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Effects of feeding high dietary thiaminase to sub-adult Atlantic salmon from three populations

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ABSTRACT

Salmonids consuming high thiaminase-containing prey fishes can develop debilitating thiamine deficiencies. Historically, salmonids within the Laurentian Great Lakes consumed low thiaminase-containing prey fishes. Currently, however, salmonids are consuming introduced high thiaminase-containing prey fishes, which may be an impediment to the persistence of native species. Here, we examined the effects of feeding high thiaminase on sub-adult (two-year-old) Atlantic salmon (*Salmo salar*) from three populations (LaHave, Sebago, and Saint-Jean) that are being used for reintroduction into Lake Ontario. The thiaminase diet, mimicking the current high thiaminase concentrations of prey fishes, was produced by mixing natural bacterial thiaminase into prepared feed. After 6 months of feeding fish the thiaminase diet, we found significant drops in thiamine in red blood cells, white muscle, and liver tissues in all three populations as compared to fish fed a control diet. Additionally for liver tissue, we found a higher reduction in thiamine for the LaHave population relative to the Sebago and Saint-Jean populations. Although the salmon fed the thiaminase diet had no change in survival or growth after 8 months, the salmon had lower swimming performance than fish fed a control diet. There were also trends for lower body condition, a less streamlined body shape, and less yellow body pigmentation when fed the thiaminase diet. The changes in these latter traits may indicate the onset of a thiamine deficiency and could negatively impact Atlantic salmon survival in the lake.

Introduction

Anthropogenic impacts on natural environments are increasingly altering prey species composition and abundance. It is becoming apparent that these impacts can lead to deficiencies in essential nutrients formerly available in prey species (Barboza et al., 2009). Because essential nutrients cannot be synthesized *de novo*, deficiencies in these nutrients can leave predator species vulnerable to metabolic dysfunction and disease. For example, habitat changes have diminished the prey resources containing vitamin A for southern sea otters (*Enhydra lutris nereis*) (St Leger et al., 2011). Subsequent vitamin A deficiencies in sea otters resulted in abnormal bone growth and a reduction in survival (St. Leger et al., 2011). Furthermore, lipid deficiencies in *Daphnia magna* caused by human-induced cyanobacteria blooms reduced the number and quality of the eggs produced (Wacker and Martin-Creuzburg, 2007). Nutrient deficiencies can have significant ecological effects, as even small reductions in individual fitness can alter community dynamics, lead to the extirpation of small populations (Hutchings, 1991), and potentially impede the restoration of native populations (Dimond and Smitka, 2005).

Thiamine (vitamin B1) is an essential, environmentally-obtained nutrient for many fish species (Halver and Hardy, 2002). Thiamine is critical for metabolism as it serves as a cofactor for several enzymes that breakdown carbohydrates and amino acids to produce energy (i.e. adenosine triphosphate, ATP) (Kawasaki and Egi, 2000). Many salmonid populations are currently experiencing thiamine deficiencies (Norrgren et al., 1993; Fisher et al., 1995; Fitzsimons et al., 1995). In the Laurentian Great Lakes and New York Finger Lakes, the source of the thiamine deficiency for salmonid fishes appears to be the consumption of introduced non-native prey fishes that contain high concentrations of thiaminase, an enzyme that degrades thiamine (Fitzsimons et al., 1998; Wistbacka et al., 2002; Honeyfield et al., 2012). Conversely, in the Baltic Sea, the thiamine deficiency in salmonids appears to be instead driven by a reduction in the transfer of thiamine from lower to higher trophic levels because of eutrophication in the environment (Sylvander et al., 2013). Fish that develop thiamine deficiencies display lethargy, 'wiggling' behavior, loss of equilibrium, and eventually cease feeding and die, all of which highlighting the importance of thiamine for energetic and metabolic function (Morito et al., 1986; Amcoff et al., 1998).

Salmonids within the Great Lakes and Finger Lakes historically consumed native prey fishes, such as cisco or lake herring (*Coregonus artedi*) and bloater (*Coregonus hoyi*), which contain low thiaminase

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concentrations (Tillitt et al., 2005; Zajicek et al., 2005). Within these lakes, the dominant prey fishes are now introduced non-native alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*), which contain high thiaminase concentrations (Tillitt et al., 2005; Zajicek et al., 2005; Honeyfield et al., 2012). A source of the thiaminase found in these introduced prey fishes is the non-pathogenic bacteria *Paenibacillus thiaminolyticus*, which has been isolated from Lake Michigan alewives (Honeyfield et al., 2002; Zajicek et al., 2009). The prey fishes can also produce thiaminase *de novo* within their bodies (Richter et al., 2012). Without consideration of the presence of thiaminase, the prey fishes themselves contain more than enough thiamine to meet the dietary requirements of salmonids (Fitzsimons et al., 1998; Tillitt et al., 2005). However, the high thiaminase concentrations of these non-native prey fishes can degrade any available thiamine in the digestive system of salmonid predators before it can be absorbed (Fitzsimons et al., 2007).

Although there is a link between the consumption of high thiaminase-containing prey fishes and the development of a thiamine deficiency (Honeyfield et al., 2005), it is less clear to what extent the ability to cope with ingested thiaminase varies within and among conspecific populations. For example, some freshwater resident populations of Atlantic salmon (*Salmo salar*) consume rainbow smelt, yet do not appear to display a thiamine deficiency (see Dimond and Smitka, 2005). Also, the extent of thiamine deficiency symptoms varies among Atlantic salmon individuals from Saint-Mary's River, Michigan (Dimond and Smitka, 2005), as well as coho salmon (*Oncorhynchus kisutch*) individuals from Platte River, Michigan (Brown et al., 2005) that typically consume alewife. These data suggest there may be some degree of variation in thiaminase tolerance both within and among populations, although additional studies under controlled conditions are needed.

