

Great Lakes Lake Trout Thiamine Status and Influencing Factors in Lake Ontario

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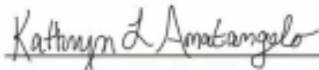
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General Introduction

The story of Great Lakes conservation efforts is one fraught with challenges. Often, the challenges are due to the sheer scale of the system and some to the complexity of the everchanging relationships among its inhabitants. The difficulty encountered in restoration of the historical apex predator of the Great Lakes, the lake trout (*Salvelinus namaycush*), is an example of the latter. Largely due to overfishing, the invasion of sea lamprey (*Petromyzon marinus*), habitat degradation, and toxic contaminants. Lake trout were extirpated from lakes Michigan, Erie, and Ontario by the middle 20th century (Morbey et al. 2008, Riley et al. 2011, Patterson et al. 2016). Several remnant populations remained in Lake Superior at Gull Island Shoal, Cat Island, Stannard Rock, Thunder Bay, Slate Islands, Isle Royale, and Munising and two remnant populations remained in Lake Huron at Parry Sound and Iroquois Bay (Morbey et al. 2008). Following successful sea lamprey control programs initiated by the Great Lakes Fishery Commission in the early 1960s, lake trout restoration efforts began (Hansen et al. 1995, Patterson et al. 2016). Stocking of hatchery bred lake trout and changes to fishing regulations in conjunction with sea lamprey control programs have since led to successful restoration self-sustaining lake trout populations to Lake Superior and to Parry Sound in Lake Huron (Hansen et al. 1995, Reid et al. 2001, Morbey et al. 2008). Lake Michigan is showing signs of recovery as well, with the catch per unit effort (CPUE) of naturally recruited adults increasing consistently from 2005 to 2022 (Lake Michigan Lake Trout Working Group 2024). Lakes Erie and Ontario however, have yet to see substantial natural recruitment into the adult

population (Lantry et al. 2020, Lake Erie Committee 2021). The process of restoration involves several steps based on the life cycle of lake trout including the building of an adult breeding population, egg deposition, fry emergence, juvenile recruitment, and adult recruitment (Lake Erie Committee 2021). Egg predation by round goby (*Neogobius melanostomus*) along with fry and juvenile predation by alewife (*Alosa pseudoharengus*) and habitat degradation can present significant challenges to recruitment (Jonas et al. 2005, Krueger et al. 2014, Hirsch et al. 2016). Lastly, thiamine (vitamin B₁) deficiency in adults and resulting progeny presents a further challenge to the generation of self-sustaining lake trout populations (Harder et al. 2018). A greater understanding of causal mechanisms of thiamine deficiency would provide managers with information necessary to meet restoration objectives.

Thiamine is produced by bacteria, fungi, and plants. It is an essential molecule for cellular metabolic function and is a cofactor in several enzymatic reactions required to sustain life (Manzetti et al. 2014, Kraft and Angert 2017). Thiamine deficiency has been noted as a condition of concern across several taxa including birds, bivalves, ray-fined fishes, and humans (Richter et al. 2012, Balk et al. 2016, Whitfield et al. 2018). Thiamine deficiency complex (TDC) in lake trout results in insufficient maternal transfer of thiamine to eggs during oogenesis (Fitzsimons et al. 1999, Fitzsimons et al. 2007). Sufficiently low total egg thiamine concentrations result in early mortality syndrome (EMS) among progeny, characterized by lethargy, spiral swimming, hyperexcitability, and death prior to the onset of exogenous feeding (Fitzsimons et al. 1999, Fitzsimons et al. 2007). Total egg thiamine concentration

thresholds have been established for direct mortality in lake trout. A 50% mortality rate (LC50) is expected among free-embryos for egg total thiamine concentrations below 2.32 nmol/g (Futia and Rinchar 2019). Additionally, Fitzsimons et al. (2007) documented 20% mortality at 2.63 nmol/g and 50% mortality at 1.57 nmol/g among lake trout free-embryos. Currently, the target threshold concentration for Great Lakes lake trout management is 4 nmol/g (Bronte et al. 2008).

TDC in lake trout of the Great Lakes has been closely tied with a diet highly reliant on alewife, an anadromous fish invasive to the Great Lakes (Fitzsimons et al. 2010, Schmidt and Morse 2011, Weber et al. 2015, Harder et al. 2018, Ladago et al. 2020). Alewife were first recorded in Lake Ontario in 1873 and expanded their range west into lakes Erie, Huron, and to Lake Michigan by 1949 (Eshenroder and Lantry 2012). Alewife were recorded in Lake Superior in 1954, but their abundance remained low due to a short growing season and low young-of-the-year survival through long winters (O’Gorman et al. 2012). The sensitivity of alewife to cold temperatures has been readily visible with large spring die-offs on the beaches of lakes Michigan and Ontario (O’Gorman et al. 2012). These die-offs have been hypothesized to be influenced by several factors, such as low food availability, spawning stress, immunosuppression, osmoregulatory stress, and homeoviscous adaptation failure (Snyder and Hennessey 2003, Lepak and Kraft 2008). Despite these die-offs, alewife became highly abundant in the lower Great Lakes throughout the 20th century (O’Gorman et al. 2012). The introduction of Pacific salmonines into the Great Lakes was implemented in part to control the alewife population, though the

restoration of lake trout would work toward the same end (O’Gorman et al. 2012). Increasingly apparent, however, was the impact alewife had in hindering natural recruitment among lake trout via TDC. Under laboratory conditions, Honeyfield et al. (2005) found that lake trout given a diet consisting of 35% alewife and 65% bloater (*Coregonus hoyi*) had a mean total egg thiamine concentration of 3.07 nmol/g, significantly lower than both a 100% bloater diet (11.57 nmol/g) and a control diet consisting of commercially available trout feed (37.01 nmol/g). Furthermore, following the 2003 collapse of alewife in Lake Huron, an increase in thiamine was observed in lake trout eggs along with an increase in wild juveniles (Riley et al. 2008, Fitzsimons et al. 2010). Interestingly, while thiamine content has been shown to vary in prey fish by age and condition, alewife have been found to contain sufficient thiamine for salmonid growth, development, and reproduction (Keinänen et al. 2012, Harder et al. 2018, Ladago et al. 2020).

Present in the visceral organs of several prey fishes including gizzard shad (*Dorosoma cepedianum*), alewife, rainbow smelt (*Osmerus mordax*), and others, thiaminase I degrades thiamine during digestion by predators, limiting the uptake of thiamine (Honeyfield et al. 2005, Tillitt et al. 2005, Fitzsimons et al. 2007). *De novo* production of thiaminase I in alewife appears likely as they have genes homologous to those in zebrafish (*Danio rerio*) which have been demonstrated to produce thiaminase I (Richter et al. 2023). However, *in vitro* thiamine degradation by *bacterial* thiaminase has been shown to be modulated by common dietary factors including inhibition by the presence of ascorbic acid and Cu^{2+} , and limitation by the

availability of co-substrates such as nicotinic acid (vitamin B₃), pyridoxine (vitamin B₆) and folic acid (vitamin B₉) (Edwards et al. 2023). The potential for these dietary factors to inhibit thiamine degradation by alewife derived thiaminase has yet to be explored. Moreover, the quantity of thiaminase I in prey fish has been shown to be variable by season, and fatty acid composition, adding difficulty to establishing quantitative connections between prey species thiaminase content and lake trout thiamine status (Tillitt et al. 2005, Honeyfield et al. 2010, Honeyfield et al. 2012).

Beyond thiaminase I, other factors have been hypothesized to exacerbate TDC. In the Baltic Sea, instances of Atlantic salmon (*Salmo salar*) suffering from M74 (thiamine deficiency) have been correlated with factors such as year class, weight, growth rate, and biomass of the native sprat (*Sprattus sprattus*) (Karlsson et al. 1999, Mikkonen et al. 2011). While sprat do contain thiaminase I, they also have relatively high lipid content and low thiamine content during early year classes 1-5 years (Keinänen et al. 2012). Alewife, like sprat, have relatively high lipid content (sprat = 1.5-15.7%, alewife = 7.3-14.7%) in comparison to other prey fish species (rainbow smelt = 3.4-6.0%, round goby = 2.2-5.8%) (Keinänen et al. 2012, Futia et al. 2019, Happel et al. 2020). A diet consisting of high amounts of highly unsaturated fatty acids can lead to excess lipid peroxidation and as thiamine can act as an antioxidant, thiamine may be excessively consumed as an antioxidant when it occurs in low quantities in comparison to lipids (Lukienko et al. 2000, Keinänen et al. 2012, 2018, Harder et al. 2018). Reduced levels of the antioxidants astaxanthin, vitamin E, and carotenoids have been associated with the development of thiamine deficiency in

salmonines, indicating excess lipid peroxidation (Palace et al. 1998a, Brown et al. 2005, Keinänen et al. 2012, 2018). Regardless of the specific mechanism behind the induction of TDC, diet composition has been clearly demonstrated as the driver of TDC in lake trout but, diet composition is not constant among all lake trout populations.

Lake trout have been documented to diverge into varying morphologies to occupy available ecological niches (ecomorphs). This is evident in the variety of documented ecomorphs in Great Bear Lake, Great Slave Lake, and Lake Superior (Muir et al. 2021). Habitat use and resulting diet preferences are readily apparent in the morphology of some ecomorphs, such as the buccal orientation of the insectivorous butterfly lake trout of Great Bear Lake or the high lipid content of the Lake Superior siscowet lake trout (Muir et al. 2021). In the Great Lakes, at least four ecomorphs have been historically present: humpers, leans, siscowets and red fins. Lean lake trout are specialized for pelagic feeding with a streamlined morphology and fast growth rate, while humper and siscowet ecomorphs with their high lipid content, larger eyes, and stout body shape, are specialized for benthic and deep-water habitats (Muir et al. 2014, 2021). Currently, The primary stocked strains of lake trout in US waters of Lake Ontario are the Seneca (SEN), Lake Champlain Domestic (LCD), and Klondike (SKW). The Seneca and Lake Champlain domestic strains are both derived from lean lake trout ecomorphs and have been shown to have a lower mortality rate associated with sea lamprey predation than the Klondike strain (Rogers et al. 2019). Klondike strain lake trout are often referred to as humper ecomorph; however, their

genealogy is somewhat more nuanced. The Klondike strain lake trout was based on gametes collected from fish near the Caribou Island complex offshore in Lake Superior and the intention was to rear the humper ecomorph (Rogers et al. 2019); however, genetic analyses of Klondike strain lake trout stocked in Lake Michigan found that they contained ~20% lean lake trout indicating a mixture of ecomorphs (Larson et al. 2021). Based on observations onboard the Research Vessel Lake Char of the way gametes were collected at Klondike Reef to make the humper broodstocks in 2011 and 2012 (Shawn Sitar, Michigan DNR, personal communication), it is highly likely that humper gametes were mixed with those from other ecomorphs including siscowets and leans since reproductive timing among lake trout ecotypes overlaps during the fall (Goetz et al. 2017, 2011). Regardless, the Klondike strain has shown distinct differences in diet preference in Lake Ontario compared to SEN or LCD strains (Heisey et al. 2023).

The term vitamin E refers to a group of lipid soluble highly active antioxidants, the tocopherols and tocotrienols (Hamre 2011). In vertebrates, α -tocopherol is the primary active vitamin E vitamer as it is preferentially retained by the tocopherol transfer protein located in the liver, while other tocopherols that are bound with a weaker affinity are excreted through the bile (Hamre 2011). The primary function of α -tocopherol is inhibition of the oxidation of polyunsaturated fatty acids (PUFA) (Hamre 2011). Other functions include modulating immune responses impacting health and disease resistance, stabilizing biological membranes, and involvement in the proliferation of some cell types (Hamre 2011). Lipid

peroxidation is initiated by the interaction of a reactive oxygen species and a phospholipid containing one or more double bonds along the phospholipid's carbon chain tail (Hamre 2011). The result is a peroxy radical which can then radicalize other phospholipids. Reactions between α -tocopherol and peroxy radicals produce a lipid hydroperoxide and a tocopheroxyl radical, ending propagation of peroxy radicals (Buettner 1993, Hamre 2011). Tocopheroxyl radicals are then thought to be recycled to tocopherol in a process facilitated by ascorbic acid (vitamin C) (Buettner 1993, Meister 1994, Hamre 2011). Results from a laboratory feeding study were consistent with ascorbic acid having a protective effect on α -tocopherol in fish (Lee and Dabrowski 2003). Hepatic α -tocopherol concentrations in fish have been shown to be representative of dietary α -tocopherol intake but hepatic α -tocopherol concentrations can also be reduced under high dietary lipid loads (Hamre 2011, Qiang et al. 2019). Hepatic α -tocopherol concentrations in Lake Ontario lake trout were significantly lower than in Lake Superior lake trout, suggesting a lower dietary α -tocopherol intake and/or a higher exposure to oxidative stress (Palace et al. 1998b). The total proportion of PUFAs in salmonine fatty acid composition has been negatively correlated with egg thiamine concentration in Lake Ontario, implicating lipid peroxidation as a source of oxidative stress consuming thiamine (Futia et al. 2019). However, in reference to Brown et al. (2005) and Keinanen et al. (2018), Futia et al. (2019) noted that this correlation was not present across all studies and was not visible for all salmonine species. These discrepancies may be due in part to the limitation of lipid peroxidation by antioxidants that are more active than thiamine,

such as α -tocopherol, when present in sufficient quantities. Interestingly, alewife in Lake Ontario have low concentrations of α -tocopherol in comparison to other prey fish (alewife = 1.6 ng/mg, rainbow smelt = 22.8 ng/mg, sculpin *Cottus spp.* = 25.3 ng/mg, round goby = 12.7 ng/mg); falling below the dietary requirement for salmon and trout of 6.7 ng/ mg (Honeyfield et al. 2012). Moreover, peroxidation potential is not equal across all PUFAs and the specific suit of PUFAs present will impact the overall risk of excess lipid peroxidation (Di Nunzio et al. 2011, 2016).

Unsaturation index (UI) indicates the degree of unsaturation in lipids as the sum of the percentage of each unsaturated fatty acid multiplied by the number of double bonds. This index is often used to determine the oxidative stability of human food and animal feed which, provides insight into the degree of intervention required to prevent oxidation of lipids, i.e., peroxidation and rancidity (Dal Bosco et al. 2022). However, Di Nunzio et al. (2011, 2016) found that living cells supplemented with docosahexaenoic acid (DHA) did not experience increased peroxidation, but cells supplemented with other PUFAs did experience increased peroxidation, despite DHA having more double bonds than the other PUFAs. This discrepancy between the presumed sensitivity of DHA to peroxidation based on its molecular structure, and the observed peroxidation sensitivity when introduced to living cells, may be due to DHA's interactions with cellular antioxidant defense systems. DHA is thought to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which is a key transcription factor thought to transmit an inducer signal to the antioxidant response element, promoting the induction of cellular antioxidant defenses (Borgonovi et al.

2023). Evidence of eicosapentaenoic acid (EPA) promoting the induction of cellular antioxidant defenses was observed by Wang et al. (2010) and is thought to act through activation of the Nrf2 pathway as well. Lastly, the sensitivity of the Nrf2 pathway to activation by oxidative stressors may be variable among ecomorphs as Goetz et al. (2016) documented a regulated Nrf2 mediated oxidative stress response in siscowet lake trout but not in lean lake trout, in response to sea lamprey parasitism, which presumably increased oxidative stress through inflammation and the response of tissues to xenobiotics.

The overall objectives of this study was: 1) to determine the thiamine status of lake trout in the Great Lakes; 2) to place long-term trends in Great Lakes lake trout egg thiamine concentrations into their relevant ecological contexts; 3) to investigate the potential influence of some biochemical and morphometric factors on lake trout egg thiamine concentrations. Chapter one focuses on graphically analyzing long-term trends in lake trout egg thiamine concentrations and the potential influence of morphometric factors. Chapter two focuses on investigating the potential role of lipid peroxidation in TDC by analyzing fatty acid compositions and α -tocopherol concentrations alongside associated egg thiamine concentrations.

Chapter 1: Great Lakes Lake Trout Thiamine Status

Abstract

Though stocking lake trout (*Salvelinus namaycush*) in the Great Lakes has found success in establishing some self-sustaining populations, a return of consistent natural recruitment of lake trout in Lakes Michigan, Erie and Ontario remains unachieved. A major impediment has been thiamine deficiency complex (TDC). Insufficient maternal deposition of thiamine (vitamin B₁) into eggs during development is a major consequence of TDC and leads to mass mortality of fry prior to their first feeding. Monitoring of egg thiamine concentrations is a critical indicator for TDC impacts on lake trout populations. Since 2001, the USGS Great Lakes Science Center in cooperation with partner agencies has monitored egg thiamine concentrations in lake trout eggs throughout the Great Lakes region. Our analysis of the resulting dataset reinforces prior findings connecting the presence of alewife (*Alosa pseudoharengus*) in the diet with reduced egg thiamine concentrations. Relationships between total length of egg producing females and total egg thiamine concentrations of lake trout eggs in Lake Ontario suggest that a shift in diet from a round goby (*Neogobius melanostomus*) dominated diet, to an alewife dominated diet, results in lower total egg thiamine concentrations among larger lake trout. Long-term trends in lake trout egg thiamine concentrations are closely linked to changes in prey base composition. Following the crash of the alewife population in Lake Huron, lake trout egg thiamine concentrations increased considerably. Similarly, reductions in site

specific alewife abundance preceded lake trout egg thiamine increases in Lake Michigan and may have helped to facilitate a recent trend of increasing natural recruitment. The introduction of alewife to Lake Champlain in 2004 led to large declines in lake trout egg thiamine concentrations in the lake, but also appears to have facilitated natural recruitment of the same lake trout population. Egg thiamine concentration from Cayuga Lake lake trout increased following the 2013 introduction of round goby, presumably offering lake trout an alternative to alewife as prey, however, lake trout egg thiamine concentrations in Cayuga Lake have returned to pre-introduction concentrations.

1. Objectives

In 2001, the Great Lake's lake trout thiamine monitoring program was initiated to assess lake trout egg thiamine concentrations throughout the Great Lakes basin. Morphometric data were collected alongside egg collections for many of the sampling efforts made in support of the monitoring program. To date, lake trout egg thiamine data collected by the monitoring program had not been centrally compiled with concurrent morphometric data. In this chapter, my first objective was to compile historical lake trout egg thiamine data with those generated during the 2021 and 2022 collection efforts and graphically analyze the resulting trends within their relevant ecological contexts. My second objective was to relate historical lake trout egg thiamine data to corresponding morphometric data and test potential influencing factors such as strain, length, weight, and age. I hypothesized that inflection points

within lake-specific long-term trends would correspond with changes in local prey base compositions; and that egg thiamine concentrations would be reduced with increased age, length, and condition factor.

