AN ABSTRACT OF THE DISSERTATION OF

Allison N. Evans for the degree of Doctor of Philosophy in Fisheries Science presented on August 25, 2016.

Title: The Ecophysiology of Thiamine Deficiency Complex: Evaluating Sources of Thiaminase in Great Lakes Food Webs

Abstract approved:

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Thiamine (vitamin B_1) is required by all living organisms for carbohydrate metabolism and synthesis of amino acids. Thiamine deficiency is responsible for several related classes of early life stage mortality disorders in salmonines, including Thiamine Deficiency Complex (TDC) in the Laurentian Great Lakes, Cayuga Syndrome in the Finger Lakes, and the M74 in the Baltic Sea. TDC is caused not by lack of sufficient intake of thiamine but rather by a diet rich in prey that contain thiaminase, a thiamine-degrading enzyme. TDC caused by ingestion of thiaminase-containing prey occurs in these wild fish populations and has also been observed in mink, foxes, seals, alligators, chickens, and ruminants.

TDC is one of several impediments to rehabilitation of Lake Trout (*Salvelinus namaycush*), the native apex predator in the Great Lakes. The proximate cause of TDC is known to be the ingestion of prey fish containing high levels of thiaminase, specifically Alewife (*Alosa pseudoharengus*) and Rainbow Smelt (*Osmerus mordax*); however, the ultimate source of thiaminase in thiaminase-containing fishes remains unknown. Evaluating potential sources of thiaminase in fishes is essential for understanding thiaminase trophodynamics and developing management strategies for mitigating TDC and facilitating rehabilitation of Lake Trout populations. Thiaminase in prey fishes is hypothesized to originate from two possible sources: either thiaminase-containing fishes acquire thiaminase from their diet (dietary acquisition hypothesis) or thiaminase-containing fishes make the thiaminase enzyme themselves (*de novo synthesis* hypothesis); these two hypotheses are not mutually

exclusive. Chapter 1 describes the causes of TDC and why characterization of the sources of thiaminase in aquatic food webs is necessary.

The three primary data chapters presented herein describe whole food web investigations that seek to determine the source of thiaminase in Great Lakes fishes. In Chapter 2, zooplankton were evaluated as a potential source of dietarily acquired thiaminase activity by comparing the thiaminase activity in bulk zooplankton to the zooplankton community composition. Three types of multivariate analysis revealed one candidate source of thiaminase activity, *Ploesoma*, an omnivorous loricate rotifer. Despite the apparent correlation between *Ploesoma* biomass and thiaminase activity in bulk zooplankton, *Ploesoma* spp. constituted sufficiently low biomass that its thiaminase activity would have to be extraordinarily high (i.e., at unprecedented levels) to constitute the major source of thiaminase in Great Lakes food webs. Furthermore, *Ploesoma* was never observed in the diet of thiaminase-containing fish. Therefore *Ploesoma* is an unlikely source of thiaminase. No other component of the zooplankton community was consistently related to thiaminase activity.

In Chapter 3, the thiaminase activity in fishes was assessed in relation to species, season, depth, and location of capture, and the dietary acquisition hypothesis was assessed directly using stomach content and fatty acid signature analysis. Thiaminase activity was higher in spring than in summer and fall. Round Goby (*Neogobius melanostomus*) thiaminase activity was higher than previously reported, and Slimy Sculpin (*Cottus cognatus*) thiaminase activity was highly variable, sometimes exceeding that of Rainbow Smelt. The dietary acquisition hypothesis was evaluated by comparing the thiaminase activity in fish viscera to both the diets and fatty acid signatures of fishes. No compelling evidence that thiaminase activity of fish viscera was consistently the result of consumption of any specific prey taxa was found using three types of multivariate analyses. Indicator Species Analysis suggested *Bythotrephes* as a potential candidate source of thiaminase, but *Bythotrephes* was not consumed by some fishes with high thiaminase activity. If the source of thiaminase activity is dietary, the source may be *Bythotrephes* for some fishes, but those that did not consume *Bythotrephes* would need to obtain thiaminase from a different source. Fatty acid analysis suggested a moderate tendency for higher thiaminase activity in pelagically-feeding rather than benthically-feeding

fishes, but several pelagically feeding fishes contained undetectable thiaminase activity and some benthically feeding fishes contained thiaminase activity comparable to or greater than pelagically feeding fishes. Together, this evidence indicated that no specific prey item was consistently related to thiaminase activity.

In Chapter 4, the *de novo* hypothesis was assessed directly using two approaches. The first approach compared the biochemical characteristics of thiaminases in Alewife, Carp (*Cyprinus carpio*), quagga mussels (*Dreissena rostriformis bugensis*), and a bacterium (*Paenibacillus thiaminolyticus*) that has been isolated from the intestines of Alewife and is known to produce thiaminase. Thiaminases in these four organisms vary in their mass, migration characteristics, tolerance to denaturation, and isoelectric points, suggesting that the source of thiaminase differs in these four taxa. The second approach identified candidate thiaminase genes in fishes using existing information from small peptide fragments from two fish thiaminases from partially purified from Carp and Red Cornetfish (*Fistularia petimba*). Candidate genes in Carp, Zebrafish (*Danio rerio*), and Alewife that were homologous to the known peptide fragments were identified, synthesized, and overexpressed. The Zebrafish candidate gene produced an active thiaminase enzyme. This finding confirms *de novo* synthesis and represents the first report of a thiaminase-encoding protein in any multicellular organism. The candidate gene identified for Alewife is predicted to produce a protein product with biochemical properties that match those determined empirically.

The dietary acquisition hypothesis was not well-supported, and findings from Chapters 2 and 3 did not converge as would be expected if the source of thiaminase was dietarily acquired. The potential for *de novo* synthesis by fishes was confirmed experimentally, which represents the first report of a gene for a thiaminase enzyme from a multicellular animal. Future research should focus on confirming that *de novo* production accounts for the thiaminase activity in fishes and understanding the physiological factors that lead to increased thiaminase production. This work is relevant to fishery managers in affected ecosystems (Great Lake, Baltic Sea, Finger Lakes, NY) and to biochemists and nutritionist interested in thiamine metabolism.

The Ecophysiology of Thiamine Deficiency Complex: Evaluating Sources of Thiaminase in Great Lakes Food Webs

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Allison N. Evans, Author

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CONTRIBUTION OF AUTHORS

Scott Heppell was involved in the overview, supervision and editing of the work conducted in all chapters. He was also involved in sample collection for chapters 2 and 3 and in advising on lab work in chapter 4. Chuck Krueger, Stephen Riley, Jacques Rinchard, and Don Tillitt were involved in the project design and sample collection for chapters 2 and 3. Jacques Rinchard was also involved in laboratory analysis for chapter 3. Cathy Richter was involved in project design, managing the daily aspects of collaboration between OSU and CERC, laboratory analysis for chapter 4, and editing of chapter 4. Stephen Riley was involved in the project design, sample collection and analysis of chapters 2 and 3. Jim Zajicek was involved the project design, sample collection, and laboratory analysis for chapters 2 and 3, and in advising on lab work for chapter 4.

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1 CHAPTER 1
INTRODUCTION

1.1 Problem description

Animal populations decline for many reasons, which can be grouped into two broad categories: direct causes, such as harvest, and indirect causes including habitat destruction and species invasions (Helfman 2007). Freshwater systems and their fauna are particularly vulnerable because of the nature with which cumulative impacts accrue in freshwater systems and because of human demand for water (Jelks et al. 2008). For North America's freshwater fishes, top reasons for severe declines in abundance include physical and chemical habitat alterations, species introductions, hybridization, and overharvest, with more than one reason contributing to population decline in the vast majority of cases where fish populations are at risk or extinct (Miller et al. 1989). Five regions in North America have suffered the greatest extinction loss, and one of those five regions is the Laurentian Great Lakes (Miller et al. 1989).

The Great Lakes and their fish communities have been exposed to a combination of overharvest, habitat alteration, and non-native species for the better part of 150 years (Bogue 2000). Classic examples of the culmination of these pressures in the Great Lakes include the functional extirpation of Atlantic Salmon (*Salmo salar*), the formerly dominant native predator in Lake Ontario, by the mid-1800s (Christie 1973), and the sequential collapse of another dominant apex native predator, Lake Trout (*Salvelinus namaycush*), in all lakes except Lake Superior between the 1940s and 1950s (Baldwin et al. 2009). A familiar trio of threats, overfishing, habitat degradation, and non-native species, worked in consort in both cases (Hansen 1999, Muir et al. 2012). Despite 50 years of aggressive management and the formulation of Lake Trout rehabilitation goals for all each of the Great Lakes (DesJardine et al. 1995, Eshenroder et al. 1995, Stewart et al. 1999, Horns et al. 2003, Ryan et al. 2003), rehabilitation of Lake Trout populations has only been fully realized in Lake Superior (Muir et al. 2012).

Failure to achieve Lake Trout rehabilitation results from a combination of many variables. These variables including stocking insufficient numbers of adults, inappropriate stocking practices, and poor survival of early life stages (Holey et al. 1995, Krueger et al. 1995a, Brown et al. 2005c, Claramunt et al. 2005, Jonas et al. 2005, Tillitt et al. 2005, Bronte et al. 2006, Bronte et al. 2007, Bronte et al. 2008).

Poor survival of early life stages represents an important bottleneck to successful Lake Trout rehabilitation (Jones et al. 1995, Eshenroder et al. 1999, Bronte et al. 2003b, Bronte et al. 2008, Morbey et al. 2008). Two mechanisms are likely responsible for poor early life stage survival: predation on Lake Trout eggs and embryos and a trophically-induced thiamine (vitamin B_1) deficiency that causes embryo mortality prior to exogenous feeding (Brown et al. 2005c, Strakosh and Krueger 2005, Bronte et al. 2008, Madenjian et al. 2008, Krueger et al. 2014). The relative importance of these two mechanisms in suppressing rehabilitation of Lake Trout remains an unanswered question, in part because of the role of the non-native Alewife (*Alosa pseudoharengus*) in both mechanisms. The effect of predation on survival of Lake Trout embryos is intuitive: predation results in decreased survival (Jones et al. 1995, Krueger et al. 1995b, Jonas et al. 2005, Strakosh and Krueger 2005, Madenjian et al. 2008, Krueger et al. 2014). The second factor, Thiamine Deficiency Complex (TDC), is a less intuitive, yet potentially important mechanism contributing to recruitment failure (Krueger et al. 1995a, Bronte et al. 2003b, Brown et al. 2005c, Bronte et al. 2008). Furthermore, these two mechanisms may interact with one another because thiamine-deficient Lake Trout embryos are less efficient foragers, have lower growth rates, and are more vulnerable to predation (Fitzsimons et al. 2009a). The dual threat posed by Alewife showcases mechanisms by which non-native species can have additive effects on native species that function at both the ecological and physiological levels.

Thiamine (vitamin B_1) deficiency is responsible for several related classes of early life stage mortality disorders in salmonines including Thiamine Deficiency Complex (TDC) in the Laurentian Great Lakes, Cayuga Syndrome in the Finger Lakes, and the M74 in the Baltic Sea (McDonald et al. 1998). Thiamine deficiency caused by ingestion of foods containing thiaminase, an enzyme that degrades the essential vitamin, thiamine. Diseases with symptoms similar to TDC have been observed in foxes (*Vulpes vulpes*), mink (*Neovison vison*), alligators (*Alligator mississippiensis*), seals (*Phoca vitulina* and *Cystophora cristata*) goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), cows (*Bos taurus*), cats (*Felis catus*), and chickens (*Gallus gallus domesticus*) (Green and Evans 1940, Smith and Proutt 1944, Yudkin 1949, Shintani 1956, Roberts and Boyd 1974, Thomas 1986, Thomas et al. 1987, Dagleish et al. 2006, Honeyfield et al. 2008, Croft et al. 2013). TDC in salmonines is characterized

by high levels of mortality between the time embryos develop fully pigmented eggs and the time at which first feeding occurs, and TDC has the capacity to cause mortality rates as high as 100% with a family. TDC is the only nutritional deficiency hypothesized to be a primary cause a species' extirpation (Ketola et al. 2000).

Several studies have established a link between the ingestion of thiaminase-containing fishes, such as Alewife and non-native Rainbow Smelt (*Osmerus mordax*), and low egg thiamine concentrations or high embryo mortality. In a comparative study of Lake Trout eggs at 18 sites in the Great Lakes basin, egg thiamine concentrations were significantly and inversely associated with the proportion of thiaminase-containing forage species in the diets of Lake Trout (Fitzsimons and Brown 1998). Additionally, Lake Trout embryo mortality was induced in the laboratory by feeding adult female Lake Trout a diet high in thiaminase-containing fish (Alewife) (Honeyfield et al. 2005b). Importantly, progeny of adult female Lake Trout fed fish low in thiaminase activity (Bloater, *Coregonus hoyi*) or a commercially available diet showed no mortality (Honeyfield et al. 2005b). The high thiaminase activity in Alewife and Rainbow Smelt, two key prey fish for affected Great Lakes salmonids, implicate these fishes as key causative factors in development of thiamine deficiency in salmonine predators in the Great Lakes (Tillitt et al. 2005).

1.2 Why is the characterization of sources of thiaminase in food webs necessary?

Although thiaminase in fishes consumed by Lake Trout and other salmonids are known to be the proximate cause of TDC in predators, the ultimate source of thiaminase in aquatic food webs remains unknown. Thiaminase activity in fishes in the Great Lakes basin has been shown to vary widely. Thiaminase activity of Bloater, Yellow Perch (*Perca flavescens*), Ninespine Stickleback (*Pungitius pungitius*), Deepwater Sculpin (*Myoxocephalus thompsoni*), and Round Goby (*Neogobius melanostomus*) have been reported to be significantly lower than thiaminase activity of Alewife and Rainbow Smelt (Tillitt et al. 2005). However, thiaminase activity of Gizzard Shad (*Dorosoma cepedianum*) and Spottail Shiner (*Notropis hudsonius*) is an order of magnitude higher than thiaminase activity of Alewife and Rainbow Smelt (Tillitt et al. 2005). Common Carp (*Cyprinus*

carpio) also has high thiaminase activity (Fujita 1954, Mazrimas et al. 1963, Bos and Kozik 2000). Generally, clupeids (e.g., Alewife and Gizzard Shad) and cyprinids (e.g., Spottail Shiner and Carp) show high thiaminase activity (Yudkin 1949), and in analysis of factors affecting the presence of thiaminase activity, taxonomy was a stronger predictor of presence of thiaminase activity in Great Lakes fishes than diet, habitat, or other factors (Riley and Evans 2008).

Although several studies have focused on the occurrence and magnitude of thiaminase activity in prey fishes (Greig and Gnaedinger 1971, Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005, Zajicek et al. 2005, Lepak et al. 2008, Lepak et al. 2013), the ultimate source of thiaminase in prey fishes remains unknown. Evaluating potential sources of thiaminase in fishes is essential to understand thiaminase trophodynamics, to develop the biochemical and genetic tools necessary to investigate movement of thiaminase through the food web, to understand the mechanisms underlying variations in thiaminase activity, and to develop management strategies for mitigation of TDC and achieving Lake Trout restoration.

Potential sources of thiaminase in thiaminase-containing fishes include *de novo* production of thiaminase by the fishes themselves (*de novo* hypothesis) and acquisition of thiaminase through diet (dietary acquisition hypothesis). The dietary acquisition hypothesis includes the acquisition of gut bacteria that could be a source of thiaminase activity. The *de novo* and dietary acquisition hypotheses are not mutually exclusive and could operate in consort with one another. Knowing whether thiaminase activity in fish results from *de novo* production, from the dietary intake of thiaminase-producing organisms such as bacteria (Fujita 1954, Campobasso et al. 1998, Sikowitz et al. 2013) or zooplankton (Zajicek et al. 2005), or both is an essential first step of the research strategy to evaluate variables affecting thiaminase activity in fishes. Characterizing the source of thiaminase in fishes will facilitate a more focused and directed evaluation of the factors that contribute to variation in thiaminase activity (Figure 1.1). For example, if *de novo* production by fishes is a major source of thiaminase, physiological studies on fishes will be necessary to determine what environmental variables govern the production of thiaminase. However, if thiaminase in fishes is acquired through the diet, research will need to

focus on identifying the organisms that produce thiaminase and the mechanisms by which thiaminase is transferred and accumulated through the food web. If thiaminase in fishes is both obtained through the diet and is produced *de novo*, a complete understanding of the variables influencing thiaminase activity in fishes will be required for both sources. Once the sources of thiaminase in Great Lakes food webs are characterized, genetic and biochemical tools can be developed to evaluate the variables influencing thiaminase activity. The variables that influence thiaminase activity must be understood to inform natural resource managers regarding predicting and mitigating TDC.

1.3 General approach

Although several studies have focused on the occurrence and magnitude of thiaminase activity in fishes (Greig and Gnaedinger 1971, Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005, Zajicek et al. 2005, Lepak et al. 2008), both the ultimate source of thiaminase and its physiological function remain unknown (Arsan and Malyarevskaya 1969, Wistbacka et al. 2009, Zajicek et al. 2009, Lepak et al. 2013, Kreinbring et al. 2014). Evaluating potential sources of thiaminase in fishes is essential for understanding thiaminase trophodynamics and developing management strategies for mitigating TDC.

I have taken a two-pronged, integrated food web approach to investigate the sources of thiaminase in Great Lakes food webs. Because the dietary acquisition and *de novo* hypothesis are not mutually exclusive, Chapters 2 and 3 explicitly test the dietary acquisition hypothesis, and Chapter 4 explicitly tests the *de novo* hypothesis. To date, explicit tests of directed hypotheses regarding the sources of thiaminase have not been conducted. Generally, evidence for or against these hypotheses has been opportunistically obtained in the course of other investigations.

In Chapter 2, I tested the hypothesis that thiaminase activity in bulk zooplankton is related to zooplankton community composition. The goal of this analysis was to identify any single zooplankton taxon (or several taxa) that was consistently abundant in samples with high thiaminase activity and absent or at low abundance in samples with low thiaminase activity across a variety of plankton communities in the Great Lakes. This work was correlational and exploratory and sought to identify

candidate taxa that should be considered for additional study. Furthermore, this work is at the base of the food web and represents a test of the capacity of taxa in the zooplankton community to constitute the source of thiaminase in Great Lakes food webs.

In Chapter 3, I tested the dietary acquisition hypothesis directly in fishes. Specifically, I tested the hypothesis that thiaminase activity in fishes is related to their diet. I used two different metrics of diet for this work, direct observation of stomach content and fatty acid profile analysis. The goal of this analysis was to identify any single prey taxon (or several taxa) in the diet of fishes that was consistently abundant in fishes with high thiaminase activity and consistently absent or at low abundance in fishes with low thiaminase activity across a variety of fish communities in the Great Lakes. As with Chapter 2, this work was correlational and exploratory and sought to identify candidate taxa that should be considered for additional study.

In Chapter 4, I tested the *de novo* hypothesis directly by both investigating the biochemical characteristics of thiaminases from several fish species and by using existing information from two known peptide fragments from fish thiaminases. Existing information was used to search for candidate fish genes encoding thiaminases and assess whether their protein product produces a thiaminase. One advantage to the approach in Chapter 4 is that biochemical methods provide useful information regardless of whether the ultimate source of thiaminase in fishes is *de novo* or is of bacterial or plankton origin. One risk of this approach is that the use of existing peptide information could result in discovery of no viable candidate genes whose gene product can be tested. This work was experimental, and, ideally, the results of this work should be congruous with the results of work in Chapters 2 and 3. For example, if Chapter 2 identified a species of zooplankton as a likely candidate source of thiaminase, the biochemical characteristics of that zooplankton's thiaminase should be consistent with the biochemical characteristics of thiaminases from fish containing that zooplankton in their diets.

One advantage to the food web approach I took was facilitation of the simultaneous sampling of multiple trophic levels (fishes and zooplankton), which allowed us to make comparisons across trophic levels and evaluate the likelihood that the

candidate taxon identified in the assessment of zooplankton was likely to have contributed to thiaminase activity in fishes. The integrated nature of these analyses and the simultaneous sampling of multiple trophic levels represent the first large-scale, vertically-integrated, multi-trophic level approach to identifying the sources of thiaminase in Great Lakes food webs.

1.4 Literature Cited

Arsan, O. M. and A. Y. Malyarevskaya. 1969. The effect of food on thiaminase activity and the content of thiamine in the liver and intestine of silver carp. Hydrobiological Journal. 5:79-81.

Baldwin, N. A., R. W. Saalfeld, M. R. Dochoda, H. J. Buettner, and R. L. Eshenroder. 2009. Commercial fish production in the Great Lakes 1867-2006. Available at http://www.glfc.org/databases/commercial/commerc.php.

Bogue, M. B. 2000. Fishing the Great Lakes : an environmental history, 1783-1933. University of Wisconsin Press, Madison, Wis.

Bos, M. and A. Kozik. 2000. Some molecular and enzymatic properties of a homogeneous preparation of thiaminase I purified from carp liver. Journal of Protein Chemistry. 19:75-84.

Bronte, C. R., J. Jonas, M. E. Holey, R. L. Eshenroder, M. L. Toneys, P. McKee, B. Breidert, R. M. Claramunt, M. P. Ebener, C. C. Krueger, G. M. Wright, and R. Hess. 2003b. Possible impediments to lake trout restoration in Lake Michigan. Lake Trout Task Group report to the Lake Michigan Committee. Great Lakes Fishery Commission. Ann Arbor, MI.

Bronte, C. R., P. C. McKee, and M. E. Holey. 2006. Relative survival of lake trout stocked at different sizes and quality in Lake Michigan. Journal of Great Lakes Research. 32:386-394.

Bronte, C. R., M. E. Holey, C. P. Madenjian, J. L. Jonas, R. M. Claramunt, P. C. McKee, M. L. Toneys, M. P. Ebener, B. Breidert, G. W. Fleischer, R. Hess, A. W. Martell, and E. J. Olsen. 2007. Relative abundance, site fidelity, and survival of adult lake trout in Lake Michigan from 1999 to 2001: Implications for future restoration strategies. North American Journal of Fisheries Management. 27:137-155.

Bronte, C. R., C. C. Krueger, M. E. Holey, M. L. Toneys, R. L. Eshenroder, and J. L. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fishery Commission Miscellaneous Publication 2008-01. 39 pages. Available at http://www.glfc.org/pubs/pub.htm#misc.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005c. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Campobasso, N., C. A. Costello, C. Kinsland, T. P. Begley, and S. E. Ealick. 1998. Crystal structure of thiaminase-I from *Bacillus thiaminolyticus* at 2.0 angstrom resolution. Biochemistry. 37:15981-15989.

Christie, W. J. 1973. A review of the changes in the fish species composition of Lake Ontario. Great Lakes Fishery Commission Technical Report:1-65.

Claramunt, R. M., J. L. Jonas, J. D. Fitzsimons, and J. E. Marsden. 2005. Influences of spawning habitat characteristics and interstitial predators on lake trout egg

deposition and mortality. Transactions of the American Fisheries Society. 134:1048-1057.

Croft, L., E. Napoli, C. K. Hung, J. St Leger, S. Gearhart, K. Heym, S. Wong, D. Sakaguchi, A. Lin, B. Puschner, and C. Giulivi. 2013. Clinical evaluation and biochemical analyses of thiamine deficiency in Pacific harbor seals (*Phoca vitulina*) maintained at a zoological facility. Javma-Journal of the American Veterinary Medical Association. 243:1179-1189.

Dagleish, M. P., I. A. P. Patterson, G. Foster, R. J. Reid, L. T. A. Brain, and D. Buxton. 2006. Polioencephalomalacia in a juvenile hooded seal (*Cystophora cristata*). Veterinary Record. 158:516-518.

DesJardine, R. L., T. K. Gorenflo, R. N. Payne, and J. D. Schrouder. 1995. Fish-community objectives for Lake Huron. Great Lakes Fishery Commission Special Publication. 95-1:1-37.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark, Jr. 1995. Fish-community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 1995-3:1-56.

Eshenroder, R. L., J. W. Peck, and C. H. Olver. 1999. Research priorities for lake trout rehabilitation in the Great Lakes: A 15-year retrospective. Great Lakes Fishery Commission Technical Report:1-40.

Fitzsimons, J. D. and S. B. Brown. 1998. Reduced egg thiamine levels in inland and Great Lakes trout and their relationship with diet. Pages 160-171 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., B. Williston, J. L. Zajicek, D. E. Tillitt, S. B. Brown, L. R. Brown, D. C. Honeyfield, D. M. Warner, L. G. Rudstam, and W. Pearsall. 2005b. Thiamine content and thiaminase activity of ten freshwater stocks and one marine stock of alewives. Journal of Aquatic Animal Health. 17:26-35.

Fitzsimons, J. D., S. B. Brown, B. Williston, G. Williston, L. R. Brown, K. Moore, D. C. Honeyfield, and D. E. Tillitt. 2009a. Influence of thiamine deficiency on lake trout larval growth, foraging, and predator avoidance. Journal of Aquatic Animal Health. 21:302-314.

Fujita, A. 1954. Thiaminase. Advances in Enzymology and Related Subjects of Biochemistry. 15:389-421.

Green, R. G. and C. A. Evans. 1940. A deficiency disease of foxes. Science. 92:154-155.

Greig, R. A. and R. H. Gnaedinger. 1971. Occurrence of thiaminase in some common aquatic animals of the United States and Canada. Special Scientific Report - Fisheries. United States Department of Commerce. Seattle, Washington, 7 pages.

Hansen, M. J. 1999. Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. Pages 417-453 *in* W. W. Taylor and C. P. Ferreri, editors. Great Lakes fisheries policy and management: a binational perspective.

Helfman, G. S. 2007. Fish conservation : a guide to understanding and restoring global aquatic biodiversity and fishery resources. Washington : Island Press, Washington.

Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown, J. E. Marsden, D. S. Lavis, M. L. Toneys, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. Journal of Great Lakes Research. 21:128-151.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. P. Ross, D. A. Carbonneau, S. P. Terrel, A. R. Woodward, T. R. Schoeb, H. F. Perceval, and J. P. Hinterkopf. 2008. Pathology, physiologic parameters, tissue contaminants, and tissue thiamine in morbid and healthy central Florida adult American alligators (*Alligator mississippiensis*). Journal of Wildlife Diseases. 44:280-294.

Horns, W. H., C. R. Bronte, T. R. Busiahn, M. P. Ebener, R. L. Eshenroder, T. Gorenflo, N. Kmiecik, W. Mattes, J. W. Peck, M. Petzold, and D. R. Schreiner. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication. 03-01:i-ii, 1-78.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries. 33:372-407.

Ji, Y. Q. and I. R. Adelman. 1998. Thiaminase activity in alewives and smelt in lakes Huron, Michigan, and Superior. Pages 154-159 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Jonas, J. L., R. M. Claramunt, J. D. Fitzsimons, J. E. Marsden, and B. J. Ellrott. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 62:2254-2264.

Jones, M. L., G. W. Eck, D. O. Evans, M. C. Fabrizio, M. H. Hoff, P. L. Hudson, J. Janssen, D. Jude, R. Ogorman, and J. F. Savino. 1995. Limitations to lake trout (*Salvelinus namaycush*) rehabilitation in the Great Lakes imposed by biotic interactions occurring at early life stages. Journal of Great Lakes Research. 21:505-517.

Ketola, H. G., P. R. Bowser, G. A. Wooster, L. R. Wedge, and S. S. Hurst. 2000. Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Transactions of the American Fisheries Society. 129:607-612.

Kreinbring, C. A., S. P. Remillard, P. Hubbard, H. R. Brodkin, F. J. Leeper, D. Hawksley, E. Y. Lai, C. Fulton, G. A. Petsko, and D. Ringe. 2014. Structure of a eukaryotic thiaminase I. Proceedings of the National Academy of Sciences. 111:137-142.

Krueger, C. C., M. L. Jones, and W. W. Taylor. 1995a. Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. Journal of Great Lakes Research. 21:547-558.

Krueger, C. C., D. L. Perkins, E. L. Mills, and J. E. Marsden. 1995b. Predation by alewives on lake trout fry in Lake Ontario: Role of an exotic species in preventing restoration of a native species. Journal of Great Lakes Research. 21:458-469.

Krueger, C. C., W. C. Shepherd, and A. M. Muir. 2014. Predation by alewife on lake trout fry emerging from laboratory reefs: Estimation of fry survival and assessment of predation potential. Journal of Great Lakes Research. 40:429-434.

Lepak, J. M., C. E. Kraft, D. C. Honeyfield, and S. B. Brown. 2008. Evaluating the effect of stressors on thiaminase activity in alewife. Journal of Aquatic Animal Health. 20:63-71.

Lepak, J. M., C. E. Kraft, and M. J. Vanni. 2013. Clupeid response to stressors: The influence of environmental factors on thiaminase expression. Journal of Aquatic Animal Health. 25:90-97.

Madenjian, C. P., R. O'Gorman, D. B. Bunnell, R. L. Argyle, E. F. Roseman, D. M. Warner, J. D. Stockwell, and M. A. Stapanian. 2008. Adverse effects of alewives on Laurentian Great Lakes fish communities. North American Journal of Fisheries Management. 28:263-282.

Mazrimas, J. A., L. L. Ingraham, R. D. Draper, and P. S. Song. 1963. Studies of thiaminase action - Hammetts Rhosigma Treatment. Archives of Biochemistry and Biophysics. 100:409-413.

McDonald, G., J. D. Fitzsimons, and D. C. Honeyfield, editors. 1998. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries. 14:22-38.

Morbey, Y. E., D. M. Anderson, and B. A. Henderson. 2008. Progress toward the rehabilitation of lake trout (*Salvelinus namaycush*) in South Bay, Lake Huron. Journal of Great Lakes Research. 34:287-300.

Muir, A. M., C. C. Krueger, and M. J. Hansen. 2012. Re-establishing lake trout in the Laurentian Great Lakes: Past, present, and future. Pages 533-588 *in* W. W. Taylor, A. J. Lynch, and N. J. Leonard, editors. Great Lakes fisheries policy and management: A binational perspective. 2nd edition. MSU Press, Lansing.

Riley, S. C. and A. N. Evans. 2008. Phylogenetic and ecological characteristics associated with thiaminase activity in Laurentian Great Lakes fishes. Transactions of the American Fisheries Society. 137:147-157.

Roberts, G. W. and J. W. Boyd. 1974. Cerebrocortical necrosis in ruminants - occurrence of thiaminase in gut of normal and affected animals and its effect on thiamine status. Journal of Comparative Pathology. 84:365-374.

Ryan, P. A., R. Knight, R. MacGregor, G. Towns, R. Hoopes, and W. Culligan. 2003. Fish-community goals and objectives for Lake Erie. Great Lakes Fishery Commission Special Publication. 03-02:1-56.

Shintani, S. 1956. On the thiaminase disease of chickens. Journal of Vitaminology. 2:23-30.

Sikowitz, M. D., B. Shome, Y. Zhang, T. P. Begley, and S. E. Ealick. 2013. Structure of a *Clostridium botulinum* C143S Thiaminase I/Thiamin Complex Reveals Active Site Architecture. Biochemistry. 52:7830-7839.

Smith, D. C. and L. M. Proutt. 1944. Development of thiamine deficiency in the cat on a diet of raw fish. Experimental Biology and Medicine. 56:1-3.

Stewart, T. J., R. E. Lange, S. D. Orsatti, C. P. Schneider, A. Mathers, and M. E. Daniels. 1999. Fish-community objectives for Lake Ontario. Great Lakes Fishery Commission Special Publication. 99-1:1-55.

Strakosh, T. R. and C. C. Krueger. 2005. Behavior of post-emergent lake trout fry in the presence of the alewife, a non-native predator. Journal of Great Lakes Research. 31:296-305.

Thomas, K. W. 1986. The effect of thiaminase-induced subclinical thiamine-deficiency on growth of weaner sheep. Veterinary Research Communications. 10:125-141.

Thomas, K. W., D. L. Turner, and E. M. Spicer. 1987. Thiamine, thiaminase, and trasketolase levels in groats with and without polioencephalomalacia. Australian Veterinary Journal. 64:126-127.

Tillitt, D. E., J. L. Zajicek, S. B. Brown, L. R. Brown, J. D. Fitzsimons, D. C. Honeyfield, M. E. Holey, and G. M. Wright. 2005. Thiamine and thiaminase status in forage fish of salmonines from Lake Michigan. Journal of Aquatic Animal Health. 17:13-25.

Wistbacka, S., L. G. Lonnstrom, E. Bonsdorff, and G. Bylund. 2009. Thiaminase activity of Crucian carp *Carassius carassius* injected with a bacterial fish pathogen, *Aeromonas salmonicida* subsp. *salmonicida*. Journal of Aquatic Animal Health. 21:217-228.

Yudkin, W. H. 1949. Thiaminase, the Chastek-paralysis factor. Physiological Reviews. 29:389-402.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

Zajicek, J. L., L. Brown, S. B. Brown, D. C. Honeyfield, J. D. Fitzsimons, and D. E. Tillitt. 2009. Variations of thiaminase I activity pH dependencies among typical Great Lakes forage fish and *Paenibacillus thiaminolyticus*. Journal of Aquatic Animal Health. 21:207-216.

1.5 Figures

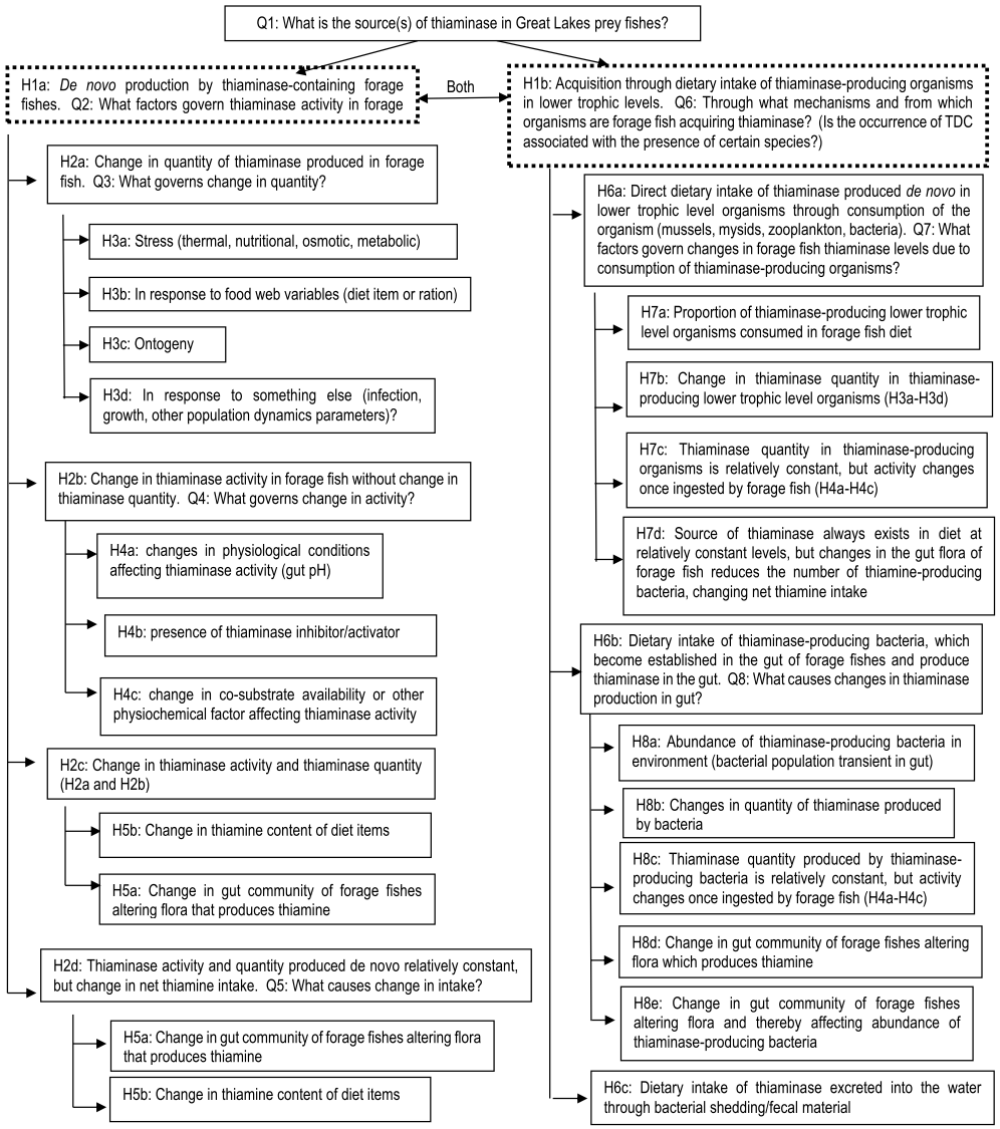

Figure 1.1. Research framework for determining the factors that control variability in thiaminase in fishes.

Research framework for determining the factors that control variability in thiaminase in fishes. This framework represents future research and illustrates why understanding the source of thiaminase in the ecosystem is a necessary precursor to evaluating the factors that control variability of thiaminase. The proposed research will address the questions outlined in the boxes with dashed borders.

2 CHAPTER 2

THIAMINASE ACTIVITY IN GREAT LAKES ZOOPLANKTON IS NOT RELATED TO ZOOPLANKTON COMMUNITY COMPOSITION

2.1 Abstract

Thiamine Deficiency Complex, a vitamin B_1 deficiency that affects survival of salmonid embryos, is caused by adult salmonids' consumption of prey fishes containing high levels of thiaminase, a thiamine-degrading enzyme. The source of thiaminase in Great Lakes prey fishes is unknown. Prey fishes may obtain thiaminase from their plankton prey, and zooplankton contain thiaminase activity. We described the distribution of thiaminase activity in three size fractions and evaluated the degree to which thiaminase varied by site, season, depth, and size fractions. We tested the hypothesis that thiaminase activity in bulk zooplankton is related to zooplankton community composition. The goal of this analysis was to identify any single zooplankton taxon (or several taxa) that were consistently abundant in samples with high thiaminase activity and absent or at low abundance in samples with low thiaminase activity across a variety of plankton communities. We compared thiaminase activity in bulk plankton tows to the species composition in tows using three multivariate procedures, Nonmetric Multidimensional Scaling, distance-based Redundancy Analysis, and Indicator Species Analysis. Only Indicator Species Analysis revealed any relationship between thiaminase activity and zooplankton community composition, and it suggested one candidate source of thiaminase activity, *Ploesoma* spp. The contribution of *Ploesoma* spp. to total plankton biomass was sufficiently low as to require extraordinary levels of thiaminase activity to constitute the major source of thiaminase in Great Lakes food webs. Our findings argue against the suggestion that a single zooplankton taxon is consistently the source of thiaminase across all plankton communities in the Great Lakes.

2.2 Introduction

Lake Trout (*Salvelinus namaycush*) was the dominant native predator in the Great Lakes prior to their functional extirpation in the 1950s through overfishing, habitat degradation, and species invasions from all lakes except Lake Superior (Hansen 1999, Krueger and Ebener 2004, Muir et al. 2012). Despite nearly 50 years of aggressive management, rehabilitation of Lake Trout populations to levels stated in lake-wide Fish Community Objectives (DesJardine et al. 1995, Eshenroder et al. 1995, Stewart et al. 1999, Horns et al. 2003, Ryan et al. 2003) has remained largely unrealized with the exception of Lake Superior (Muir et al. 2012). Failure to rehabilitate Lake Trout populations results from many factors (Muir et al. 2012), one of which is poor survival of early life stages. Two mechanisms are responsible for poor survival of early life stages: predation on Lake Trout embryos and juveniles (Jones et al. 1995, Krueger et al. 1995b, Jonas et al. 2005, Strakosh and Krueger 2005, Madenjian et al. 2008, Krueger et al. 2014), and a trophically-induced thiamine (vitamin B_1) deficiency termed Thiamine Deficiency Complex (TDC) that causes embryo mortality prior to exogenous feeding (Fisher et al. 1998b, Fitzsimons and Brown 1998, Brown et al. 2005a, Honeyfield et al. 2005a, Honeyfield et al. 2005b, Fitzsimons et al. 2007, Czesny et al. 2009).

TDC is a potentially important mechanism contributing to recruitment failure of Lake Trout (Krueger et al. 1995a, Bronte et al. 2003b, Brown et al. 2005c, Bronte et al. 2008). Adult salmonids, including Lake Trout, do not have low thiamine because they ingest too little in their diet (Fitzsimons and Brown 1998, Fitzsimons et al. 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005); rather, they become thiamine deficient because they consume prey fish that have high levels of thiaminase, a thiamine-degrading enzyme (Fisher et al. 1995, Fitzsimons et al. 1998, Ji and Adelman 1998, Fitzsimons et al. 2005b, Honeyfield et al. 2005b, Tillitt et al. 2005). Ingestion of thiaminase-containing prey fish or thiaminase-enhanced artificial diets lowers whole-body thiamine levels in adults (Brown et al. 1998a, Brown et al. 1998b, Fisher et al. 1998a, Fisher et al. 1998b, Fynn-Aikins et al. 1998, Honeyfield et al. 1998b, Ji et al. 1998, Honeyfield et al. 2005a, Houde et al. 2015), and leaves females with insufficient thiamine to allocate to their eggs. As a result, egg thiamine is reduced and embryonic mortality increases (Fisher et al. 1996, Fisher et al. 1998b,

Fitzsimons and Brown 1998, Brown et al. 2005a, Honeyfield et al. 2005a, Honeyfield et al. 2005b, Fitzsimons et al. 2007, Czesny et al. 2009).

Development of TDC in predatory salmonids is linked to persistent consumption of two non-native planktivores Alewife (*Alosa pseudoharengus*) and Rainbow Smelt (*Osmerus mordax*) that serve as forage for Lake Trout (Coble 1965, Fisher et al. 1996, Fitzsimons and Brown 1998, Ketola et al. 2000, Honeyfield et al. 2005b). Both of these fish contain high levels of thiaminase activity (Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005, Zajicek et al. 2005). The ultimate source of the thiaminase enzyme in these fishes remains unknown, and identifying the source of thiaminase in Alewife and Rainbow smelt is an essential first step in understanding why thiaminase activity in these fishes varies seasonally, annually, and spatially (Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005). The thiaminase enzyme in Alewife and Rainbow Smelt could originate from two possible sources: either planktivorous fish acquire thiaminase from their diet (dietary acquisition hypothesis) or planktivorous fish make the thiaminase enzyme themselves (*de novo* synthesis hypothesis); these two hypotheses are not mutually exclusive, as the multiple thiaminase enzymes from more than one source could contribute to the overall thiaminase activity in planktivorous fish.

The most widely held assumption is that fishes obtain thiaminase through dietary sources (Brown et al. 2005c, Tillitt et al. 2005, Bronte et al. 2008). The dietary sources of thiaminase that are most-often hypothesized to be important include thiaminase-producing heterotrophic bacteria (Honeyfield et al. 2002, Brown et al. 2005c, Tillitt et al. 2005, Kraft et al. 2014), cyanobacteria and algae (Arsan and Malyarevskaya 1969, Grigor'yeva et al. 1977, Honeyfield et al. 1998a, Honeyfield et al. 2002, Tillitt et al. 2005), and zooplankton (Fitzsimons et al. 2004a, Fitzsimons et al. 2004b, Zajicek et al. 2005). Of these potential sources, most recent research has focused on a thiaminase-producing bacterium, *Paenibacillus thiaminolyticus* (PT), because it contains a gene that has been shown to encode a thiaminase protein (Costello et al. 1996), because feeding PT to Lake Trout induces TDC (Honeyfield et al. 2005b), and because PT has been cultured from the gut of Great Lakes Alewife (Honeyfield et al. 2002). Together, this constituted suggestive evidence that PT was

the source of thiaminase in Great Lakes planktivores. Subsequent research focused on assessing the contribution of PT to thiaminase activity in both fish and zooplankton found no relationship between thiaminase activity and the abundance of PT cells or between thiaminase activity and the abundance of the PT thiaminase protein, indicating that PT is not the primary source of thiaminase in Great Lakes food webs (Richter et al. 2012). Bacterial sources of thiaminase other than PT have not been systematically assessed for contributions to thiaminase activity in Great Lakes fish or plankton, and the known ability of other bacterial taxa to produce thiaminase (Fujita 1954, Sikowitz et al. 2013, Kraft et al. 2014) suggests the potential for other taxa to be sources of thiaminase.

Cyanobacteria and algae have been proposed as sources of thiaminase for planktivorous fishes (Arsan and Malyarevskaya 1969, Honeyfield et al. 1998a, Honeyfield et al. 2002, Fitzsimons et al. 2004a, Brown et al. 2005c, Tillitt et al. 2005). Both cyanobacteria and algae have been reported as sources of thiaminase in Silver Carp (*Hypophthalmichthys molitrix*) (Arsan and Malyarevskaya 1969, Arsan 1972, Birger et al. 1973). Silver Carp fed cyanobacteria (*Microcystis aeruginosa*) had a higher level of thiaminase in their liver and intestinal tissue than those fed green algae (*Volvox aureus*) and protococcoid algae (*Protococcus* spp.) (Arsan and Malyarevskaya 1969). Algal-fed carp had higher liver and intestinal thiaminase activity than carp fed *Daphnia magna* (Arsan and Malyarevskaya 1969). Carp fed *D. magna* had higher thiaminase activity than those that were fasted. The thiaminase activity of Silver Carp fed these specific food items was approximately proportional to the thiaminase activity of the individual taxa composing the diet (Arsan and Malyarevskaya 1969). In addition, thiaminase activity in three species of fish increased when fish were grown in the presence of blue-green algae (*M. aeruginosa*), and thiamine injections were therapeutic in relieving TDC symptoms (Birger et al. 1973). In combination, this evidence suggests the potential for dietary acquisition of thiaminase from bacteria and algae.

Finally, zooplankton could be a source of thiaminase for prey fishes. The two fish species that are the causative agent of TDC in Lake Trout, Alewife and Rainbow Smelt, are both plantkivores (Honeyfield et al. 1998a, Honeyfield et al. 2002,

Fitzsimons et al. 2004a, Brown et al. 2005c, Tillitt et al. 2005); however, it should be noted that not all planktivorous fishes in the Great Lakes have high levels of thiaminase activity. In contrast to Alewife and Rainbow Smelt, Bloater (*Coregonus hoyi*), a native planktivore that was historically a main prey item for Lake Trout, contain undetectable levels of thiaminase activity (Tillitt et al. 2005). One potential explanation for both the difference in thiaminase activity among planktivores, and the spatial and temporal variability in thiaminase activity of Alewife and Rainbow Smelt, is that different planktonic prey items contain different levels of thiaminase activity. Diets of Great Lakes planktivores are known to vary across space and time (Davis et al. 2007, Bunnell et al. 2015), and thiaminase activity varies across zooplankton species. For example, *Diporeia hoyi* appears to have lower thiaminase activity than *Mysis diluviana*, and both are prey items for planktivores (Zajicek et al. 2005). Furthermore, thiaminase activity in bulk zooplankton tows was quite variable (Fitzsimons et al. 2004a, Fitzsimons et al. 2004b, Zajicek et al. 2005), suggesting that either environmental variables or zooplankton community composition causes thiaminase activity to vary. Zooplankton themselves could be the primary sources of thiaminase (i.e., they may produce their own thiaminase enzymes *de novo*), or they could serve as a conduit for delivering bacterial or algal thiaminase to planktivores by virtue of their consumption of bacteria or algae. The latter is suggested by the observation that the highest thiaminase levels in bulk zooplankton collected in Hamilton Harbor (Lake Ontario) were coincident with high abundance of blue-green algae (Fitzsimons et al. 2004b).

Understanding whether thiaminase activity in zooplankton is related to zooplankton community composition will facilitate identification of candidate sources of thiaminase activity for future investigations. To this end, our objectives were to (1) assess thiaminase activity in bulk zooplankton tows across several different size fractions across food webs where Lake Trout have differing levels of TDC and to determine whether thiaminase activity in bulk zooplankton tows is related to season, site, depth, and size fraction; (2) compare thiaminase activity among individual zooplankton taxa to empirically assess the potential for specific taxa to be sources of thiaminase activity in planktivorous fish; and (3) identify zooplankton taxa that were likely candidate sources of thiaminase activity by comparing thiaminase activity in bulk

zooplankton to the zooplankton community composition. Relative to the final objective, we sought to uncover any single zooplankton taxon (or several taxa) that was consistently abundant in samples with high thiaminase activity and absent or at low abundance in samples with low thiaminase activity across a variety of plankton communities in the Great Lakes. The overall goal of our work was to assess whether taxa in the zooplankton community to could be the source of thiaminase in Great Lakes food webs and to identify candidate taxa in the plankton community should serve as targets for future research.

2.3 Methods

2.3.1 Site selection

Zooplankton were sampled in 2007 at five different locations throughout the Great Lakes (Figure 2.1). These locations were chose to sample a broad range of zooplankton communities and TDC severities. Sturgeon Bay (Lake Michigan), Frankfort (Lake Michigan), and Port Weller (Lake Ontario) represented areas with severe TDC. Detour (Lake Huron) represented an area with moderate TDC, and Ashland (Lake Superior) represented an area with no TDC (Fitzsimons and Brown 1998, Brown et al. 2005d). In Lake Superior, egg thiamine levels typically exceed 20 nmol/g (Fitzsimons and Brown 1998), and natural recruitment of Lake Trout occurs consistently (Bronte et al. 1995a, Bronte et al. 1995b, Bronte et al. 2003a). Lake Trout egg thiamine levels for years surrounding data collection support the classification used for TDC severity (Table 2.1).

To capture seasonal and depth-driven variability in thiaminase activity and plankton community composition, we collected samples in three months (April, July, and September) and at two depths (18 m bottom depth and 100 m bottom depth) at Sturgeon Bay. These months were chosen to represent suspected key periods in the seasonal dynamics of thiaminase. We sampled as early as feasible (April) because winter and early spring are seasons in which Alewife in western Lake Michigan have the highest thiaminase levels (Ji and Adelman 1998, Fitzsimons et al. 2004a, Tillitt et al. 2005). July was chosen to maximize the likelihood of collecting cyanobacteria and algae, which have been hypothesized to be sources of thiaminase

(Arsan and Malyarevskaya 1969, Malyarevskaya et al. 1972, Stoermer 1978, Chang and Rossmann 1988). Lastly, we chose September because thiaminase activity of Alewife in Hamilton Harbor increased when water temperature dropped in late fall (Fitzsimons et al. 2004b). On each sampling date, we collected samples at both depths, resulting in 6 sampling events at Sturgeon Bay (3 months x 2 depths in each month).

To capture spatial variation in plankton community composition across the Great Lakes basin, we sampled the four remaining sites in late July and early August. At each site, mid-depth ranges were sampled to fall between the nearshore and offshore depths sampled at Sturgeon Bay. Sampling occurred at bottom depths of 55 m at Ashland, 32 m at Detour, 28 m at Frankfort, and 25 m at Port Weller. These four sampling events combined, with the six sampling events at Sturgeon Bay, represent a total of ten planned sampling events (Table 2.2). In addition to these 10 sampling events, we opportunistically collected zooplankton samples for thiaminase activity (but not community analysis) at Port Weller at 8.5 m and 75 m on the same day at which the 25 m depth was sampled. Because this sampling was opportunistic, we did not collect a full complement of samples at these depths. This resulted in 2 additional sampling events for thiaminase analysis relative to zooplankton community analysis (Table 2.2).

2.3.2 Zooplankton collection

Zooplankton were collected for two purposes: determining community composition and measuring thiaminase activity. Collection methods varied slightly for each purpose. In both cases, zooplankton were sampled using a 0.5-m diameter, 153 µm Nytex mesh net with a retrieval speed of 5 m/s. To collect zooplankton for community composition, we took three independent tows at each of the 10 sampling events (Table 2.2). Tows were depth-integrated vertical tows designed to sample the entire water column, beginning 5 m above the substrate to exclude benthos. Upon retrieval, zooplankton were carefully washed from the collecting cup of the plankton net using deionized water, narcotized with antacid tablets, and preserved in 5% sucrose formalin for subsequent analysis. Zooplankton community composition was determined as described in section 2.3.5.

To collect zooplankton for thiaminase analysis, we took three additional depth-integrated vertical tows as described for community composition. However, when plankton densities were low, a single tow was not likely to produce enough material for thiaminase analysis. Therefore, when necessary we combined the contents of multiple tows to achieve sufficient mass for thiaminase analysis. One analytical sample for thiaminase analysis occasionally consisted of pooled contents of multiple tows. One goal of the thiaminase sampling was to assess the thiaminase activity in bulk plankton samples at multiple size fractions. Therefore, upon collection, we immediately placed the contents from each tow through a series of stacked Nytex mesh sieves with mesh sizes of 125 µm, 53 µm, 25 µm. Interstitial water was drained by gravity, and the underside of each sieve was wicked with filter paper to further remove interstitial water. Samples were placed in plastic bags and immediately frozen on dry ice for thiaminase activity measurements. Our collection goal was 3 replicates per size fraction at each sampling event; however, we were not always able to collect sufficient biomass, especially for the smallest size fractions (Table 2.2). In cases where sufficient mass for the smallest fractions was obviously not obtainable using the 153 µm net, a 0.5 m-diameter, 53 µm net was used to obtain additional biomass for only the smallest size fractions. Samples from the 53 µm net were sieved through the stack of Nytex mesh sieves and the material in the 53-125 µm and 25-53 µm fractions was combined with the material for the same-sized fractions from other tows for that sampling event. All zooplankton tows for both analyses during each sampling event were taken at the same location and within a short time period (less than 1 hour) of one another.

In addition to zooplankton tows for bulk plankton thiaminase analysis, we collected individuals of large taxa to determine taxon-specific thiaminase activity. Additional zooplankton tows were conducted, as described above, and upon retrieval the entirety of the sample was placed in metal trays on ice. Large taxa for which it was practicable to identify and collect individuals (*Bythotrephes longimanus, Cercopagis pengoi,* and *Holopedium gibberum,* hereafter referred to by genus) were sorted with forceps and placed immediately in microcentrifuge tubes on dry ice. *Mysis* were

collected opportunistically from simultaneously conducted bottom trawls used for fish sampling (Chapter 3).

2.3.3 Collection of non-zooplankton taxa

Plant and algal material were only present in sufficient abundance for collection at Sturgeon Bay in the fall at 18m. We collected two species of green algae (*Chara* spp, family Characeae, and *Cladophora glomerata*, family Cladophoraceae), as well as two species of aquatic macrophytes (*Elodea canadensis*, family Hydrocharitaceae), and thin-leaf potamogeton (*Potamogeton* spp., family Potamogetonaceae) opportunistically from simultaneously conducted bottom trawls used for fish sampling (Chapter 3). We collected three samples of leaves and stems of each of these four taxa from bottom trawls using tweezers, placed them in plastic bags, and immediately froze them on dry ice for thiaminase activity measurements. Additionally, we collected one composite sample at Sturgeon Bay in April at 18m composed of mixed macroinvertebrates that included *Diporeia*, chironomids, oligochaetes, ceratopogonids, and snails.

2.3.4 Thiaminase activity

Thiaminase activity was measured using either on the bulk filtered zooplankton samples (pooled individuals of multiple taxa) or a pooled mass of large individuals from a single taxon. We pulverized samples with dry ice in ceramic mortars and pestles (Fisher Scientific). Once pulverized, the dry ice remaining in each powdered sample was allowed to sublimate at -20°C or -80°C. The frozen powdered tissue was sub-sampled and weighed into 3.6-ml Nalgene cryovials (USA Scientific, Ocala, Florida), and stored at -80°C for up to 6 months prior to analysis. We carried out all sub-sampling and weighing activities in a cold room at 4°C or –20°C using a pre-equilibrated model AE163 analytical balance (Mettler Instruments Corporation, Hightstown, New Jersey). Samples (<1 g) were homogenized directly in phosphate buffer at the time of analysis. We quantified thiaminase activity in each sample using a standard radiometric assay with nicotinic acid as the co-substrate (Zajicek et al. 2005). The limit of detection for this assay was 20 pmol thiamine degraded/g tissue/min, and all samples with thiaminase activity below the limit of detection were

assigned a thiaminase activity of 20 pmol thiamine degraded/g tissue/min, where tissue mass is reported as wet weight.

2.3.5 Zooplankton community composition

Individual organisms were identified under a dissecting microscope fitted with an ocular micrometer. All adult organisms were identified to the lowest possible taxonomic level using appropriate keys (Stemberger 1979, Balcer et al. 1984, Hudson and Lesko 2003). Rotifers, *Acanthocyclops* spp., *Alona* spp., *Bosmina* spp., and *Mesocyclops* spp. were identified to the genus level, and all other microcrustaeans were identified to the species level. Calanoid, cyclopoid, and harpacticoid nauplii were categorized to order and were considered 3 separate taxa. Copepodites were identified to order, with the exception of *Epischura lacustris*, *Limnocalanus marcurus*, *Senecella calanoides* and *Tropocyclops prasinus mexicanus*, which were classified to species, and *Mescyclops* spp., which were classified to genus. We treated copepodites that could be classified to the species or genus level as separate taxa relative to conspecific adults because of the differences in size, ecology, and the spatial and temporal distribution of copepodites and their conspecific adults. The first 20 individuals of each taxon/life-stage combination (hereafter "taxon") were measured using an ocular micrometer. We measured taxa as previously described (GLNPO 2003) with the following exceptions: *Bythotrephes* was measured from the proximal base of the spine to proximal base of the caudal kink (Garton and Berg 1990), *Mysis* was measured by antennal length (Grossnickle and Beeton 1979), *Cercopagis* was measured from the anterior tip of the head to the insertion of the third articular spine (Grigorovich et al. 2000). Dreissenid adults and veligers were measured by shell length, and fish larvae were measured by total length.

We used a split-level enumeration procedure to allow accurate enumeration of both common species and large or rare species (GLNPO 2003, Bunnell et al. 2012). We chose this procedure because of the potential influence that large bodied species have on total wet biomass calculations. Prior to splitting each sample with a Folsom plankton splitter, we examined the entire sample for any *Mysis*, *Bythotrephes*, *Cercopagis*, *Diporeia*, Dreissenid adults, and fish larvae (GLNPO 2003), which were

enumerated from the entire sample. Following splitting, we determined which dilutions constituted the 'A', 'B', 'C', and 'D' splits for the split-level sampling following described protocols (GLNPO 2003, Bunnell et al. 2012). Briefly, the dilution factor for the 'A' and 'B' splits (i.e., duplicate subsamples at the same dilution factor) was the dilution that resulted in a total of 200 to 400 individuals per split. The 'A' and 'B' splits represent the most dilute subsamples in which microcrustacean taxa (i.e., non-rotifers, non-nauplii, and non-veligers) were enumerated. The 'C' split, which was twice as concentrated as the 'A' and 'B' splits, was examined for any taxa whose counts in the 'A' and 'B' splits summed to fewer than 40 individuals (GLNPO 2003, Bunnell et al. 2012). The 'D' split, which was twice as concentrated as the 'C' split, was examined only for specific "large" taxa or taxa that were determined to be "rare" on a per-tow basis. Species treated as "large" in all samples included *Epischura lacustris*, *Holopedium*, *Leptodora kindtii*, *Limnocalanus marcrurus*, *Polyphemus pediculus*, and *Senecella calanoides*. Large species were always counted in the D split unless either (a) the sum of the counts in 'A' and 'B' splits or (b) the sum of counts in the 'A', 'B', and 'C' splits exceeded 40 individuals for a given "large" species (GLNPO 2003). For microcrustaceans, species were defined as "rare" when the combined A, B, and C splits resulted in counts of fewer than 40 individuals, in which case, those species were also counted in the D split. The determination of rarity was made on a per-tow basis for each taxon. Because of their abundance, rotifers, nauplii, and dreissenid veligers were only counted in A and B splits, and frequently the rotifer/nauplii/veliger A and B splits were more dilute than the A and B splits for microcrustaceans for any given tow. For each tow and taxon, count information from multiple splits (A-D) was averaged using a weighted average in which each split received a weighting proportional to the relative dilution of the original sample that was examined (i.e., counts from a 126-fold dilution were weighted twice as heavily as counts from a 256-fold dilution in the weighted average).

2.3.6 Conversion of zooplankton length measurements to biomass

For rotifers, mean lengths and widths were calculated arithmetically for each taxa collected in each tow. For all other taxa, the individual length measurements (mm) for each taxa in each tow were transformed to their natural logs, the mean of the log-

transformed lengths was calculated, and the geometric mean length (mm) was calculated by back-transforming the log-transformed mean to the original scale (GLNPO 2003).

Mean length measurements (arithmetic or geometric, as appropriate) were converted to wet weight biomass to facilitate a comparison between zooplankton community composition and thiaminase activity, which is calculated on a wet weight basis. When possible, a direct length-wet weight regression was used to estimate wet weight biomass directly (Table 2.3). When length-wet weight regressions were not available, we used length-dry weight equations to estimate dry weight, and dry weight was converted to wet weight (Table 2.3). Conversion of dry weight to wet weight is often achieved by applying a single percentage as a conversion factor across all individuals belonging to high-level taxonomic groupings, such as rotifers or zooplankton (Beers 1966, Omori 1969, Schindler and Noven 1971, Pace and Orcutt 1981, Lawrence et al. 1987, Johannsson et al. 2000). However, the choice of which single conversion percentage to apply is not obvious, as researchers have used percentages ranging from 5 to 19%, often with little justification for their choice, and the most appropriate percentage varies according to the size of individuals (Bottrell et al. 1976, Pauli 1989). Additionally, across taxa whose wet weights range over orders of magnitude, the percentage of dry weight in wet weight decreases as organisms increase in wet weight (Bottrell et al. 1976). Therefore, the following procedure was used to contrast a dry weight to wet weight conversion that accounts for the decreasing percentage of dry weight in wet weight as organisms increase in size: (1) data describing the relationship between wet weight and the percentage of dry weight in wet weight for cladocerans and copepods were extracted (Bottrell et al. 1976) and the *L. kindtii* wet weight data (Cummins et al. 1969) was adjusted downward by an order of magnitude to account for a 10-fold error in the original publication (Culver et al. 1985); (2) the percentage of dry weight in wet weight was regressed against wet weight (Appendix A); (3) the regression from (2) was used to predict the dry weight for individuals of a given wet weight; and (4) the calculated dry weight from (3) was regressed against the corresponding wet weight to create an equation for estimating wet weight (WW: in µg) from dry weight (DW: in µg). The

resulting regression from step 4 ($\log_{10}WW = 0.5377 + 1.52*\log_{10}DW$) was used to convert dry weight to wet weight when no other taxon-specific method was available.

2.3.7 Statistical analysis

R (R Core Team 2014) was used for all basic calculations of summary statistics, ANOVAs, and correlation analyses and PC-ORD (McCune and Mefford 2015) for all multivariate statistical analysis and to determine correlations with multivariate axes.

2.3.7.1 Assessing the relationship between thiaminase activity in bulk zooplankton samples and environmental variables

Thiaminase activity was log-transformed (natural log) prior to analysis, as its variance increased as the mean increased. The relationship between thiaminase activity in bulk zooplankton tows and four environmental variables (depth, season, fraction size, and site) was assessed using ANOVA.

To determine whether thiaminase activity is related to depth, season, and plankton size fraction, samples collected at Sturgeon Bay were compared across the three seasons, two depths, and three size fractions. Although a range of depths was sampled across all sites, depth was essentially confounded with site, with the exception of Sturgeon Bay and Port Weller, and too few samples were collected at Port Weller (8.5 and 75 m) to warrant inclusion of those data in this analysis. Therefore, we evaluated the influence of depth only at Sturgeon Bay. We constructed a full ANOVA to determine whether thiaminase activity was influenced by depth and season, while accounting for size fraction (log(*Thiaminase*) ~ *Size fraction* X *Depth* X *Season*). We treated depth, season, and size fraction as categorical variables. Variables that were not significant (see section 2.4.1) were removed from the full model and a final reduced model was constructed:

Model 2.1: log(*Thiaminase*) ~ *Size fraction* + *Season*

We used an extra-sum-of-squares F-test to determine the appropriateness of simultaneously dropping several terms from the full model. We report group means for thiaminase activity as geometric means, and sample standard deviations as

geometric standard deviations to maintain consistency with the log transformation of thiaminase activity in the models.

To evaluate the effect of site, we began by using all data from all sites in an initial full model that controlled for size fraction and season (log(*Thiaminase*) ~ *Size fraction* X *Site* X *Season*). Depth was excluded from this analysis because we found depth to not be significant in Model 2.1 (see section 2.4.1). Size fraction was included because we found that thiaminase varied by size fraction at Sturgeon Bay (see section 2.4.1). Our initial full model revealed convincing evidence of a *Size fraction* X *Site* interaction (p=0.0036), indicating that the relationship between thiaminase activity and site varied by size fraction. Therefore, we sought to conduct an analysis of the effect of site for each size fraction. However, because relatively few replicate samples were collected at the two smallest size fractions at sites other than Sturgeon Bay, we were unable to conduct separate analyses of the effect of site on thiaminase activity at each of the three size fractions (Table 2.2). Therefore, we limited the analysis of the effect of site on thiaminase activity to the >125 µm size fraction using a one-way ANOVA, and only for summer as Model 2.1 evaluates the effect of season, size fraction, and depth across size fractions.

Model 2.2: log(*Thiaminase*) ~ *Site*

For all models, we examined standard regression diagnostic plots (residual vs fit, normal Q-Q, scale-location, and leverage plots) to ensure appropriate model structure. We made *post-hoc* comparisons of the ratios of group medians using Tukey's Honest Significant Difference test.

2.3.7.2 Assessing the relationship between thiaminase activity and the zooplankton community

Selecting appropriate comparisons: We calculated the wet mass of the zooplankton community using tows collected with a 153 µm mesh net which were not sieved after collection. Therefore, we compared zooplankton community composition to the thiaminase activity in bulk zooplankton fractions that were collected in the same net (153 µm mesh) and subsequently only sieved through a 125 µm sieve. The small

difference in mesh size (28 µm) between the net and the sieve did not result in differences in the zooplankton community composition (Appendix B), so this comparison was appropriate.

The relationship between thiaminase activity and the zooplankton community was assessed by applying three multivariate methods (see below) to the 10 sampling events for which both the zooplankton community and thiaminase activity were characterized (Table 2.2). Because thiaminase is assayed on a per-gram (wet weight) basis, we converted the estimated wet biomass for each taxon to a gram-normalized equivalent on a per-tow basis; the gram-normalized wet mass therefore represents the wet mass (in mg) of each taxon that would be expected in a one gram sample from each tow. We averaged the gram-normalized wet biomass for each taxon across the three zooplankton community tows taken at each sampling event. The 10 multivariate centroids of the triplicate zooplankton community tows at each sampling event did not differ from the location (in taxa space) of the 10 sampling events when the average gram-normalized wet biomass was used to represent each sampling event (Appendix C). We averaged the thiaminase activity from the three replicate thiaminase tows for each of the 10 sampling events.

Choice of analytical methods: The three different multivariate analytical techniques we chose to assess the relationship between thiaminase activity and zooplankton community composition were: non-metric multidimensional scaling (NMS) (Kruskal 1964a, 1964b, Mather 1976), distance-based redundancy analysis (db-RDA) (Legendre and Anderson 1999, McArdle and Anderson 2001), and indicator species analysis (ISA) (Dufrene and Legendre 1997). We selected three techniques because each offers a fundamentally different conceptual method of evaluating the question of interest, and as a result, each has the potential to reach different conclusions. Each of these methods is appropriate for community data, which are typically non-normally distributed and result in a sparse matrix.

