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The effect of dietary thiaminase on cardiac function and morphology in lake trout (Salvelinus namaycush)

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

Abstract

Thiamine deficiency from the consumption of invasive, high-thiaminase prey fishes is considered to be a major barrier for lake trout restoration in the Great Lakes. In fishes, an understudied aspect of thiamine deficiency is its effect on cardiac function. I examined the effects of dietary thiaminase on cardiac function and morphology in lake trout, specifically as they relate to thermal tolerance. Two hatchery strains of lake trout (Seneca and Slate) were raised on a control or thiaminase diet for nine months. The thiaminase diet was associated with significant ventricle enlargement, impaired cardiac function, and reduced thermal tolerance; these effects were more pronounced in Slate strain fish. Similar cardiac morphological changes were observed in wild-caught lake trout from the Sudbury Basin. These results suggest that dietary thiaminase impairs cardiac function and alters cardiac morphology in fishes, and that such changes may become increasingly important as water temperatures increase through climate change.

Keywords

Thiaminase, invasive species, thermal tolerance, cardiac function, lake trout, climate change

Summary for Lay Audience

Thiamine (vitamin B1) is an essential vitamin that animals must obtain from their diet. In mammals, a deficiency of thiamine can result in severe cardiac disorders including heart failure and changes in heart size. However, little is known about how thiamine deficiency affects the cardiac system of fish. In lake trout from the Laurentian Great Lakes and Sudbury Basin, thiamine deficiency is thought to contribute to current population declines. In these ecosystems, the source of this vitamin deficiency comes from the consumption of invasive prey species — alewife and rainbow smelt — that contain high concentrations of a thiamine-degrading enzyme called thiaminase. Understanding how the consumption of thiaminase impacts cardiac function is critical in advancing ongoing lake trout management efforts, as cardiac function and morphology are integral to the thermal tolerance of fishes. In this thesis, I investigated the connection between thiaminase consumption, cardiac function, cardiac morphology, and thermal tolerance in two hatchery strains of lake trout that are currently targeted for reintroduction in the Great Lakes. I found that raising lake trout on a diet containing thiaminase for nine months resulted in impaired cardiac function, increased heart size, and reduced thermal tolerance. I also found notable differences between lake trout strains, where a strain originating from a population that historically fed on high-thiaminase prey fishes was more tolerant of the thiaminase diet. I expanded my laboratory findings to the field where I found that wild lake trout from the Sudbury Basin displayed similar cardiac structural changes in lakes where high-thiaminase prey fishes are the main forage items. Results from this research show that the consumption of dietary thiaminase from invasive species can impair cardiac function and alter cardiac morphology, which may translate to lower survival in the wild, especially as water temperatures increase with climate change.

Co-Authorship Statements

A manuscript describing the laboratory portion of this thesis has been accepted for publication in the *Canadian Journal of Zoology* with myself as primary author, and Christian A. Therrien (CT), Carlie A. Muir (CM), Shawn R. Garner (SG), and Bryan D. Neff (BN) as co-authors. All authors contributed to the conceptualization and experimental design of the work. I performed all data collection, visualization, and analysis, with contributions to statistical analysis from SG. I drafted the manuscript and all authors contributed to the final version. Supervision was provided by BN.

A manuscript describing the field portion of this thesis is being prepared for submission with Kevin Adeli (KA) as primary author, and myself, CT, and BN as co-authors. CT and I conceptualized the study, and all authors contributed to experimental design. CT performed field sample collection, with assistance from KA and I. Laboratory data collection was performed by KA, and I provided assistance and supervision. I performed all analysis, visualization, and interpretation of the data presented in this thesis.

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List of Abbreviations

AE – atrial flow end

ANOVA – analysis of variance

ATP – adenosine triphosphate

AV – atrioventricular

DFVS – Doppler flow velocity system

DSPW – Doppler signal processing workstation

EEAS – early flow end atrial flow start

EMS – early mortality syndrome

EPV – early flow peak velocity

ES – early flow start

f_H – heart rate

f_{Hmax} – maximum heart rate

f_{Hpeak} – peak heart rate

IPCC – Intergovernmental Panel on Climate Change

OCLTT – oxygen- and capacity-limited thermal tolerance

OMNRF – Ontario Ministry of Natural Resources and Forestry

PIT – passive integrative transponder

Q – cardiac output

Q_{peak} – peak cardiac output

RVM – relative ventricular mass

SD – standard deviation

SE – standard error

T^+ – free thiamine

T_{AB} – Arrhenius breakpoint temperature

T_{Arr} – arrythmia temperature

TCA – tricarboxylic acid

T_{Crit} – upper critical temperature

TD – thiamine deficiency

TDC – thiamine deficiency complex

TDP – thiamine diphosphate

TMP – thiamine monophosphate

T_{Opt} – optimum temperature

TTP – thiamine triphosphate

VM – ventricular mass

VS – ventricular shape

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Chapter 1

1 Introduction

1.1 Environmental Stressors

Ecological communities around the world are increasingly being impacted by multiple environmental stressors. An environmental stressor refers to any biotic or abiotic environmental factor that causes stress, including extreme temperatures, food availability, predatory pressure, and inter- and intra-specific competition (Schulte, 2014). In recent years, human impacts on ecosystems have grown substantially such that many organisms now face additional stressors such as habitat loss, over-exploitation, pollution, invasive species, and climate change (Christie, 1972; Rosenberg, 2003; Farrell et al., 2008; Isaksson, 2010; Kirchman et al., 2020). Predicting the cumulative effects of environmental stressors on organisms can be difficult as stressors can interact in a variety of ways in an environment. In a simplified model of two stressors acting on an organism simultaneously, there are three categories describing the outcome: additive, synergistic, or antagonistic (Folt et al., 1999). The cumulative effect of multiple stressors can be additive if the effect of the combined stressors is equal to the sum of the independent effects, synergistic if the combined effect is greater than the sum of the independent effects, or antagonistic if the combined effect is less than the sum of the independent effects (Folt et al., 1999). Of these interactions, synergistic stressor interactions are of particular concern because of their capacity to have profound, unpredictable impacts on ecosystems (Myers, 1996; Côté et al., 2016). Indeed, such interactions can result in "ecological surprises" if the cumulative effect far exceeds the predicted additive effect (Christensen et al., 2006). Thus, there is a current need to understand how environmental stressors interact in an ecosystem to accurately predict how species might respond to changes in their environment.

1.1.1 Stressors in Aquatic Ecosystems

Aquatic ecosystems are exposed to a myriad of environmental stressors, however, rising water temperatures due to climate change and the introduction of invasive species are two stressors of particular concern (Mainka and Howard, 2010). According to the Intergovernmental Panel on Climate Change (IPCC), current climate models project an average atmospheric temperature increase of 2.3-4.7°C by the year 2100 (IPCC, 2021). Consequently, lake surface water temperatures are predicted to increase dramatically across most of Canada (Sharma et al., 2007). Increases in lake surface water temperatures are predicted to modify the thermal properties of aquatic habitats such that many organisms will more frequently be exposed to temperatures that exceed their thermal optima (De Stasio et al., 1996; Ficke et al., 2007). Superimposed on the warming aquatic landscape is the increasing threat of biological invasion. Indeed, humans have greatly facilitated the spread of invasive species in aquatic ecosystems in recent years through intentional stocking, accidental releases, international trade, and through the modification of biogeographical barriers (Kolar and Lodge, 2000; Rahel, 2007). The introduction of invasive species in aquatic ecosystems has the potential to dramatically alter ecosystem structure through competition, predation, pathogen introduction, hybridization, and food web disruption (Kernan, 2015).

Most research has traditionally focused on the independent effects of invasive species and climate warming, however, recent research has suggested that it is unlikely these stressors operate in isolation (Mainka and Howard, 2010). Global climate change is predicted to increase the abundance, extent, and diversity of invasive species in aquatic ecosystems (Kolar and Lodge, 2000; Stachowicz et al., 2002; Hellmann et al., 2008; Mainka and Howard, 2010; Kernan, 2015). For example, recent increases in water temperature in the Great Lakes have created an abundance of suitable thermal habitat for potential invaders such as the grass carp (*Ctenopharyngodon idella*) — a species that is now a significant threat to the Great Lakes (Wittmann et al., 2017). In addition, a longer shipping season as a result of climate change is predicted to increase the propagative opportunities for multiple invasive species in the Great Lakes (Kolar and Lodge, 2002). Lastly, increased surface water temperatures in boreal lakes are expected to precede a range expansion of

warm water invasive species such as smallmouth bass (*Micropterus dolomieu*), whose negative impacts on native fish communities are profound and well-documented (Jackson, 2002; Jackson and Mandrak, 2002; Vander Zanden et al., 2004; Sharma et al., 2011). Altogether, interactions between these two stressors present a major challenge for the management and conservation of aquatic ecosystems.

1.2 Thiamine Deficiency: An Emerging Stressor

1.2.1 Thiamine Deficiency in Wildlife

In recent years, thiamine deficiency has been discovered in numerous wildlife populations around the world and has been hypothesized to be a significant contributing factor in worldwide biodiversity loss (Balk et al., 2016; Gilbert, 2018). Indeed, thiamine deficiency was recently listed as a significant threat to global conservation in a 2018 horizon scan for emerging issues relating to global biodiversity loss (Sutherland et al., 2018). The geographic and taxonomic extent of thiamine deficiency is widespread, with populations of birds, reptiles, fish, mammals and bivalves being affected across multiple continents (Fisher et al., 1996; Sepúlveda et al., 2004; Butler et al., 2008; Balk et al., 2009; Balk et al., 2016). For example, thiamine deficiency has been identified as a primary factor behind widespread die-offs and breeding failure of Herring Gull (*Larus argentatus*), European Starling (*Sturnus vulgaris*), and Common Eider (*Somateria mollissima*) in Europe (Balk et al., 2009). Furthermore, reports of neurological impairment and unprecedented morbidity in alligators from central Florida has been attributed to low levels of thiamine in alligator tissue (Sepúlveda et al., 2004; Honeyfield et al., 2008). To date, no general cause for thiamine deficiency in wildlife is known. In many instances, however, the thiamine-degrading enzyme thiaminase is a common factor. There are two distinct types of thiaminase, thiaminase I and thiaminase II, both of which catalyze the hydrolysis of thiamine but with different mechanisms (Jenkins et al., 2007), and both have been shown to induce thiamine deficiency in wildlife. For example, thiamine deficiency in moose from North Dakota is thought to result from grain overload, a syndrome by which a shift in diet from natural browse to agricultural crop produces a favorable rumen environment for *Clostridium perfringens*, a species of bacteria that

produces thiaminase II (Butler et al., 2008). Similarly, the consumption of prey fish that contain high concentrations of thiaminase I has been shown to induce thiamine deficiency in Pacific harbor seals (*Phoca vitulina*) in California (Croft et al., 2013). Altogether, thiamine deficiency is an emerging stressor that is hypothesized to be a significant contributing factor in large-scale wildlife mortalities and biodiversity loss (Balk et al., 2016).

1.2.2 Why is Thiamine Essential?

In animals, thiamine (vitamin B1) is an essential vitamin required for a wide range of physiological processes. Essential vitamins refer to those that cannot be endogenously synthesized and must therefore be obtained through an external source. Indeed, the monophosphorylated form of thiamine (TMP; Figure 1) can only be endogenously synthesized by some bacteria, plants and fungi, while all animals must obtain thiamine from their diet (Fitzpatrick and Thore, 2014). In many animals, the primary site for thiamine uptake is the small intestine, where free thiamine (T^+; Figure 1) is transferred to the bloodstream by a combination of concentration-dependent mechanisms (Manzetti et al., 2014). At high intestinal concentrations, thiamine passes through the intestinal membrane primarily by passive diffusion via proton channels in exchange for protons (Said et al., 1999; Manzetti et al., 2014). At low concentrations, thiamine passage is dominated by active transport via organic cation transporters and alkaline/acid phosphatases (Ferrari et al., 1978; Martel et al., 2001). In humans, approximately 47% of ingested thiamine is distributed to tissues and organs while the remainder is excreted in urine (Losa et al., 2005). Like other B-vitamins, thiamine is water soluble and cannot be stored for long periods of time. Thiamine storage can last up to 18 days in humans (Munir et al., 2001), with the greatest concentrations of thiamine existing in tissues with high metabolic requirements such as the liver, heart, and skeletal muscle (Marrs et al., 2021).

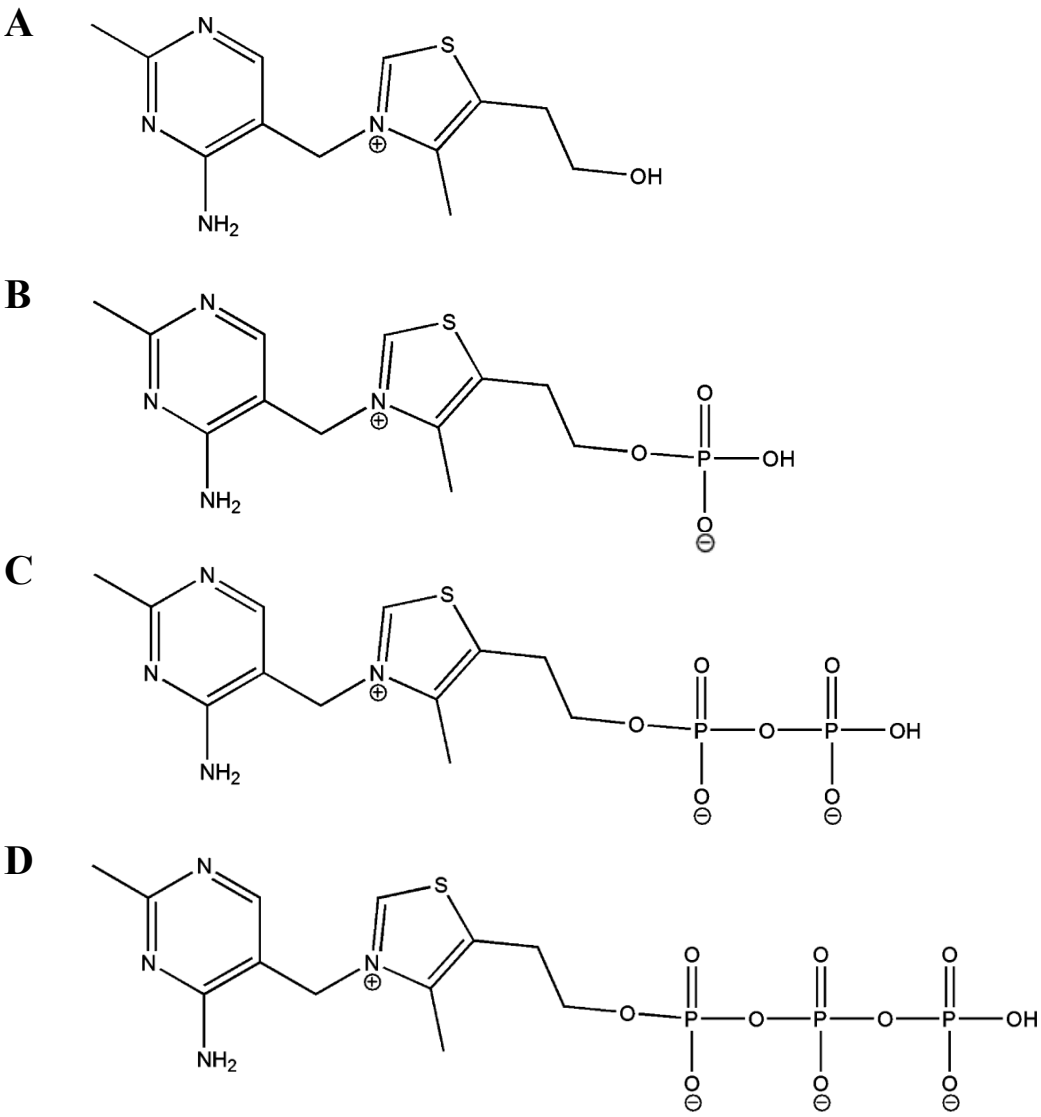

Figure 1. Naturally occurring forms of thiamine at neutral pH. (A) Free thiamine (T^+), (B) thiamine monophosphate (TMP), (C) thiamine diphosphate (TDP), (D) thiamine triphosphate (TTP). Adapted with permission from Manzetti et al. (2014). Copyright © 2014 American Chemical Society.

In most tissues, thiamine primarily exists in its metabolically relevant form, thiamine diphosphate (TDP; Figure 1C; Tillitt et al., 2005; Gangolf et al., 2010), which plays a vital role in metabolism and energy production. TDP acts as a rate-limiting cofactor for several key metabolic enzymes that catalyze the oxidative decarboxylation of α-ketoacids in the tricarboxylic acid (TCA) cycle (Depeient et al., 2006). Specifically, TDP is a cofactor for pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched chain α-ketoacid dehydrogenase (Figure 2). In the process of each of these thiamine-dependent reactions, the electron carrier nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH, which then donates electrons to the electron transport chain to facilitate adenosine triphosphate (ATP) synthesis. As such, these thiamine-dependent metabolic reactions are essential in mitochondrial ATP production. Indeed, thiamine deficiency has been shown to reduce ATP synthesis in animal tissues (McCandles et al., 1970). In addition to ATP production, TDP also plays a role in the pentose phosphate pathway where TDP is a cofactor for transketolase which aids in the synthesis of nucleic and amino acids (Racker et al., 1953).

Although thiamine is primarily known for its role in metabolism and energy production, it also serves a variety of other important functions. Particularly, thiamine plays a key role in brain function and interneuronal communication through the generation of acetylcholine, glutamate, and γ-aminobutyric acid (Perri et al., 1970; de Freitas-Silva et al., 2010; Ferreira-Vieira et al., 2016). Further, T^+ and thiamine triphosphate (TTP; Figure 1D) regulate nerve signal transmission through the activation of potassium and chloride channels (Cooper and Pincus, 1979; Bettendorff et al., 1993). Thiamine also plays an important role in the immune system, primarily through the function of T^+ as an antioxidant (Anderson, 1982; Huang et al., 2010). Multiple other immune-related processes depend on thiamine, including the release of intracellular adhesion molecules (Ottinger et al., 2012), expression of immunoglobulins (Molina et al., 1994; Zimitat and Nixon, 2001), and the anti-inflammatory response (Ke et al., 2006). Taken together, thiamine is an essential component in the diets of all animals and is vital for a variety of physiological processes including proper immune function, brain and nervous tissue function, and metabolism and energy production.

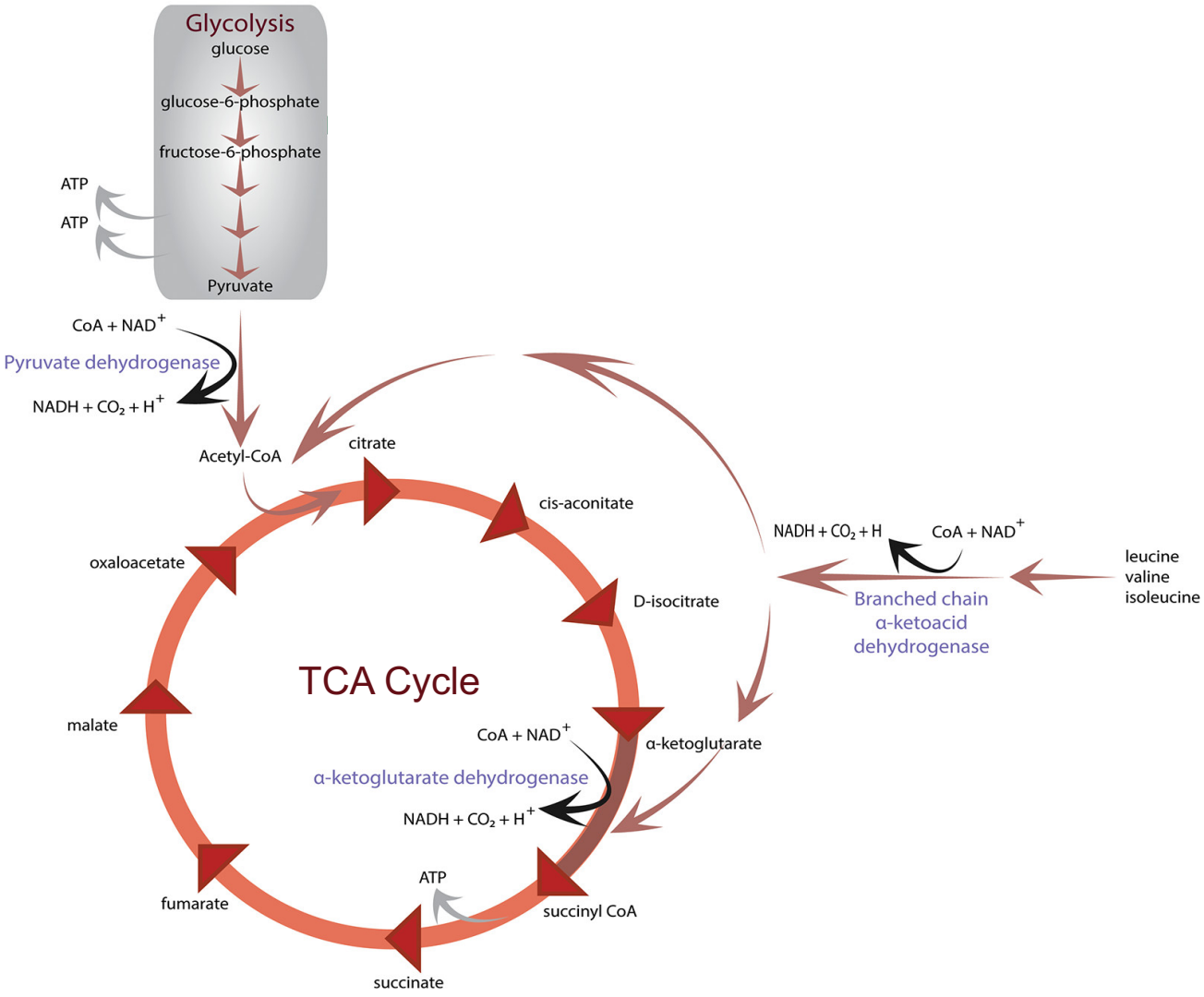

Figure 2. The role of thiamine diphosphate (TDP) in metabolism. Black arrows represent enzymatic reactions that require TDP as a cofactor and the associated enzymes are highlighted in blue. Figure adapted with permission from Kraft and Angert (2017). Copyright © 2017 University of Chicago Press.