2. Materials and Methods

2.1. Historical Data

Historical Lake Ontario lake trout egg thiamine concentrations collected as part of the Great Lakes lake trout Egg Thiamine Monitoring Program were provided by: Dale Honeyfield - USGS Eastern Ecological Science Center, Stephen Riley – USGS Great Lakes Science Center, Donald Tillitt - USGS – Columbia Environmental Research Center, Ellen Marsden University of Vermont, and Jacques Rinchar – State University of New York Brockport. These records included lake trout egg thiamine concentrations from all five of the Great Lakes, Lake Huron, Lake Erie, Lake Superior, Lake Michigan, and Lake Ontario, and from Lake Champlain along the border of New York State and Vermont, and from Cayuga Lake in New York State. These historical egg thiamine concentrations were compiled with the results of the 2021 and 2022 collection efforts in support of the Great Lakes lake trout egg thiamine monitoring program (Rinchar et al. 2022, 2023). Egg thiamine concentrations were grouped by lake, sampling location, and year for visualization of long-term trends. Collections with data on one or more morphometric factors (strain, length, weight, and age), were used for testing of morphometric factor influence on egg thiamine concentrations. In total, egg thiamine concentrations from 5878 unique

egg samples collected between 1996 and 2022 were used for graphical analysis. Of those 5878 egg samples, concurrent data on one or more morphometric factors were available for 2874 samples. Egg thiamine concentrations were determined via rapid solid-phase extraction (RSPE) for the years 2005-2020, following the method of Zajicek et al. (2005). A conversion factor of -2.0825 nmol/g was applied to RSPE determined total egg thiamine concentrations to align with values determined via HPLC (Riley et al. 2011).

2.2. Sample Collection

Lake trout were collected using bottom-set, monofilament gillnets set overnight at five sites in Lake Ontario between August and October 2021 and 2022 by the U.S. Geological Survey Lake Ontario Biological Station (USGS LOBS) and the New York State Department of Environmental Conservation (NYSDEC). Survey gillnets consisted of nine 15.2- x 2.4-m panels of 51- to 151-mm mesh (stretched measure) in 12.5-mm increments. In addition, lake trout were collected at Bald Eagle Marina in 2021 and Olcott in 2022 using monofilament gillnets using the R/V Madtom. Lake trout Collections from Lake Superior took place in Marquette, - Michigan, in 2022 using multi-filament, bottom-set gill nets with 11.4, 12.7, 14.0, 15.2 cm meshes (stretch measure) Michigan Department of Natural Resources (MIDNR) Marquette Fisheries Research Station. Additional collections in support of the Great Lakes lake trout thiamine monitoring program in 2021 (Rinchar et al. 2022) were conducted by the NYSDEC in Cayuga Lake, the University of Vermont

in Lake Champlain, the NYSDEC and USGS Lake Erie Biological Station (USGS-LEBS) in Lake Erie, U.S. Fish and Wildlife Service Fish and Wildlife Conservation Office Alpena (USFWS FWCO-Alpena) in Lake Huron, U.S. Fish and Wildlife Service Fish and Wildlife Conservation Office Green Bay (USFWS FWCO-Green Bay), Grand Traverse Bay Band of Ottawa and Chippewa Indians, Little Traverse Bay Band of Odawa Indians, Illinois Department of Natural Resources (ILDNR), Wisconsin Department of Natural Resources (WIDNR), and the USGS Great Lakes Science Center (USGS GLSC) in Lake Michigan, and the WIDNR in Lake Superior. Additional collections in 2022 were conducted by the NYSDEC in Cayuga Lake, the University of Vermont in Lake Champlain, the NYSDEC and USGS-LEBS in Lake Erie, USFWS FWCO-Alpena and MIDNR in Lake Huron, USFWS FWCO-Green Bay, Grand Traverse Bay Band of Ottawa and Chippewa Indians, Little Traverse Bay Band of Odawa Indians, ILDNR, WIDNR, Illinois Natural History Survey and the USGS Great Lakes Science Center (USGS GLSC) in Lake Michigan, and the WIDNR in Lake Superior (Rinhard et al. 2023). Upon capture, total length (mm), mass (g), and sex of lake trout were recorded. Eggs were collected from each captured mature female. Coded wire tags were removed from heads and de-coded to determine stocking time and/or location and strain identification. Samples were individually bagged, labelled, and frozen on dry ice for transport to SUNY Brockport, where they were stored at -80°C until processing. The total number of lake trout egg samples collected and processed to determine egg thiamine concentrations were 319 in 2021 and 495 in 2022.

2.3. Thiamine Analysis

Thiamine was extracted from two 1-g portions of eggs from each sample following the method of Brown et al (1998). Samples were processed in duplicate with method blanks for quality assurance. After extraction, thiamine was quantified via high performance liquid chromatography (HPLC) (Agilent Technologies 1200 series, Agilent Technologies Inc., Santa Clara, CA) following Brown et al. (1998) with modifications (Futia et al. 2017). Known concentrations of thiamine (0, 1, 2.5, 5, 10, and 30 nmol/g) were used to establish a six-point curve (Futia et al. 2017). The concentration of the three thiamine vitamers: free thiamine (TH), thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP) were calculated using their standard curves and combined to calculate total egg thiamine concentration. Thiamine concentration is expressed in nmol thiamine/g egg.

2.4. Statistical Analysis

Statistical analysis was conducted using R (R Core Team 2023: version 4.2.3). To meet assumptions of normality for analysis of variance (ANOVA), total egg thiamine was natural log transformed. All tests were considered significant at $\alpha = 0.05$. ANOVA using the R package *car* (v3.1.1; Fox and Weisberg 2019) was used to compare lake trout egg thiamine concentrations among three lake trout strains within Lake Ontario: Seneca (SEN), Lake Champlain domestic (LCD), and Klondike (SKW), using Tukey post-hoc test. A Linear mixed effect model was constructed using the *lme4* R package (v1.1.31; Bates et al. 2015) and applied to Lake Ontario

with lake trout total length (L in mm), weight (W in g), condition factor ($K = W(g) * \frac{100}{L(cm)^3}$), age, and strain as candidate fixed effects and sampling site as a random effect. Goodness of fit testing was performed using the DHARMA R package (v0.4.6; Hartig 2022).

3. Results

3.1. Long-Term Trends

Lake trout egg thiamine concentrations from 2009 to 2022 in Lake Superior were consistently above the 4 nmol/g threshold recommended by Bronte et al. (2008) (Figure 2). In the eastern basin of Lake Erie, lake trout egg thiamine concentrations were all above 4 nmol/g in 4 of the 5 years (2010, 2011, 2012, 2021 and 2022) where samples were collected. In 2012, two of the five lake trout collected in Lake Erie had egg thiamine concentrations below 4 nmol/g (Figure 3). In Lake Ontario, some individual lake trout produced eggs with thiamine concentrations below 4 nmol/g in all locations sampled in nearly every year of the time series (Figure 4). Lake trout collected at Hamlin Beach produced both the highest and lowest mean egg thiamine concentrations among Lake Ontario lake trout with a mean concentration of 13.77 nmol/g in 2022 and a mean concentration of 2.54 nmol/g in 2012. In Lake Michigan all mean total egg thiamine concentrations were below the 4 nmol/g threshold from 1996 to 2001 (Figure 5). Lake trout egg thiamine concentrations in Lake Michigan increased throughout the lake between 2004 and 2009 and remained above concentrations measured between 1996 and 2004 through to the end of the time series

(2022), but were highly variable between years and sampling sites. Mean lake trout egg thiamine concentrations for Lake Huron were at or below 4 nmol/g prior to 2005 (Figure 6). Since 2005, mean lake trout egg thiamine concentrations have not fallen below 4 nmol/g at any site in Lake Huron. Lake trout from Cayuga Lake had declining egg thiamine concentrations from 2009 to 2013, reaching their lowest mean concentration in 2013 at 1.3 nmol/g (Figure 7A), and then increased for 4 consecutive years (2014-2017), reaching their highest mean concentration in 2017 (12.24 nmol/g). Mean egg thiamine concentrations in lake trout from Cayuga Lake have since decreased, reaching 4.72 nmol/g in 2022. In Lake Champlain, the first year (2004) of lake trout egg thiamine measurements had the highest mean concentration of the lake's time series (2004-2022) at 11.1 nmol/g (Figure 7B). Mean egg thiamine concentrations in Lake Champlain then decreased continuously until reaching their lowest point in the time series in 2009 at 2.9 nmol/g. Egg thiamine concentrations have since risen in Lake Champlain with the most recent lake trout mean egg thiamine concentration to fall below 4 nmol/g occurring in 2011. However, only 2021 had no individuals with egg thiamine concentrations below 4 nmol/g in Lake Champlain, with the lowest concentration at 4.05 nmol/g.

3.2. Morphometric Factors Influencing Egg Thiamine Concentrations

Egg total thiamine concentrations were significantly different among lake trout strains in Lake Ontario (ANOVA, $F = 18.47$, $p < 0.001$) (Table 1). SKW strain lake trout had significantly higher total egg thiamine concentrations than LCD or

SEN (Tukey SKW vs. SEN $p < 0.001$; SKW:LCD $p < 0.001$). LCD and SEN strain's total egg thiamine concentrations were not significantly different (Tukey LCD vs. SEN, $p = 0.783$) (Figure 8).

Weight and condition factor were dropped from the Lake Ontario model due to collinearity with length. Length was chosen as the variable to be kept as it is the most abundantly collected of the three variables and therefore provides the largest sample size. Age was not significant ($p = 0.06$) and was dropped from the analysis. The resulting model contained strain and total length as fixed effects and sampling site as a random effect. QQ plot residual distribution was normal and passed Kolmogorov-Smirnov, dispersion, and outliers tests ($p = 0.99$; $p = 0.67$; $p = 1$, respectively) (Figure 9). Residual by predicted values were normally distributed (Figure 10). Total length had a negative main effect of $\beta = -0.143$ (SE = 0.064, $p = 0.027$) and the SKW strain had a positive main effect of $\beta = 0.293$ (SE = 0.156, $p = 0.0617$). No other strains had significant main effects.

4. Discussion

4.1. Long-Term Trends and Prey Base Changes.

Long-term trends in lake trout egg thiamine concentrations are consistently linked with the prey fish community in each of the lakes. Lake Erie has a limited population of alewife likely due to cold water temperatures in winter (O'Gorman et al. 2012). However, rainbow smelt are abundant in Lake Erie and are known to have ~50% the thiaminase activity found in alewife, though this difference was not

significant due to a high degree of variation in thiaminase activity among locations and seasons (Tillitt 2005). From 2001 to 2022, rainbow smelt have been recorded each year in 50-100% of non-empty lake trout stomachs collected in gill net surveys in the eastern basin of Lake Erie, with the majority of years having rainbow smelt recorded in >70% of non-empty stomachs (Lake Erie Committee 2023). The second most consumed prey fish species in that time series was round goby which occurred in <40% of non-empty stomachs in all but three years. The lowest mean lake trout egg thiamine concentration measured in Lake Erie was in 2012 when lake trout consumed the highest ratio of rainbow smelt to round goby, and was the only year where any sampled lake trout had egg thiamine concentrations below 4 nmol/g in Lake Erie. Overall, 2012 may present an instance in Lake Erie where TDC was induced by lake trout feeding on a diet rich in rainbow smelt. However, stomach content analysis only captures a limited window of an individual's diet prior to capture and may not be representative of the diet over the course of the induction period of TDC.

Lake Superior is located at the northern edge of the range of alewife and, in part due to consistently cold water temperatures, has historically had very low abundances of alewife (O'Gorman et al. 2012). However, rainbow smelt are abundant in Lake Superior particularly in near shore pelagic zones in a prey community that is dominated by native species (Gorman et al. 2021, Edwards 2023). Rainbow smelt are a major part of lean lake trout diets in many areas of Lake Superior, representing up to 90% of their spring diet (Ray et al. 2007). For the years 1985 to 2000, Ray et al.

(2007) reported lean lake trout diet compositions ranging between 60 to 95% rainbow smelt. Sitar (2017) reported wild origin lean lake trout diet compositions (spring and summer) consisting of up to ~60% rainbow smelt for the years 1998 to 2007, but notably larger individuals had reduced rainbow smelt intake in favor of native prey species such as Coregonines. More recently, Gerig et al. (2024) reported lean lake trout diet compositions (summer survey) consisting of ~25% to ~60% rainbow smelt for the years 2019 and 2020, but with larger individuals having higher rainbow smelt intake. Lastly, Conner et al. (1993) reported lake trout diet compositions (all seasons) in Lake Superior of ~40% to ~100% for the years 1981 to 1987. Interestingly, Conner et al. (1993) also reported seasonal variation in rainbow smelt intake with the lowest intake occurring in the months prior to spawning (June to October). The intake of thiaminase from consuming rainbow smelt in Lake Superior lake trout is seasonal (mostly in spring) and may be mitigated by the diversity of prey consumed throughout the year. However, monitoring of egg thiamine concentrations in Lake Superior is limited and continued monitoring will be necessary to determine if intermittent low egg thiamine concentrations occur in Lake Superior lake trout like that observed in Lake Erie lake trout.

The prey fish community in Lake Huron experienced dramatic declines in the early 2000's including the crash of the lake's alewife population in 2003 (Riley et al. 2008, 2010). A subsequent shift in the lake trout diet in Lake Huron was documented, transitioning from an alewife dominated diet to a diet with roughly equal intake of rainbow smelt, round goby, and other prey fishes (Madenjian et al. 2006, Roseman et

al. 2014). Lake trout egg thiamine concentrations in Lake Huron prior to the collapse of Lake Huron's alewife population were consistently under 4 nmol/g. Following the collapse of Lake Huron's alewife population, mean lake trout egg thiamine concentrations in Lake Huron increased continuously from 2004 to 2009 and have from 2009 to 2022 remained above 4 nmol/g. Additionally, recruitment of wild lake trout in Lake Huron increased following the collapse of alewife. Increased lake trout egg thiamine concentrations in Lake Huron, reductions in sea lamprey wounding rates, and increased spawner abundance all appear to be contributors to increased recruitment of wild lake trout in Lake Huron (Fitzsimons et al. 2010, He et al. 2012).

Alewife abundance in Lake Michigan decreased in the early 2000's but not to the same extent as Lake Huron (Madenjian et al. 2010, Riley et al. 2011). Riley et al. (2011) found a correlation between site specific alewife abundance and lake trout egg thiamine concentrations in Lake Michigan and noted a transition in the diet of Lake Michigan lake trout from an alewife dominated diet in 1973-1995 to a diet of mostly rainbow smelt in 2006-2008. However, while lake trout egg thiamine concentrations in Lake Michigan increased following alewife abundance reductions in 2003-2004 and have remained above concentrations measured in the late 1990's, the importance of alewife in Lake Michigan lake trout diets in 2015-2018 was similar to that of 1994-1995 (Madenjian et al. 2022). This may indicate that additional factors beyond the proportion of alewife in the diet such as variability in alewife thiaminase activity, decreased alewife lipid content and energy density, and increased consumption of round goby, contributed to increases in lake trout egg thiamine concentrations in Lake

Michigan (Madenjian et al. 2022). Regardless, CPUE of wild lake trout in Lake Michigan spring surveys increased more than tenfold from 2005 to 2022 (Lake Michigan Lake Trout Working Group 2024) (Figure 11). The proportion of wild lake trout to stocked lake trout captured, increased during this period as well but, this may largely be due to reductions in stocking (Lake Michigan Lake Trout Working Group 2024).

Alewife have maintained a robust population since invading Lake Ontario and accounted for ~85% of the prey base in Lake Ontario in 2022 (Eshenroder and Lantry 2012, Weidel et al. 2022). Temporal variability in lake trout egg thiamine concentrations has been high throughout the time series. The causes of the lake-wide temporal variability in lake trout egg thiamine concentrations are yet to be understood but may be influenced by similar factors hypothesized to be influencing Lake Michigan lake trout egg thiamine concentrations such as variability in alewife thiaminase activity and, variability in alewife lipid content and energy density. Differences in lake trout egg thiamine concentrations among strains have been documented in Lake Ontario and are related to round goby intake in proportion to alewife intake being greater for SKWs than other strains (Heisey et al. 2023).

Thiamine monitoring in Lake Champlain began in 2004, following the 2003 invasion of the alewife (Ladago et al. 2020). A precipitous decline in lake trout egg thiamine concentrations followed, reaching their lowest mean concentration in 2009. Lake trout egg thiamine concentrations have rebounded somewhat but have not returned to their 2004 high. Interestingly, natural recruitment of lake trout in Lake

Champlain was not documented until 2015 (Marsden et al. 2018). Moreover, the higher lipid content of alewife relative to other prey items in Lake Champlain, along with their lower trophic status compared to rainbow smelt, and the overall increase in pelagic prey items that the inclusion of alewife to the food web created, may have provided an energy influx necessary to increase juvenile survival and natural recruitment (Lesser et al. 2023).

In Cayuga Lake, alewife have been present since at least the late 1800's and thiamine deficiency in salmonids was present in Cayuga Lake as early as 1974 with the documentation of symptoms in fry which would later be recognized as the same deficiency as EMS in Great Lakes salmonids (Eaton 1928, Fitzsimons et al. 1998, Fisher et al. 1998). Round goby were first detected in Cayuga Lake in 2013 and were documented in the diet of the lake's lake trout population indirectly via fatty acid signature analysis in 2014 and directly via stomach content analysis in 2015 (Kraus 2018). In the four years following the discovery of round goby in Cayuga Lake (2014-2017), lake trout egg thiamine concentrations continuously increased, however, by 2021 and 2022, lake trout egg thiamine concentrations in Cayuga lake had returned to similar concentrations to those measured prior to the introduction of round goby. An initial over-proliferation of round goby to an unsustainable population size following their introduction to Cayuga Lake, followed by a reduction to a more sustainable population size would be in line with round goby population dynamics as modeled by Young (2009), and would largely explain the trend in lake trout egg thiamine concentrations in Cayuga Lake, assuming that the proportion of round goby

in the diet of lake trout in Cayuga Lake reflected these fluctuations in the round goby population.

4.2. Lake Trout Strains

Heisey et al. (2023) found that Klondike strain lake trout had significantly higher egg thiamine concentrations than Lake Champlain domestic or Seneca strain lake trout in Lake Ontario. Moreover, through analysis of lake trout fatty acid signatures Heisey et al. (2023) found that Klondike strain lake trout had a diet consisting of a higher proportion of round goby than the diets of Seneca or Lake Champlain strain lake trout in Lake Ontario. This indicates a more benthically oriented diet among Klondike strain lake trout as opposed to a more pelagically oriented diet among Seneca and Lake Champlain strain lake trout consisting of a higher proportion of alewife in the diet. Differences in egg thiamine concentrations among lake trout strains within this dataset are in line with the findings of Heisey et al. (2023) and provide further evidence of differences in feeding ecology influencing lake trout egg thiamine concentrations.

