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ARTICLE

Effects of a low-thiamine diet on reproductive traits in three populations of Atlantic salmon targeted for reintroduction into Lake Ontario[1]

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Abstract: Exotic prey fishes that have high thiaminase levels are associated with a thiamine deficiency and reduced fitness in many salmonids. If sensitivity to low thiamine availability differs among the three Atlantic salmon (*Salmo salar*) populations targeted for reintroduction into Lake Ontario, this could substantially influence their performance. We quantified the effects of a low-thiamine diet and a control diet on tissue thiamine concentrations, survival, growth rate, and reproductive traits (sperm and egg quality) in Atlantic salmon from the three candidate source populations. Fish that consumed the low-thiamine diet had comparable growth rates but lower survival and lower muscle thiamine concentrations (26 nmol·g^{-1}) than control fish (34 nmol·g^{-1}). Sperm count, velocity, motility, and longevity did not differ based on diet. Embryo survival was lower for females fed the low-thiamine diet, and the low-thiamine diet was associated with significantly lower egg thiamine concentrations (11 versus 17 nmol·g^{-1}). The effects of the low-thiamine diet did not differ among the tested populations, which suggests that source population selection is unlikely to fully overcome this potential challenge for re-establishing wild populations.

Résumé : Des poissons proies exotiques qui présentent de fortes concentrations de thiaminase sont associés à une déficience en thiamine et une réduction de l'aptitude chez de nombreux salmonidés. Si la sensibilité à une faible disponibilité de thiamine varie entre les trois populations de saumons atlantiques (*Salmo salar*) ciblées pour des efforts de réintroduction dans le lac Ontario, cela pourrait avoir une incidence significative sur leur performance. Nous avons quantifié les effets d'un régime alimentaire faible en thiamine et d'un régime alimentaire témoin sur les concentrations de thiamine dans les tissus, la survie, le taux de croissance et des caractères associés à la reproduction (qualité du sperme et des œufs) chez des saumons atlantiques des trois populations sources candidates. Les poissons au régime alimentaire faible en thiamine présentaient des taux de croissance semblables, mais des taux de survie et des concentrations de thiamine dans les muscles (26 nmol·g^{-1}) plus faibles que les poissons témoins (34 nmol·g^{-1}). Le nombre, la vitesse, la motilité et la longévité des spermatozoïdes ne variaient pas en fonction de l'alimentation. La survie des embryons était plus faible pour les femelles au régime alimentaire faible en thiamine, et ce dernier était associé à des concentrations de thiamine dans les œufs significativement plus faibles (11 contre 17 nmol·g^{-1}). Les effets du régime alimentaire faible en thiamine ne variaient pas entre les populations étudiées, ce qui porte à croire qu'il est peu probable que le choix des populations soit suffisant pour surmonter complètement cette difficulté potentielle du rétablissement de populations sauvages. [Traduit par la Rédaction]

Introduction

Atlantic salmon (*Salmo salar*) were once abundant in Lake Ontario, but were extirpated by 1898 due to habitat degradation and over-exploitation (Crawford 2001). The current efforts to reintroduce Atlantic salmon into Lake Ontario began with small-scale releases starting in 1986, which were followed by increasing stocking intensity over the last decade. However, these efforts have not yet produced a self-sustaining population. One potential challenge for the restoration of Atlantic salmon is that the prey fish community in Lake Ontario has changed considerably over the past century (Crawford 2001; Morrison 2019). Atlantic salmon in Lake Ontario historically fed primarily on lake herring (cisco, *Coregonus artedi*) and bloater (*Coregonus hoyi*), but these prey fishes have largely been replaced by introduced alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) (Beeton 2002; Morrison 2019). Relative to the native prey fishes, these introduced fishes have much higher levels of the enzyme thiaminase, which breaks down thiamine (vitamin B1) (Tillitt et al. 2005). Consequently, the ability of Atlantic salmon to tolerate novel prey fishes that contain high

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levels of thiaminase may be an important determinant of the success of the restoration program.

High dietary thiaminase has been linked to a thiamine deficiency in many animals (Harder et al. 2018). Thiamine has a critical role in metabolic function and neural development, leading to many well-documented effects of thiamine deficiency (Depeint et al. 2006). In multiple salmonids, including Atlantic salmon, thiamine deficiency has been linked to low egg survival prior to the onset of exogenous feeding and to reduced activity, abnormal movement patterns, and loss of equilibrium during multiple life stages (Fisher et al. 1995, 1996; Fitzsimons et al. 1995, 2007; Ketola et al. 2000, 2008; Brown et al. 2005). Young salmonids appear most sensitive to the effects of thiamine deficiency, probably because of their small thiamine stores and the importance of thiamine during development (Morito et al. 1986; Ketola et al. 2008). The reproductive phase is thus critically important because females that are deficient in thiamine cannot provision their eggs with sufficient thiamine. Interestingly, a study of Atlantic salmon found that the concentration of thiamine was five times higher in milt (sperm and seminal plasma) than in eggs, although the purpose of thiamine in milt was unclear, as there is little transfer of thiamine from the male to the offspring (Koski 2002). Instead, thiamine might affect sperm performance or fertilization success, although neither effect has been examined before.