1.2.3 Thiamine Deficiency: History & Manifestations

Thiamine deficiency — clinically known as beriberi in humans — was first described by Chinese physicians in 300AD when a deadly disease associated with symptoms of weakness, swelling, and extremity numbness emerged (Benedict, 2018). However, empirical evidence to suggest that the disease was diet-related did not emerge until the 1890s (Lanska, 2010), and thiamine itself was not discovered until the 1930s (Carpenter, 2012). The prevalence of beriberi increased greatly in Asia during the early 20th century, particularly in Japan where it became a national disease known as Kak'ke (Berdanier, 2021). The increased prevalence of thiamine deficiency in Japan and other Asian countries was primarily driven by a change in the processing of rice. Rice polishing, which was a novel and popular practice during the 20th century, is done by removing the outer rice husk that contains most of the plant's nutrients. As a result, excessive consumption of polished rice led to widespread thiamine deficiency across much of Asia (Berdanier, 2021). The deficiency was especially prevalent in the Japanese military, with neurological and cardiovascular symptoms frequently documented between the times of the Russo-Japanese War and World War II (Hawk, 2006). In developed countries today, thiamine deficiency is uncommon and is generally only observed in patients that suffer from chronic alcohol abuse, as excessive alcohol consumption can impair thiamine uptake (Martin et al., 2003).

Throughout the 20th century, after being recognized as a deadly disease in humans, thiamine deficiency became increasingly described in a variety of domestic animal populations. Mink and fox raised for fur production in the early 1900s were amongst the first domestic animals whose mortalities were known to be related to thiamine deficiency (Green and Evans, 1940; Stout et al., 1963). In the following decades, high mortalities of cattle, sheep, goats, and chickens had also been reported with similar sub-lethal signs that were alleviated with thiamine injection (Shintani, 1956; Edwin and Jackman, 1970; Thomas et al., 1987; Bourke et al., 2003). Similar to humans, thiamine deficiency in domestic animals was often related to diet. Indeed, large-scale die-offs of sheep in Australia were attributed to the heavy grazing of nardoo (*Marsilea drummondii*), a water fern that contains thiaminase I (McCleary and Chick, 1977).

Today, beriberi is clinically divided into two categories: wet beriberi and dry beriberi. Dry beriberi is typically characterized by central and peripheral nervous system impairment (Smith et al., 2021). During acute thiamine deficiency, dry beriberi can manifest as muscular weakness, fatigue, and memory loss (Kril et al., 1996). Prolonged and severe dry beriberi can lead to the development of Wernicke's Encephalopathy, a neuropsychiatric disease characterized by severe cognitive impairment, oculomotor abnormalities, and ataxia (Sechi and Serra, 2007). If left untreated, Wernicke's Encephalopathy can be accompanied by Korsakoff Syndrome (collectively referred to as Wernicke-Korsakoff syndrome), a memory disorder characterized by axonal degeneration, brain lesions, and cerebellum atrophy (Krill et al., 1996; Kopelman et al., 2009). Conversely, wet beriberi is characterized by cardiac impairments. The most common manifestations of wet beriberi are heart rhythm abnormalities, cardiac structural alterations, acidosis, and edema (Roman-Campos and Cruz, 2014). Severe cases of wet beriberi are clinically diagnosed as Shoshin beriberi, which is characterized by a rapid decline in systemic blood pressure, cyanosis, and cardiac failure (Roman-Campos and Cruz, 2014). In animal models, a reduction in heart rate and cardiac atrophy are among the most common cardiac-related symptoms of thiamine deficiency (Cohen et al., 1976; Cappelli et al., 1990; Oliveira et al., 2007; Roman-Campos et al., 2009; Gioda et al., 2010). Concurrent with macroscopic evidence, impaired myocyte contractility and myocyte atrophy have been documented in multiple studies in rats (Gioda et al., 2009; Roman-Campos et al., 2009; Yamasaki et al., 2010). Current evidence suggests that the reduced cardiac function documented during thiamine deficiency in mammals is facilitated by a combination of reduced ATP availability, impaired calcium release from sarcoplasmic reticulum, increased reactive oxygen species production and apoptosis, and a reduction in myocyte size (Roman-Campos and Cruz, 2014).

1.2.4 Thiamine Deficiency in Salmonids

Salmonids from the Laurentian Great Lakes are among the most well-studied wildlife populations affected by thiamine deficiency. In the Great Lakes, signs of thiamine deficiency have been observed in populations of Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*O. tshawytscha*), brown trout (*S.*

trutta), rainbow trout (*O. mykiss*), and lake trout (*Salvelinus namaycush*) (Fisher et al.,1995; Fisher et al., 1996; Marcquenski and Brown, 1997). In the Great Lakes, thiamine deficiency has been attributed to the consumption of thiaminase I (Fitzsimons and Brown, 1998). In particular, invasive alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) have been found to have high thiaminase activity relative to native prey fishes (Tillitt et al., 2005). The production of thiaminase I in these prey fish is believed to originate from gut microbiota (Honeyfield et al., 2002), however this association has been called into question (Richter et al., 2012) and recent studies suggest that the synthesis of thiaminase may be *de novo* (Richter et al., 2023). Regardless of the source of thiaminase production, alewife and rainbow smelt have become abundant since their introductions to the Great Lakes and their consumption has been directly linked to the development of thiamine deficiency in salmonids (Fitzsimons and Brown, 1998).

Signs of thiamine deficiency in the Great Lakes were first documented in 1968, when an unknown disease causing widespread mortality emerged among hatchery-raised populations of Chinook salmon, coho salmon, rainbow trout, and brown trout (Marcquenski and Brown, 1997). Early documentations of the deficiency reported high rates of mortality in sac-fry — a developmental stage prior to exogenous feeding where young fish rely on their yolk-sac for sustenance — and was aptly named early mortality syndrome (EMS). Over the next few decades, EMS became increasingly prevalent and mortality exceeded 90% in some hatchery-raised populations (Marcquenski and Brown, 1997). During this time, a syndrome named M-74 presenting with similar signs to EMS emerged in Atlantic salmon from the Baltic Sea. As with EMS, hatchery-raised Atlantic salmon sac-fry suffering from M-74 exhibited high rates of mortality (Lundström et al., 1999). In these fish, several sub-lethal impairments were also noted including ataxia and lethargy (Amcoff et al., 1998). Here, the disease was eventually linked to the consumption of sprat (*Sprattus sprattus*) and Atlantic herring (*Clupae harengus*), both of which contain high concentrations of thiaminase I, resulting in reduced egg and tissue thiamine concentrations (Karlsson et al., 1999; Wistbaka et al., 2002; Wistbaka and Byland, 2008).

Before EMS was linked to thiaminase consumption in the Great Lakes, early research focused on the possibility that environmental contaminants, such as polychlorinated biphenyls, dioxins, furans, and heavy metals, were behind the high rates of mortality (Mac et al., 1993; Fitzsimons et al., 1995). In a laboratory setting, exposure to these contaminants had previously been shown to induce a similar syndrome (Spitsbergen et al., 1991; Walker et al., 1991; Peterson et al., 1993; Walker et al., 1994), however contaminant concentrations in wild-caught fish were below the levels expected to explain the observed rate of mortality (Mac and Edsall, 1991; Fitzsimons et al., 1995). The inability to link EMS to environmental contaminants led to an alternative hypothesis that EMS was a result of a nutritional deficiency. Shortly after attention turned to a nutritional basis, Fitzsimons (1995) discovered that EMS signs were alleviated following thiamine injection while injections of the other B-vitamins were ineffective.

Since the connection of thiamine to EMS, extensive research on the lethal and sublethal effects of thiamine deficiency has occurred, with the effects collectively referred to as thiamine deficiency complex (TDC). Many of the behavioural and physical signs of TDC are analogous to symptoms observed in humans. Behaviourally, salmonids suffering from TDC present with ataxia, lethargy, and uncoordinated swimming patterns often described as “corkscrew swimming” (Fisher et al., 1995; Fitzsimons et al., 2005). In addition, TDC has been shown to decrease visual acuity and impair foraging and predator avoidance behaviours in lake trout (Carvalho et al., 2009; Fitzsimons et al., 2009a). Physically, thiamine-deficient fry exhibit edema, hemorrhaging, hydrocephalus and vascular congestion (Fisher et al., 1995; Fitzsimons et al., 2001a; Fitzsimons et al., 2001b), while thiamine-deficient juvenile and adult salmonids have a reduced growth rate (Fitzsimons et al., 2009), impaired immune response (Ottinger et al., 2012, 2014), altered body morphology and skin pigmentation, and reduced swimming performance (Houde et al., 2015a; Ketola et al., 2005, Fitzsimons et al., 2005). Many of the documented impairments associated with TDC have been attributed to the role of thiamine in the nervous system, and only indirect measures of cardiac function (i.e swimming performance) have been examined. If fish suffering from TDC exhibit similar cardiac impairments as mammals such as reductions in heart rate and cardiac atrophy, it would

have negative consequences for present and future salmonid survival and therefore warrants investigation.

1.3 A Crucial Link: Thiamine Deficiency and Thermal Tolerance

1.3.1 The Salmonid Heart: A Lynchpin for Aerobic Performance

The heart is a highly oxidative muscle that requires a high rate of ATP production to maintain its continuous mechanical work. In humans, heart tissue generates >95% of its ATP from mitochondrial oxidative phosphorylation, the majority of which is used to achieve ventricular contraction (Suga, 1990; Knaapen et al., 2007). The capacity of the heart to deliver oxygenated blood to tissues is especially important for salmonids which comprise a family of highly active fishes including trout, salmon, char, and graylings. Therefore, constraints to cardiac function as a result of TDC are expected to have pervasive impacts on the fitness of salmonids.

Like all fish, the salmonid heart is comprised of four chambers: the sinus venosus, atrium, ventricle, and the bulbous arteriosus. The atrium and ventricle are lined with excitable cells and actively contract and pump blood, while the sinus venosus and the bulbous arteriosus are chambers that function as storage units for blood entering and leaving the heart (Santer, 1985). Unlike most fish, however, the salmonid ventricle consists of two different myocardial structures: spongy and compact myocardium (Davie and Farrell, 1991). Spongy myocardium is comprised of a mesh-like network of cells that span the inner ventricle (Tota et al., 1983). Ventricles that contain only spongy myocardium are described as having either saccular- or tubular-shaped ventricles and are generally found in more sedentary fishes (Santer and Walker, 1980; Agnisola and Tota, 1994; Tota and Gattuso, 1996). Conversely, highly active fishes such as salmonids have a mixed-type ventricle that contains both spongy and compact myocardium and is described as being pyramidal-shaped (Santer and Walker, 1980; Agnisola and Tota, 1994; Tota and Gattuso, 1996). Compact myocardium consists of a dense, muscular wall that envelopes the ventricle and functions as a supplementary pump (Tota et al., 1983; Davie and Farrell, 1991; Agnisola and Tota, 1994). In fish with this ventricular

arrangement, ventricular morphology plays an important role as a determinant of cardiac capacity. Indeed, both a greater ventricular mass and a more elongated ventricle have been linked to improved upper thermal tolerance and greater swimming capability in fishes (Claireaux et al., 2005; Eliason et al., 2011; Anttila et al., 2013a).

1.3.2 Oxygen- and Capacity-Limited Thermal Tolerance

Under the Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT) hypothesis, thermal limitations of fishes are thought to be primarily set by a mismatch between tissue oxygen demand and the capacity of the cardiorespiratory system to supply adequate oxygen to tissues (Pörtner and Knust, 2007; Farell, 2009). As external temperature increases, the metabolic rate and therefore oxygen consumption of fishes increases exponentially, but this is met with capacity limitations set by the cardiorespiratory system. The difference between routine and maximum metabolic rate is known as aerobic scope and reflects an organism's ability to perform aerobic activities above standard metabolic needs (Fry, 1947; Figure 3). Aerobic scope is maximized at an organism's optimum temperature (T_{Opt}), where essential metrics related to an organism's fitness, such as growth rate and swimming ability are maximized (Gibson and Fry, 1954; Selong et al., 2001; Elliot and Elliot, 2010; Figure 3). At temperatures above T_{Opt}, aerobic scope declines due to the inability of the cardiorespiratory system to keep pace with increasing metabolic demands. The decline in aerobic scope proceeds as temperatures increase until reaching the organism's upper critical temperature (T_{Crit}) where standard metabolic requirements surpass aerobic capacity, above which fitness declines substantially (Figure 3). Thus, following the OCLTT hypothesis, cardiac function and morphology are integral to thermal tolerance and are considered to be key determinants of upper thermal limits in fishes (Farrell et al., 2008; Cooke et al., 2012).

Measuring aerobic scope to estimate T_{Opt} and T_{Crit} have proven valuable for predicting the future effects of various climate change scenarios in fishes. For example, estimating T_{Opt} has helped researchers understand the interactive effects of climate warming and ocean acidification in coral reef fishes (Munday et al., 2009). However, measuring the oxygen consumption of both resting and maximally exercised fish over a range of temperatures is

expensive and highly time consuming. Fortunately, a faster method to accurately estimate T_{Opt} and T_{Crit} using maximum heart rate (f_{Hmax}) has emerged in recent years (Casselman et al., 2012; Antilla et al., 2013b; Muir et al., 2021). In fishes, increased metabolic demand during exposure to high temperatures is supported through increases in cardiac output (Q), which in itself is mediated entirely through increases in heart rate (f_H) (Cooke et al., 2003; Clarke et al., 2005; Clarke et al., 2008; Steinhausen et al., 2008; Farrell, 2009; Gamperl et al., 2009). As temperatures approach an organism's T_{Opt}, f_{Hmax} reaches an inflection point known as the Arrhenius breakpoint temperature (T_{AB}) where the rate at which maximum heart rate (f_{Hmax}) increases with temperature slows (Casselman et al., 2012; Figure 3). This reduction in temperature-dependent increases in f_{Hmax} places a limit on oxygen availability and can result in a breakdown of cardiac function as temperatures near T_{Crit} (Figure 3). The temperature at which heart function breaks down is known as the arrythmia temperature (T_{Arr}; Clarke et al., 2008; Casselman et al., 2012; Figure 3). It has been demonstrated that T_{AB} and T_{Arr} are accurate proxies for estimating the more time-consuming measures of T_{Opt} and T_{Crit} (Casselman et al., 2012; Anttila et al., 2013b).

Altogether, ventricular morphology and cardiac function are important factors determining the thermal tolerance capabilities of fishes. It is well established that thiamine deficiency impairs cardiac function and alters ventricular morphology in mammals. Therefore, if thiamine deficiency in fish results in similar manifestations, it is anticipated that the capacity of fish to tolerate warmer water temperatures will be impacted (Figure 3).

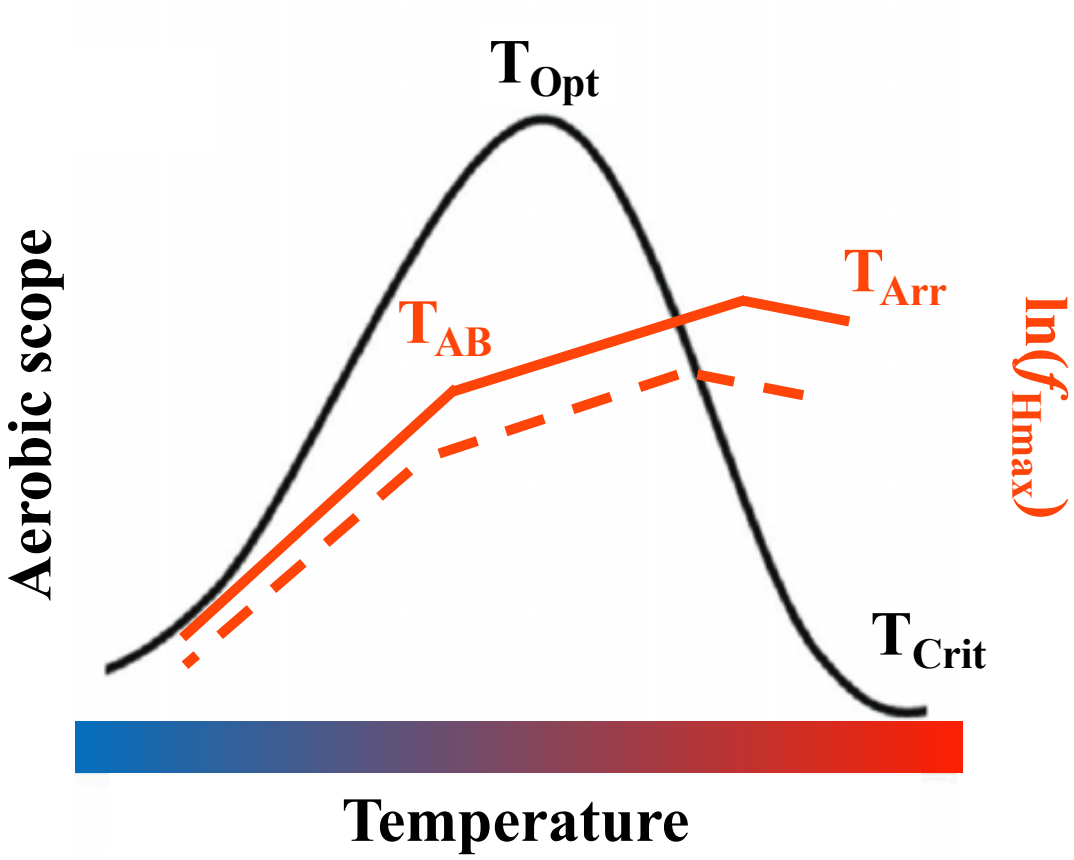

Figure 3. Aerobic scope (black line), the natural logarithm of maximum heart rate (f_{Hmax}; solid red line), and the predicted effect of thiamine deficiency on f_{Hmax} (dashed red line) as a function of temperature. T_{Opt}, optimum temperature; T_{Crit}, upper critical temperature; T_{AB}, Arrhenius breakpoint temperature; T_{Arr}, arrythmia temperature. Figure modified from Muir (2022).

1.4 Study Species: Lake Trout

1.4.1 Habits & History in the Great Lakes

Lake trout (*Salveninus namaycush*) is a cold-water species that have a widespread distribution throughout the Great Lakes region. In most lakes, lake trout spend much of the year in deep water where water temperatures range between 6-12°C (Olson et al., 1988; Bergstedt et al., 2003) and migrate to shallower water to spawn in autumn (Hanson et al., 1999). Unlike most salmonids, lake trout are iteroparous, nocturnal lake-spawners that spawn on rocky shoals throughout October and November (Gunn, 1995). Although lake trout do not migrate upriver to spawn like many of their semelparous cousins (although it is believed some strains from Lake Superior historically made river migrations (Goodier, 1981)), migration routes for lake trout can be just as impressive; typically ~50 km on average in the Great Lakes (Elrod, 1987; Kapuskinski et al., 2005; Schmalz et al., 2011; Ivanova et al., 2021). Incredibly, Ivanova et al. (2021) tracked a lake trout traveling across the length of Lake Ontario to spawn, a distance of over 200 km. In addition, the life history of lake trout is generally characterized by late maturation (6-7 years) and slow growth (Martin & Olver, 1980). Throughout much of their range, lake trout are primarily piscivorous and feed on a combination of pelagic and demersal prey fishes (Colborne et al., 2016; Mumby et al., 2018; Nawrocki et al., 2022). Historically, lake trout diets consisted predominantly of cisco (*Coregonus artedi*), bloater (*C. hoyi*), and slimy sculpin (*Cottus cognatus*) (Christie et al., 1987; Beeton, 2002, Morrison, 2019). However, a recent shift in the Great Lakes prey communities has altered lake trout diets such that invasive alewife and rainbow smelt are currently the most abundant component of lake trout diets in many parts of the Great Lakes (Ray et al., 2007; Happel et al., 2018; Luo et al., 2019; Nawrocki et al., 2022).

