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Article

Dietary thiaminase impairs cardiac function and increases heart size in lake trout (*Salvelinus namaycush* (Walbaum in Artedi, 1792))

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Abstract

The consumption of invasive, high-thiaminase prey fishes can cause thiamine deficiency, which has been hypothesized to be a major barrier for lake trout (*Salvelinus namaycush* (Walbaum in Artedi, 1792)) restoration in the Great Lakes. In fishes, an understudied aspect of thiamine deficiency is its effect on cardiac function, despite evidence of this effect in mammals. Here, parr of two strains of lake trout (Seneca and Slate) were raised on either a control or high-thiaminase diet for nine months. We then measured cardiac function and morphology, particularly as it relates to the ability of the heart to meet oxygen demands at warmer water temperatures. The thiaminase diet was associated with significant heart enlargement and reduced cardiac performance at high temperatures. These effects were observed in both strains but were more pronounced in Slate strain fish. Our data suggest that dietary thiaminase impairs cardiac function in fishes and that these impairments may become increasingly important as water temperatures increase through climate change.

Key words: thiaminase, invasive species, thermal performance, cardiac function, lake trout, *Salvelinus namaycush*

Introduction

In recent years, thiamine (vitamin B1) deficiency has increasingly been reported in wild animal populations around the world (Balk et al. 2016). Thiamine deficiency is taxonomically widespread, with populations of birds, reptiles, fish, mammals, and bivalves all known to be affected (Fisher et al. 1996; Sepúlveda et al. 2004; Butler et al. 2008; Balk et al. 2009, 2016). Referred to as thiamine deficiency complex (TDC) in salmonids, TDC has been well documented in the Laurentian Great Lakes, with signs of TDC observed in populations of Atlantic salmon (*Salmo salar* Linnaeus, 1758), coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)), chinook salmon (*Oncorhynchus tshawytscha* (Walbaum in Artedi, 1792)), brown trout (*Salmo trutta* Linnaeus, 1758), rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), and lake trout (*Salvelinus namaycush* (Walbaum in Artedi, 1792); Fisher et al. 1995, 1996; Marcquenski and Brown 1997). In the Great Lakes, thiamine deficiency has been attributed to the consumption of thiaminase I (Fitzsimmons and Brown 1998). In particular, invasive alewife (*Alosa pseudoharengus* (Wilson, 1811)) and rainbow smelt (*Osmerus mordax* (Mitchill, 1814)) 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 fishes 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 instead suggest that the synthesis of thiaminase may be de novo (Richter et al. 2023; Rowland et al., in preparation).[1] 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 (Fitzsimmons and Brown 1998).

Thiamine is an essential vitamin required by all organisms for metabolic function. The monophosphorylated form of thiamine (TMP) can be synthesized by some bacteria, plants, and fungi, while most animals must acquire thiamine from their diet (Fitzpatrick and Thore 2014). Once ingested, TMP is converted to thiamine diphosphate (Manzetti et al. 2014), which is a rate-limiting cofactor for several key metabolic enzymes in the tricarboxylic acid cycle (Depeient et al. 2006). Thiamine-dependent metabolic reactions are essential in mitochondrial ATP production, and thiamine deficiency has been shown to significantly reduce ATP synthesis in animal tissues (McCandles et al. 1970). Symptoms of low thiamine often manifest as cardiorespiratory and neurological impairments in mammals (Kril 1996; Roman-Campos and Cruz 2014). Cardiac-related symptoms of thi-

[1] Rowland, F.E., D.E. Tillitt, C.A. Richter, and D.M. Walters. (in preparation). Evolutionary and ecological correlates of thiaminase in fishes.

amine deficiency include a reduction in heart size, impaired ventricular contractility, and cardiac failure (Cohen et al. 1976; Cappelli et al. 1990; da Cunha et al. 2007; Oliveira et al. 2007; Roman-Campos et al. 2009; Gioda et al. 2010). In salmonids, known sub-lethal and secondary effects of TDC include ataxia, lethargy (Fisher et al. 1995; Brown et al. 2005*a*), impaired immune function (Ottinger et al. 2012, 2014), swimming ability (Fitzsimons et al. 2005; Ketola et al. 2005; Houde et al. 2015*a*), growth, foraging ability, predator avoidance (Fitzsimons et al. 2009), and vision (Carvalho et al. 2009). However, the extent to which TDC affects the cardiorespiratory system of salmonids has not been directly examined.

