Effects of *Ichthyophonus* on Chinook Salmon Reproductive Success in the Yukon River Drainage

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Background and Introduction

Ichthyophonus hoferi is a parasitic protozoan affecting marine and anadromous fishes, including salmonids (Kocan et al. 2004). Gross clinical signs associated with *Ichthyophonus* infection are multifocal white lesions on the heart, liver, spleen, and muscle tissue (Fig. 2 A, B). *Ichthyophonus* is likely an orally transmitted parasite with the potential to be horizontally transferred (Kocan et al. 2010). In the mid 1980's *Ichthyophonus* was identified in Chinook salmon (*Oncorhynchus tshawytscha)* by the Alaska Department of Fish and Game (ADFG) and the U.S. Fish and Wildlife Service, after fishermen noted an increase of white pustules on heart and muscle of harvested Chinook salmon. Fishermen also noted that the flesh did not dry properly and had an unpleasant fruity smell (Kocan et al. 2004).

Large scale necrosis in tissues can lead to organ failure, decreased stamina, and pre-spawning mortality (Kocan et al. 2006). *Ichthyophonus* has caused major reoccurring epizootics and mass die-offs in Atlantic herring, (*Clupea harengus),* with peaks of disease prevalence in June and November (Kramer-Schadt et al. 2010). Rainbow trout (*Oncorhynchus mykiss)* infected with *Ichthyophonus* showed significant reduction in hematocrit pointing to reduced swimming performance (Rand and Cone 1990).

In recent years, Chinook salmon stocks of Arctic-Yukon-Kuskokwim region (AYK) have had low abundance and salmon returns did not hold up to pre-season expectations based on escapement in the corresponding brood years (JTC 2011). In response, fisheries managers cancelled or restricted commercial, subsistence, and sport fishing since 2008. These actions harshly impacted U.S. subsistence fisheries along the Yukon River, but succeeded in the interim management escapement goals into Canada as part of the Pacific Salmon Treaty between the U.S. and Canada.

Yukon River Chinook salmon are undergoing one of the longest salmon migrations in the world. They must acquire considerable energy reserves before river entry to energetically prepare for this effort. Rahimian (1998) noted an association of ichthyophoniasis with reduced fish body reserves and emaciation thus complicating successful completion of the spawning migration.

Okamoto et al. (1987) showed a positive relationship between *Ichthyophonus*-related mortality and water temperature with 100% mortality occurring at 15°C to 20°C in rainbow trout. Similarly, Kocan et al. (2009) showed significantly reduced swimming performance in *Ichthyophonus*-infected rainbow trout at 15°C to 20°C. In-river conditions in the Yukon River have changed over the past 30 years, with June water temperatures having increased by approximately 2.5ºC (Horstmann-Dehn *unpublished data*).

Methods

We sampled Chinook salmon from the Salcha River tributary in summer of 2010 and 2011 (Fig. 3). In 2010, eggs and milt of infected and "healthy" individuals were stripped and cross-fertilized. In 2010, 25 males and 26 females were sampled and gametes were cross-fertilized as outlined below. Chinook salmon run in 2011 was not strong enough to sacrifice fish for gamete collection. Fish were stunned by electrofishing gear, captured with dip nets, and transferred into net holding pens until gametes were ripe. All fish were examined internally for typical clinical signs of *Ichthyophonus* infection (Fig. 2).

	Infected Male	***Ichthyophonus*-negative Male**
Infected Female	Scenario 1	Scenario 3
***Ichthyophonus*-negative Female**	Scenario 2	Scenario 4

A sub-sample of unfertilized eggs was collected from females for the determination of egg quality by analyzing eggs for total water, lipid, and crude protein. Muscle samples were taken, from the filet of the fish, for analysis of proximate composition and energy density of adults. Cardiac muscle was removed using sterile dissection techniques. The tissue was cultured in MEM-5 supplemented with 5% fetal bovine serum, penicillin, streptomycin, and gentamicin after Kocan & Hershberger (2006). The tissue was then incubated at 14°C for approximately 21 days at SUNY and examined daily for *Ichthyophonus* to confirm clinical infection. A second piece of cardiac muscle was placed in 95% ethanol. At Purdue University, the presence of *Ichthyophonus* 18S rDNA was evaluated using polymerase chain reaction (PCR) following the procedure described by Whipps et al. (2006).