We conducted this study to explore differences in tolerance for high-thiaminase prey among populations. A study of Atlantic salmon from Lake Champlain identified more than 1000 genes whose expression was associated with family-level variation in survival on a thiamine-deficient diet, suggesting that there may be additive genetic variation underlying tolerance to thiamine deficiency and consequently that this trait may have the potential to evolve in response to dietary thiamine availability (Harder et al. 2020). Three North American source populations of Atlantic salmon (LaHave River, Nova Scotia; Lac St. Jean, Quebec; and Sebago Lake, Maine) are being used for reintroduction efforts into Lake Ontario (Dimond and Smitka 2005). The Sebago and St. Jean populations are potadromous (freshwater lake migrating) and are native to lakes that contain rainbow smelt, which have high thiaminase levels (Dimond and Smitka 2005). Conversely, the LaHave population, the focus of the initial restoration efforts in Lake Ontario, is anadromous (ocean migrating) and has a diverse diet that includes capelin (*Mallotus villosus*), sand eels (Ammodytidae), krill (Euphausiacea), and amphipods (Amphipoda) (Rikardsen and Dempson 2011), which typically have lower thiaminase levels than freshwater prey fishes (Neilands 1947; Ceh et al. 1964). Consequently, it is possible that the response to dietary thiaminase differs among these populations as a result of local adaptation, albeit dietary thiaminase levels have not been directly measured for these populations. A previous study examined the effects of dietary thiaminase in subadult (2-year-old) Atlantic salmon from these three populations after they had consumed either a control diet or an experimental diet that included bacterial thiaminase for 8 months (Houde et al. 2015*b*). Across the three populations, the thiaminase diet led to a significant decline in tissue thiamine concentrations and swimming performance, but no difference in growth rate or survival. The thiaminase diet was associated with a greater decline in tissue thiamine concentrations in the LaHave population than in the Sebago and St. Jean populations (Houde et al. 2015*b*). However, the extent that thiaminase tolerance varies among populations during the critical reproductive phase has never been measured using a common experimental procedure.

Here we build on this earlier research by examining the critical reproductive phase during which the most serious effects of thiamine deficiency are expected. We hypothesized that the three populations of Atlantic salmon would differ in reproductive traits following exposure to low-thiamine and control diets and predicted that the Sebago and St. Jean populations would tolerate a low-thiamine diet better than the LaHave population due to pre-existing adaptations in their ancestral habitats. We use these data to explore the importance of using tolerance to a high-thiaminase diet as a criterion to inform broodstock selection for the Lake Ontario Atlantic salmon restoration program.

Methods

Ethics statement

Experimental methods used in this study were approved by the Western University Animal Care Committee (Protocol 2010–214). Animal care and husbandry were conducted in accordance with guidelines from the Canadian Council for Animal Care.

Experimental diets

Two diets were created: (*i*) a low-thiamine diet that included thiaminase-producing bacteria and (*ii*) a control diet that did not include thiaminase-producing bacteria. The specific composition and preparation of these diets followed Honeyfield et al. (2005) and Houde et al. (2015*b*). Both diets contained the same formulation of ingredients (see online Supplementary Table S1[2] for composition), with the exception that the low-thiamine diet included the bacteria *Paenibacillus thiaminolyticus* — isolated from Lake Michigan alewives by Honeyfield et al. (2002) — while the control diet did not include *P. thiaminolyticus*. A *P. thiaminolyticus* solution was prepared by adding 3 mL of the bacterial culture described in Houde et al. (2015*b*) to 1 L of liquid media (1.0 $g{\cdot}L^{-1}$ yeast extract and 8.0 $g{\cdot}L^{-1}$ Difco nutrient broth, Becton Dickinson, Mississauga, Ontario) and incubating the solution for 4 days at 37 °C. Dry ingredients were thoroughly mixed, and then 300 mL of the *P. thiaminolyticus* solution (low-thiamine diet) or water (control diet) were added per kilogram of dry ingredients. After 24 h, water was added until the diets had a dough-like consistency, and the diets were cold-pressed into pellets using a screw die. The resulting pellets were dried for 12 h in a food dehydrator. Diets were prepared in multiple batches and stored at –20 °C after drying. No analytical measurements of thiaminase activity were performed. Thiamine concentrations were measured in four replicates for each diet as described in the "Thiamine analysis" section below. Both diets contained negligible concentrations of thiamine monophosphate (0 $nmol{\cdot}g^{-1}$). Thiamine diphosphate concentrations were similar in the control diet (6.7 $\pm$ 1.0 $nmol{\cdot}g^{-1}$; mean $\pm$ SD) and low-thiamine diet (7.1 $\pm$ 1.7 $nmol{\cdot}g^{-1}$). The concentration of free thiamine was approximately three times higher in the control diet (13.1 $\pm$ 1.3 $nmol{\cdot}g^{-1}$) than in the low-thiamine diet (4.2 $\pm$ 1.0 $nmol{\cdot}g^{-1}$).

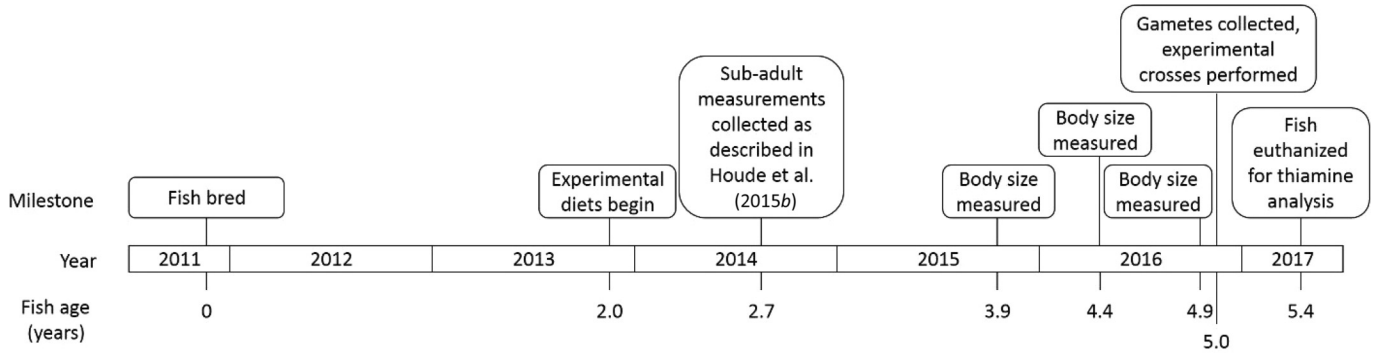
Experimental fish

Three Atlantic salmon populations were examined in this study: the Sebago Lake population from Maine, USA (43.8°N, 70.5°W), the Lac St. Jean population from Quebec, Canada (48.6°N, 72.0°W), and the LaHave River population from Nova Scotia, Canada (44.3°N, 64.4°W). The LaHave population has been maintained in the Ontario Ministry of Natural Resources and Forestry (OMNRF) hatchery system since 1995, the Sebago population since 2006, and the St. Jean population since 2007 (OMNRF 2011; Houde et al. 2015*b*).

