Fishery Data Series No. 19-22

Exploration of AYK Chinook Salmon Egg Thiamine Levels as a Potential Mechanism Contributing to Recent Low Productivity Patterns, 2014 and 2015

by

Sean Larson

and

Kathrine Howard

August 2019

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

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

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

FISHERY DATA SERIES NO. 19-22

EXPLORATION OF AYK CHINOOK SALMON EGG THIAMINE LEVELS AS A POTENTIAL MECHANISM CONTRIBUTING TO RECENT LOW PRODUCTIVITY PATTERNS, 2014 AND 2015

by
Sean Larson and Kathrine Howard,
Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

August 2019

ADF&G Fishery Data Series was established in 1987 for the publication of Division of Sport Fish technically oriented results for a single project or group of closely related projects, and in 2004 became a joint divisional series with the Division of Commercial Fisheries. Fishery Data Series reports are intended for fishery and other technical professionals and are available through the Alaska State Library and on the Internet: http://www.adfg.alaska.gov/sf/publications/. This publication has undergone editorial and peer review.

Sean Larson and Kathrine Howard,
Alaska Department of Fish and Game, Division of Commercial Fisheries,
333 Raspberry Road, Anchorage, AK 99507, USA

This document should be cited as follows:
Larson, S., and K. Howard. 2019. Exploration of AYK Chinook salmon egg thiamine levels as a potential mechanism contributing to recent low productivity patterns, 2014 and 2015. Alaska Department of Fish and Game, Fishery Data Series No. 19-22, Anchorage.

TABLE OF CONTENTS

Page

LIST OF TABLES....ii
LIST OF FIGURES....ii
ABSTRACT....1
INTRODUCTION....1
OBJECTIVES....3
STUDY AREA....3
METHODS....4
RESULTS....5
DISCUSSION....6
ACKNOWLEDGEMENTS....8
REFERENCES CITED....8
TABLES AND FIGURES....11

LIST OF TABLES

Table **Page**

1 Agencies responsible for sampling Chinook salmon, the group assigned to each sampling location, and sample sizes at each sampling location, 2014–2015. 12
2 Number of Chinook salmon whose eggs were successfully analyzed within each comparison group, 2014–2015. 12
3 Sample size (*N*), minimum, maximum, mean, and standard deviation (SD) of thiamine concentrations (nmol/g) at each sampling location, 2014. 13
4 Sample size (*N*), minimum, maximum, mean, and standard deviation (SD) of thiamine concentrations (nmol/g) at each sampling location, 2015. 13
5 Sample size (*N*), proportion of samples below 1.5 nm/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, the maximum, mean, and standard deviation (SD) of thiamine concentrations within each group, 2014. 14
6 Sample size (*N*), proportion of samples below 1.5 nm/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, and the maximum, mean, and standard deviation (SD) of thiamine concentrations within each group, 2015. 14
7 Proportions and sample sizes (*N*) of Chinook salmon sampled, across all locations, that had egg thiamine concentrations below 1.5 nmol/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, 2014, 2015, and both years combined. 14
8 Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different sections of the Yukon River, 2014. 15
9 Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different sections of the Yukon River, 2015. 15
10 Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different rivers, 2014. 15
11 Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different rivers, 2015. 15

LIST OF FIGURES

Figure **Page**

1 Map of the study area with each Chinook salmon egg sampling location identified, 2014 and 2015. 16
2 Boxplots describing total Chinook salmon egg thiamine concentrations at each collection location, 2014. 17
3 Boxplots describing total Chinook salmon egg thiamine concentrations at each collection location, 2015. 18
4 Boxplots describing total Chinook salmon egg thiamine concentrations within each group, 2014. 19
5 Boxplots describing total Chinook salmon egg thiamine concentrations within each group, 2015. 20

ABSTRACT

Chinook salmon (*Oncorhynchus tshawytscha)* in Western Alaska have experienced declines in productivity and the mechanisms driving these declines are poorly understood. Thiamine (vitamin B1) deficiency has been responsible for declines in numerous salmonid populations; its prevalence in Western Alaska Chinook salmon was investigated in 2014 and 2015. Fluorescence spectroscopy coupled with High Performance Liquid Chromatography was used to measure thiamine concentrations in Chinook salmon eggs collected from the Yukon River, Kuskokwim River, and Unalakleet River. Eggs were opportunistically collected from 398 Chinook salmon, predominately from the middle and upper Yukon River regions. In 2014, 70% of the eggs had thiamine concentrations associated with secondary effects of thiamine deficiency that can lead to mortality (1.5–8.0 nmol/g). In 2015, 58% of the eggs had thiamine concentrations associated with secondary effects of thiamine deficiency that can lead to mortality. Across both years, about 3% of eggs had thiamine concentrations associated with overt mortality (<1.5 nmol/g). Egg thiamine concentrations were lower on the Yukon River than the Kuskokwim Area and Unalakleet River and thiamine concentrations were consistently lower in eggs collected from the middle and upper Yukon River than in eggs collected from the lower Yukon River. Within the Yukon River, thiamine concentrations were consistently low in eggs collected from the Chena and Teslin rivers. These results suggested that thiamine deficiency may negatively influence productivity in Yukon River Chinook salmon.

Key words: Chinook salmon, *Oncorhynchus tshawytscha*, vitamin B1, thiamine, Yukon River, Kuskokwim River, Unalakleet River.

INTRODUCTION

Chinook salmon (*Oncorhynchus tshawytscha*) returns to Western Alaska have declined since the late 1990s. Most notably, Yukon River Chinook salmon returns have declined by approximately half of their historical size since 2007 (ADF&G 2013). In a broader geographic scope, productivity has synchronously declined in stocks across Alaska, beginning with those cohorts spawned in 2001 (ADF&G 2013). In 2004, the Alaska Board of Fisheries designated Chinook salmon in the Unalakleet River a stock of yield concern (Kent and Bergstrom 2012). In addition, there has been a sharp decline in Chinook salmon returns in the Kuskokwim River since 2010, and 2013 was the lowest return on record (Poetter 2015). Interestingly, Chinook salmon returning to the Nushagak River in Bristol Bay have not experienced declines in run sizes on the scale of Arctic-Yukon-Kuskokwim (AYK) stocks (Elison et al. 2018). Throughout the AYK region, low returns in recent years have resulted in commercial fishery closures and severe restrictions to subsistence fishing in an effort to meet escapement goals. Consequently, Chinook salmon harvests on the Yukon River were below the amount necessary for subsistence (ANS; 45,500 fish) between 2007 and 2016 (Jallen et al. 2017; JTC 2017). Similarly, Chinook salmon harvests on the Kuskokwim River were below the ANS since 2009 (Poetter and Tiernan 2017). Causes of reduced productivity and poor returns are largely unknown; however, vitamin deficiencies due to shifting food availability may play a roll.

Thiamine deficiency was first noted in reared fish systems (Halver 1989) and is currently recognized as a critical mechanism leading to population declines of top predators. Thiamine, vitamin B1, is an essential dietary nutrient that can only be obtained through certain prey sources. Animals can become thiamine deficient if they consume too many prey either low in thiamine or high in thiaminase, the enzyme that breaks down thiamine. Thiamine deficiency has led to population declines in Great Lakes salmonids (Marcquenski and Brown 1997), Baltic Sea salmon (Norrgren et al. 1998), New York Finger Lake salmonids (Fisher et al. 1996), and Florida alligators (Rice et al. 2007; Ross et al. 2009). In addition, thiamine deficiency is suspected to be the cause of recent population declines of Icelandic common eider (Balk et al. 2016), and researchers correlated the number of eggs per clutch and yolk thiamine concentration in Baltic Sea herring gulls (Balk et al. 2009).

Due to its connection with fish rearing, the best studied symptom of thiamine deficiency in fish eggs is early life stage mortality syndrome (EMS), when significant fry mortality occurs when egg thiamine levels fall below 1.5 nmol/g (Honeyfield et al. 2005; Wolgamood et al. 2005; Honeyfield et al. 2008). Although less well documented, secondary effects of thiamine deficiency in salmonid eggs have been shown to occur when thiamine levels are less than 8.0 nmol/g. For example, lake trout fry hatched from eggs with less than 4.0 nmol/g thiamine in the Great Lakes region experienced recognizable secondary effects of thiamine deficiency (Honeyfield et al. 2008). Secondary effects of egg thiamine deficiency can include impaired growth, vision, predator avoidance, prey capture and immune function, all of which can reduce or prevent recruitment into the fishery (Brown et al. 2005b; Carvalho et al. 2009; Fitzsimons et al. 2009; Ottinger et al. 2012).

