An Exploratory Assessment of Thiamine Status in Western Alaska Chinook Salmon (*Oncorhynchus tshawytscha*)

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Abstract: This study was conducted to investigate the thiamine status of Chinook salmon *Oncorhynchus tshawytscha*. Egg thiamine levels in Yukon and Kuskokwim River Chinook were examined in 2001 and 2012. Muscle and liver thiamine in Chinook, coho *O. kisutch*, chum *O. keta*, and pink *O. gorbuscha* salmon were measured in northern Bering Sea juveniles and the percentage of the diet containing thiaminase, an enzyme that destroys thiamine, was calculated. Only 23% of the eggs were thiamine replete (> 8.0 nmol·g^{-1}) in 2012. Seventy-four percent of the eggs had thiamine concentrations (1.5–8.0 nmol·g^{-1}) which can lead to mortality from secondary effects of thiamine deficiency. Only 3% of the eggs had < 1.5 nmol·g^{-1} associated with overt fry mortality. In 2001 egg thiamine in upper Yukon Chinook was 11.7 nmol·g^{-1} which was higher than that measured in 2012 (6.2 nmol·g^{-1}) and paralleled Chinook productivity. Total thiamine (nmol·g^{-1}) in Bering Sea Chinook muscle (3.8) was similar to coho (4.15), but lower than in chum (8.9) and pink salmon (9.6). Thiaminase-containing prey in Chinook (63%) and coho (36%) stomachs were elevated compared to those of chum (3%) and pink (5%) salmon. These results provide evidence of egg thiamine being less than fully replete. Thiamine deficiency was not observed in juvenile muscle tissue, but differences were present among species reflecting the percentage of diet containing thiaminase. Additional studies are recommended.

Keywords: Thiamine deficiency, thiaminase, Yukon River, Chinook salmon, survival

INTRODUCTION

Chinook salmon *Oncorhynchus tshawytscha* returns to western Alaska have declined markedly since the late 1990s. Most notably, Chinook salmon returns to the Yukon River have declined by approximately half of their 1982–1997 historical size (ADF&G 2013). In a broader geographic scope, productivity has synchronously declined in numerous stocks across Alaska, beginning with those cohorts spawned in 2001 (ADF&G 2013). Poor returns have occurred despite adequate numbers of salmon escaping fisheries to spawn. These poor returns have resulted in management actions dramatically restricting subsistence harvests, and closing or severely restricting commercial and sport fisheries. Such restrictions significantly impact Alaskan fishermen and the communities that depend on Chinook salmon for subsistence needs and economic opportunities. Causes of reduced productivity and poor returns are unknown, and have led to several initiatives to improve assessment and understanding of the mechanisms driving these declines (ADF&G 2013; Schindler et al. 2013).

Thiamine plays a critical role in bodily metabolic functions and there is evidence that thiamine deficiency can affect aquatic species survival and recruitment (Hill and Nellbring 1999; Blazer and Brown 2005; Honeyfield et al. 2008b). The role of this essential nutrient is therefore an avenue of research that may provide insight into the poor returns of western Alaska Chinook salmon. Thiamine is an essential dietary nutrient required for Krebs cycle production of ATP (Agyei-Owusu and Leeper 2009). The majority of thiamine in most organisms is found in three forms. The unphosphorylated or free thiamine (T) is readily converted to thiamine pyrophosphate (TPP), the

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active form of thiamine. Thiamine pyrophosphate is the active form of thiamine (coenzyme) required in enzymatic reactions in cells throughout the body (Depeint et al. 2006). Thiamine monophosphate (TP) is a degradation product of TPP and cannot be directly converted to TPP by fish (Gubler 1991). Free or unphosphorylated thiamine (T) is the primary form of thiamine found in fish eggs; the metabolic importance of thiamine monophosphate (TP) is less well defined (Gubler 1991) but may play role in immune function (Ottinger et al. 2014).

Thiamine deficiency causes abnormal neuromuscular signs and mortality in fish consuming diets lacking thiamine, or containing thiaminase, an enzyme that destroys thiamine (Halver 1989; NRC 2011). In the mid-1990s, thiamine deficiency was first linked to reproductive failure in wild fish (Fitzsimons 1995; Fisher et al. 1996). Honeyfield et al. (2005) demonstrated that lake trout *Salvelinus namaycush* reared on a diet containing bacterial thiaminase, or alewife *Alosa pseudoharengus* (a species known to contain thiaminase), produced low egg thiamine resulting in fry mortality. The clinical signs of the experimental lake trout fry were similar to those observed in lake-dwelling Chinook salmon, coho salmon *O. kisutch*, wild steelhead trout *O. mykiss*, brown trout *Salmo trutta*, and Atlantic salmon *Salmo salar* with high mortality rates in the Laurentian Great Lakes, New York Finger Lakes, and the Baltic Sea (Fisher et al. 1996; Marcquenski and Brown 1997; Norrgren et al. 1998). In the Great Lakes, non-native alewives were the primary source of dietary thiaminase whereas in the Baltic Sea thiamine deficiency was associated with population changes in Baltic herring *Clupea harengus* and sprat *Sprattus sprattus* known to contain thiaminase (NRC 1983). Pacific Chinook are known to feed on prey fish that contain thiaminase (Davis et al. 2005). In addition to overt fry and adult mortality observed when fish consume prey fish that contain thiaminase (Brown 2005a), low thiamine can limit spawning migration (Fitzsimons et al. 2005; Ketola et al. 2005, 2009), decrease growth, affect vision, reduce both predator avoidance and prey capture (Carvalho et al. 2009; Fitzsimons et al. 2009) and impair immune function (Ottinger et al. 2012, 2014), all of which can negatively affect population recruitment (Brown et al. 2005b). Thus, thiamine deficiency impacts multiple life stages and multiple physiological processes.

