

## **Alaska Sustainable Salmon Fund Statement of Work**

**I. Project Title:** Evaluating *Ichthyophonus*-Associated Mortality of Yukon River Canadian-Origin Chinook Salmon

**II. Project Number:** 56002

**III. Principal Investigator**

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**IV. Project Period:** 3/1/23 – 6/30/25

**V. AKSSF Objective:** Investigate salmon declines      **PCSRF Objective:** RM&E

**VI. Project Description**

1. Synopsis

*Ichthyophonus* disease (ichthyophoniasis) resulting in natural *en route* mortality of Canadian-origin Yukon River Chinook salmon (*Oncorhynchus tshawytscha*) is a leading hypothesis to explain two successive years (2020 and 2021) of failure to achieve U.S./Canada border passage objectives per the Yukon River Salmon Agreement despite adequate run sizes detected near the mouth of the Yukon River and unprecedented harvest restrictions. This project will 1) determine if ichthyophoniasis is associated with *en route* mortality; 2) estimate the lethal *Ichthyophonus* infection “threshold” range for Canadian-origin Chinook salmon to assess the risk that observed infections pose to the annual salmon run; and 3) develop a model to assess the proportion of the annual Canadian-origin Chinook salmon run past Pilot Station sonar with infections above the lethal threshold. A fish health assessment will also be conducted to assess other relevant diseases/factors that can influence the course of ichthyophoniasis.

2. Introduction

*Ichthyophonus* is a unicellular, multinucleated parasite with both fungal and animal characteristics comprised of several different populations, strains, or species of mostly marine origin. Piscivorous fish become infected by ingesting infected prey during marine residency. Amoeboid-like infectious cells invade the stomach, enter the circulatory system, and travel throughout the body until they reach blood filtering organs, especially the heart, where the parasite increases in size through asexual division to form a large multinucleated schizont (resting stage). The infection causes a chronic inflammatory response against the parasite resulting in a granuloma that encapsulates an individual or group of schizonts. Infected fish are carriers until death. In the Yukon River, salmon become infected during their marine lifestage and return to freshwater with a set number of parasites that proliferate and disseminate in the host as the fish migrates upstream to spawn. *Ichthyophonus* infection progresses to a disease state where infected tissues are replaced, disrupted, and obliterated in severe cases. Disease severity depends on general stress and health of the fish host, where it can be subclinical with no notable impact to the host, chronic with reduced physiological performance, or acute with mortality.

*Ichthyophonus* was first detected in a Yukon River Chinook salmon in 1988. Detection of the parasite followed multiple years of subsistence reports from the

middle Yukon River that some harvested Chinook salmon smelled “fruity”, did not dry properly when smoked, and had white spots in the flesh and organs. The pathogen has become a common episodic problem with resulting impacts to subsistence harvesters and the fish population. Various agency, non-agency, and academic entities have afforded much effort to understand the social and biological impacts of *Ichthyophonus* on Yukon River Chinook salmon. Past studies have relied on prevalence data (% infection in population), which is a weak metric of parasite impacts, rather than infection intensity (#s of parasites/fish in population).

Annual *Ichthyophonus* monitoring has been inconsistent and poorly supported by fishery management agencies. In 2011, the Yukon River Panel Joint Technical Committee (JTC) recommended annual monitoring and that focused research studies should be prioritized when prevalence exceeds 25%. This was not enacted, but limited voluntary annual monitoring by Mr. Stan Zuray (fisherman and biologist with Rapids Research Center) of clinically diseased fish sampled from subsistence harvest occurred in 2011–2013, 2018, and 2020 that were confirmed by histopathology conducted by Dr. Kocan with the University of Washington. In 2020, Mr. Zuray reported a high prevalence of ichthyophoniasis and concerns were further elevated post-season due to discrepancies between expected and observed Chinook salmon abundance at Eagle sonar near the U.S.-Canada border. High ichthyophoniasis and poor run sizes past the U.S.-Canada border was again observed in 2021.

Ichthyophoniasis-associated *en route* mortality is currently a leading hypothesis for the observed poor run sizes into Canada. *Ichthyophonus* monitoring and impacts on Chinook salmon inriver survival is a notable knowledge gap that hampers the ability of management to use lower river assessments to effectively manage the Canadian stock. Currently, *Ichthyophonus* infection and disease are not formally assessed and estimates of disease associated mortality are not available. The overall goal of this project is to establish a new long-term *Ichthyophonus* disease monitoring platform in the lower Yukon River to inform fishery management by determining if *Ichthyophonus* is associated with *en route* mortality and, if so, estimate annual disease-associated mortality. This approach will also build capacity and support for community-based *Ichthyophonus* monitoring.

