U.S. Fish and Wildlife Service
Office of Subsistence Management
Fisheries Resource Monitoring Program

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

Final Report for Study 01-200

Richard Kocan and Paul Hershberger*
School of Aquatic & Fishery Sciences, Box 355100
University of Washington, Seattle, WA 98195
Phone: 206-685-3275
e-mail: kocan@u.washington.edu

and

James Winton
Western Fisheries Research Center, USGS-BRD,
6505 NE 65th Street, Seattle, WA 98115
Phone: 206-526-6587
e-mail: jim_winton@usgs.gov

July 2004

*Present Address: Marrowstone Marine Station, USGS-BRD, 616 Marrowstone Point Road, Nordland, WA 98358; Phone: 360-385-1007; e-mail: phershberger@usgs.gov

TABLE OF CONTENTS

Figures

Tables

Appendices

ABSTRACT

This multi-year study confirmed that approximately 25-30% of adult chinook salmon entering the Yukon River during 1999-2002 were infected, either clinically or sub-clinically, with *Ichthyophonus*. Clinical signs of disease were minimal when fish entered the river, but increased markedly when they reached the middle Yukon River at river-mile 730. There was no difference in total infection prevalence between salmon from the early part of the run compared with the end of the run; however, in fish from the end of the run, the parasite was more frequently disseminated among multiple organs and a higher percentage of the fish had obvious clinical disease. Total infection prevalence among chinook salmon sampled from the run remained constant until they reached the upper Yukon at Whitehorse, where prevalence dropped significantly. Infection rates and disease prevalence in fish from the lower Tanana River were similar to those in the Yukon; however, female spawn-outs collected from the Chena and Salcha Rivers showed much lower infection prevalences of *Ichthyophonus*. The decrease in infection prevalence in fish near the end of their spawning migration is not thought to be due to recovery, but strongly suggests that many of the diseased fish are dying before spawning. Examination of historic temperature data suggests that rising average water temperatures during the past three decades appear to be associated with the increase in disease and potential pre-spawning mortality among Yukon River chinook. The source of infection was not determined, but *Ichthyophonus* was not found in 400 Pacific herring (*Clupea pallasi*) from the Bering Sea, nor in 120 juvenile chinook salmon from several drainages in Alaska and Canada. Freshwater burbot (*Lota lota*) from the middle Yukon River were sub-clinically infected with *Ichthyophonus*, but its origin and relationship to the chinook isolate is unknown.

Key Words: Chinook salmon, Disease, Epidemiology, Global Warming, *Ichthyophonus*, Pre-spawning Mortality, Yukon River, Alaska.

Citation: Kocan, R., P. Hershberger and J. Winton. 2003. Effects of *Ichthyophonus* on Survival and Reproductive Success of Yukon River Chinook Salmon. Federal Subsistence Fisheries Resource Monitoring Program, Final Project Report No. FIS 01-200. U. S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Information Services Division, Anchorage, Alaska.

INTRODUCTION

In the mid 1980s, subsistence fishermen along the middle Yukon River began reporting an unusual condition in a few chinook ("king") salmon (*Oncorhynchus tshawytscha*). These fish smelled mildly "fruity", did not dry properly when smoked and had white spots on their heart, liver and in the skeletal muscle. After observing the condition in increasing numbers of fish for several years, the U.S. Fish & Wildlife Service (USFWS) sent tissue samples to the Alaska Department of Fish and Game (ADF&G) and to the Bozeman (MT) Fish Technology Center (USFWS) for analysis. Based on histological evidence, both laboratories returned a diagnosis of *Ichthyophonus* infection.

Following these first reports of *Ichthyophonus* infections in Yukon River chinook salmon, fishermen and processors having more than 30 years of experience on the Yukon River observed a steady increase in the number of affected fish from the middle Yukon River (river miles 600 – 730) through 1999 (personal communication). Initially, the reports indicated that the first fish caught in late June and early July were relatively free of lesions, but by mid to late July (late in the run) fish were severely affected. More recently however, reports from fisherman indicate that fish from all parts of the run are affected, and that the severity of the condition varies from year-to-year. Fish processors buying chinook from middle Yukon River fishermen reported that as many as 20% of the salmon they purchased had to be discarded because of muscle tissue damage, presumably caused by *Ichthyophonus* (V. Umphenor, Interior Alaska Fish Processors, Fairbanks, personal communication). This description is consistent with published reports of disease in other fish species infected with *Ichthyophonus* (McVicar & Mackenzie 1972, McVicar 1999, Rahimian 1998).

Because the disease appeared to be increasing in prevalence and severity over time, and was having an economic impact, the Bering Sea Fishermen's Association initiated a study in 1999 to determine what effect, if any, *Ichthyophonus* was having on Yukon River chinook salmon. This pilot study revealed 0 of 24 (0.0%) male and 3 of 34 (8.8%) female salmon entering the Yukon River from the Bering Sea had clinical signs of Ichthyophoniasis, characterized by visible, punctate whitish nodules on the heart, liver, spleen and skeletal muscle. These lesions were confirmed by histological examination of fixed tissues to be due to *Ichthyophonus*. However, when fish from the same part of the run were sampled again at river mile (rm) 730 near the Rampart Rapids, clinical signs increased in prevalence to 7 of 46 (15.2%) male and 10 of 27 (37.0%) female salmon (Kocan and Hershberger 2000).

Culture assays for *Ichthyophonus* using *in vitro* explants of heart and liver tissue initiated at the time of visual examination revealed the presence of additional sub-clinical infections. These positive cultures brought the total infection prevalence at the mouth of the river (clinical + sub-clinical infection) to 4 of 24 (16.7%) of males and 11 of 34 (32.4%) of females. At rm 730 (Rampart Rapids) the infection prevalence increased to 10 of 46 (21.7%) males and 13 of 27 (48.1%) females, suggesting that females had a higher prevalence of infection than males (Kocan and Hershberger 2000).

To further explore the significance of the 1999 findings, a more extensive 3-year study was initiated in 2000, which included seven sample sites from the mouth of the Yukon River to

Whitehorse (Yukon Territory, Canada), the Tanana River, a major tributary of the Yukon River, and two major spawning streams, the Chena and Salcha Rivers. The underlying strategy of the expanded study was to sample fish along the length of the river and obtain temporal and spatial data on the progression of *Ichthyophonus* infection from the time fish entered the Yukon River until they spawned. Historically, chinook salmon enter the Yukon River from early June through July, the peak of the migration pulse occurring during the third to fourth week of June. During their normal migration, portions of the population divert to spawning tributaries along the Yukon River. By sampling fish as they moved upriver, we were able to monitor changes in infection and disease over time, as well as to determine whether fish leaving the mainstem of the Yukon River had the same or a different prevalence of infection or disease relative to those still en route to spawning areas farther upriver.

During the course of this study the same pattern of infection and disease was repeated each year, offering independent repetition as a test for predictable results, the closest that field studies can come to the ideal of controlled laboratory conditions. This repetition also permitted the testing of hypotheses formulated from observations made during previous years.

OBJECTIVES

1. Survey adult chinook salmon for *Ichthyophonus* infection and disease at multiple sites from Emmonak to Whitehorse
2. Determine if diseased adults are able to reach their natal streams
3. Search for the source of *Ichthyophonus* infections in Yukon River chinook salmon
4. Experimentally determine if water temperature affects the rate of growth or pathogenicity of *Ichthyophonus* in infected chinook salmon
5. Relate changes in disease severity to inter- and intra-annual water temperatures as well as with historical water temperature data available from other studies

METHODS

Sample size, location and sampling gear

The study area consisted of three components: 1) the Yukon River mainstem, 2) the Tanana River, and 3) terminal spawning areas. The Yukon River mainstem extended from river mile (rm) 24 to 1,745 and was divided into three segments, the lower (rm 0-600), middle (rm 600-1,200) and upper (rm 1,200 -1,750), while the Tanana was divided into lower river (rm 690) and upper river (rm 860-920) sections. The Chena and Salcha Rivers and Whitehorse hatchery were considered terminal spawning areas. Yukon River chinook were sampled from 1999 to 2002 from 10 sites along the Yukon, Tanana, Chena and Salcha Rivers (Table 1, Appendix I).

Salmon were sampled along the Yukon River and its tributaries using gill nets and fish wheels. Gill nets were used exclusively at the mouth of the river (rm 24) as a part of the ADF&G commercial test fishery, while upriver sites were sampled with fish wheels operated by ADF&G, USFWS, the Department of Fisheries and Oceans Canada (DFO) as well as by commercial and subsistence fishermen. The number of fish sampled at each site during each year of the study was

determined by the number of salmon caught during the sample period and the relative abundance of males and females. At the Whitehorse Rapids (rm 1,745), samples were obtained from freshly spawned chinook salmon that had returned to the mitigation hatchery operated by DFO.

Samples from adult salmon were also obtained from Chena and Salcha River terminal spawning sites on several days each year during the spawning period in conjunction with annual carcass counts. Post-spawn salmon (spawn-outs) were sampled from the Chena River during July 27-August 2, 2001 and July 28-August 7, 2002. Salcha River fish were sampled during July 28-August 7, 2002. Although *Ichthyophonus* can be cultured from post-spawn fish for up to 96 hr after death, samples were taken only from live spawn-outs or dead fish with clear eyes and firm cardiac muscle. These fish were judged to have been dead for less than 48 hours because controlled laboratory studies demonstrated that dead spawn-outs held longer than 48 hours at 12 ^{o}C developed cloudy eyes and a flaccid discolored heart (Appendix V). Chena River samples were obtained from a 12 river-mile stretch, upstream of milepost 27 on the Chena Hot Springs road, and Salcha River samples were obtained from a 20-mile stretch between 40 and 60 river miles upstream of the Highway 2 (Richardson Hwy) bridge south of Fairbanks, AK (Figure 1).

Although sample size varied depending on availability of fish, the target sample was a minimum of 60 chinook salmon from each site without regard to size. Because males were typically more abundant than females in the catch, they constituted a larger proportion of the samples at many of the sites (Appendix 1). While fish were collected by various entities as described above, all samples were taken from the fish and processed by the same team of investigators in all years to ensure consistency in the evaluation of the disease state and performance of the assays.

Identification of infected chinook salmon and classification of infections

While previous studies demonstrated that *Ichthyophonus* can be isolated from the tissues of infected fish held at ambient temperature for up to four days post mortem (unpublished data) and transmitted to susceptible fish at temperatures as high as 30 ^{o}C (Post 1987), with few exceptions, the fish collected in this study were necropsied and examined for the presence of *Ichthyophonus* within six to 12 hours of capture. At the time of examination, the sex, length and weight were recorded and a gross visual observation made of heart, liver, spleen, kidney and skeletal muscle for the presence of white punctate lesions, indicative of *Ichthyophonus* infection (Appendix II). Tissue samples were placed in growth medium for *in vitro* culture because this has been demonstrated to be the most sensitive method of detecting sub-clinical infections and of obtaining accurate infection prevalence (Rahimain and Thulin 1996, Kocan et al. 1999).

For *in vitro* culture, approximately 1 g of heart and liver tissues were placed in 5 mL of Eagle's minimum essential medium (MEM) or Leibovitz L-15 medium, both supplemented with 5% fetal bovine serum, 100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin and 100 µg mL^{-1} gentamycin. These tissues were selected because, aside from the gut which is heavily contaminated with bacteria, internal organs (especially the heart) were the first to show signs of infection. Cultures were incubated at $\leq$15 ^{o}C for 7-10 days then microscopically examined at 40X magnification for the presence of *Ichthyophonus* hyphae and spores (Appendix III). The characteristic growth and morphology of *Ichthyophonus* in culture was used to confirm the diagnosis (Okamoto et al. 1987a, Spanggaard et al. 1994, Rahimain and Thulin 1996, Kocan et al. 1998, 1999).

Representative tissue samples from selected fish at all locations were also preserved in 10% formalin for histological evaluation in order to determine the stage of the disease process and the nature of the cellular immune response of the host. Positive histopathological findings were confirmed by observation of characteristic lesions (Appendix III).

For accuracy and consistency, only fish that were confirmed to be infected with *Ichthyophonus* by *in vitro* culture or, occasionally, by histopathological observation were classified as "**infected**". Infections were classified as "**sub-clinical**" when fish were confirmed to be infected with *Ichthyophonus*, but the tissues were without visible lesions. Infections were classified as "**clinical**" when fish were confirmed to be infected with *Ichthyophonus*, and visible white punctate lesions were observed in at least one internal organ (i.e. overtly detectable in the field, but without implication as to severity or outcome). The most severe form of clinical infection was termed "**disseminated disease**" when white punctate lesions were present in multiple internal organs because a histological analysis of such lesions showed them to be representative of a disease state (i.e. adversely affecting normal tissue architecture or biological function). Fish were classified as "**negative**" if *Ichthyophonus* infection could not be confirmed by *in vitro* culture or by histopathological observation. Because no microbiological assay is perfectly sensitive, some "false negatives" probably occurred in samples from salmon having very low levels of infection. Thus, the data presented here represent minimum infection prevalence.

Sampling rationale

Based on results from the 1999 pilot study, returning Yukon River chinook salmon were treated as a single binomial population consisting of "infected and uninfected" individuals with the assumption that no additional transmission between fish occurred en route. This assumption is based on evidence that demonstrates transmission of *Ichthyophonus* results from ingestion of infected prey (McVicar & Mackenzie 1972, McVicar 1982, Slocombe 1980, Okamoto et al. 1987a, Sinderman & Chenoweth 1993, Kocan et al. 1998, McVicar 1999) and that salmon do not eat during their spawning migration. Consequently, samples taken from the population as it ascended the river to spawn could track changes in the prevalence of infection or of disease, beginning when the population entered the river and continuing until they spawned.

Miscellaneous salmon samples

Hearts from 20 adult chinook salmon captured from the lower Kuskokwim River near Bethel, AK in June 2001 in conjunction with a USFWS (Fairbanks) contaminant study (K. Mueller & A. Matz), were examined visually and by *in vitro* culture for the presence of *Ichthyophonus*.

Fifty-six adult chinook salmon hearts were collected by Ian Boyce (DFO-Canada) between June 1-13, 2002 on the mainstem Taku River between the Tulsequah River confluence and the U.S./Canada border. These hearts were evaluated by *in vitro* culture only. The Taku River empties into Stephen's Passage near the city of Juneau (S.E. Alaska).

Chum Salmon

Between June 13-20, 2000, 16 male and 42 female Yukon River chum salmon (*O. keta*) were gillnetted at river mile 24 (Emmonak, AK), and examined visually and by *in vitro* culture for the presence of *Ichthyophonus*.

Juveniles
To determine whether *Ichthyophonus* was being transmitted to chinook salmon juveniles in fresh water, 65 juveniles were captured in the Chena River by Jim Finn (USGS-BRD) April 19-21, 2002; and 57 from the Klondike River on October 6-7, 2002 by Jake Duncan (DFO). All juveniles were shipped on ice to the USGS Marrowstone Island Field Station (Nordland, WA) where they were examined for the presence of *Ichthyophonus* by *in vitro* culture.

Chinook Salmon outside Alaska
For comparison with Yukon River fish, 60 chinook salmon were sampled each year during 2000 and 2001 from the University of Washington hatchery on Lake Washington, and an additional 60 chinook were similarly examined in 2000 from the Big Beef Creek Field Research Station on Hood Canal (Washington).

Non-salmonid species
Pacific herring (*Clupea pallasi*) were captured during the spring commercial herring fishery from 2000 to 2002 and shipped on ice overnight to the University of Washington by ADF&G personnel (Paul Salomone). Herring came from Goodnews Bay (2000-2002), Norton Sound (2001) and Nelson Island (2002) (Figure 1). Each year 100 fish from each site were necropsied and examined visually for presence of white nodular lesions and assayed by *in vitro* culture for detection of *Ichthyophonus* infections.

In 2002, seven non-salmonid species from the Yukon River were visually examined and tested for the presence of *Ichthyophonus* by *in vitro* culture. The samples consisted of: six inconnu (sheefish) *Stenodus leucichthys*, three broad whitefish *Coregonus nasus*, 11 humpback whitefish *C. pidschian*, five cisco *C. artedi*, six burbot *Lota lota*, six northern pike *Esox lucius*, and nine grayling *Thymallus arcticus*. Whitefish were captured by fish wheels at rm 569 and 730; the pike and grayling were caught by hook-and-line from the Tozitna River below Tanana Village.