4.3. Lake Ontario Model

The negative relationship among length and log total egg thiamine concentration seen in the Lake Ontario model is likely indicative of the ontogenetic shift thought to occur in lake trout between ~450 mm and 600 mm in length (Fitzsimons 2022) (Table 1). This shift involves a dietary change from a round goby

dominated diet to an alewife dominated diet. The impact of strain as a fixed effect and specifically the Klondike strain, may be indicative of differences in the timing of this shift or the lack of a shift in diet among Klondikes, as Klondike strain lake trout maintain a smaller size than Seneca or Lake Champlain strain lake trout and have been shown to have a more benthically oriented diet, potentially never experiencing a dietary shift from round goby to alewife and subsequent reduction in egg thiamine concentrations (Fitzsimons 2022, Heisey et al. 2023). However, specific delineation of an ontogenetic shift in lake trout diet via egg thiamine concentrations is likely not feasible given the delayed onset of TDC following dietary changes (Honeyfield 2005). The reduction in egg thiamine concentration with increasing length is the most obvious among Klondike strain lake trout but, Klondike strain lake trout also have the best representation of smaller sizes in this dataset as they appear in spawning surveys at smaller sizes. A diet study like that of Fitzsimons (2022), but with the modern suite of lake trout strains present in Lake Ontario, may provide some clarity as to whether differences in feeding ecology among specific lake trout strains or an ontogenetic shift in diet among all strains is driving the relationship between length and lake trout egg thiamine concentration.

5. Conclusions

The long-term trends of thiamine deficiency among Great Lakes lake trout ultimately derive from changes diet. Both changes in alewife populations and in the diet of the lake trout strains chosen for stocking have been documented as significant

influences on lake trout egg thiamine concentrations. Lean lake trout continue to prove more susceptible to TDC through a higher proportion of alewife in their diet in comparison to Klondike strain lake trout. Alewife will likely continue to be a major prey for lake trout for the foreseeable future and the diversification of lake trout population through the inclusion of more benthically oriented strains may provide an avenue for some limitation of TDC impact. However, these actions would also need to be weighed against the benefits of reduced sea lamprey predation among lean lake trout. Furthermore, habitat degradation, particularly degradation of spawning reefs, remains a strong impediment to the restoration effort (Binder et al. 2018, Furgal et al. 2019). The restoration of native prey such as coregonines could provide needed diversification to the lake trout diet and potentially reduce the prevalence of TDC, though successful restoration of coregonines in the lower Great Lakes is a distant goal. Overall, management efforts to support a reduction in TDC like prey community diversification, introducing TDC tolerant lake trout strains, and habitat remediation may prove to be advantageous to the restoration effort.

Table 1. Lake Ontario linear mixed effects model summary table. Positive effects are present for the two more benthically oriented lake trout strains (SKW) and (STW). A negative effect is present for total length (cm). TTH = total egg thiamine, SKW = Klondike strain, SAW = Apostle Islands strain, SEN = Seneca strain, LCD = Lake Champlain strain, STW = Lake Superior strain, CI = confidence interval, ICC = intraclass correlation coefficient.

<i>Predictors</i>	logTTH		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(Intercept)	3.09	2.20 – 3.98	<0.001
Length cm	-0.14	-0.27 – -0.02	0.027
Strain [LCD]	-0.07	-0.38 – 0.23	0.637
Strain [SAW]	-0.07	-0.68 – 0.54	0.823
Strain [SEN]	-0.01	-0.31 – 0.29	0.948
Strain [SKW]	0.29	-0.01 – 0.60	0.062
Strain [STW]	0.29	-0.10 – 0.67	0.144
Random Effects			
σ^2	0.23		
τ_{00} Site	0.00		
ICC	0.01		
N_{Site}	18		
Observations	210		
Marginal R^2 / Conditional R^2	0.172 / 0.184		



Figure 1. Lake trout egg collection sites of the Lake Trout Egg Thiamine Monitoring Program in 2021 and 2022.

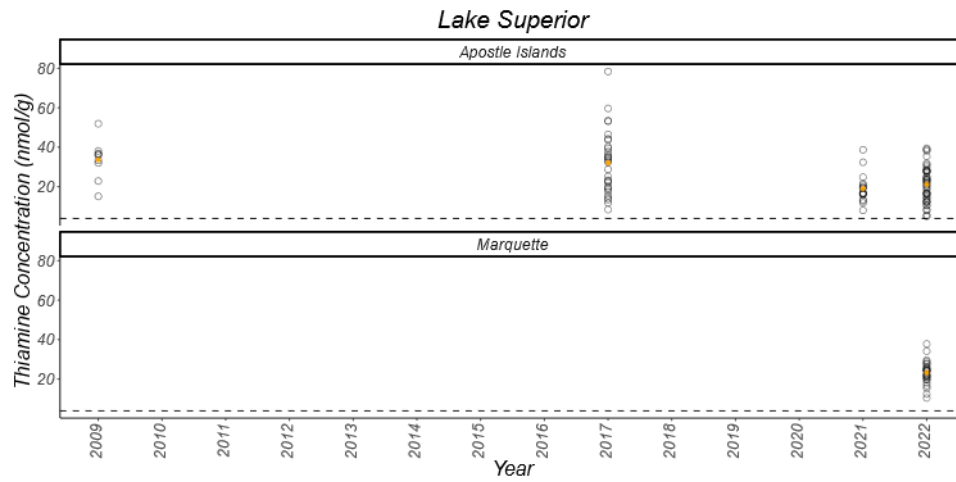


Figure 2. Total egg thiamine of lake trout eggs sampled in Lake Superior. Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

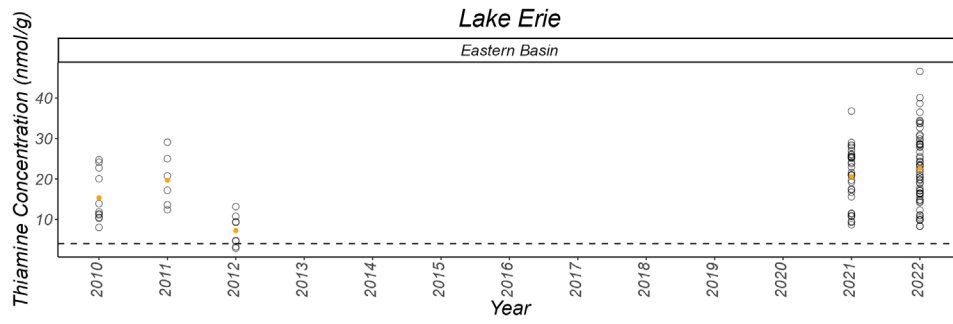


Figure 3. Total egg thiamine of lake trout eggs sampled in Lake Erie. Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

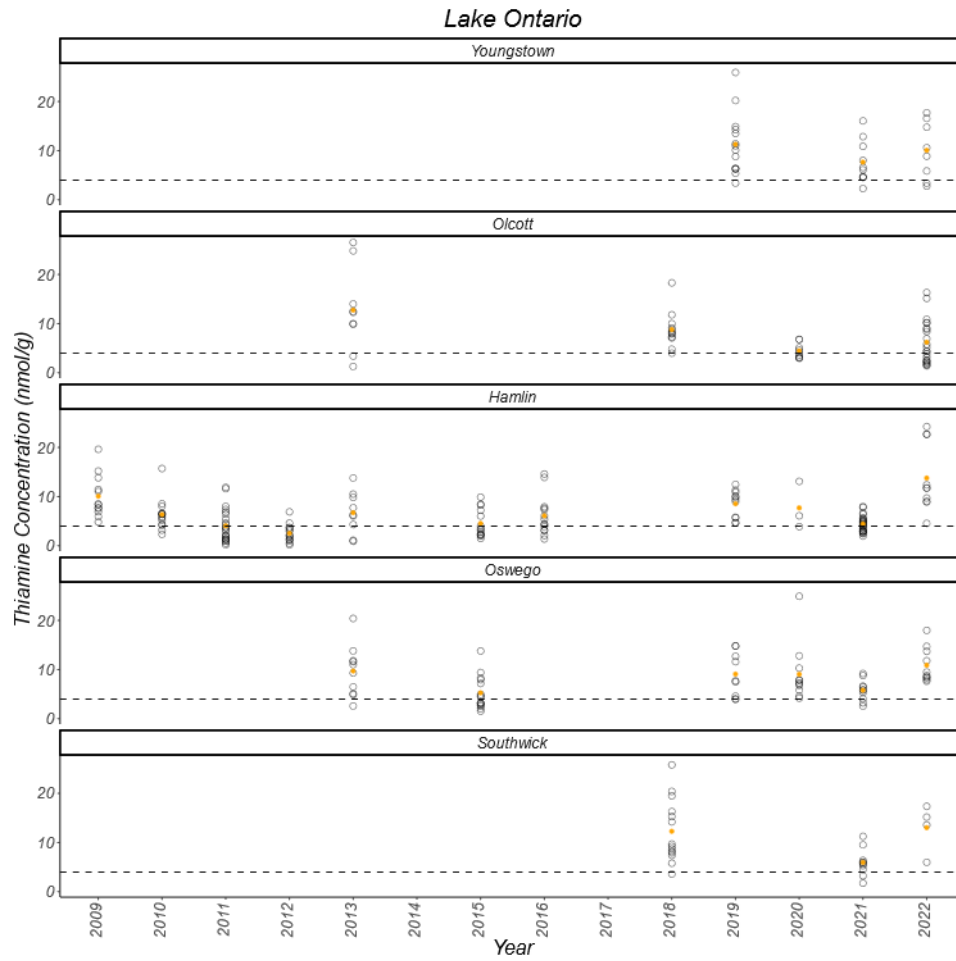


Figure 4. Total egg thiamine of lake trout eggs sampled in Lake Ontario. Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

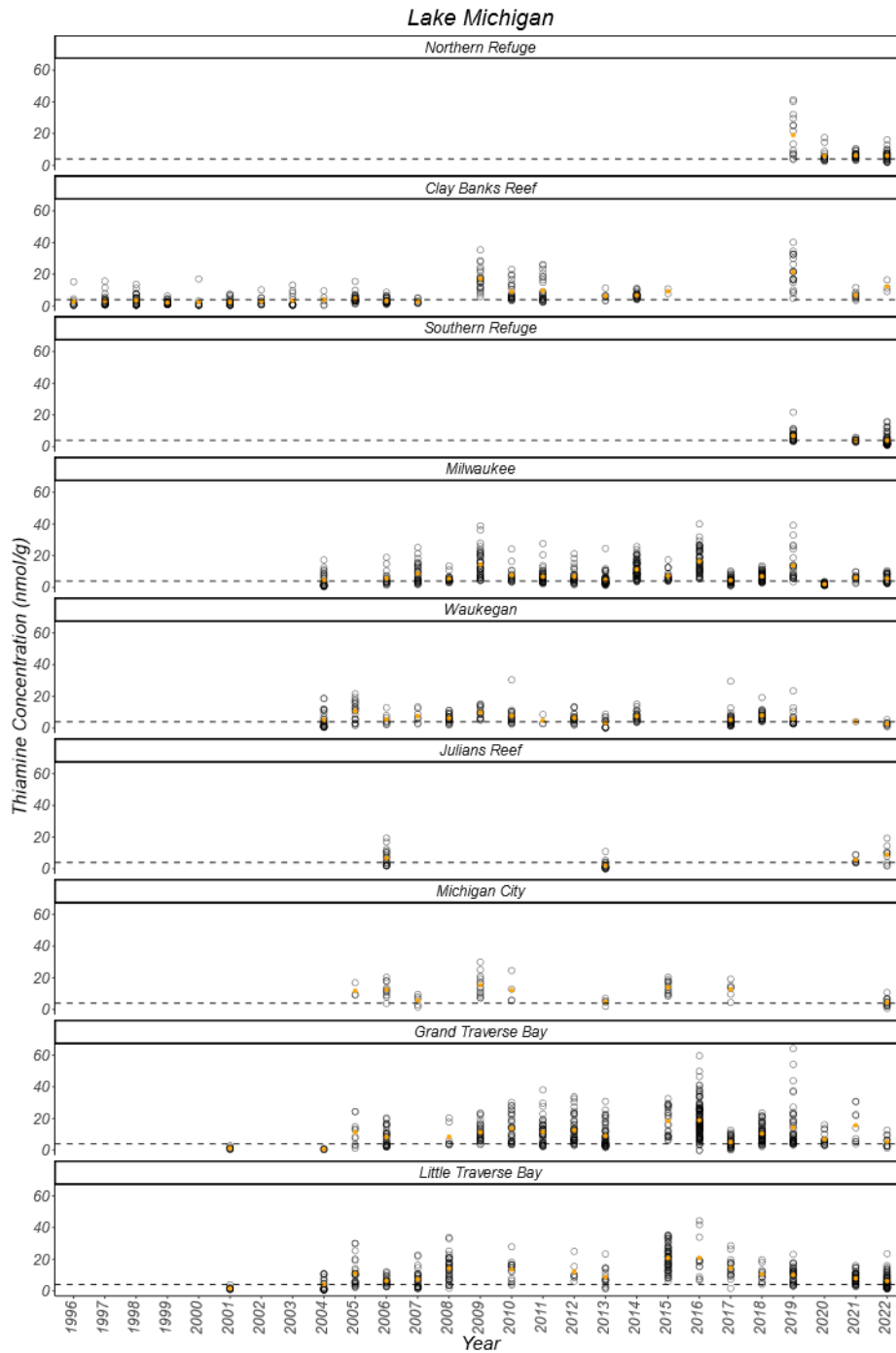


Figure 5. Total egg thiamine of lake trout eggs sampled in Lake Michigan. Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

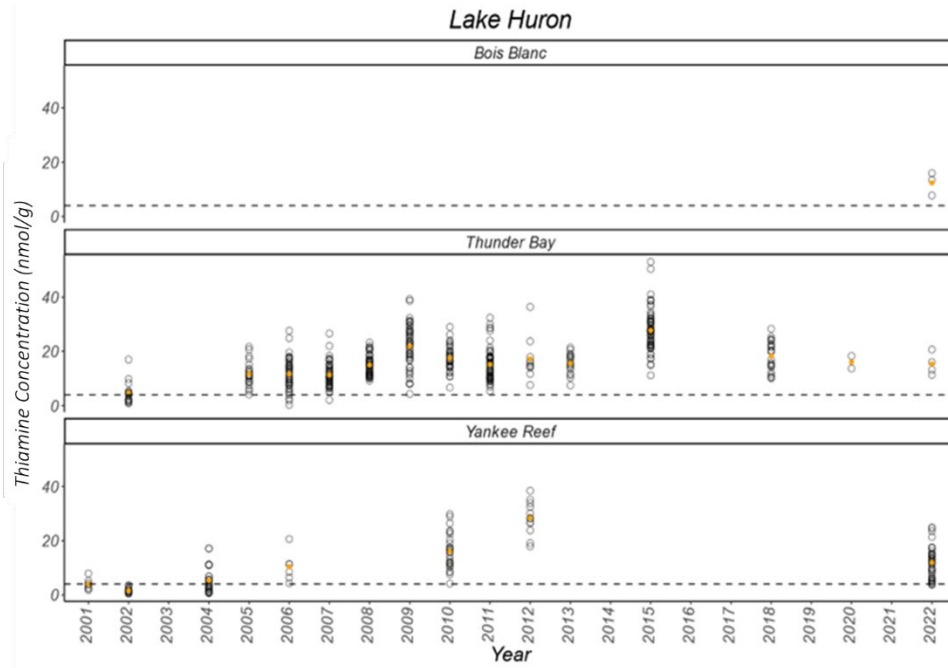


Figure 6. Total egg thiamine of lake trout eggs sampled in Lake Huron. Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

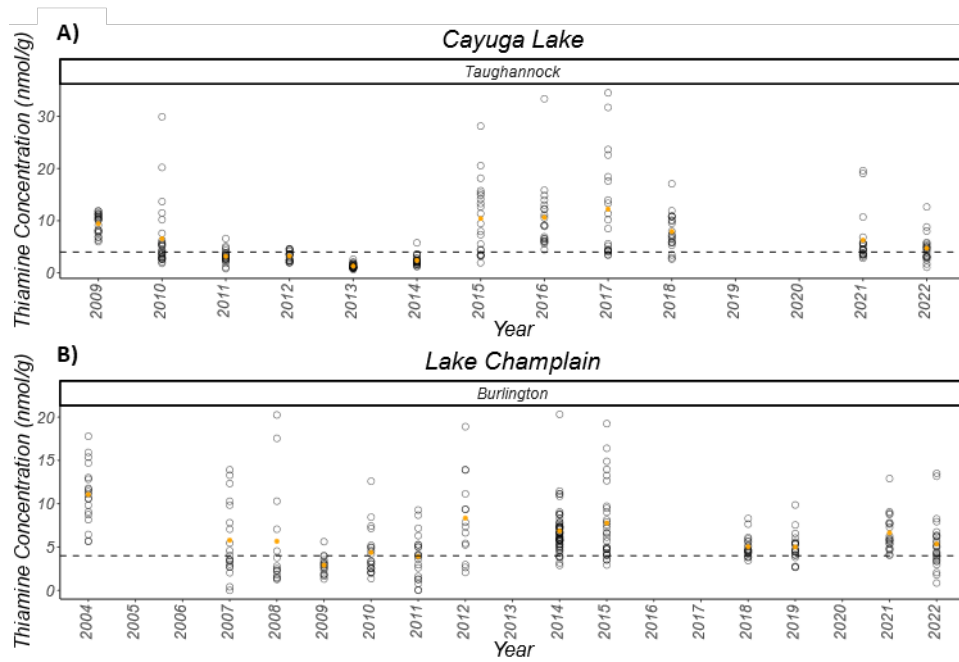


Figure 7. A) Total egg thiamine of lake trout eggs sampled in Cayuga Lake (2009-2022). B) Total egg thiamine of lake trout egg sampled in Lake Champlain (2004-2022). Open circles represent individual female lake trout, orange circles represent the mean, black dashed line represents the 4 nmol/g management target thiamine concentration.

