GREAT LAKES FISHERY COMMISSION
RESEARCH STATUS REPORT

THIAMINE DEFICIENCY COMPLEX WORKSHOP
Final Report

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GLFC-Sponsored Research Coordination Meeting on Thiamine Deficiency

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Abstract

Fry mortality which was first observed in the late 1960s in Great Lakes salmonines and in Baltic Sea salmon in 1974 has now been linked to thiamine deficiency (historically referred to as Early Mortality Syndrome, or EMS and M74, respectively). Over the past 14 years significant strides have been made in our understanding of this perplexing problem. It is now known that thiamine deficiency causes embryonic mortality in these salmonids. Both overt mortality and secondary effects of thiamine deficiency are observed in juvenile and adult animals. Collectively the morbidity and mortality (fry and adult mortality, secondary metabolic and behavior affects in juveniles and adult fish) are referred to as Thiamine Deficiency Complex (TDC). A workshop was held in Ann Arbor, MI on 6-7 November 2008 that brought together 38 federal, state, provincial, tribal and university scientists to share information, present data and discuss the latest observations on thiamine status of aquatic animals with thiamine deficiency and the causative agent, thiaminase. Twenty presentations (13 oral and 7 posters) detailed current knowledge. In Lake Huron, low alewife *Alosa pseudoharengus* abundance has persisted and egg thiamine concentrations in salmonines continue to increase, along with evidence of natural reproduction in lake trout *Salvelinus namaycush*. Lake Michigan Chinook salmon *Oncorhynchus tshawytscha* appear to have a lower thiamine requirement than other salmonids in the lake. Lake Ontario American eel *Anguilla rostrata* foraging on alewife have approximately one third the muscle thiamine compared to eels not feeding on alewife, suggesting that eels may be suffering from thiamine deficiency. Secondary effects of low thiamine exist in Great Lakes salmonines and should not be ignored. Thiaminase activity in dreissenid mussels is extremely high but a connection to TDC has not been made. Thiaminase in net plankton was found more consistently in lakes Michigan and Ontario than other lakes evaluated. The biological role of thiaminase I, associated with thiamine deficiency, remains to be determined whereas thiaminase II has been reported to be part of a salvage pathway leading to thiamine synthesis. The use of gene array technology and 3-dimensional histology is adding new understanding to the affects of thiamine deficiency. Research is needed to determine the thiamine status of species feeding on dreissenids, the environmental sources of thiaminase and the biological role of thiaminase I.

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Introduction

Historically, thiamine deficiency has affected survival of coho salmon *Oncorhynchus kisutch*, Chinook salmon *Oncorhynchus tshawytscha*, Atlantic salmon *Salmo salar*, steelhead trout *Oncorhynchus mykiss*, brown trout *Salmo trutta* and lake trout *Salvelinus namaycush* in Lakes Michigan and Ontario and to a lesser extent in Lakes Huron and Erie. Salmonid fry mortality was first observed in the late 1960s from an unknown cause (Mac et al. 1985). In 1994 and 1995 the following questions were being asked: Is this a single syndrome? Is there a genetic component? Is there a disease component? Is fry mortality caused by a dietary or water-borne contaminant? Are there signs in adult females? Is there a correlation between egg quality and fry mortality? It was known that thiamine (vitamin B_1) somehow was involved and we had a single HPLC chromatogram demonstrating the separation of three forms of thiamine (Honeyfield et al. page 12). Over the course of the last 14 years, significant strides have been made in understanding thiamine deficiency in the Great Lakes basin and a similar syndrome, M74, in Baltic Sea salmonids (Ambio 1999).

The thiamine status of salmonids has improved in Lake Huron with the major reduction in alewife population but thiamine deficiency remains apparent in Lakes Michigan and Ontario. In these two lakes, clinical symptoms of thiamine deficiency are observed in thiamine deficient fry between hatch and two weeks post swim-up (Marcquenski and Brown 1997; McDonald et al. 1998). Thiamine deficiency in salmonid fry is caused by a maternal thiamine deficiency from a diet high in thiaminase from alewife (Honeyfield et al. 2005). In addition to negative effects on fry, there is evidence of impacts due to thiamine deficiency on adults (Brown et al. 2005a; Fitzsimons et al. 2005; Ketola et al. 2005). The consequences of thiamine deficiency on salmonine reproduction, survival, behavior and physiology continue to be underappreciated and the technological tools being used to understand the effect of thiamine deficiency have become more sophisticated. As part of our effort to communicate current information about thiamine deficiency and recent progress in research studies, a workshop was held in Ann Arbor, MI, November 6-7, 2008. The workshop brought together 38 personnel from universities, natural resource agencies and federal, state, provincial and tribal groups. There were 13 oral and seven poster presentations describing the status of current understanding of thiamine deficiency in the Great Lakes basin and Baltic Sea. We report a brief synopsis of workshop presentations and provide suggestions for future studies based open discussion, presentations and recent literature.

Thiamine Status in Great Lakes Aquatic Species

Lakes Huron, Michigan and Ontario. Evidence of lake trout reproduction in Lake Huron has been welcome information to the Great Lakes fisheries community. Beginning in 2004-2005, egg total thiamine concentrations increased and bottom trawl surveys captured unclipped juvenile lake trout in Lake Huron (Riley et al. 2007; page 19). Alewife populations in Lake Huron collapsed in approximately 2003 and have not rebounded (Riley et al. 2008). The years 1983-1984 and 2003-present represent the lowest levels of alewife abundance, thus potential thiaminase intake in lake trout has been significantly reduced. Consumption of alewife has been found to cause thiamine deficiency (Honeyfield et al. 2005) and the finding of low alewife numbers along with increased egg thiamine in Lake Huron lake trout support the hypothesis that alewife consumption has serious implications as outlined by Brown et al. (2005b). Alewives continue to be a major dietary component in salmonids in Lake Michigan. In depth analysis of Lake Michigan lake trout egg thiamine content and fry survival over eight years (1996-2003) suggested an effective concentration (EC20) of thiamine required in the egg for no more than 20% fry mortality in the resultant fry was 1.7 nmol/g (Evans et al. page 15). The EC20 reported

for lake trout from Lake Ontario was higher (2.6 nmol/g; Fitzsimons et al. 2007). Based on the difference in EC20 between the two lakes, lake trout recovery might be expected in Lake Michigan before Lake Ontario. Thiamine deficiency has been suggested to be a symptom of a degraded ecosystem. In the disease modeling workshop held prior to the thiamine deficiency workshop, a report was presented on large lakes of the world. Of the large world lakes, Lake Ontario was one of three world lakes with greatest number of changing metrics and Lake Michigan was in the next lower category of lakes discussed (Hecky et al. 2008). In contrast, Lake Superior was classified as having the least number of changing metrics. Thus, it appears there may be an association between the severity of thiamine deficiency and number of changing metrics in a lake. In another study, diet analysis using a combination of stomach contents and stable isotopes found that once juvenile lake trout leave the hatchery, they experience a dramatic decline in thiamine related to but not directly proportional to the proportion of alewives in the diet (Fitzsimons et al. page 24). Alewives were the major forage fish and sculpins contributed very little to the diet. Thiamine levels in juvenile lake trout as young as age 2+ approached but generally did not fall below 500 pmol/g. Muscle thiamine at or below 500 pmol/g has been shown to be deleterious in adult fish (Brown et al. 2005a).

