

# **Chinook salmon *Ichthyophonus* Investigations**

Final Report to the Yukon River Panel

For project titled: “*Ichthyophonus* in Chinook salmon – Continuation of a baseline in Emmonak and Eagle, Alaska and potential links to fecundity.” URE 13-09

by

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## Abstract

*Ichthyophonus hoferi* is a protozoan parasite of various fish species, including salmonids, and infection has led to mass mortalities in species of economic significance. Prior evidence suggests that infection with *Ichthyophonus* leads to reduced endurance, increased pre-spawning mortality, and potentially low fecundity. Poor returns of Chinook salmon (*Oncorhynchus tshawytscha*) from adequate spawning escapements in 2007, 2008, and 2009 raise questions about involvement of disease in these declines.

Prevalence of *Ichthyophonus* in Yukon Chinook salmon at the river mouth shows a cyclic variation over time. This study continued a temporal baseline (1999-2008) established for *Ichthyophonus* prevalence at the river mouth against which to judge a potential change. *Ichthyophonus* prevalence in 2009 was 8% in Emmonak and 13% in Eagle. Total egg counts and egg quality (as determined by proximate analyses) did not differ between healthy and infected females, however only 8 of 44 females sampled were infected with *Ichthyophonus* and stock-specific differences in lipid contents in particular may confound any potential differences in egg quality.

## Introduction

*Ichthyophonus hoferi* (*Ichthyophonus* here after) is a marine-derived protozoan parasite infecting a variety of marine and anadromous fish species including salmonids (Kocan et al. 2004; Tierney and Farrell 2004; Gavryuseava 2007). While the parasite is not harmful to humans, the effects on the fish host can be devastating and mass mortalities of herring have been attributed to infection with *Ichthyophonus* (Sindermann 1965; Møllergaard and Spanggaard 1997; Kocan et al. 1999). Continued poor returns of Chinook salmon from adequate spawning escapements raise questions about the potential contribution of *Ichthyophonus* to these declines either due to pathogen-induced mortality, reduced fecundity, or the inability of fish to successfully migrate and spawn in tributaries. Prior research suggests that *Ichthyophonus* is a newly emerging parasite in the AYK region and may cause pre-spawning mortality of Yukon River Chinook salmon (Kocan et al. 2004, 2006, 2009). Moreover, Yukon River Chinook salmon appear to be more susceptible to *Ichthyophonus* than some British Columbia Chinook salmon populations (Jones and Dawe 2002) and exposure of fish with naive immune systems to *Ichthyophonus* results in high mortality (Kocan et al. 1999).

This study aims to:

- 1) Maintain the temporal baseline of *Ichthyophonus* prevalence at Emmonak and at border passage (i.e., Eagle) in Yukon Chinook salmon;
- 2) Determine fecundity in female Chinook salmon harvested in Eagle and analyze eggs for water, total lipid, and nitrogen content to evaluate if infected Chinook salmon produce a similar number of eggs and allocate the same energy stores to ova as healthy fish;

## Methods

A long-term data set (1999-2008) of *Ichthyophonus* prevalence has been established near the community of Emmonak, at the mouth of the Yukon River. In 2009, Chinook salmon *Ichthyophonus* sampling continued in Emmonak and Eagle, Alaska funded by the U.S./Canada Restoration and Enhancement Fund. The severe spring flood in both communities, but in particular in Eagle made the collection of samples challenging due to substantial loss of fishing gear and rebuilding efforts. In addition, conservation measures on the Yukon River called for a 10-day closure of the subsistence fishery in the upper Yukon River therefore reducing sampling opportunity. However, sampling in 2009 was successful and all sampling targets were reached. Chinook salmon tissues were collected near the community of Emmonak (close to the mouth of the Yukon River) as part of the Big Eddy test fishery operated by ADF&G. The Big Eddy test fish project utilizes set gillnets with an 8.5" mesh size. Samples of cardiac muscle ( $n=150$ ) were collected over the course of the Chinook salmon run from June 4 to July 8. Collection of samples over the entire run is critical as Kocan et al. (2004) noted that salmon returning early in the season seem to be relatively free of the typical clinically observed *Ichthyophonus* lesions, while fish tend to be more severely infected with these lesions later in the season.

