Final Report

Ichthyophonus-Yukon River Chinook Study

URE-13-03

Submitted by:

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November 18, 2003

Abstract

When Chinook salmon entered the Yukon River in 2003 32% were infected with Ichthyophonus, similar to what has been reported during the previous four years. However, in 2003 the number of clinically infected fish approached the total infection prevalence at all sample sites, and clinical disease appeared earlier in the run than in previous years. Fish sampled simultaneously from the north and south shore of the Yukon River at Tanana Village (river mile 690) exhibited different levels of infection and disease as predicted from previous years data. Significantly more north shore males and females exhibited signs of disease than did south shore fish. These two sub-populations ultimately migrate to geographically distinct areas; north shore fish to the upper Yukon River and Canada, and south shore fish to the Tanana River. Significantly fewer fish sampled from the Whitehorse hatchery and the Chena and Salcha Rivers (terminal spawning streams) were diseased relative to the same population sampled from the Tanana River earlier in the season. The prevalence of infection in males in 2003 was not different from that seen in females and there was no difference among size (age) groups.

Objectives:

1. Establish baseline *Ichthyophonus* infection prevalence at Emmonak for comparison with previous years samples and 2003 upriver samples.
2. Determine if Canadian-bound chinook have different infection and disease prevalence than Alaskan fish.
3. Examine spawn-outs to expand on previous years findings that *Ichthyophonus*-infected post-spawn adults are under represented on the spawning streams.

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Introduction

Studies on the effect of Ichthyophonus on Yukon River Chinook salmon have been conducted annually since 1999. During that time it was observed that each year females are more likely to be infected than males, fewer post-spawn fish exhibit clinical disease and infection than pre-spawn fish and that fish in the Yukon mainstem have higher infection and disease prevalence than those in the Tanana River. (Kocan et al 2003). Histopathology data confirms that a clear distinction exists between fish from the two rivers, with Yukon River fish exhibiting more severe tissue damage than Tanana River fish.

Each of these issues was addressed in the 2003 study. Comparisons were made between males and females, infection and disease progression was determined and Yukon and Tanana River fish were sampled simultaneously to determine whether they were actually different.

Because there are several possible explanations for the differences observed in Yukon and Tanana River fish, all variables had to be accounted for in order to formulate and test any hypothesis. The most significant variables that had to be controlled were: 1) residence time in-river, 2) distance traveled from mouth of river, and 3) proximity to spawning streams. These variables were addressed by sampling fish from river mile 690 near the confluence of the Tanana and Yukon Rivers. At this point all fish have traveled the same distance (~ 690 river miles) and if captured at the same time, should have spent the same amount of time en-route. At this point the majority of Tanana River fish have < 200 river miles before reaching their spawning streams between the mouth of the Kantishna River and the mouth of the Salcha River, while the north-shore fish have between 575 and 1,050 additional river miles to travel before reaching their spawning streams (mouth of Fortymile River to Whitehorse, YT). If proximity to spawning streams is associated with survival and pathogenicity, then fish sampled from the north and south shore of the Yukon at its confluence with the Tanana River should reveal any differences in infection prevalence and pathogenicity. This project was designed to test that hypothesis as well as continue monitoring the progression of Ichthyophoniasis in Yukon and Tanana River Chinook salmon.

Methods

Fish numbers:

In order to accomplish the field objectives set out in the original proposal, a target of 80 - 100 Chinook salmon of each sex was established for each sample site. The proposed sample sites included 1) Emmonak (set and drift nets) and the north and south shore of the Yukon River at Tanana Village (fish wheel). Mike Doxey (ADF&G) sampled spawn-outs from the Chena River on August 13 and Chris Stark (BSFA), collected Salcha River samples on August 9th, 12th and 14th. Pat Milligan of DFO-Canada supplied samples from the Whitehorse hatchery collected from August 21st to 29th.

Rationale:

The returning adults were treated as a single binomial population consisting of infected and uninfected individuals. At Emmonak the population is a mix of lower, middle and upper Yukon River fish as well as Tanana River fish. At the second collection point (Tanana Village), only the lower Yukon fish would have segregated out of the population and their contribution can calculated by comparing the infection prevalence of Emmonak fish with the combined north and south shore fish from river mile 690. The north and south shore fish were then compared with each other as well as Emmonak fish to determine any difference between the lower Yukon fish, Tanana River fish and upper Yukon-Canadian fish at that point.