NMS is a nonparametric ordination technique that seeks to reduce the dimensionality of the original multivariate community data by harnessing the correlation among taxa in the sampling events (McCune and Grace 2002). NMS arranges the position of the

sampling events in taxa-space solely based on community data and in the absence of any information about response variables (i.e., thiaminase activity). The NMS produces new ("synthetic") axes that contain information about correlated combinations of taxa. NMS is fundamentally a data reduction technique; it seeks to represent the greatest axes of variation in the community assemblage using the fewest number of dimensions while still retaining as much of the information as possible from the original *n*-dimensional community data. After conducting NMS, the ordination solution can be rotated to correspond to response variables of interest, such as thiaminase activity. We chose NMS because it first arranges sampling events along the axes representing the major sources of variation in the zooplankton community and then evaluates the degree to which these major sources of variation are related to variables such as thiaminase.

db-RDA is also an ordination technique, but it differs fundamentally from NMS in that the ordination of the community data is expressly constrained by one or more response variables. db-RDA regresses *each* of the taxa in a community separately against response variables (i.e., thiaminase activity), calculates the fitted values for each regression, and performs principle components analysis on the fitted values to find combinations of taxa that are linearly related to the response variable (Lehman and Caceres 1993). Unlike NMS, db-RDA only focuses on the elements of the variation in the community assemblage that is specifically related to response variables, which may represent a relatively small proportion of the total variation in the community assemblage. Although this technique is rarely suitable for community data because taxa do not usually responded linearly to "typical" response variables for community analysis (i.e., environmental variables) (McCune and Grace 2002), a linear relationship between taxa biomass and thiaminase activity could reasonably be expected if the mass of thiaminase-producing taxa is expected to be greatest in the samples with the highest thiaminase and lowest in the samples with the least thiaminase activity. We chose db-RDA as way to specifically identify gradients in the plankton community that are related to thiaminase, even if these gradients do not constitute the majority of the variation in the plankton community.

ISA is designed to determine which taxa are indicative of groups that are designated prior to analysis (McCune and Grace 2002). Although thiaminase activity is a quantitative variable and was treated as such in the NMS and db-RDA, quantitative thiaminase activity measurements can also be used to group the 10 sampling events into high and low thiaminase categories, which can then be interrogated for indicator species. ISA combines information on both taxa mass in each group as well as information about frequency of occurrence (McCune and Grace 2002). ISA is fundamentally different from both NMS and db-RDA because it treats the response as a categorical variable and because it integrates information about both mass and frequency of occurrence within each group into one indicator value (IV), which is used to assess the degree to which a taxon is representative of a particular group. We chose ISA because it focuses on the contributions of individual species rather than community gradients.

Data manipulations and outlier detection for multivariate analyses: The main matrix for all three multivariate analysis was identical: each row was one of the 10 sampling events, each column was a taxon, and each cell contained the average gram-normalized wet biomass for a specific taxon and sampling event. A total of 51 taxa were present in the original zooplankton community data set. Deletion of rare species often improves the ability of multivariate analyses to detect structure in community data, and generally, species that occur in fewer than 5% of the sample units are deleted prior to community analysis (McCune and Grace 2002). Six taxa (*Acanthocyclops* sp., *Eurytemora affinis*, Hydra, Nematode, *Notommata*, and Osmeridae larvae) appeared in only one of any of the 30 zooplankton community tows, and these were deleted prior to the start of analysis. In each case, the total biomass of any of these taxa constituted less than 0.32% of the total biomass in the tow. Of the remaining 45 taxa, six were present in only one of the 10 sampling units including *Alona*, Bloater larvae, *Dreissena bugensis* adults, *Daphnia mendotae*, *Daphnia retrocurva*, and *Diporeia*. These were also deleted for the main analysis, leaving 39 taxa. After taxa were removed, we to gram-renormalized the wet mass of the remaining taxa and expressing the wet biomass for each taxon as a percentage for that sampling event. The main matrix used for all three analyses was 10 sampling events (rows) X 39 taxa (columns). The second matrix varied for each

analysis and is described in the details for each procedure. Hereafter, “zooplankton community composition” refers to the gram-normalized wet mass of the 39 taxa that were used in subsequent analysis.

To detect outliers, the average distance of each sampling event from all other sampling events was calculated using Sorensen distance. Outlier analysis revealed that the Port Weller sampling event was a moderate outlier at 2.5 standard deviations from the average distance of all points from each other points. Port Weller was retained in the analysis because it represented a plankton community not otherwise represented and because its removal did not substantively change the conclusions of any of the three analyses. Arcsine square root transformation of the gram-normalized wet biomass data also did not make any substantive difference in the conclusions.

NMS procedure: We performed NMS on the main matrix in the “slow and thorough” autopilot mode in PC-ORD using Sorensen distance. We selected Sorensen (or Bray-Curtis) distance as the metric by which to measure the distance between sample units in 39-dimensional space because it is a proportional city-block measure, does not lose sensitivity with increasing heterogeneity to the extent that other metrics do, and has a maximum distance for samples containing no common species (McCune and Grace 2002). The NMS autopilot conducted 250 NMS runs with the real data from random starting configurations. Each run produced a 1-, 2-, 3-, 4-, 5-, and 6-dimensional solution from the random starting configuration for that run. The process was repeated 250 times with randomized data in which the data in each column (taxa) of the main matrix was shuffled within columns, thereby decoupling the taxa-by-sampling event relationships. To determine whether the reduction in stress achieved with the actual data is greater than that achieved with the randomized data, a p-value was calculated by comparing the proportion of times that the runs with randomized data resulted in a stress values less than or equal to the observed minimum stress in the best run with real data. We monitored the stability of the ordination by assessing the stress versus iteration trace and ensuring that the stability criterion (standard deviation of stress is less than 0.000000 in the last 10 iterations) was met (McCune and Mefford 2015).

We examined the resulting ordination graphically and rotated solutions to maximize the correspondence with the log of thiaminase activity. We evaluated the degree of correspondence between thiaminase activity and NMS axes using Pearson's correlation coefficient (*r*), which is a measure of linear correlation, Kendall's tau, which is a nonparametric rank correlation, and scatter plots of the data. The percentage of the variation in the original 39-dimensional data represented by any ordination was calculated after the ordination by calculating Pearson's coefficient of determination (r^2) between the distances in the ordination space (determined using Euclidean distance) and the distances in the original 39-dimensional space (using Sorensen distance).

db-RDA procedure: db-RDA was conducted using the same main matrix as NMS and Sorensen distance. The data in the main matrix columns were standardized but not centered to retain the effect of gram-normalizing the wet biomass data. The second matrix consisted of 10 rows (sampling event) and 1 column (log of thiaminase activity). We chose distance biplot scaling so that the distance between sample units in the ordination approximated their distance in 39-dimensional space. Because the second matrix consisted of only one variable, the result of the db-RDA is necessarily one single canonical axis. To determine whether the overall relationship between thiaminase and the resulting single ordination axis was stronger than could be expected by chance alone, we conducted a randomization test (n=998 randomizations) in which the rows of the two matrices were shuffled relative to one another, the db-RDA was conducted, and the test statistic was recorded for each randomization. A p-value was generated by determining the proportion of times the observed test statistic was greater than or equal to that resulting from the ordination of the real data. The randomization test represents a test of the null hypothesis that there is no linear relationship between thiaminase and zooplankton community composition (McCune and Mefford 2015). We assessed the amount of variation in the main matrix represented by the second matrix (R^2) based on Ezekiel-adjusted values (Peres-Neto et al. 2006).

ISA procedure: ISA was conducted using the same main matrix and Sorensen distance. The second matrix for ISA consisted of the 10 sampling events as rows and 4 columns, each of which indicated a thiaminase activity grouping for the 10 sampling events. One challenge we faced in categorizing the 10 sampling events as high or low thiaminase is several different sampling events with intermediate average thiaminase activities could be reasonably assigned to either high or low groups (Table 2.4). Therefore, we conducted four separate analyses in which the high and low thiaminase groups consisted of slightly different sampling events to evaluate the influence of placing sampling events with intermediate thiaminase values into a specific group. In the first two analyses, all 10 sampling units were assigned to either the high or low group (Table 2.4). In the last two analyses, the 10 sampling events were assigned to one of three groups (high, medium, or low thiaminase activity), and the middle group was excluded from the analysis to achieve the best contrast between the sample units containing the highest and lowest thiaminase activities. The ISA produces an indicator value (IV) for each species in each group, with a value of 100 representing perfect indication of both faithfulness (the taxa is always present in the group) and exclusivity (the taxa never occurs in other groups), whereas an IV value of zero represents no indication if faithfulness or exclusivity (McCune and Grace 2002). The highest IV for a given species in any group is designated as IV_{max}. We assessed the significance of the IVs in two ways. To determine if any given species is an indicator for a particular group, a randomization test (n=1000 randomizations) was conducted in which the sampling events were randomly reassigned to groups, the ISA was repeated, and IV_{max} for each species was calculated for each randomization. A p-value for each species was then generated by determining the proportion of times the IV_{max} calculated from the randomized data was as high as or higher than the IV_{max} calculated from the randomized data. To test the overall hypothesis of differences among the groups across all the species, the same procedure was repeated, but the sum of the IV_{max} values across all species (as opposed to the IV_{max} value for individual species) was the test statistic for which the p-value was computed (McCune and Mefford 2015). For taxa indicative of the high thiaminase group, a rank correlation analysis (Kendall's tau) was conducted to determine the taxa-specific relationship between gram-normalized wet mass and thiaminase activity.

2.4 Results

2.4.1 Relationship between thiaminase activity in bulk zooplankton tows and environmental variables

Thiaminase activity in bulk zooplankton tows ranged from undetectable (20 pmol thiamine degraded/g tissue/min) to 3,282 pmol thiamine degraded/g tissue/min (Figure 2.2) and varied according to the season, size fraction, and site.

Thiaminase activity was not related to depth (p=0.21 in the full model) at Sturgeon Bay after accounting for size fraction and season. No convincing evidence existed for two-way or three-way interactions in the full model (*Size fraction* X *Depth,* p=0.53; *Size fraction X Season*, p=0.44; *Depth X Season*, p=0.06; *Size fraction X Season X Depth*, p=0.32). Therefore, all non-significant variables were dropped, resulting in a reduced version of the initial model (log(*Thiaminase*) ~ *Size fraction* + *Season*) that performed as well as the full model (extra-sum-of-squares F-test: p=0.25).

Temporally, thiaminase activity differed by season (p<0.0001, F=23.91, df=2; Appendix D) at Sturgeon Bay after controlling for the effect of size-fraction. Thiaminase activity was higher in summer and fall than in spring (Table 2.5). Specifically, the median thiaminase activity in the summer was 9.9 times (95% CI: 3.7 to 26.7 times) higher than the median thiaminase activity in the spring, and the median thiaminase activity in the fall was 12.6 times (95% CI: 4.9 to 32.7 times) higher than that in spring (both p<0.00001). Thiaminase activity in summer and fall did not differ (p=0.75, 95% CI: 0.56 to 2.92 for the ratios of the median thiaminase activities, which includes the “no difference” value of 1.0).

Thiaminase activity at Sturgeon Bay also differed by size fraction (p<0.0001, F-13.28, df=2; Appendix D). Thiaminase activity was lowest in the smallest size fraction (25-53 µm; Table 2.5). Specifically, the median thiaminase activity in the 25-53 µm size fraction was 3.0 times (95% CI: 1.2 to 7.9 times) smaller than the median thiaminase activity in the 53-125 µm size fraction (p=0.02), and the median thiaminase activity in the 25-53 µm size fraction was 6.6 times (95% CI: 2.7 to 16.3

times) smaller than that in the >125 µm size ($p<0.00001$). Thiaminase activity in the two largest size fractions (>125 µm and 53-125 µm) did not differ ($p=0.08$, 95% CI: 0.93 to 5.15 for the ratios of the medians).

Spatially, thiaminase activity in the >125 µm size fraction differed by site during the summer ($p<0.0001$; F-13.28, df=2; Table 2.6 and Appendix E). Median thiaminase activity at Ashland and Detour did not differ ($p=0.9$) and was lower than at all other sites. Median thiaminase activity at either Ashland or Detour was between 13 and 60.5 times lower than at Port Weller, Frankfort, or Sturgeon Bay, depending on the specific pairwise comparison (Figure Appendix E.2). Thiaminase activity at Port Weller, Frankfort, and Sturgeon Bay did not differ from one another ($p>0.59$) and was higher at those locations than at Ashland or Detour (Figure Appendix E.2). The effect of site in summer in the >125 µm size fraction was: (Ashland = Detour) < (Port Weller = Frankfort = Sturgeon Bay).

For the largest size fraction, the lowest thiaminase activity occurred where egg thiamine levels are highest and natural reproduction of Lake Trout is most robust (Ashland and Detour), while the highest thiaminase activity occurred at sites with the most severe TDC (Frankfort, Sturgeon Bay, and Port Weller, all with egg thiamine ranging from 0.95 to 3.5 nmol/g). The interaction of site by size fraction ($p=0.0036$; section 2.3.7.1) in the initial full model suggests that this relationship may not hold for other size fractions.

2.4.2 Thiaminase activity in individual taxa

Thiaminase activity in *Bythotrephes* was quite variable. We did not detect thiaminase activity in *Bythotrephes* sampled at either Detour or Frankfort, but we did detect thiaminase activity (100-690 pmol/g/min) multiple times in individuals from Sturgeon Bay (Figure 2.3). The geometric mean of thiaminase activity of *Bythotrephes* at Sturgeon Bay was 302 pmol/g/min (geometric SD=2.1). We did not detect thiaminase activity in either of the samples of *Cercopagis* collected at Sturgeon Bay in the summer at 110 m but it was detectable in the sample collected at 18 m (370 pmol/g/min). Thiaminase was not detected in any of the three samples of *Holopedium*, collected only at Detour. Thiaminase activity of *Mysis* was also

variable, ranging from 88-1232 pmol/g/min with a geometric mean 212 pmol/g/min (geometric SD=2.0).

We detected thiaminase activity in every sample for each of the two algal and two plant taxa assayed (Figure 2.3). Mean thiaminase activity (geometric mean and geometric SD) was 199 pmol/g/min (SD=2.3) for *Chara*, 384 pmol/g/min (SD=2.3) for *Cladophora*, 895 pmol/p/min for *Elodea* (SD=2.6), and 203 pmol/g/min for *Potamogeton* (SD=1.7). Thiaminase activity in the single sample of mixed macroinvertebrates (a mixture of Diporeia, chironomids, oligochaetes, ceratopogonids, and freshwater snails) was 27,000 pmol/g/min.

2.4.3 Zooplankton community composition

The spring nearshore (18 m) and offshore (100m) community composition at Sturgeon Bay was dominated by Leptodiamptomids (*L. ashlandi*, *L. sicilis*, and *L. minutus*) and calanoid copepodites (likely of *L. ashlandi*, *L. sicilis*, and *L. minutus*), with these four taxa making up more than 87% and 90% of the wet biomass, respectively (Figure 2.4; all data: Appendix F). At Sturgeon Bay, the summer and fall nearshore communities were more similar to each than either were to their offshore counterpart for the same seasons. The nearshore summer and fall communities at Sturgeon Bay had the greatest number of taxa contributing to the majority of the biomass, with 9 (fall) and 10 (summer) taxa contributing to 90% of the wet biomass. In the summer, calanoid copepodites, *Dreissenid* veligers, *Conochilus*, and *Bythothrephes* each contributed to more than 10% of the wet biomass, with the Leptodiaptomids having a lesser contribution than in the spring. The fall nearshore community assemblage shared most of these taxa, but *L. ashlandi, Epischura lacustris* adults and copepodites, and *Daphnia* spp. constituted a greater proportion of the total wet biomass in the fall than in the summer, and *Bythotrephes* constituted a small proportion. The offshore communities (summer and fall) were also similar to each other at Sturgeon Bay and were less diverse than in the summer. Both communities were dominated by *L. macrurus* adults, *L. sicilis*, *Daphnia* spp., and calanoid copepodites, with these 4 taxa making up 82-84% of the total wet biomass. The summer offshore sample also contained *L. ashlandi* (7%) and *Conochilus* (3%),

whereas the fall offshore sample included *E. lacustris* adults and copepodites (7%) as well as Bythotrephes (4%).

The summer, 28 m community composition at Frankfort was more similar to the community composition at Sturgeon Bay than to other sites. Six taxa made up 90% of the biomass, including calanoid copepodites, Leptodiamptomids (*L. ashlandi*, *L. sicilis*, and *L. minutus*) *L. macrurus* adults, and Daphnia spp. The zooplankton communities at Ashland and Detour were similar to one another, with approximately 60% of the wet biomass at both consisting of just 3 taxa, *Holopedium*, calanoid copepodites, and cyclopoid copepodites. Ashland was also characterized by *Diacyclops thomasi* (9%), *L. sicilis* (9%), and *Asplancha* (6%), and whereas Detour was characterized by *L. macrurus* adults (9%), Conochilus (8%), and *Bosmina* (5%). Port Weller shared the few taxa in common with the samples from the upper Great Lakes. Two taxa, *Asplanchna* (57%) and cyclopoid copepodites (19%), made up 71% of the wet biomass of the community, with smaller contributions from *Cercopagis* (9%), *L. macrurus* adults (3%), and *Bosmina* (3%).

Community composition of the smaller size fractions were not enumerated for all samples as they were for the >125 µm fractions; inspection of the two smallest size fractions revealed that the 53-125 µm fraction was dominated by small taxa including Dreissenid veligers and calanoid nauplii, with additional components of the community including small rotifer taxa (*Kellicottia*, *Gastropus*, *Syncheata*, and *Polyarthra)* and small individuals of larger rotifer taxa (i.e.,*Conochilus, Ploesoma)*. Across all community samples (153 µm mesh net), the mean lengths of Dreissenids veligers (201 µm) and calanoid nauplii (292 µm) were greater than 125 µm, and individuals of these taxa were therefore retained in the 153 and 125 µm mesh nets and filters. The minimum lengths for veligers (62 µm) and nauplii (93 µm) were below 125 µm, and the smallest individuals in these taxa were able to pass through the 125 µm mesh net. The 25-53 µm fraction contained an occasional small *Polyarthra*, *Conochilus*, Dreissenid veliger, or nauplii, but at biomass levels approximately 1.5 to 2 orders of magnitude lower than that found in the 53-125 µm fraction, with most of the biomass consisting of phytoplankton, fragments of material that could not be identified, and presumably large bacterioplankton.

2.4.4 Relationship between thiaminase activity in bulk zooplankton tows and zooplankton community composition

The NMS autopilot recommended a 3-dimensional solution (stress =2.249, p=0.0317), which was rotated to maximize correspondence with thiaminase activity (Appendix G). Although this ordination represented 94% of the variation present in the original 39-dimensional taxa-space, the third dimension's incremental contribution to the 94.1% was only 5%. Additionally, the stress for the 2-dimensional was low (7.61), and the randomization test indicated a greater stress reduction than could be expected due to chance (p=0.04). Therefore, the 2-dimensional solution was chosen over the 3-dimensional solution as more interpretable. The 10 sampling events were successfully ordinated in taxa-space (p=0.04 for randomization test). The ordination is represented graphically in Figure 2.5; sampling events located more closely to each other have more similar zooplankton communities. The 2-dimensional solution was reached after 35 iterations, and the final stress was low (7.61). The ordination represented 88.7% of the variation present in the original 39-dimensional data. Axis 1 represented 76.8% of the variance and axis 2 represented an additional 11.9% of the variance. After rotation to maximize the correspondence axis 1 with thiaminase, the zooplankton community (as represented by the axis 1 scores) explained 0.04% of the variation in the log of thiaminase activity (r=0.205, r^2=0.04, tau=0.156). Along axis 1, the three sampling events with the highest thiaminase activity (PW Sum 20, SB Sum 18 and SB Fall 18) are separated in ordination space by the two sampling events with the lowest thiaminase activity (AS Sum 55 and DT Sum 32), and the sampling events with the highest and lowest thiaminase values are not able to be separated from one another in any other apparent non-linear grouping (Figure 2.5).

The randomization test for the db-RDA revealed no evidence for a relationship between thiaminase activity and zooplankton community composition (p=0.45). The resulting ordination, which is constrained by the thiaminase activity data in the second matrix, represented virtually none (R^2 <0.01) of the variation in the zooplankton community biomass data.

None of the four ISA groupings resulted in an overall difference between the high and low thiaminase groups across all species ($0.59<p<0.09$). In each of the four analyses, the only two taxa individually identified as associated with the high thiaminase group were *Ploesoma* and *Asplanchna*. *Ploesoma* was a significant indicator of high thiaminase in groupings 1, 2, and 3 (IV: 83.3, 91.5, and 100, respectively; p: 0.043, 0.016, and 0.028, respectively). *Asplanchna* was a significant indicator of high thiaminase activity in only grouping 2 (IV: 95.1, p=0.041). Convincing evidence of a relationship between gram-normalized wet biomass and thiaminase activity existed for *Ploesoma* (tau=0.68, p=0.009) but not for *Asplanchna* (tau=0.23, p=0.36; Appendix H).

2.5 Discussion

2.5.1 Thiaminase activity in bulk zooplankton tows and relationship to season, size fraction, and site

Overall, thiaminase activity levels were comparable with previous reports. For example, mean thiaminase activity in bulk zooplankton fractions (hereafter >153 µm for other studies and > 125 µm for this study unless otherwise specified) from western Lake Ontario (near Bronte and Hamilton Harbor) ranged from 20 to approximately 4,750 pmol/g/min (Fitzsimons et al. 2004b), with most samples ranging between 20 and 600 pmol/g/min. Similarly, mean thiaminase activity in bulk zooplankton from six Finger Lakes (New York) ranged from 180 to approximately 3,100 pmol/g/min, depending on the lake (Fitzsimons et al. 2004b).

We found that bulk zooplankton thiaminase activity was lowest in early spring at Sturgeon Bay. To the best of our knowledge, this is the first report of early-spring zooplankton thiaminase activity in the Great Lakes. Our motivation for sampling in early spring was the consistently observed higher thiaminase activity of Alewife in the winter and spring (Ji and Adelman 1998, Fitzsimons et al. 2004a, Tillitt et al. 2005), the same as we found (Chapter 3). Thiaminase activity in Rainbow Smelt, on the other hand, does not appear to vary (Ji and Adelman 1998, Tillitt et al. 2005). The seasonal patterns we observed in thiaminase activity of bulk zooplankton fractions differed from seasonal patterns of thiaminase activity in Alewife and Rainbow Smelt

(Chapter 3), which raised the possibility that zooplankton may not be the ultimate source of thiaminase reaching lake trout.

We collected samples on only one day in each of the three seasons, but it is certainly possible that thiaminase activity in zooplankton varies at small time scales. A more detailed temporal assessment of thiaminase activity in bulk zooplankton fractions (Fitzsimons et al. 2004b) found temporal patterns in zooplankton thiaminase activity, in two locations. At one location (Bronte, ON) it was measured at 1,800 pmol/g/min in mid-June, dropped to 100 pmol/g/min in mid-July, and to a low of ~20-30 pmol/g/min in late August – mid September (Fitzsimons et al. 2004b), while at the other location (Hamilton Harbor), the opposite pattern was seen; thiaminase activity was lower in mid-June and early July (25-70 pmol/g/min) than it was over 3 sampling events in late July through mid-August (300-500 pmol/g/min) (Fitzsimons et al. 2004b). Furthermore, at the second location, mean thiaminase activity in bulk zooplankton increased from approximately 25 pmol/g/min to approximately 300 pmol/g/min at two sampling events separated by approximately 7 days (Fitzsimons et al. 2004b). This suggests that temporal patterns of thiaminase activity may not be consistent across sites (even when sites are relatively close to each other and are sampled throughout the same season and year) and that thiaminase activity in bulk zooplankton tows may change on shorter temporal scales than we examined. If day-to-day variability in thiaminase is high, then any assessment of the effect of factors such as season, depth, or site requires more intensive sampling than achieved in this or other research to date.

Thiaminase activity was higher in the largest two size fractions (53-125 µm and >125 µm) at Sturgeon Bay. This suggests that on a per-mass basis, at this location, thiaminase activity of veligers, small rotifers and calanoid nauplii, as a group, is similar to that of larger crustaceous zooplankton taxa at Sturgeon Bay, but this relationship does not hold at all locations. For example, at Ashland and Detour thiaminase activity in the largest fractions was lower than or similar to that in the smaller size fractions, whereas at Sturgeon Bay, the smallest size fraction has lower thiaminase activity than the larger fractions. We obtained relatively few replicates at the lowest size fraction at sites other than Sturgeon Bay, which suggests that this

result should be interpreted cautiously. Additional intensive sampling of smaller size fractions across multiple sites is required to determine the degree to which the relationship between thiaminase activity and fraction size determined at Sturgeon Bay holds for other sites. Collecting sufficient mass of the smaller size fractions for the desired level of replication proved difficult, which is perhaps not surprising given the disappearance of the spring bloom and decline in the abundance of zooplankton in lakes Michigan and Huron (Barbiero et al. 2012). Horizontal zooplankton tows may increase the ability to collect sufficient mass of smaller size fractions, as would focusing research on seasons in which zooplankton production was expected to be highest.

The largest bulk zooplankton size fraction from Ashland and Detour had lower thiaminase activities than those sampled from Frankfort, Sturgeon Bay, and Port Weller, indicating that the lowest thiaminase activity occurred where egg thiamine levels are highest and natural reproduction of Lake Trout is most robust; however, the relationships we observed among sites may not hold for small size fractions. By way of comparison, the geometric mean thiaminase activity of bulk zooplankton in Whitefish Bay (eastern Lake Superior) in early June 1998 (Zajicek et al. 2005) was higher than we observed in late July at Ashland and higher than we observed at Detour. The geometric mean thiaminase activity in bulk zooplankton at Manistique (northern Lake Michigan) in late July of 1998 was 49 pmol/g/min (SD=33, n=3). In July of 2000, mean thiaminase activity of bulk zooplankton was approximately 120 pmol/g/min at Muskegon and approximately 320 pmol/g/min at Sturgeon Bay (Fitzsimons et al. 2004b). These thiaminase activities are on the lower end of the range of those we observed in Lake Michigan. The thiaminase activity we observed at Port Weller was generally on the high end of the range of the thiaminase activity observed in zooplankton in 2002 at Bronte and Hamilton Harbor (Fitzsimons et al. 2004b), with the thiaminase activity in our Port Weller samples exceeding all but one of the mean values for the 13 sampling events at either Hamilton Harbor or Bronte.

2.5.2 Thiaminase activity in individual taxa

The four zooplankton taxa we sampled individually were collected at too few sampling events or with too few replicates to warrant a comprehensive statistical

analysis of the effect of site, depth, or season on thiaminase activity. Thiaminase activity within each individual zooplankton taxon was variable, with no noticeably less variation in taxon-specific thiaminase activity (regardless of sampling event) as compared to that in >125 µm bulk zooplankton samples with similar mean thiaminase activity. For example, thiaminase activity across all *Mysis* sampled (geometric mean=212 pmol/g/min; geometric SD=2.0) was no less variable than the thiaminase activity in the > 125 µm bulk thiaminase fractions with similar mean thiaminase activity (Table 2.4). Within a taxon, thiaminase activity at particular sites was not necessarily reflective of the overall pattern of thiaminase activity across sites for the >125 µm bulk fractions; thiaminase activity in *Mysis* collected at Detour overlapped the thiaminase activity of *Mysis* collected at Sturgeon Bay and Port Weller, and yet the thiaminase activity of bulk fractions was lower at Detour than at Sturgeon Bay and Port Weller.

With the exception of *Mysis*, no prior reports exist of thiaminase activity in the zooplankton taxa that we sampled. Others reported thiaminase activity levels in *Mysis* that are similar to our observations (Zajicek et al. 2005). Measurements of thiaminase activity of individual zooplankton taxa are rare, likely owing to the difficulty of collecting sufficient mass for analysis. The thiaminase activity of *Diporeia* from Manistique (Zajicek et al. 2005) is generally of similar magnitude to our findings for individual taxa. Thiaminase activity has been reported for several species of *Daphnia* (Birger et al. 1973, Honeyfield et al. 2010a, Kraft et al. 2014) using assay methods that differ from ours, making direct comparisons difficult. Using the 4-nitrothiophenol (4-NTP) assay, thiaminase activity of *Daphnia pulex* (the source of which was not described) was measured as 4,600 nmol/g/min ± 1,400 (Honeyfield et al. 2010a), but note that the 4-NTP assay is approximately 10^3 more sensitive for fish than the radiometric assay that we used (Honeyfield et al. 2010a). Even considering the tentative and coarse 10^3 adjustment previously used to compare samples analyzed by the radiometric and 4-NTP assay (Honeyfield et al. 2010a, Blakeslee et al. 2015), none of the 3 cladocerans we sampled had thiaminase activity as high as that reported for *D. pulex*; however, we used nicotinic acid as a co-substrate in the radiometric assay, and if 4-NTP is a more favorable co-substrate, a direct comparison of the thiaminase activity measured by the radiometric and 4-NTP

assays should be approached cautiously. *Daphnia pulicaria* fed *Scenedesmus acutus* (chlorophyte), both of which were cultured in a laboratory, had no thiaminase activity (4-NTP assay), regardless of whether the *Scenedesmus* was grown with or without thiamine or whether the *D. pulicaria's* intestinal tracts were cleared prior to thiaminase analysis (Kraft et al. 2014). Whether the striking differences in thiaminase activity of *D. pulex* and *D. pulicaria*, both assessed with the 4-NTP assay, result from species-specific differences or environmental conditions cannot be determined without additional study but suggests the capacity for related taxa to vary widely in thiaminase activity.

Several plant taxa are known to produce thiaminase, most notably terrestrial ferns (bracken), aquatic ferns (nardoo), and horsetails (Fujita 1954, Evans 1976, McCleary and Chick 1977, Ramos et al. 2005, Kraft et al. 2014). Consumption of thiaminase-containing plants is known to cause thiamine deficiency in horses (*Equus ferus caballus*), pigs (*Sus domesticus*), and sheep (*Ovis aries*) (Evans 1976, Ramos et al. 2005). The thiaminase activity we measured in our plant taxa is of the same order of magnitude as that of thiaminase-positive ferns (126 nmol/g/min) (Kraft et al. 2014). Two taxa of green algae (*Volvox aureus* and *Protococcus* spp., both class Chlorophyceae, division Chlorophyta) have been reported to have thiaminase activity (Arsan and Malyarevskaya 1969), but the method used to quantify thiaminase activity was sufficiently different as to preclude a direct comparison with our measurements. *Cladophora*'s close physical and biological association with dreissenids (Higgins et al. 2008) combined with the prior reports of thiaminase activity in chlorophytes (Arsan and Malyarevskaya 1969) may suggest *Cladophora* as a source of thiaminase for dreissenids. The *Cladophora* specimens we sampled had mean thiaminase values 244 times lower than that of quagga mussels sampled during this same sampling event (Tillitt et al. 2009), which suggests that *Cladophora* are likely not the source of thiaminase activity in quagga mussels.

Our single, mixed macroinvertebrates sample contained the highest thiaminase activity (27,000 pmol/g/min) of all the samples we examined. This level of thiaminase activity is higher than mean whole-body thiaminase activity for most fish species for which quantitative thiaminase data are available, including Alewife and

Rainbow Smelt (Fitzsimons et al. 2005b, Tillitt et al. 2005, Honeyfield et al. 2012). Data on thiaminase activity in macroinvertebrates are scarce. Broadly, taxa represented in this sample included *Diporeia*, annelids, insects, and mollusks. The only known thiaminase data on amphipods was summarized above for *Diporeia*, and those data suggest low thiaminase activity (Zajicek et al. 2005). Several taxa of mollusks (Fujita 1954, Tillitt et al. 2009, Blakeslee et al. 2015) and one genera of insect (Nishimune et al. 2000, Okonji et al. 2012) are known to contain high levels of thiaminase.

2.5.3 Zooplankton community composition

The zooplankton community composition we report here is generally similar to that reported by others for similar locations, years, and depths. Our community composition metrics are not reported on either a density- or biomass-per-volume basis as is typical of limnological studies, because the focus of this analysis was to compare the community data to the per-gram thiaminase assay. Furthermore, community composition reported here is on a wet-weight, rather than the traditional dry-weight, basis so our community composition data should not be directly (i.e., quantitatively) compared with density or dry mass estimates of community composition from other studies.

In northern Lake Michigan (Sturgeon Bay and Frankfort), large (> 0.9 mm) taxa in summer offshore were dominated by *L. macrurus* adults, with a lower proportion of *Daphnia* spp. than seen historically, which is consistent with recent reports (Barbiero et al. 2009b). In the fall offshore samples (Sturgeon Bay only), however, the proportion of the wet biomass contributed by *Daphnia* was approximately equal to that of *L. macrurus*. Nearshore and spring samples at Sturgeon Bay did not show the same dominance by *L. macrurus* as seen in the summer and fall offshore samples. In Lake Huron, *Daphnia* spp. accounted for very little of the wet biomass, whereas copepodites, *Holopedium*, and *L. macrurus,* accounted for a large proportion of the biomass, which is consistent with 2003-2006 community composition in northern Lake Huron (Barbiero et al. 2009a). Although our collections for community composition were focused on macroplankton rather than rotifers, the most commonly encountered rotifers in lakes Michigan, Huron, and Superior

(*Conochilus*, *Kellicottia*, *Keratella*, *Polyarthra* and *Ploesoma*) were consistent with other observations (Barbiero and Warren 2011, Barbiero et al. 2012), as was our finding that *Conochilus* was both the most widespread and represented the greatest proportion of the biomass for rotifers.

The community composition at Ashland was similar to that of Detour, and the taxa contributing to the majority of the biomass are similar to previous reports (Brown and Branstrator 2004, Holbrook et al. 2006). The community composition at Port Weller was the least similar to that in the upper lakes, which is not surprising given the convergence of the trophic status and zooplankton communities in the upper lakes occurring in the early-mid 2000s (Barbiero et al. 2012). Our samples were dominated by *Asplanchna*, which is present but not typically been reported as the dominant rotifer taxa in Lake Ontario; however, our samples were taken at nearshore (25m) rather than the offshore locations (98-130 m) of other studies (Barbiero and Warren 2011, Makarewicz and Lewis 2015) and *Asplanchna* tend to be more abundant in Lake Ontario than in the upper lakes (Barbiero and Warren 2011). The taxa contributing to most of the remaining wet biomass at Port Weller were similar to those that contributed to dry mass in whole water column tows at nearshore sites in Lake Ontario in 2008 (Rudstam et al. 2015).

2.5.4 Contributions of individual taxa to thiaminase activity of bulk zooplankton tows

We did not identify an overall relationship between thiaminase activity and zooplankton community composition. The major axes of variation in zooplankton community composition characterized by NMS were at most weekly related to thiaminase activity. Visual inspection of the ordination revealed that the sampling events with the lowest thiaminase activity were relatively evenly distributed in taxa-space rather than occupying one region of taxa-space. Likewise, the sampling events with the highest thiaminase activity were spread throughout taxa-space. After rotation for correspondence with thiaminase activity, the relationship between the axis 1 scores and thiaminase activity (bottom panel, Figure 2.5) was not compelling. However, the two axes extracted by the NMS represented the two major sources of variation in community composition among these sampling events. The possibility

exists that thiaminase activity may be related to a gradient in community composition that was not one of the two gradients that best explains overall community composition. We therefore conducted a constrained ordination (db-RDA) to investigate whether any portion of the community structure is related to thiaminase activity. The db-RDA did not reveal any portion of the community that was linearly related to thiaminase activity. The ISA also failed to identify an overall difference in species composition among the high and low thiaminase groups, regardless of the way in which the sampling events with intermediate levels of thiaminase activity were assigned to groups.

Three of the four ISA groupings identified one taxon (*Ploesoma*) whose gram-normalized wet mass was consistently related to thiaminase activity. Examining the univariate relationship between *Ploesoma* biomass and thiaminase activity (Appendix H) reveals a compelling relationship in which the sampling events with the highest thiaminase activity consistently have a greater proportion of their total wet mass accounted for by *Ploesoma*, and the sampling events with no or low levels of thiaminase activity lack *Ploesoma*. This suggests *Ploesoma* as the only candidate taxon whose mass was related to thiaminase activity. *Ploesoma* spp. are large (150-600 µm, depending on the species) omnivorous loricate rotifers that consume green algae (*Chlamydomonas*) and smaller rotifers (*Polyarthra* and *Synchaeta*) (Stemberger 1979). *Ploesoma truncatum* and other *Ploesoma* spp. are widespread throughout the Great Lakes, occurring in at least half of rotifer samples collected between 1983 and 2006 in each of the Great Lakes, excepting Lake Superior, where this genus was found in approximately 25% of samples (Barbiero and Warren 2011).

Despite these suggestive results for *Ploesoma* as a primary source of thiaminase activity in Great Lake food webs, there are several reasons why *Ploesoma* may not be the culprit. *Ploesoma* accounted for only 0.8 mg/g (or 0.08%) of the entire wet biomass at Port Weller, where thiaminase activity was 1,856 pmol/g/min. If *Ploesoma* was the sole source of that thiaminase activity, the activity of *Ploesoma* would have to be 2,331,250 pmol/g/min, likely well beyond the bounds of reason, and an even higher *Ploesoma* thiaminase activity would be required at the other two sampling events where mean thiaminase activity was over 1000 pmol/g/min. Overall,

Ploesoma thiaminase activity in this situation would need to be one to two orders of magnitude higher than the highest thiaminase activity ever measured for any animal in the Great Lakes or elsewhere to produce this result. Second, several thiaminase-containing zooplankton tows contained no *Ploesoma*, so thiaminase activity cannot always be explained by the presence of *Ploesoma*. Lastly, small *Ploesoma* individuals were observed in the 53-125 µm size fractions of bulk zooplankton. Even though we might expect Ploesoma to contribute more proportional wet weight to this size fraction and therefore create higher per-gram thiaminase activity, we did not observe this result. Given these stipulations, we suggest that *Ploesoma* is not the sole source of thiaminase in Great Lakes aquatic food webs. Focused studies on this genus could confirm or refute the potential for *Ploesoma* to contribute to thiaminase activity in the Great Lakes.

2.5.5 Synthesis

Ploesoma was the only taxon identified as a consistent candidate source of thiaminase, as none of the analyses we conducted convincingly identified any other taxa or gradients within the zooplankton community as candidate thiaminase sources. This finding is perhaps not surprising given the species composition of the communities we sampled; the sampling events in which bulk zooplankton had with the highest thiaminase activity (right side of Figure 2.4) share relatively few taxa in common, and the taxa common to some sampling events with high thiaminase activity are often also present (sometimes in greater abundance) in sampling events that have little or no thiaminase activity (left side of Figure 2.4).

One challenge with our taxon-specific approach is that calanoid or cyclopoid copepodites were often one of the top three contributing taxa to wet biomass. Calanoid or cyclopoid copepodites as taxa contain multiple species and therefore grouping them together at the copepodite stage results in some loss of taxonomic resolution, but copepodites cannot be routinely identified to species using microscopy. Given that copepodites constituted 9.6 to 40.4% of total wet biomass, excluding them seemed unjustifiable. Species of copepodites that could be identified because of unique morphological characteristics we retained the highest level of

taxonomic resolution possible, but we were unable to evaluate taxon-specific copepodite contributions to thiaminase activity.

Our analysis was conducted under the assumption that, like fish, individual zooplankters have some degree of consistency with regard to the general presence or absence of thiaminase as well as the typical magnitude of thiaminase activity (Tillitt et al. 2005, Honeyfield et al. 2010a). The range of thiaminase activities we found in individual zooplankton species, either across or within sampling events, suggests that one reason for a failure to identify more taxa that are likely thiaminase sources is that there may be more spatial or temporal variability in thiaminase activity within an individual taxon than previously thought. As examples, thiaminase activity in *Bythotrephes* ranged from undetectable under some conditions or locations to levels approaching the lower end of whole-body thiaminase activity observed in Rainbow Smelt. The reason for the within-taxon variability we observed is unknown, but if such variability is commonplace among zooplankton, then our analytical approach would be unlikely to identify individual taxa as consistent and compelling candidate sources of thiaminase. The reason for variation in thiaminase activity among individuals or among species in higher organisms (fishes or zooplankton) remains unknown despite the fact that some species appear to nearly uniformly have thiaminase or not have thiaminase and some tissues routinely have more thiaminase activity than others (Fujita 1954, Tillitt et al. 2005, Zajicek et al. 2005, Honeyfield et al. 2010a, Kraft et al. 2014).

The within-taxa variability we observed in *Bythotrephes*, *Cercopagis*, and *Mysis* could be considered consistent with any proposed source of thiaminase. If the source of thiaminase in these taxa is bacterial (in the intestinal tract, for example), then within taxa variability could be explained by differences in the bacterial community associated with specific taxa at different locations (Winters et al. 2015), bacterial and algal consumption by the smaller zooplankton consumed by these predatory zooplankton, or factors that may affect the production of thiaminase by gastrointestinal bacteria, such as feeding rate, water temperature, and degree of gut fullness. If the source of thiaminase in these taxa is larger food items (phytoplankton, small zooplankton), then this variability could be explained by the

diet of these predatory zooplankton, which would be expected to vary by site, time, and across environmental conditions. If the source of thiaminase is *de novo* in each of these taxa, variability could be explained by differences in the conditions that govern *de novo* production of thiaminase, which are virtually uncharacterized. Because our samples were taken in the wild, we could not control for factors such as composition of the diet, degree of depuration, and composition of the bacterial flora, all of which are expected to vary among sampling events.

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2.7 Literature Cited

R Core Team. 2014. R: A language and environment for statistical computing. Version 3.1.0. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Arsan, O. M. and A. Y. Malyarevskaya. 1969. The effect of food on thiaminase activity and the content of thiamine in the liver and intestine of silver carp. Hydrobiological Journal. 5:79-81.

Arsan, O. M. 1972. Thiaminase activity in the liver and intestine of some freshwater fishes. Hydrobiological Journal. 6:75-77.

Balcer, M. D., N. L. Korda, and S. I. Dodson. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press, Madison, WI.

Barbiero, R. P., M. Balcer, D. C. Rockwell, and M. L. Tuchman. 2009a. Recent shifts in the crustacean zooplankton community of Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences. 66:816-828.

Barbiero, R. P., D. B. Bunnell, D. C. Rockwell, and M. L. Tuchman. 2009b. Recent increases in the large glacial-relict calanoid *Limnocalanus macrurus* in Lake Michigan. Journal of Great Lakes Research. 35:285-292.

Barbiero, R. P. and G. J. Warren. 2011. Rotifer communities in the Laurentian Great Lakes, 1983–2006 and factors affecting their composition. Journal of Great Lakes Research. 37:528-540.

Barbiero, R. P., B. M. Lesht, and G. J. Warren. 2012. Convergence of trophic state and the lower food web in Lakes Huron, Michigan and Superior. Journal of Great Lakes Research. 38:368-380.

Beers, J. R. 1966. Studies on the chemical composition of the major zooplankton groups in the Sargasso Sea off Bermuda. Limnology and Oceanography. 11:520-528.

Birger, T. I., A. Y. Malyarevskaya, and O. M. Arsan. 1973. Etiology of the Haff (Yuksov-Sartlan) disease. Hydrobiological Journal. 9:71-80.

Bisbee, J. W. 1973. Size determination in hydra - roles of growth and budding. Journal of Embryology and Experimental Morphology. 30:1-19.

Blakeslee, C. J., S. A. Sweet, H. S. Galbraith, and D. C. Honeyfield. 2015. Thiaminase activity in native freshwater mussels. Journal of Great Lakes Research. 41:516-519.

Borgmann, U. and D. M. Whittle. 1994. Particle-size-conversion efficiency, invertebrate production, and potential fish production in Lake Ontario. Canadian Journal of Fisheries and Aquatic Sciences. 51:693-700.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson, and T. Weglenska. 1976. A review of some problems is zooplankton production studies. Norwegian Journal of Zoology. 24:419-456.

Bronte, C. R., S. T. Schram, J. H. Selgeby, and B. L. Swanson. 1995a. Density-independent survival of wild lake trout in the Apostle Islands area of Lake Superior. Journal of Great Lakes Research. 21:246-252.

Bronte, C. R., J. H. Selgeby, J. H. Saylor, G. S. Miller, and N. R. Foster. 1995b. Hatching, dispersal, and bathymetric distribution of age-0 wild lake trout at the Gull Island Shoal Complex, Lake Superior. Journal of Great Lakes Research. 21:233-245.

Bronte, C. R., M. P. Ebener, D. R. Schreiner, D. S. DeVault, M. M. Petzold, D. A. Jensen, C. Richards, and S. J. Lozano. 2003a. Fish community change in Lake Superior, 1970-2000. Canadian Journal of Fisheries and Aquatic Sciences. 60:1552-1574.

Bronte, C. R., J. Jonas, M. E. Holey, R. L. Eshenroder, M. L. Toneys, P. McKee, B. Breidert, R. M. Claramunt, M. P. Ebener, C. C. Krueger, G. M. Wright, and R. Hess. 2003b. Possible impediments to lake trout restoration in Lake Michigan. Lake Trout Task Group report to the Lake Michigan Committee. Great Lakes Fishery Commission. Ann Arbor, MI.

Bronte, C. R., C. C. Krueger, M. E. Holey, M. L. Toneys, R. L. Eshenroder, and J. L. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fishery Commission Miscellaneous Publication 2008-01. 39 pages. Available at http://www.glfc.org/pubs/pub.htm#misc.

Brown, M. E. and D. K. Branstrator. 2004. A 2001 survey of crustacean zooplankton in the western arm of Lake Superior. Journal of Great Lakes Research. 30:1-8.

Brown, S. B., J. D. Fitzsimons, V. P. Palace, and L. Vandenbyllaardt. 1998a. Thiamine and early mortality syndrome in lake trout. Pages 18-25 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., D. C. Honeyfield, and L. Vandenbyllaardt. 1998b. Thiamine analysis in fish tissues. Pages 73-81 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., M. T. Arts, L. R. Brown, M. Brown, K. Moore, M. Villella, J. D. Fitzsimons, D. C. Honeyfield, D. E. Tillitt, J. L. Zajicek, M. Wolgamood, and J. G. Hnath. 2005a. Can diet-dependent factors help explain fish-to-fish variation in thiamine-dependent early mortality syndrome? Journal of Aquatic Animal Health. 17:36-47.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005b. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005c. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health. 17:59-64.

Bunnell, D. B., K. M. Keeler, E. A. Puchala, B. M. Davis, and S. A. Pothoven. 2012. Comparing seasonal dynamics of the Lake Huron zooplankton community between 1983-1984 and 2007 and revisiting the impact of *Bythotrephes* planktivory. Journal of Great Lakes Research. 38:451-462.

Bunnell, D. B., B. M. Davis, M. A. Chriscinske, K. M. Keeler, and J. G. Mychek-Londer. 2015. Diet shifts by planktivorous and benthivorous fishes in northern Lake Michigan in response to ecosystem changes. Journal of Great Lakes Research. 41, Supplement 3:161-171.

Chang, W. Y. B. and R. Rossmann. 1988. Changes in the abundance of blue-green algae related to nutrient loadings in the nearshore of Lake Michigan. Hydrobiologia. 157:271-278.

Coble, D. W. 1965. Effect of raw smelt on lake trout. Canadian Fish Culturist. 36:27-34.

Costello, C., N. Kelleher, A. Mitsuko, F. McLafferty, and T. P. Begley. 1996. Mechanistic studies on thiaminase I. Journal of Biological Chemistry. 271:3445-3452.

Culver, D. A., M. M. Boucherle, D. J. Bean, and J. W. Fletcher. 1985. Biomass of fresh-water crustacean zooplnakton from length-weight regressions. Canadian Journal of Fisheries and Aquatic Sciences. 42:1380-1390.

Cummins, K. W., R. R. Costa, R. E. Rowe, G. A. Moshiri, R. M. Scanlon, and R. K. Zajdel. 1969. Ecological energetics of a natural population of predaceous zooplankter, *Leptodora kindti*, Focke (Cladocera). Oikos. 20:189-223.

Czesny, S., J. M. Dettmers, J. Rinchard, and K. Dabrowski. 2009. Linking egg thiamine and fatty acid concentrations of Lake Michigan lake trout with early life stage mortality. Journal of Aquatic Animal Health. 21:262-271.

Davis, B. M., J. F. Savino, and L. M. Ogilvie. 2007. Diet niches of major forage fish in Lake Michigan. Pages 261-275 *in* M. Jankun, P. Brzuzan, P. Hliwa, and M. Luczynski, editors. Biology and Management of Coregonid Fishes - 2005.

DesJardine, R. L., T. K. Gorenflo, R. N. Payne, and J. D. Schrouder. 1995. Fish-community objectives for Lake Huron. Great Lakes Fishery Commission Special Publication. 95-1:1-37.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs. 67:345-366.

Dumont, H. J., I. V. d. Velde, and S. Dumont. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia. 19:75-97.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark, Jr. 1995. Fish-community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 1995-3:1-56.

Evans, W. C. 1976. Bracken thiaminase-mediated neurotoxic syndromes. Botanical Journal of The Linnean Society. 73:113-131.

Fisher, J. P., J. M. Spitsbergen, H. Symula, J. Skea, M. Babenzein, and T. Chiotti. 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the "Cayuga syndrome". Journal of Aquatic Animal Health. 7:81-94.

Fisher, J. P., J. D. Fitzsimons, G. F. Combs, and J. M. Spitsbergen. 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Transactions of the American Fisheries Society. 125:167-178.

Fisher, J. P., S. B. Brown, S. Connelly, T. Chiotti, and C. C. Krueger. 1998a. Interspecies comparisons of blood thiamine in salmonids from the Finger Lakes, and effect of maternal size on blood and egg thiamine in Atlantic salmon with and without Cayuga Syndrome. Pages 112-123 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fisher, J. P., S. B. Brown, G. W. Wooster, and P. R. Bowser. 1998b. Maternal blood, egg and larval thiamin levels correlate with larval survival in landlocked Atlantic salmon (*Salmo salar*). Journal of Nutrition. 128:2456-2466.

Fitzsimons, J. D. and S. B. Brown. 1998. Reduced egg thiamine levels in inland and Great Lakes trout and their relationship with diet. Pages 160-171 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. B. Brown, and L. Vandenbyllaardt. 1998. Thiamine levels in food chains of the Great Lakes. Pages 90-98 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, R. Barrows, C. E. Kraft, J. Lepak, D. C. Honeyfield, and D. Tillitt. 2004a. Factors influencing alewife thiaminolytic activity. Appendix 3 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 33 pages.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, J. Zajicek, D. Tillitt, and D. C. Honeyfield. 2004b. Thiaminase and thiamine in lower trophic level organisms from the Great Lakes basin. Appendix 4 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 13 pages.

Fitzsimons, J. D., B. Williston, J. L. Zajicek, D. E. Tillitt, S. B. Brown, L. R. Brown, D. C. Honeyfield, D. M. Warner, L. G. Rudstam, and W. Pearsall. 2005. Thiamine content and thiaminase activity of ten freshwater stocks and one marine stock of alewives. Journal of Aquatic Animal Health. 17:26-35.

Fitzsimons, J. D., B. Williston, G. Williston, L. Brown, A. El-Shaarawi, L. Vandenbyllaardt, D. Honeyfeld, D. Tillitt, M. Wolgamood, and S. B. Brown. 2007. Egg thiamine status of Lake Ontario salmonines 1995-2004 with emphasis on lake trout. Journal of Great Lakes Research. 33:93-103.

Fujita, A. 1954. Thiaminase. Advances in Enzymology and Related Subjects of Biochemistry. 15:389-421.

Fynn-Aikins, K., P. R. Bowser, D. C. Honeyfield, J. D. Fitzsimons, and H. G. Ketola. 1998. Effect of dietary amprolium on tissue thiamin and Cayuga syndrome in Atlantic salmon. Transactions of the American Fisheries Society. 127:747-757.

Gamble, A. E., T. R. Hrabik, J. D. Stockwell, and D. L. Yule. 2011. Trophic connections in Lake Superior Part I: The offshore fish community. Journal of Great Lakes Research. 37:541-549.

Garton, D. W. and D. J. Berg. 1990. Occurrence of *Bythotrephes cederstroemi* (Schoedler 1877) in Lake Superior, with Evidence of Demographic Variation within the Great Lakes. Journal of Great Lakes Research. 16:148-152.

GLNPO. 2003. Standard operating procedures for zooplankton analysis. U. S. Environmental Protection Agency, Great Lakes National Program Office, Report LG403. 16 pages.

Goodman, K. S. 1980. The estimation of individual dry-weight and standing crop of harpacticoid copepods. Hydrobiologia. 72:253-259.

Grigor'yeva, L. V., A. Kirpenko, V. M. Orlovskij, and V. V. Stankewich. 1977. On the antimicrobial action of toxic metabloites of some blue-green algae. Gidrobiologicheskii Zhurnal. 13:47-52.

Grigorovich, I. A., H. J. MacIsaac, I. K. Rivier, N. V. Aladin, and V. E. Panov. 2000. Comparative biology of the predatory cladoceran Cercopagis pengoi from Lake Ontario, Baltic Sea and Caspian Sea. Archiv Fur Hydrobiologie. 149:23-50.

Grossnickle, N. E. and A. M. Beeton. 1979. Antennal scale length as a measure of relative size in the opossum shrimp, *Mysis relicta* Loven. Crustaceana. 36:141-146.

Hansen, M. J. 1999. Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. Pages 417-453 *in* W. W. Taylor and C. P. Ferreri, editors. Great Lakes fisheries policy and management: a binational perspective.

Higgins, S. N., S. Y. Malkin, E. T. Howell, S. J. Guildford, L. Campbell, V. Hiriart-Baer, and R. E. Hecky. 2008. An ecological review of *Cladophora glomerata* (Chlorophyta) in the Laurentian Great Lakes. Journal of Phycology. 44:839-854.

Hillbricht-Ilkowska, A. and A. Stanczykowska. 1969. The production and standing crop of planktonic larvae of *Dreissena polymorpha* Pall. in two Mazurian lakes. Polish Archives of Hydrobiology. 16:193-203.

Holbrook, B. V., T. R. Hrabik, D. K. Branstrator, D. L. Yule, and J. D. Stockwell. 2006. Hydroacoustic estimation of zooplankton biomass at two shoal complexes in the Apostle Islands region of Lake Superior. Journal of Great Lakes Research. 32:680-696.

Honeyfield, D. C., J. D. Fitzsimons, S. B. Brown, S. V. Marcquenski, and G. McDonald. 1998a. Introduction and overview of early life stage mortality. Pages 1-7 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., K. Fynn-Aikins, J. D. Fitzsimons, and J. A. Mota. 1998b. Effect of dietary amprolium on egg and tissue thiamine concentrations in lake trout. Pages 172-177 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., J. P. Hinterkopf, and S. B. Brown. 2002. Isolation of thiaminase-positive bacteria from alewife. Transactions of the American Fisheries Society. 131:171-175.

Honeyfield, D. C., D. Beltman, M. Holey, and C. Edsall. 2005a. Investigation of thiamine and PCB association with early life stage fry mortality in lake trout from northwestern Lake Michigan in 1996-1998. Pages 182-194 *in* Health and diseases of aquatic organisms: bilateral perspectives. Proceedings of the second bilateral conference between Russian and the United States. 21-28 September 2003. Shepherdstown, West Virginia. Michigan State University, East Lansing, Michigan.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. W. Hanes, L. Brown, C. E. Kraft, and T. P. Begley. 2010. Comparison of thiaminase activity in fish using the radiometric and 4-nitrothiophenol colorimetric methods. Journal of Great Lakes Research. 36:641-645.

Honeyfield, D. C., M. E. Daniels, L. R. Brown, M. T. Arts, M. G. Walsh, and S. B. Brown. 2012. Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species. Journal of Great Lakes Research. 38:11-17.

Horns, W. H., C. R. Bronte, T. R. Busiahn, M. P. Ebener, R. L. Eshenroder, T. Gorenflo, N. Kmiecik, W. Mattes, J. W. Peck, M. Petzold, and D. R. Schreiner. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication. 03-01:1-78.

Houde, A. L. S., P. J. Saez, C. C. Wilson, D. P. Bureau, and B. D. Neff. 2015. Effects of feeding high dietary thiaminase to sub-adult Atlantic salmon from three populations. Journal of Great Lakes Research. 41:898-906.

Hudson, P. L. and L. T. Lesko. Free-living and parasitic copepods (including Branchiuraans) of the Laurentian Great Lakes: keys and details on individual species. Available at http://www.glsc.usgs.gov/greatlakescopepods/.

Ji, Y. Q. and I. R. Adelman. 1998. Thiaminase activity in alewives and smelt in lakes Huron, Michigan, and Superior. Pages 154-159 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Ji, Y. Q., J. J. Warthesen, and I. R. Adelman. 1998. Thiamine nutrition, synthesis, and retention in relation to lake trout reproduction in the Great Lakes. Pages 99-111 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Johannsson, O. E., R. Dermott, D. M. Graham, J. A. Dahl, E. Scott Millard, D. D. Myles, and J. LeBlanc. 2000. Benthic and pelagic secondary production in Lake Erie after the invasion of *Dreissena* spp. with implications for fish production. Journal of Great Lakes Research. 26:31-54.

Jonas, J. L., R. M. Claramunt, J. D. Fitzsimons, J. E. Marsden, and B. J. Ellrott. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 62:2254-2264.

Jones, M. L., G. W. Eck, D. O. Evans, M. C. Fabrizio, M. H. Hoff, P. L. Hudson, J. Janssen, D. Jude, R. Ogorman, and J. F. Savino. 1995. Limitations to lake trout (*Salvelinus namaycush*) rehabilitation in the Great Lakes imposed by biotic interactions occurring at early life stages. Journal of Great Lakes Research. 21:505-517.

Keeler, K. M., D. B. Bunnell, J. S. Diana, J. V. Adams, J. G. Mychek-Londer, D. M. Warner, D. L. Yule, and M. R. Vinson. 2015. Evaluating the importance of abiotic and biotic drivers on *Bythotrephes* biomass in Lakes Superior and Michigan. Journal of Great Lakes Research. 41, Supplement 3:150-160.

Ketola, H. G., P. R. Bowser, G. A. Wooster, L. R. Wedge, and S. S. Hurst. 2000. Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Transactions of the American Fisheries Society. 129:607-612.

Kraft, C. E., E. R. L. Gordon, and E. R. Angert. 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLOS ONE. 9:e92688.

Krueger, C. C., M. L. Jones, and W. W. Taylor. 1995a. Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. Journal of Great Lakes Research. 21:547-558.

Krueger, C. C., D. L. Perkins, E. L. Mills, and J. E. Marsden. 1995b. Predation by alewives on lake trout fry in Lake Ontario: Role of an exotic species in preventing restoration of a native species. Journal of Great Lakes Research. 21:458-469.

Krueger, C. C. and M. Ebener. 2004. Rehabilitation of lake trout in the Great Lakes: past lessons and future challenges. Pages 37-56 *in* Boreal Shield watersheds: lake trout ecosystems in a changing environment. Lewis Publishers.

Krueger, C. C., W. C. Shepherd, and A. M. Muir. 2014. Predation by alewife on lake trout fry emerging from laboratory reefs: Estimation of fry survival and assessment of predation potential. Journal of Great Lakes Research. 40:429-434.

Kruskal, J. B. 1964a. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika. 29:1-27.

Kruskal, J. B. 1964b. Nonmetric multidimensional scaling: a numerical method. Psychometrika. 29:115-129.

Landrum, P. F. 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. Aquatic Toxicology. 12:245-271.

Lawrence, S. G., D. F. Malley, W. J. Findlay, M. A. Maclver, and I. L. Delbaere. 1987. Method for estimating dry-weight of fresh-water planktonic crustaceans from measures of length and shape. Canadian Journal of Fisheries and Aquatic Sciences. 44:264-274.

Legendre, P. and M. J. Anderson. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs. 69:1-24.

Lehman, J. T. and C. E. Caceres. 1993. Food-web responses to species invasion by a predatory invertebrate - *Bythotrephes* in Lake Michigan. Limnology and Oceanography. 38:879-891.

Madenjian, C. P., R. O'Gorman, D. B. Bunnell, R. L. Argyle, E. F. Roseman, D. M. Warner, J. D. Stockwell, and M. A. Stapanian. 2008. Adverse effects of alewives on Laurentian Great Lakes fish communities. North American Journal of Fisheries Management. 28:263-282.

Makarewicz, J. C. and T. W. Lewis. 2015. Long-term changes in Lake Ontario rotifer abundance and composition: A response to *Cercopagis* predation? Journal of Great Lakes Research. 41:192-199.

Malyarevskaya, A. Y., T. I. Birger, O. M. Arsan, and V. D. Solomatina. 1972. Metabolic relationships between blue-green algae and fish. Hydrobiological Journal. 8:35-41.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. J. Wiley and Sons, New York.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology. 82:290-297.

McCleary, B. V. and B. F. Chick. 1977. Purification and properties of a thiaminase I enzyme from nardoo (*Marsilea drummondii*). Phytochemistry. 16:207-213.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR.

McCune, B. and M. J. Mefford. 2015. PC-ORD. Multivariate Analysis of Ecological Data. Version 7.359 beta. MjM Software Design, Gleneden Beach, OR, USA

Muir, A. M., C. C. Krueger, and M. J. Hansen. 2012. Re-establishing lake trout in the Laurentian Great Lakes: Past, present, and future. Pages 533-588 *in* W. W. Taylor, A. J. Lynch, and N. J. Leonard, editors. Great Lakes fisheries policy and management: A binational perspective. 2nd edition. MSU Press, Lansing.

Nalepa, T. and M. A. Quigley. 1980. The macro and meiobenthos of southeastern Lake Michigan near the mouth of the Grand River, 1976-77. Great Lakes Environmental Reasearch Laboratory, Ann Arbor, MI.

Nalepa, T. F., D. L. Fanslow, and S. A. Pothoven. 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. Journal of Great Lakes Research. 36:5-19.

Nishimune, T., Y. Watanabe, H. Okazaki, and H. Akai. 2000. Thiamin is decomposed due to *Anaphe* spp. entomophagy in seasonal ataxia patients in Nigeria. Journal of Nutrition. 130:1625-1628.

Ojaveer, H., L. A. Kuhns, R. P. Barbiero, and M. L. Tuchman. 2001. Distribution and population characteristics of *Cercopagis pengoi* in Lake Ontario. Journal of Great Lakes Research. 27:10-18.

Okonji, R. E., K. S. Bamitale, and R. O. Balogun. 2012. Biochemical properties of thiaminase from *Anaphe venata* Burtler. African Journal of Biotechnology. 11:4543-4550.

Omori, M. 1969. Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Marine Biology. 3:4-10.

Pace, M. L. and J. D. Orcutt, Jr. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography. 26:822-830.

Pauli, H.-R. 1989. A new method to estimate individual dry weights of rotifers. Hydrobiologia. 186-187:355-361.

Pearre, S. 1980. The copopod width-weight relation and its utility in food-chain research. Canadian Journal of Zoology. 58:1884-1891.

Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology. 87:2614-2625.

Ramos, J. J., L. M. Ferrer, L. Garcia, A. Fernandez, and A. Loste. 2005. Polioencephalomalacia in adult sheep grazing pastures with prostrate pigweed. Canadian Veterinary Journal-Revue Veterinaire Canadienne. 46:59-61.

Richter, C. A., A. N. Evans, M. K. Wright-Osment, J. L. Zajicek, S. A. Heppell, S. C. Riley, C. C. Krueger, and D. E. Tillitt. 2012. *Paenibacillus thiaminolyticus* is not the cause of thiamine deficiency impeding lake trout (*Salvelinus namaycush*) recruitment in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 69:1056-1064.

Riley, S. C., J. Rinchard, D. C. Honeyfield, A. N. Evans, and L. Begnoche. 2011. Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low alewife abundance. North American Journal of Fisheries Management. 31:1052-1064.

Rosen, R. A. 1981. Length dry-weight relationships of some freshwater zooplankton. Journal of Freshwater Ecology. 1:225-229.

Rudstam, L. G., K. T. Holeck, K. L. Bowen, J. M. Watkins, B. C. Weidel, and F. J. Luckey. 2015. Lake Ontario zooplankton in 2003 and 2008: Community changes and vertical redistribution. Aquatic Ecosystem Health & Management. 18:43-62.

Ryan, P. A., R. Knight, R. MacGregor, G. Towns, R. Hoopes, and W. Culligan. 2003. Fish-community goals and objectives for Lake Erie. Great Lakes Fishery Commission Special Publication. 03-02:1-56.

Schindler, D. W. and B. Noven. 1971. Vertical distribution and seasonal abundance of zooplankton in 2 shallow lakes of the Experimental Lakes Area, Northwestern Ontario. Journal of the Fisheries Research Board of Canada. 28:245-256.

Shea, M. A. and J. C. Makarewicz. 1989. Producation, biomass, and trophic interactions of *Mysis relicta* in Lake Ontario. Journal of Great Lakes Research. 15:223-232.

Sikowitz, M. D., B. Shome, Y. Zhang, T. P. Begley, and S. E. Ealick. 2013. Structure of a *Clostridium botulinum* C143S Thiaminase I/Thiamin Complex Reveals Active Site Architecture. Biochemistry. 52:7830-7839.

Smirnov, N. N. 2014. Physiology of the Cladocera. Academic Press, San Diego.

Stemberger, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency. Rept. No. EPA 600/4-79-021, 185 pages.

Stewart, T. J., R. E. Lange, S. D. Orsatti, C. P. Schneider, A. Mathers, and M. E. Daniels. 1999. Fish-community objectives for Lake Ontario. Great Lakes Fishery Commission Special Publication. 99-1:1-55.

Stoermer, E. F. 1978. Phytoplankton assemblages as indicators of water quality in the Laurentian Great Lakes. Transactions of the American Microscopy Society. 97:2-16.

Strakosh, T. R. and C. C. Krueger. 2005. Behavior of post-emergent lake trout fry in the presence of the alewife, a non-native predator. Journal of Great Lakes Research. 31:296-305.

Tillitt, D. E., J. L. Zajicek, S. B. Brown, L. R. Brown, J. D. Fitzsimons, D. C. Honeyfield, M. E. Holey, and G. M. Wright. 2005. Thiamine and thiaminase status in forage fish of salmonines from Lake Michigan. Journal of Aquatic Animal Health. 17:13-25.

Tillitt, D. E., S. C. Riley, A. N. Evans, S. J. Nichols, J. L. Zajicek, J. Rinchard, C. A. Richter, and C. C. Krueger. 2009. Dreissenid mussels from the Great Lakes contain elevated thiaminase activity. Journal of Great Lakes Research. 35:309-312.

Wieser, W. 1960. Benthic studies is Buzzard's Bay. II. The meiofauna. Limnology and Oceanography. 5:121-137.

Winnell, M. H. and D. S. White. 1984. Ecology of shallow and deep water populations of *Pontoporeia hoyi* (Smith) (Amphipoda) in Lake Michigan. Freshwater Invertebrate Biology. 3:118-138.

Winters, A. D., T. L. Marsh, T. O. Brenden, and M. Faisal. 2015. Analysis of bacterial communities associated with the benthic amphipod *Diporeia* in the Laurentian Great Lakes Basin. Canadian Journal Of Microbiology. 61:72-81.

Yan, N. D. and G. L. Mackie. 1987. Improved estimation of the dry-weight of *Holopedium gibberum* (Crustacea, Cladocera) using clutch size, a body-fat index and lake water total phosphorus concentration. Canadian Journal of Fisheries and Aquatic Sciences. 44:382-389.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

2.8 Figures

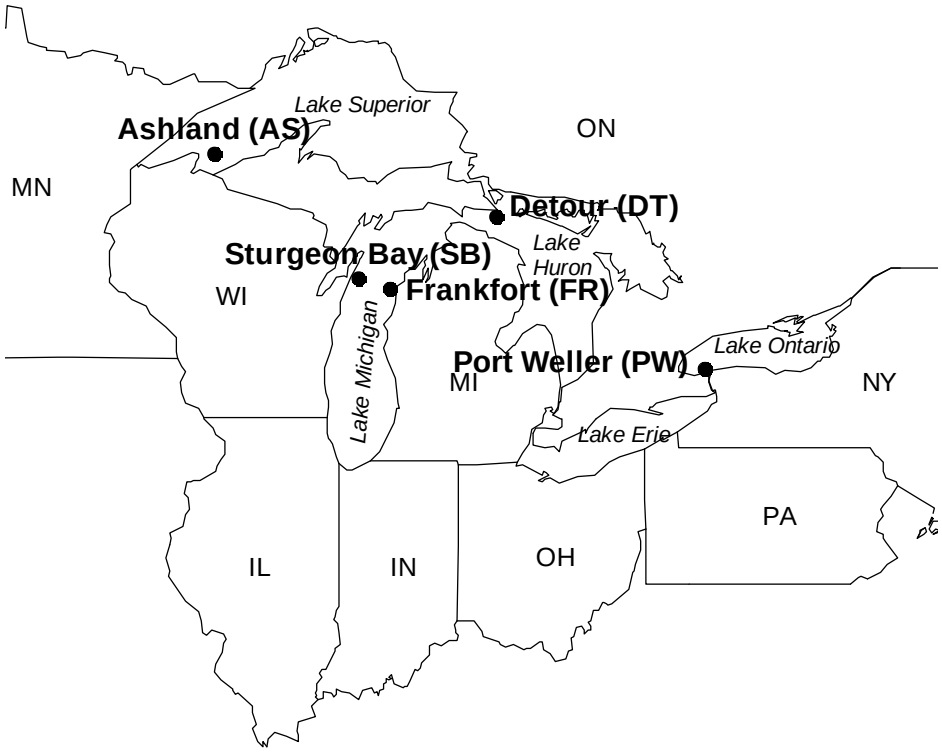

Figure 2.1 Map of sampling locations

Map showing the five sampling locations in the Great Lakes. Abbreviations for sampling locations are shown in parenthesis.

