

Final Report

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Yukon River King Salmon -*Ichthyophonus* Pilot Study

prepared by

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Abstract

When king salmon enter the Yukon River on their spawning migration in mid June, over 25% of the population are infected with *Ichthyophonus*. The percent of infected fish remains relatively constant until the fish pass river mile 1,319 at Dawson, Y.T., then it drops to 13% when they reach river mile 1,745 at Whitehorse, Y.T. When the sexes are examined separately, slightly more females are infected than males (29% vs 22%). The percent of fish exhibiting clinical signs (diseased) is 2-3% when they enter the river, but increases to over 20% at river mile 715 near Tanana, AK. Disease prevalence within the population remains constant at > 20% until fish pass Dawson, then the percent of diseased fish drops to < 9% at Whitehorse. When the sexes are examined separately, male disease prevalence is highest at Tanana (22.6%) then gradually drops to just 12.9% at Whitehorse. Females however, continue to show an increase in disease prevalence peaking at river mile 1,081 near Circle, AK, at 36.4%, then dropping to just 5.3% at Whitehorse. Data on infection and disease collected from kings at Nenana on the Tanana River more closely resembles that seen at Whitehorse than the lower and middle Yukon River.

When data collected in 1999 and 2000 are compared the prevalence of infection in males remains the same while a 15% drop in infection prevalence occurs in females. There is also a drop in the percent of infected fish showing *Ichthyophonus* infection of the muscle. This difference may be related to a 2°C lower river temperature in 2000 compared with 1999.

Significant egg resorption was seen in 25% of females but no correlation with *Ichthyophonus* infection could be made. The cause of resorption and the extent to which it affects fecundity has yet to be determined.

Attempts to experimentally infect Chinook salmon and rainbow trout with Yukon River *Ichthyophonus* isolates were essentially unsuccessful by both feeding of infected tissues and injection of cultured spores. However, other unrelated fish species were infected without difficulty.

A method for non-lethal sampling of adult spawning Chinook salmon for *Ichthyophonus* was developed using known infected fish and live returning spawners. The method consisted of taking punch biopsies of skin and muscle and culturing the biopsy tissue in vitro. A 100% correlation was made between known infected fish and cultured biopsy tissue.

Introduction

During the 1987 fishing season a local fisherman from Tanana noticed a single fish that had a very unusual fruity odor when dried in the traditional manner (W. Fliris, personal communication). The following year he noticed several more "smelly" fish, and in 1989 and 1990 he sent several fish with obvious muscle lesions to the ADF&G laboratory in Juneau where *Ichthyophonus* was identified. In 1991 samples of muscle, heart and liver suspected to be infected were forwarded to a laboratory in Oregon and the results also came back positive for *Ichthyophonus*. Since that time the numbers of fish that have been noticeably infected in the Tanana area has increased dramatically (Fliris, personal communication).

The market value of Chinook salmon (*Oncorhynchus tshawytscha*) in the middle Yukon area was also affected as a result of *Ichthyophonus* infection of muscle tissue. A commercial fish processor from Fairbanks (V. Umphenor, personal communication) estimated that as many as 20 percent of the fish he purchased in 1999 had been unusable due to lesions in the flesh. There have also been unconfirmed reports that Japanese fish buyers have noticed unacceptable fish from the lower Yukon with white spots in the flesh.

Since before the turn of the century *Ichthyophonus* has been recognized as a serious pathogen of many species of fish, including salmonids (Fish 1934, Sinderman & Rosenfield 1954; Sinderman & Scattergood 1954; Marty et al 1998; Kocan et al 1999). Reports from Scotland early in the century describe "greasers" and "smelly" haddock, which were infected with *Ichthyophonus*, possibly the same phenomenon described for Yukon kings in 1987 (Williamson, 1913).

Recently *Ichthyophonus* was reclassified from a "fungus" to that of a protist most closely related to the "rosette agent" of salmon (Spanggaard et al 1996). The rosette agent has also been implicated as a serious pathogen of Chinook salmon, especially those held in net pens (Herrell et al 1986, Arkush et al 1998). Because both of these agents are known pathogens of salmonids and are closely related, it is highly probable that *Ichthyophonus* is a significant health threat to adult salmon returning to spawn in the Yukon River.

