**Effects of *Ichthyophonus* on Survival and Reproductive Success
of Yukon River Chinook Salmon**

Annual Report for Study 01-200

This report has been prepared to assess project progress. Review comments may not be addressed in this report, but will be incorporated into the final report for this project.

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Title: Effects of *Ichthyophonus* on Survival and Reproductive Success of Yukon River Chinook Salmon

Study Number: 01-200

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Geographic Area: Yukon River main stem and selected tributaries

Information Type: Disease prevalence and intensity

Issue(s) Addressed: Role of disease in declines of chinook salmon in Yukon River

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Study Duration: May 1**,** 2001 to March 31, 2003

Abstract: Studies confirmed that approximately 25-30% of Yukon River chinook salmon enter the river infected with *Ichthyophonus*. Infection prevalence remained constant until fish reached the upper Yukon at Dawson and Whitehorse where it dramatically dropped to 10% or less. Clinical signs of disease were minimal when fish entered the river, but increased to nearly 30% when fish reached Rampart Rapids at river-mile 745. There was no difference in prevalence of infection in fish from the early part of the run compared to fish from the end of the run; however, in fish from the end of the run, the parasite was disseminated and clinical disease was apparent. Infection and disease prevalence rates in fish from the Tanana River and the mouth of the Chena River were similar to those in the Yukon River. However, female spawn-outs collected from the upper Chena River showed no evidence of *Ichthyophonus* infection. This dramatic decrease in infection prevalence in females is similar to that seen at Whitehorse in 2000 and 2001. Males showed a slight decrease in infection prevalence at Whitehorse and the Chena River. Elevated river temperatures within and among years may be an important cause of increased disease among Yukon River chinook salmon.

Key Words: Chinook, Disease, Ichthyophonus, Yukon River

Project Data: <u>*Description*</u> - Data for this study consist of information on disease prevalence and intensity from field samples obtained during surveys. <u>*Format*</u> – Survey data are stored in databases. <u>*Custodian*</u> - Dr. R. Kocan*,* School of Aquatic & Fishery Sciences, University of Washington. <u>*Availability*</u> - Access to biological data is available upon request to the custodians.

 Report Availability: Please contact either the author(s) to obtain a copy of this report.

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INTRODUCTION

A limited pilot study funded by BSFA in 1999 attempted to determine if *Ichthyophonus* was affecting the health of Yukon River Chinook salmon (*Oncorhynchus tshawytscha*). The study was prompted by observations made since the mid 1980s by Yukon River fishermen and processors who reported an increase in the number of fish exhibiting lesions in their flesh and internal organs. Initially, early season fish were reported to be relatively free of lesions, but late season fish were severely affected. Processors reported that as many as 20% of the fish they bought were discarded because of muscle tissue damage. More recently, reports by fishermen stated that fish from all parts of the run were affected and that severity varied from year-to-year.

Chinook normally enter the Yukon River during the first to second week of June, depending on when the river becomes ice-free with the last of the entering the river by the second week of August. In general, migration speed is 21-30 miles per day. Using these data is was possible to sample fish from the same portion of the run each year of the study.

Examination of chinook salmon collected at Emmonak, AK in 1999 revealed that 25-30% of all sampled fish were infected with the parasite *Ichthyophonus*, but only 4% exhibited clinical signs of disease. Subclinical infections were detectable only by explant culture or by histological examination when fish entered the river, with more females infected than males (35% vs 21%). Samples taken upriver of Tanana, AK (river-mile 720) during the second week of July, demonstrated that the infections had progressed to overt disease identifiable by visible lesions in the heart, liver, spleen and flesh of 25% of the males and 52% of the females. The severity of infection was so great that over half of the infected fish could not be used for human consumption because of damage to the flesh. Both sexes also had subclinical infections identified only after explant culture in the laboratory. River temperatures taken in 1999 revealed that the Yukon River reached temperatures as high as 18–20 $^{\circ}$C (65-68°F) from the third week of June through late July (K. Chikita, unpublished data).

To further explore the significance of the 1999 findings, a more extensive study was conducted in 2000, which included seven sample sites from the mouth of the Yukon River to Whitehorse in Yukon Territory. Approximately 800 fish were examined for length, weight, clinical infection, subclinical infection, egg resorption and other pathologic conditions. The overall sex ratio of sampled fish was 4:1 in favor of males while the overall prevalence of infection was 3:1 in favor of females. As in 1999, less than 5% of fish entering the river had clinical signs of disease and no significant change in clinical disease was noted in fish collected at Galena (rm 530). However, when the fish were sampled at rm 720, 18% exhibited clinical signs of disease in the form of lesions on the heart. However, fewer showed disseminated involvement of the liver, spleen, kidney and flesh compared with 1999. About twice as many females were affected than males. This increase in prevalence of clinical disease continued as the fish migrated upstream, reaching a peak at Dawson, YT, where 25% had clinical signs of disease. The percent of clinically affected fish dramatically declined in fish examined from Whitehorse, YT, where the overall prevalence of infection dropped to 8%. Females were also significantly smaller at Whitehorse than at all other down-river sites. Another anomalous site was Nenana, AK on the Tanana River, (rm 860) where

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fish also showed a reduction in disease prevalence, most noticeable in females (Kocan & Hershberger 2001).

The data obtained in the 2000 study confirmed the 1999 data, which showed the disease to became more severe as the fish moved upriver. One significant difference between the two years was the extent of tissue damage caused by the parasite. During 1999 a large proportion of the infected fish, beginning early in the run (1st week of July) had disseminated lesions affecting heart, liver, spleen, kidney and flesh, while in 2000 clinical disease appeared to be limited mainly to the heart during this same period. This difference was suspected to be due to differences in river conditions, such as temperature, between the two years, which affected the parasite's rate of development. There is evidence that water temperatures were warmer in 1999 than in 2000, possibly being responsible for the observed difference in severity between the two years. It has also been experimentally demonstrated that relatively small changes in ambient water temperature cause significant changes in pathogenicity of *Ichthyophonus* (Okamoto et al 1987; Halpenny et al. 2002).

OBJECTIVES

1. Repeat multi-site survey (monitoring) of chinook salmon for *Ichthyophonus* prevalence and pathogenicity from Emmonak to Whitehorse.
2. Relate changes in annual disease severity to annual changes in river conditions using new and historical water temperature data.
3. Determine if infected adults die before reaching their natal streams.
4. Attempt to find the source of *Ichthyophonus* infections in Yukon chinook.
5. Experimentally determine if water temperature affects the rate of growth or pathogenicity of *Ichthyophonus in* infected fish and compare to historical Yukon River temperatures.
6. Determine if *Ichthyophonus* can affect fecundity in experimentally infected fish.