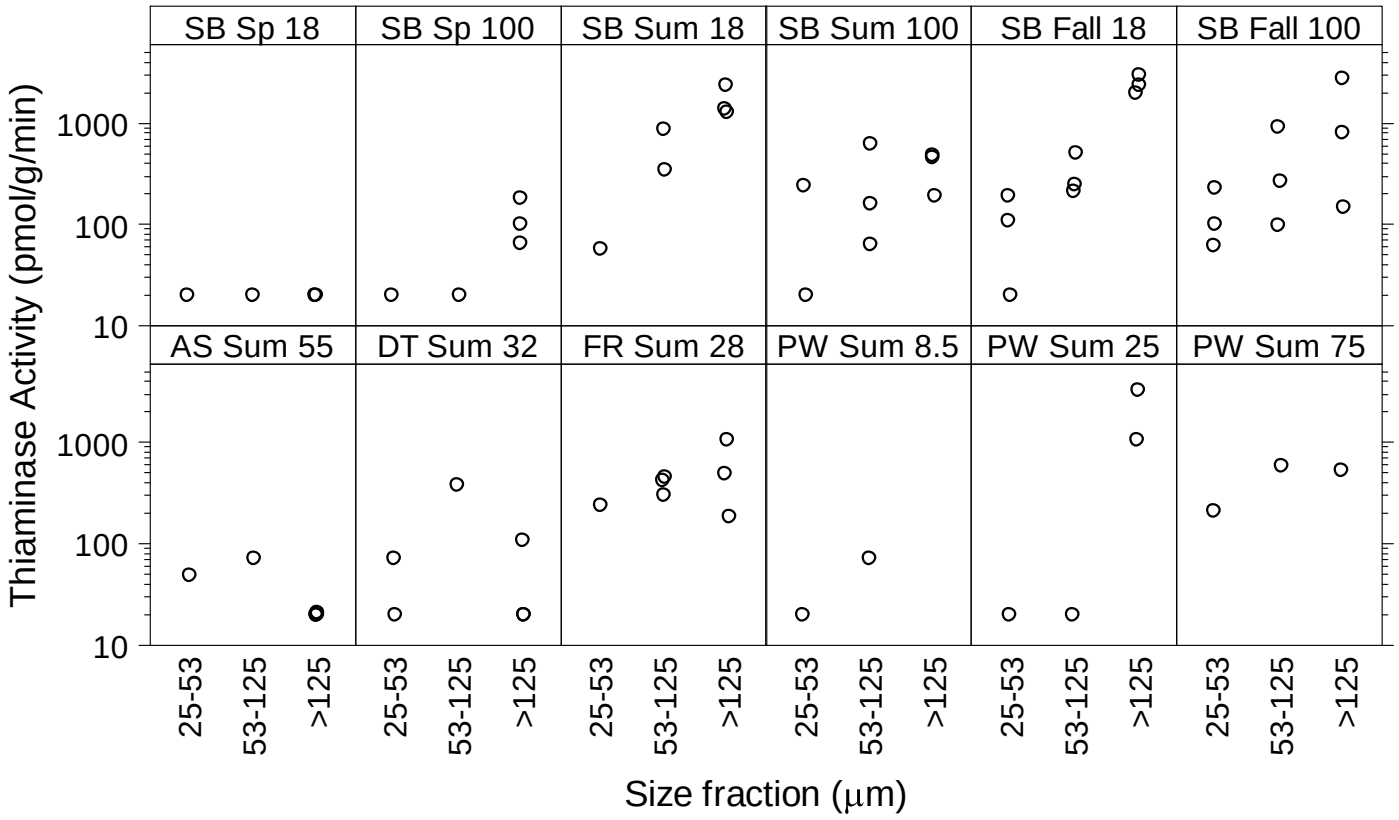

Figure 2.2 Thiaminase activity measured in three size fractions of bulk zooplankton

Thiaminase activity (pmol/g/min) measured in the three size fractions of bulk zooplankton and 12 different sampling events. Panel labels indicate the site, season, and depth (m). Thiaminase is shown on a log scale.

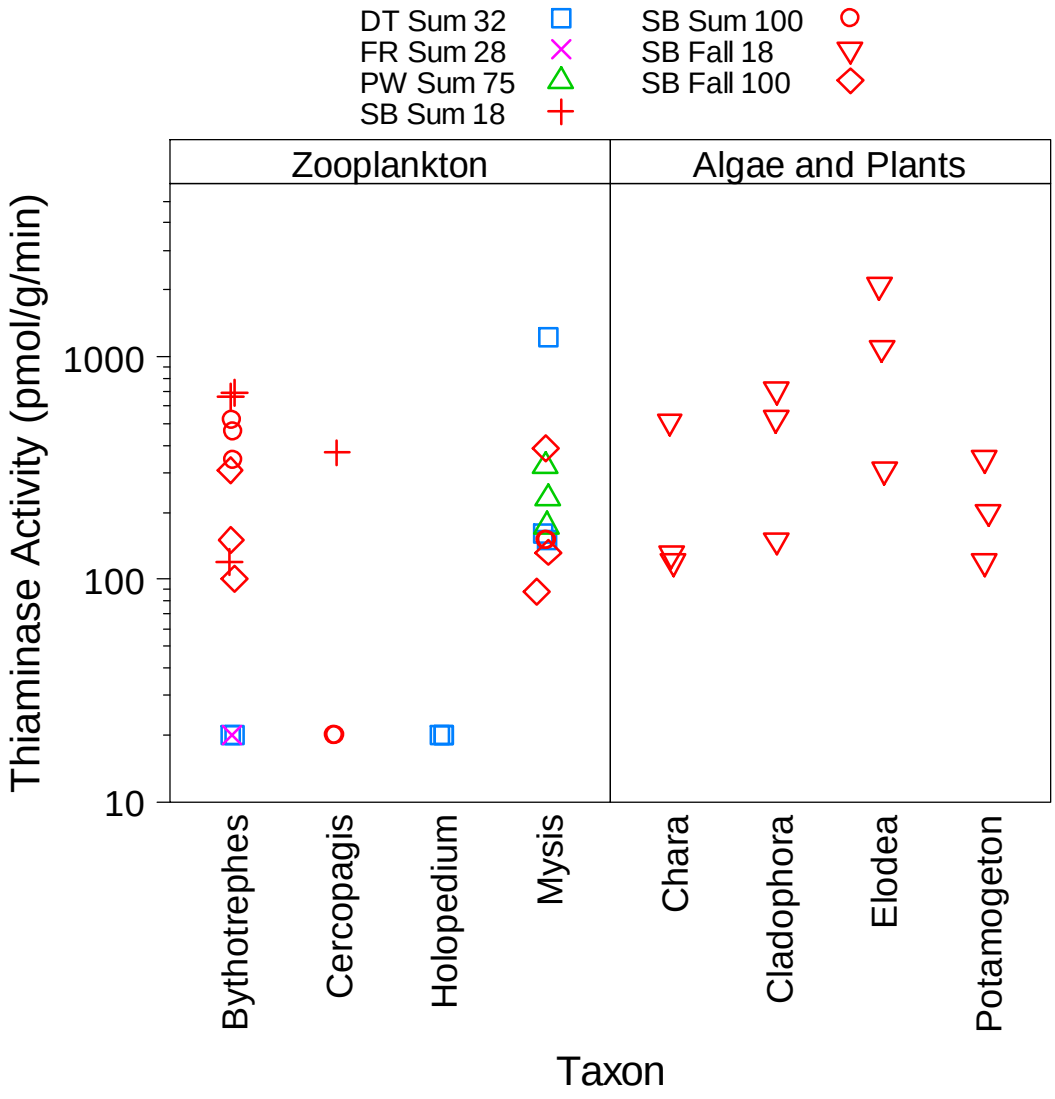

Figure 2.3 Taxon-specific thiaminase activity in zooplankton, algae, and plants

Thiaminase activity (pmol/g/min) measured in four individual zooplankton taxa, 2 algal taxa, and 2 plant taxa. Points are coded by site, season, and depth (m), with all the Sturgeon Bay samples shown in red. Thiaminase is shown on a log scale.

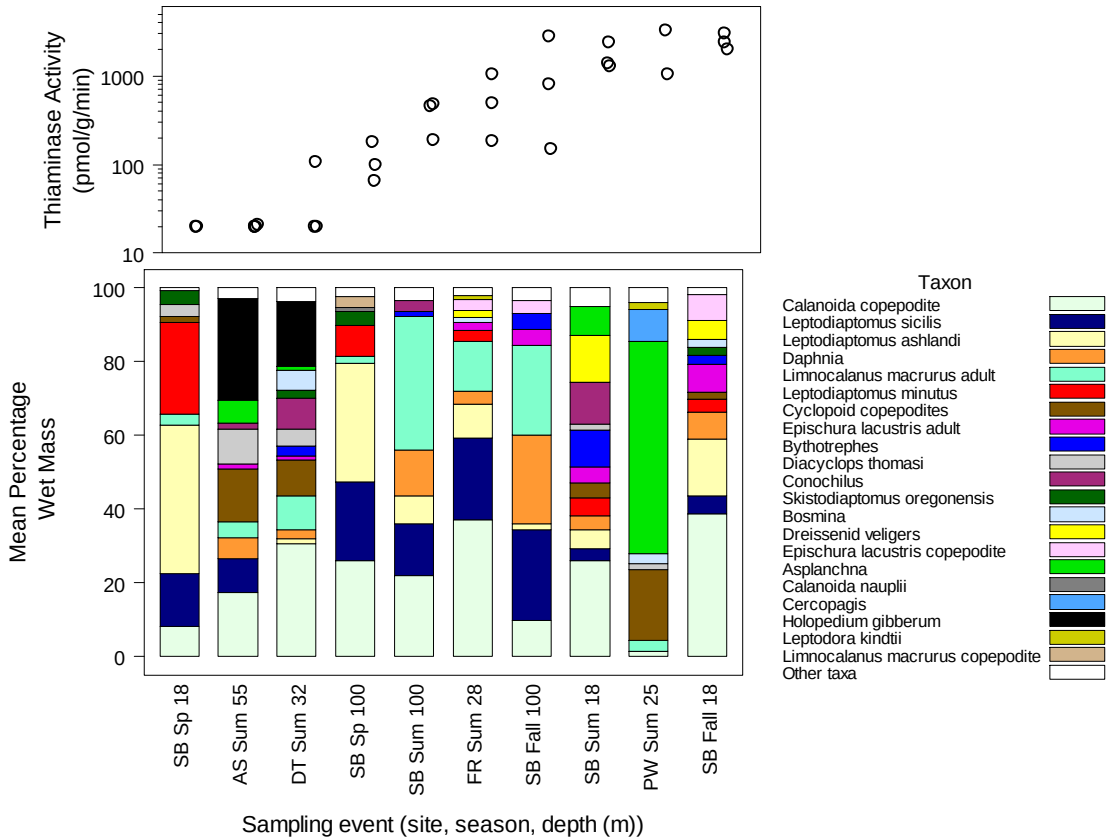

Figure 2.4. Thiaminase activity (top) and composition of the zooplankton tows (bottom) by sampling event

Top: Thiaminase activity (pmol/g/min; shown on a log scale) in the >125 µm size fraction for each of the sampling events. Bottom: The average mean percentage of wet mass by taxa for each sampling event. Data represent the 39 species that were ordinated. All taxa constituting less than 1% of the mean total wet mass for each sampling event were placed in the "Other taxa" category (see Appendix F for all data). Sampling events are ordered from lowest (left) to highest (right) mean thiaminase activity.

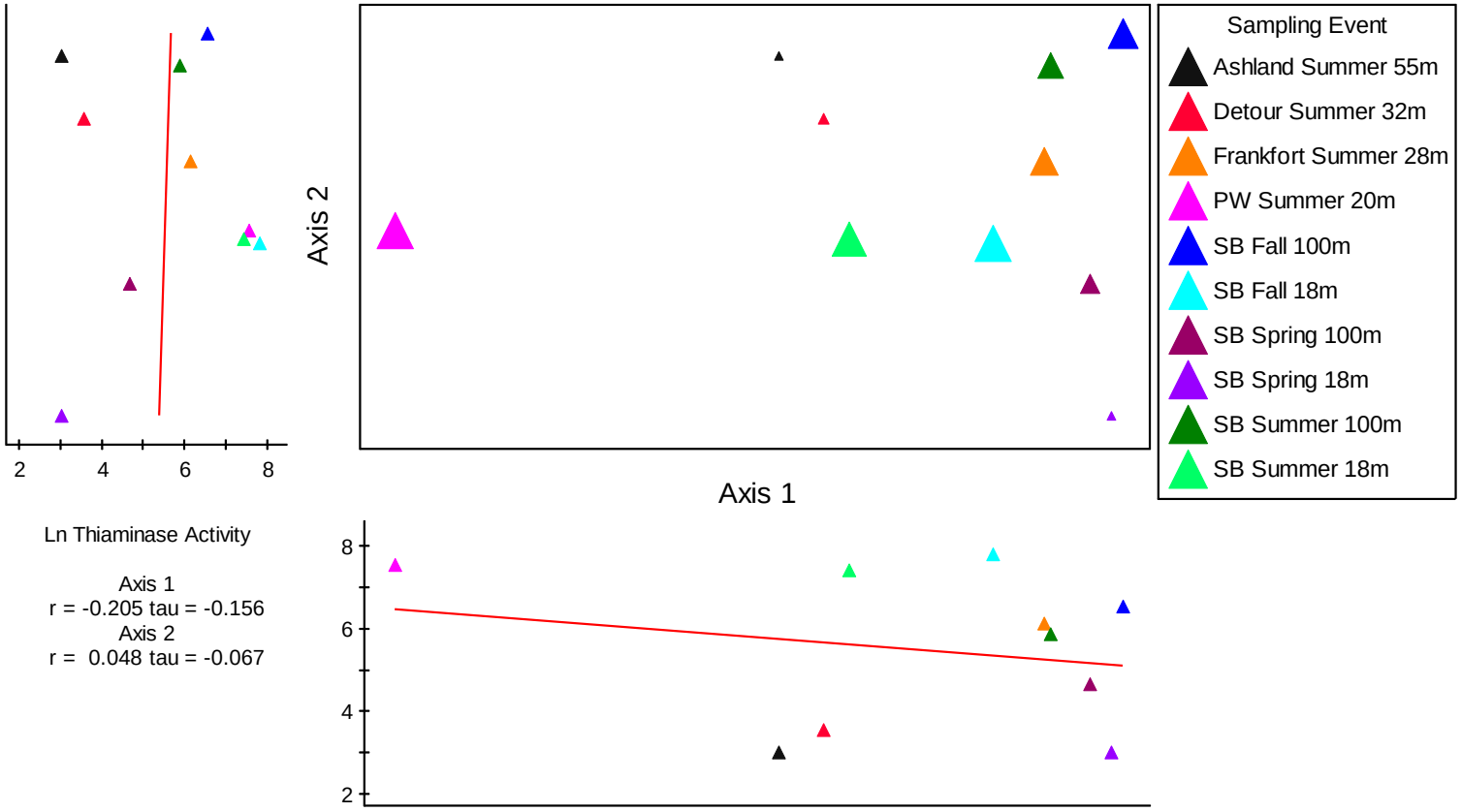

Figure 2.5. Two-dimensional ordination of 10 sampling events in taxa space

Two-dimensional ordination of 10 sampling events in taxa-space, rotated for maximum correspondence with the log of thiaminase. The center panel shows the ordination, with the size of the sampling event points representing the quantitative values for the log of thiaminase activity. The bottom scatterplot shows the relationship between the position on axis 1 and the log of thiaminase activity. The red line indicates a simple linear regression of the axis 1 scores and thiaminase activity.

2.9 Tables

Table 2.1 Egg thiamine level data supporting designation of TDC severity
Lake Trout egg thiamine levels (nmol/g) for sampling locations.

Site	Egg thiamin levels (nmol/g)	Year	Source of egg thiamine data
Ashland	<20	1993	(Fitzsimons and Brown 1998, Brown et al. 2005d)
Detour	4.48	2006	(Riley et al. 2011)
	7.38	2007	(Riley et al. 2011)
Frankfort	3.2	2006	(Riley et al. 2011)
	2.38	2007	(Riley et al. 2011)
Port Weller	2.08	2004	(Fitzsimons et al. 2007)
	3.5	2007	(Fitzsimons et al. 2007)
Sturgeon Bay	1.34	2006	(Riley et al. 2011)
	0.65	2007	(Riley et al. 2011)

Table 2.2 Number of zooplankton samples collected at each location, depth, and season

The number of samples collected at each location, depth, and season for both zooplankton community analysis and thiaminase analysis. AS=Ashland, DT=Detour, FR=Frankfort, PW=Port Weller, SB=Sturgeon Bay. *Opportunistic sampling in which a full complement of samples were not collected. Blank cells indicate no samples were collected.

Site	AS	DT	FR	PW*	PW	PW*	SB	SB	SB	SB	SB	SB
Depth (m)	55	32	28	8.5	25	75	18	100	18	100	18	100
Season	Sum	Sum	Sum	Sum	Sum	Sum	Spring	Spring	Sum	Sum	Fall	Fall
Zooplankton community analysis	3	3	3		3		3	3	3	3	3	3
Thiaminase												
Bulk Fractions												
>125 µm	4	3	3		2	1	2	3	3	3	3	3
53-125 µm	1	1	3	1	1	1	1	1	2	3	3	3
25-53 µm	1	2	1	1	1	1	1	1	1	2	3	3
Pooled individuals												
Bythotrephes		2	1						3	3		3
Cercopagis									1	2		
Holopedium		3										
Mysis		3				3				2		3
Pooled taxa												
Mixed macroinvertebrates							1					

Table 2.3 Equations used to convert zooplankton length to biomass

Equations used to convert zooplankton length to biomass. L=length in mm; WW= wet weight in mg; DW=dry weight in mg; D=width in mm; ASL=antennal scale length (*Mysis*); SL = spine length (*Bythotrephes*). Log is natural log unless specified as Log_{10}. Lack of a dry weight equation indicates that length was converted directly to wet weight.

Taxon	Dry weight equation (Source)	Wet weight equation (Source)
Rotifera		
Asplanchna		WW=(L^3)*0.23 (Bottrell et al. 1976)
Collotheca		WW=(D^3)*0.23 (Bottrell et al. 1976)
Conochilus		WW=((L*(D^2))*0.26) (Bottrell et al. 1976)
Gastropus		WW=(L^3)*0.2 (Bottrell et al. 1976)
Kellicottia		WW=(((L^3)*0.03)+((0.015*(L^3))*0.03)) (Bottrell et al. 1976)
Keratella		WW=(L^3)*0.02 (Bottrell et al. 1976)
Notommata		Used *Ploesoma* as substitute taxon
Ploesoma		WW=(L^3)*0.1 (Bottrell et al. 1976)
Polyarthra		WW=(((L^3)*0.28)+((0.1*(L^3))*0.28)) (Bottrell et al. 1976)
Synchaeta		WW=(L^3)*0.1 (Bottrell et al. 1976)
Crustacea: Cyclopoida		
Acanthocyclops		WW = 0.03493*(L^2.9878) (Pearre 1980)
Cyclopoid copepodites		WW = 0.03493*(L^2.9878) (Pearre 1980)
Diacyclops thomasi		WW = 0.03493*(L^2.9878) (Pearre 1980)
Tropocyclops adult		WW = 0.03493*(L^2.9878) (Pearre 1980)
Tropocyclops copepodite		WW = 0.03493*(L^2.9878) (Pearre 1980)
Crustacea: Calanoida		

Calanoid copepodites		WW = 0.03493*(L^2.9878) (Pearre 1980)
Calanoida nauplii	DW = 4.20*((L)^2.48) * 0.001 (Johannsson et al. 2000)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Epischura lacustris adults		WW = 0.03493*(L^2.9878) (Pearre 1980)
Epischura lacustris copepodites		WW = 0.03493*(L^2.9878) (Pearre 1980)
Eurytemora affinis		WW = 0.03493*(L^2.9878) (Pearre 1980)
Leptodiaptomus ashlandi		WW = 0.03493*(L^2.9878) (Pearre 1980)
Leptodiaptomus minutus		WW = 0.03493*(L^2.9878) (Pearre 1980)
Leptodiaptomus sicilis		WW = 0.03493*(L^2.9878) (Pearre 1980)
Limnocalanus macrurus adults		WW = 0.03493*(L^2.9878) (Pearre 1980)
Limnocalanus macrurus copepodites		WW = 0.03493*(L^2.9878) (Pearre 1980)
Mesocyclops adults		WW = 0.03493*(L^2.9878) (Pearre 1980)
Mesocyclops copepodites		WW = 0.03493*(L^2.9878) (Pearre 1980)
Senecella calanoids adult		WW = 0.03493*(L^2.9878) (Pearre 1980)
Senecella calanoides copepodite		WW = 0.03493*(L^2.9878) (Pearre 1980)
Skistodiaptomus oregonensis		WW = 0.03493*(L^2.9878) (Pearre 1980)
Crustacea: Cladocera		
Alona		WW = 0.091*(L^2.646) (Smirnov 2014)
Bosmina	DW = (2.7183^((2.5294* (log(L)))+2.7116))/1000 (Bottrell et al. 1976)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)

Bythothrephes	DW = ((10^(((log10(SL)) *1.428) + 1.67))/1000 (Garton and Berg 1990)	WW = DW /0.12 (Lehman and Caceres 1993)
Cercopagis	DW = exp((2.98*log(L))-6.42 (Ojaveer et al. 2001)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Daphnia		WW = 0.075*(L^2.925) (Smirnov 2014)
Daphnia galeata mendotae		WW = 0.075*(L^2.925) (Smirnov 2014)
Daphnia pulex		WW = 0.075*(L^2.925) (Smirnov 2014)
Daphnia retrocurva		WW = 0.075*(L^2.925) (Smirnov 2014)
Holopedium gibberum	DW = (2.7183^((3.30* (log(L))) + 2.50))/1000 (Yan and Mackie 1987)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Leptodora kindtii	DW = (2.7183^((2.67* (log(L))) - 0.822))/1000 (Rosen 1981)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Polyphemus	DW = (2.7183^((2.15* (log(L))) + 1.936))/1000 (Dumont et al. 1975).	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Crustacea: Harpacticoida		
Harpactacoida	DW = ((13.95*L) - 5.32)/1000 (Goodman 1980)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Crustacea: Malacostraca		
Diporeia	DW = (0.0067 * (L^3.0232)) (Winnell and White 1984)	DW=0.269 * WW (Landrum 1988)
Mysis	L=(6.18*ASL) + .5 (Grossnickle and Beeton 1979, Keeler et al. 2015) DW=(e^((2.86*(log(L))) - 6.1709)) (Shea and Makarewicz 1989)	WW=DW / 0.161 (Borgmann and Whittle 1994)
Mollusca		
Dreissena veligers		WW=((((58.207-(2.636*L*1000)) +(0.037*((L*1000)^2)))*0.001)/1000) (Hillbricht-Ilkowska and Stanczykowska 1969)

Dreissena bugensis adults		WW=(e^((3.062*log(L)) - 6.2795)/0.0875) modified from (Nalepa et al. 2010)
Cnidaria		
Hydra	DW = 200 ug (Bisbee 1973)	$\log_{10}$WW = 0.5377 + 1.52*$\log_{10}$DW (Bottrell et al. 1976)
Nematoda		
Nematode	DW = 0.0001 mg (Nalepa and Quigley 1980)	DW=0.25* WW (Wieser 1960)
Osteichthyes		
Bloater larva		WW = (2.7183^((3.0799*(log(L)))-12.2167))*1000 (Gamble et al. 2011a)
Burbot larva		(2.7183^((3.0799*(log(L)))-12.2167))*1000 (Gamble et al. 2011a)
Osmeridae larva		WW = (2.7183^((3.0799*(log(L)))-12.2167))*1000 (Gamble et al. 2011a)
Osteichthyes larva		WW = (2.7183^((3.0799*(log(L)))-12.2167))*1000 (Gamble et al. 2011a)

Table 2.4 Sampling event groupings for indicator species analysis

Groupings of sampling events by thiaminase activity for the four indicator species (ISA) analyses. Summary statistics (geometric mean, median, and geometric SD) of thiaminase activity for each sampling event are included. *Group was excluded from the ISA analysis.

	Thiaminase activity (pmol/g/min)						
Sampling event	Geometric mean	Median	Geometric SD	ISA Group 1	ISA Group 2	ISA Group 3	ISA Group 4
SB Sp 18	20	20	1.00	Low	Low	Low	Low
AS Sum 55	20	20	1.02	Low	Low	Low	Low
DT Sum 32	35	20	2.65	Low	Low	Low	Low
SB Sp 100	105	100	1.67	Low	Low	Low	Middle*
SB Sum 100	347	460	1.69	High	Low	Middle*	Middle*
FR Sum 28	457	487	2.38	High	Low	Middle*	Middle*
SB Fall 100	695	800	4.34	High	Low	High	Middle*
SB Sum 18	1635	1400	1.40	High	High	High	High
PW Sum 25	1856	2166	2.24	High	High	High	High
SB Fall 18	2433	2400	1.23	High	High	High	High

Table 2.5 Thiaminase activity by size fraction and season at Sturgeon Bay

The geometric mean, median, and geometric SD of thiaminase activity by size fraction and season at Sturgeon Bay.

Size fraction (µm)	Season	Thiaminase activity (pmol/g/min)			n
		Geometric mean	Median	Geometric SD	
25-53	Spring	20	20	1	2
25-53	Sum	65.3	58	3.48	3
25-53	Fall	91.7	105	2.42	6
53-125	Spring	20	20	1	2
53-125	Sum	285.7	350	2.89	5
53-125	Fall	294.4	260	2.14	6
>125	Spring	54.2	65	2.66	5
>125	Sum	753.7	890	2.55	6
>125	Fall	1300.5	2200	3.20	6

Table 2.6 Summer thiaminase activity of the >125 µm size fraction by site

The geometric mean, median, and geometric SD of thiaminase activity by site in the summer for the >125 µm size fraction of bulk zooplankton.

	Thiaminase activity (pmol/g/min)			
Site	Geometric mean	Median	Geometric SD	n
Ashland	20.2	20	1.02	4
Detour	35.1	20	2.65	3
Frankfort	456.8	487	2.38	3
Sturgeon Bay	753.7	890	2.55	6
Port Weller	1225.4	1050	2.50	3

3 CHAPTER 3

THIAMINASE ACTIVITY IN PLANKTIVOROUS FISHES IN THE GREAT LAKES IS NOT RELATED TO THE COMPOSITION OF THEIR DIET

3.1 Abstract

Thiamine Deficiency Complex, a vitamin B_1 deficiency that affects survival of salmonid embryos, is caused by adult salmonids' ingestion of fishes containing high levels of thiaminase. The source of thiaminase in Great Lakes food webs is unknown. We described thiaminase activity in 14 fish species and its relationship to capture depth, season, and location. We directly tested the hypothesis that thiaminase activity in fishes is related to their diet by comparing two metrics of diet (stomach contents and fatty acid profiles) to thiaminase activity. The goal of this analysis was to identify prey taxa in the diet of fishes that are consistently abundant in fishes with high thiaminase activity and consistently absent or at low abundance in fishes with low thiaminase activity. We found no compelling evidence that the thiaminase activity of fish viscera was related to any component of the diet. Our analysis suggested *Bythotrephes* as a potential candidate source of thiaminase, but this taxon was not consistently present in thiaminase-containing fishes. Saturated fatty acids (12:0, 17:0, and 18:0) and 20:4n-6 were associated fishes containing high thiaminase activity, suggesting that thiaminase activity was more likely to occur in pelagically feeding than benthically feeding fishes.

3.2 Introduction

Lake Trout (*Salvelinus namaycush*) was the dominant native predator in the Great Lakes until their functional extirpation in the 1950s from all lakes except Lake Superior owing to overfishing, habitat degradation, and species invasions (Hansen 1999). Despite nearly 50 years of aggressive management, rehabilitation of Lake Trout populations to levels articulated in lake-wide Fish Community Objectives (DesJardine et al. 1995, Eshenroder et al. 1995, Stewart et al. 1999, Horns et al. 2003, Ryan et al. 2003) has remained largely unrealized with the exception of Lake Superior (Muir et al. 2012). Failure to rehabilitate Lake Trout results from a combination of factors including stocking insufficient numbers of adults, inappropriate stocking practices and poor survival of early life stages (Holey et al. 1995, Krueger et al. 1995a, Brown et al. 2005c, Claramunt et al. 2005, Jonas et al. 2005, Tillitt et al. 2005, Bronte et al. 2006, Bronte et al. 2007, Bronte et al. 2008).

Poor survival of early life stages represents an important bottleneck to successful Lake Trout rehabilitation (Jones et al. 1995, Eshenroder et al. 1999, Bronte et al. 2003b, Bronte et al. 2008, Morbey et al. 2008). Two mechanisms are likely responsible for poor early life stage survival: predation on Lake Trout embryos and juveniles and a trophically-induced thiamine (vitamin B_1) deficiency which causes embryo mortality prior to exogenous feeding (Brown et al. 2005c, Strakosh and Krueger 2005, Bronte et al. 2008, Madenjian et al. 2008). The relative importance of these two mechanisms in suppressing recovery of Lake Trout remains an unanswered question, in part because of the role of the invasive Alewife (*Alosa pseudoharengus*) in both mechanisms. The effect of predation on survival of Lake Trout embryos and juveniles is intuitive: predation results in decreased survival (Jones et al. 1995, Krueger et al. 1995b, Jonas et al. 2005, Strakosh and Krueger 2005, Madenjian et al. 2008). The second factor, Thiamine Deficiency Complex (TDC), is a less intuitive, yet potentially important mechanism contributing to recruitment failure (Krueger et al. 1995a, Bronte et al. 2003b, Brown et al. 2005c, Bronte et al. 2008). Furthermore, these two mechanisms may interact with one another in an additive fashion because thiamine-deficient Lake Trout embryos are less efficient foragers, have lower growth rates, and are more vulnerable to predation (Fitzsimons et al. 2009a).

Lake Trout become thiamine deficient because they consume prey that have high levels of thiaminase, a thiamine-degrading enzyme (Fisher et al. 1995, Fitzsimons et al. 1998, Ji and Adelman 1998, Fitzsimons et al. 2005b, Honeyfield et al. 2005b, Tillitt et al. 2005). Ingestion of thiaminase-containing prey fish or artificial diets containing thiaminase lowers whole-body thiamine levels in adults (Brown et al. 1998a, Brown et al. 1998b, Fisher et al. 1998a, Fisher et al. 1998b, Fynn-Aikins et al. 1998, Honeyfield et al. 1998b, Ji et al. 1998, Honeyfield et al. 2005a, Houde et al. 2015), and leaves females with insufficient thiamine to allocate to their eggs, resulting in reduced thiamine content of eggs and subsequent embryonic mortality (Fisher et al. 1998b, Fitzsimons and Brown 1998, Brown et al. 2005a, Honeyfield et al. 2005a, Honeyfield et al. 2005b, Fitzsimons et al. 2007, Czesny et al. 2009). Development of TDC is associated with long-term consumption of diets containing a high percentage of two non-native planktivores, Alewife (*Alosa pseudoharengus*) and Rainbow Smelt (*Osmerus mordax*) (Coble 1965, Fitzsimons and Brown 1998, Honeyfield et al. 2005b). Both of these fish consistently show high levels of thiaminase activity (Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005, Zajicek et al. 2005). However, the ultimate source of the thiaminase enzyme in planktivorous fishes remains unknown. The thiaminase enzyme in Alewife and Rainbow Smelt could originate from two possible sources: either planktivorous fish acquire thiaminase from their diet (dietary acquisition hypothesis) or planktivorous fish make the thiaminase enzyme themselves (*de novo* synthesis hypothesis); these two hypotheses are not mutually exclusive, as thiaminase enzymes from multiple sources could contribute to the overall thiaminase activity in planktivorous fish. Identifying the source(s) of thiaminase in Alewife and Rainbow Smelt is an essential first step in understanding why thiaminase activity in fishes varies seasonally, annually, and spatially (Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005), and how that may inform recovery efforts for Lake Trout.

The most widely held assumption is that fishes obtain thiaminase through dietary sources (Brown et al. 2005c, Tillitt et al. 2005, Bronte et al. 2008). The most-often hypothesized dietary sources of thiaminase include thiaminase-producing bacteria (Honeyfield et al. 2002, Brown et al. 2005c, Tillitt et al. 2005, Kraft et al. 2014), cyanobacteria (Arsan and Malyarevskaya 1969, Grigor'yeva et al. 1977, Honeyfield et al. 1998a, Honeyfield et al. 2002, Tillitt et al. 2005), and zooplankton (Fitzsimons

et al. 2004a, Fitzsimons et al. 2004b, Zajicek et al. 2005). Zooplankton have been suggested as a source of thiaminase for prey fish, given that the two fish species that are the causative agent of TDC in Lake Trout are both plantkivores (Honeyfield et al. 1998a, Honeyfield et al. 2002, Fitzsimons et al. 2004a, Brown et al. 2005c, Tillitt et al. 2005). However, not all planktivorous fishes in the Great Lakes have high levels of thiaminase activity. In contrast to Alewife and Rainbow Smelt, Bloater (*Coregonus hoyi*), a native planktivore, contain essentially undetectable levels of thiaminase activity (Tillitt et al. 2005, Chapter 2). If thiaminase activity is acquired dietarily, one potential explanation for both the difference in thiaminase activity between planktivores and the spatial and temporal variability in thiaminase activity of Alewife and Rainbow Smelt is that different planktonic prey items contain different levels of thiaminase activity and that planktivore diets vary spatially, seasonally and by species. Diets of Great Lakes planktivores are known to vary based on these and other factors (Dietrich et al. 2006, Davis et al. 2007, Gamble et al. 2011b, Bunnell et al. 2015).

The dietary acquisition hypothesis is not only intuitively appealing but is also suggested by the findings that thiaminase activity is measurable in *Mysis diluviana*, *Diporeia hoyi*, *Bythotrephes*, and *Cercopagis* (Zajicek et al. 2005, Chapter 1), all of which are common prey items for Great Lakes fishes. Thiaminase activity in *Bythotrephes*, *Cergopagis*, and *Mysis* are variable, and thiaminase activity in *Mysis* has been measured at levels that approach that of the lower end of the whole-body thiaminase activity of Rainbow Smelt (Chapter 2). Given that the two species of fish whose consumption are known to cause thiamine deficiency in Lake Trout are planktivores and that at thiaminase activity has been measured in both zooplankton taxa, zooplankton could reasonably be expected to themselves be primary sources of thiaminase (i.e., they may produce their own thiaminase enzymes *de novo*). If thiaminase activity is acquired dietarily, then a functional relationship should exist between the thiaminase activity of fishes and their diet. If the source of thiaminase is of dietary origin, then those fishes with high levels of thiaminase activity should have elevated amounts of high-thiaminase producing prey in their diet, while those same prey items should be absent in the diets of fishes with low or no thiaminase activity.

Understanding the relationship between thiaminase activity and diet of fishes will facilitate identification of candidate sources of thiaminase activity for future research and has the potential to inform the often-asked question of what mechanism is responsible for the observed variation in thiaminase activity, and subsequent TDC, in fishes (Fujita 1954, Tillitt et al. 2005, Honeyfield et al. 2010b, Kraft et al. 2014). Our first objective was to assess thiaminase activity in a diversity of fishes that may serve as Lake Trout prey and assess whether thiaminase activity in fishes was related to species, season, site, and depth. Our second objective was to quantify fish diets; we selected two methods (stomach content analysis and fatty acid profile analysis) that provide information at different temporal scales and at different levels of taxonomic resolution to achieve this objective. Our final objective was to compare thiaminase activity in fishes to diet information to identify prey items that were likely candidate sources of thiaminase activity across a range of fishes in the Great Lakes.

3.3 Methods

3.3.1 Site selection and fish collection

Prey fish were sampled in 2007 at five different locations throughout the Great Lakes (Figure 2.1). We chose locations to represent a range of fish communities and known TDC severities. Sturgeon Bay (Lake Michigan), Frankfort (Lake Michigan), and Port Weller (Lake Ontario) were chosen to represent areas with severe TDC levels (Fitzsimons et al. 2007, Fitzsimons 2008, Riley et al. 2011). We chose Detour (Lake Huron) to represent an area with moderate TDC (Riley et al. 2011), and Ashland (Lake Superior) to represent an area with no TDC (Fitzsimons and Brown 1998, Brown et al. 2005d). For average Lake Trout egg thiamine levels supporting site selection, see Chapter 2.

Our sampling sought to maximize the likelihood of capturing variation in thiaminase activity of prey fishes as well as variation in the type of prey consumed by prey fishes across the Great Lakes basin. To capture seasonal and depth-driven variability, we collected samples in three months (April, July, and September of 2007) and at two depths (18 m and 100 m) at Sturgeon Bay. Detailed reasons for choosing these months are given in Chapter 2.3.1. To capture spatial variation in fish species composition, thiaminase activity of fishes, and the type of prey consumed by fishes,

we sampled the four remaining sites (Ashland, Detour, Frankfort, and Port Weller) as concurrently as possible, between late July and early August 2007. At each site, mid-depth ranges were sampled to fall between the nearshore and offshore depths sampled at Sturgeon Bay. Our target depth for each sampling event was 30m; however, depths varied slightly from the intended depth ranges owing to availability of established trawling transects or to fish availability. In some cases, we did not capture fish at the target depth after repeated trawling, and therefore we chose new depths close to the target depth. Sampling occurred at depths of 35-70 m at Ashland, 30-50 m at Detour, 24-40 m at Frankfort, and 9 m at Port Weller. Port Weller was the only sampling event for which we were unable to obtain fish at target mid-depths, and the lack of fish captured at these depths resulted in a nearshore sample instead of a mid-depth sample. These four sampling events combined with the six sampling events at Sturgeon Bay represent a total of ten planned sampling events.

In addition to these 10 scheduled sampling events, we collected fish at three of our sites in other seasons or depths opportunistically when collections were made possible by scheduled trips for other purposes. At Port Weller, we sampled at 75 m on the same day at which we sampled at 9 m depth. Because we captured relatively few fish at Frankfort and Detour in the planned summer sampling, we returned to both sites in the fall (Frankfort: September 13, 2007; Detour: October, 21, 2007) to collect fish from mid-depths. Because these samplings were not anticipated as part of the study design, we did not collect a full complement of samples at these depths.

Fish were sampled using bottom trawls from the R/V *Sturgeon* (lakes Huron and Superior), R/V *Kiyi* (Lake Superior) and R/V *Kaho* (Lake Ontario) and immediately sorted to species. Ashland was the only site where the trawl was towed cross-contour; trawls at the other sites were towed along a specified depth contour. The depth ranges reported above for the remaining sites are the result of multiple with-contour tows at a range of depths. Specific depths for capture for all species are reported in Table 3.1, but because the tows at Ashland were cross-contour, the exact depth of capture was not known.

For each sampling event, our first priority for sample collection was 10-15 individuals of each species for thiaminase analysis. Fish were individually bagged and frozen

immediately on dry ice. Our second priority for sample collection was 10-15 individuals for fatty acid analysis, and these individuals were also individually bagged and frozen on dry ice. Our last priority was collection of up to 20 more fish for stomach content analysis, and these fish were individually bagged and frozen at -20^{o}C immediately upon capture. The individuals captured were not evenly distributed with respect to species, site, season, and depth. When an insufficient number of individuals of each species was collected at each sampling event, an unbalanced design resulted, with some species at some locations/depths/seasons captured for some types of analyses but not others (Table 3.1; Figure 3.1). Fish from a total of 41 unique combinations of site, species, depth, and season (hereafter "sampling combinations") were collected for thiaminase analysis, whereas 23 sampling combinations were collected for fatty acid analysis and 17 sampling combinations were collected for gut content analysis (Table 3.1).

3.3.2 Identification of stomach contents

We thawed each individual fish at room temperature until the tissues were thawed just enough to be able to be dissect the intestinal tract with dissecting scissors. Fish length (TL: nearest mm) and weight (to 0.1g) were measured. Stomachs (from the esophagus through the intestine) were removed from fish and placed in glass vials containing 95% ethanol. The intestines were removed under a dissecting microscope prior to opening the stomach, with the exception of Round Goby, for which the intestinal contents were examined. Stomachs were opened from the distal end to the proximal end of the esophagus using dissecting scissors and tweezers. Stomach contents were rinsed out of the stomach and all prey items were removed from the wall of the stomach lining with tweezers. All prey items were counted under a Leica S8 APO dissecting scope on a Ward counting wheel, with prey items suspended in a mixture of water and iodine. All prey items were counted from every gut.

Copepod, cladoceran, and rotifer prey were identified to species when possible (Stemberger 1979, Balcer et al. 1984, Hudson and Lesko 2003), and if species could not be determined, these taxa were identified to the lowest possible taxonomic grouping (generally order). Copepodites (calanoid, cyclopoid, and harpacticoid) were identified to order and were considered 3 separate taxa. Macroinvertebrate

prey were identified to the lowest feasible taxonomic level using standard reference texts (Pennak 1989). Countable parts were considered those parts occurring only once (i.e., rami, post-abdominal claws, head capsules) or twice (i.e., Mysis eyes, dreissenid septa, and Bythotrephes mandibles). We measured ten intact individuals of each taxon from each stomach using a calibrated camera integrated into the microscope. When prey lengths were not available (i.e., no intact individuals could be measured, but countable parts were identifiable) for a given taxon consumed by a given fish species, mean lengths were substituted from other fishes of the same species collected at the same sampling event or from the grand prey mean length eaten by that fish species across all sampling events. We measured taxa as previously described (GLNPO 2003) with the following exceptions: *Bythotrephes* was measured from the proximal base of the spine to proximal base of the caudal kink (Garton and Berg 1990), and *Mysis* was measured by antennal length (Grossnickle and Beeton 1979). Dreissenids were measured by shell length.

3.3.3 Conversion of prey taxa length measurements to biomass

We calculated the mean length (in mm) as geometric means for each taxon consumed by each fish. Mean length measurements were converted to wet weight biomass to facilitate a comparison between diet and thiaminase activity, which is measured on a wet weight basis. We converted the geometric mean length for each prey taxon to wet mass (mg) on a per-fish basis using published length-weight regressions (Table 2.3 and Appendix J). When possible, we used length-wet weight regressions used to estimate wet weight biomass directly from length. When length-wet weight regressions were not available, we used length-dry weight equations to estimate dry weight, and dry weight was converted to wet weight. No adult chironomids were found intact in stomachs, and therefore no lengths were available; we substituted the average mass of chironomid pupa at each site for the mass of an adult chironomids at that site. To calculate the total wet mass of each taxon consumed by each fish, the fish-specific average mass for each taxon was multiplied by the number of that taxon consumed by that individual.

3.3.4 Thiaminase activity

We measured thiaminase activity in fish viscera rather than whole fish because thiaminase activity is highest in viscera relative to muscle and other tissue (Sealock

et al. 1943, Fujita 1954, Ji and Adelman 1998, Zajicek et al. 2005, Kraft et al. 2014) and because viscera would be expected to have the highest concentration of the source of thiaminase if thiaminase is acquired through the diet. We excised visceral tissue from the body cavity while samples were thawed just until tissues could be dissected, and we removed the entire viscera (including the stomach, intestines, liver, spleen, and gonads). Viscera were pulverized while frozen with dry ice in ceramic mortars and pestles (Fisher Scientific, Pittsburgh, Pennsylvania). Nine-spine sticklebacks were too small to analyze viscera for individual fish, and therefore we combined viscera from two to three individual fishes into one composite sample. Once pulverized, the dry ice remaining in each powdered sample was allowed to sublimate at -20°C and -80°C. The frozen powdered tissue was sub-sampled and weighed into 3.6-ml Nalgene cryovials (USA Scientific, Ocala, Florida). All sub-sampling and weighing activities were carried out in a cold room at 4°C or –20°C using a pre-equilibrated Mettler AE163 analytical balance (Mettler Instruments Corporation, Hightstown, New Jersey). Thiaminase activity was determined for each individual or composite sample using a standard radiometric assay (Zajicek et al. 2005). The limit of detection for this assay was 20 pmol thiamine degraded/g tissue/min.

3.3.5 Fatty acid profile analysis

Fish for future fatty acid profile analysis were stored at -80^{o}C until analysis was conducted. Fatty acid profiles were characterized from whole fish homogenates. Frozen samples were thawed on wet ice thawed until they could be ground with a tissue homogenizer. Homogenization occurred in a glass beaker nested in a beaker filled with ice to keep the sample cold. Total lipids were extracted from the tissue homogenate with chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Fatty acid methyl esters (FAMEs) were prepared following standard methods (Metcalfe and Schmitz 1961) and were separated by gas chromatography (Agilent 6890 Gas Chromatograph, Agilent Technologies, Inc., Wilmington, Delaware) using a 5973 mass selective detector (Agilent Technologies, Inc.), a capillary column (Omegawax™ 320, 30 m x 0.32 mm x 0.25 μm film thickness, Supleco®, Bellefonte, Pennsylvania), and an auto-injector (Hewlett Packard 7683).

Helium was used as the carrier gas at a flow of 30 ml/min. Initial temperature of the oven was 150°C for 26 minutes, which was increased to 205°C by increments of 2°C/min, then held at 205°C for 24 minutes. Mass spectrometry operating parameters were: electron multiplier 1640 V, transfer line 280°C, source and analyzer 270°C; autotune file DFTPP passed criteria, electron impact energy 70 eV. Prior to transmethylation, nonadecanoate acid (C19:0) was added to each sample as an internal standard. Individual fatty acid methyl esters were identified by comparing the retention times of authentic standard mixtures. (FAME mix 37 components, Supleco) and quantified by comparing their peak areas with that of the internal standard (Czesny and Dabrowski 1998).

3.3.6 Statistical analysis

3.3.6.1 Assessing the relationship between thiaminase activity in fishes and environmental variables

Thiaminase activity was natural log-transformed prior to analysis, as its variance increased as the mean increased. We sought to assess the relationship between thiaminase activity and depth, season, species, and site using one integrated modelling framework that would evaluate the effect of each of these factors after controlling for the others. However, each species was not consistently sampled across each depth, season, and site, (Table 3.1); temporal sampling was only conducted at Sturgeon Bay; and site and depth were often confounded at sites other than Sturgeon Bay. This resulted in an unbalanced design with respect to the four variables of interest and an inability to assess the relationship between thiaminase and one factor while controlling for the effect of other factors. Therefore, we identified all of the sampling combinations with $n \geq 3$ that allowed for an unconfounded evaluation of the effect of each variable of interest, meaning that the effect of one variable on thiaminase activity was only tested with sampling combinations that were not confounded by differences in any of the other variables of interest. We assessed the relationship between thiaminase activity and depth, season, or species individually using the appropriate subset of sampling combinations (Table 3.2). For the analysis of the effect of species, we considered the sampling combinations at 27 and 37 m at Frankfort to be similar enough in depth to analyze them together, and we similarly considered the sampling combinations at 64 and 73 m at Frankfort together. The number of comparisons used to evaluate the

effect of depth, season, and species varied (Table 3.2) owing to the design and variation in fish presence relative to the factors of interest.

We evaluated the relationship between thiaminase activity and depth, season, or species using one-way ANOVAs (models specified in Table 3.2). Standard regression diagnostics (normal qq-plots, residual vs fit plots, graphical examination of the data) were used to assess model fits (R package: car). We assessed constant variance of the residuals with respect to the fitted values using a Breusch-Pagan test and examination of spread level plots. In cases where the Breusch-Pagan test indicated non-constant variance, we used a one-way ANOVA with Welch's correction for unequal variance (Table 3.2). When ANOVA indicated a difference across depths, seasons, or species, post-hoc pair-wise comparisons were made using a Games-Howell test (Games and Howell 1976) as implemented in the R package userfriendlyscience. This multiple comparison method accounts for both unequal group size, which was common for most multiple comparisons, and unequal variance. We considered $p<0.05$ to represent convincing evidence of a difference between group medians, and $0.05<p<0.08$ to represent suggestive but inconclusive evidence of a difference and exact p-values were specified.

Assessing the effect of site proved difficult because few comparisons were unconfounded with difference in depth, season, or species; only three sites could be compared at any one time. Only two of these three-site comparisons existed, both of which included Ashland and Detour. As a result of the confounding influences, no formal statistical analysis of how thiaminase varies by site was conducted. For the six species captured most frequently, we graphically examined the relationship between thiaminase activity and season, with the understanding that site was often confounded with depth and season and that we had little ability to disentangle the effect of site from an effect of depth, season, or an interaction of depth and season.

3.3.6.2 Assessing the relationship between thiaminase activity and fish diets

Data preparation: The total wet mass of each taxon consumed by each fish was calculated as described in section 3.3.3. Empty stomachs and stomachs containing only rocks and microplastics (combined n=7) were excluded from subsequent analyses, resulting in 200 stomachs from 17 sampling combinations representing 8

species (Alewife, Bloater, Cisco, Deepwater Sculpin, Ninespine stickleback, Rainbow Smelt, Round Goby, Slimy Sculpin). A total of 47 diet items were recorded; rocks/microplastics and the one plant seed eaten by one individual were excluded from the remainder of the analyses, leaving 45 prey taxa.

We assessed the relationship between thiaminase activity and fish diets using three multivariate methods (see below) for the 17 sampling combinations having both thiaminase activity and diet data (Table 3.1). For all three analyses, we expressed the diet data both on a percentage (by mass) basis as well as on an absolute basis (total mass per stomach). This allowed us to assess the potential for total mass of prey taxa consumed to affect thiaminase activity in addition to assessing the influence of the relative proportion of prey taxa. Thiaminase activity measurements on viscera and stomach contents were necessarily taken from separate individuals; therefore, we averaged the wet biomass or proportional biomass for all individuals captured at a given site, depth, and season. We similarly averaged the thiaminase activity from individuals collected in given sampling combination.

Choice of analytical methods: We chose non-metric multidimensional scaling (NMS) (Kruskal 1964a, 1964b, Mather 1976), distance-based redundancy analysis (db-RDA) (Legendre and Anderson 1999, McArdle and Anderson 2001), and indicator species analysis (ISA) (Dufrene and Legendre 1997) to assess the relationship between thiaminase activity and diet. Each offers a fundamentally different conceptual method of evaluating the question of interest, and as a result, each has the potential to reach different conclusions. Each of these methods is appropriate for non-normally distributed data and data in a sparse matrix, both of which are typical properties of stomach content data.

A detailed treatment of the reason for choosing these three techniques is provided in section 2.3.7.2. Briefly, NMS arranges the position of each sampling combination in prey taxa-space solely based on stomach content data and in the absence of any information about response variables (i.e., thiaminase activity). After conducting NMS, the resulting ordination solution is rotated to maximally correspond to response variables of interest and evaluate the degree to which main axes of variation in the stomach contents are related to thiaminase activity. db-RDA is also an ordination

technique, but it differs fundamentally from NMS in that the ordination of the diet is expressly constrained by information about thiaminase activity. Therefore, db-RDA specifically identifies gradients in fish diets that are related to thiaminase, even if those gradients do not constitute the majority of the variation in the diets. ISA is designed to determine which taxa are indicative of groups that are designated prior to analysis (McCune and Grace 2002), and we used the quantitative thiaminase activity measurements to group the 17 sampling combination into high and low thiaminase categories, which we then interrogated for indicator species. We chose ISA because it focuses on the contributions of individual species rather than community gradients.

Data manipulations and outlier detection for multivariate analyses: The main matrix for all three multivariate analysis was identical: each row was one of the 17 sampling combinations for which we had thiaminase activity and diet data (Figure 3.1), each column was a taxon, and each cell contained the average or proportional wet biomass of each prey taxon. A total of 45 prey taxa were evaluated. Because rare species are often deleted prior to community analysis (McCune and Grace 2002) as this improves performance of multivariate analysis, we also deleted all taxa that occurred in just one of the 17 sampling combinations for NMS and db-RDA (13 taxa deleted). All taxa present in only one or two sampling combinations was deleted for ISA (resulting in 24 taxa), as taxa appearing only once or twice by definition can never yield an indicator value better than that expected by chance in the randomizations. Furthermore, this technique does not seek gradients in structure as do the two ordination techniques. For the analyses using proportional biomass, rows were re-relativized to sum of one following deletion of taxa. The main matrix used for all NMS and rd-BDA was 17 sampling combinations (rows) X 45 (or 32) taxa (columns), with the only difference for ISA being that 24 taxa were analyzed. For analyses where diet was expressed as absolute biomass in mg, biomass spanned more than 7 orders of magnitude and was log-transformed prior to analysis.

Multivariate procedures: We performed NMS, db-RDA, and ISA using PCORD (McCune and Mefford 2015) with previously described procedures (section 2.3.7.2). Sorensen distance was used for both NMS and db-RDA. For db-RDA, the second matrix consisted of 17 rows (sampling combinations) and 1 column (log of the

geometric mean thiaminase activity). The second matrix for ISA consisted of the 17 sampling combinations as rows and 4 columns, each of which indicated a thiaminase activity grouping for the 17 sampling combinations. One challenge we faced in categorizing the 10 sampling events as high or low thiaminase is several different sampling events with intermediate average thiaminase activities could be reasonably assigned to either high or low groups (Table 3.3). Therefore, we conducted four separate analyses in which the high and low thiaminase groups consisted of slightly different sampling combinations to evaluate the influence of placing sampling combinations with intermediate thiaminase values into a specific groups. In the first two analyses, all 17 sampling units were assigned to either the high or low group (Table 3.3). In the last two analyses, the 17 sampling events were assigned to one of three groups (high, medium, or low thiaminase activity), and the middle group was excluded from the analysis to achieve the best contrast between the sample units containing the highest and lowest thiaminase activities. For taxa indicative of the high thiaminase group, a rank correlation analysis (Kendall's tau) was conducted to determine the taxa-specific relationship between gram-normalized wet mass and thiaminase activity.

3.3.6.3 Assessing the relationship between thiaminase activity in fishes and fatty acid profiles

Data preparation: The quantity of each of 29 fatty acids for each of 289 fish was expressed as mg fatty acid per gram of fish tissue to assess whether variation associated with relative abundance of fatty acids is related to thiaminase activity. Thiaminase activity measurements on viscera and fatty acid profiles of whole fish were necessarily taken from separate individuals; therefore, we averaged the fatty acid proportions and thiaminase data for all individuals captured at a given site, depth, and season, resulting in fatty acid profiles that were available for 23 sampling combinations.

We assessed the relationship between thiaminase activity and fish diets using NMS and db-RDA. We did not use ISA for fatty acid profiles because fatty acid data differ in structure from diet and community data in ways that make this technique somewhat less suitable for assessing indication.

Analysis methods for fatty acids were similar to those described for diet data. Two fatty acids (14:1n-5 and 20:1n-11) were deleted prior to analysis, as both were strong outliers owing primarily to very low abundances. The main matrix used for all three analyses was 23 sampling combinations (rows) X 28 fatty acids (columns).

3.4 Results

3.4.1 Relationship between thiaminase activity and environmental variables

Thiaminase activity in Alewife was not related to depth in the summer at Sturgeon Bay ($p=0.82$; Figure 3.2; Table 3.2). Alewife were also captured in the summer at Port Weller at 9 ($n=2$) and 75 m ($n=1$; Figure 3.1), but sample size was too small for inclusion in this analysis.

Thiaminase activity was higher in spring than in summer or fall for Alewife and Deepwater Sculpin (Figure 3.3). As with depth, we had relatively few comparisons with which to evaluate the effect of season because few species were captured in all three seasons at Sturgeon Bay. Thiaminase activity was 3.4 times higher in spring than in summer and fall for offshore Alewife at Sturgeon Bay (both $p<0.00002$; Figure 3.3; Table 3.4). We saw a similar seasonal trend for offshore Deepwater Sculpin, with suggestive evidence that thiaminase activity was 4.3 higher in spring than summer ($p=0.076$) and convincing evidence that thiaminase activity was 6.4 higher in spring than in fall ($p=0.025$). Thiaminase activity in summer and fall did not differ for offshore Alewife, Deepwater Sculpin, and Rainbow Smelt at Sturgeon Bay (Figure 3.3; Table 3.4).

Thiaminase activity varied by species (Table 3.2, Figure 3.4, Figure 3.5). The nine multiple comparisons for these six species suggests a relationship of (Alewife = Rainbow Smelt) >= Slimy Sculpin > (Ninespine Stickleback = Bloater = Deepwater Sculpin) (Figure 3.4; Figure 3.5). Six species (Alewife, Rainbow Smelt, Slimy Sculpin, Ninespine Stickleback, Deepwater Sculpin, and Bloater) were captured frequently enough to be compared to one another at multiple sampling events. Of these six species, Alewife and Rainbow Smelt had the highest thiaminase activities and did not differ from each other, with geometric means ranging from 5,766 to 38,970 pmol/g/min for Alewife and 6,616 to 15,503 pmol/g/min for Rainbow Smelt

depending on the sampling event (Appendix I). Thiaminase activity in both Alewife and Rainbow Smelt was consistently and substantially (average: 207-262 times) higher than in both Deepwater Sculpin (geometric mean: 20 to 242 pmol/g/min) and Bloater (geometric mean: 28.8 to 128 pmol/g/min), whose thiaminase activity did not differ from one another and was the lowest of the six species in this comparison (Table 3.4; Figure 3.4; Appendix I).

Ninespine Stickleback thiaminase activity was generally between Alewife/Rainbow Smelt/Slimy Sculpin and Bloater (Table 3.4; Figure 3.4; Figure 3.5) and had similar thiaminase activity to Deepwater Sculpin in the one direct comparison that was available. Thiaminase activity in Ninespine Stickleback was lower (41 times; n=2 comparisons) than in Alewife; however, in one comparison in which only four Ninespine Stickleback were assessed (DT Fall 27-37 in Figure 3.4), thiaminase activity was similar to that of Alewife. Thiaminase activity in Slimy Sculpin was the most variable of any species assessed (Table 3.4; Figure 3.4; Figure 3.5). Slimy Sculpin thiaminase activity was 385 times higher than that of Deepwater Sculpin in 3 comparisons. Only one direct comparison of Slimy Sculpin and Bloater was made, which was suggestive but inconclusive ($p=0.066$) for higher thiaminase activity in Slimy Sculpin (AS Sum 50 in Figure 3.4). Together, these comparisons suggest that thiaminase activity is higher than that of Bloater/Deepwater Sculpin. Slimy Sculpin thiaminase activity was once higher than and once equal to that of Alewife and was twice lower than and once equal to that of Rainbow Smelt (Table 3.4).

Of the remaining species, the deepwater coregonids and Cisco had undetectable levels of thiaminase activity (20 pmol/g/min; Figure 3.4), which were similar to the relatively low but somewhat more variable thiaminase activity of Lake Whitefish (geometric mean: 89 pmol/g/min) and Pygmy Whitefish (geometric mean: 57 pmol/g/min). The thiaminase activity of Spoonhead Sculpin was similar to that Slimy Sculpin at Ashland. The thiaminase activity of Trout-perch (*Percopsis omiscomaycus*) (geometric means: 3,678 and 91,524 pmol/g/min) and Round Goby (geometric mean: 11,428 pmol/g/min; geometric SD: 3.6) were on the order of that found in Alewife and Rainbow Smelt (Figure 3.1). Round goby were only captured at a single sampling event in which no other species were captured, and to avoid

confounding a species comparison with site, depth, or season, its thiaminase activity was not formally compared to that of other species.

An informal comparison of thiaminase activities at different sites is shown graphically in Figure 3.5, recognizing that even among the six species captured most frequently, there are potentially confounding effects of season and depth within a species. No overwhelming effect of site is noticeable. For example, thiaminase activity at Detour was high for Slimy Sculpin relative to other sites also sampled in the summer, but this does not seem to hold for Rainbow Smelt, Alewife, or Deepwater Sculpin. An interactive effect of site, depth, and season on thiaminase activity would nullify this type of comparison, and the unbalanced nature of the design resulted in an inability to assess these types of higher-level interactions.

3.4.2 Relationship between thiaminase activity and diet

Fish diets were varied (Figure 3.6; Appendix K), as expected given the range of species, sites, depths, and seasons sampled. Cisco and Bloater, sampled only from Ashland, had diets similar to one another that consisted of principally *L. macrurus* and *L. sicilis*, which combined for over 50% of their diet. Cisco additionally consumed *Holopedium* (22%) and *Bythotrephes* (25%), whereas Bloater consumed *Mysis* (8%). The diet of Deepwater Sculpin, all of which were sampled from Sturgeon Bay at 100 m, was dominated by *Mysis* and *Diporeia* (combined >98%) regardless of the season, with the proportion of *Diporiea* being higher in summer than spring or fall. Ninespine Stickleback and Slimy Sculpin had diets that tended to vary more by site, season, and depth than other species. Ninespine Stickleback diets were diverse and were similar in nearshore summer samples at Sturgeon Bay and Frankfort, with the same four taxa contributing to most of the diet including Chironomid pupae, *Eurycercus*, calanoid copepodites, and *L. sicilis*. Spring Ninespine Stickleback diets at 100m at Sturgeon Bay were also diverse, but consisted of *Mysis* and *Diporeia*, with additional contributions from *S. calanoids*, *L. sicilis*, and *L. marcrurus*. Slimy Sculpin diets were also varied, with *Diporeia* and chironomid pupae being components of the diet at all sites, but in differing proportions. At Ashland *Diporeia* dominated the diet (77%), while at Sturgeon Bay, their diet was dominated by isopods (*C. intermedia* and *Lirceus* spp., combined 50%) and chironomid pupae (30%). At Frankfort, Chironomid pupae made up nearly half

of the biomass (42%) with *Bythotrephes* (21%) and *Eurycercus* (18%) making up the remainder. Alewife were sampled at all seasons in Sturgeon Bay at 100 m. Their spring diet was dominated by *L. sicilis* (51%) and *S. calanoids* (18%), whereas their summer and fall diet relied heavily on *Mysis* (23 and 30%) and *Bythotrephes* (48 and 69%), with *Mysis* being more important in the summer and *Bythotrephes* dominating in the fall. Round Goby were captured only at Port Weller and the majority of their diet consisted of chironomid larvae (63%) and dreissenid mussels (13%). Rainbow smelt diets were dominated by *Mysis* (92%) at Port Weller and were more diverse at Ashland, consisting of Mysis (40%), followed by nearly equal proportions (~12% each) of *Diporeia*, nematodes, fish eggs, *Bythotrephes*, and chironomid pupae or larvae. The total mass consumed across sampling combinations was variable (Appendix L), with Round Goby at Port Weller and Ninespine Stickleback at Frankfort (both summer) consuming relatively little total mass.

In all cases, NMS ordinations resulted in a 3-dimensional solution, and the final stress was low (6.5-8.9; Table 3.5). Inspection of the scree plot suggested a 3-dimensional solution was the best choice, and none of the three axes explained a trivial amount of variation, indicating retention of all three axes. All ordinations showed a greater reduction in stress than expected by chance (all $p<0.008$), represented more than 80% of the variation present in the original n-dimensional taxa-space. The two NMS ordinations based on absolute wet mass of the diet did not differ substantially from one another, either in terms of the quality of the ordination or in terms of the placement of sampling units in diet-space. They explained a relatively large proportion of the variation in the original 45- and 32-dimensional data, some of which is variation in the total wet mass consumed (as compared to the proportion of the diet; Appendix L). After rotating these ordinations to maximize the correspondence of thiaminase activity, diet explained only 5.6 to 6.8% of the variation in thiaminase activity.

The two NMS ordinations based on proportional wet mass of the diet (Figure 3.6) arranged the sampling combinations in diet space somewhat differently from one another, which is to be expected when the sample set is reduced with the exclusion of rare species. The NMS on proportional wet mass with all 45 taxa revealed little association between diet and thiaminase activity after rotation of the ordination to

maximize the correspondence with thiaminase activity (Appendix M). This ordination placed fishes that had the highest thiaminase activity (largest triangles in Appendix M, panel B) and lowest thiaminase activity (smallest triangles) relatively near each other in diet space, and this is reflected in the moderately weak ability (r=0.295; Table 3.5) of the axis of variation in diet that is most related to thiaminase activity to explain the variation in thiaminase activity (Appendix M, panel C). The final ordination based on 32 taxa and proportional biomass reveals a strong association between axis 3 and thiaminase activity (r=0.702; Table 3.5). This ordination (Figure 3.7) represented 79.6% of the original variation in 32-dimensional diet space, and axis 3 accounted for 12.4%. This axis was driven by a strong association with *Bythotrephes* (Figure 3.7, panel A, r=0.673). Axis 1 and axis 2 (not shown) were unrelated to thiaminase activity (both $r < 0.018$). The sampling combinations with the highest thiaminase activity generally occupy the higher end (right-hand side) of axis 3, whereas sampling combinations with the lowest thiaminase activity are associated with low axis 3 scores (Figure 3.7, panels B and C).

The randomization test for the four db-RDA analyses (analogous to the four NMS ordinations summarized in Table 3.5) revealed no evidence for an overall relationship between thiaminase activity and diet (all p>0.41). The resulting ordinations, which are constrained by the thiaminase activity data in the second matrix, represented virtually none (R^2 <0.01 for all permutations) of the variation originally present in the diet data.

None of the four ISA groupings revealed an overall difference in diet between the high and low thiaminase groups across all species (0.90<p<0.35; Table 3.6). The use of an absolute or relative representation of diet yielded approximately the same result (Table 3.6). The first ISA grouping (Table 3.3) in which the sampling combinations were split essentially in half, and the fourth grouping, in which sampling combinations with the middle level of thiaminase activity were excluded from the analysis, never yielded an indicator taxon. The remaining 2 groupings consistently suggested only one taxon, *Bythothrephes*, as a potential indicator of high thiaminase activity, with *Bosmina* being suggested only when diet was expressed as absolute mass (Table 3.6). The two groupings of sampling combinations (ISA groups 2 and 3; Table 3.3) that suggest *Bosmina* and *Bythotrephes* as indicators of high thiaminase

activity only differ from the two groupings that indicate no taxa (ISA groups 1 and 4) by only two sampling combinations, indicating that the analysis is particularly sensitive to the placement of the sampling combinations with intermediate thiaminase activities (all either Ninespine Stickleback or Slimy Sculpin) into particular groups. Convincing evidence of a relationship between the proportional wet biomass in diets and thiaminase activity existed for *Bosmina* (tau=0.39, p=0.046) but not for *Bythotrephes* (tau=0.19, p=0.33; Appendix N).

3.4.3 Relationship between thiaminase activity and fatty acid profiles

We generated fatty acid profiles for 23 sampling combinations representing 10 species (Alewife, Bloater, Cisco, Deepwater Sculpin, Lake whitefish, Ninespine stickleback, Rainbow Smelt, Round Goby, Slimy Sculpin, Spoonhead Sculpin) (Appendix O; Figure 3.8). The db-RDA revealed no overall relationship between fatty acids composition and thiaminase activity (p=0.14).

An NMS ordination of sampling combinations in fatty-acid space resulted in a reliable 3-dimensional solution (p=0.004) with low stress (7.3), and represented 94% of the variation in the original 28-dimensional space. Inspection of the scree plot suggested a 3-dimensional solution was the best choice, and therefore we retained all 3 dimensions. After rotating this ordination to maximize the correspondence of thiaminase activity, the proportion of the variation accounted for by the entire 3-dimensional solution was reduced to 75.3% (the percentage of the original variation represented by ordinations can change upon rotation) (McCune and Grace 2002). The axis that was maximally related to thiaminase following rotation (axis 2) revealed a moderate to strong relationship with thiaminase activity (r=0.512, tau=0.388), and axis 2 accounted for 4.6% of the variation in the original data that was explained by the ordination.

