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

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

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Abstract

The consumption of thiaminase can cause thiamine deficiency, which has been hypothesized to impede reintroduction efforts of lake trout (*Salvelinus namaycush*). In fishes, consumption of thiaminase is hypothesized to affect swimming performance and morphology because thiamine deficiency manifests as cardiac and neurological impairments. However, how those effects may differ among populations with different historical exposures to thiaminase remain understudied, despite the importance of these traits to survival and fitness. Here, juvenile lake trout from strains that originated from Seneca Lake and Slate Islands were reared in a common garden environment and received either an experimental diet containing thiaminase or a control diet. Two hundred and sixty days after the initiation of the diets, critical swim speed, morphology, and colouration were compared between strains and diets. Results indicated that, regardless of strain, the diet containing thiaminase negatively affected critical swim speed, decreased ventral-dorsal depth, and increased yellow pigmentation. While most of the negative effects of the thiaminase diet did not differ between the two strains, an increase in red pigmentation was observed in Seneca Lake fish that had received the thiaminase diet. We discuss how strain selection could help mitigate effects of thiaminase exposure on the success of reintroduction efforts for lake trout.

Keywords: thiamine deficiency, thiaminase, swimming performance, pigmentation, morphology, aquaculture

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Introduction

Thiamine (vitamin B1) is an essential water-soluble vitamin required as a cofactor for roughly 2% of all cofactor-mediated enzymatic reactions[1]. Thiamine is essential for both catabolic and anabolic central carbon metabolism and branched-chain acid synthesis[1], including glycolysis and the citric acid cycle[2], as well as proper neurological[3], immune[4], and muscular function[5]. In animals, thiamine must be acquired through the consumption of organisms that produce or contain thiamine[6]. Thiamine exists as 4 vitamers, the biologically-relevant form of which, thiamine diphosphate, mediates various enzyme complexes including pyruvate dehydrogenase, transketolase, and 2-hydroxyacyl-CoA required for carbohydrate, amino acid, and lipid metabolism[2,6–8]. Thiamine diphosphate also plays a central role in nerve function, enabling pathways associated with the production of neurotransmitters, further antioxidant mechanisms, and for the myelination of neurons[8,9]. Low concentrations of thiamine result in a syndrome referred to as thiamine deficiency[10]. Because of its role in the cardiovascular and neurological systems, symptoms of low thiamine often manifest as cardiorespiratory and neurological impairments[11–13]. In severe cases, thiamine deficiency is lethal, but it has also been associated with secondary, sub-lethal effects, such as decreased growth rates, lethargy, reduced visual acuity, reduction of cardiovascular function and hemorrhaging, and reproductive failure (reviewed in [14]). Over the past few decades, thiamine deficiency has been observed in a variety of taxa, including fishes[15], reptiles[16], and birds[17], and it has been suggested that thiamine deficiency is a contributor and threat to worldwide loss of biodiversity[17,18].

The Salmonidae subfamily Salmoninae (henceforth referred to as salmonines), which includes the Pacific salmons and trout (*Oncorhynchus* spp.), Atlantic salmon and trout (*Salmo* spp.), and charrs (*Salvelinus* spp.), has been the focus of the research on thiamine deficiency in

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fishes[14]. In the Laurentian Great Lakes, the suspected primary cause of thiamine deficiency is from thiaminase I (henceforth referred to as thiaminase) consumption from exotic prey fishes[19]. Thiaminase catalyzes the breakdown of thiamine into its precursors in the stomach of consumers, ultimately limiting thiamine uptake[20–23]. Reproductive failures and the effects of thiamine deficiency on larvae have been well-studied (see review in [14]), whereas the effects of thiamine deficiency on body development and swimming performance, particularly in juvenile salmonines, remain understudied. Authors of studies on thiamine-deficient fish have reported a range of behavioural abnormalities, including lethargy, ataxia, abnormal movement patterns, loss of equilibrium, wriggling swimming behaviour, and a reduction in the ability to ascend cascades to spawning areas. These abnormalities have been attributed to neurological and cardiac impairments including altered brain thiamine metabolism and brain lesions[24–28]. Results from other studies indicate thiamine deficient fish have physical abnormalities that could further limit survival in the wild (see review in [14]). For example, fish colouration including changes to whole body lightening and a decrease in yellow pigmentation[29,30] and less streamlined body shape[30] have been noted in thiamine deficient juvenile salmonines. These traits have been associated with survival and foraging efficiency in salmonines [31–33] and other pelagic fishes [34,35]. Most recently, a change in heart morphology and decrease in function has been observed in lake trout [also referred to as lake charr; *S. namaycush* (Walbaum, 1972)] fed a high-thiaminase diet[11,36]. In thiamine-deficient mammals, a similar change in heart morphology and function is related to further secondary physical effects, including cyanosis (a blueing of the skin's pigment[12,37,38]), a reduction in the capacity for exercise[39,40], and a decreased ability to recover post-exhaustive exercise [41]. Swimming performance is particularly important for salmonines as it is critical for prey capture and predator avoidance (reviewed in[42]), as is body morphology and colouration,

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which play roles in predator avoidance, foraging efficiency, and swimming ability[34,43,44]. Assessing how thiamine deficiency affects these traits is thus important to understand how it could influence survival of salmonines in the wild[32,45–47].

