Fishery Data Series No. 11-11

Prevalence of *Ichthyophonus* in Chinook Salmon Entering the Yukon River and at Tanana Stock Spawning Grounds, 2004–2006

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)

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

Adult Chinook salmon *Oncorhynchus tshawytscha* were sampled for *Ichthyophonus* in three sites within the Yukon River drainage in 2005 and 2006 as part of a three-year study that began in 2004. Cardiac muscle samples were collected from adult Chinook salmon at their entrance into the Yukon River mouth near the community of Emmonak and from the clear water tributaries of the Chena and Salcha rivers (spawning grounds) more than 1,500 km from the sea. Samples were collected using both explant culture and polymerase chain reaction (PCR) as part of feasibility testing of the newly developed PCR technique for *Ichthyophonus* in salmonids. Long-term emphasis of the project was to ascertain the possible effects of the parasite on reproductive success and the possibility of prespawning mortality. Prevalence of infection based on explant culture of cardiac muscle was 24.0% at Emmonak, 11.6% at Chena, and 12.1% at Salcha in 2005 and 16.3% at Emmonak, 12.8% at Chena, and 11.5% at Salcha in 2006. Agreement between the explant culture and PCR results were within 2.1% of each other over all samples. Prevalence was not significantly different between sexes as determined by explant culture of cardiac muscle at any of the sample sites in this study. Clinical signs of disease increased as the Chinook salmon migrated upstream to the spawning grounds in the Chena and Salcha rivers in 2005; however, they declined in 2006 during the migration. Spawning success was evaluated based on internal examination for three criteria: spawned out, partially spawned and unspawned. On both the Chena and Salcha rivers in 2005 and 2006, there was no significant difference between infected and uninfected Chinook salmon and female spawning success based on a measurement of expulsion of gametes. Correlation of prevalence with environmental factors is difficult at this time since the datasets are not long enough.

Keywords: *Ichthyophonus*, Yukon River, Tanana River, Salcha River, Chena River, Chinook salmon, *Oncorhynchus tshawytscha*.

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; Mendoza et al. 2002). *Ichthyophonus* has been of considerable economic concern to fishermen causing mass mortalities of Atlantic herring (*Clupea harengus*) (Mellergaard and Spanggaard 1997; Rahimian 1998) and Pacific herring (*C. pallasi*) (Marty et al. 1998; Kocan et al. 1999).

In Alaska, *Ichthyophonus* was first identified in 1988 in Chinook salmon (*Oncorhynchus tshawytscha*) of the Yukon River drainage (Anchorage Fish Pathology Laboratory disease history database, June 1988) Since then, occurrence of Ichthyophoniasis (the disease caused by the *Ichthyophonus* infection, characterized with nodular lesions in visceral organs and skeletal muscle) has been described in a variety of fish species, including sockeye (*O. nerka*) and coho salmon (*O. kisutch*) from more than 20 Alaskan locations.

In the Yukon River, since the initial discovery of *Ichthyophonus* in 1988, both fishermen and fish processors have reported an increase in the number of Chinook salmon with Ichthyophoniasis throughout the entire run. Processors in the upper Yukon River reported that as many as 20% of the purchased Chinook salmon were discarded in some years because of muscle tissue damage (Kocan et al. 2004). In 2004 and 2005 fishing seasons, approximately one percent of the Chinook salmon harvested for subsistence use was reported discarded by fishermen from surveyed communities in Alaska's Yukon Area and 10% to 20% of these discards were reported to be unpalatable due to disease (Busher et al. 2007 and 2008). Fishermen in the upper Yukon River indicated that the severity of *Ichthyophonus* in Chinook salmon (or diseases with similar clinical appearance) was variable from year-to-year. Rahimian (1998) described a passive stage (resting spores) of the parasite that was activated by a mechanism that is yet unknown. Stress (high cortisol) and increased water temperatures were shown to accelerate *Ichthyophonus* infection (Okamoto et al. 1987; Halpenny et al. 2002; Perry et al. 2004). Prevalence varied seasonally and with age in Atlantic herring with spring spawning fish being the most heavily affected (Rahimian and Thulin 1996).

Effects of *Ichthyophonus* on Chinook salmon in the Yukon River have been studied by Kocan et al. (2003 and 2004), who reported that approximately 25% to 30% of Chinook salmon entering the Yukon River were infected with *Ichthyophonus*. The prevalence remained constant until fish reached the upper Yukon River (2,792 river kilometer) where it then dropped to 10% or less. Further, only a few of the successfully spawning females sampled were infected with *Ichthyophonus* suggesting that females with Ichthyophoniasis were dying prior to spawning (Kocan et al. 2004). Kocan et al. (2006, 2009) also showed that experimentally infected salmonids suffer cardiac damage and reduced swimming stamina. This is in agreement with studies by Rahimian (1998) who histologically described massive tissue necrosis and loss of function in infected organs. These findings, however, lack information about implications of *Ichthyophonus* on fisheries management.

The Yukon River Chinook Salmon fishery is managed based on escapement goals. Under this management scheme, fisheries are restricted when the run size is too low to meet escapement goals. Typically, escapement goals are monitored at the mouth of spawning rivers below spawning grounds, in which all counted fish are *assumed* to spawn successfully. Hence, there could potentially be considerations for fisheries management if Ichthyophonus-related mortality is occurring upstream of escapement monitoring sites before spawning. If this occurs, escapement goals could be increased to account for the mortality.

The objectives of this study are to: 1) determine prevalence of *Ichthyophonus* in Chinook salmon as it pertains to conservation and management implications in the Yukon River drainage; 2) assess spawning success of infected fish and potential for prespawning mortality, from lower Yukon River and upstream in Chena and Salcha river tributaries; 3) investigate the potential correlation of environmental factors with prevalence of *Ichthyophonus* infection. This report concludes a three-year project from 2004 to 2006. As such, some results from the 2004 study (Kahler et al. 2007) are also included.

METHODS

FISH COLLECTION PROCEDURES

In 2005 and 2006, Chinook salmon were collected at three of the 2004 established locations (Emmonak, Chena and Salcha) within the Yukon River drainage (Figure 1). Sampling at Emmonak has been serving as baseline of infection prevalence for Chinook salmon entering the river (Kocan et al. 2004; Kahler et al. 2007). Chinook salmon were sampled from a test fishery operated by Alaska Department of Fish and Game (ADF&G) to avoid sacrificing additional fish. The test fishery used 8.5-inch stretch mesh set gillnets. Chinook salmon were sampled over the entire run from June 2 through July 12 in 2005 and from June 7 through July 11 in 2006. The weekly sample goals were based on 1980–2004 and 1980–2005 average run timing respectively for the Emmonak test fishery project (Appendix C1) with a target sample goal of n=105 each year.

Samples on the Chena and Salcha rivers were collected on the spawning grounds via boat surveys with spotters viewing the water and shoreline for possible carcasses for sampling. The samples were collected by hand or using a gig from the shore or bottom of the river, being careful to avoid puncturing the body cavity with the gigs to prevent sample contamination. To maximize efficiency in the search for carcasses, sampled fish were cut in half to avoid identifying them as a whole fish resulting in additional stops. Carcasses were either returned to the original location or left at water line. In 2005, samples along the Chena River were collected from July 22 through August 12 and along the Salcha River from July 17 through August 12. In 2006, the Chena and Salcha rivers sampling occurred from July 28 through August 12 and July 28 through August 14, respectively. Locations of samples on the Chena and Salcha rivers were documented with global positioning systems (GPS) in 2005 and 2006.

In 2005 and 2006, samples were limited to relatively fresh Chinook salmon carcasses, meeting criteria: clear eyes, some color in the gills (red-pink) and a firm cardiac muscle (criteria 1). These criteria were used in 2004 at Chena River, but not at Salcha River which used slightly different criteria: clear eyes and firm heart (irrespective of gill color, designated criteria 2) following Kocan et al. (2004). Because of the perceived alteration in sampling protocol in 2004 the difference between the two methods had to be examined in subsequent years of the study. Estimates of *Ichthyophonus* prevalence based on criteria 2 tends to be slightly lower than that of criteria 1 (Appendix A1).

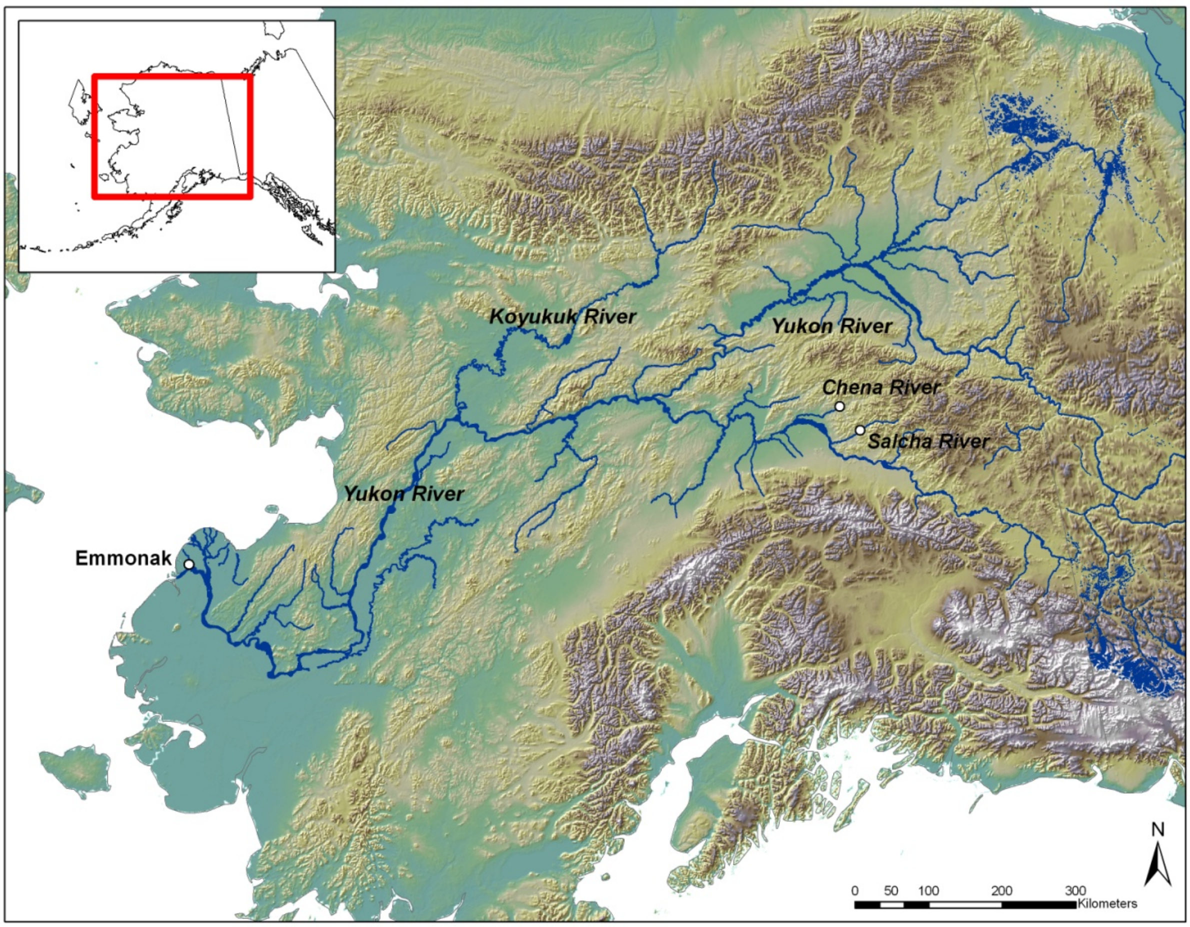

Figure 1.–Map of the Yukon River drainage showing the location of sampling sites for Chinook salmon, indicating Emmonak near the river mouth and the Chena and Salcha River spawning grounds, 2005–2006.

Age, sex, and length (ASL) data were collected from samples at Emmonak and the Chena and Salcha rivers (spawning grounds) using standard collecting procedures (Molyneaux and DuBois 1999). Sex was determined by internal examination of gonads in all of the lower river samples. In the upper river samples, determination of sex included both internal and external examination of morphology.

GROSS CLINICAL DETECTION

Clinical signs of *Ichthyophonus* infection were noted by examining for the presence of "white spots" in heart, liver, spleen, and kidney during each necropsy. However, because "white spots" are also caused by other pathogens, only fish with presence of *Ichthyophonus* confirmed by culture/PCR (polymerase chain reaction) method were determined as clinical. The "white spots", or granulomas, are an inflammatory response of fish to foreign bodies in general (Corbel 1975; Finn and Nielson 1971). The granulomas consist of lymphocytes, macrophages, neutrophils and firm connective and fibrous tissue. In the Yukon River drainage other pathogens besides *Ichthyophonus* may cause similar white spots in tissues. In this study, individuals collecting samples and identifying potential clinical signs of *Ichthyophonus* varied among the three sampling locations so individual sampler biases are possible.

PATHOLOGY SAMPLES

Collection of cardiac muscle samples followed the procedures established in 2004 (Kahler et al. 2007). Cardiac tissue samples were collected in duplicate for each Chinook salmon with one stored in culture media and one in ethanol. Presence of *Ichthyophonus* infection was tested using both explant culture and PCR methods. For the culture method, approximately 0.5 g of cardiac muscle was aseptically collected and stored in 7 ml Eagle's minimal essential medium (MEM) 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 this study). The tissue was incubated at 14˚C for a minimum of 14 days. The samples were examined every other day to monitor growth and viewed microscopically (100x magnification) for determining the presence of *Ichthyophonus* spores. For the PCR method, approximately 0.5 g cardiac muscle was stored in 95% ethanol. PCR tests for detection of *Ichthyophonus* 18S rDNA 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). The major difference between the explant culture test and the PCR test is viability of *Ichthyophonus* spores. While the explant culture test detects presence of viable *Ichthyophonus* spores, the PCR test detects presence of *Ichthyophonus* rDNA that could be viable or nonviable. A comparison of both culture and PCR tests for *Ichthyophonus* prevalence is provided in Appendix B1.

EGG RETENTION

To examine whether *Ichthyophonus* infected females spawn successfully, egg retention in Chinook salmon was measured. Eggs were collected from the body cavity of female Chinook salmon and measured in a 2000 ml graduated cylinder (by volume) with increments of 20 ml; a subsample of at least a half a cup was collected from each sample and stored in a cooler for the day. Once back at the laboratory, the subsample was again measured for volume in a 100 ml graduated cylinder with increments of 1 ml and the total number of eggs in the subsample was counted. Estimated total number of eggs retained was calculated based on the number of eggs in the subsample divided by the volume of the subsample and multiplied by the volume of the entire sample.

Chinook salmon sampled on the spawning grounds were categorized into three groups based on the amount of gametes retained in each carcass. The category “spawned out” was defined as approximately 5% or less of the gametes remaining in the body cavity. The category “partially spawned” was defined as between approximately 5% and 50% of the gametes remaining in the body cavity (some eggs, not whole skeins), while the category of “unspawned” was reserved for fish that were still gravid (whole skeins/intact milt sacs) or more than 50% of the gametes retained.

ENVIRONMENT DATA

Water temperature data were collected at select locations along the Yukon River drainage to monitor environmental factors contributing to the progression of *Ichthyophonus* once Chinook salmon enter freshwater (Figure 2). HOBO Data Logger Pro or HOBO Tidbits[1] were deployed within the Yukon River watershed to collect water temperatures, typically in conjunction with an operating fishery monitoring project. Sites on the Yukon River mainstem, that operated during the majority of the Chinook salmon migration, included locations near the following communities: Emmonak, Pilot Station, Galena, Tanana (left bank below confluence of Tanana River), Rapids, Beaver, and Eagle. In addition, temperatures were obtained from some tributaries including Henshaw Creek, Andreafsky, Anvik, Gisasa, Tanana (downstream of Nenana), Chena, Salcha, and Kantishna rivers. To maintain consistency between sampling sites, water temperatures were recorded once every hour, except for the Tanana River sites where temperatures were taken in six hour increments. Temperature collection dates varied with operational periods of fishery monitoring projects (weir, sonar, fish wheel, etc.) in the field. Water levels for selected Yukon River locations are recorded by the National Weather Service Alaska-Pacific River Forecast Center under National Oceanic and Atmospheric Administration (http://aprfc.arh.noaa.gov/ accessed November 2007). ADF&G reviews water levels at the mainstem Yukon River (Eagle and Galena) and the Salcha and Tanana rivers for use in fishery management.

In 2005, water temperatures were collected at each fish sampling location within the spawning grounds on the Chena and Salcha rivers. In 2006, both water and air temperatures were collected at each fish’s sampling location on the spawning grounds. However, daily water temperature measurements for the Salcha River were not collected or recovered throughout the season using a logger. However, water temperatures were collected with a digital hand held thermometer at each of 259 sampling sites over the course of 17 days in the field.

