The effect of exotic species on native species reintroductions: Ontario's lake trout reintroductions

by

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A thesis
presented to the University of Waterloo
in the fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Biology

Waterloo, Ontario, Canada, 2026

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public

Statement of Contributions

While the research in this thesis is my own, and the work was largely and primarily completed by me, many collaborators contributed to the project and provided valuable input to Chapters 2-5 (details below). In recognition of the contributions of others and their co-authorship on the manuscript-style chapters in this thesis, I have thus used the pronoun "we" throughout Chapters 2-5.

Chapter 2: Therrien, CA, Adeli, KA, Wolfe, PC, Matesic, S, Edwards, K, Johnston, TA, Thorn, M, Neff, BD, Swanson, HK (2026) Consumption of exotic rainbow smelt (*Osmerus mordax*) is associated with an episode of thiamine deficiency and a decline in the condition and growth of lake trout (*Salvelinus namaycush*) in the Sudbury Basin. In preparation to be submitted to *Canadian Journal of Zoology*.

This chapter was conceptualized and designed by CAT, HKS, and BDN. Samples were collected by CAT, KAA, HKS, and TAJ. Laboratory data were collected by CAT, PCW, SM, KAA, and KE. CAT conducted the statistical analyses and performed data visualization, with input and guidance from MT and HKS. The original draft of this study was written by CAT and was then reviewed and edited by HKS and BDN. All authors contributed to revisions of the final manuscript. This manuscript is in preparation for submission to a journal and has been sent to all coauthors for feedback.

Chapter 3: Therrien, CA, Baker, PM, Garner, SR, Wolfe, PC, Matesic, S, Edwards, K, Wilson, CC, Swanson, HK, Neff, BD (2025) The effect of diet-derived thiaminase on survival, growth, and liver transketolase activity in two strains of lake trout (*Salvelinus namaycush*). *Journal of Great Lakes Research,* 51: 102689.

This chapter was conceptualized by CAT, SRG, KE, CCW, and BDN and designed by CAT, SRG, HKS, and BDN. CAT, PMB, and SRG all contributed to animal husbandry. Laboratory data were collected by CAT, PMB, PCW, SM, and KE. CAT conducted the statistical analyses and performed data visualization, with input and guidance from HKS. The original draft of this study was written by CAT and was then reviewed and edited by HKS and BDN. All authors contributed to revisions of the final manuscript.

Chapter 4: Therrien, CA, Baker, PM, Garner, SR, Swanson, HK, Neff, BD (2025) Dietary thiaminase alters morphology and decreases swimming performance of lake trout (*Salvelinus namaycush*). *Scientific Reports*, 16:5493. https://doi.org/10.1038/s41598-026-35139-8

This chapter was conceptualized and designed by CAT, HKS, and BDN. CAT, PMB, and SRG all contributed to animal husbandry. Swim flume trials were conducted by CAT and PMB. Laboratory data were collected by CAT. CAT conducted statistical analyses and performed data visualization, with input and guidance from HKS. The original draft of this study was written by CAT and was then reviewed and edited by HKS and BDN. All authors contributed to revisions of the final manuscript.

Chapter 5: Therrien, CA, Wilson, CC, Louste-Fillion, J, Neff, BD, Swanson, HK (2026) First record of natural reproduction by lake trout (*Salvelinus namaycush*) in an acid-damaged lake: lessons for lake trout reintroduction programs. In preparation for submission to *Canadian Journal of Fisheries and Aquatic Sciences*.

This chapter was conceptualized and designed by CAT and HKS. Samples were collected by CAT and JLF. Fish aging was conducted by CAT and HKS. CCW conducted genetic strain determination. CAT conducted statistical analyses and performed data visualization, with input and guidance from HKS. The original draft of this study was written by CAT and was then reviewed and edited by HKS and BDN. All authors contributed to revisions of the final manuscript. This manuscript is in preparation for submission to a journal and has been sent to all coauthors for feedback.

Abstract

Reintroduction programs are important tools for reversing extirpations of fishes in freshwater environments, but only 25-50% of reintroduction programs are successful. Exotic species are present in many habitats that are targeted for restoration, and exotic species can negatively affect reintroduced species through predation, competition, and changes in the quantity and/or quality of available prey. Thiamine (vitamin B1) is an important nutrient in the diet of fishes. There is growing concern that consumption of thiaminase, an enzyme that degrades thiamine and is found in the exotic prey fish rainbow smelt (*Osmerus mordax*), may be impacting reintroductions of lake trout (*Salvelinus namaycush*) in lakes where they are present. Ongoing programs to reintroduce lake trout in Ontario, specifically in the Sudbury Basin and Lake Ontario, provide an opportunity to study how the outcome of reintroduction efforts is influenced by (1) the presence of exotic rainbow smelt; and (2) identity of source population (with specific focus on previous exposure to exotic prey species).

I used a multi-lake study to determine if presence of exotic rainbow smelt resulted in decreased tissue thiamine concentrations in lake trout and compared fish body condition and growth between lakes that do and do not contain rainbow smelt. I found that while muscle thiamine concentrations of lake trout did not differ between lakes with and without rainbow smelt, an instance of thiamine deficiency was observed in a lake that contains rainbow smelt. I also found that body condition was significantly lower in lake trout from lakes with rainbow smelt and the asymptotic length of lake trout populations declined with increasing proportion of smelt in the diet. My results suggest that consumption of exotic rainbow smelt can cause thiamine deficiency, decrease body condition and lower asymptotic length in lake trout, and ultimately may be a factor contributing to the failure of programs to reintroduce lake trout

throughout Ontario. Next, I compared the effects of diets designed to mimic the thiaminase activity of exotic prey fishes, including rainbow smelt, on survival and performance-related traits between two hatchery strains (Seneca Lake and Slate Island) of lake trout that differ in historical exposure to diet-derived thiaminase. Results indicated that, regardless of strain, the diet containing thiaminase negatively affected most performance-related traits and while most of the negative effects of the thiaminase diet did not differ between the two strains, an increase in red pigmentation and decrease in survival was observed in Seneca Lake fish that had received the thiaminase diet. By comparing the response of two hatchery strains of lake trout to a diet containing thiaminase, I found evidence that pre-existing adaptations to thiaminase differ among hatchery strains of lake trout. Of the hatchery strains I examined, the Slate Island strain is the best environment match and is likely the most suitable for reintroduction into lakes where exotic prey fishes occur. Lastly, I captured a fish of presumed wild origin from Tyson Lake, which has been subjected to reintroductions of lake trout since the 1970s but previously had no records of natural reproduction. Using a combination of methods, I confirmed the fish was, indeed, of wild origin and represents the first documentation of natural reproduction in Tyson Lake. The fish was assigned to both the Lake Manitou and Iroquois Bay strains, which suggests that these two strains of lake trout may be best suited for reintroduction into lakes in the Sudbury Basin, due to their ancestry match to extirpated populations of lake trout in the area. By incorporating multiple methods, this thesis identifies some of the major factors influencing the success of reintroductions of lake trout and advances our understanding of best practices for species reintroductions.

Acknowledgements

The work in this thesis and my progression throughout both my academic and professional careers would not be possible without the help of an extensive support network of colleagues, lab mates, friends, family, and keen volunteers. I first want to thank my supervisor Heidi Swanson and my unofficial co-supervisor Bryan Neff for all their support, encouragement, and help over the past five and a half years. This whole journey started when Zoë Lindo pulled me aside one day after class and urged me to reach out to Heidi, who she said would be a perfect fit as a doctoral supervisor. Heidi, I'm not sure why you decided to take a chance on me and my crazy project way further south than the waterbodies you normally do your research on, but I'm sure glad you did. I want to thank you for putting up with me over the years, navigating all my requests, providing career advice, portaging that 5hp motor up that crazy hill between Matagamasi and Goldie Lake, and for having faith in me. Bryan, we've been working together for over 10 years now and you've supervised all 3 of my theses. Thank you for all your help these years, from sitting with me in a car for 8 hours one day to attend a LOASRP meeting, to many successful "sampling trips" to Lake Huron, and countless manuscript revisions to address reviewer comments. You both have helped me achieve my dream career and make meaningful contributions to the management of the gamefish populations I so dearly love. For that I am grateful. I hope we can continue to collaborate in the future.

My progression through this thesis would also not be possible without the help of my internship supervisor Tom Johnston and his team at the Aquatic Research and Monitoring Section. Tom, I remember the look on your face after our first meeting when I first proposed my project. You thought I was nuts! Turns out, I am! That's exactly why I was able to get it done! I then remember your surprise when you asked me how sampling went on Bell Lake, and I told

you I was catching non-stop lake trout. I'm glad I could get you those samples! Thank you to all the ARMS staff that helped me with all my collections, spent countless days on the water with me, and who I have become good friends with over the years. Shout out to Lee Haslam, Michelle Giroux, Emily Fields, Natalie Lafleur, Charlie Leclair, and Joanna Bronson for their help in the field and for spending countless hours in a boat with me.

I would like to thank the members of my thesis and examining committees: Paul Craig and Michael Power, for your support throughout my degree; Brian Dixon, for stepping in as an advisor and examiner at the last minute; and Scott Hinch for agreeing to serve on my examination committee! Thank you to those who gave me advice and helped me resolve some of the theoretical problems I was stuck on including Michael Thorn, John Gunn, and Matthew Gilbert.

The project would have happened without the assistance of quite a few people. I am incredibly grateful to everyone who helped with sample collection and/or donated samples. Special thanks to my labmates Bronte McPhedran, Isabel Hilgendag, Peter Baker, Spencer Weinstein, Kevin Adeli, Taylor Luu, and Jennifer Zhou for making the trip to Sudbury and help collecting fish and invertebrates. Thanks to my friends Trent Sizer, Eric Boudreau, and Steven Morin for many sampling trips together and long nights processing fish. Thanks to my family members Reese Irwin, D'arcy Paquette, Brandon Legrand, and Norah Legrand-Podrucky for all their help collecting fish and invertebrates. Thank you to the many anglers who provided samples that helped make this work possible including the Lake Wanapitei Home and Campers Association, especially Jon Tomori, Denis Chaput, and Blaine Nicholls, and Doug Bruce for providing lake trout samples from Lake Panache. Special thanks to Daniel Picard for providing lake trout samples and for hosting Blaine and I for a great sampling trip to Burwash Lake.

Thanks to all my labmates from the Neff and Swanson labs for listening to my talks, providing feedback, sharing updates on the latest science, and for all the advice. Special thanks to Kevin Adeli, Peter Baker, Rosie Smith, and Kirsten Nickel for their suggestions on parts of the thesis.

This work would not have been possible without contributions from our government partners. Special thanks to the Sudbury District of the Ontario Ministry of Natural Resources (MNR), especially Jean Enneson and Denis Gendron for answering countless questions and providing grey literature on lake trout populations in the district. Thank you to the Northeast Region Broadscale Monitoring team for donating samples and to Michelle Gillespie for accommodating all my sampling requests. This work would not have been possible without help from the Fish Culture Section. Thanks to the Chatsworth Fish Culture Station for donating the lake trout that made this work possible. Special thanks to Christopher Wilson for accommodating all my requests and for answering my countless questions on the history of fish culture in Ontario. Special thanks to Jason Intini for answering all my animal husbandry questions.

Numerous funding sources made this research possible. Funding for the work in Chapter 2-5 was provided by NSERC Discovery Grants awarded to each of my supervisors Bryan Neff and Heidi Swanson (Chapters 2-5), an Early Researcher Award awarded to my supervisor Heidi Swanson (Chapter 2), an NSERC Create Grant awarded to my co-supervisor Bryan Neff (Chapter 2-5), and a GLFC Pilot project grant awarded to my co-supervisor and coauthors Bryan Neff, Chris Wilson, and Katie Edwards (Chapters 3-4). In addition to these funding sources, I received a OFAH Zone H Fisheries Research Grant for my work in Chapters 2 and 5, an NSERC Create FishCAST scholarship and additional Indigenous student grant, an NSERC Canada Graduate Scholarship, and other scholarships through the University of Waterloo and the Canadian Society of Aquatic Sciences.

I want to thank the Reid family, my cousin Pat Vincent, and my friends Niko Eusebi and Mat Stefan for letting me stay at their places while commuting between universities, which made the lab work possible.

Lastly, I want to thank my family for believing in me and for going out of their way to help me succeed. Without the love and support of my friends and family, none of this would be possible.

Science is truly a collaborative effort.

Dedication

This thesis is dedicated to my late grandmother Marie-Jeanne "*Grandmaman*" Therrien, who passed away in March 2025. She was always so supportive of my work and enjoyed hearing my countless fieldwork and fishing stories. She is greatly missed.

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List of Abbreviations

ARMS – Aquatic Research and Monitoring Section

ATP – Adenosine Triphosphate

AUP – Animal Use Protocol

BCA – Bicinchoninic Acid Assay

BsM – Broadscale Monitoring

CFEU – Cooperative Freshwater Ecology Unit

DDE - Dichlorodiphenyldichloroethylene

DNA – Deoxyribonucleic Acid

EMS – Early Mortality Syndrome

EPOC – Excess Post-exercise Oxygen Consmption

FCE – Food Conversion Efficiency

FSIS – Ontario Fish Stocking Information System

GLFC – Great Lakes Fishery Commission

GLM – General linear model

HPLC – High Performance Liquid Chromatography

HUFA – Highly Unsaturated Fatty Acids

IUCN – International Union for the Conservation of Nature

LMM – Linear Mixed Effects Model

M74 – Miljöbetinjad 1974

MES - Morpholinoethanesulfonic Acid

NADH – Nicotinamid Adenine dinucleotide

NADPH – Nicotinamid Adenine Dinucleotide Phosphate

NTP – Nitrothiophenol

NYSDEC – New York State Department of Environmental Conservation

OMECP – Ontario Ministry of Environment, Conservation, and Parks

OMNR – Ontario Ministry of Natural Resources

OMNRF – Ontario Ministry of Natural Resources and Forestry

PC – Principal Component

PCA – Principal Component Analysis

PCB - Polychlorinated Biphenyls

PIT – Passive Integrated Transponder

PUFA – Polyunsaturated Fatty Acids

RLRT – Restricted Likelihood Ratio Test

ROS – Reactive Oxygen Species

SO_2 – Sulfur Dioxide

TCA – Trichloroacetic Acid

TDP – Thiamine Diphosphate

TDC – Thiamine Deficiency Complex

TGC – Thermal Unit Growth Coefficient

TMP – Thiamine Monophosphate

TMS – Tricaine Mesylate

TTP – Thiamine Triphosphate

USFWS – United States Fish and Wildlife Service

USGS – United States Geological Survey

VBGF – von Bertalanffy Growth Function

Chapter 1

General introduction

1.1 Global biodiversity crisis

Rapid growth of the human population over the past century (Dasgupta, 2021; Rees, 2023; Steffen et al., 2015) has had profound detrimental effects on natural ecosystems, such that many can no longer sustain the abundance of wildlife they once supported (Borgelt et al., 2022; Ceballos et al., 2015; Ehrlich & Ehrlich, 1981; IPBES, 2019; Lees et al., 2022). In well-studied taxonomic groups (e.g., mammals, birds, reptiles, amphibians, fish), thousands of species and a myriad of populations have reduced in abundance or vanished (Ceballos et al., 2017; Ceballos et al., 2015; Ceballos & Ehrlich, 2002, 2023; Cowie et al., 2022; Hughes et al., 1997). Current animal extinction rates are estimated to be hundreds or thousands of times higher than those observed in the years prior to 1492 A.D., when European colonization of the Americas began (Barnosky et al., 2011; Ceballos et al., 2015; Cowie et al., 2022; Loehle & Eschenbach, 2012), and the consequent loss of biodiversity affects ecosystem services, stability, and productivity, as well as human well-being and livelihoods (Dudgeon et al., 2006; Lynch et al., 2023; Olson et al., 2002; Pejchar & Mooney, 2009; Sala et al., 2000; Tilman, 1999; Worm et al., 2006).

Declines in biodiversity are particularly high in freshwater ecosystems (Jenkins, 2003; Sala et al., 2000; Vörösmarty et al., 2010), which are among the most affected environments on Earth (Carpenter et al., 2011; Dudgeon et al., 2006; Reid et al., 2019). In 1992, it was conservatively estimated that 20% of all freshwater fish species – 1800 species in total – were already extinct or in decline (Albert et al., 2021; Moyle & Leidy, 1992). While climate change

and habitat loss are major drivers of global biodiversity loss (Dudgeon et al., 2006; Kappel, 2005), freshwater fishes are additionally threatened by water abstraction, modification of river flows, pollution, decreased habitat connectivity, and exotic invasive species (Albert et al., 2021; Birk et al., 2020; Cowx & Portocarrero Aya, 2011; Dudgeon et al., 2006; Welcomme et al., 2010). Of these factors, exotic invasive species have been identified as the most common threat associated with fish extinctions (Bellard et al., 2016; Blackburn et al., 2019; Vardakas et al., 2025).

1.2 Exotic species

An exotic invasive species (herein described as 'exotic') is a species that arrives and establishes, often with human assistance, in a habitat it had not previously occupied, spreads autonomously, and has an impact on native species and/or the ecosystem (Simberloff, 2010). Impacts of exotic species on ecosystems can be widespread, and include both direct and indirect physicochemical impacts such as ecosystem modification, resource competition, hybridization, and predation (Gallardo et al., 2016; Gutiérrez et al., 2014; Simberloff, 2010). These impacts can result in economic losses, with the damage incurred to ecosystems from exotic species estimated to exceed $185 billion USD annually in the United States and $7 billion USD annually in Canada (Colautti et al., 2006; Marbuah et al., 2014; Windle, 1997). Despite the considerable economic and ecological impacts of exotic species, the mechanisms by which exotic species impact ecosystems often remain unclear; indirect impacts of invasive species can be difficult to measure, interact synergistically with other stressors in the environment (e.g., drought, disease, habitat alterations, etc..; Doherty et al., 2015; Fournier, 2020), and can compound through time (Dostál et al., 2013; Gallardo et al., 2016; Theoharides & Dukes, 2007; Wainright et al., 2021).

Studies of species interactions in invasion and restoration ecology have concentrated largely on how native species respond to exotic predators and competitors (Carey & Wahl, 2010; Doherty et al., 2016; Gurevitch & Padilla, 2004; Sax & Gaines, 2008; Vilà & Weiner, 2004; Weis, 2011). Top-down effects of exotic species on the abundance, diversity, productivity, or community structure of native species at lower trophic levels are well-documented (e.g., Strong 1992, Atkinson 2006, Weis 2011, Britton 2023), as higher trophic level invaders tend to have broader, more severe impacts than lower trophic level invaders (Doherty et al., 2016; Sax & Gaines, 2008). Indeed, a review by Doherty et al. (2016) identified exotic predators as the causal factor for 58% of modern bird, mammal, and reptile species extinctions. Globally, an additional 1192 species are threatened by exotic predators.

Impacts of resource competition between exotic and native species that occupy similar trophic positions has also received considerable attention (e.g., Vilà and Weiner 2004, Gurevitch and Padilla 2004, Carey and Wahl 2010). Gurevitch and Padilla (2004) identified competition with exotic species as a causal factor in population declines of 53% of the native species examined in their review. For example, introductions of non-native competitors, primarily black basses (*Micropterus* spp.), have been identified as a major causal factor in declines of native brook trout (*Salvelinus fontinalis*) populations in the Northeastern United States (Whittier & Kincaid, 1999). Competition with exotic species may also alter trophic connections and result in dietary niche shifts of native species, and these dietary niche shifts can have concomitant effects on life history. For example, stocking of exotic brown trout (*Salmo trutta*) into systems in Western North America has resulted in shifts in the diet and habitat use of native cutthroat trout (*Oncorhynchus clarkii*). In systems with exotic brown trout, the diet and foraging habitat of cutthroat trout has changed from one based on large invertebrates and fishes in the lower reaches

of rivers to one more reliant on small invertebrates and zooplankton in the upper reaches of rivers – presumably because brown trout outcompete cutthroat trout for higher quality prey in the more productive lower river reaches (Budy et al., 2018; McHugh et al., 2008; McHugh & Budy, 2006). Studies have also shown that cutthroat trout that are invertivores and occupy habitats in the low-productivity, higher stream orders have much lower size-at-maturity and fecundity than those that are piscivores and occupy habitats in the higher-productivity lower stream orders (Meyer et al., 2003), which may be an impediment to ongoing reintroduction attempts for cutthroat trout (Budy et al., 2021).

Understanding how exotic prey species can affect population declines of native species has received less research attention than understanding how exotic predators and competitors can affect population declines of native species (e.g., Short and Smith 1994, Fisher et al. 1996, Innes et al. 2010). In some cases, exotic prey species have been considered as an important resource for native predator species, acting as a supplemental food source and increasing predator growth rates (Carlsson et al., 2009; Pintor & Byers, 2015). For example, predation on newly invaded round goby (*Neogobius melanostomus*) in lakes Erie and Ontario resulted in increased growth of native smallmouth bass and walleye (*Sander vitreus*; Crane et al. 2015, Crane and Einhouse 2016). Indeed, a review by Pintor and Byers (2015) revealed that when exotic species were consumed as a supplement to native prey species populations, abundance and individual growth rates of native predators increased significantly. Here, the authors attributed increases in predator abundance and growth to a greater overall availability of prey biomass (Pintor & Byers, 2015). When exotic prey replaced native prey and became the only forage available, however, individual-level predator metrics were either not significantly different (predator growth rates,

survival) from pre-invasion levels, or were significantly lower (fecundity; Pintor and Byers 2015).

Exotic prey species can alter species composition and abundance at lower trophic levels, often outcompeting and replacing native species (see review in Carlsson et al. 2009). Dramatic changes to the food web were found following invasions of zebra mussel (*Dreissena polymorpha*) and marine blue mussel (*Myutilus galloprovincialis*) into the Great Lakes and Hudson River, resulting in a near-total replacement of native species by exotics (Carlsson et al., 2009; Geller, 1999; Strayer et al., 1998). While the newly established zebra mussel became a diet item of high importance for several taxa, including turtles (Bulté & Blouin-Demers, 2008; Patterson & Lindeman, 2009), birds (Petrie & Knapton, 1999; Ross et al., 2005), fish (Colborne et al., 2015; Magoulick & Lewis, 2002), and decapods (order Decapoda; Molloy et al. 1994, zu Ermgassen and Aldridge 2011), declines in growth rate and condition of several fish species, including lake whitefish (*Coregonus clupeaformis*; Pothoven et al. 2001, Pothoven and Madenjian 2008), were noted. Since body size and condition influence survival and the number and quality of offspring in fish (Barneche et al., 2018; Birkeland & Dayton, 2005), it is likely that the change in prey base negatively affected whitefish fitness (Carlsson et al., 2009).

Fitness of native predators can also be negatively affected by the fact that exotic prey species may be of lower quality (e.g., nutritionally incomplete) than native prey species, or contain harmful chemicals (Fukuda et al., 2016; Pintor & Byers, 2015). Unsuccessful reintroduction of Atlantic salmon (*Salmo salar*) into Cayuga Lake and Lake Ontario were thought to be mediated by effects of exotic alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax;* Fitzsimons et al. 1995, Fisher et al. 1996) on fitness of stocked Atlantic salmon. Both alewife and rainbow smelt contain relatively high levels of the thiamine-degrading

enzyme thiaminase. Thiaminase has been shown to contribute to thiamine deficiency, and is associated with a range of harmful effects, including death and reproductive failures (Brown, Fitzsimons, et al., 2005; Fitzsimons et al., 2009; Fitzsimons & Brown, 1998; Futia & Rinchard, 2019).

1.3 Thiamine, thiamine deficiency, and thiaminase

Thiamine, otherwise known as thiamin (vitamin B1), is an essential water-soluble vitamin that is required as a cofactor for roughly 2% of all cofactor-mediated enzymatic reactions. Thiamine is essential for both catabolic and anabolic central carbon metabolism and synthesis of branched-chain amino acids (Monteverde et al., 2017), including glycolysis and the citric acid cycle (Bettendorf, 2013). It is also necessary for proper neurological (Bâ, 2008), immune (Abraham et al., 2026; Ottinger et al., 2014; Wen et al., 2016), and muscular function (Romanenko et al., 1994). Thiamine is a heterocyclic molecule containing pyrimidine and thiazole moieties ligated to form thiamine (Figure 1.1; Jurgenson et al. 2009). It exists as 4 vitamers: free thiamine (TH), thiamine monophosphate (TMP), thiamine diphosphate (TDP; also known as thiamine pyrophosphate [TPP]), and thiamine triphosphate (TTP; see review in Lonsdale 2006, Bettendorf 2013, Bettendorff and Wins 2013a, 2013b). In its unphosphorylated form, TH acts as an antioxidant (Lukienko et al., 2000), but has no other known physiological function (Manzetti et al., 2014). After ingestion, free thiamine may be phosphorylated into its mono-, di-, or tri- phosphate forms (Figure 1.1). TMP has no known physiological function in animal cells, and must first be either dephosphorylated to free thiamine or phosphorylated to a physiologically active form (Bettendorf, 2013; Bettendorff & Wins, 2013a; Kamath et al., 2015; Mrowicka et al., 2023). TTP is produced constantly at a low rate in animal cells and, while its precise role remains unclear, it is thought to be important for proper neuron function (Bettendorff

et al., 1993; Bettendorff & Wins, 2013b; Cooper & Pincus, 1979). TDP is considered the biologically active form of thiamine, and mediates various enzyme complexes, including pyruvate dehydrogenase, transketolase, and 2-hydroxyacyl-CoA required for carbohydrate, amino acid, and lipid metabolism (Bettendorf, 2013; Bettendorff & Wins, 2013a; Casteels et al., 2007; Manzetti et al., 2014). TDP also plays a central role in nerve function, is associated with the production of neurotransmitters and antioxidant mechanisms, and is involved in the myelination of neurons (Bettendorff & Wins, 2013a; Whitfield et al., 2018).

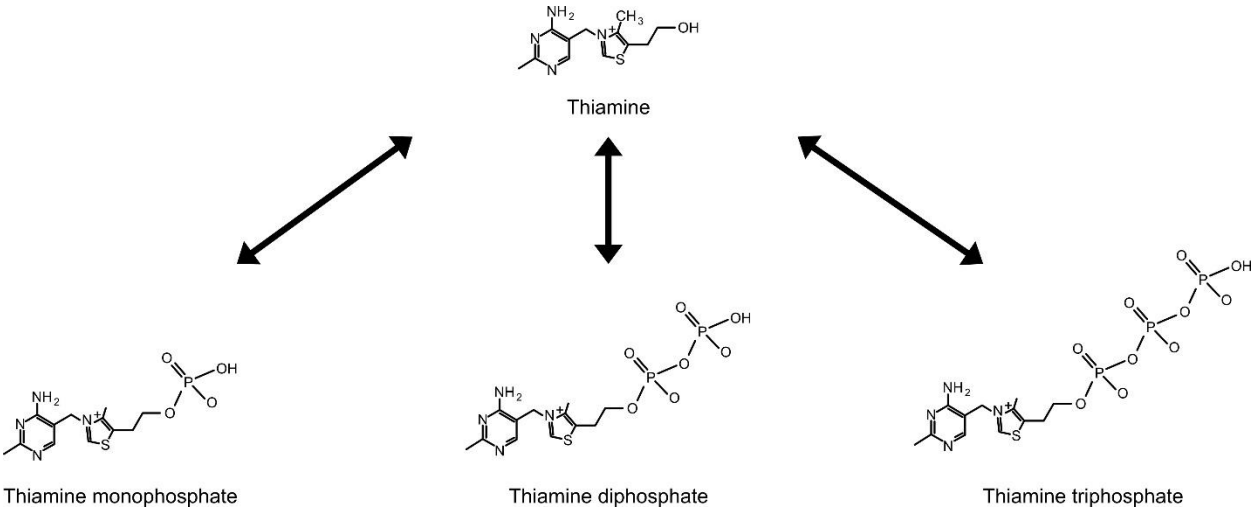


Figure 1.1 Vitamers derived from thiamine through phosphorylation. Free thiamine (thiamin) is phosphorylated into thiamine monophosphate (TMP), thiamine pyrophosphate (diphosphate; TDP), which is the active form of this vitamin and acts as a cofactor for enzymes necessary for carbohydrate metabolism, and thiamine triphosphate (TTP). Figure created by Julien Therrien and adapted from Kraft and Angert (2017).

In aquatic environments, some bacteria, archaea, eukaryotic algae, and fungi contain the necessary biosynthetic genes to produce thiamine *de novo* (prototrophs; Kraft and Angert, 2017, Paerl et al. 2018, Suffridge et al. 2020). Most other organisms lack these genes, however, and

must rely on exogenous thiamine or thiamine precursors (Kraft & Angert, 2017; Paerl et al., 2018; Suffridge et al., 2020) transferred through the diet in the pelagic food web from prototrophs to zooplankton, and then to fish at higher trophic levels (Ejsmond et al., 2019; Fridolfsson et al., 2018). There is no evidence that fish can synthesize thiamine; it is assumed that thiamine is acquired by consuming organisms that produce or contain thiamine, and that active transport of thiamine occurs in the gastrointestinal tract (Hoar & Randall, 1969; Manzetti et al., 2014). Other routes of thiamine uptake have been suggested in fish, such as gut synthesis of thiamine by bacteria, or thiamine uptake through the gills, but these are understudied (Y. Ji et al., 1998). Recently, new evidence suggests that fish eggs and sac-fry can absorb thiamine present in the environment through the egg and yolk-sac walls (Clark et al., 2023), a mechanism which has proved helpful in treating offspring of thiamine-deficient mothers through immersion in a thiamine bath (e.g., Brown et al. 2005a, Futia et al. 2017, Reed et al. 2023). Furthermore, while gut microbiota in *Drosophila melanogaster* and ruminants have been found to provision thiamine to their host when in a low-thiamine environment (Breves et al., 1979, 1981; Sannino et al., 2018), there is no evidence that this occurs in piscivorous fish, whose digestive tracts are smaller in relation to their body size and have less volume available for activity of microbiota (Wagner et al., 2009).

The production and dynamics of thiamine in aquatic ecosystems is a complex process and its availability depends on environmental factors such as pH, ultraviolet radiation, and temperature (Carlucci et al., 1969; Suffridge et al., 2023; Sylvander et al., 2013; Voelker et al., 2021). Furthermore, new research shows that anthropogenic impacts such as changes to nutrient inputs, climate change, and hydrology can reduce thiamine availability in the environment (Ejsmond et al., 2019; Suffridge et al., 2020, 2023). Food web structure affects the flow of

thiamine from lower to higher trophic levels (Ejsmond et al., 2019; Hylander et al., 2024), and thiamine content in organisms decreases as trophic position is increases (Hylander et al., 2024). Transfer of thiamine between trophic levels is less efficient in longer food webs compared to shorter food webs, and an increase in food web length can reduce overall thiamine transfer in the environment (Hylander et al., 2024). Furthermore, there is growing evidence that thiamine concentrations in phytoplankton are species-specific (Fridolfsson et al., 2018; Sylvander et al., 2013), and changes in composition of lower food web communities can lead to changes in thiamine availability (Hylander et al., 2024). Introduction of exotic species that alter food web structure may thus also alter the flow of thiamine in the environment.

Over the past few decades, thiamine deficiency has been observed in a variety of taxa, including reptiles (Sepúlveda et al., 2004), birds (Balk et al., 2009, 2016), mussels, and fishes (Balk et al., 2016; Engelhardt et al., 2020; Harder et al., 2018), and it has been suggested that thiamine deficiency is a possible contributor, and future threat, to the worldwide loss of biodiversity (Balk et al., 2016; Gilbert, 2018; Sutherland et al., 2018). For example, thiamine deficiency has been identified as a primary factor contributing to widespread die-offs and breeding failures of herring gull (*Larus argentatus*), European starling (*Sturnus vulgaris*), and common eider (*Somateria mollissima*) in Europe (Balk et al., 2009, 2016). Furthermore, thiamine deficiency has been attributed as a factor in global declines of American (*Anguilla rostrata*) and European eels (*A. anguilla*; Fitzsimons et al. 2013, Balk et al. 2016).

Globally, many salmonine (family Salmonidae, subfamily Salmoninae) populations are experiencing thiamine deficiencies (Fisher et al., 1995; Fitzsimons et al., 1995; Mantua et al., 2021; Norrgren et al., 1993; Reed et al., 2023). Low concentrations of thiamine in fish result in a syndrome that is now referred to as thiamine deficiency complex (TDC; Riley and Evans 2008).

In severe cases, thiamine deficiency is lethal (Fisher et al., 1995; Honeyfield et al., 2005), but the deficiency has also been associated with several sub-lethal effects, such as a decreased growth rates and food conversion efficiency, lethargy, reduced visual acuity, increased metabolic enzyme (e.g., transketolase) latency, reduction of cardiovascular function, hemorrhaging, and reproductive failures (reviewed in Harder et al. 2018, Baker et al. 2023, Adeli et al. 2024). Signs of thiamine deficiency in salmonines were first documented in 1968, when an unknown disease emerged and caused widespread mortality of hatchery-raised populations of Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), rainbow trout (*O. myskiss*), and brown trout (Marcquenski and Brown 1997). Initial descriptions of the disease included high mortality in sac fry (alevin) and was named early mortality syndrome (EMS). Over the next few decades, EMS became increasingly prevalent and mortality of hatchery-raised salmonines exceeded 90% in some populations (Marcquenski & Brown, 1997). At the same time, a syndrome named miljöbetingad 1974 (M74), which was similar to EMS in terms of symptoms, emerged in Atlantic salmon from the Baltic Sea, where hatchery-raised salmon populations suffered high rates of sac-fry mortality (Bylund & Lerche, 1995; Lundström et al., 1999). Elsewhere, seemingly unexplainable sac-fry mortality resulted in the loss of entire year-classes of wild-collected Atlantic salmon from Cayuga Lake in 1974, 1978, 1980-1984, and 1987 in New York State Department of Environmental Conservation (NYDEC) hatcheries, despite application of proven animal husbandry practices; this led to the coining of the term “Cayuga syndrome” (Fisher et al., 1995). It was not until later that all three syndromes – EMS, M74, and Cayuga syndrome – were linked to a thiamine deficiency (reviewed in Harder et al. 2018). In recent years, widespread sac-fry mortality and decreased reproduction has been observed in Chinook salmon of California’s central valley and the Yukon River (Honeyfield et al., 2016; Larson &

Howard, 2019; Mantua et al., 2021), as well as rainbow trout in Oregon hatcheries (Reed et al., 2023). Most recently, sublethal effects of thiamine deficiency have been reported in lake trout in the Sudbury Basin (Adeli et al., 2024).

Before EMS was linked to thiamine deficiency, early research focused on the possibility of environmental contaminants, such as polychlorinated biphenyls (PCBs), dioxins, furans, mercury, and heavy metals, being the source of the high mortality rates (Fitzsimons et al., 1995; Honeyfield et al., 1998; Mac et al., 1993; Niimi, 1983). Although exposure to these contaminants induced a similar syndrome in a laboratory setting, (McKim et al., 1976; Peterson et al., 1993; Spitsbergen et al., 1991; Walker et al., 1991, 1994), contaminant concentrations in wild-caught fish were below levels expected to induce the observed rate of mortality (Fitzsimons et al., 1995; Honeyfield et al., 1998; Mac & Edsall, 1991). The inability to link EMS to environmental contaminants led to an alternative hypothesis, which was that EMS was a nutritional deficiency. The connection was made when, out of desperation, John Fitzsimons dissolved an adult multivitamin into a tank of sac-fry plagued with EMS and found that survival improved and sub-lethal effects were reduced (Dale Honeyfield, USGS, pers. comm.). Shortly thereafter, Fitzsimons (1995) discovered that symptoms of EMS were alleviated following thiamine administration, whereas administration of other vitamins were ineffective.

To date, no general cause for thiamine deficiency is definitively known. There are two leading hypotheses: 1) the consumption of thiaminase; and 2) the consumption of high concentrations of lipids. In the Laurentian Great Lakes, New York's Finger Lakes (including Cayuga Lake), and lakes in the Sudbury Basin, the source of thiamine deficiency for salmonines appears to be consumption of exotic prey fishes that contain high concentrations of thiaminase (Fitzsimons & Brown, 1998; Honeyfield et al., 2012). Alewife and rainbow smelt, have high

thiaminase activity (Tillitt et al., 2005), are found throughout the Great Lakes Basin, and comprise a major proportion of the diets of salmonines (Kornis et al., 2020; Mumby et al., 2018; Nawrocki et al., 2022). The consumption of alewife and rainbow smelt has been associated with thiamine deficiency in several Great Lakes salmonine fishes, including lake trout, Atlantic salmon, coho salmon, and Chinook salmon (Brown, Fitzsimons, et al., 2005; Fitzsimons, Honeyfield, et al., 2021; Fitzsimons & Brown, 1998; Futia & Rinchard, 2019; Honeyfield et al., 2005). More recently, consumption of smelt has been found to induce the sub-lethal cardiac effects of thiamine deficiency in inland populations of lake trout (Adeli et al., 2024).

Thiaminases are a group of enzymes that break down thiamine in the stomach of consumers, ultimately limiting thiamine uptake (Honeyfield et al., 2005, 2010; Wolf, 1942). Thiaminases, specifically thiaminase I, catalyze the breakdown of thiamine into its precursors, specifically the replacement of the thiazol moiety (Figure 1.2; Campobasso et al. 1998). The production of thiaminase I in alewife and rainbow smelt was first believed to originate from gut microbiota, namely from the bacteria *Paenibacillus thiaminolyticus*, found in the gut of alewife (Honeyfield et al., 2002). Recent evidence suggests, however, that the synthesis of thiaminase I may also occur *de novo* (Richter et al., 2023; Rowland et al., 2023).

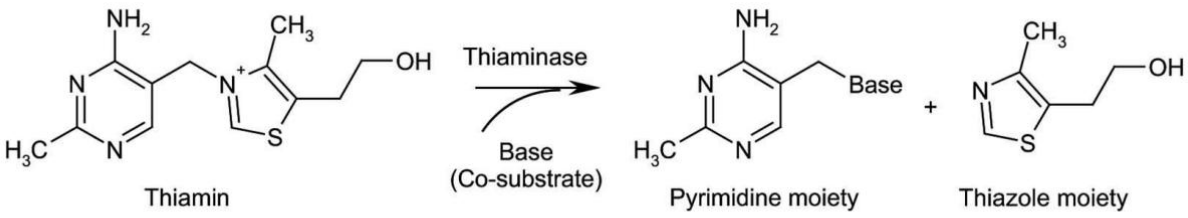


Figure 1.2 The mechanism for degradation of thiamine (thiamin) into its moieties by the enzyme thiaminase. Figure is adapted from Kraft and Angert (2017).

The factors that modulate thiaminase activity remain an ongoing area of research. Thiaminase activity has been found to vary seasonally and is influenced by environmental factors and substrate availability (Edwards et al., 2023; Lepak et al., 2013; Tillitt et al., 2005; Wistbacka et al., 2002). Thiaminase activity in alewife in the Great Lakes is greatest in summer and fall (Ji & Adelman, 1998; Tillitt et al., 2005). Previous researchers have found positive correlations between thiaminase activity and fish size and stress, and also a correlation between thiaminase activity and immune function; larger fish in poorer condition with infections had the highest thiaminase activity (Tillitt et al., 2005; Wistbacka et al., 2002, 2009; Wistbacka & Bylund, 2008). Thiaminase activity has been reported to be higher in herbivores than carnivores and to decrease with increasing trophic level, indicating that trophic and foraging characteristics might influence the thiaminase activity in the ecosystem (Boggs et al., 2019; Kraft et al., 2014; Kraft & Angert, 2017; Riley & Evans, 2008; Rowland et al., 2023). Thiaminase activity in the fish gut has been found to increase after consumption of thiaminase-producing prey (Arsan, 1970; Arsan & Malyarevskaya, 1974; Arsan & Malyarevskaya, 1969; Birger, 1973; Fitzsimons et al., 2004; Malyarevskaya et al., 1970, 1972), which may suggest that thiaminase can be transferred across trophic levels (Evans, 2016).

Alewife and rainbow smelt, which are high in thiaminase relative to native forage fishes, often become dominant forage species in lakes where they have invaded (Weidel et al. 2024, Hondrop et al. 2025, NYSDEC 2025, Ontario Ministry of Natural Resources [OMNR] unpublished results), and their consumption has been directly linked to the development of thiamine deficiency in salmonines (Fitzsimons & Brown, 1998). Conversely, recent findings from studies of salmonines in Baltic Sea and Lake Ontario suggest that thiamine deficiency

instead appears to be driven by consumption of prey fishes that are high in fatty acids but low in thiamine (Futia et al., 2019; Keinänen et al., 2012, 2018). Consumption of high amounts of easily-oxidized fatty acids can lead to oxidative stress, which may be exacerbated when thiamine is present at low concentrations relative to lipids (Keinänen et al., 2012). Specifically, highly unsaturated fatty acids (HUFAs), namely n-3 series polyunsaturated fatty acids (n-3PUFAs), are highly abundant in many fishes, such as alewife and spratt (*Sprattus sprattus*), prey fishes that are identified causes of thiamine deficiency in the Great Lakes and Baltic Sea, respectively (Vuorinen et al., 2020). HUFAs are susceptible to lipid peroxidation (Sargent et al. 2003, Keinänen et al. 2012). Lipid peroxidation, the production of a lipid peroxyl radical, is a particularly harmful form of oxidative stress and requires additional antioxidant defenses (Porter et al., 1995). Thiamine acts as both a cofactor in fatty acid metabolism and as an antioxidant, sequestering free radicals associated with lipid peroxidation (Li et al., 2014; Lukienko et al., 2000; Tylicki et al., 2018; Vuorinen et al., 2020). Ultimately, when HUFAs are present in high and thiamine in low enough concentrations, the thiamine present in the food is not great enough to support fatty acid metabolism and the fish's endogenous thiamine stores are drawn upon (Vuorinen et al., 2020). Continued consumption of high-lipid prey fishes can then lead to the utilization of the fish's thiamine stores to the point of deficiency (Vuorinen et al., 2020). Regardless of the mechanism, thiamine deficiency is an emerging stressor that is hypothesized to be a significant contributing factor in large-scale wildlife mortalities and loss of biodiversity (Balk et al., 2016).

1.4 Reintroduction of extirpated populations

Reintroduction programs, in which conspecific individuals are either translocated from the wild or released from captivity into formerly occupied habitats, have emerged as an

important tool for reversing extirpations (Armstrong & Seddon, 2008; International Union for the Conservation of Nature [IUCN], 2013; Lesage et al., 2020; Seddon, 2010). Reintroduction programs are appealing as a means of restoring biological populations and communities toward a historic baseline and have been practiced for over a century (Kleiman, 1989). Examples of successful reintroduction programs include the grey wolf (*Canis lupus*) into Yellowstone National Park (Bangs, 1996), wild turkey (*Meleagris gallopavo*) in Ontario (MacDonald et al., 2016; Nguyen et al., 2003), and duskytail darter (*Etheostoma percnurum*) in Abrams Creek, Tennessee (Shute et al., 2005). There has been a pronounced increase in the number of species targeted for reintroduction programs globally, rising from 124 species in the early 1990s to 424 species in 2005 and up to 213 animal species alone in 2019 (Resende et al., 2020; Seddon et al., 2014). Even in the absence of obvious barriers to species reintroductions, however, only 25–50% of reintroduction programs are successful (Fischer & Lindenmayer, 2000; Godefroid et al., 2011; Harding et al., 2025) and the causes or factors affecting reintroduction success are often unclear or understudied.

Reintroduction program success can be affected by physicochemical and biological changes that have taken place in the environment since the time of extirpation (Houde, Garner, et al., 2015). For example, declines in water quality at the reintroduction site since the time of extirpation was the largest factor influencing reintroduction failures of non-salmonid fishes globally (Cochran-Biederman et al., 2015). Furthermore, in a habitat suitability study for reintroduction programs in Utah, it was found that 94% of the studied streams were unacceptable for reintroductions of native species (van Wieren, 2012). One such way the environment can change is with the invasion of exotic species. As reviewed above, exotic species can either directly predate upon or act as competitors to native species targeted for reintroduction (e.g.,

Short et al. 1992, Godefroid et al. 2011, Cochran-Biederman et al. 2015). The role of exotic prey species as a causal factor for the failure of reintroduction programs has been less studied than effects of exotic predators and competitors (e.g., Short et al. 1992, Innes et al. 2010); however, there is growing concern that consumption of exotic prey fish may be impacting Salmonine reintroductions in the Laurentian Great Lakes, New York's Finger Lakes, and the Sudbury Basin (Fisher et al., 1996; Fitzsimons & Brown, 1998; Selinger et al., 2006). The consumption of thiaminase, present in exotic prey fishes, has been shown to cause thiamine deficiency in these systems (Adeli et al., 2024; Brown, Fitzsimons, et al., 2005; Fisher et al., 1996; Futia et al., 2019). While thiamine deficiency can be lethal or result in reproductive failure, there may be several sublethal effects, such as declines in growth and swimming performance, that influence the fitness of fishes in the wild (reviewed in Harder et al. 2018), and ultimate could affect the success of reintroduction programs in lakes where they cooccur.

1.5 Population selection strategies

Recently, several guidelines, strategies, and best practices have emerged for selection of source populations for reintroduction of extirpated species (Houde, Garner, et al., 2015; Figure 1.3). One such strategy, the pre-existing adaptation strategy, focuses on selecting populations with a high frequency of genotypes that should confer adaptations in the reintroduction location (Houde, Garner, et al., 2015); the premise of this strategy is that source populations may differ in viability in the reintroduction location, because of genetically based differences in individual fitness that result from local adaptations (Houde, Garner, et al., 2015). Local adaptation, the process wherein individuals of a local population exhibit higher fitness in their local environment compared to individuals from a different population and environment (Kawecki & Ebert, 2004), can lead to differences in fitness among conspecific populations of fishes across different

environments (reviewed in Fraser et al. 2011). For example, coho salmon, Chinook salmon, and sockeye salmon (*O. nerka*) populations with variable morphology that reflected their local migratory environment differed in swimming performance, aerobic scope, metabolic rates, and thermal performance (Eliason et al., 2013; Pon et al., 2007; Raby et al., 2016; Van Wert et al., 2023). Targeting source populations that have adaptations to key environmental features of reintroduction locations – e.g., presence of exotic competitors or prey – for translocation could increase the success of reintroduction programs.

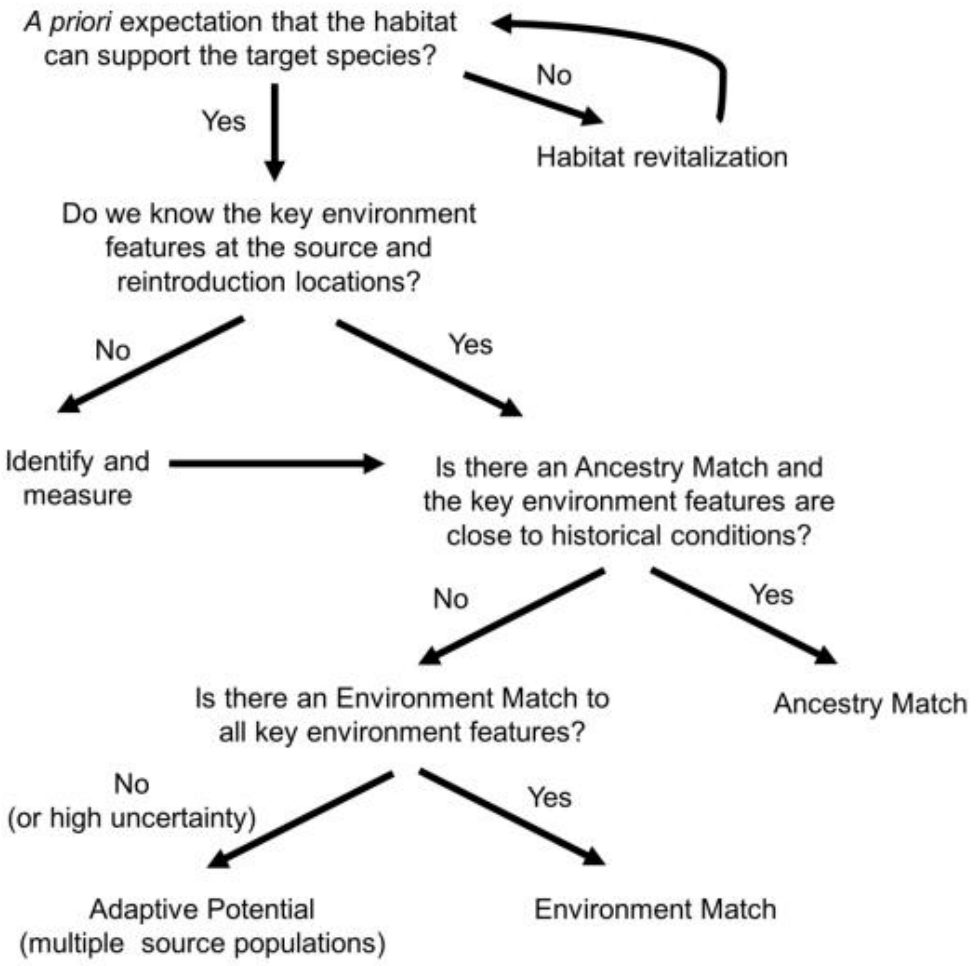


Figure 1.3 A framework for selecting source populations for reintroduction. The framework is an optimized guide for selecting source populations from Houde (2015). Reprinted with permission.

The pre-existing adaptation strategy was used to evaluate suitability of three candidate source populations of Atlantic salmon for reintroduction into Lake Ontario (Houde, Saez, et al., 2015; Houde, Wilson, et al., 2015; Mitchell et al., 2021; van Zwol et al., 2012). Source populations of Atlantic salmon were screened using a series of hatchery- and mesocosm-based studies to evaluate fitness when fed exotic prey fish species that contained high levels of

thiaminase (Houde, Saez, et al., 2015; Mitchell et al., 2021), and in the presence of multiple exotic competitors (Houde, Wilson, et al., 2015; Van Zwol et al., 2012; van Zwol et al., 2012). Populations of Atlantic salmon from lakes where rainbow smelt (a prey fish high in thiaminase) was a key prey item scored higher in performance-related metrics (e.g., body condition), than did a population of salmon from a lake where prey items were low in thiaminase (Dimond & Smitka, 2005; Houde, Saez, et al., 2015; Rikardsen & Dempson, 2011). One of the Atlantic salmon populations that fed primarily on rainbow smelt also co-existed with exotic brown trout in their native environment (Marsden et al., 2010; Pellerin & Pierce Jr., 2015); this population had higher performance-related metrics (e.g., growth) when in an experimental stream setting with brown trout than did the population that did not coexist with any exotic salmonid in its native environment (Houde, Wilson, et al., 2015; van Zwol et al., 2012). The population that fed on rainbow smelt and which coexisted with exotic brown trout in its native environment appeared to make up most adult returns of Atlantic salmon in Lake Ontario (Chris Wilson, OMNR, pers. comm.) and were successfully introduced to Lake Huron, lakes with both brown trout and rainbow smelt (Tucker et al. 2014; Roger Greil, Lake Superior State University, pers. comm.).