Blood was collected from the caudal vein in heparinized vacutainer, spun, and plasma was immediately frozen in liquid nitrogen until analysis. Blood chemistry parameters were analyzed using an Abaxis VetScan. Both a complete diagnostic and an avian reptilian rotor was used for each sample. These two rotors allowed measurements of the following parameters: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), aspertate aminotransferase (AST), blood urea nitrogen (BUN), creatine kinase (CK), creatinine (CRE), globulin (GLOB), glucose (GLU), potassium (K), sodium (Na), phosphorous (PHOS), total bilirubin (TB), total calcium (Ca), and total protein (TP). Plasma cortisol was analyzed using commercially available RIA kits. Cortisol is a leading indicator of stress. For comparison, plasma collected from two other locations on the Yukon River (Emmonak at the river mouth and Eagle at the U.S.-Canadian border) were included in this study. High levels of ALP or ALT in plasma can indicate liver damage or disease. Plasma AST is an indicator of muscle, heart, and liver damage. CK is an enzyme that catalyses the alteration of creatine and uses ATP to make phosphcreatine(PCr) and ADP. This is a reversible process, such that ATP can be generated from PCr and ADP. PCr can act as an energy pool for the quick buffering and regeneration of ATP, therefore CK is an important enzyme in tissues that consume ATP rapidly, such as skeletal muscle, brain, or spermatozoa. Together these factors can assess cell, tissue, or organ damage.

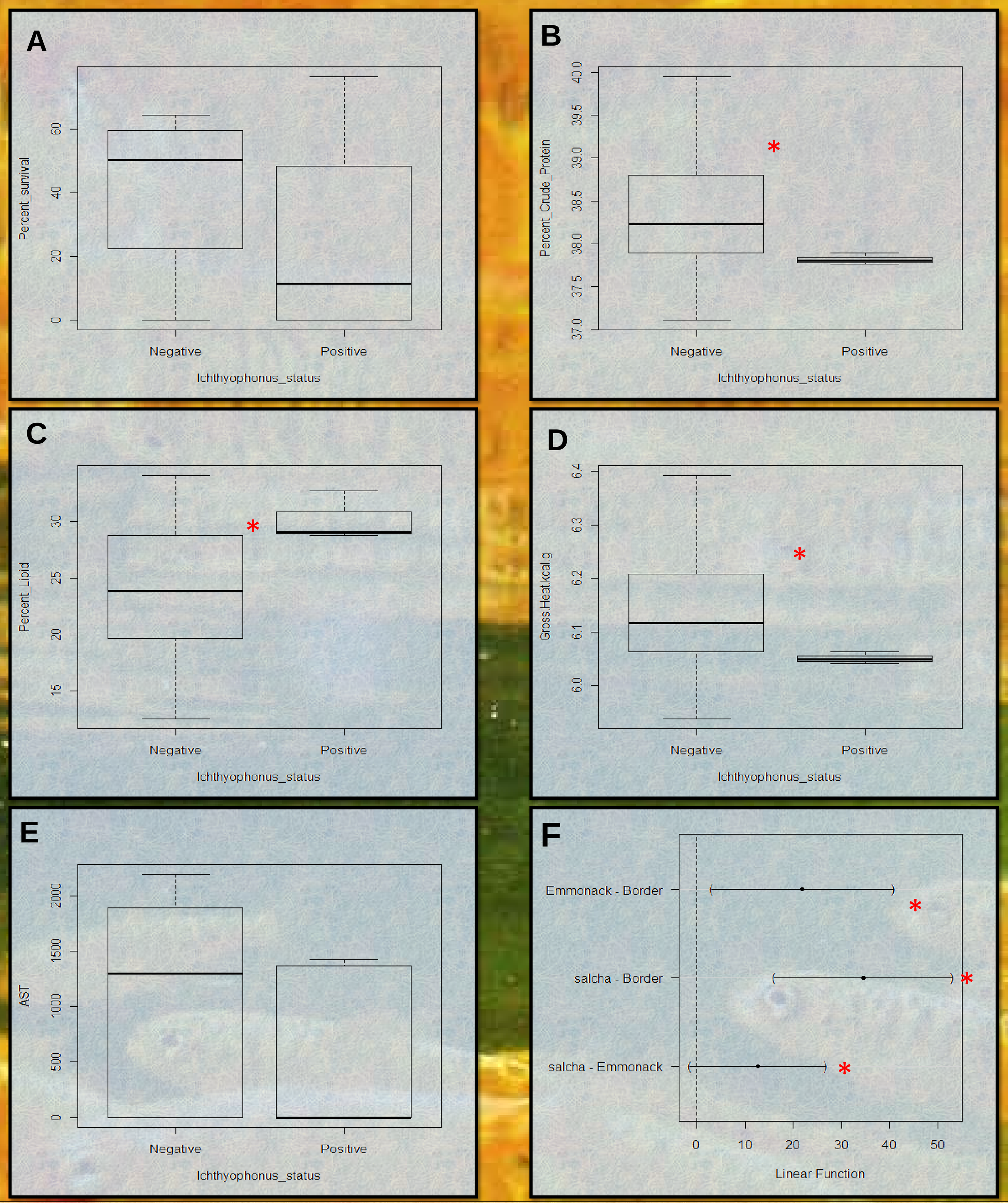

Figure 2 (A) Survival of eggs from *Ichthyophonus*-infected and 'healthy' parents. (B) Crude protein of eggs from *Ichthyophonus*-infected and 'healthy' parents. (C) Percent Lipid of eggs from *Ichthyophonus*-infected and 'healthy' parents. (D) Caloric content of eggs from *Ichthyophonus*-infected parents and 'healthy' parents. (E) AST levels from *Ichthyophonus* positive and negative fish (F) Differences in plasma cortisol levels by location, as a graphic representation of the Tukey Test. Boxes represent maximum and minimum values, and whiskers are 95% confidence intervals. * Indicates a statistically significant result

