Final Report

for

2000

Yukon River King Salmon -*Ichthyophonus* Pilot Study

prepared by

R.**M. Kocan, Ph.D. & P.K. Hershberger, Ph.D.**

School of Fisheries

Box 355100

University of Washington

Seattle WA 98195

ph (206) 685-2984

FAX (206) 685-3275

e-mail: kocan@fish.washington.edu

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U.S. Fish and Wildlife Service

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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 and1990 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 pallasi) 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 $^{\circ}$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.

Non-lethal sampling

To evaluate the possibility of using a non-lethal sampling method on migrating fish, two studies were conducted to evaluate the use of punch biopsies of skin and muscle. The initial study was designed to determine if 1/4 inch punch biopsies could accurately detect infected fish, and the second study was to evaluate the effect of the biopsies on fish survival. To do this we sampled 15 kings collected at Tanana by making visual observations, culturing heart and liver and taking punch biopsies. For consistency, the biopsies were taken from 1/2 inch below the lateral line and in line with the anterior edge of the anal fin. The visual observations and culture data from internal organs and biopsies were compared to determine the accuracy of punch biopsies.

For the fish survival study we obtained king salmon from the University of Washington hatchery as they returned to spawn in November 2000. These fish were fully colored and within several days of spawning. They were anesthetized with MS-222 (tricanemethane sulfonate) and a 1/4-inch punch biopsy taken from just below the lateral line and above the anterior edge of the anal fin. The fish were than released into the returning pond and recaptured three days later for visual examination of the biopsy wound.

Source of infection

Sixty Pacific herring (Clupea pallasi) were captured at Goodnews Bay, AK in May, 2000 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, liver and spleen tissue was cultured in MEM-10 supplemented with antibiotics and examined after 7 and 10 days for the presence of *Ichthyophonus*. Any isolates of the parasite obtained from these herring were also to be used for genetic comparison with isolates from Yukon kings.

Experimental transmission

Experimental transmission of *Ichthyophonus* to Chinook salmon and rainbow trout was attempted by feeding infected tissues and by IP injection of cultured *Ichthyophonus* spores. Infected heart and liver tissue from fish collected at Tanana and Circle was refrigerated and flown to the Marrowstone Island Marine Laboratory (USGS), WA, where the tissues were minced in culture medium and fed to 30 newly smoltified Chinook salmon being held in 70 g flowing filtered seawater tanks. A portion of the tissue was also cultured to verify that it was viable, and this was used to produce spores for IP injection.

After several passages in culture medium (Okamoto et al, 1985, 1987) infective spores of *Ichthyophonus* were fed to 10 6-inch rainbow trout and injected IP into 10 additional trout housed in 70g flowing freshwater tanks. Ten control fish were injected IP with sterile culture medium and housed similarly to the treated groups.

Results

Numbers of fish collected

A total of 470 male and 248 female king salmon were examined from 7 sites along the Yukon and Tanana rivers in 2000. At some sites however, sampling stopped when approximately equal numbers of fish (~30) of each sex were collected, so a more representative sex ratio of the population was obtained by using fish from Emmonak, Galena, Tanana, Nenana and Circle, where all fish captured during the study period were examined. These samples consisted of 409 males (69.4%) and 180 females (30.6%) for a sex ratio of 2.3 males: 1 female. The total number of fish examined at each site ranged from 60 to 177 and the number cultured for *Ichthyophonus* ranged from 60 to105 (Table 1).

Ichthyophonus

Ichthyophonus was identified by visually observing the heart, liver, spleen, muscle and skin for the presence of granulomatous white lesions (Figure 1a,b, c), by it,s growth in culture medium, and by histologic examination of stained heart and liver tissue.

Infection and disease

When the Yukon king salmon run began at Emmonak, AK (RM 26) in mid June 2000, 26% of the fish entering the river were infected with *Ichthyophonus* and < 3% exhibited clinical signs of disease. These prevalence rates were unchanged in fish sampled at Galena (RM 530), however a dramatic increase in clinical disease was detected in fish at Tanana (RM 715) during the first week of July. At this time the percent of infected fish was approximately 27% but the percent of fish exhibiting clinical signs of disease increased from < 3% to over 20%. These overall prevalence rates were similar at Circle (RM 1,081) and Dawson, Y.T. (RM 1,319). When fish were sampled at the hatchery in Whitehorse, Y.T. in late August however, the overall percent of infected fish dropped to 13% and the percent of diseased fish dropped to 8.7% (Figure 2). Overall prevalence at Nenana was 19% or 10% below the Galena and Tanana fish.

Table 1. Data summary for Yukon River kings - 2000

	Emmonak	Galena	Tanana	Nenana	Circle	Dawson	Whitehorse	totals
River miles ->	*24*	*530*	*715*	*860*	*1,081*	*1,319*	*1,745*	
Total fish examined	82	68	204	58	177	60	69	718
# males	48	50	142	47	122	30	31	470
# females	34	18	62	11	55	30	38	248
Total fish cultured	82	68	105	58	78	60	69	520
weighed and/ or measured	82	0	105	58	177	60	69	551