The ancestry matching strategy focuses on selecting the closest genetic relatives to the historical population. This approach is based on the premise that close geographic and/or genetic proximity may confer adaptations to key environmental features of the reintroduction location as the same genes may occur in both the source and extirpated populations if they were present in a recent common ancestor or were transferred between populations through gene flow (Houde, Garner, et al., 2015; Moritz, 1999). In addition, ancestry matching has the greatest potential to restore an extirpated population to its near-original state, which may be particularly valuable when restoring populations of cultural or evolutionary significance (Moritz, 1999). Typically,

several unlinked genetic markers, such as microsatellite loci or single nucleotide polymorphisms (SNPs) are used to estimate genetic similarity between source and historical populations (Beaumont & Nichols, 1997; Parker et al., 1998; Williams et al., 2014). Alternatively, geographic distance between the source and reintroduction locations can be used a proxy for genetic similarity as there is often a correlation between the two variables (Montalvo & Ellstrand, 2000; Raabová et al., 2007; Wilson & Hebert, 1998).

While ancestry matching has been used in successful translocations of other animals (e.g., bighorn sheep, Flesch et al., 2020; macroinvertebrates, Jourdan et al., 2019), its use in fish reintroductions has been limited. The ancestry matching strategy was used to evaluate the suitability of three candidate source populations of Atlantic salmon for reintroduction into Lake Ontario (Dimond & Smitka, 2005; Houde, 2015). There, source populations of Atlantic salmon were also screened based on their geographic proximity to the reintroduction location. The population of Atlantic salmon that was the closest ancestry match to Lake Ontario (Lac Saint Jean strain) had proportionally higher adult returns than other strains of Atlantic salmon in Lake Ontario tributaries (McCarthy et al. 2018; Chris Wilson, OMNR, pers. comm.). Unfortunately, despite this relative success, the use of this strain was ultimately discontinued because of poor survival in the provincial fish culture system (Jake Ruegg, OMNR, pers. comm.). The ancestry matching strategy was also used to evaluate the suitability of candidate populations of anadromous rainbow trout, Chinook salmon, coho salmon, and sockeye salmon for reintroduction into the rehabilitated upper Columbia River (Hardiman et al., 2017). Targeting source populations with adaptations to key environmental features of reintroduction locations – e.g., presence of exotic competitors or prey – or a genetic similarity to the extirpated historical population for translocation could increase the success of reintroduction programs.

1.6 Lake trout

Lake trout are a cold-water fish of considerable importance to fisheries in Ontario. In addition to being a commercially and recreationally important species, lake trout are a traditional food fish for the Cree, Anishnabek, and Métis peoples of Ontario (Preston, 1981; E. S. Rogers, 1994). Lake trout is a misnomer, as they are members of the genus *Salvelinus*, and are charr rather than trout. As a member of the charrs, they are characterized by their extreme phenotypic diversity, including wide differences in behaviour, life history, body shape, and colouration among sympatric and allopatric morphs (Muir et al., 2016; Noakes, 2008). In fact, lake trout have been considered one of the most diverse of all vertebrates on earth (Muir et al., 2016). Lake trout are the largest (>30 kg) and longest lived (>60 years) of the charrs and are a cold-water specialist (Gibson & Fry, 1954; Grande & Andersen, 1991) with a preferred temperature of 8–12 °C (Martin & Olver, 1980; Muir et al., 2021). In Ontario, the species mainly inhabits clear, deep, oligotrophic lakes with well-oxygenated bottom waters (Christie and Regier 1988, OMNR 1990, Plumb and Blanchfield 2009, Ontario Ministry of Natural Resources and Forestry [OMNRF] 2015), but can also exist in turbid lakes (Northrup et al., 2010), river systems (e.g., Nipigon or Yorston Rivers; OMNRF 2015), and shallower lakes (<17 m) with moderate levels of productivity (e.g., Elboga Lake, Ontario, Canada, OMNRF 2015).

Lake trout have particularly high visual acuity for feeding in deep, low-light conditions (Ali & Wagner, 1980) and are adapted to food deprivation by having high numbers of pyloric caeca to assist with nutrient assimilation (Vladykov, 1954). Lake trout can make use of diet items from the pelagic, profundal, and littoral zones of lakes, and will often forage outside of their preferred temperature optimum to capture prey (Muir et al. 2016, Guzzo et al. 2017, 2025, Ontario Ministry of Environment, Conservation, and Parks [MECP], unpublished results). These

adaptations, paired with their typical diet generalism, allow lake trout to thrive in low productivity environments (reviewed in Muir et al. 2021). While typically a piscivorous top predator that feeds on native species such as cisco (*C. artedi*), slimy sculpin (*Cottus cognatus*), yellow perch (*Perca flavescens*), and white sucker (*Catostomus comersonii*) in Ontario lakes, lake trout can also forage extensively on zooplankton and benthic invertebrates as alternate food sources (N. V. Martin & Olver, 1980; Muir et al., 2021). For example, in Bell Lake (Ontario, Canada), lake trout feed heavily on the abundant cisco population, while in Colin Scott Lake (Ontario, Canada), a lake without any fish species other than lake trout, lake trout feed on zooplankton, chironomids (family Chironomidae), dragonfly nymphs (infraorder Anisoptera), crayfish (*Faxonius virilis*), and terrestrial insects (Adeli, 2023; Dawe, 2023). Their flexible dietary niches also enable them to make use of novel prey items as they become available, for example with the introduction of exotic species (e.g., Rush et al. 2012).

Lake trout are only present in one percent of Ontario's lakes (~2300 lakes), yet this represents 20–25% of lakes in the world that support this species (Selinger et al. 2006, OMNRF 2015). There has been a general decline in the quality of the lake trout sport fishery (Selinger et al., 2006), and approximately 5% of the province's lake trout populations are currently listed as extirpated (Martin & Olver, 1976; OMNR, 1990; OMNRF, 2015; Selinger et al., 2006). Further, lake trout lakes in Northeastern Ontario are considered to be in poor health overall, with only 74% of lakes considered to have self-sustaining lake trout populations (OMNRF, 2015; Selinger et al., 2006). To improve the status of these fisheries, several multimillion-dollar reintroduction programs for lake trout have been undertaken (Keller et al., 2019a; Lantry et al., 2014; Selinger et al., 2006), particularly in Northeastern Ontario – near the Sudbury Basin – and in Lake Ontario.

1.7 Lake trout reintroduction in the Sudbury Basin

Historically, lake trout were widespread throughout Northeastern Ontario, including in the Sudbury Basin. Losses of populations of lake trout throughout the Sudbury Basin began to be documented in the 1940s and 50s, appearing to peak in the 1960s. An estimated 94 lakes throughout the Sudbury Basin experienced significant declines in, or a disappearance of, their lake trout fishery (Matuszek et al., 1992). These losses were linked to a single point source of acid deposition: the metal smelters in Sudbury, Ontario (Matuszek et al., 1992). Known as the nickel capital of the world, Sudbury's smelters represented one of the largest point sources of SO_2 pollution in the world (Potvin & Negusanti, 1995). Beginning in 1888 and increasing annually, SO_2 emissions peaked in 1960 when annual emissions were 2.5 million tonnes (Potvin & Negusanti, 1995). Lakes in the Sudbury basin were severely affected by the atmospheric deposition of SO_2. Over 7000 lakes acidified to the extent that pH was below 6.0, the point at which significant biological damage is expected (Neary et al., 1990). Early netting assessments in these acidified lakes indicated a loss of lake trout recruitment (e.g., death in the early developing stages of eggs, embryos and young juveniles) at pH 5.3-5.6 (Beggs & Gunn, 1987; Gunn, 1989; Matuszek et al., 1992). When lake pH was below 5.2, the entire lake trout population was usually extirpated (Beamish, 1976; Beggs & Gunn, 1987).

Since the peak in the 1960s, atmospheric SO_2 emissions from Sudbury's smelters and other sources have decreased in response to government regulations and improved technology. This decrease facilitated the chemical recovery of many lakes, beginning in the late 1970s and early 1980s (Gunn et al., 1988; Keller et al., 1986; Keller et al., 2019b; Meyer-Jacob et al., 2020). Lake trout reintroduction programs began in acid-damaged but chemically recovered lakes in the 1980s (Selinger et al., 2006). Despite nearly 1.2 million lake trout being stocked into

lakes in the Sudbury Basin for the purpose of reintroduction (OMNR 2025a), stocking has only established self-sustaining populations in ~30% of target lakes (Evans & Olver, 1995; Louste-Fillion, 2022; Selinger et al., 2006). Biological recovery of acid-damaged lakes has been slow. Various acid-sensitive invertebrates and fish have recolonized lakes, but often the lake environment has changed considerably from the conditions before acidification. For example, invasions of warm water competitors and predators, such as smallmouth bass and rock bass (*Ambloplites rupestris*), have occurred, as well as the invasion of the exotic prey fish rainbow smelt, and proliferation of native prey fish, such as cisco (see review in Gunn and Louste-Fillion 2022), are such ways in which the lake environments have changed since the time of lake trout extirpations. These changes, especially the proliferation of native cisco to abundances that result in competition with and predation upon stocked lake trout and the invasion of exotic species, are thought to be a contributing factor to populations declines and reintroduction failures of lake trout in the area (Brown, Fitzsimons, et al., 2005; Gunn et al., 1987; Selinger et al., 2006; Snucins & Gunn, 2003; Métis elder D'arcy Paquette, pers. comm.).

1.8 Lake trout reintroduction in Lake Ontario

Lake trout were once abundant in Lake Ontario, were an important predator in the pelagic food web, and had considerable commercial value (Elrod et al., 1995; Pritchard, 1931). However, the population declined to lows in the late 1880s before being extirpated in the 1950s (Christie, 1973; Elrod et al., 1995; Morrison, 2019) as a result of overharvest, invasive sea lamprey (*Petromyzon marinus*) predation, and the destruction of cobble and gravel spawning beds for aggregate (Christie, 1973; Elrod et al., 1995; Sly, 1991). Beginning in 1896, an extensive reintroduction program began, with an aim to increase the abundance of lake trout in Lake Ontario. Over 100 million fry were stocked into Lake Ontario from New York hatcheries (Elrod

et al., 1995). However, these efforts failed to result in any self-sustaining populations (Elrod et al., 1995). By the 1950s, efforts to re-establish lake trout focused on stocking fingerling and yearlings, instead of fry, but the outcome remained the same as previous attempts; failure was attributed to continued commercial harvest and sea lamprey predation (Christie, 1973; Elrod et al., 1995). The management focus then shifted to controlling exotic lamprey, and the treatment of lamprey-spawning tributaries with lampricide began in 1971 (Elrod et al., 1995). These efforts were ultimately successful in reducing lamprey biomass in Lake Ontario (Elrod et al., 1995). As a result, a new lake trout reintroduction program began in 1973 that involved stocking 1.25 million yearlings from multiple hatchery populations annually, introducing fishing restrictions, and continuing suppression of sea lamprey, in the hopes of establishing a self-sustaining population of 0.5 to 1 million adults (Elrod et al., 1995; Schneider et al., 1983).

Ongoing efforts to reintroduce lake trout in Lake Ontario have resulted in some wild recruits, but a self-sustaining population has not yet established (Lantry et al., 2019; Lantry et al., 2014; O'Malley et al., 2025). One potential challenge is marked changes in the prey fish community that have occurred over the past century (Bronte, Jonas, et al., 2003; Crawford, 2001; Morrison, 2019). Lake Ontario salmonines historically fed primarily on cisco and bloater (*C. hoyi*), but these prey fishes have largely been replaced by introduced alewife and rainbow smelt (Beeton, 2002; Miller, 1957; Morrison, 2019). Lake Ontario now has the lowest diversity of small-bodied prey fishes of all the Laurentian Great Lakes, and the bulk of prey fish biomass is comprised of alewife (Weidel et al., 2018; Weidel et al., 2019; Weidel et al., 2024; Weidel & Dunlop, 2018). All recent diet studies have identified that alewife dominate the diets of lake trout in Lake Ontario (Nawrocki et al., 2022). Unlike their native counterparts, alewife and rainbow smelt contain high levels of the enzyme thiaminase, which has been associated with thiamine

deficiency in many Great Lakes salmonids (Brown, Honeyfield, et al., 2005; Fitzsimons & Brown, 1998; Futia & Rinchard, 2019), and ultimately been determined to be a major hurdle in re-establishing lake trout populations in Lake Ontario (Lantry et al., 2014; Muir et al., 2012).

1.9 Rainbow smelt

Rainbow smelt (herein, smelt) are an anadromous forage fish native to coastal regions of North America and isolated lakes of the St. Lawrence drainage system (Evans & Loftus, 1987; Scott & Crossman, 1998). Smelt were first observed in the Laurentian Great Lakes in 1922 and spread thereafter through two invasion pathways (Hazra et al., 2025). First, smelt were introduced into Crystal Lake, which adjoins Lake Michigan, beginning in 1912 (Creaser, 1925). This population spread into Lake Michigan and dispersed thereafter (Creaser, 1925; Hazra et al., 2025). Smelt were then observed in Lake Ontario in 1929 (New York State Conservation Department, 1939). This invasion of smelt into Lake Ontario originated from either an anthropogenic introduction (Bergstedt, 1983) or via natural entry to the lake through the St. Lawrence River and subsequent dispersal (Hazra et al., 2025; Mills et al., 1993). Regardless of the exact mechanism, smelt of both the Acadian (St. Lawrence) and Atlantic (Crystal Lake) haplotype are found in the Canadian waters of all the Laurentian Great Lakes (Hazra et al., 2025). There are now also naturalized populations in at least 59 lakes in Northeastern Ontario (Dextrase & Mandrak, 2006; Evans & Loftus, 1987).

The ecological impacts of smelt invasions are difficult to predict and variable among lakes. They often become incredibly abundant in lakes they have invaded (Franzin et al., 1994). They are currently among the most numerous fishes in the Canadian waters of the Great Lakes (Hondrop et al., 2025; NYSDEC, 2025a; Vinson et al., 2024; Weidel et al., 2024). For example, they comprise 86% of the prey fish biomass of Lake Huron's North Channel (Hondrop et al.,

2025). Smelt compete with and predate upon native prey fishes species (e.g., cisco; Mercado-Silva et al. 2006, Myers et al. 2009) and often replace them completely (Hrabik et al., 1998). Smelt predate heavily on large zooplankton (e.g., *Mysis diluviana*), which often leads to their elimination, which in turn, leads to compensatory increases in the abundance of small-bodied zooplankton (Beisner et al., 2003; Brooks & Dodson, 1965; Reif & Tappa, 1966; Siegfried, 1987). Studies have shown that smelt alter food web length (Vander Zanden & Rasmussen, 1996) and disrupt trophic interactions (Lammens et al., 1985; Swanson et al., 2003) in many lakes where they have invaded. Ecological changes induced by smelt invasions have resulted in increases of dichlorodiphenyldichloroethylene (DDE), polychlorinated biphenyls (PCBs), and mercury in predatory fish tissues in some lakes (Kiriluk et al., 1995; MacCrimmon et al., 1983; Mathers & Johansen, 1985; Vander Zanden & Rasmussen, 1996) but no changes in others (Johnston et al., 2003; Swanson et al., 2003). It has been hypothesized that rainbow smelt mediated changes to trophic structure may alter how thiamine transfers between trophic levels (Hylander et al., 2024).

Smelt have a relatively low body depth–body mass ratio and do not have any defences such as dorsal spines, allowing them to be readily eaten by a wide range of predators that typically cannot consume fish due to gape constraints imposed by native prey fishes (e.g., yellow perch, cisco; Swanson et al. 2003). Due to their high fat content, predation on smelt has been associated with higher growth rates in walleye and lake trout (Evans & Loftus, 1987; MacCrimmon et al., 1983; Mathers & Johansen, 1985). However, smelt contain relatively high levels of the enzyme thiaminase (Tillitt et al., 2005), which has been shown to contribute to thiamine deficiency (Fitzsimons & Brown, 1998; Futia et al., 2019). Indeed, smelt have been implicated in lake trout recruitment declines in lakes in Maine, Ontario, Vermont, Minnesota,

(Evans & Loftus, 1987; Hassinger & Close, 1984; Labar, 1993; Riley & Marsden, 2009), and consumption of smelt has been linked to declines in egg thiamine concentrations (Fitzsimons & Brown, 1998). Further, a diet containing smelt has been associated with deleterious changes to heart morphology, which could affect the fitness of lake trout (Adeli et al., 2024).

1.10 Source populations of lake trout for reintroduction

Currently, the OMNR maintains nine hatchery populations (henceforth referred to as strains) of lake trout that differ in their history of co-occurrence with exotic species and have different diets in their native environment (Figure 1.4; Tables C-1 and C-2 in Appendix C). In Lake Ontario and the Sudbury Basin, four strains of lake trout are commonly stocked as a part of rehabilitation efforts (Lantry et al., 2014; Selinger et al., 2006). The Killala Lake strain (henceforth referred to as Killala) originates from Killala Lake and is the primary strain for lake trout reintroductions in inland Ontario lakes (Evans and Olver 1995, Christopher Wilson, OMNR, pers. comm.). In its native environment, the Killala strain typically feeds on a diet of invertebrates, perch, sculpin (*Cottus* spp.), and cisco, and encounters no exotic competitors (Therrien and Leitrants 2025, OMNR unpublished results). The Michipicoten Island strain (henceforth referred to as Michipicoten) from Michipicoten Island and the Slate Islands strain (henceforth referred to as Slate) from the Slate Islands, both originate from populations in Lake Superior and have been primarily used in reintroduction projects of lake trout in the Laurentian Great Lakes. Their use in restoration projects in inland lakes has been limited; they are primarily stocked as surplus in lakes with put-grow-take programs for recreational opportunities (Evans & Olver, 1995; Lantry et al., 2014; Selinger et al., 2006). In their native environments, both the Michipicoten and Slate strains encounter exotic competitors (Chinook salmon, coho salmon, pink salmon (*O. gorbuscha*), and rainbow trout) while feeding primarily on a diet comprising

cisco, sculpin, burbot (*Lota lota*), and invertebrates (OMNR unpublished results; Table C-2 in Appendix C). The Slate strain also encounters smelt, which made their way to the area by the 1930s (MacKay, 1963; Van Oosten, 1937); smelt now comprises a large proportion of lake trout diet in the Slate Islands (Dryer et al. 1965, Ray et al. 2007, OMNR unpublished results). Lastly, the Seneca Lake strain (henceforth referred to as Seneca) originates from Seneca Lake, New York. Similar to the strains from Lake Superior, the Seneca Lake strain has primarily used in reintroduction projects in the Great Lakes, with limited use in inland lakes. In their native environment, Seneca Lake strain lake trout encounter exotic competitors brown trout, largemouth bass (*M. salmoides*), and rainbow trout, as well as a native smallmouth bass population (NYSDEC, 2025b; Ryder, 1972). Seneca strain lake trout historically fed on a diet of native cisco, whitefish, and possibly yellow perch (Odell, 1934; Ryder, 1972). However, after alewife invaded Seneca Lake in the late 1800s, they replaced the native prey fishes as the dominant prey for lake trout in the lake (NYSDEC, 2025b; Odell, 1934).

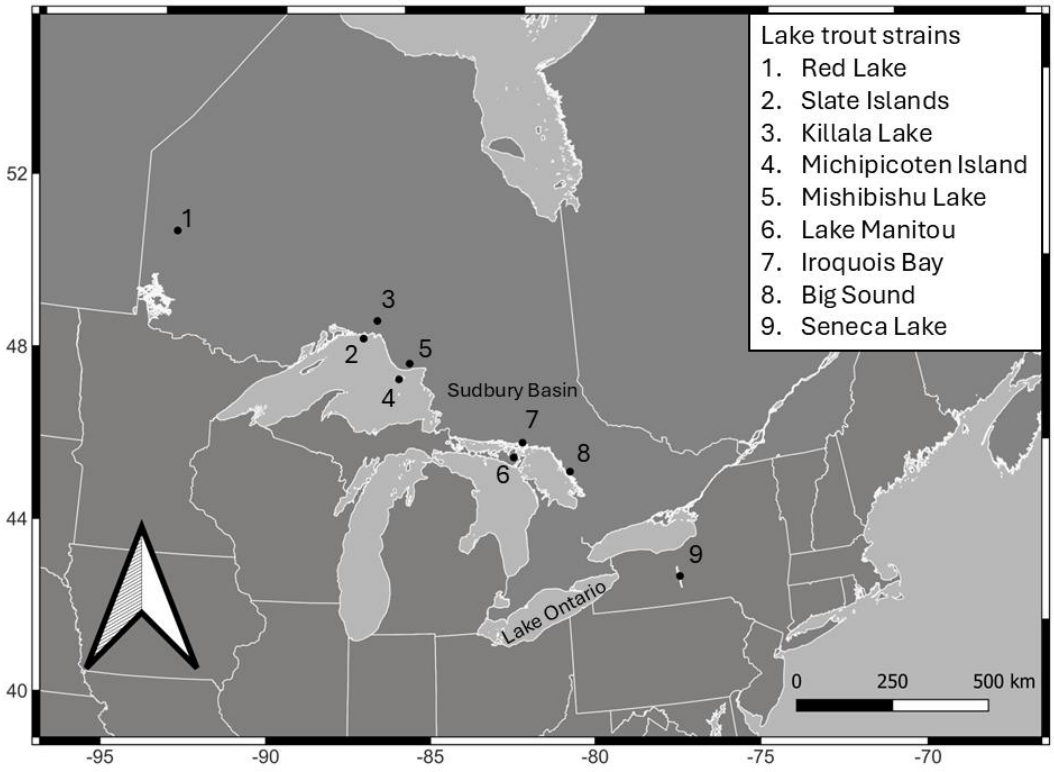


Figure 1.4 Map of the source of lake trout hatchery strains used in reintroduction programs in Ontario in reference to the Sudbury Basin, ON and Lake Ontario. Map made with QGIS version 3.36.2 with CANVEC 50K ON waterbodies and CUGIR hydrology shapefiles and EPSG:2163 projection.

Given the long history of alewife presence in Seneca Lake, which is a prey fish high in thiaminase and HUFAs, it has been suggested that the Seneca Lake strain lake trout may have a higher tolerance to exotic prey fishes that are high in thiaminase compared to the Slate, Michipicoten, and Killala strains. Indeed, Fitzsimons et al. (2021) showed that Seneca strain embryos use less thiamine as compared to embryos from other strains, and initial analyses showed that Seneca strain fish have higher survival and represent a majority of the current spawning biomass in the Great Lakes (Ebener et al., 2025; Lantry et al., 2019; Larson et al., 2021; Rogers et al., 2019). Furthermore, a study conducted by Baker et al. (2023) found that the

effects of diet-derived thiaminase on heart morphology and function were less pronounced in the Seneca strain compared to the Slate strain. However, recaptures of stocked Slate and Michipicoten fish in Lake Ontario, particularly in the Eastern Basin (MECP, unpublished results), and use of the Slate strain to successfully establish a self-sustaining population in Whiskey Lake (Ontario, Canada; MECP, unpublished results), a lake whose forage comprises rainbow smelt (Adeli et al., 2024; Dawe, 2023), might suggest these strains may also some have some resilience to diet-derived thiaminase. Although alewives are in low abundance in Lake Superior (Bronte et al., 1991) and have not been observed in the stomachs of lake trout near the Slate or Michipicoten Islands, thiaminase-containing smelt are the most frequent prey fish observed in the diet of Slate fish (OMNR, unpublished results), which may have exerted selection for genes conferring resilience to thiaminase. Among-strain differences in resilience to diet-derived thiaminase might be predicted given variability in the abundance of high-thiaminase prey fishes in the native environments of lake trout, but this has yet to be tested.

1.10 Objectives and thesis structure

Ongoing reintroduction programs for lake trout in Ontario, specifically in the Sudbury Basin and Lake Ontario, provide an opportunity to study how the success of reintroduction efforts is influenced by (1) the presence of exotic prey species; and (2) identity of source population (with specific focus on previous exposure to exotic prey species; Figure 1.5).

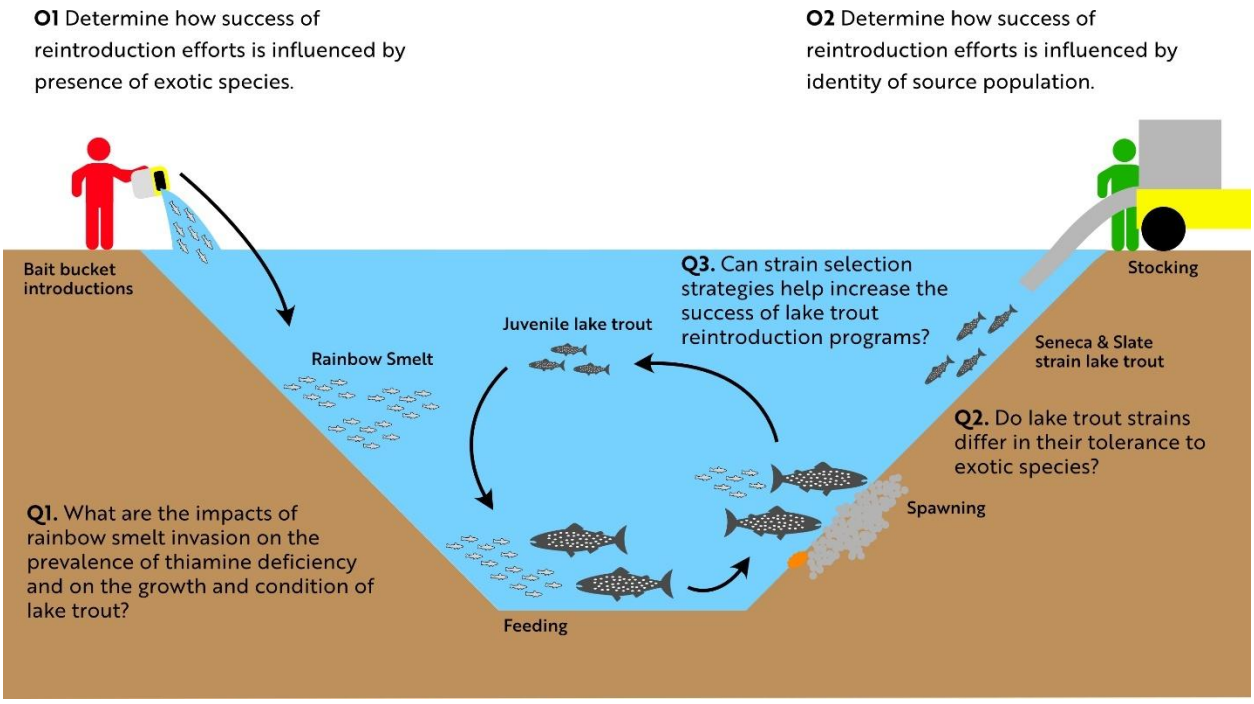


Figure 1.5 Schematic diagram of key thesis objectives and questions regarding the effects of exotic rainbow smelt (*Osmerus mordax*) on lake trout (*Salvelinus namaycush*) reintroductions in Ontario. Objectives and questions are numbered in the order they appear in the thesis. Figure prepared by Julien Therrien.

This thesis contains four data chapters. In Chapter 2, "Consumption of exotic rainbow smelt (*Osmerus mordax*) is associated with thiamine deficiency and a decline in the condition and growth of lake trout (*Salvelinus namaycush*) in the Sudbury Basin", I collected tissues from lake trout from lakes throughout the Sudbury Basin; some lakes have exotic smelt whereas others do not. I investigated whether lake trout in smelt lakes had lower tissue thiamine concentrations. I also examined how trophic ecology of lake trout in the Sudbury Basin influences their tissue thiamine concentrations. In Chapters 3 "The effect of diet-derived thiaminase on survival, growth, and liver transketolase activity in two strains of lake trout (*Salvelinus namaycush*)" and 4 "Dietary thiaminase alters morphology and decreases swimming performance of lake trout (*Salvelinus namaycush*)", I compared the effects of diet-derived thiaminase on survival and

performance-related traits between two strains (Seneca and Slate) of lake trout that differ in historical exposure to diet-derived thiaminase. Juvenile lake trout from the Seneca and Slate strains were reared in a common garden environment and received either an experimental diet containing bacterial derived-thiaminase or a control diet. Six months after the initiation of the experimental diets, survival, liver transketolase activity, growth, food conversion efficiency, swimming performance, morphology, colouration, and probability of recovery were compared between strains and treatments. Lastly, in Chapter 5,"First record of natural reproduction by lake trout (*Salvelinus namaycush*) in an acid-damaged lake: lessons for lake trout reintroduction programs", I captured an unmarked lake trout during fisheries surveys on Tyson Lake – a lake undergoing lake trout reintroduction efforts in the Sudbury Basin – and used a combination of stocking records, ageing, and strain assignment (using genotyping at 17 microsatellite loci), to describe the first instance of natural reproduction of lake trout in the lake since their extirpation in the 1970s. Tyson Lake has received plantings of three separate strains as a part of its rehabilitation program, and I discuss the implications of this finding for strain selection during restoration programs.

Fishes are among the most threatened taxa in Canada, with over 70 units (e.g., populations, subspecies, species) listed for protection under the Species At Risk Act (Species at Risk Act, 2002), and lake trout in Ontario are currently the focus of multiple, multimillion-dollar reintroduction programs that have yet to establish self-sustaining populations in 71% of the lakes targeted for reintroduction (Evans & Olver, 1995; Keller et al., 2019a; Lantry et al., 2014). The overall aim of this doctoral thesis is to contribute to the growing knowledge of best practices for reintroduction of lake trout, identify some of the major factors influencing lake trout

reintroduction success in Ontario, and provide information to fisheries managers that will allow more effective and efficient restoration, management, and conservation.

Chapter 2

Consumption of exotic rainbow smelt (*Osmerus mordax*) is associated with thiamine deficiency and a decline in the condition and growth of lake trout (*Salvelinus namaycush*) in the Sudbury Basin

This chapter is an adapted version of the following paper, in preparation for submission to the Canadian Journal of Zoology:

Therrien CA, Adeli KA, Wolfe, PC, Matesic, S, Edwards K, Johnston TA, Thorn MW, Neff BD, Swanson HK. 2026. Consumption of exotic rainbow smelt (*Osmerus mordax*) is associated with thiamine deficiency and a decline in the condition and growth of lake trout (*Salvelinus namaycush*) in the Sudbury Basin.

2.1 Introduction

Exotic invasive species (henceforth referred to as exotic species) can threaten conservation and restoration of native species via predation, competition, parasitism, habitat alteration, and hybridization (Clavero & Garcia-Berthou, 2005; Gurevitch & Padilla, 2004). Exotic species have been listed as one of the greatest threats to conservation of biodiversity (Bellard et al., 2021; Bellard, Genovesi, et al., 2016; Millennium Ecosystem Assessment, 2005). Almost 20% of the vertebrates that are in danger of extinction are threatened in some way by exotic species (McNeely, 2001). However, the role of exotic prey species as a causal factor for

population declines of native species has been less studied than effects of exotic predators and competitors (e.g., Short et al. 1992; Fisher et al. 1996; Innes et al. 2010). As prey, exotic species can be of lower quality (e.g., nutritionally incomplete) than native prey or contain harmful compounds that can lower the fitness of native predators (Fukuda et al., 2016; Pintor & Byers, 2015). For example, the cane toad (*Bufo marinus* [Linnaeus, 1758]) appears as easy prey, being found in high densities in their introduced range and often do not flee from predators (Freeland, 1986; Hudson et al., 2017; Lever, 2001). However, they contain toxic compounds that can poison and kill predators (Burnett, 1997; Lever, 2001; Phillips et al., 2003). Additionally, exotic prey items containing harmful compounds (specifically the enzyme thiaminase) have been hypothesized to be a causal factor in the failure of several salmonid (Family Salmonidae [G. Cuvier, 1816]) reintroduction attempts in the Laurentian Great Lakes and New York's Finger Lakes (Fisher et al., 1996; Fitzsimons et al., 1995).

Rainbow smelt (*Osmerus mordax* [Mitchill, 1814]; hereafter, smelt) are an anadromous forage fish native to coastal regions of North America and isolated lakes of the St. Lawrence drainage basin (Evans & Loftus, 1987; Scott & Crossman, 1998). This species has recently expanded due to a series of accidental and intentional introductions (see review in Rooney and Paterson 2009). Anadromous smelt have adopted a non-anadromous life history and now inhabit lakes throughout Ontario and Manitoba, extending to Hudson Bay (see review in Rooney and Paterson 2009). The ecological impacts of smelt invasions are difficult to predict and variable among lakes. Due to their high fat content, predation on smelt has been associated with higher growth rates in walleye (*Sander vitreus* [Mitchill, 1818]) and lake trout (*Salvelinus namaycush* [Walbaum in Artedi, 1792]; MacCrimmon et al. 1983; Mathers and Johansen 1985; Evans and Loftus 1987). However, smelt contain relatively high levels of the enzyme thiaminase (Tillitt et

al., 2005; Zajicek et al., 2009), which has been shown to contribute to thiamine deficiency (Fitzsimons & Brown, 1998; Futia et al., 2019). Thiamine deficiency is associated with a range of harmful effects. While in severe cases thiamine deficiency is lethal, it has been associated with several secondary effects such as a decrease in growth rates, lethargy, reduced visual acuity, reduction of cardiovascular function and hemorrhaging, and reproductive failures (Adeli et al., 2024; Baker et al., 2023; reviewed in Harder et al., 2018). Indeed, smelt have been implicated in lake trout recruitment declines in lakes in Maine, Ontario, Vermont, and Minnesota (Evans & Loftus, 1987; Hassinger & Close, 1984; Labar, 1993; Riley & Marsden, 2009), and consumption of smelt has been linked to declines in egg thiamine concentrations (Fitzsimons & Brown, 1998). While the nature of thiamine deficiency in the wild is episodic, with the thiamine status of individuals differing over time and space (Balk et al., 2016; Harder et al., 2018), bouts of cellular stress induced by thiamine deficiency have been linked to changes that persist even when fish recover from the deficiency (Adeli et al., 2024; Gomes et al., 2021; Mair et al., 1988). Furthermore, studies have shown that smelt alter trophic interactions in lakes where they have invaded (Lammens et al., 1985; Swanson et al., 2003), which may alter how thiamine transfers between trophic levels, limiting thiamine availability at higher trophic levels (Hylander et al., 2024).

Lake trout are native to many lakes across the Sudbury Basin (Ontario, Canada), where they are a culturally important component of recreational and subsistence fisheries (Selinger et al., 2006). In several lakes that contain lake trout, smelt have been introduced (Evans & Loftus, 1987) and often outcompete native prey fishes (Hrabik et al., 1998; Loftus & Hulsman, 1986), ultimately becoming a significant proportion of lake trout diets (Adeli et al., 2024; Johnston et al., 2003; Kirn & Labar, 1996; Ray et al., 2007). Furthermore, a diet containing smelt has been

associated with deleterious changes to heart morphology which could be affecting the fitness of lake trout (Adeli et al., 2024; Baker et al., 2023). Increases in ventricular mass, reductions in ventricular fluid content, and alterations in the allometry of myocardia have been found in lake trout populations of lakes of the Sudbury Basin and have been attributed to smelt consumption (Adeli et al., 2024). Indigenous groups in the area are also reporting a decrease in the quality of the lake trout fishery since smelt invasion (Métis elder D'arcy Paquette, pers. comm.), which could affect the traditional food supply of indigenous peoples in the Sudbury Basin.

We conducted a multi-lake study in the Sudbury Basin, to determine if the presence of smelt decreases tissue thiamine concentrations of lake trout, and to determine the prevalence of thiamine deficiency in lake trout across the Sudbury Basin. We hypothesized that the consumption of smelt affects the body condition and growth of lake trout via effects on tissue thiamine concentrations and predicted that lake trout in lakes with smelt would have lower growth and be in poorer body condition than those without smelt. Lastly, we aimed to determine if the trophic ecology of lake trout in the Sudbury Basin influences their tissue thiamine levels. We predicted that lake trout in lakes that consume the most smelt would have the lowest tissue thiamine concentrations.

2.2 Methods

2.2.1 Fish and invertebrate collection

Lake trout were captured between February 2021 and September 2023 via gillnetting and angling in 22 lakes in the Sudbury Basin (Ontario, Canada; Table 2.1), the same lakes studied in (Adeli et al., 2024). A breakdown of lake trout captures by collection method is included in Table D-1 in the Appendix D. Of the 22 lakes included in the study, 6 contained smelt and 16 did not.

Table 2.1 Lakes sampled for lake trout (*Salvelinus namaycush*) in the Sudbury Basin (Ontario, Canada).

Lake	[a]Latitude	[a]Longitude	Surface Area (ha)	Max Depth (m)	Current stocking status	[b]N	N mature[c]	Length[d] (mm)	Mass[d] (g)
Rainbow smelt present									
Bear[e]	45.778N	80.435W	682.3	36.6	Natural	15	3	445 (311-585)	662 (232-1582)
Manitou	45.778N	80.435W	10437.8	49.0	Stocked	24	18	698 (411-737)	1797 (539-3685)
Manitou 2	46.853N	80.283W	322.3	47.6	Natural	17	13	478 (290-776)	1219 (153-3841)
Panache	46.236N	81.307W	8959.0	56.4	Natural	21	4	550 (329-622)	721 (250-1588)
Wanapitei	46.440N	80.739W	13131.0	125.0	Natural	28	28	496 (445-660)	1672 (862-2722)
Whiskey	46.440N	82.336W	992.8	55.2	Stocked	12	8	496 (367-660)	1043 (360-2341)
Rainbow smelt absent									
Bell	46.129N	81.204W	217.7	26.8	Stocked	7	8	698 (642-782)	2999 (2257-4367)
Burwash	47.136N	81.053W	1058.2	52.12	Natural	28	24	478 (404-572)	878 (458-1678)
Chiniguchi	46.926N	80.707W	1198.3	44.2	Stocked	16	15	550 (485-630)	1473 (800-2300)
Colin Scott	46.830N	80.505W	43.5	43.0	Stocked	13	11	496 (426-540)	1077 (627-1316)
Elboga	47.020N	81.635W	27.2	16.2	Stocked	12	3	382 (254-583)	494 (142-1306)
Evelyn	46.896N	80.591W	110.7	24.0	Natural	11	9	435 (299-610)	791 (195-2211)
Fraleck	46.915N	80.883W	166.0	23.2	Stocked	4	2	396 (280-490)	633 (156-1108)
Kelly 27	46.782N	80.530W	17.3	17.0	Stocked	8	5	487 (306-721)	1344 (237-2934)
Mohzabong	46.965N	82.077W	1943.8	44.0	Natural	6	6	555 (486-661)	1290 (161-2049)
Nelson	46.727N	81.094W	315.8	50.3	Natural	9	6	426 (216-569)	713 (72-1712)

Paradise	46.975N	80.766W	487.4	35.0	Natural	27	4	444 (280-665)	946 (135-3752)
Rawson	46.918N	80.565W	164.1	26.0	Natural	10	4	397 (346-498)	506 (332-1116)
Savage	46.250N	81.592W	322.1	36.6	Natural	6	3	530 (296-783)	1704 (210-4706)
Shakwa	46.764N	81.978W	649.0	27.4	Natural	27	5[f]	442 (292-520)	711 (210-1180)
Tyson	46.117N	81.119W	1089.1	39.6	Stocked	9	3	573 (314-891)	2770 (252-7441)
Wavy	46.302N	81.096W	306.3	34.0	Stocked	9	11	516 (483-559)	1592 (862-2722)

a. Latitude and longitude values are for the geographical center of the waterbody

b. Number of lake trout sampled from each lake

c. Number of mature lake trout sampled from each lake

d. Total length and mass of lake trout are reported as mean with range in parentheses.

e. Bear Lake contains rainbow smelt but there was no evidence of their consumption by lake trout inferred by stomach content analysis results

f. Maturity not recorded for 17 fish from Shakwa Lake.

Thirteen of the 22 lakes had self-sustaining lake trout populations whereas 9 had populations sustained by supplemental stocking as part of efforts to reintroduce lake trout to acid-damaged lakes. In the lakes that were sampled by gillnetting, the OMNR Broad-scale Monitoring (BsM) protocol was followed (Sandstrom et al., 2018). Briefly, monofilament benthic gill nets with stretched mesh sizes ranging from 19 to 127 mms were set horizontally on the lake bottom in each of eight depth strata (1-3 m, 3-6 m, 6-12 m, 12-20 m, 20-35 m, 35-50 m, 50-75 m, +75 m). Each lake stratum was represented by at least two net replicates. Nets were set when surface temperatures were above 18 ℃ and left overnight for 16–22 hours. Water chemistry parameters were also collected for each lake and can be found in Table D-2 in Appendix D.

A total of 107 and 161 lake trout were sampled from lakes with and without smelt, respectively (Table 2.1). Fork length (± 1 mm), total length (± 1 mm) and total mass (± 1 g) were measured. For 15 fish, total length was not measured and was estimated using a linear regression between total length and fork length (Figure D-1 in Appendix D). Body condition of individual fish was estimated as the residual from the least-squares-fitted regression of log (mass) = log (a) + b log (total length) for the pooled data set (Kaufman et al., 2007; Ricker, 1973). A fish with a residual value > 0 was considered in good condition, while a fish with a residual value of <0 was considered in poor condition. Two ~ 2.5 cm^3 samples of skinless, boneless white axial muscle and gastrointestinal tracts were excised from each fish and stored on ice in the field until they could be transferred to a freezer. Sagittal otoliths were also removed, cleaned, dried, and stored for aging. One white muscle sample and all gastrointestinal tracts were frozen at -20 °C and the remaining white muscle sample and all livers were frozen at -80 °C until further processing.

To establish a lake-specific baseline signature for stable isotope ratios, primary consumers were also collected. Flat-headed mayfly nymphs (family Heptageniidae [Needham, 1901]) were chosen as the primary consumer as they were readily collected from most lakes in the Sudbury area. Mussels and snails were collected from some lakes, but large-scale acidification eliminated mollusc populations from most lakes within the Sudbury Basin (reviewed in Keller et al. 1986). Isotope values for mussels and snails are included in Table D-3 in Appendix D. Mayfly nymphs were picked from the undersides of submerged rocks in the littoral area of lakes. Approximately 50 individual mayfly nymphs (~ 5 mL of volume) were collected from each lake except Savage and Paradise lakes; logistical constraints prevented their collection in Savage Lake and no mayfly nymphs were found in Paradise Lake. Furthermore, a freezer malfunction destroyed mayfly nymph samples from Chiniguchi, Johnnie, and Kelly #27 lakes, and new samples could not be obtained. After collection, mayfly samples were frozen in bulk at -20 °C until further processing.

2.2.2 Tissue thiamine concentration

A subsample of white muscle from 59 fish (n = 59) was used to estimate tissue thiamine concentration (Table D-3 of Appendix D) using standard addition and thiochrome fluorescence. Samples were shipped on dry ice from Western University (London, ON) to Binghamton University (Binghamton, NY) and stored at -80 °C until analysis. Samples (~200 mg) were weighed into 2 mL Safe-lock® tubes (Eppendorf). The caps of the tubes were bored with a hole (~1.5 mm diameter) using an awl. Each sample was homogenized in 3 mL/g of ice-cold 5% (w/v) trichloroacetic acid (TCA) for 30 seconds using a hand-held homogenizer (TissueMiser, Biospec Products) while holding the sample on ice. Resulting slurries were boiled for 5 minutes, then cooled on ice for 10 minutes, and then vortexed with an equal volume of ice-cold 10% (v/v)

TCA. The final tissue dilution in TCA was 7.5 mL/g (concentration 133.3 mg/mL). Samples were then centrifuged at 15,000 g for 10 minutes at 4 °C. The supernatants were aliquoted and stored at -80˚C until assays could be completed. Before analysis and after thawing, the supernatants were vortexed and then centrifuged for 20 minutes at 17,000 *x g*.

Supernatants from the extracted muscle samples were diluted to 20 mg tissue/mL with HPLC-grade water and thoroughly vortexed. Thiamine diphosphate (TDP) standards were prepared in HPLC-grade water through three serial 2-fold dilutions from 200 nM to 50 nM in a black 96-well plate, including a blank (water only). TDP was used for standards as this is the predominant form of thiamine in fish muscle tissue (Brown et al., 1998). Tissue extracts (50 µL) were added to each well and mixed. A control muscle sample from a hatchery lake trout sample with undetectable thiamine levels (Chapter 3) was used as a uniform control for background fluorescence deduction across all plates. After a 30 min. incubation, 200 µL of alkaline ferricyanide (0.0075% (w/v) potassium ferricyanide diluted with 15% (w/v) sodium hydroxide) was added to all wells and rapidly mixed. The fluorescence was measured using a Varioskan plate reader (ThermoFisher) at 360/9 nm for excitation and 450/12 nm for emission.

The final spiked TDP concentrations (0-100 nM (excluding the added alkaline ferricyanide)) were plotted against the signal for each sample. The background signal from the uniform control was deleted from all. A linear regression plot was made between the spiked concentrations and the signals and the x-intercept was determined using GraphPad Prism (Version 10.2.3; GraphPad Software 2023). The negative of the x-intercept (nM) was used to calculate the TDP concentration of the sample by multiplying the nmol/L concentration from the x-intercept by the inverse of the sample concentration in the well (mg/mL) to yield the nmol/g TDP. This value using TDP as a standard was taken for estimates of total thiamine since, TDP

comprises the bulk of thiamine in muscle and liver tissue (Brown et al., 1998) and free thiamine and thiamine monophosphate (TMP) share the same spectral properties of TDP and would contribute to a lesser degree.

2.2.3 Dietary analyses

Lake trout gastrointestinal tracts were thawed, and stomachs were carefully opened with surgical scissors. Contents were removed with fine forceps. Whenever possible, consumed fish were identified to species and invertebrates were identified to Order before being counted and weighed to the nearest 0.1 g. Proportion of stomach contents represented by smelt (lake-level variable) was calculated by summing the mass of all smelt found in the stomachs and dividing by the total mass of all stomach contents for all fish in each lake (Mumby et al., 2018). The proportion of stomach contents represented by fish (lake-level variable) was also calculated by summing the mass of all fish found in stomachs (including smelt) and dividing by the total mass of all stomach contents for all fish in each lake. Prey items too digested to be identifiable were not included in the total masses for these calculations (Adeli et al., 2024; Mumby et al., 2018). The frequency of occurrence of smelt (% O_{smelt}) and other fish (% O_{fish}) was also calculated by determining the proportion of stomachs that contained either smelt or other fish from all stomachs that contained prey items in each lake (Hyslop, 1980).

2.2.4 Stable isotope analyses

White muscle samples from lake trout and bulk samples from heptageniids were freeze-dried for 48 h at – 48 °C under a vacuum pressure of 1.5×10^{-3} mbar. After freeze drying, samples of mayfly nymphs were sorted to remove any debris or foreign objects from the sample. All tissue samples were crushed into a fine powder using a Retsch MM 400 Mixer Mill (Retsch, Germany). Dry fish (mass 0.30−0.35 mg) or mayfly nymph (mass 0.40−0.45 mg) tissues were

then weighed into 3.5 mm tin capsules and submitted to the University of Waterloo Environmental Isotope Lab (Waterloo, ON). Stable carbon and nitrogen isotope ratios were analyzed using a 4010 Elemental Analyzer (Costech Instruments, Italy) with a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS). Accuracy was assessed with international reference materials (IAEA-N1 & N2, IAEA-CH3 & CH6, USGS-40 & 41) and in-house reference materials (EIL-72, EIL-3, JSEC-01). Duplicates were analyzed every tenth sample to estimate precision. C/N ratios were determined by dividing the percentage of carbon by the percentage of nitrogen in each sample.

Stable isotope ratios for each sample were expressed in standard delta notation (δ) with units of parts per mil (‰) using the following formula:

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] * 1000$,

where $R_{sample} = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ from the sample, and $R_{standard} = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ from an international standard. Accuracy of the isotope ratios were 0.1 ± 0.3 ‰ and 0.01 ± 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

$\delta^{15}N$ was used as an index of trophic position; however, among-lake differences in baseline $\delta^{15}N$ necessitate a baseline correction (Cabana & Rasmussen, 1996; Post, 2002; Vander Zanden & Rasmussen, 1999). Here we used the baseline correction in Swanson et al. (2003), who used a modified version of the model proposed by Cabana and Rasmussen (1996), but we used mayfly nymphs as a baseline instead of clams. Adjusted $\delta^{15}N$ values were used to compare the trophic position of lake trout among lakes. We did not divide by the conventional 3.4‰, since this estimate of $\delta^{15}N$ fractionation is only useful when applied to entire food webs or multiple

trophic interactions (Post, 2002). Adjusted $\delta^{15}N$ values ($\delta^{15}N_{adj}$) were calculated as follows:

$$\delta^{15}N_{adj} = \delta^{15}N_{fish} - \delta^{15}N_{baseline},$$

where $\delta^{15}N_{baseline}$ is the arithmetic mean of mayfly nymph $\delta^{15}N$ within each lake. Adjusted $\delta^{15}N$ could not be calculated for fish from five lakes with no mayfly nymph data (Chiniguchi, Johnnie, Kelly #27, Paradise, Savage). Further, two fish from Tyson Lake, two fish from Elboga Lake, and one fish from Fraleck Lake were recently stocked (< 2 years ago) and had isotopic signatures that reflected commercial fish food fed in the hatchery (high $\delta^{13}C$ and $\delta^{15}N$ for a given length). These fish were excluded from further analyses of stable isotope ratios. Sample sizes of fish with stable isotope data are included in Table D-3 of Appendix D.