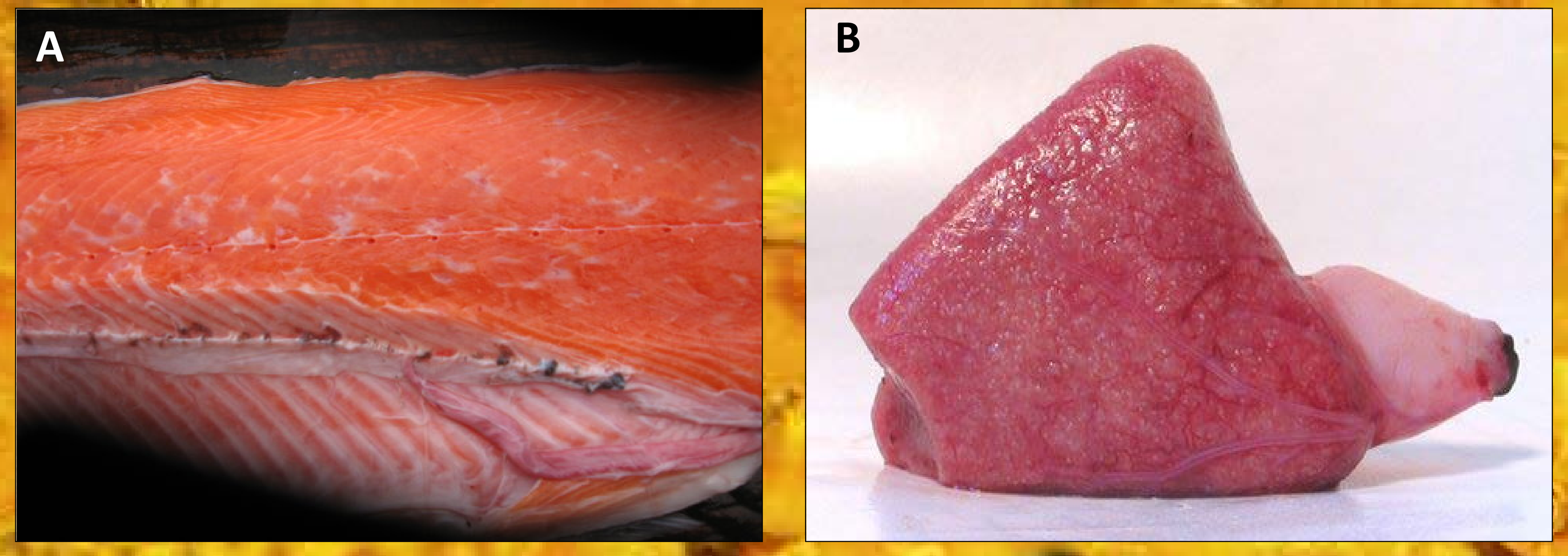

Figure 1: Clinical signs of *Ichthyophonus* infection in muscle (A) and heart (B) of Chinook salmon. White focal lesions are visible in both tissues. Photos by S. Zuray, Rapids Research and L.Horstmann-Dehn, respectively.

Hypotheses

H1: Hatching success from fertilized gametes of *Ichthyophonus*-infected parents will be negatively affected compared to 'healthy' controls

H2: *Ichthyophonus* infection will negatively affect proximate composition and energy density of eggs and fry

H3: Blood chemistry profiles and stress hormone levels in plasma of spawning adults will be different between healthy and infected fishes and can be useful as non-lethal indicators for *Ichthyophonus* infection

Acknowledgements

Funding and logistical support was provided by the Pollock Conservation Cooperative Research Center, Yukon River Panel, and ADFG. We thank C. Skaugstad, T. Redington, and A. Behr for their support in the field. We also thank L. Wilson at the UAF Palmer Research Lab for nitrogen analysis, C. Whipps at SUNY, and K. Nichols at Purdue University for *Ichthyophonus* culture and PCR. We gratefully acknowledge the assistance of B. Walker, N. Farnham, and J. Seymour with field and lab support. We thank R. Gradinger for use of his Flurescent Microscope and A. Blanchard for statistical advise. Finally we thank S. Atkinson, K. Mashburn, and A. Stevens for assistance with cortisol assays.