Here, we examine the performance of sub-adult (two-year-old) Atlantic salmon from three populations that were given prepared diets mimicking the historical diet (low thiaminase content) and the current diet (high thiaminase content) within the Great Lakes. Although several studies have examined the effects of thiamine deficiency in adult salmonids and their offspring, these effects have rarely been examined in smolt or sub-adult salmonids, the age when these fishes begin consuming high thiaminase-containing prey fishes (Morito et al., 1986; Ketola et al., 2008). The three populations we studied are being used as sources for the reintroduction of Atlantic salmon into Lake Ontario and comprise: LaHave River from Nova Scotia (hereafter referred to as LaHave), Sebago Lake from Maine (Sebago), and Lac Saint-Jean from Quebec (Saint-Jean). The recent presence of high thiaminase-containing prey fishes in Lake Ontario may be an important factor impeding restoration efforts (Dimond and Smitka, 2005). The Sebago and Saint-Jean populations are native to freshwater lakes and primarily consume rainbow smelt in their native habitats (Dimond and Smitka, 2005). By contrast, the LaHave population, which has been the focus of previous restoration efforts in Lake Ontario (Greig et al., 2003), is anadromous and has a more diverse diet of capelin (*Mallotus villosus*), sand eels (Ammodytidae), krill (Euphausiacea), and amphipods (Amphipoda) (Rikardsen and Dempson, 2011). Consequently, we anticipated that the Sebago and Saint-Jean populations would have genetic adaptations enabling them to better tolerate a high thiaminase diet relative to the LaHave population.

Methods

Study populations

Families for the LaHave ($n = 37$), Sebago ($n = 14$), and Saint-Jean ($n = 66$) populations were produced in early November 2011 using single-pair matings of mature individuals at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Harwood Fish Culture Station, Harwood, Ontario. The LaHave mature individuals originated from fertilized eggs of single-pair matings of captive LaHave adults descended from the wild source population (44°14′N 64°20′W). The OMNRF LaHave broodstock was founded from several years of wild spawn collections (1989 to 1995), and the captive adults used from the 2007 cohort were the product of two generations of post-founding hatchery breeding (OMNR, 2005). The Sebago and Saint-Jean mature individuals originated from fertilized eggs of single-pair matings of wild Sebago from Panther River (43°53′N, 70°27′W) and wild Saint Jean from Rivière-aux-Saumons (48°41′N, 72°30′W); both founding wild spawn collections were carried out in 2007. Families were transported to the OMNRF Codrington Research Facility, Codrington, Ontario in spring 2012, where they were subjected to a natural light cycle and water from a surface stream (Marsh Creek) at natural temperatures. The salmon were fed commercial pellets (Corey Aquafeeds, Fredericton, New Brunswick) until used in the experiment.

Experimental diets

Two experimental diets were formulated to be isonitrogenous, isocaloric, and to contain different concentrations of bacterial thiaminase (*P. thiaminolyticus*) isolated from Lake Michigan alewives (Honeyfield et al., 2002). These fish meal based diets (control, no thiaminase and a diet with added bacterial thiaminase) contained all required essential nutrients and were prepared as described by Honeyfield et al. (2005). The thiamine concentration measured in the individual ingredients summed to 0.9 mg/kg of complete feed. Additional thiamine hydrochloride (1.5 mg/kg) was added to the diets for a total 2.4 mg thiamine/kg feed or 8.0 nmol per gram of feed (Table 1).

The diet containing thiaminase was formulated to mimic the thiaminase activity found in alewife and other thiaminase positive prey fish that cause thiamine deficiency in Great Lakes salmonids (Tillitt et al., 2005; Honeyfield et al., 2012). We used the same strain of bacteria (# 8703) and bacterial culture conditions in the present study that previously produced signs of thiamine deficiency in lake trout (*Salvelinus namaycush*) (Honeyfield et al., 2005). *P. thiaminolyticus* cultures were prepared using liquid media (yeast extract 1.0 g/L and 8.0 g/L Difco

Table 1
Composition and proximate analysis of the experimental diets for Atlantic salmon (*Salmo salar*). Greater details on the diet composition are described in Honeyfield et al. (2005). Proximate analysis is based on dry matter basis. For the thiaminase diet, bacteria cultures were mixed into dry ingredients (300 ml/kg of feed) to produce a thiaminase activity was 6800 pmol/min/g of feed (measured by Honeyfield et al., 2005). The thiamine hydrochloride in the vitamin premix was 1.5 mg/kg of feed or 5 nmol/g of feed. Including the thiamine measured in the other ingredients besides the vitamin premix, the total thiamine was measured at 2.4 mg/kg of feed or 8.0 nmol/gram of feed.

Variable	Control (%)	Thiaminase (%)
Diet composition		
Fish meal, herring	32.0	32.0
Starch	30.0	30.0
Corn gluten meal	18.0	18.0
Blood flour	8.6	8.6
Fish oil	8.0	8.0
Dextrin	1.0	1.0
Choline chloride	0.5	0.5
Vitamin premix	0.5	0.5
Mineral premix	0.2	0.2
Ascorbyl-2-polphosphate	0.2	0.2
Betaine-HCl	1.0	1.0
Bacterial thiaminase	None	Trace
Proximate analysis		
Dry matter	81.4	80.4
Crude protein	38.7	39.4
Crude lipid	10.4	10.3
Total carbohydrates	25.2	24.0
Ash	7.1	6.7

nutrient broth, Becton Dickinson, Mississauga, Ontario) inoculated with the bacteria (3 ml inoculation per 1 L of media) and incubated for 96 h at 37 °C. The final bacteria count in the liquid media was $1.1 \times 10^8 \pm 9.2 \times 10^7$ cfu/mL. All dry ingredients were thoroughly mixed (Hobart mixer, Hobart Ltd, Don Mills, Ontario, Canada) prior to the addition of all the thiaminase bacteria liquid culture (thiaminase diet only) and water (about 400 mL of liquid per kg of mash dry weight) at the University of Guelph Fish Nutrition Research Lab, Guelph, Ontario. The mix was immediately transported to the University of Western Ontario, London, Ontario. After 24 h, more water was added until the feed was a dough-like consistency and the dough was screw pressed using a 5 mm diameter die. The resultant moist pellets were air dried at room temperature for 2 to 3 days. Thiaminase activity was estimated to be 6,800 pmol/min per gram of feed based the data provided in Honeyfield et al. (2005). No analytical measurement of thiaminase was conducted. Finished feed was transported and stored at −20 °C at the Codrington Facility until used.