The thiamine status of Chinook salmon and American eel *Anguilla rostrata* were reported at the workshop in addition to that of lake trout. Honeyfield et al. (page 19) reported that thiamine levels in Chinook muscle and liver declined over a 150 day fasting period. Muscle thiamine declined at a rate of 6.8-10.6 pmol/g muscle/day and liver thiamine declined at a rate of 73-110 pmol/g liver/day. Muscle thiamine loss was continuous over the 150 days whereas in liver the greatest loss occurred in the first 100 days. These data were collected as a follow-up to reported over-winter losses of tissue thiamine found in Lake Michigan Chinook (Honeyfield et al. 2008). The potential for other species that feed on prey containing thiaminase to develop thiamine deficiency has received less attention.

American eels are in decline. Eels from the Gulf of St. Lawrence were collected prior to their 3000 km spawning migration to the Sargasso Sea, during which no feeding is known to occur. Thiamine concentrations in eels that fed on alewives were approximately one third that of eels that had not been feeding on alewife (Fitzsimons et al. page 24). The significance of these findings remains to be determined. Although thiamine deficiency is very plausible, the fact that dams are known to block American eel migration and there are changes in oceanic circulation patterns that may alter elver food supply, the support for linking thiamine deficiency to eel declines needs more supportive data (MacGregor et al. 2008, 2009). In another presentation, the secondary effects of low thiamine in post swim-up lake trout fry were summarized by Fitzsimons et al. (page 13). In lake trout fry hatched from eggs containing less than 4 nmol/g thiamine, the secondary effects of thiamine deficiency include but are not limited to, reduced growth, foraging efficiency and predator avoidance. The impact of secondary thiamine deficiency at the population level for lake trout is currently unknown but based on the general observation that recruitment of lake trout is not evident in Lakes Michigan and Ontario, would suggest that the secondary effects of thiamine deficiency must be considered.

Links with Essential Nutrients Other than Thiamine

The question of factors other than thiamine playing a part in TDC has persisted. Although it is clear that thiamine deficiency is the major cause, Pettersson and Lignell (1998) reported that the concentration of the antioxidant astaxanthin in Baltic salmon *Salmo salar* tissues were lower in fish with M74 compared to unaffected fish. Two studies independently conducted in the Great Lakes suggest that vitamin E, an important antioxidant, may be involved in TDC. Brown et al. (page 21) reported a lower concentration of vitamin E in Lake Ontario alewife compared to round

goby *Neogobius melanostomus*, slimy sculpin *Cottus cognatus* and rainbow smelt *Osmerus mordax*. Lee et al. (page 23) reported a strong relationship between fry mortality and vitamin E in eggs collected from Lake Michigan lake trout. Thiamine and vitamin E deficiency have both independently been associated with an increased cellular oxidative stress. Thus it should not be surprising that low thiamine and low vitamin E may be additive in their effects on TDC. Other potential essential nutrients that may impact TDC were also investigated. Magnesium is an important cofactor of several enzymes including thiamine requiring enzymes. Lee et al. (page 15) found that magnesium is important in lake trout growth and development. No information is available on the magnesium status of feral lake trout at this time.

Studying Thiamine Deficiency with Advanced Technologies

The technology being used to study thiamine deficiency has progressively advanced over the years from simple to very sophisticated. Of the three most progressive technology presentations, the presentation by Allen et al. (page 14) was visually remarkable and insightful. Histological tissue sections were used for alignment and construction of the 3-D models of electron microscopy images. Results from thiamine depleted fry showed an 11.6% reduction in the optic nerve volume, a 26.5% reduction in hypothalamus volume, a 20.9% reduction in cerebellum volume, and a 5.5% reduction in overall brain size. Furthermore, there was an increase of 8.6% in olfactory nerve volume, 17.9% increase in the olfactory lobe volume, and a 26.9% larger pituitary volume observed in thiamine deficient fry. The disparity in the size of the hypothalamus and pituitary may suggest other developmental problems with thiamine deficient fry. As these results were based on only two fish, additional work is needed to determine the variability among individuals and species to fully understand the effects on brains. However, this technology has the potential to lead to new unexplored aspects of thiamine deficiency as well as confirm results from other research approaches.

Another cutting-edge presentation employing gene array technology investigated three phases (early onset, intermediate onset and late onset mortality) of M74 in Baltic salmon. Each M74-subgroup had a unique gene transcription pattern characterized by disturbances in the transcription of genes involved in cell cycle and progression of cell death. The intermediate stage of M74-mortality is associated with reduced DNA-binding of the transcription factor HIF-1 (required for normal development of vasculature and red blood cells), reduced production of a regulator protein of angiogenesis (VEGF) and decreased capillary density (Vuori and Nikinmaa page 12). These results are consistent with earlier finding of increased oxidative stress in Baltic salmon (Vuori et al. 2008). Previously, Rise et al. (2005) found 211 genes that were reproducibly down-regulated and 180 genes that had been up-regulated in thiamine deficient and replete lake trout eggs. Additional studies employing gene array technology are warranted and may potentially provide new insight about the etiology of thiamine deficiency. The third state of the art technology presentation investigated thiaminase I gene from *Paenibacillus thiaminolyticus.* Quantitative PCR assays were developed to detect most bacterial 16S rDNA, 16S rDNA of *P. thiaminolyticus* and a few closely related taxa (Richter et al. page 18). This newly developed tool can be used to look for the presence of the bacteria that produce thiaminase among a diverse community of bacteria. This is an important tool to elucidate potential sources of thiaminase associated with thiamine deficiency.

Biochemistry of Thiaminase

Recent discoveries in molecular biology provide information and insights to understand the biological role of thiaminase. There are two forms of thiaminase; Type I is the form associated

with alewife and Type II has not been directly linked with any cases of thiamine deficiency. The biological role of thiaminase II (more appropriately referred to as TenA) is a salvage enzyme involved in thiamine synthesis (Bettendorff 2007). The primary substrate for TenA was described as an environmental breakdown product of thiamine (formylaminopyrimidine), not thiamine itself. The enzyme activity for both types of thiaminase is a base-substitution end product reaction (Fig. 1). For Type I thiaminase activity, the co-substrate (base) can be any number of Lewis bases such as nicotinic acid, pyridine, cysteine, or sulfhydryl reagents. For Type II thiaminase, the co-substrate is the hydroxide ion from water. Kraft et al. (page 18) presented arguments that thiaminase I is analogous to TenA and that thiaminase I may also be involved in thiamine synthesis. First, the two reactions are similar. Secondly, the gene for thiaminase I was located on the chromosome adjacent to the gene for ThiD, a key enzyme involved in thiamine synthesis. Thirdly, a new protein expression regulatory mechanism was recently discovered that involves "riboswitches", short sequences of RNA that shut down gene expression in the presence of a substrate (Cochrane and Strobel 2008). This regulation mechanism could help explain early lab studies with *Paenibacillus thiaminolyticus* that showed repression of thiaminase I production in the presence of thiamine. These molecular insights do not yet point to a clear role for the presence of thiaminase I in nature, but they suggest that environmental conditions lacking thiamine or containing degraded thiamine should be investigated.

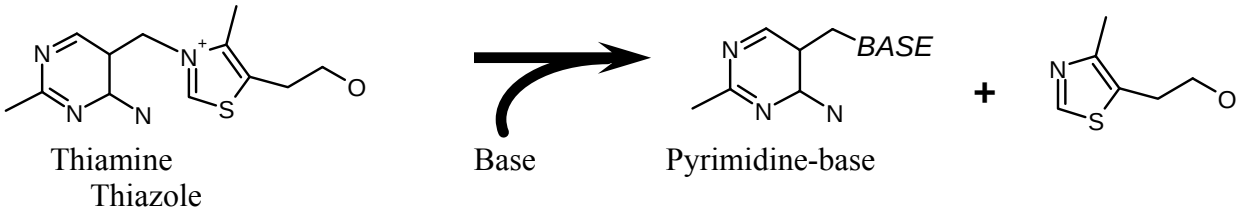

Figure 1.

