Interaction between dietary thiamine and lipid in juvenile steelhead trout

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By

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Abstract

Thiamine (vitamin B1) deficiency has been negatively affecting salmonines in the Great Lakes region. This project investigated the hypothesis that thiamine deficiency in steelhead trout is a result of a high lipid diet due to thiamine being used up as an antioxidant to prevent lipid peroxidation. Juvenile steelhead trout were fed four diets (high lipid/thiamine, high lipid/no thiamine, low lipid/thiamine, and low lipid/no thiamine) in triplicate aquaria over a six-week period. Fish were sampled every two weeks to assess survival and growth, and samples were preserved for biochemical analysis. At week six, weight and lipid content of fish fed low lipid diets differed significantly from fish fed high lipid diets regardless of the presence or absence of dietary thiamine ($P<0.05$). Thiamine concentrations in fish fed the thiamine-containing diets were significantly higher than those fed thiamine deplete diet ($P<0.05$). Mortality was the highest in fish fed low lipid/no thiamine diet, followed by fish fed the low lipid/thiamine, high lipid/no thiamine, and then high lipid/thiamine diets. Finally, fish fed the high lipid/no thiamine diet began to exhibit symptoms of thiamine deficiency during week 4 of the experiment.

Introduction

Thiamine deficiency complex (TDC), also known as early mortality syndrome (EMS) or M74 in the Baltic Sea region, is a disorder most commonly observed in salmonid species where a deficiency of vitamin B1 leads to high rates of mortality in early life stages (Harder et al. 2018). Referred to as swim-up syndrome when it was first observed, TDC causes neurological defects that impair the swimming and foraging ability of juvenile salmonids during the swim-up stage of development (Wolf 1942). In juvenile fish, TDC can cause loss of equilibrium, lethargy, unusual swimming patterns, reduced foraging rate, poor vision, and altered immune response, all leading to increased rates of early mortality (Fisher et al. 1995). Thiamine deficiency also causes adult fish to have symptoms such as decreased swimming performance, lack of coordination, and affected fish will also have decreased attempts when trying to travel over cascades during spawning migration (Houde et al. 2015; Ketola et al. 2005). Fitzsimons (1995) was the first to connect the early mortality being observed in lake trout (*Salvenlinus namaycush*) at the time to be related to a deficiency of thiamine. In subsequent years, thiamine deficiency was also observed in Atlantic salmon (*Salmo salar*), Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), steelhead trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*), being found to affect these species both in the Great Lakes and the Finger Lakes (Fisher et al. 1995, 1996; Marcquenski and Brown 1997). Because the symptoms of TDC can affect fish at both the juvenile and adult stages, survival of affected salmonine populations is reduced when TDC is prevalent.

TDC in the Great Lakes region has been observed to be connected to consumption of the prey fish, alewife (*Alosa pseudoharengus*) (Honeyfield et al. 2005). In Lake Michigan, thiaminase content of prey fish has been monitored and thiaminase of other prey fish species

such as rainbow smelt (*Osmerus mordax*) was similar to that of alewife but occurrences of TDC in salmonines was still correlated to alewife consumption (Tillitt et al. 2005). While there is evidence that TDC in salmonines is related to consumption of alewife, the biochemical mechanism behind this is debated.

Previous studies have centered around thiaminase (a thiamine degrading enzyme) activity in alewife as the biochemical mechanism for thiamine deficiency, as alewife have high thiaminase activity relative to other prey fish. The first isolated and identified bacterial source of thiaminase was found and named *Paenibacillus thiaminolyticus,* however, there is conflicting research as to whether or not this is the major source of thiaminase in alewife (Honeyfield et al. 2002). Honeyfield et al. (2002) successfully cultured *P. thiaminolyticus* in homogenized viscera of alewife, suggesting that *P. thiaminolyticus* could possibly be the source of thiaminase in alewife. However, a study quantified the contribution of *P. thiaminolyticus* to thiaminase activity at different trophic levels in the Great Lakes ecosystem and found no relationship between thiaminase activity and *P. thiaminolyticus*, suggesting that the majority of thiaminase originates from another source in alewife (Richter et al. 2012). The uncertainty surrounding the source of thiaminase in alewife and the presence of thiaminase in other salmonines' prey suggests that the thiaminase hypothesis may not fully explain the mechanism behind TDC (Harder et al. 2018).

A second hypothesis regarding the biochemical mechanism driving thiamine deficiency in salmonines is that thiamine deficiency is related to dietary lipid content. Research done on M74 in Atlantic salmon from the Baltic Sea found that an unbalanced diet containing too much fat in proportion to thiamine led to thiamine deficiency due to thiamine being used up as an antioxidant to prevent peroxidation of the lipids (Keinänen et al. 2012). While this has not been

studied as extensively in the Great Lakes region, there is a potential that the dietary lipid to thiamine ratio could impact occurrences of TDC in juvenile salmonids in this region as well.

The objective of this study is to determine if dietary lipid content has an impact on thiamine deficiency in steelhead trout in order to further investigate the biochemical mechanism behind thiamine deficiency in Great Lakes salmonid populations. I will test this hypothesis by conducting a laboratory feeding experiment where juvenile steelhead trout will be fed diets of varying lipid and thiamine levels. The survival, growth, thiamine, and lipid content of the fish will be monitored in order to assess the impact that a high fat diet has on TDC occurrence in steelhead trout.