Lake trout were historically abundant throughout their range but have faced considerable declines over the past several decades. Before their decline, lake trout held a significant economic, ecological, and cultural importance (Ryder and Kerr, 1990; Muir et al., 2012; Marin et al., 2017). As top predators, lake trout played an important ecological role in the energy cycling of benthic and pelagic zones and exerted a stabilizing influence on food webs (Ryder and Kerr, 1990). Additionally, lake trout are considered to be a bioindicator

species of overall ecosystem quality because of their sensitivity to ecosystem change (Ryder and Edwards, 1985). Economically, lake trout historically supported a highly successful commercial fishery. At its peak in the early 1900s, the Great Lakes commercial fishery generated over $220 million annually (Muir et al., 2012; Brendon et al., in review). However, by the 1950s, lake trout became extirpated from Lakes Ontario (Christie, 1972), Erie (Leach and Nepszy, 1976), and Michigan (Cuhel and Aguhilar, 2012), and were nearly extirpated from Lakes Superior (Curtis, 1990) and Huron (Berst and Spangler, 1972). The sharp declines were primarily attributed to a combination of overfishing and predation by invasive sea lamprey (*Petromyzon marinus*) (Christie, 1974). Climate change is also thought to have played a role in the decline of lake trout across their range as their life history and narrow temperature range make them particularly vulnerable to ecosystem change (Ficke et al., 2007; Guzzo & Blanchfield, 2016). Indeed, several populations of lake trout are believed to have gone extinct in eastern Ontario due to increasingly limited thermal habitat (MacLean et al., 1980).

The decline in lake trout abundance triggered dramatic ecological changes throughout the Great Lakes. In the absence of a top predator, populations of invasive alewife and rainbow smelt exploded and largely displaced native prey fishes (Christie, 1974; O'Gorman and Stewart, 1999). Indeed, alewife abundance exceeded carrying capacity in some regions, with mass die-offs occasionally polluting shorelines of Lakes Michigan and Ontario (O'Gorman and Schneider, 1976; Hatch et al., 1981). In the mid-1960s, fisheries managers began stocking Pacific salmon (*Oncorhynchus spp.*) to control alewife populations while simultaneously seeking to improve the recreational fishery (Tanner and Toddy, 2002). This strategy was initially successful in reducing alewife abundance (Madenjian et al., 2002), however, the level of piscivory was unsustainable and eventually led to a collapse of the Pacific salmon fishery in the 1980s (Holey et al., 1998). The decline in the recreational fishery generated a controversial management issue as managers were now faced with the decision on whether to manage for economics or to manage for the benefit of the ecosystem (Dettmers et al., 2012). Recognizing the importance of native fishes, lake trout restoration efforts began in most lakes by the late 1980s (Muir et al., 2012). The combination of increased lake trout stocking, reduced stocking of Pacific salmon, implementation of sea lamprey control efforts, and lake trout

commercial catch limits set the stage for the successful rehabilitation of lake trout in Lake Superior and parts of Lakes Huron and Michigan (Hansen et al., 1995; Riley et al., 2007; Patterson et al., 2016). However, despite these efforts, lake trout continue to rely almost entirely on hatchery stocking for population recruitment in Lakes Ontario and Erie (Lantry et al., 2020; Markham et al., 2022). It has been hypothesized that a primary factor contributing to the lack of restoration success is due to thiamine deficiency from the consumption of alewife and rainbow smelt, which continue to dominate prey communities in these lakes (Fitzsimons and Brown, 1998; Brown et al., 2005).

1.4.2 Lake Trout Management in Lake Ontario

Current lake trout rehabilitation efforts in Lake Ontario provide a unique opportunity to examine the effects of thiamine deficiency on cardiac function and morphology. Lake trout were functionally extirpated during the 1950s and are currently the focus of large-scale reintroduction programs (Christie, 1972; Lantry et al., 2014). Despite these restoration efforts, there is little evidence of natural reproduction and thiamine deficiency has been hypothesized as a significant contributing factor (Brown et al., 2005; Lantry et al., 2014). Indeed, thiamine concentrations in wild lake trout eggs collected from Lake Ontario are 4.7–8.8 times lower than in lake trout eggs from Lake Superior where lake trout feed primarily on native prey fishes (Fitzsimons, 1998; Fitzsimons et al., 2007; Ray et al., 2007; Gamble et al., 2011). Management strategies to restore self-sustaining populations of lake trout in Lake Ontario currently include the annual stocking of 1.3 million yearling lake trout (Lantry et al., 2014). These stocking programs predominantly release lake trout from two populations: a population from Seneca Lake, NY (Seneca strain), and a population from the Slate Islands in Lake Superior (Slate strain). Notably, these strains differ in their bathythermal preferences, survival rates, and growth rates in Lake Ontario. Adult Seneca strain lake trout typically have greater survival rates than Slate strain fish in Lake Ontario (Lantry et al. 2020), likely due to lower sea lamprey-related mortality from differences in bathymetric preferences (Schneider et al. 1996). Seneca strain lake trout typically prefer shallower, warmer water than Slate strain lake trout and juveniles have slower growth rates (Elrod et al., 1996). Perhaps most notably, these strains differ in their past history with high-thiaminase prey fishes. Seneca Lake has

long supported an abundant population of high-thiaminase prey fishes (Odell, 1934), whereas Lake Superior supports far fewer (Bronte and Hoff, 1996). Therefore, it has been suggested that local adaptation to high-thiaminase prey fishes has equipped Seneca strain lake trout with the capacity to better tolerate dietary thiaminase, possibly through reduced thiamine utilization (Fitzsimons et al., 2021). Local adaptations to dietary thiaminase have previously been identified in populations of Atlantic salmon, where populations that historically relied on high-thiaminase prey in their native lakes were more tolerant of a high-thiaminase diet (Houde et al., 2015a). Selecting a source population with pre-existing adaptations to key environmental features in the restoration location, such as high-thiaminase prey, can greatly influence the success of reintroduction efforts (Houde et al., 2015b). If Seneca strain lake trout possess adaptations for thiaminase tolerance, it would have significant implications for lake trout rehabilitation efforts and would warrant potential changes in lake trout stocking methods in the Great Lakes.

1.4.3 Lake Trout in the Sudbury Basin

Current lake trout restoration efforts in the Sudbury basin provide another unique opportunity to study the cardiac-related effects of thiamine deficiency. Sudbury is largely regarded as the nickel mining capital of the world, producing over half of world's nickel during the industries' peak in the mid 1900s (Kerfoot et al., 1960). During this time, sulphur dioxide emissions resulted in unprecedented deposition of atmospheric sulphur in over 7000 lakes within a 17000 km^2 area around Sudbury (Neary et al., 1990; Keller, 1992). The resulting lake acidification decimated wildlife at all trophic levels (Keller, 1992), and lake trout became extirpated from as many as 89 lakes by the late 1960s (Beamish and Harvey, 1972; Beggs and Gunn, 1986; Dixit et al., 1993). Since then, sulphur emissions from mining operations have dropped substantially and lake trout have become successfully re-established in some formerly acidified lakes as a result of intensive stocking efforts (Gunn and Keller, 1990; Casselman and Gunn, 1992; Luek et al., 2010). However, the recent introductions of invasive species are hypothesized to be complicating on-going lake trout restoration efforts (Therrien, 2019). Specifically, anthropogenically-mediated range expansions of non-native rainbow smelt have overlapped with several lake trout reintroduction programs in the Sudbury Basin

(Selinger et al., 2006). Indeed, lake trout collected from lakes where rainbow smelt are established have shown reduced tissue thiamine concentrations compared to lake trout from lakes where rainbow smelt are absent (Therrien et al., un-published data). Thus, the Sudbury Basin provides a valuable opportunity to investigate the cardiac-related effects of thiaminase consumption in wild-caught lake trout.

1.5 Thesis Overview & Objectives

Cardiac impairments associated with thiamine deficiency are frequently observed in mammals. Pathologically, manifestations of impaired heart rate, cardiac atrophy, and cardiac failure stem from thiamine's crucial role in cellular metabolism and energy production. However, the effects of thiamine deficiency on cardiac function have not yet been directly examined in fishes. In the context of fish thermal physiology, cardiac morphology and function play a vital role in determining the capacity in which fish can tolerate warmer water temperatures. Indeed, the Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT) hypothesis suggests that the decline in aerobic scope observed at high temperatures results from a mismatch between oxygen supply by the cardiorespiratory system and tissue oxygen demand. Therefore, it is expected that thiamine deficiency will limit the thermal tolerance of fishes if the deficiency results in comparable cardiac-related manifestations as mammals. As a cold-adapted, stenothermal species, lake trout are particularly vulnerable to environmental warming, and it is imperative to understand how these two environmental stressors might interact in the wake of global climate change.

In this thesis, I examined the effects of dietary thiaminase on cardiac function and morphology in lake trout, particularly as they related to the ability of the heart to meet increasing oxygen demands at high temperatures. I investigated and compared measures of cardiac function, morphology, and thermal tolerance between two hatchery strains of lake trout (Seneca and Slate) fed either a control diet or a diet containing bacterial-derived thiaminase. I hypothesized that dietary thiaminase would affect cardiac function, morphology, and thermal tolerance in both strains. Specifically, I predicted that lake trout raised on the thiaminase diet would have impaired cardiac function, smaller ventricles,

and reduced thermal tolerance. However, I predicted that Seneca strain lake trout would show lesser effects of the high-thiaminase diet compared to Slate strain lake trout if past differences in exposure to high-thiaminase prey fishes has led to local adaptation. Additionally, I compared cardiac morphology between lake trout from lakes where rainbow smelt have become established and where rainbow smelt remain absent in the Sudbury Basin. Given the link between rainbow smelt consumption, thiamine deficiency, and changes in cardiac morphology, I hypothesized that the presence of rainbow smelt would affect cardiac morphology in wild-caught lake trout. I predicted that lake trout collected from lakes where rainbow smelt are established would exhibit similar cardiac morphological changes to what I measured in lake trout raised on a thiaminase diet in a hatchery setting. Overall, my research had the following objectives: (1) To determine if dietary thiaminase impacts cardiac function and morphology in lake trout, (2) to determine if the resulting cardiac impairments translate to reduced thermal tolerance, (3) to compare results between two strains of lake trout currently targeted for reintroduction in Lake Ontario, and (4) to determine if wild-caught lake trout ventricles collected from the Sudbury Basin exhibit similar morphological changes as hatchery-raised fish. Altogether, addressing these objectives provides a first glimpse into how two prevalent stressors in aquatic ecosystems — warmer water temperatures and invasive high-thiaminase prey fishes — interact to threaten lake trout viability through cardiac impairments.

Chapter 2

2 Methods

2.1 Measuring Cardiac Function, Morphology, and Thermal Tolerance in Two Strains of Hatchery-raised Lake Trout

2.1.1 Experimental Animals

Families for the Slate and Seneca strains were produced in late 2019 using single pair crosses of mature individuals at the Ontario Ministry of Natural Resources (OMNRF) Dorion Fish Culture Station (Dorion, ON) and were transferred to the OMNRF Chatsworth Fish Culture Station (Chatsworth, ON) as eggs. The Seneca Lake strain originated from wild-caught adult lake trout captured in 1984 from Seneca Lake, NY (42°39'24"N, -76°53'53"W). This line was last crossed with wild individuals in 1994 (J. Intini, pers. comm., 7 October 2022). The Slate Island strain originated from wild-caught adult lake trout captured in 2004 from Lake Superior around the Slate Islands (48°39'07"N, -87°00'14"W; J. Intini, pers. comm., 7 October 2022). On March 18th, 2021, lake trout parr of the Seneca (age 13 months) and Slate (age 14 months) strains were transferred from the OMNRF Chatsworth Fish Culture Station to Western University. On the same day, between 23–30 fish of the same strain were placed into each of sixteen 73 L white polypropylene tanks, with eight tanks per strain (n=~200 fish/strain; Figure 4). Two independent recirculating water systems were used with eight tanks per system and water temperature was maintained at 9°C throughout the experiment. Dissolved oxygen levels were kept high (> 9mg L^{-1}), and treatment groups were spread equally across the two systems. Fish were given 3 weeks to acclimate to hatchery conditions, during which they were fed a commercial fish feed (Ewos Inc.) at a rate of 2% body mass day^{-1} before being anesthetized (150 mg L^{-1} MS-222 buffered with 150 mg L^{-1} sodium bicarbonate) and measured for body mass on April 12th, 2021. While anesthetized, each fish was tagged with a 1.2 mm Passive Integrated Transponder (PIT; Biomark Inc). The fish were allowed to recover for 2 weeks on a diet of commercial fish feed at a rate of 2% body mass day^{-1}. Following the recovery period, the fish received a diet consisting of a 1:1

ratio of experimental diet and commercial feed for two weeks before being fed exclusively the experimental diets. Diets were switched in stages to allow the lake trout to acclimate to the experimental diets. Control and thiaminase diets were administered to four replicate tanks for each strain. Once on the experimental diets, body mass was measured monthly and lake trout were fed daily at a rate of 2% body mass day^{-1} for the first 3 months, 1.5% body mass day^{-1} for the next 3 months, and 1% body mass day^{-1} for the remainder of the experiment.

Figure 4. Experimental hatchery setup for lake trout at Western University.

2.1.2 Experimental Diets

Control and thiaminase diets were produced following Honeyfield et al. (2005) with some modifications. Both diets contained identical ingredients (Table 1), with the addition of bacterial-derived thiaminase (from *Paenibacillus thiaminolyticus* isolated from Lake Michigan alewife; Honeyfield et al., 2002) to the thiaminase diet but not the control diet. Other sources of thiaminase also appear to be important contributors to the total thiaminase activity of some Great Lakes prey fishes (Richter et al., 2012; 2023; Rowland et al. in prep), but *P. thiaminolyticus* remains a useful source of thiaminase for experimental preparations — especially in studies focused on the effects of dietary thiaminase rather than the origin of the thiaminase activity. This diet has previously been shown to contain all the nutritional requirements for fish (Honeyfield et al., 2005; see Appendix B for full composition of vitamin and mineral premixes), including thiamine which was measured (mean ± SD) to be 7.05 ± 5.2 nmol/g in the control feed and 6.92 ± 5.8 nmol/g in the thiaminase feed. In place of herring meal, ground dried herring was used by drying ground raw pacific herring (*Clupea pallasii*) at 74°C for 48 hours. For the thiaminase diet, *P. thiaminolyticus* cultures were prepared in nutrient broth (1.0 g/L yeast extract and 8.0 g/L Difco nutrient broth (Becton Dickinson, Mississauga, ON)) and incubated for 96h at 37°C. Autoclaved nutrient broth was used in the control diets. Nutrient broths were thoroughly mixed with all dry ingredients using an electric food mixer and pelletized using an electric meat grinder. Food pellets were left to air dry at room temperature for 48h and stored at -20°C until use. Maximum storage time for diets at -20°C was 2 weeks. Here, I used the same strain and concentration (mean ± SD: $2.1 \times 10^8 \pm 6.1 \times 10^7$ CFU/mL) of *P. thiaminolyticus* that has previously been shown to reduce tissue thiamine concentrations and induce signs of thiamine deficiency in Atlantic salmon and lake trout (Houde et al., 2015a; Honeyfield et al., 2005). Experimental diets were administered for 9 months, by which signs associated with thiamine deficiency were evident in fish from the thiaminase treatment (ataxia, lethargy, increased mortality and reduced tissue thiamine levels; Therrien et al., unpublished data).

Table 1. Diet composition and proximate analysis of experimental lake trout (*Salvelinus namaycush*) diets.

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 (CFU/mL)	none	$2.1 \times 10^8 \pm 6.1 \times 10^7$
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)	7.05 ± 5.2	6.92 ± 5.8

2.1.3 Thermal Performance of Cardiac Function

After nine months on the experimental diets, test fish were anesthetized in water containing 150 mg L^{-1} of MS-222 buffered with 150 mg L^{-1} of sodium bicarbonate. Body mass was measured while fish were anesthetized. Fish were then placed ventral-side up in a temperature-controlled (9°C) holding reservoir which consisted of a short (4.5-inch diameter) segment of PVC pipe that was cut lengthwise to form a holding trough (see Muir et al., 2021; Gradil et al., 2016). Fish were held in position using a weighted Styrofoam sling. Water temperature was maintained using a recirculating water bath (VWR, Edmonton, AB, Canada), and an additional temperature probe was used in the holding reservoir to ensure that the reservoir water temperature corresponded to the set temperature of the recirculating water bath (Omega, St-Eustache, QC, Canada). A maintenance dose of anesthetic (100 mg L^{-1} of MS-222 buffered with 100 mg L^{-1} sodium bicarbonate) was present in the recirculating water bath and fish were ram-ventilated using a 2.5cm segment of rubber tubing. Each fish was maintained at 9°C in the holding reservoir for 15-minutes before starting echocardiography measurements to ensure that heart rate had stabilized after handling.

Thermal performance of cardiac function was assessed in 62 fish (Seneca control, n=12; Seneca thiaminase, n=15; Slate control, n=17; Slate thiaminase, n=18) using the Indus Doppler Flow Velocity System (DFVS; Indus Instruments, Houston, TX, USA) following the methods of Muir et al. (2021). Briefly, a 20 MHz transducer probe was held perpendicular to the ventral side of the fish, directly posterior to the gills, to measure blood flow velocity at the atrioventricular (AV) valve. Caution was taken to ensure that the probe was positioned parallel to the direction of blood flow, as the measurement error of the DFVS probe is below 1.5% when the insonation angle is within 10° of the direction of blood flow (Reddy et al., 2009). Additionally, Doppler shift is maximized when the insonation angle of the ultrasound beam approaches zero (Wang et al., 2017). Thus, to ensure proper alignment with the direction of blood flow, probe alignment was adjusted for each fish to reflect the strongest signal. Signals from the DFVS probe were digitized and displayed as real-time spectrographs using the Doppler Signal Processing Workstation (DSPW). After the 15-minute stabilization period, DFV spectrographs were

recorded to measure baseline heart rate. Pharmacological stimulation was then used to induce maximum heart rate (f_{Hmax}) in the anesthetized fish through sequential intraperitoneal injections of 1.2 mg kg^{-1} atropine sulphate (Sigma-Aldrich, St. Louis, MO, USA) and 4 μg kg^{-1} isoproterenol (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% NaCl, each followed by a 15-minute stabilization period (Casselman et al., 2012). Atropine sulphate was used to block the cholinergic response of muscarinic receptors, whereas isoproterenol was used to fully stimulate adrenergic β-receptors. Injections of 0.9% NaCl alone showed no change in heart rate. Water temperature was then increased by 1°C every 6 minutes until heartbeats became arrhythmic. After each temperature increment, DFV spectrographs were recorded (5 per temperature) and saved for later analysis. When heartbeats became arrhythmic, each fish was removed from the holding reservoir and euthanized with an overdose of MS-222 to collect heart tissue.

2.1.4 Measures of Cardiac Morphology & Atrioventricular (AV) Valve Area

Ventricles were isolated from heart samples collected from test fish by removing the atrium, sinus venosus, and bulbous arteriosus. Once isolated, the ventricle was weighed using a digital balance to determine ventricular mass (VM). Following VM measurements, hearts were placed in 10% neutral buffered formalin for 9 days before being transferred to 70% ethanol for long term storage (Perry et al., 2020). Ventricular shape (VS) was then measured using digital calipers as the quotient of ventricle width and ventricle length (Perry et al., 2020). Next, prior to AV valve measurements, hearts were immersed in 5% alcian blue stain for 1 hour followed by a 2-hour 5% acetic acid wash. Alcian blue stain selectively stains connective tissue (Nagy et al., 2009) allowing for better visualization of the AV valve. Photographs of the AV valve were taken using OPTIKA PROView (OPTIKA Srl, Ponteranica, BG, Italy; Figure 5) and AV valve diameter was measured using ImageJ (National Institutes of Health, Bethesda, MD, USA). An average AV valve diameter was calculated using two perpendicular measurements. AV valve area was then estimated using πr^2, where r represents the radius of the AV valve.

