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Ichthyophoniasis: An Emerging Disease of Chinook Salmon in the Yukon River

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Abstract.—Before 1985, *Ichthyophonus* was unreported among Pacific salmon *Oncorhynchus* spp. from the Yukon River; now it infects more than 40% of returning adult Chinook salmon *O. tshawytscha*. Overall infection prevalence reached about 45% in the Yukon River and about 30% in the Tanana River between 1999 and 2003. Mean infection prevalence was greater in females than males in the main-stem Yukon River during each of the 5 years of the study, but the infection prevalence in males increased each year until the difference was no longer significant. Clinical signs of ichthyophoniasis (presence of visible punctate white lesions in internal organs) were least at the mouth of the Yukon River (~10%) but increased to 29% when fish reached the middle Yukon River and was 22% at the upper Tanana River. However, clinical signs increased each year from 7% in 1999 to 27% in 2003 at the mouth of the river. As fish approached the upper reaches of the Yukon River (Canada) and the spawning areas of the Chena and Salcha rivers (Alaska), infection prevalence dropped significantly to less than 15% in females on the Yukon River and less than 10% for both sexes in the Chena and Salcha rivers, presumably because of mortality among infected prespawn fish. Age was not a factor in infection prevalence, nor was the position of fish within the run. The source of infection was not determined, but *Ichthyophonus* was not found in 400 Pacific herring *Clupea pallasi* from the Bering Sea or in 120 outmigrating juvenile Chinook salmon from two drainages in Alaska and Canada. Freshwater burbot *Lota lota* from the middle Yukon River were subclinically infected with *Ichthyophonus*, but the origin and relationship of this agent to the Chinook salmon isolate is unknown.

In the mid-1980s, subsistence fishermen along the middle Yukon River first reported an unusual condition in a few Chinook salmon *Oncorhynchus tshawytscha*. The fish smelled mildly ''fruity,'' did not dry properly, and had white spots on their heart, liver, and skeletal muscle. After observing the condition in increasing numbers of fish for several years, the U.S. Fish and Wildlife Service (USFWS) sent tissue samples to the Alaska Department of Fish and Game (ADFG) and to the USFWS Bozeman Fish Technology Center for analysis. After histologic evaluation, both laboratories returned a diagnosis of *Ichthyophonus* sp. Initially, reports indicated that fish caught in late June and early July (early in the run) were free of lesions, but by mid- to late July (late in the run) the fish were severely affected. More recently, however, fish from all parts of the run exhibited visible lesions and disease severity varied from year to year. Fish processors from the middle Yukon River reported as many as 20% of purchased fish were discarded because of muscle tissue damage caused by *Ichthyophonus* (Interior Alaska Fish Processors, Fairbanks, personal communication). This level of disease is consistent with reports of other fish species infected with *Ichthyophonus* (McVicar and Mackenzie 1972; Rahimian 1998).

Adult Chinook salmon enter the Yukon River from the end of May through mid-July, the migration peaking during the third week of June. During their freshwater migration, portions of the population divert to tributaries of the Yukon River. By sampling fish as they moved upriver, we were able to monitor changes in infection and disease within the same population over time, as well as determine whether fish leaving the main-stem Yu-

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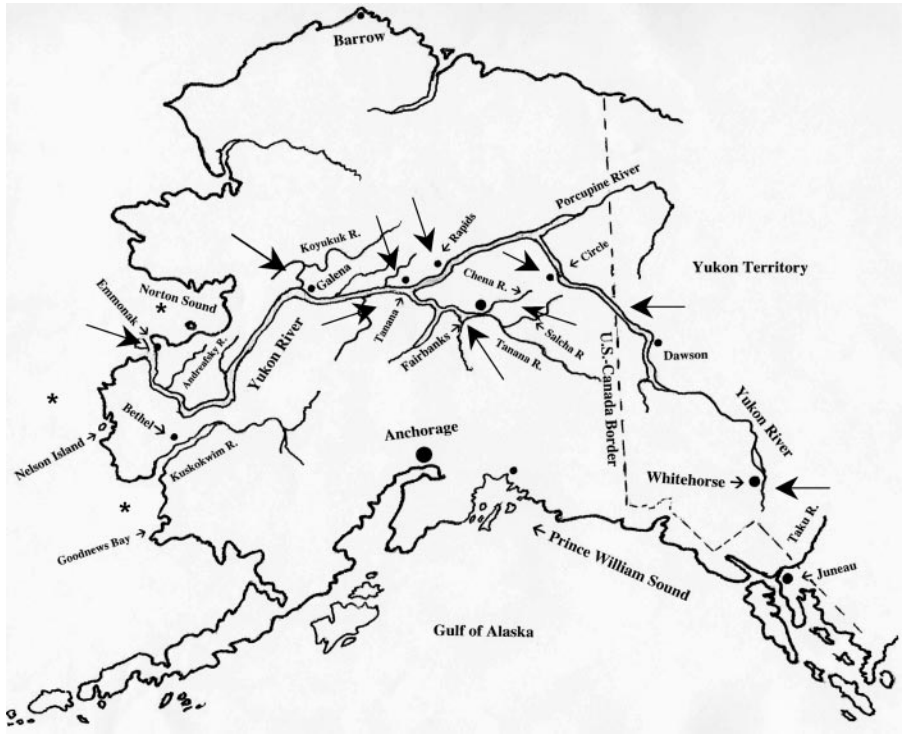

FIGURE 1.—Map of study area showing Chinook salmon sample sites along the Yukon and Tanana rivers (arrows) and Pacific herring sample sites in the Bering Sea (asterisks). See Table 1 for additional site data.

kon River had altered the prevalence of infection or disease in fish still en route to their spawning streams. During the course of the study, the same pattern of infection and disease was repeated each year at each sample site, 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.

The objectives of this study were to (1) conduct multiyear monitoring of Chinook salmon for *Ichthyophonus* over the length of the Yukon and Tanana rivers, (2) determine the fate of infected adult Chinook salmon along the length of the Yukon and Tanana rivers, and (3) identify the source of *Ichthyophonus* infections in Yukon River Chinook salmon.

Methods

Sample collection.—The study area consisted of three components: the Yukon River main stem, the Tanana River, and three terminal spawning areas. The Yukon River main stem extended from river mile (rm) 0 at the mouth of the river to rm 1,745 and was divided into three segments—lower (rm 0–600), middle (rm 600–1,200), and upper (rm 1,200–1,230) river—while the Tanana River was divided into lower (rm 695) and upper (rm 860–920) river segments (Figure 1). Fish were sampled each year from 1999 to 2003 from sites along the Yukon, Tanana, Chena, and Salcha rivers and the Whitehorse Rapids hatchery.