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

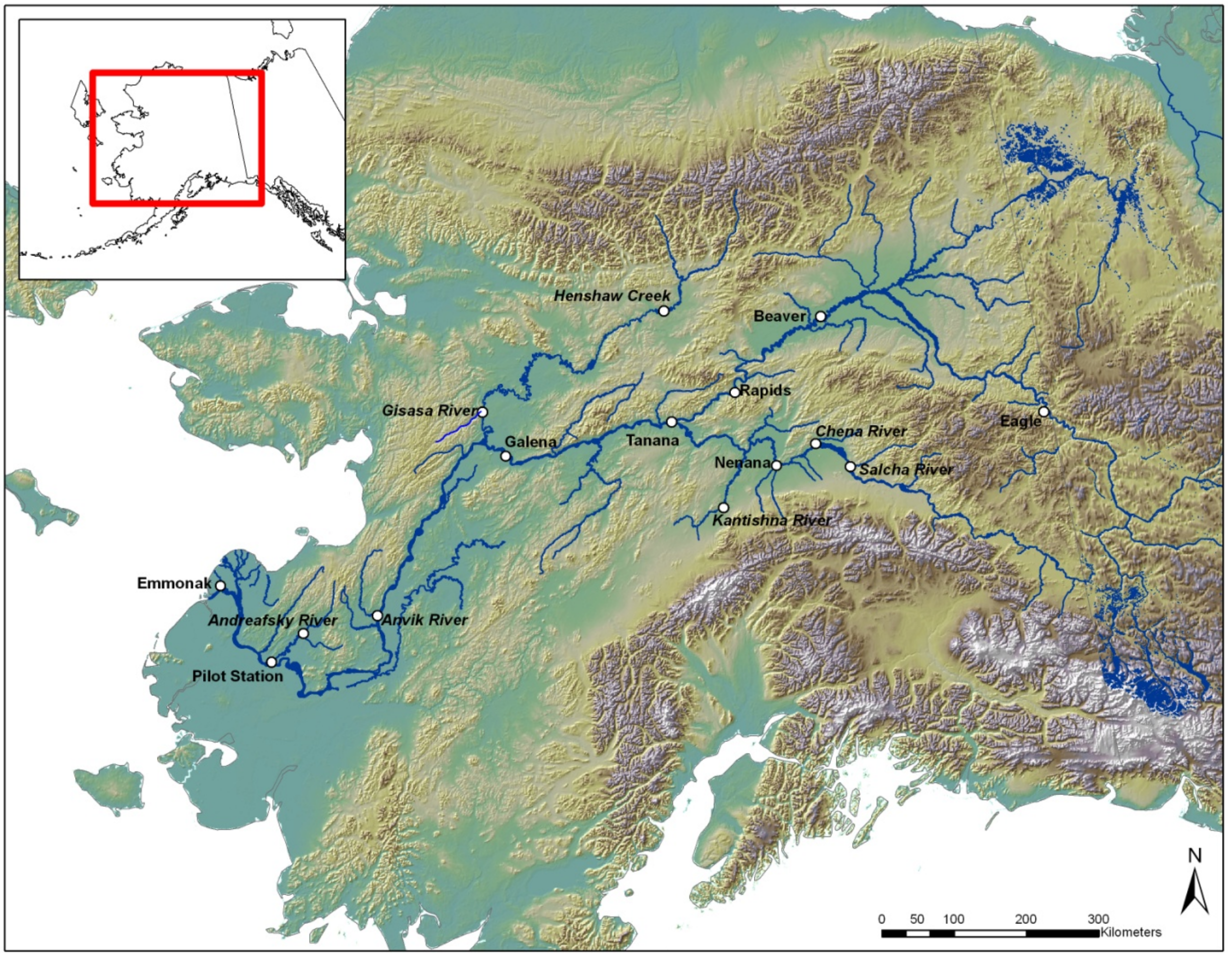


Figure 2.–Selected water temperature collection sites during Chinook salmon migration within the Yukon River drainage, Alaska, 2005 and 2006.

DATA ANALYSIS

For all three locations, presence of *Ichthyophonus* infection was determined based on explant culture method. For Chena and Salcha spawning grounds, only samples that met criteria 1 were analyzed.

To determine whether *Ichthyophonus* affects certain segments of a population, differences were examined, using chi-square tests, in infection prevalence between males and females and by age classes, where sample sizes permitted.

To determine whether *Ichthyophonus* pre-spawning mortality occurs upriver of an escapement monitoring site, infection prevalence was compared between lower and upper river spawning sites. In this analysis, the Chena River sampling sites were divided at river km 45 (Roseship boat launch), and the Salcha River sampling sites were divided at the confluence of Ninety-eight Creek at river km 58. The above were examined using a 2-sample z-test or chi-square test.

To examine whether *Ichthyophonus* infected fish spawn as successful as uninfected fish, the proportion of "spawned out" fish was compared between the two using 2-sample z-test.

RESULTS

PREVALENCE WITHIN DRAINAGE

In both 2005 and 2006, *Ichthyophonus* prevalence was higher at Emmonak than Chena and Salcha rivers (Figure 3). While the difference was significant in 2005 (p=0.001), it was not significant in 2006 (p=0.24).

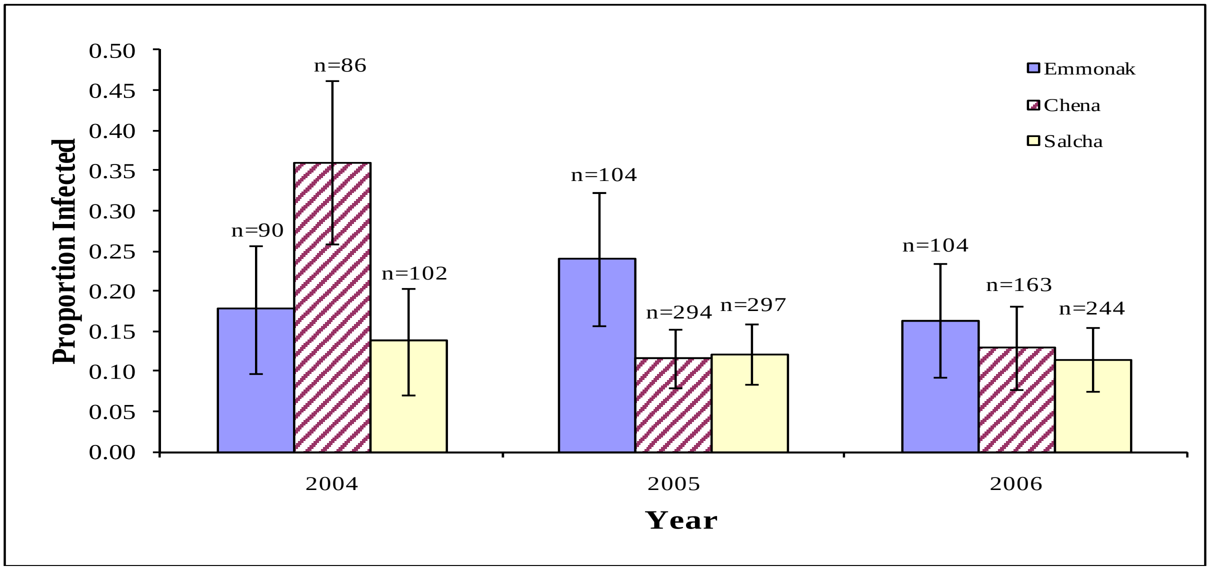


Figure 3.–Prevalence (95% CI range) of *Ichthyophonus* in Chinook salmon based on explant culture of cardiac muscle by year in Emmonak and the Chena and Salcha river spawning grounds, Alaska, 2004–2006.

Overall, females tended to have a higher prevalence of *Ichthyophonus* than males (except Chena 2006 samples); however, the difference was not significant at all three locations (2-sample z-test, p>0.05) (Figure 4).

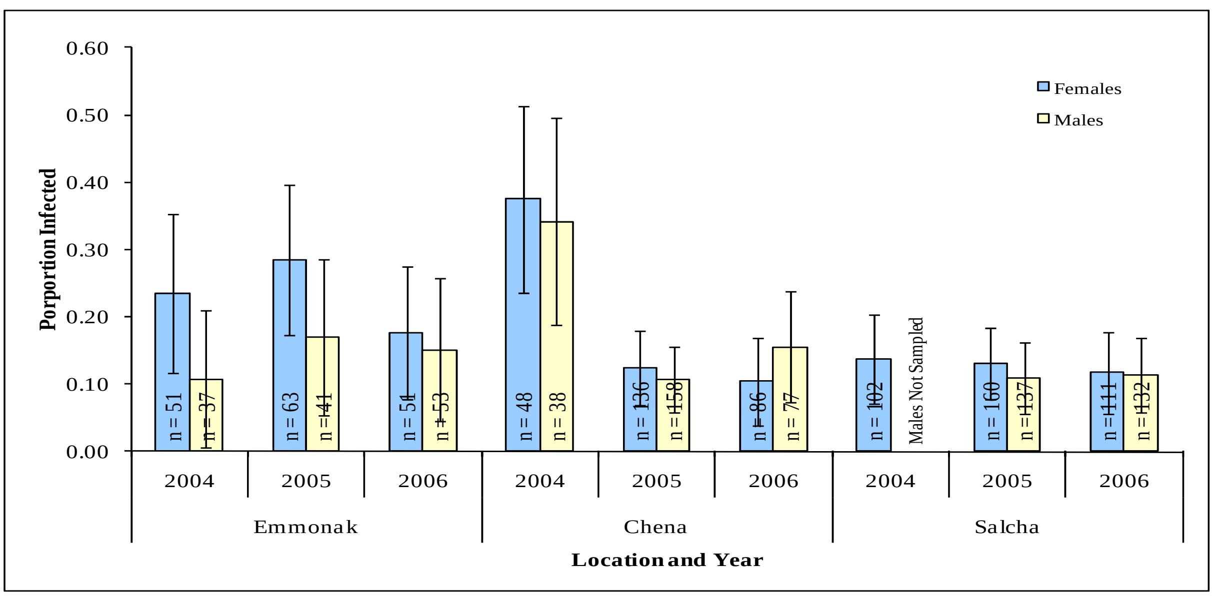


Figure 4.–Prevalence (95% CI range) of *Ichthyophonus* in Chinook salmon based on explant culture of cardiac muscle by sex at Emmonak and the Chena and Salcha river spawning grounds, Alaska, 2004–2006.

PREVALENCE AT SPAWNING RIVERS

In the Chena and Salcha rivers, Chinook salmon carcasses were collected throughout their spawning range upriver of the escapement monitoring sites (Appendices D3–D6), which resulted in similar number of samples between upstream and downstream reaches. While *Ichthyophonus* prevalence tended to be higher in the downstream reaches, there was no significant difference between upstream and downstream (Table 1) based on samples with associated GPS data.

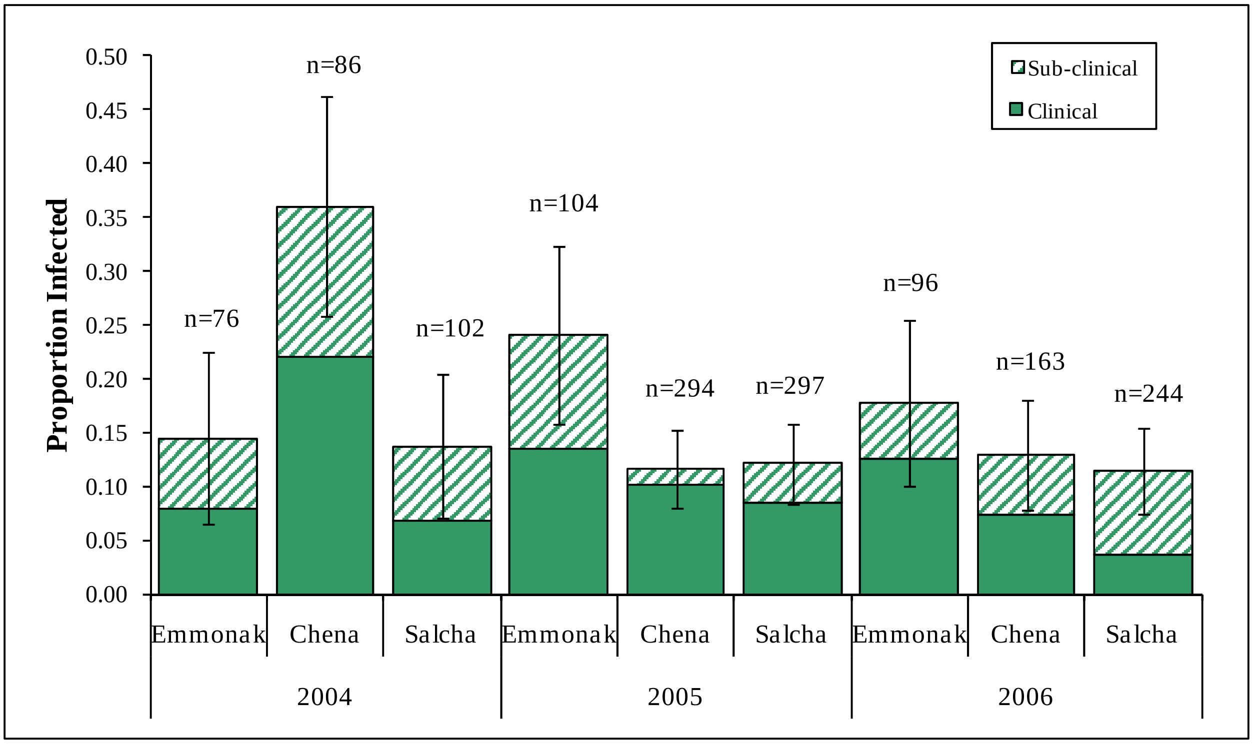
Table 1.–Comparison of *Ichthyophonus* infected Chinook salmon based on explant culture of cardiac muscle that were sampled from the downstream and upstream portions of their respective spawning areas on the Chena and Salcha rivers, 2005–2006.

	Chena River							
	Downstream			Upstream				
Year	*n*	No. Positives	Percent	*n*	No. Positives	Percent	χ^2	*p*
2005	166	27	16.3	134	16	11.9	1.13	0.29
2006	85	12	14.1	76	10	13.2	0.03	0.86
	Salcha River							
2005	167	26	15.6	133	17	12.8	0.47	0.49
2006	148	22	14.9	111	10	9.0	2.01	0.16

GROSS CLINICAL SIGNS

Cardiac muscle was the primary organ (99%) showing clinical signs (white spots or granulomas) at all sampling locations (Emmonak, Chena and Salcha). Two fish (Salcha River in 2005 and 2006) testing positive by explant culture of cardiac muscle showed clinical signs only in the spleen. The organs showing clinical signs of *Ichthyophonus* infection included 59.6% cardiac muscle, 17.0% kidney and 23.4% spleen from the explants culture samples (n=171). One hundred percent of the fish with clinical signs in the kidney also had signs in the cardiac muscle, while only 42.0% of those with signs in the kidney also had signs in the spleen.

In Emmonak, the proportion of *Ichthyophonus* infected fish with clinical signs was 56.0% and 70.6% in 2005 and 2006, respectively (Figure 5). On the spawning grounds, the proportions were 88.2% and 69.4% at the Chena and Salcha rivers, respectively in 2005. And the proportions were 57.1% and 32.1% at the Chena and Salcha rivers, respectively in 2006.



Note: Error bars are 95% CI ranges for both sub-clinical and clinical combined.

Figure 5.–Prevalence of *Ichthyophonus* in clinically and sub-clinically infected Chinook salmon, tested by explant culture from cardiac muscle from selected locations within the Yukon River drainage, Alaska in 2004–2006.

EGG RETENTION

Female Chinook salmon were categorized as fully “spawned out”, “partially spawned” and “unspawned” for a total of 654 examinations along the Chena and Salcha river combined from 2004 to 2006 (Figure 6). Physical measurements of number of eggs retained were only collected in 2005 and 2006 (Appendix C5). On average, female Chinook salmon were 830 mm in length, retaining 1,845 eggs. Fish sampled for egg retention were composed of 41%, 56%, and 3% of ages 5, 6 and 7, respectively (*n*=68). There was no difference in number of eggs retained by infected fish 1,719 (*n*=13) and uninfected fish 1,867 (*n*=70). Overall (Chena and Salcha 2004–2006 combined), there was no significant difference in percent of “spawned out” between infected (74.5%) and uninfected (79.4%) (2-sample z-test, p=0.132).

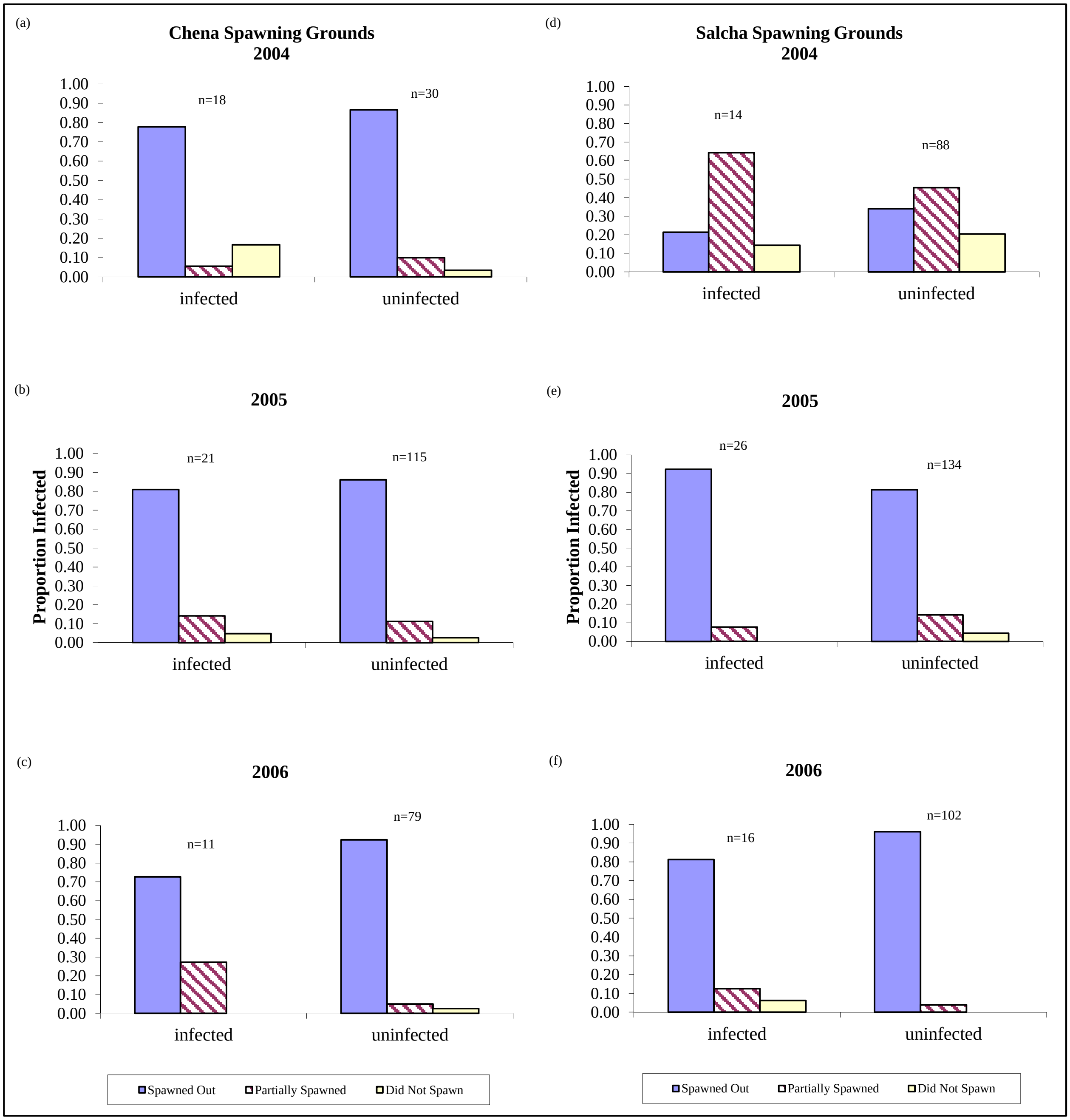
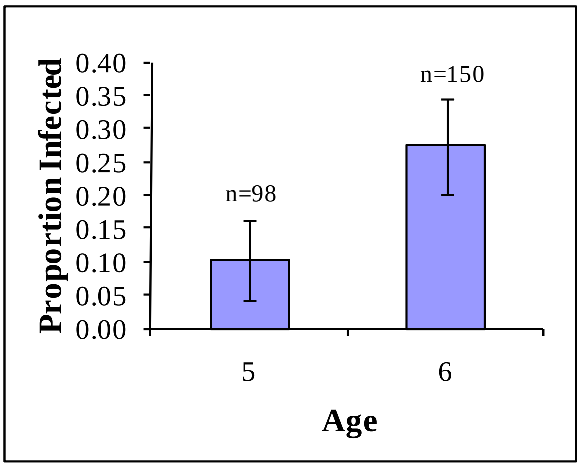


Figure 6.–Proportions by spawning category of *Ichthyophonus* infected and uninfected female Chinook salmon based on explant culture from cardiac muscle collected in the Chena (a–c) and Salcha (d–f) river spawning grounds, Alaska, 2004–2006.