METHODS

Fish Collection

Chinook salmon were sampled from the Yukon River at Emmonak and Marshal, Rampart Rapids, Tanana, Dawson Y.T. and Whitehorse, Y.T. Fish were also sampled at the confluence of the Chena and Tanana Rivers and spawn-outs were examined from the Chena River. Twenty samples were also obtained from the Kuskokwim River near Bethel, AK as part of a USFWS contaminant study. The total number of samples exceeded 700 fish. Canada's DFO supplied an additional 100 muscle biopsy samples from their test wheel just upriver from Eagle, AK. Herring were also sampled by ADF&G from Kuskokwim Bay and Norton Sound (Figure 1).
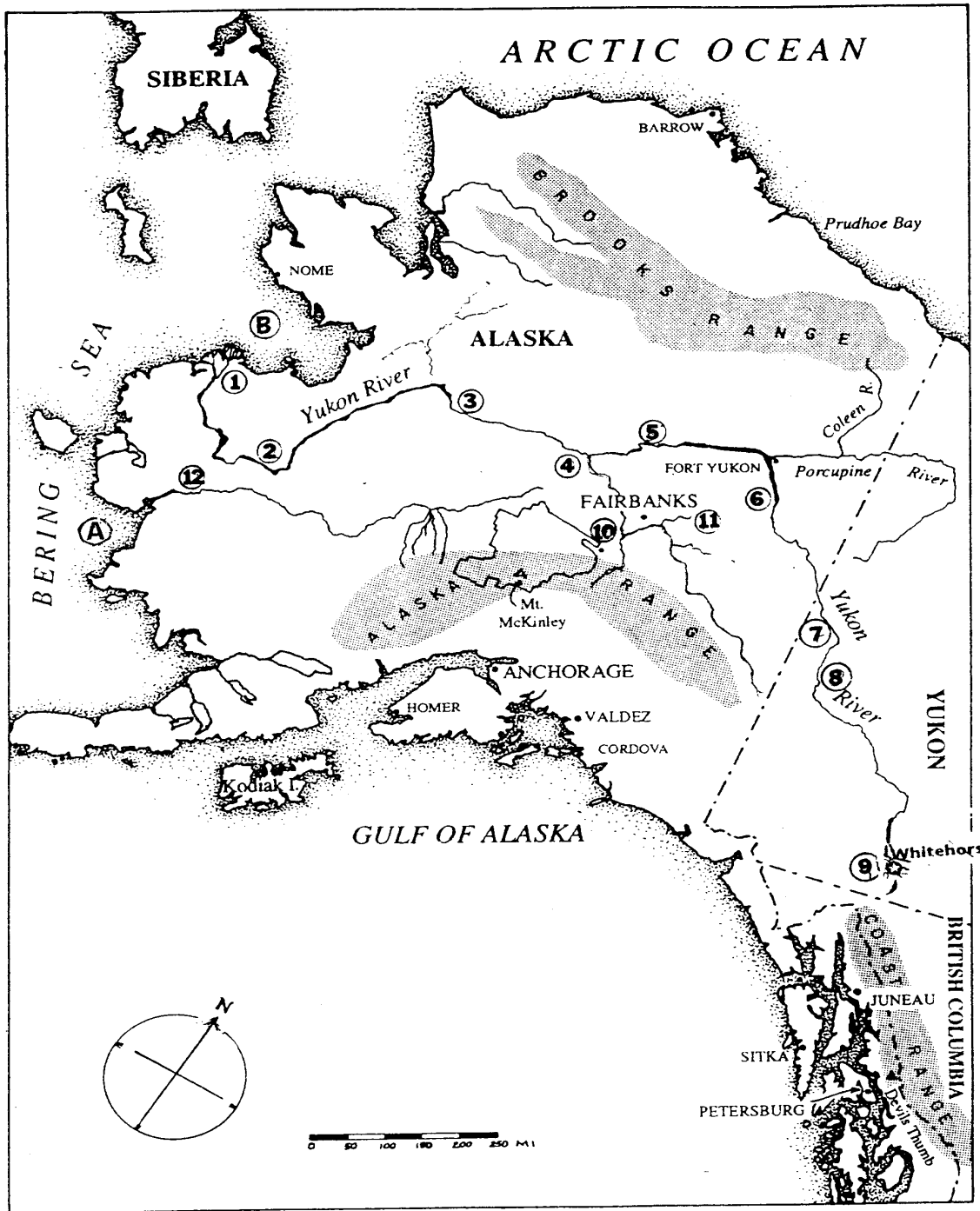
Figure 1. Sample sites for collection of chinook salmon in the Yukon, Tanana, Chena and Kuskokwim Rivers (1-12) and Pacific herring in Goodnews Bay (A) and Norton Sound (B) during 2001. Sites are: 1) Emmonak, 2) Marshal, 3) Galena, 4) Corbusier Slough (Tanana-Yukon confluence), 5) Rampart Rapids, 6) Circle, 7) DFO-White Rock test wheel, 8) Dawson, 9) Whitehorse, 10) Nenana-Tanana River confluence, 11) Chena River, 12) Kuskokwim River at Bethel.

Samples

Sex, length and weight were recorded when possible, and visual (gross) observations made on heart, liver, spleen, skein, muscle and skin. Visual examination of tissues was possible at all sites. Heart and liver tissue were collected from a subset of fish from each site and cultured in Eagle's Minimum 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 $\leq$12 ^{o}C and examined microscopically on-site for the presence of *Ichthyophonus* after 7-10 days in culture. The cultured tissue (explants) allowed us to determine the infection prevalence, while visual examination of each fish allowed us to determine the prevalence and extent of clinical disease. 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 "infection" prevalence and to confirm visual diagnoses. Representative tissue samples were also preserved in 10% formalin for histological verification of the identity of the organism. Females with skeins having attritic eggs or signs of hemorrhage were photographed and recorded.

Biopsies: Non-lethal sampling

Non-lethal sampling was conducted by sampling skin-muscle using 1/4 inch biopsy punches. Twenty-four samples were taken on July 2nd at Rampart Rapids and 50 samples taken by DFO at a test wheel upriver from the Alaska-Yukon border on July 15-18 and on August 8 – 12, 2001. Biopsies were taken from 1/2 inch below the lateral line and in line with the anterior edge of the anal fin. Biopsy tissue was placed into MEM-10 culture medium, incubated and examined as previously described. Visual observations and culture data from internal organs and biopsies were compared to determine the accuracy of punch biopsies.

Temperature effects

Disease prevalence and severity were correlated with current river temperatures[1] and with historical temperatures[2] to determine if elevated water temperature could influence the dynamics of *Ichthyophonus* disease. Water temperature changes of < 5 ^{o}C have been shown to influence the severity of *Ichthyophonus* disease in salmonids (Okamoto 1987). Consequently, because the Yukon River temperatures were significantly above that shown to be optimum for salmon, we hypothesized that elevated river temperature were responsible for the difference in disease severity observed between 1999 and 2000. To test this hypothesis we established the following predictive tests:

1. If elevated water temperature causes more severe disease, then disease severity should be greater during years when water temperatures are higher.

