this is reflected in the calculation of historical disease prevalence (2004-2013, 2018, 2020-2021). Similar methods were used in 2022 and 2023 to assess the visual disease prevalence at Rapids as part of this thesis research. Based on counting the number of lesions at Rapids and Fort Yukon, overall, the fish that were negative by laboratory diagnostic results all had a very low count for the number of lesions observed. There were only 3 cases where king salmon had visual lesions and were confirmed negative for *Ichthyophonus* infection, and all had 5 or less lesions noted. Although there were some king salmon confirmed positive for *Ichthyophonus* infection with between 3 and 5 lesions noted, these were typically low-level infections based on laboratory diagnostic tests. Therefore, when this occurs, those king salmon likely have low *Ichthyophonus* infection below the sensitivity of each diagnostic technique or it could be related to another infection that causes similar visual signs of infection.

In contrast, further upriver, the visual diagnostic accuracy drops significantly at Eagle Sonar, where it falls to 55% based on the presence of visual lesions. While this might seem less informative at first glance, it does help explain how the infection might be progressing in king salmon during migration. At Eagle Sonar, the severity of *Ichthyophonus* infection is lowest based on laboratory diagnostic tests, and the fish showing the highest level of infection which typically exhibit high visual heart lesions on the heart, may no longer be present in the population by the time the king salmon reach Eagle Sonar. The lower presence of highly infected fish in this area may be why the diagnosis based on visual lesions is less reliable. Previous research has suggested if the disease prevalence peaks in the middle river near the Rapids and decreases in the upper river or spawning locations, the trend could be interpreted as mortality due to *Ichthyophonus* infection during upriver migration (Kocan et al., 2004). However, the prevalence of infection in our study stayed consistent across all the sampling locations based on histopathology results (Chapter 2). Potentially, the reason the prevalence of infection stays consistent is that there are some fish that were undetectable by diagnostic techniques at the lower river locations, but the infection continued to progress during upriver migration and those individuals became detectable for infection by the time they reach the upriver locations. Even if some fish may have died due to *Ichthyophonus* infection, they were essentially replaced by other infected individuals, therefore, the prevalence appears to stay the same throughout the migration (Chapter 2). This interpretation of how the *Ichthyophonus* infection is progressing in king salmon during migration is based on evidence suggesting they are not able to naturally clear the infection (Hershberger et al., 2012), the infection continues to progress during upriver migration (Kocan et al., 2004; Kocan & Hershberger, 2006), limitations due to the diagnostic techniques (White et al., 2013), and reports from subsistence fishermen that helped support the hypothesis being tested. Understanding how *Ichthyophonus* infection impacts individual king salmon and how the severity of infection varies at different points along the river is crucial to suggest a linkage between in-river mortality of king salmon and *Ichthyophonus* infection during upriver migration.

From a diagnostic perspective, visual detection of heart lesions can be helpful, but it is not always accurate depending on the harvest location and may misdiagnose the actual *Ichthyophonus* infection rate. In places such as Rapids and Fort Yukon, the visual identification of lesions typical of *Ichthyophonus* infection was very reliable, but it still does not tell the whole story. It is important to pair the visual assessments for *Ichthyophonus* infection with more precise

diagnostic tools such as histopathology and qPCR. Together, all these methods can provide a more comprehensive picture of the impact of *Ichthyophonus* on each individual host. It is equally important to work with communities and people who are on the river and see changes in real time.

To assess how well the severity of *Ichthyophonus* infection is estimated by counting the number of visual lesions typical of *Ichthyophonus* infection on the heart surface, we used linear regression to compare the estimated number of lesions counted on the heart to the laboratory measure of infection severity, based on histopathology and qPCR results at the Pilot Station Sonar, Rapids, and Fort Yukon sampling sites (Figures 3.3 and 3.4). The coefficient of determination (R^2) represents how well the estimated number of visual lesions counted predicts the histopathology or qPCR infection count. There was no data collected from Eagle Sonar to estimate the number of lesions on the heart surface, since only the presence and absence of lesions was recorded.

Based on the relationship between visual signs of infection and laboratory diagnostic techniques, as the severity of *Ichthyophonus* infection increases, the estimated number of visual lesions present on the heart surface also increases. However, this relationship is not always straightforward. The distribution of infection within the tissue is random, and the size of the parasite within the tissue may vary across different river locations, which affects how accurate the visual diagnostic of *Ichthyophonus* is for estimating infection severity. This is particularly important in considering severity assessments based on qPCR data, given that parasite size and life stage likely impacts read count results. While qPCR provides a relative assessment of abundance in this approach, read counts are likely not comparable between years and studies due to inherent biases of molecular amplification techniques (Aird et al., 2011; Polz & Cavanaugh, 1998). The king salmon at Pilot Station Sonar are highly infected based on laboratory diagnostic techniques, but interestingly largely the infection has not progressed to causing visual signs of infection. As fish move upriver, the infection appears to progress with the parasites potentially growing and displacing more tissue, potentially associated with enhanced host inflammatory response which could be linked to the increase of visible lesions on the surface of the heart. This trend is observed by what is seen at the Rapids and Fort Yukon sites, where the infection has had time to develop to clinical disease.