Materials and Methods

Diet Preparation

Four diets with different lipid and thiamine levels were prepared. Two diets contained 30% of lipid, and two 10%. For each lipid level, one diet was supplemented with thiamine and the other without (Table 1) one with thiamine and one without. (Table 1). Diets were synthesized by combining wet and dry ingredients with added water (to make a paste) in a stand mixer, mixed until homogenous, pushed through the grinder of the stand mixer, and freeze dried at SUNY Brockport. Stand mixer and all attachments were thoroughly washed and dried in between each diet type preparation. Diets were sieved through 400-600 micron sieves and 600-1000 micron sieves to produce powder and pellets respectively. Diets were stored frozen until use to prevent thiamine degradation.

	LLT	HLT	LLNT	HLNT
Casein	40	40	40	40
Wheat meal	15	15	15	15
Gelatin	6	6	6	6
Cod liver oil	10	30	10	30
Thiamine	1	1	0	0
Vit no Thiamine [a]	1	1	1	1
Mineral mix [b]	3	3	3	3
Ascorbic acid	0.05	0.05	0.05	0.05
Cellulose	22.25	2.25	22.25	2.25
l-arginine	0.5	0.5	0.5	0.5
l-methionine	0.4	0.4	0.4	0.4
l-lysine	0.8	0.8	0.8	0.8
Choline chloride	1	1	1	1

Table 1. Composition of prepared diets (expressed as percent dry weight) for each diet: Low lipid with thiamine (LLT), high lipid with thiamine (HLT), low lipid without thiamine (LLNT), and high lipid without thiamine (HLNT).

[a]Dyet # 390017 Custom Vitamin Mix for Trout Diet (Dyets Inc., Bethlehem, PA), composition of vitamin mix is expressed as g/kg; vitamin D3 (400000 IU/g), 0.21; ascorbic acid, 17.1; inositol, 16.7; vitamin E (50%), 13.3; niacin (98%), 10.2; manadione, 7.3; calcium D-panthothenate, 7; riboflavin (100%), 2; vitamin B12 (0.1%), 3; biotin, 1.7; pyridoxine HCl, 1.65; folic acid, 0.67; vitamin A palmitate (500000 IU/g), 0.36; choline bitartrate, 200; dextrose, 717.42.

[b]Dyet # 200030 Modified Bernhart-Tomarelli Mineral Mix (Dyets Inc., Bethlehem, PA), composition of mineral mix is expressed as g/kg; calcium phosphate, dibasic, 735; calcium carbonate, 21; sodium chloride, 30.6; potassium phosphate, dibasic, 81; potassium sulfate, 68; sodium phosphate, dibasic, 21.4; magnesium oxide, 25; manganous carbonate, 4.212; ferric citrate, U.S.P., 11.64; zinc carbonate, 0.81; cupric carbonate, 0.333; potassium iodide, 0.0072; citric acid, 0.9978.

Fish collection

Steelhead trout for the feeding experiment were hatched and raised in the lab at SUNY Brockport. Eggs and milt were collected from steelhead trout migrating up the Salmon River at the New York State Department of Environmental Conservation's (NYSDEC) Salmon River Hatchery (Altmar, NY) during March 2019. Eggs from twenty females were fertilized individually with milt from three males. Fertilized eggs were transported back to the lab at SUNY Brockport in watertight plastic Tupperware on ice (<4 h). Once at SUNY Brockport,

fertilized eggs from each female were placed into baskets and were incubated in hatching trays in a recirculated water system. Once the eggs hatched, the embryos were transferred into flow-through aquaria and were kept separated by maternal parent. Thiamine analysis according to Futia et al. (2017) was performed on subsamples of eggs from each female to ensure that egg thiamine levels were above the threshold for deficiency (LC50= 6-7 nmol/g total thiamine) (Futia et al. 2017). All juveniles coming from parents whose eggs showed thiamine levels above the threshold were combined into larger aquaria and fish were fed a standard starter diet (Fish Starter - Ziegler) for 30 days until the start of the feeding experiment.

Feeding Experiment

Fish were randomly distributed into twelve 40-liter aquaria (4 diets x 3 replicates) with 70 fish per aquarium as part of a flow through-system using municipal water with a flow rate of 1.3 L/min per aquarium. Municipal water was dechlorinated using a carbon filter (Siemens Water Technologies, Warrendale, PA). Fish were kept in experimental aquaria and fed starter diet for one additional week prior to the start of the experiment to allow them to acclimate. Experimental diets were assigned randomly to each of the 12 aquaria.

Fish were fed 5% their body weight daily (2.5% in the morning and 2.5% in the afternoon) for 6 weeks one of the four experimental diets.