Two additional biochemical presentations on thiaminase activity were presented. Alewife and rainbow smelt both harbor thiaminase I, but feeding on rainbow smelt appears less likely to produce thiamine deficiency in predators than in fish feeding on alewife. Zajicek et al. (page 17) reported that there is a difference in thiaminase activity in rainbow smelt and alewife related to pH. In smelt, thiaminase activity is reduced at an acidic pH. Thus it appears that the acidic pH of the salmonid intestinal tract might slow the rate of thiamine destruction significantly below optimal activity at higher pH whereas alewife thiaminase remains very active at acidic pH. For this reason, lake trout feeding on rainbow smelt will have reduced thiamine concentrations but not to the same extent as feeding on alewife.

Measurement of thiaminase activity has traditionally used a C^{14}-thiamine method (Zajicek et al. 2005). A novel non-radioactive, colorimetric assay for thiaminase I was recently published (Hanes et al. 2007), although the relative activity of this assay as compared to the radioactive C^{14}-thiamine method has not been reported. The colorimetric reagent, 4-nitrothiophenol (4-NTP) disappearance is measured using a spectrophotometer fitted with 0.2 cm cell (Honeyfield et al. page 21). Regression analysis showed a strong relationship between measurements from the two assays for 49 alewife (R^2= 0.85), 37 smelt (R^2= 0.87) and 18 sculpin (R^2= 0.82). The activity in the colorimetric assay was about 1000 times higher (μmol/g/min) than radioactive method (pmol/g/min) presumably due to the higher affinity of co-substrate 4-NTP. Although it is

recommended that the assay be calibrated against the radioactive assay for each source of thiaminase, potentially this assay would allow more laboratories to measure thiaminase activity.

Thiaminase in Great Lakes Ecosystems

The presence or absence of thiaminase in Great Lakes food webs is a topic currently being investigated. Furthermore, the source of thiaminase in alewife is not known. Honeyfield et al. (2002) isolated *Paenibacillus thiaminolyticus* from alewife, but the results of the study raised even more questions. Logistic regression analysis was used to investigate relationships between the occurrence of thiaminase activity in 39 species of Great Lakes fishes and the phylogeny and ecological characteristics of each species. Fish species that were taxonomically more ancestral were more likely to show thiaminase activity than more derived species. Species that feed at lower trophic levels and occupy benthic habitats also appeared to be more likely to show thiaminase activity (Riley and Evans 2008; Evans et al. page 23).

Practically all larval fish go through a planktivorous stage during their ontogeny. Thiaminase activity in zooplankton potentially would exacerbate thiamine deficiency in lake trout fry that were not fully thiamine replete at hatch. Fitzsimons et al. (page 16) collected zooplankton (>153 um) from Lakes Superior, Michigan and Huron. Thiaminase activity was found in zooplankton from all lakes. Lake Michigan net plankton thiaminase activity was reported to average ten-fold higher than Lakes Huron or Superior. Clearly there are differences in thiaminase activity among the lake sources of net plankton but the consequences of thiaminase on young of the year salmonids or other fishes is currently not known.

Besides alewife, other non-native aquatic organisms are being investigated as potential sources of thiaminase. Tillitt et al. (page 17) reported thiaminase activity of two dreissenid mussel species. In *Dreissena bugensis* the mean thiaminase activity was 110,000 pmol/g/min (range of 33,600-148,100 pmol/g/min). In the second species, *D. polymorpha,* the mean was lower (40,000 pmol/g/min; range of 10,400-47,900 pmol/g/min) These are the highest thiaminase activities measured in any animal tissue and are much higher than any Great Lakes fish species, including alewife. Dreissenid mussels are associated with the recent profound changes observed in Great Lakes food webs. Currently there are no linkages between dreissenid thiaminase activities and thiaminase activities in higher trophic level organisms. Nevertheless, these extremely high thiaminase activities in dreissenids warrant further investigation. In another vein of research, Wright-Osment et al. (page 22) developed a recombinant his-tagged thiaminase I from the *P. thiaminolyticus* thiaminase I gene to quantify the contribution of *P. thiaminolyticus* thiaminase I to the measured thiaminase activity in food chain organisms. Two polyclonal antisera were used. Although the antisera could detect antigens in isolation, none were detected in prey fish, dreissenid mussels, or plankton with confirmed thiaminase activity. The reason for the negative findings is not clear at this time.

Recommendations

The following is the list of recommended research issues developed based on input from the group discussion during the meeting and from the literature.

1. Thiamine

- Determine thiamine status of species consuming large amounts of dreissenids. With the finding that zebra and quagga mussels are high in thiaminase, what, if any, are the effects on thiamine status of lake sturgeon, lake whitefish, freshwater drum and other species known to consume dreissenids?
- Determine the biological consequences of thiamine deficiency.
    - Quantify the secondary effects of thiamine deficiency at the population level.
    - Understand the consequences of gene product changes due to thiamine deficiency.
- Rapid method of thiamine analysis increases number of samples that can be processed in a timely manner, which is good, but eggs with low thiamine are only estimated concentrations. There is a need to modify assay to improve accuracy in eggs with less than 4 nmol/g thiamine as measured by HPLC. A validation process among labs reporting thiamine should be developed.
- Do species suffer from thiamine deficiency if they harbor high levels of thiaminase? If not, why?

2. Thiaminase

- What is the biological role of thiaminase I? Thiaminase II has been found to be a salvage pathway in the synthesis of thiamine.
- Determine source(s) of thiaminase.
    - Investigate the presence or absence of *de novo* thiaminase synthesis in alewife or other clupeids with high thiaminase.
    - Are there species of bacteria other than *Paenibacillus thiaminolyticus* in the environment that produce thiaminase?
    - What is the biological consequence of net plankton thiaminase on planktivores?

- Determine factors that modulate thiaminase activity in alewife. Is there a relationship between alewife food items and their thiaminase activity?

3. Implications of Essential Nutrients other than Thiamine on TDC

- What roles do low vitamin E, astaxanthin, or other antioxidants have in TDC? Identification of low vitamin E in alewife and in lake trout feeding on alewife coupled with what appears to be higher levels of oxidative stress raises many questions. What are the overt or latent affects of low thiamine and other antioxidants and how important are they?

Acknowledgments

We sincerely thank the Great Lakes Fishery Commission and staff for their long-standing support of thiamine deficiency research in the Great Lakes Basin. We especially want to extend our appreciation for the efforts of our speakers and other meeting attendees who took time from their busy schedules to share their work and ideas for TDC research. We thank Scott Miehls and Holly Patrick for their logistical help prior to and during the meeting.

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Abstracts of Presentations

Past, Present and Future: An Overview of Thiamine Deficiency Complex.