The fish used in this study were produced in November 2011 at the OMNRF Harwood Fish Culture Station (see Fig. 1 for experimental timeline). Additional detail about the care and rearing of these fish through the subadult stage is provided in Houde et al. (2015*b*). Briefly, the fish were transferred to the OMNRF Codrington Fisheries Research Facility in spring 2012. There the salmon were held separately by population and experienced a natural

[2]Supplementary data are available with the article at http://doi.org/10.1139/cjfas-2019-0379.

Fig. 1. Timeline showing the major experimental milestones and the age of the fish at each time.



photoperiod and seasonal temperature variation (water was supplied by Marsh Creek). The salmon were initially fed a commercial diet (Corey Aquafeeds, Fredericton, New Brunswick, Canada). In October 2013, 96 salmon from each population were anaesthetized with buffered MS-222 (tricaine methanesulfonate, 0.1 g·L^{-1}; Syndel, Nanaimo, British Columbia, Canada) and marked with uniquely numbered vinyl anchor tags (Floy Tag & Mfg. Inc., Seattle, Washington, USA). The fish were then divided equally among six tanks (n = 48 fish per tank; 16 fish from each population per tank). Over the next four weeks, the salmon were transitioned to the experimental diets, with three tanks receiving the low-thiamine diet and three tanks receiving the control diet. Fish were fed at a rate of 1% of body mass per day and maintained on these diets for the entirety of the experimental period.

We assessed the characteristics of the fish as they began to reach sexual maturity, with measurements beginning in October 2015. At that time, 35% of the surviving fish had lost the anchor tag that was used to identify their population origin. Consequently, all fish were anesthetized and marked with a uniquely numbered passive integrated transponder (PIT) tag inserted into the abdominal cavity. Anchor tags were then removed if present and used to identify population origin (n = 93). For fish that had lost their anchor tag (n = 49), a fin clip was collected for genetic determination of population origin by the OMNRF DNA lab at Trent University (Peterborough, Ontario) using 12 microsatellite loci (Houde et al. 2016). These unknown individuals were assigned to one of the three populations using Structure 2.1 (Pritchard et al. 2000) based on reference samples of at least 100 broodstock individuals from each population (see Supplementary Fig. S1[2] for additional details of the genetic assignments). All of the subsequent measurements could thus be attributed to an individual of known population origin based on its numbered PIT tag.

Body mass was measured at three times: October 2015, April 2016, and October 2016. Specific growth rate was calculated based on mass for the intervals between each pair of measurements as the natural log of mass on the second measurement date minus the natural log of mass on the first measurement date and then expressed as a percent change in body mass per day (i.e., by dividing by the number of days in the measurement interval). Body mass was thus calculated as an aggregate measure based on all fish, whereas specific growth rate was calculated as an individual-level measure using the identity of each fish.

Reproductive characteristics

Gametes were collected from mature fish between 28 October and 18 November 2016 after the fish had been fed the experimental diets for 3 years. When fish reached sexual maturity — males freely expressed milt, females had free-flowing eggs within the abdominal cavity — they were anaesthetized and gametes were expelled by gently pressing on the abdomen, taking care to avoid contamination by water, urine, or faeces. The sex of each fish was determined based on the expression of gametes at this time, with individuals classified as a mature male, a mature female, or as an immature fish of unknown sex. Egg diameter was measured for each mature female using ImageJ version 1.51 (Schneider et al. 2012) as the mean diameter of 15 eggs. A subsample of unfertilized eggs was immediately frozen at –20 °C for thiamine analysis. There were few mature females from the LaHave population, so this population was excluded from experimental components that required eggs.

Sperm were stored on ice under air for up to 6 hours before measuring sperm activity. The duration of sperm storage was not recorded for individual fish, but short-term storage (up to 24 hours) under these conditions has been associated with minimal change in sperm viability or motility across multiple salmonids (Bencic et al. 2000). Sperm from each male were activated by combining 0.2 μL of milt with 10 μL of water. Milt was placed on a 2X-CEL glass slide (Hamilton Thorne Biosciences, Massachusetts, USA) with a cover slip. Sperm activity was recorded using a digital black and white video camera (XC-ST50, Sony, Japan) connected to an external phase-contrast microscope (CX41 Olympus, Melville, New York, USA) with a 10× magnification negative-phase objective. During observations, a heat exchanger maintained the sperm at the same temperature as the hatchery water (7 °C). Following Johnson et al. (2013), HTM-CEROS sperm analysis software (version 12, Hamilton Thorne Biosciences, Beverly, Massachusetts, USA) was used to measure sperm characteristics based on 60 frames of video at 5 and 10 s postactivation. We first measured sperm count as the average number of sperm cells observed in the video (minimum sperm cell size = 3 pixels, minimum contrast = 11). Sperm motility was measured as the proportion of the sperm cells that were progressively motile. Sperm velocity was measured as the average velocity of the sperm cell over its smoothed cell path. For each sperm characteristic, the value for a male was calculated as an average based on all sperm cells at each activation time. The measured sperm characteristics are all positively associated with overall sperm performance and fertilization success (Gage et al. 2004; Rudolfsen et al. 2008; Pizzari and Parker 2009).

Sperm and egg viability were quantified using experimental crosses. The eggs of each mature female were first divided equally into four replicates of ~200 eggs. Each replicate was fertilized using the milt of a single male from the same population as the female, with two males from the high-thiaminase diet and two males from the control diet. The same four males were used to fertilize the eggs of all females from a population. Fertilizations were performed by adding ~1 mL of milt to each egg replicate and then adding stream water to activate the milt. After 1 min the eggs were rinsed with stream water and immersed for 30 min in a disinfecting bath of 0.5% Ovadine (Syndel, Nanaimo, British

Columbia, Canada). The eggs from each cross were then transferred to separate sections in flow-through Heath incubation trays. Dead or unfertilized eggs and alevins were removed and recorded every 2 days until the swim-up stage (i.e., immediately before complete yolk absorption). At this time, all alevins were euthanized with an overdose of MS-222. Survival was calculated separately for the egg stage, which spanned fertilization to hatching, and for the alevin stage, which spanned hatching to swim-up.

