Fall 12-12-2018

Causes and Impacts of Thiamine Deficiency Complex in Lake Ontario Salmonines

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Causes and Impacts of Thiamine Deficiency Complex in Lake Ontario Salmonines

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A thesis submitted to the Department of Environmental Science and Ecology of The College at Brockport – State University of New York in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science and Ecology.

12/12/2018

Department of Environmental Science and Ecology

Thesis Defense by

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Seminar Date: 12/12/2018

Thesis Defense date: 12/12/2018

Master's Degree Advisory Committee	**Approved**	**Not Approved**
Major Advisor	✓	
Committee Member	✓	
Committee Member	✓	
Graduate Director	12/13/18 **Date**	
Chairman, Environmental Science & Ecology	12/13/18 **Date**	

Acknowledgements

There are numerous people who deserve recognition for their assistance with the completion of this thesis. First and foremost, I would like to thank my major advisor, Dr. Jacques Rinchard for his continuous guidance and support throughout the entirety of this project. I'd also like to thank the rest of my thesis committee, Drs. James Haynes and Michael Connerton, for their assistance with the culmination of this written thesis. This thesis would not have been possible without help in the field and laboratory from numerous students in the Environmental Science and Ecology Department at The College at Brockport as well. These students include Nathan Barker, Thomas Bianchi, Nicholas Farese, Sage Hallenbeck, Kinsey Irvin, Jeremy Kraus, Chris Maier, and Robert Sickler.

In addition to the direct assistance from the aforementioned people, moral support was provided by many. All faculty, staff, and many of the students in the Department of Environmental Science and Ecology provided a friendly atmosphere that was conducive to the successful completion of my thesis. In addition, all of the friends that I have made during my time at Brockport including those from the Department of Environmental Science and Ecology and from Brockport's men's and women's swimming and diving teams have provided me with support and encouragement to produce the best thesis that I could. Lastly, I would like to thank my family, especially my parents. I am fortunate to have a family that has always provided me with the tools I need for success as well as the guidance for me to use those tools to their fullest potential, while maintaining constant love and support.

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Preface

For at least 50 years, salmonine species in the Great Lakes region have been suffering from a vitamin deficiency termed Thiamine Deficiency Complex (TDC) (Marcquenski and Brown 1997; Ketola et al. 1999). TDC impacts salmonines at alevin and adult stages, although the most common impacts are observed before first-feeding (Honeyfield et al. 1998; Fitzsimons et al. 1999; Lee et al. 2009). Alevins with TDC experience various neurological problems including lethargy, hyperexcitability, spiraled swimming, and ultimately death (Fitzsimons 1995). However, alevins that do not die directly from TDC may die from secondary effects that limit their feeding and predator avoidance (Fitzsimons et al. 2009; Ivan et al. 2018). Therefore, TDC can reduce offspring survival drastically, limiting wild recruitment and survival in hatcheries. However, thiamine treatments under hatchery conditions have been able to reduce TDC, increasing offspring survival significantly (Fitzsimons 1995; Koski et al. 1999; Fitzsimons et al. 2001; Lee et al. 2009; Futia et al. 2017). Adult salmonines can also experience symptoms of TDC when deficiencies are severe (Brown et al. 2005). Common symptoms include wiggling behavior while swimming, lethargy, and occasionally mortality (Brown et al. 2005; Futia et al. 2017).

Thiamine deficiency in salmonines is not limited to the Great Lakes. In New York's Finger Lakes, Lake Champlain, and the Baltic Sea, various salmonine species suffer from thiamine deficiency (Fisher et al. 1995; Bengtsson et al. 1999; J. Rinchard, The College at Brockport, Brockport, New York, personal communication, 2018). Despite thiamine deficiency being an international issue that has been occurring for

decades, the specific cause has yet to be determined. However, two hypotheses have been proposed: 1) degradation of thiamine by the thiamine-degrading enzyme, thiaminase (Honeyfield et al. 2002; Tillitt et al. 2005), and 2) increased use of thiamine as an antioxidant in response to high dietary lipid content (Lukienko et al. 2000; Keinänen et al. 2012). While both of these hypotheses are plausible, neither has been proven to cause thiamine deficiencies in wild populations.

In the Great Lakes region, alewife (*Alosa pseudoharengus*) are associated with the occurrence of TDC. Honeyfield et al. (2005) demonstrated that feeding exclusively on alewife induced TDC in adult salmonines. Furthermore, following the collapse of the alewife population in Lake Huron, wild recruitment of lake trout (*Salvelinus namaycush*) increased (Fitzsimons et al. 2010; Riley et al. 2011). Alewife have elevated thiaminase activity (Tillitt et al. 2005); however, the source of the thiaminase is unknown (Richter et al. 2012). Alewife also have greater lipid content than other abundant offshore prey fish in the Great Lakes (Happel et al. 2017); however, relationships between lipid content of prey and the occurrence of TDC in salmonines has not been proven.

In my thesis study, the extent of TDC for five salmonine species (brown trout *Salmo trutta*, Chinook salmon *Oncorhynchus tshawytscha*, coho salmon *O. kisutch*, lake trout, and steelhead trout *O. mykiss*) in Lake Ontario was determined from 2015 to 2017. The severity of TDC was determined based on thiamine concentrations in egg, liver, and muscle tissue as well as the occurrence of TDC-induced offspring mortality. Additionally, dietary influence on thiamine concentrations was evaluated. Using fatty

acid signature analysis, the inclusion of alewife, rainbow smelt (*Osmerus mordax*), and round goby (*Neogobius melanostomus*) in salmonine diets was determined. Lipid content of whole-body prey fish and various tissues of predator species were determined to investigate the influence of fat content on thiamine concentrations. Lastly, the potential effect of lipid peroxidation on salmonine thiamine concentrations was determined by comparing the proportions of fatty acids, grouped based on degrees of unsaturation (i.e., saturated, monounsaturated, and polyunsaturated), in egg and belly flap tissue with thiamine concentrations.

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Chapter 1: Extent of Thiamine Deficiency Complex in Lake Ontario Salmonines

Abstract

Thiamine Deficiency Complex (TDC) is an ongoing problem impacting salmonine health in various waterbodies including Lake Ontario. The prevalence of TDC has been variable and explanations for differences are limited. I found that Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), lake trout (*Salvelinus namaycush*), and steelhead trout (*O. mykiss*) experienced elevated offspring mortality under laboratory conditions, likely caused by TDC. No signs of TDC were observed for brown trout (*Salmo trutta*). For affected species, egg free thiamine (TH) was consistently low compared to lake trout from Lake Superior that are considered thiamine replete. In addition, species with the lowest percentages of TH in their eggs were the most susceptible to TDC, suggesting that limited thiamine reserves in the form of TH may cause TDC-induced offspring mortality. However, I found evidence that thiamine concentrations may be increasing for salmonines in Lake Ontario, potentially due to changes in their diet composition or changes in the biochemical properties of individual prey species. Lastly, I identified potential causes of thiamine deficiency and needs for future research.

Introduction

It has been over half a century since there has been continuous, successful natural reproduction of native salmonines in Lake Ontario, potentially due to Thiamine Deficiency Complex (TDC) (Elrod et al. 1995; Krueger et al. 1995; Ketola et al. 2000). In addition, introduced salmonines that provide many ecological and economic benefits for the lake (Mills et al. 2003) have experienced elevated rates of alevin mortality since the late 1960s due to TDC (Marcquenski and Brown 1997; Honeyfield et al. 1998a). Thiamine Deficiency Complex is a vitamin B_1 deficiency that results in various neurological problems and mortality, most notably with alevins (Honeyfield et al. 1998a; Fitzsimons et al. 1999; Lee et al. 2009). The deficiency was identified during the mid-1990s when its impact on salmonines in the Great Lakes basin intensified, including increased mortality of offspring, which resulted in additional research (Fisher et al. 1995; Fitzsimons 1995). Studies showed that thiamine treatments of adults, embryos, and eggs could reduce the impacts of the deficiency and allow for successful rearing of offspring under hatchery conditions (Fitzsimons 1995; Fisher et al. 1998; Koski et al. 1999; Fitzsimons et al. 2001; Lee et al. 2009). In recent years, some signs of increased wild recruitment in Lake Ontario have occurred including greater catches of young-of-year Chinook salmon (*Oncorhynchus tshawytscha*) and ages 0 through 2 lake trout (*Salvelinus namaycush*) (Bishop et al. 2018; Lantry et al. 2018). However, TDC continues to be a problem in the system as impacts remain evident (Futia et al. 2017) and self-sustaining populations of native salmonines are absent (Lantry et al. 2018), potentially limited by TDC.

After being consumed, thiamine may be transformed into multiple derivatives including unphosphorylated (free) thiamine (TH), thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP). Free thiamine may serve as a reserve for TPP as well as an antioxidant (Lukienko et al. 2000), while TPP is a coenzyme required for in-cell energy metabolism; there is no known role for TMP (Bettendorff et al. 2014). Because thiamine is highly involved with cellular metabolism, reductions in its concentration can have severe impacts, including decreased glucose oxidation and neurological disorders, ultimately leading to death (Bettendorff and Wins 2013). Adults that are thiamine deficient show abnormal behaviors including sluggish and wiggling movements, lethargy, distress, and even death (Brown et al. 2005b; Futia et al. 2017). As a result, upstream migrations have been shown to be limited (Ketola et al. 2009) and angler satisfaction may be impaired. Deficient females exhibiting these symptoms also have low reserves for egg thiamine, resulting in thiamine deficient offspring (Fisher et al. 1998). Symptoms of alevin deficiency include loss of equilibrium, hyperexcitability, and spiral swimming, which can occur before females show symptoms of the deficiency due to a higher relative requirement of thiamine in offspring (Fitzsimons 1995; Brown et al. 2005a). Symptoms exhibited by alevins may decrease foraging success, increase vulnerability to predation, and cause direct mortality, all limiting natural recruitment (Fitzsimons et al. 2009; Ivan et al. 2018). While limited wild survival may be beneficial from a management perspective by providing partial control over a potentially large component of the predatory pressure in the system, it also prevents the restoration of native salmonines.

Despite years of research, thiamine deficiencies continue to impact the salmonine fishery in Lake Ontario as well as other populations including those in the Baltic Sea (Bylund and Lerche 1995; Balk et al. 2016), New York's Finger Lakes (Fisher et al. 1995; J. Rinchard, The College at Brockport, Brockport, New York, personal communication, 2018), and Lake Champlain (J.E. Marsden, University of Vermont, Burlington, Vermont, personal communication, 2018). In addition, thiamine deficiency has recently been identified as an emerging issue in global conservation and biological diversity (Sutherland et al. 2018). The persistence of TDC may be due to important uncertainties about its cause in the wild (Harder et al. 2018). Correlative studies have shown that diets consisting of mainly alewife (*Alosa pseudoharengus*) can result in TDC (Fitzsimons et al. 2010; Riley et al. 2011), and laboratory studies have induced thiamine deficiencies in salmonines by providing alewife-rich diets (Honeyfield et al. 2005). In addition, following the collapse of alewife in Lake Huron, lake trout egg thiamine concentrations increased and coincided with large increases in wild recruitment of lake trout (Fitzsimons et al. 2010; Riley et al. 2011). However, it is unclear how alewife cause thiamine deficiency. One potential cause of TDC is the enzyme thiaminase, which is present in various prey fish in Lake Ontario and is known to degrade thiamine (Honeyfield et al. 2002; Tillitt et al. 2005, 2009; Honeyfield et al. 2012). While sources of thiaminase have been found (Honeyfield et al. 2002), they have not been shown to produce enough thiaminase to cause TDC (Richter et al. 2012). Another hypothesis is that TDC results from an imbalance in the lipid-to-thiamine concentrations in prey (Keinänen et al. 2012). When prey fish are rich in fats, such as

Baltic sprat (*Sprattus sprattus*) and alewife, tissues of consumers may become rich in lipids and susceptible to lipid peroxidation, causing thiamine to be used as an antioxidant (Keinänen et al. 2012). While this hypothesis has been supported by correlations between fat content of prey fishes and salmonine egg thiamine concentrations in the Baltic Sea region (Mikkonen et al. 2011), no evidence of such a dietary imbalance in the Great Lakes region has been observed. Lastly, additional unknown causes of TDC or additive impacts of various factors may be present.

While TDC is known to be present in Lake Ontario, the severity of its effects on some salmonines is uncertain. Therefore, the impacts on wild offspring survival and recruitment are unknown. Observed increases in wild offspring survival for some salmonine species in Lake Ontario could be indicative of reduced TDC; however, to my knowledge, recent evaluation of thiamine concentrations across various salmonine species has been limited. Thus, my main objective was to determine the extent of TDC in multiple salmonine species from Lake Ontario including brown trout (*Salmo trutta*), Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), lake trout, and steelhead trout (*Oncorhynchus mykiss*). Specifically, for each species I determined the thiamine concentrations for various tissues, the percentage of offspring dying due to TDC, and the concentrations of thiamine associated with TDC-induced mortality. In addition, I looked for correlations among liver, muscle, and egg thiamine vitamer concentrations and offspring mortality that may provide valuable information regarding the occurrence of TDC.

Materials and Methods

Fish Collection

From spring of 2015 through fall of 2017, brown trout, lake trout, steelhead/rainbow trout, Chinook salmon, and coho salmon were collected during their respective spawning periods at various locations throughout Lake Ontario and its tributaries. Steelhead trout as well as Chinook and coho salmon were collected at the Salmon River Fish Hatchery (Altmar, New York) during the annual egg take conducted by the New York State Department of Environmental Conservation (NYSDEC). Additional Chinook and coho salmon were collected directly from the Salmon River (Pulaski, NY) using Halltech HT-2000 backpack electroshockers. Brown trout were collected from Sandy Creek (Hamlin, New York), also using Halltech HT-2000 backpack electroshockers. Lastly, lake trout were collected from Lake Ontario (Hamlin and Oswego, New York) using monofilament gillnets deployed by The College at Brockport using the R/V Madtom, and the United States Geological Survey – Lake Ontario Biological Station (USGS-LOBS) using the R/V Kaho, all with approximately 24-h soak times. A reference population of lake trout with successful wild recruitment and considered thiamine replete was sampled from Lake Superior (Bayfield, Wisconsin) using monofilament gillnets with approximately 24-hr soak time by the Wisconsin Department of Natural Resources during fall 2017.

Muscle, liver, and belly flap samples were taken from all fish captured during 2015 and 2016. Egg samples were taken from all ovulating females collected during the entire sampling period (2015-2017). For fish requiring euthanasia, a blow to the

head was performed to cause rapid brain death. Tissue samples were stored on ice and transported to The College at Brockport where they were held at -80°C until processing. Subsamples of eggs were held from all ovulating females for fertilization when sperm was available. For fertilization, eggs from females were held in separate plastic containers and mixed with sperm from three separate males for approximately one minute using water from natural sources (either Lake Ontario or a surrounding tributary where fish were collected). After fertilization, eggs were rinsed with freshwater and the containers were refilled. Containers were sealed shut and placed over insulated ice for transport to The College at Brockport for incubation.

Offspring Survival

Fertilized eggs were separated by family (same maternal parent) and placed into baskets that were made of polyvinyl chloride piping with a mesh bottom. Baskets were placed in hatching trays that were irrigated with dechlorinated municipal city water run on a recirculating system and kept at a constant temperature (~8°C) using a coil water chiller (Frigid Units, Inc., Toledo, OH). The number of eggs that reached the eyed-stage were counted and remaining unfertilized eggs were counted and removed. After hatching, alevins were transferred to aquaria (30 L), remaining separated by family. Aquaria were setup with flow-through systems using dechlorinated municipal water and aerators. Daily fish husbandry was performed to provide healthy conditions for all individuals. After hatching, waste was removed from each tank and mortality and water temperature (9-12°C) were recorded daily. As salmonines have been shown to start feeding within two weeks of hatching (Ladago et al. 2016), alevins were provided dry

diet (Fish Starter 55-15, Zeigler Bros., Inc.) daily, starting 14 days post-hatch. Mortality caused by TDC was quantified based on the total number of alevins that exhibited symptoms of TDC (e.g., abnormal swimming, lethargy, hyperexcitability) and died after the swim-up stage when TDC-induced offspring mortality occurs (Fitzsimons 1995).

Thiamine Extraction from Adult Tissues

Three thiamine vitamers (TH, TMP, and TPP) were evaluated for each tissue (egg, liver, and muscle) in duplicate. Thiamine was extracted following Brown et al. (1998), using 1 g of egg tissue and 2 g each of liver and muscle tissue homogenized in a 2% trichloroacetic acid (TCA) solution. Following extraction, thiamine concentrations were quantified using HPLC (Agilent Technologies 1200 series) (Brown et al. 1998; Futia et al. 2017). A six-point standard curve with known concentrations of thiamine (0, 1, 2.5, 5, 10 and 30 nmol/g) was generated at the start of each group of samples run. Concentrations of each vitamer were then calculated based on the standard curve ($y = m*x$) and expressed as nmol/g. These concentrations were combined to determine total thiamine (TTH).

Statistical Analyses

Data were assessed using IBM SPSS 25 (SPSS Inc., Chicago, IL), SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA), and PRIMER v.6 (Primer-E, Plymouth, U.K.) with a significance level of $\alpha = 0.05$. For comparisons between and among samples, data were tested for normality using the Shapiro-Wilk test and homogeneity of the variance was tested using the Levene Statistic. Parametric tests (independent t-

test and analysis of variance [ANOVA] with Tukey post-hoc) were used unless data failed normality tests, in which case non-parametric alternatives were used (Mann-Whitney U test and Kruskal-Wallis with Dunn's post-hoc test including the Bonferroni correction for multiple testing). To compare thiamine concentrations among species across all years, a nested permutational multivariate analysis of variance (PERMANOVA) was run with year nested in species.

Linear regressions were used to determine relationships between TTH concentrations for the different tissue samples. The Spearman's rank correlation (ρ) was calculated to measure correlations between samples and significance of the relationships was determined. Three-parameter logistic correlation curves were used to compare relationships between thiamine concentrations and offspring mortality with the equation: $y = \frac{a}{1+(\frac{x}{x_o})^b}$ (a = maximum asymptote, b = inflection point (50% mortality), x_o = slope, x = thiamine concentration) (Futia et al. 2017). Using this equation, thiamine concentrations resulting in 50% mortality of offspring (EC50) were calculated.

Results

Thiamine concentrations

Total thiamine concentrations in egg, liver, and muscle tissue were highly variable. For some species, variation occurred between years and between sexes. For egg tissue, average TTH concentrations increased from 2015 to 2016 for all species. These increases were significant for Chinook salmon (Mann-Whitney U, U = 392.0, p

= 0.009) and coho salmon (Mann-Whitney U, $U = 497.5$, $p < 0.001$). Brown trout also had significant differences among 2015, 2016, and 2017 (ANOVA, $F = 7.14$, $p = 0.002$), including significant increases from 2015 to 2016 (Tukey post-hoc, $p = 0.008$) and insignificant increases from 2016 to 2017. Egg TTH concentrations continued to increase between 2015 and 2017 for steelhead trout, although these increases were not significant (Table 1). Egg TTH concentrations differed significantly among species across years (nested PERMANOVA, pseudo-$F = 6.46$, $p = 0.001$) with TTH concentrations of brown trout eggs significantly higher than those of all other species (nested PERMANOVA pair-wise comparisons, $p < 0.05$). In addition, with data from all years combined, all species except brown trout had significantly lower egg TTH concentrations compared to a reference population of lake trout collected from Lake Superior (Kruskal Wallis, $H = 147.0$, $p < 0.001$; Dunn's post-hoc, $p < 0.001$) (Figure 1).