In addition, samples of Chinook salmon ( $n=201$ ) were collected in collaboration with subsistence fishermen in the community of Eagle near the U.S.-Canadian border. Samples ( $n=100$ ) were obtained over the course of the Chinook salmon run from July 11 to July 16 (before the closure of the subsistence fishery), and from July 27 to August 2 ( $n=101$ ) after the subsistence fishery closure. Subsistence fishing gear used at Eagle included fish wheels and set gillnets with mesh sizes ranging from 6" to 8". Fishing sites varied and were dependent on the use of traditional sites. However, most subsistence gear was located on the right bank of the Yukon River in close vicinity to the community.

All Chinook salmon samples in both Emmonak and Eagle have been collected opportunistically and no fish were sacrificed for the research objectives of this project. Institutional Animal Care and Use (IACUC) protocols required by UAF as part of vertebrate research were therefore not necessary for this study.

At both locations, samples and morphometric have been obtained on shore immediately after return of the test fish crew/subsistence fishermen. Age was estimated from all fish by scale pattern analysis (ADF&G aging laboratory) with scales collected from the preferred area on the left side of the fish above the lateral line (Bales 2007). Length was measured to the nearest 5 mm from mid-eye to fork of the tail. Sex was determined by internal examination of gonads. Girth was measured to the nearest millimeter anterior to the dorsal fin using a QM2000TM circumference measuring tape and fish weights were determined on shore using a tripod and a Chatillon scale. Axillary fin clips were taken for genetics in Eagle and made available to the Department of Fisheries and Oceans in Canada.

Presence of *Ichthyophonus* 18S rDNA has been evaluated using polymerase chain reaction (PCR) with DNA extracted from cardiac muscle and following the procedure described by Whipps et al. (2006). PCR tests have been conducted at Purdue University, West Lafayette, IN for both Emmonak and Eagle samples, and at the State University of New York, College of Environmental Science and Forestry, Syracuse, NY for Emmonak

samples only. Samples from Emmonak have been analyzed by both institutions to assure inter-laboratory comparability and maintain a consistent temporal baseline at this site. The PCR method is highly sensitive and specific for *Ichthyophonus* (detection limit =  $10^{-5}$  parasite spores/reaction, Whipps et al. 2006) and samples can be stored indefinitely, thus allowing for analysis of archived samples and storage of samples in the field. PCR analysis is equally sensitive to tissue culture in identifying the presence or absence of the parasite (Whipps et al. 2006).

To address fecundity of infected and healthy Canadian-origin female Chinook salmon, both ovaries have been weighed to the nearest 0.1g following the method of Kinnison et al. (1998). Two sub-samples of fresh ova from each skein (approximately 10% of the total skein weight) have been weighed to the nearest 0.1g in the field and preserved in 10% buffered formalin for later egg counts. Mean egg weight (the ratio of sub-sample fresh weight to number of eggs in the sub-sample) has then been determined. Fecundity was defined as the ratio of total fresh skein weight and mean egg weight. Eagle was selected as the sampling site to determine fecundity, due to the high degree of variability in maturity of the mixed Chinook salmon stocks obtained in Emmonak at the Yukon River mouth.

To evaluate resource allocations of eggs, a sub-sample of roe was collected into pre-weighed vials, frozen, and shipped to the Ecophysiology Laboratory at the University of Alaska Fairbanks (UAF) where they were homogenized and freeze-dried (VirTis Sentry) to a constant weight. Water content of tissues was determined as loss of mass during the freeze-drying procedure. Tissues were then lipid-extracted using chloroform:methanol in a modified Soxhlet procedure after Schlechtriem et al. (2003). Tissue nitrogen content was measured using a CNS 2000, Leco Combustion analyzer at the soil laboratory of the Alaska Agricultural and Forestry Experiment Station in Palmer. Ash content was determined via combustion at 550°C for 8 hours in a muffle furnace. The subtractions of ash content from dry matter allowed for calculation of organic matter in the sample and further subtraction of lipid provided lean dry mass.