Lake trout were once abundant in Lake Ontario, but were functionally extirpated by the late 1800s [48] and are currently the focus of large-scale reintroduction programs[49]. One factor hypothesized to obstruct restoration efforts is the abundance of exotic prey fishes including rainbow smelt [*Osmerus mordax* (Mitchill, 1984)] and alewife [*Alosa pseudoharengus* (Wilson, 1811)] because both of these prey fishes have high thiaminase activity[50] and are known to induce thiamine deficiency[19,21]. Alewife and rainbow smelt are found throughout the Great Lakes Basin and comprise a major proportion of the diets of salmonines[51–53]. The production of thiaminase I in these prey fishes was first believed to originate from gut microbiota[54], but recent evidence suggests that the synthesis of thiaminase I may also occur *de novo*[55,56]. Currently, two hatchery populations (henceforth referred to as strains) of lake trout are commonly stocked into Lake Ontario – the Seneca Lake (42.6536 N, -76.9004 W) and Slate Islands (Lake Superior; 48.6654 N, -87.0055 W) strains[49]. Notably, these strains differ in their historical exposure to prey fishes that contain thiaminase; Seneca Lake has long supported abundant populations of alewife [57], whereas Lake Superior supports relatively few alewife[58]. Consequently, it has been suggested that the Seneca Lake strain may have a higher tolerance to thiaminase through a local adaptation to prey fishes that are high in thiaminase compared to the Slate Islands strain. Indeed, Fitzsimons et al[59] showed that Seneca Lake strain embryos use less thiamine as compared to embryos from other strains, and initial analyses show that Seneca Lake strain fish have higher survival and represent a majority of the current spawning biomass in the Great Lakes[58,60–64]. Furthermore, a previous study conducted by Baker et al.[11] found that the negative effects of diet-derived

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thiaminase on heart morphology and function were less pronounced in the Seneca Lake strain compared to the Slate Islands strain. However, how this apparent tolerance translates to swimming performance, morphology, or colouration has not yet been examined[61–64].

Given the lack of a self-sustaining lake trout population in Lake Ontario despite ongoing restoration efforts, identification of strains that possess a tolerance to thiaminase may confer some advantages in the Lake Ontario environment and may help improve restoration success[49,61]. Here, we examined the effects of diet-derived thiaminase on morphology, colouration, swimming performance, and recovery after exhaustive exercise, and compared results between the Seneca Lake and Slate Islands strains. Critical swim speed is the best ecophysiological measurement to estimate swimming performance and to predict ecological consequences of stressors outside of measurements of free-swimming individuals[42]. Lake trout are cruising predators and swimming performance is important for capturing dispersed pelagic prey items and avoiding predation[65,66]. We predicted that diet-derived thiaminase would impair swimming performance and recovery after exercise as well as change both the morphology and colouration in both strains. Based on previous research (e.g., [11,59]), we also hypothesized that the Seneca Lake strain would show lesser effects compared to the Slate Islands strain due to past differences in exposure to high-thiaminase containing prey and potential differences resulting from local adaptation to thiaminase exposure.

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Methods

Preparation of thiaminase and control diets

The experimental protocol used in this study was developed in accordance with the guidelines and regulations of the Canadian Council on Animal Care and approved by the Ontario Ministry of Natural Resources and University of Western Ontario Animal Care Committees (Protocol Number: 2018–084). The experiments carried out in this study are reported in accordance with ARRIVE guidelines[67]. Thiaminase and control diets were created based on those used by Honeyfield et al.[39]. The specific compositions and preparations are described in Baker et al.[24] and Therrien et al.[68]. Briefly, the diets were identical in composition, except that bacterial thiaminase from *Paenibacillus thiaminolyticus* (Nakamura, 1990) was added to the thiaminase diet. Full details regarding the preparation and use of *P. thiaminolyticus* can be found in Houde et al.[30] and Therrien et al.[68]. The final bacteria count in the liquid media used in the preparation of the thiaminase diets was $2.1 \times 10^8 \pm 6.1 \times 10^7$ cfu/mL, which is a concentration that has previously been shown to induce thiamine deficiency in Atlantic salmon and lake trout[21,30,68,69]. Total thiamine concentrations were similar between the thiaminase (6.92 ± 5.8 nmol/g) and control (7.05 ± 5.2 nmol/g) diets. A sample of each of the *P. thiaminolyticus* and control broth supernatants were assayed to determine thiaminase activity using the 4-NTP assay, the methods of which are included in Therrien et al.[68]. Thiaminase I activity in control diets was lower (mean ±SD; 0.73 ±1.24 pmol/min) than in thiaminase diets (mean ±SD; 10.69 ±13.82 pmol/min). Finished feed was stored at -20°C until use. Maximum storage time for the diets was 2 weeks.