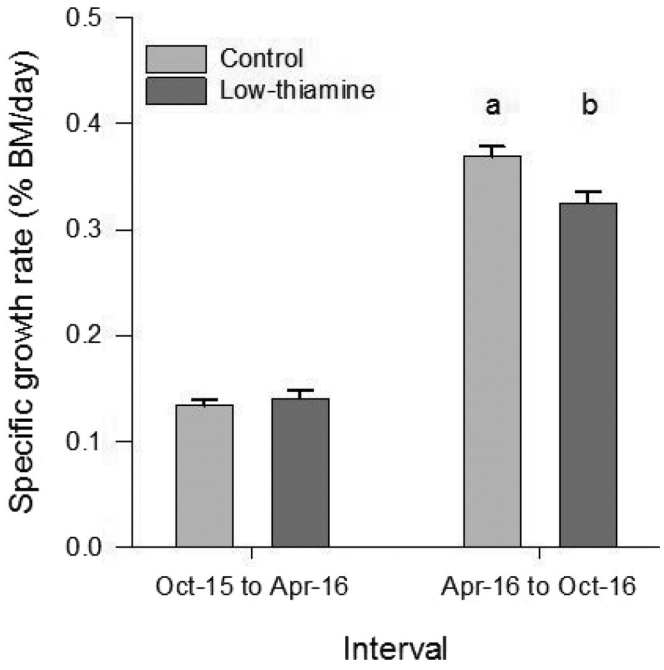
Thiamine analysis

All surviving adult salmon were euthanized with an overdose of MS-222 in April 2017, and a sample of muscle tissue was collected. Thiamine concentrations were then measured in two tissues: adult muscle (spring 2017) and unfertilized eggs (fall 2016). Some egg samples were damaged during transport, so offspring thiamine measurements are available for only a subset of the mature females in our study. All thiamine measurements were performed in the laboratory of Jacques Rinchard at the College at Brockport, State University of New York, and followed the methods of Brown et al. (1998) and Futia et al. (2017). First, 1.2–3.9 g of tissue was mixed with tricholoracetic acid, boiled in a water bath for 10 min, and centrifuged. Extracts were then washed with ethyl acetate and hexane and oxidized with sodium hydroxide and potassium ferricyanide. After extraction, high-performance liquid chromatography (HPLC) was used to determine thiamine concentrations. The system included a delivery pump, automatic sample injector, Hamilton PRP-HI column (150 mm × 4.1 mm; 5 μm mesh size) with attached guard column (25 mm × 2.3 mm; 12 to 20 mm mesh size), and a fluorometric detector (375 nm excitation wavelength and 433 nm emission wavelength). Standard curves using analytical standards of free thiamine, thiamine monophosphate, and thiamine pyrophosphate were prepared fresh daily and used to convert HPLC fluorescence values into concentrations. The concentrations of the three vitamers were then summed to determine the total thiamine concentration. A subset of the samples (n = 48) were analyzed in duplicate and showed high repeatability in the measurement of thiamine concentrations (coefficient of variation = 5.0%).

Statistical analysis

All analyses were performed in JMP (version 4.04, SAS Institute Inc., Cary, North Carolina). We first used a χ^2 test to compare survival until October 2015 between the two diets, with a second χ^2 test used to compare survival among the three populations. We next compared body mass, specific growth rate, and total thiamine concentration in muscle tissue using linear models that included sex (mature male, mature female, immature), diet, population, and the diet × population interaction as factors. Sex was included in the initial model for total thiamine concentration in muscle tissue, but the factor was nonsignificant and was removed from the final model. We examined sperm count, sperm motility, sperm velocity, and egg thiamine concentrations using linear models that included diet, population, and the diet × population interaction. The relationships between muscle thiamine concentration and sperm characteristics were examined using linear models that included population as a random factor. To examine survivorship in the experimental crosses, survivorship was first calculated as percent survival by family during the egg and alevin stages of development. Families that had 100% mortality during the egg stage were thus excluded from analyses of survivorship during the alevin stage. Survivorship was then analyzed separately for the egg and alevin stages using linear models that included paternal diet, maternal diet, and population as factors. All higher-order interaction terms were included in the initial survivorship models and then removed if nonsignificant.

Fig. 2. Specific growth rate in Atlantic salmon (*Salmo salar*) that were fed a control or low-thiamine diet. Specific growth rate is expressed as the percent change in body mass (BM) per day and was measured from October 2015 to April 2016 and from April 2016 to October 2016. Data are plotted as mean + SE. Statistical analyses were performed separately for the two intervals, with different letters above the bars used to indicate significant ($p < 0.05$) differences between groups.



Results

Adult metrics

At the first sampling point in October 2015, a significantly greater proportion of the fish fed the control diet were alive (56%, n = 80 of 144) relative to the fish fed the low-thiamine diet (42%, n = 61 of 144; χ^2 = 5.01, p = 0.025). Independent of diet, the proportion of the fish that were alive was significantly lower for the LaHave population (17%, 16 of 96) than for the Sebago (65%, 62 of 96) or St. Jean populations (66%, 63 of 96; χ^2 = 60.1, $p < 0.001$). There was little mortality after the first sampling point (see Supplementary Table S2[2]).

Body mass did not differ significantly based on diet in October 2015 ($F_{[1,134]}$ = 1.99, p = 0.16), April 2016 ($F_{[1,124]}$ = 1.96, p = 0.16), or October 2016 ($F_{[1,123]}$ = 0.01, p = 0.93; Supplementary Table S2[2]). There were significant differences in body mass among populations in October 2015 ($F_{[2,134]}$ = 12.31, $p < 0.001$), April 2016 ($F_{[2,124]}$ = 7.09, p = 0.001), and October 2016 ($F_{[2,123]}$ = 24.1, $p < 0.001$; Supplementary Table S2[2]). Fish from the Sebago population were consistently heavier than fish from the St. Jean population. Body mass did not show a significant interaction between population and diet in October 2015 ($F_{[2,134]}$ = 1.78, p = 0.17), April 2016 ($F_{[2,124]}$ = 1.96, p = 0.15), or October 2016 ($F_{[2,123]}$ = 2.08, p = 0.13; Supplementary Table S2[2]). Body mass did not differ significantly based on sex in October 2015 ($F_{[2,134]}$ = 1.31, p = 0.27), but females were significantly larger than immature fish in April 2016 ($F_{[2,124]}$ = 6.28, p = 0.003) and October 2016 ($F_{[2,123]}$ = 4.96, p = 0.008; Supplementary Table S2[2]).