Histopathology
The tissue response to *Ichthyophonus* infection was evaluated histologically. Samples of various organs were prepared for histologic examination from selected fish collected at Emmonak, Rampart Rapids, Corbusier Slough (Tanana-Yukon River confluence), Chena-Tanana River confluence, and from spawn-outs on the Chena River. These samples were fixed in 10% neutral buffered formalin for 7-10 days then transferred to 70% ethanol until processed. Samples of the organs of infected fish showing visible lesions were prepared for evaluation together with a representative number of tissues from salmon shown to have sub-clinical infections (culture positive but with no visible lesions). Host reaction was evaluated using tissue sections stained with H&E and infection intensity was determined using tissue sections stained with periodic acid Schiff (PAS). Each tissue was evaluated for visible lesions, the microscopic presence of the parasite, host reaction to the parasite and intensity of infection (Appendix II). While samples for histological analysis were obtained as rapidly as possible, it is recognized that *Ichthyophonus* hyphae and spores are known to break free of encapsulating host responses soon after death.

Non-lethal sampling (punch biopsies)
Non-lethal sampling was conducted by sampling skeletal muscle posterior to the dorsal fin using a 1/4-inch diameter dermal biopsy punch (Miltex Instrument Co., Bethpage, NY) that extracted approximately 0.5 g of tissue (Appendix IV). Biopsy tissue was placed into MEM-5 culture medium, incubated for at least 10 days and microscopically examined at 40X magnification for the presence of *Ichthyophonus*.

A pilot study was conducted on eight adult spawning chinook salmon at the University of Washington in 2000 to determine if the procedure resulted in cutaneous infections or mortality. All eight fish were biopsied on the same day, released into the hatchery's adult return pond, and upon recapture 72 h later, their wounds were examined for healing and infection.

During July 15-18 and again during August 8–12, 2001, 50 chinook salmon were biopsied then released by personnel of DFO at their test wheel on the portion of the Yukon River near the border between Alaska and the Yukon Territories. Similar samples were taken again at the DFO test wheel (Bio Island, YT) in 2002. These biopsies were able to detect infections only in fish in which the disease had spread to the skeletal muscle, an advanced stage of disseminated infection.

Tests for potential sample bias
The underlying assumption for this study was that infected and uninfected salmon were randomly distributed in time and space during the spawning migration, and that all age classes were equally likely to be infected. If any of these assumptions were incorrect, sampling biases would be considered. Sources of potential sampling biases were identified as: 1) different types of capture gear (gill net and fish wheel), 2) location of the fish within a pulse e.g. early or late in the run, 3) fish migrating near shore vs those off shore, and 4) age difference in infection prevalence.

Near shore vs off shore:
Samples from two types of gill net sets were compared in 2002 by comparing the infection prevalence in chinook salmon captured from near shore (set nets) and off shore (drift nets) using 8.5-inch mesh nets, which target larger fish. Fish were sampled at rm 24 from June17 – 30th. In addition to on shore vs off shore comparisons, infection prevalence in the first 60 chinook caught from June 17 –19 were compared with the prevalence in 60 chinook caught from June 25 – 30

Gillnet vs fish wheel:
The terrain and river conditions at the Yukon Delta preclude the use of fish wheels, so gill nets are used exclusively for the test fishery while fish wheels are used more extensively in the middle Yukon. Infection prevalence in fish captured by set nets and driftnets at the mouth of the river were compared with fish captured by fish wheels between 570 and 730 river miles upriver. Fish wheels normally target chinook salmon between 20-50 ft offshore at depths of 5-20 ft (personal communication with YR fishermen) and have historically caught proportionally more and smaller males than gillnets due to the 8.5-inch mesh gillnet used at the mouth of the river.

Position in the run:
To determine if infected and uninfected fish segregate during their spawning migration as a result of differences in stamina, infection prevalence and disease severity were compared in fish

sampled from early and late in the run as the pulse passed rm 730 in 2001 and 2002. These data also allowed us to determine if the infection prevalence or disease severity differed among salmon entering the river at different times and thus migrating through different water temperatures.

Fish size (i.e. age):
To address the possibility that infection prevalence may be biased by size/age, we divided all the salmon sampled from the lower and middle Yukon River in 2002 into five groups of increasing weight from 1 to >26 lb (277 males and 240 females). The assumption being that the larger fish were older than smaller fish, and if age influenced infection prevalence then the smallest fish would exhibit significantly different infection prevalence than the largest fish, with an infection gradient between the two extremes. A left-skewed curve would result if a higher percent of younger (smaller) salmon were infected, and a right-skewed curve would result if infection were higher in older (larger) salmon.

Statistical analyses
Chinook salmon entering the Yukon River were treated as a single binomial population consisting of infected and uninfected individuals, recognizing the population is a mix of multiple subpopulations, which ultimately segregate into spawning tributaries as the population migrates upriver. Within the large population, we defined selected groups (e.g. males vs females, upper vs lower river, early vs late run, Yukon vs Tanana, and Tanana vs Chena/Salcha) for comparison using statistical analyses. To evaluate differences between these groups, we used a standard epidemiological method whereby we established a null hypothesis (H_o), which stated no difference existed between groups. To test this hypothesis, the X^2 (chi-square) statistic with one degree of freedom was used to calculate a value from the observed data that summarizes the evidence against the H_o (Leaverton 1978, Colton, 1974, Witts, 1964). To do this, a 2 X 2 table consisting of "Groups" and "Treatments" was constructed:

	Number of		
Group	infected	uninfected	Total
A	a	b	a+b
B	c	d	c+d
Total	a+c	b+d	n

The X^2 value was computed from this table using equation (1):

$$X^2 = \frac{(|ad-bc| - n/2)^2 * n}{(a+c)(b+d)(a+b)(c+d)} \qquad (1)$$

Gender, sample site etc. comprised the groups (A & B) while "infected-uninfected" represented "successes" (a, c) and "failures" (b, d), respectively

Temperature effects
Both the prevalence and severity of ichthyophoniasis among adult chinook salmon during 1999-2002 were compared with current [(1)] and historical [(2)] river temperatures to determine if changes in water temperature were associated with changes in the dynamics of *Ichthyophonus* disease.

Differences in water temperature of < 5 ^{o}C have been shown to influence the severity of *Ichthyophonus* disease in salmonids (Okamoto et al. 1987b) and buffalo sculpin (*Enophrys bison*) (Halpenny et al. 2002) under controlled laboratory conditions.

Water temperature readings during the summers of 1999 and 2000 at Pilot Station (lower Yukon River) and the Yukon Bridge (middle Yukon River) were correlated with our data on migration stage and the *Ichthyophonus* infection levels in chinook salmon for these periods. Additional annual temperature data supplied by ADF&G, USFWS and USGS were used to compare with infection severity during the study period, as well as to determine historical changes in Yukon River water temperature. While the precision of these historical data from others and their method of collection were beyond our control, we believe them to be of sufficient accuracy to use in analysis of historic trends.

Because the Yukon River water temperature was frequently shown to be above 10-15 ºC, the optimum for chinook salmon (Piper et al. 1982), as well as above the experimentally determined lethal temperature for *Ichthyophonus*-infected trout (Okamoto et al 1987a), we hypothesized that elevated river temperatures might correlate with the differences in disease severity observed between 1999 and 2002. To test this hypothesis we established the following predictive tests:

1. If elevated water temperature was associated with more severe disease, then disease severity should be greater during years when water temperatures are higher.
2. If elevated water temperature was associated with more severe disease, then severity within years should be greater during months when water temperatures are higher.
3. If elevated water temperature was associated with more severe disease, then present Yukon River temperatures should be higher than historical temperatures, before *Ichthyophonus* was first recognized in the mid 1980s.

Laboratory temperature trials

To determine the effects of temperature on the progress and severity of *Ichthyophonus* in chinook salmon, a laboratory study was initiated at the Western Fisheries Research Center in Seattle, WA. Juvenile fall chinook salmon from the Makah National Fish Hatchery were obtained and held in 10 ºC pathogen-free fresh water until used. At the beginning of the experiment, two groups of 30 fish were injected with 0.1 mL of either a saline suspension of concentrated spores from an actively growing culture of *Ichthyophonus* (infected group) or with saline (control group). Over the next 14 days, groups of 10 infected and 10 control fish were either left at 10 ºC or removed to separate aquaria where the water temperature was slowly increased to 15 ºC or to 20 ºC. All six groups were observed daily for 60 days and mortalities were examined visually and by *in vitro* culture for the presence of *Ichthyophonus*. Upon completion of the experiment, infection and percent mortality among the groups was compared.

In a second study 10 newly smolted chinook salmon were acclimated to seawater and fed *Ichthyophonus*-infected tissue obtained from Yukon River chinook for five days. During the next 60 days fish were monitored daily, and all mortalities were examined visually and by *in vitro* culture for the presence of *Ichthyophonus*, then the survivors were necropsied and similarly examined.

RESULTS

Samples

To evaluate the prevalence of infection and the severity of disease caused by *Ichthyophonus* in Yukon River chinook salmon, samples were obtained from more than 2,500 fish between 1999 and 2002 (Appendix I). A total of 10 sites along the Yukon, Tanana, Chena and Salcha Rivers in Alaska and Yukon Territory were sampled, covering 1,745 river miles from the Yukon Delta to Whitehorse, Yukon Territory and an additional 250 river miles along the Tanana, Chena and Salcha Rivers (Figure 1, Table 1).

Infection

Yukon River

A consistent pattern of *Ichthyophonus* infection and disease severity emerged when chinook salmon were sequentially sampled over time as they progressed up the Yukon River during their annual spawning migration. *Ichthyophonus* infection prevalence from 1999 to 2002 was significantly higher in females (32.5%) than in males (25.9%) along the entire Yukon River mainstem ($X^2 = 8.78$; $P < 0.003$), excluding the Whitehorse hatchery (Figure 2). The annual infection prevalence for all fish from sample sites along the Yukon River mainstem, from 1999 to 2002 is summarized in Figure 3a. When sexes were examined separately, female infection prevalence ranged between 24.3% (2001) and 39.3% (1999), while male infection prevalence ranged from 16.9% (2001) to 31.8% (2002) (Figure 3b). The infection prevalence for all chinook at each sample site along the Yukon River is presented in Figure 4. Clinical and sub-clinical infection prevalence for males and females generally had the same pattern, but females had consistently higher infection prevalence (Figure 5).

When chinook salmon entered the Yukon River both males and females were already infected, with an infection prevalence of 19.6% and 26.9% respectively for all years combined (Figure 5). Infection prevalence increased to a high of 34.1% for males at the U.S./Canada border and 42.9% for females at Circle, AK when the peak of the run reached the middle Yukon River between 730–1,230 river miles. At rm 1,745 (Whitehorse Rapids Hatchery), mean infection prevalence declined to 14.8% for males and 15.9% for females (Figure 5).

Tanana River

Ichthyophonus infection prevalence for Tanana River chinook salmon from 2000–2002 was 29.4% for males (n = 255) and 27.7% (n = 166) for females (Figure 6). Infection prevalence was not significantly different between the lower Tanana (rm 695) and the upper Tanana (rm 860-920) ($X^2 = 1.28$; $P > 0.26$), so they were combined as a single population for future comparisons. Unlike fish sampled from the Yukon River mainstem, no difference in *Ichthyophonus* infection prevalence between Tanana River males and females was detected ($X^2 = 0.071$; $P = 0.79$). However, significantly fewer infected females were in the Tanana River compared with middle Yukon River females at rm 730 ($X^2 = 13.0$; $P < 0.005$).

When *Ichthyophonus* infection prevalence was plotted over time, beginning when salmon entered the Yukon River at Emmonak until they reached their natal spawning areas in the Chena and Salcha Rivers of the upper Tanana, a pattern similar to that observed in the Yukon River

mainstem was observed in which clinical and sub-clinical infections increased as fish moved upriver until reaching the terminal spawning areas where infection prevalence dramatically dropped (Figure 7). Clinical and sub-clinical infection prevalence for males and females had the same pattern, with no difference between sexes (Figure 8).

Clinical disease

The prevalence of clinical disease in chinook salmon entering the Yukon River was 6.4% and 9.5% for males and females, respectively, (7.8% for sexes combined, n = 451) for all four years combined. This low level of clinical disease persisted as fish passed rm 530 at Galena, but increased between rm 730 and 1,230 to 13.1% for males and 36.4% for females at rm 1,081 (Circle, AK). After fish passed rm 1,230 near the U.S./Canada border, clinical disease declined to a low of 8.5% for males and 11.0% for females at the Whitehorse Rapids hatchery, similar to the decline observed for infection prevalence (Figure 5). Similar patterns of clinical disease were observed for Tanana River chinook (Figures 7, 8).

A second pattern of disease progression emerged in a comparison between fish from early and late in the run. Although the prevalences of both infection and clinical disease in salmon from early and late in the run were similar (Figure 9a), a significant difference in the prevalence of disseminated disease was observed. None of the clinically infected fish sampled early in the run exhibited visible lesions in organs other than the heart, but 50% of males and 90% of females with clinical disease from late in the run presented with lesions in multiple organs (Figure 9b).

Terminal spawning sites

During 2001 and 2002, 603 post-spawn chinook salmon (spawn-outs) were collected from the Chena and Salcha Rivers. Of these, 277 (46%) were decomposed beyond the point from which meaningful data could be obtained. The remaining fish were either alive or had clear, bright eyes and firm cardiac muscle indicating they died less than 48 hours before sampling (Appendix V).

The *Ichthyophonus* infection prevalence for 2001 Chena River spawn-outs was 15.4% for males (n = 39) and 0% for females (n = 30). In 2002, the infection prevalence was 18.3% for males (n = 60) and 13.5% for females (n = 52). For both years combined, prevalence of *Ichthyophonus* infection among Chena River chinook was 17.2% for males (n = 99) and 8.5% for females (n = 82).

Infection prevalence for 2002 Salcha River chinook was 10.6% for males (n = 85) and 8.5% for females (n = 59). No significant difference of infection prevalence in Chena and Salcha River fish was detected in 2002 (males: $X^2 = 1.88$, P = 0.28 and females: $X^2 = 0.289$, P > 0.59). Overall clinical disease in males and females for both years was 11.0% in the Chena and 8.3% in the Salcha.

When Chena and Salcha River spawn-outs were compared with Tanana River fish, significantly fewer spawn-outs of both sexes from both spawning streams for both sample years were found to be infected ($X^2 = 34.3$, P = 0.00) (Figure 10). The same pattern was observed when males and females from the Chena and Salcha Rivers were compared with Tanana River fish in 2002; males ($X^2 = 16.1$, P=0.0001); females ($X^2 = 17.7$, P= 0.0001) (Figure 11**)**.

Histology
Of 63 tissues examined, 31 were judged clinically positive for *Ichthyophonus* by gross examination and 31 (49.2%) were histologically positive. In all but one case, visible lesions corresponded with histologically positive tissues. The lowest intensity of infection and least severe host reaction (disease) occurred in chinook salmon from the lower Yukon River and from the Tanana and Chena Rivers. The most severe host response and the greatest intensity of infection were observed in 9 of 10 fish sampled at Rampart Rapids (Table 2).

Non-lethal sampling (punch biopsies)
To determine whether punch biopsies were a feasible method of non-lethally sampling salmon, eight pre-spawn chinook salmon were biopsied at the University of Washington hatchery and their wounds monitored daily. After 72 hours, all eight fish presented with nearly complete healing of their wounds and showed no indication of infection.

Muscle biopsies taken from salmon near the U.S./Canada border in 2001 and 2002 revealed 10-20% of early run fish had infected skeletal muscle, which increased to 27-32% in late-run fish, demonstrating that significantly greater dissemination of the organism occurred in late-run fish (Table 3). This is in contrast to 6.3-6.7% positive muscle biopsies seen at Emmonak for the same years. Compared with heart cultures, the 2001 and 2002 Emmonak biopsies were only 45.4% and 27.1% efficient at detecting infected fish.

Tests for bias
Set nets vs drift nets: There was no significant difference in infection prevalence between salmon caught near shore with set nets (23.7%; n =131) and those caught off shore with drift nets (25.4%; n = 55) at Emmonak in 2002 (X^2 = 0.20; P >0.10). These two groups were then treated as one population and compared with upriver samples collected with fish wheels.

Gill nets vs fish wheels: There was no significant difference in infection prevalence between fish caught by gillnet at Emmonak and those caught with fish wheels at river mile 569 (mouth of Tanana River) and river mile 730 (Rampart Rapids) in 2002, confirming that gear type did not bias infection prevalence data (Table 4).

Early vs late run: The first 60 chinook caught from June 17 –19 were compared with 60 chinook caught from June 25 – 30 at Emmonak, and no significant difference in infection prevalence was demonstrated (X^2 = 0.73; P > 0.10).

Similarly, there was no significant difference in infection prevalence between chinook salmon sampled by fish wheels from early in the run at rm 730 (June 30-July 4) when compared with fish from late in the run (July 17-21, 2001) (males: X^2 = 0.28, n = 177; females: X^2 = 0.28, n = 63; P > 0.10). This same relationship was repeated again the following year (2002) when fish from the same location were sampled July 1-8 and July 9-15. (Males: X^2 = 0.12, n = 133) (females: X^2 = 0.95, n = 58) (P > 0.10).