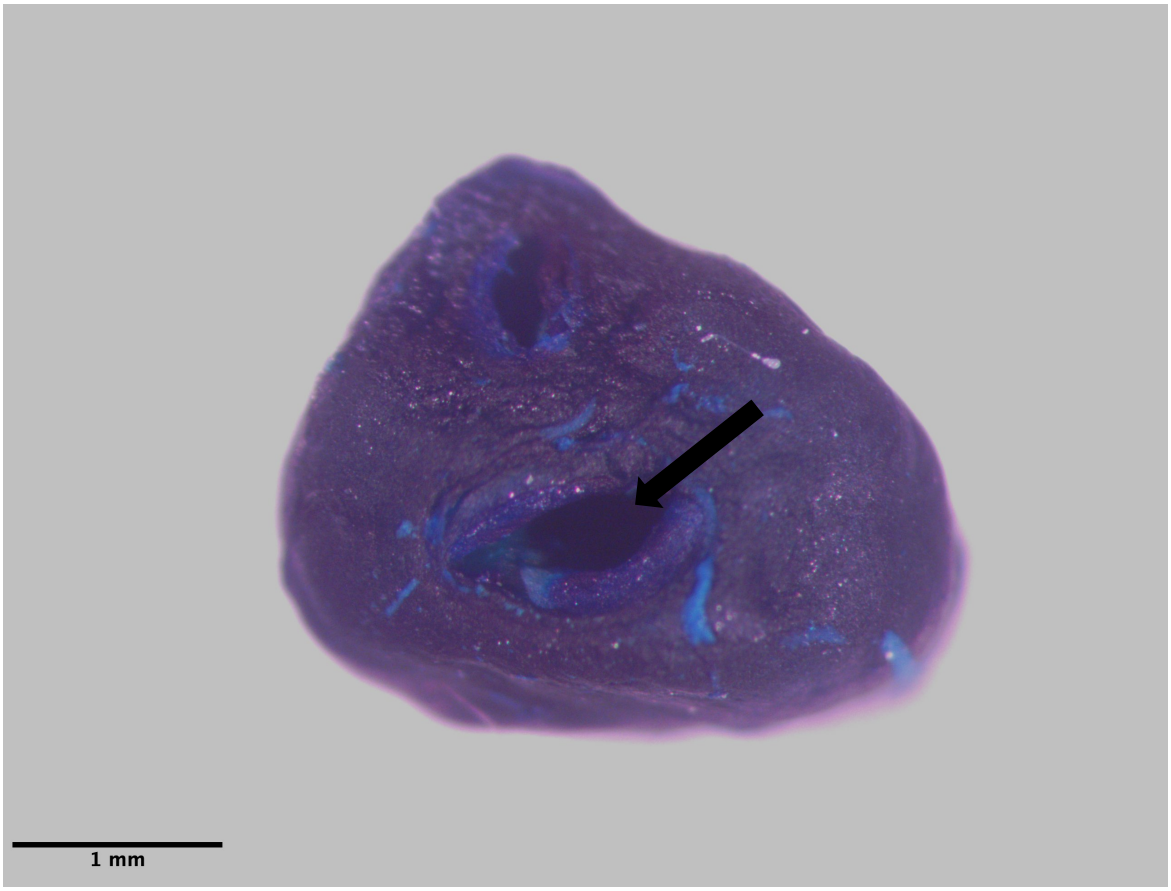

Figure 5. Dorso-anterior view of a lake trout (*Salvelinus namaycush*) atrioventricular (AV) valve (black arrow) stained with 5% alcian blue.

2.1.5 Analysis

Spectrographs were analyzed using the Doppler Signal Processing Workstation (DSPW) software using the parameters outlined in Muir et al. (2021). For each spectrograph, markers were manually placed at the 'Early Flow Start' (ES), 'Early Flow Peak Velocity' (EPV), 'Early Flow End Atrial Flow Start' (EEAS), 'Atrial Flow Peak Velocity' (APV), and 'Atrial Flow End' (AE) of each beat (n=8 beats; Figure 6). Using these markers, the software calculated an average Atrial flow (A) stroke distance and Early flow (E) stroke distance which was totaled to obtain total stroke distance (cm beat^{-1}; Figure 6). Beat markers that were manually placed at the AE of each beat were used to calculate an average heart rate (f_{Hmax}; beats min^{-1}) at each temperature using the software's 'Beat Editor' (Figure 6).

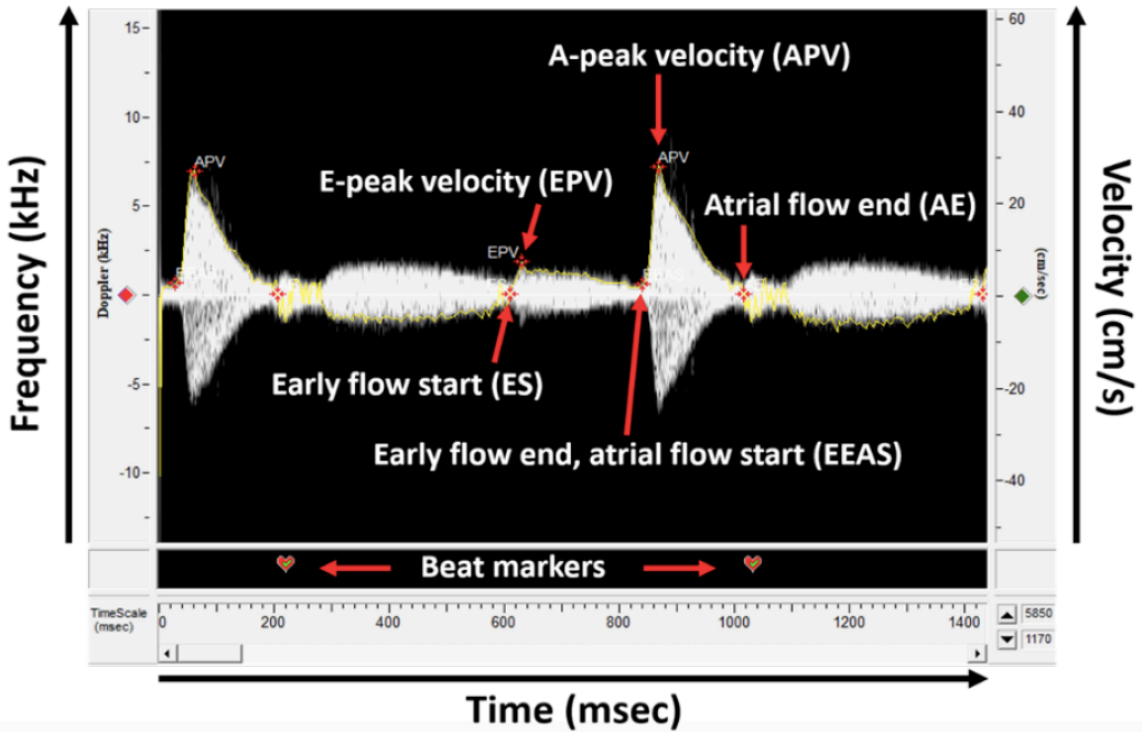

Figure 6. Parameters used to calculate atrioventricular blood flow from a Doppler spectrograph. E-stroke distance was calculated as the area under the E-wave (ES-EPV-EEAS), and A-stroke distance was calculated as the area under the A-wave (EEAS-APV-AE). Figure modified from Muir (2022).

For each fish, I estimated cardiac output (Q; mL min^{-1}) at each temperature as the product of stroke volume (mL $beat^{-1}$) and heart rate (beats min^{-1}). Stroke volume was estimated at each temperature by calculating ventricular inflow volume (cm^3 or mL $beat^{-1}$), which is the product of total stroke distance (cm $beat^{-1}$) and AV valve area (cm^2) (Lee et al., 2014). In fish, stroke volume is predominantly governed by ventricular filling pressure and ventricular inflow volume (Franklin and Davie, 1992; Keen et al., 2017). Therefore, ventricular inflow volume can be used as a proxy for stroke volume assuming the variation in ejection fraction is low across heart beats at a given temperature.

Arrhenius breakpoint temperature (T_{AB}) was identified for each fish by assessing the Arrhenius plots of f_{Hmax} as detailed in Muñoz et al. (2015). Briefly, the natural logarithm of f_{Hmax} was plotted against the inverse of temperature (K) using SigmaPlot 13.0 (Systat Software, San Jose, CA, USA). A bi-phasic line was then fitted to the data using the software's 'Dynamic Fit Wizard', and T_{AB} was calculated as the point at which the slope changed on the bi-phasic line (Yeager and Ultsch, 1989). Arrythmia temperature (T_{Arr}) was identified for each fish as the first temperature at which arrythmias were evident on the DFV spectrographs. I also recorded the highest f_{Hmax} (f_{Hpeak}) and Q (Q_{peak}) observed across all temperatures for each fish.

2.1.6 Statistical Analysis

All statistical analyses were preformed using R 1.4.2 (R Core Team, Vienna, Austria). Differences in body mass between strains at the beginning of the experiment were assessed using a two-sample t-test. Differences in body mass after 9 months were assessed using a generalized linear mixed model (package lme4; Bates et al., 2018) that included strain and treatment as main effects and tank number and water source as random effects. Differences in T_{AB}, T_{Arr}, and f_{Hpeak} were assessed using generalized linear mixed models (package lme4; Bates et al., 2018) that included strain and treatment as main effects, body mass as a covariate, and tank number and water source as random effects. A treatment × strain interaction term was also included in each model. Random effects were quantified using the intraclass correlation coefficient (ICC) method (package lme4; Bates et al., 2018) and are represented as the percentage of the total model variance accounted for by the random effects. Differences in relative ventricular mass (RVM) and peak cardiac output (Q_{peak}) were assessed by calculating the residuals from a linear regression of the natural logarithm of ventricular mass or peak cardiac output versus the natural logarithm of body mass. Differences in residuals were analyzed using generalized linear mixed models that included strain and treatment as main effects, and tank number and water source as random effects. Repeated measures ANOVAs were used to assess differences in f_{Hmax} and Q between treatments and strains across temperatures. For comparative purposes, Q was presented as per kilogram of body mass for the repeated measures ANOVA. Body mass was also included as a covariate in the repeated measures analysis of f_{Hmax}. Lastly, the relationships between f_{Hpeak}, RVM, and T_{Arr} were examined at the individual level within treatments using a Pearson's correlation test. Assumptions of all statistical tests were evaluated and confirmed prior to data analysis.

2.1.7 Animal Ethics Approval

This study was approved by the Animal Ethics Committee of the University of Western Ontario (Protocol Number: 2018–084; Appendix A).

2.2 Cardiac Morphology of Lake Trout from the Sudbury Basin

2.2.1 Sample Collection

To determine the presence or absence of exotic rainbow smelt in Sudbury Basin lakes, fish community survey data was collected and compiled for 21 lakes from the Ontario Ministry of Natural Resources and Forestry (OMNRF) Broadscale Monitoring (BsM) and Land Information Ontario (LIO) databases (Table 2). Lake trout were collected from 6 lakes that contain rainbow smelt and 15 lakes where rainbow smelt are absent by angling or using gill nets between February 2021 and September 2022 (Table 2). Immediately after capture, lake trout were euthanized with an overdose of MS-222 and body mass was measured using a digital scale. The whole heart was collected from each fish and stored at -80℃ until later analysis.

2.2.2 Cardiac Morphology Measurements

Hearts collected from wild lake trout were thawed from -80℃, and ventricular mass (VM) and shape (VS) were measured using the methods described in Chapter 2.1.4.

2.2.3 Statistical Analysis

To determine if there were differences in VS between lake trout from lakes with or without rainbow smelt, a generalized linear mixed model was used that included the presence of rainbow smelt as a main effect, body mass and strain as covariates, and lake as a random effect (package lme4; Bates et al., 2018). Random effects were quantified using the intraclass correlation coefficient (ICC) method (package lme4; Bates et al., 2018) and are presented as the percentage of the total model variance accounted for by the random effects. Differences in relative ventricular mass (RVM) were assessed by analyzing the residuals from a linear regression of the natural logarithm of ventricular mass versus the natural logarithm of body mass using a generalized linear mixed model that included the presence of rainbow smelt as a main effect, strain as a covariate, and lake as a random effect. All statistical analyses were preformed using R 1.4.2 (R Core

Team, Vienna, Austria). Assumptions of all statistical tests were evaluated and confirmed prior to data analysis.

Table 2. List of lakes sampled for lake trout (*Salveninus namaycush*) in the Sudbury Basin. Lake data were obtained from Therrien (2019), OMNRF BsM database, LIO database, or OMNRF (2015).

Name	Latitude	Longitude	Surface Area (ha)	Max Depth (m)	Rainbow smelt	Strain (if stocked)
Bear	46.1892	-81.4507	682.3	36.6	P	
Bell	46.1294	-81.2042	217.7	26.8	A	Mishibishu
Burwash	47.1311	-81.0481	1058.2		A	
Chiniguchi	46.9262	-80.7068	1198.3	44.2	A	Killala
Elboga	47.0195	-81.6348	27.2	16.2	A	Killala
Evelyn	46.8960	-80.5907	110.7	24.0	A	
Kelly #27	46.7820	-80.5298	17.3	17.0	A	Killala
Fraleck	46.8960	-80.5910	110.7	24.0	A	Killala
Manitou	45.7775	-80.4348		49.0	P	Manitou
Manitou #2	46.8529	-80.2833	322.3	47.6	P	
Mozhabong	46.9648	-82.0774	1943.8	44.0	A	
Nelson	46.7216	-81.0960	315.8	50.3	A	
Panache	46.2358	-81.3066	8959	56.4	P	
Paradise	46.9750	-80.7663	487.4	35.0	A	
Rawson	46.9176	-80.5646	164.1	26.0	A	
Savage	46.2504	-81.5920	322.1	36.6	A	
Shakwa	46.4640	-81.5915	649.0	27.4	A	
Tyson	46.1174	-81.1185	1089.1	39.6	A	Killala
Wanapitei	46.4397	-80.7390	13131.0	125.0	P	
Wavy	46.3041	-81.0915	306.3	34.0	A	Killala
Whiskey	46.4397	-82.3356	992.8	55.2	P	Manitou

Note: Table modified from Therrien, (2019); Rainbow smelt present (P) or absent (A); BsM, Broad Scale Monitoring; LIO, Land Information Ontario

Chapter 3

3 Results

3.1 Measuring Cardiac Function, Morphology, and Thermal Tolerance in Two Strains of Hatchery-raised Lake Trout

A total of 51 trials were included in my analyses, with 11 trials excluded because of an abnormal response to the pharmacological stimulants or technical issues with the water recirculator. The analyzed fish included 21 from the Seneca strain (control: n=11; thiaminase: n=10) and 30 from the Slate strain (control: n=13; thiaminase: n=17; Table 3).

3.1.1 Body Mass

At the beginning of the experiment, Slate strain lake trout had significantly greater body mass (mean ± SE: 27.2 ± 0.7g) than Seneca strain lake trout (22.3 ± 0.6g; t=-5.44, df=425, P<0.001). However, of the fish included in my thermal performance analyses after 9 months, body mass did not significantly differ between treatments (F=0.99; df=1,9; P=0.35) or strains (F=0.19; df=1,9; P=0.68), and there was no significant treatment × strain interaction (F=0.08; df=1,10; P=0.78; Table 3, 4). Tank and water source both contributed negligibly to the total variance of the model (ICC: 0% for both tank and water source; Table 4).

Table 3. Body mass and sample size of Seneca and Slate strain lake trout (*Salvelinus namaycush*) raised on either a control or thiaminase diet for 9 months.

Strain	Treatment	Body mass (g ± SE)	Sample size (n)
Seneca	Control	133 ± 16	11
Seneca	Thiaminase	116 ± 17	10
Slate	Control	119 ± 11	13
Slate	Thiaminase	108 ± 8	17

3.1.2 Thermal Performance of Cardiac Function

Maximum heart rate (f_{Hmax}) increased significantly with temperature in this study (F=200.31; df=8,14; P<0.001), and there was a significant treatment × strain × temperature interaction effect on f_{Hmax} according to a three-way repeated measures ANOVA (F=3.25; df=2,12; P<0.001). Body mass contributed negligibly to this model (F=1.76; df=1; P=0.20). In fish from the Slate strain, individuals raised on the control diet had significantly greater f_{Hmax} across all temperatures than those on the thiaminase diet (F=5.27; df=2,13; P<0.001; two-way repeated measures ANOVA; Figure 7A). There was no significant difference in f_{Hmax} across temperatures between control and thiaminase treatments in Seneca strain fish (F=0.60; df=2,12; P=0.84; two-way repeated measures ANOVA; Figure 7B).

Similarly, cardiac output (Q) per kilogram of body mass increased significantly with temperature (F=41.66; df=9,12; P<0.001). However, I did not detect a significant treatment × strain × temperature interaction effect on Q per kilogram of body mass according to a three-way repeated measures ANOVA (F=1.22; df=2,10; P=0.28). Likewise, there was no difference in Q per kilogram of body mass across temperatures between control and thiaminase treatments in Slate strain fish (F=0.88; df=2,11; P=0.56; two-way repeated measures ANOVA; Figure 8A) or Seneca strain fish (F=0.90; df=2,10; P=0.54; two-way repeated measures ANOVA; Figure 8B).

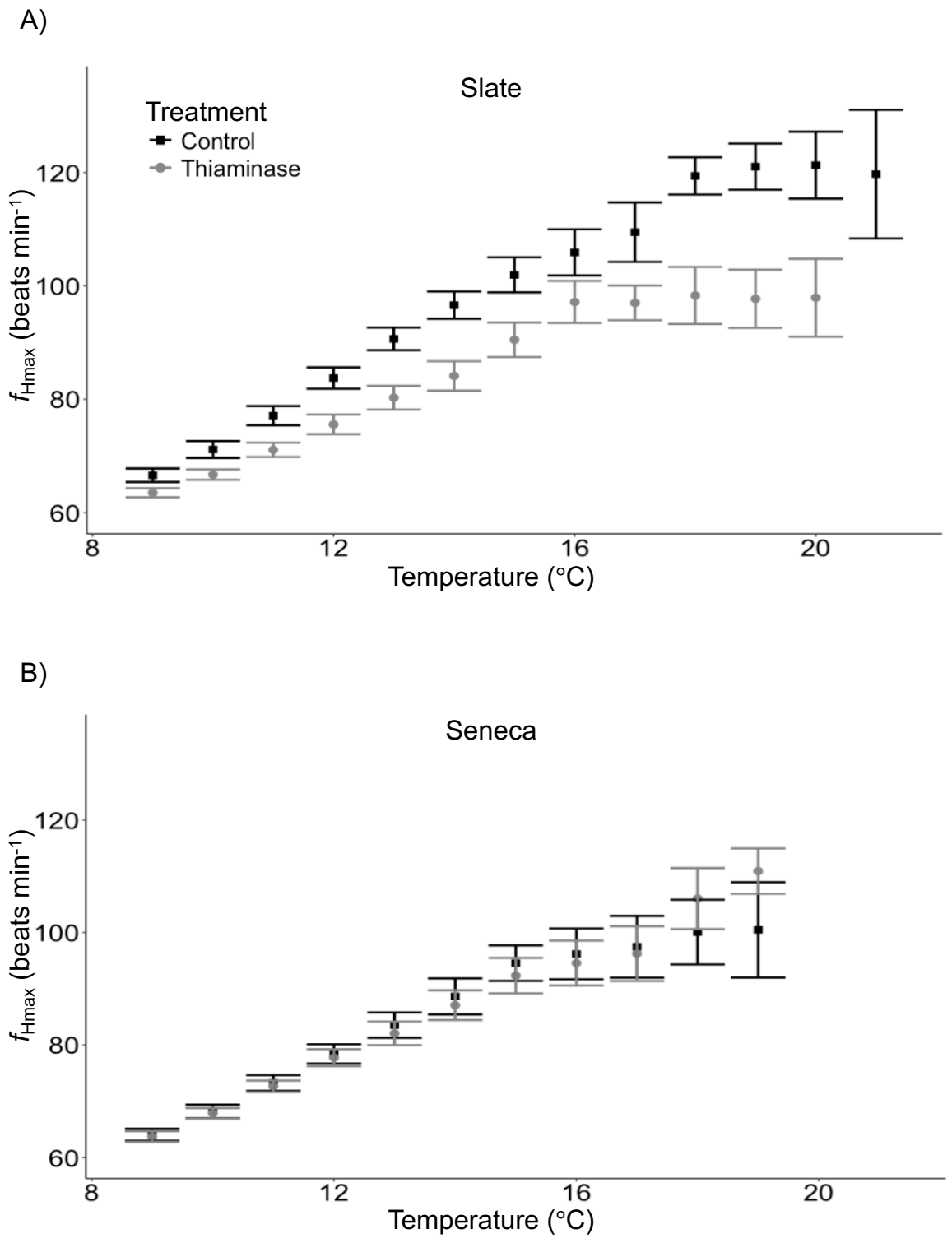

Figure 7. Effect of acute warming on maximum heart rate (f_{Hmax}) of Slate (A) and Seneca (B) strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Data are presented as means ± SE.