Constraints to cardiac function and changes to heart morphology as a result of TDC could have pervasive impacts on the fitness of salmonids. In fishes, cardiac function and morphology are important factors determining upper thermal tolerance (Farrell 2009; Casselman et al. 2012; Anttila et al. 2013). The oxygen- and capacity- limited thermal tolerance hypothesis, first described by Pörtner and Knust (2007), suggests that the decline in aerobic scope observed above an organism's optimum temperature can be explained by a mismatch between oxygen demand and the capacity of the cardiorespiratory system to supply sufficient oxygen (Farrell 2009). As temperature increases, both oxygen consumption and heart rate increase exponentially. However, increases in maximum heart rate (f_{Hmax}) slow at temperatures above a fish's optimum temperature, as indicated by the Arrhenius Breakpoint temperature (T_{AB}) placing a limitation on oxygen delivery to tissues (Casselman et al. 2012). At temperatures approaching a fish's upper thermal limit, the heart becomes arrhythmic, denoted as their arrythmia temperature (T_{Arr}), and aerobic scope collapses soon after (Casselman et al. 2012). Indeed, lower peak heart rates (f_{Hpeak}) have been linked to decreased upper thermal limits in chinook salmon and Atlantic salmon (Anttila et al. 2014; Muñoz et al. 2015), and relative ventricular mass (RVM) has been positively correlated with upper thermal limits in Atlantic salmon and European sea bass (*Dicentrarchus labrax* (Linnaeus, 1758); Anttila et al. 2013; Ozolina et al. 2016). The association between relative ventricular mass and thermal tolerance may occur because a larger ventricle has a greater capacity to provide oxygenated blood to tissues during elevated oxygen demand (Anttila et al. 2013). Taken together, relative ventricle mass and cardiac function are important factors determining the thermal tolerance of fishes.

Lake trout were extirpated from Lake Ontario 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 TDC is hypothesized to be a significant contributing factor (Brown et al. 2005*b*; Lantry et al. 2014). Indeed, both alewife and rainbow smelt comprise a large proportion of lake trout diets in Lake Ontario (Nawrocki et al. 2022). Stocking programs in Lake Ontario predominantly release two strains of lake trout: Seneca strain (Seneca Lake, NY) and Slate Island strain (Lake Superior, ON; Lantry et al. 2014). 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 of these prey species (Bronte and Hoff 1996). Consequently, it has been suggested that local adaptation to high-thiaminase prey fishes in Seneca Lake has led to Seneca strain lake trout having the capacity to better tolerate dietary thiaminase, possibly through reduced thiamine utilization (Fitzsimons et al. 2021). Local adaptations to high-thiaminase prey have previously been identified in populations of Atlantic salmon that differ in the abundance of high-thiaminase prey in their native lakes (Houde et al. 2015*a*). 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. 2015*b*).

Fish communities are increasingly being impacted by multiple environmental stressors, including climate change and invasive species. Here, we examined the interactive effects of two stressors potentially affecting lake trout in Lake Ontario in a hatchery setting: a high-thiaminase diet and increased water temperature. We compared thermal tolerance, cardiac function, and cardiac morphology between two strains of lake trout fed either a control diet or a diet containing bacterial-derived thiaminase. We predicted that dietary thiaminase, which is known to reduce tissue thiamine concentrations, would impair cardiac function, reduce relative ventricular mass, and impair thermal tolerance in both strains. However, we also hypothesized 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 has led to local adaptation.