AGE, SEX, AND LENGTH COMPOSITION

At all locations, age composition of the infected group was shifted towards older fish when compared to uninfected Chinook salmon. Older fish appear more likely to be infected with *Ichthyophonus*. The proportion of infected fish was significantly higher in age-6 compared to age-5 fish (p=0.002) at Emmonak (Figure 7a). Similarly, at Chena and Salcha rivers, older fish (age-4 through age-7) had higher *Ichthyophonus* prevalence in 2005 (p=0.0003) (Figure 7b). However, the difference was not significant in 2006 (p=0.19).

(a)



(b)

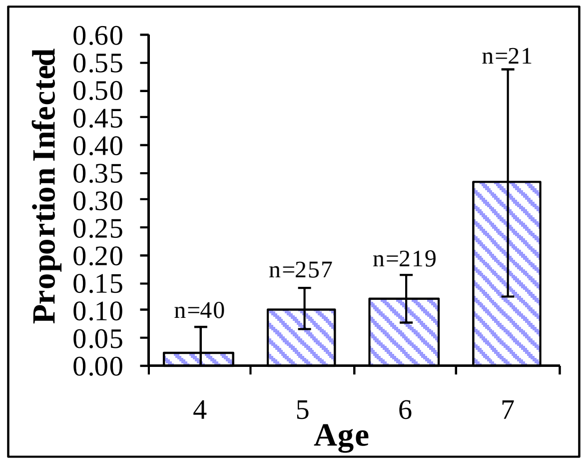


Figure 7.–Prevalence (95% CI range) of *Ichthyophonus* infected Chinook salmon tested by explant culture of cardiac muscle by age, pooled data from Emmonak samples from 2004 to 2006 (a) and pooled criteria 1 from Chena and Salcha rivers 2005 (b).

Tables 2 and 3 present the age composition of infected and uninfected fish as determined by explant culture of cardiac muscle by criteria 1 (clear eyes, some color in the gills (red-pink), and a firm heart). Mean length-at-age is given in Appendices C3 and C4.

Table 2.–Age and sex composition from *Ichthyophonus*-infected and uninfected Chinook salmon (as determined by explant culture of cardiac muscle) collected near Emmonak, and at Chena and Salcha rivers, Alaska in 2005.

Sample Location		4 Years		5 Years		6 Years		7 Years		Total	
(Source)	Sex	No.	%	No.	%	No.	%	No.	%	No.	%
Uninfected Fish											
Emmonak	Males	0	0.0	21	29.6	7	9.9	0	0.0	28	39.4
(Gillnet)	Females	1	1.4	11	15.5	30	42.3	1	1.4	43	60.6
	Subtotal	1	1.4	32	45.1	37	52.2	1	1.4	71	100.0
Chena River	Males	15	6.4	81	34.6	32	13.7	2	0.9	130	55.6
(Carcasses)	Females	0	0.0	50	21.4	50	21.4	4	1.7	104	44.4
	Subtotal	15	6.4	131	56.0	82	35.1	6	2.6	234	100.0
Salcha River	Males	24	10.0	60	24.9	30	12.4	2	0.8	116	48.1
(Carcasses)	Females	0	0.0	39	16.2	80	33.2	6	2.5	125	51.9
	Subtotal	24	10.0	99	41.1	110	45.6	8	3.3	241	100.0
All Locations	Total	40	7.3	262	48.0	229	41.9	15	2.7	546	100.0
Infected Fish											
Emmonak	Males	0	0.0	1	4.3	3	13.0	2	8.7	6	26.1
(Gillnet)	Females	0	0.0	2	8.7	14	60.9	1	4.3	17	73.9
	Subtotal	0	0.0	3	13.0	17	73.9	3	13.0	23	100.0
Chena River	Males	0	0.0	12	38.7	3	9.7	0	0.0	15	48.4
(Carcasses)	Females	1	3.2	6	19.4	7	22.6	2	6.5	16	51.6
	Subtotal	1	3.2	18	58.1	10	32.3	2	6.5	31	100.0
Salcha River	Males	0	0.0	6	19.4	6	19.4	1	3.2	13	41.9
(Carcasses)	Females	0	0.0	3	9.7	11	35.5	4	12.9	18	58.1
	Subtotal	0	0.0	9	29.1	17	54.9	5	16.1	31	100.0
All Locations	Total	1	1.2	30	35.3	44	51.8	10	11.8	85	100.0
All Samples	Total	41	6.5	292	46.3	273	43.3	25	4.0	631	100.0

Note: Infection prevalence was determined from criteria 1 samples only.

Table 3.–Age and sex composition from *Ichthyophonus*-infected and uninfected Chinook salmon (as determined by explant culture of cardiac muscle) collected near Emmonak, and at Chena and Salcha rivers, Alaska in 2006.

Sample Location		4 Years		5 Years		6 Years		7 Years		Total	
(Source)	Sex	No.	%	No.	%	No.	%	No.	%	No.	%
Uninfected Fish											
Emmonak	Males	4	5.0	28	35.0	8	10.0	0	0.0	40	39.4
(Gillnet)	Females	0	0.0	16	20.0	24	30.0	0	0.0	40	50.0
	Subtotal	4	5.0	44	55.0	32	40.0	0	0.0	80	100.0
Chena River	Males	12	9.5	40	31.7	5	4.0	1	0.8	58	46.0
(Carcasses)	Females	0	0.0	22	17.5	46	36.5	0	0.0	68	54.0
	Subtotal	12	9.5	62	49.2	51	40.5	1	0.8	126	100.0
Salcha River	Males	11	5.7	79	40.9	15	7.8	1	0.5	106	54.9
(Carcasses)	Females	0	0.0	19	9.8	65	33.7	3	1.6	87	45.1
	Subtotal	11	5.7	98	50.7	80	41.5	4	2.1	193	100.0
All Locations	Total	27	6.8	204	51.1	163	40.9	5	1.3	399	100.0
Infected Fish											
Emmonak	Males	1	5.9	6	35.5	1	5.9	0	0.0	8	47.1
(Gillnet)	Females	0	0.0	0	0.0	9	52.9	0	0.0	9	52.9
	Subtotal	1	5.9	6	35.5	10	58.8	0	0.0	17	100.0
Chena River	Males	1	6.7	4	26.7	4	26.7	0	0.0	9	60.0
(Carcasses)	Females	0	0.0	2	13.3	4	26.7	0	0.0	6	40.0
	Subtotal	1	6.7	6	40.0	8	53.4	0	0.0	15	100.0
Salcha River	Males	2	8.3	9	37.5	2	8.3	0	0.0	13	54.2
(Carcasses)	Females	0	0.0	0	0.0	10	41.7	1	4.2	11	45.8
	Subtotal	2	8.3	9	37.5	12	50.0	1	4.2	24	100.0
All Locations	Total	4	4.7	21	24.7	30	35.3	1	1.2	56	65.9
All Samples	Total	31	6.8	225	49.5	193	42.4	6	1.3	455	100.0

ENVIRONMENTAL DATA

Average daily water temperatures (June through August) ranged from 12ºC to 20ºC for the Yukon River near the community of Emmonak. Of these, the percent of days exceeding 15ºC (considered harmful) was 67%, 44% and 7% from the years 2004 through 2006 respectively (Figure 8).

In Chena and Salcha rivers, water temperature ranged from 3ºC to 16ºC and rarely exceeded 15ºC in 2005 and 2006 (Figures 9–10). As opposed to 2004 when water temperature exceeded 15ºC for 19 and 29 days for the Chena and Salcha rivers respectively. For additional water temperature data throughout the Yukon River drainage see Appendices D1 and D2.

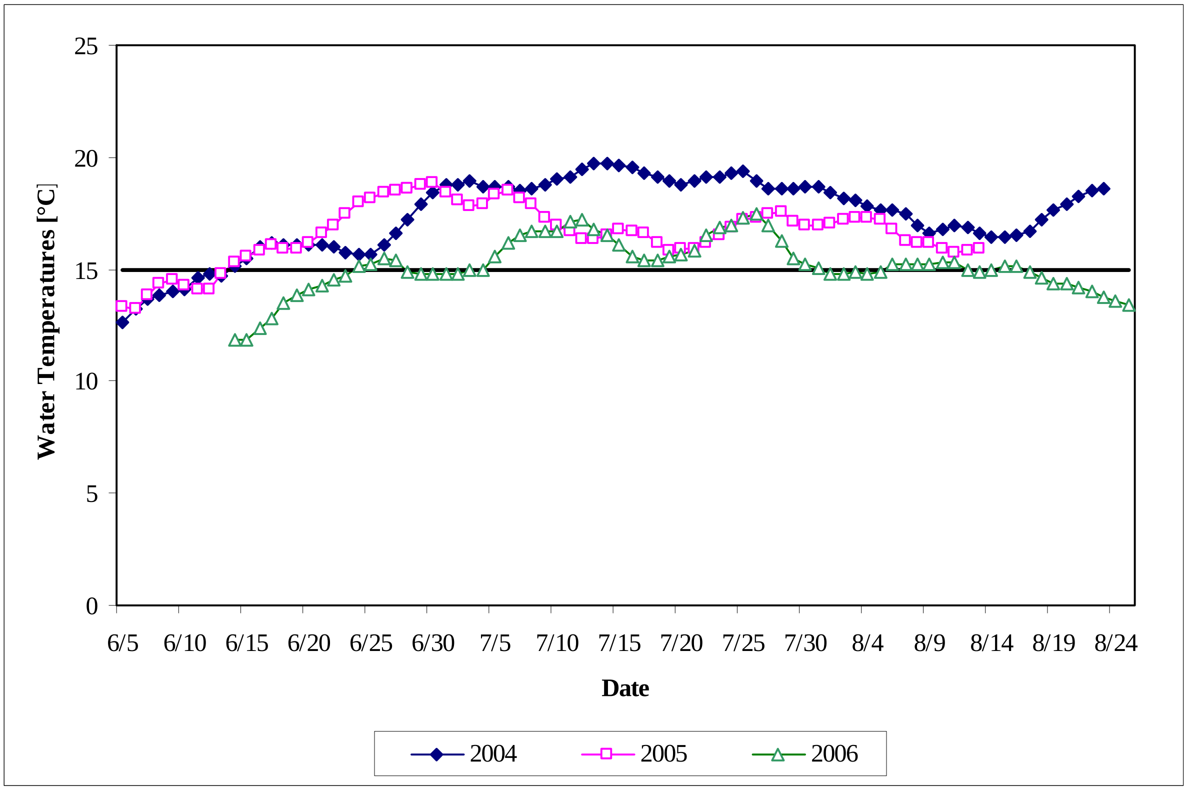


Figure 8.–Water temperatures from the Yukon River at Emmonak in 2004, 2005 and 2006.

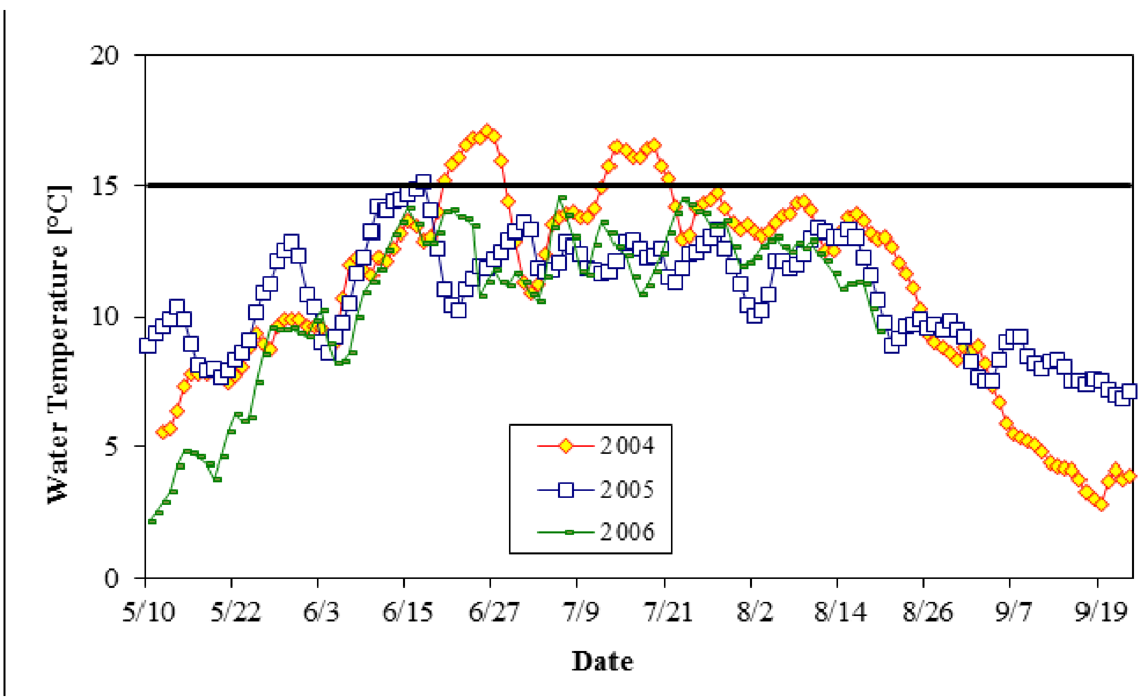


Figure 9.–Water temperatures from the Chena River in 2004, 2005 and 2006, Yukon Area.

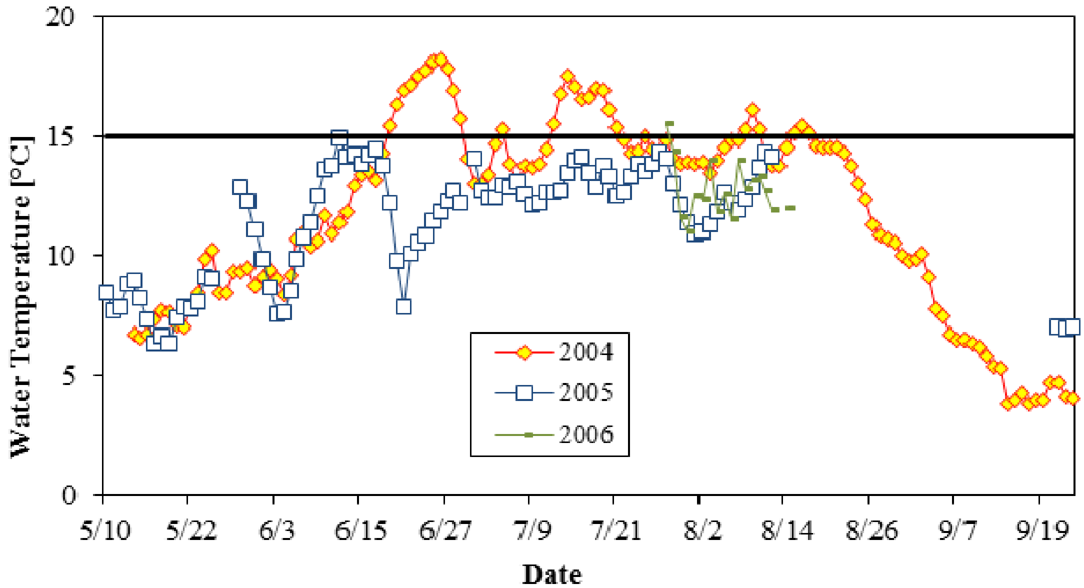


Figure 10.–Water temperatures from the Salcha River in 2004, 2005 and 2006, Yukon Area.