3.8 Concluding thoughts

By partnering with the subsistence fishermen from Tanana and Fort Yukon, we were able to learn directly from their expertise and knowledge through the entire research process, as well as holding the community meetings in collaboration with the Tribal Governments in Rampart and Fort Yukon to co-interpret project results. Unfortunately, we were not able to schedule a community visit to Tanana prior to this thesis completion date; however, will continue to attempt to work alongside communities for outcomes of this research in the future. Each community and person may have a slightly different interpretation of the findings of this research from the Yukon River, and it is important to uplift Indigenous and local voices continuously. By working directly with the fishermen on the river, using their fishing gear, and boating on the river with people who have spent most of their lives in the Yukon River drainage, a lot of valuable stories, lessons, and knowledge shaped by place are not written here, but not lost in the process. This includes lessons on how to collect samples with minimally invasive techniques to ensure the fish can still be fully utilized for subsistence use, safely working in remote field camps, and respectfully working in rural and Alaska Native communities. The success of the project at the Rapids and Fort Yukon sampling sites would not have been possible without the contributions of the subsistence fishermen who shared their time and expertise to this project. We aim to pave the way for more Tribal-led, -informed, and -designed monitoring efforts to protect salmon populations in the Yukon River and maintain culturally important practices. By pairing Indigenous, local, and western knowledge systems, we can create more holistic understandings about the health of salmon and the consequences of research findings. This project shows the power of integrating knowledge systems to better achieve the overall goals of the research. Analyzing the research in this way allows this graduate thesis to be beneficial to multiple groups, including federal and state agencies, universities, Tribal Governments, and resource users. It can also help repair the relationships between communities and researchers, as a lot of previous research practices have caused harm to communities and Indigenous peoples that are largely unacknowledged through the research process. This history creates distrust with the community and limits engagement (Herkshan et al., 2024). A community dialogue participant voiced research as an “ugly word” speaking to the historic practices and harms caused by researchers.

The hypothesis at the forefront of this work, which suggests *Ichthyophonus* could be causing mortality of king salmon during upriver migration, is largely based on observations from

Indigenous and local knowledge holders observing presence of visual signs of infection caused by *Ichthyophonus* in subsistence harvests across the Yukon River drainage, while we acknowledge it is not the only challenge salmon face. Reports from the Rapids area suggest a high presence of *Ichthyophonus* infection in king salmon, while in the upper river, near the Fort Yukon and Eagle area, *Ichthyophonus* infection was reported less, when a high *Ichthyophonus* presence was observed at Rapids in the middle river. This suggests that mortality due to *Ichthyophonus* infection may occur between the middle and upper reaches of the Yukon River in the Alaska portion of the drainage. These observations also align with earlier work by Kocan et al. (2004), which pointed to the upper reaches of the Yukon River as a hotspot for *Ichthyophonus* related mortality.

We co-interpreted thesis results with the Tribal Councils and several community members from Rampart and Fort Yukon in community meeting dialogues. Although some recognize *Ichthyophonus* should be a concern for fishery management, most do not want the disease to be labeled as the primary reason for the king salmon collapse and continued low run abundance in the Yukon River, recognizing disease is only one aspect that can be impacting the Yukon River king salmon. Additionally, some questioned what happened to the king salmon that may have died during the upriver migration. The participants suggested that people living on the river would be able to see the dead fish on the banks of the river. Therefore, participants questioned the likelihood of them dying in-river, as they did not see them washing up on the riverbanks or gravel bars. Some participants suggested the large king salmon will sink and would not be noticed due to the vastness of the Yukon River mainstem. Others suggested if mortality was occurring, the carcasses would wash up somewhere, but that has not been reported. We believe it is important to continue to report observations, and to document the possible causes of mortality if any washed-up carcasses are seen in future years and continue to explore these larger questions or research suggestions in partnership with the sovereign Tribal Governments and Yukon River residents. It is equally important to explore the possibilities raised by dialogue participants about salmon seeking colder waters during migration, the impact of marine bycatch, and the multitude of environmental stressors that impact salmon throughout their entire lifespan.

Some community members were cautious of including mortality estimates in management, as that would be one more reason hindering people from subsistence fishing since management would likely need to be more conservative to account for in-river mortality.