Infection and disease:

Fish were examined for clinical disease by visual examination and for infection prevalence by explant cultures (Kocan et al 1999). Heart, liver, spleen and muscle tissue was preserved in 10% Formalin for histologic evaluation of host response and tissue damage.

The best available data suggests that migrating salmon captured on the south shore of the Yukon River at the mouth of Corbusier Slough are essentially all Tanana River fish, while the majority of those migrating along the north shore are bound for the upper Yukon River and Canada (Bucklis, 1981, 1984). Although Bucklis' study dealt with chum salmon, it is reasonable to believe that chinook behave similarly. We have also observed that Corbusier Slough fish are dark, have hooked jaws, large teeth, pale flesh and protruding ovipositors, while the majority of fish from the north shore are either silver or "blush" and have red flesh, indicating that they are still some distance from their natal streams. Fish from the south shore were obtained from the U.S. FWS test wheel at the mouth of Corbusier Slough operated by Bill

Fliris, and from the north shore from a wheel operated by Pat Moore, a Tanana subsistence fisherman.

Capture and identification: Fish were captured by gill net at Emmonak and by fish wheel from both shores at Tanana Village. A study done in 2002 showed no difference in infection prevalence for males or females captured by gill net compared with those caught by fish wheel (X^2=1.5; P >0.05; n=371)

On-site examination: Within six hours of capture, fish were sexed, weighed, measured, and examined visually (gross examination) for clinical disease which consisted of white punctate lesions on internal organs and skeletal muscle. Samples of tissue were placed into culture medium to confirm *Ichthyophonus* infections and to evaluate the percent of subclinical infections. Other pathologic conditions, parasites and abnormalities were recorded at the time of visual examination. Data was recorded in field notebooks and transferred to electronic spreadsheets for analysis. Cultures were microscopically examined on-site after 10 days incubation.

Explant culture: Approximately 1 g of heart or skeletal muscle was placed into tissue culture medium supplemented with 5% fetal bovine serum and 2X antibiotics. Cultures were incubated on-site and microscopically evaluated for the presence of *Ichthyophonus* within 7 days of sampling. The presence or absence of growth was recorded and correlated with the visible lesions observed in the field. Correlations were also be made with sex, size, and geographic location. Representative positive cultures were shipped to the USGS laboratory on Marrowstone Island, WA and used as a source of material for experimental transmission and genetic studies.

Statistical analyses: Yukon River Chinook were treated as a binomial population consisting of infected and uninfected individuals (treatments), recognizing that the population consists of multiple sub populations, which ultimately segregate themselves as they migrate upriver. To evaluate differences between groups a null hypothesis (H_o) was established for each, which states that there is no difference between groups. To test this hypothesis we used a 2 X 2 X^2 (chi-square) *statistic*, to calculate a value from the observed data that summarizes the evidence against the H_o (Gordis 2000, Leaverton 1978, Colton, 1974, Witts, 1964). To do this we constructed a 2 X 2 table consisting of "Groups" and "Treatments":

Group	Successes	Failures	Total
A	a	b	a+b
B	c	d	d+c
Total	a+c	b+d	n

A X^2 value is computed from this table using equation (2)

$$X^2 = \frac{(|ad-bc| - n/2)^2 * n}{(a+c)(b+d)(a+b)(c+d)} \quad (2)$$

Groups can consist of gender or sample site (Male:Female; Emmonak: Rapids, etc) while "success - failure" represents "infected -uninfected" individuals in all cases. Values of $X^2 \geq 3.84$ but < 6.64 represent a P value of ≤ 0.05, while a value ≥ 6.64 represent a $P \leq 0.01$.

Infection prevalence in fish from Emmonak was compared with that of fish from the north and south shore of the Yukon River at Tanana Village. The null hypothesis (Ho) was that there is no difference between the fish at river mile 24 and those at river mile 690. The variable being tested here is that a significant proportion of infected fish divert to tributaries in the lower Yukon (Andryevski, Koyukuk, Tozitna) and change the overall make-up of the population at Tanana. We also compared the north and south shore fish using the same null hypothesis. The variable being investigated here is that Tanana River fish are different from middle Yukon and Canadian fish. This can be done by directly comparing fish from the north and south shores as well as with Emmonak fish.

Terminal spawning sites included the Whitehorse Rapids, YT (rm 1,745), , and the Chena and Salcha Rivers, AK.