The cause of the decline of Yukon River Chinook salmon has not been determined and many factors may be involved. As mentioned above, research studies in the Great Lakes and Baltic Sea have shown that thiamine deficiency has been associated with reproductive failure and declining salmonid populations. It is not known if changes in marine prey and/or migratory patterns of Chinook salmon could lead to an increase in variation in Chinook thiamine levels but it is known that Chinook salmon forage on thiaminase-positive prey species during their marine life-history period. Thiaminase-positive prey species such as capelin (*Mallotus villosus*) and Pacific herring (*Clupea pallasii*) are typically only present on the eastern Bering Sea shelf and their abundance varies with climate conditions (Andrews et al. 2015). Therefore we conducted the study reported here to gauge the potential for thiamine deficiency to occur in returning Chinook salmon because there appeared to be similarities in patterns previously observed in thiamine-deficient fish such as reduced adult migrants returning to natal spawning sites. As part of the exploratory nature of this work we measured tissue thiamine in Bering Sea juvenile salmon because there are no published thiamine data available for Alaskan salmon. The Yukon River supplies 90% percent of the juvenile Chinook salmon found in the northern Bering Sea (Murphy et al. 2009). This report provides an exploratory assessment of thiamine levels in Yukon River Chinook salmon and is the first report on the thiamine status of Pacific salmon in Alaska. The objectives of this study were to assess the potential for early life mortality and secondary effects of thiamine deficiency on fry, based on egg thiamine concentrations of Yukon River Chinook salmon, and to examine the role of diet on muscle and liver thiamine concentrations in juvenile Chinook salmon from the northern Bering Sea. Implications of thiamine deficiency on Chinook salmon stocks in the Yukon River are discussed.

Table 1. Spawning stock mean (SD) thiamine concentrations (nmol·g-1) in egg samples collected in upper Yukon River drainage (Whitehorse Hatchery) and opportunistically within the middle river drainage (Salcha, Chena and Goodpaster rivers) and in the lower river drainage (east fork of Andreafsky River) during carcass surveys and broodstock collections in 2012. In 2001 eggs were collected from upper Yukon Chinook salmon and from Kuskowim River Chinook and chum salmon. Thiamine concentrations are reported for thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free or unphosphorylated thiamine (T), and total thiamine (sum of TPP, TP, and T).

Location	Species	Year	n	TPP	TP	T	Total
Upper Yukon	Chinook	2012	28	0.2 (0.0)	0.5 (0.0)	5.6 (0.3)	6.2 (0.3)
Middle Yukon	Chinook	2012	8	0.2 (0.1)	0.4 (0.1)	7.0 (3.1)	7.5 (3.3)
Lower Yukon	Chinook	2012	2	0.2 (0.0)	0.5 (0.2)	8.5 (1.1)	9.2 (1.3)
Upper Yukon	Chinook	2001	4	0.7 (0.0)	0.6 (0.0)	8.4 (0.2)	9.6 (0.2)
Kuskokwim	Chinook	2001	10	0.7 (0.0)	1.0 (1.0)	10.1 (0.2)	11.7 (0.2)
Kuskokwim	Chum	2001	31	1.3 (0.3)	1.0 (0.2)	9.7 (1.0)	12.0 (1.3)