2.2.5 Fish aging, retrieval of broadscale monitoring survey data, and growth rate estimation

Fish age was estimated by counting annuli on thin transverse sections of otoliths. Otoliths were set in epoxy and cross-sectioned following methods published by Casselman (1983). To supplement sample sizes such that estimation of growth curves was possible, fish community survey data were obtained from MNR databases (OMNRF 2019) for 9 lakes (Chiniguchi, Colin Scott, Kelly #27, Rawson, Shakwa, Manitou, Wanapitei, Whiskey) from BsM surveys conducted by the OMNR and by the Cooperative Freshwater Ecology Unit at Laurentian University. Surveys were conducted between 2009 and 2023 (Table 2.2). Length, mass, and age data from all survey years were pooled for each lake and sample sizes are summarized in Table 2.3. Growth curves were fitted on a subsample of 10 lakes with sufficient data; 4 of these 10 lakes contained smelt whereas 6 did not contain smelt (Table 2.2). Burwash Lake was the only lake for which growth curves were estimated using fish captured in angling surveys alone.

Table 2.2 Population parameters estimated from Von Bertalanffy growth functions (VBGF) with 95% confidence intervals (CI) calculated for lake trout populations in lakes of the Sudbury Basin (Ontario, Canada). Fish were collected either by angling or with multimesh gill nets (BsM). VBGF functions were calculated using methods following Ogle (2013) in R (R core team, 2024) package FSA (version 0.9.6; Ogle 2025).

Lake	Survey year(s)	Survey type	N	Age range (years)	L_∞ (mm; estimate, LCI, UCI)	K ($year^{-1}$; estimate, LCI, UCI)	T_0 (year; estimate, LCI, UCI)
Rainbow smelt absent							
Burwash	2021, 2022, 2023	Angling	23	5–12	511.5 (479.5, 601.4)	0.51 (0.09, 1.27)	1.96 (-5.86, 4.19)
Chiniguchi	2010, 2015, 2021	BsM	54	1–20	579.4 (554.5, 614.8)	0.25 (0.20, 0.32)	0.25 (-0.57, 0.68)
Colin Scott	2020, 2021, 2022	BsM, Angling	40	1–21	526.9 (505.6, 559.6)	0.31 (0.21, 0.58)	0.26 (-0.65, 1.35)
Kelly #27	2019, 2021, 2022	BsM, Angling	15	1–14	700.3 (634.7, 981)	0.22 (0.11, 0.30)	-0.41 (-1.25, 0.16)
Rawson	2010, 2020, 2021, 2022	BsM, Angling	73	3–21	470.1 (448.1, 499.5)	0.24 (0.18, 0.32)	-0.33 (-1.15, 0.45)
Shakwa	2022	BsM, Angling	22	2–20	594.9 (469.7, 1740.3)	0.18 (0.01, 0.49)	-0.47 (-6.56, 1.11)
Rainbow smelt present							
Manitou	2011, 2016, 2021, 2022	BsM, Angling	132	2–26	731.9 (709, 764)	0.17 (0.14, 0.20)	0.01 (-0.36, 0.48)
Panache	2010, 2014, 2018, 2021	BsM, Angling	88	3–45	877.1 (703.1, 1040.65)	0.08 (0.05, 0.12)	-0.95 (-2.62, 0.42)
Wanapitei	2010, 2016, 2021, 2022	BsM, Angling	37	3–37	556.6 (520.2, 682.5)	0.38 (0.07, 0.90)	1.92 (-10.52, 3.85)
Whiskey	2010, 2014, 2019, 2021, 2022	BsM, Angling	150	2–26	812.3 (717, 1138.4)	0.08 (0.04, 0.11)	-1.69 (-4.53, -0.19)

Note. N = number of lake trout sampled from a given lake, L_∞ = asymptotic length, K = Brody growth coefficient, T_0 = length at age 0, LCI = lower 95% confidence interval, UCI = upper 95% confidence interval

Table 2.3 Summary of lake trout (*Salvelinus namaycush*) stomach content analyses for each lake in the Sudbury Basin (Ontario, Canada).

Lake	Mean mass ± SD in stomach (g)[a]					Prop RS	%O RS	Prop fish	%O fish	Empty	Prey fish[b]
	RS	Other fish	Unkwn fish	Inverts	Unkwn						
					Rainbow smelt present						
Bear[c]	0.00 ± 0.00	0.05 ± 0.15	0.39 ± 0.85	1.82 ± 3.76	0.01 ± 0.04	0.00	0.00	0.19	0.45	4	SS, CI
Manitou	5.88 ± 10.07	10.88 ± 37.30	8.03 ± 10.38	0.07 ± 0.25	0.03 ± 0.16	0.35	0.75	0.99	1.00	2	RS, CI
Manitou #2	0.71 ± 2.09	0.42 ± 1.75	2.19 ± 2.53	0.00 ± 0.00	0.04 ± 0.15	0.63	0.66	1.00	1.00	5	RS, SS
Panache	1.78 ± 4.66	6.50 ± 16.78	2.16 ± 4.01	0.03 ± 0.10	0.42 ± 1.22	0.21	0.33	0.99	0.93	7	CI, RS
Wanapitei	6.59 ± 14.28	1.80 ± 7.89	1.16 ± 4.10	1.14 ± 5.11	1.29 ± 3.12	0.69	0.72	0.89	0.96	6	RS, CI, BU
Whiskey	1.82 ± 5.11	5.00 ± 9.85	1.74 ± 2.36	0.10 ± 0.27	2.18 ± 7.55	0.26	0.50	0.99	1.00	4	RS, CI, WS
					Rainbow smelt absent						
Bell	0.00 ± 0.00	5.59 ± 12.19	13.54 ± 11.92	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	1.00	1.00	1	CI
Burwash	0.00 ± 0.00	1.29 ± 3.85	1.34 ± 3.98	0.60 ± 1.01	1.71 ± 6.63	0.00	0.00	0.81	0.63	4	YP
Chiniguchi	0.00 ± 0.00	5.70 ± 18.13	4.20 ± 11.31	0.16 ± 0.32	4.90 ± 7.40	0.00	0.00	0.98	0.50	8	YP, SB
Colin Scott	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.35 ± 6.43	0.74 ± 1.67	0.00	0.00	0.00	0.00	3	
Elboga	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.35	0.17 ± 0.40	0.79 ± 1.75	0.00	0.00	0.37	0.20	7	
Evelyn	0.00 ± 0.00	0.40 ± 1.31	0.00 ± 0.00	1.56 ± 1.85	1.22 ± 2.29	0.00	0.00	0.20	0.09	0	SB
Fraleck	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.62 ± 2.86	0.85 ± 0.99	0.00	0.00	0.00	0.00	2	
Kelly #27	0.00 ± 0.00	12.85 ± 31.60	3.40 ± 8.55	0.81 ± 1.50	0.70 ± 1.98	0.00	0.00	0.95	0.83	2	YP
Mohzabong	0.00 ± 0.00	4.50 ± 6.62	1.70 ± 2.64	0.05 ± 0.12	0.03 ± 0.07	0.00	0.00	0.99	1.00	2	YP, LW
Nelson	0.00 ± 0.00	0.84 ± 2.53	0.00 ± 0.00	4.59 ± 6.55	1.90 ± 3.65	0.00	0.00	0.16	0.20	4	YP
Paradise	0.00 ± 0.00	8.68 ± 30.73	3.27 ± 4.01	0.23 ± 0.88	0.10 ± 0.34	0.00	0.00	0.98	0.78	9	ID, WS, LW
Rawson	0.00 ± 0.00	0.00 ± 0.00	4.25 ± 13.44	1.24 ± 1.23	1.17 ± 1.77	0.00	0.00	0.77	0.13	2	
Savage	0.00 ± 0.00	17.31 ± 25.28	0.34 ± 0.54	0.00 ± 0.00	0.03 ± 0.07	0.00	0.00	1.00	1.00	2	LW, SS
Shakwa	0.00 ± 0.00	0.38 ± 1.25	1.59 ± 4.22	0.01 ± 0.08	0.92 ± 2.09	0.00	0.00	0.99	0.92	14	YP
Tyson	0.00 ± 0.00	1.01 ± 2.17	0.10 ± 0.30	0.00 ± 0.00	0.20 ± 0.31	0.00	0.00	1.00	1.00	6	CI
Wavy	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.27	0.04 ± 0.13	0.54 ± 1.10	0.00	0.00	0.67	0.50	7	

a. Mean masses are presented for rainbow smelt (RS), other fish (Other fish), unknown fish (Unkwn fish), invertebrates (Inverts) and unknown species (Unkwn). Mean values are calculated as the total mass of all focal prey items divided by the number of

sampled individuals. Sample sizes used in these calculations can be found in Table 1. Dietary proportion of rainbow smelt (Prop RS), percent occurrence of rainbow smelt (%O RS), dietary proportion of fish (Prop fish), percent occurrence of fish (%O fish), and number of empty stomachs (Empty) are calculated as described in the methods section.

b. Prey fish species listed include (up to) the top three fish most-consumed species found in the stomach, ordered from most to least consumed. Species abbreviations: BU = burbot (*Lota lota*), CI = cisco (*Coregonus artedi*), ID = Iowa darter (*Etheostoma exile*), LW = lake whitefish (*Coregonus clupeaformis*), RS = rainbow smelt (*Osmerus mordax*), SB = smallmouth bass (*Micropterus dolomieu*), SS = slimy sculpin (*Cottus cognatus*), YP = yellow perch (*Perca flavescens*), WS = white sucker (*Catostomus commersonii*)

c. Bear Lake contains rainbow smelt but there was no evidence of their consumption by lake trout.

Growth rates of each population were modelled and compared using the von Bertalanffy growth function (VBGF; Beverton 1954; Cailliet et al. 2006; Beverton and Holt 2012):

$$E[L|t] = L_\infty (1 - e^{-k(t - t0)}),$$

where E[L|t] is the expected or average fish length at time (or age) t, L_∞ is the asymptotic average length, K is the so-called Brody growth coefficient (units are in yr^{-1}), and t_0 is the theoretical time or age when the average length was zero (Cailliet et al., 2006). To compare growth rates among populations (i.e., lakes) of lake trout, the Brody growth coefficient (K) and asymptotic average length (L_∞) were extracted from fitted VBGF models for each lake.

2.2.6 Statistical analyses

All analyses were conducted using R packages and functions (version 4.3.3., R core team 2024) in the R Studio environment (Posit Team, 2025)**.** All metrics (tissue total thiamine concentration, body condition, C/N ratios, growth parameters (K, L_∞ , t_0), occurrence of fish in stomachs, dietary proportion of fish in stomachs, $\delta^{15}N$, $\delta^{13}C$) collected from individual lake trout were analyzed and compared using $\alpha = 0.05$. Linear mixed effects models fit with restricted maximum likelihood (lmm; *lmer* in the lme4 package in R; Bates et al. 2023) were used to test for differences in corrected $\delta^{15}N$, $\delta^{13}C$, C/N ratios, muscle total thiamine concentration, and body condition between lakes with and without smelt. F statistics and p values for all lmms were generated using function Anova() with type I variance tables in package car (Fox & Weisberg, 2019), and marginal and conditional R^2 values were calculated using function r.squaredglmm() in package MuMIn (Barton, 2024). In addition to the fixed effect of smelt presence/absence, fish total length was included as a fixed effect in the lmms conducted for $\delta^{15}N_{adj}$, $\delta^{13}C$, C/N ratios, and muscle TDP concentration. All mixed effects models included a random effect (intercept) of

lake. To test if the random effect explained a significant proportion of the variance, a restricted likelihood ratio test was used (function exactRLRT() in package RLRsim; Scheipl and Bolker 2025). If the random effect of lake did not explain a significant proportion of the variance of the model, then it was dropped, and a general linear model (glm) was used. When the random effect of lake was included in the final model, variation explained was quantified using the intraclass correlation coefficient method and was represented as the percentage of the variance accounted for by the random effects (Nakagawa et al., 2017). Because only one random effect was included in each lmm, percentage of the variance accounted for by the random effect was calculated by subtracting $R^2_{\text{marg.}}$ from the $R^2_{\text{cond.}}$ (Nakagawa et al. 2017).

Parameters estimated from Von Bertalanffy growth models (Brody growth coefficient, asymptotic length, and t_0) were compared between lakes with and without smelt using a Welsh two-sample t-test. Values for occurrence and dietary proportion of fish had several ties and did not fit a Gaussian distribution using a Shapiro-Wilk normality test (Wilk & Shapiro, 1968). As such, a Mann-Whitney U test was used to examine differences in the occurrence and dietary proportion of fish in the diets of lake trout in lakes with and without smelt.

The relationship between muscle thiamine concentration and corrected $\delta^{15}N$ for all fish pooled across all lakes was examined using a linear mixed effects model that was fit using restricted maximum likelihood; a random effect of lake was included. The relationship between asymptotic length and the dietary proportion of smelt for lake trout in smelt lakes was examined using a linear model. The relationship between lake trout condition and C/N ratios for fish in each of the lakes with and without smelt was examined using a linear mixed effects model that was fit using restricted maximum likelihood; a random effect of lake was included. To examine the relationship between muscle thiamine concentration and the occurrence and dietary

proportion of fish and smelt in lake trout diets across all lakes, muscle thiamine values had to be averaged to produce a mean value for each lake. Spearman rank correlations were used to examine the relationship between muscle thiamine concentration and the occurrence and dietary proportion of fish due to a high number of ties between lakes in their stomach content metrices and the non-normality of the data, whereas linear models were used to examine the relationship between muscle thiamine concentration and the occurrence and dietary proportion of smelt in lakes containing smelt.

2.3 Results

2.3.1 Tissue thiamine concentrations

Contrary to our expectation, concentrations of total thiamine in lake trout white muscle did not vary significantly between lakes with smelt (mean ± sd; 2.16 ± 1.61 nmol/g) and without smelt (3.07 ± 2.09 nmol/g; t = -1.74, df = 59, p = 0.08, R^2 = 0.06, Figure 2.1A). One fish from Lake Wanapitei (smelt lake) had a white muscle total thiamine concentration of 0.76 nmol/g, which is below previously assessed thresholds for thiamine deficiency in muscle (total thiamine <1.0nmol/g; Brown et al. 2005; Futia and Rinchard, 2019; Ottinger et al. 2014). The other three fish measured in Wanapitei had muscle thiamine concentrations from 1.25–6.38 nmol/g and were thiamine replete. There was no statistically significant relationship between muscle total thiamine concentration and fish length (t = -0.75, df = 59, p = 0.46). The random effect of lake did not explain a significant amount of the variation and was removed from the model (RLRT = 0.079, p = 0.35).

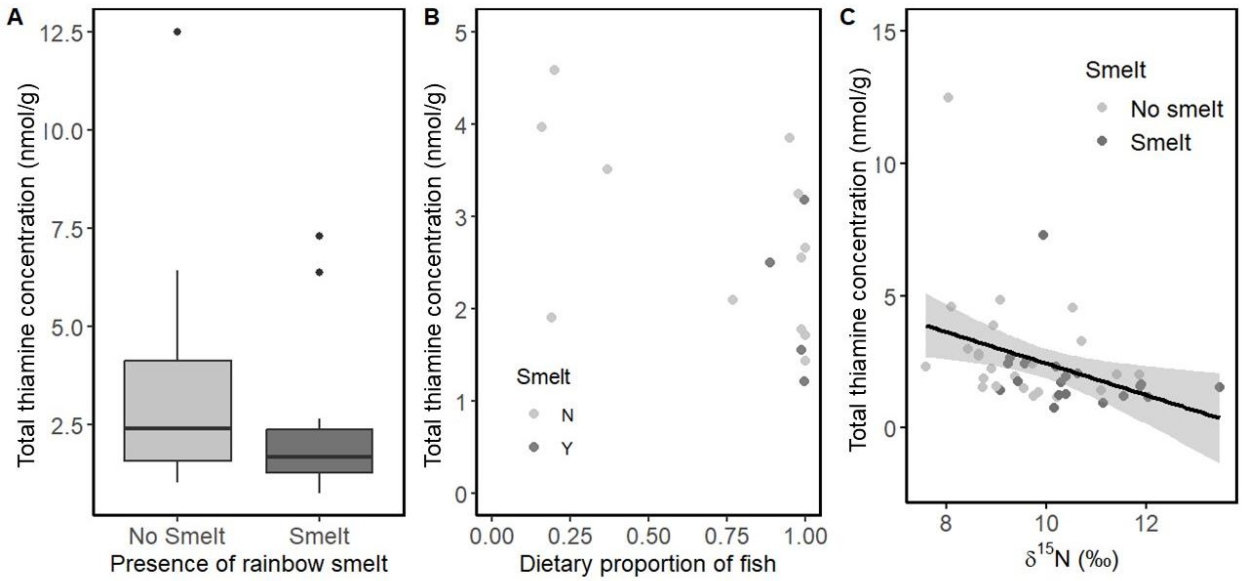


Figure 2.1 A) Concentration (nmol/g) of total thiamine in white muscle from wild lake trout from lakes with (light grey) and without smelt (dark grey) in the Sudbury Basin. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers (outside 1.5 x interquartile range). B) Relationship between mean white muscle total thiamine concentration and the dietary proportion of fish of lake trout populations with (dark grey) and without (light grey) rainbow smelt in the Sudbury Basin. C) Relationship between white muscle total thiamine concentration and white muscle corrected $\delta^{15}N$ signature of lake trout from lakes with (dark grey) and without (light grey) rainbow smelt in the Sudbury Basin. In A), there was no difference in white muscle total thiamine concentration between lake trout from lakes with and without rainbow smelt ($p = 0.08$). In B), there was a significant negative relationship between average white muscle total thiamine concentration and the dietary proportion of fish ($p = 0.016$). In C), there was a significant negative relationship between the white muscle total thiamine concentration and the white muscle corrected $\delta^{15}N$ signature ($p = 0.02$). The line of best fit equation was calculated using generalized linear mixed models with an equation of $8.89 – 0.64x$.

2.3.2 Dietary and stable isotope analyses

Results of the stomach content analyses are presented in Table 2.3. Stomach contents varied among lakes; in some lakes, stomach contents of lake trout indicated planktivory/insectivory whereas in other lakes stomach contents of lake trout indicated exclusive piscivory. Between lakes with and without smelt, there was no difference in either occurrence of

fish in the diet (% O_{fish}; W = 65.5, n = 22, p = 0.20; Table 2.3) or proportion of fish in the diet (W = 35.5, n = 22, p = 0.34). In lakes with smelt, observed consumption of smelt was lowest in Bear Lake, (occurrence of smelt [$\%O_{smelt}$] & proportion of smelt in stomach contents = 0), and highest in Wanapitei Lake, where the diet—based on stomach contents—was dominated by smelt ($\%O_{smelt}$ = 0.72, dietary proportion of smelt = 0.69).

Delta ^{15}N values of mayfly nymphs varied from -1.23 ‰ in Panache Lake to 2.39 ‰ in Fraleck Lake (Table D-4 in Appendix D). Isotope values of common prey items are included in Table D-5 in the Appendix D. A comparison of $\delta^{15}N_{adj}$ values for cisco and rainbow smelt from smelt-invaded lakes can be found in Figure D-2 in the Appendix D. Lake trout in lakes with smelt had $\delta^{15}N_{adj}$ values that were 1.47 ‰ (95% CI; 0.49−2.46 ‰) higher (on average) than those without smelt (F_1 = 8.57, n = 190, p = 0.009, $R^2_{marg.}$ = 0.35, $R^2_{cond.}$ = 0.86; Figure 2.2A). The random effect of lake explained a significant proportion of the variation (RLRT = 200.7, p < 0.001), totalling approximately 51% of the variance in the model. There was also a significant positive relationship between $\delta^{15}N_{adj}$ and lake trout length (F_1 = 53.33, n = 190, p < 0.001; Figure 4A).

Delta^{13}C in lake trout varied considerably among lakes, but there was no significant difference in $\delta^{13}C$ values between lake trout in lakes with and without smelt (F_1 = 1.87, n = 220, p = 0.19, $R^2_{marg.}$ = 0.07, $R^2_{cond.}$ = 0.08; Figure 2.2B). The random effect of lake explained a significant proportion of the variation (RLRT = 185.9, p < 0.001), totalling approximately 2% of the variance in the model. The covariate of length was not significant, indicating no relationship between $\delta^{13}C$ value and lake trout length (F_1 = 1.63, n = 220, p = 0.20; Figure 2.1B).

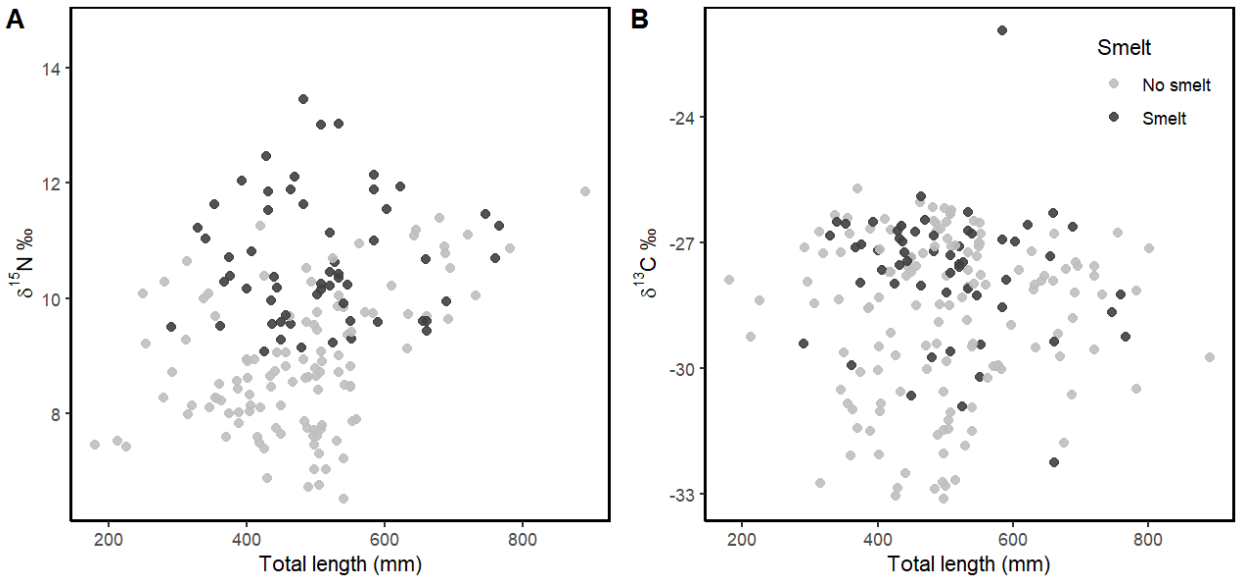


Figure 2.2 Relationship between white muscle A) corrected $\delta^{15}N$ (‰) and B) $\delta^{13}C$ (‰) isotopic signatures and total length (mm) of wild lake trout from lakes with (dark grey) and without (light grey) rainbow smelt in the Sudbury Basin. In A), there was a significant relationship between the corrected $\delta^{15}N$ signature and fish total length (p = 0.04), suggesting that with a 100 mm increase in total length, $\delta^{15}N$ increased by 0.3 ‰ (95% CI; 0.22−0.38 ‰), and a significant difference in corrected $\delta^{15}N$ signature between fish from lakes with and without rainbow smelt (p = 0.009). In B), there was no relationship between the $\delta^{13}C$ signature and fish total length (p = 0.20) and nor was there a difference in $\delta^{13}C$ signatures between fish from lakes with and without rainbow smelt (p = 0.19).

There was no difference in C/N ratios, a proxy for lipid content, between lakes with and without smelt ($F_{1, 220} = 1.82$, $p = 0.19$, $R^2_{marg.} = 0.12$, $R^2_{cond.} = 0.37$; Figure 2.3A). The covariate of length was significant, indicating a higher C/N ratio with increasing total length ($F_{1, 220} = 18.11$, df = 1, $p < 0.001$; Figure 2.3A). The random effect of lake explained a significant proportion of the variation (RLRT = 27.9, $p < 0.001$), totalling approximately 25% of the variance in the model.

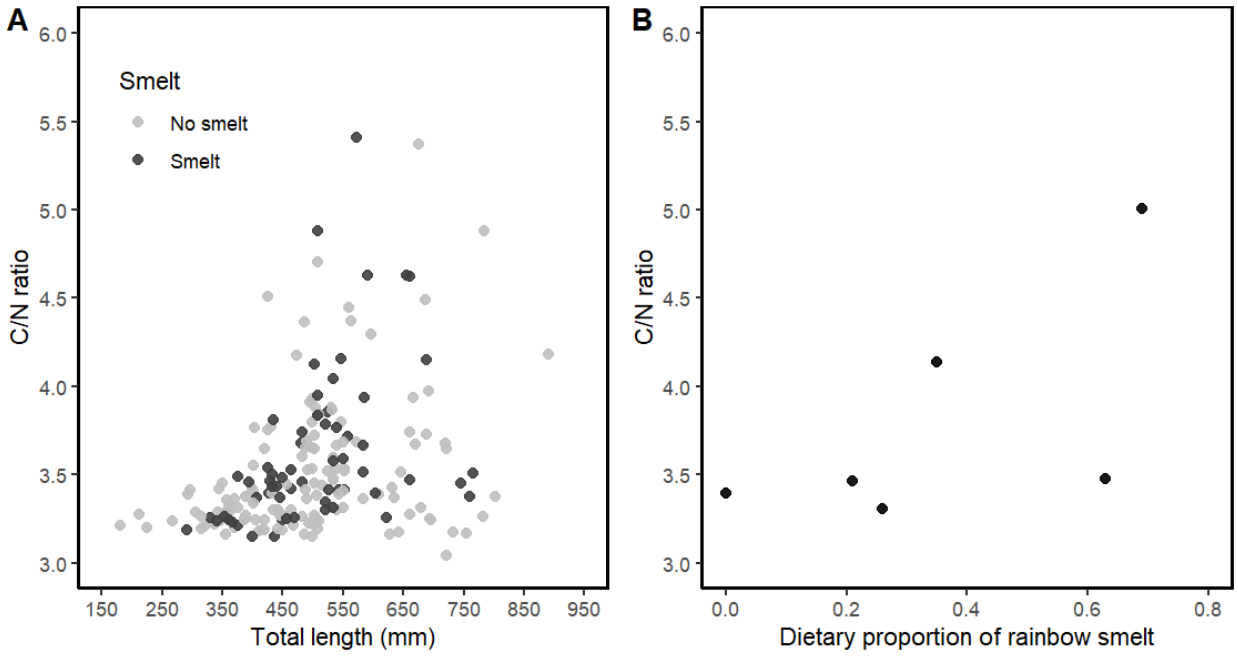


Figure 2.3 A) Relationship between white muscle C/N ratio and total length (mm) of wild lake trout from lakes with (dark grey) and without (light grey) rainbow smelt in the Sudbury Basin. B) Relationship between mean white muscle C/N ratio and the dietary proportion of rainbow smelt of lake trout populations with rainbow smelt in the Sudbury Basin. In A), there was a significant positive relationship between white muscle C/N ratio and fish total length ($p < 0.001$), but no difference in C/N ratio between fish from lakes with and without rainbow smelt ($p = 0.19$). In B), there was no relationship between average C/N ratio and the dietary proportion of smelt of lake trout populations of lakes with rainbow smelt ($p = 0.17$).

2.3.3 Body condition and growth

Growth of lake trout was positive allometric with a mass-length regression slope (b) of 3.18 (Figure 2.4A). Lake trout from lakes containing smelt had lower body condition than lake trout from lakes without smelt ($F_1 = 7.72$, n = 391, $p = 0.01$, $R^2_{marg.} = 0.08$, $R^2_{cond.} = 0.25$; Figure 2.4B). The random effect of lake explained a significant proportion of the variation (RLRT = 45.3, $p < 0.001$), totalling approximately 17% of the variance in the model. There was no relationship between lake trout muscle total thiamine and body condition ($t = 1.24$, df = 58, $p = 0.22$, $R^2_{marg.} = 0.03$). The random effect of lake did not account for a significant proportion of the

variation and was dropped from the final model (RLRT = 0.09, $p = 0.35$). In lakes without smelt, there was a significant positive relationship between condition and C/N ratio ($t = 4.04$, df = 132, $p < 0.001$, $R^2 = 0.11$). The random effect of lake did not account for a significant proportion of the variation and was dropped from the final model (RLRT = 0.95, $p = 0.14$). When lakes with smelt were analyzed, there was no relationship between condition and C/N ratio ($F_1 = 0.82$, n = 60, $p = 0.36$, $R^2_{marg.} = 0.01$, $R^2_{cond.} = 0.46$). Here, the random effect accounted for a significant proportion of the variation and was included in the final model (RLRT = 13.86, $p < 0.001$).

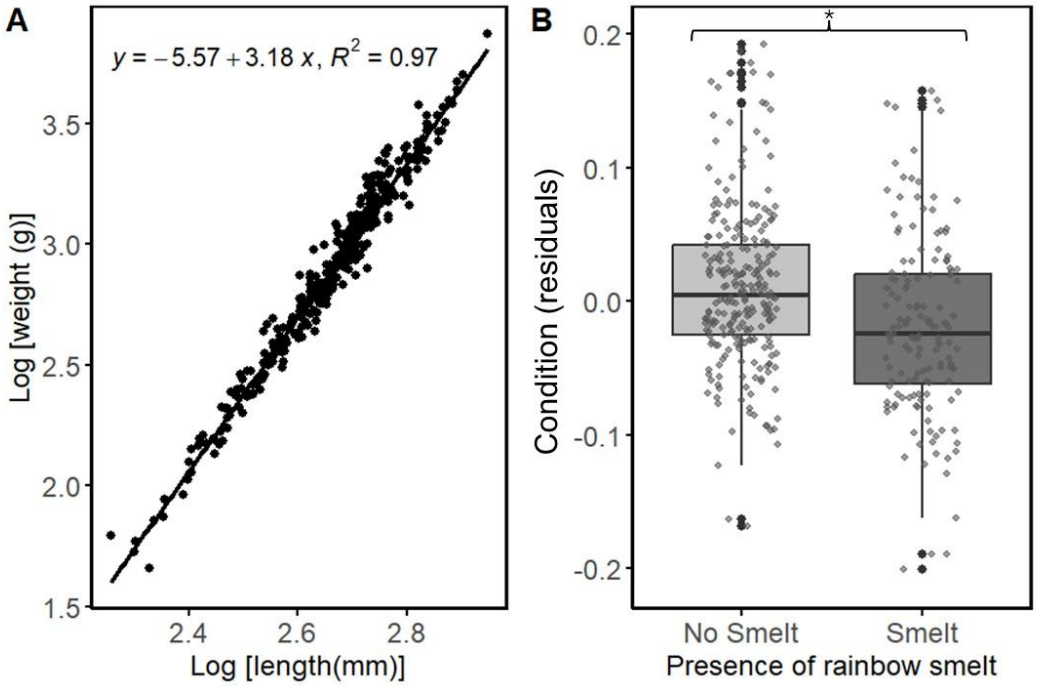


Figure 2.4 A) Relationship between log-transformed mass (g) and log-transformed total length (mm) of wild lake trout from lakes in the Sudbury Basin (n = 391). The line of best fit equation was determined using a linear model to be y = -5.57 + 3.18x. B) Residuals of the relationship between log-transformed mass (g) and log-transformed total length (mm) of wild lake trout from lakes with (light grey; n = 148) and without (dark grey; n = 243) rainbow smelt in the Sudbury Basin. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Black dots represent outliers (outside 1.5 x interquartile range). Grey dots represent individual data points. Asterisks denote significant differences in condition between fish from lakes with and without smelt (p <0.001).

Parameter estimates for fitted VBGF models varied considerably among lakes (Table 2.2) and a plot of growth curves can be found in Figure D-3 in the Appendix D. The mean asymptotic length of lake trout did not significantly differ between lakes with and without smelt (t = -2.35, df = 4.38, p = 0.07; Figure 2.5A). There was no significant difference in the Brody growth coefficients between lakes with and without smelt (t = 1.27, df = 5.6, p = 0.25; Figure 2.5B). There also was no difference in t_0 between lakes with and without smelt (t = 0.34, df = 4.49, p = 0.74).

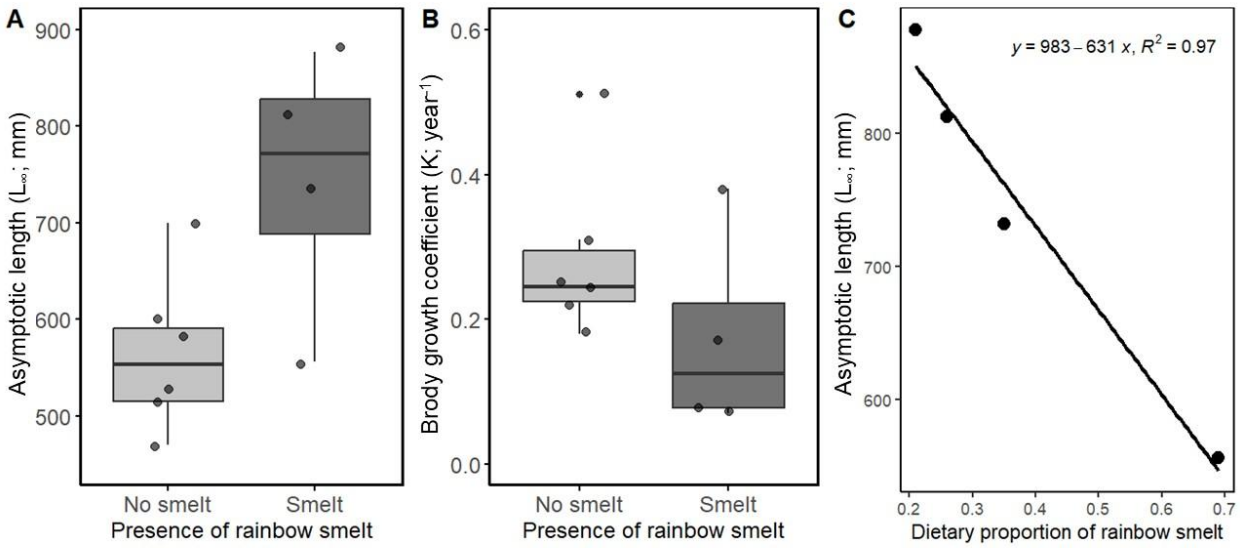


Figure 2.5 Von Bertalanffy growth function parameters A) Asymptotic length (L_∞; mm) and B) Brody growth coefficient (K; year $^{-1}$) of lake trout populations from lakes with (light grey) and without smelt (dark grey) in the Sudbury Basin. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 x interquartile range. C) Relationship between asymptotic length (L_∞; mm) and dietary proportion of rainbow smelt of lake trout populations with rainbow smelt in the Sudbury Basin. Line of best fit equation was calculated using a linear model with an equation of y = 983 - 631x. There was a significant relationship between asymptotic length and dietary proportion of rainbow smelt (p = 0.002).

2.3.4 Relationships between tissue thiamine concentration, body condition, growth, and diet

Tissue thiamine concentrations appeared to decrease with fish consumption, but the effect of smelt consumption was unclear. There was a negative relationship between muscle total thiamine concentration and both the occurrence of fish in the diet (rs = -0.58, n = 16, $p = 0.016$) and the dietary proportion of fish in the diet (rs = -0.57, n = 16, $p = 0.03$; Figure 2.1B). In lakes containing smelt, there was no relationship between muscle total thiamine concentration and the dietary proportion of smelt ($t = 0.29$, n = 6, $p = 0.42$) or the occurrence of smelt ($t = 1.38$, n = 6, $p = 0.26$), although the power of these analyses was small at 5 and 6 %, respectively. There was also a negative relationship between muscle total thiamine concentration and $\delta^{15}N_{adj}$ when fish from all lakes were pooled ($t = -2.58$, df = 51, $p = 0.01$, $R^2 = 0.14$, Figure 2.1C); a $\delta^{15}N$ increase of 1‰ was associated with a 0.59 nmol/g (95% CI; 1.05−0.14 nmol/g) decrease in muscle total thiamine concentration. The random effect of lake did not account for a significant proportion of the variation and was dropped from the final model (RLRT = 0.33, $p = 0.25$). When lake trout from lakes without smelt were excluded from the analysis, there was no relationship between $\delta^{15}N_{adj}$ and muscle total thiamine concentration ($t = -1.29$, df = 19, $p = 0.21$, $R^2 = 0.08$). The random effect of lake did not account for a significant proportion of the variation and was dropped from the final model (RLRT = 1.44, $p = 0.09$).

In lakes with smelt, there appeared to be a trend where C/N ratio increased with proportion of smelt in lake trout diets (Figure 2.3B); however, the relationship was not significant ($t = 1.61$, df = 4, $p = 0.17$, $R^2 = 0.26$). In smelt-only lakes, there was a significant negative relationship between the asymptotic length and the dietary proportion of smelt ($t = -7.99$, df = 3, $p = 0.002$, $R^2 = 0.95$; Figure 2.5C); an increase in the dietary proportion of smelt of 0.1 (10 %) corresponding to a decrease of 63.1 mm in asymptotic length (95% CI; 97.1−29.2).

2.4 Discussion

Previous researchers have reported that although smelt make up a notable proportion of the diet of thiamine-deficient salmonids in the Laurentian Great Lakes and Lake Champlain (Futia et al., 2019; Nawrocki et al., 2022; Simonin et al., 2018), alewife (*Alosa pseudoharengus* [A. Wilson, 1811]) are the driving factor behind thiamine deficiency (Brown, Honeyfield, et al., 2005; Ladago et al., 2020; Riley et al., 2011). Specifically, thiamine deficiency was only identified as a problem in these waterbodies after the contributions of alewife to the diet of salmonids began to increase. Furthermore, while smelt do have high thiaminase activity (Honeyfield et al., 2012; Tillitt et al., 2005; Zajicek et al., 2009), previous instances of thiamine deficiency have primarily been attributed to prey fishes from the families Clupeidae (G. Cuvier, 1817) and Cyprinidae [Rafinesque, 1815](Boggs et al., 2019; Fitzsimons et al., 2012; Mantua et al., 2021; Tillitt et al., 2005), even in lakes where smelt are present. Here, we found no difference in muscle total thiamine concentrations between lake trout from lakes with and without smelt, nor was thiamine deficiency widespread. We did, however, find an episode of thiamine deficiency in Lake Wanapitei, a lake whose lake trout population feeds on the highest proportion of smelt of those examined in this study and among the first instances of thiamine deficiency inferred to be attributable to smelt. This suggests that the presence of smelt alone will not lower muscle thiamine concentrations in all lake trout populations, but can occur in lakes where smelt are widely consumed.

Studies have shown that the trophic ecology of lake trout in lakes where they co-occur with smelt may be a significant predictor of muscle thiamine concentrations (Futia et al. 2019). While we did not find a generalized decrease in muscle thiamine concentration in lakes where smelt invaded, we observed a decrease in muscle thiamine concentration when there was both an

increase in the dietary proportion of fish and an increase in muscle $\delta^{15}N$. These relationships are likely driven by an increased consumption of smelt and not native prey fishes because lake trout $\delta^{15}N$ signatures were higher in lakes with smelt than without, and smelt have higher $\delta^{15}N$ signatures than native forage fishes in our study lakes. Similarly, Fitzsimons and Brown (1998) found that thiamine concentrations in lake trout eggs were lower in populations that had relatively high proportions of smelt in the diet and Futia et al. (2019) found the lowest muscle thiamine concentrations in lake trout populations consuming the highest levels of smelt and alewife. Collectively, these results suggest that the effects of smelt on thiamine concentrations may be greatest in lakes without diverse prey fish communities and where they make up the greatest proportion of the diet. The observation of thiamine replete lake trout in Lake Wanapitei suggests that occasional access to low-thiaminase prey items can rescue thiamine concentrations. Indeed, some fish in the Lake Wanapitei were found to be feeding on mysis (*Mysis diluviana* [Audzijonyte & Väinölä, 2005]), cisco (*Coregonus artedi* [Lesueur, 1818]), and burbot (*Lota lota* [Linnaeus, 1758]), which are prey items that are all low in thiaminase (Fitzsimons et al., 2012; Greig & Gnaedinger, 1971; Tillitt et al., 2005).

Recent studies have shown a strong trophic dilution of thiamine from primary producers to top consumers. This dilution suggests that changes in the lower part of the food web could affect the thiamine status of consumers occupying higher trophic levels (Hylander et al., 2024). Furthermore, studies have shown that smelt invasion can lead to the decline or elimination of large-bodied zooplankton in favour of small-bodied zooplankton (Reif & Tappa, 1966). The consequence of this shift in zooplankton size can result in less thiamine at the base of the food chain (Fridolfsson et al., 2018). This reduction can then be compounded by the trophic elongation and position of lake trout when feeding on smelt (Hrabik et al., 1998; Johnston et al.,

2003; Swanson et al., 2003, 2006; Vander Zanden & Rasmussen, 1996). Indeed, we observed this trophic elevation in lake trout in lakes with smelt that had higher $\delta^{15}N$ but similar $\delta^{13}C$ compared to lake trout in lakes without smelt. The significant negative relationship between $\delta^{15}N$ and muscle thiamine concentration of lake trout found in all lakes but that did not hold true when only lakes without smelt were examined suggests both these smelt mediated effects could be altering the flow of thiamine through the food web and to lake trout.

The growth rates and condition of lake trout have been found to rapidly increase in some inland lakes following smelt introductions yet decrease in other lakes (Evans & Loftus, 1987; Hassinger & Close, 1984; MacCrimmon et al., 1983). While we hypothesized that a diet high in smelt would negatively affect the growth of lake trout, we did not find a difference in Brody growth coefficients, asymptotic length, or t_0 between lake trout populations from lakes with and without smelt, indicating the presence of smelt alone did not affect population growth metrics. However, we did find that asymptotic length of lake trout populations decreased with increasing dietary proportion of smelt and a generalized decrease in condition of lake trout in lakes with smelt. The mechanisms by which smelt affect lake trout growth and condition are still poorly understood. Previous studies have attributed the increases in growth rates and condition after invasion of smelt to the higher fat content of smelt compared to native prey fishes and a consequent increase in energy retention at higher trophic levels (Evans and Loftus 1987; Johnson and Goettl 1999). Indeed, lake trout populations whose diet comprised a proportion of rainbow smelt less than 0.75 had the highest asymptotic lengths, which suggests that consuming smelt confers a growth benefit to lake trout, at least up to a certain consumption level. Future research is necessary to ascertain the level of smelt consumption that causes either benefits or detriments to growth. However, we did not find any difference in C/N ratios, a proxy for lipid content,

between lakes with and without smelt, indicating no general increase in lipid content because of smelt presence. Furthermore, we found no relationship between condition and C/N ratios in lakes with smelt, despite finding a positive relationship in lakes with smelt. It thus appears that lower condition in lake trout from smelt-invaded lakes is not driven by differences in proximate composition. When invasion of smelt has been observed to induce decreases in growth rates of piscivores, this has been attributed to increased competition between juvenile native piscivores and smelt — which feed at a higher trophic level than native forage fishes (Evans & Loftus, 1987; Galbraith Jr., 1967; Hassinger & Close, 1984; Johnson & Goettl Jr., 1999; Swanson et al., 2003). However, in an experimental study, lake trout fed a diet that contained thiaminase had lower growth rates and food conversion efficiency than those fed a control diet (Chapter 3). Whether diet-derived thiaminase similarly affects lake trout in the wild needs further study. Altogether, the effects of smelt consumption on growth rate of lake trout may depend on the proportion of smelt in the diet and the availability and quality of other prey, but further research is required.

2.5 Conclusion

Altogether, our study revealed that exotic smelt consumption is associated with an episode of thiamine deficiency and declines in the condition and population asymptotic length of lake trout. However, the presence of smelt alone was not associated systematic decreases in muscle thiamine concentration suggesting that thiamine deficiency is episodic and not generally widespread but can occur in a lake where smelt have invaded and are widely consumed. We also found that the trophic ecology of lake trout was a significant predictor of muscle thiamine concentrations and speculate that changes to the lower food web associated with smelt invasions may reduce thiamine transfer up the food chain. Together with the pathological cardiac

impairments induced by smelt consumption described in Adeli et al., (2024) in these same lakes, our studies suggests that smelt consumption has the potential to negatively impact lake trout populations in lakes where they've invaded.

Chapter 3

The effect of diet-derived thiaminase on survival, growth, and liver transketolase activity in two strains of lake trout (*Salvelinus namaycush*)

This chapter is an adapted version of the following paper, published in the Journal of Great Lakes Research:

Therrien CA, Baker PM, Garner SR, Wolfe PC, Matesic S, Edwards K, Wilson CC, Swanson HK, Neff BD. 2025. The effect of diet-derived thiaminase on survival, growth, and liver transketolase activity in two strains of lake trout (*Salvelinus namaycush*). Journal of Great Lakes Research, 51: 102689. https://doi.org/10.1016/j.jglr.2025.102689.

3.1 Introduction

Thiamine (vitamin B1) is an essential water-soluble vitamin required by all organisms for a range of metabolic functions, including glycolysis and the citric acid cycle (Bettendorf, 2013). Thiamine is required for proper neurological (Bâ, 2008), immune (Abraham et al., 2026; Ottinger et al., 2014; Wen et al., 2016), and muscular function (Romanenko et al., 1994). In most animals, thiamine must be acquired through the consumption of organisms that produce or contain thiamine (Manzetti et al., 2014). Low concentrations of thiamine are known to result in a

syndrome referred to as thiamine deficiency complex (TDC; Riley and Evans, 2008). TDC is also known as early mortality syndrome (EMS; Honeyfield et al., 2005) and Cayuga Lake Syndrome (Fisher et al., 1995). Globally, many salmonine (family Salmonidae, subfamily Salmoninae) populations are experiencing thiamine deficiencies (Fisher et al., 1995; Fitzsimons et al., 1995; Mantua et al., 2021; Norrgren et al., 1993; Reed et al., 2023). In the Laurentian Great Lakes, New York Finger Lakes, and Sudbury Basin, the source of thiamine deficiency for salmonines appears to be consumption of introduced prey fishes that contain high concentrations of thiaminase (Fitzsimons and Brown, 1998; Honeyfield et al., 2012), an enzyme that breaks down thiamine in the consumer's stomach when ingested, ultimately limiting thiamine uptake (Honeyfield et al., 2005, 2010; Wolf, 1942). Conversely, in the Baltic Sea, thiamine deficiency in salmonines appears to be driven by consumption of prey fishes that are high in fatty acids but low in thiamine (Keinänen et al., 2012). Consumption of high amounts of easily oxidized fatty acids, specifically highly unsaturated fatty acids (HUFAs), namely n-3 series polyunsaturated fatty acids (n-3PUFAs), can lead to oxidative stress—which has been shown in Atlantic salmon (*Salmo salar*)—and oxidative stress can be exacerbated when thiamine is present at low concentrations relative to lipids (Keinänen et al., 2012). Thiamine deficiency is associated with a range of harmful effects. In severe cases, thiamine deficiency is lethal (Fisher et al., 1995; Honeyfield et al., 2005), but deficiency has also been associated with several secondary effects, such as decreased growth rates and food conversion efficiency, lethargy, reduced visual acuity, increased enzyme (e.g., transketolase) latency, reduction of cardiovascular function and hemorrhaging, and reproductive failures (Adeli et al., 2024; Baker et al., 2023; reviewed in Harder et al., 2018).

Lake trout (*Salvelinus namaycush*) were once abundant in Lake Ontario but were functionally absent by the late 1800s (Elrod et al., 1995; Morrison, 2019). Ongoing efforts to re-establish lake trout in Lake Ontario have resulted in some wild recruits, but restoration efforts have not yet produced a self-sustaining population (B. F. Lantry et al., 2020; J. R. Lantry et al., 2014). One potential challenge to successful restoration is the marked changes in the prey fish community that have occurred over the past century (Crawford, 2001; Lantry et al., 2014; Morrison, 2019). Lake Ontario salmonines historically fed primarily on cisco (*Coregonus artedi*) and bloater (*C. hoyi*), but these prey fishes have largely been replaced by introduced alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*; Beeton, 2002; Miller, 1957; Morrison, 2019). Relative to the native prey fishes, these introduced prey fish species have higher activity of thiaminase (Tillitt et al., 2005). The source of thiaminase activity in introduced alewife and rainbow smelt was initially thought to be linked to thiaminase-producing bacteria, such as *Paenibacillus thiaminolyticus,* found in gut microbiota (Honeyfield et al., 2002; but see Richter et al., 2012). More recently, there is evidence for de novo production of thiaminase by some fishes (Richter et al., 2023; Rowland et al., 2023). Regardless of the source of thiaminase, consumption of prey fishes containing high levels of thiaminase has been linked to thiamine deficiency in many salmonines (reviewed in Harder et al., 2018). Consequently, the ability of lake trout to tolerate consumption of novel prey fishes that contain high levels of thiaminase may be an important determinant of the success of the restoration program in Lake Ontario.

Recent evidence shows that salmonine populations differ in adaptations to a range of environmental variables, and that the choice of source population can influence the success of reintroduction programs (Houde, Garner, et al., 2015). Specifically, local adaptation to high activity of dietary thiaminase has been shown among three Atlantic salmon populations that

differ in their historical exposure to high-thiaminase prey, and these differences are being incorporated into reintroduction programs for Atlantic salmon in Lake Ontario (Houde, Saez, et al., 2015; Mitchell et al., 2021; Wilson & Kidd, 2014). Furthermore, Harder et al. (2020) identified differences in expression of genes coding for heart and neurological function in Atlantic salmon families that were resilient to thiamine deficiency, which indicates that differences in tolerance to diet-derived thiaminase are heritable.

Populations of lake trout may also differ in their tolerance for high-thiaminase prey (Heisey et al., 2023), and using populations subject to historical exposure to high-thiaminase prey in stocking programs may improve the success of reintroduction efforts in the Great Lakes. Currently, several hatchery populations (henceforth referred to as strains) of lake trout originating from Great Lakes and inland sources are commonly stocked into Lake Ontario (Lantry et al., 2014; Ontario Ministry of Natural Resources [OMNR], 2025). Of these, the Seneca Lake strain (henceforth referred to as Seneca strain) is sourced from Seneca Lake, one of New York's Finger Lakes, which has supported abundant populations of alewife since the late 1800s (Miller, 1957; Odell, 1934). The Slate Islands strain (henceforth referred to as Slate strain) is sourced from Lake Superior, where alewife occur at comparatively low abundance (Bronte & Hoff, 1996; Gorman et al., 2021). It is therefore possible that the Seneca strain may be adapted to have a higher tolerance to thiaminase due to longer exposure feeding on alewife compared to the Slate strain (Gorman et al., 2021; Myers et al., 2021). Indeed, Fitzsimons et al. (2021) showed that Seneca strain embryos use less thiamine as compared to embryos from other strains, and initial analyses show that Seneca strain fish have higher survival and represent a majority of the current spawning biomass in the Great Lakes (Bronte, Ebener, et al., 2003; Bronte & Hoff, 1996; Ebener et al., 2025; Lantry et al., 2019; Larson et al., 2021; Rogers et al., 2019). Furthermore,

previous studies by Baker et al. (2023) and in Chapter 4 found that both Seneca and Slate strains showed some resilience to sublethal effects of diet-derived thiaminase such as change of colouration and heart function, and eggs from adult females of different stocked lake trout strains captured in Lake Ontario differed in total thiamine concentrations (Heisey et al., 2023). However, how this apparent tolerance to thiaminase translates to survival and growth has not yet been examined.