Specific growth rate did not differ significantly based on diet for the interval from October 2015 to April 2016 ($F_{[1,124]}$ = 0.31, p = 0.58; Fig. 2). However, from April 2016 to October 2016, specific

Table 1. Thiamine concentrations in the muscle of Atlantic salmon (*Salmo salar*) from three populations that were fed a control or low-thiamine diet.

	Control diet (C)			Low-thiamine diet (L)			Significance	
Measure	LaHave (LA)	Sebago (SE)	St. Jean (SJ)	LaHave (LA)	Sebago (SE)	St. Jean (SJ)	Diet	Population
Number	10	23	25	4	20	19		
Free thiamine	0.8±0.3	0.2±0.3	0.2±0.1	0.2±0.2	0.1±0.1	0.1±0.1	C > L	LA > SE = SJ
Thiamine monophosphate	3.1±0.5	2.0±0.8	1.4±0.4	1.8±0.3	1.5±0.5	1.0±0.3	C > L	LA > SE > SJ
Thiamine pyrophosphate	39.9±8.6	35.0±8.4	25.2±4.7	34.1±6.9	27.8±4.9	18.6±3.7	C > L	LA > SE > SJ
Total thiamine	43.8±9.2	37.1±9.3	26.8±5.2	36.1±6.7	29.4±5.3	19.7±4.0	C > L	LA > SE > SJ

Note: Concentrations are presented as mean ± SD and are measured in nanomoles thiamine per gram tissue. Data comprise the number of individuals measured; and the concentrations of free thiamine, thiamine monophosphate, and thiamine pyrophosphate; and the total across the three thiamine vitamers. Significant ($p < 0.05$) differences among diets or populations are indicated in the last two columns, showing the groups that differed.

Table 2. Sperm characteristics in Atlantic salmon (*Salmo salar*) from three populations that were fed a control or low-thiamine diet.

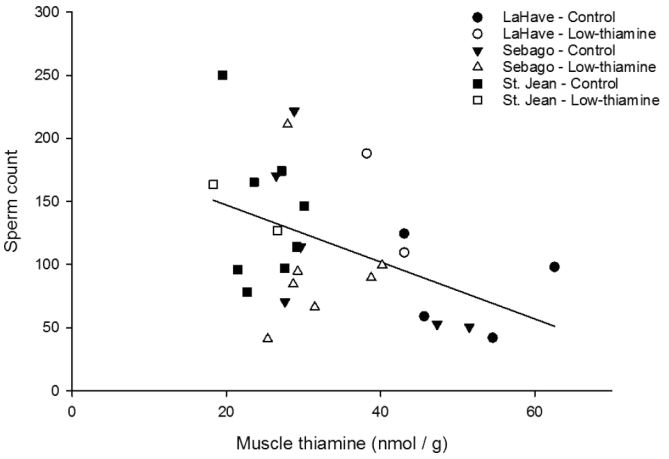
	Control diet (C)			Low-thiamine diet (L)			Significance	
Measure	LaHave (LA)	Sebago (SE)	St. Jean (SJ)	LaHave (LA)	Sebago (SE)	St. Jean (SJ)	Diet	Population
Number	4	6	10	2	7	3		
Sperm count	81±37	113±70	132±65	149±56	98±54	121±45		
Sperm motility: 5 s	75±13	79±13	72±32	84±3	74±12	75±22		
Sperm motility: 10 s	50±22	64±10	58±20	76±1	62±26	69±23		
Sperm velocity: 5 s	107±5	73±13	89±44	120±1	91±25	102±15		LA > SE
Sperm velocity: 10 s	66±11	60±6	61±13	60±3	76±16	83±13	L > C	

Note: Values are presented as mean ± SD. Data comprise the number of males whose sperm were measured, sperm count, sperm motility, and sperm velocity. Sperm motility and sperm velocity were measured at 5 and 10 s postactivation. Significant ($p < 0.05$) differences among diets or populations are indicated in the last two columns, showing the groups that differed. Sperm count was measured as the average number of sperm cells observed in the video, sperm motility as the percentage of the sperm cells that were motile, and sperm velocity as the average velocity of the sperm cells over a smoothed cell path ($\mu m \cdot s^{-1}$). Owing to a video recording failure, no measurements of sperm activity at 5 s postactivation were available for two fish fed the control diet from the St. Jean population.

growth rate was significantly higher for the fish fed the control diet than for fish fed the low-thiamine diet ($F_{[1,123]} = 6.02$, $p = 0.016$; Fig. 2). Independent of diet, there were significant differences in specific growth rate among populations for the interval from October 2015 to April 2016 ($F_{[2,124]} = 7.47$, $p = 0.001$; Supplementary Table S2[2]), with growth rate significantly higher for the LaHave population than the Sebago and St. Jean populations. There were also significant differences in specific growth rate among populations for the interval from April 2016 to October 2016 ($F_{[2,123]} = 24.36$, $p < 0.001$; Supplementary Table S2[2]), with growth rate significantly higher for the LaHave population than for both other populations and significantly higher for Sebago than for St. Jean. Specific growth rate did not show a significant interaction between population and diet for either the interval from October 2015 to April 2016 ($F_{[2,124]} = 0.24$, $p = 0.78$) or the interval from April 2016 to October 2016 ($F_{[2,123]} = 0.08$, $p = 0.93$; Supplementary Table S2[2]). There were also significant differences in specific growth rate based on sex for the interval from October 2015 to April 2016 ($F_{[2,124]} = 22.30$, $p < 0.001$; Supplementary Table S2[2]), with growth rate significantly higher for females than for males or immature fish and significantly higher for males than for immature fish. Specific growth rate did not differ significantly based on sex for the interval from April 2016 to October 2016 ($F_{[2,123]} = 0.21$, $p = 0.81$; Supplementary Table S2[2]).