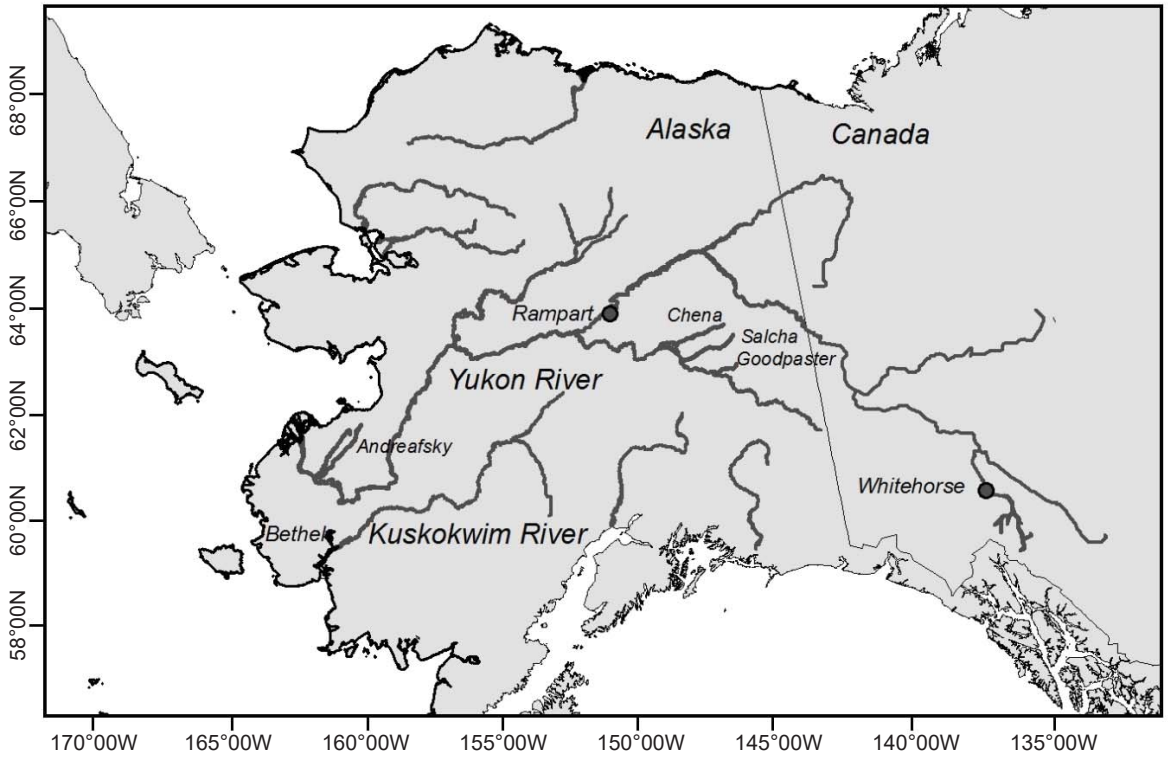

Fig. 1. Map showing where egg samples for thiamine analysis were opportunistically collected from Chinook salmon stock groups from the upper (Rampart Rapids; Whitehorse Rapids Fish Hatchery), middle (Salcha, Chena, and Goodpaster rivers) and lower (east fork of the Andreafsky River) sections of the Yukon River and from Chinook and chum salmon at Bethel on the Kuskokwim River.

METHODS

Sample Collection

Chinook salmon eggs were collected in 2012 from returning broodstock females (Table 1) at the Whitehorse Rapids Fish Hatchery and fish ladder (upper Yukon River, Canada), and from opportunistic collections during weir and carcass surveys at the following middle Yukon rivers: the Chena, Salcha, and Goodpaster, and the lower Yukon River, and the east fork of Andreafsky River above the US Fish and Wildlife Service weir (Fig. 1). Eggs were only collected from recently spawned females during carcass surveys. Eggs were frozen immediately on dry ice or kept on ice until samples could be frozen (-80°C). In 2001 Chinook salmon eggs were collected at Rampart Rapids (upper Yukon) and at Bethel on

Table 2. Mean (SD) length (mm), weight (g) and thiamine concentrations (nmol·g-1) in muscle and liver tissue of juvenile Chinook, coho, chum, and pink salmon collected from the northern Bering Sea (2012). Thiamine concentrations are reported as thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free or unphosphorylated thiamine (T), and total thiamine (sum of TPP, TP, and T).

Species	n	Length (mm)	Weight (g)	Muscle			
				TPP	TP	T	Total
Chinook	13	207 (15)	107 (29)	1.34 (0.49)	1.91 (0.33)	0.56 (0.24)	3.82 (0.68)
Coho	9	260 (14)	210 (37)	2.37 (0.40)	1.32 (0.30)	0.46 (0.20)	4.15 (0.62)
Chum	15	167 (16)	47 (13)	2.83 (1.28)	4.36 (1.38)	1.70 (0.69)	8.89 (2.51)
Pink	10	152 (11)	29 (6)	5.79 (1.43)	2.50 (0.98)	1.34 (0.89)	9.63 (2.94)
				Liver			
Chinook	12	--[1]	--	1.07 (0.48)	2.96 (1.19)	12.36 (4.78)	16.39 (5.28)
Coho	8	--	--	3.15 (1.85)	4.20 (1.66)	10.98 (3.10)	18.33 (4.33)
Chum	15	--	--	0.92 (0.18)	1.50 (0.69)	21.52 (4.94)	23.94 (4.96)
Pink	10	--	--	2.68 (0.90)	3.70 (1.89)	15.78 (3.85)	22.15 (3.98)

--[1] Fish length and weight data are listed above with muscle data

Table 3. Threshold values for overt mortality and secondary effects of thiamine deficiency in lake trout, Chinook, coho, and Atlantic salmon. Threshold values for juvenile and adult salmonid muscle are very limited and unknown for liver.

Tissue	Critical level of total thiamine		
	Overt mortality	**Secondary effects**	**Replete**
Egg	≤ 1.5 nmol/g (Honeyfield et al. 2005; Fitzsimons et al. 2007; Fisher et al. 1996)	1.5–8 nmol/g (Honeyfield et al. 2005, 2008c; Fitzsimons et al. 2009; Carvalho et al. 2009)	> 8 nmol/g (Fitzsimons et al. 2009)
Muscle	< 1 nmol/g (Brown et al. 2005a; Honeyfield et al. 2008a; Karlsson et al. 1999)	Undetermined	> 3 nmol/g (Honeyfield et al. 1998a, b; Honeyfield unpublished Chinook data; Brown et al. 2005a)

the Kuskokwim River. In addition chum salmon eggs were also collected at Bethel, Kuskokwim River, in 2001.