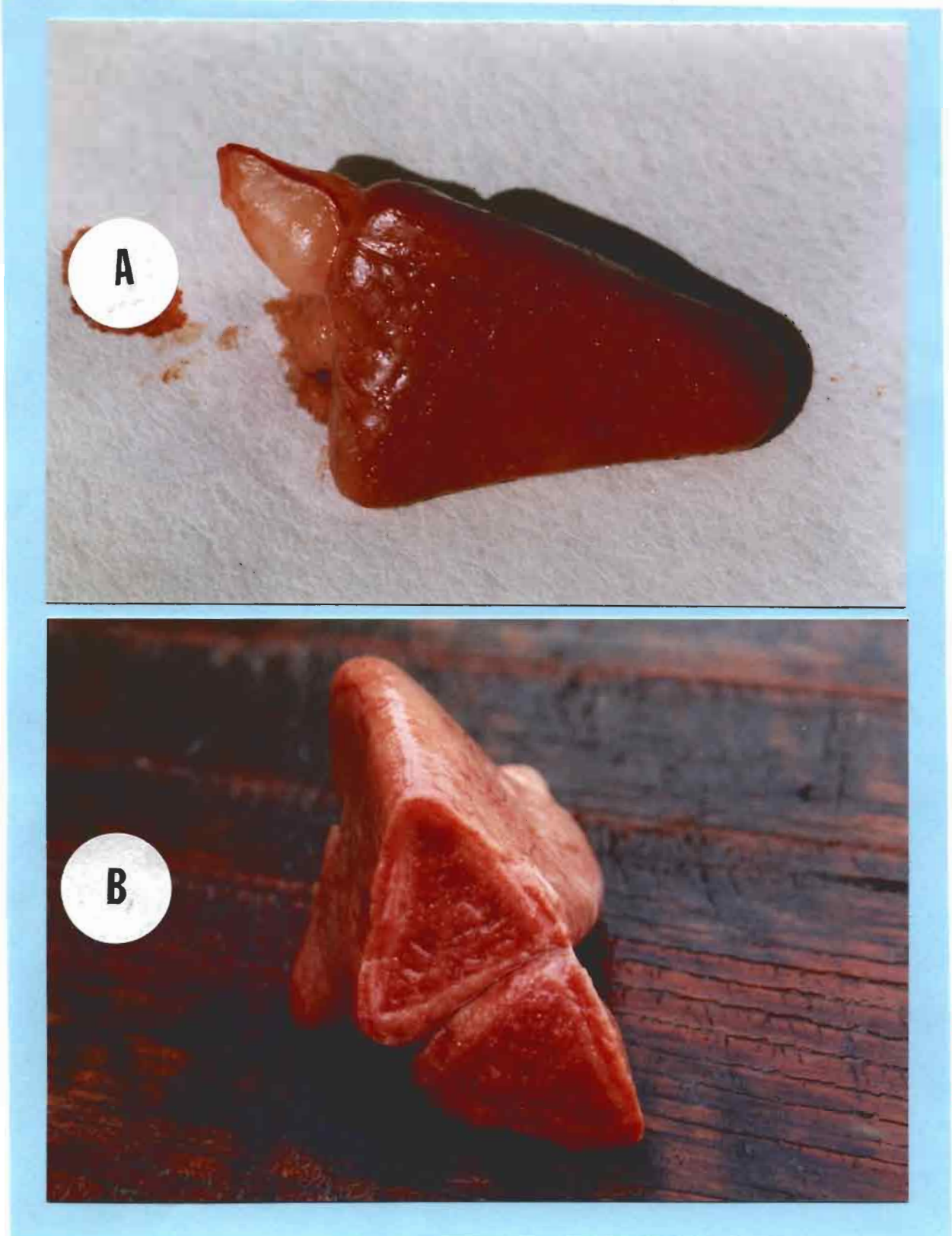

Figure 1a. Visible granulomatous white *Ichthyophonus* lesions on the surface (A) and inside (B) the heart muscle of a Yukon king salmon sampled at Circle, AK in 2000.

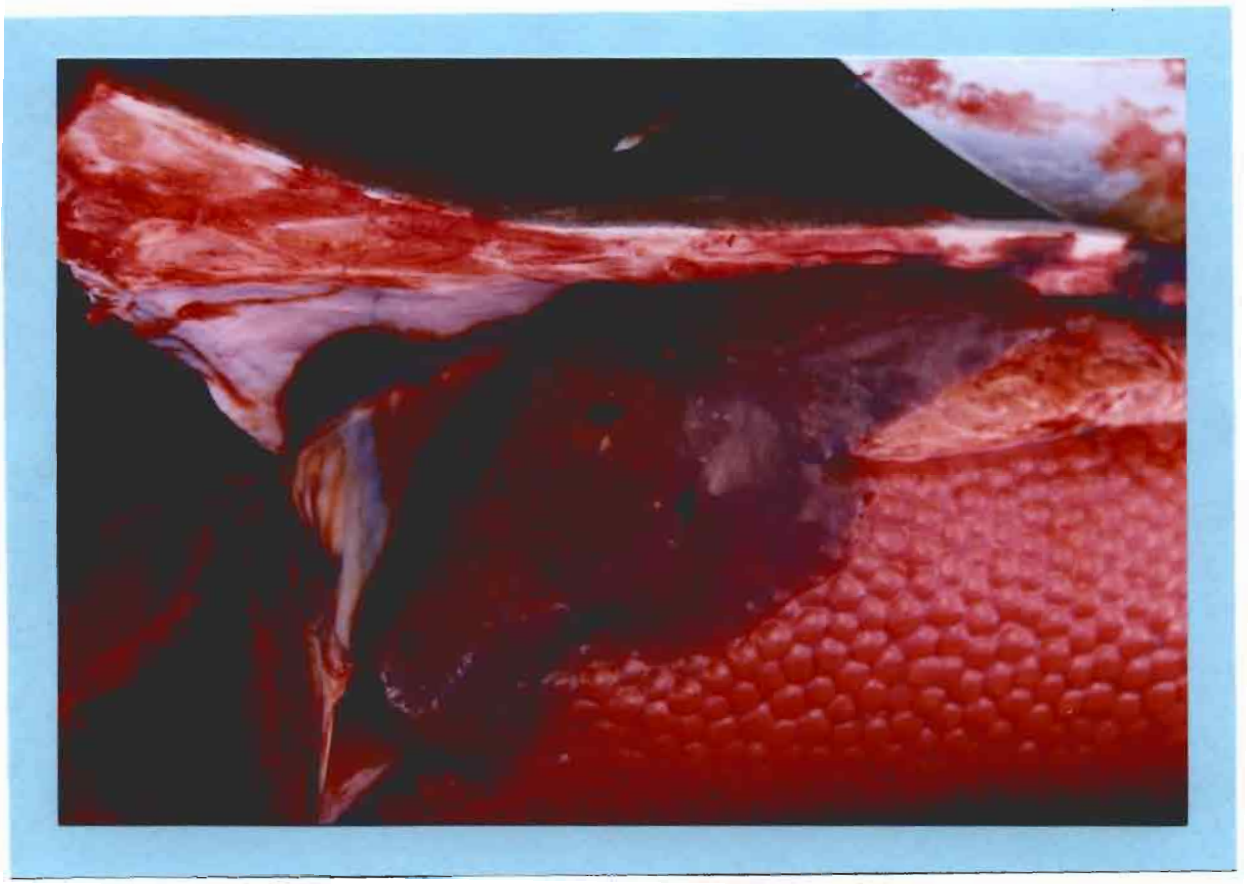

Figure 1b. Liver lesions caused by *Ichthyophonus* in a Yukon king sampled at Tanana, AK in 1999. Skein and eggs are normal.