Fish were sampled with gill nets at the mouth of the river (rm 24) and with fish wheels at upriver sites. Sampling was conducted by staff from ADFG and USFWS and by various subsistence fishers. The number of samples collected from each site was determined by the availability of fish and the relative abundance of males and females.

The three terminal spawning sites included the hatchery at Whitehorse Rapids (Yukon Territory) at rm 1,745 and the Chena and Salcha rivers, which are tributaries of the Tanana River (Alaska). Whitehorse fish were obtained for broodstock from a fishway that bypasses the Whitehorse Rapids dam near the hatchery and were collected the last week of August. Chena and Salcha river postspawn fish were sampled every 2–3 d from terminal spawning sites from the last week of July through

the third week of August. The Chena River was sampled over a 12-rm reach, upstream of milepost 27 on the Chena Hot Springs road, whereas Salcha River samples were obtained from a 20-mi reach between 40 and 60 rm upstream of the Highway 2 bridge south of Fairbanks (Figure 1).

Sampling rationale.—On the basis of data from a 1999 pilot study, we treated returning Yukon River Chinook salmon as a single binomial population consisting of ''infected'' and ''uninfected'' individuals, assumingthat no additional transmission occurred en route and that fish remained infected throughout the run. These assumptions were based on evidence that (1) fish do not recover from *Ichthyophonus* infections and (2) *Ichthyophonus* is transmitted by ingestion of infected prey (McVicar and Mackenzie 1972; Slocombe 1980; McVicar 1982; Okamoto et al. 1987b; Sindermann and Chenoweth 1993). Because salmon do not eat during their freshwater spawning migration, samples taken along the river represent a time series that tracks the relative levels of infection and disease in migrating salmon, beginning when the fish enter the river and continuing until they spawn.

Parasite identification.—Fish were necropsied and examined for the presence of *Ichthyophonus* within 12 h of capture. Sex, length, and weight were recorded and heart, liver, spleen, kidney, and skeletal muscle were inspected visually (gross observation) for the presence of punctate white lesions (hereafter referred to as ''clinical signs'' or ''visible lesions''), indicative of *Ichthyophonus* infection. Tissues from each fish were cultured in vitro, currently the most sensitive method of detecting subclinical infections and obtaining accurate infection prevalence data (Rahimian and Thulin 1996; Kocan et al. 1999). The organism can be reliably isolated from infected fish tissues at ambient temperatures for as long as 4 d postmortem (our unpublished data).

Approximately 1 g of heart tissue was cultured in 5 mL of tris-buffered Eagle's minimum essential medium supplemented with 5% fetal bovine serum, 100 IU penicillin/mL, 100 μg streptomycin/mL, and 100 μg gentamycin/mL. Cultures were incubated at 12–15°C and examined microscopically for the presence of *Ichthyophonus* after 7–14 d in culture. Data from the cultured tissues were used to determine the total infection prevalence (clinical + subclinical); prevalence of clinical signs was determined by visual examination of internal organs for visible lesions. Because of logistical constraints, more fish were visually examined than were cultured at Rampart and Circle, whereas at the U.S.-–Canada border more fish were cultured than were visually evaluated for clinical signs. The growth of *Ichthyophonus* in culture was used to confirm visual diagnoses (Okamoto et al. 1985; Spanggaard et al. 1994; Rahimian and Thulin 1996; Kocan et al. 1999) and the data obtained were used for all subsequent calculations.

Only fish confirmed to be infected with *Ichthyophonus* by in vitro culture or (occasionally) by histopathologic evaluation were classified as ''infected.'' Infections in fish were classified as ''subclinical'' when fish were confirmed to be infected with *Ichthyophonus*, but the tissues showed no visible lesions. Fish were classified as ''clinical'' when visible white punctate lesions were observed in at least one internal organ and were confirmed by in vitro culture to be infected with *Ichthyophonus*. The most severe form of clinical infection resulted in ''disseminated disease'' when white punctate lesions were present in multiple internal organs. Fish were classified as ''negative'' if *Ichthyophonus* infection could not be confirmed by in vitro culture or by histopathologic observation. Because no microbiological assay is 100% accurate, some samples from fish having very low levels of infection probably gave false negatives. Thus, the data presented here represent minimum infection prevalences.

Tests for bias.—Sources of potential bias were identified as different types of capture gear (gill net and fish wheel); location of the fish within a pulse (e.g., early or late in the run); fish migrating nearshore versus those offshore; and size and age differences in infection prevalence.

We evaluated whether bias existed between samples obtained with gill nets versus those obtained with fish wheels. Because terrain and river conditions in the Yukon Delta preclude the use of fish wheels, we used gill nets exclusively at the mouth of the river und used fish wheels more extensively in the middle and upper river. Infection prevalence in fish captured by gill nets at the mouth of the river was compared with that in fish captured by fish wheels between 570 and 730 mi upriver. Fish wheels usually target Chinook salmon between 6.1 and 15.3 m offshore at depths of 1.5−6.1 m and have historically caught proportionally more and smaller males than gill nets because the 8.5-in (21.6-cm)-mesh gill net used at the mouth of the river allows smaller fish to pass through without being caught (Yukon River fishermen, personal communication).

To evaluate potential differences in early- and late-caught fish, we compared infection prevalence

in the first 60 fish caught at rm 24 from June 17 to June 19, 2002, with the prevalence in the last 60 fish caught from June 27 to 30, 2002.

To evaluate nearshore versus offshore bias, we compared samples from two types of gill-net sets (8.5-in mesh), sampling fish at rm 24 from June 17 to June 30, 2002, using set nets (nearshore) and drift nets (offshore).

To determine whether infected and uninfected fish segregate temporally during their spawning migration, we compared infection prevalence, clinical signs, and disseminated disease in fish sampled early (June 30−July 4) and late (July 17–21) in the run as the migration pulse passed rm 730 in 2001 and 2002. These dates represent the first and last 5 d of the pulse. Pulse timing, determined from catch per unit effort at each sample station, could be estimated between stations because the migration speed of the fish is known.

To address the possibility that infection prevalence may be biased by size (i.e., 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 (0.5 to 11.8 kg) or more (348 males and 240 females). The assumption was that the largest fish were older than the smallest fish and accordingly, if age influenced infection prevalence, then the smallest fish would exhibit significantly different infection prevalence than the largest fish. 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 greater in older (larger) salmon.

We also had to determine whether there was sample bias between samples taken from live postspawn fish and those from fish determined to be dead less than 48 h. In prior pilot studies (our unpublished data), freshly spawned Chinook salmon were euthanatized and held in flowing 12°C water; the dead fish were examined for decomposition changes for 96 h. Until 48 h postmortem the eyes remained clear and the cardiac muscle was firm and normally colored. After 48 h the eyes became cloudy and the heart became discolored and flaccid, the result of autolysis of the endocardium. No fish dead longer than 48 h were used. Other pilot studies also demonstrated that *Ichthyophonus* could be successfully isolated from dead fish held at 12°C for 4 d. Spawn-outs were sampled by simultaneous drift boat and beach surveys every 2–3 d from the last week of July through mid-August in 2001 and 2002, covering the majority of the spawning activity. In 2003, severe flooding of the Chena and Salcha rivers during late July and early August restricted sampling to the third week of August, the end of the spawning period.

