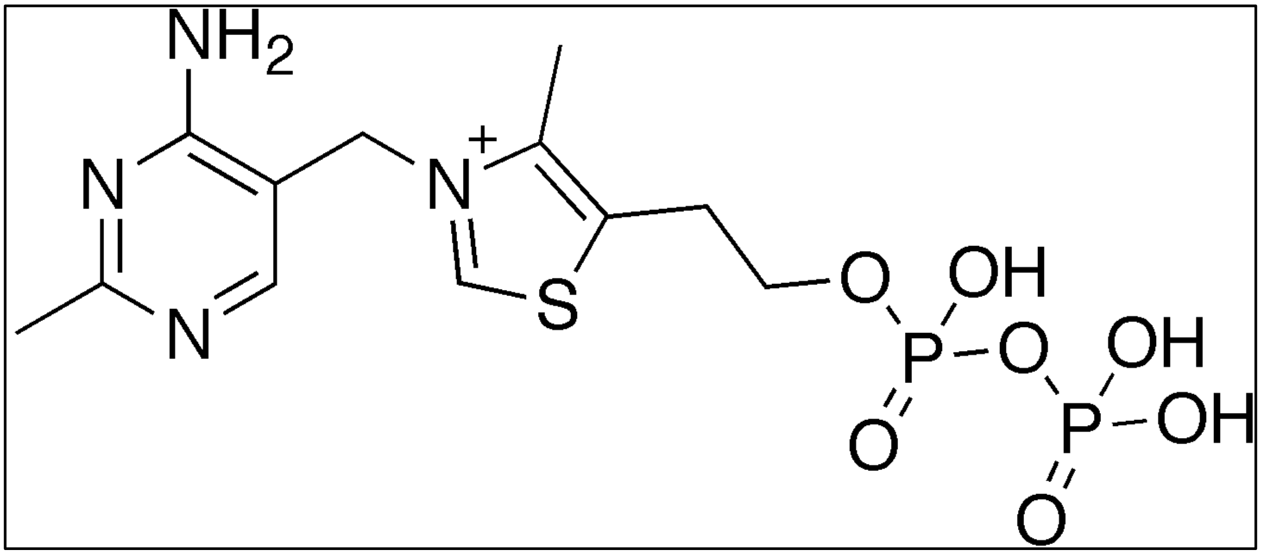
Exploration of Potential Early Life Mortality in Canadian-Origin Chinook Salmon Eggs due to Thiamine Deficiency

Project No. URE-06-14N



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Yukon River Panel
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by
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ABSTRACT

Yukon River Chinook salmon (*Oncorhynchus tshawytscha)* have experienced a marked decline in productivity and no longer support robust commercial or subsistence fisheries. The mechanisms driving this decline remain poorly understood; however, one possible explanation may involve thiamine (vitamin B1) deficiency. Thiamine deficiency has been responsible for declines in numerous salmonid populations and is often a result of shifting feeding habits. We used fluorescence spectroscopy coupled with High Performance Liquid Chromatography to measure thiamine concentrations in eggs collected from 30 female Chinook salmon returning to the Yukon River in 2013. We then compared those concentrations to critical threshold levels for thiamine deficiency established in the literature. While mean total egg thiamine concentrations were replete at most collection sites, the data were highly variable and 33% of the females used in the analysis were within the range of secondary effects of thiamine deficiency. Collectively, Yukon River Chinook salmon eggs sampled in 2013 had relatively high thiamine concentrations, but individual offspring may be susceptible to negative secondary impacts associated with thiamine deficiency. Given the high variability in thiamine concentrations, future research is needed with increased sample sizes and across multiple years to determine if interannual levels of thiamine deficiency occur on the Yukon River.

INTRODUCTION

Yukon River Chinook salmon (*Oncorhynchus tshawytscha*) have traditionally supported a lucrative in-river commercial fishery and the second largest Chinook salmon subsistence fishery in Alaska. However, the once productive Chinook salmon run on the Yukon River has declined and no longer supports a commercial fishery. Its subsistence fishery has also been severely restricted to protect the spawning population. Chinook salmon harvests on the Yukon River have been below the Amount Necessary for Subsistence (45,500 fish) since 2007 (Jallen et al. 2012; JTC 2015). In addition, between 2008 and 2013, Chinook salmon border escapement into Canada failed to meet the interim management escapement goal (IMEG) four out of six times (JTC 2015). Particularly concerning is the low productivity for Canadian origin Chinook salmon, which is far below the 1982 – 2006 average (JTC 2015). Similarly, the Kuskokwim River and Norton Sound Chinook salmon stocks have experienced poor runs and escapements in recent years. Causes of low productivity and poor returns in western Alaska Chinook salmon stocks are largely unknown, which has led to several research initiatives to improve assessment and understanding of the mechanisms driving these declines (ADF&G, 2013; Schindler, et al. 2013).