Approximately 1 g of heart tissue was cultured in 5 mL of Eagle's Minimum Essential Medium or Leibovitz L-15 Medium supplemented with 5% fetal bovine serum, 100 IU mL^{-1} penicillin,

100 μg mL^{-1} streptomycin and 100 μg mL^{-1} gentamycin. Cultures were incubated at •15 °C and examined microscopically for the presence of *Ichthyophonus* after 7-14 d in culture. Cultured tissues (explants) were used to determine the total infection prevalence (clinical + sub-clinical), while clinical disease was determined by visual examination of internal organs. The characteristic growth of *Ichthyophonus* in explant cultures was used to confirm visual diagnoses (Okamoto et al. 1985, Spanggaard et al. 1994, Rahimain and Thulin 1996; Kocan et al. 1999). For accuracy and consistency, "infection" data was based on culture or histologic confirmation of *Ichthyophonus*.

Fish were classified as: 1) "Infected" - the organism was isolated or identified from a fish, 2) "Clinical disease" or "Diseased" - Visible white punctate lesions were observed on at least one organ and confirmed to be *Ichthyophonus*, 3) "Disseminated disease" - Lesions present in multiple organs, and 4) "Sub-clinical" - Fish were infected but without visible lesions. Fish were considered "negative" if *Ichthyophonus* could not be identified by any of the above techniques, with the realization that some false negatives likely occurred in fish with very low levels of infection. Because some low-level infections were probably missed, the data represent minimum infection prevalence.

Results

Sample totals

A total of 451 male and 238 female Chinook salmon were sampled for *Ichthyophonus* during 2003. Sample sites included Emmonak (n=97), Tanana Village north shore (n=267), Tanana Village south shore (Corbusier Slough) (n=171), Nenana/Fairbanks (n=51), Chena River (n=44), Salcha River (n=27) and Whitehorse hatchery (n=37). Table I summarizes the infection and disease data from these sites.

Clinical and subclinical infections

Lower and middle Yukon River fish exhibited clinical infections approaching the total infection prevalence (e.g. clinical + subclinical). Explant cultures revealed < 5% subclinical cases at Emmonak and the two Tanana Village sample sites, while Nenana/Fairbanks and Whitehorse produced fish with 10 – 17% subclinical infections (Table I). Clinical disease data was obtained only from Chena River fish.

North and south shore comparison with Emmonak

A comparison of infection and disease between fish from Emmonak and the north and south shore of the Yukon River at Tanana Village (river mile 690) revealed no significant difference between the two sample groups (Figure 1). However, when infection prevalence was compared between north and south shore fish, a higher proportion of infected fish were identified from the north shore (Figure 2). This is similar to what Kocan et al (2002, 2003) reported for previous years when fish from the Rapids were compared with fish from the Tanana River.

Lower Yukon River to Whitehorse

Infection and disease prevalence at the Whitehorse hatchery (YT) was lower than that seen in lower and middle Yukon River fish, similar to what has been reported over the previous four years (Kocan et al 2000, 2001, 2002). The proportion of clinically diseased fish at Whitehorse decreased significantly relative to lower river sites ($X^2 = 7.79$; $P < 0.01$), leaving a higher proportion of subclinically infected fish (Figure 3).

Chena and Salcha Rivers

Total infection and disease prevalence was significantly lower in Chena and Salcha River fish when compared with Tanana River or Emmonak fish ($X^2 = 6.06$; $P < 0.025$). Clinical disease prevalence dropped from > 25% to < 5% between the Tanana River and the Chena and Salcha Rivers, leaving a higher proportion of subclinically infected post-spawn fish (Figure 4). There was no difference in disease or infection prevalence between Emmonak and Tanana River fish.

Clinical disease

From 1999 to 2000 the mean prevalence of clinical disease in fish entering the Yukon River was 2.8%. In 2001 prevalence increased to 6%, then to 11.4% in 2002 and 27.2% in 2003. During this same period clinical disease remained constant in fish sampled from the middle Yukon River (Figure 5). During this same 5-year period, there was also a steady increase in the percent of infected males in the Yukon River mainstem, increasing from a low of ~15% in 1999 to a high of >30% in 2003 (Figure 6).

Infection and fish size

A comparison of five weight groups, ranging from < 10 lb to > 26 lb showed that the smallest fish (1-10 lb) had the lowest prevalence of infection (21%) while the 21-25lb group had the highest prevalence at 48%. All other weight groups fell between 27% and 32% infection prevalence (Figure 7).