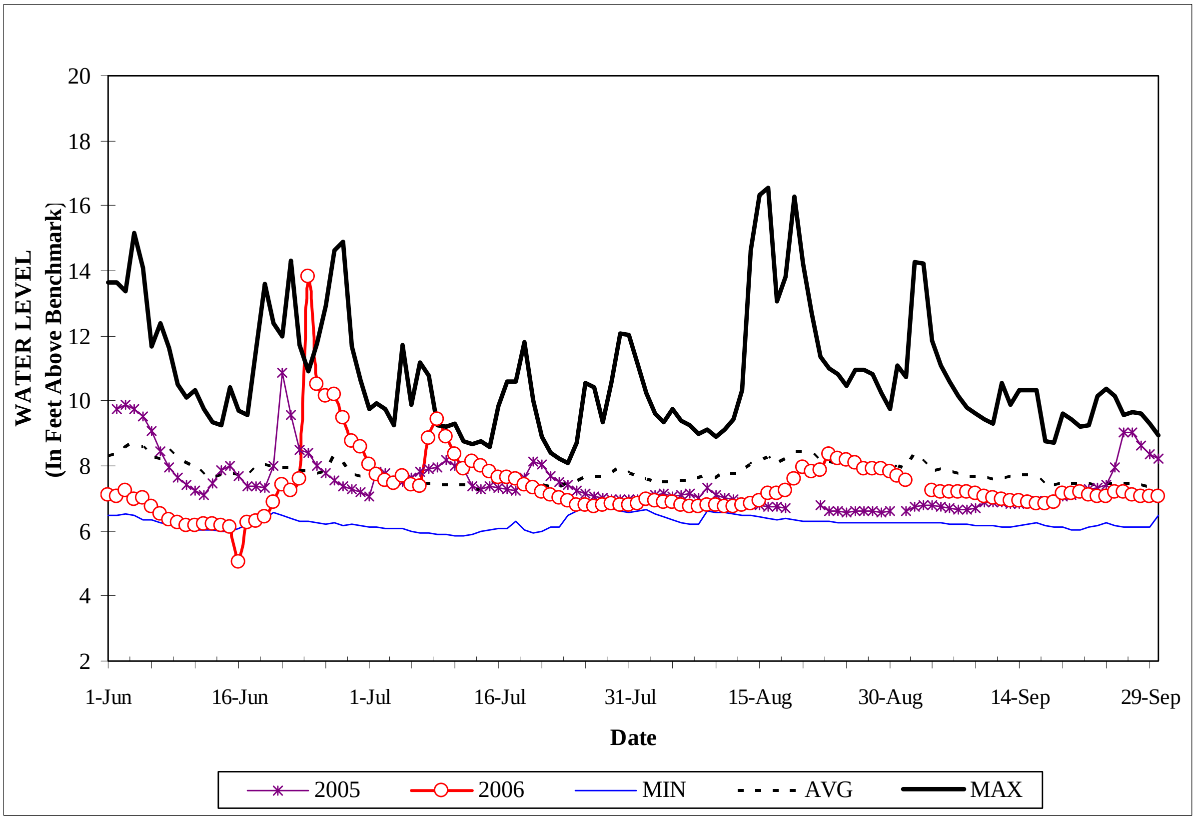


Figure 11.–Salcha River water levels in 2005 and 2006 compared to historical 1987–2005 maximums, average and minimums, Yukon Area.

In the Salcha River record low water levels were reported in 2004, compared to those reported from 1987 to 2003, during the month of August. In 2005 water levels were near the minimum recorded from late July through August (Figure 11). Water levels in 2006 continued to be low from the last week in July through mid-August. The time frames of low water correspond to the peak spawning of Chinook salmon in the Chena and Salcha rivers. The Chena River water levels are not presented in this report but the system has a similar runoff pattern to that of the Salcha River. The surges of high water are driven by rain events which typically occur in the headwaters of these two adjacent drainages at the same time.

DISCUSSION

GROSS CLINICAL SIGNS

Gross clinical signs were observed and documented at each location for most of the collections but proportions infected in the upper river were not always higher than those observed in the lower river samples (Appendix C6). Clinical infection ranged from 7.9% to 13.5% in Emmonak (38 rkm) whereas Chena River samples (1,596 rkm) ranged from 7.4% to 22.1% while the Salcha River samples (1,602 rkm) were consistently lower at 3.7% to 6.9%. Prevalence of *Ichthyophonus* in Emmonak, as determined by clinical signs of infection from 2004–2006, was within the historical range observed by Kocan et al. (2004). Observations of overall prevalence showed an increase of clinical signs from lower river sites to up river sites most consistently in 2005 and slightly in 2004, but proportions were much reduced in 2006 at up river sites (Appendix C6). The higher water temperatures in the Yukon River in June and July (17-20ºC) in 2004 and 2005 compared to relatively low temperatures observed in 2006 (Figure 8) may have led to the increase in clinical signs of infection as the fish migrated up river. Water temperatures above 15ºC are known to accelerate *Ichthyophonus* infection (Halpenny et al. 2002; Okamoto et al. 1987).

The proportions of clinical signs in the infected fish in the Chena River, where an extraordinarily high infection rate was detected in 2004, lead to concerns for contamination of samples. However, the results were found to be consistent with those collected in the following years where the proportion of infected fish with clinical signs represented 61.3%, 88.2% and 57.1% from 2004 to 2006, respectively. Clinical signs would be expected to be artificially low if contamination was affecting the prevalence of infected fish in the 2004 Chena River samples. This leads to more questions as to what other factors are affecting individual stocks since Chena and Salcha rivers that both drain the Tanana valley uplands and mirror each other in distance and timing of migration with very similar environmental conditions yet differences occur. Future work could test if there are differences in susceptibility to infection based on origin of the stock in the lower, middle and upper Yukon River stocks or broad scale separations such as Canadian vs. U.S. origin stocks.

EFFECTS OF *ICHTHYOPHONUS* ON ESCAPEMENT

The Yukon River Chinook salmon fishery is managed based on escapement counts: the number of fish arriving at the spawning grounds produced from previous successful spawning events. Although 50% of the drainage-wide Chinook salmon production occurs in Canada, the Chena and Salcha rivers are the largest individual producing systems in the Alaska portion of the drainage (JTC 2007). Both the Chena and Salcha rivers have biological escapement goals (BEG) with a range of 2,800 to 5,700 (Chena) and 3,300 to 6,500 (Salcha). Salmon escapements are evaluated by tower counts annually from early July to early August, in some years abundance estimates maybe provided with the use of mark-recapture methodologies and both systems also have aerial index areas for Chinook salmon (JTC 2007). The BEG range could potentially need to be adjusted if *Ichthyophonus* related pre-spawning mortality above the counting towers is determined to be significant.

In this regard, this study showed that Chinook salmon spawned throughout the river drainage from just upstream of the escapement monitoring site. Though infected fish tend to die in the downstream portion of the spawning rivers, the difference was not significant (Table 1). Further, the infected females tended to have a lower measure of “spawned out”; however, the difference was not significant (Figure 6). These results suggest *Ichthyophonus* infected fish spawn as successfully as uninfected fish. Simultaneously, it is acknowledged that “spawned out” does not necessarily mean spawning success because eggs from the infected females may not be as viable or develop poorly compared to those from uninfected females. Spawning success may be affected by an inability to dig an appropriate redd (i.e. to depth) including adequate time spent defending the redd. It is possible that none of eggs from the infected spawners were viable, which results in 15% reduction of the spawners at escapement. However, even in this worst case, the magnitude of reduction is smaller than the range of accuracy and precision of escapement counting (CV 30%; Audra Brase, Sport Fish Biologist, ADF&G, Fairbanks, personal communication) and the range of escapement goals (+/- 30% from mid-goal). These data suggests that effects of *Ichthyophonus* pre-spawning mortality on escapement counts are slight to negligible.

AGE, SEX, AND LENGTH COMPOSITION

The pooled data from Emmonak samples showed a significant difference between the prevalence of *Ichthyophonus* between age-5 and age-6 fish. As the fish moved up river the proportions of infected fish appear to decrease for age-6 fish. However, the significance of age difference may be affected by the collection methods. In Emmonak, the 8.5-inch mesh set gillnet test fishery tends to catch predominantly age-6 Chinook salmon (Bales 2008). In the Chena and Salcha spawning grounds, carcass sampling tends to bias toward larger fish (Zhou 2002). Nonetheless, the samples from the lower and upper river (with the exception of Chena River in 2005) followed the same trends in that age-6 fish dominated the samples in 2004 and 2005 but age-5 dominated in 2006 at all samples sites. The magnitude of the dominance of age-6 fish (Emmonak and spawning grounds samples) was greatest in 2004 where the average proportion of age-5 and age-6 fish compared was only 13.3% whereas the average proportions in 2005 and 2006 were 49.0% and 53.9%, respectively (Bales 2008).

If collection methods and effects of the inriver fishery are not an issue, the difference between that age structure from lower Yukon River and the spawning grounds could provide credence to the theory that fish infected with *Ichthyophonus* die on their way to the spawning grounds (Kocan et al. 2004). The degree of infection may be affected by factors such as accumulation of spores through exposure as the fish age, changes in feeding ecology at different life stages which may also be counteracted by acquired resistance in older fish (Rahiman and Thulin 1996). Kocan et al. (2004) suggest that infected fish are dying during the migration and in this case one would expect the composition of the infected fish to be altered. The data from this study suggest that the older fish, age-6, are succumbing to disease possibly due to differential age specific infections. Another potential cause of the disparity in prevalence by age of infected fish could be related to the inriver gauntlet fishery. This fishery has been prosecuted with large mesh gear that tends to select for older and larger individuals (Bromaghin 2005). Individuals are susceptible to harvest along the entire mainstem river such that exploitation rates are higher for fish migrating over the longest distances.

EFFECTS OF WATER TEMPERATURE ON *ICHTHYOPHONUS* INFECTION AND SEVERITY

Increased water temperatures are known to increase stress, alter immune function, and promote greater levels of infectious or parasitic diseases such as Ichthyophoniasis (United States Geological Survey 2009, Okamoto et al. 1987; Halpenny et al. 2002; Perry et al. 2004). Kocan et al (2004) proposed that the increase of the detection of *Ichthyophonus* infected fish in Yukon River Chinook salmon is due to increase in water temperature. In an experimental study, 100% of *Ichthyophonus* infected rainbow trout (*Salmo gairdneri*) died at water temperatures of 15 to 20^{o}C (Okamoto et al. 1987). In the Yukon River, Chinook salmon entering the river in late May to early July are exposed to water temperatures above 15^{o}C for at least a month during migration before reaching the natal spawning tributaries in late July and August (Appendix D1). While results of Okamoto et al. (1987) may not be directly applied to the Chinook salmon, it is likely that increased temperature affects severity of *Ichthyophonus* infection both in the Bering Sea and during migration in river. Warm anomalies in seas surface temperatures in the Bering Sea dominated from 2000 to 2005 (http://www.beringclimate.noaa.gov/reports/index.html accessed December 2010) during the collection of most of the prevalence data for the Yukon River. As for a potential relationship between *Ichthyophonus* prevalence and environmental changes, the *Ichthyophonus* prevalence data series is insufficient to discern a relationship at this time.

CONCLUSIONS

This 2005–2006 study concludes the three-year study regarding the effects of *Ichthyophonus* on Chinook salmon in the Yukon Area. The study showed that there was no significant difference between infected and uninfected female Chinook salmon that reached the spawning grounds and vacated eggs. However, it is unknown whether the eggs from the infected females are as viable or protected as those from uninfected fish. Regardless, the potential loss of infected females at the spawning grounds is too small to raise the current escapement goals. Correlations between the prevalence of *Ichthyophonus* infections and environmental factors such as temperatures in migration corridors will require longer and more consistent datasets. Testing methods using either explant culture or PCR resulted in similar estimation of *Ichthyophonus* prevalence.

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APPENDIX A

Determining Prevalence by Criteria

In 2004, Salcha carcasses samples were collected using a different set of criteria (clear eyes and firm cardiac muscle noted as "criteria 2") as opposed to clear eyes, some color in the gills, and a firm cardiac muscle noted as "criteria 1" (Kahler et al. 2007). To examine whether this affected estimates of *Ichthyophonus* prevalence, in 2005 carcass samples were collected using criteria 2 of which those meeting the criteria 1 were also recorded (Appendix Table A1). Of the samples collected, 97% of samples belonged to Criteria 1 in Chena and 53% in Salcha. Prevalence of Criteria 1 tended to be higher than those with Criteria 2; however, there was no difference in prevalence between Criteria 1 and 2 (2-sample z-test p > 0.5) for both Chena and Salcha river samples. *Ichthyophonus* prevalence of fish with white gill color was significantly lower than that with colored gills using the cultured test (7.2% vs. 12.0%, Chena and Salcha combined, 2-sample z-test, p=0.015); however there was no difference for the PCR test (11.5% vs. 13.8%, Chena and Salcha combined, 2-sample z-test, p=0.17). This suggests that the estimate of *Ichthyophonus* prevalence of Salcha River in 2004 is slightly negatively biased.

Appendix Table A1.–Number of infected Chinook salmon (positive for *Ichthyophonus*) including percent prevalence from cardiac muscle samples by test type (explant culture or polymerase chain reaction-PCR), 2005–2006.

	Criteria 2 (Total)					
				Criteria 1		
	n	Positive	% positive	n	Positive	% positive
Culture						
Chena	317	35	11.0%	294	34	11.6%
Salcha	564	56	9.9%	297	36	12.1%
PCR						
Chena	324	43	13.3%	300	40	13.3%
Salcha	571	74	13.0%	300	43	14.3%

Further, to examine the length of time it takes for fish loose gill color, chum salmon (*Oncorhynchus keta*) and small Chinook salmon were sacrificed. The chum salmon was placed in the water and a Chinook salmon was placed on the river bank of the Chena River at 14:00 on August 4, 2006. Gill colors of both fish were photographed every hour until they lost color.

After 17 hours submerged in water, the gill colors had begun fading (Appendix Figures A and B). At near 24 hours, there was still some pink in the gills (Appendix Figure C), and at 26 hours gills become completely white (Appendix Figure D). On the other hand, the gills of the fish placed on the beach remained bright red after 26 hours because they did not have water flushing through them and the blood coagulated.

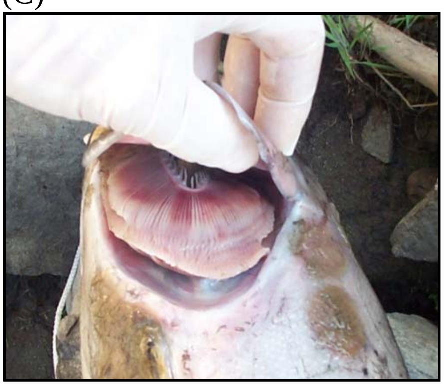
-continued-

This shows that fish collected with colored gill from submerged water are more likely to be dead within 24 hours. For beached fish, the other characteristics become more important in establishing the criteria. Kocan et al (2004) reported that eye becomes cloudy and cardiac muscle becomes discolored and flaccid after 48 hours for fish submerged in 12^{o}C flowing water. These suggest that all collected fish are dead within 24 hours for submerged and 48 hours for beached.

(A)

(B)

(C)



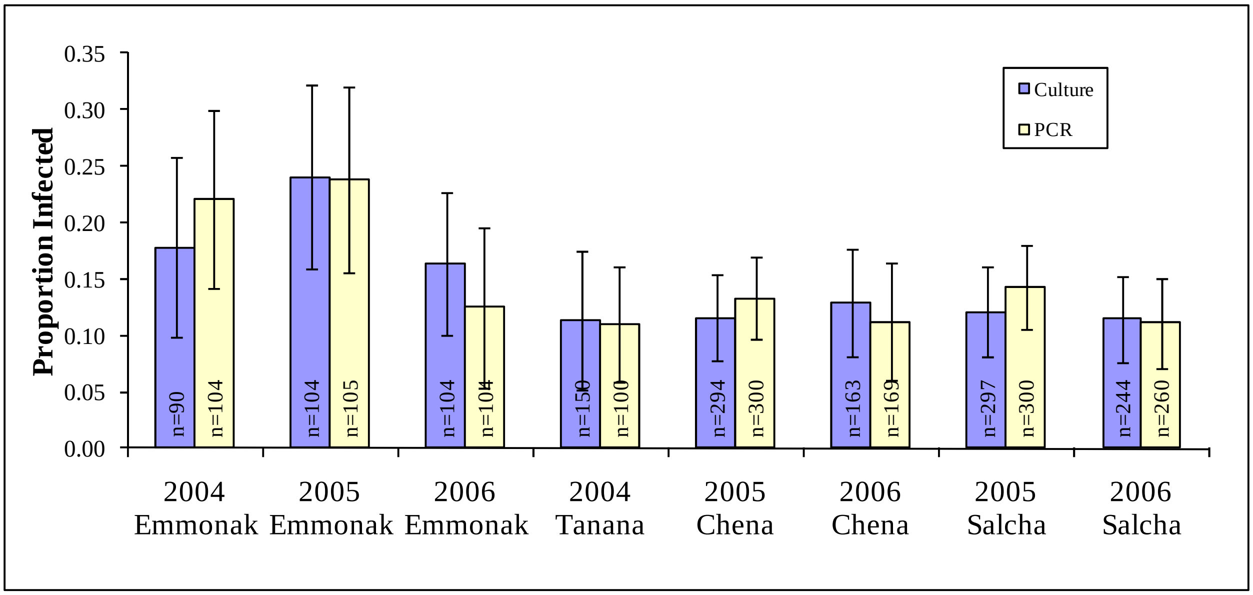
(D)

Appendix Figures.–Gill color at hour zero (A), still considered red at hour 17 (B), considered pink at hour 24 (C), and mostly white at hour 26 (D) from an expired chum salmon submerged in the Chena River, August 4-5, 2006.