2. If elevated water temperature causes more severe disease, then severity within years should be greater during months when water temperatures are higher.

3. If elevated water temperature causes more severe disease, then present Yukon River temperatures should be higher than historical temperatures, prior to the time *Ichthyophonus* was first recognized in the mid 1980's.

Experimental studies were also carried out by infecting wild buffalo sculpins and exposing them to different temperatures (10^oC and 18^oC). One hundred sculpins were divided into four groups. Two groups were injected IP with *Ichthyophonus* from cultures and two were sham injected. Half of each group was maintained at 8^oC and half at 18^oC). Subsamples from each group were necropsied and tissues taken for culture and histological examination every two weeks.

Source of infection

One hundred Pacific herring (*Clupea pallasi*) were captured at Goodnews Bay, and Unalakleet, AK in May, 2001 and shipped overnight on ice to the University of Washington. The fish were necropsied and examined visually for presence of white nodular lesions on the heart and liver, indicative of *Ichthyophonus* infection. Heart and liver tissue was also cultured in MEM-10 supplemented with antibiotics and examined after 7-10 days for the presence of *Ichthyophonus*. Any isolates of the parasite obtained from these herring were to be used for genetic comparison with isolates obtained from Yukon chinook.

RESULTS

Sample Sites

Ichthyophonus infection prevalence in over 700 sampled chinook salmon was 21.9%. Between sexes, 19.1% of males and 26.6% of females were infected when they reached Tanana (Corbusier Slough) and Rampart Rapids (rm 695–730). As in previous years, clinical signs were less apparent and occurred in ~ 5% of the fish examined at Emmonak – Marshal, but increased to 20 – 30% by the time the fish reached the Rapids. However, unlike the previous two years, sub-clinical infections were more difficult to detect at Emmonak – Marshal because of low intensity of infection. In previous years, colonies in cultured tissues were numerous and readily identified, but in 2001 it was often difficult to identify *Ichthyophonus* colonies, with only one or two colonies observed in approximately 1-2 gm of tissue. This difficulty in identifying low-level infections resulted in false negatives of infection prevalence for *Ichthyophonus* in the lower Yukon in 2001. The parasite did, however, proliferate as the fish moved upriver, with infections and disease reaching levels seen in previous years. Unlike 2000, both infection and clinical disease appeared to decrease at Dawson, which may be an artifact of excessively long storage of cultures prior to evaluation (see section on tissue biopsies). An overall low of 9.7% clinically diseased fish was recorded at Whitehorse, essentially identical to what was observed in 2000. The prevalence of infected males dropped from a high of 20.3% in the middle Yukon to 10.8% at Whitehorse and infected females dropped from 28.1% to just 8.0% (Figure 2a,b).
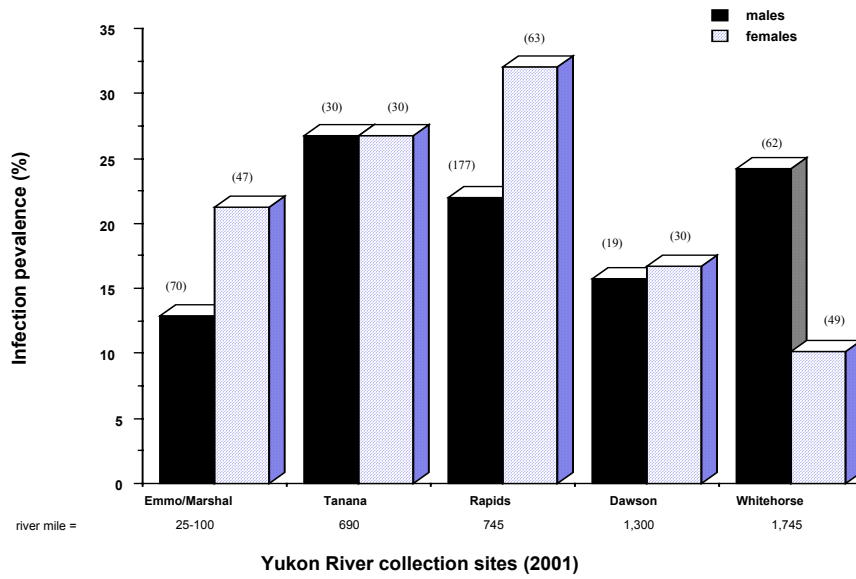
Figure 2a. Infection prevalence in chinook salmon in the Yukon River from Emmonak, AK to Whitehorse, Y.T. for 2001. (N)
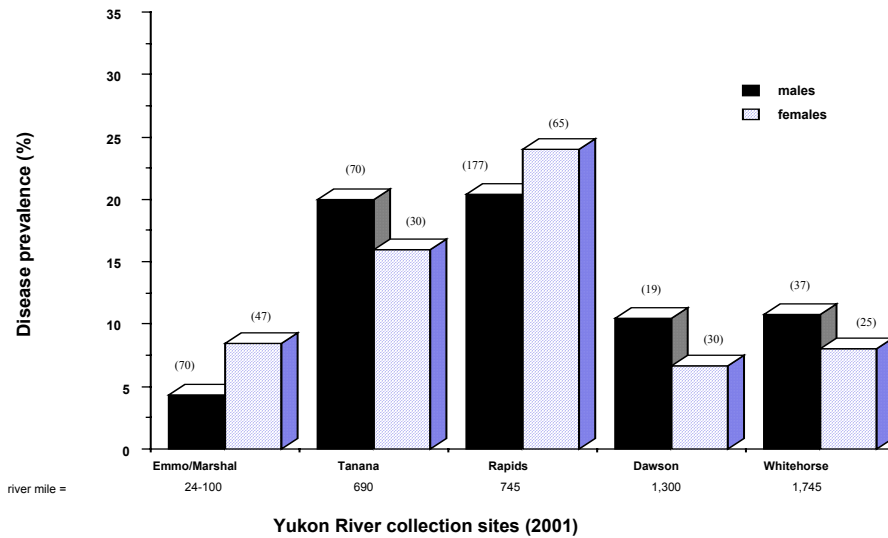

Figure 2b. Disease prevalence in chinook salmon in the Yukon River from Emmonak, AK to Whitehorse, Y.T. for 2001. (N)

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Early vs late-run infection and disease