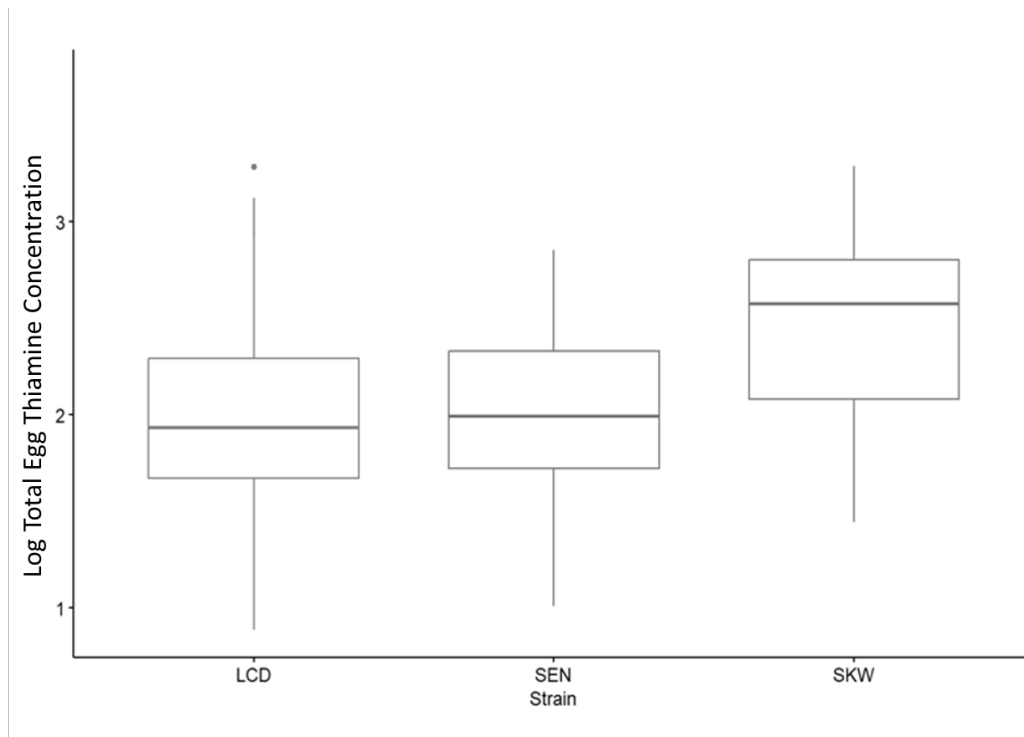


Figure 8. Box plot of total lake trout egg thiamine concentration in Lake Ontario by lake trout strain. Klondike (SKW) strain had significantly higher log total egg thiamine concentration than Seneca (SEN) and Lake Champlain (LCD) strains.

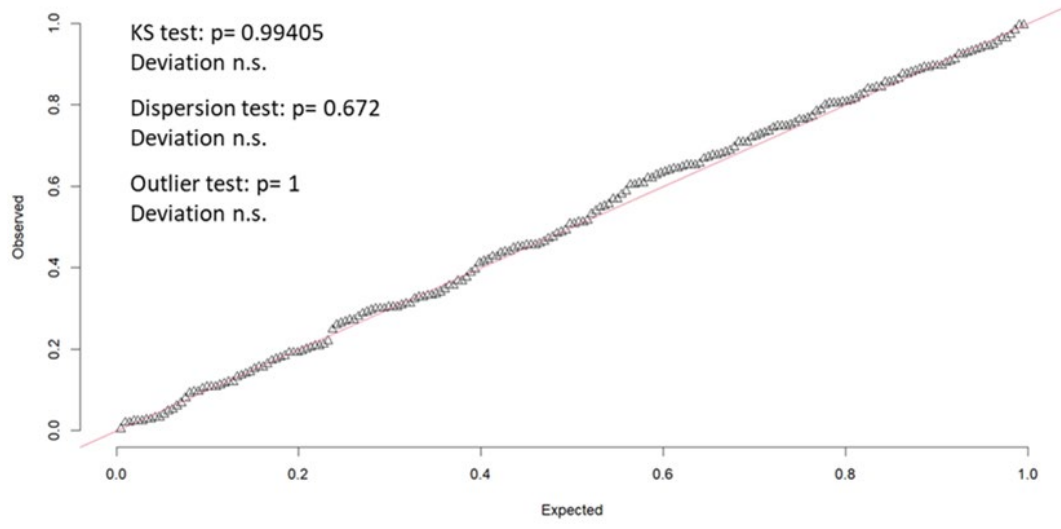


Figure 9. Q-Q plot of the Lake Ontario linear mixed effects model. Residuals are normally distributed and pass KS, dispersion, and outlier tests.

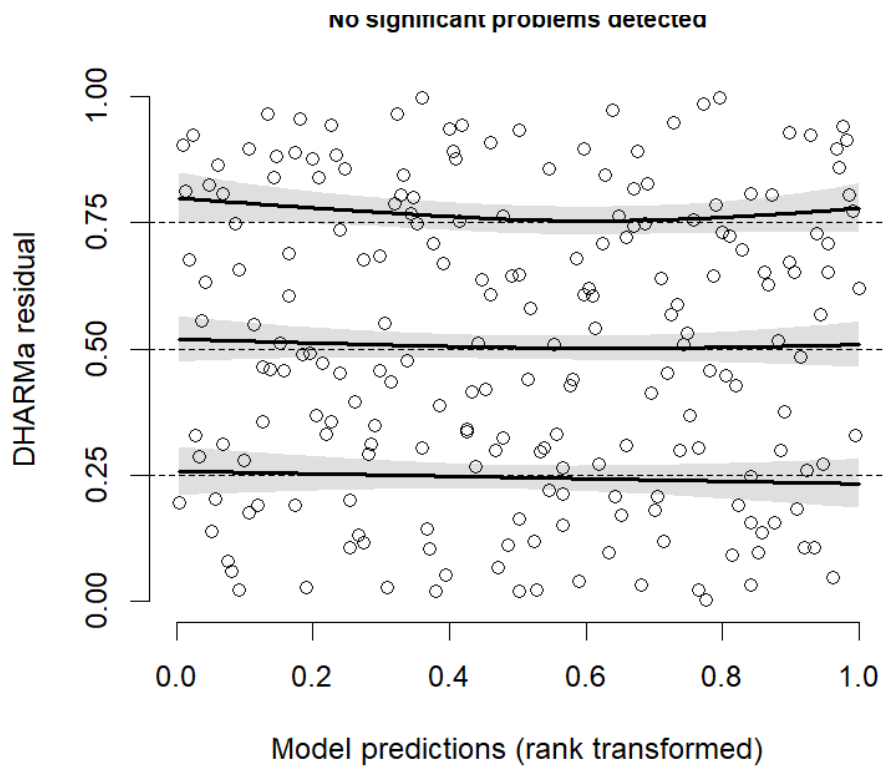


Figure 90. Residual vs predicted values for the Lake Ontario model. The deviation of the actual distribution (solid black lines) from the expected distribution (dashed black lines) was not significant, residuals are normally distributed.

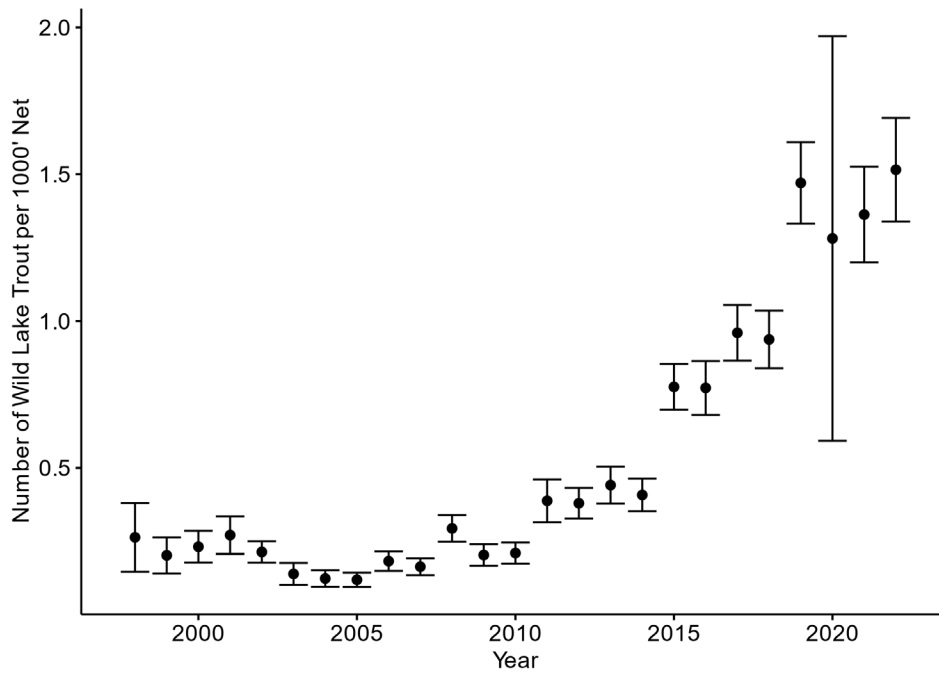


Figure 101. Lake-wide catch per unit effort (mean number of fish per 1000 ft of gill net) of wild lake trout in Lake Michigan spring surveys from 1998 to 2022. Black dots represent lake wide means and vertical bars \pm standard error. Data provided by USFWS- Green Bay FWCO. Modified from Lake Michigan Lake Trout Working Group (2024).

Chapter 2: Factors Influencing Lake Trout Egg Thiamine Concentrations in Lake Ontario

Abstract

The restoration of lake trout in the Great Lakes has been an ongoing effort for nearly 60 years. Stocking of adults has seen some success in generating self-sustaining populations in lakes with limited alewife (*Alosa pseudoharengus*) availability. Diets rich in alewife have been consistently shown to lead to thiamine deficiency complex (TDC). Insufficient maternal deposition of thiamine (vitamin B₁) into eggs during development is a major consequence of TDC and leads to mass mortality of fry prior to their first feeding. The specific mechanism behind the induction of TDC by a diet rich in alewife is yet to be fully explained. The thiamine degrading enzyme thiaminase I is present in the visceral organs of alewife and represents the leading hypothesized cause of TDC. However, oxidative stress in the form of lipid peroxidation and the consumption of thiamine through its role as an antioxidant is thought to be the mechanism behind thiamine deficiency in Atlantic Salmon (*Salmo salar*) in Northern Europe. In this chapter we explored relationships among lake trout egg thiamine concentrations, belly flap fatty acid compositions and concentrations of the primary lipid peroxidation disrupting antioxidant, α -tocopherol (vitamin E), in lake trout livers and eggs. α -tocopherol concentrations in lake trout eggs appeared to be driven by diet and have a negative relationship with length in both Lake Ontario and Lake Superior. Egg and liver α -tocopherol concentrations did not appear to have

a direct impact on egg thiamine concentrations. Integrating recent findings on the roles of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in cellular antioxidant defense activation into a calculation of peroxidation potential, led to an improved negative relationship between belly flap unsaturation index and lake trout egg thiamine concentrations, potentially implicating lipid peroxidation as a source of thiamine consumption.

1. Objectives

The first objective of this chapter was to explore the potential role of lipid peroxidation in the induction of TDC in lake trout by concurrently measuring lake trout egg thiamine concentrations, liver α -tocopherol concentrations, and egg α -tocopherol concentrations of two lake trout populations, one with TDC present, Lake Ontario, and one without any documented occurrence of TDC, Lake Superior. The resulting dataset was then compared between two populations and analyzed to identify relationships (1) between these vitamins (thiamine and α -tocopherol) and (2) between each vitamin and morphometric factors. The second objective of this chapter was to measure belly flap fatty acid compositions of Lake Ontario lake trout, determine unsaturation indexes and examine any relationships with concurrent egg and liver α -tocopherol concentrations, egg thiamine concentrations, and unsaturation indexes. I hypothesized that: 1) egg and liver α -tocopherol concentrations would have a positive relationship with egg thiamine concentrations; 2) belly flap unsaturation index would have a negative relationship with egg thiamine concentration; 3) egg

thiamine concentrations, egg α -tocopherol concentrations and liver α -tocopherol concentrations would be higher among Klondike lake trout in Lake Ontario and Lake Superior lake trout than egg thiamine concentrations, egg α -tocopherol concentrations and liver α -tocopherol concentrations among Lake Ontario lean (all lean derived strains combined) lake trout.

2. Materials and Methods

2.1. Sample Collection

Lake trout were collected using bottom-set, monofilament gillnets set overnight at five sites in Lake Ontario between August and October 2021 and 2022 by the U.S. Geological Survey Lake Ontario Biological Station (USGS LOBS) and the New York State Department of Environmental Conservation (NYSDEC). Survey gillnets consisted of nine 15.2- x 2.4-m panels of 51- to 151-mm mesh (stretched measure) in 12.5-mm increments. In addition, lake trout were collected at Bald Eagle Marina in 2021 and Olcott in 2022 using monofilament gillnets using the R/V Madtom. Lake trout Collections from Lake Superior took place in Marquette Michigan in 2022 using multi-filament, bottom-set gill nets with 11.4, 12.7, 14.0, 15.2 cm meshes (stretch measure) Michigan Department of Natural Resources (MIDNR) Marquette Fisheries Research Station. Upon capture, total length (mm) and mass (g) of female lake trout were recorded. For Lake Ontario, their livers, belly flaps, and eggs were collected whereas for Lake Superior, only livers and eggs were collected. Coded wire tags were removed from Lake Ontario lake trout heads and

read to identify strain. Livers had their gallbladder carefully detached to prevent contamination by bile. Belly flaps were sampled to assess fatty acid composition as an indicator for lake trout diet (Budge et al. 2011, Happel et al. 2020). Belly flap collections consisted of a 2 x 2 in. section removed from the underside of the fish, adjacent to the pelvic fins. Eggs and tissue samples were individually bagged, labeled, and frozen on dry ice prior to being transferred to SUNY Brockport, where they were stored at -80°C until processing.

2.2. Thiamine Analysis

For each female lake trout sampled, thiamine was extracted from 1 g of eggs following the method of Brown et al. (1998). Samples were processed in duplicate with method blanks for quality assurance. After extraction, thiamine was quantified via high performance liquid chromatography (HPLC) (Agilent Technologies 1200 series, Agilent Technologies Inc., Santa Clara, CA) following Brown et al. (1998) with modifications (Futia et al. 2017). Known concentrations of thiamine (0, 1, 2.5, 5, 10, and 30 nmol/g) were used to establish a six-point curve (Futia et al. 2017). The concentration of the three thiamine vitamers: free thiamine (TH), thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP) were calculated using their standard curves and combined to calculate total egg thiamine concentration. Thiamine concentration is expressed in nmol/g.

2.3. *α*-Tocopherol Analysis

2.3.1. *α*-Tocopherol Extraction

Egg subsamples (~10 to 20 g) and whole livers were homogenized on dry ice in a coffee grinder. Extractions of *α*-tocopherol were conducted by vortexing a 0.2 g subsample in 5.5 ml of saponification solution. Saponification solution was made by combining absolute ethanol (Thermo Fisher Scientific Inc., Waltham, MA) and nano-pure water (55:45) with 11 % (w:v) potassium hydroxide (Sigma Aldrich Inc., St. Louis, MO). Prior to vortexing, 0.2 g of L-ascorbic acid (Sigma Aldrich Inc.) was added to samples, preventing *α*-tocopherol degradation by radical oxygen species during processing. Dim light was used throughout the extraction process to minimize photodegradation of the protective ascorbic acid additions. Following vortexing, sample containers were purged with nitrogen gas, sealed, and placed in a water bath at 80°C for 15 min, then cooled in tap water for one minute. Six ml of n-hexane (Thermo Fisher Scientific Inc.) that contains 25 mg/ml of butylated hydroxytoluene (BHT) was added, samples were vortexed for 2 min and centrifuged at 1500 g for 5 min. One ml of n-hexane was removed, placed in a test tube with a spatula tip of sodium sulfate anhydrous (Thermo Fisher Scientific Inc.). An additional 2 ml of n-hexane that contained 25 mg/mL of BHT was added and the mixture was vortexed for 2 min. One ml of sample was then transferred into a syringe and filtered through a 45 mm diameter, 0.45 μ m PTFE hydrophobic filter (Agilent Technologies Inc., Santa Clara, CA). Then, 0.5 ml of sample was moved to an autosampler vial and analyzed

via HPLC (Agilent Technologies 1200 series, Agilent Technologies Inc.). Samples were processed in duplicate, and values were accepted when the CV was < 15 %. All chemicals used were HPLC grade.

2.3.2. α -Tocopherol Measurement

Hepatic and egg α -tocopherol concentrations were determined via high performance liquid chromatography following the method of Mestre Prates et al. (2006). A normal-phase silica column was used (Zorbax RX-Sil with the corresponding 12.5 mm analytical guard column, 4.6 mm ID x 250 mm, 5 mm particle size, Agilent Technologies Inc.). An excitation wavelength of 295 nm and emission wavelength of 325 nm were used for the detection of α -tocopherol. One percent isopropanol (Thermo Fisher Scientific Inc.) in n-hexane was used as the solvent with a flow rate of 1.5 ml/min. The column temperature was adjusted to 32°C and run times were 7 min. α -tocopherol concentrations were reported in mg/g.

Standard stock solution was created by combining 20 ml of absolute ethanol and 0.32 g of α -tocopherol (Sigma Aldrich Inc.). A five point (7.2, 3.6, 1.8, 0.9, 0.0 mg/ml) standard curve was then generated in n-hexane via serial dilution. Standard solutions were stored in nitrogen purged containers at -20°C. Standard solution concentrations were verified via spectrophotometry using a Beckman DU 640 Spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Spectrophotometrically determined standard solution concentrations were within 5.77 and 7.81% of expected values. Spikes and blanks were processed periodically for

quality assurance. Spikes were generated by processing 0.2 ml of 0.9 mg/ml standard solution in place of a sample. The average recovery from spikes was 91.6%. Blanks were generated by processing 0.0 mg/ml standard solution in place of a sample. Blanks were accepted if the detected α -tocopherol concentration was below 0.00000 mg/g.

2.4. Fatty Acid Analysis

Skin was removed from lake trout belly flap samples prior to belly flap tissue being ground in a coffee grinder. Lipids were extracted from homogenized belly flap samples using a 2:1 chloroform/methanol solvent containing 0.01% BHT and gravimetrically quantified (Folch et al. 1957). A known amount of nonadecanoate acid (19:0; 8 mg/50 mg of lipids) was added to each sample as an internal standard to quantify fatty acids. Extracts were then transmethylated following the method of Metcalfe and Schmitz (1961). The resulting fatty acid methyl esters (FAMES) were quantified via gas chromatography/mass spectrometry (Agilent Technologies 7693 autosampler and Agilent Technologies 5975C inert XL EI/CI mass selective detector with triple-axis detector). A 30 m x 0.25 mm Agilent J&W GC column with 0.25 μ m film thickness (Agilent Technologies Inc.) capillary column was used, with helium as a carrier gas. The oven was run at 175°C for 26 min to 205°C at 2°C per min, followed by 205°C for 24 min. The helium flow rate was 1.8 ml per minute. The source and analyzer temperatures were 230°C. Individual FAMES were identified by the comparison of standard mixture (FAME mix 37 components, Supelco, Bellefonte,

PA) retention times to known FAME spectrographic patterns. FAME spectrographic patterns were acquired from the Association Oil's Chemists' Society mass spectral library and the National Institute of Standards and Technology Mass Spectral Library provided with the GC/MS. Fatty acids are expressed as % of total fatty acid detected and as mg/g sample.