Thiamine deficiency can also impact salmonids during the adult life stage. Mortalities were observed in adult Steelhead and lake trout due to thiamine deficiency (Brown et al. 2005a), and thiamine deficiency was responsible for limited spawning migrations in adult Rainbow trout and coho salmon (Fitzsimons et al. 2005; Ketola et al. 2005). Thiamine is needed within the Krebs cycle and to produce ATP (Agyei-Owusu and Leeper 2009), which is essential for migration. Thiamine deficiency could be particularly problematic for stocks with long migration routes such as Canadian-origin Chinook salmon on the Yukon River.

Previous research, although limited in scope, has addressed thiamine deficiency in Yukon River Chinook salmon. An exploratory analysis of Yukon River Chinook salmon thiamine levels in 2012 indicated that egg thiamine concentrations may decrease with migratory distance (Honeyfield et al. 2016). Thus, Upper/Canadian-origin Chinook salmon stocks, that undertake longer migrations, may exhibit decreased thiamine levels compared to other Yukon River stocks. An expanded sampling project was conducted in 2013 and found that mean total egg thiamine concentrations were replete (thiamine ≥8.0 nmol/g) at most collection sites, but the data were highly variable and 33% (N = 10) of the females used in the analysis had measured thiamine levels that were within the range documented as producing secondary effects of thiamine deficiency (Larson et al. 2015). As a whole, Chinook salmon eggs had a relatively high thiamine concentration; however, individual offspring may have been susceptible to negative secondary impacts associated with thiamine deficiency. If Chinook salmon have diets consisting of prey that are low in thiamine, or high in thiaminase, then thiamine deficiency could be contributing to low productivity.

In 2014 and 2015, there was an opportunity to collect eggs from locations throughout Western Alaska at locations where salmon assessment already occurred. This allowed us to extend the limited thiamine related work done in the Yukon River and explore if there was evidence of thiamine deficiency in other river systems within the region. This was an exploratory study with no predetermined sampling design. The results of this work may guide more formal studies to address specific research hypotheses.

OBJECTIVES

The objectives of this study were as follows:

1. Use fluorescent spectroscopy and High-Performance Liquid Chromatography to assess thiamine levels in Chinook salmon eggs, and,
2. Test if thiamine levels differ among Chinook salmon stocks in the AYK region.

STUDY AREA

This study occurred throughout the Yukon River, Kuskokwim Area, and Unalakleet River in Western Alaska. The Yukon River watershed is the fourth largest river drainage in North America and discharge can exceed 200 km^3 of water per year (Brabets et al. 2000). This river exceeds 3,000 km in length and extends from the Bering Sea to British Columbia, Canada. Over 40 communities exist along the Yukon River and its tributaries within Alaska. As the second largest river in Alaska, the Kuskokwim River is approximately 1,130 km long within a drainage that exceeds 130,000 km^2. The Unalakleet River is approximately 210 km long and extends from the Bering Sea to the Nulato Hills. The Unalakleet River has 5 major salmon producing tributaries within its 5,400 km^2 drainage. The Yukon, Kuskokwim, and Unalakleet rivers support all 5 species of salmon.

Chinook salmon eggs were collected from 3 major river systems in Western Alaska and from Yukon River tributaries in the Yukon Territory, Canada, during 2014 and 2015 (Table 1). Sampling locations corresponded to existing assessment monitoring sites with access to large numbers of salmon carcasses and existing capacity to sample. Egg collections at sampling locations were opportunistic and may not represent non-sampled locations or the broader stock aggregate. Within the Yukon River drainage in Alaska, samples were obtained from the Andreafsky, Gisasa, Melozitna, Chena, Salcha, and Chandalar rivers. Within the Yukon River drainage in Canada, samples were obtained from the mainstem Yukon River at the Whitehorse Hatchery, the McQuesten, Nisutlin, Pelly, Teslin, Big Salmon, and Wolf rivers, and from Blind Creek. Samples were obtained from the Kogrukluk River within the Kuskokwim River drainage and the Kanektok River, which drains directly into Kuskokwim Bay adjacent to the Kuskokwim River; together, these locations are referred to as the Kuskokwim Area. Samples were also obtained from the Unalakleet River mainstem (Figure 1).

Sampling sites were assigned to various groupings for comparison; 3 within the Yukon River and one each for the Kuskokwim Area and the Unalakleet River. The Upper Yukon group (Canadian stocks) included the Whitehorse Hatchery, the McQuesten, Nisutlin, Pelly, Teslin, Big Salmon, and Wolf rivers, and Blind Creek. The Middle Yukon group included Venetie Slough and the Chena, Salcha, and Chandalar rivers. The Lower Yukon group included the East Fork Andreafsky, Gisasa, and Melozitna rivers. The Kuskokwim group included the Kanektok and Kogrukluk rivers. Finally, the Unalakleet River group included the mainstem Unalakleet River. Groupings (Lower, Middle, and Upper) for the sampling sites within the Yukon River drainage corresponded with the broad scale reporting groups developed by the ADF&G gene conservation laboratory[1].

---

[1] http://www.adfg.alaska.gov/index.cfm?adfg=fishinggeneconservationlab.main

METHODS

Sampling occurred opportunistically upriver from where the primary assessment project associated with each river was located (Figure 1). Prior to the field seasons, pre-labeled egg collection kits were mailed to the various project leaders. A minimum of 10 eggs per fish were collected from Chinook salmon during carcass surveys and hatchery egg collections. The collection of eggs during carcass sampling was largely opportunistic and was performed during annual ADF&G carcass sampling events in July and August, corresponding to when the most carcasses would be available on the spawning grounds. Samplers were advised to only collect eggs from recently spawned, dead, Chinook salmon that retained at least 10 eggs from the spawning event. Whether or not a salmon spawned recently was largely subjective and left to the discretion of the sampler; however, samplers were advised to avoid salmon that had eggs that showed signs of decomposition; i.e., pale coloration. Eggs were frozen as soon as possible and then shipped to ADF&G in coolers that contained ice packs. Target sample sizes were up to 40 female Chinook salmon per sampling site per year. Sampling spanned 2 years to account for potential annual variability in thiamine levels that could be associated with odd/even year dynamics.

Samples were processed at the National Marine Fisheries Service Auke Bay lab via fluorescence spectroscopy coupled with High Performance Liquid Chromatography (HPLC) to measure thiamine concentrations for individual Chinook salmon, following the methods of Brown et al. (1998) and similar studies. In brief, for each female Chinook salmon sampled, approximately 0.3 g of the 10+ eggs collected were homogenized in 5% trichloroacetic acid (TCA) and placed in boiling water for 5 minutes. Then 3 mL of 10% TCA was added and homogenization of the samples was repeated. The samples were allowed to sit on ice for 15 minutes before being centrifuged at 4,000 rpm for 10 minutes. The samples were then refrigerated overnight and, the following day, were washed with 2 mL of ethyl acetate:hexane (3:2 v:v) and sent through a vortexing machine. This washing process was repeated 3 times, which allowed the removal of organic material from the samples. Following the washing process, 850 µL of each sample were combined with 90 µL of 5 M NaOH and 60 µL of 3 mM potassium ferricyanide. Finally, thiamine was quantified using an HPLC equipped with a fluorescence detector using excitation and emission wavelengths of 375 nM and 433 nM, respectively.

These methods report total egg thiamine, which is the sum of free thiamine (T), thiamine monophosphate (TP), and thiamine pyrophosphate (TPP). All samples were evaluated visually for degradation and through data review. Samples with evidence of degradation (increase in TP, decrease in TPP and loss of total thiamine concentrations due to improper sample collection or handling) were discarded. As a result of egg degradation, eggs from a total of 30 females were excluded from the laboratory analysis (Table 2). Total egg thiamine concentrations for each fish and mean total egg thiamine concentrations were compared against established thresholds for thiamine deficiency for lake trout. For example, based on thiamine thresholds established for lake trout, total egg thiamine concentrations ≥ 8.0 nmol/g were considered thiamine replete, concentrations ≥ 4.0 and <8.0 nmol/g indicated a lower risk of the secondary effects of thiamine deficiency, concentrations ≥ 1.5 and <4.0 nmol/g indicated a higher risk of the secondary effects of thiamine deficiency, and concentrations <1.5 nmol/g indicated a high likelihood of overt fry mortality. Thiamine thresholds have not been evaluated for Chinook salmon and the published limits for lake trout were used as a proxy to identify if thiamine deficiency may be an issue in the AYK region.