Study strains and experimental design

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Descriptions of strains used in this study can be found in Baker et al.[11] and Therrien et al.[68]. Briefly, families for the Seneca Lake and Slate Islands strains were produced in late 2019 using single-pair matings of mature individuals at the Ontario Ministry of Natural Resources (OMNR) Dorion Fish Culture Station (Dorion, ON) and transferred as eyed eggs to the Chatsworth Fish Culture Station (Chatsworth, ON). The timeline of the experiment is presented in Table 1. On 18 March 2021, n = 200 lake trout from each of the Seneca Lake (age 13 months) and the Slate Islands (age 14 months) strains were transferred from the OMNR Chatsworth Fish Culture Station to the experimental hatchery at the University of Western Ontario (London, ON). A full description of the rearing conditions of the lake trout in the experimental hatchery can be found in Therrien et al.[68] and a figure showing the experimental hatchery can be found in Supplementary Information Fig. S1. Briefly, twenty-five fish from each strain were placed into one of sixteen 73 L white polypropylene tanks at a density of 9 g / L (i.e., 25 fish weighing 22–27g in a 73 L tank). The fish were given 27 days to acclimate to the Western University hatchery before being anesthetized in a bath of tricaine mesylate (TMS; 300 mg L^{-1}) buffered with sodium bicarbonate (300 mg L^{-1}) and measured for body mass and fork length. After measurement, fish were tagged with a unique sterile 1.2 mm Passive Integrated Transponder (PIT; Biomark Inc) in the ventral cavity using a sterile PIT tag implanter (model MK10, Biomark Inc) of which the full description of administration is found in Therrien et al.[68]. After tagging, fish were placed in a recovery tank and remained there until the return of normal behaviour, at which time they were returned to their tanks. Fish were given an additional 14 days to recover before the experiment. During this recovery time, they were fed the commercial fish feed. Four replicate tanks for each strain were then administered either a thiaminase or control diet (2 strains × 2 diets × 4 replicates; total number of experimental units = 16). Each experimental unit was randomly

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assigned to a tank and each row of 8 tanks had equal representation of each diet and strain. The feeding schedule and a full description of feed rations can be found in Therrien et al.[68].

Swimming performance and recovery

After 260 days (on 3 December 2021), critical swim speed was measured on 20 randomly selected fish from each combination of diet and strain (n = 80 fish; Table 2). Following methods described in Colborne et al.[66] and Houde et al.[30], a single fish was placed into a 40 L swim flume (Loligo Systems, Denmark) and acclimated for 3 minutes. Water velocity was increased incrementally at a rate of 0.15 m / s every 2 minutes until the fish displayed signs of fatigue (i.e., an inability to continue swimming at that speed). Critical swim speed (U_{crit}) was calculated as $U_{crit} = U_i + (T_i/T_{II} \times U_{ii})$, where U_i was the highest velocity maintained for a full 2 minute interval, T_i was the time of fatigue at last current velocity (minutes), T_{ii} was the interval length (2 minutes), and U_{ii} was the velocity increment (0.15 m/s; [30]). After fish reached exhaustion, water velocity was reduced to 0.20 m/s and fish were allowed to recover for up to 30 minutes. During this time, once a fish had resumed swimming for 5 consecutive minutes, it was considered recovered, and the trial was ended[71]. If a fish did not resume swimming for 5 consecutive minutes before the 30-minute mark, the trial was ended, and it was considered to have not recovered. For the analysis of recovery post-exhaustion, the data were converted to binary data (0 = did not recover or 1 = recovered). Every third fish did not undergo the recovery trials, instead having tissues sampled during exhaustion for a separate study. The sample size for the recovery trials was thus n = 54 (Table 2). Upon trial completion, all fish (recovered and not recovered) were euthanized with an overdose of TMS. The fish were measured for total body length and mass. Specific growth rate for each individual fish was calculated using the mass measurements and the methods of Ricker[68]. Fish were then placed on their right side and

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digitally photographed using a camera (18 MP Canon EOS Rebel T5) set at a fixed height following methods described in Muir et al.[69]. Each digital photograph contained a size and colour standard. Four fish were not photographed due to camera issues and two other photographs had corrupted colour standards and were excluded (sample size for the colour analysis was n=74).