Methods

Experimental animals

Lake trout parr (age 1+) of the Seneca and Slate strains were transferred from the Ontario Ministry of Natural Resources and Forestry Chatsworth Fish Culture Station (Chatsworth, ON) to Western University on 18 March 2021. Between 23 to 30 fish of the same strain were placed into each of 16 73 L white polypropylene tanks, with 8 tanks per strain ($n = \sim$200 fish/strain). Two independent recirculating water systems were used with eight tanks per system, and water temperature was maintained at 9 °C throughout the experiment. Treatment groups were spread equally across the two systems. Fish were given 3 weeks to acclimate to hatchery conditions before being anesthetized (150 mg/L MS-222 buffered with 150 mg/L sodium bicarbonate) and measured for body mass on 12 April 2021. While anesthetized, each fish was tagged with a 1.2 mm Passive Integrated Transponder (Biomark Inc.). The fish were allowed to recover for 2 weeks on a diet of commercial fish feed (Ewos Inc.). 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, lake trout were fed daily at a rate of

Table 1. Diet composition and proximate analysis of experimental lake trout 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	None	Trace
Proximate Analysis		
Carbohydrates	31.6	33.1
Proteins	37.0	36.8
Crude fat	22.4	18.8
Ash	2.16	4.13
Moisture	6.82	7.19
Thiamine (nmol/g)	7.05 ± 5.2	6.92 ± 5.8

2% body mass per day for the first 3 months, 1.5% body mass per day for the next 3 months, and 1% body mass per day for the remainder of the experiment.

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 (*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 Great Lakes fishes (Richter et al. 2012, 2023), 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; Table 2), including thiamine that was measured 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* Valenciennes in Cuvier and Valenciennes, 1847) at 74 °C for 48 h. *Paenibacillus 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 96 h at 37 °C. Autoclaved nutrient broth was used in the control diets. Nutrient broths were thoroughly mixed with all dry ingredients and pelletized using an electric meat grinder. Food pellets were left to air dry at room temperature for 48 h and stored at −20 °C until use. Maximum storage time for diets at −20 °C was 2 weeks. Here, we used the same strain and concentration ($2.1 \times 10^8 \pm 6.1 \times 10^7$ CFU/mL) of *P. thiaminolyticus* that has previously been shown to reduce tissue thiamine concentrations and induce symptoms of thiamine deficiency in Atlantic salmon and lake trout (Honeyfield et al. 2005; Houde et al. 2015*a*). Experimental diets were administered for 9 months, by which symptoms 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 2. Composition of the vitamin (5 g/kg; Dyets #399751) and mineral (2 g/kg; Dyets #200030) premixes used in experimental lake trout diets.

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

Note: Mixes were prepared and provided by Dyets Inc. (Bethlehem, PA, USA).

Thermal performance of cardiac function

After nine months on the experimental diets, test fish were anesthetized in water containing 150 mg/L of MS-222 buffered with 150 mg/L 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 and 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 monitor water temperature (Omega, St-Eustache, QC, Canada). A maintenance dose of anesthetic (100 mg/L of MS-222 buffered with 100 mg/L sodium bicarbonate) was present in the recirculating water bath, and fish were ram-ventilated using a 2.5 cm segment of rubber tubing. Each fish was maintained at 9 °C in the holding reservoir for 15 min before starting echocardiography measurements to ensure the 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 (DFV) System (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 valve. Signals from the transducer probe were digitized and displayed as spectrographs using the Doppler Signal Processing Workstation. After a 15 min 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 atropine sulfate (Sigma–Aldrich, St. Louis, MO, USA) and 4 μg/kg isoproterenol (Sigma–Aldrich, St. Louis, MO, USA), each followed by a 15 min stabilization period (Casselman et al. 2012). Atropine sulfate was used to block vagal tone, whereas isoproterenol was used to fully stimulate adrenergic β-receptors. Water temperature was then increased by 1 °C every 6 min 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, after which the heart was removed to determine relative ventricular mass.

Relative ventricular mass

The ventricle was isolated from the heart of each test fish by removing the atrium, sinus venosus, and bulbous arteriosus. Once isolated, the ventricle was weighed using a digital scale to determine ventricular mass. RVM was calculated using the equation RVM = (VM/BM) × 100, where VM is ventricle mass (g) and BM is body mass (g) of the same individual.