Liver TTH concentrations were generally consistent within species, although a few significant differences between sexes and between years were observed. In 2015, there were no differences in liver TTH concentrations observed between sexes; however, in 2016 male TTH concentrations were significantly greater than female TTH concentrations for brown trout (independent sample t-test, $t = -7.33$ $p < 0.001$) and coho salmon (independent sample t-test, $t = -2.96$, $p = 0.006$) (Table 2). For yearly comparisons, liver TTH concentrations increased significantly from 2015 to 2016 for female steelhead trout (independent sample t-test; $t = -3.07$; $p = 0.005$) but were relatively consistent for both sexes of all other species (Figure 2). Liver TTH

concentrations had multiple significant differences among species within sexes and years as well (Figure 2).

Muscle TTH concentrations were also relatively consistent between years and sexes with a few exceptions. Only Chinook salmon collected during 2015 had a significant difference between male and female muscle TTH concentrations (Mann-Whitney U, $U = 280.0$, $p = 0.008$). No significant differences were found between sexes for fish collected during 2016 (Table 2). However, Chinook salmon, coho salmon, and steelhead trout females had significant increases in muscle thiamine concentrations between 2015 and 2016 (Mann-Whitney U, Chinook salmon: $U = 248.0$, $p = 0.009$; coho salmon: $U = 304.5$, $p < 0.001$; steelhead trout: $U = 221.0$, $p < 0.001$), while only male coho salmon had a significant increase (Figure 3; Mann-Whitney U, $U = 278.0$, $p < 0.001$). Significant differences in muscle TTH concentrations among species within years and sexes were limited by high variation within species (Figure 3).

Percent contribution of thiamine vitamers

The percent contribution of thiamine vitamers had notable variation among species, specifically for egg thiamine. For egg thiamine, comparisons were made among the five species from Lake Ontario as well as the reference population of lake trout from Lake Superior. Overall, TH was the most abundant vitamer for three of the populations (Lake Superior lake trout, brown trout, and chinook salmon) and TPP was the most abundant for the others (Lake Ontario lake trout, steelhead trout, and coho salmon) (Table 3). While egg TMP was the least abundant vitamer for all species, concentrations had significant differences among the six populations (Table 3; Kruskal-

Wallis, H = 206.6, $p < 0.001$). For both liver and muscle tissue, TPP was the most abundant vitamer, averaging at least 75% of the TTH for all species; however, significant differences in the proportion of TPP in both liver and muscle were present among species (Table 3; Kruskal-Wallis, liver: H = 61.9, muscle: H = 60.0, $p < 0.001$). Significant differences in the percent contribution of TMP and TH among species were present in both liver and muscle tissue as well, although the differences were not consistent across species (Table 3).

Comparisons among tissue TTH

Few significant, positive correlations in TTH concentrations occurred among tissue types for all five species (Table 4). These include positive correlations for brown trout egg and muscle (Spearman's ρ = 0.38, p = 0.039) male Chinook salmon liver and muscle (Spearman's ρ = 0.45, p = 0.010), coho salmon egg and muscle (Spearman's ρ = 0.49, p = 0.002), male coho salmon liver and muscle (Spearman's ρ = 0.54, p = 0.001), lake trout egg and muscle (Spearman's ρ = 0.58, $p < 0.001$), and female steelhead trout liver and muscle (Spearman's ρ = 0.51, p = 0.004). Significant correlations between tissue types were not consistent across all species. In addition, there were no correlations between liver and egg TTH for any species (Table 4).

Relationships between thiamine concentrations and TDC-induced offspring mortality

TDC-induced offspring mortality was observed for all species except for brown trout. Therefore, brown trout were not included in the comparisons between offspring mortality and thiamine concentrations. For the other species, significant relationships occurred between TDC-induced offspring mortality and thiamine concentrations in egg

and muscle tissues, but not with liver tissue (Table 5). For egg tissue, TH and TPP concentrations were used for comparisons due to their high contribution for various species; a ratio between these two vitamers (TH:TPP) as well as TTH were also used (Figure 4). All comparisons with TH and TTH had significant relationships for each species while only coho salmon had a significant relationship between offspring mortality and TPP (Table 5). For muscle tissue, comparisons were only made between offspring mortality and TPP as well as TTH. Both TPP and TTH had significant relationships for coho salmon, lake trout, and steelhead trout, while Chinook salmon had no significant relationships between muscle thiamine concentrations and offspring mortality (Table 5).

Thiamine thresholds

Where significant relationships between offspring mortality and thiamine concentrations were found, effective concentrations of thiamine resulting in 50% mortality of offspring (EC50s) were calculated. The EC50s for both egg and muscle thiamine concentrations varied among species, with steelhead trout frequently having the highest EC50s while the EC50s for the other species were similar and considerably lower. However, rankings of the species' EC50s depended on tissue type and vitamer. For egg thiamine concentrations, Chinook salmon had the lowest EC50 for TTH while coho salmon had the lowest EC50 for TH. Meanwhile, for muscle thiamine, the ranking of species' EC50s did not change (Table 5). The prevalence of TDC within each species was quantified as the percentage of individuals with thiamine concentrations below the egg TTH and TH EC50s. For both egg TTH and TH, steelhead had the highest

prevalence of TDC, followed by coho salmon, lake trout, and finally Chinook salmon (Figure 5).

Discussion

Current thiamine status

Total thiamine concentrations were consistently low for egg, liver, and muscle tissues of all Lake Ontario salmonines, except brown trout, when compared to other studies (Brown et al. 2005b; Fitzsimons et al. 2007). In addition, for egg TTH concentrations, all species except brown trout had significantly lower TTH concentrations than the replete population from Lake Superior. This in addition to observed symptoms of TDC (i.e., elevated offspring mortality) suggests that Chinook salmon, coho salmon, lake trout, and steelhead trout from Lake Ontario were all suffering from TDC during the period when this study was conducted, while brown trout were not. There was much variability in TTH concentrations for all species, with many significant increases in TTH concentrations over time, among all tissues. Therefore, the impacts of TDC in Lake Ontario may decline in future years if these trends continue, and natural recruitment rates can be expected to increase. However, the increases observed in this study may also be due to large inter-annual variability, which could be demonstrated with future, long-term research.

Variations observed in thiamine concentrations among species are likely linked to differences in diets. Diets rich in alewife are known to result in a thiamine deficiency (Fitzsimons et al. 1999; Honeyfield et al. 2005); therefore, eating alternative prey can

limit reductions in thiamine concentrations (Brown et al. 2005a). Brown trout have highly variable diets consisting of pelagic and littoral prey (Yuille et al. 2015), which could explain the relatively high concentrations of thiamine observed in their tissues. Lake trout diet is variable as well, mainly comprised of round goby (*Neogobius melanostomus*) and alewife (Colborne et al. 2016; Happel et al. 2017; Mumby et al. 2018). In contrast, Chinook and coho salmon have a much more specific diet, mainly alewife (Yuille et al. 2015; Happel et al. 2017), which is consistent with their low thiamine concentrations as alewife-dominant diet have been shown to induce TDC (Honeyfield et al. 2005).

Temporal variation in thiamine concentrations within species may be caused by changes in the prey base in Lake Ontario. Studies have suggested that salmonines with a more diverse diet will likely have greater thiamine concentrations (Brown et al. 2005b; Honeyfield et al. 2005; Fitzsimons et al. 2007). In Lake Ontario, round goby were identified in lake trout stomachs shortly after their invasion, averaging about one-third of the stomach contents by mass (Dietrich et al. 2006). However, recent studies show that round goby may have become more important in lake trout diet, potentially contributing more than any other species (Colborne et al. 2016). Such increases in the contribution of round goby to lake trout diet over time likely resulted in higher thiamine concentrations. Varying inclusion of round goby in lake trout diet could explain the relatively large variability in their thiamine concentrations compared to the other species affected by TDC. More recently, deepwater sculpin (*Myoxocephalus thompsonii*) have had large increases in abundance in Lake Ontario (Weidel et al.

2017), potentially providing an additional alternative prey for salmonines and thereby further alleviating the deficiency. Both round goby and deepwater sculpin are benthic species and, therefore, less likely to be consumed by Chinook or coho salmon, which are pelagic feeders (Jude et al. 1987). However, efforts have been in place to increase prey fish diversity, including the restoration of pelagic coregonine species to Lake Ontario (Stewart et al. 2017). These species would provide additional prey for Chinook and coho salmon as well as the other salmonines and likely reduce the prevalence of TDC. Through long-term monitoring of salmonine diets and thiamine concentrations, variability in the severity of TDC may be better explained and potentially predicted.

Alternatively, intraspecies variation may be caused by changes in the biochemistry of prey. Thiaminase activity can be highly variable across years and sizes within species, potentially resulting in differing severities of thiamine deficiency (Tillitt et al. 2005). However, reasons for variability in thiaminase activity are unknown. Also unknown is whether changes in prey fat content relative to their thiamine concentrations can lead to variation in salmonine thiamine concentrations. Correlations between oxidative stress and reductions in thiamine-dependent processes have been demonstrated (Gibson and Zhang 2001; Vuori and Nikinmaa 2007; Keinänen et al. 2012). Salmonine antioxidant concentrations (e.g., vitamin E and thyroxine) can be reduced when thiamine deficiencies occur (Hornung et al. 1998; Palace et al. 1998); additional studies have shown that thiamine may serve as an antioxidant as well (Lukienko et al. 2000; Gibson and Zhang 2001). Therefore, if

excessive lipids from prey promote oxidative stress, thiamine reserves may be depleted. This hypothesis should be tested.

Correlations with TDC

Total thiamine concentrations in the tissue samples were low and correlations between tissues were often weak. In particular, TTH concentrations in liver and egg tissue had no strong correlations and liver and muscle concentrations only had significant correlations for males of two species and females from a third. Furthermore, liver thiamine concentrations (TTH and TPP) had no significant correlations with offspring mortality. Therefore, assessing liver thiamine concentrations alone provided no information regarding the extent of TDC in the fish studied. In contrast, both muscle and egg thiamine concentrations correlated well with the severity of TDC. Liver thiamine has been shown to be conserved more strictly than muscle and egg thiamine when individuals are deficient (Honeyfield et al. 2008). Therefore, the extent of TDC for many adults in this study may not have been severe enough to cause liver TTH concentrations to decrease and show strong correlations with other tissues or alevin mortality.

TDC-induced offspring mortality

There was notable variability in the percentage of offspring that died due to TDC within and among species. Among species differences can be explained by different thiamine requirements (i.e., thresholds and EC50s) for each species (Fitzsimons et al. 2007). No explanations for why species have differing thresholds have been demonstrated; however, susceptibility to TDC among species appears to be

consistent across studies. Fitzsimons et al. (2007) found that susceptibility to TDC decreased from coho salmon to lake trout to Chinook salmon. I found the same results, although steelhead trout were included as well, and were the most susceptible species. Interestingly, these differences in susceptibility correlate well with egg vitamer percent contributions. Species with the highest mortality (coho salmon and steelhead trout) had the lowest TH:TPP ratios, whereas Chinook salmon had the least mortality and the highest ratio. Free thiamine has been shown to be the most abundant vitamer in egg tissue, particularly for lake trout (Brown et al. 1998) and Chinook salmon (Wolgamood et al. 2005; Doyle et al. 2017). TH was the most abundant vitamer in Atlantic salmon, coho salmon, and steelhead trout, but inconsistently. Werner et al. (2011) showed yearly variation in the contribution of TH, with a TH:TPP ratio less than 1.0 for two out of five years for Atlantic salmon egg thiamine concentrations. While the authors could not explain the variation, it was hypothesized that it may affect reproduction and recruitment. Similarly, both Honeyfield et al. (1998b) and Wolgamood et al. (2005) found coho salmon with TDC had a significantly lower contribution of TH than individuals without TDC. Lastly, Futia et al. (2017) found deficient steelhead trout had very low egg TH concentrations (less than 10% of the TTH), while steelhead trout treated with thiamine had significantly higher TH concentrations that accounted for the majority of the TTH. Therefore, salmonine eggs with greater thiamine reserves in the form of TH may have less susceptibility to TDC.

Caution should be taken when using the EC50s calculated in this study as a measure of TDC. While these values provide useful information for comparing the

extent of TDC among species and years, individuals may have differing mortality rates at these concentrations. In addition, TDC can have substantial impacts at thiamine concentrations above these calculated thresholds. Sublethal thiamine concentrations may leave offspring weak and less capable of foraging and avoiding predators (Fitzsimons et al. 2009; Ivan et al. 2018). Therefore, under natural conditions, mortality rates may be even greater than those recorded during this study. Ivan et al. (2018) used an individual based model to evaluate an egg thiamine EC50 that included sublethal impacts (i.e., starvation and predation), which was calculated at 7.4 to 10.0 nmol/g. When applying this EC50 to the lake trout egg TTH concentrations from my study, only three (7%) of the individuals tested were above this threshold.

Future Work

Additional hypotheses for the causes of TDC and variations within and among species have been suggested and require further research. Kraft and Angert (2017) suggested that increased anthropogenic production of thiamine (e.g., pharmaceutical and agricultural) may have created a large influx of thiamine to the environment, which in turn resulted in excessive concentrations of thiamine degrading products. Another hypothesis proposed by Harder et al. (2018) suggested that the increasing pressure placed on salmonines and other organisms facing thiamine deficiency may have resulted in genetic adaptations allowing individuals to tolerate low thiamine availability, which could explain differences in thiamine thresholds among species.

Despite the continuing occurrence of TDC in Lake Ontario salmonines, the observed increases in thiamine concentrations are encouraging for these species. For

lake trout specifically, wild recruitment in Lake Ontario has increased substantially in recent years, although the majority of recruitment has been centered near the Niagara Bar area (Lantry et al. 2018), which is outside of the sampling area for my study. If thiamine concentrations in salmonines continue to increase, wild recruitment may increase for other areas of the lake, potentially leading to self-sustaining populations. Similarly, reduced occurrence of TDC may occur for the other salmonine species impacted by TDC as well, potentially leading to increased offspring survival and recruitment.

Greater salmonine health and survival may be detrimental to the balance between prey and predator populations if salmonine production is not controlled. However, as the ultimate cause of TDC remains unknown, it is possible that thiamine concentrations could decline unexpectedly or continue to vary among years and species. Therefore, continued monitoring of thiamine concentrations is important to identify shifts in wild recruitment success. In addition, laboratory research investigating specific causes of TDC is needed, including causes of oxidative stress and elevated lipid peroxidation associated with lipid rich prey, sources of substantial thiaminase activity, and interactions among thiamine-degrading processes. Such research would increase the mechanistic understanding of TDC and may allow managers to limit occurrences of TDC in the Laurentian Great Lakes and globally.

Acknowledgements

I thank J. Haynes and M. Connerton for their assistance reviewing this manuscript. I also thank B. Lantry (USGS – Lake Ontario Biological Station), B. Ray (Wisconsin DNR), and personnel from the NYSDEC for assisting with fish collections. Laboratory assistance was provided by undergraduate and graduate students from Rinchard's lab at The College at Brockport. Funding for this project was provided in part by the Distinguished Professors Award through The College at Brockport and a student grant from the Great Lakes Research Consortium.

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Tables

Table 1. Average total thiamine concentrations (nmol/g) and standard deviation for salmonine eggs from 2015 through 2017. Significant differences ($p < 0.05$) among species within years are represented vertically by different letters (z, y, and x) and significant differences across years within species are represented horizontally by different letters (a and b). Sample sizes (N) are included below each measurement.

	2015	2016	2017
Brown trout	$13.24 \pm 5.22^{a}_{z}$	$20.55 \pm 7.10^{b}_{z}$	$24.18 \pm 9.14^{b}_{z}$
N	13	21	14
Chinook salmon	$3.88 \pm 1.82^{a}_{yx}$	$4.53 \pm 0.89^{b}_{y}$	-
N	27	20	-
Coho salmon	$2.75 \pm 1.25^{a}_{x}$	$4.58 \pm 1.00^{b}_{y}$	-
N	29	20	-
Lake trout	$4.92 \pm 3.11_{y}$	$6.10 \pm 4.00_{y}$	-
N	29	14	-
Steelhead trout	$4.31 \pm 1.19_{y}$	$4.97 \pm 1.50_{y}$	$5.53 \pm 2.55_{y}$
N	30	20	20

Table 2. Comparisons between male and female total thiamine concentrations (nmol/g) for liver and muscle tissue within 2015 and 2016 for five salmonine species. Thiamine concentrations are displayed as averages with standard deviation. Significant differences ($p < 0.05$) between sexes for each tissue within years are denoted by different letters. Sample sizes (N) are included for each calculated value.

		2015				2016			
		Female	N	Male	N	Female	N	Male	N
Brown trout*	Liver	7.70 ± 2.94	13	-	0	5.99 ± 1.61[a]	17	14.66 ± 4.33[b]	15
	Muscle	6.93 ± 2.55	13	-	0	9.40 ± 3.64	17	7.74 ± 2.44	15
Chinook salmon	Liver	3.58 ± 1.47	22	4.52 ± 1.71	17	3.39 ± 0.78	15	3.49 ± 1.27	15
	Muscle	4.28 ± 2.19[a]	22	6.22 ± 2.64[b]	17	5.33 ± 1.18	15	5.64 ± 1.36	15
Coho salmon	Liver	5.19 ± 2.12	24	5.00 ± 2.33	20	4.27 ± 1.38[a]	15	6.05 ± 1.88[b]	15
	Muscle	4.71 ± 2.06	24	3.77 ± 1.73	20	7.30 ± 2.16	15	7.12 ± 1.65	15
Lake trout	Liver	6.16 ± 2.95	21	7.15 ± 3.06	24	6.19 ± 2.74	15	5.51 ± 2.37	15
	Muscle	7.37 ± 5.07	21	9.55 ± 6.42	24	8.31 ± 4.73	15	8.25 ± 2.55	15

Table 2 Continued

Steelhead trout*	Liver	4.67 ± 2.11	15	-	0	7.62 ± 3.07	15	7.59 ± 3.10	15
	Muscle	3.99 ± 1.43	15	-	0	9.26 ± 3.04	15	9.54 ± 6.33	15

*No comparisons were made for 2015 because no data for males were collected.

Table 3. Average percent contribution and standard deviation of thiamine vitamers (free thiamine: TH, thiamine monophosphate: TMP, and thiamine pyrophosphate: TPP) in egg, muscle, and liver samples. Data for additional egg samples from a reference population of Lake Superior lake trout are included. Significant differences ($p < 0.05$) in the percent contribution of vitamers among species for each type of tissue are denoted by different letters horizontally.

		Brown trout	Chinook salmon	Coho salmon	Lake trout	Steelhead trout	Lake Superior lake trout
Egg thiamine	TPP	34.49 ± 18.43^{c}	31.98 ± 7.30^{c}	61.59 ± 7.30^{ab}	53.56 ± 20.62^{b}	65.94 ± 11.50^{a}	66.39 ± 10.93^{a}
	TMP	5.68 ± 3.22^{c}	14.32 ± 3.59^{b}	24.63 ± 4.79^{a}	11.02 ± 3.31^{b}	12.73 ± 4.68^{b}	4.08 ± 1.10^{c}
	TH	59.78 ± 19.12^{a}	53.71 ± 12.22^{a}	13.78 ± 6.61^{c}	35.41 ± 22.10^{b}	20.89 ± 12.85^{bc}	29.54 ± 9.99^{c}
Liver thiamine	TPP	83.02 ± 7.25^{b}	84.60 ± 6.47^{ab}	77.86 ± 5.40^{c}	87.40 ± 7.89^{a}	81.80 ± 11.00^{b}	
	TMP	12.32 ± 6.20^{bc}	10.03 ± 4.53^{bc}	16.26 ± 4.16^{a}	9.35 ± 6.84^{c}	14.55 ± 9.21^{b}	
	TH	4.65 ± 2.61^{abc}	5.37 ± 3.48^{ab}	5.87 ± 2.84^{a}	3.24 ± 3.01^{d}	3.65 ± 2.75^{cd}	
Muscle thiamine	TPP	91.40 ± 2.92^{a}	85.85 ± 3.59^{ab}	89.13 ± 4.96^{ab}	88.65 ± 4.27^{b}	88.82 ± 4.90^{c}	
	TMP	7.32 ± 2.41^{c}	10.78 ± 3.98^{a}	8.01 ± 3.13^{bc}	9.13 ± 3.05^{ab}	8.33 ± 4.09^{bc}	
	TH	1.28 ± 0.99^{c}	3.36 ± 2.25^{a}	2.86 ± 2.80^{ab}	2.22 ± 2.04^{b}	2.85 ± 2.03^{a}	

Table 4. Spearman's rank correlation (rho, ρ) and sample sizes (N) for comparisons among TTH concentrations in egg, liver, and muscle tissues. For comparisons between muscle and liver TTH, males and females were separated to include correlations within each sex only. Significant correlations are denoted with an asterisk (*).