Morphology and colouration

Photographs of each lake trout were examined for body morphology and skin pigmentation using methods described by Muir et al.[69], Perreault-Payette et al.[70], and Villafuerte and Negro.[71]. Briefly, for morphology, 20 landmarks and sliding landmarks (Supplementary Information 1 Fig. S2) related to aspects of head and body depth and caudal region lengths were digitized and analyzed with the Thin Plate Spline suite (*tps*: http://life.bio.sunsysb.edu/morp). First, for each fish, a rectangular grid was overlaid to identify body curvature corresponding to 20–30–40–50% of body length using the program REVIT[76]. The body was anchored at the tip of the snout and midpoint of the hypural plate. Next, 16 homologous landmarks and four sliding landmarks were digitized with the program *tpsDig*[77]. Sliding landmarks were slid in the program *tpsUtil*[78]. These landmarks were then subjected to relative warp analysis using *tpsRelw* software[79] to get centroid sizes and principal relative warp scores. Data were imported into MorphoJ[80], and a principal component analysis (PCA) was performed to reduce the number of morphometric variables or scores and extract divergent morphometric patterns. PCA scores were exported into R (version 4.3.3.; [81]) for further analysis. For skin pigmentation, the average intensity of each colour channel (RGB [red, green, blue] colour space) was measured for the dorsal, lateral, ventral, caudal peduncle, and caudal fin regions using ImageJ version 1.8.0 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/). RGB colour space values were corrected for each photograph using both a light and a dark standard that was included in each photograph

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to account for any potential differences in lighting between photographs. RGB colour space values for skin pigmentation were then converted into LAB colour spaces in R using the function convertcolor() in package grdevices[82]. A PCA was performed in base R to reduce dimensionality.

Statistical analyses

All metrics collected of individual lake trout were analyzed in R (version 4.3.3.; [81]), using $\alpha = 0.05$ for all statistical tests. Comparisons of initial length and mass measurements were compared using a student's t-test. Linear mixed effects models fit with a restricted maximum likelihood (lmm; *lmer* in the lme4 package in R; [83]) were used to examine effects of diet type, strain, and their interaction on mass and length at experiment end, specific growth rate, critical swim speed, morphology, and colouration (PC scores). For critical swim speed, an additional fixed effect of fish body length was included in the analysis to account for differences in length among fish. Probability of recovery was analyzed using a generalized linear mixed model with a logit link using function glmer in package lme4[83] and included the fixed factors of diet-type, strain, and the diet × strain interaction. In the final model, the exponentiated coefficients reflect the odds of recovering versus not recovering, and the logit^{-1}-transformed coefficients reflect the probability of recovery. All models included a random effect for tank identity. To test if the random effect explained a significant proportion of the variance, a restricted likelihood ratio test was used (function exactRLRT() in package RLRsim; [84]). When the random effect of tank explained a significant proportion of the variation, variation explained was quantified using the intraclass correlation coefficient method and was represented as the percentage of the variance accounted for by the random effects.

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Results

Clinical signs of thiamine deficiency

After 260 days of feeding of the experimental diets, symptoms associated with thiamine deficiency were evident in fish from the thiaminase treatment including lesions of the eye, ataxia, and lethargy, as well as increased mortality and increased tissue liver transketolase latency, the results of which are presented in Therrien et al.[68] and Neff et al. [85]. Reduced muscle and tissue total thiamine concentrations were also noted and presented in Supplementary Information Fig. S3.

Comparison of length, mass, and specific growth rate

Total body length, mass, and specific growth rate are provided in Table 3. The two lake trout strains differed in their body length and mass before and after the experiment. At the start of the experiment, fish from the Slate Islands strain were both significantly heavier (t = -5.44, df = 407.63, $p < 0.001$) and longer than those from the Seneca Lake strain (t = -6.26, df = 413.49, $p < 0.001$). At the end of the experiment, fish from the Seneca Lake strain were significantly heavier ($F_{1,80} = 7.83$, $p = 0.018$) and longer ($F_{1,80} = 6.48$, $p = 0.03$) than those from the Slate Islands strain. Independent of strain, fish fed the control diet were also significantly heavier ($F_{1,80} = 5.95$, $p = 0.024$) and longer ($F_{1,80} = 6.75$, $p = 0.03$) than fish fed the thiaminase diet. For both models, the diet × strain interaction term was not significant (mass: $F_{1,80} = 1.49$, $p = 0.25$; length: $F_{1,80} = 0.65$, $p = 0.48$). Furthermore, the random effect of tank did not account for any significant variation and was excluded from the final models (mass: RLRT = 1.14×10^{-13}, $p = 0.46$; length: RLRT = 0, $p = 1$).