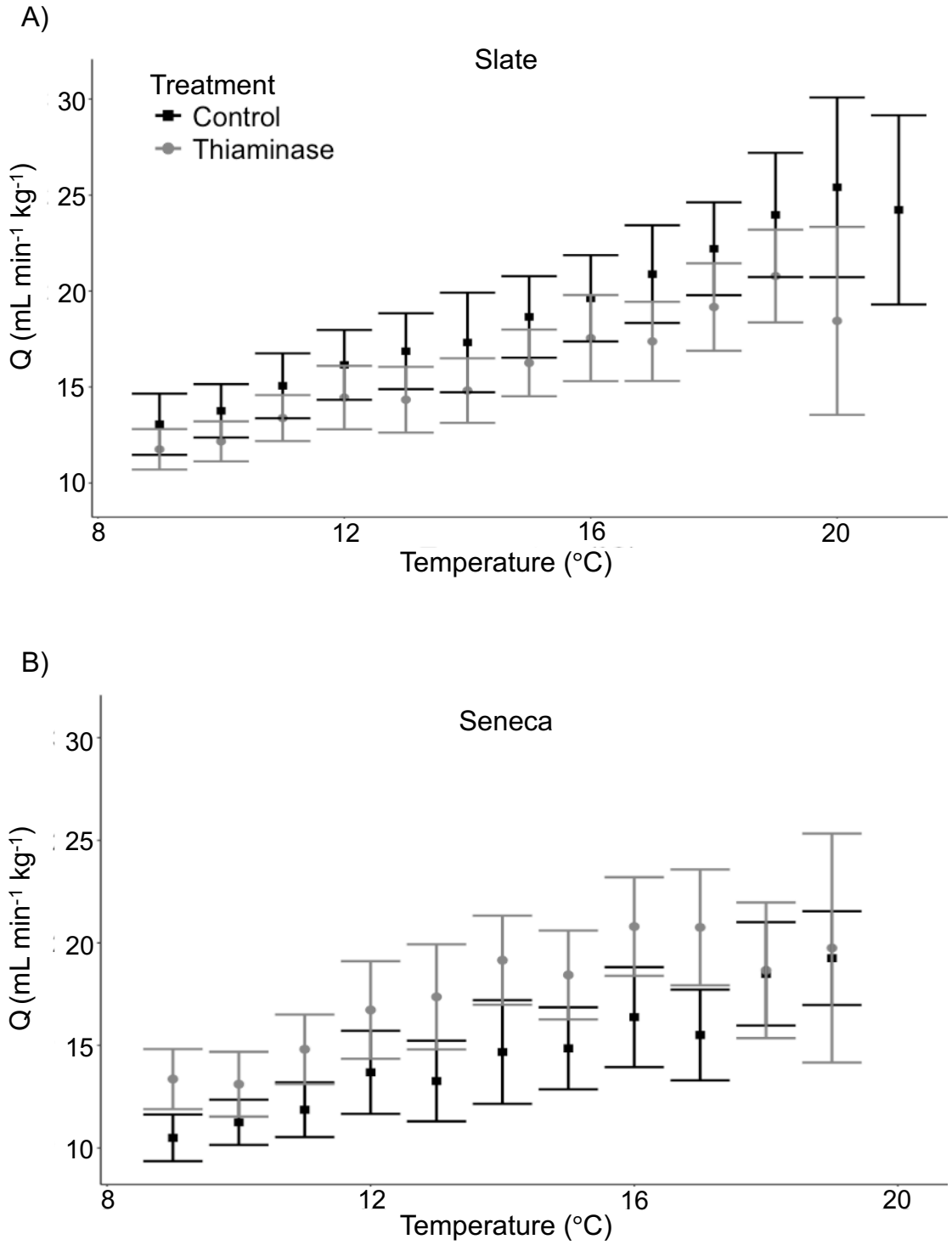

Figure 8. Effect of acute warming on cardiac output (Q) per kilogram of body mass of Slate (A) and Seneca (B) strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Data are presented as means ± SE.

3.1.3 Peak Cardiac Function

Peak heart rate (f_{Hpeak}) was significantly lower in lake trout raised on the thiaminase diet (mean ± SE: 96 ± 3 bpm) than in lake trout raised on the control diet (110 ± 4 bpm; F=9.40; df=1,9; P<0.05; Figure 9A). I did not detect any significant difference in f_{Hpeak} between strains (F=0.00060; df=1,9; P=0.98), and there was no significant treatment × strain interaction (F=2.76; df=1,10; P=0.13; Table 4). Body mass did not contribute significantly to the model (F=2.44; df=1,42; P=0.13), and the ICCs for the random effects were low to moderate (ICC: 2.6% for tank and 35.6% for water source; Table 4). In addition, f_{Hpeak} was significantly correlated with T_{Arr} at the individual level in both control and thiaminase treatment groups (R^2=0.66, P<0.001; and R^2=0.76, P<0.001, respectively). Peak cardiac output (Q_{peak}) scaled hypo-allometrically with body mass in this study (Figure 10). Using the residuals from this regression, I did not detect any difference in peak cardiac output (Q_{peak}) between treatments (F=0.07; df=1,9; P=0.80), or strains (F=0.02; df=1,10; P=0.89; Figure 9B), and there was no significant treatment × strain interaction (F=2.34; df=1,10; P=0.16; Table 4). Further, both random effects contributed negligibly to the model variance (4.3% for tank and 0% for water source; Table 4).

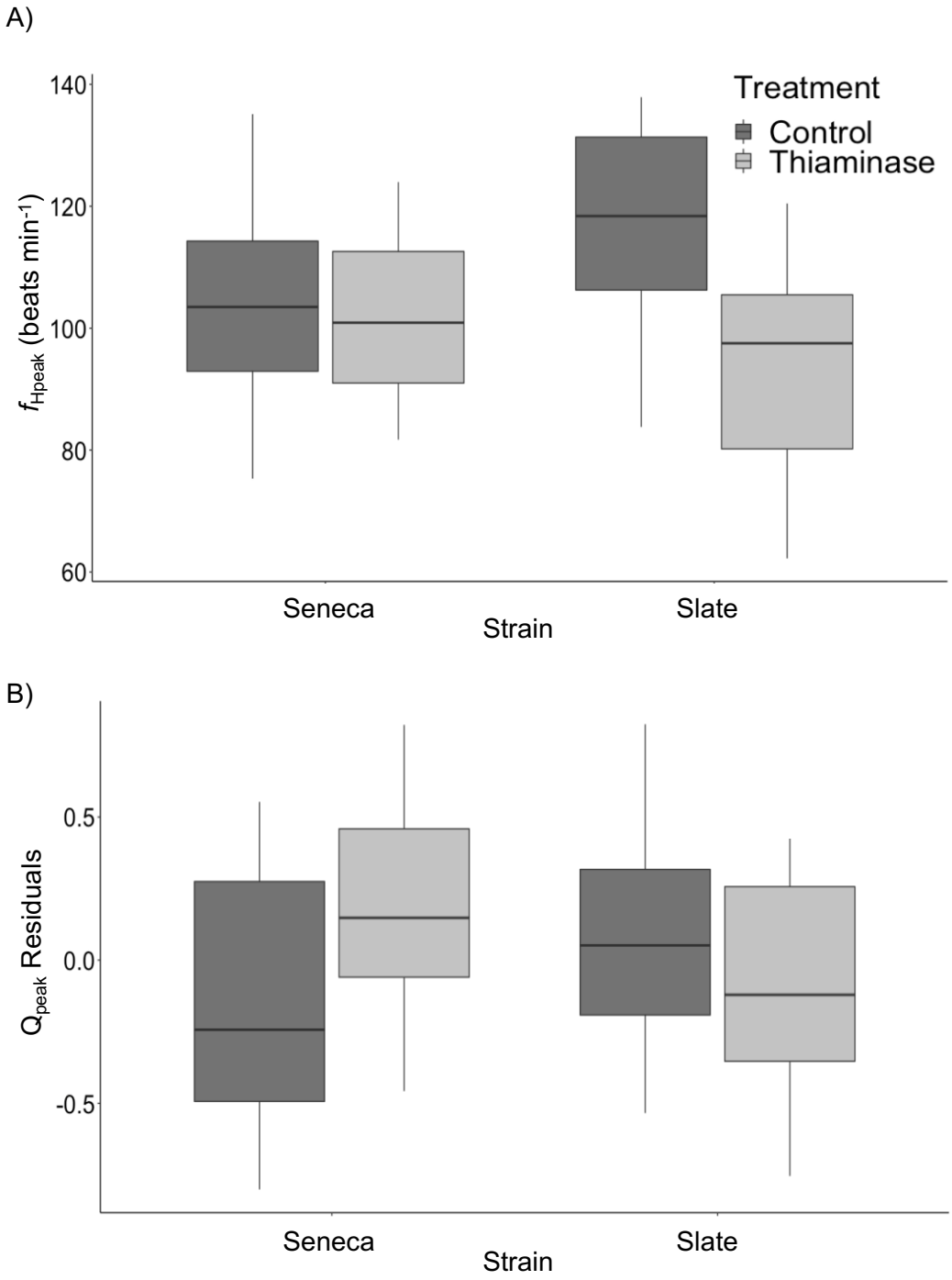

Figure 9. Peak heart rate (f_{Hpeak}) (A) and the residuals of peak cardiac output (Q_{peak}) (B) of Seneca and Slate strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Boxes show the median and the first and third quartiles. Whiskers show minimum and maximum values.

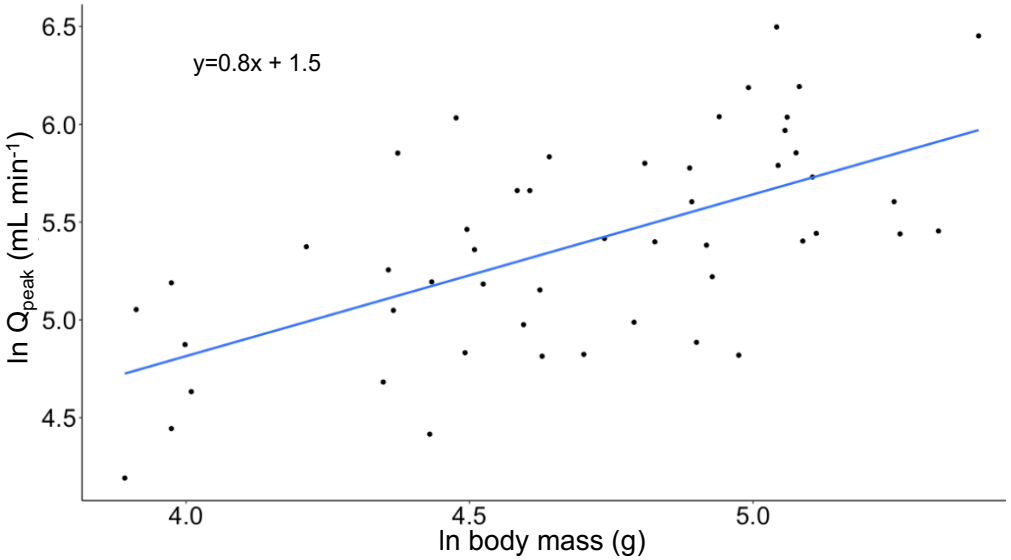

Figure 10. Allometry of peak cardiac output (Q_{peak}) versus body mass in hatchery-raised lake trout (*Salvelinus namaycush*). *N*=51.

3.1.4 Thermal Tolerance

I did not find a significant difference in arrythmia temperature (T_{Arr}) between treatments (F=2.37; df=1,10; P=0.16) or strains (F=0.067; df=1,10; P=0.80), and there was no significant treatment × strain interaction (F=0.56; df=1,10; P=0.47; Figure 11A). Body mass was not significant in this model (F=0.0040; df=1,39; P=0.95), and the ICCs for the random effects were low to moderate (15.9% for tank, 26.5% for water source; Table 4). In contrast, lake trout raised on the thiaminase diet had a significantly lower Arrhenius breakpoint temperature (T_{AB}; mean ± SE: 13.6 ± 0.3°C) compared to fish raised on the control diet (15.2 ± 0.3°C; F=12.93; df=1,9; P<0.01; Figure 11B). T_{AB} did not differ significantly between strains (F=0.24; df=1,10; P=0.64) or based on the treatment × strain interaction (F=0.96; df=1,10; P=0.35; Table 4). Body mass contributed negligibly to this model (F=1.00; df=1,43; P=0.32; Table 4), and the ICCs for the random effects were low (0% for tank, 20.0% for water source; Table 4).

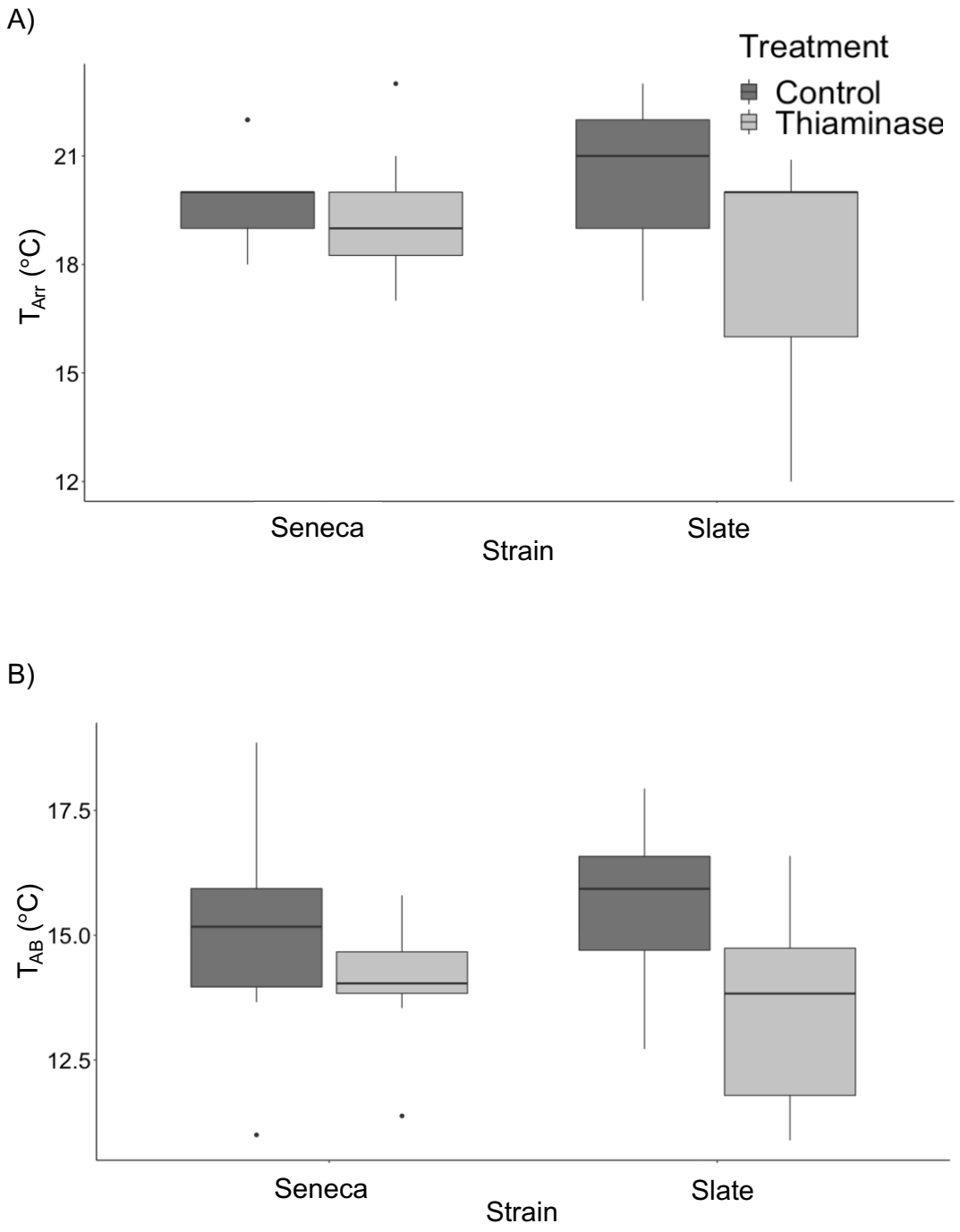

Figure 11. Arrythmia temperature (T_{Arr}) (A), and Arrhenius breakpoint temperature (T_{AB}) (B) of Seneca and Slate strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Boxes show the median and the first and third quartiles. Whiskers show minimum and maximum values. Points represent a maximum or minimum value that lies outside 1.5x the interquartile range.

Table 4. Summary of linear mixed model results for thermal tolerance, peak cardiac function, cardiac morphology, and body mass for Slate and Seneca strain lake trout (*Salvelinus namaycush*) raised on a control or thiaminase diet.

Metric	Model term	F	df	P	ß	ICC (%)	
						Tank	Water Source
T_{Arr}	Treatment	2.37	1,10	0.16	0.49	15.9	26.5
	Strain	0.067	1,10	0.80	0.35		
	Treatment × strain	0.56	1,10	0.47	1.08		
	Body mass	0.004	1,39	0.95	0.0004		
T_{AB}	Treatment	12.93	1,9	**<0.01**	1.10	0	20.0
	Strain	0.24	1,9	0.64	0.24		
	Treatment × strain	0.96	1,10	0.35	0.89		
	Body mass	1.00	1,43	0.32	0.005		
f_{Hpeak}	Treatment	9.40	1,9	**<0.05**	5.05	2.6	35.6
	Strain	0.0006	1,9	0.98	7.71		
	Treatment × strain	2.76	1,10	0.13	15.15		
	Body mass	2.44	1,42	0.13	0.08		
Q_{peak}	Treatment	0.07	1,9	0.80	0.31	4.3	0
	Strain	0.02	1,10	0.89	0.21		
	Treatment × strain	2.34	1,10	0.16	0.48		
RVM	Treatment	6.68	1,9	**<0.05**	0.09	0	0
	Strain	11.78	1,9	**<0.01**	0.13		
	Treatment × strain	0.03	1,10	0.87	0.01		
VS	Treatment	0.41	1,9	0.54	0.14	0	0
	Strain	26.38	1,9	**<0.001**	0.28		
	Treatment × strain	1.66	1,42	0.23	0.15		
	Body mass	2.17	1,9	0.15	0.0004		
Body mass	Treatment	0.99	1,9	0.35	0.15	0	0
	Strain	0.19	1,9	0.68	0.09		
	Treatment × strain	0.08	1,10	0.78	0.07		

Note: T_{Arr}, arrythmia temperature; T_{AB}, Arrhenius breakpoint temperature; f_{Hpeak}, peak heart rate; Q_{peak}, peak cardiac output; RVM, relative ventricular mass; VS, ventricular shape. P-values in bold indicate significance for $\alpha = 0.05$.

3.1.5 Cardiac Morphology

Ventricular mass (VM) scaled isometrically with body mass in this study (Figure 12). Using the residuals from this regression, RVM was significantly greater in fish raised on the thiaminase diet compared to fish raised on the control diet ($F=6.68$; $df=1,9$; $P<0.05$; Figure 13A). In addition, fish of the Seneca strain had significantly greater RVM compared to fish of the Slate strain ($F=11.78$; $df=1,9$; $P<0.01$; Figure 13A). There was no treatment × strain interaction ($F=0.03$; $df=1,10$; $P=0.87$; Table 4), and both random effects had low ICCs (0% for tank and water source; Table 4). Lastly, I did not find a significant correlation between RVM and T_{Arr} at the individual level in either control or thiaminase treatment groups ($R^2=0.004$, $P=0.77$; and $R^2=0.007$, $P=0.68$, respectively).

I did not detect any difference in ventricular shape (VS) between treatments ($F=0.41$; $df=1,9$; $P=0.54$; Table 3), however, Seneca strain lake trout had significantly wider ventricles (mean ± SE: 1.19 ± 0.040) than Slate strain lake trout (0.97 ± 0.020; $F=26.38$; $df=1,9$; $P<0.001$; Figure 13B). Body mass was not significant in this model ($F=2.17$; $df=1,42$; $P=0.15$), and there was no significant treatment × strain interaction ($F=1.66$; $df=1,9$; $P=0.23$; Table 4). Both random effects had low ICCs (0% for both tank and water source; Table 4).