Participants also shared frustration with fishery management agencies and the restrictions placed on in-river subsistence people, despite the ocean fisheries that still occur and have high reports of bycatch impacting the survival and return of salmon bound to the Yukon River. These frustrations were elevated by yet another research project that only focused on the in-river Yukon fishery with results that can potentially support more conservation measures on in-river users, who have already sacrificed all their fishing rights, despite not addressing the entire lifecycle of Yukon salmon, and the conservation equity between all user groups in order to rebuild Yukon River salmon stocks for subsistence or customary and traditional use.

This research is foundational to guide future Tribal-led efforts that monitor salmon health in the Yukon River drainage by showcasing an example of locally led monitoring from what can be seen visually in subsistence harvest on a key issue impacting Yukon River king salmon. We emphasize the value of pairing IK within western scientific findings to address complex ecological challenges in future research. We encourage other researchers interested in working with Indigenous communities to use the framework proposed by Harris and Wasilewski (2004), which emphasizes the importance of relationship, responsibility, reciprocity, and redistribution when proposing or conducting Tribal-led research. These values are crucial in ensuring that research methodologies respect and integrate Indigenous ways of knowing, build equitable partnerships between Tribal communities and researchers, as well as engaging in dialogues that can be uncomfortable and tense at times given the importance of many topics of interest to the communities and researchers. Our community dialogues in this research were surrounding one of the most cultural and societally important salmon species in the Yukon River, and we needed these values at the forefront of our work to build trust, and to listen to the Tribal member participants to explore all the possible causes of the king salmon decline to contextualize the research findings with the participants.

Reid et al. (2021) highlights another important framework based on the benefits of a "Two-Eyed Seeing" approach to pair western science with IK. This approach challenges the common narrative that IK systems are only meaningful when they align with western scientific findings. By recognizing IK as distinct and valuable in its own right, we can create research processes that respect and honor all knowledge systems. "Two-Eyed Seeing" offers a holistic approach that pairs the strengths of both Indigenous and western knowledge systems to answer critical research questions and guide resource management decisions. In this thesis research, we

paired monitoring techniques developed by a local subsistence fisherman to quantify *Ichthyophonus* infection in Yukon king salmon, worked alongside Tribal Governments and subsistence fishermen to conduct the work, and co-interpreted the findings from multiple perspectives by sharing preliminary project results. This allowed for dialogue between the researchers and community participants throughout the research process. Indigenous and local knowledge was shared throughout the harvest of fish during the sampling process, as well as how the research results were reflected upon. Community meeting participants highlighted the need to not forget about all the other potential explanations on where fish are going during migration, such as observations from Yukon River residents about the changing salmon migration patterns due to warmer water in the Yukon River mainstem, and all the other impacts salmon face during their lifespan. We paired the knowledge about what people can see in their harvest with respect to *Ichthyophonus* infection at multiple locations along the Yukon River mainstem, and how that could be critical to understanding the impacts of disease on overall salmon health. However, during this process we are not attempting to validate IK and LK about the impacts of *Ichthyophonus* on salmon health; we instead are pairing IK and LK alongside western science to help interpret our results and recognize the importance of IK and LK in shaping our research hypothesis by valuing all perspectives through dynamic inclusivity. We believe other researchers should consider pairing multiple knowledge systems not only to contribute to scientific understanding on their subject of interest, but to strengthen community engagement, foster trust, and empower communities to share their knowledge within existing research and management structures (Castleden et al., 2012; Wallerstein and Duran, 2010; Wilson et al., 2018). The inclusion of Indigenous voices in the research process ensures that the outcomes reflect the needs and priorities of the communities most affected by changes in salmon populations and health. These principles of equitable and inclusive research are essential to the management of fisheries and the stewardship of natural resources.

In conclusion, the research presented here not only sheds light on the progression of *Ichthyophonus* infection in king salmon during upriver migration from Indigenous, local, and western knowledge systems, it shows historical local-led monitoring of *Ichthyophonus* disease based on visualizing the infection within subsistence harvest and highlights the importance of incorporating multiple perspectives in understanding the health of salmon populations. Moving forward, it is vital that researchers continue to foster respectful and meaningful collaborations

with Indigenous communities when working with culturally important resources. Although we present the research here as a foundational step forward for more collaborative research and co-stewardship, we recognize future research which aims to be led, informed, and designed by Tribal Governments and Indigenous peoples must be co-developed alongside researchers from the very beginning of any research process.