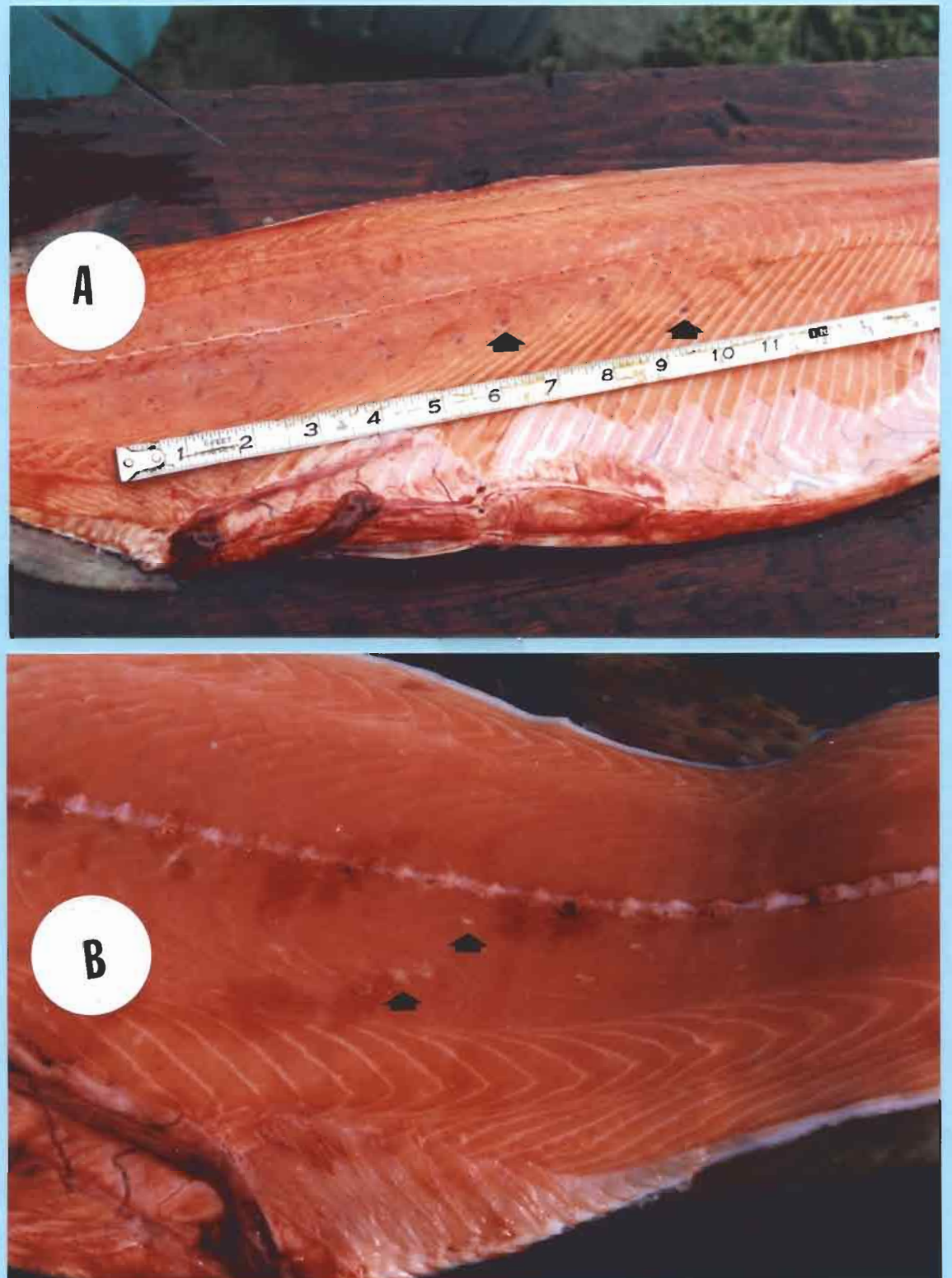

Figure 1c. ***Ichthyophonus*** **lesions in the muscle tissue of a Yukon king sampled at Tanana, AK in 2000. (A) Hemorrhage assoicated parasite, (B) No hemorrhage associated with parasite**

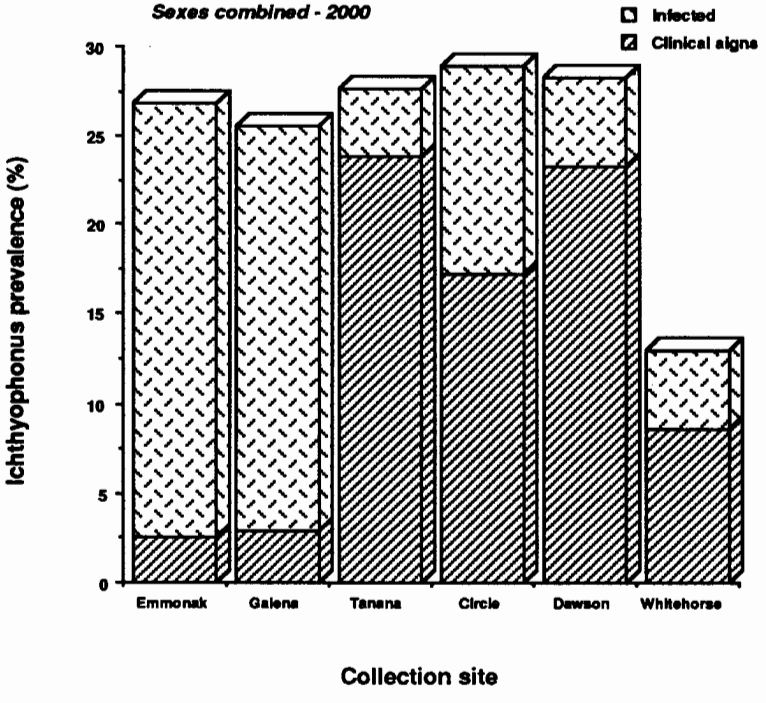

Figure 2. Prevalence of infection and disease in all Yukon River king salmon sampled from 6 Yukon River collection sites in 2000. The overall infection prevalence of fish collected at Nenana (not shown) was 19.1% with clinical disease at 5.2%, both below the overall mean for both sexes.

When the sexes were examined separately (Figure 3), the mean male prevalence rate for all sites was 21.9% (range 16.1% - 27.4%), while female prevalence for all sites was 28.7% (range 10.5% - 42.9%) (Table 2).

Fewer than 5% of the fish examined at Emmonak and Galena exhibited clinical disease, but when they reached Tanana over 20% of both males and females had classical white granulomatous lesions on their heart, liver, spleen and muscle (Figure 4). The disease prevalence rate for males declined beyond Tanana to 13%-17%. Females however, showed an increase in disease prevalence up to 36.4% at Circle and 30% atDawson, Y.T. This level of disease than dropped dramatically to only 5.3% at Whitehorse, Y.T. (Figure 4, Table 2). The overall prevalence of clinical disease at Nenana was 5.2% with 2.1% of males and 18.2% of females showing signs of disease (Table 2).

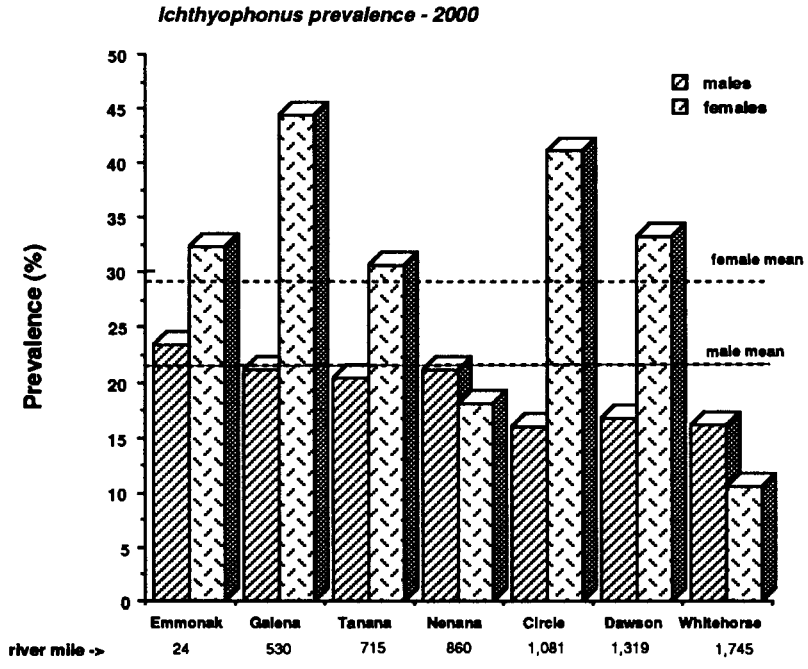

Figure 3. Percent of male and female king salmon infected with *Ichthyophonus* at 6 Yukon River sites and at Nenana on the Tanana River. The mean percent of infected males was similar among all sites, while the percent of infected females dropped dramatically between Dawson, Y.T. and Whitehorse, Y.T. Infection prevalence for Nenana males was 19.1%, similar to all sites, while female prevalence dropped to 18.2%, lower than at other Yukon River sites except Whitehorse.

Comparison of 1999 and 2000 samples

A comparison of data collected from Yukon kings at Emmonak and Tanana in 1999 and 2000 revealed little change in prevalence of infection or disease in males from year-to-year, while a 15% decline in infection prevalence occurred in females in 2000 (Figure 5).