Egg thiamine concentrations observed in the Upper/Canadian-origin Yukon stock group were compared to those from Middle and Lower Yukon River stock groups using one-way ANOVA and Tukey's Honestly Significant Difference (Tukey HSD) post-hoc pairwise comparisons to test the null hypothesis that there was no significant difference in mean egg thiamine concentration among stock groups. Similarly, ANOVA and Tukey HSD were used to compare average egg thiamine concentrations at the Yukon River, Kuskokwim Area, and Unalakleet River to test the null hypothesis that there was no significant difference in mean egg thiamine concentration among river systems. Regional differences in mean egg thiamine concentration were evaluated each year to identify any inter-annual variation in thiamine concentrations.

RESULTS

Sample sizes were highly variable across sampling locations each year. In 2014, total egg thiamine was analyzed for 204 Chinook salmon sampled across 11 sampling sites. In 2015, total egg thiamine was analyzed for 194 Chinook salmon sampled across 14 sampling sites (Table 1). Sample sizes ranged from 1 Chinook salmon at the Wolf and Melozitna rivers to 99 Chinook salmon at the Chena River. Most of the samples were collected from the Middle (n = 128) and Upper (n = 180) Yukon groups (Table 2).

Chinook salmon egg thiamine concentrations were highly variable. Average total egg thiamine concentrations were less than 8.0 nm/g in 2014 (6.1 nmol/g) and 2015 (7.6 nmol/g). Average thiamine concentrations varied among sampling locations each year (Tables 3 and 4). Furthermore, thiamine concentrations were highly variable between individuals within sampling locations (Figures 2 and 3). The lowest average thiamine concentration occurred at the McQuesten River and the highest occurred at the Unalakleet River (Table 3). Egg thiamine concentrations were, on average, greater than 8.0 nmol/g within the Unalakleet, Kuskokwim, and Lower Yukon groups whereas the Middle and Upper Yukon groups were consistently less than 8.0 nm/g (Tables 5 and 6). In 2014, 73% of Chinook salmon sampled from the middle Yukon River and 85% of Chinook salmon sampled from the upper Yukon River had egg thiamine concentrations between 1.5 and 8.0 nmol/g (Table 5). In addition, about 5% (n=10) of female Chinook salmon had egg thiamine concentrations less than 1.5 nmol/g (Table 7). Chinook salmon with the lowest egg thiamine concentrations in 2014 were sampled from the Chena, Salcha, Big Salmon, and Pelly rivers (Table 3). In 2015, 70% of Chinook salmon sampled from the middle and upper Yukon River had egg thiamine concentrations between 1.5 and 8.0 nmol/g (Table 6). Only 1% (n=2) of female Chinook salmon had egg thiamine concentrations less than 1.5 nmol/g in 2015 (Table 7). Most notable were the low egg thiamine concentrations on the Chena and Teslin rivers. Across both years, 86% of the female Chinook salmon sampled on the Chena and Teslin rivers had egg concentrations less than 8.0 nm/g (Figures 2 and 3). In addition, all Chinook salmon with egg thiamine concentrations below 1.5 nmol/g were sampled from the middle or upper Yukon River (Figure 5).

Average total egg thiamine concentrations differed across river systems and within the Yukon River. Thiamine concentrations were dependent on which section of the Yukon River Chinook salmon eggs were collected; i.e., lower, middle, or upper Yukon River in 2014 (ANOVA; F = 139, p < 0.000) and 2015 (ANOVA; F = 13, p < 0.000). Post-hoc Tukey's Honest Significant Difference (HSD) tests showed that the middle and upper Yukon River had significantly lower egg thiamine concentrations than the lower Yukon River in 2014 and the lower, middle, and upper Yukon River had significantly different egg thiamine concentrations in 2015 at the 0.05

level of significance (Tables 8 and 9). Egg thiamine concentrations in the upper Yukon River were significantly higher in 2015 relative to 2014 (*t*-test; $p < 0.000$), whereas egg thiamine concentrations in the middle Yukon River were not significantly different between years (*t*-test; $p = 0.054$). Thiamine concentrations were also dependent on which river Chinook salmon eggs were collected; i.e., the Unalakleet River, Kuskokwim Area, and Yukon River in 2014 (ANOVA; $F = 211$, $p < 0.000$) and 2015 (ANOVA; $F = 57$, $p < 0.000$). Post-hoc Tukey's HSD tests showed that the Yukon River had significantly lower egg thiamine concentrations than the Unalakleet River in 2014 and both the Unalakleet River and Kuskokwim Area in 2015 at the 0.05 level of significance (Tables 10 and 11). All other comparisons were not significant. Conversely, when pooling both years, egg thiamine concentrations were not significantly different between the Kuskokwim Area, Unalakleet River, and lower Yukon River (ANOVA; $F = 3$, $p = 0.084$). When pooled across all sampling locations, mean egg thiamine concentrations were significantly greater in 2015 than 2014 (*t*-test; $p < 0.000$).

DISCUSSION

Egg thiamine concentrations may influence productivity in Western Alaska Chinook salmon stocks. The majority of Chinook salmon sampled from the middle and upper Yukon River had egg thiamine concentrations within the range in which lake trout develop secondary effects of thiamine deficiency. The thresholds associated with thiamine deficiency and used as the basis for comparison were established for lake trout and it is unclear if these thresholds hold true for Chinook salmon in Western Alaska. Research is needed to determine if these same thresholds apply to Chinook salmon. Despite the unknowns regarding Chinook salmon specific thresholds, it has been well documented that low thiamine levels can lead to reduced fitness in animals.

Thiamine deficiency has led to productivity declines in salmonid populations. Studies of thiamine deficiency and fish have primarily focused on mortality; however, there are secondary effects that can cause declines in animal populations. These secondary effects include impaired immune response, impaired migratory ability, diminished reproductive capacity, and abnormal behavior (Balk et al. 2016). Only 3% of sampled female Chinook salmon, across all sampling locations, had egg thiamine concentrations at levels that led to overt fry mortality in lake trout (<1.5 nmol/g). The majority (64%) of Chinook salmon had egg thiamine concentration within the range that may cause secondary effects due to thiamine deficiency (1.5–8.0 nmol/g). At most sampling locations, egg thiamine concentrations tended to be lower in 2014 than 2015, which indicated an improvement in egg health from 1 year to the next. However, additional years of monitoring would be needed to identify any temporal trends in thiamine concentration.

Most of the Chinook salmon sampled from the middle and upper Yukon River had egg thiamine concentrations less than 8 nmol/g; however, some tributaries may have been more at risk to thiamine deficiency than others. Of interest were the egg thiamine concentrations from the Chena and Teslin rivers, which were consistently among the lowest observed during the study. Low egg thiamine concentrations were also observed for Chena River Chinook salmon in 2012, when about 25% of Chinook salmon sampled had egg thiamine concentrations below 1.5 nmol/g. However, sample size was considered too low for a reliable estimate at the Chena River in 2012 (Honeyfield et al. 2016). The Chena River flows through the city of Fairbanks, which is the second largest urban center in Alaska and has a long history of extreme air pollution due to the high use of wood and coal burning stoves (Nattinger et al. 2015; Wang and Hopke 2014). Residential wood burning stoves are a significant source of ambient particulate matter pollution

(Larson et al. 2004; Naeher et al. 2007). Toxic pollutants have been associated with thiamine deficiency in animal populations (Balk et al. 2009), but their relationship to thiamine deficiency in Chinook salmon remains untested.

Egg thiamine concentrations tended to be lower in Chinook salmon with longer migrations; i.e., the middle and upper Yukon River, which suggested that thiamine depletion may be associated with migratory distance. In fact, the 4 stocks with the shortest migration routes (Unalakleet, Kanektok, Kogrukluk, and Andreafsky rivers) consistently had the highest egg thiamine concentrations. Chinook salmon egg thiamine concentrations also decreased with migratory distance during exploratory egg health studies in 2001 and 2012, which indicated that there may have been a thiamine cost of migration (Honeyfield et al. 2016). Thiamine concentrations also decline through time when fish are not feeding; i.e., during migration. For example, thiamine levels in Chinook salmon muscle tissue declined significantly when Chinook salmon fasted for 150 days (Honeyfield et al. 2016). If Chinook salmon use up thiamine reserves as they travel upriver to spawn, then stocks with longer migrations; i.e., Canadian-origin Chinook salmon, may need to have larger stores of thiamine than stocks with shorter migrations. Alternatively, it may be normal for stocks with longer migrations to have lower egg thiamine concentrations. If egg thiamine monitoring continues, focus should be on Chinook salmon returning to the middle and upper Yukon Rivers, because they are associated with relatively low thiamine concentrations.