APPENDIX B

Determining Prevalence by Culture and PCR Methods

During the three years of the project (2004–2006) explant culture and PCR methods were used to test presence of *Ichthyophonus* in duplicate cardiac muscle samples from each Chinook salmon. Estimates of prevalence were generated using both methods from paired samples collected in Emmonak, Tanana (2004 only), Chena and Salcha Rivers. Comparison of the two methods resulted in no significant difference (p=0.79) in estimation of prevalence between explant culture and PCR (Appendix Figure).



Appendix Figure.–Prevalence (95% CI range) of Ichthyophonus in Chinook salmon based on cardiac muscle samples by year and testing procedure (explant culture and polymerase chain reaction-PCR) in Emmonak, Tanana, and the Chena and Salcha river spawning grounds, Alaska, 2004–2006.

The major differences between the two methods are handling of samples and viability of *Ichthyophonus*. The culture method detects presence of only viable *Ichthyophonus* that are capable of causing disease: Ichthyophonousis. For this test, the tissue samples have to be stored in cool MEM-5 solution and shipped to a lab as quickly as possible. This is difficult and costly for tissue collections in remote field conditions. On the other hand, the PCR method detects presence of *Ichthyophonus* rDNA fragments that can be viable or unviable of causing Ichthyophonousis. For this test, the tissue samples are stored in 95% ethanol, which is easy and cost saving in sampling at remote field sites. Results from PCR also cannot provide any information on the status (severity) of the infection.

At this time, the ADF&G recommends using explant culture wherever possible as it is the standard used by fish health professionals for testing *Ichthyophonus* prevalence and allows determination of the status of the infection. However, because of similarities in estimates of prevalence between the culture and PCR method, use of PCR is allowable in the field where collecting, storing, and shipping samples in a controlled environment is difficult. Further, techniques and application of PCR test method have been advancing rapidly, so the current recommendation will likely change in the future.

APPENDIX C

Appendix C1.–Chinook salmon sampling schedule for *Ichthyophonus* at Emmonak, representing the entrance into the Yukon River, 2005 and 2006.

	Emmonak (Yukon River Mouth)			
	2005		2006	
Date Ranges	Targeted Sample	Actual Sampled	Targeted Sample	Actual Sampled
5/26–5/29	1	0	1	0
5/30–6/5	5	20	4	0
6/6–6/12	15	24	13	14
6/13–6/19	24	27	22	23
6/20–6/26	28	18	28	28
6/27–7/3	19	8	21	18
7/4–7/10	10	7	11	20
7/11–7/17	3	0	4	1
Total Samples:	105	104	105	104

Appendix C2.–Chinook salmon samples collected by week for *Ichthyophonus* in the Chena and Salcha rivers, 2005 (criteria 1 only) and 2006.

	Chena River		Salcha River	
Date Ranges	Sampled 2005	Sampled 2006	Sampled 2005	Sampled 2006
7/11–7/17	0	0	1	0
7/18–7/24	2	0	33	0
7/25–7/31	67	13	202	47
8/1–8/7	204	88	64	151
8/8–8/14	27	70	0	62
Total Samples:	300	171	300	260

Appendix C3.–Chinook salmon mean length (mm) from samples collected for *Ichthyophonus* from Emmonak, and Chena and Salcha rivers, Alaska, 2005.

Sample Location (Source)	Sex	Length	4 Years	5 Years	6 Years	7 Years	Total
Emmonak (Gillnet)	Males	Mean	-	806	845	868	
		Standard Error	-	10.9	19.6	57.5	
		Minimum	-	720	745	810	
		Maximum	-	955	935	925	
		Sample Size	0	22	10	2	34
	Females	Mean	820	824	863	905	
		Standard Error	-	7.5	6.2	50.0	
		Minimum	-	780	750	855	
		Maximum	-	880	955	955	
		Sample Size	1	14	44	2	61
Chena River (Carcasses) (Criteria 1)	Males	Mean	550	733	794	908	
		Standard Error	7.6	8.2	13.6	37.5	
		Minimum	505	300	665	870	
		Maximum	600	890	980	945	
		Sample Size	15	96	37	2	150
	Females	Mean	465	786	834	883	
		Standard Error	-	5.3	6.1	18.7	
		Minimum	-	690	660	820	
		Maximum	-	870	930	940	
		Sample Size	1	57	57	6	121
Salcha River (Carcasses) (Criteria 1)	Males	Mean	560	751	841	936	
		Standard Error	7.3	7.4	15.0	54.4	
		Minimum	500	575	655	805	
		Maximum	625	895	1,010	1,070	
		Sample Size	24	67	36	4	131
	Females	Mean	-	792	840	901	
		Standard Error	-	5.7	4.7	14.3	
		Minimum	-	675	745	820	
		Maximum	-	850	935	965	
		Sample Size	0	42	91	10	143

Appendix C4.–Chinook salmon mean length (mm) from samples collected for *Ichthyophonus* from Emmonak, and Chena and Salcha rivers, Alaska, 2006.

Sample Location (Source)	Sex	Length	4 Years	5 Years	6 Years	7 Years	Total
Emmonak (Gillnet)	Males	Mean	570	769	846	-	
		Standard Error	20.2	9.4	21.2	-	
		Minimum	500	610	730	-	
		Maximum	610	885	935	-	
		Sample Size	5	34	9	0	48
	Females	Mean	-	821	864	-	
		Standard Error	-	7.5	8.4	-	
		Minimum	-	760	780	-	
		Maximum	-	865	980	-	
		Sample Size	0	16	33	0	49
Chena River (Carcasses)	Males	Mean	581	716	871	1020	
		Standard Error	16.7	13.5	29.4	-	
		Minimum	480	480	740	-	
		Maximum	720	915	1,000	-	
		Sample Size	13	46	10	1	70
	Females	Mean	-	797	872	-	
		Standard Error	-	8.7	6.8	-	
		Minimum	-	700	740	-	
		Maximum	-	870	975	-	
		Sample Size	0	25	53	0	78
Salcha River (Carcasses)	Males	Mean	565	703	809	935	
		Standard Error	15.5	7.3	27.7	-	
		Minimum	475	515	540	-	
		Maximum	660	880	985	-	
		Sample Size	14	95	18	1	128
	Females	Mean	-	806	878	930	
		Standard Error	-	9.0	4.8	38.5	
		Minimum	-	740	725	850	
		Maximum	-	885	960	1,010	
		Sample Size	0	20	79	4	103

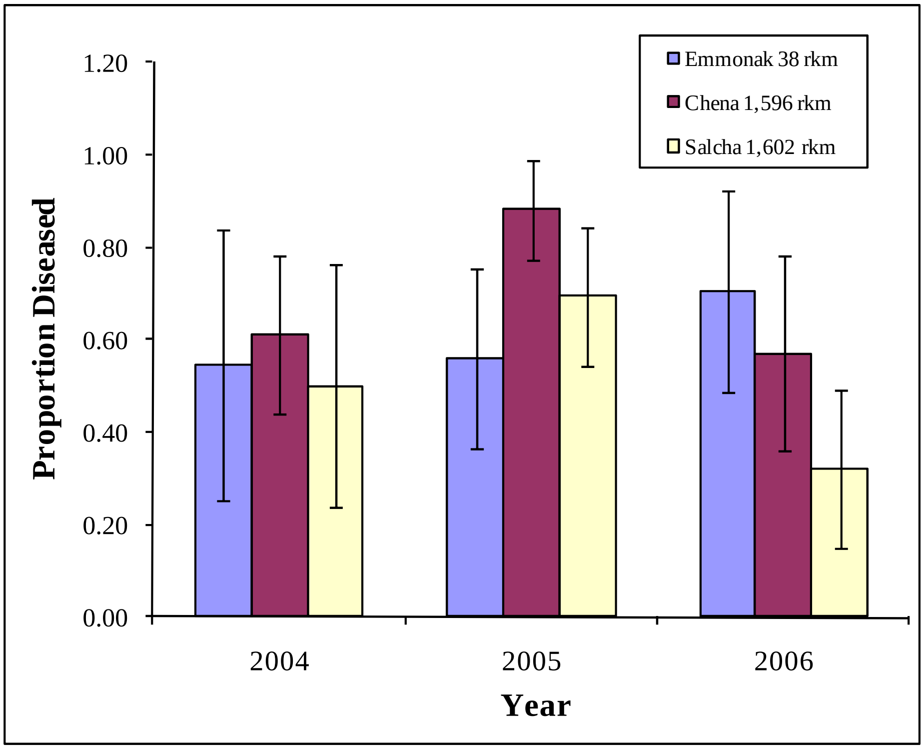
Appendix C5.–Chinook salmon egg retention data collected in the Chena and Salcha rivers, Alaska, 2005–2006.

Fish Number	Date	Length	Age	Positive Yes/No	Total Egg Volume	Egg Sub-Volume	Egg Count in Sub-Volume	Expanded Egg Count
Chena River								
1	7/27/05	900	7	Y	80	58	212	292
2	7/29/05	795	5	N	75	47	274	437
3	7/30/05	910	6	N	220	60	116	425
4	7/31/05	870	6	N	1,420	58	267	6,537
5	8/1/05	868	6	Y	80	58	223	308
6	8/1/05	815	-	N	300	60	308	1,540
7	8/3/05	875	-	N	165	60	222	611
8	8/4/05	870	6	N	95	58	203	333
9	8/5/05	760	5	N	240	49	328	1,607
10	8/5/05	700	5	N	210	47	240	1,072
11	8/4/05	820	6	N	480	45	237	2,528
12	8/5/05	790	5	N	220	62	238	845
13	8/6/05	735	-	Y	590	52	271	3,075
14	8/7/05	860	6	N	120	48	230	575
15	8/10/05	660	6	N	375	60	344	2,150
16	8/11/05	820	-	N	200	48	198	825
17	8/4/05	755	5	N	62	43	234	337
18	8/12/05	910	6	N	90	58	205	318
19	8/1/05	800	5	N	380	59	280	1,803
20	8/1/05	762	-	N	375	48	258	2,016
21	8/2/05	855	6	N	160	57	218	612
Salcha River								
22	7/22/05	750	-	N	980	70	509	7,126
23	7/23/05	770	5	N	32	32	197	197
24	7/23/05	835	6	N	260	47	187	1,034
25	7/24/05	790	5	N	440	37	182	2,164
26	7/24/05	850	5	N	540	77	286	2,006
27	7/25/05	830	5	N	600	70	224	1,920
28	7/26/05	825	6	N	280	63	221	982
29	7/26/05	675	5	N	60	60	381	381
30	7/26/05	785	5	N	260	81	392	1,258
31	7/26/05	800	5	N	760	55	262	3,620
32	7/27/05	850	6	N	25	25	192	192
33	7/28/05	845	6	N	180	69	220	574
34	7/28/05	850	6	N	640	52	210	2,585
35	7/28/05	720	-	N	66	66	168	168
36	7/28/05	890	6	N	200	56	225	804
37	7/28/05	890	-	N	41	41	135	135
38	7/28/05	850	-	N	100	48	203	423
39	7/28/05	900	6	Y	57	57	261	261
40	7/29/05	730	5	N	220	61	312	1,125
41	7/29/05	825	5	N	78	78	398	398
42	7/29/05	840	-	N	240	66	197	716
43	7/29/05	935	6	N	1,320	54	248	6,062
44	7/30/05	775	6	N	380	42	172	1,556
45	7/30/05	790	5	N	300	67	216	967
46	7/30/05	835	6	N	850	59	290	4,178

Appendix C5.–Page 2 of 2.

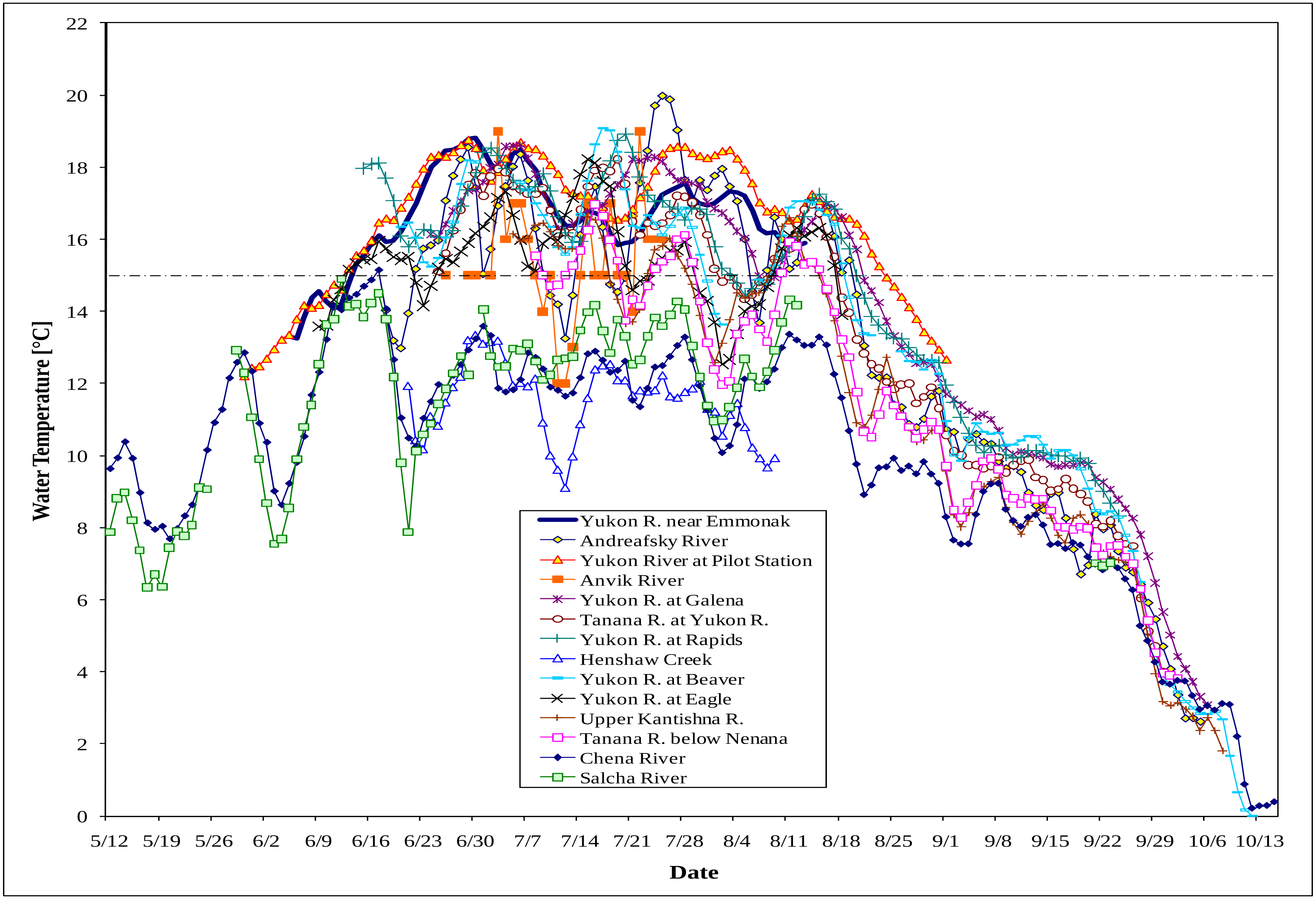
Fish Number	Date	Length	Age	Positive Yes/No	Total Egg Volume	Egg Sub-Volume	Egg Count in Sub-Volume	Expanded Egg Count
Salcha River								
47	7/30/05	860	7	N	420	68	333	2,057
48	7/30/05	845	6	N	500	58	181	1,560
49	7/30/05	800	5	N	310	66	237	1,113
50	7/30/05	810	5	N	520	56	229	2,126
51	7/30/05	875	-	N	160	54	190	563
52	8/1/05	855	6	N	680	65	227	2,375
53	8/2/05	800	5	Y	630	49	202	2,597
54	8/5/05	845	6	Y	780	44	194	3,439
55	8/4/05	820	6	N	96	96	352	352
56	8/6/05	830	-	Y	140	40	202	707
57	8/4/05	815	5	N	1,000	72	281	3,903
58	8/4/05	885	6	N	110	36	166	507
59	8/4/05	810	5	N	390	55	296	2,099
60	8/7/05	825	5	N	520	61	281	2,395
61	8/7/05	965	6	N	280	47	301	1,793
62	8/9/05	770	5	N	600	53	260	2,943
63	8/8/05	835	5	N	230	47	190	930
64	8/8/05	780	5	N	870	58	245	3,675
65	8/10/05	820	6	N	945	56	258	4,354
66	8/10/05	810	5	N	330	50	247	1,630
67	8/10/05	845	6	N	650	54	175	2,106
68	8/11/05	905	6	N	300	51	206	1,212
69	8/11/05	915	6	N	1,265	53	228	5,442
70	8/11/05	820	6	Y	340	43	201	1,589
71	8/11/05	860	5	Y	560	48	284	3,313
72	8/12/05	850	6	N	1,320	54	271	6,624
73	7/26/05	840	5	N	520	64	321	2,608
Chena River								
74	8/5/06	870	6	N	1,260	160	466	3,670
75	8/7/06	940	6	N	340	108	525	1,653
Salcha River								
76	7/28/06	815	6	N	600	27	123	2,733
77	7/30/06	830	6	Y	260	38	170	1,163
78	8/5/06	860	6	Y	300	37	145	1,176
79	8/5/06	830	-	Y	740	73	267	2,707
80	8/9/06	895	6	N	280	40	142	994
81	8/9/06	900	6	N	480	22	105	2,291

Appendix C6.–Proportion (95% CI range) of *Ichthyophonus* infected Chinook salmon (as determined by explant culture of cardiac muscle) that showed clinical signs of disease by sample location, 2004–2006, Yukon Area.