This project will also conduct a fish health assessment using qPCR and histopathology on both heart and kidney tissue. Data will be paired with those from a collaborative UAF project (and USGS project, if funded) assessing heat stress and *Ichthyophonus* disease markers to assess other relevant diseases/factors that can influence the course of *Ichthyophonus* disease.

### 3. Locations

- Pilot Station sonar test fishery: 61.933275, -162.860489
- Rampart Rapids: 65.338797, -151.07157
- Eagle sonar test fishery: 64.873278, -141.0792

## VII. Objectives

1. Determine if *Ichthyophonus* disease is associated with *en route* mortality
2. Estimate the lethal *Ichthyophonus* infection “threshold” range for Canadian-origin Chinook salmon to assess the risk that observed infections pose to the annual salmon run
3. Develop a model to assess the proportion of the annual Canadian-origin Chinook salmon run past the Pilot Station sonar that has *Ichthyophonus* infections above threshold levels associated with *en route* mortality
4. Assess other relevant diseases/factors that could influence the course of *Ichthyophonus* disease

## VIII. Methods

The long-term goal of this project is to leverage the existing Pilot Station sonar platform for annual disease monitoring. That approach differs from the historical opportunistic monitoring at Rampart Rapids, so this project will require establishing relationships between disease prevalence and severity estimates between these two sites and linking that relationship to estimates of lethal parasite “thresholds”. *Ichthyophonus* proliferates during salmonid upstream freshwater migration, so infection intensities in fish entering the river will likely positively correlate to higher infection intensities observed at upriver sites before disease-associated *en route* mortality occurs. Comparison of results from Pilot Station and Rampart Rapids will be used to define a new parameter “Ramp Rate” that represents the amount of parasite proliferation between the two sites. Ramp Rate will be needed to link future infection observations from annual monitoring at Pilot Station to expectations of *en route* mortality between Rampart Rapids and Eagle (Objectives 1 and 2). Estimates of *en route* mortality will be based on lethal parasite “thresholds” using a retrospective analysis of the frequency distribution of infection intensity for fish surviving from Rampart Rapids to Eagle, near the U.S./Canada border. Unique to this study is that fish will be sampled prior to Parasite-Associated Mortality (PAM) at Rampart Rapids to corroborate and ground truth conventional modelling approaches used in parasitology. Establishing the Ramp Rate and lethal threshold will provide data needed to annually test fish at Pilot Station to estimate the proportion of fish above the lethal threshold in a large sample (Objective 3). This project will also collect additional data on other diseases and heat stress to assess co-variants that may influence the course of ichthyophoniasis and influence the variability in key model parameters.

Samples will be collected from the Pilot Station and Eagle sonar test fisheries by ADF&G staff, and those from Rampart Rapids will be taken by USFWS with support from local experts. Samplers will be trained on proper sample collection, grossly assess clinical disease (i.e., presence of white lesions), and collect two samples of heart and kidney from each Chinook salmon for histological evaluation and qPCR analysis, respectively. This study will require a sample size of 180–200 fish per location to detect a significant difference in parasite burden between sample sites with a power of  $\geq 80\%$ . Sample size was based on literature review and a power analysis with a limited dataset from the Fish Pathology Lab’s Disease History files. Sacrificed salmon from the Eagle test fishery will be distributed to tribal members, City of Eagle residents, and Canada Department of Fisheries and Oceans for Canadian First Nations.

Infection intensity will be assessed by both qPCR with published protocols and histology using standardized methods. qPCR results will be reported as parasite gene copy #/g and histology data will be # of schizonts/mm<sup>3</sup>. DNA copy number is a proxy for parasite burden that can provide an estimate of parasite biomass. qPCR is a high-throughput test that can rapidly screen many samples to provide near real-time results. Histology broadly evaluates pathologic conditions associated with infections, and multiple sections will be evaluated to increase the detection sensitivity. Parasite enumeration and tissue volume will be measured using photomicroscopy software.

Objective 1 will be addressed by comparing infection prevalence, intensity, abundance, and Variance to Mean Ratios (VMR) from Chinook salmon sampled from all 3 sites. Declines in these metrics have been used to infer PAM in wild fish populations in the literature. The VMR of parasite infections represents the degree of parasite dispersion within the host population that is typically over-dispersed so a reduction in VMR can indicate density dependent mortality. The VMR is a normalized measure of dispersion of a probability distribution used to quantify whether a set of observed occurrences are clustered or dispersed compared to a standard statistical model. Differences in prevalence and VMRs between sample locations will be tested with Chi-squared analysis whereas infection intensity and abundance comparisons will be made using either parametric or non-parametric tests of means, whichever is appropriate for the distribution assumption of normality.