		Brown trout		Chinook salmon		Coho salmon		Lake trout		Steelhead trout	
		Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Egg	ρ	<0.00	0.38*	-0.06	0.047	-0.11	0.49*	-0.19	0.58*	0.24	0.14
	N	29	30	37	37	39	39	35	35	30	30
Liver female[†]	ρ		-0.31		0.15		0.1		0.28		0.51*
	N		30		37		39		36		30
Liver male[†]	ρ		-0.21		0.45*		0.54*		0.17		-0.02
	N		17		32		35		39		16

[†]Liver tissues were only compared to muscle tissues of the corresponding sex.

Table 5. Effective concentrations (nmol/g) of egg and muscle thiamine vitamers that result in 50% mortality of offspring due to TDC. Correlation coefficients (R) of the calculated regressions are included as well as significance ($p < 0.05$), which is denoted by an asterisk (*), for each EC50.

	Egg									Muscle				
		TH		TPP		TH:TPP		TTH			TPP		TTH	
	N	EC50	R	EC50	R	EC50	R	EC50	R	N	EC50	R	EC50	R
Chinook salmon	43	0.81	0.75*	N/A[†]	0.26	0.76	0.75*	2.06	0.62*	33	0.41	0.15	0.51	0.14
Coho salmon	40	0.26	0.81*	1.68	0.81*	0.14	0.70*	2.95	0.83*	30	4.02	0.63*	4.52	0.64*
Lake trout	13	0.38	0.88*	N/A[†]	0.10	0.34	0.88*	2.32	0.90*	13	3.43	0.91*	3.74	0.92*
Steelhead trout	44	1.58	0.56*	5.67	0.23	0.55	0.45*	6.96	0.58*	24	11.44	0.54*	12.09	0.55*

[†]Calculation produces an invalid value.

Figures

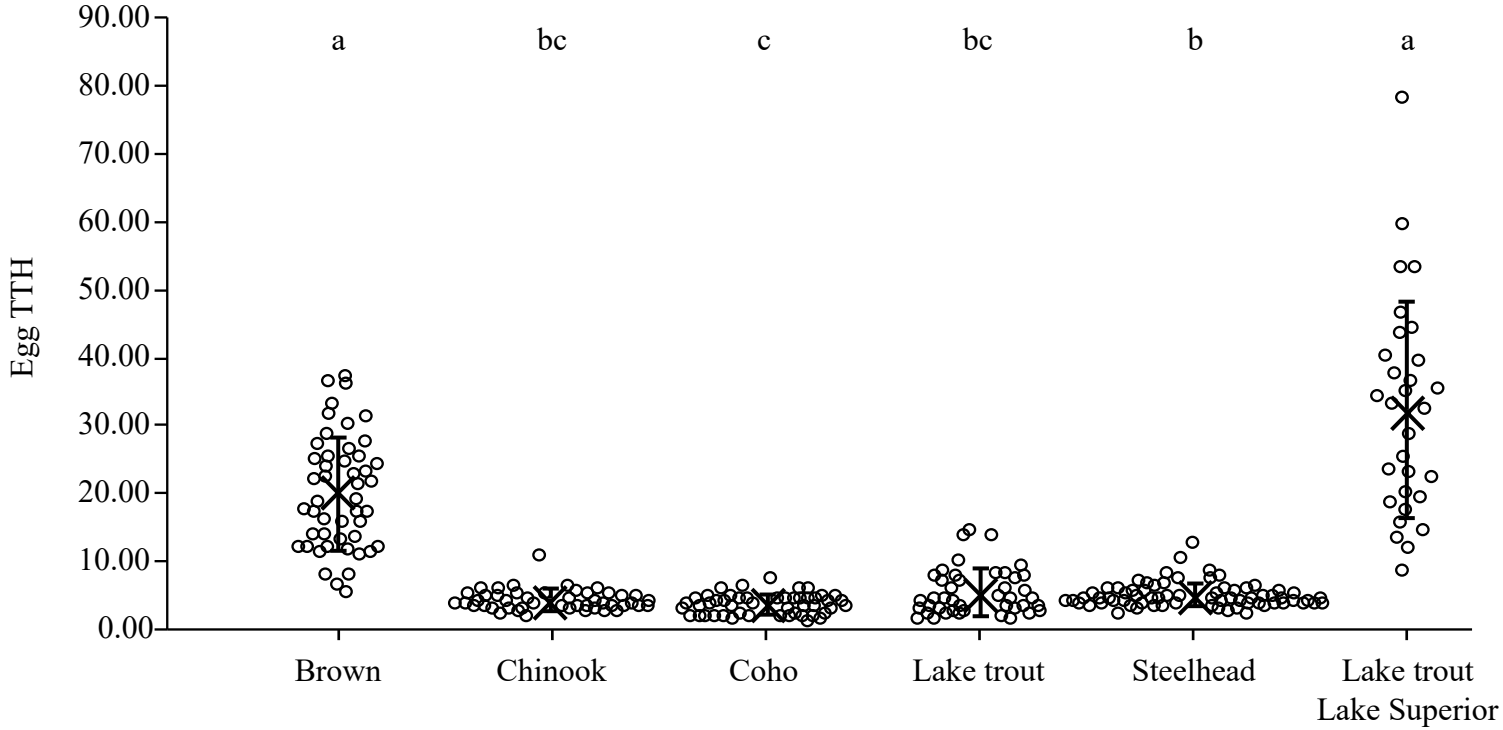

Figure 1. Egg total thiamine (TTH) concentrations for five salmonine species from Lake Ontario and a reference population of lake trout from Lake Superior. Average thiamine concentrations (X) and standard deviation are included as well as significant differences ($p < 0.05$) among populations, which are denoted by different letters.

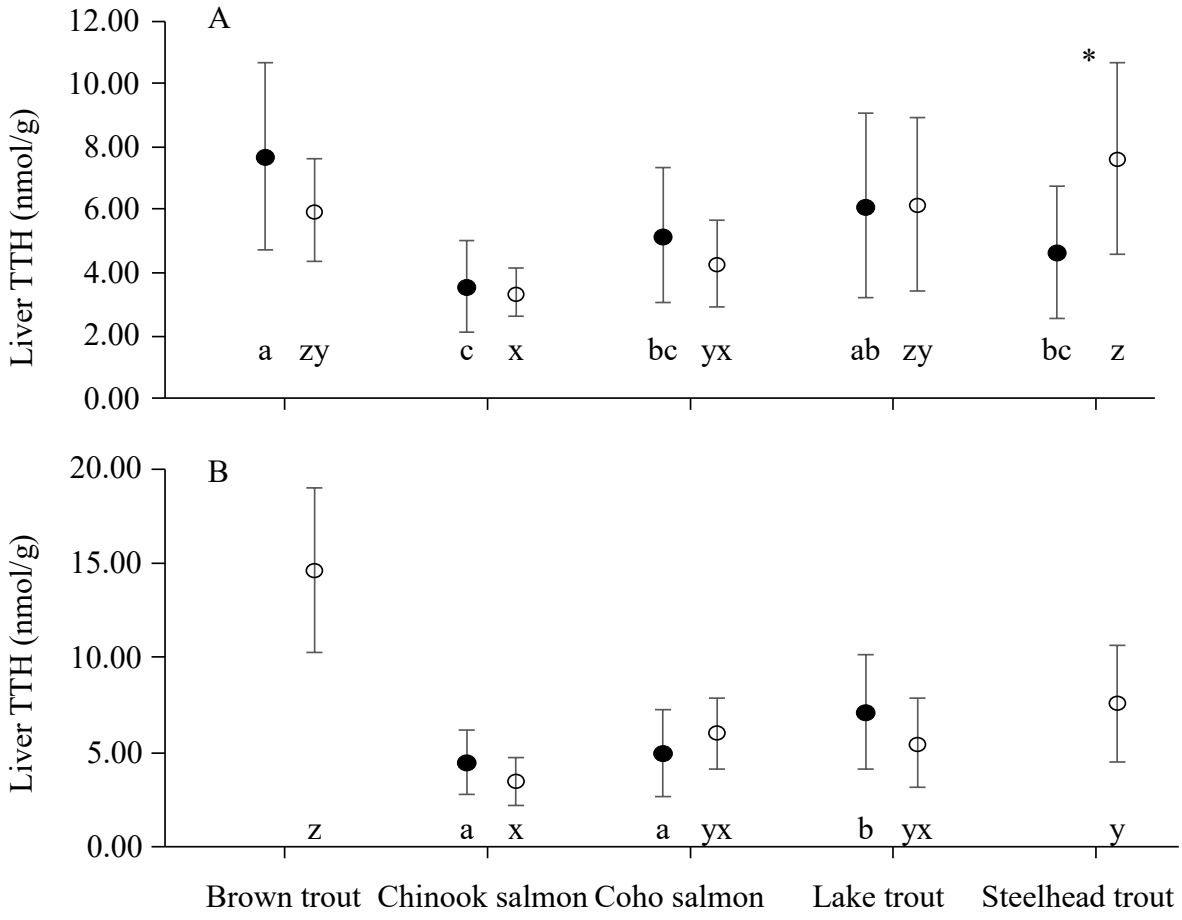

Figure 2. Average liver total thiamine (TTH) concentrations with standard deviation for female (A) and male (B) salmonines for years 2015 (●) and 2016 (○). Significant differences ($p < 0.05$) among species within years are denoted by different letters (2015: a, b, and c; 2016: z, y, and x) and significant differences between years within species are denoted by an asterisk (*).

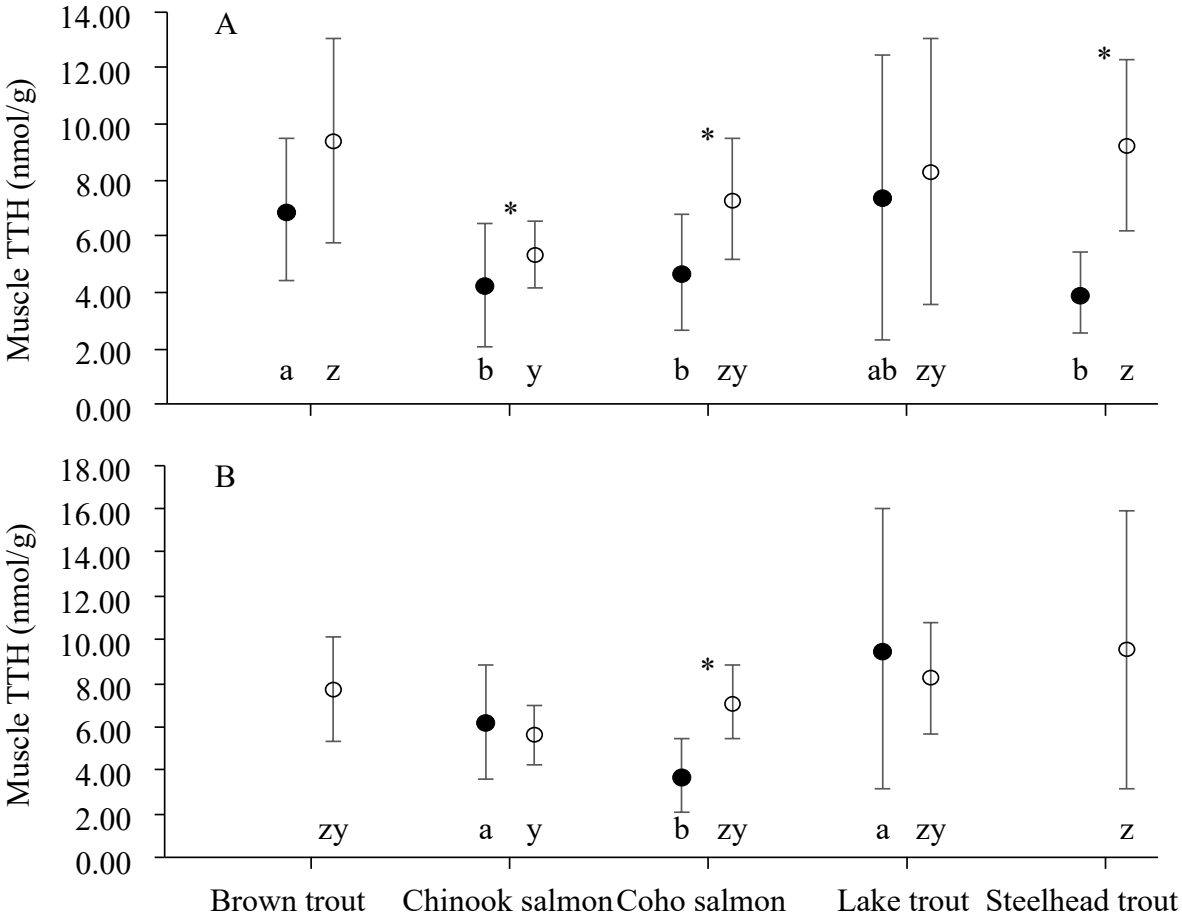

Figure 3. Average muscle total thiamine (TTH) concentrations with standard deviation for years 2015 (●) and 2016 (○). Significant differences ($p < 0.05$) among species within years are denoted by different letters (2015: a and b; 2016: z and y) and significant differences between years within species are denoted by an asterisk (*).

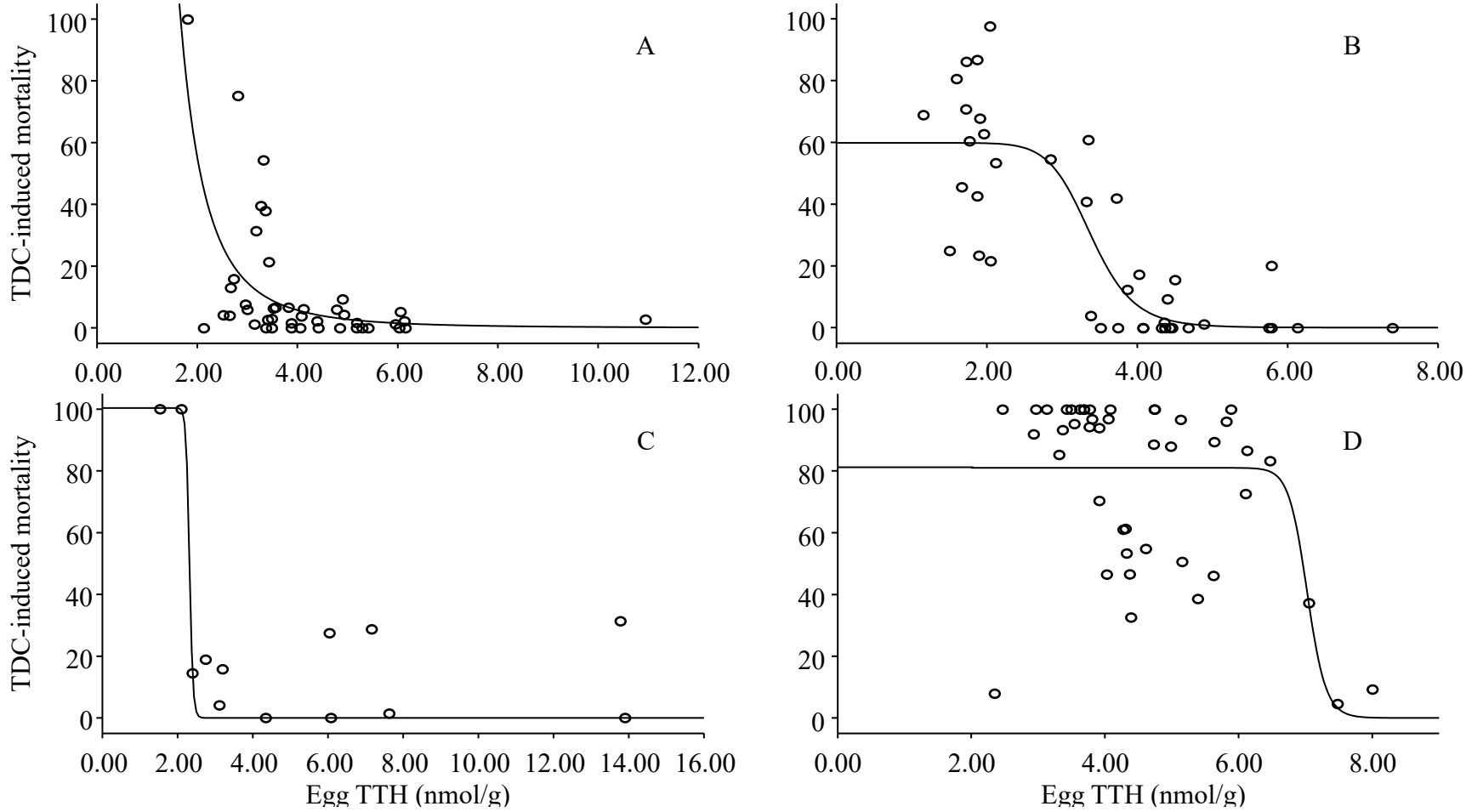

Figure 4. Three-parameter logistic correlations between egg total thiamine (TTH) and TDC-induced offspring mortality for Chinook salmon (A), coho salmon (B), lake trout (C), and steelhead trout (D).

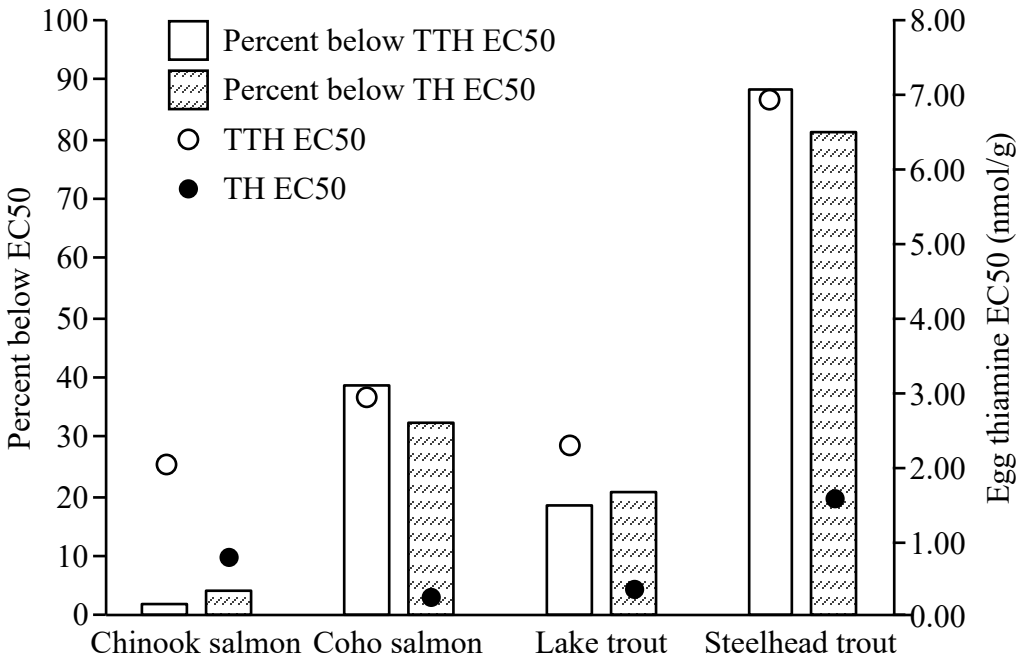

Figure 5. Percentage of individuals with egg thiamine concentrations below the calculated total thiamine (TTH) and free thiamine (TH) EC50s.