Analysis

Spectrographs from each temperature increment were analyzed using the Doppler Signal Processing Workstation software using the parameters outlined in Muir et al. (2021). For each spectrograph, beat markers were manually placed at the end of each beat ($n = 8$) and an average heart rate (f_{Hmax}; beats per minute) was calculated for each spectrograph using the software's "Beat Editor". Arrhenius breakpoint temperature (T_{AB}) was determined by assessing the Arrhenius plots of each fish 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. Arrythmia temperature (T_{Arr}) was identified for each fish as the first temperature at which arrythmias were evident on the DFV spectrographs. We also noted the highest f_{Hmax} observed across all temperatures for each fish (f_{Hpeak}).

Table 3. Body mass and sample size of Seneca and Slate strain lake trout raised on a control or thiaminase diet.

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

Note: Refer to text for statistical results.

Statistical analyses

All statistical analyses were preformed using R 1.4.1717 (R Core Team, Vienna, Austria). Differences in body mass, T_{AB}, T_{Arr}, f_{Hpeak}, and RVM 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 (not included in the analysis of body mass), 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) and are represented as the percentage of the variance accounted for by the random effects. Three-way and two-way repeated measures' ANOVAs were used to assess differences in f_{Hmax} between treatments and strains across temperatures.

Animal ethics approval

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

Results

Body mass

A total of 51 trials were included in our analyses, with 11 trials excluded because of an abnormal response to the pharmacological stimulants or technical issues with the water recirculator (Table 3). 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: $n = 17$). Among these fish, 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; $P = 0.68$; Table 3), and there was no significant treatment × strain interaction ($F = 0.08$; df = 1, 10; $P = 0.78$; Table 4). Tank and water source both contributed

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

					ICC (%)	
Metric	Model term	*F*	df	*P*	Tank	Water source
T_{Arr}	Treatment	2.37	1, 10	0.16	15.9	26.5
	Strain	0.067	1, 10	0.80		
	Treatment × strain	0.56	1, 10	0.47		
	Body mass	0.0040	1, 39	0.95		
T_{AB}	Treatment	12.93	1, 9	**<0.01**	0	20.7
	Strain	0.24	1, 10	0.64		
	Treatment × strain	0.96	1, 10	0.35		
	Body mass	1.00	1, 43	0.32		
f_{Hpeak}	Treatment	9.40	1, 9	**<0.05**	2.6	35.6
	Strain	0.00 060	1, 9	0.98		
	Treatment × strain	2.76	1, 10	0.13		
	Body mass	2.44	1, 42	0.13		
RVM	Treatment	6.48	1, 9	**<0.05**	0	5.3
	Strain	11.54	1, 9	**<0.01**		
	Treatment × strain	0.015	1, 10	0.91		
	Body mass	0.0042	1, 43	0.95		
Body mass	Treatment	0.99	1, 9	0.35	0	0
	Strain	0.19	1, 9	0.68		
	Treatment × strain	0.08	1, 10	0.78		

Note: T_{Arr}, arrythmia temperature; T_{AB}, Arrhenius breakpoint temperature; f_{Hpeak}, peak maximum heart rate (f_{Hmax}) across all temperatures; RVM, relative ventricular mass. *P* values in bold indicate significance for $\alpha = 0.05$.

negligibly to the total variance of the model (ICC: 0% for both tank and water source; Table 4).

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; $P < 0.05$; Fig. 1A). We 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 both random effects were low to moderate (ICC: 2.6% for tank and 35.6% for water source; Table 4).

Thermal performance of cardiac function

We did not find any significant differences 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; $P = 0.47$; Fig. 1B). Body mass was not significant in this model ($F = 0.0040$; df = 1, 39; $P = 0.95$), and the ICC was low for both random effects (15.9% for tank, 26.5% for water source; Table 4). In contrast, fish 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; $P < 0.01$; Fig. 1C). 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 ICC was low for both random effects (0% for tank, 20.7% for water source; Table 4).

There was a significant treatment × strain × temperature interaction effect on f_{Hmax} according to a three-way repeated measures analysis of variance (ANOVA) ($F = 3.25$; df = 2,12; $P < 0.001$). 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; Fig. 2A). 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; Fig. 2B).