To investigate the reports of fishermen and processors that late season fish were more severely affected than early season fish, we sampled fish from the Rampart Rapids at the beginning and end of the 2001 run. Early run samples were collected from June 30 through July 4, and late run samples were collected from July 17 through July 22, 2001. These samples corresponded with fish from early and late in the run as determined from CPUE data collected by the U.S. FWS at the Rapids (Figure 3). Early and late season fish had similar infection prevalence rates of 20-30% while late season fish exhibited a slightly higher prevalence of clinical disease (Figure 4a,b). As in previous years, females exhibited higher prevalence rates for both infection and disease for both early and late season fish. However, when the severity of disease was compared, we found that none of 34 clinically diseased early-run fish had more than one affected organ (e.g. heart), while 50% of male and 90% of clinically diseased late season females exhibited disseminated lesions (e.g. multiple affected organs) (Figure 5).
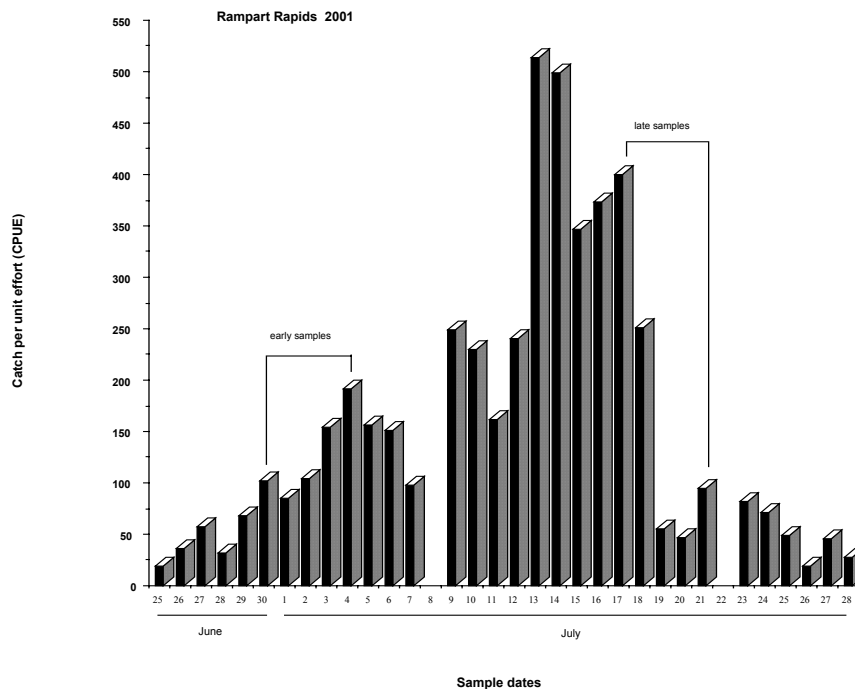

Figure 3. Catch per unit effort (CPUE) for chinook salmon at Rampart Rapids showing early and late season sample periods for 2001.
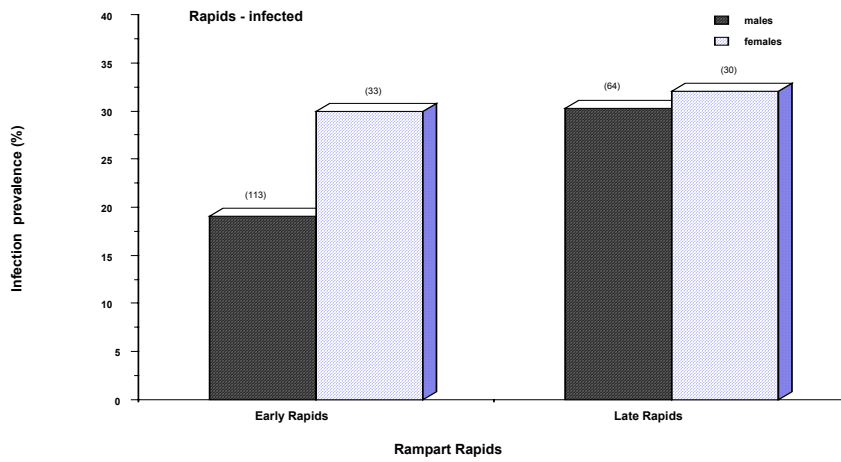
Figure 4a. Infection prevalence in chinook salmon collected at the Rampart Rapids from June 30th – July 4th (early) and July 17th- 21st, 2001 (late). (N)
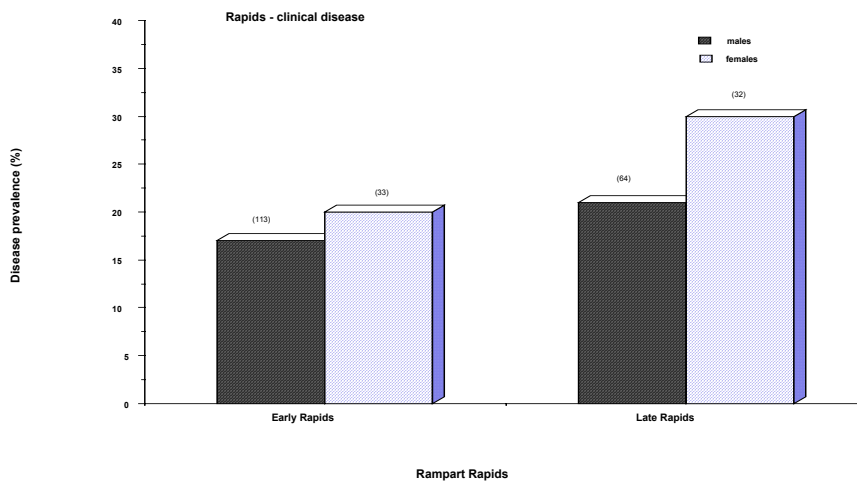

Figure 4b. Disease prevalence in chinook salmon collected at the Rampart Rapids from June 30 – July 4th (early) and July 17- 21st, 2001 (late). (N)
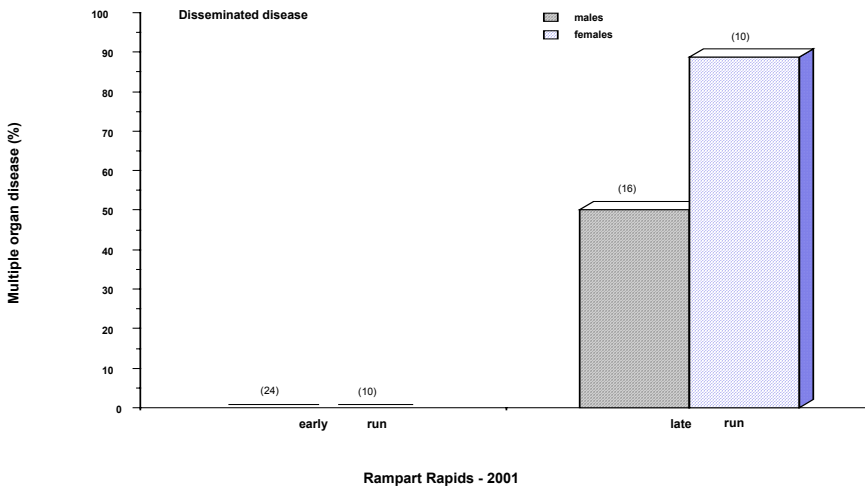
Figure 5. Disseminated disease (multiple affected organs) observed in chinook salmon collected at the Rampart Rapids from June 30 – July 4th (early) and July 17- 21st, 2001 (late). (N)

Chena River spawn-outs

Chinook salmon entering the Tanana River via Corbusier Slough, 6 miles below the town of Tanana, were collected by fish wheel from July 9 to July12, 2001. Males and females entering the Tanana River had similar infection prevalence rates of 26.7%, which remained essentially unchanged when they were sampled by gill net at the mouth of the Chena River near Fairbanks (rm 920) from July 14-15. However, spawn-outs collected by ADF&G from the upper Chena River between July 27 and August 6 had infection prevalence rates of 15.4% (6/39) for males and 0% (0/30) for females. Clinical disease occurred in 10.2% (4/39) of males and 0% of 30 females (Figure 6a,b). This decrease in the number of infected and diseased females is similar to what was observed at Whitehorse in 2000 and again in 2001.