Demonstrating an occurrence of thiamine deficiency may be done through several means: (1) Comparison of deficient animals to those known to be replete (control); (2) Control values may be known from the literature, such as in the case of muscle thiamine concentrations or latency (percentage apoenzyme, e.g., the degree of missing cofactor) of thiamine-dependent enzymes; (3) Remediation of deficient specimens by thiamine treatment that proves the specimen was deficient before the treatment; and (4) Typical clinical symptoms associated with subsequent mortality are indicative of a deficiency (Balk et al., 2016). Recently, the use of liver transketolase latency has emerged as a standard for determining thiamine deficiency in fishes (Balk et al., 2016; Engelhardt et al., 2020). Transketolase is a thiamine diphosphate-dependent enzyme in the hexose monophosphate shunt critical for sugar metabolism (Lehninger et al., 2005; Nilsson et al., 1997) and is used in the synthesis of fatty acids (Berg et al., 2012). Previous studies have shown that thiamine deficiency is characterized by high transketolase latency (> 6% latency; Balk et al., 2016; Engelhardt et al., 2020).

Here, we examined the effects of diet-derived thiaminase on liver transketolase activity, survival, growth, and food conversion efficiency in Seneca and Slate strains. We pursued transketolase activity measurements on liver samples to gain an understanding of functional thiamine usage and response to added thiamine diphosphate (TDP) as a measure of thiamine

deficiency. Thus, we predicted that diet-derived thiaminase would reduce transketolase activity and increase liver transketolase latency in both strains. To verify that the transketolase activity and latency measurements were indicative of a deficiency, we used clinical symptoms such as growth and food conversion efficiency, associated with subsequent mortality. Thus, we further predicted that a diet containing thiaminase would impair growth and food conversion efficiency and reduce survival in both strains. However, we also hypothesized that the Seneca strain would show lesser effects to a diet containing thiaminase compared to the Slate strain, if past differences in exposure to high-thiaminase containing prey have led to local adaptations.

3.2 Methods

3.2.1 Preparation of thiaminase and control diets

Thiaminase and control diets were created based on those used by Honeyfield et al. (2005). The specific compositions and preparations are described in detail by Baker et al. (2023). Briefly, the diets were identical in composition (see Tables E-1 and E-2 in Appendix E) except for whole cultures of *Paenibacillus thiaminolyticus* that were added to the thiaminase diet (strains 8703 and 8132; Honeyfield et al., 2002). *Paenibacillus thiaminolyticus* strains were isolated from the viscera of Lake Michigan alewife (Honeyfield et al., 2002), and are common in the diets of Great Lakes salmonines (Weidel et al., 2019; Weidel & Dunlop, 2018). The diet containing *P. thiaminolyticus* was formulated to mimic the thiaminase activity found in alewife and other thiaminase-positive prey fishes, such as rainbow smelt, that contribute to thiamine deficiency in Great Lakes salmonines (Honeyfield et al., 2012; Tillitt et al., 2005)**.** Full details regarding the preparation and use of *P. thiaminolyticus* can be found in Houde, Saez, et al. (2015). Briefly, 1 mL of *P. thiaminolyticus* cultures suspended in a 20% glycerol solution stored

at -80°C was added to 1 L of liquid media (1.0 g · L^{-1} yeast extract and 8.0 g · L^{-1} Difco nutrient broth, Becton Dickinson, Mississauga, Ontario) and incubated for 96 h at 37°C. After incubation, 3 mL of that *P. thiaminolyticus* culture was added to another 1 L of liquid media and incubated for another 96 h at 37°C. The final bacteria count in the liquid media was $2.1 \times 10^8 \pm 6.1 \times 10^7$ cfu/mL; this concentration has previously been shown to induce thiamine deficiency in Atlantic salmon and lake trout (Honeyfield et al., 2005; Houde, Saez, et al., 2015; Mitchell et al., 2021). For both the control and thiaminase diets, frozen whole Pacific herring (*Clupea pallasii*) were thawed and ground with a meat grinder, then dehydrated at 60°C for 48 hours until dry. Dried herring and remaining other dry ingredients (Table E-1 in Appendix E) were then thoroughly mixed before 300 mL / kg dry ingredients of one of two solutions was added: (i) whole culture *P. thiaminolyticus* solution (thiaminase diet); or (ii) autoclaved nutrient broth with no bacteria (control diet). Distilled water was added until a dough-like consistency was reached, and the diets were then screw-pressed into pellets using a 5 mm diameter die. The resulting moist pellets were air-dried at room temperature for 48 hours. Pellets of 5 mm are used in the Provincial hatchery system for fish 100 g and larger and are the appropriate size for fish used in this study (Jason Intini, OMNR, Dorion, Ontario, pers. comm.). Finished feed was stored at -20°C until use. We tested a sample of the culture supernatants for thiamine levels using a periplasmic binding method (see Appendix E-1 and Figure E-1 in Appendix E for methods). Thiamine concentrations of the *P. thiaminolyticus* culture supernatants were lower than those of the control supernatant (mean ± SD; 1000 ± 163 nmol/g versus 1178 ± 166 nmol/g). In subsamples of finished feed from each of the thiaminase and control diets, total thiamine concentrations were similar between the thiaminase (6.92 ± 5.8 nmol/g) and control (7.05 ± 5.2 nmol/g) diets (Table E-1 in Appendix E). A sample of each of the *P. thiaminolyticus* and control broth supernatants

were assayed to determine thiaminase activity using the 4-NTP assay, of which the methods are included in Appendix E-2 in Appendix E. Thiaminase I activity in control diets was lower (mean ± SD; 0.73 ± 1.24 pmol/min) than in thiaminase diets (mean ± SD; 10.69 ± 13.82 pmol/min; Figure E-2 in Appendix E). Maximum storage time for the diets was 2 weeks.

3.2.2 Study strains and experimental design

Families for the Slate and Seneca lake trout strains were produced in late 2019 using single-pair matings of mature individuals at the OMNR Dorion Fish Culture Station (Dorion, ON) and transferred as eyed eggs to the OMNR Chatsworth Fish Culture Station (Chatsworth, ON). The Seneca strain hatchery line originated from wild adult lake trout captured in 1994 from Seneca Lake (42.6536, -76.9004). The Slate strain hatchery line originated from wild, lean-morphotype adult lake trout captured in 2004 from Lake Superior, near the Slate Islands (48.6654, -87.0055).

The timeline of the experiment is shown in Table 3.1. On 18 March 2021, n = 400 (200/strain) lake trout from each of the Seneca (age 13 months) and the Slate (age 14 months) strains were transferred from the Chatsworth Fish Culture Station to the experimental hatchery at University of Western Ontario (London, ON). Twenty-five fish from each strain were placed at random into one of 16 73-L white polypropylene tanks at a density of 9 g/L (e.g., 25 fish weighing 22–27 g in a 73-L tank). The dissolved oxygen levels were kept high (> 9 mg/L), water temperature was kept constant (9.0 ± 0.8°C), and lake trout were subjected to a 12:12 light cycle. While the 12:12 light cycle may have influenced fish growth (Lundova et al., 2019; Taylor et al., 2005), all fish were subjected to the same light cycle and ultimately would not have affected the conclusions of the study. There was slight variation in water temperature between the two rows

of eight tanks, which resulted in a difference of 44°C · d^{-1} throughout the course of the experiment.

Table 3.1 Timeline of experiment using lake trout from the Seneca and Slate strains fed either a control or thiaminase diet

Date	Method	Time since experiment start
18 March 2021	Juvenile (age 1+) transferred from Chatsworth FCS to University of Western Ontario's experimental hatchery. Fish fed commercial fish feed at 2% rations.	0
14 April 2021	Length and weight measurements, PIT tagging of fish	27
28 April 2021	Feeding of experimental diets begins at 2% rations with 50% experimental diet and 50% commercial fish feed	41
12 May 2021	Feeding of 100% experimental diets at 2% rations begins	55
20 May 2021	Weighing of fish. Feeding at 2% rations continues	63
18 June 2021	Weighing of fish. Feeding at 2% rations continues	92
23 July 2021	Weighing of fish. Culls to reduce density. Feeding at 1.5% rations begins.	127
27 August 2021	Weighing of fish. Feeding at 1.5% rations continues	162
24 September 2021	Weighing of fish. Feeding at 1.5% rations continues	190
16 October 2021	Fish culled to maintain optimal densities. Feeding at 1% rations begins	212
5 November 2021	Final weighing of fish. Subset of fish removed for tissue samples. Growth rate calculated.	232
4 December 2021	Experiment ends	261

Fish were given 27 days to acclimate to the University of Western Ontario hatchery before being anesthetized in a bath of tricaine mesylate (TMS; 300 mg/L) buffered with sodium bicarbonate (300 mg/L) and measured for body mass and fork length. During this initial acclimation period, fish were fed the same commercial fish feed they received at the Chatsworth Fish Culture Station (3 mm pellet trout feed, Ewos Canada). After measurement, fish were tagged with a unique sterile 1.2 mm Passive Integrated Transponder (PIT; Biomark Inc) in the ventral cavity using a sterile PIT tag implanter (model MK10, Biomark Inc). The implanter was sterilized in 95% ethanol and allowed to soak for 30 seconds before the next injection. Needles were changed on the implanters after 10 injections. After tagging, fish were placed in a recovery tank that contained 13 ml/L of Stress Coat (API fish care) to accelerate wound healing. Fish remained in the recovery tank until normal behaviour was observed, at which time they were returned to their tanks.

Fish were given an additional 14 days to recover before commencing feeding of experimental diets. During this recovery time, they were fed the commercial trout feed. Four replicate tanks for each strain were then administered either a thiaminase or control diet (2 strains × 2 diets × 4 replicates; total number of experimental units = 16), descriptions of which are in the section below. Each experimental unit was randomly assigned to a tank, and each row of eight tanks had equal representation of each diet and strain. For the first 14 days on the experimental diets, fish were fed a ratio of 1:1 trout feed to experimental feed, before transitioning exclusively to the experimental diets. Palatability of the feed was not determined for this study. Upon food transition to the experimental diets, there was some waste feed left at the bottom of all tanks but was minimal after approximately 1 week (Christian Therrien, pers.

obs.). Feedings occurred once daily at a rate of 2% body mass per day for 86 days. Feeding rates were then reduced to 1.5% body mass per day for another 85 days before being reduced to 1% for the remainder of the study to maintain fish densities lower than 50 g/L, a density previously noted to maximize growth and minimize mortality in these two lake trout strains (Jason Intini, OMNR, pers. comm.). Once each month, all fish were removed from the tank, anesthetized as described above, and weighed; mass data were used to adjust food rations. After 127 days, five lake trout were randomly selected from each tank and euthanized in an overdose of TMS to maintain optimal densities. After 232 days, fish were weighed one final time, and a subset of remaining fish were removed for transketolase analyses. The change in mass from experiment start to end was calculated by subtracting mass after 232 days from starting mass. Mortality continued to be recorded until 261 days, after which remaining fish were used in separate studies. After being euthanized in an overdose of TMS, culled fish had their livers removed and immediately frozen at -80°C until further processing.

Because fish were weighed at repeated intervals, monthly growth rates could be calculated. For each fish, thermal unit growth coefficients (TGC) were calculated as $100 \times (S_2^{1/3} - S_1^{1/3}) / \Delta D$ (Cho, 1992), where S_2 is the size at time 2, S_1 is the size at time 1, and ΔD is the growing degree-days ($\Delta D = \Sigma$ °C per day) for each month fish were on the experimental diets. Food conversion efficiency (FCE) was also calculated for each month that fish were fed the experimental diets. Food conversion efficiency was calculated as: FCE = [weight gain (g)/food given (g)] x 100 (Salman & Eddy, 1988). Because we knew only the rations that each tank received, FCE was calculated at the level of tank. For the final analysis of FCE, data from months 1 (day 41−66) and 6 (day 212−232) of the experiment were excluded. Month 1 data were excluded because the combination of experimental and commercial trout feed pellets resulted in

inflated food conversion efficiency values. Data from month 6 were excluded because there was high mortality of Seneca strain fish, especially those fed the thiaminase diet; this led to some tanks receiving rations that were proportionally larger by mass than others, which produced inconsistent results.

3.2.3 Transketolase assays

A subsample of 33 livers was processed to determine transketolase activity (Table 3.2). The extraction procedure was adapted from Engelhardt et al. (2020). A portion of the frozen liver was weighed (~200 mg) and diced into small pieces with a stainless-steel razor and transferred to a 2 mL microcentrifuge tube. Ice-cold 0.25 M sucrose was added to the sample (3 mL/g) and the mixture was homogenized on ice using an up and down motion with a hand-held homogenizer (Bio-Spec Tissue-Tearor™) for 30 seconds at the lowest speed. The homogenizer probe was chilled between samples after washing. The slurry was then centrifuged at 10,000 g at 4°C for 10 min. The supernatant was removed and aliquoted into 100 µL portions in 1.5 mL centrifuge tubes before storage at -80°C.

Table 3.2 Sample sizes for each metric measured on lake trout from the Seneca and Slate strains fed either the control or thiaminase experimental diet. TGC and FCE were measured monthly until experiment end after 232 days. Transketolase metrics were only measured at experiment end after 232 days.

Strain (diet)	Experiment start	Thermal-unit growth coefficient (TGC)	Food conversion efficiency (FCE)	Transketolase (Basal, Supplemented, and Latency)
Seneca (control)	112	112	112	8
Seneca (thiaminase)	105	105	105	9
Slate (control)	105	105	105	8

Slate (thiaminase)	108	108	108	8
Total	*427*	*427*	*427*	*33*

Immediately preceding analysis, an aliquot was thawed on ice and centrifuged at 10,000 g for 5 min. to settle any residual solids. Five µL of this supernatant was retained for the bicinchoninic acid assay (BCA) assay. The assay protocol for transketolase activity was adapted from Jones et al. (2023, 2021). Briefly, 25 µL of the supernatant was diluted to 250 µL with 4°C 100 mM Tris, pH 7.6 and mixed before adding 30 µL to six wells of a transparent 96-well plate (Corning Inc. model 3795). Fifteen µL of either Tris buffer alone or 3.6 mg/mL TDP in Tris buffer was added to triplicate wells of the diluted supernatants. Sucrose diluted to 25 mM in Tris buffer was used as a control and treated the same way as the standards. The plate was shaken at 600 rpm for 30 seconds, sealed, then incubated at 37°C for 15 min. A mixture of NADH (256 µM), D-ribose-5-phosphate (16.26 mM), and α-GDH-TPI (0.208 U/mL) was prepared in Tris buffer containing 0.004% (w/v) Tween-20 and stored on ice. Two hundred µL of this solution was added to all wells of the plate, which was then immediately transferred to a plate reader (Varioskan, ThermoFisher) set at 37°C and read at 340 nm every minute over 60 min. to monitor NADH depletion with 5 seconds shaking at 600 rpm before each read. Following the kinetic reads, the plate was read at 900 nm and 977 nm to determine the path length.

The data were normalized by deducting the initial optical density (OD) value from each individual replicate for all time points. The slope of the plot of OD versus time (min.) was determined using a straight line fit in GraphPad Prism (version 10.1.2; GraphPad Software, 2023). All curves were examined visually for aberrations. To convert from a change in OD/min. to pmol/min., the slope was multiplied by -55,196.6. This factor was determined using the

average path length (0.787 cm), the molar extinction coefficient for NADH (5.64 $\times 10^3$ $M^{-1}cm^{-1}$), and the total assay well volume (245 µL) to solve for concentration in the Beer-Lambert law.

The specific activity of the sample was determined by taking the basal and supplemented rates and dividing by the milligrams of protein present in the assay well. To determine the concentration of protein in the assays, the microBCA™(ThermoFisher) assay was carried out on the enzyme extracts per the manufacturer's instructions, using a 1:200 dilution of the extract in HPLC grade water (JT Baker) for protein quantification against a standard curve prepared from bovine serum albumin. Samples and standards (0.94–60 µg/mL protein) were run in triplicate. The latency of transketolase activity was calculated using the following equation: latency = 100%*(*S*–*B*))/(*S*) where *S* is the supplemented and *B* is the basal transketolase activities for a given sample. A sample with high basal activity relative to supplemented activity indicating sufficient thiamine availability will have low latency, and vice versa.

A sample of muscle and livers was examined for thiamine concentration using the periplasmic binding method (Appendix E-1 in Appendix E), but unreliable readings from the detector at concentrations below 1.5 nmol/g necessitated its exclusion from this study. For readers' interest, the results of those tests have been included in Appendix E Figure E-3.

3.2.4 Statistical analyses of survival and traits

Survival and traits of individual lake trout were analyzed in R (version 4.3.3., R core team 2024), using $\alpha = 0.05$ for all statistical tests. Comparisons of initial total length and mass measurements were compared using a student's t-test. All assumptions of a student's t-test were screened for and met prior to analysis. Probability of survival curves for lake trout were generated using Cox proportional-hazard models (Cox, 1972) in the package 'survival'

(Therneau, 2023) for each strain and diet type, and compared between strains, diet types, and their interaction using the function coxph(). A random effect of tank was included in the analysis to account for potential differences in survival between tanks. Restricted likelihood ratio tests were used in the coxph() function to explain if the random effect explained a significant proportion of the variance. If a random effect did not explain a significant proportion of the variance of the model, it was dropped from the final model. When a random effect was included in the final model, variance explained was represented as the percentage of variance accounted for by random effects as calculated in the coxph() function.

Linear mixed effects models fit with restricted maximum likelihood (lmm; *lmer* in the lme4 package in R; Bates et al., 2023) were used to examine effects of diet type, strain, and their interaction on change in mass, TGC, and FCE. To account for repeated measurements on the same individual, a random effect of month was included in the models for both TGC and FCE. Since TGC was calculated at the level of the individual, a random effect of fish ID was included. A random effect of tank was also included in the analyses of TGC and change in mass to account for non-independence of fish (i.e., dominance interactions) in a tank. F statistics and p values for all lmms were generated using function Anova() with type III variance tables in package car (Fox & Weisberg, 2019). Marginal and conditional R^2 values were calculated using the function r.squaredglmm() in the package 'MuMIn' (Barton, 2024). To test if random effects explained a significant proportion of the variance, restricted likelihood ratio tests were used (function exactRLRT() in package RLRsim; Scheipl and Bolker, 2025). If a random effect did not explain a significant proportion of the variance of the model, it was dropped from the final model. When a random effect was included in the final model, variance explained was quantified using the

intraclass correlation coefficient method and was represented as the percentage of variance accounted for by random effects.

Low sample sizes for evaluations of liver transketolase activity prevented the inclusion of random effects, due to singularity issues. Generalized linear models with an identity link (glm in base R; R core team, 2024) were used to examine the effects of diet type, strain, and their interaction on basal and supplemented liver transketolase activity and latency. R^2 values for glms were calculated using McFadden's R^2 equation (Hardin & Hilbe, 2007). Here, a single R^2 value was presented. A linear model was used to examine the relationship between liver transketolase latency and TGC.

3.3 Results

3.3.1 Comparison of initial traits between strains

At the beginning of the experiment, lake trout from the Slate strain were 1 month older than those from the Seneca strain, and thus the two strains differed in initial body length and mass (Table 3.3). Fish from the Slate strain were heavier than those from the Seneca strain (t = -5.44, df = 407.63, $p < 0.001$), and they were also longer than those from the Seneca strain (t = -6.26, df = 413.49, $p < 0.001$).

At the end of the experiment, the two strains continued to differ in their mass, although the difference was opposite of what was found at the beginning of the experiment (Table 3.3). When expressed as a change in mass from experiment start to end, despite being a month younger than the Slate strain, fish from the Seneca strain grew on average 24.2 g more (95% CI; 9.5−40.0 g) than those from the Slate strain (t = -3.22, df = 1, 271, p = 0.001, R^2 = 0.08). Within each strain, there was no difference in the change in mass between fish fed the control or

thiaminase diets (t = -1.66, df =1, 271, p = 0.09). The diet × strain interaction was not significant, indicating no strain-specific effect of diet on the change in mass (t = 0.27, df = 1, 271, p = 0.79). The random effects of tank (RLRT = 0, p = 1) and row (RLRT = 0, p = 1) did not account for any of the variance and were excluded from the final model.

Table 3.3 Initial and final body lengths and masses and change in mass (mean ± SD) of lake trout from the Seneca and Slate strains of lake trout fed either the control or thiaminase experimental diet. Length is expressed in cm and both mass and change in mass are expressed in g. Superscript letters denote homogenous subsets from a t-test analysis (see text). Differences in length, mass, and change in mass at experiment end were examined with linear mixed models that do not permit post hoc tests. Descriptions of differences are found in text. The diets were administered for 232 days.

Strain	Treatment	Experiment start		Experiment end	Change in mass
		Length (cm)	Mass (g)	Mass (g)	(g)
Seneca	Control	13.9 ± 1.5[a]	25.1 ± 8.8[a]	126.3 ± 62.4	103.5 ± 57.1
	Thiaminase	13.4 ± 1.0[a]	22.1 ± 4.8[a]	114.5 ± 62.4	90.0 ± 44.4
Slate	Control	14.2 ± 1.9[b]	27.3 ± 11.6[b]	106.5 ± 45.9	79.3 ± 39.5
	Thiaminase	14.3 ± 1.7[b]	26.4 ± 8.8[b]	97.5 ± 35.3	69.5 ± 30.0

3.3.2 Survival

There were some differences in survival curves between strains of the juvenile lake trout studied (Figure 3.1). Overall, fish from the Slate strain had a risk of mortality (hazard ratio) 16% lower (95% CI; 3−87%) than fish from the Seneca strain, regardless of diet type (x^2_1 = 4.51, p =

0.03). The diet × strain interaction term was not significant, indicating that the effect of diet type on survival did not differ between the strains ($x^2_1 = 0.49$, $p = 0.50$). While there was a trend towards lower survival of the fish fed the thiaminase diet, ultimately this difference was not significant ($x^2_1 = 1.24$, $p = 0.13$). The random effect of tank was significant (likelihood ratio test = 77.2, $p < 0.001$) and explained 29% of the variance, with high mortality of Seneca fish in tanks L16, L3, L1, L12, and L13 with 12, eight, six, four, and four mortalities, respectively, and with low or no mortalities in the other tanks likely explaining this trend. Interestingly, of the five tanks with greater than two mortalities, four of them were of Seneca fish fed the thiaminase diet, and no tank with Seneca fish fed the thiaminase diet had any fewer than four mortalities. In contrast, there were only three mortalities total across all tanks of Slate fish fed the thiaminase treatment.

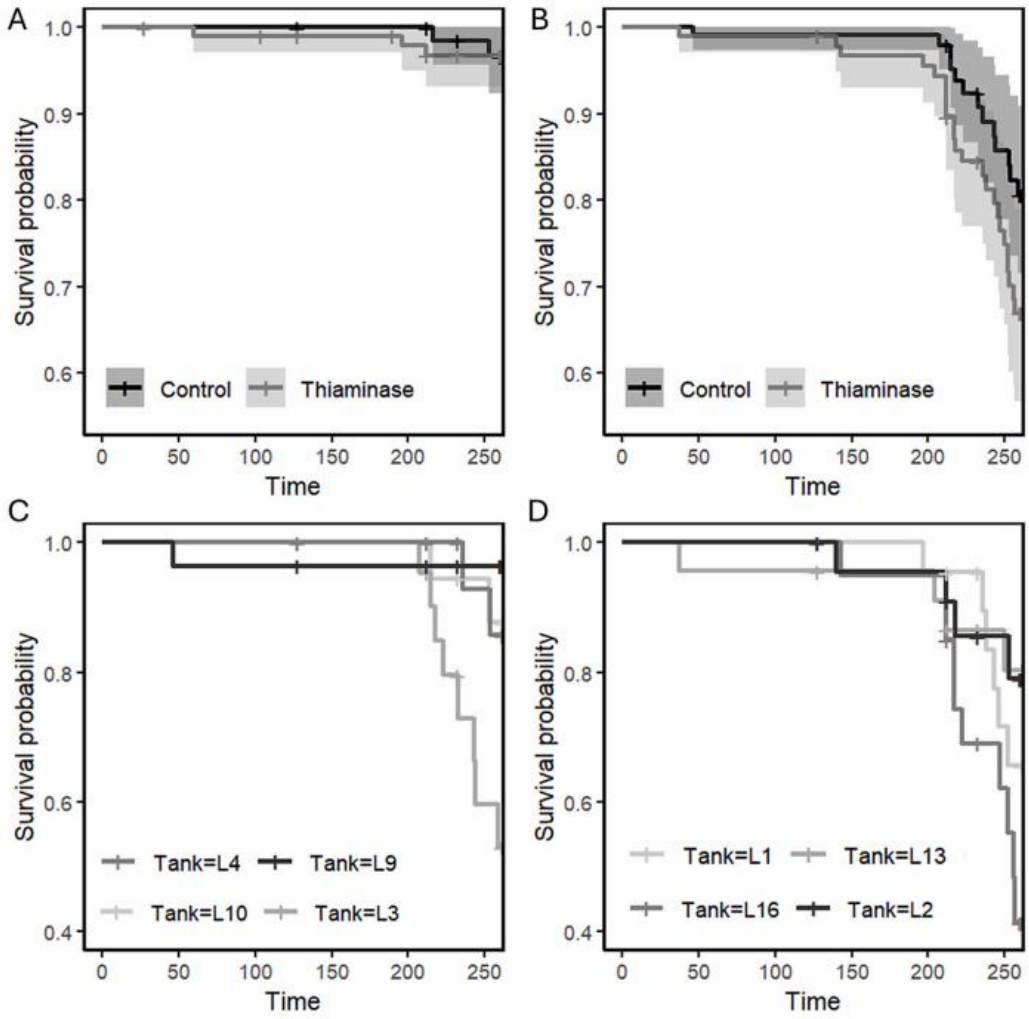


Figure 3.1 Survival probability of juvenile lake trout from the A) Slate and B) Seneca strains after 261 days fed either a control (black) or thiaminase (grey) diet. Survival probability of each tank of Seneca lake trout fed the C) control and D) thiaminase after 261 days. Bands around lines in A and B represent 95 % confidence intervals. In A and B, there was a significant difference between strains ($p = 0.03$) but no significant difference between diet groups ($p = 0.13$) or a significant interaction ($p = 0.50$). In C and D, the random effect of tank was significant ($p < 0.001$) and explained 29 % of the variance.

3.3.3 Thermal unit growth coefficient and food conversion ratio

Regardless of strain, lake trout fed the thiaminase diet grew more slowly than those fed the control diet ($F_{1,427} = 16.06$, $p = 0.002$, $R^2_{marg.} = 0.06$, $R^2_{cond.} = 0.24$; Figure 3.2A). The diet ×

strain interaction term was not significant, indicating that the effect of diet-type on growth did not differ between the strains ($F_{1,427} = 0.49$, $p = 0.50$). Growth did differ between the strains, however; Seneca strain lake trout grew more quickly than those from the Slate strain ($F_{1,427} = 66.69$, $p < 0.001$). The random effects of fish ID (RLRT = 36.4, $p < 0.001$) and month (RLRT = 146.1, $p < 0.001$) accounted for a significant amount of variation (8 and 7%, respectively) and were included in the final model. The random effect of tank did not account for a significant amount of the variance (RLRT = 2.03, $p = 0.06$) and was excluded from the final model.

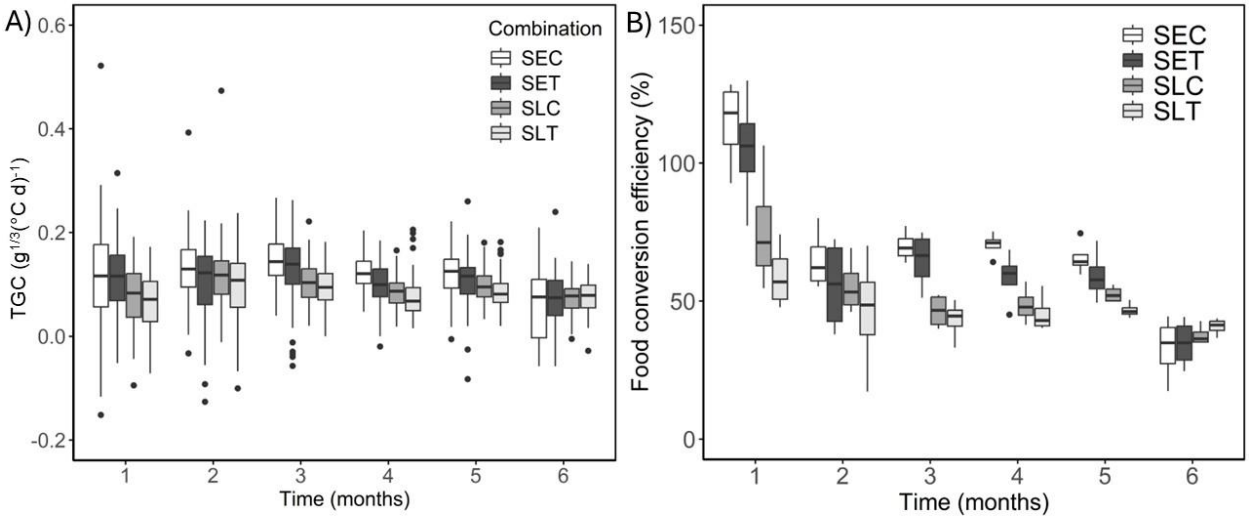


Figure 3.2 A) Thermal unit growth coefficient (TGC; $g^{1/3}(°C\ d)^{-1}$) and B) food conversion efficiency (FCE; %) of lake trout from the Slate (SL) and Seneca (SE) strains after each month fed either a control (C) or thiaminase (T) diet for 232 days. A total of 427 fish were included in the analysis of TGC (SEC, n = 112; SEC, n = 105; SLT, n = 105; SLT, n = 108). The boxplot of FCE presents a value for each tank (n = 16). Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range. In A, there was a significant difference between diet groups and between strains ($p < 0.05$) but no significant interaction ($p > 0.05$). In B, there was a significant difference between diet groups and between strains ($p < 0.05$) but no significant interaction ($p > 0.05$).

An analysis of food conversion efficiency that included data from months 1 and 6 can be found in Figure E-4 in Appendix E. Month 1 data were excluded because the combination of experimental and commercial trout feed pellets resulted in inflated food conversion efficiency values. Data from month 6 were excluded because there was high mortality of Seneca strain fish, especially those fed the thiaminase diet; this led to some tanks receiving rations that were proportionally larger by mass than others, which produced inconsistent results. When months 1 and 6 were excluded from the analysis, fish fed the thiaminase diet had a significantly lower food conversion efficiency than those fed the control diet, regardless of strain ($F_{1,16} = 4.53$, $p = 0.05$, $R^2 = 0.36$, Figure 3.2B). Food conversion efficiency also differed significantly between strains; Seneca strain lake trout had higher food conversion efficiency than Slate strain lake trout ($F_{1,16} = 19.56$, $p < 0.001$). The diet × strain interaction term was not significant, indicating that the effect of strain on food conversion efficiency was consistent regardless of diet ($F_{1,16} = 0.27$, $p = 0.61$). The random effects of tank (RLRT = 1.7×10^{-13}, $p = 0.46$) and time (RLRT = 1.7×10^{-13}, $p = 0.39$) were not significant, did not account for any of the variance, and were excluded from the final model.

3.3.4 Transketolase activity

We found that diet ($t = 0.89$, df = 1, $n = 33$, $p = 0.38$, $R^2 = 0.24$), strain ($t = 0.53$, df = 1, $n = 33$, $p = 0.60$), and their interaction ($t = 1.03$, df = 1, $n = 33$, $p = 0.31$) had no influence on basal transketolase activity of lake trout (Figure 3.3A). We also found that diet ($t = 0.08$, df = 1, $n = 33$, $p = 0.94$, $R^2 = 0.05$), strain ($t = -0.12$, df = 1, $n = 33$, $p = 0.91$), and their interaction ($t = 0.76$, df = 1, $n = 33$, $p = 0.45$) had no influence on supplemented transketolase activity of lake trout (Figure 3.3B).

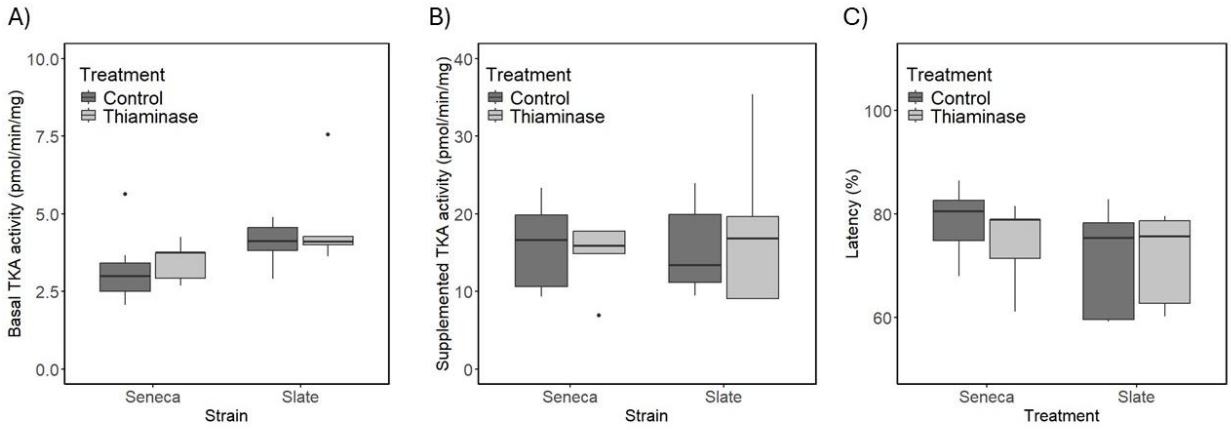


Figure 3.3 A) Basal and B) supplemented transketolase activity (pmol/min/mg) concentrations (pmol/min/mg), C) transketolase latency (%) of liver tissue from lake trout of the Seneca and Slate strains fed either a control (dark grey) or thiaminase (light grey) diet for 232 days. A total of 33 fish were included in the analysis for supplemented and basal transketolase activity and latency (Seneca control, n = 8; Seneca thiaminase, n = 9; Slate control, n = 8; Slate thiaminase, n = 8). Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5× interquartile range. For all three metrics examined, there were no differences between diet groups within each strain, nor was there a significant difference between the Slate and the Seneca strains ($p < 0.05$).

Calculated transketolase latency values of fish fed both the control and thiaminase diets were high and above the previously assumed threshold for thiamine deficiency in other fishes (> 6% latency; Figure 3.3C; see Balk et al., 2016; Engelhardt et al., 2020). Diet had no influence on the latency values (t = -1.14, df = 1, n =33, p = 0.26, R^2 = 0.08). Latency also did not differ significantly between strains (t = -1.53, df = 1, n = 33, p = 0.14), and there was no significant diet × strain interaction (t = 1.06, df = 1, n = 33, p = 0.30). There was a significant positive relationship between liver transketolase latency and growth (t = 2.22, df = 30, p = 0.03, R^2 = 0.11; Figure 3.4A); an increase in growth, as measured as thermal-unit growth coefficient (TGC), of 0.1 $g^{1/3}(°C\ d)^{-1}$ was associated with an 8.6% (95% CI; 0.7−16.6 %) increase in liver

transketolase latency. There was also a significant negative relationship between basal liver transketolase activity and growth (t = -2.21, df = 30, p = 0.03, R^2 = 0.11; Figure 3.4B); an increase in TGC of 0.1 $g^{1/3}(°C\ d)^{-1}$ was associated with a 1.3 pmol/min/g (95% CI; -24.9−1.0 pmol/min/g) decrease in basal liver transketolase activity. There was no relationship between supplemented liver transketolase and growth (t = 0.73, df = 30, p = 0.71, R^2 = -0.02).

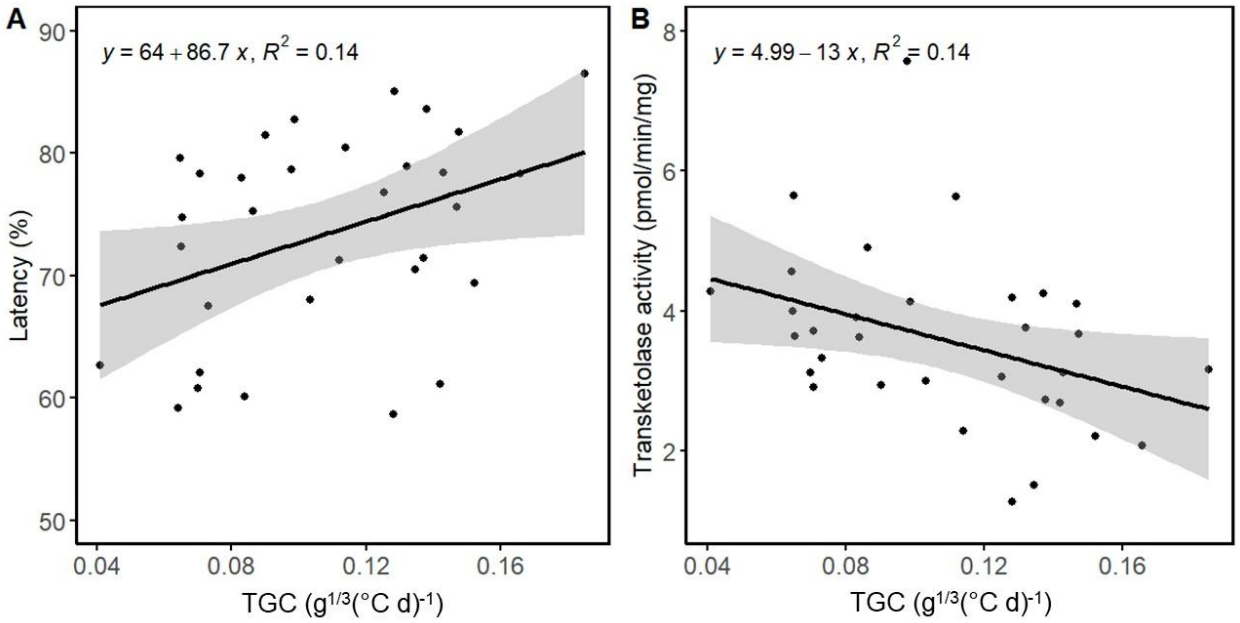


Figure 3.4 Relationship between A) transketolase latency (%), B) basal transketolase activity (pmol/min/mg), and thermal unit growth coefficient (TGC; $g^{1/3}(°C\ d)^{-1}$) of lake trout fed the experimental diets for 232 days. There was a significant relationship between both transketolase latency (p = 0.03) and basal transketolase activity (p = 0.03) and thermal unit growth coefficient. The line of best fit equation was calculated using a linear model and the grey band represents the 95% confidence interval. R^2 values presented are the multiple R-squared.

3.4 Discussion

Thiamine deficiency has been widely associated with mortality in salmonines (e.g., Balk et al., 2016; Brown, Fitzsimons, et al., 2005). In previous hatchery studies, salmonines fed a diet high in thiaminase had higher mortality compared to those fed control diets (Honeyfield et al., 2005; Mitchell et al., 2021; Morito et al., 1986). We found that fish from the Seneca strain had a lower survival probability compared to the Slate strain, regardless of diet; however, whereas there was high mortality of Seneca fish in all tanks administered the thiaminase diet, there was high mortality in only one tank fed the control. Furthermore, as mortality began to increase in the Seneca strain fish fed the thiaminase diet, symptoms associated with thiamine deficiency were evident (e.g., ataxia, lethargy, change in morphology; Chapter 4; Therrien et al., unpublished data). For Slate strain lake trout, however, we observed no difference in survival between fish fed the thiaminase diet and fish fed the control diet. It seems unlikely that Slate strain lake trout possess adaptations that increased their tolerance to mortality mediated by *P. thiaminolyticus* as these fish have not been exposed to high thiaminase prey, unlike the Seneca fish. Instead, it is possible that the Slate strain fish are better able to metabolize HUFAs without depleting thiamine to a level that induces mortality, perhaps through increased thiamine storage capabilities, increased enzyme affinity for TDP, or a lower threshold for the negative secondary effects as suggested by Baker (2023). Indeed, in their native environment, Slate Islands lake trout feed on prey fish that are higher in HUFAs (rainbow smelt, cisco) relative to Seneca fish (alewife; Futia and Rinchard, 2019; Howland et al., 2013). It is also possible that the high growth rates of the Seneca fish may have reduced their thiamine stores more quickly than their Slate counterparts and contributed to higher mortality rates. Indeed, we found a positive relationship between growth rate and liver transketolase latency. More research is needed to understand why the Slate

strain was less susceptible to mortality induced by the thiaminase diet treatment. It is also worth noting that stocked Klondike strain (Lake Superior origin) lake trout captured in Lake Ontario had a different fatty acid signature and higher egg thiamine levels than wild-caught stocked Seneca strain fish (Heisey et al., 2023), suggesting deepwater-foraging strains of lake trout may be less prone to thiamine depletion in the wild.

While the effect of the thiaminase diet on survival was strain-specific, negative effects of the thiaminase diet on growth rate and food conversion efficiency were observed in both strains. Previous authors who have examined effects of thiaminase on fish growth have reported mixed results. Atlantic salmon fed a diet containing bacterial-derived thiaminase had similar growth rates and absolute mass gains as fish fed a control diet (Houde, Saez, et al., 2015; Mitchell et al., 2021). In contrast, Carvalho et al. (2009) found that lake trout alevin with mothers fed a diet high in thiaminase had reduced foraging rates and growth compared to conspecifics fed a control diet. We predicted that the thiaminase diet would decrease the growth rate and food conversion efficiency of lake trout by inducing thiamine deficiency, because several studies have shown lower growth rates and food conversion efficiency in thiamine-deficient fish across several taxa, including sea bass (*Lates calcarifer*; Boonyaratpalin and Wanakawot, 1993), rainbow trout (*Oncorhynchus mykiss*; Morito et al., 1986), and fishes within the families Cyprinidae (Xiang et al., 2016) and Channidae (Zehra & Khan, 2018). However, the lack of difference in liver transketolase latency that we observed between the diet treatments suggests that this mechanism may not explain the differences seen in growth rate and food conversion efficiency between fish fed thiaminase and control diets.

Instead, the consumption of *P. thiaminolyticus* may have changed the gut microbiome community of those lake trout fed the thiaminase diet and influenced their growth and food

conversion efficiency, as the microbiome of salmonines have been shown to be malleable (Michl et al., 2017), and microbiome community differences have been suggested as a leading hypothesis for the differences in thiamine status between Atlantic salmon populations (Todisco, 2024). The importance of the gut microbiota on fish growth has been demonstrated in several previous studies; gut microbiota can affect growth performance, food conversion, lipid absorption, and food intake (reviewed in Butt and Volkoff, 2019). Studies have shown thiaminase activity to be concentrated in the gut (Kraft et al., 2014; Wolfe et al., 2023), and the thiaminase activity could be increased after consumption of thiaminase-containing prey fishes (Wistbacka & Bylund, 2008), all of which may be reflective of composition of the gut microbiota or change thereof. Thiamine-producing bacteria in the gut have also been identified as a potential mechanism for promoting thiamine absorption (Ji et al., 1998), and replacement of these positive bacteria with thiaminase-producing bacteria derived from the diet may have contributed to lower growth and food conversion efficiency. DNA sequence analysis of the gut microbiome of fish from both treatments are needed to further investigate this potential mechanism. Regardless, our data suggest that a diet containing thiaminase derived from *P. thiaminolyticus* results in reduced growth rates and food conversion efficiency in juvenile lake trout, regardless of strain.

A diet rich in thiaminase should induce thiamine deficiency and lead to high liver transketolase latency. We expected that fish fed the thiaminase diet would display thiamine deficiency, but we instead found that fish fed either the thiaminase or control diets had high latency values. Although unexpected, both diets used in this study contained higher lipid levels (21.1 ± 1.8%) than those used in a similar previous study (Houde, Saez, et al., 2015; 10.4 ± 0.1 %). Highly unsaturated fatty acids (HUFAs) are susceptible to lipid peroxidation due to multiple double bonds that are prone to oxidation by reactive oxygen species (ROS; Kjær et al., 2008;

Sargent et al., 2003). The Pacific herring that we used in the production of diets were high in HUFAs and were high in lipids (see Huynh et al., 2007). Lipid metabolism requires thiamine (reviewed in Harder et al., 2018), so the high concentration of HUFAs in the diets used in this study could have contributed to reduced thiamine levels in all fish. Additionally, the feed used in this experiment had higher levels of carbohydrates (32.4 ± 1.1 %) compared to those used in Houde, Saez, et al. (2015) (24.6 ± 0.9 %). Like lipid metabolism, carbohydrate metabolism is thiamine-costly; thiamine is a cofactor in the citric acid cycle and an increase in carbohydrate density can lead to an increase in thiamine turnover by the consumer (e.g., Elmadfa et al., 2001; Xu et al., 2022; Zhou et al., 2022). The higher proportions of HUFAs and carbohydrates in the diets used in this study may explain the high liver transketolase latency values observed and also explain why we did not detect differences in either liver transketolase activity or latency between the diet treatment groups. A future study could feed the two strains of lake trout diets with different concentrations of HUFAs, without the addition of *P. thiaminolyticus,* to further investigate this hypothesis. Additionally, the sample size of 33 used in this study may not have had enough power to detect differences driven by a low to medium effect size and could have further explained the lack of differences between diets. Further studies should aim to have higher sample sizes when comparing differences in transketolase activity.

The results of this study have implications for restoration efforts aimed at lake trout in Lake Ontario. Although it is unresolved whether alewife were originally native or introduced to Lake Ontario (Miller, 1957), it is the most abundant pelagic forage species in the lake (Weidel et al., 2023). Accordingly, the ability of lake trout to cope with thiaminase resulting from an alewife-heavy diet will continue to be an important element in their potential restoration (Fisher et al., 1996; Heisey et al., 2023). Strain-targeted stocking programs present a possible solution to

reduce the incidence of thiaminase-related health effects and improve survival rates of lake trout in the wild (e.g., Houde, 2015). Previous studies have identified that the Seneca strain of lake trout has higher survival rates than the Slate strain in Lake Ontario, likely due to lower mortality from sea lamprey (*Petromyzon marinus*; Schneider et al., 1995) and a presumed resilience to high-thiaminase prey. Baker et al. (2023) also found that the Seneca strain of lake trout may possess adaptations to cardiac impairments induced by consumption of diet-derived thiaminase. Here, we found that fish fed the thiaminase diet had lower survival rate in the Seneca strain but not in the Slate strain. These findings refute our prediction of an acquired adaptive response to thiaminase resistance based on exposure to alewife and suggest that Slate strain lake trout may also possess adaptations that help mitigate effects of diet-derived thiaminase or diets high in HUFAs. Together with Heisey et al. (2023) and Houde, Saez, et al. (2015)**,** our findings highlight the potential for utilizing multiple sources for rehabilitating lake trout in the Great Lakes.

3.5 Conclusions

Recent evidence shows that salmonine populations may differ in pre-existing adaptations to thiaminase and that the choice of source population may influence success of reintroduction programs (Houde, Garner, et al., 2015). Here, we confirmed the negative effects of diet-derived thiaminase on growth, food conversion efficiency, and survival compared to the control diet, and—except for survival—these effects did not differ between the two strains. Fish from the Seneca strain had lower survival when fed either the thiaminase or control diet compared to the Slate strain, with high mortality in all tanks fed the thiaminase diet compared to one tank fed the control diet; no difference in survival was found between diets fed to the Slate Islands fish. Unexpectedly, both diets resulted in liver transketolase latency values that were consistent with thiamine deficiency, and this was true for both strains, which may point to lipid peroxidation

being a contributor to thiamine deficiency, in addition to thiaminase. Our results suggest the Slate strain lake trout may also possess adaptations that help mitigate effects of diet-derived thiaminase or diets high in HUFAs, appearing more tolerant of mortality induced by the thiaminase diet than the Seneca strain. These results have implications for lake trout stocking in the Great Lakes basin given the current populations of thiaminase-positive prey fishes in the fishery.

Chapter 4

Dietary thiaminase alters morphology and decreases swimming performance of lake trout (*Salvelinus namaycush*)

This chapter is an adapted version of the following paper, published in Scientific Reports:

Therrien CA, Baker PM, Garner SR, Swanson HK, Neff BD. 2025. Dietary thiaminase alters morphology and decreases swimming performance of lake trout (*Salvelinus namaycush*). Scientific Reports, 16:5493. https://doi.org/10.1038/s41598-026-35139-8

4.1 Introduction

Thiamine (vitamin B1) is an essential water-soluble vitamin required as a cofactor for roughly 2% of all cofactor-mediated enzymatic reactions (Monteverde et al., 2017). Thiamine is essential for both catabolic and anabolic central carbon metabolism and branched-chain acid synthesis (Monteverde et al., 2017), including glycolysis and the citric acid cycle (Bettendorf, 2013), as well as proper neurological (Bâ, 2008), immune (Abraham et al., 2026; Ottinger et al., 2014; Wen et al., 2016), and muscular function (Romanenko et al., 1994). In animals, thiamine must be acquired through the consumption of organisms that produce or contain thiamine (Manzetti et al., 2014). Thiamine exists as 4 vitamers, the biologically-relevant form of which, thiamine diphosphate, mediates various enzyme complexes including pyruvate dehydrogenase,

transketolase, and 2-hydroxyacyl-CoA required for carbohydrate, amino acid, and lipid metabolism (Bettendorf, 2013; Bettendorff & Wins, 2013a; Casteels et al., 2007; Manzetti et al., 2014). Thiamine diphosphate also plays a central role in nerve function, enabling pathways associated with the production of neurotransmitters, further antioxidant mechanisms, and for the myelination of neurons (Bettendorff & Wins, 2013a; Whitfield et al., 2018). Low concentrations of thiamine result in a syndrome referred to as thiamine deficiency (Riley & Evans, 2008). Because of its role in the cardiovascular and neurological systems, symptoms of low thiamine often manifest as cardiorespiratory and neurological impairments (Baker et al., 2023; Kril, 1996; Roman-Campos & Cruz, 2014). In severe cases, thiamine deficiency is lethal, but it has also been associated with secondary, sub-lethal effects, such as decreased growth rates, lethargy, reduced visual acuity, reduction of cardiovascular function and hemorrhaging, and reproductive failure (reviewed in Harder et al., 2018). Over the past few decades, thiamine deficiency has been observed in a variety of taxa, including fishes (Fisher et al., 1996), reptiles (Sepúlveda et al., 2004), and birds (Balk et al., 2016), and it has been suggested that thiamine deficiency is a contributor and threat to worldwide loss of biodiversity (Balk et al., 2016; Gilbert, 2018).