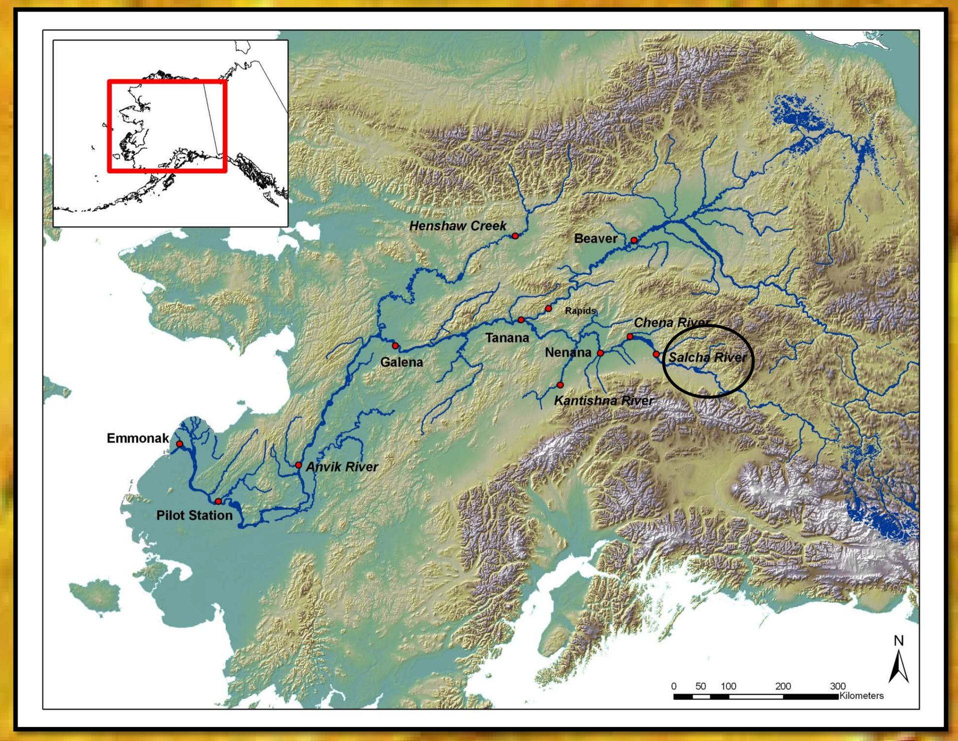

Figure 3: The Yukon River and its tributaries. Salcha River is circled.

Results

- Prevalence of *Ichthyophonus* was 7.8% in adult fish from the Salcha River in 2010 and 6.3% in 2011 and indicates continued drop in prevalence on the Yukon River.
- Average survival for *Ichthyophonus*-positive and negative egg trays is 24% and 41%, respectively ($p>0.05$, Fig. 2A).
- Eggs from *Ichthyophonus*-positive females had lower caloric density ($p=0.001$, Fig. 2D), and crude protein ($p=0.001$, Fig. 2B). Although their overall lipid content was higher ($p=0.008$, Fig. 2C).
- Once eggs hatched, crude protein, lipid, water, caloric content, and blood parameters (AST, ALT, CK, GLU, PHOS, K) were not significantly different between offspring from infected and 'healthy' parents ($p>0.05$).
- Proximate analysis of adult muscle tissue showed no significant difference (p-value>0.05) in percent crude protein, lipid, water, or caloric content.
- AST levels were significantly higher ($p=0.01$) in 'healthy' fish than in *Ichthyophonus*-infected fish (Fig. 2E)
- Plasma cortisol levels were not different between 'healthy' and infected adults ($p>0.05$). But, cortisol levels differed significantly between locations ($p=0.001$, Fig. 2F)

Conclusions

- Hatching success of eggs from *Ichthyophonus*-infected parents appeared to be lower, although results were not significant. This is likely due to sample size limitations.
  - Once hatched, yolk-sac fry have the same basic composition and chance of survival as offspring from 'healthy' parents.
- Blood parameters between *Ichthyophonus*-infected and 'healthy' fish are different, and organ damage indicators, e.g., CK and AST are unexpectedly higher in 'healthy' fish. Low plasma AST has been correlated to Vitamin B6 deficiency (Chiang et al. 2005) and is in turn associated with inflammation, i.e., *Ichthyophonus*.
- Plasma cortisol between locations was different. However, method of capture was also not directly comparable. It is possible that fish migrating for extensive distances (i.e., Eagle) may have reached adrenal fatigue, thus explaining the lowest stress hormone levels.
- It may be possible to identify sex and *Ichthyophonus* status of Chinook salmon using blood parameters. This would provide a tool for minimally-invasive monitoring and rapid turn-around of results for proactive in-season monitoring of *Ichthyophonus*-infection.

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