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Salmonid fry mortality was first observed in the late 1960s from an unknown cause. In 1994 and 1995 two workshops were sponsored by the Great Lakes Fishery Commission that set the stage for a series of studies to address the cause of the observed fry mortality based on the following questions. Is EMS a single syndrome? Is there a genetic component? Is there a disease component? Is EMS caused by a dietary or water-borne contaminant? Are there signs in adult females? Is there a correlation between egg quality and EMS? In February 1995 we knew that thiamine (vitamin B_1) was some how involved and we had a single HPLC chromatogram demonstrating the separation of three forms of thiamine. The years that followed demonstrated that EMS was caused by thiamine deficiency linked to consumption of thiaminase prey. M74 in the Baltic Sea, Cayuga syndrome in New York Finger Lakes and EMS in the Great Lakes were all caused by thiamine deficiency. The problem was not due to low intake of this essential vitamin, but its destruction. Furthermore thiamine deficiency was affecting adults and the deficiency was associated with numerous secondary affects (immune function, prey capture, growth, gene expression and more). The present workshop addresses outcome of the consumption of thiaminase prey, aspects of the foodweb, and several nuances of thiaminase. Data from Lake Huron affirms the role of alewife in TDC. There remain many unanswered questions. The future is expected to define the biological function of thiaminase I, yield insights into roles of non-native species on native populations and define interactions among disease, essential nutrients and anthropogenic ecosystem alterations as research digs ever deeper into the ecology of aquatic habitats.

Molecular Studies of M74 in Baltic Salmon - From Promoters to Populations.

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We have studied the molecular basis of M74 at several levels ranging from individual Baltic salmon yolk-sac fry to the feeding populations at different parts of Baltic Sea, and from the gene regulatory elements in DNA to the transcriptome and functional proteins. Our and previous results suggest that M74 in Baltic salmon yolk-sac fry results from oxidative stresses disturbing several different developmental pathways. The fry suffering from M74 syndrome can be divided into groups with early onset, intermediate onset and late onset mortality. Each M74-subgroup has a unique gene transcription pattern at the preclinical and clinical stage, which precedes terminal responses characterized by disturbances in the transcription of genes involved in cell cycle, and by progression of cell death. In addition, the intermediate type of M74-mortality is associated

with reduced DNA-binding of the transcription factor HIF-1 (required for normal development of vasculature and red blood cells), reduced production of VEGF protein (regulator of angiogenesis) and decreased capillary density. Since the eggs of M74-offspring-producing females have less thiamine and astaxanthin, and more oxidized fatty acids than eggs of females producing healthy offspring, oxidative stresses which adult salmon experience during their feeding migration may be decisive for the development of M74. Our recent studies demonstrate that regional and temporal differences in the oxidative stress status of feeding populations do occur, and they may help explaining the background behind the variable incidence of M74.

A Review of Sublethal Thiamine Deficiency Effects for Larval Lake Trout.

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A diet-related thiamine deficiency increases acute mortality of Great Lakes salmonines during larval stages but the consequences of the deficiency on other important early life stage processes like growth, foraging efficiency, and predator avoidance that may also increase mortality, are unknown. Accordingly, we investigated the impacts that low thiamine may exert on the specific growth rate (SGR) of first-feeding fry, the ability of first-feeding fry to capture *Daphnia*, fry emergence in the presence of a potential predator (round goby, *Neogobius melanostomus*), and predation by slimy sculpin *Cottus cognatus*, using a combination of thiamine deficient and thiamine replete wild stocks of lake trout (*Salvelinus namaycush*). From these investigations we developed predictive relationships. Specific growth rate (SGR) was related to egg thiamine concentration by an exponential relationship. From this relationship it was predicted that threshold egg thiamine concentrations associated with either a 20 or 50% reduction in SGR from a thiamine-replete stock (Lake Superior), were 8.25 and 5.10 nmol/g respectively. Foraging on *Daphnia* was also related to egg thiamine concentration by an exponential relationship. Similarly, from this relationship, it was predicted that threshold egg thiamine concentrations associated with either a 20 or 50% reduction in *Daphnia* foraging rate from a thiamine-replete stock, were 5.25 and 2.75 nmol/g respectively. The presence of a round goby significantly reduced emergence success in a hatchery stock. However, goby predation was unrelated to initial egg thiamine concentration in the families producing the swim-up fry. In contrast, sculpin predation was related although weakly, to initial egg thiamine concentration. Relative to the effects on growth, foraging, and predation, we conclude that growth effects resulting from thiamine deficiency may represent the most sensitive impact of the three, relative to acute mortality and further that sublethal effects have the potential to seriously impair recruitment. Although we acknowledge that since we used the eggs of feral fish other factors that affect growth but were not measured may also have affected growth, results with the thiamine antagonist oxythiamine support our findings on the relative sensitivity of acute mortality to growth. Recent data from Lake Huron where lake trout recruitment increased following a dramatic decline in alewife abundance, corroborates the relative increased importance of sublethal effects.

Brain Histomorphology of Thiamine Deplete and Replete Lake Trout Swimup Fry.

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The most dramatic example of acute thiamine deficiency in lake trout fry is the presentation of Early Mortality Syndrome (EMS). Observable behavioral symptoms can begin soon after hatch and by swim-up, if left untreated, result in mortality. It has been well documented that thiamine deficiency complex and the related M74 pathologies show nuclear condensation, and nuclear fragmentation in the optic lobe, hypothalamus, and cerebellum. How these known pathologies relate to overall brain histomorphology is unknown. Recent studies of salmonine fry suggest that sub-lethal, long-term behavioral effects (prey capture and predator avoidance) occur from low thiamine. If brain histomorphology is different between thiamine replete and deplete fry, can this be a possible explanation for altered behavior. The objective of this study was to quantify brain morphological differences between thiamine-deficient and thiamine-replete swim up lake trout. In the fall of 2006, individual batches of eggs were collected from 16 mature female lake trout at Clay Banks, Wisconsin in Lake Michigan. Each batch of eggs was split into two groups; one group was treated with an allithiamine bath, and the other was left untreated. Although several developmental stages were collected from each egg batch, only treated/untreated eggs from female number 2, at 890 Degree days, was used for analysis. Female number 2 was chosen because total thiamine levels for the untreated eggs, using the rapid method, were relatively low at 3.1 nmol/g of eggs. Eggs were immersion fixed in 4% paraformaldehyde, 4% glutaraldehyde, in 0.1M cacodylate buffer, dehydrated in graded EtOH series, transitioned to propylene oxide, and infiltrated and embedded in Embed-812 resin. Sectioning was done on a Reichert Jung Ultracut E, using a DiATOME diamond knife. One micrometer sections, taken every 10^{th} section mounted on a slide, 5 sections/slide for 100 slides, totaling 500 mounted sections representing 5mm of sample. Sections were stained with Toluidine Blue, images were taken on a Nikon Eclipse 80i with a Diagnostic Instruments Spot Insight digital camera. IMOD, copyright© 1994-2008 by the Boulder Laboratory for 3-Dimensional Electron Microscopy of Cells and the Regents of the University of Colorado, was used for image alignment and construction of the 3-D models. Two models were constructed, one representing whole brain, the other modeling olfactory nerve, olfactory lobe, cerebrum, optic nerve, optic lobe, hypothalamus, pituitary, and cerebellum. Results in the thiamine depleted fry show a 11.63% reduction in the optic nerve volume, a 26.45% reduction in hypothalamus volume, a 20.89% reduction in cerebellum volume, and a 5.48% reduction in overall brain size. Results in the thiamine replete fry show a 8.59% reduction in olfactory nerve volume, 17.94% reduction in the olfactory lobe volume, and a 26.89% reduction in the pituitary volume. The cerebellum functions to regulate and coordinate movement and balance. The 20.89% reduction in the cerebellum volume may further explain the TDC associated symptoms of spiral swimming, lack of coordination, disorientation, spasms, and ataxia. Low relative cerebellar volume, if maintained into adult (ratio cerebellum volume/total brain volume; treated 0.1169, depleted 0.0898), may also help explain the sub-lethal, long-term behavioral effects (prey capture and predator avoidance) that have been noted by other researchers. Lastly, the disparity in the size of the hypothalamus and pituitary may suggest other developmental problems with thiamine deficient fry. We plan to investigate growth, reproductive and metabolic aspects of adult fish after further examination of the pars distalis of the pituitary glands of both untreated and treated fry. We also plan to use this method to create 3-D histomorphology models of the thymus of thiamine replete and thiamine deplete fry. This method combines detailed quantitative cellular histology, with quantitative 3-D modeling, so direct comparisons can be made between cellular alterations and its effects on organ structure.