Experimental set-up

Atlantic salmon were adapted to experimental conditions for one year before starting the trial. Groups of 48 individually marked salmon (16 fish per population, sub-adults that were two-year-olds) were randomly distributed into six (260 L) tanks; fish from the three populations were mixed in equal numbers in each tank. Experimental diets were assigned randomly to the tanks (three tanks per diet). Salmon were maintained on water from Marsh Creek at natural temperatures and subjected to a natural light cycle.

Trials began in October 2013 when salmon were anesthetized with buffered MS-222 (tricaine methanesulfonate, 0.1 g/L), measured for fork length (nearest 0.1 cm) and mass (nearest 0.1 g). Salmon individuals had an initial body size of 56.3 ± 13.7 g (mean ± 1SD). Condition was calculated as $100 \times mass/length^3$ (Fulton, 1904). While still anesthetized, salmon were tagged with a 2 cm vinyl anchor tag on the left side just below the dorsal fin (Floy Tag & Mfg., Seattle, Washington) before being placed into the treatment tanks (Table 2). Tags were individually numbered and colored for each population and were applied using a fine fabric gun (Avery Mark III Fine Fabric Pistol Grip) with a maximum needle insertion depth of 1.5 cm. The needle was disinfected with hydrogen peroxide between individuals. The same day as tagging, salmon were given a 1% (0.01 kg/L) sodium chloride bath for 20 min for additional disinfection.

Table 2
Summary of body traits and total thiamine concentrations for three populations of sub-adult Atlantic salmon (*Salmo salar*) at the beginning of the experiment. Presented are means ± 1SD. Different uppercase letters indicate significant differences assessed using Tukey's post-hoc multiple comparisons ($p < 0.05$). For morphology, *centroid size* (used as a covariate for morphology to control for potential allometric effects of body size, see Bookstein, 1991) was included in the analysis. Morphology higher relative warp 1 (RW1) scores were associated with a more streamlined body shape. For skin pigmentation, higher principal component 1 (PC1) scores were associated with yellower body regions and higher principal component 2 (PC2) scores were associated with whiter body regions. Sample sizes are: $n = 12$ individuals for thiamine traits and $n = 96$ individuals for remaining traits for each Atlantic salmon population.

Traits	LaHave	Sebago	Saint-Jean
Length (cm)	17.1 ± 1.2^A	17.6 ± 1.5^B	16.8 ± 1.5^A
Mass (g)	52 ± 10^A	63 ± 14^B	54 ± 14^A
Condition ($100 \times g/cm^3$)	1.03 ± 0.07^A	1.12 ± 0.05^B	1.12 ± 0.06^B
Morphology (RW1)	0.018 ± 0.015^A	0.004 ± 0.011^B	0.002 ± 0.009^B
Pigmentation (PC1)	-11.4 ± 13.2^A	-6.7 ± 12.2^B	2.1 ± 13.6^C
Pigmentation (PC2)	-8.4 ± 10.3^A	-7.6 ± 10.7^A	-2.5 ± 10.9^B
Red blood cells total thiamine (nmol/g)	2.3 ± 1.2^A	1.9 ± 0.9^A	2.4 ± 1.0^A
Plasma total thiamine (nmol/mL)	0.12 ± 0.14^A	0.18 ± 0.19^A	0.26 ± 0.20^A

After a 14 day recovery period during which fish were fed a commercial diet (Corey Aquafeeds, 3 mm pellet, once a day), individual salmon were lightly anesthetized (MS-222, 0.05 g/L), placed on their right side and digitally photographed (10.3 MP Kodak Natural Color System) using a camera set at a fixed height. Each digital photograph contained a size and a color standard. Salmon were allowed to recover and were returned to their tank. A sample of extra salmon (not used in the experiment) were also sacrificed at this time point ($n = 12$ from each population) to serve as a baseline for the thiamine concentrations of red blood cells and plasma. These latter salmon were euthanized using an overdose of anesthetic until gill movement ceased; blood samples (0.5–1 mL) were then collected from the caudal peduncle posterior to the anal fin using a Heparin lined tube. Blood samples were immediately separated into plasma and red blood cells by centrifugation (1500 RCF for 5 min), frozen separately using dry ice and stored at −80 °C until thiamine analysis.

Experimental salmon recovered for another 14 days, during which they were fed a mixture of experimental diet and commercial diet (1:1). Afterward, salmon in the different treatment tanks were fed 100% their experimental diet for 8 months at 1% body mass per day from December to April and 2% body mass per day from June to August. Salmon survival was determined by removing mortalities daily from the tanks.

A subset of Atlantic salmon were sacrificed on June 10, 2014 ($n = 4$ from each population in each diet) to assess the thiamine concentrations of tissues. Baseline plasma total thiamine concentrations were at the lower end of the detection limit (mean ± 1SD, 0.18 ± 0.18 nmol/mL), so we also collected liver and white muscle tissues at this time. Liver and white muscle tissues are expected to be higher in total thiamine concentrations (see Brown et al., 1998). Liver and white muscle tissues were immediately frozen on dry ice and stored at −80 °C until thiamine analysis. The experimental protocol used in the present trial was developed in accordance with the guidelines of the Canadian Council on Animal Care as well as the Ontario Ministry of Natural Resources and University of Western Ontario Animal Care Committees.