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3.10 Figures and tables

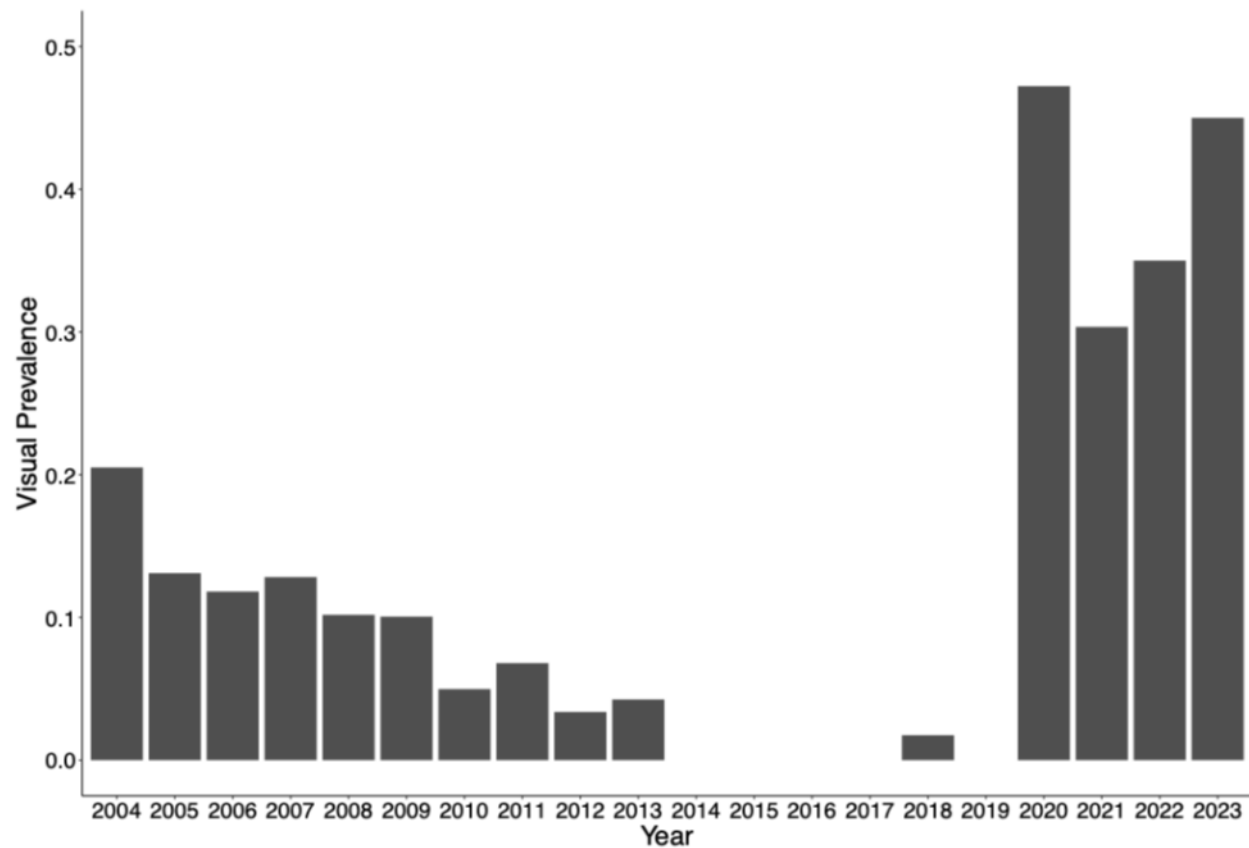


Figure 3.1. The prevalence of *Ichthyophonus* infection based on the percentage of king salmon that exhibited 3+ visual lesions on the heart surface typical of *Ichthyophonus* infection at the Rapids in the middle Yukon River near Tanana and Rampart, Alaska. Monitoring from 2004-2021 conducted by Stanley Zuray, and monitoring conducted in 2022 and 2023 as part of this graduate research using the same method of determining visual signs of infection. There was no data collected in 2014-2017, and 2019, however, Mr. Zuray noted low presence of infected king salmon during the years with no official estimate (S. Zuray, personal communication, October 14, 2021).