The Salmonidae subfamily Salmoninae (henceforth referred to as salmonines), which includes the Pacific salmons and trout (*Oncorhynchus* spp.), Atlantic salmon and trout (*Salmo* spp.), and charrs (*Salvelinus* spp.), has been the focus of the research on thiamine deficiency in fishes (Harder et al., 2018). In the Laurentian Great Lakes, the suspected primary cause of thiamine deficiency is from thiaminase I (henceforth referred to as thiaminase) consumption from exotic prey fishes (Fitzsimons & Brown, 1998). Thiaminase catalyzes the breakdown of thiamine into its precursors in the stomach of consumers, ultimately limiting thiamine uptake (Campobasso et al., 1998; Honeyfield et al., 2005, 2010; Wolf, 1942). Reproductive failures and

the effects of thiamine deficiency on larvae have been well-studied (see review in Harder et al., 2018), whereas the effects of thiamine deficiency on body development and swimming performance, particularly in juvenile salmonines, remain understudied. Authors of studies on thiamine-deficient fish have reported a range of behavioural abnormalities, including lethargy, ataxia, abnormal movement patterns, loss of equilibrium, wriggling swimming behaviour, and a reduction in the ability to ascend cascades to spawning areas. These abnormalities have been attributed to neurological and cardiac impairments including altered brain thiamine metabolism and brain lesions (Amcoff et al., 1998; Fisher et al., 1995; Fitzsimons et al., 2009; Ketola et al., 2005; Marcquenski & Brown, 1997). Results from other studies indicate thiamine deficient fish have physical abnormalities that could further limit survival in the wild (see review in Harder et al., 2018). For example, fish colouration including changes to whole body lightening and a decrease in yellow pigmentation (Houde, Saez, et al., 2015; Morito et al., 1986) and less streamlined body shape (Houde, Saez, et al., 2015) have been noted in thiamine deficient juvenile salmonines. These traits have been associated with survival and foraging efficiency in salmonines (Arostegui & Quinn, 2019; Donnelly & and Whoriskey Jr., 1991; Miyamoto, 2016) and other pelagic fishes (Gygax et al., 2018; Johnsen & Sosik, 2003). Most recently, a change in heart morphology and decrease in function has been observed in lake trout [also referred to as lake charr; *S. namaycush* (Walbaum, 1972)] fed a high-thiaminase diet (Adeli et al., 2024; Baker et al., 2023). In thiamine-deficient mammals, a similar change in heart morphology and function is related to further secondary physical effects, including cyanosis (a blueing of the skin's pigment; Barennes et al., 2015; Engbers et al., 1984; Roman-Campos & Cruz, 2014), a reduction in the capacity for exercise (Choi et al., 2013; Nozaki et al., 2009), and a decreased ability to recover post-exhaustive exercise (Suzuki & Itokawa, 1996). Swimming performance is

particularly important for salmonines as it is critical for prey capture and predator avoidance (reviewed in Plaut, 2001), as is body morphology and colouration, which play roles in predator avoidance, foraging efficiency, and swimming ability (Hyvärinen & Vehanen, 2004; Johnsen & Sosik, 2003; Taylor & and McPhail, 1985). Assessing how thiamine deficiency affects these traits is thus important to understand how it could influence survival of salmonines in the wild (Chivers et al., 2008; Day & McPhail, 1996; Miyamoto, 2016; Rodgers et al., 2013).

Lake trout were once abundant in Lake Ontario, but were functionally extirpated by the late 1800s (Morrison, 2019) and are currently the focus of large-scale reintroduction programs (Lantry et al., 2014). One factor hypothesized to obstruct restoration efforts is the abundance of exotic prey fishes including rainbow smelt [*Osmerus mordax* (Mitchill, 1984)] and alewife [*Alosa pseudoharengus* (Wilson, 1811)] because both of these prey fishes have high thiaminase activity (Tillitt et al., 2005) and are known to induce thiamine deficiency (Fitzsimons & Brown, 1998; Honeyfield et al., 2005). Alewife and rainbow smelt are found throughout the Great Lakes Basin and comprise a major proportion of the diets of salmonines (Kornis et al., 2020; Mumby et al., 2018; Nawrocki et al., 2022). The production of thiaminase I in these prey fishes was first believed to originate from gut microbiota (Honeyfield et al., 2002), but recent evidence suggests that the synthesis of thiaminase I may also occur *de novo* (Richter et al., 2023; Rowland et al., 2023). Currently, two hatchery populations (henceforth referred to as strains) of lake trout are commonly stocked into Lake Ontario – the Seneca Lake (42.6536 N, -76.9004 W) and Slate Islands (Lake Superior; 48.6654 N, -87.0055 W) strains (Lantry et al., 2014). Notably, these strains differ in their historical exposure to prey fishes that contain thiaminase; Seneca Lake has long supported abundant populations of alewife (Odell, 1934), whereas Lake Superior supports relatively few alewife (Bronte & Hoff, 1996). Consequently, it has been suggested that the

Seneca Lake strain may have a higher tolerance to thiaminase through a local adaptation to prey fishes that are high in thiaminase compared to the Slate Islands strain. Indeed, Fitzsimons et al. (2021) showed that Seneca Lake strain embryos use less thiamine as compared to embryos from other strains, and initial analyses show that Seneca Lake strain fish have higher survival and represent a majority of the current spawning biomass in the Great Lakes (Bronte, Ebener, et al., 2003; Bronte & Hoff, 1996; Ebener et al., 2025; Lantry et al., 2019; Larson et al., 2021; Rogers et al., 2019). Furthermore, a previous study conducted by Baker et al. (2023) found that the negative effects of diet-derived thiaminase on heart morphology and function were less pronounced in the Seneca Lake strain compared to the Slate Islands strain. However, how this apparent tolerance translates to swimming performance, morphology, or colouration has not yet been examined (Ebener et al., 2025; Lantry et al., 2019; Larson et al., 2021; Rogers et al., 2019).

Given the lack of a self-sustaining lake trout population in Lake Ontario despite ongoing restoration efforts, identification of strains that possess a tolerance to thiaminase may confer some advantages in the Lake Ontario environment and may help improve restoration success (Lantry et al., 2019; Lantry et al., 2014). Here, we examined the effects of diet-derived thiaminase on morphology, colouration, swimming performance, and recovery after exhaustive exercise, and compared results between the Seneca Lake and Slate Islands strains. Critical swim speed is the best ecophysiological measurement to estimate swimming performance and to predict ecological consequences of stressors outside of measurements of free-swimming individuals (Plaut, 2001). Lake trout are cruising predators and swimming performance is important for capturing dispersed pelagic prey items and avoiding predation (Holbrook et al., 2013; Vogel & Beauchamp, 1999). We predicted that diet-derived thiaminase would impair swimming performance and recovery after exercise as well as change both the morphology and

colouration in both strains. Based on previous research (e.g., Baker et al., 2023; Fitzsimons et al., 2021), we also hypothesized that the Seneca Lake strain would show lesser effects compared to the Slate Islands strain due to past differences in exposure to high-thiaminase containing prey and potential differences resulting from local adaptation to thiaminase exposure.

4.2 Methods

4.2.1 Preparation of thiaminase and control diets

Thiaminase and control diets were created based on those used by Honeyfield et al. (2005). The specific compositions and preparations are described in Baker et al. (2023) and Chapter 3. Briefly, the diets were identical in composition, except that bacterial thiaminase from *Paenibacillus thiaminolyticus* (Nakamura, 1990) was added to the thiaminase diet. Full details regarding the preparation and use of *P. thiaminolyticus* can be found in Houde, Saez, et al. (2015) and Chapter 3. The final bacteria count in the liquid media used in the preparation of the thiaminase diets was $2.1 \times 10^8 \pm 6.1 \times 10^7$ cfu/mL, which is a concentration that has previously been shown to induce thiamine deficiency in Atlantic salmon and lake trout (Honeyfield et al., 2005; Houde, Saez, et al., 2015; Mitchell et al., 2021; Chapter 3). Total thiamine concentrations were similar between the thiaminase (6.92 ± 5.8 nmol/g) and control (7.05 ± 5.2 nmol/g) diets. A sample of each of the *P. thiaminolyticus* and control broth supernatants were assayed to determine thiaminase activity using the 4-NTP assay, the methods of which are included in Chapter 3. Thiaminase I activity in control diets was lower (mean ±SD; 0.73 ±1.24 pmol/min) than in thiaminase diets (mean ±SD; 10.69 ±13.82 pmol/min). Finished feed was stored at -20°C until use. Maximum storage time for the diets was 2 weeks.

4.2.2 Study strains and experimental design

Descriptions of strains used in this study can be found in Baker et al. (2023) and Chapter 3. Briefly, families for the Seneca Lake and Slate Islands strains were produced in late 2019 using single-pair matings of mature individuals at the Ontario Ministry of Natural Resources (OMNR) Dorion Fish Culture Station (Dorion, ON) and transferred as eyed eggs to the Chatsworth Fish Culture Station (Chatsworth, ON). The timeline of the experiment is presented in Table 4.1. On 18 March 2021, n = 200 lake trout from each of the Seneca Lake (age 13 months) and the Slate Islands (age 14 months) strains were transferred from the OMNR Chatsworth Fish Culture Station to the experimental hatchery at the University of Western Ontario (London, ON). A full description of the rearing conditions of the lake trout in the experimental hatchery can be found in Chapter 3 and a figure showing the experimental hatchery can be found in Figure F-1 of Appendix F. Briefly, twenty-five fish from each strain were placed into one of sixteen 73 L white polypropylene tanks at a density of 9 g / L (i.e., 25 fish weighing 22–27g in a 73 L tank). The fish were given 27 days to acclimate to the Western University hatchery before being anesthetized in a bath of tricaine mesylate (TMS; 300 mg L^{-1}) buffered with sodium bicarbonate (300 mg L^{-1}) and measured for body mass and fork length. After measurement, fish were tagged with a unique sterile 1.2 mm Passive Integrated Transponder (PIT; Biomark Inc) in the ventral cavity using a sterile PIT tag implanter (model MK10, Biomark Inc) of which the full description of administration is found in Chapter 3. After tagging, fish were placed in a recovery tank and remained there until the return of normal behaviour, at which time they were returned to their tanks. Fish were given an additional 14 days to recover before the experiment. During this recovery time, they were fed the commercial fish feed. Four replicate tanks for each strain were then administered either a thiaminase or control diet (2 strains × 2

diets × 4 replicates; total number of experimental units = 16). Each experimental unit was randomly assigned to a tank and each row of 8 tanks had equal representation of each diet and strain. The feeding schedule and a full description of feed rations can be found in Chapter 3.

Table 4.1 Timeline of experiment using lake trout from the Seneca Lake and Slate Islands strains fed either a control or thiaminase diet.

Date	Method	Time since experiment start
18 March 2021	Juvenile (age 1+) transferred from Chatsworth FCS to Western University's experimental hatchery. Fish fed commercial fish feed at 2% rations.	0
14 April 2021	Length and mass measurements, PIT tagging of fish	27
28 April 2021	Feeding of experimental diets begins at 2% rations with 50% experimental diet and 50% commercial fish feed	41
12 May 2021	Feeding of 100% experimental diets at 2% rations begins	55
20 May 2021	Weighing of fish. Feeding at 2% rations continues	63
18 June 2021	Weighing of fish. Feeding at 2% rations continues	92
23 July 2021	Weighing of fish. Culls to reduce density. Feeding at 1.5% rations begins.	127
27 August 2021	Weighing of fish. Feeding at 1.5% rations continues	162

24 September 2021	Weighing of fish. Feeding at 1.5% rations continues	190
16 October 2021	Fish culled to maintain optimal densities. Feeding at 1% rations begins	212
5 November 2021	Weighing of fish. Feeding at 1% rations continues	232
3 December 2021	Swimming trials begin. Recovery trials and photographs occur post	260
21 December 2021	Experiment ends	278

4.2.3 Swimming performance and recovery

After 260 days (on 3 December 2021), critical swim speed was measured on 20 randomly selected fish from each combination of diet and strain (n = 80 fish; Table 4.2). Following methods described in Colborne et al.[66] and Houde, Saez, et al. (2015), a single fish was placed into a 40 L swim flume (Loligo Systems, Denmark) and acclimated for 3 minutes. Water velocity was increased incrementally at a rate of 0.15 m / s every 2 minutes until the fish displayed signs of fatigue (i.e., an inability to continue swimming at that speed). Critical swim speed (U_{crit}) was calculated as $U_{crit} = U_i + (T_i/T_{II} \times U_{ii})$, where U_i was the highest velocity maintained for a full 2 minute interval, T_i was the time of fatigue at last current velocity (minutes), T_{ii} was the interval length (2 minutes), and U_{ii} was the velocity increment (0.15 m/s; Houde, Saez, et al., 2015). U_{crit} values were then divided by fish length to get a measurement in body lengths per second ($bl \cdot s^{-1}$). After fish reached exhaustion, water velocity was reduced to 0.20 m/s and fish were allowed to recover for up to 30 minutes. During this time, once a fish had resumed swimming for 5 consecutive minutes, it was considered recovered, and the trial was ended (Lee-Jenkins et al.,

2007). If a fish did not resume swimming for 5 consecutive minutes before the 30-minute mark, the trial was ended, and it was considered to have not recovered. For the analysis of recovery post-exhaustion, the data were converted to binary data (0 = did not recover or 1 = recovered). Every third fish did not undergo the recovery trials, instead having tissues sampled during exhaustion for a separate study. The sample size for the recovery trials was thus n = 54 (Table 4.2). Upon trial completion, all fish (recovered and not recovered) were euthanized with an overdose of TMS. The fish were measured for total body length and mass. Specific growth rate for each individual fish was calculated using the mass measurements and the methods of Ricker (1975). Fish were then placed on their right side and digitally photographed using a camera (18 MP Canon EOS Rebel T5) set at a fixed height following methods described in (Muir et al. (2012). Each digital photograph contained a size and colour standard. Four fish were not photographed due to camera issues and two other photographs had corrupted colour standards and were excluded (sample size for the colour analysis was n=74).

Table 4.2 Sample sizes for each metric measured on lake trout from the Seneca Lake and Slate Islands strain of lake trout fed either the control or thiaminase experimental diet.

Strain (diet)	Critical swim speed	Probability of recovery	Morphology	Colouration
Seneca (control)	20	13	18	19
Seneca (thiaminase)	20	13	19	20
Slate (control)	20	13	19	19
Slate (thiaminase)	20	15	20	16
Total	80	54	76	74

4.2.4 Morphology and colouration

Photographs of each lake trout were examined for body morphology and skin pigmentation using methods described by Muir et al. (2012), Perreault-Payette et al. (2017), and Villafuerte & Negro (1998). Briefly, for morphology, 20 landmarks and sliding landmarks (Figure F-2 in Appendix F) related to aspects of head and body depth and caudal region lengths were digitized and analyzed with the Thin Plate Spline suite (*tps*: http://life.bio.sunsysb.edu/morp). First, for each fish, a rectangular grid was overlaid to identify body curvature corresponding to 20–30–40–50% of body length using the program REVIT (Autodesk, 2017). The body was anchored at the tip of the snout and midpoint of the hypural plate. Next, 16 homologous landmarks and four sliding landmarks were digitized with the program *tpsDig* (Rohlf, 2008). Sliding landmarks were slid in the program *tpsUtil* (Rohlf, 2015). These landmarks were then subjected to relative warp analysis using *tpsRelw* software (Rohlf, 2009) to get centroid sizes and principal relative warp scores. Data were imported into MorphoJ (Klingenber, 2011), and a principal component analysis (PCA) was performed to reduce the number of morphometric variables or scores and extract divergent morphometric patterns. PCA scores were exported into R (version 4.3.3.; R core team, 2024) for further analysis. For skin pigmentation, the average intensity of each colour channel (RGB [red, green, blue] colour space) was measured for the dorsal, lateral, ventral, caudal peduncle, and caudal fin regions using ImageJ version 1.8.0 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/). RGB colour space values were corrected for each photograph using both a light and a dark standard that was included in each photograph to account for any potential differences in lighting between photographs. RGB colour space values for skin pigmentation were then converted into LAB

colour spaces in R using the function convertcolor() in package grdevices (R core team, 2024). A PCA was performed in base R to reduce dimensionality.

4.2.5 Statistical analyses

All metrics collected of individual lake trout were analyzed in R (version 4.3.3.; R core team, 2024), using $\alpha = 0.05$ for all statistical tests. Comparisons of initial length and mass measurements were compared using a student's t-test. Linear mixed effects models fit with a restricted maximum likelihood (lmm; *lmer* in the lme4 package in R; Bates et al., 2023) were used to examine effects of diet type, strain, and their interaction on mass and length at experiment end, specific growth rate, critical swim speed, morphology, and colouration (PC scores). For critical swim speed, an additional fixed effect of fish body length was included in the analysis to account for differences in length among fish. Probability of recovery was analyzed using a generalized linear mixed model with a logit link using function glmer in package lme4 (Bates et al., 2023) and included the fixed factors of diet-type, strain, and the diet × strain interaction. In the final model, the exponentiated coefficients reflect the odds of recovering versus not recovering, and the logit^{-1}-transformed coefficients reflect the probability of recovery. All models included a random effect for tank identity. To test if the random effect explained a significant proportion of the variance, a restricted likelihood ratio test was used (function exactRLRT() in package RLRsim; Scheipl & Bolker, 2025). When the random effect of tank explained a significant proportion of the variation, variation explained was quantified using the intraclass correlation coefficient method and was represented as the percentage of the variance accounted for by the random effects.

4.3 Results

4.3.1 Clinical signs of thiamine deficiency

After 260 days of feeding of the experimental diets, symptoms associated with thiamine deficiency were evident in fish from the thiaminase treatment including lesions of the eye, ataxia, and lethargy, as well as increased mortality and increased tissue liver transketolase latency, the results of which are presented in Chapter 3 and Neff et al. (2023). Reduced muscle and tissue total thiamine concentrations and high liver transketolase latencies were also noted and presented in Figure E-3 in Appendix E and in Chapter 3.

4.3.2 Comparison of length, mass, and specific growth rate

Total body length, mass, and specific growth rate are provided in Table 4.3. The two lake trout strains differed in their body length and mass before and after the experiment. At the start of the experiment, fish from the Slate Islands strain were both significantly heavier (t = -5.44, df = 407.63, $p < 0.001$) and longer than those from the Seneca Lake strain (t = -6.26, df = 413.49, $p < 0.001$). At the end of the experiment, fish from the Seneca Lake strain were significantly heavier ($F_{1,80} = 7.83$, $p = 0.018$) and longer ($F_{1,80} = 6.48$, $p = 0.03$) than those from the Slate Islands strain. Independent of strain, fish fed the control diet were also significantly heavier ($F_{1,80} = 5.95$, $p = 0.024$) and longer ($F_{1,80} = 6.75$, $p = 0.03$) than fish fed the thiaminase diet. For both models, the diet × strain interaction term was not significant (mass: $F_{1,80} = 1.49$, $p = 0.25$; length: $F_{1,80} = 0.65$, $p = 0.48$). Furthermore, the random effect of tank did not account for any significant variation and was excluded from the final models (mass: RLRT = 1.14×10^{-13}, $p = 0.46$; length: RLRT = 0, $p = 1$).

Table 4.3 Initial and final body lengths and masses and specific growth rates (mean ± SD) of lake trout from the Seneca Lake and Slate Islands strains of lake trout fed either the control or thiaminase experimental diet.

Strain	Treatment	Experiment start		Experiment end		Specific growth rate
		Length	Mass	Length	Mass	
Seneca	Control	13.9 ± 1.5[a]	25.1 ± 8.8[a]	24.4 ± 3.3	164.6 ± 73.5	0.71 ± 0.13
	Thiaminase	13.4 ± 1.0[a]	22.1 ± 4.8[a]	22.2 ± 2.4	115.8 ± 44.6	0.62 ± 0.11
Slate	Control	14.2 ± 1.9[b]	27.3 ± 11.6[b]	22.1 ± 3.6	118.3 ± 62.8	0.55 ± 0.10
	Thiaminase	14.3 ± 1.7[b]	26.4 ± 8.8[b]	20.9 ± 2.3	97.9 ± 36.8	0.50 ± 0.13

Note. Length is expressed in cm, mass is expressed in g, and specific growth rate is expressed in $\% \cdot day^{-1}$. Superscript letters denote homogenous subsets from a t-test analysis (see text). Differences in length, mass, and specific growth rate at experiment end were examined with linear mixed models that do not permit post hoc tests. Descriptions of differences are found in text. The diets were administered for 260 days.

Specific growth rates of fish from each strain and treatment from experiment start to end are also presented in Table 4.3. Fish from the Seneca Lake strain had significantly higher growth rates ($F_{1,80} = 18.64$, $p = 0.001$) than fish from the Slate Islands strain. Independent of strain, fish fed the control diet had significantly higher growth rates ($F_{1,80} = 5.62$, $p = 0.036$) than fish fed the thiaminase diet. The diet × strain interaction term was not significant ($F_{1,80} = 0.74$, $p = 0.40$). Furthermore, the random effect of tank did not account for any significant variation and was excluded from the final models (RLRT = 0, $p = 1$).

4.3.3 Critical swim speed and probability of recovery

Fish fed the thiaminase diet had a significantly lower critical swim speed than those fed

the control diet ($F_{1,80} = 6.34$, $p = 0.027$, Figure 4.1A). This decrease in swim speed also corresponded to a lower time to reach trial end (mean ± SD; control 9.6 ± 1.5 min; thiaminase 8.3 ± 1.7 min). The fixed effect of body length was also significant in this model, showing that longer fish had faster critical swim speeds than shorter fish ($F_{1,80} = 4.00$, $p = 0.049$). Critical swim speed did not differ significantly between strains ($F_{1,80} = 2.34$, $p = 0.15$, Figure 4.1A), despite the Seneca Lake fish being longer than the Slate Islands fish (Table 4.3). The diet × strain interaction term was not significant ($F_{1,80} = 0.69$, $p = 0.42$, Figure 4.1A), indicating that the effect of diet on critical swim speed was consistent between the two strains. The random effect of tank did not explain any of the variation in the model (RLRT = 0.94, $p = 0.14$).

When corrected to body lengths per second, critical swim speed did not differ significantly between treatments ($F_{1,80} = 2.00$, $p = 0.18$; Figure 4.1B). There was a trend of faster critical swim speeds of fish from the Slate strain, but the difference was not significant ($F_{1,80} = 4.16$, $p = 0.06$, Figure 4.1B). The diet × strain interaction term was not significant ($F_{1,80} = 0.87$, $p = 0.36$, Figure 4.1B), indicating that the effect of diet on critical swim speed was consistent between the two strains. The random effect of tank did not explain any of the variation in the model (RLRT = 1.28, $p = 0.12$).

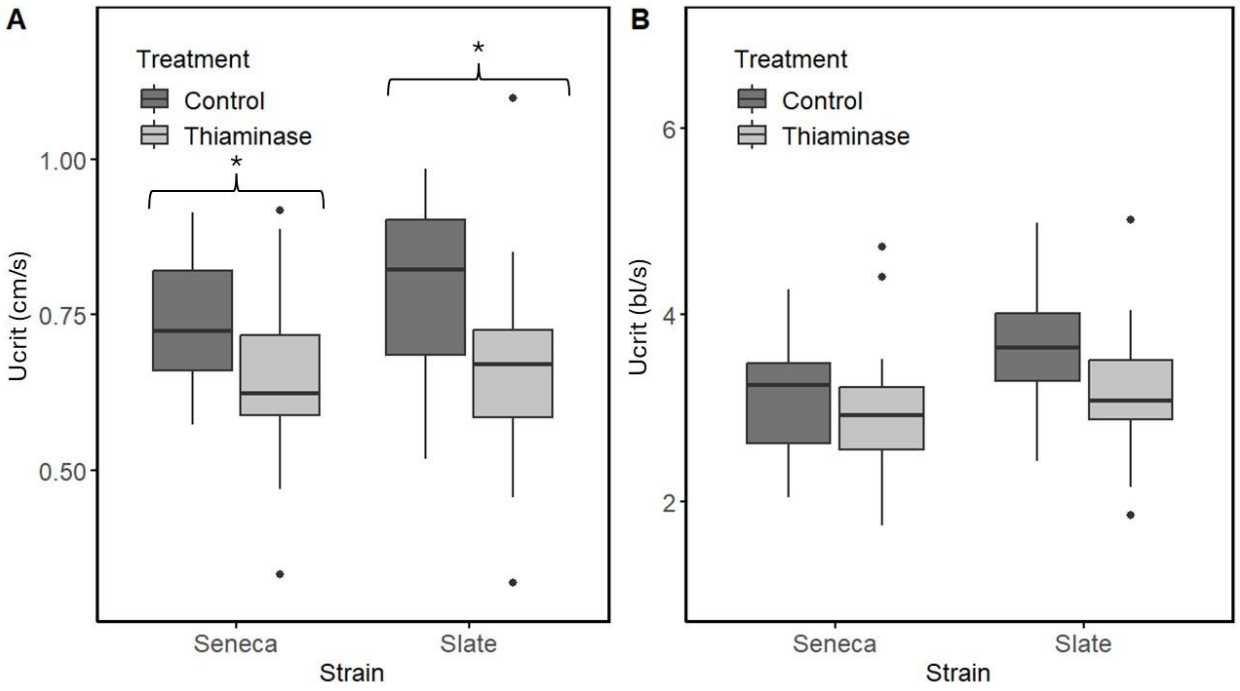


Figure 4.1 Critical swim speed (Ucrit) in A) cm/s and B) bl/s of lake trout from the Seneca Lake and Slate Islands strains fed either a control (dark grey) or high-thiaminase (light grey) diet for 6 months. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

Recovery time of the lake trout post-exhaustive exercise is included in Figure F-3 of Appendix F. A total of 9 Seneca Lake fish in the control, 5 in the thiaminase treatment, 3 Slate Island fish in the control, and 2 in the thiaminase treatment did not recover. Of the fish that did recover, the average time (± SD) across strains and treatments to recovery was 624.6 ± 206.5 (Seneca Lake control), 904.5 ± 621.3 (Seneca Lake thiaminase), 693.6 ± 406.6 (Slate Island control), and 623.8 ± 341.9 s (Slate Island thiaminase). Contrary to our expectation, fish fed the control diet tended to have a lower probability of recovery after the critical speed trials than those fed the thiaminase diet, but the difference was not statistically significant ($z = 1.55$, df = 1, 54, $p = 0.12$; Figure 4.2). The probability of recovery was significantly different between strains with fish from the Slate Islands strain having a higher probability of recovering from exhaustion

than those from the Seneca Lake strain (Figure 4.2; $z = 2.26$, df = 1, 54, $p = 0.02$). The diet × strain interaction term was not significant, indicating that the effect of diet type on critical swim speed did not differ between strains (Figure 4.2; $z = -0.47$, df = 1, 54, $p = 0.63$). The random effect of tank did not account for any of the variance in the model.

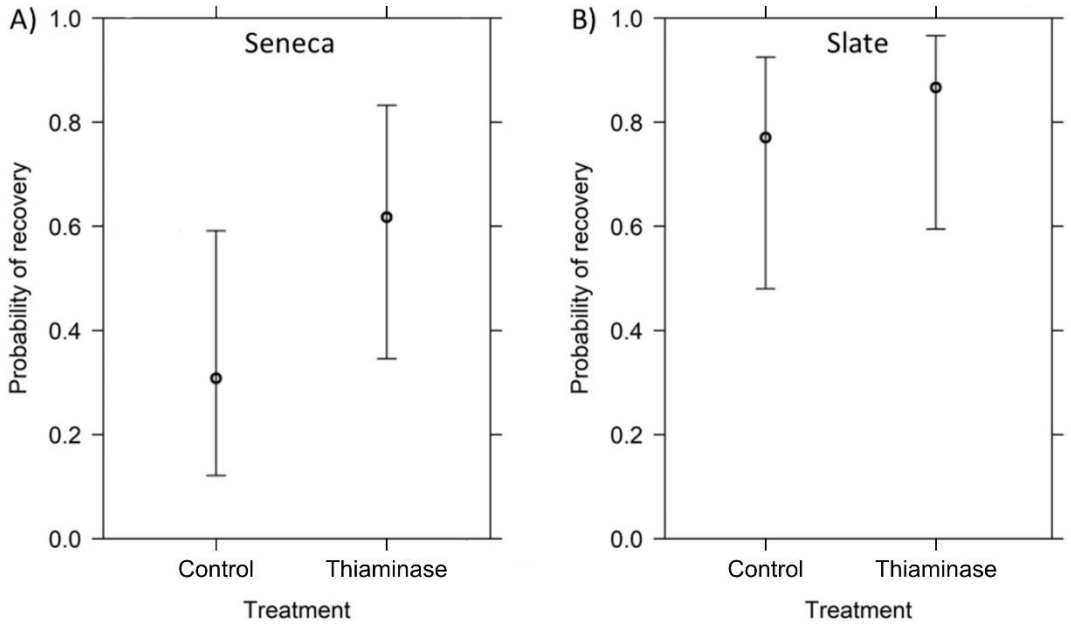


Figure 4.2 Probability of recovery after failure in the critical swim speed trials of lake trout from the (A) Seneca Lake and (B) Slate Islands strains fed either a control or thiaminase diet for 6 months. Dots denote the mean and error bars denote the 95% confidence intervals for each estimate. While there were no differences between diet groups within each strain, there was a significant difference in the probability of recovery between the Slate Islands and the Seneca Lake strains ($p < 0.05$).

4.3.4 Morphology and colouration

The morphology landmarks and skin pigmentation PCA loadings and plots are presented in Appendix F Table F-1 and figures F-4 and F-5, respectively. For morphology, we considered only principal component 1 (PC1) and principal component 2 (PC2) in further analyses. PC1 and

PC2 explained 30.8% and 15.9% of the variation, respectively, and were easily interpreted biologically. Positive PC1 scores were associated with a deeper dorsal-ventral depth and more curved body shape whereas positive PC2 scores were associated with a thinner anterior end and a longer, straighter posterior end. Fish fed the thiaminase diet had shallower dorsal-ventral depth and were less curved laterally (PC1) than those fed the control diet ($F_{1,76} = 4.91$, $p = 0.04$; Figure 4.3A). There was no effect of strain on the dorsal-ventral depth and body curvature (PC1) of the juvenile lake trout ($F_{1,76} = 3.50$, $p = 0.09$; Figure 4.3A), nor was there a significant diet × strain interaction ($F_{1,76} = 1.42$, $p = 0.26$; Figure 4.3A), indicating that differences in morphology between diet types were consistent between the two strains. The random effect of tank did not account for any of the variance in the model (RLRT = 0.03, $p = 0.38$).

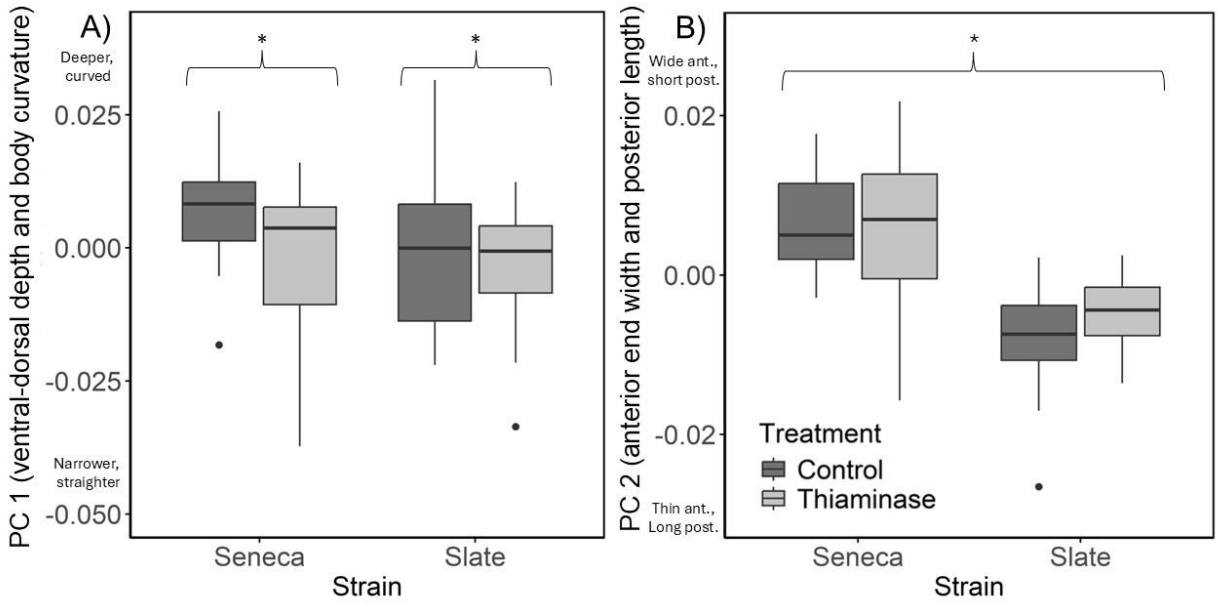


Figure 4.3 Principal components of a PCA performed on 20 morphological landmarks from lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Positive principal component 1 (PC1; A) scores were associated with a deeper dorsal-ventral depth and more curved body shape. Positive principal component 2 (PC2; B) scores were associated with a thinner anterior end and a longer posterior end. Boxplots show the median and

first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

While diet type affected body depth and curvature, it did not appear to affect anterior thickness and posterior length (PC2; $F_{1,76} = 0.14$, $p = 0.71$; Figure 4.3B). Lake trout from the Slate Islands strain had a significantly thinner anterior end and a longer posterior end than those from the Seneca Lake strain ($F_{1,76} = 31.79$, $p < 0.001$; Figure 4.3B). The diet × strain interaction term was not significant ($F_{1,76} = 1.40$, $p = 0.26$, Figure 4.3B). The random effect of tank did not explain any of the variation in the model (RLRT = 0.81, $p = 0.15$).

Examples of the colouration of lake trout in this study can be found in Figure F-6 of Appendix F. For skin pigmentation, we performed further analyses and interpretation on principal components 1 (PC1), 2 (PC2), and 3 (PC3), which explained 30.6%, 25.9%, and 16.0% of the colour variation among individuals, respectively. PC1 was negatively related to whole-body whiteness (L colour space). PC2 was associated with more yellow (positive b values) in the lateral, ventral, peduncle, and caudal regions. PC3 was associated with more green and less red (negative a values) in the caudal and peduncle body regions. We found that diet type had no influence on inferred whole-body whiteness (PC1) of lake trout ($F_{1,74} = 2.51$, $p = 0.14$; Figure 4.4A). Inferred whole-body whiteness also did not differ significantly between strains ($F_{1,74} = 2.14$, $p = 0.17$; Figure 4.4A), and there was no significant diet × strain interaction ($F_{1,74} = 0.56$, $p = 0.46$; Figure 4.4A). The random effect of tank accounted for 32 % of the variance in the model (RLRT = 7.75, $p = 0.003$).

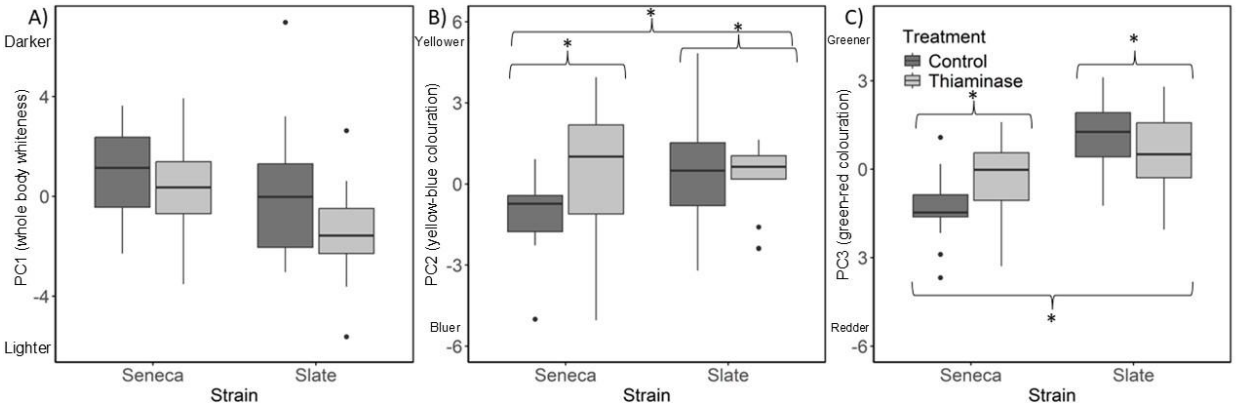


Figure 4.4 Principal components of the principal component analysis of LAB colour values from 5 body regions on lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Positive principal component 1 (PC1; A) scores were associated with darker whole body colour space values, positive principal component 2 (PC2; B) scores were associated with more yellow whole body colour spaces values, and positive principal component (PC3; C) scores were associated with a greener peduncle and fin colouration. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

Raw B (blue pigmentation) colour space values are included in Figure F-7 of Appendix F. Fish fed the thiaminase diet had more yellow colouration in the lateral, ventral, peduncle, and caudal regions (inferred from PC2) than those fed the control diet ($F_{1,74} = 5.01$, $p = 0.048$; Figure 4.4B). Inferred yellow colouration also differed significantly between strains ($F_{1,74} = 6.43$, $p = 0.03$; Figure 4.4B); lake trout from the Slate Islands strain had more yellow colouration than those from the Seneca Lake strain. The interaction term was not statistically significant, indicating that effects of diet-type on yellow colouration were consistent between the two strains ($F_{1,74} = 3.57$, p

= 0.08, Figure 4.4B). the random effect of tank did not explain any of the variation in the model (RLRT = 1.13×10^{-13}, $p = 0.45$).

Raw R (red) and a (green-red) colour space values are included in Figure F-7 of Appendix F. There was a significant effect of diet type on the red colouration of lake trout (inferred from PC3; $F_{1,74} = 5.72$, $p = 0.03$, Figure 4.4C). Anecdotally, individuals from the Seneca Lake strain in the control diet treatment appeared visually redder in colouration than those from the Slate Islands strain, especially in the caudal and fin areas (Appendix D Figure D-7). The diet × strain interaction term was significant, indicating that effect of diet type on red colouration did differ significantly between the two strains ($F_{1,74} = 6.92$, $p = 0.02$, Figure 4.4C). Lake trout from the Slate Islands strain were greener in colour than those from the Seneca Lake strain ($F_{1,74} = 33.31$, $p < 0.001$; Figure 4.4C). The random effect of tank did not explain any of the variation in the model (RLRT = 0, $p = 1$).

4.4 Discussion

A diet containing thiaminase has been reported to cause decreases in the swimming performance of salmonines (Houde, Saez, et al., 2015; Morito et al., 1986) and other fishes (e.g., eels; Balk et al., 2016). We similarly found a decline in critical swim speed, a measurement of swimming performance, in both strains of juvenile lake trout fed the thiaminase diet. These results are consistent with those of Houde, Saez, et al. (2015) who found a decrease in critical swim speed in Atlantic salmon fed a thiaminase-containing diet. Our results are also consistent with those of Morito et al. (1986), who reported a decrease in swimming performance in thiamine-deficient rainbow trout. A thiaminase-induced decline in swimming performance was previously thought to reflect either a decrease in ATP production, as thiamine enables pyruvate to enter the citric acid cycle to produce ATP (Koski et al., 2005; Morito et al., 1986), or an increase in plasma lactate that

affects muscular performance, which has been observed in juvenile rainbow trout (Fitzsimons et al., 2005; Morito et al., 1986). Recently, Baker et al. (2023) found a change in heart morphology and a decrease in cardiac function in lake trout fed the same thiaminase diet used in our study. A decrease in cardiac performance in fish fed the thiaminase diet may thus also contribute to the decline in swimming performance that we observed, as cardiac output has been directly linked to swimming performance in salmonines (Claireaux et al., 2005). Instead, a decrease in swimming performance may also be the result of a decrease in size resulting from decreased growth rates, as we found when swimming performance was corrected for size. Lake trout employ vertical and horizontal cruising to locate prey throughout the water column (Vogel & Beauchamp, 1999) and in large lakes, will make long distance movements from spawning locations to foraging ranges (Ivanova et al., 2021). Furthermore, juvenile lake trout rely on physical separation (e.g., vertical migrations) that requires swimming performance to avoid predators (Gallagher et al., 2019). Thus, a reduction in swimming performance of lake trout feeding on high-thiaminase prey fishes could reduce their ability to capture prey and avoid predation, ultimately reducing their survival in the wild.

Exhaustive exercise leads to an accumulation of lactic acid during exercise, and concomitant increases in hydrogen ions and acidosis in muscles are considered a major cause of fatigue (reviewed by Wood, 1991). Previous studies have reported a decrease in lactic and pyruvate following thiamine supplementation (Lee et al., 2023) and deficiencies in thiamine are associated with elevated lactic acid levels (Agedal et al., 2023; van der Beek et al., 1988). Thiamine supplementation has been associated with decreased recovery time and decreased muscular fatigue in mammals (Suzuki & Itokawa, 1996). Contrary to our prediction, however, a decrease in swimming performance was not associated with a decrease in the probability of

recovery, and we found no difference in the probability of recovery after exhaustive exercise between fish fed our two diets. This observation could be explained by the inability of fish fed the thiaminase diet to maintain high swim speeds and thus may be an artefact of the critical swim speed protocol. Specifically, the thiamine-deficient fish had lower critical swim speeds and remained in the critical swim speed trial for an average of 1.3 minutes less than their control counterparts so may not have exerted themselves as much as their thiamine replete counterparts. Indeed, thiamine deficiency has been associated with a decrease in Ca- and Mg-activated ATPase, as well as a decrease in neuromuscular transmission, with resulting decreased muscle contraction activity (Manzetti et al., 2014; Xu et al., 2022). A similar mechanism could have prevented the thiamine-deficient fish from reaching aerobic failure and allowed them to have similar probabilities of recovery as fish fed the control diet.

Thiamine deficiency has previously been shown to cause changes in body appearance in salmonines (Houde, Saez, et al., 2015; Morito et al., 1986). We found that juvenile lake trout had a trend towards increased whole-body lightening and increased yellow body pigmentation when fed a diet containing thiaminase. We also found a strain-specific change in red pigmentation; fish from the Seneca Lake strain had more green pigmentation whereas those from the Slate Islands strain had more red pigmentation when fed a diet high in thiaminase. Our results are consistent with Houde, Saez, et al., (2015) who reported a trend of increased whole body lightening of Atlantic salmon fed a high thiaminase diet. Body depigmentation (lightening) can occur in thiamine-deficient fish (Trzeciak, 2009) because thiamine plays a role in melanogenesis (Takenouchi et al., 1960). Specifically, it modulates the tyrosine-tyrosinase reaction (Takenouchi et al., 1960) and also has an indirect role by providing NADPH and ATP to melanocytes (Snyman et al., 2024).

Our results contrast those of Houde, Saez, et al. (2015), who found a decrease in yellow pigmentation. There, the authors attributed the decrease in yellow pigmentation to the amount of the carotenoid idoxanthin (Hatlen et al., 1998), a metabolite of astaxanthin, an antioxidant whose concentrations may decline under thiamine deficiency due to oxidative stress (Lukienko et al., 2000; Pettersson & Lignell, 1999). The increase in yellow pigmentation found here may instead be the result of jaundice, which has been previously observed in fish (Endo et al., 1992; Sakai et al., 1998). Jaundice is a symptom of hepatic dysfunction which is associated with thiamine deficiency mediated lactic acidosis (Abbas et al., 2016; De Backer, 2003). Further evidence to support this mechanism comes from the combined observation of decreased swim performance, as lactate acidosis is both a mechanism behind the reduced capacity for activity (Wood, 1991) and a symptom of hepatic dysfunction (De Backer, 2003). Additionally, an increase in yellow pigmentation may suggest a decrease in cardiac function (see Baker et al., 2023), which can decrease blood flow to the liver, ultimately reducing bilirubin clearance and causing jaundice (Shah & Sass, 2015). Decreased heart function may also explain the decrease in red pigmentation in the Seneca Lake fish fed a diet high in thiaminase as decreased blood flow to the periphery would decrease the reddening of the skin after exhaustive exercise. Indeed, the greatest difference in red colouration in Seneca strain fish was found in the fin. Vasoconstriction and reduced peripheral blood flow has been found in thiamine deficient mammals (Gioda et al., 2014; Zhao et al., 2025), although more research is needed in fish.

A diet that contains bacterially derived thiaminase may alter the morphology of lake trout. Lake trout had a shallower ventral-dorsal depth and were less curved laterally when fed the thiaminase diet, and this was observed in both strains. These results differ from those of Houde, Saez, et al., (2015) who found a general trend of a less streamlined body shape (lateral

compression, deeper ventral-dorsal depth) in Atlantic salmon fed a diet high in thiaminase. A less streamlined body shape is thought to be related to reduced swimming activity (Taylor & and McPhail, 1985) whereas an increase in a streamlined body shape and decreased ventral-dorsal depth may be related to a drop in condition because of a lower growth rate. Indeed, we found that fish from both the Seneca Lake and Slate Island strains that were fed the thiaminase diet had lower growth rates than those fed the control diet. In brown trout (*Salmo trutta* [Linnaeus, 1758]) and cisco (*Coregonus artedi* [Lesueur, 1818]), decreased body depth was associated with decreased survival as a result of increase susceptibility to large-gape predators (Hyvärinen & Vehanen, 2004; Jacobson et al., 2020). Thus, in lake trout, the decrease in ventral-dorsal depth may also reduce survival in the wild.

Finally, our results have implications for the restoration of lake trout, particularly in Lake Ontario. Strain-targeted stocking programs present a possible solution to reduce the incidence of thiaminase-related health effects of lake trout in the wild. We found that fish from the Seneca Lake strain fed the high-thiaminase diet had more yellow and less red pigmentation, potentially reflecting thiamine deficiency-induced cardiac impairment, compared to those from the Slate Islands strain. Fish from the Slate Islands strain may perform better in the Lake Ontario environment if the changes in colouration are indicative of cardiac capacity, but further research is needed to confirm this. Furthermore, Slate Islands fish had a higher probability of recovery post-exhaustive exercise and a trend towards faster swimming speeds (at a given size), which could provide an advantage in Lake Ontario, where feeding ranges are known to reach up to 4000 km^2 (Ivanova et al., 2021).

4.5 Conclusion

The consumption of thiaminase is thought to affect the swimming performance and morphology of fishes, traits important for fitness in the wild. Here, we confirmed the negative effects of diet-derived thiaminase on critical swim speed, morphology through decreased ventral-dorsal depth, and colouration, and — except for colouration — these effects did not differ between the two strains. Fish from the Seneca Lake strain had decreased red pigmentation when fed the thiaminase diet than when fed the control; no difference was found between diets fed to the Slate Island strain. Taken altogether, these findings refute our prediction of an adaptive response to thiaminase through historical exposure to high thiaminase prey and suggest that the Slate Islands strain may be better able to mitigate the effects of diet-derived thiaminase and help increase the success of lake trout restoration into Lake Ontario.

Chapter 5

First record of natural reproduction by lake trout (*Salvelinus namaycush*) in an acid-damaged lake: lessons for lake trout reintroduction programs

This chapter is an adapted version of the following paper, in preparation for submission to the Canadian Journal of Fisheries and Aquatic Science:

Therrien CA, Wilson CC, Louste-Fillion J, Neff BD, Swanson HK. 2026. First record of natural reproduction by lake trout (*Salvelinus namaycush*) in an acid-damaged lake: lessons for lake trout reintroduction programs.

5.1 Introduction

Historically, lake trout (*Salvelinus namaycush*) were widespread throughout Northeastern Ontario (Canada), including in the Sudbury Basin where they are important in recreational, commercial, and indigenous subsistence harvesting (Riley et al., 2021). Losses of lake trout populations throughout the Sudbury Basin began to be documented in the 1940s and 50s. By the 1960s, an estimated 94 lakes throughout the Sudbury Basin were experiencing significant declines in, or a disappearance of, their lake trout fishery (Matuszek et al., 1992). These losses were linked to a single point source of acid deposition – the metal smelters in Sudbury, Ontario (Matuszek et al., 1992). Known as the nickel capital of the world, Sudbury's smelters represented one of the largest point sources of SO_2 pollution in the world (Potvin & Negusanti, 1995). Beginning in 1888, SO_2 emissions increased annually and

peaked in 1960, when annual emissions reached 2.5 million tonnes (Potvin & Negusanti, 1995). Lakes in the Sudbury Basin were severely affected by the atmospheric deposition of SO_2. Over 7000 lakes were acidified to a pH below 6.0, the point at which significant biological damage occurs (Neary et al., 1990). Early netting assessments in these acidified lakes found a loss of lake trout recruitment (e.g., death in the early developing stages of eggs, embryos and young juveniles; Beggs and Gunn, 1987; Gunn, 1989; Matuszek et al., 1992) with water pH from 5.3-5.6. In lakes where the pH dropped below 5.2, entire populations were typically extirpated (Beamish, 1976; Beggs & Gunn, 1987).

Since their peak in the 1960s, atmospheric SO_2 emissions from Sudbury's smelters and other sources have been greatly reduced in response to government regulations and improved technology. This change has facilitated chemical recovery in many lakes, which began in the late 1970s (Gunn et al., 1988; Keller et al., 1986; Keller et al., 2019a; Meyer-Jacob et al., 2020). Stocking programs for lake trout then began in the 1980s (Selinger et al., 2006). Despite over 500,000 lake trout being stocked into lakes in the Sudbury Basin, self-sustaining populations have only been established in about 30 % of target lakes (Evans & Olver, 1995; Louste-Fillion, 2022; Selinger et al., 2006). Biological recovery of acid-damaged lakes has been slower than chemical recovery, and often the ecosystem in the lakes has changed considerably from the conditions before acidification. For example, the invasion of warm-water competitors and predators such as smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*), and proliferation of native prey fish such as cisco (*Coregonus artedi*) have been attributed to the lack of success of lake trout reintroduction attempts (reviewed by Gunn and Louste-Fillion, 2022).