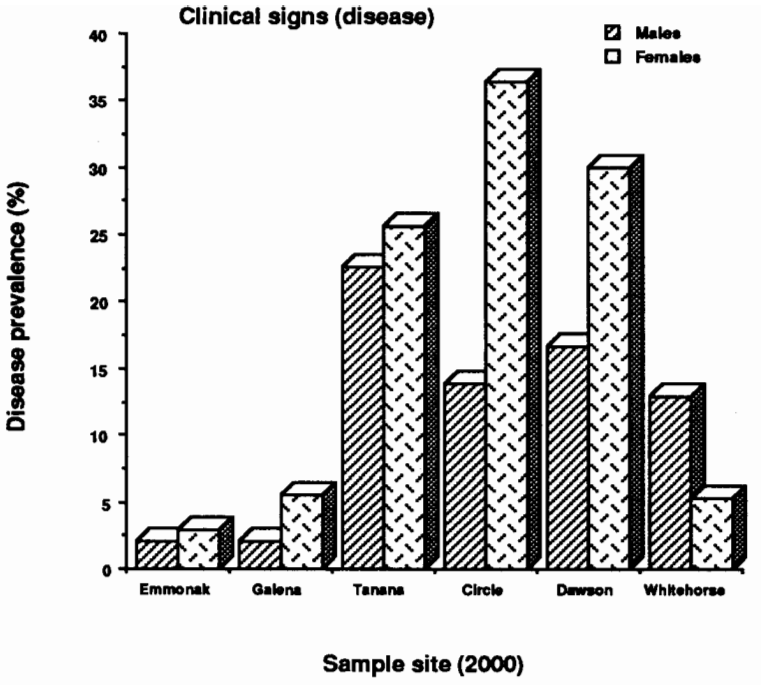

Figure 4. Prevalence of clinical signs (disease) in male and female king salmon collected from 6 sites along the Yukon River in 2000. An obvious increase in clinical disease occurred in both sexes between Galena and Tanana, while the number of diseased females declined dramatically between Dawson Y.T. and Whitehorse Y.T. Fish collected at Nenana (not shown) showed a similar drop in overall disease prevalence to just 5.2%.

Table 2 . Prevalence of infected and diseased Yukon River kings 2000

	Emmonak	Galena	Tanana	Nenana	Circle	Dawson	Whitehorse	totals
River miles ->	*24*	*530*	*715*	*860*	*1,081*	*1,319*	*1,745*	
Infected (positive/examined)								
total	22/82	18/68	29/105	11/58	22/78	17/60	9/69	128/520
%	26.8	26.5	27.6	19.0	28.2	28.3	13.0	24.6
males	12/48	11/50	17/62	9/47	7/43	7/30	5/31	68/311
%	25.0	22.0	27.4	19.1	16.3	23.3	16.1	21.9
females	10/34	7/18	12/43	2/11	15/35	10/30	4/38	60/209
%	29.4	38.9	27.9	18.2	42.9	33.3	10.5	28.7
Diseased (positive/examined)								
total	2/82	2/68	25/105	3/58	37/177	14/60	6/69	89/656
%	2.5	2.9	23.8	5.2	20.9	23.3	8.7	13.6
males	1/48	1/50	14/62	1/47	17/122	5/30	4/31	43/389
%	2.1	2.0	22.6	2.1	13.9	16.7	12.9	11.1
females	1/34	1/18	11/43	2/11	20/55	9/30	2/38	46/229
%	2.9	5.6	25.6	18.2	36.4	30.0	5.3	20.1

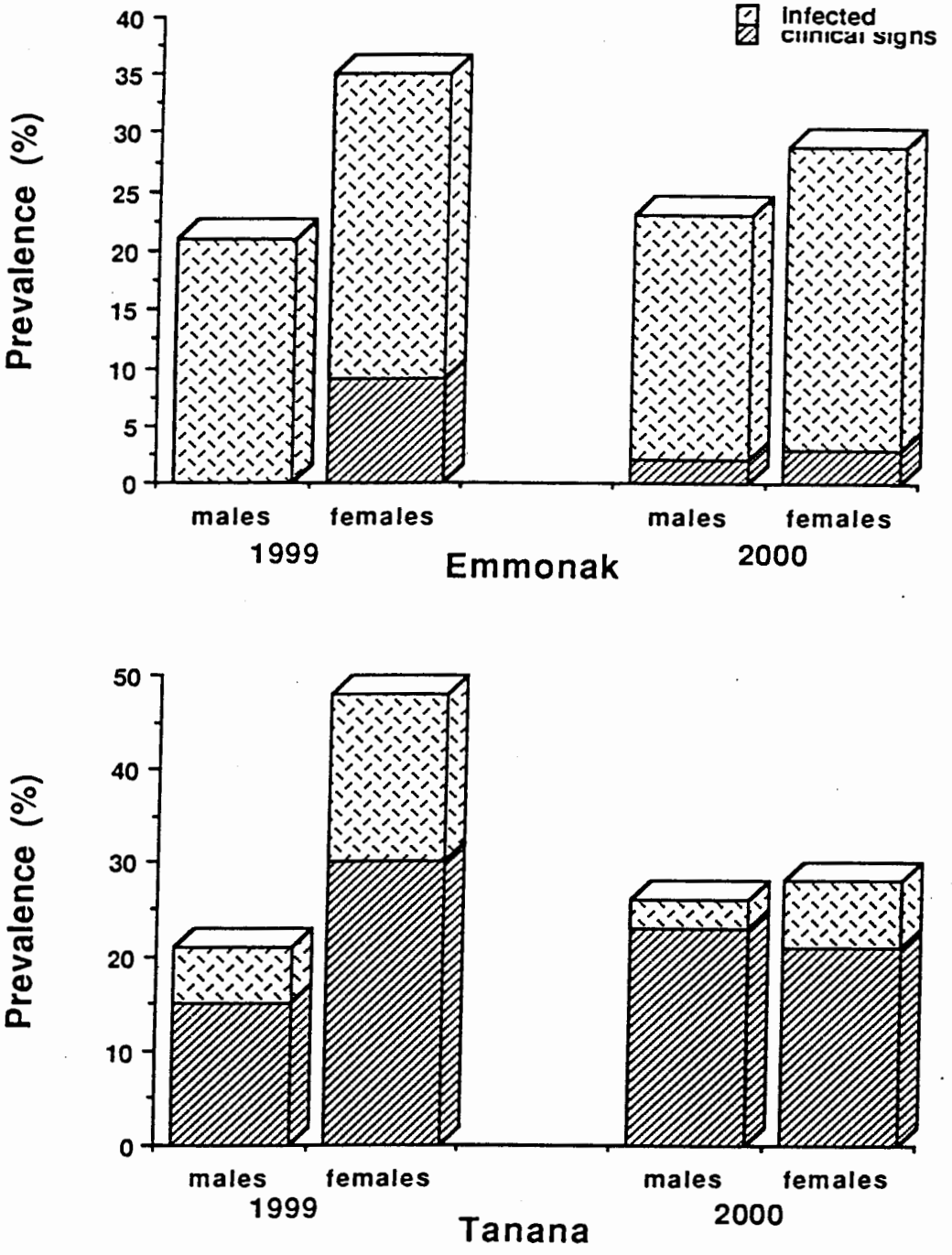

Figure 5. Comparison of infection and disease prevalence at Emmonak and Tanana from 1999 to 2000. There was no difference for infection between sites within years, but there was a drop in female infection prevalence from 1999 to 2000. No difference in prevalence was noted for males.

***Ichthyophonus* infection of muscle tissue**

The number of fish exhibiting visible *Ichthyophonus* in muscle tissue, in addition to heart and liver, was also compared between 1999 and 2000. In 1999 35% (7/18) diseased fish from Emmonak and Tanana had visible *Ichthyophonus* in their muscle tissue, while in 2000 only 19% (5/26) of the diseased fish showed any signs of muscle involvement (Figure 6). This decline in muscle involvement was supported by observations made by fishermen who traditionally fillet their fish prior to processing (Fliris; personal communication). Because of different fishermen,s methods of processing their fish we were not able to get good data on muscle involvement from other collection sites.

