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Assessment of *Ichthyophonus* in Chinook Salmon within the Yukon River Drainage, 2004

by

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Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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ABSTRACT

Ichthyophonus hoferi is a parasitic organism infecting adult Chinook salmon (*Oncorhynchus tshawytscha*) in the Yukon River. This study aimed to assess potential effects of *Ichthyophonus* on reproductive success and prespawning mortality. Tissue samples were collected from adult Chinook salmon during migration from the Yukon River mouth in Emmonak, mid-river in Tanana, and clear water tributaries of the Chena and Salcha rivers more than 1,500 km from sea. Samples were collected by both lethal (heart) and minimally invasive techniques (muscle biopsy and blood sampling) in Emmonak and Tanana for testing by explant culture, histology, and Polymerase Chain Reaction (PCR) methods. Non-lethal sampling did not detect *Ichthyophonus* infection in Chinook salmon with the same accuracy as analysis of cardiac muscle. *Ichthyophonus* prevalence was 17.8% at Emmonak, 11.3% at Tanana, 36.1% at the Chena River, and 13.7% at the Salcha River in 2004 based on heart explants. Generally, prevalence did not differ between sexes, though prevalence was significantly higher in females compared to males at the Tanana site, which utilized shore based fishing gear. Spawning success was evaluated by internal examination using 3 criteria for expulsion of gametes: fully spawned, partially spawned, and unspawned. On both the Chena and Salcha rivers there was no significant difference between infected and uninfected Chinook salmon and spawning success. The use of radio tags was evaluated to track infected and uninfected salmon to their spawning grounds. However, *Ichthyophonus* in radiotagged fish was determined via non-lethal methods, thus only identifying a limited number of salmon infected with the parasite. Further, not enough tags were recovered on the spawning grounds to evaluate their ability to migrate with infections.

Key words: *Ichthyophonus,* Yukon River, Salcha River, Chena River, Chinook salmon, radiotelemetry.

INTRODUCTION

Ichthyophonus hoferi (referred to as *Ichthyophonus* for the remainder of the manuscript) is a protozoan parasite of marine and anadromous fishes with a global distribution (McVicar 1982; Woo and Bruno 1999). Though long believed to be taxonomically related to fungi, *Ichthyophonus* is currently integrated into Mesomycetozoea (Mendoza et al. 2002). This class is highly diverse and includes other difficult to categorize organisms sharing characteristics of both animals and fungi. *Ichthyophonus* has been of considerable economic concern to fishermen in Scandinavia, where epizootics have led to mass mortalities of herring (*Clupea harengus*) (Mellergaard and Spanggaard 1997; Rahimian 1998). However, die-offs and a 50% increase in *Ichthyophonus* prevalence have also affected Pacific herring (*Clupea pallasi*) (Kocan et al. 1999; Marty et al. 1998). Recently, Criscione et al. (2002) recognized two host-specific haplotypes of *Ichthyophonus*, with one haplotype infecting both herring and salmon species, thus raising concerns about potential *Ichthyophonus*-related mortalities in Chinook salmon.

In 1988, *Ichthyophonus* was first identified in Chinook salmon *Oncorhynchus tshawytscha* within the Yukon River drainage (Alaska Department of Fish and Game (ADF&G), Anchorage Fish Pathology Laboratory disease history database, June 1988). Ichthyophoniasis has since been described in a variety of fish species, including sockeye *O. nerka* and coho salmon *O. kisutch* (Gavryuseava 2007; Tierney and Farrell 2004). Since the initial discovery of *Ichthyophonus* in the Yukon River, both fishermen and fish processors have reported an increase in the number of Chinook salmon with nodular lesions in visceral organs and skeletal muscle, characteristic of ichthyophoniasis. Focal lesions typical for *Ichthyophonus* infections are observed throughout the entire run (Kocan et al. 2004a). However, early fish are thought to be relatively lesion-free in the upper Yukon River drainage, while migrating salmon are more severely affected later in the season (Kocan et al. 2004a). Processors in the upper Yukon River reported that as many as 20%

of purchased Chinook salmon are discarded because of muscle tissue damage (Kocan et al. 2004a). Fishermen in the upper Yukon River indicated that the severity of *Ichthyophonus* in Chinook salmon (or diseases with similar clinical appearance) is variable from year-to-year. Factors influencing temporal variation of prevalence are poorly understood. Rahimian (1998) described a passive stage (resting spores) of the parasite that is activated by mechanisms yet unknown. Stress (high cortisol) and increased water temperatures are known to accelerate *Ichthyophonus* infection (Halpenny et al. 2002; Okamoto et al. 1987; Perry et al. 2004) and prevalence varied seasonally and with age in Atlantic herring, with spring spawning fish being the most heavily affected (Rahimian and Thulin 1996).

Kocan et al. (2004a) initiated studies on the effects of *Ichthyophonus* on Chinook salmon in the Yukon River. These authors reported that approximately 25–30% of Chinook salmon entering the Yukon River are infected with *Ichthyophonus* and prevalence remains constant until fish reach the upper Yukon River, where it then drops to 10% or less. Further, only a few of the successfully spawned females are infected with *Ichthyophonus*, suggesting that females with ichthyophoniasis are dying prior to spawning (Kocan et al. 2004a). More recently, Kocan et al. (2006) showed that experimentally infected Chinook salmon suffer cardiac damage and reduced swimming stamina. This is in agreement with studies by Rahimian (1998), who described massive tissue necrosis and loss of function in infected organs. These initial findings by Kocan et al. (2004a; 2006) in Yukon River salmon could have widespread fisheries management implications, such as reduced Chinook salmon spawning success and consequently the potential need to adjust escapement goals, thus warranting further research.

The objectives of this study are to: 1) determine prevalence of *Ichthyophonus* in Chinook salmon along the Yukon River in 2004; 2) compare, and evaluate non-lethal tests and methodologies used to determine *Ichthyophonus* infection; 3) assess spawning success of infected fish and estimate pre-spawning mortality from the Tanana River to the Chena and Salcha river tributaries; 4) identify locations of potential disease mortality using radiotelemetry; 5) determine prevalence of *Ichthyophonus* in juvenile (freshwater) Chinook salmon and 6) investigate the potential correlation of water temperatures and prevalence of *Ichthyophonus* infection on pre-spawning mortality.

METHODS

In 2004, tissue samples of migrating Chinook salmon were collected at selected locations to monitor *Ichthyophonus* throughout the drainage (Figure 1). Samples were collected at the Yukon River mouth (Emmonak) to maintain and monitor an existing baseline of infection prevalence for fish entering the river (Kocan et al. 2004a). Sampling at upstream sites (Tanana, Chena, and Salcha rivers) was conducted to monitor changes to *Ichthyophonus* prevalence as described by Kocan et al. (2004a) and assess spawning success of *Ichthyophonus*-positive fish. Further, paired samples were collected for histology, culture, and Polymerase Chain Reaction (PCR) analysis (as described below) to compare methodologies in their accuracy and specificity to detect *Ichthyophonus*.

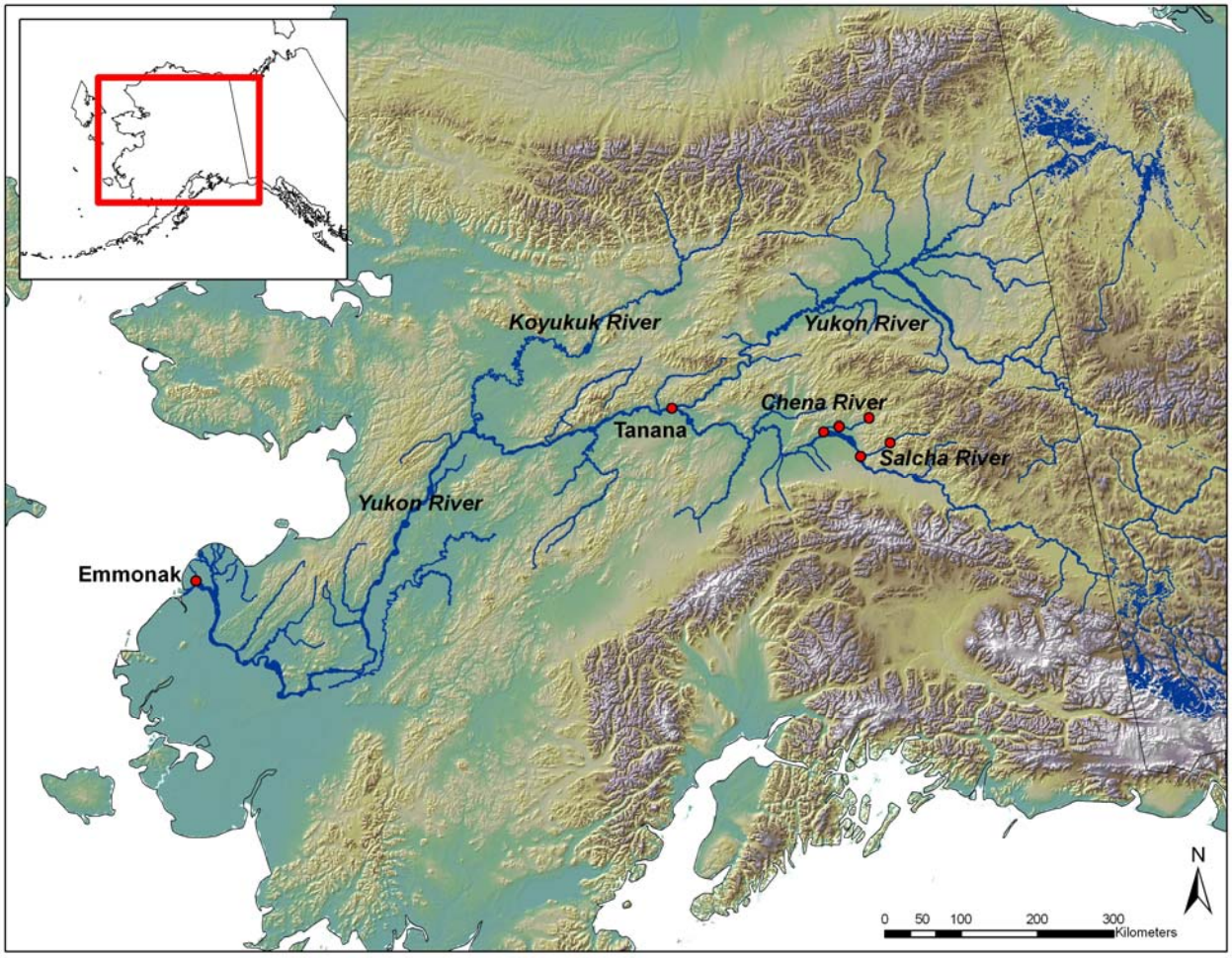


Figure 1.–Sites of tissue sample collection at the Yukon River mouth (Emmonak), confluence with the Tanana River, and escapements in the Chena and Salcha rivers, Alaska 2004.

FISH COLLECTION PROCEDURES

Yukon River Mouth-Emmonak

Emmonak is a fishing community located approximately 38 km inland on the south mouth of the Yukon River delta (Figure 1). This site was sampled to determine *Ichthyophonus* prevalence in adult Chinook salmon within mixed stocks in relation to their migratory timing and to compare results of this study with historical data sets (Kocan et al. 2004a). Paired lethal and non-lethal sampling procedures were compared for effectiveness of detection using explant culture, histology, and PCR tests to compare and determine sensitivity of each method in the lower river (Whipps et al. 2006).

Samples were collected from Chinook salmon harvested from ADF&G test fish catches in the lower Yukon River. Heart muscle was collected from freshly dead fish. Whole blood (3–5 ml) and a 0.5 g skeletal muscle punch biopsy (Miltex, 6 mm) were collected during non-lethal sampling. Muscle biopsy was taken from the left side of the fish mid-way between the lateral line and the posterior edge of the dorsal fin. Blood was sampled from the caudal vein into sodium heparin collection tubes and subsampled for different testing procedures as described below. Chinook salmon were sampled over

the course of the run from June 3 through July 15, 2004, with a sampling target of 105 fish (Appendix A1). Weekly sample sizes were based on 1980–2003 average run timing for the Emmonak test fishery.

Tanana Mouth

The Tanana River is the third largest tributary of the Yukon River with the confluence at approximately river kilometer 1,104 on the left bank. The community of Tanana is located on the right bank of the Yukon River mainstem (Figure 1). Chinook salmon are presumed to be bank orientated within the Yukon River, prior to entrance into the Tanana River, as has been noted for chum salmon (Buklis 1981). A test fish wheel project using video monitoring techniques on the mainstem Yukon River, located approximately 0.7 km below the Tanana confluence (Corbusier Slough), was the capture site for presumed Tanana-bound fish (Buklis 1981). The fish wheel was equipped with a live box and fish were sampled opportunistically throughout the day. Paired lethal and non-lethal sampling procedures were compared and used to monitor infection prevalence as fish migrate upriver to the spawning grounds in the Chena and Salcha rivers.