2.5. Statistical Analysis

Statistical analyses were done using R (R Core Team 2023: version 4.2.3). All tests had a significance level set at $\alpha = 0.05$. Kruskal-Wallis with Dunn's post-hoc test and Bonferroni correction were used to compare total egg thiamine concentrations among Lake Ontario Klondike lake trout and Lake Ontario lean lake trout collected in 2021 and 2022, as normality assumptions could not be met. T-tests were used to compare egg α -tocopherol concentrations between years for Lake Ontario samples as normality and homogeneity of variance assumptions were met. Wilcoxon rank sum tests were used to compare liver α -tocopherol concentrations between years in Lake Ontario, as normality assumptions could not be met. Linear regressions were used to determine relationships between total egg thiamine and length (mm), and total egg thiamine and egg α -tocopherol concentrations for samples collected from Lake Ontario and Lake Superior lake trout.

Due to significant differences in total egg thiamine between 2021 and 2022 among Klondike lake trout in Lake Ontario, and due to Lake Superior collections taking place in 2022, only 2022 data from Lake Ontario were used for Lake Superior

to Lake Ontario comparisons. Kruskal-Wallis with Dunn's post-hoc test and Bonferroni correction were used for comparisons of egg α -tocopherol, liver α -tocopherol, total egg thiamine, egg thiamine monophosphate, egg thiamine pyrophosphate, and egg free thiamine concentrations between Lake Ontario leans, Lake Ontario Klondikes, Lake Superior leans and Lake Superior siscowets.

Lake trout fatty acid signatures were analyzed via permutational MANOVA (Vegan:adonis) and Similarity Percentages (SIMPER), using the vegan community ecology package (v2.6-4; Oksanen et al. 2022). Unsaturation index (UI) indicates the degree of unsaturation in lipids as the sum of the percentage of each unsaturated fatty acid multiplied by the number of double bonds within that FA following the formula:
$$UI = 1 \times (\text{sum of the percentages of each fatty acid with 1 double bond; monoenoics}) + 2 \times (\text{sum of the percentages of each fatty acid with 2 double bonds (\%); dienoics}) + 3 \times (\text{sum of the percentages of each fatty acid with 3 double bonds (\%); trienoics}) + 4 \times (\text{sum of the percentages of each fatty acid with 4 double bonds (\%); tetraenoics}) + 5 \times (\text{sum of the percentages of each fatty acid with 5 double bonds (\%); pentaenoics}) + 6 \times (\text{sum of the percentages of each fatty acid with 6 double bonds (\%); hexaenoics})$$
 (Dal Bosco et al. 2022). Analysis of variance (ANOVA) with Tukey's post-hoc test was used to compare unsaturation indexes between Lake Ontario lean and Klondike lake trout across years, as normality and homogeneity of variance assumptions were met. Kruskal-Wallis with Dunn's post-hoc test and Bonferroni correction were used for comparisons of fatty acid ratios in Lake Ontario between Klondike lake trout collected in 2021, lean lake trout collected in 2021, Klondike lake trout collected in

2022 and 2022 collected lean lake trout collected in 2022, as normality assumptions could not be met. Linear regressions were used to determine relationships between egg free thiamine concentrations and belly flap unsaturation index, egg free thiamine and the ratio of 16:1n-7 to 18:1n-9, the ratio of 16:1n-7 to 18:1n-9 and length (mm), the ratio of 16:1n-7 to 18:1n-9 and PUFA, and the ratio of 16:1n-7 to 18:1n-9 and PUFA with DHA and EPA removed for samples collected from Lake Ontario lake trout.

To determine the best predictor(s) of lake trout egg free thiamine (TH) in Lake Ontario, multi-directional stepwise multiple regressions were performed using the Multi-Model Inference package (v1.47.5; Barton 2023), from samples of Klondike and lean lake trout collected in Lake Ontario with length (mm), unsaturation index (UI), ratio of 16:1n-7 to 18:1n-9 fatty acids, egg α -tocopherol concentration (mg/g), liver α -tocopherol concentration (mg/g), percent MUFA, percent saturated fatty acids, and percent PUFA as candidate independent variables and egg free thiamine (TH) concentrations as the dependent variable. Additionally, unsaturation index (UI-DE) with (DHA) and (EPA) removed, and percent PUFA after DHA and EPA were removed were included as candidate independent variables. DHA and EPA are thought to induce cellular antioxidant defenses which may counteract the peroxidation potential of DHA and EPA (Wang et al. 2010, Borgonovi et al. 2023).

3. Results

3.1. Lake Trout Egg Thiamine

Egg thiamine concentrations were determined for 122 and 27 lake trout collected from lakes Ontario and Superior, respectively. Total egg thiamine concentration ranged from 1.43 to 24.24 nmol/g in Lake Ontario and from 10.48 to 37.82 nmol/g in Lake Superior (Table 1). In Lake Ontario, total egg thiamine concentration was significantly higher among Klondike lake trout in 2022 than lean lake trout in 2022 and was significantly higher than Klondike or lean lake trout in 2021 (Kruskal-Wallis: $H = 31.36$, $df = 3$, $p < 0.05$; all post hoc Dunns comparisons $p < 0.05$) (Figure 1, Table 2). Lean lake trout in 2021 and 2022, and Klondike lake trout in 2021 did not have significantly different total egg thiamine concentrations. Lake Ontario lean lake trout had significantly lower total egg thiamine concentrations than Lake Ontario Klondikes, Lake Superior leans and siscowet lake trout (Kruskal-Wallis: $H = 52.47$, $df = 3$, $p < 0.05$; all post hoc Dunns comparisons $p < 0.05$) (Figure 2A).

Lake Ontario lean lake trout had significantly lower egg free thiamine concentrations (Figure 2B) and egg thiamine monophosphate concentrations (Figure 2D) than Lake Ontario Klondikes, Lake Superior leans, and siscowet lake trout (Kruskal-Wallis: $H = 54.18$, $df = 3$, $p < 0.05$; Kruskal-Wallis: $H = 34.57$, $df = 3$, $p < 0.05$, respectively; all post hoc Dunns comparisons $p < 0.05$). Lake Ontario lean lake trout had significantly lower egg thiamine pyrophosphate concentrations than Lake

Ontario Klondikes, but not Lake Superior leans or siscowets (Kruskal-Wallis: $H = 9.53$, $df = 3$, $p < 0.05$; Dunns: Lake Ontario lean: Lake Ontario Klondikes $p < 0.05$, Lake Ontario lean: Lake Superior leans $p = 0.31$, Lake Ontario lean: Lake Superior siscowets $p = 0.23$) (Figure 2C). Lake Ontario Klondike lake trout, Lake Superior lean, and siscowet lake trout did not have significantly different egg thiamine pyrophosphate concentrations.

In 2022, a significant negative relationship was present between lake trout length (mm) and total egg thiamine in Lake Ontario ($y = 4.77 - 0.00354$ [length]), adjusted $R^2 = 0.25$, $p < 0.05$), but not in Lake Superior ($p = 0.10$). In 2021, a significant, but much weaker negative relationship was also present between length (mm) and total egg thiamine in Lake Ontario ($y = 2.94 - 0.00154$ [length]), $R^2 = 0.05$, $p < 0.05$) (Figure 3).

3.2. Lake Trout Liver and Egg α -Tocopherol

Mean lake trout liver α -tocopherol concentration was 10.16 mg/g in Lake Superior. Mean lake trout liver α -tocopherol concentrations in Lake Ontario were 3.41 mg/g in 2021 and 3.94 mg/g in 2022 (Table 2). Lake trout liver α -tocopherol concentrations in Lake Ontario were significantly higher in 2022 than in 2021 (Wilcoxon: $Z = -799$, $p < 0.05$) (Figure 4). Lake Superior siscowet and lean lake trout had significantly higher liver α -tocopherol concentrations than the Klondike and lean lake trout from Lake Ontario but were not significantly different from each other and Lake Ontario Klondikes and leans were not significantly different from each other

(Kruskal-Wallis: $H = 36.88$, $df = 3$, $p < 0.05$; Dunns: Lake Superior leans:Lake Superior siscowets $p = 0.85$, Lake Ontario leans:Lake Ontario Klondikes $p = 0.12$, all remaining post hoc Dunns comparisons $p < 0.05$).

The mean lake trout egg α -tocopherol concentration was 0.82 mg/g in Lake Superior. Mean egg α -tocopherol concentrations Lake Ontario were 0.67 mg/g in 2021 and 0.66 mg/g in 2022. Lake Ontario egg α -tocopherol concentrations did not differ significantly between 2021 and 2022 ($p = 0.66$). Lake Superior siscowet lake trout had significantly higher egg α -tocopherol concentrations than any of the other ecomorphs (Kruskal-Wallis: $H = 22.68$, $df = 3$, $p < 0.05$; all post hoc Dunn's $p < 0.05$). Lake Superior lean lake trout had significantly higher egg α -tocopherol concentrations than Lake Ontario lean lake trout but were not significantly different from Lake Ontario Klondike lake trout (Dunns: Lake Superior leans:Lake Ontario leans $p < 0.05$, Lake Ontario Klondikes:Lake Superior leans $p = 1.0$). Lean and Klondike lake trout from Lake Ontario did not have significantly different egg α -tocopherol concentrations ($p = 0.1$) (Figure 6). There was a significant negative relationship between length (mm) and egg α -tocopherol concentration in Lake Ontario in 2022, but not in 2021 (2022: $y = 1.15 - 0.0006597[\text{Length}]$, adjusted $R^2 = 0.24$, $p < 0.05$), (2021: $p = 0.49$) (Figure 7A). There was a significant negative relationship between length (mm) and egg α -tocopherol concentration in Lake Superior ($y = 1.53 - 0.0009526[\text{length}]$, adjusted $R^2 = 0.48$, $p < 0.05$) (Figure 7B).

In Lake Ontario there was a significant positive relationship between egg α -tocopherol concentration and log total egg thiamine concentrations in both 2021 ($y =$

-1.28 + 10.96 [egg α -tocopherol], adjusted $R^2 = 0.13$, $p = 0.05$) and 2022 ($y = -8.97 + 27.91$ [egg α -tocopherol], adjusted $R^2 = 0.30$, $p < 0.05$) (Figure 8). There was no significant relationship between egg α -tocopherol concentration and total egg thiamine in Lake Superior ($p = 0.07$).

3.3. Lake Trout Fatty Acid Composition

Palmitic (16:0), oleic acid (18:1n-9) and docosahexaenoic acid (22:6n-3) were the most abundant saturated, monounsaturated, and polyunsaturated fatty acids, respectively, in belly flap lipids of lean and Klondike lake trout (table 3). Belly flap fatty acid signatures in Lake Ontario differed between ecomorphs, between years and there was an interaction between ecomorph and year (adonis: $r^2 = 0.17$, pseudo-F = 24.12, $df = 1$, $p < 0.05$; $r^2 = 0.09$, pseudo-F = 12.93, $df = 1$, $p < 0.05$; $r^2 = 0.25$, pseudo-F = 3.50, $df = 1$, $p < 0.05$). SIMPER analysis showed that the fatty acids with the largest contribution to differences between Klondike and lean lake trout and between years were 16:1n-7 and 16:0, 18:1n-9, and 22:6n-3. Palmitoleic acid (16:1n-7) and 16:0 made up a larger proportion of belly flap lipids in Klondike lake trout than lean lake trout and were a larger proportion of belly flap lipids in both Klondikes and leans in 2022 than in 2021. Oleic acid (18:1n-9) and 22:6n-3 made up a larger proportion of belly flap lipids in lean lake trout than Klondike lake trout and were a larger proportion of belly flap lipids in both Klondikes and leans in 2021 than in 2022.

Lake Ontario Lean lake trout had significantly higher unsaturation indexes than Klondike lake trout in 2022, but not in 2021 (ANOVA: $F = 27.47$, $df = 3$, $p <$

0.05; Tukey: 2022 SKW:2022 Lean $p < 0.05$, 2021 SKW:2021 Lean $p = 0.15$) (Figure 9). In Lake Ontario, Klondike lake trout had a significantly higher 16:1n-7 to 18:1n-9 ratio than lean lake trout in both 2021 and 2022 (Kruskal-Wallis: $H = 37.99$, $df = 3$, $p < 0.05$; all post hoc Dunns $p < 0.05$) (Figure 10). There was a significant negative relationship between egg free thiamine and belly flap unsaturation index (UI) ($y = 3.83 - 23.8[UI] + 5.65[UI]^2$, adjusted $R^2 = 0.42$, intercept $p < 0.05$, UI 1st degree $p < 0.05$, UI 2nd degree $p = 0.061$) (Figure 11A). The same significant negative relationship remained once DHA and EPA were removed from the unsaturation index ($y = 3.83 - 24.2[UI-DE] + 13.9[UI-DE]^2$, adjusted $R^2 = 0.55$, intercept $p < 0.05$, UI-DE 1st degree $p < 0.05$, UI-DE 2nd degree $p = 0.06$) (Figure 11B).

A significant positive relationship was present between egg free thiamine and the 16:1n-7 to 18:1n-9 ratio in belly flaps ($y = -8.082 + 35.672 [16:1n-7/18:1n-9]$, adjusted $R^2 = 0.43$, $p < 0.05$) (Figure 12). There was also a significant negative relationship between length (mm) and the belly flap 16:1n-7 to 18:1n-9 ratio ($y = 0.7095 - 0.0005299 [length]$, adjusted $R^2 = 0.38$, $p < 0.05$) (Figure 13). Finally, a significant negative relationship was present in belly flaps between the 16:1n-7 to 18:1n-9 ratio and the proportion of PUFA and the proportion of PUFA minus DHA and EPA ($y = 51.2 - 30.8[16:1n-7/18:1n-9]$, adjusted $R^2 = 0.41$, $p < 0.05$; $y = 37.4 - 32.5[16:1n-7/18:1n-9]$, adjusted $R^2 = 0.72$, respectively) (Figure 14A,B).

3.4. Egg Free Thiamine Models

The final Klondike lake trout model, the final lean lake trout model, and the final lean and Klondike combined model, all had normally distributed QQ plot residuals, and passed Kolmogorov-Smirnov, dispersion, and outliers tests (Klondike model: $p = 0.92$; $p = 1$; $p = 1$, respectively; Lean model: $p = 0.23$; $p = 0.82$; $p = 1$, respectively; Combined model: $p = 0.06$; $p = 0.84$; $p = 0.53$, respectively). Thus, meeting the assumptions of normally distributed residuals, independence of observations, constant variance, and linearity to the response.

The final model for determining egg free thiamine concentrations among Lake Ontario Klondike lake trout only included one variable, belly flap unsaturation index with DHA and EPA removed (UI-DE) ($y = 85.4 - 0.621[\text{UI} - \text{DE}]$, adjusted $R^2 = 0.71$, $p < 0.05$) (Figure 15). The final model for determining egg free thiamine concentrations among Lake Ontario lean lake trout included two variables, egg α -tocopherol concentration and PUFA ($y = 17.1481 - 0.4643[\text{PUFA}] + 7.6565[\text{Egg } \alpha\text{-tocopherol concentration}]$, adjusted $R^2 = 0.33$, PUFA $p < 0.05$, Egg α -tocopherol concentration $p < 0.05$). The final model for determining egg free thiamine (TH) concentrations among Lake Ontario lean and Klondike lake trout combined included two variables, belly flap unsaturation index with DHA and EPA removed as a two degree polynomial, and egg α -tocopherol concentration ($y = -1.89 - 19.96[\text{UI} - \text{DE}] + 15.2[\text{UI} - \text{DE}]^2 + 8.63[\text{Egg } \alpha\text{-tocopherol concentration}]$, adjusted $R^2 = 0.59$, Intercept $p = 0.32$, UI-DE 1st degree $p < 0.05$, UI-DE 2nd degree $p < 0.05$, Egg α -Tocopherol $p < 0.05$).

4. Discussion

4.1. Lake Trout Egg Thiamine Concentrations and Diet

Thiamine deficiency remains a persistent concern for lake trout in Lake Ontario. Their egg thiamine concentrations averaged just above the 4 nmol/g concentration recommended by Bronte et al. (2008). A significant increase in egg thiamine concentrations was observed in 2022. However, 26% of individual fish still fell below the 4 nmol/g threshold, demonstrating that individual variability in egg thiamine concentrations remains as persistent as temporal variability population wide. In part, the intrapopulation variability shown here appeared to be explained by differences in diet as expressed by the 16:1n-7 and 18:1n-9 ratio in belly flaps of egg bearing females. Palmitoleic acid (16:1n-7) has been previously associated with benthic feeding prey such as deepwater sculpin (*Myoxocephalus thompsonii*) and round goby, while 18:1n-9 has been associated with pelagic prey items such as alewife and rainbow smelt (Happel et al. 2017, 2020, Futia et al. 2021, Heisey et al. 2023). Population wide, total egg thiamine concentrations increased with an increasing 16:1n-7 to 18:1n-9 ratio, indicating that increased benthic prey intake is associated with higher total egg thiamine concentrations. Furthermore, the 16:1n-7 to 18:1n-9 ratio had a negative relationship with length providing evidence of an ontogenetic shift from a more benthically derived diet to a more pelagically derived diet. These trends become clearer when the differences among lake trout ecomorphs are considered.

The significantly higher 16:1n-7 to 18:1n-9 ratio found in the belly flaps of Klondike lake trout, when compared to lean lake trout in 2021 and 2022, indicates a more benthically oriented diet among Klondike lake trout. Klondike lake trout also had significantly higher total egg thiamine concentrations in both 2021 and 2022. Furthermore, the difference in total egg thiamine concentrations and 16:1n-7 to 18:1n-9 ratio between Klondike lake trout and the two lean derived strains, increased simultaneously from 2021 to 2022. These findings are in line with those of Heisey et al. (2023), who demonstrated the same pattern of diverging 16:1n-7 to 18:1n-9 ratio and total egg thiamine concentrations between Klondike lake trout and the lean derived Seneca and Lake Champlain Domestic strains. The Klondike strain has been repeatedly shown to have consistently higher total egg thiamine concentrations than its lean strain counterparts in Lake Ontario.

4.2. α -Tocopherol and Lake Trout Diet

Lower liver α -tocopherol concentrations among Lake Ontario lean and Klondike lake trout when compared to Lake Superior lake trout, indicate that despite the diet differences between Lake Ontario Klondikes and leans, both are experiencing a lower effective intake of α -tocopherol than the Lake Superior lake trout population. The higher liver α -tocopherol concentrations among Lake Superior lake trout in comparison to Lake Ontario lake trout are in line with the findings of Palace et al. (1998b). Liver α -tocopherol concentrations we measured among Lake Superior lake trout were 10.16 ± 5.26 mg/g and Lake Ontario were 3.26 ± 2.41 mg/g while Palace

et al. (1998b) reported liver α -tocopherol concentrations between ~ 6 and ~ 0.8 mg/g in Lake Superior lake trout and ~ 3 and ~ 0.5 mg/g in Lake Ontario lake trout.