Aquaria was cleaned daily with temperature and mortality recorded daily as well. At weeks 0, 2, 4, and 6, subsamples of 8 fish were taken randomly from each tank to be weighed, measured, and preserved at -80 degrees Celsius for future biochemical analysis. Samples of each diet were also analyzed for total thiamine and lipid concentration. Total weight of the remaining fish was recorded in order to adjust feeding rate. After week 4, individuals which began

exhibiting behavior linked with thiamine deficiency (spiral swimming, swimming upside down) were determined to be moribund and were also preserved for later biochemical analysis.

Biochemical Analysis

Total thiamine in whole fish was extracted according to Brown et al. 1998 and was measured using a high-performance liquid chromatography (HPLC) system (Agilent Technologies 1200 Series) according to Futia et al. (2017).

Lipid was extracted from 4 whole fish per tank per sampling and measured gravimetrically according to the method described by Folch et al. (1957). Lipid samples were transmethylated to produce fatty acid methyl esters (FAMEs) according to Metcalfe and Schmitz (1961) and were expressed as mg of fatty acid/g of sample. Internal standard (nonadecanoate acid; 19:0 Nu-Check Prep Inc., Elysian, MN) of a known amount was added to each sample. Fatty acid signatures of individual fish were determined using a gas chromatography-mass spectrometry (GC/MS) system (Agilent 7890A GC and 5975C inert XL EI/CI MSD, Agilent Technologies, Inc) with an Agilent J&W column with 30 m × 0.250 mm × 0.50 μm thickness with helium as the carrier gas and run conditions set according to Happel et al. (2017). The concentration of each FAME was determined by identifying peak area and comparing retention times with retention times of authentic standard mixtures (FAME mix 37 components, Sigma-Aldrich, St. Louis, MO). Fatty acids were referred to according to International Union of Pure and Applied Chemistry Nomenclature.

Moribund fish were analyzed in the same way, except multiple fish of the same diet treatment were combined for each sample due to the small sizes of the fish.

Statistical Analysis

Univariate statistics were performed using “tidyverse” and “ggplot2” packages in R (Wickham 2017; Kassambara 2018). Difference in weight, mortality, lipid content, and thiamine concentration among dietary treatments were assessed using a one-way ANOVA ($P<0.05$). A two-way ANOVA ($P<0.05$) was used to assess interaction effect of thiamine and lipid on mortality. Prior to statistical analysis, percent data (mortality and lipid content) were arcsin transformed, and normality and homogeneity of variance were confirmed (Shapiro-Wilk test; Levene’s test). Multivariate statistics were performed using Primer v.6 software. Non-metric multidimensional scaling (nMDS) to display fatty acid signatures (based on mg of fatty acid/g of sample) was performed using Bray-Curtis similarity. Analysis of similarities (ANOSIM) was used to compare dissimilarities in fatty acid signatures between diet types at the end of the experiment. R values <0.05 were considered to be an excellent representation of relationships with extremely low chance of misleading interpretation. Additionally, a similarity percentages analysis (SIMPER) was used to compare the proportions of individual fatty acids among samples by generating percent dissimilarity among samples and identifying the fatty acids most responsible for dissimilarities.

Excel 2016 was used to display regressions between select fatty acids (arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and oleic acid (18:1n-9)) and total thiamine concentrations across all diet treatments at week 6 and for moribund fish.

Results

Growth, thiamine concentration, and lipid concentration

At the end of the experiment (week 6), mass of fish fed low lipid diets differed significantly (ANOVA; $P<0.05$) from fish fed high lipid diets regardless of the presence or absence of thiamine in the diet (Figure 1). Fish fed high lipid diets grew significantly more than fish fed low lipid diets by week 6. Percent weight gain and percent specific growth rate were not found to be significantly different for any diet type (Table 2). The percent lipid content of whole fish tissue varied among the diet treatments (Figure 2A). The fish fed low lipid diets had significantly less percent lipid in their tissues than the fed either high lipid diets. Percent lipid content was not significantly between thiamine or no thiamine diet types of the same lipid level (ANOVA; $P<0.05$). Total thiamine concentrations (as nmol/g of whole fish tissue) in fish fed the thiamine-containing diets were significantly higher (ANOVA; $P<0.05$) than the concentrations in fish fed thiamine deplete diets (Figure 2B). No significant difference was found between low lipid with no thiamine and low lipid with thiamine or between high lipid with thiamine and high lipid with no thiamine. Percent mortality was the highest in fish fed low lipid/no thiamine diet, followed by fish fed the low lipid/thiamine diet, the high lipid/no thiamine diet, and then high lipid/thiamine diet with only the low lipid/no thiamine diet and the high lipid/thiamine diet being significantly different (ANOVA; $P<0.05$); lipid content, but not thiamine content of diet, had a significant impact on mortality (Figure 2C). The interaction between thiamine and lipid on mortality was not found to be significant (ANOVA; $P>0.05$).

Fatty acid signature analysis

Fatty acid signatures from fish consuming low lipid/no thiamine versus low lipid/thiamine and high lipid/no thiamine versus high lipid/thiamine were not significantly different while the rest of the comparisons were significantly different (ANOSIM, $R<0.001$; Global $R = 0.605$). Average dissimilarities as determined by SIMPER revealed the diets having

the most differences in comparison to other diets (Table 3). Oleic acid (18:1n-9) was included in the top three fatty acids contributing to the most dissimilarity for every diet type comparison. Diets of the same lipid level were most similar to each other compared to dissimilarities between diets of different lipid levels. (Table 4).