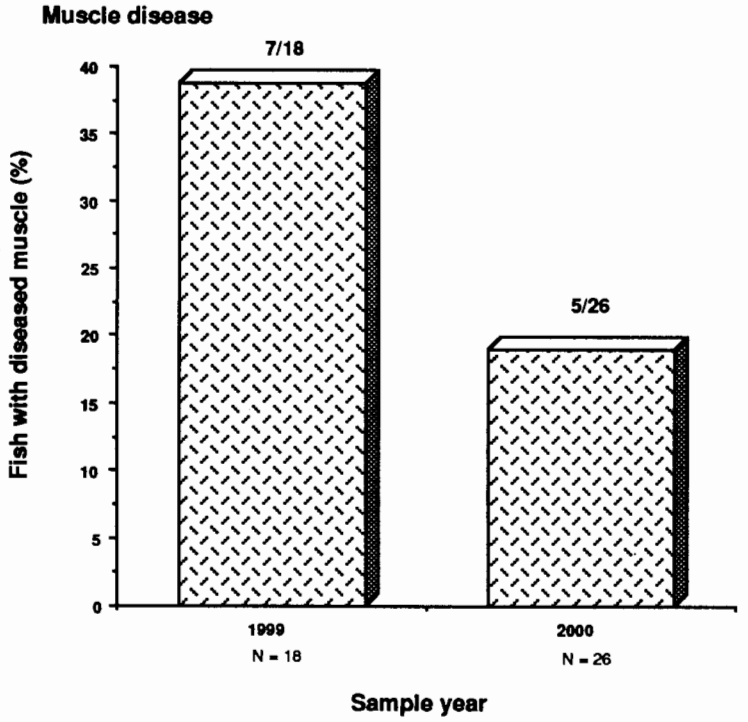

Figure 6. The percent of diseased Yukon king salmon with visible *Ichthyophonus* in muscle tissue in 1999 was about double that seen in 2000. A 2 °C higher water temperature in 1999 may account for the difference in tissue distribution of the parasite between years.

Weight

Weights were obtained from 463 fish. Because various fishermen handled their catch differently it was not possible to obtain weights from fish at Galena and at some sites weights were obtained only from fish that were cultured for *Ichthyophonus*. The mean weight of 290 males was 12.5 + 4.90 pounds while the weight of 173 females was 18.0 + 4.96 pounds (Figure 7). When each site was examined separately however, the mean weight of males remained constant for all Yukon sites, while female weight steadily dropped from 22 pounds at Emmonak to 12.5 pounds at Whitehorse (Figure 8).

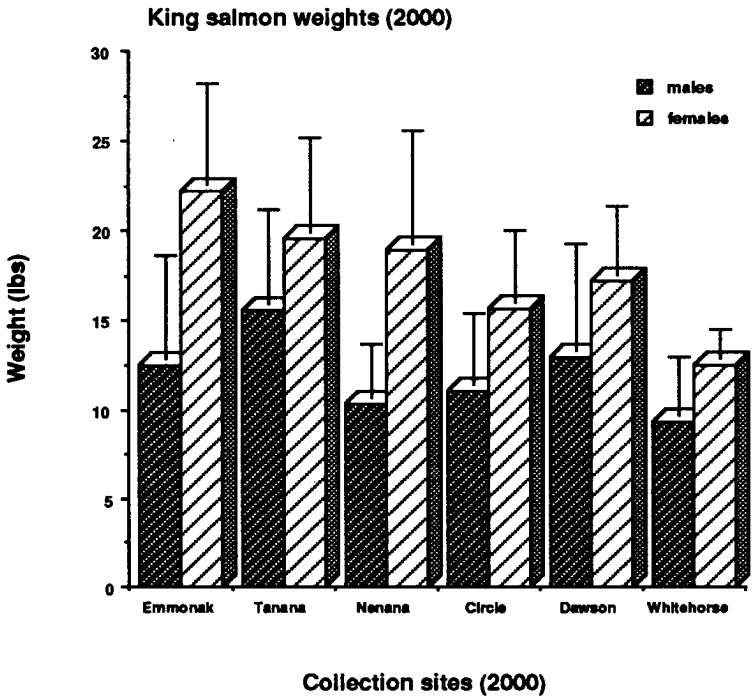

Figure 8. The mean weight of male and female Yukon king salmon from 6 Yukon River sites in 2000. River-wide weights for males were significantly less than females ($P < 0.05$) Bars = 1 SD

Egg resorption

Observations made on skeins from females sampled at Tanana, Circle and Whitehorse revealed that attritic eggs were evident in over 25% of females. However, no correlation could be made with *Ichthyophonus* infection (Table 3), with many infected females showing no signs of attritic eggs (Figure 1b, 9). Hemorrhage was also observed in some skeins but phenomenon this was relatively rare.

Experimental transmission

None of the 30 Chinook smolts fed tissues obtained from infected Yukon kings or the 10 fish injected IP with spores developed *Ichthyophonus* infections after 120 days.

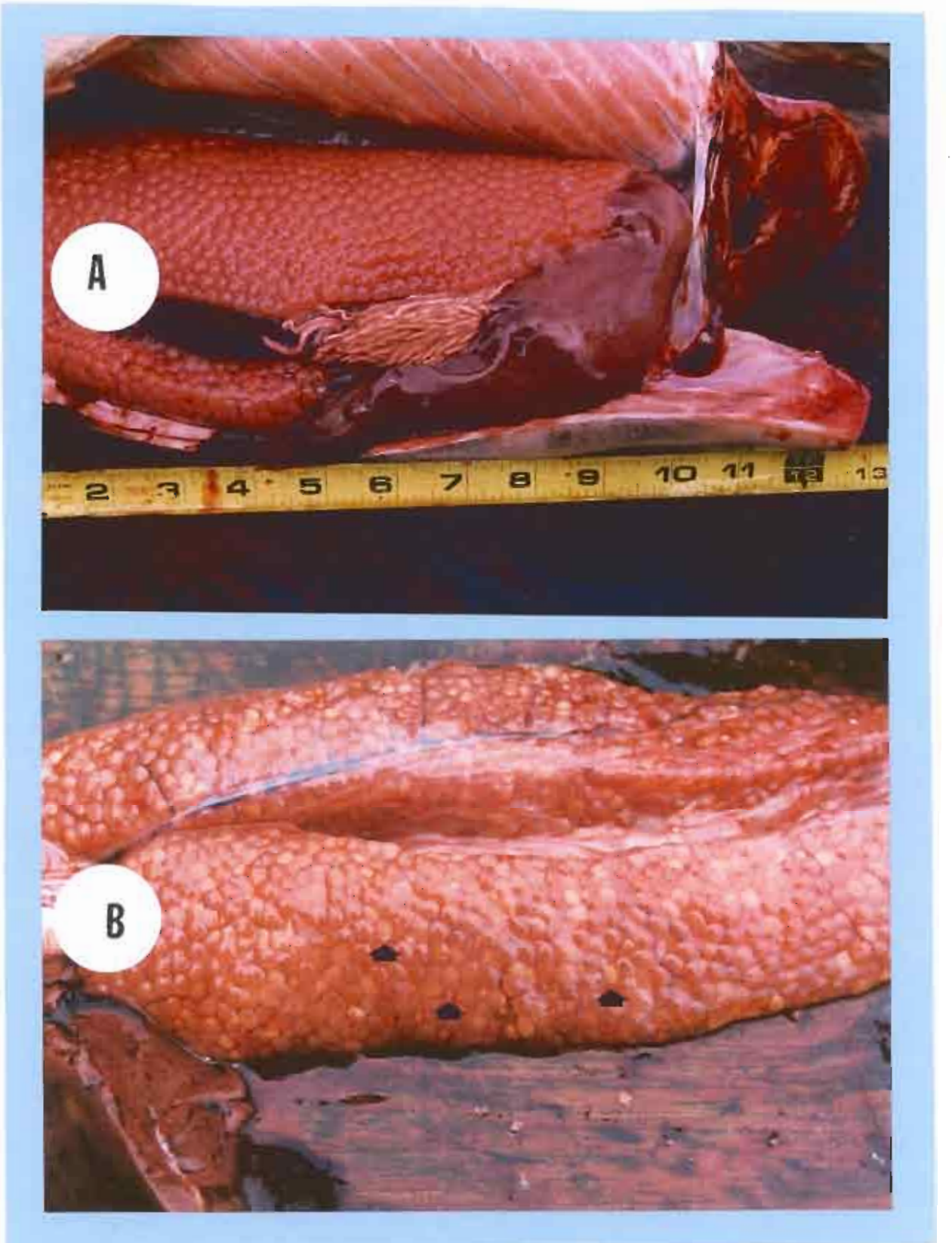

Figure 9. Normal (A) and abnormal (B) skeins from Yukon king females showing whitish eggs of varying sizes characteristic of egg resorption. Sample taken at Tanana in 2000.