Jacks

In 2000 and 2001 *Ichthyophonus* was isolated from "jacks" and many of these showed signs of clinical disease. We defined a jack as being less than 10 pounds and compared their overall prevalence in the sampled population with their infection prevalence. Although several small females ("jills") were observed, over 95% of the fish < 10 lb were males.

In 2001, 29% (68 / 237) of the sampled fish weighed < 10 lb and were classified as jacks. Of these 68 jacks, 19.0% (13 / 68) were infected with *Ichthyophonus*, representing 5.5% of the total sampled chinook population. These numbers were compared with those obtained from the previous year's samples and revealed that 15% (23/161) of fish less than 10 lb were also infected with *Ichthyophonus* in 2000 indicating no difference in infection from year-to-year (Figure 7) ($X^2 = 0.51$; $P > 0.05$)
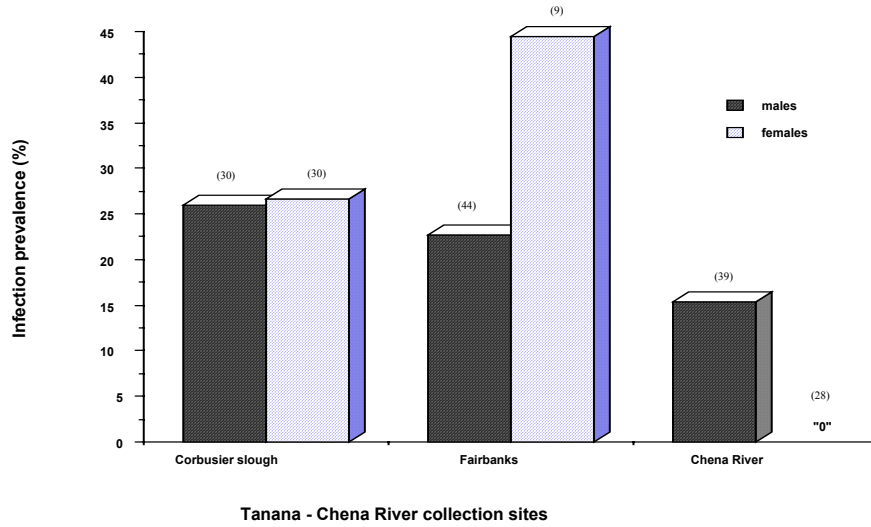
Figure 6a. Infection prevalence in chinook salmon from the Tanana and Chena Rivers, 2001. Chena River samples obtained from live spawn-outs. (N)
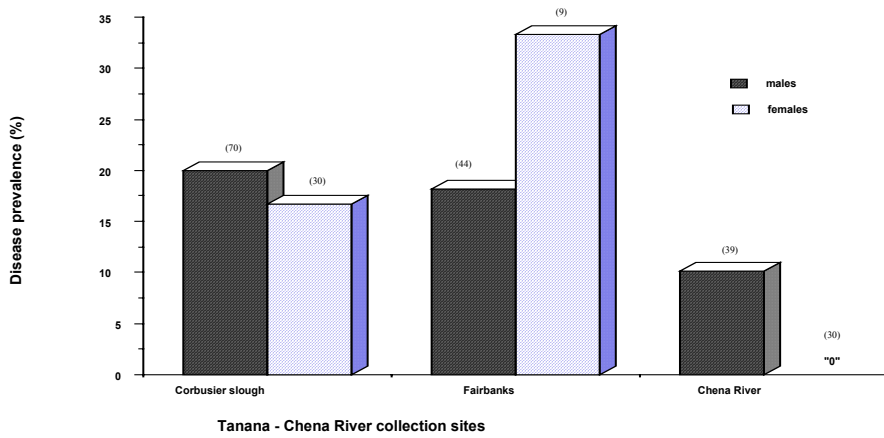
Figure 6b. Disease prevalence in chinook salmon from the Tanana and Chena Rivers, 2001. Chena River samples obtained from spawn-outs. (N)
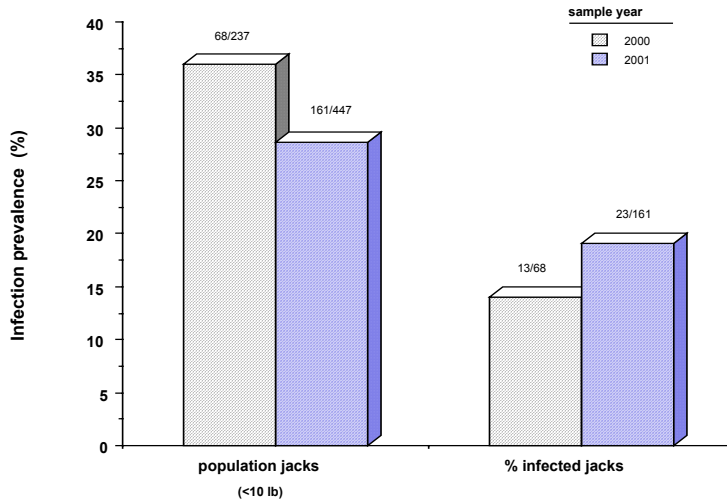
Figure 7. Percent chinook "jacks" (< 10 lb) in samples collected in 2000 and 2001 and the percent infected with *Ichthyophonus.* ($X^2 = 0.51$; $P > 0.05$)

Biopsies: Non-lethal sampling

The infection prevalence of 24 fish sampled at the Rampart Rapids on July 2nd was 29.2% (7/24) detected by standard explant culture. Muscle biopsies of the same fish revealed only 16.7% (4/24) positive, representing 12.5% (3/24) false negatives.