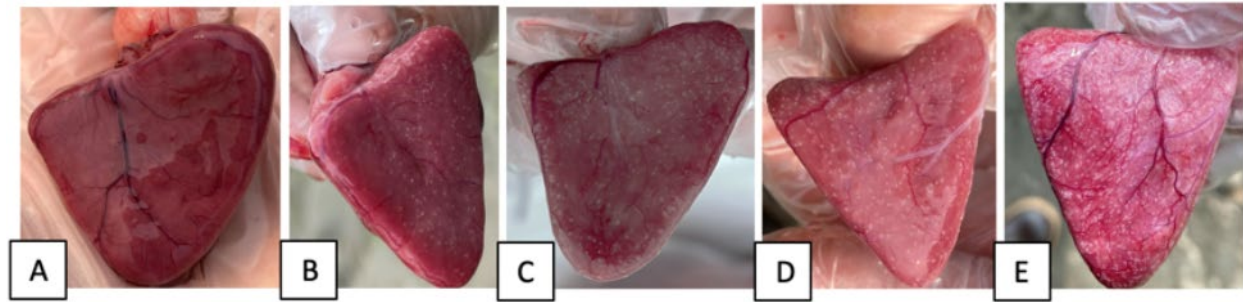


Figure 3.2. Estimating the number of visible lesions on king salmon heart muscle typical of *Ichthyophonus* infection. Examples of *Ichthyophonus* infection severity: (a) normal heart muscle, no infection; (b) 3-99 visual lesions present; (c) 100-499 visual lesions present; (d) 500-999 visual lesions present; (e) 1000+ visual lesions present. This is only an example to show various stages for the number of visible lesions in king salmon harvest at the Rapids and does not categorize infection severity.

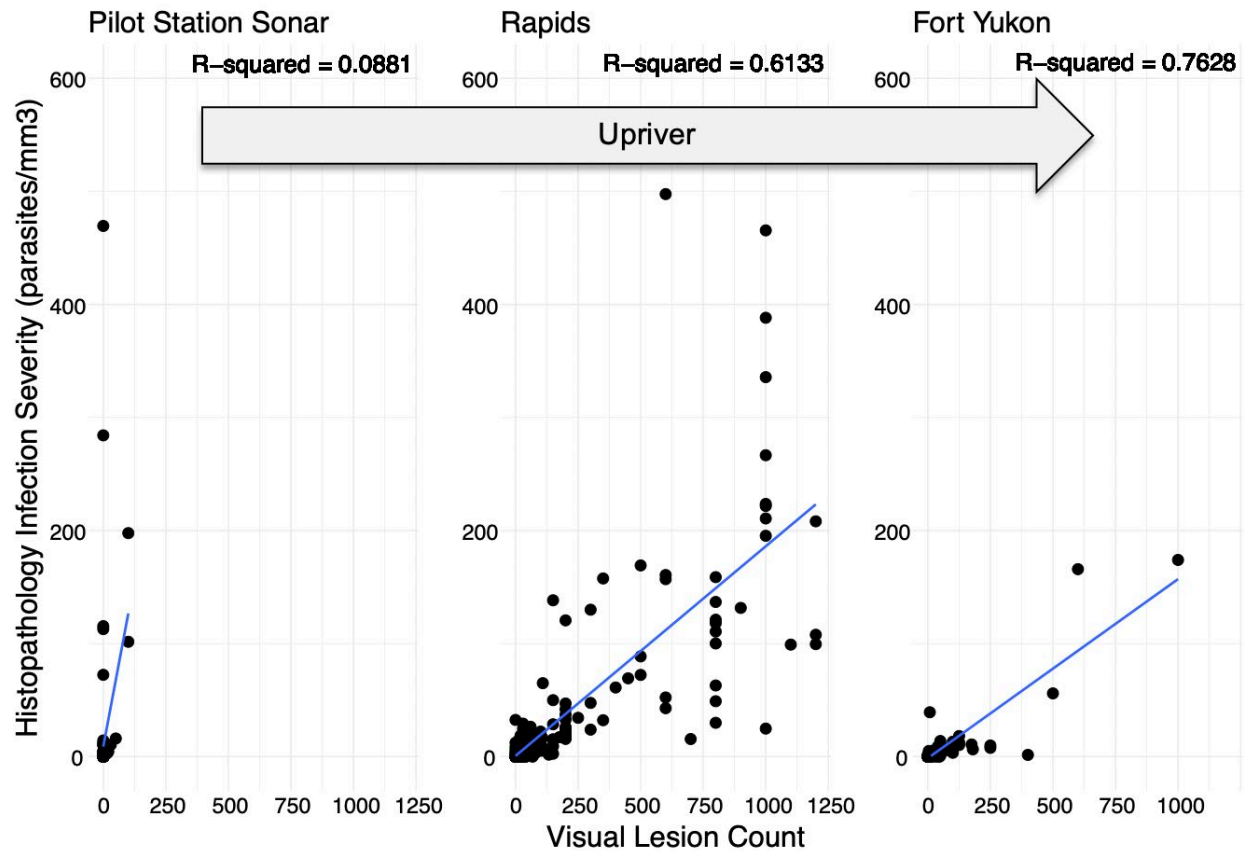


Figure 3.3. Linear Regression for *Ichthyophonus* infection severity based on histopathology compared to the estimated visual lesions counted on king salmon heart surfaces. Sites are arranged from downriver to upriver, left to right.