Tyson Lake is one lake where recovery of native lake trout has been slow. A large (1143.1 ha) oligotrophic lake, Tyson Lake historically had been a popular spot for cottagers and campers and has supported a recreational fishery for lake trout and northern pike (*Esox*

lucius; Ontario Ministry of Natural Resources (OMNR) unpublished report). It is situated in the La Cloche mountain range and is surrounded by quartzite and granite bedrock, common among lakes in the mountain range. The granite bedrock exacerbated the effects of SO_2 deposition by preventing percolation into the surrounding soil and instead concentrating it in the lakes (Beamish, 1976; see review in Keller et al., 2019). As a result, by 1971 the pH of Tyson Lake reached a low of 5.14 (see Table G-1 in Appendix G), resulting in the loss of the native populations of lake trout, burbot (*Lota lota*), lake whitefish (*C. clupeaformis*), sculpin (*Cottus* spp.), darters (*Etheostoma* spp.), and cyprinids (family Cyprinidae; OMNR unpublished data). To prevent a total loss of the lake trout fishery in Tyson Lake, stocking of lake trout occurred in the 1950s and 1980s, but these efforts failed to produce a self-sustaining population (Selinger et al., 2006).

Chemical recovery of Tyson Lake continued into the 2000s and became part of the Northeastern Ontario lake trout recovery program, with regular plantings of 3500−4000 yearling (10−19 months) or sub-adult (>20 months) lake trout occurring annually in the hopes of establishing a self-sustaining population (Selinger et al., 2006; OMNR unpublished results). The Killala Lake (49.08°, -86.52°) hatchery strain of lake trout has primarily been used in the plantings and has been the primary strain used for lake trout rehabilitation in inland Ontario lakes. Single plantings of surplus adult broodstock lake trout from each of the Slate Island (48.67°, -87.02°) and the Michipicoten Island (47.71°, -85.80°) strains occurred in 2003 and 2004. These two strains both originate from populations in Lake Superior and have primarily been used in rehabilitation projects of lake trout in the Laurentian Great Lakes. Their use in inland lakes has been limited and primarily included stocking as surplus in lakes with put-grow-take programs for recreational opportunities. These reintroduction efforts have been successful in creating a recreational lake trout fishery in Tyson Lake.

The pH of Tyson Lake is now 6.56, some acid-sensitive species (i.e. *Hexaginea* spp., *Faxonius propinquus,* family Hirunidae) have recovered, and acidity no longer appears to be a limiting factor for lake trout reintroduction (Louste-Fillion, 2022). Despite this, while a hatchery-supported recreational fishery has been established in Tyson Lake, natural reproduction has not been recorded. Failed natural reproduction was suspected because no unclipped fish (OMNR clips fins on a 5-year rotating cycle prior to release) had been captured during netting surveys or reported by anglers. In 2019, a large, unclipped adult lake trout (body length = 685 mm, body mass = 2600 g) was collected during a broadscale monitoring survey (BsM) carried out by the OMNR and the Cooperative Freshwater Ecology Unit (CFEU). Because genetic determination of the origin of this fish did not occur, it could not be confirmed that it was of wild origin. Instead, the lack of unclipped fins could have been due to improper fin clipping, fin regeneration, or misidentification of clips. In summer 2021, we collected another unclipped adult lake trout in an angling survey. Here, we used fish ageing and genetic strain assignment to confirm the wild origin of this fish and describe the first documented occurrence of natural reproduction of lake trout in Tyson Lake since their extirpation in the late 1970s. We discuss implications for future lake trout reintroduction programs.

5.2 Methods

5.2.1 Fish collection

Lake trout were collected from Tyson Lake (Figure 5.1) during four surveys from 2019–2023 that employed three separate protocols. First, a broadscale monitoring survey (BsM) was carried out from 8–12 August 2019. Briefly, monofilament gill nets with mesh sizes that ranged from 19 mm to 127 mm were set horizontally in each of the lake depth strata (i.e. 1–3 m, 3–6 m, 6–12 m; OMNRF 2019). Each lake stratum was represented by at least 2 net replicates

(spatial and temporal; range = 2–7, median = 6). Nets were set overnight, for between 16-22 hours, when surface temperatures were above 18°C. Further description of the BsM protocol can be found in Sandstrom et al., (2018). Next, angling surveys were carried out on 8 June and 19 August 2021. Trolling spoons and crankbaits were trolled behind the boat at 3.05–4.35 km/h and at different depths using downriggers and cannonballs, dipsy divers (Luhr-Jensen, Oshawa, ON), and steel line. Main lake points, shoals, and bathymetric contour lines at depths of 10.5–24.5 m were targeted during angling surveys. Lastly, qualitative surveys were carried out from 20–30 September 2022 and 2–5 October 2023 by the OMNR Aquatic Research and Monitoring Section (ARMS). Qualitative surveys followed a protocol similar to the BsM surveys but with nets set in deeper strata to increase lake trout catches. Fork length, total length, and mass were measured for all captured lake trout in all surveys. Sagittal otoliths were removed for age determination. The membranous sac surrounding the otolith was removed before drying and subsequent processing. A sample of skinless, boneless white axial muscle, whole livers, and whole brains were excised from each fish and stored on ice in the field until they could be transferred to a −80 °C freezer. No tissues for genetic strain assessment were collected from the 2019 BsM survey.

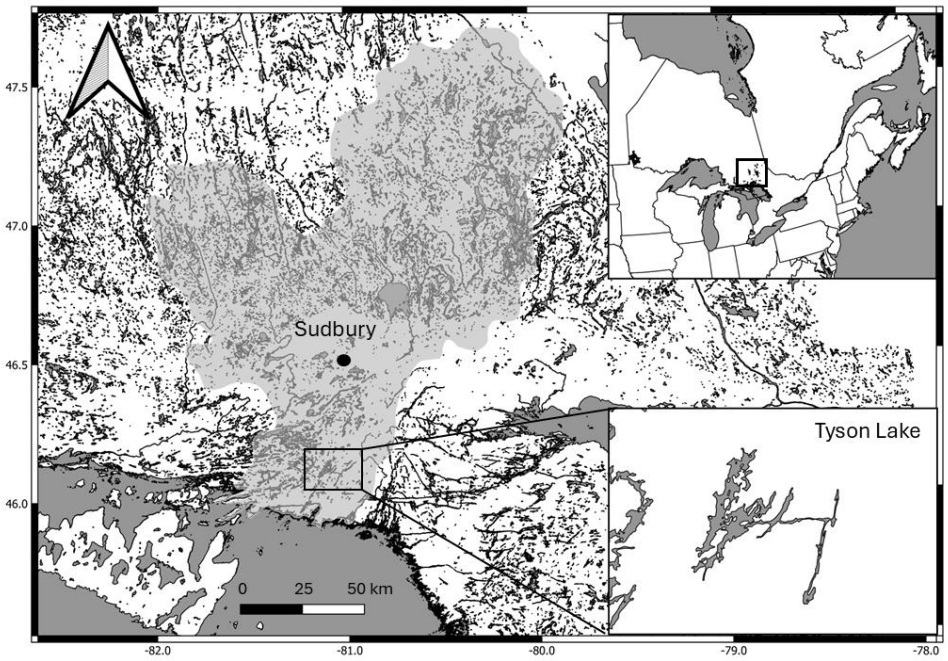


Figure 5.1 Map of Tyson Lake within the Sudbury Basin in Ontario, Canada. Grey shaded area represents the acid deposition zone from nickel mining and smelting operations in the Sudbury Basin (Neary et al., 1990). Map made with QGIS version 3.36.2 with CANVEC 50K ON waterbodies shapefile and EPSG:2163 projection.

5.2.2 Strain assignment

The complete history of lake trout plantings in Tyson Lake was obtained from the Ontario Fish Stocking Information System (FSIS) and from paper records in the OMNR Sudbury district office (see Table 5.1). To assign fish to strain, all captured lake trout that had a clipped fin were presumed to be from the Killala strain as all plantings of lake trout into Tyson Lake of clipped fish were of the Killala strain. The error rate of fin clipping (missed or wrong clip) is 1.47 ± 0.11 % (mean ± SD; Jason Intini unpublished data).

Table 5.1 Summary of the history of lake trout (*Salvelinus namaycush*) stocking in Tyson Lake. All data were retrieved from historical records in the Sudbury District office of the OMNR or from the Ontario Fish Stocking Information System (FSIS).

Year	Life stage	Spawn year	Clip	Strain	Number (Percentage of total since 1990)
1956	-	-	-	-	2000
1958	-	-	-	-	2500
1981	-	-	-	-	3500
1983	-	-	-	-	3000
1984	-	-	RP	-	13700
1985	-	-	-	-	13800
1986	-	-	-	-	7500
1987	-	-	-	-	8000
1988	-	-	-	-	8000
1989	-	-	-	-	3000

2002	Sub-adult	1999	LP	Killala Lake	4100
2003	Adult	1995	None	Slate Island	209
2003	Adult	1996	None	Slate Island	379
2004	Adult	1992	None	Michipicoten Island	187
2004	Yearling	2001	Adipose	Killala Lake	3350
2005	Sub-adult	2002	LV	Killala Lake	3100
2008	Sub-adult	2005	RV	Killala Lake	4500
2012	Sub-adult	2009	RP	Killala Lake	3500
2014	Sub-adult	2011	Adipose	Killala Lake	3000
2015	Yearling	2013	LP	Killala Lake	4000
2017	Sub-adult	2014	RP	Killala Lake	4000
2019	Sub-adult	2016	Adipose	Killala Lake	4000
2021	Sub-adult	2018	LP	Killala Lake	3700
2022	Yearling	2020	LV	Killala Lake	3500

Total		Overall	105525
		Since 1990	41525
		Killala Lake	40750 (98.13 %)
		Michipicoten Island	187 (0.45 %)
		Slate Island	588 (1.41 %)

Note. Dash indicates missing data. Life stage, spawn year, clip, and strain were not recorded for most plantings prior to 1990. Yearling indicates fish stocked from 10-19 months, sub-adult indicates immature fish stocked ≥ 20 months, and adult indicates fully mature fish that were typically stocked at the end of their production cycle (> 7 years). Acronyms for fin clippings are: LP, left pectoral; RP, right pectoral; RV, right ventral; and, LV, left ventral.

A subsample of muscle, liver, and brain tissue collected from captured unclipped fish (n = 1) and a subsample of clipped fish (n = 6) were sent on dry ice from the University of Western Ontario (London, Ontario) to the OMNR DNA lab at Trent University (Peterborough, Ontario) for genetic determination of strain. Genomic DNA was extracted from lake trout tissue samples using a crude lysis extraction method (see Wilson et al. 2007). Muscle tissues samples were originally used, but poor initial extractions led to either liver or brain tissue samples being used (Table 5.2). DNA samples were amplified at 17 DNA microsatellite loci (Msu05; Msu02; Sfo1; Msu13; Ogo1a; Msu10; Msu09; Sco215; Ssa85; Msu03; Msu06; Msu08; SfoC88; Msu01; Sco19; Sfo12; Msu11). The heat cycle parameters were amplification at 95°C for 3 min, and then 35 cycles at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 60 s. The extension time on the final cycle was 5 min. Amplified products were electrophoresed using an AB 3730 DNA Sequencer along with LIZ 500 size standards (Applied Biosystems). Genotypes at each locus were scored using GenoTyper 4.0 (Applied Biosystems) and confirmed by manual proofreading. Individual lake trout were assigned to different strains using Structure 2.1 (J. K. Pritchard et al., 2000), based on 410, 556, 83, 574, 33, 635, and 431 reference samples of brood-stock individuals from the Big Sound, Iroquois Bay, Killala Lake, Lake Manitou, Michipicoten Island, Mishibishu Lake, Seneca Lake, and Slate Islands strains, respectively, using the Bayesian assignment algorithm from Rannala & Mountain, (1997).

Table 5.2 Summary of lake trout captured in Tyson Lake from the 2019-2023 broadscale monitoring (BsM), qualitative, and angling surveys.

Survey	Number captured	Number clipped	Total length (mm; mean, min, max)	Mass (g; mean, min, max)	Age (mean, min, max)
2019 BsM	14	13[a]	537, 260, 688	2034, 288, 4320	9, 2, 17
2021 Angling	3	2	577, 314, 891	2956, 252, 7441	8, 4, 14
2022 Qualitative	8	8	438, 250, 687	1182, 106, 3150	6, 3, 10
2023 Qualitative	4	4[b]	370, 311, 467	586, 212, 1206	5, 4, 6

[a] Clipping information was not recorded on for two individual lake trout, but survey briefing notes indicated that only one fish was unclipped.

[b] Clipping information was not recorded for the lake trout in this survey but technicians that conducted the survey confirmed they were all clipped (Natalie Lafleur, OMNR, pers. comm.).

5.2.3 Otolith processing, age interpretation, and assessment of accuracy

Otoliths were sectioned transversely and polished using methods by Casselman (1983) with some modifications. Briefly, otoliths were viewed under a dissecting microscope to determine if they were vateritic. If both sagittal otoliths were in good condition, the right otolith was preferentially aged. Out of 29 fish from all four surveys, only two fish from the 2019 BsM survey had two vateritic otoliths; these fish could not be aged. Otoliths were embedded in a resin form (50 mm × 10 mm × 3 mm) with epoxy (2:1 ratio; Cold cure, System Three Resins, Lacey, WA). Embedded otoliths cured for 72 hours at room temperature until the epoxy had fully hardened. A 0.35 mm-thick transverse section of the embedded otolith was cut through the nucleus (e.g., origin of growth) perpendicular to the sulcus, using a Buehler IsoMet low-speed saw (Buehler, IL) and a pair of Buehler IsoMet diamond wafering blades (15HC; Buehler, IL). Sections were mounted on glass microscope slides using a clear adhesive (Gorilla Glue Clear, Gorilla Glue, Cincinnati, OH) prior to successively polishing with silicon carbide abrasive discs (1000-8000 grit).

Age of lake trout was interpreted by two different readers for fish captured during the qualitative and angling surveys, and by a single reader for fish captured during the 2019 BsM survey. All readers had experience interpreting ages of lake trout from otoliths prior to this study. Age interpretations were made directly by viewing otolith sections under a Leica M80 compound microscope (Leica, Germany) at 20-30x magnification with transmitted light. Annuli were counted along the dorsal lobe or the section of the dorsal lobe adjacent to the sulcus (Figure 5.2). Annuli were identified as translucent bands (bright with transmitted light) immediately following opaque regions (dark with transmitted light) as read from the nucleus to the margin. Photographs of otoliths for digital reference were taken with a IC80 HD camera mounted to the microscope

with the program LAS X (Leica, 2023) using transmitted light at 25x magnification. Because all lake trout used in this study were captured during the summer, the outside edge of otoliths was not interpreted as annuli.



Figure 5.2 Otolith used for age interpretation of a large, unclipped lake trout (fish LAK-TYS-003) collected on 21 August 2021 during an angling survey of Tyson Lake. Numbers denote interpreted age at each annulus.

For lake trout with a clipped fin, interpreted ages were compared to the stocking schedule (Table 5.1) to determine their true age. Clipping information was not collected for five fish and another two fish had unclipped fins; true age could thus not be determined for a total of seven fish. The accuracy of age interpretations was assessed between the interpreted ages and the true ages following methods in Osborne et al. (2022). Briefly, an age-difference plot of the interpreted and true ages was generated and compared for all otoliths where a true age could be identified

using the ageBias() function. Within the ageBias() function in the package FSA (Ogle, 2023) in R (R core team, 2024), one-sample *t*-tests, adjusted using the Holm method for multiple comparisons (Holm, 1979) were performed to test if the mean difference between each age interpretation and corresponding true ages were significantly different than 0 for each age-class (i.e. group of fish with same true age; Ogle, 2023). A Bowker's unpooled test of symmetry was also performed in package FSA to test if age interpretations were systematically biased relative to true age (Bowker, 1948).

5.3 Results

A total of 29 lake trout were captured during the four surveys (Table 5.2). Of the 29 lake trout captured, 27 had been clipped and were assigned to the Killala strain. In the 2019 BsM survey, an unclipped fish (total length = 685 mm, mass = 2600 g) with an assessed age of 15 was captured. Unfortunately, a tissue sample was not collected from this fish, so genetic strain assignment could not be performed. A second unclipped lake trout (LAK-TYS-003; total length = 891 mm, mass = 7441 g) with an assessed age of 14 (Figure 5.2) was captured during the 2021 angling surveys.

Table 5.3 Strain assignments of lake trout captured in Tyson Lake from 2021−2023. Individual lake trout were assigned to different strains using Structure 2.1 (Pritchard et al., 2000), based on reference samples of at least 100 brood-stock individuals from each strain using the Bayesian assignment algorithm from Rannala and Mountain (1997).

Fish	Fin clip	Tissue	Most likely match	Score (%)	Second most likely match	Score (%)
LAK-TYS-003	No	Brain	Lake Manitou	70.9	Iroquois Bay	29.1
22 Tyson 1	Yes	Liver	Killala Lake	100	-	-
22 Tyson 3	Yes	Liver	Killala Lake	100	-	-
22 Tyson 4	Yes	Liver	Killala Lake	100	-	-
22 Tyson 6	Yes	Liver	Killala Lake	100	-	-
22 Tyson 14	Yes	Muscle	Killala Lake	100	-	-
22 Tyson 15	Yes	Muscle	Killala Lake	100	-	-

Strain assignments of all lake trout can be found in Table 5.3. All clipped fish assigned to the Killala strain with 100% probability, matching the stocking records. The unclipped lake trout of interest, LAK-TYS-003 was assigned to the Lake Manitou strain with a 70.9% probability, and the Iroquois Bay strain with a 29.1% probability. The high probability of assignment to the Lake Manitou and Iroquois Bay strains – strains that do not appear to have been stocked in the lake according to record (Table 5.1) – suggest that this fish is likely the descendant of fish from stocking prior to 2002, or a descendant of Tyson Lake's original lake trout population.

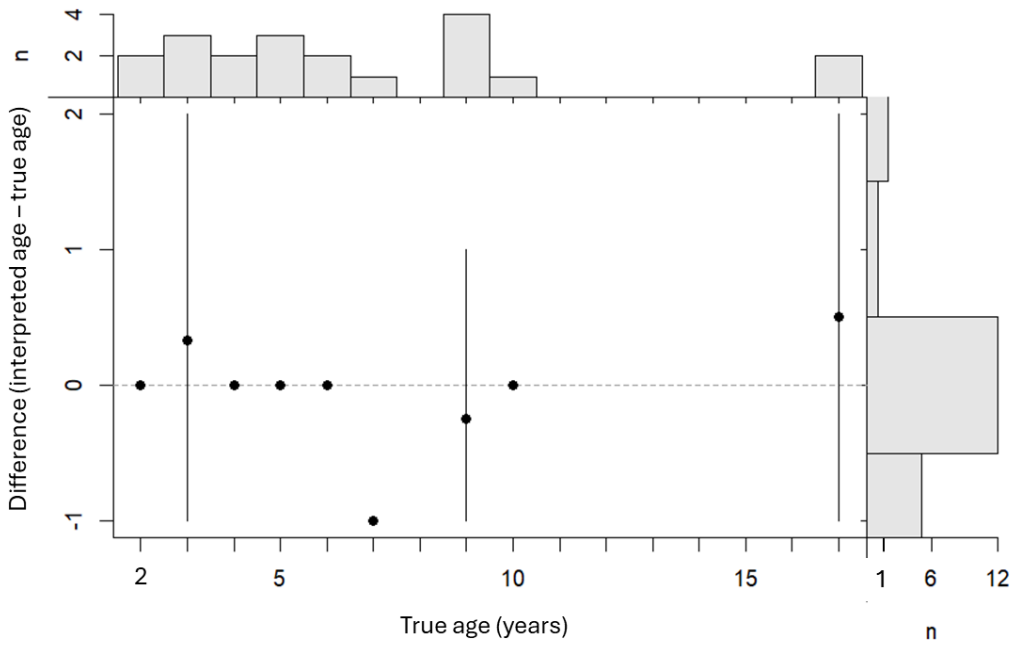


Figure 5.3 Age-difference plots comparing the difference in age interpretations between interpreted age and the true age for captured lake trout from Tyson Lake (Ontario, Canada). Black points represent mean difference values and bars represent the range of differences between true and interpreted ages. The vertical histogram (right side of the plot) depicts the frequency at which a given level of agreement occurred between age interpretations for the same otolith. The horizontal histogram (top of the plot) depicts the sample size of fish for each true age.

Age interpretations matched the true ages for 65 % (n = 12/20) of aged fish and accuracy was within +/- 1 year for 90 % (n= 18/20) of aged fish (Figure 5.3). Accuracy was within +/- 2 years for all aged fish. Age difference plots revealed that underestimates of age were the most likely error (Figure 5.3). Results of one sample t-tests did not reveal significant differences between age interpretations for any age class and any interpretations (Table 5.4). Further, Bowker's tests did not reveal systematic bias in the interpreted age (n = 20, x^2_7= 8.00, P = 0.33).

Table 5.4 Age differences between the interpreted and true ages for each year class of lake trout from Tyson Lake, Ontario, Canada. Age differences and one-sample t-tests were calculated using the ageBias() function (Ogle, 2023) in R (R core team, 2024).

True age	n	Mean ± SE age interpretation (min, max)	*t* value[c]	*p* value[c]
2	2	2[a] (2, 2)		
3	3	3.33 ± 0.88 (2, 5)	0.88	0.37
4	2	4[a] (4, 4)		
5	3	5 (5, 5)		
6	2	6[a] (6, 6)		
7	1	6[b]		
9	4	8.75 (8, 10)	0.48	1.00
10	1	10[b]		
17	2	17.50[a] (16, 19)		

[a] No SE due to n=2

[b] No SE, min, or max due to n=1

[c] *t* and *p* could only be calculated for age classes with $n \geq 3$ or with variation in interpreted age

Fish from the last stocking of unclipped Slate Island and Michipicoten Island fish in 2003 would have been age 24 at the time of capture of LAK-TYS-003 in 2021. Because all fish were within 2 years of the true age, the maximum age this fish could be is 16, several years younger

than 24. Paired with the genetic assignment, we are inferring that this unclipped fish reflects natural reproduction of lake trout in Tyson Lake.

5.4 Discussion

Lake trout have been stocked in Tyson Lake since the 1950s, and a revised rehabilitation program was initiated in 2000. The program was launched to update available information regarding the status of acid-damaged lakes, to evaluate the overall health and sustainability of surviving lake trout populations, and to stock lake trout in 33 lakes in Northeastern Ontario where native populations were lost to acidification (Selinger et al., 2006). Here, we describe the first confirmed occurrence of natural reproduction of lake trout in Tyson Lake since their extirpation in the late 1970s. Genetic analysis of a tissue sample from the unclipped fish indicated assignment to Manitou Lake and Iroquois Bay strains with high confidence; these strains were never stocked in Tyson Lake. Furthermore, age of this fish was assessed as 14 years, which is 10 years younger than the last and only stocking of unclipped fish into Tyson Lake. The extent of natural reproduction in Tyson Lake, along with the factors that influence reproductive success, are unknown and warrant further investigation.

The assignment of the captured unclipped fish to the Manitou and Iroquois strain with 70.9 and 29.1% probability, respectively, leaves the fish's ancestry unresolved. It is possible that the fish represents a descendant of mixed ancestry of Lake Manitou and Iroquois Bay strains from plantings when strain was not recorded, which occurred most recently during 1981-1989. While some recaptures of adult lake trout, likely of hatchery origin, occurred in the 1970s and 1980s, it seems unlikely many persisted as the 1986 stocking assessment recaptured only juvenile lake trout stocked that year (OMNR unpublished data). Although the ancestry of the plantings prior to 2002 is unknown, eggs have been collected from wild-caught adults from the

Manitou strain and stocked in Lake Manitou and other area lakes since 1959, so it is plausible fish from the Manitou strain were involved in the plantings in Tyson lake prior to 2002 (OMNR, 2011). Furthermore, a brood line from the lake trout population in Iroquois Bay was founded in 1982 (OMNR, 2011). It is possible that this strain could also have been stocked into Tyson Lake in 1989, as that brood line would have come into production that year (Jason Intini, OMNR, pers. comm.).

It is also unlikely that this fish is the result of movement of lake trout from another lake, whether naturally or human assisted. Much like Tyson Lake, all other lakes in the surrounding watersheds were acidified and lost their lake trout populations by the 1970s (Neary et al., 1990). The only other lake upstream from Tyson Lake undergoing reintroduction efforts, Broker Lake, received plantings of adult Seneca strain fish in 1994 and juvenile Simcoe strain fish in 2001 and 2002 (OMNR, 2025). The unclipped fish did not assign to either of those strains. Most of the other lakes in proximity to Tyson Lake were stocked with fish from the Killala Lake, Mishibishu Lake, and Michipicoten Lake strains (OMNR, 2025). The only other lakes stocked with Iroquois Bay strain fish, Great Mountain Lake and Long Lake, are 18-22 km close to Tyson Lake but are separated from Tyson Lake by the LaCloche mountain range and are in different watersheds with no roads across, making the movement of fish from one lake to the other unlikely. Given that lake trout inhabit the hypolimnion of lakes with low avian predation (Martin & Olver, 1980; Muir et al., 2021), and adults spawn on shoals at night (Esteve et al., 2008), opposite of when diurnal avian predators are foraging, it is also unlikely that an avian predator moved fish from another lake.

Alternatively, it is possible that the captured unclipped fish is a survivor from the original population. The fish's partial assignment to two geographically proximate populations could

reflect shared ancestry from postglacial colonization and subsequent divergence through genetic drift. Indeed, Wilson & Hebert (1996) found that lake trout from Lake Manitou and Iroquois Bay (and Northeastern lakes generally) were descended from different lineages than those from Killala Lake, Slate Islands, and Michipicoten Island (and Northwestern Ontario lakes generally), suggesting their dispersal from separate glacial refugia and intraspecific divergence. Without genetic information from the presumed extirpated Tyson Lake population, we cannot confirm the origin of the unclipped fish with certainty. What can be stated definitively is that reproduction of fish from plantings whose origin is known (Killala Lake, Slate Islands, Michipicoten Island) has not been found to occur in Tyson Lake since the Northeastern Ontario lake trout recovery program began in 2002.

Considering lake trout reintroductions in the region more broadly, competition with and predation by cisco may be a key factor that has prevented the establishment of self-sustaining lake trout populations (Selinger et al., 2006; Snucins & Gunn, 2003). Competition for food between juvenile lake trout and cisco can reduce growth and abundance of lake trout in lakes where reintroduction attempts are ongoing (Gunn et al., 1987; Selinger et al., 2006; Snucins & Gunn, 2003; Trippel & Beamish, 1989). Stocking of large adult lake trout is a method that could reduce the negative impact of cisco and increase the reintroduction success. Indeed, Snucins and Gunn (2003) found that stocking of adult lake trout in Bell and Johnnie Lakes (Killarney, Ontario, Canada) led to a sharp decline in the abundance of cisco, and resulted in natural reproduction of lake trout in one of the lakes. The last plantings of adult lake trout in Tyson Lake occurred in 2003 and 2004. Given that the two unclipped fish captured in Tyson Lake in 2019 and 2021 were from the 2003- and 2007-year classes, it is possible that successful reproduction was able to occur those years due to the suppression of the cisco population by the stocked lake

trout adults. Given that our evidence of natural reproduction in Tyson Lake aligns with the last stocking of adult fish, plantings of adults should continue to be considered as a strategy to re-establish lake trout populations co-occurring with cisco populations.

Several guidelines, strategies, and best practices have emerged for source population selection for reintroduction programs (e.g., Houde, Garner, et al., 2015). One strategy referred to as environment matching targets source populations with adaptations to key environmental features of a reintroduction location; e.g., an affinity for a common prey item or similar game fish community. This approach has been used to screen the suitability of several strains of Atlantic salmon for reintroduction into Lake Ontario (Dimond & Smitka, 2005; Houde, 2015; van Zwol et al., 2012). The three strains of lake trout that historically have been stocked in Tyson Lake (Killala Lake, Slate Island, Michipicoten Island) may differ in their adaptations to key environmental features (data on fish community characteristics, population metrics, and lake trout diets for all three strains in their native environment can be found in Tables G-2 and G-3 in Appendix G) but may not be environmental matches to Tyson Lake. While the Killala Lake strain has been used to successfully re-establish self-sustaining lake trout populations in acidified lakes in the Sudbury basin where plankton, benthic invertebrates, and small-bodied littoral fishes are the predominant prey base, this strain has not been successfully re-introduced into lakes where cisco are present (Louste-Fillion, 2022; Selinger et al., 2006). Both the Slate Islands and Michipicoten Island strains have been found to reproduce in some Northeastern Ontario lakes (Snucins & Gunn, 2003, Christian Therrien, unpublished results) where cisco comprise the primary forage base (Adeli et al., 2024), but have not been able to establish self-sustaining populations in inland lakes with abundant and diverse warm-water fish communities (Louste-Fillion, 2022; Selinger et al., 2006). Despite the success of these three strains in lakes elsewhere

in the Northeastern Ontario, there has been no evidence of successful re-introduction of any of the Killala Lake, Slate Island, or Michipicoten strain lake trout in Tyson Lake since their plantings in 2002. Instead, the Lake Manitou and Iroquois Bay strains may represent alternative strains that should be prioritized for stocking in Tyson Lake. These two strains represent the closest geographic relatives to the ancestral Tyson Lake population and may be better environment matches to Tyson Lake, as both strains encounter a more robust warm-water fish community and more littoral competitors in their native environments than the strains currently being stocked (see Table G-3 in Appendix G). Furthermore, both the Lake Manitou and Iroquois Bay strains also feed on cisco in their native environments (Adeli et al., 2024, OMNR unpublished results), the prey fish that comprises the bulk of the diet of lake trout in Tyson Lake (Adeli et al., 2024). Taken altogether, we suggest that the Lake Manitou and Iroquois Bay strains could provide an alternative to the other three strains of lake trout currently stocked to help increase the success of lake trout reintroduction into Tyson Lake.

Our finding of natural reproduction of lake trout in Tyson Lake reinforces the need for expanded surveys of spawning areas to document the spatial and temporal extent of natural reproduction in Tyson Lake. As of writing, Tyson Lake continues to be a part of the Northeastern Ontario lake trout rehabilitation plan (OMNRF, 2015) with regular plantings of the Killala Lake and Slate Island strain scheduled to continue (Jean Enneson, OMNR, pers. comm.). It is necessary to understand the extent of natural reproduction in Tyson Lake and characteristics of these lake trout to inform future management plans. Future reintroduction efforts should consider the evolutionary history of lake trout strains and their performance in lakes of a similar environment (environment match) prior to selecting a strain for stocking. Lastly, reproduction of lake trout following plantings of adult lake trout in Tyson, Bell, and Johnnie Lakes, all lakes with

abundant populations of cisco, provide some evidence of a potential top-down control of cisco through predation by adults that might relax competition and predation pressures on juvenile and larval lake trout. Future research should aim to test these hypotheses in lakes with abundant cisco populations.

Chapter 6

General Discussion

6.1 General discussion

Exotic prey fishes have been suggested to be a causal factor in the failure of several attempts to reintroduce salmonines (Family Salmonidae, subfamily Salmoninae; Brown et al., 2005; Fisher et al., 1996), but the role of exotic prey in the failure of reintroduction programs has received less attention than the role of exotic predators and competitors (e.g., Fisher et al., 1996; Innes et al., 2010; Short et al., 1992). In this thesis, I investigated (1) how success of reintroduction efforts are influenced by presence of exotic rainbow smelt (*Osmerus mordax*; herein referred to as smelt; Figure 6.1) and (2) how success of reintroduction efforts are influenced by identity of source population (with specific focus on previous exposure to exotic prey species). Reintroductions programs for lake trout (*Salvelinus namaycush*) were used as model systems. Lake trout in Ontario are currently the focus of multiple, multimillion-dollar reintroduction programs. Several source strains of lake trout have been stocked, but self-sustaining populations have not been established in the majority of the lakes targeted for reintroduction (Evans & Olver, 1995; Keller et al., 2019a; Lantry et al., 2014).

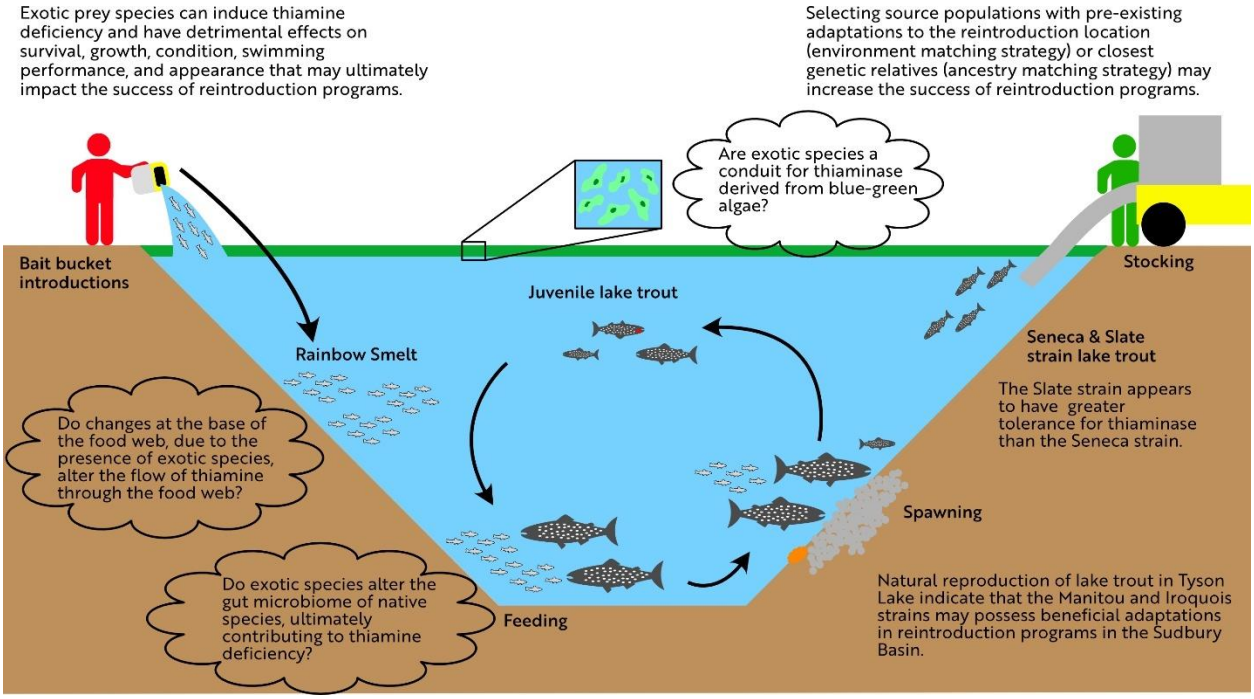


Figure 6.1 Conceptual diagram of key thesis findings and new questions (bubbles) regarding the effects of exotic rainbow smelt (*Osmerus mordax*) on reintroductions of lake trout (*Salvelinus namaycush*) in Ontario. Figure prepared by Julien Therrien.

The Sudbury Basin in Ontario, Canada, is one region where much effort has been, and continues to be, directed toward re-establishment of self-sustaining lake trout populations. Many populations of lake trout were lost to acidification of lakes that occurred because of nickel smelting operations through the 1900s, and reintroduction efforts have only been successful in ~30% of target lakes (Evans and Olver 1995; Sellinger et al. 2006; Louste-Fillion 2022). In several regional lakes that contain lake trout, smelt have been introduced (Evans & Loftus, 1987) and are thought to be a contributing factor to populations declines and reintroduction failures of lake trout (Fisher et al. 1996; Brown et al. 2005; Métis elder D'arcy Paquette, pers. comm.). In Chapter 2, I conducted a multi-lake study in the Sudbury Basin to compare concentrations of

thiamine in fish tissue, prevalence of thiamine deficiency, growth of lake trout, and condition of lake trout in lakes with and without exotic smelt. There, I described one of the first instances where thiamine deficiency in lake trout was inferred to be associated with the presence of exotic smelt. Further, I found that presence of smelt was associated with lower condition of lake trout, and that asymptotic length of lake trout populations decreased with increasing dietary proportion of smelt. Ancillary results in Chapter 3 and Chapter 4, where hatchery experiments were used to investigate effects of diet-derived thiaminase on lake trout, suggested that consumption of thiaminase-containing rainbow smelt could be a contributing factor in the failure of lake trout reintroductions in the Laurentian Great Lakes. In this final discussion chapter, I discuss how exotic prey fishes may affect the success of reintroduction programs (Section 6.2) and provide some recommendations for future research (Section 6.4).

Given the low success rates for reintroduction programs in all taxa (Fischer & Lindenmayer, 2000; Godefroid et al., 2011; Harding et al., 2025), several guidelines, strategies, and best practices have emerged for selection of source populations (Houde, Garner, et al., 2015). Targeting source populations with adaptations that are well-matched to key environmental features of reintroduction locations (environment matching strategy), or a genetic similarity to the extirpated historical population (ancestry matching strategy) are two such strategies (Houde, Garner, et al., 2015) that I considered in the context of this thesis. Of the nine source strains of lake trout that are currently maintained in the Ontario provincial fish culture system for reintroduction programs in Ontario lakes (see Table C-1 in Appendix C), I directly examined four strains (Slate, Seneca, Killala Lake, and Michipicoten Island) and indirectly assessed two additional strains (Lake Manitou and Iroquois Bay; Figure 6.2). Using results of a comparison of relative performance of two lake trout strains fed a diet designed to mimic the thiaminase activity

of exotic prey fishes (Chapters 3 and 4), I inferred that source strains may differ in their tolerance to exotic prey fishes that contain thiaminase. Below, I discuss which of the current strains in the Ontario fish culture program may be best suited for reintroduction into lakes that contain exotic prey fishes (Section 6.3.1).

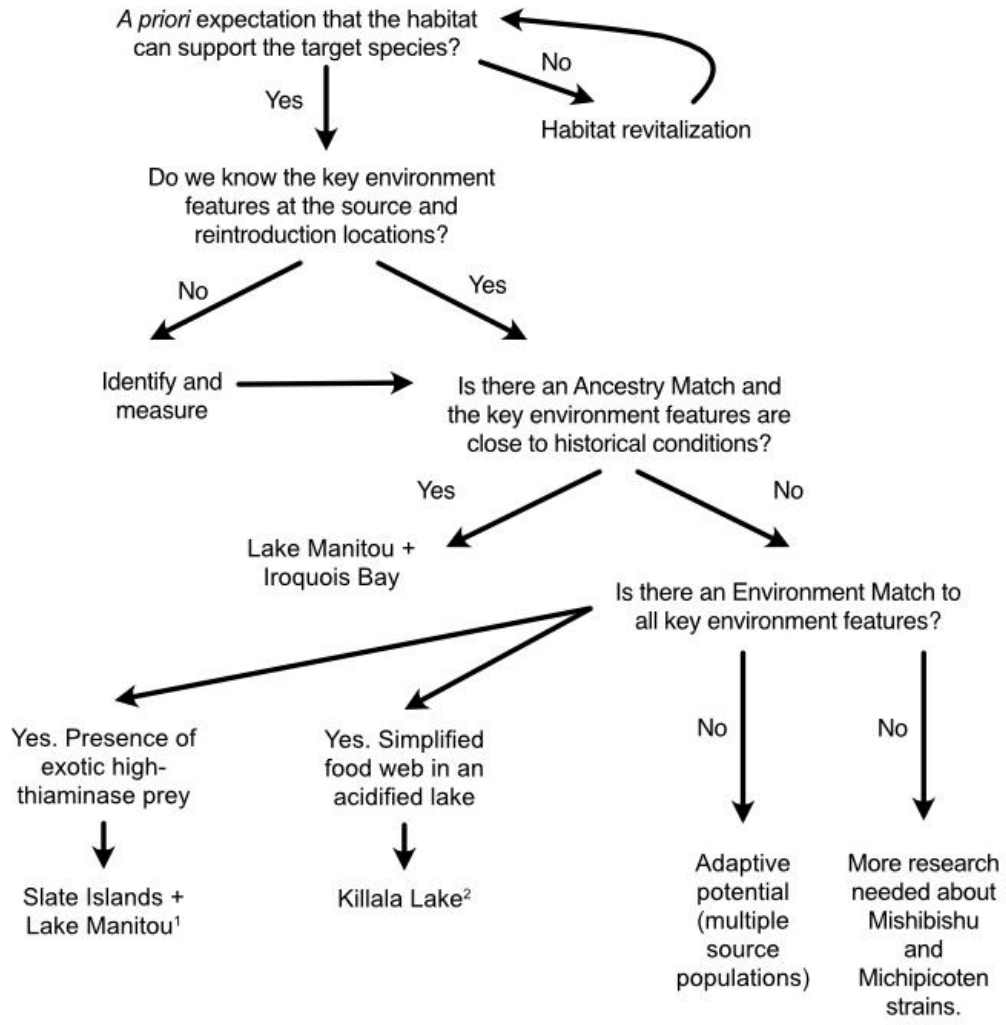


Figure 6.2 A framework for selecting source populations of lake trout for reintroduction to lakes in the Sudbury Basin. The framework is an optimized guide for selecting source populations and is modified and reprinted with permission from Houde (2015). Modified figure produced by Julien Therrien. [1]More research needed [2]Described in Selinger et al. (2006) and Louste-Fillion (2022)

In Chapter 5, I report capture of a fish thought to be of wild origin in Tyson Lake, a lake where the lake trout population was extirpated by the 1970s. Despite the fact that more than 105000 lake trout representing 3 strains have been stocked since extirpation, it was thought that reintroduction efforts have failed to produce any wild recruits. Using a combination of fish aging, comparison to historical stocking records, and genotyping at 17 microsatellite loci, I described the first instance of natural reproduction of lake trout in Tyson Lake since extirpation (Chapter 5) and suggest that selecting source populations that are the closest genetic relative to the source population may improve the success of lake trout reintroductions. Below, in Section 6.3.2, I discuss how future reintroduction programs should consider the evolutionary history of lake trout strains prior to selecting a source population. Finally, in Section 6.4, I present knowledge gaps and research needs to better understand the impacts that exotic species may have on the reintroduction of native species and to evaluate source population selection strategies.

6.2 The role of exotic prey fishes in affecting success of reintroduction programs

In Chapter 2, I described one of the first episodes of thiamine deficiency in lake trout that were primarily feeding on smelt, an exotic species known to contain the enzyme thiaminase (Evans, 2016; Gordon, 2012; Tillitt et al., 2005). While thiamine deficiency can be lethal or result in reproductive failure, there may also be several sublethal effects, such as declines in growth and swimming performance, that influence the fitness of fishes in the wild (reviewed in Harder et al. 2018). Additionally, some cardiac and neurological effects associated with thiamine deficiency may persist and affect future fitness, even following recovery from thiamine deficiency (Adeli et al., 2024; Gomes et al., 2021; Mair et al., 1988). Whereas lake trout are not currently being stocked in Lake Wanapitei (the lake where the episode of thiamine deficiency

was identified), annual plantings of lake trout have occurred in lakes Manitou and Whiskey, lakes where reintroduction efforts for lake trout are ongoing and where smelt have invaded, since 2001 and 1985, respectively (Ontario Ministry of Natural Resources [OMNR], 2025a), and reintroduction success has been mixed. My results are consistent with the hypothesis that consuming smelt may cause thiamine deficiency in lake trout, which may decrease the probability of success of reintroduction programs.

In Chapters 3 and 4, I confirmed that diet-derived thiaminase has negative effects on colouration, morphology, swimming performance, survival, growth, and food conversion efficiency. These negative effects could decrease the fitness of consumers if they impact energy allocation or the timing of key life-history events (e.g., maturation; Brown et al., 2005). For instance, a reduction in swimming capacity and changes in colouration and morphology can impact the foraging ability of juvenile salmonines (Holbrook et al., 2013) and risk of predation (Johnsen & Sosik, 2003; Taylor & and McPhail, 1985). In the pelagic environment of the Great Lakes, background matching (which is mediated by colouration) is one of the most important mechanisms of predator avoidance for pelagic fishes (Johnsen & Sosik, 2003; Stevens & Merilaita, 2011). Studies have shown that fishes that were not optimally colour-matched to their environment were detected by predators at nearly twice the distance and had survival rates up to 25% lower than background-matched conspecifics (Arostegui & Quinn, 2019; Johnsen & Sosik, 2003; Miyamoto, 2016)**.** Furthermore, swimming performance is important for salmonines in general and is particularly important for lake trout (reviewed in Plaut 2001) because lake trout employ vertical and horizontal cruising to locate prey throughout the water column (Vogel & Beauchamp, 1999) and rely on physical separation (e.g., vertical migrations) to avoid predators (Gallagher et al., 2019). Thus, a change in colouration and reduction in swimming performance

of juvenile lake trout, due to consumption of exotic prey fishes, could reduce their ability to capture prey and avoid predation, which would ultimately reduce their fitness in the wild and affect success of reintroduction programs.

Effects of exotic prey species on growth and morphology may also impact individual fitness in restoration programs. Growth can affect survival (Gunn et al., 1987; Hesthagen & Johnsen, 1992) and predation rates (Hostetter et al., 2012, 2015) of salmonines. For fishes with predators that are gape-limited, increased individual growth can result in a shorter period of susceptibility to predators (Persson et al., 1996; Sogard, 1997). I found that the consumption of thiaminase resulted in a shallower body depth of juvenile lake trout (Chapter 4), which may also increase the susceptibility of juvenile lake trout to predation. In brown trout (*Salmo trutta*) and cisco (*Coregonus artedi*), decreased body depth was associated with lower survival due to increased susceptibility to large-gape predators (Hyvärinen & Vehanen, 2004; Jacobson et al., 2020). Decreases in juvenile growth and changes in morphology (body depth) may therefore also reduce survival of lake trout and affect success of reintroduction programs.

For reintroduction programs to be successful, survival and reproductive output must be maximized (Ferrer et al., 2018; Fiumera et al., 2004; Komers & Curman, 2000; Ruzzante et al., 2016). In salmonines, body size (total length and body mass) and condition can affect the number and quality of offspring (Barneche et al., 2018; Birkeland & Dayton, 2005). Fecundity has previously been found to increase with body size in several populations of female lake trout (e.g., Lake Ontario, Lake Superior; Eschmeyer, 1955; Goetz et al., 2021; Martin & Olver, 1980; O'Gorman et al., 1998; Peck, 1988). Decreases in condition, growth, and asymptotic length caused by consumption of exotic prey species (Chapters 2 and 3) could therefore decrease fitness of lake trout. Indeed, decreases in size-at-maturity and fecundity of cutthroat trout (*Oncorhynchus clarkii*)

due to the presence of exotic species has been identified as a potential impediment for successful reintroduction in Western North America (Budy et al., 2020). Altogether, the presence of exotic prey fishes that contain thiaminase may directly limit reintroduction success through mortality due to thiamine deficiency and indirectly limit reintroduction success through reductions in reproductive output via effects on growth and condition.

Effects of exotic species on reintroduction efforts could be exacerbated by other stressors and cumulative effects. Stressors including exotic species, commercial and recreational fishing pressure, water level manipulations, high water temperatures, and infectious agent loads were found to act cumulatively to contribute to salmonine declines across the Northern Hemisphere (Bronte et al., 2003; Gillson et al., 2022; Hinch et al., 2021; Lantry et al., 2014; Miller et al., 2014; Teffer et al., 2019). In the Sudbury Basin, recreational fishing pressure and associated mortality rate of lake trout is high (Selinger et al., 2006). In fact, 32% of lakes with lake trout that were surveyed by Selinger et al. (2006) in Northeastern Ontario experienced angling effort above sustainable levels. Additionally, several lakes with smelt in the Sudbury Basin (e.g., Panache, Manitou, and Wanapitei) undergo water level manipulations for production of hydroelectricity and flood prevention. Water level manipulation was identified as a potential factor in declines of lake trout in lakes in Northeastern Ontario (Selinger et al., 2006) because, as shown in other managed systems, winter drawdowns may limit spawning success of lake trout (Ontario Power Generation, 2024). Effects of exotic species on reintroduction efforts should be considered in conjunction with other stressors.

6.3 Applying strain selection strategies to Ontario's lake trout reintroduction programs

6.3.1 Environment matching strategy

Selecting populations for reintroduction that are adapted to receiving lake environments should improve reintroduction success (Houde, Garner, et al., 2015; Jones, 2003, 2013; Krueger et al., 1981; Moritz, 1999). In Chapters 3 and 4, survival and some performance-related metrics differed between lake trout strains when exposed to diet-derived thiaminase. Consumption of thiaminase resulted in a lower survival rate in the Seneca strain but no difference in the Slate strain, suggesting that the Slate strain may possess adaptations that help mitigate the secondary effects of diet-derived thiaminase.

Because the Slate strain of lake trout have not been exposed to thiaminase-containing alewife (unlike the Seneca strain fish), I originally predicted that Seneca-strain fish would be better adapted to a diet high in thiaminase and would perform better in the experiments conducted in Chapters 3 and 4. My results were inconsistent with this prediction, but may still reflect and be explained by environmental adaptations. In their native environment, Slate strain lake trout feed on prey fishes that are relatively higher in highly unsaturated fatty acids (HUFAs; e.g., smelt, cisco) than Seneca strain lake trout (Futia et al., 2019; Howland et al., 2013). In Chapter 3, I suggested that Slate strain fish may be better able to metabolize HUFAs without depleting thiamine to a level that induces mortality, perhaps through increased thiamine storage capabilities, increased enzyme affinity for thiamine diphosphate (TDP), or a lower threshold for negative secondary effects as suggested by Baker, (2023). Also, while levels are lower than those observed in alewife, smelt contain thiaminase (Evans, 2016; Gordon, 2012; Tillitt et al., 2005).

Smelt first invaded Lake Superior in the 1930s (MacKay, 1963; Van Oosten, 1937) and have been a component of lake trout diets ever since (Dryer et al., 1965; Ray et al., 2007), including the Slate Islands, where smelt were the most frequent prey fish observed in the diet of lake trout (see Table C-2 in Appendix C). Exposure to prey with high thiaminase and HUFA in their native environment may have selected genes for resiliency to thiaminase or low thiamine in the Slate strain, but further research is needed to confirm this.