Of the 10 rainbow trout fed *Ichthyophonus* spores, 6 were dead after 2 weeks, while 2 of the fish injected IP were dead. The parasite was not grossly visible in any of the dead

fish and culture of heart and liver tissues revealed two fish infected with *Ichthyophonus* from the IP injected group. No morbidity or mortality occurred in the 10 control fish after 21 days.

Table 3. Prevalence of egg attrition in female kings observed in 2000

Site	females with attritic eggs / females examined (%)	*Ichthyophonus* positive females / females with attritic eggs (%)
Tanana	8/39 (20.5%)	2/8 (25.0%)
Circle	13/55 (23.6%)	7/13 (53.8%)
Whitehorse	6/37 (16.2%)	2/6 (33.3%)
Sites combined	27/131 (20.6%)	11/27 (40.7%)

Source of infection (Goodnews Bay herring)

Visual examination and primary tissue culture of heart, liver and spleen from 60 herring collected at Goodnews Bay, AK in May 2000 revealed no *Ichthyophonus*. About 10% of the fish had nematode worms in the body cavity but no potential pathogens were observed.

Non-lethal sampling

Three of the 15 randomly sampled Tanana kings were positive for *Ichthyophonus* by both gross examination and tissue culture of heart and liver tissue. These same 3 fish were also positive by punch biopsy tissue culture.

Punch biopsies on the 8 live fish sampled at the University of Washington hatchery resulted in no mortality after 7 days and the 1/4 inch wound produced by the biopsy was essentially healed after 3 days

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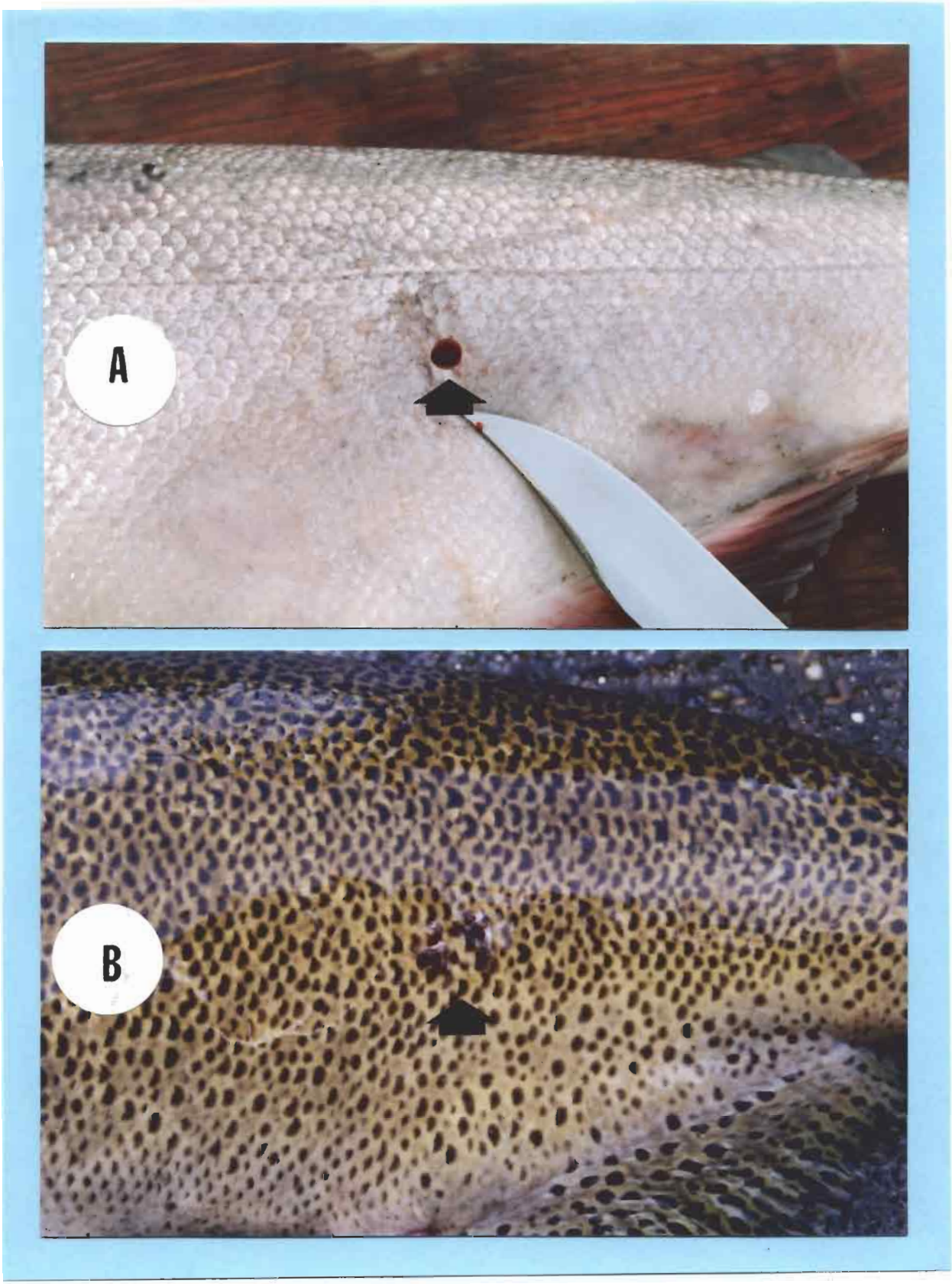

Figure 10. Wound produced by 1/4" punch biopsy (A) and healed wound (B) 72 hours after biopsy was taken from spawning king salmon.

Discussion

In 1999 we observed that king salmon entering the Yukon River at Emmonak were infected with *Ichthyophonus* and a small percentage had clinical signs of disease. When they reached river mile 715 at Tanana, AK over half of the infected fish were exhibiting clinical signs of disease. Our hypothesis at that time was that the disease became progressively more severe as the fish moved upriver in response to some physical or chemical factors in the river, or in response to changing hormonal and immunologic condition of the fish. A subsequent study conducted in 2000 expanded on the 1999 study by adding 4 additional sites on the Yukon and one on the Tanana River at Nenana. The final sample site on the Yukon River was at the hatchery in Whitehorse, Y.T. at RM 1,745. This site and the one at Nenana were considered to be near-terminal spawning sites. Data collected included infection and disease prevalence, sex, length, weight, and condition of eggs within the skein.

Infection:

The results of the 2000 study confirmed that fish entering the river are infected with *Ichthyophonus* and that the infection prevalence remains constant at about 25% until they pass Dawson, Y.T. at river mile 1,319. When the fish arrived at Whitehorse, Y.T. (RM 1,745) a dramatic drop in the percent of infected fish occurred, from >28% at Dawson to 13% at Whitehorse (Figure 2). This same phenomenon was observed at Nenana (RM 860) on the Tanana River, where the percent of infected fish dropped to just 19% while ~ 27% of the fish at Galena (RM 530) and Tanana (RM 715) were infected (Table 2).

When male and female data are examined separately, the pattern of infection for each sex is distinctly different (Figure 3). The overall mean infection prevalence for males is

about 22% and remains constant at all sample sites except Whitehorse, where it falls to 16%. Females however, exhibit a much higher prevalence of infection, > 30% at all sites, except Whitehorse and Nenana where infection prevalence in females drops to just 10.5% and 18.2% respectively (Table 2).