Total thiamine concentrations were significantly lower in the muscle of Atlantic salmon fed the low-thiamine diet than in salmon fed the control diet ($F_{[1,95]} = 21.2$, $p < 0.001$; Table 1). Total thiamine concentrations in muscle also differed significantly among all three populations ($F_{[2,95]} = 39.6$, $p < 0.001$; Table 1), being lowest in the St. Jean population, intermediate in the Sebago population, and highest in the LaHave population. Total thiamine concentrations in the muscle did not show a significant interaction between population and diet ($F_{[2,95]} = 0.02$, $p = 0.98$; Table 1). Similar patterns were observed when the three thiamine vitamers were instead compared individually (analysis not shown).

Fig. 3. Relationship between muscle thiamine concentrations and sperm count in Atlantic salmon (*Salmo salar*) from three populations that were fed a control or low-thiamine diet. Sperm count was measured as the average number of sperm cells observed during the video observations of sperm activity. The line shows a significant ($p < 0.05$) linear regression.



Reproductive metrics

Sperm count did not differ significantly based on diet ($F_{[1,26]} = 0.33$, $p = 0.57$), population ($F_{[2,26]} = 0.35$, $p = 0.71$), or the interaction between diet and population ($F_{[2,26]} = 1.03$, $p = 0.37$; Table 2). Sperm motility at 5 s postactivation did not differ significantly based on diet ($F_{[1,24]} = 0.11$, $p = 0.74$), population ($F_{[2,24]} = 0.37$, $p = 0.69$), or the interaction between diet and population ($F_{[2,24]} = 0.56$, $p = 0.58$; Table 2). Sperm motility at 10 s postactivation similarly did not differ significantly based on diet ($F_{[1,26]} = 2.20$, $p = 0.15$),

Table 3. Diameter and thiamine concentrations in the eggs of Atlantic salmon (*Salmo salar*) from females from two populations that were fed a control or low-thiamine diet.

	Control diet (C)		Low-thiamine diet (L)		Significance	
Measure	Sebago (SE)	St. Jean (SJ)	Sebago (SE)	St. Jean (SJ)	Diet	Population
Number (diameter/thiamine)	7/5	12/5	9/6	6/1		
Diameter	6.73±0.26	6.31±0.35	6.46±0.22	6.11±0.13	SE > SJ	C > L
Free thiamine	15.9±3.5	15.1±2.9	10.0±1.9	5.5		C > L
Thiamine monophosphate	0.3±0.03	0.3±0.03	0.3±0.06	0.3		
Thiamine pyrophosphate	0.9±0.2	0.9±0.1	1.0±0.3	0.9		
Total thiamine	17.1±3.6	16.4±2.9	11.3±2.0	6.7		C > L

Note: Values are presented as mean ± SD and are measured in mm for egg diameter and in nanomoles thiamine per gram tissue for thiamine concentrations. Data comprise the number of females whose eggs were measured for each trait; egg diameter; and the concentrations of free thiamine, thiamine monophosphate, thiamine pyrophosphate, and the total across the three thiamine vitamers. Significant ($p < 0.05$) differences among diets or populations are indicated in the last two columns, showing the groups that differed.

population ($F_{[2,26]} = 0.00$, $p = 1.00$), or the interaction between diet and population ($F_{[2,26]} = 0.93$, $p = 0.41$; Table 2).

Sperm velocity did not differ significantly based on diet at 5 s postactivation ($F_{[1,24]} = 3.10$, $p = 0.09$), but at 10 s postactivation sperm velocity was significantly higher for fish fed the low-thiamine diet than for fish fed the control diet ($F_{[1,26]} = 4.58$, $p = 0.042$; Table 2). Sperm velocity differed significantly among populations at 5 s postactivation ($F_{[2,24]} = 4.84$, $p = 0.017$), but did not differ significantly among populations at 10 s postactivation ($F_{[2,26]} = 0.94$, $p = 0.40$; Table 2). Finally, sperm velocity did not show a significant interaction between diet and population at either 5 s ($F_{[2,24]} = 0.05$, $p = 0.95$) or 10 s postactivation ($F_{[2,26]} = 2.31$, $p = 0.12$; Table 2).

There was a significant negative relationship between muscle thiamine concentration and sperm count ($F_{[1,26]} = 6.60$, $p = 0.017$; Fig. 3). Muscle thiamine concentration was not significantly related to sperm motility at 5 s postactivation ($F_{[1,25]} = 0.02$, $p = 0.89$) or 10 s postactivation ($F_{[1,25]} = 0.18$, $p = 0.67$). There was also no significant relationship between muscle thiamine concentration and sperm velocity at 5 s postactivation ($F_{[1,25]} = 0.04$, $p = 0.84$) or 10 s postactivation ($F_{[1,25]} = 0.001$, $p = 0.97$).

Egg diameter was significantly lower in the eggs of Atlantic salmon fed the low-thiamine diet than in salmon fed the control diet ($F_{[1,30]} = 6.04$, $p = 0.020$; Table 3). Egg diameter was significantly higher in the Sebago population than in the St. Jean population ($F_{[1,30]} = 16.1$, $p < 0.001$; Table 3). Egg diameter did not show a significant interaction between population and diet ($F_{[1,30]} = 0.19$, $p = 0.67$; Table 3).

Total thiamine concentrations were significantly lower in the eggs of Atlantic salmon fed the low-thiamine diet than in salmon fed the control diet ($F_{[1,13]} = 19.1$, $p < 0.001$; Table 3). Total thiamine concentrations in eggs did not differ significantly between populations ($F_{[1,13]} = 0.16$, $p = 0.16$; Table 3). Total thiamine concentrations in the eggs did not show a significant interaction between population and diet ($F_{[1,13]} = 1.16$, $p = 0.30$; Table 3). Similar patterns were observed when the three thiamine vitamers were instead compared individually (analysis not shown).