As a result of reports of diseased Yukon kings over a 10-year period, and the high probability that *Ichthyophonus* is a serious pathogen of Chinook (king) salmon, the Bering Sea Fishermen,s Association (BSFA) funded a pilot study in 1999. The study was designed to determine the extent of *Ichthyophonus*, role in causing the reported condition in Yukon kings. Pathologic examination of Chinook salmon collected at Emmonak (river mile 26) and Tanana (river mile 715) revealed that approximately 30% of all sampled fish were infected with the parasite *Ichthyophonus* at the time they entered the river in late June, with 4% exhibiting overt signs of disease. Subclinical infections were detectable only by primary tissue culture or by histologic examination when fish entered the river, with more females infected than males (35% vs 21%). None of the males and only 4% of the females exhibited visible

lesions when they entered the river. Samples of the same run taken the second week of July 1999 at Tanana demonstrated that the infections had progressed to overt disease identifiable by visible lesions in 25% of males and 52% of females. At Tanana, additional subclinical infections were also identified in fish of both sexes by primary tissue culture. Several helminth parasites were observed, but not in numbers or frequency to be a health problem (Kocan & Hershberger 1999).

Based on these data, a more extensive study was conducted in 2000, which involved sampling fish from 6 stations along the Yukon River from Norton Sound to Whitehorse Y.T., and 1 station on the Tanana River at Nenana, AK. The major objectives of this study were:

- 1) To determine the % of fish are carrying *Ichthyophonus* when they enter the river.
- 2) ... if clinical signs of disease (lesions) are present when fish enter the river.
- 3) ... if a change in infection prevalence occurs as the fish migrate upriver.
- 4) ... if the percent of diseased fish changed as the fish migrate upriver.
- 5) ... if the organism impacted the survival or fecundity of spawning fish.

Additional laboratory studies were also initiated because conclusive proof of an organism's pathogenicity requires that experimental infections of known specific-pathogen-free host organisms be carried out to confirm Koch's Postulates. Previous studies have confirmed the pathogenicity of *Ichthyophonus* for Pacific herring (*Clupea pallasii*) and the coast range sculpin, (*Cottus aleuticus*) (Kocan et al 1999). However, no studies to date have conclusively demonstrated that *Ichthyophonus* is pathogenic for Chinook salmon. Current laboratory studies involve 1) Transmission and 2) Temperature effects on growth and pathogenicity.

Methods

Fish collection

ADF& G personnel captured fish by gill net at Emmonak from June 22-29, while fish at Galena (July 1-3), Nenana (July 11), Tanana (July 2-9), and Circle (July 13-19) were caught by subsistence fishermen using both gill net and fish wheels. Fish from Dawson (July 13-19) were captured by fish wheel during a test fishery, while at Whitehorse (Aug 19-28) wild fish were sampled by personnel from the Department of Fisheries and Oceans, Canada, as they were captured at a hatchery.

Samples collected

Sex, length and weight were recorded when possible, and visual (gross) observations were made on heart, liver, spleen, skein, muscle and skin. The processing methods used by different fishermen prevented our obtaining all of the above data from some sample sites. At Tanana and Circle all fish captured during the collection period were examined. Consequently, these two sites offer the best data for sample population estimates. At all sites however, visual examination of all fish was possible and heart and liver tissue were collected from a subset of these fish and cultured in Eagles Minimal Essential Medium supplemented with 5% fetal bovine serum, 100 IU mL⁻¹ penicillin, 100 IU mL⁻¹ streptomycin and 100 IU mL⁻¹ gentamycin.. Cultures were incubated at 12 °C and examined microscopically for the presence of hyphae and spores after 7 and 10 days.

The cultured tissue enabled us to determine the infection rate while visual examination of each fish allowed us to determine the extent of disease progression. The presence of white spots on the heart, liver and muscle tissue were considered clinical signs of "disease" while positive identification of *Ichthyophonus* in culture was used to determine subclinical "infection" prevalence. Fish were recorded as "infected" if they had subclinical or clinical signs (disease), while fish were recorded as „diseased% only if they exhibited visible lesions (clinical signs).

Representative tissue samples were also preserved in 10% Formalin for later histologic verification of the identity of the organism. Females with skeins having attritic eggs or signs of hemorrhage were photographed and recorded.

Cultured as well as infected tissues were shipped overnight to the USGS laboratory at Marrowstone Island, WA where they were examined microscopically and the number of positive cultures recorded. The fresh infected tissues (heart and liver) were minced and fed to Puget Sound Chinook salmon smolts while portions of the same tissues were cultured to verify the parasite,s viability and to supply material for future experimental transmission and genetic comparison studies.