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 pustules 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 of 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. Rohmann (1998) noted an association of *Ichthyophonus* 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 a significantly reduced swimming performance in *Ichthyophonus*-infected rainbow trout at 15°C to 20°C. In river conditions the Yukon River have changed over the past 30 years, with June water temperatures having by increased approximately 2.5°C. (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 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.

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 creatinine (CRE), globulin (GLOB), glucose (GLU), potassium (K), sodium (Na), phosphorous (PHOS), total bilirubin (ALT), amylase (AMY), aspertate aminotransferase (AST), blood urea nitrogen (BUN), creatine kinase (CK), and inorganic phosphorus (P). Blood was collected from the caudal vein; plasma was placed immediately in 95% ethanol. At Purdue University, the presence of 18S rDNA was evaluated using polymerase chain reaction (PCR) following the procedure described by Whipps et al. (2006). Blood chemistry parameters were analyzed using an Abaxis VetScan. Both a non-lethal indicator for *Ichthyophonus*-infection will negatively affect proximate composition and caloric content 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 graph representation of the Tukey Test. Boxes represent maximum and minimum values, and whiskers are 95% confidence intervals. *Indicates a statistically significant result.

**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 pre-spawned crustacean on *Ichthyophonus*-infected adults will be different between healthy and infected fishes and can be useful as non-lethal indicators for *Ichthyophonus* infection

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**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 Assistance with cortisol assays.

**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 graph representation of the Tukey Test. Boxes represent maximum and minimum values, and whiskers are 95% confidence intervals. *Indicates a statistically significant result.

**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. 2C), 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 offsprings from infected and ‘healthy’ parents (p>0.05).**
- **Proximate analysis of adult muscle tissue showed no significant difference.**
- **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 offspings from ‘healthy’ parents.**
- **Blood chemistry parameters were different between ‘healthy’ and infected fish; however, calculated creatinine, lipid, water, or caloric content were not difference between parents with and without *Ichthyophonus* infection.**
- **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 internal 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.**

**References**