A retrospective analyses on fish that survive to be sampled at Eagle will be performed to address Objective 2. This approach has been used to infer PAM in many host-parasite systems. Typically, researchers do not have access to fish prior to PAM so statistics have been developed to estimate the distribution of parasites prior to mortality based on observed data in sampled survivors and the assumption that the parasite persists within the host. The key concept of this approach is that the distribution of parasites in the host population pre- and post- mortality are compared. Data from the sampled survivors are used to estimate the parameters of the distribution, which is then compared to the observed parasite distribution in the surviving fish. If PAM has occurred, then the two distributions would be significantly different due to the loss of heavily infected fish at the tail end of the distribution. Therefore, an iterative procedure of truncating the expected distribution is performed until the observed distribution significantly fits the truncated expected distribution. The infection intensity where these two distributions fit then becomes the estimated lethal threshold for infections. Unique to this study is that data will be obtained from fish sampled at Rampart Rapids where historically the heaviest infections occur prior to declines in infection, so there is an opportunity to compare infections in pre-mortality fish to the lethal threshold derived from survivors using this model to further strengthen conclusions.

Objective 3 will be accomplished by using lethal thresholds generated from Objective 2 and parasite burden estimated annually from Pilot Station after adjusting for the Ramp Rate of infection between Pilot Station and Rampart Rapids. Infections at Pilot Station will be multiplied by the Ramp Rate and these adjusted infection intensities would then be compared to the estimated threshold levels. The proportion of fish above that threshold

in the representative samples tested at Pilot Station would reflect a similar proportion of fish at risk for disease-associated mortality for the larger population.

For Objective 4, a fish health assessment using qPCR and histopathology on both heart and kidney tissue will be conducted and data will be paired with those from a collaborative UAF project (and USGS project, if funded) assessing heat stress and *Ichthyophonus* disease markers. This is needed to assess other relevant diseases/factors as co-variants of ichthyophoniasis.

Success in meeting these objectives will be determined by results from statistical testing and comparing model outputs with fish abundance estimates annually.

## **IX. Benefits**

This project contributes to sustaining salmon populations by researching the leading hypothesis of ichthyophoniasis as a main contributor for the dramatic difference in Chinook abundance estimates between sonar projects on the Yukon River. Clarifying how this disease causes *en route* mortality and developing a model and methods for ongoing monitoring will facilitate management of the stock and maximize subsistence fishing opportunity on both sides of the border.

## **X. Products, Milestones, and Timelines**

- January-May 2023 & 2024: Continue community outreach and discussions with local stakeholders
- February-March 2023 & 2024: Order sampling supplies
- April-May 2023 & 2024: Renew required permits and cooperative agreements; prepare and ship sampling supplies
- June-August 2023 & 2024: Sample test fisheries for *Ichthyophonus* starting in June at Pilot Station sonar, late June in Rampart Rapids, and early July at Eagle sonar with each location sampling for about 1.5 months; sampling will be complete by mid-August
- July-September 2023 & 2024: Return samples to the lab for analysis
- September-December 2023 & 2024: Perform laboratory and statistical analysis
- October-December 2023 & 2024: Develop mortality model
- January-March 2024 & 2025: Write annual project reports
- Periodically in 2024 & 2025: Present results to stakeholders, Yukon River JTC, American Fisheries Society meetings, and other venues
- December 2025: Provide peer reviewed final report to AKSSF

## **XI. Project Budget**

| <b>Summary Budget</b> | <b>Total</b> |
|-----------------------|--------------|
| 100 Personnel         | \$124,567    |
| 200 Travel            | \$4,948      |
| 300 Contractual       | \$16,740     |
| 400 Supplies          | \$59,454     |
| 500 Equipment         | \$0          |

|              |           |
|--------------|-----------|
| Subtotal     | \$205,709 |
| 600 Indirect | \$35,887  |
| Total        | \$241,596 |

This project funds the following entities:

- ADF&G CF: \$160,610
- USFWS: \$80,986

#### **ADF&G CF Budget**

| <b>ADF&amp;G CF Budget</b>   | <b>Total</b> |
|------------------------------|--------------|
| 100 Personnel                | \$76,855     |
| 200 Travel                   | \$1,868      |
| 300 Contractual              | \$14,600     |
| 400 Supplies                 | \$46,544     |
| 500 Equipment                | \$0          |
| Subtotal                     | \$139,867    |
| Expenses subject to indirect | \$76,855     |
| 600 Indirect                 | \$20,743     |
| Total                        | \$160,610    |

#### **ADF&G CF Budget Narrative:**

##### Line 100: Personnel (\$76,855)

The ADF&G CF Admin Project System was used to estimate personnel costs. The monthly total salaries include salary, premium pay (overtime, swing shift, and grave shift), and benefits.