A second group of salmon was sampled and analyzed via non-lethal (muscle punch biopsy) PCR methods as described below. In addition, these fish were implanted with radio tags (inserted into the stomach through the mouth) to allow their tracking to locations of potential mortality events (due to *Ichthyophonus* infection) during their migration to the spawning grounds. Chinook salmon caught and held in the live box over night were used only for lethal sampling due to increased physiological stress of capture and holding (Cleary 2003). The target sample size was 150 fish from the subsistence harvests and an additional 100 samples were targeted for non-lethal procedures. Samples were collected from July 2 through July 9, 2004 (Appendix A2).

Chena/Salcha Mouth

The Chena and Salcha rivers are both clear groundwater runoff tributaries located on the right bank of the Tanana River with the confluences at approximately river kilometers 1,472 and 1,544, respectively (Figure 1). Sampling at multiple locations within the 2 rivers assisted in monitoring fish as they approached the spawning grounds. Non-lethal samples were taken in the lower Chena and Salcha rivers. The sampling targets for the individual drainages were based on collection over the entire run using historical average run timing (Appendix A3 and A4). The sampling target at the mouth of the Chena River was 100 fish. Samples were collected on July 6 and July 8, 2004. In addition, fish were caught by local sport fishermen using rod and reel and made available for collection of heart muscle. The Salcha sampling site is located downstream of an escapement enumeration tower within 1 kilometer of the confluence with the Tanana River. Live salmon were collected primarily by fishing with rod and reel for non-lethal sampling procedures. Samples were collected on July 5 through July 29, 2004, with a sampling target of 150 fish.

Lower Chena

Representative sampling over the entire run at the Chena and Salcha rivers was based on historical average run timing (Appendix A3 and A4). Fish were captured in a set gillnet, with mesh size of 8 inches by 29 meshes deep and 60 ft long, set in an eddy located approximately 24.6 river kilometers upstream from the mouth of the Chena River. The net was attended at all times while fishing; when a fish was captured the net was pulled into the boat, the fish was placed in a tote of water, and carefully untangled and removed from the net. After non-lethal sampling, all fish were released 50 meters upstream of sampling and fishing sites. Salmon were sampled from July 5 to July 22, 2004 with a sampling target of 150 fish.

Chena/Salcha Spawning Grounds

Fresh carcasses were collected by hand or using a gig with a sampling target of 150 fish for each drainage. Collectors were careful to avoid puncturing the body cavity with gigs to limit sample contamination. To assure collection of relatively fresh carcasses, Chena River samples were collected using the criteria "clear eyes" and "some pink in the gills", while Salcha River samples were gathered utilizing "clear eyes" and "firm heart." On the spawning grounds, radiotagged fish were recovered, and *Ichthyophonus* prevalence was determined from carcasses. Spawning success was evaluated using criteria described below. Chena River sample dates were July 23 through August 10, and Salcha River samples were collected from July 21 to August 8, 2004.

Juvenile Collections

Samples of juvenile Chinook salmon consisted of whole body and cardiac tissue and were provided by archived collections from the ADF&G Gene Conservation Laboratory. Fish were harvested on August 22–26, 1992. Samples had been stored at -70ºC since time of collection. All samples were collected from the Canadian portion of the Yukon River drainage, i.e., Blind Creek (n=145), Nordenskiold River (n=69), Sidney Creek (n=149), and McQueston River (n=100). Archived samples were analyzed using PCR (see below).

Morphometrics

Age, sex, and length (ASL) data were collected from samples at Emmonak, the Tanana mouth (radio tagged salmon) and the Chena and Salcha rivers (spawning grounds) using standard collecting procedures (Molyneaux and DuBois 1999). Sex was determined by internal examination during lethal sampling and via examination of external secondary sex characteristics as part of non-lethal sampling.

On the spawning grounds, evaluation of spawning success was based on gamete retention by visual internal examination. Chinook salmon were categorized into 3 groups. Fish were classified as "spawned out" where the cavity contained ≤ 10% remnants of gametes (empty skeins/milt sacs). The category "partially spawned out" was defined by ≤ 50% of the gametes retained in the cavity (some eggs, not whole skeins), while those fish categorized as "did not spawn" were still gravid (whole skeins/intact milt sacs).

GROSS CLINICAL SIGNS

Clinical signs of *Ichthyophonus* infection were noted by examining visceral organs. Ichthyophoniasis is commonly identified by the presence of "white spots" in infected tissues (Figure 2). However, white lesions, or focal granulomas, are an inflammatory response of fish to foreign bodies in general, and do not necessarily reflect infection with *Ichthyophonus* (Corbel 1975; Finn and Nielson 1971). Granulomas consist of lymphocytes, macrophages, neutrophils, and firm connective and fibrous tissue. Other pathogens causing visually similar white lesions in tissues are larval forms of cestodes and nematodes, as well as, protozoan parasites in the class Myxosporea genus *Henneguya* (Dykova and Lom 1978; Fish 1939). Fish were examined for clinical signs of *Ichthyophonus* infection during lethal tissue collection at the various locations (Emmonak, Tanana, and Chena and Salcha rivers).

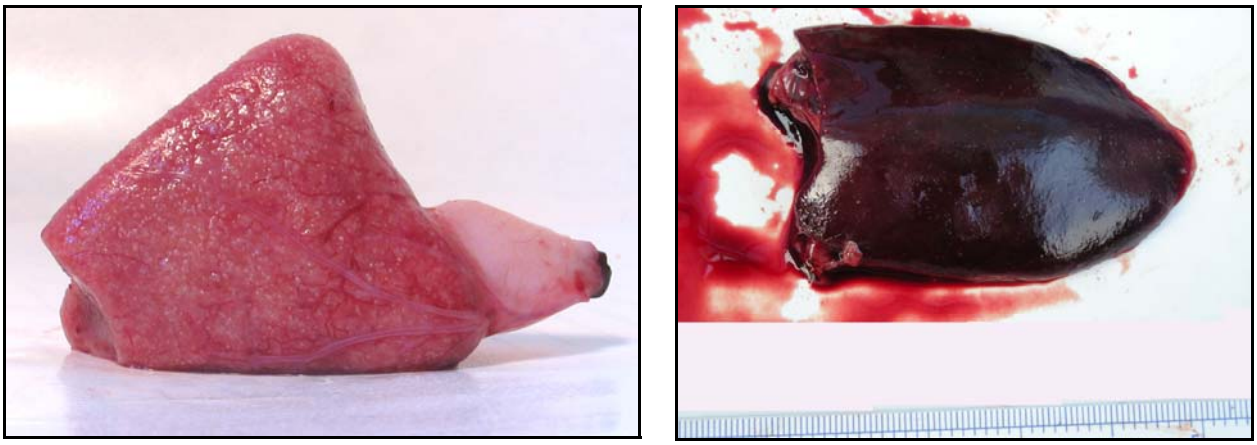


Figure 2.–White focal lesions on a) heart muscle and b) spleen of Chinook salmon. These gross clinical signs are typical for *Ichthyophonus* infection, but are also a general inflammatory response of fish to foreign bodies. *Ichthyophonus* has not yet been confirmed in this sample.

PATHOLOGY

Whole blood was subsampled for different testing procedures. An aliquot of 0.5 ml blood was stored in 95% ethanol for polymerase chain reaction (PCR) analysis and shipped at room/ambient temperature. A sub-sample of 0.5 ml was stored in cryovials and shipped on dry ice, and 0.5 ml of blood was transferred into cryovials and shipped frozen to be analyzed by PCR. Remaining blood samples were refrigerated (approximately 12 C) in the collection tube and shipped cool but not frozen to be analyzed by culture. A portion of the skeletal muscle sample was aseptically stored in 7 ml Eagle's Minimal Essential Medium (MEM)[1] supplemented with 5% fetal bovine serum, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, and 100 µg ml^{-1} gentamicin (referred to as MEM-5 for the remainder of the manuscript) for culture. Subsamples were stored in 95% ethanol for PCR testing. For histological examination, muscle samples were stored in a tissue cassette in 10% buffered formalin then transferred to 70% isopropyl alcohol prior to histological processing. Approximately 0.5 g of cardiac muscle (lethal sampling) was aseptically collected and cultured in MEM-5, 0.5 g cardiac tissue was stored in 95% ethanol for PCR testing, and a 0.5 g sample was collected for histology and stored in tissue cassettes as described above.

For explant culture, tissue was incubated at 14 C for a minimum of 14 days. The samples were periodically examined microscopically (100X magnification) for the presence of *Ichthyophonus*. PCR tests for detection of *Ichthyophonus* DNA in blood and tissue samples were performed using the procedures established by the Center for Fish Disease Research (Oregon State University) and the ADF&G pathology laboratory (Whipps et al. 2006). Histology samples were processed using standard procedures (Short and Meyers 2000). Briefly, tissues were prepared in 6 µm sections, placed on glass slides, and stained with hematoxylin and eosin. Results were reported as number of typical *Ichthyophonus* spores present per mm^2 of tissue observed in the densest area of the section using a slide micrometer (Marty et al. 1998).

[1] Product names used in this report are included for scientific completeness, but do not constitute a product endorsement.

RADIOTELEMETRY

Chinook salmon were captured by fish wheel as they approached the mouth of the Tanana River. Morphometric data (age, sex, and length) were collected as part of the tagging process. Only fish with no visible wounds or lesions were selected for radio tagging. Chinook salmon were tagged with a pulse-coded radio transmitter (Advanced Telemetry Systems). Additionally, a uniquely numbered 35 cm long external florescent yellow spaghetti tag was attached below the dorsal fin as a secondary mark. A minimally invasive muscle punch was taken for PCR testing prior to release. All methods and standards used were established by Eiler et al. (2004) for both tagging and tracking procedures.

Primary tracking was via aerial surveys in conjunction with 6 remote tracking sites (3 on mainstem Tanana and 3 on tributaries). In addition, radiotagged Chinook salmon were tracked using handheld units in the terminal study areas by boat and automobile. The tagging goal was 100 fish at the Tanana site with an expected recovery of 30 fish in both the Chena and Salcha rivers.

ENVIRONMENTAL DATA

An effort was made to obtain water temperature data at selected locations along the Yukon River drainage to monitor potential factors contributing to the progression of *Ichthyophonus* once fish enter the freshwater system (Figure 3). Temperatures were collected using HOBO Data Logger Pro or HOBO Tidbits deployed in conjunction with an operating fishery monitoring project. Therefore, time periods for collected temperature data were variable. Sites on the Yukon River mainstem included Emmonak, Pilot Station, Galena and Rapids. In addition, temperatures were obtained from some tributaries such as Anvik River, Tanana River, and upper Kantishna River. Water levels for selected Yukon River locations were recorded by the National Weather Service Alaska-Pacific River Forecast Center (http://aprfc.arh.noaa.gov), and ADF&G monitors water levels on the mainstem Yukon River at Galena and Eagle, the Tanana River at Nenana, and the Salcha River for fishery management purposes.

STATISTICAL ANALYSIS

Ichthyophonus prevalence in adult Chinook salmon was evaluated assuming a population of infected and uninfected individuals. A 2 X 2 chi-square statistic with 1 degree of freedom (Zar 1996) was used to test for differences in infection prevalence between males and females at each location, between each possible pair of locations, and between lower and upper river sampling sites. Due to sample size limitations, a 2 X 3 Fisher-Freeman-Halton test (R Development Core Team 2006) was used to test for differences in spawning success between infected and uninfected females. A 2 X 4 chi-square statistic with 3 degrees of freedom (Zar 1996) was used to test for differences in infection prevalence between the dominant age classes.

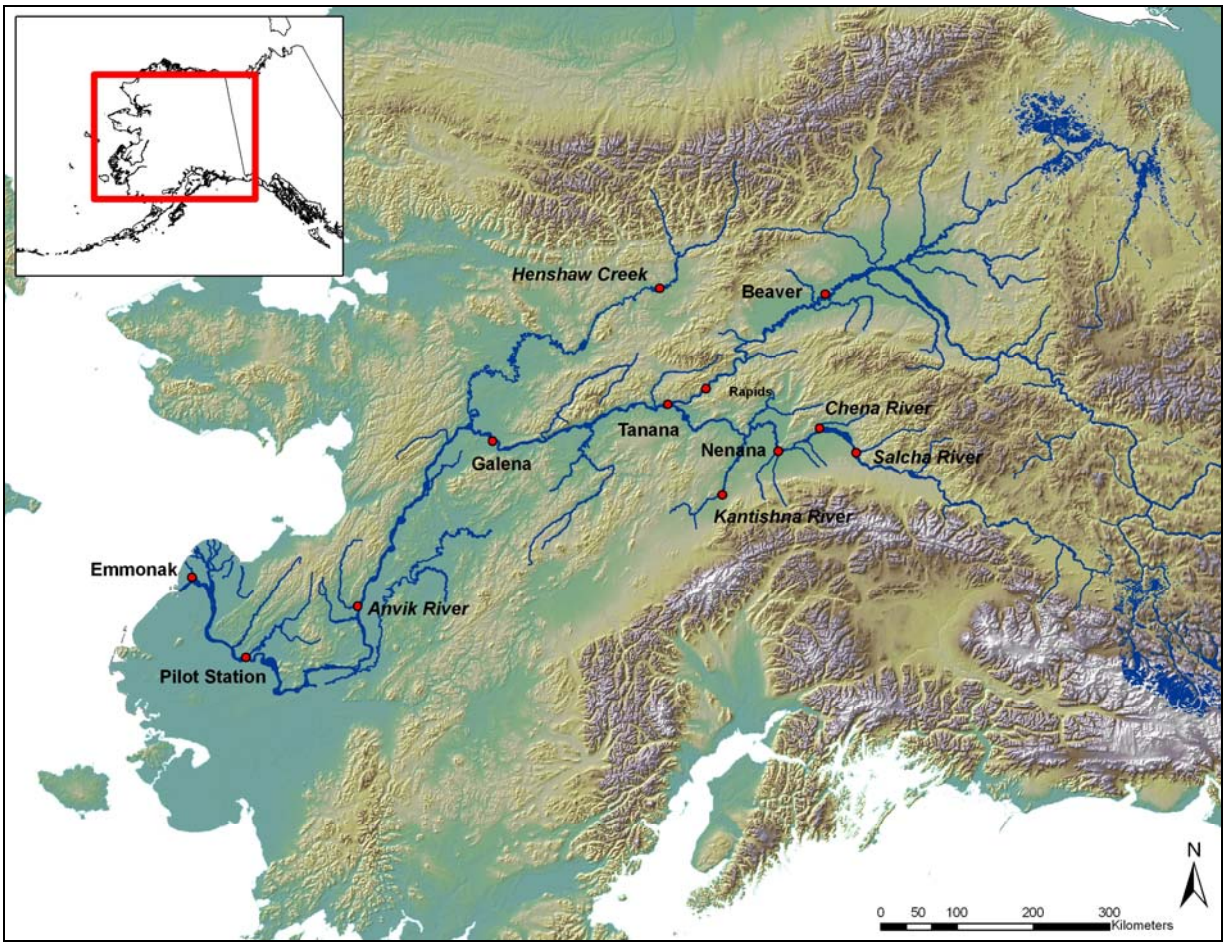


Figure 3.–Selected water temperature collection sites during Chinook salmon migration within the Yukon River drainage, Alaska, 2004.