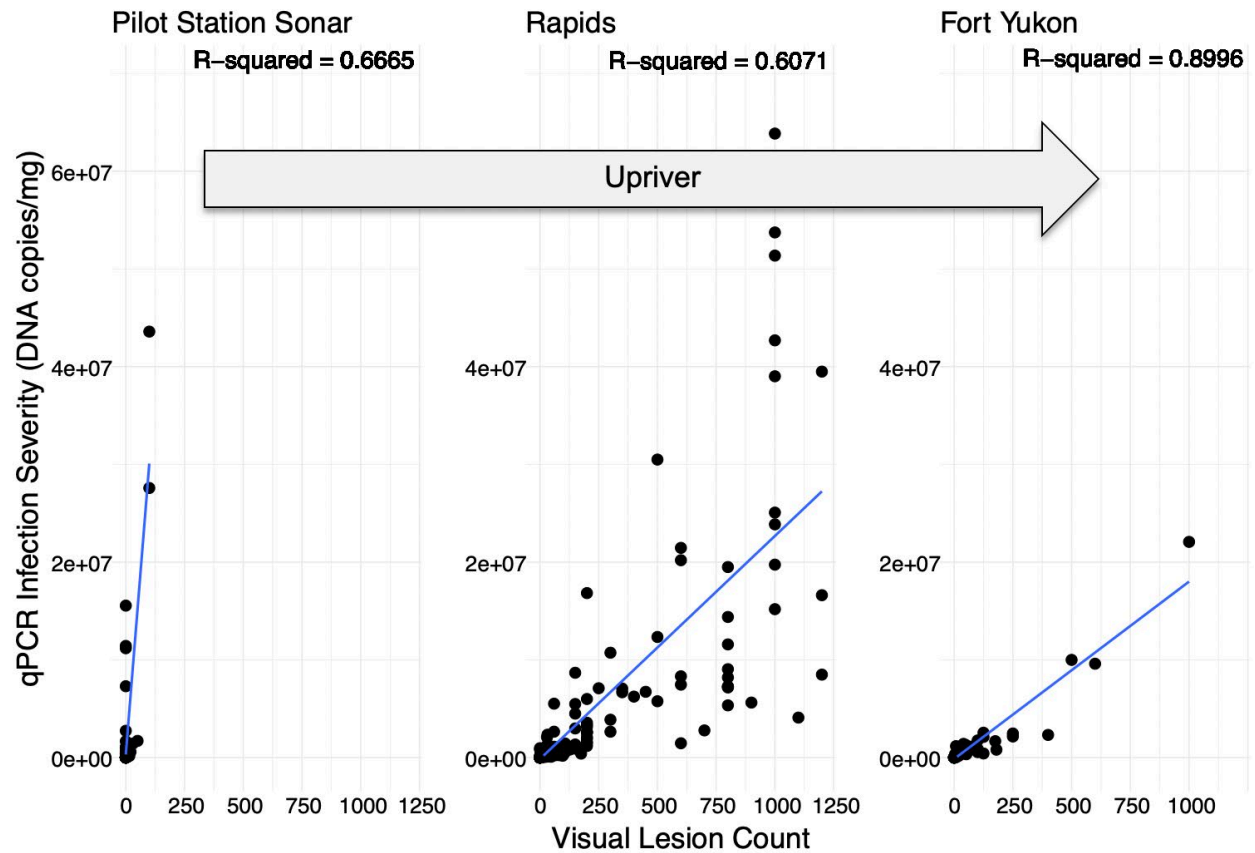


Figure 3.4. Linear Regression for *Ichthyophonus* infection severity based on qPCR compared to the estimated visual lesions counted on king salmon heart surfaces. Sites are arranged from downriver to upriver, left to right.

Table 3.1. A table to compare the presence (Yes) and absence (No) of visual lesions on king salmon heart surfaces and the confirmation of *Ichthyophonus* infection across Yukon River locations.

Sampling site	Visual lesions	<i>Ichthyophonus</i> infection	n	Number of lesions
Pilot Station Sonar	No	No	206	unknown
	No	Yes	108	
	Yes	No	6	
	Yes	Yes	38	
Rapids	No	No	217	3 and 4
	No	Yes	12	
	Yes	No	2	
	Yes	Yes	149	
Fort Yukon	No	No	57	5
	No	Yes	1	
	Yes	No	1	
	Yes	Yes	31	
Eagle Sonar	No	No	30	
	No	Yes	9	
	Yes	No	0	
	Yes	Yes	11	

Table 3.2. A table to compare the percentage agreement and disagreement between visual lesions typical of *Ichthyophonus* infection and the confirmation of *Ichthyophonus* infection across Yukon River locations. Overall agreement calculation example (Rapids): 149 king salmon had visual lesions and confirmed *Ichthyophonus* infection representing the numerator. 2 king salmon had visual lesions and did not have confirmed *Ichthyophonus* infection. 12 king salmon did not have visual lesions but had confirmed *Ichthyophonus* infection. The denominator represents the 163 king salmon with either visual lesions or confirmed infection.