Size (i.e. age) vs infection prevalence: No difference in infection prevalence relative to weight/age was observed when 588 adult chinook from the lower and middle Yukon River (rm

24–730) were divided into five weight groups ranging from 1 lb to >26 lb and compared for infection prevalence (Figure 12).

Other rivers and salmonid species

Kuskokwim River: Ichthyophonus was identified in 3 of 20 (15%) adult chinook salmon from the Kuskokwim River (K. Meuller, personal communication) confirming its presence in this river system.

Taku River: Hearts from 56 adult chinook salmon were collected from the Taku River between June 1 and June 13, 2002. Of these, 23% (13 of 56) were positive for *Ichthyophonus* by *in vitro* culture.

Juveniles: Ichthyophonus was not detected by *in vitro* culture in 57 juvenile chinook salmon sampled from the Klondike River (Yukon Territory) in October of 2002, nor from 65 juveniles sampled in April, 2002 from the Chena River.

Puget Sound chinook salmon: Ichthyophonus was not detected by *in vitro* culture in 180 adult spawning chinook salmon from two watersheds in Puget Sound between 2000 and 2001. Thirty males and 30 females were examined each year from the University of Washington hatchery adult return pond and found to be free of *Ichthyophonus*. An additional 60 chinook from the Big Beef Creek Field Station on Hood Canal (Washington) were also found to be free of *Ichthyophonus* by *in vitro* culture. No record of *Ichthyophonus* in Puget Sound salmon could be found in the literature, although the pathogen is present among herring and rockfish in the region (Hershberger et al. 2002, Kent et al. 2001, Kocan et al 1999).

Chum salmon: Sixty chum salmon were examined at Emmonak, AK during the ADF&G test fishery from June 17-21, 2000. Visual examination of heart, liver and *in vitro* culture of these tissues revealed 0 of 16 infected males (0%) and 4 of 42 infected females (9.5%). Only sub-clinical infections were observed.

Non-salmonids

Of the seven non-salmonid species from the Yukon River that were examined for *Ichthyophonus* infection by *in vitro* culture, only burbot were found to be infected (1 of 4 males, 1 of 2 females). These fish were caught by hook-and-line at rm 730 from July 9–15, 2002 in the vicinity of several fish camps. *Ichthyophonus* was not detected in the other six species examined, but broad and humpback whitefish and Inconnu (sheefish) had raised white spots on their hearts, superficially resembling *Ichthyophonus*. *In vitro* culture and histological examination of cardiac tissues from these fish revealed the organism was not *Ichthyophonus*, however, the identity of the organism was not determined.

Temperature

From 1999 through 2002, the temperature of the Yukon River ranged from 8-12 ^{o}C during the first week of June and increased to 15-20^{o}C during the last two weeks of June and all of July (Figure 13**)**. Based on a ten-year average, chinook salmon entering the Yukon River at the beginning of the annual migration encounter colder water temperatures in early June, while those migrating during the peak and at the end of the run encountered warmer temperatures in late June

and July (Figure 14**).** During late June and July water temperatures exceeded 15 ^{o}C, a temperature known to be lethal for *Ichthyophonus*-infected rainbow trout (Okamoto et al 1987b). Disseminated disease reached its highest prevalence during 2001 (Figure 15) the year with highest water temperatures (Figure 13). Only once, in early July 2002, did the Yukon River water temperature drop below 15 ^{o}C.

Since 1975, the mean monthly Yukon River temperatures at Emmonak have increased during June from <10 ºC to 15 ºC, while mean temperatures for July increased from 15 ºC to more than 20 ºC (Figure 16).

Laboratory exposure and temperature trials

No fish in any of the control or exposed groups died or became infected at any of the three temperatures during the 60-day test period. The reasons were unknown, but may have been due to loss of viability of the inoculum (spore preparation) or to differences in the susceptibilities of Puget Sound and Yukon River chinook salmon to *Ichthyophonus*.

Similarly, none of the seawater adapted chinook salmon that were fed infected tissue acquired infections, although half of the fish did die during the 60 day observation period. *Ichthyophonus* could not be isolated from fish that died or from survivors after 60 days, even though viable *Ichthyophonus* was isolated from the infected tissues used to feed these fish.

DISCUSSION

Prior to the mid 1980s, *Ichthyophonus* was unreported from the Yukon River. However in the last decade, the organism appears to have become firmly established in adult Yukon River chinook salmon, causing levels of disease that are affecting subsistence and commercial fishing and, perhaps, the resource itself. It is not known whether *Ichthyophonus* is a recently introduced pathogen or if it has historically been present and is only now emerging as a disease entity because of changes in the feeding ecology of salmon in the Bering Sea or changes in environmental factors in the Yukon River. As its title implies, this study was only able to address the portion of the chinook life cycle that occurs in the Yukon River and its tributaries and was not able to evaluate the potentially important effects of *Ichthyophonus* on the seawater phase of these fish. Similarly, this study focused on *Ichthyophonus* and, while our investigation did reveal a few other disease conditions (Appendix V), these were not judged to be a significant source of mortality. We did not perform specific assays for viruses or other known pathogens of chinook salmon; however, our gross and histopathological examinations revealed no evidence of other infectious conditions that might be adversely affecting survival of these fish.

Infection and disease

A consistent pattern of *Ichthyophonus* infection and subsequent disease occurred in mainstem Yukon River chinook salmon each year from 1999-2002. When adult salmon entered the river in June of each year they were already infected with *Ichthyophonus*. As they migrated upriver, the percentage of infected individuals remained relatively constant (25-35%), with a consistent increase at the U.S./Canada border (rm 1,230), most likely caused by pre-patent infections

reaching detectable levels. Clinical disease exhibited a different pattern, increasing from ~5% at the mouth of the river to over 30% between river mile 730 and 1,230. As fish reached river mile 1,745 near the upper reaches of the Yukon River, the prevalence of both infection and of disease dropped to less than 15% in fish sampled from the Whitehorse hatchery. Over the first 1,200 miles of the Yukon River, infection prevalence was significantly higher in females than males for all years, but this pattern reversed at the Whitehorse hatchery (rm 1,745), for two of the three years chinook salmon were sampled, corresponding with the decrease in infection and disease prevalence at this site.

The relatively stable infection prevalence over the first 1,200 river miles supports our hypothesis that fish become infected before entering the Yukon River. This hypothesis is consistent with experimental evidence that shows *Ichthyophonus*-infected chinook salmon develop signs of disease 25 to 35 days following exposure, with mortality occurring between 25 and 60 days post-exposure (Kocan et al. 1999, Jones & Dawe 2002).

Among infected salmon in the lower Yukon, the low percentage of fish that exhibited clinical signs of disease (e.g. white punctate lesions) may be indicative of infections that are either newly acquired or older infections that have been held in a sub-clinical state by the fish immune system. In either case, changing river conditions could influence the proliferation of the parasite and/or the success of the salmon immune system, leading to the appearance of clinical disease. The higher water temperatures observed in late June and early July could effect such changes because *Ichthyophonus* is known to be most pathogenic at temperatures above 15 ^{o}C (Okamoto et al 1987b, Company et al. 1999, Halpenny et al. 2002) and the parasite proliferates more rapidly at higher temperatures (Sitja-Bobadilla & Alvarez-Pelletero 1990, Spanggaard et al. 1994, Kishio 1999). These data strongly suggest that increased water temperature is responsible for the rapid increase in clinical disease observed in fish sampled from the middle Yukon River.

Decline in infection & disease prevalence at Whitehorse

A dramatic drop in both infection and disease prevalence occurred each year from 2000 to 2002 at the Whitehorse hatchery (rm 1,745). The mean infection prevalence decreased from 34.3% at rm 1,230 to 15% at Whitehorse. A similar decrease in clinical disease was also observed (Figure 4, Appendix I). Since the decline in infection and disease was consistent from year-to-year, sampling error and statistical anomaly were ruled out as probable causes. However, before Whitehorse chinook were sampled at the time of artificial spawning, the fish were captured and held at the hatchery for varying lengths of time, unlike the conditions they would experience if allowed to continue to their natal streams above the adjacent fishway. To account for the effect of variables associated with confinement in the hatchery and to explore this phenomenon further, we sought a natural spawning area that was similar to the Whitehorse hatchery in migration distance, but without the associated variables. To this end, we examined the Chena and Salcha Rivers, two terminal spawning tributaries of the Tanana River.

Tanana River

Although *Ichthyophonus* was present in 25-30% of males and females in the Tanana River, the female infection prevalence was not significantly different from males, unlike that in the mainstem Yukon River. The prevalence of infection in Tanana River females was, in fact,

significantly lower than that of females in the middle Yukon River (rm 730-1,230) that had migrated a similar distance.

While we cannot completely rule out the effects of different stocks, we believe the difference in infection and disease prevalence in Tanana River females may be caused by diseased fish dropping out of the population as they approach their natal streams. Essentially all of the chinook entering the Tanana River were dark, with males having hooked jaws and females having protruding ovipositors, while in the mainstem Yukon upriver from the mouth of the Tanana, silver and "blush" fish predominate, with few apparent morphological changes. Tanana River fish have only 100-250 river miles to swim before reaching their natal streams, while mainstem chinook must migrate an additional 575-1,050 river miles before reaching their upper Yukon River natal streams. If proximity to spawning streams (e.g. greater sexual maturity) negatively affects survival of *Ichthyophonus*-infected fish, then Tanana River fish should begin to drop out of the population before Yukon River mainstem chinook salmon. What is clear from this study is that as adult chinook in the Tanana system reached their spawning areas, a significant proportion of the infected fish were unaccounted for.

Chena and Salcha Rivers

To address the unexplained low prevalence of infection and disease observed in the upper Yukon River at Whitehorse, chinook salmon from two Tanana River tributaries, the Chena and Salcha Rivers, were sampled. These sites were chosen because they are similar to the upper Yukon River in migration distance and proximity to their spawning areas, but the influences of confinement and hatchery residence were eliminated. These two tributaries are responsible for 70-80% of the total Tanana River escapement, thus reducing the possibility that a significant number of infected fish were diverting to other tributaries and were not being sampled (U.S./Canada Joint Technical Committee 2000; C. Stark, personal communication).

Post-spawn fish of both sexes from the Chena and Salcha Rivers exhibited infection prevalence significantly lower than those from the Tanana River for the same years, similar to what was observed in the upper Yukon River. Surprisingly, a majority of the infected fish observed in the Tanana River failed to appear in either the Chena or Salcha Rivers. In a summary of *Ichthyophonus* in North Atlantic herring, Sinderman and Chenoweth (1993) hypothesized that the low prevalence of *Ichthyophonus* observed during and following an epizootic may reflect high mortality among infected individuals. If this hypothesis is correct, and Yukon River chinook are experiencing an epizootic of Ichthyophoniasis en route to their spawning areas, then the low prevalence of infection among post-spawn chinook could be caused by mortality in pre-spawn fish before they reach their spawning areas. Because samples were collected from fish in the terminal spawning areas at frequent intervals during the 3-week spawning period and because it seems unlikely that infected fish would have a significantly different time of arrival at these terminal areas given that they were still present within the pulse of fish sampled in the upper Tanana, we do not believe that the failure to find predicted numbers of infected fish on the spawning grounds is indicative of a sampling bias.

No direct evidence was found to explain the fate of the missing infected upper Yukon and Tanana River chinook, but based on published experimental and historical field data, it is very improbable that *Ichthyophonus* infections in the fish had been eliminated, leading to the

conclusion that the missing fish had died. *Ichthyophonus* has been shown to cause heavy mortality in numerous species (McVicar 1982, Mellergaard & Spanggaard 1997, Patterson 1996, Powles et al. 1968, Rahimian & Thulin 1996, Rucker 1953, Sindermann & Chenoweth 1993). Surveys of herring (Scattergood 1948, Sindermann 1963, Sindermann & Chenoweth 1993) and yellowtail flounder (Powles et al. 1968, Ruggieri et al. 1971) have shown that following *Ichthyophonus* epizootics in the North Atlantic a dramatic decline occurred in the infection prevalence of *Ichthyophonus* equal to or exceeding the proportions observed in Yukon River chinook. These declines were attributed to diseased fish being removed from the population, leaving mostly uninfected individuals.

Histological examination of tissues from infected chinook salmon further supports the hypothesis that more severely affected or diseased fish were dying prior to spawning. This was evident from the pattern of tissue damage and host response that were more severe in chinook sampled from the middle portion of the mainstem Yukon River than in fish from either the Tanana River or the Chena and Salcha Rivers. If the more severely affected fish died first, the less severely infected individuals would predominate among the survivors. *Ichthyophonus* infections are not known to spontaneously resolve, although under favorable conditions the cellular and humoral responses of the host may be able to contain, but not eliminate, the parasite (McVicar 1982, McVicar and McLay 1985). However, the stressful conditions in the Yukon River and the hormonal changes associated with spawning appeared to result in the inability of the immune system to contain the infection, resulting in disseminated disease. The progressive nature of the disease under these conditions suggests that severely infected individuals may succumb before reaching their natal streams.

The significant decrease in infection prevalence seen in the Chena and Salcha Rivers in 2001 and 2002 closely paralleled that seen at the Whitehorse hatchery from 2000 to 2002, suggesting the two phenomena may have a similar cause. Since a spontaneous recovery from *Ichthyophonus* infection of the hatchery fish is improbable, and sampling error was ruled out, the most parsimonious explanation is that a significant proportion of infected fish died before reaching the Whitehorse Rapids. Definitive proof of *Ichthyophonus*-related mortality would come from finding dead pre-spawn *Ichthyophonus*-infected chinook salmon before they reached their terminal spawning streams. Finding dead salmon in the Yukon mainstem or Tanana is unlikely, however, because of turbidity and the fact that dead salmon sink.

During 1997-1999, a problem in the Whitehorse Rapids fishway (rm 1,745) made possible the observation of upper Yukon River pre-spawn chinook for an extended period. The configuration of baffles in the fishway was believed to impede migrating spawners at a time when water temperatures reached 18 ºC. During this period, chinook salmon were reported to suffer higher than normal mortality. The 1999 JTC technical report states: "*The 1997 through 1999 seasons have demonstrated what are believed to be record numbers of (chinook) mortalities in the fishway*." with the majority of the mortalities occurring among females (U.S./Canada Yukon River Joint Technical Committee, 1999 pg. 44 & 2000 pg. 46). When the baffle configuration was modified in 2000 to make passage easier, chinook mortality returned to normal. If *Ichthyophonus* was causing mortality in the upper Yukon River chinook near the Whitehorse Rapids, retention of adult fish in the fishway increased the probability of detecting dying fish. If these fish had passed the fishway without delay, the mortalities may have gone unnoticed.

Unfortunately, because no pathologic examination of the dead salmon was conducted, the cause of death is not known; however, salmon with disseminated *Ichthyophonus* infections could be expected to have poor cardiac function with resulting difficulties in digging spawning redds, ascending steep gradients or surviving physically stressful conditions such as those encountered in the Whitehorse Rapids fishway.

Disease progression (dissemination)

Although there was no significant difference in the prevalence of infection between fish sampled from early versus late in the run, a clear difference in severity of disease was observed between the two groups. Among the clinically infected fish, those sampled from early in the run exhibited visible lesions primarily in the heart, while clinically infected fish from late in the run had overt, disseminated disease affecting multiple internal organs. This finding is consistent with earlier reports from fishermen and processors who indicated an increased presence of white lesions in fish caught late in the run (V. Umphenor, Interior Alaska Fish Processors, Fairbanks, personal communication). Initially, it was believed that diseased salmon could not maintain the same swimming pace as uninfected fish, causing them to lag behind the main migration pulse, thus increasing the probability of finding fish with infected flesh late in the run. However, we found that the prevalence of infection and clinical disease did not differ between fish from early and late in the run.

In contrast, we did find that the most severe form of disease occurred in fish sampled later in the run. The observation was confirmed by muscle biopsy data collected by DFO near the U.S./Canada border during 2001 and 2002. These data revealed a mean prevalence for infected muscle of 15% for early run fish (mid July) and 31% for late run fish (mid August). Because the prevalence of infection or the prevalence of disease were not different between early and late run chinook sampled 500 miles down river, the muscle biopsies confirmed a more extensive spread of the parasite to the skeletal muscle in fish sampled late in the run. Taken together, these findings seem best explained by the effects of higher water temperatures on the disease process that would have affected the late run chinook to a greater degree.

The high proportion of fish with infected muscle at the U.S./Canada border also confirmed that the organism progressively disseminates throughout the organs as the fish migrate. At Emmonak the percent of fish showing positive muscle cultures was just 6.2-6.7%, which was 27-45% of the infection prevalence determined from cardiac tissue culture. In contrast, when the fish reached the border, 15% to 30% of the fish cultured positive for *Ichthyophonus* by muscle biopsy. This clearly shows that although there was little change in the overall infection prevalence, dissemination of the parasite increased significantly as the fish approached their natal streams.