The Role of Interaction between Dietary Sources of Vitamin B1 (Thiamine) and Magnesium in Inducing Early Mortality Syndrome (EMS) in the Lake Trout (*Salvelinus namaycush*).

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The evidence in mammals suggests that thiamine deficiency in diet is exacerbated by low magnesium. This relationship was not examined in fish. Therefore, the purpose of this study was to investigate the effects of dietary thiamine and magnesium (Mg) levels in lake trout to better understand the mechanism leading to early mortality syndrome (EMS). Progenies from 3 lake trout females with high levels of thiamine (6.4±2.8 nmol/g) in the unfertilized eggs, collected in L. Michigan, were combined for this feeding trial. Semi-purified diets were prepared to accomplish 2 × 2 factorial design (thiamine × Mg) that were either devoid or supplemented with thiamine and/or Mg (TM++, TM+-, TM-+, and TM--, respectively). A commercial starter diet was also used as the reference diet. Lake trout alevins at the swim-up stage were distributed to glass tanks at the density of 99 fish/tank (four replicates per treatment). All groups were fed one of the four experimental or a commercial diet beginning at the swim-up stage and terminated after 7 week duration. At the completion of the feeding trail, fish fed TM+- showed the highest growth performance among treatments. The fish fed semi-purified diets showed a high mortality (34-65%) compared to the fish fed a commercial diet (4%). Among those semi-purified diet groups, TM-- treatment had an increased mortality rate (65%) in comparison to other treatments (34-37%). Fish were sampled for analysis of thiamine and Mg and for histopathological observation of the brain and retina. In our recent studies on effects of thiamine deficiency in lake trout alevins at swim-up stage, we found that in some affected individuals all strata of the brain, olfactory lobe and retina contained necrotic neurons. The glycogen depletion in the liver of alevins was also noticed when thiamine levels in unfertilized eggs were below 0.73 nmol/g. The histopathological results in the analyzed fish in response to the dietary thiamine and/or Mg deficiencies will be discussed in the future.

Exploring the Relationship between Lake Trout Egg Thiamine Concentration and Fry Survival in Lake Michigan from 1996 through 2003.

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Low levels of egg thiamine in Great Lakes salmonines result in decreased survival of salmonine fry between hatch and first-feeding. We quantified the relationship between egg thiamine levels in lake trout (*Salvelinus namaycush*) and fry mortality from lake trout collected near Sturgeon Bay, WI, Lake Michigan. From 1996 through 2003, eggs from adult female lake trout were collected and fertilized by sperm pooled from several males. Eggs from 124 individual females were collected, and fry were reared to approximately 1100 cumulative temperate units. The percentage of surviving fry and observations of symptoms of Thiamine Deficiency Complex were collected as response variables. Total egg thiamine concentration was determined by HPLC and was used as the explanatory variable. Fry survival was variable and ranged from 0% to 100%. Mean total thiamine concentration in egg lots (family groups) with EMS was 0.76 nmol/g compared to 4.4 nmol/g in egg lots with no EMS. Regression techniques were used to quantify the relationship between egg thiamine and mortality. This monitoring data serves as a reference point for fishery biologists and assisted in establishing the relationship between thiamine deficiency and mortality. Our analysis will help managers assess the risk of mortality associated with specific levels of thiamine in lake trout eggs.

Factors Affecting Thiaminase Activity and Thiamine Concentration in Great Lakes Net Plankton.

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The finding of relatively high thiaminase activity in zooplankton based on limited sampling in Lake Ontario and the Finger Lakes raised the possibility for thiamine deficiency in larval fish in the Great Lakes. Practically all larval fish go through a planktivorous stage during their ontogeny. There was however, little information on either spatial or temporal variability of thiaminase activity in zooplankton or the factors responsible. To address these deficiencies we collected zooplankton (>153 um) from two depths (1 and 30 m), in spring and summer, from ten separate locations in each of lakes Superior, Michigan and Huron. Samples were analyzed for species composition, thiaminase activity, thiamine, stable isotopes, carotenoids, lipid, and fatty acids. For each collection water samples were also collected for chlorophyll A, nutrients, temperature, and water clarity as well as phytoplankton and zooplankton species composition and abundance. Thiaminase activity was found in zooplankton from all lakes, bit it was only in Lake Michigan where it was consistently detected on both dates and at both depths, and where the highest overall activity was observed. Thiaminase activity in zooplankton from Lakes Michigan was on average ten-fold higher than in zooplankton from lakes Huron or Superior. In contrast, thiamine concentrations in zooplankton from lakes Michigan and Huron were on average two fold higher than for Lake Superior. Thiaminase activity across all lakes was correlated with measures of water clarity, nutrients, thiamine, carotenoids, and fatty acids. Of these variables, we found thiaminase activity was related to chlorophyll A, nitrogen, thiamine, total carotenoids, pentadecanoic acid, 11-octadecenoic acid, and the sum of omega 3 and polyunsaturated fatty acids using stepwise regression analysis. Similarly thiamine concentration across all lakes was correlated with measures of water clarity, chlorophyll A, nutrients, $\delta^{15}N$, thiaminase activity,

carotenoids, and heptadecanoic acid. Of these variables, we found thiaminase activity was related to water clarity, chlorophyll A, $\delta^{15}N$, nitrogen, total carotenoids, and heptadecanoic acid. Although this work highlights the potential for relatively high dietary exposure to thiaminase by larval fish, particularly in Lake Michigan, additional work is required to understand the implications to their thiamine status.

Dreissenid Mussels from the Great Lakes Contain Elevated Thiaminase Activity.

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Dreissenid mussels are considered the major causative factor for the profound changes observed in food webs and energy pathways in Great Lakes ecosystems over the past two decades. Here we present evidence that two Dreissenid mussel species (*Dreissena bugensis* and *D. polymorpha*) contain thiaminase activity that is 5-100 fold greater than observed in Great Lakes fishes. Thus, Dreissenids constitute a significant and previously unknown pool of thiaminase in the Great Lakes food web compared to other known sources of this thiamine (vitamin B_1) -degrading enzyme. Thiaminase in forage fish of the Great Lakes has been causally linked to thiamine deficiency in salmonines. We currently do not know if there are linkages between thiaminase activities observed in Dreissenids and the thiaminase activities in higher trophic levels of the Great Lakes food web. However, the extreme thiaminase activities observed in Dreissenids from the Great Lakes may represent a serious unanticipated negative effect of these exotic species on Great Lakes ecosystems.

The pH-Dependence of Thiaminase Activity from Great Lakes Forage Fish.