Thiamine analysis

We focussed our thiamine analysis on the red blood cells, white muscle, and liver tissues. Thiamine concentrations of red blood cells, white muscle, and liver tissues were determined using the method developed by Brown et al. (1998). Samples of red blood cells (100–200 mg), white muscle or liver (300 mg) tissue were mixed with tricholoracetic acid, boiled for 10 min, centrifuged (14,000 RCF for 15 min), washed with ethyl acetate and hexane, and kept at −20 °C until oxidized. Washed extracts were oxidized with sodium hydroxide and potassium ferricyanide to their corresponding thiochromes. The thiochrome fluorescence of thiamine pyrophosphate, thiamine monophosphate, and free thiamine was measured using reverse-phase high-performance liquid chromatography (HPLC) with a Poroshell 120 column (100 × 4.6 mm, 2.7 μm mesh size; Agilent, Mississauga, Ontario) and a fluorescence detector at Agriculture Canada, London, Ontario. Sample HPLC area units were compared to a standard linear relationship of known HPLC area units against known thiamine standard concentrations.

Morphology and skin pigmentation

Photographs of the salmon were examined for body morphology and skin pigmentation using the methods described by Fraser et al. (2010) and Villafuerte and Negro (1998). For morphology, 21 landmarks related to aspects of head and body depth and caudal region lengths were measured using *tpsDig* software (Rohlf, 2008) and these landmarks were subjected to a relative warp analysis using *tpsRelw* software (Rohlf, 2009) to get the centroid sizes and principal relative warp

scores. For skin pigmentation, the average color of red, green, and blue pixels (RGB color space) were measured for the dorsal, lateral, ventral, caudal peduncle, and caudal fin body regions using ImageJ version 1.47 (NIH, Bethesda, MD, available at www.rsbweb.nih.gov/ij/). RGB color space values for skin pigmentation, i.e. dorsal, ventral, lateral, caudal peduncle, and caudal fin body regions, were converted into XYZ color space values, and then converted into LAB color space values using color conversion formulas of EasyRGB (available at: http://www.easyrgb.com/). Principal component analysis (PCA) with the covariance matrix in R 3.0.1 (available at http://www.r-project.org/) was used to simplify LAB color space values into a smaller number of variables.

For morphology, we considered only relative warp 1 which explained 30.4% of the variation among individuals and could be easily interpreted biologically: positive relative warp 1 scores were associated with a more streamlined body shape. For skin pigmentation, we considered principal components 1 and 2 which explained 39.0% and 22.6% of the variation among individuals, respectively. Principal component 1 was positively related to the yellowness of the lateral, ventral, and caudal peduncle body regions. Principal component 2 was positively related to the whiteness of the lateral, ventral, caudal peduncle, and dorsal body regions. The morphology landmarks and thin plate splines and skin pigmentation PCA loadings are presented in Electronic Supplementary Material (ESM) Table S1.

Swimming performance

Atlantic salmon were measured for critical swimming speed between July 23 and August 4 using the methods described in Colborne et al. (2011). Briefly, an individual was placed into an acrylic swim flume (Loligo Systems, Denmark) and acclimated for a period of 3 min. Water flow speed was then increased incrementally at 0.3 m/s every 2 min until the individual displayed signs of fatigue. Critical swimming speed (U_{crit}) was calculated as $U_{crit} = U_i + (T_i / T_{ii} \times U_{ii})$, where U_i is the highest velocity maintained for a full 2 minute interval, T_i is the time of fatigue at last current velocity (minutes), T_{ii} is the interval length (2 min), and U_{ii} is the velocity increment (0.3 m/s). To account for size influences on swimming performance, we used an Aitchinson (1986) log-ratio correction to produce relative swimming performance scores (also see Colborne et al., 2011) calculated as $rsp_i = [\ln(sp_i) - \ln(centroid_i)] / 2 + K$, where for individual i, rsp_i is the relative swimming performance, sp_i is the critical swimming speed, $centroid_i$ is the centroid size, and K is the minimum rsp_i included so that all rsp_i values are positive. Fatigued salmon were lightly anesthetized, measured for length and mass, and then digitally photographed as described above. Thermal-unit growth coefficient (TGC) was calculated as $100 \times (S_2^{1/3} - S_1^{1/3}) / \Delta D$ (Cho, 1992), where S_2 is the size at time 2, S_1 is the size at time 1, and ΔD is the growing degree-days ($\Delta D = \sum$ °C per day) from the initial body size measurements.

Statistical analysis of traits

Traits of individual Atlantic salmon were analyzed in R, using a significance threshold of $\alpha = 0.05$ for all statistical tests. Changes in traits (final–initial values for individuals) were used for analyses of body condition, morphology, and skin pigmentation. Linear mixed-effects models (*lmer* in the lmerTest package of R) were used to examine effects for normally distributed data and binomial mixed-effects models were used for survival (coded as 1 for alive and 0 for dead). Mixed-effects models contained fixed effects for *population*, *diet*, and *population* × *diet* and a random effect for *tank* identity. A linear discriminant analysis (*lda* in the MASS package of R) was then used to examine the effect of diet on the three populations. Five traits were included in the analysis (liver thiamine concentrations; relative swimming performance; and changes in morphology, skin pigmentation, and body condition) because these traits displayed differences between diets. Linear discriminant components were examined for correlations to variables and a two-way ANOVA was used to examine *population*, *diet*, and *population* × *diet* effects.

Results

Population comparison of initial traits

The three Atlantic salmon populations initially differed in body length, mass, condition, morphology, and skin pigmentation (Table 2). Sebago salmon were longer and heavier than LaHave and Saint-Jean salmon. Both Sebago and Saint-Jean salmon had higher condition than LaHave salmon, whereas LaHave salmon had a more streamlined body shape than the other two populations. For pigmentation, Saint-Jean salmon had yellower and whiter body regions than LaHave and Sebago salmon. Despite these phenotypic differences, the three Atlantic salmon populations did not initially differ in baseline red blood cells or plasma total thiamine concentrations (Table 2). Total thiamine concentrations derivatives – thiamine pyrophosphate, thiamine monophosphate, and free thiamine – are presented in ESM Table S2.