Identifying causes for Chinook salmon declines can be challenging because Chinook salmon spend most of their lives feeding in the open ocean. During this time, western Alaskan Chinook salmon migrate into the Bering Sea shelf and central basin (Myers et al. 2010) and primarily eat squid, euphausiids, and fish; including, capelin, northern lampfish, Pacific sand lance, and walleye pollock (Davis et al. 2005). The marine food web is influenced by numerous physical and ecological factors, which further complicates our understanding of Chinook salmon foraging patterns. Changes to the marine food web in the Bering Sea could be a result of natural, cyclical fluctuations in water temperatures such as the Pacific Decadal Oscillation (PDO) or the El Niño-Southern Oscillation (ENSO) (www.beringclimate.noaa.gov/data). However, changes to marine communities may also result from the direct and indirect effects of climate change (Brander 2010). With these changes, 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 upon returning to the Yukon River.

Thiamine (vitamin B1) is an essential dietary nutrient which cannot be synthesized by fish and is obtained or lost through certain prey sources. Thiamine deficiency occurs in fish that consume prey either low in thiamine or high in thiaminase, an enzyme that breaks down thiamine. While thiamine deficiency was first noted as problematic in reared fish systems (Halver 1989), since the late 1990s, it has been recognized as a critical mechanism leading to population declines of top predators, including Great Lakes salmonines (Marcquenski and Brown 1997), Baltic sea salmon (Norrgren et al. 1998), New York Finger Lake salmonines (Fisher et al. 1996), and Florida alligators (Rice et al. 2007; Ross et al. 2009). Due to its inherent link within fish culture, the best studied symptom of thiamine deficiency in eggs is early life stage mortality syndrome (EMS), where 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). Secondary effects of thiamine deficiency in salmonid eggs have been less well documented but have been demonstrated to occur when thiamine levels are below replete (8 nmol/g). Lake trout fry hatched from eggs with less than 4 nmol/g thiamine in the Great Lakes region experienced marked secondary effects of thiamine deficiency (Honeyfield et al. 2008). Symptoms of secondary effects of egg thiamine deficiency include adverse effects on 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 also has the capacity to impair adults; death was observed in adult Steelhead and Lake trout due to thiamine deficiency (Brown et al. 2005a) and thiamine deficiency was found to limit spawning migration of adult Rainbow trout and coho salmon (Fitzsimons et al. 2005; Ketola et al. 2005). A potential explanation for these effects is that thiamine is required within the Krebs cycle and for the production of ATP (Agyei-Owusu & Leeper 2009), which is essential for migration. This could be particularly problematic for stocks with long migration routes like Canadian-origin Chinook salmon. An exploratory analysis on Yukon River Chinook salmon thiamine levels in 2012 indicated that egg thiamine concentrations may decrease with migratory distance (Figure 1). If Yukon River Chinook salmon prey are low in thiamine or high in thiaminase then it could impact their reproductive potential and be a contributing factor in recent declines in productivity of Yukon River Chinook salmon.

OBJECTIVE

Assess thiamine concentrations in Chinook salmon eggs collected from the Yukon River and compare them to critical thresholds established in the literature.

STUDY AREA

Chinook salmon eggs were primarily collected from the upper reaches of the Yukon River in Canada; however, samples were also collected during carcass surveys on the Salcha River within the Tanana River drainage and from the Rapids Research Center fish wheel (rkm 1,169) along the mainstem Yukon River near Rampart Rapids (Figure 2). Salmon sampled at Rampart Rapids are assumed to be predominately Canadian-origin fish. Eggs were also collected near Whitehorse, Canada during daily operations at the Whitehorse Rapids Hatchery (rkm 2,808). Additional sampling occurred during carcass surveys conducted on the Swift, Wolf, and Nordenskiold rivers and on Blind and Tatchun creeks (Figure 2; Table 1).

METHODS

Project partners collected egg samples from the 2013 Chinook salmon run. At least 10 eggs were collected from each recently spawned female during carcass surveys at a fish wheel near Rampart Rapids (39 females), egg collections for the Whitehorse hatchery (36 females), and carcass surveys on the Swift River (4 females), Nordenskiold River (2 females), Blind Creek (2 females), Salcha River (5 females), Tatchun Creek (2 females), and Wolf River (4 females) (Figure 2; Table 1). All samples were frozen immediately after being collected and were ultimately shipped to the National Marine Fisheries Service (NMFS) facility in Juneau, Alaska.

Researchers at the NMFS lab used fluorescence spectroscopy coupled with High Performance Liquid Chromatography to measure thiamine concentrations, following the methods of Brown et al. (1998) and similar studies. In brief, approximately 0.3 g of eggs 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 10,000 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 three times which allowed for the removal of organic material from the samples. Then, 850 uL of each sample were combined with 90 uL of 5 M NaOH and 60 uL 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 run in duplicate and were evaluated for degradation visually 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 included in 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 salmonids, primarily Lake Trout. Total egg thiamine concentrations $\geq$ 8.0 nmol/g are thiamine replete, concentrations $\geq$ 4.0 and $<$ 8.0 nmol/g indicate a lower risk of the secondary effects of thiamine deficiency, concentrations $\geq$ 1.5 and $<$ 4.0 nmol/g indicate a higher risk of the secondary effects of thiamine deficiency, and concentrations $<$ 1.5 nmol/g indicate a high likelihood of overt fry mortality. Given the low sample sizes at our collection locations, mean total egg thiamine was reported for three different groups; the Salcha River, the Whitehorse Hatchery, and the Upper Yukon River (Rampart Rapids, Swift River, Nordenskiold River, Blind Creek, Tatchun Creek, and Wolf River).