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The sources of thiaminase in the Great Lakes food web remain unknown. We investigated the pH-dependencies of the thiaminase I activities observed in forage fish to provide insights into potential thiaminase sources and to optimize catalytic assay conditions. We measured thiaminase I activities of crude extracts made from five forage fish species and two strains of bacteria over a range of pH. ^{14}C-Thiazole-labeled thiamine and nicotinic acid were used as substrate and cosubstrate, respectively. The clupeids, alewife *Alosa pseudoherengus* and gizzard shad

Dorosoma cepedianum, had very similar thiaminase I pH-dependencies with apparent maximal activities between pH 5.0 and 5.4. Rainbow smelt *Osmerus mordax* and spottail shiner *Nortropis hudsonius* had maximal activities between pH 5.5 and 6.4. In contrast, the thiaminase I activity pH-dependence profiles of two cultured strains of *Paenibacillus thiaminolyticus* were identical, yet very different from the forage fish. The profiles for the bacteria each had two apparent maxima at pH 6.1 and 6.5 and sharp transitions between low and high activities with 50% maximal activity over a very narrow range of pH between 6.1 and 6.8. Incubation of *P. thiaminolyticus* extracts with extracts from bloater *Coregonus hoyi* (normally bloater contain low or no detectable thiaminase I activity) caused the pH-dependence profile of *P. thiaminolyticus*-derived thiaminase I to resemble that of the rainbow smelt and spottail shiner. Therefore, fish extracts can alter the pH profile of bacterial thiaminase I activity, which has implications on our understanding of the source of thiaminase in the Great Lakes food web.

The Ecological Role of Thiaminase in Aquatic Ecosystems: Mechanistic Insights from Molecular Biology.

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Recent discoveries in molecular biology provide insights that should advance our understanding of the role of thiaminase I in an ecological context, particularly with regard to environmental conditions likely to produce high thiaminase levels in fishes and other organisms. The first key insight comes from recognizing thiaminase II (more appropriately referred to as TenA) as a salvage enzyme involved in thiamine synthesis by microbes. The primary substrate for TenA was described in 2005 as an environmental breakdown product of thiamine (formylaminopyrimidine), not thiamine itself. This discovery of the function of thiaminase II has led several authors to speculate that thiamine is not the target substrate for thiaminase I, a view bolstered in a 2008 publication showing that both TenA and thiaminase I function in a similar mechanistic fashion. The second line of evidence that the primary function of thiaminase I is associated with thiamine synthesis, not thiamine degradation, comes from microbial gene sequences. In two published whole genome microbial DNA sequences, the gene for thiaminase I was located on the chromosome adjacent to the gene for ThiD, a key enzyme involved in thiamine synthesis (specifically, pyrimidine phosphorylation). It is also potentially relevant that sequences for TenA and thiaminase I have not been found in the same organism, suggesting that they provide similar functions that are not duplicated. The third key insight comes from the recent discovery of a new protein expression regulatory mechanism involving "riboswitches", short sequences of RNA that shut down gene expression in the presence of a substrate. One of the first and best characterized riboswitches is the thiamin pyrophosphate binding riboswitch (THI- box) that regulates thiamine synthesis, i.e. shutting down microbial thiamine synthesis in the presence of thiamine pyrophosphate. This gene regulation mechanism could help explain early (1960s) lab studies of *Paenibacillus thiaminolyticus* that showed repression of thiaminase I production in the presence of thiamine. Although these molecular insights do not yet point to a clear role for the presence of thiaminase I in nature, they strongly suggest that environmental conditions leading to the production of thiaminase I will only be understood by recognizing that this enzyme proliferates under localized conditions in which thiamine is scarce and thiamine synthesis is occurring.

Quantitative PCR Assays for the Bacterial Thiaminase I Gene and the Thiaminase-Producing Bacteria *Paenibacillus thiaminolyticus.*

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The thiaminase I enzyme produced by the Gram-positive bacterium *Paenibacillus thiaminolyticus* isolated from viscera of Lake Michigan alewife *Alosa pseudoharengus* currently is the best-established source of thiaminase linked to thiamine (vitamin B_1) deficiency in early mortality syndrome (EMS) in larvae of Great Lakes salmonines. Diets of alewife or isolated strains of *P. thiaminolyticus* mixed in a semipurified diet and fed to lake trout have been shown to produce EMS in fry. In order to aid studies of the sources of *P. thiaminolyticus* and thiaminase I, we have developed and characterized quantitative PCR assays for the thiaminase I gene and the 16S rRNA gene of *P. thiaminolyticus*. Sequencing of the thiaminase I gene from four strains of *P. thiaminolyticus* isolated from Great Lakes alewife gut showed that the four genes were identical to each other, but were not identical to the previously sequenced strain NCIB9632, from which the thiaminase I gene was first isolated. Sequencing of the 16S rDNA confirmed that the Great Lakes alewife strains are *P. thiaminolyticus*. Quantitative PCR (Q-PCR) assays were established to detect most bacterial 16S rDNA, 16S rDNA of *P. thiaminolyticus* and a few closely related taxa, and the thiaminase I gene. The Q-PCR assays are linear over at least four orders of magnitude and are predicted to detect the thiaminase I gene of *P. thiaminolyticus* from as few as 1000 *P. thiaminolyticus* cells/sample. This method is expected to be adaptable to many sample types, including fish tissue, water and soil.

Chinook Tissue Thiamine and Fatty Acids after Fasting 150 Days at 5 C.

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The effect of winter environmental conditions on the nutritional status of Lake Michigan Chinook salmon has not been investigated extensively. A laboratory study was conducted to determine the effect of fasting for 150 days in cold water (5 C) on tissue stores of fatty acids and thiamine. Body weight and length of fish did not change during the study but tissue lipid and thiamine decreased. Percent water in whole fish and muscle increased (linear $P< 0.0001$; quadratic $P< 0.0001$) with time where as percent lipid decreased. The omega-3 fatty acid, C22:6n3, increased in whole body tissue (linear $P<0.0307$; quadratic $P<0.0349$) during 150 day study. The change in concentration of fatty acids was more prevalent in muscle than in liver or whole body tissues. Experimental simulated over-winter loss of thiamine was calculated to be 6.8-10.6 pmol/g/day in muscle and 73-110 pmol/g/day in liver tissue. Thus thiamine status in Chinook would naturally be lower in the spring. These changes in nutritionally important components could adversely affect survival.

Alewife Abundance Affects Thiaminase Impacts on Reproduction by Lake Trout in Lake Huron.

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Thiamine Deficiency Complex (TDC) results in high levels of early life stage mortality of lake trout and may be a significant impediment to restoration of lake trout populations in the Laurentian Great Lakes. Invasive alewives and rainbow smelt contain high concentrations of thiaminase, make up an important part of the diet of Great Lakes salmonines, and are thought to be an important vector of thiaminase to lake trout. A recent drastic decline in alewife abundance in Lake Huron has been followed by increases in thiamine levels in lake trout eggs and widespread evidence of natural reproduction by lake trout. Similar changes have not been observed in Lake Michigan, where alewives remain abundant. Our results support the contention that alewives may be an important vector of thiaminase to Great Lakes lake trout.

Differences in Thiamine Egg Levels Measured by HPLC and the Rapid Method.

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Thiamine concentrations have been routinely analyzed in eggs of several species (e.g., lake trout, coho salmon, Chinook salmon, Atlantic salmon, walleye) from the Great Lakes to evaluate potential thiamine deficiency leading to Thiamine Deficiency Complex. Currently, two methods are used in research laboratories to determine the thiamine concentration, high performance liquid chromatography (HPLC, Brown et al. 1998) and the rapid solid-phase extraction fluorometric method (RSPE, Zajicek et al. 2005). In this presentation, we will compare both methods and stress their limitations. Although the HPLC method is reliable and accurate, discrepancies among laboratories have been observed. The RPSE method appears to be a cheap and reliable method, however, a correction factor is required at low thiamine concentrations to ensure comparison to the HPLC method.