Chapter 2: Relationships between dietary components and thiamine concentrations of Lake Ontario salmonines

Abstract

Thiamine Deficiency Complex (TDC) is an unsolved problem that limits offspring survival of various salmonine species in the Laurentian Great Lakes and other aquatic systems. High fat content of prey has been hypothesized as a cause of the deficiency; however, no direct relationship has been demonstrated. I demonstrate that alewife (*Alosa pseudoharengus*) have higher lipid content (%) than other abundant prey in Lake Ontario and that abundance of alewife in the diets of five salmonine species (brown trout *Salmo trutta*, Chinook salmon *Oncorhynchus tshawytscha*, coho salmon *O. kisutch*, lake trout *Salvelinus namaycush*, and steelhead trout *O. mykiss*), determined by fatty acid signature analysis, correlates positively with the extent of TDC in the predators. In addition, the proportions of polyunsaturated fatty acids (PUFAs) in belly flap tissue had negative correlations with egg and/or muscle thiamine concentrations (nmol/g) for the iteroparous salmonine species studied. As PUFAs have the greatest potential for lipid peroxidation, it is possible that oxidative stress rates were elevated in these species, resulting in increased use of thiamine as an antioxidant and thereby reducing thiamine reserves. While these correlations were notable, they accounted for less than 50% of the variability in salmonine thiamine concentrations, suggesting that fat content alone is not the only cause of TDC.

Introduction

Salmonine species in the Great Lakes basin have suffered from a thiamine (vitamin B_1) deficiency, known as Thiamine Deficiency Complex (TDC), since the 1960s (Marcquenski and Brown, 1997) and potentially even earlier (Ketola et al., 1999). Similarly, salmonines in the Baltic Sea have been affected by thiamine deficiency, referred to as M74, which was first observed in the mid-1970s (Bengtsson et al., 1999). Adults that are thiamine deficient show abnormal behaviors (e.g., sluggish and wiggling movements, lethargy, and distress) and may experience mortality with extreme deficiencies (Brown et al., 2005a; Futia et al., 2017). Females exhibiting these symptoms have low thiamine reserves for egg tissue, resulting in thiamine deficient alevins. Deficient offspring frequently experience neurological disorders (e.g., loss of equilibrium, hyperexcitability, and spiral swimming), which can decrease foraging efficiency and increase vulnerability to predation (Fitzsimons et al., 2009a; Ivan et al., 2018). Direct alevin mortality may occur as well, causing up to 100% of offspring to die (Brown et al., 2005a; Futia et al., 2017).

In the Great Lakes basin, TDC has been associated with elevated consumption of alewife (*Alosa pseudoharengus*), despite thiamine concentrations of alewife being comparable to other species that do not induce the deficiency (Tillitt et al., 2005). Honeyfield et al. (2005) showed that feeding on alewife for a minimum of two years induced thiamine deficiencies in lake trout (*Salvelinus namaycush*) eggs and offspring. In addition, in both Lakes Michigan and Huron, thiamine concentrations in lake trout eggs increased following declines in alewife populations, likely due to shifts in lake

trout diet (Fitzsimons et al., 2010; Riley et al., 2011). Incorporation of round goby (*Neogobius melanostomus*) in lake trout diet has been suggested to ameliorate TDC by limiting thiamine reductions (Fitzsimons et al., 2009b). Why an alewife-dominant diet results in reduced thiamine concentrations remains unknown, but two hypotheses have been proposed. In the Great Lakes, multiple non-native prey species including alewife and rainbow smelt (*Osmerus mordax*) have been shown to have high activities of the enzyme thiaminase, which is capable of degrading thiamine (Honeyfield et al., 2002; Tillitt et al., 2005). Therefore, high consumption of thiaminase-rich prey may result in thiamine depletion. In the Baltic Sea region, an alternative hypothesis has been proposed to explain the occurrence of M74, suggesting the deficiency is caused by insufficient thiamine concentrations in prey species in proportion to their fat content (Keinänen et al., 2012). Because thiamine is required for energy metabolism and the synthesis of fatty acids (Boxer and Stetten, 1944; Harder et al., 2018), the amount required may be partially dependent on the amount of lipids consumed. In addition, fats are susceptible to lipid peroxidation, which has been related to declines in thiamine concentrations due to the antioxidant potential of the vitamin (Lukienko et al., 2000).

Because TDC is related to diet, determining prey-predator interactions may provide valuable information explaining observed differences in thiamine concentrations. Fatty acid signature (FAS) analysis has been used successfully to understand the diet of aquatic animals (Napolitano, 1999; Budge et al., 2002; Iverson et al., 2009). Fatty acids with a carbon chain longer than 14 remain in their basic form during digestion and if they are not initially used for energy, they are stored in adipose

tissue of predators with little modifications (Budge et al., 2002; Iverson et al., 2004). Adipose rich belly flap tissue of predator species has been shown to provide the most accurate FAS for predicting diet (Budge et al., 2011). Studying FASs can also provide insight about the effects of fatty acid concentrations on thiamine concentrations. Different types of fatty acids have varying degrees of unsaturation (i.e., presence of double bonds), which changes their susceptibility to lipid peroxidation. Potential for lipid peroxidation is greatest in unsaturated fatty acid (monounsaturated fatty acids, MUFAs, and polyunsaturated fatty acids, PUFAs), which contain double bonds, whereas saturated fatty acids (SAFAs) have no double bonds and low susceptibility to peroxidation. PUFAs contain two or more double bonds in their carbon chain making them particularly vulnerable to prooxidants (Sargent et al., 2002). Fish generally have high concentrations of PUFAs relative to other organisms, with the greatest requirements during early development stages and breeding periods (Tocher, 2010). As a result, proper maintenance of the concentrations of fatty acids is critical for proper growth, development, and reproduction. Multiple studies have evaluated correlations between fatty acid and thiamine concentrations (Pickova et al., 1998; Brown et al., 2005b; Honeyfield et al., 2008; Czesny et al., 2009, 2012; Keinänen et al., 2018). Overall, it appears that PUFAs, particularly docosahexaenoic acid (22:6n-3), may have a negative relationship with free thiamine (TH) concentrations (Pickova et al., 1998; Czesny et al., 2009, 2012; Keinänen et al., 2017), although this finding is not consistent across all studies. Therefore, additional research is required to confirm the influence of these fatty acids on thiamine.

In Lake Ontario, the extent of TDC was recently determined for brown trout (*Salmo trutta*), Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), lake trout, and steelhead trout (*O. mykiss*); Atlantic salmon (*Salmo salar*) were not included in the study. Results of the study showed that all species, except brown trout, were suffering from TDC (Futia and Rinchard, submitted). In the current study, my objectives were to determine the diet for the same five salmonine species through FAS analyses. In addition, I evaluated the hypothesis that higher prey fat content results in lower thiamine concentrations. To test this hypothesis, I compared lipid content of prey and predators, and in predators I determined correlations between predators' thiamine concentrations and the lipid content of various tissues. As the presence of TDC can be predicted based on egg free thiamine, egg total thiamine (TTH), and muscle TTH (Futia and Rinchard, submitted), I used these thiamine concentrations for comparisons with fatty acid profiles and lipid content to determine potential associations with TDC.

Methods

Fish Collection

Five salmonine species including brown trout, lake trout, steelhead trout, Chinook salmon, and coho salmon were collected during their respective spawning periods at various locations throughout Lake Ontario and its tributaries between spring 2015 and fall 2017. Brown trout were captured in Sandy Creek (Hamlin, NY) by backpack electrofishing (Halltech HT-2000 backpack electroshockers). Chinook

salmon, coho salmon, and steelhead trout were collected at the New York State Department of Environmental Conservation (NYSDEC) Salmon River Fish Hatchery (Altmar, NY) during their annual egg take. Additional Chinook and coho salmon were captured in the fall at the Salmon River (Pulaski, NY) through backpack electrofishing. Lastly, lake trout were collected from Lake Ontario (Hamlin and Oswego, NY) at spawning time using monofilament gillnets with approximately 24-h soak times. Belly flap and muscle samples were taken from all salmonines captured during 2015. In addition, egg samples were collected from all mature females throughout the sampling period. Following collection, tissue samples were held on ice and transported to The College at Brockport – State University of New York (hereafter called Brockport) for storage at -80°C until processing. Three prey fishes (alewife, rainbow smelt, and round goby) were collected by the United States Geological Survey – Lake Ontario Biological Station (USGS-LOBS), R/V Kaho, and NYSDEC, R/V Seth Green, outside of Rochester, NY during spring and fall of 2015 and 2016 using 3N1 bottom trawls at depths varying from 15 to 185 m (Weidel et al., 2017). Directly after collection, whole fish were put on ice for transport to Brockport, where they were stored at -80°C until processing.

Thiamine Extraction

Thiamine concentrations from this study are taken from Futia and Rinchard (submitted). Thiamine was extracted from all egg and muscle samples in duplicate following the procedure of Brown et al. (1998). One g of egg tissue and 2 g of muscle tissue were used for each replicate. Samples were homogenized in a 2% trichloroacetic

acid solution. After extraction, the concentrations of three thiamine vitamers (i.e., TH, thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP)) were quantified using high performance liquid chromatography (Agilent Technologies 1200 series) as described by Futia et al. (2017), in which peak areas were calculated based on a standard curve. Vitamer concentrations were calculated as nmol/g and combined to represent the TTH concentration.

Lipid Extraction and Fatty Acid Analysis

Total lipids were determined gravimetrically for all salmonine and prey fish samples following the methods of Folch et al. (1957). For salmonines, 1 g of belly flap and egg tissue (for females only) as well as 2 g of muscle tissue were used. Belly flap samples were skinned, then ground to make homogenized parts. For prey fishes, samples of whole-body tissue were used; individuals were ground with a blender to homogenize body contents, and 1 g samples were collected. Lipids were extracted from each sample in 2:1 chloroform/methanol solvent containing 0.01% butylated hydroxytoluene, used as an antioxidant (Folch et al., 1957).

Following lipid extraction, belly flap, egg, and prey fish samples were prepared for fatty acid analysis. A known amount of nonadecanoate acid (19:0, Nu-Check Prep Inc., Elysian, MN) was added to each sample based on the amount of total lipids present (8 mg/50 mg of lipids) to serve as an internal standard. Samples were then transmethylated to produce fatty acid methyl esters (FAMEs) following Metcalfe and Schmitz (1961). Fatty acids profiles were determined using an Agilent Technologies 7890A gas chromatograph (GC) system with Agilent Technologies 7693 autosampler

and Agilent Technologies 5975C inert XL EI/CI mass selective detector with triple-axis detector (Agilent Technologies, Inc., Santa Clara, CA). The column used was an Agilent J&W GC column with 30 m × 0.250 mm × 0.50 µm thickness and helium was used as the carrier gas. Run conditions were set following Happel et al. (2017). Individual fatty acid methyl esters (FAMEs) were identified by comparing their retention times to those of authentic standard mixtures (FAME mix 37 components, Supelco) with known spectrographic patterns of FAMEs. The number of FAMEs identified in each type of sample (i.e., belly flap tissue, egg tissue, and whole-body prey) was dependent on the detectability of each FAME, which differed among sample types. Therefore, 26, 25, and 24 FAMES were identified in egg, belly flap and prey fish samples, respectively. For statistical tests among different types of samples, only FAMEs detected in all samples were used. The area of each FAME was calculated in proportion to that of the internal standard and was reported as a percentage of the total value of all FAMEs. Fatty acids are henceforth written in International Union of Pure and Applied Chemistry (IUPAC) nomenclature – carbon chain length:number of double bonds and the position of the first double bond (n-x) relative to the terminal methyl group.

Statistical Analyses

Data collected during this study were assessed using Microsoft Excel v16.0, IBM SPSS 17.0 Statistics Version 25, and PRIMER v.6. For univariate statistics, data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. For normal data, comparison among groups were made

with analysis of variance (ANOVA) followed by Tamahane's post-hoc test. Non-parametric data were analyzed using non-parametric alternatives including Mann-Whitney U for between sample comparisons and Kruskal-Wallis with Dunn's post hoc test for comparisons among multiple groups. In addition, correlations between quantitative data were assessed with Spearman's rank correlation (rho, ρ) to evaluate the strength of linear regressions.

Fatty acid signatures were compared using multivariate analyses. Analysis of similarity (ANOSIM) was conducted to determine the relative strength of the dissimilarity among multiple groups (Global R) as well as the strength of pairwise comparisons (R). Values of R between 0.25 and 0.5 were considered different with some overlap, 0.5 to 0.75 as different, and 0.75 to 1.0 as highly different. The proportions of individual fatty acids were compared among samples using similarity percentage analysis (SIMPER) to determine the overall percent dissimilarity among samples as well as to identify the contribution of each fatty acid on the dissimilarity. Additionally, Principal component analyses (PCA) were also conducted to reduce the number of variables by generating principal components (PC). The PCs accounting for over 60% of the variability of the original data cumulatively were included. The analysis provided visuals of the separation among species and identified the fatty acids driving the separations among them. The two principal components incorporating the greatest amount of the original variation were included in the analyses, and the loading values for fatty acids with the strongest correlations to those components are provided. Lastly, fatty acid proportions were used to create Bray-Curtis similarity matrices for

additional analyses. Using these values, fatty acid signatures were plotted using non-metric multidimensional scaling (nMDS) to display dissimilarity among samples. For all statistical analyses, a significance level of $\alpha = 0.05$ was used.

Results

Variation in salmonine fatty acid composition

Belly flap FASs from the five salmonine species separated well based on the proportions of 24 fatty acids (Table 1; ANOSIM, Global $R = 0.55$, $p < 0.001$). This separation was well represented by PCA analysis with the first two components accounting for over 80% of the variation (PC1 = 70.5%, PC2 = 12.5%). The first principal component was mainly driven by 16:1n-7 (-0.28), 18:1n-9 (-0.61), and 22:6n-3 (0.70), while the second was driven by 16:1n-7 (-0.70), 18:0 (0.35), and 18:1n-9 (0.44) (Figure 1; PCA). Slight differences were observed in the FAS between sampling years for coho salmon, lake trout, and steelhead trout (ANOSIM, coho salmon: $R = 0.26$, $p < 0.001$, lake trout: $R = 0.29$, $p < 0.001$, steelhead trout: $R = 0.28$, $p < 0.001$), although these differences were insignificant compared to the differences observed among salmonine species. In addition, differences occurred within semelparous species (i.e., Chinook and coho salmon) based on sex, while no differences occurred for iteroparous species (Figure 2; ANOSIM, Chinook salmon: $R = 0.42$, $p < 0.001$, coho salmon: $R = 0.35$, $p < 0.001$). Female Chinook and coho salmon had greater proportions of 22:6n-3 than males, while males had a greater proportion of 18:1n-9 than females

(Table 2; SIMPER). In addition, FASs among semelparous females were less similar than the FASs of the males (Table 2; SIMPER).

Proportions of belly flap fatty acids in females based on degrees of unsaturation (i.e., SAFAs, MUFAs, and PUFAs) varied among species (Figure 3). The proportions of SAFAs had some significant differences (Kruskal-Wallis, $H = 116.6$, $p < 0.001$) among species, although all proportions were between 20 and 25%, meaning there was not likely any biological significance to these differences. MUFAs and PUFAs had large differences in their relative importance among species; MUFAs had significantly greater contributions in brown and lake trout than the other salmonines, and PUFAs had the greatest contributions in Chinook salmon, coho salmon, and steelhead trout (Kruskal-Wallis, MUFAs: $H = 148.5$, $p < 0.001$, PUFAs: $H = 119.0$, $p < 0.001$). Lastly, the proportions of SAFAs, MUFAs, and PUFAs remained relatively consistent across varying lipid content (%) for each species.

Egg FASs also had strong separation among the five salmonine species based on the proportions of 26 fatty acids (Tables 3 and 4; ANOSIM, Global $R = 0.75$, $p < 0.001$). Using PCA analysis, over 70% of the variability was explained with the first two components (PC1 = 49.2%, PC2 = 21.9%). PC1 was mainly driven by 16:1n-7 (0.32), 18:1n-9 (0.60), and 20:5n-3 (0.47), while PC2 was mainly driven by 16:0 (0.74), 18:0 (0.43), and 22:6n-3 (-0.35) (Figure 4; PCA). Pairwise comparisons among species showed that brown trout and Chinook salmon had the greatest dissimilarity (SIMPER, 15.25%), while coho salmon and steelhead trout had the least (SIMPER, 6.70%). Annual changes in egg FASs occurred as well but were limited to Chinook and coho

salmon (ANOSIM, Chinook salmon: $R = 0.36$, $p < 0.001$, coho salmon: $R = 0.84$, $p < 0.001$). These variations were mainly driven by a decrease in the proportion of 22:6n-3 from 2015 to 2016. However, the average dissimilarity of the FASs between years was low for both species compared to differences among species (SIMPER, Chinook salmon: 5.93%, coho salmon: 5.80%). Despite these differences, eggs from all species generally had the same fatty acids accounting for the majority (>50%) of their FAS. For coho salmon, lake trout, and steelhead trout, the same four fatty acids accounted for the majority of the FAS and had the same ranking (high to low): 22:6n-3, 18:1n-9, 16:0 and 20:5n-3 (Table 3). Chinook salmon had the same four fatty acids comprising the majority of their egg FAS, but 18:1n-9 was more abundant than 22:6n-3, similar to brown trout. However, 20:5n-3 had a much lower contribution for brown trout, while 18:1n-7 was more important based on SIMPER analysis when compared to the other species (Table 3).

Prey fatty acid composition

Whole-body FASs of alewife, rainbow smelt, and round goby had strong separation based on the proportions of 24 fatty acids (Table 5; ANOSIM, Global $R = 0.55$, $p < 0.001$), which was well represented by PCA analysis. Using two principal components, over 65% of the variability among samples was explained (PC1 = 46.9%, PC2 = 20.2%) with the fatty acids 18:1n-9 (-0.48), 18:3n-3 (-0.23) and 22:6n-3 (0.74) driving PC1 and 16:0 (0.35), 16:1n-7 (-0.84), and 22:6n-3 (0.28) driving PC2 (Figure 5A; PCA). Within species, there were seasonal and annual variations in the FAS (Table 5). Alewife and round goby had similar variation with differences across seasons

(spring and fall) for both years (ANOSIM, alewife 2015: $R = 0.75$, $p < 0.001$, alewife 2016: $R = 0.63$, $p < 0.001$, round goby 2015: $R = 0.62$, $p < 0.001$, round goby 2016: $R = 0.70$, $p < 0.001$), as well as differences across years within seasons (ANOSIM, alewife spring: $R = 0.85$, $p < 0.001$, alewife fall: $R = 0.75$, $p < 0.001$, round goby spring: $R = 0.83$, $p < 0.001$, round goby fall: $R = 0.59$, $p < 0.001$). Rainbow smelt had seasonal variation as well within both years (ANOSIM, 2015: $R = 0.48$, $p < 0.001$, 2016: $R = 0.89$, $p < 0.001$), but annual variation only occurred across samples collected during the fall (ANOSIM, $R = 0.82$, $p < 0.001$). Although these variations were present, differences among species were greater, allowing all individuals within species to be pooled for among-species comparisons.