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Specific growth rates of fish from each strain and treatment from experiment start to end are also presented in Table 3. Fish from the Seneca Lake strain had significantly higher growth rates ($F_{1,80} = 18.64$, $p = 0.001$) than fish from the Slate Islands strain. Independent of strain, fish fed the control diet had significantly higher growth rates ($F_{1,80} = 5.62$, $p = 0.036$) than fish fed the thiaminase diet. The diet × strain interaction term was not significant ($F_{1,80} = 0.74$, $p = 0.40$). Furthermore, the random effect of tank did not account for any significant variation and was excluded from the final models (RLRT = 0, $p = 1$).

Critical swim speed and probability of recovery

Fish fed the thiaminase diet had a significantly lower critical swim speed than those fed the control diet ($F_{1,80} = 6.34$, $p = 0.027$, Fig. 1). This decrease in swim speed also corresponded to a lower time to reach trial end (mean ± SD; control 9.6 ± 1.5 min; thiaminase 8.3 ± 1.7 min). The fixed effect of body length was also significant in this model, showing that longer fish had faster critical swim speeds than shorter fish ($F_{1,80} = 4.00$, $p = 0.049$). Critical swim speed did not differ significantly between strains ($F_{1,80} = 2.34$, $p = 0.15$, Fig. 1), despite the Seneca Lake fish being longer than the Slate Islands fish (Table 2). The diet × strain interaction term was not significant ($F_{1,80} = 0.69$, $p = 0.42$, Fig. 1), indicating that the effect of diet on critical swim speed was consistent between the two strains. The random effect of tank did not explain any of the variation in the model (RLRT = 0.94, $p = 0.14$).

Recovery time of the lake trout post-exhaustive exercise is included in Supplementary Information Fig. S4. A total of 9 Seneca Lake fish in the control, 5 in the thiaminase treatment, 3 Slate Island fish in the control, and 2 in the thiaminase treatment did not recover. Of the fish that did recover, the average time (± SD) across strains and treatments to recovery was 624.6 ± 206.5 (Seneca Lake control), 904.5 ± 621.3 (Seneca Lake thiaminase), 693.6 ± 406.6 (Slate Island

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control), and 623.8 ± 341.9 s (Slate Island thiaminase). Contrary to our expectation, fish fed the control diet tended to have a lower probability of recovery after the critical speed trials than those fed the thiaminase diet, but the difference was not statistically significant (z = 1.55, df = 1, 54, p = 0.12; Fig. 2). The probability of recovery was significantly different between strains with fish from the Slate Islands strain having a higher probability of recovering from exhaustion than those from the Seneca Lake strain (Fig. 2; z = 2.26, df = 1, 54, p = 0.02). The diet × strain interaction term was not significant, indicating that the effect of diet type on critical swim speed did not differ between strains (Fig. 2; z = -0.47, df = 1, 54, p = 0.63). The random effect of tank did not account for any of the variance in the model.

Morphology and colouration

The morphology landmarks and skin pigmentation PCA loadings and plots are presented in Supplementary Information Table S1 and figures S5 and S6, respectively. For morphology, we considered only principal component 1 (PC1) and principal component 2 (PC2) in further analyses. PC1 and PC2 explained 30.8% and 15.9% of the variation, respectively, and were easily interpreted biologically. Positive PC1 scores were associated with a deeper dorsal-ventral depth and more curved body shape whereas positive PC2 scores were associated with a thinner anterior end and a longer, straighter posterior end. Fish fed the thiaminase diet had shallower dorsal-ventral depth and were less curved laterally (PC1) than those fed the control diet ($F_{1,76}$ = 4.91, p = 0.04; Fig. 3A). There was no effect of strain on the dorsal-ventral depth and body curvature (PC1) of the juvenile lake trout ($F_{1,76}$ = 3.50, p = 0.09; Fig. 3A), nor was there a significant diet × strain interaction ($F_{1,76}$ = 1.42, p = 0.26; Fig. 3A), indicating that differences in morphology between diet types were consistent between the two strains. The random effect of tank did not account for any of the variance in the model (RLRT = 0.03, p = 0.38).