Clinical disease:

During the salmon,s upriver migration *Ichthyophonus* becomes progressively more pathogenic causing clinical signs of disease in heart, liver, spleen and muscle tissue. The percent of all fish exhibiting clinical signs of disease between Emmonak (RM) 26 and Galena (RM 530) remains stable at < 3%. However, at Tanana (RM 715) over 80% of the infected fish exhibit clinical signs of disease, characterized

by white granulomatous nodules on the heart, liver, spleen and muscle. During the 600 mile migration from Tanana to Dawson the percent of infected fish remained constant at about 25%, while diseased fish remained at approximately 80% of the infected fish or > 20% of the entire population. At Whitehorse the percent of all fish exhibiting clinical signs of disease dropped to just 8.7%.

When sexes were examined separately, a greater percent of females exhibited clinical signs of disease at all sites except Whitehorse. There was a large increase in percent of diseased males and females between Galena (RM 530) and Tanana (RM 715), where disease prevalence increased from 3-5% to 22-25%. Similar to what was observed for infection prevalence, clinical disease in females declined significantly between Dawson (30%) and Whitehorse (5%), and for the first time the percent of diseased females dropped below that of males. The percent of diseased males at Whitehorse (12.9%) remained essentially the same as that observed at Circle (13.9%) and Dawson (16.7%). The same decrease in clinical disease was also observed at Nenana where overall clinical signs for both sexes was only 5.2% while 23.8% of the fish examined at Tanana were diseased.

Two hypotheses have been proposed to explain the sharp decline in prevalence of both infection and disease observed at Whitehorse. One hypothesis proposes that a large proportion of diseased fish segregate out of the Yukon main channel between Dawson and Whitehorse, leaving a segment of the population with a low infection/disease prevalence to continue to Whitehorse. The second hypothesis proposes that diseased fish are dying as they approach their spawning areas, thus leaving a segment of the population with a lower disease prevalence to continue on to Whitehorse.

For the first hypothesis (population segregation) to be correct the following assumptions are necessary:

Populations originating in different spawning streams or watersheds are differentially infected.

If infection occurs in freshwater then *Ichthyophonus* transmission must occur in natal streams.

If infection occurs in natal streams then transmission rates must differ among natal streams.

If infection occurs in the open sea, then fish originating in different natal streams are differentially exposed to different sources of infection or infected prey while at sea.

For the second hypothesis (mortality of diseased fish) to be correct the following assumptions are necessary:

All populations have the same probability of being infected

Diseased fish die before reaching their terminal spawning areas

To test these hypotheses in 2001, a component of the study will examine spawned out fish from several Yukon River tributaries (Chena and Koyukuk) to determine if infection/disease prevalence is lower at terminal spawning sites relative to the Yukon River mainstream. An attempt to examine outmigrating smolts will also be made to determine if they became infected in freshwater, as well as to examine Bering Sea herring for the presence of *Ichthyophonus*, to determine if they are the source of *Ichthyophonus* infection.

Comparison of 1999 with 2000:

Data on infection and disease collected from Emmonak and Tanana in 2000 was compared with data collected from the same sites in 1999. The overall percent of infected fish between sites was similar for both years (Figure 5). The prevalence of infection in males was similar between years while there was a decrease in percent of infected females from 1999 to 2000. At this time it is not possible to determine if the decrease in percent infected females is real or an artifact of sampling.

Muscle infection:

When *Ichthyophonus* produces clinical signs of disease in the muscle tissue of infected fish it results in an economic loss as well as having biological impact. Fillets containing *Ichthyophonus* lesions do not dry or smoke properly (Fliris; personal communication) and are usually discarded by commercial processors (Umphenor; personal communication). A similar condition was reported from haddock near Scotland and described as „smelly‰ haddock or „greasers‰. These conditions were attributed to infection of the haddock by *Ichthyophonus*, similar to what was observed in Yukon kings (Williamson 1913).

Since data on muscle involvement was collected from Emmonak and Tanana in 1999 and 2000, we compared the prevalence of these lesions in infected fish between years and found that it dropped from nearly 40% of infected fish in 1999 to about 20% in 2000 (Figure 6). This was confirmed by local fishermen who reported that they encountered far fewer fish with diseased muscle in 2000 than they did in 1999. There was no commercial fishery in 2000, so no observations were made by processors.

An explanation for the apparent drop in muscle involvement may be linked to river temperature from year to year. *Ichthyophonus* is known to exhibit increased pathogenicity in experimentally infected fish as temperature increases. Okamoto et al (1987) reported 100% mortality of rainbow trout at 20 oC and just 10% mortality at 15 oC, a difference of just 5 degrees. In 1999 Yukon River temperatures exceeded 19 oC at Tanana during the first week of July, but only reached 17 ˆ 17.5 oC in 2000. A data set collected by an independent investigator (Chikita, K. unpublished data) at the Yukon bridge from June through August showed a similar 2 oC difference between years (Figure 11). Temperatures at the bridge reached 18 oC by mid June in 1999 but did not reach this level until mid July in 2000. Pilot Station temperatures were similarly high in 1999 but did not reach 18 oC until one week later than at the Yukon

bridge. A 2-degree difference in temperature could account for a difference in pathogen growth rate and distribution. Since experimental studies have not been conducted on king salmon infected with *Ichthyophonus* we can not correlate mortality with tissue involvement at this time. It is assumed that heart and liver pathology would be more likely to be associated with mortality than muscle infection.

Weight

Females were significantly heavier than males (t-test; $P < 0.05$) when all sites are combined. However, when males and females were compared by site, male weights remained constant among all sites while females lost about 10 pounds between Emmonak and Whitehorse. If this weight loss were due to selective removal of larger fish during the fishery, then both sexes would be expected to decline proportionally. Since females became smaller while males did not, then some mechanism other than fishing should be considered to explain the loss of larger females.

Egg resorption:

Although a high proportion of female kings showed signs of egg attrition (25%) there was no correlation between attrition and infection by *Ichthyophonus* (Table 3). The high proportion of females with attritic eggs may indicate that another unrelated problem is affecting normal egg development.

The effect of egg resorption on fecundity could be determined by artificially fertilizing eggs from affected and normal females with sperm from pooled males and comparing the hatching success between groups. The hatchery at Whitehorse would be an ideal location for such a study since they have the facilities as well as access to affected and unaffected females.