Dietary interactions between prey and salmonines

When comparing the FASs among all prey and salmonine belly flap (excluding semelparous females due to variability unrelated to diet discussed later), dissimilarity among species remained high (Figure 6; ANOSIM, Global $R = 0.72$, $p < 0.001$). Therefore, predator diet could be classified based on correlations with prey fatty acids. Three fatty acids (16:1n-7, 18:1n-9, and 22:6n-3) most frequently had large contributions towards the dissimilarity among all salmonines and prey (Table 6; SIMPER). In addition, 16:1n-7 and 18:1n-9 were strong factors accounting for dissimilarity among both the prey and salmonines (16:1n-7: PC1 = -0.35, PC2 = 0.74; 18:1n-9: PC1 = -0.71, PC2 = -0.38) FASs (Figures 5 A and B; PCA). These two fatty acids also had the greatest contribution to the dissimilarity between alewife and round goby FASs (SIMPER), with alewife having a greater proportion of 18:1n-9 (alewife:

18.0%, round goby: 13.3%) and round goby having more 16:1n-7 (alewife: 3.4%, round goby: 9.6%). Therefore, a ratio of these two fatty acids (18:1n-9/16:1n-7) was compared among the three prey species. All species were significantly different from each other, with the ratio being highest for alewife and lowest for round goby (Figure 7; Kruskal-Wallis, $H = 214.1$, $p < 0.001$; Dunn's post hoc, $p < 0.001$). The ratio was then determined for salmonine belly flap to compare the influence of alewife and round goby in their diets. Chinook and coho salmon had the greatest ratio, suggesting they have the greatest proportions of alewife in their diet. Steelhead had the next highest proportion but was only significantly lower than Chinook salmon (Kruskal-Wallis, $H = 198.3$, $p < 0.001$; Dunn's post hoc, $z = 54.90$, $p = 0.01$). Both brown trout and lake trout had ratios that were significantly lower than the other three species (Dunn's post hoc, $p < 0.001$), indicating a lower proportion of alewife in their diet.

Lipid content of salmonine and prey species

The percent lipid content of belly flap, egg, and muscle tissue for females varied across the five species of salmonines studied (Figure 8; Kruskal-Wallis, belly flap: $H = 144.5$, $p < 0.001$, egg: $H = 142.3$, $p < 0.001$, muscle: $H = 114.7$, $p < 0.001$). Interspecific variation in lipid content was similar for belly flap and muscle tissue, being highest in lake and brown trout and significantly lower in Chinook and coho salmon (Dunn's post hoc, $p < 0.015$). In contrast, egg lipid content was highest in Chinook and coho salmon, while brown and lake trout had significantly lower egg lipid content (Dunn's post hoc, $p < 0.001$). Steelhead trout had intermediate lipid content for all tissues. Lastly, belly flap and muscle lipid content had significant positive

correlations for all species except steelhead trout, while egg lipid content had non-significant correlations with muscle lipid content for all species as well as belly flap lipid content for all species, except Chinook salmon (Table 7; Spearman's rank correlation). Intraspecific variation in tissue lipid content across years was limited for each species (Table 8). Brown trout had the most variation for belly flap and muscle tissue with significant decreases in lipid content between 2015 and 2016 (Mann-Whitney U, belly flap: $U = 59.0$, $p = 0.031$; muscle: $U = 47.0$, $p = 0.007$). Lake trout belly flap lipid content also changed significantly but increased from 2015 to 2016 contrary to the brown trout (Mann-Whitney U, $U = 425.0$, $p = 0.001$). Chinook salmon, coho salmon, and steelhead trout had non-significant differences in lipid content for all three types of tissue across years.

Lipid content varied among prey species (Figure 9; Kruskal-Wallis, $H = 148.6$, $p < 0.001$), being greatest in alewife (11.0 ± 3.7%) and significantly lower (Dunn's post hoc, $p < 0.001$) in both rainbow smelt (4.7 ± 1.3%) and round goby (4.0 ± 1.8%), which did not differ from each other. Within species, lipid content varied temporally from spring 2015 through fall 2016 (Figure 9; ANOVA). For alewife, lipid content tended to be higher in the fall than the spring and also had a general increase from spring 2015 through fall 2016. Similarly, rainbow smelt lipid content tended to be greater in the fall than the spring, although it remained fairly consistent across years. Round goby had significantly lower lipid content in the fall than the spring for both years (ANOVA, $F = 47.6$; Tamahane's post hoc, $p < 0.001$) and a decrease in lipid content across years.

Comparisons between lipid content and thiamine concentrations

TDC is mainly observed in offspring because of insufficient maternal reserves, so thiamine concentrations were only compared to female lipid content. Correlations between tissue (belly flap, egg, and muscle) lipid content and thiamine concentrations (egg TH, egg TTH, and muscle TTH) were generally low for all species except brown trout (Table 9). Brown trout had significant correlations between all thiamine concentrations and lipid content of belly flap as well as muscle tissue. In addition, these correlations were all negative (i.e., higher lipid content was correlated with lower thiamine concentrations). Both Chinook salmon and steelhead trout had significant correlations with egg lipid content and thiamine concentrations: egg TH and egg TTH for Chinook salmon and egg TH and muscle TTH for steelhead trout. These correlations were positive.

Comparisons between fatty acid composition and thiamine concentrations

There were multiple significant correlations between the proportions of belly flap SAFAs, MUFAs, and PUFAs and egg and muscle thiamine concentrations; however, most of these correlations were for brown and lake trout (Table 10; Spearman's rank correlation). PUFAs had the greatest number of notable correlations with thiamine concentrations, including negative correlations with all thiamine concentrations for brown and lake trout. Additionally, coho salmon and steelhead trout had positive correlations between PUFAs and muscle TTH that were significant. As for PUFAs, brown and lake trout had significant correlations between MUFAs and all thiamine concentrations; however, these correlations were positive. There was only one

significant correlation with SAFAs, which was with brown trout egg TTH and was negatively correlated.

Correlations between the proportions of SAFAs, MUFAs, and PUFAs in egg tissue and thiamine concentrations differed among the five salmonine species as well but were generally weak (Table 11). Egg SAFAs had the most significant correlations with thiamine concentrations, which were all negative. These included all thiamine concentrations for coho salmon as well as egg TH and egg TTH for steelhead trout. There were three significant, positive correlations between egg MUFAs and thiamine concentrations, including brown trout egg TH and egg TTH as well as lake trout egg TTH. Lastly, there were three significant correlations between egg PUFAs and thiamine concentrations, including negative correlations with brown trout egg TH and egg TTH, and a positive correlation with coho salmon muscle TTH.

Discussion

Salmonine diet and relationships with thiamine concentrations

For dietary analyses including belly flap FASs, semelparous females were excluded due to high variability that is unlikely related to diet. Many studies have demonstrated that Chinook and coho salmon have diets consistently comprised of alewife (Brandt, 1986; Yuille et al., 2015; Happel et al., 2017; Mumby et al., 2018). In addition, male Chinook and coho salmon FASs had less variability than the females. Therefore, it is unlikely for females to have high FAS variability caused by differences in diet among individuals. Instead, it is possible that females of

semelparous species have elevated biosynthesis of 22:6n-3 from essential fatty acid precursors (Tocher, 2003) and/or preferential conservation of 22:6n-3 during pre-spawning degeneration.

Based on the belly flap FASs of the five salmonine species (excluding semelparous females) and three prey species studied, dietary preferences differed among the salmonines. The differences were mainly due to variations among the proportions of 16:1n-7 and 18:1n-9, which have been used in freshwater and marine systems as markers of benthic and pelagic origins, respectively (Kelly and Scheibling, 2012; Croizier et al., 2016; Happel et al., 2017). In Lake Ontario, alewife (pelagic species) and round goby (benthic species) have been shown to account for the majority of salmonine diet (Colborne et al., 2016; Happel et al., 2017; Mumby et al., 2018). Therefore, the fatty acids of these two species likely account for the majority of the differences observed in the salmonine FASs. In the present study, alewife had the highest proportion of 18:1n-9 among the three prey species, suggesting the use of this fatty acid as a marker for alewife consumption, while 16:1n-7 was highly abundant in round goby relative to the other prey fish, indicating it is likely a good index of round goby abundance in diet. Happel et al. (2017) had the same results and used a ratio of these two fatty acids to differentiate the inclusion of alewife and round goby in diets of various predators. Here, I compared the same ratio among salmonine species and found Chinook salmon predominantly fed on alewife compared to the other salmonines, while coho salmon and steelhead trout incorporated a large amount of alewife in their diets, but not as exclusively as Chinook salmon. In contrast, lake trout and brown trout have

more variable diets, likely incorporating higher proportions of round goby as shown in recent studies for Lake Ontario (Colborne et al., 2016; Happel et al., 2017; Mumby et al., 2018).

Combined with the thiamine concentrations reported by Futia and Rinchard (submitted), these dietary results provide further evidence that increased consumption of alewife results in lowered thiamine concentrations, with thiamine concentrations being lower for species with a higher ratio of 18:1n-9 to 16:1n-7. However, due to differing thiamine requirements among species (Brown et al., 2005b; Fitzsimons et al., 2007; Futia and Rinchard, submitted), the extent of TDC would vary among species with similar ratios. Interestingly, brown trout did not experience any thiamine deficiency despite having a ratio greater than lake trout, which do have reduced egg and muscle thiamine concentrations. The elevated ratio for brown trout is due to a high proportion of 18:1n-9 in their belly flap. Brown trout have been shown to feed on a high proportion of alewife (Mumby et al., 2018); however, their high thiamine concentrations and 18:1n-9 to 16:1n-7 ratio suggest that brown trout may have other sources of 18:1n-9 such as *de novo* biosynthesis (Nelson, 1992) or different prey sources. Mumby et al. (2018) found brown trout also had the highest proportion of round goby in their diets based on stable isotope analysis and suggested that they may be feeding on other nearshore prey as well. Alternatively, brown trout may have differing biochemical properties (e.g., lipid content or lipid peroxidation potential) that allow thiamine concentrations to remain high when feeding on alewife. Lastly, the higher concentrations of thiamine observed in brown trout may be due to a limited

perspective of diet provided by FAS analyses. These analyses give insight to the diet of an organism based on what it was feeding on in the previous weeks to months (Budge et al., 2006). Therefore, if individuals were feeding on alternative prey (i.e., round goby) much earlier in the year (i.e., winter or spring), their thiamine concentrations may be elevated and remain sufficient despite shifts in their diet to mainly feeding on alewife since inducing a thiamine deficiency for thiamine replete individuals can take up to two years (Honeyfield et al., 2005). Such variation in long-term diet may explain observed intraspecific differences in thiamine concentrations for other species (i.e., lake trout) as well.

Impact of lipid content and fatty acid composition on thiamine concentrations

The significant correlations between salmonine tissue lipid content and thiamine concentrations had few similarities across species and tissues. All significant correlations for belly flap and muscle tissue were negative and only occurred for brown trout, while positive correlations occurred between egg lipid content and thiamine concentrations of Chinook salmon and steelhead trout. However, the correlations with egg lipid content were inconsistent, with only egg TH having a significant correlation for both species. The results for the brown trout are similar to those observed by Keinänen et al. (2012) for Atlantic salmon. In their study, thiamine deficient Atlantic salmon from the Baltic Sea were associated with diets comprised of prey fish with greater fat content. Thus, brown trout feeding on prey with higher lipid content, such as alewife, would be expected to have the greatest lipid content and, therefore, have declines in their thiamine concentrations. However, these declines were not large

enough to induce TDC in the fish studied. In addition, the relatively low amount of variability in thiamine concentrations accounted for by correlations with lipid content suggests that other factors may be causing inconsistencies and having an influence on thiamine concentrations.

One potential explanation for decreased thiamine concentrations with increased lipid content in brown trout is the increased requirement of thiamine with greater lipid metabolism. TPP acts as a cofactor during lipid metabolism (Harder et al., 2018); therefore, increased lipid content resulting in greater rates of lipid metabolism may cause thiamine to be depleted. A second possible cause of limited thiamine concentrations with increased lipid content is the greater potential for lipid peroxidation, particularly when lipids are mainly composed of PUFAs. PUFAs are particularly sensitive to oxidative stress caused by the reaction between free radicals and unsaturated fatty acids. In addition, this process generates peroxides that react with additional unsaturated fatty acids. This sequence may go through multiple cycles before termination or until a reaction with an antioxidant (Hsieh and Kinsella, 1989). Free thiamine has been shown to act as an antioxidant by reacting with free radicals to inhibit lipid peroxidation (Lukienko et al., 2000; Gibson and Zhang, 2001); therefore, fish experiencing oxidative stress may have a greater need for TH. As a result, thiamine reserves may be reduced. Shangari et al. (2005) found a similar relationship for rats demonstrating thiamine deficiency induced by oxidative stress. Studies have also linked thiamine deficiencies with oxidative stress, demonstrating multiple ways that oxidative stress can impact the deficiency (Hazell et al., 2013). Lastly, oxidative stress

may become even more influential when fish spawn, as oxidative stress has been shown to increase while enzymatic activities of certain antioxidants decrease during the starvation period prior to spawning (Furné et al., 2009).

As previously mentioned, the potential for lipid peroxidation is greatest for PUFAs (Hsieh and Kinsella, 1989). Therefore, increases in these fatty acids would result in reduced thiamine concentrations, which occurred to some extent with increases in belly flap and egg PUFA concentrations of brown trout and lake trout. Conversely, the positive correlations between MUFAs and thiamine concentrations in brown and lake trout may be due to the limited peroxidation potential of these fatty acids. Still, none of these correlations account for the majority of the variation in thiamine concentrations observed. In addition, there were conflicting results for coho salmon and steelhead trout, which had positive correlations between muscle TTH and PUFAs, although these correlations were weak as well.

It is important to note that correlations with belly flap and muscle tissue may have been limited, particularly in semelparous species, due to the deterioration of fat reserves associated with pre-spawning starvation in the fall. As a result, lipid content and fatty acid concentrations would lower overall. Lipid content has also been shown to influence fatty acid concentrations directly; higher total lipids through consumption of more fatty prey results in elevated concentrations of PUFAs, regardless of the specific diet (Kainz et al., 2017). Thus, feeding on prey with the highest lipid content (i.e., alewife) would likely increase concentrations of PUFAs in the predator, potentially leading to the lower thiamine concentrations observed. Through controlled

feeding experiments, such relationships could be evaluated. Interestingly, the average proportions of PUFAs for each species correspond well with the thiamine concentrations observed in these salmonines from Futia and Rinchard (submitted), with the lowest thiamine concentrations being in species that had the highest proportions of PUFAs. These results are contradictory to findings by Keinänen et al. (2018), in which muscle PUFA concentrations were significantly lower in years when M74 was present compared to years when it was not present.

Despite the aforementioned correlations, no brown trout experienced thiamine deficiencies equivalent to the other species (Futia and Rinchard, submitted). In addition, thiamine concentrations were low regardless of the lipid content for all salmonine species other than brown trout. Furthermore, while greater lipid content increases the potential for thiamine deficiency, the muscle lipid content of the salmonines from this study were comparable to those of Lake Superior where thiamine deficiency does not occur (Neff et al., 2012). Therefore, it is unlikely that lipid content alone causes thiamine deficiencies, and other factors account for the majority of the deficiency. However, it is important to note that my recorded lipid content may low due to pre-spawning starvation (Madenjian et al., 2000), thereby limiting the correlations with thiamine concentrations.

With regard to PUFA concentrations, variability has been observed among the Great Lakes, with concentrations of muscle PUFAs in lake trout being greatest in Lakes Michigan and Ontario, where thiamine deficiencies are most prominent (Brown et al., 2005c), and much lower in Lake Superior (Pantazopoulos et al., 2013). Still, increased

concentrations of PUFAs may not correlate perfectly with increased lipid peroxidation and thus greater requirements for antioxidants such as free thiamine, as increased numbers of reactive oxygen species may be needed as well. However, it is possible that anthropogenic and environmental factors that induced oxidative stress may be increasing, which could lead to greater peroxidation. Examples of such factors in aquatic systems include pharmaceutical agents, which have caused greater oxidative stress by inducing lipid peroxidation and altering antioxidant enzymes in *Daphnia magna* (Gómez-Oliván et al., 2014), as well as numerous environmental conditions including changes in temperature and oxygen levels (Lushchak, 2011). Contaminants including PCBs, pesticides, dioxins, and trace metals are also potential causes of oxidative stress; however, studies have found no relationships between any of these contaminants and thiamine deficiency (Fitzsimons et al., 1995). To determine which factor(s) cause(s) thiamine concentrations to decline, further research is needed. Additionally, research evaluating differences in concentrations of alternative antioxidants (e.g., vitamin E) between fish experiencing TDC and those that are not may help explain the influence of lipid peroxidation.

When looking at egg fatty acid results, there was limited variability both within and among species, suggesting the fatty acids incorporated into eggs were less influenced by diet than those incorporated into belly flap. In addition, correlations between egg fatty acid proportions and thiamine concentrations were minimal, which was also reported by Brown et al. (2005b). In addition, Czesny et al. (2012) found negligible differences in the egg fatty acid signatures of lake trout between two

locations in Lake Michigan with largely different prey assemblages: one mainly round goby while the other was mainly alewife. However, the occurrence of TDC was significantly greater in the location that contained a greater proportion of alewife. Lastly, Honeyfield et al. (2009) conducted a controlled feeding experiment in which lake trout fed various diets including different ratios of alewife and bloater (*Coregonus hoyi*) all had adequate fatty acid concentrations to support developing embryos, despite lower egg thiamine concentrations in lake trout fed alewife only. Because of the limited influence of diet on salmonine egg composition, any influence that fat content or fatty acid composition may have on egg thiamine concentrations likely occurs prior to egg deposition.

Despite limited variability in egg FASs among salmonines, symptoms of oxidative stress have been shown to vary between thiamine deficient and thiamine replete fish (Palace et al., 1998; Pickova et al., 1998, 2003; Brown et al., 2005b). Decreased carotenoids, particularly astaxanthin, have been associated with lower egg thiamine concentrations in lake trout and coho salmon, although no differences were found between deficient and healthy Chinook salmon (Palace et al., 1998; Brown et al., 2005b). Reasons for this difference among species are unknown but may explain the lower thiamine requirement observed for Chinook salmon (Fitzsimons et al., 2007; Futia and Rinchard, submitted). In addition to differences in carotenoid levels, increased levels of oxidation products have been observed in Atlantic salmon eggs from Baltic Sea affected with thiamine deficiency that also had low astaxanthin content and a higher content of phospholipid 22:6n-3 (Pickova et al., 2003; Keinänen et al. 2017).

Therefore, oxidative stress may still occur in eggs during embryo development, although specific causes and impacts of oxidative stress are unknown.

Conclusions

My FAS results show that the diet of Lake Ontario salmonines varies, with Chinook salmon, coho salmon, and steelhead trout feeding predominantly on alewife, while brown and lake trout incorporate greater proportions of alternative prey, particularly round goby. Due to these differences, thiamine concentrations varied among the salmonine species. My results show that increased consumption of alewife likely results in elevated concentrations of PUFAs, which have high potential for lipid peroxidation. In addition, PUFA concentrations often had negative correlations with thiamine concentrations, potentially due to thiamine being used to counter lipid peroxidation. However, it is undetermined if these reductions are capable of inducing TDC as correlations accounted for less than 50% of the variation observed in thiamine concentrations. In addition, for this hypothesis to be supported, oxidative stress would need to be more prevalent in thiamine deficient fish than healthy fish in other systems (i.e., Lake Superior) and from other time periods (i.e., prior to the occurrence of TDC). Additional research is needed to determine if such increases in oxidative stress occur, and what factors are driving it. Lastly, alternative factors such as thiaminase may be involved with reductions in thiamine concentrations, and they may have additive effects with lipid peroxidation, causing thiamine concentrations to be depleted further.