RESULTS

LETHAL AND NON-LETHAL SAMPLING

Results for *Ichthyophonus* prevalence in heart and muscle tissue are summarized in Table 1 using 3 different screening techniques at 2 locations.

Non-lethal samples collected from Chinook salmon during the Tanana radiotelemetry study and those sampled in the lower Chena and Salcha rivers are shown in Table 2. Non-lethal PCR muscle samples collected in Tanana and the lower Chena were comparable ($p=0.97$) and there was no significant difference ($p=0.12$) between the Chena and Salcha rivers using PCR techniques. However, these sites differed significantly ($p=0.006$) when analyzed by culture.

Table 1.–Samples of Chinook salmon by laboratory test method and tissue type in Emmonak and the Tanana River, Alaska in 2004. Number of infected salmon (positive) and prevalence are shown.

	Emmonak			Tanana		
Sample Type	Number Positives	Number Samples	Prevalence [%]	Number Positives	Number Samples	Prevalence [%]
Culture						
Heart [a]	16	90	17.78	17	150	11.33
Muscle	ND	ND	-	14	150	9.33
Histology						
Heart	15	101	14.85	11	149	7.38
Muscle	5	101	4.95	8	149	5.37
PCR						
Heart [b]	23	104	22.12	11	100	11.00
Muscle	11	104	10.58	11	150	7.33

Note: ND = No Data.

[a] Test used to determine prevalence from Tanana samples.

[b] Test used to determine prevalence from Emmonak samples.

Table 2.–Non-lethal skeletal muscle samples tested by culture and PCR, from Chinook salmon collected from Tanana radiotelemetry fish and lower Chena and Salcha rivers, Alaska in 2004. Number of infected salmon (positive) and prevalence are shown.

		Muscle Culture			Muscle PCR		
Sample Site	Method	Number Positives	Number Sampled	Prevalence [%]	Number Positives	Number Sampled	Prevalence [%]
Tanana	Fish Wheel	ND	ND	-	9	109	8.26
Lower Chena	Gillnet	13	96	13.50	9	96	9.40
Lower Salcha	Rod and Reel	2	99	2.02	3	100	3.00

ND = No Data.

As part of developing non-lethal sampling techniques blood storage methods were evaluated. The blood of 12 fish, 4 from each storage method (i.e., refrigerated, frozen at -20C, dry ice then transferred to -10C and ethanol) was analyzed for the parasite via PCR. Samples were further selected from 3 "infection" categories, i.e., negative, low positive and high positive, as determined by spore densities in histological slides (Whipps et al. 2006). Even though, two-thirds of fish were expected to test positive (based on histology), only 25% were positive for *Ichthyophonus* in blood using any storage method. This is suggestive of low sensitivity or specificity, making blood not ideal for *Ichthyophonus* screening.

PREVALENCE WITHIN DRAINAGE

In order to compare *Ichthyophonus* prevalence within the drainage, results from heart explants were used as this was the only method utilized consistently at all sampling sites. *Ichthyophonus* prevalence was 17.8% at Emmonak, 11.3% at Tanana, 36.0% at the Chena River, and 13.7% at the Salcha River. At Emmonak, *Ichthyophonus* prevalence was not significantly different (p=0.21) between females (23.5%) and males (10.8%; Figure 4). Similarly, at the Chena River spawning grounds, *Ichthyophonus* prevalence for females (37.5%) was not significantly different from males (34.2%; p=0.93) (Figure 4). However, the Tanana fish wheel infection prevalence for females (26.1%) was statistically different from males (8.7%; p=0.04) (Figure 4).

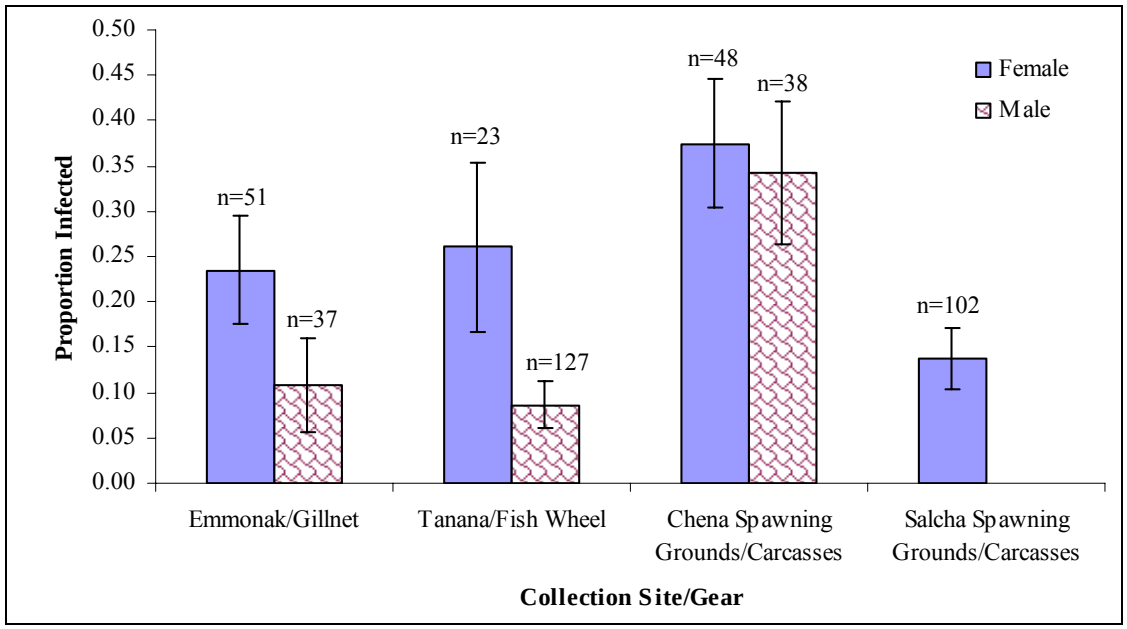


Figure 4.–*Ichthyophonus* prevalence of Chinook salmon by sex collected at specific locations using different gear types within the Yukon River drainage, Alaska in 2004.

Note: Prevalence was determined by heart culture. Error bars are standard deviations.

As males were not sampled on the Salcha River spawning grounds, comparisons of *Ichthyophonus* prevalence were only conducted for females. No significant differences were detected in *Ichthyophonus* prevalence of females between sampling sites, with the exception of the Chena and Salcha spawning grounds (p=0.002).

Ichthyophonus was not identified in archived samples collected from juvenile Chinook salmon (n=463) in the upper Yukon River. No adult Chinook salmon archived tissues were tested for historic prevalence.

GROSS CLINICAL SIGNS

Cardiac muscle was the primary organ exhibiting clinical signs (white spots or granulomas) at Emmonak. Typical clinical signs of ichthyophoniasis that correlated with actual presence of the parasite (as determined by culture, histology, or PCR) were observed in 9.62% of fish sampled at Emmonak (Figure 5). Of 150 Chinook salmon sampled at Tanana, a total of 21 fish had typical clinical signs in the heart, kidney and/or spleen. Seventeen fish (11.3%) tested positive for *Ichthyophonus* based on heart culture and of those, 16 (10.7%) had clinical signs of the disease, implying different causes of clinical disease in 5 fish.

On the Chena River spawning grounds, granulomas were observed in the heart, kidney, spleen and/or liver, although they were most commonly identified in cardiac muscle. On the Chena River, clinical signs of ichthyophoniasis correlated with actual *Ichthyophonus* infection, as determined by laboratory tests, for all Chinook salmon sampled. On the Salcha River, 7 Chinook salmon with clinical signs were positive for *Ichthyophonus*, while visible granulomas of 33 salmon were attributed to other infections or diseases. Prevalence of clinical signs ranged from 8.0% at lower (i.e., Emmonak) and middle river (i.e., Tanana) sites to 22.1% at the Chena River spawning grounds (Figure 5a).

(a) (b)

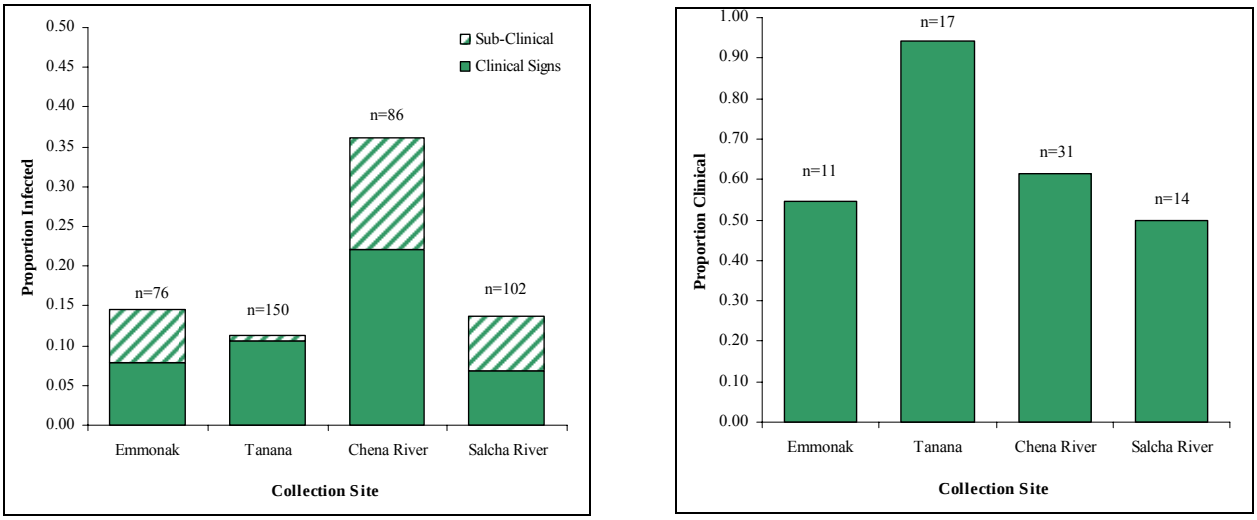


Figure 5.–Prevalence of *Ichthyophonus* in (a) clinically (visible) and sub-clinically infected fish and (b) clinical signs as a proportion of total infected fish, tested by heart explants from Chinook salmon samples collected at selected locations within the Yukon River drainage, Alaska in 2004.