Temperature effects

While the prevalence of *Ichthyophonus* infection did not appear to be linked to river conditions, the development, spread and pathogenicity of the organism within the host appeared to be correlated with Yukon River water temperature. No difference in infection prevalence was detected between fish from early and late in the run, but a dramatic difference in disease severity was demonstrated by the dissemination of the parasite throughout the host's organs late in the run. These data are consistent with reports of local fishermen and processors who reported chinook salmon from early in the run, when temperatures were low, had normal appearing flesh,

but an increased portion of the fish from late in the run, when temperatures exceeded 15 ºC, had "white spots" in their flesh and the fillets did not dry or smoke properly. During the late 1990s, fishermen began reporting higher numbers of affected salmon from early in the run, a condition which has since returned to one of just the late run fish being affected (W. Fliris, personal communication). However during the period of our study, disseminated disease was highest during 2001, the year with the highest water temperatures (Figures 13, 15).

The most obvious difference between early and late run fish was that salmon entering the Yukon River prior to mid June encountered water temperatures between 8 ºC and 14 ºC, while those entering after the middle of June encounter water temperatures ranging from 15–20 ºC, which is known to increase the mortality rate in *Ichthyophonus*-infected fish. Because salmon were sampled from the same site for early and late run comparison, the distance and time en route was similar, eliminating these as potential variables. Thus, the difference in water temperature encountered by fish early in the run compared with fish from late in the run, could account for the difference in disease severity observed between early and late run fish.

The concept of temperature affecting pathogenesis in poikilothermic species is not new. Barrow (1958) and Barrow & Stockton (1960) demonstrated that the ambient temperature of the host influences the pathogenicity of trypanosomes infecting newts and amoebae infecting snakes. Studies on *Ichthyophonus* in several fish species clearly show the effects of higher temperatures on disease progression in a fish host (Okamoto et al. 1987b, Halpenny et al. 2002).

Field observations alone cannot implicate nor exonerate elevated temperature as the cause of increased disease severity in *Ichthyophonus*-infected fish. To accurately describe the effects of temperature on fish infected with *Ichthyophonus,* controlled studies must be performed where temperature, infectious dose, exposure time and duration are controlled.

Source of infection

At this point, no evidence indicates where or when Yukon River chinook became infected, however, because *Ichthyophonus* is primarily a saltwater pathogen, the marine environment is the most probable site of infection. Initially, infected herring were suspected as the source of infection, but after three years of sampling Bering Sea herring and finding no infected fish, this hypothesis had to be questioned. It was curious that, even in our limited sampling, no infected herring were found north of the Aleutian Islands because populations of herring from Washington State, British Columbia and the Gulf of Alaska are known to be heavily infected with *Ichthyophonus* (Marty et al. 1998, Hershberger et al. 2002, Jones and Dawe 2002). Perhaps the Aleutian Islands form a barrier to the spread of the strain or species of *Ichthyophonus* that infects Pacific herring. This supposition leads to the possibility that another forage fish or invertebrate is the marine source of infection for Yukon River chinook salmon in the Bering Sea (Sinderman & Scattergood 1954, Karisbakk et al. 2000) or that some fraction of the Yukon chinook feed south of the Aleutian chain where infected herring are present.

An alternative possibility is that Yukon River chinook may be infected as juveniles in fresh water. Although the parasite was not found in 120 juveniles from two widely separated Yukon River tributaries, it was identified in 2 of 6 Yukon River burbot. It is not known if the parasite is endemic in burbot, or if burbot become infected by eating infected salmon tissue. Numerous

reports written about freshwater species becoming infected and suffering high mortality rates after eating *Ichthyophonus*-infected marine fish make introduction into a freshwater drainage highly probable (Slocombe 1980, Chun & Kim 1981, Tung et al. 1986, Athanassopoulou 1992, Galuppi et al 1994, Kocan et al. 1999). If the burbot is a newly established host for *Ichthyophonus*, it has the potential to become a source of infection for juvenile salmon and other freshwater species in the Yukon River and its tributaries, thus exacerbating the current situation.

If the *Ichthyophonus* identified in Taku River chinook were the same strain as that in Yukon River chinook, it could indicate the pathogen may have a wider geographic range within Alaskan waters than is currently known, perhaps affecting multiple susceptible species. Based on recently published studies identifying distinct genetic differences in isolates of *Ichthyophonus* (Rand et al. 2000, Kent et al. 2001), the Yukon and Taku River isolates cannot be presumed to be the same until proven so by host susceptibility studies and genetic comparison. Nevertheless, a recent genetic analysis of *Ichthyophonus* isolates from rockfish in Puget Sound, Washington revealed these isolates were indistinguishable from the strain of *Ichthyophonus* in the Yukon River, indicating a potentially wide geographic distribution of this strain of the pathogen along the west coast of North America (A. Hart, Western Fisheries Research Center, unpublished data).

Although Yukon River mainstem females had consistently higher infection and disease prevalence rates than males, no obvious cause for the difference could be identified. Initially, it was thought that because females spent more years at sea, and therefore were exposed for a longer time, the probability of infection was increased. When no difference in infection prevalence was detected in small (young) and large (old) fish, this hypothesis was abandoned. However, an alternate hypothesis is that both sexes are exposed to the same source of infection, but females spend more time feeding at this source before entering the river. If females spent more time feeding just before entering the river, and the site of infection was the Yukon Delta, this behavior would increase the probability of female exposure, thus explaining the higher infection prevalence in females. This hypothesis is supported by the observation that males constitute a greater proportion of the population early in the run while females predominate late in the run. Whatever the mechanism, the difference in infection prevalence between males and females was consistent in Yukon River mainstem salmon for all four years of the study.

Directions for future studies

Results from this study suggest future research areas that will provide further information about the epidemiology and ecology of *Ichthyophonus* infections in Yukon River chinook salmon. These studies include, but are not limited to:

1. Effects of water temperature on the pathogenicity of *Ichthyophonus* for chinook salmon
2. Effects of corticosteroids on the progression *Ichthyophonus* disease in chinook salmon
3. Sources or reservoirs of *Ichthyophonus* infection for Yukon River chinook & burbot
4. Differences among strains of *Ichthypohonus* present in marine/freshwater fish
5. Progression of infection and pathogenesis of *Ichthyophonus* in chinook salmon
6. Fate of infected fish as they near or arrive at terminal spawning areas
7. How the severity of disease based upon macroscopic changes and histology correlates with clinical parameters and performance measures (e.g. hematocrit, cardiac function, swimming stamina)

SUMMARY

Between 1999 and 2002, more that 2500 Yukon River chinook salmon were examined for the presence of *Ichthyophonus* infection and the resulting disease. Overall infection prevalence in the Yukon River peaked at river mile (rm) 1,230 at ~40%, while on the Tanana River infection peaked at ~30% at rm 900. More females than males were infected at all sites and all years in the Yukon River, while in the Tanana River there was no difference between male and female infection prevalence. The prevalence of clinical disease among the infected salmon increased from ~5% at the mouth of the Yukon River to 35% at rm 731 on the Yukon River, and to 22% at rm 900 on the Tanana River. When the fish approached the upper reaches of the Yukon River at rm 1,745 (Whitehorse, Y.T.) and the spawning areas of the Chena and Salcha Rivers in Alaska, the prevalence of infection dropped significantly to <15%. Likewise, the prevalence of clinical disease dropped to ~12% at rm 1,745 on the Yukon River and < 10% on the Chena and Salcha Rivers (rm 970 -1,015). Position of fish within the run did not affect infection or disease prevalence, but a higher percentage of the fish at the end of the run exhibited disseminated disease affecting multiple organs. This was verified by muscle biopsies taken at the U.S./Canada border (rm 1,220). Increasing river temperature from mid June through mid July occurred during the second half of the run and appeared to be associated with the increase in disseminated disease. *Ichthyophonus* was also detected in chinook salmon in the Kuskokwim and Taku Rivers during limited surveys. The source of infection could not be determined, but no *Ichthyophonus*-infected herring could be found north of the Aleutian Islands, although all populations of herring sampled south of the Aleutians are reported to be infected at relatively high levels. The discovery of infected burbot in the Yukon River demonstrated the presence of *Ichthyophonus* in a freshwater Yukon River species. We are uncertain if this infection is endemic or was acquired through ingestion of infected salmon tissues. A limited survey of pike, grayling, sheefish and juvenile salmon revealed no infected individuals, however 6% of chum salmon were found to be infected, but without clinical signs of disease. Analysis of historic data indicates that rising average water temperatures during the past three decades may be an important cause of increased disease and consequent pre-spawning mortality among Yukon River chinook salmon.

CONCLUSIONS

1. *Ichthyophonus* sp. is an important pathogen of Yukon River adult chinook salmon
2. Clinical disease from *Ichthyophonus* infection increased as chinook salmon move upriver
3. More Yukon River females than males were infected during 1999-2002
4. No significant age- or size-related difference was detected in infection prevalence
5. *Ichthyophonus* caused severe disease in the heart, liver, spleen, kidney and skeletal muscle
6. Chinook salmon late in the run had significantly more affected organs than fish from early in the run
7. Significantly fewer infected chinook salmon were found at upriver spawning areas
8. At least one non-salmonid Yukon River species, the burbot (*Lota lota)* was infected with *Ichthyophonus*
9. Water temperatures above 15 ºC appeared to be associated with increased disease
10. Rising average water temperatures in the Yukon River in the past three decades may be an important cause of increased disease and pre-spawning mortality among chinook salmon

ACKNOWLEDGEMENTS

The authors are indebted to G. Sanders for providing histology photographs and the following for providing data and assistance in the field: Dave Daum, Russ Holder, Tevis Underwood, U.S. Fish & Wildlife Service, Fairbanks; Mike Doxey, Gene Sandone, Tom Vania, Steve Hayes, and Paul Salmone, Alaska Department of Fish & Game; Bill Fliris & Stan Zuray, Tanana, AK; Virgil Umphenor, Fairbanks, AK; Brian Asplund, Circle, AK; Pat Milligan, DFO, Yukon, Canada; Frank Shipley, USGS-BRD, Seattle, WA. Three peer reviewers provided valuable suggestions for improving this report. The U.S. Fish and Wildlife Service, Office of Subsistence Management, provided $177,580 in funding support for this project through the Fisheries Resource Monitoring Program, under an Inter-agency agreement FWS# 70181-1-N115. Additional funds were obtained from the U.S. Fish and Wildlife Service, Office of Restoration and Enhancement under contract # URE-13-01, 02.

LITERATURE CITED

Athanassopoulou, F. 1992. Ichthyophoniasis in sea bream, *Sparus aurata* (L.), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), from Greece. Journal of Fish Diseases 15:437-441.

Barrow, J.H., Jr. 1958. The biology of *Trypanosoma diemyctyli*, Tobey. III. Factors influencing the cycle of *Trypanosoma diemyctyli* in the vertebrate host *Triturus v. viridescens*. Journal of Protozoology 5:161-170.

Barrow, J.H. Jr., and S.S. Stockton 1960. The influence of temperature on the host-parasite relationships of several species of snakes infected with *Entamoeba invadens*. Journal of Protozoology 7:373-383.

Chun, S.K., and Y.G. Kim. 1981. Infection by an *Ichthyophonus sp*. fungus in rainbow trout *Salmo gairdneri*. Bulletin of the Korean Fisheries Society 14:37-42.

Colton, T. 1974. Statistics in Medicine. Little Brown, Boston.

Company, R., A. Sitja-Bobadilla, M.J. Pujalte, E. Garay, P. Alvarez-Pellitero, and J. Perez-Sanchez. 1999. Bacterial and parasitic pathogens in cultured common dentex, *Dentex dentex* L. Journal of Fish Diseases 22:299-309.

Galuppi, R., H. Cappellaro, M.L. Fioravanti, and M.P. Tampieri. 1994. *Ichthyophonus* infection in farm-raised rainbow trout (*Oncorhynchus mykiss*): Observations of the ecological agents in the host and in vitro. Parassitologia 36: suppl. p. 63.

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. American Fisheries Society Fish Health Section Newsletter 30(2):17-20.

Heike, S-P., and T. Wahl. 2002. First report of *Ichthyophonus hoferi* infection in wild brown trout (*Salmo trutta*) in Switzerland. Bulletin of the European Association of Fish Pathologists 22:225-228.

Hershberger, P.K., K. Stick, B. Bui, C. Carroll, B. Fall, C. Mork, J.A. Perry, E. Sweeney, J. Wittouck, J. Winton, and R. Kocan. 2002. Incidence of *Ichthyophonus hoferi* in Puget Sound fishes and its increase with age of Pacific herring. Journal of Aquatic Animal Health 14:50-56.

Jones, S.R.M., and S.C. Dawe. 2002. *Ichthyophonus hoferi* Plehn & Mulsow in British Columbia stocks of Pacific herring, *Clupea pallasi* Valenciennes, and its infectivity to Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). Journal of Fish Diseases 25:415-421.

Karisbakk, E. J.A. Balbuena, K. Hodneland, A.M. Kvenseth, J. Mullins, A. Tolonen, T. Torgersen, and A. Nylund. 2000. Parasite induced mortality in herring. Institutt for Fiskeri- og Marinbiologi Rapport Bergen No. 7.

Kent, M.L., V. Watral, S.C. Dawe, P. Reno, J.R. Heidell, and S.R.M. Jones. 2001. *Ichthyophonus* and *Mycobacterium*-like bacterial infections in commercially important rockfish, *Sebastes* spp., in the eastern North Pacific Ocean. Journal of Fish Diseases 24:427-431.

Kishio, H. 1999. Important fungal diseases of fish and shellfish. Fourth symposium on diseases in Asian aquaculture: Aquatic Animal Health for Sustainability. Nov. 22-26. Cebu International Convention Center, Cebu City, Philippines.

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

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

Kocan, R.M., P.K. Hershberger, and J.R. Winton. 1998. Controlled field and laboratory studies of VHSV and *Ichthyophonus* in Pacific herring. Exxon Valdez Oil Spill restoration project. Annual Report, Sect. II: Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound. G.D.Marty *et al*. Alaska Department of Fish & Game.

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.

Leaverton, P.E. 1978. A Review of Biostatistics. 2nd ed. Chap. 4, Statistical Inference, pp. 35-62. Little, Brown and Company, Boston. MA

McVicar, A.H. 1981. An assessment of Ichthyophonus disease as a component of natural mortality in plaice populations in Scottish waters. International Council for Exploration of the Sea, Document C.M. 1981/G49.

McVicar A.H. 1982. *Ichthyophonus* infections of fish. Pages 243-269 *in* R.J. Roberts editors. Microbial diseases of fish. Academic Press, London.

McVicar, A.H. 1999. *Ichthyophonus* and related organisms. Pages 661-687 *in* P.T.K. Woo and D.W. Bruno editors. Fish Diseases and Disorders Volume 3, Viral, Bacterial and Fungal Infections. CABI Publishing, New York.

McVicar, A.H., and K. Mackenzie. 1972. A fungus disease of fish. Scottish Fisheries Bulletin 37:27-28.

McVicar, A.H., and H.A. McLay. 1985. Tissue response of plaice, haddock, and rainbow trout to the systemic fungus *Ichthyophonus*. Pages 329-346 *in* A.E. Ellis editor. Fish and Shellfish Pathology. Academic Press, London.

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

Okamoto, N., K. Nakase, and T. Sano 1987b. Relationships between water temperature, fish size, infective dose and *Ichthyophonus* infection in rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 53:581-584.

Patterson, K.R. 1996. Modeling the impact of disease-induced mortality in an exploited population: The outbreak of the fungal parasite *Ichthyophonus hoferi* in the North Sea herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences 53:2870-2887.

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler, and J.R. Leonard. 1982. Fish Hatchery Management. U.S. Fish & Wildlife Service, Wash. D.C.

Post, G. 1987. Textbook of Fish Health. TFH Publications, Neptune City, NJ. page 88.

Powles, P.M., D.G. Garnett, G.D. Ruggieri, and R.F. Nigrelli. 1968. Ichthyophonus infection in yellowtail flounder (*Limanda ferruginea*) off Nova Scotia. Journal of the Fisheries Research Board of Canada 25:597-598.

Rahimian, H. 1998. Pathology and morphology of *Ichthyophonus hoferi* in naturally infected fishes off the Swedish west coast. Diseases of Aquatic Organisms 34:109-123.

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.

Rand, T.G., K. White, J.J. Cannone, R.R. Gutell, C.A. Murphy, and M.A. Ragan. 2000. *Ichthyophonus irregularis* sp. nov. from the yellowtail flounder *Limanda ferruginea* from the Nova Scotia shelf. Diseases of Aquatic Organisms 41:31-36.

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

Ruggieri, G.D., R.F. Nigrelli, P.M. Powles, and D.G. Garnett. 1971. Epizootics in yellowtail flounder, *Limanda ferruginea* Storer, in the western North Atlantic caused by *Ichthyophonus*, an ubiquitous parasitic fungus. Zoologica 55:57-61.