Non-Yukon River salmon.—In 2001, hearts from 20 adult Chinook salmon from the lower Kuskokwim River were examined visually and by in vitro culture for the presence of *Ichthyophonus*. An additional 56 adult Chinook salmon hearts were collected from fish in the Taku River (in southeastern Alaska near the Canadian border) and evaluated by in vitro culture only. Sixty-five juvenile Chinook salmon were captured from the Chena River in April 2002 and 57 from the Klondike River in October 2002. All were examined for the presence of *Ichthyophonus* by in vitro culture.

For comparison with Yukon River fish, 120 Chinook salmon (60 per year) from the University of Washington hatchery were sampled from 2000 to 2001, and an additional 60 fish were similarly examined in 2000 from the Big Beef Creek Field Research Station on Hood Canal, Washington.

Nonsalmonids.—Pacific herring *Clupea pallasi* were sampled 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 and by in vitro culture.

In 2002, seven nonsalmonid species from the Yukon River were examined for the presence of *Ichthyophonus* by visual examination and in vitro culture: 6 sheefish *Stenodus leucichthys*, 3 broad whitefish *Coregonus nasus*, 11 humpback whitefish *C. pidschian*, 5 ciscoes *C. artedi*, 6 burbot *Lota lota*, 6 northern pike *Esox lucius*, and 9 grayling *Thymallus arcticus*.

Statistical analysis.—Chinook salmon entering the Yukon River were treated as a single binomial population consisting of infected and uninfected individuals, recognizing that the population is a mix of multiple subpopulations that ultimately segregate into spawning tributaries as the metapopulation migrates upriver. Within this population, we defined various groups, for example, males versus females, upper versus lower Yukon River, Yukon River versus Tanana River, and Tanana River versus Chena−Salcha rivers. Having established a null hypothesis (H_0) stating that there was no difference between groups, we evaluated the groups using a 2×2 chi-square test with one degree of freedom (Witts 1964; Colton 1974; Leaverton 1978; Gordis 2000).

Results

From 1999 to 2003, a total of 3,327 adult Chinook salmon were sampled from the lower, middle,

TABLE 1.—Sample sites, dates, and total Chinook salmon sampled from 1999 to 2003.

Nearest town	River miles[a]	Dates sampled	Years sampled	Number sampled		
				Males	Females	Total
Yukon River						
Emmonak	24	Jun 17–30	1999–2003	305	238	543
Galena	530	Jul 01–03	2000	50	18	68
Rampart Rapids	730	Jun 30–Jul 21	1999–2003	527	318	845[b]
Circle	1,080	Jul 13–19	2000	122	55	177[b]
U.S.–Canada border[c]	1,220	Jul 13–Aug 10	2000–2002	261	205	466[b]
Whitehorse[d]	1,745	Aug 19–Sep 2	2000–2003	106	94	200[b]
Total				1,371	928	2,299
Tanana River						
Tanana	695	Jul 9–12	2001–2003	190	137	327
Nenana–Fairbanks	860–920	Jul 9–25	2000–2003	201	115	316
Total				391	252	643
Chena River[e]	980	Jul 26–Aug 13	2001–2003	125	100	225
Salcha River[e]	1,025	Jul 28–Aug 14	2002–2003	99	71	170
Total				224	161	385
Grand total						3,327

[a] From the mouth of the Yukon River.
[b] More fish were visually examined than were cultured at Rampart and Circle, and more fish were cultured than visually examined at the U.S.–Canada border and Whitehorse.
[c] Samples from Department of Fisheries and Oceans Canada test fishery at U.S.–Canada border.
[d] Sampled from Whitehorse hatchery.
[e] Spawn-outs sampled during Alaska Department of Fish and Game annual carcass counts.

and upper Yukon River as well as from three terminal spawning sites (Table 1).

Characteristics of the Infection

Infection in Yukon River Chinook salmon.—A repetitive pattern of clinical and subclinical infection occurred each year when fish were sequentially sampled over time as they progressed up the Yukon River during their freshwater spawning migration. From 1999 to 2003, overall infection prevalence in females ranged from 24% to 40%, whereas the prevalence in males increased annually from 20.0% in 1999 to 34.0% in 2003. The infection prevalence in the Yukon River main stem for both sexes over the 5-year study was 29.8 ± 5.71% (mean ± SD; Figure 2).

When fish entered the Yukon River, both sexes were infected. When the run reached the middle Yukon River between rm 730–1,230, infection prevalence peaked at 34.0% for males and 42.8% for females. At rm 1,745 (Whitehorse Rapids), infection prevalence declined to 17.1 ± 5.3% for males and 15.4 ± 9.2% for females (Figure 3). Although infection prevalence in the Yukon main stem (excluding Whitehorse) was greater in females each year, the difference was significant in 1999 and 2000 but only marginally significant in 2001. The percent of infected males increased each year until infection prevalence in males approached that in females by 2003 (Figure 2). Even with the increasing infection prevalence in males, the combined data for all 5 years revealed significantly more infected females (34.0 ± 1.78%) than males (27.7 ± 1.32%) along the Yukon River main stem ($\chi^2 = 15.1$; $n = 1{,}918$, $P < 0.001$; Table 2).

Infection in Tanana River Chinook salmon.—The mean infection prevalence for Tanana River fish from 2000 to 2003 was 27.9% ± 2.3% for males and 31.0% ± 2.9% for females. Infection prevalence was not different between the lower and the upper Tanana River fish ($X^2 = 1.71$; $n = 643$, $P = 0.19$), so they were treated as a single population for statistical purposes. Unlike fish from the Yukon River main stem, Tanana River males and females exhibited no difference in infection prevalence ($X^2 = 0.82$; $n = 643$, $P = 0.36$; Table 2).

Plotting the infection prevalence in Tanana River fish from their entry into the Yukon River at Emmonak until they reached their natal spawning steams on the Chena and Salcha rivers, yielded a pattern similar to that observed in the Yukon River main stem, showing a decrease in the proportion of infected postspawn fish (Figure 4).