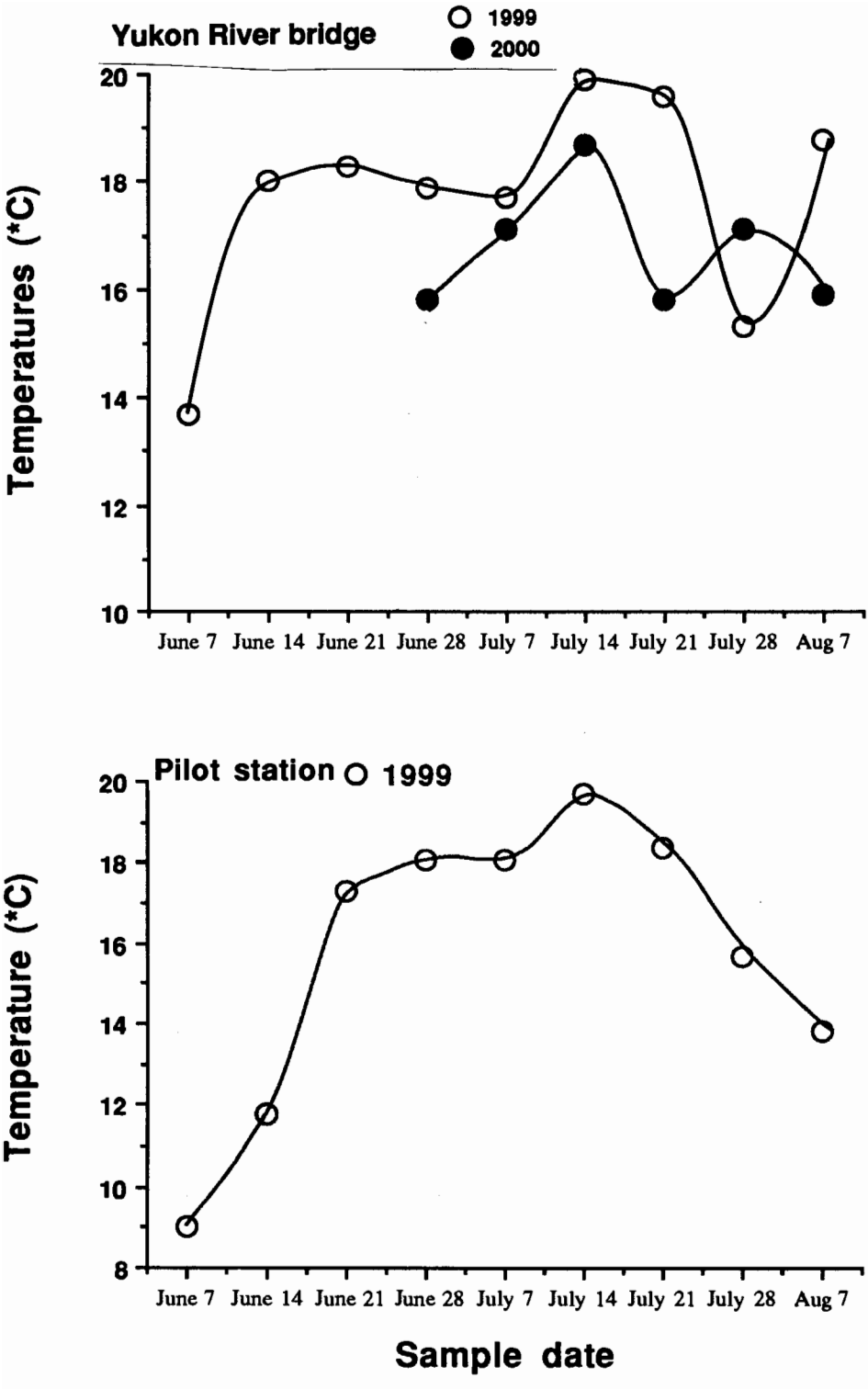

Figure 11. Yukon River temperatures taken at the Yukon bridge and Pilot Station in 1999 and 2000.

Experimental transmission:

We were unable to establish an infection of *Ichthyophonus* in newly smolted Chinook salmon of Puget Sound origin, even though the parasite was determined to be viable by

primary tissue culture. It is possible that the infective stage of the parasite was no longer present in the tissues when they arrived at the University of Washington, or that Puget Sound kings are refractory to the Yukon River isolate. It is also possible that the life stage of the salmon at the time of exposure is critical to successful infection. Since we do not know when Yukon River kings become infected, this hypothesis can not be tested at this time.

If heart and liver tissue from Yukon kings could be examined for the presence of *Ichthyophonus* while the fish are still at sea, prior to entering the Yukon River, it would give some clue as to when the fish were infected. It is possible that they become infected before leaving the river or that they become infected while in the saltwater phase of their life cycle.

There has been one report of *Ichthyophonus* being inadvertently transmitted to rainbow trout in Western Washington (Rucker 1953) and numerous reports occur in the European literature of successful infection of rainbow trout with *Ichthyophonus* spores or infected tissue (McVicar 1982, 1990; McVicar & McLay 1985). We have on rare occasions been successful in infecting rainbow trout with isolates from either Puget Sound herring or Yukon River kings. We have however, successfully transmitted the Puget Sound strain to other fresh and saltwater species by both feeding and IP injection without difficulty (Kocan et al 1999). Why rainbow trout are essentially refractory to this parasite is unclear at this time, but it is possible that several species of *Ichthyophonus* exist and we are working with one or more species that do not affect rainbow trout.

Source of infection (Goodnews Bay herring):

Because herring are known to be infected with *Ichthyophonus* worldwide and that Chinook salmon feed on herring, it was hypothesized that examination of herring from the Bering Sea or Norton Sound might give some clue as to the origin of the *Ichthyophonus* affecting the Yukon kings. Previous studies in Prince William Sound demonstrated that a high proportion of herring could occasionally be infected with *Ichthyophonus*. Approximately 80,000 tons (60%) of spawning Pacific herring (Clupea pallasi) failed to return to Prince William Sound, Alaska in 1993, following which, the prevalence of *Ichthyophonus* in

survivors reached 27%, more than double that seen in previous years (Marty et al. 1998). Although epizootics of *Ichthyophonus*, have occurred sporadically in herring (Clupea harengus) in North Atlantic since 1898 (Sinderman 1958; Sinderman & Chenoweth 1993) and more recently in the North Sea and Baltic Sea (Rahimian & Thulin 1996, Mellergaard & Spanggaard 1997), similar epizootics have not been reported from the North Pacific.

We had originally proposed to genetically compare isolates of the parasite from both Yukon kings and Bering Sea herring to determine if they were genetically related and thus possibly establish a source of infection for Yukon kings (Clark and Lanigan. 1993). However, none of the 60 herring examined from Goodnews Bay in 2000 were infected with *Ichthyophonus*. These fish may have recovered from previous infection or may never been exposed to the parasite. Neither of these explanations seems probable however, since this is the first time the authors have seen a population of herring that was not infected with the parasite. Normally, the older the fish the more heavily they are infected. These fish being approximately 1 pound were obviously an older stock and should have been heavily infected.

Since we were unable to obtain any *Ichthyophonus* of Bering Sea origin, we could not do the genetic comparison proposed. However, a comparison of the Yukon isolate and an isolate from Puget Sound is being conducted, and a second group of herring from the Bering Sea will be examined in 2001 to determine if this stock is actually free of *Ichthyophonus* or if the 2000 sample was unusual occurrence.

Nonlethal sampling:

The nonlethal sampling study demonstrated that we could successfully isolate and identify the parasite in live fish using a 1/4 inch punch biopsy. The procedure does not appear to have any detrimental effects on Chinook salmon in fresh water. Consequently, the procedure is now ready to be tested in the field in conjunction with tagging studies, where the relative survival rate of infected vs uninfected kings could be determined

Conclusions

1) Approximately 25% of the Chinook (king) salmon returning to the Yukon River in 1999 and 2000 were infected with *Ichthyophonus*.

2) Over 20% of the king salmon reaching river mile 715 at Tanana exhibited clinical signs of disease, which rose to over 30% when they reached Circle and Dawson.

3) Overall, slightly more females than males were infected, but females were twice as likely to exhibit clinical signs of disease.

4) The prevalence of infection and disease remained constant in both males and females until they passed Dawson, Y.T. at river mile 1,319.

5) Female king salmon exhibited a dramatic decrease in both infection and disease when they reached Whitehorse, Y.T. while males showed little change over the entire course of their migration. Mortality of diseased females is the most probable explanation for this phenomenon.

6) The percent of fish showing clinical signs of disease in the muscle decreased approximately 50% between 1999 and 2000.

7) The water temperature of the Yukon River decreased approximately 2 oC from 1999 to 2000, and may have influenced *Ichthyophonus* distribution in fish muscle.

8) The mean weight of female king salmon decreased approximately 50% between Emmonak and Whitehorse, while males exhibited no change in weight over the same period.

9) It is possible to obtain non-lethal biopsies of skin/muscle from king salmon and to use them to screen live fish for the presence of *Ichthyophonus* in the field.

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