Moribund Fish

A total of seven HLNT and one LLNT-fed samples containing multiple moribund fish per sample were preserved and analyzed. The LLNT and HLNT combined fish samples had a tissue thiamine concentration of 11.1 nmol/g and 10.46 ± 1.30 nmol/g, respectively (Table 5). Fatty acid composition of moribund fish tissue was found and expressed as mg of fatty acid/mg sample (Table 6). Moribund sample fatty acid compositions were similar to the fatty acid compositions of the normal fish.

The relationships between fatty acid compositions of arachidonic acid, EPA, DHA, and oleic acid and total thiamine concentration in fish from all diet treatments at week 6 were positive except for the relationship between total thiamine concentrations and oleic acid concentrations which was negative (Figure 4). All relationships had low R^2 values, signifying that the relationships were weak. The relationship between the same 4 fatty acid concentrations and total thiamine concentrations in moribund samples was positive for all fatty acids, but the R^2 values were also low for this, signifying that the relationships were weak (Figure 5). The relationship between the ratio of oleic acid: arachidonic acid and total thiamine was negative for both moribund samples and fish from all diet treatments at week 6 (Figure 6).

Discussion

While the interaction was not significant, the mortality across diets showed an unexpected pattern with mortality grouping neither by lipid or thiamine. If mortality had been entirely dependent on either dietary lipid or thiamine separately, then it would be expected that mortality between diets would be significantly different based on one of those factors. However, this did not happen suggesting that both affected mortality in this experiment. Further investigation is needed though, as the interaction was not significant. Although the thiamine concentrations between low lipid/no thiamine and high lipid/no thiamine were not significantly different, fish fed the low lipid/no thiamine diet began to exhibit symptoms of thiamine deficiency (loss of equilibrium, erratic swimming) during week 4 of the experiment.

The majority of the moribund fish that had been exhibiting symptoms of TDC came from the HLNT treatment. While thiamine analysis of the HLNT moribund fish revealed that they were above LC50 levels for egg thiamine (6-7 nmol/g), the fish still exhibited severe symptoms suggesting that the threshold may be higher than originally thought (Futia and Rinchard 2019). Further research into species-specific thiamine threshold levels is needed.

Fatty acid compositions of moribund fish and normal fish were similar. However, the relationship between total thiamine concentration and oleic acid was weakly negative for normal fish, suggesting that there may have been degradation of oleic acid from presence of high thiamine levels. Keinänen et al. (2012) found that the strongest negative correlations between fatty acid concentration and total thiamine concentration was with the ratio of oleic acid: arachidonic acid. The relationship between oleic acid: arachidonic acid and total thiamine concentration in this experiment were also found to be negative and this relationship was the strongest observed for moribund samples, relative to other comparisons. While this relationship was strong relative to other comparisons, it was still weak correlation. But as oleic acid was the

fatty acid responsible for the most dissimilarity in every diet type comparison, there is a possibility that there might have been a stronger correlation with a longer experiment.

Results of this experiment up to this point have been altogether inconclusive. The relationship between oleic acid: arachidonic acid and total thiamine concentrations is not strong enough to be considered conclusive evidence. Possible explanations for the lack of difference in response between the two diets with no thiamine could be that a) the juvenile fish still had leftover thiamine in their tissues from feeding off of their yolk sacs or b) a stressor may need to be placed on the fish to induce oxidative stress in order to observe lipid depletion as a result of peroxidation over a short time span (also could increase experiment length). While evidence supports the high lipid hypothesis in the Baltic Sea, it has not yet been proven to be the biochemical mechanism for thiamine deficiency in other regions such as the Great Lakes (Keinänen et al. 2012). A negative relationship between tissue lipid content and tissue thiamine concentration has been observed in brown trout, but the decreased thiamine concentrations was not thought to be enough to induce TDC (Futia 2018). Given that there is a current lack of controlled laboratory experiments that investigate high dietary lipid content as a potential cause for TDC in the Great Lakes region, there is a need for more research. Although no conclusions could be drawn from the data collected from this experiment, it could be modified and redone to provide more evidence as to whether or not dietary lipid content is related to TDC in Great Lakes salmonids.