Clinical signs.—From 1999 to 2001, the mean prevalence of clinical infections (visible lesions) in fish entering the Yukon River ranged from 2% to 8%. In 2002, the prevalence of clinical signs increased to 11.4%, then increased again in 2003 to 29.3%. During this same period clinical infection remained constant in fish sampled from the

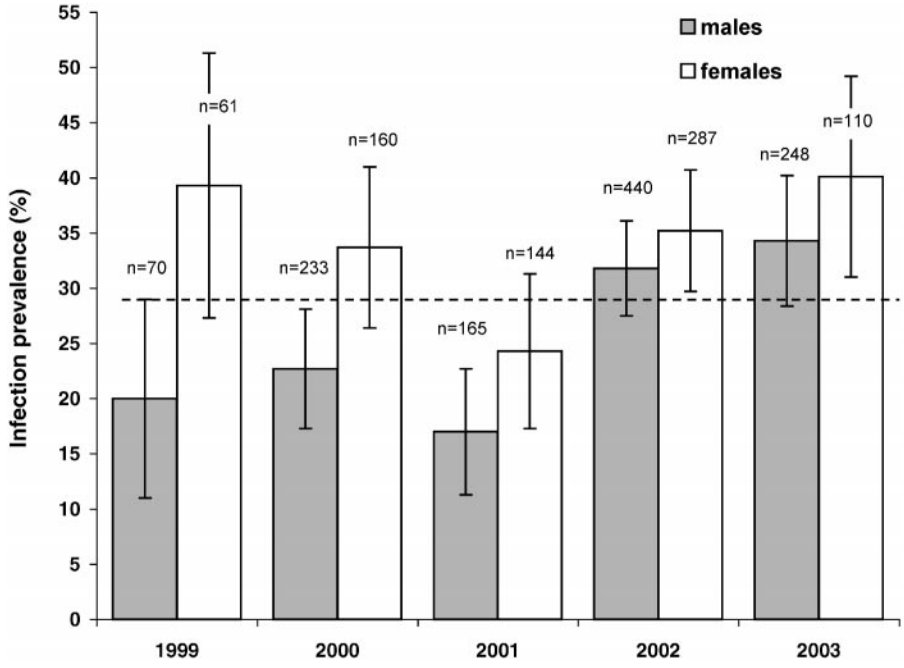

FIGURE 2.—*Ichthyophonus* infection prevalence, confirmed by in vitro culture, in Yukon River main-stem Chinook salmon (excluding Whitehorse) from 1999 to 2003. Bars = 95% confidence intervals; the dashed line = the mean 5-year infection prevalence.

middle Yukon River, rising slightly in 2003 (Figure 5). Each year the prevalence of clinical infections approached total infection levels as the fish passed rm 1,080–1,230, after which it declined to a low of 11.1% and 12.9% for males and females, respectively, at the Whitehorse Rapids Hatchery, similar to the decline observed for infection prevalence (Figure 3). A similar pattern of declining clinical infections was also observed in postspawn fish from the Tanana River (Figure 4).

Disseminated disease.—A second pattern of disease progression emerged when fish from early and late in the run were compared. Although the prevalence of clinical and subclinical infection in fish from early and late in the run were not significantly different in 2001 ($\chi^2 = 0.68$; $n = 419$, $P = 0.41$), we observed a significant difference in the proportion of fish with disseminated disease. None of the fish sampled early in the run exhibited visible lesions in any organ other than the heart, whereas 50% of males and 90% of females with clinical infections from late in the run presented with lesions in multiple organs. The heart and spleen were the primary target organs, followed by the kidney, liver, and finally the skeletal muscle.

As fish migrated upriver, *Ichthyophonus* also became more disseminated, ultimately spreading to the skeletal muscle. Only 6.8% of the fish sampled at rm 24 in 2001 and 2002 had detectable parasites in skeletal muscle, but when they reached the U.S.–Canada border (rm 1,230) muscle biopsies revealed that 10–20% of fish from early in the run had infected skeletal muscle. This incidence increased significantly to 27–32% in fish from late in the run ($\chi^2 = 8.18$; $n = 198$, $P < 0.001$; Table 3).

Terminal Spawning Sites

From 2000 to 2003, Chinook salmon broodstock were sampled from the Whitehorse Rapids Hatchery (rm 1,745) in late August. These fish were obtained earlier from a fishway that diverts fish around a dam just upriver from the hatchery.

Clinical and subclinical infection prevalence for males was 9.5 ± 3.49% and 17.0 ± 4.50%, respectively, whereas prevalences in females were 11.0 ± 3.47% and 15.9 ± 7.42%. A comparison of infection prevalence in Whitehorse males with that of males from the two previous downriver sites indicated no significant difference ($\chi^2 = 2.5$; $n = 410$, $P = 0.11$). However, infection prevalence in females was significantly lower at Whitehorse than at the two downriver sample sites ($\chi^2 = 16.4$; $n = 334$, $P < 0.0001$; Figure 3).

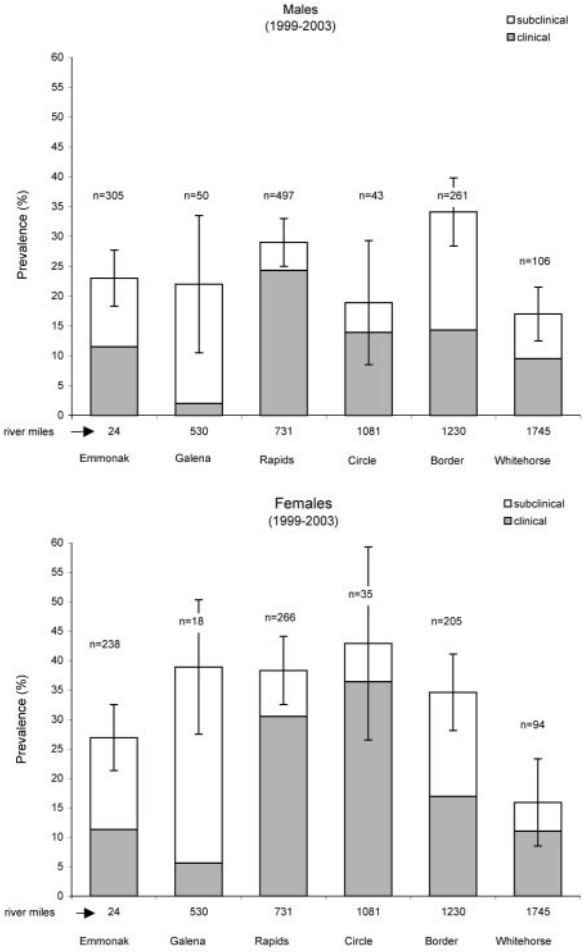

FIGURE 3.—Progression of clinical and subclinical infection in Yukon River Chinook salmon from the river mouth to Whitehorse from 1999 to 2003. All infections were confirmed by in vitro culture; bars = SDs.

TABLE 2.—Comparison of *Ichthyophonus*-infected males and females from the main-stem Yukon and main-stem Tanana rivers from 1999 to 2003.