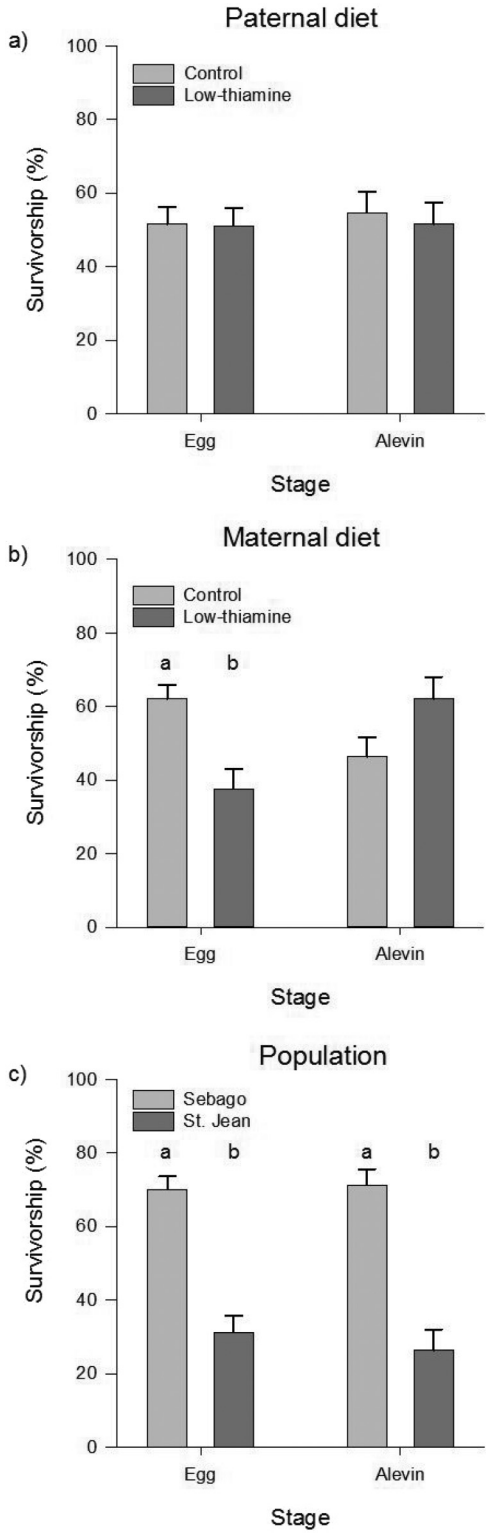
Examining the experimental crosses, there was no effect of paternal diet on family survivorship at the egg stage ($F_{[1,120]} = 0.04$, $p = 0.84$) or alevin stage ($F_{[1,84]} = 0.07$, $p = 0.79$; Fig. 4*a*). Maternal diet, however, had a significant effect on family survivorship at the egg stage ($F_{[1,120]} = 29.2$, $p < 0.001$; Fig. 4*b*); survivorship was lower for the eggs of females fed a low-thiamine diet than for the eggs of females fed a control diet. Maternal diet was not associated with family survivorship at the alevin stage ($F_{[1,84]} = 3.05$, $p = 0.084$; Fig. 4*b*). Population had a significant effect on family survivorship at both the egg stage ($F_{[1,120]} = 63.7$, $p < 0.001$) and alevin stage ($F_{[1,84]} = 34.8$, $p < 0.001$; Fig. 4*c*), with higher survivorship in the Sebago population than the St. Jean population. All higher-order interactions were nonsignificant ($p > 0.05$) and were removed from the final models of survivorship.

Discussion

High levels of dietary thiaminase are often associated with thiamine deficiency and a range of negative consequences (Harder et al. 2018). In our study, the low-thiamine diet containing bacterial thiaminase was associated with a decline in total thiamine levels in muscle of about 7.5 nmol·g^{-1}, and across populations the concentrations averaged 20–36 nmol·g^{-1} on the low-thiamine diet. In an earlier study of the same populations at the subadult stage (Houde et al. 2015*b*), the low-thiamine diet was associated with a reduction in muscle thiamine levels of ~6 nmol·g^{-1}, with the populations averaging 3–6 nmol·g^{-1} on the low-thiamine diet. A previous study of adult coho salmon (*Oncorhynchus kisutch*), steelhead trout (*Oncorhynchus mykiss*), and lake trout (*Salvelinus namaycush*) in the Great Lakes found that total thiamine levels in muscle averaged about 0.5 to 8 nmol·g^{-1}, with external symptoms of thiamine deficiency (e.g., wiggling and lethargy) typically observed when muscle concentrations were less than 1 nmol·g^{-1} (Brown et al. 2005). Thiamine concentrations in the current study were thus relatively high compared with previous studies, although the cause of this difference is not clear. Despite this, the significant difference in thiamine concentrations between diets suggests that the low-thiamine diet may still be associated with symptoms of thiamine deficiency.

One potential consequence of thiamine deficiency is reduced growth rate, as was observed in rainbow trout (*O. mykiss*) exposed to a low-thiamine experimental diet (Morito et al. 1986). In contrast, a low-thiamine diet was not associated with a difference in growth rate in subadult Atlantic salmon (Houde et al. 2015*b*). In adult Atlantic salmon, we found that a low-thiamine diet was associated with a significant reduction in specific growth rate during one of the two measurement intervals. However, body mass did not differ between the two diets at any point. Body mass might obscure differences between diets if many of the smaller fish fed the low-thiamine diet died, leading to similar body size (calculated as an aggregate value based only on the surviving fish), but not growth rate (calculated at the level of individual fish). However, the fish that died were slightly larger at the start of our experiment than the fish that survived, which suggests that selective mortality cannot explain the difference between growth metrics. Instead, it appears that the effects of a low-thiamine diet on growth were minor in our study. The strong effect observed in rainbow trout likely reflects the near zero concentration of thiamine in the experimental diet in that study, which was associated with acute clinical symptoms that included loss of appetite and eventually death (Morito et al. 1986). Increased mortality associated with a low-thiamine diet was also observed in our study of adult Atlantic salmon, albeit less frequently and after longer exposure to a low-thiamine diet than in the rainbow trout study. Together these data suggest that thiamine-

Fig. 4. Survivorship of experimental crosses at the egg and alevin stage in Atlantic salmon (*Salmo salar*) from two populations that were fed a control or low-thiamine diet. Survivorship is shown as a function of paternal diet (*a*), maternal diet (*b*), and population (*c*). Data are plotted as mean + SE. Statistical analyses were performed separately for the egg and alevin stage, with different letters above the bars used to indicate significant ($p < 0.05$) differences between groups.



deficient diets can reduce survival in fishes, but that these diets do not have a strong influence on adult growth rate outside of cases in which serious clinical symptoms are also present (e.g., Morito et al. 1986).