Discussion

Results obtained from the 2003 Yukon River Chinook salmon Ichthyophonus study were similar to that observed over the past four years. Fish enter the Yukon River already infected with Ichthyophonus, and as they migrate upriver clinical disease increases until it approaches total infection. Fish sampled from spawning streams and the Whitehorse hatchery again presented with clinical disease and total infection prevalence significantly lower than that seen in the mainstem Yukon and Tanana Rivers. Although there is no conclusive proof as to what happend to the diseased fish, it is certain that they were underrepresented among post-spawn fish.

There was no significant difference in diseased or infected fish between the mouth of the river at Emmonak (rm 24) and at Tanana Village (rm 690) ($X^2 = 0.06$; $P > 0.10$) indicating no differential diversion of infected fish to lower Yukon River tributaries. However, fish from the north shore of the Yukon River at rm 690 did have a higher prevalence of infection and disease when compared with fish sampled simultaneously from the south shore (Table I, Figure2). The difference between males was significant ($X^2 = 5.01$; $P = 0.02$), while the difference between females was not ($X^2 = 1.37$; $P > 0.05$). However, this same pattern of a higher percentage of upper Yukon-bound females being infected than Tanana River females has occurred since

2001 (Kocan et al 2002). Previous comparisons showed that significantly more males and females sampled at the Rapids (rm 730) were infected than were Tanana River fish (Kocan et al 2002). North shore fish are most likely to be bound for the upper Yukon River and Canada, while south shore fish at rm 690 are essentially all Tanana River fish and much closer to their natal streams.

A comparison of mainstem Yukon and Tanana River fish with fish from Whitehorse and the Chena and Salcha Rivers revealed a pattern of lower disease and infection prevalence in post-spawn fish, similar to that observed over the past four years. The greatest difference observed was in the proportion of fish exhibiting clinical disease, which droped from a high of 43% in the Tanana mainstem fish to a low of 14.1% in post-spawn fish. This pattern of loss of Ichthyophonus-infected fish has occurred every year since 2002, and appears to be the result of the loss of pre-spawn fish as they approach their natal streams.

The most dramatic change in the pattern of Ichthyophonus progression observed over the past five years has been the steady increase in the percent of infected males, from a low of ~15% in 1999 to over 30% in 2003. This increase in the number of infected males resulted in an overall increase in infection prevalence when values from both sexes were pooled, even though female infection prevalence has not increased significantly since the study began. A second change in disease progression was the steady increase in the percent of fish exhibiting clinical disease when they entered the river at Emmonak. In 1999 and 2000 the percent of fish exhibiting clinical signs at the mouth of the Yukon River was < 5%. This increased each year until by 2003 over 27% of the fish entering the River showed clear signs of clinical disease; that is, 87% of all infected fish were already clinically positive when they entered the river. In 2003 subclinical infections exceeded clinical infections by less than 5% in the mainstem Yukon and Tanana Rivers. This is in contrast to previous years when as many as 30% of the infected fish were subclinical. The cause of the increase in clinical cases early in the run and it's high prevalence throughout the remainder of the run, may be related to increased parasite virulence, time of exposure or changing environmental conditions that favor growth of the parasite.

When size classes were compared for infection prevalence, the smallest fish (< 10 lb) exhibited the lowest infection prevalence (21%), while the 21-25 lb weight class had the highest

infection prevalence (47%). Since 57% of all fish sampled from the lower Tanana River were < 10 lb, this explains why lower Tanana River fish appeared to be less heavily infected than upper river fish (Appendix I).

Conclusions

1. Ichthyophonus continues to be present in Yukon River Chinook salmon
2. Ichthyophonus appears to be increasing in prevalence in males
3. Clinical signs of disease are appearing earlier every year
4. Clinical signs of disease are approaching total infection prevalence
5. Diseased and infected fish continue to be absent from terminal spawning areas
6. Infection and disease continues to be distributed over all weight classes

Acknowledgments

The authors are indebted to the following for providing data and assistance in field collections: Mike Doxey and Tracy Lingnau, (Alaska Department of Fish & Game); Bill Fliris & Patrick Moore (Tanana, AK); Virgil Umphenor, (Interior Alaska Processors, (Fairbanks). This research was funded by the Office of Restoration and Enhancement (URE13-03).