Sampling site	Category	Yes	No	Overall Agreement
Pilot Station Sonar	Agree	38/ 44 (86.4%)	206/314 (65.6%)	38/152 (25.0%)
	Disagree	6/ 44 (13.6%)	108/314 (34.4%)	
Rapids	Agree	149/151 (98.7%)	217/229 (94.8%)	149/163 (91.4%)
	Disagree	2/151 (1.3%)	12/229 (5.2%)	
Fort Yukon	Agree	31/ 32 (96.9%)	57/ 58 (98.3%)	31/ 33 (93.9%)
	Disagree	1/ 32 (3.1%)	1/ 58 (1.7%)	
Eagle Sonar	Agree	11/ 11 (100.0%)	30/ 39 (76.9%)	11/ 20 (55.0%)
	Disagree	0/ 11 (0.0%)	9/ 39 (23.1%)	

Chapter 4: General Conclusion

Overall, this thesis intended to uplift Indigenous Knowledge (IK) and pair it with western fisheries research to co-interpret research outcomes. However, during this process, we did not seek to validate the IK or local knowledge (LK) shared about the impacts of *Ichthyophonus* on salmon health. Instead, we paired IK and LK systems with western science to interpret our results and recognize the importance of multiple knowledge systems in shaping our research hypotheses by uplifting all perspectives. The overall objectives for this thesis research aimed to be solution-oriented by (1) conducting a research study to investigate the impacts of *Ichthyophonus* on Yukon River king salmon during upriver spawning migration, (2) co-interpreting research findings with Tribal Government Councils and community members to explore the health of Yukon River king salmon by combining multiple knowledge systems (Indigenous, local, and western), and (3) co-develop research recommendations moving forward for a more complete assessment of the health of king salmon in the Yukon River.

The second chapter of this thesis addresses the first objective. The main takeaway of Chapter 2, which explored the association between ichthyophoniasis disease progression and in-river mortality of adult king salmon during spawning migration provides evidence to strongly suggest a linkage between *Ichthyophonus* and in-river mortality. Chapter 2 and 3 covers the second and third objectives. The main takeaway of Chapter 3, where we attempted to co-interpret the linkage between *Ichthyophonus* disease progression and in-river mortality during community dialogue meetings highlights the importance of disease not becoming a scapegoat to dismiss the impacts of marine bycatch and all the other factors that impact salmon throughout their lifespan. Additionally, Chapter 3 highlights the importance of future community-based and Tribal-led monitoring and research that includes IK and youth developed by each community's values and goals.

In future research that aims to center tribal-led or community-based research, I recommend ideas are further refined and developed alongside Yukon River communities and Tribal Governments from the beginning of the research proposal process. I sought to implement methodology within this research after being handed a project that had already been set in motion to better engage communities and Tribal Governments, but I do believe future research can be more collaborative with Tribal governments and communities by working together from the developmental stages. The overall information gained through this graduate thesis can

ultimately be helpful to better inform equitable fishery management decisions focused on restoring salmon runs and health. To achieve these objectives throughout the research process, I aimed to co-interpret the graduate thesis research outcomes utilizing methodologies that respect and integrate Indigenous ways of knowing, as well as build equitable partnerships between communities and myself as a researcher. My intent was to uplift multiple knowledge systems together not only to contribute to the scientific understanding of the impact the parasitic pathogen *Ichthyophonus* has on Yukon River king salmon during upriver migration, but also to strengthen community engagement, foster trust, and empower the communities I worked alongside to be included in the research process. This collaboration with several community members helped share their knowledge and ensure their participation in the research process to gather information that could inform the management of fisheries (Castleden et al., 2012; Wallerstein & Duran, 2010; Wilson et al., 2018).