Scattergood, L.W. 1948. A report on the appearance of the rungus *Ichthyosporidium hoferi* in the herring of the northwestern Atlantic. U.S. Fish Wildlife Service Special Scientific Report on Fisheries 58:1-33.

Sindermann 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. International Council for the Exploration of the Sea Meeting Papers, ICES-CM 1993/F:41, Copenhagen (Denmark) 39pp.

Sindermann 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 Department of Sea and Shore Fisheries, Augusta, Maine.

Sitja-Bobadilla, and A., P. Alvarez-Pellitero. 1990. First report of *Ichthyophonus* disease in wild and cultured sea bass *Dicentrachus labrax* from the Spanish Mediterranean area. Diseases of Aquatic Organisms 8:145-150.

Slocombe, R.F. 1980. Induction of Ichthyosporidiosis in brown trout (*Salmo trutta*) by feeding of raw mullet. Australian Veterinary Journal 56:585-587.

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.

Tung, M-C, S-T Huang, and S-S Tsai. 1986. An epizootic in largemouth bass, *Micropterus salmoides* Lacepede, caused by *Ichthyophonus* in freshwater pond in southern Taiwan. Coa Fish. Ser. Taipei, no. 8, 6-13.

United States/Canada Yukon River Joint Technical Committee. 1999. Yukon River salmon season review for 1999 and Technical Committee Report. Anchorage, AK, Oct. 1999. p. 53.

United States/Canada Yukon River Joint Technical Committee. 2000. Yukon River salmon season review for 1999 and Technical Committee Report. Anchorage, AK, Oct. 2000. p. 68.

Witts, L.J. (ed.). 1964. Medical Surveys and Clinical Trials. 2nd ed. London: Oxford Univ. Press.

FOOTNOTES

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

[2] 1975 – 2002 temperature data supplied by Tim Brabbits, U.S. Geological Survey, Fairbanks; Ryan Sollee, Alaska Department of Fish and Game, Anchorage; and David Daum, U.S. Fish and Wildlife Service, Fairbanks, Alaska.

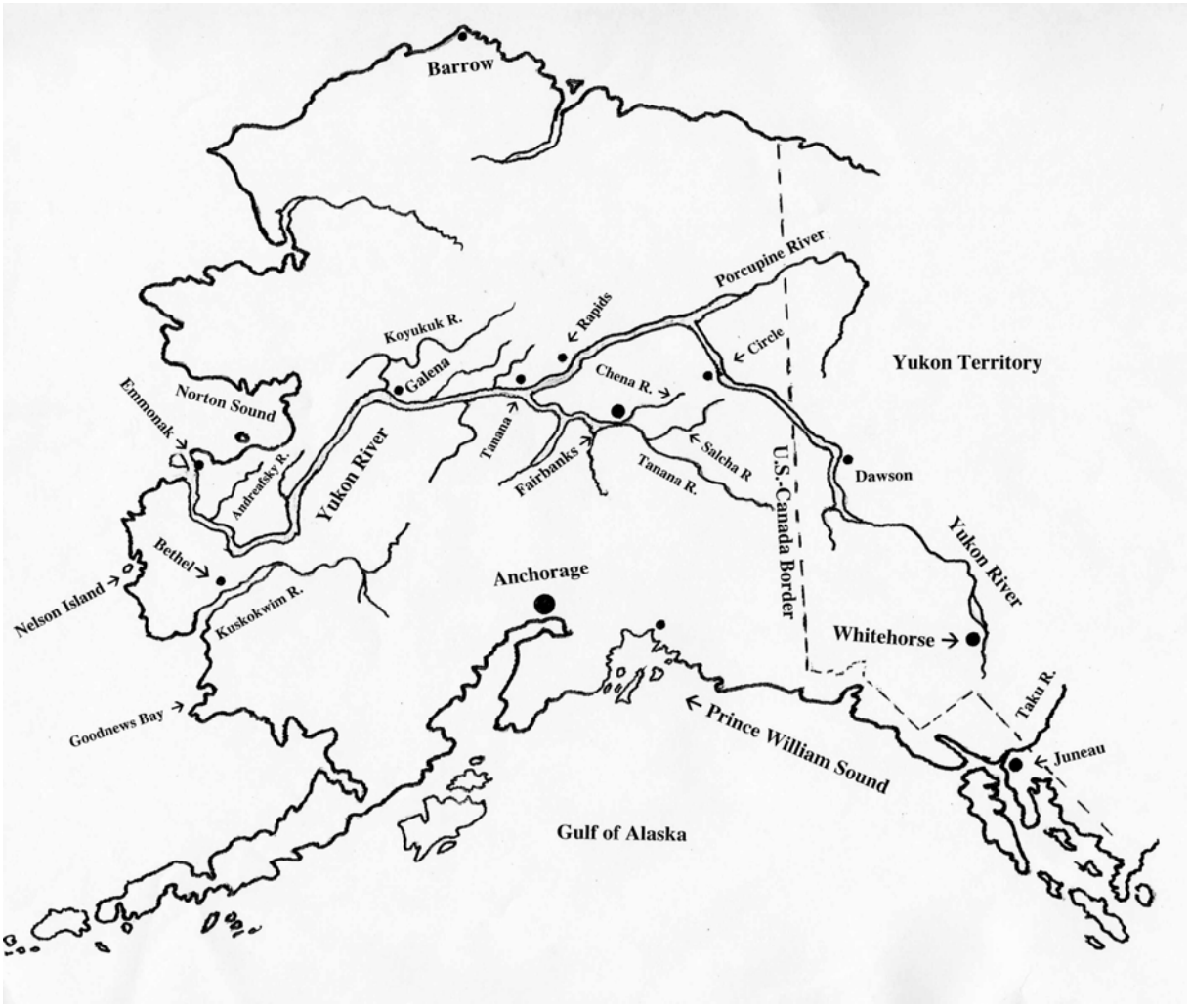

Figure 1. Map of Alaska showing sample sites along the Yukon and Tanana Rivers. (See Table 1 for site data)

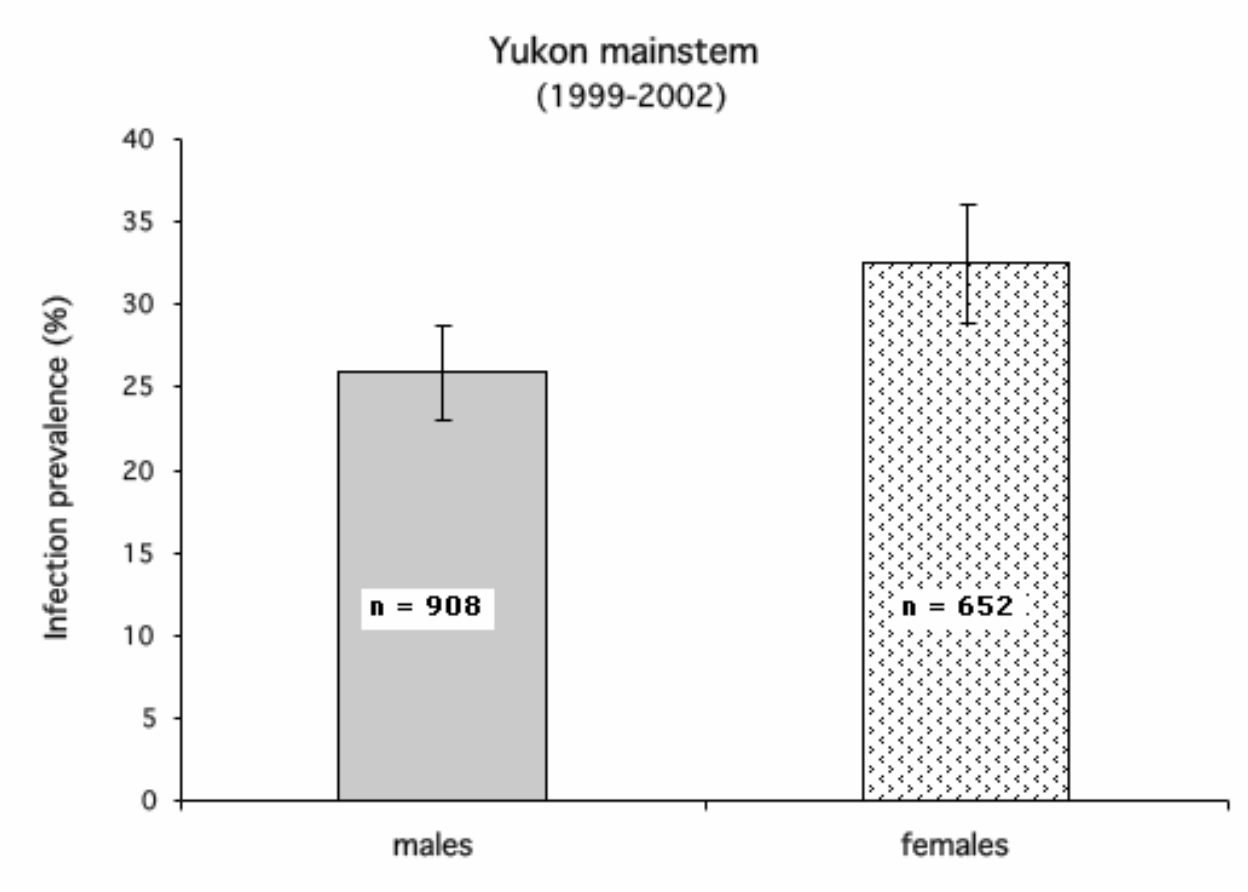

Figure 2. *Ichthyophonus* infection prevalence in male and female chinook salmon from the Yukon River mainstem (Emmonak, Galena, Rapids, Circle and U.S./Canada Border) all years combined. Infection prevalence in females was significantly greater than in males. (X^2=8.78, df=1, P=0.003) (bars = 95% conf.)

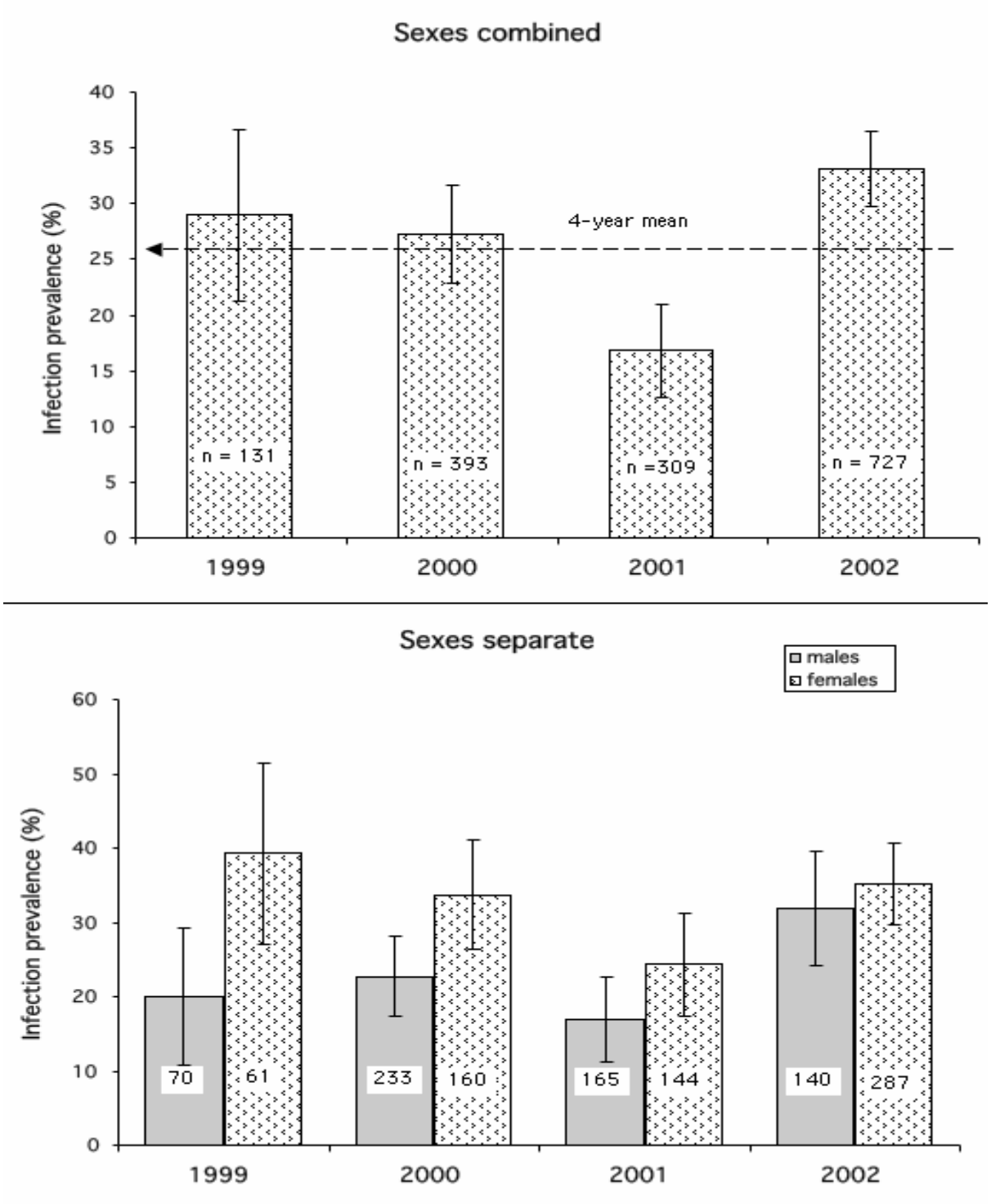

Figure 3. Annual infection prevalence in chinook salmon from the Yukon River mainstem (Emmonak, Galena, Rapids, Circle and the U.S./Canada Border) for all sites combined. Infection prevalence for sexes combined and by sex (bars = 95% conf.)

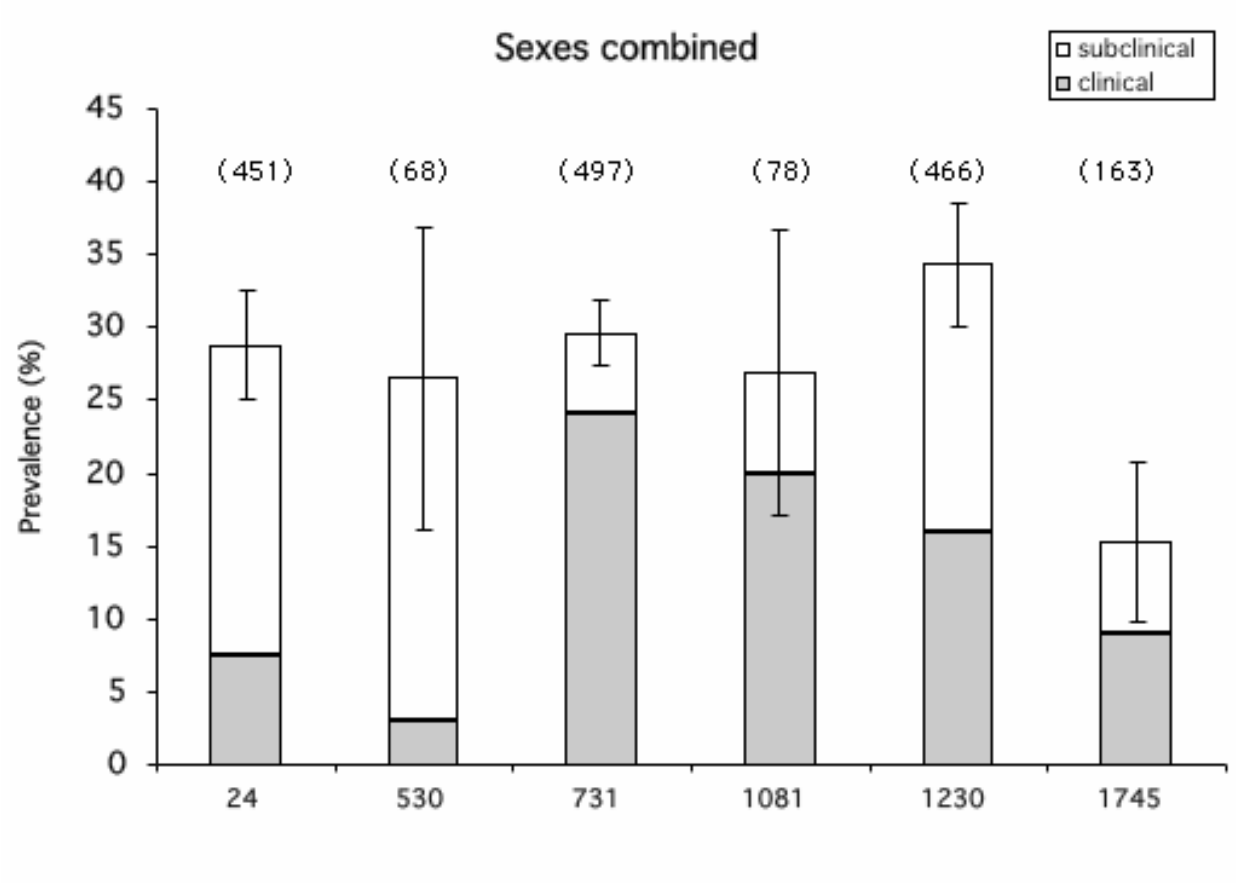

Figure 4. Mean 4-year *Ichthyophonus* infection and clinical disease prevalence in all fish over the length of the Yukon River. Samples were collected sequentially as the fish moved upriver during a 10-week period beginning the 3rd week of June through the 1st week of September. (n) (bars = 95% conf.)