Cardiac morphology

RVM was significantly greater in fish raised on the thiaminase diet (0.090 ± 0.002%) compared to fish raised on the control diet (0.083 ± 0.002%; $F = 6.48$; df = 1, 9; $P < 0.05$; Fig. 3). In addition, fish of the Seneca strain had significantly greater RVM (0.093 ± 0.003%) compared to fish of the Slate strain (0.082 ± 0.002%; $F = 11.54$; df = 1, 9; $P < 0.01$; Fig. 3). Body mass was not significant in this model ($F = 0.0042$; df = 1, 43; $P = 0.95$), and there was no significant treatment × strain interaction ($F = 0.015$; df = 1, 10; $P = 0.91$; Table 4). Both ran-

Fig. 1. Peak maximum heart rate (f_{Hpeak}) (A), arrythmia temperature (T_{Arr}) (B), and Arrhenius breakpoint temperature (T_{AB}) (C) of Seneca and Slate strain lake trout raised on a control or thiaminase 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 1.5× the interquartile range.

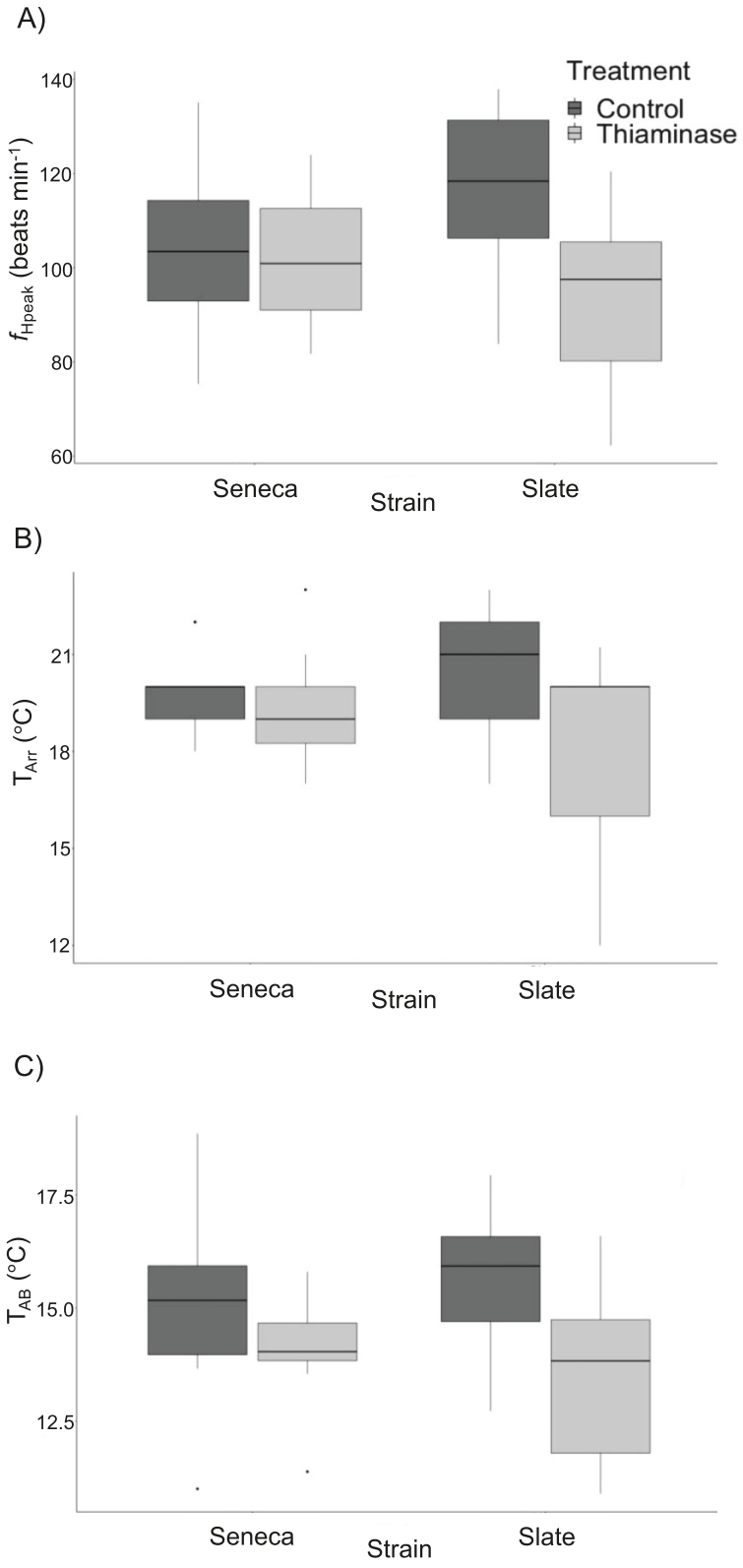
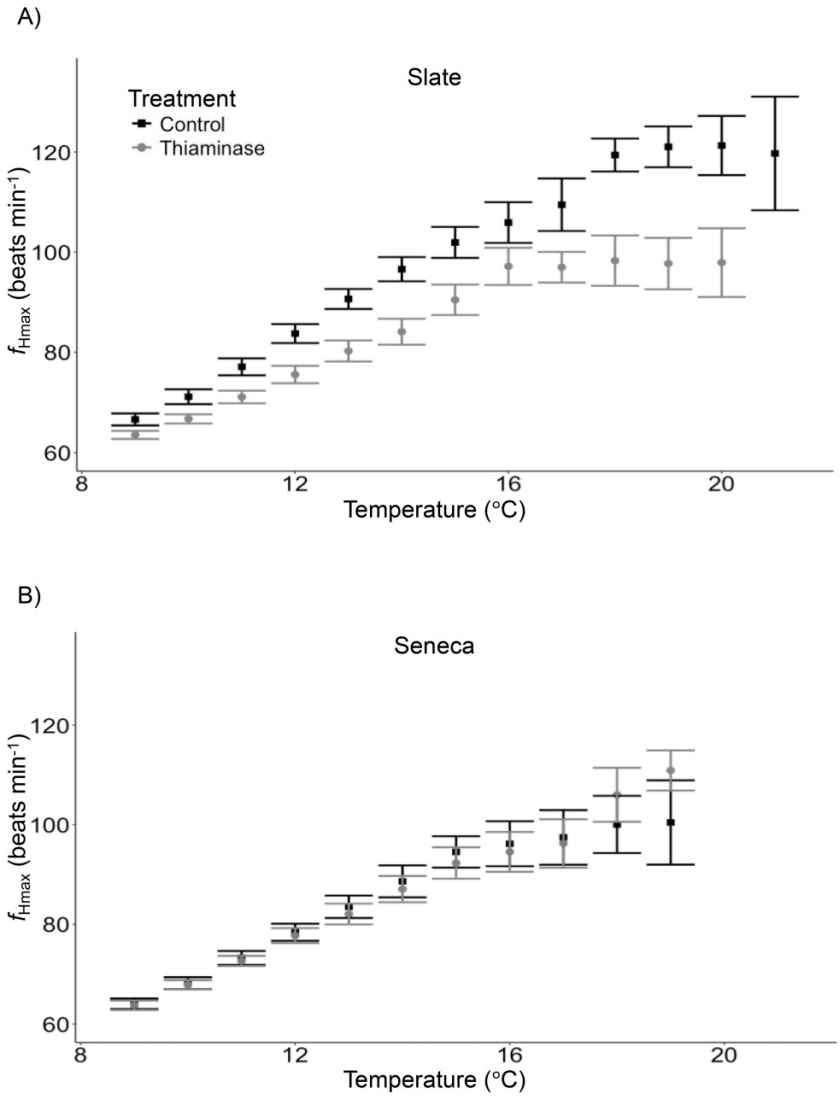


Fig. 2. Effect of acute warming on maximum heart rate (f_{Hmax}) of Slate (A) and Seneca (B) strain lake trout raised on a control (Slate: $n = 13$; Seneca: $n = 11$; dark grey) or thiaminase diet (Slate: $n = 17$; Seneca: $n = 10$; light grey). Data are presented as means ± SE. Data at temperatures with less than three data points were not included in this figure.