Sample combinations with fatty acid signatures similar to one another are located more closely to each other in the ordination space than those with dissimilar fatty acid signatures (Figure 3.9). Increased proportions of saturated fatty acids (12:0, 17:0, and 18:0) along with 20:4n-6 were associated with high levels of thiaminase, and increased proportions of monounsaturated fatty acids (18:1n-9, 20:1n-9, and 21:1n-9). Because the axis of interest represented only a small proportion of the

variation accounted for by the entire 3-dimensional ordination, we additionally forced the NMS to adopt a 2-dimensional solution. The rotated solution for this ordination represented more variation in the fatty acid signatures (88% of the variation in the original matrix), but the association between thiaminase activity and fatty acids was somewhat reduced (r=0.368, tau=0.222). The same gradient of fatty acids was indicative of high and low thiaminase activity as that described for the 3-dimensional ordination.

3.5 Discussion

3.5.1 Relationship between thiaminase activity and environmental variables

Although our comparisons were limited, our data suggest no effect of depth on thiaminase activity. Our motivation for assessing the relationship between depth and thiaminase activity was to determine whether offshore food webs differ from nearshore food webs in affecting thiaminase activity of prey fish and to evaluate the consequences of any differences for the risk of TDC development in Lake Trout relative to nearshore versus offshore stocking practices. To our knowledge, the effect of depth of capture on thiaminase activity has not been previously undertaken. In an evaluation of thiaminase activity in Alewife as it relates to limnological factors in seven Finger Lakes (NY), thiaminase activity was strongly and positively correlated with maximum lake depth, total lake area, and total lake volume, all of which were correlated with one another (Fitzsimons et al. 2005b). In this case, fish were apparently all captured in nearshore areas (Fitzsimons et al. 2005b), and therefore the correlation between lake depth and thiaminase activity is not necessarily informative relative to depth of capture across a wide range of depths.

Our findings agree with these previous reports that thiaminase activity in Alewife is consistently higher in spring than in summer or fall (Ji and Adelman 1998, Fitzsimons et al. 2004a, Tillitt et al. 2005). Thiaminase activity in Alewife was 3.4 times higher in spring than in fall or summer, which is similar to the effect sizes (~ 1.5, ~ 2, and 2.4 times) reported previously for Lake Michigan (Ji and Adelman 1998, Tillitt et al. 2005). We are aware of no case in which Alewife have been sampled in spring, summer, and fall that has failed to show higher thiaminase activity in spring. This contrasts with our finding that thiaminase activity in bulk zooplankton (>125 µm) at

Sturgeon Bay is 10-12 times lower in spring than in the summer or fall (Chapter 1). Deepwater Sculpin show a similar seasonal pattern, with fish captured in the spring having, on average, 5.5 times higher thiaminase than those captured in summer or fall. Not all spring-caught fish show elevated thiaminase activity. Spring-caught Rainbow Smelt in Lake Michigan did not differ from that in the summer or fall for the same years and sites in which the effect of season was evident for Alewife (Tillitt et al. 2005). This suggests the potential for an interaction between season and species, and additional winter and early spring sampling is required to ascertain the uniformity of this effect across species.

Higher spring thiaminase activity in Alewife suggests the possibility that thiaminase activity is increased because of physiological or ecological factors such as exposure to cold water temperatures, lower feeding rates, or what is assumed to be the general induction of stress related to the declining energy density and lack of growth exhibited by Alewife over the winter (Flath and Diana 1985, Stewart and Binkowski 1986, Madenjian et al. 2006b). The relationships of all three of these factors to thiaminase activity have been explicitly tested experimentally in a laboratory (food deprivation, salinity stress and netting stress) or ponds (cold water exposure), and none of the treatments resulted in an increase in thiaminase activity relative to controls (Lepak et al. 2008, Lepak et al. 2013). These findings, combined with low spring zooplankton thiaminase levels, leaves open the question of what mechanism causes the increased spring Alewife thiaminase activity.

Our thiaminase values are somewhat higher than those reported by others, especially for species containing high thiaminase activity (Fitzsimons et al. 2005b, Tillitt et al. 2005, Honeyfield et al. 2012) (see reference lines in Figure 3.4). However, we assessed thiaminase activity only in fish viscera rather than whole-body homogenates, and thiaminase activity in visceral tissue is higher than in other tissue types (Sealock et al. 1943, Fujita 1954, Ji and Adelman 1998, Zajicek et al. 2005, Kraft et al. 2014), so this finding was not unexpected. Our thiaminase activity levels in visceral tissue were, on average, 3.5 times higher than that previously reported for whole-body thiaminase in Alewife from lakes Michigan and Ontario (Fitzsimons et al. 2005b, Tillitt et al. 2005) and 4.7 times higher than whole-body levels in Rainbow Smelt from Lake Michigan (Tillitt et al. 2005); this difference should

be considered when comparing thiaminase activities presented here to those reported by others.

The rank order of thiaminase activity for our six most commonly captured species is the same as those reported by others (Ji and Adelman 1998, Tillitt et al. 2005, Honeyfield et al. 2012). We found that Slimy Sculpin had highly variable thiaminase activity, and generally occupied a position between that of Alewife/Rainbow Smelt and Ninespine Stickleback/Bloater/Deepwater Sculpin. Thiaminase activity in Slimy Sculpin from Lake Ontario showed similar variability in relationship to that in Alewife and Rainbow Smelt; thiaminase activity was as high or higher than that of Alewife and Rainbow Smelt in 2006 but substantially lower in 2007 (Honeyfield et al. 2012). Across four sampling locations in Lake Ontario, the sampling location with the highest mean thiaminase activity had 81-fold higher thiaminase activity than the sampling location with the minimum mean thiaminase (Figure 3.4). Similarly, in the five sampling events in which we captured slimy sculpin, the maximum mean thiaminase activity (DT Sum 37; Figure 3.4) was a comparable 99-fold higher than that of the minimum mean for this species (SB Fall 100). This high degree of variability makes it difficult to determine the position of Slimy Sculpin relative to other species. Interestingly, other species with high thiaminase activity appear to have less variability. The ratio of the maximum to minimum mean Alewife thiaminase activity was 7.2 for our samples and 1.6 in Lake Ontario samples (Honeyfield et al. 2012) and was 2.2 (our samples) and 1.4 (Lake Ontario) for Rainbow Smelt. Thus, slimy sculpin may represent a species with less predictable thiaminase activity as compared to Alewife and Rainbow Smelt.

Our findings represent the first report of thiaminase activity in deepwater coregonids, Spoonhead Sculpin, and Trout-perch. Thiaminase activity was undetectable in Kiyi and Shortjaw Cisco, which were part of the ciscoes species flock that was historically a major food source for Lake Trout. With respect to other *Coregonus* spp., our results are in agreement with previous reports suggesting that Cisco and Bloater are essentially thiaminase-negative (Deutsch and Hasler 1943, Tillitt et al. 2005). However, our results for deepwater coregonids are based on very small sample sizes, and thus caution is advised in making generalizations about these species without additional samples. Lake whitefish and round whitefish have been reported

as thiaminase-positive (Deutsch and Hasler 1943), and our results for Lake Whitefish and Pygmy Whitefish indicate that these two species have generally low thiaminase activity which is comparable to that of other coregonids. Thiaminase activity in Spoonhead Sculpin was more similar to that of Slimy Sculpin than Deepwater Sculpin in the one sampling even in which Spoonhead Sculpin were captured. Fourhourn Sculpin *(Myoxocephalus quadricornis*) from Lake Michigan was reported as thiaminase-positive (Greig and Gnaedinger 1971) but no assessment of its thiaminase activity relative to that of other species was provided. Trout-perch had higher thiaminase activity than Alewife in the one sampling event in which both were captured (Figure 3.4). Although thiaminase activity in all individual trout perch captured was sufficiently high to indicate the potential to cause TDC upon ingestion, Trout-perch are not considered to be a dominant or even minor component of Lake Trout diets (Dietrich et al. 2006, Madenjian et al. 2006a, Jacobs et al. 2010, Gamble et al. 2011b, Rush et al. 2012, Roseman et al. 2014, Colborne et al. 2016), and therefore Trout-perch would not be expected to currently constitute a likely reproductive threat to Lake Trout.

The thiaminase activity in Round Goby has been reported to span 3 orders of magnitude, which is even more disparate than that reported for Slimy Sculpin. The first report of thiaminase activity in Round Goby (six individuals from Lake Michigan in 1998-2000) suggested that this species was thiaminase-negative, with undetectable (< 20 pmol/g/min) thiaminase activity, making Round Goby thiaminase activity similar to that of Bloater and Deepwater Sculpin (Tillitt et al. 2005). The second, and only other, report of thiaminase activity for Round Goby came from Lake Ontario, with thiaminase activity in 20 individuals ranging from 135 to 57,700 pmol/g/min in 2006 (mean ~12,500 pmol/g/min) and from 0 to 4,970 pmol/g/min in 2007 (mean ~1,000 pmol/g/min) (Honeyfield et al. 2012). Thiaminase activity in the Round Goby we sampled from Port Weller ranged from 1,726 to 54,256 pmol/g/min (mean: 20,508 pmol/g/min; Figure 3.1), which was not as variable nor as low as that reported from Lake Ontario (Honeyfield et al. 2012). At Port Weller, Alewife (n=2) captured in the same sampling event had thiaminase activities that fell in the middle of the range we observed for Round Goby, and although we could not include Round Goby in formal cross-species comparisons, Round Goby had thiaminase activity comparable to Alewife and Rainbow Smelt captured at other locations (Figure 3.1).

Unlike Trout-perch, Round Goby are becoming increasingly common in the diets of Lake Trout, rendering them a potential source of concern relative to their ability to induce TDC. In eastern Lake Ontario in 2003 and 2004, Round Goby was the second most abundant food item consumed by Lake Trout, with Round Goby making up 36% of biomass and Alewife making up 56% of the biomass in Lake Trout stomachs (Dietrich et al. 2006). In 2008 and 2010, Lake Trout diets on the southern shore of Lake Ontario were estimated (stable isotope mixing models) to consist principally of Round Goby (54-68% of diet), followed by Alewife (22%) and Rainbow Smelt (8-20%) (Rush et al. 2012, Colborne et al. 2016). If Round Goby have thiaminase activity as high as or higher than Alewife and Rainbow Smelt, then the reduction of Alewife in the diet of Lake Trout would not be expected to provide any relief with respect to the likelihood for development of TDC. The initial report of low thiaminase activity in Round Goby (Tillitt et al. 2005) prompted a modelling analysis in which the authors concluded that the recruitment benefit achieved by Lake Trout in terms of reduced likelihood of TDC of the then-presumed low thiaminase activity associated with Round Goby was expected to be outweighed by the negative effects of Round Goby predation on eggs and fry (Fitzsimons et al. 2009b). However, the emerging finding that Round Goby thiaminase can be as high as or higher than that of Alewife may mean that exchanging Round Goby for Alewife is neutral or detrimental relative to risk of TDC. Together, Alewife, Round Goby, and Smelt constituted 95% (2008) and 96% (2010) of the diet of Lake Trout in Lake Ontario (Colborne et al. 2016), and domination of Lake Trout diets by a trio of high-thiaminase containing prey is potentially no better with regard to TDC risk than a diet of high in only Alewife. However, if some Round Goby individuals contain low thiaminase activity, Round Goby could be preferable to Alewife with regards to TDC development, as no case of Alewife individuals lacking thiaminase activity have been reported. The progeny of Lake Trout presumed to feed on a diet of Round Goby had higher thiamine levels in the yolk at hatching and higher survival relative to progeny of Lake Trout presumed to feed on Alewife (Jaroszewska et al. 2009), but the diet of adult lake trout was not directly investigated in this study.

The reason for highly variability in Round Goby thiaminase activity is unknown (Honeyfield et al. 2010a, Honeyfield et al. 2012). Given their role as a primary prey

item for Lake Trout, the reasons for the observed variability should be examined and a more thorough investigation of the among-individual variation in thiaminase activity of Round Goby should be conducted. Our samples were visceral tissue and would therefore be expected to include any items in the gut at the time of analysis. Round Goby are known to consume dreissenids (Ray and Corkum 1997, French and Jude 2001, Walsh et al. 2007, Andraso et al. 2011, Bunnell et al. 2015). Additionally, a comparison of the effect of feeding Lake Trout Alewife, Round Goby, and Smelt is warranted to evaluate the potential for any differences in the biochemical nature of the thiaminase enzymes in these fishes (Zajicek et al. 2009) to differentially affect Lake Trout upon ingestion in a way that is not accounted for by the radiometric measurement of thiaminase activity. For example, if different thiaminase enzymes are produced *de novo* by different species, each enzyme may be differentially susceptible to digestion or may have differing pH optima that make some more thiamine-destructive in the gut of Lake Trout than others (Zajicek et al. 2009).

We had little ability to evaluate the effect of site directly without confounding the analysis with differences in depth, season, or species, but no consistent pattern in thiaminase activity with regard to site appears to occur in our samples once the variation in species is considered (Figure 3.5). The effect of season (3.4 and 5.5 times; Table 3.4) was relatively small compared to the one-to-two order of magnitude effect sizes seen for some of the cross-species comparisons (31 to 385; Table 3.4). Given that thiaminase activity in bulk zooplankton (>125 µm) at Ashland and Detour was 13- to 60-fold lower (Chapter 2) than that at Frankfort, Port Weller, and Sturgeon Bay, no similar pattern was evident among planktivores sampled simultaneously at these locations. Thiaminase activity did not vary by site for Rainbow Smelt or Bloater from Lake Michigan but did differ for Alewife (Tillitt et al. 2005), and the differences for Alewife were did not reflect any patterns relative to regions of the lake. Similarly, thiaminase activity in Rainbow Smelt did not differ in lakes Superior, Michigan, or Huron (Ji and Adelman 1998). Thiaminase activity for Alewife or Rainbow Smelt did not vary across sites in Lake Ontario, but did differ across the same sites for Round Goby and Slimy Sculpin (Honeyfield et al. 2012). This suggests a species by site interaction in which some species have spatially highly variable thiaminase activity while others do not.

3.5.2 Relationship between thiaminase activity and diet

The diets we describe are generally similar to those described from these species assessed in similar years, locations, and seasons (Dietrich et al. 2006, Gamble et al. 2011b, Bunnell et al. 2015). A complete comparison of the diet represented by these 17 sampling combinations relative to other spatio- and temporally- congruent assessments is beyond the scope of this paper, the focus of which is the relationship of diet to thiaminase activity. Some brief notable comparisons to previous findings include the lack of seasonal variability and diversity in Deepwater Sculpin diets as noted by others sampling diets across seasons at Sturgeon Bay (Bunnell et al. 2015). Our samples contained a higher proportion of *Diporeia* than that reported from samples collected in 2010 (Bunnell et al. 2015), and *Diporeia* may have been more prevalent in 2007 than they were in 2010 given the dramatic and ongoing decline in their abundance (Nalepa et al. 2007, Nalepa et al. 2009). Ninespine stickleback diets are rarely described in the Great Lakes, with this being only the third report (Gamble et al. 2011b, Bunnell et al. 2015). These two reports vary in the degree to which *Diporeia* is consumed, with Bunnell et al. (2015) observing no *Diporeia* consumption and Gamble et al. (2011) observing dominance of *Diporeia* in the spring. Our data suggest that Ninespine Stickleback diets may vary substantially across time and/or space, with *Diporeia* constituting a relatively small portion of the diet offshore at Sturgeon Bay in the spring and nearshore in the summer, with no *Diporeia* consumption observed at Frankfort. Chironomoids and *Eurycercus* were in summer diets, a finding that was observed by Bunnell et al. (2015) but not Gamble et al. (2011). One notable difference between the diets we observed and those reported by others was the large proportion of isopods in Slimy Sculpin from nearshore summer samples at Sturgeon Bay. This site is well-represented regarding diet surveys (Davis et al. 2007, Mychek-Londer et al. 2013, Bunnell et al. 2015); isopods were not reported in any prior reports and our data suggest that they can be a dominant component of the diet.

Since the first reports that zooplankton contain thiaminase activity (Fitzsimons et al. 2004b, Zajicek et al. 2005), zooplankton have been suggested as a potential source of thiaminase activity in planktivorous Rainbow Smelt and Alewife (Fitzsimons et al. 2004a, Fitzsimons et al. 2004b, Zajicek et al. 2005). This seemingly intuitive suggestion is appealing in part because predatory fish are known to develop

thiamine deficiency from consuming prey fishes with high thiaminase-activity (Honeyfield et al. 2005b). We uncovered no compelling, large-scale relationships between diet of fishes and their thiaminase activity. Both the NMS and some ISA analyses did suggest two potential taxa that merit further investigation, *Bythotrephes* and *Bosmina*.

If *Bythotrephes* was the primary source of thiaminase, such a result would not account for any of the thiaminase activity in Port Weller Rainbow Smelt, which did not consume *Bythotrephes*. Additionally, the proportional contribution of *Bythotrephes* to the diet of low-thiaminase Bloater and Cisco from Ashland was as large as or larger than that of Round Goby and Alewife, both of which had visceral thiaminase activity in excess of 11,300 pmol/g/min. Slimy Sculpin had high thiaminase levels at Frankfort and relatively large consumption of *Bythothrephes*, but concomitantly sampled *Bythothrephes* had undetectable levels of thiaminase. Presumably, then, *Bythotrephes* cannot contribute to thiaminase activity of these Slimy Sculpin. Lastly, *Bythotrephes* was not identified as a potential source of thiaminase activity in bulk plankton tows (Chapter 1), which would be expected if it were a consistent and compelling source of thiaminase in aquatic food webs.

Although the rank correlation between the proportion of *Bosmina* in the wet biomass of diets and thiaminase activity was suggestive, *Bosmina* constitutes such a small proportion of the total wet mass of the diet that its thiaminase activity would have to be seemingly unrealistically high to account for the thiaminase activity in the highest-thiaminase samples. Likewise, although *Ploesoma* was the only candidate zooplankton species whose proportional abundance was correlated with thiaminase activity in bulk plankton tows (Chapter 2), *Ploesoma* was not recorded in any fish stomach that we examined. Our diet analysis assumes that stomach content analysis represents recent consumption, and therefore we cannot rule out the possibility that *Ploesoma* or *Bythotrephes* were consumed in the recent past by thiaminase-containing fishes that did not contain these taxa upon capture. In the case of *Bythotrephes*, spines are often retained in stomachs and would be expected to provide an indication of recent consumption, but no similarly retained structures exist for *Ploesoma*.

Our finding of no compelling evidence for one single (or several correlated) prey taxa that are (a) consistently abundant in the diets of fish with high levels of thiaminase and (b) consistently absent or in low abundance in the diets of fish with low levels of thiaminase suggests that either (1) some prey taxa are sources of thiaminase at some seasons locations and depths but not others (i.e., the production of thiaminase by zooplankton and macroinvertebrates is highly variable and no individual taxa is uniformly the source for dietarily acquired thiaminase), (2) the source of thiaminase in prey fishes is not dietary, or (3) some combination of both. This is consistent with our finding that thiaminase activity in bulk plankton tows cannot be attributed to any obvious source (Chapter 1).

3.5.3 Relationship between thiaminase activity and fatty acids

Fatty acids are potentially useful food chain biomarkers. Their ability to provide species-level details such as those provided by stomach content analysis is limited in the absence of controlled feeding studies that develop calibration coefficients for use in specialized applications (Iverson et al. 2004, Budge et al. 2006, Budge et al. 2012, Happel et al. 2016). However, fatty acids can still provide a general indication of diet tendency (Czesny et al. 2011, Happel et al. 2015a, Happel et al. 2015b). Fatty acid profiles of individual species of Great Lakes fishes tend to fall along an axis ranging from benthic, as indicated by 16:1n-7 and 18:3n-3, to pelagic, as indicated by 22:6n-3 and 20:4n-6 (Czesny et al. 2011, Happel et al. 2015a). The fatty acids that were associated with the highest thiaminase levels included 20:4n-6, which is considered a marker for a pelagic diet. This association is sensible given that Alewife and Rainbow Smelt are generally pelagic feeders and generally have high thiaminase. In our samples, Bloater and Ciscos were exceptions to the generality that pelagic feeders may be more likely to contain thiaminase activity. The stomach content analysis clearly indicated pelagic feeding by these species (Figure 3.6), and yet they had lower proportions of 20:4n-6 than Alewife (Appendix O), which was reflected in their position in the fatty acid ordination. Likewise, lower levels of thiaminase activity were associated with the benthic marker 18:3n-3, and this marker was generally associated with benthivores such as Deepwater Sculpin. High levels of 16:1n-7 is linked to high chironomid and isopod consumption (Happel et al. 2015b). In our samples, Deepwater Sculpin were highly enriched for 16:1n-7 (Czesny et al. 2011), but the diet data showed no evidence of chironomid or isopod consumption. The two

sampling combinations that showed evidence of chironomids consumption had levels of 16:1n-7 that were higher than average percentage but not as high as Deepwater Sculpin.

We are aware of only one other study that has specifically investigated the relationship between fatty acids and thiaminase activity in Alewife (Honeyfield et al. 2010b). They found that high thiaminase activity was related to *low* levels of saturated fatty acids, whereas in our collections, high thiaminase activity was related to *increased* levels of saturated fatty acids. However, Honeyfield et al.'s (2010) finding that higher levels of 18:1n-9 were associated with lower levels of thiaminase activity, is consistent with our finding that 18:1n-9 was the single fatty acid that was best correlated with low levels of thiaminase activity. One important reason our findings may differ from those of Honeyfield et al. (2010) with respect to saturated fatty acids is because our study is cross-species whereas theirs focused solely on Alewife. For example, we assessed the relationship between thiaminase activity and fatty acids over nearly 4 orders of magnitude of thiaminase activity, whereas Honeyfield et al. (2010) assessed Alewife whose thiaminase activity varied over just one order of magnitude.

Site-to-site variation, as well as seasonal variation in fatty acid profiles exists, but these variations were generally small in comparison to those attributable to species differences (Czesny et al. 2011). Our samples were also taken from a suit of locations, times, and species, and caution is therefore advised when interpreting fatty acid data. Fish species may differ in their ability to synthesize particular fatty acids, and this potential source of variation, in addition to that attributable to season and location, suggest that interpreting fatty acid profiles may be more complex than our sampling design will support. Many of the factors for which it would be desirable to assess for variation (i.e., site versus depth, or site versus season) are confounded in our design. Broadly, in our analysis, fatty acids suggested that planktivory is more likely to be associated with high thiaminase, but until additional controlled studies are conducted, identifying specific taxa in diets will require identification of stomach contents.

One additional potentially useful feature of fatty acids profiles is that fatty acids not only reflect the content of the diet, but they can also provide information about the physiological status of an organism; the fatty acid content of any consumer is a function of both the dietary source of fatty acids and the individual's physiological status. Fatty acid metabolism can affect the physiological status of copepods (Ahlgren et al. 2005) as well as top predators (Ballantyne et al. 2003). This feature makes fatty acids potentially more complex to interpret but also provides insights into organismal metabolism that may not be obtainable through more typical methods, such as stomach content analysis.

In summary, we found that thiaminase activity varied by season and by species, but were unable to fully evaluate the degree to which thiaminase activity varies by depth or across site. Variation in thiaminase according to some or all of these factors exists, and the underlying reasons for that variation remain poorly understood. We found no compelling evidence that the thiaminase activity, as measured in fish viscera, is consistently the result of consumption (or lack thereof) of any specific prey taxa. *Bythotrephes* was suggested as a potential candidate sources of thiaminase, however not all high-thiaminase fishes consumed Bythotrephes and some low-thiaminase fishes did. Stomach content data provided only a snapshot in time, whereas biomarkers such a fatty acids have the potential to provide a time-integrated measure of general diet tendencies along with an indicator of the metabolic status of an organism. Our finding that diets of planktivores was not related to thiaminase activity was consistent with our previous finding that no zooplankton taxa was associated with thiaminase activity in bulk plankton and suggests that zooplankton may not be the source of thiaminase in these food webs.

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3.7 Literature Cited

Ahlgren, G., L. Van Nieuwerburgh, I. Wanstrand, M. Pedersen, M. Boberg, and P. Snoeijs. 2005. Imbalance of fatty acids in the base of the Baltic Sea food web - a mesocosm study. Canadian Journal of Fisheries and Aquatic Sciences. 62:2240-2253.

Andraso, G. M., M. T. Ganger, and J. Adamczyk. 2011. Size-selective predation by round gobies (*Neogobius melanostomus*) on dreissenid mussels in the field. Journal of Great Lakes Research. 37:298-304.

Arsan, O. M. and A. Y. Malyarevskaya. 1969. The effect of food on thiaminase activity and the content of thiamine in the liver and intestine of silver carp. Hydrobiological Journal. 5:79-81.

Balcer, M. D., N. L. Korda, and S. I. Dodson. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press, Madison, WI.

Ballantyne, A. P., M. T. Brett, and D. E. Schindler. 2003. The importance of dietary phosphorus and highly unsaturated fatty acids for sockeye (*Oncorhynchus nerka*) growth in Lake Washington - a bioenergetics approach. Canadian Journal of Fisheries and Aquatic Sciences. 60:12-22.

Bronte, C. R., J. Jonas, M. E. Holey, R. L. Eshenroder, M. L. Toneys, P. McKee, B. Breidert, R. M. Claramunt, M. P. Ebener, C. C. Krueger, G. M. Wright, and R. Hess. 2003b. Possible impediments to lake trout restoration in Lake Michigan. Lake Trout Task Group report to the Lake Michigan Committee. Great Lakes Fishery Commission. Ann Arbor, MI.

Bronte, C. R., P. C. McKee, and M. E. Holey. 2006. Relative survival of lake trout stocked at different sizes and quality in Lake Michigan. Journal of Great Lakes Research. 32:386-394.

Bronte, C. R., M. E. Holey, C. P. Madenjian, J. L. Jonas, R. M. Claramunt, P. C. McKee, M. L. Toneys, M. P. Ebener, B. Breidert, G. W. Fleischer, R. Hess, A. W. Martell, and E. J. Olsen. 2007. Relative abundance, site fidelity, and survival of adult lake trout in Lake Michigan from 1999 to 2001: Implications for future restoration strategies. North American Journal of Fisheries Management. 27:137-155.

Bronte, C. R., C. C. Krueger, M. E. Holey, M. L. Toneys, R. L. Eshenroder, and J. L. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fishery Commission Miscellaneous Publication 2008-01. 39 pages. Available at http://www.glfc.org/pubs/pub.htm#misc.

Brown, S. B., J. D. Fitzsimons, V. P. Palace, and L. Vandenbyllaardt. 1998a. Thiamine and early mortality syndrome in lake trout. Pages 18-25 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., D. C. Honeyfield, and L. Vandenbyllaardt. 1998b. Thiamine analysis in fish tissues. Pages 73-81 *in* G. McDonald, J. D. Fitzsimons, and D. C.

Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., M. T. Arts, L. R. Brown, M. Brown, K. Moore, M. Villella, J. D. Fitzsimons, D. C. Honeyfield, D. E. Tillitt, J. L. Zajicek, M. Wolgamood, and J. G. Hnath. 2005a. Can diet-dependent factors help explain fish-to-fish variation in thiamine-dependent early mortality syndrome? Journal of Aquatic Animal Health. 17:36-47.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005c. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005d. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health. 17:59-64.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Marine Mammal Science. 22:759-801.

Budge, SuzanneÂ M., SarahÂ N. Penney, and SantoshÂ P. Lall. 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. Canadian Journal of Fisheries and Aquatic Sciences. 69:1033-1046.

Bunnell, D. B., B. M. Davis, M. A. Chriscinske, K. M. Keeler, and J. G. Mychek-Londer. 2015. Diet shifts by planktivorous and benthivorous fishes in northern Lake Michigan in response to ecosystem changes. Journal of Great Lakes Research. 41, Supplement 3:161-171.

Claramunt, R. M., J. L. Jonas, J. D. Fitzsimons, and J. E. Marsden. 2005. Influences of spawning habitat characteristics and interstitial predators on lake trout egg deposition and mortality. Transactions of the American Fisheries Society. 134:1048-1057.

Coble, D. W. 1965. Effect of raw smelt on lake trout. Canadian Fish Culturist. 36:27-34.

Colborne, S. F., S. A. Rush, G. Paterson, T. B. Johnson, B. F. Lantry, and A. T. Fisk. 2016. Estimates of lake trout (*Salvelinus namaycush*) diet in Lake Ontario using two and three isotope mixing models. Journal of Great Lakes Research. 42:695-702.

Czesny, S. and K. Dabrowski. 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). Aquatic Living Resources. 11:371-378.

Czesny, S., J. M. Dettmers, J. Rinchard, and K. Dabrowski. 2009. Linking egg thiamine and fatty acid concentrations of Lake Michigan lake trout with early life stage mortality. Journal of Aquatic Animal Health. 21:262-271.

Czesny, S. J., J. Rinchard, S. D. Hanson, J. M. Dettmers, and K. Dabrowski. 2011. Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. Canadian Journal of Fisheries and Aquatic Sciences. 68:1211-1230.

Davis, B. M., J. F. Savino, and L. M. Ogilvie. 2007. Diet niches of major forage fish in Lake Michigan. Pages 261-275 *in* M. Jankun, P. Brzuzan, P. Hliwa, and M. Luczynski, editors. Biology and Management of Coregonid Fishes - 2005.

DesJardine, R. L., T. K. Gorenflo, R. N. Payne, and J. D. Schrouder. 1995. Fish-community objectives for Lake Huron. Great Lakes Fishery Commission Special Publication. 95-1:1-37.

Deutsch, H. F. and A. D. Hasler. 1943. Distribution of a vitamin B1 destructive enzyme in fish. Proceedings of the Society for Experimental Biology and Medicine. 53:63-65.

Dietrich, J. P., B. J. Fvlorrison, and J. A. Hoyle. 2006. Alternative ecological pathways in the Eastern Lake Ontario food web-round goby in the diet of lake trout. Journal of Great Lakes Research. 32:395-400.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs. 67:345-366.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark, Jr. 1995. Fish-community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 1995-3:1-56.

Eshenroder, R. L., J. W. Peck, and C. H. Olver. 1999. Research priorities for lake trout rehabilitation in the Great Lakes: A 15-year retrospective. Great Lakes Fishery Commission Technical Report:1-40.

Fisher, J. P., J. M. Spitsbergen, H. Symula, J. Skea, M. Babenzein, and T. Chiotti. 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the "Cayuga syndrome". Journal of Aquatic Animal Health. 7:81-94.

Fisher, J. P., S. B. Brown, S. Connelly, T. Chiotti, and C. C. Krueger. 1998a. Interspecies comparisons of blood thiamine in salmonids from the Finger Lakes, and effect of maternal size on blood and egg thiamine in Atlantic salmon with and without Cayuga Syndrome. Pages 112-123 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fisher, J. P., S. B. Brown, G. W. Wooster, and P. R. Bowser. 1998b. Maternal blood, egg and larval thiamin levels correlate with larval survival in landlocked Atlantic salmon (*Salmo salar*). Journal of Nutrition. 128:2456-2466.

Fitzsimons, J. 2008. Egg thaimine data from Port Weller, ON. Personal communication (e-mail) to S. C. Riley.

Fitzsimons, J. D. and S. B. Brown. 1998. Reduced egg thiamine levels in inland and Great Lakes trout and their relationship with diet. Pages 160-171 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. B. Brown, and L. Vandenbyllaardt. 1998. Thiamine levels in food chains of the Great Lakes. Pages 90-98 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, R. Barrows, C. E. Kraft, J. Lepak, D. C. Honeyfield, and D. Tillitt. 2004a. Factors influencing alewife thiaminolytic activity. Appendix 3 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 33 pages.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, J. Zajicek, D. Tillitt, and D. C. Honeyfield. 2004b. Thiaminase and thiamine in lower trophic level organisms from the Great Lakes basin. Appendix 4 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of

low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 13 pages.

Fitzsimons, J. D., B. Williston, J. L. Zajicek, D. E. Tillitt, S. B. Brown, L. R. Brown, D. C. Honeyfield, D. M. Warner, L. G. Rudstam, and W. Pearsall. 2005b. Thiamine content and thiaminase activity of ten freshwater stocks and one marine stock of alewives. Journal of Aquatic Animal Health. 17:26-35.

Fitzsimons, J. D., B. Williston, G. Williston, L. Brown, A. El-Shaarawi, L. Vandenbyllaardt, D. Honeyfeld, D. Tillitt, M. Wolgamood, and S. B. Brown. 2007. Egg thiamine status of Lake Ontario salmonines 1995-2004 with emphasis on lake trout. Journal of Great Lakes Research. 33:93-103.

Fitzsimons, J. D., S. B. Brown, B. Williston, G. Williston, L. R. Brown, K. Moore, D. C. Honeyfield, and D. E. Tillitt. 2009a. Influence of thiamine deficiency on lake trout larval growth, foraging, and predator avoidance. Journal of Aquatic Animal Health. 21:302-314.

Fitzsimons, J. D., M. Clark, and M. Keir. 2009b. Addition of round gobies to the prey community of Lake Ontario and potential implications to thiamine status and reproductive success of Lake Trout. Aquatic Ecosystem Health & Management. 12:296-312.

Flath, L. E. and J. S. Diana. 1985. Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Transactions of the American Fisheries Society. 114:328–337.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226:497-509.

French, J. R. P. and D. J. Jude. 2001. Diets and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. Journal of Great Lakes Research. 27:300-311.

Fujita, A. 1954. Thiaminase. Advances in Enzymology and Related Subjects of Biochemistry. 15:389-421.

Fynn-Aikins, K., P. R. Bowser, D. C. Honeyfield, J. D. Fitzsimons, and H. G. Ketola. 1998. Effect of dietary amprolium on tissue thiamin and Cayuga syndrome in Atlantic salmon. Transactions of the American Fisheries Society. 127:747-757.

Gamble, A. E., T. R. Hrabik, D. L. Yule, and J. D. Stockwell. 2011b. Trophic connections in Lake Superior Part II: The nearshore fish community. Journal of Great Lakes Research. 37:550-560.

Games, P. A. and J. F. Howell. 1976. Pairwise Multiple Comparison Procedures with Unequal N's and/or Variances: A Monte Carlo Study. Journal of Educational and Behavioral Statistics. 1:113-125.

Garton, D. W. and D. J. Berg. 1990. Occurrence of *Bythotrephes cederstroemi* (Schoedler 1877) in Lake Superior, with Evidence of Demographic Variation within the Great Lakes. Journal of Great Lakes Research. 16:148-152.

GLNPO. 2003. Standard operating procedures for zooplankton analysis. U. S. Environmental Protection Agency, Great Lakes National Program Office, Report LG403. 16 pages.

Greig, R. A. and R. H. Gnaedinger. 1971. Occurrence of thiaminase in some common aquatic animals of the United States and Canada. Special Scientific Report - Fisheries. United States Department of Commerce. Seattle, Washington, 7 pages.

Grigor'yeva, L. V., A. Kirpenko, V. M. Orlovskij, and V. V. Stankewich. 1977. On the antimicrobial action of toxic metabloites of some blue-green algae. Gidrobiologicheskii Zhurnal. 13:47-52.

Grossnickle, N. E. and A. M. Beeton. 1979. Antennal scale length as a measure of relative size in the opossum shrimp, *Mysis relicta* Loven. Crustaceana. 36:141-146.

Hansen, M. J. 1999. Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. Pages 417-453 *in* W. W. Taylor and C. P. Ferreri, editors. Great Lakes fisheries policy and management: a binational perspective.

Happel, A., S. Creque, J. Rinchard, T. Höök, H. Bootsma, J. Janssen, D. Jude, and S. Czesny. 2015a. Exploring yellow perch diets in Lake Michigan through stomach content, fatty acids, and stable isotope ratios. Journal of Great Lakes Research. 41, Supplement 3:172-178.

Happel, A., J. Lafountain, S. Creque, J. Rinchard, T. Höök, H. Bootsma, J. Janssen, D. Jude, and S. Czesny. 2015b. Spatio-temporal description of spottail shiner (*Notropis hudsonius*) fatty acid profiles in Lake Michigan's southern basin. Journal of Great Lakes Research. 41, Supplement 3:179-184.

Happel, A., L. Stratton, R. Pattridge, J. Rinchard, and S. Czesny. 2016. Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. Freshwater Biology.

Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown, J. E. Marsden, D. S. Lavis, M. L. Toneys, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. Journal of Great Lakes Research. 21:128-151.

Honeyfield, D. C., J. D. Fitzsimons, S. B. Brown, S. V. Marcquenski, and G. McDonald. 1998a. Introduction and overview of early life stage mortality. Pages 1-7 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., K. Fynn-Aikins, J. D. Fitzsimons, and J. A. Mota. 1998b. Effect of dietary amprolium on egg and tissue thiamine concentrations in lake trout. Pages 172-177 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., J. P. Hinterkopf, and S. B. Brown. 2002. Isolation of thiaminase-positive bacteria from alewife. Transactions of the American Fisheries Society. 131:171-175.

Honeyfield, D. C., D. Beltman, M. Holey, and C. Edsall. 2005a. Investigation of thiamine and PCB association with early life stage fry mortality in lake trout from northwestern Lake Michigan in 1996-1998. Pages 182-194 *in* Health and diseases of aquatic organisms: bilateral perspectives. Proceedings of the second bilateral conference between Russian and the United States. 21-28 September 2003. Shepherdstown, West Virginia. Michigan State University, East Lansing, Michigan.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. W. Hanes, L. Brown, C. E. Kraft, and T. P. Begley. 2010a. Comparison of thiaminase activity in fish using the radiometric and 4-

nitrothiophenol colorimetric methods. Journal of Great Lakes Research. 36:641-645.

Honeyfield, D. C., D. E. Tillitt, J. D. Fitzsimons, and S. B. Brown. 2010b. Variation in Lake Michigan alewife (*Alosa pseudoharengus*) thiaminase and fatty acids composition. Journal of Freshwater Ecology. 25:65-71.

Honeyfield, D. C., M. E. Daniels, L. R. Brown, M. T. Arts, M. G. Walsh, and S. B. Brown. 2012. Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species. Journal of Great Lakes Research. 38:11-17.

Horns, W. H., C. R. Bronte, T. R. Busiahn, M. P. Ebener, R. L. Eshenroder, T. Gorenflo, N. Kmiecik, W. Mattes, J. W. Peck, M. Petzold, and D. R. Schreiner. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication. 03-01:i-ii, 1-78.

Houde, A. L. S., P. J. Saez, C. C. Wilson, D. P. Bureau, and B. D. Neff. 2015. Effects of feeding high dietary thiaminase to sub-adult Atlantic salmon from three populations. Journal of Great Lakes Research. 41:898-906.

Hudson, P. L. and L. T. Lesko. Free-living and parasitic copepods (including Branchiuraans) of the Laurentian Great Lakes: keys and details on individual species. Available at http://www.glsc.usgs.gov/greatlakescopepods/.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs. 74:211-235.

Jacobs, G. R., C. P. Madenjian, D. B. Bunnell, and J. D. Holuszko. 2010. Diet of lake trout and burbot in Northern Lake Michigan during spring: Evidence of ecological interaction. Journal of Great Lakes Research. 36:312-317.

Jaroszewska, M., B. J. Lee, K. Dabrowski, S. Czesny, J. Rinchard, P. Trzeciak, and B. Wilczynska. 2009. Effects of vitamin B-1 (thiamine) deficiency in lake trout (*Salvelinus namaycush*) alevins at hatching stage. Comparative Biochemistry And Physiology A-Molecular & Integrative Physiology. 154:255-262.

Ji, Y. Q. and I. R. Adelman. 1998. Thiaminase activity in alewives and smelt in lakes Huron, Michigan, and Superior. Pages 154-159 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Ji, Y. Q., J. J. Warthesen, and I. R. Adelman. 1998. Thiamine nutrition, synthesis, and retention in relation to lake trout reproduction in the Great Lakes. Pages 99-111 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Jonas, J. L., R. M. Claramunt, J. D. Fitzsimons, J. E. Marsden, and B. J. Ellrott. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 62:2254-2264.

Jones, M. L., G. W. Eck, D. O. Evans, M. C. Fabrizio, M. H. Hoff, P. L. Hudson, J. Janssen, D. Jude, R. Ogorman, and J. F. Savino. 1995. Limitations to lake trout (*Salvelinus namaycush*) rehabilitation in the Great Lakes imposed by biotic interactions occurring at early life stages. Journal of Great Lakes Research. 21:505-517.

Kraft, C. E., E. R. L. Gordon, and E. R. Angert. 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLOS ONE. 9:e92688.

Krueger, C. C., M. L. Jones, and W. W. Taylor. 1995a. Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. Journal of Great Lakes Research. 21:547-558.

Krueger, C. C., D. L. Perkins, E. L. Mills, and J. E. Marsden. 1995b. Predation by alewives on lake trout fry in Lake Ontario: Role of an exotic species in preventing restoration of a native species. Journal of Great Lakes Research. 21:458-469.

Kruskal, J. B. 1964a. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika. 29:1-27.

Kruskal, J. B. 1964b. Nonmetric multidimensional scaling: a numerical method. Psychometrika. 29:115-129.

Legendre, P. and M. J. Anderson. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs. 69:1-24.

Lepak, J. M., C. E. Kraft, D. C. Honeyfield, and S. B. Brown. 2008. Evaluating the effect of stressors on thiaminase activity in alewife. Journal of Aquatic Animal Health. 20:63-71.

Lepak, J. M., C. E. Kraft, and M. J. Vanni. 2013. Clupeid response to stressors: The influence of environmental factors on thiaminase expression. Journal of Aquatic Animal Health. 25:90-97.

Madenjian, C. P., J. D. Holuszko, and T. J. Desorcie. 2006a. Spring-summer diet of lake trout on Six Fathom Bank and Yankee Reef in Lake Huron. Journal of Great Lakes Research. 32:200-208.

Madenjian, C. P., S. A. Pothoven, J. M. Dettmers, and J. D. Holuszko. 2006b. Changes in seasonal energy dynamics of alewife (*Alosa pseudoharengus*) in Lake Michigan after invasion of dreissenid mussels. Canadian Journal of Fisheries and Aquatic Sciences. 63:891-902.

Madenjian, C. P., R. O'Gorman, D. B. Bunnell, R. L. Argyle, E. F. Roseman, D. M. Warner, J. D. Stockwell, and M. A. Stapanian. 2008. Adverse effects of alewives on Laurentian Great Lakes fish communities. North American Journal of Fisheries Management. 28:263-282.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. J. Wiley and Sons, New York.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology. 82:290-297.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR.

McCune, B. and M. J. Mefford. 2015. PC-ORD. Multivariate Analysis of Ecological Data. Version 7.359 beta. MjM Software Design, Gleneden Beach, OR, USA

Metcalfe, L. D. and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Analytical Chemistry. 33:363-364.

Morbey, Y. E., D. M. Anderson, and B. A. Henderson. 2008. Progress toward the rehabilitation of lake trout (*Salvelinus namaycush*) in South Bay, Lake Huron. Journal of Great Lakes Research. 34:287-300.

Muir, A. M., C. C. Krueger, and M. J. Hansen. 2012. Re-establishing lake trout in the Laurentian Great Lakes: Past, present, and future. Pages 533-588 *in* W. W. Taylor, A. J. Lynch, and N. J. Leonard, editors. Great Lakes fisheries policy and management: A binational perspective. 2nd edition. MSU Press, Lansing.

Mychek-Londer, J. G., D. B. Bunnell, W. Stott, J. S. Diana, J. R. P. French, and M. A. Chriscinske. 2013. Using diets to reveal overlap and egg predation among

benthivorous fishes in Lake Michigan. Transactions of the American Fisheries Society. 142:492-504.

Nalepa, T. F., D. L. Fanslow, S. A. Pothoven, A. J. Foley, and G. A. Lang. 2007. Long-term trends in benthic macroinvertebrate populations in Lake Huron over the past four decades. Journal of Great Lakes Research. 33:421-436.

Nalepa, T. F., D. L. Fanslow, and G. A. Lang. 2009. Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. Freshwater Biology. 54:466-479.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States: protozoa to mollusca. 3rd edition. Wiley, New York.

Ray, W. J. and L. D. Corkum. 1997. Predation of zebra mussels by round gobies, *Neogobius melanostomus*. Environmental Biology of Fishes. 50:267-273.

Riley, S. C., J. Rinchard, D. C. Honeyfield, A. N. Evans, and L. Begnoche. 2011. Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low alewife abundance. North American Journal of Fisheries Management. 31:1052-1064.

Roseman, E. F., J. S. Schaeffer, E. Bright, and D. G. Fielder. 2014. Angler-caught piscivore diets reflect fish community changes in Lake Huron. Transactions of the American Fisheries Society. 143:1419-1433.

Rush, S. A., G. Paterson, T. B. Johnson, K. G. Drouillard, G. D. Haffner, C. E. Hebert, M. T. Arts, D. J. McGoldrick, S. M. Backus, B. F. Lantry, J. R. Lantry, T. E. D. Schaner, and A. T. Fisk. 2012. Long-term impacts of invasive species on a native top predator in a large lake system. Freshwater Biology. 57:2342-2355.

Ryan, P. A., R. Knight, R. MacGregor, G. Towns, R. Hoopes, and W. Culligan. 2003. Fish-community goals and objectives for Lake Erie. Great Lakes Fishery Commission Special Publication. 03-02:1-56.

Sealock, R. R., A. H. Livermore, and C. A. Evans. 1943. Thiamine inactivation by the fresh-fish or Chastek-paralysis factor. Journal of the American Chemical Society. 65:935-940.

Stemberger, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency. Rept. No. EPA 600/4-79-021, 185 pages.

Stewart, D. J. and F. P. Binkowski. 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: An energetics-modeling synthesis. Transactions of the American Fisheries Society. 115:643-661.

Stewart, T. J., R. E. Lange, S. D. Orsatti, C. P. Schneider, A. Mathers, and M. E. Daniels. 1999. Fish-community objectives for Lake Ontario. Great Lakes Fishery Commission Special Publication. 99-1:1-55.

Strakosh, T. R. and C. C. Krueger. 2005. Behavior of post-emergent lake trout fry in the presence of the alewife, a non-native predator. Journal of Great Lakes Research. 31:296-305.

Tillitt, D. E., J. L. Zajicek, S. B. Brown, L. R. Brown, J. D. Fitzsimons, D. C. Honeyfield, M. E. Holey, and G. M. Wright. 2005. Thiamine and thiaminase status in forage fish of salmonines from Lake Michigan. Journal of Aquatic Animal Health. 17:13-25.

Walsh, M. G., D. E. Dittman, and R. O'Gorman. 2007. Occurrence and food habits of the round goby in the profundal zone of southwestern Lake Ontario. Journal of Great Lakes Research. 33:83-92.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

Zajicek, J. L., L. Brown, S. B. Brown, D. C. Honeyfield, J. D. Fitzsimons, and D. E. Tillitt. 2009. Variations of thiaminase I activity pH dependencies among typical Great Lakes forage fish and *Paenibacillus thiaminolyticus*. Journal of Aquatic Animal Health. 21:207-216.

3.8 Figures

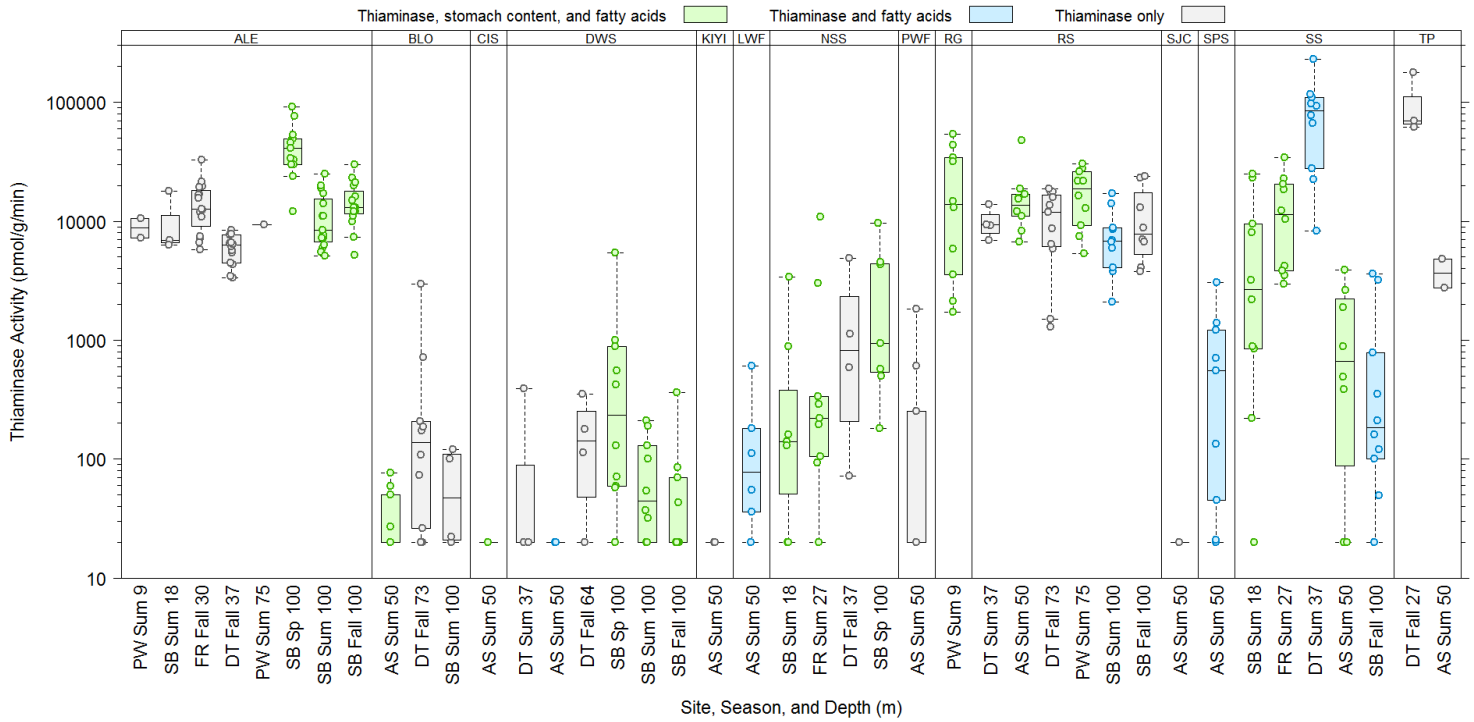

Figure 3.1 Thiaminase activity in fish viscera paneled by species

Thiaminase activity (pmol/g/min) in fish viscera from the 41 unique combinations of site, season, and depth (m) for each species. Site: AS=Ashland, DT=Detour, FR=Frankfort, PW=Port Weller, SB=Sturgeon Bay. Season: Sp=Spring, Sum=Summer, Fall=Fall. Species abbreviations are as is Table 3.1. Ashland tows were cross-contour (35-70 m) and were assigned 50 m depth for graphing purposes. Within each panel, sampling events are arranged by depth. Box and point colors characterize the types of analysis for which samples were collected from each sampling event.

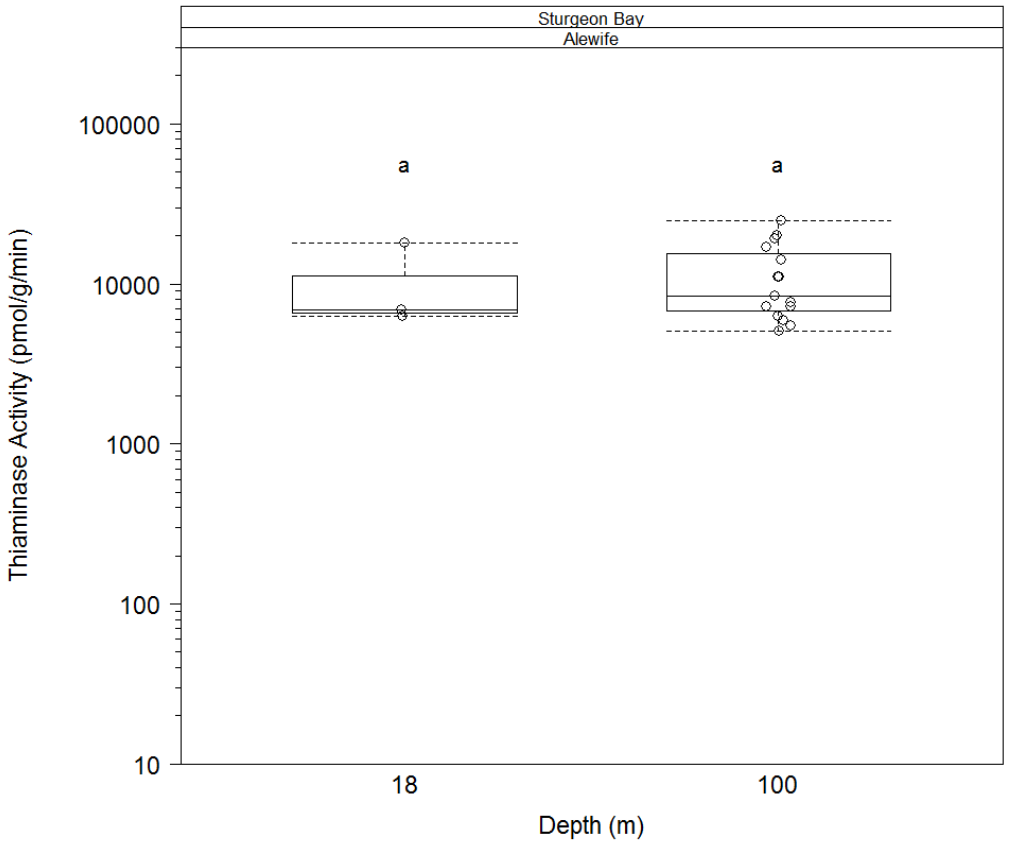

Figure 3.2 Effect of depth on thiaminase activity in Alewife viscera

Thiaminase activity in Alewife at two depths (Sturgeon Bay; summer). Sampling combinations with different letters are significantly different.

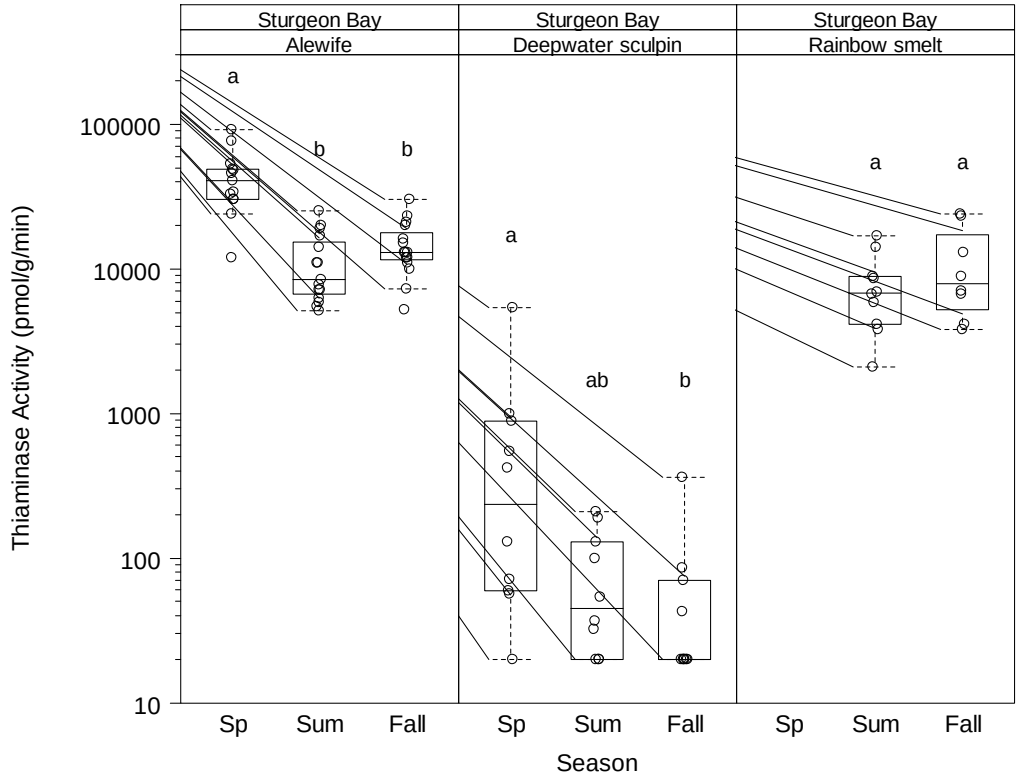

Figure 3.3 Effect of season on thiaminase activity in viscera of three fish species

Thiaminase activity in different seasons in three different species (Alewife, Deepwater Sculpin, and Rainbow Smelt) at Sturgeon Bay (100 m) in the summer. Sampling combinations within each panel with different letters are significantly different.

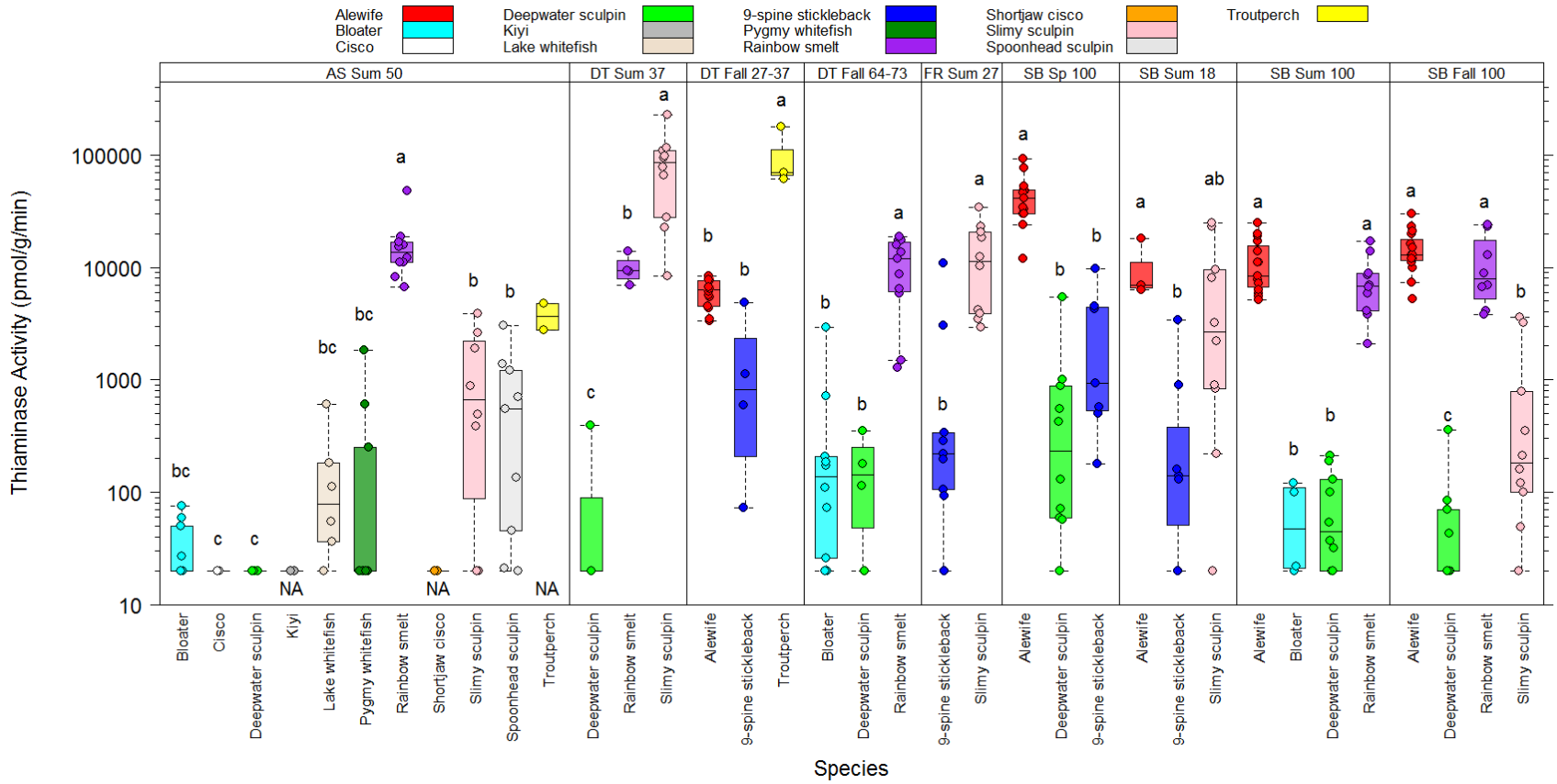

Figure 3.4 Effect of species on thiaminase activity in fish viscera

Thiaminase activity in different species captured at the same site, season, and depth (see panels). Sampling combinations within each panel with different letters are significantly different. Sampling combinations marked "NA" were excluded from the within-panel ANOVAs because n<3. Ashland tows were cross-contour (35-70 m) and were assigned 50 m depth for graphing purposes.

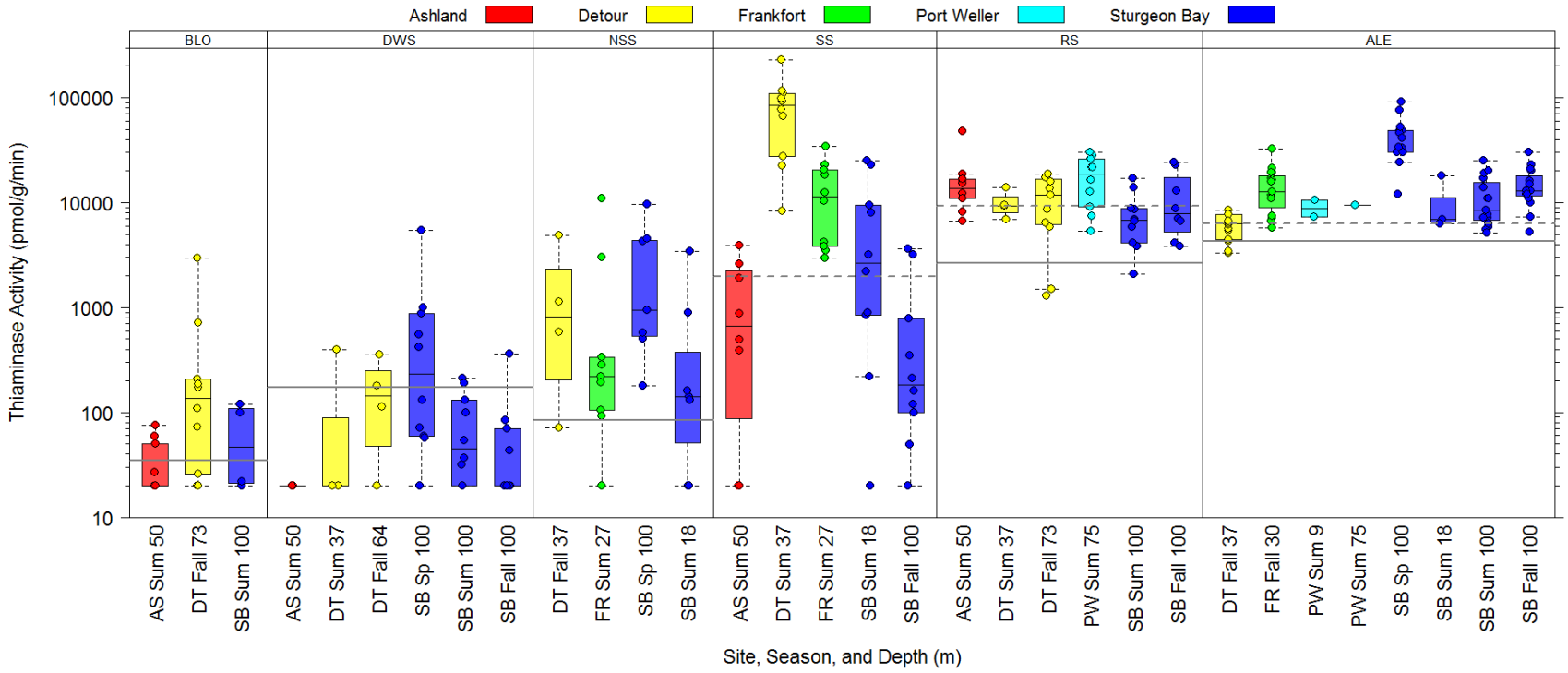

Figure 3.5 Effect of site on thiaminase activity in fish viscera

Thiaminase activity in different species color-coded by site at potentially differing seasons and depths (see X-axis for site, season, and depth combinations). Ashland tows were cross-contour (35-70 m) and were assigned 50 m depth for graphing purposes. Solid grey lines are mean thiaminase activity from summer samples collected at 11 sites in Lake Michigan from 1998-2000 (Tillitt et al. 2005) and dotted grey lines are the average thiaminase activity from fall samples collected from 4 regions of Lake Ontario in 2006 and 2007 (Honeyfield et al. 2012). Species abbreviations are as is Table 3.1.

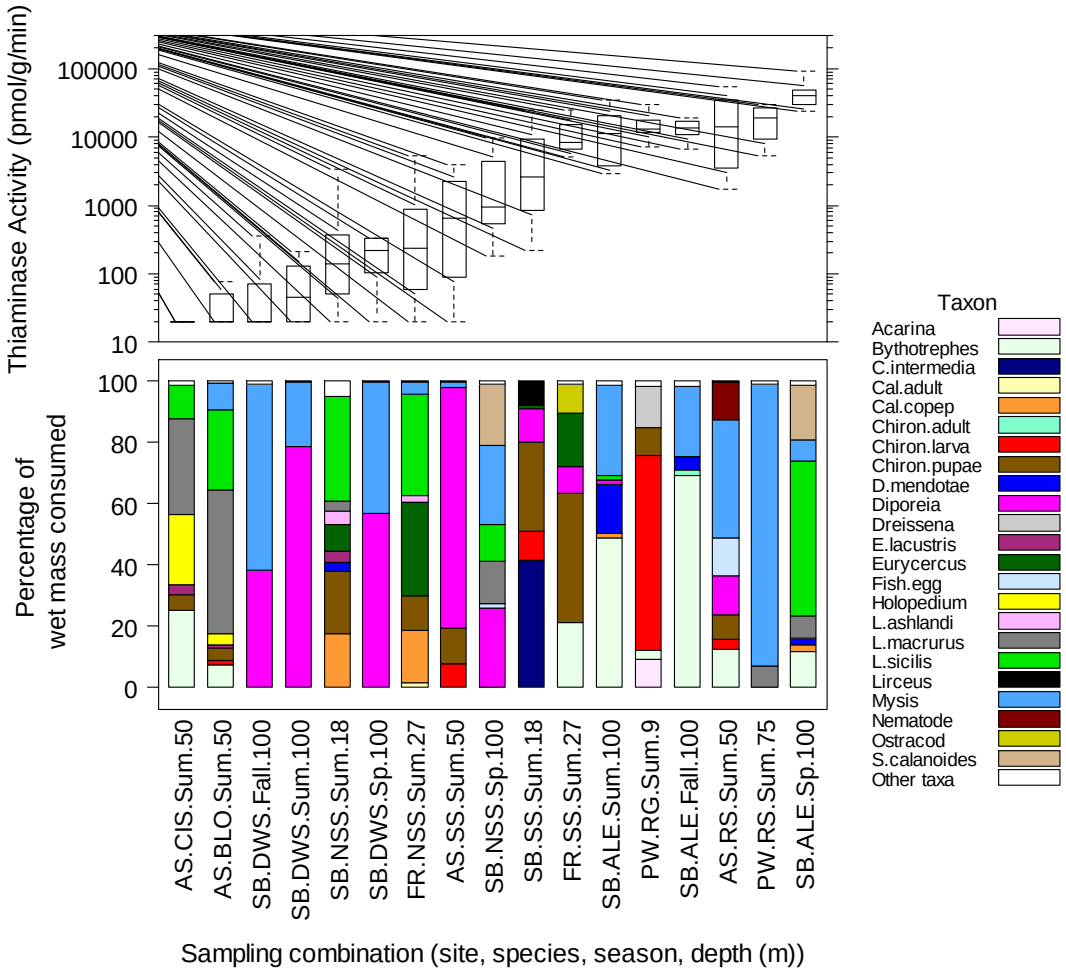

Figure 3.6 Average percentage of the wet biomass of prey taxa consumed and thiaminase activity for 17 sampling combinations

Average percentage of the wet biomass of each prey taxa consumed (bottom panel) and thiaminase activity (pmol/g/min; top panel) for 17 sampling combinations. All taxa that individually constitute less than one percent of the average wet biomass per sampling event are grouped together as “other taxa”.