In Chapter 4, I found a higher probability of recovery following exhaustive exercise and faster critical swim speeds at a given size of Slate strain fish than Seneca strain fish, regardless of diet treatment. Together with the higher maximum heart rates of Slate strain fish found by Baker et al. (2023), this suggests that fish from the Slate strain have a higher aerobic scope than those from the Seneca strain. Aerobic scope has been found to differ among other conspecific salmonine populations due to differences in local migratory environments (Eliason et al., 2011, 2013; Lee et al., 2003; Van Wert et al., 2023). Salmonine populations with longer, more physically challenging migrations typically have a larger aerobic scope, increased ability to recover from exercise, and greater swimming performance than conspecifics with shorter, less challenging migrations (Anttila et al., 2019; Cooke et al., 2012; Eliason et al., 2011; Van Wert et al., 2023). Lean morphotype lake trout in Lake Superior have been found to travel an average of 52.6 km and up to 410 km between tagging and recapture locations (Eschmeyer et al., 1953; Kapuscinski et al., 2005). These large movements have been associated with migratory movements of prey in Lake Superior, such as those of coregonines (genus *Coregonus*; Stockwell et al., 2014) and of *Mysis* spp. (Ahrenstorff et al., 2011). While movement distances of lake trout in Seneca Lake have not been quantified, lake trout are presumed to move less than conspecifics in Lake Superior (e.g., Slate and Michipicoten Island strains), owing to Seneca Lake's smaller

size. Lake trout from the Slate strain may thus have evolved to have higher aerobic scope relative to the Seneca strain, due to the greater distances required to search for prey and reach spawning locations. Further research to investigate this is necessary, however.

Higher aerobic scope could confer some advantages to lake trout from the Slate strain during reintroduction programs. Higher capacity for activity could provide an advantage in large Ontario lakes, especially Lake Ontario, where lake trout are known to make large migratory movements between spawning and feeding areas (Ivanova et al., 2021) or ascend the Niagara River to reach spawning substrate (Gatch et al., 2021). Higher aerobic scope could also confer some resilience to diet-derived thiaminase. Salmonine populations that undergo more challenging migrations and have higher aerobic scopes are typically more adept at conserving energy than those with shorter migrations (Crossin et al., 2004; Hinch et al., 2005; Pon et al., 2007). The inferred higher activity requirements of Slate strain fish in their native environment could thus result in improved energy conservation, including thiamine stores, relative to Seneca strain conspecifics. However, care must be taken to ensure that aerobic scope and other traits of interest are examined throughout the course of the strain's life history, Eliason et al. (2017) found, for example, that differences in aerobic scope between populations that were observed at the juvenile stage were opposite to those observed at the adult stage. Future studies should compare the aerobic scope among candidate populations during all life stages to identify the best environment match prior to reintroduction programs, and determine the link between thiamine utilization and aerobic scope in a controlled study.

Adaptation to captive-breeding environments can result in the accumulation of alleles that are deleterious to individuals released into the wild, ultimately reducing their fitness (Frankham, 2008; Warwick et al., 2024). These genetic changes may be especially problematic

when a population has experienced multiple generations of captive breeding (Araki et al., 2007; Lynch & O'Hely, 2001), because reductions in fitness typically increase with the number of generations in captivity (see review in Fraser, 2008). The Seneca strain fish with apparent resilience to diet-derive thiaminase that were used by Fitzsimons et al. (2021) originated from eggs collected from wild fish in 1995, 1997, and 2000. At the time the experiments in this thesis were conducted, the Seneca strain had been captively bred in Ontario since 1994 and for three or four generations (Christopher Wilson, OMNR, pers. comm.). In contrast, the Slate strain had been captively bred for two or three generations (Jason Intini, OMNR, pers. comm.). The lower performance of the Seneca strain relative to the Slate strain when exposed to diet-derived thiaminase may therefore be explained by a reduction in performance due to domestic selection. Reintroduction guidelines suggest avoiding source populations that may have suffered from domestication or an extensive time in captive environments (Houde, Garner, et al., 2015; Montalvo et al., 1997; Weeks et al., 2011) and future reintroduction programs should consider the time in captivity of candidate source populations.

There has been mixed success in the reintroduction of fish from these two strains (Slate and Seneca) in locations where exotic prey fishes are present. The Slate strain has had relatively low reproductive success in Lakes Ontario, Huron, Michigan, and Erie (Ebener et al., 2025; Markham et al., 2022; Perkins et al., 1995; Scribner et al., 2018), which are lakes where exotic prey fishes comprise the largest proportion of lake trout diets (Hondrop et al., 2025; NYSDEC, 2025a; Vinson et al., 2024; Weidel et al., 2024). In contrast, adult lake trout from the Seneca strain typically have greater survival rates than the Slate strain and represent the majority of the spawning biomass in Lakes Erie, Michigan, and Ontario (Ebener et al., 2025; Lantry et al., 2019; Larson et al., 2021; Rogers et al., 2019). This success has been partially attributed to lower

mortality from sea lamprey (*Petromyzon marinus*) compared to other strains, rather than resilience to thiaminase (Bronte et al., 2007; Larson et al., 2021; Rogers et al., 2019; Schneider et al., 1996). Indeed, thiamine concentrations in eggs of Seneca strain fish that were collected from spawning reefs in Lake Ontario were not significantly different from those in eggs of Slate strain fish (Heisey et al., 2023; Osborne et al., 2026). In contrast, reintroduction of lake trout from the Slate strain has resulted in naturally reproducing populations of lake trout in Whiskey Lake (Algoma, Ontario, Canada; Ministry of Environment, Conservation, and Parks [MECP] unpublished results) and in Birch Lake (Algoma, Ontario, Canada; Denis Gendron, OMNR, pers. comm.), where smelt are established and comprise a substantial proportion of the diet of lake trout (Adeli et al., 2024; Denis Gendron, OMNR, pers. comm.). The success of Slate strain fish in inland lakes where the prey base is composed of exotic prey fishes, together with the findings of the hatchery experiment (Chapters 3 and 4), suggests that Slate strain fish may possess adaptations that confer some tolerance to diet-derived thiaminase, although more research is needed. Regardless, future reintroduction programs should consider the relative performance of source strains in environments with similar features to the reintroduction location (e.g., environment match) when selecting a strain for stocking.

With models predicting surface temperatures of freshwater lakes in Canada to increase by as much as 18 °C by 2100 (Sharma et al., 2007), climate change is predicted to have adverse effects for cold-water fishes (Ficke et al., 2007; Stefan et al., 1998). Future reintroduction programs should consider the relative performance of source strains in projected future as well as current conditions. While Baker et al. (2023) found no difference in thermal tolerance between the Seneca and Slate strains, thermal tolerance has been found to differ between salmonine populations (e.g., Eliason et al., 2017; Gradil et al., 2016). Although critical for predicting the

vulnerability of species to climate change, thermal performance among strains has not been thoroughly characterized in lake trout and warrants further research.

6.3.2 Ancestry matching strategy

When planning reintroduction programs, guidelines often suggest selecting the closest genetic relatives (e.g., ancestry match) to the historical population (Houde, Garner, et al., 2015; Krueger et al., 1981; Moritz, 1999; Smith et al., 2024). Selecting close genetic relatives as source populations for translocations was effective at reestablishing self-sustaining populations of brook trout (*S. fontinalis*) in watersheds of the Southern Appalachian Mountains (USA; Smith et al., 2024) and slimy sculpin (*Cottus cognatus*) populations in the Driftless Region of Southeastern Minnesota (USA; Huff et al., 2010). This approach was also used in successful reintroductions of other animal taxa, including bighorn sheep (*Ovis canadensis*; Flesch et al., 2020) and macroinvertebrates (Jourdan et al., 2019). In Chapter 5, I identified the potential of the Lake Manitou [henceforth referred to as Manitou] and Iroquois Bay [henceforth referred to as Iroquois] strains of lake trout as ancestry matches for reintroduction programs in the Sudbury Basin (Figure 6.2). Iroquois Bay and Lake Manitou are two geographically proximate populations to Tyson Lake, the lake where I discovered the first instance of natural reproduction of lake trout since their extirpation from the lake in the 1970s. Iroquois, Manitou, and Tyson Lake lake trout are descended from the same glacial refugia (Wilson & Hebert, 1996). The shared ancestry of the Iroquois and Manitou populations with the extirpated populations of lake trout historically present in lakes in the Sudbury Basin may confer adaptations to key environmental features in the reintroduction locations and improve reintroduction success. There is no evidence of natural reproduction in the three strains of lake trout that were stocked into Tyson Lake — Killala Lake (henceforth referred to as Killala), Michipicoten Island (henceforth

referred to as Michipicoten), and Slate — strains that are neither environment or ancestry matches to Tyson Lake. Partial assignment of the unclipped fish to the Manitou and Iroquois strains may indicate that these strains possess adaptations that would be beneficial in reintroduction programs in the Sudbury Basin.

6.3.3. Use of other strains in reintroduction programs

Although the two other strains examined in this thesis — Killala and Michipicoten — are not environment matches for systems with exotic prey fishes or ancestry matches to historical populations in the Sudbury Basin and Lake Ontario, they may have potential for other reintroduction programs in Ontario (Figure 6.2). Currently, the Killala strain is the primary strain stocked into Ontario's inland lakes (OMNR, 2025a). Reintroduction of the Killala strain has led to self-sustaining lake trout populations in acidified Sudbury Lakes where plankton, benthic invertebrates, and small-bodied littoral fishes are the predominant prey base, but reintroduction has been unsuccessful in lakes where cisco or smelt are present (Louste-Fillion, 2022; Selinger et al., 2006). The low reproductive success of the Killala strain in stocking programs in Lake Ontario (Marsden et al., 1993; Perkins et al., 1995), paired with its historical presence in systems without high-thiaminase prey fishes, suggests a potential vulnerability to diet-derived thiaminase that warrants further investigation. The lack of reproduction by Killala fish in Tyson Lake, where the pelagic prey base is dominated by cisco and regular plantings have occurred since 2002, provides further evidence of the low performance of this strain in reintroduction environments that support cisco and exotic prey fishes.

The Michipicoten strain, which has been used in reintroduction programs in the Laurentian Great Lakes and as surplus for put-grow-take recreational opportunities in inland lakes, was not recaptured and did not show signs of natural reproduction in Tyson Lake. Despite

this, the Michipicoten strain appears to have high performance (growth and survival), including documented reproduction, in reintroduction programs in lakes with exotic competitors (smallmouth bass [*Micropterus dolomieu*]) and abundant cisco in the Sudbury Basin (e.g. Bell and Johnnie Lakes; Snucins and Gunn, 2003; Christian Therrien, MECP, unpublished results). In their native environment, lake trout from the Michipicoten strain feed on cisco and encounter exotic competitors (Table C-3 of Appendix C), and thus this strain could be an environment match for reintroduction locations with these habitat characteristics. Although lake trout from the Michipicoten strain have not been observed feeding on high-thiaminase prey (Table C-3 of Appendix C), the close proximity of their native environment to the Slate Islands suggests that they may be closely related to the Slate strain (Ihssen et al., 1988; Wilson & Hebert, 1996), and may share some of the genes possessed by the Slate strain that confer resilience to thiaminase. The Michipicoten strain made up a large percentage (20.7%) of recaptured fish in the Eastern Basin of Lake Ontario (Neff et al., 2023; Therrien et al., 2023), yet accounted for only 5.44% of the lake trout stocked in Lake Ontario since 1998 (Great Lakes Fisheries Commission, 2025). Thus, the Michipicoten strain likely possesses some traits that would be beneficial in Ontario reintroduction locations, but further investigation is required to assess whether their success in the Eastern Basin is associated with resilience to thiaminase.

6.4 Future directions

6.4.1 Caveats and knowledge gaps

The hatchery experiment described in Chapters 3 and 4 involved rearing fish under two diets: control and thiaminase. To better identify the causal mechanism of thiamine deficiency induced by the experimental diets, a third diet could be added that would contain no measurable thiamine (no-thiamine diet). Previous studies that included diets formulated with thiamine

concentrations of 0 mg/kg were able to induce thiamine deficiency in Ya-fish (*Schizothorax prenanti*), Indian major carp (*Cirrhinus mrigala*), and spotted snakehead (*Channa punctata*; Xiang et al. 2016; Zehra and Khan 2017, 2018). Given that there were no measurable differences in thiamine concentrations between the control and thiaminase diets that I used, a reduction of thiamine in food by *P. thiaminolyticus* prior to consumption by lake trout in the thiaminase treatment may not be the cause of thiamine deficiency, in contrast to the mechanism proposed by Houde, Saez, et al. (2015) and Mitchell et al. (2021). The addition of a no-thiamine diet could help to determine if thiamine deficiency is instead induced some other mechanism, which would support the suggestion (described below) that changes to the gut microbiome of fishes cause thiamine deficiency. Furthermore, supply chain issues due to the COVID-19 pandemic limited the available supply of commercial herring meal and an alternative protein base was needed to form the base of the experimental diets described in Chapters 3 and 4. I used pacific herring (*Clupea pallasii*) that I ground and dehydrated. When the ground herring was mixed with other ingredients and pelletized, the composition of pellets was more heterogeneous than those used in previous diet studies (e.g., Houde, Saez, et al., 2015; Mitchell et al., 2021). This heterogeneity could have contributed to the observed variation in thiamine and thiaminase concentrations among diet subsamples (Chapter 3). Future diet studies should ensure that food pellets are homogeneous to reduce variability in thiamine and thiaminase content among pellets.

Another opportunity for building on findings of Chapter 4 would be to collect alternate measurements of recovery of fish post-exhaustive exercise. Authors of several studies have shown that recovery after exhaustive exercise takes much longer than 30 mins (e.g., Lee-Jenkins et al., 2007; reviewed in Wood, 1991). The method used to determine recovery in this study does not give any information into the metabolic recovery of the fish in the swim trials. Future studies

should increase the cut-off of the recovery threshold beyond 1 hour, like that done in Lee-Jenkins et al. (2007), and collect data on metrics that reflect metabolic recovery, such as measures of lactate post-exhaustion. Respirometry could also be used to determine how much excess post-exercise oxygen consumption (EPOC) was required for full recovery [e.g., Lee et al. (2003), Kieffer et al., (2011), and Heuer et al., (2026)].

An opportunity for strengthening the findings of Chapter 2 is to increase the sample size of lakes that contain smelt. Smelt are only found in 14 lakes in the Sudbury basin (OMNR 2025), and five of these lakes contain no lake trout or small populations of lake trout (e.g., limited catchability). Efforts were made to include one additional lake with smelt, Little Panache, but no lake trout were captured during angling surveys and Indigenous fishers in the area report that no lake trout have been caught in Little Panache Lake since 2004 (Andy Nootchetai, Atikameksheng Anishnawbek, pers. comm.). Including lakes with smelt populations from outside the Sudbury Basin could increase sample sizes, but would also risk introducing confounding factors (e.g., differences in fish community, water chemistry), which could influence comparisons to lakes without smelt from the Sudbury Basin. Future studies should employ province-wide sampling, like that done by Swanson et al. (2006), to increase sample sizes and better determine how smelt invasion affects lake trout throughout their Ontario range.

Given the episodic nature of thiamine deficiency (Balk et al., 2016), the lack of differences in muscle thiamine concentration in Chapter 2 may also be an artefact of study design. In my study, most fish were sampled during the spring and summer months (May-August), rather than the fall spawning season, and a combination of immature and mature fish were examined. In other systems, thiamine deficiency has been reported to be more prevalent during bouts of starvation related to spawning and thiamine routing from muscle to eggs in

mature fish (Honeyfield et al., 2016; Larson & Howard, 2019; Todisco et al., 2024; Vuorinen et al., 2020). It may be that thiamine deficiency and lower muscle thiamine concentrations become more prevalent during spawning season in lakes with smelt, and future studies should aim to examine egg and liver thiamine concentrations of mature fish from these populations during the fall spawning period.

In addition to the above questions that relate directly from the results of my thesis, there are three primary knowledge gaps that have been identified in the literature and this thesis, and which warrant investigation. First, it was previously thought that thiamine deficiency in fish fed a thiaminase diet was due to the digestion of thiamine in the food by *Paenibacillus thiaminolyticus*, and that this would result in low food thiamine concentrations. However, I observed thiamine deficiency and other negative effects observed in fish fed diets containing *P. thiaminolyticus* despite no measurable difference in food thiamine concentration between the thiaminase and the control diets. These results indicate that an alternative hypothesis for the causal mechanism of thiamine deficiency is warranted. Consumption of *P. thiaminolyticus* may have changed the composition of the gut microbiome of lake trout fed the thiaminase diet, which could have affected absorption and uptake in the gut. Wistbacka and Bylund (2008) found that the thiaminase activity of prey fishes remained active in the viscera of gillnetted Atlantic salmon who had consumed prey fishes prior to their capture**,** and several studies have shown that thiaminase activity is concentrated in the gut (Kraft et al., 2014; Wolfe et al., 2023), which may reflect composition of the gut microbiota. Thiamine-producing bacteria in the gut may promote thiamine absorption (Ji et al., 1998) and some gut microbiota in *Drosophila melanogaster* and ruminants have been found to provision thiamine to their hosts in low thiamine environments (Breves et al., 1979, 1981; Sannino et al., 2018). Further research is needed to understand how

thiaminase-producing bacteria derived from the diet may contribute to thiamine deficiency. DNA sequence analysis of the gut microbiome of fish from both diet treatments in Chapters 3 and 4 could identify if the consumption of *P. thiaminolyticus* changed the bacterial community in the guts of fishes. If there are differences in microbiomes between diet treatments, measuring thiaminase activity in the gut could then indicate if thiaminase production by bacteria in the gut is a contributing factor of thiamine deficiency. Lastly, a comparison of gut microbiomes between fish feeding on high-thiaminase exotic prey fishes (e.g., smelt, alewife) in the wild and those feeding on native prey fishes could further determine the importance of changes to the gut microbiome and effects on the induction of thiamine deficiency.

The second knowledge gap is how multiple factors interact to influence the movement of thiamine through the food web. It has been suggested that the composition of phytoplankton and zooplankton communities could affect the thiamine status of biota at higher trophic levels (Hylander et al., 2024), and may contribute to the global prevalence of thiamine deficiency (Ejsmond et al., 2019; Hylander et al., 2024; Majaneva et al., 2020). Given that exotic species can alter food web dynamics and increase the trophic position of native predators (see review in Gallardo et al. 2016; Jacobs et al. 2017; Wainright et al. 2021), changes to trophic dynamics induced by the invasion of exotic species may also be a causal factor for thiamine deficiency in North American lakes. Future studies should aim to investigate how invasions of exotic species have altered the lower trophic levels of food webs, and if these changes are affecting the flow of thiamine to native consumers at higher trophic levels. Finally, the impacts should be assessed to identify if they are sufficient to affect the success of reintroductions of native species.

The final knowledge gap is how blooms of blue-green algae and invasions of exotic species may interact to potentially induce thiamine deficiency. The only episode of thiamine

deficiency that has been observed in the Sudbury Basin was in Lake Wanapitei, which was the only lake in the study to have had confirmed blooms of blue-green algae (MECP, unpublished results). Previous studies have shown that thiaminase activity in zooplankton communities was highest in areas with blooms of blue-green algae (Fitzsimons et al., 2004), and that thiaminase activity of fishes increased in the presence of blue-green algae (Arsan, 1970; Arsan & Malyarevskaya, 1974; Arsan & Malyarevskaya, 1969; Birger, 1973; Malyarevskaya et al., 1970, 1972). Although the exact mechanism is unknown, it has been suggested that zooplankton accumulate thiaminase by consuming blue-green algae, which are then consumed by exotic prey fishes (Evans, 2016). Future studies should aim to measure thiaminase activity in exotic prey fishes during algal blooms in lakes where thiamine deficiency is known to occur.

6.4.2 Strain screening and post-reintroduction monitoring

Prior to commencing reintroduction efforts, prospective source populations should be screened to ensure that environmental features at source locations are well-matched with the reintroduction environment *a priori* (Anderson et al., 2014; Houde, 2015; Krueger et al., 1995). Two factors, presence of exotic prey fishes that contain thiaminase and competition with native cisco, have been implicated as likely impediments to successful reintroduction of lake trout in Ontario lakes (Krueger et al., 1995; Muir et al., 2012; Selinger et al., 2006; Snucins & Gunn, 2003). Experiments measuring the relative fitness of different source populations in laboratory, eco-evolutionary mesocosm studies, or in experimental lakes (i.e. Experimental Lakes Area, Ontario, Canada) can inform reintroduction efforts (Gunn & Sein, 2000; Hendry et al., 2024; Houde, Saez, et al., 2015; Houde, Wilson, et al., 2015; Mitchell et al., 2021; Snucins & Gunn, 2003). Future investigations should include assessments of the relative abundance (e.g., catch per

unit effort) of exotic and native prey fishes and comparisons of fish community composition between source and reintroduction locations.

Resilience to diet-derived thiaminase from exotic prey fishes should be thoroughly examined in two additional strains of lake trout (Figure 6.2). The Mishibishu Lake (henceforth referred to as Mishibishu) strain are hatchery-reared lake trout that originated from egg collections from the river-spawning lake trout population of the Dog (Nimoosh) River (Algoma, Ontario) that flows into northeastern Lake Superior (Ihssen et al., 1988; Loftus, 1958). Reintroductions using the Mishibishu strain have successfully established self-sustaining populations (Evans & Olver, 1995), including in lakes where competition with cisco is known to occur (Snucins & Gunn, 2003). Furthermore, the Mishibishu strain was observed among adult returns to the Niagara River (a major lake trout spawning site in the Lake Ontario basin) during the short period they were stocked in Lake Ontario and may have established a population there (Gatch et al., 2021). This strain has unique life history characteristics; as river-spawners, they may have higher aerobic scope (Jones et al., 2018; Loftus, 1958). As discussed above (Section 6.3.1), which could result in improved energy conservation, including thiamine stores, and confer some resilience to thiaminase. Further investigation is warranted.

The Manitou strain, as discussed above, is a potential ancestry match for lakes where lake trout have been extirpated in the Sudbury Basin. The strain also appears to have high performance (growth rate, condition, muscle thiamine concentrations) in lakes with smelt, compared to other strains of lake trout in the Sudbury Basin (Chapter 2). The Manitou strain is the sole genetic contributor to the population of lake trout in Lake Simcoe (Chris Wilson, OMNR, unpublished results), where the pelagic forage base primarily comprises exotic smelt (OMNR, unpublished results). The Manitou strain may possess adaptations to diet-derived

thiaminase that translate to high performance in other lakes where exotic prey fishes occur. Further study would be useful to determine whether the Manitou and Mishibishu strains possess adaptations for thiaminase resilience and whether these adaptations translate to improved survival or performance in reintroduction locations where exotic prey fishes occur.

The observation of natural reproduction of lake trout in Tyson Lake (Chapter 5) reinforces the need for expanded post-release monitoring of reintroduction programs. Of the 33 lakes where stocking of lake trout has occurred in the Northeastern Ontario lake trout recovery program (Selinger et al., 2006), evidence of natural reproduction of lake trout has only been observed in 21 lakes and reintroductions have been deemed successful in only 16 lakes (Selinger et al. 2006; Louste-Fillion 2022; John Gunn, Cooperative Fisheries Ecology Unit [CFEU], pers. comm.). At the time of writing, 22 lakes are part of the Northeastern Ontario lake trout recovery plan (Ontario Ministry of Natural Resources and Forestry [OMNRF] 2015), and regular plantings of the Killala strain are scheduled to continue (Jean Enneson, OMNR, pers. comm.). Monitoring is an essential component of any reintroduction program and permits assessment of whether reintroduction programs are successful and if program goals have been achieved (Anderson et al., 2014; Fischer & Lindenmayer, 2000; George et al., 2009; Godefroid et al., 2011; Menges, 2008; Menges et al., 2016). Monitoring also provides essential information for the adaptive management of ongoing reintroduction programs and provides data that are essential for the planning of future programs (Anderson et al., 2014). Lastly, monitoring can identify factors that limit survival and reproductive success, and provide insight that guides alternative reintroduction strategies (Anderson et al., 2014; Cochran-Biederman et al., 2015). Post-release monitoring could have identified natural reproduction in Tyson Lake earlier and allowed for a switch from plantings of the Killala strain to the Manitou and/or Iroquois strains, potentially

improving program success. In the future, if one or two generations of plantings fail to produce a self-sustaining population, then post-release monitoring and/or further research should be conducted to identify factors that may be limiting reintroduction success and identify if other source strains may perform better in the reintroduction environment (Anderson et al., 2014; Houde, 2015).

Monitoring programs also provide opportunities for managers to engage with the public and maintain public support. Considered an essential precursor for the success of reintroduction programs, positive public support should be maintained for the duration of the programs (Friedrich et al., 2014; Hiroyasu et al., 2019; International Union for the Conservation of Nature [IUCN], 2013; Treves et al., 2013, 2013; Williams et al., 2019). Unfortunately, reintroduction programs that continue for years with limited success may be perceived as wasteful and can shift public support against the programs (Best, 2003; Malakoff, 2008; McCarthy, 2007; Watkins et al., 2021). A lack of post-stocking monitoring and adaptive management has been identified as a causal factor in the loss of public enthusiasm for some lake trout reintroductions in Ontario (e.g., Black Sturgeon Lake; Peter Addison, OMNR, pers. comm.).

6.4.3 Management recommendations

The findings presented in this thesis have implications for the conservation of native fishes. Exotic species have been identified as one of the greatest threats to biodiversity, which is supported by my finding in Chapter 2 that the invasion of smelt has negative impacts on native lake trout. Given the high cost and limited success of smelt removal (Dahlsten, 1986; Simberloff, 2002), efforts must be made to prevent further dispersal of smelt. Human transfer is the primary mechanism behind the continued range expansion of smelt (Hrabik & Magnuson, 1999). To prevent further dispersal of smelt into unaffected lakes, greater public education is required

regarding the risk of releasing live smelt and disposing of cleaned smelt (Hrabik & Magnuson, 1999; Litvak & Mandrak, 1993). At the time of writing, smelt are not listed as a restricted or prohibited species under Ontario's Invasive Species Act (Province of Ontario, 2015), although they are listed as a restricted baitfish species in the current fishing regulations (OMNR, 2025c). Further prohibition of possession of live smelt and disposal of dead smelt could help limit their dispersal into unaffected waters.

In lakes where smelt have become established, efforts must be made to limit cumulative effects that may affect resilience of populations of lake trout. In lakes where thiamine deficiency has occurred after introduction of exotic prey species (smelt in Lake Wanapitei, smelt and alewife in Great Lakes), the exotic species were very abundant (Weidel and Dunlop 2018; Weidel et al. 2018; OMNR unpublished results). Maintaining healthy communities of forage fishes is critical; consumption of diverse prey species can limit thiamine deficiency (Brown, Honeyfield, et al., 2005; Futia et al., 2019; Riley et al., 2011) and maintain high growth rates (Chapter 2). Further, efforts to limit human-induced mortality would lower the pressures on populations of lake trout that may already be stressed/affected by smelt. Lastly, eutrophication and blooms of blue-green algae should be prevented. As described above, Lake Wanapitei, the only lake where thiamine deficiency was detected, is also the only lake to have confirmed blooms of blue-green algae (MECP, unpublished results). The presence of algal blooms, paired with the high reliance on smelt by lake trout in Lake Wanapitei, may be a contributing factor to the occurrence of thiamine deficiency in the lake.

6.5 Conclusion

I determined that exotic prey fishes may be a causal factor for the failure of programs to reintroduce lake trout throughout Ontario (Figure 6.1). The negative effects of exotic prey fishes

may be further exacerbated by other stressors in the environment and may contribute to failure of reintroduction programs. I also found evidence that pre-existing adaptations to thiaminase may differ among strains of lake trout and that the choice of source strain may influence success of reintroduction programs (Houde, Garner, et al., 2015). My results suggest that, of the source strains I examined, the Slate strain is likely the most suitable for reintroduction into lakes where exotic prey fishes occur. The Manitou and Iroquois strains may be best suited for reintroduction into lakes in the Sudbury Basin, due to their ancestry match to extirpated populations of lake trout in the area, although more research is warranted. Future screening of other source strains that are used in Ontario's fish culture program, as well as results from post-stocking monitoring, could reveal additional source strains that are good candidates for reintroductions into the Sudbury Basin and into Lake Ontario. This thesis advances our understanding of best practices for species reintroductions, identifies some of the major factors influencing the success of lake trout reintroduction programs in Ontario, and provides information to fisheries managers that will contribute to ongoing efforts to conserve lake trout fisheries.

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Appendix A: Experimental Protocol Approval Records

The experimental protocol used in this study was developed in accordance with the guidelines and regulations of the Canadian Council on Animal Care and approved by the Ontario Ministry of Natural Resources and University of Western Ontario Animal Care Committees (Protocol Number: 2018–084). The experiments carried out in this study are reported in accordance with ARRIVE guidelines (Kilkenny et al., 2010).

University of Western Ontario

Animal Use Protocol #2018-084 (2018-2022) for Chapters 2-5

Animal Use Protocol for #2022-0061 (2022-2026) for Chapters 2 and 5

"Behavioural and molecular ecology of fishes"

Ontario Ministry of Natural Resources

Aquatic Research and Monitoring Section

Animal Use Protocol #ARMS_ACC97C for Chapters 2 and 5

Ontario Ministry of Natural Resources

Sudbury District

License to Collect Fish for Scientific Purposes #1097689 (2012-2023) for Chapters 2 and 5

Ontario Ministry of Environment, Conservation, and Parks

Ontario Parks – Northeast Zone

Research authorization # RA_Neff_NEZ_Aug_2021 for Chapter 2

PI :	Neff, Bryan
Protocol #	2018-084
Status :	Approved (w/o Stipulation)
Approved :	07/01/2018
Expires :	07/01/2022
Title :	Behavioural and molecular ecology of fishes

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Protocol Introduction

The questions on this page activate specific sections within the AUP form.

Note that species selection is part of this introductory page

Does this AUP involve teaching?

Yes ○ No ◉

Is the animal work on this project shared by another Animal Care Committee?

Yes ◉ No ○

Will you be using hazards?

Appendix B: Permission to Reproduce Published Materials

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Appendix C: Supplementary Materials for Chapters 1 and 6

Table C-1 Lake trout (*Salvelinus namaycush*) strains maintained in Ontario's provincial fish culture system.

Strain	Origin	Latitude	Longitude	Type	Diet	Exotic competitors	References
Big Sound	Big Sound, Lake Huron	45.34°	-80.13°	Great Lakes	BL, CI, RS	RT	Reid et al. 2001, Hondrop et al. 2025 , OMNR unpublished results
Iroquois Bay	Iroquois Bay, Lake Huron	46.10°	-81.65°	Great Lakes	AW, BL, CI, RS	CH, SB[b]	Hondrop et al. 2025, OMNR unpublished results
Killala	Killala Lake, Ontario	49.08°	-86.52°	Inland	CI, IN, YP	None	Therrien and Leitrants 2025, OMNR unpublished results
Manitou	Manitou Lake, Ontario	45.77°	-81.98°	Inland	CI, RS	SB[c]	Adeli 2023, Dawe 2023, OMNR unpublished results
Michipicoten	Michipicoten Island, Lake Superior	47.71°	-85.80°	Great Lakes	CI, IN, SU	CH, CO, PS, RT	Vinson et al. 2024, OMNR unpublished results
Mishibishu	Nimoosh River, Lake Superior[d]	48.07°	-85.41°	Great Lakes	CI	None	OMNR 2025
Red Lake	Red Lake, Ontario	51.04°	-93.91°	Inland	CI, RS	None	OMNR 2025
Slate Islands	Slate Islands, Lake Superior	48.67°	-87.02°	Great Lakes	BU, CI, RS, SU	CH, CO, PS, RT	OMNR unpublished results

Seneca Lake	Seneca Lake, New York	42.65°	-76.89°	Finger Lakes	AW, RS	AS, BR, LB[c], SB[b]	Ryder 1972, NYSDEC 2025

Notes

d. Species nomenclature: AS = Atlantic salmon (*Salmo salar*), AW = alewife (*Alosa pseudoharengus*), BL = bloater (*Coregonus hoyi*), BR = brown trout (*S. trutta*), BU = burbot (*Lota lota*), CH = Chinook salmon (*Oncorhynchus tshawytscha*), CI = cisco (*C. artedi*), CO = coho salmon (*O. kisutch*), IN = invertebrates, LB = largemouth bass (*Micropterus salmoides*), PS = pink salmon (*O. gorbuscha*), RS = rainbow smelt (*Osmerus mordax*), RT = rainbow trout (*O. mykiss*), SB = smallmouth bass (*M. dolomieu*), SS = sculpin (*Cottus* spp.), YP = yellow perch (*Perca flavescens*)

e. Indicates the smallmouth bass population in the lake is native.

f. Indicates the origin of the population in the lake is unknown

g. Strain originates from a population in Lake Superior that spawned in the Nimoosh River but was stocked into Mishibishu Lake and wild collections now occur from the lake.

Table C-2 Summary of stomach content from surveys of the Tyson Lake, Lake Manitou, Slate Islands, and Michipicoten Island populations of lake trout (*Salvelinus namaycush*). Data presented are from Adeli et al., (2024) and unpublished data from the OMNR.

Strain	n	Empty	Unknown	Unidentified fish	Rainbow smelt	Cisco	Sculpin[a]	Burbot
Tyson Lake	9	3	3	1	0	2	0	0
Lake Manitou[b]	24	2	1	15	9	2	0	0
Lake Superior								
Slate Islands (Superior)	144	44	8	67	18	2	3	2
Michipicoten[c,d] Island (Superior)	210	47	19	51	0	2	3	2

Note. Rainbow smelt (*Osmerus mordax*), cisco (*Coregonus artedi*), and burbot (*Lota lota*).

[a]No distinguishment in the survey was made between deepwater (*Myoxocephalus thompsonii*), slimy (*Cottus cognatus*), and spoonhead (*Cottus ricei*) sculpins, and were grouped together in the diet analyses.

[b]The stomach of two fish contained invertebrates of the genus *Hexagenia* and one fish contained Chironomidae larvae.

[c]Most of the diet was comprised of invertebrates with 25 fish having some sort of unidentified invertebrate, 1 containing Simuliidae, 8 containing Insecta, 5 containing Annelida, 1 containing Nematoda, 12 containing Hemiptera, 18 containing Coleoptera, 25 containing Mysida, and 1 containing Trichoptera.

[d]The stomach of one fish also contained a ninespine stickleback (*Pungitis pungitis*).

Appendix D: Supplementary Materials for Chapter 2

Table D-1 Capture method of lake trout caught in lakes with and without smelt in the Sudbury basin.

	Method	
Lake	Angling	Gillnetting[a]
Rainbow smelt present		
Bear	15	0
Manitou	25	30
Manitou-2	29	0
Panache	22	0
Wanapitei	28	18
Whiskey	12	0
Rainbow smelt absent		
Bell	8	0
Burwash	31	0
Chiniguchi	0	16
Colin Scott	13	0
Elboga	11	0
Evelyn	14	0
Fraleck	0	4
Kelly #27	6	2
Mozhabong	7	0
Nelson	0	10

Paradise	0	29
Rawson	10	0
Savage	6	0
Shakwa	10	31
Tyson	3	12
Wavy	0	11

Note [a]Fish captured were caught with either North American (NA1; OMNR 2019) or Summer Profundal Index Netting (OMNR 2019) nets.

Table D-2 Water quality of lake trout lakes in and around the Sudbury Basin monitored as a part of the BsM program (2009–2015) or CRADL program (2019–2022). All data were retrieved from the Ontario Ministry of Natural Resources BsM database or Louste-Fillion (2022).

Lake Name	Survey year	pH	Calcium	Potassium	Alkalinity	Total Phosphorus (ug/L)	Sulphate	Nitrate/Nitrite (ug/L)	Dissolved organic carbon	Conductivity (*u* mhos/cm)	Secchi depth (m)
Rainbow smelt present											
Bear[a]											
Manitou	2016	8.01	31.0	0.74	117	5.20	13.9	2	4.30	266	6.1
Manitou-2	2022	6.94	4.53	0.26	11.1	6.44	4.96	130	2.17	40.2	7
Panache	2014	7.33	5.76	0.61	13.7	4.70	8.30	46	3.60	67.4	4.5
Wanapitei	2015	6.89	6.88	0.33	16.7	3.60	6.15	214	5.10	56.8	4.0
Whiskey	2019	6.97	12.9	1.16	7.04	3.10	29.3	324	2.60	99.6	5.1
Rainbow smelt absent											
Bell	2019	6.5	1.42	0.27	2.07	7.60	3.65	8	6.20	18.6	3.6
Burwash[a]											
Chiniguchi	2015	6.21	1.54	0.28	1.72	2.80	5.55	22	1.80	21.3	6.1
Colin Scott[b]	2020	6.49	1.38		5.00	3.10	5.20		1.9		9.8
Elboga[b]	2019	6.26	1.72		5.56	6.00	3.55		7.20		2.2
Evelyn[a]											
Fairbank	2016	6.91	6.70	0.43	15.5	5.00	8.40	44	1.50	61.4	9.0
Fraleck	2019	6.34	1.40	0.33	1.82	4.60	4.20	12	4.90	18.3	2.8
Kelly #27[b]	2019	6.46	1.48		3.20	3.10	5.75		2.50		11.5
Johnnie	2019	6.31	1.16	0.25	1.91	4.80	3.65	18	4.90	16.5	5.45
Mozhabong	2015	6.53	1.78	0.21	4.12	3.60	3.75	30	3.20	21.1	4.3

Nelson	2019	6.53	1.88	0.33	3.45	4.00	5.30	22	2.00	22.8	6
Paradise[b]	2020	6.74	1.40		5.00	7.8	4.90		3.10		6.6
Rawson	2019	6.31	2.64	0.20	1.78	4.10	6.90	6	3.20	23.4	5.6
Savage[a]											
Shakwa[a]											
Tyson	2019	6.51	1.26	0.34	2.25	10.60	4.15	6	5.50	21.5	3.7
Wavy	2019	5.74	0.96	0.24	0.60	5.20	4.40	4	4.40	17.2	5.1

Note. All values in mg/L unless otherwise noted. [a]No water chemistry data collected for Bear, Burwash, Evelyn, Savage, or Shakwa lakes. [b]Potassium, nitrate/nitrite, and conductivity was not collected for Colin Scott, Elboga, Kelly #27, and Paradise Lakes.

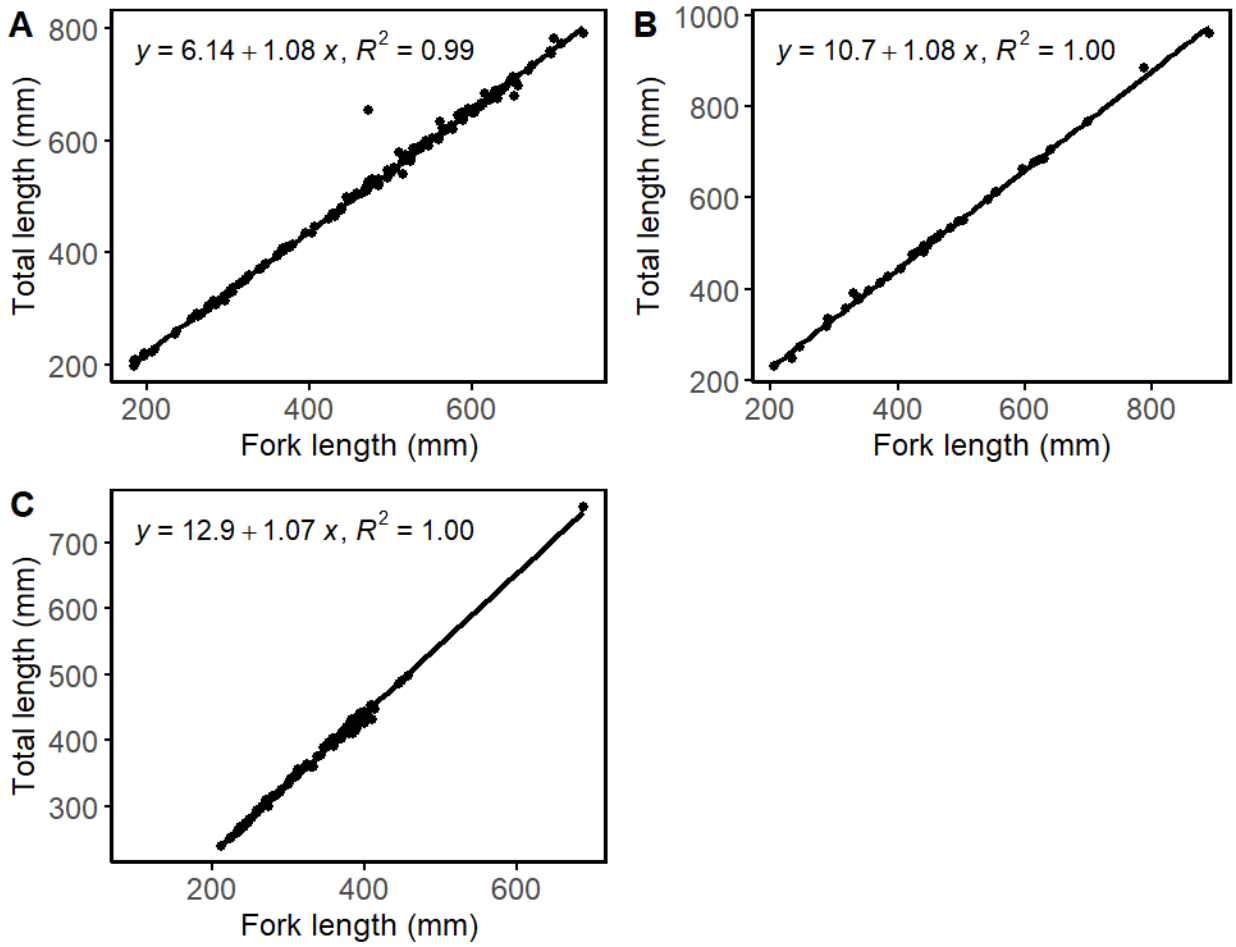


Figure D-1 Relationship between total length (mm) and fork length (mm) of wild lake trout captured from A) Manitou Lake (45.78°, -80.44°) from broadscale monitoring and angling surveys between 2011 to 2022, B) Mozhabong Lake (46.96°, -82.07°) during broadscale monitoring and angling surveys between 2010 and 2023, and C) Rawson Lake (46.91°, -80.56°) from broadscale monitoring and angling surveys between 2010 and 2022.

Table D-3 Sample sizes for each metric measured on lake trout (*Salvelinus namaycush*) from lakes in the Sudbury Basin

Lake	Muscle thiamine concentration	Body condition	Stable isotopes	
			C/N ratio and $\delta^{13}C$	Corrected $\delta^{15}N$
Rainbow smelt present				
Bear	5	15	13	13
Manitou	5	46	10	10
Manitou-2	0	29	10	10
Panache	4	21	16	16
Wanapitei	4	30	14	14
Whiskey	4	7	11	11
Rainbow smelt absent				
Bell	2	8	8	8
Burwash	0	24	14	14
Chiniguchi	0	16	8	0
Colin Scott	0	13	12	12
Elboga	5	11	6	6
Evelyn	5	14	10	10
Fairbank	0	8	7	7
Fraleck	0	4	2	2
Johnnie	0	11	8	0
Kelly #27	4	8	6	6
Mozhabong	2	6	6	6
Nelson	3	10	3	3

Paradise	3	29	2	0
Rawson	4	10	10	10
Savage	3	6	6	0
Shakwa	4	41	20	20
Tyson	2	15	9	9
Wavy	0	9	9	9
Total	59	391	220	190

Table D-4 Summary of stable isotopes for benthic invertebrates (families Heptageniidae, Chironomidae, Unionidae, snails) and bulk seston for each sample lake in the Sudbury Basin (Ontario, Canada). Presented are the mean, min, and max values.

	Heptageniidae			Bulk seston			Chironomidae			Unionidae			Snails[a]		
Lake	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Rainbow smelt present															
Bear	1	-13.08	-0.45	1	-31.20	3.10	1	-31.32	2.61	10	-28.92 (-29.22, -28.38)	0.86 (0.57, 1.22)	1	-15.83	0.55
Manitou	1	-24.1	2.33	1	-30.48	2.93	1	-39.94	1.61	11	-28.60 (-30.34, -27.21)	2.37 (1.93, 2.82)			
Manitou-2	3	-25.38 (-27.40, -24.11)	0.29 (-0.23, 0.97)	1	-37.15	2.02	1	-30.6	2.56	10	-31.85 (-32.72, -30.86)	2.44 (1.51, 3.70)			
Panache	1	-21.59	-1.23	1	-23.09	0.47	2	-29.34 (-30.86, -27.81)	5.15 (4.6, 5.7)	16	-28.26 (-30.69, -26.42)	1.91 (1.03, 2.93)	1	-15.32	-0.26

Wanapitei	1	-25.74	1.99	1	-31.03	3.17	1	-35.92	5.11	-28.92 (-30.84, -28.34)	2.49 (1.92, 3.16)	3	-15.55 (-16.25, -14.37)	1.64 (1.22, 2.11)
Whiskey	1	-19.45	1.52	2	-29.97 (-31.6, -28.33)	5.15 (4.76, 5.54)	1	-30.18	7.83			1	-12.14	2.16
Rainbow smelt absent														
Bell	1	-20.80	0.74	1	-30.20	5.12	1	-33.52	5.01					
Burwash	1	-21.72	0.44	1	-29.44	1.97	2	-29.66 (-29.82, -29.49)	5.80 (5.65, 5.95)					
Chiniguchi				1	-33.90	2.09	1	-37.76	4.26					
Colin Scott	1	-25.37	0.24	1	-35.13	1.78	1	-31.24	3.44					
Elboga	1	-25.82	1.47	1	-33.46	3.14	1	-34.36	6.18					
Evelyn	1	-24.80	0.69	1	-32.67	3.28	1	-44.45	0.63					

Fraleck	2	-28.09 (-28.23, -27.96)	2.39 (2.25, 2.54)	1	-31.90	1.06	3	-31.09 (-33.67, -25.98)	3.9 (0.49, 5.94)			
Kelly #27							1	-31.70	3.86			
Matagamasi							1	-33.88	5.31			
Mozhabong	1	-25.38	-0.37	1	-33.37	1.79	1	-34.94	3.43	5	-31.64 (-32.25, -31.2)	1.93 (1.44, 2.24)
Nelson	1	-24.88	-1.08	2	-33.35 (-34.02, -32.67)	1.14 (1.14, 1.14)	1	-33.06	3.4			
Paradise				1	-37.52	6.15	1	-33.54	7.66	5	-31.38 (-31.88, -30.73)	2.39 (2.26, 2.52)
Rawson	1	-23.74	-0.13	1	-32.97	1.76	1	-34.05	4.98			
Savage												

Shakwa	2	-23.28 (-23.59, -22.96)	1.42 (1.16, 1.69)	1	-32.66	3.81	1	-29.17	3.34	3	-28.51 (-28.71, -28.12)	2.98 (2.86, 3.21)
Tyson	2	-23.54 (-23.57, -23.51)	0.56 (0.37, 0.76)	2	-29.45 (-29.88, -29.02)	4.89 (4.53, 5.25)	1	-30.01	5.77			
Wavy	1	-25.77	0.45	2	-27.61 (-28.24, -26.97)	1.18 (1.07, 1.29)	1	-31.69	2.22			

Notes. Blanks indicate missing data. [a] Snails included the Lymnaeidae, Planorbidae, and Valvatidae families.

Table D-5a. Summary of stable isotopes for prey fishes commonly consumed by lake trout from lakes in the Sudbury Basin (Ontario, Canada). Data were collected during fish collection as a part of this study or from the Boreal Food Webs database (OMNR 2024). Presented are the mean, min, and max values.

	Burbot			Cisco			Lake whitefish			Rainbow smelt		
Lake	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Rainbow smelt present												
Bear[a]												
Manitou	3	-24.17 (-25.19, -23.12)		[b]			[c]			[b]		
Manitou-2	[c]			10	-31.52 (-33.78, -30.01)	7.83 (6.62, 9.53)	[d]			6	-31.17 (-31.25, -31.04)	6.96 (6.65, 7.39)
Panache	[c]			10	-26.64 (-27.79, -26.02)	7.08 (5.43, 9.10)	[c]			6	-27.00 (-29.07, -26.07)	8.61 (7.97, 9.53)

Wanapitei	[b]	13	-27.56 (-28.20, -26.91)	9.31 (8.26, 10.22)	[c]	11	-26.93 (-27.28, -25.95)	9.30 (8.47, 10.15)
Whiskey[a]								
Rainbow smelt absent								
Bell	[d]	5	-29.91 (-32.02, -28.43)	8.05 (7.54, 8.65)	[c]	[d]		
Burwash[a]								
Chiniguchi	[d]	[d]			[d]	[d]		
Colin Scott	[d]	[d]			[d]	[d]		
Elboga	[d]	[d]			[d]	[d]		
Evelyn[a]								

Fraleck	[d]			[d]		[d]		[d]
Kelly #27	[d]			[d]		[d]		[d]
Matagamasi	[d]			[d]		[d]		[d]
Mozhabong[a]								
Nelson	1	-24.33	7.84	[d]		[d]		[d]
Paradise	[c]			[d]		3	-31.09 (-32.15, -30.22)	6.98 (6.59, 7.28)	[d]
Rawson	[d]			[d]		[d]		[d]
Savage[a]								
Shakwa	[d]			[d]		[d]		[d]

Tyson	[d]	8	-29.41 (-30.38, -26.79)	8.45 (7.54, 9.75)	[d]	[d]
Wavy	[d]	[d]			[d]	[d]

Notes. Burbot (*Lota lota*), cisco (*Coregonis artedi*), lake whitefish (*Coregonis clupeaformis*), and rainbow smelt (*Osmerus mordax*). Only prey fishes with a fork length less than 301mm were included in this table. [a] Prey fish were not collected in this lake. [b]Species not collected at this lake. [c]No individuals under 300mm collected. [d]Species does not occur at this lake

Table D-5b. Summary of stable isotopes for prey fishes commonly consumed by lake trout from lakes in the Sudbury Basin (Ontario, Canada). Data were collected during fish collection as a part of this study or from the Boreal Food Webs database (OMNR 2024). Presented are the mean, min, and max values.