Egg α -tocopherol concentrations among ecomorphs followed a similar pattern to total egg thiamine concentrations in that, Klondike lake trout from Lake Ontario had higher egg α -tocopherol concentrations than Lake Ontario lean strains. Moreover, the negative relationship between egg α -tocopherol and length would indicate the influence of changes in diet with increased length however, Klondikes had slightly lower, but similar liver α -tocopherol concentrations to Lake Ontario lean lake trout, despite higher concentrations of α -tocopherol in round goby (12.74 ng/mg) compared to alewife (1.6 ng/mg) and the higher intake of round goby by Klondikes compared to leans (Honeyfield et al. 2012, Heisley et al. 2023). Overall, this finding demonstrates a disconnect between dietary intake of α -tocopherol as represented by liver α -tocopherol concentration (Hamre 2011, Qiang et al. 2019), and egg deposition of α -tocopherol in Lake Ontario lake trout.

Higher liver and egg α -tocopherol among Lake Superior siscowet lake trout may have been due to their greater potential for α -tocopherol storage given their higher lipid content in comparison to the other ecomorphs, as α -tocopherol is soluble in lipid (Hamre 2011, Muir et al. 2021). Siscowets have a lipid metabolism that differs from lean ecomorphs as they have lower circulating lipid levels than leans and store lipids in both liver and muscle. In contrast, leans have higher carbohydrate storage in liver and muscle and higher circulating glucose levels (Goetz et al. 2014).

The increased lipid content in siscowets may also require more α -tocopherol and thiamine to prevent excess lipid peroxidation.

4.3. α -Tocopherol and Lake Trout Egg Thiamine Concentrations

The significant increase of liver α -tocopherol and significant reduction of the unsaturation indexes observed in the Lake Ontario lake trout population as a whole between 2021 and 2022 may indicate a reduced risk or prevalence of excess lipid peroxidation in 2022 over 2021. These observations coincided with increased total egg thiamine concentrations in 2022 over 2021. However, Klondikes had the largest increase in total egg thiamine concentrations, but simultaneously had reduced liver α -tocopherol concentrations, implying that total egg thiamine concentrations are not directly driven by dietary intake of α -tocopherol as represented by liver α -tocopherol concentrations.

A large year class of one year old alewife was present in Lake Ontario in 2021 (Weidel et al. 2022). Given the smaller size of Klondike lake trout compared to lean lake trout, younger alewife may be a more size appropriate prey than larger, older alewife. This appears to be demonstrated by the change in 16:1n-7 to 18:1n-9 ratios among Klondikes from 2021 to 2022. The ratio of 16:1n-7 to 18:1n-9 among Klondikes was lower in 2021 compared to 2022 which indicates higher alewife consumption in 2021 than in 2022 when there was no longer a large young year class of alewife present in Lake Ontario. Higher intake of alewife by smaller lake trout in 2021 compared to 2022 would reduce the overall difference in diet between small and

large lake trout in 2021 compared to 2022. This potential reduced difference in diet across lake trout lengths in 2021 compared to 2022 may explain (1) the lack of a relationship between egg α -tocopherol and length in 2021, while this relationship is present in 2022, (2) the weaker relationship between egg thiamine concentrations and length in 2021 compared to 2022, and (3) the weaker relationship between egg thiamine concentrations and egg α -tocopherol concentrations in 2021 compared to 2022. Taken together, this provides evidence that egg α -tocopherol concentrations in Lake Ontario are reduced under high alewife consumption, though it is notable that liver α -tocopherol concentrations do not follow this trend.

Differences in diet driven by length may also explain the relationship between egg α -tocopherol and length among Lake Superior lake trout as well. However, the relationship between egg thiamine concentrations and egg α -tocopherol concentrations was not present in Lake Superior, demonstrating that decreasing egg α -tocopherol concentrations does not dictate a decrease in egg thiamine concentrations. Overall, the relationship between egg thiamine and α -tocopherol concentrations is inconsistent and our analysis further supports the conclusion of Rinhard et al. (2021), α -tocopherol does not appear to be directly associated with TDC. Modulation of an additional variable, such as unsaturation index or fatty acid composition, is needed to link egg and liver α -tocopherol concentrations and egg thiamine concentrations.

4.4. Polyunsaturated Fatty Acids and Lake Trout Egg Thiamine Concentrations

Excess peroxidation driving reductions in egg thiamine concentrations should be most visible in changes to free thiamine (TH) as lipid hydroperoxide produced from the lipid peroxidation chain reaction oxidizes free thiamine (Lukienko et al. 2000). Unsaturation index, egg α -tocopherol and PUFA all represent potential contributors to a condition of excess lipid peroxidation and all were significant variables related to the concentration of egg free thiamine in our analyses. Moreover, the removal of DHA and EPA from the calculation of the unsaturation index provides an improvement in the quantification of *in vivo* peroxidation potential. The resulting improvement in the relationship between UI and TH may indicate that lipid peroxidation is a cause of thiamine consumption contributing to TDC. However, the relationship between the ratio of 16:1n-7 to 18:1n-9 and PUFA is also improved by the removal of DHA and EPA from the calculation of PUFA which obscures the connection between TH and the peroxidative potential of the remaining PUFAs in the calculation, as their proportion in belly flaps is closely tied to alewife consumption. Without disentangling *in vivo* peroxidation potential from alewife consumption, distinction between peroxidation induced and thiaminase induced reductions in lake trout egg thiamine concentrations does not appear feasible from observational study alone.

It is interesting that much of the improvement of the relationship between 16:1n-7 to 18:1n-9 ratio and PUFA when DHA and EPA were excluded, occurred among the lower half of the ratio which represents individuals with higher alewife

consumption (Happel et al. 2017, 2020, Futia et al. 2021, Heisey et al. 2023). This could be due to annual variation in alewife DHA content, or a difference in DHA content in the young alewife present in large number in 2021 compared to the older alewife who dominated the alewife population in Lake Ontario in 2022 (Weidel et al. 2022). The variation in DHA content in lake trout belly flaps may also be due to biosynthesis, as salmonids and most freshwater fish are known to be capable of synthesizing EPA and DHA from other long-chain PUFAs such as α -linoleic acid (18:3n-3), and some intermediary fatty acids (Olsen 1999, Meyer et al. 2004, Arts and Kohler 2009, Bou et al. 2017). Alewife appear unable to synthesize DHA and EPA due to a deficiency in $\Delta 5$ desaturase which, is thought to be common among marine fish and was cited by Snyder and Hennessey (2003) as a potential cause of lower levels of DHA among mortalities of alewife undergoing cold-water trials (Olsen 1999, Tocher et al. 2003). Sprat, another member of the Clupeidae family, and thought to be the culprit behind M74 syndrome in Atlantic salmon, experienced decreasing size at age off the Crimean coast in the Black Sea from the 1980s to the 2000s (Yuneva et al. 2016). Decreasing growth rates among this population of sprat are thought to be in part due to an inability to biosynthesize sufficient DHA to compensate for climate and eutrophication induced reductions in local phytoplankton DHA production (Yuneva et al. 2016). Snyder et al. (2011) demonstrated lower growth rates among alewife fed corn oil (no DHA or EPA), over alewife fed fish oil (contains DHA and EPA) which may indicate a similar susceptibility to variation in phytoplankton DHA production impacting growth and overall health.

5. Conclusions

Lake trout in Lake Ontario are at a higher risk of excess lipid peroxidation than those of the Lake Superior reference population. The dynamics of α -tocopherol are different between the two lakes, with Lake Superior lake trout liver α -tocopherol concentrations being predictive of their respective egg α -tocopherol concentrations, while Lake Ontario lake trout did not have this relationship between egg and liver α -tocopherol concentrations. The difference in α -tocopherol egg deposition among ecomorphs in Lake Ontario and Lake Superior may demonstrate a greater retention or intake of α -tocopherol among lake trout with more benthically oriented diets. However, α -tocopherol intake as represented by hepatic concentrations and α -tocopherol concentrations in eggs do not appear to be driving egg thiamine concentrations. Moreover, the removal of DHA and EPA from the calculation of belly flap unsaturation indexes improves the quantification of *in vivo* peroxidation potential. This more accurate quantification of *in vivo* peroxidation potential then leads to a stronger relationship between lake trout egg free thiamine and belly flap unsaturation index, thus implicating excess lipid peroxidation as a source of free thiamine consumption. However, current understanding of the degree to which the peroxidation potential of individual fatty acids when integrated into living tissue deviates from the peroxidation potential inferred from their molecular structure is highly limited. As research in this area advances, more targeted methods of predicting *in vivo* peroxidation may become available and could clarify the role, if any, of excess lipid peroxidation in TDC induction. Lastly, while the potential role of α -tocopherol

and PUFA dynamics in TDC induction are intriguing and warranting of further study, both correlated with indications of alewife consumption, and their impact on egg thiamine concentrations may also be attributed to thiaminase content of alewife. Moreover, considering Richter et al. (2023)'s demonstration of a high likelihood of *de novo* thiaminase production in alewife, it appears unlikely that excess lipid peroxidation would be the sole cause of TDC in lake trout.

Table 1. Characteristics (mean \pm standard deviation) of female lake trout collected from Lake Ontario (2021-2022) and Lake Superior (2022).

Characteristics	Ontario (2021-2022)	Superior (2022)
Number of Samples (N)	122	27
Length (mm)	728 \pm 77	747 \pm 137
Total Egg Thiamine (TTH) (nmol/g)	7.40 \pm 4.77	23.28 \pm 5.96
Egg Free Thiamine (TH) (nmol/g)	3.40 \pm 3.70	17.90 \pm 5.36
Egg Thiamine Monophosphate (TMP) (nmol/g)	1.09 \pm 0.62	1.91 \pm 0.37
Egg Thiamine Pyrophosphate (TPP) (nmol/g)	2.90 \pm 1.15	3.47 \pm 1.14
Liver α -Tocopherol (mg/g)	3.26 \pm 2.41	10.16 \pm 5.26
Egg α -Tocopherol (mg/g)	0.67 \pm 0.10	0.82 \pm 0.18
Ratio 16:1n-7/18:1n-9	0.32 \pm 0.07	-
Unsaturation Index (UI)	214.92 \pm (0.87)	-

- not measured

Table 2. Concentrations of α -tocopherol and thiamine, 16:1n-7 to 18:1n-9 ratio and unsaturation index of female Lake Ontario lake trout collected in 2021 and 2022.

Data are mean \pm standard deviation.

Year	2021	2022
Number of Samples (N)	68	54
Liver α -Tocopherol (mg/g)	2.69 \pm 1.52	3.90 \pm 3.01
Egg α -Tocopherol (mg/g)	0.67 \pm 0.09	0.66 \pm 0.12
Total Egg Thiamine (nmol/g)	5.63 \pm 2.78	9.55 \pm 5.74
Ratio 16:1n-7/18:1n-9	0.31 \pm 0.05	0.34 \pm 0.08
Unsaturation Index (UI)	220.15 \pm 8.85	208.08 \pm 9.39

Table 3. Fatty acids (expressed as % average percent detected \pm standard deviation) in belly flaps of female Lake Ontario lean and Klondike lake trout in 2021 and 2022.

SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

Fatty acids	Lean Lake Trout	Klondike Lake Trout
Number of Samples (N)	93	24
Percent Lipid	37.65 \pm 9.19	47.67 \pm 7.01
12:0	0.04 \pm 0.01	0.04 \pm 0.01
14:0	3.10 \pm 0.26	3.04 \pm 0.18
15:0	0.42 \pm 0.04	0.42 \pm 0.04
16:0	11.67 \pm 0.92	13.04 \pm 1.15
16:1n-9	1.05 \pm 0.12	0.89 \pm 0.11
16:1n-7	6.95 \pm 1.23	9.28 \pm 1.38
17:0	0.43 \pm 0.06	0.41 \pm 0.04
17:1	0.78 \pm 0.13	0.82 \pm 0.10
18:0	2.99 \pm 0.30	3.20 \pm 0.35
18:1n-9	23.04 \pm 1.35	22.95 \pm 0.99
18:1n-7	4.26 \pm 0.37	4.72 \pm 0.46
18:2n-6	4.70 \pm 0.46	3.93 \pm 0.42
18:3n-3	3.72 \pm 0.48	3.28 \pm 0.48
18:4n-3	1.43 \pm 0.31	1.41 \pm 0.25
20:0	0.17 \pm 0.02	0.14 \pm 0.02
20:1	2.79 \pm 0.39	2.67 \pm 0.43
20:2n-6	1.12 \pm 0.18	0.97 \pm 0.18
20:3n-6	0.55 \pm 0.11	0.41 \pm 0.08
20:4n-6	4.09 \pm 0.23	3.77 \pm 0.28
20:3n-3	1.11 \pm 0.19	0.88 \pm 0.18
20:4n-3	2.79 \pm 0.43	2.16 \pm 0.46
20:5n-3	5.08 \pm 0.70	4.97 \pm 0.58
22:1n-11	0.25 \pm 0.04	0.20 \pm 0.05
21:5n-3	0.27 \pm 0.06	0.23 \pm 0.08
22:4n-6	1.50 \pm 0.30	1.29 \pm 0.15
22:5n-6	1.99 \pm 0.25	1.92 \pm 0.20
22:5n-3	4.52 \pm 0.55	3.79 \pm 0.44
22:6n-3	9.22 \pm 0.97	9.16 \pm 0.74
SAFA	22.38 \pm 1.21	23.79 \pm 1.33
MUFA	35.54 \pm 2.06	38.03 \pm 2.47
PUFA	42.08 \pm 2.54	38.177 \pm 3.39

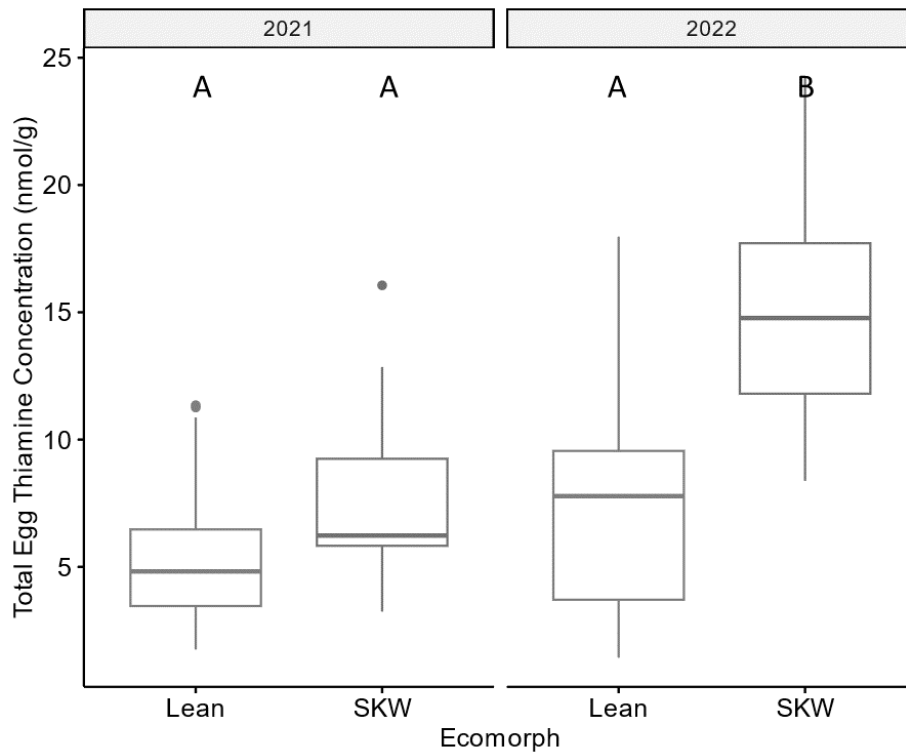


Figure 1. Box plot of total egg thiamine concentrations (nmol/g) collected from Lake Ontario lean and Klondike (SKW) lake trout in 2021 and 2022. Different letters denote significant differences among groups (Kruskal-Wallis $p < 0.05$; Dunns, $p < 0.05$).

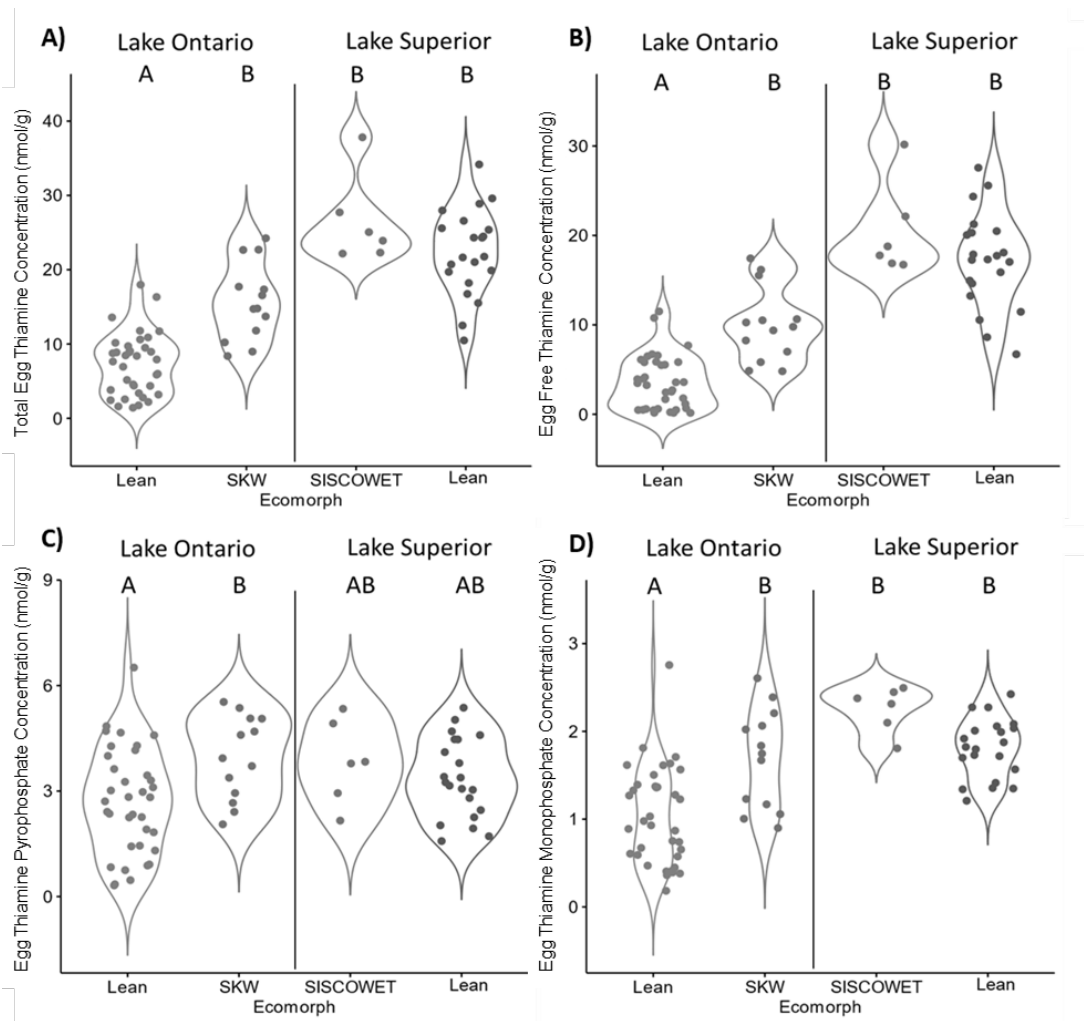


Figure 2. Violin plot of total egg thiamine, egg free thiamine, egg thiamine pyrophosphate and egg thiamine monophosphate concentrations (nmol/g) of Lake Ontario lean lake trout, Lake Ontario Klondike (SKW) lake trout, Lake Superior siscowet and Lake Superior lean lake trout in 2022. Different letters denote significant differences among ecomorphs (Kruskal-Wallis $p < 0.05$; Dunns: $p < 0.05$). Comparisons were made among all four groups for each thiamine vitamer and total thiamine.