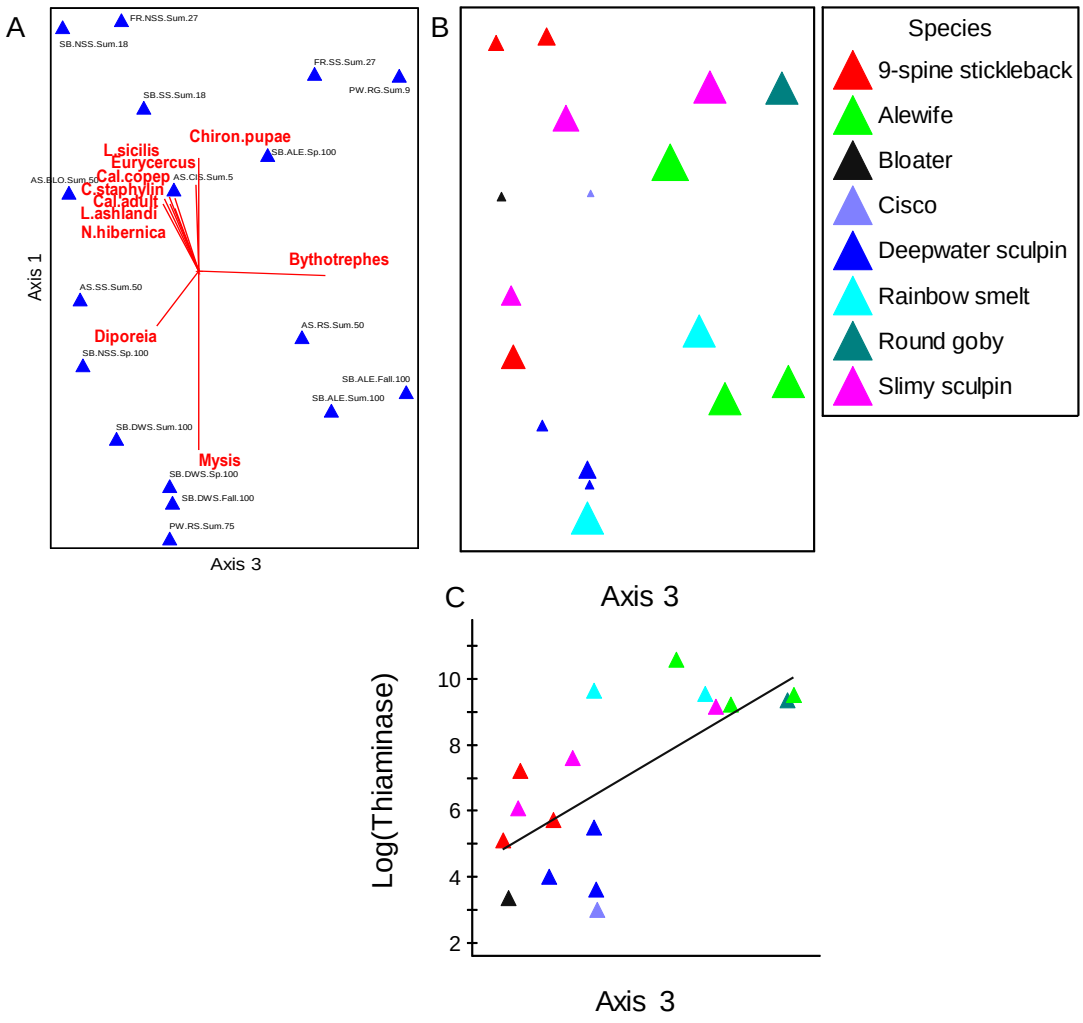

Figure 3.7 NMS ordination of diet and the relationship with thiaminase

NMS ordination of 17 sampling combinations based on 32 taxa represented as proportional biomass. Each point represents one of the 17 sampling combinations, which are labeled in panel A. Sampling combinations located closely to each other have diets more similar that those located farther apart from one another. Diet-space is defined by gradients driven by species shown in panel A. The vectors indicate both the direction and strength (length of vector) of the influence of the species on diet-space. Panel B is the same arrangement of points in diet-space shown in panel A, but the size of the points in panel B is proportional to thiaminase activity. The correlation between the axis 1 score and the log of thiaminase is illustrated in panel C. Axis 2 of this 3-dimensional ordination is not shown, but was unrelated to thiaminase activity (r=0.02).

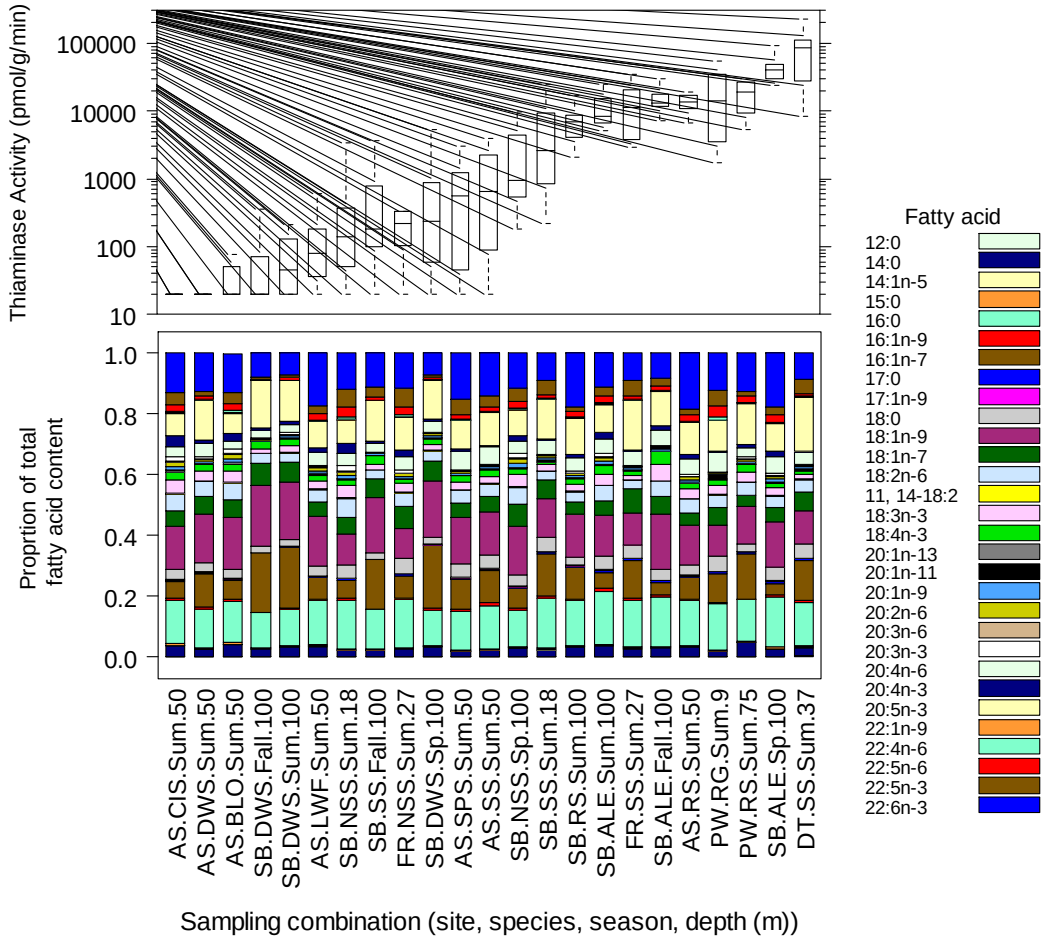

Figure 3.8 Average proportional fatty acid content and thiaminase activity for 23 sampling combinations

Average proportion by mass of the total fatty acid content (bottom panel) and thiaminase activity (pmol/g/min; top panel) for 17 sampling combinations. .

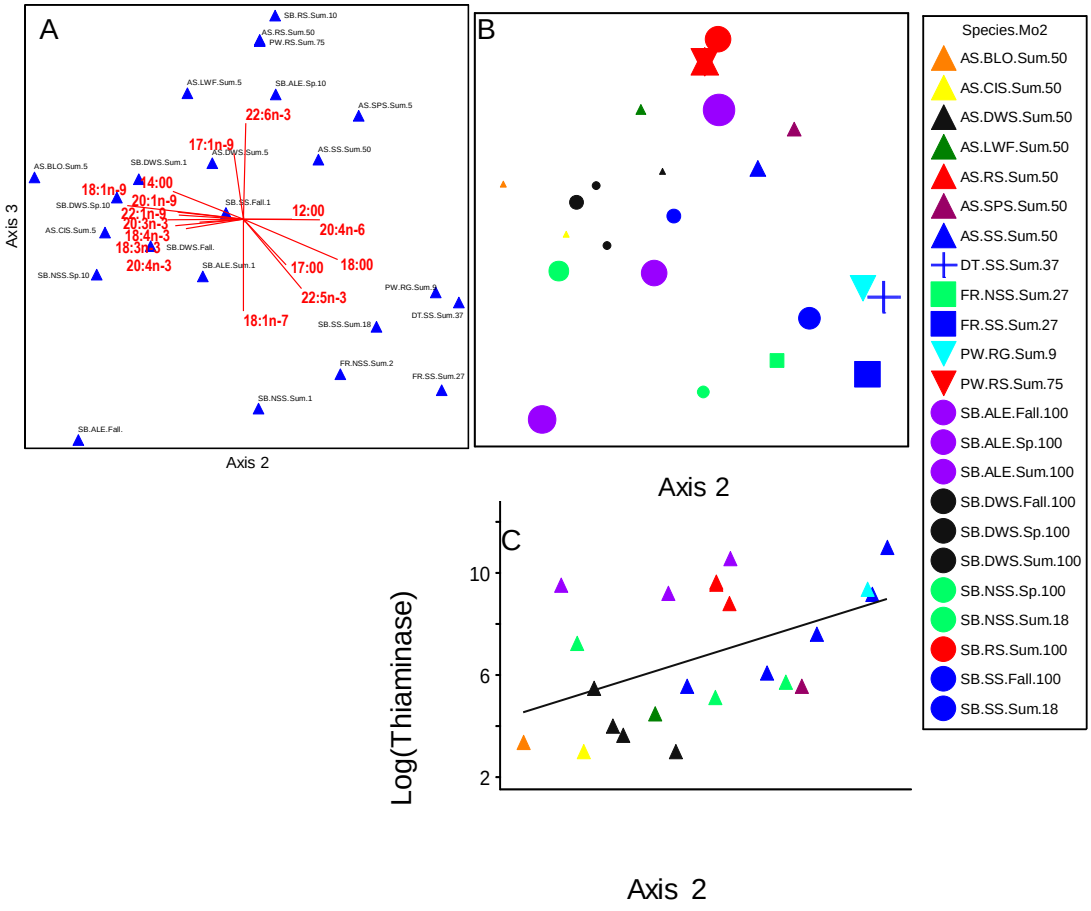

Figure 3.9 NMS ordination of diet and the relationship with thiaminase

NMS ordination of 23 sampling combinations based on 28 fatty acids represented as proportional biomass. Each point represents one of the 23 sampling combinations, which are labeled in panel A and in the legend. Diet-space is defined by gradients driven by species shown in panel A. The vectors indicate both the direction and strength (length of vector) of the influence of the specific fatty acids in the ordination space. Panel B is the same arrangement of points in diet-space shown in panel A, but the size of the points in panel B is proportional to thiaminase activity. The correlation between the axis 2 score and the log of thiaminase is illustrated in panel C. Axis 1 of this 3-dimensional ordination is not shown, but was unrelated to thiaminase activity.

3.9 Tables

Table 3.1 Number of fish collected at each location, depth, and season by species for 3 types of analysis

The number of each species collected at each location, depth, and season for thiaminase, fatty acid, and gut content analysis. Blank cells indicate no samples were collected. *Opportunistic sampling events. **Tows at Ashland were cross-contour, so the exact depth of occupancy upon capture was not known. Species abbreviations are listed on first occurrence.

Site	Species (Abbreviation)	Season	Depth	Thiaminase	Fatty Acids	Gut Content
Ashland	Bloater (BLO)	Summer	Mid (35-70 m)**	10	14	10
Ashland	Cisco (CIS)	Summer	Mid (35-70 m)	4	16	10
Ashland	Deepwater Sculpin (DWS)	Summer	Mid (35-70 m)	5	4	
Ashland	Kiyi (KIYI)	Summer	Mid (35-70 m)	2		
Ashland	Lake Whitefish (LWF)	Summer	Mid (35-70 m)	6	15	
Ashland	Pygmy Whitefish (PWF)	Summer	Mid (35-70 m)	10		
Ashland	Rainbow Smelt (RS)	Summer	Mid (35-70 m)	10	15	8
Ashland	Shortjaw Cisco (SJC)	Summer	Mid (35-70 m)	2		
Ashland	Slimy Sculpin (SS)	Summer	Mid (35-70 m)	8	9	10
Ashland	Spoonhead Sculpin (SPS)	Summer	Mid (35-70 m)	9	5	
Ashland	Trout-perch (TP)	Summer	Mid (35-70 m)	2		
*Detour	Alewife (ALE)	Fall	Mid (37 m)	14		
*Detour	Bloater	Fall	Mid (73 m)	10		
Detour	Deepwater Sculpin	Summer	Mid (37 m)	3		
*Detour	Deepwater Sculpin	Fall	Mid (64 m)	4		
*Detour	Ninespine Stickleback (NSS)	Fall	Mid (37 m)	4		
Detour	Rainbow Smelt	Summer	Mid (37 m)	4		

*Detour	Rainbow Smelt	Fall	Mid (73 m)	11		
Detour	Slimy Sculpin	Summer	Mid (37 m)	10	11	
*Detour	Trout-perch	Fall	Mid (27 m)	3		
*Frankfort	Alewife	Fall	Mid (30 m)	15		
Frankfort	Ninespine Stickleback	Summer	Mid (27 m)	9	13	11
Frankfort	Slimy sculpin	Summer	Mid (27 m)	10	16	10
Port Weller	Alewife	Summer	Near (9 m)	2		
*Port Weller	Alewife	Summer	Off (75 m)	1		
*Port Weller	Rainbow Smelt	Summer	Off (75 m)	10	15	33
Port Weller	Round Goby (RG)	Summer	Near (9m)	10	15	11
Sturgeon Bay	Alewife	Spring	Off (110 m)	13	15	9
Sturgeon Bay	Alewife	Summer	Near (18 m)	3		
Sturgeon Bay	Alewife	Summer	Off (110 m)	15	15	10
Sturgeon Bay	Alewife	Fall	Off (110 m)	15	15	10
Sturgeon Bay	Bloater	Summer	Off (110 m)	4		
Sturgeon Bay	Deepwater Sculpin	Spring	Off (110 m)	10	15	10
Sturgeon Bay	Deepwater Sculpin	Summer	Off (110 m)	10	15	10
Sturgeon Bay	Deepwater Sculpin	Fall	Off (110 m)	10	15	10
Sturgeon Bay	Ninespine Stickleback	Spring	Off (110 m)	7	15	10
Sturgeon Bay	Ninespine Stickleback	Summer	Near (18 m)	7	14	18
Sturgeon Bay	Rainbow Smelt	Summer	Off (110 m)	10	5	
Sturgeon Bay	Rainbow Smelt	Fall	Off (110 m)	8		
Sturgeon Bay	Slimy Sculpin	Summer	Near (18 m)	10	15	10
Sturgeon Bay	Slimy Sculpin	Fall	Off (110 m)	10	2	

Table 3.2 Comparisons used to evaluate the effect of depth, season, and species on thiaminase activity

Comparisons used to evaluate the effect of depth, season, and species on thiaminase activity. Specific sampling units used to make each comparison are listed, along with the model, the test used, the resulting model statistics, and the figure showing the result of the multiple comparison tests among sampling combinations for each model. For a summary of the multiple comparisons, see Table 3.4.

	Sampling combinations	Model	Test	p, F, df	Result of multiple comparison test
Depth					
	ALE SB Sum 18 ALE SB Sum 100	Log(Thiaminase) ~ Depth	ANOVA	p = 0.816 F = 0.0512 df = 1, 16	Figure 3.2
Season					
	ALE SB Sp 100 ALE SB Sum 100 ALE SB Fall 100	Log(Thiaminase) ~ Season	ANOVA	p < 0.00001 F = 28.8 df = 2, 40	Figure 3.3
	DWS SB Sp 100 DWS SB Sum 100 DWS SB Fall 100	Log(Thiaminase) ~ Season	ANOVA with unequal variances	p = 0.03 F = 4.28 df = 2, 17	Figure 3.3
	RS SB Sum 100 RS SB Fall 100	Log(Thiaminase) ~ Season	ANOVA	p = 0.32 F = 1.02 df =1, 16	Figure 3.3
Species					
	BLO AS Sum 50 CIS AS Sum 50 DWS AS Sum 50 LWF AS Sum 50 PWF AS Sum 50 RS AS Sum 50 SJC AS Sum 50 SS AS Sum 50 SPS AS Sum 50	Log(Thiaminase) ~ Species	ANOVA	p < 0.00001 F = 22.6 df =7, 54	Figure 3.4

DWS DT Sum 37 RS DT Sum 37 SS DT Sum 37	Log(Thiaminase) ~ Species	ANOVA	p < 0.00001 F = 54.3 df = 2, 14	Figure 3.4
ALE DT Fall 27-37 NSS DT Fall 27-37 TP DT Fall 27-37	Log(Thiaminase) ~ Species	ANOVA with unequal variances	p < 0.0069 F = 29.4 df = 2, 4	Figure 3.4
BLO DT Fall 64-73 DWS DT Fall 64-73 RS DT Fall 64-73	Log(Thiaminase) ~ Species	ANOVA	p < 0.00001 F = 32.4 df = 2, 22	Figure 3.4
NSS FR Sum 27 SS FR Sum 27	Log(Thiaminase) ~ Species	ANOVA with unequal variances	p < 0.0004 F = 24.7 df =1, 11	Figure 3.4
ALE SB Sp 100 DWS SB Sp 100 NSS SB Sp 100	Log(Thiaminase) ~ Species	ANOVA with unequal variances	p < 0.00003 F = 52 df=2, 10	Figure 3.4
ALE SB Sum 18 NSS SB Sum 18 SS SB Sum 18	Log(Thiaminase) ~ Species	ANOVA	p < 0.01 F = 5.4 df = 2, 17	Figure 3.4
ALE SB Sum 100 BLO SB Sum 100 DWS SB Sum 100 RS SB Sum 100	Log(Thiaminase) ~ Species	ANOVA with unequal variances	p < 0.00001 F = 102 df =3, 10	Figure 3.4
ALE SB Fall 100 DWS SB Fall 100 RS SB Fall 100 SS SB Fall 100	Log(Thiaminase) ~ Species	ANOVA with unequal variances	p < 0.00001 F = 110 df=3, 16	Figure 3.4

Table 3.3 Groupings of sampling combinations for indicator species analysis of diet data

Groupings of sampling combinations by thiaminase activity for the four indicator species (ISA) analyses. Summary statistics (geometric mean, median, and geometric SD) of thiaminase activity for each sampling event is included. *Group was excluded from the ISA analysis.

Site	Species	Season	Depth (m)	Thiaminase activity (pmol/g/min)			ISA Group 1	ISA Group 2	ISA Group 3	ISA Group 4
				Geometric mean	Median	Geometric SD				
Ashland	CIS	Sum	50	20	20	1	Low	Low	Low	Low
Ashland	BLO	Sum	50	29	20	1.7	Low	Low	Low	Low
Sturgeon Bay	DWS	Fall	100	38	20	2.65	Low	Low	Low	Low
Sturgeon Bay	DWS	Sum	100	55	46	2.56	Low	Low	Low	Low
Sturgeon Bay	NSS	Sum	18	166	140	6.49	Low	Low	Low	Low
Sturgeon Bay	DWS	Sp	100	242	275	5.56	Low	Low	Low	Low
Frankfort	NSS	Sum	27	310	219	6.57	Low	Low	Low	Middle*
Ashland	SS	Sum	50	436	687	7.84	Low	Low	Low	Middle*
Sturgeon Bay	NSS	Sp	100	1,370	940	4.26	High	Low	Middle*	Middle*
Sturgeon Bay	SS	Sum	18	1,998	2,700	9.22	High	Low	Middle*	Middle*
Frankfort	SS	Sum	27	9,552	11,370	2.47	High	High	High	High
Sturgeon Bay	ALE	Sum	100	9,970	8,400	1.68	High	High	High	High
Port Weller	RG	Sum	9	11,429	13,892	3.6	High	High	High	High
Sturgeon Bay	ALE	Fall	100	13,517	13,000	1.56	High	High	High	High
Ashland	RS	Sum	50	14,044	13,764	1.72	High	High	High	High
Port Weller	RS	Sum	75	15,503	19,052	1.83	High	High	High	High
Sturgeon Bay	ALE	Sp	100	38,970	41,000	1.68	High	High	High	High

Table 3.4 Summary of multiple comparisons resulting from models in Table 3.2.

Summary of multiple comparisons resulting from the models in Table 3.2 The results of the comparison indicate how the elements of the pairwise comparison are related, and the number in parentheses is indicated if each of a given comparison did not produce the same result. Ratios of the median thiaminase activity for each pairwise comparison are given for cases where thiaminase activity differs. When more than one pairwise comparison was available, the average of ratios of the medians for that result is reported.

	Number of comparisons	Result of comparison (number)	Ratios of medians
Depth			
18m-100m	1	18m=100m	
Season			
Sp-Sum-Fall	2	ALE: Sp>(Sum=Fall) DWS: Sp=Sum*; Sp>Fall, Sum=Fall *p=0.076	ALE: 3.4 DWS: 5.5
Sum-Fall	1	Sum=Fall	
Species			
ALE-RS	2	ALE=RS	
ALE-SS	2	ALE=SS; ALE>SS	 52
ALE-NSS	3	ALE>NSS (2) ALE=NSS (1)	41
ALE-DWS	3	ALE>DWS	233
ALE-BLO	1	ALE>BLO	207
RS-SS	3	RS>SS (2); RS<SS;	33.5 0.15 (SS=6.3x RS)
RS-NSS	0		
RS-DWS	5	RS>DWS	262
RS-BLO	3	RS>BLO	230
SS-NSS	2	SS>NSS; SS=NSS** **p=0.0619	31
SS-DWS	3	SS>DWS	385
SS-BLO	1	SS=BLO*** ***p=0.066	
NSS-DWS	1	NSS=DWS	
NSS-BLO	0		
DWS-BLO	3	DWS=BLO	

Table 3.5 Summary statistics for NMS ordinations of sampling units in diet-space

Summary of NMS ordinations of sampling units in diet-space. *Tests whether the reduction in stress is better than that achieved after randomized data. **Percent variance in the initial n-dimensional data represented by the ordinations. ***Correlation coefficient between thiaminase activity and the axis scores for the axis rotated to maximally correspond to thiaminase activity. A visual representation of the highlighted ordination is shown in Figure 3.7.

Expression of diet:	Dimensions	Number of prey taxa in main matrix	NMS summary statistics			Correlations with thiaminase activity		
			*p**	*Stress*	*% variance***	*r****	*r2*	*tau*
Absolute wet mass	3	45	0.004	6.83	92.8	0.238	0.056	0.147
Absolute wet mass	3	32	0.004	6.56	93.6	0.262	0.068	0.147
Proportional wet mass	3	45	0.008	7.89	84.0	0.295	0.087	0.015
Proportional wet mass	3	32	0.004	8.90	79.6	0.702	0.492	0.368

Table 3.6 Summary statistics for ISA analysis

Summary statistics for ISA analysis.

ISA Group	Expression of diet	Number of taxa	Overall p-value	Taxa indicating high thiaminase	Indicator Value	p-value for individual taxa as indicators
Group 1	Relative	24	0.96	None		
Group 2	Relative	24	0.78	Bythotrephes	75	p=0.005
Group 3	Relative	24	0.92	Bythotrephes	68.2	p=0.02
Group 4	Relative	24	0.98	None		
Group 1	Absolute	24	0.83	None		
Group 2	Absolute	24	0.56	Bosmina	62.5	p=0.010
				Bythotrephes	69	p=0.012
Group 3	Absolute	24	0.72	Bosmina	60.6	p=0.022
				Bythotrephes	65.8	p=0.034
Group 4	Absolute	24	0.90	None		

4 CHAPTER 4

DE NOVO SYNTHESIS OF THIAMINASE I IN VERTEBRATES: DEFINITIVE PROOF IN ZEBRAFISH AND THE CASE FOR *DE NOVO* SYNTHESIS BY ALEWIFE

4.1 Abstract

Thiaminase is a vitamin B_1-degrading enzyme that causes thiamine deficiency in Great Lakes salmonids. Thiamine deficiency leads to high early life stage mortality in Lake Trout, which impedes achievement of Lake Trout (*Salvelinus namaycush*) rehabilitation goals for Great Lake's populations. The source of thiaminase in Great Lakes food webs is unknown. We tested the hypothesis that fish make their own thiaminases *de novo* using two approaches. First, we compared the biochemical characteristics of thiaminases from different taxonomic groups of aquatic organisms. Secondly, we used existing information from two known fish thiaminase peptide fragments to search for candidate fish genes that encode homologous thiaminases; additionally, we determined whether the protein products encoded by the candidate genes produced active thiaminase enzymes. The biochemical characteristics of thiaminases differed across the fishes examined. This finding indicated that each fish is capable of making its own thiaminase and argued against all thiaminase-containing fishes obtaining thiaminase from some common food web source. Furthermore, we identified candidate thiaminase genes in Carp (*Cyprinus carpio*), Alewife (*Alosa pseudoharengus)*, and Zebrafish (*Danio rerio*). Empirically measured biochemical properties from Alewife thiaminase were exact matches to the predictions from the candidate gene. The candidate Zebrafish gene was cloned and overexpressed in *E. coli* and produced an active thiaminase enzyme. This constitutes the first report of a gene encoding a thiaminase in any multicellular organism. Our findings supported the *de novo* hypothesis and indicated that the primary source of thiaminase activity in Great Lakes fishes results from a gene in fishes that encodes a thiaminase enzyme. Multiple sources of thiaminase (*de novo* and dietarily acquired thiaminase) cannot be definitively eliminated, and additional research is necessary to confirm that *de novo* production accounts for the majority of thiaminase activity in fishes.

4.2 Introduction

Lake Trout (*Salvelinus namaycush*) populations have declined substantially since the 1950s, and rehabilitation of these populations is a goal for all of the Laurentian Great Lakes (DesJardine et al. 1995, Eshenroder et al. 1995, Stewart et al. 1999, Horns et al. 2003, Ryan et al. 2003). Recruitment failure of Lake Trout in the Great Lakes likely results from a combination of factors, one of which includes poor survival of early life stages (Holey et al. 1995, Krueger et al. 1995a, Brown et al. 2005c, Claramunt et al. 2005, Jonas et al. 2005, Tillitt et al. 2005, Bronte et al. 2006, Bronte et al. 2007, Bronte et al. 2008). One factor contributing to poor survival of early life stages is Thiamine Deficiency Complex (TDC), a thiamine (vitamin B1) deficiency that causes increased mortality of embyros and sublethal effects in juvenile and adult Lake Trout (Fisher et al. 1995, Fitzsimons 1995, Fisher et al. 1996, Marcquenski and Brown 1997, Brown et al. 2005d, Fitzsimons et al. 2005a, Honeyfield et al. 2005b, Ketola et al. 2005, Wolgamood et al. 2005, Werner et al. 2006, Fitzsimons et al. 2007). TDC occurs in Lake Trout because females ingest prey fish (notably Alewife, *Alosa pseudoharengus*, and Rainbow Smelt, *Osmerus mordax*) that contain high levels of thiaminase, a thiaminolytic enzyme. Thiaminase activity in prey fish varies spatially and temporally (Ji and Adelman 1998, Fitzsimons et al. 2005b, Tillitt et al. 2005), and the cause of this variability not only remains unknown. Attempts to induce elevated levels of thiaminase activity in response to factors hypothesized to cause increased thiaminase activity have not revealed specific underlying causes for variation in thiaminase activity (Wistbacka et al. 2002, Lepak et al. 2008, Wistbacka and Bylund 2008, Wistbacka et al. 2009, Honeyfield et al. 2012, Lepak et al. 2013, Kraft et al. 2014). Understanding the spatial and temporal variation in thiaminase activity is essential for developing effective management strategies for TDC, and this understanding necessitates identification of the sources of thiaminase.

The ultimate source of the thiaminase enzyme in fishes remains unknown. The two dominant hypotheses are the dietary acquisition hypothesis, which suggests that high levels of thiaminase could occur if thiaminase-containing fishes consume thiaminase in their diet, and the *de novo* hypothesis, which suggests that fish have a gene that encodes for a thiaminase enzyme themselves. These two hypotheses are not mutually exclusive; one species of fish may produce thiaminase enzymes *de novo* and another may obtain thiaminase from the diet. Alternatively, total

thiaminase activity in an individual could result from a combination of *de novo* and dietary sources. Dietary acquisition of thiaminase is an appealing idea because predators become thiamine-deficient as a result of ingesting thiaminase-containing prey and because lower trophic levels of food webs contain thiaminase activity. However, two main forms of evidence exist to better support the *de novo* hypothesis: ecological and biochemical (protein and genetic) evidence.

Little ecological evidence has been collected with the specific intention of determining the source of thiaminase in fishes. In an evaluation of factors affecting the presence of thiaminase activity, taxonomy was a stronger predictor of thiaminase activity in Great Lakes fishes than diet, habitat, or other factors (Riley and Evans 2008). To date, two studies sought to test specific mechanisms hypothesized to affect thiaminase activity (stress, food deprivation, or water temperature) in clupeids, but none of the experimental treatments resulted in an increase in thiaminase activity relative to controls (Lepak et al. 2008, Lepak et al. 2013). Interestingly, these studies revealed one commonality associated with reliably increased thiaminase activity. Alewife held in the laboratory had 2- to 3-fold increased thiaminase activity relative to the source population, and these fish were in good condition, growing, and fed commercial trout pellets that had been heated to denature any potential thiaminases (Lepak et al. 2008). These fish essentially showed increased thiaminase activity despite being exposed to no known dietary source of thiaminase. Gizzard shad had increased thiaminase activity in pond experiments when higher-quality food was available than when low-quality food was available, but their thiaminase activity was not related to the experimental treatment (temperature change) (Lepak et al. 2013). These findings suggest the possibility of *de novo* synthesis of thiaminase in response to food quality, but they do not rule out a bacterial source of thiaminase in gut flora whose production of thiaminase is also related to food quality.

Protein chemistry investigations provide additional compelling evidence for *de novo* synthesis of thiaminase. Crude enzyme preparations of thiaminase from bacteria, Carp, and a variety of crustaceans suggest that thiaminase enzymatic properties (km, substrate specificity, and co-substrate specificity) vary depending on the source (Fujita 1954). Carp and shellfish thiaminases are more different from bacterial thiaminase than from each other, and bacterial thiaminases are usually more similar

to one another than to thiaminases of other taxa (Fujita 1954). These biochemical differences suggest that fish and shellfish have thiaminases that differ from bacteria, which suggests *de novo* production and that fish and shellfish do not primarily ingest or accumulate thiaminase of bacterial origin.

A thiaminase has been fully or partially purified from at least two bacterial taxa, *Paenibacillus thiaminolyticus* (hereafter, PT) (Wittliff and Airth 1968) and *Clostridium botulinum* (Sikowitz et al. 2013); an amoeba (*Naeglaria gruberi*) (Kreinbring et al. 2014); a mollusk (*Anodonta cygnea*) (Puzach et al. 1984); a silkworm (*Anaphe venata*) (Okonji et al. 2012); and two fishes: Carp (*Cyprinus Carpio*) (Bos and Kozik 2000) and red cornetfish (*Fistularia petimba*) (Nishimune et al. 2008). Biochemically, the properties of the purified enzymes from different organisms differ from one another, suggesting that the enzymes are unique to each organism. Furthermore, the pH optima for thiaminases from Alewife and Gizzard Shad (*Dorosoma cepedianum*) were similar to one another but were more acidic than that of PT, indicating a non-PT source of thiaminase in these fishes (Zajicek et al. 2005). In some marine fish species, optimum pH for thiaminases varies among tissues within species (Ishihara et al. 1973). Lastly, short (<20 amino acids) peptide fragments have been sequenced for both the Carp and Red Cornetfish thiaminases, and neither showed significant sequence similarity to the bacterial enzymes (Bos and Kozik 2000, Nishimune et al. 2008). Taken together, the protein chemistry and sequence evidence suggests that fish thiaminases are different from bacterial thiaminases and are possibly produced *de novo*. Given our inability to uncover any compelling lower trophic level dietary source of thiaminase despite efforts to do so (Chapter 2, Chapter 3, Richter et al. 2012), direct exploration of *de novo* synthesis was warranted.

Our overall goal of this research was to determine if fishes produce their own thiaminase. We adopted two independent yet complementary approaches to achieve this goal. The first was an unbiased, or naïve, approach in which we sought to empirically determine biochemical characteristics of thiaminases from fishes and assess whether these characteristics are consistent with a *de novo* or dietary source of thiaminase. This approach requires no *a priori* knowledge in terms of the nature of the sources of thiaminase and has the potential to reveal multiple sources of

thiaminase in fishes, should multiple sources exist. The disadvantages to this approach is that they are labor intensive, can be challenging if the enzyme is unstable or has multiple subunits that must remain intact to be functional, and does not take in informed approach. Our second approach was that informed approach, in which we used existing protein sequence information from PT, Carp, and Red Cornetfish to identify candidate genes in fishes that may encode thiaminases. This approach takes advantage of existing information, thereby potentially facilitating a more rapid evaluation of the potential for *de novo* production of thiaminase by fishes. However, it risks producing no useful result if thiaminases are not well-conserved or if there is insufficient coverage of fish genomes to identify candidate genes from known sequence information.

4.3 Methods

4.3.1 Organism selection and collection/culture

We investigated the biochemical properties of thiaminases of four wild-type organisms. PT was chosen as a positive control and also as a comparator to determine whether other organisms have thiaminases similar to that of PT. Alewife was chosen because of their ecological relevance to the Great Lakes and because recently available next-gen sequence data (Czesny et al. 2012) provided genomic resources. Carp was chosen because of its availability in the Great Lakes, its history of association with thiaminase-induced thiamine deficiency (Green et al. 1941), and because of work on Carp thiaminase was available for comparison (Bos and Kozik 2000). Quagga mussels (*Dreissena rostriformis bugensis*) were chosen because of their high levels of thiaminase activity (Tillitt et al. 2009) and their ecological dominance in the Great Lakes (Bunnell et al. 2009). Pure cultures of PT strain 8188 (Honeyfield et al. 2002) were cultured at 37 °C in Terrific Broth (MO BIO Laboratories, Carlsbad, California) in either a shaking incubator or in a beveled flask with a stir bar and were harvested after 48-80 h of culture. These conditions reliably resulted in thiaminase production, whereas Terrific Broth made from scratch (Sambrook and Russell 2001) failed to induce thiaminase production under identical conditions. Upon harvest, cultures were frozen whole in 50mL Falcon tubes at -80°C. Adult Carp were captured from Lake Erie using short-set gill nets. Adult

Alewife and quagga mussels were collected from Sturgeon Bay, Lake Michigan using bottom trawls (section 3.3.1). All fish and mussels were flash-frozen between slabs of dry ice, and stored at -80°C.

4.3.2 Protein extraction

Starting materials for protein extraction varied depending on the taxon. For wild-type PT cultures, thiaminase is excreted extracellularly, so cultures were spun at 14,000*g* and culture supernatant was concentrated using amicon-ultra 10kDa molecular weight cut-off (MWCO) filters (EMD Millipore, Billerica, MA). To extract recombinant proteins that were overexpressed in *Escherichia coli* (see below) cells were lysed in 1X BugBuster (Millipore) according to the manufacturer's instructions in the presence of benzonase. Whole Carp and Alewife were thawed until they could just be dissected. Preliminary trial extractions on Alewife stomach and intestines, spleen, and gills revealed similar results and revealed that gills and spleen tissue produced the cleanest protein preparations. Therefore subsequent extractions for Carp and Alewife used gill tissue. Quagga mussels were thawed just sufficiently to be husked from their shell and were used whole. Animal tissues were placed in ice-cold (4°C) beakers and containing cold extraction buffer (16 mM K_3HPO_4, 84 mM KH_2PO_4, 100 mM NaCl, pH 6.5 with 1 mM DTT, 2 mM EDTA, 3 mM Pepstatin, 1X Protease inhibitor cocktail (Sigma), and 1 mM AEBSF). All extractions were carried out at 4°C in pre-chilled glassware. Samples were mechanically homogenized using a rotor-stator tissue grinder. Samples were stirred gently for several hours to overnight at 4°C, centrifuged at 14,000*g* to remove debris, and strained through cheesecloth to remove any insoluble lipids. Extracts were then subjected to 30-75% ammonium sulfate precipitation. Pellets from the precipitation were resuspended in 100 mM KPO buffer with 100 mM NaCl, centrifuged to remove any remaining debris, and stored in 30% glycerol at -20 °C.

4.3.3 Polyacrylamide Gel Electrophoresis (PAGE)

Four types of PAGE were run as part of this study. Native PAGE was used to compare the migration characteristics of multiple thiaminases. Proteins migrate in native PAGE based on a combination of both size and charge, and therefore thiaminases with different mass and charge properties should show different migration patterns. Native PAGE was run using either pre-cast TGX gels (BioRad,

Hercules, California) of varying percentage (7.5% to 12% or 8-16% gradient gels) or on hand-cast gels cast (TGX FastCast, BioRad) according to the manufacturer's instructions. Blue-native PAGE was used to estimate the mass of thiaminases in their native conformation. Blue-native PAGE (Schagger and Vonjagow 1991) gels were run using the NativePage Novex Bis-Tris system (Life Technologies) or hand-cast equivalents (Schamel 2001). Either light or dark blue cathode buffer could be used, but for thiaminases with lower levels of activity, light blue cathode buffer facilitated visualization of the activity stain as compared to dark blue cathode buffer. Standard denaturing SDS-PAGE was used to estimate the molecular mass of thiaminases after denaturation. Denaturing SDS-PAGE was run using one of three relatively equivalent methods: pre-cast TGX gels (BioRad) according to the manufacturer's instructions, hand-cast Tris-HCl gels using standard Laemmli chemistry (Simpson et al. 2009) with an operating pH of approximately 9.5, or hand-cast Bis-Tris gels (MOPS buffer) with an operating pH of approximately 7. For all denaturing and non-denaturing SDS-PAGE applications, standard Laemmli sample buffer was used, and samples were heated to 75°C for 15 minutes to facilitate denaturation followed by brief centrifugation to eliminate any precipitated debris. Lastly, non-denaturing PAGE was used as an alternative to denaturing PAGE for thiaminases that could not be renatured (i.e., their activity could not be reconstituted) following a denaturing SDS-PAGE. Non-denaturing PAGE was conducted using any of the three aforementioned gel chemistries with SDS-containing running buffers including reductant (DTT), but samples were not heated prior to application to the gel. Samples for non-denaturing PAGE were allowed to incubate in sample buffer at room temperature for 30 minutes prior to gel loading. This preserves the charge-shift induced by SDS but does not result in protein denaturation, facilitating in-gel analysis of thiaminase activity after separation. To visualize proteins following electrophoresis, we stained gels with Coomassie stain (CBR-250 at 1g/L in methanol/acetic acid/water (4:5:1) and destained with methanol/acetic acid/water (1.7:1:11.5). Mini-gels were run on BioRad's mini-protean gel rigs. Midi-gels (16 cm length) were run on Hoefer's SE660, and large-format gels (32 cm length) were run on a BioRad's Protean Slab Cell. Mini-gels were generally run at room temperature, and midi- and large-format gels were run at 4°C. Blue-native PAGE was always run at 4°C.

4.3.4 In-gel activity assay and renaturation procedures

In-gel activity stains were used to investigate enzymatic function of differing isoforms of a protein and to assess the relationship between protein function and mass (Rivoal et al. 2002, Manchenko 2003, Baba et al. 2012). After electrophoresis, gels were stained for thiaminase activity using a previously described diazo-coupling reaction (Abe et al. 1986, Abe et al. 1987). We began by using the published protocol (Abe et al. 1987) as follows: after electrophoresis, gels were rinsed 3 times in water. Gels were then incubated for 2, 30-minute periods in 25 mM sodium phosphate buffer with DTT added to a concentration of 1 mM, and then a third time for 30 minutes with no DTT as DTT interferes with the development of the stain in the diazo reaction. Gels were then immersed in a solution of 0.89 mM thiamine-HCl and co-substrate (1.45 mM pyridoxine, 24 mM nicotinic acid, or 20 mM pyridine) made in sodium phosphate buffer and incubated for 10 minutes with gentle agitation. Gels were briefly rinsed in water and placed in a lidded container and incubated at 37°C for 30 minutes. The diazo stain (Abe et al. 1986, Abe et al. 1987) was then applied, allowed to incubate on the gel for five minutes with gentle agitation, and the rinsed three times (5 minutes each) with water at room temperature to remove any surface staining residue.

Coomassie stain was applied to visualize total protein following the activity stain; incubation time on the Coomassie stain was be increased half again the standard time in order to achieve good staining, but if the activity stain was light, incubating gels too long in Coomassie stain hampered the ability to see the formerly-visible activity stain, even after destaining (for example, Figure 4.1, panel B). We modified this protocol for the non-denaturing SDS-PAGE, especially when assessing activity of very active thiaminases (i.e., recombinant proteins). In those cases, activity was so high that the activity clearing could be large enough so as to not be able to identify a single band of interest. After electrophoresis, we rinsed the gel briefly (1-2 minutes) in 25 mM sodium phosphate buffer to remove residual SDS, followed by a 1-5 minute incubation at room temperature in the sodium phosphate-thiamine-co-substrate solution. The gel was rinsed for 30 seconds in water and stained immediately with the diazo stain.

After a native-PAGE, blue-native PAGE, or non-denaturing SDS-PAGE, no renaturation procedure was necessary. Following SDS-PAGE, we renaturated Alewife and PT thiaminases in the sodium phosphate buffer that preceded the activity stain. However, this procedure was not successful for renaturing thiaminases from Carp and quagga mussels. Therefore we tried several renaturation methods others have used successfully for renaturing phosphatases and kinases (Kameshita et al. 1997, Bischoff et al. 1998). Procedures included incubating gels overnight, for several days, or up to a week at room temperature and at 4°C in solutions of 2.5% Triton X-100, 20% isopropanol, 8M urea, 0.02% Tween 20, and sequential combinations of these treatments as suggested by others (Kleiner and Stetler-Stevenson 1994, Kameshita et al. 1997, Bischoff et al. 1998, Baba et al. 2012). The theory behind use of this suite of reagents is that after a denaturing separation with SDS, partial renaturation may have occurred and incorrect partial folding prevents proper enzymatic activity. For some proteins, treatment with a strong denaturant such as urea facilitates complete denaturation after the SDS separation and then allows proper refolding from that completely denatured state. When we treated gels with strong denaturants, gels were then incubated for hours to days in 25-100 mM sodium phosphate buffer (pH 4.2-6.8) to allow renaturation. None of these methods successfully renatured Carp or quagga mussel thiaminases.

4.3.5 Isoelectric focusing

4.3.5.1 In-gel

Isoelectric focusing (IEF) separates proteins based their isoelectric point (pI), or the pH at which they are neutrally charged. IEF was conducted either in a gel matrix (i.e., an immobilized pH gradient strip; IPG) or in a liquid matrix. We conducted in-gel IEF using a Multifor II (GE Healthcare Life Sciences) with consumables (IPG strips, IPG buffers, other reagents) purchased from GE Healthcare Life Sciences. IGP strips ranged in size from 7 to 13 cm depending on the application. Prior to rehydration, all protein preparations were desalted in low-salt (~5 to 10 mM) sodium or potassium phosphate buffer (pH 6.5) using 10kDA MWCO filter. This also served to concentrate protein in the protein extracts if required. All samples were applied using with rehydration loading with sample volumes and protein concentrations recommended by the manufacturer.

For standard denaturing in-gel IEF, rehydration solution consisted of 8M urea, 2% CHAPS, 2% IPG buffer of the appropriate pH-range, 1% bromophenol blue, and 18 mM DTT. Rehydration was conducted overnight at room temperature in an acrylic glass re-swelling tray with sliding lid. Isoelectric focusing was conducted in the drystrip tray on top of the cooling tray of the Multiphor II with silicon between the cooling tray and drystrip tray. Standard IEF was conducted with the water running through the cooling tray set to 20°C. IPG strips were covered in silicon oil prior to running. IEF was conducted at maximum of 2 mA total current and 5W total power, with an EPS3500 XL power supply in gradient mode. Voltage gradients were based on standard protocols recommended by the manufacturer (Healthcare 2009). We also performed in-gel IEF under native conditions followed by activity staining of IPG strips to determine PIs of thiaminases. Protocols were essentially the same as those for denaturing conditions, with the following exceptions: (1) Urea was eliminated and the CHAPS concentration was reduced to 0.5% in the rehydration solution; (2) rehydration was conducted at ~ 14°C by placing the re-swelling tray over a expanded polystyrene plate on ice in a cooler; and (3) the water in the cooling tray was cooled to 4°C.

4.3.5.2 In-liquid

We conducted in-liquid IEF for several applications. We used in-liquid IEF as an enrichment technique (i.e., to separate thiaminases from other proteins) as well as to investigate the behavior of different isoforms of the Carp thiaminase. We conducted IEF using a Rotofor (BioRad) with consumables (IPG buffers, other reagents) purchased from BioRad according to the manufacturer's instructions. We also conducted liquid IEF under non-denaturing conditions, in which case typical focusing solution included no urea, 2% pH 3-10 biolyte (pH range as appropriate for the specific application), 0.5% CHAPS, 20% glycerol, and 5 mM DTT. We found that the addition of glycerol helped retain activity but that it also increased focusing times. The Rotofor was run at a constant 15W with a maximum current of 20mA current and voltage set for a maximum of 2000V. Samples containing 8M urea (denaturing) were cooled to only 14°C during focusing to avoid urea precipitation, while samples lacking urea were cooled to 4°C during focusing. Protein extracts that were in salt solutions greater than 10 mM were desalted directly in focusing solution using a 10 kDA MWCO filter. Focusing runs were allowed to proceed until the voltage stabilized

(which was often under 2000V) and fractions were harvested with the needle array and vacuum pump. The Rotofor produces 20 fractions, and treatment of the fractions varied according to the downstream application. When the Rotofor was used for general enrichment via re-fractionation, 5-8 fractions in the pI range of the thiaminase of interest were pooled and were re-fractionated with no additions to the focusing solution. When the Rotofor was used to investigate specific isoform behavior, 1 or 2 fractions with adjoining pHs were pooled. After fractionation or re-fractionation, if active enzyme preparations were needed for downstream applications, ampholytes were removed by addition of NaCl to 1M and then samples were desalted into phosphate buffer of appropriate strength for downstream application using a 10kD MWCO filter.

4.3.6 Two-dimensional Electrophoresis (2DE)

2DE is a technique in which proteins are separated in one dimension based on pI and in another dimension based on a second property, such as mass. 2DE was performed by combining in-gel IEF with either denaturing SDS-PAGE, non-denaturing SDS-PAGE, or native PAGE. IPG strips were incubated TRIS-buffered equilibration solution (Gorg et al. 2007) either with 6M urea, SDS, and iodacetamide (denaturing) or without urea, SDS, and iodacetamide (non-denaturing) for 20 minutes. Low melting point agarose was used to solidify IGP strips in place. Agarose was cooled to just above the gelling temperature, as hot agarose inactivated thiaminase activity.

4.3.7 Identification of candidate gene sequences

Two sets of peptide fragments were available from prior studies of fish thiaminases. One was the n-terminal sequence (20 amino acids) reported for Carp (Bos and Kozik 2000). The other was the n-terminal (18 amino acid) and two internal n-terminal fragments found after trypsin digestion (both 11 amino acids) reported for the Red Cornetfish (Nishimune et al. 2008). We searched the NCBI database for teleost proteins with similar amino acid sequences or for genes that would encode proteins with similar sequences, and resulting homologues were characterized as candidate genes and subsequently expressed.

4.3.8 Expression of candidate genes

All identified candidate genes (see section 4.4.2.1) were cloned and overexpressed to determine whether they produced functional thiaminases. Three separate expression systems were investigated, and all identified candidate genes were expressed in the three systems. For all expression systems, we used recombinant PT thiaminase as a positive control. Candidate genes were synthesized (Integrated DNA Technologies, Inc., Coralville, Iowa) and placed into the pET52b vector (EMD Millipore). The plasmid was transformed into *E. coli* (Rosetta 2(DE3)pLysS Singles™ Competent Cells, EMD Millipore) according to the manufacturer's instructions, and expression of the candidate gene was induced by the addition of IPTG. This system was successful for one of the candidate gene sequences.

In an effort to achieve expression of the remaining candidate genes, we cloned the putative thiaminase genes into the pKLAC2 vector (Invitrogen; GENEART) and over-expressed them in yeast (*Kluyveromyces lactis*) using the *K. lactis* Protein Expression Kit (New England Biolabs). This second expression system was also not successful in producing active thiaminase enzymes from candidate genes. As a result, we moved to a third expression system, an *in vitro* transcription/translation system based on rabbit cells (TnT T7 Quick Coupled Transcription/Translation System, Promega). This approach is known to produce only small quantities of protein, and it also did not that produce any evidence of thiaminase activity for any candidate genes. When gene products of the expected size were found in inclusion bodies, we applied a number of methods (urea stepdown, commercially available refolding kits) to resolubilize precipitated proteins from inclusion bodies and aid in the proper re-folding of proteins to recover thiaminase activity.

4.4 Results

4.4.1 Unbiased approach

4.4.1.1 Size characteristics of wild-type thiaminases and renaturation characteristics

The relative molecular mass of putative wild-type Alewife thiaminase was determined to be ~24,000, as identified by SDS-PAGE followed by the activity assay, while the relative molecular mass of the PT thiaminase was found to be the known size of

42,000 (Figure 4.1). We were unable to estimate the denatured molecular mass of thiaminases from Carp or quagga mussels because none of the renaturation methods we employed were successful for these thiaminases, rendering the activity assay ineffective. Using blue-native PAGE, we estimated the native size of the holoenzymes to be the expected 42,000 for PT, ~250,000 for Carp and ~ 480,000 for quagga mussels (Figure 4.2).

We electrophoresed both Carp and quagga mussel protein extracts on native gels with standard procedures (sensu Figure 4.3) and subjected them to these renaturation procedures after electrophoresis to investigate the influence of the renaturation reagents on thiaminase activity. Thiaminase activity was easily detectable for Carp but was not detectable for quagga mussels.

4.4.1.2 Native migration rates of wild-type thiaminases

All four thiaminases migrated differently on native-PAGE (Figure 4.3), and the relative migration speeds were highly repeatable from one protein preparation to the next. Our results illustrated that the PT thiaminase was the most mobile and migrated the fastest, followed by the Alewife thiaminase. The thiaminase of quagga mussels migrated more slowly, and Carp thiaminase migrated the slowest.

4.4.1.3 Isoelectric point and isoform investigations

In-gel native IEF followed by activity staining revealed different pIs for all wild-type thiaminases investigated (Figure 4.4). In native conformation, we found that the pI of PT thiaminase is approximately 4.5-4.7, which is in exact agreement with the pI of purified recombinant PT thiaminase (reported to be 4.6) (Costello et al. 1996). We also noticed an unexpected, and somewhat less intense clearing on the activity stained IPG strip for PT in the pH~10 region. A higher-resolution native-native 2D gel also showed at least three isoforms of thiaminase in PT culture supernatant (Figure 4.5). Two of the three isoforms had pIs of approximately 9 and 10, and the two basic isoforms along with the expected acidic form all migrated to approximately the same location in the second dimension. We inferred that all three isoforms were approximately the same mass given that 1D gels and denaturing gels produce only one band with thiaminase activity rather than several. Application of this same protein source to a standard denaturing 2D gel followed by in-gel renaturation was

unable to show evidence of the 2 basic isoforms (Appendix P), indicating that the two basic isoforms exist only when isoelectric focusing is conducted under native conditions.

The pI of Quagga mussel thiaminase was in the range of 5.1 to 5.6, whereas that of the Alewife thiaminase was approximately 5.0 (Figure 4.4). The Carp thiaminase showed at least two zones of activity, one at pH7 and one at pH 9. Zones with potentially lesser activity occurred from approximately pH 5-6, but the two most intense areas of activity were more basic than 7. Given the wide range of apparent pIs of Carp isoforms, we fractionated a larger amount of Carp protein and separated it on a native gel to confirm the existence of multiple isoforms and to rule out poor focusing as the reason for the observations relative to extended pI range shown in Figure 4.4. At least four distinct isoforms of Carp thiaminase were evident, with pIs ranging from 5.5 to 10.6 (Figure 4.6, panel A). No thiaminase activity was apparent in fractions with pH < 5.1. This result was consistent with the in-gel IEF (Figure 4.4.). The lack of thiaminase activity at pH < 5.1 was not due to a lack of protein in those fractions (Figure 4.6, panel B). Thus, the relatively wide clearing for Carp thiaminase activity shown in Figure 4.4 was likely the result of the combined effect of the four isoforms of thiaminase that have somewhat overlapping pIs. The basic nature of the Carp proteins meant that substantial run times were required to move them into the gel. Separation of the isoforms (Figure 4.6) required a minimum of 3 hours of electrophoresis at 100 V.

4.4.1.4 Co-substrate utilization

Co-substrate utilization assays showed that Carp, Alewife, and quagga mussels could not use pyridoxine but could use nicotinic acid and pyridine (Figure 4.7; data for all species not shown). In all cases, pyridine resulted in greater activity than nicotinic acid. PT thiaminase was able to use all three co-substrates investigated.

4.4.2 Informed approach

4.4.2.1 Identification of candidate gene sequences

The peptide fragment reported by Bos and Kozik (2000) lead to the identification of one homologous gene sequence from Carp (Appendix Q). We identified this gene

from a Carp cDNA that, when translated, would produce a protein with an N-terminal sequence that identically matched the 20 amino acids of the Carp thiaminase reported by Bos and Kozik (2000). This gene sequence encoded a protein of molecular mass 44,000, which is incongruous with the mass of 55,000 on SDS-PAGE that was reported during the purification (Bos and Kozik 2000). No homologue to this gene was located in other fish species.

The n-terminal peptide fragment reported by Nishimune et al. (2008) led to the identification of a second candidate gene, with homologues identified in Carp, Alewife, and Zebrafish (Appendix Q). All three candidate genes based on the peptide fragment reported by Nishimune et al. (2008) encoded proteins of molecular mass 23,500 (Carp), 23,600 (Zebrafish), and 24,600 (Alewife). In total, this resulted in four candidate genes: two in Carp (one based on the homology to peptide sequences reported by Bos and Kozik (2000) and one based on the homology to peptide sequences reported by Nishimune et al. (2008), one in Alewife (based on the homology to peptide sequences reported by Nishimune et al. 2008), and one in zebrafish (based on the homology to peptide sequences reported by Nishimune et al. 2008). We had not originally planned to target Zebrafish as an organism of interest, but given that they are known to produce thiaminase, their status as a model system, and that their entire genome was sequenced, we added zebrafish to take advantage of the genetic and proteomic information afforded by such a well-studied organism.

4.4.2.2 Expression of candidate genes

The candidate gene from Zebrafish that was homologous to the peptide fragment reported by Nishimune et al. (2008) produce an active thiaminase enzyme (Figure 4.8). Because the recombinant protein from the candidate Zebrafish gene (hereafter designated rZF) produced an active thiaminase, we were able to biochemically characterize it using the methods used for wild-type fishes and mussels. None of the other candidate genes produced an active thiaminase despite our efforts to use multiple expression systems to obtain active thiaminase enzymes from these gene constructs. In the bacterial expression system, the gene products for each of the candidate genes were produced, but they were found in inclusion bodies and were not soluble (Figure 4.8), and therefore could not be tested for thiaminase activity. Interestingly, the pKLAC2 expression system in yeast expressed the positive control

protein (which is not a thiaminase) very effectively, but the system did not make the thiaminase protein from Carp. We had difficulty recovering any yeast cells that had the putative thiaminase gene inserted into them, and we suspect that the yeast cells containing the thiaminase gene were killed by the action of the thiaminase before the yeast colony could grow to a suitable size. This is a common problem when trying to grow yeast or bacteria with encoding genes that create a protein or toxin that has undesirable effects on the host organism.

4.4.2.3 Biochemical characterization of rZF

The mass of the active rZF thiaminase was estimated to be ~25,000 by SDS-PAGE (Figure 4.8), and the mass of the rZF on blue-native PAGE was estimated to range from 25,000 to 150,000 (Figure 4.2). The activity of the rZF thiaminase on blue-native PAGE was so high that it was difficult to know which band constituted activity. Non-denaturing SDS-PAGE revealed active thiaminase in discrete bands at 25,000 and ~23,000 in standard Laemmli chemistry and at 25,000 and 75,000 in BioRad's TGX chemistry. Because this protein is recombinant and the protein product is known to have a mass of 25,000, we suspect the 75,000 represents a trimeric form. We suspect the clear and discrete 150,000 (Figure 4.2) represents a hexameric form. The standard native PAGE migration rate of rZF thiaminase was similar to that of Carp (data not shown). Native IEF revealed that the recombinant zebrafish thiaminase showed at least two isoforms, one (or more) isoform(s) with a pI in the 3-6 range and one with a pI of approximately 10 (Figure 4.8). Co-substrate utilization for rZF thiaminase is reported in Table 4.1.

4.4.2.4 Alignments

Given our empirical finding that the size of the Alewife thiaminase enzyme is of similar size as the Red Cornetfish thiaminase and that the candidate Alewife thiaminase gene codes for a protein that is predicted to be the same size and pI as the functional Alewife thiaminase (Table 4.2), we conducted a detailed alignment of the amino acid sequences reported from red cornetfish thiaminase with the genetic information available from Carp, Zebrafish, Alewife, PT, and the recently reported thiaminase from *N. gruberi* (Figure 4.10). The aligned proteins have similar predicted molecular masses of 24,000. The Zebrafish thiaminase and proteins encoded by the two putative thiaminase sequences from Carp and Alewife have

homology to the hemeO protein superfamily (cl15243), which includes bacterial and mammalian heme oxygenases. These enzymes are active in the degradation of heme and recycling of iron. Additional putative fish thiaminases were detected in EST libraries from multiple fish species, including *Ictalurus punctatus* (Channel Catfish, UGID:3688761), *Ictalurus furcatus* (Blue Catfish, UGID:3965793) and *Pimephales promelas* (Fathead Minnow, UGID:4220552). Surprisingly, the putative fish thiaminase proteins show a weak but significant alignment to bacterial TenA (Figure 4.11), a thiamine salvage enzyme (Jenkins et al. 2007). Specifically, the Alewife predicted protein shares 27% sequence identity and 45% sequence similarity with *Stigmatella aurantiaca* tena/thi-4 (ZP_01462836, Expect = 1e-15). The Carp and zebrafish putative thiaminases have similar degrees of homology to TenA.

4.5 Discussion

We report here the first discovery of a thiaminase enzyme from the genome of any organism other than a bacterium (Costello et al. 1996) or a unicellular ameboflagellate (Kreinbring et al. 2014). We have demonstrated that Zebrafish are capable of *de novo* thiaminase synthesis. The thiaminase of Zebrafish is homologous to the candidate gene identified in Alewife, and the empirically derived mass and pI of the wild-type Alewife thiaminase exactly match that predicted for the candidate Alewife thiaminase gene, demonstrating convergence across these two approaches. This alone presents a compelling case for *de novo* synthesis of thiaminase by Alewife, a primary prey item for Lake Trout in the Great Lakes. The empirically determined biochemical characteristics of putative Alewife, Carp, and quagga mussel thiaminases provide additional evidence to strengthen the case for *de novo* synthesis in both fish and mussels.

Our findings indicate that the Alewife thiaminase cannot be a monomer of the PT thiaminase, further confirming previous findings that PT is not the source of thiaminase in Alewife (Richter et al. 2012). Size estimates from denaturing SDS-PAGE revealed that the putative wild-type Alewife thiaminase is smaller than the PT thiaminase and that the wild-type Alewife thiaminase is of the same size as that predicted from the Alewife gene that is homologous to the Red Cornetfish thiaminase. Because the entire PT thiaminase gene sequence is known (Costello et

al. 1996) and the structure has been crystalized (Campobasso et al. 1998), there is no question that the 42kDa mass of the PT protein represents the holoenzyme.

Because the wild-type Carp thiaminase is resistant to renaturation, we currently have no estimate of the denatured size of the putative wild-type Carp thiaminase for comparison to the predicted size of the protein products encoded by the two candidate thiaminase genes we identified in Carp. In-gel renaturation of enzymes after denaturation is a technique used most commonly with phosphatases but is also used in studies of enzymes with other functions (Kameshita et al. 1997, Bischoff et al. 1998). A survey of enzymes that are able to be renatured after SDS-PAGE revealed no obvious patterns associated with the source taxa, catalytic function, or subcellular location (Bischoff et al. 1998); some enzymes are apparently more inherently amenable to renaturation than others (Bischoff et al. 1998). Thus, even the propensity of an enzyme to renature may be informative relative to the source of the protein. Notably, Alewife and PT thiaminases were able to be renatured under moderate conditions, but the two cyprinid thiaminases (Carp and rZF) were not able to be renatured after heat denaturation by any of the procedures we tested. Because renaturation (even after non-denaturing SDS-PAGE) did not produce and active thiaminase from wild-type Carp, we are not able to effectively evaluate which (if any) of the two candidate sequences for Carp encodes for a thiaminase. The candidate sequence identified from Bos and Kozik (2000) was not related to the sequence identified from Nishimune et al. (2008), and the broad pI range we identified empirically does not allow for discrimination of whether neither, one, or both of those candidate sequences could be Carp thiaminases.

In addition to size and native migration patterns, each putative wild-type thiaminase we investigated had a unique pI. Carp appears to have four isoforms that vary in pI across a rather wide range. Given the presumed oligomerization seen with the rZF thiaminase, it is possible that one or more of what we termed Carp “isoforms” is an oligomer of the same monomer. The Alewife thiaminase focused in a very narrow range on IEF therefore appears to consist of only one isoform. The pI for quagga mussel thiaminase appeared somewhat wide (5.1 to 5.6), but given that the focusing was done under native conditions rather than conditions that maximize dissociation

among proteins, the result for quagga mussels could simply be residual activity left from focusing that did not quite reach completion.

We intended to use PT as a methodological control for testing native IEF, as little information was available regarding native IEF (Yokoyama et al. 2009). We were surprised to find two heretofore unreported basic isoforms of PT thiaminase. To our knowledge, multiple isoforms of thiaminase in *P. thiaminolyticus* have not been demonstrated before. Typically, denaturing IEF is used, and the use of denaturants can mask the differences in ionization that create unique isoforms (Salaman and Williams 1971). The gel shown in Figure 4.5 and Appendix P speak to this phenomenon, producing differing results despite originating from the same protein preparation. Interestingly, the rZF thiaminase is predicted to have pI of 10, and yet the majority of the activity is clearly in the acidic range. Prediction algorithms for pIs are not as refined as those for mass, especially for basic proteins. Additionally, the use of the native IEF can result in observed pI that differ relative to those obtained when using denaturing IEF with urea because the unfolding of the protein that occurs with urea can expose ionizable groups that are not as easily exposed in the native conformation (Salaman and Williams 1971).

Conducting co-substrate utilization tests with in-gel assays did not reveal any differential use of a given co-substrate by different Carp isoforms for the three co-substrates that we assessed. However, different isoforms of the Red Cornetfish thiaminase used the same co-substrates differentially, with the pI 7-9 isoform degrading three times more thiamine with pyridine than the pI 5.7 isoform (Nishimune et al. 2008). In-gel activity assays would be another way to assess these differences. Generally co-substrate utilization tests are conducted on aqueous total protein preparations, which do not allow resolution of differences in co-substrate use among isoforms unless isoforms are first isolated via IEF. Understanding co-substrate usage has practical applications for questions about variation in thiaminase among fish species. The two most widely used methods to assess thiaminase in Great Lakes fishes are the radiometric method (Zajicek et al. 2005) and the 4-nitrothiolphenol (4-NTP) method (Kraft et al. 2014). The radiometric method can accommodate any co-substrate (nicotinic acid is usually the choice because it appears to be broadly used by fishes), but the 4-NTP method must use 4-NTP as the

co-substrate. Red Cornetfish thiaminase did not use nicotinic acid as a co-substrate (Nishimune et al. 2008), and presumably by the radiometric assay, Red Cornetfish would appear thiaminase-negative with the standard procedure used to assess thiaminase activity in Great Lakes fishes. Differences in co-substrate utilization by different species may contribute to reasons why the thiaminase activity in some fish species does not seem to be correlated for the radiometric and 4-NTP assays (Honeyfield et al. 2010a).

Three of the four candidate genes we identified did not produce soluble, active thiaminases. The lack of thiaminase activity could have resulted either because the candidate genes do not, in fact, encode thiaminases, or because of improper post-translational processing of the candidate thiaminases in the expression systems we used. Improper post-translational processing of the eukaryotic proteins by prokaryotic expression systems is not uncommon. We expect that the protein products produced by the candidate genes in Alewife and Carp that are homologous to the Red Cornetfish thiaminase do encode thiaminases for two reasons. First, the alignments show a close relationship between the Zebrafish gene that we have shown to produce a thiaminase, and the candidate Carp and Alewife genes homologous to the sequence reported by Nishimune et al. (2008). Secondly, the candidate gene for Alewife is expected to produce a protein whose size and pI is an exact match to those we observed empirically. We suspect that technical reasons associated with either expressing a eukaryotic gene in a prokaryotic host or expressing a protein that degrades an essential nutrient prevented us from being able to demonstrate thiaminase activity at this time.

Taken together, our biochemical and genetic findings suggest that each of the organisms we have investigated encodes a thiaminase *de novo*. An alternative explanation that we cannot definitively rule out is that that Carp and Alewife obtain thiaminase from production by intestinal microbial flora. That the Zebrafish thiaminase we have discovered is responsible for most or all of the thiaminase activity in Zebrafish should be confirmed experimentally in light of our findings. Without this confirmation, we cannot rule out the possibility that thiaminase produced by bacteria in the gut of fish also contributes to, or perhaps even constitutes the majority of, the thiaminase activity in some thiaminase-containing fishes (Kraft et al.

2014). Although our previous work investigating zooplankton and macroinvertebrates as dietary sources of thiaminase (Chapter 1 and Chapter 3) suggest that no consistent source of thiaminase across the Great Lakes, with the exception of PT (Richter et al. 2012), we have not undertaken a thorough investigation of other bacterial taxa known to produce thiaminase (Sikowitz et al. 2013, Kraft et al. 2014). However, we have definitively proven the capacity for *de novo* synthesis in fishes, and with regard to Alewife specifically, we view the expectation that the candidate gene identified in Alewife encodes a thiaminase as strongly implicating *de novo* synthesis as the primary source of thiaminase activity in Alewife. Nonetheless, multiple sources of thiaminase activity are not mutually exclusive.

The *in-vivo* function of the Zebrafish protein has yet to be determined. The Zebrafish gene contains three intronic sequences, and the zebrafish cDNAs that encode putative thiaminases are located on two separate zebrafish chromosomes; one chromosomal copy encodes a cDNA with a known 3" untranslated region and the other encodes a cDNA with a known 5" untranslated region. The physiological function of thiaminase is "a long-standing unsolved problem in thiamin physiology" (Soriano et al. 2008). The function of thiaminase can be more easily unraveled with modern genetic and proteomic tools now that the gene encoding the protein is known and a recombinant version of the protein is available. Knowing that fish are capable of producing a thiaminase *de novo* should drive future investigations for questions of fishery management importance such as understanding the factors that cause thiaminase activity in fish to vary.

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4.7 Literature Cited

Abe, M., T. Nishimune, S. Ito, M. Kimoto, and R. Hayashi. 1986. A simple method for the detection of 2 types of thiaminase-producing colonies. Fems Microbiology Letters. 34:129-133.