Similar biopsies taken at the DFO fish test wheel near the Yukon border revealed 10% (5/50) positive biopsies collected from July 15-18 and 30.6% (15/49) positive biopsies collected from August 8-12 (Figure 8). Explant cultures were not taken from these fish for infection confirmation, so it is not possible to evaluate the efficiency of these biopsies. However, the increase in the prevalence of fish with infected muscle indicates that significantly more fish had developed disseminated (infecting multiple organs) infections. ($X^2 = 7.86$; $P < 0.01$). Based on down-river infection prevalence, it appeared that essentially all of the infected fish that reached the U.S.-Canada border had developed disseminated infections affecting the skeletal muscle.

Ichthyophonus-positive biopsies (%)

35

30 (50)

25

20

15

10 (50)

5

0

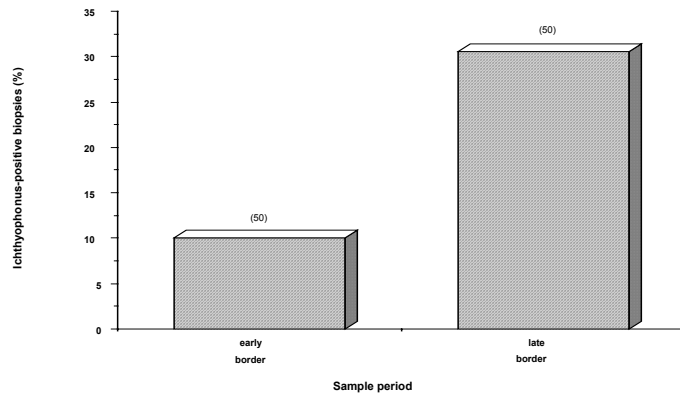early border   late border

Sample period

Figure 8. Muscle biopsies taken at DFO Whiterock test wheel (AK-Yukon border). The number of fish with *Ichthyophonus* in muscle tissue increased by 3X in 21 days ($X^2 = 7.86$; P< 0.01).

Temperature effects on disease severity

1. A comparison of mean water temperatures for 1999 and 2000 shows that the Yukon River was 2-3 ^{o}C higher in 1999, the year when disseminated disease was greatest; supporting the first hypothesis of more severe disease in years when water temperature is warmer (annual report 2000)

2. A comparison of June and July water temperatures for 1999 and 2000 shows that Yukon River water temperatures were consistently higher in July than in June, confirming that late season fish were exposed to higher water temperatures than early season fish. In 1999 and 2000, the only data available on early and late run disease severity were observations by fishermen and processors, who both observed higher levels of infected fillets at the end of the run relative to the early part of the run. In 2001 early and late run data were collected from fish at Rampart Rapids, showing that late run fish had more disseminated lesions than early run fish (Figure 5). Temperature data for 2001, supplied by the USGS again confirms that July water temperatures were higher than June temperatures.

3. Historical Yukon River temperature data shows that prior to the decade of the 1980s, the Yukon River was several degrees cooler from late June through July, than it has been during the decade of the 1990s (Figure 9). *Ichthyophonus* was first reported in chinook salmon in the Yukon River in the mid 80's and has increased in severity through the present time. High temperatures appear to have leveled off at about 19 ^{o}C to 20 ^{o}C for the past decade.
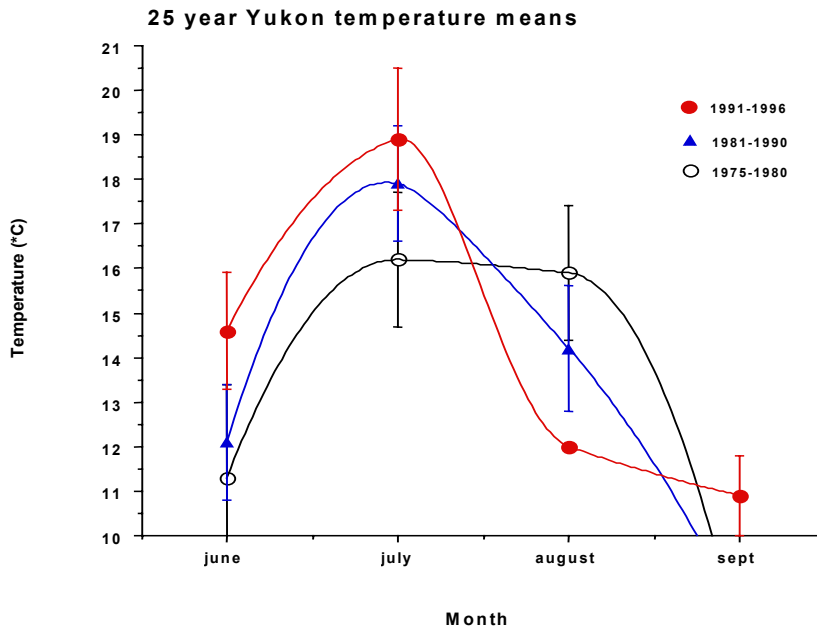
25 year Yukon temperature means

Figure 9. Changes in Yukon River summer temperatures since 1975 recorded at Pilot Station on the lower Yukon River (river mile 122).

Experimental temperature studies

Table 1 summarizes the data obtained following exposure of *Ichthyophonus*-infected buffalo sculpins (*Enophrys bison*) to high and low temperatures similar to those encountered in the Yukon River from June through July. At the end of 14 days there was no difference between treatment groups, but by days 20 and 26, both clinical disease and overall infection were significantly elevated in the high temperature groups.

Table 1. Influence of temperature on the pathogenicity of *Ichthyophonus* in sculpins.

Days post-challenge	Temperature Group	% Clinical Infection	% Total (Clinical + Subclinical)
14	(10 °C)	20% (1/5)	60% (3/5)
	16-18 °C	14% (1/7)	71% (5/7)
20	(10 °C)	14% (1/7)	57% (4/7)
	16-18 °C	75% (6/8)	100% (8/8)
26	(10 °C)	0% (0/7)	29% (2/7)
	16-18 °C	50% (9/18)	67% (12/18)

Adapted from "Halpenny et al" (2002).

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Kuskokwim River

Twenty chinook salmon from the lower Kuskokwim River near Bethel, AK were examined in conjunction with a contaminant study being conducted by the USFWS. One of these fish cultured positive for *Ichthyophonus* and had clinical lesions on the heart and liver. This confirms that *Ichthyophonus* is present in the Kuskokwim River and that it can develop to clinical disease. Data on prevalence and disease impact has not been collected, but is proposed for future sampling.

Source of infection

Pacific herring (*Clupea pallasi*) from Puget Sound north to Prince William Sound and Kodiak Island are known to be infected with *Ichthyophonus*, (Marty et al, 1998, Kocan et al, 1999) and herring are a major food item for salmon. Consequently, we hypothesized that the source of *Ichthyophonus* in Yukon River chinook salmon might be infected herring. To test this hypothesis, we proposed to genetically compare isolates of the organism from Bering Sea herring and Yukon chinook. Attempts to isolate and identify *Ichthyophonus* from Bering Sea herring for two consecutive years were unsuccessful. One hundred herring were collected each year for the past two years (2000, 2001) from Kuskokwim Bay and shipped to the University of Washington where they were examined visually and by explant culture for the presence of *Ichthyophonus*. A similar sample of 100 fish was also collected from Norton Sound near the village of Unalakleet in 2001 and similarly examined for *Ichthyophonus*. None of the 300 fish submitted for analysis was visually or culture-positive for *Ichthyophonus*.