Thiamine concentrations

The baseline red blood cells total thiamine concentrations were not significantly different from that of salmon fed the control diet after 6 months ($t = -0.22$, df = 22, $p = 0.828$); however, they were significantly different and higher from those of the salmon fed the thiaminase diet at 6 months ($t = -6.22$, df = 45, $p < 0.001$; Table 2; Fig. 1). The total thiamine concentrations in plasma were nearly undetectable for the thiaminase diet (data not shown).

Significant diet but not population effects were also detected for red blood cells, white muscle, and liver total thiamine concentrations (Table 3; Fig. 1). Atlantic salmon fed the thiaminase diet had lower total thiamine concentrations in red blood cells, white muscle, and liver than those fed the control diet. We also detected a diet by population interaction for liver total thiamine concentrations with LaHave salmon having a larger decrease in liver total thiamine concentrations than Sebago and Saint-Jean salmon. The diet by population interaction for total thiamine concentrations in red blood cells and white muscle was not significant (Table 3; Fig. 1). Despite this latter finding, there were significant correlations between liver and red blood cells ($r = 0.75$, df = 22, $p < 0.001$) or white muscle ($r = 0.62$, df = 22, $p = 0.001$) total thiamine concentrations across all fish. There were also significant correlations between skin pigmentation (PC1) and total thiamine concentrations in red blood cells ($r = 0.54$, df = 22, $p = 0.006$), white muscle ($r = 0.73$, df = 22, $p < 0.001$), or liver ($r = 0.63$, df = 22, $p = 0.001$); PC1 largely reflected the amount of yellow in the skin pigmentation. There were no significant correlations between total thiamine concentrations in any of the tissues and body length, mass, condition, morphology, or PC2 of the skin pigmentation (Pearson correlations, $p > 0.16$ for all).

Diet effects on traits

Significant population, but not diet, effects were detected for the survival of sub-adult Atlantic salmon (Table 4; Fig. 2) with the LaHave population exhibiting lower survival than the Sebago and Saint-Jean populations independent of diet treatment. Significant population effects were also detected for changes in skin pigmentation; LaHave salmon had whiter body regions than Saint-Jean salmon with Sebago salmon being intermediate (Table 4; Fig. 2). There was a trend for all populations to have a less streamlined body shape and less yellow body pigmentation in the thiaminase diet. Significant diet effects were detected for the relative swimming performance of sub-adult Atlantic salmon; for all three populations, Atlantic salmon had lower relative

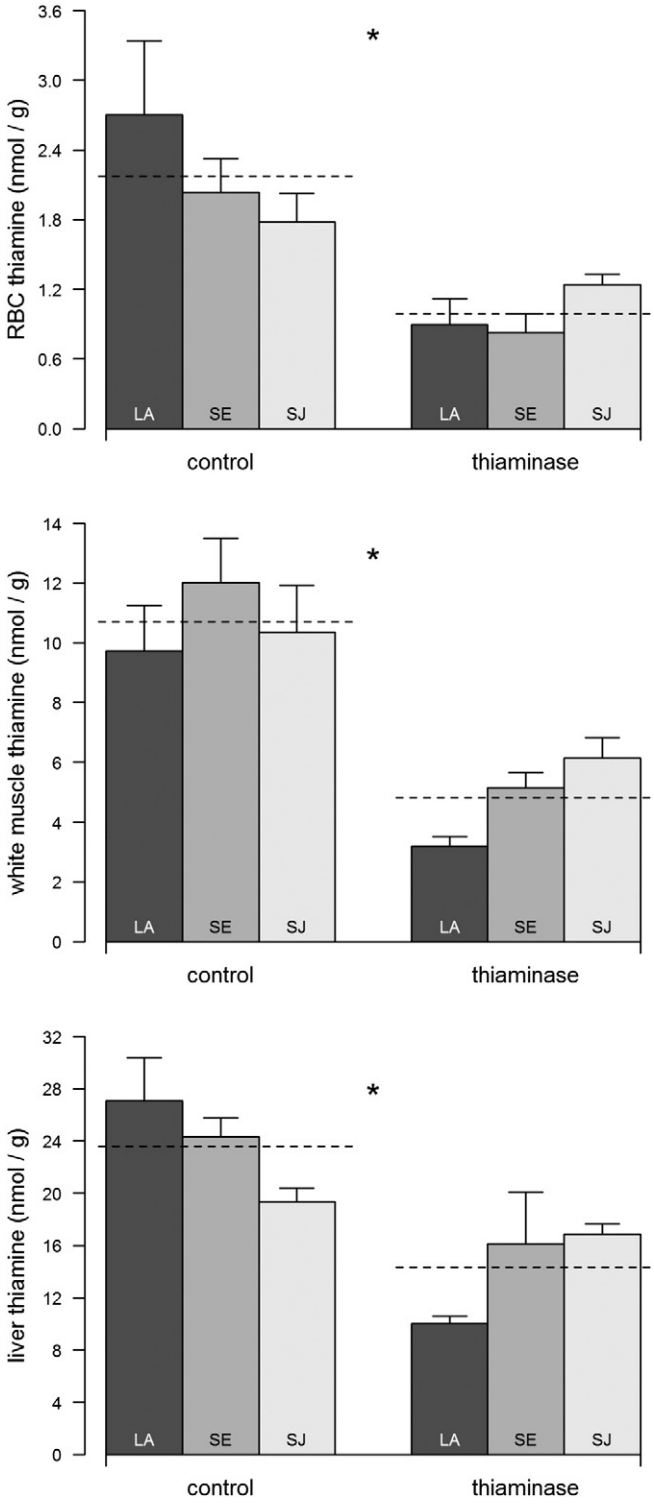

Fig. 1. Total thiamine concentrations in red blood cells (RBC), white muscle, and liver by diet for three populations of Atlantic salmon (*Salmo salar*). Displayed are means ± 1SE for diets. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. Dashed lines show the means for the diets across all populations. Asterisks indicate significant differences between diets ($p < 0.05$). Total thiamine concentrations derivatives, thiamine pyrophosphate, thiamine monophosphate, and free thiamine, are presented in the ESM Table S3.

swimming performance in the thiaminase compared to control diet (Table 4; Fig. 2).