- Elizabeth Lee, Fisheries Geneticist I (PCN 11-5073), will coordinate with project staff for genetic sampling, analyze genetic data, summarize genetic findings, and complete reporting:
  - FY24: 0.5 months @ \$8,884/month = \$4,442
  - FY25: 0.5 months @ \$9,152/month = \$4,576
- Jodi Estrada, Fishery Biologist II (PCN 11-1933), will supervise laboratory procedures and conduct quality control analyses:
  - FY24: 0.25 months @ \$8,132/month = \$2,033
  - FY25: 0.25 months @ \$8,376/month = \$2,094
- Zach Pechacek, Fishery Biologist I (PCN 11-7018), will prepare samples for genotyping and conduct sequencing to produce genetic data:
  - FY24: 0.5 months @ \$8,070/month = \$4,035
  - FY25: 0.5 months @ \$8,314/month = \$4,157
- Davis Stewart, Laboratory Technician (PCN 11-5281), will: prepare field kits for sample collection and coordinate shipping of field kits; perform DNA extractions and qPCR testing of samples; provide support with preparing histological sections for the Fish Pathologist; and analyze qPCR data and summarize results for the Fish Pathologist for final reporting:
  - FY23: 0.35 months @ \$7,572/month = \$2,650
  - FY24: 0.88 months @ \$7,799/month = \$6,863
  - FY25: 0.53 months @ \$8,033/month = \$4,258

- Kevin Sailors, Fish and Wildlife Technician (FWT) II (PCN 11-1528), will sample Chinook salmon harvested at the Pilot Station test fishery during June and July annually:
  - FY23: 1.0 month @ \$10,785/month = \$10,785
  - FY24: 1.5 months @ \$11,109/month = \$16,664
  - FY25: 0.5 months @ \$11,442/month = \$5,721
- Payton Russel, FWT II (PCN 11-5193), will sample Chinook salmon harvested at the Eagle test fishery and assist with fish preservation for community distribution annually in August (ADF&G are covering July costs as an in-kind contribution):
  - FY24: 0.5 months @ \$8,450/month = \$4,225
  - FY25: 0.5 months @ \$8,704/month = \$4,352

Line 200: Travel: (\$1,868)

Davis Stewart, Franklin Woitel, or Jayde Ferguson from the Anchorage ADF&G Fish Pathology Lab will travel from Anchorage to Fairbanks to train field crew on *Ichthyophonus* sampling both years of the project:

- Airfare: 1 RT ticket/year @ \$264/ticket x 2 years = \$528
- Per diem: 2 days/year @ \$45/day x 2 years = \$180
- Lodging: 1 night/year @ \$260/day x 2 years = \$520

Payton Russel will travel from Fairbanks to Eagle to conduct field sampling both years of the project:

- Airfare: 1 RT ticket/year @ \$320/ticket x 2 years = \$640

Line 300: Contractual: (\$14,600)

- Genetics lab equipment maintenance contract: 500 samples/year @ \$2.00/sample x 2 years = \$2,000
- Eagle Village contract to implement fish distribution of harvested fish to tribal members, City of Eagle residents, and Canada Department of Fisheries and Oceans for Canadian First Nations (includes 28 days of work, boat rental, and fuel purchases): 2 years @ \$6,300/year = \$12,600.

Line 400: Supplies (\$48,073)

- Genetics lab consumables for tissue collection and preservation: 400 samples @ \$1.00/sample = \$400
- Genetics lab consumables for DNA extraction: 400 samples @ \$1.83/sample = \$732
- Genetics lab consumables for sequencing: 1,000 samples @ \$4.49/sample = \$4,490
- Fish Pathology Lab consumables for tissue collection and preservation of 1,200 heart and kidney samples each year (field kits):
  - FY23: \$4,210 (includes one-time purchase of pH meter)
  - FY24: \$2,271
- Fish Pathology Lab consumables for DNA extraction and qPCR of 1,200 heart and kidney samples:
  - FY24: \$18,350 (includes one-time sequencing of isolate)
  - FY25: \$10,737
- Fish Pathology Lab consumables for histology of 1,200 heart and kidney samples:
  - FY24: \$2,526 (some items adequate to cover FY25 needs)

- FY25: \$1,727
- Fish tagging supplies for use at Pilot Station and Eagle so that each fish has a unique ID number for joining samples collected on the boat versus onshore:
  - FY23: \$915 (includes one-time purchase of tag applicator)
  - FY24: \$675
- Fish sampling supplies for use at Pilot Station and Eagle (e.g., gloves, sample collection bags, scale sampling materials): 2 years @ \$520/year = \$1,040

Line 600: (\$20,743)

ADF&G's negotiated indirect rate for FY23 is 26.99% on salaries and wages.