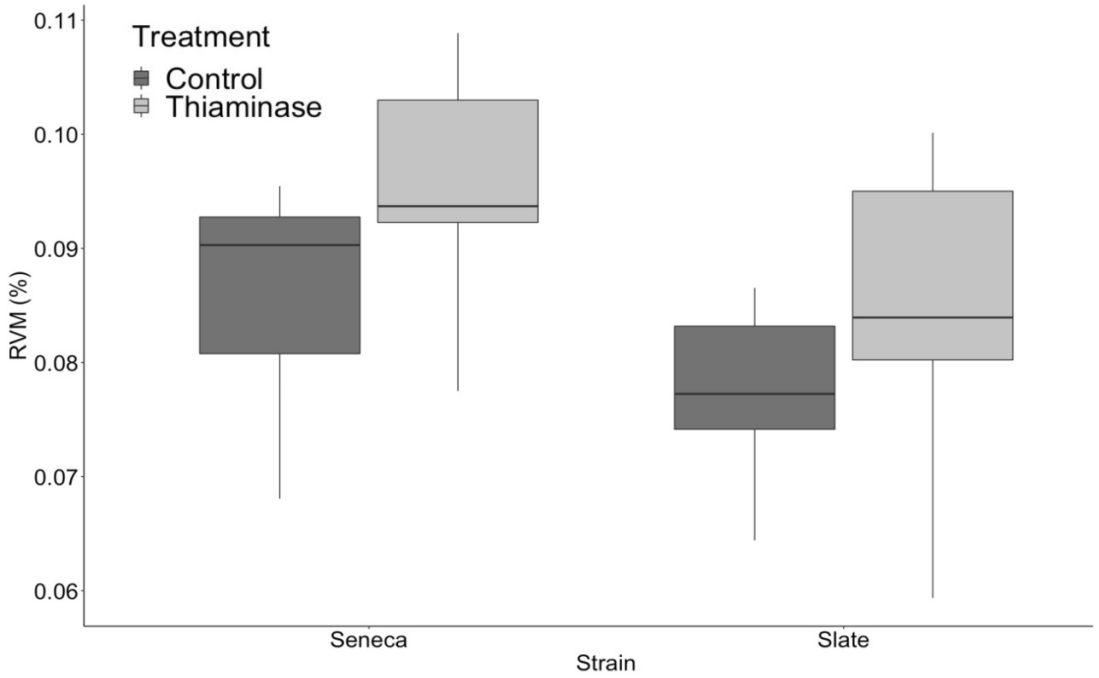
dom effects had low ICCs (0% for tank, 5.3% for water source; Table 4).

Discussion

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 our knowledge, our study is the first to directly examine the cardiorespiratory effects of thiamine deficiency in a fish. We show 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. Our results are consistent with studies that have shown a reduced heart rate in rats during thiamine deficiency (Yoshitoshi et al. 1961; Davies and Jennings 1970; Oliveira et al. 2007). Interestingly, all of the fish that displayed abnormal reactions to the pharmacological stimulants 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. Cardiac impairments associated with thiamine deficiency have been 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, our study provides some of the first evidence suggesting that thiamine deficiency induces cardiac impairments in a fish.

Cardiac structural alterations, including changes in heart mass, have often accompanied impaired heart function in mammals during thiamine deficiency (Roman-Campos and

Fig. 3. Relative ventricular mass (RVM) of Seneca and Slate strain lake trout raised on a control or thiaminase diet. Box plots show the median and first and third quartiles. Whiskers show minimum and maximum values.



Cruz 2014). Contrary to our prediction of a reduced relative ventricular mass in fish from the thiaminase treatment, we found that relative ventricular mass 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. Our results are 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 (water retention) due to impaired ion-pump function (Tanaka et al. 2003; Klein et al. 2004).