Abe, M., S. Ito, M. Kimoto, R. Hayashi, and T. Nishimune. 1987. Molecular studies on thiaminase I. Biochimica Et Biophysica Acta. 909:213-221.

Baba, H., Y. Masuda, N. Sueyoshi, and I. Kameshita. 2012. In-gel phosphatase assay using non-denaturing two-dimensional electrophoresis. Journal of Biochemistry. 152:557-563.

Bischoff, K. M., L. Shi, and P. J. Kennelly. 1998. The detection of enzyme activity following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Analytical Biochemistry. 260:1-17.

Bos, M. and A. Kozik. 2000. Some molecular and enzymatic properties of a homogeneous preparation of thiaminase I purified from carp liver. Journal of Protein Chemistry. 19:75-84.

Bronte, C. R., P. C. McKee, and M. E. Holey. 2006. Relative survival of lake trout stocked at different sizes and quality in Lake Michigan. Journal of Great Lakes Research. 32:386-394.

Bronte, C. R., M. E. Holey, C. P. Madenjian, J. L. Jonas, R. M. Claramunt, P. C. McKee, M. L. Toneys, M. P. Ebener, B. Breidert, G. W. Fleischer, R. Hess, A. W. Martell, and E. J. Olsen. 2007. Relative abundance, site fidelity, and survival of adult lake trout in Lake Michigan from 1999 to 2001: Implications for future restoration strategies. North American Journal of Fisheries Management. 27:137-155.

Bronte, C. R., C. C. Krueger, M. E. Holey, M. L. Toneys, R. L. Eshenroder, and J. L. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fishery Commission Miscellaneous Publication 2008-01. 39 pages. Available at http://www.glfc.org/pubs/pub.htm#misc.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005c. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005d. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health. 17:59-64.

Bunnell, D. B., C. P. Madenjian, J. D. Holuszko, J. V. Adams, and J. R. P. French. 2009. Expansion of *Dreissena* into offshore waters of Lake Michigan and potential impacts on fish populations. Journal of Great Lakes Research. 35:74-80.

Campobasso, N., C. A. Costello, C. Kinsland, T. P. Begley, and S. E. Ealick. 1998. Crystal structure of thiaminase-I from *Bacillus thiaminolyticus* at 2.0 angstrom resolution. Biochemistry. 37:15981-15989.

Claramunt, R. M., J. L. Jonas, J. D. Fitzsimons, and J. E. Marsden. 2005. Influences of spawning habitat characteristics and interstitial predators on lake trout egg

deposition and mortality. Transactions of the American Fisheries Society. 134:1048-1057.

Costello, C., N. Kelleher, A. Mitsuko, F. McLafferty, and T. P. Begley. 1996. Mechanistic studies on thiaminase I. Journal of Biological Chemistry. 271:3445-3452.

Czesny, S., J. Epifanio, and P. Michalak. 2012. Genetic divergence between freshwater and marine morphs of alewife (*Alosa pseudoharengus*): A 'next-generation' sequencing analysis. PLOS ONE. 7:e31803.

DesJardine, R. L., T. K. Gorenflo, R. N. Payne, and J. D. Schrouder. 1995. Fish-community objectives for Lake Huron. Great Lakes Fishery Commission Special Publication. 95-1:1-37.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark, Jr. 1995. Fish-community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 1995-3:1-56.

Fisher, J. P., J. M. Spitsbergen, H. Symula, J. Skea, M. Babenzein, and T. Chiotti. 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the "Cayuga syndrome". Journal of Aquatic Animal Health. 7:81-94.

Fisher, J. P., J. D. Fitzsimons, G. F. Combs, and J. M. Spitsbergen. 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Transactions of the American Fisheries Society. 125:167-178.

Fitzsimons, J. D. 1995. The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. Journal of Great Lakes Research. 21:286-289.

Fitzsimons, J. D., B. Williston, P. Amcoff, L. Balk, C. Pecor, H. G. Ketola, J. P. Hinterkopf, and D. C. Honeyfield. 2005a. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine-deficient coho salmon. Journal of Aquatic Animal Health. 17:48-58.

Fitzsimons, J. D., B. Williston, J. L. Zajicek, D. E. Tillitt, S. B. Brown, L. R. Brown, D. C. Honeyfield, D. M. Warner, L. G. Rudstam, and W. Pearsall. 2005b. Thiamine content and thiaminase activity of ten freshwater stocks and one marine stock of alewives. Journal of Aquatic Animal Health. 17:26-35.

Fitzsimons, J. D., B. Williston, G. Williston, L. Brown, A. El-Shaarawi, L. Vandenbyllaardt, D. Honeyfeld, D. Tillitt, M. Wolgamood, and S. B. Brown. 2007. Egg thiamine status of Lake Ontario salmonines 1995-2004 with emphasis on lake trout. Journal of Great Lakes Research. 33:93-103.

Fujita, A. 1954. Thiaminase. Advances in Enzymology and Related Subjects of Biochemistry. 15:389-421.

Gorg, A., A. Klaus, C. Luck, F. Weiland, and W. Weiss. 2007. Two-dimensional electrophoresis with immobilized pH gradients for proteome analysis. Technische Universitat Munchen. 166 pages. Available at http://proteomik.wzw.tum.de/fileadmin/documents/2D_Manual-Gorg_2007.pdf.

Green, R. G., W. E. Carlson, and C. A. Evans. 1941. A deficiency disease of foxes produced by feeding fish. Journal of Nutrition. 21:243-256.

Healthcare, G. 2009. Immobiline DryStrip. Instructions 28-9537-55 AA. GE Healthcare.

Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown, J. E. Marsden, D. S. Lavis, M. L. Toneys, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. Journal of Great Lakes Research. 21:128-151.

Honeyfield, D. C., J. P. Hinterkopf, and S. B. Brown. 2002. Isolation of thiaminase-positive bacteria from alewife. Transactions of the American Fisheries Society. 131:171-175.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. W. Hanes, L. Brown, C. E. Kraft, and T. P. Begley. 2010a. Comparison of thiaminase activity in fish using the radiometric and 4-nitrothiophenol colorimetric methods. Journal of Great Lakes Research. 36:641-645.

Honeyfield, D. C., M. E. Daniels, L. R. Brown, M. T. Arts, M. G. Walsh, and S. B. Brown. 2012. Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species. Journal of Great Lakes Research. 38:11-17.

Horns, W. H., C. R. Bronte, T. R. Busiahn, M. P. Ebener, R. L. Eshenroder, T. Gorenflo, N. Kmiecik, W. Mattes, J. W. Peck, M. Petzold, and D. R. Schreiner. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication. 03-01:i-ii, 1-78.

Ishihara, T., H. Kinari, and M. Yasuda. 1973. Studies on thiaminase I in marine fish - II. Distribution of thiaminase in marine fish. Bulletin of the Japanese Society of Scientific Fisheries. 39:55-59.

Jenkins, A. H., G. Schyns, S. Potot, G. X. Sun, and T. P. Begley. 2007. A new thiamin salvage pathway. Nature Chemical Biology. 3:492-497.

Ji, Y. Q. and I. R. Adelman. 1998. Thiaminase activity in alewives and smelt in lakes Huron, Michigan, and Superior. Pages 154-159 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Jonas, J. L., R. M. Claramunt, J. D. Fitzsimons, J. E. Marsden, and B. J. Ellrott. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 62:2254-2264.

Kameshita, I., A. Ishida, S. Okuno, and H. Fujisawa. 1997. Detection of protein phosphatase activities in sodium dodecyl sulfate-polyacrylamide gel using peptide substrates. Analytical Biochemistry. 245:149-153.

Ketola, H. G., T. L. Chiotti, R. S. Rathman, J. D. Fitzsimons, D. C. Honeyfield, P. J. Van Dusen, and G. E. Lewis. 2005. Thiamine status of Cayuga Lake rainbow trout and its influence on spawning migration. North American Journal of Fisheries Management. 25:1281-1287.

Kleiner, D. E. and W. G. Stetler-Stevenson. 1994. Quantitative zymography - detection of picogram quantities of gelatinases. Analytical Biochemistry. 218:325-329.

Kraft, C. E., E. R. L. Gordon, and E. R. Angert. 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLOS ONE. 9:e92688.

Kreinbring, C. A., S. P. Remillard, P. Hubbard, H. R. Brodkin, F. J. Leeper, D. Hawksley, E. Y. Lai, C. Fulton, G. A. Petsko, and D. Ringe. 2014. Structure of a eukaryotic thiaminase I. Proceedings of the National Academy of Sciences. 111:137-142.

Krueger, C. C., M. L. Jones, and W. W. Taylor. 1995a. Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. Journal of Great Lakes Research. 21:547-558.

Lepak, J. M., C. E. Kraft, D. C. Honeyfield, and S. B. Brown. 2008. Evaluating the effect of stressors on thiaminase activity in alewife. Journal of Aquatic Animal Health. 20:63-71.

Lepak, J. M., C. E. Kraft, and M. J. Vanni. 2013. Clupeid response to stressors: The influence of environmental factors on thiaminase expression. Journal of Aquatic Animal Health. 25:90-97.

Manchenko, G. P. 2003. Handbook of detection of enzymes on electrophoretic gels. 2nd ed. edition. Boca Raton : CRC Press, Boca Raton.

Marcquenski, S. V. and S. B. Brown. 1997. Early mortality syndrome (EMS) in salmonid fishes from the Great Lakes. Pages 135-152 *in* R. M. Rolland, M. Gilbertson, and R. E. Peterson, editors. Chemically induced alterations in functional development and reproduction of fishes. SETAC (Society of Environmental Toxicology and Chemistry), Pensacola, FL.

Nishimune, T., Y. Watanabe, and H. Okazaki. 2008. Studies on the polymorphism of thiaminase I in seawater fish. Journal of Nutritional Science and Vitaminology. 54:339-346.

Okonji, R. E., K. S. Bamitale, and R. O. Balogun. 2012. Biochemical properties of thiaminase from *Anaphe venata* Burtler. African Journal of Biotechnology. 11:4543-4550.

Puzach, S. S., Z. V. Gorbach, and Y. M. Ostrovskii. 1984. Isolation, purification, and certain properties of thiaminase I from *Anodonta cygnea*. Biochemisty-Moscow. 49:1010-1016.

Richter, C. A., A. N. Evans, M. K. Wright-Osment, J. L. Zajicek, S. A. Heppell, S. C. Riley, C. C. Krueger, and D. E. Tillitt. 2012. *Paenibacillus thiaminolyticus* is not the cause of thiamine deficiency impeding lake trout (*Salvelinus namaycush*) recruitment in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 69:1056-1064.

Riley, S. C. and A. N. Evans. 2008. Phylogenetic and ecological characteristics associated with thiaminase activity in Laurentian Great Lakes fishes. Transactions of the American Fisheries Society. 137:147-157.

Rivoal, J., C. R. Smith, T. F. Moraes, D. H. Turpin, and W. C. Plaxton. 2002. A method for activity staining after native polyacrylamide gel electrophoresis using a coupled enzyme assay and fluorescence detection: Application to the analysis of several glycolytic enzymes. Analytical Biochemistry. 300:94-99.

Ryan, P. A., R. Knight, R. MacGregor, G. Towns, R. Hoopes, and W. Culligan. 2003. Fish-community goals and objectives for Lake Erie. Great Lakes Fishery Commission Special Publication. 03-02:1-56.

Salaman, M. R. and A. R. Williams. 1971. Isoelectric focusing of proteins in native and denatured states - anomalous behavior of plasma albumin. Biochemical Journal. 122:93-99.

Sambrook, J. and D. W. Russell. 2001. Molecular cloning: a laboratory manual. Third edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schagger, H. and G. Vonjagow. 1991. Blue native electrophoresis for isolation of membrane-protein complexes in enzymatically active form. Analytical Biochemistry. 199:223-231.

Schamel, W. W. A. 2001. Two-Dimensional Blue Native Polyacrylamide Gel Electrophoresis *in* Current Protocols in Cell Biology. John Wiley & Sons, Inc.

Sikowitz, M. D., B. Shome, Y. Zhang, T. P. Begley, and S. E. Ealick. 2013. Structure of a *Clostridium botulinum* C143S Thiaminase I/Thiamin Complex Reveals Active Site Architecture. Biochemistry. 52:7830-7839.

Simpson, R. J., P. D. Adams, and E. Golemis. 2009. Basic methods in protein purification and analysis: a laboratory manual. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Soriano, E. V., K. R. Rajashankar, J. W. Hanes, S. Bale, T. P. Begley, and S. E. Ealick. 2008. Structural similarities between thiamin-binding protein and thiaminase-I suggest a common ancestor. Biochemistry. 47:1346-1357.

Stewart, T. J., R. E. Lange, S. D. Orsatti, C. P. Schneider, A. Mathers, and M. E. Daniels. 1999. Fish-community objectives for Lake Ontario. Great Lakes Fishery Commission Special Publication. 99-1:1-55.

Tillitt, D. E., J. L. Zajicek, S. B. Brown, L. R. Brown, J. D. Fitzsimons, D. C. Honeyfield, M. E. Holey, and G. M. Wright. 2005. Thiamine and thiaminase status in forage fish of salmonines from Lake Michigan. Journal of Aquatic Animal Health. 17:13-25.

Tillitt, D. E., S. C. Riley, A. N. Evans, S. J. Nichols, J. L. Zajicek, J. Rinchard, C. A. Richter, and C. C. Krueger. 2009. Dreissenid mussels from the Great Lakes contain elevated thiaminase activity. Journal of Great Lakes Research. 35:309-312.

Werner, R. M., B. Rook, and R. Greil. 2006. Egg-thiamine status and occurrence of early mortality syndrome (EMS) in Atlantic salmon from the St. Marys River, Michigan. Journal of Great Lakes Research. 32:293-305.

Wistbacka, S., A. Heinonen, and G. Bylund. 2002. Thiaminase activity of gastrointestinal contents of salmon and herring from the Baltic Sea. Journal of Fish Biology. 60:1031-1042.

Wistbacka, S. and G. Bylund. 2008. Thiaminase activity of Baltic salmon prey species: a comparision of net- and predator-caught samples. Journal of Fish Biology. 72:787-802.

Wistbacka, S., L. G. Lonnstrom, E. Bonsdorff, and G. Bylund. 2009. Thiaminase activity of Crucian carp *Carassius carassius* injected with a bacterial fish pathogen, *Aeromonas salmonicida* subsp. *salmonicida*. Journal of Aquatic Animal Health. 21:217-228.

Wittliff, J. L. and R. L. Airth. 1968. The extracellular thiaminase I of *Bacillus thiaminolyticus*. I. Purification and physicochemical properties. Biochemistry. 7:736-744.

Wolgamood, M., J. G. Hnath, S. B. Brown, K. Moore, S. V. Marcquenski, D. C. Honeyfield, J. P. Hinterkopf, J. D. Fitzsimons, and D. Tillit. 2005. Temporal and spatial variation of early mortality syndrome in salmonids from lakes Michigan and Huron. Journal of Aquatic Animal Health. 17:65-76.

Yokoyama, R., Y. Iwafune, H. Kawasaki, and H. Hirano. 2009. Isoelectric focusing of high-molecular-weight protein complex under native conditions using agarose gel. Analytical Biochemistry. 387:60-63.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

4.8 Figures

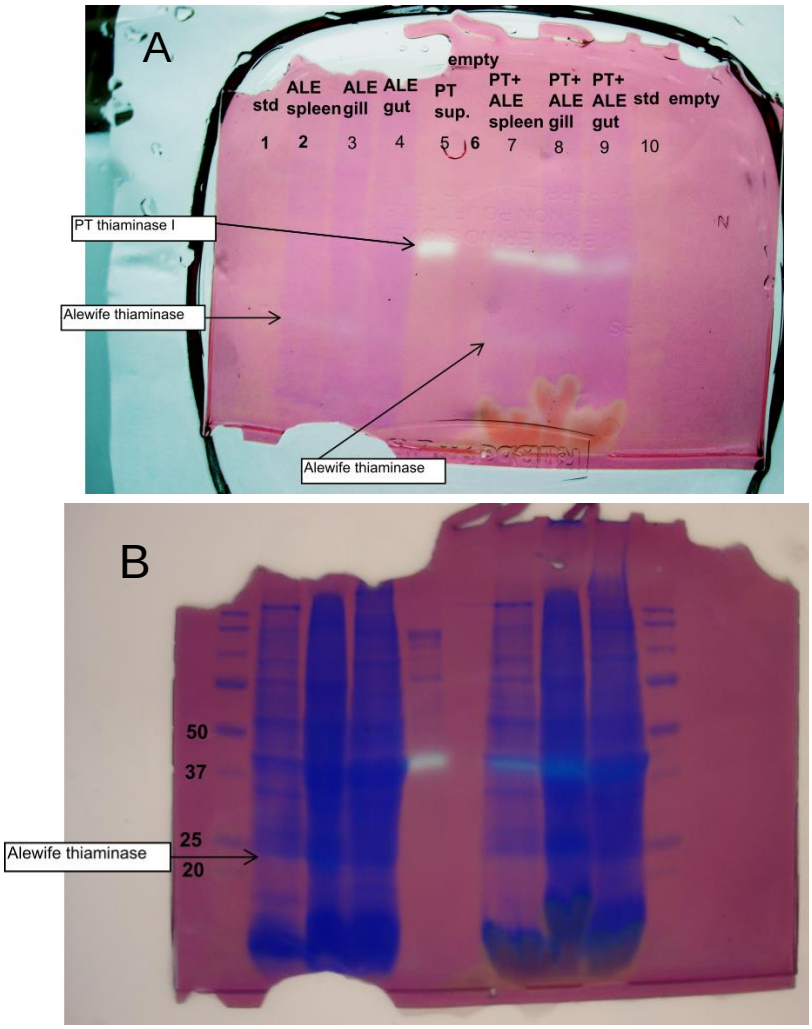

Figure 4.1 Size determination for Alewife thiaminase

Two images of the same SDS-PAGE gel showing the size determination for the Alewife and PT thiaminases. Panel A: Activity stain in which with the location of the thiaminases noted by the white clearing showing thiamine degradation against the red background showing remaining thiamine. Pyridine was used as the co-substrate for the thiaminase activity assay. Panel B: Coomassie blue staining of the gel in Panel A to reveal the location of the thiaminases relative to the molecular weight standards, which are marked on the gel in kDa. ALE= Alewife. Three different preparations of Alewife tissue (gill, spleen, and gut) are shown.

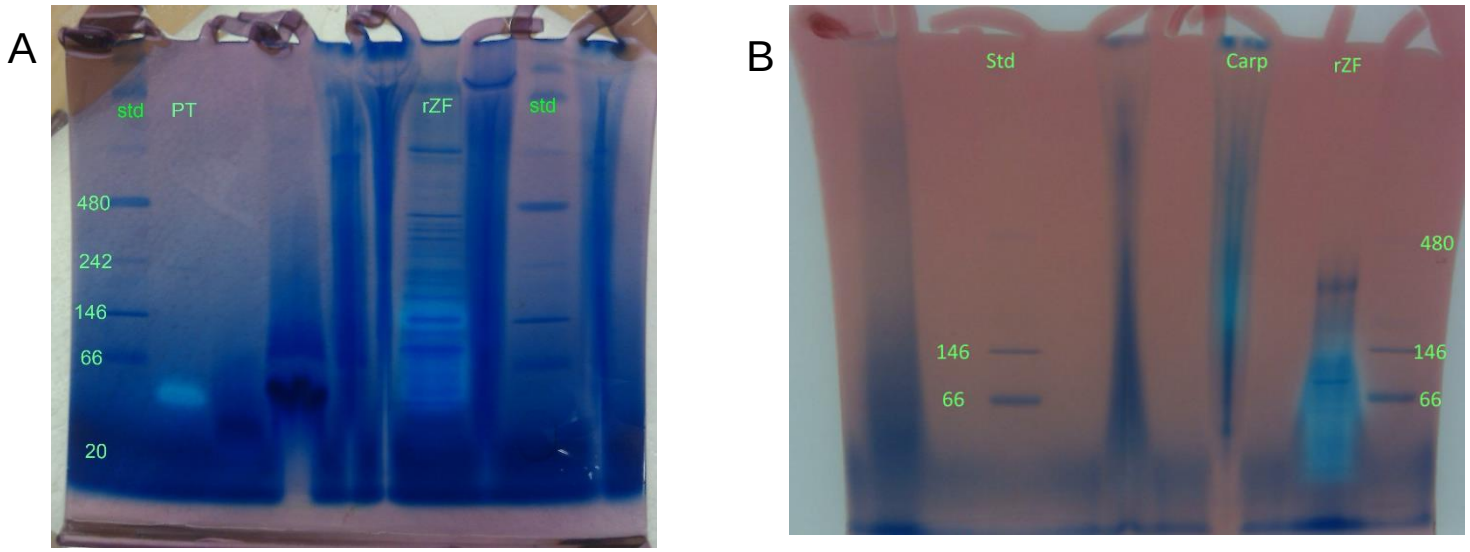

Figure 4.2 Holoenzyme size asssessment by blue-native PAGE thiaminases

Native-PAGE gel stained with activity stain showing the holoenzyme size determinations for PT (panel A), Carp (panel B), and recombinant Zebrafish (panel A and B) thiaminases. The location of the white clearing indicates thiaminases against the red, thiamine-containing background. The gel in panel A used dark blue cathode buffer, and the gel in panel B used light blue cathode buffer. Pyridine was used as the co-substrate for the thiaminase activity assay.

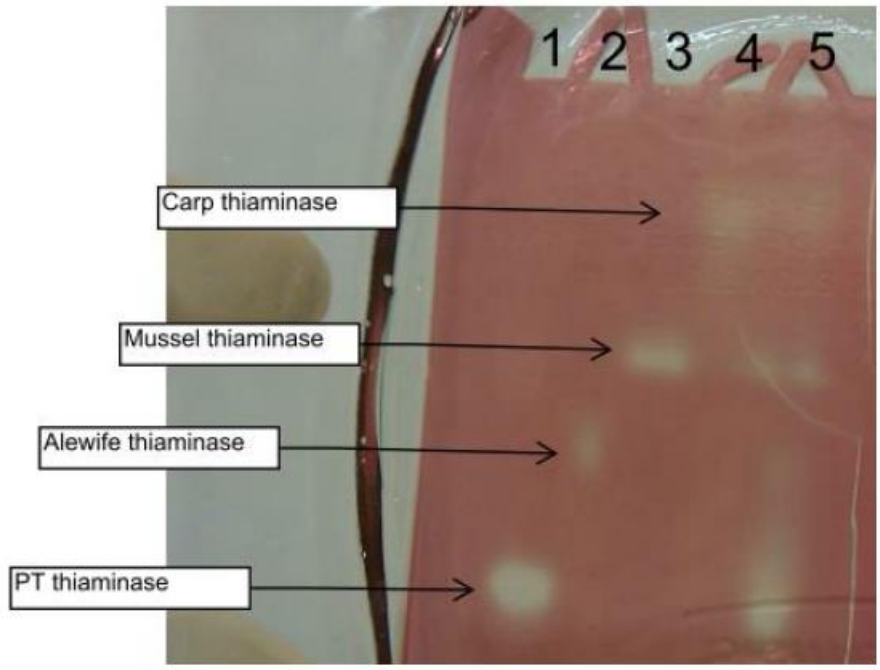

Figure 4.3 Migration speed comparison among thiaminases

Native-PAGE gel stained with activity stain showing the size determination for the Alewife and PT thiaminases. . Lane 1: PT; Lane 2: Alewife; Lane 3: quagga mussel; Lane 4: Carp; Lane 5: mixture of PT, Alewife, quagga mussel, and Carp protein preparations. The location of the white clearing indicates thiaminases against the red, thiamine-containing background. Nicotinic acid was used as the co-substrate for the thiaminase activity assay.

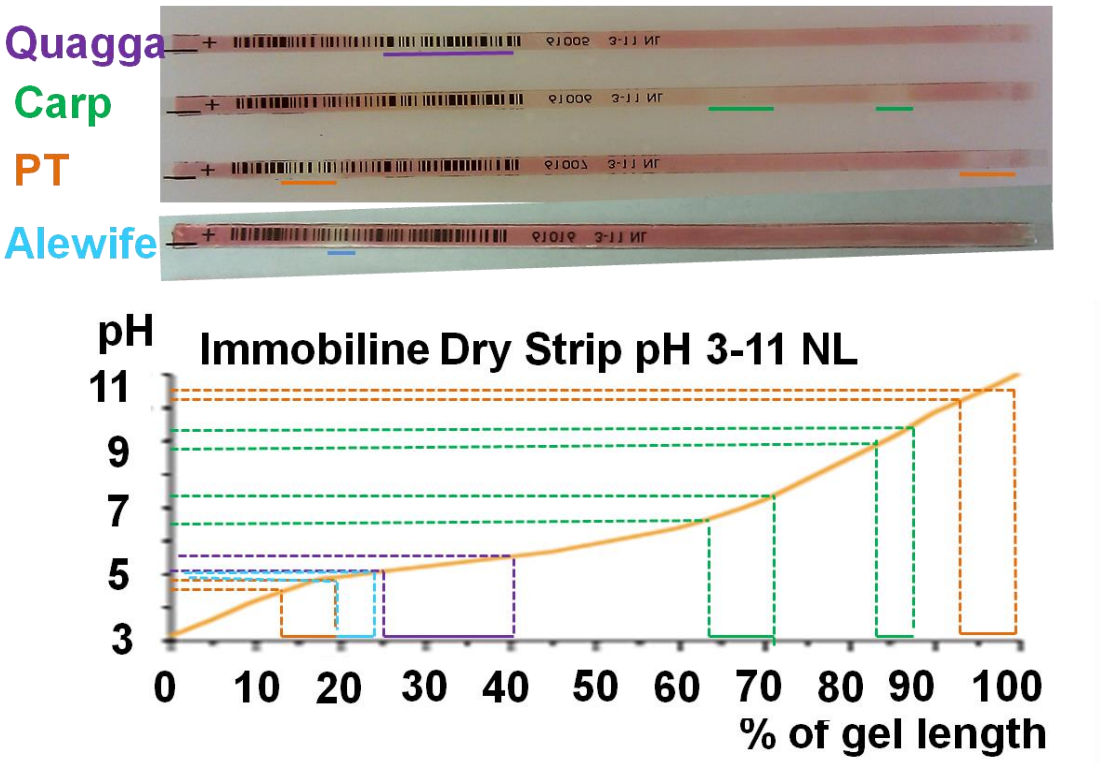

Figure 4.4 Native isoelectric point comparison among thiaminases

Activity staining of native isoelectric focusing of four thiaminases. After focusing, the isoelectric focusing strips are stained with the activity stain; white areas on the red background indicate thiaminase activity (underlined areas). Top panel: each organism's thiaminase is focused on a separate gel. Bottom panel: the position of the thiaminase activity on the strip is compared to the pH gradient of the strip to estimate the pI of the thiaminase(s) for each organism.

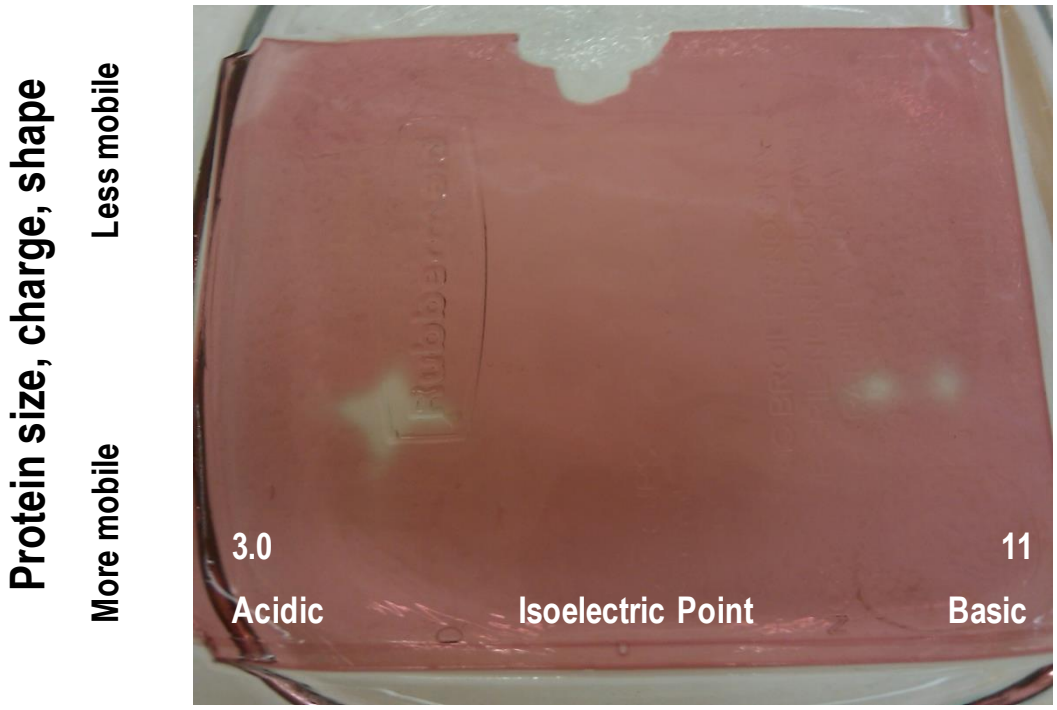

Figure 4.5 Native-native 2D gel of PT culture supernatant

A native-native 2-dimensional gel of PT culture supernatant. The first dimension (X direction) is isoelectric focusing under native conditions, and the second dimension (Y direction) is a native-PAGE, separating proteins on the basis of a combination of mass, charge, and size. The result clearly shows 3 isoforms of thiaminase in native conformation (indicated by the white clearing). Pyridine was used as the co-substrate for the thiaminase assay. The two basic isoforms are at pIs of ~ 9 and ~10.

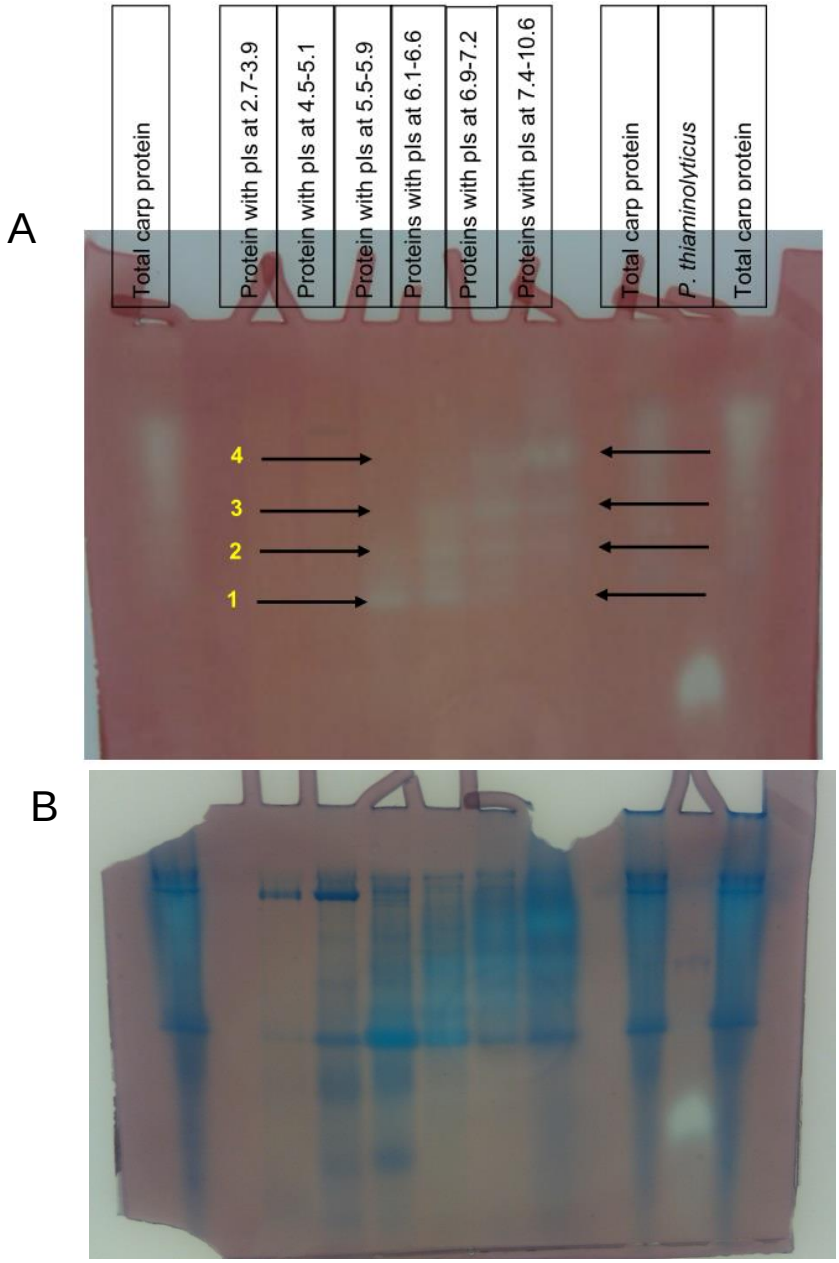

Figure 4.6 Native 2D gel of previously fractionated Carp protein preparations

A native gel of Carp proteins that were fractioned in-liquid, with specific fractions applied to each lane of the gel. The pIs of the fractions from in-liquid IEF are noted above each lane. Liquid isoelectric focusing was conducted under native conditions. The arrows show the four distinct isoforms of Carp, with pIs ranging from 5.5 to 10.6. Pyridine was used as the co-substrate for the thiaminase activity assay. Panel A and B are the same gel, with A showing only the activity stain and B showing the total protein stain. Activity was assayed with pyridine as the co-substrate in pH 5.2 citrate-phosphate buffer.

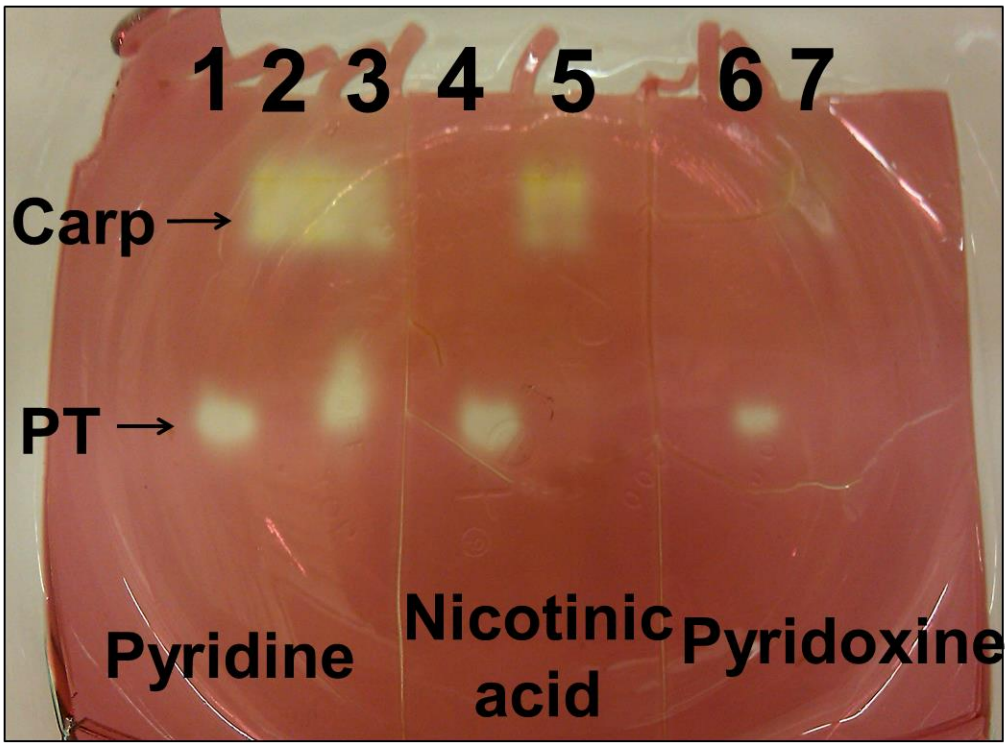

Figure 4.7 Native gel showing co-substrate utilization

A native gel of Carp and PT thiaminase showing differential co-substrate utilization patterns. Lanes 1-3 were assayed with pyridine as the co-substrate, lanes 4-5 were assayed with nicotinic acid as the co-substrate, and lanes 6-7 were assayed with pyridoxine as the co-substrate.

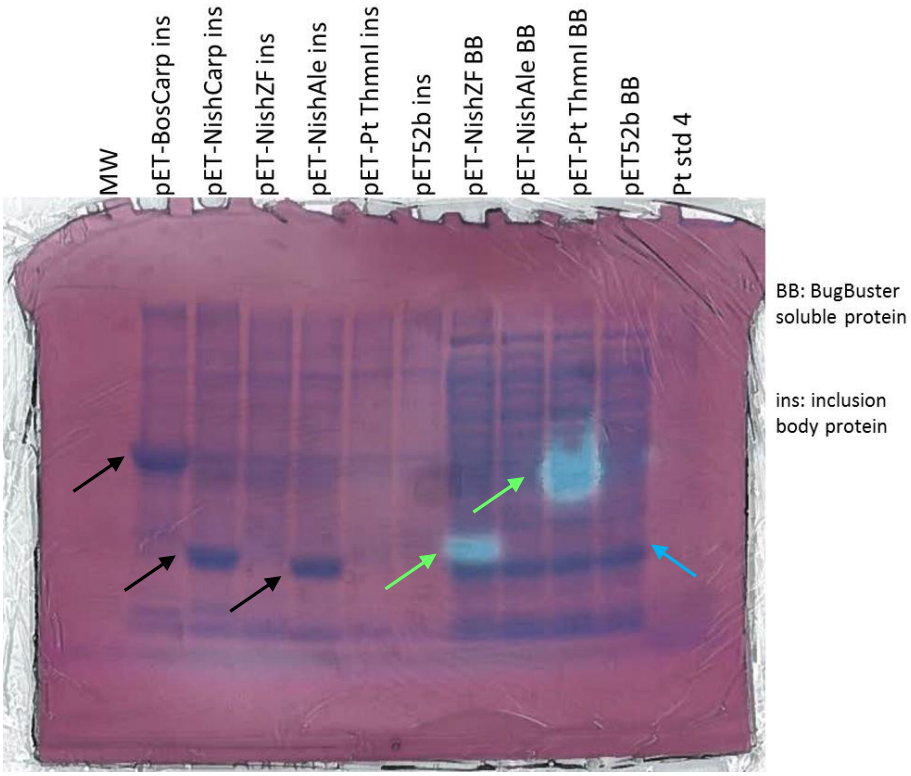

Figure 4.8 SDS-PAGE showing production of thiaminase by one candidate gene

An SDS-PAGE gel of the protein products produced by candidate genes overexpressed in *E. coli*. Lanes marked "ins" are preparations of insoluble protein from inclusion bodies illustrating that proteins of the appropriate size are being produced by the bacterial expression systems (black arrows). Lanes marked "BB" are preparations of soluble proteins and illustrate that the only recombinant genes gene that produces a soluble and active thiaminase are the Zebrafish gene that is homologous to the sequence of Nishimune et al. (2008) and the PT thiaminase that served as the positive control (green arrows). The vector-only control produces no thiaminase activity, as expected.

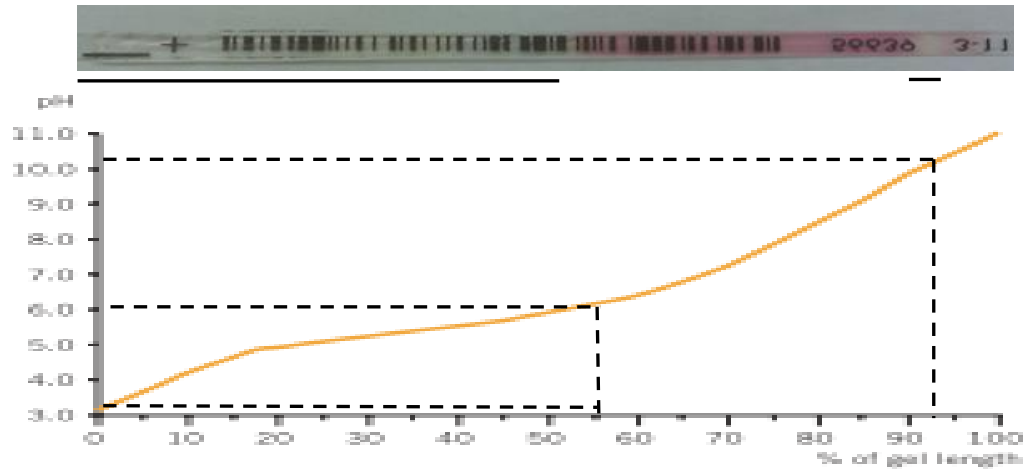

Figure 4.9 Native isoelectric point comparison among thiaminases

Activity stain of native isoelectric focusing of rZF thiaminases. After focusing, the isoelectric focusing strips are stained with the activity stain; white areas on the red background indicate thiaminase activity (underlined areas). Top panel: in-gel IEF. Bottom panel: the position of the thiaminase activity on the strip is compared to the pH gradient of the strip to estimate the pI of the thiaminase(s).

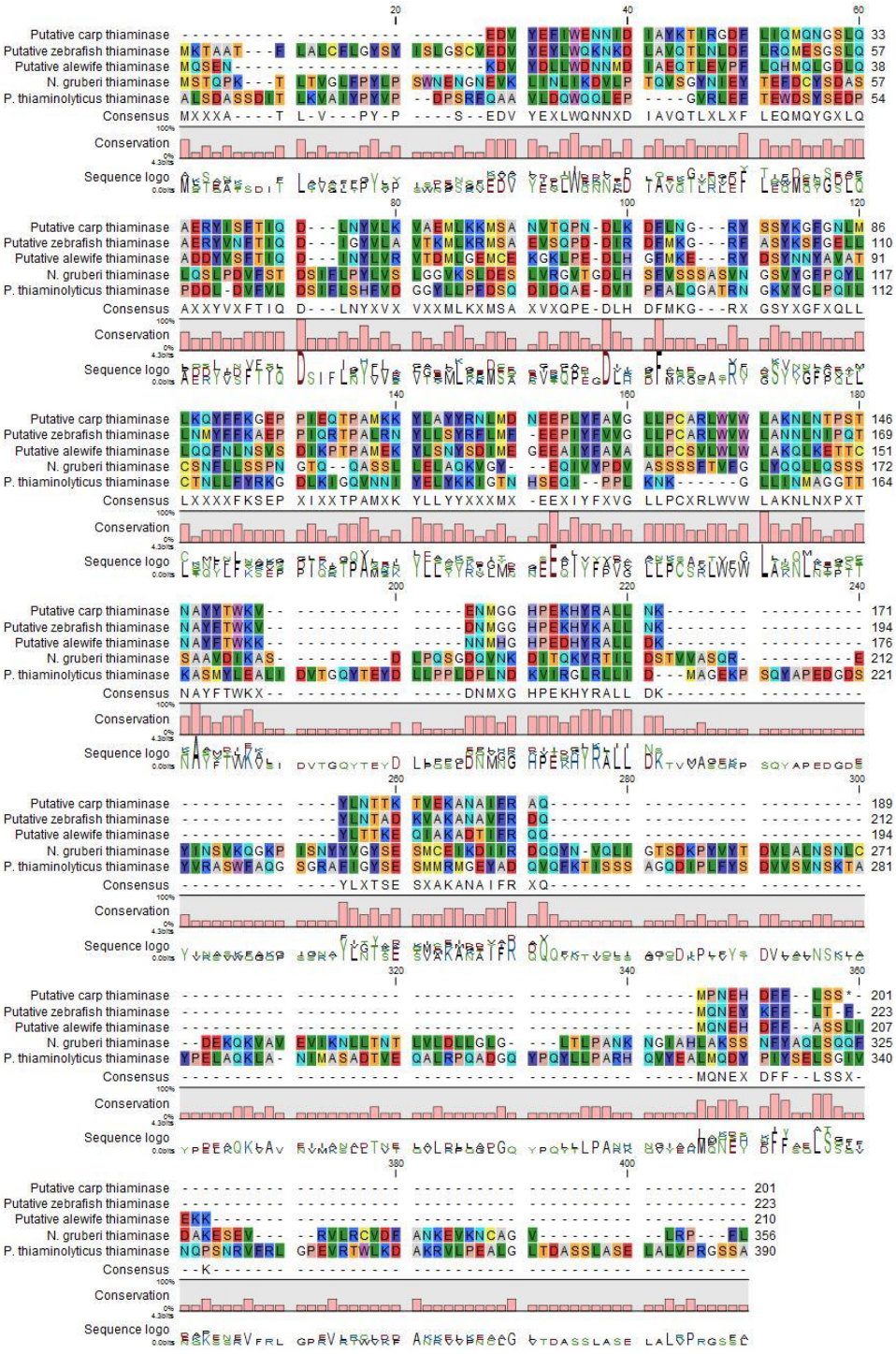

Figure 4.10 Alignments of known and putative thiaminases

Alignment of the putative Carp thiaminase, the thiaminase-encoding rZF gene sequence, the putative Alewife thiaminase, and the known thiaminases from PT and amoeba *N. gruberi*.

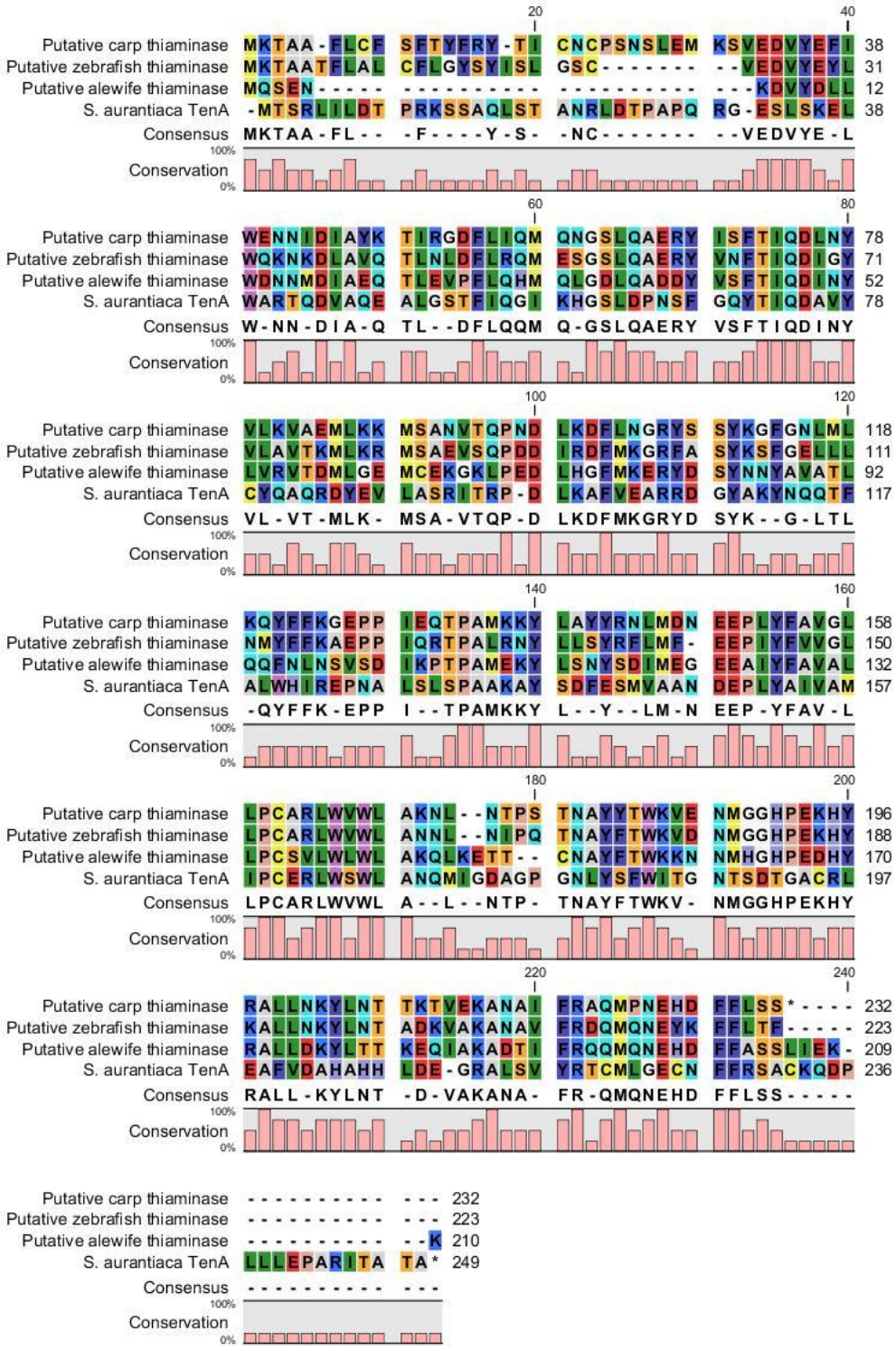

Figure 4.11 Alignments of known and candidate thiaminases to TenA

Protein sequence alignment of the full-length protein predicted from C. Carpio EST (EX880964.1), the *D. rerio* hypothetical protein (XM_002661366), putative Alewife protein, and TenA, a thiamine salvage enzyme, from *Stigmatella aurantiaca*.

4.9 Tables

Table 4.1 Summary of empirically determined characteristics of thiaminases

Summary of empirically determined characteristics of thiaminases. Blank cells means that the parameter of interest was not measured in other studies. Data in gray cells are from this study.

	Pt	*Pt*	Carp	Carp	Alewife	Quagga Mussel	Recombinant Zebrafish	Red cornetfish
Source of information	Previous studies*	This study	Bos and Kozik (2000)	This study	This study	This study	This study	Nishimune et al. 2008
Molecular Mass (M_r) when denatured	42,000	42,000 (Figure 4.1)	55,000	Sensitive to denaturation	~24,000 (Figure 4.1)	Sensitive to denaturation	~25,000 (on non-denaturing SDS-PAGE)	22,000 to 25,000
Relative Molecular Mass (M_r) in native confirmation	42,000	42,000 (Figure 4.2)	85,000–110,000	~ 250,000	Not assessed (Figure 4.2)	>480,000	~25,000 to ~ 100,000 indicating oligomerization	106,000 (by gel filtration)
Migration rate in native gel		Fastest (1) (Figure 4.3)		Slowest (4) (Figure 4.3)	Second fastest (2) (Figure 4.3)	Second slowest (3) (Figure 4.3)	Comparable to Carp (slowest; 4) (Figure 4.3)	
In-gel denaturation/ renaturation possible?	Yes	Yes		No	Yes	No	Only when not treated with heat	Yes
Number of isoforms (IEF)	1	3 (Figure 4.4;) Figure 4.5)	1	~4 (Figure 4.4; Figure 4.6)	1 (Figure 4.4)	1 (Figure 4.4)	2	2
pI of native thiaminase	4.6	4.6, ~9, ~10 (Figure 4.4; Figure 4.5)		Overlapping ~5.5-10.6 (Figure 4.6)	5.0 (Figure 4.4)	5.1-5.6 (Figure 4.4)	3-6; 10	5.7; ~7-9
Co-substrates tested with thiamine as a substrate (Figure 4.7)								
Pyridoxine	Yes	Yes	Not tested	No	No	No	Not tested	No
Nicotinic Acid	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Pyridine	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

*(Fujita et al. 1952, Wittliff and Airth 1968, Abe et al. 1987, Costello et al. 1996, Campobasso et al. 1998)

Table 4.2 Comparison of predicted biochemical characteristics derived from candidate gene sequences to empirically determined characteristics

Comparison of predicted biochemical characteristics derived from candidate gene sequences to empirically determined characteristics. Gray cells indicate predictions from candidate gene sequences and white cells indicate empirically determined characteristics.

Species	**Carp**			**Alewife**		**Zebrafish**	
Source of information	Predicted properties of protein encoded by candidate gene homologous to Bos and Kozik (2000)	Predicted properties of protein encoded by candidate gene homologous to Nishimune et al. (2008)	Empirical observation	Predicted properties of protein encoded by candidate gene homologous to Nishimune et al. (2008)	Empirical observation	Predicted properties of protein encoded by candidate gene homologous to Nishimune et al. (2008)	Empirical observation from recombinant protein
Mass when denatured	44,300	23,500	Unknown	24,600	~23,000-25,000	23,600	~24,000
Predicted isoelectric point	6.7	8.7	At least 4 isoforms, ranging from 5.6 to 10.6	5.0	5.0	9.0	3-6 and 10

5 CHAPTER 5
CONCLUSIONS

5.1 Synthesis

Herein, we have undertaken the most exhaustive investigation to date expressly aimed at identifying the sources of thiaminase in aquatic food webs. This work was part of a larger collaboration that sought to investigate the sources of thiaminase by adopting of a whole food web approach. This approach was unique relative to other research and was timely given the state of knowledge regarding the causes of TDC. The problem of TDC began at the top of food web, with the first observations of early life stage mortality in Lake Trout in the late 1980s and early 1990s (Mac et al. 1985, Marcquenski 1995). When thiamine, but none of the other B vitamins, was found to be therapeutic and rescue the phenotype (Fitzsimons 1995), the search for the cause of the deficiency began in earnest. By 2005, the proximate cause of thiamine deficiency in Lake Trout had been ascribed to ingestion of the thiaminase-containing prey fish, Alewife and Rainbow Smelt (Brown et al. 2005c), with lines of evidence coming from physiological studies illustrating the effect thiamine treatment (Hornung et al. 1998, Fitzsimons et al. 2001, Brown et al. 2005b, Fitzsimons et al. 2005a), assessments of thiamine status in Lake Trout (Brown et al. 2005d), correlations between thiamine status and diet in wild populations (Fitzsimons and Brown 1998), and controlled feeding studies (Honeyfield et al. 2005b).

Although *thiamine* levels in food webs were investigated early on as the potential cause for TDC in Lake Trout (Fitzsimons et al. 1998), *thiaminase* in food webs had not been studied in an integrated fashion, largely because a compelling body of evidence was only just becoming robust enough to be certain the proximate cause was ingestion of thiaminase-containing fishes. Thiaminase levels in fishes had been investigated to arrive at the level of understanding that existed in 2005, and at that time just one published report of thiaminase in lower trophic levels existing, consisting of two samples of *Diporeia*, two samples of bulk zooplankton, and two samples of *Mysis* (Zajicek et al. 2005). Our approach featured simultaneous sampling of bacteria (Richter et al. 2012), mussels (Tillitt et al. 2009), zooplankton (Chapter 2), prey fishes (Chapter 3; Chapter 4) and Lake Trout eggs (Riley et al. 2011) to provide a fully integrated perspective on thiaminase in Great Lakes food webs.

We eliminated PT as a source of thiaminase early in our investigations when neither the abundance of PT nor the abundance of PT thiaminase was related to thiaminase activity in prey fishes (Richter et al. 2012). We also identified the exceptionally high thiaminase levels in zebra and quagga mussels (Tillitt et al. 2009), a finding which would not have been possible without an integrated food web approach. Having eliminated PT as the likely source of thiaminase, we sought to identify candidate sources of thiaminase in zooplankton (Chapter 2). Options for investigating thiaminase in individual species of zooplankton are limited, as samples must be frozen as quickly as possible, leaving little room for selecting individuals of species other than those of the largest taxa. We adopted a correlational approach, recognizing that this approach would only provide an answer to the question if the source of thiaminase across different Great Lakes food webs was consistent. If there were a single taxon that was in high abundance in zooplankton samples containing high thiaminase and in low abundance in samples containing low thiaminase, we predicted that this approach would find it. Despite the initially appealing finding that *Ploesoma* biomass in bulk zooplankton tows was related to thiaminase activity, the very low percentage of zooplankton biomass accounted for by *Ploesoma* suggests that that from a practical perspective, this taxon was unlikely to be a major source of thiaminase activity.

Failing to find any plankton taxa that could realistically be expected to be the source of thiaminase in bulk plankton tows, we moved to assessing whether fish diets were related to thiaminase activity in fishes (Chapter 3). Just as some fishes may have thiaminase but not be primary food for Lake Trout (Trout-perch, for example) and therefore not contribute to TDC, some zooplankton may contain thiaminase but not be major contributors to the diets of thiaminase-containing fishes. Prior work showing that although fish species vary in their thiaminase activity, for the highest and lowest thiaminase species, the within-species variation is generally less than the among-species variation was an important concept in both formulating this approach as well as suggesting the possibility of *de novo* synthesis of thiaminase. The interaction of the fish diet portion of this work and the zooplankton community portion of this work in Chapter 2 was useful in further assessing the likely role of *Ploesoma* as a source of thiaminase activity in planktivores, and the ability to say that

thiaminase-containing fishes simultaneously sampled with the zooplankton tows ate no *Ploesoma* was one strength of the integrated food web approach we adopted.

In Chapter 3, we showed that there was no species whose abundance was consistently high when in thiaminase-containing fish and consistently low or absent in fish with low or no thiaminase activity. The only taxon suggested by multiple analytical methods was *Bythotrephes*, and although it is plausible that *Bythotrephes* could be the source of thiaminase for fishes ingesting it, *Bythotrephes* could not be the source of thiaminase in fishes that did not prey on *Bythotrephes*. Evaluating the consistency with which *Bythotrephes* could contribute to thiaminase activity in prey fish was aided by our integrated food web approach. Knowing directly from the work at the zooplankton level in Chapter 2 that the *Bythotrephes* had highly variable thiaminase activity and that its thiaminase activity was undetectable at some sites where it was consumed allowed us to evaluate the correlation suggested by the diet analysis from a practical perspective. Statistically, the fatty acid analysis showed a far greater degree of correlation with thiaminase activity than the stomach content data. Knowing now that *de novo* synthesis of thiaminase occurs in fish, the fatty acid correlations may be more explainable as either a reaction to thiaminase production by fishes or, perhaps more likely, an indicator of the conditions that induce production of thiaminase in fishes.

The pursuit of the *de novo* synthesis question in Chapter 4 was somewhat risky. The thiaminases in fishes (whatever their origin) could be unstable or otherwise intractable with which to work. We could find biochemical evidence of different thiaminases in different fishes, and yet there may be no genomic or proteomic data would available for comparison with the biochemical data. Such a result would still leave our primary question of interest unanswered. Biochemical evidence for different sources of thiaminase in different fishes does not rule out the possibility that fishes acquired thiaminase dietarily from separate lower trophic level organisms. When we began this work, the only gene definitively known that produced a thiaminase was that of PT. The *C. botulinum* work became available in 2013 and the *N. gruberi* work in 2014.

In combination, the evidence presented here not only proves the capacity for *de novo* synthesis in fishes but also strongly argues for general *de novo* production of thiaminase in other portions of the aquatic food web (i.e., mussels). This seems a likely mechanism for fishes given that they are one of a sporadic grouping of taxa containing thiaminases. The source of thiaminase in ruminants (Roberts and Boyd 1974, Boyd and Walton 1977) and birds (Shintani 1956) seems much more likely to be bacterial, as thiaminases are not known to occur in vertebrates other than fishes.

The need for this research was clear. Recent work in fish biology (Lepak et al. 2008, Honeyfield et al. 2010a, Honeyfield et al. 2012, Lepak et al. 2013) and in basic thiamine metabolism have speculated about both the *in-vivo* physiological function of and reason for the existence of thiaminases (Jenkins et al. 2007, Jenkins et al. 2008, Soriano et al. 2008, Fitzpatrick and Thore 2014, Kraft et al. 2014, Kreinbring et al. 2014). One increasingly common suggestion after the discovery that prokaryotic thiaminase IIs function in thiamine salvage (Toms et al. 2005, Jenkins et al. 2007, Jenkins et al. 2008) is that the thiaminase Is in fishes also play a role in salvaging and recycling thiamine ring structures. This suggestion is particularly interesting in light of the observations that high-quality (presumably thiamine-rich) food is associated with increased thiaminase production in fishes (Lepak et al. 2008, Lepak et al. 2013). However, this would seem somewhat at odds with repeated observations Alewife captured spring have higher thiaminase than in summer or fall. We observed the highest levels of thiaminase in Alewife in the spring as compared to summer or fall, and Alewife at this time of year consumed less total mass of food than at any other season.

Identifying the first known thiaminase gene from a multicellular organism should propel future research on the questions of what causes variation in thiaminase activity (i.e., Figure 1.1) but also should inspire structural studies to determine whether vertebrate thiaminases retain major structural similarities to other thiaminases despite obvious sequence differences, such as the structural similarities maintained by PT and *N. gruberi* (Kreinbring et al. 2014). This work has primary relevance not only for the fishery management community in the Great Lakes, but also animal nutritionists, nutritional chemists, and biochemists interested in thiamine metabolism.

5.2 Literature Cited

Boyd, J. W. and J. R. Walton. 1977. Cerebrocortical necrosis in ruminants: an attempt to identify source of thiaminase in affected animals. Journal of Comparative Pathology. 87:581-589.

Brown, S. B., L. R. Brown, M. Brown, K. Moore, M. Villella, J. D. Fitzsimons, B. Williston, D. C. Honeyfield, J. P. Hinterkopf, D. E. Tillit, J. L. Zajicek, and M. Wolgamood. 2005b. Effectiveness of egg immersion in aqueous solutions of thiamine and thiamine analogs for reducing early mortality syndrome. Journal of Aquatic Animal Health. 17:106-112.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005c. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005d. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health. 17:59-64.

Fitzpatrick, T. B. and S. Thore. 2014. Complex behavior: from cannibalism to suicide in the vitamin B-1 biosynthesis world. Current Opinion in Structural Biology. 29:34-43.

Fitzsimons, J. D. 1995. The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. Journal of Great Lakes Research. 21:286-289.

Fitzsimons, J. D. and S. B. Brown. 1998. Reduced egg thiamine levels in inland and Great Lakes trout and their relationship with diet. Pages 160-171 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. B. Brown, and L. Vandenbyllaardt. 1998. Thiamine levels in food chains of the Great Lakes. Pages 90-98 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., L. Vandenbyllaardt, and S. B. Brown. 2001. The use of thiamine and thiamine antagonists to investigate the etiology of early mortality syndrome in lake trout (*Salvelinus namaycush*). Aquatic Toxicology. 52:229-239.

Fitzsimons, J. D., B. Williston, P. Amcoff, L. Balk, C. Pecor, H. G. Ketola, J. P. Hinterkopf, and D. C. Honeyfield. 2005a. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine-deficient coho salmon. Journal of Aquatic Animal Health. 17:48-58.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. W. Hanes, L. Brown, C. E. Kraft, and T. P. Begley. 2010a. Comparison of thiaminase activity in fish using the radiometric and 4-nitrothiophenol colorimetric methods. Journal of Great Lakes Research. 36:641-645.

Honeyfield, D. C., M. E. Daniels, L. R. Brown, M. T. Arts, M. G. Walsh, and S. B. Brown. 2012. Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species. Journal of Great Lakes Research. 38:11-17.

Hornung, M. W., L. Miller, R. E. Peterson, S. V. Marcquenski, and S. B. Brown. 1998. Efficacy of thiamine, astaxanthin, β-carotene, and thyroxine treatments in reducing early mortality syndrome in Lake Michigan salmonid embryos. Pages 124-134 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Jenkins, A. H., G. Schyns, S. Potot, G. X. Sun, and T. P. Begley. 2007. A new thiamin salvage pathway. Nature Chemical Biology. 3:492-497.

Jenkins, A. L., Y. Zhang, S. E. Ealick, and T. P. Begley. 2008. Mutagenesis studies on TenA: A thiamin salvage enzyme from *Bacillus subtilis*. Bioorganic Chemistry. 36:29-32.

Kraft, C. E., E. R. L. Gordon, and E. R. Angert. 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLOS ONE. 9:e92688.

Kreinbring, C. A., S. P. Remillard, P. Hubbard, H. R. Brodkin, F. J. Leeper, D. Hawksley, E. Y. Lai, C. Fulton, G. A. Petsko, and D. Ringe. 2014. Structure of a eukaryotic thiaminase I. Proceedings of the National Academy of Sciences. 111:137-142.

Lepak, J. M., C. E. Kraft, D. C. Honeyfield, and S. B. Brown. 2008. Evaluating the effect of stressors on thiaminase activity in alewife. Journal of Aquatic Animal Health. 20:63-71.

Lepak, J. M., C. E. Kraft, and M. J. Vanni. 2013. Clupeid response to stressors: The influence of environmental factors on thiaminase expression. Journal of Aquatic Animal Health. 25:90-97.

Mac, M. J., C. C. Edsall, and J. G. Seelye. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. Journal of Great Lakes Research. 11:520-529.

Marcquenski, S. V. 1995. Characterization of Early Mortality Syndrome (EMS) in salmonids from the Great Lakes. Second workshop on reproductive disturbances in fish, Stockholm, Sweden. 73-75 pages.

Richter, C. A., A. N. Evans, M. K. Wright-Osment, J. L. Zajicek, S. A. Heppell, S. C. Riley, C. C. Krueger, and D. E. Tillitt. 2012. *Paenibacillus thiaminolyticus* is not the cause of thiamine deficiency impeding lake trout (*Salvelinus namaycush*) recruitment in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 69:1056-1064.

Riley, S. C., J. Rinchard, D. C. Honeyfield, A. N. Evans, and L. Begnoche. 2011. Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low alewife abundance. North American Journal of Fisheries Management. 31:1052-1064.

Roberts, G. W. and J. W. Boyd. 1974. Cerebrocortical necrosis in ruminants - occurrence of thiaminase in gut of normal and affected animals and its effect on thiamine status. Journal of Comparative Pathology. 84:365-374.

Shintani, S. 1956. On the thiaminase disease of chickens. Journal of Vitaminology. 2:23-30.

Soriano, E. V., K. R. Rajashankar, J. W. Hanes, S. Bale, T. P. Begley, and S. E. Ealick. 2008. Structural similarities between thiamin-binding protein and thiaminase-I suggest a common ancestor. Biochemistry. 47:1346-1357.

Tillitt, D. E., S. C. Riley, A. N. Evans, S. J. Nichols, J. L. Zajicek, J. Rinchard, C. A. Richter, and C. C. Krueger. 2009. Dreissenid mussels from the Great Lakes

contain elevated thiaminase activity. Journal of Great Lakes Research. 35:309-312.

Toms, A. V., A. L. Haas, J. H. Park, T. P. Begley, and S. E. Ealick. 2005. Structural characterization of the regulatory proteins TenA and TenI from *Bacillus subtilis* and identification of TenA as a thiaminase II. Biochemistry. 44:2319-2329.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

6 Bibliography

R Core Team. 2014. R: A language and environment for statistical computing. Version 3.1.0. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Abe, M., T. Nishimune, S. Ito, M. Kimoto, and R. Hayashi. 1986. A simple method for the detection of 2 types of thiaminase-producing colonies. Fems Microbiology Letters. 34:129-133.

Abe, M., S. Ito, M. Kimoto, R. Hayashi, and T. Nishimune. 1987. Molecular studies on thiaminase I. Biochimica Et Biophysica Acta. 909:213-221.

Ahlgren, G., L. Van Nieuwerburgh, I. Wanstrand, M. Pedersen, M. Boberg, and P. Snoeijs. 2005. Imbalance of fatty acids in the base of the Baltic Sea food web - a mesocosm study. Canadian Journal of Fisheries and Aquatic Sciences. 62:2240-2253.

Andraso, G. M., M. T. Ganger, and J. Adamczyk. 2011. Size-selective predation by round gobies (*Neogobius melanostomus*) on dreissenid mussels in the field. Journal of Great Lakes Research. 37:298-304.

Arsan, O. M. and A. Y. Malyarevskaya. 1969. The effect of food on thiaminase activity and the content of thiamine in the liver and intestine of silver carp. Hydrobiological Journal. 5:79-81.

Arsan, O. M. 1972. Thiaminase activity in the liver and intestine of some freshwater fishes. Hydrobiological Journal. 6:75-77.

Baba, H., Y. Masuda, N. Sueyoshi, and I. Kameshita. 2012. In-gel phosphatase assay using non-denaturing two-dimensional electrophoresis. Journal of Biochemistry. 152:557-563.

Balcer, M. D., N. L. Korda, and S. I. Dodson. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press, Madison, WI.

Baldwin, N. A., R. W. Saalfeld, M. R. Dochoda, H. J. Buettner, and R. L. Eshenroder. 2009. Commercial fish production in the Great Lakes 1867-2006. Available at http://www.glfc.org/databases/commercial/commerc.php.