	Males			Females				
Year	*n*	No. positive	%	*n*	No. positive	%	χ^2	*P*
Yukon River main-stem								
1999	70	14	20.0	61	24	39.3	5.16	0.008
2000	233	53	23.2	160	54	33.8	5.86	0.015
2001	165	28	16.9	144	35	24.3	2.56	0.08
2002	440	140	31.8	287	101	35.2	2.47	0.12
2003	248	85	34.3	110	45	40.9	2.03	0.15
Total	1,156	320	27.7	762	259	34.0	15.1	<0.001
Tanana River main-stem								
2000	48	9	18.7	11	2	18.2	0.215	0.724
2001	74	18	24.3	39	12	30.8	0.925	0.336
2002	133	48	36.1	116	32	27.6	1.68	0.195
2003	136	34	25.0	86	32	37.2	4.36	0.037
Total	391	109	27.9	252	78	31.0	0.82	0.364

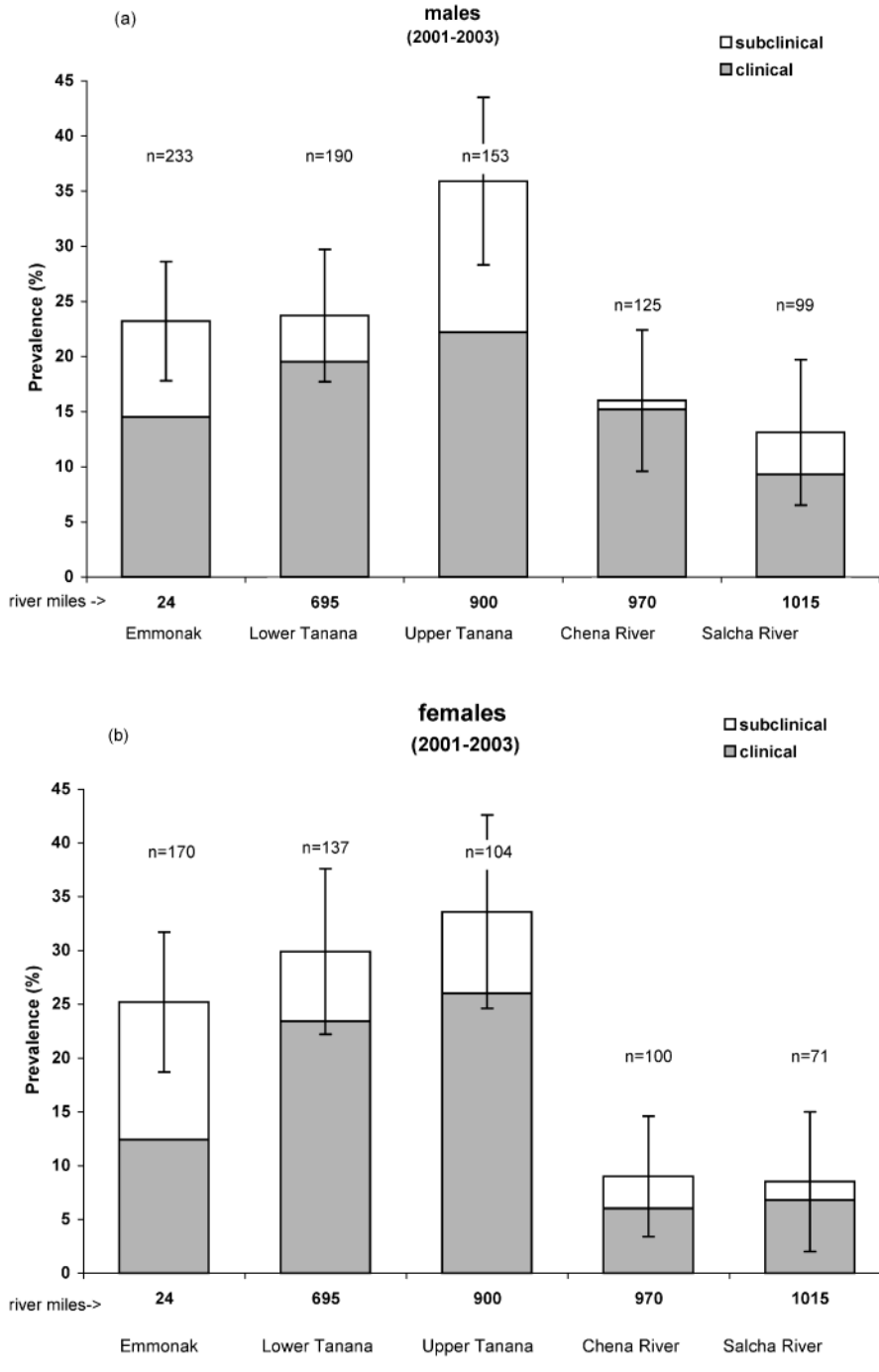

FIGURE 4.—Progression of clinical and subclinical *Ichthyophonus* infection from 2001 to 2003 in Tanana River Chinook salmon from the mouth of the Yukon River to the spawning reaches of the Chena and Salcha rivers. All infections were confirmed by in vitro culture; bars = SDs.

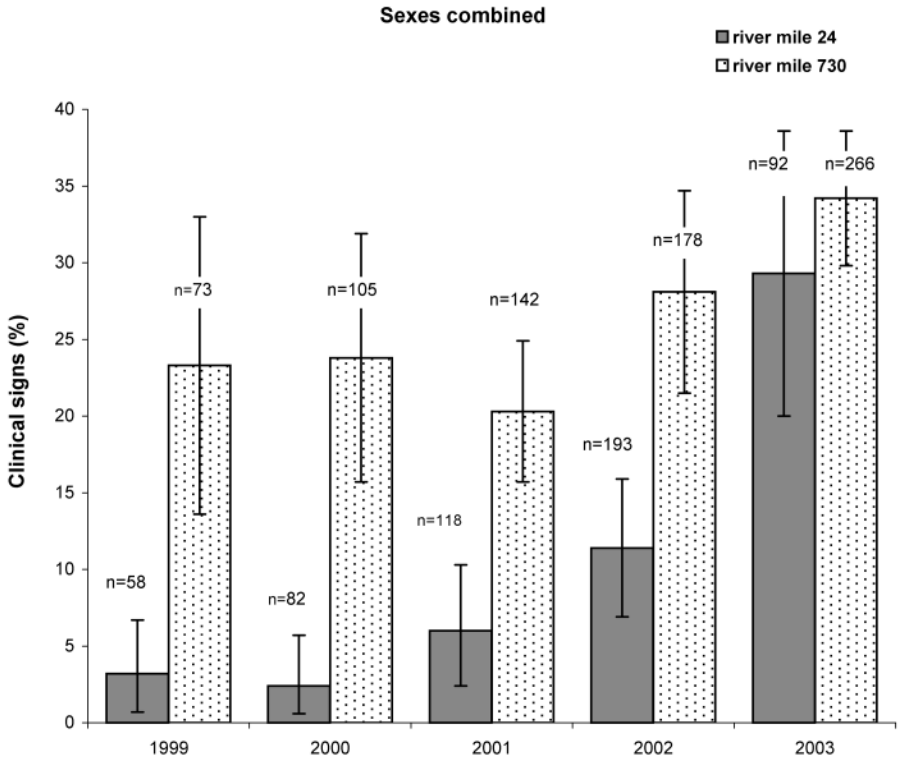

FIGURE 5.—Prevalence of clinical ichthyophoniasis (visible punctate white lesions) from 1999 to 2003 in Chinook salmon at the mouth of the Yukon River (rm 24) and at rm 730. Bars = 95% confidence intervals.