Significant population but not diet effects were also detected for the thermal-unit growth coefficient of body length and mass and changes in body condition of sub-adult Atlantic salmon; although, there was a trend for Atlantic salmon to be in lower condition in the thiaminase than control diet, the differences were not significant (Table 4; Fig. 2). Independent of diet, LaHave and Sebago salmon had a higher thermal-unit growth coefficient of length and mass than Saint-Jean salmon. Sebago salmon maintained a better condition relative to LaHave and Saint-Jean salmon.

There were no significant relationships between changes in morphology and changes in skin pigmentation as measured by either PC1 or PC2 within each diet (Pearson correlations, $p > 0.12$ for all).

Table 3
Summary of model results comparing total thiamine concentrations of red blood cells, white muscle, and liver by diet across three populations of Atlantic salmon (*Salmo salar*). Linear mixed-effects results are given. Fixed effects were diet and population and a random effect was tank identity.

Tissue	df	*F*-statistic	*p*-value
Red blood cells			
population	2, 18	0.72	0.498
diet	1, 18	18.92	<0.001
population × diet	2, 18	1.87	0.195
White muscle			
population	2, 16.4	1.41	0.272
diet	1, 16.4	13.03	0.002
population × diet	2, 16.4	0.94	0.412
Liver			
population	2, 18	0.48	0.625
diet	1, 18	24.64	<0.001
population × diet	2, 18	5.30	0.015

Table 4
Summary of model results comparing survival, swimming performance, and body traits by diet across three populations of Atlantic salmon (*Salmo salar*). Displayed are binomial mixed-effects results for survival and linear mixed-effects results for the remaining traits. Changes in traits (final–initial values for individuals) were used for analyses of morphology, skin pigmentation, and condition. TGC is thermal-unit growth coefficient. Diet, population, and diet by population were treated as fixed effects; tank identity was treated as a random effect for the tests.

Trait	df	*F*-statistic	*p*-value
Survival			
Population	2, 277.9	42.99	<0.001
Diet	1, 4.0	0.00	1
Population × diet	2, 277.9	0.00	1
Relative swim performance			
Population	2, 223.1	0.31	0.732
Diet	1, 4.1	8.19	0.045
Population × diet	2, 223.1	0.29	0.750
Morphology (RW1)			
Population	2, 225.5	1.76	0.174
Diet	1, 225.5	3.45	0.064
Population × diet	2, 225.5	2.09	0.126
Pigmentation (PC1)			
Population	2, 224.1	2.18	0.115
Diet	1, 4.0	5.66	0.076
Population × diet	2, 224.1	0.02	0.977
Pigmentation (PC2)			
Population	2, 224.1	5.49	0.005
Diet	1, 4.0	0.13	0.741
Population × diet	2, 224.1	1.46	0.234
TGC of length			
Population	2, 212.4	53.94	<0.001
Diet	1, 4.1	0.54	0.503
Population × diet	2, 212.4	3.03	0.050
TGC of mass			
Population	2, 223.5	36.08	<0.001
Diet	1, 4.1	0.02	0.713
Population × diet	2, 223.5	2.34	0.015
Condition			
Population	2, 223.9	17.33	<0.001
Diet	1, 4.1	4.99	0.088
Population × diet	2, 223.9	0.06	0.938