POSTER ABSTRACTS

Comparison of Thiaminase I Activity in Prey Fish Measured by Two Methods.

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A novel non-radioactive, colorimetric assay for thiaminase I was published in 2007, though the relative activity of this assay as compared to the radioactive C^{14}-thiamine method has not been reported. The standard radioactive assay has been optimized to measure thiaminase I activity associated with thiamine deficiency in aquatic top predators. The objective of the study was to measure and compare thiaminase activity by both methods in Lake Ontario alewife, rainbow smelt, and sculpin. In order to produce repeatable results several modifications were made to the published colorimetric assay, including a change in pH, sample clean-up, assay temperature control, an increase in concentration of color reagent, 4-nitrothiophenol (4-NTP) and use of a spectrophotometer fitted with 0.2 cm cell. Regression analysis showed a strong relationship between measurements from the two assays for 49 alewife (R^2= 0.85), 37 smelt (R^2= 0.87) and 18 sculpin (R^2= 0.82). However, relative activity of the assays and the slope of the lines differed. The activity in the colorimetric assay was about 1000 times higher (µmol/g/min) than radioactive method (pmol/g/min) and the slope of the line for sculpin was steeper than for alewife or smelt. This difference may be reflective of the higher affinity of 4-NTP in the reaction than the co-substrate used in the radioactive assay. No 4-NTP thiaminase activity was found in bloater, lake trout, steelhead or chinook salmon. We also evaluated changes in thiaminase I activity from a known microbial source, *Paenibacillus thiaminolyticus* bacteria. In all cases the 4-NTP assay results reflected the previously observed trends in thiaminase I production. However, at present time we cannot explain the observed relative differences in thiaminase activity among species of fish, therefore recommend that the assay be calibrated against radioactive assay for each source of thiaminase.

Nutritional Composition of Lake Ontario Prey Fish.

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Research has shown that thiamine deficiency is a significant impediment to salmonine reproduction but little is known about other essential nutrients. Thiamine and thiaminase content of alewife and rainbow smelt have been reported but we lack information in other prey fish found in Lake Ontario. The content of other vitamins and fatty acids in Lake Ontario prey fish have not been reported. The objective of the research was to collect the major species of Lake Ontario prey fish (alewife, rainbow smelt, round goby, three spine stickleback, emerald shiner, spottail shiner, slimy sculpin, lake herring and round whitefish for nutritional component analysis. Prey

fish were collected from four primary sites; east – eastern area between Fair Haven and Oswego, Rochester - near Rochester, 30 Mile Island – near Olcott and north – near Toronto. Not all target species could be collected. In 2006, samples collected included 48 alewife, 32 smelt, 24 sculpin, 12 goby, and 8 stickle-back. Alewife total thiamine (13.6 nmol/g) was higher than other species (6.2- 9.0 nmol/g). Unexpected high thiaminase activity (pmol/g/min) within goby (12,491), sculpin (1,988) and smelt (9,238) was found. Samples for thiaminase collected in 2007 also found thiaminase activity in goby. In contrast with 2006 data, lower thiaminase activity was recorded in smelt. Thiaminase activity in 2006 alewife (6,315) and stickle back (55) was within normal ranges. Tocopherol (ng/mg) was low in alewife (1.59) compared to goby (12.74), sculpin (25.29), and smelt (22.81). No stickle back sample was available for tocopherol content or other vitamins. Differences existed among species content of retinol, retinoic derivatives, fatty acids and carotenoids. Astaxanthin was low and similar among species (0-0.31 ng/mg). This study found important difference in thiamine, tocopherol, retinol, carotenoids and fatty acids among prey fish species. This suggests that other nutritional deficiencies may be influencing the health and well-being of Lake Ontario salmonines.

Screening Method for Bacterial Thiaminase I Protein in Extracts of Fish, Dreissenid Musssels and Plankton.

Maureen K. Wright-Osment[1]*, Catherine A. Richter[1], James L. Zajicek[1], Stephen C. Riley[2], Allison N. Evans[3], S. Jerrine Nichols[2], Jacques Rinchard[2], Charles C. Krueger[3] Dale C. Honeyfield[4], John D. Fitzsimons[5], and Donald E. Tillitt[1]

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The thiaminase I enzyme produced by the gram-positive bacterium *Paenibacillus thiaminolyticus* has been implicated as one of the causative factors of thiamine (vitamin B_1) deficiency in Great Lakes salmonines. Efforts to quantify the contribution of *P. thiaminolyticus* thiaminase I to the measured thiaminase activity in food chain organisms in the Great Lakes region have been hampered by an inability to detect and quantify this specific protein. To accomplish this, we developed a recombinant his-tagged thiaminase I from the *P. thiaminolyticus* thiaminase I gene. Synthetic antigenic peptides derived from the known amino acid sequence of thiaminase I, and the purified recombinant protein were used to produce two polyclonal antisera. The antisera were used in western blots to screen protein extracts from samples of fish, dreissenid mussels, and plankton with known thiaminase activities. Both antisera recognized bacterial thiaminase I, detecting as little as 4 ng of the purified recombinant protein. Both antisera also detected a protein of similar molecular weight in extracts of *P. thiaminolyticus* and in fish tissue samples spiked with recombinant bacterial thiaminase I protein. Thus, the anti-thiaminase western blot assay is sensitive and specific for thiaminase I protein produced by *P. thiaminolyticus*. However, neither antiserum consistently exhibited specific binding to a protein of the expected molecular weight in unspiked samples of alewife, dressenids, or plankton even when thiaminase activity was present. This unexpected result suggests the existence of non-*P. thiaminolyticus* sources of thiaminase activity.

Tocopherol(s) in Early Life History of Lake Trout.

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The purpose of this study was to investigate the relationship between thiamine and tocopherol concentrations in lake trout as the plausible explanation to physiological causes of early mortality syndrome (EMS). Eggs from 32 and 11 females captured in L. Michigan in 2006 and 2007, respectively, were fertilized individually with the sperm of several males. The embryos were transported to Columbus laboratory and incubated in a semi-recirculation system at 5-9˚C. Eggs were collected after ovulation prior to fertilization for thiamine and tocopherol analysis (HPLC system using fluorescent detector), and then during characteristic stages of embryonic development. Fractional cumulative mortality (FCM) was assessed for the following periods of early life history: fertilization to eyed-embryo, eyed-embryo to hatching, hatching to swim-up, and swim-up until 42 days. Thiamine and tocopherol concentrations in unfertilized eggs showed positive correlation in both years. FCM in lake trout alevin correlated with tocopherol as well as thiamine levels in the unfertilized eggs. At the 50% FCM between swim-up and 42 days, total thiamine and α-tocopherol concentrations amounted to 0.82 nmol/g and 165.5 μg/g, respectively, in unfertilized egg. Stored thiamine and tocopherol concentrations in lake trout eggs were dramatically decreased at the swim-up stage and at the hatching stage, respectively. We conclude that tocopherol is positively impacting enhanced thiamine concentrations. This is contrary to observations made with L. Michigan coho and chinook salmon eggs viability (negative relationship). We hypothesize that tocopherol would not directly influence the outbreak of EMS, but will indirectly influence the utilization and function of thiamine. A further study needs to clarify the relationship between thiamine and tocopherol and its derivatives for better understanding of EMS.

Thiaminase Status is Related to Phylogeny and Ecological Characteristics of Great Lakes Fishes.