The specific mechanisms controlling thiamine content in Chinook salmon in Western Alaska remain unclear. Toxic pollutants can contribute to thiamine deficiency in animals (Balk et al. 2009). However, Western Alaska Chinook salmon spend most of their lives feeding in the Bering Sea and away from concentrated pollutants. Although egg thiamine concentrations for Chinook salmon returning to the Chena River were consistently low, the relationship between thiamine deficiency and pollution in that region is unknown. Thiamine deficiency in Chinook salmon is predominately a result of marine diet high in thiaminase or low in thiamine. Although it is understood that Chinook salmon eat primarily squid, euphausiids, and fish, prey availability can fluctuate (Davis et al. 2005). Changing ocean conditions can influence the biochemistry of marine waters important for Chinook salmon foraging. A survey along the northwest coast of the U.S. indicated that there are large areas of water that are depleted of thiamine, which can have a bottom-up influence on the marine food web (Sanudo-Wilhelmy et al. 2012). Changes to the food web in the Bering Sea, where Western Alaska Chinook salmon spend most of their lives, could be a result of natural, cyclical, fluctuations in water temperatures such as the Pacific Decadal Oscillation or the El Niño-Southern Oscillation. Similarly, climate change may directly and indirectly affect marine communities (Brander 2010). Marine diet studies of salmon in the Bering Sea are expensive and logistically challenging, therefore direct observations of diet patterns and food web changes are infrequent and often opportunistic in geographic scope. Although the ultimate cause of diet or food web changes may be difficult to study, the proximate cause of thiamine deficiency in eggs may be a more tangible approach to address marine research needs. As food webs fluctuate, it is essential that adult Chinook salmon maintain a nutritious, high fat, diet while in the ocean to prepare for their extensive upstream spawning migration and the production of viable offspring. Research involving stable isotope analysis is needed to determine how diets may differ between thiamine deficient and thiamine replete Chinook salmon, and what might account for any differences. Low thiamine concentrations in Chinook salmon, and consequently their eggs, probably influence an individual's fitness and warrants consideration when examining quality of escapement. Implications of this research could extend beyond the Western Alaska Chinook salmon stocks and may provide insight into currently unmonitored changes in the marine ecosystem.

ACKNOWLEDGEMENTS

This study was a collaborative effort among several agencies; including, the Alaska Department of Fish and Game, the National Oceanic and Atmospheric Administration, and the Department of Fisheries and Oceans Canada. In addition, several important contributors have generously donated their time and effort in collecting samples for this project. Among these are James Savereide, Lisa Stuby, and Kevin Schaberg of the Alaska Department of Fish and Game, Chris Stark of the Bering Sea Fishermen's Association, Jeremy Mears of the U.S. Fish and Wildlife Service, Wes Jones and Renae Ivanoff of the Norton Sound Economic Development Corporation, Trix Tanner and Sean Collins of the Department of Fisheries and Oceans Canada, and Lawrence Vano of the Whitehorse Rapids Hatchery. We also thank Dale Honeyfield and Jim Murphy for providing their expertise. We gratefully thank Paige Drobny with Spearfish Research for assisting with the report writing and sample collection. Finally, we thank the North Pacific Research Board for providing funding for this study.

REFERENCES CITED

Agyei-Owusu, K., and F. J. Leeper. 2009. Thiamin diphosphate in biological chemistry: analogues of thiamin diphosphate in studies of enzymes and riboswitches. FEBS Journal 276: 2905-2916.

ADF&G Chinook Salmon Research Team. 2013. Chinook salmon assessment and research plan. 2013. Alaska Department of Fish and Game, Special Publication No. 13-01, Anchorage.

Balk, L., P. Å. Hägerroth, G. Åkerman, M. Hanson, U. Tjärnlund, T. Hansson, G. T. Hallgrimsson, Y. Zebühr, D. Broman, T. Mörner, and H. Sundberg. 2009. Wild birds of declining European species are dying from a thiamine deficiency syndrome. Proceedings of the National Academy of Sciences, 106(29):12001-12006.

Balk, L., P. Å. Hägerroth, H. Gustavsson, L. Sigg, G. Åkerman, Y. R. Muñoz, D. C. Honeyfield, U. Tjärnlund, K. Oliveira, K. Ström, and S. D. McCormick. 2016. Widespread episodic thiamine deficiency in Northern Hemisphere wildlife. Scientific reports, 6.

Brabets, T. P., B. Wang, and R. H. Meade. 2000. Environmental and hydrologic overview of the Yukon River Basin, Alaska and Canada (No. 99-4204). US Dept. of the Interior, US Geological Survey; Branch of Information Services [distributor].

Brander, K. 2010. Impacts of climate change on fisheries. Journal of Marine Systems 79: 389-402.

Brown, S. B., D. C. Honeyfield, and L. Vandenbyllaardt. 1998. Thiamine analysis in fish tissues. [*In*] G. McDonald, J. Fitzsimons, and D. C. Honeyfield, editors. Early life stage mortality syndrome in fishes of the Great Lakes and the Baltic Sea. American Fisheries Society, Symposium 21, Bethesda, Maryland.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005a. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health 17: 59-64.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005b. Implications of thiamine deficiency in Great Lakes salmonines. Journal of Aquatic Animal Health 17:113-124.

Carvalho, P. S. M., D. E. Tillitt, J. L. Zajicek, R. A. Claunch, D. C. Honeyfield, J. D. Fitzsimons, and S. B. Brown. 2009. Thiamine deficiency effects on the vision and foraging ability of lake trout fry. Journal of Aquatic Animal Health 21: 315-325.

Davis, N. D., M. Fukuwaka, J. L. Armstrong, and K. W. Myers. 2005. Salmon food habits studies in the Bering Sea, 1960 to present. NPAFC Technical Report No. 6: 24-28.

Elison, T., P. Salomone, T. Sands, G. Buck, K. Sechrist, and, D. Koster. 2018. 2017 Bristol Bay annual management report. Alaska Department of Fish and Game, Fishery Management Report No. 18-11, Anchorage.

REFERENCES CITED (Continued)

Fisher, J. P., J. D. Fitzsimons, G. G. Combs, and J. M. Spitzbergan. 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes trout. Transactions of American Fishery Society 125: 167-178.

Fitzsimons, J. D., S. B. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, D. C. Honeyfield, and D. E. Tillitt. 2009. Influence of thiamine deficiency on lake trout larval growth, foraging, and predator avoidance. Journal of Aquatic Animal Health 21: 302-314.

Fitzsimons, J. D., B. Williston, P. Amcoff, L. Balk, C. Pecor, H. G. Ketola, J. P. Hinterkopf, and D. C. Honeyfield. 2005. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine-deficient coho salmon. Journal of Aquatic Animal Health 17:48-58.

Halver, J. E. 1989. Fish nutrition, Academic Press, San Diego, CA.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health 17:4-12.

Honeyfield, D. C., J. M. Murphy, K. G. Howard, W. W. Strasburger, and A. C. Matz. 2016. An exploratory assessment of thiamine status in Western Alaska Chinook salmon (Oncorhynchus tshawytscha). North Pacific Anadromous Fish Commission Bulletin No. 6, 21-31.

Honeyfield, D. C., A. K. Peters, and M. L. Jones. 2016. Thiamine and lipid utilization in fasting Chinook salmon. North Pacific Anadromous Fish Commission. Bulletin 6: 13-19.

Honeyfield, D. C., D. E. Tillitt, and S. C. Riley. 2008. Thiamine deficiency complex workshop report, Ann Arbor, MI., November 6-7, 2008, pg. 1-27. Great Lakes Fishery Commission report. http://www.glfc.org/research/reports/TDC%20workshop_2008.htm

Jallen, D. M., S. K. S. Decker, and T. Hamazaki. 2017. Subsistence and personal use salmon harvests in the Alaska portion of the Yukon River drainage, 2015. Alaska Department of Fish and Game, Fishery Data Series No. 17-39, Anchorage.

JTC (Joint Technical Committee of the Yukon River US/Canada Panel). 2017. Yukon River salmon 2016 season summary and 2017 season outlook. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 3A17-01, Anchorage.