RESULTS

Mean (SD) egg thiamine concentration (T + TP + TPP) was 9.5 (3.9) nmol/g for all female Chinook salmon sampled in 2013. Each egg sample was above the minimum threshold for overt mortality; however, 10 samples (33%) were in the range associated with the deleterious secondary effects of thiamine deficiency; two at the Salcha River, two at Blind Creek, two at Wolf River, and four at the Whitehorse Hatchery (Figure 3). Mean egg thiamine concentrations were less than 8 nmol/g (thiamine replete) at Blind Creek (6.42 nmol/g) and Salcha River (7.93 nmol/g) and greater than 8 nmol/g at all other collection locations, including the Upper Yukon River group. The lowest individual egg thiamine concentrations occurred at the Salcha River; two of the five samples (40%) had thiamine concentrations < 4.0 nmol/g (Figure 4; Table 2). Egg thiamine concentrations were also the most variable at the Salcha River (SD = 4.83 nmol/g). Four (31%) of the Whitehorse Rapids samples and four (33%) of the Upper Yukon River samples had thiamine concentrations that were less than replete (>8 nmol/g). The highest mean egg thiamine concentration occurred within the Upper Yukon River group (10.67 nmol/g) (Figure 5; Table 3).

DISCUSSION

In an attempt to determine if thiamine deficiency could be impacting Yukon River Chinook salmon productivity, we measured thiamine concentrations in eggs collected from the 2013 Chinook salmon run and compared them against critical thresholds established in the literature. Collectively, egg thiamine concentrations were within replete levels; i.e., mean egg thiamine concentrations were > 8nmol/g at all but two collection locations; however, after grouping the data from Blind Creek, Rampart Rapids, Swift River, and Tatchun Creek, the Salcha River was the only location/group with below replete mean egg thiamine concentration. While averaging thiamine concentrations offers a way to observe differences in thiamine concentrations among collection locations, a superior measure of the potential impact of thiamine deficiency is the proportion of female Chinook salmon with egg thiamine concentrations below critical thresholds. For example, even though all three groups (Salcha River, Whitehorse Hatchery, and Upper Yukon River) had relatively high mean thiamine concentrations, roughly a third of all females sampled had egg thiamine concentrations less than replete and within the range associated with the deleterious secondary effects of thiamine deficiency. These samples came from Blink Creek, Wolf River, Whitehorse Hatchery, and the Salcha River. Female Chinook salmon at the Salcha River appear to be the most at risk of being impacted by thiamine deficiency, given that 40% of the samples collected from the Salcha River had thiamine concentrations less than 4 nmol/g (higher risk from deleterious secondary effects of thiamine deficiency). While acknowledging that our sample sizes were small, some female Chinook salmon sampled in 2013 may be at risk from the deleterious secondary effects of thiamine deficiency.

Sample sizes were relatively low due to the opportunistic nature of the egg collections and the poor Chinook salmon run size in 2013. At each location, egg collection came secondary to other forms of data collection; i.e., age-sex-length recording during carcass surveys. Project collaborators also had difficulty sampling a sufficient number of female Chinook salmon because the 2013 run was so low. In fact, the Canadian mainstem border passage estimate for Yukon River Chinook salmon in 2013 was the lowest on record; approximately 31,000 fish crossed the border (JTC 2015). Increasing our sample sizes in the future will be possible as long as 1) there are directed egg collections, 2) the samples are frozen immediately and remain frozen, and 3) the

Chinook salmon run increases and a sufficient number of salmon make it to the spawning grounds.

An understanding of the interannual variability in egg thiamine concentrations is needed to predict which years Chinook salmon may be most impacted by thiamine deficiency. Roughly 67% of the female Chinook sampled in 2013 had eggs that were thiamine replete. Alternately, a preliminary analysis completed on Yukon River Chinook salmon eggs collected in 2012 indicated that only 23% of the females sampled had eggs with fully replete thiamine levels. The 2012 study was also limited with only 38 samples included in the analysis. Additional years of data collection are needed to determine if interannual variability in the incidence of thiamine deficiency is significant within the Yukon River. If it is, there may be potential for it to influence Chinook salmon productivity if years with a higher proportion of thiamine deficient female salmon correlate with years of lower brood productivity. This may be addressed through an ongoing study titled "Exploration of AYK Chinook salmon egg thiamine levels as a potential mechanism contributing to recent low productivity patterns". This study is funded through the North Pacific Research Board (NPRB) and greatly increases the size and scope of thiamine research in the Yukon River. In 2014, Chinook salmon egg were collected from multiple locations within the lower, middle, and upper Yukon River, including the Chena and Salcha Rivers on the Tanana River, as well as from the Unalakleet, Kuskokwim, and Nushagak rivers. These collections will continue in 2015 and results are expected to become available in 2016. This study will build upon the work presented here and will allow researchers to make a more definitive assessment of thiamine deficiency in Yukon River Chinook salmon and help determine if there is interannual variability in the prevalence of thiamine deficiency.