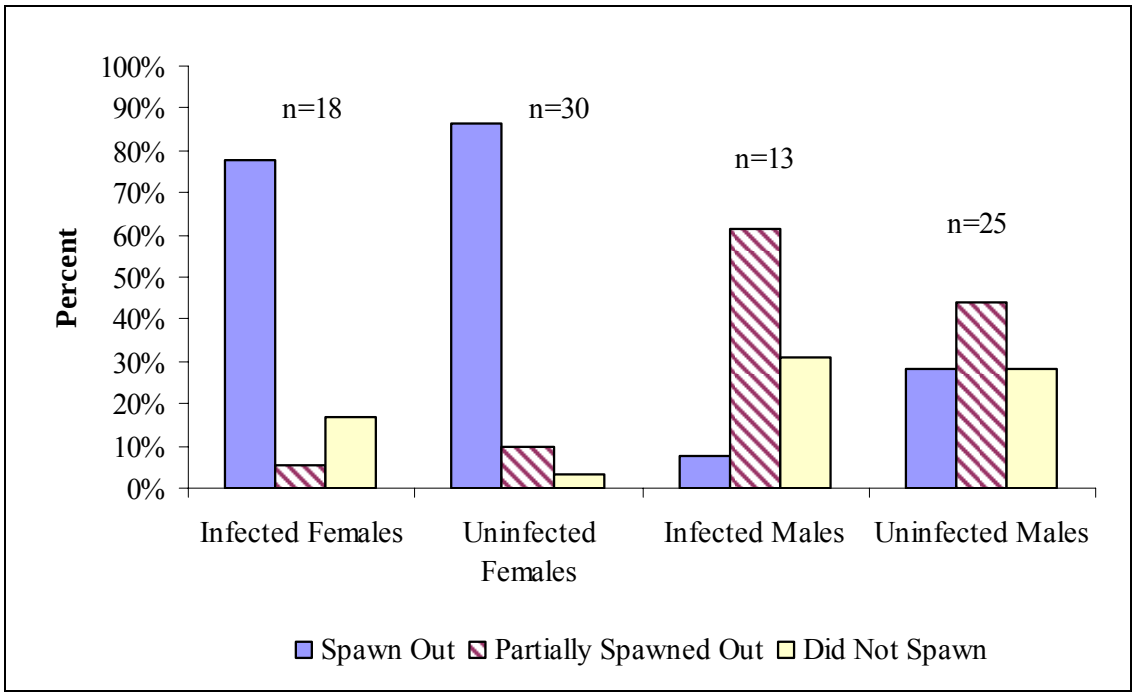
SPAWNING SUCCESS

Global Positioning System (GPS) locations of sampled carcasses on the Chena River are mapped in Appendix B5. To test for differences in *Ichthyophonus* prevalence between upper and lower Chena River sampling sites, the river was divided into one downstream (lower) and one upstream (upper) unit at river mile 28 (designated ROSEHIP 28MI on map 2). Sampling trips in the middle river area were typically conducted upstream or downstream from the Rosehip boat launch. The sampling areas represented approximately 52 river km downstream and 36 river km upstream of the Rosehip location. The proportion of infected Chinook salmon in the lower Chena River (39.6%) was not significantly different from the upper Chena River (29.4%) ($p=0.46$; n=53 lower and n=34 upper). Therefore, data were pooled for analysis at the spawning grounds to increase sample size and statistical power.

The prevalence of *Ichthyophonus* (based on heart culture) at the Chena River was 55.8%, 26.7% and 17.4% for Chinook salmon categorized as fully spawned, partially spawned and unspawned, respectively. Differences between infected and uninfected males and females in the 3 categories were not significant, though sample sizes were small ($p=0.40$ and $p=0.38$, respectively). In the spawned out category, 77.8% of the females were infected, while uninfected represented 86.7% of the category (7.7% and 28.0% for infected and uninfected males, respectively). For partially spawned females, 5.6% were infected and 10.0% were uninfected (61.5% infected and 44.0% uninfected males). However, 16.7% of the infected females did not spawn (30.8% males) compared to 3.3% of the uninfected females (28.0% males) (Figure 6a).

Only females were collected on the Salcha River. Relative occurrence of *Ichthyophonus* by category (as analyzed by heart culture) was 21.4% (34.1% uninfected), 64.3% (45.5% uninfected) and 14.3% (20.5%) for fully spawned, partially spawned and unspawned Chinook salmon (Figure 6b). There was no difference in gamete expulsion (based on established categories) between infected and uninfected females ($p=0.45$).

(a)



(b)

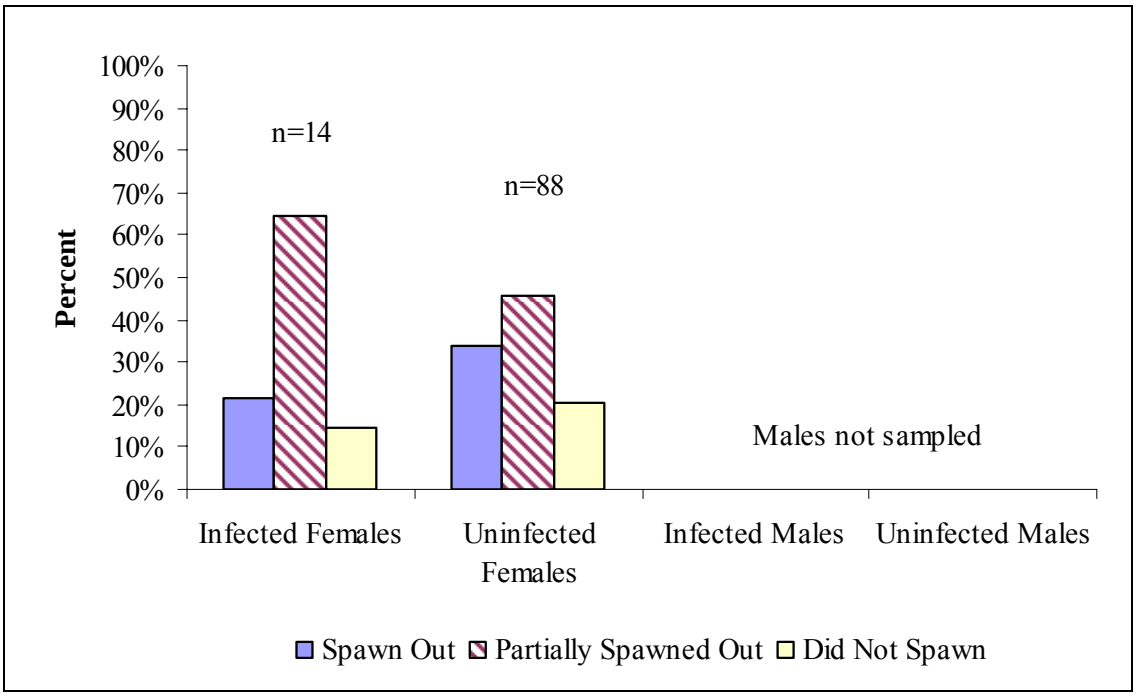


Figure 6.–Proportions by spawning category of infected and uninfected Chinook salmon by sex, collected in the Chena (a) and Salcha (b) river spawning grounds, Alaska, 2004.

The prevalence of *Ichthyophonus* (based on heart culture) at the Chena River was 55.8%, 26.7% and 17.4% for Chinook salmon categorized as fully spawned, partially spawned and unspawned, respectively. Differences between infected and uninfected males and females in the 3 categories were not significant, though sample sizes were small (p=0.40 and p=0.38, respectively).

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AGE, SEX, AND LENGTH COMPOSITION

Age, sex, and length (ASL) data were collected in Emmonak, and on the Chena and Salcha rivers spawning grounds. Sex was determined by internal examination of fish. Table 3 shows the age composition of infected and uninfected fish as determined by heart explants. At all locations, age composition of the infected group was shifted towards older fish when compared to uninfected Chinook salmon. Older fish were more likely to be infected with *Ichthyophonus* at all locations. However, sample sizes in separate age classes were not sufficient for statistical analysis (Table 3).

Sex was the only ASL variable recorded during lethal sampling at the Tanana fish wheel (Table 3). For radiotagged Chinook salmon captured at the Tanana fish wheel site, sex was determined by external examination (n=79) of body morphology with 65% females. The sex of 31 radiotagged fish could not be determined. Age composition of the samples (n=97) was dominated by the age-6 class (52.6%) followed by 39.2% age-5, 7.2% age-4, and 1.3% age-7. Thirteen fish could not be aged. Mean length at age is given in Appendix B1.

RADIOTELEMETRY

Muscle punch biopsy samples were collected for PCR from 109 Chinook salmon radio tagged in Tanana; *Ichthyophonus* prevalence was 8.3% (Table 2). Of the 9 fish that tested positive for *Ichthyophonus* by muscle PCR, 78% were located in known spawning tributaries, while the location of 22% remained unknown. Nine radiotagged Chinook salmon were located in the Chena River and 10 within the Salcha River. Tissues from 1 of the tagged fish recoveries were collected from each river system, though neither showed clinical signs of ichthyophoniasis and both were negative for *Ichthyophonus.* Three radio tags were recovered from the Tanana River commercial fishery and were harvested near the community of Nenana. Samples were collected from 2 recovered radiotagged fish and both were negative for *Ichthyophonus*.

Table 3.–Chinook salmon age and sex composition from uninfected and infected samples collected of *Ichthyophonus* from Emmonak and the Tanana, and Chena and Salcha rivers, Alaska in 2004.

Sample Location	Sample		4 Years		5 Years		6 Years		7 Years		Total	
(Source)	Size	Sex	No.	%	No.	%	No.	%	No.	%	No.	%
Uninfected Fish												
Emmonak	57	Males	8	14.0	9	15.8	13	22.8	0	-	30	52.6
(Gillnet)		Females	0	-	1	1.8	25	43.9	1	1.8	27	47.4
		Subtotal	8	14.0	10	17.5	38	66.7	1	1.8	57	100.0
Tanana	133	Males	ND	-	ND	-	ND	-	ND	-	116	87.2
(Fish Wheel)		Females	ND	-	ND	-	ND	-	ND	-	17	12.8
		Subtotal	ND	-	ND	-	ND	-	ND	-	133	100.0
Chena River	49	Males	10	20.4	4	8.2	7	14.3	0	-	21	42.9
(Carcasses)		Females	0	-	4	8.2	23	46.9	1	2.0	28	57.1
		Subtotal	10	20.4	8	16.3	30	61.2	1	2.0	49	100.0
Salcha River	82	Males	ND	-	ND	-	ND	-	ND	-	ND	-
(Carcasses)		Females	0	-	2	2.4	79	96.3	1	1.2	82	100.0
		Subtotal	0	-	2	2.4	79	96.3	1	1.2	82	100.0
All Locations		Total	18	9.6	20	10.6	147	78.2	3	1.6	188	100.0
Infected Fish												
Emmonak	11	Males	0	-	1	9.1	2	18.2	0	-	3	27.3
(Gillnet)		Females	0	-	0	0.0	7	63.6	1	9.1	8	72.7
		Subtotal	0	-	1	9.1	9	81.8	1	9.1	11	100.0
Tanana	17	Males	ND	-	ND	-	ND	-	ND	-	11	64.7
(Fish Wheel)		Females	ND	-	ND	-	ND	-	ND	-	6	35.3
		Subtotal	ND	-	ND	-	ND	-	ND	-	17	100.0
Chena River	31	Males	2	6.5	3	9.7	6	19.4	2	6.5	13	41.9
(Carcasses)		Females	0	-	1	3.2	16	51.6	1	3.2	18	58.1
		Subtotal	2	6.5	4	12.9	22	71.0	3	9.7	31	100.0
Salcha River	14	Males	ND	-	ND	-	ND	-	ND	-	ND	-
(Carcasses)		Females	0	-	0	-	13	92.9	1	7.1	14	100.0
		Subtotal	0	-	0	-	13	92.9	1	7.1	14	100.0
All Locations		Total	2	3.6	5	8.9	44	78.6	5	8.9	56	100.0
All Samples	394	Total	20	8.2	25	10.2	191	78.3	8	3.3	244	100.0

Note: Infection prevalence was determined by heart culture. ND = No Data.

Of the 110 tags deployed, 5% of fish were last located downstream from the tagging site, 2% were harvested and 11% could not be relocated after release (Figure 7). Approximately 47% of the tags were located along the Tanana River mainstem, however, several radio tags in this area could be attributed to harvest, as tags seemed more concentrated near communities or fish camps. In addition to 17 tags (15.5%) recovered in the Chena and Salcha river tributaries, 22 radio tags (20.0%) were located in known spawning tributaries (Appendix B2).

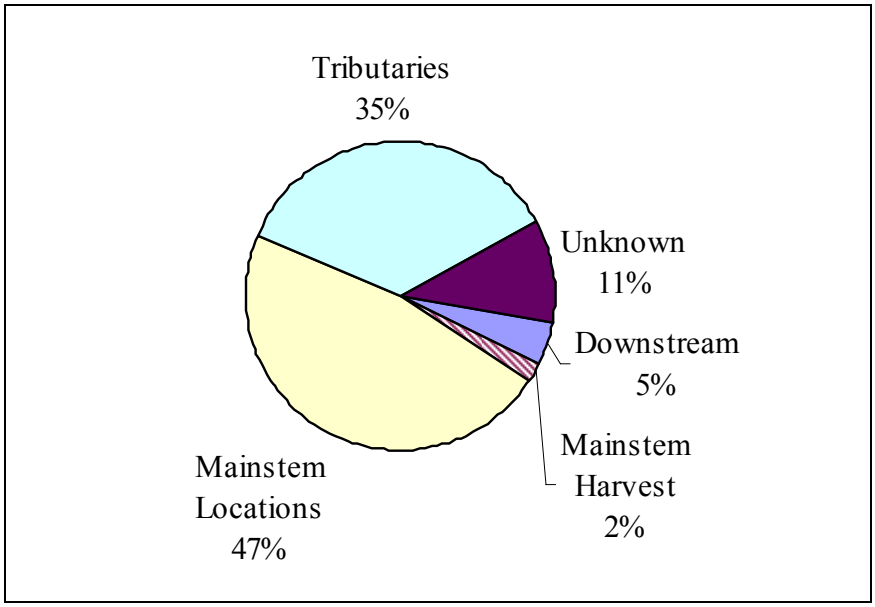

Figure 7.–Final locations by proportion of radiotagged Chinook salmon deployed at the confluence of the Tanana River, Alaska in 2004.