Ballantyne, A. P., M. T. Brett, and D. E. Schindler. 2003. The importance of dietary phosphorus and highly unsaturated fatty acids for sockeye (*Oncorhynchus nerka*) growth in Lake Washington - a bioenergetics approach. Canadian Journal of Fisheries and Aquatic Sciences. 60:12-22.

Barbiero, R. P., M. Balcer, D. C. Rockwell, and M. L. Tuchman. 2009a. Recent shifts in the crustacean zooplankton community of Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences. 66:816-828.

Barbiero, R. P., D. B. Bunnell, D. C. Rockwell, and M. L. Tuchman. 2009b. Recent increases in the large glacial-relict calanoid *Limnocalanus macrurus* in Lake Michigan. Journal of Great Lakes Research. 35:285-292.

Barbiero, R. P. and G. J. Warren. 2011. Rotifer communities in the Laurentian Great Lakes, 1983–2006 and factors affecting their composition. Journal of Great Lakes Research. 37:528-540.

Barbiero, R. P., B. M. Lesht, and G. J. Warren. 2012. Convergence of trophic state and the lower food web in Lakes Huron, Michigan and Superior. Journal of Great Lakes Research. 38:368-380.

Beers, J. R. 1966. Studies on the chemical composition of the major zooplankton groups in the Sargasso Sea off Bermuda. Limnology and Oceanography. 11:520-528.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society. 18:308-343.

Birger, T. I., A. Y. Malyarevskaya, and O. M. Arsan. 1973. Etiology of the Haff (Yuksov-Sartlan) disease. Hydrobiological Journal. 9:71-80.

Bisbee, J. W. 1973. Size determination in hydra - roles of growth and budding. Journal of Embryology and Experimental Morphology. 30:1-19.

Bischoff, K. M., L. Shi, and P. J. Kennelly. 1998. The detection of enzyme activity following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Analytical Biochemistry. 260:1-17.

Blakeslee, C. J., S. A. Sweet, H. S. Galbraith, and D. C. Honeyfield. 2015. Thiaminase activity in native freshwater mussels. Journal of Great Lakes Research. 41:516-519.

Bogue, M. B. 2000. Fishing the Great Lakes : an environmental history, 1783-1933. University of Wisconsin Press, Madison, Wis.

Borgmann, U. and D. M. Whittle. 1994. Particle-size-conversion efficiency, invertebrate production, and potential fish production in Lake Ontario. Canadian Journal of Fisheries and Aquatic Sciences. 51:693-700.

Bos, M. and A. Kozik. 2000. Some molecular and enzymatic properties of a homogeneous preparation of thiaminase I purified from carp liver. Journal of Protein Chemistry. 19:75-84.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson, and T. Weglenska. 1976. A review of some problems is zooplankton production studies. Norwegian Journal of Zoology. 24:419-456.

Boyd, J. W. and J. R. Walton. 1977. Cerebrocortical necrosis in ruminants: an attempt to identify source of thiaminase in affected animals. Journal of Comparative Pathology. 87:581-589.

Bronte, C. R., S. T. Schram, J. H. Selgeby, and B. L. Swanson. 1995a. Density-independent survival of wild lake trout in the Apostle Islands area of Lake Superior. Journal of Great Lakes Research. 21:246-252.

Bronte, C. R., J. H. Selgeby, J. H. Saylor, G. S. Miller, and N. R. Foster. 1995b. Hatching, dispersal, and bathymetric distribution of age-0 wild lake trout at the Gull Island Shoal Complex, Lake Superior. Journal of Great Lakes Research. 21:233-245.

Bronte, C. R., M. P. Ebener, D. R. Schreiner, D. S. DeVault, M. M. Petzold, D. A. Jensen, C. Richards, and S. J. Lozano. 2003a. Fish community change in Lake Superior, 1970-2000. Canadian Journal of Fisheries and Aquatic Sciences. 60:1552-1574.

Bronte, C. R., J. Jonas, M. E. Holey, R. L. Eshenroder, M. L. Toneys, P. McKee, B. Breidert, R. M. Claramunt, M. P. Ebener, C. C. Krueger, G. M. Wright, and R. Hess. 2003b. Possible impediments to lake trout restoration in Lake Michigan. Lake Trout Task Group report to the Lake Michigan Committee. Great Lakes Fishery Commission. Ann Arbor, MI.

Bronte, C. R., P. C. McKee, and M. E. Holey. 2006. Relative survival of lake trout stocked at different sizes and quality in Lake Michigan. Journal of Great Lakes Research. 32:386-394.

Bronte, C. R., M. E. Holey, C. P. Madenjian, J. L. Jonas, R. M. Claramunt, P. C. McKee, M. L. Toneys, M. P. Ebener, B. Breidert, G. W. Fleischer, R. Hess, A. W. Martell, and E. J. Olsen. 2007. Relative abundance, site fidelity, and

survival of adult lake trout in Lake Michigan from 1999 to 2001: Implications for future restoration strategies. North American Journal of Fisheries Management. 27:137-155.

Bronte, C. R., C. C. Krueger, M. E. Holey, M. L. Toneys, R. L. Eshenroder, and J. L. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fishery Commission Miscellaneous Publication 2008-01. 39 pages. Available at http://www.glfc.org/pubs/pub.htm#misc.

Brown, M. E. and D. K. Branstrator. 2004. A 2001 survey of crustacean zooplankton in the western arm of Lake Superior. Journal of Great Lakes Research. 30:1-8.

Brown, S. B., J. D. Fitzsimons, V. P. Palace, and L. Vandenbyllaardt. 1998a. Thiamine and early mortality syndrome in lake trout. Pages 18-25 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., D. C. Honeyfield, and L. Vandenbyllaardt. 1998b. Thiamine analysis in fish tissues. Pages 73-81 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Brown, S. B., M. T. Arts, L. R. Brown, M. Brown, K. Moore, M. Villella, J. D. Fitzsimons, D. C. Honeyfield, D. E. Tillitt, J. L. Zajicek, M. Wolgamood, and J. G. Hnath. 2005a. Can diet-dependent factors help explain fish-to-fish variation in thiamine-dependent early mortality syndrome? Journal of Aquatic Animal Health. 17:36-47.

Brown, S. B., L. R. Brown, M. Brown, K. Moore, M. Villella, J. D. Fitzsimons, B. Williston, D. C. Honeyfield, J. P. Hinterkopf, D. E. Tillit, J. L. Zajicek, and M. Wolgamood. 2005b. Effectiveness of egg immersion in aqueous solutions of thiamine and thiamine analogs for reducing early mortality syndrome. Journal of Aquatic Animal Health. 17:106-112.

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield, and D. E. Tillitt. 2005c. Implications of thiamine deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health. 17:113-124.

Brown, S. B., D. C. Honeyfield, J. G. Hnath, M. Wolgamood, S. V. Marcquenski, J. D. Fitzsimons, and D. E. Tillitt. 2005d. Thiamine status in adult salmonines in the Great Lakes. Journal of Aquatic Animal Health. 17:59-64.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Marine Mammal Science. 22:759-801.

Budge, SuzanneÂ M., SarahÂ N. Penney, and SantoshÂ P. Lall. 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. Canadian Journal of Fisheries and Aquatic Sciences. 69:1033-1046.

Bunnell, D. B., C. P. Madenjian, J. D. Holuszko, J. V. Adams, and J. R. P. French. 2009. Expansion of *Dreissena* into offshore waters of Lake Michigan and potential impacts on fish populations. Journal of Great Lakes Research. 35:74-80.

Bunnell, D. B., K. M. Keeler, E. A. Puchala, B. M. Davis, and S. A. Pothoven. 2012. Comparing seasonal dynamics of the Lake Huron zooplankton community between 1983-1984 and 2007 and revisiting the impact of *Bythotrephes* planktivory. Journal of Great Lakes Research. 38:451-462.

Bunnell, D. B., B. M. Davis, M. A. Chriscinske, K. M. Keeler, and J. G. Mychek-Londer. 2015. Diet shifts by planktivorous and benthivorous fishes in northern Lake Michigan in response to ecosystem changes. Journal of Great Lakes Research. 41, Supplement 3:161-171.

Campobasso, N., C. A. Costello, C. Kinsland, T. P. Begley, and S. E. Ealick. 1998. Crystal structure of thiaminase-I from *Bacillus thiaminolyticus* at 2.0 angstrom resolution. Biochemistry. 37:15981-15989.

Chang, W. Y. B. and R. Rossmann. 1988. Changes in the abundance of blue-green algae related to nutrient loadings in the nearshore of Lake Michigan. Hydrobiologia. 157:271-278.

Christie, W. J. 1973. A review of the changes in the fish species composition of Lake Ontario. Great Lakes Fishery Commission Technical Report:1-65.

Claramunt, R. M., J. L. Jonas, J. D. Fitzsimons, and J. E. Marsden. 2005. Influences of spawning habitat characteristics and interstitial predators on lake trout egg deposition and mortality. Transactions of the American Fisheries Society. 134:1048-1057.

Coble, D. W. 1965. Effect of raw smelt on lake trout. Canadian Fish Culturist. 36:27-34.

Colborne, S. F., S. A. Rush, G. Paterson, T. B. Johnson, B. F. Lantry, and A. T. Fisk. 2016. Estimates of lake trout (*Salvelinus namaycush*) diet in Lake Ontario using two and three isotope mixing models. Journal of Great Lakes Research. 42:695-702.

Costello, C., N. Kelleher, A. Mitsuko, F. McLafferty, and T. P. Begley. 1996. Mechanistic studies on thiaminase I. Journal of Biological Chemistry. 271:3445-3452.

Croft, L., E. Napoli, C. K. Hung, J. St Leger, S. Gearhart, K. Heym, S. Wong, D. Sakaguchi, A. Lin, B. Puschner, and C. Giulivi. 2013. Clinical evaluation and biochemical analyses of thiamine deficiency in Pacific harbor seals (*Phoca vitulina*) maintained at a zoological facility. Javma-Journal of the American Veterinary Medical Association. 243:1179-1189.

Culver, D. A., M. M. Boucherle, D. J. Bean, and J. W. Fletcher. 1985. Biomass of fresh-water crustacean zooplnakton from length-weight regressions. Canadian Journal of Fisheries and Aquatic Sciences. 42:1380-1390.

Cummins, K. W., R. R. Costa, R. E. Rowe, G. A. Moshiri, R. M. Scanlon, and R. K. Zajdel. 1969. Ecological energetics of a natural population of predaceous zooplankter, *Leptodora kindti*, Focke (Cladocera). Oikos. 20:189-223.

Czesny, S. and K. Dabrowski. 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). Aquatic Living Resources. 11:371-378.

Czesny, S., J. M. Dettmers, J. Rinchard, and K. Dabrowski. 2009. Linking egg thiamine and fatty acid concentrations of Lake Michigan lake trout with early life stage mortality. Journal of Aquatic Animal Health. 21:262-271.

Czesny, S., J. Epifanio, and P. Michalak. 2012. Genetic divergence between freshwater and marine morphs of alewife (*Alosa pseudoharengus*): A 'next-generation' sequencing analysis. PLOS ONE. 7:e31803.

Czesny, S. J., J. Rinchard, S. D. Hanson, J. M. Dettmers, and K. Dabrowski. 2011. Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. Canadian Journal of Fisheries and Aquatic Sciences. 68:1211-1230.

Dagleish, M. P., I. A. P. Patterson, G. Foster, R. J. Reid, L. T. A. Brain, and D. Buxton. 2006. Polioencephalomalacia in a juvenile hooded seal (*Cystophora cristata*). Veterinary Record. 158:516-518.

Davis, B. M., J. F. Savino, and L. M. Ogilvie. 2007. Diet niches of major forage fish in Lake Michigan. Pages 261-275 *in* M. Jankun, P. Brzuzan, P. Hliwa, and M. Luczynski, editors. Biology and Management of Coregonid Fishes - 2005.

Dermott, R. M. and C. G. Paterson. 1974. Determining dry weight and percentage dry matter of chironomid larvae. Canadian Journal of Zoology. 52:1243-1250.

DesJardine, R. L., T. K. Gorenflo, R. N. Payne, and J. D. Schrouder. 1995. Fish-community objectives for Lake Huron. Great Lakes Fishery Commission Special Publication. 95-1:1-37.

Deutsch, H. F. and A. D. Hasler. 1943. Distribution of a vitamin B1 destructive enzyme in fish. Proceedings of the Society for Experimental Biology and Medicine. 53:63-65.

Dietrich, J. P., B. J. Fvlorrison, and J. A. Hoyle. 2006. Alternative ecological pathways in the Eastern Lake Ontario food web-round goby in the diet of lake trout. Journal of Great Lakes Research. 32:395-400.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs. 67:345-366.

Dumont, H. J., I. V. d. Velde, and S. Dumont. 1975. The Dry Weight Estimate of Biomass in a Selection of Cladocera, Copepoda and Rotifera from the Plankton, Periphyton and Benthos of Continental Waters. Oecologia. 19:75-97.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark, Jr. 1995. Fish-community objectives for Lake Michigan. Great Lakes Fishery Commission Special Publication 1995-3:1-56.

Eshenroder, R. L., J. W. Peck, and C. H. Olver. 1999. Research priorities for lake trout rehabilitation in the Great Lakes: A 15-year retrospective. Great Lakes Fishery Commission Technical Report:1-40.

Evans, W. C. 1976. Bracken thiaminase-mediated neurotoxic syndromes. Botanical Journal of The Linnean Society. 73:113-131.

Fisher, J. P., J. M. Spitsbergen, H. Symula, J. Skea, M. Babenzein, and T. Chiotti. 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the "Cayuga syndrome". Journal of Aquatic Animal Health. 7:81-94.

Fisher, J. P., J. D. Fitzsimons, G. F. Combs, and J. M. Spitsbergen. 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Transactions of the American Fisheries Society. 125:167-178.

Fisher, J. P., S. B. Brown, S. Connelly, T. Chiotti, and C. C. Krueger. 1998a. Interspecies comparisons of blood thiamine in salmonids from the Finger Lakes, and effect of maternal size on blood and egg thiamine in Atlantic salmon with and without Cayuga Syndrome. Pages 112-123 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fisher, J. P., S. B. Brown, G. W. Wooster, and P. R. Bowser. 1998b. Maternal blood, egg and larval thiamin levels correlate with larval survival in landlocked Atlantic salmon (*Salmo salar*). Journal of Nutrition. 128:2456-2466.

Fitzpatrick, T. B. and S. Thore. 2014. Complex behavior: from cannibalism to suicide in the vitamin B-1 biosynthesis world. Current Opinion in Structural Biology. 29:34-43.

Fitzsimons, J. 2008. Egg thaimine data from Port Weller, ON. Personal communication (e-mail) to S. C. Riley.

Fitzsimons, J. D. 1995. The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. Journal of Great Lakes Research. 21:286-289.

Fitzsimons, J. D. and S. B. Brown. 1998. Reduced egg thiamine levels in inland and Great Lakes trout and their relationship with diet. Pages 160-171 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., S. B. Brown, and L. Vandenbyllaardt. 1998. Thiamine levels in food chains of the Great Lakes. Pages 90-98 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Fitzsimons, J. D., L. Vandenbyllaardt, and S. B. Brown. 2001. The use of thiamine and thiamine antagonists to investigate the etiology of early mortality syndrome in lake trout (*Salvelinus namaycush*). Aquatic Toxicology. 52:229-239.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, R. Barrows, C. E. Kraft, J. Lepak, D. C. Honeyfield, and D. Tillitt. 2004a. Factors influencing alewife thiaminolytic activity. Appendix 3 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 33 pages.

Fitzsimons, J. D., S. Brown, G. Fodor, B. Williston, L. Brown, K. Moore, J. Zajicek, D. Tillitt, and D. C. Honeyfield. 2004b. Thiaminase and thiamine in lower trophic level organisms from the Great Lakes basin. Appendix 4 *in* Investigations into the causes of thiamine deficiency in Great Lakes salmonids and the effects of low thiamine on swim-up fry behavior. Completion Report to the Great Lakes Fishery Trust, 13 pages.

Fitzsimons, J. D., B. Williston, P. Amcoff, L. Balk, C. Pecor, H. G. Ketola, J. P. Hinterkopf, and D. C. Honeyfield. 2005a. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine-deficient coho salmon. Journal of Aquatic Animal Health. 17:48-58.

Fitzsimons, J. D., B. Williston, J. L. Zajicek, D. E. Tillitt, S. B. Brown, L. R. Brown, D. C. Honeyfield, D. M. Warner, L. G. Rudstam, and W. Pearsall. 2005b. Thiamine content and thiaminase activity of ten freshwater stocks and one marine stock of alewives. Journal of Aquatic Animal Health. 17:26-35.

Fitzsimons, J. D., B. Williston, G. Williston, L. Brown, A. El-Shaarawi, L. Vandenbyllaardt, D. Honeyfeld, D. Tillitt, M. Wolgamood, and S. B. Brown. 2007. Egg thiamine status of Lake Ontario salmonines 1995-2004 with emphasis on lake trout. Journal of Great Lakes Research. 33:93-103.

Fitzsimons, J. D., S. B. Brown, B. Williston, G. Williston, L. R. Brown, K. Moore, D. C. Honeyfield, and D. E. Tillitt. 2009a. Influence of thiamine deficiency on lake trout larval growth, foraging, and predator avoidance. Journal of Aquatic Animal Health. 21:302-314.

Fitzsimons, J. D., M. Clark, and M. Keir. 2009b. Addition of round gobies to the prey community of Lake Ontario and potential implications to thiamine status and

reproductive success of Lake Trout. Aquatic Ecosystem Health & Management. 12:296-312.

Flath, L. E. and J. S. Diana. 1985. Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Transactions of the American Fisheries Society. 114:328–337.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226:497-509.

French, J. R. P. and D. J. Jude. 2001. Diets and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. Journal of Great Lakes Research. 27:300-311.

Fujita, A. 1954. Thiaminase. Advances in Enzymology and Related Subjects of Biochemistry. 15:389-421.

Fynn-Aikins, K., P. R. Bowser, D. C. Honeyfield, J. D. Fitzsimons, and H. G. Ketola. 1998. Effect of dietary amprolium on tissue thiamin and Cayuga syndrome in Atlantic salmon. Transactions of the American Fisheries Society. 127:747-757.

Gamble, A. E., T. R. Hrabik, J. D. Stockwell, and D. L. Yule. 2011a. Trophic connections in Lake Superior Part I: The offshore fish community. Journal of Great Lakes Research. 37:541-549.

Gamble, A. E., T. R. Hrabik, D. L. Yule, and J. D. Stockwell. 2011b. Trophic connections in Lake Superior Part II: The nearshore fish community. Journal of Great Lakes Research. 37:550-560.

Games, P. A. and J. F. Howell. 1976. Pairwise Multiple Comparison Procedures with Unequal N's and/or Variances: A Monte Carlo Study. Journal of Educational and Behavioral Statistics. 1:113-125.

Garton, D. W. and D. J. Berg. 1990. Occurrence of *Bythotrephes cederstroemi* (Schoedler 1877) in Lake Superior, with Evidence of Demographic Variation within the Great Lakes. Journal of Great Lakes Research. 16:148-152.

GLNPO. 2003. Standard operating procedures for zooplankton analysis. U. S. Environmental Protection Agency, Great Lakes National Program Office, Report LG403. 16 pages.

Goodman, K. S. 1980. The estimation of individual dry-weight and standing crop of harpacticoid copepods. Hydrobiologia. 72:253-259.

Gorg, A., A. Klaus, C. Luck, F. Weiland, and W. Weiss. 2007. Two-dimensional electrophoresis with immobilized pH gradients for proteome analysis. Technische Universitat Munchen. 166 pages. Available at http://proteomik.wzw.tum.de/fileadmin/documents/2D_Manual-Gorg_2007.pdf.

Green, R. G. and C. A. Evans. 1940. A deficiency disease of foxes. Science. 92:154-155.

Green, R. G., W. E. Carlson, and C. A. Evans. 1941. A deficiency disease of foxes produced by feeding fish. Journal of Nutrition. 21:243-256.

Greig, R. A. and R. H. Gnaedinger. 1971. Occurrence of thiaminase in some common aquatic animals of the United States and Canada. Special Scientific Report - Fisheries. United States Department of Commerce. Seattle, Washington, 7 pages.

Grigor'yeva, L. V., A. Kirpenko, V. M. Orlovskij, and V. V. Stankewich. 1977. On the antimicrobial action of toxic metabloites of some blue-green algae. Gidrobiologicheskii Zhurnal. 13:47-52.

Grigorovich, I. A., H. J. MacIsaac, I. K. Rivier, N. V. Aladin, and V. E. Panov. 2000. Comparative biology of the predatory cladoceran Cercopagis pengoi from Lake Ontario, Baltic Sea and Caspian Sea. Archiv Fur Hydrobiologie. 149:23-50.

Grossnickle, N. E. and A. M. Beeton. 1979. Antennal scale length as a measure of relative size in the opossum shrimp, *Mysis relicta* Loven. Crustaceana. 36:141-146.

Hansen, M. J. 1999. Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. Pages 417-453 *in* W. W. Taylor and C. P. Ferreri, editors. Great Lakes fisheries policy and management: a binational perspective.

Happel, A., S. Creque, J. Rinchard, T. Höök, H. Bootsma, J. Janssen, D. Jude, and S. Czesny. 2015a. Exploring yellow perch diets in Lake Michigan through stomach content, fatty acids, and stable isotope ratios. Journal of Great Lakes Research. 41, Supplement 3:172-178.

Happel, A., J. Lafountain, S. Creque, J. Rinchard, T. Höök, H. Bootsma, J. Janssen, D. Jude, and S. Czesny. 2015b. Spatio-temporal description of spottail shiner (*Notropis hudsonius*) fatty acid profiles in Lake Michigan's southern basin. Journal of Great Lakes Research. 41, Supplement 3:179-184.

Happel, A., L. Stratton, R. Pattridge, J. Rinchard, and S. Czesny. 2016. Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. Freshwater Biology.

Healthcare, G. 2009. Immobiline DryStrip. Instructions 28-9537-55 AA. GE Healthcare.

Helfman, G. S. 2007. Fish conservation : a guide to understanding and restoring global aquatic biodiversity and fishery resources. Washington : Island Press, Washington.

Higgins, S. N., S. Y. Malkin, E. T. Howell, S. J. Guildford, L. Campbell, V. Hiriart-Baer, and R. E. Hecky. 2008. An ecological review of *Cladophora glomerata* (Chlorophyta) in the Laurentian Great Lakes. Journal Of Phycology. 44:839-854.

Hillbricht-Ilkowska, A. and A. Stanczykowska. 1969. The production and standing crop of planktonic larvae of *Dreissena polymorpha* Pall. in two Mazurian lakes. Polish Archives of Hydrobiology. 16:193-203.

Hislop, J. R. G. and M. A. Bell. 1987. Observations on the size, dry-weight and energy content of the eggs of some demersal fish speciecs from British marine waters. Journal of Fish Biology. 31:1-20.

Hoar, W. S. and D. J. Randall, editors. 1969. Reproduction and growth, bioluminescence, pigments and poisons. Academic Press, New York.

Holbrook, B. V., T. R. Hrabik, D. K. Branstrator, D. L. Yule, and J. D. Stockwell. 2006. Hydroacoustic estimation of zooplankton biomass at two shoal complexes in the Apostle Islands region of Lake Superior. Journal of Great Lakes Research. 32:680-696.

Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown, J. E. Marsden, D. S. Lavis, M. L. Toneys, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. Journal of Great Lakes Research. 21:128-151.

Honeyfield, D. C., J. D. Fitzsimons, S. B. Brown, S. V. Marcquenski, and G. McDonald. 1998a. Introduction and overview of early life stage mortality. Pages 1-7 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., K. Fynn-Aikins, J. D. Fitzsimons, and J. A. Mota. 1998b. Effect of dietary amprolium on egg and tissue thiamine concentrations in lake trout. Pages 172-177 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Honeyfield, D. C., J. P. Hinterkopf, and S. B. Brown. 2002. Isolation of thiaminase-positive bacteria from alewife. Transactions of the American Fisheries Society. 131:171-175.

Honeyfield, D. C., D. Beltman, M. Holey, and C. Edsall. 2005a. Investigation of thiamine and PCB association with early life stage fry mortality in lake trout from northwestern Lake Michigan in 1996-1998. Pages 182-194 *in* Health and diseases of aquatic organisms: bilateral perspectives. Proceedings of the second bilateral conference between Russian and the United States. 21-28 September 2003. Shepherdstown, West Virginia. Michigan State University, East Lansing, Michigan.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek, and S. B. Brown. 2005b. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health. 17:4-12.

Honeyfield, D. C., J. P. Ross, D. A. Carbonneau, S. P. Terrel, A. R. Woodward, T. R. Schoeb, H. F. Perceval, and J. P. Hinterkopf. 2008. Pathology, physiologic parameters, tissue contaminants, and tissue thiamine in morbid and healthy central Florida adult American alligators (*Alligator mississippiensis*). Journal of Wildlife Diseases. 44:280-294.

Honeyfield, D. C., J. W. Hanes, L. Brown, C. E. Kraft, and T. P. Begley. 2010a. Comparison of thiaminase activity in fish using the radiometric and 4-nitrothiophenol colorimetric methods. Journal of Great Lakes Research. 36:641-645.

Honeyfield, D. C., D. E. Tillitt, J. D. Fitzsimons, and S. B. Brown. 2010b. Variation in Lake Michigan alewife (*Alosa pseudoharengus*) thiaminase and fatty acids composition. Journal of Freshwater Ecology. 25:65-71.

Honeyfield, D. C., M. E. Daniels, L. R. Brown, M. T. Arts, M. G. Walsh, and S. B. Brown. 2012. Survey of four essential nutrients and thiaminase activity in five Lake Ontario prey fish species. Journal of Great Lakes Research. 38:11-17.

Horns, W. H., C. R. Bronte, T. R. Busiahn, M. P. Ebener, R. L. Eshenroder, T. Gorenflo, N. Kmiecik, W. Mattes, J. W. Peck, M. Petzold, and D. R. Schreiner. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication. 03-01:i-ii, 1-78.

Hornung, M. W., L. Miller, R. E. Peterson, S. V. Marcquenski, and S. B. Brown. 1998. Efficacy of thiamine, astaxanthin, β-carotene, and thyroxine treatments in reducing early mortality syndrome in Lake Michigan salmonid embryos. Pages 124-134 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Houde, A. L. S., P. J. Saez, C. C. Wilson, D. P. Bureau, and B. D. Neff. 2015. Effects of feeding high dietary thiaminase to sub-adult Atlantic salmon from three populations. Journal of Great Lakes Research. 41:898-906.

Hudson, P. L. and L. T. Lesko. Free-living and parasitic copepods (including Branchiuraans) of the Laurentian Great Lakes: keys and details on individual species. Available at http://www.glsc.usgs.gov/greatlakescopepods/.

Ishihara, T., H. Kinari, and M. Yasuda. 1973. Studies on thiaminase I in marine fish - II. Distribution of thiaminase in marine fish. Bulletin of the Japanese Society of Scientific Fisheries. 39:55-59.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs. 74:211-235.

Jacobs, G. R., C. P. Madenjian, D. B. Bunnell, and J. D. Holuszko. 2010. Diet of lake trout and burbot in Northern Lake Michigan during spring: Evidence of ecological interaction. Journal of Great Lakes Research. 36:312-317.

Jaroszewska, M., B. J. Lee, K. Dabrowski, S. Czesny, J. Rinchard, P. Trzeciak, and B. Wilczynska. 2009. Effects of vitamin B-1 (thiamine) deficiency in lake trout (*Salvelinus namaycush*) alevins at hatching stage. Comparative Biochemistry And Physiology A-Molecular & Integrative Physiology. 154:255-262.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries. 33:372-407.

Jenkins, A. H., G. Schyns, S. Potot, G. X. Sun, and T. P. Begley. 2007. A new thiamin salvage pathway. Nature Chemical Biology. 3:492-497.

Jenkins, A. L., Y. Zhang, S. E. Ealick, and T. P. Begley. 2008. Mutagenesis studies on TenA: A thiamin salvage enzyme from *Bacillus subtilis*. Bioorganic Chemistry. 36:29-32.

Ji, Y. Q. and I. R. Adelman. 1998. Thiaminase activity in alewives and smelt in lakes Huron, Michigan, and Superior. Pages 154-159 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Ji, Y. Q., J. J. Warthesen, and I. R. Adelman. 1998. Thiamine nutrition, synthesis, and retention in relation to lake trout reproduction in the Great Lakes. Pages 99-111 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Johannsson, O. E., R. Dermott, D. M. Graham, J. A. Dahl, E. Scott Millard, D. D. Myles, and J. LeBlanc. 2000. Benthic and pelagic secondary production in Lake Erie after the invasion of *Dreissena* spp. with implications for fish production. Journal of Great Lakes Research. 26:31-54.

Jonas, J. L., R. M. Claramunt, J. D. Fitzsimons, J. E. Marsden, and B. J. Ellrott. 2005. Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 62:2254-2264.

Jones, M. L., G. W. Eck, D. O. Evans, M. C. Fabrizio, M. H. Hoff, P. L. Hudson, J. Janssen, D. Jude, R. Ogorman, and J. F. Savino. 1995. Limitations to lake trout (*Salvelinus namaycush*) rehabilitation in the Great Lakes imposed by biotic interactions occurring at early life stages. Journal of Great Lakes Research. 21:505-517.

Kameshita, I., A. Ishida, S. Okuno, and H. Fujisawa. 1997. Detection of protein phosphatase activities in sodium dodecyl sulfate-polyacrylamide gel using peptide substrates. Analytical Biochemistry. 245:149-153.

Keeler, K. M., D. B. Bunnell, J. S. Diana, J. V. Adams, J. G. Mychek-Londer, D. M. Warner, D. L. Yule, and M. R. Vinson. 2015. Evaluating the importance of

abiotic and biotic drivers on *Bythotrephes* biomass in Lakes Superior and Michigan. Journal of Great Lakes Research. 41, Supplement 3:150-160.

Ketola, H. G., P. R. Bowser, G. A. Wooster, L. R. Wedge, and S. S. Hurst. 2000. Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Transactions of the American Fisheries Society. 129:607-612.

Ketola, H. G., T. L. Chiotti, R. S. Rathman, J. D. Fitzsimons, D. C. Honeyfield, P. J. Van Dusen, and G. E. Lewis. 2005. Thiamine status of Cayuga Lake rainbow trout and its influence on spawning migration. North American Journal of Fisheries Management. 25:1281-1287.

Kleiner, D. E. and W. G. Stetler-Stevenson. 1994. Quantitative zymography - detection of picogram quantities of gelatinases. Analytical Biochemistry. 218:325-329.

Kraft, C. E., E. R. L. Gordon, and E. R. Angert. 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLOS ONE. 9:e92688.

Kreinbring, C. A., S. P. Remillard, P. Hubbard, H. R. Brodkin, F. J. Leeper, D. Hawksley, E. Y. Lai, C. Fulton, G. A. Petsko, and D. Ringe. 2014. Structure of a eukaryotic thiaminase I. Proceedings of the National Academy of Sciences. 111:137-142.

Krueger, C. C., M. L. Jones, and W. W. Taylor. 1995a. Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. Journal of Great Lakes Research. 21:547-558.

Krueger, C. C., D. L. Perkins, E. L. Mills, and J. E. Marsden. 1995b. Predation by alewives on lake trout fry in Lake Ontario: Role of an exotic species in preventing restoration of a native species. Journal of Great Lakes Research. 21:458-469.

Krueger, C. C. and M. Ebener. 2004. Rehabilitation of lake trout in the Great Lakes: past lessons and future challenges. Pages 37-56 *in* Boreal Shield watersheds: lake trout ecosystems in a changing environment. Lewis Publishers.

Krueger, C. C., W. C. Shepherd, and A. M. Muir. 2014. Predation by alewife on lake trout fry emerging from laboratory reefs: Estimation of fry survival and assessment of predation potential. Journal of Great Lakes Research. 40:429-434.

Kruskal, J. B. 1964a. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika. 29:1-27.

Kruskal, J. B. 1964b. Nonmetric multidimensional scaling: a numerical method. Psychometrika. 29:115-129.

Landrum, P. F. 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. Aquatic Toxicology. 12:245-271.

Lawrence, S. G., D. F. Malley, W. J. Findlay, M. A. Maclver, and I. L. Delbaere. 1987. Method for estimating dry-weight of fresh-water planktonic crustaceans from measures of length and shape. Canadian Journal of Fisheries and Aquatic Sciences. 44:264-274.

Legendre, P. and M. J. Anderson. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs. 69:1-24.

Lehman, J. T. and C. E. Caceres. 1993. Food-web responses to species invasion by a predatory invertebrate - *Bythotrephes* in Lake Michigan. Limnology and Oceanography. 38:879-891.

Lepak, J. M., C. E. Kraft, D. C. Honeyfield, and S. B. Brown. 2008. Evaluating the effect of stressors on thiaminase activity in alewife. Journal of Aquatic Animal Health. 20:63-71.

Lepak, J. M., C. E. Kraft, and M. J. Vanni. 2013. Clupeid response to stressors: The influence of environmental factors on thiaminase expression. Journal of Aquatic Animal Health. 25:90-97.

Leuven, R., T. C. M. Brock, and H. A. M. Vandruten. 1985. Effects of preservation on dry-free and ash-free dry-weight biomass of some common aquatice macro-invertebrates. Hydrobiologia. 127:151-159.

Lynch, M., L. J. Weider, and W. Lampert. 1986. Measurement of the carbon balance in *Daphnia*. Limnology and Oceanography. 31:17-33.

Mac, M. J., C. C. Edsall, and J. G. Seelye. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. Journal of Great Lakes Research. 11:520-529.

Madenjian, C. P., J. D. Holuszko, and T. J. Desorcie. 2006a. Spring-summer diet of lake trout on Six Fathom Bank and Yankee Reef in Lake Huron. Journal of Great Lakes Research. 32:200-208.

Madenjian, C. P., S. A. Pothoven, J. M. Dettmers, and J. D. Holuszko. 2006b. Changes in seasonal energy dynamics of alewife (*Alosa pseudoharengus*) in Lake Michigan after invasion of dreissenid mussels. Canadian Journal of Fisheries and Aquatic Sciences. 63:891-902.

Madenjian, C. P., R. O'Gorman, D. B. Bunnell, R. L. Argyle, E. F. Roseman, D. M. Warner, J. D. Stockwell, and M. A. Stapanian. 2008. Adverse effects of alewives on Laurentian Great Lakes fish communities. North American Journal of Fisheries Management. 28:263-282.

Makarewicz, J. C. and T. W. Lewis. 2015. Long-term changes in Lake Ontario rotifer abundance and composition: A response to *Cercopagis* predation? Journal of Great Lakes Research. 41:192-199.

Malyarevskaya, A. Y., T. I. Birger, O. M. Arsan, and V. D. Solomatina. 1972. Metabolic relationships between blue-green algae and fish. Hydrobiological Journal. 8:35-41.

Manchenko, G. P. 2003. Handbook of detection of enzymes on electrophoretic gels. 2nd ed. edition. Boca Raton : CRC Press, Boca Raton.

Marcquenski, S. V. 1995. Characterization of Early Mortality Syndrome (EMS) in salmonids from the Great Lakes. Second workshop on reproductive disturbances in fish, Stockholm, Sweden. 73-75 pages.

Marcquenski, S. V. and S. B. Brown. 1997. Early mortality syndrome (EMS) in salmonid fishes from the Great Lakes. Pages 135-152 *in* R. M. Rolland, M. Gilbertson, and R. E. Peterson, editors. Chemically induced alterations in functional development and reproduction of fishes. SETAC (Society of Environmental Toxicology and Chemistry), Pensacola, FL.

Mather, P. M. 1976. Computational methods of multivariate analysis in physical geography. J. Wiley and Sons, New York.

Mazrimas, J. A., L. L. Ingraham, R. D. Draper, and P. S. Song. 1963. Studies of thiaminase action - Hammetts Rhosigma Treatment. Archives of Biochemistry and Biophysics. 100:409-413.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology. 82:290-297.

McCleary, B. V. and B. F. Chick. 1977. Purification and properties of a thiaminase I enzyme from nardoo (*Marsilea drummondii*). Phytochemistry. 16:207-213.

McCune, B. and J. B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR.

McCune, B. and M. J. Mefford. 2015. PC-ORD. Multivariate Analysis of Ecological Data. Version 7.359 beta. MjM Software Design, Gleneden Beach, OR, USA

McDonald, G., J. D. Fitzsimons, and D. C. Honeyfield, editors. 1998. Early life stage morality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Bethesda, Maryland.

Metcalfe, L. D. and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Analytical Chemistry. 33:363-364.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries. 14:22-38.

Morbey, Y. E., D. M. Anderson, and B. A. Henderson. 2008. Progress toward the rehabilitation of lake trout (*Salvelinus namaycush*) in South Bay, Lake Huron. Journal of Great Lakes Research. 34:287-300.

Muir, A. M., C. C. Krueger, and M. J. Hansen. 2012. Re-establishing lake trout in the Laurentian Great Lakes: Past, present, and future. Pages 533-588 *in* W. W. Taylor, A. J. Lynch, and N. J. Leonard, editors. Great Lakes fisheries policy and management: A binational perspective. 2nd edition. MSU Press, Lansing.

Mychek-Londer, J. G., D. B. Bunnell, W. Stott, J. S. Diana, J. R. P. French, and M. A. Chriscinske. 2013. Using diets to reveal overlap and egg predation among benthivorous fishes in Lake Michigan. Transactions of the American Fisheries Society. 142:492-504.

Nalepa, T. and M. A. Quigley. 1980. The macro and meiobenthos of southeastern Lake Michigan near the mouth of the Grand River, 1976-77. Great Lakes Environmental Reasearch Laboratory, Ann Arbor, MI.

Nalepa, T. F., D. L. Fanslow, S. A. Pothoven, A. J. Foley, and G. A. Lang. 2007. Long-term trends in benthic macroinvertebrate populations in Lake Huron over the past four decades. Journal of Great Lakes Research. 33:421-436.

Nalepa, T. F., D. L. Fanslow, and G. A. Lang. 2009. Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. Freshwater Biology. 54:466-479.

Nalepa, T. F., D. L. Fanslow, and S. A. Pothoven. 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. Journal of Great Lakes Research. 36:5-19.

Nishimune, T., Y. Watanabe, H. Okazaki, and H. Akai. 2000. Thiamin is decomposed due to *Anaphe* spp. entomophagy in seasonal ataxia patients in Nigeria. Journal of Nutrition. 130:1625-1628.

Nishimune, T., Y. Watanabe, and H. Okazaki. 2008. Studies on the polymorphism of thiaminase I in seawater fish. Journal of Nutritional Science and Vitaminology. 54:339-346.

Ojaveer, H., L. A. Kuhns, R. P. Barbiero, and M. L. Tuchman. 2001. Distribution and population characteristics of *Cercopagis pengoi* in Lake Ontario. Journal of Great Lakes Research. 27:10-18.

Okonji, R. E., K. S. Bamitale, and R. O. Balogun. 2012. Biochemical properties of thiaminase from *Anaphe venata* Burtler. African Journal of Biotechnology. 11:4543-4550.

Omori, M. 1969. Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Marine Biology. 3:4-10.

Pace, M. L. and J. D. Orcutt, Jr. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography. 26:822-830.

Pauli, H.-R. 1989. A new method to estimate individual dry weights of rotifers. Hydrobiologia. 186-187:355-361.

Pearre, S. 1980. The copopod width-weight relation and its utility in food-chain research. Canadian Journal of Zoology. 58:1884-1891.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States: protozoa to mollusca. 3rd edition. Wiley, New York.

Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology. 87:2614-2625.

Puzach, S. S., Z. V. Gorbach, and Y. M. Ostrovskii. 1984. Isolation, purification, and certain properties of thiaminase I from *Anodonta cygnea*. Biochemisty-Moscow. 49:1010-1016.

Ramos, J. J., L. M. Ferrer, L. Garcia, A. Fernandez, and A. Loste. 2005. Polioencephalomalacia in adult sheep grazing pastures with prostrate pigweed. Canadian Veterinary Journal-Revue Veterinaire Canadienne. 46:59-61.

Ray, W. J. and L. D. Corkum. 1997. Predation of zebra mussels by round gobies, *Neogobius melanostomus*. Environmental Biology of Fishes. 50:267-273.

Richter, C. A., A. N. Evans, M. K. Wright-Osment, J. L. Zajicek, S. A. Heppell, S. C. Riley, C. C. Krueger, and D. E. Tillitt. 2012. *Paenibacillus thiaminolyticus* is not the cause of thiamine deficiency impeding lake trout (*Salvelinus namaycush*) recruitment in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences. 69:1056-1064.

Riley, S. C. and A. N. Evans. 2008. Phylogenetic and ecological characteristics associated with thiaminase activity in Laurentian Great Lakes fishes. Transactions of the American Fisheries Society. 137:147-157.

Riley, S. C., J. Rinchard, D. C. Honeyfield, A. N. Evans, and L. Begnoche. 2011. Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low alewife abundance. North American Journal of Fisheries Management. 31:1052-1064.

Rivoal, J., C. R. Smith, T. F. Moraes, D. H. Turpin, and W. C. Plaxton. 2002. A method for activity staining after native polyacrylamide gel electrophoresis using a coupled enzyme assay and fluorescence detection: Application to the analysis of several glycolytic enzymes. Analytical Biochemistry. 300:94-99.

Roberts, G. W. and J. W. Boyd. 1974. Cerebrocortical necrosis in ruminants - occurrence of thiaminase in gut of normal and affected animals and its effect on thiamine status. Journal of Comparative Pathology. 84:365-374.

Roseman, E. F., J. S. Schaeffer, E. Bright, and D. G. Fielder. 2014. Angler-caught piscivore diets reflect fish community changes in Lake Huron. Transactions of the American Fisheries Society. 143:1419-1433.

Rosen, R. A. 1981. Length dry-weight relationships of some freshwater zooplankton. Journal of Freshwater Ecology. 1:225-229.

Rudstam, L. G., K. T. Holeck, K. L. Bowen, J. M. Watkins, B. C. Weidel, and F. J. Luckey. 2015. Lake Ontario zooplankton in 2003 and 2008: Community changes and vertical redistribution. Aquatic Ecosystem Health & Management. 18:43-62.

Rush, S. A., G. Paterson, T. B. Johnson, K. G. Drouillard, G. D. Haffner, C. E. Hebert, M. T. Arts, D. J. McGoldrick, S. M. Backus, B. F. Lantry, J. R. Lantry,

T. E. D. Schaner, and A. T. Fisk. 2012. Long-term impacts of invasive species on a native top predator in a large lake system. Freshwater Biology. 57:2342-2355.

Ryan, P. A., R. Knight, R. MacGregor, G. Towns, R. Hoopes, and W. Culligan. 2003. Fish-community goals and objectives for Lake Erie. Great Lakes Fishery Commission Special Publication. 03-02:1-56.

Sage, R. D. 1982. Wet and dry-weight estimates of insects and spiders based on length. The American Midland Naturalist. 108:407-411.

Salaman, M. R. and A. R. Williams. 1971. Isoelectric focusing of proteins in native and denatured states - anomalous behavior of plasma albumin. Biochemical Journal. 122:93-99.

Sambrook, J. and D. W. Russell. 2001. Molecular cloning: a laboratory manual. Third edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schagger, H. and G. Vonjagow. 1991. Blue native electrophoresis for isolation of membrane-protein complexes in enzymatically active form. Analytical Biochemistry. 199:223-231.

Schamel, W. W. A. 2001. Two-Dimensional Blue Native Polyacrylamide Gel Electrophoresis *in* Current Protocols in Cell Biology. John Wiley & Sons, Inc.

Schindler, D. W. and B. Noven. 1971. Vertical distribution and seasonal abundance of zooplankton in 2 shallow lakes of the Experimental Lakes Area, Northwestern Ontario. Journal of the Fisheries Research Board of Canada. 28:245-256.

Sealock, R. R., A. H. Livermore, and C. A. Evans. 1943. Thiamine inactivation by the fresh-fish or Chastek-paralysis factor. Journal of the American Chemical Society. 65:935-940.

Shea, M. A. and J. C. Makarewicz. 1989. Producation, biomass, and trophic interactions of *Mysis relicta* in Lake Ontario. Journal of Great Lakes Research. 15:223-232.

Shintani, S. 1956. On the thiaminase disease of chickens. Journal of Vitaminology. 2:23-30.

Sikowitz, M. D., B. Shome, Y. Zhang, T. P. Begley, and S. E. Ealick. 2013. Structure of a *Clostridium botulinum* C143S Thiaminase I/Thiamin Complex Reveals Active Site Architecture. Biochemistry. 52:7830-7839.

Simpson, R. J., P. D. Adams, and E. Golemis. 2009. Basic methods in protein purification and analysis: a laboratory manual. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Smirnov, N. N. 2014. Physiology of the Cladocera. Academic Press, San Diego.

Smith, D. C. and L. M. Proutt. 1944. Development of thiamine deficiency in the cat on a diet of raw fish. Experimental Biology and Medicine. 56:1-3.

Soriano, E. V., K. R. Rajashankar, J. W. Hanes, S. Bale, T. P. Begley, and S. E. Ealick. 2008. Structural similarities between thiamin-binding protein and thiaminase-I suggest a common ancestor. Biochemistry. 47:1346-1357.

Stemberger, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency. Rept. No. EPA 600/4-79-021, 185 pages.

Stewart, D. J. and F. P. Binkowski. 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: An energetics-modeling synthesis. Transactions of the American Fisheries Society. 115:643-661.

Stewart, T. J., R. E. Lange, S. D. Orsatti, C. P. Schneider, A. Mathers, and M. E. Daniels. 1999. Fish-community objectives for Lake Ontario. Great Lakes Fishery Commission Special Publication. 99-1:1-55.

Stoermer, E. F. 1978. Phytoplankton assemblages as indicators of water quality in the Laurentian Great Lakes. Transactions of the American Microscopy Society. 97:2-16.

Strakosh, T. R. and C. C. Krueger. 2005. Behavior of post-emergent lake trout fry in the presence of the alewife, a non-native predator. Journal of Great Lakes Research. 31:296-305.

Thomas, K. W. 1986. The effect of thiaminase-induced subclinical thiamine-deficiency on growth of weaner sheep. Veterinary Research Communications. 10:125-141.

Thomas, K. W., D. L. Turner, and E. M. Spicer. 1987. Thiamine, thiaminase, and trasketolase levels in groats with and without polioencephalomalacia. Australian Veterinary Journal. 64:126-127.

Tillitt, D. E., J. L. Zajicek, S. B. Brown, L. R. Brown, J. D. Fitzsimons, D. C. Honeyfield, M. E. Holey, and G. M. Wright. 2005. Thiamine and thiaminase status in forage fish of salmonines from Lake Michigan. Journal of Aquatic Animal Health. 17:13-25.

Tillitt, D. E., S. C. Riley, A. N. Evans, S. J. Nichols, J. L. Zajicek, J. Rinchard, C. A. Richter, and C. C. Krueger. 2009. Dreissenid mussels from the Great Lakes contain elevated thiaminase activity. Journal of Great Lakes Research. 35:309-312.

Toms, A. V., A. L. Haas, J. H. Park, T. P. Begley, and S. E. Ealick. 2005. Structural characterization of the regulatory proteins TenA and TenI from *Bacillus subtilis* and identification of TenA as a thiaminase II. Biochemistry. 44:2319-2329.

Walsh, M. G., D. E. Dittman, and R. O'Gorman. 2007. Occurrence and food habits of the round goby in the profundal zone of southwestern Lake Ontario. Journal of Great Lakes Research. 33:83-92.

Werner, R. M., B. Rook, and R. Greil. 2006. Egg-thiamine status and occurrence of early mortality syndrome (EMS) in Atlantic salmon from the St. Marys River, Michigan. Journal of Great Lakes Research. 32:293-305.

Wieser, W. 1960. Benthic studies is Buzzard's Bay. II. The meiofauna. Limnology and Oceanography. 5:121-137.

Winnell, M. H. and D. S. White. 1984. Ecology of shallow and deep water populations of *Pontoporeia hoyi* (Smith) (Amphipoda) in Lake Michigan. Freshwater Invertebrate Biology. 3:118-138.

Winters, A. D., T. L. Marsh, T. O. Brenden, and M. Faisal. 2015. Analysis of bacterial communities associated with the benthic amphipod *Diporeia* in the Laurentian Great Lakes Basin. Canadian Journal Of Microbiology. 61:72-81.

Wistbacka, S., A. Heinonen, and G. Bylund. 2002. Thiaminase activity of gastrointestinal contents of salmon and herring from the Baltic Sea. Journal of Fish Biology. 60:1031-1042.

Wistbacka, S. and G. Bylund. 2008. Thiaminase activity of Baltic salmon prey species: a comparision of net- and predator-caught samples. Journal of Fish Biology. 72:787-802.

Wistbacka, S., L. G. Lonnstrom, E. Bonsdorff, and G. Bylund. 2009. Thiaminase activity of Crucian carp *Carassius carassius* injected with a bacterial fish pathogen, *Aeromonas salmonicida* subsp. *salmonicida*. Journal of Aquatic Animal Health. 21:217-228.

Wittliff, J. L. and R. L. Airth. 1968. The extracellular thiaminase I of *Bacillus thiaminolyticus*. I. Purification and physicochemical properties. Biochemistry. 7:736-744.

Wolgamood, M., J. G. Hnath, S. B. Brown, K. Moore, S. V. Marcquenski, D. C. Honeyfield, J. P. Hinterkopf, J. D. Fitzsimons, and D. Tillit. 2005. Temporal and spatial variation of early mortality syndrome in salmonids from lakes Michigan and Huron. Journal of Aquatic Animal Health. 17:65-76.

Yan, N. D. and G. L. Mackie. 1987. Improved estimation of the dry-weight of *Holopedium gibberum* (Crustacea, Cladocera) using clutch size, a body-fat index and lake water total phosphorus concentration. Canadian Journal of Fisheries and Aquatic Sciences. 44:382-389.

Yokoyama, R., Y. Iwafune, H. Kawasaki, and H. Hirano. 2009. Isoelectric focusing of high-molecular-weight protein complex under native conditions using agarose gel. Analytical Biochemistry. 387:60-63.

Yudkin, W. H. 1949. Thiaminase, the Chastek-paralysis factor. Physiological Reviews. 29:389-402.

Zajicek, J. L., D. E. Tillitt, D. C. Honeyfield, S. B. Brown, and J. D. Fitzsimons. 2005. A method for measuring total thiaminase activity in fish tissues. Journal of Aquatic Animal Health. 17:82-94.

Zajicek, J. L., L. Brown, S. B. Brown, D. C. Honeyfield, J. D. Fitzsimons, and D. E. Tillitt. 2009. Variations of thiaminase I activity pH dependencies among typical Great Lakes forage fish and *Paenibacillus thiaminolyticus*. Journal of Aquatic Animal Health. 21:207-216.

7 Appendices

Appendix A. Regression for converting zooplankton dry weight to wet weight

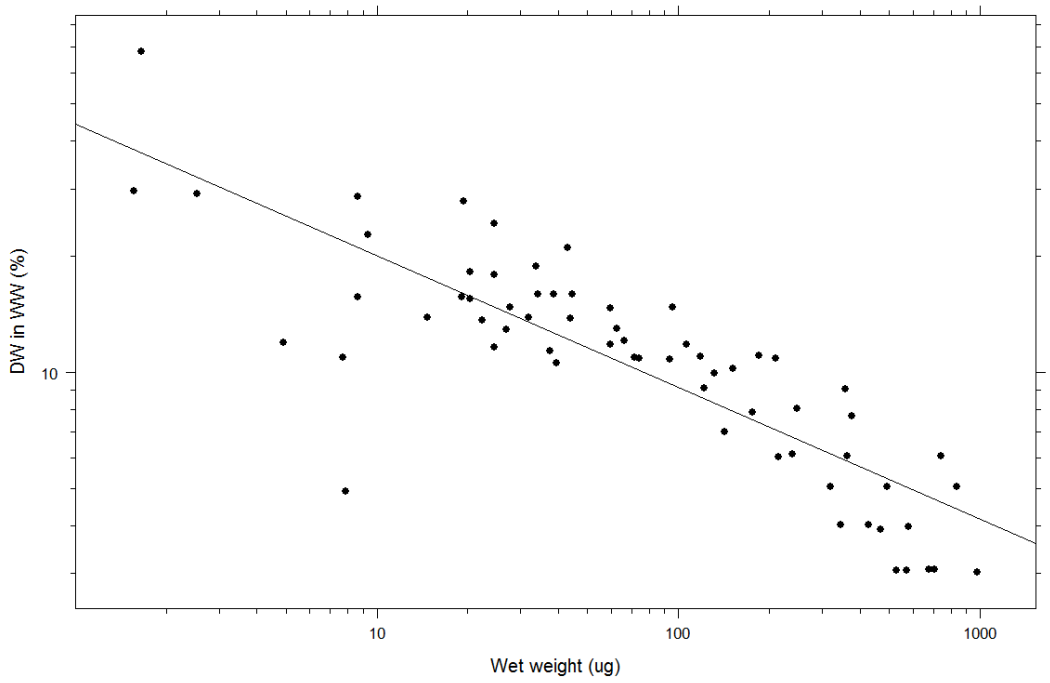

Figure Appendix A.1. Regression of wet weight on the percentage of dry weight in wet weight

Regression of wet weight on the percentage of dry weight in wet weight. For methods, see section 2.3.6.

Appendix B. Effect of sieving 153 µm tows with a 125 µm filter

Methods for evaluating the effect of sieving the 153 µm tow with 125 µm tows filter:

To ensure that this small difference (28 µm) in mesh size did not result in differences in community composition, we enumerated one zooplankton sample from each of two sampling events for which the a standard 153 µm mesh community tow sieved through the 125 µm mesh prior to enumeration. Enumeration occurred as described in section 2.3.5. We compared the community composition (gram-normalized wet mass) estimated from the fractionated (125 µm mesh) tows to that estimated from the standard unfractionated (153 µm mesh) tows using a Mann-Whitney-Wilcoxon test on nested ranks for each of the two samples, as implemented by the nestedRanksTest package in R. Additionally, the frequency of occurrence and importance of taxa with mean lengths between 125 µm and 153 µm were assessed.

Results:

A nested ranks test revealed no difference in the gram-normalized mass of taxa between the two filter sizes at either 18 m ($p=0.95$) or 100 m ($p=0.65$) (Figure Appendix B.1). The results were virtually identical in terms of p-values regardless of whether or not the taxa that were jointly absent from the samples filtered with the 125 µm and 153 µm mesh were included or excluded in the comparison.

Only 2 taxa had mean lengths between 125 and 153 µm: Synchaeta and Conochilus. Synchaeta was only in 7 of the 30 samples and constituted a maximum of 0.06% of any sample mass. Conochilus was more widespread, occurring in 26 of the 30 samples. Conochilus is colonial, and although the mean lengths of individuals was 138 µm, colonies would be expected to have a functional colonial length of larger than 125 µm and therefore would be expected to be retained in both the 125 and 153 µm filters.

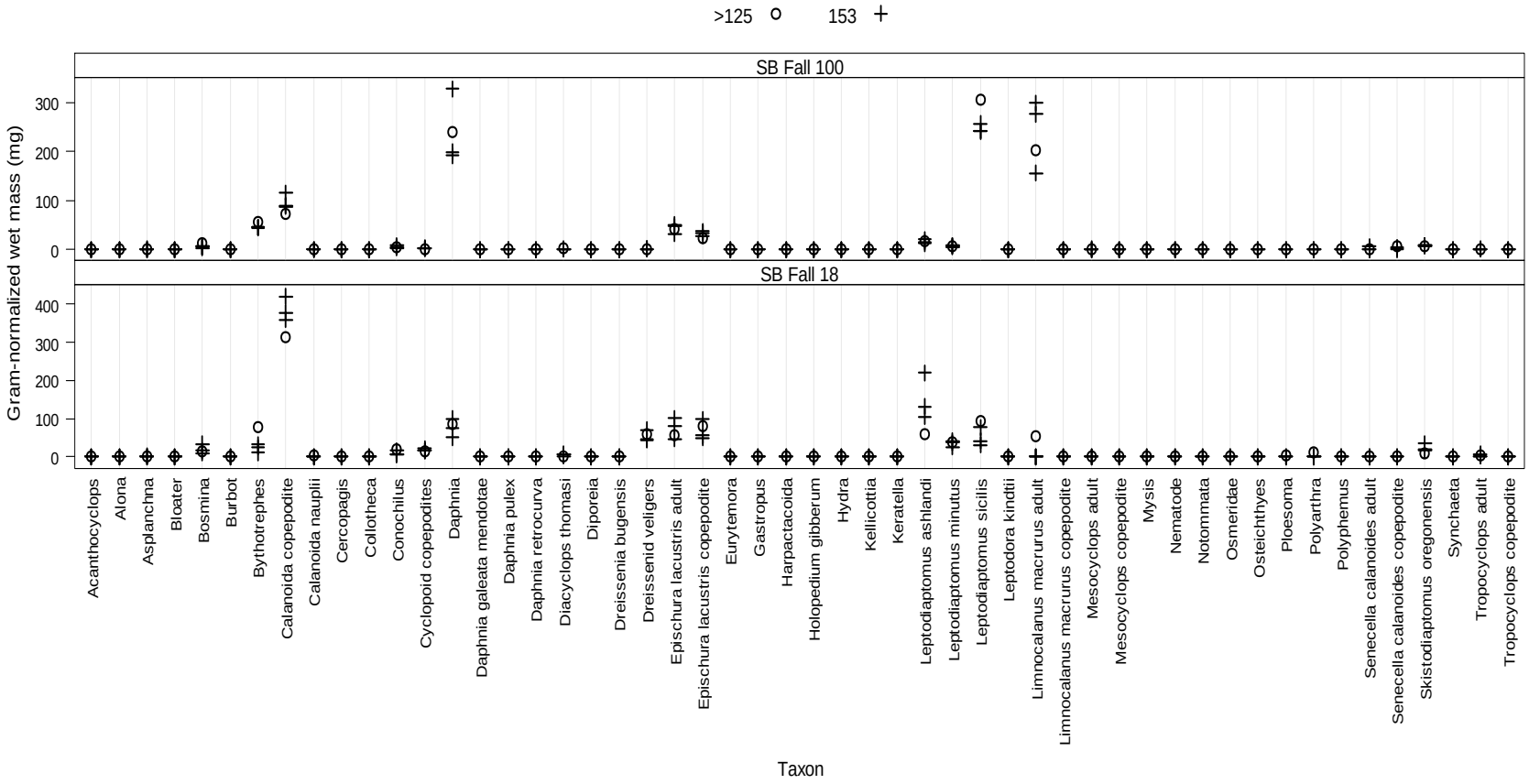

Figure Appendix B.1. Effect of sieving 153 µm tows with a 125 µm filter on community composition

Gram-normalized wet mass (mg) for each taxon in two samples form Sturgeon Bay that were enumerated after filtering with tow mesh sized (125 µm and 153 µm). Points are coded by mesh size and and panels are sorted by sampling event (site, season, and depth in meters).

Appendix C. Comparison of zooplankton community replicates and averages in species-space

Methods for evaluating the relationship between sample event centroids and the location of sample events when represented by the average gram-normalized mass:

The 3 replicates for each of the 10 sampling events were ordinated using NMS (PCORD) on the "slow and thorough" autopilot setting with Sorensen distance. The main matrix consisted of the 30 individual replicates as rows and gram-normalized biomass of 39 species as columns. The resulting ordination was rotated to maximize the correspondence with the log of thiaminase activity, and triplicates samples were each assigned the average log thiaminase values for their sampling event. Ordination results were examined graphically and the centroids for each sampling event were calculated as average position of each of the 3 triplicate points in the ordination space in PCORD. The same 10 sampling events were also ordinated in the same manner. The main matrix consisted of the 10 sampling events as rows and average gram-normalized biomass of 39 species for each sampling event as columns, and the final ordination was rotated to maximize the correspondence with the average of the log of thiaminase activity.

To determine whether the centroids from the ordination based on 30 samples produced the same representation of sampling events in species-space as the ordination of the 10 sampling events based on averaging triplicate samples at each site, the 10 centroids from the 30 sample ordination and the 10 points from the ordination of the averages for each sampling event were compared using a Procrustes rotation (package vegan, R), which allows a graphical representation of the best match of two ordinations by scaling, rotating, and reflection to compare two ordinations. A permutation test (n=999 permutations; function protest in package vegan) was used to assess the degree of similarity between the two ordinations.

Results:

The ordination based on the 30 sample-unit matrix that included the replicates at each sampling event were located near each other in species-space and that the centroids for each sampling event were reasonable representations of the location of each sampling events (Figure Appendix C.1). A 2-dimensional solution was recommended. Stress was low (10.74) and the 2-dimensional solution explained

83.9% of the variation in the original 39-dimensional species-space. The p-values for the randomization test cannot be interpreted accurately for this ordination because of the psuedoreplication associated with the dependence among rows. For the ordination of the 10 sample events, a 3-dimensional solution was recommended by the PCORD autopilot, but because the third axis explained only an additional 5% of the variation, the stress was for the 2-dimensional was low (7.61), and the randomization test indicated sufficient structure (p=0.044), the 2-dimensional solution was chosen (Figure Appendix C.2). Procrustes rotation revealed that the two ordinations did not differ, with the Procrustes sum of squares = 0.0688 and p=0.001 for the permutation test (Figure Appendix C.3).

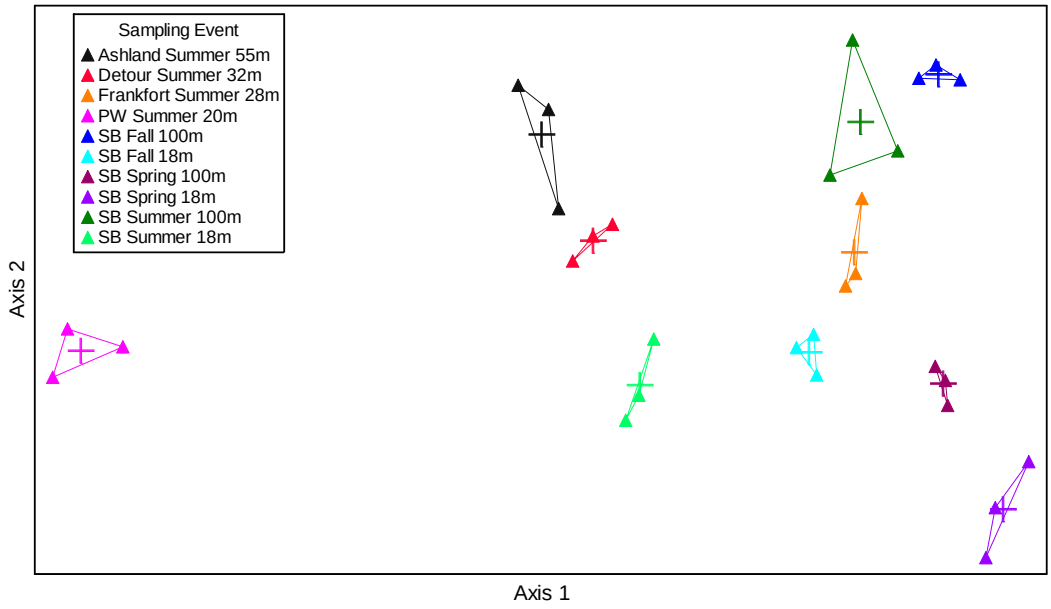

Figure Appendix C.1. Ordination of zooplankton community replicates in species-space

NMS ordination of 30 samples in species-space, with each replicate shown individually as a triangle and the centroids shown as pluses. Convex hulls are superimposed as lines.

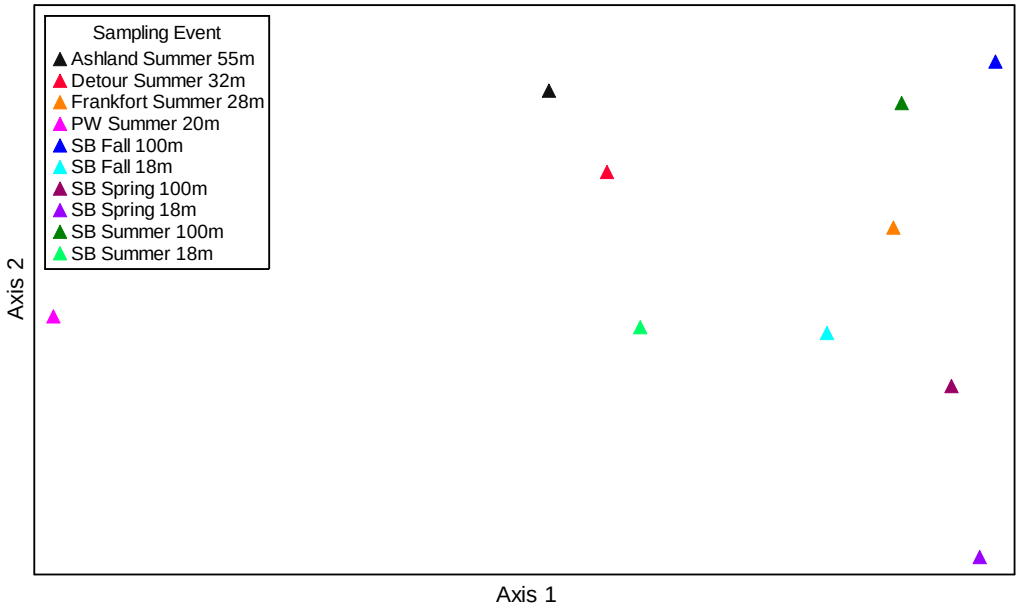

Figure Appendix C.2. Ordination of zooplankton community averages in species-space

NMS ordination of 10 sampling events using average gram-normalized wet mass in species-space, with each sampling event shown individually as a triangle.

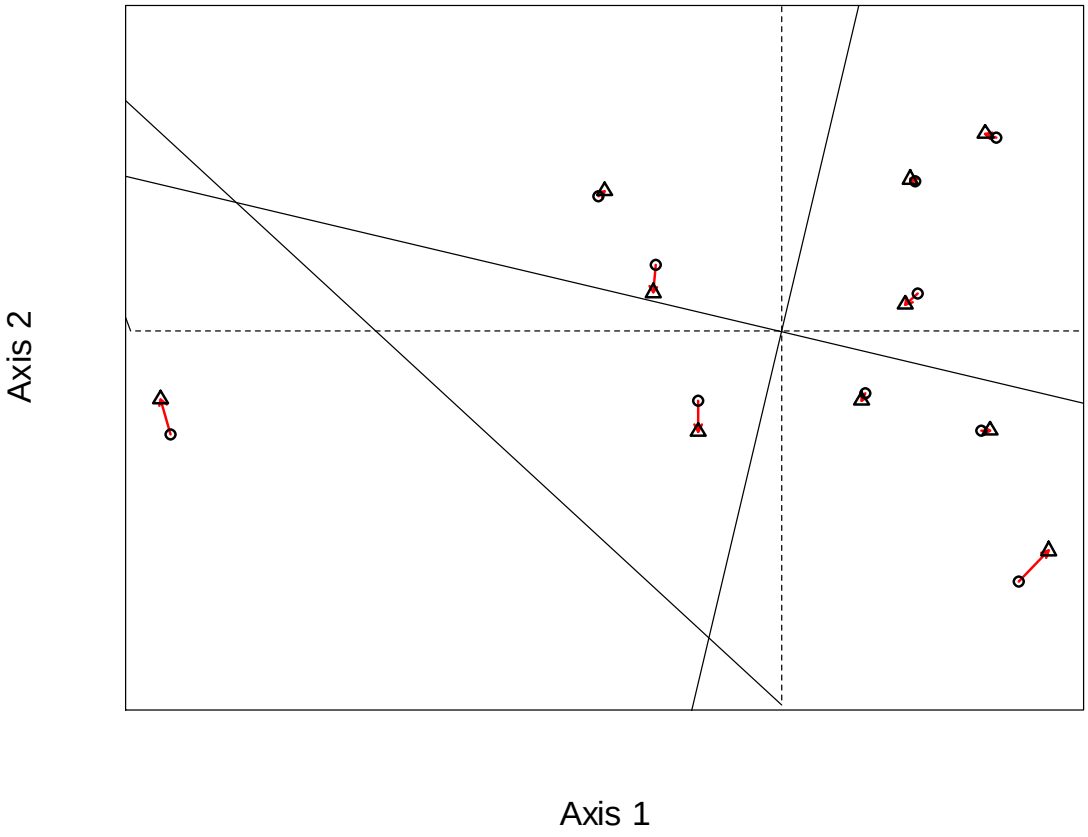

Figure Appendix C.3. Procrustes rotation comparing the positions of sampling events in species-space for centroids of replicates and averages of replicates

Procrustes rotation comparing the positions of sampling events in species-space for the centroids of replicates and averages of replicates. Centroids from the 30-sample ordination (Figure Appendix C.1) are represented as triangles, the points from the 10-sample ordination (Figure Appendix C.2) are represented as circles, and the red arrowheads show the rotation required to achieve the best match of the two ordinations.