Egg health is an important aspect of the quality of escapement and may influence productivity changes in salmon populations. The objective of this study was to measure Chinook salmon egg thiamine concentrations from opportunistic collections within the Yukon River to determine the potential for thiamine deficiency to influence Yukon River Chinook salmon productivity. Mean thiamine concentrations of the 30 egg samples that were examined indicate a low likelihood that Yukon River Chinook salmon from the 2013 year class will be significantly impacted by thiamine deficiency (i.e., mostly replete egg samples and there was no evidence of overt fry mortality). However, individual egg thiamine concentrations were highly variable at some collection locations, particularly for the Salcha River, and roughly a third were below thiamine replete levels. Increased sample sizes will be needed to adequately assess egg thiamine status at these locations. The critical thresholds associated with deleterious secondary effects of thiamine deficiency were established for Lake trout and were used for comparison in this study; therefore, research is needed to determine if these same thresholds apply to Chinook salmon. Future studies investigating thiamine deficiency in Yukon River Chinook salmon may be warranted if sample sizes can be increased through a more rigorous, multi-year, egg collection effort. Future studies would also benefit from the inclusion of stable isotope analysis to determine how diets differ between thiamine deficient and thiamine replete Chinook salmon.

ACKNOWLEDGEMENTS

This project is a collaborative effort among several agencies, including the Alaska Department of Fish and Game, U.S. Geological Service and the National Oceanic and Atmospheric Administration. In addition, several important contributors have generously donated their time and effort in collecting samples for this project. Among these are Chris Stark of the Bering Sea Fishermen's Association, Stan Zuray of Rapids Research Center, Trix Tanner and Sean Collins of the Department of Fisheries and Oceans Canada, and Lawrence Vano of the Whitehorse Rapids Hatchery. We acknowledge and thank these contributors for their help. Finally, we thank the Joint Technical Committee and the Yukon River Panel for providing funding for this study. Note: This article reflects the views of the authors and should not be construed to represent FDA's views or policies.

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FIGURE

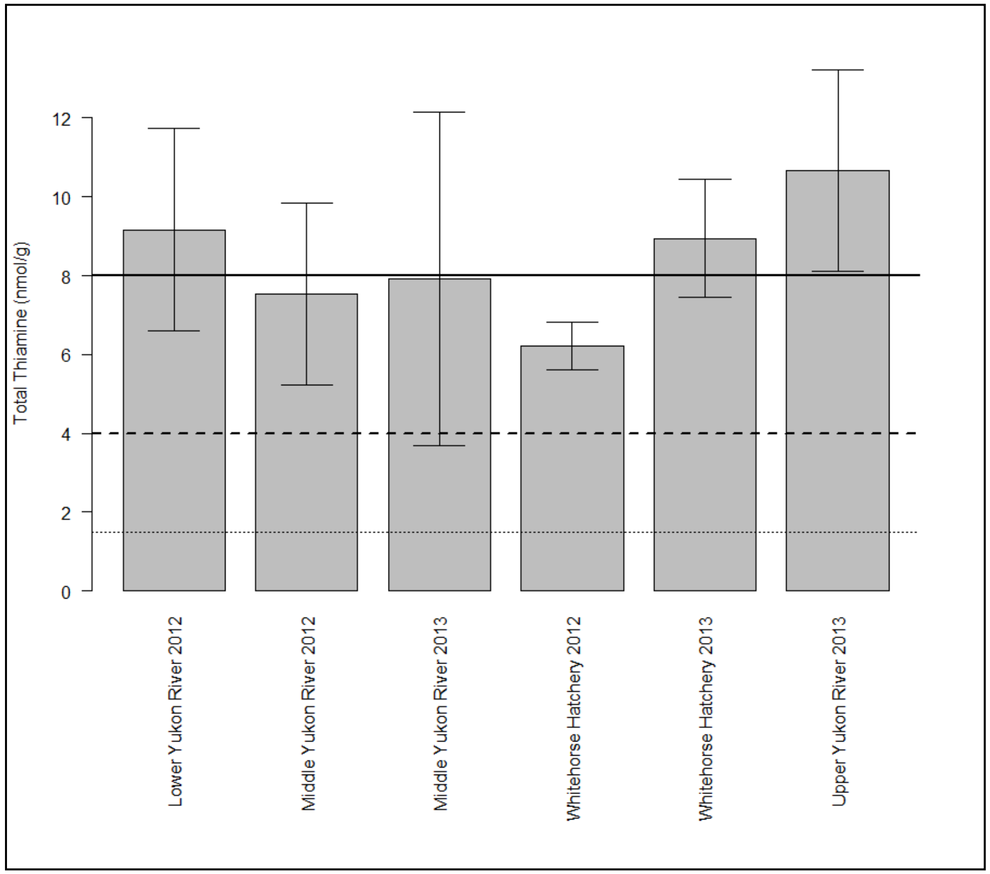

Figure 1. Mean egg thiamine concentrations (nmol/g) in lower Yukon River, middle Yukon River, and hatchery Yukon River Chinook salmon in 2012 and in middle Yukon River, hatchery, and upper Yukon River Chinook salmon in 2013. Error bars indicate 95% confidence intervals. The solid line designates total 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. Thiamine concentrations below the solid line indicate that salmon may have some risk of being negatively impacted by thiamine deficiency. The 2012 data is from Honeyfield et al., In Prep; D. Honeyfield, U. S. Geological Survey, Wellsboro, PA (personal communication).