An additional, basin-wide Chinook salmon radiotelemetry project operated at river kilometer 341 near the community of Russian Mission (Eiler et al. 2006). Of the 2,107 radiotagged fish released in the lower Yukon River, 68 were tracked to final locations within the Salcha River drainage and 9 of those were recovered. Of the 7 tagged fish that were sampled for *Ichthyophonus*, 3 tested positive. Distribution of the radiotagged fish upstream of Nenana River had similar proportions returning to both the Chena and Salcha rivers from both the basin-wide study (Eiler et al. 2006) as well as this study. However, a larger proportion of fish were tracked within the Kantishna and Tolovana river drainages than the basin-wide study. The contract fisherman noted some drastic changes in the river channel affecting the fishing site that could possibly affect sampling of specific stocks.

ENVIRONMENTAL DATA

HOBO data loggers were deployed at selected locations within the Yukon River drainage. Figure 8 illustrates average daily water temperatures in 2004 from 4 primary *Ichthyophonus* sampling sites (Emmonak, Tanana, and the Chena and Salcha rivers). Water temperatures at the Tanana mouth were taken every 6 hours, while temperature at other sites was recorded hourly. Additional water temperature data, throughout the Yukon River drainage, is compiled in Appendix B3 and B4. Low water levels were observed over the majority of the season with record low water levels (1987–2003) in the Salcha River in August 2004 (Figure 9).

DISCUSSION

LETHAL AND NON-LETHAL SAMPLING

Both lethal and non-lethal sampling techniques were evaluated as monitoring tools to detect *Ichthyophonus* in Chinook salmon. Non-lethal screening has many advantages over the typical lethal sampling of cardiac muscle, such as repeated sampling of fish over time to observe the progression of the disease, survival of infected fish, and individual spawning success. Various tissue types, storage, and testing methods were evaluated. Cardiac muscle is frequently the first tissue affected by *Ichthyophonus* and is then disseminated through the body (Jones and Dawe 2002; Rahimian and Thulin 1996; Spanggaard et al. 1995) and cardiac muscle was utilized in this study to maintain baselines established by Kocan et al. (2004a). Paired tissue samples were collected at Emmonak and Tanana and used to compare lethal and non-lethal samples to identify *Ichthyophonus*-positive fish. However, skeletal muscle and blood were less sensitive and reliable than cardiac muscle in detecting *Ichthyophonus* infection. Similarly, Whipps et al. (2006) recommended the analysis of cardiac muscle over skeletal muscle for future *Ichthyophonus* studies. Further, PCR analysis of heart tissue was highly sensitive and specific for *Ichthyophonus* and comparable to established procedures using culture and histology (Whipps et al. 2006). In addition, samples collected for PCR can be archived and stored indefinitely in ethanol, thus making this method ideal for field collections where storage, controlled environment, and timely sample treatments can be problematic.

Muscle punches were taken from Chinook salmon in the lower Chena and Salcha rivers. As mentioned above, detection of the parasite in muscle tissue was unreliable and *Ichthyophonus* can only be found if the disease is more advanced and disseminated throughout the body. However, the significant difference in prevalence, as determined in skeletal muscle, between the Salcha and Chena rivers (2.0% and 13.5%, respectively) was unexpected, though the use of different gear types at both locations may account for this peculiarity. Rod and reel gear was used on the lower Salcha River, while set gillnets were utilized on the lower Chena River. The introduction of catch biases using different gear types is a commonly known problem in fisheries management (Bromaghin 2004, 2005; Olin et al. 2004; Quang and Geiger 2002). Holst (1996) strongly cautioned over-interpretation of *Ichthyophonus* prevalence data in herring stocks caught with different gear types and recommended the use of low selectivity gear for estimation of *Ichthyophonus* prevalence. Similarly, the difference in prevalence between the Chena and Salcha rivers is likely due to gear selectivity, as rod and reel gear may introduce a sampling bias towards more vigorous fish compared to those captured in set gillnets.

PREVALENCE WITHIN THE DRAINAGE

Ichthyophonus prevalence at the mouth of the Yukon River varied with time, ranging from 25.9% in 1999 (Kocan et al. 2004a) to approximately 33% in 2003 (Kocan and Hershberger 2006). Prevalence in 2004 at Emmonak (17.8%, this study) was lower than reported for all but 1 of the previous 5 years. At the Tanana fish wheel (confluence of the Tanana River), prevalence in 2004 was 11.3%, lower than in 2000 (Kocan et al. 2004a) and less than half of the infection prevalence reported from 2001 to 2003 (Kocan et al. 2004a; Kocan and Hershberger 2006). *Ichthyophonus* prevalence on the Chena River in 2004 was the highest on record for this site (36.1%), compared to 8.7% and 16.1% in 2001 and 2002, respectively (Kocan et al. 2004b). Prevalence at the Salcha River in 2004 was comparable to 2002 (Kocan et al. 2004a). If diseased

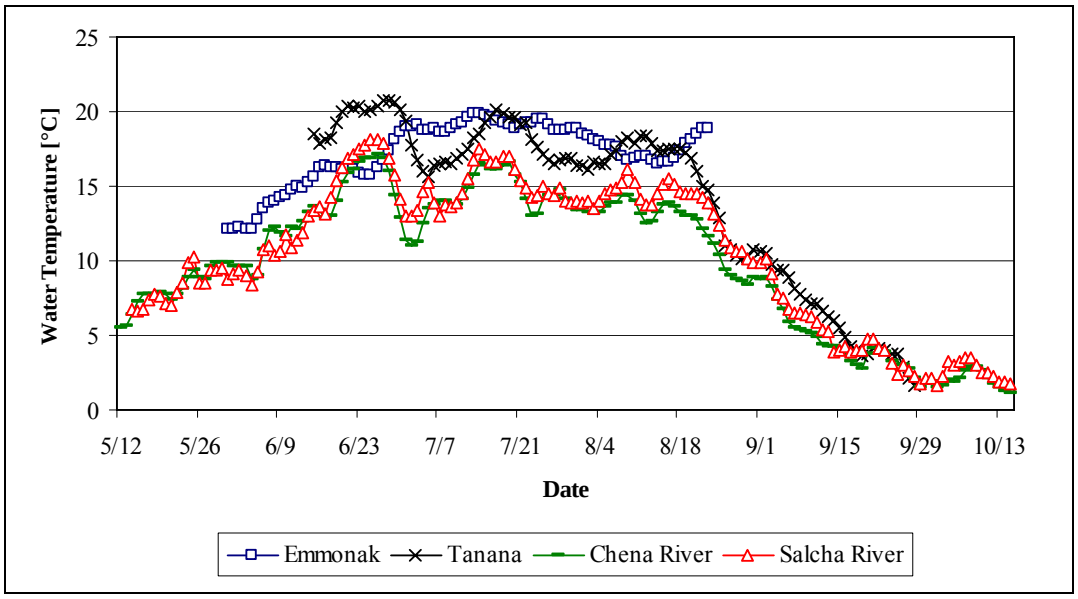

Figure 8.–Water temperatures [ºC] from Emmonak (mouth), Tanana (mouth), and Chena and Salcha rivers, Alaska, 2004.

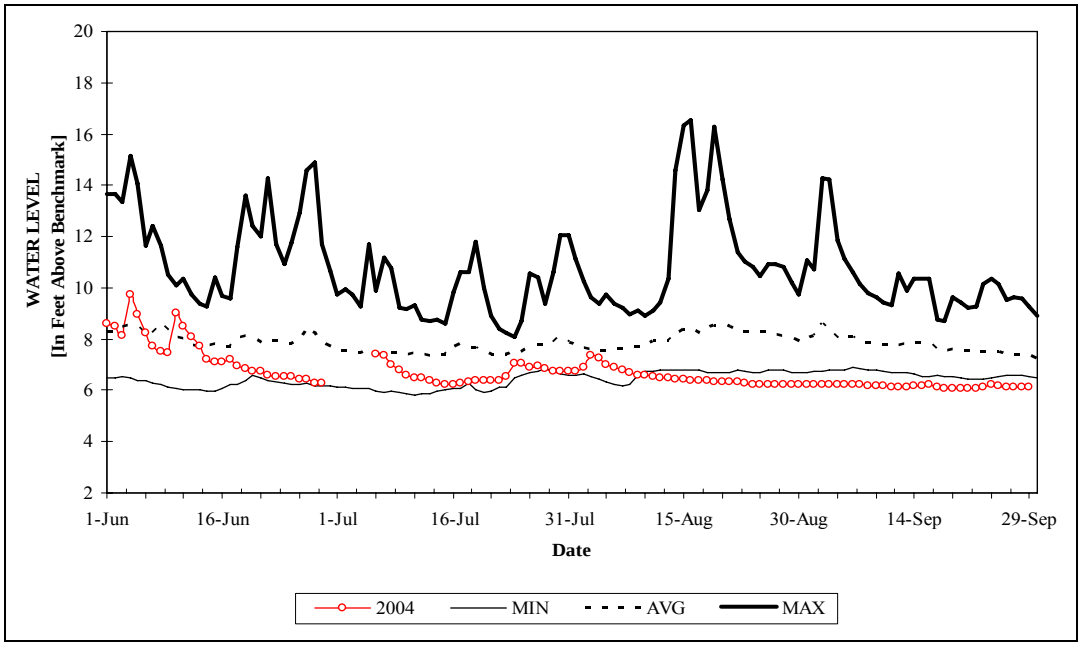

Figure 9.–Salcha River water level in 2004 compared to historical 1987–2003 maximum, average and minimum, Alaska, 2004.

fish from both Chena and Salcha rivers in 2002 (Kocan et al. 2004b) are combined (12.5%), it can be assumed that a minimum of 14.6% (prevalence at Tanana confluence minus the pooled prevalence of Chena and Salcha rivers) of infected fish spawned at other tributaries, died prior to spawning or biases are introduced to carcass sampling (Zhou 2002). Similarly, pooled prevalence at the Chena and Salcha rivers spawning grounds in 2004 was 23.9%, while prevalence at the Tanana fish wheel was only 11.3%. This suggests a possible biased sampling of the spawning grounds (Zhou 2002), or infected salmon were underrepresented at the Tanana fish wheel site. The possibility of cross-contamination in samples from the Chena River in 2004 (i.e., 6 successive samples tested *Ichthyophonus*-positive) was considered and a sequential run test for randomness was performed. The results indicated that there was not enough evidence to reject the null hypothesis that detection of positive samples did not occur randomly ($p=0.07$).

Prevalence may exhibit temporal fluctuations possibly due to environmental factors that are still poorly understood. For example, within spawning tributaries of the Chena and Salcha rivers, temperature fluctuations are seasonally erratic due to effects of sudden high water from rain events or exposure to intense sun during low water. The possible combination of environmental factors such as low water and high temperatures could have an effect on fish behavior. For example, in 2004, movement patterns at the mouth of the Salcha River were atypical, with fish milling/spawning and expiring lower in the system than usual (T. C. Stark, Fishery Biologist, Bering Sea Fishermen's Association, Fairbanks; personal communication). Okamoto et al. (1987) showed a positive correlation of *Ichthyophonus*-related mortality with water temperature. In their experiments, these authors described a dramatic increase in mortality between 10°C and 15°C, with mortality being 100% at 15°C to 20°C. In contrast, experimentally infected rainbow trout (*Salmo gaidrneiri*) did not die at lower temperatures, i.e., 4°C (Okamoto et al. 1987). Further, increased stress response (corticosteroids) has been known to accelerate the course of the infection (Perry et al. 2004). While corticosteroids accelerate disease dissemination, it is not a host response to infection with *Ichthyophonus* (Rand and Cone 1990). It is therefore likely that fish in the marine environment may be infected with *Ichthyophonus* at a dormant stage without, or limited mortality, while fish entering the river system would suffer temperature and nutritional stress and prepare for spawning migration, thus accelerating the infection. Given the rising temperatures in the Yukon River over past decades (Kocan et al. 2004a), prevalence of *Ichthyophonus* and potential pre-spawning mortality should be closely monitored.

In addition, Jones and Dawe (2002) suggested Yukon Chinook salmon may be more susceptible to *Ichthyophonus* than some British Columbia stocks, i.e., Little and Big Qualicum. However, *Ichthyophonus* prevalence of Chinook salmon in the Yukon River system was similar to those reported for the Kuskokwim River (15%) and the Taku River (23.2%) in 2001 (Kocan et al. 2004a). Prevalence in other fish species is highly variable and ranges from ~70% in Puget Sound herring (Kocan et al. 1999) to 2% in Auke Bay pollock (*Theragra chalcogramma*) (Eaton et al. 1991).

Kocan et al. (2004a) reported decreasing *Ichthyophonus* prevalence during upriver migration, with 1999–2003 average infection prevalence dropping from ~30% in the Yukon mainstem to ~16% at the Whitehorse hatchery. These authors argued that this decrease is indicative of pre-spawning mortality, though it is equally likely that the "missing" fish branched off the mainstem to terminal spawning areas. It is also conceivable that some Yukon Chinook salmon stocks are more susceptible than others to *Ichthyophonus* infection, warranting further study.