Works Cited

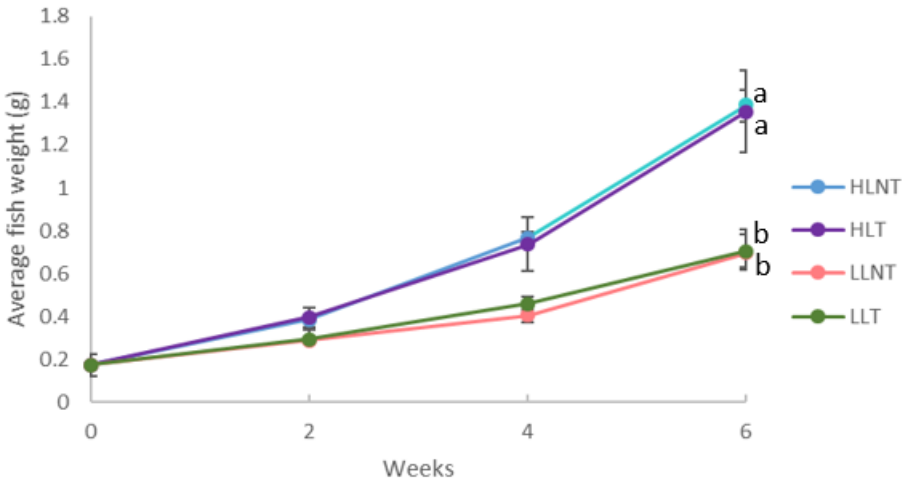

Figure 1. Average fish weight with standard deviation over the 6-week experiment for low lipid/no thiamine (LLNT), low lipid/thiamine (LLT), high lipid/no thiamine (HLNT), and high lipid/thiamine (HLT) diets. Significant differences indicated by different letters (ANOVA, $P < 0.05$).

Table 2. Feeding experiment weight gain (WG) and specific growth rate (SGR) for the duration of the experiment.

Diet	WG (%)	SGR (%/day)
LLNT	174.4	4.2
LLT	174.4	4.2
HLNT	175.1	4.2
HLT	175.1	4.2

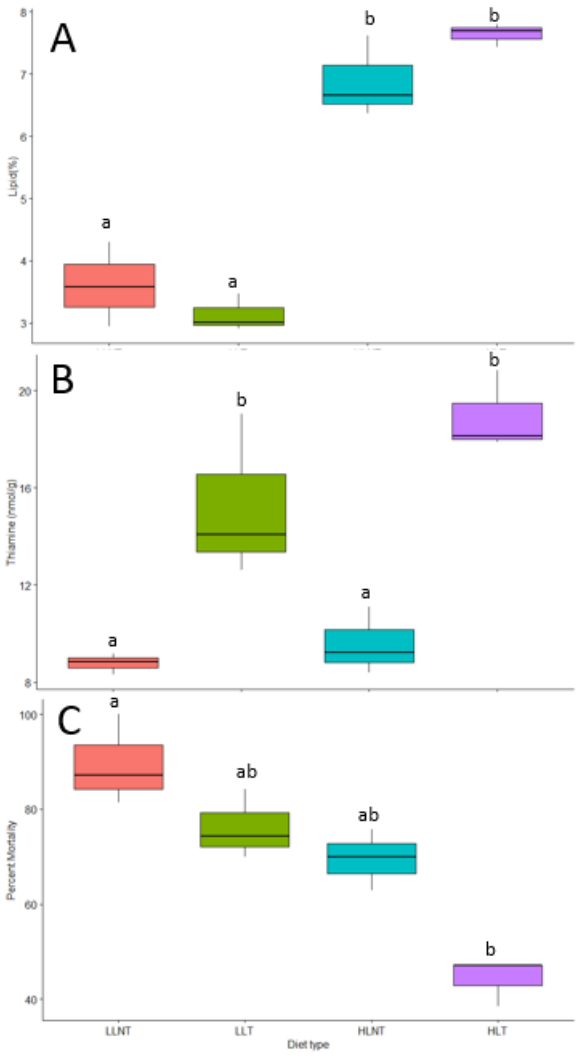

Figure 2. Fish lipid content (A), thiamine content (B) and mortality (C) week 6 for low lipid/no thiamine (LLNT), low lipid/thiamine (LLT), high lipid/no thiamine (HLNT), and high lipid/thiamine (HLT) diets. Significant differences indicated by different letters (ANOVA, $P < 0.05$).

Table 3. Average dissimilarities as calculated via SIMPER analysis, with the top three fatty acids contributing to the dissimilarity. Diets are abbreviated as low lipid/no thiamine (LLNT), low lipid/thiamine (LLT), high lipid/no thiamine (HLNT), and high lipid/thiamine (HLT).

Comparisons	Average percent dissimilarity	Major FA responsible for differences
LLT vs. LLNT	15.31%	C18:1n-9, C16:0, C16:1n-7
LLT vs. HLNT	36.80%	C18:1n-9, C22:6n-3, C20:1
LLNT vs. HLNT	31.51%	C22:6n-3, C20:1, C18:1n-9
LLT vs. HLT	36.67%	C18:1n-9, C22:6n-3, C20:1
LLNT vs. LLT	31.88%	C22:6n-3, C20:1, C18:1n-9
HLNT vs. HLT	10.31%	C18:1n-9, C16:0, C22:6n-3