Although I recognize this research as a foundational step forward to co-stewardship, I acknowledge the process I present here had its shortcomings. Although I attempted to implement measures to reduce research harm and be inclusive of multiple knowledge systems in my thesis outcomes, I wish I could have engaged more Yukon River residents to be part of the research process, and engaged more Yukon River Tribal Governments, and organizations such as the Yukon River Intertribal Fish Commission (YRITFC) and Yukon River Drainage Fisheries Association (YRDFA) which have immense drainage-wide representation of Yukon River communities, fisherman, and leaders. I do have the opportunity as part of the larger research team to increase collaboration on future project reports as this thesis is based on the first two years of data, as part of a larger 3-year drainage-wide project led by the Alaska Department of Fish and Game (ADF&G) and United States Fish and Wildlife Service (USFWS). I acknowledge the concerns I have heard about lethal sampling during historic low salmon run abundance, and share the same concerns, the decision to be part of the research that required lethal sampling of fish during these times was not taken lightly. Moreover, I would like to acknowledge that the thesis research presented here does not represent all the other paired samples taken from the fish utilized in this study to monitor fish health more generally in the Yukon River, including important samples taken for investigating heat stress, egg productivity and nutrient levels, blood samples for potential non-lethal stress biomarkers, or the impact of multiple stressors interacting. I ultimately wanted to be part of a research project that was set in motion and help conduct it in

the best way possible in collaboration with the subsistence fishermen and communities. The initial concern about the resurgence of *Ichthyophonus* and its impact on king salmon migration success was raised from a local fisherman.

The success of the project at the Rapids and Fort Yukon sampling locations would not have been possible without the contributions of the subsistence fishermen who gave their time and expertise to the successful completion of this project. By highlighting the importance of this type of inclusive research, I aim to advocate for more Tribal-led, -informed, and -designed efforts to monitor and protect salmon populations in the Yukon River. This type of advocacy was raised as an important outcome in this research from community meetings. By integrating IK, LK, and western systems of knowledge, we can create more holistic understandings about the health of salmon and the consequences of research findings. This project shows the power of integrating multiple knowledge systems to better achieve the overall goals of the research. Analyzing the research in this way allows this graduate thesis to be beneficial to multiple groups, including federal and state agencies, universities, Tribal Governments, and resource users, as well as repair relationships between communities and researchers. Many previous research practices have caused harm to communities and Indigenous peoples that remain largely unacknowledged, creating distrust and limiting engagement (Herkshan et al., 2024). I tried to reduce harm as much as possible by being transparent throughout the research process by engaging Tribal Governments, Yukon fishery organizations who help inform management and research, and work with community subsistence fishermen throughout the entire research process. I shared preliminary thesis project results with two Yukon River Tribal councils and communities, and plan to follow-up with the final report to not make the same damaging mistake described in community meetings of past research practices. I valued and embraced the journey of researching Yukon salmon health with relational accountability to the fish, the communities and Indigenous peoples of the Yukon River who continue to practice traditional stewardship to care for our salmon relatives throughout the whole graduate thesis process.

I encourage future researchers to work alongside Tribal Councils and communities when planning research or identifying research needs to better co-develop and conduct research throughout the entire research process. I encourage researchers not only to be accountable to their funding timelines but also to be accountable to the communities and Tribal Governments near the location they are working. Research that occurs without follow-up or report-back to the

community on what has been found or done is seen as extractive and places distrust between the community and research entities (Peltier, 2018). These sentiments were shared with me during the community visits to discuss project results. There was interest from participants for Tribal Governments to have their own way of monitoring natural resources, beyond collaboration or being told what is happening from other organizations. Additionally, participants highlighted the need to include youth mentorship in projects to teach the next generation the importance of maintaining healthy natural resource populations and their cultural connection to land and place. By increasing the representation of IK and Tribal governance in research and western management processes, I see this as an important step toward co-stewardship of land and water resources, ensuring that Indigenous voices and knowledges are heard and documented to influence decision-making bodies. However, the idea of research and monitoring could be different depending on the methodology utilized, and I encourage researchers and western science-based agencies to not attempt to validate traditional and Indigenous knowledge systems but to be inclusive of multiple knowledge systems to inform decision-making processes.

I dream about the day when fishery management processes are based on a metaphorical fishing net, held afloat by Tribal Governance, with buoys representing the Tribes. The net mesh is made up of Tribal leaders, advocates, allies, managers, researchers, communities, culture, youth, Indigenous values, IK, LK, western knowledge, and all the pieces that make the processes whole. The net mesh is bonded together by knots based on reciprocal relationships built through respect, and common interest of restoring, and rebuilding the fishery management systems for the benefit of the people and fish to maintain spiritual and cultural practices alongside thriving natural resource populations. The fishing net is anchored by the original and present stewards, the people of the land. Together in this type of management system, we can restore balance, rebuild relationships and respect for the land which continues to provide the ways of life that are connected to our souls by building the fishing net together. In this way, we can work on rebuilding relationship to one another and our environments, the natural resource populations, and kinship to our salmon relatives and restore our relations with them as living beings once again in a fishing net that we all built together (Tamamta – “All of us”). There will always be snags to navigate in any process, but we will have the fishing net in place to repair and think of our future generations.

4.1. References

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