TABLE 3.—Infection prevalence in Chinook salmon skeletal muscle from fish at the mouth of the Yukon River and from early- and late-run fish at the U.S.–Canada border.

Sample site and year	Sample dates	*n*	% positive
Emmonak[a]			
2001	Jun 19–22	79	6.3
2002	Jun 17–30	186	6.9
2001 + 2002		265	6.8
U.S.–Canada border			
2001			
Early	Jul 15–18	50	10.0
Late	Aug 8–12	49	27.1
2002			
Early	July 20–25	49	20.4
Late	Aug 4–20	50	32.0
2001–2002			
Early[b]	Jul 15–25	99	15.1
Late	Aug 4–20	99	31.0

[a] Samples taken after the peak of the run (e.g., late-run fish).
[b] $\chi^2 = 8.2$, $P < 0.001$ for July versus August fish.

From 2001 through 2003, more than 800 postspawn Chinook salmon were sampled from the Chena and Salcha rivers. Of these, 413 (~52%) had not decomposed beyond the point from which meaningful data could be obtained (e.g., more than 48 h postmortem). The infection prevalence for Chena River spawn-outs in 2001 was 15.4% for males ($n = 39$) and 0% for females ($n = 30$), whereas the infection prevalence in 2002 was 20.0% for males ($n = 60$) and 13.5% for females ($n = 52$). In 2003, the infection prevalence was 11.5% for males ($n = 26$) and 11.1% for females ($n = 18$). For all years combined, infection prevalence in Chena River fish was 18.2% for males ($n = 125$) and 8.5% for females ($n = 99$) (Figure 4).

Infection prevalence in Salcha River fish was 10.5% in males ($n = 85$) and 6.8% in females ($n = 59$) in 2002; by 2003 the prevalence was 28.6% in males ($n = 14$) and 8.3% in females ($n = 12$). No significant difference between Chena River and Salcha River fish was detected (males: $\chi^2 = 3.44$; $n = 224$, $P = 0.06$; females: $\chi^2 = 0.08$; $n = 171$, $P = 0.78$). Overall infection in Chena and Salcha

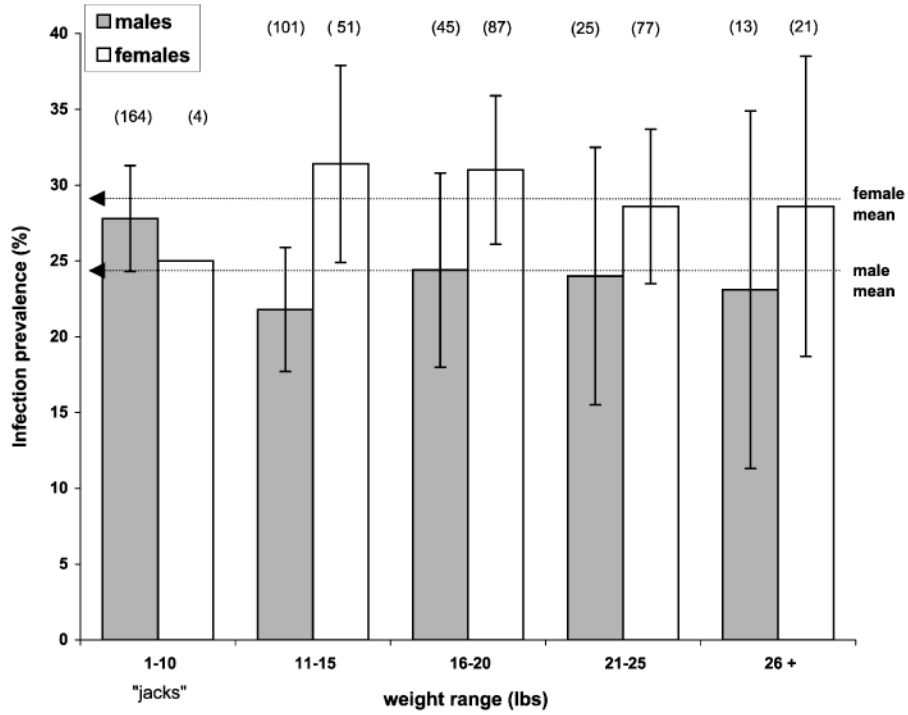

FIGURE 6.—*Ichthyophonus* infection prevalence relative to weight (age) in Chinook salmon from the Yukon River main stem in 2002. Bars = 95% confidence intervals.

River fish for all years was 14.6% for males and 7.8% for females. Compared with the same population of prespawn fish sampled from the Tanana River, the number of infected postspawn fish of both sexes in both the Chena and Salcha rivers was significantly lower ($\chi^2 = 33.8$; $n = 979$, $P < 0.0001$; Figure 4).

Tests for Bias

Nearshore versus offshore sampling.—No difference in infection prevalence was detected between salmon caught nearshore with set nets (23.7%; $n = 131$) and those caught offshore with drift nets (25.4%; $n = 55$) in the lower Yukon River ($\chi^2 = 0.20$; $P > 0.10$). We therefore treated these two groups as one population and compared them with upriver samples collected with fish wheels.

Gill nets versus fish wheels.—No difference in infection prevalence was detected between fish caught by gill net at Emmonak (25.0%, $n = 193$) and those caught with fish wheels at rm 569 (mouth of the Tanana River: 27.0%, $n = 96$, $P > 0.05$) and rm 730 (Rampart Rapids: 33.7%, $n = 178$, $P > 0.05$), confirming that gear type did not bias infection prevalence data. This also confirmed that infection prevalence did not change between the lower and middle Yukon.

Early versus late run.—No difference in infection prevalence was demonstrated between the first 60 fish caught with gill nets at Emmonak on June 17–19 and the last 60 fish caught on June 27–30 ($\chi^2 = 0.73$; $n = 120$, $P = 0.39$). There was also no difference in infection prevalence between fish sampled by fish wheels from early in the run at rm 730 (June 30–July 4, 2001) compared with fish from late in the run (July 17–21, 2001; males: $\chi^2 = 0.28$, $n = 177$; females: $\chi^2 = 0.28$, $n = 63$; $P = 0.60$). This relationship was repeated the following year (2002) when fish from the same location were sampled on July 1–7 and July 9–15 (males: $\chi^2 = 0.12$, $n = 133$; females: $\chi^2 = 0.95$, $n = 58$; $P = 0.31$).