Acknowledgements

I thank J. Haynes and M. Connerton for their assistance reviewing this manuscript. Additionally, I thank personnel from the USGS – LOBS and NYSDEC for their assistance with fish collections for this project. Laboratory assistance was provided by undergraduate and graduate students from Dr. Rinchard's lab at The College at Brockport. This work was supported in part by the Distinguished Professors Award through The College at Brockport and a student grant from the Great Lakes Research Consortium.

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Tables

Table 1. Fatty acid content (expressed as % fatty acids detected) in belly flap of male and female salmonine species. Results are mean ± standard deviation and sample sizes are included in brackets for each sex within species.

		Brown trout		Chinook salmon		Coho salmon		Lake trout		Steelhead trout	
		Female (30)	Male (17)	Female (37)	Male (33)	Female (39)	Male (35)	Female (51)	Male (40)	Female (30)	Male (15)
SAFA	14:0	2.82 ± 0.53	2.79 ± 0.57	2.42 ± 0.89	3.78 ± 0.86	3.01 ± 0.73	3.91 ± 0.27	3.44 ± 0.25	3.48 ± 0.27	3.22 ± 0.49	3.65 ± 0.32
	15:0	0.28 ± 0.06	0.30 ± 0.07	0.28 ± 0.06	0.39 ± 0.07	0.30 ± 0.06	0.39 ± 0.04	0.42 ± 0.04	0.43 ± 0.02	0.36 ± 0.06	0.39 ± 0.05
	16:0	14.22 ± 1.05	14.00 ± 1.09	15.42 ± 1.42	14.41 ± 1.39	14.01 ± 1.30	13.97 ± 1.37	12.68 ± 0.89	13.29 ± 0.89	13.47 ± 1.13	14.67 ± 0.98
	17:0	0.26 ± 0.06	0.27 ± 0.08	0.52 ± 0.07	0.53 ± 0.09	0.38 ± 0.07	0.42 ± 0.05	0.33 ± 0.04	0.33 ± 0.03	0.42 ± 0.08	0.40 ± 0.04
	18:0	5.55 ± 0.73	5.34 ± 1.00	6.38 ± 0.53	6.17 ± 0.63	5.22 ± 0.61	4.80 ± 0.58	3.08 ± 0.41	3.14 ± 0.42	4.85 ± 0.46	4.90 ± 0.46
MUFA	16:1n-9	0.62 ± 0.19	0.78 ± 0.25	0.73 ± 0.26	1.12 ± 0.28	0.87 ± 0.17	1.00 ± 0.19	0.72 ± 0.25	0.73 ± 0.26	0.94 ± 0.16	1.05 ± 0.17
	16:1n-7	7.87 ± 1.56	7.53 ± 2.13	2.19 ± 0.58	2.98 ± 0.70	2.90 ± 0.65	3.51 ± 0.38	8.23 ± 1.44	8.32 ± 1.46	4.00 ± 0.80	3.97 ± 0.48
	17:1n-7	0.36 ± 0.07	0.42 ± 0.06	0.30 ± 0.08	0.45 ± 0.16	0.30 ± 0.08	0.39 ± 0.06	0.75 ± 0.08	0.73 ± 0.06	0.46 ± 0.10	0.43 ± 0.07
	18:1n-9	28.39 ± 2.46	29.73 ± 3.28	18.80 ± 4.91	26.23 ± 4.74	21.25 ± 3.15	24.54 ± 2.19	24.67 ± 1.81	24.43 ± 1.35	24.95 ± 2.67	23.76 ± 1.85

Table 1 Continued

	18:1n-7	4.62 ± 0.77	4.42 ± 0.56	2.04 ± 0.49	2.82 ± 0.57	3.13 ± 0.43	3.15 ± 0.40	3.95 ± 0.41	3.81 ± 0.38	2.98 ± 0.33	3.30 ± 0.12
	20:1*	4.01 ± 0.45	3.39 ± 0.29	1.96 ± 0.87	3.18 ± 0.79	2.65 ± 0.63	3.17 ± 0.47	2.63 ± 0.27	2.38 ± 0.22	2.68 ± 0.42	2.30 ± 0.27
PUFA	18:2n-6	2.88 ± 0.79	3.13 ± 1.14	4.89 ± 0.72	5.85 ± 0.99	4.88 ± 0.69	5.31 ± 0.43	4.20 ± 0.50	4.15 ± 0.56	4.93 ± 0.49	4.73 ± 0.35
	18:3n-3	1.85 ± 0.65	1.96 ± 0.90	3.74 ± 0.53	3.63 ± 0.57	3.19 ± 0.54	3.33 ± 0.47	3.68 ± 0.55	3.66 ± 0.54	3.16 ± 0.58	3.43 ± 0.35
	18:4n-3	0.46 ± 0.17	0.56 ± 0.23	0.70 ± 0.19	0.90 ± 0.30	0.79 ± 0.28	1.09 ± 0.28	1.43 ± 0.31	1.49 ± 0.27	0.82 ± 0.30	1.16 ± 0.19
	20:2n-6	0.62 ± 0.25	0.65 ± 0.39	0.60 ± 0.22	0.91 ± 0.27	1.02 ± 0.22	1.32 ± 0.17	0.95 ± 0.18	0.95 ± 0.20	0.90 ± 0.15	1.03 ± 0.10
	20:3n-6	0.43 ± 0.12	0.40 ± 0.17	0.55 ± 0.10	0.57 ± 0.09	0.51 ± 0.08	0.57 ± 0.07	0.53 ± 0.07	0.49 ± 0.06	0.63 ± 0.12	0.59 ± 0.06
	20:4n-6	3.66 ± 0.42	3.72 ± 0.44	5.52 ± 1.24	3.84 ± 0.75	4.36 ± 0.76	3.86 ± 0.19	3.91 ± 0.20	3.89 ± 0.21	4.56 ± 0.36	4.21 ± 0.32
	20:3n-3	0.50 ± 0.25	0.50 ± 0.38	0.47 ± 0.15	0.64 ± 0.19	0.82 ± 0.23	1.11 ± 0.16	0.96 ± 0.23	0.99 ± 0.28	0.77 ± 0.26	1.07 ± 0.15
	20:4n-3	1.57 ± 0.72	1.62 ± 1.04	2.15 ± 0.37	2.36 ± 0.44	2.41 ± 0.50	2.85 ± 0.43	2.55 ± 0.54	2.56 ± 0.60	2.78 ± 0.60	3.29 ± 0.33
	20:5n-3	3.12 ± 0.60	3.20 ± 0.60	5.62 ± 1.40	3.17 ± 0.97	4.24 ± 0.76	3.49 ± 0.60	4.91 ± 0.69	5.07 ± 0.60	4.07 ± 0.90	4.64 ± 0.35
	22:4n-6	1.63 ± 0.34	1.35 ± 0.22	0.72 ± 0.21	0.78 ± 0.15	1.11 ± 0.36	1.27 ± 0.29	1.30 ± 0.20	1.22 ± 0.25	1.58 ± 0.41	1.08 ± 0.15
	22:5n-6	1.27 ± 0.77	1.71 ± 0.29	1.17 ± 0.27	0.91 ± 0.24	1.33 ± 0.32	1.44 ± 0.22	1.72 ± 0.27	1.62 ± 0.28	1.64 ± 0.36	1.27 ± 0.12

Table 1 Continued

22:5n-3	5.42 ± 0.71	4.85 ± 0.52	4.63 ± 0.54	3.73 ± 0.45	6.79 ± 1.03	5.50 ± 0.69	4.47 ± 0.40	3.93 ± 0.31	5.62 ± 0.53	4.73 ± 0.48
22:6n-3	7.58 ± 0.98	7.40 ± 0.78	18.20 ± 5.34	10.65 ± 6.18	14.54 ± 3.83	9.62 ± 1.13	8.48 ± 0.76	8.93 ± 0.56	10.21 ± 2.97	9.95 ± 1.10

*20:1n-9 and 20:1n-11 are combined and represented by 20:1

Table 2. Differences between and within the fatty acid signatures (FAS) of male and female Chinook and coho salmon. The proportions for the two fatty acids most responsible for the differences (ANOSIM) are included for each sex of both species, along with their percent contributions to the overall difference (SIMPER). R values explaining the differences between sexes (ANOSIM) are included within parentheses following each species' name. Differences in FASs within sex (SIMPER) are presented as percent similarity.

	Chinook salmon (0.42)				Coho salmon (0.35)			
	Fatty acid	Female (%)	Male (%)	Contribution (%)	Fatty acid	Female (%)	Male (%)	Contribution (%)
Between sexes	22:6n-3	18.16	10.61	26.69	22:6n-3	14.51	9.59	23.65
	18:1n-9	18.75	26.14	23.97	18:1n-9	21.2	24.47	18.17
Within sex		87.67	88.70			90.14	93.72	

Table 3. Fatty acid content (expressed as % fatty acids detected) of egg tissue for five salmonine species. Results are mean ± standard deviation and sample sizes are included in brackets for each sex within species.

		Brown trout (48)	Chinook salmon (46)	Coho salmon (49)	Lake trout (35)	Steelhead trout (49)
SAFA	12:0	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
	14:0	1.61 ± 0.28	2.35 ± 0.33	2.37 ± 0.19	1.89 ± 0.39	2.08 ± 0.31
	15:0	0.24 ± 0.04	0.33 ± 0.04	0.32 ± 0.02	0.32 ± 0.09	0.33 ± 0.03
	16:0	13.54 ± 0.66	12.03 ± 1.55	12.76 ± 0.89	11.35 ± 3.20	14.28 ± 0.80
	17:0	0.23 ± 0.06	0.37 ± 0.05	0.32 ± 0.03	0.22 ± 0.07	0.40 ± 0.04
	18:0	5.92 ± 0.43	5.30 ± 0.76	5.20 ± 0.33	3.34 ± 1.03	6.50 ± 0.53
	20:0	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.05 ± 0.01
MUFA	16:1n-9	0.70 ± 0.09	0.92 ± 0.10	0.87 ± 0.07	1.24 ± 0.21	0.65 ± 0.06
	16:1n-7	6.16 ± 1.29	3.94 ± 0.46	4.02 ± 0.28	5.46 ± 1.14	3.94 ± 0.42
	17:1n-7	0.36 ± 0.06	0.55 ± 0.06	0.45 ± 0.07	0.51 ± 0.06	0.43 ± 0.06
	18:1n-9	19.73 ± 1.95	17.33 ± 1.40	14.98 ± 0.82	18.55 ± 1.89	15.31 ± 1.21
	18:1n-7	6.62 ± 1.11	3.11 ± 0.17	3.52 ± 0.18	5.36 ± 0.68	3.50 ± 0.15
	20:1*	1.91 ± 0.25	0.93 ± 0.13	1.31 ± 0.16	1.87 ± 0.30	1.10 ± 0.15
PUFA	18:2n-6	2.05 ± 0.62	3.68 ± 0.36	3.10 ± 0.34	2.66 ± 0.32	3.03 ± 0.33
	18:3n-3	2.12 ± 0.68	3.96 ± 0.48	3.70 ± 0.53	3.08 ± 0.44	3.61 ± 0.42
	18:4n-3	0.21 ± 0.08	1.18 ± 0.17	0.84 ± 0.09	0.69 ± 0.13	0.70 ± 0.12

Table 3 Continued

20:2n-6	0.63 ± 0.25	0.63 ± 0.08	0.79 ± 0.06	0.96 ± 0.18	0.74 ± 0.11
20:3n-6	0.40 ± 0.10	0.53 ± 0.08	0.43 ± 0.07	0.34 ± 0.04	0.46 ± 0.05
20:4n-6	5.97 ± 0.43	6.91 ± 0.42	6.98 ± 0.50	6.20 ± 0.52	7.32 ± 0.61
20:3n-3	0.66 ± 0.30	0.99 ± 0.11	1.15 ± 0.09	1.09 ± 0.25	1.05 ± 0.21
20:4n-3	1.83 ± 0.85	3.55 ± 0.50	2.69 ± 0.33	2.15 ± 0.48	3.34 ± 0.55
20:5n-3	5.24 ± 0.71	9.96 ± 0.63	9.41 ± 0.57	7.26 ± 0.61	8.82 ± 0.73
22:4n-6	0.87 ± 0.19	0.24 ± 0.09	0.28 ± 0.06	0.48 ± 0.10	0.44 ± 0.09
22:5n-6	0.80 ± 0.41	0.79 ± 0.11	0.78 ± 0.11	0.90 ± 0.21	0.84 ± 0.14
22:5n-3	6.23 ± 0.64	4.97 ± 0.32	6.91 ± 0.47	5.31 ± 0.52	5.39 ± 0.52
22:6n-3	15.92 ± 1.51	15.39 ± 1.38	16.77 ± 1.78	18.75 ± 1.57	15.66 ± 1.12

*20:1n-9 and 20:1n-11 are combined and represented by 20:1

Table 4. Pairwise comparisons of egg fatty acid signatures using ANOSIM and SIMPER analyses for five salmonine species. SIMPER results demonstrate the percent dissimilarity of FASs between species and ANOSIM results (R) represent the strength of the dissimilarity. Species names are abbreviated as follows: brown trout, BT; Chinook salmon, CK; coho salmon, CO; lake trout, LT; and steelhead trout, ST.

	BT, CK	BT, CO	BT, LT	BT, ST	CK, CO	CK, LT	CK, ST	CO, LT	CO, ST	LT, ST
ANOSIM (R)	0.95	0.92	0.63	0.91	0.65	0.83	0.70	0.84	0.65	0.88
SIMPER (%)	15.25	14.03	11.81	13.49	7.51	12.38	7.69	11.34	6.70	12.50

Table 5. Fatty acid content (expressed as % fatty acids detected) in whole-bodied prey fishes during spring and fall of 2015 and 2016. Results are mean ± standard deviation and sample sizes are included in brackets for each sex within species.

		Alewife				Rainbow smelt				Round goby			
		Spring 2015 (30)	Fall 2015 (13)	Spring 2016 (20)	Fall 2016 (25)	Spring 2015 (20)	Fall 2015 (22)	Spring 2016 (19)	Fall 2016 (28)	Spring 2015 (24)	Fall 2015 (24)	Spring 2016 (24)	Fall 2016 (19)
SAFA	14:0	2.86 ± 0.52	3.53 ± 0.45	5.58 ± 1.14	4.68 ± 0.42	3.95 ± 0.95	4.11 ± 0.52	4.06 ± 0.63	5.10 ± 0.44	2.06 ± 0.39	1.83 ± 0.40	2.75 ± 0.41	2.06 ± 0.58
	15:0	0.41 ± 0.06	0.51 ± 0.07	0.66 ± 0.18	0.53 ± 0.05	0.45 ± 0.06	0.55 ± 0.10	0.50 ± 0.05	0.60 ± 0.06	0.38 ± 0.04	0.58 ± 0.07	0.52 ± 0.09	0.48 ± 0.08
	16:0	17.91 ± 1.20	18.66 ± 1.14	18.85 ± 4.39	18.85 ± 0.87	16.25 ± 0.98	17.55 ± 1.36	16.89 ± 1.45	16.94 ± 1.37	14.27 ± 0.65	16.99 ± 0.84	16.36 ± 4.36	17.03 ± 0.71
	17:0	0.55 ± 0.10	0.65 ± 0.11	0.56 ± 0.16	0.58 ± 0.08	0.49 ± 0.34	0.81 ± 0.10	0.38 ± 0.06	0.49 ± 0.11	0.74 ± 0.44	0.68 ± 0.09	0.33 ± 0.06	0.44 ± 0.09
	18:0	3.87 ± 0.32	3.76 ± 0.30	2.89 ± 0.66	3.53 ± 0.43	2.44 ± 0.38	3.47 ± 0.42	2.34 ± 0.25	2.90 ± 0.43	3.20 ± 0.36	4.82 ± 0.90	3.08 ± 0.65	5.02 ± 0.96
MUFA	16:1n-9	0.85 ± 0.14	1.44 ± 0.22	1.08 ± 0.21	1.14 ± 0.11	0.63 ± 0.10	0.86 ± 0.14	0.73 ± 0.15	0.96 ± 0.16	0.50 ± 0.07	0.56 ± 0.09	0.60 ± 0.08	0.57 ± 0.06
	16:1n-7	2.54 ± 0.30	3.52 ± 0.35	3.48 ± 0.43	4.18 ± 0.60	6.32 ± 1.46	5.28 ± 1.41	7.92 ± 1.45	6.63 ± 1.62	9.41 ± 1.90	7.85 ± 1.75	12.93 ± 1.26	7.60 ± 2.40
	17:1n-7	0.32 ± 0.06	0.50 ± 0.20	0.62 ± 0.10	0.63 ± 0.04	0.42 ± 0.11	0.32 ± 0.06	0.63 ± 0.10	0.70 ± 0.09	0.40 ± 0.06	0.52 ± 0.07	0.84 ± 0.11	0.73 ± 0.11
	18:1n-9	15.60 ± 2.51	18.72 ± 2.66	17.06 ± 3.63	21.09 ± 1.75	13.46 ± 1.69	13.60 ± 1.41	14.96 ± 2.70	15.94 ± 2.06	12.65 ± 1.17	12.31 ± 1.11	15.39 ± 1.36	12.85 ± 1.52
	18:1n-7	3.17 ± 0.26	3.31 ± 0.24	3.58 ± 0.40	3.52 ± 0.27	2.91 ± 0.17	2.93 ± 0.21	3.37 ± 0.10	3.37 ± 0.18	4.35 ± 0.42	4.67 ± 0.76	5.33 ± 0.57	3.72 ± 0.66
	20:1*	1.39 ± 0.31	1.32 ± 0.21	1.94 ± 0.24	1.94 ± 0.41	1.25 ± 0.20	1.30 ± 0.28	0.89 ± 0.30	0.72 ± 0.24	1.47 ± 0.35	2.64 ± 0.70	1.91 ± 0.36	1.35 ± 0.37
PUFA	18:2n-6	3.49 ± 0.61	4.09 ± 0.25	4.46 ± 0.40	4.76 ± 0.30	3.58 ± 0.83	4.50 ± 0.50	3.59 ± 0.53	6.51 ± 0.72	2.72 ± 0.36	3.48 ± 0.65	2.95 ± 0.37	3.96 ± 0.68
	18:3n-3	4.01 ± 0.73	6.34 ± 1.13	4.56 ± 0.58	5.45 ± 0.38	3.08 ± 0.65	4.41 ± 0.91	3.65 ± 0.95	7.28 ± 0.94	2.79 ± 0.53	3.19 ± 0.93	3.32 ± 0.49	2.85 ± 0.56