Muscle and liver tissue of juvenile salmon collected during surface trawl surveys in the northern Bering Sea as part of the 2012 Arctic Ecosystem Integrated Survey (Andrews 2012) were collected and stored frozen (Table 2). Although several river drainages contribute to juvenile Chinook salmon stocks in the northern Bering Sea, the Yukon River is the primary (90%) source of juvenile Chinook salmon (Murphy et al. 2009). Coho, chum and pink juvenile salmon were included in this analysis to provide species-level contrasts between tissue thiamine concentrations and their diets. Whole juvenile fish were frozen at -20°C onboard the vessel, shipped on dry ice to Wellsboro, PA, and stored at -80°C until analysis.

Food habits of juvenile salmon were summarized from diet information collected in the northern Bering Sea during similar surveys between 2009 and 2013. Diet data from 2009–2011 and 2013 were used. Due to the highly digested state of the stomach contents, and possibly human error, the 2012 diet data were unusable. The proportional weight of the i^{th} prey species at the j^{th} station (Stomach Content Index, $SCI_{i,j}$) was scaled by predator body weight and subsequently weighted by the predator catch at each station, calculated as:

$$SCI_{i,j} = \sum_{j} \frac{Prey_{i,j}}{Pred_j} C_j,$$

where $Prey_{i,j}$ is the total weight of the i^{th} prey species at station j, $Pred_j$ is the total predator weight at station j, and C_j is the predator catch at station j. Prey specific $SCI_{i,j}$ values for each predator species were summed across stations by year and divided by the total $SCI_{i,j}$ for all prey items. Predator diets were calculated from the average proportion of prey items across years. Based on published data, prey items were classified as thiaminase positive when thiaminase I (Wittliff and Airth 1968) activity was reported > 2.5 nmol·g^{-1}·min^{-1} (Ceh et al. 1964; NRC 1983; Zajicek et al. 2005). We applied literature values of thiaminase activity for diet prey species from other locations in this report. Thiaminase activity in Alaska prey items has yet to be reported. Thiaminase activity > 2.5 nmols·g^{-1}·min^{-1} has been shown to reduce the thiamine status of the consumer. Species with thiaminase activity < 1.5 nmols·g^{-1}·min^{-1} have not been associated with thiamine deficiency.

Thiamine Measurement and Analysis

Thiamine concentrations were measured in egg, muscle, and liver tissue by high pressure liquid chromatography following the methods of Brown et al. (1998). This method reports thiamine pyrophosphate (TPP), thiamine monophosphate (TP) and free thiamine (T). Total thiamine is the sum of the three forms. All samples were run in duplicate. Samples were evaluated for degradation visually and by data QA/QC. Samples with evidence of degradation (increase in TP, decrease in TPP, and loss of total concentrations due to improper sample collection or handling such as refreezing thawed samples) were discarded.

Total thiamine levels were evaluated in four ways: (1) Yukon River Chinook salmon eggs and juvenile salmon muscle thiamine concentrations from the northern Bering Sea shelf were compared to reference critical levels for salmonine eggs and muscle tissues; (2) 2012 Yukon River Chinook salmon egg thiamine concentrations were compared to 2001 Chinook salmon egg collections from Yukon and Kuskokwim rivers; (3) Chinook salmon egg thiamine levels from upper, middle and lower Yukon stocks were compared to each other; and (4) prevalence of known thiaminase-positive prey fish species (capelin, rainbow smelt *Osmerus mordax,* and Pacific herring) was compared among the diets of juvenile salmon predator species and related to average muscle thiamine levels detected in each species of juvenile salmon.

To estimate the potential impact tissue concentration of thiamine may pose on the Yukon River Chinook, threshold or critical thiamine concentrations of egg and muscle were drawn from other studies listed in Table 3. Eggs containing < 1.5 nmols·g^{-1}of thiamine were considered to result in > 20% overt fry mortality (ED 20) (Fitzsimons et al. 2007). Egg thiamine concentrations from 1.5 to 8.0 nmols·g^{-1} have been linked to secondary effects of low thiamine such as poor growth, reduced predator avoidance, reduced prey capture and immune dysfunction. These secondary effects of thiamine deficiency increase the probability of mortality. Eggs with concentrations above 8.0 nmol·g^{-1} total thiamine were considered thiamine replete. Threshold levels for muscle tis-