Relative ventricular mass and cardiac function are important factors determining the thermal tolerance of fishes (Anttila et al. 2013; 2014). Interestingly, the greater relative ventricular mass observed in fish from the thiaminase treatment in our study was not associated with any advantage in thermal tolerance. Instead, we 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 the arrythmia temperature, although it was not statistically significant. In thiamine-replete fish, previous studies have demonstrated that individuals with larger ventricles have a higher capacity to tolerate elevated temperatures (Anttila et al. 2013; 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 (Eliason et al. 2011; Muir et al. 2022). Our results suggest that the increased relative ventricular mass in fish from the thiaminase treatment does not reflect an increase in compact myocardium but instead may be the result of edema, a symptom that has been clinically reported in the brain, liver, and heart of thiamine-deficient humans (Watanabe et al. 1981; Hazell and Butterworth. 2009; Helali et al. 2019; Smith et al. 2021). Conversely, our findings of a decreased peak heart rate in fish from the thiaminase treatment are consistent with previous research that has demonstrated a positive correlation between peak heart rate and upper thermal tolerance (Anttila et al. 2014; Muñoz et al. 2015; Safi et al. 2019). Regardless, these findings represent the first evidence to suggest that dietary thiaminase, which is known to result in thiamine deficiency, hinders thermal tolerance in a juvenile salmonid.

Lake trout are a stenothermal species that typically prefer water temperatures between 8 and 12 °C (Christie and Reiger 1988), making them particularly susceptible to the effects of climate change (Chu et al. 2005; Sharma et al. 2011; Williams et al. 2015). Metabolic optimum temperatures for lake trout have previously been estimated to be between 15 and 17 °C (Gibson and Fry 1954; Evans 2007), which is consistent with the average 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), which is also comparable to the average T_{Arr} of control fish in this study. Indeed, climate change is predicted to have adverse consequences for cold-water fishes, primarily through altering the thermal profiles of 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). Increases in surface water temperatures are predicted to strengthen thermal stratification in temperate lakes, which can reduce oxygen concentrations in the hypolimnion where lake trout reside during the summer months (Stefan et al. 1998; Ficke et al. 2007). In Canada, some of the highest water temperatures in lakes are predicted to occur in ON (Sharma et al. 2007), where approximately 25% of the lakes that contain lake trout exist globally (OMNRF 2015). Our results of a reduced thermal tolerance in thiaminase-fed juvenile 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 invasive species (Rahel et al. 2008). Interestingly, the effects of dietary thiaminase are likely to be greatest in the Great Lakes, while the temperature effects of climate change will largely be prevalent for 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 non-native rainbow smelt have overlapped with several lake trout reintroduction programs (Selinger et al. 2006).

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). 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 mortality from sea lamprey (*Petromyzon marinus* Linnaeus, 1758) (Schneider et al. 1996). In this study, we found notable differences in thiaminase tolerance between strains. Perhaps most noteworthy, we found a reduction in f_{Hmax} 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, we 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 our prediction, these findings suggest that Seneca strain lake trout may possess local adaptations that help mitigate the effects of dietary thiaminase. Furthermore, we found no differences in T_{Arr}, T_{AB}, or f_{Hpeak} between strains. Taken together, our results suggest that Seneca strain lake trout may possess beneficial genetic adaptations for thiaminase tolerance that could translate to improved survival in the current and potential future environmental conditions in Lake Ontario. However, further study would be useful to determine whether these adaptions translate to improved survival.

In summary, we show for the first time that an experimental diet that contains thiaminase, which is known to reduce tissue thiamine concentrations, was associated with impaired cardiac function, greater relative ventricular mass, and reduced thermal tolerance in lake trout. Furthermore, maximum heart rate was reduced at higher temperatures in Slate but not in Seneca strain lake trout raised on the thiaminase diet. Seneca strain lake trout appear more tolerant of the thiaminase diet than the Slate strain suggesting adaptions to dietary thiaminase. These results have implications for lake trout stocking in the Great Lakes basin and elsewhere given climate change projections of increased water temperatures and current populations of thiaminase-positive prey fishes in the fishery.

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Competing interests

The authors declare there are no competing interests.

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Supplementary material

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