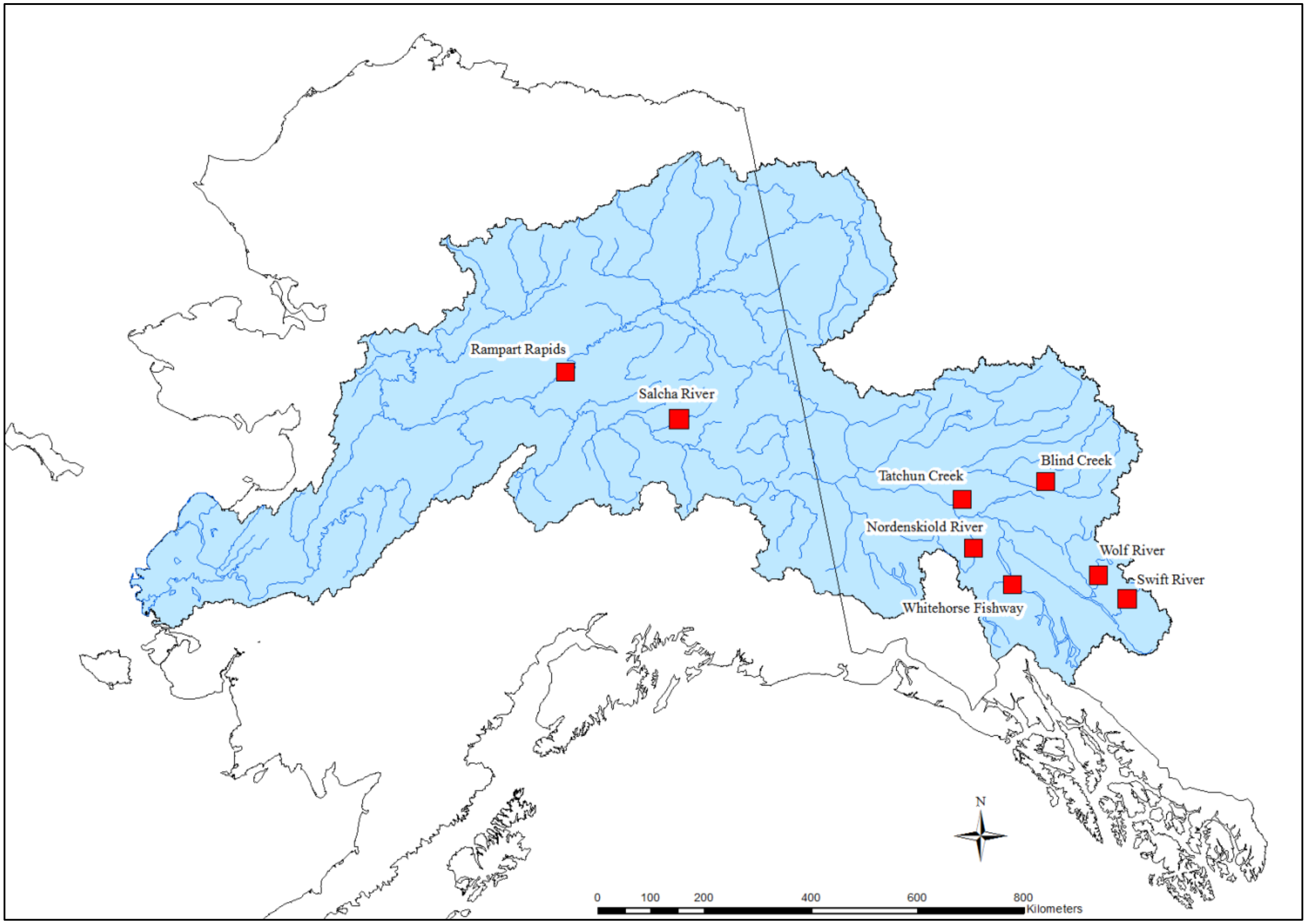

Figure 2. Map of the Yukon River watershed showing Chinook salmon egg collection locations in 2013. Chinook salmon sampled at Rampart Rapids came from predominantly mixed Canadian-origin stocks, while Chinook salmon sampled on the Salcha River were U.S. stocks.