## Results and Discussion

Sex composition of the acquired samples was 63.3% and 21.9% female for Emmonak and Eagle, respectively, as determined by internal examination of gonads. Fish sampled at Emmonak had a mean length of  $851 \pm 63$  mm (mid-eye to tail fork), mean weight of  $21.8 \pm 5$  lbs, and the mean girth was  $518 \pm 44$  mm. At Eagle, the mean length was  $725 \pm 118$  mm and mean weight was  $11.9 \pm 6$  lbs; mean girth was  $379 \pm 72$  mm. Age was estimated by scale pattern analysis with scales collected from the preferred area on the left side of the fish above the lateral line (Bales 2007). At Emmonak, age-6 (89.3%) fish were strongly represented, followed by age-5 (6%). Age composition of the fish sampled in Eagle was 25.4% age-4, 29.4% age-5, and 38.3% age-6 fish. Proportions of age-3 and age-7 fish were small in Eagle (0.5% and 1.5%, respectively). Due to advanced resorption of scales at later migratory stages, 5.0% of Chinook salmon collected in Eagle could not be aged.

Heart samples were fixed in 95% ethanol at time of collection and were analyzed for the presence of *Ichthyophonus* 18s rDNA using polymerase chain reaction (PCR) following

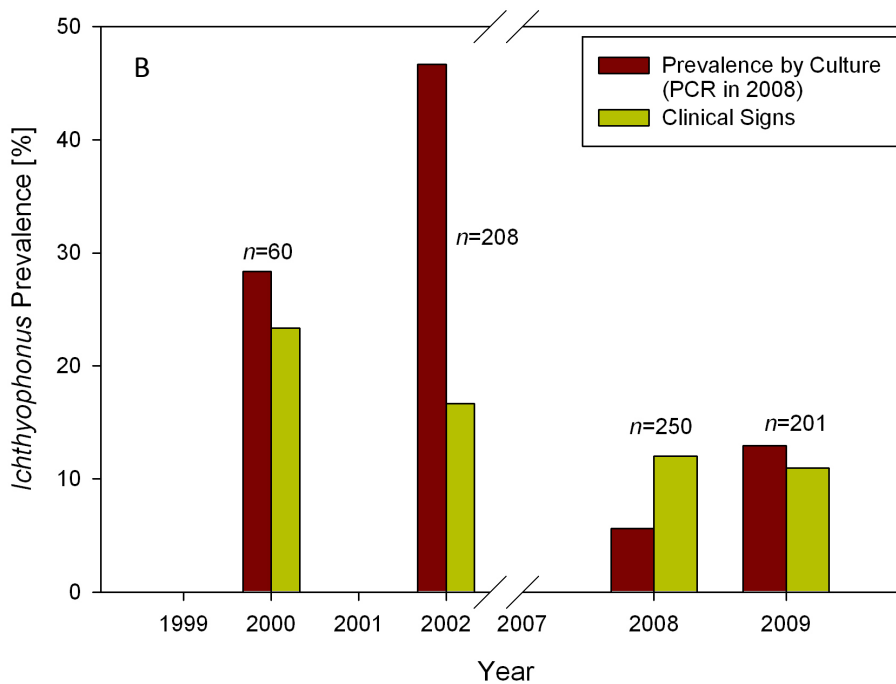
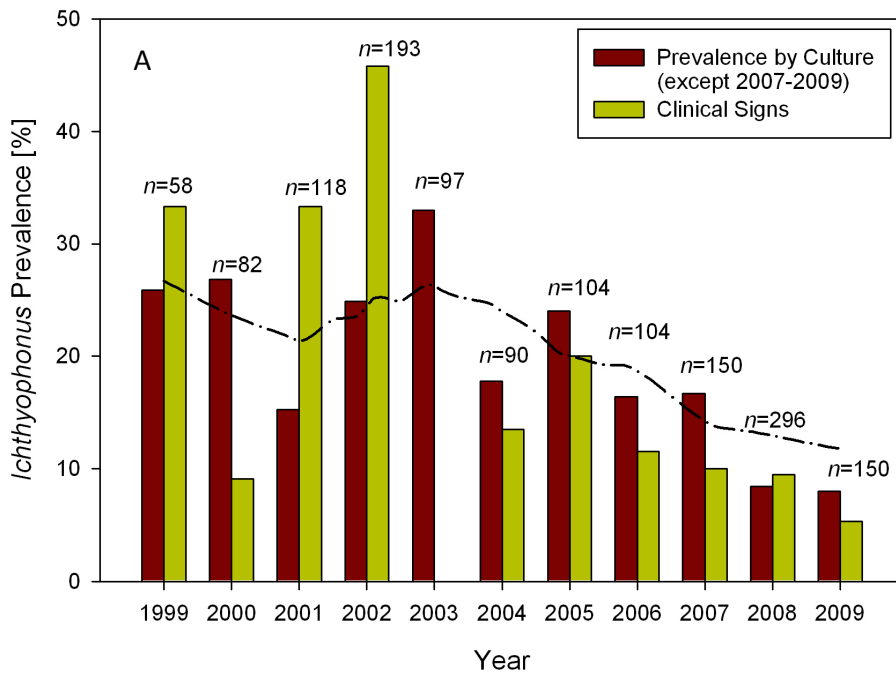
the procedure described by Whipps et al. (2006). Clinical signs typical for *Ichthyophonus* infection were noted at the time of collection in 5.3% (8 of 150) of fish sampled in Emmonak. Clinical signs of *Ichthyophonus* infection were recorded in 11% (22 of 201) of Chinook salmon collected in Eagle. However, white, granulomatous lesions are a general inflammatory response of fish to foreign bodies and do not necessarily establish actual infection with the parasite (Corbel 1975). PCR analysis of tissues obtained in Emmonak indicated a low infection prevalence of 8% (12 of 150) and 13% in Eagle (26 of 201). *Ichthyophonus* prevalence over time for both Emmonak and Eagle is provided in Figure 1. Cyclic *Ichthyophonus* epizootics have been described in herring (Sindermann, 1965) and a similar cyclic pattern is noticeable in the Chinook salmon time series data from Emmonak. While reasons for this temporal variability are poorly understood, a potential correlation with ocean conditions appears likely. For three consecutive years, the average summer and winter water temperatures in the Eastern Bering Sea have been cold (NOAA mooring M2, NOAA's Pacific Marine Environmental Laboratory) and coincide with a noticeable drop in *Ichthyophonus* prevalence over this time period (Figure 1). Environmental change can have direct impacts on the physiological response