As mentioned above, it is difficult to interpret data from fish obtained by different gear types with their own selectivity bias. The Tanana fish wheel site uses video monitoring during the fishing season. Small fish (<700mm) are primarily males ("jacks"), but may include some smaller age-4 female Chinook salmon (Fliris and Daum 2004). Age and sex are factors to consider when discussing *Ichthyophonus* prevalence, and a high catch of small fish may account for the relatively low prevalence observed at the Tanana fish wheel in 2004. On the other hand, fish infected with *Ichthyophonus* exhibit behavioral changes due to the highly necrotic effect of the parasite in host tissues, such as cardiac damage and reduced swimming stamina (Kocan et al. 2006; Tierney and Farrell 2004). It is likely that infected fish will swim close to shore to conserve energy, and thus fish wheels may be selective for less vigorous fish (Bromaghin et al. 2007).

An important variable is also the time from infection to death or the rate at which the disease is disseminated. Jones and Dawe (2002) indicated that clinical disease was visible 35 to 47 days after exposure and Kocan and Hershberger (2006) noted a significant difference between macroscopic signs of infection between Canada-bound salmon and Tanana fish. At travel speeds of approximately 55 km per day, salmon reach Tanana at approximately 19 days after entry in the river and the Canadian border after about 35 days, thus clinical signs should be most prominent in the Canadian fish. This is in agreement with Kocan et al. (2004a). These authors also stated that *Ichthyophonus* prevalence was statistically different between salmon early in the run and fish that enter the river system later, with late Chinook salmon being more heavily infected. All these variables have to be taken into account and it becomes challenging to make inferences about disease-related mortality based on comparisons between different locations, temperatures, gear selectivity, testing methodologies, run timing, and potential differences in stock susceptibility.

GROSS CLINICAL SIGNS

Identification of *Ichthyophonus* via laboratory analysis requires either DNA sequencing or culturing of the organism for approximately 2 weeks. At this time, these techniques are not suitable to report prevalence of the parasite inseason to provide ad hoc management recommendations. Thus, the use of clinical signs to instantly and accurately identify *Ichthyophonus* in a field environment was evaluated. Internal organs (heart, kidney, spleen) were visually examined for characteristic signs of ichthyophoniasis, i.e., white nodular lesions.

Accuracy of correctly identifying *Ichthyophonus* based on visual inspection alone varied markedly between sampling sites, ranging from 17.5% on the Salcha River spawning grounds (meaning 82.5% of fish were false positives, displaying clinical pathology without carrying the parasite) to 100% accuracy on the Chena River spawning grounds. This study further showed (based on heart culture from Emmonak, Tanana, Chena and Salcha rivers) that percentages for infected Chinook salmon but without characteristic signs of the disease (false negatives) were 11.1%, 0.7%, 14%, and 6.9% respectively (Figure 5). This indicates that correct identification of *Ichthyophonus*-positive fish via typical clinical signs is unreliable as they are non-specific immune responses similar to those induced by other fish diseases and parasitic infections (Dykova and Lom 1978; Fish 1939). Rahimian and Thulin (1996) also indicated that prevalence, as determined by histology, is on average 4.5 times more accurate than using clinical signs alone. These authors further noted that the ratio between micro- and macroscopic examination is variable, with larger fish being more likely to show clinical signs.

However, as *Ichthyophonus* becomes more disseminated, clinical signs are more obvious. Clinical signs in Chinook salmon entering the Yukon River are generally less severe, but lesions may be visible in the heart. Fish sampled further upriver present white lesions on multiple organs (heart, kidney, spleen). Although, if an increase in global ocean water temperatures were to occur, the disease could potentially be accelerated, causing clinical signs to become more obvious. Their use for fisheries management may be revisited in the future.

SPAWNING SUCCESS

Management of Chinook salmon fisheries is predicated on indices of escapements based on the number of fish arriving at the spawning grounds. The Chena and Salcha rivers are the main producers for the middle Yukon River stock (Eiler et al. 2004) and both systems have biological escapement goals (BEG) representing the greatest potential for maximum sustained yields. The BEG range for the Chena and Salcha rivers is 2,800 to 5,700 and 3,300 to 6,500 Chinook salmon. The escapements are evaluated by tower counts, conducted annually on each system; they can be further refined by mark–recapture abundance estimates (Brase and Doxey 2006). *Ichthyophonus* related prespawning mortality is of significant management concern once Chinook salmon have passed the counting towers. If significant die-offs occur prior to successful spawning, adjustments to BEG's may be necessary.

A large percentage of both infected and uninfected Chinook salmon from the Chena and Salcha rivers were fully or partially spawned (Figure 6). However, evaluation criteria (spawned out, partially spawned, or not spawned) are difficult to apply consistently between sites and assessment of male gonads proved particularly challenging. Also, this study did not address several other variables directly related to spawning success, such as energy stores of individual eggs, hatching and survival, successful defense of redds, etc. Rahimian (1998) suggested that fish infected with *Ichthyophonus* were in poor body condition as indicated by thin appearance with low body fat. It was therefore conceivable that salmon infected with *Ichthyophonus* do not allocate the same energy stores to eggs, or use energy stored in gonads to reach the spawning grounds in addition to body-fat reserves. Further, Groot and Margolis (1991) reported that the average duration of redd residency for female Chinook salmon was 15–16 days. Offspring of infected adults may be at a disadvantage if spawners are physically unable to spend sufficient time to defend redds. Additionally, infection with *Ichthyophonus* is known to trigger stress responses in other fish species (Hershberger et al. 2006), and Pankhurst and Van Der Kraak (2000) have shown that increased corticosteroids (stress hormones) negatively impact vitellogenesis. However, this study indicates that at least some infected female Chinook salmon, migrating past escapement enumeration projects, deposited their eggs. Assuming that egg deposition signified successful spawning, biological escapement goals on the Chena and Salcha rivers may not need to be re-evaluated at this time. Nevertheless, future studies should explore the effect of *Ichthyophonus* on egg quality and juvenile survival.

AGE, SEX, AND LENGTH COMPOSITION

Ichthyophonus was not detected in any of the archived samples from juvenile (freshwater) Chinook salmon in this study. PCR techniques test for the presence of parasite DNA and storage (archived at -70ºC) has no impact on the accurate analysis of the samples, though long-term storage in ethanol is recommended (Taggart et al. 1992). However, Hershberger et al. (2006) described ichthyophoniasis in age-0 (approximately 4 months posthatch) herring captured in

Puget Sound. This indicates marine rather than freshwater origin of the parasite. Although the source of *Ichthyophonus* remains unknown, it is likely transmitted via ingestion of infected prey. Low pH (i.e., passage through the stomach) stimulates hyphal growth (Spanggaard et al. 1994) and fish experimentally fed infected prey acquired *Ichthyophonus* (Kocan et al. 1999). Further, an *Ichthyophonus*-like pathogen causing abnormal coloring was described in the copepod *Calanus* (Torgersen et al. 2002) and it is known that Chinook salmon selectively feed on pigmented prey (Schabetsberger et al. 2003). Therefore, the life cycle of the parasite may require at least 1 intermediate host.

An increase of *Ichthyophonus* prevalence with age, as indicated in Table 4, has also been reported in other species (Hershberger et al. 2002; Rahimian and Thulin 1996). Conversely, Kocan et al. (2004a) did not find a positive correlation of prevalence with age, though these authors used weight as a proxy for age. Lauckner (1984), Reno (1998) and Kocan et al. (1999) indicated that a threshold number of spores need to be ingested to cause *Ichthyophonus* infection. This suggests a gradual accumulation of spores with age and therefore an increased prevalence in the older age classes. On the other hand, Rahiman and Thulin (1996) speculated that older fish may have acquired a resistance to *Ichthyophonus*, while most young, immunologically naïve fish die from the infection. This is in agreement with experimental feeding trials by Kocan et al. (1999), who documented 80% *Ichthyophonus*-related mortality in herring with unchallenged immune systems. Lastly, the positive correlation of disease prevalence with age may be related to heterogeneous feeding ecology between age classes and consequently different exposure to a reservoir host. For example, juvenile Chinook salmon consume age-0 sandlance, rockfish and crustacean prey (Brodeur and Pearcy 1990), while the size of prey increased with increasing length of Chinook salmon (Brodeur 1991).

Kocan et al. (2004a) theorized that fish infected with *Ichthyophonus* die on their way to the spawning ground, and this in turn would have a larger impact on older age classes with higher parasite prevalence. More recently, concerns have been voiced that Chinook salmon on the Yukon River are becoming smaller (Bigler et al. 1996; Hyer and Schleusner 2005), thus age, sex, and length data (ASL) should accompany *Ichthyophonus* samples to further assess and monitor this potential relationship of age and *Ichthyophonus* prevalence. Further, ASL data may elucidate any biases that are introduced by different gear types, so that more appropriate comparisons can be made.

RADIOTELEMETRY

Prevalence of *Ichthyophonus* in Chinook salmon was monitored throughout each migratory stage, however comparison between different methodologies, tissue, and gear types make direct comparisons between sites difficult, as mentioned above. Radiotelemetry was evaluated as a tool to track individual infected and non-infected salmon to their final destination. A large percentage of Chinook salmon within the Tanana River spawn in the Chena and Salcha rivers, thus it is probable that a significant portion of radiotagged salmon would migrate to these tributaries (Eiler et al. 2004). In this study, Chinook salmon responded well to fish wheel capture, tagging, and biopsy procedures as the majority of the salmon continued their upriver movements with expected migration speeds. Tracking of radiotelemetry fish indicated that migration rates from the Tanana site to the spawning grounds was comparable to lower Yukon tagged fish at the same locations (Eiler et al. 2006), at an average of 40 km/day mid-Tanana, then slowing down to an average of 25 km/day as they approached the spawning tributaries. Although 5% of tagged fish

exhibited downstream movement (i.e., alternate spawning location, handling stress, tag regurgitations, post-handling mortality), this number was similar to other Yukon River studies (Milligan et al. 1985). As discussed above, detection of *Ichthyophonus* in muscle punches is unreliable and only infections disseminating to this tissue are identifiable. Therefore, determination of *Ichthyophonus* in radiotagged Chinook salmon only represented a minimum estimate of actual prevalence (8.3% in this study).

Recovery and sampling of spawned-out carcasses required transmission of a mortality signal. This signal is only transmitted after fish remained stationary for 24 hours and carcasses are often carried by currents, making recovery and sample collection challenging while tissues are fresh. Although sample sizes are small, 7 of 9 *Ichthyophonus*-positive salmon (as determined by PCR from muscle punches) were located on known spawning grounds, suggesting that the majority of infected Chinook are not dying during their migration. Dissemination to muscle tissue is generally associated with severe *Ichthyophonus* infection (Kocan et al. 2006; Marty et al. 1998). In this study, *Ichthyophonus*-positive fish (as determined by muscle punch) should have exhibited cardiac necrosis yet some infected fish arrived at the spawning grounds. However, radiotagging studies generally select the most robust fish (without obvious lesions or loss of ability to maintain equilibrium) for implantation of transmitters to keep handling mortality at a minimum (J. H. Eiler, Biologist, NOAA, Juneau; personal communication). This inevitably introduces biases to the selected fish as parasite infection has debilitating effects on salmon health, including tissue necrosis and cardiac muscle damage (Kocan et al. 2006). *Ichthyophonus* prevalence in radiotagged fish should therefore be interpreted with caution.

CONCLUSIONS

This study demonstrated that cardiac muscle is the most reliable tissue type for *Ichthyophonus* monitoring. Further, PCR analysis of cardiac muscle is highly specific and sensitive for *Ichthyophonus* and is an adequate alternative to histology and culture techniques. Gear type is likely the main bias inadvertently introduced to *Ichthyophonus* studies. For example, fish wheels tend to catch a larger number of small males or less vigorous (potentially diseased) fish that migrate closer to shore. This may lead to a presumably higher prevalence of *Ichthyophonus* in "weaker" fish (i.e., females) or large fish with shore preference. Similarly, clinical signs associated with the parasite seem to increase upriver, though gear types differ, thus making direct comparisons difficult. Additionally, clinical pathology associated with *Ichthyophonus* is ambiguous and therefore the correct identification of the parasite using this non-specific immune response is unreliable. Results of this study show no difference in spawning success, as determined by egg expulsion, between infected and uninfected females. Further, *Ichthyophonus* prevalence is potentially correlated to age and sex and it is recommended to include ASL data collection as part of any future *Ichthyophonus* sampling protocol.

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APPENDIX A

Appendix A1.–Chinook salmon sampling schedule for *Ichthyophonus* at Emmonak, Alaska, 2004.

Emmonak (Lower Yukon River)		
Date Ranges	Targeted Sample	Actual Sampled
5/26–5/29	1	0
5/30–6/5	6	10
6/6–6/12	16	13
6/13–6/19	25	16
6/20–6/26	27	30
6/27–7/3	18	17
7/4–7/10	9	16
7/11–7/17	2	2
Total Samples:	105	104
Sampling Goal:	103	

Appendix A2.–Chinook salmon sampling schedule for *Ichthyophonus* at Tanana, Alaska, 2004.