Kent, S. M., and D. J. Bergstrom. 2012. Norton Sound Subdistrict 5 (Shaktoolik) and Subdistrict 6 (Unalakleet) king salmon stock status and action plan, 2013: a report to the Alaska Board of Fisheries. Alaska Department of Fish and Game, Special Publication No. 12-28, Anchorage.

Ketola, H. G., T. L. Chiotti, R. S. Rathman, J. D. Fitzsimons, D. C. Honeyfield, P. J. Van Dusen, and G. E. Lewis. 2005. Thiamine status of Cayuga Lake rainbow trout and its influence on spawning migration. North American journal of Fisheries Management 25:1281-1287.

Larson, S., C. Fugate, K. Howard, D. Honeyfield, J. Murphy, and L. Schaufler. 2015. Exploration of potential early life mortality in Canadian-origin Chinook salmon eggs due to thiamine deficiency. Yukon River Panel's Restoration and Enhancement Fund, URE-06-14N.

Larson, T., T. Gould, C. Simpson, L. J. S. Liu, C. Claiborn, and J. Lewtas. 2004. Source apportionment of indoor, outdoor, and personal PM2. 5 in Seattle, Washington, using positive matrix factorization. Journal of the Air and Waste Management Association 54(9):1175-1187.

Marcquenski, S. V., and S. B. Brown. 1997. Early mortality syndrome in the Great Lakes. Pages 135–152 in R. M. Rolland, M. Gilbertson, and R. E. Peterson, editors. Chemically induced alterations in functional development and reproduction in fishes. SETAC, Pensacola, Florida.

Naeher, L. P., M. Brauer, M. Lipsett, J. T. Zelikoff, C. D. Simpson, J. Q. Koenig, and K. R. Smith. 2007. Woodsmoke health effects: a review. Inhalation toxicology 19(1); 67-106.

REFERENCES CITED (Continued)

Nattinger, K., W. R. Simpson, and D. Huff. 2015. Compositional analysis of fine particulate matter in Fairbanks, Alaska. In AGU Fall Meeting Abstracts.

Norrgren, L., P. Amcoff, H. Börjeson, and P. O. Larsson. 1998. Reproductive disturbances in Baltic fish: A review. Pages 8-17 [*In*] G. McDonald, J. Fitzsimons, and D. C. Honeyfield, editors. Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, MD.

Ottinger, C. A., D. C. Honeyfield, C. L. Densmore, and L. R. Iwanowicz. 2012. Impact of thiamine deficiency on T-cell dependent and T-cell independent antibody production in post-yearling lake trout (Salvelinus namaycush). Journal of Aquatic Animal Health 24:258-273.

Poetter, A. D. 2015. Kuskokwim River salmon stock status and Kuskokwim area fisheries, 2015; a report to the Alaska Board of Fisheries. Alaska Department of Fish and Game, Special Publication No. 15-21, Anchorage.

Poetter, A. D., and A. Tiernan. 2017. 2016 Kuskokwim area management report. Alaska Department of Fish and Game, Fishery Management Report No. 17-50, Anchorage.

Rice, A. N., J. P. Ross, A. R. Woodward, D. A. Carbonneau, and H. F. Percival. 2007. Alligator diet in relation to alligator mortality on Lake Griffin, FL. Southeastern Naturalist 6:97-110.

Ross, J. P., D. C. Honeyfield, S. B. Brown, L. R. Brown, A. R. Waddle, M. E. Welker, and T. R. Schoeb. 2009. Gizzard shad thiaminase activity and its effect on the thiamine status of captive American alligators *Alligator mississippiensis*. Journal of Aquatic Animal Health 21:239-248.

Sañudo-Wilhelmy, S. A., L. S. Cutter, R. Durazo, E. A. Smail, L. Gómez-Consarnau, E. A. Webb, M. G. Prokopenko, W. M. Berelson, and D. M. Karl. 2012. Multiple B-vitamin depletion in large areas of the coastal ocean. Proceedings of the National Academy of Sciences, 109(35):14041-14045.

Wang, Y., and P. K. Hopke. 2014. Is Alaska truly the great escape from air pollution?–long term source apportionment of fine particulate matter in Fairbanks, Alaska. Aerosol and Air Quality Research 14(7):1875-1882.

Wolgamood, M., J. G. Hnath, S. B. Brown, K. Moore, S. V. Marcquenski, D. C. Honeyfield, J. P. Hinterkopf, J. D. Fitzsimons, and D. E. Tillitt. 2005. Temporal and spatial variation of early mortality syndrome in salmonids from Lakes Michigan and Huron. Journal of Aquatic Animal Health 17:65-76.

TABLES AND FIGURES

Table 1.–Agencies responsible for sampling Chinook salmon, the group assigned to each sampling location, and sample sizes (number of Chinook salmon whose eggs were successfully analyzed) at each sampling location, 2014–2015.

Location	Agency	Group	2014	2015	Total
Unalakleet River	NSEDC	Unalakleet	16	19	35
Kanektok River	ADF&G	Kuskokwim	0	13	13
Kogrukluk River	ADF&G	Kuskokwim	1	4	5
Andreafsky River	USFWS/Spearfish Research	Lower Yukon	18	11	29
Gisasa River	USFWS/Spearfish Research	Lower Yukon	0	7	7
Melozitna River	USFWS/Spearfish Research	Lower Yukon	0	1	1
Chena River	ADF&G	Middle Yukon	65	34	99
Salcha River	BSFA/ADF&G	Middle Yukon	16	10	26
Chandalar River	ADF&G	Middle Yukon	0	3	3
McQuesten River	DFO	Upper Yukon	2	0	2
Nisutlin River	DFO	Upper Yukon	0	3	3
Pelly River	DFO	Upper Yukon	6	0	6
Teslin River	DFO	Upper Yukon	30	20	50
Big Salmon River	DFO	Upper Yukon	14	7	21
Blind Creek	DFO	Upper Yukon	0	20	20
Whitehorse Hatchery	Yukon Energy	Upper Yukon	35	42	77
Wolf River	DFO	Upper Yukon	1	0	1
Total			204	194	398

Note: NSEDC = Norton Sound Economic Development Corporation, ADF&G = Alaska Department of Fish and Game, USFWS = United Stated Fish and Wildlife Service, BSFSA = Bering Sea Fisherman's Association, and DFO = Department of Fisheries and Oceans (Canada). Sample sizes do not include Chinook salmon eggs that were too degraded to analyze.

Table 2.–Number of Chinook salmon whose eggs were successfully analyzed within each comparison group, 2014–2015.

Group	2014	2015	Total
Unalakleet	16	19	35
Kuskokwim	1	17	21
Lower Yukon	18	19	37
Middle Yukon	81	47	136
Upper Yukon	88	92	180
Total	204	194	398

Note: Sample sizes do not include Chinook salmon eggs that were too degraded to analyze.

Table 3.–Sample size (*N*), minimum, maximum, mean, and standard deviation (SD) of thiamine concentrations (nmol/g) at each sampling location, 2014.

Location	*N*	Min	Max	Mean	SD
Unalakleet River	16	7.72	14.16	10.88	1.88
Kogrukluk River	1	10.11	10.11	10.11	-
Andreafsky River	18	5.99	13.84	9.26	2.42
Chena River	65	0.51	10.58	4.54	2.45
Salcha River	16	0.25	10.30	6.51	3.04
McQuesten River	2	1.68	6.25	3.97	3.24
Pelly River	6	0.43	8.56	5.24	3.68
Teslin River	30	2.28	8.93	4.82	1.58
Big Salmon River	14	1.44	8.07	4.09	2.01
Whitehorse Hatchery	35	4.64	9.13	6.84	1.10
Wolf River	1	6.53	6.53	6.53	–

Table 4.–Sample size (*N*), minimum, maximum, mean, and standard deviation (SD) of thiamine concentrations (nmol/g) at each sampling location, 2015.

Location	*N*	Min	Max	Mean	SD
Unalakleet River	19	5.70	12.86	9.69	2.14
Kanektok River	13	3.18	15.05	9.47	3.77
Kogrukluk River	4	9.04	10.99	10.08	0.99
Andreafsky River	11	6.00	11.23	8.82	1.57
Gisasa River	7	6.20	11.86	8.94	2.07
Melozitna River	1	5.94	5.94	5.94	-
Chena River	34	0.53	11.66	5.22	2.58
Salcha River	10	4.07	10.69	7.49	2.46
Chandalar River	3	7.50	8.53	8.18	0.59
Nisutlin River	3	8.08	10.16	8.97	1.07
Teslin River	20	1.49	10.36	6.28	2.35
Big Salmon River	7	5.20	7.82	6.42	1.08
Blind Creek	20	4.74	12.10	7.08	1.95
Whitehorse Hatchery	42	5.08	10.44	7.53	1.35

Table 5.–Sample size (*N*), proportion of samples below 1.5 nm/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, the maximum, mean, and standard deviation (SD) of thiamine concentrations within each group, 2014.