of fish to parasite or pathogen exposure as the inflammatory and stress responses in general are temperature dependent in poikilotherms (Finn and Nielsen 1971, Strange et al. 1977). In addition, temperature appears to influence the activity of *Ichthyophonus* in the host (e.g., higher overall parasite load with increased temperature, Kocan et al. 2009), and therefore has noticeable effects on host stamina, in particular once they enter the warmer river systems. In the light of rapidly changing Arctic and sub-Arctic ecosystems, temporal trends (such as disease monitoring near Emmonak) become crucial in documenting and understanding response and adaptation potential of salmonid populations as well as identifying mechanisms preceding these changes.

The effect of *Ichthyophonus* on salmon health, egg quality, and juvenile survival remains poorly understood. Fish egg and embryo vitality is correlated to body condition of spawning females. Condition is in turn dependent on physiological status and energy demands and generally fish exposed to stress or disease show an increase in energetic costs (King et al. 2003; Rand et al. 2006). Lipids may therefore be re-routed from gonads of *Ichthyophonus*-positive fish to complete the spawning migration and then they may either produce less or lower quality eggs. Collections in Eagle were therefore paired with egg counts and egg quality (as determined by proximate analyses; % water, % lipid, % Nitrogen, and ash) data to assess fecundity, gonad energy storage, and potential links to *Ichthyophonus* infection (Table 1). In 2009, 44 females (22% of sampled fish) were available for study showing a significant correlation between fecundity and girth and fecundity and weight ( $p < 0.0001$ , Figure 2). While total egg count was significantly lower in *Ichthyophonus*-positive compared to healthy females ( $p = 0.03$ ), this difference did not persist when data were normalized for girth or weight ( $p = 0.47$ , difference in slope and  $p = 0.11$ , difference in intercept). However only 8 of the 44 females sampled were infected with *Ichthyophonus* (based on culture) illustrating the need for larger sample sizes to further investigate the effect of disease on fecundity. In addition, egg quality (as determined by proximate analyzes) was investigated between healthy and infected females in 2009. No statistical differences were found in the parameters analyzed to determine egg quality between healthy and *Ichthyophonus*-positive females. However, samples sizes were small and stock-specific variability, in particular in lipid contents of ova may have to be considered.