Tanana (Middle Yukon River)				
Date Ranges	Targeted Sample	Actual Lethal Sampled	Actual Non-Lethal Sample	Actual Total Sampled
6/20–6/26	0	0	0	0
6/27–7/3	142	60	5	65
7/4–7/10	108	90	105	195
Total Samples:	250	150	110	260
Sampling Goal:	250			

Appendix A3.–Chinook salmon sampling schedule for *Ichthyophonus* at various sites in the Chena River, Alaska, 2004.

Chena River						
	Mouth		Lower		Spawning Grounds	
Date Ranges	Targeted Sample	Actual Sampled	Targeted Sample	Actual Sampled	Targeted Sample	Actual Sampled
6/27–7/3	4	0	5	0	3	0
7/4–7/10	13	2	15	23	13	0
7/11–7/17	41	0	58	25	53	0
7/18–7/24	32	0	53	48	56	18
7/25–7/31	11	0	22	0	28	33
8/1–8/7	2	0	4	0	4	35
8/8–8/14	0	0	1	0	1	1
Total Samples:	103	2	158	96	158	87
Sampling Goal:	100		150		150	

Appendix A4.–Chinook salmon sampling schedule for *Ichthyophonus* at various sites in the Salcha River, Alaska, 2004.

Salcha River				
	Mouth		Spawning Grounds	
Date Ranges	Targeted Sample	Actual Sampled	Targeted Sample	Actual Sampled
6/27–7/3	4	0	1	0
7/4–7/10	16	31	9	0
7/11–7/17	49	44	40	0
7/18–7/24	58	12	62	18
7/25–7/31	22	13	32	34
8/1–8/7	4	0	6	37
8/8–8/14	1	0	1	15
Total Samples:	154	100	151	104
Sampling Goal:	150		150	

APPENDIX B

Appendix B1.–Chinook salmon mean length (mm) from samples collected for *Ichthyophonus* from Emmonak, Tanana, and Chena and Salcha rivers, Alaska, 2004.

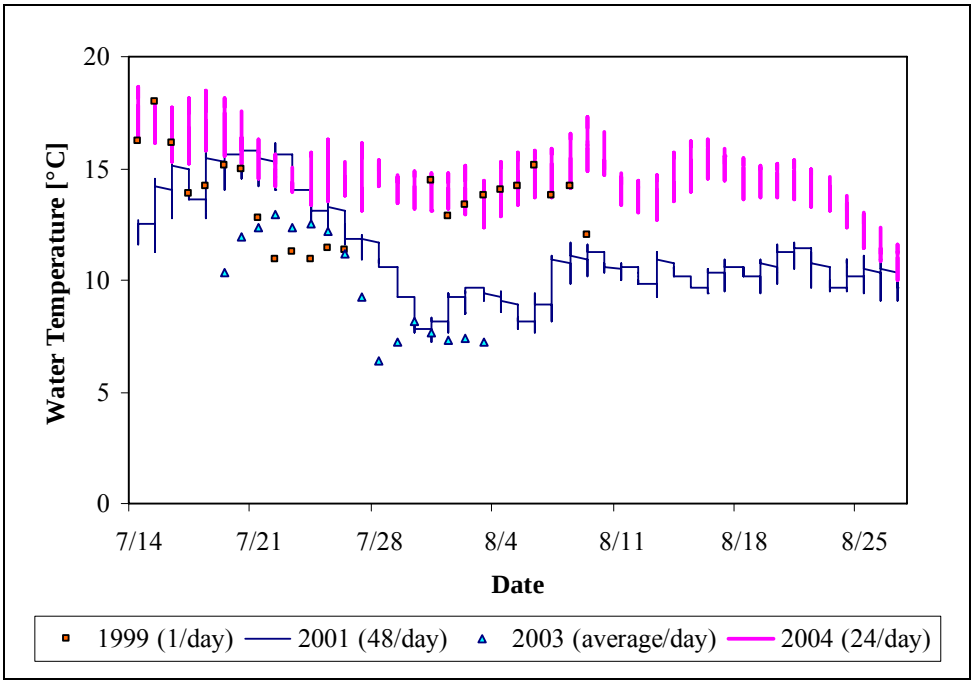
Sample Location (Source)	Sex	Length	4 Years	5 Years	6 Years	7 Years	Total
Emmonak	Males	Mean	599	777	846	-	
(Gillnet)		Standard Error	16.6	17.5	11.0	-	
		Minimum	535	625	755	-	
		Maximum	680	840	920	-	
		Sample Size	8	11	17	0	36
	Females	Mean	-	800	859	933	
		Standard Error	-	-	5.3	27.5	
		Minimum	-	-	810	905	
		Maximum	-	-	920	960	
		Sample Size	0	1	37	2	40
Tanana	Males	Mean	624	708	836	-	
(Fish Wheel)		Standard Error	11.4	12.9	43.0	-	
(Non-Lethal)		Minimum	600	610	725	-	
		Maximum	650	805	1015	-	
		Sample Size	4	17	6	0	27
	Females	Mean	-	749	855	910	
		Standard Error	-	14.3	8.9	-	
		Minimum	-	690	730	-	
		Maximum	-	800	975	-	
		Sample Size	0	6	39	1	46
Chena River	Males	Mean	606	692	815	975	
(Carcasses)		Standard Error	9.4	27.2	23.6	75.0	
		Minimum	560	580	695	900	
		Maximum	670	790	945	1050	
		Sample Size	12	7	13	2	34
	Females	Mean	-	819	882	943	
		Standard Error	-	16.7	5.6	22.5	
		Minimum	-	765	820	920	
		Maximum	-	870	980	965	
		Sample Size	0	5	39	2	46
Salcha River	Females	Mean	-	825	882	935	
(Carcasses)		Standard Error	-	5.0	4.3	65.0	
		Minimum	-	820	780	870	
		Maximum	-	830	1020	1000	
		Sample Size	0	2	93	2	97

Appendix B2.–Radiotelemetry tag final locations, grouped by tributaries and harvest areas on mainstem Tanana River, Alaska, 2004.

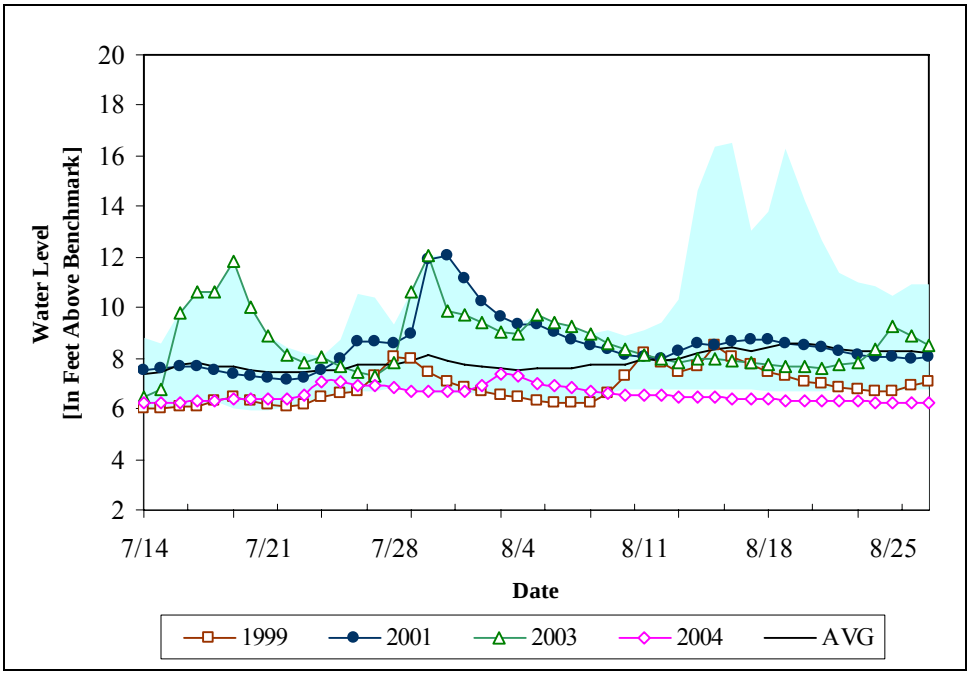
Drainage	Area Located	Number of Tags/(Harvested)	Percent of Total
Kantishna River	Kantishna River	3	0.03
	Clear Creek	2	0.02
	Bear Paw River	1	0.01
	Lake Minchumina	1	0.01
	Toklat River	5	0.05
	Barton Creek	2	0.02
Chatanika River	Tolovana River	5	0.05
	Chatanika River	2	0.02
Chena River	Chena River	7 (2)	0.06
Salcha River	Salcha River	10	0.09
Goodpaster River	Goodpaster River	1	0.01
	Tributary Locations Subtotal	39	0.35
Tanana River Mainstem			
	Tanana Village to Manley Hot Springs	11	0.10
	Manley Hot Springs to Nenana	33 (2)	0.30
	Nenana to Chena River	6 (1)	0.05
	Chena River to Salcha River	2	0.02
	Reported in Commercial Fishery	2	0.02
	Subtotal	54	0.49
Yukon River–Downstream from tagging site		5	0.05
Unknown		12	0.11
	Outside Tanana River Subtotal	17	0.15
	Total	110	

Appendix B3.–Water temperatures [ºC] in 1999, 2001, 2003 and 2004 (a) and water levels 1999, 2001, 2003, and 2004 (b) compared to historical 1987–2003 maximum, average, and minimums, Salcha River, Alaska.

(a)

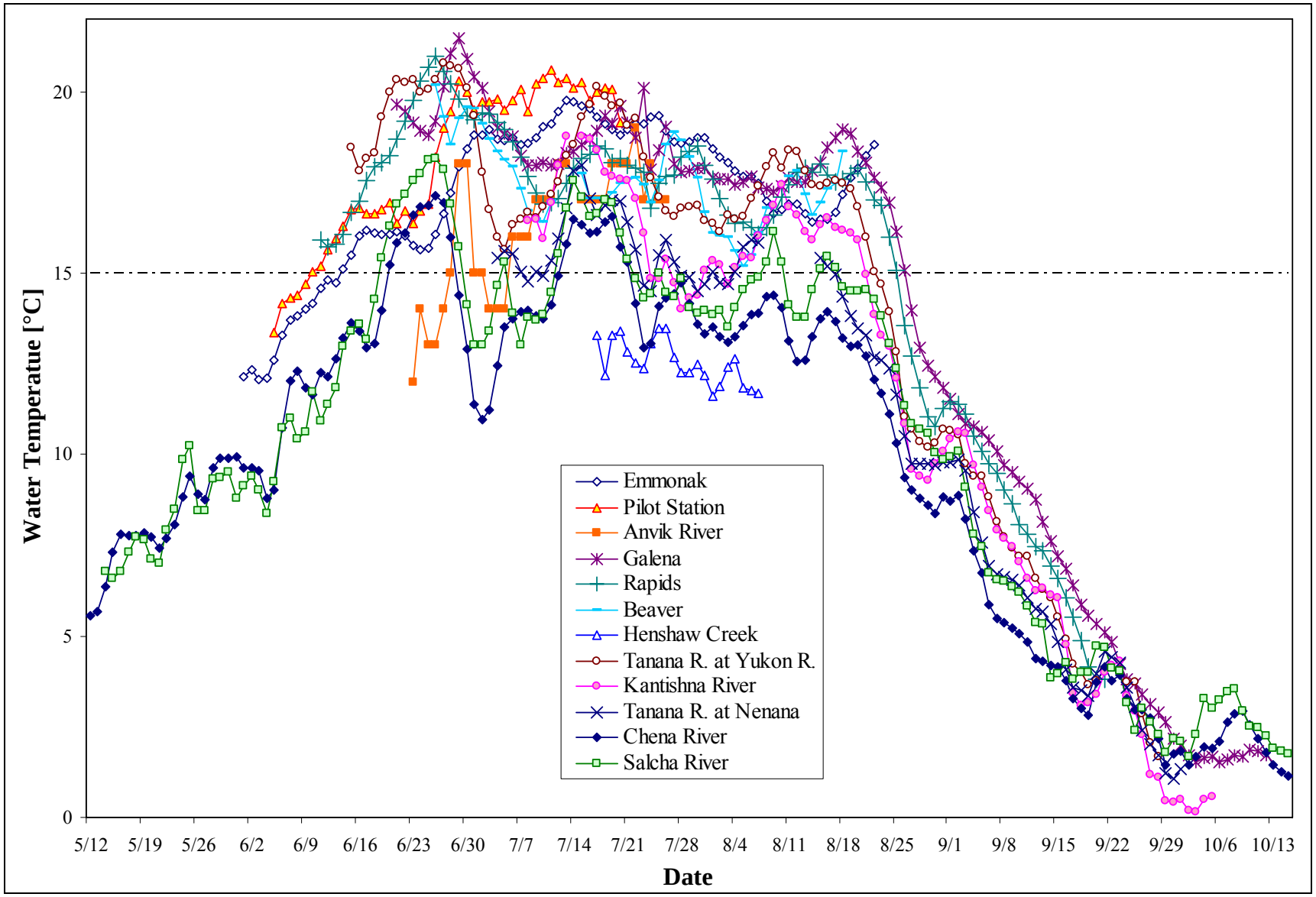


(b)