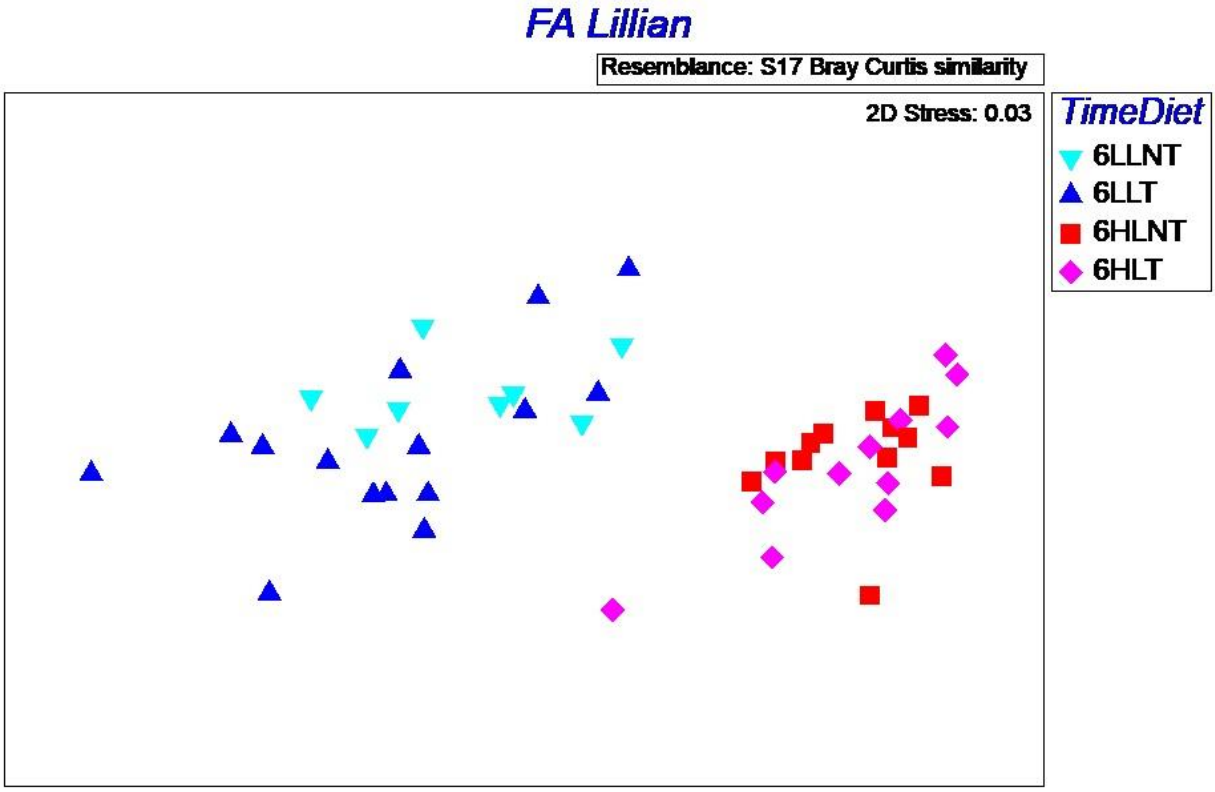

Figure 3. nMDS plot of fatty acid signatures from fish at week 6. Diet types are abbreviated as 6LLNT (low lipid/no thiamine), 6LLT (low lipid/thiamine), 6HLNT (high lipid/no thiamine), and 6HLT (high lipid/thiamine). Stress value of 0.03 is <0.05, indicating an excellent representation of relationships.

Table 4. Average ± standard deviation of each fatty acid (expressed as mg fatty acid/ g of sample) for fish fed HLNT (high lipid/no thiamine), HLT (high lipid/thiamine), LLNT (low lipid/ no thiamine) and LLT (low lipid/thiamine) diets at week 6. Fatty acid types are separated by saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA).

		HLNT	HLT	LLNT	LLT
SAFA	14:0	0.93 ± 0.04	0.91 ± 0.09	0.58 ± 0.19	0.48 ± 0.07
	15:0	0.093 ± 0.004	0.094 ± 0.02	0.039 ± 0.008	0.034 ± .004
	16:0	3.83 ± 0.30	3.77 ± 0.48	2.85 ± 0.57	2.36 ± 0.13
	17:0	0.056 ± 0.002	0.055 ± 0.010	0.028 ± 0.005	0.024 ± 0.003
	18:0	1.19 ± 0.08	1.10 ± 0.10	1.06 ± 0.17	0.83 ± 0.03
MUFA	16:1n-9	0.17 ± 0.01	0.16 ± 0.01	0.24 ± 0.06	0.19 ± 0.03
	16:1n-7	1.60 ± 0.06	1.60 ± 0.13	1.16 ± 0.37	0.89 ± 0.16
	18:1n-9	6.46 ± 0.48	6.39 ± 0.80	5.08 ± 1.04	3.85 ± 0.32
	18:1n-7	1.58 ± 0.56	1.17 ± 0.11	0.75 ± 0.17	0.59 ± 0.01
	20:1	2.86 ± 0.18	2.85 ± 0.30	0.93 ± 0.31	0.76 ± 0.17
	21:5n-3	0.084 ± 0.006	0.091 ± 0.025	0.017 ± 0.007	0.017 ± 0.006
PUFA	18:2n-6	1.02 ± 0.07	1.02 ± 0.11	0.40 ± 0.11	0.36 ± 0.08
	18:3n-3	0.34 ± 0.02	0.35 ± 0.06	0.09 ± 0.03	0.08 ± 0.02
	18:4n-3	0.39 ± 0.04	0.42 ± 0.09	0.09 ± 0.03	0.08 ± 0.02
	20:2n-6	0.13 ± 0.004	0.12 ± 0.02	0.08 ± 0.03	0.06 ± 0.01
	20:3n-6	0.050 ± 0.0007	0.048 ± 0.009	0.032 ± 0.010	0.032 ± 0.003
	20:4n-6	0.16 ± 0.01	0.16 ± 0.02	0.14 ± 0.03	0.143 ± 0.004
	20:3n-3	0.051 ± 0.003	0.053 ± 0.013	0.012 ± 0.003	0.013 ± 0.004
	20:4n-3	0.202 ± 0.009	0.222 ± 0.063	0.045 ± 0.018	0.047 ± 0.014
	20:5n-3	1.22 ± 0.14	1.35 ± 0.21	0.28 ± 0.08	0.29 ± 0.06
	22:4n-6	0.029 ± 0.003	0.027 ± 0.008	0.024 ± 0.009	0.023 ± 0.002
	22:5n-6	0.34 ± 0.03	0.38 ± 0.09	0.10 ± 0.03	0.11 ± 0.02
	22:6n-3	4.57 ± 0.36	4.51 ± 0.36	2.09 ± 0.26	2.08 ± 0.18