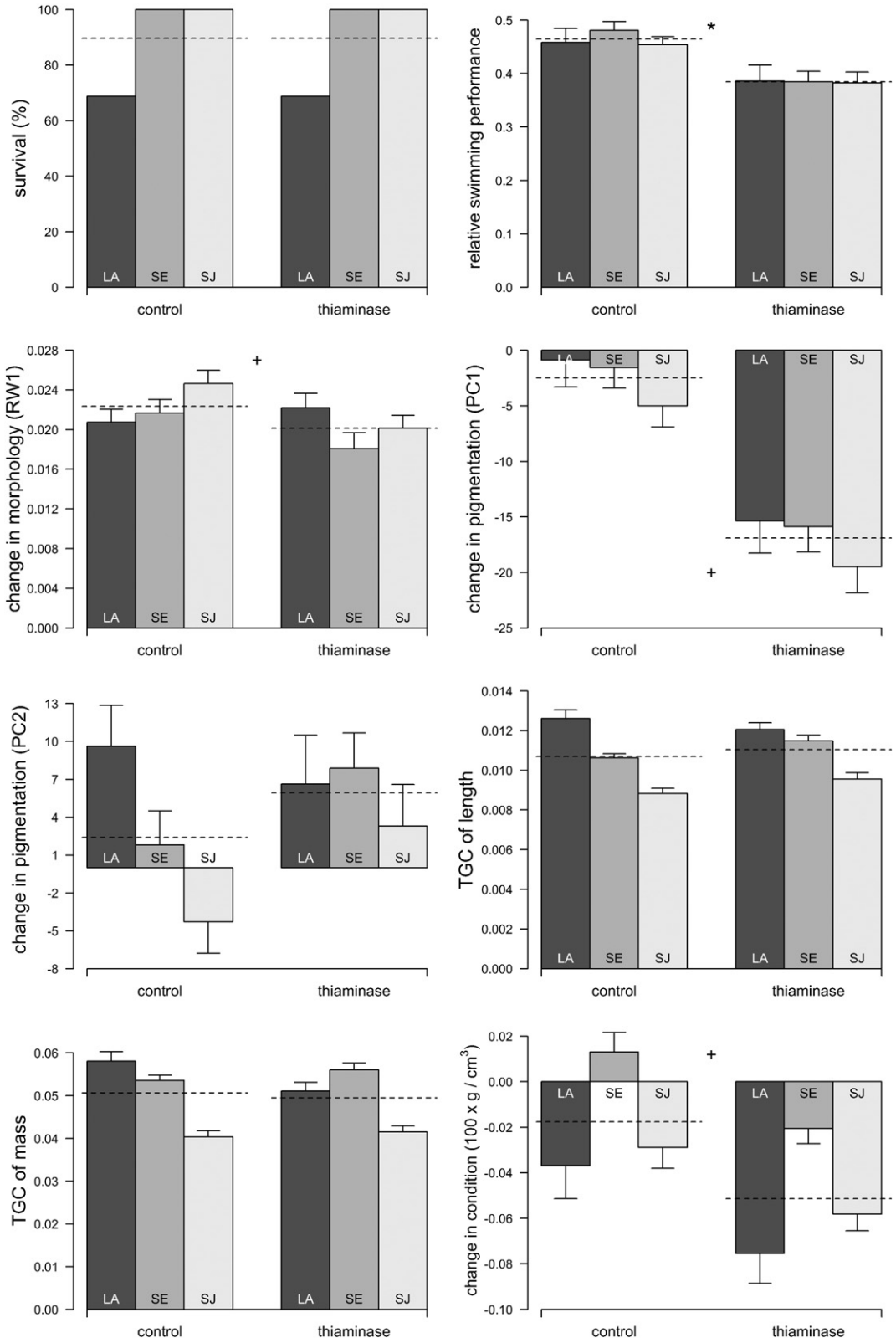

Fig. 2. Survival, swimming performance, and body traits by diet for three populations of Atlantic salmon (*Salmo salar*). Displayed are means ± 1SE for diets. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. TGC is thermal-unit growth coefficient. Dashed lines show the means for the diets across all populations. Asterisks indicate significant differences between diets ($p < 0.05$) and crosses indicate trends between diets ($p < 0.1$). For morphology, positive relative warp 1 (RW1) scores were associated with a more streamlined body shape. For skin pigmentation, principal component 1 (PC1) was positively related to the yellowness of the body regions, and principal component 2 (PC2) was positively related to the whiteness of the body regions.

There were also no significant relationships between relative swimming performance and changes in body condition or changes in skin pigmentation as measured by either PC1 or PC2 within each diet (Pearson correlations, $p > 0.10$ for all).

Linear discriminant analysis

We considered linear discriminant components 1 and 2 (LD1, LD2), which explained 80.1% and 12.8% of the variation among the six groups (two diets by three populations), respectively. LD1 was positively related to liver thiamine concentrations, relative swimming performance, and changes in skin pigmentation (PC1) and body condition; LD2 was positively related to relative swimming performance and changes in morphology, skin pigmentation (PC1), and body condition. The linear discriminant loadings are presented in ESM Table S4.

Significant population, diet, and population by diet effects were detected for LD1 (two-way ANOVA, $p < 0.001$ for all) and significant diet and population by diet effects were detected for LD2 (two-way ANOVA, $p < 0.002$ for both; Fig. 3). Generally, within the control diet, LaHave salmon had higher LD1 values but lower LD2 values than Sebago and Saint-Jean salmon. The thiaminase diet also affected LaHave salmon more so than the other two populations, resulting in the opposite pattern — within the thiaminase diet, LaHave salmon had lower LD1 values and higher LD2 values than Sebago and Saint-Jean salmon (Fig. 3).

Discussion

Atlantic salmon migrate into Lake Ontario as smolts and become sub-adults, remaining in the lake environment until they mature. During this time, high thiaminase-containing prey fishes may form a significant part of their diet due to the presence of alewife and rainbow smelt and near-absence of the historical prey fishes (Tillitt et al., 2005; Zajicek et al., 2005; Honeyfield et al., 2012). We fed sub-adult (two-year-old) Atlantic salmon from three populations an artificial diet that mimicked the current high thiaminase content of prey fishes (Honeyfield et al., 2005) in an 8 month trial. These sub-adult Atlantic salmon had lower thiamine concentrations in tissues and lower swimming performance, but showed no change in survival or growth. This result is in contrast to Morito et al. (1986), who observed juvenile rainbow trout (*O. mykiss*) mortality after about 3 months of consuming low thiamine content diets (thiamine content of <2 mg/kg or 6.5 nmol/g in feed).

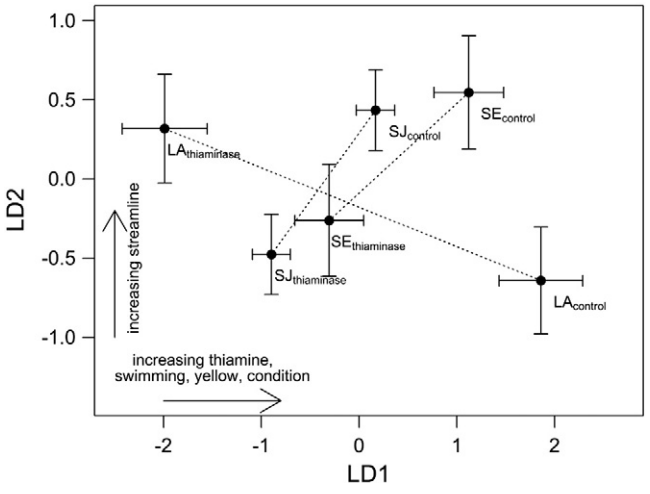

Fig. 3. Canonical plot of the first two linear discriminant components (LD1, LD2) separating six groups (two diets by three populations) for Atlantic salmon (*Salmo salar*). Displayed are the centroids with 95% confidence intervals for the groups. Population symbols are LA = LaHave salmon, SE = Sebago salmon, SJ = Saint-Jean salmon. Dashed lines connect the two diet centroids for each population.