Allison N. Evans, Great Lakes Fishery Commission, Ann Arbor, MI (Present address: Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR)
Stephen C. Riley, USGS/BRD Great Lakes Science Center, Ann Arbor, MI

Although thiaminase has been found in a variety of Great Lakes fishes, the ultimate source of thiaminase in Great Lakes fishes is currently unknown. We used logistic regression analysis to investigate relationships between the occurrence of thiaminase activity in 39 species of Great Lakes fishes and the phylogeny and ecological characteristics of each species. Fish species that were taxonomically more ancestral were more likely to show thiaminase activity than more derived species. Species that feed at lower trophic levels and occupy benthic habitats also appeared to be more likely to show thiaminase activity; these variables were correlated with taxonomy, which was the most important predictor of thiaminase activity. Further analyses of the relationship between quantitative measures of thiaminase activity and the ecological characteristics of Great Lakes fish species would provide greater insight into potential sources and pathways of thiaminase in Great Lakes food webs.

Possible Juvenile Lake Trout Recruitment Failure in Lake Ontario Resulting from a Dreissenid-Facilitated Diet Shift Causing Thiamine Deficiency.

John Fitzsimons[1] and Dale Honeyfield[2]

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Declines in *Diporeia* in Lake Ontario associated with colonization of the lake by dreissenids were associated with a decline in slimy sculpin, historically the primary forage species for juvenile lake trout. Alewives likely replaced sculpins in the diet, and while little is known of the effects of an alewife diet on juvenile lake trout thiamine status, there has been a catastrophic decline in survival indices of juvenile lake trout in recent years for which the causes remain unclear. To assess the current thiamine status of Lake Ontario lake trout we used muscle thiamine concentrations to evaluate the ontogeny of the thiamine deficiency during 2005-2006, and assessed the effect of diet, basin, and season. We compared thiamine levels in Lake Ontario lake trout to lake trout from Lake Superior where alewives are considerably less abundant, and to lake trout in Spray Lake in Alberta, where alewives are absent. Diet was determined using a combination of stomach contents and stable isotope mixing models. Thiamine levels were related to known threshold effect levels. Based on our results, once juvenile lake trout leave the hatchery, they experience a dramatic decline in thiamine status related to but not directly proportionate to the proportion of alewives in the diet. Mixing models confirmed that alewives were the major forage fish and that sculpins contributed very little to the diet. Thiamine levels in juvenile lake trout at ages as young as 2+ approached but generally did not fall below threshold effect level for acute mortality. Since acute mortality is a relatively insensitive measure of thiamine deficiency effects, we suspect that other sublethal effects may be occurring (e.g. reduced, growth, foraging, predator avoidance) but more work is required to confirm this supposition.

Thiamine Status of Lake Ontario American eel *Anguilla rostrata*: Could a Thiamine Deficiency Be Contributing to Population Decline?

John Fitzsimons[1], Scott Brown[2, 6], Lisa Brown[2], Guy Verreault[3], Jesse Lepak[4], and Ken Drouillard[5]

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[6]Deceased

The Lake Ontario-St. Lawrence River population of the American eel (*Anguilla rostrata*) is in a state of serious decline and in danger of extinction. Because eels are panmictic, conditions in this population have the potential to affect the species throughout its range. Several hypotheses have been advanced to explain this decline but none satisfactorily explains the current state of the

population. We assessed the potential that a diet-induced thiamine deficiency associated with consumption of alewives (*Alosa pseudoharengus*), and that already affects salmonines throughout the Great Lakes Basin, could be affecting eels as well. We collected eels throughout the Lake Ontario-St. Lawrence corridor, both before, during, and after residence in Lake Ontario, and analyzed muscle samples for thiamine, mirex, and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$). We used muscle mirex concentrations to assess relative period of residence in Lake Ontario and used muscle stable isotope analysis to infer diet within Lake Ontario using mixing models. Our results indicate that with increased time spent in Lake Ontario, there is a co-occurring decline in thiamine status which remains relatively unchanged during the downstream migration from Lake Ontario to the Gulf of St. Lawrence. Alewives appear responsible for the thiamine status of Lake Ontario eels based on stable isotope analysis and the decline in thiamine is probably related to the high thiaminase content of Lake Ontario alewives. Similar results were obtained for Chesapeake Bay eels where diet was also dominated by thiaminase-rich prey. For eels in the Gulf of St. Lawrence about to begin a 3000 km spawning migration to the Sargasso Sea during which no feeding occurs, mean thiamine levels were approximately one third that of eels that had apparently not been feeding on alewives. Moreover, thiamine levels in alewife consuming eels were just above the threshold for pathological effects. With the likely further decline in thiamine levels during migration since maintaining swimming activity requires the thiamine dependent enzyme pyruvate dehydrogenase, additional effects on growth, reproduction and survival seem highly likely.

Roster of Attendees to EMS Task Meeting, Ann Arbor, MI, November 6-7, 2008

NAME	ORGANIZATION	LOCATION	EMAIL
Jean Adams	USGS Great Lakes Science Center	Antigo, WI	jvadams@usgs.gov
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Agenda EMS Workshop November 6-7 2008, Ann Arbor, MI
Sponsored by the Great Lakes Fishery Commission

November 6, 2008
Poster presentations
Dale Honeyfield……. Comparison of thiaminase I activity in prey fish measured by two methods.
Dale Honeyfield……..Nutritional composition of Lake Ontario prey fish.
Wright-Osment……Screening method for bacterial thiaminase I protein in extracts of fish, dreissenid musssels, and plankton.
Bong Lee ……Tocopherol(s) in early life history of lake trout.
Allison Evans…….Thiaminase status is related to phylogeny and ecological characteristics of Great Lakes fishes.
John Fitzsimons…….Possible juvenile lake trout recruitment failure in Lake Ontario resulting from a dreissenid-facilitated diet shift causing thiamine deficiency.

November 7, 2008
Oral presentations
8:00 Honeyfield/Tillitt/Riley – Welcome/Introduction/Over-view to the TDC Workshop.
8:30 Kristiina Vuori – Molecular studies of M74 in Baltic salmon - from promoters to populations.
9:00 John Fitzsimons – A review of sublethal thiamine deficiency effects for larval lake trout.
9:20 Jeff Allen – Brain histomorphology of thiamine deplete and replete lake trout swimup fry.
9:40 Bong Joo Lee – Effects of dietary thiamine and magnesium on survival, growth and histopathologies in lake trout alevins fed starter diets.

10:00 BREAK
10:20 Allison Evans – Exploring the relationship between lake trout egg thiamine concentration and fry survival in Lake Michigan.
10:40 John Fitzsimons – Factors affecting thiaminase activity and thiamine concentration in Great Lakes net plankton.
11:00 Don Tillitt – Dreissenids from the Great Lakes contain elevated thiaminase.
11:20 Jim Zajicek – The pH-dependence of thiaminase activity from Great Lakes forage fish.
11:40 Cliff Kraft – The ecological role of thiaminase in aquatic ecosystems: mechanistic insights from molecular biology.

12:00 – 1:30 LUNCH
1:30 Cathy Richter – Quantitative PCR assays for the bacterial thiaminase I gene and the thiaminase-producing bacteria Paenibacillus thiaminolyticus.
1:50 Dale Honeyfield – Chinook over-winter changes in thiamine and fatty acids.
2:10 Stephen Riley – Lake trout egg thiamine monitoring in lakes Huron and Michigan.
2:30 Jacques Rinchard – Differences in thiamine levels measured by HPLC and the rapid method.
3:00 BREAK
3:30 – 5:00 Wrap-up discussions