DISCUSSION

Infection and disease prevalence

The prevalence of *Ichthyophonus* infection and clinical disease in Yukon chinook has remained constant at 25-30% for the past 3 years, while the severity of disease has varied from year-to-year and from the beginning to the end of each years run. The prevalence of infection in jacks (< 10 lb) has also remained constant for the past two years. This consistent infection prevalence suggests that a portion of the Yukon chinook are being exposed to a source of infection with essentially all fish becoming infected, while the remaining fish are feeding on uninfected prey. When the two groups of fish return to the Yukon River, they mix, thus producing a constant 25-30% adult infection prevalence from year-to-year and 15% prevalence in fish under 10 lb.

Early vs late run infection and disease

Subsistence fishermen and processors regularly reported that late-run fish were more likely to be infected than early-run fish. Because of this, it was hypothesized that the sicker infected fish migrated more slowly and thus were over represented in the later part of the run. To test this hypothesis we examined fish from early and late in the run at Rampart Rapids and found that infection prevalence and clinical disease was not different between the two groups of fish (Figure 4a,b). Since fish were sampled from extreme ends of the run we concluded that *Ichthyophonus* had no effect on their stamina or position within the run. In light of this finding we concluded that the fish reaching the spawning streams early would be no less likely to be infected than fish entering later in the run.

Effect of Ichthyophonus on spawning fish

The effect of *Ichthyophonus* infection on chinook salmon is most obvious as they approach their spawning streams. Based on data from the Tanana River and Whitehorse, Y.T. collected in 2000, and from Whitehorse and the Chena River in 2001, it appears that *Ichthyophonus*-infected females are dying prior to spawning. In 2000 and 2001 a dramatic drop in the percent of infected females occurred at Whitehorse, and a similar drop in infected females occurred at Nenana in 2000. Although there are a number of possible explanations for this decline, we suspected that the females were dying as they approached their spawning streams. To test this hypothesis, we examined fish as they entered the Tanana River, at the mouth of the Chena River and again as spawn-outs in the upper Chena River. Although no infected pre-spawn dead females were observed, there were no infected females among the 30 spawn-outs examined from the Chena River during the first week of August. This was unexpected since nearly 30% of the females were found to be infected when they entered the Tanana River and at the confluence of the Chena River. The prevalence of infection in males did not seem to decline significantly, which is similar to what was recorded at Whitehorse in 2000 and 2001.

Temperature effects

The severity of infection from year-to-year and within the same year appears to be temperature related. The most sever disease observed during this study occurred in 1999 when river temperatures reached 20 ^{o}C (68 ^{o}F) in late June and July, which is three degrees higher than what was recorded in the subsequent two years when disease severity was lower. The most severely diseased fish also occur during the late run when river temperatures reach their peak. Although the prevalence of infection and clinical disease is similar in both early and late run fish, clinical disease in early run fish is restricted to the heart, while late run fish exhibit a much more disseminated form of the disease, which affects multiple organs, including the muscle. It is highly probable that because of this muscle involvement that fishermen and processors were more aware of *Ichthyophonus*-infected fish taken later in the run.

Because disease severity can be correlated with elevated river temperature, and elevated temperature is known to increase the pathogenicity of *Ichthyophonus* (Okamoto 1987), we hypothesized a cause-and-effect relationship between river temperature and disease due to infection by *Ichthyophonus*. This hypothesis is supported by several observations: 1) The most severe disease occurred in 1999, the warmest year during this study, 2) Late run fish are more severely infected than early run fish, corresponding to the warmer period of the migration, and 3) Current river temperatures are higher than pre-1980s temperatures.

Historical temperature data going back several decades shows that in the mid 1980s Yukon River temperatures began to increase, and have remained approximately 4-5 ^{o}C higher during the decade of the 1990s. If *Ichthyophonus* has always been enzootic in Yukon chinook, this would explain why it was not observed prior to the mid 1980s, and why it has persisted and become more severe through the last decade. Since there is no evidence that *Ichthyophonus* was present in the Yukon River prior to the1980s, this is only one of several hypotheses explaining the recent rise in infection and disease in Yukon chinook. However, the correlation of disease severity with present elevated water temperatures remains valid.

Biopsy samples

The use of biopsies to identify *Ichthyophonus* infections appears to have limited value when the fish enter the River, but is more reliable as they approach their spawning areas. Early in the run and in the lower river where most infections are either subclinical or restricted to the heart tissue, many false negatives were be encountered. However, later in the run when the disease had spread to numerous tissues, biopsies appeared to be very predictive of overall infection prevalence. This also appeared to be true for upper Yukon fish, but this has yet to be confirmed by controlled sampling. In 2002 we expect to test the hypothesis that late run and upper Yukon fish can be sampled accurately with just punch biopsies.

Source of infection (herring?)

Although no *Ichthyophonus*-infected herring were found in 300 Bering Sea herring over a two-year period, it is still possible that infected herring are the source of infection for Yukon chinook. If 25-30% of the chinook leaving the Yukon River each year migrate through False Pass and feed south of the Aleutian Islands, they would be at risk of encountering infected herring around Kodiak Island and Prince William Sound. After feeding on these infected herring, they would migrate back through False Pass and rejoin the remainder of the Yukon chinook that fed on uninfected Bering Sea herring, thus making up the 25–30% of the infected chinook consistently observed in the Yukon River. Historically this may not have been a problem, but with increasing water temperatures in the Yukon over the past 20 years, the parasite was able to proliferate faster than normal, thus overwhelming the fish's immune system.

CONCLUSIONS

As in previous years, Yukon River chinook salmon entering the river in June were found to be infected with *Ichthyophonus*. Although >20% of sampled fish were infected, only about 5% showed clinical signs of disease when they entered the River. By the time the fish reached Tanana and the Rampart Rapids, most of the infected fish presented with clinical signs of disease, with significantly more females than males being infected. Fish sampled early in the run exhibited clinical lesions only in the heart, while 50% of male and 90% of late season females with clinical disease exhibited disseminated lesions affecting multiple organs.

The prevalence of infection and disease in fish sampled at the mouth of the Tanana River (Corbusier Slough) and later at the mouth of the Chena River, were similar to that found in fish sampled at the Rampart Rapids. However, when spawn-outs were sampled from the Chena River in late July and early August, infections were found only in males. No infection or disease was detected in 30 females, suggesting that they were removed from the population prior to spawning.