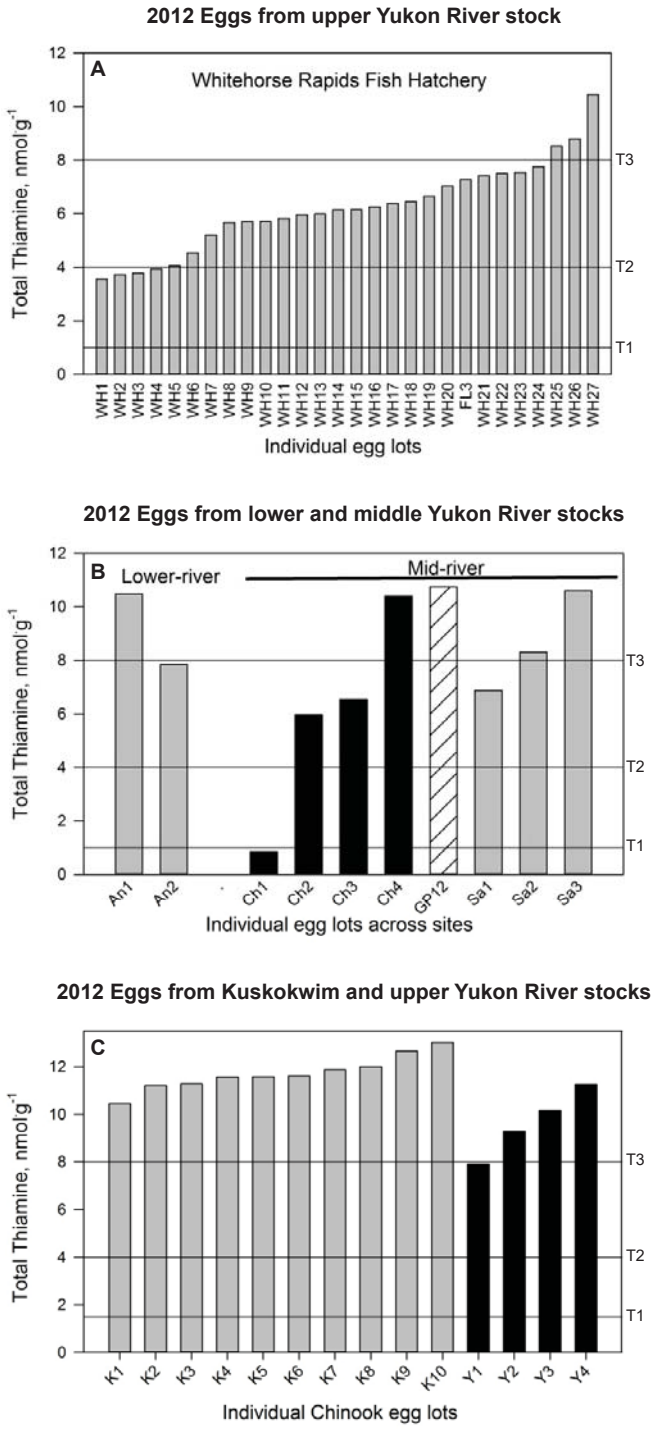

Fig. 2. Total thiamine concentration (nmol·g-1) in Chinook eggs collected in 2012 from the upper Yukon River (panel A), the middle and lower Yukon River sections (panel B) at Salcha, (Sa), Chena (Ch), Goodpaster (GP) and east fork of the Andreafsky (An) Rivers and in 2001 eggs (panel C) collected from Kuskokwim River (K) and upper Yukon River (Y) Chinook salmon. The right axes show critical levels of total thiamine concentrations. T3 > 8.0 nmol/g thiamine replete; T2 < 4.0 nmol/g secondary effects; T1 < 1.5 nmol/g fry mortality.

sue thiamine concentration are less well-defined in the literature than the threshold for eggs. Thiamine deficient mortality in lake trout, Atlantic salmon, coho salmon and rainbow trout occur when muscle tissue is below 1.0 nmol·g^{-1} (Karlsson et al. 1999; Brown et al. 2005a). In this report we used the threshold for potential fish mortality from thiamine deficiency at < 1 nmol·g^{-1} muscle thiamine.

RESULTS

Egg Thiamine Concentrations

Average total thiamine concentrations in the majority of Chinook salmon eggs were above the concentration known to cause overt fry mortality (Table 3). Overall, three percent of females sampled had egg thiamine concentrations low enough to result in overt fry mortality; however, 74% of females sampled had egg thiamine concentrations in the range in which lake trout develop secondary effects of thiamine deficiency (1.5–8.0 nmol·g^{-1}). Only a quarter of the egg lots were fully thiamine-replete (Fig. 2; Table 3). Although the number of samples at the lower and mid-river sites may limit conclusive statements, averages reported here provide a basis for further research. Average egg thiamine concentration decreased with increasing migratory distance of adults (lower, 9.2; middle 7.5; and upper Yukon River drainage 6.2 nmol·g^{-1}). The predominant form of thiamine present in egg samples was either unphosphorylated or free thiamine (92%) (Table 1). Samples from 2012 exhibited much lower egg thiamine concentrations than those obtained in 2001 (Table 1). Average total thiamine in 2001 Chinook salmon eggs (9.6 nmol·g^{-1}) collected at Rampart Rapids (upper Yukon) were lower than Chinook salmon eggs (11.7 nmol·g^{-1}) from Bethel, Kuskokwin River. Furthermore, 2001 Chinook salmon egg thiamine from Bethel, Kuskowim River was similar to concentrations observed in chum salmon eggs collected from the same location in the same year (12.0 nmol·g^{-1}).

Whitehorse Hatchery (Fig. 2a; Table 1) was the only location with a sufficient number of egg samples to support a site-specific summary. Low sample numbers at other sampling sites were due to the opportunistic and exploratory nature of the collections (Fig. 2b; Table 1). None of the females sampled at the Whitehorse Hatchery had egg thiamine concentrations low enough to cause overt fry mortality (Table 3). However, thiamine concentration in 25 of 28 (89%) egg lots was in the range associated with the deleterious secondary effects of thiamine deficiency in lake trout. Only 3 of 28 (11%) egg samples at the Whitehorse Hatchery were fully thiamine-replete. At the Chena River site (Fig. 2) one of four females sampled (25%) had egg thiamine concentrations sufficiently low to produce overt fry mortality however, the sample size is very low for a reliable population estimate. Five of the 10 females sampled at Yukon River sites other than the Whitehorse Hatchery had fully replete egg thiamine levels, and four of 10 females contained egg

thiamine levels in the range where secondary effects of thiamine deficiency are known to occur in salmonids.