Maternal thiamine deficiency has been widely associated with offspring mortality in salmonids (e.g., Fisher et al. 1995, 1996; Fitzsimons et al. 1995, 2007; Ketola et al. 2000; Brown et al. 2005). Consistent with these previous studies, we found that a low-thiamine diet led to significantly higher offspring mortality during the egg to alevin stage. Egg thiamine concentrations were lower for the low-thiamine diet (7–11 nmol·g^{-1}) than for the control diet (16–17 nmol·g^{-1}), consistent with thiamine deficiency being the cause of the increased mortality. Previous research has suggested a critical threshold for egg thiamine concentrations of ~1 nmol·g^{-1} in salmonids (Fisher et al. 1996; Honeyfield et al. 2005), and the present study indicates that negative effects may be seen at even higher egg thiamine concentrations. An individual-based model for lake trout similarly showed that median lethal egg thiamine concentrations may be as high as 7–10 nmol·g^{-1} (Ivan et al. 2018). In our study, the low-thiamine diet was also associated with smaller egg size, similar to a study that found that wild-caught Atlantic salmon that exhibited symptoms of thiamine deficiency had smaller eggs than salmon that did not exhibit symptoms (Amcoff et al. 2000). Smaller egg size might be an adaptive response to low maternal thiamine levels, as it would enable the eggs to have higher thiamine concentrations than if the same maternal thiamine resources were distributed among larger eggs. However, low maternal thiamine levels still led to reduced egg thiamine concentrations and lower survival, suggesting that any benefit to smaller egg size is unable to fully mitigate the effects of maternal thiamine deficiency. Overall, our data add to the strong evidence in salmonids that a low-thiamine diet is associated with increased mortality prior to the onset of exogenous feeding.

Thiamine has previously been detected in the milt of Atlantic salmon, but its function is not well-characterized (Koski 2002). We tested the hypothesis that thiamine promotes sperm performance and successful fertilization. If this hypothesis is true, we predicted that a low-thiamine diet would result in reduced sperm performance and lower fertilization success. Our data did not support these predictions. We found no significant effect of diet on sperm count or sperm motility, and sperm velocity was actually higher for fish fed the low-thiamine diet at one of the two measurement points. Male diet was likewise unrelated to fertilization success, with no difference in mortality during the egg to alevin stage (or during the alevin to fry stage). At the individual level, we observed a negative relationship between muscle thiamine concentration and sperm count. This relationship may result from a mobilization of thiamine resources to support sperm production, although this mechanism is speculative. Regardless, our data do not support the hypothesis that thiamine promotes sperm performance or successful fertilization, meaning that the importance of thiamine in milt remains uncertain.

We hypothesized that susceptibility to thiamine deficiency might differ among populations if those populations are locally adapted to consume high-thiaminase prey fishes at different rates, and we predicted that local adaptation would lead to differences among populations in their response to the two experimental diets. Independent of the experimental diets, we observed significant differences in muscle thiamine concentrations among the three populations, with the highest concentration in the LaHave population, intermediate in the Sebago population, and lowest in the St. Jean population. However, the effects of the low-thiamine and control diets on muscle thiamine concentrations and growth rate did not differ among the three populations, and the effects of the experimental diets on egg thiamine concentrations and offspring mortality did not differ between the Sebago and St. Jean populations. These results do not provide evidence for local

adaptation to dietary thiamine deficiency among the three populations we tested. The absence of differences among populations may relate in part to the low numbers of fish from the LaHave population, which was predicted to be the most susceptible to a low-thiamine diet. The LaHave population had poor survival regardless of diet, leading to large sample sizes for only the St. Jean and Sebago populations, which share a similar diet in their native habitats and were not predicted to differ in susceptibility to thiamine deficiency. The cause of the low survival for the LaHave population is not clear, as this population has a long history of successful rearing in the OMNRF fish culture system, including at the facility used for our study. The three populations are typically reared in population-specific tanks, whereas our study reared fish in mixed-population tanks, so it is possible that the low survival of the LaHave population was a consequence of interactions with Sebago and St. Jean populations. Alternatively, the intensity of selection associated with dietary thiaminase may differ insufficiently among the studied populations or have been present for an insufficiently long time to have led to local adaptation. Future studies seeking to identify local adaptation to dietary thiaminase would benefit from assessing more individuals and additional populations that differ in predicted susceptibility to dietary thiaminase, as well as collecting wild individuals directly from the populations of interest. Regardless of our low power to detect differences in susceptibility to thiamine deficiency among populations, our data indicate that dietary thiaminase has negative effects on multiple traits even in Atlantic salmon populations with prior exposure to high-thiaminase prey fishes.

Thiamine deficiency is a disorder that affects many Great Lakes salmonids and has been linked to the consumption of introduced prey fishes that are high in the enzyme thiaminase (Harder et al. 2018), with negative consequences for Atlantic salmon in particular (Ketola et al. 2000). Our research quantified the effects of dietary thiamine levels in multiple populations of Atlantic salmon and was conducted in large part to address the objective of the Lake Ontario Atlantic Salmon Restoration Program to identify the source population that is best-suited for reintroduction (i.e., following Houde et al. 2015*a*). Previous research, for example, has identified differences in growth and survival among Atlantic salmon populations when in competition with other salmonids, which may help to inform source population selection for reintroduction into Lake Ontario (Houde et al. 2015*c*). In the current study, with few fish available from the LaHave population — historically stocked at the highest frequency — we were unable to fully evaluate the contribution of dietary thiaminase to reintroduction outcomes. However, a low-thiamine diet led to high mortality even in the populations (Sebago and St. Jean) that naturally consume high-thiaminase prey fishes, so source population selection is unlikely to entirely overcome issues associated with thiaminase consumption in the Great Lakes. With thiaminase in naturalized invasive species an ongoing concern for Great Lakes fisheries management (Ketola et al. 2000; Zimmerman and Krueger 2009), restoration of native forage species would benefit rehabilitation efforts for multiple species.

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