#### **USFWS Budget**

| <b>USFWS Budget</b> | <b>Total</b> |
|---------------------|--------------|
| 100 Personnel       | \$47,712     |
| 200 Travel          | \$3,080      |
| 300 Contractual     | \$2,140      |
| 400 Supplies        | \$12,910     |
| 500 Equipment       | \$0          |
| Subtotal            | \$65,842     |
| 600 Indirect        | \$15,144     |
| Total               | \$80,986     |

#### **USFWS Budget Narrative:**

Line 100: Personnel (\$47,712)

The following non-permanent employees will be hired for the project:

- A Biological Technician (GS-6, TBD) will work alongside another technician and supervisory staff to conduct sampling activities at Rampart Rapids and assist with preserving fish as needed for fish distribution: 4 weeks/year @ \$1,542/week x 2 years = \$12,336.
- A second Biological Technician (GS-6, TBD) will work alongside the other technician and supervisory staff to conduct sampling activities at Rampart Rapids and assist with preserving fish as needed for fish distribution: 4 weeks/year @ \$1,542/week x 2 years = \$12,336.
- Stan Zuray, Biologist with Rapids Research Center, will oversee fishwheel installation and operation to capture fish at Rampart Rapids for sampling; assist as needed with sampling activities and fish distribution logistics; and provide camp accommodations for other staff: 6 weeks/year @ \$960/week x 2 years = \$11,520.
- A local fish capture expert (TBD) will assist with fishwheel installation and operation to capture fish at Rampart Rapids for sampling and assist as needed with sampling activities: 6 weeks/year @ \$960/week x 2 years = \$11,520.

Line 200: Travel: (\$3,080)

Two technicians will travel from Fairbanks to Tanana to conduct sampling activities:

- Round trip airfare: 2 tickets/year @ \$500/ticket x 2 years = \$2,000

- Travel per diem: 2 travel days/year @ \$60/day x 2 people x 2 years = \$480
- Incamp per diem: 30 days/year @ \$5/day x 2 people x 2 years = \$600

Line 300: Contractual: (\$2,140)

- Shipping supplies to sample Rampart Rapids: 2 shipments/year @ \$250/shipment x 2 years = \$1,000
- Barge shipments of 450 gallons of unleaded gas from Fairbanks to Rapids: 1 shipment/year @ \$570/shipment x 2 years = \$1,140

Line 400: Supplies (\$12,910)

- Fish sampling supplies for use at Rampart Rapids (e.g., fish collection supplies for fishwheels and gillnets as needed, boat parts, gloves, sample collection bags, fish transport materials): 2 years @ \$2,000/year = \$4,000
- Food for sampling crew: 30 days/year @ \$30/day x 2 people x 2 years = \$3,600
- Fuel for local fishermen (Zuray and TBD) to operate boats and generators required to facilitate harvest of fish for sampling: 450 gallons regular unleaded gas/year @ \$5.90/gal x 2 years = \$5,310

Line 600: Indirect (\$15,144)

USFWS's indirect rate through FY22 is 23% on total direct costs.

**XII. Match Budget**

| <b>ADF&amp;G CF Match Budget</b> | <b>Total</b> |
|----------------------------------|--------------|
| 100 Personnel                    | \$84,559     |
| 200 Travel                       | \$0          |
| 300 Contractual                  | \$0          |
| 400 Supplies                     | \$0          |
| 500 Equipment                    | \$0          |
| Total                            | \$84,559     |

Line 100: Personnel (\$84,559)

The ADF&G Admin Project System was used to estimate personnel costs. The monthly totals include salary and benefits.

- Jayde Ferguson, Fishery Pathologist (PCN 11-5089), will oversee histology and qPCR sample prep/testing, read histology slides, conduct report writing, and present results:
  - FY24: 2 months @ \$13,218/month = \$26,436
  - FY25: 2 months @ \$13,599/month = \$27,198
- Franklin Woitel, Microbiologist I (PCN 11-5355), will assist with sample preparation and process histology slides:
  - FY24: 2 months @ \$8,224/month = \$16,448
  - FY25: 1.7 months @ \$8,477/month = \$14,413