		Proportion						
Group	*N*	<1.5	1.5-8.0	>8.0	Min	Max	Mean	SD
Unalakleet	16	0.00	0.06	0.94	7.72	14.16	10.88	1.88
Kuskokwim	1	0.00	0.00	1.00	10.11	10.11	10.11	–
Lower Yukon	18	0.00	0.39	0.61	5.99	13.84	9.26	2.42
Middle Yukon	81	0.09	0.73	0.19	0.25	10.58	4.93	2.68
Upper Yukon	88	0.03	0.85	0.11	0.43	9.13	5.54	2.02

Table 6.–Sample size (*N*), proportion of samples below 1.5 nm/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, and the maximum, mean, and standard deviation (SD) of thiamine concentrations within each group, 2015.

		Proportion						
Group	*N*	<1.5	1.5-8.0	>8.0	Min	Max	Mean	SD
Unalakleet	19	0.00	0.21	0.79	5.70	12.86	9.69	2.14
Kuskokwim	17	0.00	0.24	0.76	3.18	15.05	9.61	3.31
Lower Yukon	19	0.00	0.42	0.58	5.94	11.86	8.71	1.80
Middle Yukon	47	0.01	0.70	0.28	0.53	11.66	5.89	2.68
Upper Yukon	92	0.01	0.70	0.29	1.49	12.10	7.13	1.80

Table 7.–Proportions and sample sizes (*N*) of Chinook salmon sampled, across all locations, that had egg thiamine concentrations below 1.5 nmol/g thiamine, between 1.5 and 8.0 nmol/g thiamine, and above 8.0 nmol/g thiamine, 2014, 2015, and both years combined.

	2014		2015		Both years	
	Proportion	*N*	Proportion	*N*	Proportion	*N*
Less than 1.5	0.05	10	0.01	2	0.03	12
1.5-8.0	0.70	142	0.58	113	0.64	255
Greater than 8	0.25	52	0.41	79	0.33	131

Table 8.–Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different sections of the Yukon River, 2014.

Groups compared			Difference	*P*-Value
Middle Yukon	-	Lower Yukon	4.33	<0.000
Upper Yukon	-	Lower Yukon	3.73	<0.000
Upper Yukon	-	Middle Yukon	0.60	0.224

Note: The outlined group had a significantly lower mean thiamine concentration than the comparison group at the 0.05 level of significance.

Table 9.–Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different sections of the Yukon River, 2015.

Groups compared			Difference	*P*-Value
Middle Yukon	-	Lower Yukon	2.82	<0.000
Upper Yukon	-	Lower Yukon	1.59	0.009
Upper Yukon	-	Middle Yukon	2.24	0.004

Note: The outlined group had a significantly lower mean thiamine concentration than the comparison group at the 0.05 level of significance.

Table 10.–Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different rivers, 2014.

Rivers Compared			Difference	*P*-Value
Unalakleet River	-	Kuskokwim Area	0.77	0.996
Yukon River	-	Kuskokwim Area	4.48	0.201
Yukon River	-	Unalakleet River	5.25	<0.000

Note: The outlined group had a significantly lower mean thiamine concentration than the comparison group at the 0.05 level of significance.

Table 11.–Results from Tukey's Honestly Significant Difference test comparing egg thiamine concentrations within different rivers, 2015.

Rivers Compared			Difference	*P*-Value
Unalakleet River	-	Kuskokwim Area	0.09	0.994
Yukon River	-	Kuskokwim Area	2.66	<0.000
Yukon River	-	Unalakleet River	2.75	<0.000

Note: The outlined group had a significantly lower mean thiamine concentration than the comparison group at the 0.05 level of significance.

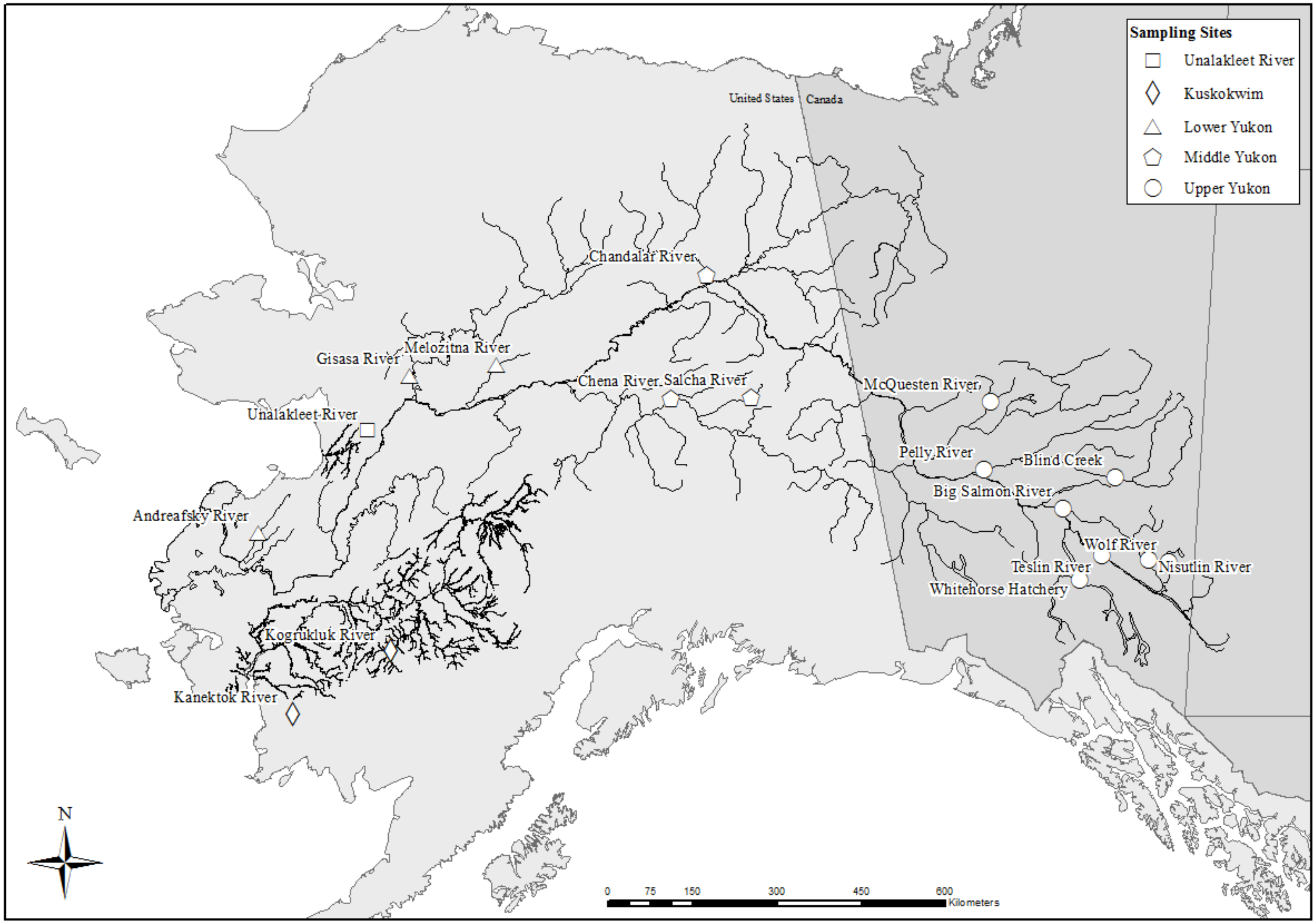

Figure 1.–Map of the study area with each Chinook salmon egg sampling location identified, 2014 and 2015.