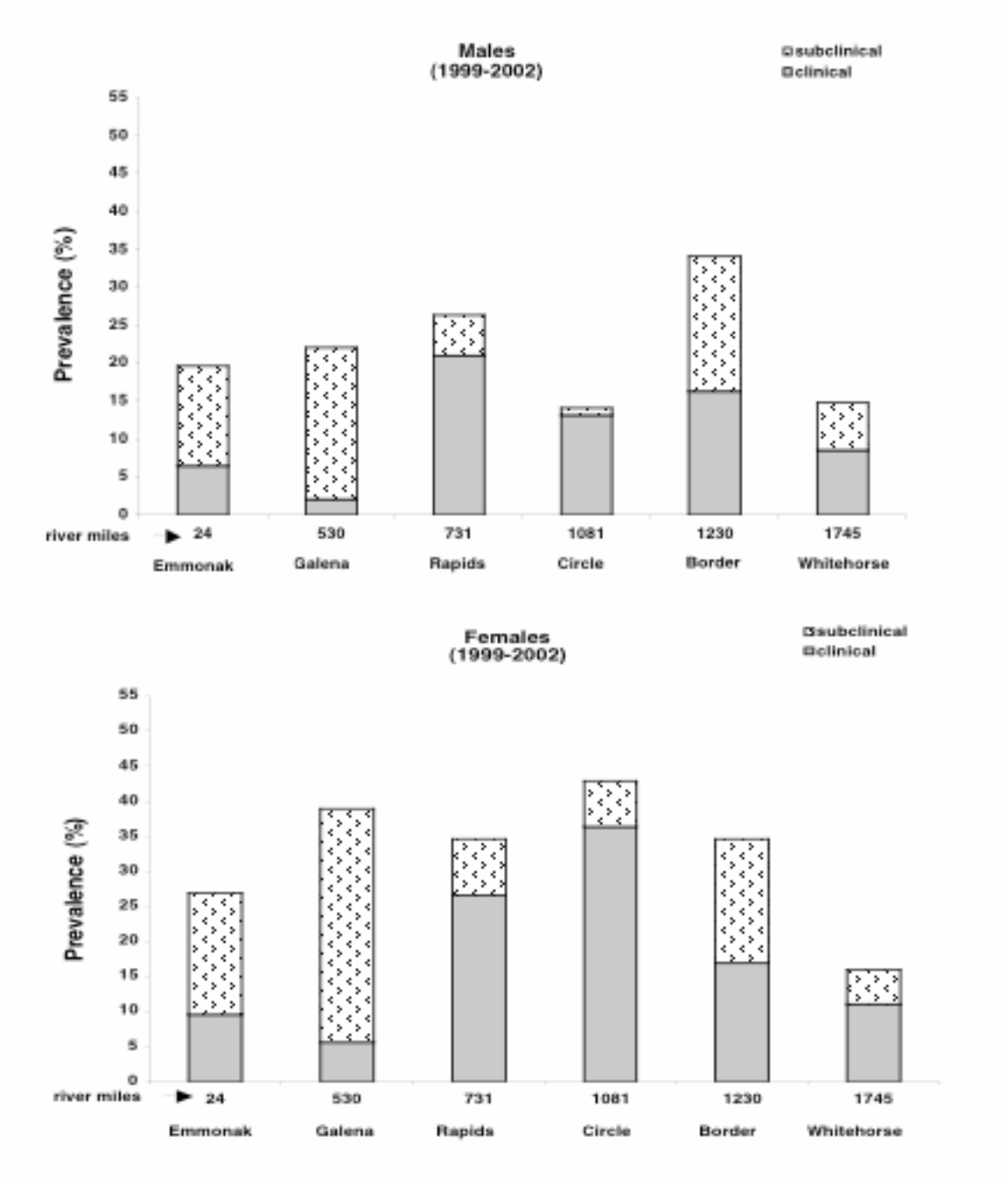

Figure 5. Clinical and sub-clinical *Ichthyophonus* infection prevalence in male and female chinook salmon over the length of the Yukon River for all years combined. Samples were collected sequentially as the fish moved upriver during a 10-week period beginning the 3rd week of June through the 1st week of September. (see Appendix I for n values)

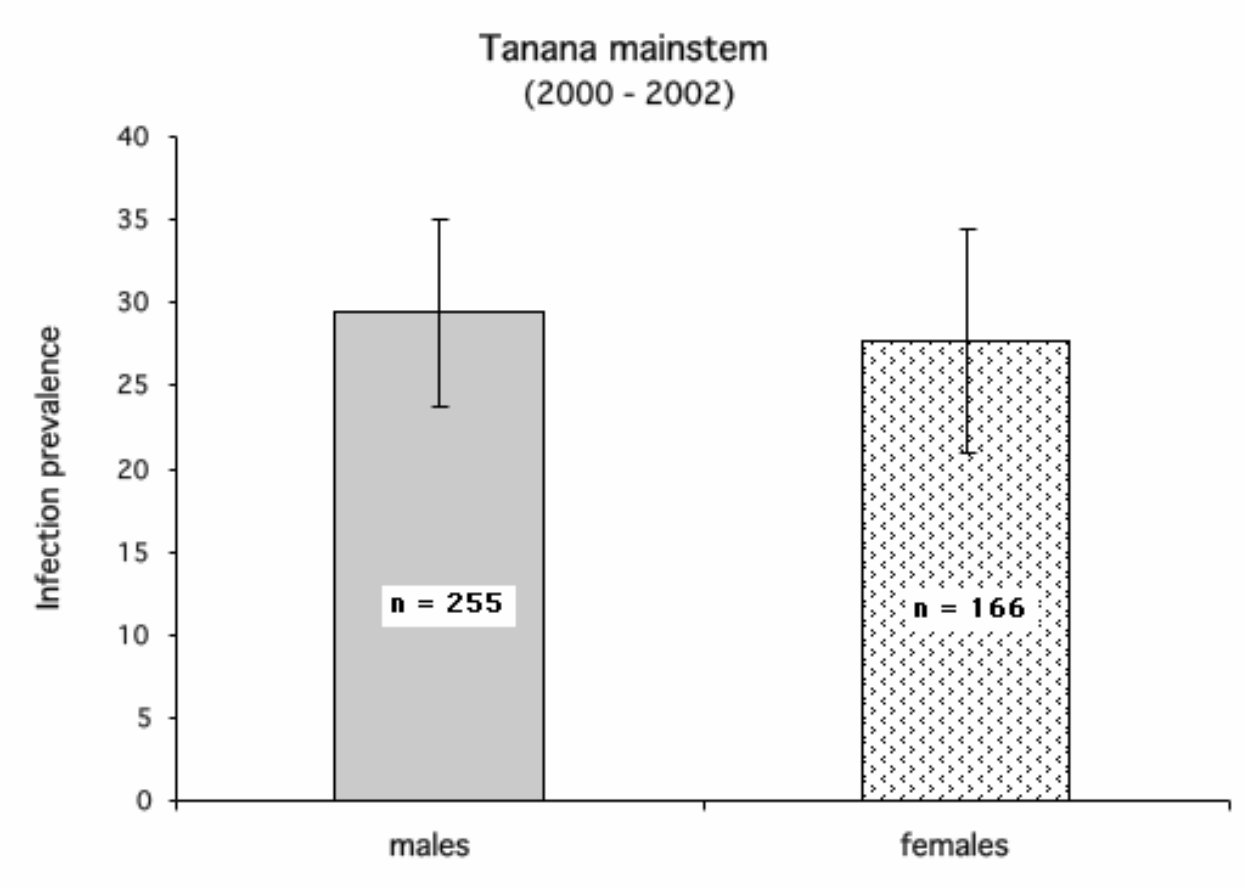

Figure 6. *Ichthyophonus* infection prevalence in male and female chinook salmon from the Tanana River mainstem, a major tributary of the Yukon River. There was no difference between male and female infection prevalence. (X^2=0.071, df=1, P=0.789) (bars = 95% conf.)

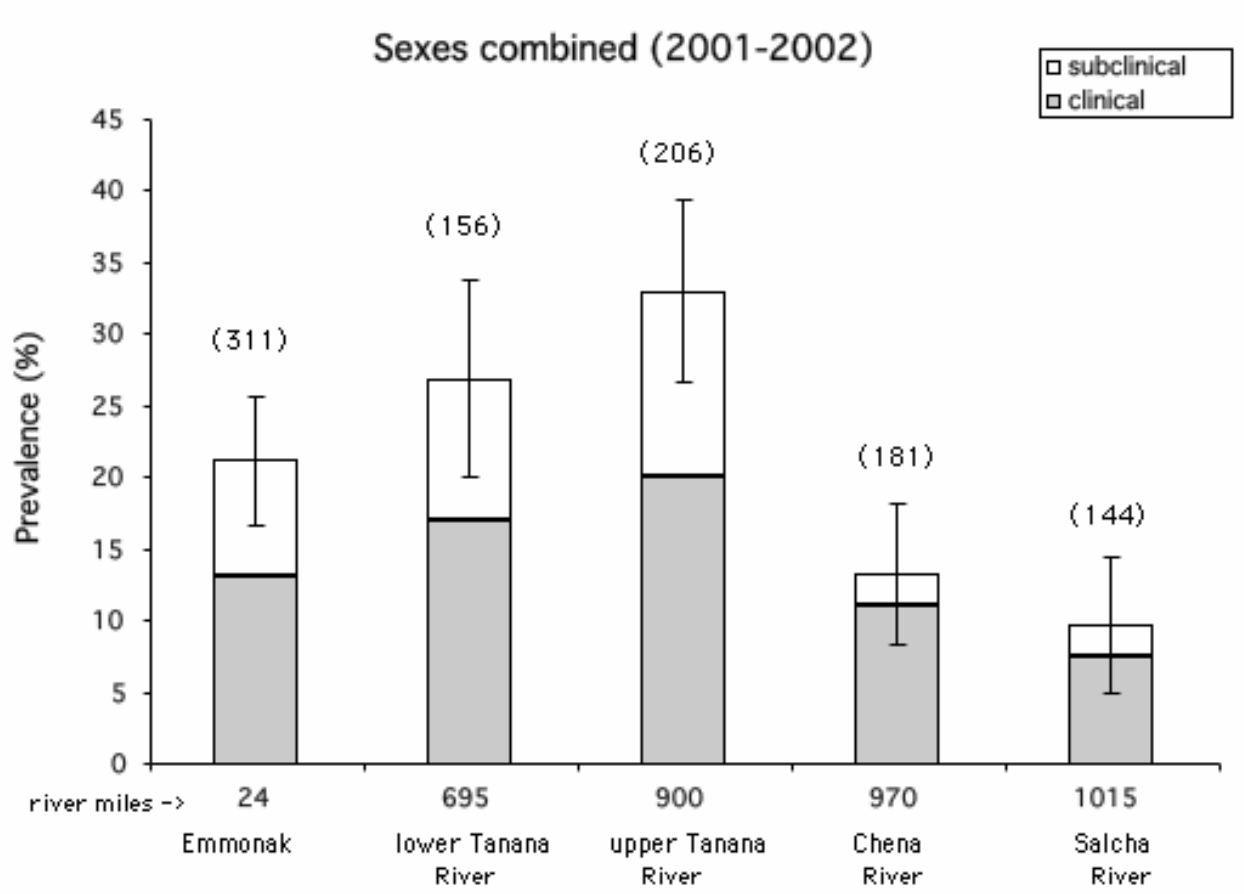

Figure 7. Clinical and sub-clinical *Ichthyophonus* infection prevalence in chinook salmon sampled from the Yukon, Tanana, Chena and Salcha Rivers from 2001-2002. (n) (bars = 95% conf.).

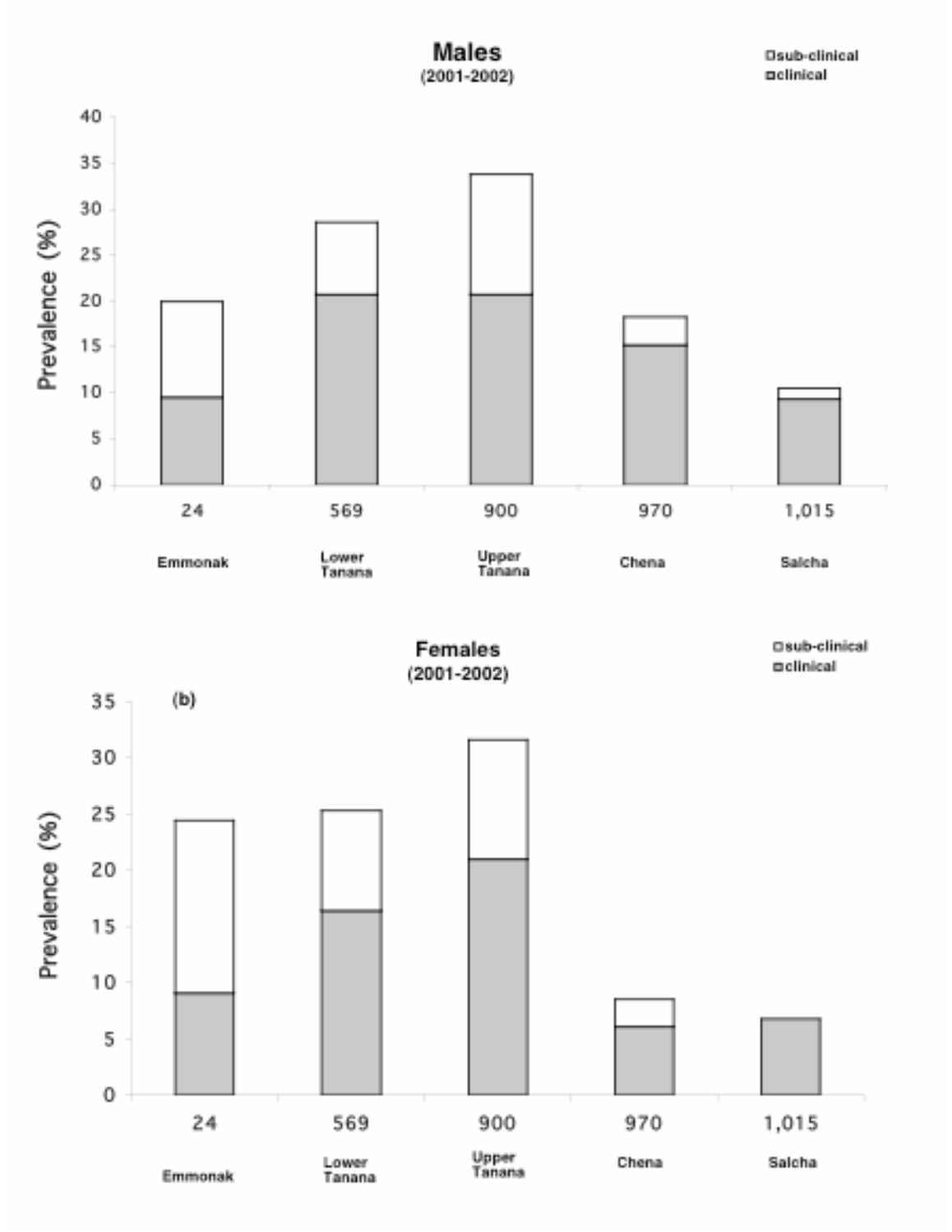

Figure 8. Clinical and sub-clinical *Ichthyophonus* infection prevalence in chinook salmon from the Yukon, Tanana, Chena and Salcha Rivers both years combined. (see Appendix I for n values)