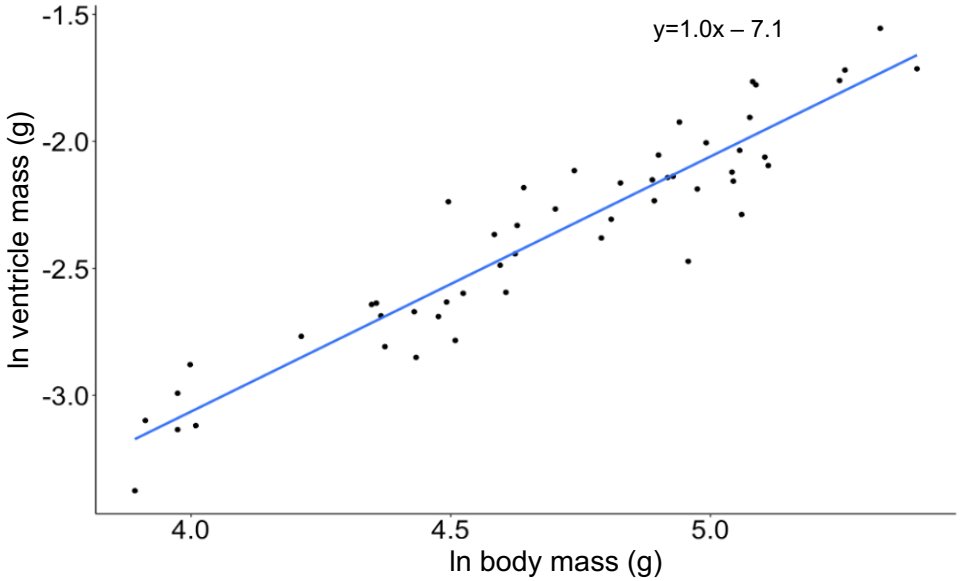

Figure 12. Allometry of ventricle mass versus body mass in hatchery-raised lake trout (*Salvelinus namaycush*). *N*= 51.

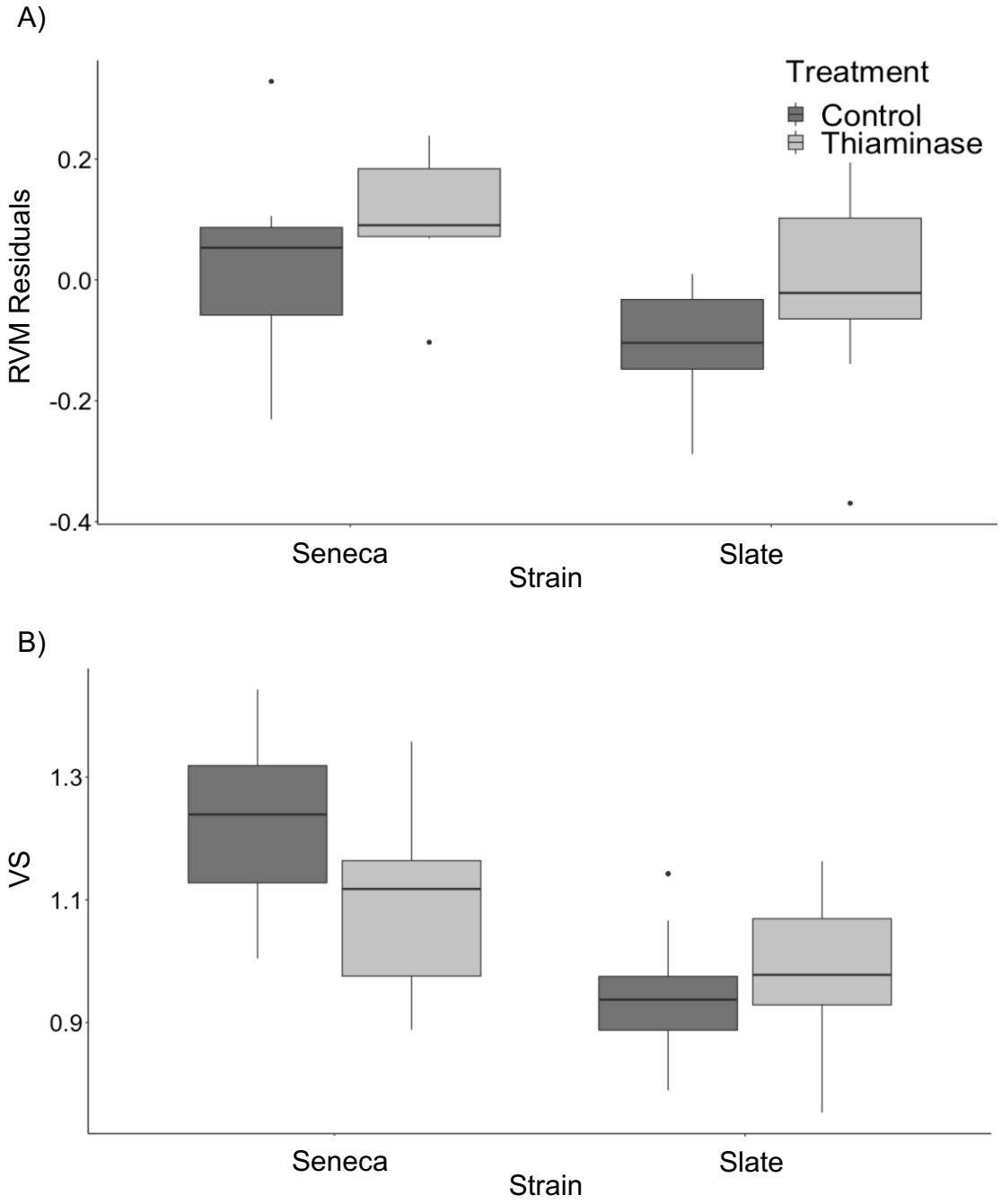

Figure 13. Residuals of relative ventricular mass (RVM) (A) and ventricular shape (VS) (B) of Seneca and Slate strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Points represent a maximum or minimum value that lies outside 1.5x the interquartile range.

3.2 Cardiac Morphology of Lake Trout from the Sudbury Basin

3.2.1 Sample collection

A total of 229 lake trout were collected across 21 lakes in the Sudbury Basin, with at least 3 fish collected per lake. Of these fish, 96 came from lakes where rainbow smelt are present, and 133 came from lakes where rainbow smelt are absent.

3.2.2 Body mass

Body mass was measured in 202 lake trout (rainbow smelt absent, n=123; rainbow smelt present, n=79; Table 5). Of these fish, body mass did not differ significantly between lakes with or without rainbow smelt ($F=0.36$; $df=1,11$; $P=0.56$), however strain was significant in this model ($F=7.92$; $df=4,15$; $P<0.01$) such that Mishibishu strain fish had a significantly greater body mass than other lake trout strains. The ICC for lake was low (13.2%; Table 7).

Table 5. Body mass and sample size of lake trout (*Salvelinus namaycush*) collected from lakes with and without rainbow smelt (RS) in the Sudbury Basin.

RS present?	Body mass (g ± SE)	Sample size (n)
Yes	1250 ± 103	79
No	1175 ± 93	123

3.2.3 Cardiac Morphology

Of the 229 fish collected, hearts were obtained from 225 fish (Table 6). Ventricular mass (VM) was measured in 200 fish (rainbow smelt absent, n=121; rainbow smelt present, n=79; Table 6). Twenty-five fish could not be included in this analysis due to missing body mass data or physical damage to the ventricle during dissection. Similar to hatchery-raised lake trout, body mass scaled isometrically with VM in lake trout from the Sudbury Basin (Figure 14). Using the residuals from this regression, RVM did not significantly differ between lakes with and without rainbow smelt, however it was

approaching significance (F=3.47; df=1,12; P=0.06; Figure 15A). Strain was not significant in this model (F=0.63; df=1,17; P=0.65), and the ICC was low for lake (12.5%; Table 7).

Ventricular shape (VS) was measured in a total of 174 fish (rainbow smelt absent, n=89; rainbow smelt present, n=85; Table 6). VS could not be calculated in 51 fish due to physical damage to the heart during dissection or damage during storage. Of the fish included, VS was significantly lower (ventricles were significantly more elongated) in lake trout collected from lakes with rainbow smelt (mean ± SE: 0.78 ± 0.01) than lakes without rainbow smelt (0.81 ± 0.01; F=6.97; df=1,9; P<0.05; Figure 15B). Body mass was not significant in this model (F=3.16; df=1,140; P=0.08; Table 6), strain was not significant in this model (F=1.87; df=4,17; P=0.16), and the ICC for lake was low (5.4%; Table 6).

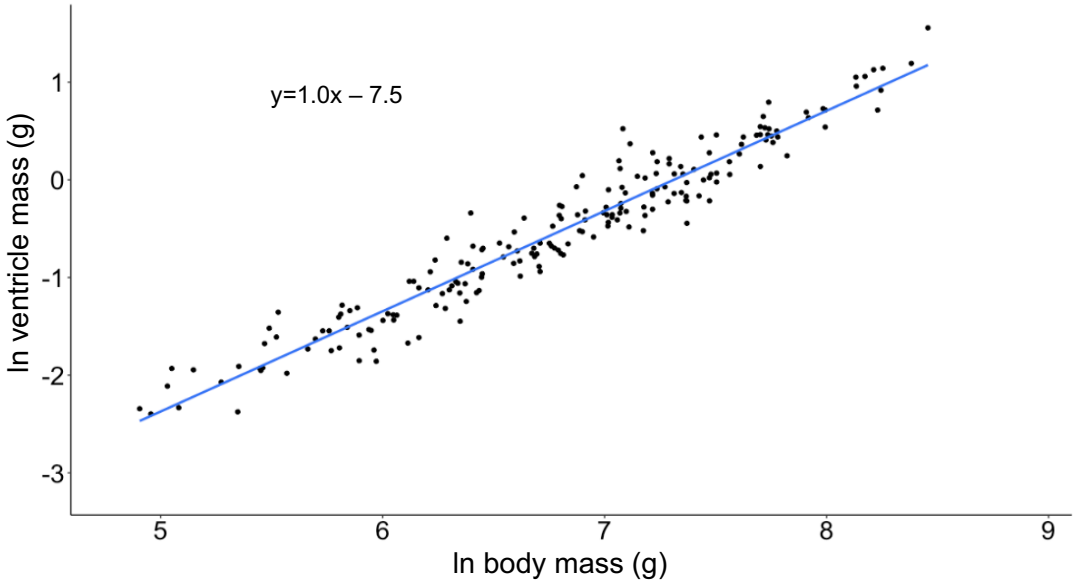

Figure 14. Allometry of ventricle mass versus body mass in wild lake trout (*Salvelinus namaycush*) collected from the Sudbury Basin. *N*=200.

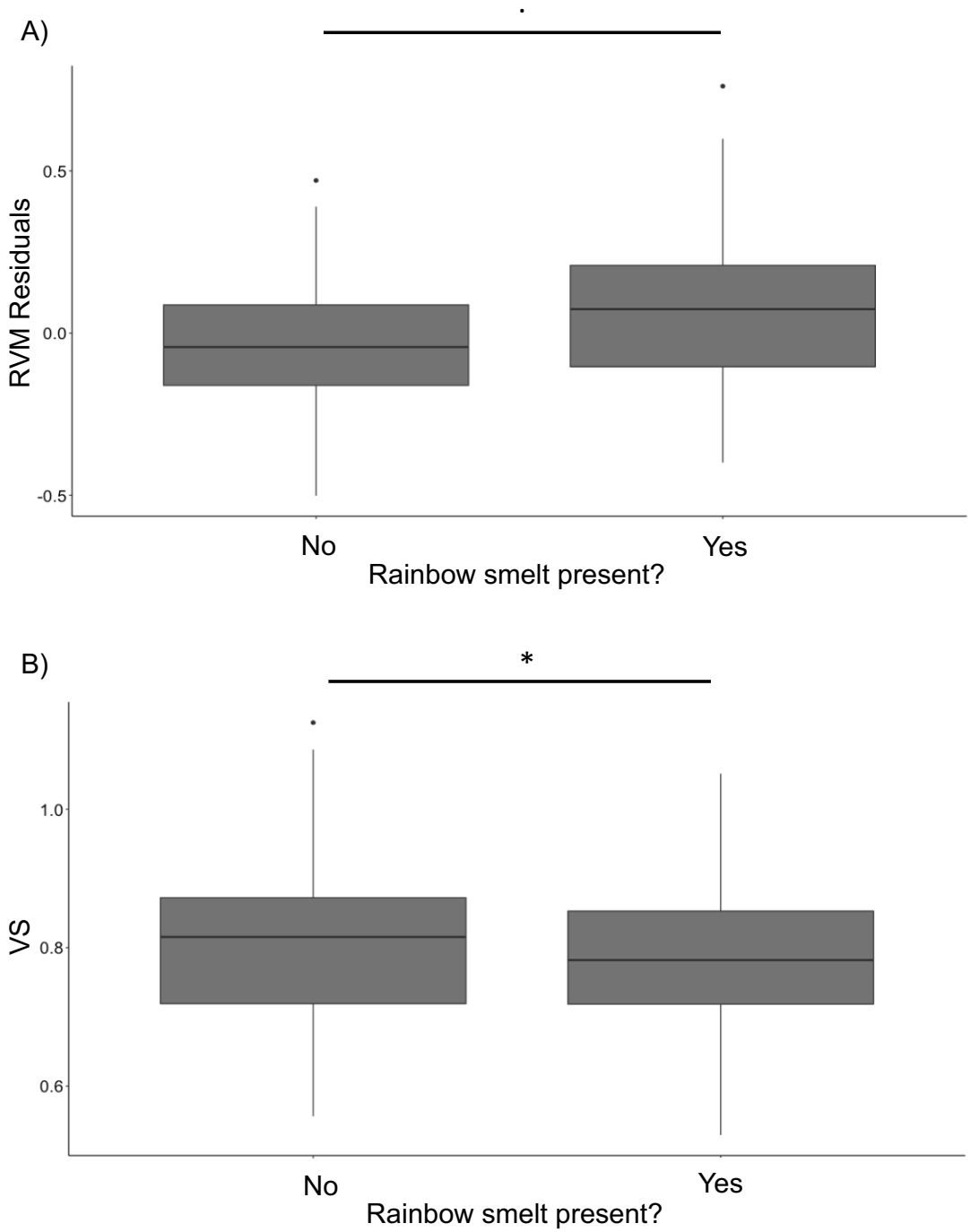

Figure 15. Residuals of relative ventricular mass (RVM) and ventricular shape (VS) of lake trout (*Salvelinus namaycush*) collected from lakes with and without rainbow smelt in the Sudbury Basin. Boxplots show the median and the first and third quartiles. Whiskers show minimum and maximum values. Points represent a maximum or minimum value that lies outside 1.5x the interquartile range. An asterisk (*) represents a significant difference ($p<0.05$) between lakes with and without rainbow smelt, and a dot (.) represents a p-value approaching significance ($0.10 > p > 0.05$).

Table 6. Sample sizes by lake of lake trout (*Salveninus namaycush*) collected in the Sudbury Basin.

Lake Name	Number of hearts collected (n)	RVM sample size (n)	VS sample size (n)
Bear	15	15	14
Bell	7	7	6
Burwash	12	7	10
Chiniguchi	15	15	7
Elboga	9	9	5
Evelyn	7	4	7
Fraleck	3	3	3
Kelly #27	5	5	3
Manitou	19	19	16
Manitou #2	15	15	13
Mozhabong	4	4	2
Nelson	9	9	6
Panache	14	11	11
Paradise	20	20	15
Rawson	8	8	5
Savage	3	3	3
Shakwa	20	19	10
Tyson	4	4	3
Wanapitei	19	11	19
Wavy	7	7	7
Whiskey	10	5	9

Note: RVM, relative ventricular mass; VS, ventricular shape.

Table 7. Summary of linear mixed model results for cardiac morphological metrics of lake trout (*Salvelinus namaycush*) collected from lakes with and without rainbow smelt in the Sudbury Basin.

Metric	Model term	F	df	P	ICC (%) Lake
RVM	Rainbow smelt	3.47	1,12	0.06	12.5
	Strain	0.63	4,17	0.55	
VS	Rainbow smelt	6.97	1,9	**<0.05**	5.4
	Strain	1.87	4,17	0.16	
	Body mass	3.16	1, 140	0.08	
Body mass	Rainbow smelt	0.36	1,11	0.56	13.2
	Strain	7.92	4,15	**<0.01**	

Note: RVM, relative ventricular mass; VS, ventricular shape. P-values in bold indicate significance for $\alpha = 0.05$.

Chapter 4

4 Discussion

4.1 Functional and Structural Effects of Dietary Thiaminase on the Heart

In mammals, thiamine deficiency is often associated with impaired cardiac function (Roman-Campos and Cruz 2014), yet this relationship has received limited attention in other taxa. To my knowledge, my study is the first to directly examine the cardiac-related effects of thiamine deficiency in a fish. In agreement with my prediction, I demonstrate that the consumption of bacterial-derived thiaminase can impair cardiac function. Lake trout raised on a diet containing thiaminase for 9 months displayed a 13% decline in peak heart rate (f_{Hpeak}) compared to fish fed a control diet. My results are consistent with studies that have shown a reduced heart rate in mammals during thiamine deficiency (Yoshitoshi et al. 1961; Davies and Jennings 1970; Oliveira et al. 2007). Interestingly, reduced cardiac function in fish from the thiaminase treatment may explain the abnormal responses of fish to the pharmacological stimulants, as all of the fish that displayed abnormal reactions were from the thiaminase treatment. Instead of an increase in heart rate after injection, these fish displayed either a reduction in heart rate or immediate arrythmia. This is potentially linked to the cardiac impairments associated with thiamine deficiency, such that fish with a less-fit heart struggled to reach f_{Hmax} when stimulated. In contrast to a reduced f_{Hpeak}, lake trout from the thiaminase treatment did not display any reduction in peak cardiac output (Q_{peak}). It is possible that the greater relative ventricular mass in fish from the thiaminase treatment ameliorated any potential in reduction cardiac output from a reduced heart rate, since larger ventricles have a greater capacity to pump larger volumes of blood. However, I believe that the lack of difference in cardiac output between treatments resulted from the high variance in my stroke volume measurements (Appendix C). Stroke volume and heart rate are the two components that comprise cardiac output, and stroke volume did not change with temperature in this study (Appendix C). This indicates that increases in cardiac output during acute warming in this

study were driven entirely by increases in maximum heart rate - a common finding in fishes (Farrell, 2009; Eliason and Anttila, 2017). Because thiaminase-fed lake trout of the Slate strain demonstrated a reduction in maximum heart rate compared to the control group, I suspect that cardiac output was also reduced, but the variation introduced from my stroke volume measurements masked this difference. Cardiac impairments associated with thiamine deficiency have been largely attributed to a variety of factors including limited ATP production (McCandles et al., 1970), increased levels of reactive oxygen species (Gioda et al., 2010), impaired calcium release from sarcoplasmic reticulum (Oliveira et al., 2007), and lactic acidosis (Klein et al., 2004; Karapinar et al., 2008). Regardless of the specific cause, my study provides some of the first evidence to suggest that the cardiac impairments associated with thiamine deficiency in mammals are also present in fish.

Cardiac structural alterations have often accompanied impaired heart function in mammals during thiamine deficiency (Roman-Campos and Cruz, 2014). Contrary to my prediction of a reduced relative ventricular mass (RVM) in fish from the thiaminase treatment, I found that RVM was greater in lake trout raised on the thiaminase diet than in lake trout raised on the control diet. In studies with rats there has been reports of both increased heart size (Yoshitoshi et al. 1961; McCandles et al. 1970) and decreased heart size (Cohen et al. 1976; Oliveira et al. 2007; Roman-Campos et al. 2009; Gioda et al. 2010) during thiamine deficiency. My results are most similar to those seen in humans, where heart enlargement due to thiamine deficiency is associated with a disease clinically known as Shoshin beriberi (Meurin 1996; Chisolm-Straker and Cherkas 2013). The mechanisms underlying heart enlargement during thiamine deficiency are unknown. However, it has been suggested that a reduction in ATP availability in tissues can result in edema (fluid retention) due to impaired ion-pump function (Tanaka et al. 2003; Klein et al. 2004). It is also possible that the greater RVM observed in thiaminase-fed lake trout is the result of an indirect response to ameliorate reductions in oxygen delivery due to reductions in f_{Hmax}. However, this conclusion would only be supported in Slate strain fish, as there was no difference in f_{Hmax} between treatments in Seneca strain fish. Furthermore, I did not detect any difference in ventricular shape (VS) between treatments. However, there did appear to be a trend towards having longer ventricles in fish from the

thiaminase group, particularly in lake trout of the Seneca strain. Similar structural changes reflecting more elongated ventricles have been reported in both thiamine deficient rats and humans (Yoshitoshi et al., 1961; Park et al., 2007; Coelho et al., 2008). Dilated cardiomyopathy — a condition where the muscular wall of a ventricle stretches and becomes weaker — has been previously reported during thiamine deficiency and is thought to be the result of impaired energy production and elevated ventricular diastolic pressure (Mendoza et al., 2003; Park et al., 2007; Panigrahy et al., 2020). It is possible that the elongated hearts observed in Seneca strain lake trout from the thiaminase treatment are the result of dilated cardiomyopathy.

In a hatchery-setting, I demonstrated that administering an experimental diet that mimicked a diet based on high-thiaminase prey fishes altered cardiac morphology in lake trout. Given this demonstrated change in cardiac morphology, I predicted that wild lake trout collected from lakes where rainbow smelt have invaded in the Sudbury Basin would show similar effects. In agreement with my prediction, lake trout collected from lakes with rainbow smelt trended towards having a greater relative ventricular mass compared to lake trout from lakes where rainbow smelt are absent. Surprisingly, I also found that ventricles were more elongated in lake trout from lakes with rainbow smelt present compared to lakes without rainbow smelt. These results represent the first instance of cardiac-related effects of thiamine deficiency being reported in wild fish and further support my laboratory findings of thiaminase-induced increases in relative ventricular mass and possible changes in ventricle shape.