Table 5. Thiamine tissue concentration for moribund samples collected as they displayed symptoms of thiamine deficiency for low lipid/no thiamine (LLNT) and high lipid/no thiamine (HLNT) diet treatments.

Diet	Total thiamine (nmol/g)
LLNT	11.1
HLNT	10.46 ± 1.30

Table 6. Average ± standard deviation of each fatty acid (expressed as mg fatty acid/ mg of lipid sample) for moribund fish fed HLNT (high lipid/no thiamine) and LLNT (low lipid/ no thiamine). Fatty acid types are separated by saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). Only one sample was obtained for LLNT, therefore no standard deviation was calculated.

		LLNT	HLNT
SAFA	14:0	0.439	1.065 ± 0.240
	15:0	0.025	0.100 ± 0.019
	16:0	2.537	4.359 ±1.070
	17:0	0.016	0.0173 ± 0.004
	18:0	0.838	1.139 ± 0.256
MUFA	16:1n-9	0.243	0.171 ± 0.036
	16:1n-7	0.973	1.753 ± 0.395
	18:1n-9	4.222	7.291 ± 1.882
	18:1n-7	0.551	1.371 ± 0.311
	20:1	0.294	2.881 ± 0.664
PUFA	21:5n-3	0.002	0.069 ± 0.035
	18:2n-6	0.185	1.116 ± 0.237
	18:3n-3	0.029	0.359 ± 0.077
	18:4n-3	0.031	0.417 ± 0.087
	20:2n-6	0.043	0.128 ± 0.026
	20:3n-6	0.017	0.049 ± 0.013
	20:4n-6	0.129	0.174 ± 0.035
	20:3n-3	0.012	0.051 ± 0.014
	20:4n-3	0.001	0.193 ± 0.050
	20:5n-3	0.134	1.330 ± 0.279
	22:4n-6	0.004	0.026 ± 0.012
	22:5n-6	0.059	0.328 ± 0.063
	22:6n-3	1.637	5.026 ± 1.049

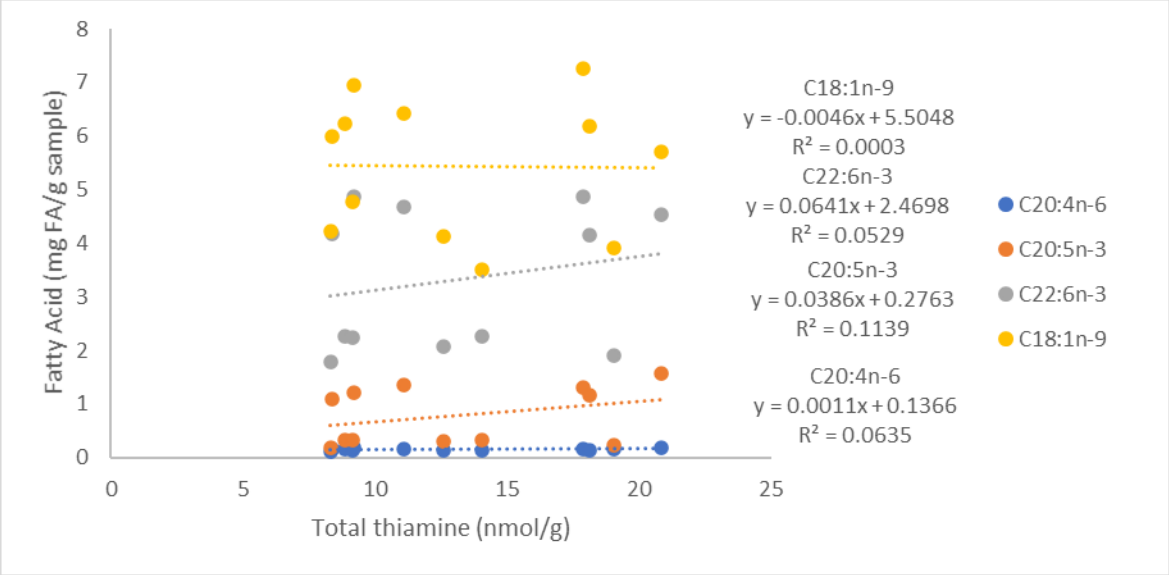

Figure 4. Relationship between fatty acid concentrations (as mg FA/ g sample) of arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and oleic acid (18:1n-9) and thiamine concentrations (nmol/g) of fish from all diet treatments at week 6.