On the other hand, adult lake trout took more than 2 years on a similar bacterial thiaminase diet to ours to show an effect of thiamine deficiency (Honeyfield et al., 2005). Atlantic salmon thus appear to be able to tolerate a high thiaminase diet for at least 8 months without showing an effect on survival. However, there were trends for lower body condition, a less streamlined body shape, and less yellow body pigmentation when fed the thiaminase diet. These latter changes may be important because they have been shown to negatively impact Atlantic salmon survival (Taylor and McPhail, 1985; Taylor, 1991; Sutton et al., 2000; Garcia de Leaniz et al., 2007). In addition, Atlantic salmon survival may be reduced in the wild when exposed to pathogens. A thiamine deficiency has been associated with a decline in immunity components for lake trout (Ottinger et al., 2012, 2014). A longer-term study is warranted to investigate survival in a natural environment and across the entire lake-phase life stage (2 to 4 years).

Although there was no effect of the thiaminase diet on survival, there were several indicators of thiamine deficiency in the Atlantic salmon. We detected a decline in the swimming performance of sub-adult Atlantic salmon fed the thiaminase diet. Morito et al. (1986) similarly found that the first signs of thiamine deficiency in the juvenile rainbow trout were changes in swimming behavior (also see Amcoff et al., 1998; Brown et al., 2005; Fitzsimons et al., 2005). Thiamine is important for energy production, as it is enables pyruvate to enter the citric acid cycle to produce ATP (Morito et al., 1986; Koski et al., 2005). In addition, plasma lactate can increase as a result of thiamine deficiency in juvenile rainbow trout, which affects muscle performance (Morito et al., 1986; Fitzsimons et al., 2012). Because swimming is energetically costly, the Atlantic salmon fed the high thiaminase diet in the present study may have had lower swimming performance due to a reduction in ATP production or a build-up of lactate caused by a thiamine deficiency, although neither lactate nor ATP production were measured in this study.

Other indicators of a thiamine deficiency may be changes in body appearance. We found a trend suggesting that sub-adult Atlantic salmon have less yellow body pigmentation when fed a thiaminase diet. Yellow pigmentation can be related to the amount of the carotenoid idoxanthin, a metabolite of astaxanthin (Hatlen et al., 1998). These data would suggest that thiamine deficiency may have been a contributing factor in the low astaxanthin observed in Baltic salmon with the deficiency (Pettersson and Lignell, 1999). Because thiamine can act as an anti-oxidant (Lukienko et al., 2000), a thiamine deficiency may cause oxidative stress in the bodies of Atlantic salmon, resulting in the decline of other anti-oxidants such as astaxanthin (Pettersson and Lignell, 1999). Body de-pigmentation may also be related to a lack of essential fatty acids (Leclercq et al., 2010). The lower liver thiamine concentration that we detected in the present study has been previously associated with lower liver lipid content in Chinook salmon (*O. tshawytscha*) (Honeyfield et al., 2008). Juvenile Chinook salmon fed diets lacking linolein have decreased skin pigmentation because of a lower number of melanin-producing cells in the skin (Nicolaides and Woodall, 1962) and we also found a trend for lower condition and a trend for a less streamlined body shape in fish fed the thiaminase diet. But, a less streamlined body shape could also be a developmental effect related to reduced swimming activity (Taylor and McPhail, 1985). Nevertheless, our data suggest that changes in all of skin pigmentation, body shape, and body condition could serve as indicators of a thiamine deficiency in Atlantic salmon.

Although all three populations that we studied had similar responses to the thiaminase diet, we found that the LaHave population had a greater reduction in thiamine concentrations in the liver relative to the Sebago and Saint-Jean populations. The liver is a storage tissue for thiamine (Depeint et al., 2006), so our results suggest that fish from the LaHave population may be using more of their thiamine stores than the Sebago and Saint-Jean populations. We also found that the Sebago population was able to maintain better condition relative to the LaHave and Saint-Jean populations when fed a high thiaminase

diet. These results do not appear to reflect competitive differences among the populations. Similar growth and condition differences among the populations have been observed when the populations were reared separately as juvenile and adults in common-garden environments (Houde et al., 2015a,b; Houde et al. unpublished data). Instead, we predicted that freshwater resident populations, such as the Sebago and Saint-Jean populations, should have adaptations to higher thiaminase in their diets from consuming primarily rainbow smelt (Dimond and Smitka, 2005) relative to anadromous populations, such as the LaHave population, that consume a more diverse diet (Rikardsen and Dempson, 2011). Our results suggest genetic differences in thiaminase tolerance among populations because the populations were reared in the same environment. Given that the LaHave population has been in captive breeding for longer than the Sebago and Saint-Jean populations (3 generations of captive breeding for the LaHave population vs. single-pair matings using wild fish for the other two populations), the results from the present study might also reflect selection relaxation for tolerance to thiaminase resulting from several generations of consuming a commercial diet that lacks any thiaminase.

Finally, our results have implications for the restoration efforts of an extirpated species. The restoration of Atlantic salmon into Lake Ontario may be impeded by a diet of high thiaminase-containing prey fishes (Dimond and Smitka, 2005; COSEWIC, 2006, 2010). We found that a thiaminase diet mimicking a current Lake Ontario diet of prey fishes negatively impacted the swimming performance and body appearance of sub-adult Atlantic salmon relative to a control diet that mimicked a more historical diet of low thiaminase-containing prey fishes. Although we found no direct effect of the diets on survival during the 8 months trial, the Atlantic salmon fed a high thiaminase diet had less total thiamine in tissues, tended to be in lower condition and had a less streamlined body shape, all of which are indicators of lower potential survival (e.g. Taylor and McPhail, 1985; Sutton et al., 2000; Taylor, 1991; Garcia de Leaniz et al., 2007). The restoration of native prey fishes, containing lower thiaminase, may have to be considered for Lake Ontario to increase the health of salmonids in the lake (also see Fitzsimons and O'Gorman, 2006). As the Sebago and Saint-Jean populations retained more thiamine in their tissues when fed the high thiaminase diet, they may have higher resistance to thiamine deficiency under natural conditions than the LaHave population. If so, this may have a significant effect on adult survival and recruitment in Lake Ontario, with implications for the restoration effort. Considering other populations of Atlantic salmon with high resistance to thiamine depletion may provide additional alternatives to increase the success of Atlantic salmon restoration into Lake Ontario.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jglr.2015.06.009.

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