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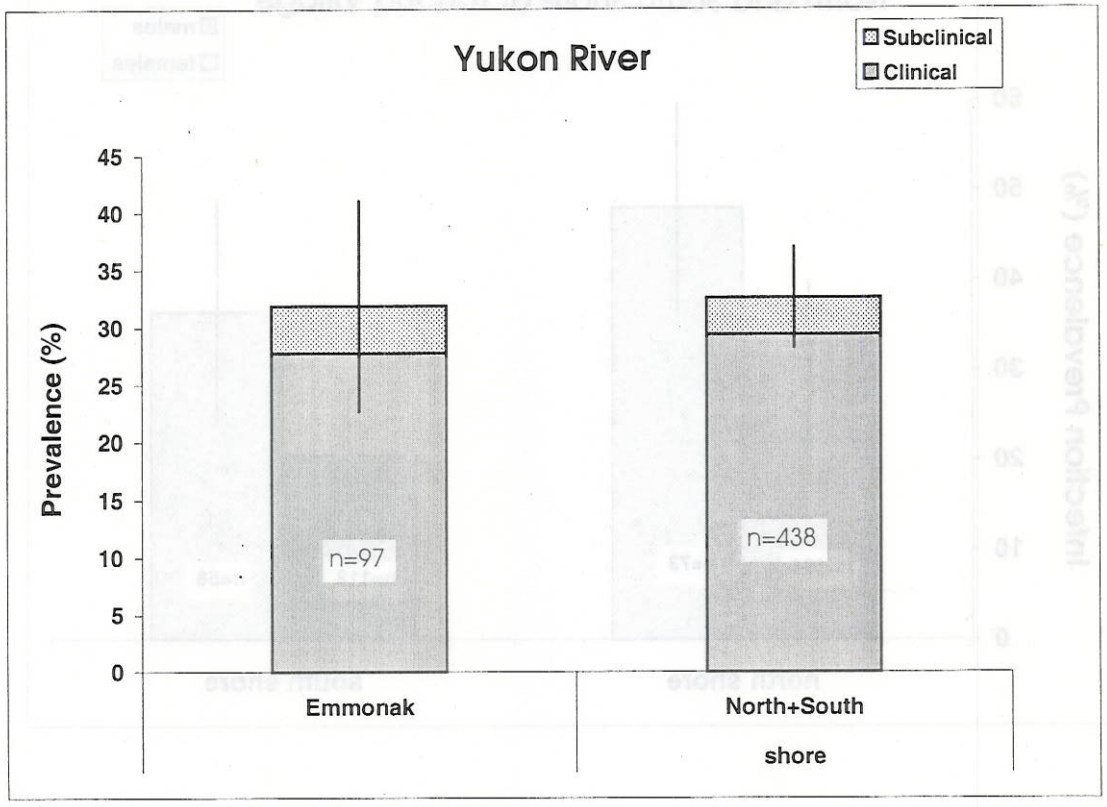

Figure 1. Comparison of Ichthyophonus-infected fish from Emmonak and Tanana Village (lower and middle Yukon). No difference between infection and disease in the two populations was detected.