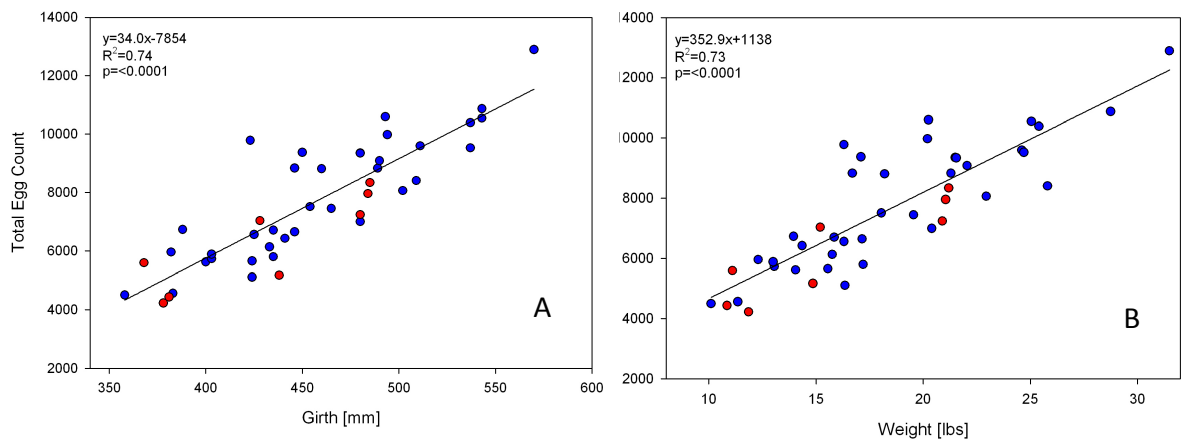
**Table 1:** Proximate analyses of ova from healthy and *Ichthyophonus*-infected Chinook salmon harvested in Eagle, Alaska in 2009.

	% Water	%Lipid	% Nitrogen	Lean Dry Mass [g%]	Lean Organic Mass [g%]	Crude Protein [g%]
<b>Healthy</b>	57.2 ± 1.5	22.6 ± 4.1	10.2 ± 0.3	20.2 ± 4.2	16.2 ± 4.3	20.3 ± 1.3
<b>Range</b>	54.0 - 59.8	15.9 - 32.3	9.6 - 10.9	10.2 - 27.3	4.7 - 23.4	17.7 - 22.4
<b>Infected</b>	56.5 ± 1.3	21.9 ± 3.3	10.2 ± 0.4	21.7 ± 3.3	17.7 ± 2.9	20.9 ± 1.4
<b>Range</b>	54.8 - 59.2	17.6 - 26.7	9.6 - 10.7	17.0 - 25.9	13.7 - 21.3	19.6 - 22.9
<b>p-value</b>	0.20	0.63	0.88	0.36	0.35	0.32

testing  $H_0$ : no difference between healthy and infected Chinook



**Figure 1:** Time-series of *Ichthyophonus* prevalence at Emmonak (A) and Eagle (B) based on heart culture and PCR in Chinook salmon ( $n$  = sample size). LOESS non-parametric smoothing (dashed line) was applied to visualize temporal trends of parasite prevalence. Data from 1999 to 2003 is based on studies by Kocan et al. (2004), Kocan and Hershberger (2006) in Eagle and Emmonak and data from 2004-2006 in Emmonak after Kahler et al. (2007).



**Figure 2:** Total egg count versus girth (A) and weight (B) in Yukon River Chinook salmon caught during the subsistence harvest in Eagle, Alaska in 2009. Red symbols indicate *Ichthyophonus*-positive females as determined by culture. Linear regression parameters are provided in the graph.



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