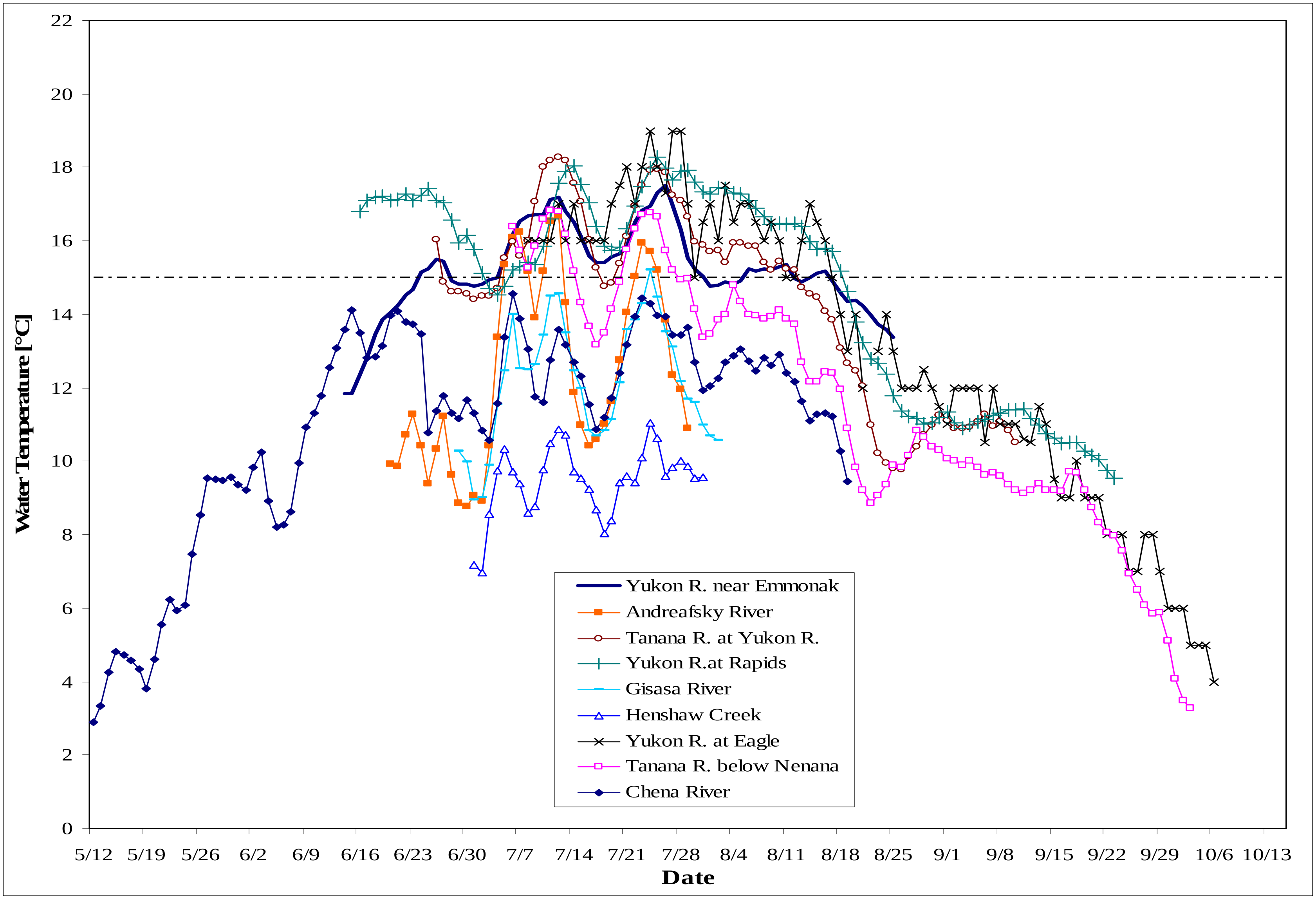


APPENDIX D

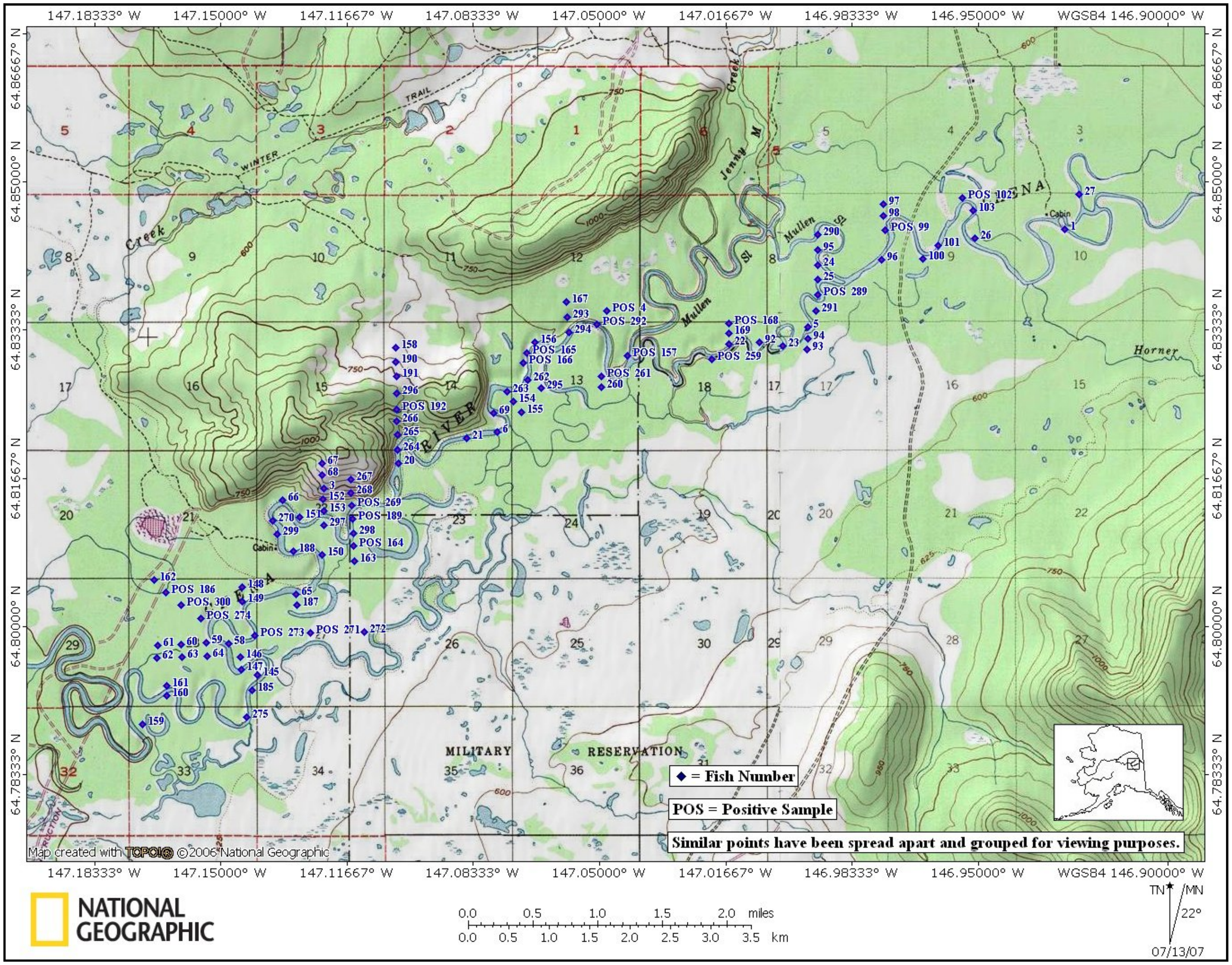
Appendix D1.–Water temperatures [°C] collected within the Yukon River drainage near communities or within tributaries, Alaska 2005.



Appendix D2.–Water temperatures [ºC] collected within the Yukon River drainage near communities or within tributaries, Alaska 2006.



Appendix D3.–Locations of Chinook salmon carcasses collected for *Ichthyophonus* sampling (criteria 1) in the Chena River, Alaska 2005 (Map 1).



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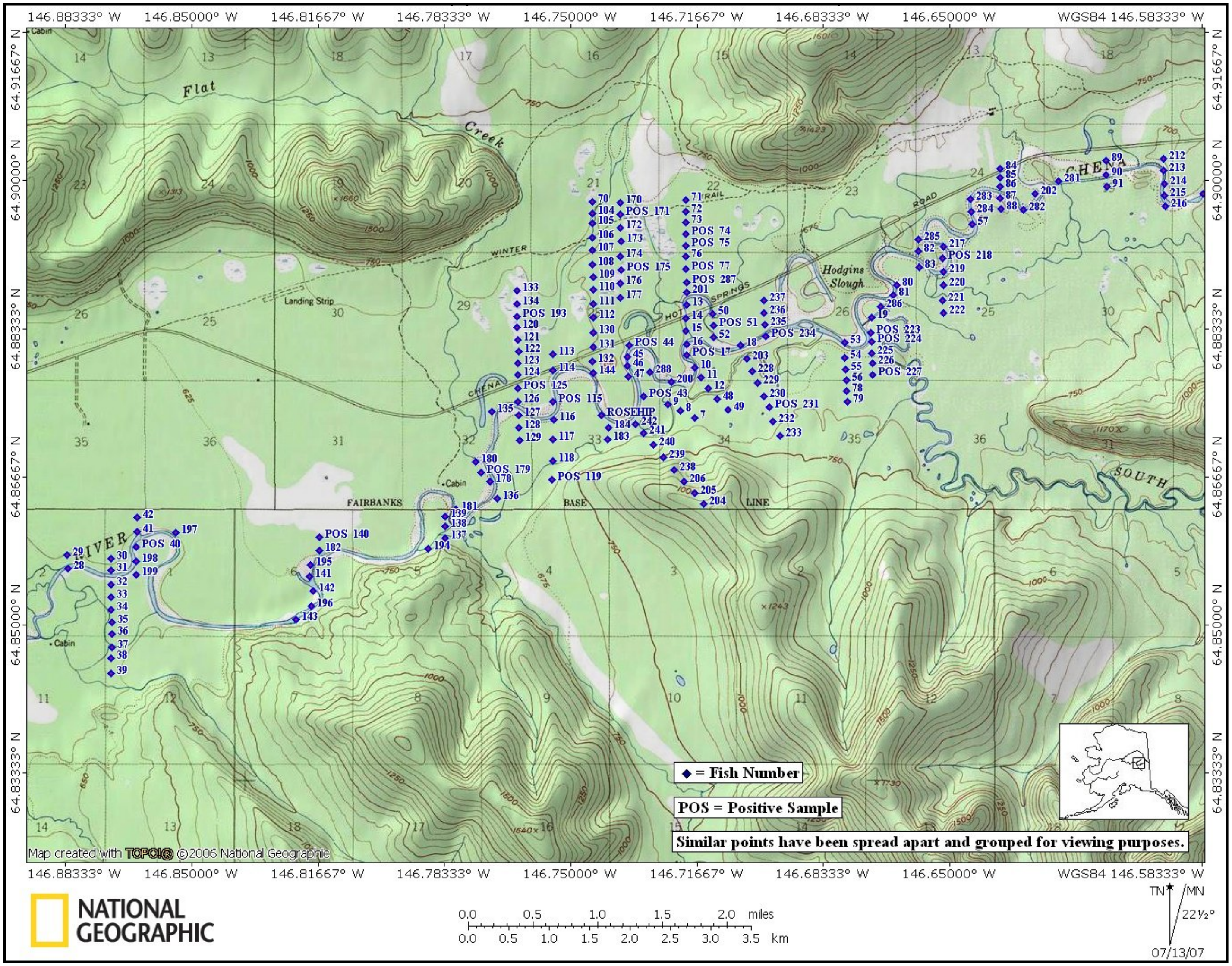
Appendix D3.–Page 2 of 3. (Map 2)



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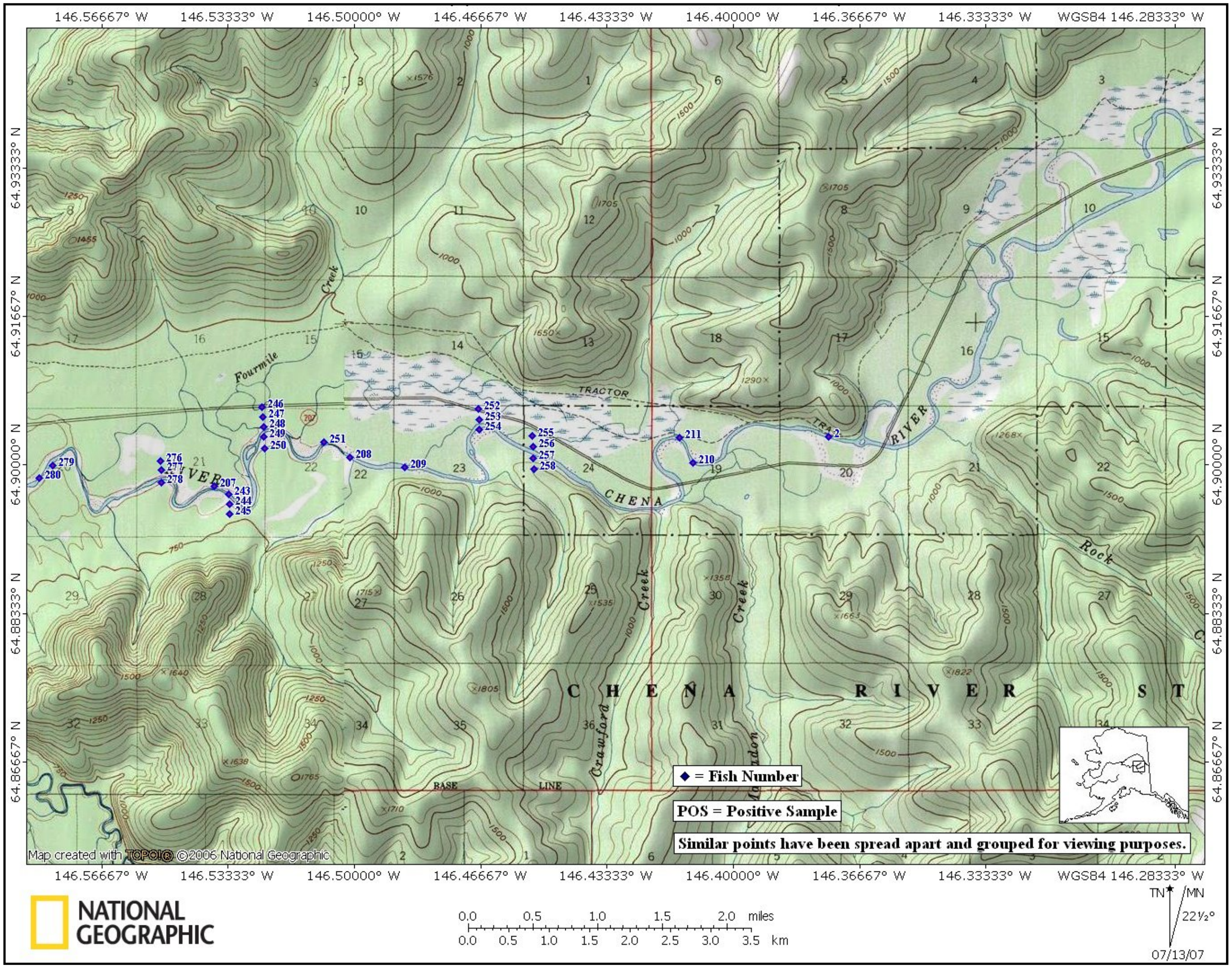
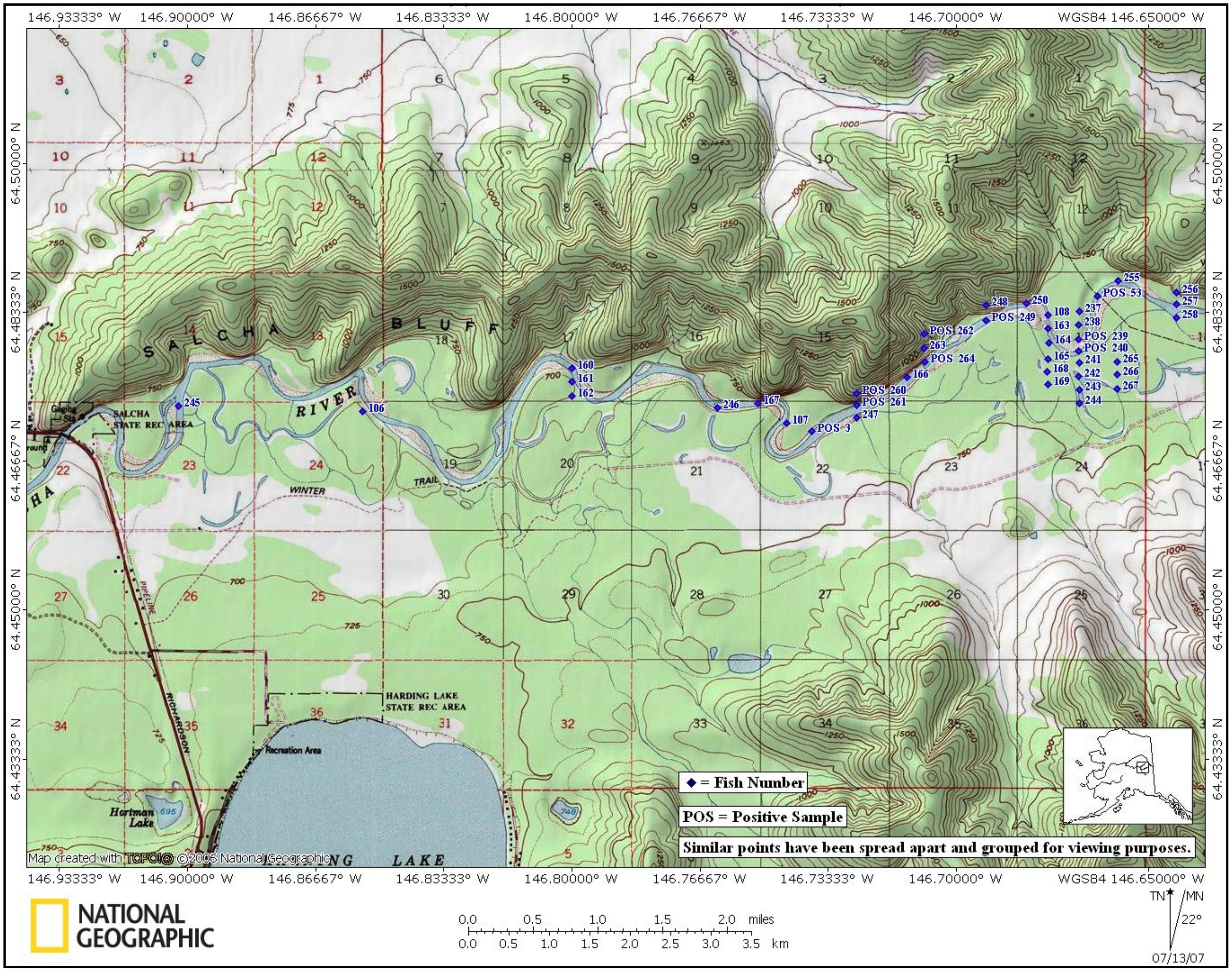
Appendix D3.–Page 3 of 3. (Map 3)



Appendix D4.–Locations of Chinook salmon carcasses collected for *Ichthyophonus* sampling (criteria 1) in the Salcha River, Alaska 2005 (Map 1).