	Smallmouth bass			Slimy sculpin			White sucker			Yellow perch		
Lake	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Rainbow smelt present												
Bear[a]												
Manitou	6	-22.67 (-24.17, -21.41)	8.96 (8.2, 9.98)	[b]			[c]			[b]		
Manitou-2	5	-25.98 (-27.72, -22.19)	6.95 (6.64, 7.77)	7	-27.75 (-31.27, -22.62)	7.23 (6.4, 8.37)	7	-28.56 (-30.26, -26.61)	6.54 (5.72, 9.82)	1	-24.31	5.5
Panache	3	-23.32 (-24.76, -20.81)	8.36 (7.80, 9.13)	10	-26.65 (-26.88, -26.02)	8.68 (7.93, 9.31)	10	-23.14 (-24.14, -21.49)	6.43 (5.92, 7.51)	7	-23.63 (-27.08, -19.68)	5.90 (5.46, 6.73)

Wanapitei	3	-21.19 (-23.51, -19.06)	9.15 (8.57, 9.57)	16	-25.02 (-26.19, -23.26)	8.74 (7.38, 10.79)	14	-21.73 (-27.13, -13.20)	7.68 (5.51, 9.19)	2	-19.76 (-19.46, -18.89)	7.10 (7.02, 7.18)
Whiskey[a]												
Rainbow smelt absent												
Bell	7	-26.32 (-26.67, -25.63)	8.10 (7.23, 8.98)	[d]			7	-29.72 (-30.27, -28.68)	7.44 (6.71, 9.18)	[c]		
Burwash[a]												
Chiniguchi	8	-27.90 (-29.55, -26.30)	6.99 (6.21, 7.89(	[d]			7	-26.83 (-29.09, -24.84)	6.37 (5.72, 7.71)	2	-26.3 (-27.07, -25.53)	4.93 (4.7, 5.16)
Colin Scott	[d]			[d]			[d]			[d]		
Elboga	[d]			3	-31.79 (-33.59, -30.22)	8.81 (8.54, 9.32)	5	-31.30 (-32.53, -28.69)	7.86 (7.36, 8.03)	[d]		

Evelyn[a]											
Fraleck	5	-26.91 (-27.72, -25.77)	7.84 (7.49, 8.42)	[d]	2	-28.33 (-28.73, -27.94)	5.62 (5.28, 5.95)	[b]			
Kelly #27	[d]			[d]	[d]			2	-27.84 (-28.70, -26.89)	4.63 (3.98, 5.29)	
Matagamasi	6	-26.25 (-26.58, -25.92)	6.88 (6.45, 7.28)	[d]	3	-27.02 (-27.64, -26.53)	4.67 (4.44, 5.04)	14	-27.14 (-28.69, -25.5)	6.47 (5.43, 7.31)	
Mozhabong[a]											
Nelson	3	-26.76 (-27.14, -26.56)	6.29 (6.21, 6.37)	[d]	7	-25.27 (-27.08, -22.96)	5.13 (4.78, 5.39)	7	-27.54 (-28.66, -26.21)	5.54 (4.52, 6.71)	
Paradise	3	-26.16 (-26.87, -25.36)	7.31 (7.28, 7.32)	[d]	1	-27.95	5.66	[b]			
Rawson	23	-26.31 (-27.51, -24.56)	8.18 (6.59, 9.41)	[d]	[c]			[b]			

Savage[a]										
Shakwa	[d]			[d]		3	-26.38 (-28.21, -25.27)	6.65 (6.09, 7.46)	7	-27.96 (-29.67, -25.91)	6.73 (6.04, 7.61)
Tyson	8	-26.10 (-27.11, -24.9)	7.70 (6.46, 8.93)	[d]		[c]			7	-27.68 (-29.39, -26.35)	6.73 (5.1, 7.44)
Wavy	[d]			[d]		[d]			7	-28.02 (-30.55, -26.32)	6.61 (5.82, 7.64)

Notes. Smallmouth bass (*Micropterus dolomieu*), slimy sculpin (*Cottus cognatus*), white sucker (*Catostomus comersonii*), and yellow perch (*Perca flavescens*). Only prey fishes with a fork length less than 301mm were included in this table. [a] Prey fish were not collected in this lake. [b]Species not collected at this lake. [c]No individuals under 300mm collected. [d]Species does not occur at this lake

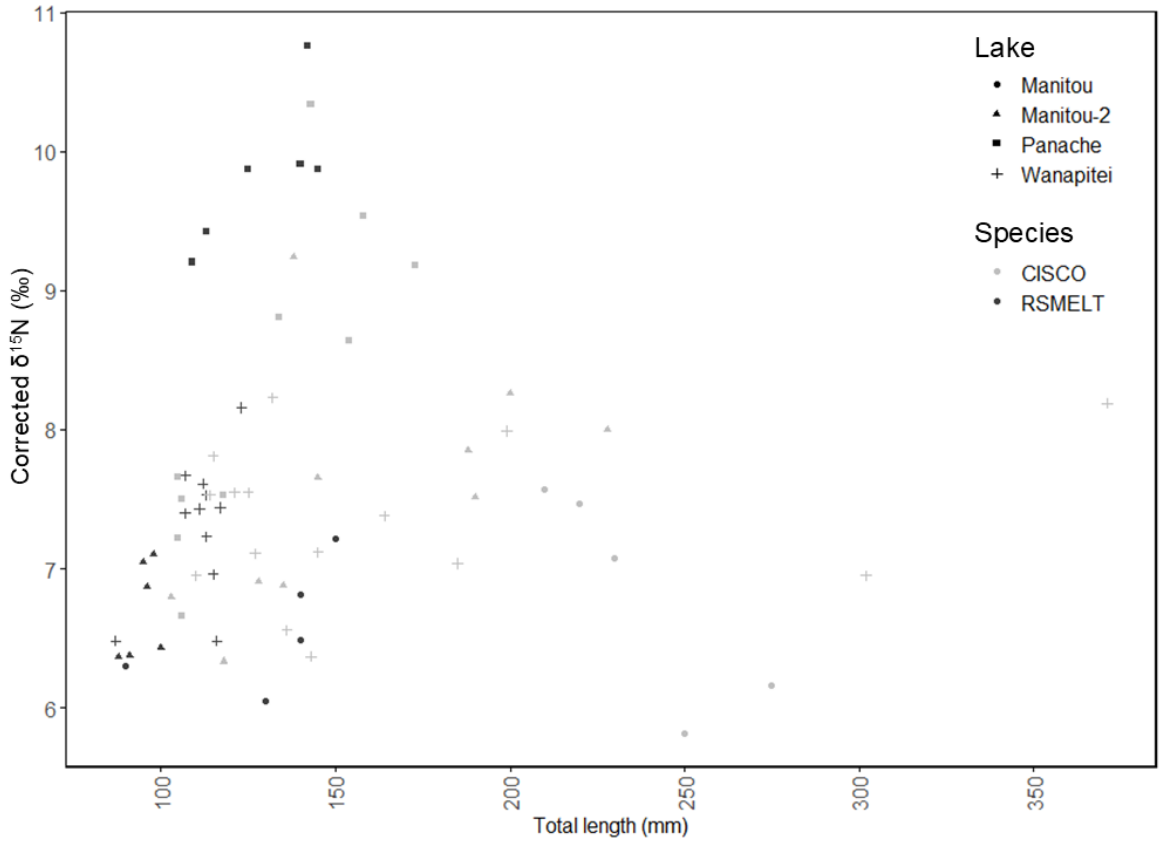


Figure D-2 Relationship between white muscle corrected $\delta^{15}N$ (‰) isotopic signatures and total length (mm) of wild cisco (light grey) and rainbow smelt (RSMELT; dark grey) from Manitou (circle), Manitou-2 (triangle), Panache (square), and Wanapitei (cross) Lakes in the Sudbury Basin. When analyzed with a linear mixed models with species and total length as fixed and lake as a random effect, there was a significant relationship between the corrected $\delta^{15}N$ signature and fish total length ($F_1 = 10.01$, n = 69, p = 0.001, $R^2_{marg.} = 0.06$, $R^2_{cond.} = 0.68$), suggesting that with a 100 mm increase in total length, $\delta^{15}N$ increased by 0.6 ‰ (95% CI; 0.2−1.1 ‰). Smelt were also significantly higher in corrected $\delta^{15}N$ signatures than cisco ($F_1 = 4.28$, n = 69, p = 0.04) by approximately 0.45 ‰ (95% CI; 0.02−0.88 ‰) at a given length. The random effect of lake accounted for approximately 62 % of the variance.

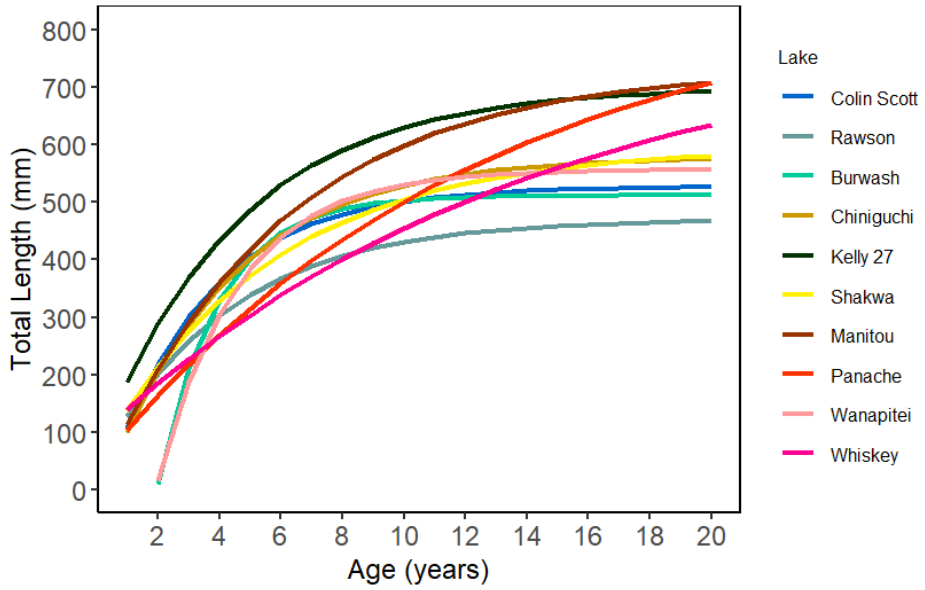


Figure D-3 Von Bertalanffy growth function (VBGF) curves of lake trout populations in the Sudbury basin. VBGF functions were calculated using methods following (Ogle, 2013) in R (R core team, 2024) package FSA (version 0.9.6; Ogle 2025).

Appendix E: Supplementary Materials for Chapter 3

Table E-1 Diet composition and proximate analysis of experimental lake trout (*Salvelinus namaycush*) diets. Adapted with permission from Baker et al. (2023).

Variable	Control (g/kg)	Thiaminase (g/kg)
Diet Composition		
Dry ground herring	320	320
Corn starch	300	300
Corn gluten meal	180	180
Blood flour	86	86
Menhaden oil	80	80
Betaine-HCl	10	10
Dextrin	10	10
Choline chloride	5	5
Vitamin premix	5	5
Mineral premix	2	2
Ascorbic acid	2	2
Nutrient broth	300 mL	300 mL
Difco nutrient broth	2.7	2.7
Yeast extract	0.3	0.3
ddH_2O	100 mL	100 mL
Bacterial thiaminase	none	trace
Proximate Analysis	%	%
Carbohydrates	31.6	33.1
Proteins	37.0	36.8
Crude fat	22.4	18.8
Ash	2.16	4.13
Moisture	6.82	7.19
Thiamine (nmol/g)	6.92 ± 5.8	7.05 ± 5.2

Table E-2 Composition of the vitamin (5 g/kg; Dyets #399751) and mineral (2 g/kg; Dyets #200030) premixes used in experimental lake trout (Salvelinus namaycush) diets. Mixes were prepared and provided by Dyets Inc. (Bethlehem, PA, USA). Adapted with permission from Baker et al. (2023).

Ingredient	Concentration (g/kg)
Vitamin premix	
Niacin	0.025
Calcium Pantothenate	0.05
Pyridoxine HCl	0.015
Thiamin HCl	0
Riboflavin	0.0175
Folic acid	0.005
Biotin	0.000375
Vitamin E Acetate (500 iu/g)	0.25
Vitamin B12 (0.1%)	0.025
Vitamin D3 (400,000 iu/g)	0.015
Vitamin A Palmitate (250,000 iu/g)	0.025
Vitamin K1 Premix (10 mg/g)	1.375
Dextrose	3.197125
Total	5
Mineral premix	
Calcium Phosphate, dibasic	1.47
Calcium Carbonate	0.042
Sodium Chloride	0.0612
Potassium Phosphate, dibasic	0.162
Potassium Sulfide	0.136
Sodium Phosphate, dibasic	0.0428
Magnesium Oxide	0.05
Manganous Carbonate	0.008424
Ferric Citrate, U.S.P.	0.02328
Zinc Carbonate	0.00162
Cupric Carbonate	0.000666
Potassium Iodide	0.0000144
Citric acid	0.0019956
Total	2

Appendix E-1. Methods for thiamine concentration determination

Two methods were used for thiamine analysis. Thiamine analyses on the culture supernatants was carried out using a previously developed competitive binding assay using the *Escherichia coli* thiamine periplasmic binding protein (Edwards et al., 2016), which was previously established for use in culture media as a sample matrix (Sannino et al., 2018). Culture supernatants dilutions were made 10-fold in 990 mM MES, 20 mM NaCl, pH 6.5, then 8 µL of this was added to 392 µL 0.3% (v/v) TCA in 50 mM MES, 200 mM NaCl, pH 6.5 (Edwards et al., 2016). We also analyzed the food by homogenizing samples at 50 mg/mL in 7.5% TCA as above, neutralizing the samples at a 5-fold dilution with 990 mM MES, 20 mM NaCl, pH 6.5, then 8 µL of this was added to 392 µL 0.3% (v/v) TCA in 50 mM MES, 200 mM NaCl, pH 6.5. This assay was also used to determine thiamine concentrations in an initial set of Slate strain muscle samples after consuming both diets for 127 days. 16 µL of these sample extracts in 7.5% TCA was neutralized with 64 µL 990 mM MES, 20 mM NaCl, pH 6.5, then diluted with 320 µL 50 mM MES, 200 mM NaCl, pH 6.5 to a final tissue concentration of 2 mg/mL. The sample diluent and dilution for each sample type matched the composition of the standard diluent and was chosen to be within dilution range of the assay. A five-parameter logistic fit was used to fit the response to thiamine standard concentrations (Figure E-1).

Subsequent samples were assayed using an in-house developed digestion assay for thiamine quantification. The extractions were carried out as above, except using 3.75 mL/g tissue 5% (v/v) TCA and 3.75 mL/g tissue 10% (v/v) TCA. The neutralized extract was digested using recombinant thiaminase I from *Clostridium botulinum* at a final concentration of 1 µg/mL in the presence of 1 mM pyridoxine, serving as a cosubstrate for the enzyme, for 24 hours, alongside a similarly diluted thiaminase-free control. The thiamine concentration was determined by

converting thiamine to thiochrome using alkaline potassium ferricyanide (0.0075% (w/v) in 15% sodium hydroxide) and comparing it to thiamine standards prepared in the same composition of TCA, buffer, and alkaline ferricyanide. The muscle matrix has a significant signal suppression either due to interference with thiochrome formation or fluorescence quenching. The thiamine concentration determined against matrix-free thiamine standards must be multiplied by 4.7 to correct this suppression in muscle. The signal suppression in liver versus matrix-free thiamine standards was less significant, and these values needed to be multiplied by 1.3. Most muscle tissue samples after 232 days analyzed did not produce a detectable thiochrome signal below ~1.5 nmol/g owing to the high background signal. As such, most muscle thiamine concentrations in most fish analyzed after 232 days are likely thiamine deficient. We note that while HPLC-based methods can provide speciation and a breakdown of thiamine forms, the thiamine periplasmic binding protein detects thiamine and its mono and diphosphates and only an overall value can be reported. Efforts to validate this competitive binding method against HPLC analysis and establish it as a high throughput option for fish tissue analysis are ongoing.

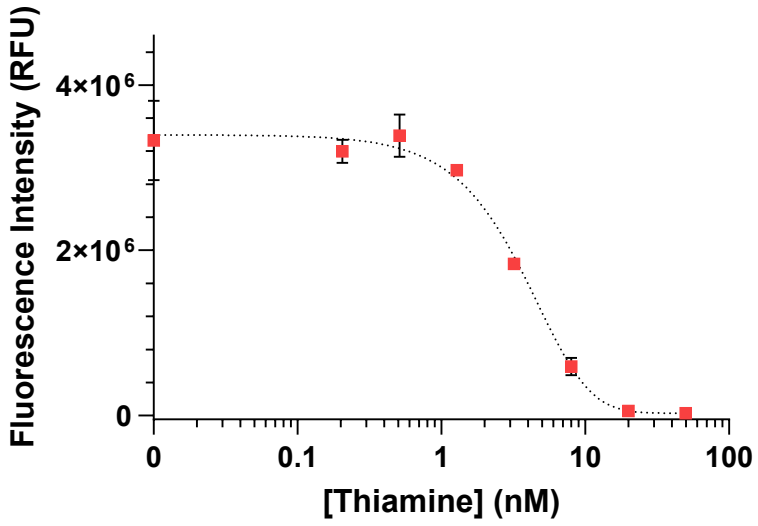


Figure E-1 Calibration curve for the thiamine standards run in triplicate using the thiamine periplasmic binding assay. Data was determined from standards fitted using a 5-parameter logistic fit.

Appendix E-2. Thiaminase assays

Thiaminase activity in a subsample of culture supernatants was determined by the 4-NTP assay, carried out as described in Wolfe et al., (2023) against a control of recombinant thiaminase from *C. botulinum* at a pH of 5.9. The assay relies on the degradation of 4-NTP, a co-substrate of thiaminase I, monitored by decline in its absorbance at 411 nm in the presence and absence of added thiamine. Samples were measured in triplicate for rates of 4-NTP breakdown I endogenous samples (basal) relative to thiamine supplemented. Slopes between 5 and 14 minutes were used for data analysis. The difference in these rates indicating the thiamine-specific 4-NTP breakdown is the thiaminase activity. Thiaminase activity values were not standardized to the protein concentrations in the supernatant.

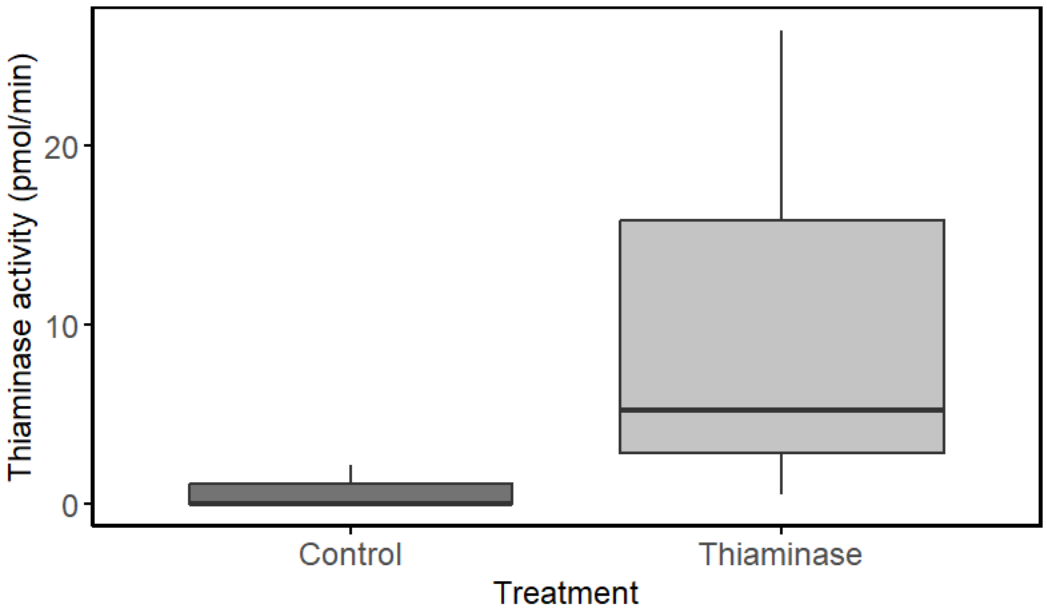


Figure E-2 Thiaminase activity (pmol/min) in control (dark gray) and *P. thiaminolyticus* (thiaminase; light grey) supernatants measured by the 4-NTP assay. Boxplots show the median and first and third quartiles of the technical replicates. Whiskers show minimum and maximum values of technical replicates. 3 technical replicates were run on a sample of both control and thiaminase supernatants. Thiaminase activity values were not standardized to the protein concentrations in the supernatant.

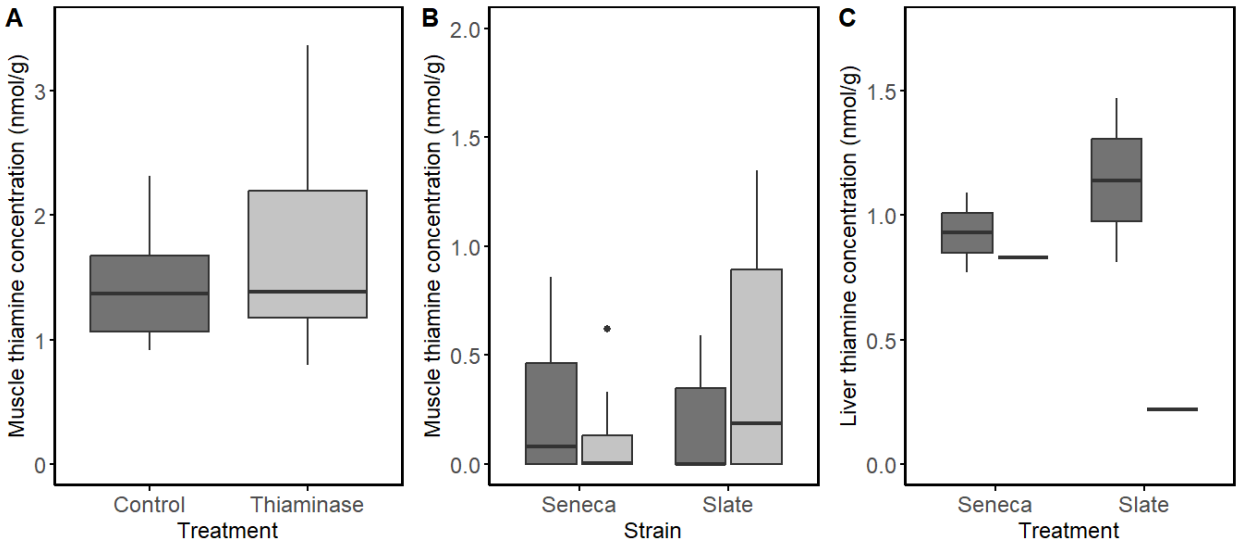


Figure E-3 Tissue thiamine concentrations of A) muscle from Slate strain fish fed either a control (dark grey) or thiaminase (light grey) diet after 127 days, B) muscle, and C) liver from fish of Seneca and Slate Island strain fish fed either a control or thiaminase diet after 232 days. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1.

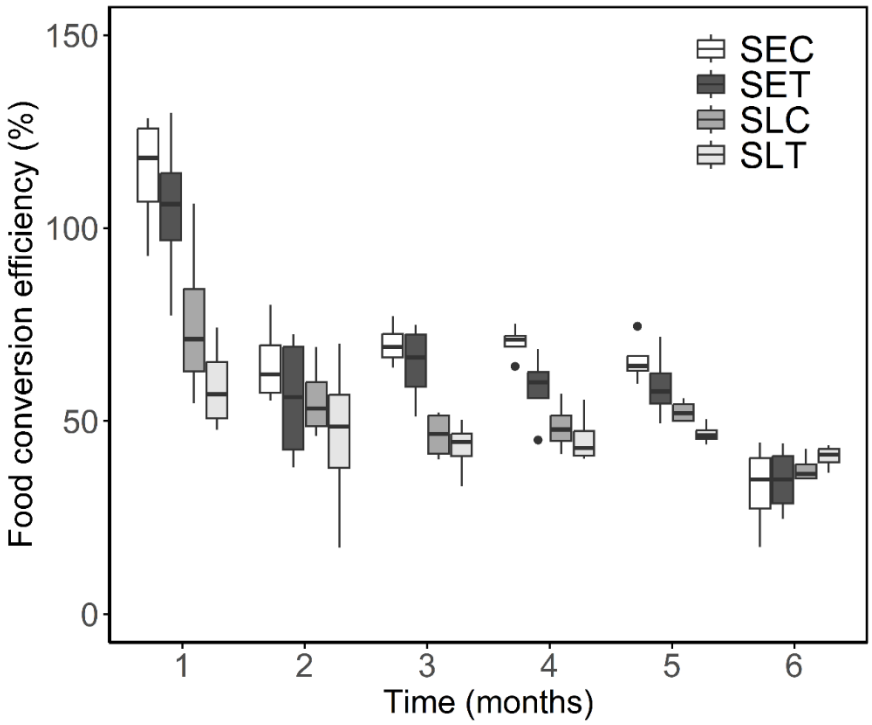


Figure E-4 Food conversion efficiency (%) of lake trout from the Slate (SL) and Seneca (SE) strains after each month fed either a control (C) or thiaminase-added (T) diet. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 x interquartile range greater than Q3 or smaller than Q1. There was a significant difference between strains ($p < 0.05$) but not between diet groups, nor was there a significant interaction ($p > 0.05$).

Appendix F: Supplementary Materials for Chapter 4



Figure F-1 Experimental hatchery at the University of Western Ontario (London, ON, Canada). Shown are the two separate recirculation loops each feeding eight 73 L tanks.

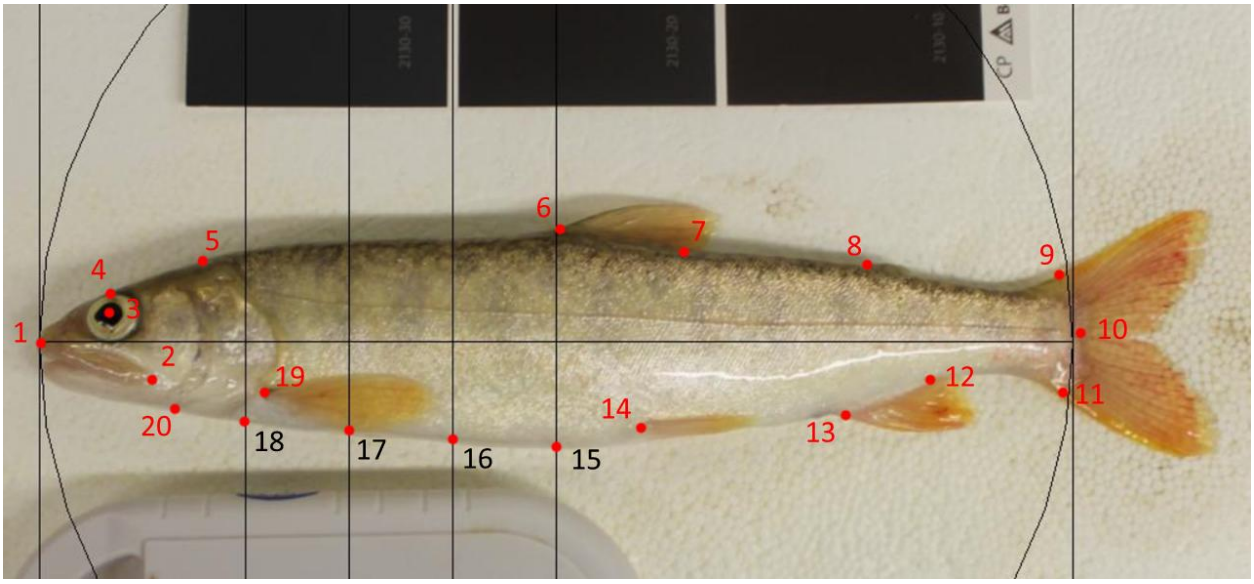


Figure F-2 Landmark and semi-landmark positions digitized on juvenile lake trout. Homologous landmarks are represented by red text and semi-landmarks by black text. Sixteen homologous landmarks and four semi-landmarks were placed on each fish body; (1) tip of the snout, (2) posterior tip of the maxillary, (3) center of the eye, (4) top of the head above the center of the eye, (5) posterior of neurocranium above the edge of opercula, (6) anterior insertion of dorsal fin, (7) posterior insertion of dorsal fin, (8) anterior insertion of adipose fin, (9) dorsal insertion of caudal fin, (10) midpoint of hypural plate, (11) ventral insertion of caudal fin, (12) posterior insertion of anal fin, (13) anterior insertion of anal fin, (14) anterior insertion of pelvic fin, (15) 50% of body length, (16) 40% of body length, (17) 30% of body length, (18) 20% of body length, (19) anterior insertion of pectoral fin, (20) connection (isthmus) between branchiostegals.

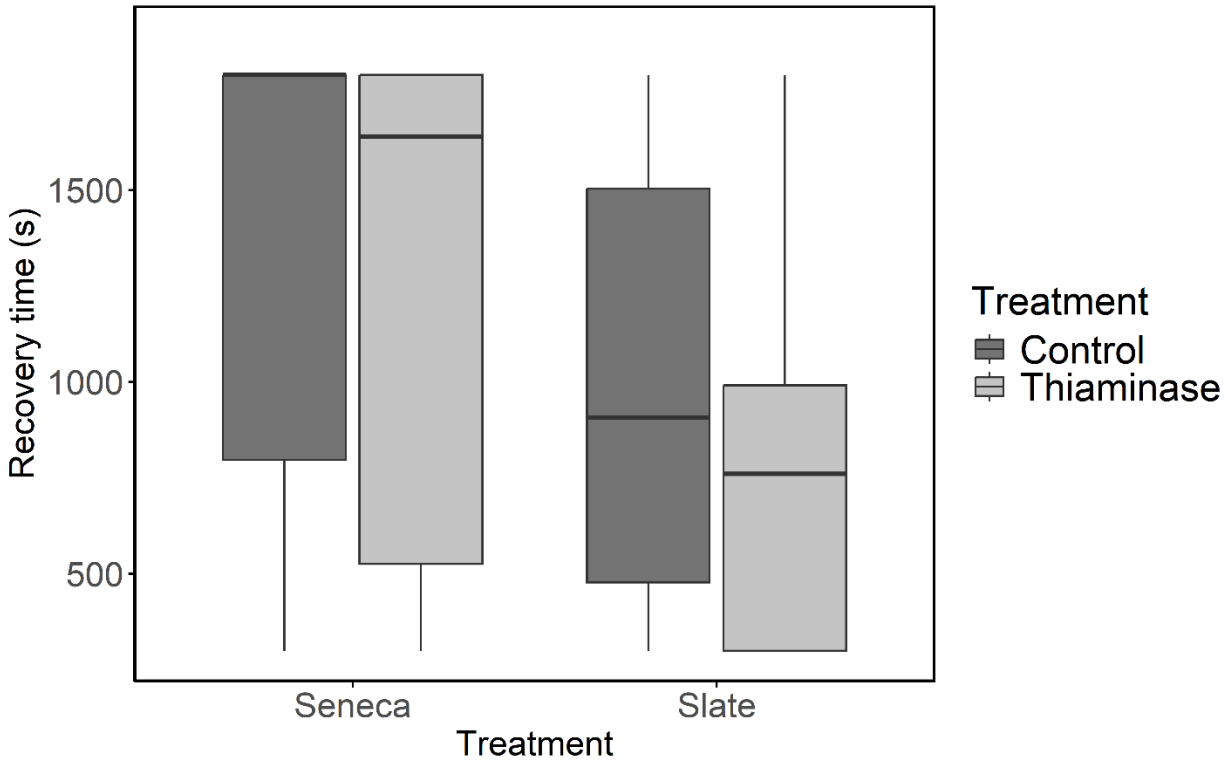


Figure F-3 Recovery time (s) of lake trout post exhaustive exercise from the Seneca and Slate Island strains fed either a control (dark grey) or thiaminase (light grey) diet for 6 months. Recovery trials were ended if a fish did not recover by 1800 s. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values.

Table F-1 Loadings of the first two principal components of the LAB colour space values for the body regions of juvenile lake trout (*Salvelinus namaycush*). L is a black to white colour scale, A is a green to red colour scale, and B is a blue to yellow colour scale.

Variable	PC1	PC2	PC3
dorsal L	-0.344	-0.155	-0.297
dorsal A	0.150	-0.055	-0.061
dorsal B	-0.159	0.040	-0.432
lateral L	-0.388	-0.088	-0.201
lateral A	0.294	-0.243	-0.099
lateral B	0.136	0.420	-0.152
ventral L	-0.302	0.061	-0.158
ventral A	0.260	-0.331	-0.197
ventral B	0.220	0.371	-0.115
caudal peduncle L	-0.344	-0.183	-0.172
caudal peduncle A	0.221	-0.092	-0.488
caudal peduncle B	0.050	0.449	-0.210
caudal fin L	-0.388	0.047	0.047
caudal fin A	0.221	-0.195	-0.457
caudal fin B	0.014	0.439	-0.220

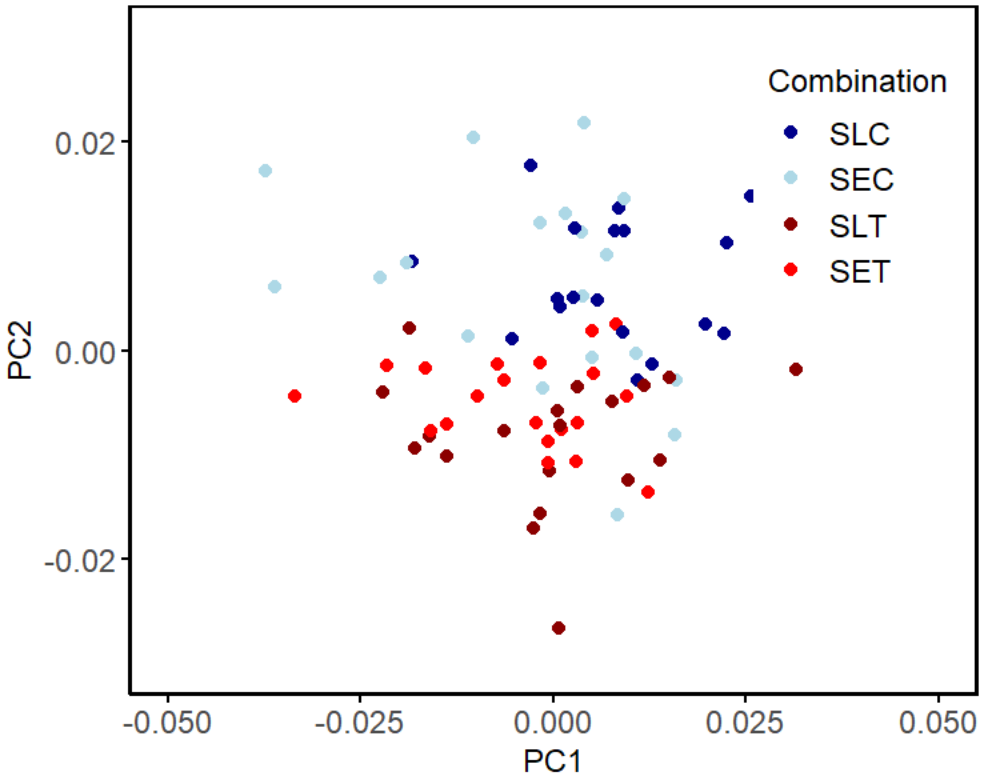


Figure F-4 Principal components 1 and 2 of a PCA performed on 20 morphological landmarks from lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Dots in light blue are fish of the Seneca Lake strain fed the control diet (SEC), dark blue represent fish of the Slate Islands strain fed the control diet (SLC), light red represents fish of the Seneca Lake strain fed a the thiaminase diet (SET), and dark red represents fish of the Slate Island strain fed the thiaminase diet (SLT). Positive principal component 1 (PC1; A) scores were associated with a deeper dorsal-ventral depth and more curved body shape. Positive principal component 2 (PC2; B) scores were associated with a thinner anterior end and a longer posterior end.

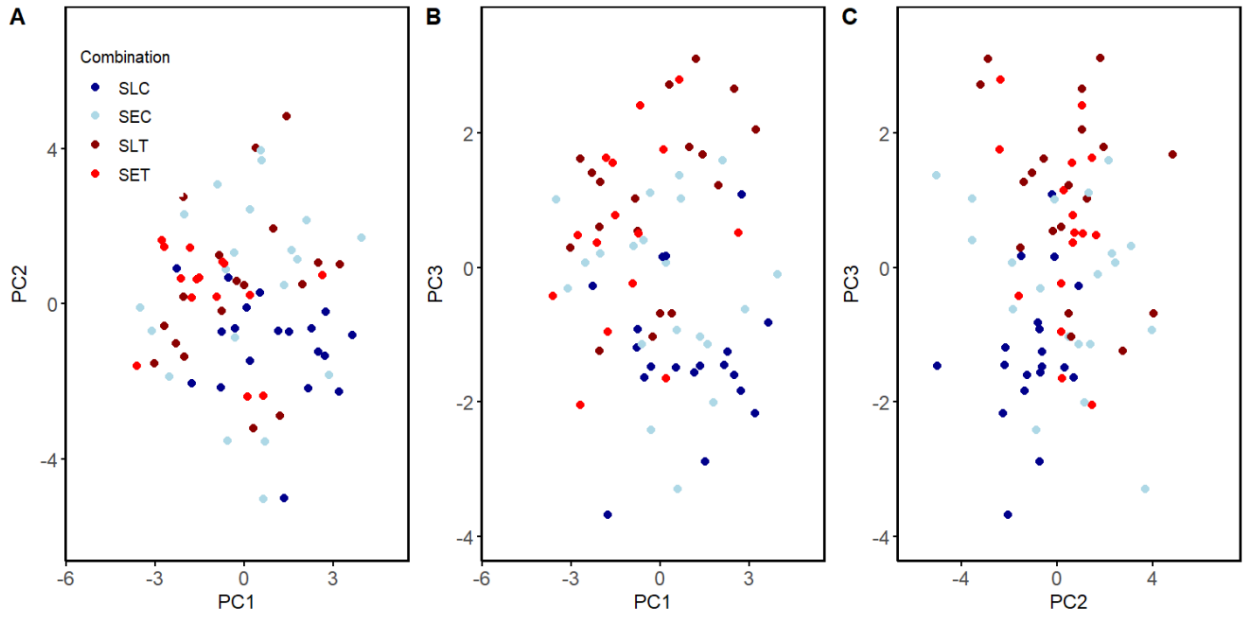


Figure F-5 Plots of the principal components A) 2 and 1, B) 3 and 1, and C)3 and 2 from the principal component analysis of LAB colour values from 5 body regions on lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Dots in light blue are fish of the Seneca Lake strain fed the control diet (SEC), dark blue represent fish of the Slate Islands strain fed the control diet (SLC), light red represents fish of the Seneca Lake strain fed a the thiaminase diet (SET), and dark red represents fish of the Slate Island strain fed the thiaminase diet (SLT). Positive principal component 1 (PC1; A) scores were associated with darker whole body colour space values, positive principal component 2 (PC2; B) scores were associated with more yellow whole body colour spaces values, and positive principal component (PC3; C) scores were associated with a greener peduncle and fin colouration.

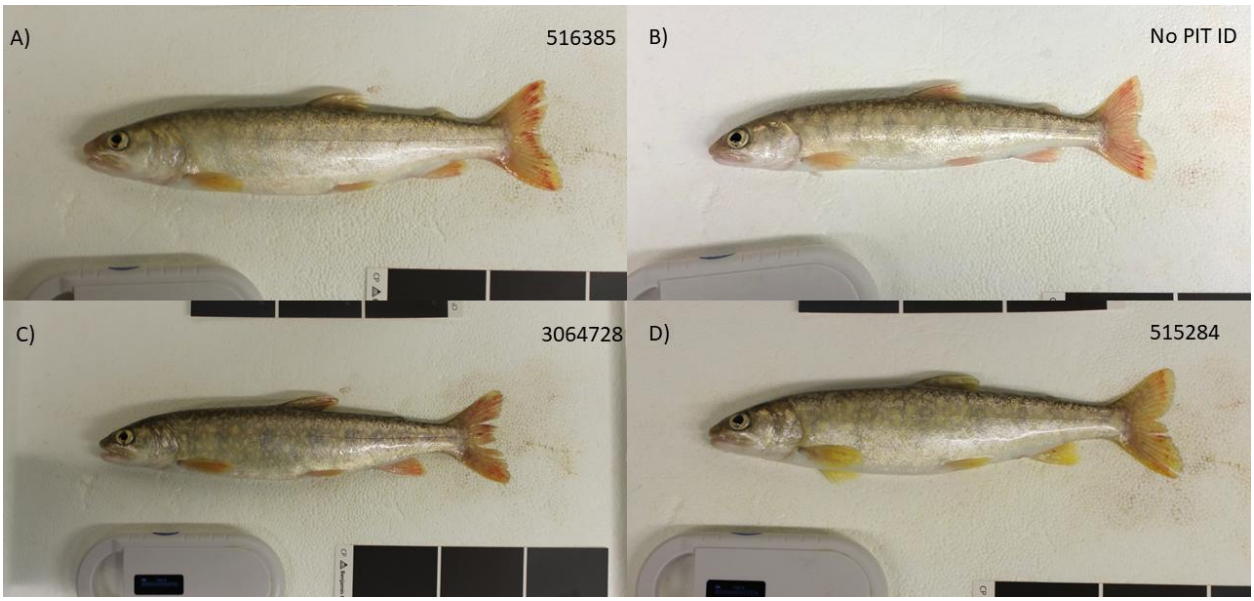


Figure F-6 Photographs of juvenile lake trout for morphological and colourmetric analyses. A) Is an example of a fish from the Seneca strain in the control treatment, B) is a fish from the Seneca strain in the thiaminase treatment, C) is a fish from the Slate strain in the control treatment, and D) is a fish from the Slate strain in the thiaminase treatment.

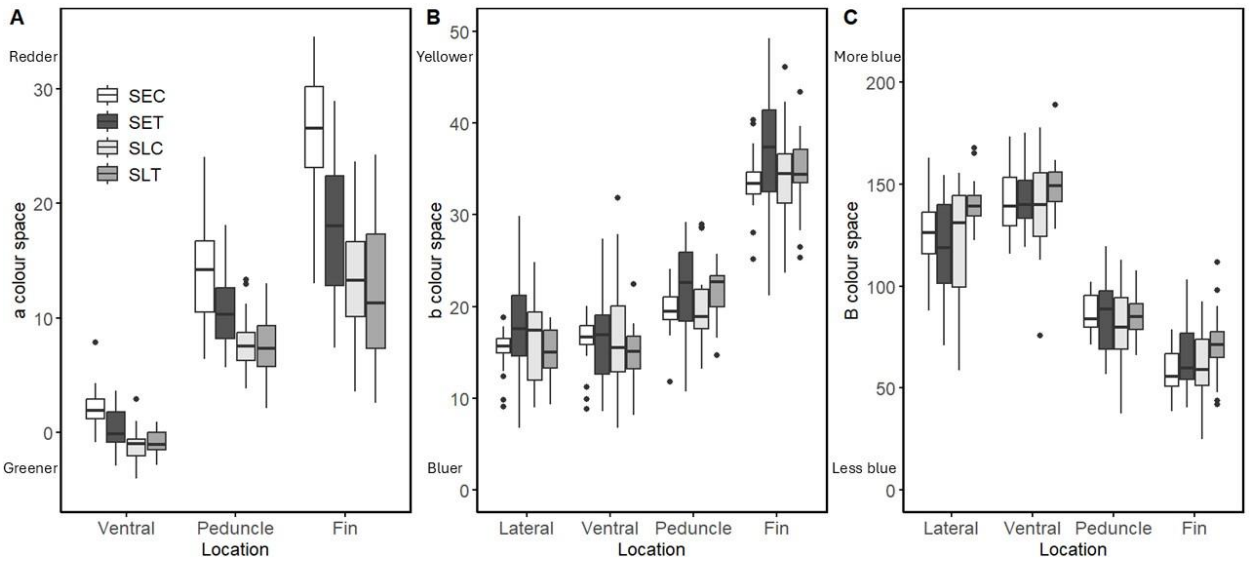


Figure F-7 Raw A) a (red-green), B) b (yellow-blue), and C) B (blue) colour space values from body regions on lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Positive a colour space values (A) scores are associated with more red pigmentation, positive b colour space values (B) scores were associated with more yellow pigmentation, and positive B colour space values (C) scores were associated with more blue pigmentation. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1.

Appendix G: Supplementary Materials for Chapter 5

Table G-1 Summer (June-August) surface water pH of Tyson Lake from 1971−2022. Data from Louste-Fillion (2022), Polkinghorne and Gunn (1981), and Ontario Ministry of the Environment, Conservation, and Parks unpublished data.

Year	pH
1971	5.14
1981	5.29
1982	5.46
1983	5.42
1984	5.56
1985	5.6
1986	5.7
1987	5.75
1988	5.62
1989	5.45
1990	5.61
1991	5.64
1992	5.42
1993	5.83
1994	5.75
1995	5.97
1996	5.83
1997	6.29
1999	6.33
2000	6.27
2001	6.25
2002	6.15
2003	6.23
2004	6.2
2005	6.41
2006	6.41
2007	6.42
2008	6.48
2009	6.29
2010	6.54
2011	6.59
2012	6.54
2013	6.42
2014	6.34
2015	6.54
2016	6.43
2017	6.56
2018	6.51
2019	6.39
2022	6.56

Note. Water samples taken once annually

Table G-2 Characteristics of lake trout (Salvelinus namaycush) source populations for the Killala lake, Lake Manitou, Iroquois Bay, Slate Islands, and Michipicoten Island (Ontario, Canada) hatchery strains. Data presented are unpublished data from the OMNR and Christian Therrien.

Lake	N	Total length (mm)	Mass (g)	Age	K[a] (yr^{-1})	L_∞[b] (mm)
Inland						
Killala Lake	51	621 (243, 899)	2187 (88, 6700)	15 (5, 30)	0.143	740
Lake Manitou	132	698 (411-737)	1797 (539-3685)	(2, 26)	0.17	731.9
Huron						
Iroquois Bay	36	407 (138, 768)	1013 (60, 4500)	-	-	-
Superior						
Slate Island	3147	475 (173, 895)	1088 (30, 7795)	13 (1, 43)	0.143	574
Michipicoten Island	210	480 (201, 811)	1098 (55, 5940)	-	-	-

Note. Length, mass, and age data show the mean and in parentheses (min, max). Dash indicates missing data. Both the [a]Brody coefficient or the exponential rate of approach to the asymptotic size (Schnute & Fournier, 1980) and [b]asymptotic age length parameters of Von Bertalanffy growth functions calculated for all three populations (Cailliet et al., 2006). Age data was unavailable for the Iroquois Bay and Michipicoten Island populations so Von Bertalanffy growth functions could not be calculated.

Table G-3 Fish community composition of Tyson Lake (Ontario, Canada), the origin lakes of the lake trout (Salvelinus namaycush) strains stocked into Tyson Lake, and of the Iroquois Bay and Lake Manitou populations of lake trout. Data for fish species assemblages were obtained from Land Information Ontario (LIO), unpublished data from the OMNR, Adeli et al., (2024), and Vinson et al., (2022).

Lake	Piscivores[a]	Benthivores[b]	Littoral prey[c]	Pelagic prey[d]
Tyson Lake	LB, LT, NP, RB, SB	BB, WS	BG, GS, PS, YP	CI
Inland Lakes				
Killala Lake	BU, LT, NP	LW, LS, RW, WS	YP	CI
Lake Manitou	BT, BU, LT, NP, RB, SB, WA	BB, LW, WS	MS, PS, YP	CI, RS
Huron				
Iroquois Bay	CH, LB, LG, LT, MU, NP, RB, SB	CC, LW	BG, CS, PS, YP,	AL, CI, DS, RS
Superior				
Slate Island	BT, BU, CH, CO, LT, NP, PS, RT	LW, LS, PW, RW	NS	BL, CI, RS, SP
Michipicoten Island	BU, LT, CH, CO, LT, PS, RT	LS, LW, RW	NS	CI, DS, RS, SC, SS, SP

a. Piscivore species codes: brook trout (*Salvelinus fontinalis*; **BT**), burbot (*Lota lota*; **BU**), Chinook salmon (*Oncorhynchus tshawytscha;* **CH**), coho salmon (*O. kisutch;* **CO**), largemouth bass (*Micropterus salmoides*; **LB**), longnose gar (*Lepisosteus osseus*; **LG**), lake trout (*S. namaycush*; **LT**), muskellunge (*Esox masquinongy*; **MU**), northern pike (*E. lucius*; **NP**), pink salmon (*O. gorbuscha;* **PS**), rock bass (*Ambloplites rupestris*; **RB**), rainbow trout (*O. mykiss*; **RT**), smallmouth bass (*Micropterus dolomieu*; **SB**), walleye (*Sander vitreus*; **WA**).

b. Benthivore species codes: brown bullhead (*Ameiurus nebulosus*; **BB**), common carp (*Cyprinus carpio*; **CC**), lake whitefish (*Coregonus clupeaformis*; **LW**), longnose sucker (*Catostomus catostomus*; **LS**), pygmy whitefish (*P. coulterii;* **PW**), round whitefish (*Prosopium cylindraceum*; **RW**), white sucker (*Catostomus commersonii*; **WS**).

c. Littoral prey species codes: bluegill (*Lepomis macrochirus*; **BG**), common shinner (*Luxilus cornutus*; **CS**), golden shiner (*Notemigonus crysoleucas*; **GS**), mimic shiner (*Paranotropis volucellus*; **MS**), ninespine stickleback (*Pungitius pungitius*; **NS**), pumpkinseed *(Lepomis gibbosus*; **PS**), yellow perch (*Perca flavescens*; **YP**)

d. Pelagic prey species codes: alewife (*Alosa pseudoharengus*; **AL**), bloater (*C. hoyi*; **BL**), cisco (*C. artedi*; **CI**), deepwater sculpin (*Myoxocephalus thompsonii*; **DS**), shortjaw cisco

(*C. zenithicus*; **SC**), rainbow smelt (*Osmerus mordax*; **RS**), slimy sculpin (*Cottus cognatus*; **SS**), spoonhead sculpin (*Cottus ricei*; **SP**).