Table 5 Continued

18:4n-3	2.04 ± 0.46	3.48 ± 0.31	2.36 ± 0.34	3.66 ± 0.64	1.67 ± 0.42	2.54 ± 0.38	2.17 ± 0.76	4.10 ± 0.32	2.22 ± 0.38	1.82 ± 0.60	2.64 ± 0.40	1.72 ± 0.50
20:2n-6	0.61 ± 0.23	0.38 ± 0.22	1.38 ± 0.31	0.59 ± 0.12	1.01 ± 0.24	0.95 ± 0.26	0.86 ± 0.22	0.80 ± 0.21	0.51 ± 0.09	0.41 ± 0.08	0.42 ± 0.10	0.39 ± 0.07
20:3n-6	0.22 ± 0.04	0.39 ± 0.14	0.26 ± 0.03	0.26 ± 0.02	0.16 ± 0.05	0.21 ± 0.06	0.11 ± 0.03	0.13 ± 0.02	0.17 ± 0.06	0.15 ± 0.04	0.09 ± 0.03	0.13 ± 0.05
20:4n-6	6.35 ± 0.98	6.13 ± 0.88	3.64 ± 0.66	4.14 ± 0.32	4.60 ± 0.82	5.17 ± 0.52	4.20 ± 0.69	4.69 ± 0.50	6.16 ± 0.66	6.62 ± 1.42	4.71 ± 0.82	7.96 ± 1.16
20:3n-3	0.75 ± 0.29	0.62 ± 0.53	1.87 ± 0.44	0.71 ± 0.21	1.08 ± 0.30	1.25 ± 0.54	0.94 ± 0.25	1.05 ± 0.39	0.45 ± 0.09	0.27 ± 0.06	0.32 ± 0.08	0.26 ± 0.07
20:4n-3	1.75 ± 0.36	1.57 ± 0.52	3.08 ± 0.44	1.82 ± 0.25	1.38 ± 0.41	1.40 ± 0.43	1.33 ± 0.31	1.30 ± 0.32	0.51 ± 0.08	0.42 ± 0.08	0.52 ± 0.11	0.42 ± 0.09
20:5n-3	11.17 ± 1.23	9.64 ± 0.66	7.71 ± 0.92	7.92 ± 0.55	13.33 ± 1.97	11.16 ± 1.80	11.58 ± 1.08	9.69 ± 0.84	12.80 ± 1.74	8.17 ± 1.57	8.49 ± 1.28	9.67 ± 1.49
22:4n-6	0.42 ± 0.14	0.31 ± 0.14	0.95 ± 0.13	1.09 ± 0.39	1.27 ± 0.66	0.31 ± 0.06	1.60 ± 0.33	0.97 ± 0.23	0.80 ± 0.39	0.96 ± 0.17	2.37 ± 0.62	3.10 ± 1.03
22:5n-6	1.89 ± 0.41	0.91 ± 0.24	1.98 ± 0.50	0.66 ± 0.21	1.08 ± 0.38	1.89 ± 0.46	0.47 ± 0.17	0.80 ± 0.38	2.79 ± 0.78	3.17 ± 0.53	0.16 ± 0.06	0.15 ± 0.05
22:5n-3	2.49 ± 0.33	1.89 ± 0.28	2.42 ± 0.35	1.85 ± 0.18	1.45 ± 0.34	1.68 ± 0.39	0.98 ± 0.23	1.28 ± 0.38	3.99 ± 0.86	3.85 ± 0.42	3.33 ± 0.57	3.16 ± 0.81
22:6n-3	15.36 ± 2.53	8.33 ± 2.71	9.01 ± 1.43	6.40 ± 1.16	17.77 ± 3.51	13.76 ± 2.15	15.85 ± 2.54	7.04 ± 1.52	14.65 ± 2.42	14.05 ± 2.52	10.67 ± 1.33	14.40 ± 2.73

*20:1n-9 and 20:1n-11 are combined and represented by 20:1

Table 6. Three major fatty acids driving the differences between species' belly flap fatty acid signatures (based on the proportions of individual fatty acids), determined by SIMPER analysis, and their corresponding contribution (percentage) to the dissimilarity. The fatty acid with the greatest contribution is in bold font. Species names are abbreviated as follows: brown trout, BT; Chinook salmon, CK; coho salmon, CO; lake trout, LT; steelhead trout, ST; alewife, ALE; rainbow smelt, RS; and round goby, RG.

	BT, CK	BT, CO	BT, LT	BT, ST	CK, CO	CK, LT	CK, ST	CO, LT	CO, STL	LT, ST	ALE, RS	ALE, RG	RS, RG
16:1n-7	**15.5**	15.4	7.0	13.7	3.2	**17.7**	4.6	**22.1**	4.6	**20.3**	9.9	**15.3**	10.2
18:1n-9	12.6	**16.8**	**17.3**	**16.6**	**18.8**	13.0	**17.0**	9.8	**16.9**	11.2	13.5	12.4	6.8
22:6n-3	10.7	7.8	5.3	9.8	16.9	11.0	16.8	5.8	11.5	8.8	**17.8**	12.3	**12.8**

Table 7. Spearman's rank correlation (rho, ρ) for pairwise comparisons of egg, muscle, and belly flap lipid content. Sample size (n) is included for each correlation and significant correlations are marked with an asterisk (*).

	Belly flap*Egg		Belly flap*Muscle		Egg*Muscle	
	ρ	n	ρ	n	ρ	n
Brown trout	0.21	30	0.75*	30	0.19	30
Chinook salmon	0.34*	37	0.66*	37	0.20	37
Coho salmon	0.06	39	0.65*	39	0.02	39
Lake trout	-0.13	35	0.39*	35	-0.02	29
Steelhead trout	-0.38	15	0.40	25	-0.40	15

Table 8. Yearly changes in average lipid content (± standard deviation) of belly flap, egg, and muscle tissue from females of five salmonine species. Sample size (n) is included for each average. Significant differences in tissue lipid content across years for each species are denoted by different letters (i.e., a and b).

	Brown trout			Chinook salmon		Coho salmon		Lake trout		Steelhead trout	
	2015	2016	2017	2015	2016	2015	2016	2015	2016	2015	2016
Belly flap	19.8 ± 7.8[a]	13.9 ± 8.5[b]		1.7 ± 1.4	1.4 ± 0.4	3.8 ± 3.7	2.7 ± 2.1	25.4 ± 9.2[a]	37.0 ± 10.6[b]	6.9 ± 3.8	8.5 ± 5.1
n	13	17		22	15	24	15	36	15	12	15
Egg	9.6 ± 0.7	9.2 ± 0.9	8.8 ± 0.8	12.0 ± 0.9	12.1 ± 0.9	10.4 ± 1.4	10.5 ± 0.9	8.6 ± 1.2	7.9 ± 1.6	9.0 ± 1.0	9.6 ± 0.9
n	13	21	14	27	20	29	20	21	14	25	20
Muscle	6.7 ± 2.9[a]	3.8 ± 2.4[b]		1.4 ± 0.6	1.2 ± 0.3	1.9 ± 0.6	1.6 ± 0.4	9.9 ± 6.0	8.8 ± 3.4		3.3 ± 2.0
N	13	17		22	14	24	15	21	14		15

Table 9. Spearman's rank correlation (rho, ρ) between tissue thiamine concentrations (nmol/g) and lipid content (%) for females of five salmonine species. Significant correlations are denoted by an asterisk (*). Thiamine vitamers are abbreviated as follows: free thiamine, TH; total thiamine, TTH.

		Belly flap lipid content			Egg lipid content			Muscle lipid content		
		Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH
Brown trout	ρ	-0.50*	-0.46*	-0.55*	-0.18	-0.21	-0.13	-0.52*	-0.45*	-0.44*
	n	30	30	30	48	48	30	30	30	30
Chinook salmon	ρ	0.01	0.17	0.16	0.33*	0.33*	0.17	0.12	0.19	0.20
	n	37	37	37	47	47	37	37	37	37
Coho salmon	ρ	-0.06	-0.01	-0.07	0.15	0.20	0.20	-0.03	-0.08	-0.21
	n	39	39	39	49	49	39	39	39	39
Lake trout	ρ	0.14	0.20	0.20	0.19	0.11	-0.13	0.00	-0.04	0.26
	n	43	43	36	35	35	29	35	35	35
Steelhead trout	ρ	0.10	0.02	0.08	0.39*	0.15	0.47*	-0.16	-0.18	-0.45
	n	27	27	27	45	45	25	15	15	15

Table 10. Spearman's rank correlation (rho, ρ) between the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in belly flap tissue of females from five salmonine species with egg free thiamine (TH), egg total thiamine (TTH) and muscle total thiamine. Significant correlations are denoted by an asterisk (*). Sample sizes for each species are included in parentheses following their common name.

	Saturated fatty acids			Monounsaturated fatty acids			Polyunsaturated fatty acids		
	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH
Brown trout (30)	-0.27	-0.37*	0.08	0.67*	0.55*	0.43*	-0.53*	-0.40*	-0.38*
Chinook salmon (37)	-0.31	-0.28	-0.47	-0.08	-0.01	-0.01	0.11	0.02	0.06
Coho salmon (39)	-0.44	-0.44	-0.30	-0.09	-0.02	-0.29	0.28	0.20	0.40*
Lake trout (43)	0.07	-0.12	0.16	0.58*	0.47*	0.47*	-0.56*	-0.41*	-0.48*
Steelhead trout (30)	-0.23	0.13	-0.18	0.02	-0.07	-0.26	0.06	0.05	0.42*

Table 11. Spearman's rank correlation (rho, ρ) between the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in egg tissue from five salmonine species with egg free thiamine (TH), egg total thiamine (TTH) and muscle total thiamine. Significant correlations are denoted by an asterisk (*).

	Saturated fatty acids			Monounsaturated fatty acids			Polyunsaturated fatty acids			Sample size		
	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH	Egg TH	Egg TTH	Muscle TTH
Brown trout	-0.21	-0.23	0.06	0.58*	0.45*	0.11	-0.49*	-0.36*	-0.10	48	48	30
Chinook salmon	-0.22	-0.26	0.19	0.05	0.00	-0.17	0.16	0.23	0.05	46	46	36
Coho salmon	-0.40*	-0.37*	-0.36*	0.16	0.25	-0.01	0.26	0.15	0.34*	49	49	39
Lake trout	-0.01	0.02	0.22	0.33	0.40*	0.11	-0.24	-0.32	-0.19	30	30	29
Steelhead trout	-0.49*	-0.32*	-0.15	-0.10	0.01	-0.37	0.26	0.06	0.32	45	45	25

Figures

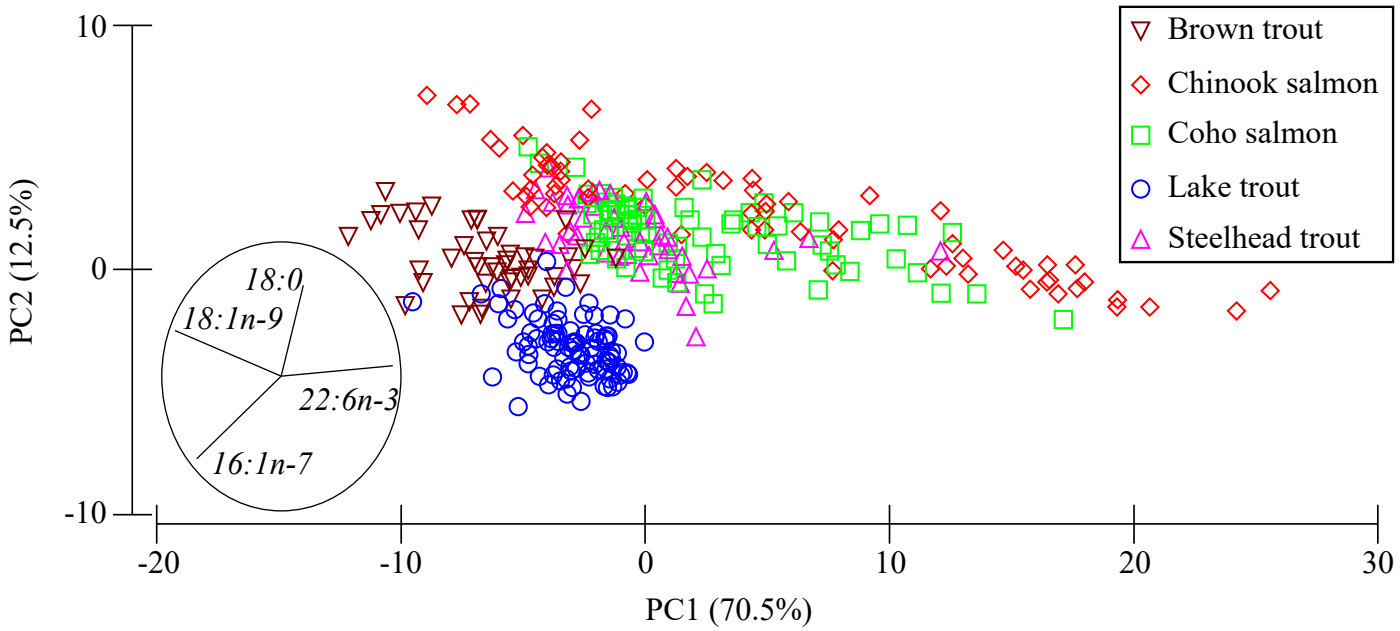

Figure 1. Principal component analyses of belly flap fatty acid signatures for five salmonine species based on the proportions of fatty acids. Vectors for the fatty acids with the greatest contributions to PC 1 and 2 based on loading values are included. The percent variation accounted for by each principal component (PC) is included in parentheses on the axis title.

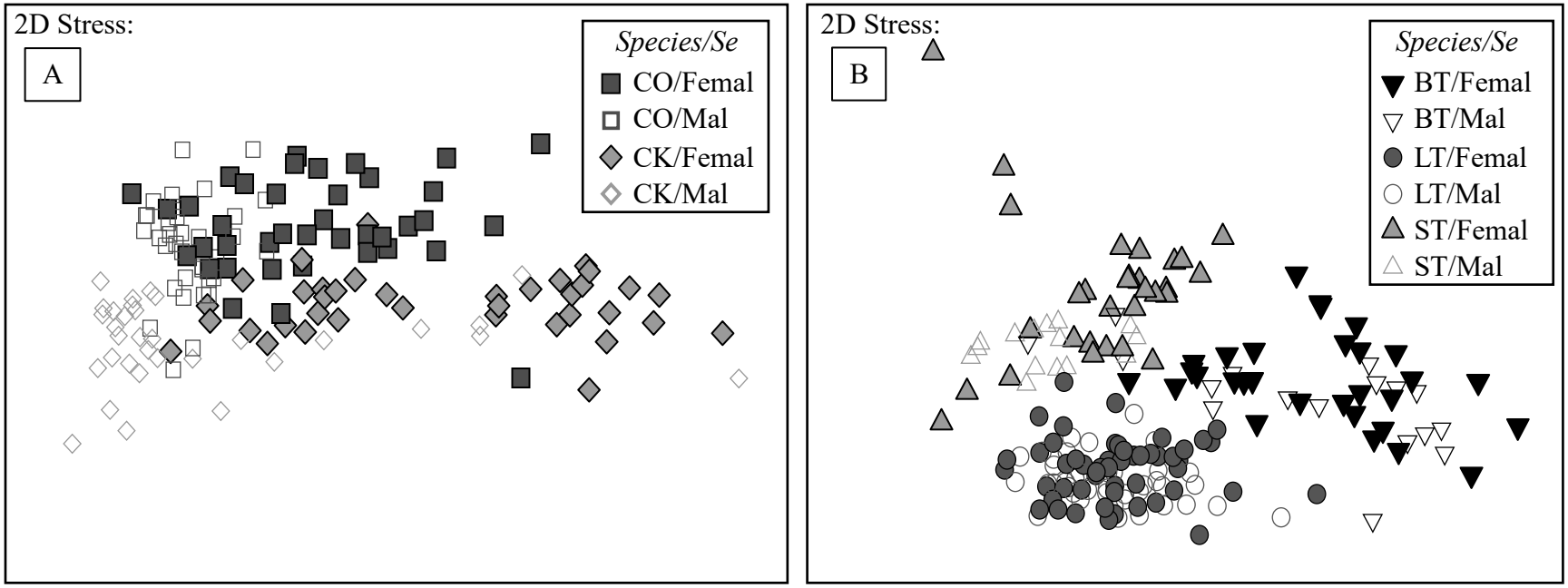

Figure 2. Belly flap tissue fatty acid signatures based on percent detection for females (closed symbols) and males (open symbols) of semelparous (A) and iteroparous (B) species represented on nMDS plots. Species names are abbreviated as follows: brown trout, BT; Chinook salmon, CK; coho salmon, CO; lake trout, LT; and steelhead trout, ST.

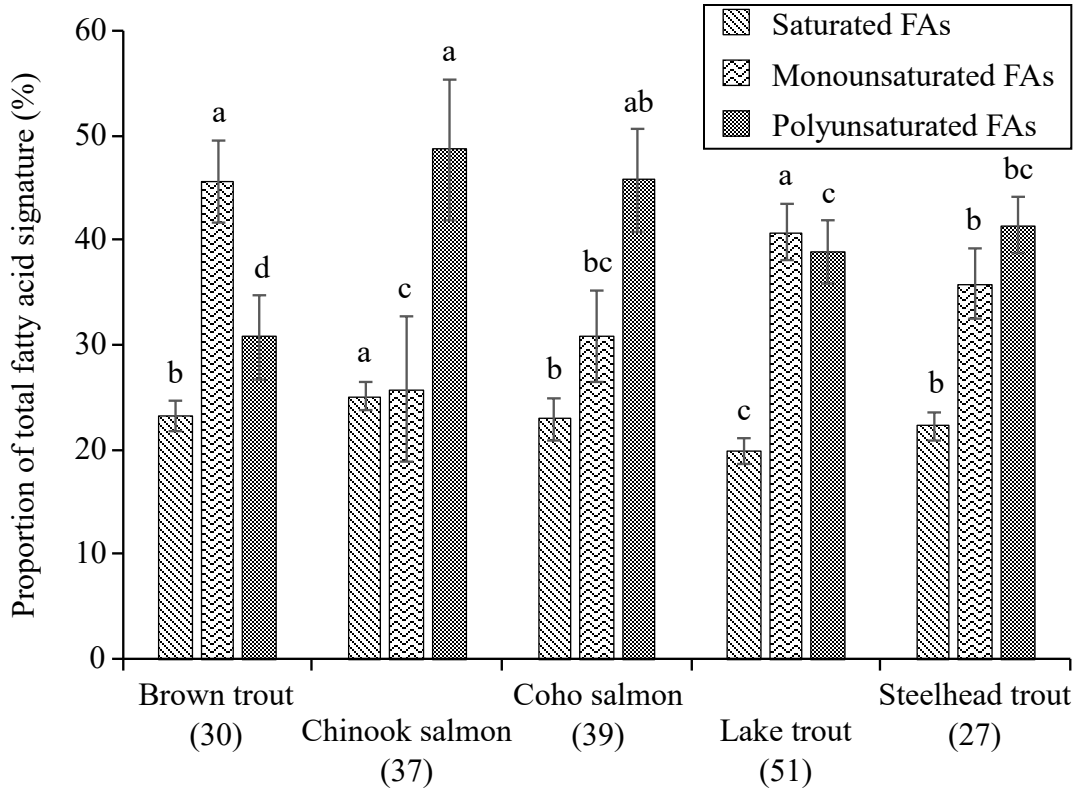

Figure 3. Average proportions of saturated fatty acids (FAs), monounsaturated FAs, and polyunsaturated FAs in belly flap tissue from females of five salmonine species. Error bars represent standard deviation for each corresponding average. Sample sizes are included in parenthesis below species' names and significant differences among species within each type of FA are denoted by different letters (i.e., a, b, c, and d).