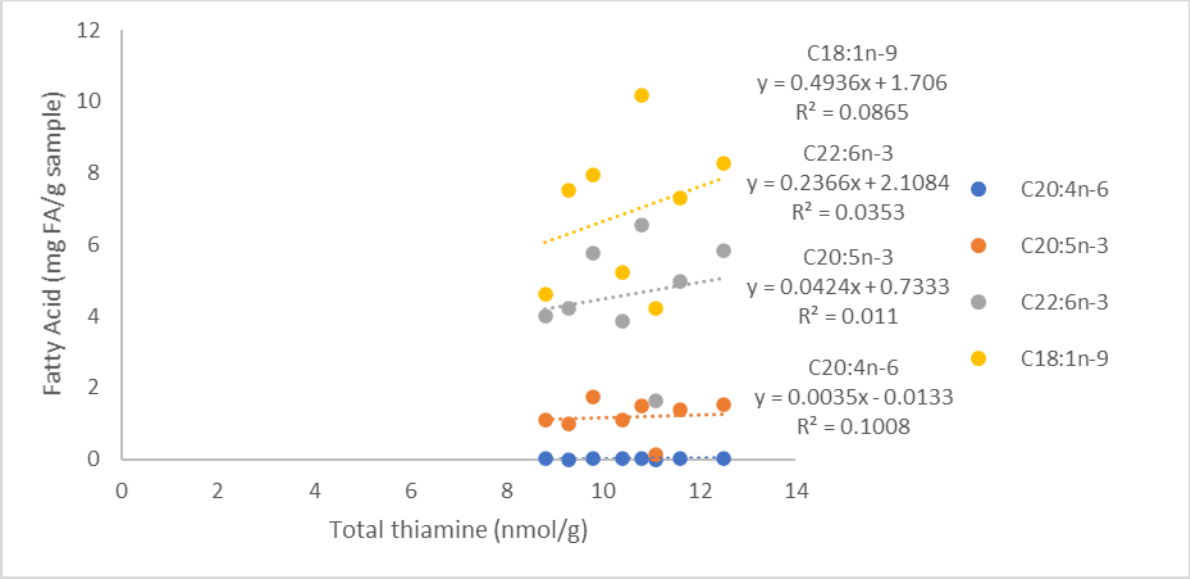

Figure 5. Relationship between fatty acid concentrations (as mg FA/ g sample) of arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and oleic acid (18:1n-9) and thiamine concentrations (nmol/g) of moribund samples.

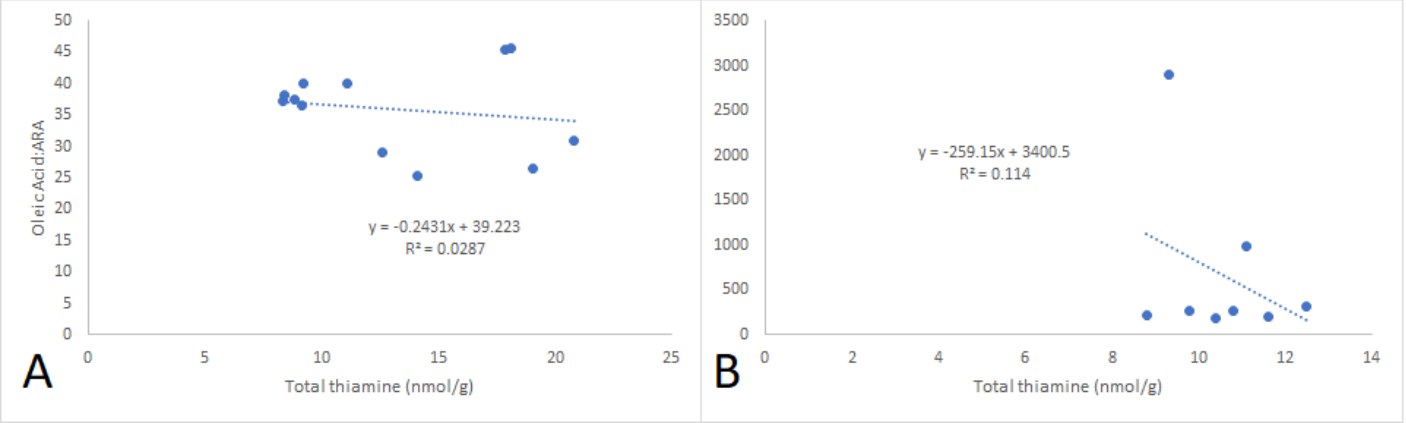

Figure 6. Relationship of the ratio of oleic acid (mg FA/g sample): arachidonic acid (mg FA/g sample) and thiamine concentrations (nmol/g) for fish of all diet treatments at week 6 (A) and moribund samples (B).

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