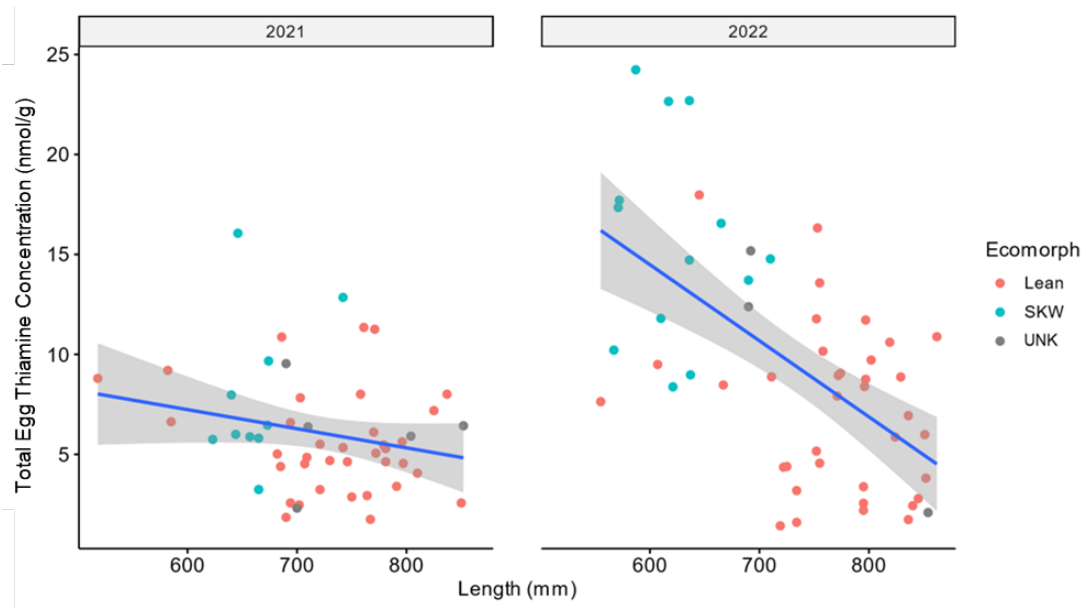


Figure 3. Relationships between total egg thiamine concentrations (nmol/g) of lake trout eggs collected from Lake Ontario in 2021 and 2022, and total length (mm). SKW: Klondike strain lake trout, UNK: lake trout with unknown strain. (Both linear regressions: $p < 0.05$).

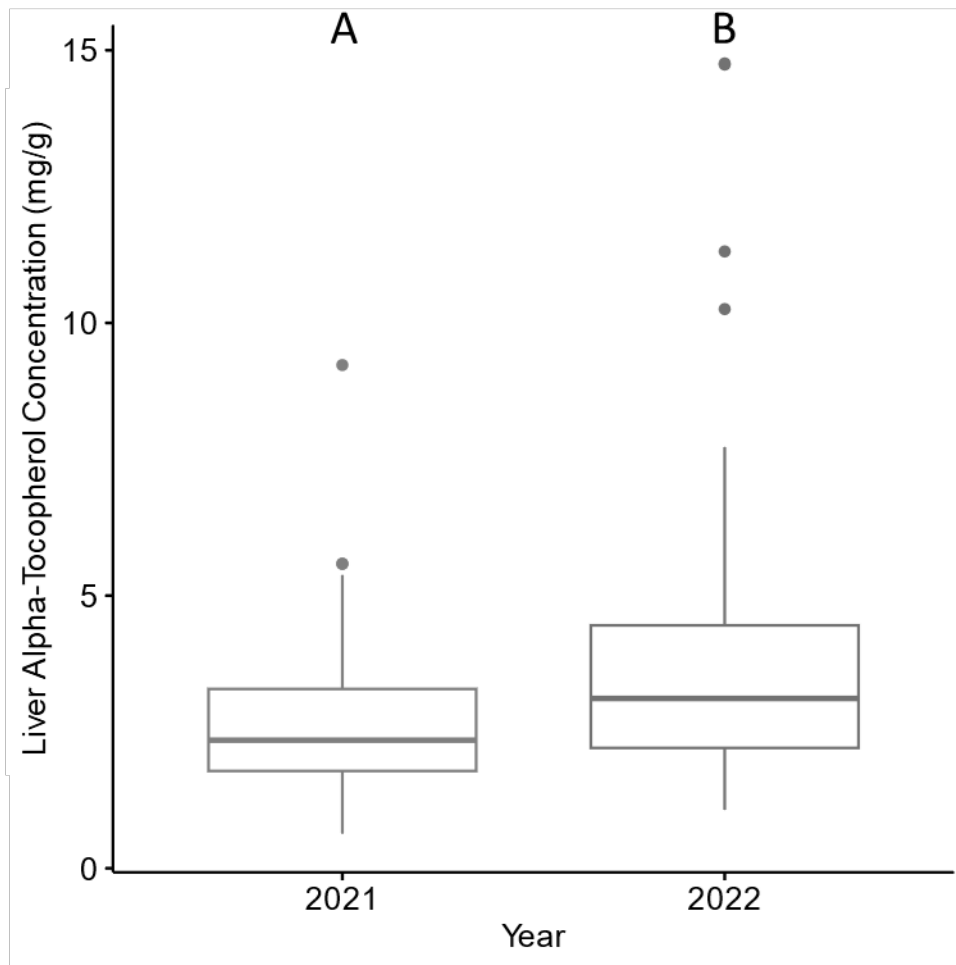


Figure 4. Box plot of α -tocopherol concentrations (mg/g) in lake trout livers collected from Lake Ontario in 2021 and 2022. Different letters denote significant differences between years (Wilcoxon: $p < 0.05$).

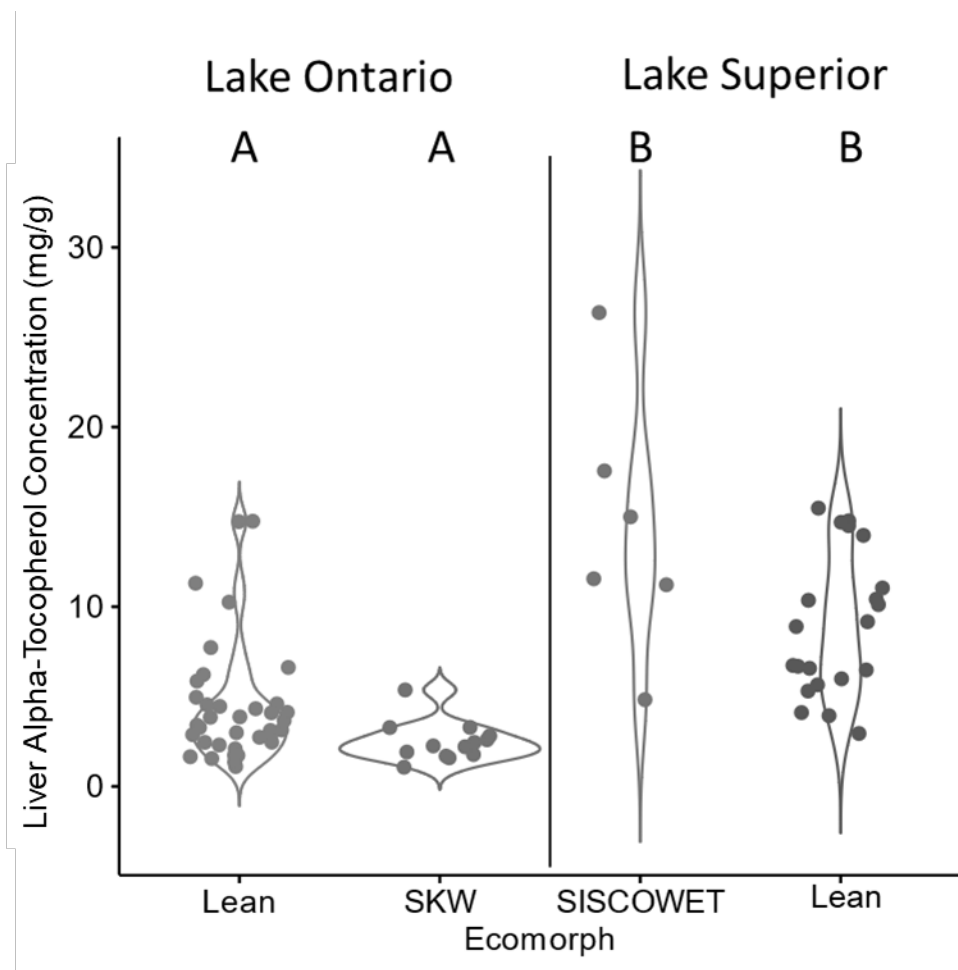


Figure 5. Violin plot of α -tocopherol concentrations (mg/g) in livers of Lake Ontario lean and Klondike lake trout and Lake Superior lean and siscowet lake trout. Different letters denote significant differences among ecomorphs (Kruskal-Wallis $p < 0.05$; Dunns: $p < 0.05$). Comparisons were made across all four groups.

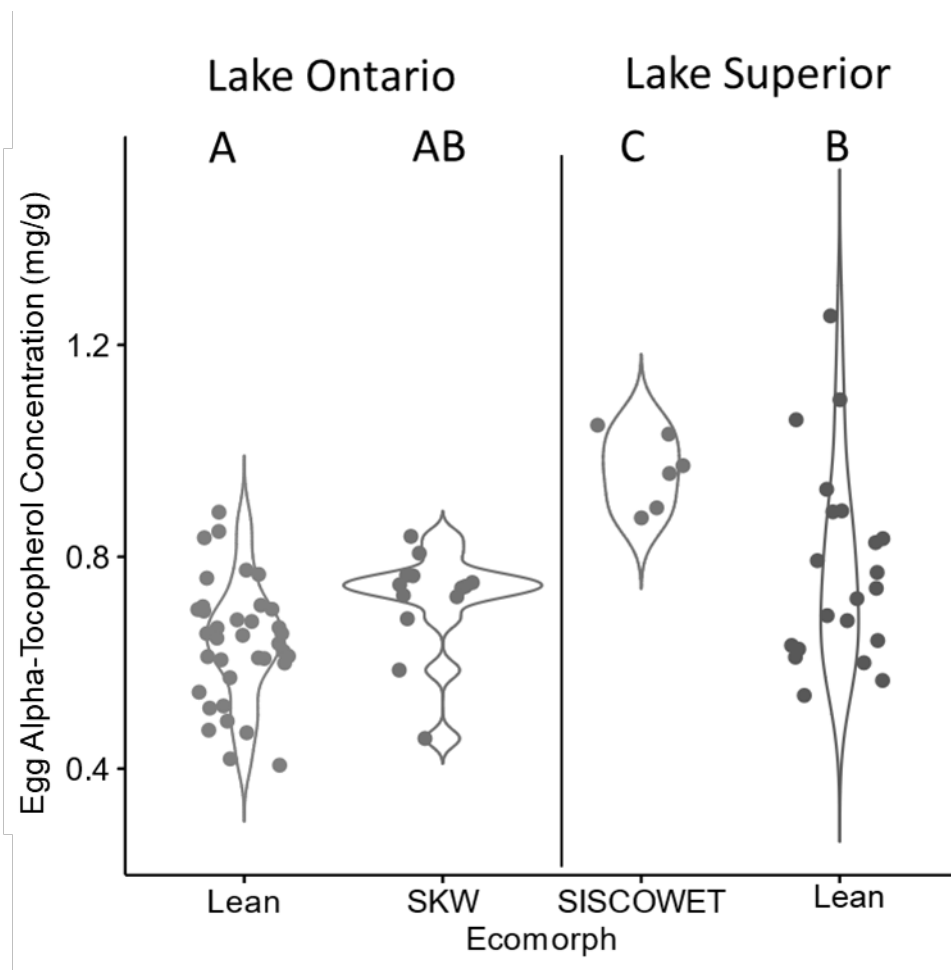


Figure 6. Violin plot of α -tocopherol concentrations (mg/g) in eggs of Lake Ontario lean and Klondike lake trout and Lake Superior lean and siscowet lake trout. Different letters denote significant differences among ecomorphs (Kruskal-Wallis $p < 0.05$; Dunns: $p < 0.05$). Comparisons were made across all four groups.

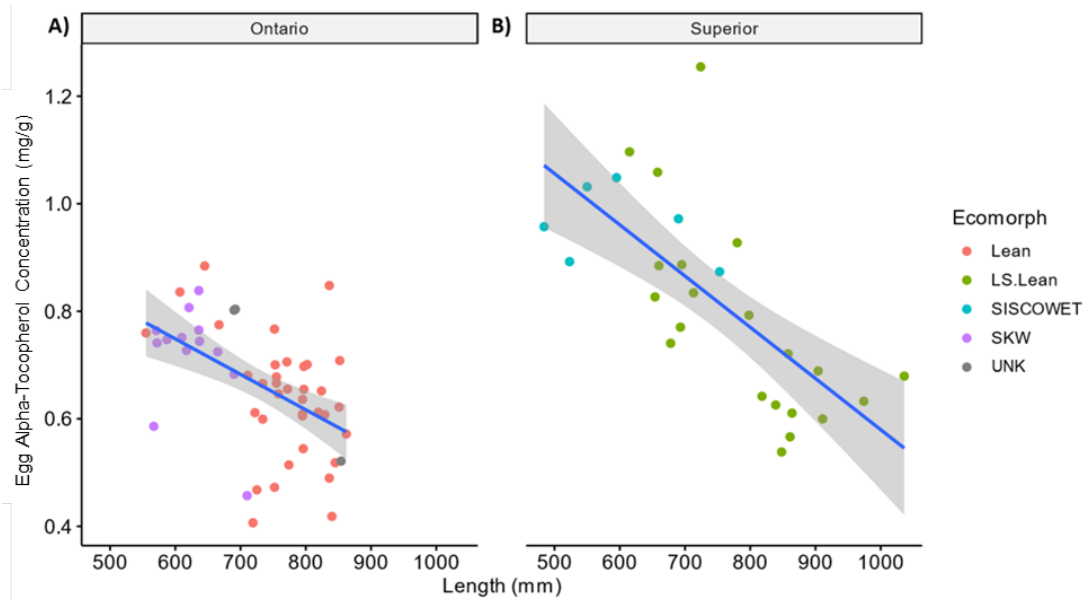


Figure 7. Relationships between α -tocopherol concentrations (mg/g) of lake trout eggs collected from Lakes Ontario and Superior in 2022, and total length (mm). Both linear regressions were significant ($p < 0.05$). LS Lean: lean lake trout from Lake Superior, SKW: Klondike strain lake trout from Lake Ontario, UNK: lake trout with ecomorph not determined.

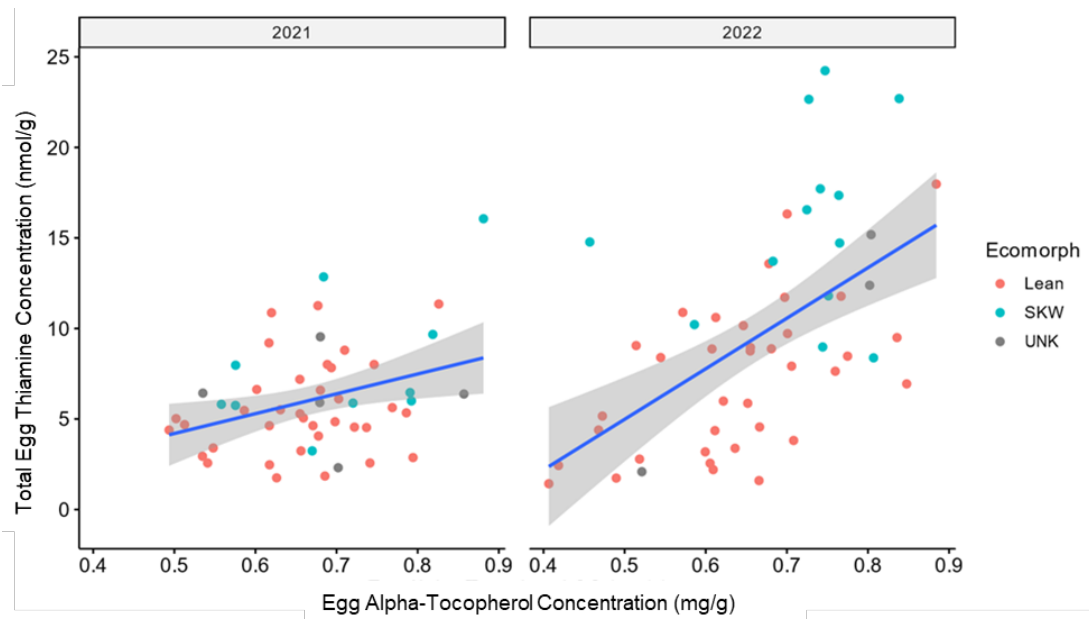


Figure 8. Relationships between total egg thiamine concentrations (nmol/g) and α -tocopherol concentrations (mg/g) of lake trout eggs collected from Lake Ontario in 2021 and 2022. Both linear regressions were significant ($p < 0.05$). SKW: Klondike strain lake trout from Lake Ontario, UNK: lake trout with ecomorph not determined.