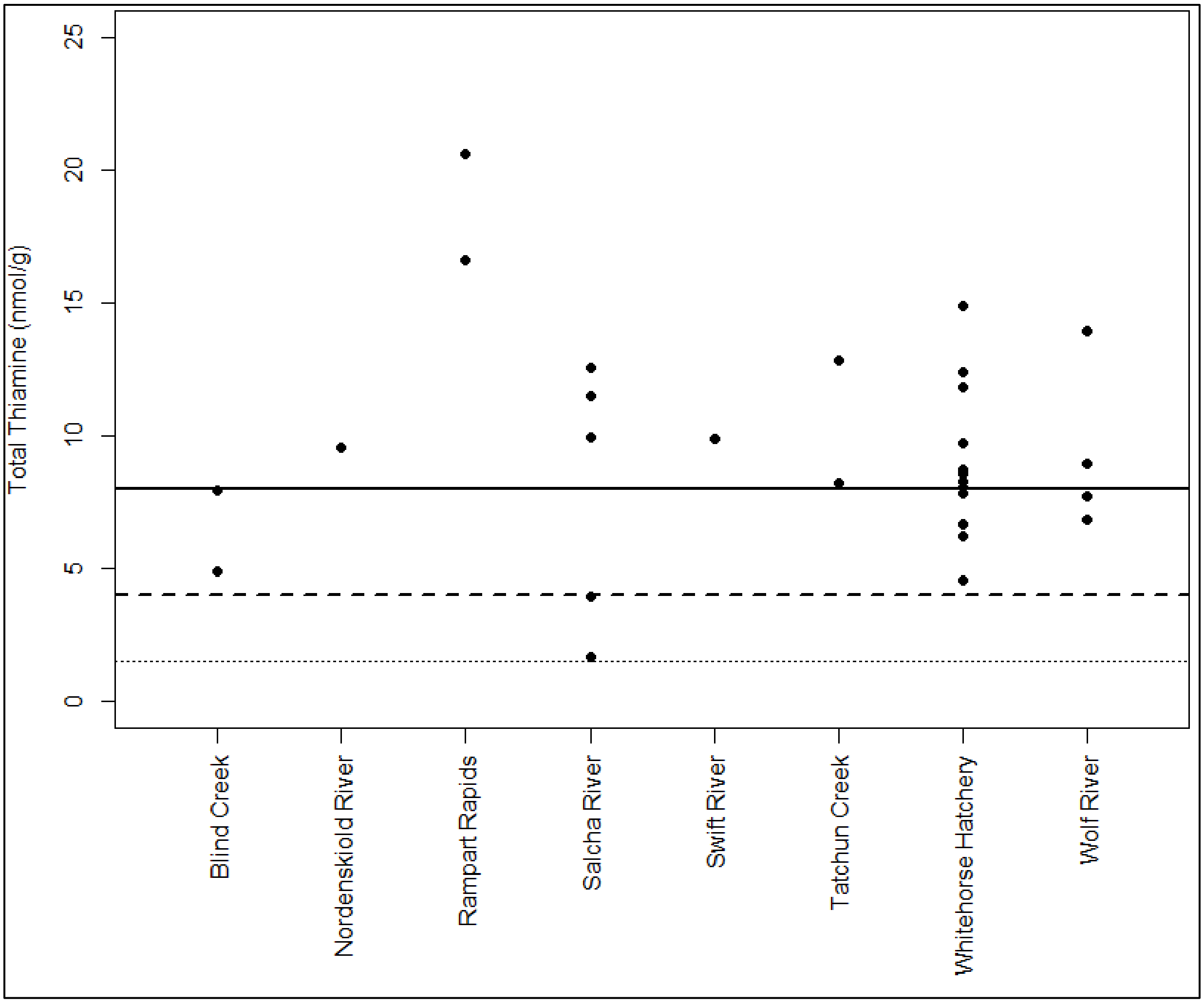

Figure 3. Total egg thiamine concentrations for each female Chinook salmon sampled at each of the eight collection locations. The solid line designates total egg thiamine concentrations of 8.0 nmol/g (thiamine replete) and the middle dashed line and bottom dotted line designate total egg thiamine concentrations of 4.0 (severe secondary effects) and 1.5 nmol/g (overt mortality), respectively. Eggs with thiamine concentrations below the solid line may have some risk of being negatively impacted by thiamine deficiency.