Size (i.e., age) versus infection prevalence.—We observed no difference in infection prevalence relative to weight/age among 348 males and 240 females from the lower and middle Yukon River. The weight groups observed ranged from 1 lb. to 26 lb. or more (Figure 6).

Infection in Other Fish

Non-Yukon River salmon.—*Ichthyophonus* was identified in 3 of 20 (15%) adult Chinook salmon

from the Kuskokwim River near Bethel, Alaska, and from 13 of 56 (23.2%) Chinook salmon from the Taku River in southeast Alaska. None of the juveniles from the Chena or Klondike rivers and none of the 180 Chinook salmon from Puget Sound were positive for *Ichthyophonus*.

Nonsalmonid species.—*Ichthyophonus* was not detected in 400 Pacific herring examined from three areas of the Bering Sea each year from 2000 to 2002 (Norton Sound, Goodnews Bay, and Nelson Island; see Figure 1).

Of seven nonsalmonid species collected from the Yukon River, only burbot were infected with *Ichthyophonus* (1/4 males, 1/2 females). *Ichthyophonus* was not seen in six other species examined, but broad and humpback whitefish and sheefish frequently exhibited raised punctate white spots on their hearts, superficially resembling *Ichthyophonus*. Explant cultures and histologic examination of cardiac tissue from these fish confirmed that the organism was not *Ichthyophonus*; however, the organism was not further identified.

Discussion

Before the mid-1980s ichthyophoniasis was unknown in Pacific salmon and had not been reported from any species from the Yukon River. Since then, the disease has emerged to become firmly established in adult Yukon River Chinook salmon, increasing to levels that impact subsistence and commercial fishing, as well as the resource itself. We do not know whether *Ichthyophonus* is a recently introduced pathogen to the Yukon River drainage or has been present historically but is only now emerging as a disease entity because of changing conditions in the Bering Sea, the Yukon River, or both. If *Ichthyophonus* is responsible for significant prespawn mortality, as the data suggest, this represents a case of a parasite limiting the reproductive success of a host species.

Clinical and Subclinical Infections

Chinook salmon entered the Yukon River with both clinical and subclinical infections that exhibited the same pattern of development in main-stem Yukon River Chinook salmon each year from 1999 to 2003. The percent of infected males remained unchanged from the river mouth to Whitehorse, Yukon Territory, whereas the percentage of infected females was significantly less by the time they reached Whitehorse (Figure 3).

Clinical infections (visible lesions) exhibited a more complex pattern of development. The annual pattern was a major conversion of subclinical to clinical cases between the lower and middle Yukon, exceeding 25% in males and 35% in females in the middle Yukon. A second pattern of clinical infection was an annual increase in visible lesions at the mouth of the river, beginning with about 5% in 1999 and increasing annually to 27% in 2003. Because no change in overall infection prevalence occurred in the middle Yukon River during the same 5-year period (Figure 5), clinical disease appeared earlier each year.

The observed patterns of clinical and subclinical infection observed among and within years were predictable based on known developmental patterns of similar infectious agents. The stable infection prevalence over the first 1,200 river miles indicates that fish become infected before entering the Yukon River and is consistent with experimental evidence that *Ichthyophonus*-infected fish develop visible lesions about 30 d after exposure (Kocan et al. 1999; Jones and Dawe 2002). The initial low numbers of fish with visible lesions in the lower Yukon River probably represent newly acquired, subclinical infections that have not yet progressed to visible lesions. The higher water temperatures in the Yukon River in late June and early July (18–20°C) might influence parasite proliferation and thus lead to an increase in clinical infections while fish are en route. These data are consistent with reports that *Ichthyophonus* is most pathogenic at temperatures at and above 15°C (Okamoto et al. 1987a; Company et al. 1999; Halpenny et al. 2002) and that the parasite proliferates more rapidly at higher temperatures (Sitja-Bobadilla and Alvarez-Pellitero 1990; Spanggaard and Huss 1996). The regular increase in visible lesions observed at the mouth of the river from 1999 through 2003 may be the result of changing ocean conditions in the Bering Sea or Yukon Delta.

Disseminated Disease

Although the clinical and subclinical infections in fish from early and late in the run were similar, early- and late-run fish exhibited a dramatic difference in the extent of dissemination of the organism throughout fish tissues. Only 6.8% of fish sampled at Emmonak had identifiable *Ichthyophonus* in their skeletal muscle in 2001 and 2002. In contrast, fish sampled early in the run from the middle Yukon River exhibited visible lesions primarily in the heart, whereas fish from late in the run had multiple infected organs, including skeletal muscle. This finding was further confirmed by muscle biopsy data collected by the Department of Fisheries and Oceans Canada near the U.S,–

Canada border during 2001 and 2002. These data revealed a significantly lower prevalence of infected skeletal muscle in mid-July (10–20%) than in mid-August (27–32%) (Table 3). These data convincingly demonstrate that *Ichthyophonus* became more disseminated as the fish migrate upriver and that dissemination of the parasite to skeletal muscle was significantly higher in fish from late in the run. These observations are consistent with earlier reports by fishers and processors who reported that a large proportion of fish caught late in the run had to be discarded because of *Ichthyophonus* in the fillets.

Terminal Spawning Areas

Significantly fewer clinically and subclinically infected female Chinook salmon were observed among fish sampled from the Whitehorse Rapids hatchery (rm 1,745), where they were intercepted from a fishway as they approached their upriver spawning sites. Mean infection prevalence in females from 2000 to 2003 decreased from a high of 35–40% between the middle Yukon River (rm 731–1,230) to 15% or less at Whitehorse (rm 1,745). A similar but nonsignificant decrease was also observed in males. Because the decline in infection and disease at Whitehorse was consistent from year to year, sampling error was ruled out as a probable explanation. However, numerous variables—such as the 500–1,000 mi between the middle Yukon River and Whitehorse, numerous spawning tributaries along this route, artificial selection of fish for broodstock, and residence under hatchery conditions—prevented establishing a convincing hypothesis to explain the missing fish. To test the hypothesis that infected fish were dropping out of the population as they approached their terminal spawning streams, we selected the Chena and Salcha rivers as alternative terminal spawning sites because the variables associated with the Whitehorse samples were not present. These tributaries are in the upper Tanana River drainage and an estimated 75–85% of Tanana River fish spawn in these two rivers, eliminating the possibility of a significant portion of the infected fish diverting to other streams. Only 70–100 mi. separate the last sample station on the Tanana River and the mouths of the Chena and Salcha rivers, no artificial selection occurs, hatchery residency is absent, and only postspawn fish were sampled. By eliminating these variables, we felt that the Chena and Salcha rivers were good models to explain the decrease in spawning females in the upper Yukon River at the Whitehorse Rapids.