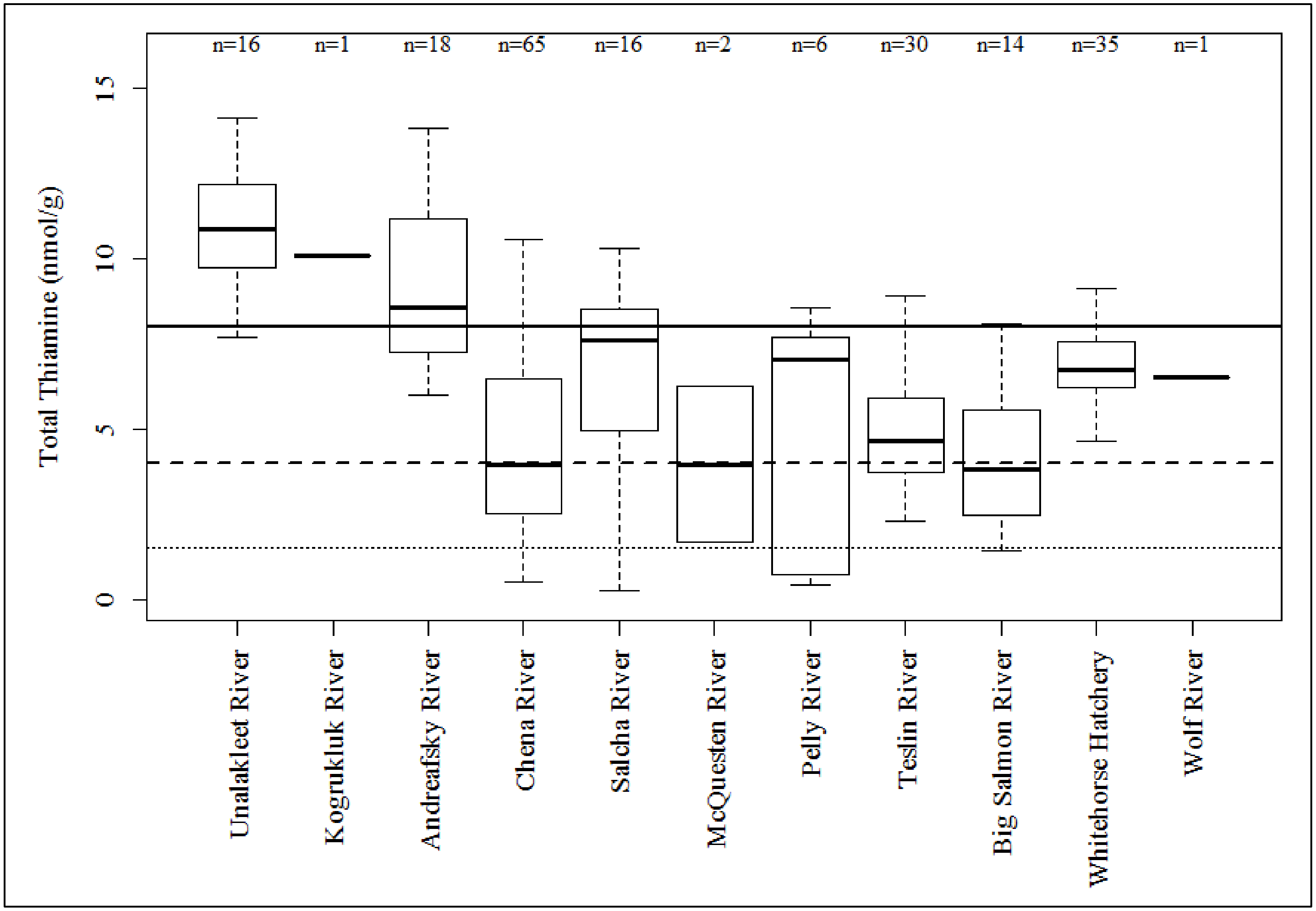

Figure 2.–Boxplots describing total Chinook salmon egg thiamine concentrations at each collection location, 2014.

Note: The box represents the interquartile range, the median is shown as the solid black line, and the bars represent the minimum and maximum values. The solid line designates total egg thiamine concentrations of 8.0 nmol/g (thiamine replete) and the middle-dashed line and bottom dotted line designate total egg thiamine concentrations of 4.0 (severe secondary effects) and 1.5 nmol/g (overt mortality), respectively. Sample sizes are shown at the top of the graph.

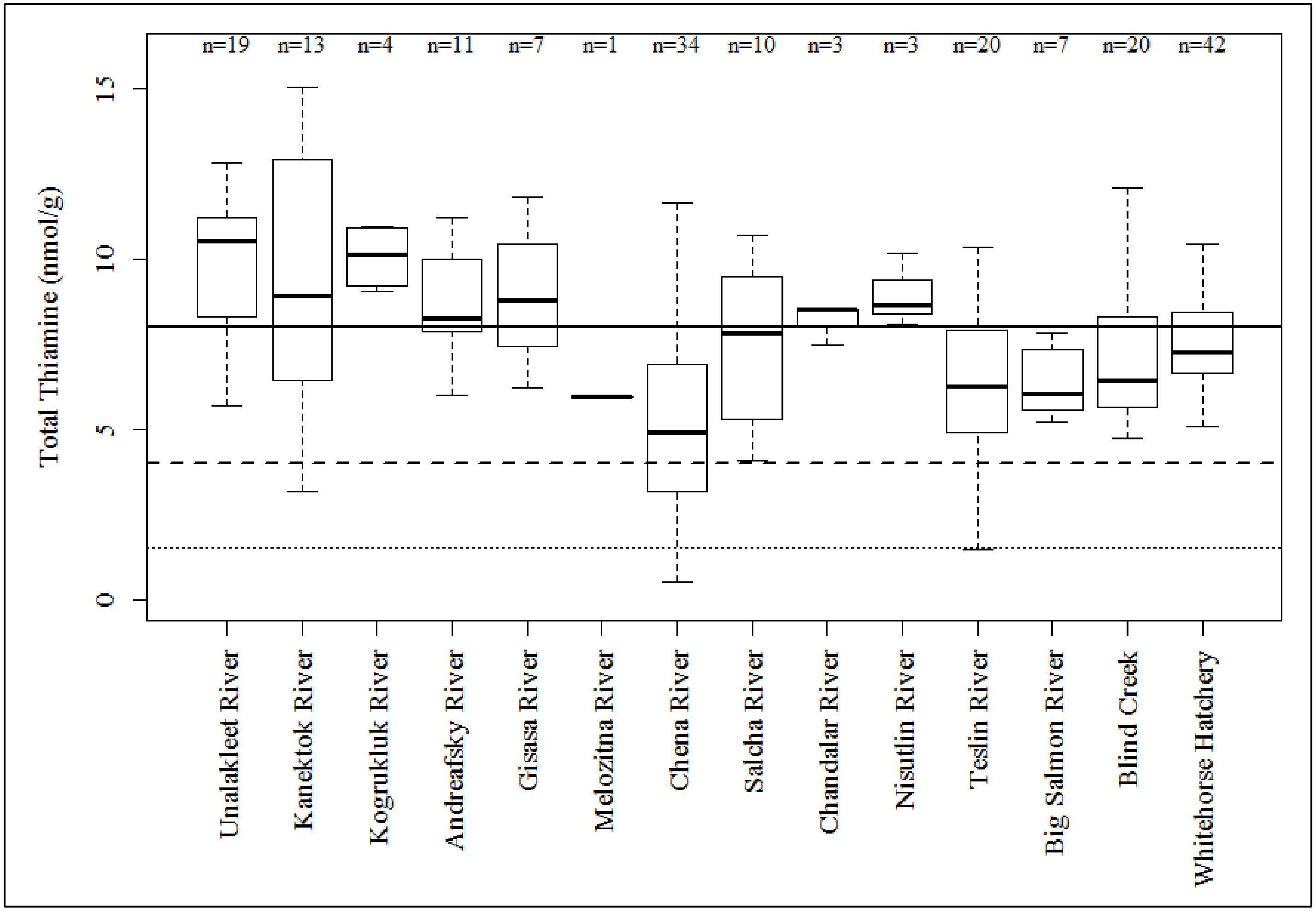

Figure 3.–Boxplots describing total Chinook salmon egg thiamine concentrations at each collection location, 2015.

Note: The box represents the interquartile range, the median is shown as the solid black line, and the bars represent the minimum and maximum values. The solid line designates total egg thiamine concentrations of 8.0 nmol/g (thiamine replete) and the middle-dashed line and bottom dotted line designate total egg thiamine concentrations of 4.0 (severe secondary effects) and 1.5 nmol/g (overt mortality), respectively. Sample sizes are shown at the top of the graph.