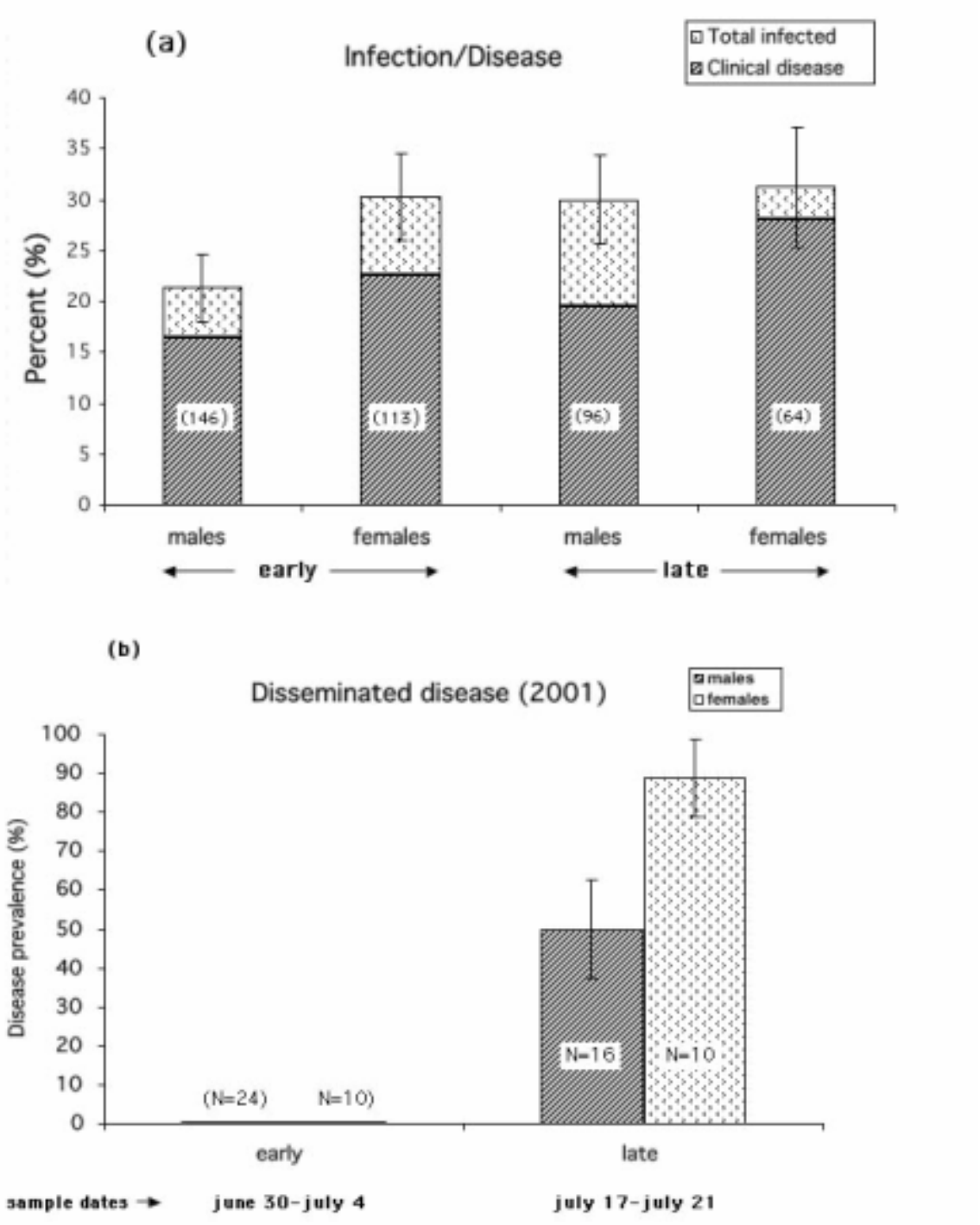

Figure 9. Comparison of infection and clinical disease prevalence between fish from early and late in the 2001 annual migration (a), and disseminated disease in clinically infected fish from the same population (b). (bars = 95% conf.)

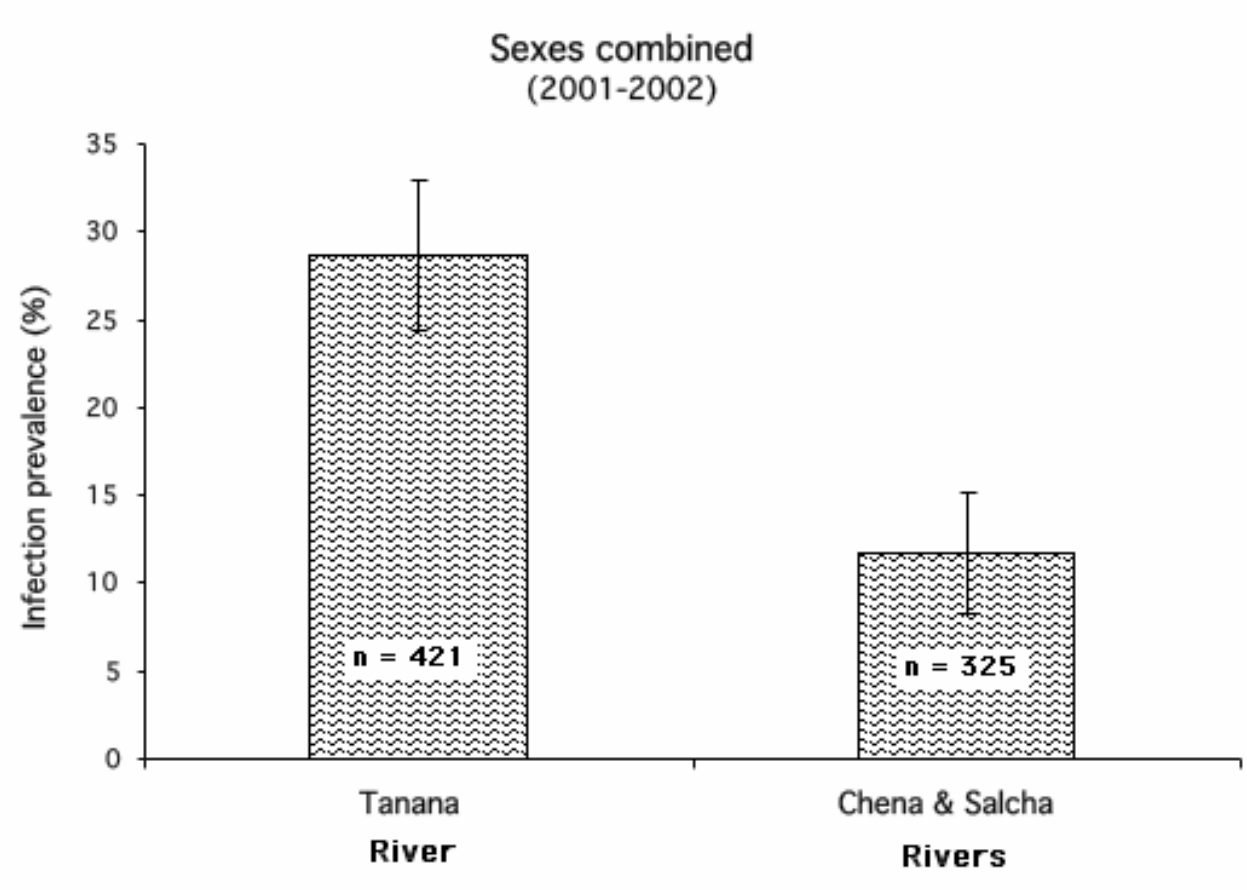

Figure 10. Mean *Ichthyophonus* infection prevalence in chinook salmon sampled from the Tanana, Chena and Salcha Rivers in 2001 and 2002. Significantly fewer infected fish were found in both the Chena and Salcha Rivers than the Tanana River. (X^2=34.3, df=1, P=0.00) (bars = 95% conf.)

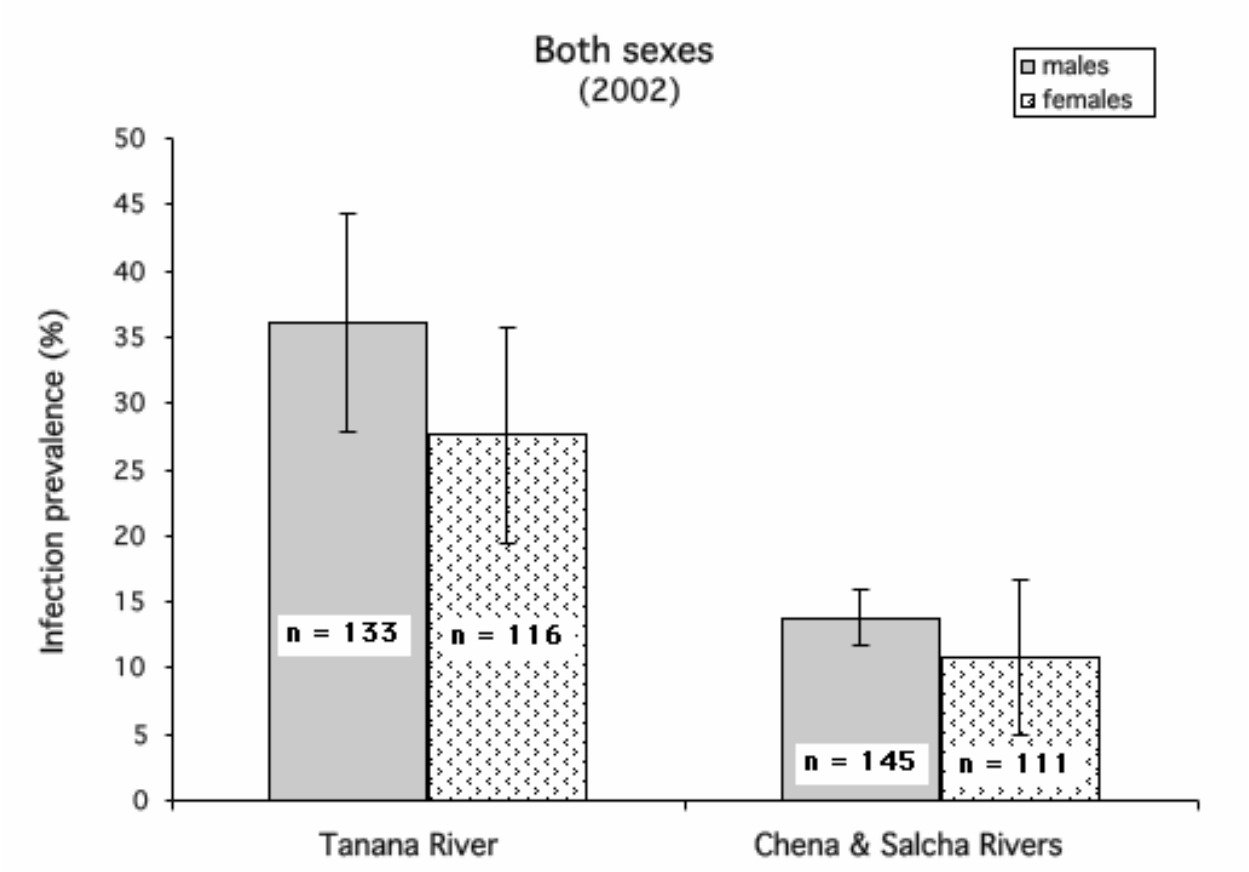

Figure 11. Comparison of *Ichthyophonus* infection prevalence in male and female chinook salmon sampled from the Tanana River and spawned-out male and female chinook sampled from the Chena and Salcha Rivers. Significantly fewer infected fish of both sexes were identified from both the Chena and Salcha Rivers relative to the Tanana. (Males: X^2=16.1, df=1, P=0.000; Females: X^2=17.7, df =1, P=0.0001) (bars = 95% conf.)

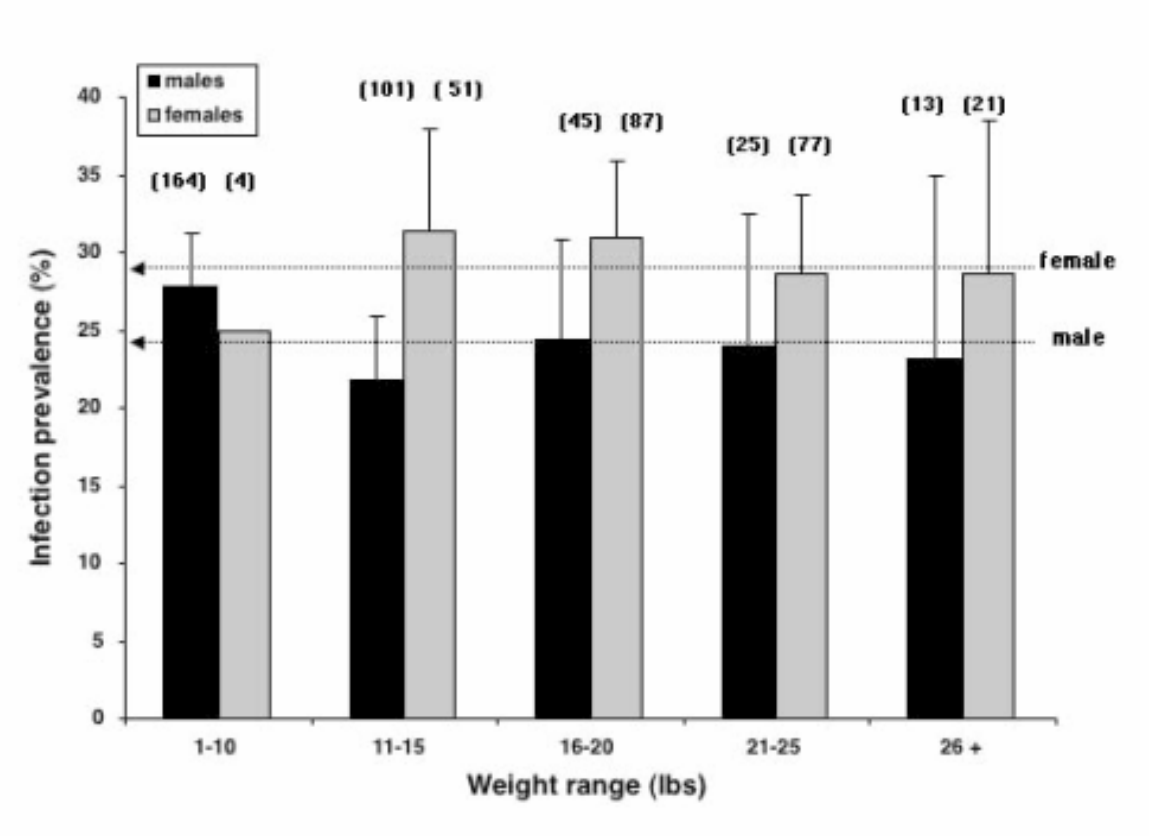

Figure 12. Comparison of *Ichthyophonus* infection prevalence in chinook salmon by sex and weight in Yukon River mainstem (Emmonak, Rapids and the U.S./Canada Border). Population mean: males 24.2 ± 2.24; females 28.9 ± 2.55. There was no correlation between fish size (age) and infection prevalence. (n) (bars = 95% conf.)

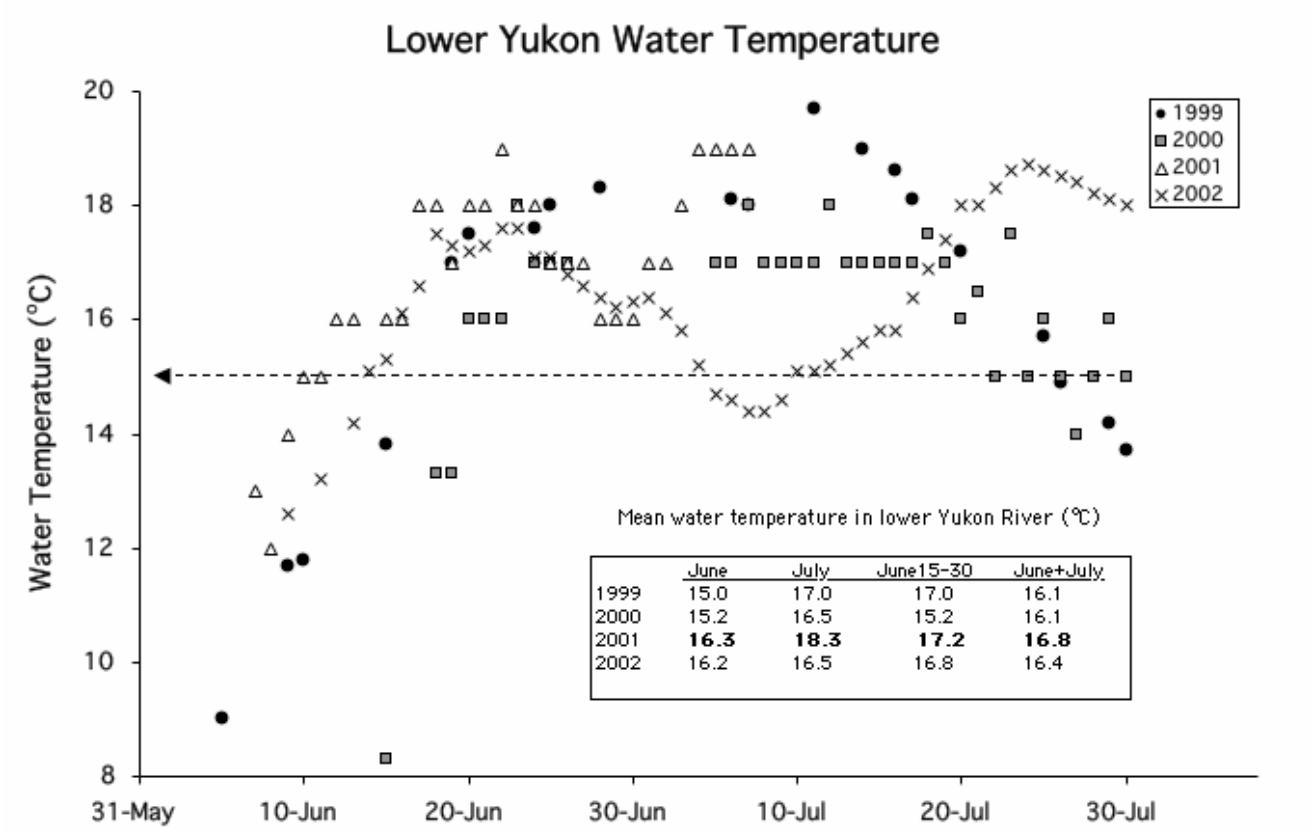

Figure 13. Lower Yukon River temperatures during the 1999-2002 annual chinook spawning run. Highest monthly temperatures occurred in 2001 (bold type in table).