Appendix D. Effect of season, depth, and size fraction on thiaminase activity at Sturgeon Bay

Table Appendix D.1. ANOVA table for the effect of season and size fraction on thiaminase activity at Sturgeon Bay

ANOVA table for the effect of season and size fraction on thiaminase activity at Sturgeon Bay.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Size fraction	2	23.97	11.987	13.28	4.78E-05
Season	2	43.15	21.577	23.91	2.47E-07
Residuals	36	32.49	0.902		

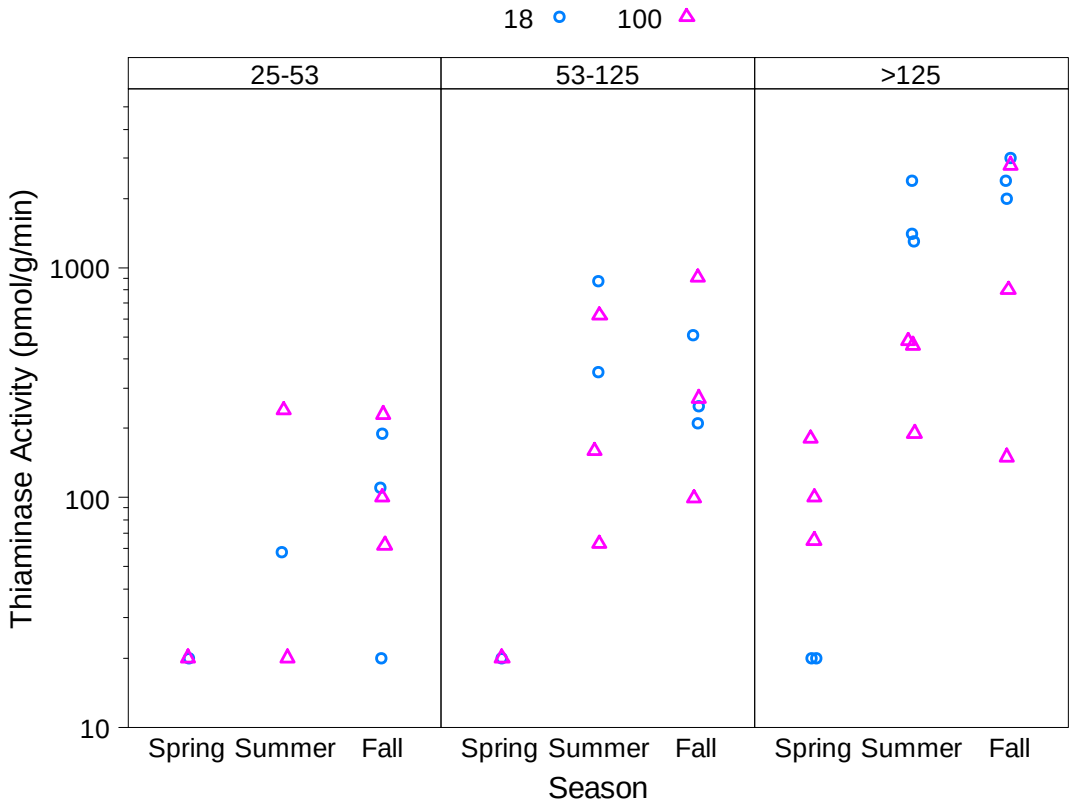

Figure Appendix D.1. Thiaminase activity by season at Sturgeon Bay

Thiaminase activity at Sturgeon Bay, with points coded by depth and panels sorted by size fraction.

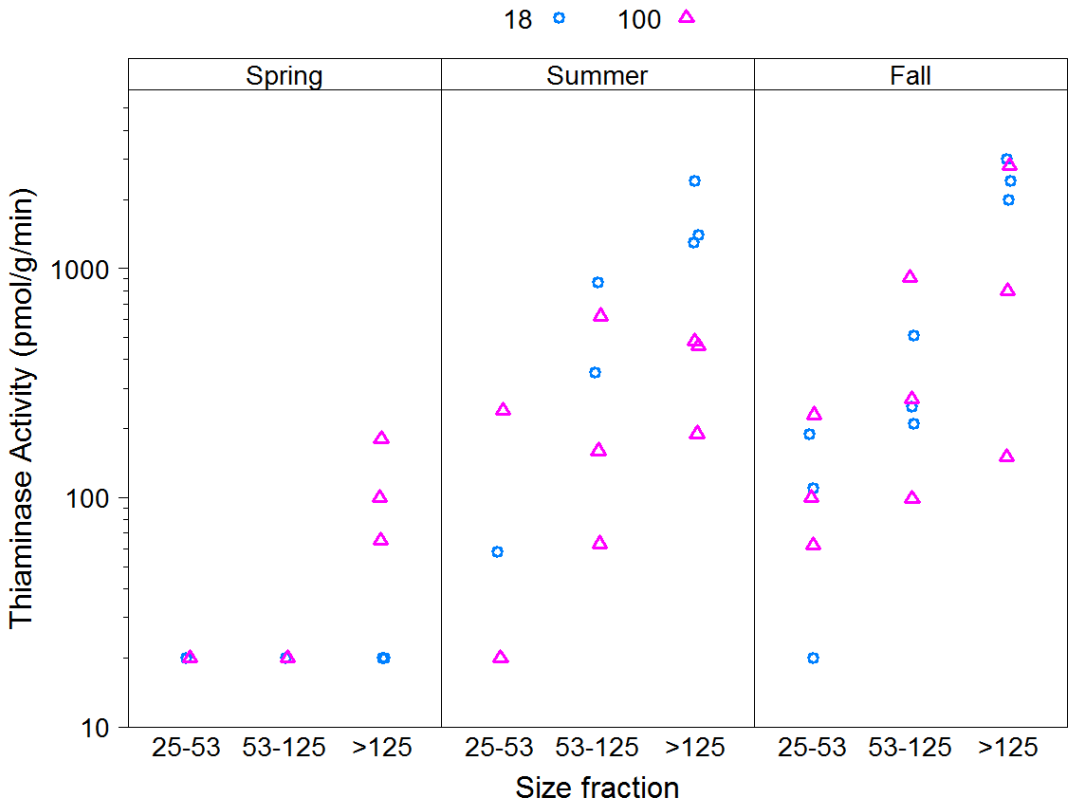

Figure Appendix D.2. Thiaminase activity by size fraction at Sturgeon Bay

Thiaminase activity at Sturgeon Bay, with points coded by depth and panels sorted by season.

Appendix E. Effect of site on thiaminase activity

Table Appendix E.1. ANOVA table for the effect of site on summer thiaminase activity in the >125 µm size fraction

ANOVA table for the effect of site on summer thiaminase activity in the >125 µm size fraction.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	4	52.42	13.104	19	0.0000135
Residuals	14	9.46	0.676		

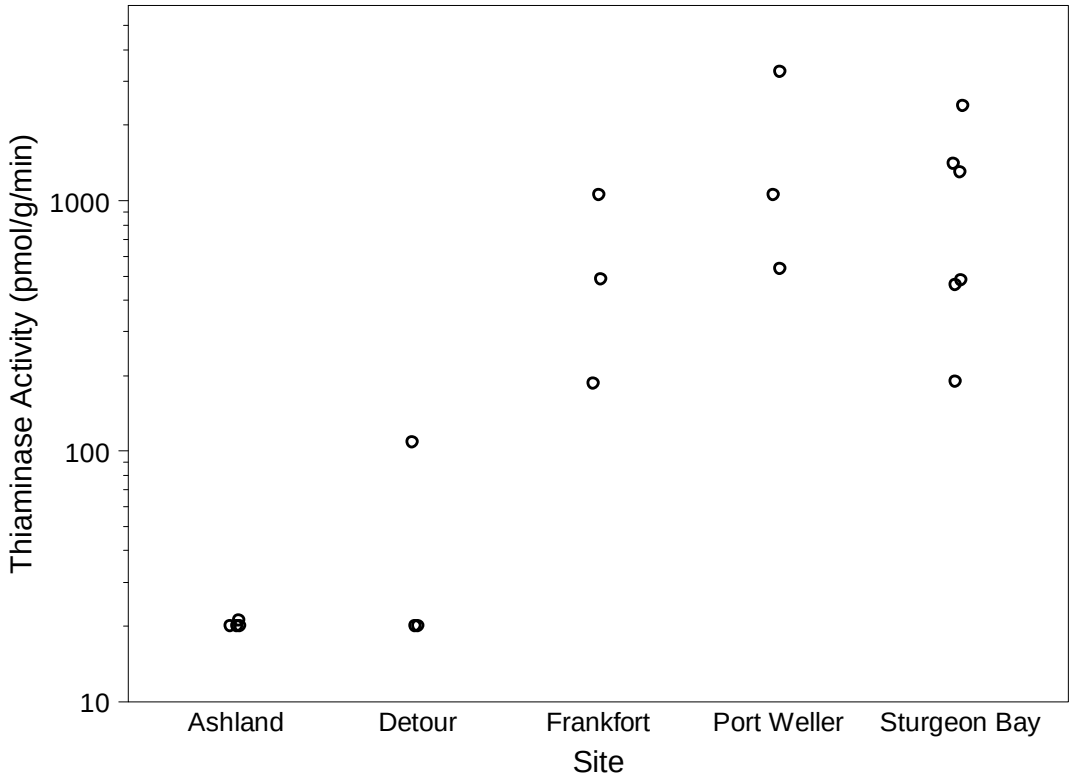

Figure Appendix E.1. Summer thiaminase activity in the >125 µm size fraction

Summer thiaminase activity in bulk zooplankton tows from the >125 µm size fraction.

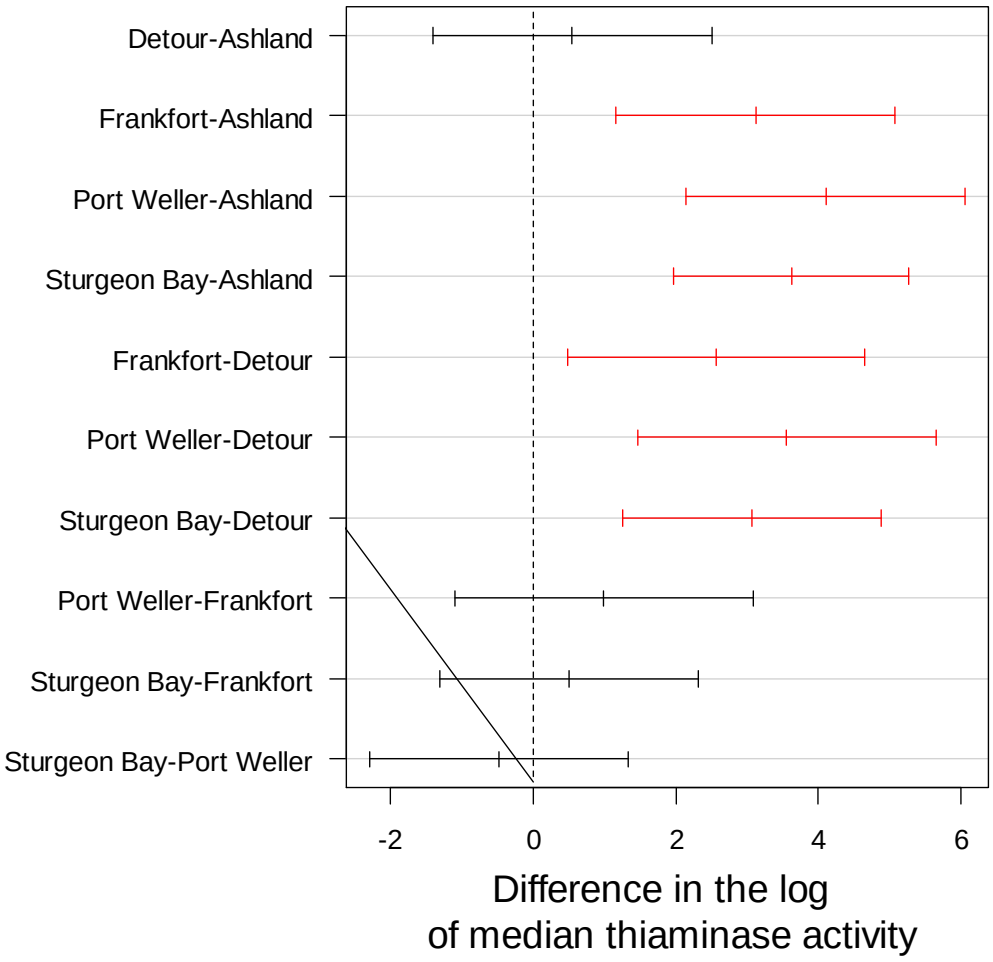

Figure Appendix E.2. 95% Confidence intervals for *Site* based on Model 2.2

Graph showing pairwise 95% confidence intervals for the effect of site on summer thiaminase activity based on parameter estimates from Model 2.2. Note: the difference shown on the X-axis represents the pairwise difference on the log scale. Red confidence intervals show the pairwise comparisons in which the thiaminase activities differs, and confidence intervals that include zero indicate no difference at the 95% family-wise confidence level.

Appendix F. Average gram-normalized wet mass of each taxon by sampling event

Table Appendix F.1. Average gram-normalized wet mass of each taxon by sampling event

Average wet mass (in mg) expected in a one-gram sample of each taxon by sampling event. AS=Ashland, DT=Detour, FR=Frankfort, PW=Port Weller, SB=Sturgeon Bay. Note that only 39 of these taxa were used in multivariate analyses.

Site	AS	DT	FR	PW	SB	SB	SB	SB	SB	SB
Season	Summer	Summer	Summer	Summer	Spring	Spring	Summer	Summer	Fall	Fall
Depth (m)	55	32	28	25	18	100	18	100	18	100
Acanthocyclops spp.	0	0	0	0	0	0	0	0.616	0	0
Alona spp.	0	0	0	0	0.172	0	0	0	0	0
Asplanchna	63.091	11.866	0	545.039	0	0	79.975	2.697	0.656	0.27
Bloater larvae	2.836	0	0	0	0	0	0	0	0	0
Bosmina spp.	6.594	52.258	13.949	26.758	0.538	0.226	2.96	2.381	19.913	5.149
Burbot larvae	0.444	0.115	0	0	0	0	0	0	0	0
Bythotrephes longimanus	5.394	27.544	2.075	0	0	0	102.036	14.19	23.072	44.757
Calananoid copepodites	173.871	305.381	369.839	13.172	80.031	193.798	260.451	219.184	385.704	97.681
Calananoid nauplii	2.988	1.658	0.458	0.224	1.219	7.729	3.094	0.246	0.49	0.448
Cercopagis pengoi	0	0	0.007	84.104	0	0	0	0	0	0
Collotheca	0.331	0	1.589	0	0	0	0.083	0	0	0
Conochilus	16.671	83.678	9.024	0.718	0.079	0.001	114.492	28.738	9.779	5.209
Cyclopoid copepodites	142.075	97.964	0.552	181.42	15.67	1.959	39.496	1.198	17.683	2.717
Daphnia mendotae	0	0	0	37.785	0	0	0	0	0	0
Daphnia pulex	0	0	0.767	0	0	0	5.358	0	0	0
Daphnia retrocurva	0	0	0	13.414	0	0	0	0	0	0
Daphnia spp.	57.354	22.138	35.643	0	3.882	1.436	39.075	123.909	75.18	239.361
Diacyclops thomasi	94.385	45.289	0	14.239	34.258	4.809	15.069	0.953	4.591	0.722
Diporeia hoyi	0	0	0	0	0	1.513	0	0	0	0
Dreissena bugensis	0	0	0	0	0	250.032	0	0	0	0
Dreissena veligers	1.508	6.403	20.845	4.398	0	0.422	125.86	4.867	53.087	0.886

Epischura lacustris adult	14.39	12.017	19.192	0	0	0	43.216	3.242	76.245	43.571
Epischura lacustris copepodite	0	4.932	27.812	0	0	0.081	7.658	1.156	67.997	33.012
Eurytemora affinis	0	0	0.455	0	0	0	0	0	0	0
Harpactacoid	0	0	0.139	0.311	1.107	0	0	0	0	0
Holopedium gibberum	275.101	175.678	0	1.183	0	0	0	0	0	0
Hydra	0	0	0	0	0	1.057	0	0	0	0
Kellicottia	2.132	0.286	0.141	2.598	0.001	0.001	0.66	1.113	0.073	0.016
Keratella	0.08	1.035	0.005	0.68	0	0	0.045	0	0	0
Leptodiaptomus ashlandi	0	14.911	93.033	1.115	402.851	241.082	51.171	74.836	152.676	15.801
Leptodiaptomus minutus	0	9.958	29.671	2.516	247.211	62.138	47.603	2.366	34.687	7.148
Leptodiaptomus sicilis	90.555	7.651	221.117	8.934	144.754	161.253	31.496	140.613	49.893	246.328
Leptodora kindti	0	0	11.652	17.721	0	0	0.778	0	0	0
Limnocalanus macrurus adult	43.425	91.686	136.024	28.873	30.428	14.286	8.772	363.103	0	243.524
Limnocalanus macrurus copepodite	0	0	0	0	0.816	22.578	0	1.726	0	0
Mesocyclops spp. adult	0	5.209	0	0.092	0	0	6.472	0	0	0.319
Mesocyclops spp. copepodite	0	0	0	0	0	0	6.784	0.177	0.313	0
Mysis diluviana	5.21	0	0	0	0	0	0	0	0	0.033
Nematode	0	0	0	0.007	0	0	0	0	0	0
Notommata	0	0	0.002	0	0	0	0	0	0	0
Osmeridae larvae	0.824	0	0	0	0	0	0	0	0	0
Osteichthyes larvae	0	0.132	0.008	0	0	0.289	0	0.69	0	0.037
Ploesoma	0	0	0.277	0.847	0	0	0.364	0	0.18	0.035
Polyarthra	0.094	0	0	0.105	0	0	0.029	0	0	0
Polyphemus pediculus	0	0	0.076	5.593	0	0	1.064	0	0	0
Senecella calanoids adult	0	0	0	0	0	0	0	5.024	0	1.928
Senecella calanoids copepodite	0	0	0	0	0	3.568	0	2.627	0	2.655
Skistodiaptomus.oregonensis	0.635	22.194	5.316	7.841	36.509	29.791	5.574	4.337	24.101	7.714
Synchaeta	0.01	0.016	0	0.31	0	0	0.025	0.01	0	0
Tropocyclops prasinus mexicanus adult	0	0	0.332	0	0.473	1.724	0.34	0	3.68	0.663
Tropocyclops prasinus mexicanus copepodite	0	0	0	0	0	0.224	0	0	0	0.017

Appendix G. 3-dimensional NMS ordination of taxa rotated to correspond to thiaminase activity

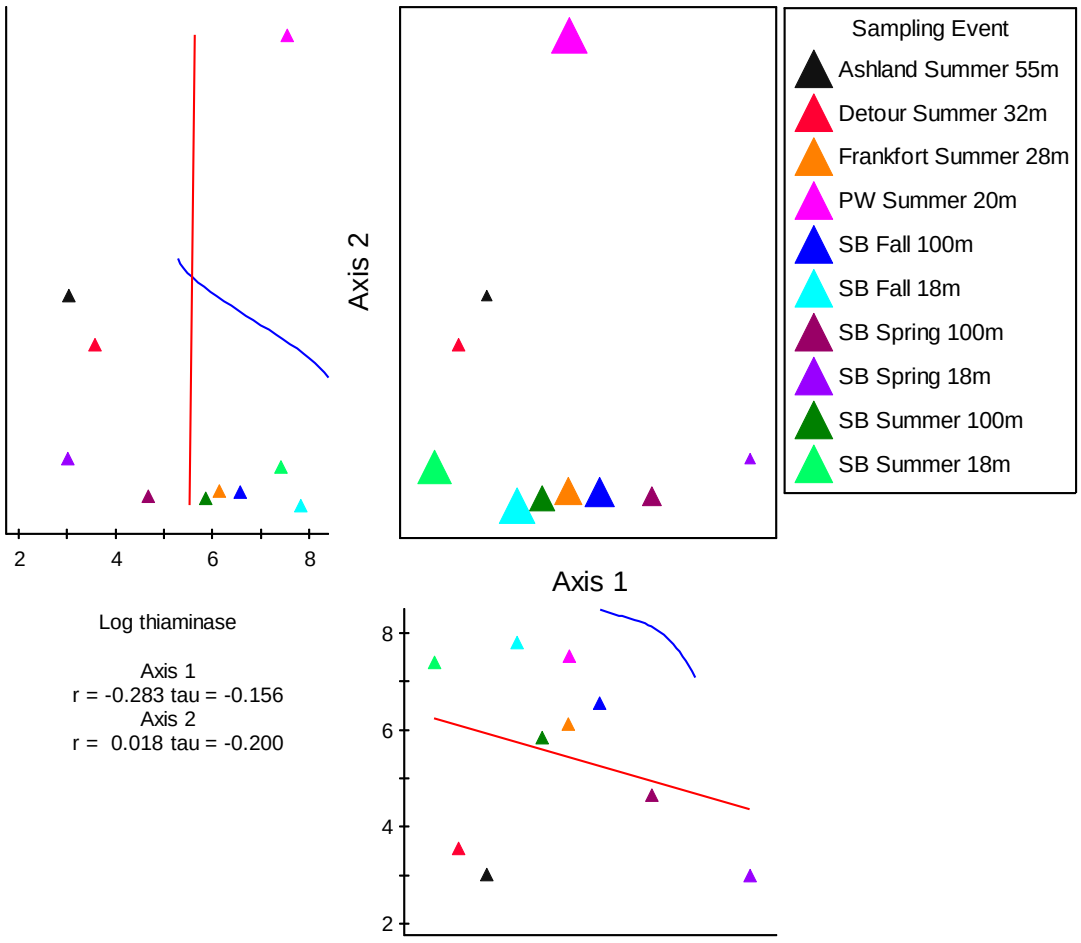

Figure Appendix G.1. 3-dimensional NMS ordination of taxa rotated to correspond to thiaminase activity

First two dimensions of the 3-dimensional NMS solution of 10 sampling events in taxa-space, rotated for maximum correspondence with the log of thiaminase. Only the first and second axes are shown. The third axis was unrelated to thiaminase activity (r=0.041, tau = -0.067). Point size in the ordination panel represents thiaminase activity, and the bottom scatterplot shows the relationship between axis 1 and the log of thiaminase activity.

Appendix H. Relationship between thiaminase activity and gram-normalized wet biomass for *Asplanchna* and *Ploesoma*

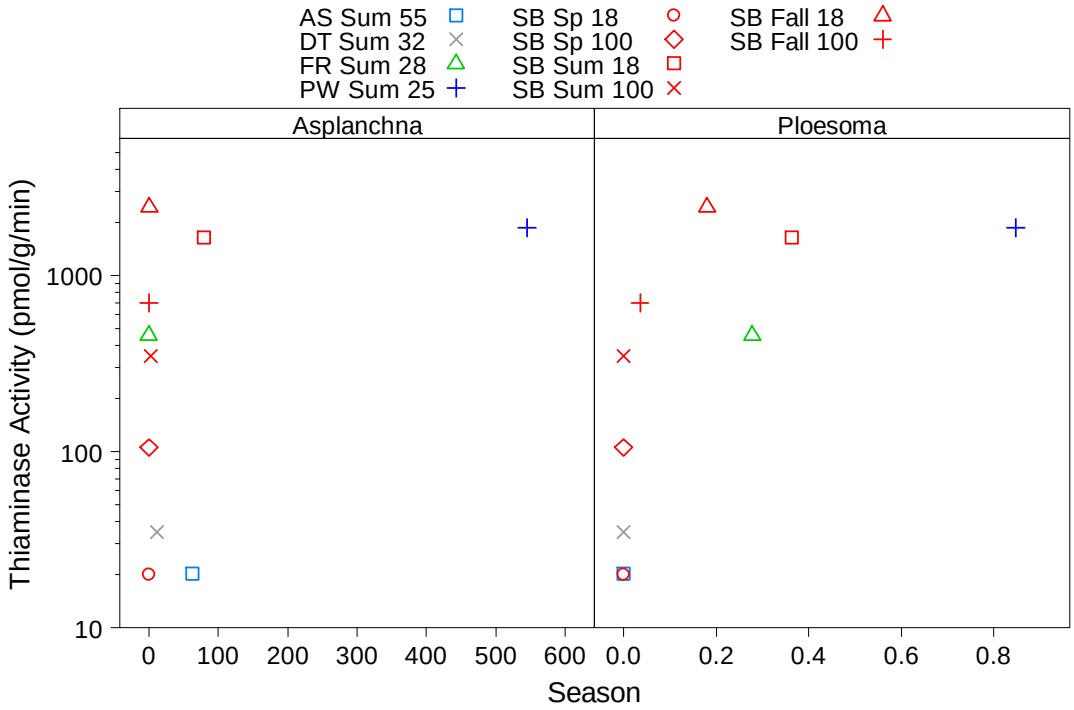

Figure Appendix H.1. Relationship between thiaminase activity and gram-normalized wet biomass for *Asplanchna* and *Ploesoma*

The relationship between thiaminase activity (pmol/g/min) and the gram-normalized wet biomass (mg) for *Asplanchna* and *Ploesoma*, the two taxa with discriminating ability for the high thiaminase groups in the ISA.

Appendix I. Summary statistics for thiaminase activity in fish viscera

Table Appendix I.1. Summary statistics for thiaminase activity in fish viscera

Geometric mean, geometric standard deviation (SD), median, range, and sample size for thiaminase activity measurements in fish viscera (pmol/g/min) in the 41 combinations (site, species, season, and depth) of sampled fishes.

				Thiaminase activity (pmol/g/min)				
Site	**Species**	**Season**	**Depth**	**Geo-metric Mean**	**Geo-metric SD**	**Median**	**Range**	**n**
Ashland	Bloater	Summer	Mid (35-70 m)**	29	1.70	20	20-76	10
Ashland	Cisco	Summer	Mid (35-70 m)	20	1.00	20	20-20	4
Ashland	Deepwater Sculpin	Summer	Mid (35-70 m)	20	1.00	20	20-20	5
Ashland	Kiyi	Summer	Mid (35-70 m)	20	1.00	20	20-20	2
Ashland	Lake Whitefish	Summer	Mid (35-70 m)	89	3.41	83	20-606	6
Ashland	Pygmy Whitefish	Summer	Mid (35-70 m)	57	5.75	20	20-1835	10
Ashland	Rainbow Smelt	Summer	Mid (35-70 m)	14044	1.72	13764	6707-48121	10
Ashland	Shortjaw Cisco	Summer	Mid (35-70 m)	20	1.00	20	20-20	2
Ashland	Slimy Sculpin	Summer	Mid (35-70 m)	436	7.84	687	20-3899	8
Ashland	Spoonhead Sculpin	Summer	Mid (35-70 m)	258	6.76	551	20-3053	9
Ashland	Trout-perch	Summer	Mid (35-70 m)	3647	1.48	3788	2767-4808	2
*Detour	Alewife	Fall	Mid (37 m)	5766	1.35	6291	3328-8392	14
*Detour	Bloater	Fall	Mid (73 m)	128	4.95	141	20-2951	10
Detour	Deepwater Sculpin	Summer	Mid (37 m)	54	5.58	20	20-393	3
*Detour	Deepwater Sculpin	Fall	Mid (64 m)	109	3.40	147	20-352	4
*Detour	Ninespine Stickleback	Fall	Mid (37 m)	695	5.76	858	72-4879	4

Detour	Rainbow Smelt	Summer	Mid (37 m)	9553	1.33	9302	6904-13941	4
*Detour	Rainbow Smelt	Fall	Mid (73 m)	8062	2.60	11887	1292-18721	11
Detour	Slimy Sculpin	Summer	Mid (37 m)	61036	2.65	85684	8305-229649	10
*Detour	Rainbow Smelt	Fall	Mid (27 m)	91524	1.79	69655	61466-179068	3
*Frankfort	Alewife	Fall	Mid (30 m)	12953	1.64	12644	5740-32783	15
Frankfort	Ninespine Stickleback	Summer	Mid (27 m)	310	6.57	219	20-10874	9
Frankfort	Slimy sculpin	Summer	Mid (27 m)	9552	2.47	11370	2942-34208	10
Port Weller	Alewife	Summer	Near (9 m)	8761	1.30	8911	7283-10538	2
*Port Weller	Alewife	Summer	Mid (75 m)	9416	NA	9416	9416-9416	1
*Port Weller	Rainbow Smelt	Summer	Mid (75 m)	15503	1.83	19052	5352-30289	10
Port Weller	Round Goby	Summer	Near (9m)	11429	3.60	13892	1726-54256	10
Sturgeon Bay	Alewife	Spring	Off (110 m)	38970	1.68	41000	12000-92000	13
Sturgeon Bay	Alewife	Summer	Near (18 m)	9215	1.79	6900	6300-18000	3
Sturgeon Bay	Alewife	Summer	Off (110 m)	9970	1.68	8400	5100-25000	15
Sturgeon Bay	Alewife	Fall	Off (110 m)	13517	1.56	13000	5200-30000	15
Sturgeon Bay	Bloater	Summer	Off (110 m)	48	2.61	61	20-120	4
Sturgeon Bay	Deepwater Sculpin	Spring	Off (110 m)	242	5.56	275	20-5400	10
Sturgeon Bay	Deepwater Sculpin	Summer	Off (110 m)	55	2.56	46	20-210	10
Sturgeon Bay	Deepwater Sculpin	Fall	Off (110 m)	38	2.65	20	20-360	10
Sturgeon Bay	Ninespine Stickleback	Spring	Off (110 m)	1370	4.26	940	180-9700	7
Sturgeon Bay	Ninespine Stickleback	Summer	Near (18 m)	166	6.49	140	20-3400	7
Sturgeon Bay	Rainbow Smelt	Summer	Off (110 m)	6616	1.86	6800	2100-17000	10
Sturgeon Bay	Rainbow Smelt	Fall	Off (110 m)	9078	2.03	7900	3800-24000	8
Sturgeon Bay	Slimy Sculpin	Summer	Near (18 m)	1998	9.22	2700	20-25000	10
Sturgeon Bay	Slimy Sculpin	Fall	Off (110 m)	257	5.41	185	20-3600	10

Appendix J. Equations used to convert prey taxa lengths to biomass

Table Appendix J.1. Equations used to convert prey taxa length to biomass

Equations used to convert prey taxa length to wet biomass. L = body length in mm (diameter for fish eggs); WW= wet weight in mg; DW=dry weight in mg; D=width in mm; HCW=head capsule width (Chironomid larvae); Log is natural log unless specified as Log_{10}. Lack of a dry weight equation indicates that length was converted directly to wet weight. See Table 2.3 for length-weight regressions used for additional zooplankton taxa.

Taxon	Dry weight equation (Source)	Wet weight equation (Source)
Chelicerata		
Acarina		WW = exp(-6.504 + (0.45919*L) + (-0.0068653*(L^2))) (Sage Full publication date:
Crustacea: Cladocera		
Ephippia	DW = (2.9 * (L^2.60))/1000 (Lynch et al. 1986)	log_{10}WW = 0.5377 + 1.52*log_{10}DW (Bottrell et al. 1976)
Eurycercus lamellatus	DW = 0.0111	log_{10}WW = 0.5377 + 1.52*log_{10}DW (Bottrell et al. 1976)
Crustacea: Calanoida		
Unidentified calanoid adults		WW = 0.03493*(L^2.9878) (Pearre 1980)
Skistodiaptomus pallidus		WW = 0.03493*(L^2.9878) (Pearre 1980)
Crustacea: Cyclopoida		
Acanthocyclops		WW = 0.03493*(L^2.9878) (Pearre 1980)
Unidentified cyclopoid adults		WW = 0.03493*(L^2.9878) (Pearre 1980)
Crustacea: Malacostra		
Isopoda	DW = (0.0054*(L^2.948)) (Benke et al. 1999)	WW = DW*100 / 20.3 (Leuven et al. 1985)
Crustacea: Ostracoda		
Ostracods	DW = 0.0212 mg (Nalepa and Quigley 1980)	WW = DW / 0.18 (Beers 1966)
Insecta		
Chironomid larvae	DW = 1.9574 * (HCW^2.589) (Benke et al. 1999)	WW= DW / 0.11 (Dermott and Paterson 1974)
Chironomid pupae		WW = (exp(-8.503 + (0.69325*L) + (-0.017613*(L^2))))*1000 (Sage Full publication
Osteichthyes		
Fish eggs	DW= 0.043 * (L^2.941)) (Hislop and Bell 1987)	WW = DW/0.315 (Hoar and Randall 1969)

Literature Cited

Beers, J. R. 1966. Studies on the chemical composition of the major zooplankton groups in the Sargasso Sea off Bermuda. Limnology and Oceanography. 11:520-528.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society. 18:308-343.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson, and T. Weglenska. 1976. A review of some problems is zooplankton production studies. Norwegian Journal of Zoology. 24:419-456.

Dermott, R. M. and C. G. Paterson. 1974. Determining dry weight and percentage dry matter of chironomid larvae. Canadian Journal of Zoology. 52:1243-1250.

Hislop, J. R. G. and M. A. Bell. 1987. Observations on the size, dry-weight and energy content of the eggs of some demersal fish speciecs from British marine waters. Journal of Fish Biology. 31:1-20.

Hoar, W. S. and D. J. Randall, editors. 1969. Reproduction and growth, bioluminescence, pigments and poisons. Academic Press, New York.

Leuven, R., T. C. M. Brock, and H. A. M. Vandruten. 1985. Effects of preservation on dry-free and ash-free dry-weight biomass of some common aquatice macro-invertebrates. Hydrobiologia. 127:151-159.

Lynch, M., L. J. Weider, and W. Lampert. 1986. Measurement of the carbon balance in *Daphnia*. Limnology and Oceanography. 31:17-33.

Nalepa, T. and M. A. Quigley. 1980. The macro and meiobenthos of southeastern Lake Michigan near the mouth of the Grand River, 1976-77. Great Lakes Environmental Reasearch Laboratory, Ann Arbor, MI.

Pearre, S. 1980. The copopod width-weight relation and its utility in food-chain research. Canadian Journal of Zoology. 58:1884-1891.

Sage, R. D. 1982. Wet and dry-weight estimates of insects and spiders based on length. The American Midland Naturalist. 108:407-411.

Appendix K. verage proportional biomass of prey taxa consumed for each of 17 sampling combinations

Table Appendix K.1. Average proportional biomass (by mass) of prey taxa consumed for each of 17 sampling combinations

Average proportional biomass (by mass) of prey taxa consumed for each of 17 sampling combinations. Species abbreviations are as in Table 3.1. All taxa constituting <0.005% of the average proportional biomass are designated as "0.00". An empty cell means the taxon was not found in any individual stomach for that sampling combination.

Site	AS	AS	AS	AS	FR	FR	PW	PW	SB	SB	SB	SB	SB	SB	SB	SB	SB
Season	Sum	Sum	Sum	Sum	Sum	Sum	Sum	Sum	Sp	Sum	Fall	Sp	Sum	Fall	Sp	Sum	Sum
Depth	50	50	50	50	27	27	75	9	100	100	100	100	100	100	100	18	18
Species	BLO	CIS	RS	SS	NSS	SS	RS	RG	ALE	ALE	ALE	DWS	DWS	DWS	NSS	NSS	SS
Acathocyclops brevispinosis																0.00	
Acathocyclops venustoides																0.01	
Acarina								9.11									
Bryocamptus minutus																0.00	
Bosmina							0.00	0.00	0.02	0.04	0.19					0.12	
Bythotrephes	7.46	25.25	12.48			21.36		2.85	11.79	48.78	69.41						
Canthocamptus assimilis																0.03	
Caecidotea intermedia																0.80	41.76
Canthocamptus staphylinoides					0.02											0.02	
Calanoid adult	0.24				1.54			0.04	0.27	0.24	0.09				0.35	0.94	
Calanoid copep	0.05	0.07			17.06		0.00		2.20	1.51	0.01					17.44	
Chironomid adult										0.01	1.70						
Chironomid larva	1.34	0.23	3.27	7.78		0.78		63.84									9.30
Chironomid pupae	3.94	5.15	8.08	11.70	11.35	42.10	0.18	9.09								20.67	29.16
Cyclopoid adult										0.00						0.12	
Cyclopoidcopep		0.00								0.04						0.02	
D. mendotae		0.53							2.05	16.01	4.39					2.64	
Diacycops thomasi	0.01	0.01				0.07	0.48	0.07		0.02	0.00					0.48	
Diporeia			12.50	78.63		8.62		0.75		1.54		56.78	78.75	38.42	25.96	0.52	10.72
Dreissena								13.51									
Eucycolops elegans																0.03	

Epischura lacustris	1.29	3.22					0.01			0.01	0.17				3.63	
Ephippia		0.01		0.00							0.12	0.00				
Eurycercus lamellatus			0.06		30.49	17.70		0.70							8.97	1.20
Fish eggs			12.43	0.02						0.04		0.37	0.10	0.87	1.34	
Harpacticoid adult				0.00	0.02			0.01							0.21	
Harpacticoid copepodite						0.00		0.00							0.04	
Holopedium gibberum	3.42	22.91														
Kellicottia							0.00									
Leptodiaptomus ashlandi					2.09				0.91	0.08	0.00				0.52	4.33
Limnocalanus macrurus	46.92	31.31					7.12		7.23	0.68	0.32				13.92	3.11
Leptodiaptomus minutus					0.01				0.01						0.04	0.40
Leptodiaptomus sicilis	26.35	10.77	0.02		33.31		0.36		50.59	1.29	0.74				11.89	34.16
Leptodiaptomus siciloides																0.03
Lirceus spp.																7.86
Mesocyclops edax										0.01						0.75
Mysis diluviana	8.76	0.52	38.58	1.86	4.10		91.86		7.00	29.70	22.85	42.86	21.16	60.72	26.00	
Nitokra hibernica					0.02											0.01
Nematode			12.57	0.00												
Ostracod		0.00				9.36										0.17
Paracyclops chiltoni																0.00
Paracyclops poppei																0.08
Senecella.calanoides	0.23								17.92						19.93	
Skistodiaptomus oregonensis									0.01	0.01					0.04	0.27
Skistodiaptomus pallidus										0.00						

Appendix L. Average wet biomass of prey taxa consumed per stomach and thiaminase activity for 17 sampling combinations

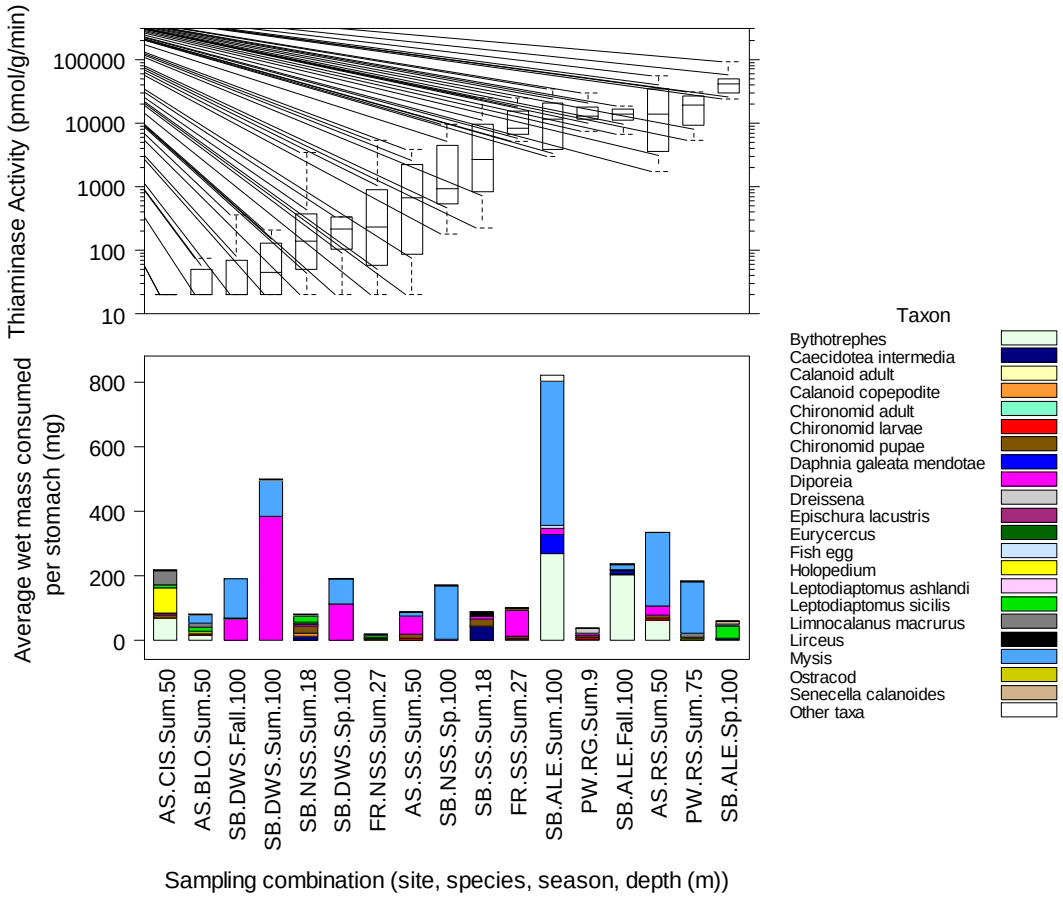

Figure Appendix L.1. Average wet biomass (mg) of prey taxa consumed and thiaminase activity for 17 sampling combinations

Average wet biomass (in mg) of each prey taxa consumed (bottom panel) and thiaminase activity (pmol/g/min; top panel) for 17 sampling combinations. All taxa that individually constitute less than one percent of the average wet biomass per sampling event are grouped together as “other taxa”.

Appendix M. NMS ordination of 45 taxa represented as proportional wet biomass and the relationship with thiaminase

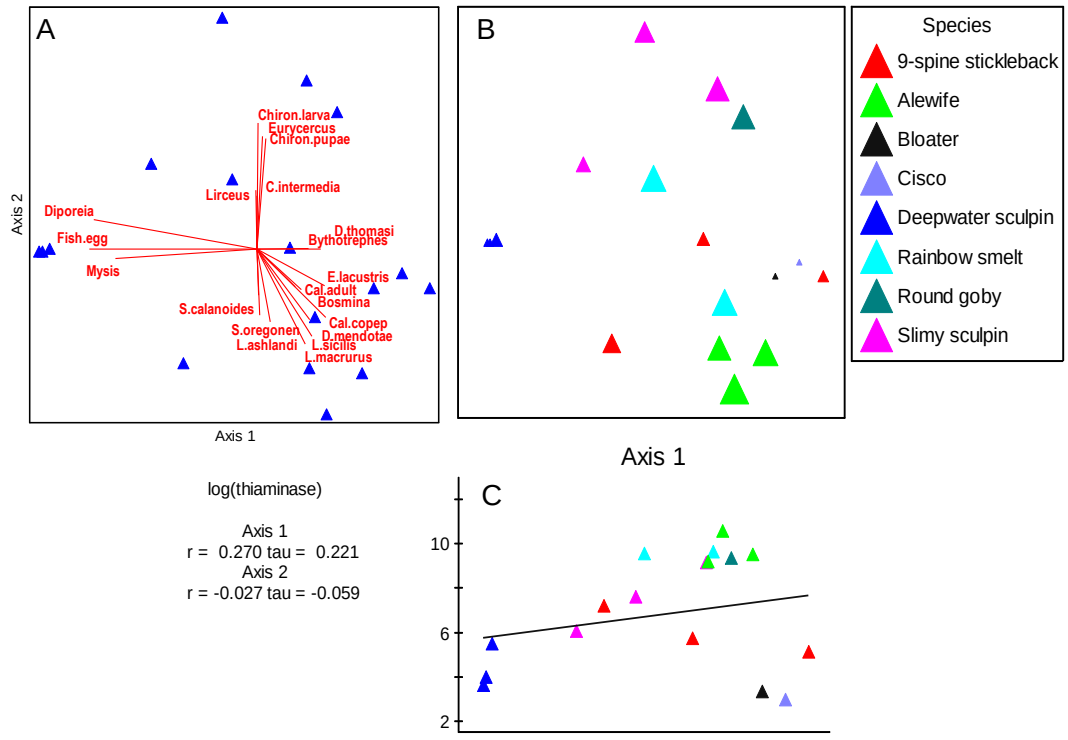

Figure Appendix M.1. NMS ordination of 45 taxa represented as proportional wet biomass and the relationship with thiaminase

NMS ordination of 17 sampling combinations in diet-space based on proportional wet biomass of 45 prey taxa. Points represent the 17 sampling combinations in diet-space. Diet-space is defined by the species shown in panel A, with vectors indicating the direction and strength (length of vector) associated with that portion of diet-space. The size of the points in panel B is proportional to thiaminase activity. The correlation between the axis 1 score and the log of thiaminase is illustrated in panel C. Axis 3 of this 3-dimensional ordination is not shown, but was unrelated to thiaminase activity (r~0.03).

Appendix N. Relationship between the proportional wet biomass of Bosmina and *Bythotrephes* in diet and thiaminase activity wet biomass and the relationship with thiaminase

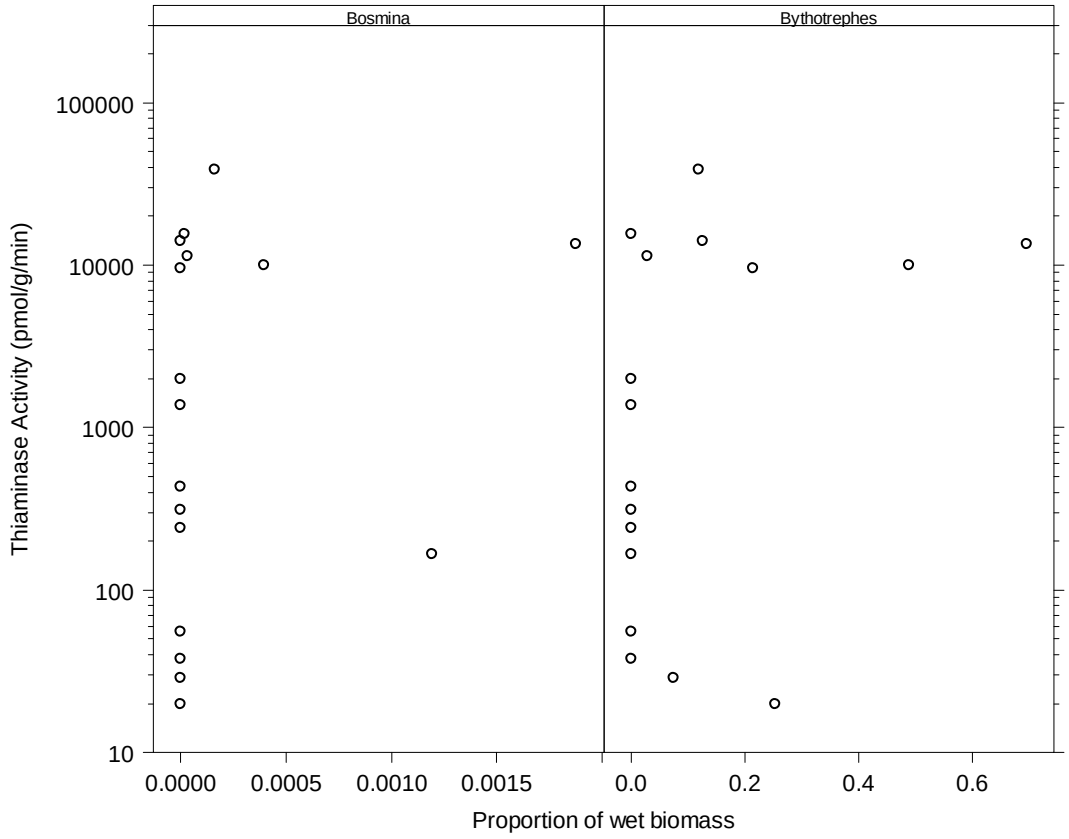

Figure Appendix N.1. Relationship between the proportional wet biomass of Bosmina and *Bythotrephes* in diet and thiaminase activity

The relationship between thiaminase activity (pmol/g/min) and the proportion of wet biomass in diets for *Bosmina* and *Bythotrephes*, the two taxa with discriminating ability for some high thiaminase groups in the ISA.

Appendix O. Average fatty acid (%) content for 23 sampling combinations.

Table Appendix O.1. Average fatty acid (%) content for 23 sampling combinations

Average fatty acid content (% of total fatty acid mass) for 23 sampling combinations.

	AS	AS	AS	AS	AS	AS	AS	DT	FR
	BLO Sum 50	CIS Sum 50	DWS Sum 50	LWF Sum 50	RS Sum 50	SPS Sum 50	SS Sum 50	SS Sum 37	NSS Sum 27
12:0	0.0	0.0	0.1	0.1	0.1	0.0	0.2	0.5	0.0
14:0	4.2	3.9	2.4	3.4	3.2	1.8	2.2	2.6	2.5
14:1n-5	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.3	0.0
15:0	0.5	0.6	0.4	0.4	0.4	0.4	0.4	0.3	0.3
16:0	13.7	14.4	13.0	14.6	14.8	12.7	14.2	14.2	16.1
16:1n-9	0.4	0.6	0.6	0.4	0.5	0.9	0.8	0.6	0.5
16:1n-7	6.4	5.4	11.0	7.3	7.4	9.6	10.8	13.3	7.3
17:0	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.5	0.7
17:1n-9	0.3	0.3	0.3	0.2	0.3	0.4	0.3	0.1	0.0
18:0	2.9	3.3	3.1	3.2	3.4	4.4	4.3	4.5	5.0
18:1n-9	17.1	14.1	15.9	16.2	13.2	15.4	14.3	11.1	10.1
18:1n-7	6.1	5.0	5.7	4.9	3.7	4.8	4.9	6.1	7.1
18:2n-6	5.2	5.7	5.1	3.9	4.9	4.2	4.2	4.2	4.5
11, 14-18:2	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.3	0.2
18:3n-3	3.6	4.2	3.1	2.6	3.0	2.4	2.5	1.3	2.9
18:4n-3	2.2	2.7	2.3	1.9	2.0	2.0	2.1	1.2	1.4
20:1n-13	0.7	0.6	0.2	0.3	0.2	0.2	0.2	0.4	0.3
20:1n-11	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.4	0.0
20:1n-9	1.1	1.0	0.7	1.0	0.8	0.7	0.7	0.7	0.7
20:2n-6	1.3	1.4	0.7	0.9	0.9	0.5	0.5	0.5	0.9
20:3n-6	0.4	0.5	0.1	0.3	0.2	0.1	0.1	0.1	0.3
20:3n-3	1.4	1.6	0.5	0.6	0.8	0.4	0.3	0.2	1.1
20:4n-6	2.5	3.2	4.3	4.1	5.0	6.2	5.5	4.1	4.3
20:4n-3	2.8	3.6	1.1	1.5	1.3	0.6	0.6	0.4	2.2
20:5n-3	6.4	7.3	13.3	8.7	10.7	9.6	11.0	17.7	10.9
22:1n-9	0.4	0.3	0.1	0.2	0.1	0.1	0.1	0.0	0.1
22:4n-6	0.6	0.6	0.1	0.4	0.3	0.3	0.2	0.4	0.5
22:5n-6	2.2	2.2	1.4	1.9	2.3	1.6	1.2	0.7	2.5
22:5n-3	3.7	3.9	1.3	2.7	1.5	4.8	3.9	4.6	6.4
22:6n-3	12.9	12.8	12.6	17.2	18.5	15.2	13.8	8.7	11.4

Table Appendix O.1. (Continued)

	FR SS Sum 27	PW RS Sum 7	PW RG Sum 9	SB ALE Sp 100	SB ALE Sum 100	SB ALE Fall 100	SB DWS SP 10	SB DWS Sum 100	SB DWS Fall 100
12:0	0.1	0.2	0.0	0.0	0.1	0.0	0.1	0.1	0.1
14:0	2.6	4.8	1.8	2.8	3.8	3.1	3.4	3.3	2.7
14:1n-5	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
15:0	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.2
16:0	15.2	13.5	15.4	16.5	17.5	16.4	11.6	12.0	11.8
16:1n-9	0.7	0.3	0.4	0.6	0.8	0.7	0.6	0.5	0.0
16:1n-7	12.5	14.7	9.2	3.8	5.3	3.9	20.6	19.8	19.5
17:0	0.6	0.7	0.7	0.5	0.7	0.6	0.2	0.3	0.0
17:1n-9	0.2	0.0	0.0	0.4	0.2	0.0	0.3	0.3	0.0
18:0	4.3	2.7	5.3	4.5	4.4	3.8	2.2	1.9	2.0
18:1n-9	10.7	12.3	10.2	14.9	13.5	18.1	18.6	18.9	20.0
18:1n-7	7.9	3.8	6.0	4.7	4.6	5.6	6.5	6.5	7.5
18:2n-6	2.7	4.2	4.0	3.7	5.1	5.3	3.4	3.2	3.0
11, 14-	0.3	0.1	0.2	0.1	0.1	0.0	0.2	0.2	0.1
18:3n-3	1.5	3.2	3.0	2.8	3.7	5.3	2.0	2.1	1.6
18:4n-3	1.2	2.6	1.8	1.3	3.0	4.5	1.8	2.3	2.3
20:1n-13	0.3	0.3	0.4	0.3	0.4	0.0	0.3	0.3	0.1
20:1n-11	0.2	0.0	1.3	0.1	0.0	0.0	0.0	0.0	0.0
20:1n-9	0.6	0.8	0.3	1.2	0.9	0.8	0.8	0.7	0.5
20:2n-6	0.4	0.7	0.4	0.9	1.0	0.5	0.5	0.5	0.3
20:3n-6	0.1	0.1	0.1	0.2	0.3	0.0	0.1	0.1	0.0
20:3n-3	0.3	0.8	0.2	0.8	1.1	0.6	0.4	0.5	0.3
20:4n-6	4.7	3.0	6.3	5.5	4.9	4.9	3.3	2.8	2.6
20:4n-3	0.4	1.1	0.4	1.6	2.1	1.7	0.9	1.1	0.8
20:5n-3	16.2	13.4	10.4	9.0	9.3	11.3	12.8	13.4	15.5
22:1n-9	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0
22:4n-6	0.4	0.2	0.9	0.3	0.4	0.1	0.1	0.1	0.0
22:5n-6	1.0	2.1	3.7	2.7	2.4	1.7	0.7	0.8	0.6
22:5n-3	5.4	1.5	5.0	2.5	2.7	2.3	0.8	1.0	0.7
22:6n-3	8.8	12.6	12.3	17.6	11.3	8.4	7.1	7.0	7.7

Table Appendix O.1. (Continued)

	FR NSS SP 100	SB NSS SP 100	SB RS Sum 100	SB SS Sum 18	SB SS Fall 100
12:0	0.0	0.0	0.1	0.2	0.1
14:0	2.9	2.4	3.5	2.2	2.4
14:1n-5	0.1	0.0	0.0	0.2	0.0
15:0	0.4	0.3	0.3	0.4	0.3
16:0	12.0	15.9	14.6	16.3	13.1
16:1n-9	0.7	0.6	0.5	0.8	0.0
16:1n-7	6.6	6.0	10.6	13.9	16.2
17:0	0.3	0.8	0.3	0.7	0.0
17:1n-9	0.3	0.0	0.3	0.1	0.0
18:0	3.4	4.2	2.6	4.5	2.4
18:1n-9	16.2	10.2	14.2	12.9	18.0
18:1n-7	7.1	5.6	3.9	6.1	6.4
18:2n-6	5.4	6.3	3.6	2.9	2.8
11, 14-18:2	0.4	0.2	0.1	0.2	0.0
18:3n-3	4.0	4.1	2.3	1.9	1.9
18:4n-3	1.8	1.7	1.7	1.0	2.8
20:1n-13	0.5	0.4	0.3	0.3	0.0
20:1n-11	0.1	0.0	0.1	0.3	0.0
20:1n-9	1.2	1.0	0.9	0.8	0.6
20:2n-6	1.5	1.4	0.8	0.5	0.2
20:3n-6	0.3	0.3	0.1	0.1	0.0
20:3n-3	1.6	1.9	0.7	0.3	0.2
20:4n-6	3.9	3.8	4.3	4.9	2.9
20:4n-3	2.0	3.3	1.1	0.4	0.7
20:5n-3	8.3	7.9	12.0	12.9	13.7
22:1n-9	0.2	0.2	0.0	0.0	0.0
22:4n-6	0.6	0.6	0.2	0.4	0.0
22:5n-6	2.1	3.5	1.9	1.0	0.8
22:5n-3	4.3	5.8	1.6	4.7	3.5
22:6n-3	11.4	11.7	17.6	9.0	11.0

Appendix P. Denaturing 2D gel of PT culture supernatant

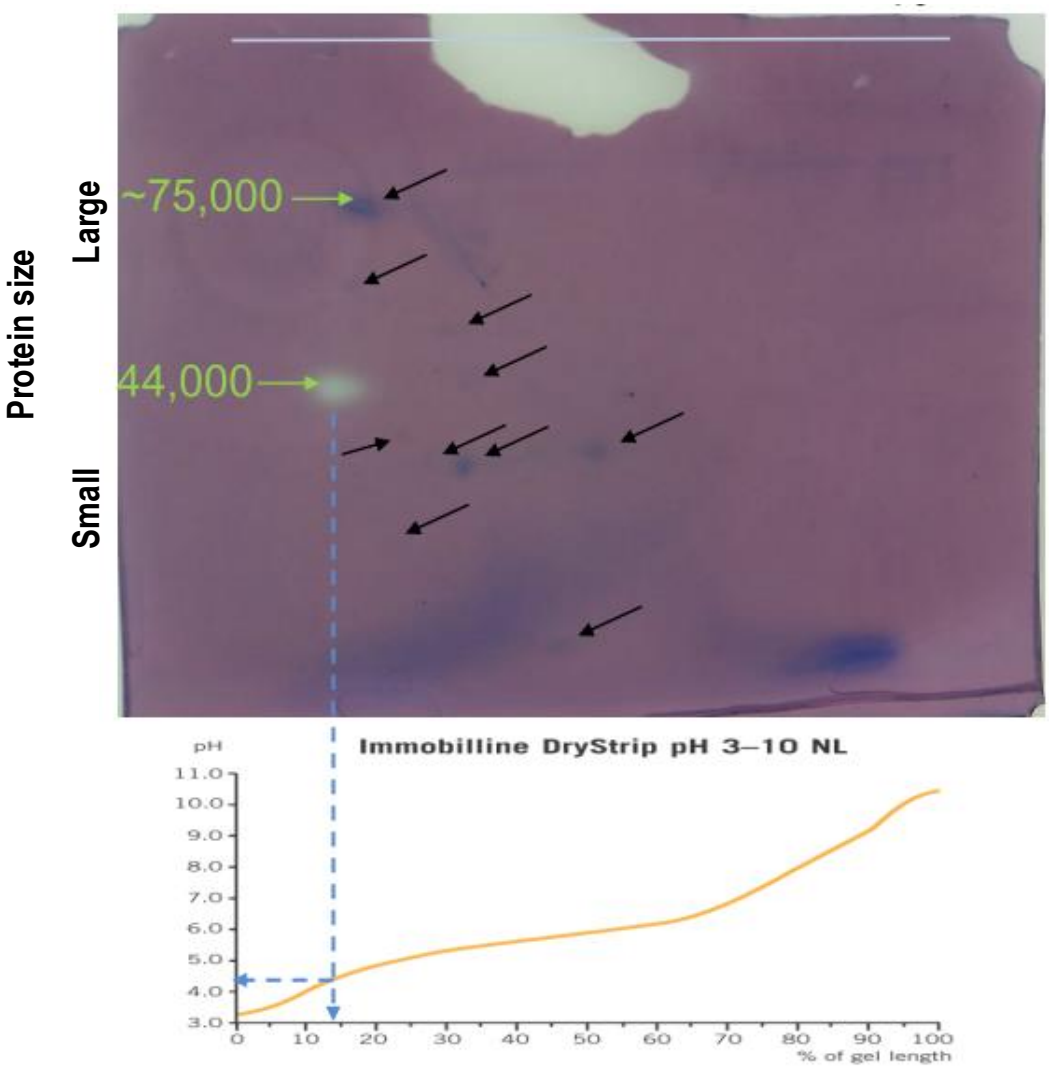

Figure Appendix P.1. Denaturing 2D gel of PT culture supernatant

A 2-dimensional gel (first dimension: denaturing IEF over pH 3-10, second dimension: a denaturing SDS-PAGE) showing thiaminase from PT culture supernatant. The vertical axis shows protein size and the PT thiaminase is known have a Mr of 44,000. The horizontal axis shows the isoelectric point, with proteins having an acidic isoelectric point on left and a basic isoelectric point on the right. The thiaminase enzyme is represented by the white clearing on the red (thiamine-indicating) gel directly above the isoelectric point of 4.6. Black arrows represent other (non-thiaminase) proteins (stained blue).

Appendix Q. Candidate gene sequences for thiaminases

Sequence homologous to: Carp thiaminase reported by Bos and Kozik (2000)
Organism containing gene: Carp
Sequence:

TCGAAAAGGGTGCACTTGAAGTCCTCTTTCAGGGACCCGGGTACCAGGATAAG
TTGCCATCTTTGATCAAGATGAACAACGATTTCGCTTTCCACTTGTACAAGAGAT
TGGTGGAAATGCCAGAATACCAATCCAAGAACATTTTCTTCTCTCCATTCTCTGT
GTCCATGGCTTTGTCTGAATTGTCTTTGGGTGCTGGTGGTGAAACAAAAGAACA
ATTATTGTCTGGTATCGGTCATAACTCCTCTGTTTTCTCTACTGAAGAAATGCAT
CAAATGTTCCATTCTTTGTTGGAAGAAATCGATCAAAGAACCGGTGTGGATATTA
ACGTTGGTTCTGCATTATACGCTTCCGATAAGTTGAAGTTGTTGCCAGAATTCTT
GAAAGAAATCAAAGAATTCTACCATTCTGATGGTTTCACCGTGGATTTCTCTGTG
AAAGAAACTTTGGATAAGATCAACACCTACGTGAAAGAAAAGACCCATGGTAAG
ATTGATCAAGCTGTGGATGATTTGGAATCCGATACTTTGATGTTCTTGTTGACCT
ACATCTACTTCAAGGGAAAATGGGATATGCCATTCAACCCATCTAAGACCTCTC
AATCTAGATTCCATGTTGATGCTGAAACTACCGTTCCAGTTCAAATGATGCATCA
GTACAAGTCCTTGAAGGTGTACTACGATGTTGAATTGACCTCTAAGGTGTTGTG
TTTGGATTACAACGATTCTTTCTCCATGTTCTTGGCTGTTCCAGATACTGATAGA
CCAGCTAAGACTATCAAGGATTTGGAAATGGCTATCTCTAGACAACATATCGAA
AAGTGGAGATCTGCTGTGAGAAAGAGAAAGACTGATATCTTCGTTCCAAAGTTG
TCCTTGAAAACCACCTACTCATTGAAGGATATCTTGAAGGGTATGGGTATGGCT
GATATGTTCTCTTACAGAGCTAACTTCACTGGTATCTCCGAAGAAAACATGTTGA
TCTCAAAGGTGTTGCATAAGGCTTCATTGGATATCGACGAAAAGGGTACTACTG
CTGCTGCTGTTACTACTGTTGATTTCAGACCAATGTCTTACTCTCCATTGGATAC
CTTGTCTTTCGATAGACCATTCATGATCTTCATCACCGATCAAAAGATGACCACC
TCTTCATCTTTGGAAAAGTTGTCTATTAGAAGAAAGAACTCCAACGTGGCTTTCA
AGATGTCTGAGCTCGCTCTGGTGCCACGCGGTAGTTCCGCTCATCACCACC

Sequence homologous to: Red Cornetfish thiaminase reported by Nishimune et al. 2008
Organism containing gene: Alewife
Sequence:

CCATGGCACAATCCGAAAACAAGGATGTGTACGATTTGTTGTGGGATAACAACA
TGGATATCGCTGAACAAACTTTGGAAGTGCCATTCTTGCAACATATGCAATTGG
GTGATTTGCAAGCTGATGATTACGTTTCTTTCACCATCCAAGATATCAACTACTT
GGTTAGAGTGACCGATATGTTGGGTGAAATGTGTGAAAAAGGTAAATTGCCTGA
AGATTTGCATGGTTTCATGAAGGAAAGATACGATTCCTACAACAACTACGCTGTT
GCTACATTGCAACAATTCAACTTGAACTCCGTGTCTGATATTAAGCCAACTCCAG
CTATGGAAAAGTACTTGTCTAACTACTCCGATATCATGGAAGGTGAAGAAGCTA
TCTATTTCGCTGTTGCTTTGTTGCCATGTTCTGTTTTGTGGTTGTGGTTGGCTAA
GCAATTGAAAGAAACTACCTGTAACGCTTACTTCACCTGGAAAAAGAACAACAT
GCATGGTCATCCAGAAGATCATTACAGAGCTTTGTTGGATAAGTACTTGACCAC
CAAAGAACAAATCGCTAAGGCTGATACCATCTTCAGACAACAAATGCAAAACGA
ACATGATTTCTTCGCTTCATCTTTGATCGAAAAAAAGGAGCTC

Sequence homologous to: Red Cornetfish thiaminase reported by Nishimune et al. 2008

Organism containing gene: Carp

Sequence:

CCATGGAAGACGTCTACGAATTCATTTGGGAAAACAACATCGATATCGCTTACA
AGACTATCAGAGGTGATTTCTTGATCCAAATGCAAAACGGTTCATTGCAAGCTG
AAAGATATATCTCCTTCACCATCCAAGATTTGAATTACGTTTTGAAGGTTGCTGA
AATGTTGAAAAAGATGTCTGCTAACGTTACCCAACCTAACGATTTGAAGGATTTC
TTGAACGGTAGATACTCCTCTTATAAGGGTTTCGGTAACTTGATGTTGAAGCAAT
ACTTCTTCAAAGGTGAACCACCAATCGAACAAACTCCAGCTATGAAGAAATACTT
GGCTTACTACAGAAACTTGATGGATAACGAAGAACCATTATACTTCGCTGTTGG
TTTGTTGCCATGTGCTAGATTGTGGGTTTGGTTGGCTAAGAATTTGAACACTCCA
TCAACTAACGCTTACTACACTTGGAAGGTTGAAAACATGGGTGGTCATCCAGAA
AAACATTACAGAGCTTTGTTGAACAAGTACTTGAACACTACCAAGACCGTTGAAA
AGGCTAACGCTATTTTCAGAGCACAAATGCCAAACGAACATGATTTCTTTTTGTC
CTCTGAGCTC

Sequence homologous to: Red Cornetfish thiaminase reported by Nishimune et al. 2008

Organism containing gene: Zebrafish

Sequence:

CCATGGAAGACGTCTACGAATACTTGTGGCAAAAGAACAAGGATTTGGCTGTTC
AAACCTTGAACTTGGATTTCTTGAGACAAATGGAATCTGGTTCCTTGCAAGCTGA
AAGATACGTTAACTTCACCATCCAAGATATCGGTTACGTTTTGGCTGTTACCAAG
ATGTTGAAAAGAATGTCTGCTGAAGTGTCCCAACCAGATGATATTAGAGATTTCA
TGAAGGGTAGATTCGCTTCTTACAAGTCTTTCGGTGAGTTGTTGTTGAATATGTA
CTTCTTCAAGGCTGAACCACCAATCCAAAGAACTCCAGCTTTGAGAAATTACTTG
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GCCATGTGCTAGATTGTGGGTTTGGTTGGCTAACAATTTGAACATCCCACAAAC
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AATGCTGTTTTCAGAGATCAAATGCAAAACGAGTACAAGTTCTTCTTGACCTTCG
AGCTC