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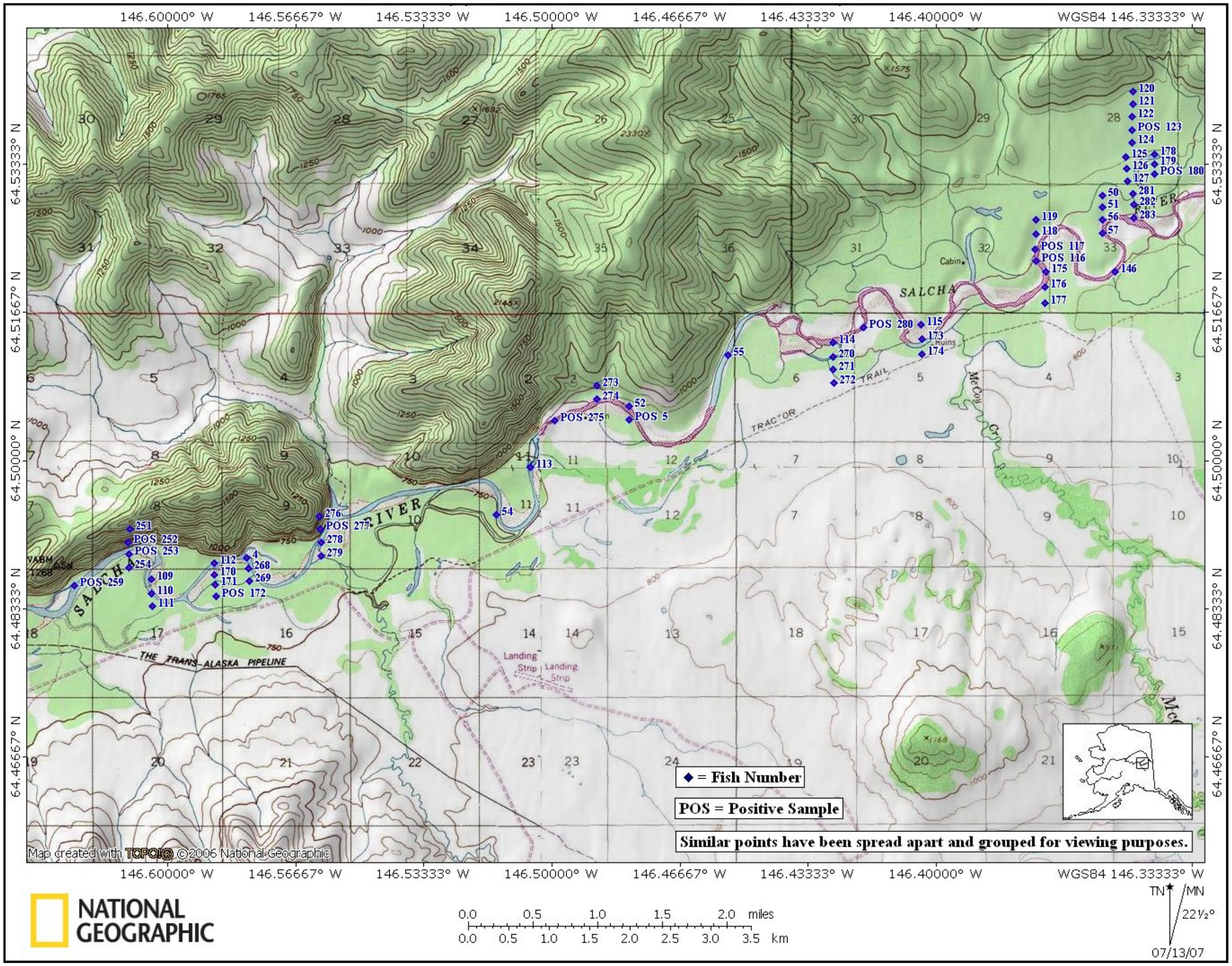
Appendix D4.–Page 2 of 4. (Map 2)



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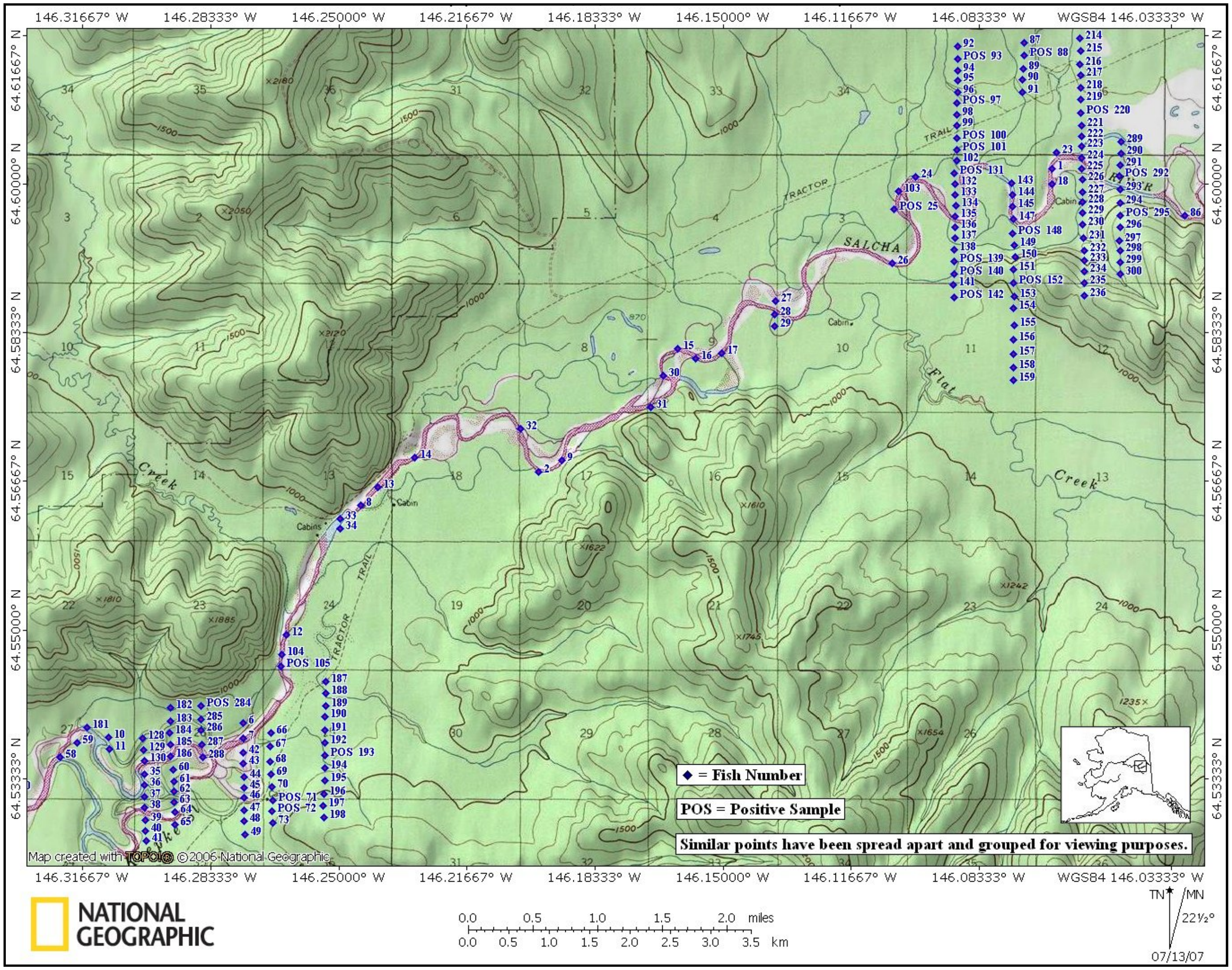

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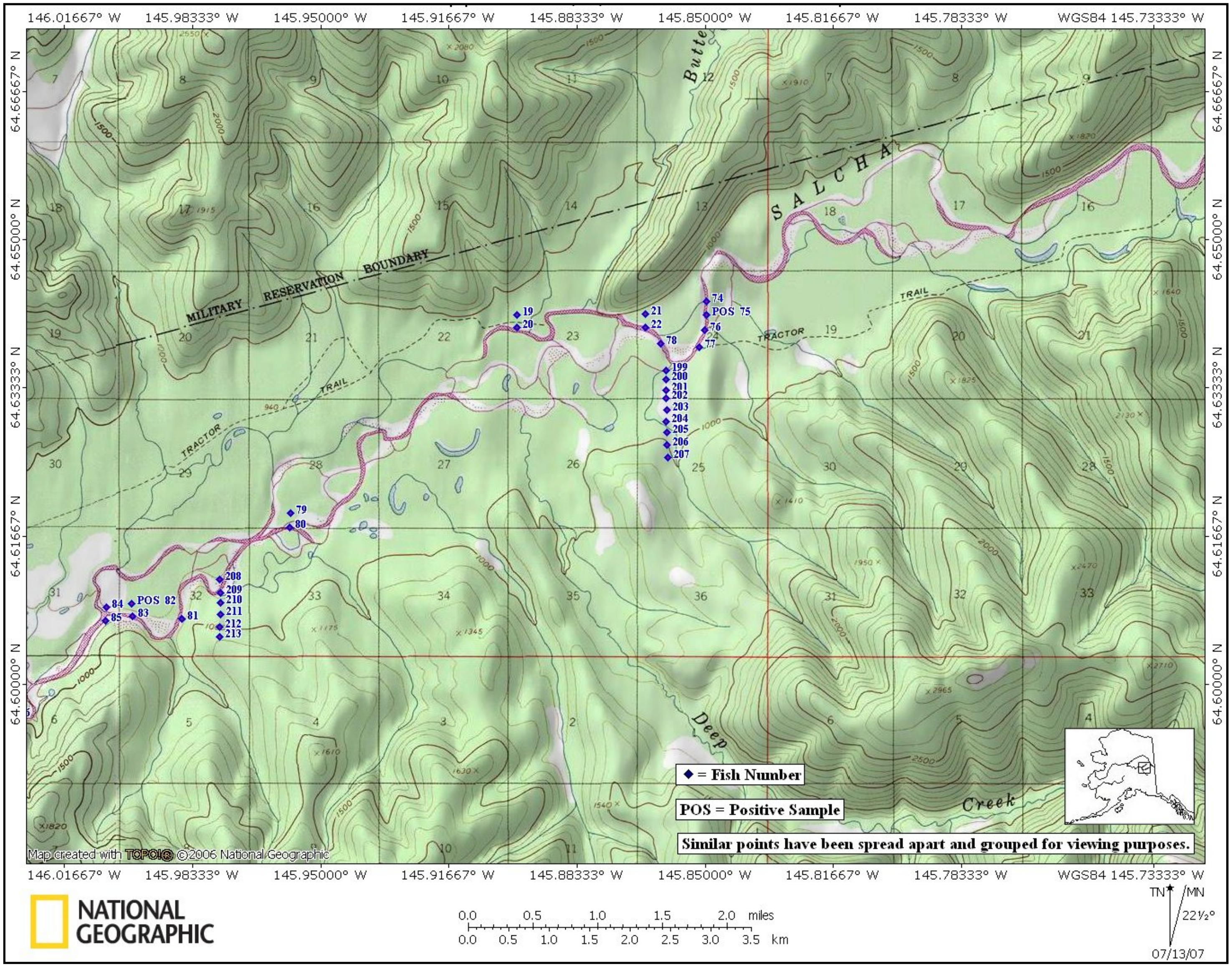
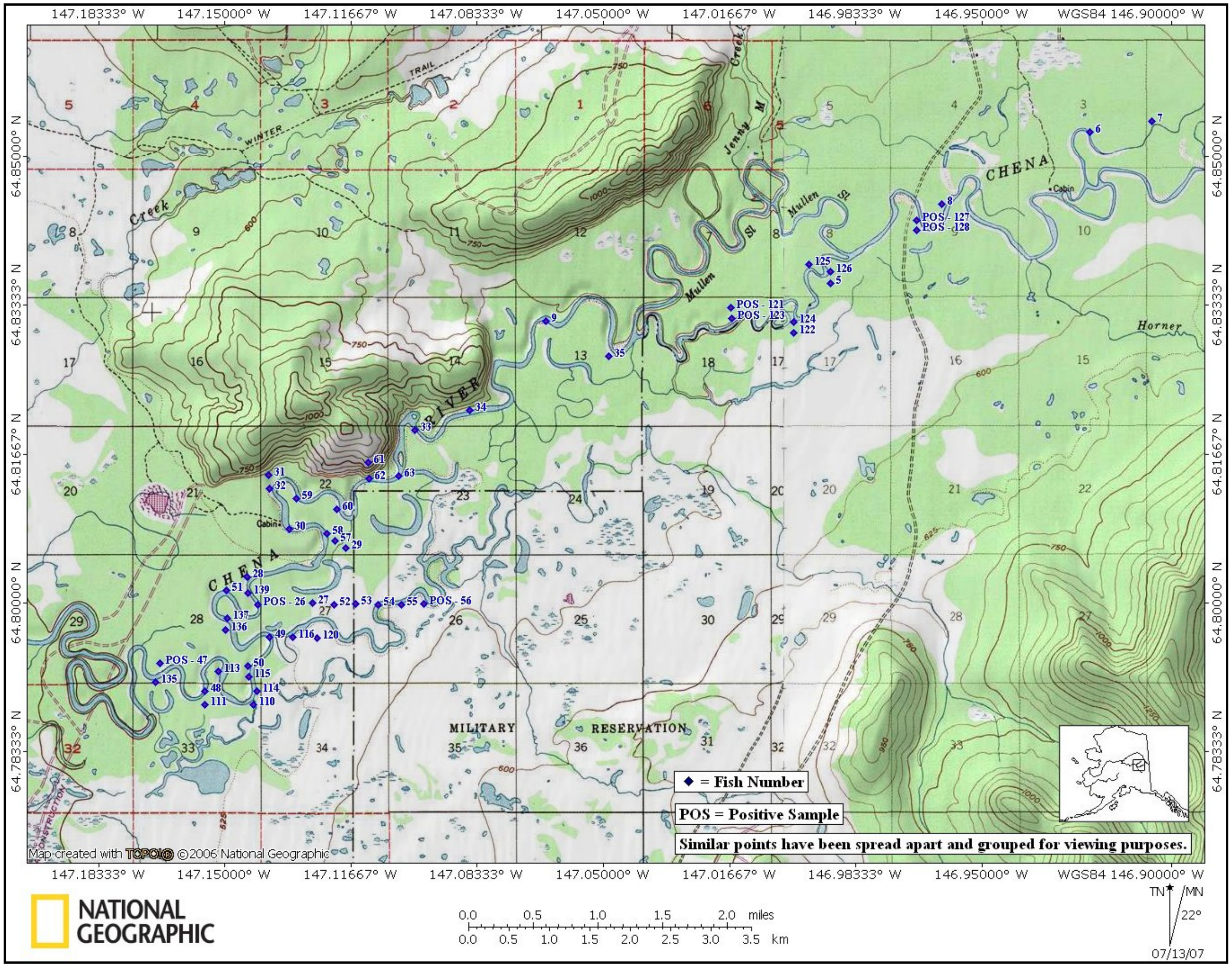


Appendix D5.–Locations of Chinook salmon carcasses collected for *Ichthyophonus* sampling in the Chena River, Alaska 2006 (Map 1).