Juvenile Thiamine Concentrations and Diet

Concentrations of total thiamine (nmol·g^{-1}) in muscle and liver of juvenile Chinook (3.8 and 16.4) and coho (4.2 and 18.3) salmon were lower than those found in juvenile chum (8.9 and 23.9) and pink (9.6 and 22.2) salmon. Total muscle and liver thiamine from juvenile salmon (Table 2) tended to reflect the proportion of thiaminase-positive forage fish consumed (Table 4; Fig 3).

Invertebrate prey items were the main component of juvenile chum and pink salmon diets at 90% and 66%, respectively; while Chinook and coho salmon juveniles consumed almost entirely teleost prey (96%). In the present study thiaminase-positive species (Pacific herring, capelin, and rainbow smelt) made up 63% of the Chinook salmon diet. One-third of the coho salmon diet consisted of one thiaminase-positive species, capelin. In contrast chum and pink salmon diets contained few (3–5%) known thiaminase-containing prey fish species.

Table 4. Percent mean catch weighted diet composition (SD) of juvenile Chinook, coho, chum, and pink salmon in the northern Bering Sea (2009–2011, 2013).

Prey organism	Thiaminase[1]	Chinook	Coho	Chum	Pink[2]
Invertebrates					
Amphipod	-	2 (1)	1 (1)	4 (8)	1 (1)
Appendicularia	Unk			60 (19)	5 (6)
Chaetognath	Unk			4 (9)	6 (11)
Copepod	-			3 (5)	9 (8)
Decapod	Unk	2 (1)	1 (1)	1 (1)	1 (1)
Euphausiid	Unk	1 (1)		2 (3)	13 (22)
Mysid	-			1 (1)	
Polychaeta	Unk				
Pteropod	Unk				1 (1)
Other Inv	Unk		2	15 (15)	30 (24)
Teleosts					
Capelin	+	58 (22)	32 (26)	2 (2)	5 (3)
Flatfish	Unk	4 (4)	2 (3)	1 (2)	
Herring	+	4 (3)	4 (6)	1 (1)	
Pollock	Unk		6 (10)		
Rainbow smelt	+	<1 (1)			
Saffron cod	Unk			1 (1)	
Sandlance	Unk	16 (12)	49 (27)	3 (4)	25 (33)
Sculpin	-				2 (2)
Unid fish[3]	Unk	13 (10)	3 (5)	2 (1)	2 (2)
Total diet	% thiaminase	63 (30)	36 (25)	3 (2)	5 (3)

[1]Thiaminase, + positive; - negative; Unk, unknown or not reported (Ceh et al. 1964; NRC 1983; Zajicek et al. 2005)
[2]2013 pink salmon diet data not available.
[3]Unidentifiable fish species

DISCUSSION

An exploratory analysis of thiamine in western Alaska Chinook salmon was completed to determine if thiamine could be a factor contributing to their recent decline in survival. We examined thiamine levels in the egg and juvenile stages, both known to be critical periods in their overall survival. Here we provide evidence to suggest that thiamine appears to be low and there may be a link between thiamine and reduced productivity in Yukon River Chinook salmon stocks which merits further investigation. Over the past few decades, total recruitment failure was observed in Lake Michigan lake trout, associated with thiamine deficiency. Although other factors may have been involved in the observed stock declines, thiaminase associated with alewife played a key role (Honeyfield et al. 2005; Bronte et al. 2008). Reduced Chinook and coho salmon fry survival was also documented at the same time in eggs collected from adults eating alewife. The importance of thiamine in lake trout reproduction can be seen with the recent increase in recruitment coinciding with egg thiamine concentrations exceeding 4 nmol·g^{-1} (Hanson et al. 2013), however for full and normal recruitment, egg thiamine will need to be > 8 nmol·g^{-1}.

Although the data are limited, egg thiamine levels appear consistent with productivity patterns observed in upper Yukon River Chinook salmon. Productivity levels in 2001 were more than twice that observed in the past 6–7 years (JTC 2013). Similarly, mean egg thiamine levels in 2001 (9.6–11.7 nmol·g^{-1}) were nearly twice as high as in 2012 (Table 1). Egg thiamine concentrations in 2001 Chinook salmon were fully thiamine-replete and these eggs contained thiamine levels similar to those in chum salmon, which consume less thiaminase-positive prey fish (Table 4; Fig. 3; Davis et al. 2005). These data suggest that low thiamine may be a contributing factor in the decline of Yukon River Chinook. However many unanswered question remain and these findings may only be coincidental. However higher egg thiamine concentrations and higher productivity observed in 2001, compared to 2012, clearly merits further investigation.