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While diet type affected body depth and curvature, it did not appear to affect anterior thickness and posterior length (PC2; $F_{1,76} = 0.14$, $p = 0.71$; Fig. 3B). Lake trout from the Slate Islands strain had a significantly thinner anterior end and a longer posterior end than those from the Seneca Lake strain ($F_{1,76} = 31.79$, $p < 0.001$; Fig. 3B). The diet × strain interaction term was not significant ($F_{1,76} = 1.40$, $p = 0.26$, Fig. 3B). The random effect of tank did not explain any of the variation in the model (RLRT = 0.81, $p = 0.15$).

Examples of the colouration of lake trout in this study can be found in Supplementary Information 1 Fig. S7. For skin pigmentation, we performed further analyses and interpretation on principal components 1 (PC1), 2 (PC2), and 3 (PC3), which explained 30.6%, 25.9%, and 16.0% of the colour variation among individuals, respectively. PC1 was negatively related to whole-body whiteness (L colour space). PC2 was associated with more yellow (positive b values) in the lateral, ventral, peduncle, and caudal regions. PC3 was associated with more green and less red (negative a values) in the caudal and peduncle body regions. We found that diet type had no influence on inferred whole-body whiteness (PC1) of lake trout ($F_{1,74} = 2.51$, $p = 0.14$; Fig. 4A). Inferred whole-body whiteness also did not differ significantly between strains ($F_{1,74} = 2.14$, $p = 0.17$; Fig. 4A), and there was no significant diet × strain interaction ($F_{1,74} = 0.56$, $p = 0.46$; Fig. 4A). The random effect of tank accounted for 32 % of the variance in the model (RLRT = 7.75, $p = 0.003$).

Raw B (blue pigmentation) colour space values are included in Supplementary Information Fig. S8. Fish fed the thiaminase diet had more yellow colouration in the lateral, ventral, peduncle, and caudal regions (inferred from PC2) than those fed the control diet ($F_{1,74} = 5.01$, $p = 0.048$; Fig. 4B). Inferred yellow colouration also differed significantly between strains ($F_{1,74} = 6.43$, $p = 0.03$; Fig. 4B); lake trout from the Slate Islands strain had more yellow colouration than those from the Seneca Lake strain. The interaction term was not statistically significant, indicating that effects of

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diet-type on yellow colouration were consistent between the two strains ($F_{1,74} = 3.57$, $p = 0.08$, Fig. 4B). the random effect of tank did not explain any of the variation in the model (RLRT = 1.13×10^{-13}, $p = 0.45$).

Raw R (red) and a (green-red) colour space values are included in Supplementary Information Fig. S8. There was a significant effect of diet type on the red colouration of lake trout (inferred from PC3; $F_{1,74} = 5.72$, $p = 0.03$, Fig. 4c). Anecdotally, individuals from the Seneca Lake strain in the control diet treatment appeared visually redder in colouration than those from the Slate Islands strain, especially in the caudal and fin areas (Supplementary Information Fig. S7). The diet × strain interaction term was significant, indicating that effect of diet type on red colouration did differ significantly between the two strains ($F_{1,74} = 6.92$, $p = 0.02$, Fig. 4C). Lake trout from the Slate Islands strain were greener in colour than those from the Seneca Lake strain ($F_{1,74} = 33.31$, $p < 0.001$; Fig. 4C). The random effect of tank did not explain any of the variation in the model (RLRT = 0, $p = 1$).

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Discussion

A diet containing thiaminase has been reported to cause decreases in the swimming performance of salmonines[29,30] and other fishes (e.g., eels; [17]). We similarly found a decline in critical swim speed, a measurement of swimming performance, in both strains of juvenile lake trout fed the thiaminase diet. These results are consistent with those of Houde et al.[18] who found a decrease in critical swim speed in Atlantic salmon fed a thiaminase-containing diet. Our results are also consistent with those of Morito et al.[17], who reported a decrease in swimming performance in thiamine-deficient rainbow trout. A thiaminase-induced decline in swimming performance was previously thought to reflect either a decrease in ATP production, as thiamine enables pyruvate to enter the citric acid cycle to produce ATP[29,86], or an increase in plasma lactate that affects muscular performance, which has been observed in juvenile rainbow trout[29,87]. Recently, Baker et al.[11] found a change in heart morphology and a decrease in cardiac function in lake trout fed the same thiaminase diet used in our study. A decrease in cardiac performance in fish fed the high-thiaminase diet may thus also contribute to the decline in swimming performance that we observed, as cardiac output has been directly linked to swimming performance in salmonines[88]. Lake trout employ vertical and horizontal cruising to locate prey throughout the water column[65] and in large lakes, will make long distance movements from spawning locations to foraging ranges[89]. Furthermore, juvenile lake trout rely on physical separation (e.g., vertical migrations) that requires swimming performance to avoid predators[90]. Thus, a reduction in swimming performance of lake trout feeding on high-thiaminase prey fishes could reduce their ability to capture prey and avoid predation, ultimately reducing their survival in the wild.