4.2 Dietary Thiaminase and Thermal Tolerance

The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis suggests that upper temperature tolerance in fish is limited by the capacity of the cardiorespiratory system to meet tissue oxygen demands (Pörtner and Knust, 2007), and that relative ventricular mass and peak heart rate are important factors determining the thermal tolerance capabilities of fishes (Anttila et al., 2013a; Anttila et al., 2014). Therefore, I hypothesized that if dietary thiaminase impaired cardiac function and reduced relative ventricular mass in lake trout as it does in mammals, fish raised on the thiaminase diet

would have a reduced ability to tolerate warmer water temperatures. Interestingly, I found that lake trout fed dietary thiaminase had a greater relative ventricular mass, but that the greater relative ventricle mass was not associated with any advantage in thermal tolerance. Instead, I found that lake trout raised on a thiaminase diet had an Arrhenius breakpoint temperature that was 1.6°C lower than that of the control group. A similar trend was seen with Arrythmia temperature, although it was not statistically significant. In thiamine-replete fish, individuals with larger ventricles have been shown to have a higher capacity to tolerate elevated temperatures (Anttila et al. 2013a; Ozolina et al. 2016). It has been suggested that the improved thermal tolerance capabilities in fish with larger ventricles may be mediated by an increased percentage of compact myocardium, a trait that is associated with greater oxygen delivery in fish that are warm-acclimated and whose life histories are more metabolically demanding (Eliason et al., 2011; Muir et al., 2022). My results suggest that the increased relative ventricular mass in the thiaminase treatment does not reflect an increase in compact myocardium but instead may be the result of edema (fluid retention), a symptom which has been clinically reported in the brain, liver, and heart of humans diagnosed with beriberi (Watanabe et al., 1981; Hazell and Butterworth., 2009; Helali et al., 2019; Smith et al., 2021). In humans, cardiac edema is associated with a multitude of negative functional implications including reduced ventricular contractility and congestive heart failure (Fattal-Valevski, 2011, Helali et al., 2019).

Lastly, I found considerable support for the OCLTT hypothesis in this thesis. Metabolic optimum temperatures for lake trout have previously been estimated to be between 15-17°C (Gibson and Fry, 1954; Evans, 2007), which is consistent with the average Arrhenius breakpoint temperature (T_{AB}) of control fish observed in this study. The upper critical temperature for lake trout has previously been estimated to be 23.5°C (Gibson and Fry, 1954; Evans, 2007), which is also comparable to the average arrythmia temperature (T_{Arr}) of control fish in this study. The close association between the rate transition temperatures for maximum heart rate (T_{AB} and T_{Arr}) measured in this study and the known optimum (T_{Opt}) and upper critical temperature (T_{Crit}) of lake trout further supports the notion that T_{AB} and T_{Arr} can be used to estimate T_{Opt} and T_{Arr}, respectively — as has previously been demonstrated in studies with coho salmon (Casselman et al., 2012;

Anttila et al., 2013b). Furthermore, my findings of a decreased peak heart rate accompanied by reduced thermal tolerance in fish from the thiaminase treatment together with a significant correlation between peak heart rate and Arrythmia temperature at the individual level are consistent with previous research (Anttila et al., 2014; Muñoz et al., 2015; Safi et al., 2019). Taken together, these findings represent the first evidence to suggest that dietary thiaminase may hinder thermal tolerance in a salmonid.

4.3 A Cold-Adapted Fish in Hot Water

Lake trout is a cold water, stenothermal species that typically prefer water temperatures between 8-12°C (Christie and Reiger, 2011). Being a cold-adapted species with a narrow thermal window, lake trout are particularly susceptible to the effects of global climate change (Chu et al., 2005; Sharma et al., 2011; Kovach et al., 2019). Indeed, climate change is predicted to have adverse consequences for cold-water fishes, primarily through the alteration of thermal profiles in freshwater lakes (Stefan et al. 1998; Ficke et al. 2007). Climate models predict surface temperatures of freshwater lakes in Canada to increase as much as 18°C by 2100 (Sharma et al. 2007), and an increase of as much as 3°C is expected to reduce the range and abundance of cold-water salmonids by 20% (Casselman, 2002). Increases in surface water temperatures are predicted to increase the strength and duration of thermal stratification in temperate lakes which can reduce dissolved oxygen concentrations in the hypolimnion where lake trout reside during the summer months (Christie and Regier 1988; Stefan et al. 1998; Ficke et al. 2007). A reduction in dissolved oxygen concentrations could exacerbate the thermal effects of thiamine deficiency in lake trout as environmental hypoxia has previously been linked to reductions in thermal tolerance in fish (Pörtner and Lannig, 2009). In Canada, some of the highest surface water temperatures in lakes are predicted to occur in Ontario (Sharma et al. 2007), where approximately 25% of the lakes that contain lake trout exist globally (OMNRF 2015). My results of a reduced thermal tolerance in thiaminase-fed lake trout suggest that the effects of climate change may be exacerbated by the presence of invasive high-thiaminase prey fishes. This potential interaction presents an even greater challenge in the efforts of lake trout conservation and restoration, particularly as climate change continues to drive the range expansions of aquatic invasive species (Rahel et al. 2007).

Anecdotally, the negative effects of dietary thiaminase on lake trout are likely to be most pervasive in the Great Lakes, while the greatest temperature effects of climate change on lake trout will likely occur in inland lakes at higher latitudes. Thus, many lake trout populations may not experience both stressors at the same time, at least not initially. However, this concern may be particularly heightened in areas such as inland lakes of the Sudbury Basin where range expansions of rainbow smelt have overlapped with several lake trout restoration programs (Selinger et al. 2006).

4.4 Strain Differences and Management Implications

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. In Lake Ontario, approximately 500,000 yearling lake trout are stocked in Canadian waters every year (Lantry et al., 2014). Of these fish, about 60% are of the Seneca strain and 25% are of the Slate strain (Lantry et al., 2014). In this study, I found notable differences in thiaminase tolerance between strains. Perhaps most noteworthy, I found a significant strain-specific difference in maximum heart rate (f_{Hmax}) between treatments across temperature profiles. A strong reduction in f_{Hmax} was observed across all temperatures in the thiaminase treatment for the Slate strain compared to the control group, while no difference between treatments was observed in fish of the Seneca strain at any temperature. Though not statistically significant, I also found that Slate strain lake trout in the thiaminase treatment demonstrated a greater reduction relative to the control group in both thermal tolerance metrics and peak heart rate compared to Seneca strain fish. In agreement with my prediction, these findings suggest that Seneca strain lake trout may possess local adaptations that help mitigate the effects of dietary thiaminase. These results are in agreement with recent findings from Fitzsimons et al. (2021), who demonstrated that Seneca strain lake trout have reduced thiamine utilization and a higher tolerance for thiamine deficiency compared to other lake trout strains. Of fish fed the control diet, I did not find any differences in thermal tolerance or peak cardiac function between the two strains. However, I did find strain-specific differences in relative ventricular mass, where Seneca strain lake trout had larger ventricles than Slate strain fish relative to body mass. It is possible that this difference is the result of population-specific

temperature preferences. Since Seneca strain lake trout typically inhabit warmer, shallower water compared to Slate strain fish (Elrod et al., 1996), a larger ventricle could help facilitate heightened oxygen delivery during extended foraging bouts in water temperatures above their thermal optima. In addition, I found that Seneca strain fish had wider hearts than Slate strain fish. Because fish with wider ventricles are typically associated with more sedentary habits, I suspect that this difference in VS is the result of a more-metabolically demanding lifestyle in Slate strain fish as they historically inhabited a much larger body of water than Seneca strain lake trout. Altogether, these results suggest that Seneca strain lake trout may possess beneficial adaptations that could translate to improved survival in the current and potential future environmental conditions in Lake Ontario.

4.5 Connecting the Dots: Linking Cardiac Impairments to Whole-body Metrics

Prior to this study, there has been little direct mechanistic evidence to explain the observed physiological and behavioural impairments associated with thiamine deficiency in salmonids. Here, I propose that impairments to cardiac function and changes in cardiac morphology may contribute to some of the impairments observed at the whole-body level during thiamine deficiency. To this point, a variety of whole-body metrics were measured throughout this study by Chris Therrien (CT; Therrien et al., un-published data; Appendix D). In addition to the various cardiac implications and reduced thermal tolerance observed in my study, CT found that lake trout fed dietary thiaminase for 6 months had reduced swimming performance, a slower growth rate, changes in skin pigmentation, lower tissue thiamine concentrations, and reduced survival (Therrien et al., un-published data; Appendix D). Contrary to our prediction of increased thiaminase-tolerance in Seneca strain lake trout and to some of the findings from my study, reductions in survival and white muscle thiamine concentrations were only observed in Seneca strain lake trout from the thiaminase treatment, whereas Slate strain lake trout did not show any difference between treatment groups. These seemingly contradictory findings show some striking resemblances to manifestations of thiamine deficiency in humans. In humans, thiamine

deficiency typically manifests as either dry beriberi (no cardiac impairments), or wet beriberi (cardiac impairments; Fattal-Valevski, 2011). This division in clinical diagnoses is thought to occur due to differences in pre-existing health conditions among patients (i.e. patients with previous heart injuries/conditions are more likely to develop wet beriberi; Wiley and Gupta, 2019). It is possible that Seneca and Slate strain lake trout differ in their manifestations of thiamine deficiency, where Seneca strain fish display signs analogous to dry beriberi and Slate strain fish show signs analogous to wet beriberi; however, genetic differences between strains likely underly this difference. Seneca strain lake trout in the thiaminase treatment did not show any reductions in cardiac function compared to control fish but had reduced survival and swimming performance. Other aspects of thiamine deficiency such as neuropathy and immune function impairments may be more prominent than cardiac impairments in Seneca strain lake trout, and likely drive the reductions in whole-body metrics measured by CT. However, further investigations are needed to confirm if neurological and immune system impairments are evident in this strain during thiamine deficiency. Surprisingly, the cardiac impairments observed in Slate strain lake trout from the thiaminase treatment in my study did not translate to reduced survival but were associated with reduced swimming performance (Appendix D) and thermal tolerance. This suggests that the cardiac impairments associated with thiamine deficiency might be detrimental during periods of physiological stress (e.g. vigorous exercise and acute heat stress) and less important when at rest. Preliminary data on white muscle and liver thiamine concentrations follow a similar pattern, where there was a reduction of tissue thiamine in Seneca strain lake trout of the thiaminase treatment and no difference between treatments in Slate strain fish (Appendix D). This could be the result of preferential tissue thiamine allocation, which has been suggested to occur in coho salmon during thiamine injection (Fitzsimons et al., 2005). It is possible that thiamine is preferentially allocated to heart tissue to maintain cardiac function under low-thiamine conditions in Seneca strain fish and more-so to liver and muscle tissue in Slate strain fish which would explain the strain-specific differences in cardiac impairments and tissue thiamine concentrations between treatments. Interestingly, CT found an increase in blue colouration in lake trout raised on the thiaminase diet. Similar findings have been reported in human patients suffering from thiamine deficiency

(clinically reported as cyanosis) and is linked to reduced energy production and deteriorating heart function (Engbers et al., 1984; Roman-Campos and Cruz, 2014; Barennes et al., 2015). Taken together, my study is the first to provide direct evidence suggesting that cardiac impairments due to thiamine deficiency may be a contributing factor underlying some of the whole-body manifestations of thiamine deficiency in lake trout.

4.6 Limitations and Future Directions

A number of questions remain unanswered regarding the mechanism behind dietary thiaminase and thiamine deficiency that were beyond the scope of this thesis. While it has been shown that bacterially derived thiaminase from the viscera of alewife and rainbow smelt can contribute to thiamine deficiency in salmonids (Honeyfield et al., 2002; Honeyfield et al., 2005), the mechanisms by which dietary thiaminase contributes to thiamine deficiency in wild salmonids are still largely debated. It has been suggested that the primary mechanism by which dietary thiaminase contributes to thiamine deficiency is through the incorporation of thiaminase-producing bacteria from ingested prey fishes into the gut microbiota of salmonids where it interferes with thiamine uptake (Wistbacka and Bylund., 2008). However, recent studies have argued that the synthesis of thiaminase in prey fishes may actually be de novo (Richter et al., 2012; Richter et al., 2022; Rowland et al. in prep). Although I did not conduct any experiments on gut microbiota, Therrien et al. (un-published data) found that tissue thiamine concentrations were reduced in lake trout fed dietary thiaminase while the thiamine content of the control and thiaminase diets were indistinguishable, thereby providing support for the hypothesis that gut microbiota play an important role in facilitating thiamine deficiency in salmonids when bacterial-derived thiaminase is consumed. To this end, a limitation of this study is that a low thiamine diet wasn't administered to control for the effects of bacterial community changes in the lake trout gut. Future research may wish to instead administer low- and high-thiamine diets to more directly address questions of thiamine deficiency in salmonids. However, as the importance of gut microbiota in salmonid-related thiamine deficiency becomes increasingly apparent (Ji et al., 1998; Wistbacka and Bylund., 2008), future research should also address questions regarding changes in the gut microbiota of

thiaminase-fed salmonids to further understand the mechanism by which thiaminase contributes to thiaminase deficiency.

Another challenge in understanding the effects of dietary thiaminase comes from the ecological relevance of an experimental thiaminase diet. The thiaminase diet formulated by Honeyfield et al. (2005) that provided a framework for the diet used in this study mimics a lake trout diet that consists entirely of high-thiaminase prey fishes. Although this appears to still be the case in much of Lake Ontario where alewife and rainbow smelt continue to dominate the diets of lake trout (Nawrocki et al., 2022), results from this study may have a limited capacity to be applied to instances where lake trout feed on a greater diversity of prey. For instance, it has been suggested that the invasion of round goby (*Neogobus melanostomus*) in the Great Lakes could provide alleviation from the effects of thiamine deficiency in lake trout if their abundance in lake trout diets continues to increase (Fitzsimons et al., 2009b). Future studies are needed that address questions pertaining to dietary proportions of high-thiaminase prey fishes and thresholds for cardiac-related signs of thiamine deficiency.

Lastly, questions remain regarding the mechanisms behind the apparent thiaminase-tolerance observed in Seneca strain lake trout from my study. Evidence of genetic adaptations to low thiamine conditions at the population level have been demonstrated in Atlantic salmon, where strains that consume high-thiaminase prey in their native lakes showed smaller reductions in tissue thiamine concentrations than strains with a diet lacking thiaminase (Houde et al. 2015a). In lake trout, Fitzsimons et al. (2021) demonstrated that Seneca strain fish have reduced thiamine utilization compared to other strains, which may explain the smaller reductions in thermal tolerance and cardiac function metrics measured in my study. However, the reason for this difference in thiamine utilization remains unknown. Local adaptation to dietary thiaminase likely involves the selection of genes associated with enzymes in biochemical pathways that are dependent on thiamine, such as the TCA cycle, or genes associated with pathways of thiamine modification or transport. In Atlantic salmon, over 3600 genes have shown differential expression during thiamine deficiency — many of which are associated with cardiac function and development (Harder et al., 2020). Furthermore, nearly 1500 genes

were highly associated with strain-specific survival during thiamine deficiency (Harder et al., 2020). There are a number of possible mechanisms by which strains could adapt to low thiamine conditions (Harder et al., 2018). One possibility involves selection acting on sequence variation in the genes encoding the various thiamine-dependant enzymes involved in carbohydrate metabolism and energy production (i.e. pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched chain α-ketoacid dehydrogenase). If sequence variation in the genes encoding these enzymes results in altered binding affinities for thiamine diphosphate (TDP), and if a greater binding affinity results in an increased use-efficiency of TDP, this variation could be selected on in low thiamine environments. Second, variation in genes associated with the expression of thiamine pyrophosphokinase, the enzyme that converts free thiamine to TDP, could be subject to selection if variation in these genes results in an increased expression in thiamine pyrophosphokinase and if an increased expression increases the availability of TDP. Future studies are needed to identify the particular adaptations associated with thiaminase tolerance in Seneca strain lake trout. Additionally, future investigations into thiaminase tolerance in other strains of lake trout, particularly those that rely on high-thiaminase prey in their native lakes, will be worthwhile in advancing lake trout conservation efforts in the Great Lakes and elsewhere.

4.7 Conclusion

With the threats of anthropogenically mediated biological invasion and climate change on the rise, it is becoming increasingly important to understand how these stressors might interact to better protect and manage aquatic ecosystems. In this study, I show for the first time that the consumption of a diet containing bacterial-derived thiaminase from invasive species can impair cardiac function, alter cardiac morphology and reduce thermal tolerance in a salmonid. Contrary to my prediction, I found that lake trout raised on a thiaminase diet had a greater relative ventricular mass and more elongated ventricles than lake trout in the control group. Lastly, I provide evidence to support the hypothesis that Seneca strain lake trout possess local adaptions to high-thiaminase prey fishes. Across temperature profiles, I observed a significant difference in f_{Hmax} between treatments in Slate strain fish, while this difference was not observed between treatments in Seneca

strain fish. As Seneca strain lake trout maintained cardiac function when fed the thiaminase diet, stocking a higher proportion of Seneca strain fish may provide an alternative method to improve lake trout survival in Lake Ontario. However, whole-body data suggests that Seneca strain lake trout may manifest other non-cardiac related signs of thiamine deficiency that translate to lower survival and performance. Altogether, this study provides a first assessment of how two prevalent stressors in the Great Lakes — warmer water temperatures and invasive high-thiaminase prey fishes — might interact to threaten current and future lake trout viability through cardiac impairments.

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Appendices

Appendix A. Animal Use Protocol

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

Table of Contents

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. Composition of Vitamin and Mineral Premixes Used in Experimental Diets

Table B.1. Composition of vitamin (5g/kg; Dyets #399751) and mineral (2g/kg; Dyets #200030) premixes used in experimental lake trout (*Salvelinus namaycush*) diets. Mixes were prepared and provided by Dyets Inc. (Bethlehem, PA, USA).

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 C. Thermal Performance of Stroke Volume

C.1 Methods

See section 2.1.5 for detailed methods on how stroke volume was measured in my study. Repeated measures ANOVAs were used to assess differences in stroke volume between treatments and strains across temperatures.

C.2 Results

Stroke volume did not change with temperature in this study ($F=1.14$; $df=1,12$; $P=0.33$; Figure C.1), and I did not detect a significant treatment × strain × temperature interaction effect on stroke volume according to a three-way repeated measures ANOVA ($F=0.55$; $df=1,10$; $P=0.85$).

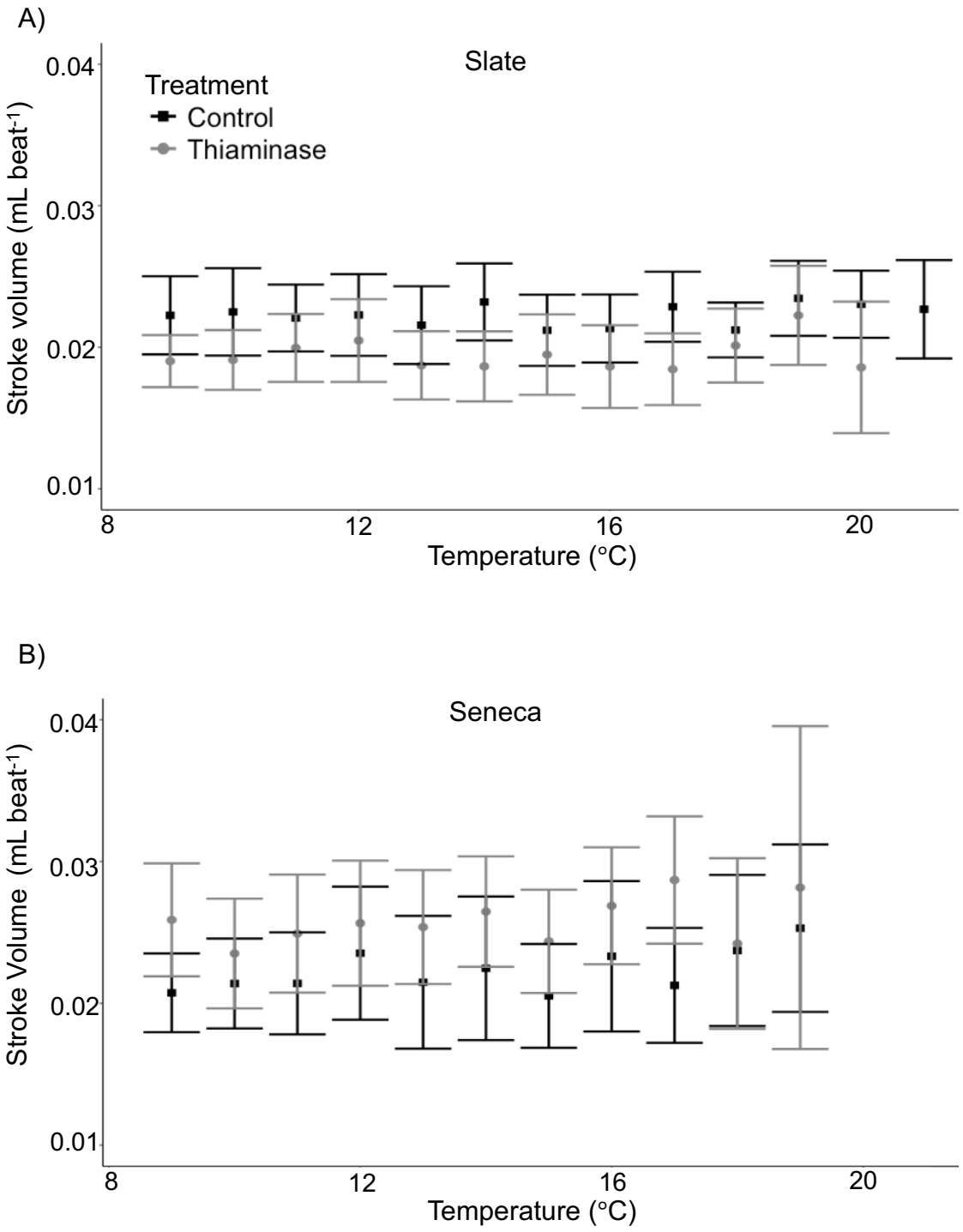

Figure C.1. Effect of acute warming on stroke volume of Slate (A) and Seneca (B) strain lake trout (*Salvelinus namaycush*) raised on a control (dark grey) or thiaminase (light grey) diet. Data are presented as means ± SE.