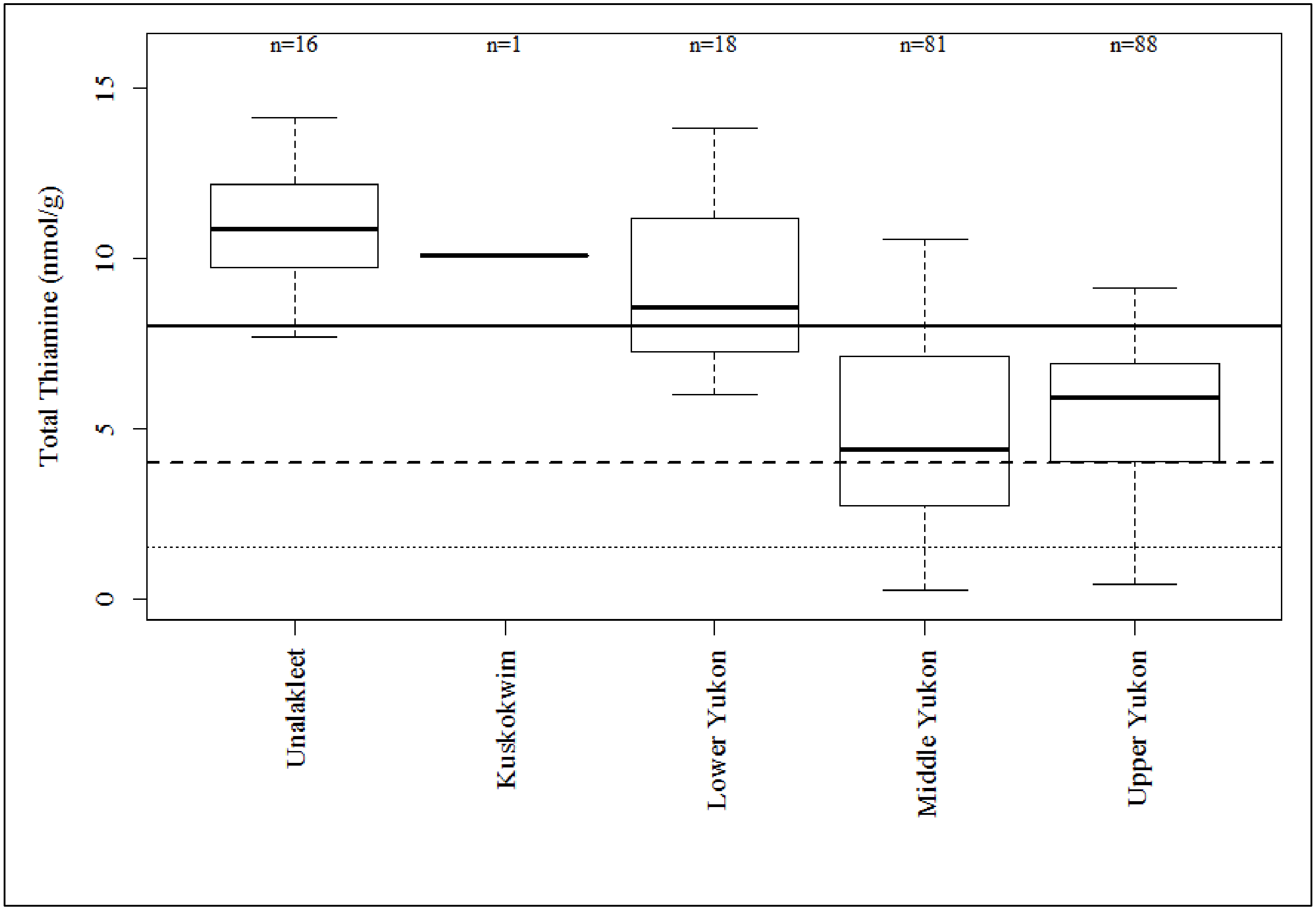

Figure 4.–Boxplots describing total Chinook salmon egg thiamine concentrations within each group, 2014.

Note: The box represents the interquartile range, the median is shown as the solid black line, and the bars represent the minimum and maximum values. The solid line designates total egg thiamine concentrations of 8.0 nmol/g (thiamine replete) and the middle-dashed line and bottom dotted line designate total egg thiamine concentrations of 4.0 (severe secondary effects) and 1.5 nmol/g (overt mortality), respectively. Sample sizes are shown at the top of the graph.

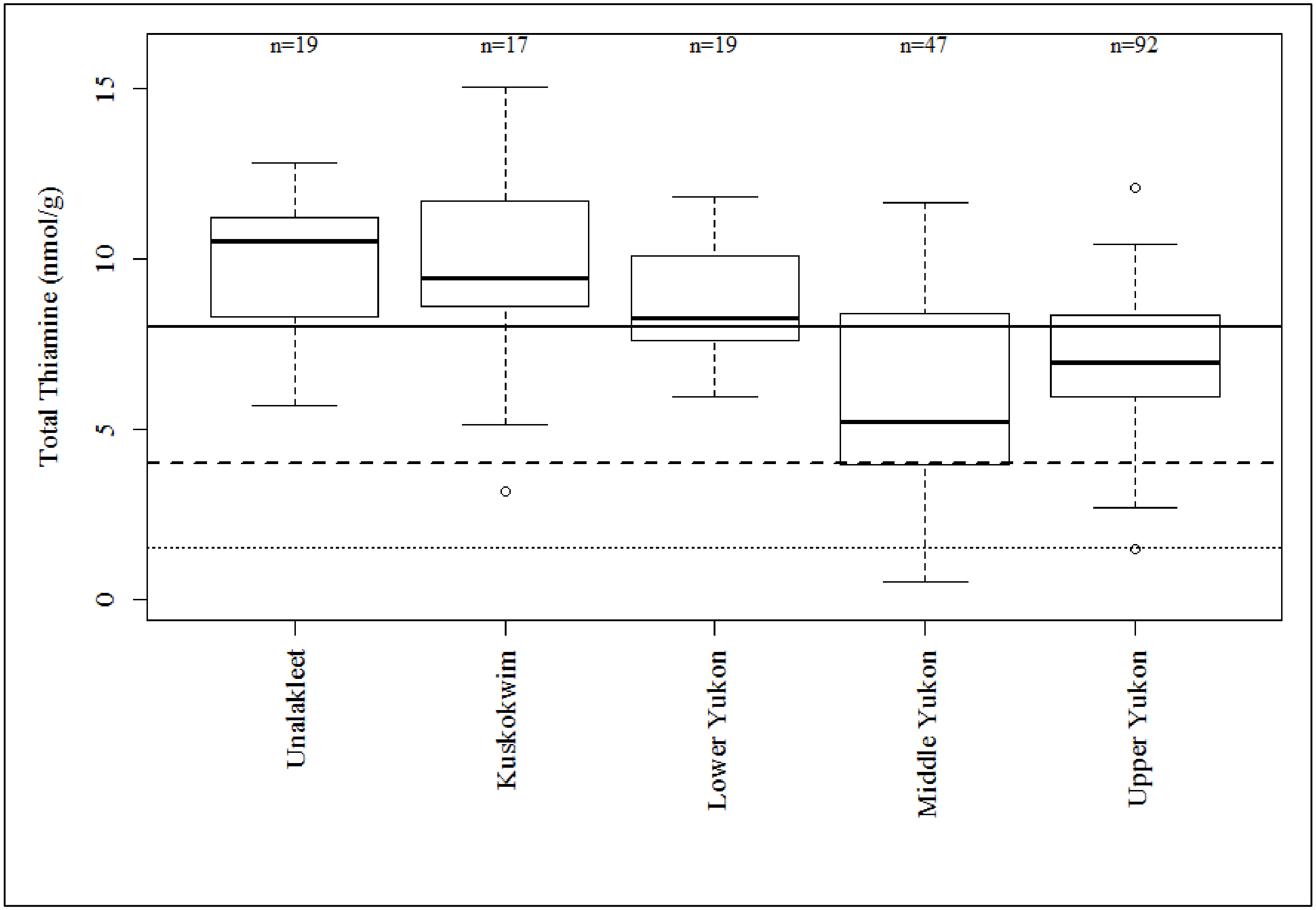

Figure 5.–Boxplots describing total Chinook salmon egg thiamine concentrations within each group, 2015.

Note: The box represents the interquartile range, the median is shown as the solid black line, and the bars represent the minimum and maximum values. The solid line designates total egg thiamine concentrations of 8.0 nmol/g (thiamine replete) and the middle-dashed line and bottom dotted line designate total egg thiamine concentrations of 4.0 (severe secondary effects) and 1.5 nmol/g (overt mortality), respectively. Sample sizes are shown at the top of the graph.