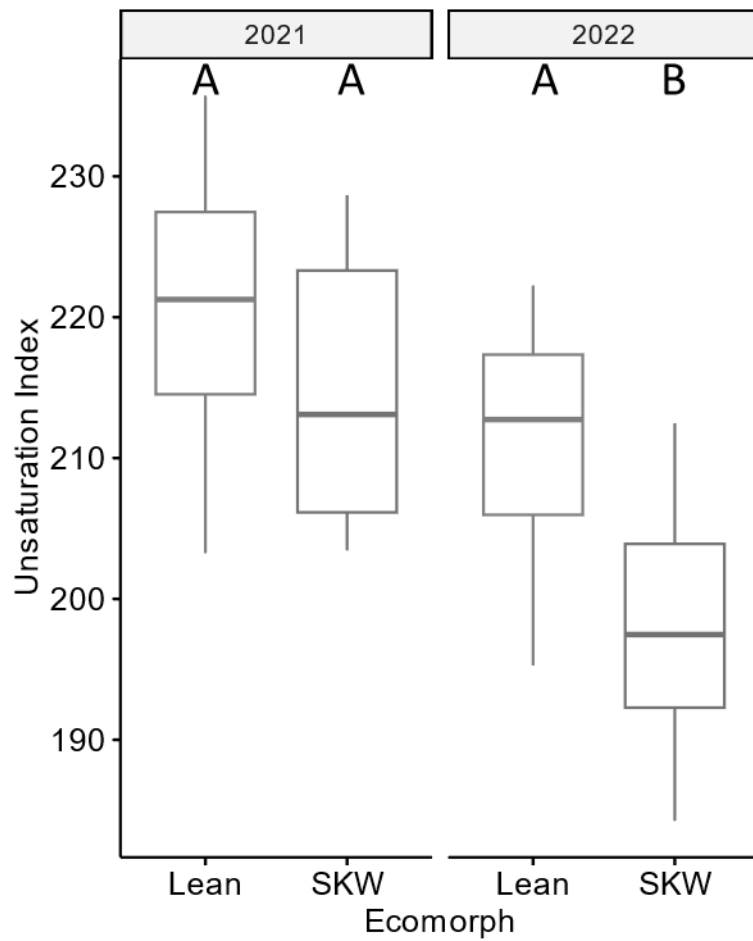


Figure 9. Box plot of unsaturation indexes of belly flaps from lean and Klondike (SKW) lake trout collected from Lake Ontario in 2021 and 2022. Different letters denote significant differences among groups (ANOVA: $p < 0.05$; Tukey: $p < 0.05$).

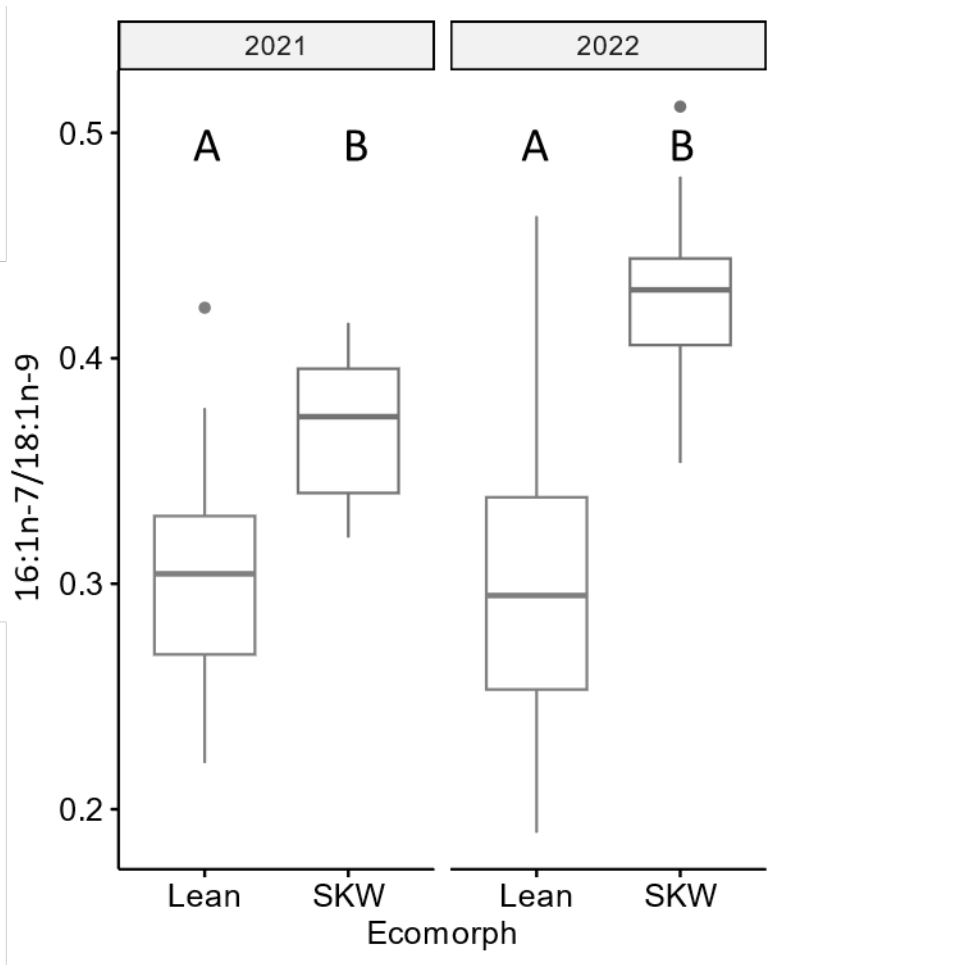


Figure 10. Box plot of the ratio of 16:1n-7 (palmitoleic acid) to 18:1n-9 (oleic acid) in the belly flap of Klondike (SKW) and lean lake trout collected in Lake Ontario in 2021 and 2022. Letters denote significant differences among groups (Kruskal-Wallis $p < 0.05$; Dunns: $p < 0.05$).

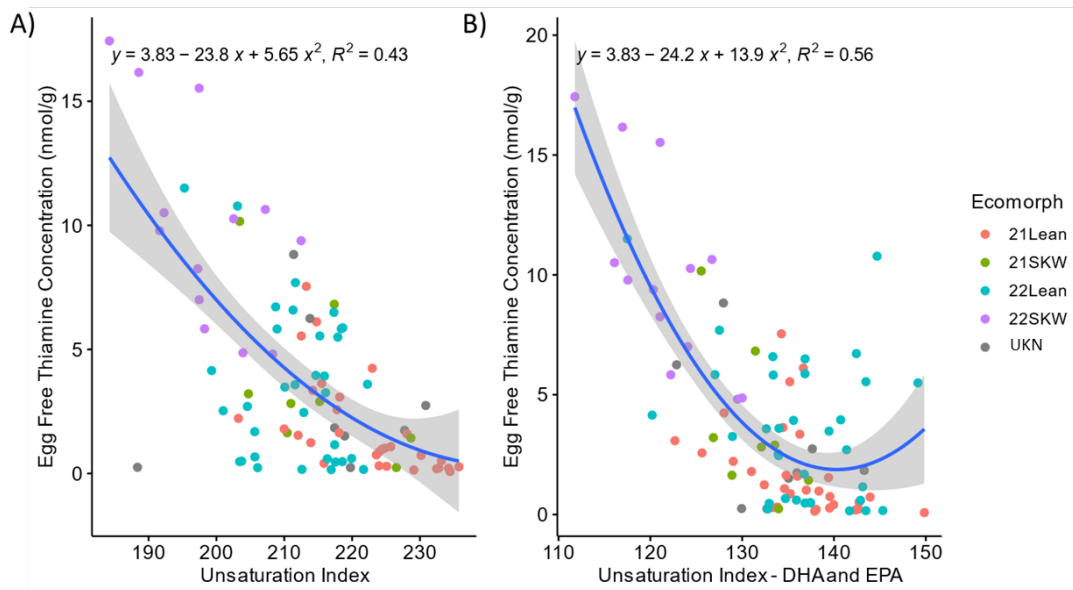


Figure 11. A) Relationship between egg free thiamine concentrations (nmol/g) and belly flap unsaturation index of Lake Ontario lake trout collected in 2021 and 2022. B) Relationship between egg free thiamine concentrations (nmol/g) and belly flap unsaturation index minus docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) of Lake Ontario lake trout collected in 2021 and 2022. Both polynomial regressions: $p < 0.05$. SKW: Klondike strain lake trout from Lake Ontario, UNK: lake trout with ecomorph not determined.

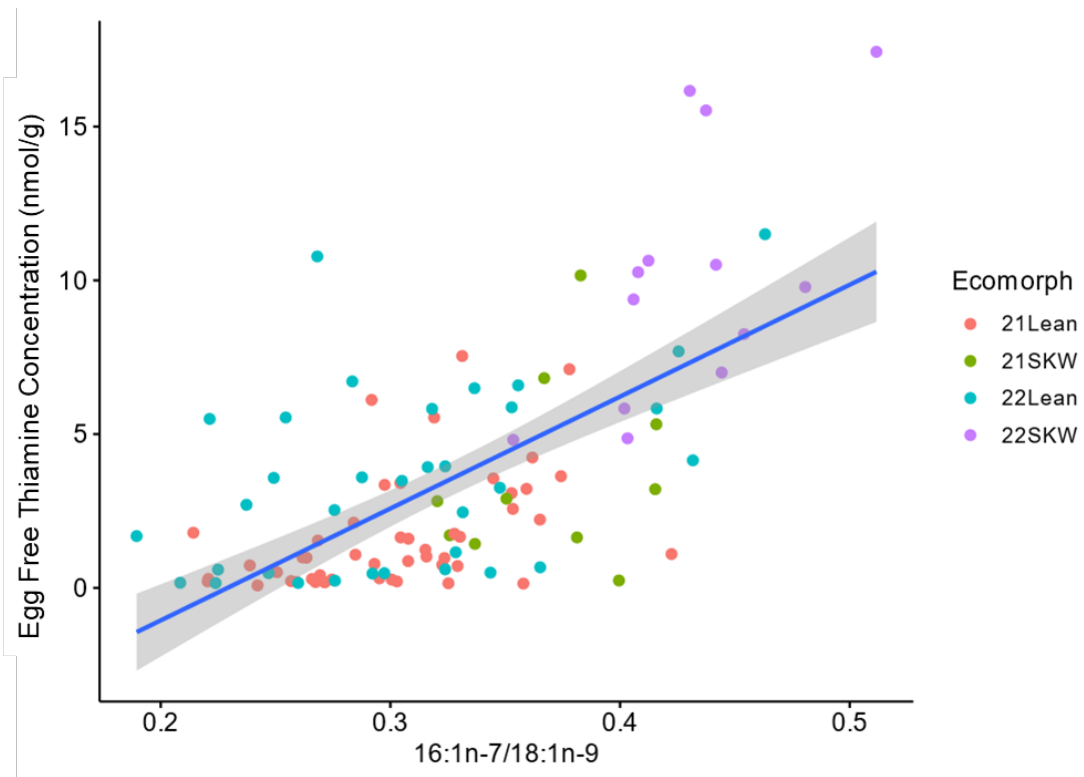


Figure 12. Relationship between egg free thiamine concentrations (nmol/g) and the ratio of 16:1n-7 to 18:1n-9 in the belly flaps of Lake Ontario lake trout collected in 2021 and 2022. The linear regression was significant ($p < 0.05$). SKW: Klondike strain lake trout from Lake Ontario.

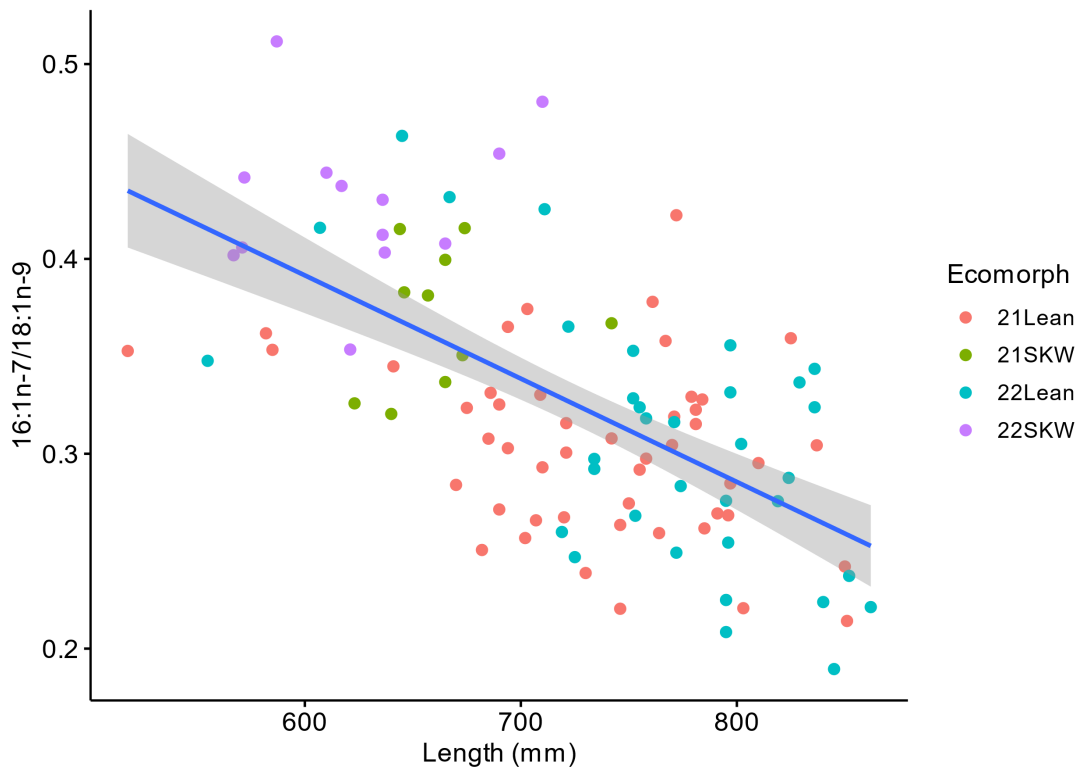


Figure 13. Relationship between length (mm) and the ratio of 16:1n-7 to 18:1n-9 in the belly flaps of Lake Ontario lake trout collected in 2021 and 2022. The linear regression was significant ($p < 0.05$). SKW: Klondike strain lake trout from Lake Ontario.

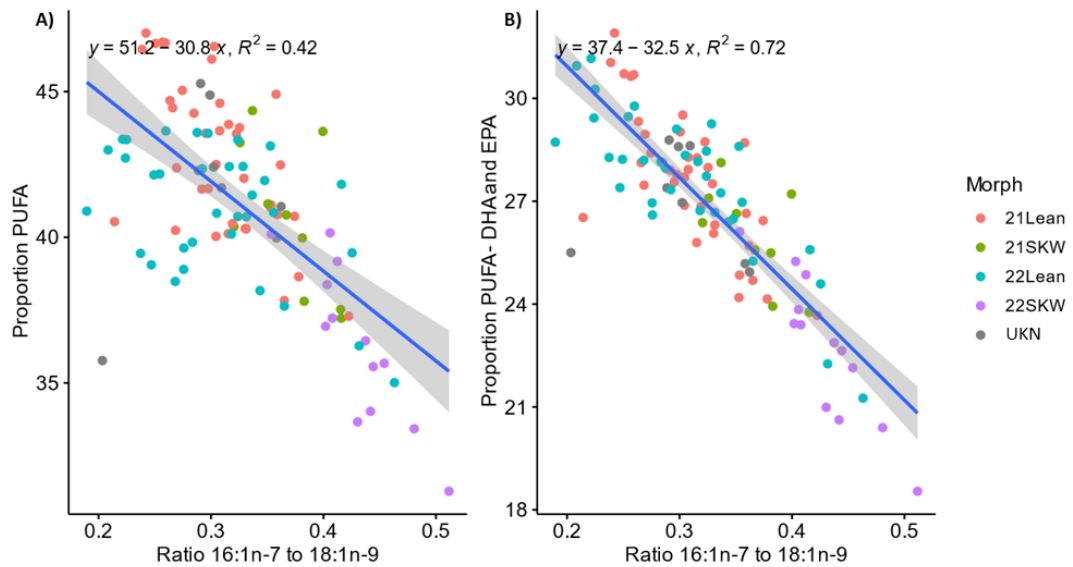


Figure 14. A) Relationship between proportion of PUFA to the ratio of 16:1n-7 to 18:1n-9 in the belly flaps of Lake Ontario lake trout collected in 2021 and 2022. B) Relationship between proportion of PUFA minus docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and the ratio of 16:1n-7 to 18:1n-9 in the belly flaps of Lake Ontario lake trout collected in 2021 and 2022. Both linear regressions were significant ($p < 0.05$). SKW: Klondike strain lake trout from Lake Ontario, UNK: lake trout with ecomorph not determined.

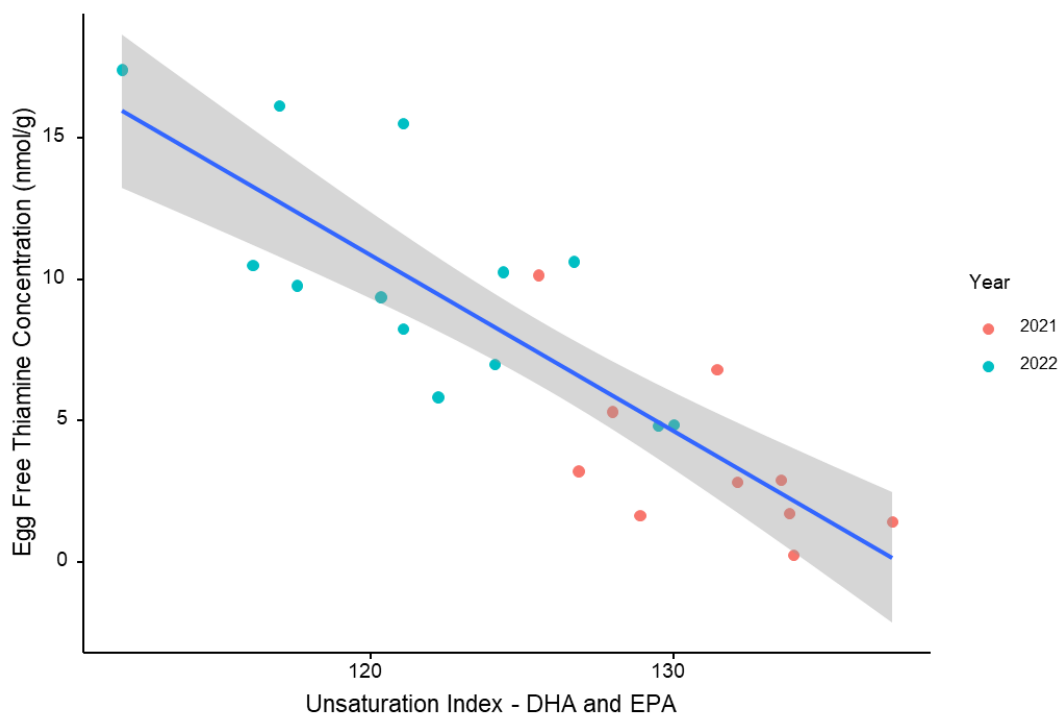


Figure 15. Relationship between egg free thiamine concentrations (nmol/g) and belly flap unsaturation index minus docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) of Lake Ontario Klondike strain lake trout collected in 2021 and 2022. The linear regression was significant ($p < 0.05$).

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