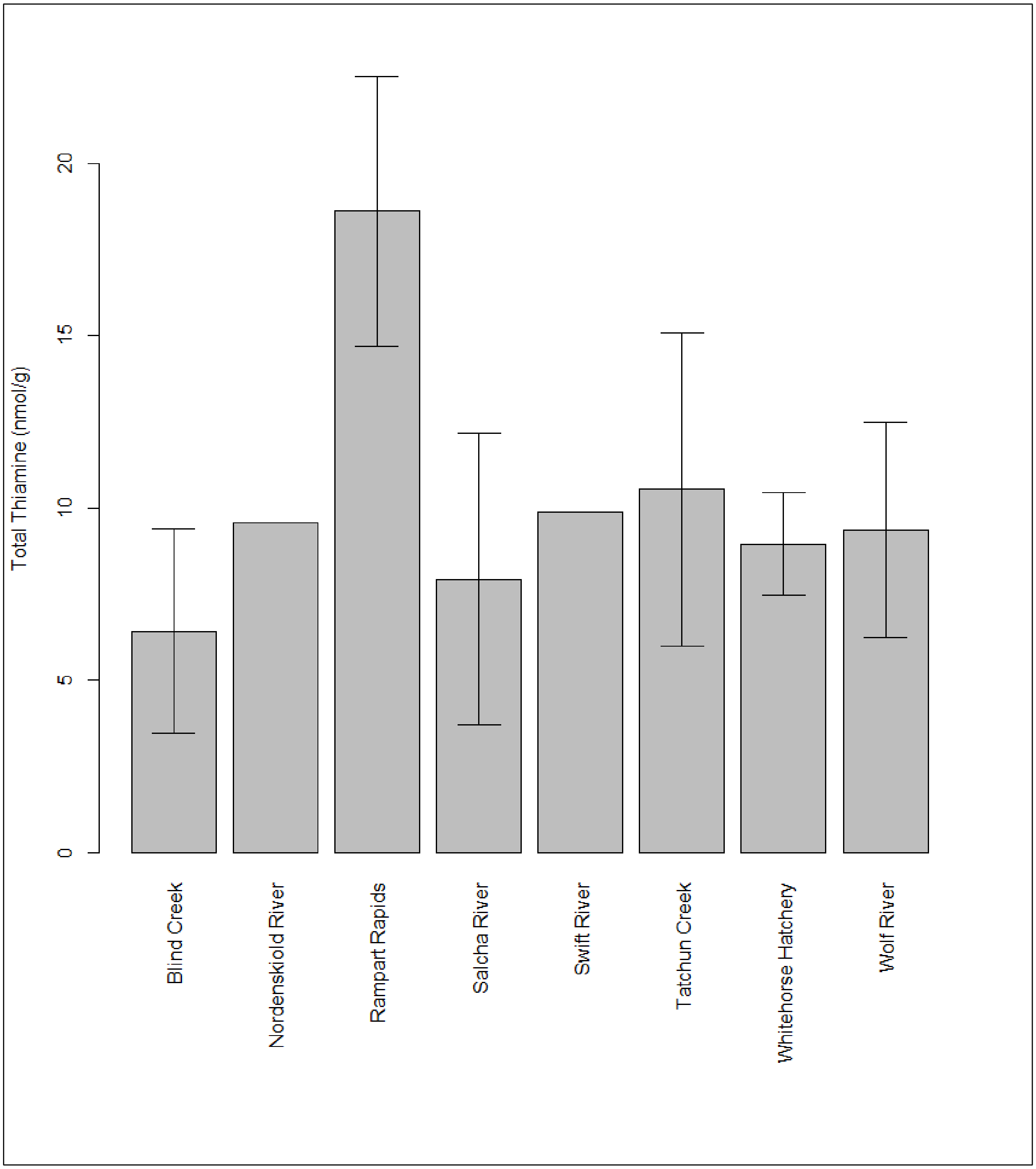

Figure 4. Mean total egg thiamine (nmol/g) at each collection location within the Yukon River drainage. Only one egg sample was collected from the Nordenskiold and Swift rivers. Error bars indicate 95% confidence intervals.