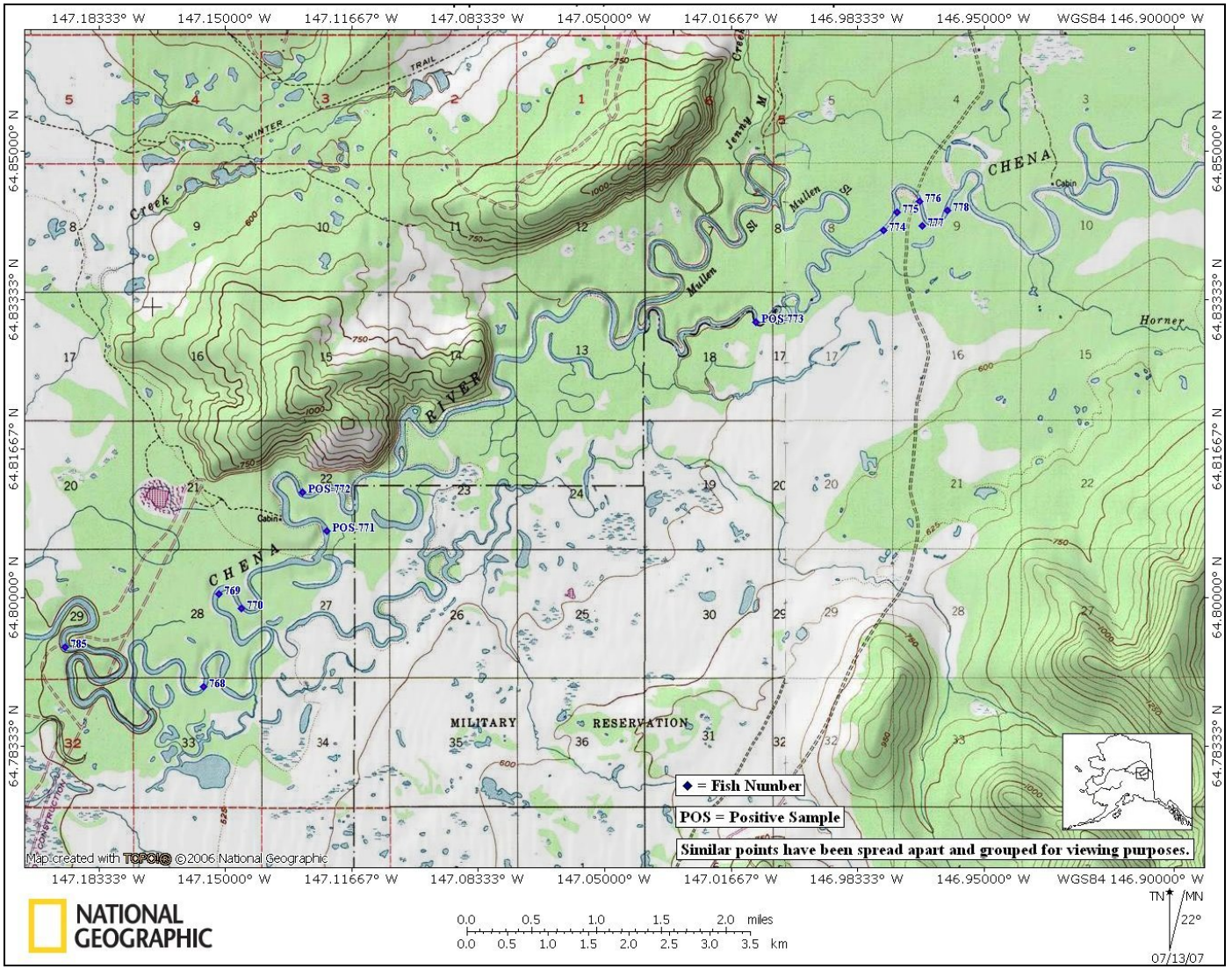


Note: Temperatures in 1999 were obtained daily, in 2001 they were taken half-hourly, in 2003 they were collected every 3 minutes but provided as average per day and in 2004 they were measured hourly.

Appendix B4.–Water temperatures [ºC] collected within the Yukon River drainage near communities or within tributaries, Alaska, 2004.



Appendix B5.–Locations of Chinook salmon carcasses collected for *Ichthyophonus* sampling in the Chena River, Alaska, 2004.



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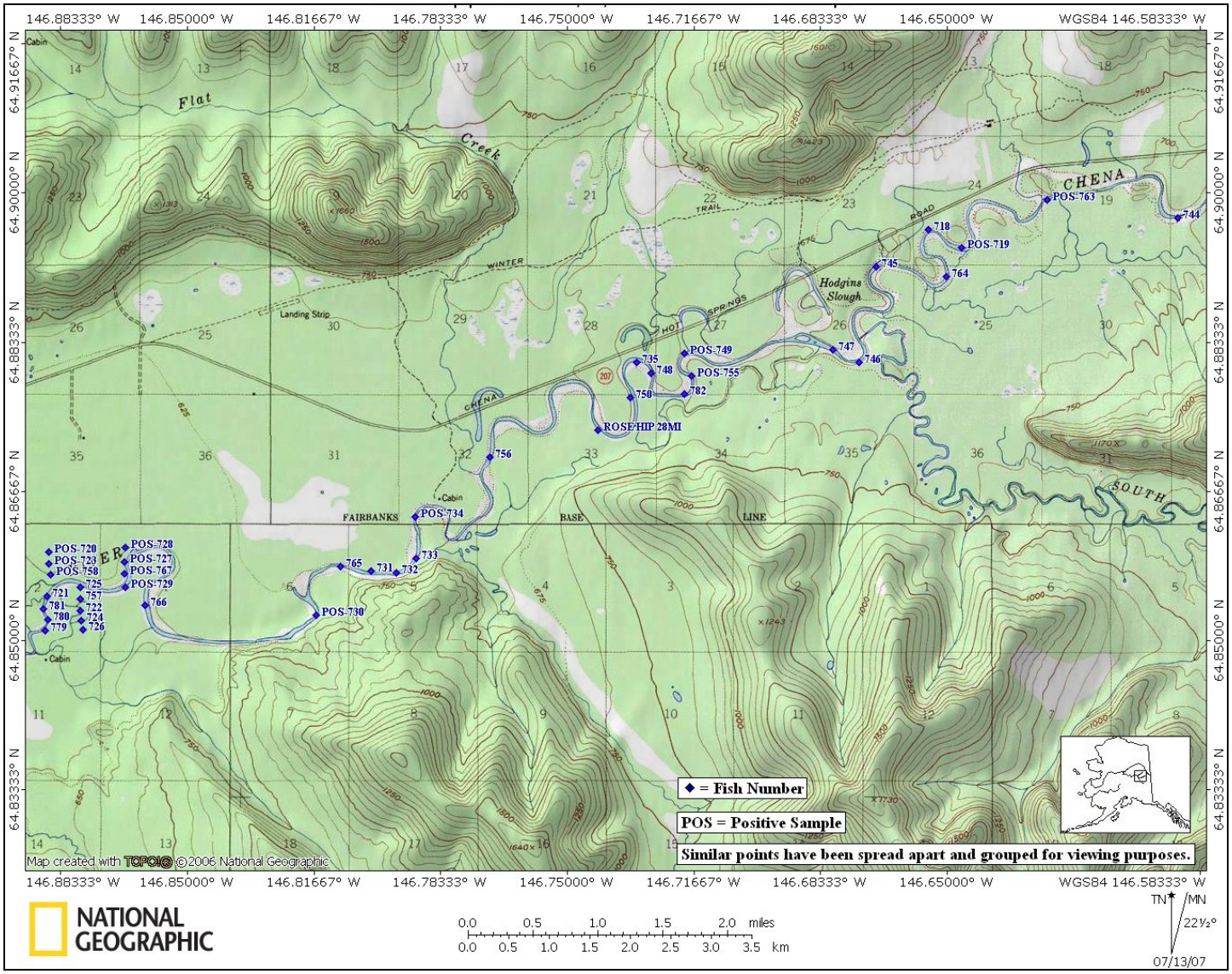
Appendix B5.–Page 2 of 2. (Map 2)



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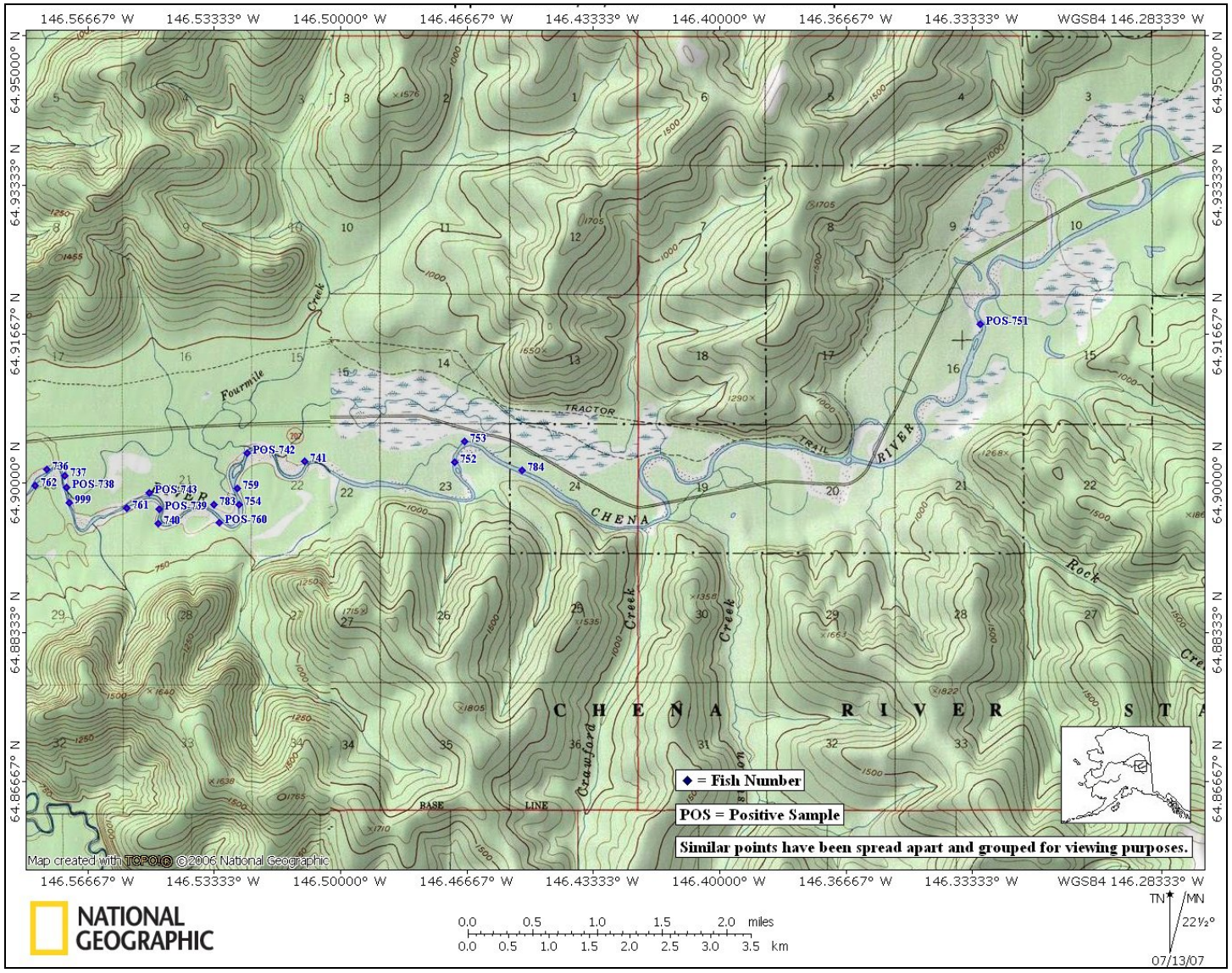
Appendix B5.–Page 3 of 3. (Map 3)



Appendix B6.–*Ichthyophonus* prevalence of Chinook salmon by sex from lower river to upriver sampling sites, based on described detection methods, Alaska, 2004.

	Total Sampled [a]				Males				Females			
Sample Size	Number Positive	n	Prevalence [%]		Number Positive	n	Prevalence [%]		Number Positive	n	Prevalence [%]	
Emmonak	23	104	22.1	[b]	5	43	11.6		18	59	30.5	
Tanana Non-Lethal	9	109	8.3	[c]	2	28	7.1		5	51	9.8	
Tanana Lethal	17	150	11.3	[d]	11	127	8.7		6	23	26.1	
Lower Chena	13	96	13.5	[e]	2	30	6.7		11	66	16.7	
Chena Spawning Grounds	31	86	36.1	[d]	13	38	34.2		18	48	37.5	
Lower Salcha	3	100	3.0	[f]	ND	ND	-		-	-	-	
Salcha Spawning Grounds	14	102	13.7	[d]	ND	ND	-		14	102	13.7	
Total	110	747	14.7		33	266	12.4		72	349	20.6	

Note: n = sample size, % = percent positive, ND= No Data.

[a] Total sample size was not classified by sex.

[b] Detection by heart polymerase chain reaction (PCR).

[c] Detection by muscle PCR.

[d] Detection by heart culture.

[e] Detection by culture muscle.

[f] Detection by muscle PCR.