Average Yukon River Chinook salmon egg thiamine concentrations decreased with migratory distance of females in both 2012 and 2001. These results suggest that there is a thiamine cost of migration that was not previously recognized in studies with severely thiamine-deficient rainbow trout, Chinook or coho salmon (Fitzsimons et al. 2005; Ketola et al. 2005, 2009). Based on egg concentrations, Chinook salmon in the current study were not as severely thiamine-depleted as in the above-mentioned studies. If Chinook adults returning to Whitehorse Hatchery started with the same thiamine levels as these juvenile Chinook, (Table 3) the expected tissue cost or loss of thiamine from

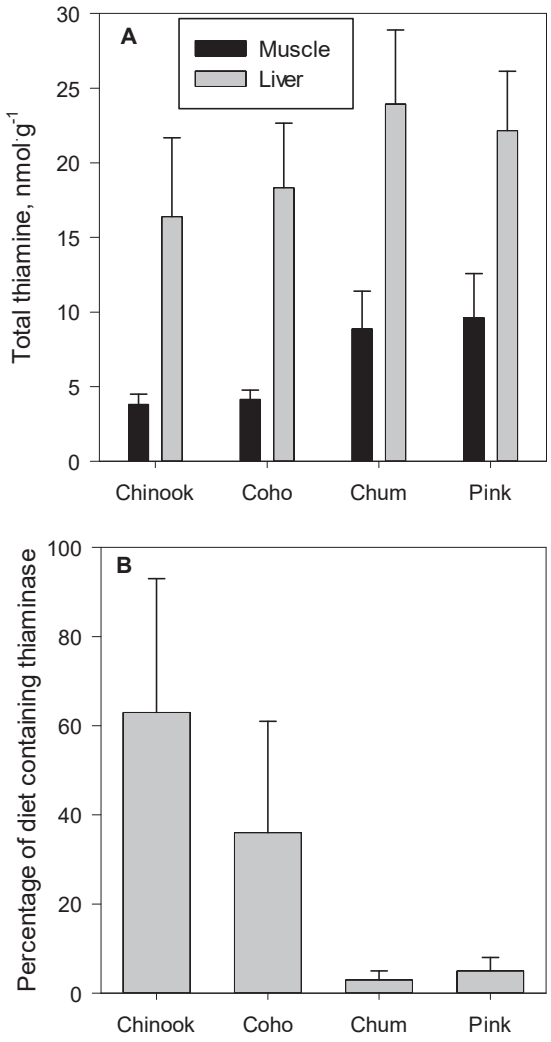

Fig. 3. Samples collected in 2012 from Bering Sea juvenile salmon (Panel A) muscle and liver total thiamine and the (Panel B) percentage of prey fish in the diet containing thiaminase.

basal fasting metabolism would be approximately 0.42 and 8.8 nmol·g^{-1} in muscle and liver, respectively. These resting metabolic thiamine (100 days at 5˚C) costs in fasting Chinook salmon were calculated to be 5.3 pmol·g^{-1}·day^{-1} loss from muscle and 110 pmol·g^{-1}·day^{-1} from liver tissue (Honeyfield et al. 2016). It must be pointed out that these values do not include the cost of thiamine associated with strenuous swimming. Thiamine required for strenuous swimming has not been determined and is yet another fertile area of investigation.

Juvenile muscle and liver thiamine concentrations (Table 2) measured in four salmon species from the northern Bering Sea were considered adequate and above the critical threshold levels for salmonids (Table 3). However, the thiamine requirements for northern Bering Sea juvenile salmon to avoid negative effects of thiamine deficiency have not been determined. We measured both muscle and liver thiamine to provide a better assessment of a fish's overall thiamine status. When muscle thiamine is < 1.0 nmol·g^{-1}, the thiamine status of the fish is considered to be low, but the fish may not be in imminent danger of death if its liver stores have not been fully exhausted. In one study there was little evidence of thiamine deficient behavior (lethargy) in Chinook salmon with ≤ 0.5 nmol·g^{-1} total muscle thiamine, suggesting that Chinook may be more tolerant of low thiamine; however the study did not measure liver concentration (Honeyfield et al. 2008a). More work is needed to refine threshold tissue values for effects of low thiamine on sub-adult and adult mortality. Only limited data describe secondary effects over a range of thiamine concentrations in sub-adult or adult salmonids (see Ottinger et al. 2012 on immune dysfunction). Also there are no previous studies on thiamine status of northern Bering Sea or Gulf of Alaska salmon for comparison.

Our interpretation of thiamine data is limited by not having specific reference thiamine values for western Alaska Chinook salmon. Although there are differences between Chinook salmon and lake trout, the use of lake trout thiamine muscle thresholds values to estimate thiamine susceptibility could be argued to be an overestimate of the severity of thiamine deficiency. Sufficient data exist to substantiate that the threshold for egg thiamine concentration and fry mortality are lower in Chinook than in lake trout (Fitzsimons et al. 2007). For all other tissues there are insufficient data to differentiate thresholds among the salmonid species. More research is needed to determine thiamine requirements/thresholds of Chinook. Despite differences among species, a common theme has been observed when thiamine deficiency is present. This often includes reproductive failure, a decline in populations, and multiple hypotheses offered to explain the reproductive failures observed (see Hill and Nellbring 1999; Brown et al. 2005b; Honeyfield et al. 2008b; Balk et al. 2009).