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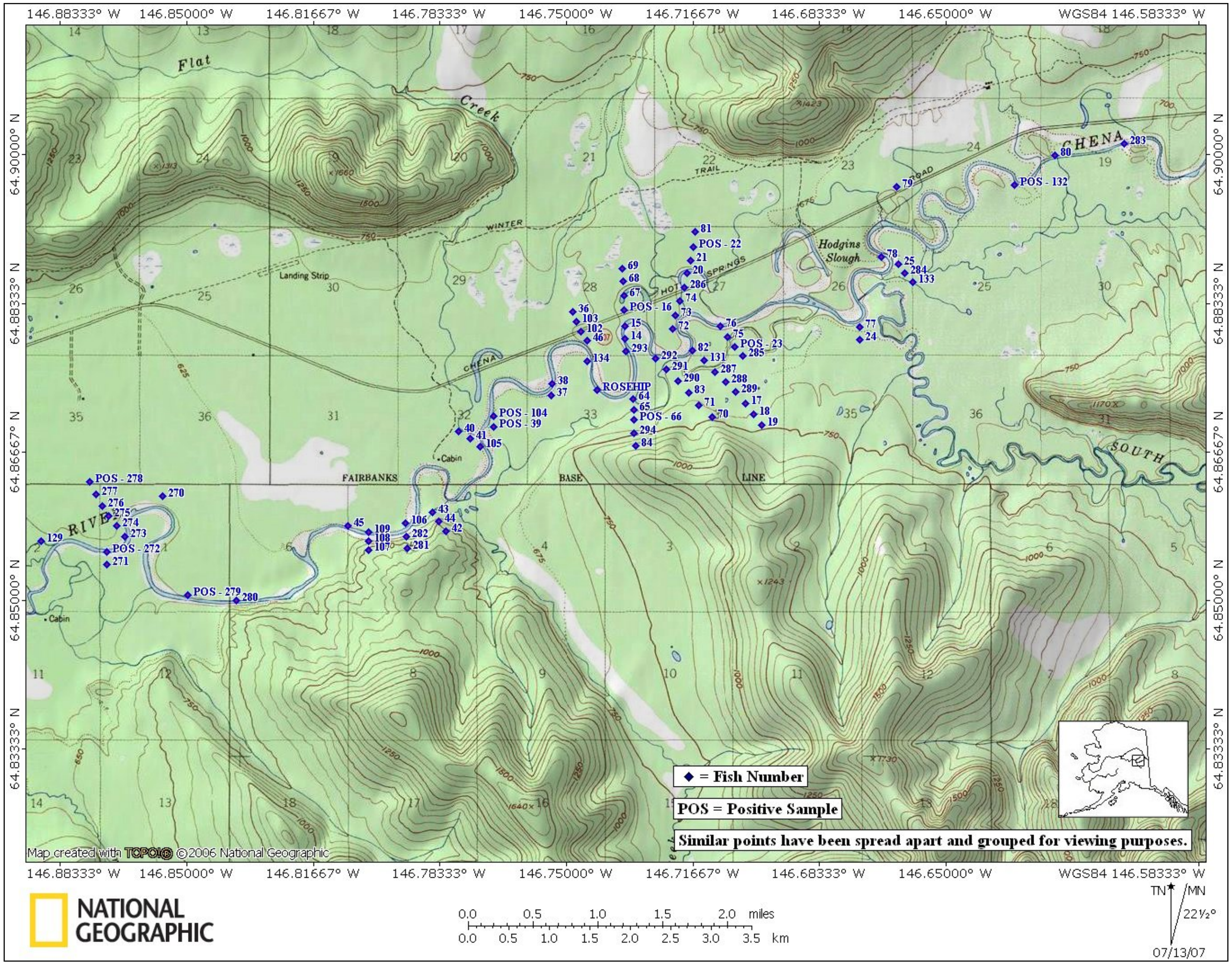
Appendix D5.–Page 2 of 3. (Map 2)



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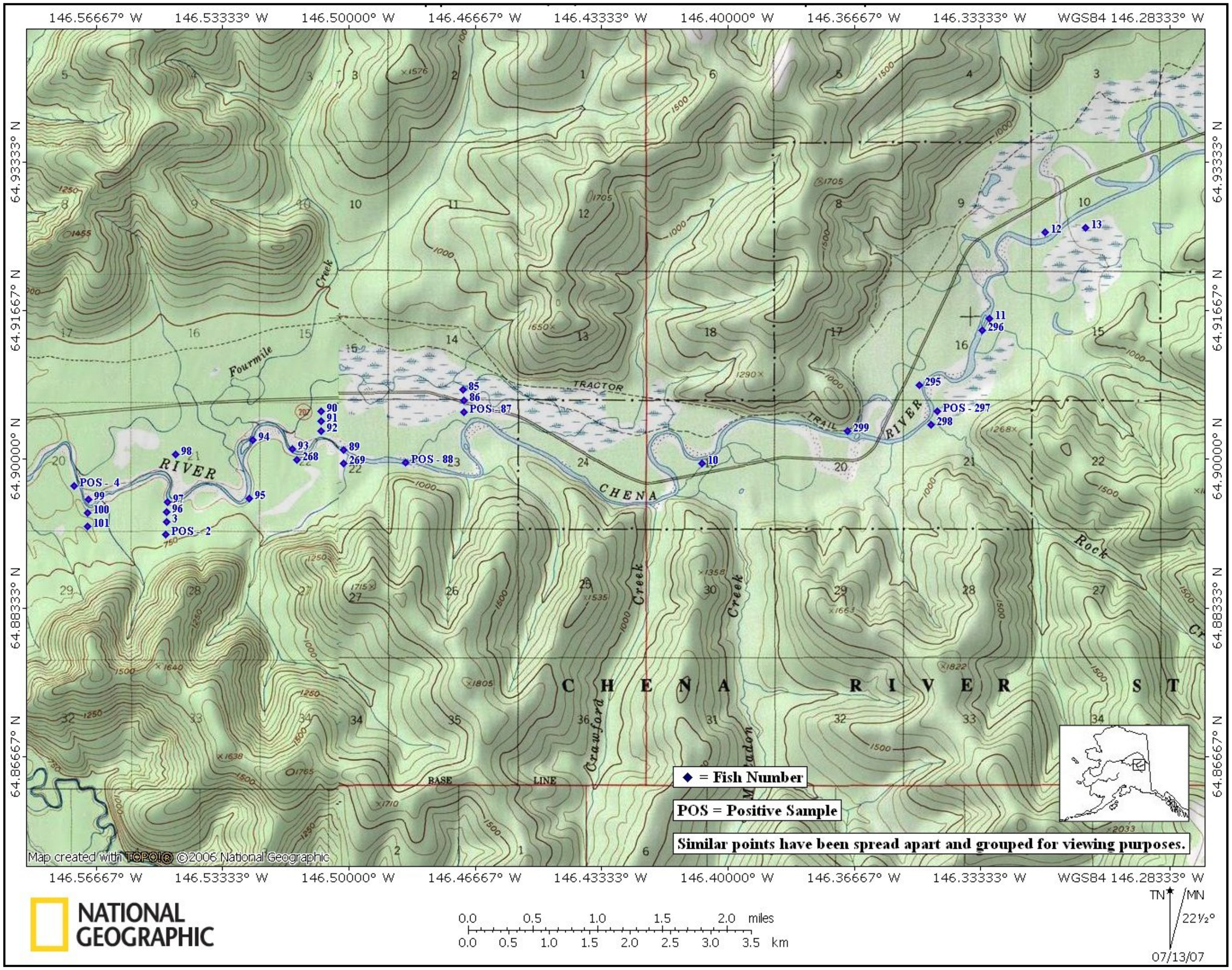
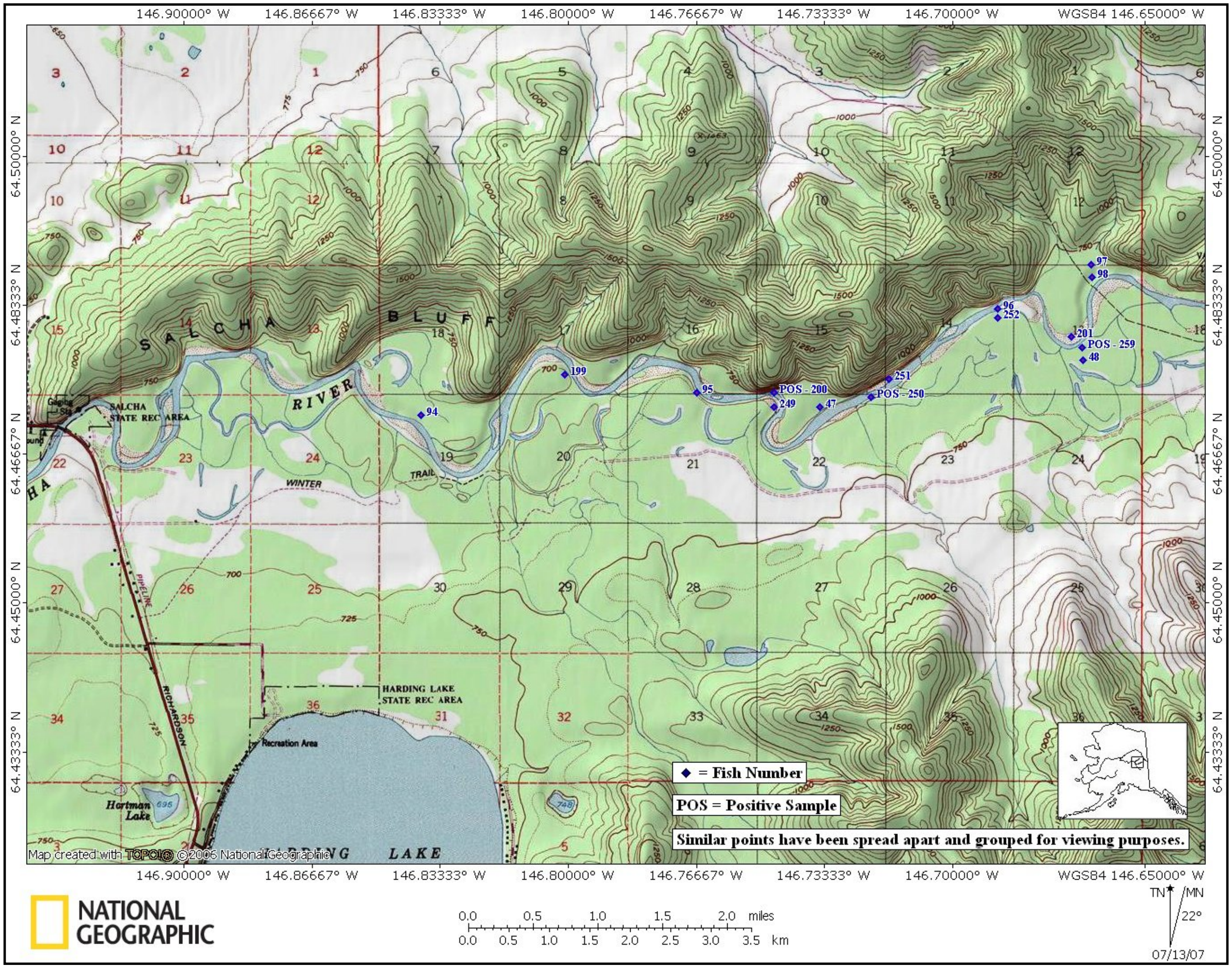
Appendix D5.–Page 3 of 3. (Map 3)



Appendix D6.–Locations of Chinook salmon carcasses collected for *Ichthyophonus* sampling in the Salcha River, Alaska 2006 (Map 1).



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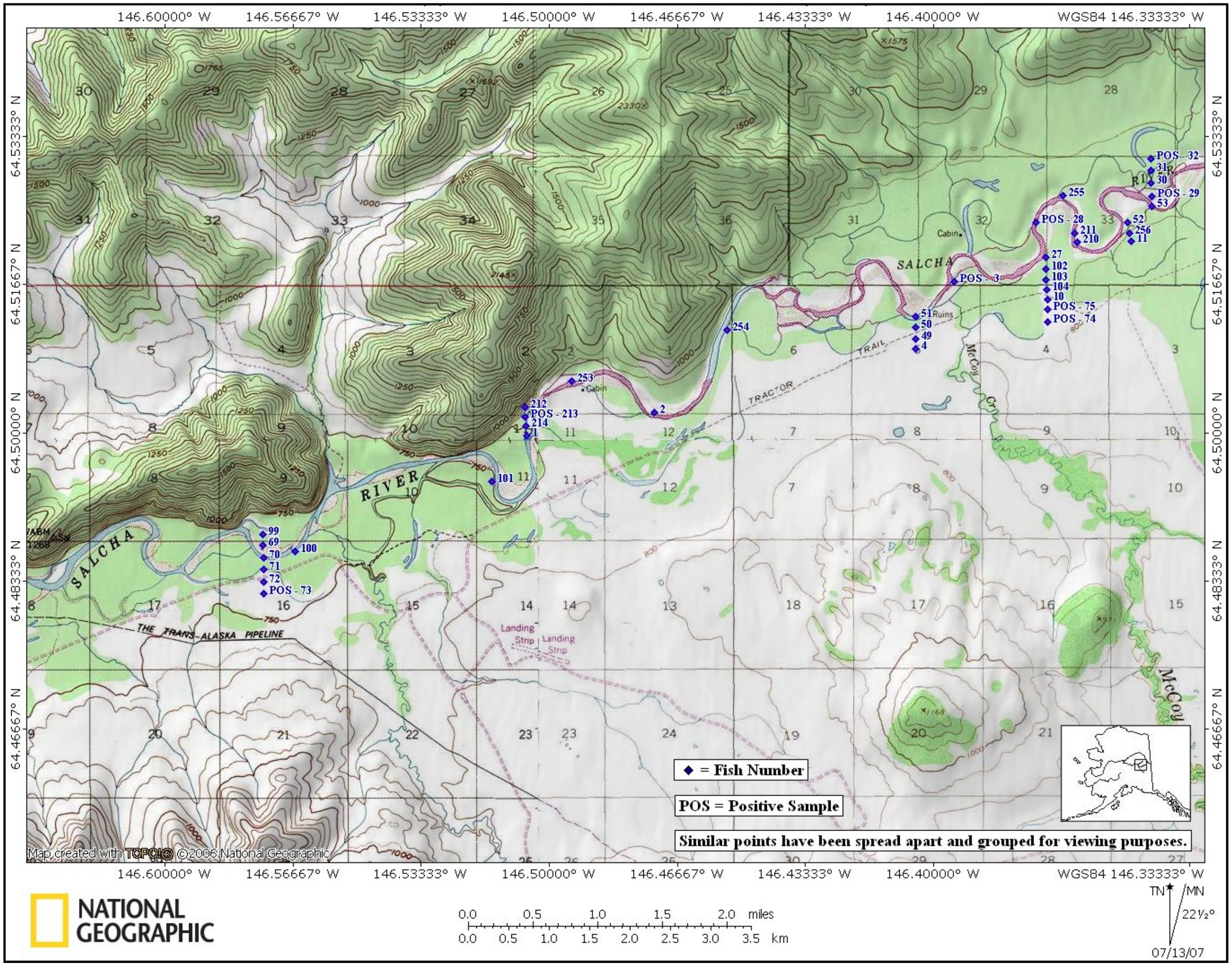
Appendix D6.–Page 2 of 4. (Map 2)



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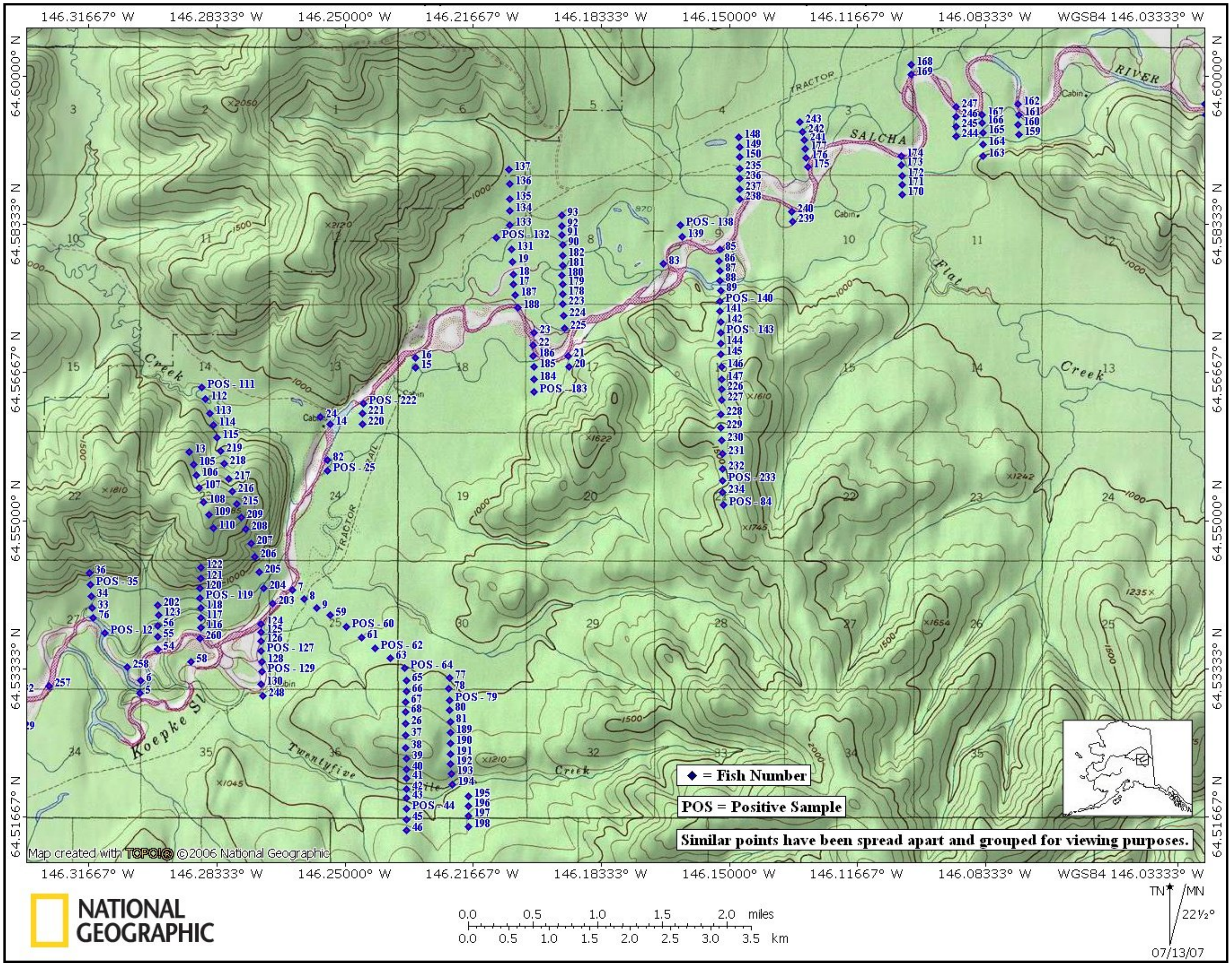
Appendix D6.–Page 3 of 4. (Map 3)



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Appendix D6.–Page 4 of 4. (Map 4)