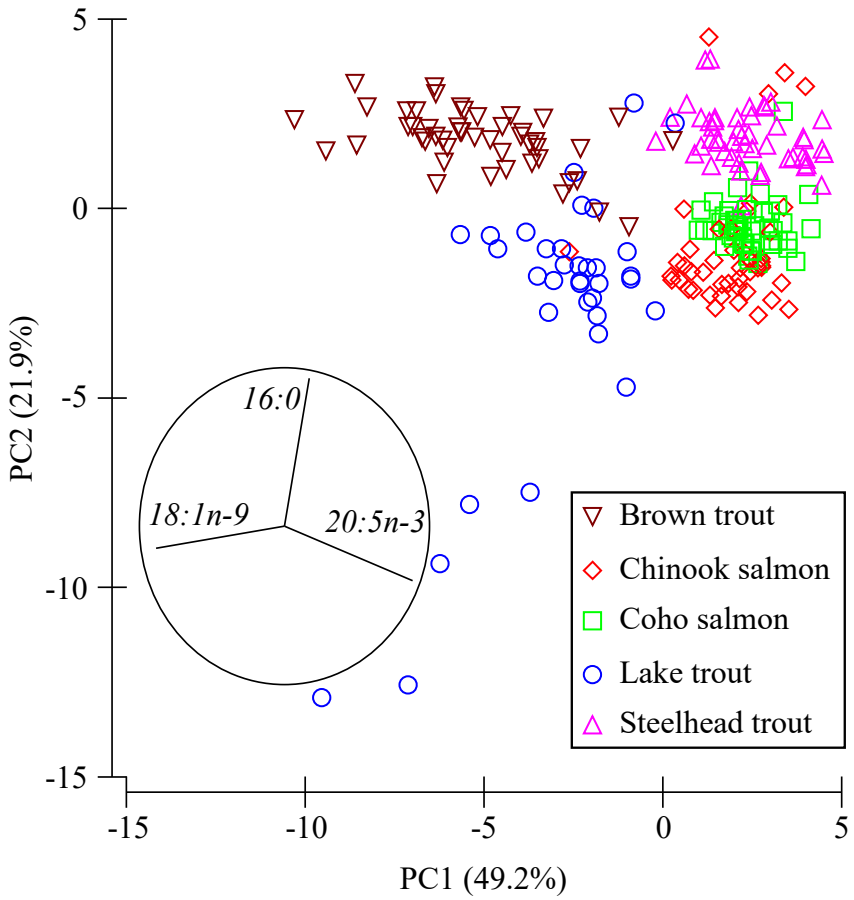

Figure 4. Principal component analyses of egg fatty acid signatures of five salmonine species based on the proportions of fatty acids. Vectors for the fatty acids with the greatest contributions to PC 1 and 2 based on loading values are included. The percent variation accounted for by each principal component (PC) is included in parentheses on the axis title.

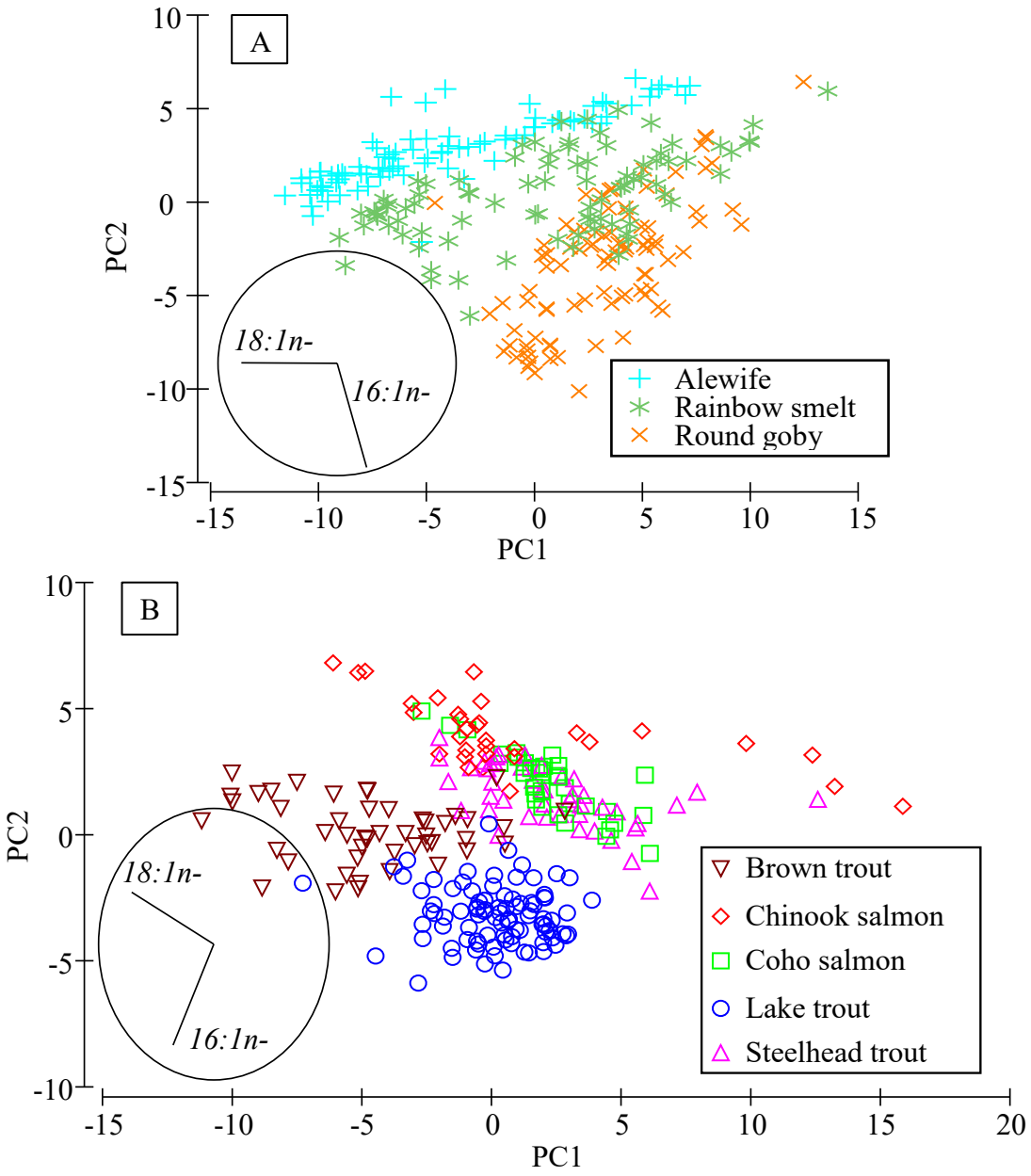

Figure 5. Principal component analyses of whole-body prey fish (A) and salmonine belly flap, excluding semelparous females, (B) fatty acid signatures based on the proportions of fatty acids. Vectors for 16:1n-7 and 18:1n-9 are included based on their loading values. The percent variation accounted for by each principal component (PC) is included in parentheses on the axis title.

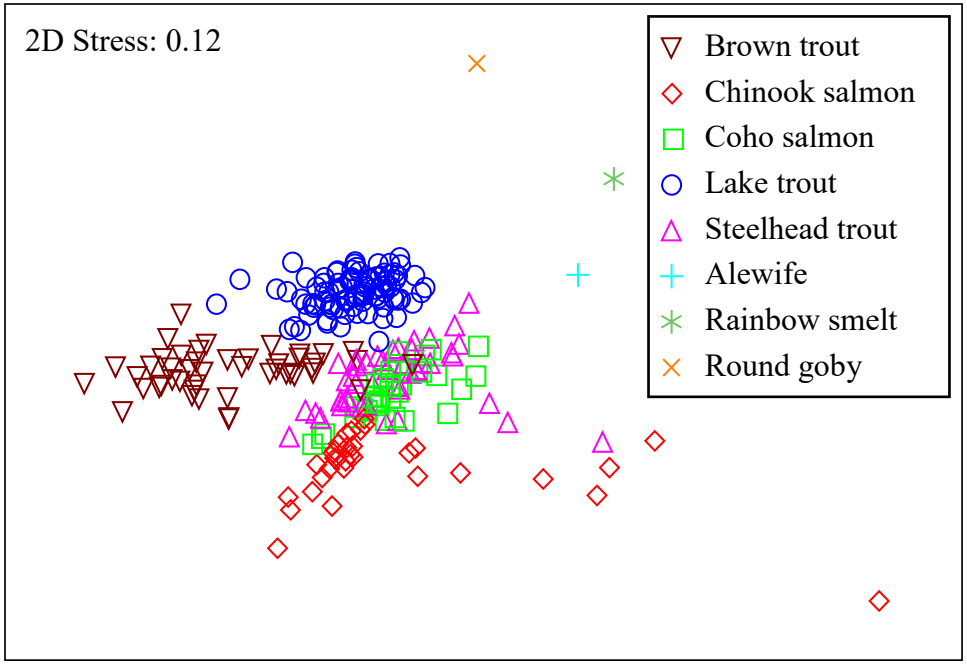

Figure 6. Belly flap tissue fatty acid signatures based on percent detection for five salmonine species as well as the average FAS of whole-bodied alewife, rainbow smelt, and round goby represented on an nMDS plot. Females of semelparous species are excluded.

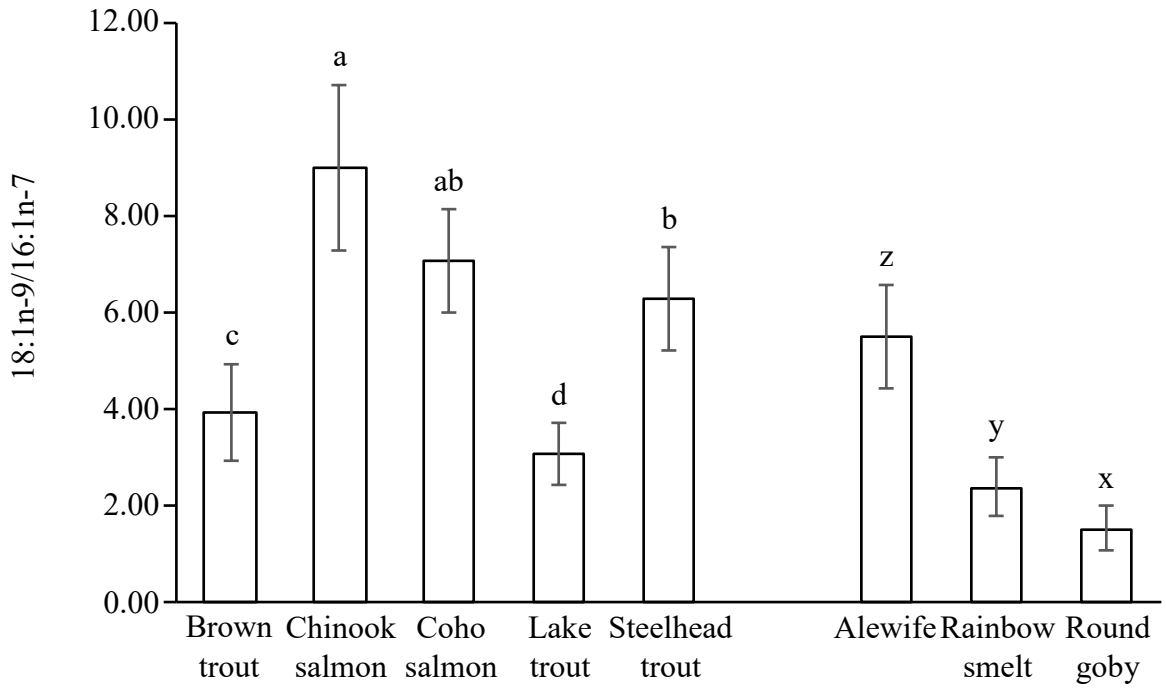

Figure 7. Average ratios of the percent contributions of 18:1n-9 to 16:1n-7 in belly flap fatty acid signatures for five salmonine species and whole-body FAS of three prey species. Ratios of females are excluded for semelparous species. Significant differences among salmonines are denoted by differences in the letters a, b, c, and d, while differences among prey species are denoted by differences in the letters z, y, and x.

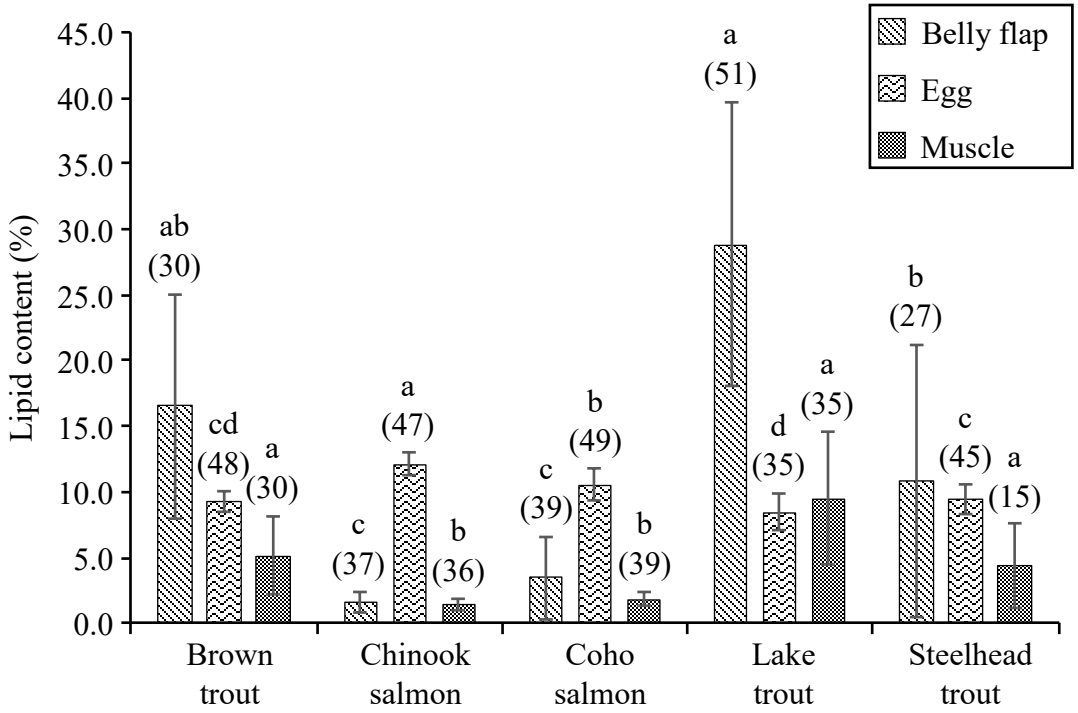

Figure 8. Average lipid content and standard deviation (error bars) of belly flap, egg, and muscle tissue from females of five salmonine species. Sample sizes are included in parentheses and significant differences in lipid content among species within each type of tissue are denoted by different letters (i.e., a, b, c, and d).

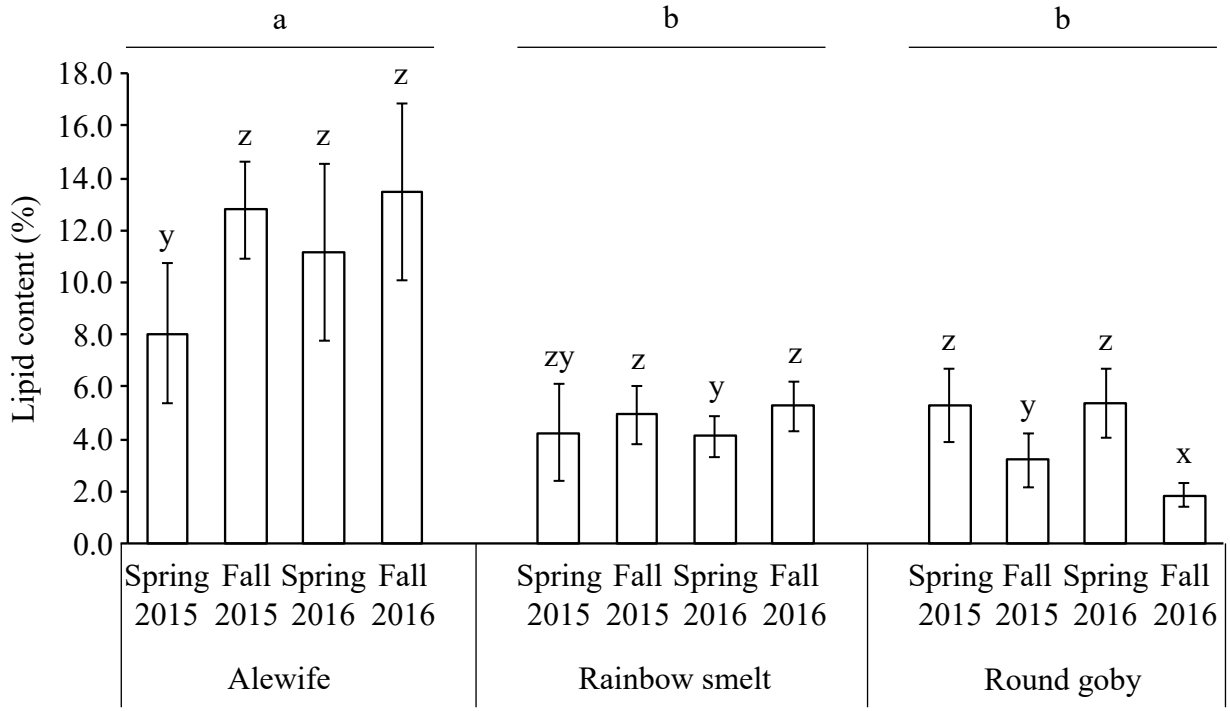

Figure 9. Average lipid content (%) for whole-bodied alewife, rainbow smelt, and round goby collected during the spring and fall of 2015 and 2016. Significant differences among species and temporally within species are represented by different letters (species: a and b; temporal: z, y, and x).

Conclusion

TDC continues to impact salmonine species in Lake Ontario. Of the five species studied, all but brown trout had reductions in thiamine concentrations that resulted in elevated offspring mortality. However, thiamine concentrations have yearly variability, and had increasing trends for all species in recent years. This coupled with observed increases in natural recruitment for some salmonine species in the lake suggest that the extent of TDC may be decreasing. While such changes would be beneficial for restoration purposes, increases in the abundance of predators in the lake could lead to an imbalanced prey-predator relationship leading to the collapse of the fishery. Therefore, it is important that future monitoring of the extent of TDC in salmonines be continued, as well as determining the presence of wild adult salmonines and the potential for self-sustaining populations.

Determining the cause(s) of TDC may help managers understand the potential for successful natural recruitment. As thiamine concentrations have yearly variability, identifying the factors responsible for the differences would provide valuable information regarding yearly wild recruitment. The results from this study support that salmonine thiamine concentrations in Lake Ontario are related to consumption of alewife; however, the specific driver(s) of TDC remain(s) uncertain. My results demonstrate significant negative correlations between PUFAs and thiamine concentrations, suggesting that high proportions of these fatty acids (e.g., 22:6n-3) may lead to lower thiamine concentrations. However, high proportions of PUFAs did not always induce TDC. Specifically, brown trout were able to maintain sufficient thiamine

concentrations for healthy survival, regardless of the proportion of PUFAs. In addition, while these correlations were present, there were some inconsistencies among species and the correlations left much of the variability in thiamine concentrations unexplained. Therefore, it is likely that other factors are involved in causing TDC in salmonines from Lake Ontario.

My study highlights several opportunities for future studies and experiments. Salmonines used for this study were collected during their spawning periods, which is generally associated with a period of starvation. Therefore, my results may be limited due to the temporary change in fish diet. Thus, studies evaluating salmonine diet, lipid content, and thiamine concentrations during other seasons may provide further insight regarding the causes of TDC as well as intraspecific and temporal differences. Additionally, future work should investigate the occurrence of lipid peroxidation in salmonines. In this study, the occurrence of lipid peroxidation was based on the assumption that peroxidation is directly proportional to the amount of PUFAs (i.e., increased PUFAs directly increases lipid peroxidation). Therefore, comparing concentrations of the end products of lipid peroxidation and thiamine concentrations could provide a more direct analysis for determining potential relationships between these two variables. Lastly, as previously mentioned, future work is needed for monitoring the thiamine concentrations for these salmonine species as well as their wild production. Changes in thiamine concentrations may have drastic impacts on the populations of these fish and, therefore, large impacts on the entire Lake Ontario

fishery. Thus, until TDC is eliminated, continued monitoring will provide valuable information for successful management.