Postspawn fish of both sexes from the Chena and Salcha rivers exhibited levels of visible lesions and total infection that were significantly lower than prespawn fish from the Tanana River (Figure 4). More than 60% of the infected Tanana River fish (20% of total fish) at rm 900 failed to appear on the spawning reaches of either the Chena or Salcha rivers. Although no direct evidence (e.g., dead prespawn fish) was found to confirm the fate of the missing infected upper Tanana River fish, the existing data lead us to hypothesize that the missing fish died en route.

This level of loss is similar to that reported for *Ichthyophonus* infections in Atlantic herring *Clupea harengus* in the North Atlantic (Sindermann and Chenoweth 1993), who hypothesized that the low prevalence of *Ichthyophonus* observed during and after an epizootic reflected high mortality among infected individuals. *Ichthyophonus* has been shown to cause substantial mortality in several species (Rucker 1953; Powles et al. 1968; McVicar 1981, 1982; Sindermann and Chenoweth 1993; Patterson 1996; Rahimian and Thulin 1996; Mellergaard and Spanggaard 1997). Surveys of herring (Scattergood 1948; Sindermann 1958; Sindermann and Chenoweth 1993) and yellowtail flounder *Pleuronectes ferrugineus* (Powles et al. 1968; Ruggieri et al. 1971) have shown that *Ichthyophonus* epizootics in the North Atlantic resulted in dramatic declines in the infection prevalence of *Ichthyophonus* in these species that were equal to, or exceeded, the decreases we observed in Yukon River Chinook salmon. These declines were attributed to the death of diseased fish, leaving a population of mostly uninfected individuals. Similarly, if Yukon River Chinook salmon are experiencing an epizootic of ichthyophoniasis en route to their natal spawning streams, then the low prevalence of infection and disease among postspawn fish may reflect mortality in fish before they reached their terminal spawning areas.

The significant decrease in prevalence of *Ichthyophonus*-infected fish from the Chena and Salcha rivers from 2001 to 2003 closely paralleled that seen in female broodstock from the Whitehorse Rapids Hatchery from 2000 to 2003, suggesting that the two phenomena are mechanistically related. Definitive proof of *Ichthyophonus*-related mortality would be the finding of infected dead prespawn fish with visible lesions. This would be difficult to obtain, however, because dead salmon sink rapidly and the visibility in the Yukon and Tanana Rivers is poor. Nonetheless, some experimental evidence does demonstrate that *Ichth-*

yophonus is lethal for Yukon River Chinook salmon (Jones and Dawe 2002).

Sex Difference in Infection

Although Yukon River main-stem females had consistently higher rates of infection and disease prevalence than males, no obvious cause for the difference could be identified. Initially, we thought that females spent more years at sea, thus increasing their probability of becoming infected. When no difference in infection prevalence was detected between small (young) and large (old) fish, however, this hypothesis was abandoned. An alternative 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 spend more time feeding just before entering the river, and if the site of infection is near the mouth of the Yukon River, 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 Yukon River migrants early in the run, whereas females predominate late in the run. Whatever the mechanism, the difference in infection prevalence between males and females in the Yukon main stem was consistent for all 5 years of the study. However, the increasing numbers of infected males in 2002 and 2003 may reflect a changing pattern in male–female exposure risk.

Unlike Yukon River fish, there was no significant difference in infection prevalence between the sexes in Tanana River fish. Infection prevalence in Tanana River females was, in fact, significantly lower than that of middle Yukon River females (rm 730–1,230), which had migrated a similar distance. The difference in infection and disease prevalence in Tanana River females compared with Yukon River fish might be explained if diseased fish begin to drop out of the population as they approach their natal streams. Essentially all of the fish entering the Tanana River were dark, the males having hooked jaws and the females having protruding ovipositors; in the Yukon River above the Tanana River, however, silver and ''blush'' fish predominate, with few morphological changes. At this point Tanana River fish have only 100–250 river miles to travel before reaching their natal streams, whereas Yukon River main-stem fish still have 575–1,050 river miles before reaching the spawning areas of the upper Yukon River. Tanana River fish would be expected to show a greater rate of mortality earlier than Yukon River main-stem fish if *Ichthyophonus*-related mortality were linked to proximity to spawning areas.

Source of Infection

There is no evidence as to where or when Yukon River Chinook salmon become infected. Herring were initially suspected as the source of infection, but after 3 years of sampling Bering Sea herring and finding no *Ichthyophonus*, this hypothesis had to be reevaluated. If herring are the source of infection, some portion of Yukon River Chinook salmon may feed south of the Aleutian Islands, where infected herring are known to occur. Although no infected herring were found north of the Aleutian Islands, all populations 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 Aleutians form a physical barrier to the spread of *Ichthyophonus* to Bering Sea herring. Alternatively, it is possible that another forage fish or invertebrate is the source of infection for Yukon River Chinook salmon in the Bering Sea.

The herring–Chinook salmon connection seems reasonable given the report of Criscione et al. (2002) that *Ichthyophonus* isolates from Yukon River Chinook salmon and Pacific herring are genetically indistinguishable. In addition, Jones and Dawe (2002) reported that juvenile Yukon River Chinook salmon (but not British Columbia Chinook salmon) succumbed to a Canadian herring isolate. Interestingly, we could not find *Ichthyophonus* in Chinook salmon from two watersheds in Puget Sound, nor could we find a reference to naturally occurring *Ichthyophonus* in any species of Pacific salmon, even though British Columbia and Puget Sound herring are infected at levels as high as 50–70% and constitute a major food item for Chinook salmon. Clearly, more studies are needed to resolve the issue of *Ichthyophonus* strain or species identity.

Another, but less probable, hypothesis for the source of infection is that Yukon River salmon become infected as juveniles in freshwater. However, examination of 120 out-migrating juvenile Chinook salmon from two widely separated Yukon River tributaries revealed no infected fish. However, *Ichthyophonus* was identified in 2/6 (33%) Yukon River burbot from the middle Yukon River (rm 730). Whether the parasite is endemic in burbot or they became infected after eating infected salmon tissue is unknown. Some freshwater species become infected and suffer high mortality af-

ter eating *Ichthyophonus*-infected fish, thus making introduction of the parasite into a freshwater drainage highly probable (Slocombe 1980; Chun and Kim 1981; Tung et al. 1986; Athanassopoulou 1992; Galuppi et al. 1994; Kocan et al. 1999; Heike and Wahl 2002). If the burbot is a new host for *Ichthyophonus*, it has the potential to become a source of infection for juvenile salmon and other nonsalmonid species in the Yukon River and its tributaries.

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