Appendix D. The Effect of Dietary Thiaminase on Whole-body Performance Metrics

All data presented in Appendix D were collected, analyzed, visualized, and interpreted by Chris Therrien (CT).

D.1 Tissue thiamine concentrations

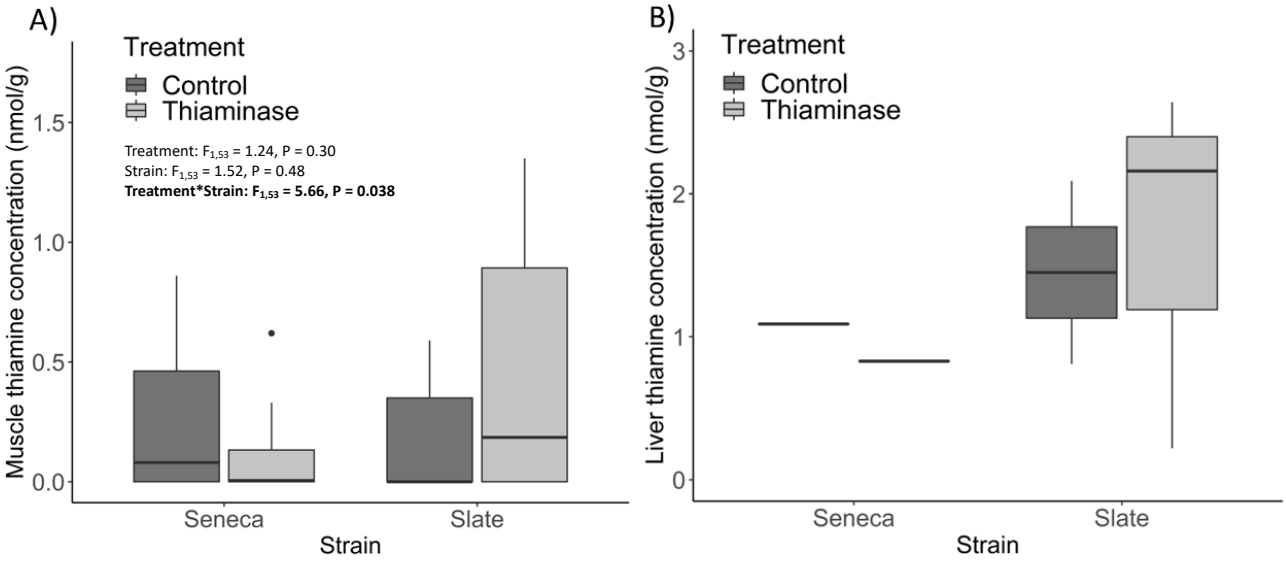

Figure D.1. A) White muscle thiamine concentration (nmol/g) and B) liver thiamine concentration (nmol/g) of juvenile lake trout from the Seneca and Slate Island strains fed either a control (dark grey) or thiaminase (light grey) diet for 6 months. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 x interquartile range. Note: Only 7 samples have been analyzed to date for liver thiamine concentrations and no inferential statistics could be implemented.

D.2 Survival

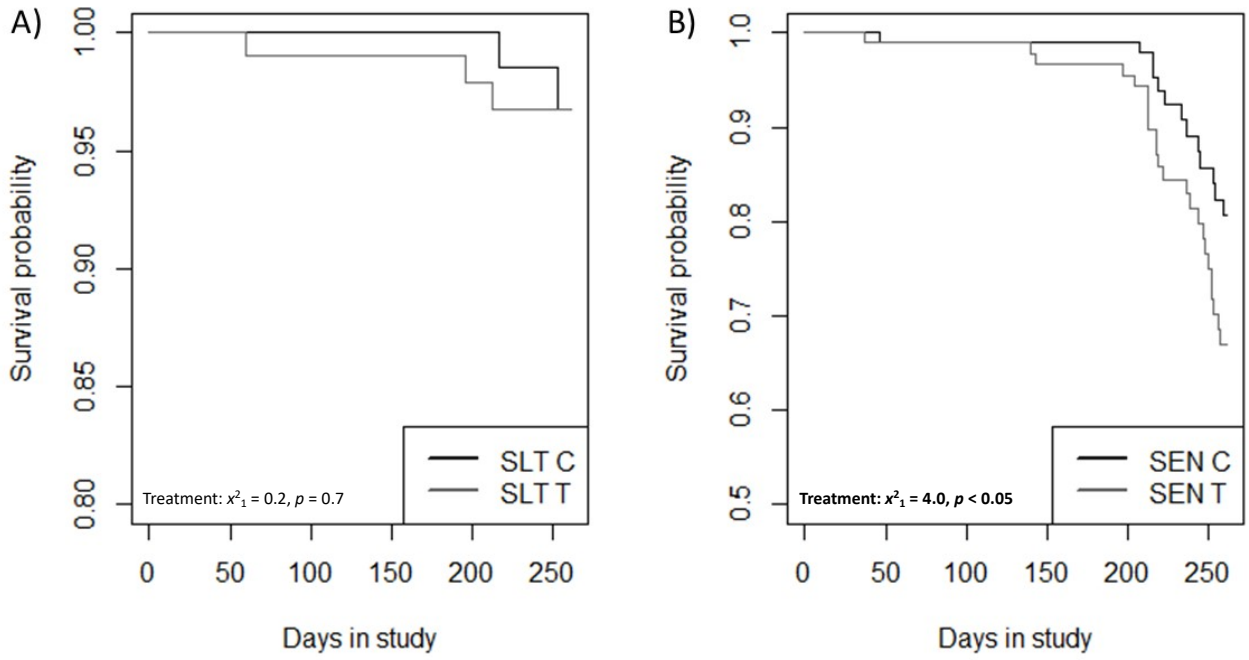

Figure D.2. Kaplan-meier survival curves of lake trout from the A) Slate Island (SLT) and B) Seneca Lake (SEN) strains after 9 months fed either a control (C) or thiaminase (T) diet.

D.3 Swim Performance

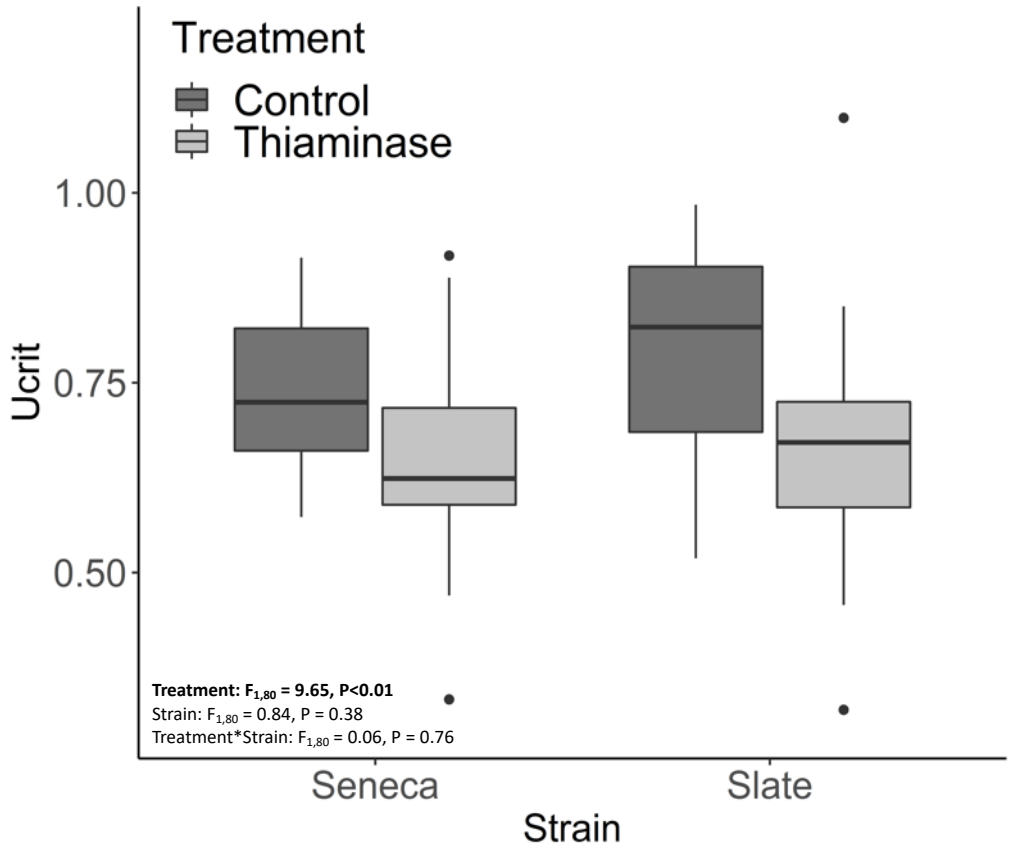

Figure D.3. Critical swim speed (Ucrit) of lake trout from the Seneca and Slate strains fed either a control (dark grey) or thiaminase (light grey) diet for 6 months. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 x interquartile range.

D.4 Growth Rate

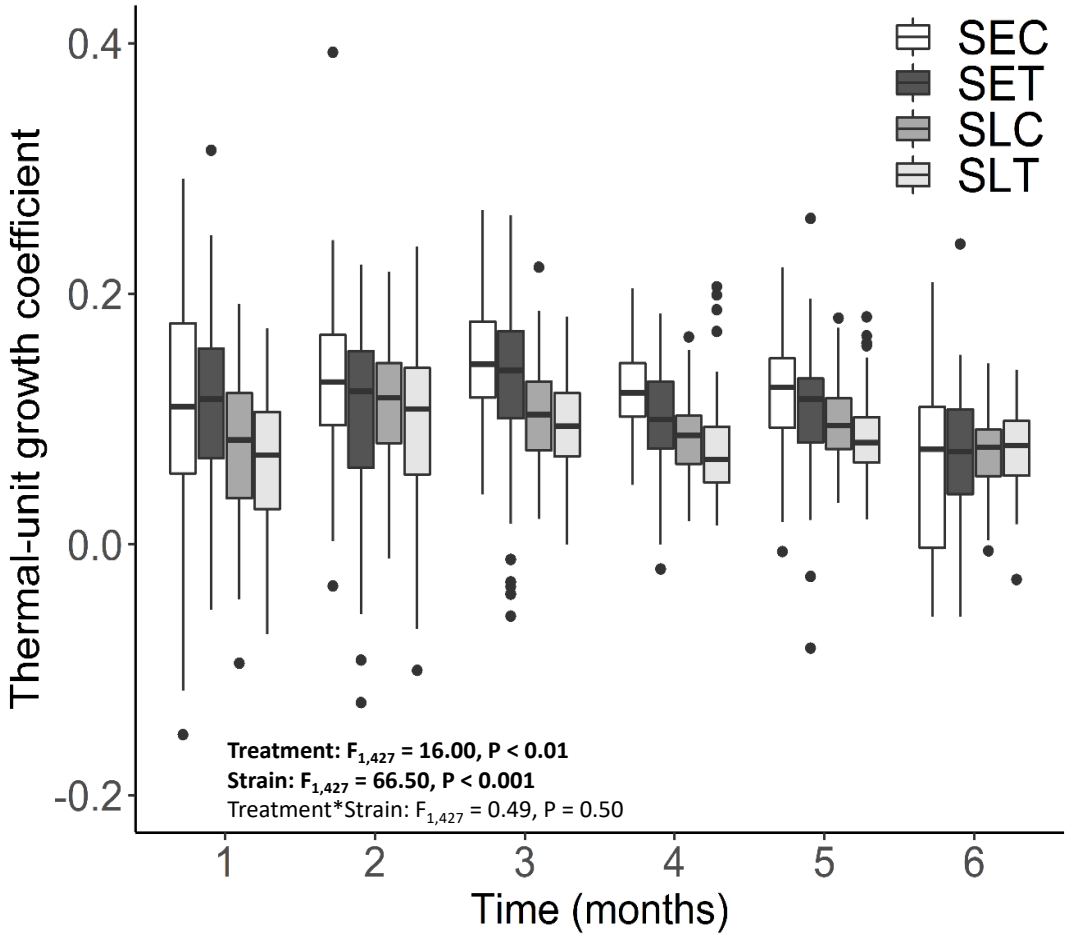

Figure D.4. Thermal-unit growth coefficient of lake trout from the Slate (SL) and Seneca (SE) strains after 6 months of being fed either a control (C) or thiaminase (T) diet.

D.5 Colouration

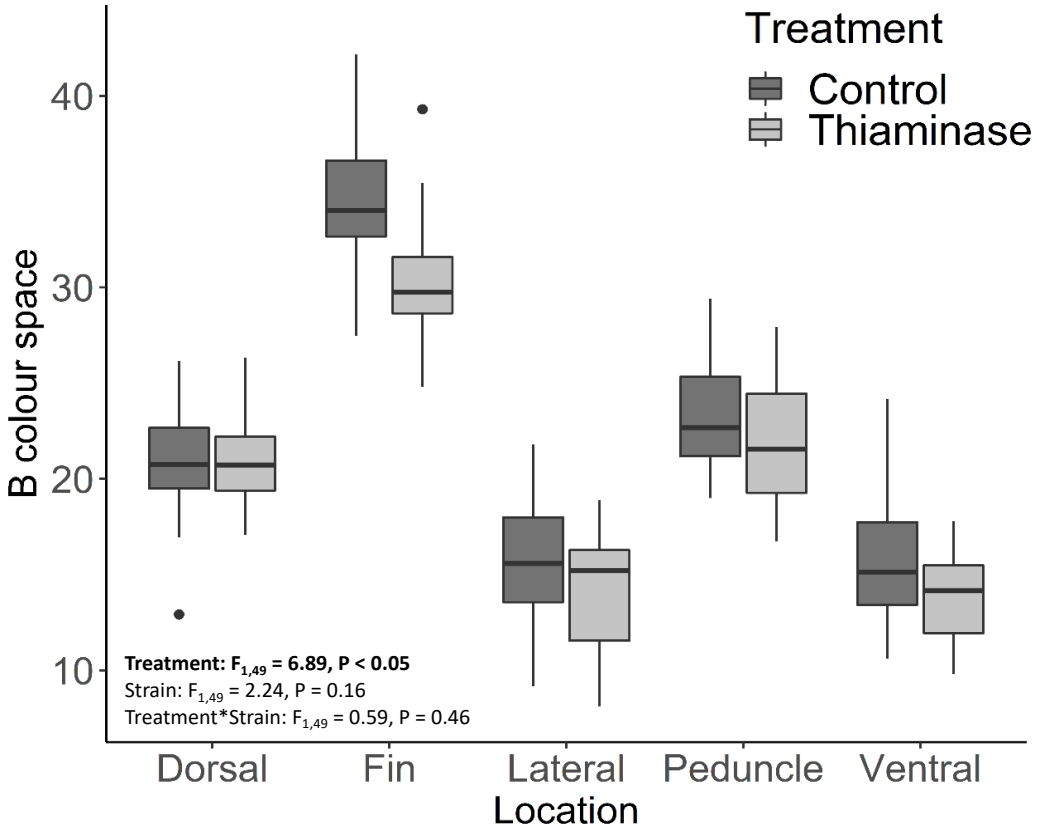

Figure D.5. B colour space (blue-yellow) of lake trout fed either a control (dark grey) or thiaminase (light grey) diet for 6 months. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 x interquartile range.

Curriculum Vitae

Name: Peter Baker

Post-secondary Education and Degrees:

Western University
London, Ontario, Canada
2021-2023 M.Sc.

Western University
London, Ontario, Canada
2017-2021 B.Sc. (Hons)

Honours and Awards

Graduate Travel Award, Western University, 2023

Queen Elizabeth II Graduate Scholarship in Science and Technology (QEII-GSST), Western University, 2022

L. Margolis Scholarship, Canadian Society of Zoologists, 2022

FishCAST Graduate Fellowship, NSERC, 2021

Terence Laverty Memorial Gold Medal, Western University, 2021

UWO In-Course Scholarships, Western University, 2020

Undergraduate Student Research Award, NSERC, 2019

Dean's Honor List, Western University, 2018-2021

Western Scholarship of Excellence, Western University, 2017

Related Work Experience

Graduate Teaching Assistant
Western University
2021-2023

Fisheries Biologist (Intern)
Ontario Ministry of Natural Resources & Forestry
2022

Research Assistant
Western University
2020-2021

Environmental Field Technician
Grey-Bruce Public Health Unit

2019

Environmental Assistant
Ontario Ministry of the Environment, Conservation, & Parks
2018

Related Volunteer Experience

Co-Chair/Co-Founder
Western Biodiversity Inventory
2022-present

Ecological Advisor
Ecological Community Advisory Committee, City of London, ON
2022-present

Regional Representative
American Fisheries Society, Ontario Chapter - Student Subunit
2022-present

Presentations:

Baker, P.M., Therrien, C.A.., Garner, S.R. & Neff, B.D. (2023) Heart on fire: dietary thiaminase impairs cardiac function and alters heart size in lake trout. Annual Meeting of the Society of Canadian Aquatic Sciences (SCAS), Montreal, QC, CA [Oral].

Baker, P. M. (2022). Where are the lake trout? A look into the history of lake trout in the Great Lakes, and how a vitamin deficiency might threaten their future. Fisheries Management and Conservation Careers in Science and Technology (FishCAST) Annual General Meeting, (Virtual) [Oral].

Baker, P.M., Therrien, C.A., Garner, S.R. & Neff, B.D. (2021) The effect of dietary thiaminase on two strains of lake trout (*Salvelinus namaycush*) currently stocked in Lake Ontario. Western Undergraduate Student Research Internship Conference (Virtual) [Poster].

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North America. Annual Meeting of the International Charr Symposium, Nikko, Japan [Oral].

Adeli, K., Baker, P.M., Therrien, C.A. & Neff, B.D. (2023). The effects of an invasive high-thiaminase prey fish on heart morphology of lake trout (*Salvelinus namaycush*) in the Sudbury Basin. Ontario Biology Day, St. Cathrines, ON, CA [Oral].

Therrien, C.A., Baker, P.M., Garner, S.R., Edwards, K., Wilson, C., Swanson, H. & Neff, B.D. (2022). Effects of dietary thiaminase on two strains of lake trout. Annual Meeting of the American Fisheries Society, Spokane, WA, USA [Oral].

Publications:

Baker, P.M., Therrien, C.A., Muir, C.A., Garner, S.R. & Neff, B.D. (2023). Dietary thiaminase impairs cardiac function and alters heart size in lake trout (*Salvelinus namaycush*). *Canadian Journal of Zoology*. https://doi.org/10.1139/cjz-2023-0012

Baker, P.M. (2023). Deadly deficiency: A brief history of the Great Lakes lake trout collapse. *Ontario Out of Doors*, *55*(5), 26-27.

Baker, P.M., Samuels, B. & Hain, T.J.A. (In prep). Using citizen science to document biodiversity on campus: A one-year case study.

Adeli, K.A., Baker, P.M., Therrien, C.A., Garner, S.R. & Neff, B. D (In prep). The effect of invasive rainbow smelt on lake trout cardiac morphology in the Sudbury Basin.

Therrien, C.A., Baker, P.M., Garner, S.R., Edwards, K., Wilson, C., Swanson, H. & Neff, B.D. (In prep). Effects of dietary thiaminase on two strains of lake trout.