We tested the hypothesis that *Ichthyophonus* infection was influenced by water temperature and found that every prediction made was supported by the data. eg. 1) The most severe disease was observed in 1999, the warmest year encountered during this study. 2) Late season fish exhibited more disseminated disease and were exposed to higher temperatures than early season fish. 3) The Yukon River has become steadily warmer since the mid 1980s, when *Ichthyophonus* was first reported from Yukon chinook.

As in previous years, no evidence of *Ichthyophonus* could be found in Bering Sea herring sampled at Good News Bay and at Norton Sound, suggesting that: 1) Another forage species is the source of infection. 2) Some portion of the Yukon chinook may be feeding in areas containing infected herring, possibly south of the Aleutian Islands. 3) Chinook are not being infected in salt water.

1) *Ichthyophonus* is firmly established in Yukon River chinook salmon.
2) *Ichthyophonus* has become more pathogenic over the past 15 years, possibly due to an increase in river temperature.
3) Female chinook salmon are more likely to be infected and have a higher prevalence of disease than males.
4) *Ichthyophonus*-infected females have a high probability of dying before they spawn.
5) Kuskokwim River chinook salmon are also infected with *Ichthyophonus*.
6) The source of *Ichthyophonus* infection is not known, but does not appear to be Bering Sea herring.

RECOMMENDATIONS

1. Establish a monitoring station in the lower Yukon to determine the prevalence of *Ichthyophonus* early in the season.
2. Use real-time water temperatures at Pilot Station to predict upriver disease severity.
3. Use potential female mortality in calculating biological escapement success.
4. Attempt to identify the source of infection; species and fresh or salt water.

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LITERATURE CITED

Fish F.F. 1934. A fungus disease in fishes of the Gulf of Maine. Parasitology 26:1-20.

Halpenny, C.M., R.M. Kocan, J.R. Winton, J.A. Perry, and P.K. Hershberger 2002. Elevated temperature exacerbates *Ichthyophonus* infections in buffalo sculpin. Fish Health Section Newsletter 30:2:17-20.

Harrell L.W., R.A. Elston, T.M. Scott, and M.T. Wilkinson 1986. A significant new systemic disease of net-pen reared chinook salmon (*Oncorhynchus tshawytscha*) brood stock. Aquaculture 55: 249-262.

Kocan R.M., P. Hershberger, T. Mehl, N. Elder, M. Bradley, D. Wildermuth, and K. Stick 1999. Pathology of *Ichthyophonus* hoferi for laboratory-reared Pacific herring *Clupea pallasi* and its early appearance in wild Puget Sound herring. Diseases of Aquatic Organisms 35:23-29.

Kocan, R.M. and P.K. Hershberger 1999. Yukon River *chinook* salmon-*Ichthyophonus* pilot study. 15 pp. Final Report to Bering Sea Fishermen's Association, Anchorage, AK.

Kocan, R.M. and P.K. Hershberger 2001. Yukon River King Salmon – Ichthyophonus Pilot Study (2000) Final report to U.S. Fish & Wildlife Service. pp. 33.

McVicar A.H. 1982. *Ichthyophonus* infections of fish. In: R.J. Roberts (ed) Microbial diseases of fish. Academic Press, London, p 243-269.

McVicar A.H. and H.A. McLay 1985. Tissue response of plaice, haddock, and rainbow trout to the systemic fungus *Ichthyophonus*. In: A.E. Ellis (ed) Fish and Shellfish Path. Academic Press, London, p 329-346.

McVicar A.H. 1990. Epidemiology/epizootiology: A basis for control of disease in mariculture. In: F.O. Perkins and T.C. Cheng [eds] Pathology in marine science, Academic Press, Inc., New York, p 397-405.

Marty G.D., E.F. Freiberg, T.R. Meyers, J. Wilcock, T.B. Farver and D.E. Hinton 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Diseases of Aquatic Organisms 32:15-40.

Mellergaard, S. and B. Spanggaard 1997. An *Ichthyophonus hoferi* epizootic in herring in the North Sea, the Skagerrak, the Kattegat and the Baltic Sea. Diseases of Aquatic Organisms 28:191-199.

Okamoto N., K. Nakase, H. Suzuki Y. Nakai, K. Fujii and T. Sano 1985. Life history and morphology of *Ichthyophonus hoferi* in vitro. Fish Pathology 20:273-285.

Okamoto, N., K. Nakase, Y. Nakai, H. Suzuki and T. Sano 1987. Experimental oral infection of rainbow trout with cultivated spherical bodies of *Ichthyophonus hoferi*. Nippon Suisan Gakkaishi 53:407-409.

Rahimian, H. and J. Thulin 1996. Epizootiology of *Ichthyophonus hoferi* in herring populations off the Swedish west coast. Diseases of Aquatic Organisms 27:187-195.

Rucker, R.R. 1953. An epizootic among rainbow trout. Progressive Fish-Culturist 15: 179-181.

Sinderman C.J. 1958. An epizootic in Gulf of Saint Lawrence fishes. Transactions of the North American Wildlife Conference 23:349-360.

Sindermann C.J. and J.F. Chenoweth 1993. The fungal pathogen *Ichthyophonus hoferi* in sea herring *Clupea harengus*: A perspective from the Western North Atlantic. In: International Council for the Exploration of the Sea, C.M. 1993/F:41, Pelagic Fish Committee. pp 1-39.

Sinderman C.J. and A. Rosenfield 1954. Diseases of fishes of the Western North Atlantic: I. Diseases of the Sea Herring. Research Bulletin No. 18. Dept. of Sea and Shore Fisheries, Augusta, Maine, p 1-23.

Sinderman C.J., and L.W. Scattergood 1954. Diseases of fishes of the Western North Atlantic: II. *Ichthyosporidium* disease of the sea herring (*Clupea harengus*). Research Bulletin No. 19 Dept. of Sea and Shore Fisheries, Augusta, Maine, p 1-40.

Spanggaard, B., L. Gram, N. Okamoto and H.H. Huss 1994. Growth of the fish-pathogenic fungus, *Ichthyophonus hoferi*, measured by conductimetry and microscopy. Journal of Fish Diseases 17:145-153.

Spanggaard, B. and H.H. Huss 1996. Growth of the fish parasite Ichthyophonus hoferi under food relevant conditions. International Journal of Food Science and Technology 31:427-432.

Spanggaard, B., P. Skouboe, L. Rossen and J.W. Taylor 1996. Phylogenetic relationships of the intercellular fish pathogen *Ichthyophonus hoferi* and fungi, choanoflagellates and the rosette agent. Marine Biology 126: 109-115.

Footnotes

[1] 1999 & 2000 temperature data supplied by Chikita, Kazuhisa, Laboratory of Hydrology Division of Earth and Planetary Sciences, Hokkaido University, Sapporo, Japan (060-0810).

[2] Current temperature data supplied by Tim Brabbits, U.S. Geological Survey, Fairbanks, AK.