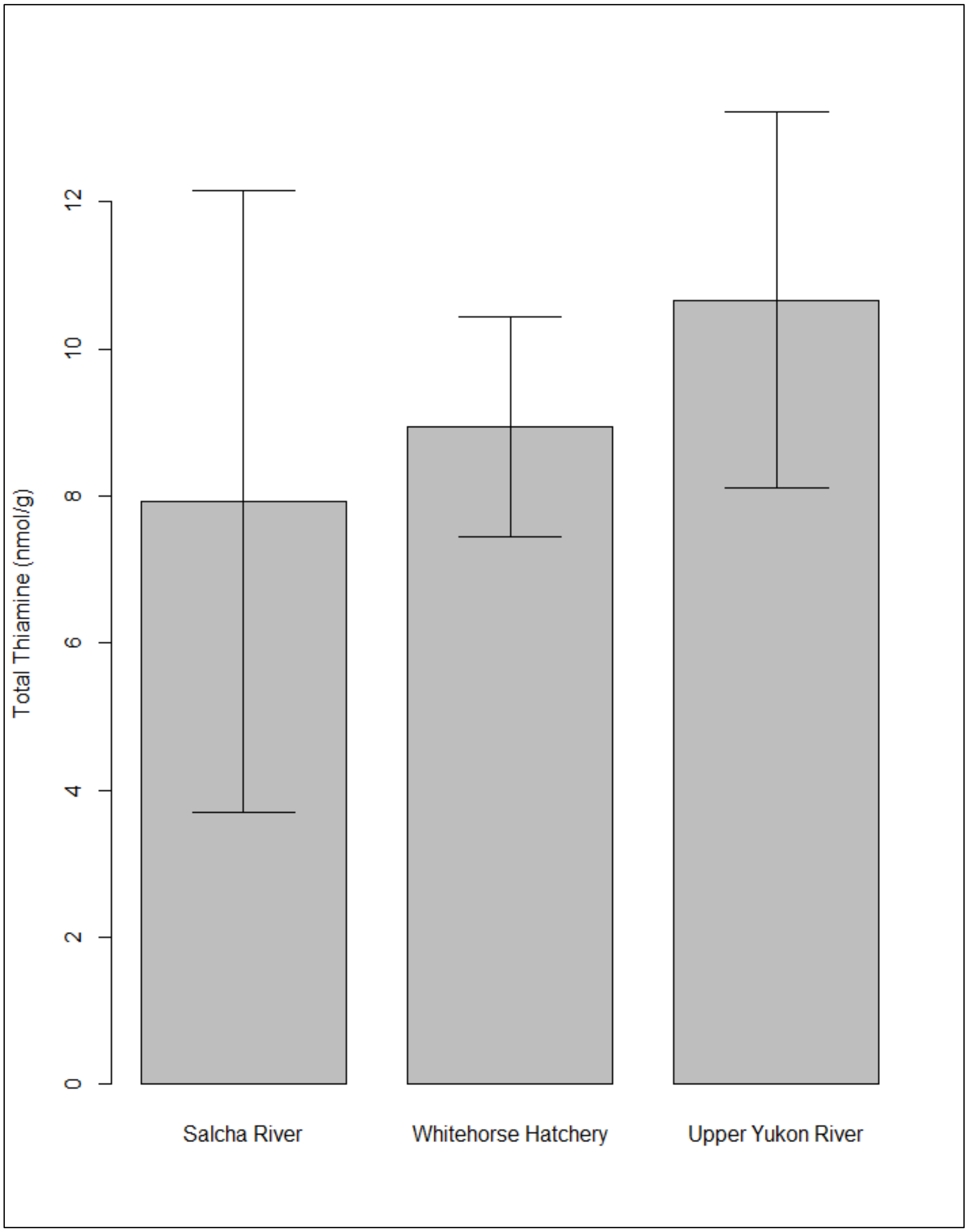

Figure 5. Mean total egg thiamine (nmol/g) for Chinook salmon eggs collected in 2013. Due to small sample sizes at collection locations, the data were combined into three main groups; the Salcha River, the Whitehorse Hatchery, and the Upper Yukon River (Rampart Rapids, Swift River, Nordenskiold River, Blind Creek, Tatchun Creek, and Wolf River). Error bars indicate 95% confidence intervals.

TABLES

Table 1. Collection locations, collection methods, and the agencies responsible for Chinook salmon egg collections in 2013. Rampart Rapids and the Salcha River were the only collection location within the U.S. reaches of the Yukon River; however, it was assumed that most of the fish caught at Rampart Rapids were bound for spawning locations in Canada.

Collection Location	Collection Methods	Agency
Salcha River (Tanana River; U.S.)	Carcass Survey	Bering Sea Fisherman's Association
Rampart Rapids (Mainstem; U.S.)	Fish Wheel	Rapids Research Center
Whitehorse (Mainstem; Canada)	Hatchery	Yukon Energy
Swift River	Carcass Survey	Department of Fisheries and Oceans
Nordenskiold River	Carcass Survey	Department of Fisheries and Oceans
Blind Creek	Carcass Survey	Department of Fisheries and Oceans
Tatchun Creek	Carcass Survey	Department of Fisheries and Oceans
Wolf River	Carcass Survey	Department of Fisheries and Oceans

Table 2. Mean and standard deviations (SD), reported in nmol/g, for thiamine pyrophosphate (TPP), thiamine monophosphate (TP), and free thiamine (T) for Chinook salmon eggs collected from each of the eight sampling locations in 2013. Total thiamine is the sum of TPP, TP, and T, and is used to compare against the known critical values associated with thiamine deficiency. The percentages of egg lots with less than replete thiamine levels (< 8 nmol/g) are also shown.

		TPP		TP		T		Total		% of egg lots
Sampling Location	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	< 8 nmol/g
Blind Creek	2	0.26	0.05	0.45	0.11	5.72	1.99	6.42	2.14	100.0
Nordenskiold River	1	0.17	-	0.6	-	8.79	-	9.56	-	0.0
Rampart Rapids	2	0.25	0.06	1.39	0.11	16.97	2.77	18.61	2.82	0.0
Salcha River	5	0.25	0.06	0.4	0.14	7.28	4.65	7.93	4.83	40.0
Swift River	1	0.16	-	0.72	-	9.01	-	9.89	-	0.0
Tatchun Creek	2	0.29	0.04	0.78	0.24	9.47	3.06	10.54	3.27	0.0
Whitehorse Hatchery	13	0.28	0.12	0.72	0.12	7.95	2.65	8.94	2.75	30.8
Wolf River	4	0.21	0.09	0.6	0.09	8.55	3.17	9.36	3.18	50.0

Table 3. Mean and standard deviations (SD), reported in nmol/g, for thiamine pyrophosphate (TPP), thiamine monophosphate (TP), and free thiamine (T) for Chinook salmon eggs collected in 2013. Due to small sample sizes at collection locations, the data were combined into three main groups; the Salcha River, the Whitehorse Hatchery, and the Upper Yukon River (Rampart Rapids, Swift River, Nordenskiold River, Blind Creek, Tatchun Creek, and Wolf River). Total thiamine is the sum of TPP, TP, and T, and is used to compare against the known critical values associated with thiamine deficiency. The percentages of egg lots with less than replete thiamine levels (< 8 nmol/g) are also shown.

		TPP		TP		T		Total		% of egg lots
Sampling Location	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	< 8 nmol/g
Salcha River	5	0.25	0.06	0.40	0.14	7.28	4.65	7.93	4.83	40.0
Whitehorse Hatchery	13	0.28	0.12	0.72	0.12	7.95	2.65	8.94	2.75	30.8
Upper Yukon River	12	0.23	0.07	0.75	0.33	9.69	4.21	10.67	4.52	33.3