Exhaustive exercise leads to an accumulation of lactic acid during exercise, and concomitant increases in hydrogen ions and acidosis in muscles are considered a major cause of

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fatigue (reviewed by [90]). Previous studies have reported a decrease in lactic and pyruvate following thiamine supplementation[92] and deficiencies in thiamine are associated with elevated lactic acid levels[93,94]. Thiamine supplementation has been associated with decreased recovery time and decreased muscular fatigue in mammals[41]. Contrary to our prediction, however, a decrease in swimming performance was not associated with a decrease in the probability of recovery, and we found no difference in the probability in recovery after exhaustive exercise between fish fed our two diets. This observation could be explained by the inability of fish fed the thiaminase diet to maintain high swim speeds and thus may be an artefact of the critical swim speed protocol. Specifically, the thiamine-deficient fish had lower critical swim speeds and remained in the critical swim speed trial for an average of 1.3 minutes less than their control counterparts so may not have exerted themselves as much as their thiamine replete counterparts. Indeed, thiamine deficiency has been associated with a decrease in Ca- and Mg-activated ATPase, as well as a decrease in neuromuscular transmission, with resulting decreased muscle contraction activity[6,95]. A similar mechanism could have prevented the thiamine-deficient fish from reaching aerobic failure and allowed them to have similar probabilities of recovery as fish fed the control diet.

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Thiamine deficiency has previously been shown to cause changes in body appearance in salmonines[29,30]. We found that juvenile lake trout had a trend towards increased whole body lightening and increased yellow body pigmentation when fed a high-thiaminase diet. We also found a strain-specific change in red pigmentation; fish from the Seneca Lake strain had more green pigmentation whereas those from the Slate Islands strain had more red pigmentation when fed a diet high in thiaminase. Our results are consistent with Houde et al.[30] who reported a trend of increased whole body lightening of Atlantic salmon fed a high thiaminase diet. Body depigmentation (lightening) can occur in thiamine-deficient fish[96] because thiamine plays a role

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in melanogenesis[97]. Specifically, it modulates the tyrosine-tyrosinase reaction[97] and also has an indirect role by providing NADPH and ATP to melanocytes[98].

Our results contrast those of Houde et al.[30], who found a decrease in yellow pigmentation. There, the authors attributed the decrease in yellow pigmentation to the amount of the carotenoid idoxanthin[99], a metabolite of astaxanthin, an antioxidant whose concentrations may decline under thiamine deficiency due to oxidative stress[100,101]. The increase in yellow pigmentation found here may instead be the result of jaundice, which has been previously observed in fish[102,103]. Jaundice is a symptom of hepatic dysfunction which is associated with thiamine deficiency mediated lactic acidosis[104,105]. Further evidence to support this mechanism comes from the combined observation of decreased swim performance, as lactate acidosis is both a mechanism behind the reduced capacity for activity[91] and a symptom of hepatic dysfunction[105]. Additionally, an increase in yellow pigmentation may suggest a decrease in cardiac function (see Baker et al.[11]), which can decrease blood flow to the liver, ultimately reducing bilirubin clearance and causing jaundice[106]. Decreased heart function may also explain the decrease in red pigmentation in the Seneca Lake fish fed a diet high in thiaminase as decreased blood flow to the periphery would decrease the reddening of the skin after exhaustive exercise. Indeed, the greatest difference in red colouration in Seneca strain fish was found in the fin. Vasoconstriction and reduced peripheral blood flow has been found in thiamine deficient mammals[107,108], although more research is needed in fish.

A diet high in bacterially derived thiaminase may alter the morphology of lake trout. Lake trout had a shallower ventral-dorsal depth and were less curved laterally when fed the thiaminase diet, and this was observed in both strains. These results differ from those of Houde et al.[30] who found a general trend of a less streamlined body shape (lateral compression, deeper

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ventral-dorsal depth) in Atlantic salmon fed a diet high in thiaminase. A less streamlined body shape is thought to be related to reduced swimming activity[44] whereas an increase in a streamlined body shape and decreased ventral-dorsal depth may be related to a drop in condition because of a lower growth rate. Indeed, we found that fish from both the Seneca Lake and Slate Island strains that were fed the thiaminase diet had lower growth rates than those fed the control diet. In brown trout (*Salmo trutta* [Linnaeus, 1758]) and cisco (*Coregonus artedi* [Lesueur, 1818]), decreased body depth was associated with decreased survival as a result of increase susceptibility to large-gape predators[43,109]. Thus, in lake trout, the decrease in ventral-dorsal depth may also reduce survival in the wild.