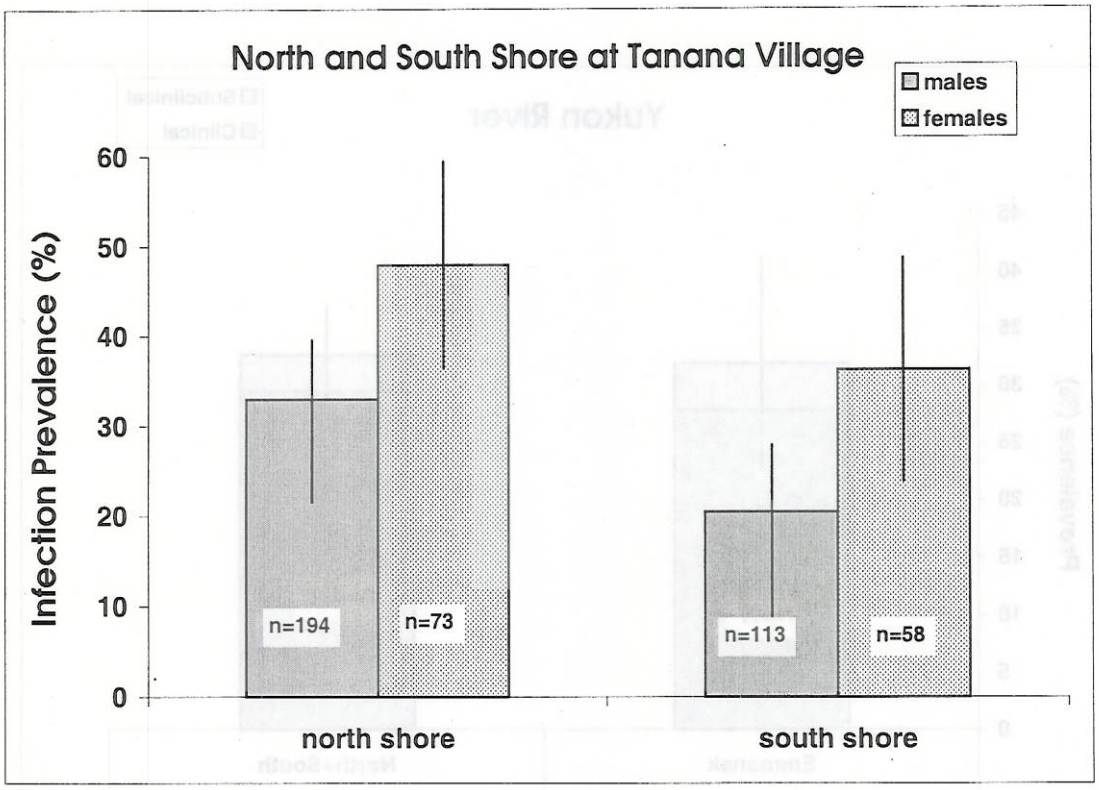

Figure 2. Comparison of infection in north and south shore Chinook salmon at Tanana Village (river mile 690). There were more infected males and females detected from the north shore. Bars = 95% conf.

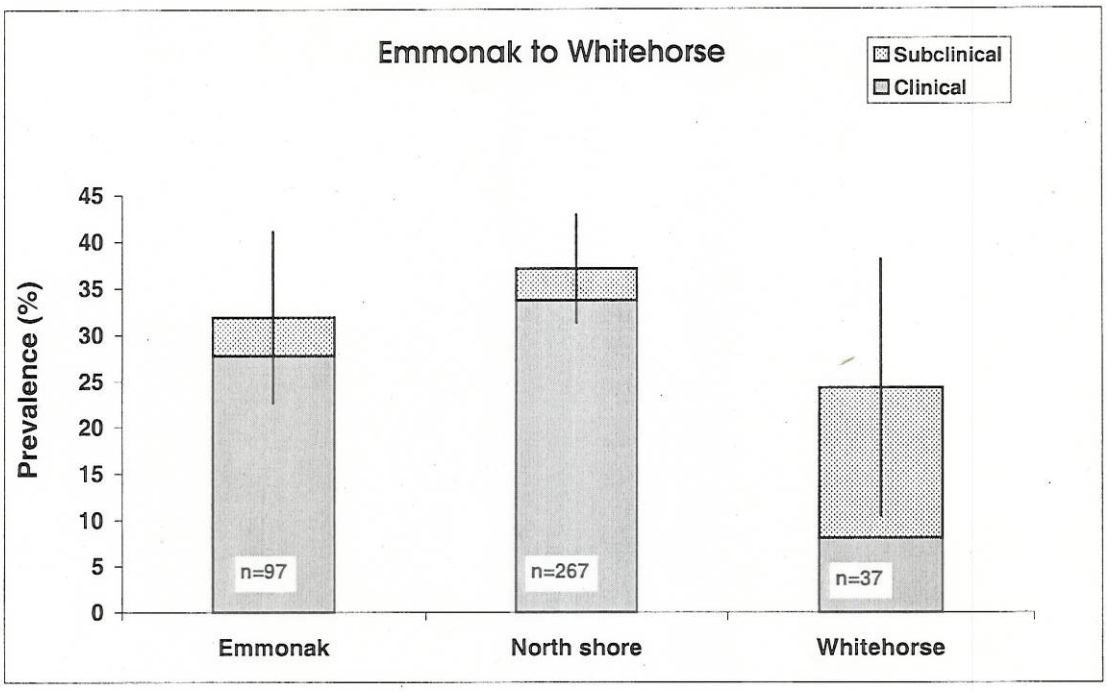

Figure 3. Change in infection and disease prevalence from Emmonak to Whitehorse. A significant drop in the percent of infected and diseased fish occurred at Whitehorse, similar to what was observed from 2000 to 2002. Bars = 95% conf.