The relationship between juvenile salmon diet, specifically the prevalence of thiaminase-positive prey fish in the diet, and thiamine status is evident in this study. Juvenile Chinook and coho had lower muscle and liver total thiamine concentrations than chum or pink salmon. Juvenile Chinook salmon are known to be highly piscivorous and are much more selective for fish as prey than other salmon species (Brodeur et al. 2007; Daly et al. 2010; Weitkamp and Sturdevant 2010). Juvenile Chinook had the highest yearly average proportion of thiaminase-positive prey fish (63%) in their stomachs followed by coho salmon at 36% (Table 4), yet Chinook and coho muscle and liver total thiamine were similar. Although diet (thiaminase-positive prey) and thiamine data were not available for 2012, Brodeur et al. (2007) found that juvenile salmon diet exhibits minimal variation on an annual scale, especially in the case of Chinook and coho salmon. In both Chinook and coho the distinct lack of invertebrate prey, only 4% of stomach content, illustrates this consistent selectivity for teleost prey items.

Oceanographic features of the northern Bering Sea play an important role in juvenile Yukon River Chinook salm-

on diets. The northern Bering Sea is a unique habitat for juvenile Chinook salmon (Murphy et al. 2013). During colder years, a cold pool extends through the middle of the northern Bering Sea (Stabeno et al. 2012) which represents a thermal barrier to juvenile Chinook salmon. This barrier seems to shape the near-shore distribution of juvenile Chinook salmon (Murphy et al. 2013). Forage fish species in the northern Bering share this near-shore distribution and this spatial overlap may be a contributing factor in the high levels of thiaminase-positive prey items in the juvenile Chinook salmon diet. Over 80% of the Chinook diets from this area were comprised of small forage fish, primarily age-0 capelin (Cook and Sturdevant 2013). Typically, the dietary composition of juvenile Chinook in other regions (California Coastal Current, WA, OR, BC, and the Columbia River plume) also tend to be predominantly piscivorous, (Schabetsberger et al. 2003; Brodeur et al. 2007; Daly et al. 2009, 2010) but focused on different taxonomic groups (e.g. *Sebastes* spp., Hexagrammidae, and *Engraulis mordax*).

Previous studies with alewife, rainbow smelt, and Baltic herring *Clupea harengus* found thiaminase activity to be highly variable (Wistbacka et al. 2002; Tillitt et al. 2005; Honeyfield et al. 2012). More importantly, Wistbacka and Bylund (2008) suggested that Baltic salmon with a high incidence of thiamine deficiency (referred to as M74) targeted herring with higher thiaminase activity. The variability in thiaminase concentration in Alaska prey items is unknown. Likewise the effect of variable thiaminase activity on thiamine concentrations in juvenile Chinook or coho salmon has not been determined. Therefore knowledge of thiamine and thiaminase activity of potential prey items is important information, not only for Alaskan salmon species, but also for other top predators found to have low thiamine (Balk et al. 2009).

Significant environmental changes have been reported in the aquatic ecosystem off the Alaska coast (Grebmeier et al. 2006; Doney et al. 2012; Moore et al. 2014). Ecosystem shifts and changes in fish populations can and have led to thiamine deficiency in top predators. Thiamine deficiency directly impacts top predator metabolic functions and low thiamine in its inhabitants appears to be an indicator of ecosystem distress or dysfunction as seen in the Great Lakes Basin, Baltic Sea, and in Florida lakes (Hill and Nellbring 1999; Blazer and Brown 2005; Honeyfield et al. 2008b). In each of these ecosystems, abnormal food web dynamics produced direct and indirect thiamine-deficient mortality in top predators. Non-native alewife containing thiaminase became the dominant prey item for lake trout, Chinook and coho salmon in the Great Lakes basin as coregonid prey species without thiaminase either declined or disappeared. Thiamine deficiency (M74) in Baltic Sea salmon occurred with a shift in marine populations of Baltic cod *Gadus morhua*, sprat, and Atlantic herring (Hill and Nellbring 1999), such that salmon with a high incidence of M74 were selectively feeding on herring containing the highest thiaminase levels (Wistbacka and Bylund 2008). In the third case, an increase in American gizzard shad *Dorosoma cepedianum* containing thiaminase in the Florida alligator (*Alligator mississippiensis*) diet resulted in thiamine deficient mortality. Shad populations increased due to changes in water quality (Rice et al. 2007; Ross et al. 2009). In these examples, a stressed ecosystem and a change in dietary thiaminase led to a population decline in its top predator. While thiamine deficiency is a problem in and of itself, thiamine deficiency may also be an indicator of a stressed ecosystem.

The research reported here provides an exploratory assessment of the thiamine status of Yukon River Chinook salmon and suggests that additional studies on thiamine status are justified. The data showed no overwhelming evidence for acute thiamine deficiency in eggs leading to overt fry mortality, but egg thiamine values were sufficiently low enough to lead to secondary effects of thiamine deficiency such as immune dysfunction (Ottinger et al. 2012). Low thiamine is unlikely to be the only factor involved in Chinook salmon declines from Alaska to California but interactions with thiamine are highly probable. Little is known about food web dynamics in the open ocean or its effect on Chinook thiamine stores. Collecting thiamine and thiaminase activity data in all environmental compartments (water, primary productive organisms, prey items and top predators) across life stages is recommended.

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