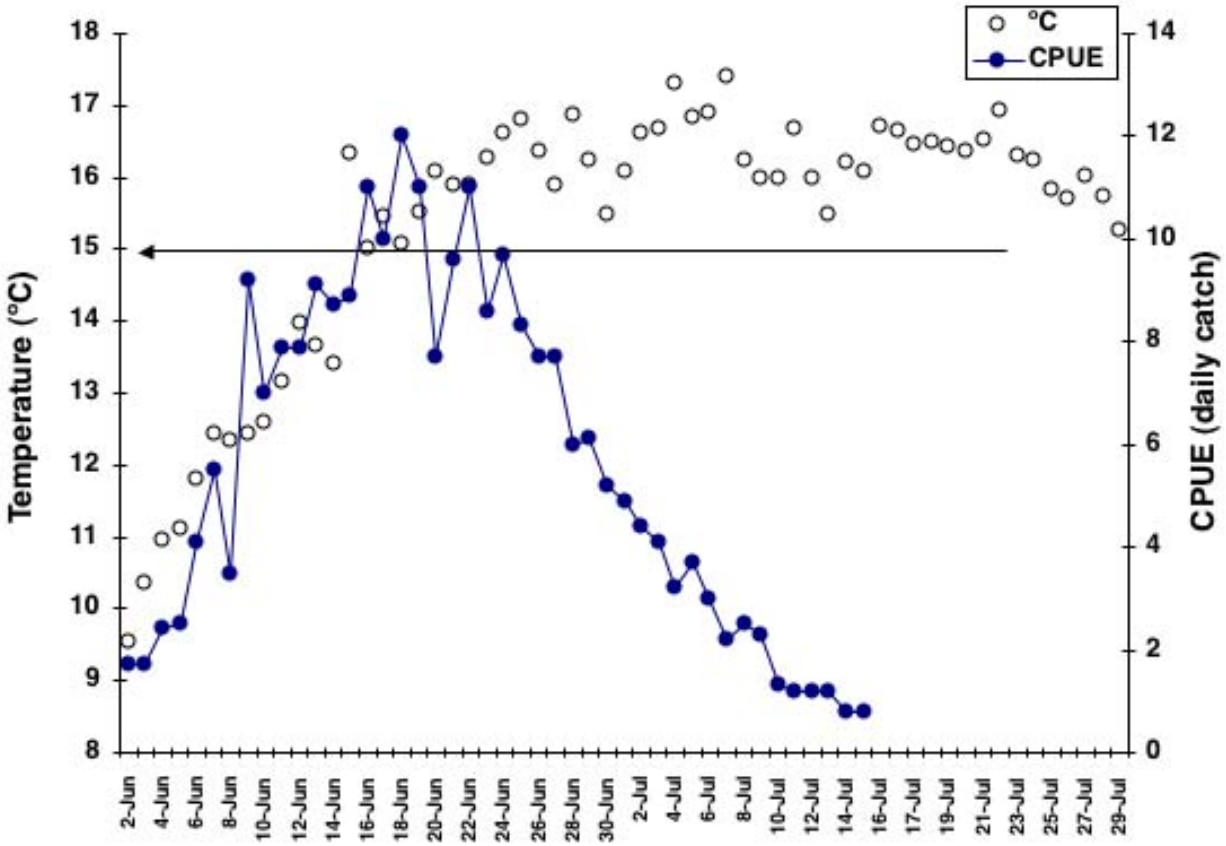

Figure 14. Ten-year mean chinook catch per unit effort (CPUE) at the mouth of the Yukon River relative to water temperatures observed from 1997-2002. Temperatures exceed 15 ^{o}C from mid June through the end of July, corresponding to the peak and the 2nd half of the chinook run. Horizontal line represents the known lethal temperature for *Ichthyophonus*-infected trout.

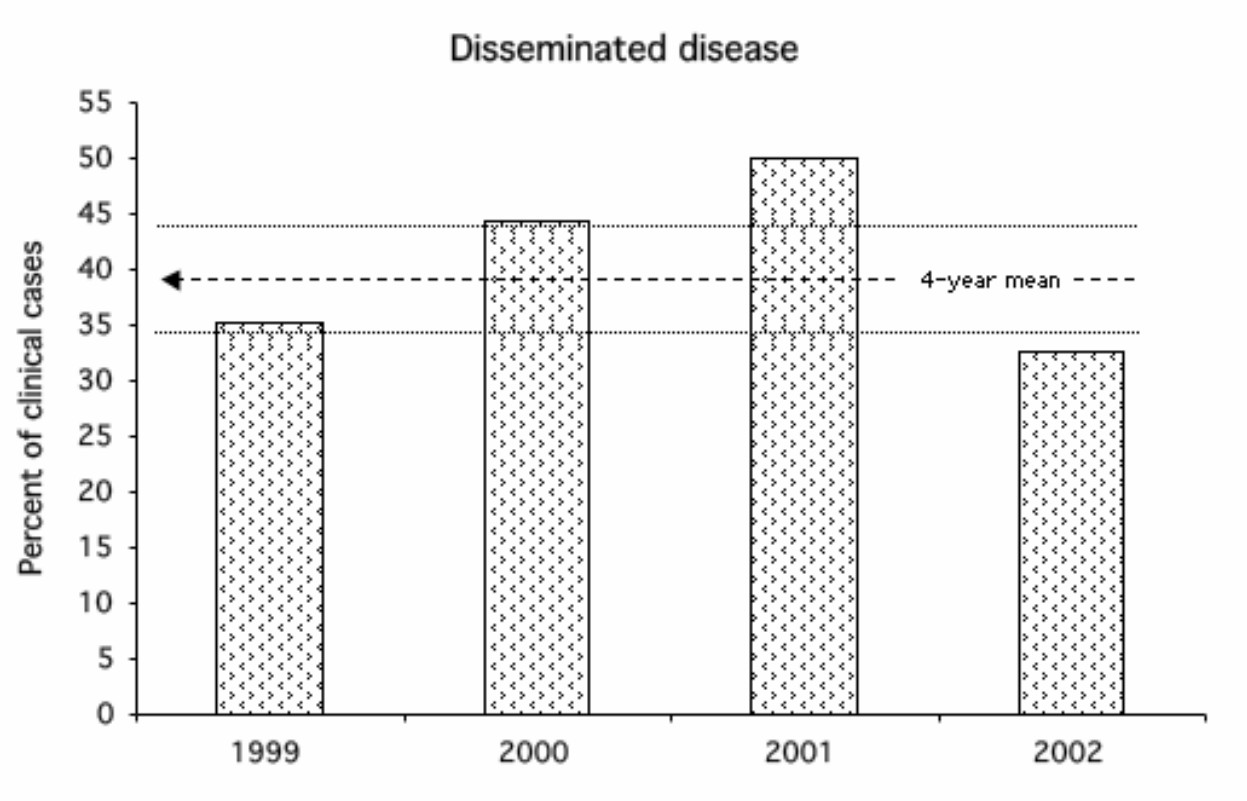

Figure 15. Percent of clinical cases exhibiting disseminated disease (e.g. multiple infected organs) from 1999 to 2002. The highest temperatures in the lower Yukon River for all months occurred in 2001, corresponding to the year with the highest prevalence of disseminated disease (see Fig. 13 for temperatures). Arrow = 4-year mean dissemination prevalence ± s.d.

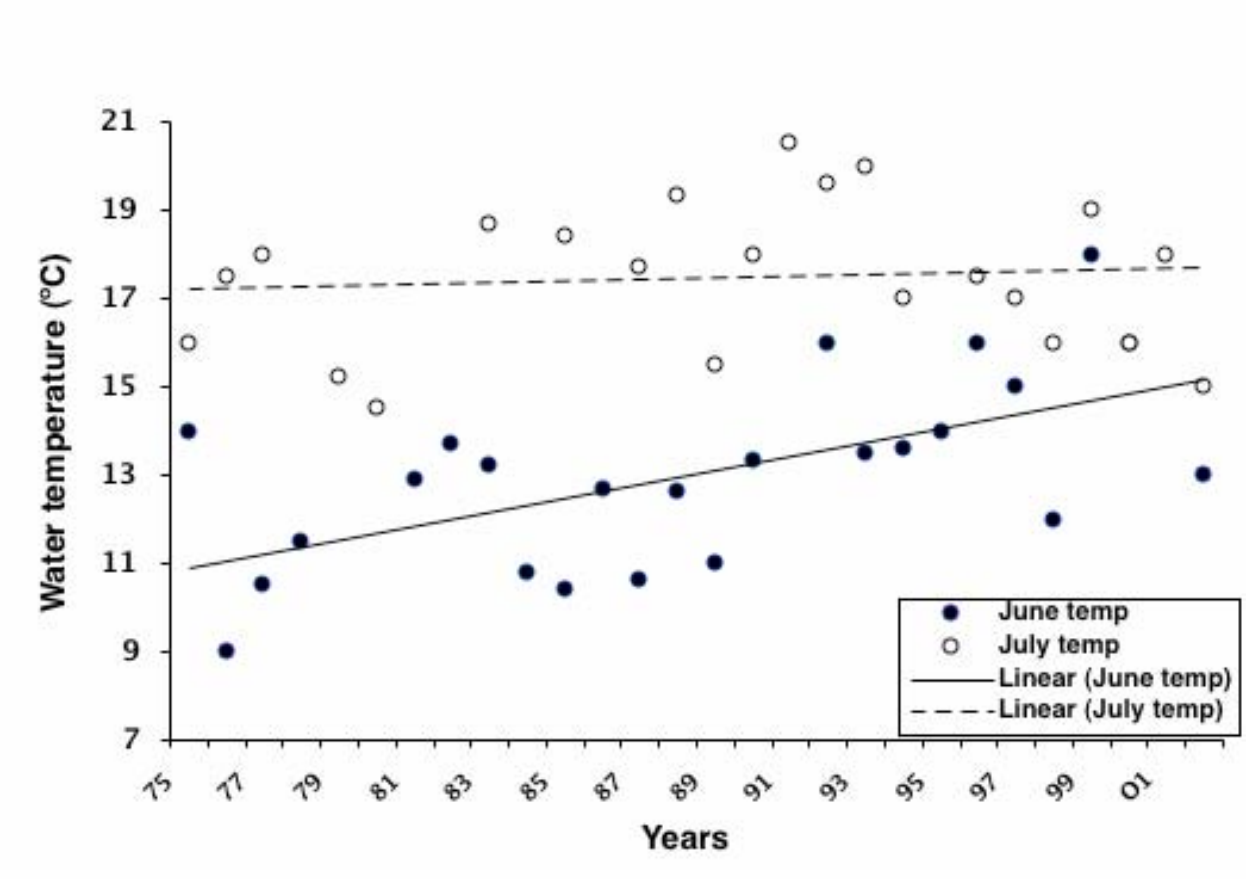

Figure 16. Mean Yukon River temperature at Emmonak (river mile 24) from 1975 to 2002, corresponding to the annual chinook salmon spawning migration. June temperatures show a mean increase from < 11 ºC to ~ 15 ºC ,while July mean temperatures remained relatively constant at 17 ºC. (Trend lines: solid=June, dashed=July).

Table 1. Sample sites: 1999 through 2002.

	River mile[1]	Dates Sampled	Years Sampled
Yukon River			
Emmonak	24	6/17-6/30	1999-2002
Galena	530	7/1-7/3	2000
Rampart Rapids	730	6/30-7/21	1999-2002
Circle	1,080	7/13-7/19	2000
U.S./Canada [2] Border	1,220	7/13-8/10	2000-2002
Whitehorse	1,745	8/19-9/2	2000-2002
Tanana River			
Lower Tanana	690	7/9-7/12	2001-2002
Upper Tanana (Nenana, Fairbanks)	860, 920	7/9-7/25	2000-2002
Chena River [3]	>980	7/26-8/7	2001-2002
Salcha River [3]	1,025	7/26-8/7	2002

1) River mile from mouth of the Yukon River
2) Samples from DFO (Canada) test fishery
3) Spawn-outs sampled on multiple days over 12-20 river miles

Table 2. Histopathology of chinook heart tissue infected with *Ichthyophonus*.

Site	N	Clinically [1] Positive (%)		Histologically [2] Positive (%)		Host [3] Reaction	Intensity [4]
Emmonak	9	4	(44)	3	(44)	minimal	2-3
				1		moderate-severe	4+
Rampart Rapids	29	10	(34)	1	(34)	minimal	2-3
				9		moderate-severe	4+
Lower Tanana	16	10	(63)	5	(63)	none	0-1
				2		minimal	2-3
				3		moderate-severe	4+
Upper Tanana	4	2	(50)	1	(75)	none	0-1
				2		minimal	2-3
Chena River	5	4	(80)	2	(80)	none	< 1
				1		none-minimal	0-1
				1		minimal-moderate	2-3

[1] Visible lesions on heart recorded during field observation.
[2] *Ichthyophonus* observed microscopically in histology sections examined independently.
[3] Minimal – peripheral necrosis without fibrosis
Minimal to moderate – peripheral necrosis with fibrosis
Moderate to severe - peripheral necrosis, fibrosis and inflammation
[4] Number of organisms per 10X microscopic field

Table 3. Infection prevalence (%) detected in skeletal muscle using punch biopsies of early and late run chinook salmon at the U.S./Canada border.

Sample Site (year)		N	% positive	X^2	P
Whiterock (2001)					
Early	(July 15-18)	50	10.0 %		
				7.86	< 0.01
Late	(August 8-12)	49	27.1 %		
Bio Island (2002)					
Early	(July 20-25)	49	20.4 %		
				2.37	> 0.05
Late	(Aug 4-20)	50	32.0 %		
Combined years	(2001 + 2002)				
Early	(July 15-25)	99	15.1 %		
				11.8	< 0.005
Late	(Aug 4-20)	99	31.0 %		

Table 4. Comparison of gear type for sample bias [1]

Comparison	N	% infected	X^2	P
Gill nets (8.5 inch mesh)				
Set nets [2]	131	23.7	0.20	> 0.10
Drift nets [2]	55	25.4		
Gill net vs fish wheel				
Males				
Emmonak	109	23.8	0.96	> 0.10
Tanana	47	29.5		
Emmonak	109	23.8	0.92	> 0.10
Rapids	119	28.6		
Females				
Emmonak	84	26.2	0.001	> 0.95
Tanana	49	24.5		
Emmonak	84	26.2	2.07	> 0.10
Rapids	59	38.9		

1 Nets used exclusively at Emmonak & fish wheels used at Tanana and Rapids.
2 Sexes combined, Emmonak, 2002

Appendix I. Four-year summary of infection and disease prevalence in Yukon and Tanana River chinook salmon. (see attached file)

Appendix II. Examples of gross and microscopic lesions caused by *Ichthyophonus.* (see attached file)

Appendix III. *In vitro* culture and histology of *Ichthyophonus* in cardiac muscle. (see attached file)

Appendix IV. Dynamics of disease progression. (see attached file)

Appendix V. Other “conditions” observed in Yukon chinook (1999 – 2002). (see attached file)

Appendix VI. Sampling spawn-outs on the Chena & Salcha Rivers. (see attached file)

Appendix VII. Bibliography of *Ichthyophonus* literature. (see attached file)

The U.S. Fish and Wildlife Service, Office of Subsistence Management conducts all programs and activities free from discrimination on the basis of sex, color, race, religion, national origin, age, marital status, pregnancy, parenthood, or disability. For information on alternative formats available for this publication please contact the Office of Subsistence Management to make necessary arrangements. Any person who believes she or he has been discriminated against should write to: Office of Subsistence Management, 3601 C Street, Suite 1030, Anchorage, AK 99503; or O.E.O., U.S. Department of Interior, Washington, D.C. 20240.