Finally, our results have implications for the restoration of lake trout, particularly in Lake Ontario. Strain-targeted stocking programs present a possible solution to reduce the incidence of thiaminase-related health effects of lake trout in the wild. We found that fish from the Seneca Lake strain fed the high-thiaminase diet had more yellow and less red pigmentation, potentially reflecting thiamine deficiency-induced cardiac impairment, compared to those from the Slate Islands strain. Fish from the Slate Islands strain may perform better in the Lake Ontario environment if the changes in colouration are indicative of cardiac capacity, but further research is needed to confirm this. Furthermore, Slate Islands fish had a higher probability of recovery post-exhaustive exercise and a trend towards faster swimming speeds (at a given size), which could provide an advantage in Lake Ontario, where feeding ranges are known to reach up to 4000 km^2 [89].

Conclusion

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

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Author statements

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Competing interests

The authors declare no competing interests.

Author contributions

C.T., B.N. and S.G. all helped conceive the original idea and methodology. C.T. collected the data, conducted data processing, and drafted the manuscript. P.B. also helped carry out the swim flume trials. C.T., P.B., and S.G. all helped with animal husbandry. C.T. and H.K.S. contributed to data analysis. All authors discussed the results and contributed to the final manuscript. Supervision was provided by H.K.S. and B.N.

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Data availability

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All data generated or analysed during this study are included in this published article and its supplementary information files.

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Tables

Table 1. Timeline of experiment using lake trout from the Seneca Lake and Slate Islands strains fed either a control or thiaminase diet.

Date	Method	Time since experiment start
18 March 2021	Juvenile (age 1+) transferred from Chatsworth FCS to Western University's experimental hatchery. Fish fed commercial fish feed at 2% rations.	0
14 April 2021	Length and mass measurements, PIT tagging of fish	27
28 April 2021	Feeding of experimental diets begins at 2% rations with 50% experimental diet and 50% commercial fish feed	41
12 May 2021	Feeding of 100% experimental diets at 2% rations begins	55
20 May 2021	Weighing of fish. Feeding at 2% rations continues	63
18 June 2021	Weighing of fish. Feeding at 2% rations continues	92
23 July 2021	Weighing of fish. Culls to reduce density. Feeding at 1.5% rations begins.	127
27 August 2021	Weighing of fish. Feeding at 1.5% rations continues	162
24 September 2021	Weighing of fish. Feeding at 1.5% rations continues	190

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

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Table 2. Sample sizes for each metric measured on lake trout from the Seneca Lake and Slate Islands strain of lake trout fed either the control or thiaminase experimental diet.

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

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Table 3. Initial and final body lengths and masses and specific growth rates (mean ± SD) of lake trout from the Seneca Lake and Slate Islands strains of lake trout fed either the control or thiaminase experimental diet.

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

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

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Figure captions

Fig. 1. Critical swim speed (*Ucrit*) of lake trout from the Seneca Lake and Slate Islands strains fed either a control (dark grey) or high-thiaminase (light grey) diet for 6 months. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

Fig. 2. Probability of recovery after failure in the critical swim speed trials of lake trout from the (A) Seneca Lake and (B) Slate Islands strains fed either a control or thiaminase diet for 6 months. Dots denote the mean and error bars denote the 95% confidence intervals for each estimate. While there were no differences between diet groups within each strain, there was a significant difference in the probability of recovery between the Slate Islands and the Seneca Lake strains ($p < 0.05$).

Fig. 3. Principal components of a PCA performed on 20 morphological landmarks from lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Positive principal component 1 (PC1; A) scores were associated with a deeper dorsal-ventral depth and more curved body shape. Positive principal component 2 (PC2; B) scores were associated with a thinner anterior end and a longer posterior end. Boxplots show the median and first and third quartiles. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

Fig. 4. Principal components of the principal component analysis of LAB colour values from 5 body regions on lake trout from the Seneca Lake or Slate Islands strains fed either a control or thiaminase diet for 6 months. Positive principal component 1 (PC1; A) scores were associated

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with darker whole body colour space values, positive principal component 2 (PC2; B) scores were associated with more yellow whole body colour spaces values, and positive principal component (PC3; C) scores were associated with a greener peduncle and fin colouration. Whiskers show minimum and maximum values. Dots represent outliers according to 1.5 × interquartile range greater than Q3 or smaller than Q1. Asterisks denote a significant difference ($p < 0.05$).

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